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(54) Title: POLYPEPTIDES FOR USE IN THE SYNTHESIS OF BIOACTIVE PHENOLIC COMPOUNDS

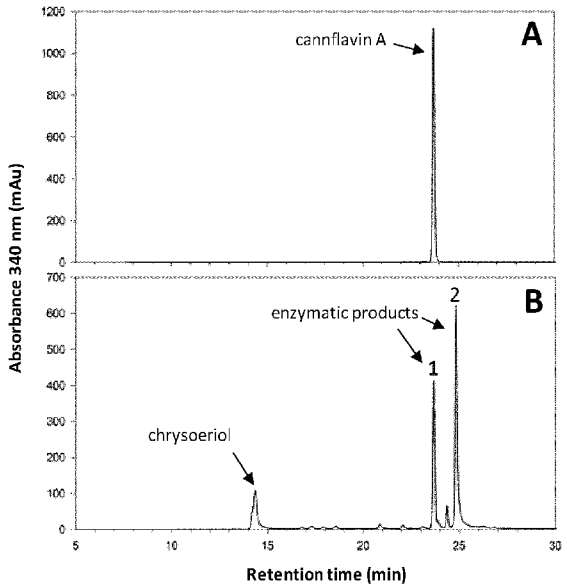

Figure 1

(57) Abstract: Described herein is a polypeptide encoding a prenyltransferase for prenylating a polyphenol and a polypeptide encoding an O-methyltransferase for methylating a polyphenol. For example, the polypeptide comprises or consists of the sequence of SEQ ID NO: 1, 2, 3, 4, 5, and/or 6, and/or a polypeptide listed in Table 1, and/or SEQ ID NO: 7-30, or a variant thereof having at least 80% sequence identity to SEQ ID NO: 1, 2, 3, 4, 5, and/or 6, and/or the polypeptide listed in Table 1, and/or SEQ ID NO: 7-30, or a fragment of the polypeptide or the variant thereof.

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POLYPEPTIDES FOR USE IN THE SYNTHESIS OF BIOACTIVE PHENOLIC COMPOUNDS

Field

The present invention relates to polypeptides. More specifically, the present invention is, in embodiments, concerned with the use of polypeptides encoding enzymes capable of modifying phenolic molecules and related products, methods, and uses.

Background

Natural product compounds of the polyphenol class often possess anti-oxidant, anti-inflammatory, anti-microbial, anti-viral, and anti-cancer activities (Kikuchi et al. 2019; Kumar and Pandey 2013; Rasouli et al. 2017). Production of these compounds relies on the phenylpropanoid pathway which starts with the synthesis of hydroxycinnamic acids, a C6-C3 carbon backbone synthesized from either phenylalanine or tyrosine. From them, flavonoids (C6-C3-C6) and stilbenes or bibenzyls (C6-C2-C6) can be synthesized. Because of their wide distribution in plants and their health-promoting properties, flavonoids are a well-studied group of compounds. In plants, flavonoids, including chalcones, flavanones, flavones, flavonols and isoflavones, share a basic flavan structure of 15 carbon atoms derived from a C6-C3-C6 skeleton. Modification of primary and secondary metabolites by glycosylation, methylation or prenylation is one of nature's means to modulate their bioactivity and contributes substantially to the large diversity of secondary metabolites present in plants, fungi, and bacteria. Therefore, it is not surprising that flavonoids are usually found naturally as derivatives, e.g., glycosides, methyl ethers and prenylated forms (Koirala et al. 2016; Xiao et al. 2014; Yang et al. 2015).

Over 1000 natural products with one or more prenyl groups have been isolated predominantly from higher plants. Prenylation has been detected on most flavonoids, though prenylated flavonones are the most common subclass and prenylated flavanols are the rarest subclass, with C-prenylation being much more common than O-prenylation. C-prenylation occurs more frequently on ring A at C-6/C-8 positions and on ring B at C-3' and C-5'. Prenylation at ring C is rare in natural prenylated flavonoids. In terms of prenyl groups, 3,3-dimethylallyl group (5 C) is the most common example presented in nature, though geranyl (C 10) and farnesyl (C 15) flavonoids are also well known in prenylated flavonoids (Barron & Ibrahim, 1996).

In plants, O-methylated flavonoids (or methoxyflavonoids) are more widely distributed than the C-methylated forms, and the methoxyl group is often present on the C-2′, 3′, 4′, 5′, 3, 5, 6, 7, and 8 positions of flavonoids (Bandyukova and Avanesov, 1971). Because methylated forms of flavonoids present higher metabolic stability and increased membrane transport in the intestine and liver than the hydroxylated counterparts, resulting in greater oral bioavailability, it has been suggested that they also have stronger anticancer potential (Bernini et al. 2011; Walle 2007).

In the case of prenylated plant polyphenols, studies on chemical structures revealed that prenylation enhances their biological activity compared with those non-prenylated forms. For example, the prenylated phenylpropanoids drupanin, artepillin C and baccharin, which are p-coumaric acid derivatives, have been shown to induce apoptotic events in a colon cancer cell line SW480 and human leukemia cell line HL60 (Akao et al 2008); and only oral administration of these prenylated phenylpropanoids causes a significant reduction in tumor growth to mice with sarcoma S-180 (Mishima et al 2005). The mechanism for their enhanced biological activities relies on better membrane permeability due to the lipophilicity of the prenyl moiety, whereby they engage in improved interaction with biological targets such as cell membranes, transporters and other proteins. (Maitrejean et al. 2000; Murakami et al. 2000).

Stilbenoids and dihydrostilbenoids (bibenzyls), which are usually classified as phytoalexins, are antimicrobial compounds used by plants to protect themselves against fungal infection and toxins. Similar to flavonoids, many stilbenoids, such as the recognized resveratrol, pterostilbene, and piceatannol, have important biological effects. Among their biological activities, they have been shown to offer promise in cancer prevention and treatment, cardioprotection, neuroprotection, anti-diabetic properties, and anti-inflammation (Akinwumi et al. 2018; Xiao et al. 2008).

Actinobacteria is a large phylum of terrestrial and aquatic Gram-positive bacteria. Their main representative genera are a source of many antibiotics. Soluble prenyltransferases presenting relaxed substrate specificity and displaying regiospecificity in prenyl group transfer and prenyl chain selectivity have been identified in Actinobacteria, especially among the *Streptomyces* genus (Bonitz et al. 2011). For example, in *Streptomyces sp*. CL190, biosynthesis of the anti-oxidant naphterpin includes prenylation of flaviolin with a geranyl group by the prenyltransferase NphB. This enzyme was shown to have broad substrate specificity, and also is able to prenylate several plant polyphenols including 1,6-dihydroxynaphthalene (DHN), naringenin, daidzein, genistein, resveratrol, and olivetol (Kuzuyama et al. 2005). Interestingly, other genes encoding prenyltransferases (namely SCO7190, NovQ, and CloQ) have also been identified from other *Streptomyces* species (*S. coelicolor A3*, *S. niveus*, respectively) and also show loose substrate specificity (Kumano et al. 2008; Ozaki et al. 2009).

U.S. Patent Application Publication No. 2006/0183211 describes a novel aromatic prenyltransferase, Orf2 from *Streptomyces* sp. strain CL190, involved in naphterpin biosynthesis. This prenyltransferase catalyzes the formation of a C—C bond between a prenyl group and a compound containing an aromatic nucleus and also displays C—O bond formation activity. Numerous crystallographic structures of the prenyltransferase have been solved and refined and provide a mechanistic basis for understanding prenyl chain length determination and aromatic co-substrate recognition in this structurally unique family of aromatic prenyltransferases.

U.S. Patent Application Publication No. 2019/0352679 describes the use of enzyme combinations or recombinant microbes comprising the same to make isoprenoid precursors, isoprenoids and derivatives thereof including prenylated aromatic compounds. Novel metabolic pathways exploiting Claisen, aldol, and acyioin condensations are used instead of the natural mevalonate (MVA) pathway or 1-deoxy-d-xylulose 5-phosphate (DXP) pathways for generating isoprenoid precursors such as isopentenyl pyrophosphate (IPP), dimethylallyl pyrophosphate (DMAPP), and geranyl pyrophosphate (GPP).

U.S. Patent Application Publication No. 2006/0137207 describes novel flavonoid compounds having antioxidant activity. The compounds have been shown to exhibit anti-oxidative properties in biological systems and their utility in a sunscreen or skincare composition or to treat conditions involving oxidative damage, especially curative or prophylactic treatment of Alzheimer's disease or ischaemia-reperfusion injury, is described.

U.S. Patent Application Publication No. 2019/0100549 describes compounds useful in the treatment of many diseases such as a skin disease, an allergy, an autoimmune disease, a cardiovascular disease, a lung disease, asthma, a bacterial, viral or parasitic disease, metabolic syndrome, cancer, Alzheimer's disease or diabetes and are furthermore useful in the preparation of cosmetics and for use in food and animal feed.

U.S. Patent Application Publication No. 2018/0135029 describes a method for producing flavonoids, comprising the steps: (a) providing of a transgenic microorganism, containing (i) a first nucleic acid section (A), comprising or consisting of a gene coding for a CYP450 oxidase, (ii) a second nucleic acid section (B), comprising or consisting of a gene coding for a plant O-methyltransferase, and (b) adding of one or more flavanones to the transgenic microorganism, (c) the conversion of the substrate flavanones by the transgenic microorganism to the corresponding flavonoids, and optionally (d) isolating and purifying of the final products.

U.S. Patent Application Publication No. 2007/0150984 describes a genetic sequence encoding a polypeptide having methyltransferase activity and the use of the genetic sequence and/or the polypeptide to modify one or more phenotypic characteristics of a plant. More particularly, the methyltransferase of the present invention acts on flavonoids, preferably wherein the flavonoid is an anthocyanin. Described is a polypeptide having S-adenosyl-L-methionine:anthocyanin 3′-O-methyl-transferase or S-adenosyl-L-methionine:anthocyanin 3′,5′-O-methyltransferase activity. Further described is a genetic sequence encoding a polypeptide having methyltransferase activity derived from *Petunia, Torenia Fuchsia* or *Plumbago* or botanically related plants.

U.S. Patent Application Publication No. 2016/0273006 describes a biosynthetic method of making pterostilbene including expressing a 4-coumaratexoenzyme A ligase (4CL) in a cellular system, expressing a stilbene synthase (STS) in the cellular system, expressing a resveratrol O-methyltransferase (ROMT) in the cellular system, feeding p-coumaric acid to the cellular system, growing the cellular system in a medium, and producing pterostilbene.

U.S. Patent No. 7732666 relates to an O-methyltransferase gene cloned from sorghum, the sorghum O-methyltransferase-3 gene, SbOMT3. Quantitative real-time RT-PCR and recombinant enzyme studies with putative O-methyltransferase sequences obtained from an EST data set from sorghum have led to the identification of the novel root hair-specific O-methyltransferase designated SbOMT3. Transgenic plants which express SbOMT3 can convert resveratrol into pterostilbene in planta. SbOMT3 is also involved in the biosynthesis of sorgoleone.

There is a need to provide a useful alternative to overcome at least some of the deficiencies of the prior art.

Description of the Drawings

The present invention will be further understood from the following description with reference to the Figures:

Figure 1. Evidence for cannflavin A synthesis by NphB. (**A**) Representative HPLC chromatogram for an authentic cannflavin A standard. (**B**) Representative chromatogram of the product from a cell-free enzyme assay with recombinant NphB plus chrysoeriol and GPP. Note that the assay produces two major products; the first major peak elutes at the same time as the cannflavin A standard while the second peak elutes approximately 1 min later. The first major peak ("1") which corresponds to cannflavin A elution time was collected and processed for mass spectrometry analysis.

Figure 2. Mass spectrometry evidence for the synthesis of cannflavin A from chrysoeriol by NphB. (**A**) Q-TOF mass spectra of a cannflavin A standard (upper panel). The lower panel shows the collision-induced dissociation (CID)-Q-TOF mass spectral fragmentation pattern of such standard. (**B**) The first peak, which corresponds to an enzymatic product with the same retention time as cannflavin A on the HPLC (**Figure 1B**), was collected offline. The upper panel shows that the mass spectrum of such a product (6-geranyl chrysoeriol) is consistent with the pattern of a cannflavin A standard ([M+H]+ 437). The CID-Q-TOF mass spectral fragmentation pattern of the enzymatic product from this assay (bottom panel) also resembles that of the cannflavin A standard in (A, bottom panel), indicating that prenylation of chrysoeriol with GPP by NphB produces cannflavin A.

Figure 3. Selective methylation of flavonoids by O-methyltransferases form Cannabis. (**A**) Three flavones (apigenin, luteolin and chrysoeriol) and two flavonols (quercetin and kaempferol) that typically accumulate in *C. sativa* were tested as potential substrates for CsOMT6 and CsOMT21. Relative enzymatic activity and substrate preference of (**B**) CsOMT6 and (**C**) CsOMT21. The selected flavones and flavonols (as numbered in A) were provided to recombinant CsOMT6 or CsOMT21 that were purified by Ni2+-affinity chromatography in enzyme assays along with [^{14}C]-SAM as a methyl donor. Data are means ± SD from three

independent experiments and are presented as relative activity compared to that observed with the preferred substrate for each enzyme.

Figure 4. Methylation of bibenzyls by O-methyltransferases from Cannabis. (**A**) Four bibenzyl compounds (dihydroresveratrol, tristin, gigantol and batatasin III) were tested as potential substrates for CsOMT1, CsOMT3, CsOMT5, and CsOMT13. (**B**) Relative enzymatic activity of the four OMTs. The four selected bibenzyl compounds were provided to purified recombinant CsOMT1, CsOMT3, CsOMT5 or CsOMT13 in enzyme assays along with [^{14}C]-SAM as a methyl donor. Data are means from three independent experiments and are shown as relative activity compared to that of CsOMT1 with dihydroresveratrol.

Figure 5. Selective methylation of a bibenzyl by CsOMT1. (**A**) Phenolic compounds representing a bibenzyl (dihydroresveratrol), stilbenoids (resveratrol and pinosylvin), and a hydroxycinamic acid (caffeic acid) and in its reduced form (dihydrocaffeic acid) were tested as substrates for CsOMT1. (**B**) Relative enzymatic activity and substrate preference of CsOMT1. The five compounds were provided to purified recombinant CsOMT1 in enzyme assays along with [^{14}C]-SAM as a methyl donor. Data are means from three independent experiments and are shown as relative activity compared to that of CsOMT1 with dihydroresveratrol.

Figure 6. Enzymatic methylation activity of CsOMT1. (**A**) Reaction catalyzed by CsOMT1. (**B**) Representative chromatogram showing the reaction products resolved by HPLC that illustrates the separation of the methylated dihydroresveratrol from its corresponding substrate (dihydroresveratrol, DHR). The identity of 3-O-methyl-dihydroresveratrol (pinobistilbene) was structurally determined by NMR. (**C**) Kinetic analysis of CsOMT1. Recombinant CsOMT1 was assayed under standard assay conditions at the indicated concentrations of dihydroresveratrol. Kinetic parameters were determined by non-linear regression analysis using the Michaelis-Menten kinetics model of the SigmaPlot 12.3 software.

Summary

In accordance with an aspect, there is provided a polypeptide encoding a prenyltransferase for prenylating a polyphenol.

In an aspect, the prenyltransferase is a microbial prenyltransferase.

In an aspect, the polypeptide is comprising or consisting of a polypeptide having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the sequence of any one or more of SEQ ID NO: 1-6 and/or a polypeptide listed in Table 1, or a fragment of any thereof.

In an aspect, the polypeptide is comprising or consisting of the sequence of any one or more of SEQ ID NO: 1-6 and/or a polypeptide listed in Table 1.

In an aspect, the polypeptide is comprising or consisting of the sequence of any one or more of SEQ ID NO: 1-6.

In an aspect, the polypeptide prenylates the polyphenol using a prenyl donor.

In an aspect, the prenyl donor is IPP, FPP, GPP, and/or DMAPP, or a variant or derivative thereof.

In an aspect, the polyphenol is a flavonoid, stilbenoid, and/or bibenzyl, or a derivative thereof.

In an aspect, the flavonoid is a flavone, such as apigenin, luteolin, chrysoeriol, chrysin, acacetin, baicalein, baicalin, vitexin, wogonin, orientin, oroxylin A. rutin, or tangeritin; a flavonol such as quercetin, kaempferol, galangin, myricetin, tamarixetin, fisetin, or casticin; a flavanone such as naringenin, hesperetin, pinocembrin, hesperidin, or eriodictyol; a flavanonol such as taxifolin; a flavanol such as catechin, or epicatechin; an isoflavone such as genistein, or daidzein; an anthocyanin such as cyanidin, chrysanthemin, pelargonidin, delphinidin, or malvidin; or any combination thereof.

In an aspect, the stilbenoid is resveratrol, piceatannol, pterostilbene, pinosylvin, gnetol, oxyresveratrol, pinostilbene, or any combination thereof.

In an aspect, the bibenzyl is a dihydrostilbenoid such as dihydroresveratrol, combretastatin, dihydropiceatannol, dihydrognetol, dihydropinosylvin, gigantol, pinobistilbene, batatasin III, crepidatin, moscatilin, crysotoxine, chrysotobibenzyl, amoenylin, tristin, cumulating, or any combination thereof.

In an aspect, the prenyltransferase prenylates the flavonoid to produce 8-prenyl kaempferol, isocannflavin B, cannflavin C, 6-prenylnaringenin, 6-prenylapigenin, neougonin A, neougonin B, and/or kuraridin.

In an aspect, the prenyltransferase prenylates the stilbenoid to produce arachidins, isorhapontigenin, rhapontigenin, pawhuskin A, aglaiabbrevin E, amorphastilbol, or longistylins.

In an aspect, the prenyltransferase prenylates the bibenzyl to produce canniprene, cannabistilbene, dihydrolongistylins, amorfrutin 1/A, or amorfrutin B.

In an aspect, the prenyltransferase prenylates chrysoeriol using GPP to produce cannflavin A.

In an aspect, the prenyltransferase prenylates chrysoeriol using DMAPP to produce cannflavin B.

In accordance with an aspect, there is provided a polypeptide encoding an O-methyltransferase for methylating a polyphenol.

In an aspect, the O-methyltransferase is a plant O-methyltransferase.

In an aspect, the O-methyltransferase is a Cannabis sativa O-methyltransferase.

In an aspect, the polypeptide is comprising or consisting of a polypeptide having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the sequence of any one or more of SEQ ID NO: 7-30, or a fragment of any thereof.

In an aspect, the polypeptide is comprising or consisting of the sequence of any one or more of SEQ ID NO: 7-30.

In an aspect, the polypeptide methylates the polyphenol using a methyl donor.

In an aspect, the methyl donor is S-adenosyl methionin, or a variant or derivative thereof.

In an aspect, the polyphenol is a flavonoid, stilbenoid, and/or bibenzyl, or a derivative thereof.

In an aspect, the flavonoid is a flavone, such as apigenin, luteolin, chrysoeriol, chrysin, acacetin, baicalein, baicalin, vitexin, wogonin, orientin, oroxylin A. rutin, or tangeritin; a flavonol such as quercetin, kaempferol, galangin, myricetin, tamarixetin, fisetin, or casticin; a flavanone such as naringenin, hesperetin, pinocembrin, hesperidin, or eriodictyol; a flavanonol such as taxifolin; a flavanol such as catechin, or epicatechin; an isoflavone such as genistein, or daidzein; an anthocyanin such as cyanidin, chrysanthemin, pelargonidin, delphinidin, or malvidin; or any combination thereof.

In an aspect, the stilbenoid is resveratrol, piceatannol, pterostilbene, pinosylvin, gnetol, oxyresveratrol, or any combination thereof.

In an aspect, the bibenzyl is a dihydrostilbenoid such as dihydroresveratrol, combretastatin, dihydropiceatannol, dihydrognetol, dihydropinosylvin, gigantol, batatasin III, crepidatin, moscatilin, crysotoxine, chrysotobibenzyl, amoenylin, tristin, cumulating, or any combination thereof.

In an aspect, the O-methyltransferase methylates a flavonoid to produce chrysoeriol, acacetin, tamarixetin, or methylquercetin.

In an aspect, the O-methyltransferase methylates a stilbenoid to produce pinostilbene, isorhapontigenin, rhapontigenin, or any combination thereof.

In an aspect, the O-methyltransferase methylates a bibenzyl to produce gigantol, tristin, orpinobistilbene.

In an aspect, the polypeptide, variant, or fragment comprises up to about 100, about 150, about 200, about 250, about 300, about 350, about 360, about 370, about 380, about 390, about 400, about 410, about 420, about 430, about 440, about 450, or about 500 amino acids

In an aspect, the polypeptide is synthetic.

In an aspect, the polypeptide is recombinant.

In accordance with an aspect, there is provided a nucleic acid encoding the polypeptide described herein.

In an aspect, the nucleic acid is cDNA.

In accordance with an aspect, there is provided a vector comprising the nucleic acid described herein.

In accordance with an aspect, there is provided a host cell comprising the vector described herein.

In accordance with an aspect, there is provided a host cell expressing the polypeptide described herein.

In an aspect, the host cell is a bacterial cell (e.g., E. coli or Agrobacterium tumefaciens), a yeast cell (e.g., S. cerevisiae), an algal cell, or a plant cell (e.g., Nicotiana spp.).

In an aspect, the host cell is provided in combination with the polyphenol.

In an aspect, the polyphenol is provided in the host cell culture medium.

In an aspect, the polyphenol is expressed by the host cell.

In an aspect, the host cell is provided in combination with a prenyl donor and/or a methyl donor.

In an aspect, the prenyl donor and/or methyl donor is provided in the host cell culture medium.

In an aspect, the prenyl donor and/or methyl donor is expressed by the host cell.

In accordance with an aspect, there is provided an expression system comprising the polypeptide; the nucleic acid, the vector; or the host cell described herein.

In an aspect, the expression system further comprises the polyphenol and a prenyl donor and/or methyl donor.

In accordance with an aspect, there is provided a system for prenylating and/or methylating a polyphenol the system comprising the polypeptide described herein.

In an aspect, the polypeptide is in a batch solution.

In an aspect, the polypeptide is immobilized in a support matrix.

In an aspect, the polypeptide is in a cell.

In an aspect, the system is cell-free.

In accordance with an aspect, there is provided a method for prenylating and/or methylating a polyphenol, wherein the method comprises contacting the polyphenol with the polypeptide described herein.

In an aspect, the method is carried out in the system described herein.

In an aspect, the method is a recombinant method comprising expressing the polypeptide described herein in a cell in the presence of the polyphenol and a prenyl donor and/or methyl donor.

In an aspect, the method is in combination with a synthetic chemical catalysis method.

In an aspect, the method comprises a single synthesis step.

In an aspect, the method is carried out in combination with an enzymatic reaction.

In an aspect, the method comprises a combined enzymatic O-methylation and prenylation step.

In accordance with an aspect, there is provided a method of producing cannflavin A, cannflavin B, isocannflavin B, the method comprising carrying out a combined enzymatic O-methylation and prenylation of a flavonoid.

In accordance with an aspect, there is provided a method of producing a longistylin, the method comprising carrying out a combined enzymatic O-methylation and prenylation of a stilbenoid.

In accordance with an aspect, there is provided a method of producing canniprene, cannabistilbene, dihydrolongistylin, amorfrutin 1/A, or amorfrutin B, the method comprising carrying out a combined enzymatic O-methylation and prenylation of a bibenzyl.

In accordance with an aspect, there is provided a synthetic chemical catalysis method of producing cannflavin A and/or cannflavin B, the method comprising using GPP and DMAPP in a single synthesis step from chrysoeriol or in combination with an enzymatic reaction such as the O-methylation of luteolin.

In accordance with an aspect, there is provided a prenylated and/or methylated polyphenol produced by the method described herein.

In an aspect, the polyphenol is substantially pure, for example, at least about 95%, about 96%, about 97%, about 98%, about 99%, about 99.5%, or about 99.9% pure.

In an aspect, the polyphenol is cannflavin A and/or cannflavin B.

In accordance with an aspect, there is provided a cosmetic composition comprising the polyphenol described herein and at least one cosmetically acceptable carrier.

In accordance with an aspect, there is provided a pharmaceutical composition comprising the polyphenol described herein and at least one pharmaceutically acceptable carrier.

In accordance with an aspect, there is provided a natural health product comprising the polyphenol described herein, such as a supplement, beverage, or food.

In accordance with an aspect, there is provided a use of the polyphenol described herein in a cosmetic, pharmaceutical, or natural health product.

Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from the detailed description.

Detailed Description

The enhanced and distinct biological activities of modified polyphenols have prompted the utilization of plants containing these as medicinal plants and also as ingredients in products for food industries, breweries, and cosmetic companies. Some show promise as important compounds for the development of nutraceuticals and new pharmacological agents for the treatment of different medical conditions. However, there are several obstacles that have limited the potential applications of these compounds:

- In many cases, prenylated and/or methylated flavonoids often exist at trace levels in natural sources or are present in a limited number of plant species.
- Plant extraction of polyphenols often poses a significant challenge for cost-effective production due to the high cost associated with downstream processing and purification

from complicated mixtures. Isolation from native plant sources also depends on external factors (e.g. agricultural, climate and geographic variations), which results in inconsistent quality needed for drug ingredients.

- *In vivo* synthesis in heterologous plant or microbial systems is usually regarded as not sustainable because of low production rates.
- Genetic reconstruction of biosynthetic routes raises several challenges in terms of the assembly of a genetic pathway. Although yeast has proven to be an amenable organism for replicating biosynthesis of various plant pathways, only a few have been reconstructed successfully (Paddon and Keasling 2014; Thodey et al. 2014). For example, compartmentalization, expression of integral membrane-bound plant enzymes, branched pathways and specific regulatory mechanisms affecting synthesis are among the major challenges to microorganisms for producing secondary natural products.
- In many cases, organic chemical synthesis is not an amenable cost-effective approach.

Described herein are prenyltransferases and methyltransferases that can serve as alternate production catalysts for the in vitro and in vivo prenylation and/or methylation of natural products of plant origin, such as polyphenols, such as flavonoids, stilbenoids and bibenzyls. In addition, the approaches described herein provide the basis for exploring novel prenylation and methylation chemistry and bioactivity of natural and novel synthetic prenylated or methylated aromatic compounds by means of structure-based enzyme engineering.

Thus, described herein are polypeptides and related methods for the synthesis of polyphenols using prenyltransferases and methyltransferases or variants of such enzymes. These are typically derived from microbial, such as bacterial or fungal, or plant sources, such as *Cannabis sativa*. The use of enzymes with potential prenyltranferase activity is described herein, for example, NphB, HypSc, CloQ, NovQ, Fur7, and PpzP and related sequences. In vitro catalysis systems, designed to utilize these enzymes and thereby produce substantially pure prenylated polyphenols, such as flavonoids, stilbenoids or bibenzyls, are described. For example, NphB or an enzyme with a similar amino acid sequence which prenylates chrysoeriol using GPP or DMAPP to produce cannflavin A or cannflavin B, respectively.

Like cannflavin A and cannflavin B, these and other prenylated flavonoids (for example, 8-prenyl kaempferol, isocannflavin B, cannflavin C, 6-prenylnaringenin, 6-prenylapigenin, neougonin A and B), stilbenoids (for example, pawhuskin A aglaiabbrevin E, amorphastilbol, longistylins) or bibenzyls (for example, canniprene, cannabistilbene, dihydrolongistylins, amorfrutin 1/A, amorfrutin B) may find use in anti-inflammatory compositions and methods.

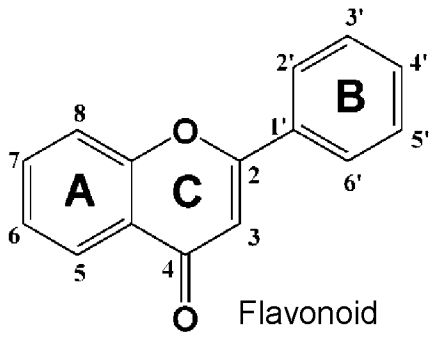
In addition, the use of plant enzymes with O-methyltransferase activity is described, for example CsOMT1-24. Methylation by these methyltransferases can produce pure polyphenols such as flavonoids (for example, chrysoeriol, acacetin, tamarixetin, methylquercetin), stilbenoids, or bibenzyls (for example gigantol and pinobistilbene), bioactive compounds that

can find direct use or can be employed as substrates for further prenylation through an in vitro method or by chemical synthesis.

Definitions

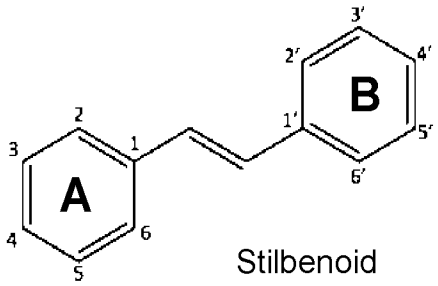
Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. See, e.g. Singleton et al., Dictionary of Microbiology and Molecular Biology 2nd ed., J. Wiley & Sons (New York, N.Y. 1994); Sambrook et al., Molecular Cloning. A Laboratory Manual, Cold Springs Harbor Press (Cold Springs Harbor, NY 1989), each of which is incorporated herein by reference. For the purposes of the present invention, the following terms are defined below.

As used herein, the term “flavonoid” includes, for example, flavanone, flavone, flavonol, flavanonol, isoflavone, anthocyan, and chalcone, as well as derivatives thereof, such as prenylated or methylated derivatives thereof, unless otherwise specified. Examples of flavonoids include naringenin, apigenin, luteolin, myricetin, quercetin, catechin, daidzein, genistein, kaempferol, pelargonidin, delphinidin, and cyanidin. Flavonoids may be of natural or synthetic origin and have the following general structure. Numbers in the ring structures indicate the positions of hydroxyl groups and where modifications such as prenylation, methylation or glycosylation can occur. Usually, the position of such modifications and hydroxyl groups determine than bioactivity of the molecule.



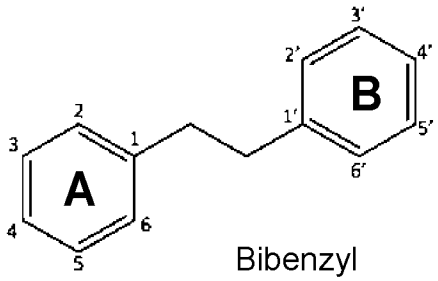
Flavonoid

The term “stilbenoid” includes any hydroxylated derivatives of stilbene, as well as derivatives thereof, such as methylated or prenylated derivatives thereof unless otherwise specified. Examples of stilbenoids include resveratrol, piceatannol, pterostilbene, pinosylvin and gnetol. Stilbenoids may be of natural or synthetic origin and have the following general structure. Numbers in the ring structures indicate the positions of hydroxyl groups and where modifications such as prenylation, methylation or glycosylation can occur. Usually, the position of such modifications and hydroxyl groups determines than bioactivity of the molecule.



Stilbenoid

The term “bibenzyl” includes any dihydrostilbene derivatives, as well as derivatives thereof, such as methylated or prenylated derivatives thereof unless otherwise specified. Examples of bibenzyls include dihydroresveratrol, combretastatin, dihydropiceatannol, gigantol, tristin, batatasin III, crepidatin, and amoenylin. Bibenzyls may be of natural or synthetic origin and have the following general structure. Numbers in the ring structures indicate the positions of hydroxyl groups and where modifications such as prenylation, methylation or glycosylation can occur. Usually, the position of such modifications and hydroxyl groups determines than bioactivity of the molecule.



Bibenzyl

“Variants” of the sequences described herein are biologically active sequences that have a peptide sequence that differs from the sequence of a native or wild-type sequence, by virtue of an insertion, deletion, modification and/or substitution of one or more amino acids within the native sequence. Such variants generally have less than 100% sequence identity with a native sequence. Ordinarily, however, a biologically active variant will have an amino acid sequence with at least about 70% sequence identity with the sequence of a corresponding naturally occurring sequence, typically at least about 75%, more typically at least about 80%, even more typically at least about 85%, even more typically at least about 90%, and even more typically of at least about 95%, 96%, 97%, 98%, or 99% sequence identity. The variants nucleotide fragments of any length that retain a biological activity of the corresponding native sequence. Variants also include sequences wherein one or more amino acids are added at either end of, or within, a native sequence. Variants also include sequences where a number of amino acids are deleted and optionally substituted by one or more different amino acids.

As used herein, "treatment" or “therapy” is an approach for obtaining beneficial or desired clinical results. For the purposes described herein, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of the extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression,

amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" and “therapy” can also mean prolonging survival as compared to expected survival if not receiving treatment or therapy. Thus, "treatment" or “therapy” is an intervention performed with the intention of altering the pathology of a disorder. Specifically, the treatment or therapy may directly prevent, slow down or otherwise decrease the pathology of a disease or disorder such as inflammation, or may render the inflammation more susceptible to treatment or therapy by other therapeutic agents.

The terms "therapeutically effective amount", "effective amount" or "sufficient amount" mean a quantity sufficient, when administered to a subject, including a mammal, for example, a human, to achieve a desired result, for example, an amount effective to treat inflammation. Effective amounts of the polyphenolic compounds described herein may vary according to factors such as the disease state, age, sex, and weight of the subject. Dosage or treatment regimens may be adjusted to provide the optimum therapeutic response, as is understood by a skilled person.

Likewise, an “effective amount” of the polyphenolic compounds described herein refers to an amount sufficient to function as desired, such as to treat inflammation.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

The term “pharmaceutically acceptable” means that the compound or combination of compounds is compatible with the remaining ingredients of a formulation for pharmaceutical use, and that it is generally safe for administering to humans according to established governmental standards, including those promulgated by the United States Food and Drug Administration.

"Carriers" as used herein include cosmetically or pharmaceutically acceptable carriers, excipients, or stabilizers that are non-toxic to the cell or subject being exposed thereto at the dosages and concentrations employed. Often the pharmaceutically acceptable carrier is an aqueous pH buffered solution. Examples of pharmacologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, and dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol and sorbitol; salt-forming counterions such as sodium; and/or non-ionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

In understanding the scope of the present application, the articles “a”, “an”, “the”, and “said” are intended to mean that there are one or more of the elements. Additionally, the term "comprising" and its derivatives, as used herein, is intended to be open-ended terms that

specify the presence of the stated features, elements, components, groups, integers, and/or steps, but do not exclude the presence of other unstated features, elements, components, groups, integers and/or steps. The foregoing also applies to words having similar meanings such as the terms, "including", "having" and their derivatives.

It will be understood that any embodiments described as “comprising” certain components may also “consist of” or “consist essentially of,” wherein “consisting of” has a closed-ended or restrictive meaning and “consisting essentially of” means including the components specified but excluding other components except for materials present as impurities, unavoidable materials present as a result of processes used to provide the components, and components added for a purpose other than achieving the technical effect of the invention. For example, a composition defined using the phrase “consisting essentially of” encompasses any known pharmaceutically acceptable additive, excipient, diluent, carrier, and the like. Typically, a composition consisting essentially of a set of components will comprise less than 5% by weight, typically less than 3% by weight, more typically less than 1% by weight of non-specified components.

It will be understood that any component defined herein as being included may be explicitly excluded from the claimed invention by way of proviso or negative limitation. For example, in embodiments, THC, cannabinoids, and/or terpenes are explicitly excluded from the compositions and methods described herein.

In addition, all ranges given herein include the end of the ranges and also any intermediate-range points, whether explicitly stated or not.

Finally, terms of degree such as "substantially", "about" and "approximately" as used herein mean a reasonable amount of deviation of the modified term such that the end result is not significantly changed. These terms of degree should be construed as including a deviation of at least ±5% of the modified term if this deviation would not negate the meaning of the word it modifies.

Polypeptides

Described herein, in aspects, are polypeptides encoding enzymes that are typically prenyltransferases. These polypeptides are typically derived from microbes such as bacteria or fungus and find use in converting polyphenols, such as flavonoid compounds, into compounds with medicinal activity and/or precursors to such compounds. For example, described herein are polypeptides comprising one or more of the following sequences, variants thereof, or fragments of the polypeptides or variants. Variants can be of natural origin such as orthologous sequences from related bacterial or fungal species, or of non-natural versions obtained synthetically or by mutations. In the following sequences, amino acid sequences and GenBank accession numbers of these enzymes and their relatives are shown. Also described herein are orthologous

sequences similar to SEQS NO:1-6 found in nature with their respective GenBank accession numbers (see Table 1).

SEQ ID NO: 1

>NphB / Orf2 / BAE00106 [Streptomyces sp. CL190]
MSEAADVERVYAAMEEAAGLLGVACARDKIYPLLSTFQDTLVEGGSVVVFSMASGRHSTELDFSISVPTSHGDPYAT
VVEKGLFPATGHPVDDLLADTQKHLPVSMFAIDGEVTGGFKKTYAFFPTDNMPGVAELSAIPSMPPAVAENAELFAR
YGLDKVQMTSMDYKKRQVNLYFSELSAQTLEAESVLALVRELGLHVPNELGLKFCKRSFSVYPTLNWETGKIDRLCF
AVISNDPTLVPSSDEGDIEKFHNYATKAPYAYVGEKRTLVYGLTLSPKEEYYKLGAYYHITDVQRGLLKAFDSLED

SEQ ID NO: 2

>HypSc / SCO7190 / NP_631248 [*Streptomyces coelicolor* A3(2)]
MPTGRTTDLALFLSDLEAYAKLAEVTFDKRAVEQVVDVFAEQFATGTITVRTTTHEAANRSVNFRYMYPDSPHDPVE
IARAHGLLPDADPAVMSLLAEVTEKIPLWWGLDASVGHGVQKVWAFFEQPLEFGEIASLENTPHSLRDHRERFGEAR
IDRFAIMGFDFRDNTTNLYSEMVSPGYFEQEEVARMIRDVGSLPPDNEEIERCRGAINVYYTFDWNSPQARRLCFAV
PSRDGEFPSHLHPLAARFAAEAPVQAERRELIFNPTFGARGSYLKMEADYTGDAASRVFGYWNR

SEQ ID NO: 3

>CloQ / AAN65239 [*Streptomyces roseochromogenus* subsp. oscitans]
MPALPIDQEFDCERFRADIRATAAAIGAPIAHRLTDTVLEAFRDNFAQGATLWKTTSQPGDQLSYRFFSRLKMDTVS
RAIDAGLLDAAHPTLAVVDAWSSLYGGAPVQSGDFDAGRGMAKTWLYFGGLRPAEDILTVPALPASVQARLKDFLAL
GLAHVRFAAVDWRHHSANVYFRGKGPLDTVQFARIHALSGSTPPAAHVVEEVLAYMPEDYCVAITLDLHSGDIERVC
FYALKVPKNALPRIPTRIARFLEVAPSHDVEECNVIGWSFGRSGDYVKAERSYTGNMAEILAGWNCFFHGEEGRDHD
LRALHQHTESTMGGAR

SEQ ID NO: 4

>NovQ / AAF67510 [*Streptomyces niveus*]
MPALPMNQEFDRERFRVDLRATAAAIGAPVTPRVTDTVLETFRDNFAQGATLWKTTSQPGDQLSYRFFSRLKMDTVG
RAVDAGLLDGTHPTVPIVEDWSDLYGGTPVQSADFDAGRGMAKTWLYFGGLRPAEDILSVPALPAPVQARLKDFLGL
GLAHVRFAAVDWRHRSANVYFRGQGPLDTAQFARVHALSGGTPPAADVVAEVLAYVPEDYCVAITLDLHTGAIDRVC
FYALKVPKDARPRVPARIATFLEVAPSHDPEECNVIGWSFGRSGDYVKAERSYTGNMTEILSGWNCFFHGEEGRDHD
LRALQDTGSITGGAR

SEQ ID NO: 5

>Fur7 / Q2L6E3.1 [Streptomyces sp. KO-3988]
MPGTDDVAVDVASVYSAIEKSAGLLDVTAAREVVWPVLTAFEDVLEQAVIAFRVATNARHEGDFDVRFTVPEEVDPY
AVALSRSLIAKTDHPVGSLLSDIQQLCSVDTYGVDLGVKSGFKKVWVYFPAGEHETLARLTGLTSMPGSLAGNVDFF
TRYGLADKVDVIGIDYRSRTMNVYFAAPSECFERETVLAMHRDIGLPSPSEQMFKFCENSFGLYTTLNWDTMEIERI
SYGVKTENPMTFFARLGTKVEHFVKNVPYGVDTQKMVYAAVTSSGEEYYKLQSYYRWRSVSRLNAAYIAARDKEST

SEQ ID NO: 6

>PpzP / C4PWA1.1 [*Streptomyces anulatus*]
MSESAELTELYSAIEETTRVVGAPCRRDTVRPILTAYEDVIAQSVISFRVQTGTSDAGDLDCRFTLLPKDMDPYATA
LSNGLTAKTDHPVGSLLEEVHRQFPVDCYGIDFGAVGGFKKAWSFFRPDSLQSASDLAALPSMPSGVSENLGLFDRY
GMTDTVSVVGFDYAKRSVNLYFTGASPESFEPRGIQAILRECGLPEPSDELLRFGEEAFAIYVTLSWDSQKIERVTY
SVNTPDPMALPVRIDTRIEQLVKDAPLGSAGHRYVYGVTATPKGEYHKIQKYFQWQSRVEKMLTADAG

Table 1. Accessions with significant similarity amino acid sequences to SEQS NO:1-6

Nº	Accession	Description	% Ident.
1	WP_057602682.1	hypothetical protein [Streptomyces sp. Root1310] NphB	100.00%

2	1ZB6_A	Chain A, Aromatic Prenyltransferase [Streptomyces sp.] HypSc	100.00%
3	WP_023545098.1	4-hydroxyphenylpyruvate 3-DMAPP transferase [Streptomyces roseochromogenus], CloQ	100.00%
4	WP_079127921.1	hypothetical protein [Streptomyces] NovQ	100.00%
5	Q2L6E3.1	Furaquinocin biosynthesis prenyltransferase [Streptomyces sp. KO-3988] Fur7	100.00%
6	C4PWA1.1	Dihydro-PCA dimethylallyltransferase; [Streptomyces anulatus] PpzP	100.00%
7	2XLQ_A	Chain A, CLOQ [Streptomyces roseochromogenus subsp. oscitans]	100.00%
8	2XM7_A	Chain A, CLOQ [Streptomyces roseochromogenus subsp. oscitans]	99.69%
9	WP_069622146.1	hypothetical protein [Streptomyces niveus]	98.76%
10	EST19414.1	hypothetical protein M877_36355 [Streptomyces niveus NCIMB 11891]	98.74%
11	AFS18550.1	ABBA prenyltransferase Ptf_St [Streptomyces tendae]	97.38%
12	WP_078566427.1	prenyltransferase [Streptomyces sp. CNS654]	90.82%
13	WP_109499705.1	hypothetical protein [Streptomyces sp. Act143]	89.84%
14	WP_017621683.1	hypothetical protein [Nocardiopsis gilva]	89.81%
15	KUN17719.1	hypothetical protein AQJ23_40425 [Streptomyces antibioticus]	88.93%
16	KPI30857.1	Aromatic prenyltransferase, Orf2 [Actinobacteria bacterium OV320]	83.39%
17	WP_037872080.1	aromatic prenyltransferase [Streptomyces sp. NRRL S-37]	81.64%
18	WP_114664513.1	prenyltransferase [Streptomyces sp. GSSD-12]	81.31%
19	WP_067123092.1	prenyltransferase [Streptomyces yokosukanensis]	80.27%
20	WP_114021785.1	prenyltransferase [Candidatus Streptomyces philanthi]	80.07%
21	WP_015662653.1	hypothetical protein [Streptomyces davaonensis]	78.43%
22	WP_114016600.1	prenyltransferase [Streptomyces sp. LHW50302]	78.43%
23	GCB50864.1	hypothetical protein SNL152K_8211 [Streptomyces sp. NL15-2K]	76.08%
24	WP_124443591.1	prenyltransferase [Streptomyces sp. NL15-2K]	74.58%
25	WP_078582630.1	hypothetical protein [Streptomyces sp. URHA0041]	73.11%
26	WP_073501789.1	hypothetical protein [Streptomyces paucisporeus]	73.11%
27	WP_078869665.1	hypothetical protein [Streptomyces sp. NRRL B-1347]	71.95%
28	WP_063792633.1	prenyltransferase [Streptomyces atriruber]	67.58%
29	WP_150163952.1	prenyltransferase [Streptomyces venezuelae]	66.56%
30	A2AXG5.1	Flaviolin linalyltransferase [Streptomyces cinnamonensis], Fnq26	66.21%
31	WP_150181843.1	prenyltransferase [Streptomyces venezuelae]	65.89%
32	WP_101421563.1	prenyltransferase [Streptomyces sp. CMB-StM0423]	65.80%
33	WP_047018069.1	hypothetical protein [unclassified Streptomyces]	65.57%
34	WP_052770383.1	hypothetical protein [unclassified Streptomyces]	64.47%
35	WP_125936269.1	prenyltransferase [Streptomyces sp. WAC 06738]	64.14%
36	WP_101422827.1	hypothetical protein [Streptomyces sp. CMB-StM0423]	63.82%
37	AKH86873.1	hypothetical protein AA958_18620 [Streptomyces sp. CNQ-509]	63.57%
38	WP_063790017.1	prenyltransferase [Streptomyces sp. MMG1121]	60.88%
39	WP_078627616.1	hypothetical protein [Streptomyces sp. CNH099]	60.53%
40	AQU65790.1	hypothetical protein BBN63_05560 [Streptomyces niveus]	60.33%
41	WP_147874937.1	hypothetical protein [Streptomyces sp. IB2014 016-6]	60.33%
42	WP_142270720.1	prenyltransferase [Streptomyces sp. SLBN-115]	59.86%
43	WP_030308521.1	hypothetical protein [Streptomyces sp. NRRL F-6131]	59.08%
44	WP_053649621.1	hypothetical protein [Streptomyces sp. XY431]	58.94%
45	WP_097275187.1	prenyltransferase [Streptomyces sp. TLI_55]	58.89%
46	WP_132804080.1	prenyltransferase [Streptomyces sp. BK042]	58.74%
47	WP_045940576.1	hypothetical protein [Streptomyces sp. NRRL S-495]	58.61%
48	WP_103552886.1	prenyltransferase [Streptomyces populi]	58.16%
49	WP_030397806.1	hypothetical protein [Kitasatospora]	58.09%
50	WP_067237424.1	prenyltransferase [Streptomyces longwoodensis]	58.01%
51	WP_137304658.1	prenyltransferase [Streptomyces lasaliensis]	57.95%
52	WP_093861400.1	hypothetical protein [Streptomyces sp. TLI_053]	57.76%
53	WP_026220570.1	aromatic prenyltransferase [Streptomyces vitaminophilus]	57.45%
54	4EE8_A	Chain A, Prenyltransferase [Streptomyces cinnamonensis]	57.10%
55	ADQ43372.1	prenyltransferase [Streptomyces cinnamonensis]	57.10%
56	WP_130469611.1	prenyltransferase [Actinopolyspora sp. DSM 45956]	56.79%
57	WP_150173938.1	prenyltransferase [Streptomyces venezuelae]	56.77%
58	WP_063784116.1	prenyltransferase [Streptomyces sp. SBT349]	56.66%
59	WP_146478482.1	prenyltransferase [Streptomyces sp. SSL-25]	56.00%
60	RZU69833.1	aromatic prenyltransferase Orf2 [Actinopolyspora sp. DSM 45956]	55.99%
61	4EE6_A	Chain A, Prenyltransferase [Streptomyces cinnamonensis]	55.78%
62	WP_092625972.1	prenyltransferase [Actinopolyspora mzabensis]	55.76%
63	4EE6_B	Chain B, Prenyltransferase [Streptomyces cinnamonensis]	55.45%
64	WP_137303681.1	prenyltransferase [Streptomyces galbus]	55.05%
65	AEW22941.1	WT5.12c [Streptomyces sp. WT5]	54.97%
66	WP_139642050.1	prenyltransferase [Streptomyces sedi]	54.85%

67	WP_027750390.1	aromatic prenyltransferase [Streptomyces sp. CNH287]	54.85%
68	WP_055569452.1	hypothetical protein [Streptomyces atriruber]	54.79%
69	WP_079307203.1	prenyltransferase [Streptomyces sp. GKU 895]	54.73%
70	WP_139674960.1	prenyltransferase [unclassified Streptomyces]	54.70%
71	WP_037700793.1	aromatic prenyltransferase [Streptomyces atratus]	54.33%
72	WP_091645455.1	prenyltransferase [Micromonospora pallida]	54.05%
73	WP_111241504.1	prenyltransferase [Jishengella endophytica]	53.74%
74	WP_122184786.1	prenyltransferase [Streptomyces triticirhizae]	53.69%
75	WP_143644462.1	prenyltransferase [Streptomyces sp. IB201691-2A2]	53.25%
76	WP_095564968.1	prenyltransferase [Plantactinospora sp. KBS50]	53.00%
77	WP_103783028.1	prenyltransferase [Streptomyces sp. Ru71]	52.63%
78	WP_135330626.1	prenyltransferase [Streptomyces sp. MZ04]	52.50%
79	WP_119294080.1	prenyltransferase [Streptomyces sp. YIM 130001]	52.45%
80	OPG09817.1	prenyltransferase [Streptomyces sp. GKU 895]	52.31%
81	WP_078616106.1	hypothetical protein [Streptomyces sp. 303MFCol5.2]	52.30%
82	WP_132158260.1	prenyltransferase [Actinomadura sp. 7K507]	51.99%
83	WP_079307242.1	prenyltransferase [Streptomyces sp. GKU 895]	51.96%
84	WP_073827930.1	prenyltransferase [Micromonospora sp. TSRI0369]	51.21%
85	WP_040687408.1	aromatic prenyltransferase [Nocardia vinacea]	51.19%
86	WP_150215285.1	prenyltransferase [Streptomyces venezuelae]	51.16%
87	WP_083940321.1	prenyltransferase [Saccharomonospora saliphila]	51.04%
88	WP_104112297.1	prenyltransferase [Micromonospora chalcea]	50.87%
89	EWM63041.1	hypothetical protein MCBG_00174 [Micromonospora sp. M42]	50.87%
90	WP_030499012.1	aromatic prenyltransferase [Micromonospora purpureochromogenes]	50.87%
91	WP_027748955.1	hypothetical protein [Streptomyces sp. CNH287]	50.79%
92	WP_067309254.1	prenyltransferase [Micromonospora rifamycinica]	50.71%
93	WP_064445539.1	prenyltransferase [Micromonospora sp. NBRC 110037]	50.52%
94	WP_063043013.1	prenyltransferase [Nocardia pseudovaccinii]	50.17%
95	WP_117400900.1	prenyltransferase [Actinomadura sp. LHW52907]	49.83%
96	WP_091651703.1	prenyltransferase [Micromonospora pallida]	49.83%
97	WP_084467029.1	prenyltransferase [Nocardia arthritidis]	49.49%
98	WP_145922251.1	prenyltransferase [Micromonospora sp. HM134]	49.29%
99	WP_141955840.1	prenyltransferase [Actinoallomurus bryophytorum]	49.17%
100	WP_063813137.1	prenyltransferase [Nocardia anaemiae]	49.15%
101	WP_083864015.1	prenyltransferase [Nocardia exalbida]	49.15%
102	WP_121435675.1	prenyltransferase [Actinomadura pelletieri]	49.13%
103	WP_116072936.1	prenyltransferase [Asanoa ferruginea]	49.13%
104	WP_020673231.1	hypothetical protein [Amycolatopsis nigrescens]	49.11%
105	WP_104379267.1	prenyltransferase [Nocardia nova]	48.99%
106	WP_037748550.1	aromatic prenyltransferase [unclassified Streptomyces]	48.97%
107	WP_101420943.1	prenyltransferase [Streptomyces sp. CMB-StM0423]	48.97%
108	WP_145782929.1	prenyltransferase [Streptomyces sp. CNZ287]	48.92%
109	AXO35195.1	hypothetical protein MicB006_2914 [Micromonospora sp. B006]	48.76%
110	WP_049572089.1	hypothetical protein [Streptomyces sp. SBT349]	48.67%
111	WP_145899200.1	prenyltransferase [Streptomyces sp. CNZ289]	48.62%
112	WP_047016268.1	aromatic prenyltransferase [Streptomyces sp. CNQ-509]	48.56%
113	WP_063619318.1	prenyltransferase [Streptomyces sp. CNH099]	48.56%
114	WP_099926184.1	hypothetical protein [Streptomyces sp. 70]	48.51%
115	WP_084499306.1	prenyltransferase [Nocardia gamkensis]	48.46%
116	WP_027752515.1	aromatic prenyltransferase [Streptomyces sp. CNH099]	48.44%
117	WP_018839517.1	hypothetical protein [Streptomyces sp. CNQ766]	48.40%
118	WP_104366678.1	prenyltransferase [Nocardia nova]	48.32%
119	WP_100301149.1	prenyltransferase [Streptomyces sp. CNZ306]	48.28%
120	WP_121433960.1	prenyltransferase [Actinomadura pelletieri]	48.21%
121	WP_063015362.1	hypothetical protein [Corynebacteriales]	47.99%
122	WP_073502277.1	prenyltransferase [Streptomyces paucisporeus]	47.95%
123	WP_063790015.1	prenyltransferase [Streptomyces sp. MMG1121]	47.95%
124	WP_137233316.1	hypothetical protein [Streptomyces sp. BPSDS2]	47.88%
125	WP_065481562.1	hypothetical protein [Streptomyces sp. PTY087I2]	47.76%
126	WP_145815899.1	prenyltransferase [Micromonospora sagamiensis]	47.75%
127	WP_150521200.1	cupin domain-containing protein [Streptomyces subrutilus]	47.74%
128	WP_039778124.1	aromatic prenyltransferase [Nocardia cerradoensis]	47.65%
129	WP_030517845.1	hypothetical protein [Nocardia sp. NRRL WC-3656]	47.65%
130	WP_027740846.1	aromatic prenyltransferase [Streptomyces sp. CNT371]	47.59%
131	WP_125503295.1	prenyltransferase [unclassified Streptomyces]	47.57%

132	WP_100203158.1	prenyltransferase [Streptomyces carminius]	47.57%
133	WP_101424043.1	prenyltransferase [Streptomyces sp. CMB-StM0423]	47.48%
134	WP_051956317.1	prenyltransferase [Streptomyces atratus]	47.33%
135	WP_063066486.1	hypothetical protein [Nocardia violaceofusca]	47.32%
136	WP_063007124.1	hypothetical protein [Nocardia kruczakiae]	47.32%
137	WP_104364109.1	prenyltransferase [Nocardia nova]	47.32%
138	SUA47165.1	Aromatic prenyltransferase Orf2 [Nocardia africana]	47.32%
139	WP_117399009.1	prenyltransferase [Actinomadura sp. LHW52907]	47.28%
140	WP_049575632.1	aromatic prenyltransferase [Streptomyces sp. SBT349]	47.19%
141	WP_131544751.1	hypothetical protein [Streptomyces sp. IBTA2]	47.19%
142	WP_036499104.1	hypothetical protein [Nocardia aobensis]	47.18%
143	WP_143644477.1	prenyltransferase [Streptomyces sp. IB201691-2A2]	47.16%
144	WP_083880814.1	prenyltransferase [Nocardia araoensis]	47.10%
145	WP_084489194.1	prenyltransferase [Nocardia niwae]	47.10%
146	WP_125619124.1	hypothetical protein [Streptomyces sp. WAC04770]	47.06%
147	WP_063920851.1	hypothetical protein [Nocardia mikamii]	46.98%
148	WP_051866258.1	prenyltransferase [Streptomyces griseus]	46.98%
149	WP_053560640.1	hypothetical protein [Streptomyces sp. CFMR 7]	46.86%
150	WP_106954687.1	prenyltransferase [Nocardia sp. MDA0666]	46.64%
151	XP_025437791.1	hypothetical protein BO95DRAFT_456766 [Aspergillus brunneoviolaceus CBS 621.78]	46.46%
152	WP_084823458.1	prenyltransferase [Nocardia beijingensis]	46.42%
153	WP_097275149.1	prenyltransferase [Streptomyces sp. TLI_55]	46.40%
154	RBL81021.1	hypothetical protein DDE05_47290 [Streptomyces cavourensis]	46.34%
155	WP_073499477.1	prenyltransferase [Streptomyces paucisporeus]	46.26%
156	WP_031521063.1	aromatic prenyltransferase [Streptomyces sp. NRRL F-5123]	46.24%
157	WP_082412569.1	hypothetical protein [Actinobacteria bacterium OV320]	46.23%
158	TCR75458.1	aromatic prenyltransferase Orf2 [Streptomyces sp. BK042]	46.18%
159	SDJ65986.1	Aromatic prenyltransferase Orf2 [Actinopolyspora mzabensis]	46.15%
160	XP_025503118.1	hypothetical protein BO66DRAFT_325070 [Aspergillus aculeatinus CBS 121060]	46.13%
161	WP_040717943.1	hypothetical protein [Nocardia veterana]	46.10%
162	WP_091459402.1	prenyltransferase [Micromonospora inyonensis]	46.08%
163	WP_141973439.1	prenyltransferase [Actinomadura hallensis]	45.58%
164	WP_079073831.1	hypothetical protein [Streptomyces sp. Root1310]	45.55%
165	WP_078890532.1	hypothetical protein [unclassified Streptomyces]	44.52%
166	XP_001589743.1	hypothetical protein SS1G_09465 [Sclerotinia sclerotiorum 1980 UF-70]	44.37%
167	WP_101421570.1	hypothetical protein [Streptomyces sp. CMB-StM0423]	44.26%
168	WP_147874947.1	hypothetical protein [Streptomyces sp. IB2014 016-6]	44.22%
169	PQE07084.1	Aromatic prenyltransferase protein [Rutstroemia sp. NJR-2017a BVV2]	44.11%
170	WP_078616079.1	hypothetical protein [Streptomyces sp. 303MFCol5.2]	44.00%
171	WP_078899560.1	hypothetical protein [Streptomyces]	43.88%
172	WP_078074323.1	hypothetical protein [Streptomyces niveus]	43.88%
173	WP_135330628.1	hypothetical protein [Streptomyces sp. MZ04]	43.58%
174	WP_063826174.1	hypothetical protein [Streptomyces antibioticus]	43.54%
175	WP_117358562.1	hypothetical protein [Actinomadura sp. NEAU-G17]	43.45%
176	PVH76557.1	hypothetical protein DL98DRAFT_535616 [Cadophora sp. DSE1049]	43.45%
177	WP_047018064.1	hypothetical protein [Streptomyces sp. CNQ-509]	43.39%
178	WP_145784670.1	hypothetical protein [Streptomyces sp. CNZ287]	43.39%
179	AQU65798.1	hypothetical protein BBN63_05610 [Streptomyces niveus]	43.33%
180	MWA08000.1	hypothetical protein [Streptomyces sp. BA2]	43.23%
181	CDH35382.1	aromatic prenyltransferase [Streptomyces iakyrus]	43.20%
182	WP_069463857.1	hypothetical protein [Streptomyces rubidus]	43.15%
183	WP_066951135.1	hypothetical protein [Microtetraspora fusca]	43.10%
184	KJK34409.1	hypothetical protein UK15_36180 [Streptomyces variegatus]	43.07%
185	TVY84701.1	4-hydroxyphenylpyruvate 3-dimethylallyltransferase [Lachnellula suecica]	43.00%
186	TGO19921.1	hypothetical protein BTUL_0002g01700 [Botrytis tulipae]	43.00%
187	WP_031138137.1	hypothetical protein [Streptomyces xanthophaeus]	42.71%
188	KAA8569914.1	hypothetical protein EYC84_002254 [Monilinia fructicola]	42.66%
189	XP_001560463.1	hypothetical protein BCIN_06g02600 [Botrytis cinerea B05.10]	42.66%
190	WP_063619313.1	hypothetical protein [Streptomyces sp. CNH099]	42.57%
191	WP_133260072.1	hypothetical protein [Streptacidiphilus pinicola]	42.52%
192	KKY13521.1	hypothetical protein UCRPC4_g06967 [Phaeomoniella chlamydospora]	42.41%
193	WP_018840564.1	hypothetical protein [unclassified Streptomyces]	42.33%
194	TGO66701.1	hypothetical protein BOTNAR_0056g00400 [Botryotinia narcissicola]	42.32%
195	WP_078564382.1	hypothetical protein [Streptomyces sp. CNQ329]	42.32%
196	AFI64508.1	Wt3.9 [Streptomyces sp. WT3]	42.27%

197	CCD48995.1	similar to gi|310689659|pdb|2XM7|A Chain A [Botrytis cinerea T4]	42.22%
198	WP_148356240.1	hypothetical protein [Actinomadura syzygii]	42.21%
199	WP_143644478.1	hypothetical protein [Streptomyces sp. IB201691-2A2]	42.16%
200	WP_126635119.1	hypothetical protein [Streptomyces hyalinus]	42.16%
201	KUN17777.1	hypothetical protein AQJ23_40535 [Streptomyces antibioticus]	42.05%
202	WP_018844883.1	hypothetical protein [unclassified Streptomyces]	42.00%
203	WP_103786661.1	hypothetical protein [Streptomyces sp. Ru71]	41.98%
204	TGO79002.1	hypothetical protein BELL_0046g00010 [Botrytis elliptica]	41.98%
205	TGO61486.1	hypothetical protein BCON_0027g00760 [Botryotinia convoluta]	41.98%
206	WP_078904276.1	hypothetical protein [Streptomyces xanthophaeus]	41.98%
207	OTA08152.1	hypothetical protein A9Z42_0091200 [Trichoderma parareesei]	41.89%
208	WP_109499726.1	hypothetical protein [Streptomyces sp. Act143]	41.86%
209	RAG82183.1	hypothetical protein DN069_28980 [Streptacidiphilus pinicola]	41.84%
210	GAO81753.1	hypothetical protein AUD_0713 [Aspergillus udagawae]	41.84%
211	WP_078982086.1	hypothetical protein [Streptomyces scabrisporus]	41.81%
212	THV47184.1	hypothetical protein BGAL_0329g00100 [Botrytis galanthina]	41.78%
213	WP_100302114.1	hypothetical protein [Streptomyces sp. CNZ306]	41.67%
214	WP_037751911.1	hypothetical protein [Streptomyces sp. CNQ-525]	41.67%
215	WP_103552883.1	hypothetical protein [Streptomyces populi]	41.61%
216	KQX72267.1	hypothetical protein ASD48_39950 [Streptomyces sp. Root1310]	41.61%
217	WP_053666646.1	hypothetical protein [Streptomyces sp. MMG1121]	41.58%
218	SKA30833.1	Aromatic prenyltransferase Orf2 [Marinactinospora thermotolerans DSM 45154]	41.58%
219	WP_078763101.1	hypothetical protein [Marinactinospora thermotolerans]	41.58%
220	WP_130454911.1	hypothetical protein [Streptomyces sp. CNZ288]	41.33%
221	WP_145901163.1	hypothetical protein [Streptomyces sp. CNZ289]	41.33%
222	WP_027745939.1	hypothetical protein [Streptomyces sp. CNT371]	41.33%
223	WP_055569113.1	hypothetical protein [Streptomyces atriruber]	41.31%
224	KAB8298687.1	hypothetical protein EYC80_000864 [Monilinia laxa]	41.30%
225	WP_146478483.1	hypothetical protein [Streptomyces sp. SSL-25]	41.16%
226	WP_122199365.1	hypothetical protein [Actinomadura sp. NEAU-Ht49]	41.14%
227	WP_150213418.1	hypothetical protein [Streptomyces venezuelae]	41.10%
228	TGO83018.1	hypothetical protein BPOR_0718g00070 [Botrytis porri]	40.96%
229	WP_143023025.1	hypothetical protein [Lentzea jiangxiensis]	40.82%
230	XP_001210907.1	predicted protein [Aspergillus terreus NIH2624]	40.82%
231	WP_130468244.1	hypothetical protein [Actinopolyspora sp. DSM 45956]	40.78%
232	WP_049571866.1	hypothetical protein [Streptomyces sp. SBT349]	40.67%
233	TGO41762.1	hypothetical protein BHYA_0017g00730 [Botrytis hyacinthi]	40.61%
234	WP_084510537.1	hypothetical protein [Nocardia lijiangensis]	40.33%
235	TGO19972.1	hypothetical protein BPAE_0327g00150 [Botrytis paeoniae]	40.27%
236	AGS49802.1	hypothetical protein [uncultured bacterium esnapd16.1]	40.21%
237	WP_142270708.1	hypothetical protein [Streptomyces sp. SLBN-115]	40.13%
238	WP_150163951.1	hypothetical protein [Streptomyces venezuelae]	40.07%
239	WP_150181842.1	hypothetical protein [Streptomyces venezuelae]	40.00%
240	WP_078627940.1	hypothetical protein [Streptomyces sp. CNH099]	40.00%
241	KOV57748.1	hypothetical protein ADK64_38125 [Streptomyces sp. MMG1121]	40.00%
242	XP_024752719.1	hypothetical protein BBK36DRAFT_1111187 [Trichoderma citrinoviride]	39.93%
243	WP_101420456.1	hypothetical protein [Streptomyces sp. CMB-StM0423]	39.93%
244	WP_091373094.1	hypothetical protein [Alloactinosynnema album]	39.93%
245	TEY79915.1	hypothetical protein BOTCAL_0040g00320 [Botryotinia calthae]	39.87%
246	MWA09138.1	prenyltransferase [Streptomyces sp. BA2]	39.86%
247	XP_020123439.1	hypothetical protein UA08_01803 [Talaromyces atroroseus]	39.67%
248	XP_002847323.1	NovQ [Microsporum canis CBS 113480]	39.65%
249	WP_078627401.1	hypothetical protein [Streptomyces sp. CNH099]	39.60%
250	WP_043384953.1	hypothetical protein [Streptomyces luteus]	39.54%
251	WP_104481745.1	cupin domain-containing protein [Actinokineospora auranticolor]	39.40%
252	TQL97062.1	aromatic prenyltransferase Orf2 [Actinoallomurus bryophytorum]	39.27%
253	WP_157429804.1	hypothetical protein [Actinomadura oligospora]	39.25%
254	WP_146478483.1	hypothetical protein [Streptomyces sp. SSL-25]	39.19%
255	WP_141955833.1	hypothetical protein [Actinoallomurus bryophytorum]	39.14%
256	WP_141955837.1	hypothetical protein [Actinoallomurus bryophytorum]	39.02%
257	PMD17248.1	hypothetical protein NA56DRAFT_648701 [Pezoloma ericae]	38.75%
258	WP_063784117.1	hypothetical protein [Streptomyces sp. SBT349]	38.56%
259	WP_084496655.1	hypothetical protein [Nocardia amamiensis]	38.51%
260	WP_078899998.1	hypothetical protein [Streptomyces sp. SBT349]	38.19%
261	WP_092625293.1	hypothetical protein [Actinopolyspora mzabensis]	38.02%

262	CAL34106.1	putative prenyltransferase [Streptomyces cinnamonensis]	37.99%
263	WP_078560068.1	hypothetical protein [Streptomyces sp. CNT371]	37.67%
264	TCO55794.1	aromatic prenyltransferase Orf2 [Actinocrispum wychmicini]	37.65%
265	KJZ73749.1	hypothetical protein HIM_06867 [Hirsutella minnesotensis 3608]	37.63%
266	CEL03594.1	hypothetical protein ASPCAL04746 [Aspergillus calidoustus]	37.50%
267	OOQ89743.1	hypothetical protein PEBR_07177 [Penicillium brasilianum]	37.41%
268	PYH97268.1	hypothetical protein BO71DRAFT_468204 [Aspergillus ellipticus CBS 707.79]	37.41%
269	KAE9377664.1	hypothetical protein N431DRAFT_527667 [Chalara longipes BDJ]	37.37%
270	KUL90715.1	hypothetical protein ZTR_00003 [Talaromyces verruculosus]	37.16%
271	GAM39384.1	hypothetical protein TCE0_034r10891 [Talaromyces cellulolyticus]	36.64%
272	PCH00031.1	hypothetical protein PENOC_055060 [Penicillium sp. 'occitanis']	36.64%
273	WP_069462248.1	hypothetical protein [Streptomyces rubidus]	36.51%
274	PQE21594.1	4-hydroxyphenylpyruvate 3-DMAPP transferase protein [Rutstroemia sp. NJR-2017a]	36.46%
275	OQD84936.1	hypothetical protein PENANT_c011G06136 [Penicillium antarcticum]	36.15%
276	PQE13805.1	Chain A like protein [Rutstroemia sp. NJR-2017a BBW]	36.11%
277	RAO64565.1	hypothetical protein BHQ10_000577 [Talaromyces amestolkiae]	35.96%
278	CRG83396.1	hypothetical protein PISL3812_00747 [Talaromyces islandicus]	35.91%
279	KAA8647038.1	putative secondary metabolism biosynthetic enzyme [Aspergillus tanneri]	35.89%
280	XP_018062656.1	hypothetical protein LY89DRAFT_339943 [Phialocephala scopiformis]	35.62%
281	KIM97227.1	hypothetical protein OIDMADRAFT_20473 [Oidiodendron maius Zn]	35.52%
282	PQE15082.1	Aromatic prenyltransferase protein [Rutstroemia sp. NJR-2017a BVV2]	35.02%
283	KJX93611.1	Chain A like protein [Zymoseptoria brevis]	34.71%
284	KFX50527.1	Aromatic prenyltransferase NovQ [Talaromyces marneffei PM1]	34.65%
285	XP_024705102.1	hypothetical protein P170DRAFT_358697 [Aspergillus steynii IBT 23096]	33.67%
286	KIM92739.1	hypothetical protein OIDMADRAFT_139131 [Oidiodendron maius Zn]	33.57%
287	PVH96444.1	hypothetical protein DM02DRAFT_569563 [Periconia macrospinosa]	33.33%
288	MAR13547.1	hypothetical protein [Blastopirellula sp.]	32.75%
289	RDW66932.1	hypothetical protein BP5796_09681 [Coleophoma crateriformis]	32.00%
290	RDW62099.1	hypothetical protein BP6252_11532 [Coleophoma cylindrospora]	30.87%
291	POS72278.1	hypothetical protein DHEL01_v209327 [Diaporthe helianthi]	27.36%
292	ROW05052.1	hypothetical protein VSDG_00518 [Valsa sordida]	27.36%
293	ROV99468.1	hypothetical protein VMCG_06385 [Valsa malicola]	26.71%
294	KKY34764.1	hypothetical protein UCDDA912_g05266 [Diaporthe ampelina]	26.69%
295	KUI68523.1	4-hydroxyphenylpyruvate 3-dimethylallyltransferase [Valsa mali]	25.26%

Also described herein are polypeptides encoding enzymes that are typically *O*-methyltransferases. These polypeptides are typically derived from plants and find use in converting phenolic compounds into compounds with medicinal activity and/or precursors to such compounds. For example, described herein are polypeptides comprising one or more of the following sequences, variants thereof, or fragments of the polypeptides or variants. In the following sequences, amino acid sequences and GenBank accession numbers of *O*-methyltransferases from *Cannabis sativa* are shown.

SEQ ID NO: 7

>CsOMT1 [PK03555]
MEADGEDAVLRGQVEIWKYMLSFADSMALKCAVELQLADIIHSHTSPITLSQIASAIPGATSPDLSCLARIMRLLVR
RRIFTQHQKPKSDGEEEEALYGPTHSSRWLLTKTNDHDQLTLAPMILMENDPRLMAPWHCFSRCVKEGGVAFKKAHN
GQSIWEFGAENPEINKLFNDAMECTAKVVMKAILSHYSDGGFSDIKSMVDVGGGTGGSISEIVRSYPHIKGINFDLP
HVIATAPPYSGVSHVGGDMFRSVPTADAIFMKWILHDWSDEDCVKILKNCRKAIAEESGKVIIVESVLEEESNNNNN
NNEVFGDTALMLDLVMVAHTTGGKERTQKQWKTILEQGGFPRYNFIKIKALPSIIEAYPN

SEQ ID NO: 8

>CsOMT2 [PK03696]
SELLFQAQTHLYNHTLSYISSMCLKCAIELGIPDIINNHGQPHIPLPQLVSSLRLPPTKTDILRRLMRPLVHFGYFT
TTKVVINSQNKEEEEEEVDAYGLTSSSKLLFVNNNGNNKIPSMSTIVCLQLDQAFMTPWHSLGNWLRKDEATTLFES
AHEMSFWEYTSKNTKFGHLFNEAMADDSKLMLKLVIEDVKPVFEGLTSLVDVGGGTGEVCKILTQVFPHLKCSVLEL

SHVVANLPNAQNLKFIEGDMFQAIPPANAVLLKWILHNWSDEECVTILKKCREAIASNEGGKVVIIDVVINSKKDEH
EVNEAKISFDLMMMVLFNGRERSQKEWENLFFKAGFTRYKITPIFGLESLIEVFP

SEQ ID NO: 9

>CsOMT3 [PK04621]
MASAVKGAILAIDNEASFQDQAEIWKYMLNFGDSMALKCAVELRLPDILHSADGPMTLSEIAAAIPNAPSPQASHLF
RVLRLLVRRKIFTSEEDKVSGETLYGPTKLSSWLLHEPAPTSDSDASIMTLAPMLIMENHPWHVDPWHKLSEFIREG
GLAPFEKEHGYMFDFAAKNPLYNKLLNNAMACTARITIRTLLSHCGDDLFNGVGSVVDVGGGTGRFISEIVKSYPHI
KGINFDRSHVISTAPAYPGVTHIGGDMFQEVPSADAVVTKWVLHDWGDELCVKLLKNCRKAIPEKGGKVIIVDIVVE
ADGEGLFDDTGVVFDVLMLAHNTGGKERTEKEWKSLLDQSGFPRYKITKIPALQSVIEAYPN

SEQ ID NO: 10

>CsOMT4 [PK05994]
MMSSINDNTITTTQQLSLGYVNLYKHMLSYASSMALKSAVELGIPDIIFNKGKAQTISLHELVSALQIPPSRTNFLR
RVMRVLVHSGIFTNEKGYNDDKEEEEVYGLTPSSKLLLTNGNNSEVPSVGPFVLSVLEPVTVTAFHLIGNWLKNEDS
PATPFHLANDDGLGLYEYWGKNTDGFGDRLNEGMESDSGTLKFVLKNFKSTFEGITTLVDVGGNTGTMCKMLIEAFP
HLKCSVFDLPYVVEANSHNNTENLKFIEGDMFQTIPEADAILFKLVLCGCSDDESIKILKNCREAISRNGKGKVLII
ENNVINSEKDELLELEAKLYFDMLLLASVTGRERSKKDWENIFFQAGFTHYEITPMFGLEALIEVFP

SEQ ID NO: 11

>CsOMT5 [PK07724]
MDALSRDQAEIFEHMFSFVDSMALKCAVELRIADIIHSQDCPISLTQIASKIITNSHNSPMISSSPDNNTMLYLNRI
MTSLVRKKIFTAQYDHDQNNNQTVLYYGVTSKSRWLLRDAKPSLAPLILMENHPIQMAPWHYFSHIIKDQEGSATAY
EKAHGCGIFELASVNGELNKIFNDGMACLGEMVMGAILPAYDVFGCMGSLVDVGGGIGGDLAEIVKSHPHIKGINFD
LPHVTATAPESNGVTHVAGNMFESVPSADAIFIKWVLHDWCDEECVKILRNCRKAIPEKNGKVIIVEIVLKDSSQNK
ENDDVFDETRMIFDMVMMAHTCRGKERTEFEWKKLLEEAGFPRYKITKIPAIPSIIEAYPF

SEQ ID NO: 12

>CsOMT6 [PK08183]
MAPTQISEELEASLFAMQLAGTSSILPMVLKTALELDLLEIIAMAGPNAFLSPSDIAAQLPTNNPNASMMLDRMLRL
LASYNVLTYLLRDKVTSDGKVLVERLYGLAPLSKFLTKNEDGASIAPLCLMVQDKVFMESWYHLKDAILDGGIAFDK
AHGMPAFKYNQIDKRFNKIFNKGMFDHSSITMKKILETYKGFEGLNSMVDVGGGSGAVLSMIVTKYPSIKGINFDLH
HVIEDAPPFPGVAHVGGDMFVSVPKGDAIFIKWICHDWSDEDCLKLLKNCYDAVPHHGKVIVAEFILSVAPDSSLAA
KCTAHSDMIMLVDHGGKERTQKEFEELAKAAGFKGFKVVCNAFNTYIMEFLKTN

SEQ ID NO: 13

>CsOMT7 [PK08793]
MAVETHKDELIWIPKEDEEERARVDIYKYIFGFVEMAVVKCAIELGIADAIESHGRPMSLLELSSALGCAAPALHRI
MRFLTNRKLFKEIRINENVQDSEQPSLYAQTALSRLILRSGEKSMATFVLMESSPPMLAPWHGLSARVKTEVDDSSA
PSPFEVANGKDVWSYAAANPGHSQLINEAMACNARVTVAAILDGCLDVFDGIGTIVDVGGGNGTALRMLVRACPWIR
GINFDLPHVVSVALKSEGVEHVGGDMFKFVPKADAAFLMSVLHDWEDDECIQILKKCREAIPGDKGKVIMVECVIEE
NNNNVEEKHEELELKDVGLFLDMVMIAHTNKGKERTLEEWAYVLAQAGFNRYNIRAINAIYSIIEAFPN

SEQ ID NO: 14

>CsOMT8 [PK10317]
MAKMQEGGDHHDELTCRAKEEDEQEEERARIDIYKYVFGFVEMAVVKCAIELGIADTIECHGRPMSLKELSSALGCT
PHNIHRIMRFLVNRRIFKEIKNDIVNDEGAGTLYVQTSLSRLLIKSGERSMASFVLMESSKPMLAPWHCLSSRLKAE
VIDNSLTPFEEANGQDLWSYTAANPEHSQLLNEAMACNARVTVAAILDSCLEVFDGVGSIVDVGGGNGTAMQLLVKG
CPWIKEGILFDLPHVVSVALKSDRVVHVGGDMFDSVPKADAAFLMWVLHDWEDKDCIQILKNCREAISEKGKVIIVE
SVIENNKEQNNGMKKDELEFKDVGLFLDMVMMAHTNKGKERTLDQWVYVLHQAGFTRYNVRSIKGAISSLIEAFPI

SEQ ID NO: 15

>CsOMT9 [PK10819]
MEKLKSFSYLNNNIDLAMNEENNTKLLGAQAHIWNQIFNFINSMCLKCAVELGIPDIINNYGKPMTISQLTLALSIN
KNKFHCLYRLMRLLTHSGFFALEKVEIEGEKEEEGYVITEASKLLLKDNPMSVTPFLLLVLNPILTKSFDVLDTWFQ
NDSPTPFDTANGRTFWDYGSHEPKLVQLFNDGMASDARLVTSVVIEKCKGVFEGVERLVDVGGGTGTVAKSIATAFP
QIECSVLDLPHVVADLEDENNLKFIGGDMFVEIPTADVVLLKWILHDWNDEESVKILKNCREAVYKSKKKSGKLIII
DMNIKNDNNENSFETQLFFDMLMMALVSGRERNEKEWSKLFKDAGFSRYKITPILGLRFVIEVYP

SEQ ID NO: 16

>CsOMT10 [PK11500]
MDGIQEGDHHDELTLRLNEKEEEERARIDIYKYVFGFVEMAVVKCAIELGIADTIESHGRPISLLDLSSALSCNPHN
LHRIMRFLVNRRIFKEIKNDTVNDKGCLYVQTSLSRLLIKSGERSMASFVLMESSNPMLAPWHGLSARVKAEATDAL
TPFEAANGVDVWSYAAANPDHSQLINEAMACNARVTVAAILNGCLDVFDGVGSIVDVGGGNGTTLQLLVKGCPWINQ
GINFDLPHVVSVALKSDGVVHVGGDMFDSVPKADAAFLMWVLHDWGDQECIQILKKCKEAIPEKGKVIIVESVIENN
KLEENVMKKELELKDVGLFLDMTMMAHTNKGKERSLDEWVYVLHQAGFTRYNVRSIDGAVSSVIEAFPA

SEQ ID NO: 17

>CsOMT11 [PK12774]
MGSISENTTTLELSQGYVNVYKHMLSYASSMALKCAIQLHIPDIIFTKGKDQTITLPELASALQIPPSRISCLRRVM
RVLVHSGIFSNKNQHDDDKTEEEEVYGLTSSSKILLTGGNNNGVPSVGGYVLAVLEPVTVTAFHLIGSWLKKESPRT
PFHLANDEGLSLYEYWGKNIDGFGDRLDEGMESDSGVLKFVLKDLKSSFEDITTLVDVGGNTGSMCMMLIEEFPHLK
CTVFDLPYAIEANSHNSTSNLKFVEGDMFQSTFPEADAFLLKSVLSGCSDEECVKILKNCGEAISRNGGGKVMVIDN
NVINTKNDEAAELEAKLYFDMLFMTALTGRERTKKDYENIFYQAGFTRIKITRMFGLKSLIEGFL

SEQ ID NO: 18

>CsOMT12 [PK13022]
MTTPTQMSEELEANYLFAMKLASATVLPMVLKTALELGLLEIIVMAGPGAFLSPSNIVAQLPTKNPNAPVMLDRMLR
LLASYNVLTYSIRDGERLYGMTPLSKFLTKNEGGLSIAPLCHMDQDKVIIDCWYHMKDAVLDGGIPFNKAHGMPIFE
YTQRDQRLNKIVNRAMSTLSTIIMKNILETYNGFKGLNSIVDVGGGTGATLSMIIAKYPSIKGINFDLHHVIQHAPP
LPGVEHVSGDMFVSVPKGDAIFMKRICHDWSDEECLKLLKNCYDALDDDGKVIVEELIVPAAPDSSPSTKNSFHYDI
LMMVNLNGKERTQKEYEQLAMEAGFKAFKIHCIAFNSYIMEFLAKGPKVFWSVRVPPLL

SEQ ID NO: 19

>CsOMT13 [PK15692]
MESSQLRGQELICQLIFSYYNTMALKCAVELRIADIIHSHGKPITISQIASDIQSNSNSKSPINIDNLFRIMRILVR
KGVFIEHHDDDHGDSTISLYGLCDSSRCLLWDFDSSLVPFILLNTHPLMMASSHNFGKSVIGDKGNPFENDQDVWSF
ASNNPIFNKLFNDAMISGSHMVLRHVLSTYKDSFNCIKGTMVDVGGGVGQVISEIVKSHRHIKGINFDLPHVIATAP
TYDGVTHVGGDMFESIPSADVVFLKWIIHGWNDDACVKILKNCRKAIGEKKNGKIIIVDMVLDPNSNEIFQETRLAM
DLVMLANSNNGKERTELEWKKLLNEAGFSRYKITKNQNILDIIEAFPF

SEQ ID NO: 20

>CsOMT14 [PK17162]
MGSINENTITTTQELSQGYVNLYKHMLSYASSMALKSAVELGIPDIIFTKGKAQTISLHELVSALQIPPSRTNFLRR
VMRVLVHSGIFTNEKGYNDDKEEEEVYGLTPSSKLLLTGGKNNGVPSVGPYVLAVLEPVTVTAFGSIGNWLKKESPT
TPFHLANDEGLSLYEYWGKNTDGFGDRLNEGMESDSGVLKFVLKDFKSVFEGITTLVDVGGNTGLMCKMLLEAFPHL
QCSVYDLAYAVDANSHNNTQNLKFIEGDMFQTVPQADAILFKCVLSGCSDEECTKILKNCRDAISRNGGGKVLIIDN
NVINSKTEDHLAMETLLYFDMLMMTALTGRERTKKDWEKIFFEAGFSSCKVTPMFGVKTLIEVFP

SEQ ID NO: 21

>CsOMT15 [PK19674.1]
MGSELEGTTEVVVDLKRKQEEESFCYAAQLLNTNVLTKSLQTTIELGIFDIIAKAGEGGKLSAREIVAQLPTNNPDA
PMMVDRILRMLASYSVLVCSVVADDQRAYSLNNVSKCFVTNEDGVSLGPLMLLLEDKVFSDSWSQLKGAILEGGIPF
NRFHGMNAFEYPALDSRFNKVFNRAMQSMTTMLAKQTIESYKGFENLKQLVDVGGGLGVTLKEITSTYPHIKGINFD
LPHVVQHAPSYPGVEHVGGDMFESVPSGDAIFMKWILHDWSDEQCVKVLKNCYKAIPENGKVIVMEGLLPMLPEASY
GDNIMSKTDVLMMTQNPGGKERSKQEFQALASGAGFNGIRFECCVSGFWIMEFFK

SEQ ID NO: 22

>CsOMT16 [PK19674.2]
MAPPSEELANTPQIVNDERKQEEENFAYAAQLVNSSVLSMSLQSAIELGVFDIIAKAGDAAKLSAQDIVAQMPTTNP
DAPRMLDRILRMLASHSVLACSLENEDLRVYCLNDVSKLFVTNEDGVSLGPLMLLQDKVFLDSWSQLKGAILEGGIP
FNRVHGMHAFEYPSLDQKFNQVFNKAMYNQTTLVLKKILEVYKGFENLEKVVDVGGGLGGTLNQITSKYPHIKGINF
DLPHVVEHAPSYPGVEHVGGDMFESVPTGAIFMKWILHDWSDEHCLKLLKNCYKAIPDNGNVIVMEAILPTIPETNS
ADRCTSQTDVLMMTQNPGGKERSKQEFQALASGAGFNGIRFECCVSGFWIMEFFK

SEQ ID NO: 23

>CsOMT17 [PK19674.3]
MMGSDQLEVIVDLKRPKQEESFCYALQLLSTNILIKSLQATVELGIFDIIAKAGEGSKLSAAEIVAQLPTNNPDAVM
MVDRILRMLAGHSVLTCSVVADNPRVYSHNTVSKCFVTDEDGVSLGSLISLLDDKVYSDSWSQLKGAILEGGIPFNR
LHGMNSFEYTALDSRFNKVFNRAMQSMTTMIAKQTIESYKGFENLKQLVDVGGGLGVTLKEITSTYPHIKGINFDLP
HVVQHAPSYPGVEHVGGDMFESVPSGDAIFMKWILHDWSDEQCVKVLKNCYKAIPENGKVIVMEGLLPMLPEASYGD
NIMSKTDVLMMTQNPGGKERSKQEFQALASGAGFNGIRFECCVSGFWIMEFFK

SEQ ID NO: 24

>CsOMT18 [PK19674.4]
MAPPSEELANTPQIVNDERKQEEENFAYAAQLVNSSVLSMSLQSAIELGVFDIIAKAGDAAKLSAQDIVAQMPTTNP
DAPRMLDRILRMLASHSVLACSLENEDLRVYCLNDVSKLFVTNEDGVSLGPLMSLLQDKVFLDSWSQLKGAILEGGI
PFNRVHGMHAFEYPSLDQKFNQVFNKAMYNQTTLVLKKILEVYKGFENLEKVVDVGGGLGGTLNQITSKYPHIKGIN
FDLPHVVEHAPSYPGVEHVGGDMFESVPTGDAIFMKWILHDWSDEHCLKLLKNCYKAIPDNGNVIVMEAILPTIPET
NSADRCTSQTDVLMMTQNPGGKERSKKEFLALATGAGFSGIRFECFVCNFMIMEFYK

SEQ ID NO: 25

>CsOMT19 [PK19715]
MEKSRNSSSHVDLVVNEDNNTKLLRAQAHIWNHICKFINSMSLKCAIELGIPDIVNNHGKPMTISQLTLALPINKNK
SHCLYRLMRLLTHSGFFALEKTEIKGEEEEEGYVITEASKLLLKDNPMSVTPLLLVLLDPTLTKPYDVLSTWFRNDD
STPFVTTNGMAIWDYYSHEPKLAQSFNEAMASDARLVTSVLIEKCKGVFEGVDSLVDVGGGTGTVAKSIATTFPQIQ
CSVLDLPHVVAGLQGEKNLNFIAGDMFVEVPTAQVVLLKWILHDWSDENSVKILKKCKEAITKSGKKIGKVVVIDMI
IENEKGEIDDESYETQLFMDMTMTLVSGRERNEKELSKLFKDAGFSHYKITPILGLRSLIEIYP

SEQ ID NO: 26

>CsOMT20 [PK23308]
MEKLKSFRHLNNNIDLVLNEENSIELLRAQGHIWNQIFNFINSMSLKCAIQLGIPDIINNYGKPMTISQLKLALPIN
QKKSSCVYRLMRILTHSNFFALQKVEGREGEEEEEGYVITDASKLLLKDNPMSVTPFLLAMLDPVITKPWDFLSNWF
QNDDPTPFDTANGMTFWDYGSHQPNLARFFNDAMASDARLVTSVVIEKCRWVFEGVESLVDVGGGTGTVATTIATSF
PQIQCSVLDLPHVVADLQGANNLVNFIGGDMFVEVPPAEVVLLKWILHDWNDEESVKILKKCKEAITKNNKKGGKVI
IIDMKVENEKDEDDESYETQLFFDMLMMALVTGKERNEKEWAKLFKDAGFSDYKITPILGLRSLIEVYP

SEQ ID NO: 27

>CsOMT21 [PK24150]
MGSTGIETQMTPTQISDEEANLFAMQLASASVLPMVLKAALELDLLEIIAKAGPGAFLSPSDIAQQLPTQNPDAPVM
LDRMLRLLASYNVVTYSLRERETAEEEGKVERLYGLAPVSKYLTKNEDGVSIAPLCLMNQDKVLMESWYHLKDAVLD
GGIPFNKAYGMTAFEYHGTDQRFNKIFNRGMSDHSTITMKKILETYKGFEGLNSIVDVGGGTGAVVNMIVSKYPTIK
GINFDLPHVIEDAPPLTGVEHVGGDMFVSVPKGDAIFMKWICHDWSDEHCLKFLKNCHAALPEHGKVIVAECILPVA
PDSSLATKSTVHIDVIMLAHNPGGKERTEKEFEALAKGAGFKGFKVHCNAFNTHIMEFLKTI

SEQ ID NO: 28

>CsOMT22 [PK27112]
MNLIMGEGELVSCRELVEAQELIYNCSLSHIKPMSLKCAIELGIPDIIHNHGQPITLSKLISSLPIHPSKAHCIHRL
MRILVHFGFFTTQLLLPQQQEETYSLTLASKLFLKDCPIKATPFFLVQLNPLLLKPWHFLSTWLQKGEDDDDHPSTP
FEMANGINFWDGVGNDPMVKYMFTEAMATDSYLMSKVIVEEGKEVFEGLSSLVDVGGGTGIMANAIVEAFTNIKCTV
LDLPYIVADLKGTHNLNFVEGDMFKKIPSANAVLLKWTLHDWNDEEVVVILKKCREAIWSKDKGGKVIVIDMVIDDD
EEPKSSVETQLCFDMLMMVNLTGKERNEKEWENLFLAAGFSHYKINPIVGFRSLIEVFP

SEQ ID NO: 29

>CsOMT23 [PK27154]
MEKLKSFRHLNNNIDLVLNEENSIELLGAQGHIWNQIFNFINSMSLKCAIQLGIPDIVNNYRKPMTISQLVLALPIN
QKKSPCVYRLMRILIHSGFFALQKVEGGGEGEEEEGYVITDASKLLLKDNPMSVTPFLLSMLDPVMTKPWDFLSNWF
QNDDPTPFDTANGMTFWDYGSHQPNLARFFNDAMASDARLVTSVVIEKCKWVFEGVESLVDVGGGTGTVATSIATNF
PQIQCTVLDLPHVVADLQGGNNLNFVGGDMFVEVPTAEVVLLKWILHDWNDEESVKILKKCKEAIMKSKKKGGKVII
IDMKVENEKDEDDESYETQLFFDMLMMTLLTGKERNEKEWAKLFKDAGFSDYKITPILGLRSVIEVYP

SEQ ID NO: 30

>CsOMT24 [PK29262]
MQKGQKGCQINQIPMSIERNNVEEDESFFYAVELRSSVVLPMSLYATIELGVFEILAKAGDGAKLSSSDIASHLPTE
NPDAPMMLDRILTLLASHSVLDCVVVGEGSSMRKLYSLSPVSKHFLPKEDGVSSHALMKLGLDKVSLESWFELKNAV
LEGGTSFKRAHGMNVFEYGKSDSRFGEVFNSAMYNQAKIVTKKIIESYKGFENNIKTLVDVGGGFGVTVSLIVSKYP
QIKAINFDLPHVIKNAPTYPGVEHVGGDMFEKIPNGDAIFMKWILHDWNDEDCVKILKKCYEAIPSNGKVIVVDMVV
PIMAETTHKAKSIFQLDLVMLSQNPGGKERNQHEFQAIANAAGFSTINFACSIENVKVIEFIK

The variants of the polypeptides described herein may have any degree of sequence identity to the polypeptides, provided they retain some degree of native activity, for example prenyltransferase or O-methyltransferase activity. For example, the variants typically have at least about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 99.5% sequence identity.

Likewise, the fragments of the polypeptides or variants described herein may have any length, provided they retain some degree of native activity, for example, prenyltransferase or O-methyltransferase activity. For example, the fragments may be missing about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, about 50, about 60, about 70, about 80, about 90, about 100, about 125, about 150, about 175, about 200, or about 250 amino acid residues as compared to the polypeptide in question.

The polypeptides, variants, and fragments described herein may also be fused to other polypeptides and could, therefore comprise additional amino acid residues, such as for example about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, about 50, about 60, about 70, about 80, about 90, about 100, about 125, about 150, about 175, about 200, about 250, about 300, about 400, about 500, about 600, about 700, about 800, about 900, or about 1000 or more additional amino acids.

The polypeptides described herein may comprise, consist of, or consist essentially of the sequences of SEQ ID NO:1-30, or those represented by the accessions in Table 1, and typically encode an enzyme. Typically, the enzyme prenylates a polyphenol, such as a flavonoid,

stilbenoid or bibenzyl in the presence of GPP, DMAPP or FPP. In other aspects, the enzyme adds a methyl radical to a polyphenol, such as a flavonoid, stilbenoid or bibenzyl.

The polypeptides described herein are typically expressed in a host cell or organism, such as a bacterium, an archaeon, a yeast, a protozoon, an alga, a fungus, or a plant, including single cells and cell cultures of any thereof for enzymatically acting on a molecule present in the host cell or organism or its cell culture medium. The polypeptides described herein may instead be used in a cell-free system for acting on a molecule present in the system.

In embodiments, the hosts described herein endogenously express and/or are engineered to express at least one nucleic acid coding for an aromatic prenyltransferase polypeptide that is suitable for prenylating a polyphenol, such as a flavonoid, a stilbenoid or a bibenzyl or variants thereof using geranyl diphosphate (GPP), or dimethylallyl diphosphate (DMAPP), or farnesyl diphosphate (FPP) (or variants of either thereof) to produce a prenylated polyphenol, such as a prenylated flavonoid, a stilbenoid or a bibenzyl, or variants thereof. In other embodiments, the engineered host expresses at least one nucleic acid coding for an O-methyltransferase polypeptide that is suitable for methylating a polyphenol, such as a flavonoid, a stilbenoid or a bibenzyl or variants thereof using a methyl (-CH3) donor molecule such as S-adenosyl methionine to produce a methylated polyphenol, such as a methoxy-flavonoid, methoxy-stilbenoid or methoxy-bibenzyl, or variants thereof.

Host cells described herein can be any cell capable of producing at least one protein described herein and include bacterial, fungal (including yeast), animal, algal, and plant cells. The cells may be prokaryotic or eukaryotic. Typical host cells are bacterial, yeast, algal and plant cells. In a typical embodiment, the plant cell is a seed cell, in particular, a cell in a cotyledon or endosperm of a seed. In one embodiment, the cell is a bacterial cell. An example of a bacterial cell useful as a host cell of the present invention is *Escherichia coli*, *Synechococcus* spp. (also known as *Synechocystis* spp.), for example, *Synechococcus elongatus*. Examples of algal cells useful as host cells of the present invention include, for example, *Chlorella* sp., *Chlamydomonas* sp. (for example, *Chlamydomonas reinhardtii*), *Dunaliella* sp., *Haematococcus* sp., *Schizochytrium* sp., and *Volvox* sp.

Further exemplary prokaryotic and eukaryotic host cell species are described in more detail below. However, it will be appreciated that other species not specifically described may be suitable.

For example, a recombinant host can be an Ascomycete. A recombinant host can be of a genus selected from the group consisting of *Aspergillus, Candida, Pichia*, *Saccharomyces*, and *Zygosaccharomyces*. A recombinant host can be a photosynthetic microorganism. A recombinant host can be a cyanobacterium selected from the group consisting of *Synechocystis, Synechococcus, Athrospira* (*Spirulina*), *Anabaena, Rhodopseudomonas*. For example, the organism can be of a genus selected from the group consisting of

Chlamydomonas, Dunaliella, Chlorella, Botryococcus, Nannochloropsis, Physcomitrella and *Ceratodon*.

Thus, it will be understood that the polypeptides described herein can be expressed in a variety of expression host cells e.g., bacteria, yeasts, mammalian cells, plant cells, and algal cells, or cell-free expression systems. In one embodiment, described herein are expression vectors comprising the coding DNA sequence for the polypeptides described herein for the expression and purification of the recombinant polypeptide produced from a protein expression system using host cells selected from, e.g., bacteria, mammalian, insect, yeast, or plant cells. In some embodiments, the nucleic acid can be subcloned into a recombinant expression vector that is appropriate for the expression of fusion polypeptide in bacteria, mammalian, yeast, or plant cells or a cell-free expression system such as the wheat germ cell-free expression system or a rabbit reticulocyte expression system. Examples of expression vectors and host cells are the Pichia expression vectors pPICZα, pPICZ, pFLDα and pFLD (Invitrogen) for expression in *P. pastoris* and vectors pMETα and pMET for expression in *P. methanolica*; pYES2/GS and pYD1 (Invitrogen) vectors for expression in yeast *S. cerevisiae*; pET system vectors (Novagen); pGEX (Promega) for expression in *E. coli*; pBIN and pCAMBIA vectors for expression in plant cells; the strong CMV promoter-based pcDNA3.1 (Invitrogen) and pCINEO vectors (Promega) for expression in mammalian cell lines such as CHO, COS, HEK-293, Jurkat, and MCF-7; replication incompetent adenoviral vector vectors pADENO-X™, pAd5F35, pLP-ADENO™-X-CMV (CLONTECH®), pAd/CMV/V5-DEST, pAd-DEST vector (Invitrogen) for adenovirus-mediated gene transfer and expression in mammalian cells; pLenti4/V5-DEST™, pLenti6/V5-DEST™, and pLenti6.2/V5-GW/lacZ (Invitrogen) for lentivirus-mediated gene transfer and expression in mammalian cells; BACpak6 baculovirus (Clontech) and pFASTBAC™ HT (Invitrogen) for the expression in *S. frugiperda* 9 (Sf9), Sf11, Tn-368 and BTI-TN-5B4-1 insect cell lines.

In some embodiments, cell-free systems can include in vitro enzymatic reactions performed in tubes, columns, chips or any other solid support or surface where the prenyltransferase or O-methyltransferase polypeptide described herein is present in solution or immobilized in a resin or another solid support matrix. A range of reversible physical adsorption and ionic linkages, to irreversible stable covalent bonds exist to produce immobilized enzymes. Such techniques include: (a) physical adsorption (for example with cellulose crystals, sol-gel silica, hydroxyapatite, activated carbon, TiO_2 nanoparticles, polyethersulphone membrane, or Ni-/Co-/Zn-nitrilotriacetic acid-agarose); (b) entrapment (for example with agarose or chitosan); and (c) covalent attachment/cross-linking (for example using polyaniline, polystyrene, polyvinyl alcohol, polypropylene, silica gel, bentonite, magnetic nanoparticles, multi-walled carbon nanotubes, reduced graphene oxide, cellulose-poly(acrylic acid) fibers, graphene oxide-Fe3O4, polyacrylonitrile-multi-walled carbon nanotubes, silica-graphene oxide particles).

Thus, in embodiments, the host cells or cell-free systems described herein are suitable for producing a substantially pure prenylated and/or methylated flavonoid, a prenylated and/or methylated stilbenoid or a prenylated and/or methylated bibenzyl, in the presence of at least an aromatic prenyltransferase or an O-methyltransferase polypeptide, such as those represented by SEQ ID:1-30 or those represented by the accessions in Table 1 (or fragments or variants thereof). In embodiments, the host cells or cell-free systems can include a previous modification step such as a nucleic acid coding for an O-methyltransferase polypeptide that is suitable for methylating luteolin to produce chrysoeriol which can then be prenylated by a polypeptide described herein into a substantially pure Cannflavin, such as Cannflavin A and/or B. It will be understood that an O-methyltransferase may be optional when a Cannflavin precursor such as chrysoeriol is produced and/or available to the host cell, the host cell or cell-free system may also comprise a polypeptide for an O-methyltransferase. Likewise, genes encoding one or more enzymes involved in the upstream production of luteolin, apigenin, or naringenin, such as F3'H, FNS, CHI, CHS, 4CL, C4H, or PAL, may also be expressed in the host cells or present in an in vitro system and/or sources of these precursor molecules may be provided exogenously or endogenously. In embodiments, production of pure cannflavin, such as cannflavin A and/or B can also include combining the methylation of luteolin with a subsequent prenylation of chrysoeriol with GPP or DMAPP achieved by means of an organic chemistry synthesis method.

Also described herein are compositions comprising a prenylated and/or methylated flavonoid, a prenylated and/or methylated stilbenoid, or a prenylated and/or methylated bibenzyl obtainable or obtained by one of the methods as disclosed above, and to the use of said composition as a medicinal agent, such as an anti-inflammatory or anti-cancer agent, for pharmacological purposes and/or cosmetic purposes.

Provided herein are compositions comprising substantially pure prenylated and/or methylated flavonoid (for example cannflavin A or cannflavin B), a prenylated and/or methylated stilbenoid, or a prenylated and/or methylated bibenzyl, which are, for example, at least about 95%, about 96%, about 97%, about 98%, about 99%, about 99.5%, or about 99.9% pure.

The compositions comprising a modified flavonoid, stilbenoid or bibenzyl described herein may be formulated for use by a subject, such as a mammal, including a human. Such compositions may comprise about 0.00001% to about 99% by weight of the active and any range there-in-between. For example, typical doses may comprise from about 0.1 µg to about 100 µg of the molecules described herein per 300 mg dose, such as about 0.5 µg, about 1 µg, about 2 µg, about 3 µg, about 4 µg, about 5 µg, about 6 µg, about 7 µg, about 8 µg, about 9 µg, about 10 µg, about 25 µg, about 50 µg, or about 75 µg per 300 mg dose, such as from about 0.1 µg to about 10 µg, or from about 1 µg to about 5 µg, or from about 1 µg to about 2 µg per 300 mg dose (and all related increments and percentages by weight).

The prenylated and/or methylated molecules described herein may be used in any suitable amount, but are typically provided in doses comprising from about 1 to about 10000

ng/kg, such as from about 1 to about 1000, about 1 to about 500, about 10 to about 250, or about 50 to about 100 ng/kg, such as about 1, about 10, about 25, about 50, about 75, about 100, about 150, about 200, about 250, about 300, or about 500 ng/kg. Similar amounts, higher amounts, or lower amounts could be used for administration.

The prenylated and/or methylated molecules described herein may be administered over a period of hours, days, weeks, or months, depending on several factors, including the severity and type of the inflammation or other condition being treated, whether a recurrence is considered likely, or to prevent the inflammation or other condition, etc. The administration may be constant, e.g., constant infusion over a period of hours, days, weeks, months, etc. Alternatively, the administration may be intermittent, e.g., the molecules may be administered once a day over a period of days, once an hour over a period of hours, or any other such schedule as deemed suitable.

The compositions described herein can be prepared by per se known methods for the preparation of pharmaceutically or cosmetically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in "Handbook of Pharmaceutical Additives" (compiled by Michael and Irene Ash, Gower Publishing Limited, Aldershot, England (1995)). On this basis, the compositions include, albeit not exclusively, solutions of the substances in association with one or more pharmaceutically acceptable vehicles or diluents, and may be contained in buffered solutions with a suitable pH and/or be iso-osmotic with physiological fluids. In this regard, reference can be made to U.S. Patent No. 5,843,456 (the entirety of which is incorporated herein by reference).

Pharmaceutically acceptable carriers are well known to those skilled in the art and include, for example, sterile saline, lactose, sucrose, calcium phosphate, gelatin, dextrin, agar, pectin, peanut oil, olive oil, sesame oil, cannabis oil, and water. Furthermore, the composition may comprise one or more stabilizers such as, for example, carbohydrates including sorbitol, mannitol, starch, sucrose, dextrin and glucose, proteins such as albumin or casein, and buffers like alkaline phosphates.

The prenylated and/or methylated molecules described herein can, in embodiments, be administered for example, by parenteral, intravenous, subcutaneous, intradermal, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, intrarectal, intravaginal, aerosol, oral, topical, or transdermal administration. Typically, the compositions of the invention are administered orally or topically directly to the site of inflammation or in a cosmetic oil, lotion, cream, or gel to a desired body location, such as the face.

It is understood by one of skill in the art that the produced molecules described herein can be used in conjunction with known therapies for prevention and/or treatment of

inflammation in subjects and/or with compositions for preventing the signs of aging or other cosmetic compositions. Similarly, the produced modified molecules described herein can be combined with one or more other pharmaceutical or natural health products, such as cannabinoids, terpenes, or other natural or synthetic compounds. The produced molecules described herein may, in embodiments, be administered in combination, concurrently or sequentially, with conventional treatments for inflammation, including non-steroidal anti-inflammatory drugs, for example. The prenylated and/or methylated molecules described herein may be formulated together with such conventional treatments when appropriate. Other uses of these prenylated and/or methylated molecules can be found due to their anticipated antiatherosclerotic, anticancer, antiviral, antimicrobial or hepatoprotective activities.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

Methods

Chemicals and reagents. Authentic flavonoid standards for chrysoeriol, apigenin, luteolin, kaempherol and quercetin, dihydroresveratrol, tristin, gigantol, batatasin III, resveratrol, pinosylvin, caffeic acid and dihydrocaffeic acid can be purchased from specialized chemical companies such as Toronto Research Chemicals, Indofine Chemical Company or Sigma-Aldrich. The trans-prenyl diphosphates: dimethyllalyl diphosphate (DMAPP), isopentenyl diphosphate (IPP), and geranyl diphosphate (GPP) can be obtained from Echelon Biosciences. Radiolabeled S-[Methyl-14C] adenosyl-L-methionine (58.0 mCi mmol−1) can be obtained from PerkinElmer.

Expression of recombinant prenyltransferases in E. coli. In typical aspects, open reading frames encoding soluble aromatic prenyltransferases from bacteria or fungi, for example NphB, HypSC, CloQ, NovQ, Fur7, or PpzP are synthesized commercially. This cDNA is amplified by PCR and then inserted into an expression vector system (for example, Novagen's pET28) which introduces an N-terminal 6x His tag to the coding sequence. The construct is then introduced into a bacterial host (for example *E. coli* BL21-CodonPlus (DE3)-RIPL) cell. Bacterial cells expressing recombinant NphB are cultured for several hours in the presence of IPTG to induce recombinant protein expression. The bacterial cells are collected by centrifugation, re-suspended, and then disrupted by sonication. Crude protein extracts are applied and purified with a Ni^{2+} affinity matrix (for example a HisTrap HP column). Afterwards, the enzyme is eluted

with high concentrations (250 to 400 uM) of imidazole and then equilibrated in a suitable buffer. The purified NphB protein can be frozen prior to use.

Enzymatic prenylation reactions. Typically, enzyme reactions can be carried out in assay tubes by mixing purified prenyltransferase enzyme, the flavonoid, stilbenoid or bibenzyl substrate (for example, chrysoeriol, kaempferol, or quercetin), a prenyl donor group (GPP, or DMAPP), $MgCl_2$, and Tris-HCl buffer and incubated in a 30°C water-bath overnight. Reactions can be stopped by adding 20% formic acid and then extracted with ethyl acetate. For analysis, usually, reactions are dried under N_2 and then resuspended in methanol before separating them on a HPLC with a reverse-phase column (for example Spherisorb ODS2) and eluted with a linear gradient of methanol:water.

Recombinant protein expression of CsOMTs in E. coli. Open reading frames corresponding to O-methyltransferases from *C. sativa* are synthesized by commercially. These cDNAs are amplified by PCR using a high fidelity DNA polymerase and cloned and expressed following the method described above for prenyltransferase genes.

O-methyltransferase enzyme assays. Assays for determining O-methyltransferase enzyme activity are performed using purified recombinant protein incubated in a final reaction volume of 100 µL of 1mM substrate and radiolabeled adenosyl-L-methionine in 50 mM Tris-HCl, pH 7.5, 5 mM MgCl2, and 10% (v/v) glycerol for 30 min at 37°C. The enzymatic products are extracted with four volumes of ethyl acetate and quantified using a scintillation counter. For reaction product identification, assays are scaled up to a final volume of 500 µL containing 50 to 200 µg of recombinant protein, 2 mM of substrate and 2 mM S-adenosyl-L-methionine in the same buffer as above. Enzymatic products are extracted as above, evaporated to dryness under N_2 gas, and resuspended in 100 µL of methanol. Samples are analyzed by HPLC with a Spherisorb ODS2 reverse-phase column and eluted over a 20 min gradient from 45% to 95% methanol with 0.1% formic acid (v/v) followed by 100% methanol for 10 min. The eluted products are detected by absorption at the 210-350 nm range and quantified relative to authentic standards. Mass spectral analysis of the enzymatic products is performed as described below.

Mass spectrometry analysis of enzymatic reaction products. The prenylated or methylated products can be purified by HPLC as described above. Generally, samples are evaporated under nitrogen and then re-suspended in methanol prior to liquid chromatography mass spectrometry (LC-MS) analysis (for example HPLC liquid chromatography interfaced with a Q-TOF mass spectrometer). During such analysis, the mass-to-charge ratio is typically scanned across the m/z range of 100-3000 m/z in an extended dynamic range positive-ion MS mode. Chromatograms can be analyzed by using a software that compares MS patterns from standard libraries or known standards used in the laboratory. Fragmentation patterns of the various parent (molecular) ions obtained using collision energies of 5 to 20 V from the

recovered peak products (modified compounds) are usually also compared with fragmentation patterns of standard molecules.

In typical aspects, nuclear magnetic resonance (NMR) spectroscopy, is used as a preeminent technique for determining the structure of the prenylated or methylated compounds that can be obtained (for example for determining the position of the GPP, DMAPP, or methyl group in one of the flavonoid, stilbenoid or bibenzyl rings). After the enzymatic reaction products from the enzymatic assays are resolved by HPLC, the compounds are eluted and subsequently collected. Usually, approximately 0.5 mg of each compound is evaporated to dryness under N_2 gas, resuspended in acetone-d6, and analyzed using ^{1}H and ^{13}C NMR. NMR spectra are collected on a spectrometer (for example a Bruker AVANCE III 600 MHz equipped with a 5 mm TCI cryoprobe).

In an exemplary extraction step, this or a similar in vitro cell-free system described herein can be suitable for producing a substantially pure prenylated flavonoid, a prenylated stilbenoid or a prenylated bibenzyl in the presence of at least an aromatic prenyltransferase described herein. The prenylated product can undergo further purification if necessary depending on the in vitro system selected before being destined to be used in preparations.

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Examples

Example 1

This example describes a general method for using the prenyltransferase NphB from Streptomyces sp. strain CL190 to prenylate chrysoeriol using GPP and to produce cannflavin A. A synthetic cDNA sequence of NphB was sub-cloned in the pET28a vector system (Novagen) which introduced an N-terminal 6x His tag to the coding sequence and then the construct was introduced into E. coli BL21-CodonPlus (DE3)-RIPL cells. Bacterial cells expressing recombinant NphB were cultured in 1 L of LB media at 37°C to an OD_{600} of 0.6. Isopropyl-β-D-thiogalactoside (IPTG) was then added to a final concentration of 1 mM and the cells were incubated at 16°C for an additional 18 h to induce recombinant protein expression. The bacterial cells were collected by centrifugation, re-suspended in 20 mM Tris-HCl, pH 8.0, 500 mM KCl (Buffer A), and then disrupted by sonication. Crude protein extracts were centrifuged at 12,000 x g for 10 min at 4°C to remove unbroken cells and debris and the supernatant was applied to a HisTrap HP column. After washing the column with the same buffer, the NphB enzyme bound to

the Ni^{2+} affinity matrix was eluted with one column volume of Buffer A containing 400 mM imidazole, and then immediately desalted on PD-10 columns (GE Healthcare) equilibrated with 50 mM Tris-HCl, pH 7.5, 5 mM MgCl2, and 10% (v/v) glycerol. The purified NphB protein was quantified and stored at -80°C prior to use.

Enzymatic reactions were carried out as follows: purified NphB (200 µg), chrysoeriol (400 µM), GPP (800 µM), $MgCl_2$ (10 mM), Tris-HCl pH 9.0 (100 mM) were mixed in a total volume of 500 µL and were incubated in a 30°C water-bath overnight. Reactions were terminated with 20 µL of 20% formic acid and extracted twice with ethyl acetate, dried under N_2 gas, and then resuspended in 200 µL of methanol. Samples were run on an HPLC with a Spherisorb ODS2 reverse-phase column and eluted with a 20 min linear gradient from 45% to 95% methanol in water containing 0.1% formic acid (v/v). The HPLC chromatogram of the product showed a first major peak that eluted at the same time as the cannflavin A standard (**FIGURE 1**) which was collected and processed for mass spectrometry analysis.

The prenylated products that were produced by NphB in vitro, were purified by HPLC as described above. Samples were evaporated under nitrogen and then re-suspended in methanol prior to liquid chromatography-mass spectrometry (LC-MS) analysis performed on an Agilent 1200 HPLC interfaced with an Agilent UHD 6530 Q-TOF mass spectrometer. A C18 cartridge column (Agilent Rapid Resolution 2.1 x 30 mm, 3.5µm) was used at 30°C with 1:1 water and acetonitrile as solvents, both with 0.1 % formic acid. The flow rate was maintained at 0.4 mL/min. The mass spectrometer electrospray capillary voltage was maintained at 4.0 kV and the drying gas temperature at 250°C with a flow rate of 8 L/min. Nebulizer pressure was 30 psi and the fragmentor was set to 160 V. Nitrogen was used as both nebulizing, drying gas, and collision-induced dissociation gas. The instrument was externally calibrated with the ESI TuneMix (Agilent). The mass-to-charge ratio was scanned across the m/z range of 100-3000 m/z in 4 GHz extended dynamic range positive-ion MS mode. Chromatograms were analyzed within Agilent Qualitative Analysis software B 08.0. Fragmentation patterns of the various parent (molecular) ions were obtained using collision energies of 5, 10 and 20 V, with 20 V being optimal. Those fragmentation patterns obtained from the recovered peak products (geranylation of chrysoeriol) were compared with those of Cannflavin A. Q-TOF mass spectra of a cannflavin A standard shows that the mass spectra of the prenylated product (6-geranyl chrysoeriol) is consistent with the pattern of a cannflavin A standard ([M+H]+ 437) (**FIGURE 2A** and **2B**, upper panels) and mass spectral fragmentation pattern of the enzymatic product from the assay (bottom panel) also resembles that of the cannflavin A standard in (A, bottom panel), indicating that prenylation of chrysoeriol with GPP by NphB produces cannflavin A (**FIGURE 2A** and **2B**, bottom panels).

Example 2

This example describes a general method for using O-methyltransferases from *Cannabis sativa* and S-adenosyl-methionine to produce methylated flavonoids, specifically a flavone and a flavonol. Synthetic cDNA sequences of CsOMT6 and csOMT21 were sub-cloned in the pET28a vector system (Novagen) to produce a 6x His-protein fusion and then the construct was introduced into E. coli BL21-CodonPlus (DE3)-RIPL cells. Bacterial cells expressing recombinant proteins were cultured in 1 L of LB media at 37°C to an OD_{600} of 0.6. Isopropyl-β-D-thiogalactoside (IPTG) was then added to a final concentration of 1 mM and the cells were incubated at 16°C for an additional 18 h to induce recombinant protein expression. The bacterial cells were collected by centrifugation, re-suspended in 20 mM Tris-HCl, pH 8.0, 500 mM KCl (Buffer A), and then disrupted by sonication. Crude protein extracts were centrifuged at 12,000 x g for 10 min at 4°C to remove unbroken cells and debris and the supernatant was applied to a HisTrap HP column. After washing the column with the same buffer, the enzymes bound to the Ni^{2+} affinity matrix were eluted with one column volume of Buffer A containing 400 mM imidazole, and then immediately desalted on PD-10 columns (GE Healthcare) equilibrated with 50 mM Tris-HCl, pH 7.5, 5 mM MgCl2, and 10% (v/v) glycerol. The purified proteins were quantified and stored at -80°C prior to use.

Enzymatic reactions for determining O-methyltransferase activity of CsOMT6 and CsOMT21 were carried out using ~2 µg of purified recombinant enzyme incubated in a final reaction volume of 100 µL containing 1mM substrate (luteolin, quercetin, kaempferol, apigenin and chrysoeriol, **FIGURE 3A**) and 6.9 µM S-[Methyl-^{14}C] adenosyl-L-methionine in 50 mM Tris-HCl, pH 7.5, 5 mM MgCl2, and 10% (v/v) glycerol for 30 min at 37°C. The enzymatic products were extracted with four volumes of ethyl acetate and quantified using a scintillation counter (Model LS6500, Beckman). For the identification of reaction products, the assays were scaled up to a final volume of 500 µL using ~50 µg of recombinant protein, 2 mM substrate and 2 mM S-adenosyl-L-methionine in 50 mM Tris-HCl, pH 7.5, 5 mM MgCl2, and 10% (v/v) glycerol for 60 min at 37°C. The enzymatic products were extracted as above, evaporated to dryness under N_2 gas, and resuspended in 100 µL of methanol. Samples were analyzed by HPLC with a Spherisorb ODS2 reverse-phase column (250 mm x 4.6 mm, 5 µm) and eluted over a 20 min gradient from 45% to 95% methanol with 0.1% formic acid (v/v) followed by 100% methanol for 10 min. The eluted products were detected by absorption at the 210-350 nm range and quantified relative to authentic standards. Next, mass spectral analyses of the enzymatic products were performed as described in Example 1. With CsOMT6, preferred methylation of quercetin was detected (**FIGURE 3B**), whereas recombinant CsOMT21 was able to methylate luteolin and quercetin but with less efficiency (**FIGURE 3C**), indicating that the selected OMTs from cannabis present preferred substrate specificity for flavonoid compounds.

Example 3

This example describes a method for using O-methyltransferases from *Cannabis sativa* to produce methylated bibenzyls using S-adenosyl-methionine. Similar to what was described in the previous example, cDNA sequences of CsOMT1, CsOMT3, CsOMT5 and CsOMT13 were sub-cloned in the same expression vector and recombinant proteins were produced in E. coli and purified using the same method as above.

In this example, the activity of these four O-methyltransferases was tested with the bibenzyl compounds dihydroresveratrol, tristin, gigantol and batatasin III as potential substrates (**FIGURE 4A**). The higher O-methyltransferase activity was observed with CsOMT1 when dihydroresveratrol (DHR) was used as substrate. CsOMT1 also displayed activity with batatasin III, although this was about 25% of that with DHR (**FIGURE 4B**). Similar levels of substrate conversion to a methylated form were observed with CsOMT3 with DHR as substrate and CsOMT13 with DHR and gigantol product (**FIGURE 4B**). CsOMT5 did not show any preference with the selected substrates nor produced any specific product. To evaluate whether CsOMT1 can methylate stilbenoids, the oxidized form of DHR, resveratrol, along with pinosylvin and similar phenolic molecules such as caffeic acid and its reduced form, dihydrocaffeic acid (**FIGURE 5A**) were used in similar enzymatic assays. Only residual activity (lower than 5% of that with DHR) was observed with the two stilbenoids tested (**FIGURE 5B**), indicating that CsOMT1 has specific preference to bibenzyl compounds, specifically towards DHR. To further validate and characterize CsOMT1 methyltransferase activity, the identity of the methylated product of the reaction was structurally determined by NMR as pinobistilbene, which indicated that the modification on DHR occurred in position 3 of the ring that has the two hydroxyl groups (ring A, **FIGURE 6A**). **FIGURE 6B** shows a representative chromatogram of the reaction products resolved by HPLC which illustrates the separation of the substrate (DHR) from its methylated form. Enzyme kinetics assays were also performed to characterize CsOMT1 activity, which also confirmed the efficient O-methyltransferase activity of the recombinant enzyme with dihydroresveratrol as substrate (**FIGURE 6C**).

The above disclosure generally describes the present invention. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

All publications, patents and patent applications cited above are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

Although preferred embodiments of the invention have been described herein in detail, it will be understood by those skilled in the art that variations may be made thereto without departing from the spirit of the invention or the scope of the appended claims.

Claims

1. A polypeptide encoding a prenyltransferase for prenylating a polyphenol.

2. The polypeptide of claim 1, wherein the prenyltransferase is a microbial prenyltransferase.

3. The polypeptide of claim or 2, comprising or consisting of a polypeptide having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the sequence of any one or more of SEQ ID NO: 1-6 and/or a polypeptide listed in Table 1, or a fragment of any thereof.

4. The polypeptide of claim 3, comprising or consisting of the sequence of any one or more of SEQ ID NO: 1-6 and/or a polypeptide listed in Table 1.

5. The polypeptide of claim 4, comprising or consisting of the sequence of any one or more of SEQ ID NO: 1-6.

6. The polypeptide of any one of claims 1 to 5, wherein the polypeptide prenylates the polyphenol using a prenyl donor.

7. The polypeptide of claim 6, wherein the prenyl donor is IPP, FPP, GPP, and/or DMAPP, or a variant or derivative thereof.

8. The polypeptide of any one of claims 1 to 7, wherein the polyphenol is a flavonoid, stilbenoid, and/or bibenzyl, or a derivative thereof.

9. The polypeptide of claim 8, wherein the flavonoid is a flavone, such as apigenin, luteolin, chrysoeriol, chrysin, acacetin, baicalein, baicalin, vitexin, wogonin, orientin, oroxylin A. rutin, or tangeritin; a flavonol such as quercetin, kaempferol, galangin, myricetin, tamarixetin, fisetin, or casticin; a flavanone such as naringenin, hesperetin, pinocembrin, hesperidin, or eriodictyol; a flavanonol such as taxifolin; a flavanol such as catechin, or epicatechin; an isoflavone such as genistein, or daidzein; an anthocyanin such as cyanidin, chrysanthemin, pelargonidin, delphinidin, or malvidin; or any combination thereof.

10. The polypeptide of claim 8, wherein the stilbenoid is resveratrol, piceatannol, pterostilbene, pinosylvin, gnetol, oxyresveratrol, pinostilbene, or any combination thereof.

11. The polypeptide of claim 8, wherein the bibenzyl is a dihydrostilbenoid such as dihydroresveratrol, combretastatin, dihydropiceatannol, dihydrognetol, dihydropinosylvin, gigantol, pinobistilbene, batatasin III, crepidatin, moscatilin, crysotoxine, chrysotobibenzyl, amoenylin, tristin, cumulating, or any combination thereof.

12. The polypeptide of claim 8, wherein the prenyltransferase prenylates the flavonoid to produce 8-prenyl kaempferol, isocannflavin B, cannflavin C, 6-prenylnaringenin, 6-prenylapigenin, neougonin A, neougonin B, and/or kuraridin.

13 The polypeptide of claim 8, wherein the prenyltransferase prenylates the stilbenoid to produce arachidins, isorhapontigenin, rhapontigenin, pawhuskin A, aglaiabbrevin E, amorphastilbol, or longistylins.

14 The polypeptide of claim 8, wherein the prenyltransferase prenylates the bibenzyl to produce canniprene, cannabistilbene, dihydrolongistylins, amorfrutin 1/A, or amorfrutin B.

15. The polypeptide of 9, wherein the prenyltransferase prenylates chrysoeriol using GPP to produce cannflavin A.

16. The polypeptide of claim 9, wherein the prenyltransferase prenylates chrysoeriol using DMAPP to produce cannflavin B.

17. A polypeptide encoding an O-methyltransferase for methylating a polyphenol.

18. The polypeptide of claim 17, wherein the O-methyltransferase is a plant O-methyltransferase.

19. The polypeptide of claim 18, wherein the O-methyltransferase is a *Cannabis sativa* O-methyltransferase.

20. The polypeptide of any one of claims 17 to 19, comprising or consisting of a polypeptide having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the sequence of any one or more of SEQ ID NO: 7-30, or a fragment of any thereof.

21. The polypeptide of claim 20, comprising or consisting of the sequence of any one or more of SEQ ID NO: 7-30.

22. The polypeptide of any one of claims 17 to 21, wherein the polypeptide methylates the polyphenol using a methyl donor.

23. The polypeptide of claim 22, wherein the methyl donor is S-adenosyl methionin, or a variant or derivative thereof.

24. The polypeptide of any one of claims 17 to 23, wherein the polyphenol is a flavonoid, stilbenoid, and/or bibenzyl, or a derivative thereof.

25. The polypeptide of claim 24, wherein the flavonoid is a flavone, such as apigenin, luteolin, chrysoeriol, chrysin, acacetin, baicalein, baicalin, vitexin, wogonin, orientin, oroxylin A. rutin, or tangeritin; a flavonol such as quercetin, kaempferol, galangin, myricetin, tamarixetin, fisetin, or casticin; a flavanone such as naringenin, hesperetin, pinocembrin, hesperidin, or eriodictyol; a flavanonol such as taxifolin; a flavanol such as catechin, or epicatechin; an isoflavone such as genistein, or daidzein; an anthocyanin such as cyanidin, chrysanthemin, pelargonidin, delphinidin, or malvidin; or any combination thereof.

26. The polypeptide of claim 24, wherein the stilbenoid is resveratrol, piceatannol, pterostilbene, pinosylvin, gnetol, oxyresveratrol, or any combination thereof.

27. The polypeptide of claim 24, wherein the bibenzyl is a dihydrostilbenoid such as dihydroresveratrol, combretastatin, dihydropiceatannol, dihydrognetol, dihydropinosylvin, gigantol, batatasin III, crepidatin, moscatilin, crysotoxine, chrysotobibenzyl, amoenylin, tristin, cumulating, or any combination thereof.

28. The polypeptide of claim 24, wherein the O-methyltransferase methylates a flavonoid to produce chrysoeriol, acacetin, tamarixetin, or methylquercetin.

29. In an aspect, the O-methyltransferase methylates a stilbenoid to produce pinostilbene, isorhapontigenin, rhapontigenin, or any combination thereof.

30. The polypeptide of claim 24, wherein the O-methyltransferase methylates a bibenzyl to produce gigantol, tristin, or pinobistilbene.

31. The polypeptide of any one of claims 1 to 30, wherein the polypeptide, variant, or fragment comprises up to about 100, about 150, about 200, about 250, about 300, about 350, about 360, about 370, about 380, about 390, about 400, about 410, about 420, about 430, about 440, about 450, or about 500 amino acids

32. The polypeptide of any one of claims 1 to 31, wherein the polypeptide is synthetic.

33. The polypeptide of any one of claims 1 to 32, wherein the polypeptide is recombinant.

34. A nucleic acid encoding the polypeptide of any one of claims 1 to 33.

35. The nucleic acid of claim 34, wherein the nucleic acid is cDNA.

36. A vector comprising the nucleic acid of claim 34 or 35.

37. A host cell comprising the vector of claim 36.

38. A host cell expressing the polypeptide of any one of claims 1 to 33.

39. The host cell of claim 37 or 38, wherein the host cell is a bacterial cell (e.g., E. coli or Agrobacterium tumefaciens), a yeast cell (e.g., S. cerevisiae), an algal cell, or a plant cell (e.g., Nicotiana spp.).

40. The host cell of any one of claims 37 to 39, in combination with the polyphenol.

41. The host cell of claim 40, wherein the polyphenol is provided in the host cell culture medium.

42. The host cell of claim 40 or 41, wherein the polyphenol is expressed by the host cell.

43. The host cell of any one of claims 37 to 42, in combination with a prenyl donor and/or a methyl donor.

44. The host cell of claim 43, wherein the prenyl donor and/or methyl donor is provided in the host cell culture medium.

45. The host cell of claim 43 or 44, wherein the prenyl donor and/or methyl donor is expressed by the host cell.

46. An expression system comprising the polypeptide of any one of claims 1 to 33; the nucleic acid of claim 34 or 35, the vector of claim 36, or the host cell of any one of claims 37 to 45.

47. The expression system of claim 46, further comprising the polyphenol and a prenyl donor and/or methyl donor.

48. A system for prenylating and/or methylating a polyphenol the system comprising the polypeptide of any one of claims 1 to 33.

49. The system of any one of claims 46 to 48, wherein the polypeptide is in a batch solution.

50. The system of any one of claims 46 to 49, wherein the polypeptide is immobilized in a support matrix.

51. The system of any one of claims 46 to 50, wherein the polypeptide is in a cell.

52. The system of any one of claims 46 to 50, wherein the system is cell-free.

53. A method for prenylating and/or methylating a polyphenol, wherein the method comprises contacting the polyphenol with the polypeptide of any one of claims 1 to 33.

54. The method of claim 53, carried out in the system of any one of claims 46 to 52.

55. The method of claim 53 or 54, wherein the method is a recombinant method comprising expressing the polypeptide of any one of claims 1 to 33 in a cell in the presence of the polyphenol and a prenyl donor and/or methyl donor.

56. The method of any one of claims 53 to 55, in combination with a synthetic chemical catalysis method.

57. The method of any one of claims 53 to 56, comprising a single synthesis step.

58. The method of any one of claims 53 to 57, wherein the method is carried out in combination with an enzymatic reaction.

59. The method of any one of claims 53 to 58, comprising a combined enzymatic O-methylation and prenylation step.

60. A method of producing cannflavin A, cannflavin B, isocannflavin B, the method comprising carrying out a combined enzymatic O-methylation and prenylation of a flavonoid.

61. A method of producing a longistylin, the method comprising carrying out a combined enzymatic O-methylation and prenylation of a stilbenoid.

62. A method of producing canniprene, cannabistilbene, dihydrolongistylin, amorfrutin 1/A, or amorfrutin B, the method comprising carrying out a combined enzymatic O-methylation and prenylation of a bibenzyl.

63. A synthetic chemical catalysis method of producing cannflavin A and/or cannflavin B, the method comprising using GPP and DMAPP in a single synthesis step from chrysoeriol or in combination with an enzymatic reaction such as the O-methylation of luteolin.

64. A prenylated and/or methylated polyphenol produced by the method of any one of claims 53 to 63.

65. The polyphenol of claim 64, wherein the polyphenol is substantially pure, for example, at least about 95%, about 96%, about 97%, about 98%, about 99%, about 99.5%, or about 99.9% pure.

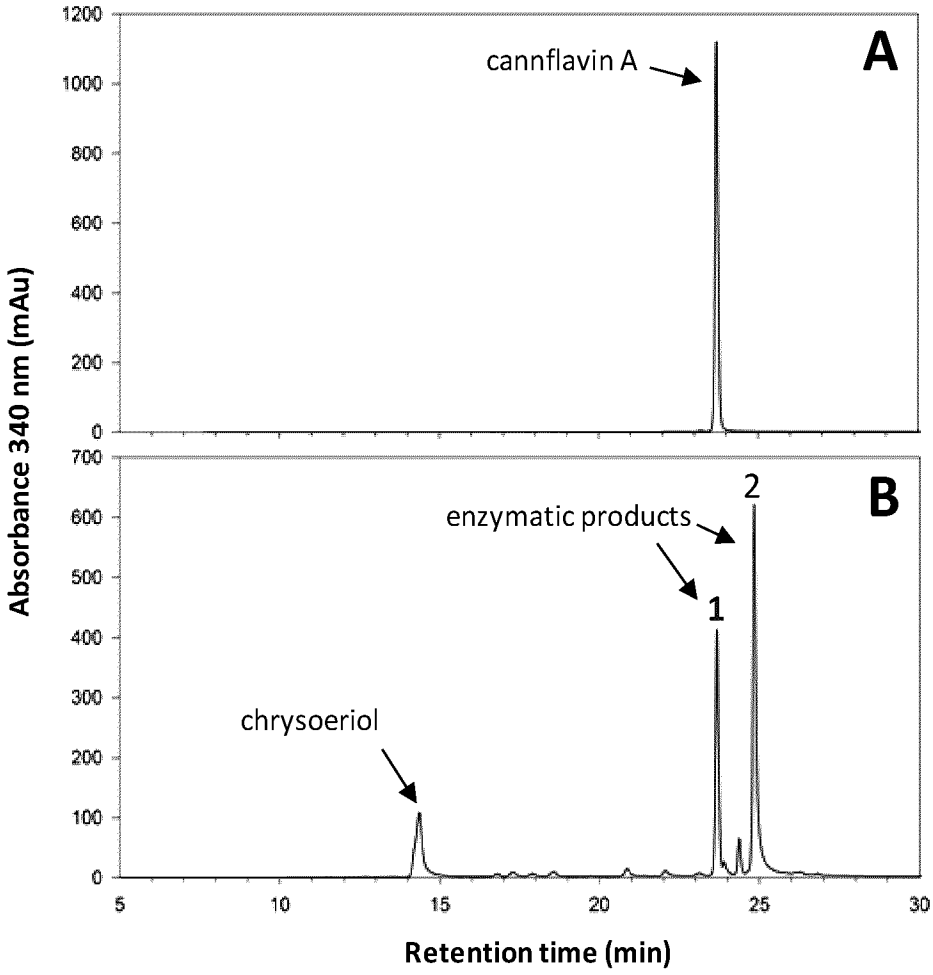
66. The polyphenol of claim 64 or 65, wherein the polyphenol is cannflavin A and/or cannflavin B.

67. A cosmetic composition comprising the polyphenol of any one of claims 64 to 66 and at least one cosmetically acceptable carrier.

68. A pharmaceutical composition comprising the polyphenol of any one of claims 64 to 66 and at least one pharmaceutically acceptable carrier.

69. A natural health product comprising the polyphenol of any one of claims 64 to 66, such as a supplement, beverage, or food.

70. Use of the polyphenol of any one of claims 64 to 66 in a cosmetic, pharmaceutical, or natural health product.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Absorbance 340 nm (mAu)
Retention time (min)
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Figure 1

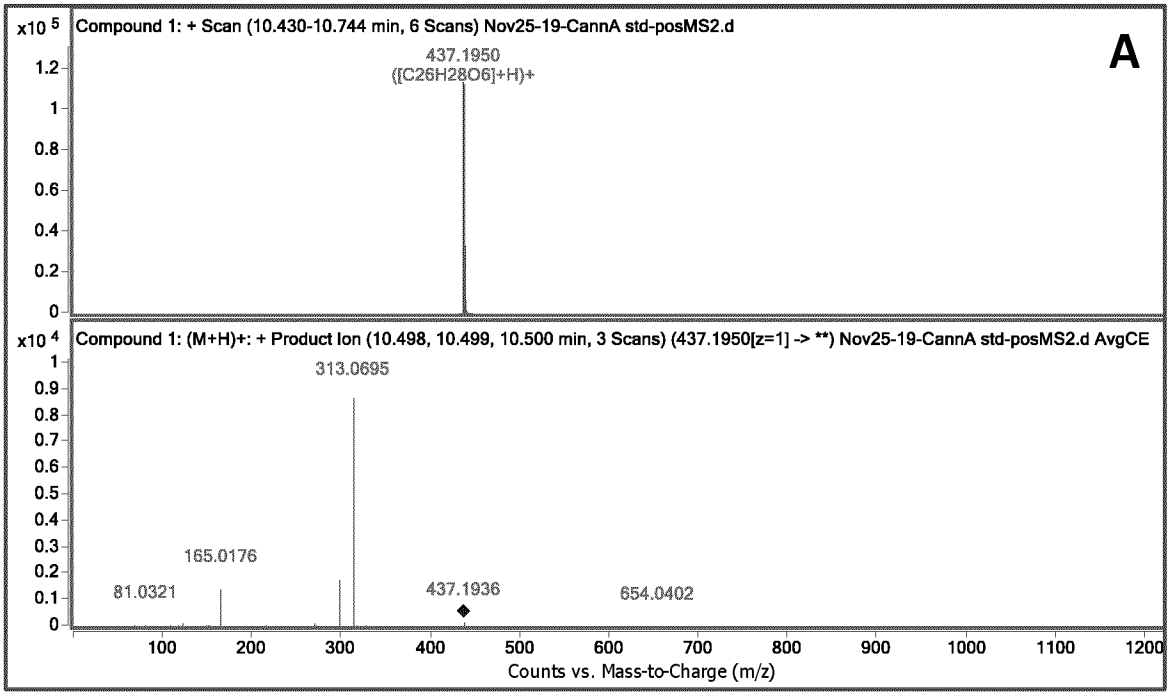
A
Compound 1: + Scan (10.430-10.744 min, 6 Scans) Nov25-19-CannA std-posMS2.d
437.1950
([C26H28O6]+H)+
Compound 1: (M+H)+: + Product Ion (10.498, 10.499, 10.500 min, 3 Scans) (437.1950[z=1] -> **) Nov25-19-CannA std-posMS2.d AvgCE
313.0695
165.0176
81.0321
437.1936
654.0402
Counts vs. Mass-to-Charge (m/z)
B
Compound 1: + Scan (10.357-10.908 min, 13 Scans) Dec09-19-CP-1.d
336.3265
437.1960
(M+H)+
124.0872
229.2161
502.3155
707.6648
938.2933
Compound 1: (M+H)+: + Product Ion (10.392, 10.401, 10.410, 10.429 ... min, 6 Scans) (437.1963[z=1] -> **) Dec09-19-CP-1.d AvgCE
313.0704
165.0181
69.0698
437.1945
1001.2147
Counts vs. Mass-to-Charge (m/z)

Figure 2

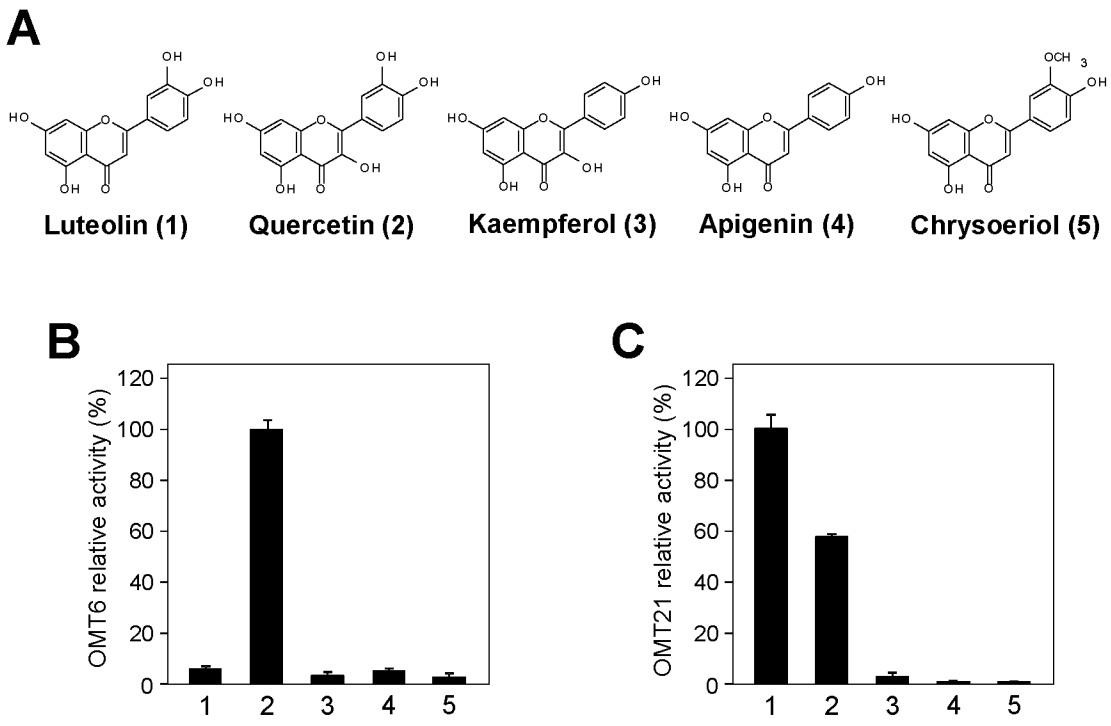
A
Luteolin (1)
Quercetin (2)
Kaempferol (3)
Apigenin (4)
Chrysoeriol (5)
B
OMT6 relative activity (%)
C
OMT21 relative activity (%)
0
20
40
60
80
100
120
1
2
3
4
5

Figure 3

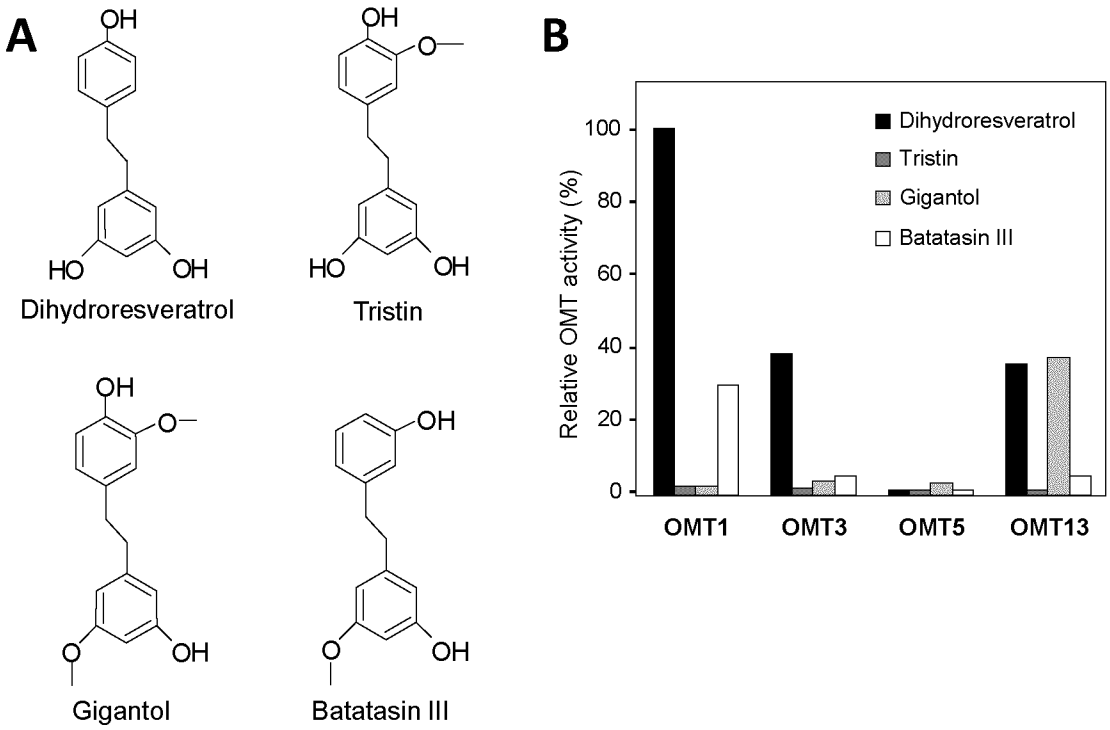
A
OH
HO
OH
Dihydroresveratrol
OH
O
HO
OH
Tristin
OH
O
O
OH
Gigantol
OH
O
OH
Batatasin III
B
Relative OMT activity (%)
100
80
60
40
20
0
Dihydroresveratrol
Tristin
Gigantol
Batatasin III
OMT1
OMT3
OMT5
OMT13

Figure 4

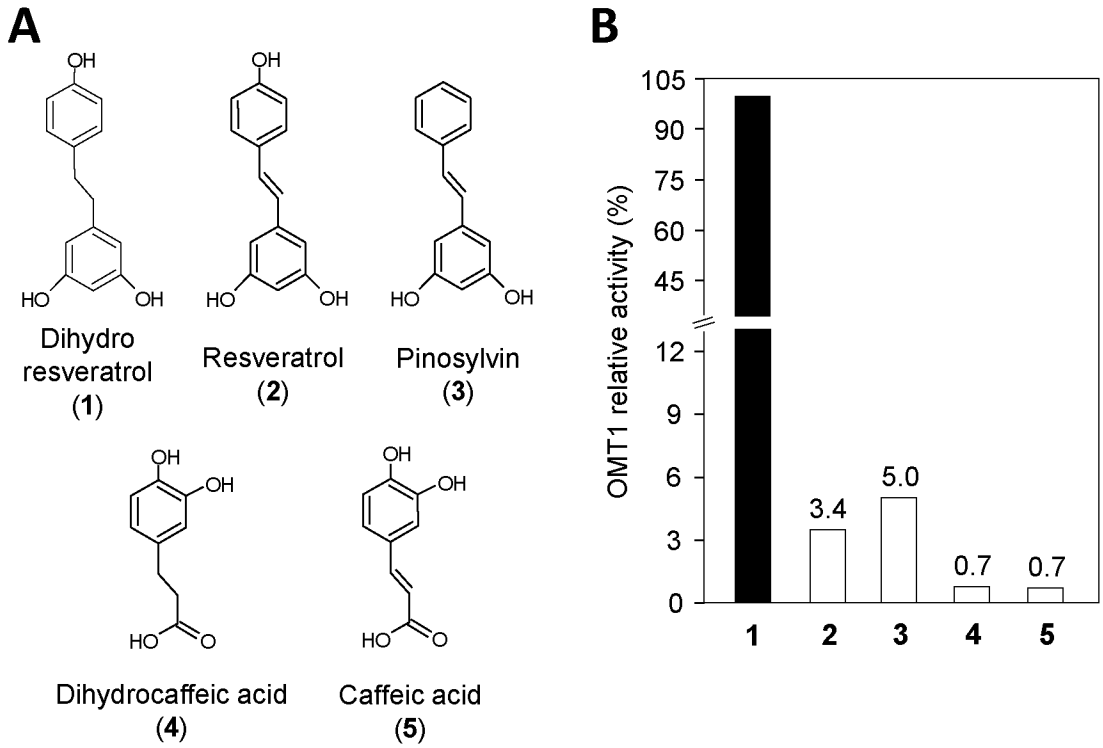
A
OH
HO
OH
Dihydro resveratrol (1)
Resveratrol (2)
Pinosylvin (3)
O
Dihydrocaffeic acid (4)
Caffeic acid (5)
B
OMT1 relative activity (%)
105
90
75
60
45
12
9
6
3
0
3.4
5.0
0.7
0.7
1
2
3
4
5

Figure 5

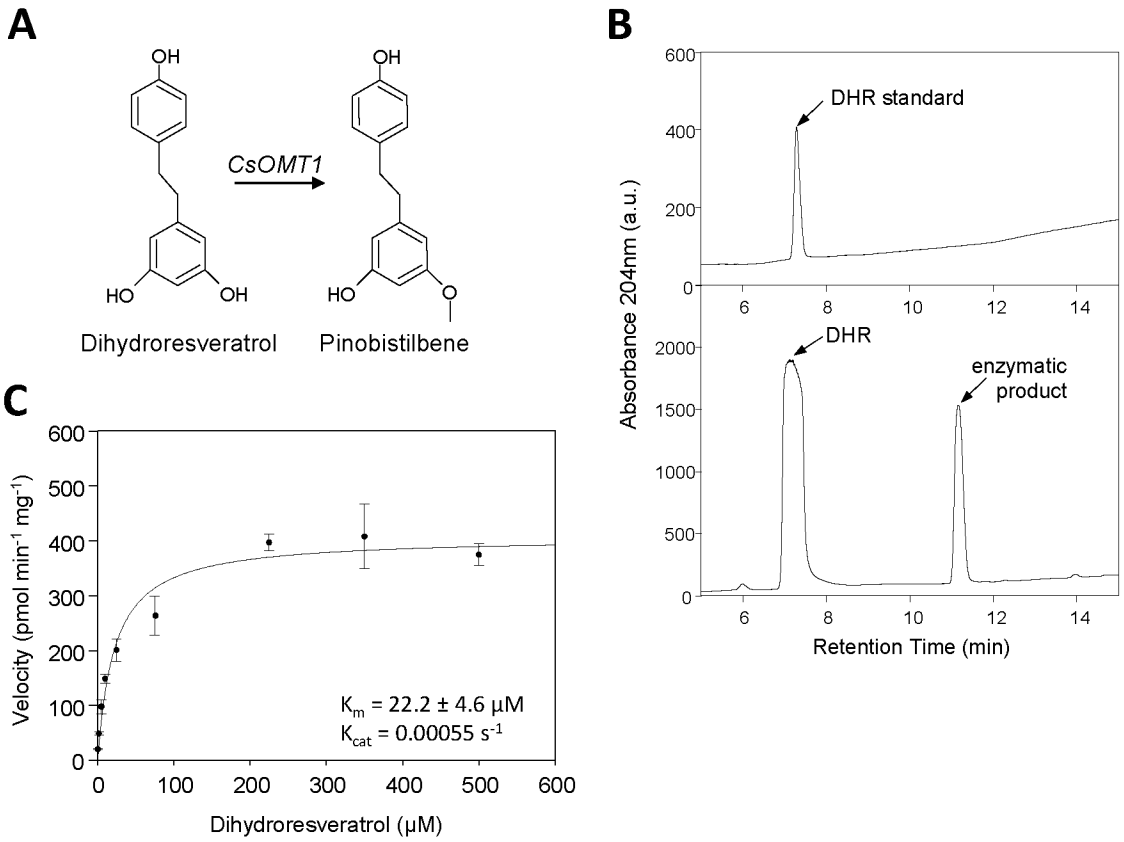
A
OH
OH
CsOMT1
HO
OH
HO
O
Dihydroresveratrol
Pinobistilbene
B
DHR standard
DHR
enzymatic product
Absorbance 204nm (a.u.)
Retention Time (min)
C
Velocity (pmol min-1 mg-1)
Km = 22.2 ± 4.6 µM
Kcat = 0.00055 s-1
Dihydroresveratrol (µM)

Figure 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2021/051682

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***C12P 17/06*** (2006.01) , ***A61K 8/49*** (2006.01) , ***A61K 31/352*** (2006.01) , ***C07D 311/30*** (2006.01) , ***C12N 9/10*** (2006.01) , ***C12N 15/54*** (2006.01), ***C12N 15/63*** (2006.01), ***C12P 7/22*** (2006.01)

CPC: , A61K 8/498 (2020.01) , A61K 31/352 (2020.01) , C07D 311/30 (2020.01) , C12N 9/1085 (2020.01) , C12N 15/52 (2020.01), C12N 15/63, C12P 7/22 (2020.01), C12P 17/06 (2020.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: ALL

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Databases used: Questel Orbit, Scopus, STNext
Search terms: Prenylation, prenyltransferase, polyphenol, flavonoid, flavonol, prenyl*, combrestatin, longistylins, prenylapigenin, dihydrostilbenoid, dihydroresveratrol, dihydropiceatannol, dihydrognetol, dihydropinosylvin, gigantol, pinobistilbene, batatasin, crepidatin, moscatilin, crysotoxine, chrysotobibenzyl, amoenylin, tristin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 2019183152 A1 (MENDEZ M. *et al*) 26 September 2019 (29-09-2019) *whole document*	1-10, 31-48, 51-55 11-16, 49, 50, 56-59
Y	PERVEEN S. and AL-TAWEEL A.J., Phenolic Compounds from the Natural Sources and Their Cytotoxicity", Chapter 2 in *Phenolic Compounds - Natural Sources Importance and Applications*, Edited by Marcos Soto-Hernandez, Mariana Palma-Tenango and Maria del Rosario Garcia-Mateos, 15 March 2017 (15-03-2017), retrieved on 14 February 2022 (14-02-2022) from URL http://dx.doi.org/10.5772/66898 pp 1-32 *whole document*	11-14
Y	YANG, X. *et al.,* "Prenylated flavonoids, promising nutraceuticals with impressive biological activities", Trends in Food Science & Technology, 1 July 2015 (01-07-2015), vol. 44, issue 1, pp 93-104. ISSN: 0924-2244 *whole document*	11-14

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

*	Special categories of cited documents:
"A"	document defining the general state of the art which is not considered to be of particular relevance
"D"	document cited by the applicant in the international application
"E"	earlier application or patent but published on or after the international filing date
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O"	document referring to an oral disclosure, use, exhibition or other means
"P"	document published prior to the international filing date but later than the priority date claimed
"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&"	document member of the same patent family

Date of the actual completion of the international search 16 February 2022 (16-02-2022)	Date of mailing of the international search report 02 March 2022 (02-03-2022)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Sandhya Khurana (819) 635-3884

Box No. II **Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III **Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Groups 1-301: Claims 1-16 (completely); claims 31-59 (partially): A polypeptide encoding a prenyltransferase having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the sequence of any one or more of SEQ ID NO: 1-6 and/or a polypeptide listed in Table 1, or a fragment of any thereof, and nucleic acids, vectors, host cells, and expression systems encoding or containing same; and methods thereof, wherein each particular SEQ ID NO. is considered to define a distinct inventive concept.

Continued on page 7

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Claims 1-16 (completely) and claims 31-59 (partially) as they relate to SEQ ID NO. 1

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☒ forming part of the international application as filed:

☒ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO2020/19066 A1 (Tariq A., *et al*) 30 January 2020 (30-01-2020) *whole document*	15, 16, 49, 50, and 56-59
A	CHEN, X. *et al*, "A systematic review on biological activities of prenylated flavonoids", *Pharmaceutical Biology*, published 21 November 2013 (21-11-2013), vol. 52, no. 1, pp 655-660, ISSN: 1388-0209 (Print) 1744-5116 (Electronic) *whole document*	1-16, and 31-59

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2021/051682

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2019183152A1	26 September 2019 (26-09-2019)	CA3094161A1	26 September 2019 (26-09-2019)
		US2020123511A1	23 April 2020 (23-04-2020)
		US10894952B2	19 January 2021 (19-01-2021)
		US2021301267A1	30 September 2021 (30-09-2021)
WO202019066 A1	30 January 2020 (30-01-2020)	None	30 January 2020 (30-01-2020)

Continuation of Box III

Groups 302-327: Claims 17-30 (completely); claims 31-59 (partially): A polypeptide encoding a methyltransferase having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the sequence of any one or more of SEQ ID NO: 7-30 or a fragment thereof, and nucleic acids, vectors, host cells, expression systems encoding or containing same, and methods thereof, wherein each particular SEQ ID NO. is considered to define a distinct inventive concept.

Group 328: Claim 60 (completely): A method of producing cannflavin A, cannflavin B, isocannflavin B comprising carrying out a combined enzymatic O-methylation and prenylation of a flavonoid.

Group 329: Claim 61 (completely): A method of producing a longistylin, comprising carrying out a combined enzymatic O-methylation and prenylation of a stilbenoid.

Group 330: Claim 62 (completely): A method of producing canniprene, cannabistilbene, dihydrolongistylin, amorfrutin 1/A, or amorfrutin B, the method comprising carrying out a combined enzymatic O-methylation and prenylation of a bibenzyl.

Group 331: Claim 63 (completely): A synthetic chemical catalysis method of producing cannflavin A and/or cannflavin B, the method comprising using GPP and DMAPP in a single synthesis step from chrysoeriol or in combination with an enzymatic reaction such as the O-methylation of luteolin.

Group 332: Claims 64-70 (completely): Substantially pure, prenylated and/or methylated polyphenols, and the use thereof in cosmetic and pharmaceutical compositions, and natural health products.

The present application relates to polypeptides encoding enzymes involved in the prenylation, and in the methylation of polyphenols. However there is no technical relationship between the identified groups as the polypeptides encoding a prenyltransferase and a methyltransferase do not share a common property or activity, and fail to share a common structure. Therefore the group of polynucleotide molecules claimed does not meet the requirement of unity of invention and are considered to lack unity *a priori*.

The claims must be limited to one inventive concept as set out in PCT Rule 13.