(12) STANDARD PATENT (11) Application No. **AU 2012342114 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Methods and materials for enzymatic synthesis of mogroside compounds

(51) International Patent Classification(s)
C12P 19/18 (2006.01) ***C12P 33/00*** (2006.01)

(21) Application No: **2012342114** (22) Date of Filing: **2012.11.19**

(87) WIPO No: **WO13/076577**

(30) Priority Data

(31) Number	(32) Date	(33) Country
61/563,303	**2011.11.23**	**US**

(43) Publication Date: **2013.05.30**
(44) Accepted Journal Date: **2017.12.21**

(71) Applicant(s)
Evolva SA

(72) Inventor(s)
Liu, Yaoquan;Lee, Jung Yeop;Khare, Monika

(74) Agent / Attorney
FPA Patent Attorneys Pty Ltd, Level 43 101 Collins Street, Mebourne, VIC, 3000, AU

(56) Related Art
WO 2008/062165

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 May 2013 (30.05.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/076577 A1

(51) International Patent Classification:
C12P 19/18 (2006.01) *C12P 33/00* (2006.01)

(21) International Application Number:
PCT/IB2012/002857

(22) International Filing Date:
19 November 2012 (19.11.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/563,303 23 November 2011 (23.11.2011) US

(71) Applicant: **EVOLVA SA** [CH/CH]; Duggingerstrasse 23, CH-4153 Reinach (CH).

(72) Inventors: **LIU, Yaoquan**; 2440 Embarcadero Way, Palo Alto, CA 94303 (US). **LEE, Jung, Yeop**; 10243 Alpine Dr. #2, Cupertino, CA 95014 (US). **KHARE, Monika**; 19980 Pear Tree Lane, Cupertino, CA 95014 (US).

(74) Agent: **HØIBERG A/S**; St. Kongensgade 59 A, DK-1264 Copenhagen K (DK).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

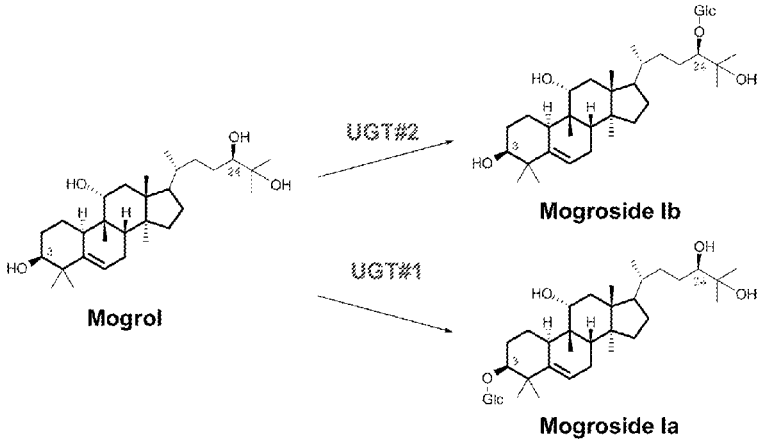
— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: METHODS AND MATERIALS FOR ENZYMATIC SYNTHESIS OF MOGROSIDE COMPOUNDS

FIGURE 2



(57) Abstract: Methods and materials for enzymatic synthesis of mogroside compounds described.

WO 2013/076577 A1

2012342114 31 Jan 2017

METHODS AND MATERIALS FOR ENZYMATIC SYNTHESIS OF MOGROSIDE COMPOUNDS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority from U.S. Serial No. 61/563,303, filed November 23, 2011, the disclosure of which is incorporated by reference in its entirety.

TECHNICAL FIELD

This invention relates to methods and materials for enzymatic synthesis of mogroside compounds, and more particularly to glycosylating mogrol using Uridine-5'-diphospho (UDP) dependent glucosyltransferases (UGTs) to produce various mogroside compounds.

BACKGROUND

Reference to any prior art in the specification is not an acknowledgment or suggestion that this prior art forms part of the common general knowledge in any jurisdiction or that this prior art could reasonably be expected to be understood, regarded as relevant, and/or combined with other pieces of prior art by a skilled person in the art.

Mogrosides are a family of triterpene glycosides isolated from fruits of *Siraitia grosvenorii* (Swingle), also known as *Momordica grosvenori* (Swingle). Extracts of the fruits are commercially used as natural sweeteners. Four major compounds, Mogroside V, Mogroside IV, Siamenoside I, and 11-Oxomogroside V, have been identified from the fruits of *Siraitia grosvenorii* (Swingle) that are responsible for the sweetness of the fruits. See FIG. 1. Mogroside V is the most abundant of these four compounds at approximately 0.57% (w/w) of the dry fruit, followed by Mogroside IV and Siamenoside I, each of which contain four glucose moieties. 11-Oxomogroside V has a ketone group instead of a hydroxyl at C-11. *See, e.g.*, Takemoto, *et al., Yakugaku Zasshi*, 103, 1151-1154; 1155-1166; 1167-1173, (1983); Kasai, *et al.*, *Agric. Biol. Chem.* 53, 3347-3349 (1989); Matsumoto, *Chem. Pharm. Bull.* 38, 2030-2032 (1990); and Prakash, *et al.*, *J. Carbohydrate Chem.* 30, 16-26 (2011).

All mogrosides share the same triterpene core, named mogrol. The aglycone mogrol is glycosylated with different numbers of glucose moieties to form various mogroside compounds. Mogroside is thought to be synthesized in the following manner: synthesis of cucurbitadienol from the common triterpene precursor squalene; P450 oxidations of cucurbitadienol to produce the aglycone mogrol; and glycosylation of

mogrol to add the five glucoses one after another to produce mogroside V. See, Tang, *et al.*, *BMC Genomics*, 12, 343 (2011). Both intermediates cucurbitadienol and mogrol exist in the fruit as they have been isolated as minor products. See Ukiya, *et al.*, *J. Agric. Food Chem.* 50, 6710-6715 (2002). Glycoside intermediates exist in both 11-hydroxy and 11-oxo series, and gradually change from mogroside I to mogroside V as fruits ripen, which indicates that the triterpene core is fully oxidized by P450 enzymes before the subsequent glycosylations. However, the enzymes responsible for producing mogrosides have not been identified.

SUMMARY

In one aspect, this document features a method of producing a mogroside compound. The method includes incubating mogrol with a Uridine-5'-diphospho (UDP) dependent glucosyltransferase (UGT) to produce a mogroside compound (e.g., mogroside Ia, mogroside Ib, or a mogroside compound glycosylated at C25-OH). The UGT can be selected from the group consisting of 73C3, 73C6, 85C2, 73C5, and 73E1. The UGTs can be recombinantly produced or can be in a cell lysate of a recombinant host.

This document also features a method of producing a mogroside compound. The method includes contacting mogrol with a cell lysate prepared from a recombinant host expressing a UGT to produce a mogroside compound (e.g., mogroside Ia, mogroside Ib, or a mogroside compound glycosylated at C25-OH). The UGT can be selected from the group consisting of 73C3, 73C6, 85C2, 73C5, and 73E1.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials aredescribed below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting. Other features and advantages of the invention will be apparent from the following detailed

description. Applicants reserve the right to alternatively claim any disclosed invention using the transitional phrase "comprising," "consisting essentially of," or "consisting of," according to standard practice in patent law.

DESCRIPTION OF DRAWINGS

FIG. 1 contains the chemical structure of Mogroside V, Mogroside IV, Siamenoside I, and 11-Oxomogroside V.

FIG. 2 is a depiction of the biosynthesis of mogroside Ia and mogroside Ib from mogrol using UGTs.

FIG. 3 contains the amino acid sequences of the followings UGTs: UGT73C3, UGT73C5, UGT73C6, UGT73E1, and UGT85C2 (SEQ ID NOs: 1-5, respectively).

FIG. 4 is a schematic of the products obtained from mogroside V after incubation with a pectinase and/or a cellulase.

DETAILED DESCRIPTION

This document provides methods and materials for glycosylating mogrol using one or more Uridine-5'-diphospho (UDP) dependent glucosyltransferases (UGTs). As indicated below, at least five UGTs have been identified that glycosylate the aglycone mogrol. See FIG. 2. Each of the UGTs identified herein are in glycosyltransferase family I. UGTs 73C3, 73C6, 85C2 and 73E1 glycosylate at the C24-OH position (UGT#2 in FIG. 2), while UGT73C5 glycosylates at both the C3-OH (UGT#1 in FIG. 2) and C24-OH position (UGT#2). UGTs 73C3, 73C5, and 73C6 are from *Arabidopsis thaliana*. UGT 73E1 and 85C2 are from *Stevia rebaudiana*. The amino acid sequences of UGTs 73C3, 73C5, 73C6, 73E1, and 85C2 (SEQ ID NOs:1-5) are set forth in FIG. 3.

UGT polypeptides described herein can be produced using any suitable method. For example, UGT polypeptides can be produced by chemical synthesis. Alternatively, a UGT polypeptide described herein can be produced by standard recombinant technology using heterologous expression vectors encoding that UGT polypeptide. Expression vectors can be introduced into host cells (e.g., by transformation or transfection) for expression of the encoded polypeptide, which then can be purified. Expression systems

that can be used for small or large scale production of UGT polypeptides include, without limitation, microorganisms such as bacteria (e.g., *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA, or cosmid DNA expression vectors containing the nucleic acid molecules described herein. Useful expression systems also include insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the nucleic acid molecules described herein, and plant cell systems infected with recombinant virus expression vectors (e.g., tobacco mosaic virus) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing the nucleic acid molecules described herein. UGT polypeptides also can be produced using mammalian expression system harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., the metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter and the cytomegalovirus promoter), along with the nucleic acids described herein. UGT polypeptides can have an N-terminal or C-terminal tag as discussed below.

This document also provides isolated nucleic acids encoding the UGT polypeptides. An "isolated nucleic acid" refers to a nucleic acid that is separated from other nucleic acid molecules that are present in a genome, including nucleic acids that normally flank one or both sides of the nucleic acid in a genome. The term "isolated" as used herein with respect to nucleic acids also includes any non-naturally-occurring nucleic acid sequence, since such non-naturally-occurring sequences are not found in nature and do not have immediately contiguous sequences in a naturally-occurring genome.

An isolated nucleic acid can be, for example, a DNA molecule, provided one of the nucleic acid sequences normally found immediately flanking that DNA molecule in a naturally-occurring genome is removed or absent. Thus, an isolated nucleic acid includes, without limitation, a DNA molecule that exists as a separate molecule (e.g., a chemically synthesized nucleic acid, or a cDNA or genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other sequences as well as DNA that is incorporated into a vector, an autonomously replicating plasmid, a virus (e.g., any paramyxovirus, retrovirus, lentivirus, adenovirus, or herpes virus), or into the genomic

DNA of a prokaryote or eukaryote. In addition, an isolated nucleic acid can include an engineered nucleic acid such as a DNA molecule that is part of a hybrid or fusion nucleic acid. A nucleic acid existing among hundreds to millions of other nucleic acids within, for example, cDNA libraries or genomic libraries, or gel slices containing a genomic DNA restriction digest, is not considered an isolated nucleic acid.

In some embodiments, a nucleic acid sequence encoding a UGT polypeptide can include a tag sequence that encodes a "tag" designed to facilitate subsequent manipulation (e.g., to facilitate purification or detection), secretion, or localization of the encoded polypeptide. Tag sequences can be inserted in the nucleic acid sequence encoding the UGT polypeptide such that the encoded tag is located at either the carboxyl or amino terminus of the UGT polypeptide. Non-limiting examples of encoded tags include green fluorescent protein (GFP), glutathione S transferase (GST), HIS tag, and Flag™ tag (Kodak, New Haven, CT). Other examples of tags include a chloroplast transit peptide, a mitochondrial transit peptide, an amyloplast peptide, signal peptide, or a secretion tag.

Functional homologs

Functional homologs of the polypeptides described above are also suitable for use in the methods and recombinant hosts described herein. A functional homolog is a polypeptide that has sequence similarity to a reference polypeptide, and that carries out one or more of the biochemical or physiological function(s) of the reference polypeptide. A functional homolog and the reference polypeptide may be natural occurring polypeptides, and the sequence similarity may be due to convergent or divergent evolutionary events. As such, functional homologs are sometimes designated in the literature as homologs, or orthologs, or paralogs. Variants of a naturally occurring functional homolog, such as polypeptides encoded by mutants of a wild type coding sequence, may themselves be functional homologs. Functional homologs can also be created via site-directed mutagenesis of the coding sequence for a polypeptide, or by combining domains from the coding sequences for different naturally-occurring polypeptides ("domain swapping"). Techniques for modifying genes encoding functional

UGT polypeptides described herein are known and include, *inter alia*, directed evolution techniques, site-directed mutagenesis techniques and random mutagenesis techniques, and can be useful to increase specific activity of a polypeptide, alter substrate specificity, alter expression levels, alter subcellular location, or modify polypeptide:polypeptide interactions in a desired manner. Such modified polypeptides are considered functional homologs. The term "functional homolog" is sometimes applied to the nucleic acid that encodes a functionally homologous polypeptide.

Functional homologs can be identified by analysis of nucleotide and polypeptide sequence alignments. For example, performing a query on a database of nucleotide or polypeptide sequences can identify homologs of UGT polypeptides. Sequence analysis can involve BLAST, Reciprocal BLAST, or PSI-BLAST analysis of nonredundant databases using an UGT amino acid sequence as the reference sequence. Amino acid sequence is, in some instances, deduced from the nucleotide sequence. Those polypeptides in the database that have greater than 40% sequence identity are candidates for further evaluation for suitability as a UGT polypeptide. Amino acid sequence similarity allows for conservative amino acid substitutions, such as substitution of one hydrophobic residue for another or substitution of one polar residue for another. If desired, manual inspection of such candidates can be carried out in order to narrow the number of candidates to be further evaluated. Manual inspection can be performed by selecting those candidates that appear to have domains present in UGT polypeptides, *e.g.*, conserved functional domains.

Conserved regions can be identified by locating a region within the primary amino acid sequence of a polypeptide that is a repeated sequence, forms some secondary structure (*e.g.*, helices and beta sheets), establishes positively or negatively charged domains, or represents a protein motif or domain. See, *e.g.*, the Pfam web site describing consensus sequences for a variety of protein motifs and domains on the World Wide Web at sanger.ac.uk/Software/Pfam/ and pfam.janelia.org/. The information included at the Pfam database is described in Sonnhammer *et a*l., *Nucl. Acids Res.*, 26:320-322 (1998); Sonnhammer *et al.*, *Proteins*, 28:405-420 (1997); and Bateman *et al.*, *Nucl. Acids Res.*, 27:260-262 (1999). Conserved regions also can be determined by aligning sequences of

the same or related polypeptides from closely related species. Closely related species preferably are from the same family. In some embodiments, alignment of sequences from two different species is adequate.

Typically, polypeptides that exhibit at least about 40% amino acid sequence identity are useful to identify conserved regions. Conserved regions of related polypeptides exhibit at least 45% amino acid sequence identity (*e.g.*, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% amino acid sequence identity). In some embodiments, a conserved region exhibits at least 92%, 94%, 96%, 98%, or 99% amino acid sequence identity. Sequence identity can be determined as set forth above.

Methods of Producing Mogroside Compounds

Mogroside compounds can be produced by incubating a mogrol substrate with one or more of the UGT polypeptides described herein, resulting in the production of a mogroside product. In some embodiments, the reaction mixture contains a plurality of UGT polypeptides such that a plurality of glycosylations occur in the reaction vessel. In other embodiments, the reaction mixture contains a single UGT polypeptide and one or more glycosylations catalyzed by that polypeptide take place. For example, a first reaction vessel can comprise a substrate and one or more UGT polypeptides for producing an intermediate, which can be introduced into a second reaction vessel containing one or more other UGT polypeptides to produce a subsequent intermediate or a mogroside product. The product produced in the second reaction vessel then can be recovered.

Each of the UGT polypeptides can be a purified polypeptide, e.g., can be added to a reaction mixture as a solution containing 80%, 90%, 95%, or greater than 99% by weight of the desired UGT. Alternatively, the UGT polypeptide(s) can be present in a cell lysate prepared from a recombinant host expressing the UGT(s), and can be added to a reaction mixture as a cell lysate for incubation with the mogrol substrate.

Levels of products, substrates and intermediates can be determined by extracting samples from the reaction vessel for analysis according to published methods.

Mogroside compounds can be recovered from the reaction vessel using various techniques known in the art.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1 -Purification of mogroside V

Mogroside V was purified from commercially available monk fruit extracts (PureLo®, Swanson) as follows. Three bottles of PureLo® (240 grams) were dissolved in water (900 mL), then loaded on a column of HP-20 resin (400 gram resin). The column was washed with water (2.5 liters); then further washed with 20% methanol-water. The product was eluted with methanol. After evaporation of solvents and drying under high vacuum, mogroside V (2.5 grams, ~80% purity, 11-oxomogroside V was the major impurity) was obtained.

Example 2 - Enzymatic synthesis of mogrol from mogroside V

Mogroside V (300 mg) was dissolved in 0.1M sodium acetate buffer (pH 4.5, 100mL), and crude pectinase from *Aspergillus niger* (25mL, Sigma P2736) was added. The mixture was stirred at 50°C for 48 hours. The reaction mixture was extracted with ethyl acetate (2x100ml). The organic extract was dried under vacuum then purified with preparative HPLC. Pure mogrol (40 mg) was obtained and its structure confirmed by NMR and mass spectroscopy. See FIG. 4.

Example 3 - Enzymatic synthesis of mogrol 3-O-glucoside (mogroside Ia) and mogrol 24-O-glucoside (mogroside Ib) from mogroside V

Mogroside V (300 mg) was dissolved in 0.1M sodium acetate buffer (pH 4.5, 100ml), and crude pectinase from *Aspergillus niger* (25ml, Sigma P2736) was added. The mixture was stirred at 50°C for 6.5 hours. The reaction mixture was extracted with ethyl acetate (2x100ml). The organic extract was dried under vacuum then purified with

preparative HPLC. Pure mogroside Ia (11.0 mg) and mogroside Ib (8.0 mg) were obtained. Their structures were confirmed by NMR and mass spectroscopy. See FIG. 4.

Example 4 - In vitro UGT screening and reactions

In vitro reactions of mogrol with a panel of 230 UGT enzymes were performed and the products were analyzed with LC-MS. The *in vitro* UGT rection mixtures included 4X Tris buffer, mogrol (250 μM), UDP-glucose (750 μM) and 1% alkaline phosphatase. Five μl of each partially purified UGT enzyme or crude enzyme extract was added to the reaction, and the reaction volume brought to 50 μl with water. The reactions were incubated overnight at 30°C and performed in sterilized 96 well plates. After the incubation, 25 μL of DMSO were added into each reaction and the reaction plates were centrifuged for 5 min. Forty μL samples were taken from each well and filtered, and were used for LC-MS analysis.

UGTs 73C3, 73C6 and 85C2 were found to convert all the mogrol substrate to mogroside Ib. UGT 73C5 makes both mogroside Ia and Ib. In the reaction with UGT 73E1, although the reaction was not complete, mogroside Ib was found as the major product, together with a new glycosylated mogrol (neither mogroside Ia nor Ib; exact mass shown as a mogroside I, presumably caused by a glycosylation event on C25-OH).

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

2012342114 08 Sep 2017

The claims defining the invention are as follows:

1. A method of producing a mogroside compound, comprising contacting and glycosylating mogrol with a Uridine-5'-diphospho (UDP) dependent glucosyltransferase (UGT) polypeptide that is:

(a) UGT73C3 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:1;

(b) UGT73C5 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:2;

(c) UGT73C6 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:3;

(d) UGT73E1 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:4; or

(e) UGT85C2 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:5.

2. A method of producing a mogroside compound, comprising contacting and glycosylating mogrol with a cell lysate prepared from a recombinant host expressing a UGT polypeptide that is:

(a) UGT73C3 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:1;

(b) UGT73C5 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:2;

(c) UGT73C6 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:3;

(d) UGT73E1 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:4; or

(e) UGT85C2 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:5.

2012342114 08 Sep 2017

3. A method of producing a mogroside compound, comprising:

(a) contacting and glycosylating a mogrol with a one or more Uridine-5'-diphospho (UDP) dependent glucosyltransferase (UGT) polypeptides;

(b) contacting and glycosylating a mogrol with a cell lysate prepared from a recombinant host expressing a one or more UGT polypeptides;

(c) (i) contacting and glycosylating a mogrol in a first reaction vessel with a one or more Uridine-5'-diphospho (UDP) dependent glucosyltransferase (UGT) polypeptides;

(ii) contacting and glycosylating the intermediate compound in a second reaction vessel with the one or more UGT polypeptides; and

(iii) recovering the one or more mogroside compounds produced in step (ii); or

(d) (i) contacting and glycosylating a mogrol in a first reaction vessel with a cell lysate prepared from a recombinant host expressing a one or more UGT polypeptides;

(ii) contacting and glycosylating the intermediate compound in a second reaction vessel with the one or more UGT polypeptides; and

(iii) recovering the one or more mogroside compounds produced in step (ii), wherein the UGT polypeptide is:

(a) UGT73C3 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:1;

(b) UGT73C5 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:2;

(c) UGT73C6 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:3;

(d) UGT73E1 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:4; and

(e) UGT85C2 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:5.

2012342114 08 Sep 2017

4. The method of any one of claims 1 to 3, wherein:

(a) the glycosylation is effected by the activity of the polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:2 and the mogroside compound is mogroside Ia;

(b) the glycosylation is effected by the activity of one or more polypeptides having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs:1-5 and the mogroside compound is mogroside Ib; or

(c) the glycosylation is effected by the activity of the polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:4 and the mogroside compound is a mogroside compound glycosylated at C25-OH.

5. The method of any one of claims 1 to 3, wherein the UGT polypeptide is a recombinant UGT polypeptide.

6. The method of claim 1, wherein the UGT polypeptide is produced by chemical synthesis.

7. The method of claim 5, wherein the recombinant UGT polypeptide is produced in a cell lysate of a recombinant host.

8. The method of any one of claims 1 to 3, 5 or 7, wherein the UGT polypeptide contains a tag sequence located at either a carboxyl or an amino terminus of the UGT polypeptide.

9. The method of claim 8, wherein the tag sequence comprises a green fluorescent protein (GFP), a glutathione S transferase (GST), a HIS tag, a Flag tag, a chloroplast transit peptide, a mitochondrial transit peptide, an amyloplast peptide, a signal peptide, or a secretion tag.

10. The method of any one of claims 2, 3 or 7, wherein the recombinant host is a microorganism transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors.

11. The method of any one of claims 2, 3 or 7, wherein the recombinant host is an insect cell system infected with recombinant virus expression vectors.

2012342114 08 Sep 2017

12. The method of any one of claims 2, 3 or 7, wherein the recombinant host is a plant cell system infected with recombinant virus expression vectors or transformed with recombinant virus expression vectors.

13. The method of any one of claims 2, 3 or 7, wherein the recombinant host is a mammalian expression system harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells or from mammalian viruses.

14. A method for producing a mogroside compound, comprising:

(a) adding one or more recombinant polypeptides capable of catalyzing glycosylation of mogrol to produce a mogroside compound and having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs:1-5 and a plant-derived or synthetic mogrol to a reaction mixture; and synthesizing the mogroside compound thereby; and

(b) recovering the mogroside compound produced in step (a) from the reaction mixture.

15. A method for producing a mogroside compound, comprising:

(a) adding a cell lysate prepared from a recombinant host expressing one or more recombinant polypeptides capable of catalyzing glycosylation of mogrol to produce a mogroside compound and having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs:1-5 and a plant-derived or synthetic mogrol to a reaction mixture; and synthesizing the mogroside compound thereby; and

(b) recovering the mogroside compound produced in step (a) from the reaction mixture.

16. A method of producing a mogroside compound, comprising:

(a) (i) adding one or more recombinant or synthetic polypeptides capable of catalyzing glycosylation of mogrol to produce a mogroside compound and having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs:1-5 and a plant-derived or synthetic mogrol to a reaction mixture; and producing the mogroside compound;
wherein the one or more recombinant or synthetic polypeptides is produced in a recombinant host or by chemical synthesis; and

2012342114 08 Sep 2017

(ii) recovering the mogroside compound produced in step (a)(i) from the reaction mixture; or

(b) (i) adding a cell lysate prepared from a recombinant host expressing one or more recombinant polypeptides capable of catalyzing glycosylation of mogrol to produce a mogroside compound and having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs:1-5 and a plant-derived or synthetic mogrol to a reaction mixture; and synthesizing the mogroside compound; and

(ii) recovering the mogroside compound produced in step (b)(i) from the reaction mixture;

(c) (i) contacting and glycosylating mogrol in a first reaction vessel with a one or more recombinant polypeptides capable of catalyzing glycosylation of mogrol to produce a mogroside compound and having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs:1-5;

(ii) contacting and glycosylating the intermediate compound in a second reaction vessel with the one or more recombinant polypeptides capable of catalyzing glycosylation of mogrol to produce a mogroside compound and having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs:1-5; and

(iii) recovering the one or more mogroside compound produced in step (ii); or

(d) (i) contacting and glycosylating mogrol in a first reaction vessel with a cell lysate prepared from a recombinant host expressing a one or more recombinant polypeptides capable of catalyzing glycosylation of mogrol to produce a mogroside compound and having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs:1-5;

(ii) contacting and glycosylating the intermediate compound in a second reaction vessel with the one or more UGT polypeptides; and

(iii) recovering the one or more mogroside compound produced in step (ii).

2012342114 08 Sep 2017

17. A method for transferring a sugar moiety to a C24' position, C3' position, both C24' and C3' positions, or C25' position of mogrol, comprising contacting the mogrol with a recombinant polypeptide having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs: 1-5, and a UDP-sugar under suitable reaction conditions for the transfer of the sugar moiety to the mogrol,

wherein a mogroside Ia, mogroside Ib, a mogroside compound glycosylated at C25-OH, an isomer thereof, and/or a mogroside composition thereof is produced upon transfer of the sugar moiety.

18. The method of claim 17, wherein:

(a) the sugar moiety is glucose, the glycosylation is effected by the activity of the polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:2, and mogroside Ia is produced upon transfer of the glucose moiety to mogrol;

(b) the sugar moiety is glucose, the glycosylation is effected by the activity of one or more the polypeptides having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs:1-5, and mogroside Ib is produced upon transfer of the glucose moiety to mogrol; or

(c) the sugar moiety is glucose, the glycosylation is effected by the activity of the polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:4, and mogroside I is produced upon transfer of the glucose moiety to mogrol.

19. The method of claim 17, wherein the mogroside compound is mogroside Ia;

wherein the glycosylation is effected by the activity of the polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:2.

20. The method of claim 17, wherein the mogroside compound is mogroside Ib;

wherein the glycosylation is effected by the activity of one or more the polypeptides having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs:1-5.

2012342114 08 Sep 2017

21. The method of claim 17, wherein the mogroside compound is a mogroside compound glycosylated at C25-OH;

wherein glycosylation is effected by the activity of the polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:4.

FIGURE 1

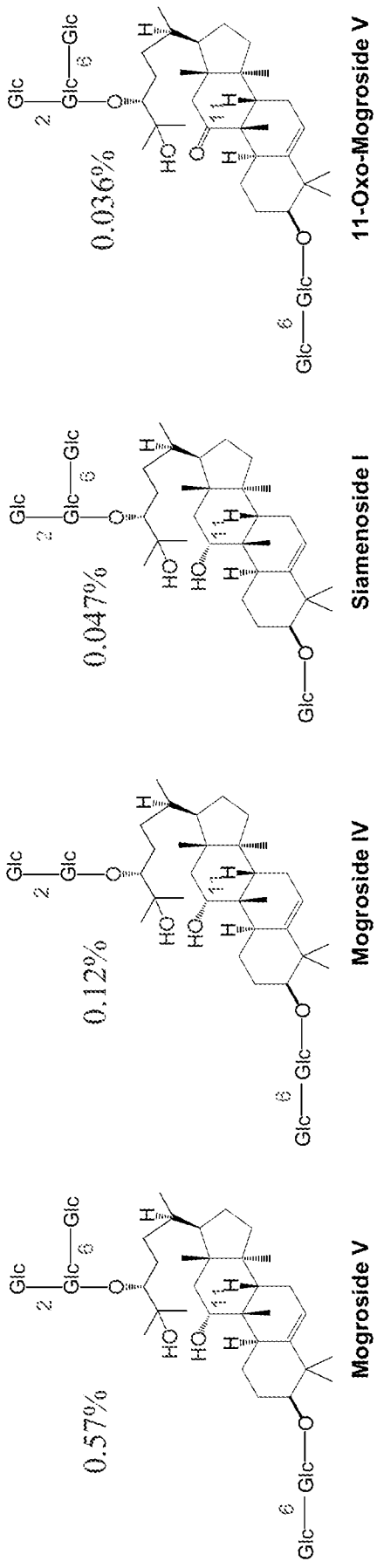

0.57%
Mogroside V
0.12%
Mogroside IV
0.047%
Siamenoside I
0.036%
11-Oxo-Mogroside V

FIGURE 2

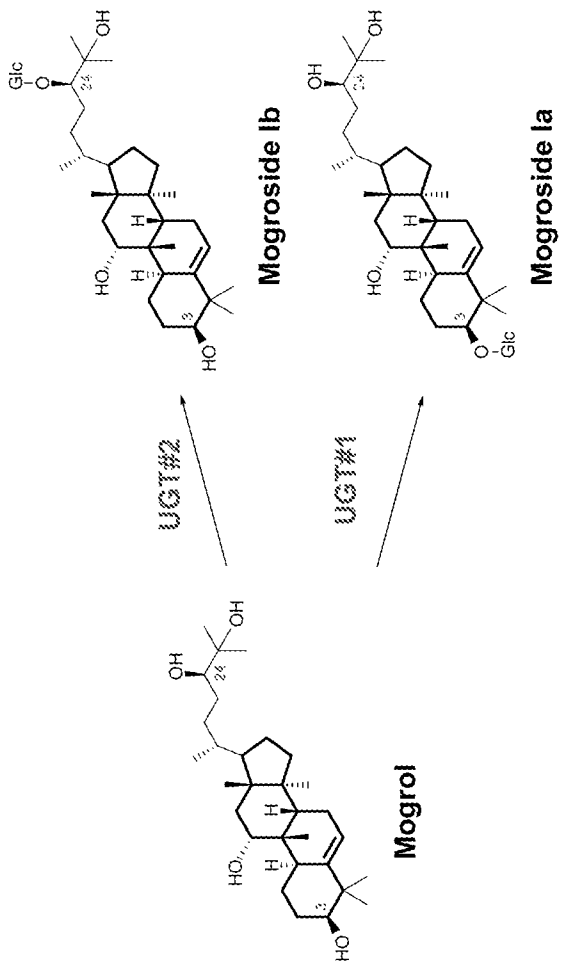


FIGURE 3

UGT73C3 (SEQ ID NO: 01)

MATEKTHQFHPSLHFVLFPFMAQGHMIPMIDIARLLAQRGVTITIVTTPHNAARFKNVLNRAIESGLAINILHVKFPYQEFGLPEGKENIDS
LDSTELMVPFFKAVNLLEDPVMKLMEEMKPRPSCLISDWCLPYTSIIAKNFNIPKIVFHGMGCFNLLCMHVLRRNLEILENVKSDEEYFL
VPSFPDRVEFTKLQLPVKANASGDWKEIMDEMVKAEYTSYGVIVNTFQELEPPYVKDYKEAMDGKVWSIGPVSLCNKAGADKAERGS
KAAIDQDECLQWLDSKEEGSVLYVCLGSICNLPLSQLKELGLGLEESRRSFIWVIRGSEKYKELFEWMLESGFEERIKERGLLIKGWAP
QVLILSHPSVGGFLTHCGWNSTLEGITSGIPLITWPLFGDQFCNQKLVVQVLKAGVSAGVEEVMKWGEEDKIGVLVDKEGVKKAVEEL
MGDSDDAKERRRRVKELGELAHKAVEKGGSSHSNITLLLQDIMQLAQFKN

UGT73C5 (SEQ ID NO: 02)

MVSETTKSSPLHFVLFPFMAQGHMIPMVDIARLLAQRGVIITIVTTPHNAARFKNVLNRAIESGLPINLVQVKFPYLEAGLQEGQENIDSL
DTMERMIPFFKAVNFLEEPVQKLIEEMNPRPSCLISDFCLPYTSKIAKKFNIPKILFHGMGCFCLLCMHVLRKNREILDNLKSDKELFTVP
DFPDRVEFTRTQVPVETYVPAGDWKDIFDGMVEANETSYGVIVNSFQELEPAYAKDYKEVRSGKAWTIGPVSLCNKVGADKAERGNK
SDIDQDECLKWLDSKKHGSVLYVCLGSICNLPLSQLKELGLGLEESQRPFIWVIRGWEKYKELVEWFSESGFEDRIQDRGLLIKGWSP
QMLILSHPSVGGFLTHCGWNSTLEGITAGLPLLTWPLFADQFCNEKLVVEVLKAGVRSGVEQPMKWGEEEKIGVLVDKEGVKKAVEEL
MGESDDAKERRRRAKELGDSAHKAVEEGGSSHSNISFLLQDIMELAEPNN

UGT73C6 (SEQ ID NO: 03)

MAFEKNNEPFPLHFVLFPFMAQGHMIPMVDIARLLAQRGVLITIVTTPHNAARFKNVLNRAIESGLPINLVQVKFPYQEAGLQEGQENM
DLLTTMEQITSFFKAVNLLKEPVQNLIEEMSPRPSCLISDMCLSYTSEIAKKFKIPKILFHGMGCFCLLCVNVLRKNR

FIGURE 3 – CONTINUED

EILDNLKSDKEYFIVPYFPDRVEFTRPQVPVETYVPAGWKEILEDMVEADKTSYGVIVNSFQELEPAYAKDFKEARSGKAWTIGPVSLC
NKVGVDKAERGNKSDIDQDECLEWLDSKEPGSVLYVCLGSICNLPLSQLLELGLGLEESQRPFIWVIRGWEKYKELVEWFSESGFEDR
IQDRGLLIKGWSPQMLILSHPSVGGFLTHCGWNSTLEGITAGLPMLTWPLFADQFCNEKLVVQILKVGVSAEVKEVMKWGEEEKIGVL
VDKEGVKKAVEELMGESDDAKERRRRAKELGESAHKAVEEGGSSHSNITFLLQDIMQLAQSNN

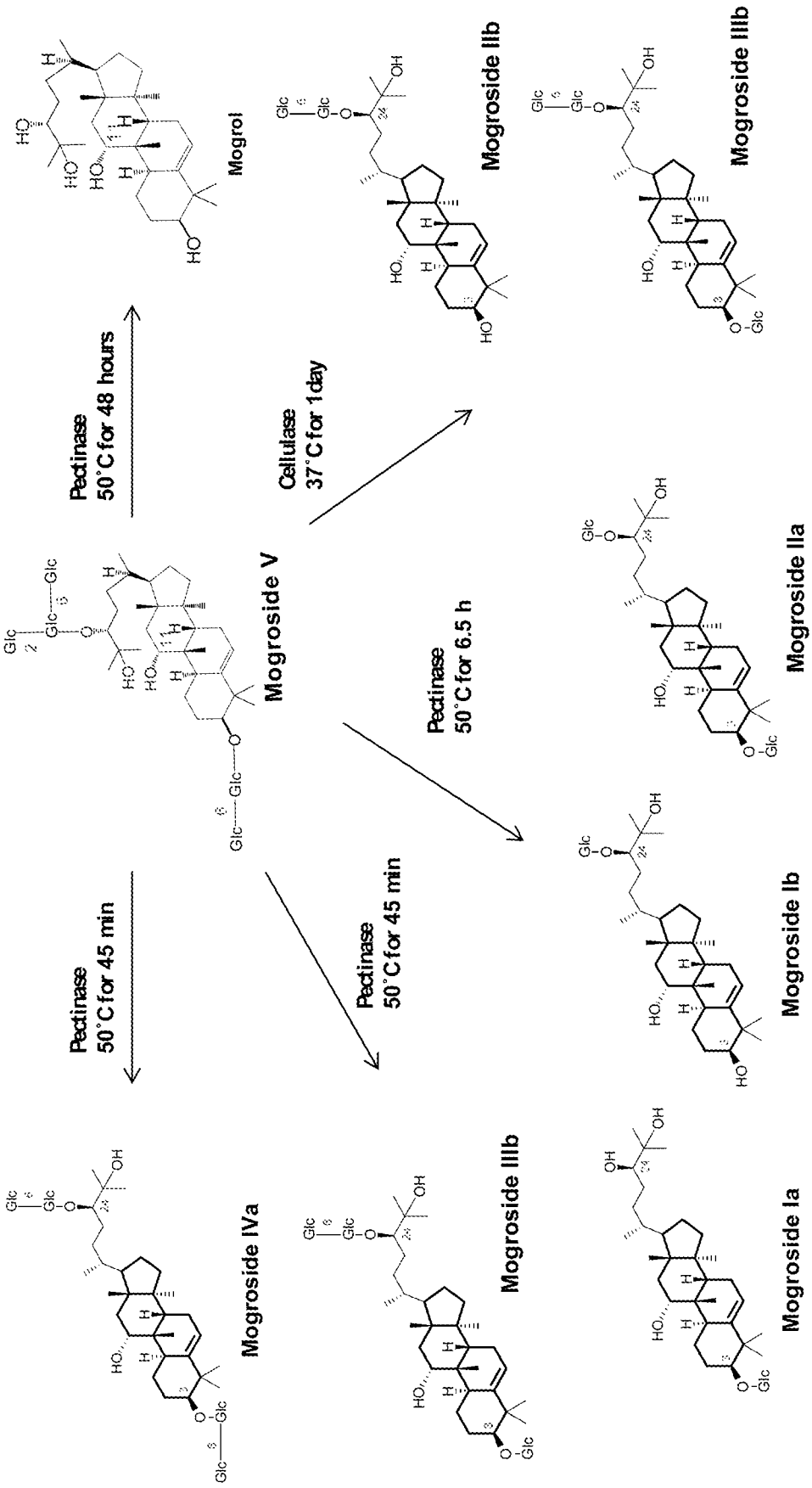
UGT73E1 (SEQ ID NO: 04)

MSPKMVAPPTNLHFVLFPLMAQGHLVPMVDIARILAQRGATVTIITTPYHANRVRPVISRAIATNLKIQLLELQLRSTEAGLPEGCESFDQ
LPSFEYWKNISTAIDLLQQPAEDLLRELSPPPDCIISDFLFPWTTDVARRLNIPRLVFNGPGCFYLLCIHVAITSNILGENEPVSSNTERVV
LPGLPDRIEVTKLQIVGSSRPANVDEMGSWLRAVEAEKASFGIVVNTFEELEPEYVEEYKTVKDKKMWCIGPVSLCNKTGPDLAERGN
KAAITEHNCLKWLDERKLGSVLYVCLGSLARISAAQAIELGLGLESINRPFIWCVRNETDELKTWFLDGFEERVRDRGLIVHGWAPQVLI
LSHPTIGGFLTHCGWNSTIESITAGVPMITWPFFADQFLNEAFIVEVLKIGVRIGVERACLFGEEDKVGVLVKKEDVKKAVECLMDEDED
GDQRRKRVIELAKMAKIAMAEGGSSYENVSSLIRDVTETVRAPH

UGT85C2 (SEQ ID NO: 05)

MDAMATTEKKPHVIFIPFPAQSHIKAMLKLAQLLHHKGLQITFVNTDFIHNQFLESSGPHCLDGAPGFRFETIPDGVSHSPEASIPIRESL
LRSIETNFLDRFIDLVTKLPDPPTCIISDGFLSVFTIDAAKKLGIPVMMYWTLAACGFMGFYHIHSLIEKGFAPLKDASYLTNGYLDTVIDW
VPGMEGIRLKDFPLDWSTDLNDKVLMFTTEAPQRSHKVSHHIFHTFDELEPSIIKTLSLRYNHIYTIGPLQLLLDQIPEEKKQTGITSLHGY
SLVKEEPECFQWLQSKEPNSVVYVNFGSTTVMSLEDMTEFGWGLANSNHYFLWIIRSNLVIGENAVLPPELEEHIKKRGFIASWCSQE
KVLKHPSVGGFLTHCGWGSTIESLSAGVPMICWPYSWDQLTNCRYICKEWEVGLEMGTKVKRDEVKRLVQELMGEGGHKMRNKAK
DWKEKARIAIAPNGSSSLNIDKMVKEITVLARN

FIGURE 4


Mogrol
Mogroside IIb
Mogroside IIIb
Pectinase
50°C for 48 hours
Cellulase
37°C for 1day
Mogroside V
Pectinase
50°C for 6.5 h
Mogroside IIa
Pectinase
50°C for 45 min
Pectinase
50°C for 45 min
Mogroside Ib
Mogroside IVa
Mogroside IIIb
Mogroside Ia

SEQUENCE LISTING

<110> Evolva SA

<120> METHODS AND MATERIALS FOR BIOSYNTHESIS
OF MOGROSIDE COMPOUNDS

<130> 30998-0013WO1

<150> 61/563,303
<151> 2011-11-23

<160> 5

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 496
<212> PRT
<213> Arabidopsis thaliana

<400> 1
Met Ala Thr Glu Lys Thr His Gln Phe His Pro Ser Leu His Phe Val
 1   5   10   15
Leu Phe Pro Phe Met Ala Gln Gly His Met Ile Pro Met Ile Asp Ile
            20   25   30
Ala Arg Leu Leu Ala Gln Arg Gly Val Thr Ile Thr Ile Val Thr Thr
        35   40   45
Pro His Asn Ala Ala Arg Phe Lys Asn Val Leu Asn Arg Ala Ile Glu
    50   55   60
Ser Gly Leu Ala Ile Asn Ile Leu His Val Lys Phe Pro Tyr Gln Glu
65   70   75   80
Phe Gly Leu Pro Glu Gly Lys Glu Asn Ile Asp Ser Leu Asp Ser Thr
                85   90   95
Glu Leu Met Val Pro Phe Phe Lys Ala Val Asn Leu Leu Glu Asp Pro
            100   105   110
Val Met Lys Leu Met Glu Glu Met Lys Pro Arg Pro Ser Cys Leu Ile
        115   120   125
Ser Asp Trp Cys Leu Pro Tyr Thr Ser Ile Ile Ala Lys Asn Phe Asn
    130   135   140
Ile Pro Lys Ile Val Phe His Gly Met Gly Cys Phe Asn Leu Leu Cys
145   150   155   160
Met His Val Leu Arg Arg Asn Leu Glu Ile Leu Glu Asn Val Lys Ser
                165   170   175
Asp Glu Glu Tyr Phe Leu Val Pro Ser Phe Pro Asp Arg Val Glu Phe
            180   185   190
Thr Lys Leu Gln Leu Pro Val Lys Ala Asn Ala Ser Gly Asp Trp Lys
        195   200   205
Glu Ile Met Asp Glu Met Val Lys Ala Glu Tyr Thr Ser Tyr Gly Val
    210   215   220
Ile Val Asn Thr Phe Gln Glu Leu Glu Pro Pro Tyr Val Lys Asp Tyr
225   230   235   240
Lys Glu Ala Met Asp Gly Lys Val Trp Ser Ile Gly Pro Val Ser Leu
                245   250   255
Cys Asn Lys Ala Gly Ala Asp Lys Ala Glu Arg Gly Ser Lys Ala Ala
            260   265   270
Ile Asp Gln Asp Glu Cys Leu Gln Trp Leu Asp Ser Lys Glu Glu Gly
        275   280   285
Ser Val Leu Tyr Val Cys Leu Gly Ser Ile Cys Asn Leu Pro Leu Ser
    290   295   300
Gln Leu Lys Glu Leu Gly Leu Gly Leu Glu Glu Ser Arg Arg Ser Phe
305   310   315   320
Ile Trp Val Ile Arg Gly Ser Glu Lys Tyr Lys Glu Leu Phe Glu Trp
                325   330   335
Met Leu Glu Ser Gly Phe Glu Glu Arg Ile Lys Glu Arg Gly Leu Leu
            340   345   350

```
Ile Lys Gly Trp Ala Pro Gln Val Leu Ile Leu Ser His Pro Ser Val
        355                 360                 365
Gly Gly Phe Leu Thr His Cys Gly Trp Asn Ser Thr Leu Glu Gly Ile
    370                 375                 380
Thr Ser Gly Ile Pro Leu Ile Thr Trp Pro Leu Phe Gly Asp Gln Phe
385                 390                 395                 400
Cys Asn Gln Lys Leu Val Val Gln Val Leu Lys Ala Gly Val Ser Ala
                405                 410                 415
Gly Val Glu Glu Val Met Lys Trp Gly Glu Glu Asp Lys Ile Gly Val
            420                 425                 430
Leu Val Asp Lys Glu Gly Val Lys Lys Ala Val Glu Glu Leu Met Gly
        435                 440                 445
Asp Ser Asp Asp Ala Lys Glu Arg Arg Arg Arg Val Lys Glu Leu Gly
    450                 455                 460
Glu Leu Ala His Lys Ala Val Glu Lys Gly Gly Ser Ser His Ser Asn
465                 470                 475                 480
Ile Thr Leu Leu Leu Gln Asp Ile Met Gln Leu Ala Gln Phe Lys Asn
                485                 490                 495
```

<210> 2
<211> 495
<212> PRT
<213> Arabidopsis thaliana

<400> 2

```
Met Val Ser Glu Thr Thr Lys Ser Ser Pro Leu His Phe Val Leu Phe
 1               5                  10                  15
Pro Phe Met Ala Gln Gly His Met Ile Pro Met Val Asp Ile Ala Arg
            20                  25                  30
Leu Leu Ala Gln Arg Gly Val Ile Ile Thr Ile Val Thr Thr Pro His
        35                  40                  45
Asn Ala Ala Arg Phe Lys Asn Val Leu Asn Arg Ala Ile Glu Ser Gly
    50                  55                  60
Leu Pro Ile Asn Leu Val Gln Val Lys Phe Pro Tyr Leu Glu Ala Gly
65                  70                  75                  80
Leu Gln Glu Gly Gln Glu Asn Ile Asp Ser Leu Asp Thr Met Glu Arg
                85                  90                  95
Met Ile Pro Phe Phe Lys Ala Val Asn Phe Leu Glu Glu Pro Val Gln
            100                 105                 110
Lys Leu Ile Glu Glu Met Asn Pro Arg Pro Ser Cys Leu Ile Ser Asp
        115                 120                 125
Phe Cys Leu Pro Tyr Thr Ser Lys Ile Ala Lys Lys Phe Asn Ile Pro
    130                 135                 140
Lys Ile Leu Phe His Gly Met Gly Cys Phe Cys Leu Leu Cys Met His
145                 150                 155                 160
Val Leu Arg Lys Asn Arg Glu Ile Leu Asp Asn Leu Lys Ser Asp Lys
                165                 170                 175
Glu Leu Phe Thr Val Pro Asp Phe Pro Asp Arg Val Glu Phe Thr Arg
            180                 185                 190
Thr Gln Val Pro Val Glu Thr Tyr Val Pro Ala Gly Asp Trp Lys Asp
        195                 200                 205
Ile Phe Asp Gly Met Val Glu Ala Asn Glu Thr Ser Tyr Gly Val Ile
    210                 215                 220
Val Asn Ser Phe Gln Glu Leu Glu Pro Ala Tyr Ala Lys Asp Tyr Lys
225                 230                 235                 240
Glu Val Arg Ser Gly Lys Ala Trp Thr Ile Gly Pro Val Ser Leu Cys
                245                 250                 255
Asn Lys Val Gly Ala Asp Lys Ala Glu Arg Gly Asn Lys Ser Asp Ile
            260                 265                 270
Asp Gln Asp Glu Cys Leu Lys Trp Leu Asp Ser Lys Lys His Gly Ser
        275                 280                 285
Val Leu Tyr Val Cys Leu Gly Ser Ile Cys Asn Leu Pro Leu Ser Gln
    290                 295                 300
Leu Lys Glu Leu Gly Leu Gly Leu Glu Glu Ser Gln Arg Pro Phe Ile
305                 310                 315                 320
Trp Val Ile Arg Gly Trp Glu Lys Tyr Lys Glu Leu Val Glu Trp Phe
                325                 330                 335
```

```
Ser Glu Ser Gly Phe Glu Asp Arg Ile Gln Asp Arg Gly Leu Leu Ile
            340                 345                 350
Lys Gly Trp Ser Pro Gln Met Leu Ile Leu Ser His Pro Ser Val Gly
        355                 360                 365
Gly Phe Leu Thr His Cys Gly Trp Asn Ser Thr Leu Glu Gly Ile Thr
    370                 375                 380
Ala Gly Leu Pro Leu Leu Thr Trp Pro Leu Phe Ala Asp Gln Phe Cys
385                 390                 395                 400
Asn Glu Lys Leu Val Val Glu Val Leu Lys Ala Gly Val Arg Ser Gly
                405                 410                 415
Val Glu Gln Pro Met Lys Trp Gly Glu Glu Glu Lys Ile Gly Val Leu
            420                 425                 430
Val Asp Lys Glu Gly Val Lys Lys Ala Val Glu Glu Leu Met Gly Glu
        435                 440                 445
Ser Asp Asp Ala Lys Glu Arg Arg Arg Arg Ala Lys Glu Leu Gly Asp
    450                 455                 460
Ser Ala His Lys Ala Val Glu Glu Gly Gly Ser Ser His Ser Asn Ile
465                 470                 475                 480
Ser Phe Leu Leu Gln Asp Ile Met Glu Leu Ala Glu Pro Asn Asn
                485                 490                 495


<210> 3
<211> 495
<212> PRT
<213> Arabidopsis thaliana

<400> 3
Met Ala Phe Glu Lys Asn Asn Glu Pro Phe Pro Leu His Phe Val Leu
 1                5                  10                  15
Phe Pro Phe Met Ala Gln Gly His Met Ile Pro Met Val Asp Ile Ala
            20                  25                  30
Arg Leu Leu Ala Gln Arg Gly Val Leu Ile Thr Ile Val Thr Thr Pro
        35                  40                  45
His Asn Ala Ala Arg Phe Lys Asn Val Leu Asn Arg Ala Ile Glu Ser
    50                  55                  60
Gly Leu Pro Ile Asn Leu Val Gln Val Lys Phe Pro Tyr Gln Glu Ala
65                  70                  75                  80
Gly Leu Gln Glu Gly Gln Glu Asn Met Asp Leu Leu Thr Thr Met Glu
                85                  90                  95
Gln Ile Thr Ser Phe Phe Lys Ala Val Asn Leu Leu Lys Glu Pro Val
            100                 105                 110
Gln Asn Leu Ile Glu Glu Met Ser Pro Arg Pro Ser Cys Leu Ile Ser
        115                 120                 125
Asp Met Cys Leu Ser Tyr Thr Ser Glu Ile Ala Lys Lys Phe Lys Ile
    130                 135                 140
Pro Lys Ile Leu Phe His Gly Met Gly Cys Phe Cys Leu Leu Cys Val
145                 150                 155                 160
Asn Val Leu Arg Lys Asn Arg Glu Ile Leu Asp Asn Leu Lys Ser Asp
                165                 170                 175
Lys Glu Tyr Phe Ile Val Pro Tyr Phe Pro Asp Arg Val Glu Phe Thr
            180                 185                 190
Arg Pro Gln Val Pro Val Glu Thr Tyr Val Pro Ala Gly Trp Lys Glu
        195                 200                 205
Ile Leu Glu Asp Met Val Glu Ala Asp Lys Thr Ser Tyr Gly Val Ile
    210                 215                 220
Val Asn Ser Phe Gln Glu Leu Glu Pro Ala Tyr Ala Lys Asp Phe Lys
225                 230                 235                 240
Glu Ala Arg Ser Gly Lys Ala Trp Thr Ile Gly Pro Val Ser Leu Cys
                245                 250                 255
Asn Lys Val Gly Val Asp Lys Ala Glu Arg Gly Asn Lys Ser Asp Ile
            260                 265                 270
Asp Gln Asp Glu Cys Leu Glu Trp Leu Asp Ser Lys Glu Pro Gly Ser
        275                 280                 285
Val Leu Tyr Val Cys Leu Gly Ser Ile Cys Asn Leu Pro Leu Ser Gln
    290                 295                 300
Leu Leu Glu Leu Gly Leu Gly Leu Glu Glu Ser Gln Arg Pro Phe Ile
305                 310                 315                 320
```

```
Trp Val Ile Arg Gly Trp Glu Lys Tyr Lys Glu Leu Val Glu Trp Phe
                325                 330                 335
Ser Glu Ser Gly Phe Glu Asp Arg Ile Gln Asp Arg Gly Leu Leu Ile
            340                 345                 350
Lys Gly Trp Ser Pro Gln Met Leu Ile Leu Ser His Pro Ser Val Gly
        355                 360                 365
Gly Phe Leu Thr His Cys Gly Trp Asn Ser Thr Leu Glu Gly Ile Thr
    370                 375                 380
Ala Gly Leu Pro Met Leu Thr Trp Pro Leu Phe Ala Asp Gln Phe Cys
385                 390                 395                 400
Asn Glu Lys Leu Val Val Gln Ile Leu Lys Val Gly Val Ser Ala Glu
                405                 410                 415
Val Lys Glu Val Met Lys Trp Gly Glu Glu Glu Lys Ile Gly Val Leu
            420                 425                 430
Val Asp Lys Glu Gly Val Lys Lys Ala Val Glu Glu Leu Met Gly Glu
        435                 440                 445
Ser Asp Asp Ala Lys Glu Arg Arg Arg Arg Ala Lys Glu Leu Gly Glu
    450                 455                 460
Ser Ala His Lys Ala Val Glu Glu Gly Gly Ser Ser His Ser Asn Ile
465                 470                 475                 480
Thr Phe Leu Leu Gln Asp Ile Met Gln Leu Ala Gln Ser Asn Asn
                485                 490                 495
```

<210> 4
<211> 495
<212> PRT
<213> Stevia rebaudiana

<400> 4

```
Met Ser Pro Lys Met Val Ala Pro Pro Thr Asn Leu His Phe Val Leu
 1               5                  10                  15
Phe Pro Leu Met Ala Gln Gly His Leu Val Pro Met Val Asp Ile Ala
            20                  25                  30
Arg Ile Leu Ala Gln Arg Gly Ala Thr Val Thr Ile Ile Thr Thr Pro
        35                  40                  45
Tyr His Ala Asn Arg Val Arg Pro Val Ile Ser Arg Ala Ile Ala Thr
    50                  55                  60
Asn Leu Lys Ile Gln Leu Leu Glu Leu Gln Leu Arg Ser Thr Glu Ala
65                  70                  75                  80
Gly Leu Pro Glu Gly Cys Glu Ser Phe Asp Gln Leu Pro Ser Phe Glu
                85                  90                  95
Tyr Trp Lys Asn Ile Ser Thr Ala Ile Asp Leu Leu Gln Gln Pro Ala
            100                 105                 110
Glu Asp Leu Leu Arg Glu Leu Ser Pro Pro Pro Asp Cys Ile Ile Ser
        115                 120                 125
Asp Phe Leu Phe Pro Trp Thr Thr Asp Val Ala Arg Arg Leu Asn Ile
    130                 135                 140
Pro Arg Leu Val Phe Asn Gly Pro Gly Cys Phe Tyr Leu Leu Cys Ile
145                 150                 155                 160
His Val Ala Ile Thr Ser Asn Ile Leu Gly Glu Asn Glu Pro Val Ser
                165                 170                 175
Ser Asn Thr Glu Arg Val Val Leu Pro Gly Leu Pro Asp Arg Ile Glu
            180                 185                 190
Val Thr Lys Leu Gln Ile Val Gly Ser Ser Arg Pro Ala Asn Val Asp
        195                 200                 205
Glu Met Gly Ser Trp Leu Arg Ala Val Glu Ala Glu Lys Ala Ser Phe
    210                 215                 220
Gly Ile Val Val Asn Thr Phe Glu Glu Leu Glu Pro Glu Tyr Val Glu
225                 230                 235                 240
Glu Tyr Lys Thr Val Lys Asp Lys Lys Met Trp Cys Ile Gly Pro Val
                245                 250                 255
Ser Leu Cys Asn Lys Thr Gly Pro Asp Leu Ala Glu Arg Gly Asn Lys
            260                 265                 270
Ala Ala Ile Thr Glu His Asn Cys Leu Lys Trp Leu Asp Glu Arg Lys
        275                 280                 285
Leu Gly Ser Val Leu Tyr Val Cys Leu Gly Ser Leu Ala Arg Ile Ser
    290                 295                 300
```

```
Ala Ala Gln Ala Ile Glu Leu Gly Leu Gly Leu Glu Ser Ile Asn Arg
305                 310                 315                 320
Pro Phe Ile Trp Cys Val Arg Asn Glu Thr Asp Glu Leu Lys Thr Trp
                325                 330                 335
Phe Leu Asp Gly Phe Glu Glu Arg Val Arg Asp Arg Gly Leu Ile Val
            340                 345                 350
His Gly Trp Ala Pro Gln Val Leu Ile Leu Ser His Pro Thr Ile Gly
        355                 360                 365
Gly Phe Leu Thr His Cys Gly Trp Asn Ser Thr Ile Glu Ser Ile Thr
    370                 375                 380
Ala Gly Val Pro Met Ile Thr Trp Pro Phe Phe Ala Asp Gln Phe Leu
385                 390                 395                 400
Asn Glu Ala Phe Ile Val Glu Val Leu Lys Ile Gly Val Arg Ile Gly
                405                 410                 415
Val Glu Arg Ala Cys Leu Phe Gly Glu Glu Asp Lys Val Gly Val Leu
            420                 425                 430
Val Lys Lys Glu Asp Val Lys Lys Ala Val Glu Cys Leu Met Asp Glu
        435                 440                 445
Asp Glu Asp Gly Asp Gln Arg Arg Lys Arg Val Ile Glu Leu Ala Lys
    450                 455                 460
Met Ala Lys Ile Ala Met Ala Glu Gly Gly Ser Ser Tyr Glu Asn Val
465                 470                 475                 480
Ser Ser Leu Ile Arg Asp Val Thr Glu Thr Val Arg Ala Pro His
                485                 490                 495


<210> 5
<211> 481
<212> PRT
<213> Stevia rebaudiana

<400> 5
Met Asp Ala Met Ala Thr Thr Glu Lys Lys Pro His Val Ile Phe Ile
 1                  5                  10                  15
Pro Phe Pro Ala Gln Ser His Ile Lys Ala Met Leu Lys Leu Ala Gln
            20                  25                  30
Leu Leu His His Lys Gly Leu Gln Ile Thr Phe Val Asn Thr Asp Phe
        35                  40                  45
Ile His Asn Gln Phe Leu Glu Ser Ser Gly Pro His Cys Leu Asp Gly
    50                  55                  60
Ala Pro Gly Phe Arg Phe Glu Thr Ile Pro Asp Gly Val Ser His Ser
65                  70                  75                  80
Pro Glu Ala Ser Ile Pro Ile Arg Glu Ser Leu Leu Arg Ser Ile Glu
                85                  90                  95
Thr Asn Phe Leu Asp Arg Phe Ile Asp Leu Val Thr Lys Leu Pro Asp
            100                 105                 110
Pro Pro Thr Cys Ile Ile Ser Asp Gly Phe Leu Ser Val Phe Thr Ile
        115                 120                 125
Asp Ala Ala Lys Lys Leu Gly Ile Pro Val Met Met Tyr Trp Thr Leu
    130                 135                 140
Ala Ala Cys Gly Phe Met Gly Phe Tyr His Ile His Ser Leu Ile Glu
145                 150                 155                 160
Lys Gly Phe Ala Pro Leu Lys Asp Ala Ser Tyr Leu Thr Asn Gly Tyr
                165                 170                 175
Leu Asp Thr Val Ile Asp Trp Val Pro Gly Met Glu Gly Ile Arg Leu
            180                 185                 190
Lys Asp Phe Pro Leu Asp Trp Ser Thr Asp Leu Asn Asp Lys Val Leu
        195                 200                 205
Met Phe Thr Thr Glu Ala Pro Gln Arg Ser His Lys Val Ser His His
    210                 215                 220
Ile Phe His Thr Phe Asp Glu Leu Glu Pro Ser Ile Ile Lys Thr Leu
225                 230                 235                 240
Ser Leu Arg Tyr Asn His Ile Tyr Thr Ile Gly Pro Leu Gln Leu Leu
                245                 250                 255
Leu Asp Gln Ile Pro Glu Glu Lys Lys Gln Thr Gly Ile Thr Ser Leu
            260                 265                 270
His Gly Tyr Ser Leu Val Lys Glu Glu Pro Glu Cys Phe Gln Trp Leu
        275                 280                 285
```

```
Gln Ser Lys Glu Pro Asn Ser Val Val Tyr Val Asn Phe Gly Ser Thr
    290                 295                 300
Thr Val Met Ser Leu Glu Asp Met Thr Glu Phe Gly Trp Gly Leu Ala
305                 310                 315                 320
Asn Ser Asn His Tyr Phe Leu Trp Ile Ile Arg Ser Asn Leu Val Ile
                325                 330                 335
Gly Glu Asn Ala Val Leu Pro Pro Glu Leu Glu Glu His Ile Lys Lys
            340                 345                 350
Arg Gly Phe Ile Ala Ser Trp Cys Ser Gln Glu Lys Val Leu Lys His
        355                 360                 365
Pro Ser Val Gly Gly Phe Leu Thr His Cys Gly Trp Gly Ser Thr Ile
    370                 375                 380
Glu Ser Leu Ser Ala Gly Val Pro Met Ile Cys Trp Pro Tyr Ser Trp
385                 390                 395                 400
Asp Gln Leu Thr Asn Cys Arg Tyr Ile Cys Lys Glu Trp Glu Val Gly
                405                 410                 415
Leu Glu Met Gly Thr Lys Val Lys Arg Asp Glu Val Lys Arg Leu Val
            420                 425                 430
Gln Glu Leu Met Gly Glu Gly Gly His Lys Met Arg Asn Lys Ala Lys
        435                 440                 445
Asp Trp Lys Glu Lys Ala Arg Ile Ala Ile Ala Pro Asn Gly Ser Ser
    450                 455                 460
Ser Leu Asn Ile Asp Lys Met Val Lys Glu Ile Thr Val Leu Ala Arg
465                 470                 475                 480
Asn
```