Request for Reconsideration after Final Action

The table below presents the data as entered.

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LITERAL ELEMENT	CBDWATER
STANDARD CHARACTERS	YES
USPTO-GENERATED IMAGE	YES
MARK STATEMENT	The mark consists of standard characters, without claim to any particular font style, size or color.
ARGUMENT(S)	
Please see the actual argument text attached within the Evidence section.	
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DESCRIPTION OF EVIDENCE FILE	pdf files containing response to final office action including request for reconsideration and exhibits

GOODS AND/OR SERVICES SECTION (005)(current)

INTERNATIONAL CLASS	005
DESCRIPTION	

Dietary supplemental drinks; Liquid nutritional supplement; all of the foregoing comprised of cannabidiol

FILING BASIS	Section 1(b)

GOODS AND/OR SERVICES SECTION (005)(proposed)

INTERNATIONAL CLASS	005
TRACKED TEXT DESCRIPTION	

Dietary supplemental drinks; Liquid nutritional supplement; ~~all of the foregoing comprised of cannabidiol~~; all of the foregoing comprised of cannabidiol, and all of the foregoing being lawful under state and federal laws

FINAL DESCRIPTION	

Dietary supplemental drinks; Liquid nutritional supplement; all of the foregoing comprised of cannabidiol, and all of the foregoing being lawful under state and federal laws

FILING BASIS	Section 1(b)

GOODS AND/OR SERVICES SECTION (032)(current)

INTERNATIONAL CLASS	032
DESCRIPTION	

Bottled drinking water containing CBD; Bottled water comprised of CBD; Flavored bottled water containing CBD; Flavored waters containing CBD; Soda water comprised of CBD; Sparkling water comprised of CBD

FILING BASIS	Section 1(b)

GOODS AND/OR SERVICES SECTION (032)(proposed)

INTERNATIONAL CLASS	032

TRACKED TEXT DESCRIPTION

Bottled drinking water containing CBD; Bottled water comprised of CBD; Flavored bottled water containing CBD; Flavored waters containing CBD; Soda water comprised of CBD; ~~Sparkling water comprised of CBD~~; <u>Sparkling water comprised of CBD, all of the foregoing being lawful under state and federal laws</u>

FINAL DESCRIPTION

Bottled drinking water containing CBD; Bottled water comprised of CBD; Flavored bottled water containing CBD; Flavored waters containing CBD; Soda water comprised of CBD; Sparkling water comprised of CBD, all of the foregoing being lawful under state and federal laws

FILING BASIS	Section 1(b)

GOODS AND/OR SERVICES SECTION (035)(current)

INTERNATIONAL CLASS	035

DESCRIPTION

Promoting, advertising and marketing of the brands, products, services and online websites of individuals, businesses and nonprofit organizations

FILING BASIS	Section 1(b)

GOODS AND/OR SERVICES SECTION (035)(proposed)

INTERNATIONAL CLASS	035

TRACKED TEXT DESCRIPTION

~~Promoting, advertising and marketing of the brands, products, services and online websites of individuals, businesses and nonprofit organizations~~; <u>Promoting, advertising and marketing of the brands, products, services and online websites of individuals, businesses and nonprofit organizations, all of the foregoing being lawful under state and federal laws</u>

FINAL DESCRIPTION

Promoting, advertising and marketing of the brands, products, services and online websites of individuals, businesses and nonprofit organizations, all of the foregoing being lawful under state and federal laws

FILING BASIS	Section 1(b)

SIGNATURE SECTION

RESPONSE SIGNATURE	/Jane S. Berman/
SIGNATORY'S NAME	Jane S. Berman
SIGNATORY'S POSITION	Attorney of Record, Illinois Bar Member
SIGNATORY'S PHONE NUMBER	3128404333
DATE SIGNED	11/09/2018
AUTHORIZED SIGNATORY	YES
CONCURRENT APPEAL NOTICE FILED	YES

FILING INFORMATION SECTION

SUBMIT DATE	Fri Nov 09 15:53:48 EST 2018
TEAS STAMP	USPTO/RFR-XX.XX.XXX.XX-20 181109155348764315-871943 61-610d0b5635f821c6df0ca7 eb64fe4c4b2f60a1a70a92ea1 faa8461dceb0c7fe8-N/A-N/A -20181109154202087181

Request for Reconsideration after Final Action

To the Commissioner for Trademarks:

Application serial no. **87194361** CBDWATER(Standard Characters, see https://tmng-al.uspto.gov/resting2/api/img/87194361/large) has been amended as follows:

ARGUMENT(S)
In response to the substantive refusal(s), please note the following:

Please see the actual argument text attached within the Evidence section.

EVIDENCE
Evidence in the nature of pdf files containing response to final office action including request for reconsideration and exhibits has been attached.
Original PDF file:
evi_241215541-20181109154202087181_._Response87194361_Nov_9_2018.pdf
Converted PDF file(s) (15 pages)
Evidence-1
Evidence-2
Evidence-3
Evidence-4
Evidence-5
Evidence-6
Evidence-7
Evidence-8
Evidence-9
Evidence-10
Evidence-11
Evidence-12
Evidence-13
Evidence-14
Evidence-15
Original PDF file:
evi_241215541-20181109154202087181_._EXH_A_Nov_9_2018.pdf
Converted PDF file(s) (4 pages)
Evidence-1
Evidence-2
Evidence-3
Evidence-4
Original PDF file:
evi_241215541-20181109154202087181_._EXH_B_PART_1_Nov_9_2018.pdf
Converted PDF file(s) (85 pages)
Evidence-1
Evidence-2
Evidence-3
Evidence-4
Evidence-5
Evidence-6
Evidence-7
Evidence-8
Evidence-9
Evidence-10
Evidence-11

Original PDF file:

Converted PDF file(s) (50 pages)

Original PDF file:
evi_241215541-20181109154202087181_._EXH_B_PART_3_Nov_9_2018.pdf
Converted PDF file(s) (58 pages)

Original PDF file:
evi_241215541-20181109154202087181_._EXH_C_Nov_9_2018.pdf
Converted PDF file(s) (33 pages)

CLASSIFICATION AND LISTING OF GOODS/SERVICES
Applicant proposes to amend the following class of goods/services in the application:
Current: Class 005 for Dietary supplemental drinks; Liquid nutritional supplement; all of the foregoing comprised of cannabidiol
Original Filing Basis:
Filing Basis: Section 1(b), Intent to Use: *For a trademark or service mark application:* As of the application filing date, the applicant had a bona fide intention, and was entitled, to use the mark in commerce on or in connection with the identified goods/services in the application. *For a collective trademark, collective service mark, or collective membership mark application:* As of the application filing date, the applicant had a bona fide intention, and was entitled, to exercise legitimate control over the use of the mark in commerce by members on or in connection with

the identified goods/services/collective membership organization. ***For a certification mark application:*** As of the application filing date, the applicant had a bona fide intention, and was entitled, to exercise legitimate control over the use of the mark in commerce by authorized users in connection with the identified goods/services, and the applicant will not engage in the production or marketing of the goods/services to which the mark is applied, except to advertise or promote recognition of the certification program or of the goods/services that meet the certification standards of the applicant.

Proposed:
Tracked Text Description: Dietary supplemental drinks; Liquid nutritional supplement; ~~all of the foregoing comprised of cannabidiol~~; <u>all of the foregoing comprised of cannabidiol, and all of the foregoing being lawful under state and federal laws</u>

Class 005 for Dietary supplemental drinks; Liquid nutritional supplement; all of the foregoing comprised of cannabidiol, and all of the foregoing being lawful under state and federal laws
Filing Basis: Section 1(b), Intent to Use: ***For a trademark or service mark application:*** As of the application filing date, the applicant had a bona fide intention, and was entitled, to use the mark in commerce on or in connection with the identified goods/services in the application. ***For a collective trademark, collective service mark, or collective membership mark application:*** As of the application filing date, the applicant had a bona fide intention, and was entitled, to exercise legitimate control over the use of the mark in commerce by members on or in connection with the identified goods/services/collective membership organization. ***For a certification mark application:*** As of the application filing date, the applicant had a bona fide intention, and was entitled, to exercise legitimate control over the use of the mark in commerce by authorized users in connection with the identified goods/services, and the applicant will not engage in the production or marketing of the goods/services to which the mark is applied, except to advertise or promote recognition of the certification program or of the goods/services that meet the certification standards of the applicant.

Applicant proposes to amend the following class of goods/services in the application:
Current: Class 032 for Bottled drinking water containing CBD; Bottled water comprised of CBD; Flavored bottled water containing CBD; Flavored waters containing CBD; Soda water comprised of CBD; Sparkling water comprised of CBD
Original Filing Basis:
Filing Basis: Section 1(b), Intent to Use: ***For a trademark or service mark application:*** As of the application filing date, the applicant had a bona fide intention, and was entitled, to use the mark in commerce on or in connection with the identified goods/services in the application. ***For a collective trademark, collective service mark, or collective membership mark application:*** As of the application filing date, the applicant had a bona fide intention, and was entitled, to exercise legitimate control over the use of the mark in commerce by members on or in connection with the identified goods/services/collective membership organization. ***For a certification mark application:*** As of the application filing date, the applicant had a bona fide intention, and was entitled, to exercise legitimate control over the use of the mark in commerce by authorized users in connection with the identified goods/services, and the applicant will not engage in the production or marketing of the goods/services to which the mark is applied, except to advertise or promote recognition of the certification program or of the goods/services that meet the certification standards of the applicant.

Proposed:
Tracked Text Description: Bottled drinking water containing CBD; Bottled water comprised of CBD; Flavored bottled water containing CBD; Flavored waters containing CBD; Soda water comprised of CBD; ~~Sparkling water comprised of CBD~~; <u>Sparkling water comprised of CBD, all of the foregoing being lawful under state and federal laws</u>

Class 032 for Bottled drinking water containing CBD; Bottled water comprised of CBD; Flavored bottled water containing CBD; Flavored waters containing CBD; Soda water comprised of CBD; Sparkling water comprised of CBD, all of the foregoing being lawful under state and federal laws
Filing Basis: Section 1(b), Intent to Use: ***For a trademark or service mark application:*** As of the application filing date, the applicant had a bona fide intention, and was entitled, to use the mark in commerce on or in connection with the identified goods/services in the application. ***For a collective trademark, collective service mark, or collective membership mark application:*** As of the application filing date, the applicant had a bona fide intention, and was entitled, to exercise legitimate control over the use of the mark in commerce by members on or in connection with the identified goods/services/collective membership organization. ***For a certification mark application:*** As of the application filing date, the applicant had a bona fide intention, and was entitled, to exercise legitimate control over the use of the mark in commerce by authorized users in connection with the identified goods/services, and the applicant will not engage in the production or marketing of the goods/services to which the mark is applied, except to advertise or promote recognition of the certification program or of the goods/services that meet the certification standards of the applicant.

Applicant proposes to amend the following class of goods/services in the application:
Current: Class 035 for Promoting, advertising and marketing of the brands, products, services and online websites of individuals, businesses and nonprofit organizations
Original Filing Basis:
Filing Basis: Section 1(b), Intent to Use: ***For a trademark or service mark application:*** As of the application filing date, the applicant had a bona fide intention, and was entitled, to use the mark in commerce on or in connection with the identified goods/services in the application. ***For a collective trademark, collective service mark, or collective membership mark application:*** As of the application filing date, the applicant had a

bona fide intention, and was entitled, to exercise legitimate control over the use of the mark in commerce by members on or in connection with the identified goods/services/collective membership organization. *For a certification mark application:* As of the application filing date, the applicant had a bona fide intention, and was entitled, to exercise legitimate control over the use of the mark in commerce by authorized users in connection with the identified goods/services, and the applicant will not engage in the production or marketing of the goods/services to which the mark is applied, except to advertise or promote recognition of the certification program or of the goods/services that meet the certification standards of the applicant.

Proposed:
Tracked Text Description: ~~Promoting, advertising and marketing of the brands, products, services and online websites of individuals, businesses and nonprofit organizations~~; Promoting, advertising and marketing of the brands, products, services and online websites of individuals, businesses and nonprofit organizations, all of the foregoing being lawful under state and federal laws

Class 035 for Promoting, advertising and marketing of the brands, products, services and online websites of individuals, businesses and nonprofit organizations, all of the foregoing being lawful under state and federal laws
Filing Basis: Section 1(b), Intent to Use: *For a trademark or service mark application:* As of the application filing date, the applicant had a bona fide intention, and was entitled, to use the mark in commerce on or in connection with the identified goods/services in the application. *For a collective trademark, collective service mark, or collective membership mark application:* As of the application filing date, the applicant had a bona fide intention, and was entitled, to exercise legitimate control over the use of the mark in commerce by members on or in connection with the identified goods/services/collective membership organization. *For a certification mark application:* As of the application filing date, the applicant had a bona fide intention, and was entitled, to exercise legitimate control over the use of the mark in commerce by authorized users in connection with the identified goods/services, and the applicant will not engage in the production or marketing of the goods/services to which the mark is applied, except to advertise or promote recognition of the certification program or of the goods/services that meet the certification standards of the applicant.

SIGNATURE(S)
Request for Reconsideration Signature
Signature: /Jane S. Berman/   Date: 11/09/2018
Signatory's Name: Jane S. Berman
Signatory's Position: Attorney of Record, Illinois Bar Member

Signatory's Phone Number: 3128404333

The signatory has confirmed that he/she is an attorney who is a member in good standing of the bar of the highest court of a U.S. state, which includes the District of Columbia, Puerto Rico, and other federal territories and possessions; and he/she is currently the owner's/holder's attorney or an associate thereof; and to the best of his/her knowledge, if prior to his/her appointment another U.S. attorney or a Canadian attorney/agent not currently associated with his/her company/firm previously represented the owner/holder in this matter: (1) the owner/holder has filed or is concurrently filing a signed revocation of or substitute power of attorney with the USPTO; (2) the USPTO has granted the request of the prior representative to withdraw; (3) the owner/holder has filed a power of attorney appointing him/her in this matter; or (4) the owner's/holder's appointed U.S. attorney or Canadian attorney/agent has filed a power of attorney appointing him/her as an associate attorney in this matter.

The applicant is filing a Notice of Appeal in conjunction with this Request for Reconsideration.

Serial Number: 87194361
Internet Transmission Date: Fri Nov 09 15:53:48 EST 2018
TEAS Stamp: USPTO/RFR-XX.XX.XXX.XX-20181109155348764
315-87194361-610d0b5635f821c6df0ca7eb64f
e4c4b2f60a1a70a92ea1faa8461dceb0c7fe8-N/
A-N/A-20181109154202087181

RESPONSE TO FINAL OFFICE ACTION

U.S. Trademark Application Serial No.:	**87194361**
Mark:	**CBDWATER** **(Word mark in standard characters)**
Applicant:	**Canna Farm LLC**
Final Office Action Issued On:	**May 10, 2018**
Attorney Docket No.:	**CAN20-00101**

1.　　Under 37 C.F.R. §2.63(b)(3), Applicant files this timely request for reconsideration seeking to overcome the substantive refusals and to comply with outstanding requirements before the deadline for filing an appeal. Applicant is also filing a timely notice of appeal under 37 C.F.R. §2.63(b)(1).

2.　　Applicant requests reconsideration of the final substantive refusals to register and the final requirement for information set forth in the final Office action issued May 10, 2018 (the "Final Action"). The response also addresses matters maintained and continued from the first non-final Office action issued January 5, 2017 (the "First Action") and the second non-final Office action issued October 3, 2017 (the "Second Action"). As evidence provided by Applicant in its first Response filed July 5, 2017 (the "First Response") and in its second Response filed April 3, 2018 (the "Second Response") is considered to remain applicable to the present refusals and requests, the contents of the First Response and the Second Response are herein incorporated by reference.

REPLY TO FINAL REFUSAL: NO LAWFUL USE IN COMMERCE

3.   As to the goods in Class 005, as amended, namely: "dietary supplemental drinks; liquid nutritional supplement; all of the foregoing comprised of cannabidiol, and all of the foregoing being lawful under state and federal laws," and as to the goods in Class 032, as amended, namely: "bottled drinking water containing CBD; bottled water comprised of CBD; flavored bottled water containing CBD; flavored waters containing CBD; soda water comprised of CBD; sparkling water comprised of CBD, all of the foregoing being lawful under state and federal laws," it is respectfully submitted that the refusal to register should be reconsidered and withdrawn. Applicant has a bona fide intent to lawfully use the applied-for mark in commerce, in compliance with Trademark Act Sections 1 and 45, 15 U.S.C. §§1051, 1127. Applicant's identification of goods excludes any items that are not lawful under state and federal laws. Thus, the goods to which the proposed mark will be applied are, by definition, lawful under the federal Controlled Substances Act (CSA), 21 U.S.C. §§801-971.

4.   The record fails to establish that Applicant's Class 005 and Class 032 goods will be unlawful. Applicant's amended identification of goods in Class 005 and Class 032 explicitly excludes any item that is derived from unlawful sources. The Declaration of Robert DiVito (attached hereto as **EXHIBIT A**) provides facts that refute the examining attorney's presumptions on this point. Applicant intends to synthesize, or otherwise to obtain, the cannabidiol referenced in the identification of goods in a manner that is in full accordance and compliance with all state and federal laws and regulations, and to sell its Class 005 and Class 032 goods in a manner that is in full accordance and compliance with all state and federal laws and regulations. In contrast to the examining attorney's finding, based entirely on the examining attorney's speculation, Exhibit A provides factual evidence that the goods will be lawful.

5.   Because the record fails to show that Applicant's Class 005 and Class 032 goods will be unlawful, Applicant submits that the refusal to register under Trademark Act Sections 1

and 45, 15 U.S.C. §§1051, 1127 is premature. The examining attorney should, at the least, defer the refusal until the time of submission of a statement of actual use and specimens.

6.   The examining attorney has erred in dismissing the possibility of lawful sourcing of cannabidiol as merely "theoretical." It is submitted this dismissal is not based on the evidence of record. Scientific advances have been made in synthesizing, sourcing, extracting, and concentrating cannabidiol since publication of the outdated reports relied upon by the examining attorney (Mölleken & Husmann (1997); S.A. Ross et al (2000)). For example, attached hereto as **EXHIBIT B** are selected references identifying non-cannabis sources of cannabidiol that are lawful under state and federal law.

a.   Poulos et al, U.S. Patent No. 10,093,949, discloses a method of producing cannabidiol using genetically engineered yeast.

b.   Medical Marijuana, Inc. distributes a cannabidiol oil sourced from hops (accessed at https://www.medicalmarijuanainc.com/humulus/;

see also https://dabsmagazine.com/you-dont-even-need-cannabis-to-make-organic-cbd-anymore/;

https://merryjane.com/are-fears-of-canadian-cannabis-shortages-justified-october-2-18; and

https://westword.com/cbd-derived-from-the-hop-plant-not-cannabis-now-offered-by-peak-health-foundation-10578719).

c.   Murphy, U.S. Patent App. Pub. No. US 2018/0153948 A1, discloses improved systems and methods for extracting from non-*cannabis* plant species cannabinoids (which includes cannabidiol) or similar compounds having affinity for cannabinoid receptors.

     d.   Winnicki, et al., U.S. Patent App. Pub. No. US 2018/0179564 A1, discloses improved methods and apparatus for biosynthetic production of cannabinoids (which includes cannabidiol) using yeast species.

It is submitted that, by the time of submission of Applicant's statement of use as to the listed goods in Class 005 and Class 032, even more lawful sources of cannabidiol will be available due to the swift advance of scientific research in this area exemplified by the references in Exhibit B.

     7.   Further showing that Applicant's Class 005 and Class 032 goods will be lawful when sold are the reports cited in HEMP INDUSTRIES ASSOCIATION, et al., v. DRUG ENFORCEMENT ADMINISTRATION, et al., 2017 WL 1346354 (C.A.9), 8 (Brief of Petitioners at 7), already of record. The reports summarize studies indicating that cannabinoids such as cannabidiol may be derived from non-cannabis plant sources such as coneflower (Echinacea), oxeye (Heliopsis helianthoides), electric daisy (Acmella oleracea), Helichrysum umbraculigerum, liverwort (Radula marginata), black pepper (Piper nigrum), chocolate (Theobrama cacao) plants, Echinacea purpurea, Echinacea angustifolia, Acmella oleracea, Helichrysum umbraculigerum, and Radula marginata, with lipophilic alkamides (alkylamides) from Echinacea species being the best-known). The examining attorney has erred in disregarding these studies showing that cannabinoids may be sourced from non-cannabis plants, on grounds that Ninth Circuit denied the petition in question. The examining attorney deferred to the Ninth Circuit's decision to deny the petition, and to the "DEA's authority to amend and interpret the regulations for which [it] is primarily responsible for administering."

     8.   First, with regard to the examining attorney's mention of the DEA's authority to amend and interpret regulations, Applicant points out that the DEA recently placed drug products in a finished dosage formulation that has been approved by the U.S. FDA that contains cannabidiol derived from cannabis on the least-restrictive schedule, Schedule V, of the

Controlled Substances Act. *See FDA-APPROVED DRUG EPIDIOLEX PLACED IN SCHEDULE V OF CONTROLLED SUBSTANCES ACT, DOJ 18-1265,* 2018 WL 4628300 (D.O.J., D.E.A., Sept. 27, 2018); *Schedules of Controlled Substances: Placement in Schedule V of Certain FDA-Approved Drugs Containing Cannabidiol; Corresponding Change to Permit Requirements,* 83 FR 48950-02, 2018 WL 4632087 (September 28, 2018). This change in scheduling of an FDA-approved drug product containing cannabis-derived cannabidiol shows that it is possible, and even likely, that DEA will undertake other scheduling changes affecting cannabidiol in the near future. Thus, recent regulatory changes by the DEA shows it is improper for the examining attorney to presume that Applicant will be unable to legally perfect its intent-to-use basis as to its Class 005 goods in the future. The examining attorney should, at the least, defer the refusal until the time of submission of a statement of actual use and specimens.

9. Second, the Ninth Circuit's denial of the *Hemp Industries Assn.* petition, and in turn, the examining attorney's dismissal of the cited reports, fail to diminish the scientific findings in the cited reports. The reports included:

a. Bauer et al., CB Receptor Ligands from Plants, 8 *Curr. Topics in Med. Chem.* 173, 173-86 (2008);

b. R. Bauer, P. Remiger, TLC and HPLC Analysis of Alkamides in Echinacea Drugs, 55 *Planta Medica* 367, 367-71 (1989);

c. Stefan Raduner et al., Alkylamides from Echinacea Are a New Class of Cannabinomimetics: Cannabinoid Type 2 Receptor-Dependent and -Independent Immunomodulatory Effects, 281 *J. of Bio. Chem.* 14,192, 14,192-206 (2006);

d. Nigel B. Perry et al., Alkamide Levels in Echinacea purpurea: A Rapid Analytical Method Revealing Differences among Roots, Rhizomes, Stems, Leaves and Flowers, 63 *Planta Medica* 58, 58-62 (1997);

e. Xian-guo He et al, Analysis of alkamides in roots and achenes of Echinacea purpurea by liquid chromatography-electrospray mass spectrometry, 815 *J. of Chromatography A* 205, 205-11 (1998);

f. Allesia Ligresti et al., Kavalactones and the endocannabinoid system: The plant-derived yangonin is a novel CB1 receptor ligand, 66 *Pharmacological Research* 163, 163-169 (2012);

g. G. Korte al., Tea catechins' affinity for human cannabinoid receptors, 17 *Phytomedicine* 19, 19-22 (2010);

h. Jurg Gertsch et al., Beta-caryophyllene is a dietary terpene, 105 *Proceedings of Nat'l Academy of Sciences* 9099, 9099-9104;

i. Giovanni Pcioni et al., Truffles contain endocannabinoid metabolic enzymes and anandamide, 110 *Phytochemistry* 104, 104-10 (2015).

10. The reports cited above support Applicant's factual statements of its bona fide intent to lawfully source and sell "dietary supplemental drinks; liquid nutritional supplement; all of the foregoing comprised of cannabidiol, and all of the foregoing being lawful under state and federal laws."

11. The examining attorney cited *In Re Morgan Brown*, 119 U.S.P.Q.2d 1350, 2016 WL 4140917 (T.T.A.B. July 14, 2016) in support of the examining attorney's determination that "[b]ecause the identification is unrestricted as to the source of the CBD used to be used in the goods, it is proper to presume that applicant will use CBD obtained from Cannabis sativa L, an unlawful source." Because the identification of Class 005 and Class 032 goods in the present application has been restricted to goods that are "lawful under state and federal laws," the analysis of *Brown* is inapplicable to the present application, and reliance upon *Brown* is unsupported by the present record.

12.   In *Brown*, the Board found there was unrebutted evidence of record that the applicant's mark was actually "being used in connection with sales of a specific substance (marijuana) that falls within both the services identification and the prohibitions of the CSA." *Brown*, 2016 WL 4140917, at *5. The Board thus found that "Applicant's retail store services include sales of a good that is illegal under federal law, and therefore encompasses a use that is unlawful. *Id.* Unlike in *Brown,* there is no *per se* violation of law of record in the present application, as Applicant has established that it intends to source its cannabidiol only from lawful sources. *See* Exhibit A.   Further, it is an impossibility for the Applicant to perfect its intent-to-use basis by declaring use as to any goods prohibited by the CSA, or by any other laws, because the identification of goods by its own terms excludes all unlawful goods. The goods identification at issue in *Brown* lacked the explicit exclusion of unlawful goods contained in the description of Class 005 and Class 032 goods in the instant application.

13.   The examining attorney cited *In Re JJ206, LLC, DBA Juju Joints*, 120 U.S.P.Q.2d 1568, 2016 WL 7010624 (T.T.A.B. Oct. 27, 2016) in support of the conclusion that "[b]ecause the identified goods and/or services consist of or include items or activities that are prohibited under the Controlled Substances Act, applicant cannot have a bona fide intent to lawfully use the applied-for mark in commerce in connection with the goods." In *In re JJ206*, one of the two applications at issue was based upon an intent to use the mark in commerce for "smokeless cannabis vaporizing apparatus, namely, oral vaporizers for smoking purposes; vaporizing cannabis delivery device, namely, oral vaporizers for smoking purposes." *JJ206*, 2016 WL 7010624, at *1. The other application was based on actual use of the mark in commerce for "smokeless marijuana or cannabis vaporizer apparatus, namely, oral vaporizers for smokers; vaporizing marijuana or cannabis delivery device, namely, oral vaporizers for smoking purposes." *Id.* Thus, in both applications, the applicant explicitly identified its goods as vaporizing devices for cannabis or marijuana. The Board found that, "because Applicant's

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identified goods constitute illegal drug paraphernalia under the CSA, Applicant's use and intended use of the applied-for marks on these goods is unlawful, and cannot serve as the basis for federal registration. *Id.,* at *5.

14. In contrast to *JJ206,* in the instant application, Applicant explicitly excludes from the identification any unlawful goods, specifying that the goods are "lawful under state and federal laws." Unlike the situation in *JJ206* where it was "'a legal impossibility' for the applicant to have the requisite bona fide intent to use the mark," *id.,* at *2, in the instant application, it is an impossibility for the Applicant to perfect its intent-to-use basis by declaring use as to any goods prohibited by the CSA, or by any other laws, because the identification of goods by its own terms excludes all unlawful goods. Applicant cannot submit, and the Office cannot accept, a statement of use and specimens in this application declaring use for unlawful goods, as such have been explicitly excluded from the identification of goods. Thus, the "legal impossibility" found in *JJ206* does not exist in the present application. *JJ206* must be distinguished on this basis. Applicant respectfully submits that the examining attorney's reliance upon *JJ206* in determining that Applicant cannot have a bona fide intent to lawfully use the applied-for mark in commerce in connection with the goods is thus based on error, unsupported by the record.

15. Applicant notes that the *JJ206* and *Brown* decisions cited by the examining attorney were followed by *In re PharmaCann LLC,* 2017 WL 2876812 (T.T.A.B. June 16, 2017), but the Board there affirmed a refusal of intent-to-use applications covering "retail store services featuring medical marijuana," in International Class 35, and "dispensing of pharmaceuticals featuring medical marijuana," in International Class 44. *Id.,* at *1. But like the analyses in *JJ206* and *Brown,* the analysis in *PharmaCann* cannot support the examining attorney's refusal to register in the instant application. This is because the services descriptions in *PharmaCann* explicitly reference marijuana, and thus directly implicate violation of the

CSA. The services descriptions in *PharmaCann* also lack the exclusion of unlawful goods contained in the description of Class 005 goods in the instant application.

REPLY TO REQUIREMENT FOR INFORMATION

16.   The following is submitted in response to the examining attorney's determination that "applicant did not provide a satisfactory response to Question 4 in the January 5, 2017 or October 3, 2017 Office Actions." The Final Action continued and made final the requirement for response to Question 4:

> *4.   Do or will the identified services in Class 35 enable applicant or consumers to disseminate, distribute, purchase, buy, sell, trade or barter any goods which contain as an ingredient marijuana, cannabis, hemp, marijuana-based, cannabis-based or hemp-based preparations, or marijuana, cannabis or hemp-based extracts or derivatives, synthetic marijuana, or any other illegal controlled substances?*

The examining attorney has characterized this complex, multi-part question as "a simple 'yes' or 'no' question," in the Final Action, but it is not a simple 'yes' or 'no' question. Nonetheless, Applicant will attempt in the following to comply with the examining attorney's requirement for a simple 'yes' or 'no' answer to Question 4.

17.   The identified services in Class 035 will not enable applicant or consumers to disseminate, distribute, purchase, buy, sell, trade or barter any goods which contain as an ingredient marijuana, cannabis, hemp, synthetic marijuana, or any other illegal controlled substances. Applicant has no intent to provide any services that will enable applicant or consumers to violate any laws, including the CSA.

18.   The identified services in Class 035 will not enable applicant or consumers to disseminate, distribute, purchase, buy, sell, trade or barter any goods which contain as an ingredient marijuana-based, cannabis-based or hemp-based preparations, or marijuana, cannabis

or hemp-based extracts or derivatives, to the extent that any of the foregoing are unlawful under any laws, including the CSA. Applicant has no intent to provide any services that will enable applicant or consumers to violate any laws, including the CSA.

19.   Many preparations, extracts, or derivatives falling within the list in the examining attorney's "Question 4" are not illegal controlled substances. For example, reference is made to U.S. Trademark Reg. Nos. 5363541, 5139076, 5589298, 5543497, 5193224, 5288805, and 5498192 (*see* TESS records and registration certificates copied in **EXHIBIT C,** attached). These registrations list examples of goods that contain hemp-based preparations, extracts, or derivatives that may be sold in U.S. commerce without violating any laws, including the CSA. The goods listed in these registrations provide examples of the sorts of products that fall within the examiner's Question 4 categories and yet do not implicate the CSA or violate any laws. Thus, Applicant's services of "Promoting, advertising and marketing of the brands, products, services and online websites of individuals, businesses and nonprofit organizations" may involve promoting, advertising and marketing of such goods while not indicating the violation of any laws. As Applicant has explicitly excluded any unlawful goods from its identifications of goods and recitations of services in this application, it is submitted that Applicant's response to Question 4 should now be accepted.

20.   Other preparations, extracts or derivatives falling within the list in the examining attorney's "Question 4" that are not illegal controlled substances may be exemplified by the plant extracts referenced in the scientific journal reports listed in Para. 9 above. Also, Exhibit B illustrates other preparations, extracts or derivatives that fall within the examining attorney's list in Question 4 but that are not illegal controlled substances.

21.   Although the Final Action is not clear, it appears that the examining attorney has already accepted as adequate Applicant's prior responses to the other requirements for

information (other than Question 4), and so it is respectfully submitted that all requirements for information have now been adequately addressed.

SECTION 2(E)(1) REFUSAL TO REGISTER AS TO ALL CLASSES

22.   Applicant incorporates by reference all evidence of record and all prior arguments traversing refusal to register on grounds of Section 2(e)(1) descriptiveness. Applicant maintains and preserves all its arguments for appeal.

23.   The examining attorney has engaged in impermissible dissection of the coined mark in making the finding of descriptiveness. CBDWATER is a unitary coined mark, originated and created by Applicant for use in connection with its Class 5, 32, and 35 goods and services. The mark is fanciful insofar as CBD is known to be water-insoluble, and so the telescoped mark CBDWATER has an incongruous, nonsensical meaning as applied to the listed goods and services. "Cannabidiol is insoluble in water but soluble in organic solvents such as pentane."   Wikipedia   entry,   "Cannabidiol," https://en.wikipedia.org/wiki/Cannabidiol (accessed on April 3, 2018).

24.   With respect to the Class 35 services of "promoting, advertising and marketing of the brands, products, services and online websites of individuals, businesses and nonprofit organizations, all of the foregoing being lawful under state and federal laws" there is nothing in the mark that conveys an idea of the qualities or characteristics of the promotional, advertising, and marketing services.

25.   As to the Class 5 goods of "dietary supplemental drinks; Liquid nutritional supplement, all of the foregoing comprised of cannabidiol, and all of the foregoing being lawful under state and federal laws," the mark is at best suggestive of the goods as it fails to immediately convey an immediate idea of "dietary supplemental drinks" or "liquid nutritional supplement."

26.   As to Class 32, the mark is incongruous and cannot be immediately understood to apply to the listed goods, as it is known that CBD is insoluble in water. *See* Wikipedia article, *supra.* Thus, it is submitted that the refusal to register the mark on grounds of mere descriptiveness under Section 2(e)(1) should be reconsidered and withdrawn as to all three classes.

27.   A term is merely descriptive of goods or services within the meaning of Section 2(e)(1) of the Trademark Act only if it forthwith conveys an immediate idea of an ingredient, quality, characteristic, feature, function, purpose, or use of the goods or services. *In re Gyulay,* 3 USPQ2d 1009 (Fed. Cir. 1987); *In re Abcor Development Corp.*, 200 USPQ 215, 217-18 (CCPA 1978); *J.S. Paluch Co., Inc. v. Irwin,* 215 USPQ 533, 536 (TTAB 1982); *In re Tennis in the Round, Inc.*, 199 USPQ 496, 498 (TTAB 1978). Under this standard, a mark is suggestive, rather than descriptive, if it requires imagination, thought, and perception to reach a conclusion as to the nature of the covered services or goods. *In re Mayer-Beaton Corp.,* 223 USPQ 1347, 1349 (TTAB 1984); *Plyboo Am., Inc. v. Smith & Fong Co.,* 51 USPQ2d 1633, 1640 (TTAB 1999). The Examining Attorney bears the burden of proving that Applicant's mark is merely descriptive of the cited goods or services. TMEP Section 1209.02. And any doubt as to the mere descriptiveness of a word or phrase must be resolved in an applicant's favor. *In re Conductive Systems, Inc.*, 220 USPQ 84, 86 (TTAB 1983); *In re Gourmet Bakeries, Inc.*, 173 USPQ 565 (TTAB 1972).

28.   A mark that suggests a number of things, but falls short of describing the goods with "any degree of particularity," is not merely descriptive. See *In re TMS Corp. of the Americas*, 200 USPQ 57, 59 (TTAB 1978). Rather, "[i]f information about the product or service given by the term used as a mark is indirect or vague, then this indicates that the term is being used in a 'suggestive' not descriptive manner." 2 J. Thomas McCarthy, MCCARTHY ON TRADEMARKS AND UNFAIR COMPETITION, Section 11:19. To be merely descriptive, a

mark must immediately convey some particular and clear idea about the goods and services. See, e.g., *In re Hutchinson Technology*, 7 USPQ2d 1490, 1493 (Fed. Cir. 1988) (finding that "technology is a very broad term which includes many categories of goods" and thus "does not convey an immediate idea of the 'ingredients, qualities, or characteristics of the goods' listed in [the application]").

29.   The term "CBD" has numerous meanings in various contexts, and CBDWATER, the unitary mark, has even more possible meanings. In this instance, consumers must exercise some imagination to draw this connection and meaning from the term relative to Applicant's products - the very definition of a "suggestive" and not "descriptive" mark. This is underscored by the fact that CBD and WATER telescoped into a unitary mark CBDWATER is a nonsensical and fanciful term, as the two literally do not mix, as CBD is known to be insoluble in water. And so, any possible association between Applicant's mark and Applicant's goods, and in particular Applicant's Class 35 services, is far less immediate than the association between marks and covered goods and services previously held to be suggestive. Suggestive examples include: SUGAR & SPICE for bakery products, *In re Colonial Stores, Inc.,* 394 F.2d 549 (CCPA 1968); SNO-RAKE for a snow removal hand tool, *In re Shutts*, 217 U.S.P.Q. 363 (TTAB 1983); WET/DRY BROOM for electric vacuum cleaners, *In re Shop-Vac Corp.,* 219 U.S.P.Q. 470 (TTAB 1983); HANDI WIPES for dusting cloths, *In re Colgate-Palmolive Co.,* 149 U.S.P.Q. 793 (TTAB 1966). Each of these marks is far more direct than Applicant's mark in conveying a characteristic or quality of the relevant goods or services, yet each was found capable of registration on the Principal Register.

30.   It is notable that the Examining Attorney has offered only minimal evidence of either "Competitors' Use" or "Competitors' Need" tests in rendering the descriptiveness refusal, particularly with respect to the Class 5 goods and the Class 35 services. The explanation is simple. Such evidence is *de minimus* at most. Competitors have no "need" to use the phrase

CBDWATER to identify the Applicant's listed Class 5, 32, and 35 goods and services, because that phrase conveys no clear, definitive, or descriptive information about them.

31.   Recognizing that "the suggestive/descriptive dichotomy can require the drawing of fine lines and often involves a good measure of subjective judgment," the TTAB in *Shutts* held that "it is clear that such doubts are to be resolved in favor of applicants." *In re Shutts*, 217 USPQ 363, 365 (TTAB 1983). The Examining Attorney should follow *Shutts* and resolve any doubts about the descriptiveness of the CBDWATER mark in favor of Applicant.

32.   As to Class 035, additional reference is made to Exhibit C to this Response as evidence of non-descriptiveness of the services as applied to promoting, advertising and marketing of goods such as those listed in the registrations of Exhibit C. The registrations contain examples of the types of goods that Applicant intends to promote, advertise and market when it provides the services listed in the Class 035 recitation in the future. The examples of the types of goods reveal that the mark is not merely descriptive as to such goods.

33.   Thus, as to all three classes of goods and services, it is submitted that the refusal to register the mark on grounds of mere descriptiveness under Section 2(e)(1) should be reconsidered and withdrawn.

CONCLUSION

34.   It is respectfully submitted that all requirements raised by the examining attorney have been addressed. Reconsideration and withdrawal of all refusals to register are hereby respectfully requested in view of the arguments, amendments, and evidence presented in and with this Response, the First Response and the Second Response. It is submitted that the application is in condition for approval for publication, and early, favorable treatment of the application is requested. The examining attorney is encouraged to contact the undersigned counsel for Applicant with any additional requests for information.

Respectfully submitted,

Signature:_____/Jane S. Berman/_____

Jane S. Berman

Attorney of Record, Illinois Bar Member

Date: November 9, 2018
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EXHIBIT A

Begins on the following page

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

U.S. Trademark Application Serial No.:   87194361

Mark: **CBDWATER**
                          (Word mark in standard characters)

Applicant: **Canna Farm LLC**

Attorney Docket No.:   **CAN20-00101**

DECLARATION OF ROBERT DIVITO

I, Robert DiVito, declare the following:

1.   I am a Manager of Canna Farm LLC, the Applicant in the above-referenced trademark and service mark application, Application No. 87/194,361 for the mark CBDWATER (the "Mark").

2.   I have personal knowledge of the matters set forth in this Declaration, and I am competent to make the following statements.

3.   The Applicant Canna Farm LLC has a bona fide intention to use the Mark in the future on the goods in Classes 005 and 032 identified in the application, and in connection with the services in Class 035 recited in the application, in a way that fully complies with the Controlled Substances Act (the "CSA") and all other applicable laws.

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4. The Applicant intends to use the Mark only on lawful goods and services. Accordingly, the Applicant has amended the identification of goods and recitation of services in this Application to the following:

In Int. Class 005: Dietary supplemental drinks; liquid nutritional supplement; all of the foregoing comprised of cannabidiol, and all of the foregoing being lawful under state and federal laws

In Int. Class 032: Bottled drinking water containing CBD; Bottled water comprised of CBD; Flavored bottled water containing CBD; Flavored waters containing CBD; Soda water comprised of CBD; Sparkling water comprised of CBD, all of the foregoing being lawful under state and federal laws

In Int. Class 035: Promoting, advertising and marketing of the brands, products, services and online websites of individuals, businesses and nonprofit organizations, all of the foregoing being lawful under state and federal laws

5. The identified goods in this application in Classes 005 and 032 will not include as an ingredient any illegal controlled substances at the time of their sale. The goods in Classes 005 and 032 that will be sold under the Mark will include cannabidiol or cannabidiol-like compounds, as set forth in the identifications of goods for these classes in this application. The Applicant intends to synthesize, or otherwise to obtain, the cannabidiol or cannabidiol-like compounds in accordance with all applicable laws and regulations, including the CSA. Applicant intends to source the cannabidiol or cannabidiol-like compounds only from lawful sources and only from lawful source materials.

6. Because this application is pending as an intent to use application, the source materials to be used for extracting or synthesizing cannabidiol to be used in the listed goods in Classes 005 and 032 to be sold under the Mark remain uncertain at this point in time. The

Applicant will make sure the goods in Classes 005 and 032, and the services in Class 035, will not be unlawful at the time of their sale, and hence, will not be unlawful at the time that the Mark is used in commerce.

7.   Applicant has no intent to provide any services that will enable applicant or consumers to violate any laws, including the CSA.

8.   The undersigned being warned that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements and the like may jeopardize the validity of the application or document or any registration resulting therefrom, declares that all statements made of his/her own knowledge are true; and all statements made on information and belief are believed to be true.

Signature: _____

Name: Robert DiVito

Dated: _____ 11/6/2018 _____

EXHIBIT B – PART 1

Begins on the following page

(12) **United States Patent**   (10) **Patent No.:**   **US 10,093,949 B2**

Poulos et al.   (45) **Date of Patent:**   *Oct. 9, 2018

(54) **PRODUCTION OF CANNABIDIOLIC ACID IN YEAST**

(71) Applicant: **Librede Inc.**, Sherman Oaks, CA (US)

(72) Inventors: **Jason L. Poulos**, Los Angeles, CA (US); **Anthony N. Farnia**, Pasadena, CA (US)

(73) Assignee: **Librede Inc.**, Sherman Oaks, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **15/815,651**

(22) Filed: **Nov. 16, 2017**

(65) **Prior Publication Data**

US 2018/0073043 A1   Mar. 15, 2018

Related U.S. Application Data

(63) Continuation of application No. 14/795,816, filed on Jul. 9, 2015, now Pat. No. 9,822,384.

(60) Provisional application No. 62/024,099, filed on Jul. 14, 2014.

(51) **Int. Cl.**
C12P 7/42	(2006.01)
C12N 1/14	(2006.01)
C12N 15/00	(2006.01)
C12N 9/88	(2006.01)
C12N 9/10	(2006.01)
C12N 9/00	(2006.01)
C12N 15/81	(2006.01)

(52) **U.S. Cl.**
CPC *C12P 7/42* (2013.01); *C12N 9/1029* (2013.01); *C12N 9/1085* (2013.01); *C12N 9/88* (2013.01); *C12N 9/93* (2013.01); *C12N*

15/81 (2013.01); *C12Y 203/01206* (2015.07); *C12Y 205/01* (2013.01); *C12Y 504/99* (2013.01); *C12Y 602/01* (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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Primary Examiner — Yong D Pak

(74) *Attorney, Agent, or Firm* — Carr & Ferrell LLP
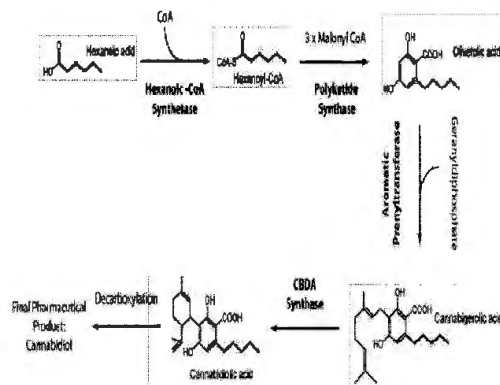
(57)   **ABSTRACT**

Exemplary embodiments provided herein include genetically engineering microorganisms, such as yeast or bacteria, to produce cannabinoids by inserting genes that produce the appropriate enzymes for the metabolic production of a desired compound.

1 Claim, 8 Drawing Sheets

Specification includes a Sequence Listing.

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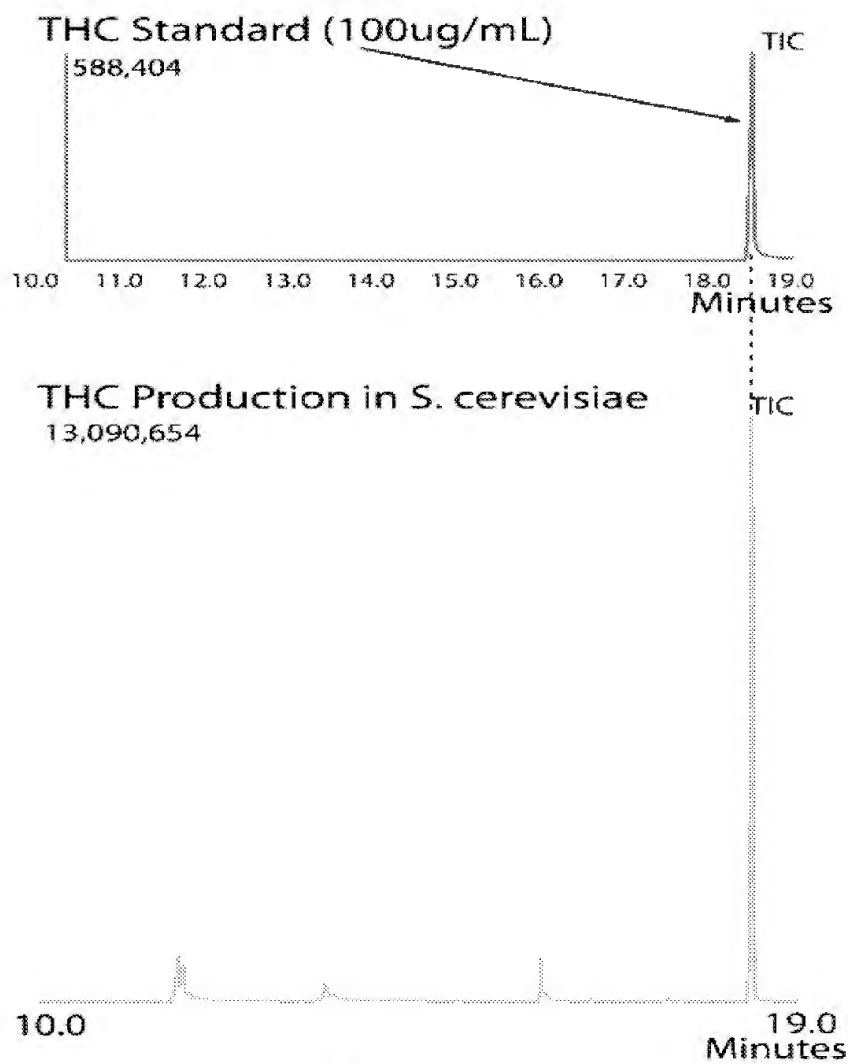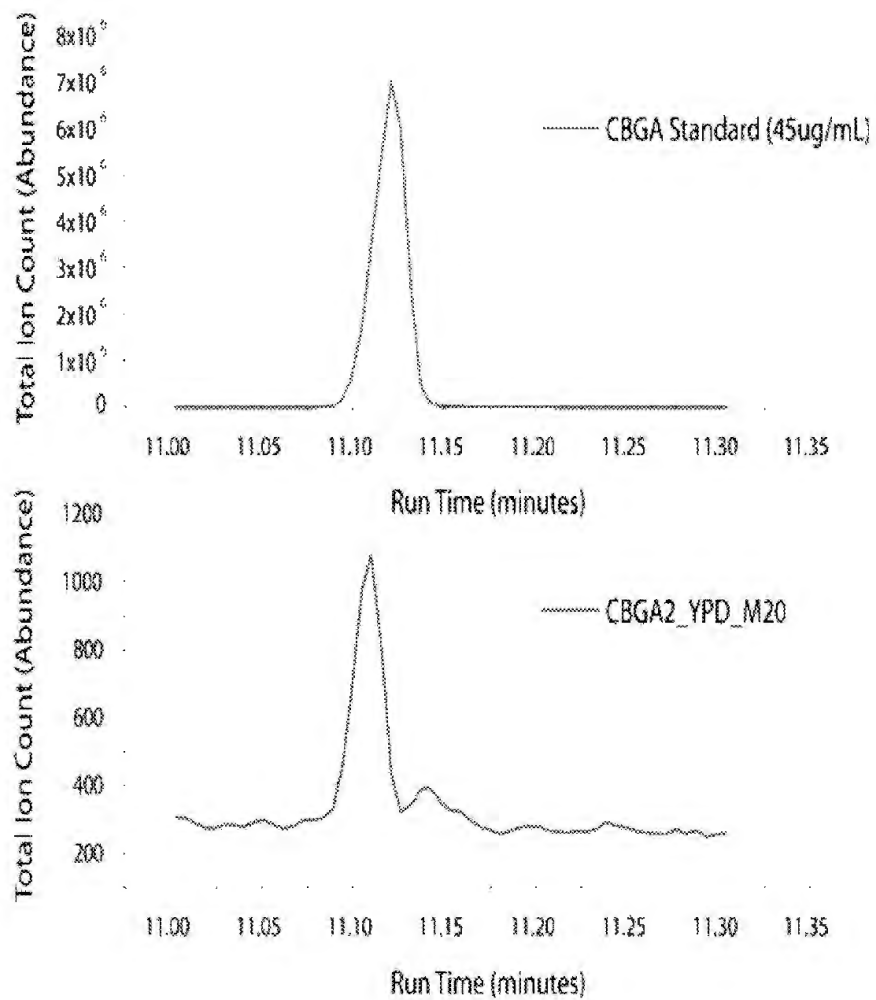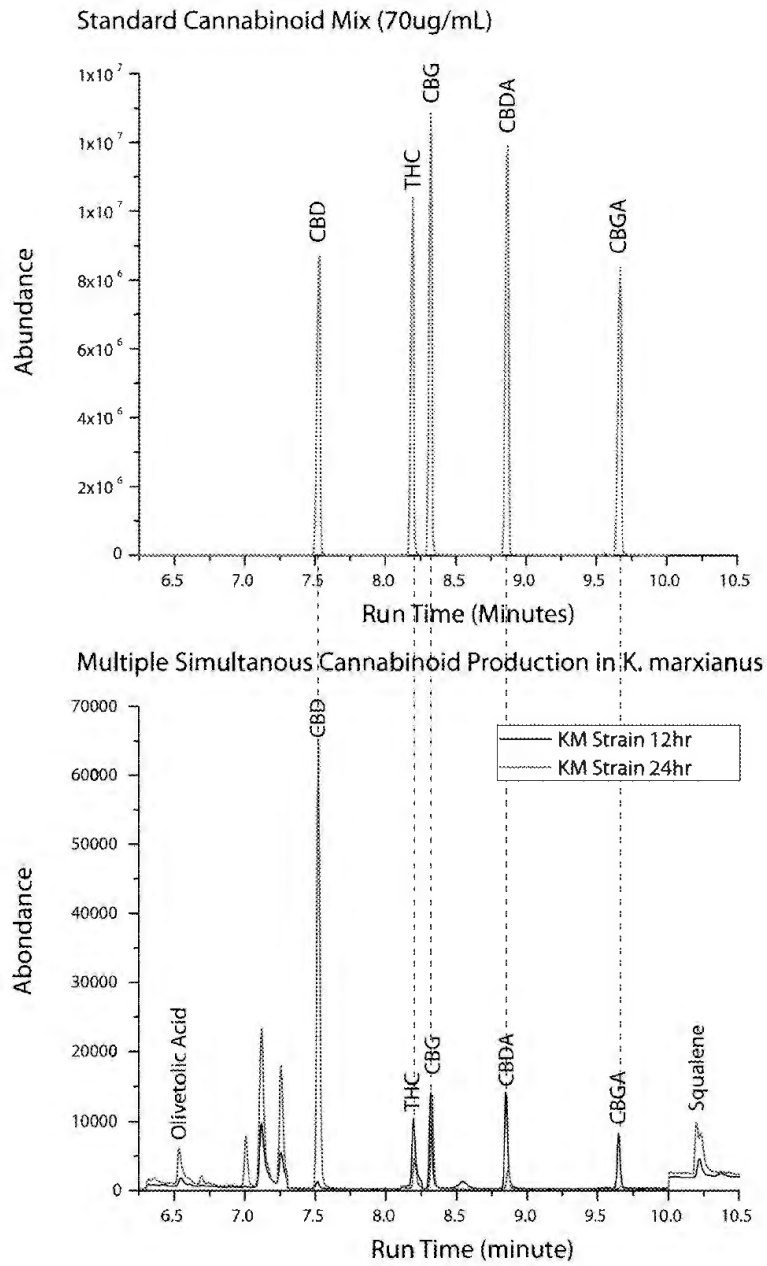
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FIG. 1

THC Standard (100ug/mL)   TIC
588,404

10.0   11.0   12.0   13.0   14.0   15.0   16.0   17.0   18.0   19.0
Minutes

THC Production in S. cerevisiae   TIC
13,090,654

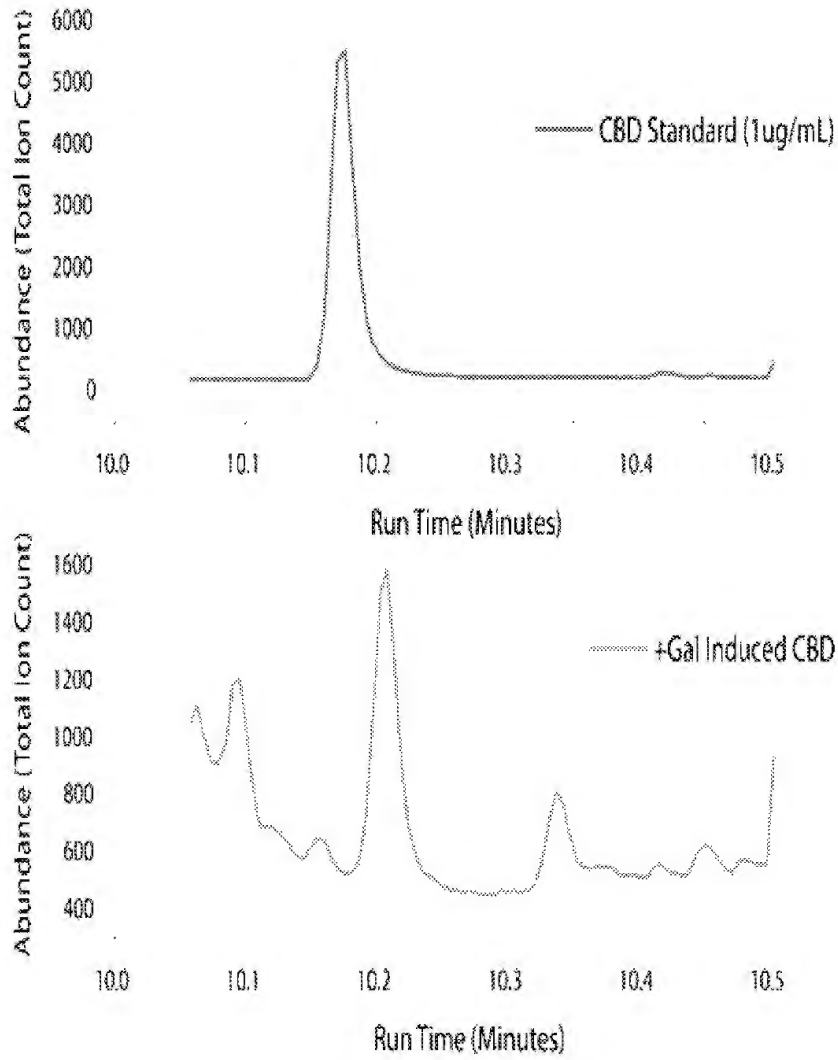10.0   19.0
Minutes
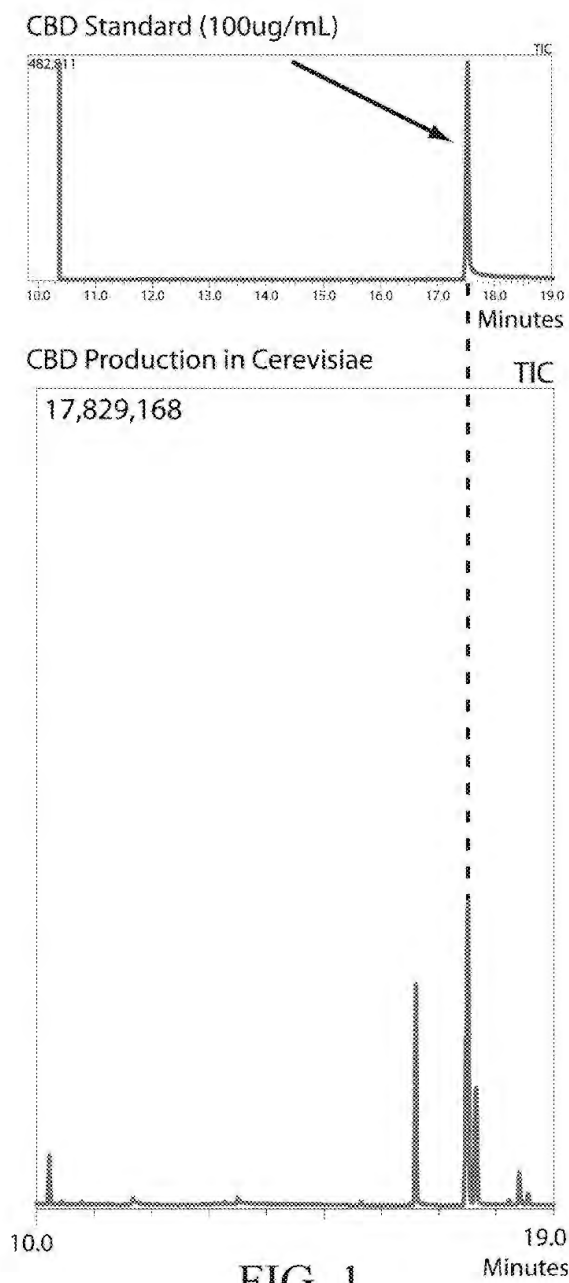
FIG. 2

FIG. 3

FIG. 4

FIG. 5
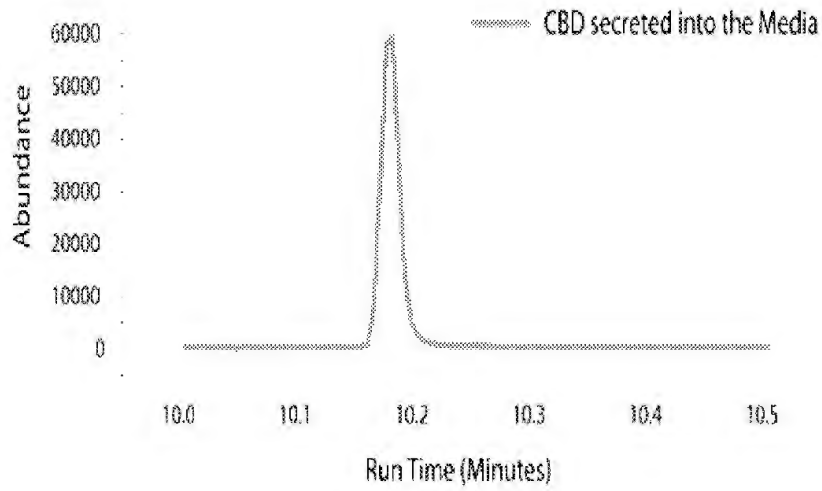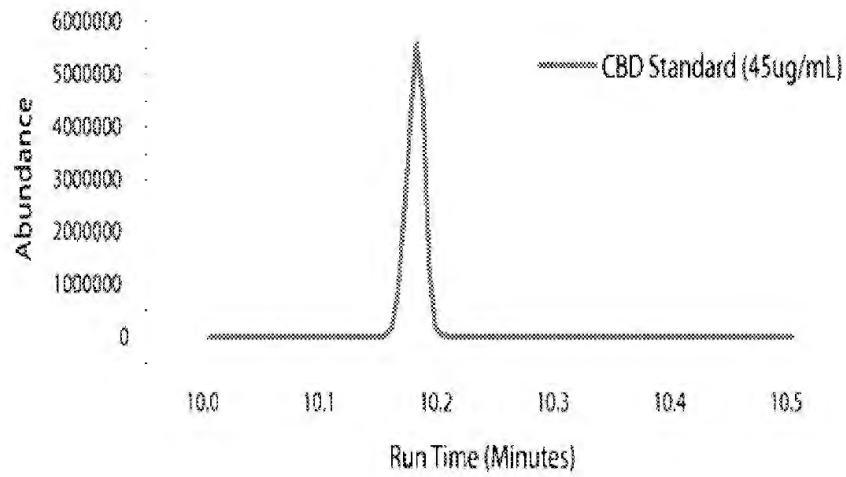
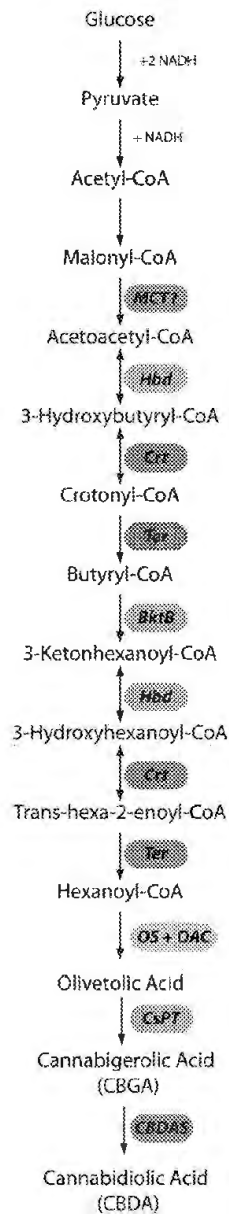FIG. 6
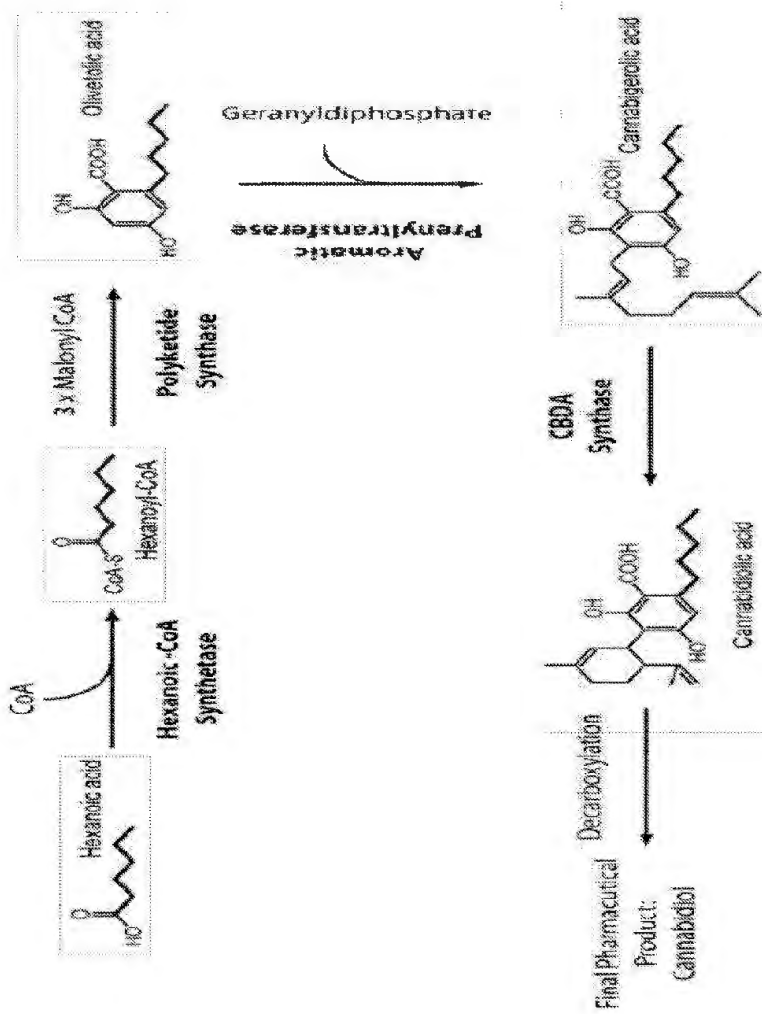
FIG. 7

Librede's Pathway from
Glucose to CBD

Glucose

↓ +2 NADH

Pyruvate

↓ + NADH

Acetyl-CoA

↓

Malonyl-CoA

↓ MCT1

Acetoacetyl-CoA

↓ Hbd

3-Hydroxybutyryl-CoA

↓ Crt

Crotonyl-CoA

↓ Ter

Butyryl-CoA

↓ BktB

3-Ketonhexanoyl-CoA

↓ Hbd

3-Hydroxyhexanoyl-CoA

↓ Crt

Trans-hexa-2-enoyl-CoA

↓ Ter

Hexanoyl-CoA

↓ OS + DAC

Olivetolic Acid

↓ CsPT

Cannabigerolic Acid
(CBGA)

↓ CBDAS

Cannabidiolic Acid
(CBDA)

FIG. 8

PRODUCTION OF CANNABIDIOLIC ACID IN YEAST

CROSS-REFERENCE TO RELATED APPLICATIONS

This continuation application claims the priority benefit of U.S. Non-Provisional patent application Ser. No. 14/795,816 filed on Jul. 9, 2015 titled "Production of Cannabinoids in Yeast," which will issue on Nov. 21, 2017 as U.S. Pat. No. 9,822,384, which in turn claims the benefit and priority of U.S. Provisional Patent Application Ser. No. 62/024,099 filed Jul. 14, 2014, titled "Terpenophenolic Production in Microorganisms," which is incorporated by reference herein.

REFERENCE TO SEQUENCE LISTINGS

The present application is filed with sequence listing(s) attached hereto and incorporated by reference, including Appendix 1B titled "Sequence IDs".

FIELD OF THE INVENTION

This invention relates to molecular biology, and more specifically to the transformation of yeast cells and the production of cannabinoids.

SUMMARY OF THE INVENTION

Exemplary embodiments provided herein include genetically engineering microorganisms, such as yeast or bacteria, to produce cannabinoids by inserting genes that produce the appropriate enzymes for the metabolic production of a desired compound.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows exemplary experimental data achieved in connection with Example 1 of Appendix 1A titled "Additional Examples" for cannabidiol ("CBD")/cannabidiolic acid ("CBDA") production in *S. cerevisiae.*

FIG. 2 shows exemplary experimental data achieved in connection with Example 2 of Appendix 1A titled "Additional Examples" for tetrahydrocannabinol ("THC")/tetra-hydrocannabinolic acid ("THCA") production in *S. cerevisiae.*

FIG. 3 shows exemplary experimental data achieved in connection with Example 3 of Appendix 1A titled "Additional Examples" for cannabigerol ("CBG")/cannabigerolic acid ("CBGA") production in *S. cerevisiae.*

FIG. 4 shows exemplary experimental data achieved in connection with Example 4 of Appendix 1A titled "Additional Examples" for CBGA, CBDA, CBD, CBG and THC production in *K. marxianus.*

FIG. 5 show exemplary experimental data achieved in connection with Example 5 of Appendix 1A titled "Additional Examples" for galactose induced CBD production in *S. cerevisiae.*

FIG. 6 shows exemplary experimental data achieved in connection with Example 6 of Appendix 1A titled "Additional Examples" for secretion of CBD into media by *S. cerevisiae.*

FIG. 7 shows an exemplary metabolic pathway for the production of cannabinoids by *Cannabis sativa.*

FIG. 8 shows an exemplary biosynthetic route for the production of CBDA from glucose.

DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

The present application relates to the field of cannabinoid production in yeasts. Cannabinoids are a general class of chemicals that act on cannabinoid receptors and other target molecules to modulate a wide range of physiological behavior such as neurotransmitter release. Cannabinoids are produced naturally in humans (called endocannabinoids) and by several plant species (called phytocannabinoids) including *Cannabis sativa.* Cannabinoids have been shown to have several beneficial medical/therapeutic effects and therefore they are an active area of investigation by the pharmaceutical industry for use as pharmaceutical products for various diseases.

Currently the production of cannabinoids for pharmaceutical or other use is done by chemical synthesis or through the extraction of cannabinoids from plants that are producing these cannabinoids, for example *Cannabis sativa.* There are several drawbacks to the current methods of cannabinoid production. The chemical synthesis of various cannabinoids is a costly process when compared to the extraction of cannabinoids from naturally occurring plants. The chemical synthesis of cannabinoids also involves the use of chemicals that are not environmentally friendly, which can be considered as an additional cost to their production. Furthermore, the synthetic chemical production of various cannabinoids has been classified as less pharmacologically active as those extracted from plants such as *Cannabis sativa.* Although there are drawbacks to chemically synthesized cannabinoids, the benefit of this production method is that the end product is a highly pure single cannabinoid. This level of purity is preferred for pharmaceutical use. The level of purity required by the pharmaceutical industry is reflected by the fact that no plant extract based cannabinoid production has received FDA approval yet and only synthetic compounds have been approved.

In contrast to the synthetic chemical production of cannabinoids, the other method that is currently used to produce cannabinoids is production of cannabinoids in plants that naturally produce these chemicals; the most used plant for this is *Cannabis sativa.* In this method, the plant *Cannabis sativa* is cultivated and during the flowering cycle various cannabinoids are produced naturally by the plant. The plant can be harvested and the cannabinoids can be ingested for pharmaceutical purposes in various methods directly from the plant itself or the cannabinoids can be extracted from the plant. There are multiple methods to extract the cannabinoids from the plant *Cannabis sativa.* All of these methods typically involve placing the plant, *Cannabis sativa* that contains the cannabinoids, into a chemical solution that selectively solubilizes the cannabinoids into this solution. There are various chemical solutions used to do this such as hexane, cold water extraction methods, CO2 extraction methods, and others. This chemical solution, now containing all the different cannabinoids, can then be removed, leaving behind the excess plant material. The cannabinoid containing solution can then be further processed for use.

There are several drawbacks of the natural production and extraction of cannabinoids in plants such as *Cannabis sativa.* Since there are numerous cannabinoids produced by *Cannabis sativa* it is often difficult to reproduce identical cannabinoid profiles in plants using an extraction process. Furthermore, variations in plant growth will lead to different

levels of cannabinoids in the plant itself making reproducible extraction difficult. Different cannabinoid profiles will have different pharmaceutical effects which are not desired for a pharmaceutical product. Furthermore, the extraction of cannabinoids from *Cannabis sativa* extracts produces a mixture of cannabinoids and not a highly pure single pharmaceutical compound. Since many cannabinoids are similar in structure it is difficult to purify these mixtures to a high level resulting in cannabinoid contamination of the end product.

Disclosed herein are strategies for creating cannabinoids in microorganisms such as yeast and methods to produce various cannabinoids in yeast from a simple sugar source. The general methods involve genetically engineering yeast to produce various cannabinoids, where the main carbon source available to the yeast is a sugar (glucose, galactose, fructose, sucrose, honey, molasses, raw sugar, etc.). Genetic engineering of the microorganism involves inserting various genes that produce the appropriate enzymes and/or altering the natural metabolic pathway in the microorganism to achieve the production of a desired compound. Through genetic engineering of microorganisms these metabolic pathways can be introduced into these microorganisms and the same metabolic products that are produced in the plant *Cannabis sativa* can be produced by the microorganisms. The benefit of this method is that once the microorganism is produced, the production of the cannabinoid is low cost and reliable, only a specific cannabinoid is produced or a subset is produced, depending on the organism. The purification of the cannabinoid is straight forward since there is only a single cannabinoid or a selected few cannabinoids present in the microorganism. The process is a sustainable process which is more environmentally friendly than synthetic production.

FIG. 1 shows exemplary experimental data achieved in connection with Example 1 of Appendix 1A titled "Additional Examples" for cannabidiol ("CBD")/cannabidiolic acid ("CBDA") production in *S. cerevisiae*.

FIG. 1 shows gas chromatography—mass spectrometry of cannabidiol (CBD) produced in *S. cervisiae*. After processing the yeast cells, as described in Example 1 of Appendix 1A, the whole cell ethyl acetate extract is analyzed for the presences of CBD. The samples were prepared in a way similar to that shown in Appendix A1 except that no MSTFA derivatization was used in this sample (therefore CBDA turns into CBD upon heating), the oven protocol was also slightly different than that shown in Appendix 1A. Initially, a standard CBD solution is run (100 ug/mL; TOP). After running the standard the inventors determined the run time of 17.5 minutes. After running the standard the inventors repeated the GC-MS experiment with their whole cell extract (BOTTOM). At a run time of 17.5 minutes, the inventors saw the same peak as in the standard. Mass spectrometry analysis of the peaks showed identical mass for the two samples (standard and extract) indicating the presence of CBD in their whole cell extract.

FIG. 2 shows exemplary experimental data achieved in connection with Example 2 of Appendix 1A titled "Additional Examples" for tetrahydrocannabinol ("THC")/tetrahydrocannabinolic acid ("THCA") production in *S. cerevisiae*.

FIG. 2 shows gas chromatography—mass spectrometry of tetrahydrocannabinol (THC) produced in *S. cervisiae*. After processing the yeast cells, as described in Example 2 of Appendix 1A, the whole cell ethyl acetate extract was analyzed for the presences of THC. The samples were prepared in a way similar to that shown in Appendix 1A

except that no MSTFA derivatization was used in this sample (therefore THCA turns into THC upon heating), the oven protocol was also slightly different than that shown in Appendix 1A. Initially, a standard CBD solution was run (100 ug/mL; TOP). After running the standard the inventors determined the run time of 18.5 minutes. After running the standard the inventors repeated the GC-MS experiment with their whole cell extract (BOTTOM). At a run time of 18.5 minutes, the inventors saw the same peak as in the standard. Mass spectrometry analysis of the peaks showed identical mass for the two samples (standard and extract) indicating the presence of THC in their whole cell extract.

FIG. 3 shows exemplary experimental data achieved in connection with Example 3 of Appendix 1A titled "Additional Examples" for cannabigerol ("CBG")/cannabigerolic acid ("CBGA") production in *S. cerevisiae*.

FIG. 3 shows gas chromatography—mass spectrometry of cannabigerolic acid (CBGA) produced in *S. cervisiae*. After processing the yeast cells, as described in Example 3 of Appendix 1A, the whole cell ethyl acetate extract was analyzed for the presences of CBGA. The samples were prepared in a way as described in Appendix 1A, but the oven protocol was also slightly different than that shown in Appendix 1A. Initially, a standard CBGA solution was run (45 ug/mL; TOP). After running the standard, the inventors determined the run time of 11.1 minutes. After running the standard, the inventors repeated the GC-MS experiment with their whole cell extract (BOTTOM). At a run time of 11.1 minutes, the inventors saw the same peak as in the standard. Mass spectrometry analysis of the peaks showed identical mass for the two samples (standard and extract) indicating the presence of CBGA in their whole cell extract.

FIG. 4 shows exemplary experimental data achieved in connection with Example 4 of Appendix 1A titled "Additional Examples" for CBGA, CBDA, CBD, CBG and THC production in *K. marxianus*.

FIG. 4 shows gas chromatography—mass spectrometry of cannabinoid production (CBGA, CBDA, CBD, CBG, THC) produced in *K. marxianus*. After processing the yeast cells, as described in Example 4 of Appendix 1A, the whole cell ethyl acetate extract was analyzed for the presence of cannabinoids. The samples were prepared in a way as described in Appendix 1A, but the oven protocol was also slightly different than that shown in Appendix 1A. Initially, a standard solution containing CBD, CBG, THC, CBDA, and CBGA was run (70 ug/mL each; TOP). After running the standard, the inventors determined the run time for each compounds. After running the standard, the inventors repeated the GC-MS experiment with their whole cell extract (BOTTOM). At each run time the inventors saw the same peaks as in the standard. Mass spectrometry analysis of the peaks showed identical mass for the two samples (standard and extract) indicating the presence of cannabinoids in their whole cell extract.

FIG. 5 show exemplary experimental data achieved in connection with Example 5 of Appendix 1A titled "Additional Examples" for galactose induced CBD production in *S. cerevisiae*.

FIG. 5 shows gas chromatography—mass spectrometry of induced cannabidiol (CBD) production in *S. cervisiae*. After processing yeast cells, as described in Example 5 of Appendix 1A, the whole cell ethyl acetate extract was analyzed for the presences of CBD. The samples were prepared in a way as described in Appendix 1A. Initially, a standard solution containing CBD was run (1 ug/mL; TOP). After running the standard the inventors determined the run time for CBD as 10.2 minutes. After running the standard, the inventors

repeated the GC-MS experiment with their whole cell extract (BOTTOM). At 10.2 minutes, the inventors saw the same peak as in the standard. Mass spectrometry analysis of the peaks showed identical mass for the two samples (standard and extract) indicating the presence of CBD in their whole cell extract.

FIG. **6** shows exemplary experimental data achieved in connection with Example 6 of Appendix 1A titled "Additional Examples" for secretion of CBD into media by *S. cerevisiae.*

FIG. **6** shows gas chromatography—mass spectrometry of induced cannabidiol production (CBD) produced in *S. cerevisiae* and secreted into the media. After processing the growth media, as described in Example 6 of Appendix 1A, the media ethyl acetate extract was analyzed for the presence of CBD. The samples were prepared in a way as described in Appendix 1A. Initially, a standard solution containing CBD was run (45 ug/mL; TOP). After running the standard the inventors determined the run time for CBD as 10.2 minutes. After running the standard, the inventors repeated the GC-MS experiment with their whole cell extract (BOTTOM). At 10.2 minutes, the inventors saw the same peak as in the standard. Mass spectrometry analysis of the peaks showed identical mass for the two samples (standard and extract) indicating the presence of CBD in their whole cell extract.

Biosynthetic Production of Cannabidiolic Acid (CBDA) in *S. cerevisiae*

Through genetic engineering the inventors have reconstituted the cannabidiolic acid (CBDA) metabolic pathway found in *Cannabis sativa* into *S. cerevisiae* (a species of yeast).

Producing CBGA is an initial step in producing many cannabinoids from *Cannabis sativa* in *S. cerevisiae.* Once CBGA is produced a single additional enzymatic step is required to turn CBGA into many other cannabinoids (CBDA, THCA, CBCA, etc.). The acidic forms of the cannabinoids can be used as a pharmaceutical product or the acidic cannabinoids can be turned into their neutral form for use, for example Cannabidiol (CBD) is produced from CBDA through decarboxylation. The resulting cannabinoid products will be used in the pharmaceutical/nutraceutical industry to treat a wide range of health issues.

FIG. **7** shows an exemplary metabolic pathway for the production of cannabinoids by *Cannabis sativa.*

The biosynthetic route for the production of cannabidiolic acid in *Cannabis sativa* is shown in FIG. **7**. The pathway begins with the conversion of Hexanoic acid (a simple fatty acid) to Hexanoyl-CoA by Hexanoyl-CoA Synthetase. Hexanoyl-CoA is converted to Oleviolic acid (OA), a polyketide, by a Polyketide synthase. OA is then prenylated with the monoterpene geranyl diphosphate to cannabigerolic acid by an Aromatic prenyltransferase. Finally, cannabidiolic acid (CBDA) is produced by cyclizing cannabigerolic acid via CBDA synthase (CBDAs). In summary, it takes four enzymatic steps to produce CBDA from Hexanoic acid. The inventors have engineered this metabolic pathway into *S. cerevisiae* (a species of yeast) for the production of CBDA.

There are a few key differences between plant polyketide and terpene biosynthesis when compared to yeast. Yeast does not contain many of the enzymes and fatty acids required for the production of CBDA. Moreover, yeast do not express high levels of geranyl diphosphate (GPP), a chemical required for the production of cannabigerolic acid, the precursor to CBDA. Yet, through genetic engineering many of the required enzymes can be added and the production of GPP can be increased. In order to add the required

enzymes for CBDA production in yeast the inventors created plasmids that contain the essential genes in the CBDA biosynthetic pathway. The inventors have transformed these genes into *S. cerevisiae.*

Synthesis of Fusion Genes Required for CBDA Production in *S. cerevisiae*

The genome of *Cannabis sativa* has been investigated and the acyl-activating enzymes CsAAE1 was determined to convert hexanoic acid to hexanoyl-CoA (Step 1 in FIG. **7**). The inventors have overexpressed CsAAE1 in yeast while simultaneously supplementing the growth media with Hexanoic acid. By supplementing the media with hexanoic acid, the inventors ensured that the yeast have the required starting materials for the production of hexanoyl-CoA.

The next enzymatic step that was engineered into the yeast strain was for the production of Olivetolic acid (OA) from hexanoyl-CoA. This step requires the substrates hexanoyl-CoA and 3 malonyl-CoA molecules, with the malonyl-CoA molecule produced by yeast naturally. Olivetolic acid production requires two enzymes for the condensation and subsequent cyclization of malonyl-CoA with hexanoyl-CoA. This process requires the tetraketide synthase, olivetolic synthase (OS), and the polyketide cyclase, olivetolic acid cyclase (OAC). In some embodiments, stoichiometric amounts of both of these enzymes are preferred; as it has been experimentally determined that OAC binds a chemical intermediate made by OS. In various embodiments, in order to ensure the proper amounts of OS and OAC the inventors have created a single gene that is a fusion of OS, a self cleaving T2A peptide, and the OAC gene (OS-T2A-OAC) and in certain cases an HA tag was inserted at the C-terminus of OAC to verify protein expression. This entire fusion protein was produced in yeast and the self cleaving peptide is spliced in vivo to produce OS and OAC.

The next enzymatic step requires the production of geranyl pyrophosphate (GPP). In yeast the prenyltransferace Erg20 condenses isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAP) to geranyl pyrophosphate (GPP) and feranyl pyrophosphate (FPP) naturally. While only these two products are produced in yeast, a greater quantity of FPP when compared to GPP is produced. More GPP is required for the production of CBDA. In order to increase the production of GPP compared to FPP the inventors inserted a mutant prenyltransferase, Erg20(K179E) in the yeast strain. This mutant has been shown to shift the ratio of GPP:FPP to 70:30. This Erg20(K179E) mutant was placed on a fusion gene with CsAAE1, the enzyme for hexanoyl-CoA, and a self-cleaving peptide, T2A (CsAAE1-T2A-Erg20(K179E). We also added a FLAG tag to the C-terminus of the Erg20p (K197E) enzyme (CsAAE1-T2A-Erg20(K179E)-FLAG) to verify expression of this fusion protein in yeast in certain yeast strains. After production in yeast the self-cleaving peptide was cut producing CsAAE1 and Erg20(K179E).

Once the inventors verified that they had enough GPP to prenylate Olivetolic acid to cannabigerolic acid the inventors inserted the aromatic prenyltransferase (CsPt1) gene into the yeast. In this final enzymatic step the inventors placed the cannabidiolic acid synthase (CBDAs) gene into yeast for the conversion of cannabigerolic acid to CBDA. Similar to the inventors' previous approach, they introduced a single gene containing CsPt1, a self-cleaving peptide T2A, CBDs, and in certain cases a MYC tag was inserted at the C-terminus of CBDs in order to verify production of each enzyme (CsPt1-T2A-CBDs-MYC).

Creation of a Stable Yeast Strain Producing the Metabolic Pathway for CBDA

Three stable transformations of *S. cerevesaie* where created utilizing selection for leucine, uracil and tryptophan. The inventors first transformed an auxotrophic yeast strain (his3D1/leu2/trp1-289/ura3-52) with the CsAAE1-T2A-Erg20(K197E)-FLAG gene in an integrating vector. 5 µg of CsAAE1-T2A-Erg20(K197E)-FLAG in a vector containing a gene for tryptophan depletion resistance was linearized with the restriction enzyme EcoRV, transformed into chemically competent InVSc1, and grown on Yeast Nitrogen Base without amino acids and 0.5% ammonium sulfate (YNBA) agar plates supplemented with histidine, leucine, tryptophan, 1% glucose and 2% lactic acid are grown at 30° C. until colonies are formed. Any yeast colonies that did not incorporate the plasmid, that contains the CsAAE1-T2A-Erg20 (K197E)-FLAG gene died since the starting yeast strain is a tryptophan auxotroph. All colonies, with successful plasmid incorporation, where picked and grown in YNBA supplemented with histidine, leucine and uracil, 1% glucose and 2% lactic acid. All colonies were screened for protein production by taking whole cell extracts of each induced clone and the total protein was subjected to SDS-PAGE followed by western blotting against the c-terminal tag of Erg20(K197E). Positive clones where stored at –80° C. in glycerol stocks. The highest expressing clone was taken for the second transformation and was be designated as VscG-PHA.

Using the VscGPHA strains the inventors added 5 µg of OS-T2A-OAC-HA in a vector containing a gene for leucine depletion resistance. This plasmid was linearized with the restriction enzyme AseI and transformed into chemically competent VscGPHA and grown on YNBA agar plates supplemented with histidine and uracil, 1% glucose and 2% lactic acid and grown at 30° C. until colonies were formed. Any yeast colonies that did not incorporate the plasmid that contains the OS-T2A-OAC-HA gene died since the VscGPHA is a leucine auxotroph. All colonies, with successful plasmid incorporation, were picked and grown in YNBA supplemented with histidine, and leucine. All colonies were screened for protein production by taking whole cell extracts of each induced clone and subjected the total protein to SDS-PAGE followed by western blotting against the c-terminal HA tag of OAC. Positive clones were stored at –80° C. in glycerol stocks. The highest expressing clone was taken for the second stable transformation and was designated VscGPHOA.

The final stable transformation was done in a similar way as the previous transformation. The CsPT-T2A-CBDAs-MYC gene was placed in the vector containing a gene for uracil depletion resistance 5 µg of this plasmid was linearized with EcorV and transformed into chemically competent VscGPHOA. Transformed VscGPHOA was grown in YNBA supplemented with histidine, 1% glucose and 2% lactic acid. Any yeast colonies that did not incorporate the plasmid that contains the CsPT-T2A-CBDAs-MYC gene died since they lacked leucine. All colonies were picked and grown in YNBA supplemented with histidine, 1% glucose and 2% lactic acid. All colonies were screened for protein production by taking whole cell extracts of each induced clone and subjecting the total protein to SDS-PAGE followed by western blotting against the c-terminal Myc tag of CBDAs. Positive clones are stored at –80° C. in glycerol stocks. The highest expressing CBDAs was taken for the final strain and designated VscCBDA.

Production of CBDA in Yeast

To initiate the reconstituted metabolic pathway of CBDA a colony of VscCBDA was freshly streaked on a plate of a frozen glycerol stock of VscCBDA. A small culture of VscCBDA was grown in YNBA supplemented with 0.05% histidine, 1% glucose, 2% lactic acid, and 0.03% hexanoic acid overnight at 30° C. The overnight culture was transferred to 1 L of YNBA supplemented with 0.05% histidine, 1% glucose, 2% lactic acid, and 0.03% hexanoic acid and was grown at 30° C. until mid log phase. Cells were pelleted by centrifugation then washed with 200 ml of phosphate buffered saline (PBS) and repelleted. Pelleted cells were resuspended with 1 L of YNBA supplemented with 0.05% histidine, 2% galactose, and 0.03% hexanoic acid and grown at 30° C. overnight.

Overnight 1 L cultures were pelleted by centrifugation, resuspended, washed one time in PBS and pelleted. Cell pellets were resuspended in 40% (wt/vol) KOH and 50% (vol/vol) ethanol solution and boiled for 10 minutes. Metabolite extraction was done by extracting from the boiled extracts 3 times with hexane, then 3 times with ethyl acetate. The spent supernatant broth was extracted in a similar fashion as described above. Organic phases of extracts of each sample were pooled then dried by a rotary evaporator and stored for liquid chromatography mass spectrometry (LC-MS) and gas chromatography mass spectrometry (GC-MS) analysis to confirm and quantitate how much CBDA is produced from strain VscCBDA.

Biosynthetic Production of Cannabidiolic Acid (CBDA) in *K. marxianus*

Through genetic engineering the inventors have reconstituted the cannabidiolic acid (CBDA) metabolic pathway found in *Cannabis sativa* into *K. marxianus* (a species of yeast). Producing CBGA is an initial step in producing many cannabinoids from *Cannabis sativa* in *K. marxianus*. Once CBGA is produced a single additional enzymatic step is required to turn CBGA into many other cannabinoids (CBDA, THCA, CBCA, etc.). The acidic forms of the cannabinoids can be used as a pharmaceutical product or the acidic cannabinoids can be turned into their neutral form for use, for example Cannabidiol (CBD) is produced from CBDA through decarboxylation. The resulting cannabinoid products will be used in the pharmaceutical/nutraceutical industry to treat a wide range of health issues. Synthesis of fusion genes required for CBDA production in *k. Marxianus*

FIG. **8** shows an exemplary biosynthetic route for the production of CBDA from glucose.

The biosynthetic route for the production of cannabidiolic acid in *Cannabis sativa*, from glucose to CBDA is shown in FIG. **8**. The pathway begins with the conversion of glucose to malonyl-CoA through a series of steps that are common to many strains of yeast. The conversion of malonyl-CoA to Acetoacetyl-CoA is conducted by the enzyme MCT1, an acyl-carrier-protein. Acetoacetyl-CoA is converted to 3-Hydroxybutyryl-CoA by the enzyme 3-hydroxybutyryl-CoA dehydrogenase (Hbd) from *Clostridium acetobutylicum*. Next, 3-Hydroxybutyryl-CoA is converted into Crotonyl-CoA by the enzyme crotonase (Crt) from *Clostridium acetobutylicum* and the conversion of Crotonyl-CoA to Butyryl-CoA is controlled by the enzyme trans-enoyl-CoA reducatase (Ter) from *Treponema denticola*. The Butyryl-CoA is converted to 3-Ketonhexanoyl-CoA by the enzyme β-ketothiolase (Bktb) from *Ralstonia Eutropha*. 3-Ketonhexanoyl-CoA is converted to 3-Hydroxyhexanoyl-CoA by the enzyme Hbd. Hydroxyhexanoyl-CoA is converted to Trans-hexa-2-enoyl-CoA by the enzyme Crt. Trans-hexa-2-enoyl-CoA is converted to Hexanoyl-CoA by the enzyme Ter. Hexanoyl-CoA, with 3 malonyl-CoAs, is converted to

Oleviolic acid (OA) by a Polyketide synthase and cyclase, OA and OAC respectively. OA is then prenylated with the monoterpene geranyl diphosphate to cannabigerolic acid by an Aromatic prenyltransferase, CsPT. Finally, cannabidiolic acid (CBDA) is produced by cyclizing cannabigerolic acid via CBDA synthase (CBDAS). We have engineered this metabolic pathway into *K. marxianus* (a species of yeast) for the production of CBDA (FIG. 8).

There are a few key differences between plant polyketide and terpene biosynthesis when compared to yeast. Yeast does not contain many of the enzymes and fatty acids required for the production of CBDA. Moreover, yeast do not express high levels of geranyl diphosphate (GPP), a chemical required for the production of cannabigerolic acid, the precursor to CBDA. Yet, the inventors through genetic engineering created many of the required enzymes that can be added so the production of GPP was increased. In order to add the required enzymes for CBDA production in yeast the inventors created plasmids that contain the essential genes in the CBDA biosynthetic pathway. The inventors have transformed these genes into *K. marxianus*.

Creation of a Stable *K. marxianus* Strain Producing the Metabolic Pathways for Hexonyl-coA and CBDA

Two stable transformations of *K. marxianus* were created utilizing selection for uracil and G418 (Genenticin). The inventors first transformed an auxotrophic *K. marxianus* strain (ATCC 17555 KM5) with 5 different genes needed to produce high levels of hexanoyl-CoA. After functional conformation of the genes required for hexanoyl-CoA the inventors did a second transformation with the genes responsible for CBDA production. The molecular biology methods required for biosynthetic production of CBDA in *K. marxianus* are outlined below.

Gene names Crt, Bktb, MCT1, TeR, Hbd, Erg20p(K179E) were codon optimized, synthesized and subcloned into puc57 and p426 ATCC with the restriction enzymes SpeI and SalI.

Genes Crt, Bktb, MCT1, TeR, Hbd, and Erg20p(K179E) were amplified via PCR using the primers GPD_F and URA_R and all 6 amplicons were electroporated into *K. marxianus* ATCC 17555 KM5 at a concentration of 200 nM and selected onto yeast nitrogen base (YNB) supplemented with amino acid dropout mix (DO supplement—Ura Clonetech 630416) 2% glucose, and 2% Agar plates.

Gene integration and functional gene expression of Crt, Bktb, MCT1, TeR, Hbd, and Erg20p(K179E) was validated by genomic PCR and RT-PCR methods respectively. The final strain produced containing the functional expression of Crt, Bktb, MCT1, TeR, Hbd, and Erg20p(K179E) was labeled kMarxHex1.

Gene names CBDAs, CsPt, OS, and OAC were codon optimized and synthesized by Genscript. The codon optimized gene sequences of CBDAs and CsPt were cloned in frame with a nucleotide sequence containing a self cleaving T2A peptide and designated as CstTCbds. The codon optimized gene sequences of OS and OAC were cloned in frame with a nucleotide sequence containing a self cleaving T2A peptide and designated as OSTOc. CsTCbds and OSTOc were cloned in frame with an *S. cerevisiae* internal ribosomal entry site (IRES), Ure2, into a galactose inducible vector and the final gene sequence pcen/arsGal-OSTOc-IRES-CstTCbds plasmid can be seen below. The plasmid pcen/arsGal-OSTOc-IRES-CstTCbds was used to synthesize a functional gene fragment that expresses the enzymes CBDAs, CsPt, OS, and OAC by using the primers GalI-RES_F, GalIRES_R.

The Gibson Assembly method was used to subclone the PCR fragment from [0057] into the plasmid HO-poly-KanMx4-HO (ATCC 87804) using the primers KmX-IRES_F and KmXIRES_R to create the plasmid pHOOSCstKnMxHO.

The plasmid pHOOSCstKnMxHO was digested with NotI and transformed into kMarxHex1 using standard electroporation methods. The selection of stable integrants was done with yeast nitrogen base (YNB) supplemented with amino acid dropout mix (DO supplement—Ura Clonetech 630416) 2% glucose, 1 mg/ml G418 (Gibco) and 2% Agar plates.

Gene integration and functional gene expression of pHOOSCstKnMxHO validated by genomic PCR and RT-PCR methods respectively. The final strain produced containing the functional expression of Crt, Bktb, MCT1, TeR, Hbd, Erg20p(K179E) CBDAs, CsPt, OS, and OAC was labeled k.MarxCBDA.

Production of CBDA in *K. marxianus*

To initiate the reconstituted metabolic pathway of CBDA, a colony from *K. marx* CBDA was freshly streaked onto an agar plate from a frozen glycerol stock of k.MarxCBDA. A small culture of VscCBDA was grown in YNBA base (YNB) supplemented with amino acid dropout mix (DO supplement—Ura Clonetech 630416) 2% glucose, 1 mg/ml G418 (Gibco) and 2% Agar plates was grown overnight at 30° C. The overnight culture was transferred to 1 L of YNBA supplemented with base (YNB) supplemented with amino acid dropout mix (DO supplement—Ura Clonetech 630416) 2% glucose, and 1 mg/ml G418 (Gibco) and was grown at 30° C. until mid log phase. Cells were pelleted by centrifugation then washed with 200 ml of phosphate buffered saline (PBS) and repelleted. Pelleted cells were resuspended with 1 L of YNBA supplemented with base (YNB) supplemented with amino acid dropout mix (DO supplement—Ura Clonetech 630416) 2% galactose, and 1 mg/ml G418 (Gibco) and grown at 30° C. overnight.

Processing CBDA for Analysis of Cannabinoid Production

Overnight 1 L cultures were pelleted by centrifugation, re-suspended, washed one time in PBS and pelleted. The process for extracting cannabinoids from the yeast generally follows the following basic steps:

1. Remove the yeast cells from the media by centrifugation or filtration.
2. Lysis the cells using either chemical or mechanical methods or a combination of methods. Mechanical methods can include a French Press or glass bead milling or other standard methods. Chemical methods can include enzymatic cell lysis, solvent cell lysis, or detergent based cell lysis.
3. Perform a liquid-liquid extraction of the cannabinoids form the cell lysate using the appropriate chemical solvent. An appropriate solvent is any solvent where the cannabinoids are highly soluble in this solvent and the solvent is not miscible in water. Examples of this are hexane, ethyl acetate, and cyclohexane. Preferred solvents can be straight or branched alkane chains (C5-C8) work well; mixtures of these solvents can also be use.

Protocol Used for Cannabinoid Extraction from Yeast Cell Lysate

1. After lysising the cells using any mechanical technique, add 1 mL of 4M KCl, pH2.0 to each 1 mL of cell lysate.
2. Add 1-2 mLs of ethyl acetate for each 1 mL of cell lysate.
3. Rigorously mix for 1 min.

4. Centrifuge the mixture for 5 min at 1000×g.
5. Remove the top ethyl acetate layer. Cannabinoids are present in this layer.
   a. The ethyl acetate can be removed under vacuum if desired.
   b. Cannabinoids can be further purified through liquid chromatography methods if desired.

Protocol Used for Cannabinoid Extraction from Growth Media (for Secreted Cannabinoid Samples)
1. Add 1 mL of ethyl acetate for every 1 mL of growth media.
2. Rigorously mix for 1 min.
3. Centrifuge the mixture for 5 min at 1000×g.
4. Remove the top ethyl acetate layer. Cannabinoids are present in this layer.
   a. The ethyl acetate can be removed under vacuum if desired.
   b. Cannabinoids can be further purified through liquid chromatography methods if desired.

Protocol Used for GC-MS Analysis of Cannabinoid Extracts for *k. Marx* CBDA
1. Remove solvent from samples under vacuum.
2. Re-suspend dry samples in either 100 uL of dry hexane or dry ethyl acetate

3. Add 20 uL of N-Methyl-N-(trimethylsilyl) trifluoroac-etamide (MSTFA)
4. Briefly mix
5. Heat solution to 60° C. for 10-15 minutes
6. GC-MS Method
   a. Instrument Agilent 6890-5975 GC-MS (Model Number: Agilent 19091S-433)
   b. Column HP-5MS 5% Phenyl Methyl Siloxane
   c. OVEN:
      i. Initial temp: 100° C. (On) Maximum temp: 300° C.
      ii. Initial time: 3.00 min Equilibration time: 0.50 min
      iii. Ramps:

#	Rate	Final temp	Final time
1 -	30.00	280	1.00
2 -	70.00	300	5.00
3 -	0.0 (Off)		

   iv. Post temp: 0° C.
   v. Post time: 0.00 min
   vi. Run time: 15.29 min

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<400> SEQUENCE: 8

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<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Codon optimized

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catatgtagt gttgaagaaa catgaaattg cccagtattc ttaacccaac tgcacagaac     420

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gggcacagtt aagccgctaa aggcattatc cgcaagtac aatttttta tcttcgaaga     720

cagaaaattt gctgacattg gtaatacagt caaattgcag tactctgcgg gtgtatacag     780

aatagcagaa tgggcagaca ttacgaatgc acacggtgtg gtgggcccag gtattgttag     840

cggtttgaag caggcggcag aagaagtaac aaaggaacct agaggccttt tgatgttagc     900

agaattgtca tgcaagggct ccctatctac tggagaatat actaagggta ctgttgacat     960

tgcgaagagc gacaaagatt ttgttatcgg ctttattgct caaagagaca tgggtggaag    1020

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cagaaaagca ggctgggaag catatttgag aagatgcggc cagcaaaact aaaaaactgt   1260

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gttattaccc tgcggtgtga aataccgcac agatgcgtaa ggagaaaata ccgcatcagg   1380

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acgtcaaagg gcgaaaaacc gtctatcagg gcgatggccc actacgtgaa ccatcaccct   1620

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cccgatttag agcttgacgg ggaaagccgg cgaacgtggc gagaaaggaa gggaagaaag   1740

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ttgcataaca ctaacttaat ttcatggtcc ttgatgttca aggcattttt cttttttagtt   4620

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gaagcattta tcagggttat tgtctcatga gcggatacat atttgaatgt atttagaaaa    7800

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<211> LENGTH: 3353
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized
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<400> SEQUENCE: 14

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aattatggtg ctgcaacccc tcaaacttgg atcaacatcg ctaaccatat cttgtcacca    180

gatttgcctt tctccttaca ccaaatgttg ttttatggtt gctacaagga tttcggtcca    240

gccccacctg cttggattcc agaccctgaa aaagtcaagt caactaattt gggtgctttg    300

ttggaaaaga gaggtaaaga atttttgggt gtaaagtaca aagatccaat ttcttctttt    360

tctcacttcc aagaattttc tgttagaaac cctgaagtct attggagaac agtattgatg    420

gatgaaatga aaattagttt ctctaaggac ccagaatgta tcttgagaag agatgacatc    480

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<210> SEQ ID NO 15
<211> LENGTH: 1553
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 15

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agtgaacaca tgactcaatt gaaggaaaag tttagaaaaa tatgtgataa gtctatgatc    180

agaaagagaa actgcttctt gaacgaagaa catttgaagc aaaatccaag attggtagaa    240

cacgaaatgc aaacattgga tgccagacaa gacatgttag ttgtcgaagt tcctaaattg    300

ggtaaagatg cttgtgcaaa agccattaag gaatggggtc aaccaaagtc aaagatcact    360

catttgattt ttacaagtgc atctactaca gatatgcctg gtgcagacta ccactgtgcc    420

aaattgttag gtttgtcacc atccgttaag agagtcatga tgtatcaatt aggttgctac    480

ggtggtggta ctgttttgag aatcgctaag gatattgcag aaaacaacaa gggtgccaga    540

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ttggaattgt tagttggtca agctatcttc ggtgacggtg ctgctgctgt tattgttggt    660

gcagaacctg acgaatctgt tggtgaaaga ccaatatttg aattagtcag tacaggtcaa    720

accatcttgc ctaattctga aggtacaatt ggtggtcata taagagaagc aggtttgatc    780

ttcgatttgc acaaagacgt tccaatgtta atctctaaca acatagaaaa gtgtttgata    840

gaagcattca ctcctatagg tatctcagat tggaactcta ttttctggat aacacatcca    900

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gctatgaaag atgtatactg gggtaaagac gttacacaaa agaaagaaga aggttataca    1380

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gctcacgttg gttttggtga cgtttacaga tccttctggg aaaaaattgtt gatcttcgat    1500

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<210> SEQ ID NO 16
<211> LENGTH: 2918
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized
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<400> SEQUENCE: 16
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acaaatcaaa ctgaaccacc tgaaagtgat aatcactctg ttgccacaaa aattttgaac    300

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attaatcaaa tatacgattt gcacatcgac agaattaaca aaccagattt gcctttggct    540

tcaggtgaaa tatccgtcaa tactgcatgg atcatgtcta tcatagtagc cttgttcggt    600

ttgatcatca caattaaaat gaagggtggt ccattgtaca tcttcggtta ctgtttcggt    660

atcttcggtg gtattgtcta ttccgtacca cctttttagat ggaaacaaaa ccctagtact    720

gccttttttgt tgaatttctt agctcatatc atcacaaact tcaccttcta ctacgcttca    780

agagctgctt taggtttgcc attcgaattg agaccttcat tcacattttt gttggcattc    840

atgaaaagta tgggttctgc attagccttg atcaaggatg cctctgacgt tgaaggtgac    900

acaaagttcg gtattagtac cttggcttct aagtacggtt caagaaattt gactttgttc    960

tgctccggta tcgtttttgtt aagttacgtc gcagccattt tggcaggtat catttggcca   1020

caagccttta attctaacgt tatgttgttg tcacatgcca tcttggcttt ctggttgatc   1080

ttgcaaacta gagatttcgc tttgacaaat tatgaccctg aagcaggtag aagattctac   1140

gagtttatgt ggaaattgta ctacgctgaa tatttggtat acgtttttat tgaaggtaga   1200

ggttcttttgt tgacctgtgg tgacgttgaa gaaaatccag gtcctatgaa atgttcaact   1260

ttctcctttt ggttcgtatg caagatcatc ttctttttct tttcctttaa catccaaaca   1320

agtatcgcaa acccaagaga aaactttttg aagtgcttct cacaatacat acctaataac   1380

gccaccaatt tgaagttggt ttacactcaa aacaacccat tgtacatgtc cgtcttgaac   1440

agtacaatcc ataatttgag attcacttct gataccactc caaaacctt ggtcattgta    1500

accctagtc atgtatctca catccaaggt actatcttat gttctaaaaa ggttggtttg   1560
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caaattagaa ctagatccgg tggtcatgat agtgaaggca tgtcatacat ctcccaagtt   1620

ccattcgtta tcgttgattt gagaaacatg agatcaatta aaatagacgt acactcacaa   1680

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aatgaaaact tatcattggc tgctggttac tgtccaacag tttgcgcagg tggtcatttt   1800

ggtggtggtg gttatggtcc tttaatgaga aactacggtt tggccgctga taacataatc   1860

gacgctcatt tggtaaatgt tcacggtaaa gttttggata gaaagtctat gggtgaagac   1920

ttattttggg ctttgagagg tggtggtgca gaatcattcg gtatcatagt tgcttggaag   1980

ataagattag tcgcagtacc aaagtctact atgttctcag tcaaaaagat aatggaaatc   2040

catgaattag ttaaattggt caataagtgg caaaacatcg catacaagta cgataaggac   2100

ttgttgttga tgactcattt catcacaaga aacatcaccg ataaccaagg taaaaataag   2160

actgctatcc acacatactt ttcttcagtt ttcttgggtg gtgtcgattc cttagtagac   2220

ttgatgaata agtcttttcc agaattaggt attaagaaaa ctgattgtag acaattgtct   2280

tggatcgaca ccatcatctt ttattcaggt gttgtcaact acgatacaga caacttcaac   2340

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agtgaatctg ccattccatt tcctcataga gctggtatct tatacgaatt gtggtacatt   2580

tgttcatggg aaaagcaaga agataacgaa aagcacttaa actggattag aaacatctat   2640

aacttcatga ctccatacgt ttctaaaaac cctagattgg catatttgaa ctacagagat   2700

ttggacatcg gtattaacga tccaaagaat cctaacaact atacccaagc tagaatttgg   2760

ggtgaaaaat acttcggtaa aaatttcgat agattagtaa aggttaagac attggttgac   2820

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caaaaattaa taagtgaaga agatttgtaa gcggccgc                           2918


<210> SEQ ID NO 17
<211> LENGTH: 167
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 17

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caacaggcat tttcggatat gagtcacgtg gagtattcca gaattacaaa attttttcaa    120

gaacaaccac tggagggata tacccttttc tctcacaggt ctgcgcc                  167


<210> SEQ ID NO 18
<211> LENGTH: 1518
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 18

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ataagaatag ataatactag aacttggaca tacgatgacg cctttgcttt atctggtaga    120

atagcatcag ctatggatgc tttgggtatc agaccaggtg acagagtcgc agttcaagta    180
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gaaaaatccg ctgaagcatt gatcttgtat ttggcttgtt tgagaagtgg tgcagtttat      240

ttgccattga atactgccta cacattagct gaattggatt acttcatagg tgacgcagaa      300

cctagattgg ttgtagtcgc ctcttcagcc agagctggtg tagaaacaat tgctaaacca      360

agaggtgcaa tagtcgaaac cttagatgct gctggttctg gtagtttgtt agatttggcc      420

agagacgaac ctgctgattt tgttgacgct tcaagatcag ccgatgactt agccgctatt      480

ttgtacacct ctggtactac aggtagatca aagggtgcta tgttgactca tggtaatttg      540

ttgtcaaacg cattaacctt gagagatttc tggagagtta ctgccggtga cagattaatc      600

cacgctttgc caatttttca tactcacggt ttattcgttg ctaccaacgt aactttgtta      660

gcaggtgcct ccatgttctt gttgagtaag ttcgatccag aagaaatatt atctttgatg      720

cctcaagcta ctatgttgat gggtgtccca acattctacg ttagattgtt acaatcacct      780

agattagata agcaagctgt tgcaaacatc agattgttta tatccggtag tgctccattg      840

ttagcagaaa cccatactga atttcaagca agaacaggtc acgccatttt agaaagatac      900

ggtatgacag aaaccaatat gaacacttct aacccttatg aaggtaaaag aatagctggt      960

acagttggtt ttccattgcc tgatgtcaca gttagagtaa ccgacccagc cactggttta     1020

gctttgccac ctgaacaaac tggtatgatc gaaattaaag gtccaaacgt ttttaagggt     1080

tactggagaa tgcctgaaaa gactgctgct gagtttactg ctgatggttt ctttatctct     1140

ggtgacttag gtaaaattga tagagacggt tatgtccata ttgttggtcg tggtaaagat     1200

ttggttatat ccggtggtta taacatctac cctaaggaag tagaaggtga aatagatcaa     1260

atcgaaggtg ttgtagaatc agctgtaata ggtgtcccac atcctgattt tggtgaaggt     1320

gttacagcag tcgttgtaag aaaaccaggt gctgcattag atgaaaaggc aattgtttct     1380

gccttacaag acagattggc tagatacaag caaccaaaga gaataatctt cgcagaagat     1440

ttgcctagaa atactatggg taaagtacaa aagaacatct tgagacaaca atacgccgac     1500

ttatacacca gaacctga                                                    1518
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<210> SEQ ID NO 19
<211> LENGTH: 2092
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 19

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cctattaagt actcttacaa caactttcca tcaaaacatt gttcaaccaa gtccttccac      180

ttacaaaata agtgctccga aagtttgtct atagctaaga actctatcag agctgcaact      240

acaaatcaaa ctgaaccacc tgaaagtgat aatcactctg ttgccacaaa aattttgaac      300

ttcggtaaag catgttggaa gttgcaaaga ccatacacca taatcgcttt tacttcttgt      360

gcatgcggtt tattcggtaa agaattgttg cataacacta acttaatttc atggtcctat      420

gaactgctcc gcattctctt tctggttcgt ctgtaaaata atcttcttct tcttgtcctt      480

caacatccaa atctccatcg caaatcccaca agaaaacttt ttgaagtgtt tctccgaata      540

catcccaaac aaccctgcta acccaaagtt tatatatact caacatgatc aattgtacat      600

gtccgttttg aacagtacca tccaaaattt gagattcact tctgacacta caccaaaacc      660

tttagtcatt gttacacctt ccaatgttag tcacattcaa gcttctatat tgtgctctaa      720
```

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gaaagtaggt ttgcaaatca gaactagatc aggtggtcat gatgcagaag gcatgtctta    780

catctcacaa gttccattcg ttgtagtcga tttgagaaat atgcattcca taaagatcga    840

cgttcacagt caaacagcat gggtagaagc aggtgccacc ttgggtgaag tttactactg    900

gatcaacgaa aagaatgaaa acttttcttt ccctggtggt tactgtccaa cagtaggtgt    960

cggtggtcac tttctggtg gtggttatgg tgcattgatg agaaactacg gtttagctgc    1020

agataatatt atagacgccc atttggttaa cgtagatggt aaagttttgg acagaaagtc    1080

tatgggtgaa gatttgtttt gggccataag aggtggtggt ggtgaaaatt tcggtatcat    1140

tgccgcttgg aaaattaagt tagtcgctgt tccttccaaa agtactattt tctctgtcaa    1200

aaagaacatg gaaatccacg gtttggttaa gttgtttaat aagtggcaaa acatcgctta    1260

caagtacgat aaggacttgg ttttgatgac ccatttcatc actaaaaata ttacagataa    1320

ccatggtaaa aataagacca ctgttcacgg ttattttct tcaattttcc atggtggtgt    1380

agattctttg gttgatttga tgaataagtc attcccagaa ttgggtatta aaaagacaga    1440

ttgcaaggaa ttttcttgga tagacacaac catcttctat tcaggtgttg taaacttcaa    1500

caccgctaac ttcaaaaagg aaatcttgtt ggatagatcc gctggtaaaa agaccgcttt    1560

ttctattaaa ttggactacg ttaagaaacc aatccctgaa actgcaatgg tcaagatatt    1620

ggaaaagttg tacgaagaag atgtaggtgt cggcatgtac gttttgtatc catacggtgg    1680

tattatggaa gaaatatctg aatcagccat accatttcct cacagagctg gtatcatgta    1740

tgaattatgg tacacagcct catgggaaaa gcaagaagat aacgaaaagc atatcaactg    1800

ggtcagatcc gtttacaact tcactacacc ttacgttagt caaaacccaa gattggcata    1860

tttgaactac agagatttgg acttaggtaa aactaaccct gaatctccaa ataactatac    1920

acaagcaaga atttggggtg aaaagtactt tggtaaaaat ttcaacagat tagttaaagt    1980

aaagactaaa gccgacccta acaactttt cagaaacgaa caatccatcc acctttgcc    2040

acctcaccac cacgaacaaa aattaataag tgaagaagat ttgtaagtcg ac    2092
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<210> SEQ ID NO 20
<211> LENGTH: 7904
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 20
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cagcttgtct gtaagcggat gccgggagca gacaagcccg tcagggcgcg tcagcgggtg    120

ttggcgggtg tcggggctgg cttaactatg cggcatcaga gcagattgta ctgagagtgc    180

accatatcga ctacgtcgta aggccgtttc tgacagagta aaattcttga gggaactttc    240

accattatgg gaaatggttc aagaaggtat tgacttaaac tccatcaaat ggtcaggtca    300

ttgagtgttt tttattgtt gtattttttt tttttagag aaaatcctcc aatatcaaat    360

taggaatcgt agtttcatga ttttctgtta cacctaactt tttgtgtggt gccctcctcc    420

ttgtcaatat taatgttaaa gtgcaattct ttttccttat cacgttgagc cattagtatc    480

aatttgctta cctgtattcc tttactatcc tcctttttct ccttcttgat aaatgtatgt    540

agattgcgta tatagtttcg tctaccctat gaacatattc cattttgtaa tttcgtgtcg    600

tttctattat gaatttcatt tataaagttt atgtacaaat atcataaaaa aagagaatct    660
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ttttaagcaa ggatttttctt aacttcttcg gcgacagcat caccgacttc ggtggtactg    720

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tcaatggcct taccttcttc aggcaagttc aatgacaatt tcaacatcat tgcagcagac    840

aagatagtgg cgatagggtc aaccttattc tttggcaaat ctggagcaga accgtggcat    900

ggttcgtaca aaccaaatgc ggtgttcttg tctggcaaag aggccaagga cgcagatggc    960

aacaaaccca aggaacctgg gataacggag gcttcatcgg agatgatatc accaaacatg   1020

ttgctggtga ttataatacc atttaggtgg gttgggttct taactaggat catggcggca   1080

gaatcaatca attgatgttg aaccttcaat gtagggaatt cgttcttgat ggtttcctcc   1140

acagtttttc tccataatct tgaagaggcc aaaacattag ctttatccaa ggaccaaata   1200

ggcaatggtg gctcatgttg tagggccatg aaagcggcca ttcttgtgat tctttgcact   1260

tctggaacgg tgtattgttc actatcccaa gcgacaccat caccatcgtc ttcctttctc   1320

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aattgtggct tgattggaga taagtctaaa agagagtcgg atgcaaagtt acatggtctt   1440

aagttggcgt acaattgaag ttctttacgg atttttagta aaccttgttc aggtctaaca   1500

ctacggtac cccatttagg accacccaca gcacctaaca aaacggcatc aaccttcttg   1560

gaggcttcca gcgcctcatc tggaagtggg acacctgtag catcgatagc agcaccacca   1620

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tattgctgaa atgtaaaagg taagaaaagt tagaaagtaa gacgattgct aaccacctat   1860

tggaaaaaac aataggtcct taaataatat tgtcaacttc aagtattgtg atgcaagcat   1920

ttagtcatga acgcttctct attctatatg aaaagccggt tccggcctct caccttttcct   1980

tttttctccca atttttttcagt tgaaaaaggt atatgcgtca ggcgacctct gaaattaaca   2040

aaaaatttcc agtcatcgaa tttgattctg tgcgatagcg ccctgtgtg ttctcgttat    2100

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gccaaacaac caattacttg ttgagaaata gagtataatt atcctataaa tataacgttt   2280

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gaatagaccg agatagggtt gagtgttgtt ccagtttgga acaagagtcc actattaaag   2640

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cagctggcga gggggggatg tgctgcaagg cgattaagtt gggtaacgcc agggttttcc   3060
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cagttcggtg taggtcgttc gctccaagct gggctgtgtg cacgaacccc ccgttcagcc    6360

cgaccgctgc gccttatccg gtaactatcg tcttgagtcc aacccggtaa gacacgactt    6420

atcgccactg gcagcagcca ctggtaacag gattagcaga gcgaggtatg taggcggtgc    6480

tacagagttc ttgaagtggt ggcctaacta cggctacact agaaggacag tatttggtat    6540

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 21

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accacgcttt tcaattcaat tcatcatttt tttttattc ttttttttga tttcggtttc     240

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

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gtaaaatcgg tacagatatt caagacaata agtgcagttg ggttattaac aaggctttgg    4920

aattagcatc tgccgaacaa agaaagactt tggatgaaaa ctacggtaaa aaggactcag    4980

ttgctgaagc aaagtgtaag aaaattttta atgatttgaa gattgaacaa ttgtaccatg    5040

aatacgaaga atccatcgct aaagacttaa aggcaaagat tagtcaagtt gatgaatcaa    5100

gaggttttaa agccgacgtt ttgacagctt tcttgaataa ggtctacaag agatcaaagt    5160

gatgggctgc aggaattcga tatcaagctt atcgataccg tcgacctcga gtcatgtaat    5220

tagttatgtc acgcttacat tcacgccctc cccccacatc cgctctaacc gaaaaggaag    5280

gagttagaca acctgaagtc taggtcccta tttatttttt tatagttatg ttagtattaa    5340

gaacgttatt tatatttcaa attttttcttt tttttctgta cagacgcgtg tacgcatgta    5400

acattatact gaaaaccttg cttgagaagg ttttgggacg ctcgaaggct ttaatttgcg    5460

gccggtaccc agcttttgtt ccctttagtg agggttaatt ccgagcttgg cgtaatcatg    5520

gtcatagctg tttcctgtgt gaaattgtta tccgctcaca attccacaca acataggagc    5580

cggaagcata aagtgtaaag cctggggtgc ctaatgagtg aggtaactca cattaattgc    5640

gttgcgctca ctgcccgctt tccagtcggg aaacctgtcg tgccagctgc attaatgaat    5700

cggccaacgc gcggggagag gcggtttgcg tattgggcgc tcttccgctt cctcgctcac    5760

tgactcgctg cgctcggtcg ttcggctgcg gcgagcggta tcagctcact caaaggcggt    5820

aatacggtta tccacagaat caggggataa cgcaggaaag aacatgtgag caaaaggcca    5880

gcaaaaggcc aggaaccgta aaaaggccgc gttgctggcg tttttccata ggctcggccc    5940

ccctgacgag catcacaaaa atcgacgctc aagtcagagg tggcgaaacc cgacaggact    6000

ataaagatac caggcgttcc cccctggaag ctccctcgtg cgctctcctg ttccgaccct    6060

gccgcttacc ggatacctgt ccgcctttct cccttcggga agcgtggcgc tttctcaatg    6120

ctcacgctgt aggtatctca gttcggtgta ggtcgttcgc tccaagctgg gctgtgtgca    6180

cgaacccccc gttcagcccg accgctgcgc cttatccggt aactatcgtc ttgagtccaa    6240

cccggtaaga cacgacttat cgccactggc agcagccact ggtaacagga ttagcagagc    6300

gaggtatgta ggcggtgcta cagagttctt gaagtggtgg cctaactacg gctacactag    6360

aaggacagta tttggtatct gcgctctgct gaagccagtt accttcggaa aaagagttgg    6420

tagctcttga tccggcaaac aaaccaccgc tggtagcggt ggtttttttg tttgcaagca    6480

gcagattacg cgcagaaaaa aaggatctca agaagatcct ttgatctttt ctacggggtc    6540

tgacgctcag tggaacgaaa actcacgtta agggattttg gtcatgagat tatcaaaaag    6600

gatcttcacc tagatccttt taaattaaaa atgaagtttt aaatcaatct aaagtatata    6660

tgagtaaact tggtctgaca gttaccaatg cttaatcagt gaggcaccta tctcagcgat    6720
```

```
ctgtctattt cgttcatcca tagttgcctg actgcccgtc gtgtagataa ctacgatacg    6780

ggagggctta ccatctggcc ccagtgctgc aatgataccg cgagacccac gctcaccggc    6840

tccagattta tcagcaataa accagccagc cggaagggcc gagcgcagaa gtggtcctgc    6900

aactttatcc gcctccatcc agtctattaa ttgttgccgg gaagctagag taagtagttc    6960

gccagttaat agtttgcgca acgttgttgc cattgctaca ggcatcgtgg tgtcacgctc    7020

gtcgtttggt atggcttcat tcagctccgg ttcccaacga tcaaggcgag ttacatgatc    7080

ccccatgttg tgaaaaaaag cggttagctc cttcggtcct ccgatcgttg tcagaagtaa    7140

gttggccgca gtgttatcac tcatggttat ggcagcactg cataattctc ttactgtcat    7200

gccatccgta agatgctttt ctgtgactgg tgagtactca accaagtcat tctgagaata    7260

gtgtatgcgg cgaccgagtt gctcttgccc ggcgtcaata cgggataata ccgcgccaca    7320

tagcagaact ttaaaagtgc tcatcattgg aaaacgttct tcggggcgaa aactctcaag    7380

gatcttaccg ctgttgagat ccagttcgat gtaacccact cgtgcaccca actgatcttc    7440

agcatctttt actttcacca gcgtttctgg gtgagcaaaa acaggaaggc aaaatgccgc    7500

aaaaaaggga ataagggcga cacggaaatg ttgaatactc atactcttcc tttttcaata    7560

ttattgaagc atttatcagg gttattgtct catgagcgga tacatatttg aatgtattta    7620

gaaaaataaa caaatagggg ttccgcgcac atttccccga aaagtgccac ctgacgtcta    7680

agaaaccatt                                                            7690
```

<210> SEQ ID NO 23
<211> LENGTH: 2163
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 23

```
atgggtaaaa actataagtc cttggattca gtcgttgcct cagatttcat cgcattgggt      60

atcacctcag aagtagcaga aacattacat ggtagattgg cagaaatcgt ttgtaattat     120

ggtgctgcaa cccctcaaac ttggatcaac atcgctaacc atatcttgtc accagatttg     180

cctttctcct tacaccaaat gttgttttat ggttgctaca aggatttcgg tccagcccca     240

cctgcttgga ttccagaccc tgaaaaagtc aagtcaacta atttgggtgc tttgttggaa     300

aagagaggta aagaattttt gggtgtaaag tacaaagatc caatttcttc tttttctcac     360

ttccaagaat tttctgttag aaaccctgaa gtctattgga gaacagtatt gatggatgaa     420

atgaaaatta gtttctctaa ggaccccagaa tgtatcttga agagagatga catcaacaac     480

ccaggtggtt ctgaatggtt acctggtggt tacttgaact cagctaaaaa ttgcttgaac     540

gtaaactcca ataagaaatt gaacgatact atgatcgttt gggagacgga gggtaacgat     600

gacttgcctt tgaataagtt gacattagat caattgagaa agagagtttg gttggttggt     660

tatgcattgg aagaaatggg tttagaaaaa ggttgtgcaa tagccatcga tatgccaatg     720

catgttgatg ctgttgttat atatttggcc atagtattgg ctggttacga agttgtctct     780

atagcagatt cattttccgc ccctgaaatc tcaactagat tgagattatc caaagctaag     840

gcaattttca cacaagatca catcatcaga ggtaaaaaga gaataccatt gtattcaaga     900

gtagttgaag ctaaatcccc aatggcaata gttatccctt gtagtggttc taacattggt     960

gcagaattga gagatggtga catatcttgg gattactttt tagaaagagc caaggagttt    1020
```

```
aaaaactgcg agtttactgc cagagaacaa cctgttgatg cttatactaa catcttattc    1080

tccagtggta ctacaggtga accaaaagca attccttgga cacaagccac cccattgaag    1140

gctgctgctg atggttggtc tcatttggat attagaaaag gtgacgttat agtatggcca    1200

actaatttgg gttggatgat gggtccttgg ttggtttatg ctagtttgtt aaatggtgca    1260

tctattgcct tgtacaacgg tagtccttta gtctctggtt tcgctaaatt tgttcaagat    1320

gcaaaggtca caatgttggg tgtcgtacca tctattgtaa gatcatggaa atccacaaat    1380

tgtgtttcag gttacgattg gtccaccata agatgctttt cttcatccgg tgaagcctct    1440

aatgtagacg aatatttgtg gttaatgggt agagctaact acaagccagt tatagaaatg    1500

tgtggtggta cagaaatcgg tggtgctttt tctgctggtt cattttttgca agctcaatct    1560

ttaagttctt tttcatccca atgtatgggt tgcaccttgt acatattaga taagaacggt    1620

tacccaatgc ctaaaaataa gccaggtatc ggtgaattgg cattaggtcc tgttatgttt    1680

ggtgcctcaa aaacattgtt aaacggtaat catcacgatg tctatttcaa gggtatgcca    1740

accttgaatg gtgaagtatt gagaagacat ggtgacattt tcgaattgac ctctaacggt    1800

tactaccatg cacacggtag agccgatgac actatgaaca tcggtggtat caaaattagt    1860

tctatcgaaa tcgaaagagt ctgtaatgaa gtagatgaca gagtttttga aaccactgct    1920

attggtgttc cacctttggg tggtggtcca gaacaattgg tcatattttt cgtattgaag    1980

gattcaaacg acacaaccat tgatttgaac caattgagat tatcctttaa cttgggtttg    2040

caaaagaaat tgaacccatt attcaaagtt actagagttg tcccattgtc atccttacct    2100

agaactgcaa caaacaagat catgagaaga gttttgagac aacaattcag tcatttcgaa    2160

tga                                                                   2163
```

<210> SEQ ID NO 24
<211> LENGTH: 1059
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 24

```
atggcttcag aaaaggaaat aagaagagaa agattcttga acgtattccc aaagttagtt      60

gaagaattga acgctagttt gttagcttat ggtatgccta aagaagcctg cgattggtat     120

gctcactctt taaactacaa tactccaggt ggtaaattga atagaggttt gagtgtagtt     180

gatacttatg ctatcttgtc taacaaaacc gttgaacaat taggtcaaga agaatacgaa     240

aaggtcgcta tcttgggttg gtgtattgaa ttgttgcaag catacttttt ggttgccgat     300

gacatgatgg ataagtctat aacaagaaga ggtcaaccat gctggtacaa agttccagaa     360

gttggtgaaa tagccataaa tgatgctttt atgttggaag ccgctatcta taaattgttg     420

aagtcacatt tcagaaacga aaagtactac atcgatatta ccgaattatt ccacgaagtt     480

actttccaaa cagaattggg tcaattgatg gatttgataa ctgcacctga agataaagtt     540

gacttgtcaa agttttcctt gaagaaacat tcattcatcg tcacctttga aactgcttat     600

tactccttct atttgccagt cgccttggct atgtacgtag ctggtattac tgatgaaaaa     660

gacttgaagc aagcaagaga tgtttttgata cctttgggtg aatacttcca aatccaagat     720

gactacttag actgtttcgg tactccagaa caaataggta aaatcggtac agatattcaa     780

gacaataagt gcagttgggt tattaacaag gctttggaat tagcatctgc cgaacaaaga     840

aagactttgg atgaaaacta cggtaaaaag gactcagttg ctgaagcaaa gtgtaagaaa     900
```

```
atttttaatg atttgaagat tgaacaattg taccatgaat acgaagaatc catcgctaaa        960

gacttaaagg caaagattag tcaagttgat gaatcaagag gttttaaagc cgacgttttg       1020

acagctttct tgaataaggt ctacaagaga tcaaagtag                              1059


<210> SEQ ID NO 25
<211> LENGTH: 1158
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 25

atgaaccatt tgagagccga aggtcctgcc tccgtattag ccataggtac agccaaccca         60

gaaaacatat tgatccaaga tgaatttcct gattattact tcagagttac caagagtgaa        120

cacatgactc aattgaagga aaagtttaga aaaatatgtg ataagtctat gatcagaaag        180

agaaactgct tcttgaacga agaacatttg aagcaaaatc caagattggt agaacacgaa        240

atgcaaacat tggatgccag acaagacatg ttagttgtcg aagttcctaa attgggtaaa        300

gatgcttgtg caaaagccat taaggaatgg ggtcaaccaa agtcaaagat cactcatttg        360

atttttacaa gtgcatctac tacagatatg cctggtgcag actaccactg tgccaaattg        420

ttaggtttgt caccatccgt taagagagtc atgatgtatc aattaggttg ctacggtggt        480

ggtactgttt tgagaatcgc taaggatatt gcagaaaaca caagggtgc cagagtatta        540

gctgtttgtt gcgacattat ggcttgcttg tttagaggtc caagtgattc tgacttggaa        600

ttgttagttg gtcaagctat cttcggtgac ggtgctgctg ctgttattgt tggtgcagaa        660

cctgacgaat ctgttggtga aagaccaata tttgaattag tcagtacagg tcaaaccatc        720

ttgcctaatt ctgaaggtac aattggtggt catataagag aagcaggttt gatcttcgat        780

ttgcacaaag acgttccaat gttaatctct aacaacatag aaaagtgttt gatagaagca        840

ttcactccta taggtatctc agattggaac tctattttct ggataacaca tccaggtggt        900

aaagccattt tggataaggt tgaagaaaaa ttggatttga agaaagaaaa gtttgtagat        960

agtagacatg ttttatctga acacggtaac atgtcttcat ccactgtctt gttcgtaatg       1020

gatgaattga aaagagatc attagaagag ggtaaatcta ctactggtag cggttttgaa        1080

tggggtgtct tatttggttt cggtcctggt ttgaccgtcg aaagagtagt tgtcagatca        1140

gtaccaatta aatattag                                                     1158


<210> SEQ ID NO 26
<211> LENGTH: 315
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 26

atggccgtca agcatttgat agtattgaag tttaaagatg aaatcacaga agctcaaaag         60

gaagaatttt tcaagaccta cgttaatttg gtcaacatta tacctgctat gaaagatgta        120

tactggggta aagacgttac acaaaagaaa gaagaaggtt atacacacat tgtcgaagta        180

accttcgaat cagttgaaac tatccaagat tacatcattc atccagctca cgttggtttt        240

ggtgacgttt acagatcctt ctgggaaaaa ttgttgatct tcgattacac cccaagaaag        300

ttaaagccaa aataa                                                        315
```

<210> SEQ ID NO 27
<211> LENGTH: 1188
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 27

```
atgggtttat catccgtctg tactttctcc ttccaaacta actatcatac cttattgaat      60

cctcacaaca acaatccaaa aacatcattg ttgtgttaca gacatccaaa gacacctatt     120

aagtactctt acaacaactt tccatcaaaa cattgttcaa ccaagtcctt ccacttacaa     180

aataagtgct ccgaaagttt gtctatagct aagaactcta tcagagctgc aactacaaat     240

caaactgaac cacctgaaag tgataatcac tctgttgcca caaaaatttt gaacttcggt     300

aaagcatgtt ggaagttgca aagaccatac accataatcg cttttacttc ttgtgcatgc     360

ggtttattcg gtaaagaatt gttgcataac actaacttaa tttcatggtc cttgatgttc     420

aaggcatttt tctttttagt tgccatcttg tgcatcgctt cattcaccac tacaattaat     480

caaatatacg atttgcacat cgacagaatt aacaaaccag atttgccttt ggcttcaggt     540

gaaatatccg tcaatactgc atggatcatg tctatcatag tagccttgtt cggtttgatc     600

atcacaatta aaatgaaggg tggtccattg tacatcttcg gttactgttt cggtatcttc     660

ggtggtattg tctattccgt accacctttt agatggaaac aaaaccctag tactgccttt     720

ttgttgaatt tcttagctca tatcatcaca aacttcacct tctactacgc ttcaagagct     780

gctttaggtt tgccattcga attgagacct tcattcacat ttttgttggc attcatgaaa     840

agtatgggtt ctgcattagc cttgatcaag gatgcctctg acgttgaagg tgacacaaag     900

ttcggtatta gtaccttggc ttctaagtac ggttcaagaa atttgacttt gttctgctcc     960

ggtatcgttt gttaagtta cgtcgcagcc attttggcag gtatcatttg gccacaagcc    1020

tttaattcta acgttatgtt gttgtcacat gccatcttgg cttttctggt gatcttgcaa    1080

actagagatt tcgctttgac aaattatgac cctgaagcag gtagaagatt ctacgagttt    1140

atgtggaaat gtactacgc tgaatatttg gtatacgttt ttatttag                  1188
```

<210> SEQ ID NO 28
<211> LENGTH: 1635
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 28

```
atgaaatgtt caactttctc cttttggttc gtatgcaaga tcatcttctt tttcttttcc      60

tttaacatcc aaacaagtat cgcaaaccca agagaaaact ttttgaagtg cttctcacaa     120

tacataccta ataacgccac caatttgaag ttggtttaca ctcaaaacaa cccattgtac     180

atgtccgtct tgaacagtac aatccataat ttgagattca cttctgatac cactccaaaa     240

cctttggtca ttgtaacccc tagtcatgta tctcacatcc aaggtactat cttatgttct     300

aaaaaggttg gtttgcaaat tagaactaga tccggtggtc atgatagtga aggcatgtca     360

tacatctccc aagttccatt cgttatcgtt gatttgagaa acatgagatc aattaaaata     420

gacgtacact cacaaactgc ttgggttgaa gctggtgcaa cattgggtga agtatactac     480

tgggttaacg aaaagaatga aaacttatca ttggctgctg gttactgtcc aacagtttgc     540

gcaggtggtc attttggtgg tggtggttat ggtcctttaa tgagaaacta cggtttggcc     600
```

```
gctgataaca taatcgacgc tcatttggta aatgttcacg gtaaagtttt ggatagaaag     660

tctatgggtg aagacttatt ttgggctttg agaggtggtg gtgcagaatc attcggtatc     720

atagttgctt ggaagataag attagtcgca gtaccaaagt ctactatgtt ctcagtcaaa     780

aagataatgg aaatccatga attagttaaa ttggtcaata agtggcaaaa catcgcatac     840

aagtacgata aggacttgtt gttgatgact catttcatca caagaaacat caccgataac     900

caaggtaaaa ataagactgc tatccacaca tacttttctt cagttttctt gggtggtgtc     960

gattccttag tagacttgat gaataagtct tttccagaat taggtattaa gaaaactgat    1020

tgtagacaat tgtcttggat cgacaccatc atcttttatt caggtgttgt caactacgat    1080

acagacaact tcaacaaaga aatattattg gatagatccg caggtcaaaa cggtgccttt    1140

aaaattaagt tagactacgt taaaaagcca atacctgaat cagttttcgt ccaaatctta    1200

gaaaaattgt acgaagaaga tattggtgca ggcatgtacg ccttgtatcc atacggtggt    1260

ataatggacg aaatcagtga atctgccatt ccatttcctc atagagctgg tatcttatac    1320

gaattgtggt acatttgttc atgggaaaag caagaagata acgaaaagca cttaaactgg    1380

attagaaaca tctataactt catgactcca tacgtttcta aaaaccctag attggcatat    1440

ttgaactaca gagatttgga catcggtatt aacgatccaa agaatcctaa caactatacc    1500

caagctagaa tttggggtga aaaatacttc ggtaaaaatt tcgatagatt agtaaaggtt    1560

aagacattgg ttgacccaaa caacttcttt agaaacgaac aatccattcc acctttacct    1620

agacatagac actga                                                    1635
```

```
<210> SEQ ID NO 29
<211> LENGTH: 1638
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 29
```

```
atgaactgct ccgcattctc tttctggttc gtctgtaaaa taatcttctt cttcttgtcc      60

ttcaacatcc aaatctccat cgcaaatcca caagaaaact ttttgaagtg tttctccgaa     120

tacatcccaa acaaccctgc taacccaaag tttatatata ctcaacatga tcaattgtac     180

atgtccgttt tgaacagtac catccaaaat ttgagattca cttctgacac tacaccaaaa     240

cctttagtca ttgttacacc ttccaatgtt agtcacattc aagcttctat attgtgctct     300

aagaaagtag gtttgcaaat cagaactaga tcaggtggtc atgatgcaga aggcatgtct     360

tacatctcac aagttccatt cgttgtagtc gatttgagaa atatgcattc cataaagatc     420

gacgttcaca gtcaaacagc atgggtagaa gcaggtgcca ccttgggtga agtttactac     480

tggatcaacg aaaagaatga aaactttttct ttccctggtg gttactgtcc aacagtaggt     540

gtcggtggtc acttttctgg tggtggttat ggtgcattga tgagaaacta cggtttagct     600

gcagataata ttatagacgc ccatttggtt aacgtagatg gtaaagtttt ggacagaaag     660

tctatgggtg aagatttgtt ttgggccata agaggtggtg gtggtgaaaa tttcggtatc     720

attgccgctt ggaaaattaa gttagtcgct gttccttcca aaagtactat tttctctgtc     780

aaaaagaaca tggaaatcca cggtttggtt aagttgttta ataagtggca aaacatcgct     840

tacaagtacg ataaggactt ggttttgatg acccatttca tcactaaaaa tattacagat     900

aaccatggta aaaataagac cactgttcac ggttattttt cttcaatttt ccatggtggt     960
```

-continued

```
gtagattctt tggttgattt gatgaataag tcattcccag aattgggtat taaaaagaca      1020

gattgcaagg aattttcttg gatagacaca accatcttct attcaggtgt tgtaaacttc      1080

aacaccgcta acttcaaaaa ggaaatcttg ttggatagat ccgctggtaa aaagaccgct      1140

tttttctatta aattggacta cgttaagaaa ccaatccctg aaactgcaat ggtcaagata     1200

ttggaaaagt tgtacgaaga agatgtaggt gtcggcatgt acgttttgta tccatacggt      1260

ggtattatgg aagaaatatc tgaatcagcc ataccatttc ctcacagagc tggtatcatg      1320

tatgaattat ggtacacagc ctcatgggaa aagcaagaag ataacgaaaa gcatatcaac      1380

tgggtcagat ccgtttacaa cttcactaca ccttacgtta gtcaaaaccc aagattggca      1440

tatttgaact acagagattt ggacttaggt aaaactaacc ctgaatctcc aaataactat      1500

acacaagcaa gaatttgggg tgaaaagtac tttggtaaaa atttcaacag attagttaaa      1560

gtaaagacta aagccgaccc taacaacttt ttcagaaacg aacaatccat cccacctttg      1620

ccacctcacc accactaa                                                   1638


<210> SEQ ID NO 30
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 30

aggaaacgaa gataaatctc gagtttatca ttatcaatac tg                         42


<210> SEQ ID NO 31
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 31

ggaaaaatca gtcaaggcaa attaaagcct tcgagcg                               37


<210> SEQ ID NO 32
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 32

gatggggggat ccactagttc tagaatc                                         27


<210> SEQ ID NO 33
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 33

tgatgggctg caggaattcg atatc                                            25


<210> SEQ ID NO 34
<211> LENGTH: 38
<212> TYPE: DNA
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gtgcccgtcg actcattcga aatgactgaa ttg                                  33
```

What is claimed is:

1. A method for making cannabidiolic acid, the method comprising:

transforming *S. cervisiae* with a first nucleotide sequence comprising the nucleotide sequence of SEQ. ID. NO.1 expressing an acyl-activating enzyme;

transforming the *S. cervisiae* with a second nucleotide sequence comprising the nucleotide sequence of SEQ. ID. NO.22 expressing a mutant prenyltransferase;

transforming the *S. cervisiae* with a third nucleotide sequence comprising the nucleotide sequence of SEQ. ID. NO.25 expressing olivetolic synthase;

transforming the *S. cervisiae* with a fourth nucleotide sequence comprising the nucleotide sequence of SEQ. ID. NO.26 expressing olivetolic acid cyclase;

transforming the *S. cervisiae* with a fifth nucleotide sequence comprising the nucleotide sequence of SEQ. ID. NO.27 expressing aromatic prenyltransferase; and

transforming the *S. cervisiae* with a sixth nucleotide sequence comprising the nucleotide sequence of SEQ. ID. NO.28 expressing cannabidiolic acid synthase.

* * * * *

EXHIBIT B – PART 2

Begins on the following page

MERRY JANE

Monday, October 22, 2018 | 2:28 PM

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California Company Creates

The new extract is made from humulus, a variety of hop plant that naturally cross-pollinated with wild cannabis in India, and thus is entirely legal to sell.

Photo via iStock/ Targrid

Cannabidiol, more popularly known as CBD, is one of the hottest new trends in medicine and wellness, and patients and physicians across the country are uncovering new uses for this natural extract, ranging from the treatment of epilepsy to pain relief and anxiety. Although the medical evidence confirming the safety and efficacy of these treatments is overwhelming, the DEA still classifies CBD as a Schedule I drug in the U.S., since it's typically derived from prohibited cannabis or hemp plants. This has left CBD in a legal grey area, as it has been approved in some form by 30 U.S. states, and is widely available for sale throughout the rest of the country despite the federal ban.

The U.S. Food and Drug Administration has even approved a CBD-based epilepsy medication, but it seems likely that the price of this drug will be exponentially higher than other commercially-available CBD products. There are currently several different bills circulating in Congress that would authorize most forms of CBD federally by way of legalizing hemp, protecting states' rights to legalize marijuana, or by completely legalizing cannabis nationwide, but conservative Republicans have been finding ways to block all avenues of cannabis reform.

While these bills slowly make their way through the legislative process, a San Francisco-based company just discovered another interesting way around the problem — by creating a new CBD extract sourced from unquestionably legal plants.

Peak Health Foundation just unveiled Real Scientific Humulus Oil (RHSO-K), a CBD oil derived from a new strain of the humulus plant. Humulus is a variety of hop, not cannabis or hemp, and hence this oil is entirely legal in the U.S. Dr. Bomi Joseph, director of Peak Health, discovered the plant in the Silk Road region of India. Although hop plants do not traditionally contain cannabinoids, this particular variety of humulus has naturally cross-pollinated with cannabis, leading to some humulus strains with high concentrations of CBD and even THC.

Joseph told *Westword* that John Sullivan, a British governor of India in the mid-1800s, ordered his soldiers to collect and farm a variety of plants used for natural medicine, including hops, from around the country. This bit of history provided a

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The Doggfather's fresh tome of dope cuisine drops on October 23rd, so don't cheat yourself; treat yourself.

CULTURE

clue, and Joseph headed to India to locate the noted plant. "I knew that there was a chance of this [cannabis-humulus cross-pollination] actually happening," said Joseph, who began to take samples of humulus plants in order to test his hypothesis. "We looked at thousands of samples before we found one or two that had CBD in it," Joseph said, but once identifying them, "we were in good shape."

Peak Health selected the humulus strains with the largest natural quantities of CBD, and then cross-bred them in order to arrive at a new variety of hops with even higher amounts of cannabidiol, the kriya brand humulus. This specific plant, which is patented by Joseph, has an 18% concentration of CBD, making it ripe for production of medicinal oils. "This is certainly going to help change the dialogue for not only many parents whose children have epilepsy, but various other world markets which still, of course, consider cannabis part of the United Nations single convention treaty on narcotics," Stuart Titus, CEO of Medical Marijuana Inc., the company that is distributing this new product, said to *Westword*.

Published on August 1, 2018

Chris Moore 👥

Chris Moore is a New York-based writer who has written for Mass Appeal while also mixing records and producing electronic music.

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Hop-based CBD, Real Scientific Humulus Oil.   Kathryn Reinhardt, CMW Media

World's First Non-Cannabis CBD Oil Is High on Hops

shait CBD down in doughnuts, slurp it up with CBD-infused lattes, lather it on with lotions, gulp it down in capsules and, of course, puff it the old-fashioned way with high-CBD pot strains. But while the CBD craze consumes Colorado, CBD remains illegal in many American markets, since it is still labeled by the DEA's Schedule I as having "no currently accepted medical use and a high potential for abuse."

But there is a loophole: for CBD that is not derived from cannabis. And the Peak Health Foundation took advantage of that loophole to create Real Scientific Humulus Oil (RHSO-K), a CBD oil derived from the kriya brand humulus plant. Because that plant is a variety of hop, not cannabis, the oil is legal in this country.

RELATED STORIES

- The Clear Comes to Colorado — in CBD Form
- Eight Denver Spots Where You Can Add CBD to Food and Drinks
- The FDA Just Approved a CBD Drug. What Does That Mean for Colorado?

Discovered by Bomi Joseph in the Silk Road region of northern India, kriya brand humulus is naturally endowed with a high concentration of CBD because the hop plants cross-pollinate with wild cannabis plants that grow nearby. Peak Health, a San Francisco holistic medicine center where Joseph is the director, extracts a CBD oil from these plants that's dubbed ImmunAG.

Though his discovery and cross-breeding of kriya brand humulus may be a fresh development, the plant's story dates back to the mid-1800s when John Sullivan was a British governor in the southern part of India. Sullivan was ahead of

Dr. Bomi Joseph   Kathryn Reinhardt, CMW Media

his time, Joseph says: "He believed in natural health; he believed in natural curing. And he was powerful, right, he had the British government, they ruled India. He could do what he wanted. He made an estate called Stone House in a place called Ooty – it's a cool-climate, hilled station in the southern part of India – and he had the British soldiers bring plants from all over the country and plant them there."

Sullivan's Stone House became a sanatorium for the British. When they felt sick or in need of some rest and relaxation, they would go there, taking solace in the hills. Years later, researchers identified a variety of humulus yunnanensis at Stone House that was useful in treating malaria.

"That got my attention," Joseph says, "because normally when people talk about yunnanensis, they talk about China, the Yunnan province. So the fact that in the southern part of India, where my family is historically from, you find this humulus yunnanensis, I was like, 'How the hell did it get there?'"

He was determined to find out. Then Ari Cohen, one of his colleagues at Peak Health, found a reference to the yunannensis plant at a symposium given by India's Central Food Technological Research Institute. Their analysis of the plant discovered traces of cannabinoids.

Joseph cites this as his first tip. "I knew that there was a chance of this [cannabis-humulus cross-pollination] actually happening," Joseph recalls, so he headed to northern India and started searching. "In the beginning it was hard, because the native tribes people there, they're all sitting and looking at me like, 'What is this crazy guy doing?' They're like the porters, we had hired them and they're wandering around chewing betel nut, drinking their rice wine and sitting around looking at me. For a few weeks it was crazy, but then I finally showed them what we're looking for. Once they got it, they were just taking me here, taking me there, showing me this, showing me that. I was like, 'No, no, no,' but then we found it. It started getting faster and faster. Once they found some and we found some, then we started getting samples. But we looked at thousands of samples before we found one or two that had CBD in it."

A mature pod on a kriya humulus plant
Kathryn Reinhardt, CMW Media

After that, though, "We were in good shape," Joseph says. "Then it was just a matter of grunt work and effort," cross-breeding the plants (in which CBD is a recessive trait) until they'd created a dependable, high-CBD concentration variety.

Joseph's kriya brand humulus is a variety of humulus yunnanensis, one of three species of the humulus genus. Distinct from humulus lupulus – a different species of hop, the one from which the female flowers (known as hops, plural) are used to make beer – humulus yunnanensis is native to the Yunnan province in southern China, along the Indian border. Here, the plant was able to cross-pollinate with wild cannabis, as both genera are members of the same family of flowering plants, cannabaceae. This endowed kriya brand humulus with trace amounts of CBD and, in some cases, THC. Avoiding the latter, Joseph and his team meticulously selected and cross-bred plants with high concentrations of CBD until they arrived at a variety – kriya brand humulus – with an 18 percent CBD concentration. Joseph holds a patent for this as well as the

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modification of any other humulus plant to produce CBD and cannabinoids.

Through a partnership with distributor Medical Marijuana Inc. (which previously made headlines as the first publicly traded cannabis company in the U.S.), what's now known as ImmunAG is combined with medium-chain triglyceride oil to form RSHO-K. Last month, Medical Marijuana Inc. made the product available to consumers nationwide via its online store.

Since it's free of THC and the cumbersome legal baggage of cannabis, RSHO-K gives Stuart Titus, CEO of Medical Marijuana Inc., high hopes. Beyond simply filling gaps in the U.S. CBD market, he expects the product to have an international impact. "This is certainly going to help change the dialogue for not only many parents whose children have epilepsy," he says, "but various other world markets which still, of course, consider cannabis part of the United Nations single convention treaty on narcotics."

Looking back, Joseph is grateful for his luck. "If John Sullivan hadn't planted it and if a mention had not been made of it, I don't know if we would have had a clue," he says. "He did something that made it stick out and that led us to it. I'm sitting here in my office in Los Gatos, a fancy little place. I've got 500 megabit WiFi speeds; I can Google anything. But the reality is, we haven't studied more than 4 percent of all the plants that are out there. If I want to go beyond the 4 percent, I've got to go to the Amazon jungles, the Himalayan mountains; there's no other way. We've got to go get bitten by mosquitoes, chewed up by leeches and deal with the heat and humidity, there's no other way."

Nick Maahs is an editorial intern for Westword during summer 2018. Raised in Denver, he attends Whitman College in Walla Walla, Washington, where he tries to escape his small-town blues by studying English and writing for the student-run paper, *The Wire*.
CONTACT: Nick Maahs
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DENVER DEALS NEAR YOU

Juicy Fruit is a favorite among users who started toking in the '70s and '80s   Herbert Fuego

Tokers of the Old-School: Dispensaries Carrying Classic Strains

HERBERT FUEGO | OCTOBER 22, 2018 | 9:25AM

Thanks to legalization, we've never had more choices when buying pot; commercial breeders are constantly toying with the boundless combinations of cannabis genetics, looking for a way to get an edge up on the competition. But all these new options can make strain shopping tricky, especially for newcomers to this state encountering an embarrassment of riches.

Unlike in Colorado, tokers in non-legal states have had to purchase whatever was available where they grew up, giving someone from Illinois or Nebraska a larger affinity for Northern Lights, for example, than someone from the West Coast. And for anyone getting back into cannabis use after taking off for a few decades, such classic strains as AK-47, Hindu Kush or Maui Wowie provide a sense of comfort...if they can find them.

RELATED STORIES

- Hemp Sold in Dispensaries? DORA Recommends That and More!
- Colorado Marijuana Will Likely Require Heavy Metals Testing in 2019
- Ghostly Ganja: Ten Weed Strains for Halloween Lovers

Fortunately, despite the wide array of strains available in Colorado, a lot of the old, reliable strains are more available than you might think. Here are ten dispensaries that carry a boatload of classic buds, from Colombian Gold to East Coast Sour Diesel.

Botanico
3054 Larimer Street
303-297-2273
botanicommj.com

Diego Pellicer
2949 West Alameda Avenue
720-787-4909
co.diego-pellicer.com

Good Chemistry
Three metro locations
goodchem.org

Green Cross of Cherry Creek
3400 South Oneida Way
303-321-4201
greencrosscc.com

Healing House Denver
2383 South Downing Street
720-379-3816
healinghousefamily.com

The Kind Room carries a long list of old-school strains.   Scott Lentz

The Joint by Cannabis
4735 West 38th Avenue
303-455-0079
thejoint.life

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Sacred Seed
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Silver Stem Fine Cannabis

Herbert Fuego is the resident stoner at *Westword*, ready to
answer all your marijuana questions.
CONTACT: Herbert Fuego

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UPCOMING EVENTS

Casey Donahew Wed., Nov. 21, 8:30pm TICKETS	**Denver Broncos** Sun., Nov. 25, 2:25pm TICKETS
Smithfield and Elvie Shane Fri., Nov. 23, 8:30pm TICKETS	**Mark Chesnutt** Fri., Nov. 30, 8:30pm TICKETS
Chris Lane Sat., Nov. 24, 8:30pm TICKETS	**Alice 105.9's Alice in Winterland** Tue., Dec. 4, 6:00pm TICKETS
Comedian Alonzo Bodden Fri., Nov. 30, 7:30pm TICKETS	**A Drag Queen Christmas - The N...** Fri., Dec. 7, 7:00pm TICKETS

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US 20180153948A1

(54) **SYSTEMS AND METHODS FOR EXTRACTION OF NATURAL PRODUCTS**

(71) Applicant: **METAGREEN**, Los Angeles, CA (US)

(72) Inventor: **Randall B. Murphy**, Glenmoore, PA (US)

Publication Classification

(51) **Int. Cl.**
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A61K 31/352	(2006.01)
B01D 11/02	(2006.01)

(52) **U.S. Cl.**
CPC *A61K 36/185* (2013.01); *A61K 31/352* (2013.01); *A61K 2236/37* (2013.01); *A61K 2236/333* (2013.01); *B01D 11/0288* (2013.01)

(57) **ABSTRACT**

Described herein, inter alia, are processes, methods, and compositions useful for the extraction of natural products from source materials.

FIGURE 1

Extraction Efficiency of Mixtures of R22/DME

FIGURE 2

Effects of different gases on extraction efficiency
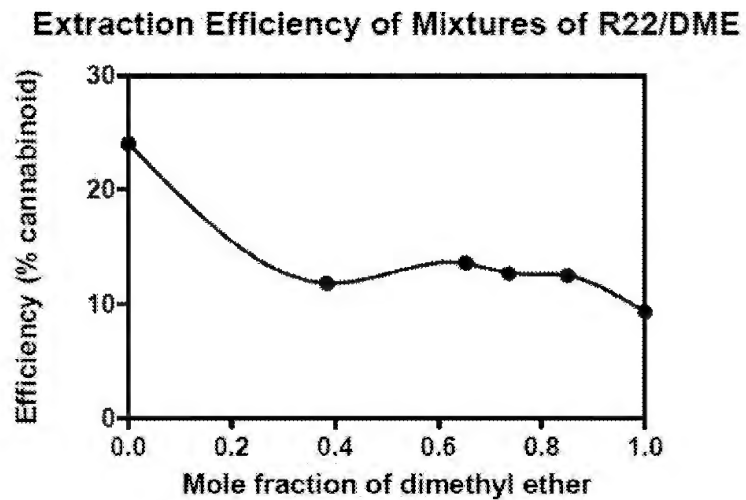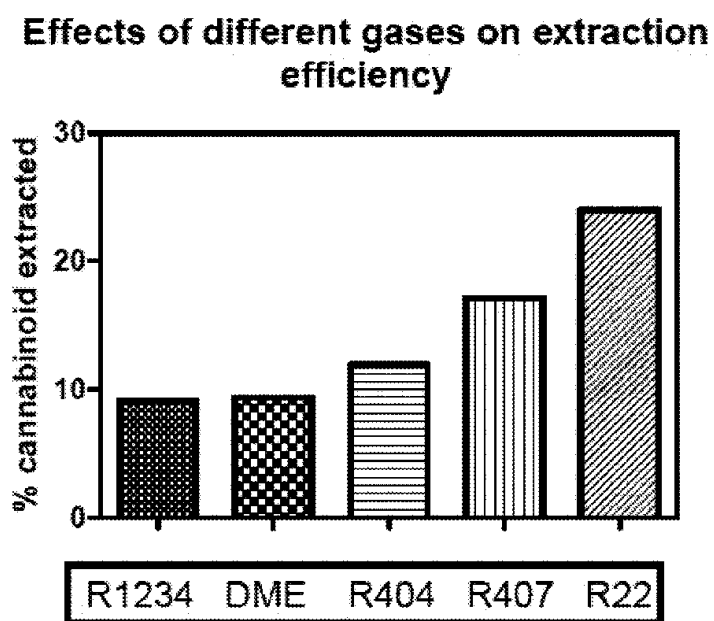
SYSTEMS AND METHODS FOR EXTRACTION OF NATURAL PRODUCTS

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/431,351, filed Dec. 7, 2016, which is incorporated herein by reference in entirety and for all purposes.

BACKGROUND OF THE INVENTION

[0002] A major problem in pharmaceutical chemistry relates to extraction of useful substances from plants or animals where such useful substances are employed for the formulation of a pharmaceutical or a nutraceutical. Various processes exist for the extraction of natural products from plant, animal, fungi, bacteria, or virus, but each of these processes suffer from one or more deficiencies. There is an unmet need for a new methodology which can safely and selectively extract desired medicinally or nutritionally valuable components from natural materials.

BRIEF SUMMARY OF THE INVENTION

[0003] In a first aspect, is provided a method of extracting a natural organic compound from a natural material, the method including contacting the natural material with an extraction fluid thereby extracting the natural organic compound from the natural material into the extraction fluid to form an extracted fluid solution. The extraction fluid includes a fluorophilic compound and a hydrofluorocarbon.
[0004] In another aspect, is provided a fluid including chlorodifluoromethane and dimethylether.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] FIG. 1 Graph depicting the efficiency of mixtures of fluorocarbon R22 and dimethyl ether (DME) to extract total cannabinoids in a single 30-minute extraction at 26° C.
[0006] FIG. 2 Graph demonstrating that the efficiency of pure materials to extract cannabinoids in a single 30-minute procedure at 26° C. can be determined.

DETAILED DESCRIPTION OF THE INVENTION

I. DEFINITIONS

[0007] The abbreviations used herein have their conventional meaning within the chemical and biological arts. The chemical structures and formulae set forth herein are constructed according to the standard rules of chemical valency known in the chemical arts.
[0008] Where substituent groups are specified by their conventional chemical formulae, written from left to right, they equally encompass the chemically identical substituents that would result from writing the structure from right to left, e.g., —CH$_2$O— is equivalent to —OCH$_2$—.
[0009] The term "alkyl," by itself or as part of another substituent, means, unless otherwise stated, a straight (i.e., unbranched) or branched carbon chain (or carbon), or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include mono-, di- and multivalent radicals, having the number of carbon atoms designated (i.e., C$_1$-C$_{10}$ means one to ten carbons). Alkyl is an uncyclized

chain. Examples of saturated hydrocarbon radicals include, but are not limited to, groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, (cyclohexyl) methyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butynyl, and the higher homologs and isomers. An alkoxy is an alkyl attached to the remainder of the molecule via an oxygen linker (—O—). An alkyl moiety may be an alkenyl moiety. An alkyl moiety may be an alkynyl moiety. An alkyl moiety may be fully saturated. An alkenyl may include more than one double bond and/or one or more triple bonds in addition to the one or more double bonds. An alkynyl may include more than one triple bond and/or one or more double bonds in addition to the one or more triple bonds.

[0010] The term "alkylene," by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from an alkyl, as exemplified, but not limited by, —CH$_2$CH$_2$CH$_2$CH$_2$—. Typically, an alkyl (or alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer carbon atoms being preferred herein. A "lower alkyl" or "lower alkylene" is a shorter chain alkyl or alkylene group, generally having eight or fewer carbon atoms. The term "alkenylene," by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from an alkene.

[0011] The term "heteroalkyl," by itself or in combination with another term, means, unless otherwise stated, a stable straight or branched chain, or combinations thereof, including at least one carbon atom and at least one heteroatom (e.g., O, N, P, Si, and S), and wherein the nitrogen and sulfur atoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) (e.g., N, S, Si, or P) may be placed at any interior position of the heteroalkyl group or at the position at which the alkyl group is attached to the remainder of the molecule. Heteroalkyl is an uncyclized chain. Examples include, but are not limited to: —CH$_2$—CH$_2$—O—CH$_3$, —CH$_2$—CH$_2$—NH—CH$_3$, —CH$_2$—CH$_2$—N(CH$_3$)—CH$_3$, —CH$_2$—S—CH$_2$—CH$_3$, —CH$_2$—CH$_2$, —S(O)—CH$_3$, —CH$_2$—CH$_2$—S(O)$_2$—CH$_3$, —CH=CH—O—CH$_3$, —Si(CH$_3$)$_3$, —CH$_2$—CH=N—OCH$_3$, —CH=CH—N(CH$_3$)—CH$_3$, —O—CH$_3$, —O—CH$_2$—CH$_3$, and —CN. Up to two or three heteroatoms may be consecutive, such as, for example, —CH$_2$—NH—OCH$_3$ and —CH$_2$—O—Si(CH$_3$)$_3$. A heteroalkyl moiety may include one heteroatom (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include two optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include three optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include four optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include five optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include up to 8 optionally different heteroatoms (e.g., O, N, S, Si, or P). The term "heteroalkenyl," by itself or in combination with another term, means, unless otherwise stated, a heteroalkyl including at least one double bond. A heteroalkenyl may optionally include more than one double bond and/or one or more triple bonds in additional to the one or more double bonds. The term "heteroalkynyl," by itself or

in combination with another term, means, unless otherwise stated, a heteroalkyl including at least one triple bond. A heteroalkynyl may optionally include more than one triple bond and/or one or more double bonds in additional to the one or more triple bonds.

[0012] Similarly, the term "heteroalkylene," by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from heteroalkyl, as exemplified, but not limited by, —CH_2—CH_2—S—CH_2—CH_2— and —CH_2—S—CH_2—CH_2—NH—CH_2—. For heteroalkylene groups, heteroatoms can also occupy either or both of the chain termini (e.g., alkyleneoxy, alkylenedioxy, alkyleneamino, alkylenediamino, and the like). Still further, for alkylene and heteroalkylene linking groups, no orientation of the linking group is implied by the direction in which the formula of the linking group is written. For example, the formula —$C(O)_2R'$— represents both —$C(O)_2R'$— and —$R'C(O)_2$—. As described above, heteroalkyl groups, as used herein, include those groups that are attached to the remainder of the molecule through a heteroatom, such as —$C(O)R'$, —$C(O)NR'$, —$NR'R''$, —OR', —SR', and/or —SO_2R'. Where "heteroalkyl" is recited, followed by recitations of specific heteroalkyl groups, such as —$NR'R''$ or the like, it will be understood that the terms heteroalkyl and —$NR'R''$ are not redundant or mutually exclusive. Rather, the specific heteroalkyl groups are recited to add clarity. Thus, the term "heteroalkyl" should not be interpreted herein as excluding specific heteroalkyl groups, such as -$NR'R''$ or the like.

[0013] The terms "cycloalkyl" and "heterocycloalkyl," by themselves or in combination with other terms, mean, unless otherwise stated, cyclic versions of "alkyl" and "heteroalkyl," respectively. Cycloalkyl and heterocycloalkyl are not aromatic. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Examples of heterocycloalkyl include, but are not limited to, 1-(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and the like. A "cycloalkylene" and a "heterocycloalkylene," alone or as part of another substituent, means a divalent radical derived from a cycloalkyl and heterocycloalkyl, respectively.

[0014] The terms "halo" or "halogen," by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as "haloalkyl" are meant to include monohaloalkyl and polyhaloalkyl. For example, the term "halo(C_1-C_4)alkyl" includes, but is not limited to, fluoromethyl, difluoromethyl, trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

[0015] The term "acyl" means, unless otherwise stated, —$C(O)R$ where R is a substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

[0016] The term "aryl" means, unless otherwise stated, a polyunsaturated, aromatic, hydrocarbon substituent, which can be a single ring or multiple rings (preferably from 1 to 3 rings) that are fused together (i.e., a fused ring aryl) or linked covalently. A fused ring aryl refers to multiple rings fused together wherein at least one of the fused rings is an aryl ring. The term "heteroaryl" refers to aryl groups (or rings) that contain at least one heteroatom such as N, O, or S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. Thus, the term "heteroaryl" includes fused ring heteroaryl groups (i.e., multiple rings fused together wherein at least one of the fused rings is a heteroaromatic ring). A 5,6-fused ring heteroarylene refers to two rings fused together, wherein one ring has 5 members and the other ring has 6 members, and wherein at least one ring is a heteroaryl ring. Likewise, a 6,6-fused ring heteroarylene refers to two rings fused together, wherein one ring has 6 members and the other ring has 6 members, and wherein at least one ring is a heteroaryl ring. And a 6,5-fused ring heteroarylene refers to two rings fused together, wherein one ring has 6 members and the other ring has 5 members, and wherein at least one ring is a heteroaryl ring. A heteroaryl group can be attached to the remainder of the molecule through a carbon or heteroatom. Non-limiting examples of aryl and heteroaryl groups include phenyl, naphthyl, pyrrolyl, pyrazolyl, pyridazinyl, triazinyl, pyrimidinyl, imidazolyl, pyrazinyl, purinyl, oxazolyl, isoxazolyl, thiazolyl, furyl, thienyl, pyridyl, pyrimidyl, benzothiazolyl, benzoxazoyl benzimidazolyl, benzofuran, isobenzofuranyl, indolyl, isoindolyl, benzothiophenyl, isoquinolyl, quinoxalinyl, quinolyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxalinyl, 5-quinoxalinyl, 3-quinolyl, and 6-quinolyl. Substituents for each of the above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below. An "arylene" and a "heteroarylene," alone or as part of another substituent, mean a divalent radical derived from an aryl and heteroaryl, respectively. A heteroaryl group substituent may be —O— bonded to a ring heteroatom nitrogen.

[0017] Spirocyclic rings are two or more rings wherein adjacent rings are attached through a single atom. The individual rings within spirocyclic rings may be identical or different. Individual rings in spirocyclic rings may be substituted or unsubstituted and may have different substituents from other individual rings within a set of spirocyclic rings. Possible substituents for individual rings within spirocyclic rings are the possible substituents for the same ring when not part of spirocyclic rings (e.g. substituents for cycloalkyl or heterocycloalkyl rings). Spirocyclic rings may be substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkyl or substituted or unsubstituted heterocycloalkylene and individual rings within a spirocyclic ring group may be any of the immediately previous list, including having all rings of one type (e.g. all rings being substituted heterocycloalkylene wherein each ring may be the same or different substituted heterocycloalkylene). When referring to a spirocyclic ring system, heterocyclic spirocyclic rings means a spirocyclic rings wherein at least one ring is a heterocyclic ring and wherein each ring may be a different ring. When referring to

a spirocyclic ring system, substituted spirocyclic rings means that at least one ring is substituted and each substituent may optionally be different.

[0018] The term "oxo," as used herein, means an oxygen that is double bonded to a carbon atom.

[0019] The term "alkylarylene" as an arylene moiety covalently bonded to an alkylene moiety (also referred to herein as an alkylene linker). In embodiments, the alkylarylene group has the formula:

or
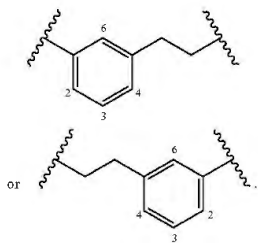
[0020] An alkylarylene moiety may be substituted (e.g. with a substituent group) on the alkylene moiety or the arylene linker (e.g. at carbons 2, 3, 4, or 6) with halogen, oxo, $-N_3$, $-CF_3$, $-CCl_3$, $-CBr_3$, $-CI_3$, $-CN$, $-CHO$, $-OH$, $-NH_2$, $-COOH$, $-CONH_2$, $-NO_2$, $-SH$, $-SO_2CH_3$, $-SO_3H$, $-OSO_3H$, $-SO_2NH_2$, $-NHNH_2$, $-ONH_2$, $-NHC(O)NHNH_2$, substituted or unsubstituted C_1-C_5 alkyl or substituted or unsubstituted 2 to 5 membered heteroalkyl). In embodiments, the alkylarylene is unsubstituted.

[0021] Each of the above terms (e.g., "alkyl," "heteroalkyl," "cyclalkyl," "heterocycloalkyl," "aryl," and "heteroaryl") includes both substituted and unsubstituted forms of the indicated radical. Preferred substituents for each type of radical are provided below.

[0022] Substituents for the alkyl and heteroalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) can be one or more of a variety of groups selected from, but not limited to, $-OR'$, $=O$, $=NR'$, $=N-OR'$, $-NR'R''$, $-SR'$, -halogen, $-SiR'R''R'''$, $-OC(O)R'$, $-C(O)R'$, $-CO_2R'$, $-CONR'R''$, $-OC(O)NR'R''$, $-NR''C(O)R'$, $-NR'-C(O)NR''R'''$, $-NR''C(O)_2R'$, $-NR-C(NR'R''R''')=NR''''$, $-NR-C(NR'R'')=NR'''$, $-S(O)R'$, $-S(O)_2R'$, $-S(O)_2NR'R''$, $-NRSO_2R'$, $-NR'NR''R'''$, $-ONR'R''$, $-NR'C(O)NR''NR'''R''''$, $-CN$, $-NO_2$, $-NR'SO_2R''$, $-NR'C(O)R''$, $-NR'C(O)-OR''$, $-NR'OR''$, in a number ranging from zero to (2 m'+1), where m' is the total number of carbon atoms in such radical. R, R', R'', R''', and R'''' each preferably independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl (e.g., aryl substituted with 1-3 halogens), substituted or unsubstituted heteroaryl, substituted or unsubstituted alkyl, alkoxy, or thioalkoxy groups, or arylalkyl groups. When a compound described herein includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''', and R'''' group when more than one of these groups is present. When R' and

R'' are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 4-, 5-, 6-, or 7-membered ring. For example, $-NR'R''$ includes, but is not limited to, 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term "alkyl" is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (e.g., $-CF_3$ and $-CH_2CF_3$) and acyl (e.g., $-C(O)CH_3$, $-C(O)CF_3$, $-C(O)CH_2OCH_3$, and the like).

[0023] Similar to the substituents described for the alkyl radical, substituents for the aryl and heteroaryl groups are varied and are selected from, for example: $-OR'$, $-NR'R''$, $-SR'$, -halogen, $-SiR'R''R'''$, $-OC(O)R'$, $-C(O)R'$, $-CO_2R'$, $-CONR'R''$, $-OC(O)NR'R''$, $-NR''C(O)R'$, $-NR'-C(O)NR''R'''$, $-NR''C(O)_2R'$, $-NR-C(NR'R''R''')=NR''''$, $-NR-C(NR'R'')=NR'''$, $-S(O)R'$, $-S(O)_2R'$, $-S(O)_2NR'R''$, $-NRSO_2R'$, $-NR'NR''R'''$, $-ONR'R''$, $-NR'C(O)NR''NR'''R''''$, $-CN$, $-NO_2$, $-R'$, $-N_3$, $-CH(Ph)_2$, fluoro(C_1-C_4)alkoxy, and fluoro(C_1-C_4) alkyl, $-NR'SO_2R''$, $-NR'C(O)R''$, $-NR'C(O)-OR''$, $-NR'OR''$, in a number ranging from zero to the total number of open valences on the aromatic ring system; and where R', R'', R''', and R'''' are preferably independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl. When a compound described herein includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''', and R'''' groups when more than one of these groups is present.

[0024] Substituents for rings (e.g. cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene) may be depicted as substituents on the ring rather than on a specific atom of a ring (commonly referred to as a floating substituent). In such a case, the substituent may be attached to any of the ring atoms (obeying the rules of chemical valency) and in the case of fused rings or spirocyclic rings, a substituent depicted as associated with one member of the fused rings or spirocyclic rings (a floating substituent on a single ring), may be a substituent on any of the fused rings or spirocyclic rings (a floating substituent on multiple rings). When a substituent is attached to a ring, but not a specific atom (a floating substituent), and a subscript for the substituent is an integer greater than one, the multiple substituents may be on the same atom, same ring, different atoms, different fused rings, different spirocyclic rings, and each substituent may optionally be different. Where a point of attachment of a ring to the remainder of a molecule is not limited to a single atom (a floating substituent), the attachment point may be any atom of the ring and in the case of a fused ring or spirocyclic ring, any atom of any of the fused rings or spirocyclic rings while obeying the rules of chemical valency. Where a ring, fused rings, or spirocyclic rings contain one or more ring heteroatoms and the ring, fused rings, or spirocyclic rings are shown with one or more floating substituents (including, but not limited to, points of attachment to the remainder of the molecule), the floating substituents may be bonded to the heteroatoms. Where the ring heteroatoms are shown bound to one or more hydrogens (e.g. a ring nitrogen with two bonds to ring atoms and a third bond to a hydrogen) in the

structure or formula with the floating substituent, when the heteroatom is bonded to the floating substituent, the substituent will be understood to replace the hydrogen, while obeying the rules of chemical valency.

[0025] Two or more substituents may optionally be joined to form aryl, heteroaryl, cycloalkyl, or heterocycloalkyl groups. Such so-called ring-forming substituents are typically, though not necessarily, found attached to a cyclic base structure. In one embodiment, the ring-forming substituents are attached to adjacent members of the base structure. For example, two ring-forming substituents attached to adjacent members of a cyclic base structure create a fused ring structure. In another embodiment, the ring-forming substituents are attached to a single member of the base structure. For example, two ring-forming substituents attached to a single member of a cyclic base structure create a spirocyclic structure. In yet another embodiment, the ring-forming substituents are attached to non-adjacent members of the base structure.

[0026] Two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally form a ring of the formula -T-C(O)—(CRR')q-U-, wherein T and U are independently —NR—, —O—, —CRR'—, or a single bond, and q is an integer of from 0 to 3. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -A-(CH$_2$)r-B—, wherein A and B are independently —CRR'—, —O—, —NR—, —S—, —S(O) —, —S(O)$_2$—, —S(O)$_2$NR'—, or a single bond, and r is an integer of from 1 to 4. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula —(CRR')$_s$—X'—(C"R"R''')$_d$—, where s and d are independently integers of from 0 to 3, and X' is —O—, —S—, —S(O)—, —S(O)$_2$—, or —S(O)$_2$NR'—. The substituents R, R', R", and R''' are preferably independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

[0027] As used herein, the terms "heteroatom" or "ring heteroatom" are meant to include oxygen (O), nitrogen (N), sulfur (S), phosphorus (P), and silicon (Si).

[0028] A "substituent group," as used herein, means a group selected from the following moieties:

[0029] (A) oxo, halogen, CF$_3$, CN, OH, NH$_2$, COOH, CONH$_2$, NO$_2$, SH, —SO$_3$H, —SO$_4$H, —SO$_2$NH$_2$, —NHNH$_2$, —ONH$_2$, —NHC=(O)NHNH$_2$, —NHC= (O)NH$_2$, —NHSO$_2$H, —NHC=(O)H, —NHC(O)—OH, NHOH, OCF$_3$, OCHF$_2$, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

[0030] (B) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, substituted with at least one substituent selected from:

[0031] (i) oxo, halogen, CF$_3$, CN, OH, NH$_2$, COOH, CONH$_2$, NO$_2$, SH, —SO$_3$H, —SO$_4$H, —SO$_2$NH$_2$, —NHNH$_2$, —ONH$_2$, —NHC=(O)NHNH$_2$, —NHC= (O) NH$_2$, —NHSO$_2$H, —NHC=(O)H, —NHC(O)— OH, NHOH, OCF$_3$, OCHF$_2$, unsubstituted alkyl, unsub-

stituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

[0032] (ii) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, substituted with at least one substituent selected from:

[0033] (a) oxo, halogen, CF$_3$, CN, OH, NH$_2$, COOH, CONH$_2$, NO$_2$, SH, —SO$_3$H, —SO$_4$H, —SO$_2$NH$_2$, —NHNH$_2$, —ONH$_2$, —NHC=(O)NHNH$_2$, —NHC= (O) NH$_2$, —NHSO$_2$H, —NHC=(O)H, —NHC(O)— OH, NHOH, OCF$_3$, OCHF$_2$, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

[0034] (b) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, substituted with at least one substituent selected from: oxo, halogen, CF$_3$, CN, OH, NH$_2$, COOH, CONH$_2$, NO$_2$, SH, —SO$_3$H, —SO$_4$H, —SO$_2$NH$_2$, —NHNH$_2$, —ONH$_2$, —NHC=(O)NHNH$_2$, —NHC= (O) NH$_2$, —NHSO$_2$H, —NHC=(O)H, —NHC(O)— OH, NHOH, OCF$_3$, OCHF$_2$, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl.

[0035] A "size-limited substituent" or " size-limited substituent group," as used herein, means a group selected from all of the substituents described above for a "substituent group," wherein each substituted or unsubstituted alkyl is a substituted or unsubstituted C$_1$-C$_{20}$ alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 20 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C$_3$-C$_8$ cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 8 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C$_6$-C$_{10}$ aryl, and each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 10 membered heteroaryl.

[0036] A "lower substituent" or "lower substituent group," as used herein, means a group selected from all of the substituents described above for a "substituent group," wherein each substituted or unsubstituted alkyl is a substituted or unsubstituted C$_1$-C$_8$ alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 8 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C$_3$-C$_7$ cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 7 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C$_6$-C$_{10}$ aryl, and each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 9 membered heteroaryl.

[0037] In some embodiments, each substituted group described in the compounds herein is substituted with at least one substituent group. More specifically, in some embodiments, each substituted alkyl, substituted heteroalkyl, substituted cycloalkyl, substituted heterocycloalkyl, substituted aryl, substituted heteroaryl, substituted alkylene, substituted heteroalkylene, substituted cycloalkylene, substituted heterocycloalkylene, substituted arylene, and/or substituted heteroarylene described in the compounds herein are substituted with at least one substituent group. In other embodiments, at least one or all of these groups are substituted with at least one size-limited substituent group.

In other embodiments, at least one or all of these groups are substituted with at least one lower substituent group.

[0038] In other embodiments of the compounds herein, each substituted or unsubstituted alkyl may be a substituted or unsubstituted C_1-C_{20} alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 20 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C_3-C_8 cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 8 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C_6-C_{10} aryl, and/or each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 10 membered heteroaryl. In some embodiments of the compounds herein, each substituted or unsubstituted alkylene is a substituted or unsubstituted C_1-C_{20} alkylene, each substituted or unsubstituted heteroalkylene is a substituted or unsubstituted 2 to 20 membered heteroalkylene, each substituted or unsubstituted cycloalkylene is a substituted or unsubstituted C_3-C_8 cycloalkylene, each substituted or unsubstituted heterocycloalkylene is a substituted or unsubstituted 3 to 8 membered heterocycloalkylene, each substituted or unsubstituted arylene is a substituted or unsubstituted C_6-C_{10} arylene, and/or each substituted or unsubstituted heteroarylene is a substituted or unsubstituted 5 to 10 membered heteroarylene.

[0039] In some embodiments, each substituted or unsubstituted alkyl is a substituted or unsubstituted C_1-C_8 alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 8 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C_3-C_7 cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 7 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C_6-C_{10} aryl, and/or each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 9 membered heteroaryl. In some embodiments, each substituted or unsubstituted alkylene is a substituted or unsubstituted C_1-C_8 alkylene, each substituted or unsubstituted heteroalkylene is a substituted or unsubstituted 2 to 8 membered heteroalkylene, each substituted or unsubstituted cycloalkylene is a substituted or unsubstituted C_3-C_7 cycloalkylene, each substituted or unsubstituted heterocycloalkylene is a substituted or unsubstituted 3 to 7 membered heterocycloalkylene, each substituted or unsubstituted arylene is a substituted or unsubstituted C_6-C_{10} arylene, and/or each substituted or unsubstituted heteroarylene is a substituted or unsubstituted 5 to 9 membered heteroarylene. In some embodiments, the compound is a chemical species set forth in the Examples section, figures, or tables below.

[0040] In embodiments, a substituted or unsubstituted moiety (e.g., substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted alkylene, substituted or unsubstituted heteroalkylene, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkylene, substituted or unsubstituted arylene, and/or substituted or unsubstituted heteroarylene) is unsubstituted (e.g., is an unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, unsubstituted alkylene,

unsubstituted heteroalkylene, unsubstituted cycloalkylene, unsubstituted heterocycloalkylene, unsubstituted arylene, and/or unsubstituted heteroarylene, respectively). In embodiments, a substituted or unsubstituted moiety (e.g., substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted alkylene, substituted or unsubstituted heteroalkylene, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkylene, substituted or unsubstituted arylene, and/or substituted or unsubstituted heteroarylene) is substituted (e.g., is a substituted alkyl, substituted heteroalkyl, substituted cycloalkyl, substituted heterocycloalkyl, substituted aryl, substituted heteroaryl, substituted alkylene, substituted heteroalkylene, substituted cycloalkylene, substituted heterocycloalkylene, substituted arylene, and/or substituted heteroarylene, respectively).

[0041] In embodiments, a substituted moiety (e.g., substituted alkyl, substituted heteroalkyl, substituted cycloalkyl, substituted heterocycloalkyl, substituted aryl, substituted heteroaryl, substituted alkylene, substituted heteroalkylene, substituted cycloalkylene, substituted heterocycloalkylene, substituted arylene, and/or substituted heteroarylene) is substituted with at least one substituent group, wherein if the substituted moiety is substituted with a plurality of substituent groups, each substituent group may optionally be different. In embodiments, if the substituted moiety is substituted with a plurality of substituent groups, each substituent group is different.

[0042] In embodiments, a substituted moiety (e.g., substituted alkyl, substituted heteroalkyl, substituted cycloalkyl, substituted heterocycloalkyl, substituted aryl, substituted heteroaryl, substituted alkylene, substituted heteroalkylene, substituted cycloalkylene, substituted heterocycloalkylene, substituted arylene, and/or substituted heteroarylene) is substituted with at least one size-limited substituent group, wherein if the substituted moiety is substituted with a plurality of size-limited substituent groups, each size-limited substituent group may optionally be different. In embodiments, if the substituted moiety is substituted with a plurality of size-limited substituent groups, each size-limited substituent group is different.

[0043] In embodiments, a substituted moiety (e.g., substituted alkyl, substituted heteroalkyl, substituted cycloalkyl, substituted heterocycloalkyl, substituted aryl, substituted heteroaryl, substituted alkylene, substituted heteroalkylene, substituted cycloalkylene, substituted heterocycloalkylene, substituted arylene, and/or substituted heteroarylene) is substituted with at least one lower substituent group, wherein if the substituted moiety is substituted with a plurality of lower substituent groups, each lower substituent group may optionally be different. In embodiments, if the substituted moiety is substituted with a plurality of lower substituent groups, each lower substituent group is different.

[0044] In embodiments, a substituted moiety (e.g., substituted alkyl, substituted heteroalkyl, substituted cycloalkyl, substituted heterocycloalkyl, substituted aryl, substituted heteroaryl, substituted alkylene, substituted heteroalkylene, substituted cycloalkylene, substituted heterocycloalkylene, substituted arylene, and/or substituted heteroarylene) is substituted with at least one substituent group, size-limited substituent group, or lower substituent group; wherein if the

substituted moiety is substituted with a plurality of groups selected from substituent groups, size-limited substituent groups, and lower substituent groups; each substituent group, size-limited substituent group, and/or lower substituent group may optionally be different. In embodiments, if the substituted moiety is substituted with a plurality of groups selected from substituent groups, size-limited substituent groups, and lower substituent groups; each substituent group, size-limited substituent group, and/or lower substituent group is different.

[0045] Certain compounds of the present invention possess asymmetric carbon atoms (optical or chiral centers) or double bonds; the enantiomers, racemates, diastereomers, tautomers, geometric isomers, stereoisometric forms that may be defined, in terms of absolute stereochemistry, as (R)-or (S)- or, as (D)- or (L)- for amino acids, and individual isomers are encompassed within the scope of the present invention. The compounds of the present invention do not include those that are known in art to be too unstable to synthesize and/or isolate. The present invention is meant to include compounds in racemic and optically pure forms. Optically active (R)- and (S)-, or (D)- and (L)-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers.

[0046] As used herein, the term "isomers" refers to compounds having the same number and kind of atoms, and hence the same molecular weight, but differing in respect to the structural arrangement or configuration of the atoms.

[0047] The term "tautomer," as used herein, refers to one of two or more structural isomers which exist in equilibrium and which are readily converted from one isomeric form to another. It will be apparent to one skilled in the art that certain compounds of this invention may exist in tautomeric forms, all such tautomeric forms of the compounds being within the scope of the invention.

[0048] Unless otherwise stated, structures depicted herein are also meant to include all stereochemical forms of the structure; i.e., the R and S configurations for each asymmetric center. Therefore, single stereochemical isomers as well as enantiomeric and diastereomeric mixtures of the present compounds are within the scope of the invention.

[0049] Unless otherwise stated, structures depicted herein are also meant to include compounds which differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of a hydrogen by a deuterium or tritium, or the replacement of a carbon by ^{13}C- or ^{14}C-enriched carbon are within the scope of this invention.

[0050] The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive isotopes, such as for example tritium (^3H), iodine-125 (^{125}I), or carbon-14 (^{14}C). All isotopic variations of the compounds of the present invention, whether radioactive or not, are encompassed within the scope of the present invention.

[0051] It should be noted that throughout the application that alternatives are written in Markush groups, for example, each amino acid position that contains more than one

possible amino acid. It is specifically contemplated that each member of the Markush group should be considered separately, thereby comprising another embodiment, and the Markush group is not to be read as a single unit.

[0052] The terms "a" or "an," as used in herein means one or more. In addition, the phrase "substituted with a[n]," as used herein, means the specified group may be substituted with one or more of any or all of the named substituents. For example, where a group, such as an alkyl or heteroaryl group, is "substituted with an unsubstituted C_1-C_{20} alkyl, or unsubstituted 2 to 20 membered heteroalkyl," the group may contain one or more unsubstituted C_1-C_{20} alkyls, and/or one or more unsubstituted 2 to 20 membered heteroalkyls.

[0053] Moreover, where a moiety is substituted with an R substituent, the group may be referred to as "R-substituted." Where a moiety is R-substituted, the moiety is substituted with at least one R substituent and each R substituent is optionally different. Where a particular R group is present in the description of a chemical genus (such as Formula (I)), a Roman alphabetic symbol may be used to distinguish each appearance of that particular R group. For example, where multiple R^{13} substituents are present, each R^{13} substituent may be distinguished as R^{13A}, R^{13B}, R^{13C}, R^{13D}, etc., wherein each of R13A, R^{13B}, R^{13C}, R^{13D}, etc. is defined within the scope of the definition of R^{13} and optionally differently.

[0054] Descriptions of compounds of the present invention are limited by principles of chemical bonding known to those skilled in the art. Accordingly, where a group may be substituted by one or more of a number of substituents, such substitutions are selected so as to comply with principles of chemical bonding and to give compounds which are not inherently unstable and/or would be known to one of ordinary skill in the art as likely to be unstable under ambient conditions, such as aqueous, neutral, and several known physiological conditions. For example, a heterocycloalkyl or heteroaryl is attached to the remainder of the molecule via a ring heteroatom in compliance with principles of chemical bonding known to those skilled in the art thereby avoiding inherently unstable compounds.

[0055] The compounds of the present invention may exist as salts, such as with pharmaceutically acceptable acids. The present invention includes such salts. Non-limiting examples of such salts include hydrochlorides, hydrobromides, phosphates, sulfates, methanesulfonates, nitrates, maleates, acetates, citrates, fumarates, proprionates, tartrates (e.g., (+)-tartrates, (−)-tartrates, or mixtures thereof including racemic mixtures), succinates, benzoates, and salts with amino acids such as glutamic acid, and quaternary ammonium salts (e.g. methyl iodide, ethyl iodide, and the like). These salts may be prepared by methods known to those skilled in the art.

[0056] The neutral forms of the compounds are preferably regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound may differ from the various salt forms in certain physical properties, such as solubility in polar solvents.

[0057] Certain compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the present invention. Certain compounds of the present invention may exist in multiple crystalline or amor-

phous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

[0058] "Contacting" is used in accordance with its plain ordinary meaning and refers to the process of allowing at least two distinct species (e.g. chemical compounds including biomolecules or cells) to become sufficiently proximal to react, interact or physically touch. It should be appreciated; however, the resulting reaction product can be produced directly from a reaction between the added reagents or from an intermediate from one or more of the added reagents that can be produced in the reaction mixture.

[0059] The term "contacting" may include allowing two species to react, interact, or physically touch, wherein the two species may be a compound as described herein and a protein or enzyme. In some embodiments contacting includes allowing a compound described herein to interact with a protein or enzyme that is involved in a signaling pathway.

[0060] "Selective" or "selectivity" or the like of a compound refers to the compound's ability to discriminate between molecular targets.

[0061] "Specific", "specifically", "specificity", or the like of a compound refers to the compound's ability to cause a particular action, such as inhibition, to a particular molecular target with minimal or no action to other proteins in the cell.

[0062] "Fungi" are defined as any of a kingdom (Fungi) of saprophytic and parasitic spore-producing eukaryotic typically filamentous organisms formerly classified as plants that lack chlorophyll and include molds, rusts, mildews, smuts, mushrooms, and yeasts. Several other groups that historically have been associated with fungi, such as the slime molds and water molds are now not considered to be fungi. The phyla of fungi are distinguished primarily by their sexual reproductive structures. Yeasts do not form a single taxonomic or phylogenetic grouping. Some yeast such as Candida albicans are human pathogens.

[0063] Bacteria constitute a large domain of prokaryotic microorganisms. Bacteria have a number of shapes, ranging from spheres to rods and spirals. Bacterial cells are typically 0.5-5. μm in length. The bacterial cell is surrounded by a cell membrane, which encloses the contents of the cell and acts as a barrier to hold nutrients, proteins and other essential components of the cytoplasm within the cell. In some bacteria, a cell wall is present on the outside of the cell membrane. As they are prokaryotes, bacteria do not usually have membrane-bound organelles in their cytoplasm, and thus contain few large intracellular structures. They lack a true nucleus, mitochondria, chloroplasts and the other organelles present in eukaryotic cells.

[0064] A virus is a small infectious agent that replicates only inside the living cells of other organisms. Viruses can infect all types of life forms, from animals and plants to microorganisms, including bacteria and archaea.

[0065] The term "animal" is used in accordance with its well understood common meaning and represents a multicellular, eukaryotic organism of the kingdom Animalia (also called Metazoa). Animals are divided into various subgroups, some of which are: vertebrates (birds, mammals, amphibians, reptiles, fish); molluscs (clams, oysters, octopuses, squid, snails); arthropods (millipedes, centipedes, insects, spiders, scorpions, crabs, lobsters, shrimp); annelids (earthworms, leeches); sponges; and jellyfish.

[0066] The term "aromatic compound" refers to an organic compound including at least one aromatic moiety (e.g., aryl or heteroaryl).

[0067] As used herein, a "fluorophilic" component is any fluorinated compound such as a linear, branched, cyclic, saturated, or unsaturated fluorinated hydrocarbon. The fluorophilic component can optionally include at least one heteroatom (e.g., O, N, S, P, Si; in the backbone of the component). In some cases, the fluorophilic compound may be highly fluorinated, i.e., at least 30%, at least 50%, at least 70%, or at least 90% of the hydrogen atoms of the component are replaced by fluorine atoms. The fluorophilic component may include a fluorine to hydrogen ratio of, for example, at least 0.2:1, at least 0.5:1, at least 1:1, at least 2:1, at least 5:1, or at least 10:1. In some such embodiments, at least 30%, at least 50%, at least 70%, or at least 90% but less than 100% of the hydrogen atoms of the component are replaced by fluorine atoms. In other cases, the fluorophilic component is perfluorinated, i.e., the component contains fluorine atoms but contains no hydrogen atoms. Fluorophilic components compatible with the present invention may have low toxicity, low surface tension, and the ability to dissolve and transport gases. Examples of types of fluorophilic components include but are not limited to hydrofluorocarbons, chlorofluorocarbons, and perfluorocarbons.

[0068] As used herein, a "fluorophilic compound" refers to a class of compounds that includes hydrocarbon and fluorocarbon. In embodiments, fluorophilic compounds does not include the chlorofluorocarbon (e.g. R-11(Trichlorofluoromethane), CCl_3F; R-12 (Dichlorodifluoromethane), CCl_2F_2; or R-13 (Chlorotrifluoromethane),$CClF_3$). In embodiments, the fluorophilic compound is dimethyl ether, methyl ethyl ether, methyl n-propyl ether, methyl isopropyl ether, methyl-n-butyl ether, diethyl ether, methyl tert-butyl ether, or ethyl tert-butyl ether.

[0069] A "fluorocarbon compound" is a compound including fluorine and carbon, but not hydrogen (e.g., no carbon-hydrogen bonds). In embodiments, a fluorocarbon compound is an FC—fluorocarbon compound ("FC"), which consists solely of fluorine and carbon. In embodiments, a fluorocarbon compound is a chlorofluorocarbon (CFC) compound, wherein FC and CFC are common terms used to define refrigerants [see, for example, Downing, Ralph C. Fluorocarbon refrigerants handbook Prentice Hall (1988)]. Examples of fluorocarbon compounds include fluoroether compounds, fluoroketone compounds, fluoroaromatic compounds and fluoroolefin compounds. In embodiments, fluorocarbon compounds also include compounds wherein one or more optional substituents therein may be selected from one or more of bromine, chlorine and iodine. Fluorocarbon molecules may have various structures, including straight or branched chain or cyclic structures. The chemical properties of certain of these compounds, because of the unusual polarity of the carbon-fluorine bond, are unexpected. For example, perfluorotrimethyl iodide, a gas which boils at −22.5° C., is relatively stable in the absence of light (which can produce heterolysis and subsequent free radical formation) even in the presence of water below 100° C. or moderately basic solutions. Brominated perfluorocarbons such as 1-bromo-heptadecafluorooctane ($C_8F_{17}Br$), sometimes designated perfluorooctyl bromide α, ω-dibromo-F-butane; 1-bromopenta-decafluoroheptane ($C_7F_{15}Br$); 1-bromo-nonafluorobutane (C_4F_9Br); and 1-bromotridecafluorohexane ($C_6F_{13}Br$) are quite stable under extraction

conditions. It is also contemplated that fluorocarbons having nonfluorinated substituents, such as perfluorooctyl chloride, or perfluorooctyl hydride may be used in the apparatus, methods, and compositions described herein, as well as similar compounds having different numbers of carbon atoms, e.g., 2-8 carbon atoms. Those skilled in the art will appreciate that esters, thioesters, amines, amides, and other variously modified fluorocarbon-hydrocarbon compounds are also encompassed within the definition of fluorocarbon materials suitable for use in the present invention. Certain perfluorinated compounds are relatively inert and have unexpected properties; for example perfluorotributylamine is not at all basic. See, for example, Hong, Angela C., Cora J. Young, Michael D. Hurley, Timothy J. Wallington, and Scott A. Mabury. "Perfluorotributylamine: A novel long-lived greenhouse gas." Geophysical Research Letters 40, no. 22 (2013): 6010-6015, which is incorporated herein by reference for all purposes.

[0070] A "hydrofluorocarbon compound" is a compound including fluorine, carbon and at least one hydrogen atom (e.g., at least one carbon-hydrogen bond). In embodiments, a hydrofluorocarbon compound is an HFC-hydrofluorocarbon compound ("HFC"), which consists solely of fluorine, carbon and hydrogen. In embodiments, a hydrofluorocarbon compound is a hydrochlorofluorocarbon (HCFC) compound. HFC and HCFC are common terms used to define refrigerants (see Downing, Ralph C. supra.). Non-limiting examples of hydrofluorocarbons include trifluoromethane (HFC-23), difluoromethane (HFC-32), pentafluoroethane (HFC-125), 1,1,2,2-tetrafluoroethane (HFC-134), 1,1,1,2-tetrafluoroethane (HFC-134a), 1,1,1-trifluoroethane (HFC-143a), 1,1-difluoroethane (HFC-152a) and fluoroethane (HFC-161). Hydrofluorocarbon compounds may be hydrofluoroether compounds, hydrofluoroketone compounds, hydrofluoroaromatic compounds or hydrofluoroolefin compounds. Non-limiting examples of hydrofluorocarbon compounds include methyl nonafluoroisobutyl ether, methyl nonafluorobutyl ether, ethyl nonafluoroisobutyl ether, ethyl nonafluorobutyl ether, and 3-ethoxy-1,1,1,2,3,4,4,5,5,6,6,6-dodecafluoro-2-trifluoromethylhexane. In embodiments, hydrofluorocarbon compounds include compounds wherein one or more optional substituents may be one or more bromine, chlorine, or iodine. In embodiments, the hydrofluorocarbon is chlorodifluoromethane, methyl nonafluoroisobutyl ether, methyl nonafluorobutyl ether, ethyl nonafluoroisobutyl ether, ethyl nonafluorobutyl ether, 3-ethoxy-1,1,1,2,3,4,4,5,5,6,6,6-dodecafluoro-2-trifluoromethylhexane, trifluoromethane (HFC-23), difluoromethane (HFC-32), pentafluoroethane (HFC-125), 1,1,2,2-tetrafluoroethane (HFC-134), 1,1,1,2-tetrafluoroethane (HFC-134a), 1,1,1-trifluoroethane (HFC-143a), 1,1-difluoroethane (HFC-152a), (1,1,1,3,3,3-hexafluoro-2-(fluoromethoxy)propane, 1,2,2,2-tetrafluoroethyl difluoromethyl ether, 2-chloro-1,1,2,-trifluoroethyl difluoromethyl ether, 1-chloro-2,2,2-trifluoroethyl difluoromethyl ether, 2,2-dichloro-1,1-difluoromethyl ether, or fluoroethane (HFC-161).

[0071] Within the class of fluorophilic compounds, hydrofluorocarbon compounds, and fluorocarbon compounds, "optionally substituted" indicates that one or more fluorine or hydrogen atoms may be replaced with an independently selected alkane, alkene, alkoxy, fluoroalkoxy, perfluoroalkoxy, fluoroalkyl, perfluoroalkyl, aryl or heteroaryl group or compound (e.g., one or more hydrogens on the carbon chain of the group or compound may be independently

substituted with one or more of independently selected substitutents (e.g., substituent groups). For example, a substituted C_2H_5 group may, without limitation, be —CF_2CF_3, —CH_2CH_2OH or —CF_2CF_2I.

[0072] A "refrigerant" is a substance that is capable of removing heat from its surroundings (e.g., when it changes phase from liquid to vapor (i.e. when it evaporates)). Refrigerants may add heat to its surroundings in a complementary reaction (e.g., when it changes phase from vapor to liquid (i.e. when it condenses)) (e.g., FC-fluorocarbon, an HFC-hydrofluorocarbon, a chlorofluorocarbon, a hydrochlorofluorocarbon, an alkane, an alkene, or an aromatic compound; or ammonia, carbon dioxide or other gases such as hydrogen, oxygen, nitrogen and argon). Refrigerant substances may contain oxygen, or bromine, chlorine or iodine, as described above, for example, in relation to hydrofluorocarbon and fluorocarbon compounds.

[0073] An "azeotrope", or an "azeotropic" or "constant boiling" is a mixture of two or more components whose proportions cannot be altered by simple distillation. When boiled, the vapor has the same proportions of the constituents as the un-boiled mixture. In embodiments, a constant boiling mixture is a "near-azeotropic" mixture, which is a mixture that maintains a substantially constant vapor pressure even after evaporative losses, thereby exhibiting constant boiling behavior. Azeotropic and constant boiling mixtures also include mixtures wherein the boiling points of two or more of the components thereof are separated by only about 5° C. or less.

[0074] The "critical pressure" of a substance is the pressure required to liquefy a gas at its critical temperature, which is the temperature at and above which vapor of the substance cannot be liquefied, regardless of how much pressure is applied.

[0075] As used herein, "nonaqueous" is meant to define material such as a fluid that is immiscible with water. That is, a liquid that when mixed with water will form a stable two-phase mixture. The non-aqueous phase need not be liquid, but can be a solid or semi-solid lipid or other nonpolar substance that is not soluble in water. In some instances, the nonaqueous phase can include a lipophilic component (e.g., a hydrocarbon) or a fluorinated component (e.g., a fluorocarbon). The aqueous phase can be any liquid miscible with water; that is, any liquid that, when admixed with water, can form a room-temperature, single-phase solution that is stable. In some cases, the aqueous phase can comprise one or more physiologically acceptable reagents and/or solvents, etc. Non-limiting examples of aqueous phase materials include (besides water itself) methanol, ethanol, DMF (dimethylformamide), or DMSO (dimethyl sulfoxide).

[0076] The term "chirality" refers to the geometric property of a rigid object (or spatial arrangement of points or atoms) of being non-superimposable on its mirror image. If the object is superimposable on its mirror image the object is described as being achiral.

[0077] The term "chiral center" refers to an atom holding a set of ligands in a spatial arrangement, which is not superimosable on its mirror image. A chirality center may be considered a generalized extension of the concept of the asymmetric carbon atom to central atoms of any element. Each chiral center (*C) is labeled R or S according to a system by which its substituents are each designated a

priority according to the Cahn Ingold Prelog priority rules (CIP), based on atomic number.

[0078] The term, "enantiomer" refers to one of a pair of optical isomers containing one or more asymmetric carbons whose molecular configurations have left- and right-hand (chiral) forms. Enantiomers have identical physical properties, except for the direction of rotation of the plane of polarized light. Enantiomers have identical chemical properties except toward optically active reagents.

[0079] An "ionic liquid" is a salt in liquid form. In embodiments, an ionic liquid includes an organic compound (e.g., and counter ion), typically a salt of an organic acid and an organic base, which may exist in a zwitterionic form, which is present in a liquid state (e.g., at room temperature or substantially close to room temperature, wherein room temperature is defined as a range of temperatures from about 4° C. to about 50° C., and most typically between 15° C. and 30° C., and more typically about 25° C.). In embodiments, an ionic liquid differs from most salts in that it has a very low melting point, and tends to be liquid over a wide temperature range. In embodiments, an ionic liquid is not soluble in non-polar hydrocarbons; is immiscible with water, depending on the anion; or is highly ionizing (but has a low dielectric strength). In embodiments, an ionic liquid has essentially no vapor pressure, most are air and water stable, and they can either be neutral, acidic or basic. In embodiments, the properties of an ionic liquid can be tailored by varying the cation and anion. In embodiments, the cation or anion of an ionic liquid can be any cation or anion such that the cation and anion together form an organic salt that is liquid at or below about 100° C. In embodiments, an ionic liquid is formed by reacting a nitrogen-containing heterocyclic ring, preferably a heteroaromatic ring, with an alkylating agent (for example, an alkyl halide) to form a quaternary ammonium salt, and performing ion exchange or other suitable reactions with various Lewis acids or their conjugate bases to form the ionic liquid. Examples of suitable heteroaromatic rings include substituted pyridines, imidazole, substituted imidazole, pyrrole and substituted pyrroles. In embodiments, these rings can be alkylated with virtually any straight, branched or cyclic C_1-C_{20} alkyl group (e.g., the alkyl groups are C_1-C_{16} groups). In embodiments, various triarylphosphines, thioethers and cyclic and non-cyclic quaternary ammonium salts may also be used for this purpose. In embodiments, counterions that may be used include chloroaluminate, bromoaluminate, gallium chloride, tetrafluoroborate, tetrachloroborate, hexafluorophosphate, nitrate, trifluoromethane sulfonate, methylsulfonate, p-toluenesulfonate, hexafluoroantimonate, hexafluoroarsenate, tetrachloroaluminate, tetrabromoaluminate, perchlorate, hydroxide anion, copper dichloride anion, iron trichloride anion, zinc trichloride anion, as well as various lanthanum, potassium, lithium, nickel, cobalt, manganese, or other metal-containing anions. In embodiments, ionic liquids may be synthesized by salt metathesis, by an acid-base neutralization reaction or by quaternizing a selected nitrogen-containing compound; or they may be obtained commercially from several companies such as Merck (Darmstadt, Germany) or BASF (Mount Olive, N.J.). Representative examples of useful ionic liquids are described in sources such as Clare, Bronya, Amal Sirwardana, and Douglas R. MacFarlane. "Synthesis, purification and characterization of ionic liquids." In Ionic Liquids, pp. 1-40. Springer Berlin Heidelberg, 2010; Valderrama, J. O., and P. A. Robles.

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[0080] The term "non-ideal mixture" or "non-ideal fluid" refers to a fluid mixture wherein the enthalpy of mixing is non-zero and the volume change upon mixing is non-zero. In embodiments, a non-ideal mixture displays a vapor pressure lower than expected from Raoult's law (negative deviation), which may be evidence of adhesive forces between different components of the mixture being stronger than the average cohesive forces between the like components. When cohesive forces between like components are stronger than between different components, the vapor pressure is greater than expected from Raoult's law (positive deviation).

[0081] The term "supercritical fluid" refers to any substance at a temperature and pressure above its critical point, where distinct liquid and gas phases do not exist. It can effuse through solids like a gas, and dissolve materials like a liquid.

[0082] The term "essential oil" refers to a concentrated liquid (e.g., hydrophobic) containing volatile aroma compounds from plants. Essential oils may also be called volatile oils, ethereal oils, aetherolea, or simply as the oil of the plant from which they were extracted, such as oil of clove. An oil

is "essential" in the sense that it contains the "essence of" the plant's fragrance—the characteristic fragrance of the plant from which it is derived.

[0083] The term "natural" as used for "natural product" and "natural organic compound" refers to something that is found in, or isolated from, nature and is not itself synthetic, artificial, and/or man-made. In some embodiments, a "natural product" is a molecule, compound, or substance that is produced by a living organism, i.e., is found in nature. In some embodiments, natural products are isolated from natural sources that are produced by pathways of primary and secondary metabolism. In some embodiments, natural products are isolated from natural sources that are produced where expression of the product is influenced artificially. In some embodiments, natural products are isolated from natural sources that are produced through over-expression of the natural product. In some embodiments, a "natural organic compound" is a purified organic (carbon-containing chemical) compound isolated from natural sources. In some embodiments, a natural organic compound is isolated from natural sources including plant, animal, fungus, virus, and bacteria. In some embodiments, natural organic compounds are isolated from natural sources that have been genetically modified or engineered, including plant, animal, fungus, virus, and bacteria. In some embodiments, natural organic compounds are isolated from natural sources through extraction. In some embodiments, natural organic compounds are isolated from natural sources through extraction methods as described herein.

[0084] The term "flavonoid" (or bioflavonoid) refers to a class of plant and fungus secondary metabolites. Flavonoids have the general structure of a 15-carbon skeleton, which consists of two phenyl rings and a heterocyclic ring. In some embodiments, flavonoids are classified as flavonoids (or bioflavonoids), isoflavonoids (derived from 3-phenyl-chromen-4-one (3-phenyl-1,4-benzopyrone) structure), and neoflavonoids (derived from 4-phenylcoumarine (4-phenyl-1,2-benzopyrone) structure). The term "prenylflavonoid" (or prenylated flavonoids) refers to a subclass of flavonoids. Chemically, they have a prenyl group attached to the flavonoid backbone.

[0085] The term "kavalactone" refers to a class of lactone compounds found in the kava plant. In some embodiments, kavalactones possess a wide variety of pharmacological effects including analgesic, anticonvulsant, amnestic, nootropic, and sedative activity.

[0086] The term "salvorin" refers to a terpenoid with psychotropic properties found in the *Salvia divinorum* plant.

[0087] The term "terpene" refers to a large class of hydrocarbon organic compound found in a variety of plants, including conifers, and some insects, such as termites and swallowtail butterflies. Terpenes are derived biosynthetically from units of isoprene, which has the molecular formula C_5H_8. The basic molecular formulae of terpenes are multiples of that, $(C_5H_8)_n$, where n is the number of linked isoprene units. This is called the isoprene rule or the C5 rule. In some embodiments, terpenes are the primary constituents of the essential oils of many flowers and plants. Terpenes may include monoterpenes, diterpenes, sesquiterpenes, triterpenes, sesterterpenes, norterpenes, nortriterpenes, and norsesquiterpenes. Diterpenes refers to diterpene acids, esters, alkaloids (such as indolo-terpenes) which are composed of two isoprene units.
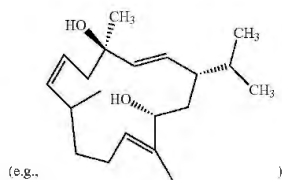
[0088] In embodiments, the terpene is a camphor or a derivative, analog, or prodrug thereof

(e.g.,)
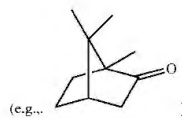
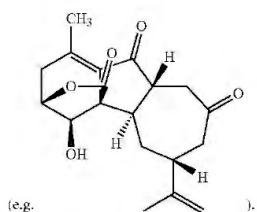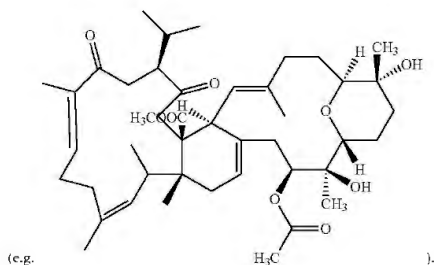[0089] In embodiments, the terpene is a carvone or a derivative, analog, or prodrug thereof

(e.g.,).
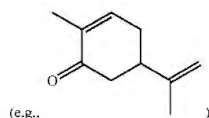
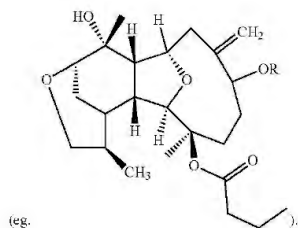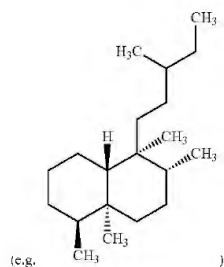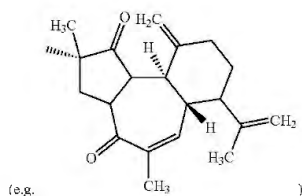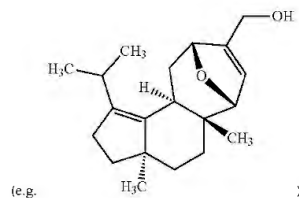[0090] In embodiments, the terpene is a limonene or a derivative, analog, or prodrug thereof
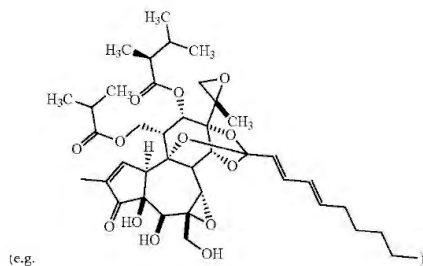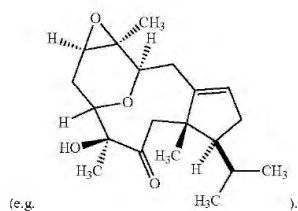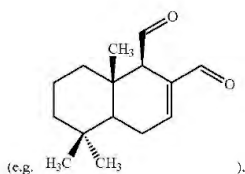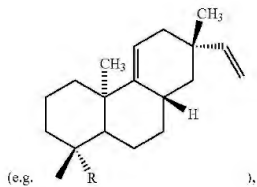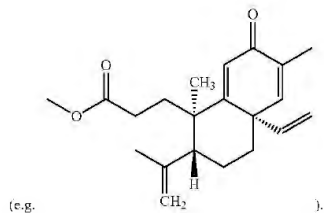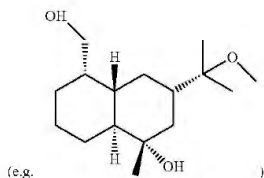
(e.g.,).
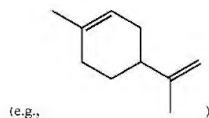
[0091] In embodiments, the terpene is a linalool or a derivative, analog, or prodrug thereof
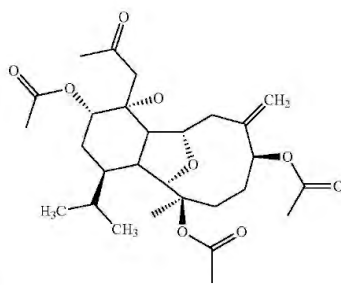
(e.g.,).
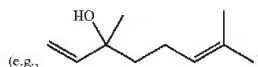
[0092] In embodiments, the terpene is a geraniol or a derivative, analog, or prodrug thereof

(e.g.).
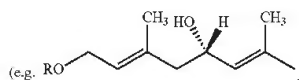
[0093] In embodiments, the terpene is a pinene or a derivative, analog, or prodrug thereof

(e.g.).

11
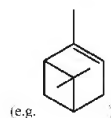
[0094] In embodiments, the terpene is a ionone or a derivative, analog, or prodrug thereof

(e.g.,).
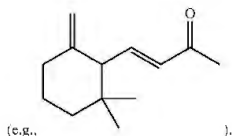
[0095] In embodiments, the terpene is a iridoid or a derivative, analog, or prodrug thereof (e.g.,

).
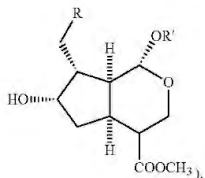
[0096] In embodiments, the terpene is a abietane or a derivative, analog, or prodrug thereof

(e.g.,).
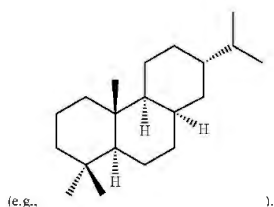
[0097] In embodiments, the terpene is a atisane or a derivative, analog, or prodrug thereof

(e.g.,).
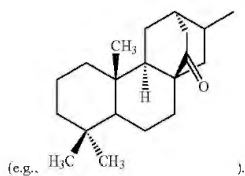
[0098] In embodiments, the terpene is a basmane or a derivative, analog, or prodrug thereof

(e.g.,).
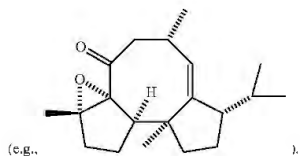
[0099] In embodiments, the terpene is a briarane or a derivative, analog, or prodrug thereof

(e.g.,),
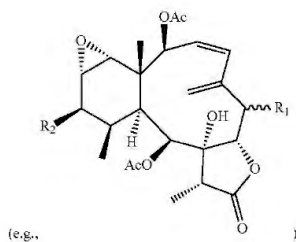
wherein Ac refers to an acetyl group; ∿∿∿ refers to a bond designating a mix of stereoisomers; R_1 refers to a moiety selected from —Cl and —OAc (acetic acid) and R_2 is a moiety selected from —OH, —OAc, —OCOCH$_2$CH (CH$_3$)$_2$, —OCOCH$_3$, —OCOCH$_2$CH$_3$ (propionate).

[0100] In embodiments, the terpene is a carophyllene or a derivative, analog, or prodrug thereof

(e.g.,).
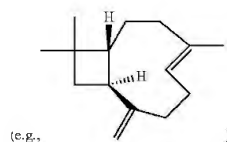
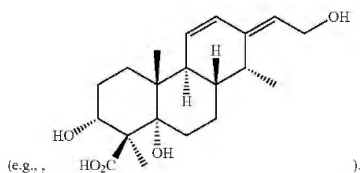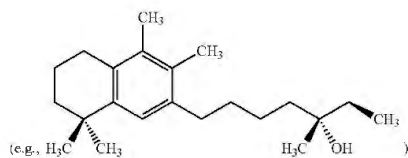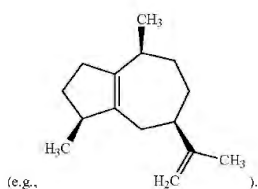[0101] In embodiments, the terpene is a casbane or a derivative, analog, or prodrug thereof

(e.g.).
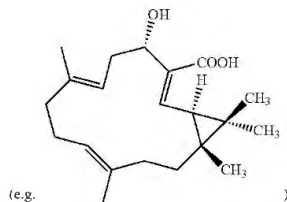
[0102] In embodiments, the terpene is a cassane or a derivative, analog, or prodrug thereof

(e.g.).

12
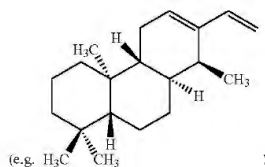
[0103]   In embodiments, the terpene is a cembranoid or a derivative, analog, or prodrug thereof

(e.g.,   )

[0104]   In embodiments, the terpene is a norcembranoid or a derivative, analog, or prodrug thereof

(e.g.   ).

[0105]   In embodiments, the terpene is a bicembrane or a derivative, analog, or prodrug thereof

(e.g.   ).

[0106]   In embodiments, the terpene is a cladiellane or a derivative, analog, or prodrug thereof
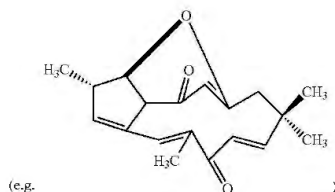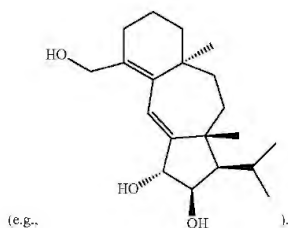
(eg.   ).

[0107]   In embodiments, the terpene is a clerodane or a derivative, analog, or prodrug thereof

(e.g.   ).

[0108]   In embodiments, the terpene is a curcusone or a derivative, analog, or prodrug thereof

(e.g.   ).

[0109]   In embodiments, the terpene is a cyathane or a derivative, analog, or prodrug thereof
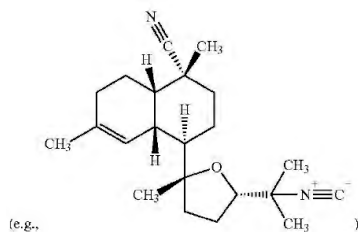
(e.g.   ).

[0110]   In embodiments, the terpene is a daphnane or a derivative, analog, or prodrug thereof

(e.g.   ).

[0111] In embodiments, the terpene is a dolabellane or a derivative, analog, or prodrug thereof

(e.g.).

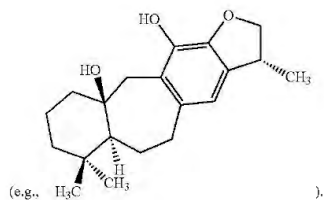[0112] In embodiments, the terpene is a drimane or a derivative, analog, or prodrug thereof

(e.g.).

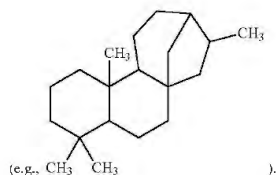[0113] In embodiments, the terpene is a pimane or a derivative, analog, or prodrug thereof

(e.g.),

wherein R is a moiety selected from —H, —OH, —CH₂OH, —CO₂CH₃, —CHO, —CH₂OAc, —COOH, and —CH₃.

[0114] In embodiments, the terpene is a ent-pimane or a derivative, analog, or prodrug thereof

(e.g.).

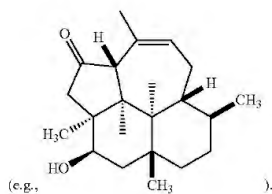[0115] In embodiments, the terpene is a eudesmane or a derivative, analog, or prodrug thereof

(e.g.).

[0116] In embodiments, the terpene is a eunicellin or a derivative, analog, or prodrug thereof

(e.g.).

[0117] In embodiments, the terpene is a franchetine or a derivative, analog, or prodrug thereof

(e.g. .).
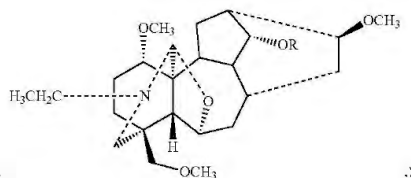
wherein R represents a benzyl group.

[0118] In embodiments, the terpene is a gibberelin or a derivative, analog, or prodrug thereof

(e.g.).
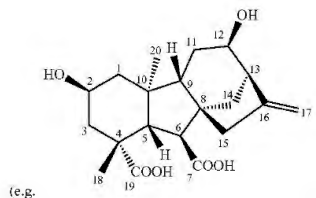
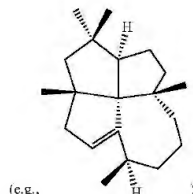[0119] In embodiments, the terpene is a grayane or a derivative, analog, or prodrug thereof

(e.g., ,).

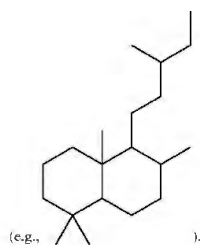[0120] In embodiments, the terpene is a guaiene or a derivative, analog, or prodrug thereof

(e.g.,).

[0121] In embodiments, the terpene is a Guanacastane or a derivative, analog, or prodrug thereof

(e.g.,).

[0122] In embodiments, the terpene is a icetaxane or a derivative, analog, or prodrug thereof
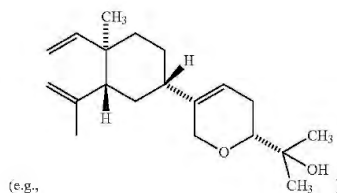
(e.g.,).

[0123] In embodiments, the terpene is a isofregenedane or a derivative, analog, or prodrug thereof

(e.g.,).

[0124] In embodiments, the terpene is a jatrophane or a derivative, analog, or prodrug thereof

(e.g.).

[0125] In embodiments, the terpene is a kalihinene or a derivative, analog, or prodrug thereof

(e.g.,).

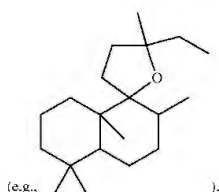[0126] In embodiments, the terpene is a kaurane or a derivative, analog, or prodrug thereof

(e.g.,).

[0127] In embodiments, the terpene is a kempane or a derivative, analog, or prodrug thereof

(e.g.,).

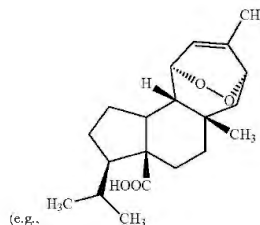[0128] In embodiments, the terpene is a labdane or a derivative, analog, or prodrug thereof

(e.g.,).

[0129] In embodiments, the terpene is a grindelane labdane or a derivative, analog, or prodrug thereof

(e.g.,).

[0130] In embodiments, the terpene is a lathyrane or a derivative, analog, or prodrug thereof

(e.g.,).

[0131] In embodiments, the terpene is a laurenene or a derivative, analog, or prodrug thereof

(e.g.,).

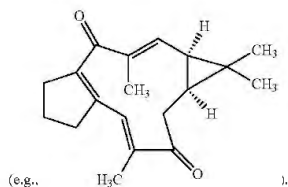[0132] In embodiments, the terpene is a lobane (e.g., lobatriene) or a derivative, analog, or prodrug thereof
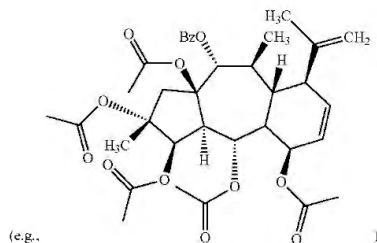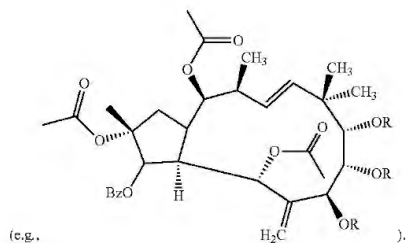
(e.g.,).

[0133] In embodiments, the terpene is a mulinane or a derivative, analog, or prodrug thereof
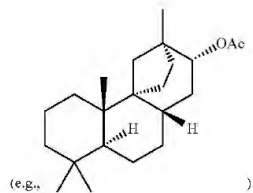
(e.g., .

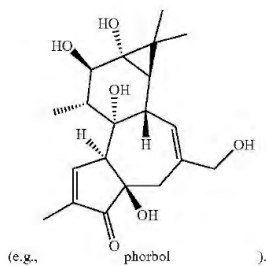[0134] In embodiments, the terpene is a myrsinol or a derivative, analog, or prodrug thereof

(e.g.,).

[0135] In embodiments, the terpene is a pepluane or a derivative, analog, or prodrug thereof
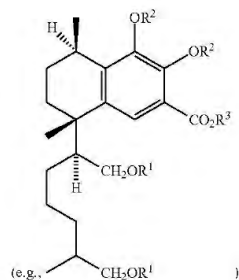
(e.g., [structure]).

[0136] In embodiments, the terpene is a phorbol or a derivative, analog, or prodrug thereof

(e.g., phorbol).

[0137] In embodiments, the terpene is a rosane or a derivative, analog, or prodrug thereof

(e.g., [structure]).

[0138] In embodiments, the terpene is a sclaerol or a derivative, analog, or prodrug thereof

(e.g., [structure]).

[0139] In embodiments, the terpene is a scopadulane or a derivative, analog, or prodrug thereof

(e.g., [structure]).

[0140] In embodiments, the terpene is a serrulatane or a derivative, analog, or prodrug thereof

(e.g., [structure]).

wherein R_1 is a moiety selected from —H, —OH, —N, —NH, —NOH, —CH$_3$, —CO-heteroaryl, —CH$_2$OCOC$_4$H$_3$N$_2$, —CNOH, —CH$_2$, and

[structure]

and R_2 is a moiety selected from —H, —NO$_2$, —CH$_3$, and halogen.

[0141] In embodiments, the terpene is a spatane or a derivative, analog, or prodrug thereof

(e.g., [structure] , [structure]),

wherein R is a moiety selected from —H, —OH, —CH$_2$CH$_3$ (CH$_2$)$_4$(CH$_3$)$_2$, and —CH$_3$.

[0142] In embodiments, the terpene is a stemodane or a derivative, analog, or prodrug thereof

(e.g.,),

wherein R is a moiety selected from —H, —OH, —CH₃, and —OCH₃; R₁ is a moiety selected from —H, —OH, —CH₂OH, and —CH₃; and R₂ is a moiety selected from —H, —OH, and —O.
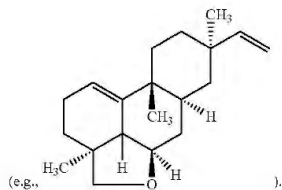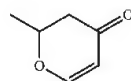
[0143] In embodiments, the terpene is a taxane or a derivative, analog, or prodrug thereof (e.g.,

).

[0144] In embodiments, the terpene is a tigliane or a derivative, analog, or prodrug thereof

(e.g.,),
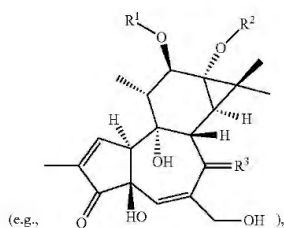
wherein R₁ is a moiety selected from —H, —CH₃, —COC₁₁H₂₃, —CH₂OH, —Bz, —OAc, 2-methylbutryrl, and

R₂ is a moiety selected from —CH3, —COCH3, —Bz, —OAc, isobutryryl, and 2-methylbutryrl; and R₃ is a moiety selected from —O, and —CH₂.

[0145] In embodiments, the terpene is a tormesane (e.g., tormesolane) or a derivative, analog, or prodrug thereof

(e.g.,).
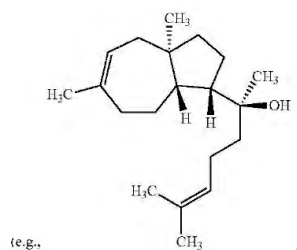
[0146] In embodiments, the terpene is a valparane or a derivative, analog, or prodrug thereof

(e.g.,).
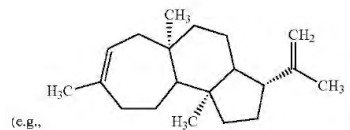
[0147] In embodiments, the terpene is a vibsane or a derivative, analog, or prodrug thereof
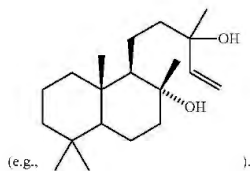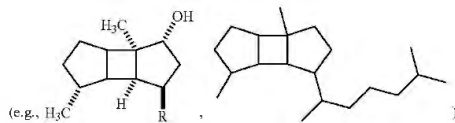

(e.g.,).

(−)-5-epi-Vibsanin E [(−)-2]
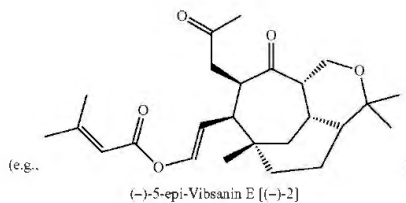
[0148] In embodiments, the terpene is a xenicane or a derivative, analog, or prodrug thereof

(e.g.,).
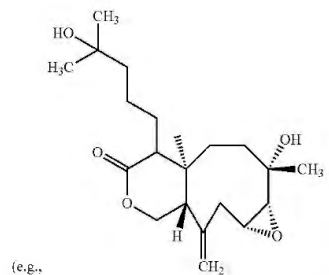
[0149] In embodiments, the terpene is a bakkane or a derivative, analog, or prodrug thereof

(e.g.,   ).
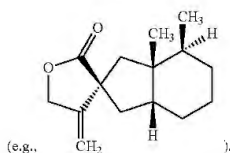
[0150] 101441 In embodiments, the terpene is a bisabo-lane or a derivative, analog, or prodrug thereof

(e.g.,   ).
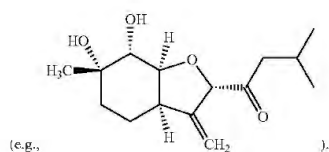
[0151] In embodiments, the terpene is a zizanoic acid or a derivative, analog, or prodrug thereof

(e.g., HO₂C   ).
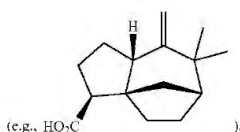
[0152] In embodiments, the terpene is a drimenol or a derivative, analog, or prodrug thereof

(e.g., H₃C   ).
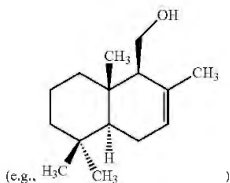
[0153] In embodiments, the terpene is a isolongifolene or a derivative, analog, or prodrug thereof

(e.g.,   ).
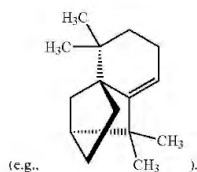
[0154] In embodiments, the terpene is a tirotundin or a derivative, analog, or prodrug thereof

(e.g.,   ).
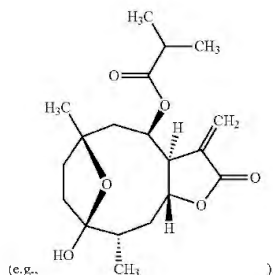
[0155] In embodiments, the terpene is a clovane or a derivative, analog, or prodrug thereof

(e.g.,   ).

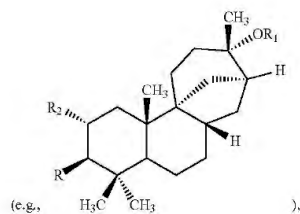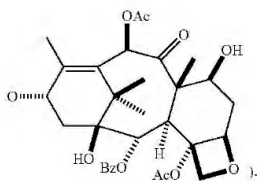[0156] In embodiments, the terpene is a germacrane or a derivative, analog, or prodrug thereof

(e.g.,   ).
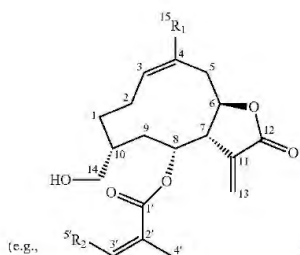
wherein R_1 is a moiety selected from —H, —OH, and —CH₃ and R_2 is a moiety selected from —CH₃.

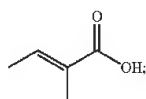[0157] In embodiments, the terpene is a sesterterpene or a derivative, analog, or prodrug thereof

(e.g.,

Ophiobolin A
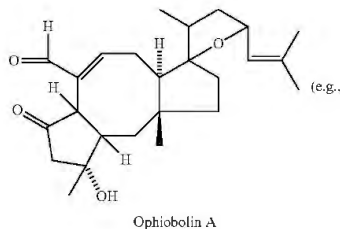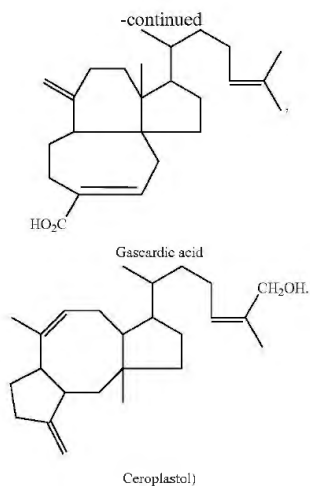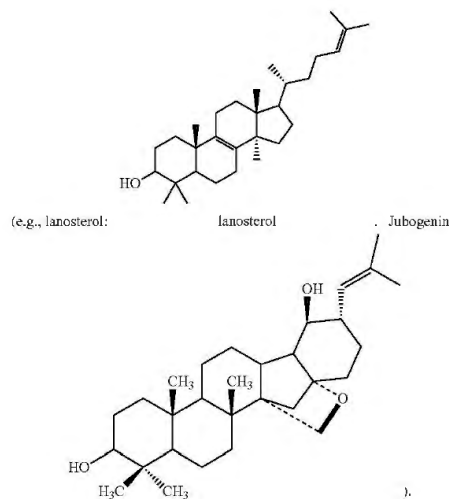
-continued

Gascardic acid

Ceroplastol)

[0158] In embodiments, the terpene is a triterpene or a derivative, analog, or prodrug thereof

(e.g., lanosterol: lanosterol   . Jubogenin

).

[0159] The term "humulone" (or α-lupulic acid) refers to a prevalent member of the class of compounds known as alpha acids, which provide a characteristically bitter flavor. Humulone is a phloroglucinol derivative with three isoprenoid side-chains. Two side-chains are prenyl groups and one is an isovaleryl group. The acidity of the ring enol moieties that give rise to its designation as an acid lie in the vinylogous relationship with the ring and side chain carbonyl functional groups.

[0160] The term "humulene" (also known as a-humulene or a-caryophyllene), refers to a monocyclic sesquiterpene

($C_{15}H_{24}$), containing an 11-membered ring and consisting of three isoprene units containing three non-conjugated C=C double bonds, two of them being triply substituted and one being doubly substituted. It is found in the essential oils of *Humulus lupulus*. Humulene is an isomer of β-caryophyllene, and the two are often found together as a mixture in many aromatic plants.

[0161] The term "caryophyllene" (or (−)-β-caryophyllene), refers to a bicyclic sesquiterpene that is a constituent of many essential oils, especially clove oil, the oil from the stems and flowers of *Syzygium aromaticum* (cloves), the essential oil of *Cannabis sativa*, rosemary, and hops. It is usually found as a mixture with isocaryophyllene (the cis double bond isomer) and α-humulene. Caryophyllene possesses both a cyclobutane ring and as a trans-double bond in an 8-membered ring.

[0162] The term "lupulone" (or β-lupulic acid) refers to a beta acid found in *Humulus lupulus* (Hops). Lupulones are sensitive to oxidative decomposition; their break down creates flavors that may adversely affect the taste of beer.

[0163] The term "myrcene" (or β-myrcene) refers to an olefinic organic hydrocarbon, classified as a monoterpene. It is a component of the essential oil of several plants including bay, cannabis, ylang-ylang, wild thyme, parsley, and hops.

[0164] The term "alkaloid" refers to a group of naturally occurring chemical compounds that mostly contain basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties. In addition to carbon, hydrogen and nitrogen, alkaloids may also contain oxygen, sulfur, chlorine, bromine, and phosphorus. Alkaloids are produced by a large variety of organisms including bacteria, fungi, plants, and animals.

[0165] The term "cannabinoid" refers to a class of chemical compounds that act on cannabinoid receptors. These are G-protein-coupled receptors denoted by the terms CB_1 and CB_2 receptors. The structure of the CB1 receptor has been determined; see Hua, T., Vemuri, K., Pu, M., Qu, L., Han, G. W., Wu, Y., Zhao, S., Shui, W., Li, S., Korde, A. and Laprairie, R. B., 2016. Crystal structure of the human cannabinoid receptor CB 1. *Cell*, 167(3), pp. 750-'762, which is incorporated herein by reference for all purposes. The anatomical distribution of these receptors is complex, but broadly CB1 receptors in the central nervous system mediate many of the effects of cannabinoids in the brain, whereas CB2 receptors in the periphery mediate anti-inflammatory and related actions of cannabinoids. See Munro, S., Thomas, K. L. and Abu-Shaar, M., 1993. Molecular characterization of a peripheral receptor for cannabinoids. *Nature*, 365(6441), p. 61. which is incorporated herein by reference for all purposes

[0166] There are at least 113 different cannabinoids isolated from cannabis, exhibiting varied effects. Many of these compounds are structurally related to Δ9-tetrahydrocannabinol (THC). Classes of natural cannabinoids isolated from cannabis include cannabigerol, cannabichromene, cannabidiol, tetrahydrocannabinol, cannabinol, cannabielsoin, iso-tetrahydrocannabinol, cannabicyclol, and cannabicitran. Examples of well-studied cannabinoids include cannabinol (CBN), tetrahydrocannabinol (THC), and cannabinol (CBN).

II. METHODS

[0167] The methods provided herein include processes, methods, and compositions for the extraction of natural

products from plant material employing pure fluorocarbon liquids or gases and optionally admixtures of fluorocarbon and non-fluorocarbon gases and liquids. In some embodiments, the extraction may be carried out in a highly selective manner such that specific components consisting of pure compounds or defined mixtures thereof may be extracted from plant or animal material without extracting undesired materials, obviating the need for subsequent purification steps following the extraction, wherein said specific components are valuable in the preparation of pharmaceutical and nutraceutical compositions which are useful in the prevention and treatment of various diseases and syndromes in humans and animals.

[0168] In a first aspect, is provided a method of extracting a natural organic compound from a natural material, the method including contacting the natural material with an extraction fluid thereby extracting the natural organic compound from the natural material into the extraction fluid to from an extracted fluid solution. In an embodiment, the extraction fluid includes a fluorophilic compound and a hydrofluorocarbon. In another embodiment, the extraction fluid is a non-ideal fluid.

[0169] In one embodiment, the natural material is a material derived from a plant, an animal, a fungi, a bacteria or a virus. In another embodiment, the natural material is a material derived from a plant. In another embodiment, the natural material is a material derived from an animal. In another embodiment, the natural material is a material derived from a fungus. In another embodiment, the natural material is a material derived from a bacteria. In another embodiment, the natural material is a material derived from a virus.

[0170] In one embodiment, the plant is *Piper methysticum, Cannabis* spp., *Salvia* spp., *Banisteriopsis caapi, Psychotria viridis* (chacruna), *Diplopterys cabrerana, Peganum harmala, Humulus lupulus* or mixture thereof. In another embodiment, the plant is *Piper methysticum.* In another embodiment, the plant is *Cannabis* spp. In another embodiment, the plant is *Salvia* spp. In another embodiment, the plant is *Banisteriopsis caapi.* In another embodiment, the plant is *Psychotria viridis* (chacruna). In another embodiment, the plant is *Diplopterys cabrerana.* In another embodiment, the plant is *Peganum harmala.* In another embodiment, the plant is *Humulus lupulus.* In another embodiment, the *Cannabis* spp. plant is *Cannabis Sativa.*

[0171] In one embodiment, the plant is *Echinacea purpurea, Echinacea angustifolia, Acmella oleracea, Helichrysum umbraculigerum,* or *Radula marginata.* In another embodiment the plant is an *Echinacea* spp. In another embodiment, the plant is *Echinacea purpurea.* In another embodiment, the plant is *Echinacea angustifolia.* In another embodiment, the plant is *Acmella oleracea.* In another embodiment, the plant is *Helichrysum umbraculigerum.* In another embodiment, the plant is *Radula marginata.*

[0172] In one embodiment, the natural organic compound is a biologically active organic compound. In another embodiment, the natural organic compound is an aromatic compound. In another embodiment, the natural organic compound forms part of an aromatic oil or essential oil. In another embodiment, the natural organic compound forms an aromatic oil. In another embodiment, the natural organic compound forms an essential oil. In another embodiment, the natural organic compound is a component of an aromatic oil. In another embodiment, the natural organic compound is

a component of an essential oil. In another embodiment, the natural organic compound is caffeine. In one embodiment, the natural organic compound is a terpene, a humulone, a lupulone, a myrcene, a humulene, a caryophyllene, an alkaloid, a flavonoid, a cannabinoid, menthol, capsaicin, anise or camphor. In another embodiment, the natural organic compound is a terpene. In another embodiment, the natural organic compound is a humulone. In another embodiment, the natural organic compound is a lupulone. In another embodiment, the natural organic compound is a myrcene. In another embodiment, the natural organic compound is a humulene. In another embodiment, the natural organic compound is a caryophyllene. In another embodiment, the natural organic compound is an alkaloid. In another embodiment, the natural organic compound is a flavonoid. In another embodiment, the natural organic compound is menthol. In another embodiment, the natural organic compound is capsaicin. In another embodiment, the natural organic compound is anise. In another embodiment, the natural organic compound is camphor. In one embodiment, the natural organic compound is xanthohumol, 8-prenylnaringenin or isoxanthohumol. In another embodiment, the natural organic compound is xanthohumol. In another embodiment, the natural organic compound is 8-prenylnaringenin. In another embodiment, the natural organic compound is isoxanthohumol. In another embodiment, the natural organic compound is a prenylflavonoid. In another embodiment, the natural organic compound is a kavalactone or a salvorin.

[0173] In one embodiment, the natural organic compound is a cannibinoid. In another embodiment, the natural organic compound is tetrahydrocannabinol, cannabidiol or cannabinol. In another embodiment, the natural organic compound is cannabidiol. In another embodiment, the natural organic compound is cannabinol. In another embodiment, the natural organic compound is tetrahydrocannabinol.

[0174] In another embodiment, the natural organic compound is cannabigerol, cannabichromene, cannabicyclol, cannabivarin, tetrahydrocannabivarin, cannabidivarin, cannabichromevarin, cannabigerovarin, cannabigerol monomethyl ether, tetrahydrocannbinolic acid, or cannabidiolic acid. In another embodiment, the natural organic compound is cannabigerol. In another embodiment, the natural organic compound is cannabichromene. In another embodiment, the natural organic compound is cannabicyclol. In another embodiment, the natural organic compound is cannabivarin. In another embodiment, the natural organic compound is tetrahydrocannabivarin. In another embodiment, the natural organic compound is cannabidivarin. In another embodiment, the natural organic compound is cannabichromevarin. In another embodiment, the natural organic compound is cannabigerovarin. In another embodiment, the natural organic compound is cannabigerol monomethyl ether. In another embodiment, the natural organic compound is tetrahydrocannbinolic acid. In another embodiment, the natural organic compound is cannabidiolic acid.

[0175] In embodiments, at least 20 Kg of the natural organic compound is present in the extracted fluid solution. In embodiments, at least 10 Kg of the natural organic compound is present in the extracted fluid solution. In embodiments, at least 5 Kg of the natural organic compound is present in the extracted fluid solution. In embodiments, at least 4 Kg of the natural organic compound is present in the extracted fluid solution. In embodiments, at least 3 Kg of the

natural organic compound is present in the extracted fluid solution. In embodiments, at least 2 Kg of the natural organic compound is present in the extracted fluid solution. In embodiments, at least 1 Kg of the natural organic compound is present in the extracted fluid solution. In embodiments, at least 500 g of the natural organic compound is present in the extracted fluid solution. In embodiments, at least 400 g of the natural organic compound is present in the extracted fluid solution. In embodiments, at least 300 g of the natural organic compound is present in the extracted fluid solution. In embodiments, at least 200 g of the natural organic compound is present in the extracted fluid solution. In embodiments, at least 100 g of the natural organic compound is present in the extracted fluid solution. In embodiments, about 20 Kg of the natural organic compound is present in the extracted fluid solution. In embodiments, about 10 Kg of the natural organic compound is present in the extracted fluid solution. In embodiments, about 5 Kg of the natural organic compound is present in the extracted fluid solution. In embodiments, about 4 Kg of the natural organic compound is present in the extracted fluid solution. In embodiments, about 3 Kg of the natural organic compound is present in the extracted fluid solution. In embodiments, about 2 Kg of the natural organic compound is present in the extracted fluid solution. In embodiments, about 1 Kg of the natural organic compound is present in the extracted fluid solution. In embodiments, about 500 g of the natural organic compound is present in the extracted fluid solution. In embodiments, about 400 g of the natural organic compound is present in the extracted fluid solution. In embodiments, about 300 g of the natural organic compound is present in the extracted fluid solution. In embodiments, about 200 g of the natural organic compound is present in the extracted fluid solution. In embodiments, about 100 g of the natural organic compound is present in the extracted fluid solution. In embodiments, about 100 g to about 15 Kg of the natural organic compound is present in the extracted fluid solution. In embodiments, about 100 g to about 10 Kg of the natural organic compound is present in the extracted fluid solution. In embodiments, about 100 g to about 5 Kg of the natural organic compound is present in the extracted fluid solution. In embodiments, about 100 g to about 1 Kg of the natural organic compound is present in the extracted fluid solution. In embodiments, about 500 g to about 15 Kg of the natural organic compound is present in the extracted fluid solution. In embodiments, about 1 Kg to about 10 Kg of the natural organic compound is present in the extracted fluid solution. In embodiments, about 1 Kg to about 5 Kg of the natural organic compound is present in the extracted fluid solution.

[0176] In embodiments, the extraction fluid does not include supercritical CO_2. In embodiments, extraction fluid does not include argon. In embodiments, the extraction fluid does not include xenon. In embodiments, the extraction fluid does not include nitrous oxide.

[0177] In an embodiment, the extraction fluid includes trifluorethanol or hexafluoroisopropanol. In an embodiment, the extraction fluid includes trifluorethanol. In an embodiment, the extraction fluid includes hexafluoroisopropanol.

[0178] In embodiments, the extraction fluid is above about 15° C. In embodiments, the extraction fluid is above about 20° C. In embodiments, the extraction fluid is from about 15° C. to about 35° C. In embodiments, the extraction fluid

is from about 20° C. to about 30° C. In embodiments, the extraction fluid is about 15° C. In embodiments, the extraction fluid is about 16° C. In another embodiment, the extraction fluid is about 17° C. In another embodiment, the extraction fluid is about 18° C. In another embodiment, the extraction fluid is about 19° C. In another embodiment, the extraction fluid is about 20° C. In another embodiment, the extraction fluid is about 21° C. In another embodiment, the extraction fluid is about 22° C. In another embodiment, the extraction fluid is about 23° C. In another embodiment, the extraction fluid is about 24° C. In another embodiment, the extraction fluid is about 25° C. In another embodiment, the extraction fluid is about 26° C. In another embodiment, the extraction fluid is about 27° C. In another embodiment, the extraction fluid is about 28° C. In another embodiment, the extraction fluid is about 29° C. In another embodiment, the extraction fluid is about 30° C. In another embodiment, the extraction fluid is about 31° C. In another embodiment, the extraction fluid is about 32° C. In another embodiment, the extraction fluid is about 33° C. In another embodiment, the extraction fluid is about 34° C. In another embodiment, the extraction fluid is about 35° C.

[0179] In one embodiment, the hydrofluorocarbon is a hydrofluoroether, a hydrofluoroketone, a hydrofluoroaromatic or a hydrofluoroolefin. In another embodiment, the hydrofluorocarbon is a hydrofluoroether. In another embodiment, the hydrofluorocarbon is a hydrofluoroketone. In another embodiment, the hydrofluorocarbon is a hydrofluoroaromatic. In another embodiment, the hydrofluorocarbon is a hydrofluoroolefin.

[0180] In one embodiment, the hydrofluorocarbon is chlorodifluoromethane, methyl nonafluoroisobutyl ether, methyl nonafluorobutyl ether, ethyl nonafluoroisobutyl ether, ethyl nonafluorobutyl ether, 3-ethoxy-1,1,1,2,3,4,4,5,5,6,6,6-dodecafluoro-2-trifluoromethylhexane.trifluoromethane (HFC-23), difluoromethane (HFC-32), pentafluoroethane (HFC-125), 1,1,2,2-tetrafluoroethane (HFC-134), 1,1,1,2-tetrafluoroethane (HFC-134a), 1,1,1-trifluoroethane (HFC-143a), 1,1-difluoroethane (HFC-152a) or fluoroethane (HFC-161). In another embodiment, the hydrofluorocarbon is chlorodifluoromethane. In another embodiment, the hydrofluorocarbon is methyl nonafluoroisobutyl ether. In another embodiment, the hydrofluorocarbon is methyl nonafluorobutyl ether. In another embodiment, the hydrofluorocarbon is ethyl nonafluoroisobutyl ether. In another embodiment, the hydrofluorocarbon is ethyl nonafluorobutyl ether. In another embodiment, the hydrofluorocarbon is 3-ethoxy-1,1,1,2,3,4,4,5,5,6,6,6-dodecafluoro-2-trifluoromethylhexane.trifluoromethane (HFC-23). In another embodiment, the hydrofluorocarbon is difluoromethane (HFC-32). In another embodiment, the hydrofluorocarbon is pentafluoroethane (HFC-125). In another embodiment, the hydrofluorocarbon is 1,1,2,2-tetrafluoroethane (HFC-134). In another embodiment, the hydrofluorocarbon is 1,1,1,2-tetrafluoroethane (HFC-134a). In another embodiment, the hydrofluorocarbon is 1,1,1-trifluoroethane (HFC-143a). In another embodiment, the hydrofluorocarbon is 1,1-difluoroethane (HFC-152a). In another embodiment, the hydrofluorocarbon is fluoroethane (HFC-161).

[0181] In one embodiment, the fluorophilic compound is dimethyl ether.

[0182] In one embodiment, the method includes, prior to contacting, freezing the natural material at a temperature from about 0° C. to about −60° C. (e.g., at about 0 to −50,

0 to −40, 0 to −30, 0 to −20, 0 to −10, 0, −1, −2, −3, −4, −5, −6, −7, −8, −9, −10, −15, −20, −25, −30, −35, −40, −45, or −50° C.). In embodiments, freezing the natural material uses a freezer. In embodiments, freezing the natural material uses a blast freezer. In embodiments, freezing the natural material uses compressed cryogenic gas (e.g., CO_2, N_2, He). In another embodiment, the mole fraction of the fluorophilic compound is at least four-fold (e.g., at least 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 15-fold, 20-fold, or 25-fold) greater than the mole fraction of the hydrofluorocarbon. In embodiments, the method includes a recirculating pump to administer the extraction fluid to the natural material. In embodiments, the method includes volatilizing the extraction fluid. In embodiments, the natural organic compound is not volatilized when the extraction fluid is volatilized. In embodiments, the method includes extracting the volatilized extraction fluid. In embodiments, the method includes chilling the extracted volatilized extraction fluid (e.g., with a heat exchanger). In embodiments, the method includes compressing the chilled extracted volatilized extraction fluid (e.g., to an extraction liquid). In embodiments, the method includes warming the extraction liquid resulting from chilling the extracted volatilized extraction fluid. In embodiments the method includes recirculating the warmed extraction liquid resulting from chilling the extracted volatilized extraction fluid (e.g. continuously for a fixed amount of time). In embodiments the method includes warming the recirculated extraction fluid to a temperature range from about 40° C. to about 80° C. (e.g., to about 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80° C.). In embodiments the method includes warming the recirculated extraction fluid to a temperature of about 80° C. In embodiments the method includes collecting portions of the recirculating extraction fluid while warming the recirculating extraction fluid to a temperature of about 80° C. (e.g., from 40 to 80° C.). In another embodiment, the method includes separating the extraction fluid from the natural material by volatizing the extraction fluid to form a volatilized extraction fluid. In another embodiment, the method includes chilling and compressing the volatilized extraction fluid to form a liquid extraction fluid. In another embodiment, the method includes recirculating the liquid extraction fluid to the natural material. In another embodiment, the method includes collecting separated fractions of the liquid extraction fluid. In embodiments, extraction fluid includes a mole fraction of a fluorocarbon at least four-fold (e.g., at least 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 15-fold, 20-fold, or 25-fold) greater than the mole fraction of the hydrofluorocarbon. In embodiments, extraction fluid includes an additional component (e.g., fluorophilic compound, fluorophilic amine, alcohol, or non-fluorinated hydroxy-alkyl, non-fluorinated hydroxy-cycloalkyl, or non-fluorinated hydroxyl-aryl, inert gas (e.g., SF_6, CO_2, N_2O, CH_4, C_2H_6, argon)). In embodiments, the mole fraction of the additional component of the extraction fluid is at least four fold (e.g., at least 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 15-fold, 20-fold, or 25-fold) less than the mole fraction of the hydrofluorocarbon.

[0183] In an aspect, is provided a fluid including chlorodifluoromethane and dimethylether. In one embodiment, the fluid is a non-ideal fluid.

[0184] In an aspect is provided an apparatus for the extraction of natural products (e.g., of medicinal, nutraceutical, health-promoting, or pharmacological, or other value) from a plant, animal, bacterial, fungal, or viral material, or mixtures thereof (e.g., which contain multiple natural products). In embodiments, the desired materials are separated from the undesired materials (e.g., leaving the undesired materials in the unextracted residue. In embodiments, the apparatus frees the desired material in solution. In embodiments, the natural product is further purified (e.g., by fractional distillation, flash chromatography, preparative high pressure liquid chromatography on normal and reverse phase media, countercurrent liquid chromatography, liquid-liquid extraction, co-solvent precipitation, or crystallization).

[0185] In an aspect is provided a method (process) for the extraction of natural products (e.g., of medicinal, nutraceutical, health-promoting, or pharmacological, or other value) from a plant, animal, bacterial, fungal, or viral material, or mixtures thereof (e.g., which contain multiple natural products). In embodiments, the desired materials are separated from the undesired materials (e.g., leaving the undesired materials in the unextracted residue. In embodiments, the apparatus frees the desired material in solution. In embodiments, the natural product is further purified (e.g., by fractional distillation, flash chromatography, preparative high pressure liquid chromatography on normal and reverse phase media, countercurrent liquid chromatography, liquid-liquid extraction, co-solvent precipitation, or crystallization).

[0186] In as aspect is provided a composition for the extraction of natural products (e.g., of medicinal, nutraceutical, health-promoting, or pharmacological, or other value) from a plant, animal, bacterial, fungal, or viral material, or mixtures thereof (e.g., which contain multiple natural products). In embodiments, the desired materials are separated from the undesired materials (e.g., leaving the undesired materials in the unextracted residue. In embodiments, the apparatus frees the desired material in solution. In embodiments, the natural product is further purified (e.g., by fractional distillation, flash chromatography, preparative high pressure liquid chromatography on normal and reverse phase media, countercurrent liquid chromatography, liquid-liquid extraction, co-solvent precipitation, or crystallization).

[0187] In embodiments, the composition includes a hydrofluorocarbon or fluorocarbon. In embodiments, the composition includes a hydrofluorocarbon and fluorocarbon. In embodiments, the composition includes a hydrofluorocarbon. In embodiments, the composition includes a fluorocarbon. In some embodiments, the composition includes hydrofluorocarbons, fluorocarbons, and optionally other substances selected from the alkanes, alkenes, alkynes, alcohols, and aromatic hydrocarbons. In embodiments, the composition includes at least one fluorocarbon which is Freon™ 134a (1,1,1,2-Tetrafluoroethane).

[0188] In embodiments, the method of extraction includes fractional distillation, flash chromatography, preparative high pressure liquid chromatography on normal and reverse phase media, countercurrent liquid chromatography, liquid-liquid extraction, co-solvent precipitation, crystallization, or combinations thereof. In embodiments, the method of extraction includes fractional distillation. In embodiments, the method of extraction includes flash chromatography. In

embodiments, the method of extraction includes preparative high pressure liquid chromatography on normal and reverse phase media. In embodiments, the method of extraction includes fractional distillation, countercurrent liquid chromatography. In embodiments, the method of extraction includes liquid-liquid extraction. In embodiments, the method of extraction includes co-solvent precipitation. In embodiments, the method of extraction includes crystallization. Instruments for extraction may include mass spectrometer (MS), gas chromatograph (GC), GC-mass spectrometer, liquid chromatography mass spectrometer, high pressure liquid chromatograph, and combinations thereof.

[0189] Described herein is a multiplicity of co-solvents, provided that the principal extraction medium is either a fluorocarbon or a hydrofluorocarbon in admixture with another fluorophilic compound. Mixtures of fluorocarbons, hydrofluorocarbons, and optionally alkanes, including but not limited to straight chain, branched chain, cycloalkanes, and alkylcycloalkanes, possess the previously unexpected ability to extract specific components of high commercial and health-related value from plant, animal, fungi, bacteria, or virus material. In some embodiments, solvents useful in the methods of the present invention include fluorocarbons and hydrofluorocarbons. Furthermore, forming the continuous phase from mixtures of fluorocarbons is also contemplated herein. In embodiments, the instant conversion of a discontinuous phase to a continuous phase represents the conditions where the maximum solubility of desired, highly hydrophobic plant, animal, fungi, bacteria, or virus materials may be maximally evidenced.

[0190] In embodiments, the extraction fluid (e.g. including extraction components) includes trifluorethanol. In embodiments, the extraction fluid (e.g. including extraction components) includes hexafluoroisopropanol. In embodiments, the extraction fluid (e.g. including extraction components) includes chlorodifluoromethane and dimethylether. In embodiments, the extraction fluid (e.g. including extraction components) includes chlorodifluoromethane. In embodiments, the extraction fluid (e.g. including extraction components) includes dimethylether. In embodiments, the extraction fluid (e.g. including extraction components) includes a liquid-gas mixture. In embodiments, the extraction fluid (e.g. including extraction components) is a non-ideal fluid. In embodiments, extraction fluid (e.g. including extraction components) does not include supercritical CO_2, argon, xenon, or nitrous oxide. In embodiments, extraction fluid (e.g. including extraction components) does not include supercritical CO_2. In embodiments, extraction fluid (e.g. including extraction components) does not include argon. In embodiments, extraction fluid (e.g. including extraction components) does not include xenon. In embodiments, extraction fluid (e.g. including extraction components) does not include nitrous oxide. In embodiments, the extraction fluid (e.g. including extraction components) includes a hydrofluorocarbon

[0191] In some embodiments, the extraction fluid includes a hydrofluorocarbon and a fluorophilic compound. The hydrofluorocarbon may include but is not limited to: a hydrofluoroether, a hydrofluoroketone, a hydrofluoroaromatic or a hydrofluoroolefin, chlorodifluoromethane, methyl nonafluoroisobutyl ether, methyl nonafluorobutyl ether, ethyl nonafluoroisobutyl ether, ethyl nonafluorobutyl ether, 3-ethoxy-1,1,1,2,3,4,4,5,5,6,6,6-dodecafluoro-2-trifluoromethylhexane.trifluoromethane (HFC-23), difluoromethane

(HFC-32), pentafluoroethane (HFC-125), 1,1,2,2-tetrafluoroethane (HFC-134), 1,1,1,2-tetrafluoroethane (HFC-134a), 1,1,1-trifluoroethane (HFC-143a), 1,1-difluoroethane (HFC-152a) or fluoroethane (HFC-161). The fluorophilic compound may include dimethyl ether.

[0192] In some embodiments, the mole fraction of the fluorophilic compound is at least four-fold greater than the mole fraction of the hydrofluorocarbon. The mole fraction of the fluorophilic compound may be at least four fold greater, five-fold greater, six fold greater, seven fold greater, eight fold greater, nine fold greater, ten-fold greater, twenty fold greater, fifty fold greater, seventy five-fold greater, or one hundred fold greater than the mole fraction of the hydrofluorocarbon.

[0193] In some embodiments, the mole fraction of dimethyl ether is at least four-fold greater than the mole fraction of the chlorodifluoromethane. The mole fraction of dimethyl ether may be at least four fold greater, five-fold greater, six fold greater, seven fold greater, eight fold greater, nine fold greater, ten-fold greater, twenty fold greater, fifty fold greater, seventy five-fold greater, or one hundred fold greater than the mole fraction of the chlorodifluoromethane.

[0194] In some embodiments, the extraction fluid is above about 15° C. In embodiments, the extraction fluid is above about 20° C. In embodiments, the extraction fluid is from about 15° C. to about 35° C. In embodiments, the extraction fluid is from about 20° C. to about 30° C. In embodiments, the extraction fluid is above about 15° C., 16° C., 17° C., 18° C., 19° C., 20° C., 21° C., 22° C., 23° C., 24° C., 25° C., 26° C., 27° C., 28° C., 29° C., 30° C., 31° C., 32° C., 33° C., 34° C., or 35° C. In some embodiments, the extraction fluid is above 15° C. In embodiments, the extraction fluid is above 20° C. In embodiments, the extraction fluid is from 15° C. to 35° C. In embodiments, the extraction fluid is from 20° C. to 30° C. In embodiments, the extraction fluid is above 15° C., 16° C., 17° C., 18° C., 19° C., 20° C., 21° C., 22° C., 23° C., 24° C., 25° C., 26° C., 27° C., 28° C., 29° C., 30° C., 31° C., 32° C., 33° C., 34° C., or 35° C.

[0195] In embodiments, an ionic liquid includes a cation described by one or more of the following formulae:
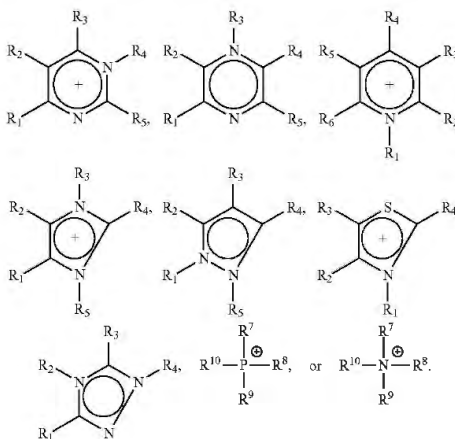
R_1, R_2, R_3, R_4, R_5, R_6, R^7, R^8, R^9, and R^{10} are independently halogen, $—CX_3$, $—CHX_2$, $—CH_2X$, $—CN$, $—OH$, $—NH_2$, $—COOH$, $—CONH_2$, $—NO_2$, $—SH$, $—SO_3H$, $—SO_4H$, $—SO_2NH_2$, $—NHNH_2$, $—ONH_2$, $—NHC═(O)NHNH_2$, $—NHC═(O)$ NH_2, $—NHSO_2H$, $—NHC═(O)H$, $—NHC$ $(O)—OH$, $—NHOH$, $—OCX_3$, $—OCHX_2$, $—OCH_2X$, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl. Two of R_1, R_2, R_3, R_4, R_5, R_6, R^7, R^8, R^9, and R^{10} may independently optionally be joined to form a substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl.

[0196] In embodiments, R_1, R_2, R_3, R_4, R_5, R_6, R^7, R^8, R^9, and R^{10} are independently substituted (e.g., substituted with a substituent group, a size-limited substituent group, or lower substituent group) or unsubstituted alkyl, substituted (e.g., substituted with a substituent group, a size-limited substituent group, or lower substituent group) or unsubstituted heteroalkyl, substituted (e.g., substituted with a substituent group, a size-limited substituent group, or lower substituent group) or unsubstituted cycloalkyl, substituted (e.g., substituted with a substituent group, a size-limited substituent group, or lower substituent group) or unsubstituted heterocycloalkyl, substituted (e.g., substituted with a substituent group, a size-limited substituent group, or lower substituent group) or unsubstituted aryl, or substituted (e.g., substituted with a substituent group, a size-limited substituent group, or lower substituent group) or unsubstituted heteroaryl. In embodiments, two of R_1, R_2, R_3, R_4, R_5, R_6, R^7, R^8, R^9, and R^{10} may independently optionally be joined to form a substituted (e.g., substituted with a substituent group, a size-limited substituent group, or lower substituent group) or unsubstituted cycloalkyl, substituted (e.g., substituted with a substituent group, a size-limited substituent group, or lower substituent group) or unsubstituted heterocycloalkyl, substituted (e.g., substituted with a substituent group, a size-limited substituent group, or lower substituent group) or unsubstituted aryl, or substituted (e.g., substituted with a substituent group, a size-limited substituent group, or lower substituent group) or unsubstituted heteroaryl. In embodiments, wherein R^1, R^2, R^3, R^4, R^5 and R^6 are each independently selected from the group consisting of: (i) H; (ii) halogen; (iii) $—CH_3$, $—C_2H_5$, or C_3 to C_{25} straight-chain, branched or cyclic alkane or alkene, optionally substituted with at least one member selected from the group consisting of Cl, Br, F, I, OH, NH_2 and SH; (iv) $—CH_3$, $—C_2H_5$, or C_3 to C_{25} straight-chain, branched or cyclic alkane or alkene comprising one to three heteroatoms selected from the group consisting of O, N, Si and S, and optionally substituted with at least one member selected from the group consisting of Cl, Br, F, I, OH, NH2 and SH; (v) C_6 to C_{20} unsubstituted aryl, or C_3 to C_{25} unsubstituted heteroaryl having one to three heteroatoms independently selected from the group consisting of O, N, Si and S; and (vi) C_6 to C_{25} substituted aryl, or C_3 to C_{25} substituted heteroaryl having one to three heteroatoms independently selected from the group consisting of O, N, Si and S; wherein said substituted aryl or substituted heteroaryl has one to three substituents independently selected from the group consisting of: 1. $—CH_3$, $—C_2H_5$, or C_3 to C_{25} straight-chain, branched or cyclic alkane or alkene, optionally substituted

with at least one member selected from the group consisting of Cl, Br, F, I, OH, NH_2 and SH, 2. OH, 3. NH_2, and 4. SH. In embodiments, R^7, R^8, R^9, and R^{10} are each independently selected from the group consisting of: (vii) $—CH_3$, $—C_2H_5$, or C_3 to C_{25} straight-chain, branched or cyclic alkane or alkene, optionally substituted with at least one member selected from the group consisting of Cl, Br, F, I, OH, NH_2 and SH; (viii) $—CH_3$, $—C_2H_5$, or C_3 to C_{25} straight-chain, branched or cyclic alkane or alkene comprising one to three heteroatoms selected from the group consisting of O, N, Si and S, and optionally substituted with at least one member selected from the group consisting of Cl, Br, F, I, OH, NH_2 and SH; (ix) C_6 to C_{25} unsubstituted aryl, or C_3 to C_{25} unsubstituted heteroaryl having one to three heteroatoms independently selected from the group consisting of O, N, Si and S; and (x) C_6 to C_{25} substituted aryl, or C_3 to C_{25} substituted heteroaryl having one to three heteroatoms independently selected from the group consisting of O, N, Si and S; wherein said substituted aryl or substituted heteroaryl has one to three substituents independently selected from the group consisting of: (1) $—CH_3$, $—C_2H_5$, or C_3 to C_{25} straight-chain, branched or cyclic alkane or alkene, optionally substituted with at least one member selected from the group consisting of Cl, Br, F, I, OH, MI_2 and SH, (2) OH, (3) NH_2, and (4) SH; and wherein optionally at least two of R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, and R^{10} can together form a cyclic or bicyclic alkanyl or alkenyl group. In embodiments, an ionic liquid includes fluorinated cations wherein at least one member selected from R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, and R^{10}, as described above, includes F.

[0197] In embodiments, is included the extraction of medicinally, pharmaceutically, or other economically valuable organic compounds, including but not limited to terpenes, alkaloids, and essential oils (e.g., for preparation of flavors and fragrances). In embodiments, the extraction includes use of two different fluorophilic compounds, specifically a fluorocarbon and a hydrofluorocarbon. In embodiments, is included the extraction of medicinally, pharmaceutically, or other economically valuable organic compounds, including but not limited to terpenes, alkaloids, and essential oils (e.g., for preparation of flavors and fragrances). In embodiments, the extraction includes use of three different chemical compounds, including two different fluorophilic compounds (e.g., a fluorocarbon and a hydrofluorocarbon), and an alkane. In embodiments, is included the extraction of medicinally, pharmaceutically, or other economically valuable organic compounds, including but not limited to terpenes, alkaloids, and essential oils (e.g., for preparation of flavors and fragrances). In embodiments, the extraction includes use of three different fluorophilic chemical compounds, including two different fluorophilic compounds (e.g., a fluorocarbon and a hydrofluorocarbon), and a fluorinated ether. In some embodiments, the mixture is a fluorocarbon and an ionic liquid.

[0198] In embodiments, a natural organic compound is extracted from a natural material (e.g., plant, animal, fungi, bacteria, or virus). In embodiments, the natural material is a plant. In embodiments, the plant is *Piper methysticum*, *Cannabis* spp., *Salvia* spp., *Banisteriopsis caapi*, *Psychotria viridis* (chacruna), *Diplopterys cabrerana*, *Peganum harmala*, *Humulus lupulus* or mixtures thereof.

[0199] In some embodiments, are methods of extracting natural organic compounds from natural materials. In some embodiments, natural organic compound includes biologi-

cally active organic compound. In some embodiments, the natural organic compound includes aromatic oil and/or essential oil. In embodiments, the natural organic compound is caffeine, terpene, a humulone, a lupulone, a myrcene, a humulene, a caryophyllene, an alkaloid, a flavonoid, a cannabinoid, menthol, capsaicin, anise, camphor, xanthohumol, 8-prenylnaringenin, isoxanthohumol, prenylflavonoid, kavalactone, or a salvorin. In embodiments, the natural product is cannabinoid. In embodiments, the cannabinoid is tetrahydrocannabinol, cannabidiol, or cannabinol. In embodiments, the natural organic compound is tetrahydrocannabinol.

[0200] In embodiments, the plant, animal, fungi, bacteria, or virus material used is cannabis. Multiple medicinal uses have been found for the active ingredients of cannabis, either *Cannabis* spp. but most commonly *Cannabis sativa.* Other plants than *Cannabis* spp. may contain useful cannabinoid activity, or may possess compounds, typically terpeneoid in character, which possess micromolar or higher affinity for the CB_1 or CB_2 cannabinoid receptors present in a man, animal, or bird.

[0201] Cannabinoids present in cannabis include the ingredients tetrahydrocannabinol, cannabinol, cannabidiol, and cannabichromene. The medicinal uses of cannabis include but are not limited to: epilepsy [Porter, Brenda E., and Catherine Jacobson. "Report of a parent survey of cannabidiol-enriched cannabis use in pediatric treatment-resistant epilepsy." *Epilepsy & Behavior* 29, no. 3 (2013): 574-577, which is incorporated herein by reference for all purposes]; pain [Cooper, Ziva D., Sandra D. Comer, and Margaret Haney. "Comparison of the analgesic effects of dronabinol and smoked marijuana in daily marijuana smokers." *Neuropsychopharmacology* 38, no. 10 (2013): 1984-1992; Kahan, Meldon, Anita Srivastava, Sheryl Spithoff, and Lisa Bromley. "Prescribing smoked cannabis for chronic noncancer pain Preliminary recommendations." *Canadian Family Physician* 60, no. 12 (2014): 1083-1090; Wilsey, Barth, Thomas Marcotte, Reena Deutsch, Ben Gouaux, Staci Sakai, and Haylee Donaghe. "Low-dose vaporized cannabis significantly improves neuropathic pain." *The Journal of Pain* 14, no. 2 (2013): 136-148, which are incorporated herein by reference for all purposes], specifically as evidenced in the treatment of nausea and pain associated with cancer and chemotherapy [United States Patent Document U.S. Pat. No. 8,119,697, Anti-Nausea and Anti Vomiting Activity of Cannabadiol Compounds, which is incorporated herein by reference for all purposes]; viral infection [Molina, Patricia E., Peter Winsauer, Ping Zhang, Edith Walker, Leslie Birke, Angela Amedee, Curtis Vande Stouwe et al. "Cannabinoid administration attenuates the progression of simian immunodeficiency virus." *AIDS research and human retroviruses* 27, no. 6 (2011): 585-592, which is incorporated herein by reference for all purposes]; AIDS-related pain and Wasting; multiple sclerosis [Svendsen, Kristina B., Troels S. Jensen, and Flemming W. Bach. "Does the cannabinoid dronabinol reduce central pain in multiple sclerosis? Randomized double blind placebo controlled crossover trial." *Bmj* 329, no. 7460 (2004): 253, which is incorporated herein by reference for all purposes] arthritis; rheumatism; glaucoma [Hingorani, Tushar, Waseem Gul, Mahmoud Elsohly, Michael A. Repka, and Soumyajit Majumdar. "Effect of ion pairing on in vitro transcorneal permeability of a Δ9-tetrahydrocannabinol prodrug: Potential in glaucoma therapy." *Journal of pharmaceutical sciences* 101, no.

2 (2012): 616-626, which is incorporated herein by reference for all purposes]; migraines; muscle spasticity; chemical dependency. Prior art further suggests that cannabis and its components have utility in oncology [Chakravarti, Bandana, Janani Ravi, and Ramesh K. Ganju. "Cannabinoids as therapeutic agents in cancer: current status and future implications." *Oncotarget* 5, no. 15 (2014): 5852], Parkinson's disease [see, for example, More, Sandeep Vasant, and Dong-Kug Choi. "Promising cannabinoid-based therapies for Parkinson's disease: motor symptoms to neuroprotection." *Molecular neurodegeneration* 10, no. 1 (2015): 1-26, which is incorporated herein by reference for all purposes], Within this embodiment, extracted material may be used as a composition to treat post-herpetic neuralgia, shingles, burns, actinic keratosis, oral cavity sores, oral ulcers, post-episiotomy pain, psoriasis, pruritus, contact dermatitis, eczema, bullous dermatitis herpetiformis, exfoliative dermatitis, mycosis fungoides, pemphigus, severe erythema multiforme, seborrheic dermatitis, psoriatic arthritis, diabetic neuropathy, ankylosing spondylitis, Reiter's syndrome, gout, chondrocalcinosis, joint pain secondary to dysmenorrhea, fibromyalgia [Fiz, Jimena, Marta Duran, Dolors Capella, Jordi Carbonell, and Magi Farr& "Cannabis use in patients with fibromyalgia: effect on symptoms relief and health-related quality of life." (2011): e18440, which is incorporated herein by reference for all purposes], musculoskeletal pain, neuropathic-postoperative complications, polymyositis, acute nonspecific tenosynovitis, bursitis, epicondylitis, post-traumatic osteoarthritis, synovitis, juvenile rheumatoid arthritis, contact eczema, allergies (not otherwise specified), phototoxic reactions, inflammatory and itching dermatoses, rosacea, perioral dermatitis, acne, acne, psoriasis, mosquito and other insect bites, skin atrophy, allergic rhinitis, conjunctivitis, otitis, bronchial asthma, Crohn's disease, ulcerative colitis, sarcoidosis, inflammatory-rheumatic diseases of the soft tissue or joints, mycoses, or combinations thereof.

[0202] In embodiments, the plant is *Cannabis* spp. In embodiments, the apparatus, method, or composition includes Freon™ 134a (1,1,1,2-Tetrafluoroethane). In embodiments, the apparatus, method, or composition includes Freon™ 134a (1,1,1,2-Tetrafluoroethane) and optionally one or more of carbon dioxide, nitrous oxide, sulfur hexafluoride, trifluoromethane, trifluoromethyl iodide, or tetrafluoromethane. In embodiments, the apparatus, method, or composition includes two extraction components (e.g., one component used in the extraction consists of Freon™ 134a (1,1,1,2-Tetrafluoroethane) and another is selected from the optional group of gases consisting of carbon dioxide, nitrous oxide, sulfur hexafluoride, trifluoromethane, trifluoromethyl iodide, or tetrafluoromethane; one component used in the extraction is Freon™ 134a (1,1,1,2-Tetrafluoroethane) within the range of 20 mol-% to 99 mol-% and another is selected from the optional group of gases consisting of carbon dioxide, nitrous oxide, sulfur hexafluoride, trifluoromethane, trifluoromethyl iodide, or tetrafluoromethane in the range independently from 80 mol-% to 1 mol-%; one component used in the extraction is Freon™ 134a (1,1,1,2-Tetrafluoroethane) within the range of 80 mol-% to 90 mol-% and another is selected from the optional group of gases consisting of carbon dioxide, nitrous oxide, sulfur hexafluoride, trifluoromethane, trifluoromethyl iodide, or tetrafluoromethane in the range independently from 10 mol-% to 20 mol-%; or one component used in the

extraction consists of Freon™ 134a (1,1,1,2-Tetrafluoroethane) within the range of 80 mol-% to 90 mol-% and another is selected from the optional group of gases consisting of carbon dioxide, nitrous oxide, sulfur hexafluoride, trifluoromethane, trifluoromethyl iodide, or tetrafluoromethane in the range independently from 9 mol-% to 19 mol-% where ethanol is present in the range from 1 mol-% to 10 mol-%).

[0203] In embodiments, the plant material used is *Salvia* spp. Multiple medicinal uses have been found for the active ingredients of *Salvia* spp. in particular achieving sedation and tranquilization in psychiatric and neurological disorders and treatment of insomnia [see for example Perron, Brian E., Brian K. Ahmedani, Michael G. Vaughn, Joseph E. Glass, Arnelyn Abdon, and Li-Tzy Wu. "Use of Salvia divinorum in a nationally representative sample." *The American journal of drug and alcohol abuse* 38, no. 1 (2012): 108-113; Potter, David N., Diane Damez-Werno, William A. Carlezon, Bruce M. Cohen, and Elena H. Chartoff. "Repeated exposure to the κ-opioid receptor agonist salvinorin A modulates extracellular signal-regulated kinase and reward sensitivity." *Biological psychiatry* 70, no. 8 (2011): 744-753; Teksin, Zeynep S., Insong J. Lee, Noble N. Nemieboka, Ahmed A. Othman, Vijay V. Upreti, Hazem E. Hassan, Shariq S. Syed, Thomas E. Prisinzano, and Natalie D. Eddington. "Evaluation of the transport, in vitro metabolism and pharmacokinetics of Salvinorin A, a potent hallucinogen." *European Journal of Pharmaceutics and Biopharmaceutics* 72, no. 2 (2009): 471-477, which are incorporated herein by reference for all purposes]. Salvinorin A and other closely related salvinorins have substantial activity at the nanomolar level on kappa-type opioid receptors, which are involved, among other things, in analgesia. Accordingly, the extracts obtained from *Salvia* spp. are of utility of the treatment and amelioration of the disease process as well as the symptomatology of a variety diseases and pathological conditions, such as pain, especially neuropathic pain and cancer-breakthrough pain, which would normally be responsive to opioids.

[0204] In embodiments, the plant is *Salvia* spp. In embodiments, the apparatus, method, or composition includes Freon™ 134a (1,1,1,2-Tetrafluoroethane). In embodiments, the apparatus, method, or composition includes Freon™ 134a (1,1,1,2-Tetrafluoroethane) and optionally one or more of carbon dioxide, nitrous oxide, sulfur hexafluoride, trifluoromethane, trifluoromethyl iodide, or tetrafluoromethane. In embodiments, the apparatus, method, or composition includes two extraction components (e.g., one component used in the extraction is Freon™ 134a (1,1,1,2-Tetrafluoroethane) and another is selected from the optional group of gases consisting of carbon dioxide, nitrous oxide, sulfur hexafluoride, trifluoromethane, trifluoromethyl iodide, or tetrafluoromethane; one component used in the extraction is Freon™ 134a (1,1,1,2-Tetrafluoroethane) within the range of 20 mol-% to 99 mol-% and another is selected from the optional group of gases consisting of carbon dioxide, nitrous oxide, sulfur hexafluoride, trifluoromethane, trifluoromethyl iodide, or tetrafluoromethane in the range independently from 80 mol-% to 1 mol-%; one component used in the extraction is Freon™ 134a (1,1,1,2-Tetrafluoroethane) within the range of 80 mol-% to 90 mol-% and another is selected from the optional group of gases consisting of carbon dioxide, nitrous oxide, sulfur hexafluoride, trifluoromethane, trifluoromethyl iodide, or tetrafluoromethane in the range independently from 10 mol-% to 20 mol-%; one

component used in the extraction is Freon™ 134a (1,1,1,2-Tetrafluoroethane) within the range of 80 mol-% to 90 mol-% and another is selected from the optional group of gases consisting of carbon dioxide, nitrous oxide, sulfur hexafluoride, trifluoromethane, trifluoromethyl iodide, or tetrafluoromethane in the range independently from 9 mol-% to 19 mol-% where ethanol is present in the range from 1 mol-% to 10 mol-%.

[0205] In embodiments, the plant material used is *Banisteriopsis caapi, Psychotria viridis* (chacruna), *Diplopterys cabrerana* (also known as chaliponga and chagropanga), *Peganum harmala,* or any mixture thereof. Multiple medicinal uses have been found for the active ingredients of *Banisteriopsis caapi, Psychotria viridis* (chacruna), *Diplopterys cabrerana* (also known as chaliponga and chagropanga), *Peganum harmala,* or any mixture thereof, in particular achieving sedation and tranquilization in psychiatric and neurological disorders and treatment of insomnia. See, for example, Riba, Jordi, Marta Valle, Gloria Urbano, Mercedes Yritia, Adelaida Morte, and Manel J. Barbanoj. "Human pharmacology of ayahuasca: subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics." Journal of Pharmacology and Experimental Therapeutics 306, no. 1 (2003): 73-83; Rivier, Laurent, and Jan-Erik Lindgren. "'Ayahuasca,' the South American hallucinogenic drink: An ethnobotanical and chemical investigation." Economic Botany 26, no. 2 (1972): 101-129, which are incorporated herein by reference for all purposes. Accordingly, the extracts obtained from *Banisteriopsis caapi, Psychotria viridis* (chacruna), *Diplopterys cabrerana* (also known as chaliponga and chagropanga), *Peganum harmala,* or any mixture thereof. Such mixtures are of utility of the treatment and amelioration of the disease process as well as the symptomatology of the diseases and pathological conditions, especially in the treatment of psychiatric disorders, such as depression.

[0206] In embodiments, the plant is *Banisteriopsis caapi, Psychotria viridis* (chacruna), *Diplopterys cabrerana* (also known as chaliponga and chagropanga), *Peganum harmala,* or any mixture thereof. In embodiments, the plant is *Banisteriopsis caapi.* In embodiments, the plant is *Psychotria viridis* (chacruna). In embodiments, the plant is *Diplopterys cabrerana* (also known as chaliponga and chagropanga). In embodiments, the plant is *Peganum harmala.* In embodiments, the apparatus, method, or composition includes Freon™ 134a (1,1,1,2-Tetrafluoroethane). In embodiments, the apparatus, method, or composition includes Freon™ 134a (1,1,1,2-Tetrafluoroethane) and optionally one or more of carbon dioxide, nitrous oxide, sulfur hexafluoride, trifluoromethane, trifluoromethyl iodide, or tetrafluoromethane. In embodiments, the apparatus, method, or composition includes two extraction components (e.g., one component used in the extraction is Freon™ 134a (1,1,1,2-Tetrafluoroethane) and another is selected from the optional group of gases consisting of carbon dioxide, nitrous oxide, sulfur hexafluoride, trifluoromethane, trifluoromethyl iodide, or tetrafluoromethane; one component used in the extraction is Freon™ 134a (1,1,1,2-Tetrafluoroethane) within the range of 20 mol-% to 99 mol-% and another is selected from the optional group of gases consisting of carbon dioxide, nitrous oxide, sulfur hexafluoride, trifluoromethane, trifluoromethyl iodide, or tetrafluoromethane in the range independently from 80 mol-% to 1 mol-%; one component used in the extraction is Freon™ 134a (1,1,1,2-Tetrafluoroethane)

within the range of 80 mol-% to 90 mol-% and another is selected from the optional group of gases consisting of carbon dioxide, nitrous oxide, sulfur hexafluoride, trifluoromethane, trifluoromethyl iodide, or tetrafluoromethane in the range independently from 10 mol-% to 20 mol-%; one component used in the extraction is Freon™ 134a (1,1,1,2-Tetrafluoroethane) within the range of 80 mol-% to 90 mol-% and another is selected from the optional group of gases consisting of carbon dioxide, nitrous oxide, sulfur hexafluoride, trifluoromethane, trifluoromethyl iodide, or tetrafluoromethane in the range independently from 9 mol-% to 19 mol-% where ethanol is present in the range from 1 mol-% to 10 mol-%.

[0207] In embodiments, the plant material used is kava (*Piper myristicum*). Multiple medicinal uses have been found for the active ingredients of kava (*Piper myristicum*), in particular achieving sedation and tranquilization in psychiatric and neurological disorders and treatment of insomnia and anxiety. See, for example, Volz, Hans-Peter, and M. Kieser. "Kava-kava extract WS 1490 versus placebo in anxiety disorders: A randomized placebo-controlled 25-week outpatient trial." *Pharmacopsychiatry* (1997). Sarris, J., D. J. Kavanagh, G. Byrne, K. M. Bone, J. Adams, and G. Deed. "The Kava Anxiety Depression Spectrum Study (KADSS): a randomized, placebo-controlled crossover trial using an aqueous extract of *Piper methysticum.*" *Psychopharmacology* 205, no. 3 (2009): 399-407, which are incorporated herein by reference for all purposes. However, there have been reports of hepatotoxicity due to Kava [Clouatre, Dallas

[0208] L. "Kava: examining new reports of toxicity." *Toxicology letters* 150, no. 1 (2004): 85-96, which is incorporated herein by reference for all purposes]; better extraction procedures could offer the promise of ameliorating these difficulties if only the active kavalactones, which account for much of the biological activity, could be extracted in a substantially purer form. The extracts obtained from kava are of utility of the treatment and amelioration of the disease process as well as the symptomatology of the diseases and pathological conditions.

[0209] In embodiments, the plant is *Piper methysticum* (Kava). In embodiments, the apparatus, method, or composition includes Freon™ 134a (1,1,1,2-Tetrafluoroethane). In embodiments, the apparatus, method, or composition includes Freon™ 134a (1,1,1,2-Tetrafluoroethane) and optionally one or more of carbon dioxide, nitrous oxide, sulfur hexafluoride, trifluoromethane, trifluoromethyl iodide, or tetrafluoromethane. In embodiments, the apparatus, method, or composition includes two extraction components (e.g., one component used in the extraction is Freon™ 134a (1,1,1,2-Tetrafluoroethane) and another is selected from the optional group of gases consisting of carbon dioxide, nitrous oxide, sulfur hexafluoride, trifluoromethane, trifluoromethyl iodide, or tetrafluoromethane; one component used in the extraction consists of Freon™ 134a (1,1,1,2-Tetrafluoroethane) within the range of 20 mol-% to 99 mol-% and another is selected from the optional group of gases consisting of carbon dioxide, nitrous oxide, sulfur hexafluoride, trifluoromethane, trifluoromethyl iodide, or tetrafluoromethane in the range independently from 80 mol-% to 1 mol-%; one component used in the extraction is Freon™ 134a (1,1,1,2-Tetrafluoroethane) within the range of 80 mol-% to 90 mol-% and another is selected from the optional group of gases consisting of

carbon dioxide, nitrous oxide, sulfur hexafluoride, trifluoromethane, trifluoromethyl iodide, or tetrafluoromethane in the range independently from 10 mol-% to 20 mol-%.

III. EXAMPLES

[0210] The following examples illustrate certain specific embodiments of the invention and are not meant to limit the scope of the invention.

[0211] Embodiments herein are further illustrated by the following examples and detailed protocols. However, the examples are merely intended to illustrate embodiments and are not to be construed to limit the scope herein. The contents of all references and published patents and patent applications cited throughout this application are hereby incorporated by reference.

[0212] A problem in pharmaceutical chemistry relates to extraction of useful substances from plants or animals where such useful substances are employed for the formulation of a pharmaceutical or a nutraceutical. For example, morphine is a pharmaceutically highly useful material. All morphine used today originates in natural opium, which is obtained exclusively by extraction from *Papaver somniferum* (opium poppies), which is supplied primarily from India, Afghanistan, and Turkey where the poppies contain up to 20% of morphine in their latex. There are many total synthetic pathways to morphine but to date there is no reported synthesis of the alkaloid that would show much promise for a large-scale manufacturing [Zezula, Josef, and Tomas Hudlicky. "Recent progress in the synthesis of morphine alkaloids." Synlett 3 (2005): 388-405, which is incorporated herein by reference for all purposes]. Thus the supply of morphine remains dependent upon extraction of plant material.

[0213] Alternatively, the extracted useful substance may be employed as a synthetic intermediate in the manufacture of other drugs which are formulated as a pharmaceutical dosage form or a nutraceutical. As an example, thebaine is an opium alkaloid which is also extracted from opium poppies which itself is somewhat toxic and convulsant and when administered as a drug has no medical value. However, thebaine is used as the key intermediate for the synthesis of most of the non-natural opiates used in clinical practice. See, for example, Schiff, Paul L. "Opium and its alkaloids." American Journal of Pharmaceutical Education 66.2 (2002): 188-196; Tolstikova, T G. A V Bolkunov, E A Morozova, and S E Tolstikov. "Thebaine as a Precursor of Opioid Analgesic Agents." Chemistry for Sustainable Development 17 (2009) 109-126, which are incorporated herein by reference for all purposes.

[0214] Plants may be shrubs, trees, roots, berries, or other components of normal terrestrial plants, or they can be plants present in freshwater aquatic or marine environments. For examples of the latter, see Rocha, Fabiola Dutra, Angelica Ribeiro Soares, Peter John Houghton, Renato Crespo Pereira, Maria Auxiliadora Coelho Kaplan, and Valeria Laneuville Teixeira. "Potential cytotoxic activity of some Brazilian seaweeds on human melanoma cells." Phytotherapy Research 21, no. 2 (2007): 170-175, which is incorporated herein by reference for all purposes.

[0215] Although in many cases plants represent the major source of naturally occurring compounds which are useful in the prevention and treatment of diseases in humans and animals other natural sources can be important as a source of these materials. For example, many drugs have been

derived from marine natural products [Jha, Rajeev Kumar, and Xu Zi-rong. "Biomedical compounds from marine organisms." Marine drugs 2.3 (2004): 123-146, which is incorporated herein by reference for all purposes].

[0216] Also, many marine drugs are extracted from organisms. See, for example, Thornburg, Christopher C., T. Mark Zabriskie, and Kerry L. McPhail. "Deep-Sea Hydrothermal Vents: Potential Hot Spots for Natural Products Discovery?" Journal of natural products 73, no. 3 (2010): 489-499, Pettit, George R., Jun-ping Xu, Zbigniew A. Cichacz, Michael D. Williams, Ann-Christine Dorsaz, Daniel C. Brune, Michael R. Boyd, and Ronald L. Cerny. "Antineoplastic agents 315. Isolation and structure of the marine sponge cancer cell growth inhibitor phakellistatin 5." Bioorganic & Medicinal Chemistry Letters 4, no. 17 (1994): 2091-2096; Yosief, Tesfamariam, Amira Rudi, and Yoel Kashman. "Asmarines A F, novel cytotoxic compounds from the marine sponge *Raspailia* species." Journal of natural products 63, no. 3 (2000): 299-304. Numata, Atsushi, Taro Amagata, Katsuhiko Minoura, and Tadayoshi Ito. "Gymnastatins, novel cytotoxic metabolites produced by a fungal strain from a sponge." Tetrahedron letters 38, no. 32 (1997): 5675-5678; Kobayashi, Jun'ichi, Shinji Takeuchi, Masami Ishibashi, Hideyuki Shigemori, and Takuma Sasaki. "Plakotenin, a new cytotoxic carboxylic acid from the okinawan marine sponge plakortis Sp." Tetrahedron letters 33, no. 18 (1992): 2579-2580. ; Washida, Kazuto, Tomoyuki Koyama, Kaoru Yamada, Masaki Kita, and Daisuke Uemura. "Karatungiols A and B, two novel antimicrobial polyol compounds, from the symbiotic marine dinoflagellate *Amphidinium* sp." Tetrahedron letters 47, no. 15 (2006): 2521-2525, Kwon, Hak Cheol, Christopher A. Kauffman, Paul R. Jensen, and William Fenical. "Marinomycins A D, antitumor-antibiotics of a new structure class from a marine actinomycete of the recently discovered genus '*Marinispora*'." Journal of the American Chemical Society 128, no. 5 (2006): 1622-1632, which are incorporated herein by reference for all purposes. Drugs may be extracted from many sources. See, for example, Yan, Yong-Ming, Jun Ai, Yan-Ni Shi, Zhi-Li Zuo, Bo Hou, Jie Luo, and Yong-Xian Cheng. "(±)-Aspongamide A, an N-Acetyldopamine Trimer Isolated from the Insect Aspongopus chinensis, Is an Inhibitor of p-Smad3." Organic letters 16, no. 2 (2014): 532-535, Whitehouse, M. W., A. G. Turner, C. K. C. Davis, and M. S. Roberts. "Emu oil (s): a source of non-toxic transdermal anti-inflammatory agents in aboriginal medicine." Inflammopharmacology 6, no. 1 (1998): 1-8, which are incorporated herein by reference for all purposes.

[0217] It should further be noted that typically natural products, including but not limited to marine natural products, and natural products from terrestrial plants, may contain multiple chiral centers which manifest optical activity. In general, this complicates the total synthesis of these natural products from commercially available achiral molecules. Although many methods exist in modern organic chemistry to perform enantioselective or chiral synthetic steps in high yield as well as to form multiple chiral centers in the correct stereochemical relation to each other in a single chemical step, many of these procedures are not well scalable from milligram scale in the laboratory to kilogram scale in production. Accordingly, semisynthesis is commonly employed. For example although there are many total syntheses of Paclitaxel, an important cancer drug, it is still produced from cells maintained in plant tissue culture derived from the pacific yew tree, *Taxus brevifolia* as none

of the total syntheses are practical at large scale and there is not much possibility that they will ever be.

[0218] Many methods have been developed for extraction of natural products most particularly from plant, animal, fungi, bacteria, or viruses, where this has been historically important long before the advent of modern medicine in the preparation of materials required for the fragrance industry, such as the formulation of perfumes. These have been reviewed. See, for example, Wang, Lijun, and Curtis L. Weller. "Recent advances in extraction of nutraceuticals from plants." Trends in Food Science & Technology 17, no. 6 (2006): 300-312, which is incorporated herein by reference for all purposes. The oldest methods, which are still used, involve steam distillation, co-distillation with a solvent, typically ethanol, or Soxhlet extraction with an organic solvent. In these processes, the water, ethanol or organic solvent which co-distills with the desired mixture of natural products is removed, leaving the final product, typically as an oil, which may in its impure state may still be highly desired in the fragrance industry. For example Otto of roses is produced by steam distillation of rose (Rosa damascene) petals and is an important item of commerce in the perfume industry. However, steam distillation is an extremely inefficient and laborious process in practice, and it cannot be applied effectively to molecules which are somewhat polar in aqueous solution, as is, for example, the case with most alkaloids.

[0219] Extraction with organic solvents has been used for obtaining desired valuable substances from natural product plant, animal, fungi, bacteria, or virus material. This could involve Soxhlet extraction [De Castro, M D Luque, and F. Priego-Capote. "Soxhlet extraction: Past and present panacea." Journal of Chromatography A 1217, no. 16 (2010): 2383-2389, which is incorporated herein by reference for all purposes] whereby solvent is heated under reflux and the refluxed solvent is passed over a porous thimble containing the natural product. The natural product material is continually washed with fresh solvent in this approach, allowing the removal of much more product than would otherwise be possible because the amount in solution is not limited by equilibrium solubility of the solute in the solvent. While this approach is useful with highly soluble material, especially at the laboratory scale it becomes physically inefficient from the point of view of solvent and material handling at greater than a kilo scale. To some this can be addressed by using a different design, such as a fluidized bed extractor.

[0220] Extraction with hydrocarbon gases is also a useful technique, especially for the isolation of extremely hydrophobic materials such as waxes and oils [U.S. Pat. No. 5,405,633, Process for the extraction of fats and oils; European Patent Office Document EP0711508A1, Verfahren zur Extraktion von natürlichen Aromen aus fett-und ölhaltigen Naturstoffen; Nobre, Beatriz P., Luisa Gouveia, Patricia G S Matos, Ana F. Cristino, Antonio F. Palavra, and Rui L. Mendes. "Supercritical extraction of lycopene from tomato industrial wastes with ethane." Molecules 17, no. 7 (2012): 8397-8407]; which are incorporated herein by reference. Propane versus supercritical CO_2, is ten times more efficient at extracting carotenoids from pepper. See, for example, Daood, H. G., V. Illés, M. H. Gnayfeed, B. Mészáros, G. Horváth, and P. A. Biacs. "Extraction of pungent spice paprika by supercritical carbon dioxide and subcritical propane." The Journal of supercritical fluids 23, no. 2 (2002): 143-152, which is incorporated herein by reference for all

purposes. However, these processes which employ ethane, propane, or butane although they can be worked safely still present a prima facie serious risk of fire or explosion, and great care must be taken on an industrial scale to avoid this. In "backyard" extraction of cannabis by this approach, many serious fires and explosions have occurred. See, for example, Downs, D. "Don't Try This At Home: Butane Hash Oil Penalties Stiffen", East Bay Express Aug. 11, 2015, which is incorporated herein by reference for all purposes. Also, although solvent extraction processes are used on a commercial scale, the extraction solvents which are currently used in these processes are not wholly satisfactory. Thus, when solvents such as hexane are used to extract aromatic oils, such as are used in the food and cosmetic industries, from plant matter containing those oils, unwanted materials contained in the plant, animal, fungi, bacteria, or virus, e.g. high molecular weight waxes, tend to be eluted along with the desired oil. This then can necessitate a further costly purification step [U.S. Pat. No. 2,467,403, Solvent extraction of castor oils, which is incorporated herein by reference for all purposes].

[0221] Halogenated solvents, such as dichloromethane or bromomethane, have been used for extraction of natural products [U.S. Pat. No. 2,294,811 Crystallized glucoside from red squill; U.S. Pat. No. 2,472,121, Decaffeinated soluble coffee; US 2010/0314240, Process of extracting aromatic compounds from plants using bromomethane as a solvent, which are incorporated herein by reference for all purposes]. By the use of a phase transfer catalyst even relatively polar alkaloids can be efficiently extracted [U.S. Pat. No. 4,818,533, Production of high purity alkaloids, which is incorporated herein by reference for all purposes]. However, the use of these halogenated solvents has been diminished greatly in recent years for substances of pharmaceutical or nutraceutical activity due to concerns about solvent residues, even at the parts-per-million levels, in the final product, since these halogenated solvents are known to be toxic and some such as chloroform and bromomethane to be putative carcinogens.

[0222] More recently, steam distillation, co-distillation with a solvent, typically ethanol, or Soxhlet extraction have been substantially superseded except in "niche" applications by a method employing extraction with supercritical gases, most particularly supercritical carbon dioxide (CO_2). This method employs CO_2 under substantial temperature and pressure, typically greater than 200 atmospheres and at a temperature between 40° C. and 80° C. This has recently been reviewed. See, for example, De Melo, M. M. R., A. J. D. Silvestre, and C. M. Silva. "Supercritical fluid extraction of vegetable matrices: applications, trends and future perspectives of a convincing green technology." The Journal of Supercritical Fluids 92 (2014): 115-176, which is incorporated herein by reference for all purposes. Originally, this methodology was adopted on an industrial scale for decaffeination of coffee, when it was recognized that it was highly undesirable to utilize chlorinated solvents, such as dichloromethane, which leave trace solvent residues. See U.S. Pat. No. 2,472,121, Decaffeinated soluble coffee, which is incorporated herein by reference for all purposes. The methodology is described in U.S. Pat. No. 4,820,537 Method for decaffeinating coffee with a supercritical fluid; U.S. Pat. No. 5,288,511 Supercritical carbon dioxide decaffeination of acidified coffee, which are incorporated herein by reference for all purposes. The caffeine may be economically recov-

ered [U.S. Pat. No. 4,996,317 Caffeine recovery from supercritical carbon dioxide, which is incorporated herein by reference for all purposes].

[0223] Though this approach has become widely used for extraction of plants, it suffers from a number of important deficiencies. In the first place, the temperatures and pressures which are required result in the need for specialized pressure vessels and high pressure pumps, and although such equipment is commercially available, is can become extremely expensive if a large scale (hundreds of kilograms of plant, animal, fungi, bacteria, or virus material per batch) is required. Since many natural products are present at concentrations in the plant, animal, fungi, bacteria, or virus which are relatively low, in many cases a few percent by weight or less, multiple large batches of raw plant, animal, fungi, bacteria, or virus material frequently need to be processed if a few to tens of kilo/day quantities of extract are required. In many cases, economic requirements dictate hundreds of kilos per day to be produced of extract in order for the extractive process to be run in a manner which is profitable. At this scale, supercritical CO_2 extraction becomes extremely expensive in terms of capital requirements for plant, animal, fungi, bacteria, or virus construction. Furthermore, because CO_2's vapor pressure at room temperature is greater than sixty times normal atmospheric pressure, the use of CO_2 in a process creates a potential safety hazard relative to the same process operated at one atmosphere operation. Clearly, there is a need for processes which can be run in more conventional equipment at significantly lower pressures and temperatures.

[0224] Many extraction processes which are generically termed "supercritical" in nature with regard to CO_2 pressures and temperatures are actually not carried out at temperatures above the critical temperature and critical pressure of the gas, but are actually carried out at temperatures slightly to substantially below these pressures and temperatures. However, below the critical point temperature and pressure the efficiency of extraction of valuable natural products by CO_2 is markedly reduced. It is therefore commonly the case that so-called "supercritical" CO_2 extraction is not as efficient as would be the case if it were truly carried out under supercritical conditions. This limitation, which is not solely a semantic one, also contributes to a deficiency of the CO_2 extraction procedure which must be recognized. An advantage of CO_2 under subcritical conditions is that the solubility of undesired waxes is substantially lower at slightly reduced pressures [Jha, Sujit Kumar, and Giridhar Madras. "Modeling the solubilities of high molecular weight n-alkanes in supercritical carbon dioxide." Fluid phase equilibria 225 (2004): 59-62, which is incorporated herein by reference for all purposes].

[0225] It is generally recognized that liquid CO_2, regardless of whether it is used as the extracting fluid at fully supercritical conditions or at lower pressures and temperatures, is not a completely inert solvent. Carbon dioxide is relatively inert towards reactive compounds, but CO_2's relative inertness should not be confused with complete inertness. For example, an attempt to conduct a hydrogenation in CO_2 over a platinum catalyst at 303 K will lead to the production of carbon monoxide CO, which itself could be quite reactive under the conditions of supercritical CO_2 extraction. Simple salts such as NaCl, KCl, and LiCl which are invariably present in plant material, especially if the said plant material has been heated, will serve efficiently to

catalyze the addition of CO_2 to activated systems at one atmosphere. See, for example, Kihara, Nobuhiro, Nobutaka Hara, and Takeshi Endo. "Catalytic activity of various salts in the reaction of 2,3-epoxypropyl phenyl ether and carbon dioxide under atmospheric pressure." The Journal of Organic Chemistry 58, no. 23 (1993): 6198-6202, which is incorporated herein by reference for all purposes. Although it might be suggested that it is improbable that plant material would contain epoxides, this is not true as many terpenes will oxidize to produce epoxides; this could in fact be accelerated under supercritical CO_2 conditions if care is not taken to exclude O_2 from the system prior to pressurizing with CO_2. In the presence of simple inorganic catalysts such as zeolites, terpenes such as limonene are efficiently converted to epoxides by oxygen. See, for example, Bhattacharjee, Samiran, and James A. Anderson. "Epoxidation by Layered Double Hydroxide-Hosted Catalysts. Catalyst Synthesis and Use in the Epoxidation of R-(+)-Limonene and (−)-α-Pinene Using Molecular Oxygen." Catalysis letters 95, no. 3-4 (2004): 119-125, which is incorporated herein by reference for all purposes. Other materials that possess catalytic activity similar to the zeolites, such as clays, which could be present to some degree in plant material could serve as catalysts in a similar manner.

[0226] It should be however recognized that even a small amount of addition of CO_2 to a reactive moiety could result in multiple reactions under the conditions of supercritical extraction of a nature which would yield some amount of polymeric tarry material of indefinite character. Indeed, such tars do, to some degree, invariably occur in the course of supercritical CO_2 extraction. Supercritical CO_2 is, in fact, generally recognized as an excellent solvent in which to perform polymerization reactions [Kendall, Jonathan L., Dorian A. Canelas, Jennifer L. Young, and Joseph M. DeSimone. "Polymerizations in supercritical carbon dioxide." Chemical Reviews99, no. 2 (1999): 543-564, which is incorporated herein by reference for all purposes]. The presence of such tars and their removal represents a serious deficiency in supercritical CO_2 extraction of natural products, as commonly it is necessary to employ a secondary purification process following the extraction to remove them. Typically, this is realized by a method such as high vacuum fractional distillation, molecular distillation, or flash chromatography [Still, W. Clark, Michael Kahn, and Abhijit Mitra. "Rapid chromatographic technique for preparative separations with moderate resolution." The Journal of Organic Chemistry 43, no. 14 (1978): 2923-2925, which is incorporated herein by reference for all purposes]. These processes are expensive and furthermore result invariably in some measure of loss of the desired product. For example, aromatic oils contained in certain plants are complex substances containing a large number of individual compounds some of which are relatively volatile or relatively thermally unstable. Consequently, high distillation temperatures can tend to result in a loss of product either through evaporation of the more volatile compounds or thermal degradation of the more thermally unstable compounds. It would therefore be highly desirable to have a process which did not yield such undesired polymeric tarry materials.

[0227] Carbon dioxide will add to olefins at 1 atmosphere and 60° C. in the presence of a free radical initiator and a strong base; under conditions of elevated pressure this could be expected to occur in the presence of weaker bases (e.g. Potassium carbonates and hydroxides) which could be pres-

ent in plant derived material, especially if it has been heated [Eghbali, Nicolas, and Chao-Jun Li. "Conversion of carbon dioxide and olefins into cyclic carbonates in water." Green Chem. 9, no. 3 (2007): 213-215, which is incorporated herein by reference for all purposes]. CO_2 will add to heterocyclic systems in the presence of catalytic amounts of copper halides at 1 atmospheres pressure and a temperature of 80° C. [Zhang, Liang, Jianhua Cheng, Takeshi Ohishi, and Zhaomin Hou. "Copper-Catalyzed Direct Carboxylation of C—H Bonds with Carbon Dioxide." Angewandte Chemie 122, no. 46 (2010): 8852-8855, which is incorporated herein by reference for all purposes].

[0228] All plant, animal, fungi, bacteria, or virus materials which serve as the raw feedstock for extraction contain a significant amount of water. Even if these materials are dried in vacuo, they nonetheless inherently possess some water which is only released at the elevated temperatures and pressures which are required to carry out the supercritical CO_2 extraction process. In completely pure CO_2, water has substantial solubility: at pressures and temperatures just below the critical point the mole fraction of water in CO_2 is about 0.02 [King Jr, Allen Dupree, and C. R. Coan. "Solubility of water in compressed carbon dioxide, nitrous oxide, and ethane. Evidence for hydration of carbon dioxide and nitrous oxide in the gas phase." Journal of the American Chemical Society 93, no. 8 (1971): 1857-1862, which is incorporated herein by reference for all purposes]. This paper further teaches that the water in the liquid CO_2 medium is almost completely solvated and is in the form of carbonic acid. Carbonic acid has a pKa of 3.45 [Adamczyk, Katrin, Mirabelle Prémont-Schwarz, Dina Pines, Ehud Pines, and Erik T J Nibbering. "Real-time observation of carbonic acid formation in aqueous solution." Science 326, no. 5960 (2009): 1690-1694, which is incorporated herein by reference for all purposes] and in a nonaqueous system such as liquid CO_2 it probably exists substantially in the form of the gas-phase dimer [Bernard, Jürgen, Markus Seidl, Ingrid Kohl, Klaus R. Liedl, Erwin Mayer, Óscar Gálvez, Hinrich Grothe, and Thomas Loerting. "Spectroscopic Observation of Matrix-Isolated Carbonic Acid Trapped from the Gas Phase." Angewandte Chemie International Edition 50, no. 8 (2011): 1939-1943, which is incorporated herein by reference for all purposes] in which it will effectively possess greater acidic character. Carbonic acid in this medium at elevated pressures and temperatures possesses substantial reactivity.

[0229] The critical pressure of CO_2 is significantly higher than that for alkanes or fluorocarbons. This anomalously high critical pressure is due to the fact that CO_2 has a high quadripole moment. It has been suggested that CO_2 may prove to be a solvent whose strength would rival or surpass that of alkanes and ketones. Because early models employed to calculate CO_2's solvent power relied on a direct relationship between the Hildebrandt solubility parameter (δ) and the square root of the critical pressure the solubility parameter of CO_2 was over-predicted by 20-100%, leading to early inflated claims as to its potential.

[0230] Supercritical CO_2 processes can benefit, in many cases, from the addition of various cosolvents. For example, addition of a few percent of methanol to CO_2 will result in dramatic increases in solubility of slightly polar materials, such as for example acridine [Brennecke, Joan F., and Charles A. Eckert. "Phase equilibria for supercritical fluid process design." AIChE Journal 35, no. 9 (1989): 1409-

1427, which is incorporated herein by reference for all purposes]. The addition of a small amount of isopropanol to the supercritical CO$_2$ system has been carefully shown to dramatically increase the recovery of the sugar tagatose [Montañés, Fernando, Tiziana Fornari, Pedro J. Martín-Álvarez, Nieves Corzo, Agustín Olano, and Elena Ibáñez. "Selective recovery of tagatose from mixtures with galactose by direct extraction with supercritical CO$_2$ and different cosolvents." Journal of agricultural and food chemistry 54, no. 21 (2006): 8340-8345, which is incorporated herein by reference for all purposes]. While this approach is of principal utility with regard to the extraction of substances of intermediate hydrophobicity, it can be used, to advantage for very hydrophobic systems. For example, it has been reported that different very hydrophobic fractions containing useful antioxidant activity can be obtained from a bark extract by varying small amounts of ethanol which are added to the supercritical extraction [Braga, Mara E M, Rosa M S Santos, Ines J. Seabra, Roselaine Facanali, Marcia O M Marques, and Herminio C. de Sousa. "Fractioned SFE of antioxidants from maritime pine bark." The Journal of Supercritical Fluids 47, no. 1 (2008): 37-48, which is incorporated herein by reference for all purposes].

[0231] One approach to improvement of certain of the deficiencies of the supercritical CO$_2$ extraction system is to utilize a different supercritical gas. Unfortunately, many other gases have inconvenient critical properties. For example Argon has a critical pressure of 705 psi which can be attained easily but a critical temperature of only 151° K, which is so cold that it will not be a very effective solvent. Xenon would be an extremely good solvent but it is prohibitively expensive although but its critical pressure is high (847 psi) although its critical temperature is close to room temperature. Nitrous oxide is attractive because its critical temperature is just above room temperature (36° C.) although its critical pressure is rather high (1044 psi). Furthermore, nitrous oxide is potentially reactive to sensitive organic material in the presence of water, and the limited literature data suggest that it generally it is not as good a solvent in practice as CO$_2$.

[0232] Fluorocarbons are a chemical class selected from the field of fluorophilic compounds that are clear, colorless, odorless, nonflammable liquids that are essentially insoluble in water. In addition, fluorocarbon liquids are denser than water and soft tissue, have low surface tension and, for the most part, low viscosity.

[0233] Fluorocarbons have been used as solvents [U.S. Pat. No. 2,410,101, U.S. Pat. No. 2,449,671]. Chlorofluorocarbon Freon™ gases have been used in the extraction of perfume components [U.S. Pat. No. 3,150,050]. Chlorofluorocarbons have been used in the extraction of caffeine in coffee [U.S. Pat. No. 3,669,679]. However, this process employed a single class of fluorocarbon and no examples of mixtures of gases are therein cited.

[0234] Combinations of supercritical CO$_2$ and Freon™ solvents have been used for spice extraction of active materials [U.S. Pat. No. 4,490,398]. U.S. Patents U.S. Pat. No. 6,455,087 and U.S. Pat. No. 6,649,205 also describe potential uses of fluorocarbons in solvent extraction methods.

[0235] Dielectric constant is not an important parameter in determining the interaction of hydrofluorocarbons and fluorocarbons in the extraction process and its sole use in the selection of cosolvents is not supported by the present scientific literature (see supra). For example, for fluoroethanes the Kamlet-Taft parameters, which do depend upon the dielectric constant, albeit not in a simple, monotonic manner, do not appear to be strongly predictive of the microscopic thermodynamic behavior of the system [Lagalante, Anthony F., Robert L. Hall, and Thomas J. Bruno. "Kamlet-Taft solvatochromic parameters of the sub-and supercritical fluorinated ethane solvents." The Journal of Physical Chemistry B 102, no. 34 (1998): 6601-6604, which is incorporated herein by reference for all purposes]. A much better choice of parameter would exemplified by one which is experimentally determined, such as the partial molal free energy of mixing. This parameter which can be readily measured [for example see Duce, Celia, Maria Tinè, L. Lepori, E. Matteoli, B. Marongiu, and Alessandra Piras. "A comparative study of thermodynamic properties of binary mixtures containing perfluoroalkanes." Journal of Thermal Analysis and Calorimetry 92, no. 1 (2008): 145-154, which is incorporated herein by reference for all purposes]. The second virial coefficients of the solution, which were measured in the work of Scott supra, or most localized energetic calculations of the mixtures using a mixed approach of Monte Carlo dynamics and Kohn-Sham quantum calculations (DFT) incorporating explicit electron correlation. These studies show that aggregation behavior of hydrofluorocarbons in fluorocarbon binary (or tertiary) mixtures represents the most important component of the prediction of solubility of hydrophobic organic compounds in these mixtures. A useful way to think of this is that the solution of a component in a fluorophilic mixture is controlled by the clustering of a component (typically a hydrofluorocarbon) around the solute on the dynamic timescale of order-disorder in the fluid system [as described in Gerig, John T. "Selective solvent interactions in a fluorous reaction system. "Journal of the American Chemical Society 127, no. 25 (2005): 9277-9284, which is incorporated herein by reference for all purposes]. This can also be approached experimentally [Binks, B. P., P. D. I. Fletcher, S. N. Kotsev, and R. L. Thompson. "Adsorption and aggregation of semifluorinated alkanes in binary and ternary mixtures with hydrocarbon and fluorocarbon solvents." Langmuir 13, no. 25 (1997): 6669-6682; Ruckenstein, E., and I. Shulgin. "Aggregation in binary solutions containing hexafluorobenzene." The Journal of Physical Chemistry B 103, no. 46 (1999): 10266-10271, which are incorporated herein by reference for all purposes] or spectroscopically, for example, with the use of small angle X-ray scattering [Brady, George W. "Cluster Formation in Perfluoroheptane-iso-Octane Systems near the Consolute Temperature." The Journal of Chemical Physics 32, no. 1 (1960): 45-51, which is incorporated herein by reference for all purposes], or even through surface tension measurements [McLure, I. A., B. Edmonds, and M. Lal. "Extremes in surface tension of fluorocarbon+hydrocarbon mixtures." Nature 241, no. 107 (1973): 71-71, which is incorporated herein by reference for all purposes]. Hydrogen bond donation is important, yet dielectric constant in a non-hydrogen bonding solvent has little to do with this critical parameter [Williams, Thomas D., Michael Jay, Hans-Joachim Lehmler, Michael E. Clark, Dennis J. Stalker, and Paul M. Bummer. "Solubility enhancement of phenol and phenol derivatives in perfluorooctyl bromide." Journal of pharmaceutical sciences 87, no. 12 (1998): 1585-1589, which is incorporated herein by reference for all purposes]. In a highly polar system as in water one can build a model for a dielectric constant based

upon a hydrogen bound network [see for example Suresh, S. J., and V. M. Naik. "Hydrogen bond thermodynamic properties of water from dielectric constant data." The Journal of Chemical Physics 113, no. 21 (2000): 9727-9732, which is incorporated herein by reference for all purposes]. Using a classical approach [Oster, Gerald, and John G. Kirkwood. "The influence of hindered molecular rotation on the dielectric constants of water, alcohols, and other polar liquids." The Journal of Chemical Physics 11, no. 4 (1943): 175-178, which is incorporated herein by reference for all purposes]. Furthermore, the fact that CO_2 does not possess a dipole moment (although it has a quadripole moment) reinforces the lack of utility of the bulk dielectric constant as a metric within the context of the present invention.

[0236] While the processes described in the aforementioned documents are advantageous in some circumstances, there is a limit to the types of materials that can be extracted. Deficiencies are present in the use of steam, alcohols, supercritical or subcritical CO_2, or pure fluorocarbons to extracted valuable material from plant sources.

[0237] Surprisingly it has been found that mixtures of fluorocarbons and hydrofluorocarbons at or very close to the point where the two components are immiscible, although while still remaining partially or approximately miscible, but usually within a range of pressures and temperatures well below the critical temperature and pressure of the individual components, possess dramatically altered and improved solvent properties as compared with the individual components, if the mixtures are of a binary nature, or of binary mixtures of the individual components if the mixtures are of a ternary nature. [For example, see also Shin, Jungin, Moon Sam Shin, Won Bae, Youn-Woo Lee, and Hwayong Kim. "High-pressure phase behavior of carbon dioxide+heptadecafluoro-1-decanol system." The Journal of Supercritical Fluids 44, no. 3 (2008): 260-265, Morgado, Pedro, Jana Black, J. Ben Lewis, Christopher R. Iacovella, Clare McCabe, Luis F G Martins, and Eduardo J M Filipe. "Viscosity of liquid systems involving hydrogenated and fluorinated substances: Liquid mixtures of (hexane+perfluorohexane)." Fluid Phase Equilibria 358 (2013): 161-165, which is incorporated herein by reference for all purposes.]

[0238] It has not previously been recognized that the extrema points of solutions of hydrofluorocarbons in fluorocarbons, and most particularly those hydrofluorocarbons and fluorocarbons under which the desired conditions of pressure and temperature result in a barely miscible system, would possess the unique ability to tunably extract, e.g. to extract in a manner which is tunable the desired hydrophobic organic materials from complex natural product mixtures. Described herein are processes, methods, and compositions related to discovery of extraction of natural products from plant material employing pure fluorocarbon liquids or gases and optionally admixtures of fluorocarbon and non-fluorocarbon gases and liquids. Extraction may be carried out in a highly selective manner such that specific components consisting of pure compounds or defined mixtures thereof may be extracted from said plant or animal material without extracting undesired materials.

Example 1

[0239] An extraction vessel is charged with 10 Kg. of *Cannabis Sativa* "trim". This material is obtained when harvesting cannabis flower, all the non-flower material which does not contain many trichomes is essentially a "waste product" from the production of the flower. It is most commonly the material which is used for cannabinoid extraction. This botanical material is contained in a cloth bag, which is placed within the extraction vessel in order to contain the material from dispersion through the extraction system. The vessel is evacuated. Subsequently, a premixed liquid phase which contains R-22 fluorocarbon (mole fraction 0.4), R-134a fluorocarbon (0.2 mole fraction) dimethyl ether (0.3 mole fraction), ethanol (0.05 mole fraction) and isobutane (0.05 mole fraction) is circulated through the plant material. The pressure is increased to 1.3 Mpa and the temperature to 45° C. and the circulation is continued for a 40-min period. At the end of this time, the liquid phase is pumped in the sealed system to a flash evaporator. The gases are removed and reprocessed through 3A molecular sieves $(2/3K_2O.1/3Na_2O.Al_2O_3.2\ SiO_2.9/2\ H_2O)$ and are compressed using a Corkin compressor into a storage vessel. The product oil from the flash evaporator is collected and assayed for cannabinoid content on an Agilent 1200 series HPLC with diode array detector

[0240] Over a four-hour period while the extraction is carried out, the Freon™ turns bright green due to chlorophyll and other pigments which it contains that have been extracted from it. At the end of the four hour period, the Freon™ is compressed and recovered in a storage tank. It is regenerated by passing through a column of 4A molecular sieves to remove water and terpenes which may be present.

[0241] The extracted material may be separated in the form of multiple components as a function of time during the extraction period. These multiple components contain different chemically distinct fractions, comprised of different approximate mixtures of compounds. Such mixtures may be precisely characterized in terms of composition and quantified in terms of concentration using analytical methodology well known to one normally skilled in the Art, such as Gas Chromatography, High Pressure Liquid Chromatography, Superfluid Critical Liquid Chromatography, Ultrahigh Resolution High Performance Liquid Chromatography, and the like. For the purposes of the present example the total amount of extracted material is quantified.

[0242] Data for Example 1.

Data:

[0243]

Run Number	Oil Recovered	% tetrahydrocannabinol
1	1.72 kg	68
2	2.02	66
3	1.98	82.3
4	2.18	71.5
5	2.26	71.3
6	1.92	64
Mean	2.01	70.5

Example 2

[0244] An extraction vessel is charged with 10 Kg. of *Cannabis Sativa* "trim". This material is obtained when harvesting cannabis flower, all the non-flower material which does not contain many trichomes is essentially a "waste product" from the production of the flower. It is most commonly the material which is used for cannabinoid extraction. This botanical material is contained in a cloth

bag, which is placed within the extraction vessel in order to contain the material from dispersion through the extraction system. The vessel is evacuated. Subsequently, a premixed liquid phase which contains R-22 fluorocarbon (mole fraction 0.6), R-134a fluorocarbon (0.2 mole fraction), and dimethyl ether (0.2 mole fraction), is circulated through the plant material. The pressure is increased to 1.3 Mpa and the temperature to 45° C. and the circulation is continued for a 40-min period. At the end of this time, the liquid phase is pumped in the sealed system to a flash evaporator. The gases are removed and reprocessed through 3A molecular sieves $(2/3K_2O.1/3Na_2O.Al_2O_3.2 SiO_2.9/2 H_2O)$ and are compressed using a Corkin compressor into a storage vessel. The product oil from the flash evaporator is collected and assayed for cannabinoid content on an Agilent 1200 series HPLC with diode array detector

[0245] Over a four-hour period while the extraction is carried out, the Freon™ turns bright green due to chlorophyll and other pigments which it contains that have been extracted from it. At the end of the four hour period, the Freon™ is compressed and recovered in a storage tank. It is regenerated by passing through a column of 4A molecular sieves to remove water and terpenes which may he present.

[0246] The extracted material may be separated in the form of multiple components as a function of time during the extraction period. These multiple components contain different chemically distinct fractions, comprised of different approximate mixtures of compounds. Such mixtures may he precisely characterized in terms of composition and quantified in terms of concentration using analytical methodology well known to one normally skilled in the Art, such as Gas Chromatography, High Pressure Liquid Chromatography, Superfluid Critical Liquid Chromatography, Ultrahigh Resolution High Performance Liquid Chromatography, and the like. For the purposes of the present example the total amount of extracted material is quantified.

Run Number	Oil Recovered	% tetrahydrocannabinol
1	2.2 Kg	68
2	2.4	72
3	2.1	76
4	2.0	62
Mean	2.175 Kg	69.5

Example 3

[0247] An extraction vessel is charged with about 50 Kg. of *Cannabis Sativa* "trim". This material is obtained when harvesting cannabis flower, all the non-flower material which does not contain many trichomes is essentially a "waste product" from the production of the flower. It is most commonly the material which is used for cannabinoid extraction. This botanical material is contained in a cloth bag, which is placed within the extraction vessel in order to contain the material from dispersion through the extraction system. The vessel is evacuated. Subsequently, a premixed liquid phase which contains R-22 fluorocarbon (mole fraction 0.4), R-134a fluorocarbon (0.2 mole fraction) dimethyl ether (0.3 mole fraction), ethanol (0.05 mole fraction) and isobutane (0.05 mole fraction) is circulated through the plant material. The pressure is increased to 1.3 Mpa and the temperature to 45° C. and the circulation is continued for a 40-min period. At the end of this time, the liquid phase is

pumped in the sealed system to a flash evaporator. The gases are removed and reprocessed through 3A molecular sieves $(2/3K_2O.1/3Na_2O.Al_2O_3.2 SiO_2.9/2 H_2O)$ and are compressed using a Corkin compressor into a storage vessel. The product oil from the flash evaporator is collected and assayed for cannabinoid content on an Agilent 1200 series HPLC with diode array detector

[0248] Over a four-hour period while the extraction is carried out, the Freon™ turns bright green due to chlorophyll and other pigments which it contains that have been extracted from it. At the end of the four hour period, the gases is compressed and recovered in a storage tank. It is regenerated hy passing through a column of 3A molecular sieves to remove water and terpenes which may he present.

[0249] The extracted material may be separated in the form of multiple components as a function of time during the extraction period. These multiple components contain different chemically distinct fractions, comprised of different approximate mixtures of compounds. Such mixtures may be precisely characterized in terms of composition and quantified in terms of concentration using analytical methodology well known to one normally skilled in the Art, such as Gas Chromatography, High Pressure Liquid Chromatography, Superfluid Critical Liquid Chromatography, Ultrahigh Resolution High Performance Liquid Chromatography, and the like. For the purposes of the present example the total amount of extracted material is quantified.

[0250] Run 1

[0251] Charge: 48.72 Kg

[0252] Extraction yields 7.82 Kg of oil, 68% by weight total cannabinoids

[0253] Run 2

[0254] Extraction yields 779 Kg of oil, 72% cannabinoids by weight

Example 4

[0255] An extraction vessel is charged with 10 Kg. of *Cannabis Sativa* "flower". This botanical material is contained in a cloth bag, which is placed within the extraction vessel in order to contain the material from dispersion through the extraction system. The vessel is evacuated. Subsequently, a premixed liquid phase which contains R-22 fluorocarbon (mole fractiou 0.4), R-134a fluorocarbon (0.2 mole fraction) dimethyl ether (0.3 mole fraction), ethanol (0.05 mole fraction) and isobutane (0.05 mole fraction) is circulated through the plant material. The pressure is increased to 1.3 Mpa and the temperature to 45° C. and the circulation is continued for a 40-min period. At the end of this time, the liquid phase is pumped in the sealed system to a flash evaporator. The gases are removed and reprocessed through 3A molecular sieves $(2/3K_2O.1/3Na_2O.Al_2O_3.2 SiO_2.9/2 H_2O)$ and are compressed using a Corkin compressor into a storage vessel. The product oil from the flash evaporator is collected and assayed for cannabinoid content on an Agilent 1200 series HPLC with diode array detector

[0256] Over a four-hour period while the extraction is carried out, the Freon™ turns bright green due to chlorophyll and other pigments which it contains that have been extracted from it. At the end of the four hour period, the gases is compressed and recovered in a storage tank. It is regenerated by passing through a column of 3A molecular sieves to remove water and terpenes which may be present.

[0257] The extracted material may be separated in the form of multiple components as a function of time during the

extraction period. These multiple components contain different chemically distinct fractions, comprised of different approximate mixtures of compounds. Such mixtures may be precisely characterized in terms of composition and quantified in terms of concentration using analytical methodology well known to one normally skilled in the Art, such as Gas Chromatography, High Pressure Liquid Chromatography, Superfluid Critical Liquid Chromatography, Ultrahigh Resolution High Performance Liquid Chromatography, and the like. For the purposes of the present example the total amount of extracted material is quantified.

[0258]   Run 1: 2.4 Kg of oil, 78% cannabinoids

[0259]   Run 2: 2.2 Kg of oil, 82% cannabinoids

[0260]   Run3: 2.6 Kg of oil, 79% cannabinoids

Example 5

[0261]   A stainless steel tube about 10" in length and 1.25" in diameter was equipped with sanitary flanges at each end, to which could be affixed a pressure transducer and a sight glass. A sample of amount 500 mg to 5 gm was placed in the tube contained in an inert polypropylene mesh bag. The entire apparatus was placed on a toploading balance, and by means of a flexible hose gases could be added to the vessel. By means of the change in weight, different gases could be added in known ratios. The apparatus, after filling, could then be maintained in a constant temperature hath for any desired period of time, and at the end of this time the apparatus could be opened, the bag removed and the gas volatilized, and the extracted residue dissolved in a suitable solvent (generally acetone) in a quantitative manner. The acetone could then be transferred to a tared roundbottom flask, and solvent removed on a rotary evaporator under vacuum. The amount of the residue in the flask corresponds to the total soluble mass extracted, and this is then quantified by weighing the flask. After determining this weight, the gummy residue could be redissolved in a suitable solvent (generally methanol) in a volumetric flask and aliquots of this material analyzed by HPLC to determine the amounts of cannabinoids (eg. THC, CBD, THCA, and so forth). HPLC analysis is carried out using an Agilent 1100 Series Separation Module, with an Agilent diode array detector, using Agilent Chemstation Software. The column used is a Restek Raptor ARC-18 2.7 μm, 4.6×150 mm column, equipped with a Guard Cartridge (Restek Catalog #9304A0252) or equivalent). Samples are injected and eluted with an isocratic solvent system A/B (25/75). Mobile phase A consists of 0.1% Trifluoroacetic acid (TFA) in H_2O (chromatography quality). Mobile phase B consists of 0.1% TFA in chromatography grade acetonitrile. The Detector Wavelength is 220 nm, Flow Rate is 1.5 mL/min, Injection Volume is 10 and Column Temp is 45.0° C. Under these conditions the Run time is about 9 minutes. Typically, a standards calibration curve of THC of nine concentrations (5-200 ppm) is run daily.

[0262]   Using these conditions, the efficiency of mixtures of fluorocarbon R22 and dimethyl ether (DME) to extract total cannabinoids in a single 30-minute extraction at 26° C. is shown in FIG. 1.

[0263]   Using the same approach, the efficiency of the pure materials to extract cannabinoids in a single 30-minute procedure at 26° C. can be determined and this is shown in FIG. 2.

Embodiments

[0264]   Embodiments contemplated herein include the following.

Embodiment 1

[0265]   A method of extracting a natural organic compound from a natural material, said method comprising contacting said natural material with an extraction fluid thereby extracting said natural organic compound from said natural material into said extraction fluid to from an extracted fluid solution, wherein said extraction fluid comprises a fluorophilic compound and a hydrofluorocarbon.

Embodiment 2

[0266]   The method of embodiment 1, wherein said extraction fluid is a non-ideal fluid.

Embodiment 3

[0267]   The method of embodiments 1 or 2, wherein the natural material is a material derived from a plant, an animal, a fungi, a bacteria or a virus.

Embodiment 4

[0268]   The method of embodiments 1 or 2, wherein the natural material is a material derived from a plant.

Embodiment 5

[0269]   The method of embodiments 1 or 2, wherein the plant is *Piper methysticum, Cannabis* spp., *Salvia* spp., *Banisteriopsis caapi, Psychotria viridis* (chacruna), *Diplopterys cabrerana, Peganum harmala, Humulus lupulus* or mixture thereof.

Embodiment 6

[0270]   The method of embodiments 1 or 2, wherein the plant is *Cannabis Sativa.*

Embodiment 7

[0271]   The method of one of embodiments 1 to 6, wherein the natural organic compound is a biologically active organic compound.

Embodiment 8

[0272]   The method of one of embodiments 1 to 6, wherein the natural organic compound is an aromatic compound.

Embodiment 9

[0273]   The method of one of embodiments 1 to 6, wherein the natural organic compound forms art of an aromatic oil or essential oil.

Embodiment 10

[0274]   The method of one of embodiments 1 to 6, wherein the natural organic compound is caffeine.

Embodiment 11

[0275]   The method of one of embodiments 1 to 6, wherein the natural organic compound is a terpene, a humulone, a

lupulone, a myrcene, a humulene, a caryophyllene, an alkaloid, a flavonoid, a cannibinoid, menthol, capsaicin, anise or camphor.

Embodiment 12

[0276] The method of one of embodiments 1 to 6, wherein the natural organic compound is xanthohumol, 8-prenylnaringenin or isoxanthohumol.

Embodiment 13

[0277] The method of one of embodiments 1 to 6, wherein the natural organic compound is a prenylflavonoid.

Embodiment 14

[0278] The method of one of embodiments 1 to 6, wherein the natural organic compound is a kavalactone or a salvorin.

Embodiment 15

[0279] The method of one of embodiments 1 to 6, wherein the natural organic compound is a cannibinoid.

Embodiment 16

[0280] The method of one of embodiments 1 to 6, wherein the natural organic compound is tetrahydrocannabinol, cannabidiol or cannabinol.

Embodiment 17

[0281] The method of one of embodiments 1 to 6, wherein the natural organic compound is tetrahydrocannabinol.

Embodiment 18

[0282] The method of one of embodiments 1 to 17, wherein at least 5,000 g of said natural organic compound is present in said extracted fluid solution.

Embodiment 19

[0283] The method of one of embodiments 1 to 18, wherein said extraction fluid does not comprise supercritical CO2.

Embodiment 20

[0284] The method of one of embodiments 1 to 18, wherein said extraction fluid does not comprise argon.

Embodiment 21

[0285] The method of one of embodiments 1 to 18, wherein said extraction fluid does not comprise xenon.

Embodiment 22

[0286] The method of one of embodiments 1 to 18, wherein said extraction fluid does not comprise nitrous oxide.

Embodiment 23

[0287] The method of one of embodiments 1 to 22, wherein said extraction fluid further comprises trifluorethanol or hexafluoroisopropanol.

Embodiment 24

[0288] The method of one of embodiments 1 to 23, wherein said extraction fluid is above about 15° C.

Embodiment 25

[0289] The method of one of embodiments 1 to 23, wherein said extraction fluid is above about 20° C.

Embodiment 26

[0290] The method of one of embodiments 1 to 23, wherein said extraction fluid is from about 15° C. to about 35° C.

Embodiment 27

[0291] The method of one of embodiments 1 to 23, wherein said extraction fluid is from about 20° C. to about 30° C.

Embodiment 28

[0292] The method of one of embodiments 1 to 27, wherein the hydrofluorocarbon is a hydrofluoroether, a hydrofluoroketone, a hydrofluoroaromatic or a hydrofluoroolefin.

Embodiment 29

[0293] The method of one of embodiments 1 to 27, wherein the hydrofluorocarbon is chlorodifluoromethane, methyl nonafluoroisobutyl ether, methyl nonafluorobutyl ether, ethyl nonafluoroisobutyl ether, ethyl nonafluorobutyl ether, 3-ethoxy-1,1,1,2,3,4,4,5,5,6,6,6-dodecafluoro-2-trifluoromethylhexane.trifluoromethane (HFC-23), difluoromethane (HFC-32), pentafluoroethane (HFC-125), 1,1,2,2-tetrafluoroethane (HFC-134), 1,1,1,2-tetrafluoroethane (HFC-134a), 1,1,1-trifluoroethane (HFC-143a), 1,1-difluoroethane (HFC-152a) or fluoroethane (HFC-161).

Embodiment 30

[0294] The method of one of embodiments 1 to 29, wherein the fluorophilic compound is dimethyl ether.

Embodiment 31

[0295] The method of one of embodiments 1 to 30, wherein the extraction fluid is a liquid-gas mixture fluid.

Embodiment 32

[0296] The method of one of embodiments 1 to 31, further comprising, prior to said contacting, freezing the natural material at a temperature from about 0° C. to about −60° C.

Embodiment 33

[0297] The method of one of embodiments 1 to 32, wherein the mole fraction of the fluorophilic compound is at least four-fold greater than the mole fraction of the hydrofluorocarbon.

Embodiment 34

[0298] The method of one of embodiments 1 to 33, further comprising separating said extraction fluid from said natural material by volatizing said extraction fluid to form a volatilized extraction fluid.

Embodiment 35

[0299] The method of embodiment 34, further comprising chilling and compressing the volatilized extraction fluid to form a liquid extraction fluid.

Embodiment 36

[0300] The method of embodiments 34 or 35, further comprising recirculating the liquid extraction fluid to the natural material.

Embodiment 37

[0301] The method of one of embodiments 34 to 36, further comprising collecting separated fractions of the liquid extraction fluid.

Embodiment 38

[0302] A fluid comprising chlorodifluoromethane and dimethylether.

Embodiment 39

[0303] The fluid of embodiment 38, wherein said fluid is a non-ideal fluid.

Embodiment P1

[0304] A process for extraction of natural products of medicinal, pharmacological, or other value from plant, animal, fungi, bacteria, or virus mixtures consisting of

[0305] (a) freezing the plant, animal, fungi, bacteria, or virus material to a temperature between 0° C. and −60° C. by the use of a blast freezer or a compressed cryogenic gas

[0306] (b) passing a fluid over the plant, animal, fungi, bacteria, or virus material with the use of a recirculating pump, whereby the fluid consists of three components:

[0307] (i) a fluorophilic compound

[0308] (ii) a hydro fluorocarbon

[0309] (iii) a third component which is a fluorophilic amine, alcohol, or nonfluorinated alkanol

[0310] (iv) wherein the mole fraction of the fluorocarbon is at least four-fold greater than the mole fraction of the hydrofluorocarbon, and the mole fraction of the third component is four-fold less than the hydrofluorocarbon

[0311] (c) volatilizing the fluorophilic compound which has been passed over the plant, animal, fungi, bacteria, or virus material using a heated column, whereby the extracted plant, animal, fungi, bacteria, or virus material solubilized by the fluid remains at the bottom of the said column and the fluorophilic compound is extracted in a gaseous form at one end of said column

[0312] (d) chilling the volatilized fluorophilic compound with a heat exchanger and compressing the fluorophilic compound to the liquid state

[0313] (e) warming the liquefied fluorophilic compound in a controlled manner

[0314] (f) recirculating the liquefied warm fluorophilic compound back through the plant, animal, fungi, bacteria, or virus material in a continuous manner

[0315] (g) slowly increasing the temperature of the liquefied recirculated fluorophilic compound which flows back over the plant, animal, fungi, bacteria, or virus material, thereby increasing the temperature of

the said plant, animal, fungi, bacteria, or virus material from the temperature range of freezing to a temperature range between 40° C. and 80° C.

[0316] (h) collecting separated fractions at the bottom of the volatilizing column as the temperature is thereby increased.

Embodiment P2

[0317] A process according to embodiment P1, wherein said fluorocarbon is 1,1,1,2 tetrafluoroethane.

Embodiment P3

[0318] A process according to embodiment P1, wherein said fluorocarbon is tetrafluoromethane.

Embodiment P4

[0319] A process according to embodiment P1, wherein said fluorocarbon is hexafluoroethane.

Embodiment P5

[0320] A process according to embodiment P1, wherein said fluorocarbon is trifluoromethyl iodide.

Embodiment P6

[0321] A process according to embodiment P1, wherein said fluorocarbon is perfluorocyclobutane.

Embodiment P7

[0322] A process according to embodiment P1, wherein said fluorocarbon is perfluorotributylamine.

Embodiment P8

[0323] A process according to embodiment P1, wherein said fluorocarbon is perfluoro-n-propane.

Embodiment P9

[0324] A process for extraction of natural products of medicinal, pharmacological, or other value from plant, animal, fungi, bacteria, or virus mixtures consisting of

[0325] (a) freezing the plant, animal, fungi, bacteria, or virus material to a temperature between 0° C. and −60° C. by the use of a blast freezer or a compressed cryogenic gas

[0326] (b) passing a fluid over the plant, animal, fungi, bacteria, or virus material with the use of a recirculating pump, whereby the fluid consists of three components:

[0327] (v) a fluorophilic compound

[0328] (vi) a hydrofluorocarbon

[0329] (vii) a third component which is an inert gas

[0330] (c) volatilizing the fluorophilic compound which has been passed over the plant, animal, fungi, bacteria, or virus material using a heated column, whereby the extracted plant, animal, fungi, bacteria, or virus material solubilized by the fluid remains at the bottom of the said column and the fluorophilic compound is extracted in a gaseous form at one end of said column

[0331] (d) chilling the volatilized fluorophilic compound with a heat exchanger and compressing the fluorophilic compound to the liquid state

[0332] (e) warming the liquefied fluorophilic compound in a controlled manner

[0333] (f) recirculating the liquefied warm fluorophilic compound back through the plant, animal, fungi, bacteria, or virus material in a continuous manner

[0334] (g) slowly increasing the temperature of the liquefied recirculated fluorophilic compound which flows back over the plant, animal, fungi, bacteria, or virus material, thereby increasing the temperature of the said plant, animal, fungi, bacteria, or virus material from the temperature range of freezing to a temperature range between 40° C. and 80° C.

[0335] (h) collecting separated fractions at the bottom of the volatilizing column as the temperature is thereby increased.

Embodiment P10

[0336] A process according to embodiment P9, wherein said fluorocarbon is 1,1,1,2 tetrafluoroethane and the inert gas is SF_6.

Embodiment P11

[0337] A process according to embodiment P9, wherein said fluorocarbon is 1,1,1,2 tetrafluoroethane and the inert gas is CO_2.

Embodiment P12

[0338] A process according to embodiment P9, wherein said fluorocarbon is 1,1,1,2 tetrafluoroethane and the inert gas is N_2O.

Embodiment P13

[0339] A process according to embodiment P9, wherein said fluorocarbon is 1,1,1,2 tetrafluoroethane and the inert gas is CH_4.

Embodiment P14

[0340] A process according to embodiment P9, wherein said fluorocarbon is 1,1,1,2 tetrafluoroethane and the inert gas is C_2H_6.

Embodiment P15

[0341] A process according to embodiment P9, wherein said fluorocarbon is tetrafluoromethane and the inert gas is SF_6.

Embodiment P16

[0342] A process according to embodiment P9, wherein said fluorocarbon is tetrafluoromethane and the inert gas is CO_2.

Embodiment P17

[0343] A process according to embodiment P9, wherein said fluorocarbon is tetrafluoromethane and the inert gas is N_2O.

Embodiment P18

[0344] A process according to embodiment P9, wherein said fluorocarbon is tetrafluoromethane and the inert gas is CH_4.

Embodiment P19

[0345] A process according to embodiment P9, wherein said fluorocarbon is tetrafluoromethane and the inert gas is C_2H_6.

Embodiment P20

[0346] A process according to embodiment P9, wherein said fluorocarbon is perfluorocyclobutane and the inert gas is SF_6.

Embodiment P21

[0347] A process according to embodiment P9, wherein said fluorocarbon is perfluorocyclobutane and the inert gas is CO_2.

Embodiment P22

[0348] A process according to embodiment P9, wherein said fluorocarbon is perfluorocyclobutane and the inert gas is N_2O.

Embodiment P23

[0349] A process according to embodiment P9, wherein said fluorocarbon is perfluorocyclobutane and the inert gas is CH_4.

Embodiment P24

[0350] A process according to embodiment P9, wherein said fluorocarbon is perfluorocyclobutane and the inert gas is C_2H_6.

Embodiment P25

[0351] A process for extraction of natural products of medicinal, pharmacological, or other value from plant, animal, fungi, bacteria, or virus mixtures consisting of

[0352] (a) freezing the plant, animal, fungi, bacteria, or virus material to a temperature between 0° C. and −60° C. by the use of a blast freezer or a compressed cryogenic gas

[0353] (b) passing a fluid over the plant, animal, fungi, bacteria, or virus material with the use of a recirculating pump, whereby the fluid consists of three components:

[0354] (i) a fluorophilic compound

[0355] (ii) a hydrofluorocarbon

[0356] (iii) an ionic liquid

[0357] (c) volatilizing the fluorophilic compound which has been passed over the plant, animal, fungi, bacteria, or virus material using a heated column, whereby the extracted plant, animal, fungi, bacteria, or virus material solubilized by the fluid remains at the bottom of the said column and the fluorophilic compound is extracted in a gaseous form at the top of said column

[0358] (d) chilling the volatilized fluorophilic compound with a heat exchanger and compressing the fluorophilic compound to the liquid state

[0359] (e) warming the liquefied fluorophilic compound in a controlled manner

[0360] (f) recirculating the liquefied warm fluorophilic compound back through the plant, animal, fungi, bacteria, or virus material in a continuous manner

[0361] (g) slowly increasing the temperature of the liquefied recirculated fluorophilic compound which

flows back over the plant, animal, fungi, bacteria, or virus material, thereby increasing the temperature of the said plant, animal, fungi, bacteria, or virus material from the temperature range of freezing to a temperature range between 40° C. and 80° C.

[0362]　(h) collecting separated fractions at the bottom of the volatilizing column as the temperature is thereby increased.

What is claimed is:

1. A method of extracting a natural organic compound from a natural material, said method comprising contacting said natural material with an extraction fluid thereby extracting said natural organic compound from said natural material into said extraction fluid to form an extracted fluid solution, wherein said extraction fluid comprises a fluorophilic compound and a hydrofluorocarbon.

2. The method of claim 1, wherein the natural material is a material derived from a plant, an animal, a fungi, a bacteria or a virus.

3. The method of claim 2, wherein the plant is *Piper methysticum, Cannabis* spp., *Salvia* spp., *Banisteriopsis caapi, Psychotria viridis* (chacruna), *Diplopterys cabrerana, Peganum harmala, Humulus lupulus* or mixture thereof.

4. The method of claim 2, wherein the plant is *Cannabis Sativa.*

5. The method of one of claims 1, wherein the natural organic compound is a biologically active organic compound, an aromatic compound, or forms part of an aromatic oil or essential oil.

6. The method of one of claims 1, wherein the natural organic compound is caffeine, a terpene, a humulone, a lupulone, a myrcene, a humulene, a caryophyllene, an alkaloid, a flavonoid, a cannabinoid, menthol, capsaicin, anise, camphor, xanthohumol, 8-prenylnaringenin, isoxanthohumol, a prenylflavonoid, a kavalactone, a salvorin, a cannabinoid, tetrahydrocannabinol, cannabidiol, or cannabinol.

7. The method of one of claims 1, wherein at least 5000 g of said natural organic compound is present in said extracted fluid solution.

8. The method of one of claims 1, wherein said extraction fluid does not comprise supercritical CO_2.

9. The method of one of claims 1, wherein said extraction fluid does not comprise argon, xenon, or nitrous oxide.

10. The method of one of claims 1, wherein said extraction fluid further comprises trifluorethanol or hexafluoroisopropanol.

11. The method of one of claims 1, wherein said extraction fluid is above about 15° C.

12. The method of one of claims 1, wherein the hydrofluorocarbon is a hydrofluoroether, a hydrofluoroketone, a hydrofluoroaromatic or a hydrofluoroolefin.

13. The method of one of claims 1, wherein the hydrofluorocarbon is chlorodifluoromethane, methyl nonafluoroisobutyl ether, methyl nonafluorobutyl ether, ethyl nonafluoroisobutyl ether, ethyl nonafluorobutyl ether, 3-ethoxy-1, 1,1,2,3,4,4,5,5,6,6,6-dodecafluoro-2-trifluoromethylhexane, trifluoromethane (HFC-23), difluoromethane (HFC-32), pentafluoroethane (HFC-125), 1,1,2,2-tetrafluoroethane (HFC-134), 1,1,1,2-tetrafluoroethane (HFC-134a), 1,1,1-trifluoroethane (HFC-143a), 1,1-difluoroethane (HFC-152a), (1,1,1,3,3,3-hexafluoro-2-(fluoromethoxy)propane, 1,2,2,2-tetrafluoroethyl difluoromethyl ether, 2-chloro-1,1,2,-trifluoroethyl difluoromethyl ether, 1-chloro-2,2,2-trifluoroethyl difluoromethyl ether, 2,2-dichloro-1,1-difluoromethyl ether, or fluoroethane (HFC-161).

14. The method of one of claims 1, wherein the fluorophilic compound is dimethyl ether, methyl ethyl ether, methyl n-propyl ether, methyl isopropyl ether, methyl-n-butyl ether, diethyl ether, methyl tert-butyl ether, or ethyl tert-butyl ether.

15. The method of one of claims 1, further comprising, prior to said contacting, freezing the natural material at a temperature from about 0° C. to about −60° C.

16. The method of one of claims 1, wherein the mole fraction of the fluorophilic compound is at least four-fold greater than the mole fraction of the hydrofluorocarbon.

17. The method of one of claims 1, further comprising separating said extraction fluid from said natural material by volatizing said extraction fluid to form a volatilized extraction fluid.

18. The method of claim 17, further comprising chilling and compressing the volatilized extraction fluid to form a liquid extraction fluid.

19. The method of claim 17, further comprising recirculating the liquid extraction fluid to the natural material.

20. A fluid comprising chlorodifluoromethane and dimethylether.

*　*　*　*　*

EXHIBIT B – PART 3

Begins on the following page

US 20180179564A1

(54) **APPARATUS AND METHODS FOR BIOSYNTHETIC PRODUCTION OF CANNABINOIDS**

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(73) Assignee: **Teewinot Technologies Limited,** Dublin (IE)

(21) Appl. No.: **15/881,197**

(22) Filed: **Jan. 26, 2018**

Related U.S. Application Data

(63) Continuation of application No. 15/433,270, filed on Feb. 15, 2017, now Pat. No. 9,879,292, which is a continuation of application No. 15/232,405, filed on Aug. 9, 2016, now Pat. No. 9,587,212, which is a continuation of application No. 15/158,565, filed on May 18, 2016, now Pat. No. 9,512,391, which is a continuation of application No. 14/835,444, filed on Aug. 25, 2015, now Pat. No. 9,394,510.

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Publication Classification

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\textit{C12M 1/36} & (2006.01) \\
\textit{C12P 7/22} & (2006.01) \\
\textit{C12P 7/42} & (2006.01) \\
\textit{C12N 9/02} & (2006.01) \\
\textit{C07C 37/74} & (2006.01) \\
\textit{C07C 37/01} & (2006.01) \\
\textit{C07C 29/80} & (2006.01) \\
\textit{C12M 1/00} & (2006.01) \\
\textit{C12M 1/40} & (2006.01)
\end{array}
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(52) **U.S. Cl.**
CPC *C12P 17/06* (2013.01); *C12M 41/32* (2013.01); *C12M 47/10* (2013.01); *C12M 41/40* (2013.01); *C12M 41/30* (2013.01); *C12M 41/48* (2013.01); *C12P 7/22* (2013.01); *C12P 7/42* (2013.01); *C12Y 121/03* (2013.01); *C12N 9/0004* (2013.01); *C12Y 121/03008* (2015.07); *C07C 37/74* (2013.01); *C07C 37/01* (2013.01); *C07C 29/80* (2013.01); *C12M 29/04* (2013.01); *C12M 21/18* (2013.01); *C12M 41/26* (2013.01); *C12M 41/12* (2013.01); *C12M 29/00* (2013.01); *C12Y 121/03007* (2015.07)

(57) **ABSTRACT**

The present invention provides an apparatus and methods for producing tetrahydrocannabinolic acid (THCA), cannabichromenic acid (CBCA) and cannabichromenic acid (CBCA) in different ratios. The apparatus comprises: (i) a bioreactor comprising (a) an automated supply system configured to deliver a first automated supply of cannabigerolic acid (CBGA), a cannabinoid acid synthase, and a reaction mixture; and (b) a second automated system to cease the reaction; (ii) a controller configured to modify a property of the reaction mixture to produce the desired products; and (iii) an extractor configured to recover the tetrahydrocannabinolic acid (THCA), cannabichromenic acid (CBCA) or cannabidiolic acid (CBDA) and cannabichromenic acid.

FIGURE 1
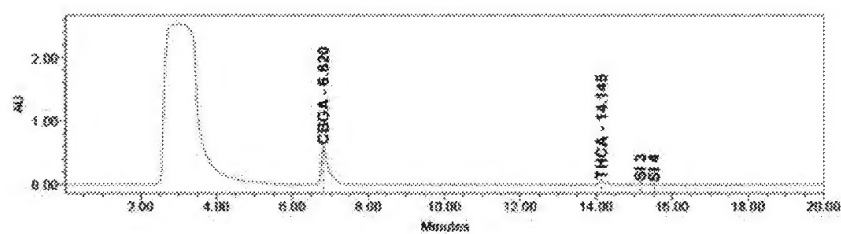
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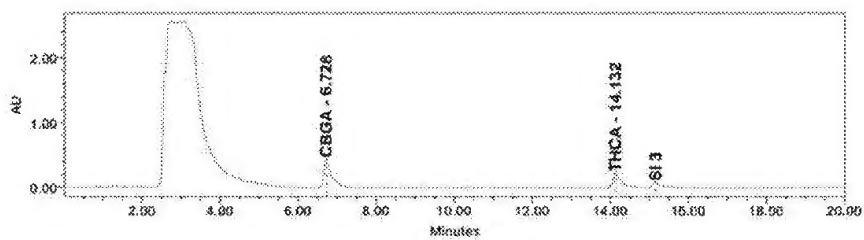
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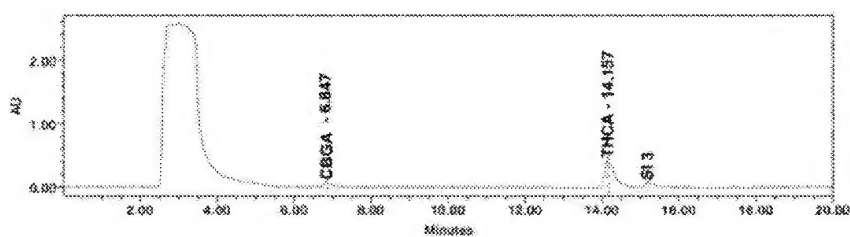
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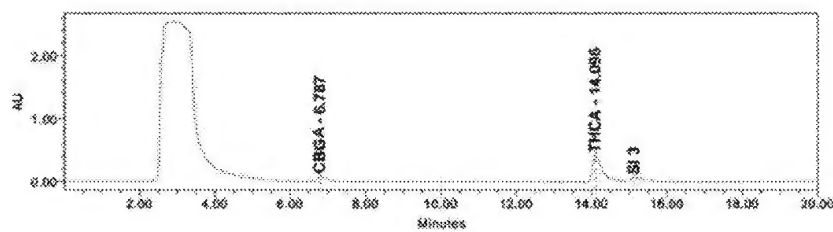
FIGURE 5

FIGURE 6

FIGURE 7

100

FIGURE 8

FIGURE 9

Provide starting materials — 901

Distribute starting materials — 902

Cause reaction — 903

Adjust pH? — 904

Generate products — 905

Add hexane — 906

Remove hexane — 907

Recover products — 908
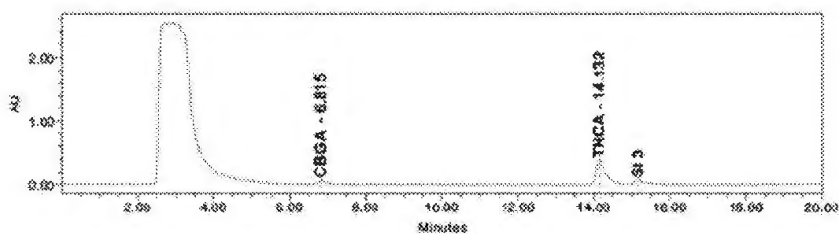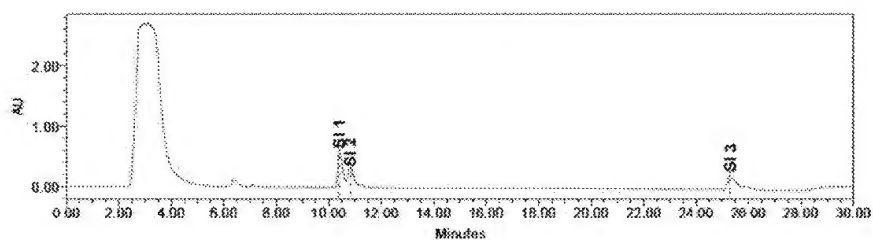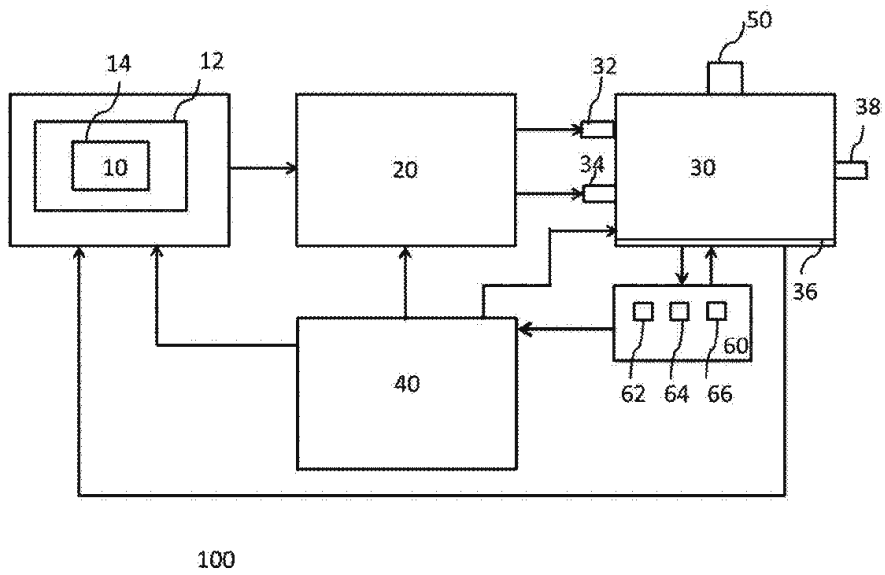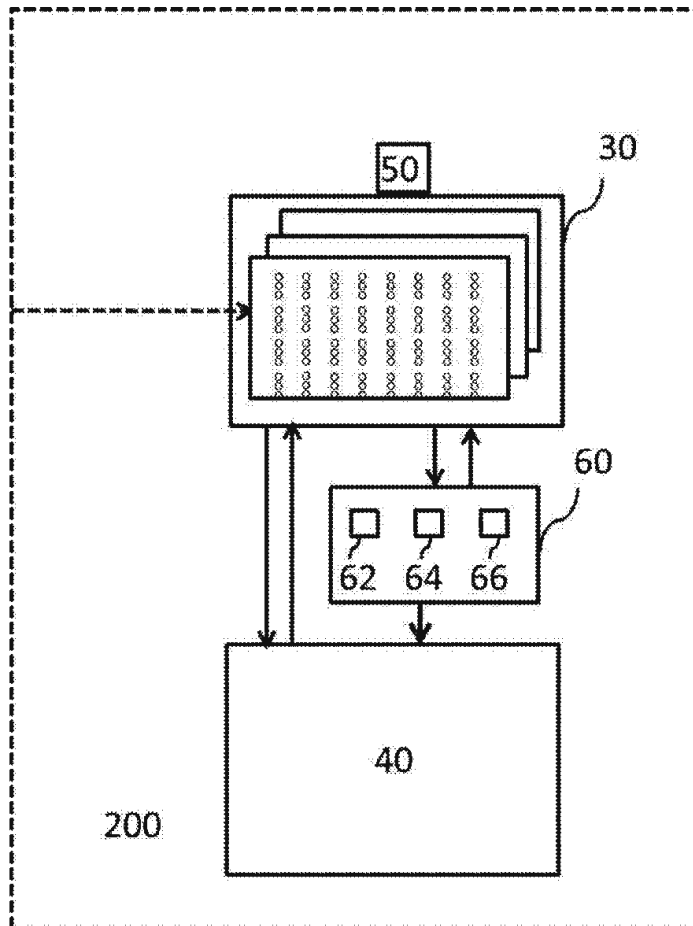
Re-suspend products — 909
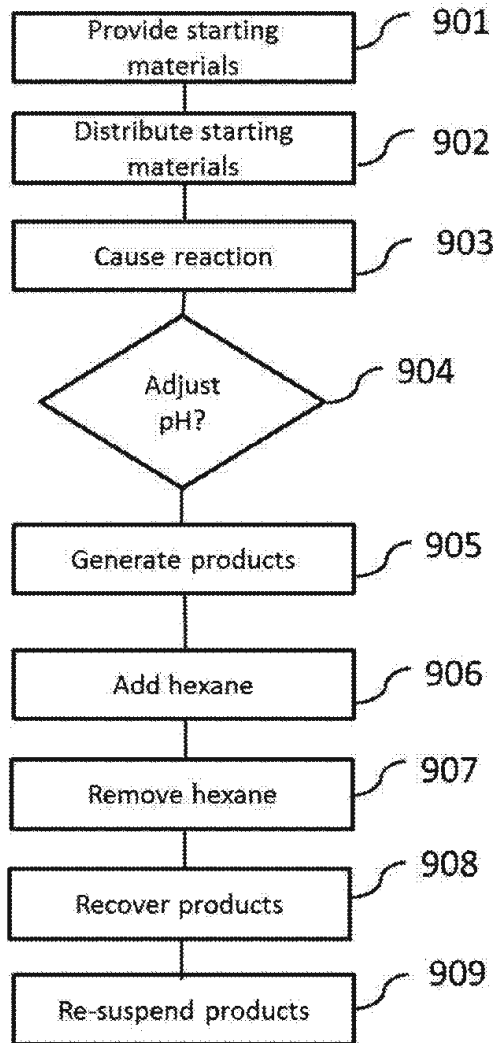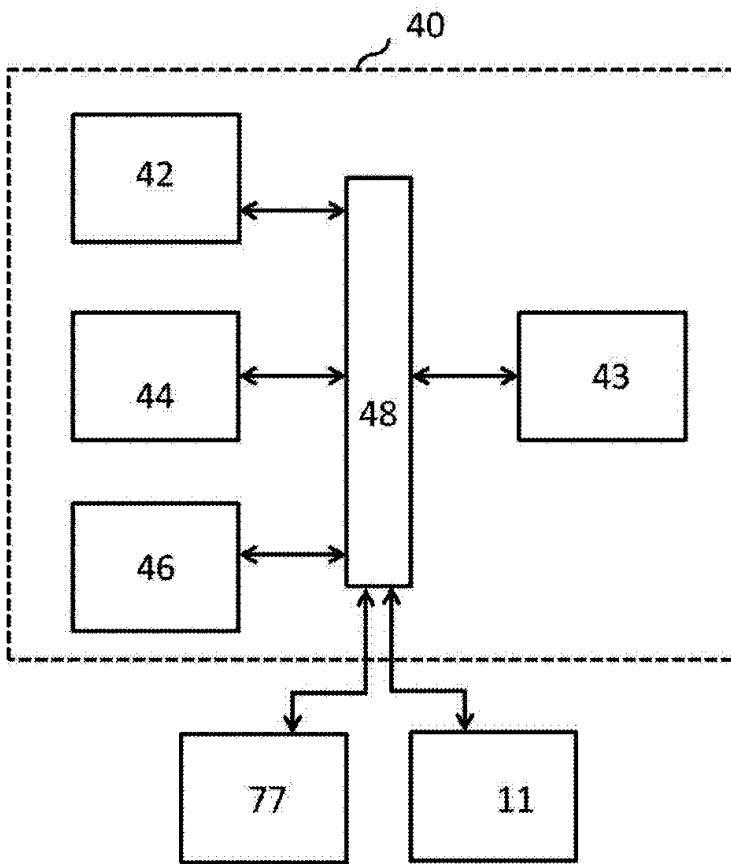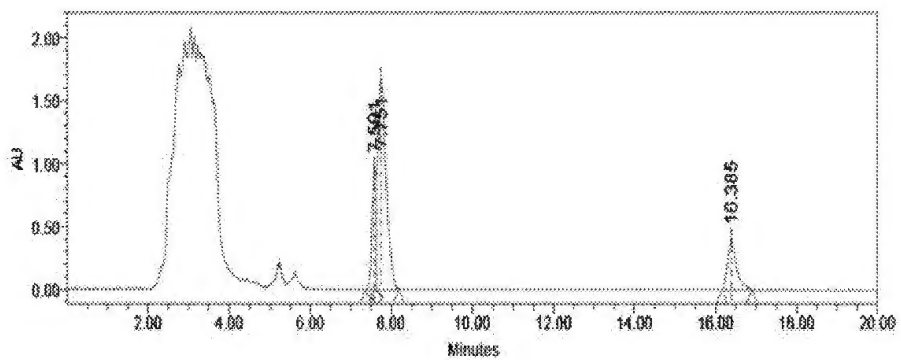
FIGURE 10

FIGURE 11

FIGURE 12
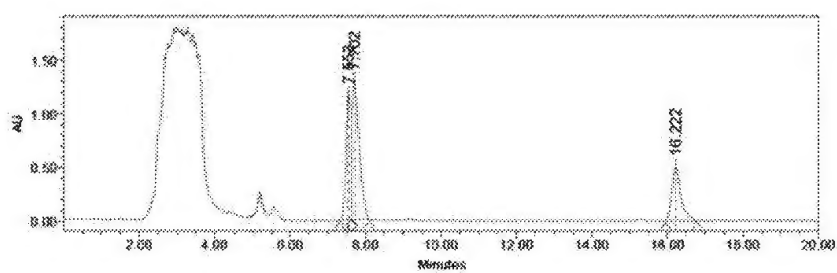
FIGURE 13

FIGURE 14

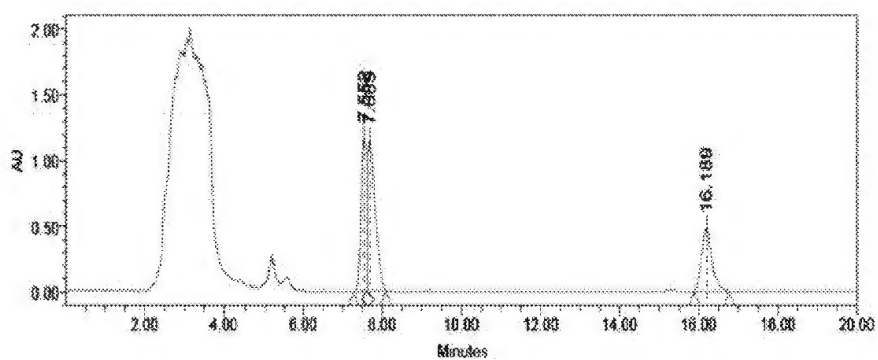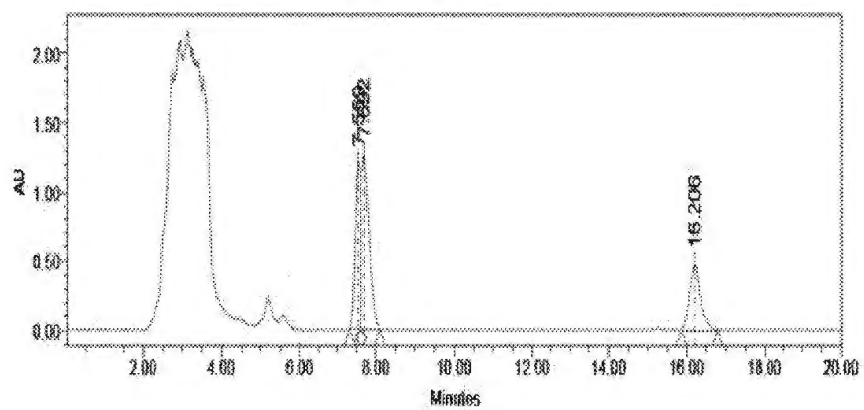## FIGURE 15
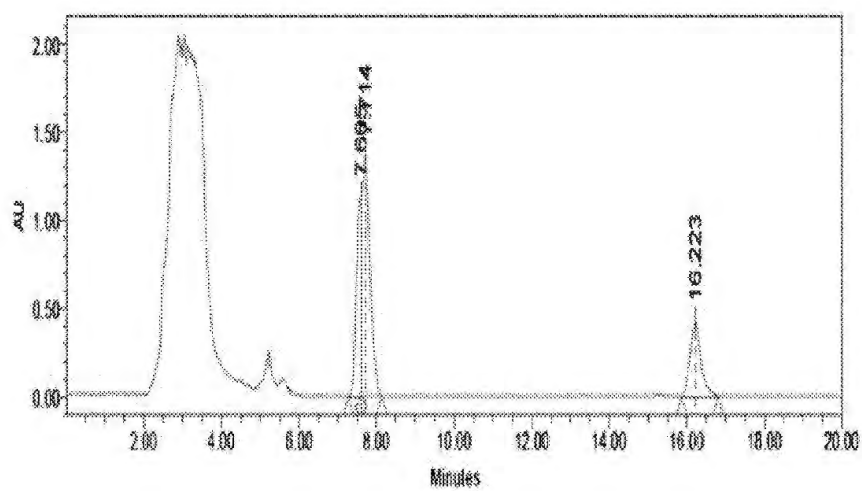
FIGURE 16
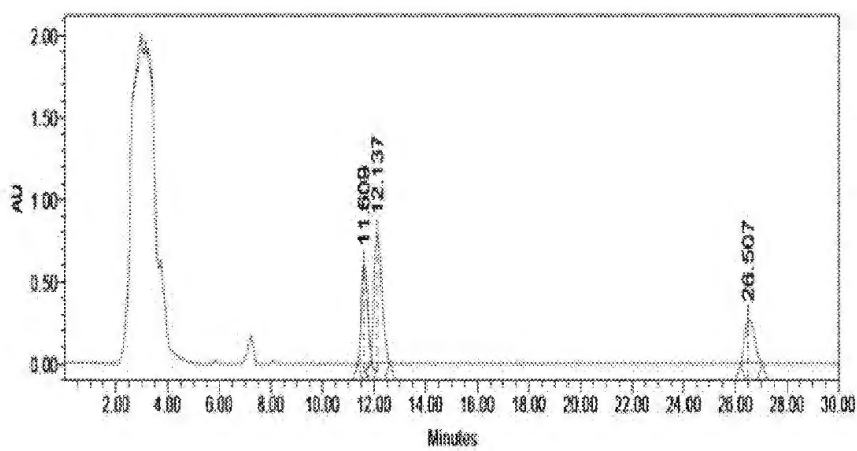
FIGURE 17
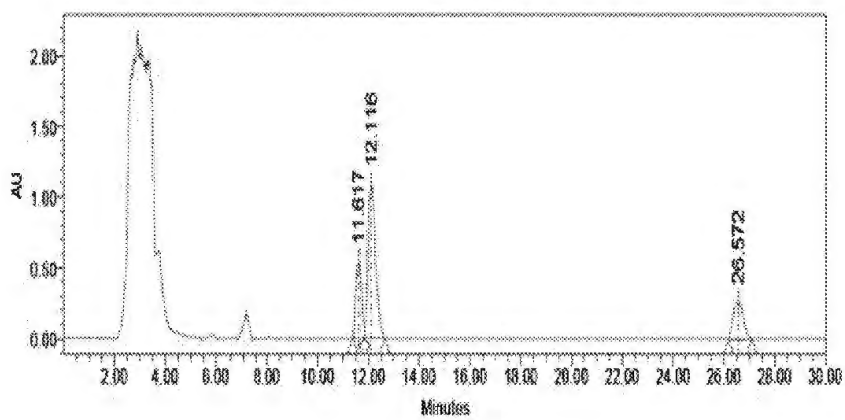
APPARATUS AND METHODS FOR BIOSYNTHETIC PRODUCTION OF CANNABINOIDS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a Continuation of U.S. application Ser. No. 15/433,270 filed Feb. 15, 2017, incorporated herein by reference in its entirety, which is a Continuation of U.S. application Ser. No. 15/232,405 filed Aug. 9, 2016, now U.S. Pat. No. 9,587,212 issued Mar. 7, 2017, incorporated herein by reference in its entirety, which is a Continuation of U.S. application Ser. No. 15/158,565, filed May 18, 2016, now U.S. Pat. No. 9,512,391 issued Dec. 6, 2016, incorporated herein by reference in its entirety, which is a Continuation of U.S. application Ser. No. 14/835,444, filed Aug. 25, 2015, now U.S. Pat. No. 9,394,510 issued Jul. 19, 2016, incorporated herein by reference in its entirety, which claims priority from Provisional U.S. Application 62/041,521, filed Aug. 25, 2014, incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to the biosynthesis of cannabinoids. Specifically, the present invention relates to the production and manipulation of enzymes involved in the synthesis of cannabinoids, and to the simultaneous synthesis of various cannabinoids in different ratios.

BACKGROUND OF THE INVENTION

[0003] Cannabinoids are terpenophenolic compounds found in *Cannabis sativa*, an annual plant belonging to the Cannabaceae family. The plant contains more than 400 chemicals and approximately 70 cannabinoids. The latter accumulate mainly in the glandular trichomes. The most active of the naturally occurring cannabinoids is tetrahydrocannabinol (THC), which is used for treating a wide range of medical conditions, including glaucoma, AIDS wasting, neuropathic pain, treatment of spasticity associated with multiple sclerosis, fibromyalgia and chemotherapy-induced nausea. THC is also effective in the treatment of allergies, inflammation, infection, epilepsy, depression, migraine, bipolar disorders, anxiety disorder, drug dependency and drug withdrawal syndromes.

[0004] Additional active cannabinoids include cannabidiol (CBD), an isomer of THC, which is a potent antioxidant and anti-inflammatory compound known to provide protection against acute and chronic neuro-degeneration; cannabigerol (CBG), found in high concentrations in hemp, which acts as a high affinity α_2-adrenergic receptor agonist, moderate affinity 5-HT_{1A} receptor antagonist and low affinity CB1 receptor antagonist, and possibly has anti-depressant activity; and cannabichromene (CBC), which possesses anti-inflammatory, anti-fungal and anti-viral properties. Many phytocannabinoids have therapeutic potential in a variety of diseases and may play a relevant role in plant defense as well as in pharmacology. Accordingly, biotechnological production of cannabinoids and cannabinoid-like compounds with therapeutic properties is of uttermost importance. Thus, cannabinoids are considered to be promising agents for their beneficial effects in the treatment of various diseases.

[0005] Despite their known beneficial effects, therapeutic use of cannabinoids is hampered by the high costs associated with the growing and maintenance of the plants in large scale and the difficulty in obtaining high yields of cannabinoids. Extraction, isolation and purification of cannabinoids from plant tissue is particularly challenging as cannabinoids oxidize easily and are sensitive to light and heat. In addition, although it has been hypothesized that CBCA is predominantly synthesized from CBGA by the enzyme CBCA synthase, the enzyme has not been isolated or cloned. There is therefore a need for developing methodologies that allow large-scale production of cannabinoids for therapeutic use. The present invention addresses this need.

SUMMARY OF THE INVENTION

[0006] It is therefore an object of the invention to provide solutions to the aforementioned deficiencies in the art. To this end the invention provides a method of producing one or more cannabinoids or cannabinoid analogs comprising the steps of: (a) selecting a compound according to Formula I:
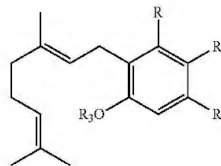
Formula I

(b) selecting a cannabinoid acid synthase as a catalyst for transforming the compound according to Formula I into one or more cannabinoids or cannabinoid analogs; (c) reacting the compound of Formula I with the cannabinoid acid synthase in a reaction mixture comprising a solvent and an amphiphilic compound; (d) isolating from the reaction mixture one or more cannabinoid acids or cannabinoid analogs produced in step (c); and (e) optionally decarboxylating the cannabinoid acids or cannabinoid analogs isolated in step (c); wherein R is selected from —OH, halogen, —SH, or a —NR_aR_b group; R_1 and R_2 are each independently selected from the group consisting of —H, —$C(O)R_a$, —OR_a, an optionally substituted C_1-C_{10} linear or branched alkylene, an optionally substituted C_2-C_{10} linear or branched alkenylene, an optionally substituted C_2-C_{10} linear or branched alkynylene, an optionally substituted C_3-C_{10} aryl, an optionally substituted C_3-C_{10} cycloalkyl, (C_3-C_{10})aryl-(C_1-C_{10})alkylene, (C_3-C_{10})aryl-(C_2-C_{10})alkenylene, and (C_3-C_{10})aryl-(C_1-C_{10})alkynylene, or R_1 and R_2 together with the carbon atoms to which they are bonded form a C_5-C_{10} cyclic ring; R_3 is selected from the group consisting of H, —$C(O)R_a$ and C_1-C_{10} linear or branched alkyl; and R_a and R_b are each independently —H, —OH, —SH, —NH_2, (C_1-C_{10}) linear or branched alkyl, or a C_3-C_{10} cycloalkyl.

[0007] Preferably, the cannabinoid acid synthase is cannabidiolic acid (CBDA) synthase or tetrahydrocannabinolic acid (THCA) synthase. In a preferred aspect of the invention, the C_5-C_{10} cyclic ring comprises one or more heteroatoms selected from oxygen, sulfur or nitrogen. In another preferred aspect of the invention, R_2 is a linear alkylene selected from the group consisting of CH_3, C_2H_5, C_3H_7,

2

C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15} and C_8H_{17}. Preferably, R_2 is a C_2-C_{10} alkenylene selected from the group consisting of
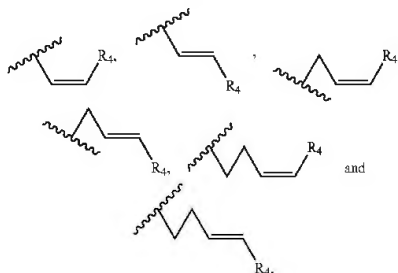
and R_4 is a linear alkylene selected from the group consisting of CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15} and C_8H_{17}. In another preferred aspect, R_2 is a C_2-C_{10} linear or branched alkynylene selected from the group consisting of
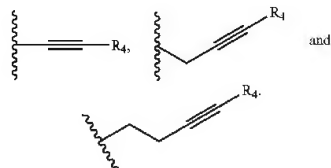
[0008]   In an additional preferred embodiment, R_2 is
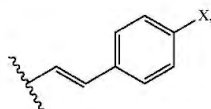
wherein X is —OH, —SH, or —NR_aR_b, and wherein R_a and R_b are each independently —H, —OH, —SH, —NH_2, (C_1-C_{10}) linear or branched alkyl, or a C_3-C_{10} cycloalkyl. Most preferably, R is —OH, R_1 is —COOH, R_2 is C_5H_{11} and R_3 is —H.

[0009]   In one embodiment, the cannabinoid acid synthase is a recombinant cannabinoid acid synthase obtained by generating one or more copies of a cannabinoid acid synthase gene and overexpressing a protein encoded by the cannabinoid acid synthase gene. In a preferred aspect of the invention, one or more copies of a cannabinoid acid synthase gene are generated in vivo and the method comprises step (i) of integrating one or more copies of the cannabinoid acid synthase gene into the genome of an eukaryotic host to scale up protein expression. Preferably, the eukaryotic host is *Pichia pastoris* and the cannabinoid acid synthase gene is codon optimized with an alpha secretion sequence to target protein secretion and tagged with six tandem histidine residues (SEQ ID NO: 9). Step (i) may comprise linearizing the cannabinoid acid synthase gene by digestion with one or more restriction enzymes; extracting the cannabinoid acid

synthase gene by gel extraction; ligating the cannabinoid acid synthase gene into a *Pichia pastoris* plasmid; and electroporating the plasmid into bacterial cells to generate one or more cannabinoid acid synthase gene copy colonies.

[0010]   In a preferred aspect of the invention, the solvent is DMSO, and the concentration of DMSO in the reaction mixture is 20% (w/v). In an additional preferred aspect, the amphiphilic compound is a surfactant or a cyclodextrin. In a preferred embodiment, the cyclodextrin is α-cyclodextrin, β-cyclodextrin or γ-cyclodextrin. Even more preferably, the cyclodextrin is sulfobuthylether β-cyclodextrin sodium salt or randomly methylated β-cyclodextrin, and the concentration of cyclodextrin in the reaction mixture is between 2 and 28 mg/ml. In a most preferred embodiment, the concentration of cyclodextrin in the reaction mixture is 8 mg/ml.

[0011]   In one embodiment, the cannabinoids or cannabinoid analogs are single enantiomers with an enantiomeric purity of at least 95%, and preferably of at least 99%.

[0012]   In a preferred embodiment, the cannabinoid acid synthase is THCA synthase and the one or more cannabinoids or cannabinoid analogs are tetrahydrocannabinol (THCA), cannabichromene (CBCA), THCA and CBCA, or analogs thereof. In a preferred aspect, the amphiphilic compound is cyclodextrin and the mass:mass ratio of cyclodextrin to the compound of Formula I is 28:1 or the molar ratio of cyclodextrin to the compound of Formula I is 7.3:1. Preferably, step (c) of the reaction is performed at a pH in a range between 3.8 and 7.2, and the method produces THCA, CBCA, or THCA and CBCA in a ratio as shown in the following table at each specified pH:

pH	THCA	CBCA
4	1	0
5	2.33	1
6	1	5.67
7	0	1
8	0	0

[0013]   Preferably, 98% of the compound of Formula I is converted into one or more cannabinoids or cannabinoid analogs within two hours.

[0014]   In a different embodiment, the cannabinoid acid synthase is CBDA synthase and the method produces cannabidiol (CBDA), cannabichromene acid (CBCA), CBDA and CBCA, or analogs thereof. Preferably, the amphiphilic compound is cyclodextrin and the mass:mass ratio of cyclodextrin to the compound of Formula I is 11:1 or the molar ratio of cyclodextrin to the compound of Formula I is 4:1. Preferably, step (c) is performed at a pH in a range between 3.8 and 7.2. In a preferred embodiment, the method produces CBDA, CBCA, or CBDA and CBCA in a ratio as shown in the following table at each specified pH:

pH	CBDA	CBCA
4.2	2.5	1
5	1.13	1
5.2	1	1.17
5.4	1	2.45
5.8	1	6.14
6.2	1	28.13
6.8	0	0

[0015] Most preferably, 98% of the compound of Formula I is converted into one or more cannabinoids or cannabinoid analogs within two hours.

[0016] In a different embodiment, the invention provides a method of producing one or more cannabinoids or cannabinoid analogs according to Formula II

wherein the method comprises the steps of: (a) reacting a compound according to Formula III with a compound according to Formula IV;

III

IV
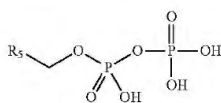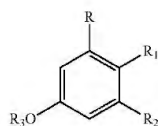
in the presence of an enzyme that catalyzes the reaction of the Formula III and Formula IV compounds to form a Formula II compound; (b) reacting the Formula II compound with a cannabinoid acid synthase in a reaction mixture comprising a solvent and an amphiphilic compound to produce one or more cannabinoids or cannabinoid analogs; (c) isolating from the reaction mixture one or more cannabinoids or cannabinoid analogs produced in step (b); and (e) optionally decarboxylating the one or more cannabinoids or cannabinoid analogs isolated in step (c); wherein R is selected from —OH, halogen, —SH, or a —NR_aR_b group; R_1 and R_2 are each independently selected from the group consisting of —H, —$C(O)R_a$, —OR_a, an optionally substituted linear or branched (C_1-C_{10})alkylene, an optionally substituted linear or branched (C_2-C_{10})alkenylene, an optionally substituted linear or branched (C_2-C_{10})alkynylene, an optionally substituted C_3-C_{10} aryl, an optionally substituted C_3-C_{10} cycloalkyl, (C_3-C_{10})aryl-(C_1-C_{10})alkylene, (C_3-C_{10})aryl-(C_2-C_{10})alkenylene, and (C_3-C_{10})aryl-(C_1-C_{10})alkynylene, or R_1 and R_2 together with the carbon atoms to which they are bonded form a C_5-C_{10} cyclic ring; R_3 is selected from the group consisting of H, —$C(O)R_a$ and C_1-C_{10} linear or branched alkyl; R_5 is selected from the group consisting of a linear or branched (C_1-C_{10})alkylene, a linear or branched (C_2-C_{10})alkenylene, a linear or branched (C_2-C_{10})alkynylene, —C(O)—(C_1-C_{10})alkylene, —C(O)—(C_2-C_{10})alkenylene and —C(O)—(C_2-C_{10})alkynylene; wherein any alkylene, alkenylene, alkynylene, aryl, arylalkylene, or cycloalkyl group is further substituted with one or more groups selected from the group consisting of —OH, halogen, —NR_bR_c, —$C(O)R_a$, —$C(O)NR_bR_c$, (C_1-C_{10})

alkyl, —CN, (C_1-C_4)alkoxy, (C_1-C_4)haloalkyl, and (C_1-C_4)hydroxyalkyl; and R_a, R_b and R_c are each independently —H, —OH, —SH, —NH_2, (C_1-C_{10}) linear or branched alkyl, or a C_3-C_{10} cycloalkyl.

[0017] In one embodiment, R_5 is a (C_2-C_{10})alkenylene selected from the group consisting of
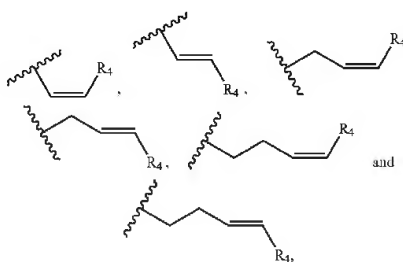
and R_4 is a linear alkylene selected from the group consisting of CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15} and C_8H_{17}. In a preferred aspect of the invention, R_5 is
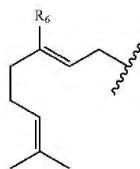
and R_6 is selected from (C_1-C_{10})alkylene, (C_2-C_{10})alkenylene, —OH, —SH, NO_2, F, Cl, Br, —NH_2, or —NHR_a.

[0018] In another preferred embodiment, the cannabinoid acid synthase is a recombinant cannabinoid acid synthase obtained by generating one or more copies of a cannabinoid acid synthase gene and by overexpressing a protein encoded by the cannabinoid acid synthase gene. Preferably, one or more copies of a cannabinoid acid synthase gene are generated in vivo and the method comprises step (i) of integrating one or more copies of the cannabinoid acid synthase gene into the genome of a eukaryotic host to scale up protein expression. Preferably, the eukaryotic host is *Pichia pastoris* and the cannabinoid acid synthase gene is codon optimized with an alpha secretion sequence to target protein secretion and tagged with six tandem histidine residues (SEQ ID NO: 9). Step (i) may comprise linearizing the cannabinoid acid synthase gene by digestion with one or more restriction enzymes; extracting the cannabinoid acid synthase gene by gel extraction; ligating the cannabinoid acid synthase gene into a *Pichia pastoris* plasmid; and electroporating the plasmid into bacterial cells to generate one or more cannabinoid acid synthase gene copy colonies.

[0019] In a preferred aspect of the invention, the solvent is DMSO, and the concentration of DMSO in the reaction mixture is 20% (w/v). In an additional preferred aspect, the amphiphilic compound is a surfactant or a cyclodextrin. In a preferred embodiment, the cyclodextrin is α-cyclodextrin, β-cyclodextrin or γ-cyclodextrin. Even more preferably, the

cyclodextrin is sulfobuthylether β-cyclodextrin sodium salt or randomly methylated β-cyclodextrin, and the concentration of cyclodextrin in the reaction mixture is between 2 and 28 mg/ml. In a most preferred embodiment, the concentration of cyclodextrin in the reaction mixture is 8 mg/ml.

[0020] In one embodiment, the cannabinoids or cannabinoid analogs are single enantiomers with an enantiomeric purity of at least 95%, and preferably of at least 99%.

[0021] In a preferred embodiment, the cannabinoid acid synthase is THCA synthase and the one or more cannabinoids or cannabinoid analogs are tetrahydrocannabinol (THCA), cannabichromene (CBCA), THCA and CBCA, or analogs thereof. In a preferred aspect, the amphiphilic compound is cyclodextrin and the mass:mass ratio of cyclodextrin to the compound of Formula I is 28:1 or the molar ratio of cyclodextrin to the compound of Formula I is 7.3:1. Preferably, step (c) of the reaction is performed at a pH in a range between 3.8 and 7.2, and the method produces THC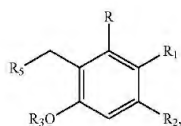A, CBCA, or THCA and CBCA in different ratios as described above. Preferably, 98% of the compound of Formula I is converted into one or more cannabinoids or cannabinoid analogs within two hours.

[0022] In a different embodiment, the cannabinoid acid synthase is CBDA synthase and the method produces cannabidiol (CBDA), cannabichromene acid (CBCA), CBDA and CBCA, or analogs thereof. Preferably, the amphiphilic compound is cyclodextrin and the mass:mass ratio of cyclodextrin to the compound of Formula I is 11:1 or the molar ratio of cyclodextrin to the compound of Formula I is 4:1. Preferably, step (c) is performed at a pH in a range between 3.8 and 7.2, and the method produces CBDA, CBCA, or CBDA and CBCA in different ratios as described above. Most preferably, 70% of the compound of Formula I is converted into one or more cannabinoids or cannabinoid analogs within two hours.

[0023] In yet another embodiment, the invention provides a method for producing a tetrahydrocannabinol, cannabichromene, or both tetrahydrocannabinol and cannabichromene, or their analogs, wherein the method comprises the steps of: (a) selecting a compound according to Formula I;
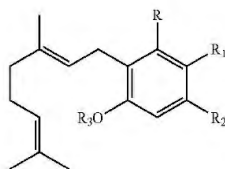
Formula I

(b) reacting the compound of Formula I with a tetrahydrocannabinolic acid (THCA) synthase in a reaction mixture comprising a solvent and an amphiphilic compound; (c) modifying at least one property of the reaction mixture to obtain a tetrahydrocannabinol, a cannabichromene, or both tetrahydrocannabinol and cannabichromene, or their analogs as products; (d) isolating tetrahydrocannabinol, cannabichromene, or both tetrahydrocannabinol and cannabichromene, or their analogs from the reaction mixture; and (e) decarboxylating the tetrahydrocannabinolic acid, cannabichromenic acid, or both tetrahydrocannabinolic acid and

cannabichromenic acid, or their analogs; wherein R is selected from —OH, halogen, —SH, or a —NR$_a$R$_b$ group; R$_1$ and R$_2$ are each independently selected from the group consisting of —H, —C(O)R$_a$, —OR$_a$, an optionally substituted C$_1$-C$_{10}$ linear or branched alkylene, an optionally substituted C$_2$-C$_{10}$ linear or branched alkenylene, an optionally substituted C$_2$-C$_{10}$ linear or branched alkynylene, an optionally substituted C$_3$-C$_{10}$ aryl, an optionally substituted C$_3$-C$_{10}$ cycloalkyl, (C$_3$-C$_{10}$)aryl-(C$_1$-C$_{10}$)alkylene, (C$_3$-C$_{10}$)aryl-(C$_2$-C$_{10}$)alkenylene, and (C$_3$-C$_{10}$)aryl-(C$_1$-C$_{10}$)alkynylene, or R$_1$ and R$_2$ together with the carbon atoms to which they are bonded form a C$_5$-C$_{10}$ cyclic ring; R$_3$ is selected from the group consisting of H, —C(O)R$_a$ and C$_1$-C$_{10}$ linear or branched alkyl; and R$_a$ and R$_b$ are each independently —H, —OH, —SH, —NH$_2$, (C$_1$-C$_{10}$) linear or branched alkyl, or a C$_3$-C$_{10}$ cycloalkyl.

[0024] In a preferred embodiment, the THCA synthase is a recombinant THCA synthase obtained by generating one or more copies of a THCA synthase gene and by overexpressing a protein encoded by the THCA synthase gene. Preferably, one or more copies of the THCA synthase gene are generated in vivo and the method comprises step (i) of integrating one or more copies of the cannabinoid acid synthase gene into the genome of a eukaryotic host to scale up protein expression. Preferably, the eukaryotic host is *Pichia pastoris* and the THCA synthase gene is codon optimized with an alpha secretion sequence to target protein secretion and tagged with six tandem histidine residues (SEQ ID NO: 9). Step (i) may comprise linearizing the cannabinoid acid synthase gene by digestion with one or more restriction enzymes; extracting the cannabinoid acid synthase gene by gel extraction; ligating the cannabinoid acid synthase gene into a *Pichia pastoris* plasmid; and electroporating the plasmid into bacterial cells to generate one or more cannabinoid acid synthase gene copy colonies.

[0025] In a preferred aspect of the invention, the solvent is DMSO, and the concentration of DMSO in the reaction mixture is 20% (w/v). In an additional preferred aspect, the amphiphilic compound is a surfactant or a cyclodextrin. In a preferred embodiment, the cyclodextrin is α-cyclodextrin, β-cyclodextrin or γ-cyclodextrin. Even more preferably, the cyclodextrin is sulfobuthylether β-cyclodextrin sodium salt or randomly methylated β-cyclodextrin, and the concentration of cyclodextrin in the reaction mixture is between 2 and 28 mg/ml. In a most preferred embodiment, the concentration of cyclodextrin in the reaction mixture is 8 mg/ml.

[0026] In a preferred aspect of the invention, the amphiphilic compound is cyclodextrin and the mass:mass ratio of cyclodextrin to the compound of Formula I is 28:1 or the molar ratio of cyclodextrin to the compound of Formula I is 7.3:1. Preferably, step (c) of modifying at least one property of the reaction mixture comprises modifying the pH of the reaction mixture in a range between 3.8 and 7.2, and the method produces THCA, CBCA, or THCA and CBCA in different ratios as described above. Preferably, 98% of the compound of Formula I is converted into one or more cannabinoids or cannabinoid analogs within two hours.

[0027] In a different embodiment, the invention provides a method for producing a cannabidiol, cannabichromene, or both cannabidiol and cannabichromene, or their analogs comprising the steps of: (a) selecting a compound according to Formula I;
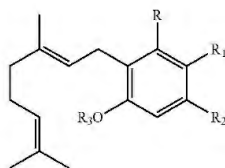
Formula I

(b) reacting the compound of Formula I with a cannabinodiolic acid (CBDA) synthase in a reaction mixture comprising a solvent and an amphiphilic compound; (c) modifying at least one property of the reaction mixture to obtain a cannabidiol, a cannabichromene, or both cannabidiol and cannabichromene, or their analogs as products; (d) isolating cannabidiol, cannabichromene, or both cannabidiol and cannabichromene, or their analogs from the reaction mixture; and (e) decarboxylating the cannabidiol, cannabichromene, or both cannabidiol and cannabichromene, or their analogs; wherein R is selected from —OH, halogen, —SH, or a —NR_aR_b group; R_1 and R_2 are each independently selected from the group consisting of —H, —$C(O)R_a$, —OR_a, an optionally substituted C_1-C_{10} linear or branched alkylene, an optionally substituted C_2-C_{10} linear or branched alkenylene, an optionally substituted C_2-C_{10} linear or branched alkynylene, an optionally substituted C_3-C_{10} aryl, an optionally substituted C_3-C_{10} cycloalkyl, (C_3-C_{10})aryl-(C_1-C_{10})alkylene, (C_3-C_{10})aryl-(C_2-C_{10})alkenylene, and (C_3-C_{10})aryl-(C_1-C_{10})alkynylene, or R_1 and R_2 together with the carbon atoms to which they are bonded form a C_5-C_{10} cyclic ring; R_3 is selected from the group consisting of H, —$C(O)R_a$ and C_1-C_{10} linear or branched alkyl; and R_a and R_b are each independently —H, —OH, —SH, —NH_2, (C_1-C_{10}) linear or branched alkyl, or a C_3-C_{10} cycloalkyl.

[0028]   Preferably, the CBDA synthase is a recombinant CBDA synthase obtained by generating one or more copies of a CBDA synthase gene and by overexpressing a protein encoded by the CBDA synthase gene. In a preferred aspect of the invention, one or more copies of a CBDA synthase gene are generated in vivo and the method comprises step (i) of integrating one or more copies of the CBDA synthase gene into the genome of a eukaryotic host to scale up protein expression. Preferably, the eukaryotic host is *Pichia pastoris* and the CBDA synthase gene is codon optimized with an alpha secretion sequence to target protein secretion and tagged with six tandem histidine residues (SEQ ID NO: 9). Step (i) may comprise linearizing the CBDA synthase gene by digestion with one or more restriction enzymes; extracting the CBDA synthase gene by gel extraction; ligating the CBDA synthase gene into a *Pichia pastoris* plasmid; and electroporating the plasmid into bacterial cells to generate one or more cannabinoid acid synthase gene copy colonies.

[0029]   In a preferred aspect of the invention, the solvent is DMSO, and the concentration of DMSO in the reaction mixture is 20% (w/v). In an additional preferred aspect, the amphiphilic compound is a surfactant or a cyclodextrin. In a preferred embodiment, the cyclodextrin is α-cyclodextrin, β-cyclodextrin or γ-cyclodextrin. Even more preferably, the cyclodextrin is sulfobuthylether β-cyclodextrin sodium salt or randomly methylated β-cyclodextrin, and the concentration of cyclodextrin in the reaction mixture is between 2 and 28 mg/ml. In a most preferred embodiment, the concentration of cyclodextrin in the reaction mixture is 8 mg/ml.

[0030]   In a preferred aspect of the invention, the amphiphilic compound is cyclodextrin and the mass:mass ratio of cyclodextrin to the compound of Formula I is 28:1(w/w) or the molar ratio of cyclodextrin to the compound of Formula I is 7.3:1. Preferably, step (c) of modifying at least one property of the reaction mixture comprises modifying the pH of the reaction mixture in a range between 3.8 and 7.2, and the method produces CBDA, CBCA, or CBDA and CBCA in different ratios as described above. In a preferred embodiment, 98% of the compound of Formula I is converted into one or more cannabinoids or cannabinoid analogs within two hours.

[0031]   In a different embodiment, the invention provides a system for producing one or more cannabinoids or cannabinoid analogs, comprising: a fermentor holding a medium and a plurality of cells, wherein the cells are configured to produce and secrete a cannabinoid synthase; a bioreactor containing a reactant in a reaction mixture comprising a solvent and an amphiphilic compound, the reactant configured to interact with cannabinoid acid synthase to form a first cannabinoid and a second cannabinoid; and a control mechanism configured to control a condition of the bioreactor, wherein the condition of the bioreactor influences a quantity formed of the first cannabinoid relative to a quantity formed of a second cannabinoid, and wherein the first and second cannabinoids are each one a cannabinoid or a cannabinoid analog.

[0032]   In a preferred embodiment, the bioreactor is a column bioreactor containing nickel, and the cannabinoid acid synthase includes a tag configured to bond to nickel. In some embodiments, the bioreactor is a column bioreactor containing both nickel and another metal.

[0033]   In one embodiment, the reactant in the system is a compound according to Formula I;
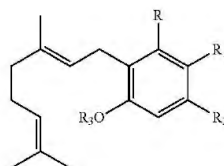
Formula I

Wherein R is selected from —OH, halogen, —SH, or a —NR_aR_b group; R_1 and R_2 are each independently selected from the group consisting of —H, —$C(O)R_a$, —OR_a, an optionally substituted C_1-C_{10} linear or branched alkylene, an optionally substituted C_2-C_{10} linear or branched alkenylene, an optionally substituted C_2-C_{10} linear or branched alkynylene, an optionally substituted C_3-C_{10} aryl, an optionally substituted C_3-C_{10} cycloalkyl, (C_3-C_{10})aryl-(C_1-C_{10})alkylene, (C_3-C_{10})aryl-(C_2-C_{10})alkenylene, and (C_3-C_{10})aryl-(C_1-C_{10})alkynylene, or R_1 and R_2 together with the carbon atoms to which they are bonded form a C_5-C_{10} cyclic ring; R_3 is selected from the group consisting of H, —$C(O)R_a$ and C_1-C_{10} linear or branched alkyl; and R_a and R_b are each independently —H, —OH, —SH, —NH_2, (C_1-C_{10}) linear or branched alkyl, or a C_3-C_{10} cycloalkyl.

[0034] In one preferred embodiment, the cannabinoid acid synthase is cannabidiolic acid (CBDA) synthase and the first and the second cannabinoids are one or both of cannabidiolic acid and cannabichromenic acid or their analogs.

[0035] In another preferred embodiment, the cannabinoid acid synthase is tetrahydrocannabinolic acid (THCA) synthase and the first and the second cannabinoids are one or both of tetrahydrocannabinolic acid and cannabichromenic acid, or their analogs.

[0036] Preferably, the cannabinoid acid synthase interacts with the reactant in the bioreactor to form both the first cannabinoid and the second cannabinoid, and the condition of the bioreactor is a function of at least one of pH, solvent, temperature, pressure, and flow rate.

[0037] In a preferred embodiment, a change in the condition of the bioreactor is configured to cause a shift from: 1) formation of the first cannabinoid in greater quantities relative to the second cannabinoid to 2) formation of the second cannabinoid in greater quantities relative to the first cannabinoid.

[0038] Preferably the solvent in the system is DMSO, and the concentration of DMSO in the reaction mixture is 20% (w/v).

[0039] In yet another preferred embodiment, the amphiphilic compound in the system is a surfactant or a cyclodextrin. Preferably, the cyclodextrin is α-cyclodextrin, β-cyclodextrin or γ-cyclodextrin. Even more preferably, the cyclodextrin is sulfobuthylether β-cyclodextrin sodium salt or randomly methylated β-cyclodextrin, and the concentration of cyclodextrin in the reaction mixture is between 2 and 28 mg/ml. Most preferably, the concentration of cyclodextrin in the reaction mixture is 8 mg/ml.

[0040] In one aspect of the invention, the amphiphilic compound is cyclodextrin and the mass:mass ratio of cyclodextrin to the compound of Formula 1 is 28:1 (w/w), or the molar ratio of cyclodextrin to the compound of Formula 1 is 7.3:1. In a preferred embodiment, 98% of the compound of Formula 1 in the system is converted into one or more cannabinoids or cannabinoid analogs within two hours.

[0041] In a preferred aspect of the invention, the cannabinoid acid synthase in the system is CBDA synthase and the change in the condition of the bioreactor comprises modifying the pH of the reaction mixture in a range between 3.8 and 7.2. Preferably, the method produces CBDA, CBCA, or CBDA and CBCA in different ratios as described above.

[0042] In another preferred embodiment, the cannabinoid acid synthase is THCA synthase and the change in the condition of the bioreactor comprises modifying the pH of the reaction mixture in a range between 3.8 and 7.2. Preferably, the method produces THCA, CBCA, or THCA and CBCA in different ratios as described above.

[0043] In yet another embodiment, the invention provides a method for producing at least one cannabinoid or cannabinoid analog, that includes the steps of: providing cannabigerol, a cannabinoid acid synthase, and a reaction mixture comprising a solvent and an amphiphilic compound via an automated delivery system; reacting the cannabigerol with the cannabinoid acid synthase in the reaction mixture; adding a solvent via the automated delivery system to cease the reaction; removing the solvent; and recovering the at least one cannabinoid or cannabinoid analog produced by the reaction. Preferably, the reaction mixture comprises DMSO and the cannabinoid acid synthase is CBDA synthase or THCA synthase. Even more preferably, the step of

reacting the cannabigerol with the cannabinoid acid synthase comprises controlling the pH of the reaction mixture via a controller. Thus, in a preferred aspect of the invention, the method further comprises controlling the pH of the reaction mixture to produce a predetermined quantity of at least a first cannabinoid or first cannabinoid analog and controlling the pH of the reaction mixture to produce the predetermined quantity of the first cannabinoid or first cannabinoid analog and a predetermined quantity of a second cannabinoid or second cannabinoid analog. In a preferred embodiment, the first cannabinoid or first cannabinoid analog is THCA or CBDA and the second cannabinoid or second cannabinoid analog is CBCA. In another preferred embodiment, the first cannabinoid or first cannabinoid analog is THCA or CBDA and the second cannabinoid or second cannabinoid analog is CBCA.

[0044] In another embodiment, the invention provides a method for producing at least one cannabinoid or cannabinoid analog, that comprises: reacting cannabigerol with cannabinoid acid synthase in a reaction mixture comprising a solvent and an amphiphilic compound; adding a solvent to cease the reaction; removing the solvent; and recovering the cannabinoid or cannabinoid analog produced by the reaction. In a preferred aspect of the invention, the step of reacting the cannabigerol with the cannabinoid acid synthase comprises controlling the pH of the reaction mixture. Preferably, the pH of the reaction mixture is controlled by adjusting the pH of the reaction mixture to achieve a predetermined ratio of a first cannabinoid or first cannabinoid analog to a second cannabinoid or second cannabinoid analog. Even more preferably, the reaction mixture comprises DMSO and the cannabinoid acid synthase is CBDA synthase or THCA synthase. In one embodiment, the first cannabinoid or first cannabinoid analog is THCA or CBDA and the second cannabinoid or second cannabinoid analog is CBCA.

[0045] In an additional embodiment, the invention provides an apparatus that comprises an automated supply system configured to deliver a first automated supply of cannabigerol, a cannabinoid acid synthase, and a reaction mixture comprising a solvent and an amphiphilic compound; a bioreactor configured to receive the first supply and permit reaction of the cannabigerol and cannabinoid acid synthase in the reaction mixture, and a second automated supply of a solvent so as to cease the reaction; and an extractor configured to remove the solvent and recover at least a first cannabinoid or cannabinoid analog. The apparatus may further comprise a controller configured to adjust at least one property of the reaction mixture so as to produce the first cannabinoid or first cannabinoid analog and a second cannabinoid or second cannabinoid analog in a predetermined ratio. The controller may be also configured to determine a first quantity of the first cannabinoid or first cannabinoid analog and a second quantity of a second cannabinoid or second cannabinoid analog, and adjust at least one property of the reaction mixture so as to produce the first quantity of the first cannabinoid or first cannabinoid analog and the second quantity of a second cannabinoid or second cannabinoid. Preferably, the reaction mixture comprises DMSO and the cannabinoid acid synthase is CBDA synthase or THCA synthase. In a preferred aspect of the invention, the first cannabinoid or first cannabinoid analog is THCA or CBDA and the second cannabinoid or second cannabinoid analog is CBCA.

[0046] In yet another embodiment, the invention provides an apparatus for producing tetrahydrocannabinolic acid (THCA) and cannabichromenic acid (CBCA) or cannabidiolic acid (CBDA) and cannabichromenic acid (CBCA) in different ratios comprising: a bioreactor comprising an automated supply system configured to deliver (a) a first automated supply of cannahigerol, a cannabinoid acid synthase, and a reaction mixture comprising a solvent and an amphiphilic compound, wherein the solvent is one or more of dimethyl sulfoxide (DMSO), dimethyl formamide (DMF) and iso-propyl alcohol and the concentration of the solvent in the reaction mixture is between 5% and 30% (w/v), and wherein the amphiphilic compound is a surfactant or a cyclodextrin and the concentration of the amphiphilic compound in the reaction mixture is between 2 and 28 mg/ml; and (b) a second automated supply of solvent to cease the reaction; an extractor configured to remove the solvent and recover tetrahydrocannabinolic acid (THCA) and cannabichromenic acid (CBCA) or cannabidiolic acid (CBDA) and cannabichromenic acid (CBCA) from the reaction mixture; and a controller configured to modify the pH of the reaction mixture to produce tetrahydrocannabinolic acid (THCA) and cannabichromenic acid (CBCA) or cannabidiolic acid (CBDA) and cannabichromenic acid (CBCA) in different ratios, and adjust the concentration of the amphiphilic compound in the reaction mixture to affect the conversion rate of cannabigerolic acid (CBGA) into tetrahydrocannabinolic acid (THCA) and cannabichromenic acid (CBCA) or into cannabidiolic acid (CBDA) and cannabichromenic acid (CBCA) in different ratios. In a preferred embodiment, the cannabinoid acid synthase is tetrahydrocannabinolic acid synthase (THCA synthase) or cannabidiolic acid synthase (CBDA synthase). In one aspect of the invention, the cannabinoid synthase is immobilized on a solid support. In another aspect of the invention, the cannabinoid synthase is a recombinant cannabinoid synthase, and the apparatus further comprises a system to produce the recombinant cannabinoid synthase in large scale. Preferably, the pH is in the range from about 3.8 to about 8.0.

[0047] In a preferred aspect of the invention, the solvent is one or more of dimethyl sulfoxide (DMSO), dimethyl formamide (DMF) and iso-propoyl alcohol, and the concentration of the solvent in the reaction mixture is between 5% and 30% (w/v). In another preferred aspect of the invention, the amphiphilic compound is a surfactant or a cyclodextrin. The cyclodextrin can be α-cyclodextrin, β-cyclodextrin or γ-cyclodextrin. In one aspect of the invention, the cyclodextrin is sulfobuthylether β-cyclodextrin sodium salt or randomly methylated β-cyclodextrin, and the concentration of cyclodextrin in the reaction mixture is between 2 and 28 mg/ml. Preferably, the concentration of cyclodextrin in the reaction mixture is 8 mg/ml.

[0048] In one embodiment, the tetrahydrocannabinolic acid (THCA) and cannabichromenic acid (CBCA) or cannabidiolic acid (CBDA) and cannabichromenic acid (CBCA) are single enantiomers with an enantiomeric purity of at least 95%.

[0049] In one aspect of the invention, the cannabinoid synthase is THCA synthase and the amphiphilic compound is cyclodextrin. Preferably, the mass:mass ratio of cyclodextrin to cannabigerolic acid (CBGA) is 28:1 or the molar ratio of cyclodextrin to cannabigerolic acid (CBGA) is 7.3:1. In a preferred aspect of the invention, the apparatus produces

tetrahydrocannabinolic acid (THCA) and cannabichromenic acid (CBCA) in the following ratios:

pH	THCA	CBCA
4	1	0
5	2.33	1
6	1	5.67
7	0	1

[0050] Preferably, 98% of the cannabigerolic acid CBGA is converted into tetrahydrocannabinolic acid (THCA) and cannabichromenic acid (CBCA) within two hours.

[0051] In a different aspect of the invention, the cannabinoid acid synthase is CBDA synthase and the amphiphilic compound is cyclodextrin. Preferably, the mass:mass ratio of cyclodextrin to the cannabigerolic acid (CBGA) is 11:1 or the molar ratio of cyclodextrin to the cannabigerolic acid (CBGA) is 4:1. In a preferred aspect of the invention, the apparatus produces cannabidiolic acid (CBDA) and cannabichromenic acid (CBCA) in the following ratios:

pH	CBDA	CBCA
4.2	2.5	1
5	1.13	1
5.2	1	1.17
5.4	1	2.45
5.8	1	6.14
6.2	1	28.13
6.8	0	0

[0052] Preferably, 98% of the cannabigerolic acid (CBGA) is converted into cannabidiolic acid (CBDA) and cannabichromenic acid (CBCA) within two hours.

[0053] The foregoing general description and the detailed description are exemplary and explanatory and are intended to provide further explanation of the invention as claimed. For detailed understanding of the invention, reference is made to the following detailed description of the preferred embodiments, taken in conjunction with the accompanying drawing. Other objects, advantages and novel features will be readily apparent to those skilled in the art from the following detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0054] FIG. 1 illustrates the effect of 10% DMSO and no cyclodextrin on THCA synthase activity. 100 μl THCA synthase in crude fermentation supernatant (10× concentrated) were reacted with 50 μd 2 mg/ml CBGA in 350 μl citrate buffer at pH 4.85. Peaks (from left to right): #1 CBGA (86.51%), #2 THCA (10.4%), #3 CBCA (3.09%).

[0055] FIG. 2 illustrates the effect of 20% DMSO and no cyclodextrin on THCA synthase activity. 100 μl THCA synthase in crude fermentation supernatant (10× concentrated) were reacted with 50 μl 2 mg/ml CBGA in 300 μl citrate buffer at pH 4.85 in the presence of 50 μl DMSO. Peaks (from left to right): #1 CBGA (54.18%), #2 THCA (32.39%), #3 CBCA (13.43%).

[0056] FIG. 3 illustrates the effect of 10% DMSO and 20% cyclodextrin on THCA synthase activity. 100 μl THCA synthase in crude fermentation supernatant (10× concentrated) were reacted with 50 μd 2 mg/ml CBGA in 350 μl

citrate buffer at pH 4.85 containing 2 mg cyclodextrin. Peaks (from left to right): #1 CBGA (10.33%), #2 THCA (72. 37%), #3 CBCA (17.3%).

[0057] FIG. 4 illustrates the effect of 10% DMSO and 40% cyclodextrin on THCA synthase. 150 µl THCA synthase in crude fermentation supernatant (10× concentrated) were reacted with 75 µl 2 mg/ml CBGA in 525 µl citrate buffer at pH 4.85 containing 3 mg cyclodextrin. Peaks (from left to right): #1 CBGA (11.50%), #2 THCA (72.08%), #3 CBCA (16.42%).

[0058] FIG. 5 illustrates the effect of 10% DMSO and 60% cyclodextrin on THCA synthase activity. 200 µl THCA synthase in crude fermentation supernatant (10× concentrated) were reacted with 100 µl 2 mg/ml CBGA in 700 µl citrate buffer at pH 4.85 containing 4 mg cyclodextrin. Peaks (from left to right): #1 CBGA (10.36%), #2 THCA (73. 98%), #3 CBCA (15.65%).

[0059] FIG. 6 illustrates the effect of 10% DMSO and 20 mg/ml cyclodextrin on CBDA synthase activity. 200 µl CBDA synthase in crude fermentation supernatant (10× concentrated) were reacted with 100 µl 4 mg/ml CBGA in 700 µl citrate buffer at pH 4.85 containing 4 mg cyclodextrin. Peaks (from left to right): #1 CBDA (40.63%), #2 CBGA (28.43%), #3 CBCA (30.95%).

[0060] FIG. 7 is a block diagram of a system for producing cannabinoids and/or cannabinoid analogs.

[0061] FIG. 8 is a block diagram of a system for producing cannabinoids and/or cannabinoid analogs.

[0062] FIG. 9 is a flow diagram illustrating a method for producing cannabinoids.

[0063] FIG. 10 is a block diagram of a controller.

[0064] FIG. 11 illustrates the effect of 0 mg/ml cyclodextrin on CBDA synthase reaction conversion rate and product ratio. 50 µl of 10× concentrated fermentation supernatant were reacted with 25 µl of 5 mg/ml CBGA in 175 µl of citrate buffer pH 4.8. Peaks (Left to right): CBDA (17.99%), CBGA (65.72%), CBCA (16.30%).

[0065] FIG. 12 illustrates the effect of 2 mg/ml cyclodextrin on CBDA synthase reaction conversion rate and product ratio. 50 µl of 10× concentrated fermentation supernatant were reacted with 25 µl of 5 mg/ml CBGA in 175 µl of citrate buffer pH 4.8 containing 2 mg/ml cyclodextrin. Peaks (Left to right): CBDA (29.53%), CBGA (47.40%), CBCA (23.08%).

[0066] FIG. 13 illustrates the effect of 8 mg/ml cyclodextrin on CBDA synthase reaction conversion rate and product ratio. 50 µl of 10× concentrated fermentation supernatant were reacted with 8.25 µl of 5 mg/ml CBGA in 175 µl of citrate buffer pH 4.8 containing 8 mg/ml cyclodextrin. Peaks (Left to right): CBDA (33%), CBGA (41.98%), CBCA (25.02%).

[0067] FIG. 14 illustrates the effect of 12 mg/ml cyclodextrin on CBDA synthase reaction conversion rate and product ratio. 50 µl of 10× concentrated fermentation supernatant were reacted with 25 µl of 5 mg/ml CBGA in 175 µl of citrate buffer pH 4.8 containing 12 mg/ml cyclodextrin. Peaks (Left to right): CBDA (30.63%), CBGA (45.22%), CBCA (24.15%).

[0068] FIG. 15 illustrates the effect of 16 mg/ml cyclodextrin on CBDA synthase reaction conversion rate and product ratio. 50 µl of 10× concentrated fermentation supernatant were reacted with 25 µl of 5 mg/ml CBGA in 175 µl

of citrate buffer pH 4.8 containing 16 mg/ml cyclodextrin. Peaks (Left to right): CBDA (28.54%), CBGA (49.63%), CBCA (21.84%).

[0069] FIG. 16 illustrates the effect of 20 mg/ml cyclodextrin on CBDA synthase reaction conversion rate and product ratio. 50 µl of 10× concentrated fermentation supernatant were reacted with 25 µl of 5 mg/ml CBGA in 175 µl of citrate buffer pH 4.8 containing 20 mg/ml cyclodextrin. Peaks (Left to right): CBDA (29.05%), CBGA (50.04%), CBCA (20.91%).

[0070] FIG. 17 illustrates the effect of 28 mg/ml cyclodextrin on CBDA synthase reaction conversion rate and product ratio. 50 µl of 10× concentrated fermentation supernatant were reacted with 25 µl of 5 mg/ml CBGA in 175 µl of citrate buffer pH 4.8 containing 28 mg/ml cyclodextrin. Peaks (Left to right): CBDA (22.09%), CBGA (59.60%), CBCA (18.32%).

DETAILED DESCRIPTION OF THE INVENTION

[0071] The present invention provides a system and methods for large scale simultaneous enzymatic production of different cannabinoids or cannabinoid analogs, as well as methods for cloning, expressing and purifying enzymes that catalyze large scale simultaneous synthesis of THCA, CBDA, CBCA or analogs thereof under various pH, temperature and aqueous/lipophilic conditions.

Definitions

[0072] As used herein, unless otherwise stated, the singular forms "a," "an," and "the" include plural reference. Thus, for example, a reference to "a cell" includes a plurality of cells, and a reference to "a molecule" is a reference to one or more molecules.

[0073] As used herein, "about" will be understood by persons of ordinary skill in the art and will vary to some extent depending upon the context in which it is used. If there are uses of the term which are not clear to persons of ordinary skill in the art, given the context in which it is used, "about" will mean up to plus or minus 10% of the particular term.

[0074] The term "alkyl" refers to a straight or branched chain, saturated hydrocarbon having the indicated number of carbon atoms. For example, (C_1-C_{10}) alkyl is meant to include but is not limited to methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tert-butyl, pentyl, isopentyl, neopentyl, hexyl, isohexyl, and neohexyl, etc. An alkyl group can be unsubstituted or optionally substituted with one or more substituents as described herein below.

[0075] The term "alkenyl" refers to a straight or branched chain unsaturated hydrocarbon having the indicated number of carbon atoms and at least one double bond. Examples of a (C_2-C_{10}) alkenyl group include, but are not limited to, ethylene, propylene, 1-butylene, 2-butylene, isobutylene, sec-butylene, 1-pentene, 2-pentene, isopentene, 1-hexene, 2-hexene, 3-hexene, isohexene, 1-heptene, 2-heptene, 3-heptene, isoheptene, 1-octene, 2-octene, 3-octene, 4-octene, and isooctene. An alkenyl group can be unsubstituted or optionally substituted with one or more substituents as described herein below.

[0076] The term "alkynyl" refers to a straight or branched chain unsaturated hydrocarbon having the indicated number of carbon atoms and at least one triple bond. Examples of a

(C_2-C_{10}) alkynyl group include, but are not limited to, acetylene, propyne, 1-butyne, 2-butyne, 1-pentyne, 2-pentyne, 1-hexyne, 2-hexyne, 3-hexyne, 1-heptyne, 2-heptyne, 3-heptyne, 1-octyne, 2-octyne, β-octyne and 4-octyne. An alkynyl group can be unsubstituted or optionally substituted with one or more substituents as described herein below.

[0077] The term "alkoxy" refers to an —O-alkyl group having the indicated number of carbon atoms. For example, a (C_1-C_6) alkoxy group includes —O-methyl, —O-ethyl, —O-propyl, —O-isopropyl, —O-bntyl, —O-sec-butyl, —O-tert-butyl, —O-pentyl, —O-isopentyl, —O-neopentyl, —O-hexyl, —O-isohexyl, and —O-neohexyl.

[0078] The term "aryl" refers to a 3- to 14-member monocyclic, bicyclic, tricyclic, or polycyclic aromatic hydrocarbon ring system. Examples of an aryl group include naphthyl, pyrenyl, and anthracyl. An aryl group can be unsubstituted or optionally substituted with one or more substituents as described herein below.

[0079] The terms "alkylene," "alkenylene," and "arylene," alone or as part of another substituent, means a divalent radical derived from an alkyl, cycloalkyl, alkenyl, aryl, or heteroaryl group, respectively, as exemplified by —$CH_2CH_2CH_2CH_2$—. For alkylene, alkenyl, or aryl linking groups, no orientation of the linking group is implied.

[0080] The term "halogen" and "halo" refers to —F, —Cl, —Br or —I.

[0081] The term "heteroatom" is meant to include oxygen (O), nitrogen (N), and sulfur (S).

[0082] A "hydroxyl" or "hydroxy" refers to an —OH group.

[0083] The term "hydroxyalkyl," refers to an alkyl group having the indicated number of carbon atoms wherein one or more of the alkyl group's hydrogen atoms is replaced with an —OH group. Examples of hydroxyalkyl groups include, but are not limited to, —CH_2OH, —CH_2CH_2OH, —$CH_2CH_2CH_2OH$, —$CH_2CH_2CH_2CH_2OH$, —$CH_2CH_2CH_2CH_2CH_2OH$, —$CH_2CH_2CH_2CH_2CH_2CH_2OH$, and branched versions thereof.

[0084] The term "cycloalkyl" refer to monocyclic, bicyclic, tricyclic, or polycyclic, β- to 14-membered ring systems, which are either saturated, unsaturated or aromatic. The heterocycle may be attached via any heteroatom or carbon atom. Cycloalkyl include aryls and hetroaryls as defined above. Representative examples of cycloalky include, but are not limited to, cycloethyl, cyclopropyl, cycloisopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclopropene, cyclobutene, cyclopentene, cyclohexene, phenyl, naphthyl, anthracyl, benzofuranyl, and benzothiophenyl. A cycloalkyl group can be unsubstituted or optionally substituted with one or more substituents as described herein below.

[0085] The term 'nitrile or cyano" can be used interchangeably and refer to a —CN group which is bound to a carbon atom of a heteroaryl ring, aryl ring and a heterocycloalkyl ring.

[0086] The term "amine or amino" refers to an —NR_cR_d group wherein R_c and R_d each independently refer to a hydrogen, (C_1-C_8)alkyl, aryl, heteroaryl, heterocycloalkyl, (C_1-C_8)haloalkyl, and (C_1-C_6)hydroxyalkyl group.

[0087] The term "alkylaryl" refers to C_1-C_8 alkyl group in which at least one hydrogen atom of the C_1-C_8 alkyl chain is replaced by an aryl atom, which may be optionally substituted with one or more substituents as described herein

below. Examples of alkylaryl groups include, but are not limited to, methylphenyl, ethylnaphthyl, propylphenyl, and butylphenyl groups.

[0088] "Arylalkylene" refers to a divalent alkylene wherein one or more hydrogen atoms in the C_1-C_{10} alkylene group is replaced by a (C_3-C_{14})aryl group. Examples of (C_3-C_{14})aryl-(C_1-C_{10})alkylene groups include without limitation 1-phenylbutylene, phenyl-2-butylene, 1-phenyl-2-methylpropylene, phenylmethylene, phenylpropylene, and naphthylethylene.

[0089] "Arylalkenylene" refers to a divalent alkenylene wherein one or more hydrogen atoms in the C_2-C_{10} alkenylene group is replaced by a (C_3-C_{14})aryl group.

[0090] The term "arylalkynylene" refers to a divalent alkynylene wherein one or more hydrogen atoms in the C_2-C_{10} alkynylene group is replaced by a (C_3-C_{14})aryl group.

[0091] The terms "carboxyl" and "carboxylate" include such moieties as may be represented by the general formulas:
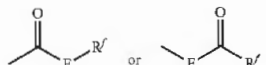
[0092] E in the formula is a bond or O and R^f individually is H, alkyl, alkenyl, aryl, or a pharmaceutically acceptable salt. Where E is O, and R^f is as defined above, the moiety is referred to herein as a carboxyl group, and particularly when R^f is a hydrogen, the formula represents a "carboxylic acid". In general, where the expressly shown oxygen is replaced by sulfur, the formula represents a "thiocarbonyl" group.

[0093] Unless otherwise indicated, "stereoisomer" means one stereoisomer of a compound that is substantially free of other stereoisomers of that compound. Thus, a stereomerically pure compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, for example greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, or greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, or greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound.

[0094] The present invention provides methods for the enzymatic synthesis of cannabinoids or cannabinoid analogs in a cell-free environment. Also described is an apparatus for the ex vivo manufacture of cannabinoids and analogs of cannabinoids. The term "analog" refers to a compound that is structurally related to naturally occurring cannabinoids, but whose chemical and biological properties may differ from naturally occurring cannabinoids. In the present context, analog or analogs refer compounds that may not exhibit one or more unwanted side effects of a naturally occurring cannabinoid. Analog also refers to a compound that is

derived from a naturally occurring cannabinoid by chemical, biological or a semi-synthetic transformation of the naturally occurring cannabinoid.

[0095] Cannabinoid compounds include, but are not limited to, cannabinol, cannabidiol, Δ9-tetrahydrocannabinol, Δ8-tetrahydrocannabinol, 11-hydroxy-tetrahydrocannabinol, 11-hydroxy-Δ9-tetrahydrocannabinol, levonantradol, Δll-tetrahydrocannabinol, tetrahydrocannabivarin, dronabinol, amandamide and nabilone, as well as natural or synthetic molecules that have a basic cannabinoid structure and are modified synthetically to provide a cannabinoid analog.

[0096] The present invention also provides methods for the large scale cloning and expression of the enzymes that play a role in the biosynthesis of cannabinoids and for the use of an eukaryotic expression system for the production of biosynthetic enzymes that can be used for the manufacture of cannabinoids and cannabinoid analogs. Yeast as well as eukaryotic and prokaryotic cells are suitable for the cloning and expression of the cannabinoid acid synthase enzymes and include without limitation E. coli, yeast and baculovirus hosts. Thus, the present invention discloses a method for the large-scale production of several cannabinoid acid synthase enzymes including, but not limited to, tetrahydrocannahinolic acid (THCA) synthase and cannabidiolic acid (CBDA) synthase, using the pink Pichia yeast expression system. Accordingly, large scale production of these enzymes can be carried out by transforming yeast with a DNA construct that comprises a gene for a cannabinoid synthase, generating one or more copies of the cannabinoid acid synthase gene and overexpressing a protein encoded by the cannabinoid acid synthase gene.

[0097] The nucleic acid sequence of the THCA synthase gene is represented by SEQ ID NO: 1 and encodes a polypeptide sequence set forth in SEQ ID NO: 2. The codon optimized nucleic acid sequence of the THCA synthase gene for Pichia pastoris expression is represented by SEQ ID NO: 3 and encodes a polypeptide sequence set forth in SEQ ID NO: 4, which is the THCA synthase amino acid sequence comprising the alpha secretion sequence of Pichia pastoris. "THCA synthase expression" refers to the biosynthesis of a gene product encoded by SEQ ID NO: 1 or by SEQ ID NO: 3, or a variant, fragment or portion of SEQ ID NO: 1 or SEQ ID NO: 3. "THCA synthase expression" also refers to the biosynthesis of a polypeptide comprising SEQ ID NO: 2 or SEQ ID NO: 4, or a variant, fragment or portion of a polypeptide comprising SEQ ID NO: 2 or SEQ ID NO: 4. "THCA synthase overexpression" denotes an increase in THCA synthase expression. THCA overexpression affects an increase in THCA or CBCA content for a plant or cell in which the overexpression occurs. THCA overexpression refers to upregulated biosynthesis of a gene product encoded by SEQ ID NO: 1 or by SEQ ID NO: 3, or any variant, fragment or portion of SEQ ID NO: 1 or SEQ ID NO: 3.

[0098] The nucleic acid sequence of the CBDA synthase gene (codon optimized for Pichia pastoris expression) is represented by SEQ ID NO: 5 and encodes a polypeptide sequence set forth in SEQ ID NO: 6. The codon optimized nucleic acid sequence of the CBDA synthase gene for Pichia pastoris expression is represented by SEQ ID NO: 7 and encodes a polypeptide sequence set forth in SEQ ID NO: 8, which is the CBDA synthase amino acid sequence comprising the alpha secretion sequence of Pichia pastoris. "CBDA synthase expression" refers to the biosynthesis of a gene product encoded by SEQ ID NO: 5 or by SEQ ID NO: 7, or a variant, fragment or portion of SEQ ID NO: 5 or SEQ ID NO: 7. "CBDA synthase expression" also refers to the biosynthesis of a polypeptide comprising SEQ ID NO: 6 or SEQ ID NO: 8, or a variant, fragment or portion of a polypeptide comprising SEQ ID NO: 6 or SEQ ID NO: 8. "CBDA synthase overexpression" denotes an increase in CBDA synthase expression. CBDA overexpression affects an increase in CBDA or CBCA content for a plant or cell in which the overexpression occurs. CBDA overexpression refers to upregulated biosynthesis of a gene product encoded by SEQ ID NO: 5 or by SEQ ID NO: 7, or any variant, fragment or portion of SEQ ID NO: 5 or SEQ ID NO: 7.

[0099] The present invention encompasses any nucleic acid, gene, polynucleotide, DNA, RNA, mRNA, or cDNA molecule that is isolated from the genome of a plant species, or produced synthetically, that increases biosynthesis of cannabinoids or cannabinoid analogs. Additionally, expression of such cannabinoid acid synthase sequence produces cannabinoids or cannabinoid analogs in a non-cannabinoid producing cell, including yeast, prokariotic cells and eukariotic cells, such as a non-cannabinoid producing plant cell, a bacteria cell, an insect cell, or an yeast cell. The DNA or RNA may be double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also called the anti-sense strand.

[0100] It is understood that THCA synthase and CBDA synthase include the sequences set forth in SEQ ID NOs: 1, 3, 5 and 7, respectively, as well as nucleic acid molecules comprising variants, fragments or portions of SEQ ID NOs: 1, 3, 5 and 7, with one or more bases deleted, substituted, inserted, or added, wherein a variant of anyone of SEQ ID Nos: 1, 3, 5 and 7 codes for a polypeptide with cannabinoid or cannabinoid analog biosynthesis activity. Accordingly, sequences having "base sequences with one or more bases deleted, substituted, inserted, or added" retain physiological activity even when the encoded amino acid sequence has one or more amino acids substituted, deleted, inserted, or added. Physiological activity of the encoded amino acid sequences may be tested using conventional enzymatic assays known in the art. Additionally, multiple forms of THCA synthase and CBDA synthase may exist, which may be due to post-translational modification of a gene product, or to multiple forms of the respective THCA synthase and CBDA synthase. Nucleotide sequences that have such modifications and that code for cannabinoid or cannabinoid analog biosynthesis enzymes are included within the scope of the present invention.

[0101] For example, the poly A tail or 5'- or 3'-end, nontranslation regions may be deleted, and bases may be deleted to the extent that amino acids are deleted. Bases may also be substituted, as long as no frame shift results. Bases also may be "added" to the extent that amino acids are added. It is essential, however, that any such modification does not result in the loss of cannabinoid acid or cannabinoid acid analog biosynthesis enzyme activity. A modified DNA in this context can be obtained by modifying the DNA base sequences of the invention so that amino acids at specific sites are substituted, deleted, inserted, or added by site-specific mutagenesis, for example, and that still retain cannabinoid acid or cannabinoid acid analog biosynthesis enzyme activity. Cannabinoid acid or cannabinoid acid analog biosynthesis enzyme activity of the encoded amino acid sequences may be assayed as described above.

[0102] A cannabinoid or cannabinoid analog biosynthesis sequence can be synthesized ab initio from the appropriate bases, for example, by using an appropriate protein sequence disclosed herein as a guide to create a DNA molecule that, though different from the native DNA sequence, results in the production of a protein with the same or similar amino acid sequence. This type of synthetic DNA molecule is useful when introducing a DNA sequence into a non-plant cell, coding for a heterologous protein, that reflects different (non-plant) codon usage frequencies and, if used unmodified, can result in inefficient translation by the host cell.

[0103] By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, recombinant DNA molecules contained in a DNA construct are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or DNA molecules that are purified, partially or substantially, in solution. Isolated RNA molecules include in vitro RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules, according to the present invention, further include such molecules produced synthetically.

[0104] "Exogenous nucleic acid" refers to a nucleic acid, DNA or RNA, which has been artificially introduced into a cell. Such exogenous nucleic acid may be a copy of a sequence which is naturally found in the cell into which it was introduced, or fragments thereof.

[0105] In contrast, "endogenous nucleic acid" refers to a nucleic acid, gene, polynucleotide, DNA, RNA, mRNA, or cDNA molecule that is present in the genome of a plant or organism that is to be genetically engineered. An endogenous sequence is "native" to, i.e., indigenous to, the plant or organism that is to be genetically engineered.

[0106] "Heterologous nucleic acid" refers to a nucleic acid, DNA or RNA, which has been introduced into a cell which is not a copy of a sequence naturally found in the cell into which it is introduced. Such heterologous nucleic acid may comprise segments that are a copy of a sequence which is naturally found in the cell into which it has been introduced, or fragments thereof.

[0107] A "chimeric nucleic acid" comprises a coding sequence or fragment thereof linked to a transcription initiation region that is different from the transcription initiation region with which it is associated in cells in which the coding sequence occurs naturally.

[0108] The present application is directed to such nucleic acid molecules which are at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a nucleic acid sequence described in any of SEQ ID NO: 1, 3, 5 and 7. Preferred are nucleic acid molecules which are at least 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequence shown in any of SEQ ID NO: 1, 3, 5 and 7. Differences between two nucleic acid sequences may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

[0109] As a practical matter, whether any particular nucleic acid molecule is at least 95%, 96%, 97%, 98% or 99% identical to a reference nucleotide sequence refers to a comparison made between two molecules using standard algorithms well known in the art and can be determined conventionally using publicly available computer programs such as the BLAST algorithm.

[0110] The present invention further provides nucleic acid molecules comprising the nucleotide sequence of SEQ ID NOs: 1, 3, 5 and 7, respectively, which encode an active cannabinoid or cannabinoid analog biosynthesis enzyme, wherein the enzyme has an amino acid sequence that corresponds to SEQ ID NOs: 2, 4, 6, and 8, respectively, or a variant, fragment or portion of anyone of SEQ ID Nos: 2, 4, 6 and 8, and wherein the protein of the invention encompasses amino acid substitutions, additions and deletions that do not alter the function of the cannabinoid or cannabinoid analog biosynthesis enzyme.

[0111] A "variant" is a nucleotide or amino acid sequence that deviates from the standard, or given, nucleotide or amino acid sequence of a particular gene or protein. The terms "isoform," "isotype," and "analog" also refer to "variant" forms of a nucleotide or an amino acid sequence. An amino acid sequence that is altered by the addition, removal, or substitution of one or more amino acids, or a change in nucleotide sequence, may be considered a "variant" sequence. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. A variant may have "nonconservative" changes, e.g., replacement of a glycine with a tryptophan. Analogous minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted may be found using computer programs well known in the art.

[0112] The invention contemplates genetically engineering "non-cannabinoid or cannabinoid analog producing cells" with a nucleic acid sequence encoding an enzyme involved in the production of cannabinoids or cannabinoid analogs. Non-cannabinoid or cannabinoid analog producing cells refer to a cell from any organism that does not produce a cannabinoid or cannabinoid analog. Illustrative cells include but are not limited to plant cells, as well as insect, mammalian, yeast, fungal, algal, or bacterial cells.

[0113] "Fungal cell" refers to any fungal cell that can be transformed with a gene encoding a cannabinoid or cannabinoid analog biosynthesis enzyme and is capable of expressing in recoverable amounts the enzyme or its products. Illustrative fungal cells include yeast cells such as *Saccharomyces cerivisae* and *Pichia pastoris*. Cells of filamentous fungi such as *Aspergillus* and *Trichoderma* may also be used.

[0114] Cannabinoid acid synthase gene sequences may be obtained from a publicly available database. In a preferred aspect of the invention, one or more copies of a cannabinoid acid synthase gene are generated in vivo and the method comprises integrating one or more copies of the cannabinoid acid synthase gene into the genome of a eukaryotic host, such as *Pichia pastoris*, to scale up protein expression. Preferably, the cannabinoid acid synthase gene is codon optimized with an alpha secretion sequence to target protein secretion or tagged with six tandem histidine (SEQ ID NO: 9) residues at the 3' end to facilitate purification. This process comprises linearizing the cannabinoid acid synthase gene by digestion with one or more restriction enzymes; extracting the cannabinoid acid synthase gene by gel extraction; ligating the cannabinoid acid synthase gene into a

Pichia pastoris plasmid; and electroporating the plasmid into bacterial cells to generate one or more cannabinoid acid synthase gene copy colonies.

[0115] Thus, in one embodiment, one or more copies of alpha-CBDA synthase and alpha-THCA synthase sequences, for example, are generated by modification as described above, insertion into pPink-HC vector (Invitrogen®) and transformation into *E. coli* cells. The transformed cells may be stored as agar stabs for future use. Prior to transformation of yeast cells, the vector containing the cannabinoid acid synthase gene of interest (GOI) is isolated from the agar stabs containing the transformed *E. coli* cells, linearized using PmeI or SpeI restriction enzymes and the linearized plasmids thus obtained are electroporated into *Pichia pastoris* pepB deficient mutant cells using PichiaPink™ Yeast Expression Systems (Invitrogen®). Linearization with the restriction enzyme PmeI directs the insert into the AOX1 promoter region of the *Pichia* genome, whereas linearization with the restriction enzyme SpeI directs the insert into the TRP gene.

[0116] The transformed yeast cells may be grown on adenine-deficient selective plates and the colonies thus formed may be screened to identify positive transformants. Screening methods include, but are not limited to, color screening methodology. Typically, cells having 6-10 copies of the gene of interest are desired for obtaining large amounts of recombinant protein, for example, about 1.0 g to about 2.0 g of protein per liter of culture.

[0117] In one embodiment, individual white colonies of yeast cells carrying the THCA synthase gene or the CBDA synthase gene, for example, are separately cultured in flasks using BMGY medium, followed by induction by growth in BMMY medium, to induce the expression of THCA synthase or CBDA synthase as further described below. Briefly, the medium containing the enzyme in each culture is separated from the cells, reacted with a known amount of substrate and the product is analyzed. Cultures of transformants showing greater than 20% conversion are used for the commercial synthesis of cannabinoids or cannabinoid analogs pursuant to methods of the invention.

[0118] The cannabinoid acid synthase enzymes, THCA synthase and CBDA synthase, obtained using the PichiaPink™ Yeast Expression system described above, can be used for the manufacture of cannabinoids or cannabinoid analogs. The cannabinoid or cannabinoid analogs thus obtained are isolated, purified and used as therapeutics. In a further embodiment, the cannabinoids or cannabinoid analogs thus obtained undergo a decarboxylation step.

[0119] Cannabinoid synthases according to the invention include, but are not limited to, cannabidiolic acid (CBDA) synthase and tetrahydrocannabinolic acid (THCA) synthase.

[0120] In one embodiment, the invention provides a method for producing a cannabinoid or a cannabinoid analog by selecting a Formula I compound and a cannabinoid acid synthase as a catalyst for transforming the Formula I compound to a cannabinoid or a cannabinoid analog.

[0121] In Formula I, R can be selected from hydroxyl (—OH), halogen, thiol (—SH), or a —NR_aR_b group. Substituent groups R_1 and R_2 are each independently selected from the group consisting of —H, —$C(O)R_a$, —OR_a, an optionally substituted C_1-C_{10} linear or branched alkylene, an optionally substituted C_2-C_{10} linear or branched alkenylene, an optionally substituted C_2-C_{10} linear or branched alkynylene, an optionally substituted C_3-C_{10} aryl, an optionally substituted C_3-C_{10} cycloalkyl, (C_3-C_{10})aryl-(C_1-C_{10})alkylene, (C_3-C_{10})aryl-(C_2-C_{10})alkenylene, and (C_3-C_{10})aryl-(C_1-C_{10})alkynylene. Alternatively, R_1 and R_2 together with the carbon atoms to which they are bonded form a C_5-C_{10} cyclic ring. For compounds according to Formula I, R_3 is selected from the group consisting of H, —$C(O)R_a$ and C_1-C_{10} linear or branched alkyl and R_a and R_b are each independently —H, —OH, (C_1-C_{10}) linear or branched alkyl, —SH, —NH_2, or a C_3-C_{10} cycloalkyl.

[0122] R_2 can be a linear alkylene or a branched alkylene. Exemplary of linear alkylenes include without limitation CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15} and C_8H_{17}. Illustrative of branched alkylenes are groups selected from, iso-propyl, sec-butyl, iso-butyl, neopentyl, 2-methyl hexyl, or 2,3-dimethyl hexyl groups. In some embodiments, R_2 can be an optionally substituted linear or branched alkylene in which one or more hydrogen atoms is replaced without limitation with a group selected from chlorine, fluorine, bromine, nitro, amino, hydroxyl, phenyl, or benzyl group.

[0123] In one embodiment, R_1 and R_2 together with the ring carbon atoms to which they are bonded form a C_5-C_{10} cyclic ring. For such Formula I compounds one or more carbon atoms of the ring can be substituted with a heteroatom selected from oxygen, sulfur or nitrogen.

[0124] In another embodiment, R_2 is a C_2-C_{10} alkenylene and is selected from the group consisting of
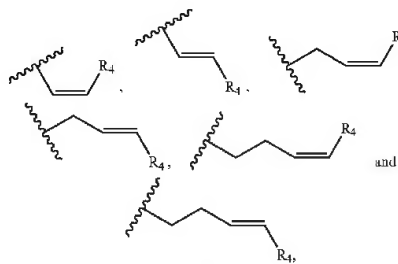
with R_4 being a linear or branched alkylene as described above. When R_2 is a C_2-C_{10} linear or branched alkynylene, R_2 can be

Formula I
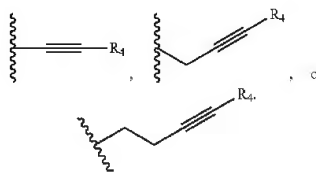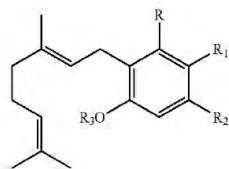
Alternatively, R_2 in Formula I is

[0125]
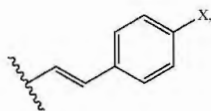
substituent X is a group selected from

—OH, —SH, or NR_aR_b and groups R_a and R_b. are as defined above.

[0126] In one embodiment, the cannabinoids and/or cannabinoid analogs synthesized according to the invention have a carboxylic acid (—COOH) group as the R_1 substituent and may undergo an optional decarboxylation step prior to their use as pharmaceutical or nutraceutical agents. Examples of cannabinoids or cannabinoid analogs having a carboxylic acid group include, but are not limited to, compounds obtained by reacting a compound of Formula I in which R is —OH, R_1 is —COOH, R_2 is C_5H_{11} and R_3 is —H with a cannabinoid acid synthase obtained as described above.

[0127] The synthesis, isolation and purification of cannabinoids or cannabinoid analogs can be improved by immobilization of a cannabinoid acid synthase to a solid support, or by encapsulation of the synthase within a liposome. In one aspect, the enzyme is immobilized to a solid support. Without being bound to any theory, the inventors of the present application have unexpectedly discovered that immobilization facilitates use and recovery of the enzyme catalyst, purification of the desired product, and preservation of the enantiomeric excess (ee) of the final product, and provides an overall improvement in the yield of the product. Furthermore, immobilization permits recycling and reuse of the immobilized enzyme which significantly reduces the costs associated with the manufacture of pharmaceutical grade cannabinoids or cannabinoid analogs. Typically, the enantiomeric purity of the cannabinoids and/or cannabinoid analogs produced according to the invention is from about 90% ee to about 100% ee, for instance, a cannabinoid or a cannabinoid analog produced using the inventive methodology can have an enantiomeric purity of about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee and about 99% ee.

[0128] Typically, the enzyme to be immobilized can be absorbed onto a solid support, adsorbed onto a support, covalently linked to a support or can be immobilized onto a solid support through ionic interactions. In one embodiment, the cannabinoid acid synthase is covalently linked to a solid support. Suitable strategies for linking an enzyme to a solid support are well known in the biochemical art and include covalent linkages between an appropriately functionalized support and a side chain of an amino acid group or through covalent linkages using appropriately functionalized linkers or spacers to separate the support from the enzyme. The term "linker" refers to any group that separates the support from the enzyme. Accordingly, a linker is a group that is covalently tethered at one end to a group on the surface of the support and is attached to the enzyme at the other end. Illustrative linkers include (C_1-C_{10})alkylene linker polymers

of ethylene glycol such as a —$(OCH_2—CH_2)_n$—O—group, where n is an integer from 0 to 10, —(C_1-C_{10})alkylene-NH—, —(C_1-C_{10})alkylenesiloxy, or a —(C_1-C_{10})alkylene-C(O)—.

[0129] Supports suitable for immobilizing enzymes include, but are not limited to, Amberlite resins, Duolite resins, acrylic resins such as Eupergit® C, DEAE-Sephadex and gels made using polyvinyl alcohol.

[0130] Cannabinoids exert different physiological properties and are known to lessen pain, stimulate appetite and have been tested as candidate therapeutics for treating a variety of disease conditions such as allergies, inflammation, infection, epilepsy, depression, migraine, bipolar disorders, anxiety disorder, and glaucoma. The physiological effects exerted by cannabinoids is affected by their ability to stimulate or deactivate the cannabinoid receptors, for instance the CB1, CB2 and CB3 receptors. Accordingly, the present invention provides the means to modulate cannabinoid receptor activity and their pharmaceutical properties by modifying the cannabinoid and/or cannabinoid analog binding interactions and the orientation of a ligand within the cannabinoid receptors active site by determining the nature and orientation of substituent groups attached to the cannabinoids and/or cannabinoid analogs produced according to the invention.

[0131] Thus, in one embodiment the invention provides a method for the manufacture of cannabinoids and cannabinoid analogs that have structurally distinct and diverse substituent groups attached to a central core and thus exhibit different pharmaceutically beneficial properties. Structural diversity is accomplished by contacting an appropriately substituted Formula III compound with a Formula IV compound in the presence of an enzyme, such as GPP olivetolate geranyltransferase (a polyketide synthase), to produce a compound of Formula II. Scheme 1 below structurally illustrates the protocol for synthesizing a Formula II compound pursuant to this embodiment.

Scheme 1

[0132]
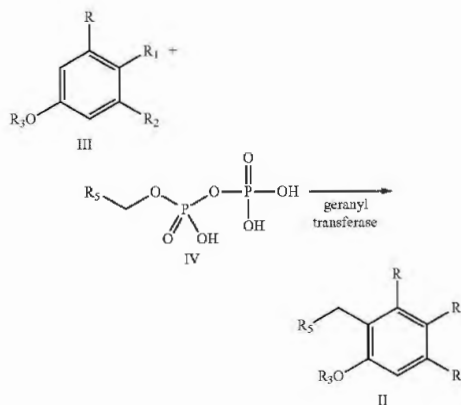
[0133] Different compounds of Formula II that serve as substrates for the manufacture of cannabinoids and/or cannabinoid analogs according to the invention may be obtained by varying the nature and type of substituent groups at R, R_1, R_2, R_3 and R_5, in the compounds of Formulas III and IV. According to this embodiment, therefore, different cannabinoids and/or cannabinoid analogs may be obtained by reacting a compound of Formula II with a cannabinoid acid synthase, for example, THCA synthase or CBDA synthase obtained as described above, followed by isolation and decarboxylation of the obtained product to give a cannabinoid or a cannabinoid analog.

[0134] In Formula III, R can be selected from hydroxyl (—OH), halogen, thiol (—SH), or a —NR_aR_b group. Substituents R_1 and R_2 are each independently selected from the group consisting of —H, —$C(O)R_a$, —OR_a, an optionally substituted linear or branched (C_1-C_{10})alkylene, an optionally substituted linear or branched (C_2-C_{10})alkenylene, an optionally substituted linear or branched (C_2-C_{10})alkynylene, an optionally substituted C_3-C_{10} aryl, an optionally substituted C_3-C_{10} cycloalkyl, (C_3-C_{10})aryl-(C_1-C_{10})alkylene, (C_3-C_{10})aryl-(C_2-C_{10})alkenylene, and (C_3-C_{10})aryl-(C_1-C_{10})alkynylene.

[0135] In certain embodiments R_1 and R_2 together with the carbon atoms to which they are bonded form a C_5-C_{10} cyclic ring and R_3 is selected from the group consisting of H, —$C(O)R_a$ and C_1-C_{10} linear or branched alkyl.

[0136] R_5 in Formula IV can be a linear or branched (C_1-C_{10})alkylene, a linear or branched (C_2-C_{10})alkenylene, a linear or branched (C_2-C_{10})alkynylene, —$C(O)$—(C_1-C_{10})alkylene, —$C(O)$—(C_2-C_{10})alkenylene and —$C(O)$—(C_2-C_{10})alkynylene. For Formulae II, III and IV compounds any alkylene, alkenylene, alkynylene, aryl, arylalkylene, or cycloalkyl group can be further substituted with one or more groups selected from the group consisting of —OH, halogen, —NR_bR_c, —$C(O)R_a$, —$C(O)NR_bR_c$, (C_1-C_{10})alkyl, —CN, (C_1-C_4)alkoxy, (C_1-C_4)haloalkyl, and (C_1-C_4)hydroxyalkyl with R_a, R_b and R_c each independently being selected from —H, —OH, or (C_1-C_{10}) linear or branched alkyl, —SH, —NH_2, or a C_3-C_{10} cycloalkyl.

[0137] According to one embodiment, R_5 in Formula IV can be a (C_2-C_{10})alkenylene selected from
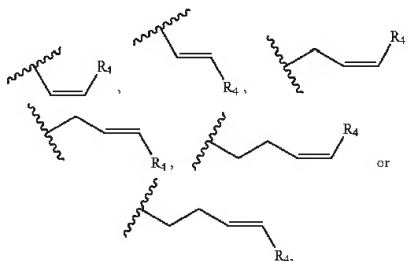
with R_4 being a linear alkylene selected from the group consisting of CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15} and C_8H_{17}. For certain Formula IV compounds R_5 is
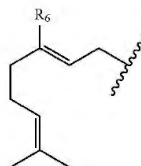
and group R_6 is selected from (C_1-C_{10})alkylene, (C_2-C_{10})alkenylene, —OH, —SH, NO_2, F, Cl, Br, —NH_2, or a —NHR_a where R_a is as defined above.

[0138] A recombinant cannabinoid acid synthase obtained by overexpressing a protein encoded by a recombinant cannabinoid acid synthase gene as described above is reacted with a substrate according to Formula I or with a substrate according to Formula II as described above in a reaction mixture comprising a solvent and an amphiphilic compound to produce one or more cannabinoids or cannabinoid analogs. The cannabinoids or cannabinoid analogs thus formed are isolated from the reaction mixture and optionally decarboxylated. Preferably, the recombinant cannabinoid acid synthase is a recombinant CBDA synthase or a recombinant THCA synthase obtained by the method described above. In a preferred aspect of the invention, the solvent in the reaction mixture is a non-aqueous solvent, such as dimethyl sulfoxide (DMSO), dimethyl formamide (DMF), or iso-propoyl alcohol. The concentration of the solvent in the reaction mixture may vary between 10% and 30% (v/v). The inventors of the present application have unexpectedly discovered that the concentration of the non-aqueous solvent in the reaction mixture affects the rate of the reaction as well as the ratio between the different cannabinoid products. Thus, the table below shows that in a reaction driven by the THCA synthase, the presence of DMSO in a concentration of 20% (v/v) in the reaction mixture increases the rate of the reaction by 2.5-fold and causes the reaction to produce THCA and CBCA in a ratio of 5:1, whereas the presence of DMSO in a concentration of 10% (v/v) in the reaction mixture produces THCA and CBCA in a ratio of 10:1. Accordingly, in a preferred aspect of the invention, the non-aqueous solvent in the reaction mixture is DMSO and the concentration of DMSO in the reaction mixture is most preferably 20% (v/v).

TABLE 1

Effect of DMSO Concentration on Reaction Rate and Products		
DMSO	Rate of Reaction	THCA:CBCA
0%	1X	
10%	1.2X	10:1
20%	2.5X	5:1
25%	—	1:1
30%	0.3X	

[0139] In an additional preferred embodiment of the invention, the reaction mixture also comprises an amphiphilic compound. Preferably, the amphiphilic compound is a surfactant or a cyclodextrin. Surfactants may include, but are not limited to, cationic surfactants, ionic surfactants and anionic surfactants. Most preferably, the reaction mixture contains a cyclodextrin.

[0140] Cyclodextrins are natural cyclic oligosaccharides consisting of six or more 1-4 linked α-anhydro-glucose moieties, which may be produced from starch through an enzymatic reaction. Cyclodextrins are classified according to the number of glucose units as α-cyclodextrin (six units),

β-cyclodextrin (seven units) and γ-cyclodextrin (eight units). The structure of the cyclodextrin is shown below:

α-CD

β-CD

γ-CD
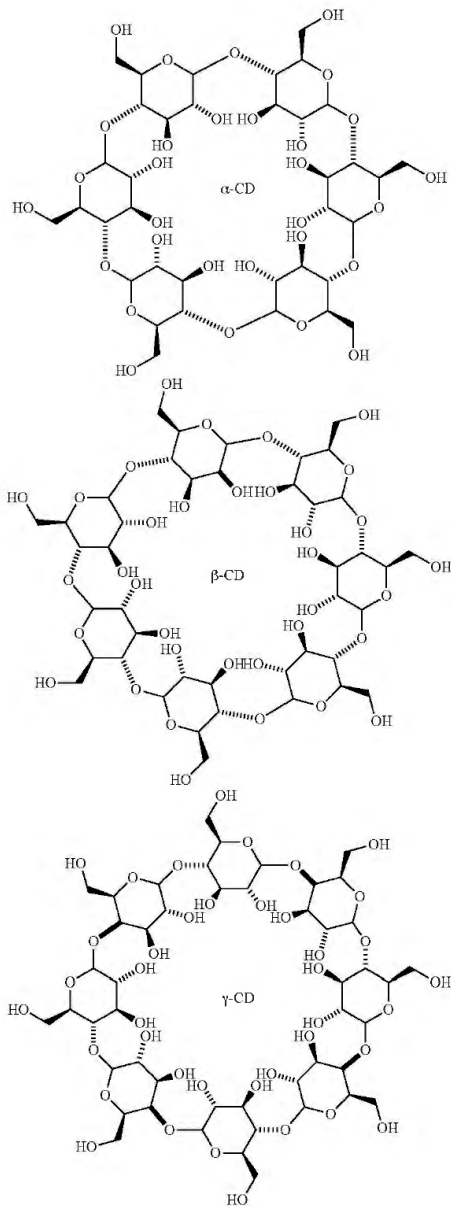
[0141] The secondary hydroxyl groups on the exterior side of the cyclodextrin molecule are hydrophilic, whereas the primary hydroxyl groups form the hydrophobic central cavity. Without being bound to any theory, it is believed that the hydrophobic central cavity in cyclodextrin incorporates the substrate in the reaction mixture as a guest molecule and the complex thus formed protects and stabilizes the substrate, although no covalent or ionic bonds are formed.

[0142] The inventors of the present application have unexpectedly discovered that the concentration of cyclodextrin in the reaction mixture affects the conversion rate of the substrate into the products as well as the ratio between the different products of the reaction, as shown in the table below. shows the effect of cyclodextrin concentration in the reaction mixture on CBDA synthase reaction at pH 4.85.

[0143] As shown in the table below, increasing the concentration of cyclodextrin from 0 mg/ml to 28 mg/ml in the CBDA synthase enzyme reaction increases the conversion rate of CBGA to CBDA and CBCA, with the highest conversion rates seen when cyclodextrin concentrations were 8 mg/ml and 12 mg/ml (20% higher conversion rate comparing to no cyclodextrin added to the reaction).

[0144] Addition of cyclodextrin also slightly changes the ratio of CBDA:CBCA at pH 5. The highest CBDA:CBCA ratio (CBDA:CBCA 1.41:1) was observed when cyclodextrin concentration was 20 mg/ml and the lowest CBDA:CBCA ratio (CBDA:CBCA 1.04:1) was observed when cyclodextrin concentration was 16 mg/ml.

TABLE 2

Effect of Cyclodextrin on CBDA Synthase Reaction Conversion Rate and Product Ratio

Cyclodextrin concentration	Conversion rate	CBDA:CBCA ratio	FIG.
0 mg/ml	40%	1.13:1	11
2 mg/ml	57%	1.24:1	12
4 mg/ml	N/A	N/A	N/A
8 mg/ml	61%	1.28:1	13
12 mg/ml	60%	1.33:1	14
16 mg/ml	50%	1.04:1	15
20 mg/ml	53%	1.41:1	16
28 mg/ml	45%	1.24:1	17

[0145] The cyclodextrin may be α-cyclodextrin, β-cyclodextrin or γ-cyclodextrin. In some embodiments, the cyclodextrin is sulfobuthylether β-cyclodextrin sodium salt or randomly methylated β-cyclodextrin. When present in the reaction mixture, the cyclodextrin is in a concentration of from about 0.001 to about 30 mg/ml. Preferably, the concentration of cyclodextrin in the reaction mixture is between 2 and 28 mg/ml. In a most preferred embodiment, the concentration of cyclodextrin in the reaction mixture is 8 mg/ml.

[0146] As shown in the table below and in FIGS. 11-17, with no cyclodextrin, increasing the amount of DMSO from 10% to 20% increased the conversion of CBGA from 13.5% to 45.8% overnight, and changed the ratio of THCA:CBCA from 3.33:1 to 2.24:1. Including cyclodextrin in the reaction with 10% DMSO, increased the conversion of CBGA to 89.7% and gave a ratio of 4.2:1 THCA:CBCA. Increasing the concentration of cyclodextrin to 40% or 60% gave the same results.

Condition	CBGA	THCA	CBCA	THCA:CBCA	Figure ID
10% DMSO, no cyclodextrin	86.51%	10.40%	3.0900%	3.37:1	1
20% DMSO, no cyclodextrin	54.18%	32.39%	13.43%	2.41:1	2
10% DMSO, 20% cyclodextrin	10.33%	72.37%	17.300%	4.20:1	3
10% DMSO, 40% cyclodextrin	11.50%	72.08%	16.42%	4.39:1	4
10% DMSO, 60% cyclodextrin	10.36%	73.98%	15.65%	4.73:1	5

[0147] The cannabinoids or cannabinoid analogs produced according to the methods of the invention are may be single enantiomers with an enantiomeric purity of at least 95%, and preferably of at least 99%.

[0148] The inventors of the present application have also unexpectedly discovered that the pH of the reaction mixture affects the ratio between the different cannabinoid products obtained. Accordingly, in a preferred embodiment, the pH of the reaction mixture is modified to obtain the cannabinoid products and/or cannabinoid analog products in the desired ratio. Thus, when reacted with a compound of Formula I according to the invention, THCA synthase may produce tetrahydrocannabinol (THCA), cannabichromene (CBCA), THCA and CBCA, or analogs thereof in different ratios, according to the pH of the reaction. Preferably, the reaction is performed at a pH in a range between 3.8 and 7.2, and the method produces THCA, CBCA, or THCA and CBCA in a ratio as shown in the following table at each specified pH:

TABLE 3

Effect of pH on THCA Synthase Reaction Products

pH	THCA	CBCA
4	1	0
5	2.33	1
6	1	5.67
7	0	1

[0149] In summary, changing the pH of the THCA synthase enzyme reaction affects the products. At pH 4 THCA is the only product. At pH 5 the ratio of THCA:CBCA is 2.33:1. At pH 6 the ratio is reversed and the product mix is THCA:CBCA 1:5.67. At pH 7 CBCA is the only product. Under these conditions, 98% of the compound of Formula I is converted into one or more cannabinoids or cannabinoid analogs within two hours.

[0150] Similarly, when reacted wit a compound of Formula I, CBDA synthase may produce cannabidiol (CBDA), cannabichromene acid (CBCA), CBDA and CBCA, or analogs thereof in different ratios, according to the pH of the reaction. Preferably, the reaction is performed at a pH in a range between 3.8 and 7.2, and the method produces CBDA, CBCA, or CBDA and CBCA in a ratio as shown in the following table at each specified pH:

TABLE 4

Effect of pH on CBDA Synthase Reaction Products

pH	CBDA	CBCA
4.2	2.5	1
5	1.13	1

TABLE 4-continued

Effect of pH on CBDA Synthase Reaction Products

pH	CBDA	CBCA
5.2	1	1.17
5.4	1	2.45
5.8	1	6.14
6.2	1	28.13
6.8	0	0

[0151] In summary, changing the pH of the CBDA synthase enzyme reaction affects the products. At pH 4.2 the CBDA:CBCA ratio is 2.5:1. At pH 5 the ratio of CBDA:CBCA is 1.13:1. At pH 6.8 there is no product forming from CBDA synthase enzyme reaction. Under these conditions, 70% of the compound of Formula I is converted into one or more cannabinoids or cannabinoid analogs within two hours.

[0152] The invention also provides a method of producing one or more cannabinoids or cannabinoid analogs according to Formula II

wherein the method comprises the steps of: (a) reacting a compound according to Formula III with a compound according to Formula IV;

III

IV

in the presence of an enzyme that catalyzes the reaction of the Formula III and Formula IV compounds to form a

17
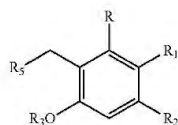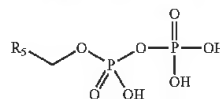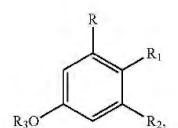
Formula II compound; (b) reacting the compound of Formula II with a cannabinoid acid synthase in a reaction mixture comprising a solvent and an amphiphilic compound as described above to produce one or more cannabinoids or cannabinoid analogs; (c) isolating from the reaction mixture one or more cannabinoids or cannabinoid analogs produced in step (b); and (e) optionally decarboxylating the one or more cannabinoids or cannabinoid analogs isolated in step (c). R in Formula III may be selected from —OH, halogen, —SH, or a —NR_aR_b group; R_1 and R_2 are each independently selected from the group consisting of —H, —C(O) R_a, —OR_a, an optionally substituted linear or branched (C_1-C_{10})alkylene, an optionally substituted linear or branched (C_2-C_{10})alkenylene, an optionally substituted linear or branched (C_2-C_{10})alkynylene, an optionally substituted C_3-C_{10} aryl, an optionally substituted C_3-C_{10} cycloalkyl, (C_3-C_{10})aryl-(C_1-C_{10})alkylene, (C_3-C_{10})aryl-(C_2-C_{10})alkenylene, and (C_3-C_{10})aryl-(C_1-C_{10})alkynylene, or R_1 and R_2 together with the carbon atoms to which they are bonded form a C_5-C_{10} cyclic ring; R_3 is selected from the group consisting of H, —C(O)R_a and C_1-C_{10} linear or branched alkyl. R_5 in Formula IV may be selected from the group consisting of a linear or branched (C_1-C_{10})alkylene, a linear or branched (C_2-C_{10})alkenylene, a linear or branched (C_2-C_{10})alkynylene, —C(O)—(C_1-C_{10})alkylene, —C(O)—(C_2-C_{10})alkenylene and —C(O)—(C_2-C_{10})alkynylene; wherein any alkylene, alkenylene, alkynylene, aryl, arylalkylene, or cycloalkyl group is further substituted with one or more groups selected from the group consisting of —OH, halogen, —NR_bR_c, —C(O)R_a, —C(O)NR_bR_c, (C_1-C_{10}) alkyl, —CN, (C_1-C_4)alkoxy, (C_1-C_4)haloalkyl, and (C_1-C_4) hydroxyalkyl; and R_a, R_b and R_c are each independently —H, —OH, —SH, —NH_2, (C_1-C_{10}) linear or branched alkyl, or a C_3-C_{10} cycloalkyl.

[0153] In one embodiment, R_5 is a (C_2-C_{10})alkenylene selected from the group consisting of
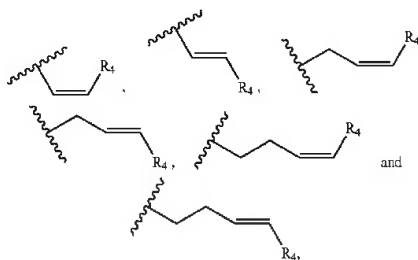
and R_4 is a linear alkylene selected from the group consisting of CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15} and C_8H_{17}. In a preferred aspect of the invention, R_5 is
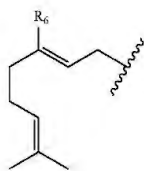
and R_6 is selected from (C_1-C_{10})alkylene, (C_2-C_{10})alkenylene, —OH, —SH, NO_2, F, Cl, Br, —NH_2, or —NHR_a.

[0154] In yet another embodiment, the invention provides a method for producing a tetrahydrocannabinol, cannabichromene, or both tetrahydrocannabinol and cannabichromene, or their analogs, wherein the method comprises the steps of: (a) selecting a compound according to Formula I;

Formula I

(b) reacting the compound of Formula I with a tetrahydrocannabinolic acid (THCA) synthase in a reaction mixture comprising a solvent and an amphiphilic compound as described above; (c) modifying at least one property of the reaction mixture, such as the pH of the reaction, the nature and/or concentration of the non-aqueous solvent and/or the concentration of an amphiphilic compound, such as cyclodextrin, to obtain a tetrahydrocannabinol, a cannabichromene, or both tetrahydrocannabinol and cannabichromene, or their analogs as products as described above; (d) isolating tetrahydrocannabinol, cannabichromene, or both tetrahydrocannabinol and cannabichromene, or their analogs from the reaction mixture; and (e) decarboxylating the tetrahydrocannabinol, cannabichromene, or both tetrahydrocannabinol and cannabichromene, or their analogs. R in Formula I may be selected from —OH, halogen, —SH, or a —NR_aR_b group; R_1 and R_2 are each independently selected from the group consisting of —H, —C(O)R_a, —OR_a, an optionally substituted C_1-C_{10} linear or branched alkylene, an optionally substituted C_2-C_{10} linear or branched alkenylene, an optionally substituted C_2-C_{10} linear or branched alkynylene, an optionally substituted C_3-C_{10} aryl, an optionally substituted C_3-C_{10} cycloalkyl, (C_3-C_{10})aryl-(C_1-C_{10})alkylene, (C_3-C_{10})aryl-(C_2-C_{10})alkenylene, and (C_3-C_{10})aryl-(C_1-C_{10})alkynylene, or R_1 and R_2 together with the carbon atoms to which they are bonded form a C_5-C_{10} cyclic ring; R_3 is selected from the group consisting of H, —C(O)R_a and C_1-C_{10} linear or branched alkyl; and R_a and R_b are each independently —H, —OH, —SH, —NH_2, (C_1-C_{10}) linear or branched alkyl, or a C_3-C_{10} cycloalkyl.

[0155] In a different embodiment, the invention also provides a method for producing a cannabidiol, cannabichromene, or both cannabidiol and cannabichromene, or their analogs comprising the steps of: (a) selecting a compound according to Formula I;

Formula I

(b) reacting the compound of Formula I with a cannabino-diolic acid (CBDA) synthase in a reaction mixture comprising a solvent and an amphiphilic compound as described above; (c) modifying at least one property of the reaction mixture, such as the pH of the reaction, the nature and/or concentration of the non-aqueous solvent and/or the concentration of an amphiphilic compound, such as cyclodextrin, to obtain a cannabidiol, a cannabichromene, or both cannabidiol and cannabichromene, or their analogs as products; (d) isolating cannabidiol, cannabichromene, or both cannabidiol and cannabichromene, or their analogs from the reaction mixture; and (e) decarboxylating the cannabidiol, cannabichromene, or both cannabidiol and cannabichromene, or their analogs. R in Formula I may be selected from —OH, halogen, —SH, or a —NR$_a$R$_b$ group; R$_1$ and R$_2$ are each independently selected from the group consisting of —H, —C(O)R$_a$, —OR$_a$, an optionally substituted C$_1$-C$_{10}$ linear or branched alkylene, an optionally substituted C$_2$-C$_{10}$ linear or branched alkenylene, an optionally substituted C$_2$-C$_{10}$ linear or branched alkynylene, an optionally substituted C$_3$-C$_{10}$ aryl, an optionally substituted C$_3$-C$_{10}$ cycloalkyl, (C$_3$-C$_{10}$)aryl-(C$_1$-C$_{10}$)alkylene, (C$_3$-C$_{10}$)aryl-(C$_2$-C$_{10}$)alkenylene, and (C$_3$-C$_{10}$)aryl-(C$_1$-C$_{10}$)alkynylene, or R$_1$ and R$_2$ together with the carbon atoms to which they are bonded form a C$_5$-C$_{10}$ cyclic ring; R$_3$ is selected from the group consisting of H, —C(O)R$_a$ and C$_1$-C$_{10}$ linear or branched alkyl; and R$_a$ and R$_b$ are each independently —H, —OH, —SH, —NH$_2$, (C$_1$-C$_{10}$) linear or branched alkyl, or a C$_3$-C$_{10}$ cycloalkyl. Thus, the present inventors have devised methods that produce different cannabinoids and/or cannabinoid analogs in the desired ratio and in a controlled manner, by varying the pH of the reaction, the nature and/or concentration of the non-aqueous solvent and/or the concentration of an amphiphilic compound, such as cyclodextrin, in the reaction mixture.

Apparatus and Methods for Producing Cannabinoids or Cannabinoid Analogs

[0156] An apparatus or system is provided for producing one or more cannabinoids or cannabinoid analogs according to the methods of the invention. The apparatus may comprise a fermentor, a filter, a bioreactor, and a control mechanism. FIG. 7 depicts an apparatus 100 configured to produce at least one cannabinoid and/or at least one cannabinoid analog according to an embodiment. As shown in FIG. 7, the apparatus 100 includes a fermentor 10, a filter 20, a bioreactor 30, and a control mechanism (controller) 40. The fermentor 10 holds cell culture medium 12 and a plurality of cells 14. The cells 14 produce and secrete a cannabinoid acid synthase. The cells 14 grown in the fermentor 10 for the manufacture of a cannabinoid acid synthase can be yeast, prokaryotic or eukaryotic cells that have been genetically modified to include a nucleic acid sequence or a gene that encodes a cannabinoid acid synthase protein. In certain embodiments, the nucleic acid sequence that encodes a cannabinoid acid synthase protein is modified to include a yeast alpha secretion sequence at its 5' end and to incorporate a 6-residue histidine tag (SEQ ID NO: 9) at its 3' end. The addition of the yeast alpha secretion sequence permits secretion of the cannabinoid acid synthase protein into the medium 12 used for eukaryotic cell growth. Following production of cannabinoid acid synthase in the fermentor 10,

the supernatant comprising the medium 12 and cells 14 (and cannabinoid synthase), is transported along a pathway to the filter 20.

[0157] The filter 20 may filter the supernatant to at least partially separate the cells 14 from the medium 12 containing the expressed enzyme. Typically, the filter 20 separates at least 80% of the total cells 14 from the medium. In some embodiments, the filter 20 separates at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the total cells 14 from the medium 12. Following filtration, the cells 14 are transported back to the fermentor 10. In one embodiment, the filter 20 can be a filtration and purification system that includes multiple filters and reservoirs to purify the cannabinoid synthase.

[0158] After passing through the filter 20, the cannabinoid acid synthase flows into the bioreactor 30 and enters the bioreactor 30 through an inlet 32. The bioreactor 30 also includes an inlet 34 for reactants, such as the substrate CBGA or other substrates according to the Formula I compound described above.

[0159] In some embodiments, the bioreactor 30 can be a column bioreactor having a support 36. The support 36 may be a solid support that is impregnated with divalent metal ions or a support whose surface is functionalized with divalent metal ions. Typically, sepharose, agarose or other biopolymers are used as supports for binding divalent metal ions such as nickel, cobalt, magnesium and manganese. Such supports have a strong affinity for the histidine tag that is present on the expressed cannabinoid acid synthase and can be used to sequester the synthase and separate it from other non-essential proteins and debris that may interfere or impede cannabinoid synthesis.

[0160] The bioreactor 30 used for synthesizing cannabinoids is configured for batch and continuous synthetic processes to permit commercial production of pharmaceutically useful cannabinoids. In one embodiment, the bioreactor 30 is configured for batch synthesis in which the composition of the medium, concentration of the enzyme and substrate are fixed at the beginning of the process and not allowed to change during catalysis. Synthesis is terminated when the concentration of the desired product in the medium of the bioreactor 30 reaches a predetermined value or the concentration of substrate falls below a predetermined level, such as to a level where there is no detectable catalytic conversion of substrate to product.

[0161] In one embodiment, therefore, the His-tagged cannabinoid acid synthase is sequestered onto a nickel containing resin support within the bioreactor 30 prior to the introduction of a known amount of substrate, for example, cannabigerolic acid (CBGA), or a compound of Formula I or Formula II into the bioreactor 30. In an alternate embodiment, CBGA or a compound of Formula I or Formula II can be present within the bioreactor 30 having a nickel resin support prior to the introduction of the medium containing a cannabinoid acid synthase into the bioreactor 30.

[0162] The progress of the reaction within the bioreactor 30 can be monitored periodically or continuously. For instance, an optical monitoring system 50 may be utilized to detect the concentration of product in the medium within the bioreactor as a function of time. Alternatively, the decrease in the concentration of substrate can be monitored to signal termination of synthesis. The cannabinoid product thus produced can be readily recovered from the medium using

standard solvent extraction or chromatographic purification methods. The monitoring system **50** may be part of or may interact with a control mechanism **40** (a controller) described further below.

[0163] An alternative to the batch process mode is the continuous process mode in which a defined amount of substrate and medium are continuously added to the bioreactor **30** while an equal amount of medium containing the cannabinoid product is simultaneously removed from the bioreactor **30** to maintain a constant rate for formation of product. The medium can enter the bioreactor **30** through the inlet **32** and exit the bioreactor through an outlet **38**.

[0164] The conditions of the bioreactor can be controlled using a control mechanism **40**. The control mechanism **40** may be coupled to the bioreactor **30** or, alternatively, may interact with the bioreactor **30** wirelessly or remotely. The control mechanism **40** may also be used to control the conditions of the fermentor **10**, such the oxygen level, agitation, pH, and feed rate. The control mechanism **40** may also control the flow of materials (e.g. by controlling at least one pump) into and out of the fermentor **10**, filter **20**, and bioreactor **30**. In some embodiments, the control mechanism **40** is configured to control the conditions of at least one of the fermentor **10**, the filter **20** and the bioreactor **30** based on information obtained from the optical monitoring system **50**.

[0165] The control mechanism **40** may include a processing circuit having a processor and memory device. The processor and memory are configured to complete or facilitate the various processes and functions described in the present application, such as controlling the pH, temperature, and pressure of the bioreactor **30**, or altering the flow rate of medium into or out of the bioreactor **30**. In some embodiments, for facilitating the control of pH, temperature, pressure and flow rate, the control mechanism **40** may be configured to communicate with at least one sensor in a sensor suite **60**. The sensor suite **60** may include a pH sensor **62**, a temperature sensor **64**, and a pressure sensor **66**. The control mechanism **40** may include a proportional-integral-derivative (PID) controller for feedback-based control. The control mechanism **40** may be further configured to regulate the flow rate of materials into and out of the fermentor **10**, the filter **20** and the bioreactor **30** via pulse width modulation (PWM) techniques.

[0166] FIG. **10** depicts the control mechanism **40**. The control mechanism **40** includes a processor **43** coupled to a communication bus **48**. The control mechanism **40** further includes a main memory **42**, such as a random access memory (RAM) or other dynamic storage device, coupled to the bus **48** for storing information, and configured to store instructions to be executed by the processor **43**. The main memory **42** is further configured to store temporary variables and intermediate information during execution of instructions by the processor **43**. The control mechanism **40** may additionally include a read only memory (ROM) **44** or other static storage device connected to the bus **48** for storing information and instructions. Additionally, a storage device **46**, such as a solid state device, magnetic disk or optical disk, may be coupled to the bus **48** for persistently storing information and instructions.

[0167] Furthermore, the control mechanism **40** may be coupled (via the bus **48**) to a display **77**, such as a liquid crystal display, or active matrix display, for displaying information to a user. In some embodiments, an input device **11**, such as a keyboard, may also be coupled to the bus **48** for communicating information, and to convey commands to the processor **43**. In some embodiments, the input device **11** has a touch screen display.

[0168] In some embodiments, the bioreactor **30** is not a column reactor. Instead, as shown in FIG. **8**, the bioreactor **30** comprises a plurality of microtiter plates and is provided in a system **200**. The system **200**, like the system **100**, includes a controller **40** configured to control the bioreactor **30**. The controller **40** may control the environmental conditions of the bioreactor **30** and the supply of materials to the bioreactor **30**, and may also control operations performed on the plurality of microtiter plates.

[0169] In some embodiments, each of the microtiter plates of system **200** has 96 wells. In other embodiments, at least one microtiter plate has 384 wells, 1,536 wells, 3456 wells, or 9600 wells. In embodiments with 96-well microtiter plates, an enzyme reaction may take place in each of the 96 wells. The reaction in each well make take place in a volume of 0.5 ml or in a volume exceeding 0.5 mL. The apparatus described above are configured to produce cannabinoid acids or cannabinoid acid analogs, specifically, THCA and CBCA or CBDA and CBCA, by implementing the techniques described below in reference to FIG. **9**. FIG. **9** illustrates an automated method (**900**) for producing cannabinoids or cannabinoid analogs according to an embodiment. The method includes providing cannabinoid CBG, DMSO, and a cannabinoid biosynthetic enzyme in each of the wells (**901**). The cannabinoid biosynthetic enzyme may be, for example, THCA synthase. The cannabinoid biosynthetic enzyme is produced by the fermentor **10** by growing yeast transformed with a gene encoding THCA synthase, as described above. The cannabinoid CBG, on the other hand, is chemically synthesized. The cannabinoid CBG, the DMSO and cannabinoid biosynthetic enzyme may be considered to be the 'starting materials' introduced into the bioreactor to ultimately yield at least one cannabinoid or cannabinoid analog. The cannabinoid CBG, DMSO, and cannabinoid biosynthetic enzyme may be provided in each of the wells via automatic pipetting. In other words, an apparatus such as the systems **100**, **200** may comprise mechanized componentry that may be controlled, for example, by the control mechanism **40**, so as to deliver an appropriate amount of at least one of the cannabinoid CBG, DMSO, and cannabinoid biosynthetic enzyme to each well of the microtiter plates. In some implementations, operations shown in FIG. **9** may be performed iteratively by virtue of such automation. For example, an automated dispensing system or automated delivery system may be configured as a supply mechanism and used to deliver at least one of CBG, DMSO and THCA synthase and a solvent. In some implementations, the systems **100**, **200** may be configured with a programmable sample changer configured to automate sample preparation and transfer. The programmable sample changer may be, for example, the Gilson **223** Sample Changer produced by Gilson, Inc. of Middleton, Wis., USA, and may be operable with peristaltic and/or syringe pumps.

[0170] The method further includes reacting the cannabinoid CBG and the cannabinoid biosynthetic enzyme such as THCA synthase in the DMSO once these materials are distributed in the wells (**902**). The method further includes, in some implementations, determining a ratio of THCA to CBCA or a ratio of CBDA to CBCA to be produced by the reaction (**903**). In some implementations, the control mechanism **40** determines a quantity of THCA and a quantity of

CBCA to be produced, or a quantity of CBDA and a quantity of CBCA to be produced. Next, the method includes determining whether the pH of the reaction mixture requires adjusting in order to yield the predetermined ratio of THCA to CBCA or the predetermined ratio of CBDA to CBCA (904). Specifically, the pH may be adjusted by altering the composition of the reaction mixture to obtain a desired ratio of THCA:CBCA or a desired ratio of CBDA:CBCA. The reaction culminates in the production of THCA and CBCA or CBDA and CBCA (905).

[0171] The method further includes automatically pipetting a solvent into each well of the microtiter plate (906). The addition of the solvent results in cessation of the reaction. The method additionally includes, following the introduction of the solvent into the wells and cessation of the reaction, recovering cannabinoids or cannabinoid analogs in the solvent layer.

[0172] Once the reaction has ceased, the resulting solvent layer is removed (907), and the cannabinoids or cannabinoid analogs are recoverable. More specifically, the cannabinoids or cannabinoid analogs are recoverable from a solvent fraction present in each of the wells via vacuum evaporation or ethanol extraction (908). In some embodiments, a rotary evaporator is used to remove the solvent. The rotary evaporator may be an automated rotary evaporator such as the fully automated POWERVAP® rotary evaporator produced by Genser Scientific Instruments of Rothenburg ob der Tauber, Germany. Upon removal of the solvent, the cannabinoids or cannabinoid analogs are left in the bottoms of the wells. The method further includes re-suspending the cannabinoids or cannabinoid analogs (909). The cannabinoids or cannabinoid analogs may be re-suspended in ethanol, liposomes, or lipid micelles.

[0173] The process illustrated in FIG. 9 permits recovery of cannabinoids or cannabinoid analogs that may be readily formulated into pharmaceuticals and marijuana-infused products including beverages, confectionery, and cosmetics, among other examples. The cannabinoids or cannabinoid analogs may be readily purified via HPLC for pharmaceutical applications.

[0174] In at least one implementation, 0.5 mg of buffered CBG, buffered THCA synthase or buffered CBDA synthase with or without stabilizer, and DMSO are automatically pipetted into each of a plurality of wells of a microtiter plate. The DMSO that is added may have a final concentration of 20%, in some implementations. The ensuing reaction in each of the plurality of wells generally yields approximately 0.5 mg of cannabinoids when incubated for 2 hours, 4 hours, 12 hours, and 24 hours. Thus, for a microtiter plate including 96 wells, the system 100 produces about 48 mg of cannabinoids. It follows that the amount of cannabinoids produced 'scales up' when a plurality of microtiter plates are used. For example, if 21 microtiter plates are used, each having 96 wells, then 1008 mg (about 1 gram of cannabinoids) may be produced according to the techniques described above. Using 315 microtiter plates produces approximately 150 grams of cannabinoids. In some implementations, volumes greater than 0.5 mg may be used for the reaction mixture.

[0175] In some embodiments, the processor of the controller can be implemented as a general purpose processor, an application specific integrated circuit (ASIC), one or more field programmable gate arrays (FPGAs), a group of processing components, or other suitable electronic processing components. The memory device (e.g., memory,

memory unit, storage device, etc.) is one or more devices (e.g., RAM, ROM, Flash memory, hard disk storage, etc.) for storing data and/or computer code for completing or facilitating the various processes and functions described above. The memory device may be or include volatile memory or non-volatile memory. The memory device may include database components, object code components, script components, or any other type of information structure for supporting the various activities and information structures described in the present application. According to one embodiment, the memory device is communicably connected to the processor via the processing circuit and includes computer code for executing (e.g., by the processing circuit and/or processor) one or more processes described herein.

[0176] The present disclosure contemplates methods, apparatus and program products on any machine-readable media for accomplishing various operations, such as controlling the conditions of the bioreactor. The embodiments of the present disclosure may be implemented using existing computer processors, or by a special purpose computer processor for an appropriate system, incorporated for this or another purpose, or by a hardwired system. Embodiments within the scope of the present disclosure include program products comprising machine-readable media for carrying or having machine-executable instructions or data structures stored thereon. Such machine-readable media can be any available media that can be accessed by a general purpose or special purpose computer or other machine with a processor. By way of example, such machine-readable media can comprise RAM, ROM, EPROM, EEPROM, CD-ROM or other optical disk storage, magnetic disk storage, other magnetic storage devices, solid state storage devices, or any other medium which can be used to carry or store desired program code in the form of machine-executable instructions or data structures and which can be accessed by a general purpose or special purpose computer or other machine with a processor. When information is transferred or provided over a network or another communications connection (either hardwired, wireless, or a combination of hardwired or wireless) to a machine, the machine properly views the connection as a machine-readable medium. Thus, any such connection is properly termed a machine-readable medium. Combinations of the above are also included within the scope of machine-readable media. Machine-executable instructions include, for example, instructions and data which cause a general purpose computer, special purpose computer, or special purpose processing machines to perform a certain function or group of functions.

[0177] The control mechanism may further include additional devices, such as a keyboard and display, to allow a user to interact with the control mechanism to control the conditions of the bioreactor. For example, the display may include a screen to allow a user to monitor changes in pH, temperature, pressure, and flow rate of the bioreactor, or to monitor any other condition of the system for producing cannabinoids or cannabinoid analogs. The present invention is further described by the following examples which are not meant to limit the scope of the claims.

EXAMPLES

[0178] A. Molecular Cloning, Screening and Expression of Protein from High Yield Yeast Transformants

1. Restriction Digestion.

[0179] THCA α plasmid DNA and CBDA α plasmid DNA were linearized by digesting each plasmid with Pme I or Spe

I restriction enzymes at 37° C. for two hours. Linearized plasmids were verified on 0.8% agarose gel by electrophoresis. Qiagen Gel Extraction kit was used to extract the linearized plasmid from the agarose gel and the plasmids were frozen at −20° C. until use. 2. Preparation of Electrocompetent Yeast Cells.

Electrocompetent PichiaPink (pPink) cells were made by inoculating 10 mL of YPD media with a glycerol stock of a genetically engineered Ade2, pep4 knockout pPink yeast strain 2. These cells were grown overnight in a 125 ml baffled flask at 28° C., using a shaker spinning at 270 rpm until the OD_{600} of the culture reached a value of 1.3 units indicating log phase growth. This culture was then added to 100 ml of YPD media and allowed to incubate overnight under the same conditions. The OD_{600} was checked hourly and after a 12 hour incubation period reached a value of 1.3 units.

[0180] After reaching log phase growth the cells were transferred to a 500 ml centrifuge tube and spun down for 5 minutes at 4° C. and 2500 rpm. The YPD broth was decanted and 250 ml of sterile ice-cold water was added and the cells re-suspended. The cells were then centrifuged at 4° C., 2500 rpm for another 5 minutes, re-suspended with an additional 250 ml of water to ensure removal of all YPD media and centrifuged under the same conditions again. The water was then decanted and 50 ml of sterile ice-cold water was added and the cells re-suspended and centrifuged under the same conditions. The water was then decanted and 10 ml of sterile, ice-cold 1M sorbitol was added and the cells re-suspended. The suspension was then transferred to a sterile 15 ml conical tube and centrifuged under the same conditions as before. The 1M sorbitol was then decanted, 300 µl of sterile ice-cold 1M sorbitol was added and the cells were re-suspended and placed on ice for use.

3. Electroporation

[0181] The previously frozen linearized plasmid DNA was thawed on ice and 80 µl of the electrocompetent pPink cells were added to the tube. This volume was then transferred to a 0.2 cm electroporation cuvette and incubated on ice for 5 minutes. The cuvette was then pulsed at 1640 V, 200Ω, and 25 µF for a total pulse time of approximately 4 minutes. Immediately after pulsing, 1 ml of YPDS media was added to the cuvette and mixed by pipetting. The cuvette was then placed in a 28° C. incubator, without shaking, for 2 hours, after which 300 µl was spread onto fresh PAD plates. The PAD plates were then placed into the 28° C. incubator for approximately 7-10 days and inspected each day for cell growth.

4. Screening

[0182] White colonies are indicative of positive expression of the gene of interest, whereas red colonies indicate no expression. All white colonies were selected and re-streaked onto fresh PAD plates and allowed to grow for 3-5 days until individual colonies appeared. A single colony was then used to inoculate 10 ml of BMGY in a 125 ml baffled flask and placed into an incubator overnight shaking at 28° C. and 270 rpm. When the OD_{600} reached 1.2-1.5 (after 1:10 dilution in water) the inoculum was transferred to a 50 ml conical tube and centrifuged at 2500 rpm for 5 minutes. The BMGY was decanted and 1 ml of BMMY was added. The tubes were

then covered with air porous tape to allow for sterile air exchange and placed into the shaking incubator at 28° C. and 270 rpm.

[0183] After 24 hours 100 µl of the sample were removed and 100 µl of 40% methanol were added. The removed portion was then centrifuged at 12,000 rpm for 5 minutes and the supernatant and pellet were saved as T=1 (day 1) samples. This procedure was then repeated after 48 hours (T=2). After 72 hours (T=3) the remaining sample was harvested as the final time point. The T=3 supernatant was then spun through an Amicon 30 kD protein filter and run on an SDS-PAGE for visualization of protein.

5. Enzymatic Conversion

[0184] Samples that had greater than 20% conversion of CBGA to CBDA over 4-24 hours were then scaled up. Briefly, enzymatic conversion reaction was as follows: 25 µl of cell free supernatant from the T=3 samples was incubated for 2 hours at 30° C., with 25 µl of a 1 mg/ml CBGA stock in DMSO in 200 µl of pH 4.8, 100 mM citrate buffer. Reaction yielded a final concentration of CBGA of 0.1 mg/ml at pH 5.0.

[0185] For Scale-Up, a single colony was used to inoculate 10 ml of BMGY in a 125 ml baffled flask which was incubated overnight at 28° C. and 270 rpm. The OD_{600} was measured after 24 hours, and once it reached 1.2 the 10 ml suspension was then used to inoculate 90 ml of BMGY in a 1 L baffled flask. The suspension was then allowed to incubate overnight at 28° C. and 270 rpm. When the OD_{600} reached 1.2-1.5 the inoculum was then transferred to a 500 ml centrifuge bottle and pelleted at 2500 rpm for 5 minutes.

[0186] The BMGY was decanted and the cell pellet washed with 10 ml of BMMY. After 2 washings the pellet was re-suspended with 10 ml of BMMY, transferred to a 500 ml baffled flask and allowed to incubate overnight at 28° C. and 270 rpm. After 24 hours 1 ml of the sample was removed (T=1) and 1 ml of 40% methanol was added. This was repeated after 48 and after 72 hours the full sample volume was harvested, separated and analyzed.

[0187] Table 5 below shows the results of small scale screening samples with greater than 20% conversion of CBGA to THCA that were selected for scale-up.

TABLE 5

Small Scale Screening Samples with Greater than 20% Conversion of CBGA to THCA

Sample ID	% Conversion of CBGA to THCA in reaction containing 0.1 mg/ml CBGA.
Spe THC #3	20.6
Spe THC #4	28.7
Spe THC #22	20.6
Spe THC #23	18.7
Pme THC #5	32.5
Pme THC(2) #1	29.1
Pme THC(2) #2A	27.2
Pme THC(2) #25	31.6
Pme THC(2) #36	27.7
Pme THC(2) #41	32.5
Pme THC(2) #42	27.6
Pme THC(2) #46	40.7
Pme THC(2) #51	26.8
Pme THC(3) #1	55.2

TABLE 5-continued

	Small Scale Screening Samples with Greater than 20% Conversion of CBGA to THCA
Sample ID	% Conversion of CBGA to THCA in reaction containing 0.1 mg/ml CBGA.
Pme THC(3) #11	35.0
Pme THC(3) #17	69.9
Pme THC(3) #19	36.8
Pme THC(3) #20	34.3

6. Cloning Strategy for Generating Multi-Copy GOI Inserts In Vitro.

[0188]   An alternate yeast expression system was used to obtain transformed cells having one or more copies of the gene of interest. The multi-copy *Pichia* Expression Kit from Invitrogen was used to construct new plasmids that could generate multi-copy gene inserts in vitro or in vivo.

In Vitro Generation of Multi-Copy Inserts

[0189]   To generate multi-copy GOI inserts in vitro, the pAO815 vector was used to clone the gene of interest. α-CBDA synthase and α-THCA synthase were cut with EcoR I and Bam HI from pPink-HC plasmid by incubating 100 ng of the pPink-HC vector containing the α-CBDA synthase gene or the α-THCA synthase gene with 1 μl of EcoR I buffer, 1 μl of each restriction enzyme (10 units/μl) and 1 μl of BSA in 20 μl total reaction volume at 37° C. for 2 hr. 100 ng of pAO815 vector was also digested with Eco R I and Bam HI enzymes following the same protocol.

[0190]   After digestion, the GOI and vectors mixture were run on a 0.8% agarose gel at 95 V for 1 hr. Bands of correct size were excised and extracted from the gel with Invitrogen gel extraction kit. The linearized vector and gene inserts were ligated using T4 DNA ligase protocol from NEB®. Upon ligation, the circular vector containing the gene of interest was transformed into *E. coli* Top 10 F⁻ cells to harvest plasmid by electroporation at 1500 V, 200Ω and 25 μF for 4 milliseconds. The transformed cells were then mixed with 250 μl of SOC medium (provided with One Shot® Top 10 Electrocomp™ *E. coli* from Invitrogen) and plated on a LB-Amp100 plate at 37° C. overnight. The next morning, positive colonies were identified with colony PCR protocol with 5' AOX1 and 3'AOX1 primers. Positive colonies containing the gene of interest were grown in liquid LB-Amp100 media overnight at 37° C. The next day plasmid mini-preps were done with Invitrogen's fast prep kit and the concentration of the plasmid was analyzed on 0.8% agarose gel before further amplification.

[0191]   The recombinant pAO815 plasmid containing the alpha-THCA synthase and alpha-CBDA synthase genes was divided into 2 batches, one batch was used as a vector in which was inserted a second copy of the gene of interest and one batch was used for extracting the alpha-THCA synthase or alpha-CBDA synthase genes. The vector batch was first digested with Bam HI following NEB's single digest protocol. The second batch was digested with Bgl II and Bam HI restriction enzymes. The linearized vector and genes were purified on a 0.8% agarose gel and extracted. The vector and genes were then ligated following NEB's T4 DNA ligase protocol and then transformed into *E. coli* Top10 F⁻ cells by electroporation as described above. The

cells were incubated at 37° C. overnight and then screened for the correct gene insert by PCR. Gene sequences were confirmed by sequencing. The multi-copy plasmids were linearized at the His4 sequence region by restriction enzyme digestion and transformed into competent *Pichia pastoris* strain G115 (his4, Mut+) cells. The transformed cells were grown on His-plates for screening. Screening was done on His⁻ plates to confirm integration of the plasmid at the His site of the *Pichia Pastoris* genome. Positive colonies were chosen for methanol induction of protein, time points protein SDS-gel and enzyme assay.

In Vivo Generation of Multi-Copy Inserts

[0192]   To generate multi-copy GOI inserts in vivo, the pPIC-3.5K vector was used as the backbone to carry and insert one or more copies of the α-CBDA synthase gene or the α-THCA synthase gene into the *Pichia pastoris* GS115 strain genome. α-CBDA synthase and α-THCA synthase genes were excised out with Pme I and Bam HI from pPink-HC plasmid, separated from the pPink-HC backbone on a 0.8% agarose gel at 95 V for 1 hr and extracted from the gel with Qiagen or Invitrogen gel extraction kit. pPIC-3.5K plasmid was digested by PmeI and BamHi from NEB, run on 0.8% agarose gel and extracted from the gel with Qiagen or Invitrogen gel extraction kit.

[0193]   The linearized vector and gene inserts were ligated together using Invitrogen T4 DNA ligase protocol from NEB®. Ligated circular recombinant plasmids were electroporated into *E. coli* Top 10 F⁻ strain and the cells were plated on LB-Amp-100 plates. The plates were incubated were incubated overnight at 37° C. for colonies to form. Colony PCR was applied to verify successful transformation and colonies bearing pPIC-3.5K-alpha-THCA synthase or pPIC-3.5K-alpha-CBDA synthase were re-streaked on new LB-Amp-100 plates to generate more plasmids.

[0194]   pPIC-3.5K-alpha-THCA synthase and pPIC-3.5K-alpha-CBDA synthase were inserted into GS115 strain by electroporation as described above. The transformed GS115 cells were then plated on YPD-geneticin plates with 0.25 mg/mml-3 mg/ml geneticin to select for one or more THCA synthase gene and CBDA synthase gene copy colonies. Colonies grown on 3 mg/ml YPD-geneticin plates were selected for THCA synthase and CBDA synthase production screeuing.

Results

[0195]   The conversion rate from CBGA to THCA and CBCA was greater than 90% in two hours using crude fermentation supernatant (FIGS. 3 and 5).

[0196]   The conversion rate from CBGA to CBDA and CBCA was greater than 70% overnight using crude fermentation supernantant. (FIG. 6).

7. Enzyme Purification

[0197]   The cannabinoid acid synthase enzymes thus obtained were purified by size exclusion chromatography (SEC) using a 2.2 cm inner diameter column and 5 ml supernatant in a column volume to crude enzyme supernatant ratio of 20:1. Briefly, 10 g of dry sephadex beads were measured and added to a Pyrex glass container. 100 ml of 50 mM Phosphate buffer pH 7.4 were added to Bio-GEL P-100 beads with excess amount and let sit for more than 12 hours. (P-100 beads swollen 12× when completely hydrated).

Using a vacuum pump, the hydrated P-100 beads and another 1 L pH 7.4 50 mM Phosphate buffer were de-gassed to cause the beads to settle in the excess buffer. The buffer was poured off, and 100 mL de-gassed Phosphate buffer were poured into the beads, such that the beads settled again. These steps were repeated two more times. The hydrated P-100 was then poured into a glass column until 2-5 cm of the gel bed was formed, then more gel was poured to the desired height and let it settle. The column thus formed was stored at 4° C. 5 mL of either THCA or CBDA synthase crude supernatant was run through the column at 4° C. and the fractions were collected at 5 mL/fraction for 25 fractions. All fractions were saved, stored at 4° C. and analyzed for enzyme activity and by SDS-PAGE gel to examine purification efficiency and resolution.

B. Cannabinoid and Cannabinoid Analog Enzymatic Production

1. Enzymatic Assay Conditions

[0198] The Standard CBDA synthase enzyme/THCA synthase enzyme reaction assay conditions were as follows: enzyme reaction was conducted in a 1.5 ml Eppendorf snap cap tube. 25 µl substrate, such as CBGA, dissolved in DMSO at 1.0 mg/ml in 200 µl of 100 mM citrate buffer pH 4.85 was incubated with 25 µl enzyme solution at 30° C. for 2 hours. The reaction was terminated by the addition of 250 µl MeOH and analyzed by HPLC.

[0199] Enzyme activity was tested under a variety of conditions as follows:

[0200] 1. Different solvents and conditions were tested to enhance substrate solubility and delivery, including but not limited to DMSO, DMF, IPA, cyclodextrin (CD), SDS, Triton-X.

[0201] 2. Assays were run at pH's 4, 5, 6, 7, and 8.

[0202] 3. Enzyme assays were run in either Sodium phosphate buffer or Citrate buffer with or without SDS or Triton-X

[0203] 4. Enzyme assays were run under a variety of ionic strengths

[0204] 5. Results of incubation times between 2 hrs to 4 days were compared.

Results

[0205] Table 6 below shows that DMSO, DMF, IPA and cyclodextrin facilitated solubilization of cannabinoids. Cyclodextrin solubilized up to 20-25 g/L of CBGA for conversion. Enzymatic rate was enhanced when 20% DMSO (v/v) was added to the reaction mixture and THCA synthase produced both THCA and CBCA in the reaction (Table 7).

TABLE 6

Effects of solvents on THCA Synthase Activity

Reaction Condition Studies	Parameters	% CBGA conversion	THCA:CBCA
100 mM Solvents	100 mM Cit 50 ug Enzyme in DMF	84	1.066:1
	100 mM Cit 50 ug Enzyme in DMSO	85	7.96:1

TABLE 6-continued

Effects of solvents on THCA Synthase Activity

Reaction Condition Studies	Parameters	% CBGA conversion	THCA:CBCA
	100 mM Cit 50 ug Enzyme in CD	81	12.34:1
	400 mM cit 50 ug Enzyme in IPA	61	11.9:1
	100 mM NaP 50 ug Enzyme in 20 ul CD	79	1.11:1
	100 mM NaP 50 ug Enzyme in 20 ul SDS	72	1.22:1
	100 mM Cit 50 ug Enzyme + SDS in CD	8	10.45:1

TABLE 7

Effects of DMSO Concentration on THCA Synthase Rate and Product Ratio

DMSO	FASTER	THCA:CBCA
0%	1 X	
10%	1.2 X	10:1
20%	2.5 X	5:1
25%	—	1:1
30%	0.3 X	

[0206] The effect of pH on THCA Synthase activity is shown in Tables 8 and 9 below.

TABLE 8

Effects of pH on THCA Synthase Activity

pH	THCA	CBCA
4	1	0
5	2.33	1
6	1	5.67
7	0	1

[0207] In summary, changing the pH of the THCA synthase enzyme reaction affects the products. At pH 4 THCA is the only product. At pH 5 the ratio of THCA:CBCA is 2.33:1. At pH 6 the ratio is reversed and the product mix is THCA:CBCA 1:5.67. At pH 7 CBCA is the only product.

TABLE 9

Effects of pH and Cyclodextrin on THCA Synthase Activity

Reaction Condition	Parameters	% CBGA conversion	THCA CBCA
pH exchange	400 mM Cit pH 5.0, 50 µg Enzyme in CD	59	14.9:1
	400 mM Cit pH 6.5, 50 µg Enzyme iu CD	42	1.1:1
	400 mM NaPi pH 5.0, 50 µg Enzyme in CD	59	17.37:1
	400 mM NaPi pH 6.5 50 µg enzyme 20 µg in CD	65	1.11:1

[0208] The effect of pH on CBDA synthase activity is shown in Table 10 below.

TABLE 10

Effects of pH on CBDA Synthase Activity

pH	CBDA	CBCA
4.2	2.5	1
5	1.13	1
5.2	1	1.17
5.4	1	2.45
5.8	1	6.14
6.2	1	28.13
6.8	0	0

[0209] In summary, changing the pH of the CBDA synthase enzyme reaction affects the products. At pH 4.2 CBDA:CBCA ratio is 2.5:1. At pH 5 the ratio of CBDA:CBCA is 1.13:1. At pH 6.8 there is no product forming from CBDA synthase enzyme reaction.

[0210] These results clearly show that it is possible to control the ratio of THCA:CBCA produced by the THCA synthase by controlling the pH of the enzymatic reaction. Enzyme assays were run in either Sodium phosphate buffer or Citrate buffer with or without SDS or Triton-X.

[0211] The effect of different concentrations of cyclodextrin on cannabinoid acid synthase activity was evaluated. The results for the CBDA synthase at pH 4.85 are shown in Table 11 below.

TABLE 10

Effect of Cyclodextrin on CBDA Synthase Reaction Conversion Rate and Product Ratio

Cyclodextrin concentration	Conversion rate	CBDA:CBCA ratio
0 mg/ml	40%	1.13:1
2 mg/ml	57%	1.24:1
4 mg/ml	N/A	N/A
8 mg/ml	61%	1.27:1
12 mg/ml	60%	1.33:1
16 mg/ml	50%	1.04:1
20 mg/ml	53%	1.0:1
28 mg/ml	45%	1.24:1

[0212] These results clearly show that the concentration of cyclodextrin in the reaction mixture affects the enzymatic conversion rate of the substrate into the products as well as the ratio between the different products of the reaction.

[0213] These experiments also showed that the optimal cyclodextrin (CD):CBGA ratio in the enzyme reaction mix was 11:1 (mass:mass) or 4:1 (molar ratio) for CBDA synthase, and that the optimal cyclodextrin (CD):CBGA ratio in the enzyme reaction mix was 28:1 (mass:mass) or 7.3:1 (molar ratio) for THCA synthase CD:CBGA. The presence of cyclodextrin in the reaction mix in such concentration resulted in 98% conversion in 2 hours (data not shown).

B. Cannabinoid Extraction and Purification

[0214] Cannabinoids and cannabinoid analogs obtained from the enzymatic reactions with the cannabinoid acid synthase as described above were extracted by solvent extraction as follows:

[0215] Solvent was added to the reaction mix at a ratio of 1:3 (v/v), the mixture was vigorously vortexed at room temperature for 2 minutes and centrifuged at 3200 g for 10 minutes. The solvent fraction was separated and stored in a glass vial. These steps were repeated and all extractions were combined and analyzed by HPLC.

C. Production of Cannabinoid acid synthase Enzymes by Fermentation

[0216] Cannabinoid acid synthase enzymes were produced by fermentation following Invitrogen 'Pichia Fermentation Process Guidelines'. Some modifications were as follows:

[0217] A. Inoculum Flask Preparation

[0218] From a frozen glycerol stock of Pichia strain GS115 (Mut+, Arg+, His−), a YPD plate was inoculated. After 48 hours a single colony on YPD was used to inoculate 300 ml of BMGY, in a 2 L baffled flask. The culture was grown at 28° C., 270 rpm, until OD_{600} reached 2-6 (approximately 15 hours).

[0219] B. Fermentor Preparation/Batch Glycerol

[0220] After sterilization and cooling of the 3.5 L of Basal Salts Medium in the Glass vessel of the BioFlo 3000 Fermentor, the temperature was set to no less than 27° C. and no more than 30° C. Aeration and Agitation were set to the PID mode (dissolved Oxygen dependent). pH was continually adjusted to 6.5 with 30% NH_4OH. The Fermentor was inoculated with 300 ml of the culture generated above. 200 ml of 20% casamino acids prepared in sterile 100 mM, pH 6.5 Phosphate Buffer, were added. The dissolved oxygen was adjusted to be maintained above 20%. After the glycerol from the BMGY medium was completely consumed (approximately 24 hours), a 10 ml sample was taken at the end of this first fermentation stage and analyzed for cell growth (OD600) and wet cell weight. The pellet was frozen at −80° C. for later analysis of protein. The sampling was repeated at end of each stage.

[0221] C. Glycerol Fed-Batch Phase

[0222] 50% w/v glycerol with 12 ml PTM trace salts per liter of glycerol solution was added to increase cell biomass. Feed rate was set to 18.15 ml/hr./liter initial fermentation volume. Glycerol feed was continued until wet cell weight reached 180-220 g/liter and DO spike was used to monitor the end point of glycerol fed batch phase.

[0223] D. Methanol Fed-Batch Phase

[0224] Methanol induction was initiated after all glycerol was consumed to induce the AOX1 promoter and express the cannabinoid synthases. 100% methanol with 12 ml PTM trace salts per liter of methanol was added. Feed rate was initially set at 3.6 ml/hr./liter initial fermentation volume. Agitation, aeration and oxygen feed were adjusted for the next two hours to maintain the DO above 20%. A steady DO reading inferred a full adaptation to methanol at which point methanol feed was doubled to 7.3 ml/hr./liter. After 2 hours methanol feed was further increased to 10.9 ml/hr./liter initial fermentation volume. After about 2 hours or at the first sign of foaming, 20-50 μl Sterile Pure Anti-Foam 204, Sigma were added so as to keep the headspace of the fermentor clear and prevent the foam from interfering with the agitation and various feeds. Additional 20-50 μl aliquots were added as needed approximately once a day or every other day of the entire run of fermentation. Once 10.9 ml/hr./liter was established, enzyme activity was measured and monitored every 8 hours thereafter. Fermentation was stopped 5 days after initial inoculation or upon reaching a plateau in protein concentration.

[0225] E. Harvesting Cells and Supernatant

[0226] At harvest time, the final fermentation volume was almost double the initial volume. The cell density was

increased to ~400 g/liter wet cells. The 7 liter of culture was collected into 500 ml centrifuge bottles and centrifuged at 10,000 RPM for 15 min to separate cells from the supernatant. The supernatant was concentrated 10× using Tangential Flow Filtration. A sample of supernatant was loaded onto a polyacrylamide gel for protein analysis. THCA synthase was around 80 KDa. 30 Kda TFF filter was used to concentrate the fermentation supernatant 10×. A portion of the TFF concentrated supernatant was loaded onto a nickel column for purification of the enzyme. A portion of the original fermentation supernatant was fractioned by ammonium sulfate precipitation (45%-75%).

[0227] F. Standard Enzyme Activity Assay

[0228] In 200 µl of 100 mM pH 4.8 Citrate buffer; 25 µl Substrate (CBGA) dissolved in DMSO at 1 mg/mL concentration; and 25 µl enzyme (supernatant) were added in a 1.5 mL Eppendorf snap cap tube. The tube was incubated at 30° C. for 2 hours and the reaction was terminated by adding 250 µl MeOH. Activity of the enzymes was analyzed by HPLC. E. Concentration/Purification of Cannabinoid Acid Synthase Enzymes from Fermentation

[0229] After fermentation the cells were separated from the supernatant by centrifugation at 10,000 RPM×15 min. The enzyme was then concentrated and purified as follows: the supernatant was concentrated 10× using Tangential Flow Filtration and then fractionated using ammonium sulfate precipitation; the protein fraction salting out between 45%-75% (NH4)2SO4 contained the synthase. The TFF filtered supernatant was loaded onto a nickel column for purification of the enzyme.

F. Chemical Synthesis of Cannabinoid Substrates

A. Synthesis of Geraniol
(3,7-Dimethylocta-2,6-dien-1-ol,)

[0230]
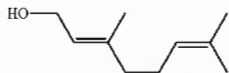
[0231] Geraniol was obtained by distillation of palmarosa oil. Palmarosa oil (New Directions Aromatics) was distilled under reduced pressure and the fractions that distil between 139-145° C. and under a reduced pressure of 25 mm Hg were pooled to obtain pure geraniol.

B. Synthesis of Olivetol

[0232]
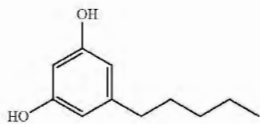
[0233] Olivetol was synthesized using a published procedure (Focella, A, et al., *J. Org. Chem., Vol.* 42, No. 21, (1977), p. 3456-3457).

1. Methyl 6-N-Pentyl-2-hydroxy-4-oxo-cyclohex-2-ene-1-carboxylate

[0234]
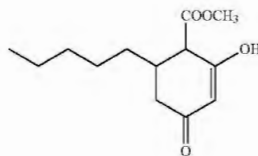
[0235] To a stirring solution of sodium methoxide (32.4 g, 0.60 mol) and dimethyl malonate (90 g, 0.68 mol) in 230 mL of anhydrous methanol was added portion wise 75 g (0.48 mol) of 90% β-nonen-2-one. The reaction mixture was then refluxed for 3 h under N_2 and allowed to cool to room temperature. The solvent was distilled under reduced pressure and the residue dissolved in 350 mL of water. The slurry of white crystals and the almost clear solution was extracted thrice with 80 mL of chloroform. The aqueous layer was acidified to pH 4 with concentrated HCl and the white precipitate that formed was allowed to stand overnight prior to filtration. The crystals were dried at 50° C. under high vacuum for 5 hours to yield 106.5 g (0.4416 mol) (92%) of methyl 6-n-Pentyl-2-hydroxy-4-oxo-cyclohex-2-ene-1-carboxylate (mp 96-98 C). The product was recrystallized using a mixture of petroleum ether:ethyl acetate (9:1), and gave 94 g of pure methyl 6-n-Pentyl-2-hydroxy-4-oxo-cyclohex-2-ene-1-carboxylate (melting point of 98-100 C).

2. 1-N-Pentyl-3,5-dihydroxybenzene (Olivetol)

[0236]
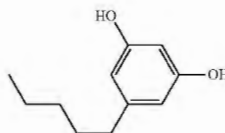
[0237] To a stirring ice-cooled solution of methyl 6-N-pentyl-2-hydroxy-4-oxo-cyclohex-2-ene-1-carboxylate (58.4 g, 0.24 mol) dissolved in 115 mL dimethylformamide was added dropwise 37.9 g (0.23 mol) of bromine dissolved in 60 mL of dimethylformamide. At the end of the addition (ca. 90 min) the reaction mixture was slowly heated to 80° C. during which time the evolution of carbon dioxide became quite vigorous.

[0238] The reaction was maintained at this temperature until gas evolution had ceased following which the reaction was further heated to 160° C. and held at this temperature for approximately 10 hours. After heating, the reaction was allowed to cool and the solvent DMF was removed under reduced pressure. The residue thus obtained was treated with water (80 mL) and extracted twice with 250 mL of ether. The combined ether layers were washed with water, then washed with 2×80 mL of a 10% solution of sodium bisulfite, 2×80 mL of a 10% solution of acetic acid, and then again with water.

[0239] After drying over anhydrous sodium sulfate the solvent was removed under reduced pressure to give 46.8 g of a viscous oil. The oil was distilled under reduced pressure to give 30.3 g (0.168 mol) (69.3%) of olivetol as product. HPLC analysis indicated 97.5% purity.

C. Synthesis of CBG

[0240] CBG was synthesized following the protocol disclosed by Taura et al., (1996), The Journal of Biological Chemistry, Vol. 271, No. 21, p. 17411-17416.

1. Synthesis of 2-[(2E)-3,7-dimethylocta-2,6-dienyl]-5-pentyl-benzene-1,3-diol (Cannabigerol (CBG))

[0241]
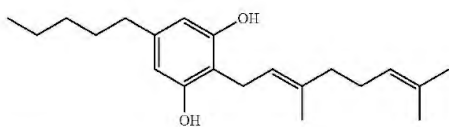
[0242] Geraniol (3 g, 0.0194 mol) and olivetol (2 g, 0.0111 mol) were dissolved in 400 mL of chloroform containing 80 mg of p-toluenesulfonic acid as catalyst and the reaction mixture was stirred at room temperature for 12 h in the dark. After 12 hours, the reaction mixture was washed with saturated sodium bicarbonate (400 mL) and then with H_2O (400 mL). The chloroform layer was concentrated at 4° C. under reduced pressure, and the residue obtained was chromatographed on a 2.0 cm×25 cm silica gel column using benzene (1000 mL) as the eluent to give 1.4 g (0.00442 mol)(39.9%) CBG as product.

[0243] Alternatively crude CBG was purified as follows. To a 250 mL beaker was added 7.25 g crude CBG and 50 mL benzene. The flask was swirled to dissolve the CBG and 50 g silica gel was added, along with a stir bar. The solution was stirred overnight, and then poured into a 44 cm×2.75 cm column. The column was eluted with 300 mL benzene. The eluent, approximately 70 mL fractions were assayed for CBG. Fractions 1, 2, and 3 (~230 mL) that contained CBG were combined and the solvent removed under pressure to give 6.464 g residue containing >80% CBG, having a purity suitable for use in the next synthetic step.

[0244] In one embodiment, crude CBG was purified by mixing 7.25 g crude CBG residue with a slurry of silica gel (50 mL), in a 250 ml Beaker. This mixture was slowly agitated for 1 hour and then vacuum filtered using a fine mesh filter paper. The filter cake was washed with 250 ml benzene until a clear filtrate was obtained. The solvent from the filtrate was removed under reduced pressure to give 6.567 g of a residue having >80% CBG.

A. Synthesis of Methylmagnesium Carbonate (MMC)

[0245] Methylmagnesium Carbonate (MMC) was synthesized following the protocol disclosed by Balasubrahman-yam et al., (1973), *Organic Synthesis, Collective Volume V*, John Wiley & Sons, Inc., p. 439-444.

[0246] A dry 2 liter, three necked flask was fitted with a mechanical stirrer, a condenser, and a 1 litre, pressure-equalizing addition funnel, the top of which was fitted with a gas inlet tube. A clean, dry magnesium ribbon (40.0 g, 1.65 mol) was placed in the flask and the system was flushed with nitrogen prior to the addition of anhydrous methanol (600 mL). The evolution of hydrogen gas was controlled by cooling the reaction mixture externally. When hydrogen evolution had ceased, a slow stream of nitrogen was passed through the system and the condenser was replaced by a total condensation-partial take-off distillation head. The nitrogen flow was stopped and the bulk of the methanol distilled from the solution under reduced pressure. Distillation was stopped when stirring of the pasty suspension of magnesium methoxide was no longer practical. The system was again flushed using nitrogen and the outlet from the distillation head was attached to a small trap containing mineral oil so that the volume of gas escaping from the reaction system could be estimated.

[0247] Anhydrous dimethylformamide (DMF)(700 mL) was added to the reaction flask, and the resulting suspension was stirred vigorously while a stream of anhydrous carbon dioxide was passed into the reaction vessel through the gas inlet tube attached to the addition funnel. The dissolution of carbon dioxide was accompanied by an exothermic reaction with the suspended magnesium methoxide. When no more CO_2 is absorbed, the colorless solution was heated under a slow stream of CO_2 gas until the temperature of the liquid distilling reached 140° C., indicating that residual methanol had been removed from the reaction mixture. The reaction mixture was flushed using a slow stream of nitrogen to aid in cooling the mixture to room temperature under an inert atmosphere. This yielded a solution having 536 mg MMC/mL of DMF.[8]

B. Synthesis of CBGA

[0248] 6-carboxylic acid-2-[(2E)-3,7-dimethylocta-2,6-dienyl]-5-pentyl-benzene-1,3-diol, Cannabigerolic Acid (CBGA) was prepared as follows. To a 10 mL conical flask was added 1 mL of a DMF solution of MMC. To this solution was added 2-[(2E)-3,7-dimethylocta-2,6-dienyl]-5-pentyl-benzene-1,3-diol (120 mg, 0.379 mmol). The flask was heated at 120° C. for 1 hour, following which the reaction mixture was dissolved in 100 mL of chloroform: methanol (2:1) solution. The pH of this solution was adjusted with dilute HCl to pH 2.0, and then partitioned using 50 mL H_2O.

[0249] The organic layer was dried over sodium sulfate and the solvent was removed by evaporation. HPLC analysis of the crude reaction showed ~40% conversion of CBG to CBGA.

[0250] Alternatively, 3.16 g (10 mmols) of CBG (or any other neutral cannabinoid), 8.63 g (100 mmols) magnesium methylate and 44 g (1 mol) of dry ice were sealed in a pressure compatible vessel. The vessel is heated to 50° C., and the temperature held at this value for three hours. Following heating, the vessel is cooled to room temperature and slowly vented.

[0251] The reaction mixture was dissolved in 100 mL of a chloroform: methanol (2:1) solvent. The pH of this solution was adjusted with dilute HCl to pH 2.0 and this solution was then partitioned using 50 mL of H_2O. The organic layer was dried over sodium sulfate and the solvent was removed by evaporation. HPLC analysis of crude reaction mixture showed ~85% conversion of CBG to CBGA using this protocol.

[0252] Crude CBGA was purified by chromatography using a 2.0 cm×25 cm silica gel column. The product was eluted using a mixture of n-hexane:ethyl acetate (2:1) (1000 mL), to obtain 45 mg (0.125 mmol)(37.5%) of the desired product.

[0253] Alternatively, ultra high purity CBGA was obtained by chromatographing the crude using LH-20 lipophilic resin as the medium. 400 g of LH-20 Sephadex resin was first swollen using 2 L of DCM:chloroform (4:1) solvent. The swollen resin was gravity packed in a 44×2.75 cm column. The column was loaded with 2.1 g of crude CBGA dissolved in a minimum amount of DCM:chloroform (4:1) solvent and eluted with 1.7 L of the same solvent. 100 mL fractions were collected. The unreacted CBG was eluted as a yellow/orange solution using this solvent system. After the passage of about 1.7 L of this solvent, no more yellow/orange fraction were observed and the eluting solvent was changed to 100% acetone to elute the bound CBGA.

[0254] The fractions containing CBGA were pooled and the solvent was removed to obtain 0.52 g CBGA (~90% recovery). Increasing the volume of DCM:chloroform (4:1) solvent passed through the column prior to eluting with acetone, yielded CBGA having purity greater than 99.5%.

C. Synthesis of CBG V

[0255] CBGV was synthesized as follows.

A. Methyl 6-N-Propyl-2-hydroxy-4-oxo-cyclohex-2-ene-1-carboxylate

[0256]
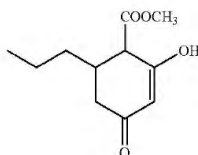
[0257] Briefly, 3-hepten-2-one (30.1 g, 0.25 mol) was added dropwise to a dry methanolic (125 mL dry MeOH), solution of diethyl malonate (52.016 g, 0.323 mol) and sodium methoxide (16.206 g, 0.3 mol). The crude product weighed 46.315 g upon drying at 45° C. overnight in a vacuum oven. The crude product was dissolved in petroleum ether (300 mL). After stirring, any undissolved material was filtered from the solution prior to the addition of ethyl acetate (30 mL), to precipitate CBGV. The precipitate was filtered and dried overnight at 44° C. in a vacuum oven. A total of 33.569 g (0.157 mol) (52.3%) of the desired product was recovered.

B. 1-N-Propyl-3,5-dihydroxybenzene

[0258]
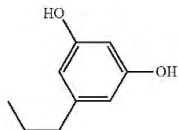
[0259] A procedure similar to the one described above for the synthesis of olivetol was used to manufacture the titled compound, except that methyl 6-N-propyl-2-hydroxy-4-oxo-cyclohex-2-ene-1-carboxylate was used as the starting material. Briefly, to a stirring ice cold DMF solution of methyl 6-N-propyl-2-hydroxy-4-oxo-cyclohex-2-ene-1-carboxylate was added a DMF solution of bromine. Following the addition of bromine the reaction mixture was heated to 80° C. Heating was accompanied by the generation and release of carbon dioxide gas. After gas evolution has ceased, the temperature of the reaction was increased to 160° C. and heating was continued for 10 hours. The reaction was then cooled and DMF was removed under reduced pressure. The crude mixture was diluted with water and subjected to solvent extraction using diethyl ether. The titled compound was obtained by removing the ether and distilling the oil that remains.

C. 2-[(2E)-3,7-dimethylocta-2,6-dienyl]-5-propyl-benzene-1,3-diol, (CBGV)

[0260] The synthesis of CBGV proceeded by adding p-toluenesulfonic acid to a chloroform solution of geraniol and 1-N-Propyl-3,5-dihydroxybenzene. After stirring the reaction at room temperature in the dark for 12 hours, water was added to partition the crude product into the chloroform layer. The chloroform layer was then washed with saturated sodium bicarbonate, dried and the organic solvent removed prior to purification as described above for the synthesis of CBG.

D. 6-carboxylic acid-2-[(2E)-3,7-dimethylocta-2,6-dienyl]-5-propyl-benzene-1,3-diol (CBGVA)

[0261] 6-carboxylic acid-2-[(2E)-3,7-dimethylocta-2,6-dienyl]-5-propyl-benzene-1,3-diol, cannabigerolic Acid (CBGVA) was prepared as follows. Methyl magnesium carbonate (MMC) was prepared as described above. To a DMF solution of MMC in a flask was added 2-[(2E)-3,7-dimethylocta-2,6-dienyl]-5-propyl-benzene-1,3-diol. The flask was heated at 120° C. for 1 hour, following which the reaction mixture was dissolved in a 2:1 mixture of chloroform:methanol. The pH of this solution was adjusted with dilute HCl to pH 2.0, and the reaction mixture was extracted using H_2O. The organic layer was dried over sodium sulfate and the solvent was removed by evaporation.

[0262] G. Large Scale Enzymatic Production of Cannabinoids

[0263] 100 ml of a 10 mM sodium phosphate buffer (pH 5.0) were placed in a glass reaction vessel equipped with oxygen gas sparger and a stirrer. To this solution 35 g/l of either 2-hydroxypropyl-β-cyclodextrin (HPβCD; Kleptose® HPB), a sulfobutylether β-cyclodextrin sodium salt (SBEβCD; Captisol®), or a randomly methylated β-cyclodextrin (RMβCD) were added. The CD was added in small 5 g portions to ensure full dissolution.

[0264] 2.5 g of a cannabinoid acid synthase substrate, for example, CBGA or CBGV-A or a Formula I, II or V compound, were added to the buffered cyclodextrin solution. The molar ratio of CD to substrate was about 4:1. 60 mg of purified synthase were added to the solution and the reaction mixture was incubated at 30° C. for 8 hours. Progress of the reaction was periodically monitored by HPLC, and using an enzymatic assay to detect and quantify the evolution of hydrogen peroxide.

[0265] After 8 hours, greater than 90% of a CBGA substrate was converted to THCA and CBCA. The ratio of THCA to CBCA was approximately 10:1 at an acidic pH of 5.0. The ratio of the CBC isomers was 5:1.

[0266] The aqueous solution was diluted 10:1 with 95% EtOH. This causes cyclodextrin to precipitate out leaving the cannabinoids in solution. The cyclodextrin was vacuum filtered, washed with 1 L of 90% EtOH, and dried to permit its reuse in a future reaction.

[0267] Concentration of the ethanolic solution containing the cannabinoids followed suspension of the residue in DCM:chlorofrom (4:1) solvent yields ~25 g crude orange-yellow residue.

[0268] H. Large Scale Purification of Cannabinoids

[0269] Purification of cannabinoids synthesized using a method of this technology was accomplished chromatographically using LH-20 lipophilic resin. Briefly, 4000 g of the resin was swollen using 20 L of DCM:chloroform (4:1). The swollen resin was gravity packed in a 44×27.5 cm

column. The volume of the swollen resin is ~1350 mL. The column was loaded with 25 g crude residue dissolved in a minimum amount of the solvent and then washed with 4 L DCM:chloroform (4:1) solvent to elute CBG. No cannabinoid acids were eluted from the column during this elution.

[0270] Gradient elution with a 1:1 to 0:1 DCM:acetone solvent was used to elute the cannabinoid acids. Each step of the gradient used one column volume (4 L) of solvent. CBCA eluted first, followed by CBGA, and then THCA. The purity of each cannabinoid was >99.5%.

[0271] The pure cannabinoids can further be processed to their neutral or "active" form by heating the acid forms at 90° C. under vacuum. Decarboxylation was quantitative to give the neutral cannabinoid. If necessary, recrystallization can be performed to obtain pharmaceutical grade cannabinoids.

[0272] Those of skill in the art will recognize that numerous modifications and changes may be made to the exemplary designs and embodiments described herein and that the invention is not limited to such embodiments.

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Phe Pro His Arg Ala Gly Ile Met Tyr Glu Leu Trp Tyr Thr Ala Ser
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Trp Glu Lys Gln Glu Asp Asn Glu Lys His Ile Asn Trp Val Arg Ser
    450              455              460

Val Tyr Asn Phe Thr Thr Pro Tyr Val Ser Gln Asn Pro Arg Leu Ala
465              470              475              480

Tyr Leu Asn Tyr Arg Asp Leu Asp Leu Gly Lys Thr Asn His Ala Ser
            485              490              495

Pro Asn Asn Tyr Thr Gln Ala Arg Ile Trp Gly Glu Lys Tyr Phe Gly
            500              505              510

Lys Asn Phe Asn Arg Leu Val Lys Val Lys Thr Lys Val Asp Pro Asn
            515              520              525

Asn Phe Phe Arg Asn Glu Gln Ser Ile Pro Pro Leu Pro Pro His His
    530              535              540

His
545
```

<210> SEQ ID NO 3
<211> LENGTH: 1830
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polynucleotide

<400> SEQUENCE: 3

atgagattcc catccatctt cactgctgtt ttgttcgctg cttcttccgc tttggctgct   60

ccagttaaca ctactactga ggacgagact gctcagattc cagctgaagc tgttattggt   120

tactccgact tggaaggtga cttcgacgtt gctgttttgc cattctccaa ctccactaac   180

aacggtttgt tgttcatcaa cactacaatc gcttccattg ctgctaaaga agagggagtt   240

tccttggaga agagagaggc tgaagctaac ccaagagaaa acttcttgaa gtgtttttcc   300

aagcacatcc caaacaacgt tgctaaccct aagttggttt acactcagca cgaccagttg   360
```

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```
tacatgtcct tgttgaactc cacaatccag aacttgagat tcatctccga cactactcca    420

aagccattgg ttatcgttac tccatccaac aactcccaca tccaggctac tatcttgtgt    480

tccaagaagg ttggattgca gatcagaaca agatccggtg gtcatgacgc tgaaggtatg    540

tcctacattt cccaggttcc attcgttgtt gttgacttga gaaacatgca ctccatcaag    600

atcgacgttc actcccaaac tgcttgggtt gaagctggtg ctactttggg tgaagtttac    660

tactggatca acgagaagaa cgagaacttg tccttcccag gtggttactg tccaactgtt    720

ggtgttggtg gtcactttc tggtggtggt tacggtgctt tgatgagaaa ctacggattg    780

gctgctgaca acatcatcga cgctcacttg gttaacgttg acggtaaggt tttggacaga    840

aagtccatgg gtgaggactt gttctgggct attagaggtg gtggtggtga gaacttcggt    900

attattgctg cttggaagat caagttggtt gctgttccat ccaagtccat tatcttctcc    960

gttaagaaaa acatggaaat cccacggtttg gttaagttgt ttaacaagtg gcagaacatt   1020

gcttacaagt acgacaagga cttggttttg atgactcact tcatcactaa gaacatcact   1080

gacaaccacg gtaagaacaa gactactgtt cacggttact tctcttccat cttccacggt   1140

ggtgttgatt ccttggttga tttgatgaac aagtctttcc cagagttggg tatcaagaaa   1200

actgactgta aagagttctc ctggatcgac acaacaatct tctactccgg tgttgttaac   1260

ttcaacactg ctaactttaa gaaagagatc ttgttggaca gatccgctgg taaaaagact   1320

gctttctcca ttaagttgga ctacgttaag aagccaatcc cagagactgc tatggttaag   1380

attttggaga agttgtacga agaggacgtt ggtgctggta tgtacgtttt gtacccatac   1440

ggtggtatca tggaagaaat ctccgagtcc gctattccat tcccacacag agctggtatt   1500

atgtacgagt gtggtacac tgcttcttgg gagaagcaag aggacaacga aaagcacatc   1560

aactgggtta gatccgttta caacttcact actccttacg tttcccagaa cccaagattg   1620

gcttacttga actacagaga cttggacttg ggtaagacta accacgcttc cccaaacaat   1680

tacacacagg ctagaatctg gggtgaaaag tacttcggaa agaactttaa cagattggtt   1740

aaggttaaga ctaaggttga ccctaacaac ttcttcagaa acgagcagtc catcccacca   1800

ttgccaccac atcatcatca ccatcactaa                                     1830
```

<210> SEQ ID NO 4
<211> LENGTH: 609
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide

<400> SEQUENCE: 4

```
Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser
1               5                   10                  15

Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp Glu Thr Ala Gln
            20                  25                  30

Ile Pro Ala Glu Ala Val Ile Gly Tyr Ser Asp Leu Glu Gly Asp Phe
        35                  40                  45

Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu
    50                  55                  60

Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val
65                  70                  75                  80
```

-continued

```
Ser Leu Glu Lys Arg Glu Ala Glu Ala Asn Pro Arg Glu Asn Phe Leu
                85                  90                  95

Lys Cys Phe Ser Lys His Ile Pro Asn Asn Val Ala Asn Pro Lys Leu
            100                 105                 110

Val Tyr Thr Gln His Asp Gln Leu Tyr Met Ser Leu Leu Asn Ser Thr
        115                 120                 125

Ile Gln Asn Leu Arg Phe Ile Ser Asp Thr Thr Pro Lys Pro Leu Val
    130                 135                 140

Ile Val Thr Pro Ser Asn Asn Ser His Ile Gln Ala Thr Ile Leu Cys
145                 150                 155                 160

Ser Lys Lys Val Gly Leu Gln Ile Arg Thr Arg Ser Gly Gly His Asp
                165                 170                 175

Ala Glu Gly Met Ser Tyr Ile Ser Gln Val Pro Phe Val Val Val Asp
            180                 185                 190

Leu Arg Asn Met His Ser Ile Lys Ile Asp Val His Ser Gln Thr Ala
        195                 200                 205

Trp Val Glu Ala Gly Ala Thr Leu Gly Glu Val Tyr Tyr Trp Ile Asn
    210                 215                 220

Glu Lys Asn Glu Asn Leu Ser Phe Pro Gly Gly Tyr Cys Pro Thr Val
225                 230                 235                 240

Gly Val Gly Gly His Phe Ser Gly Gly Gly Tyr Gly Ala Leu Met Arg
                245                 250                 255

Asn Tyr Gly Leu Ala Ala Asp Asn Ile Ile Asp Ala His Leu Val Asn
            260                 265                 270

Val Asp Gly Lys Val Leu Asp Arg Lys Ser Met Gly Glu Asp Leu Phe
        275                 280                 285

Trp Ala Ile Arg Gly Gly Gly Gly Glu Asn Phe Gly Ile Ile Ala Ala
    290                 295                 300

Trp Lys Ile Lys Leu Val Ala Val Pro Ser Lys Ser Thr Ile Phe Ser
305                 310                 315                 320

Val Lys Lys Asn Met Glu Ile His Gly Leu Val Lys Leu Phe Asn Lys
                325                 330                 335

Trp Gln Asn Ile Ala Tyr Lys Tyr Asp Lys Asp Leu Val Leu Met Thr
            340                 345                 350

His Phe Ile Thr Lys Asn Ile Thr Asp Asn His Gly Lys Asn Lys Thr
        355                 360                 365

Thr Val His Gly Tyr Phe Ser Ser Ile Phe His Gly Gly Val Asp Ser
    370                 375                 380

Leu Val Asp Leu Met Asn Lys Ser Phe Pro Glu Leu Gly Ile Lys Lys
385                 390                 395                 400

Thr Asp Cys Lys Glu Phe Ser Trp Ile Asp Thr Thr Ile Phe Tyr Ser
                405                 410                 415

Gly Val Val Asn Phe Asn Thr Ala Asn Phe Lys Lys Glu Ile Leu Leu
            420                 425                 430

Asp Arg Ser Ala Gly Lys Lys Thr Ala Phe Ser Ile Lys Leu Asp Tyr
        435                 440                 445

Val Lys Lys Pro Ile Pro Glu Thr Ala Met Val Lys Ile Leu Glu Lys
    450                 455                 460

Leu Tyr Glu Glu Asp Val Gly Ala Gly Met Tyr Val Leu Tyr Pro Tyr
465                 470                 475                 480

Gly Gly Ile Met Glu Glu Ile Ser Glu Ser Ala Ile Pro Phe Pro His
```

33

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```
                485              490              495

Arg Ala Gly Ile Met Tyr Glu Leu Trp Tyr Thr Ala Ser Trp Glu Lys
            500              505              510

Gln Glu Asp Asn Glu Lys His Ile Asn Trp Val Arg Ser Val Tyr Asn
            515              520              525

Phe Thr Thr Pro Tyr Val Ser Gln Asn Pro Arg Leu Ala Tyr Leu Asn
        530              535              540

Tyr Arg Asp Leu Asp Leu Gly Lys Thr Asn His Ala Ser Pro Asn Asn
545              550              555              560

Tyr Thr Gln Ala Arg Ile Trp Gly Glu Lys Tyr Phe Gly Lys Asn Phe
                565              570              575

Asn Arg Leu Val Lys Val Lys Thr Lys Val Asp Pro Asn Asn Phe Phe
            580              585              590

Arg Asn Glu Gln Ser Ile Pro Pro Leu Pro Pro His His His His His
        595              600              605

His
```

<210> SEQ ID NO 5
<211> LENGTH: 1635
<212> TYPE: DNA
<213> ORGANISM: Cannabis sativa

<400> SEQUENCE: 5

```
atgaagtgct caacattctc cttttggttt gtttgcaaga taatattttt cttttttctca      60

ttcaatatcc aaacttccat tgctaatcct cgagaaaact tccttaaatg cttctcgcaa     120

tatattccca ataatgcaac aaatctaaaa ctcgtataca ctcaaaacaa cccattgtat     180

atgtctgtcc taaattcgac aatacacaat cttagattca cctctgacac aaccccaaaa     240

ccacttgtta tcgtcactcc ttcacatgtc tctcatatcc aaggcactat tctatgctcc     300

aagaaagttg gcttgcagat tcgaactcga agtggtggtc atgattctga gggcatgtcc     360

tacatatctc aagtcccatt tgttatagta gacttgagaa acatgcgttc aatcaaaata     420

gatgttcata gccaaactgc atgggttgaa gccggagcta cccttggaga agtttattat     480

tgggttaatg agaaaaatga gaatcttagt ttggcggctg ggtattgccc tactgtttgc     540

gcaggtggac actttggtgg aggaggctat ggaccattga tgagaaacta tggcctcgcg     600

gctgataata tcattgatgc acacttagtc aacgttcatg gaaaagtgct agatcgaaaa     660

tctatggggg aagatctctt ttgggcttta cgtggtggtg gagcagaaag cttcggaatc     720

attgtagcat ggaaaattag actggttgct gtcccaaagt ctactatgtt tagtgttaaa     780

aagatcatgg agatacatga gcttgtcaag ttagttaaca aatggcaaaa tattgcttac     840

aagtatgaca aagatttatt actcatgact cacttcataa ctaggaacat tacagataat     900

caagggaaga ataagacagc aatacacact tacttctctt cagttttcct tggtggagtg     960

gatagtctag tcgacttgat gaacaagagt tttcctgagt ggggtattaa aaaaacggat    1020

tgcagacaat tgagctggat tgatactatc atcttctata gtggtgttgt aaattacgac    1080

actgataatt ttaacaagga aattttgctt gatagatccg ctgggcagaa cggtgctttc    1140

aagattaagt tagactacgt taagaaacca attccagaat ctgtatttgt ccaaattttg    1200

gaaaaattat atgaagaaga tataggagct gggatgtatg cgttgtaccc ttacggtggt    1260

ataatggatg agatttcaga atcagcaatt ccattccctc atcgagctgg aatcttgtat    1320
```

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```
gagttatggt acatatgtag ttgggagaag caagaagata acgaaaagca tctaaactgg    1380

attagaaata tttataactt catgactcct tatgtgtcca aaaatccaag attggcatat    1440

ctcaattata gagaccttga tataggaata aatgatccca agaatccaaa taattacaca    1500

caagcacgta tttgggggtga gaagtatttt ggtaaaaatt ttgacaggct agtaaaagtg    1560

aaaaccctgg ttgatcccaa taactttttt agaaacgaac aaagcatccc acctcttcca    1620

cggcatcgtc attaa                                                     1635
```

<210> SEQ ID NO 6
<211> LENGTH: 544
<212> TYPE: PRT
<213> ORGANISM: Cannabis sativa

<400> SEQUENCE: 6

```
Met Lys Cys Ser Thr Phe Ser Phe Trp Phe Val Cys Lys Ile Ile Phe
1               5                   10                  15

Phe Phe Phe Ser Phe Asn Ile Gln Thr Ser Ile Ala Asn Pro Arg Glu
            20                  25                  30

Asn Phe Leu Lys Cys Phe Ser Gln Tyr Ile Pro Asn Asn Ala Thr Asn
        35                  40                  45

Leu Lys Leu Val Tyr Thr Gln Asn Asn Pro Leu Tyr Met Ser Val Leu
    50                  55                  60

Asn Ser Thr Ile His Asn Leu Arg Phe Thr Ser Asp Thr Thr Pro Lys
65                  70                  75                  80

Pro Leu Val Ile Val Thr Pro Ser His Val Ser His Ile Gln Gly Thr
                85                  90                  95

Ile Leu Cys Ser Lys Lys Val Gly Leu Gln Ile Arg Thr Arg Ser Gly
            100                 105                 110

Gly His Asp Ser Glu Gly Met Ser Tyr Ile Ser Gln Val Pro Phe Val
        115                 120                 125

Ile Val Asp Leu Arg Asn Met Arg Ser Ile Lys Ile Asp Val His Ser
    130                 135                 140

Gln Thr Ala Trp Val Glu Ala Gly Ala Thr Leu Gly Glu Val Tyr Tyr
145                 150                 155                 160

Trp Val Asn Glu Lys Asn Glu Asn Leu Ser Leu Ala Ala Gly Tyr Cys
                165                 170                 175

Pro Thr Val Cys Ala Gly Gly His Phe Gly Gly Gly Gly Tyr Gly Pro
            180                 185                 190

Leu Met Arg Asn Tyr Gly Leu Ala Ala Asp Asn Ile Ile Asp Ala His
        195                 200                 205

Leu Val Asn Val His Gly Lys Val Leu Asp Arg Lys Ser Met Gly Glu
    210                 215                 220

Asp Leu Phe Trp Ala Leu Arg Gly Gly Gly Ala Glu Ser Phe Gly Ile
225                 230                 235                 240

Ile Val Ala Trp Lys Ile Arg Leu Val Ala Val Pro Lys Ser Thr Met
                245                 250                 255

Phe Ser Val Lys Lys Ile Met Glu Ile His Glu Leu Val Lys Leu Val
            260                 265                 270

Asn Lys Trp Gln Asn Ile Ala Tyr Lys Tyr Asp Lys Asp Leu Leu Leu
        275                 280                 285

Met Thr His Phe Ile Thr Arg Asn Ile Thr Asp Asn Gln Gly Lys Asn
```

-continued

```
          290                    295                    300

Lys Thr Ala Ile His Thr Tyr Phe Ser Ser Val Phe Leu Gly Gly Val
305                    310                    315                    320

Asp Ser Leu Val Asp Leu Met Asn Lys Ser Phe Pro Glu Leu Gly Ile
                    325                    330                    335

Lys Lys Thr Asp Cys Arg Gln Leu Ser Trp Ile Asp Thr Ile Ile Phe
                340                    345                    350

Tyr Ser Gly Val Val Asn Tyr Asp Thr Asp Asn Phe Asn Lys Glu Ile
            355                    360                    365

Leu Leu Asp Arg Ser Ala Gly Gln Asn Gly Ala Phe Lys Ile Lys Leu
        370                    375                    380

Asp Tyr Val Lys Lys Pro Ile Pro Glu Ser Val Phe Val Gln Ile Leu
385                    390                    395                    400

Glu Lys Leu Tyr Glu Glu Asp Ile Gly Ala Gly Met Tyr Ala Leu Tyr
                    405                    410                    415

Pro Tyr Gly Gly Ile Met Asp Glu Ile Ser Glu Ser Ala Ile Pro Phe
                420                    425                    430

Pro His Arg Ala Gly Ile Leu Tyr Glu Leu Trp Tyr Ile Cys Ser Trp
            435                    440                    445

Glu Lys Gln Glu Asp Asn Glu Lys His Leu Asn Trp Ile Arg Asn Ile
        450                    455                    460

Tyr Asn Phe Met Thr Pro Tyr Val Ser Lys Asn Pro Arg Leu Ala Tyr
465                    470                    475                    480

Leu Asn Tyr Arg Asp Leu Asp Ile Gly Ile Asn Asp Pro Lys Asn Pro
                    485                    490                    495

Asn Asn Tyr Thr Gln Ala Arg Ile Trp Gly Glu Lys Tyr Phe Gly Lys
                500                    505                    510

Asn Phe Asp Arg Leu Val Lys Val Lys Thr Leu Val Asp Pro Asn Asn
            515                    520                    525

Phe Phe Arg Asn Glu Gln Ser Ile Pro Pro Leu Pro Arg His Arg His
        530                    535                    540
```

<210> SEQ ID NO 7
<211> LENGTH: 1833
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polynucleotide

<400> SEQUENCE: 7

```
atgagattcc catccatctt cactgctgtt ttgttcgctg cttcttccgc tttggctgct      60

ccagttaaca ctactactga ggacgagact gctcagattc cagctgaagc tgttattggt     120

tactccgact tggaaggtga cttcgacgtt gctgttttgc cattctccaa ctccactaac     180

aacggtttgt tgttcatcaa cactacaatc gcttccattg ctgctaaaga agagggagtt     240

tccttggaga agagagaggc tgaagctaac ccaagagaaa acttcttgaa gtgttttttcc     300

cagtacatcc aaacaacgc tacaaacttg aagttggttt acactcagaa caacccattg     360

tacatgtccg ttttgaactc cacaatccac aacttgagat tcacttccga cactactcca     420

aagccattgg ttatcgttac tccatcccac gtttcccaca tccagggtac tattttgtgt     480

tccaagaagg ttggattgca gatcagaaca agatccggtg gtcacgactc tgaaggtatg     540
```

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```
tcctacattt cccaggttcc tttcgttatc gttgacttga gaaacatgag atccatcaag     600

atcgacgttc actcccagac tgcttgggtt gaagctggtg ctactttggg tgaagtttac     660

tactgggtta acgagaagaa cgagaacttg tccttggctg ctggttactg tccaactgtt     720

tgtgctggtg gtcatttcgg tggtggtggt tatggtccat tgatgagaaa ctacggtttg     780

gctgctgaca acatcatcga cgctcacttg gttaacgttc acggtaaggt tttggacaga     840

aagtccatgg gtgaggactt gttctgggct ttgagaggtg gtggtgctga atccttcggt     900

attatcgttg cttggaagat cagattggtt gctgttccaa agtccactat gttctccgtt     960

aagaaaatca tggaaatcca cgaattggtt aagttggtta acaagtggca gaacattgct    1020

tacaagtacg acaaggattt gttgttgatg actcacttca tcactagaaa catcactgac    1080

aaccagggta agaacaagac tgctatccac acttacttct cttccgtttt cttgggtggt    1140

gttgactcct tggttgattt gatgaacaag tccttcccag agttgggtat caagaaaact    1200

gactgtagac agttgtcctg gatcgacact atcatcttct actccggtgt tgttaactac    1260

gacacagaca acttcaacaa agagatcttg ttggacagat ccgctggaca gaacggtgct    1320

ttcaagatca agttggacta cgttaagaag ccaatcccag agtccgtttt cgttcagatt    1380

ttggagaagt tgtacgaaga ggacatcggt gctggtatgt acgctttgta cccatacggt    1440

ggtatcatgg acgaaatttc cgagtccgct attccattcc cacacagagc tggtatcttg    1500

tacgagttgt ggtacatctg ttcttgggag aagcaagagg acaacgagag gcacttgaac    1560

tggatcagaa acatctacaa cttcatgact ccatacgttt ccaagaaccc aagattggct    1620

tacttgaact acagagactt ggacatcgga atcaacgacc aaagaaccc taacaactac     1680

actcaggcta gaatctgggg tgaaaagtac ttcggtaaga acttcgacag attggttaag    1740

gttaagactt tggttgaccc aaacaatttc ttcagaaacg agcagtccat ccctccattg    1800

ccaagacata gacatcatca ccatcaccac taa                                  1833
```

```
<210> SEQ ID NO 8
<211> LENGTH: 610
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide

<400> SEQUENCE: 8

Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser
1               5                   10                  15

Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp Glu Thr Ala Gln
            20                  25                  30

Ile Pro Ala Glu Ala Val Ile Gly Tyr Ser Asp Leu Glu Gly Asp Phe
        35                  40                  45

Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu
    50                  55                  60

Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val
65                  70                  75                  80

Ser Leu Glu Lys Arg Glu Ala Glu Ala Asn Pro Arg Glu Asn Phe Leu
                85                  90                  95

Lys Cys Phe Ser Gln Tyr Ile Pro Asn Asn Ala Thr Asn Leu Lys Leu
            100                 105                 110
```

-continued

```
Val Tyr Thr Gln Asn Asn Pro Leu Tyr Met Ser Val Leu Asn Ser Thr
        115                 120                 125

Ile His Asn Leu Arg Phe Thr Ser Asp Thr Thr Pro Lys Pro Leu Val
    130                 135                 140

Ile Val Thr Pro Ser His Val Ser His Ile Gln Gly Thr Ile Leu Cys
145                 150                 155                 160

Ser Lys Lys Val Gly Leu Gln Ile Arg Thr Arg Ser Gly Gly His Asp
                165                 170                 175

Ser Glu Gly Met Ser Tyr Ile Ser Gln Val Pro Phe Val Ile Val Asp
            180                 185                 190

Leu Arg Asn Met Arg Ser Ile Lys Ile Asp Val His Ser Gln Thr Ala
        195                 200                 205

Trp Val Glu Ala Gly Ala Thr Leu Gly Glu Val Tyr Tyr Trp Val Asn
    210                 215                 220

Glu Lys Asn Glu Asn Leu Ser Leu Ala Ala Gly Tyr Cys Pro Thr Val
225                 230                 235                 240

Cys Ala Gly Gly His Phe Gly Gly Gly Gly Tyr Gly Pro Leu Met Arg
                245                 250                 255

Asn Tyr Gly Leu Ala Ala Asp Asn Ile Ile Asp Ala His Leu Val Asn
            260                 265                 270

Val His Gly Lys Val Leu Asp Arg Lys Ser Met Gly Glu Asp Leu Phe
        275                 280                 285

Trp Ala Leu Arg Gly Gly Gly Ala Glu Ser Phe Gly Ile Ile Val Ala
    290                 295                 300

Trp Lys Ile Arg Leu Val Ala Val Pro Lys Ser Thr Met Phe Ser Val
305                 310                 315                 320

Lys Lys Ile Met Glu Ile His Glu Leu Val Lys Leu Val Asn Lys Trp
                325                 330                 335

Gln Asn Ile Ala Tyr Lys Tyr Asp Lys Asp Leu Leu Leu Met Thr His
            340                 345                 350

Phe Ile Thr Arg Asn Ile Thr Asp Asn Gln Gly Lys Asn Lys Thr Ala
        355                 360                 365

Ile His Thr Tyr Phe Ser Ser Val Phe Leu Gly Gly Val Asp Ser Leu
    370                 375                 380

Val Asp Leu Met Asn Lys Ser Phe Pro Glu Leu Gly Ile Lys Lys Thr
385                 390                 395                 400

Asp Cys Arg Gln Leu Ser Trp Ile Asp Thr Ile Ile Phe Tyr Ser Gly
                405                 410                 415

Val Val Asn Tyr Asp Thr Asp Asn Phe Asn Lys Glu Ile Leu Leu Asp
            420                 425                 430

Arg Ser Ala Gly Gln Asn Gly Ala Phe Lys Ile Lys Leu Asp Tyr Val
        435                 440                 445

Lys Lys Pro Ile Pro Glu Ser Val Phe Val Gln Ile Leu Glu Lys Leu
    450                 455                 460

Tyr Glu Glu Asp Ile Gly Ala Gly Met Tyr Ala Leu Tyr Pro Tyr Gly
465                 470                 475                 480

Gly Ile Met Asp Glu Ile Ser Glu Ser Ala Ile Pro Phe Pro His Arg
                485                 490                 495

Ala Gly Ile Leu Tyr Glu Leu Trp Tyr Ile Cys Ser Trp Glu Lys Gln
            500                 505                 510

Glu Asp Asn Glu Lys His Leu Asn Trp Ile Arg Asn Ile Tyr Asn Phe
```

-continued

```
         515                520                525

Met Thr Pro Tyr Val Ser Lys Asn Pro Arg Leu Ala Tyr Leu Asn Tyr
    530                535                540

Arg Asp Leu Asp Ile Gly Ile Asn Asp Pro Lys Asn Pro Asn Asn Tyr
545                550                555                560

Thr Gln Ala Arg Ile Trp Gly Glu Lys Tyr Phe Gly Lys Asn Phe Asp
                565                570                575

Arg Leu Val Lys Val Lys Thr Leu Val Asp Pro Asn Asn Phe Phe Arg
                580                585                590

Asn Glu Gln Ser Ile Pro Pro Leu Pro Arg His Arg His His His His
        595                600                605

His His
    610


<210> SEQ ID NO 9
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      6xHis tag

<400> SEQUENCE: 9

His His His His His His
1               5
```

1.-6. (canceled)

7. A system for producing cannabinoids wherein the system comprises:

(i) a bioreactor comprising cells stably transformed with one or more cannabinoid acid synthase genes;

(ii) a reaction mixture; and

(iii) a controller configured to modify one or more reaction conditions to modulate the ratio of the cannabinoid products.

8. The system of claim 1, wherein the one or more cannabinoid acid synthase genes comprises a tetrahydrocannabinolic acid (THCA) synthase gene.

9. The system of claim 2, wherein the THCA synthase gene comprises a nucleic acid sequence of SEQ ID NO: 1, SEQ ID NO: 3, or a variant or fragment thereof.

10. The system of claim 2, wherein the THCA synthase gene comprises a nucleic acid sequence that encodes a polypeptide of SEQ ID NO: 2, SEQ ID NO: 4, or a variant or fragment thereof.

11. The system of claim 1, wherein the one or more cannabinoid acid synthase genes comprises a cannabidiolic acid (CBDA) synthase gene.

12. The system of claim 5, wherein the CBDA synthase gene comprises a nucleic acid sequence of SEQ ID NO: 5, SEQ ID NO: 7, or a variant or fragment thereof.

13. The system of claim 5, wherein the CBDA synthase gene comprises a nucleic acid sequence that encodes a polypeptide of SEQ ID NO: 6, SEQ ID NO: 8, or a variant or fragment thereof.

14. The system of claim 1, wherein the cannabinoid acid synthase gene is codon optimized with an alpha secretion sequence.

15. The system of claim 1, wherein the cannabinoid acid synthase gene is tagged with six tandem histidine residues (SEQ ID NO: 9).

16. A method for producing a cannabinoid product comprises:

(1) reacting a CBDA synthase in a reaction mixture with a compound according to Formula I:

Formula I

wherein R_1 is H or —COOH and R_2 is a linear or branched CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, or C_8H_{17} group;

(2) recovering the cannabinoid product.

17. The method of claim 10, further comprises producing the CBDA synthase.

18. The method of claim 11, wherein the CBDA synthase is a recombinant synthase.

19. The method of claim 10, wherein the CBDA synthase is encoded by a nucleic acid sequence of SEQ ID NO: 5, SEQ ID NO: 7, or a variant or fragment thereof.

20. The method of claim 10, wherein the CBDA synthase comprises a polypeptide of SEQ ID NO: 6, SEQ ID NO: 8, or a variant or fragment thereof.

21. The system of claim **10**, wherein the CBDA synthase is encoded by a cannabinoid acid synthase gene that is tagged with six tandem histidine residues (SEQ ID NO: 9).

22. The method of claim **10**, wherein the CBDA synthase is encoded by a cannabinoid acid synthase gene that is codon optimized with an alpha secretion sequence.

23. The method of claim **10**, wherein the pH in the reaction mixture is in the range from about 4.2 to about 6.2.

24. The method of claim **10**, wherein the reaction mixture comprises a solvent that comprises dimethyl sulfoxide (DMSO), dimethyl formamide (DMF), iso-propoyl alcohol, or combination thereof.

25. The method of claim **18**, wherein the solvent comprises DMSO.

26. The method of claim **18**, the concentration of the solvent in the reaction mixture is between 5% and 30% (w/v).

27. A method for producing a cannabinoid product comprises:

(1) reacting a THCA synthase in a reaction mixture with a compound according to Formula I:

Formula I

wherein R_1 is H or —COOH and R_2 is a linear or branched CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, or C_8H_{17} group;

(2) recovering the cannabinoid product so produced.

28. The method of claim **21**, further comprises producing the THCA synthase.

29. The method of claim **22**, wherein the THCA synthase is a recombinant synthase.

30. The method of claim **21**, wherein the THCA synthase is encoded by a nucleic acid sequence of SEQ ID NO: 1, SEQ ID NO: 3, or a variant or fragment thereof.

31. The method of claim **21**, wherein the wherein the THCA synthase comprises a polypeptide of SEQ ID NO: 2, SEQ ID NO: 4, or a variant or fragment thereof.

32. The system of claim **21**, wherein the THCA synthase is encoded by a cannabinoid acid synthase gene that is tagged with six tandem histidine residues (SEQ ID NO: 9).

33. The method of claim **21**, wherein the THCA synthase is encoded by a cannabinoid acid synthase gene that is codon optimized with an alpha secretion sequence.

34. The method of claim **21**, wherein the pH in the reaction mixture is in the range from about 3.8 to about 7.2.

35. The method of claim **21**, wherein the reaction mixture comprises a solvent that comprises dimethyl sulfoxide (DMSO), dimethyl formamide (DMF), iso-propoyl alcohol, or combination thereof.

36. The method of claim **29**, wherein the solvent comprises DMSO.

37. The method of claim **29**, the concentration of the solvent in the reaction mixture is between 5% and 30% (w/v).

* * * * *

EXHIBIT C

Begins on the following page

<div align="right">

HEMPCHEW

</div>

US Serial Number:	86668083	**Application Filing Date:**	Jun. 19, 2015
US Registration Number:	5139076	**Registration Date:**	Feb. 07, 2017
Filed as TEAS RF:	Yes	**Currently TEAS RF:**	Yes
Register:	Supplemental		
Mark Type:	Trademark		
Amended to Principal Register:	No	**Date Amended to Current Register:**	Dec. 06, 2016

Status: Registered. The registration date is used to determine when post-registration maintenance documents are due.

Status Date: Feb. 07, 2017

Mark Information

Mark Literal Elements: HEMPCHEW

Standard Character Claim: Yes. The mark consists of standard characters without claim to any particular font style, size, or color.

Mark Drawing Type: 4 - STANDARD CHARACTER MARK

Goods and Services

Note: The following symbols indicate that the registrant/owner has amended the goods/services:

- Brackets [..] indicate deleted goods/services;
- Double parenthesis ((..)) identify any goods/services not claimed in a Section 15 affidavit of incontestability; and
- Asterisks *..* identify additional (new) wording in the goods/services.

For: chewing gum; confectionery, namely, hard candies, chewable candies, lozenges, and lollipops; all the foregoing contain hemp, hemp extract, or hemp oil

International Class(es):	030 - Primary Class	**U.S Class(es):**	046
Class Status:	ACTIVE		
Basis:	1(a)		
First Use:	Dec. 05, 2016	**Use in Commerce:**	Dec. 05, 2016

Basis Information (Case Level)

Filed Use:	No	**Currently Use:**	Yes	**Amended Use:**	No
Filed ITU:	Yes	**Currently ITU:**	No	**Amended ITU:**	No
Filed 44D:	No	**Currently 44D:**	No	**Amended 44D:**	No
Filed 44E:	No	**Currently 44E:**	No	**Amended 44E:**	No
Filed 66A:	No	**Currently 66A:**	No		
Filed No Basis:	No	**Currently No Basis:**	No		

Current Owner(s) Information

Owner Name: Axim Biotechnologies, Inc.

Owner Address: 18 E 50th St., 5th Floor
New York, NEW YORK 10022
UNITED STATES

Legal Entity Type:	CORPORATION	**State or Country Where Organized:**	NEVADA

Attorney/Correspondence Information

Attorney of Record

Attorney Name: Khanh T. Glatzel

Attorney Primary Email Address: klglatzel@gmail.com

Docket Number: T015-US

Attorney Email Authorized: Yes

Correspondent

Correspondent Name/Address: Khanh T. Glatzel
Premium IP Services, P.C.
1637 E. Valley Pkwy, #182
Escondido, CALIFORNIA 92027
UNITED STATES

Phone: 619-889-2990

Correspondent e-mail: kenny.glatzel@preips.com khanhtmhuynh@yahoo.com.au

Correspondent e-mail Authorized: Yes

Domestic Representative - Not Found

Prosecution History

Date	Description	Proceeding Number
Jul. 20, 2018	TEAS CHANGE OF CORRESPONDENCE RECEIVED	
Mar. 08, 2018	TEAS CHANGE OF CORRESPONDENCE RECEIVED	
Jan. 08, 2018	TEAS CHANGE OF CORRESPONDENCE RECEIVED	
Feb. 07, 2017	REGISTERED-SUPPLEMENTAL REGISTER	
Jan. 03, 2017	LAW OFFICE PUBLICATION REVIEW COMPLETED	70633
Dec. 29, 2016	ASSIGNED TO LIE	70633
Dec. 13, 2016	NOTICE OF ACCEPTANCE OF AMENDMENT TO ALLEGE USE E-MAILED	
Dec. 11, 2016	APPROVED FOR REGISTRATION SUPPLEMENTAL REGISTER	
Dec. 11, 2016	USE AMENDMENT ACCEPTED	83184
Dec. 07, 2016	TEAS/EMAIL CORRESPONDENCE ENTERED	88889
Dec. 06, 2016	CORRESPONDENCE RECEIVED IN LAW OFFICE	88889
Dec. 06, 2016	TEAS REQUEST FOR RECONSIDERATION RECEIVED	
Dec. 07, 2016	AMENDMENT TO USE PROCESSING COMPLETE	88889
Dec. 07, 2016	USE AMENDMENT FILED	88889
Dec. 06, 2016	TEAS AMENDMENT OF USE RECEIVED	
Jun. 07, 2016	ATTORNEY/DOM.REP.REVOKED AND/OR APPOINTED	
Jun. 07, 2016	TEAS REVOKE/APP/CHANGE ADDR OF ATTY/DOM REP RECEIVED	
Jun. 07, 2016	NOTIFICATION OF FINAL REFUSAL EMAILED	
Jun. 07, 2016	FINAL REFUSAL E-MAILED	
Jun. 07, 2016	FINAL REFUSAL WRITTEN	83184
May 10, 2016	TEAS/EMAIL CORRESPONDENCE ENTERED	88889
May 09, 2016	CORRESPONDENCE RECEIVED IN LAW OFFICE	88889
May 09, 2016	TEAS RESPONSE TO OFFICE ACTION RECEIVED	
Nov. 12, 2015	NOTIFICATION OF NON-FINAL ACTION E-MAILED	6325
Nov. 12, 2015	NON-FINAL ACTION E-MAILED	6325
Nov. 12, 2015	NON-FINAL ACTION WRITTEN	83184
Nov. 03, 2015	ASSIGNED TO EXAMINER	83184
Aug. 07, 2015	ATTORNEY/DOM.REP.REVOKED AND/OR APPOINTED	
Aug. 07, 2015	TEAS REVOKE/APP/CHANGE ADDR OF ATTY/DOM REP RECEIVED	
Jul. 21, 2015	ASSIGNED TO EXAMINER	68110
Jul. 07, 2015	ASSIGNED TO EXAMINER	83184
Jun. 26, 2015	NOTICE OF PSEUDO MARK E-MAILED	
Jun. 25, 2015	NEW APPLICATION OFFICE SUPPLIED DATA ENTERED IN TRAM	
Jun. 23, 2015	NEW APPLICATION ENTERED IN TRAM	

TM Staff and Location Information

TM Staff Information - None

File Location

Current Location: PUBLICATION AND ISSUE SECTION

Date in Location: Feb. 07, 2017

United States of America

United States Patent and Trademark Office

HEMPCHEW

Reg. No. 5,139,076

Registered Feb. 07, 2017

Int. Cl.: 30

Trademark

Supplemental Register

Axim Biotechnologies, Inc. (NEVADA CORPORATION)
18 E 50th St., 5th Floor
New York, NY 10022

CLASS 30: chewing gum; confectionery, namely, hard candies, chewable candies, lozenges, and lollipops; all the foregoing contain hemp, hemp extract, or hemp oil

FIRST USE 12-5-2016; IN COMMERCE 12-5-2016

THE MARK CONSISTS OF STANDARD CHARACTERS WITHOUT CLAIM TO ANY PARTICULAR FONT STYLE, SIZE OR COLOR

SER. NO. 86-668,083, FILED P.R. 06-19-2015; AM. S.R. 12-06-2016
LINDSEY HEATHER BEN, EXAMINING ATTORNEY

Michelle K. Lee

Director of the United States
Patent and Trademark Office

REQUIREMENTS TO MAINTAIN YOUR FEDERAL TRADEMARK REGISTRATION

WARNING: YOUR REGISTRATION WILL BE CANCELLED IF YOU DO NOT FILE THE DOCUMENTS BELOW DURING THE SPECIFIED TIME PERIODS.

Requirements in the First Ten Years*
What and When to File:

- *First Filing Deadline:* You must file a Declaration of Use (or Excusable Nonuse) between the 5th and 6th years after the registration date. See 15 U.S.C. §§1058, 1141k. If the declaration is accepted, the registration will continue in force for the remainder of the ten-year period, calculated from the registration date, unless cancelled by an order of the Commissioner for Trademarks or a federal court.

- *Second Filing Deadline:* You must file a Declaration of Use (or Excusable Nonuse) and an Application for Renewal between the 9th and 10th years after the registration date.* See 15 U.S.C. §1059.

Requirements in Successive Ten-Year Periods*
What and When to File:

- You must file a Declaration of Use (or Excusable Nonuse) and an Application for Renewal between every 9th and 10th-year period, calculated from the registration date.*

Grace Period Filings*

The above documents will be accepted as timely if filed within six months after the deadlines listed above with the payment of an additional fee.

*ATTENTION MADRID PROTOCOL REGISTRANTS: The holder of an international registration with an extension of protection to the United States under the Madrid Protocol must timely file the Declarations of Use (or Excusable Nonuse) referenced above directly with the United States Patent and Trademark Office (USPTO). The time periods for filing are based on the U.S. registration date (not the international registration date). The deadlines and grace periods for the Declarations of Use (or Excusable Nonuse) are identical to those for nationally issued registrations. See 15 U.S.C. §§1058, 1141k. However, owners of international registrations do not file renewal applications at the USPTO. Instead, the holder must file a renewal of the underlying international registration at the International Bureau of the World Intellectual Property Organization, under Article 7 of the Madrid Protocol, before the expiration of each ten-year term of protection, calculated from the date of the international registration. See 15 U.S.C. §1141j. For more information and renewal forms for the international registration, see http://www.wipo.int/madrid/en/.

NOTE: Fees and requirements for maintaining registrations are subject to change. Please check the USPTO website for further information. With the exception of renewal applications for registered extensions of protection, you can file the registration maintenance documents referenced above online at h ttp://www.uspto.gov.

NOTE: A courtesy e-mail reminder of USPTO maintenance filing deadlines will be sent to trademark owners/holders who authorize e-mail communication and maintain a current e-mail address with the USPTO. To ensure that e-mail is authorized and your address is current, please use the Trademark Electronic Application System (TEAS) Correspondence Address and Change of Owner Address Forms available at http://www.uspto.gov.

<div align="right">VITACANNABIS</div>

US Serial Number:	86754656	**Application Filing Date:**	Sep. 11, 2015
US Registration Number:	5498192	**Registration Date:**	Jun. 19, 2018
Filed as TEAS RF:	Yes	**Currently TEAS RF:**	Yes
Register:	Principal		
Mark Type:	Trademark		

Status: Registered. The registration date is used to determine when post-registration maintenance documents are due.

Status Date: Jun. 19, 2018

Publication Date: Aug. 30, 2016

Notice of Allowance Date: Oct. 25, 2016

Mark Information

Mark Literal Elements: VITACANNABIS

Standard Character Claim: Yes. The mark consists of standard characters without claim to any particular font style, size, or color.

Mark Drawing Type: 4 - STANDARD CHARACTER MARK

Goods and Services

Note: The following symbols indicate that the registrant/owner has amended the goods/services:

- Brackets [..] indicate deleted goods/services;
- Double parenthesis ((..)) identify any goods/services not claimed in a Section 15 affidavit of incontestability; and
- Asterisks *..* identify additional (new) wording in the goods/services.

For: Dietary and nutritional supplements; hemp powder for use as a nutritional supplement; nutritional supplement meal replacement powders for medical purposes; nutritional supplement meal replacement bars for medical purposes; nutritional supplement meal replacement drink mixes for medical purposes and dietary supplement drink mixes; nutraceuticals for use as a dietary supplement; topical creams, gels, salves, sprays, balms and ointments for analgesic purposes; nutrition supplements in drop form, capsule form and in liquid form; edible hemp oil for use as a dietary supplement; hemp oil as a nutritional supplement; all of the foregoing containing hemp or hemp-based extracts or oils as an ingredient

International Class(es): 005 - Primary Class

U.S Class(es): 006, 018, 044, 046, 051, 052

Class Status: ACTIVE

Basis: 1(a)

First Use: Mar. 14, 2018

Use in Commerce: Mar. 14, 2018

Basis Information (Case Level)

Filed Use:	No	**Currently Use:**	Yes	**Amended Use:**	No
Filed ITU:	Yes	**Currently ITU:**	No	**Amended ITU:**	No
Filed 44D:	No	**Currently 44D:**	No	**Amended 44D:**	No
Filed 44E:	No	**Currently 44E:**	No	**Amended 44E:**	No
Filed 66A:	No	**Currently 66A:**	No		
Filed No Basis:	No	**Currently No Basis:**	No		

Current Owner(s) Information

Owner Name:	HDDC Holdings LLC		
Owner Address:	10525 Vista Sorrento Pkwy Suite 200 San Diego, CALIFORNIA 92121 UNITED STATES		
Legal Entity Type:	LIMITED LIABILITY COMPANY	State or Country Where Organized:	NEVADA

Attorney/Correspondence Information

Attorney of Record

Attorney Name:	Khanh T. Glatzel	Docket Number:	HD-T016-US
Attorney Primary Email Address:	kenny.glatzel@preips.com	Attorney Email Authorized:	Yes

Correspondent

Correspondent Name/Address:	Khanh T. Glatzel Premium IP Services, P.C. 10525 Vista Sorrento Pkwy Suite 200 San Diego, CALIFORNIA 92121 UNITED STATES		
Phone:	619-889-2990		
Correspondent e- mail:	kenny.glatzel@preips.com khanhtmhuynh@yahoo.com.au	Correspondent e- mail Authorized:	Yes

Domestic Representative - Not Found

Prosecution History

Date	Description	Proceeding Number
Sep. 11, 2018	APPLICANT/CORRESPONDENCE CHANGES (NON-RESPONSIVE) ENTERED	88888
Sep. 11, 2018	TEAS CHANGE OF OWNER ADDRESS RECEIVED	
Jul. 20, 2018	TEAS CHANGE OF CORRESPONDENCE RECEIVED	
Jun. 19, 2018	REGISTERED-PRINCIPAL REGISTER	
May 12, 2018	NOTICE OF ACCEPTANCE OF STATEMENT OF USE E-MAILED	
May 11, 2018	ALLOWED PRINCIPAL REGISTER - SOU ACCEPTED	
Apr. 20, 2018	STATEMENT OF USE PROCESSING COMPLETE	74055
Apr. 03, 2018	USE AMENDMENT FILED	74055
Apr. 19, 2018	CASE ASSIGNED TO INTENT TO USE PARALEGAL	74055
Apr. 03, 2018	TEAS STATEMENT OF USE RECEIVED	
Dec. 06, 2017	ATTORNEY/DOM.REP.REVOKED AND/OR APPOINTED	
Dec. 06, 2017	TEAS REVOKE/APP/CHANGE ADDR OF ATTY/DOM REP RECEIVED	
Oct. 20, 2017	NOTICE OF APPROVAL OF EXTENSION REQUEST E-MAILED	
Oct. 18, 2017	EXTENSION 2 GRANTED	98765
Oct. 18, 2017	EXTENSION 2 FILED	98765
Oct. 18, 2017	TEAS EXTENSION RECEIVED	
Apr. 14, 2017	NOTICE OF APPROVAL OF EXTENSION REQUEST E-MAILED	
Apr. 11, 2017	EXTENSION 1 GRANTED	98765
Apr. 11, 2017	EXTENSION 1 FILED	98765
Apr. 11, 2017	TEAS EXTENSION RECEIVED	
Apr. 11, 2017	TEAS CHANGE OF CORRESPONDENCE RECEIVED	
Oct. 25, 2016	NOA E-MAILED - SOU REQUIRED FROM APPLICANT	
Aug. 30, 2016	OFFICIAL GAZETTE PUBLICATION CONFIRMATION E-MAILED	
Aug. 30, 2016	PUBLISHED FOR OPPOSITION	
Aug. 10, 2016	NOTIFICATION OF NOTICE OF PUBLICATION E-MAILED	
Jul. 25, 2016	LAW OFFICE PUBLICATION REVIEW COMPLETED	68171
Jul. 21, 2016	APPROVED FOR PUB - PRINCIPAL REGISTER	
Jun. 28, 2016	TEAS/EMAIL CORRESPONDENCE ENTERED	68171
Jun. 28, 2016	CORRESPONDENCE RECEIVED IN LAW OFFICE	68171
Jun. 23, 2016	ASSIGNED TO LIE	68171

Jun. 16, 2016	TEAS RESPONSE TO OFFICE ACTION RECEIVED	
Jun. 16, 2016	TEAS CHANGE OF CORRESPONDENCE RECEIVED	
Jun. 07, 2016	ATTORNEY/DOM.REP.REVOKED AND/OR APPOINTED	
Jun. 07, 2016	TEAS REVOKE/APP/CHANGE ADDR OF ATTY/DOM REP RECEIVED	
Dec. 16, 2015	NOTIFICATION OF NON-FINAL ACTION E-MAILED	6325
Dec. 16, 2015	NON-FINAL ACTION E-MAILED	6325
Dec. 16, 2015	NON-FINAL ACTION WRITTEN	82107
Dec. 15, 2015	ASSIGNED TO EXAMINER	82107
Dec. 07, 2015	APPLICANT/CORRESPONDENCE CHANGES (NON-RESPONSIVE) ENTERED	88888
Dec. 07, 2015	TEAS CHANGE OF OWNER ADDRESS RECEIVED	
Oct. 05, 2015	ASSIGNED TO EXAMINER	76072
Oct. 05, 2015	ASSIGNED TO EXAMINER	82107
Sep. 16, 2015	NEW APPLICATION OFFICE SUPPLIED DATA ENTERED IN TRAM	
Sep. 15, 2015	NEW APPLICATION ENTERED IN TRAM	

TM Staff and Location Information

TM Staff Information - None

File Location

Current Location: PUBLICATION AND ISSUE SECTION   **Date in Location:** May 11, 2018

United States of America

United States Patent and Trademark Office

VITACANNABIS

Reg. No. 5,498,192

Registered Jun. 19, 2018

Int. Cl.: 5

Trademark

Principal Register

HDDC Holdings LLC (NEVADA LIMITED LIABILITY COMPANY)
525 B Street, Suite 2200
San Diego, CALIFORNIA 92101

CLASS 5: Dietary and nutritional supplements; hemp powder for use as a nutritional supplement; nutritional supplement meal replacement powders for medical purposes; nutritional supplement meal replacement bars for medical purposes; nutritional supplement meal replacement drink mixes for medical purposes and dietary supplement drink mixes; nutraceuticals for use as a dietary supplement; topical creams, gels, salves, sprays, balms and ointments for analgesic purposes; nutrition supplements in drop form, capsule form and in liquid form; edible hemp oil for use as a dietary supplement; hemp oil as a nutritional supplement; all of the foregoing containing hemp or hemp-based extracts or oils as an ingredient

FIRST USE 3-14-2018; IN COMMERCE 3-14-2018

THE MARK CONSISTS OF STANDARD CHARACTERS WITHOUT CLAIM TO ANY PARTICULAR FONT STYLE, SIZE OR COLOR

SER. NO. 86-754,656, FILED 09-11-2015

Director of the United States
Patent and Trademark Office

REQUIREMENTS TO MAINTAIN YOUR FEDERAL TRADEMARK REGISTRATION

WARNING: YOUR REGISTRATION WILL BE CANCELLED IF YOU DO NOT FILE THE DOCUMENTS BELOW DURING THE SPECIFIED TIME PERIODS.

Requirements in the First Ten Years*
What and When to File:

- *First Filing Deadline:* You must file a Declaration of Use (or Excusable Nonuse) between the 5th and 6th years after the registration date. See 15 U.S.C. §§1058, 1141k. If the declaration is accepted, the registration will continue in force for the remainder of the ten-year period, calculated from the registration date, unless cancelled by an order of the Commissioner for Trademarks or a federal court.

- *Second Filing Deadline:* You must file a Declaration of Use (or Excusable Nonuse) and an Application for Renewal between the 9th and 10th years after the registration date.* See 15 U.S.C. §1059.

Requirements in Successive Ten-Year Periods*
What and When to File:

- You must file a Declaration of Use (or Excusable Nonuse) and an Application for Renewal between every 9th and 10th-year period, calculated from the registration date.*

Grace Period Filings*

The above documents will be accepted as timely if filed within six months after the deadlines listed above with the payment of an additional fee.

*ATTENTION MADRID PROTOCOL REGISTRANTS: The holder of an international registration with an extension of protection to the United States under the Madrid Protocol must timely file the Declarations of Use (or Excusable Nonuse) referenced above directly with the United States Patent and Trademark Office (USPTO). The time periods for filing are based on the U.S. registration date (not the international registration date). The deadlines and grace periods for the Declarations of Use (or Excusable Nonuse) are identical to those for nationally issued registrations. See 15 U.S.C. §§1058, 1141k. However, owners of international registrations do not file renewal applications at the USPTO. Instead, the holder must file a renewal of the underlying international registration at the International Bureau of the World Intellectual Property Organization, under Article 7 of the Madrid Protocol, before the expiration of each ten-year term of protection, calculated from the date of the international registration. See 15 U.S.C. §1141j. For more information and renewal forms for the international registration, see http://www.wipo.int/madrid/en/.

NOTE: Fees and requirements for maintaining registrations are subject to change. Please check the USPTO website for further information. With the exception of renewal applications for registered extensions of protection, you can file the registration maintenance documents referenced above online at h ttp://www.uspto.gov.

NOTE: A courtesy e-mail reminder of USPTO maintenance filing deadlines will be sent to trademark owners/holders who authorize e-mail communication and maintain a current e-mail address with the USPTO. To ensure that e-mail is authorized and your address is current, please use the Trademark Electronic Application System (TEAS) Correspondence Address and Change of Owner Address Forms available at http://www.uspto.gov.

US Serial Number:	87036912	**Application Filing Date:**	May 13, 2016
US Registration Number:	5193224	**Registration Date:**	Apr. 25, 2017
Filed as TEAS RF:	Yes	**Currently TEAS RF:**	Yes
Register:	Supplemental		
Mark Type:	Trademark		
Amended to Principal Register:	No	**Date Amended to Current Register:**	Mar. 02, 2017

Status: Registered. The registration date is used to determine when post-registration maintenance documents are due.

Status Date: Apr. 25, 2017

Mark Information

Mark Literal Elements: NATURAL COLORADO WELLNESS

Standard Character Claim: Yes. The mark consists of standard characters without claim to any particular font style, size, or color.

Mark Drawing Type: 4 - STANDARD CHARACTER MARK

Goods and Services

Note: The following symbols indicate that the registrant/owner has amended the goods/services:

- Brackets [..] indicate deleted goods/services;
- Double parenthesis ((..)) identify any goods/services not claimed in a Section 15 affidavit of incontestability; and
- Asterisks *..* identify additional (new) wording in the goods/services.

For: Nutritional supplements containing essential hemp oils found in plant extracts

International Class(es):	005 - Primary Class	**U.S Class(es):**	006, 018, 044, 046, 051, 052
Class Status:	ACTIVE		
Basis:	1(a)		
First Use:	May 12, 2016	**Use in Commerce:**	May 12, 2016

Basis Information (Case Level)

Filed Use:	Yes	**Currently Use:**	Yes	**Amended Use:**	No
Filed ITU:	No	**Currently ITU:**	No	**Amended ITU:**	No
Filed 44D:	No	**Currently 44D:**	No	**Amended 44D:**	No
Filed 44E:	No	**Currently 44E:**	No	**Amended 44E:**	No
Filed 66A:	No	**Currently 66A:**	No		
Filed No Basis:	No	**Currently No Basis:**	No		

Current Owner(s) Information

Owner Name: CWB Holdings, Inc.

Owner Address: Suite 700
1720 S. Bellaire Street
Denver, COLORADO 80222
UNITED STATES

Legal Entity Type:	CORPORATION	State or Country Where Organized:	COLORADO

Attorney/Correspondence Information

Attorney of Record

Attorney Name:	Ryan M. Kaiser
Attorney Primary Email Address:	trademark@amintalati.com	**Attorney Email Authorized:** Yes

Correspondent

Correspondent Name/Address: Ryan M. Kaiser
Amin Talati Upadhye LLP
100 S. Wacker Dr.
Suite 2000
Chicago, ILLINOIS 60606
UNITED STATES

Phone:	3124661033	**Fax:**	3128847352

Correspondent e-mail: trademark@amintalati.com james@amintalati.com   **Correspondent e-mail Authorized:** Yes

Domestic Representative - Not Found

Prosecution History

Date	Description	Proceeding Number
Mar. 29, 2018	ATTORNEY/DOM.REP.REVOKED AND/OR APPOINTED	
Mar. 29, 2018	TEAS REVOKE/APP/CHANGE ADDR OF ATTY/DOM REP RECEIVED	
Apr. 25, 2017	REGISTERED-SUPPLEMENTAL REGISTER	
Mar. 18, 2017	LAW OFFICE PUBLICATION REVIEW COMPLETED	69712
Mar. 18, 2017	ASSIGNED TO LIE	69712
Mar. 02, 2017	TEAS CHANGE OF CORRESPONDENCE RECEIVED	
Mar. 02, 2017	APPROVED FOR REGISTRATION SUPPLEMENTAL REGISTER	
Mar. 02, 2017	EXAMINER'S AMENDMENT ENTERED	88888
Mar. 02, 2017	NOTIFICATION OF EXAMINERS AMENDMENT E-MAILED	6328
Mar. 02, 2017	EXAMINERS AMENDMENT E-MAILED	6328
Mar. 02, 2017	EXAMINERS AMENDMENT -WRITTEN	78449
Sep. 03, 2016	NOTIFICATION OF NON-FINAL ACTION E-MAILED	6325
Sep. 03, 2016	NON-FINAL ACTION E-MAILED	6325
Sep. 03, 2016	NON-FINAL ACTION WRITTEN	78449
Aug. 29, 2016	ASSIGNED TO EXAMINER	78449
May 18, 2016	NEW APPLICATION OFFICE SUPPLIED DATA ENTERED IN TRAM	
May 17, 2016	NEW APPLICATION ENTERED IN TRAM	

TM Staff and Location Information

TM Staff Information - None

File Location

Current Location:	PUBLICATION AND ISSUE SECTION	**Date in Location:**	Apr. 25, 2017

United States of America

United States Patent and Trademark Office

NATURAL COLORADO WELLNESS

Reg. No. 5,193,224

Registered Apr. 25, 2017

Int. Cl.: 5

Trademark

Supplemental Register

CWB Holdings, Inc. (COLORADO CORPORATION)
Suite 700
1720 S. Bellaire Street
Denver, CO 80222

CLASS 5: Nutritional supplements containing essential hemp oils found in plant extracts

FIRST USE 5-12-2016; IN COMMERCE 5-12-2016

THE MARK CONSISTS OF STANDARD CHARACTERS WITHOUT CLAIM TO ANY PARTICULAR FONT STYLE, SIZE OR COLOR

SER. NO. 87-036,912, FILED P.R. 05-13-2016; AM. S.R. 03-02-2017
ODESSA B BIBBINS, EXAMINING ATTORNEY

Director of the United States
Patent and Trademark Office

REQUIREMENTS TO MAINTAIN YOUR FEDERAL TRADEMARK REGISTRATION
WARNING: YOUR REGISTRATION WILL BE CANCELLED IF YOU DO NOT FILE THE DOCUMENTS BELOW DURING THE SPECIFIED TIME PERIODS.

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- *Second Filing Deadline:* You must file a Declaration of Use (or Excusable Nonuse) and an Application for Renewal between the 9th and 10th years after the registration date.* See 15 U.S.C. §1059.

Requirements in Successive Ten-Year Periods*
What and When to File:

- You must file a Declaration of Use (or Excusable Nonuse) and an Application for Renewal between every 9th and 10th-year period, calculated from the registration date.*

Grace Period Filings*

The above documents will be accepted as timely if filed within six months after the deadlines listed above with the payment of an additional fee.

*ATTENTION MADRID PROTOCOL REGISTRANTS: The holder of an international registration with an extension of protection to the United States under the Madrid Protocol must timely file the Declarations of Use (or Excusable Nonuse) referenced above directly with the United States Patent and Trademark Office (USPTO). The time periods for filing are based on the U.S. registration date (not the international registration date). The deadlines and grace periods for the Declarations of Use (or Excusable Nonuse) are identical to those for nationally issued registrations. See 15 U.S.C. §§1058, 1141k. However, owners of international registrations do not file renewal applications at the USPTO. Instead, the holder must file a renewal of the underlying international registration at the International Bureau of the World Intellectual Property Organization, under Article 7 of the Madrid Protocol, before the expiration of each ten-year term of protection, calculated from the date of the international registration. See 15 U.S.C. §1141j. For more information and renewal forms for the international registration, see http://www.wipo.int/madrid/en/.

NOTE: Fees and requirements for maintaining registrations are subject to change. Please check the USPTO website for further information. With the exception of renewal applications for registered extensions of protection, you can file the registration maintenance documents referenced above online at h ttp://www.uspto.gov.

NOTE: A courtesy e-mail reminder of USPTO maintenance filing deadlines will be sent to trademark owners/holders who authorize e-mail communication and maintain a current e-mail address with the USPTO. To ensure that e-mail is authorized and your address is current, please use the Trademark Electronic Application System (TEAS) Correspondence Address and Change of Owner Address Forms available at http://www.uspto.gov.

<div align="right">

GREEN ROADS

</div>

US Serial Number:	87080056	**Application Filing Date:**	Jun. 22, 2016
US Registration Number:	5288805	**Registration Date:**	Sep. 19, 2017
Filed as TEAS RF:	Yes	**Currently TEAS RF:**	Yes
Register:	Principal		
Mark Type:	Trademark		

TM5 Common Status Descriptor:

LIVE/REGISTRATION/Issued and Active

The trademark application has been registered with the Office.

Status: Registered. The registration date is used to determine when post-registration maintenance documents are due.

Status Date: Sep. 19, 2017

Publication Date: Jul. 04, 2017

Mark Information

Mark Literal Elements: GREEN ROADS

Standard Character Claim: Yes. The mark consists of standard characters without claim to any particular font style, size, or color.

Mark Drawing Type: 4 - STANDARD CHARACTER MARK

Related Properties Information

Parent Of: 87975187

Goods and Services

Note: The following symbols indicate that the registrant/owner has amended the goods/services:

- Brackets [..] indicate deleted goods/services;
- Double parenthesis ((..)) identify any goods/services not claimed in a Section 15 affidavit of incontestability; and
- Asterisks *..* identify additional (new) wording in the goods/services.

For: dietary and nutritional supplements; neutraceuticals for use as a dietary supplement drink mix; nutraceuticals for use as dietary supplement; nutritional supplements in drop form, capsule form, and liquid form; edible hemp oil for use as a dietary supplement; all of the foregoing containing hemp oil and only naturally occurring amounts of CBD

International Class(es):	005 - Primary Class	**U.S Class(es):**	006, 018, 044, 046, 051, 052
Class Status:	ACTIVE		
Basis:	1(a)		
First Use:	Nov. 18, 2014	**Use in Commerce:**	Nov. 18, 2014

Basis Information (Case Level)

Filed Use:	Yes	**Currently Use:**	Yes
Filed ITU:	No	**Currently ITU:**	No
Filed 44D:	No	**Currently 44E:**	No
Filed 44E:	No	**Currently 66A:**	No
Filed 66A:	No	**Currently No Basis:**	No

Filed No Basis: No

Current Owner(s) Information

Owner Name: Green Roads of Florida, LLC.

Owner Address: 6891 Stirling Road
Davie, FLORIDA UNITED STATES 33314

Legal Entity Type: LIMITED LIABILITY COMPANY   **State or Country** FLORIDA
Where Organized:

Attorney/Correspondence Information

Attorney of Record

Attorney Name: Grace R Neibaron

Attorney Primary grace@neibaronlaw.com   **Attorney Email** Yes
Email Address: **Authorized:**

Correspondent

Correspondent Grace R Neibaron
Name/Address: Hoban Law Group
235 Foss Creek Circle
Healdsburg, CALIFORNIA UNITED STATES 95448

Phone: 7078436645

Correspondent e- grace@neibaronlaw.com davidk@hoban.law   **Correspondent e-** Yes
mail: **mail Authorized:**

Domestic Representative - Not Found

Prosecution History

Date	Description	Proceeding Number
Sep. 19, 2017	REGISTERED-PRINCIPAL REGISTER	
Jul. 27, 2017	ATTORNEY/DOM.REP.REVOKED AND/OR APPOINTED	
Jul. 27, 2017	TEAS REVOKE/APP/CHANGE ADDR OF ATTY/DOM REP RECEIVED	
Jul. 04, 2017	OFFICIAL GAZETTE PUBLICATION CONFIRMATION E-MAILED	
Jul. 04, 2017	PUBLISHED FOR OPPOSITION	
Jun. 14, 2017	NOTIFICATION OF NOTICE OF PUBLICATION E-MAILED	
May 31, 2017	LAW OFFICE PUBLICATION REVIEW COMPLETED	70633
May 26, 2017	APPROVED FOR PUB - PRINCIPAL REGISTER	
May 15, 2017	TEAS/EMAIL CORRESPONDENCE ENTERED	70633
May 15, 2017	CORRESPONDENCE RECEIVED IN LAW OFFICE	70633
May 04, 2017	TEAS REQUEST FOR RECONSIDERATION RECEIVED	
Apr. 20, 2017	NOTIFICATION OF FINAL REFUSAL EMAILED	
Apr. 20, 2017	FINAL REFUSAL E-MAILED	
Apr. 20, 2017	FINAL REFUSAL WRITTEN	82107
Mar. 24, 2017	DIVISIONAL PROCESSING COMPLETE	
Feb. 20, 2017	DIVISIONAL REQUEST RECEIVED	
Mar. 20, 2017	CASE ASSIGNED TO INTENT TO USE PARALEGAL	66530
Mar. 15, 2017	TEAS/EMAIL CORRESPONDENCE ENTERED	70633
Mar. 15, 2017	CORRESPONDENCE RECEIVED IN LAW OFFICE	70633
Mar. 09, 2017	ASSIGNED TO LIE	70633
Feb. 21, 2017	TEAS RESPONSE TO OFFICE ACTION RECEIVED	
Feb. 20, 2017	TEAS REQUEST TO DIVIDE RECEIVED	
Sep. 09, 2016	NOTIFICATION OF NON-FINAL ACTION E-MAILED	6325
Sep. 09, 2016	NON-FINAL ACTION E-MAILED	6325
Sep. 09, 2016	NON-FINAL ACTION WRITTEN	82107
Sep. 02, 2016	ASSIGNED TO EXAMINER	82107
Jul. 08, 2016	ASSIGNED TO EXAMINER	76072
Jul. 07, 2016	ASSIGNED TO EXAMINER	82107

Jun. 28, 2016   NEW APPLICATION OFFICE SUPPLIED DATA ENTERED IN TRAM
Jun. 25, 2016   NEW APPLICATION ENTERED IN TRAM

TM Staff and Location Information

TM Staff Information - None

File Location

Current Location: PUBLICATION AND ISSUE SECTION   **Date in Location:** Sep. 19, 2017

United States of America

United States Patent and Trademark Office

GREEN ROADS

Reg. No. 5,288,805

Registered Sep. 19, 2017

Int. Cl.: 5

Trademark

Principal Register

Green Roads of Florida, LLC. (FLORIDA LIMITED LIABILITY COMPANY)
6891 Stirling Road
Davie, FL 33314

CLASS 5: dietary and nutritional supplements; neutraceuticals for use as a dietary supplement drink mix; nutraceuticals for use as dietary supplement; nutritional supplements in drop form, capsule form, and liquid form; edible hemp oil for use as a dietary supplement; all of the foregoing containing hemp oil and only naturally occurring amounts of CBD

FIRST USE 11-18-2014; IN COMMERCE 11-18-2014

THE MARK CONSISTS OF STANDARD CHARACTERS WITHOUT CLAIM TO ANY PARTICULAR FONT STYLE, SIZE OR COLOR

SER. NO. 87-080,056, FILED 06-22-2016
ROBERT J STRUCK, EXAMINING ATTORNEY

Joseph Matol

Performing the Functions and Duties of the
Under Secretary of Commerce for
Intellectual Property and Director of the
United States Patent and Trademark Office

REQUIREMENTS TO MAINTAIN YOUR FEDERAL TRADEMARK REGISTRATION

WARNING: YOUR REGISTRATION WILL BE CANCELLED IF YOU DO NOT FILE THE DOCUMENTS BELOW DURING THE SPECIFIED TIME PERIODS.

Requirements in the First Ten Years*
What and When to File:

- *First Filing Deadline:* You must file a Declaration of Use (or Excusable Nonuse) between the 5th and 6th years after the registration date. See 15 U.S.C. §§1058, 1141k. If the declaration is accepted, the registration will continue in force for the remainder of the ten-year period, calculated from the registration date, unless cancelled by an order of the Commissioner for Trademarks or a federal court.

- *Second Filing Deadline:* You must file a Declaration of Use (or Excusable Nonuse) and an Application for Renewal between the 9th and 10th years after the registration date.* See 15 U.S.C. §1059.

Requirements in Successive Ten-Year Periods*
What and When to File:

- You must file a Declaration of Use (or Excusable Nonuse) and an Application for Renewal between every 9th and 10th-year period, calculated from the registration date.*

Grace Period Filings*

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NOTE: A courtesy e-mail reminder of USPTO maintenance filing deadlines will be sent to trademark owners/holders who authorize e-mail communication and maintain a current e-mail address with the USPTO. To ensure that e-mail is authorized and your address is current, please use the Trademark Electronic Application System (TEAS) Correspondence Address and Change of Owner Address Forms available at http://www.uspto.gov.

CANNABITES

US Serial Number:	87276686	**Application Filing Date:**	Dec. 21, 2016
US Registration Number:	5543497	**Registration Date:**	Aug. 21, 2018
Filed as TEAS RF:	Yes	**Currently TEAS RF:**	Yes
Register:	Principal		
Mark Type:	Trademark		
Status:	Registered. The registration date is used to determine when post-registration maintenance documents are due.		
Status Date:	Aug. 21, 2018		
Publication Date:	Jun. 05, 2018		

Mark Information

Mark Literal Elements: CANNABITES

Standard Character Claim: Yes. The mark consists of standard characters without claim to any particular font style, size, or color.

Mark Drawing Type: 4 - STANDARD CHARACTER MARK

Goods and Services

Note: The following symbols indicate that the registrant/owner has amended the goods/services:

- Brackets [..] indicate deleted goods/services;
- Double parenthesis ((..)) identify any goods/services not claimed in a Section 15 affidavit of incontestability; and
- Asterisks *..* identify additional (new) wording in the goods/services.

For: dietary and nutritional supplements for pets; edible hemp oil animal treats for use as a dietary supplement; nutritional food additives for culinary purposes; all of the foregoing containing edible hemp seed oil and vitamins for animals

International Class(es):	005 - Primary Class	**U.S Class(es):**	006, 018, 044, 046, 051, 052
Class Status:	ACTIVE		
Basis:	1(a)		
First Use:	Dec. 20, 2016	**Use in Commerce:**	Dec. 20, 2016

Basis Information (Case Level)

Filed Use:	Yes	**Currently Use:**	Yes	**Amended Use:**	No
Filed ITU:	No	**Currently ITU:**	No	**Amended ITU:**	No
Filed 44D:	No	**Currently 44D:**	No	**Amended 44D:**	No
Filed 44E:	No	**Currently 44E:**	No	**Amended 44E:**	No
Filed 66A:	No	**Currently 66A:**	No		
Filed No Basis:	No	**Currently No Basis:**	No		

Current Owner(s) Information

Owner Name: Victory Gardens, LLC

Owner Address: 785 La Mirada Ave
Encinitas, CALIFORNIA 92024
UNITED STATES

Legal Entity Type:	LIMITED LIABILITY COMPANY	**State or Country**
		Where Organized: CALIFORNIA

Attorney/Correspondence Information

Attorney of Record

Attorney Name:	Pollie Gautsch		
Attorney Primary Email Address:	pollie@gandalegal.com	**Attorney Email Authorized:**	Yes

Correspondent

Correspondent Name/Address:	POLLIE GAUTSCH G&A LEGAL, APC 665 SAN RODOLFO 124-209 SOLANA BEACH, CALIFORNIA 92075 UNITED STATES

Phone:	858-344-5905	**Fax:**	760-454-4673
Correspondent e-mail:	pollie@gandalegal.com	**Correspondent e-mail Authorized:**	Yes

Domestic Representative - Not Found

Prosecution History

Date	Description	Proceeding Number
Aug. 21, 2018	REGISTERED-PRINCIPAL REGISTER	
Jun. 05, 2018	OFFICIAL GAZETTE PUBLICATION CONFIRMATION E-MAILED	
Jun. 05, 2018	PUBLISHED FOR OPPOSITION	
May 16, 2018	NOTIFICATION OF NOTICE OF PUBLICATION E-MAILED	
Apr. 27, 2018	APPROVED FOR PUB - PRINCIPAL REGISTER	
Apr. 04, 2018	TEAS/EMAIL CORRESPONDENCE ENTERED	88889
Apr. 03, 2018	CORRESPONDENCE RECEIVED IN LAW OFFICE	88889
Apr. 03, 2018	TEAS RESPONSE TO OFFICE ACTION RECEIVED	
Oct. 26, 2017	NOTIFICATION OF NON-FINAL ACTION E-MAILED	6325
Oct. 26, 2017	NON-FINAL ACTION E-MAILED	6325
Oct. 26, 2017	NON-FINAL ACTION WRITTEN	82107
Oct. 02, 2017	TEAS/EMAIL CORRESPONDENCE ENTERED	66213
Oct. 02, 2017	CORRESPONDENCE RECEIVED IN LAW OFFICE	66213
Sep. 26, 2017	TEAS RESPONSE TO OFFICE ACTION RECEIVED	
Sep. 15, 2017	TEAS/EMAIL CORRESPONDENCE ENTERED	88889
Sep. 14, 2017	CORRESPONDENCE RECEIVED IN LAW OFFICE	88889
Sep. 14, 2017	TEAS RESPONSE TO OFFICE ACTION RECEIVED	
Sep. 06, 2017	NOTIFICATION OF NON-FINAL ACTION E-MAILED	6325
Sep. 06, 2017	NON-FINAL ACTION E-MAILED	6325
Sep. 06, 2017	NON-FINAL ACTION WRITTEN	82107
Aug. 08, 2017	TEAS/EMAIL CORRESPONDENCE ENTERED	66213
Aug. 08, 2017	CORRESPONDENCE RECEIVED IN LAW OFFICE	66213
Aug. 02, 2017	ASSIGNED TO LIE	66213
Jul. 03, 2017	TEAS RESPONSE TO OFFICE ACTION RECEIVED	
Apr. 26, 2017	NOTIFICATION OF NON-FINAL ACTION E-MAILED	6325
Apr. 26, 2017	NON-FINAL ACTION E-MAILED	6325
Apr. 26, 2017	NON-FINAL ACTION WRITTEN	82107
Apr. 19, 2017	ASSIGNED TO EXAMINER	82107
Jan. 09, 2017	ASSIGNED TO EXAMINER	76072
Jan. 09, 2017	ASSIGNED TO EXAMINER	82107
Jan. 04, 2017	NEW APPLICATION OFFICE SUPPLIED DATA ENTERED IN TRAM	
Dec. 24, 2016	NEW APPLICATION ENTERED IN TRAM	

TM Staff and Location Information

Current Location: PUBLICATION AND ISSUE SECTION   **Date in Location:** Aug. 21, 2018

United States of America

United States Patent and Trademark Office

CANNABITES

Reg. No. 5,543,497

Registered Aug. 21, 2018

Int. Cl.: 5

Trademark

Principal Register

Victory Gardens, LLC (CALIFORNIA LIMITED LIABILITY COMPANY)
785 La Mirada Ave
Encinitas, CALIFORNIA 92024

CLASS 5: dietary and nutritional supplements for pets; edible hemp oil animal treats for use as a dietary supplement; nutritional food additives for culinary purposes; all of the foregoing containing edible hemp seed oil and vitamins for animals

FIRST USE 12-20-2016; IN COMMERCE 12-20-2016

THE MARK CONSISTS OF STANDARD CHARACTERS WITHOUT CLAIM TO ANY PARTICULAR FONT STYLE, SIZE OR COLOR

SER. NO. 87-276,686, FILED 12-21-2016

Director of the United States
Patent and Trademark Office

REQUIREMENTS TO MAINTAIN YOUR FEDERAL TRADEMARK REGISTRATION

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Requirements in the First Ten Years*
What and When to File:

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Requirements in Successive Ten-Year Periods*
What and When to File:

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stoned cold

US Serial Number:	87426938	**Application Filing Date:**	Apr. 26, 2017
US Registration Number:	5363541	**Registration Date:**	Dec. 26, 2017
Filed as TEAS Plus:	Yes	**Currently TEAS Plus:**	Yes
Register:	Principal		
Mark Type:	Trademark		
Status:	Registered. The registration date is used to determine when post-registration maintenance documents are due.		
Status Date:	Dec. 26, 2017		
Publication Date:	Oct. 10, 2017		

Mark Information

Mark Literal Elements: STONED COLD

Standard Character Claim: Yes. The mark consists of standard characters without claim to any particular font style, size, or color.

Mark Drawing Type: 4 - STANDARD CHARACTER MARK

Goods and Services

Note: The following symbols indicate that the registrant/owner has amended the goods/services:

- Brackets [..] indicate deleted goods/services;
- Double parenthesis ((..)) identify any goods/services not claimed in a Section 15 affidavit of incontestability; and
- Asterisks *..* identify additional (new) wording in the goods/services.

For: Medicated lip balm and medicated skin care preparations comprised of hemp seed oil and menthol

International Class(es):	005 - Primary Class	**U.S Class(es):**	006, 018, 044, 046, 051, 052
Class Status:	ACTIVE		
Basis:	1(a)		
First Use:	Mar. 01, 2017	**Use in Commerce:**	Apr. 11, 2017

Basis Information (Case Level)

Filed Use:	Yes	**Currently Use:**	Yes	**Amended Use:**	No
Filed ITU:	No	**Currently ITU:**	No	**Amended ITU:**	No
Filed 44D:	No	**Currently 44D:**	No	**Amended 44D:**	No
Filed 44E:	No	**Currently 44E:**	No	**Amended 44E:**	No
Filed 66A:	No	**Currently 66A:**	No		
Filed No Basis:	No	**Currently No Basis:**	No		

Current Owner(s) Information

Owner Name: LOVEBUD, LLC

DBA, AKA, Formerly: DBA LOVEBUD

Owner Address: 578 Washington Blvd., #507

Marina del Rey, CALIFORNIA 90292
UNITED STATES

Legal Entity Type: LIMITED LIABILITY COMPANY   **State or Country**   CALIFORNIA
Where Organized:

Attorney/Correspondence Information

Attorney of Record - None

Correspondent

Correspondent   LOVEBUD, LLC
Name/Address: 578 WASHINGTON BLVD., #507
MARINA DEL REY, CALIFORNIA 90292
UNITED STATES

Phone: 424-781-4135

Correspondent e-   becky@lovebudshop.com   **Correspondent e-**   Yes
mail: **mail Authorized:**

Domestic Representative - Not Found

Prosecution History

Date	Description	Proceeding Number
Dec. 26, 2017	REGISTERED-PRINCIPAL REGISTER	
Oct. 10, 2017	OFFICIAL GAZETTE PUBLICATION CONFIRMATION E-MAILED	
Oct. 10, 2017	PUBLISHED FOR OPPOSITION	
Sep. 20, 2017	NOTIFICATION OF NOTICE OF PUBLICATION E-MAILED	
Sep. 01, 2017	APPROVED FOR PUB - PRINCIPAL REGISTER	
Aug. 24, 2017	TEAS/EMAIL CORRESPONDENCE ENTERED	68171
Aug. 24, 2017	CORRESPONDENCE RECEIVED IN LAW OFFICE	68171
Aug. 22, 2017	TEAS RESPONSE TO OFFICE ACTION RECEIVED	
Aug. 16, 2017	COMBINED EXAMINER'S AMENDMENT/PRIORITY ACTION ENTERED	68171
Aug. 16, 2017	ASSIGNED TO LIE	68171
Aug. 15, 2017	NOTIFICATION OF EXAMINER'S AMENDMENT/PRIORITY ACTION E-MAILED	6326
Aug. 15, 2017	EXAMINER'S AMENDMENT/PRIORITY ACTION E-MAILED	6326
Aug. 15, 2017	EXAMINERS AMENDMENT AND/OR PRIORITY ACTION - COMPLETED	72008
Jul. 24, 2017	ASSIGNED TO EXAMINER	72008
Jul. 22, 2017	ASSIGNED TO EXAMINER	91238
Jun. 05, 2017	NEW APPLICATION OFFICE SUPPLIED DATA ENTERED IN TRAM	
Apr. 29, 2017	NEW APPLICATION ENTERED IN TRAM	

TM Staff and Location Information

TM Staff Information - None

File Location

Current Location: PUBLICATION AND ISSUE SECTION   **Date in Location:** Dec. 26, 2017

United States of America

United States Patent and Trademark Office

stoned cold

Reg. No. 5,363,541

Registered Dec. 26, 2017

Int. Cl.: 5

Trademark

Principal Register

LOVEBUD, LLC (CALIFORNIA LIMITED LIABILITY COMPANY), DBA LOVEBUD
578 Washington Blvd., #507
Marina Del Rey, CALIFORNIA 90292

CLASS 5: Medicated lip balm and medicated skin care preparations comprised of hemp seed oil and menthol

FIRST USE 3-1-2017; IN COMMERCE 4-11-2017

THE MARK CONSISTS OF STANDARD CHARACTERS WITHOUT CLAIM TO ANY PARTICULAR FONT STYLE, SIZE OR COLOR

SER. NO. 87-426,938, FILED 04-26-2017

Joseph Matal

Performing the Functions and Duties of the
Under Secretary of Commerce for
Intellectual Property and Director of the
United States Patent and Trademark Office

REQUIREMENTS TO MAINTAIN YOUR FEDERAL TRADEMARK REGISTRATION

WARNING: YOUR REGISTRATION WILL BE CANCELLED IF YOU DO NOT FILE THE DOCUMENTS BELOW DURING THE SPECIFIED TIME PERIODS.

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What and When to File:

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Mark: PRIME MY BODY

PRIME *my* BODY

US Serial Number:	87794756	**Application Filing Date:**	Feb. 12, 2018
US Registration Number:	5589298	**Registration Date:**	Oct. 23, 2018
Filed as TEAS RF:	Yes	**Currently TEAS RF:**	Yes
Register:	Principal		
Mark Type:	Trademark		

Status: Registered. The registration date is used to determine when post-registration maintenance documents are due.

Status Date: Oct. 23, 2018

Publication Date: Aug. 07, 2018

Mark Information

Mark Literal Elements: PRIME MY BODY

Standard Character Claim: No

Mark Drawing Type: 3 - AN ILLUSTRATION DRAWING WHICH INCLUDES WORD(S)/ LETTER(S)/NUMBER(S)

Description of Mark: The mark consists of the distressed stylized word "PRIME"; next to the cursive stylized word "MY" in a circle; next to that is the stylized word "BODY".

Color(s) Claimed: Color is not claimed as a feature of the mark.

Design Search Code(s): 26.01.21 - Circles that are totally or partially shaded.

Goods and Services

Note: The following symbols indicate that the registrant/owner has amended the goods/services:

- Brackets [..] indicate deleted goods/services;
- Double parenthesis ((..)) identify any goods/services not claimed in a Section 15 affidavit of incontestability; and
- Asterisks *..* identify additional (new) wording in the goods/services.

For: Skin moisturizers; Cosmetic preparations, namely, firming creams

International Class(es):	003 - Primary Class	**U.S Class(es):**	001, 004, 006, 050, 051, 052
Class Status:	ACTIVE		
Basis:	1(a)		
First Use:	Jul. 2013	**Use in Commerce:**	Jul. 2013

For: Dietary and nutritional supplements; Protein supplements; Hemp oil for use as a dietary supplement

International Class(es):	005 - Primary Class	**U.S Class(es):**	006, 018, 044, 046, 051, 052
Class Status:	ACTIVE		
Basis:	1(a)		
First Use:	Jul. 2013	**Use in Commerce:**	Jul. 2013

Basis Information (Case Level)

Filed Use:	Yes	**Currently Use:**	Yes	**Amended Use:**	No
Filed ITU:	No	**Currently ITU:**	No	**Amended ITU:**	No
Filed 44D:	No	**Currently 44D:**	No	**Amended 44D:**	No
Filed 44E:	No	**Currently 44E:**	No	**Amended 44E:**	No

	Filed 66A:	No		Currently 66A:	No
	Filed No Basis:	No		Currently No Basis:	No

Current Owner(s) Information

Owner Name:	Digital Media Group - US, LLC		
Owner Address:	1501 LBJ Freeway, Suite 500 Dallas, TEXAS 75234 UNITED STATES		
Legal Entity Type:	LIMITED LIABILITY COMPANY	State or Country Where Organized:	NEVADA

Attorney/Correspondence Information

Attorney of Record

Attorney Name:	Joshua M. Gerben, Esq.	Docket Number:	06869-0002
Attorney Primary Email Address:	jgerben@gerbenlawfirm.com	Attorney Email Authorized:	Yes

Correspondent

Correspondent Name/Address:	JOSHUA M. GERBEN, ESQ. GERBEN LAW FIRM, PLLC 1050 CONNECTICUT AVE. NW SUITE 500 WASHINGTON, DISTRICT OF COLUMBIA 20036 UNITED STATES		
Phone:	202-294-2287		
Correspondent e- mail:	jgerben@gerbenlawfirm.com eperrott@gerbenlaw firm.com	Correspondent e- mail Authorized:	Yes

Domestic Representative - Not Found

Prosecution History

Date	Description	Proceeding Number
Oct. 23, 2018	REGISTERED-PRINCIPAL REGISTER	
Aug. 07, 2018	OFFICIAL GAZETTE PUBLICATION CONFIRMATION E-MAILED	
Aug. 07, 2018	PUBLISHED FOR OPPOSITION	
Jul. 18, 2018	NOTIFICATION OF NOTICE OF PUBLICATION E-MAILED	
Jun. 29, 2018	APPROVED FOR PUB - PRINCIPAL REGISTER	
Jun. 22, 2018	TEAS/EMAIL CORRESPONDENCE ENTERED	73797
Jun. 22, 2018	CORRESPONDENCE RECEIVED IN LAW OFFICE	73797
Jun. 20, 2018	ASSIGNED TO LIE	73797
Jun. 06, 2018	TEAS RESPONSE TO OFFICE ACTION RECEIVED	
May 31, 2018	NOTIFICATION OF EXAMINER'S AMENDMENT/PRIORITY ACTION E-MAILED	6326
May 31, 2018	EXAMINER'S AMENDMENT/PRIORITY ACTION E-MAILED	6326
May 31, 2018	COMBINED EXAMINER'S AMENDMENT/PRIORITY ACTION AUTOMATIC ENTRY	93972
May 31, 2018	EXAMINERS AMENDMENT AND/OR PRIORITY ACTION - COMPLETED	93972
May 25, 2018	ASSIGNED TO EXAMINER	93972
Feb. 22, 2018	NOTICE OF DESIGN SEARCH CODE E-MAILED	
Feb. 21, 2018	NEW APPLICATION OFFICE SUPPLIED DATA ENTERED IN TRAM	
Feb. 15, 2018	NEW APPLICATION ENTERED IN TRAM	

TM Staff and Location Information

TM Staff Information - None

File Location

Current Location:	PUBLICATION AND ISSUE SECTION	Date in Location:	Oct. 23, 2018

United States of America

United States Patent and Trademark Office

PRIME MY BODY

Reg. No. 5,589,298

Registered Oct. 23, 2018

Int. Cl.: 3, 5

Trademark

Principal Register

Digital Media Group - US, LLC (NEVADA LIMITED LIABILITY COMPANY)
1501 Lbj Freeway, Suite 500
Dallas, TEXAS 75234

CLASS 3: Skin moisturizers; Cosmetic preparations, namely, firming creams

FIRST USE 7-00-2013; IN COMMERCE 7-00-2013

CLASS 5: Dietary and nutritional supplements; Protein supplements; Hemp oil for use as a dietary supplement

FIRST USE 7-00-2013; IN COMMERCE 7-00-2013

The mark consists of the distressed stylized word "PRIME"; next to the cursive stylized word "MY" in a circle; next to that is the stylized word "BODY".

SER. NO. 87-794,756, FILED 02-12-2018

Director of the United States
Patent and Trademark Office

REQUIREMENTS TO MAINTAIN YOUR FEDERAL TRADEMARK REGISTRATION

WARNING: YOUR REGISTRATION WILL BE CANCELLED IF YOU DO NOT FILE THE DOCUMENTS BELOW DURING THE SPECIFIED TIME PERIODS.

Requirements in the First Ten Years*
What and When to File:

- *First Filing Deadline:* You must file a Declaration of Use (or Excusable Nonuse) between the 5th and 6th years after the registration date. See 15 U.S.C. §§1058, 1141k. If the declaration is accepted, the registration will continue in force for the remainder of the ten-year period, calculated from the registration date, unless cancelled by an order of the Commissioner for Trademarks or a federal court.

- *Second Filing Deadline:* You must file a Declaration of Use (or Excusable Nonuse) and an Application for Renewal between the 9th and 10th years after the registration date.* See 15 U.S.C. §1059.

Requirements in Successive Ten-Year Periods*
What and When to File:

- You must file a Declaration of Use (or Excusable Nonuse) and an Application for Renewal between every 9th and 10th-year period, calculated from the registration date.*

Grace Period Filings*

The above documents will be accepted as timely if filed within six months after the deadlines listed above with the payment of an additional fee.

*ATTENTION MADRID PROTOCOL REGISTRANTS: The holder of an international registration with an extension of protection to the United States under the Madrid Protocol must timely file the Declarations of Use (or Excusable Nonuse) referenced above directly with the United States Patent and Trademark Office (USPTO). The time periods for filing are based on the U.S. registration date (not the international registration date). The deadlines and grace periods for the Declarations of Use (or Excusable Nonuse) are identical to those for nationally issued registrations. See 15 U.S.C. §§1058, 1141k. However, owners of international registrations do not file renewal applications at the USPTO. Instead, the holder must file a renewal of the underlying international registration at the International Bureau of the World Intellectual Property Organization, under Article 7 of the Madrid Protocol, before the expiration of each ten-year term of protection, calculated from the date of the international registration. See 15 U.S.C. §1141j. For more information and renewal forms for the international registration, see http://www.wipo.int/madrid/en/.

NOTE: Fees and requirements for maintaining registrations are subject to change. Please check the USPTO website for further information. With the exception of renewal applications for registered extensions of protection, you can file the registration maintenance documents referenced above online at h ttp://www.uspto.gov.

NOTE: A courtesy e-mail reminder of USPTO maintenance filing deadlines will be sent to trademark owners/holders who authorize e-mail communication and maintain a current e-mail address with the USPTO. To ensure that e-mail is authorized and your address is current, please use the Trademark Electronic Application System (TEAS) Correspondence Address and Change of Owner Address Forms available at http://www.uspto.gov.