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Andersen-Ranberg et al.

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(54) PRODUCTION OF OXYGENATED DITERPENOID COMPOUNDS

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(71) Applicant: Københavns Universitet, Copenhagen K (DK)

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(57) ABSTRACT

Disclosed is a method for production of oxygenated diterpenoid compounds, such as triptophenolide, triptonide and triptolide, by inserting genes encoding particular cytochrome P450 enzymes and expressing the genes in selected host cells for synthesis of the compounds. Further disclosed are particular cytochrome P450 enzymes suitable for this synthesis.

Specification includes a Sequence Listing.

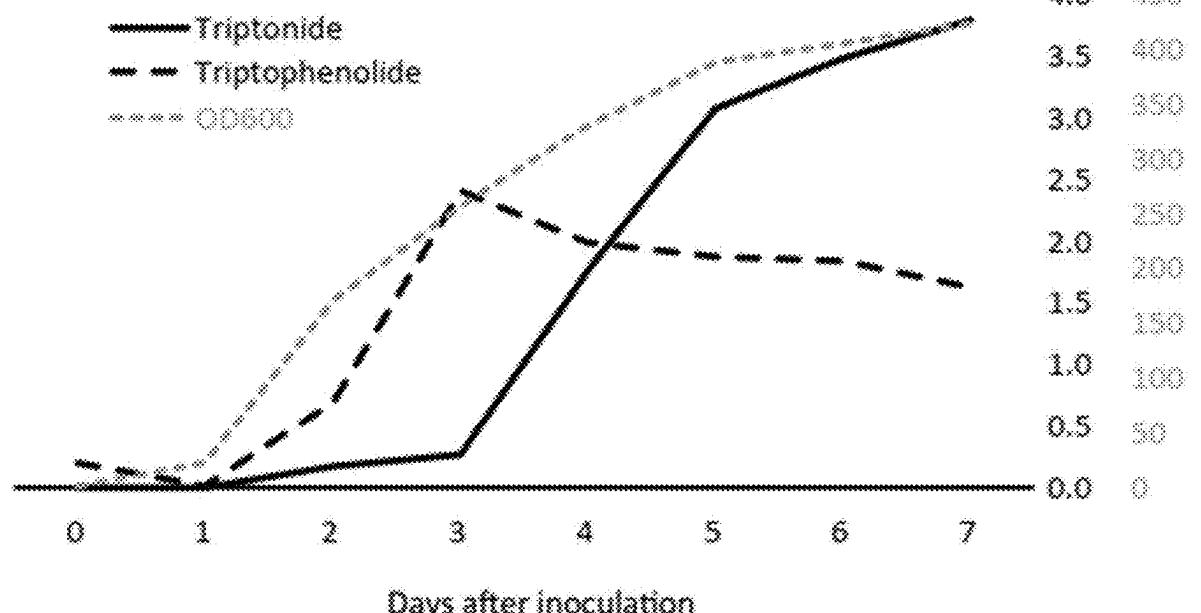


Figure 1

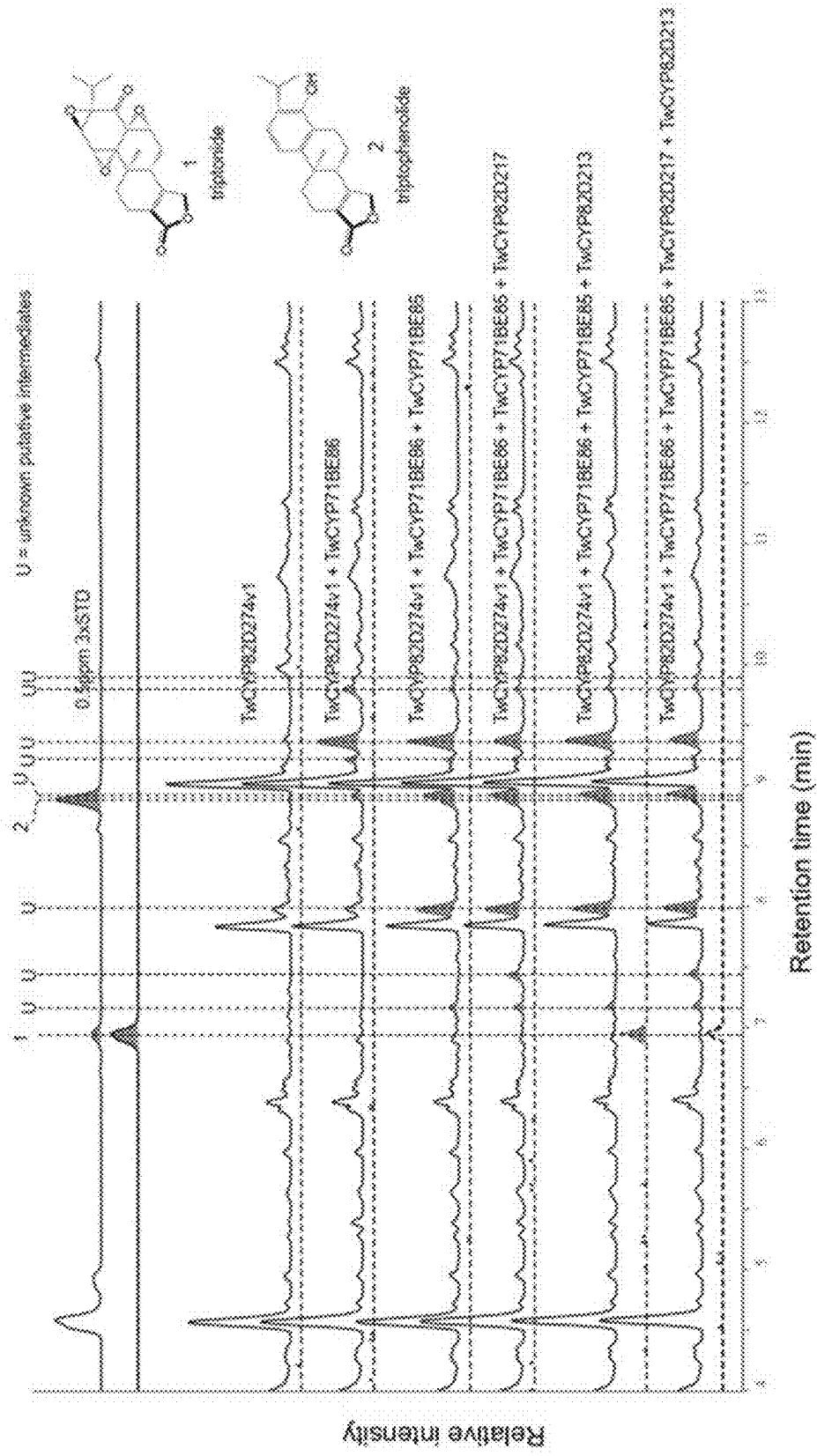


Figure 2

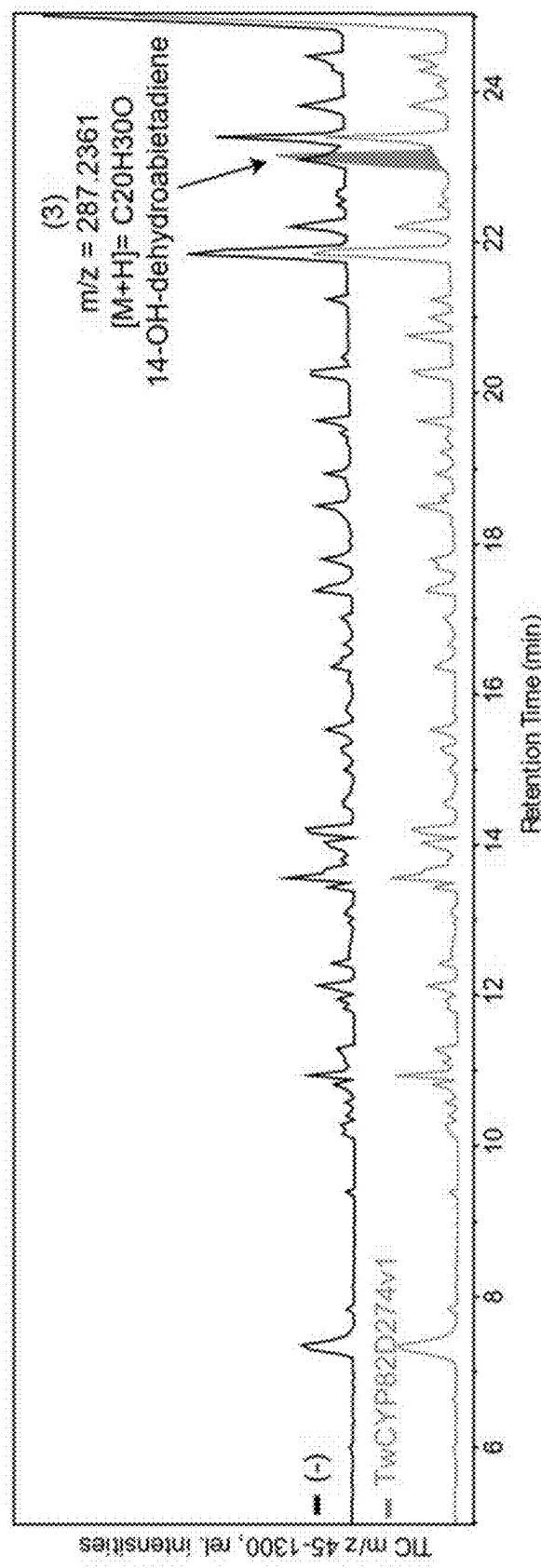
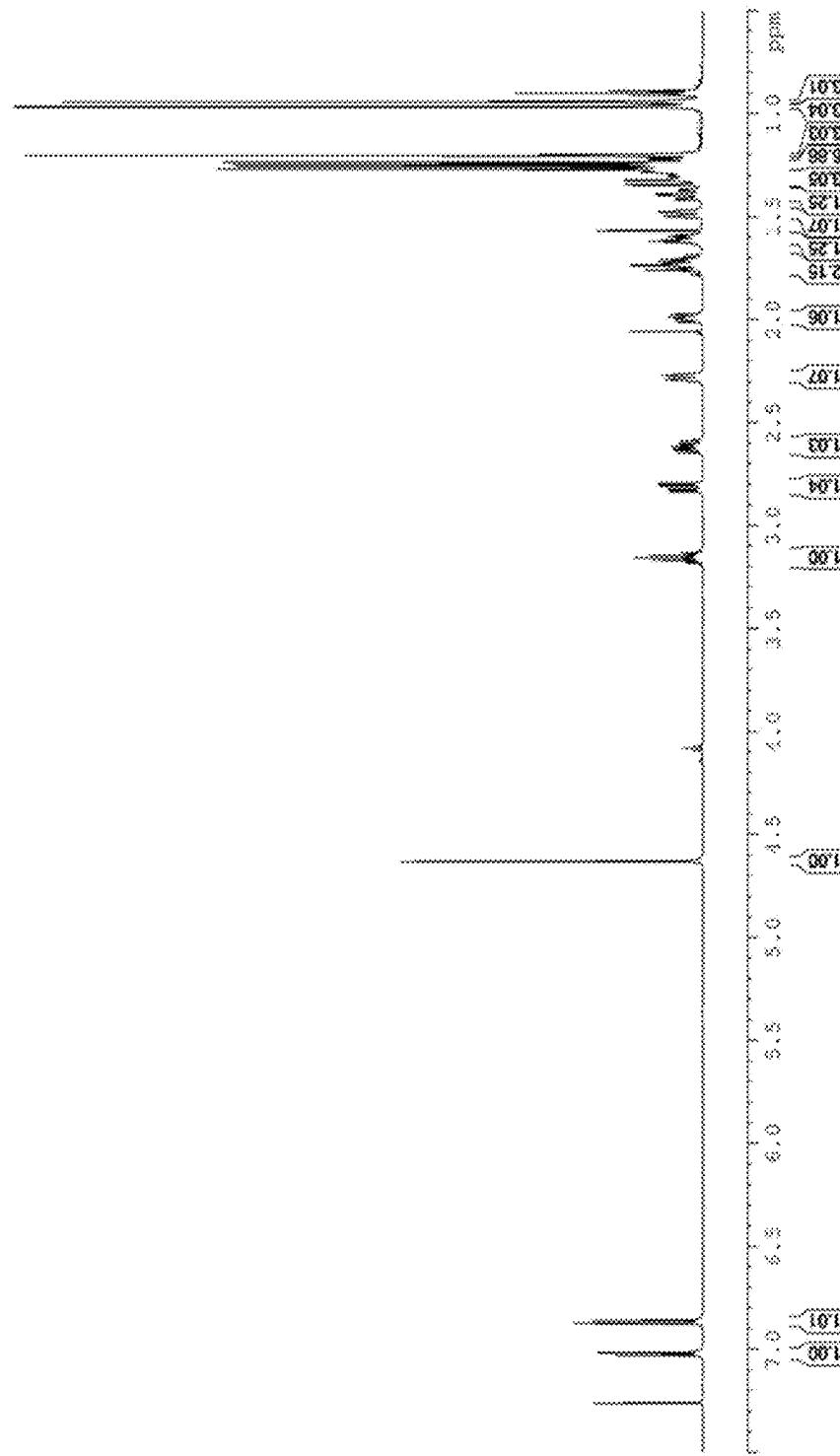


Figure 3.



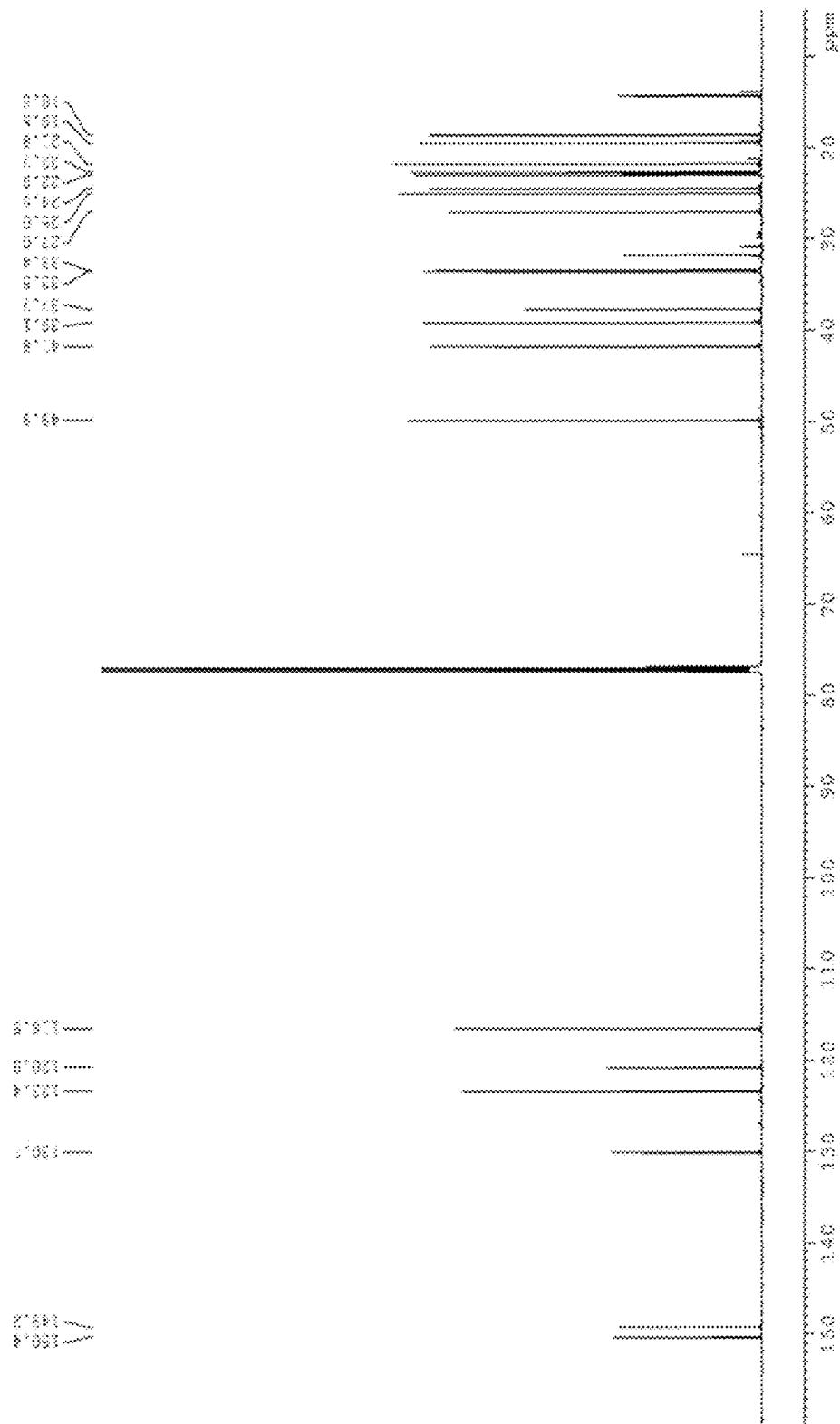
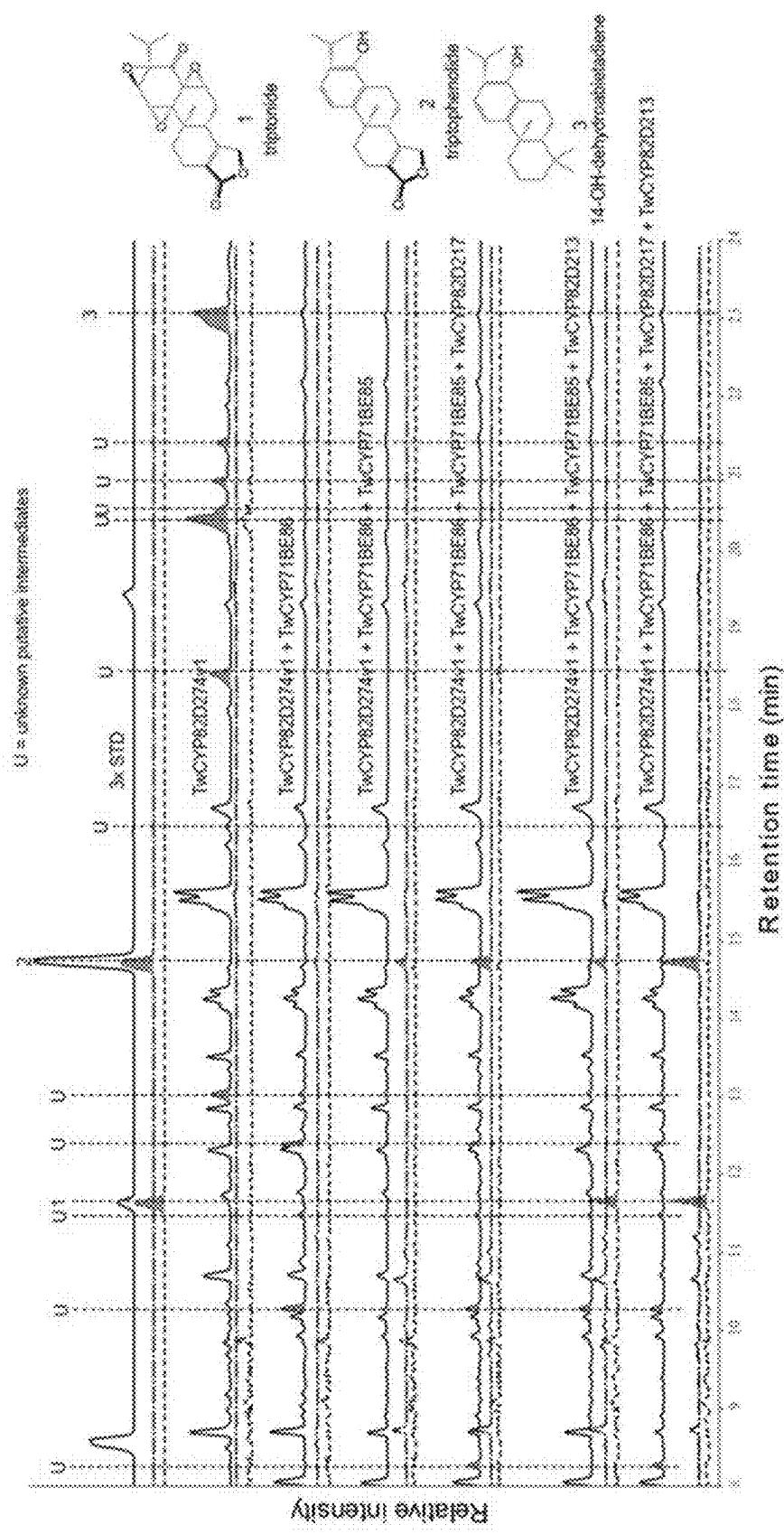
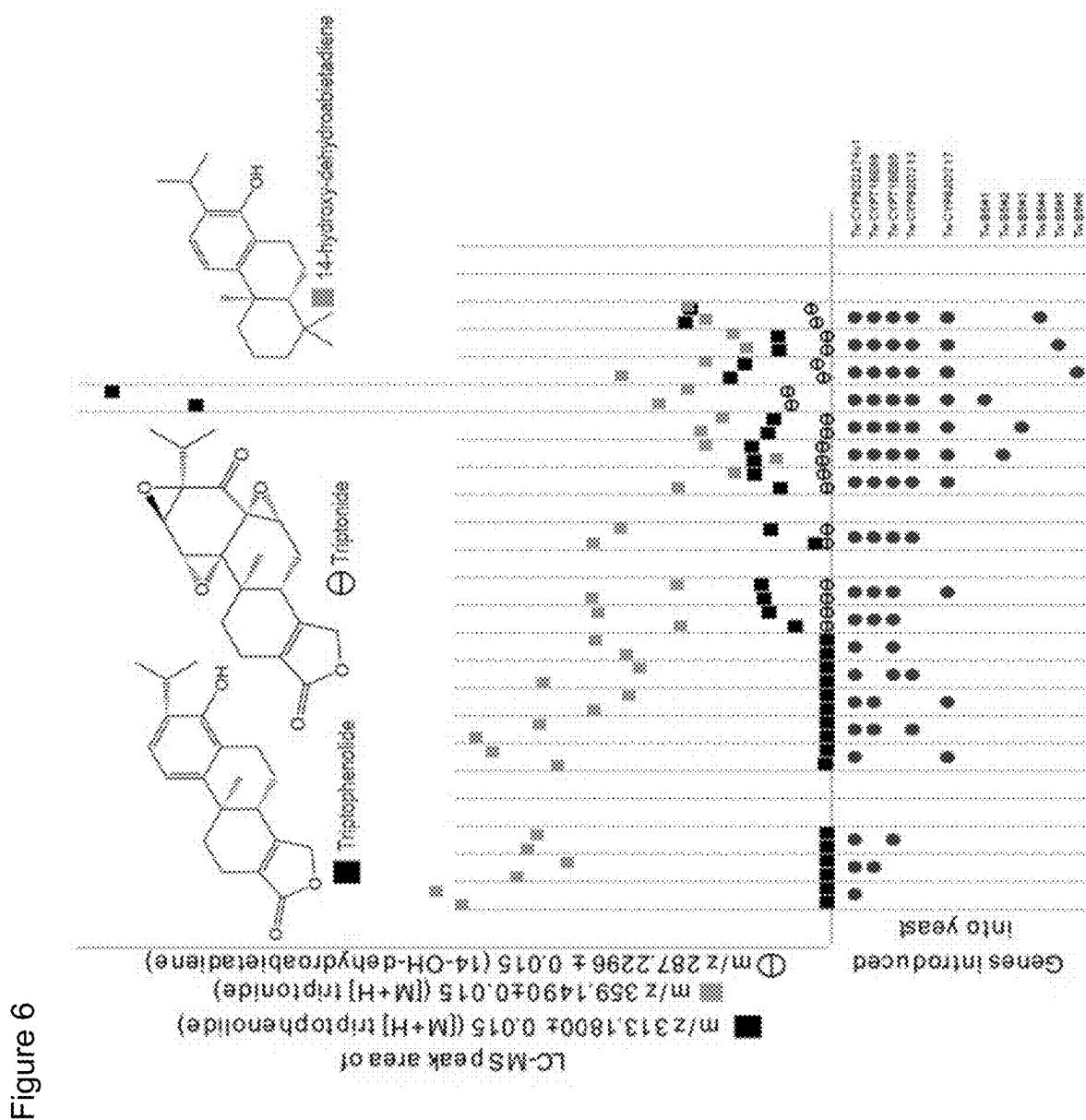


Figure 4

Figure 5





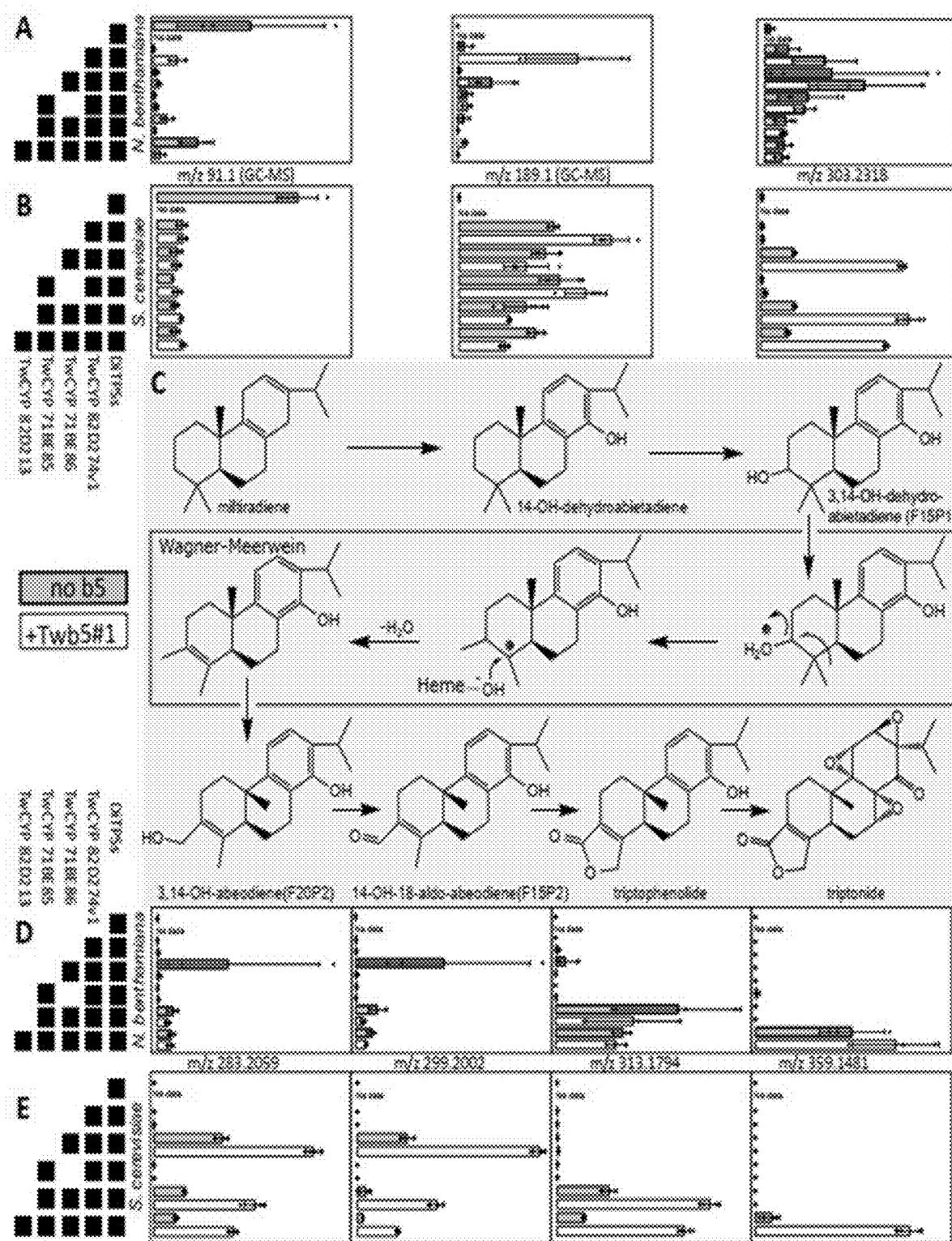
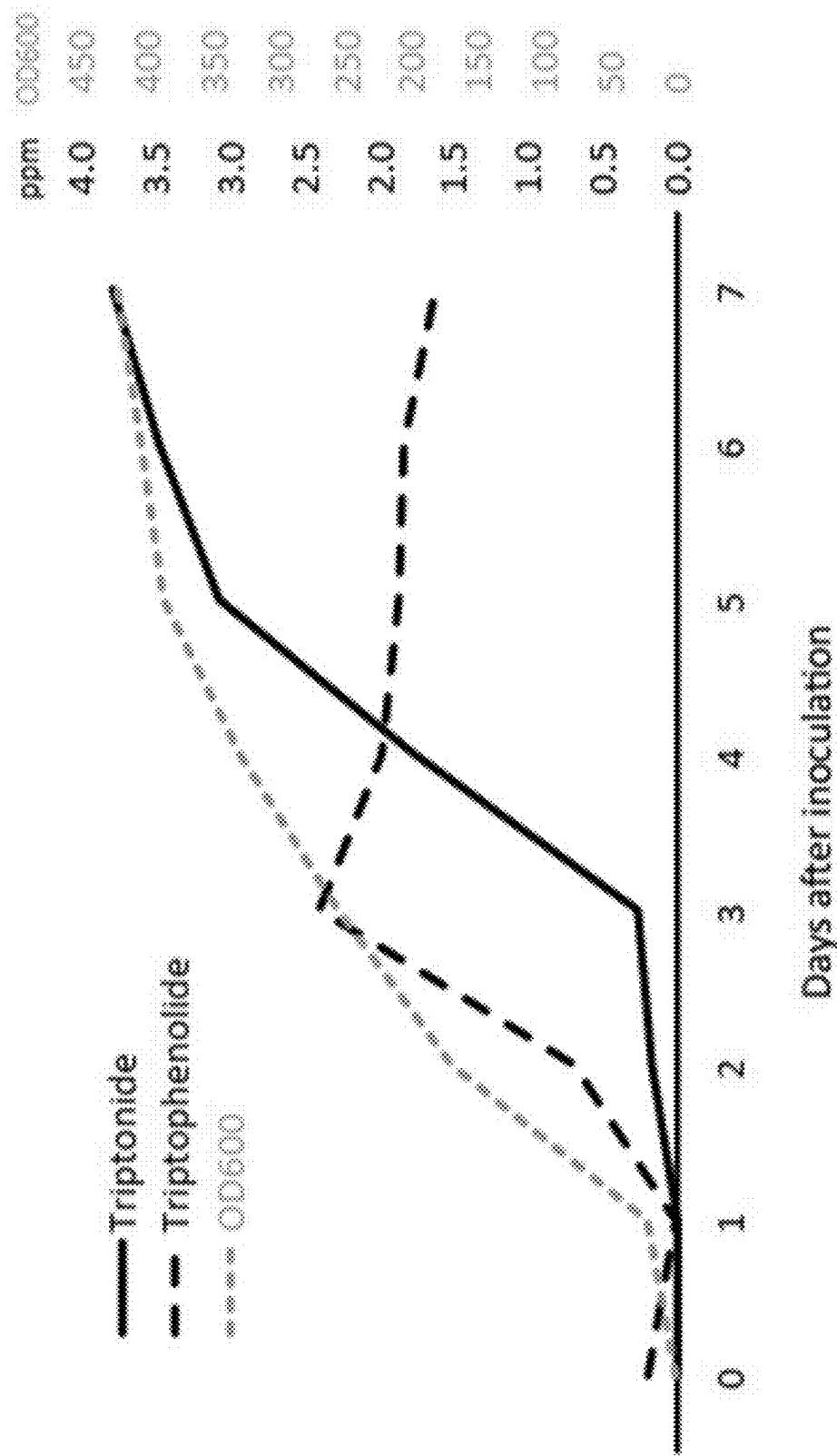


Figure 7

Figure 8



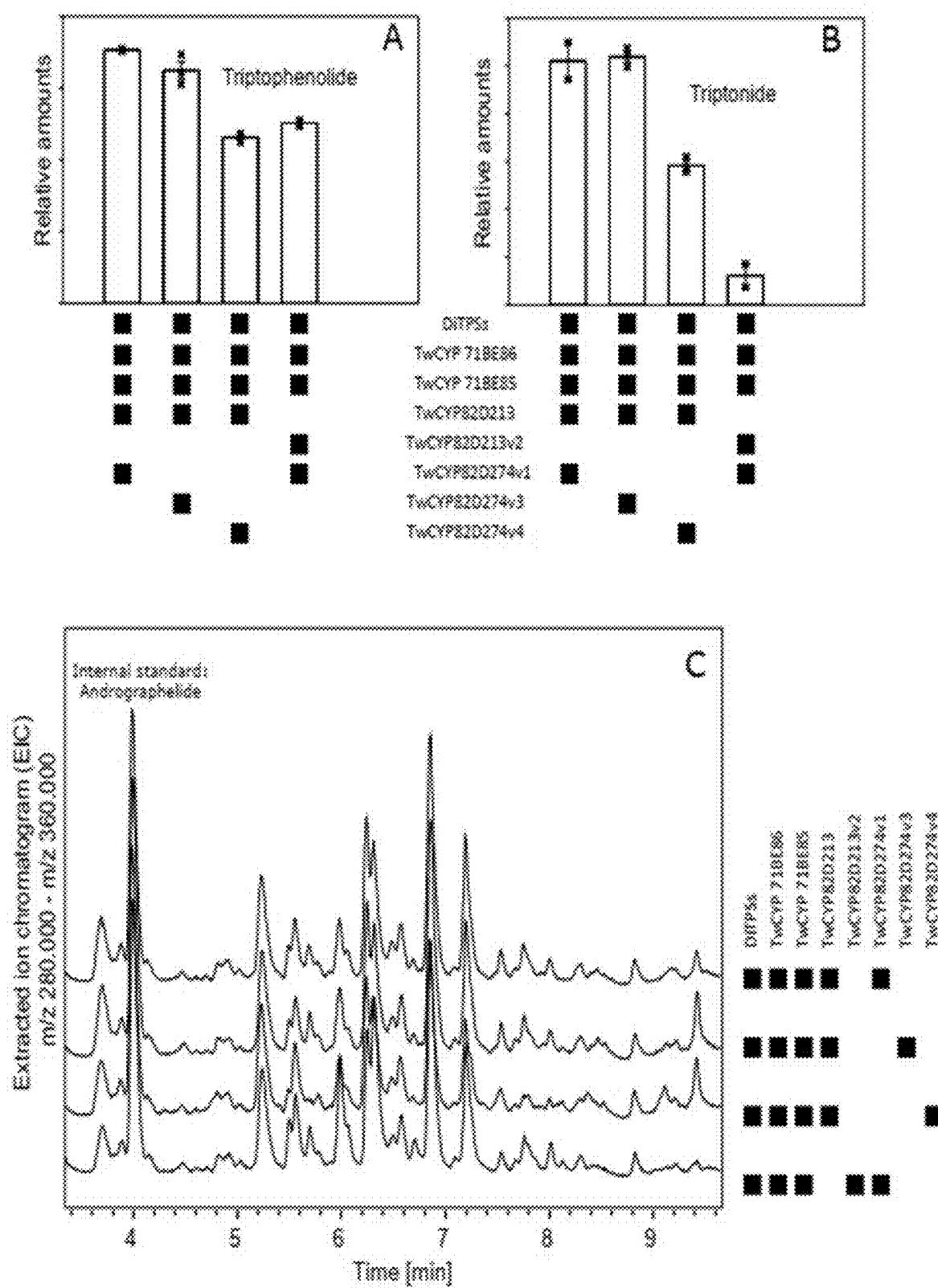
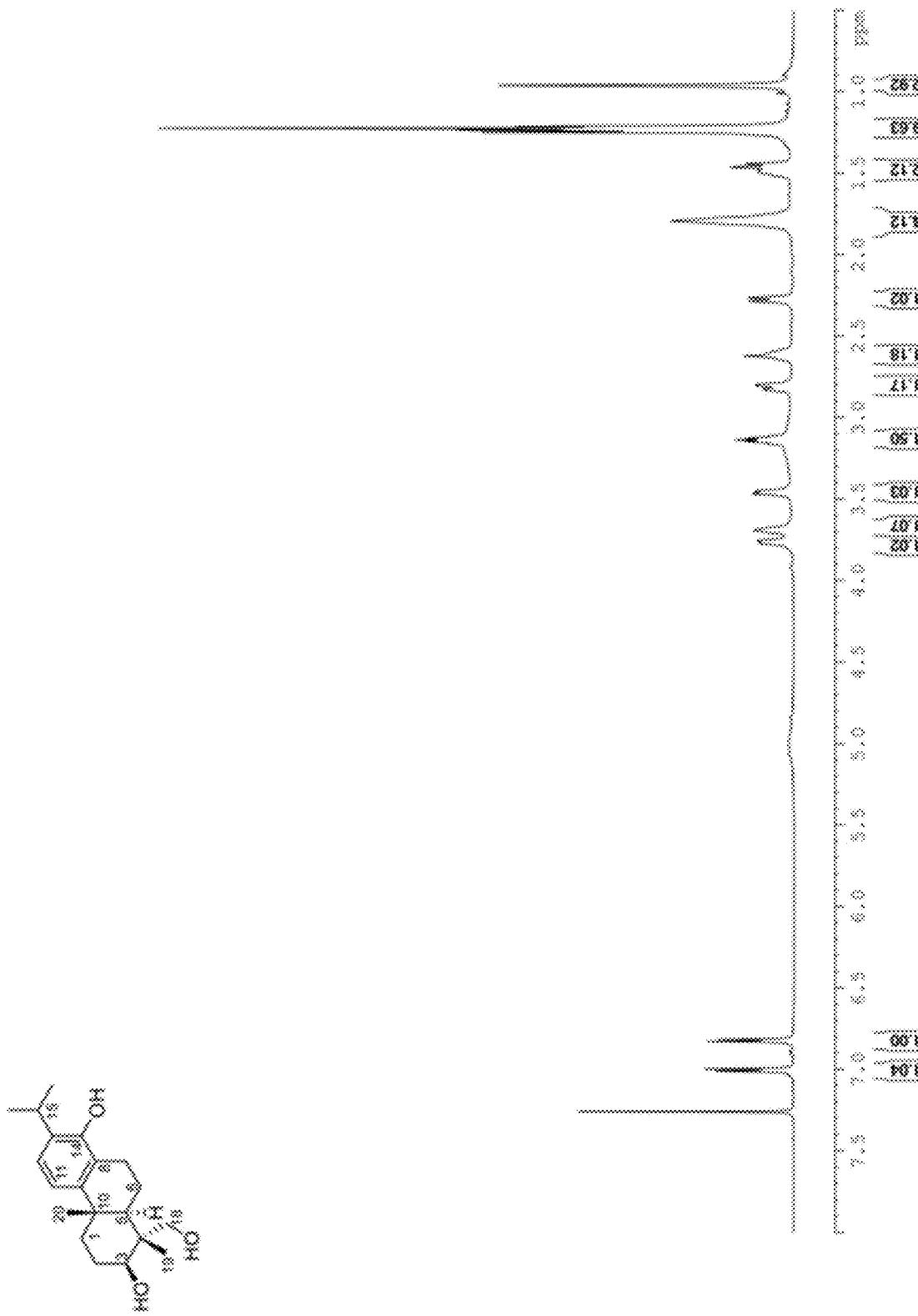


Figure 9

Figure 10



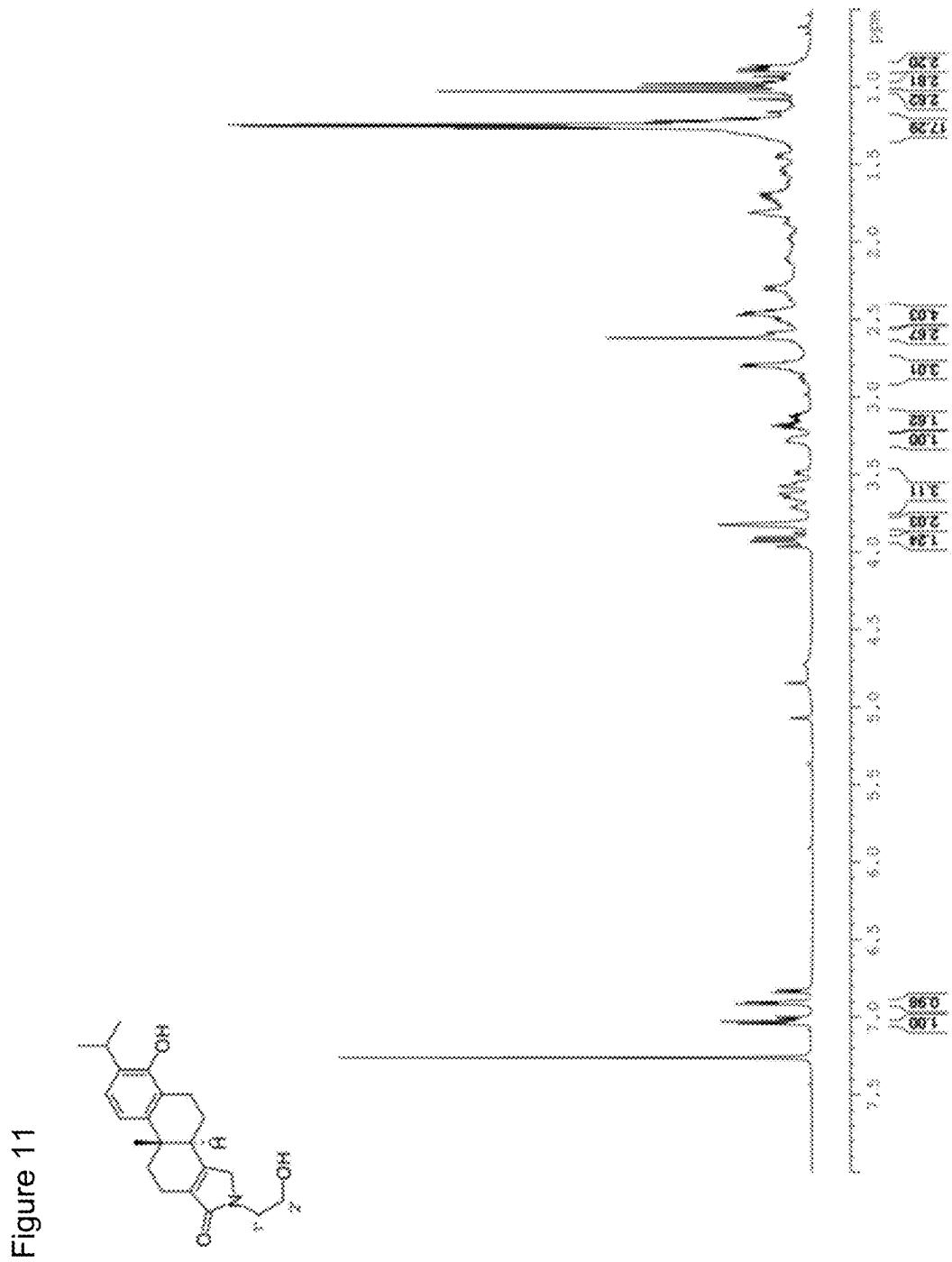


Figure 12

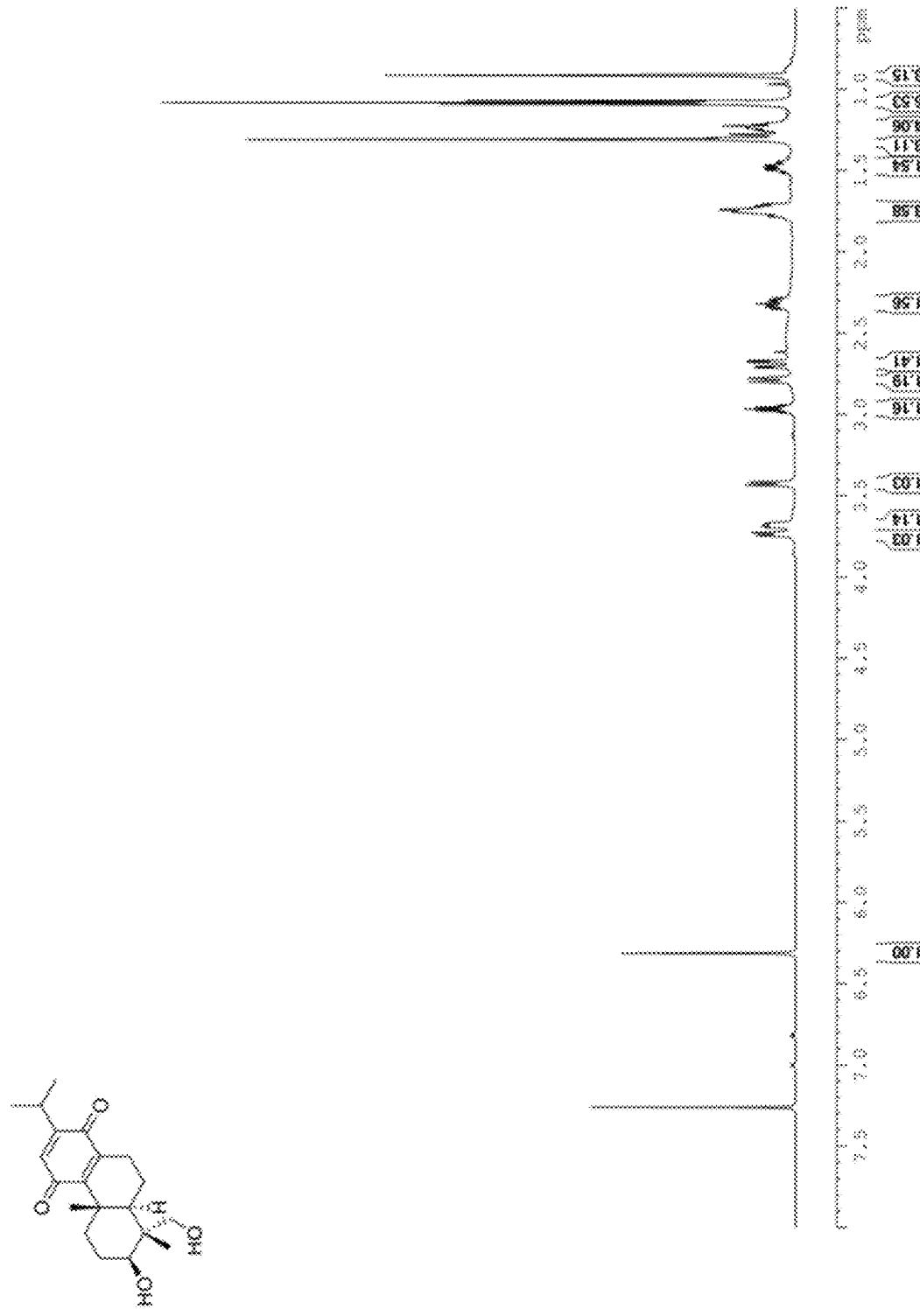


Figure 13

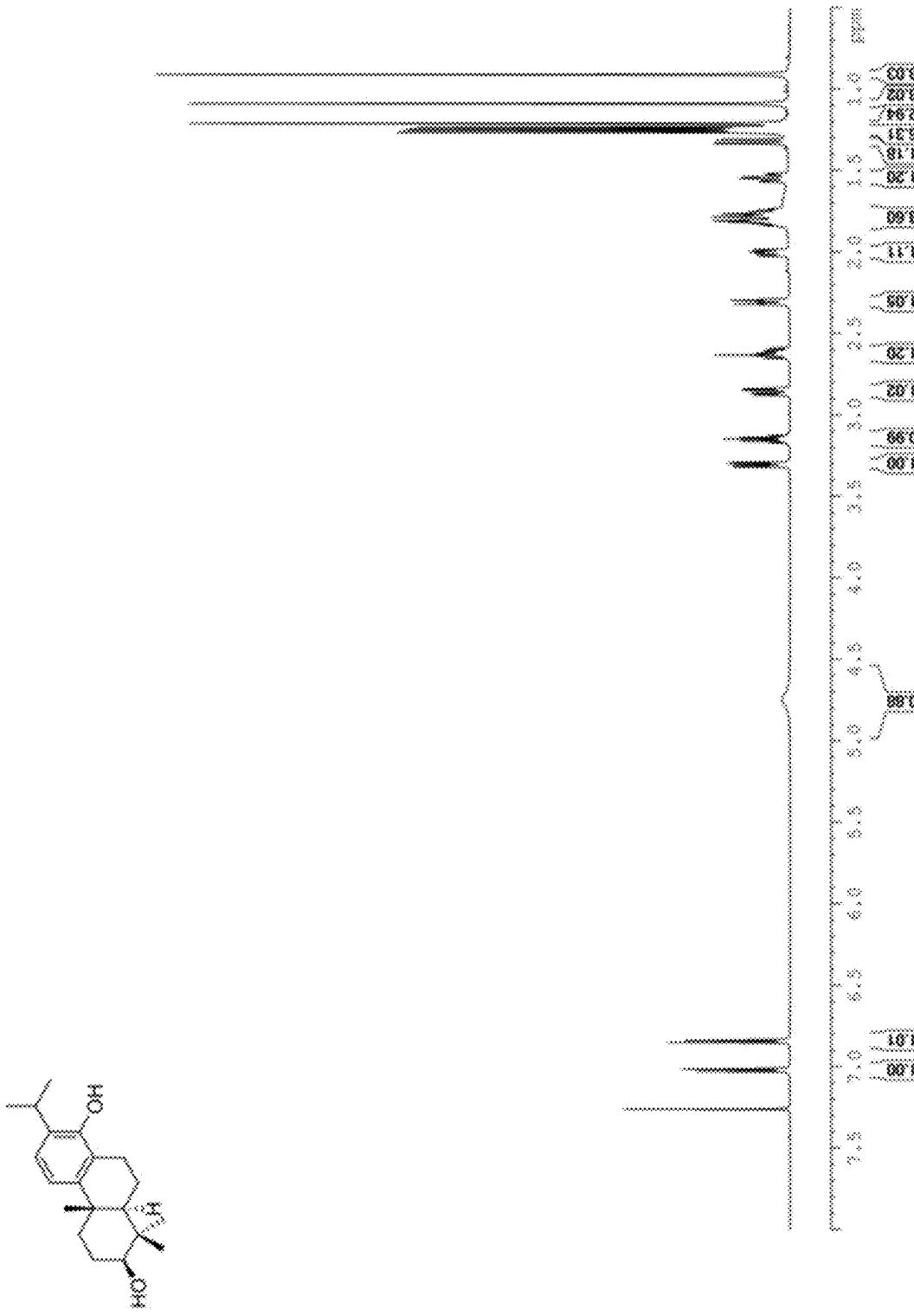
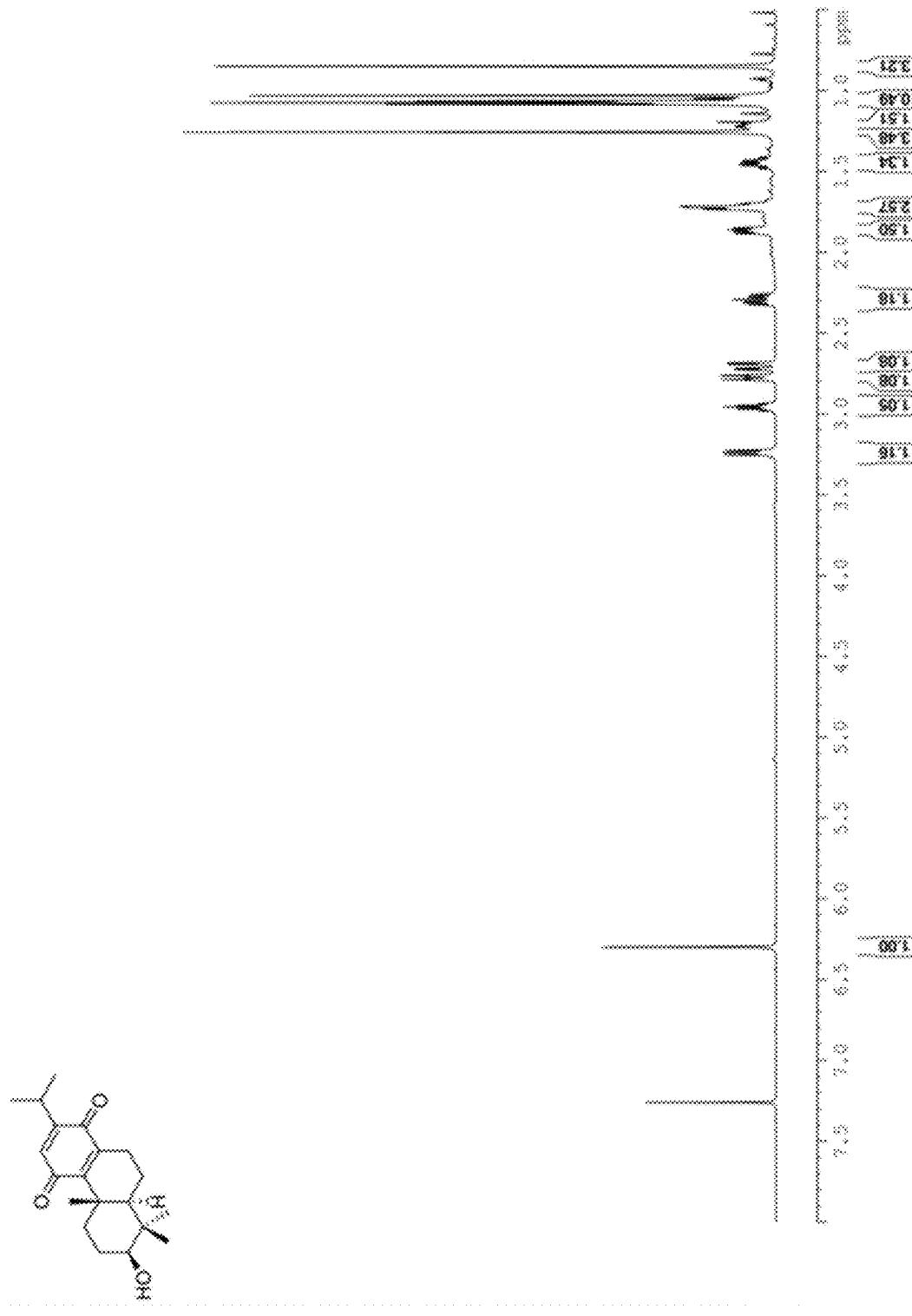


Figure 14



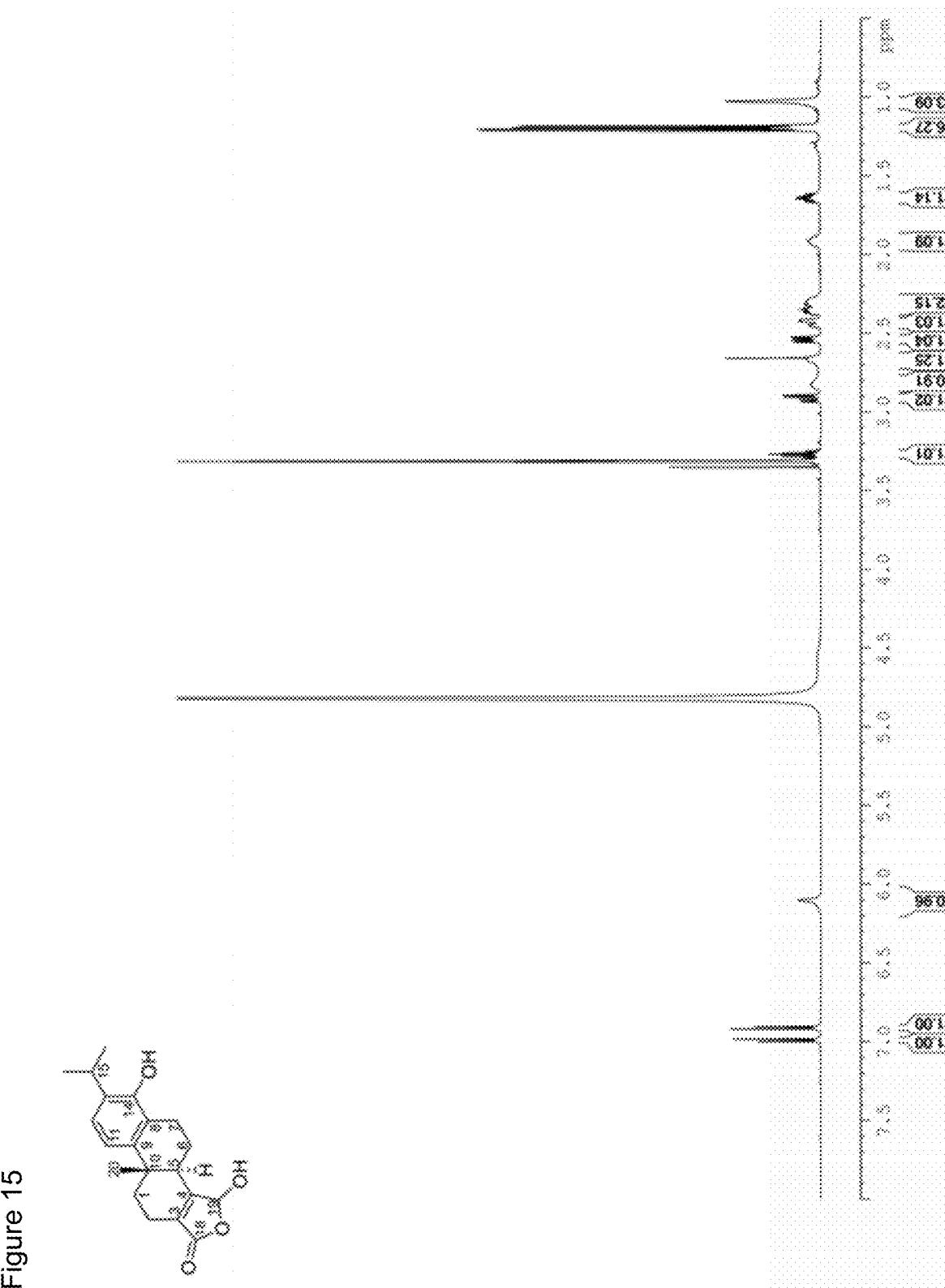


Figure 16

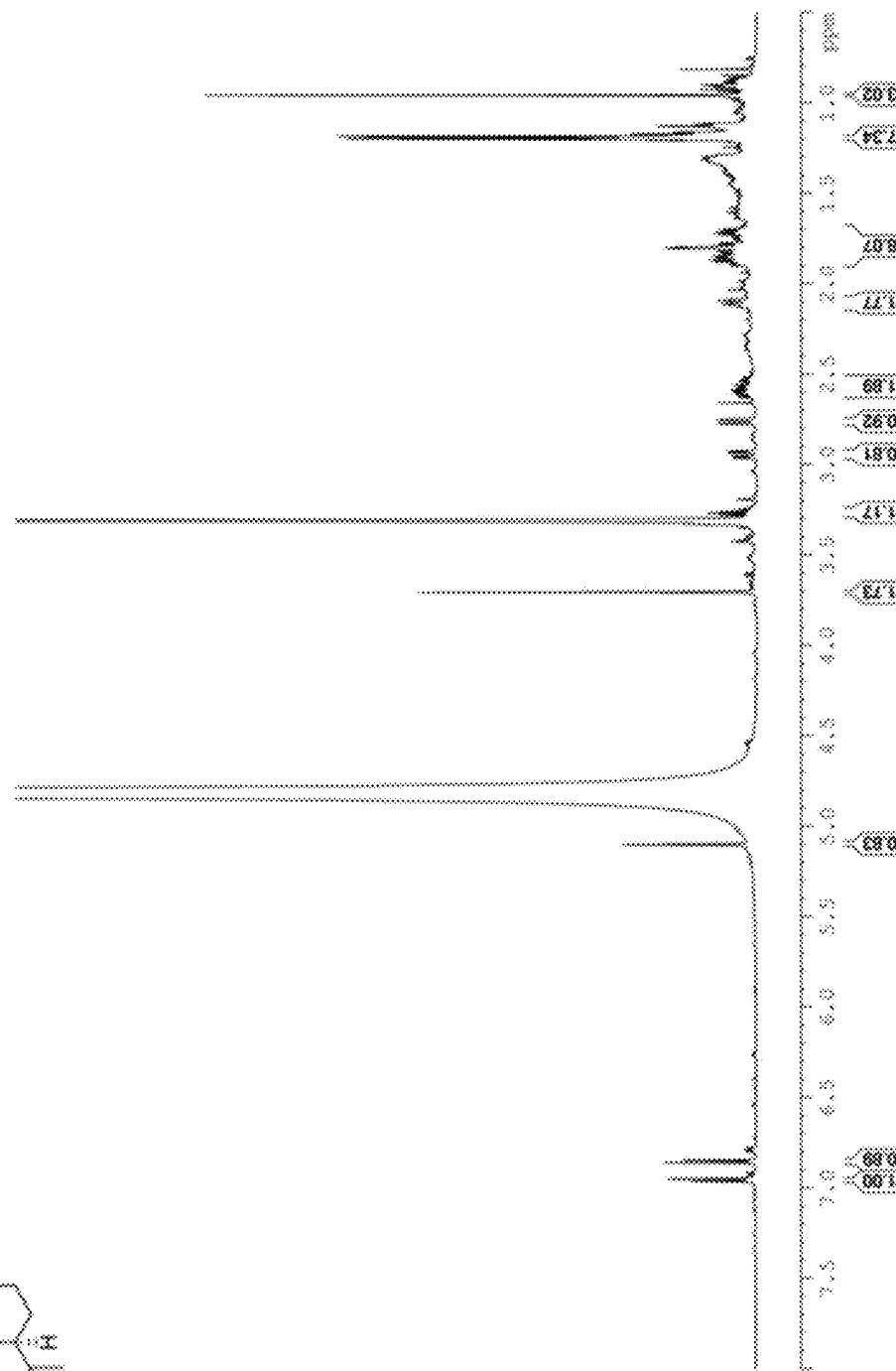
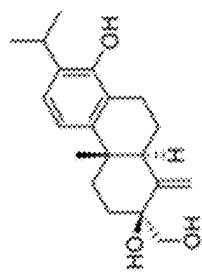


Figure 17

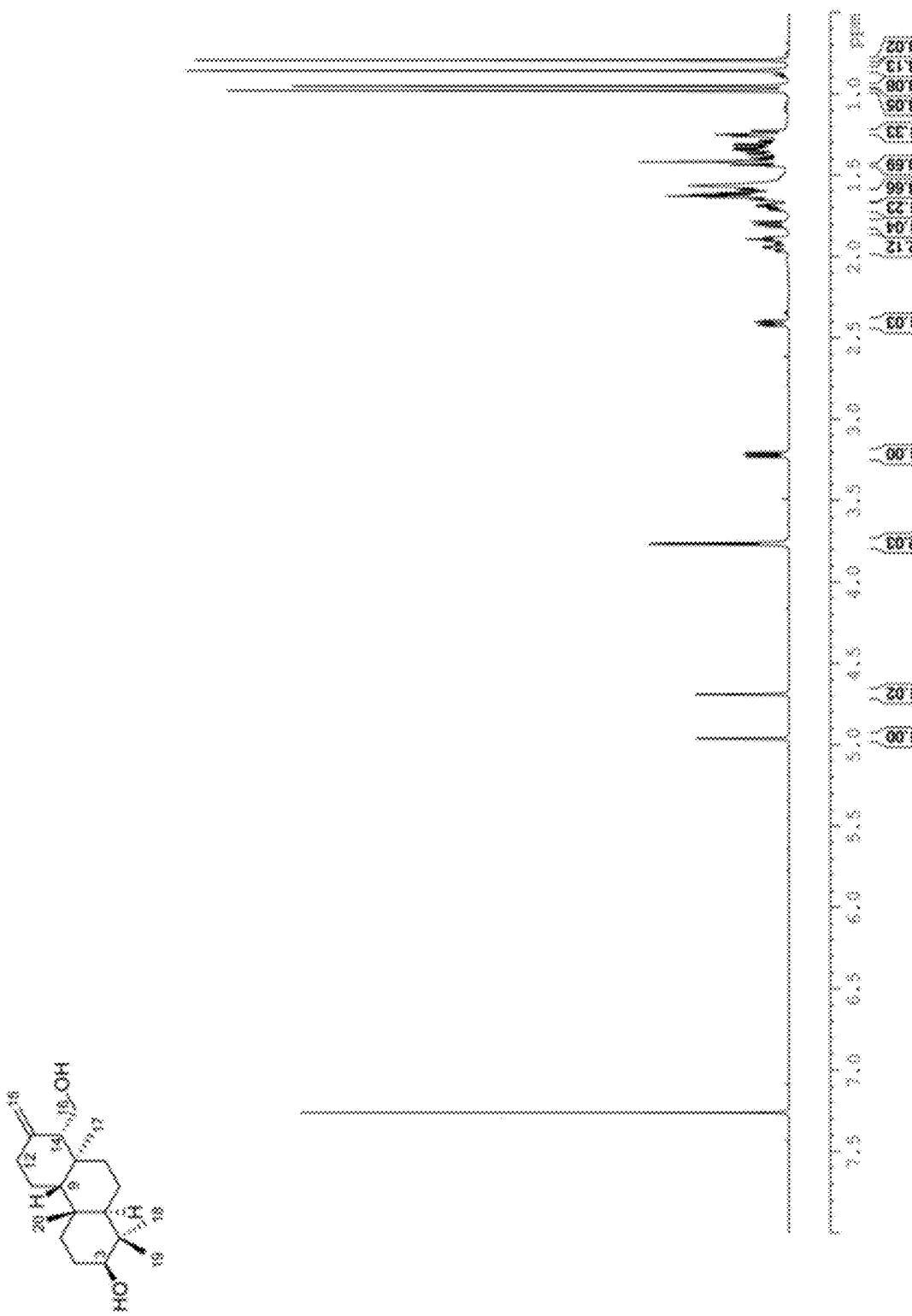


Figure 18

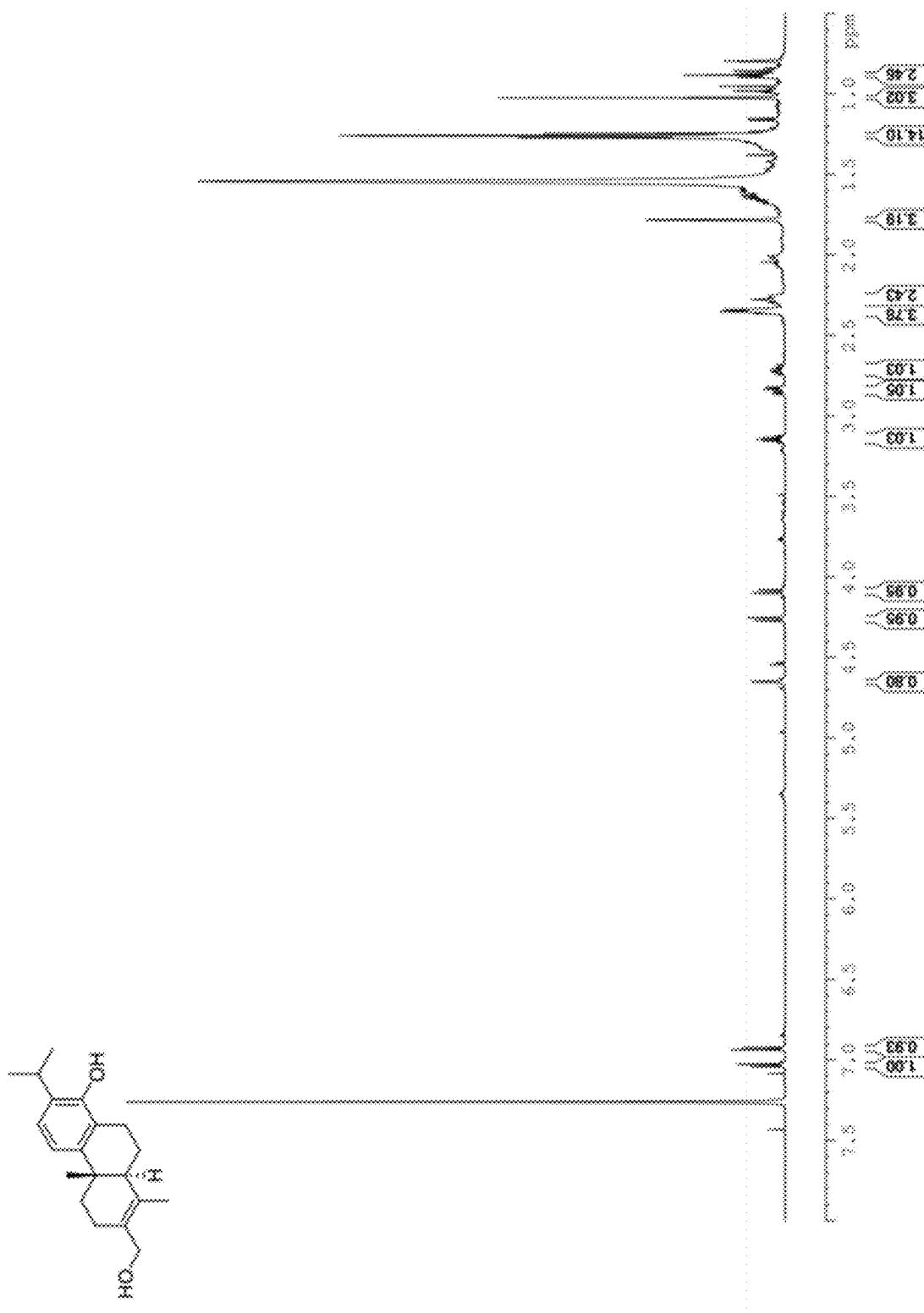
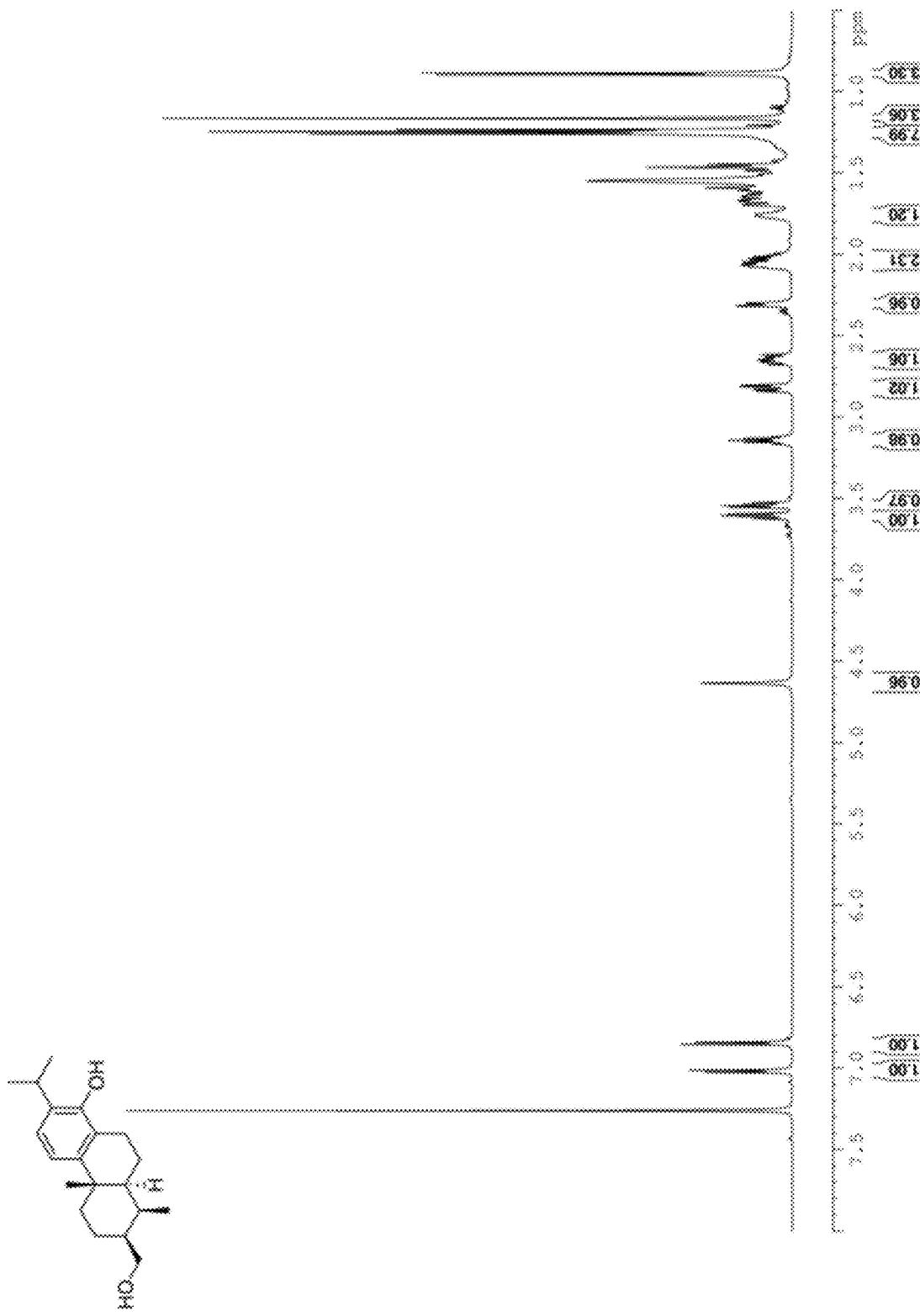


Figure 19



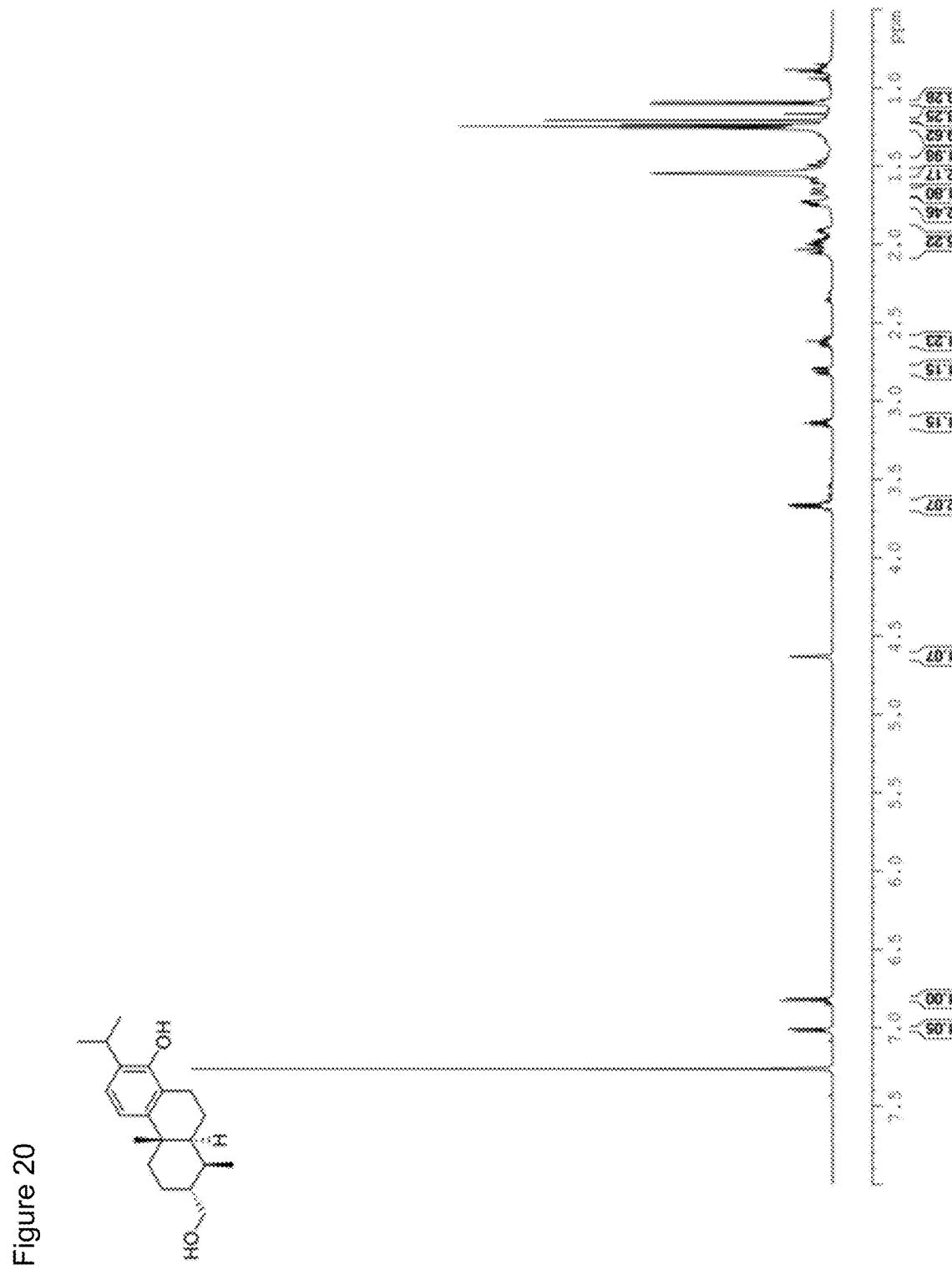


Figure 21

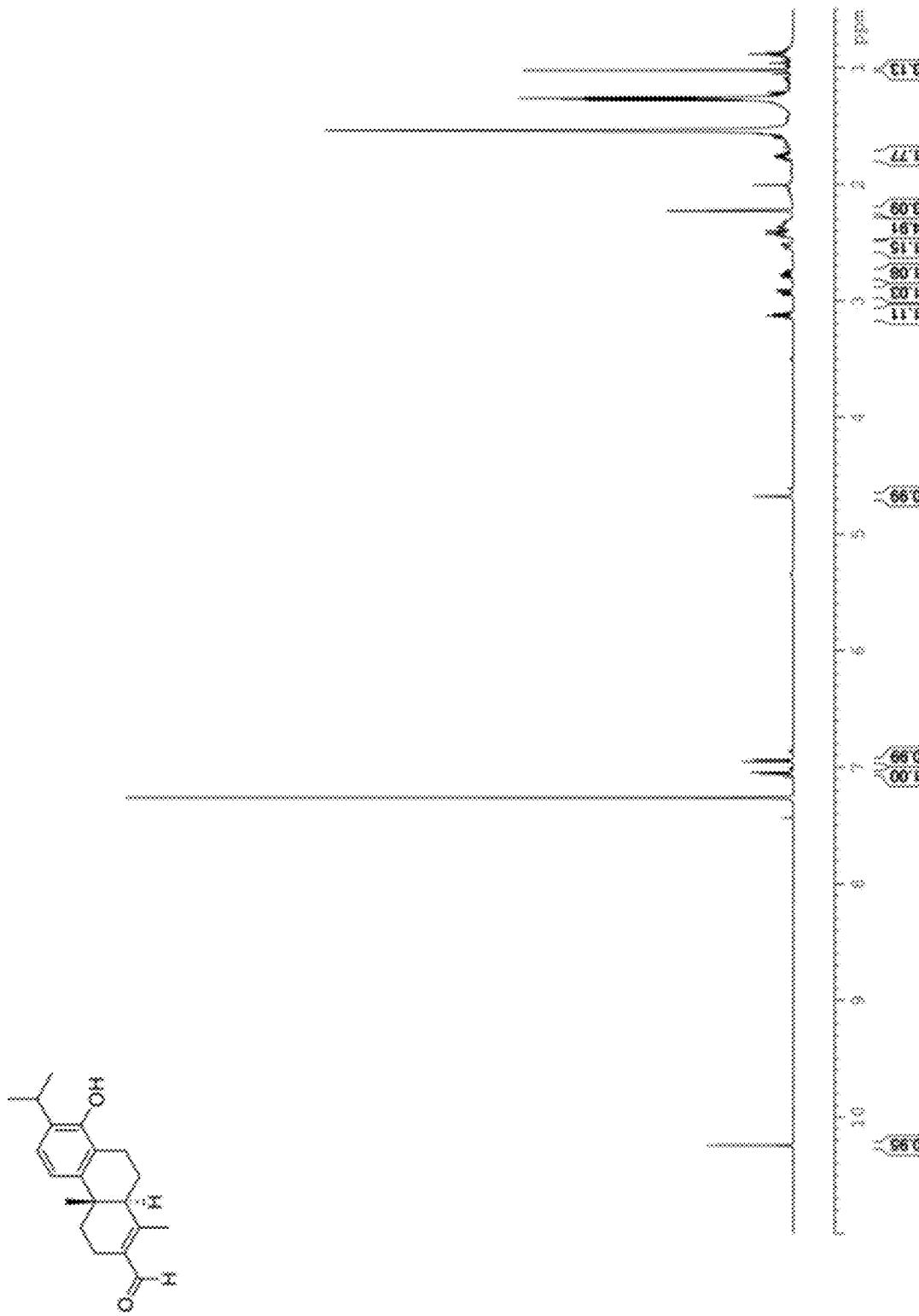


Figure 22

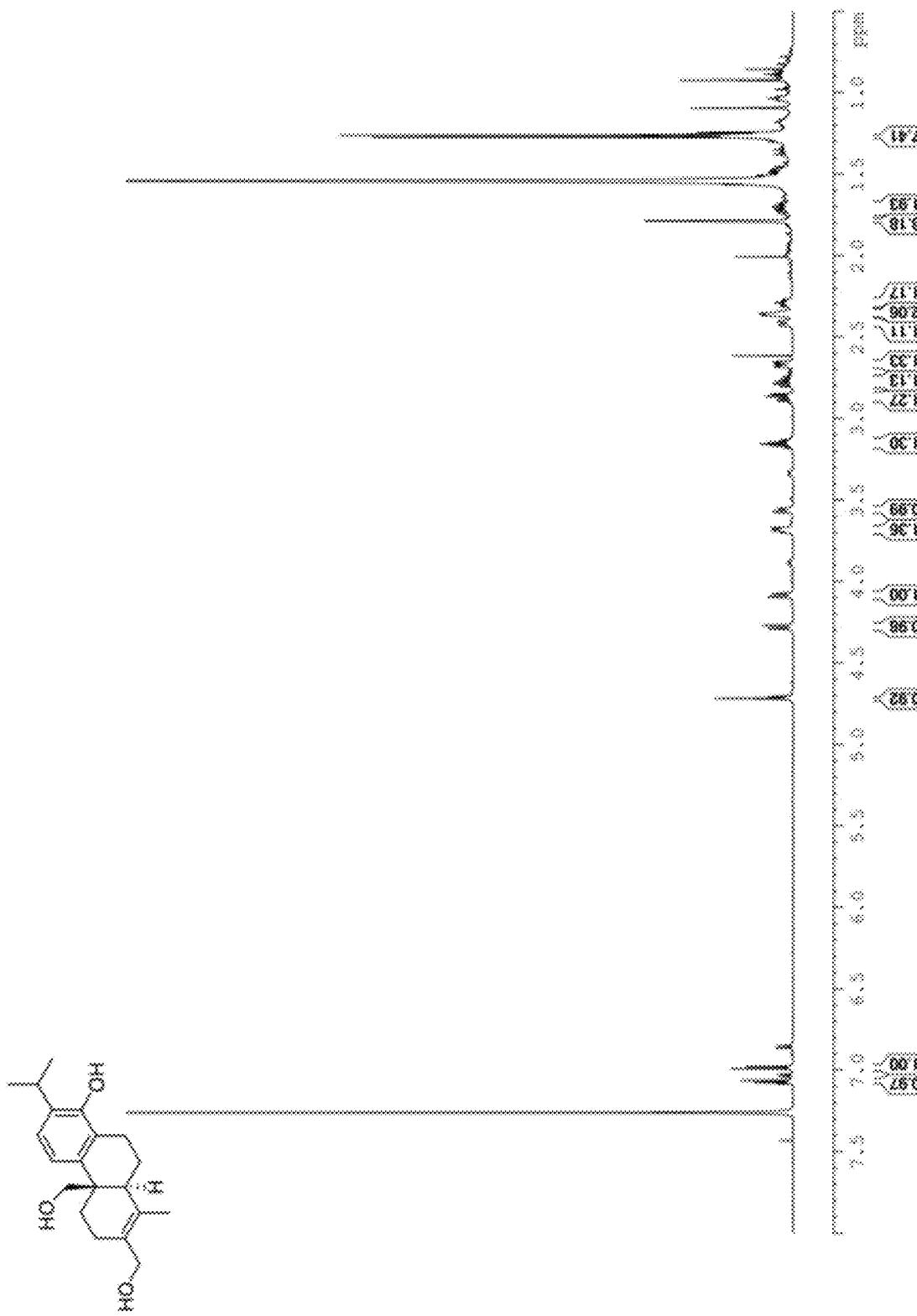


Figure 23

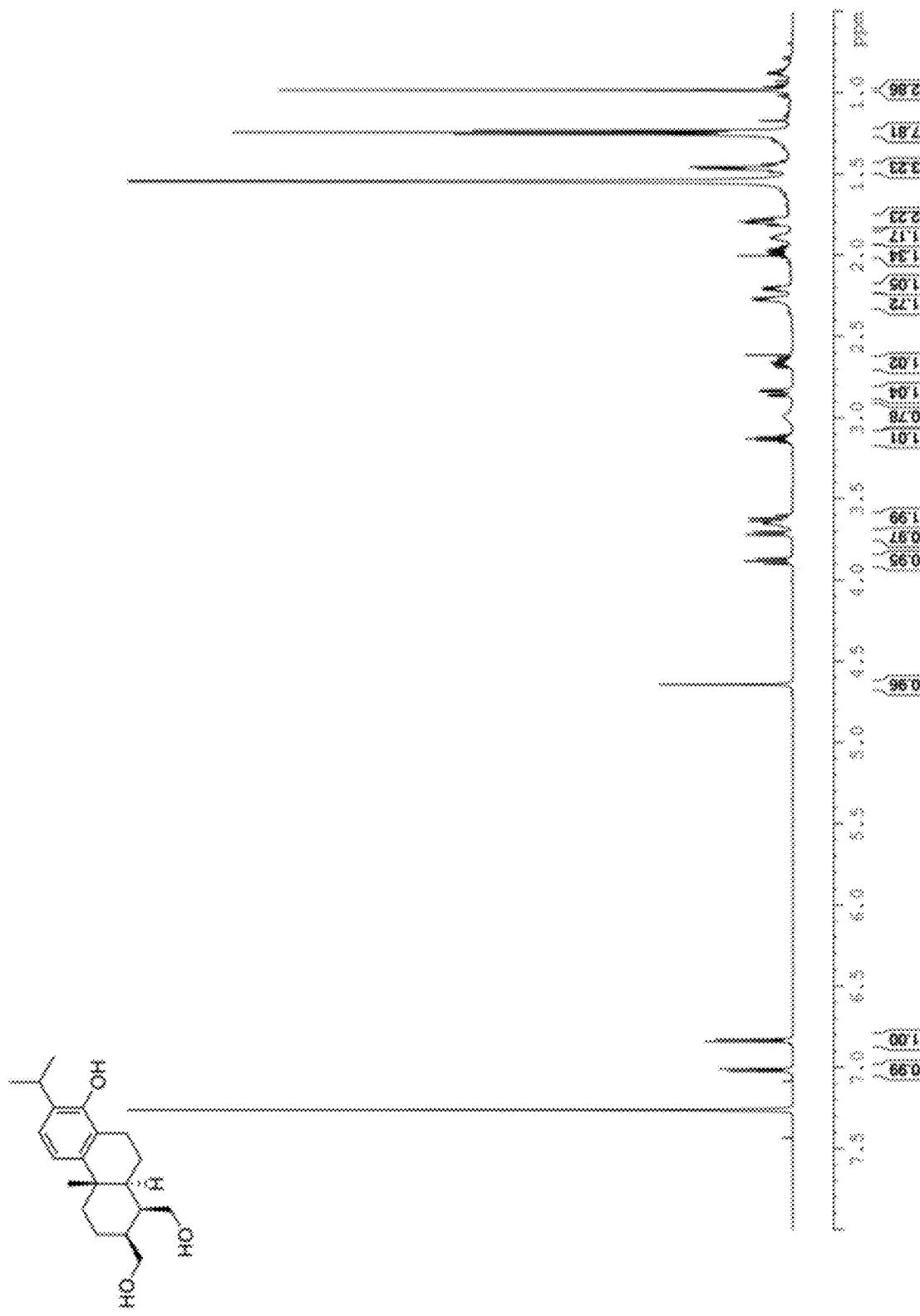


Figure 24

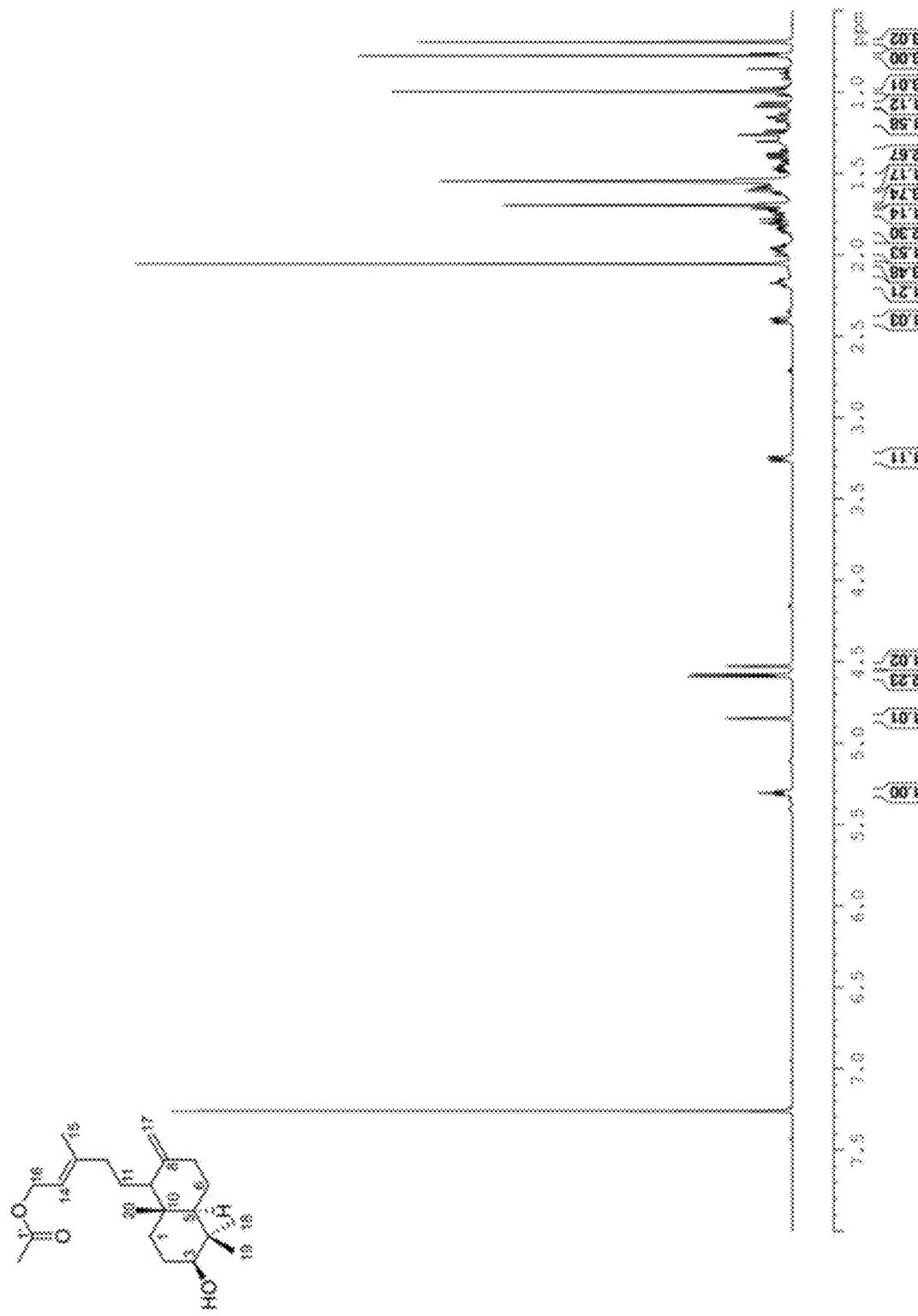
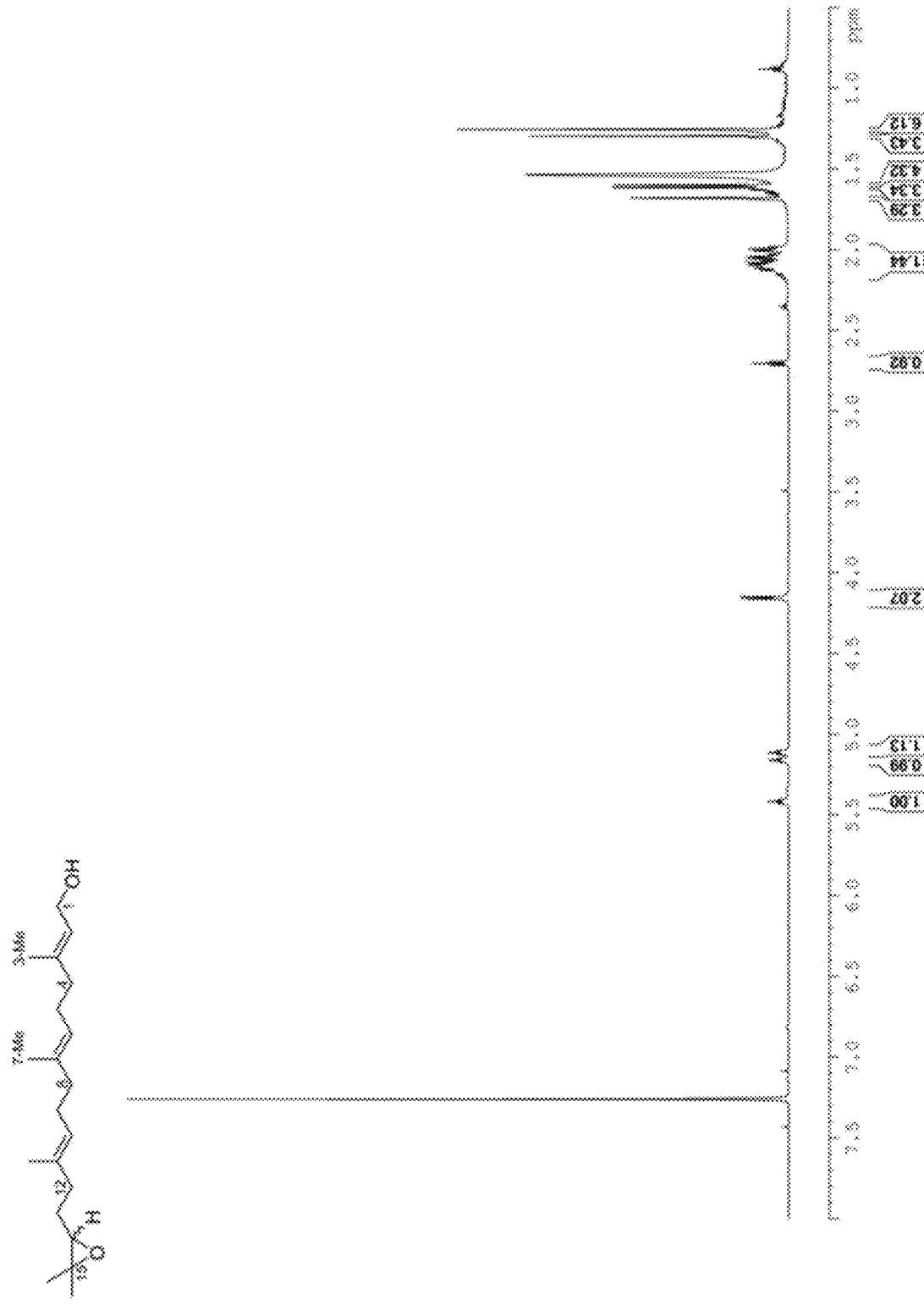
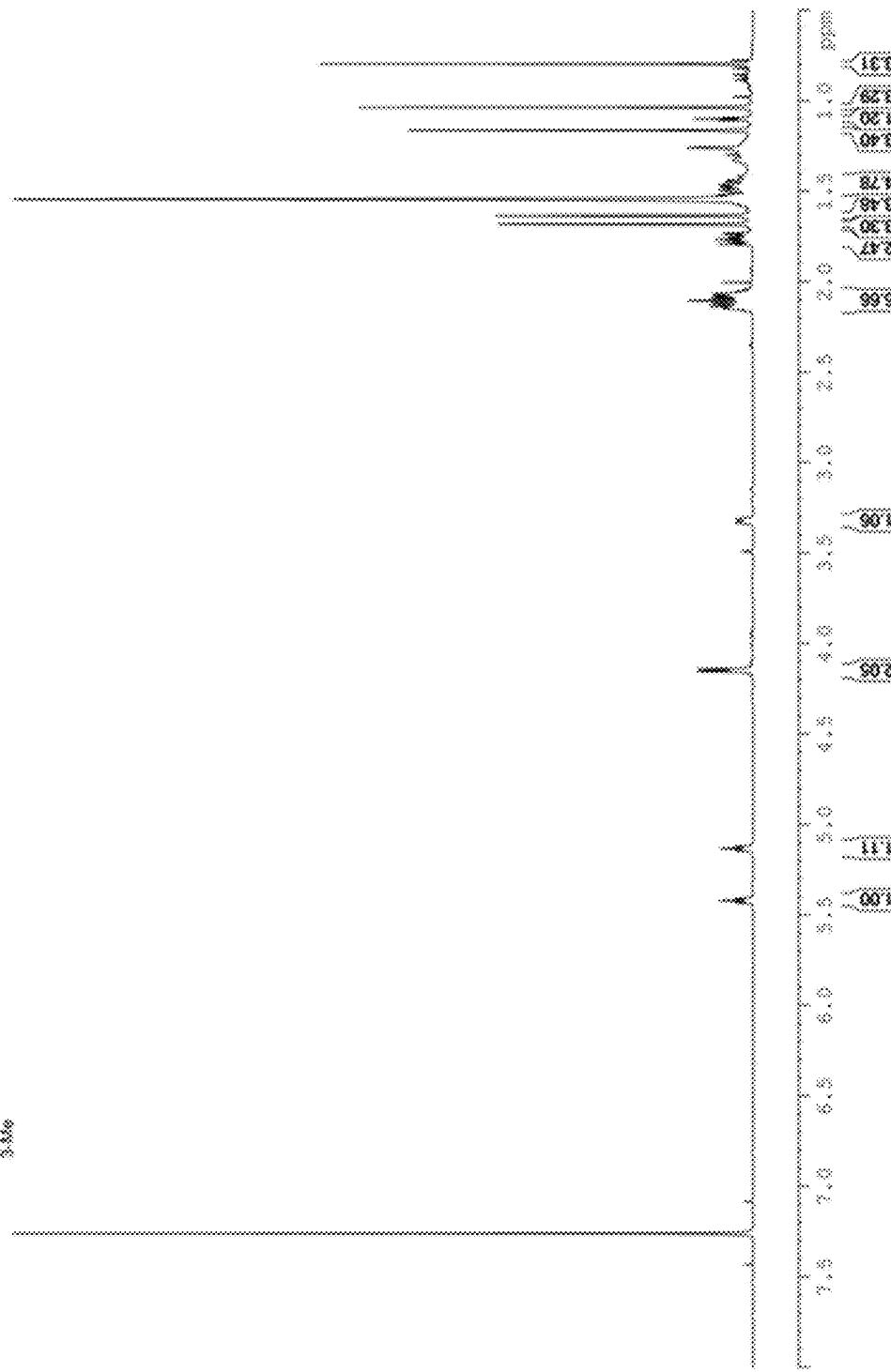
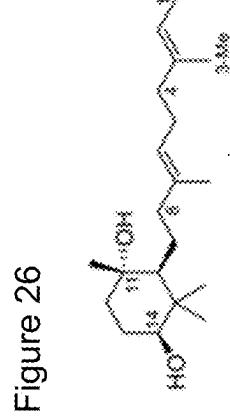


Figure 25





PRODUCTION OF OXYGENATED DITERPENOID COMPOUNDS

REFERENCE TO SEQUENCE LISTING

[0001] The present application contains a sequence listing in computer readable form, which is incorporated by reference.

FIELD OF INVENTION

[0002] The invention relates to the production of oxygenated diterpenoid compounds in recombinant cells, such as yeast cells. The oxygenated diterpenoid compounds are useful as intermediate or final compounds in the synthesis of useful bioactive compounds for use in e.g. pharmaceutical treatment of diseases such as cancer. The invention further relates to genes, enzymes and cells, such as yeast cells, particularly suited for production of such compounds.

BACKGROUND FOR THE INVENTION

[0003] Terpenes is a diverse group of compounds generated from a basic 5-carbon structure, isoprene (2-methyl-1, 3-butadiene). Diterpenes are compounds having a 20-carbon structure, generated by the action of an enzyme, diterpene synthase, that converts the compound geranyl-geranyl-diphosphate (GGPP) into a diterpene structure, which can be further modified forming a vast range of diterpene or diterpenoid compounds. Diterpenes, diterpenoids, derivatives thereof are widely used, e.g. as pharmaceuticals, cosmetics, nutraceuticals, flavors, fragrances and pesticides. Methods for increasing the production of these compounds in natural or engineered cells are abundant in the art. The Chinese medicinal plant, *Tripterygium wilfordii*, is known to produce several sesquiterpenoids, diterpenoids and triterpenoids with potential pharmacological properties, including the diterpenoid compounds triptonide and triptolide. Triptolide, an oxygenated diterpenoid compound, and derivatives thereof, has been identified as potential valuable pharmacological compounds and is under investigation as immunosuppressant and for treatment of cancer. Triptolide may further be used in treatment against COVID-19. Triptonide may be useful as male contraceptive agent.

[0004] Using engineered microorganisms for producing valuable molecules from renewable feedstock is a desirable alternative from conventional means of production. However, achieving economically viable yield, titers and productivity is a major roadblock towards industrialisation.

[0005] N L Hansen et al (2017) in *The Plant Journal* 2017, 89, 429-441, described a diterpene synthase capable of converting GGPP into the dipterpane, miltiradiene, which is a precursor for triptolide. The findings were confirmed by P Su et al (2018) in *The plant Journal* 2018, 50-65; and by J Guo et al. PNAS 2013, 110, 12108-12113.

[0006] The complete pathway for converting miltiradiene into other diterpenoid compounds, such as triptolide, has not yet been elucidated.

[0007] Cytochrome P450 enzymes (CYPs) are involved in the biosynthesis of terpenoids, and for many cytochrome P450 enzymes nothing is known with respect to the substrate they are acting on, which compounds they are generating, or their role in the biosynthesis of specific compounds.

[0008] US20190270971A1 discloses methods for increasing productivity of microbial host cells that functionally express p450 enzymes. The document describes how P450

genes can be modified in order to improve performance in microorganisms, such as yeast, and it mentions that co-expression with cytochrome P450 reductase can be beneficial to improve the yield. It is mentioned that triptolide may be the subject of P450 chemistry, but the document does not provide any link between triptolide and any specific P450 enzymes or cytochromes.

[0009] CN 108395997A describes yeast with increased GGPP production. The yeast is transformed with different diterpene synthases and P450 enzymes to synthesize diterpenoid compounds. The scientist team behind this patent is also behind more patents and patent applications disclosing the synthesis of different di- and triterpenoid compounds using suitable terpene synthases and P450 enzymes e.g. CN 108866029 (friedelin), CN107058419 (Kauren-type) and WO 2020029564 (Fridelin and amyrins). CN 110747178A describes the P450 gene TwCYP728B70 as encoding a Cytochrome P450 enzyme having a role in triptolide synthesis.

SHORT DESCRIPTION OF THE INVENTION

[0010] The inventors have solved the problem of providing an improved method of producing oxygenated diterpenoid compounds, such as triptophenolide, triptonide and triptolide. In a first aspect the invention relates to a method for producing an oxygenated diterpenoid compound is disclosed, the method comprising the steps of:

[0011] a. providing a host cell capable of producing miltiradiene and/or dehydroabietadiene;

[0012] b. transforming the host cell with a first gene encoding an enzyme having cytochrome P450 activity;

[0013] c. growing the transformed cell under conditions leading to expression of the transformed gene; whereby the oxygenated diterpenoid compound is formed;

wherein:

[0014] the first gene encoding an enzyme having cytochrome P450 activity encodes a polypeptide comprising an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity to SEQ ID NO: 1 (TwCYP82D274v1), SEQ ID NO: 2 (TwCYP82D274v2), SEQ ID NO: 74 (TwCYP82D274v3) or SEQ ID NO: 75 (TwCYP82D274v4), or the mature polypeptide thereof.

[0015] In a second aspect, the invention relates to methods for producing oxygenated diterpenoid compound, and comprises transforming the host cell with the first gene encoding an enzyme having cytochrome P450 activity and further with a second gene encoding a second enzyme having cytochrome P450 activity and with a third gene encoding a third enzyme having cytochrome P450 activity wherein:

[0016] the second gene encoding an enzyme having cytochrome P450 activity encodes a polypeptide comprising an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity to SEQ ID NO: 3 (TwCYP71BE85) or the mature polypeptide thereof; and

[0017] the third gene encoding an enzyme having cytochrome P450 activity encodes a polypeptide com-

prising an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity to SEQ ID NO: 4 (TwCYP71BE85) or the mature polypeptide thereof.

[0018] In a third aspect, the invention relates to methods for producing oxygenated diterpenoid compound, wherein said method comprises that the host cell is transformed with the first, second and third genes encoding enzymes having cytochrome P450 activities, and further with a fourth gene encoding a fourth enzyme having cytochrome P450 activity;

[0019] wherein:

[0020] the fourth gene encoding a fourth enzyme having cytochrome P450 activity encodes a polypeptide comprising an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity to SEQ ID NO: 5 (TwCYP82D213v1) or to SEQ ID NO: 76 (TwCYP82D213v2), or the mature polypeptide thereof.

[0021] According to the invention the useful oxygenated diterpenoid compounds, triptophenolide, triptonide or triptolide, may be provided.

[0022] The invention further relates to polypeptides, poly-nucleotides, plasmids and expression constructs as well as recombinant host cells useful in the methods of the invention.

SHORT DESCRIPTION OF THE DRAWINGS

[0023] FIG. 1 shows LCMS profiles of extracts from *N. benthamiana* leafs expressing miltidiene biosynthesis genes and selected *T. wilfordii* CYPs.

[0024] TwCYPs was co-expressed with CfDXS, CfGGPPS, CfTPS1 and CfTPS3. "3xSTD" represents a LCMS run of a sample of three mixed authentic standards: Triptolide, triptonide, and triptophenolide. Solid lines represent ion chromatograms at range m/z 280-380. Dashed lines (- - -) represent extracted ion chromatograms at m/z 313.1800±0.015 which corresponds to the parental ion of triptophenolide [M+H]. Dashed lines (- - -) represents extracted ion chromatograms at m/z 359.1490±0.015 which corresponds to the parental ion of triptonide [M+H]. LC protocol 1 used. For more details see example 1.

[0025] FIG. 2 shows LCMS profiles of extracts of genetically engineered *Saccharomyces cerevisiae* (*S. cerevisiae*) strain.

[0026] In the background strain (-), genes encoding the diterpene biosynthetic enzymes SPGGPPS7, CfTPS1, CfTPS3 and TwCPR1 were integrated into genome of wild type *S. cerevisiae*. In the TwCYP82D274v1 strain, the diterpene biosynthetic enzymes were expressed with TwCYP82D274v1, resulting in the formation of compound (3) identified as 14-OH-dehydroabietadiene marked in grey. LC method 1 was used for the analysis. For more details see example 3.

[0027] FIG. 3 shows the ¹H NMR spectrum of 14-OH-dehydroabietadiene in CDCl₃ at 599.85 MHz. For more details see example 4.

[0028] FIG. 4 shows the ¹³C NMR spectrum of 14-OH-dehydroabietadiene in CDCl₃ at 150.83 MHz. For more details see example 4.

[0029] FIG. 5 shows LCMS profiles of extracts of yeast having denoted gene combinations genome integrated.

[0030] All yeast strains have genome integrated spGGPPS7, CfTPS1, CfTPS3 and TwCPR1. "0.5 ppm 3xSTD" represents a LCMS run of a sample of three mixed authentic standards: Triptolide, triptonide and triptophenolide. Non-dashed lines represent ion chromatograms at range m/z 280-380. Dashed lines represents extracted ion chromatograms at m/z 359.1490±0.015 which corresponds to the parental ion of triptonide ([M+H]⁺). LC protocol 2 used. For more details see example 5.

[0031] FIG. 6 shows co-expression of TwCYPs and different variants of B5 proteins isolated from *Tripterygium wilfordii*

[0032] Levels of triptophenolide, triptonide and 14-OH-dehydroabietadiene quantified from cultures of engineered *S. cerevisiae* strains. Each column represents an engineered yeast strain and their output of selected compounds. The genes integrated into each of the individual strains are denoted in the lower panel. Quantification was based on the areas of the peaks representing each of the compounds of interest. Individual scales applies for each of the compounds. For more details see example 5.

[0033] FIG. 7 shows relative quantity (bars) of key intermediates in the proposed biosynthetic pathway of triptonide, when established in vivo, via heterologous gene expression in *N. benthamiana* (panel A and D) and *S. cerevisiae* (panel B and E, strains listed in table 3). Gene expression is indicated by black squares to the left, while relative quantity is indicated by bars (average of 3-4 biological replicates; black diamond squares) with white- and grey fill color distinguishing expression and no expression of Twb5 #1, respectively. Error bars represent standard deviation. "DTPSs" reflects CfTPS1 and CfTPS3. In quantification of peak areas, the signature mass tolerance was ±0.1m/z for GCMS (miltadiene and 14-OH-dehydroabietadiene) and ±0.005m/z for LCMS (all other compounds). Panel C: A hypothesized biosynthetic pathway from miltadiene to triptonide in vivo in *N. benthamiana* and *S. cerevisiae* that include a Wagner-Meerwein rearrangement reaction to account for a methyl shift of C-19 or C-18 to C-3 in the abietane carbon backbone.

[0034] FIG. 8 shows accumulation over 7 days of triptophenolide and triptonide produced with yeast strain NVJ8.15 when grown in bioreactor. Level of triptonide (solid black line) and triptophenolide (dotted black line) shows absolute quantity (ppm, w/v) in samples of the culture taken each day. Biomass was quantified by absorbance at 600 nm (grey dotted line).

[0035] FIG. 9 shows yeast strains expressing the genes needed for triptonide biosynthesis, but with genes variants substituting TwCYP82D274v1 or TwCYP82D213v1, retain the ability to produce triptophenolide (panel A) and triptonide (panel B) and results in similar LCMS profiles (panel C). Genes present in the engineered strains are represented by the black squares. Panel A and B: Bars represent the average relative quantity (2-3 biological replicates, crosses) with error bars showing std. error. From left to right, bars represent yeast strains: NVJ10-1, NVJ10-3, NVJ10-6, NVJ10-8 (see table 3). Panel C: EICs (m/z 280-360) of LCMS analyzed yeast cultures. From top and down pairs of chromatograms represent yeast strains NVJ10-1, NVJ10-3, NVJ10-6 and NVJ10-8.

SHORT DESCRIPTION OF THE SEQUENCES

- [0036] SEQ ID NO: 1 shows the amino acid sequence of a cytochrome P450 enzyme derived from *T. wilfordii*. The enzyme is also known as TwCYP82D274v1.
- [0037] SEQ ID NO: 2 shows the amino acid sequence of a cytochrome P450 enzyme derived from *T. wilfordii*. The enzyme is also known as TwCYP82D274v2. SEQ ID NO: 2 differs from SEQ ID NO: 1 in only three positions, and it is therefore assumed that SEQ ID NO: 1 and SEQ ID NO: 2 represent different alleles of the same gene.
- [0038] SEQ ID NO: 3 shows the amino acid sequence of a cytochrome P450 enzyme derived from *T. wilfordii*. The enzyme is also known as TwCYP71BE85.
- [0039] SEQ ID NO: 4 shows the amino acid sequence of a cytochrome P450 enzyme derived from *T. wilfordii*. The enzyme is also known as TwCYP71BE86.
- [0040] SEQ ID NO: 5 shows the amino acid sequence of a cytochrome P450 enzyme derived from *T. wilfordii*. The enzyme is also known as TwCYP82D213v1.
- [0041] SEQ ID NO: 6 shows the amino acid sequence of a cytochrome P450 enzyme derived from *T. wilfordii*. The enzyme is also known as TwCYP82D217.
- [0042] SEQ ID NO: 7 shows the amino acid sequence of a cytochrome P450 enzyme derived from *T. wilfordii*. The enzyme is also known as TwCYP82D275.
- [0043] SEQ ID NO: 8 shows the amino acid sequence of a cytochrome B5 enzyme derived from *T. wilfordii*. This enzyme is also known as TwB5 #1.
- [0044] SEQ ID NO: 9 shows the amino acid sequence of a cytochrome P450 reductase enzyme derived from *T. wilfordii*. This enzyme is also known as TwCPR1.
- [0045] SEQ ID NO: 10-66 show PCR primers as further described in Example 2.
- [0046] SEQ ID NO: 67 shows the amino acid sequence of a diterpene synthase TPS1 derived from *Plectranthus barbatus*. The enzyme is also known as CfTPS1.
- [0047] SEQ ID NO: 68 shows the amino acid sequence of a diterpene synthase TPS3 derived from *Plectranthus barbatus*. The enzyme is also known as CfTPS3.
- [0048] SEQ ID NO: 69 shows the amino acid sequence of a terpene synthase TPS9, derived from *T. wilfordii*. The enzyme is also known as TwTPS9.
- [0049] SEQ ID NO: 70 shows the amino acid sequence of a terpene synthase derived from *T. wilfordii*. The enzyme is also known as TwTPS27.
- [0050] SEQ ID NO: 71 shows the amino acid sequence of a copalyl diphosphate synthase CPS1 derived from *Salvia miltiorrhiza*. The enzyme is also known as SmCPS.
- [0051] SEQ ID NO: 72 shows the amino acid sequence of a miltiradiene synthase KSL1 derived from *Salvia miltiorrhiza*. The enzyme is also known as SmKSL.
- [0052] SEQ ID NO: 73 shows the amino acid sequence of a geranyl geranyl diphosphate synthase derived from *Synechococcus* sp. The enzyme is also known as SpGGPPs7v1.
- [0053] SEQ ID NO: 74 shows the amino acid sequence of a cytochrome P450 enzyme derived from *T. wilfordii*. The enzyme is also known as TwCYP82D274v3.
- [0054] SEQ ID NO: 75 shows the amino acid sequence of a cytochrome P450 enzyme derived from *T. wilfordii*. The enzyme is also known as TwCYP82D274v4.
- [0055] SEQ ID NO: 76 shows the amino acid sequence of a cytochrome P450 enzyme derived from *T. wilfordii*. The enzyme is also known as TwCYP82D213v2.

[0056] SEQ ID NO: 77 shows the truncated amino acid sequence of a diterpene synthase TPS1 derived from *Plectranthus barbatus*. The amino acid sequence was truncated to remove a transit peptide. The enzyme is also known as CfTPS1.

[0057] SEQ ID NO: 78 shows the truncated amino acid sequence of a diterpene synthase TPS3 derived from *Plectranthus barbatus*. The amino acid sequence was truncated to remove a transit peptide. The enzyme is also known as CfTPS3.

[0058] SEQ ID NO: 79 shows the amino acid sequence of a DXS enzyme derived from *Plectranthus barbatus*. The enzyme is also known as CfDXS.

[0059] SEQ ID NO: 80 shows the amino acid sequence of a truncated HMGR enzyme derived from *S. cerevisiae*. The enzyme is also known as SctHMGR.

[0060] SEQ ID NO: 81 shows the amino acid sequence of a geranyl geranyl diphosphate synthase derived from *Synechococcus* sp. The enzyme is also known as SpGGPPs7v2.

DETAILED DESCRIPTION OF THE INVENTION

[0061] According to a first aspect of the invention, a method for producing an oxygenated diterpenoid compound is disclosed, the method comprising the steps of:

[0062] a. providing a host cell capable of producing miltiradiene and/or dehydroabietadiene;

[0063] b. transforming the host cell with a first gene encoding an enzyme having cytochrome P450 activity;

[0064] c. growing the transformed cell under conditions leading to expression of the transformed gene; whereby the oxygenated diterpenoid compound is formed;

wherein:

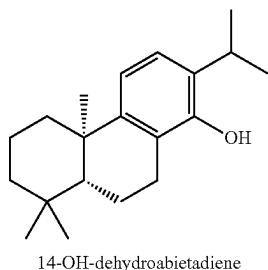
[0065] the first gene encoding an enzyme having cytochrome P450 activity encodes a polypeptide comprising or consisting of SEQ ID NO:1 or an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity more preferred at least 98% sequence identity to SEQ ID NO: 1 (TwCYP82D274V1) or the mature polypeptide thereof.

[0066] The polypeptide having SEQ ID NO: 1 is a preferred example of a first gene having cytochrome P450 activity, the polypeptides having SEQ ID NO: 2, SEQ ID NO: 74 and SEQ ID NO: 75 are other examples of such a polypeptide.

[0067] Thus, the enzyme encoded by the first gene has the ability to convert miltiradiene and/or dehydroabietadiene into 14-OH-dehydroabietadiene by inserting an OH group in position 14 of the diterpene skeleton of miltiradiene.

[0068] In some embodiments, the synthesis of 14-OH-dehydroabietadiene takes place via the compound 14-OH-miltiradiene that subsequently is converted into 14-OH-dehydroabietadiene. However, the invention is not limited to any particular mechanism for converting miltiradiene into 14-OH-dehydroabietadiene.

[0069] Using the method according to the first aspect of the invention leads to the formation of the oxygenated diterpenoid compound, 14-OH-dehydroabietadiene,



that is a useful intermediate in the synthesis of oxygenated diterpenoid compounds of pharmaceutical use, including well known compounds such as triptophenolide, triptonide and triptolide.

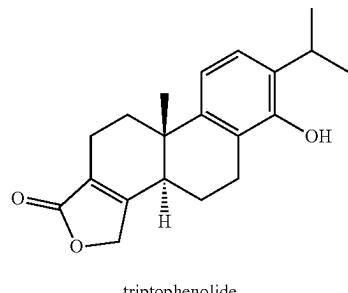
[0070] In a second aspect of the invention, the method step b. comprises transforming the host cell with the first gene encoding an enzyme having cytochrome P450 activity and further with a second gene encoding a second enzyme having cytochrome P450 activity and a third gene encoding a third enzyme having cytochrome P450 activity

[0071] wherein:

[0072] the second gene encoding an enzyme having cytochrome P450 activity encodes a polypeptide comprising or consisting of SEQ ID NO:4 or an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity to SEQ ID NO: 4 (TwCYP71BE86) or the mature polypeptide thereof; and

[0073] the third gene encoding an enzyme having cytochrome P450 activity encodes a polypeptide comprising or consisting of SEQ ID NO: 3 or an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity to SEQ ID NO: 3 (TwCYP71BE85) or the mature polypeptide thereof.

[0074] In this second aspect, the host cell preferably further produces the oxygenated diterpenoid compound, triptophenolide, (3bR,9bS)-6-hydroxy-9b-methyl-7-propan-2-yl-3,3b,4,5,10,11-hexahydronaphtho[2,1-e]isobenzofuran-1-one.



[0075] Triptophenolide is a valuable compound that has been identified as an antiandrogen. In addition, it may be useful as a starting point for further modifications leading to further bioactive compounds.

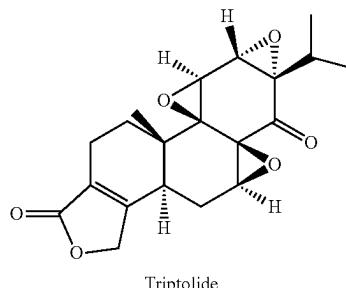
[0076] In a preferred embodiment of the second aspect of the invention, the host cell is further transformed with a fifth gene encoding a polypeptide having cytochrome B5 activity and comprising or consisting of SEQ ID NO:8 or an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity to SEQ ID NO: 8 (TwB5 #1) or the mature polypeptide thereof. It has surprisingly been found that expressing the polypeptide having cytochrome B5 activity in the same cell that expresses the first, second and third genes encoding enzymes having cytochrome P450 activity, leads to a significantly higher production of the oxygenated diterpenoid compound. The production is increased at least 50% compared with the production of a similar cell without the polypeptide having cytochrome B5 activity, preferably increased at least 100%, preferably at least 200% or even more.

[0077] In a third aspect of the invention, the host cell is transformed with the first, second and third genes encoding enzymes having cytochrome P450 activities, and further with a fourth gene encoding a fourth enzyme having cytochrome P450 activity;

[0078] wherein:

[0079] the fourth gene encoding a fourth enzyme having cytochrome P450 activity encodes a polypeptide comprising or consisting of SEQ ID NO:5 or an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity to SEQ ID NO: 5 (TwCYP82D213v1) or the mature polypeptide thereof. The polypeptide having SEQ ID NO: 5 is a preferred example of a fourth gene having cytochrome P450 activity; the polypeptide having SEQ ID NO: 76 is another example of such a polypeptide.

[0080] In the third aspect of the invention, the transformed eukaryotic cell preferably produces the oxygenated diterpenoid compound, triptonide.



[0081] The compound triptonide has been reported to have a strong inhibition activity in cancers (Fulu Dong et al 2019, The Prostate, Volume 19, issue 11, pages 1284-1293). The compound is also useful as male contraceptive agent. Fur-

ther, the compound is useful as a starting point for further modifications leading to further bioactive compounds.

[0082] In a preferred embodiment of the third aspect of the invention, the host cell is further transformed with a fifth gene encoding a polypeptide having cytochrome B5 activity and comprising or consisting of SEQ ID NO:8 or an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity to SEQ ID NO: 8 (TwB5 #1) or the mature polypeptide thereof. It has surprisingly been found that expressing the polypeptide having cytochrome B5 activity in the same cell that expresses the first, second, third and fourth genes encoding enzymes having cytochrome P450 activity, leads to a significantly higher production of the oxygenated diterpenoid compound. The production is increased at least 50% compared with the production of a similar cell without the polypeptide having cytochrome B5 activity, preferably increased at least 100%, preferably at least 200% or even more.

[0083] In a further preferred embodiment of the third aspect of the invention, the host cell is further transformed with a sixth gene encoding a fifth enzyme having cytochrome P450 activity and/or a seventh gene encoding a sixth enzyme having cytochrome P450 activity wherein:

[0084] the sixth gene encoding a fifth enzyme having cytochrome P450 activity encodes a polypeptide comprising or consisting of SEQ ID NO:6 or an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity to SEQ ID NO: 6 (TwCYP82D217) or the mature polypeptide thereof; and

[0085] the seventh gene encoding a sixth enzyme having cytochrome P450 activity encodes a polypeptide comprising an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity to SEQ ID NO: 7 (TwCYP82D275) or the mature polypeptide thereof. It has surprisingly been found that expressing the sixth and/or seventh genes encoding enzymes having cytochrome P450 activity, leads to a higher production of the oxygenated diterpenoid compound. Preferably, the production is increased at least 10% compared with the production of a similar cell without sixth and/or seventh genes encoding enzymes having cytochrome P450 activity, preferably increased at least 20%, even more preferred at least 50% or even more.

[0086] The first, second, third, fourth, fifth, sixth and seventh gene may be comprised in one or more nucleic acid molecules, such as one or more heterologous nucleic acids. The heterologous nucleic acid encoding the first enzyme having cytochrome P450 activity may herein be referred to as the "first heterologous nucleic acid". The heterologous nucleic acid encoding the second enzyme having cytochrome P450 activity may herein be referred to as the "second heterologous nucleic acid". The heterologous nucleic acid encoding the third enzyme having cytochrome P450 activity may herein be referred to as the "third heterologous nucleic acid". The heterologous nucleic acid encoding the fourth enzyme having cytochrome P450 activity may herein be referred to as the "fourth heterologous nucleic

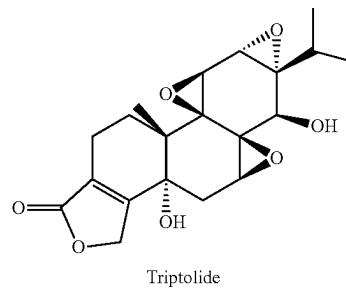
acid". The heterologous nucleic acid encoding the fifth enzyme having cytochrome P450 activity may herein be referred to as the "fifth heterologous nucleic acid". The heterologous nucleic acid encoding the sixth enzyme having cytochrome P450 activity may herein be referred to as the "sixth heterologous nucleic acid". The heterologous nucleic acid encoding the enzyme having cytochrome B5 activity may herein be referred to as the "seventh heterologous nucleic acid". This does not imply that the recombinant host cell must comprises seven heterologous nucleic acids in total; in some embodiments, the cell comprises only one or more of the first, second, third, fourth, fifth, sixth and seventh heterologous nucleic acids.

[0087] The oxygenated diterpenoid compounds produced according to the methods of the invention may be further modified by biological or chemical synthesis. In connection with this, biological synthesis is understood as a method where the host cell comprising the genes of the invention is further provided with one or more additional genes encoding further enzymes having the capability of modifying the oxygenated diterpenoid compounds produced according to the methods of the invention.

[0088] Chemical modification of the oxygenated diterpenoid compounds produced according to the methods of the invention may be performed directly on the culture broth before recovery of the oxygenated diterpenoid compounds or it may be performed on the recovered oxygenated diterpenoid compounds.

[0089] Reduction of triptenide to triptolide can be achieved by organic synthesis. An example of such synthesis is the reduction by a nucleophilic attack by a hydride on C-14 ketone. For this reaction Sodium borohydride is a suitable agent for catalyzing this reaction at neutral pH in the appropriate solvent e.g. water or MetOH.

[0090] In one preferred embodiment, triptenide produced according to the methods of the invention is converted into the compound triptolide, that is reported to be an immunosuppressant and is under investigation for use in cancer therapy.



The Host Cell

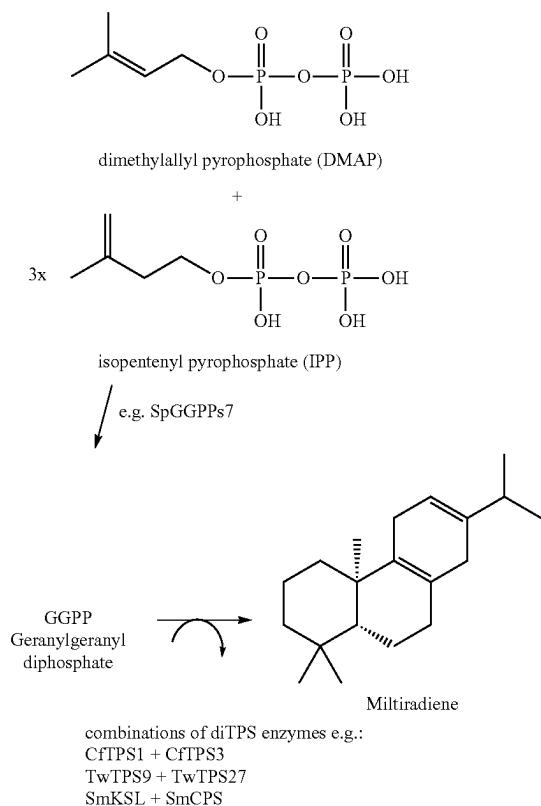
[0091] The host cell capable of producing miltiradiene and/or dehydroabietadiene may in principle be any such cell. The cell may be a cell that naturally produces miltiradiene and/or dehydroabietadiene or it may be a cell that has been engineered to produce one or both of these compounds.

[0092] It is believed that miltiradiene, at least under some circumstances, may be spontaneously converted into dehydroabietadiene, and the invention may therefore be performed using a cell producing miltiradiene that is spontaneously converted into dehydroabietadiene or it may be performed in a cell comprising an enzyme that facilitates the

conversion of miltiradiene to dehydroabietadiene (see a J. Zi, et al., *Organic & Biomolecular Chemistry* 2013, 11, 7650-7652).

[0093] The synthesis of miltiradiene in general begins with the formation of GGPP. GGPP may be synthesized by condensation of one dimethylallyl pyrophosphate (DMAP) molecule and three isopentenyl pyrophosphate (IPP) molecules and is typically catalyzed by a geranylgeranyl diphosphate synthase e.g. the SpGGPPs7 enzyme derived from *Synechococcus* sp.; and having the amino acid sequence shown in SEQ ID NO: 73 or SEQ ID NO: 81.

[0094] GGPP is converted into miltiradiene by action of a diterpene synthase or by the combined action of two or more diterpene synthases, e.g. a combination of two diterpene synthases, CfTPS1 and CfTPS3, or CfTPS1 as set forth in SEQ ID NO: 77 and CfTPS3 as set forth in SEQ ID NO: 78, derived from *Plectranthus barbatus* and having the amino acid sequences of SEQ ID NO: 67 and 68; a combination of two diterpene synthases, TwTPS9 and TwTPS27, derived from *T. wilfordii* and having the amino acid sequences of SEQ ID NO: 69 and 70; or a combination of a copalyl diphosphate synthase, SmCPS derived from *Salvia miltiorrhiza* and having the amino acid sequence of SEQ ID NO: 71, and a miltiradiene synthase, SmKSL derived from *Salvia miltiorrhiza* and having the amino acid sequence of SEQ ID NO: 72.



Prior art published:
N. L. Hansen, et al., *The Plant Journal* 2017, 89, 429-441.
P. Su, et al., *The Plant Journal* 2018, 93, 50-65.
J. Guo, et al., *PNAS* 2013, 110, 12108-12113.

[0095] One preferred way to provide a host cell producing miltiradiene and/or dehydroabietadiene is selecting a host cell producing GGPP and transforming it with a diterpene synthase catalyzing the transformation of GGPP into miltiradiene. Alternatively, a host cell that have been genetically engineered to produce GGPP may be used as a starting point. Techniques for transforming a host cell with a diterpene synthase catalyzing the transformation of GGPP into miltiradiene is known in the prior art, e.g. in NL Hansen et al (2017) in *The Plant Journal* 2017, 89, 429-441 (Incorporated herein by reference), P Su et al (2018) in *The plant Journal* 2018, 50-65 and in: J. Guo, et al., *Proceedings of the National Academy of Sciences* 2013, 110, 12108-12113, and the procedures and methods disclosed in these publications are also useful for providing a host cell for use in the present invention.

[0096] The host cell may be a prokaryotic cell, such as a eubacterial or archaeabacterial cell; or a eukaryotic cell, such as a plant cell, an animal cell, an insect cell, a fungal cell or a yeast cell.

[0097] Practically all eukaryotic cells produce GGPP for their biosynthesis, but in some embodiments a eukaryotic cell produces an increased amount of GGPP, which may increase the production of miltiradiene, compared with a similar eukaryotic that does not produce increased amounts of GGPP. Methods for increasing the GGPP production in a eukaryotic cell has also been described in the prior art.

[0098] The host cell may be a unicellular organism, or it may be comprised within a multicellular organism, e.g. a plant. Examples of suitable plants or plant cells for use as host cells according to the invention includes corn (*Zea mays*), canola (*Brassica napus*, *Brassica rapa* ssp.), alfalfa (*Medicago sativa*), rice (*Oryza sativa*), rye (*Secale cereale*), sorghum (*Sorghum bicolor*, *Sorghum vulgare*), sunflower (*Helianthus annuus*), wheat (*Triticum aestivum* and other species), triticale, rye (*Secale*) soybean (*Glycine max*), tobacco (*Nicotiana tabacum*), potato (*Solanum tuberosum*), peanuts (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), sweet potato (*Ipomoea batatas*), cassava (*Manihot esculenta*), coffee (*Coffea* spp.), coconut (*Cocos nucifera*), pineapple (*Anana comosus*), citrus (*Citrus* spp.) cocoa (*Theobroma cacao*), tea (*Camellia senensis*), banana (*Musa* spp.), avocado (*Persea americana*), fig (*Ficus carica*), guava (*Psidium guajava*), mango (*Mangifera indica*), olive (*Olea europaea*), papaya (*Carica papaya*), cashew (*Anacardium occidentale*), macadamia (*Macadamia intergrifolia*), almond (*Prunus amygdalus*), apple (*Malus* spp.), pear (*Pyrus* spp.), plum and cherry tree (*Prunus* spp.), ribes (currant etc.), Vitis, Jerusalem artichoke (*Helianthemum* spp.), non-cereal grasses (Grass family), sugar and fodder beets (*Beta vulgaris*), chicory, oats, barley, vegetables, or ornamentals, crop plants (for example, cereals and pulses, maize, wheat, potatoes, tapioca, rice, sorghum, millet, cassava, barley, pea, sugar beets, sugar cane, soybean, oilseed rape, sunflower and other root, tuber or seed crops. Other important plants may be fruit trees, crop trees, forest trees or plants grown for their use as spices or pharmaceutical products (*Mentha* spp., *clove*, *Artemesia* spp., *Thymus* spp., *Lavendula* spp., *Allium* spp., *Hypericum*, *Catharanthus* spp., *Vinca* spp., *Papaver* spp., *Digitalis* spp., *Rawolfia* spp., *Vanilla* spp., *Petrosilium* spp., *Eucalyptus*, *tea tree*, *Picea* spp., *Pinus* spp., *Abies* spp., *Juniperus* spp.). Horticultural plants which can be used with the present invention may

include lettuce, endive, and vegetable brassicas including cabbage, broccoli, and cauliflower, carrots, and carnations and geraniums.

[0099] The plant can also be tobacco, cucurbits, carrot, strawberry, sunflower, tomato, pepper, or chrysanthemum.

[0100] Further examples of plants include grain plants for example oil-seed plants or leguminous plants. Seeds of interest include grain seeds, such as corn, wheat, barley, sorghum, rye, etc. Oil-seed plants include cotton soybean, safflower, sunflower, *Brassica*, maize, alfalfa, palm, coconut, etc. Leguminous plants include beans and peas. Beans include guar, locust bean, fenugreek, soybean, garden beans, cowpea, mung bean, lima bean, fava bean, lentils, chickpea.

[0101] Particular preferred plant species include *Physcomitrella* sp., such as *P. patens*; *Arabidopsis* sp., such as *A. thaliana*; *Nicotiana* sp., such as *N. benthamiana*; *Chlamydomonas* sp., such as *C. reinhardtii*; and *Nannochloropsis* sp., such as *N. oceanica*.

[0102] Examples of suitable eukaryotic cell for use according to the invention include fungal cells such as *Agaricus*, *Aspergillus*, *Candida*, *Eremothecium*, *Fusarium*/*Gibberella*, *Kluyveromyces*, *Laetiporus*, *Lentinus*, *Phaffia*, *Phanerochaete*, *Pichia*, *Physcomitrella*, *Rhodoturula*, *Saccharomyces*, *Schizosaccharomyces*, *Sphaereloma*, *Xanthophyllomyces* or *Yarrowia*. Exemplary species from such genera include *Lentinus tigrinus*, *Laetiporus sulphureus*, *Phanerochaete chrysosporium*, *Pichia pastoris*, *Cyberlindnera jadinii*, *Physcomitrella patens*, *Rhodoturula glutinis*, *Rhodoturula mucilaginosa*, *Phaffia rhodozyma*, *Xanthophyllomyces dendrorhous*, *Fusarium fujikuroi*/*Gibberella fujikuroi*, *Candida utilis*, *Candida glabrata*, *Candida albicans*, and *Yarrowia lipolytica*.

[0103] In some embodiments, a host cell can be an Ascomycete such as *Gibberella fujikuroi*, *Kluyveromyces lactis*, *Schizosaccharomyces pombe*, *Aspergillus niger*, *Yarrowia lipolytica*, *Ashbya gossypii*, or *S. cerevisiae*.

[0104] In some embodiments, the host cell can be an algae cell such as *Blakeslea trispora*, *Dunaliella salina*, *Haematococcus pluvialis*, *Chlorella* sp., *Undaria pinnatifida*, *Sargassum*, *Laminaria japonica*, *Scenedesmus almeriensis*.

[0105] In some embodiments, a host cell can be a prokaryote such as *Bacillus* cells, for example *Bacillus subtilis*; *Escherichia* cells, for example, *Escherichia coli* cells; *Lactobacillus* cells; *Lactococcus* cells; *Streptomyces* cells, *Streptococcus* cells, *Cornebacterium* cells; *Acetobacter* cells; *Acinetobacter* cells; or *Pseudomonas* cells.

[0106] In some embodiments, the host cell can be a cyanobacterial cell such as *Synechocystis* sp. or *Synechococcus* sp.

[0107] In one embodiment, a host cell that is suitable for growth in a fermenter is selected. Growing the recombinant host cell according to the invention is a convenient way of growing the host cell for production of the oxygenated diterpenoid compounds of the invention.

[0108] In another embodiment, the host cell is a phototropic cell and the cell is cultivated in a green house or photobioreactor.

The Genes and Enzymes

[0109] The recombinant host cell of the present invention is capable of producing miltiradiene and/or dehydroabietadiene. Miltiradiene may be spontaneously converted into

dehydroabietadiene or it may be converted by an enzyme that facilitates the conversion of miltiradiene to dehydroabietadiene.

[0110] As described herein above, the synthesis of miltiradiene usually begins with the formation of GGPP by condensation of one dimethylallyl pyrophosphate (DMAP) molecule and three isopentenyl pyrophosphate (IPP) molecules by a geranylgeranyl diphosphate synthase.

[0111] Recombinant host cells and heterologous nucleic acids that encode enzymes that catalyze the synthesis of GGPP in recombinant host cells are generally known in the art, see e.g. WO 2015/113570. In addition, many host organisms are capable of producing GGPP intrinsically and heterologous nucleic acids may thus not always be necessary for production of GGPP.

[0112] In some embodiments, the recombinant host cell comprises a heterologous nucleic acid encoding a geranylgeranyl diphosphate synthase, such as the geranylgeranyl diphosphate synthase SpGGPPs7 as set forth in SEQ ID NO: 73 or SEQ ID NO: 81, or a functional homologue thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto, or the mature polypeptide thereof.

[0113] Subsequently, GGPP may be converted into miltiradiene by the action of one or more diterpene synthases, copalyl diphosphate synthases and/or miltiradiene synthases.

[0114] In some embodiments, the recombinant host cell comprises one or more heterologous nucleic acids encoding one or more diterpene synthases, such as the diterpene synthases CfTPS1 (SEQ ID NO: 67) and CfTPS3 (SEQ ID NO: 68), or CfTPS1 (SEQ ID NO: 77) and CfTPS3 (SEQ ID NO: 78), or respective functional homologues thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto, or the mature polypeptides thereof.

[0115] In some embodiments, the recombinant host cell comprises one or more heterologous nucleic acids encoding one or more diterpene synthases, such as the diterpene synthases TwTPS9 (SEQ ID NO: 69) and TwTPS27 (SEQ ID NO: 70), or respective functional homologues thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto, or the mature polypeptides thereof.

[0116] In some embodiments, the recombinant host cell comprises a combination of one or more copalyl diphos-

phate synthases and one or more miltiradiene synthases, such as a combination of the copalyl diphosphate synthases SmCPS (SEQ ID NO: 71) and the miltiradiene synthase SmKSL (SEQ ID NO: 72), or respective functional homologues thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto, or the mature polypeptides thereof.

[0117] In an even further aspect, the invention relates to polypeptides having cytochrome P450 enzyme activity and comprising an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, preferably at least 96% sequence identity, preferably at least 97% sequence identity, preferably at least 98% sequence identity or even 100% sequence identity to one of the sequences SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7 or the mature polypeptide thereof.

[0118] In a further aspect, the invention relates to a polypeptide having cytochrome B5 activity and comprising an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, preferably at least 96% sequence identity, preferably at least 97% sequence identity, preferably at least 98% sequence identity or even 100% sequence identity to SEQ ID NO: 8, or the mature polypeptide thereof.

[0119] The invention also relates to polynucleotide sequences or genes encoding polypeptides having cytochrome P450 enzyme activity and comprising an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, preferably at least 96% sequence identity, preferably at least 97% sequence identity, preferably at least 98% sequence identity or even 100% sequence identity to one of the sequences SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7 or the mature polypeptide thereof, or encoding a polypeptide having cytochrome B5 activity and comprising an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, preferably at least 96% sequence identity, preferably at least 97% sequence identity, preferably at least 98% sequence identity or even 100% sequence identity to SEQ ID NO: 8, or the mature polypeptide thereof.

[0120] In preferred embodiments, one or more of the first, second, third, fourth, fifth and sixth enzymes having cytochrome P450 activity comprises or consists of an amino acid sequence according to any one of SEQ ID NO: 1 (TwCYP82D274v1), SEQ ID NO: 2 (TwCYP82D274v2), SEQ ID NO: 74 (TwCYP82D274v3), SEQ ID NO: 75 (TwCYP82D274v4), SEQ ID NO: 3 (TwCYP71BE85), SEQ ID NO: 4 (TwCYP71BE86), SEQ ID NO: 5 (TwCYP82D213v1) and SEQ ID NO: 76 (TwCYP82D213v2), and respective functional homologs thereof having at least 80% sequence identity, preferably at

least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, preferably at least 96% sequence identity, preferably at least 97% sequence identity, preferably at least 98% sequence identity, preferably at least 99% sequence identity thereto, or the mature polypeptide thereof.

[0121] In some embodiments, the first heterologous nucleic acid encoding a first enzyme having cytochrome P450 activity encodes TwCYP82D274 as set forth in SEQ ID NO: 1 (TwCYP82D274v1), SEQ ID NO: 2 (TwCYP82D274v2), SEQ ID NO: 74 (TwCYP82D274v3), SEQ ID NO: 75 (TwCYP82D274v4), or a functional homologue thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto.

[0122] In some embodiments, the second heterologous nucleic acid encoding a second enzyme having cytochrome P450 activity encodes the cytochrome P450 enzyme TwCYP71BE86 as set forth in SEQ ID NO: 4, or a functional homologue thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto.

[0123] In some embodiments, the third heterologous nucleic acid encoding a third enzyme having cytochrome P450 activity encodes the cytochrome P450 enzyme

[0124] TwCYP71BE85 as set forth in SEQ ID NO: 3, or a functional homologue thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto.

[0125] In some embodiments, the fourth heterologous nucleic acid encoding a fourth enzyme having cytochrome P450 activity encodes the cytochrome P450 enzyme TwCYP82D213v1 as set forth in SEQ ID NO: 5 or TwCYP82D213v2 as set forth in SEQ ID NO: 76 (TwCYP82D213v2), or a functional homologue thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto.

[0126] In some embodiments, the fifth heterologous nucleic acid encoding a fifth enzyme having cytochrome P450 activity encodes the cytochrome P450 enzyme TwCYP82D217 as set forth in SEQ ID NO: 6, or a func-

tional homologue thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto.

[0127] In some embodiments, the sixth heterologous nucleic acid encoding a sixth enzyme having cytochrome P450 activity encodes the cytochrome P450 enzyme TwCYP82D275 as set forth in SEQ ID NO: 7, or a functional homologue thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto.

[0128] In some embodiments, the seventh heterologous nucleic acid encoding an enzyme having cytochrome B5 activity encodes the cytochrome B5 enzyme TwB5 #1 as set forth in SEQ ID NO: 8, or a functional homologue thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto.

[0129] In some embodiments is provided a recombinant host cell

[0130] i. wherein the host cell is capable of producing miltiradiene and/or dehydroabietadiene; and

[0131] ii. comprises a heterologous nucleic acid encoding TwCYP82D274 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 74 or SEQ ID NO: 75, or a functional homolog thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto, or the mature polypeptide thereof,

wherein said cell is capable of producing 14-hydroxydehydroabietadiene.

[0132] In some embodiments is provided a recombinant host cell

[0133] i. wherein the host cell is capable of producing miltiradiene and/or dehydroabietadiene; and

[0134] ii. comprises a heterologous nucleic acid encoding TwCYP82D274 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 74 or SEQ ID NO: 75, or a functional homolog thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such

as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto, or the mature polypeptide thereof; and

[0135] iii. comprises a heterologous nucleic acid encoding TwCYP71BE86 of SEQ ID NO: 4 or a functional homolog thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto, or the mature polypeptide thereof.

wherein said cell is capable of producing 14-hydroxydehydroabietadiene, 3,14-dihydroxydehydroabietadiene, 3,14-dihydroxyabeodiene and/or 14-hydroxy-18-aldo-abeoediene.

[0136] In some embodiments is provided a recombinant host cell

[0137] i. wherein the host cell is capable of producing miltiradiene and/or dehydroabietadiene; and

[0138] ii. comprises a heterologous nucleic acid encoding TwCYP82D274 SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 74 or SEQ ID NO: 75, or a functional homolog thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto, or the mature polypeptide thereof.

[0139] iii. comprises a heterologous nucleic acid encoding TwCYP71BE86 of SEQ ID NO: 4 or a functional homolog thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto, or the mature polypeptide thereof; and

[0140] iv. comprises a heterologous nucleic acid encoding TwCYP71BE85 of SEQ ID NO: 3 or a functional homolog thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto, or the mature polypeptide thereof,

wherein said cell is capable of producing triptonide with a titer that is at least 2-fold, such as at least 3-fold, such as at least 4-fold, such as at least 5-fold higher than an identical yeast cell, except wherein said yeast said does not express said TwB5 #1 or said functional homolog thereof.

[0154] In some embodiments is provided a recombinant host cell

[0155] i. wherein the host cell is capable of producing miltiradiene and/or dehydroabietadiene; and

[0156] ii. comprises a heterologous nucleic acid encoding TwCYP82D274 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 74 or SEQ ID NO: 75, or a functional homolog thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto, or the mature polypeptide thereof;

[0157] iii. comprises a heterologous nucleic acid encoding TwCYP71BE86 of SEQ ID NO: 4 or a functional homolog thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto, or the mature polypeptide thereof;

[0158] iv. comprises a heterologous nucleic acid encoding TwCYP71BE85 of SEQ ID NO: 3 or a functional homolog thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto, or the mature polypeptide thereof;

[0159] v. comprises a heterologous nucleic acid encoding TwCYP82D213 of SEQ ID NO: 5 or SEQ ID NO: 76, or a functional homolog thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto, or the mature polypeptide thereof; and

[0160] vi. comprises a heterologous nucleic acid encoding TwB5 #1 of SEQ ID NO: 8 or a functional homolog thereof having at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least

86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto, or the mature polypeptide thereof,

[0161] wherein said cell is capable of growing in a fermentation medium and where said fermentation medium after 7 days of fermentation comprises:

[0162] at least 3 ppm triptonide and/or

[0163] at least 1 ppm triptophenolide.

[0164] Aforementioned recombinant host cells may be capable of producing miltiradiene and/or dehydroabietadiene for several different reasons. For example, the host cells may endogenously be capable of producing miltiradiene. Alternatively, the recombinant host cell may comprise one or more heterologous nucleic acid sequences encoding one or more enzymes involved in the production of miltiradiene, such as the diterpene biosynthetic enzymes SPGGPPS7 of SEQ ID NO: 73 or SEQ ID NO: 81, CfTPS1 of SEQ ID NO: 67, CfTPS1 of SEQ ID NO: 77, CfTPS3 of SEQ ID NO: 68, CfTPS3 of SEQ ID NO: 78 and/or TwCPR1 of SEQ ID NO: 9, or respective functional homologs thereof having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, preferably at least 97% sequence identity, preferably at least 98% sequence identity, preferably at least 99% sequence identity thereto, or the mature polypeptides thereof.

[0165] Functional homologues of the first (e.g. TwCYP82D274), second (e.g. TwCYP71BE86), third (e.g. TwCYP71BE85), fourth (e.g. TwCYP82D213), fifth (e.g. TwCYP82D217) and sixth (e.g. TwCYP82D275) enzymes having cytochrome P450 activity and the enzyme having cytochrome B5 activity (e.g. TwB5 #1) may be verified by expressing the relevant protein in a yeast cell and assessing whether they are able to produce specific compounds as described herein below.

[0166] A yeast cell expressing a functional homolog of TwCYP82D274 (SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 74 or SEQ ID NO: 75) and further expressing

[0167] i. the diterpene biosynthetic enzymes SPGGPPS7v2 (SEQ ID NO: 81), CfTPS1 (SEQ ID NO: 77), CfTPS3 (SEQ ID NO: 78) and TwCPR1 (SEQ ID NO: 9), is preferably capable of producing 14-hydroxydehydroabietadiene.

[0168] A yeast cell expressing a functional homolog of TwCYP71BE86 (SEQ ID NO: 4) and further expressing

[0169] i. the diterpene biosynthetic enzymes SPGGPPS7v2 (SEQ ID NO: 81), CfTPS1 (SEQ ID NO: 77), CfTPS3 (SEQ ID NO: 78) and TwCPR1 (SEQ ID NO: 9); and

[0170] ii. TwCYP82D274 (SEQ ID NO: 1 or SEQ ID NO: 2),

is preferably capable of producing 14-hydroxydehydroabietadiene, 3,14-dihydroxydehydroabietadiene, 3,14-dihydroxyabeodiene and 14-hydroxy-18-aldo-abediene.

[0171] A yeast cell expressing a functional homolog of TwCYP71BE85 (SEQ ID NO: 3) and further expressing

[0172] i. the diterpene biosynthetic enzymes SPGGPPS7v2 (SEQ ID NO: 81), CfTPS1 (SEQ ID NO: 77), CfTPS3 (SEQ ID NO: 78) and TwCPR1 (SEQ ID NO: 9);

[0173] ii. TwCYP82D274 (SEQ ID NO: 1 or SEQ ID NO: 2); and

[0174] iii. TwCYP71BE86 (SEQ ID NO: 4),

is preferably capable of producing 14-hydroxydehydroabietadiene, 3,14-dihydroxydehydroabietadiene, 3,14-dihydroxyabeodiene, 14-hydroxy-18aldo-abeodiene and triptophenolide.

[0175] A yeast cell expressing a functional homolog of TwCYP82D213 (SEQ ID NO: 5 or SEQ ID NO: 76) and further expressing

[0176] i. the diterpene biosynthetic enzymes SPGGPPS7v2 (SEQ ID NO: 81), CfTPS1 (SEQ ID NO: 77), CfTPS3 (SEQ ID NO: 78) and TwCPR1 (SEQ ID NO: 9);

[0177] ii. TwCYP82D274 (SEQ ID NO: 1 or SEQ ID NO: 2);

[0178] iii. TwCYP71BE86 (SEQ ID NO: 4); and

[0179] iv. TwCYP71BE85 (SEQ ID NO: 3),

is preferably capable of producing 14-hydroxydehydroabietadiene, 3,14-dihydroxydehydroabietadiene, 3,14-dihydroxyabeodiene, 14-hydroxy-18aldo-abeodiene, triptophenolide and triptonide.

[0180] A yeast cell expressing a functional homolog of TwB5 #1 (SEQ ID NO: 8) and further expressing

[0181] i. the diterpene biosynthetic enzymes SPGGPPS7v2 (SEQ ID NO: 81), CfTPS1 (SEQ ID NO: 77), CfTPS3 (SEQ ID NO: 78) and TwCPR1 (SEQ ID NO: 9);

[0182] ii. TwCYP82D274 (SEQ ID NO: 1 or SEQ ID NO: 2);

[0183] iii. TwCYP71BE86 (SEQ ID NO: 4);

[0184] iv. TwCYP71BE85 (SEQ ID NO: 3); and

[0185] v. TwCYP82D213 (SEQ ID NO: 5 or SEQ ID NO: 76)

is preferably capable of producing triptonide with a titer that is at least 2-fold, such as at least 3-fold, such as at least 4-fold, such as at least 5-fold higher than an identical yeast cell, except wherein said yeast said does not express said functional homolog of TwB5 #1.

[0186] In preferred embodiments, the enzyme having cytochrome B5 activity comprises or consists of an amino acid sequence according to SEQ ID NO: 8 (TwB5 #1), or a functional homolog thereof having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, preferably at least 96% sequence identity, preferably at least 97% sequence identity, preferably at least 98% sequence identity, preferably at least 98% sequence identity thereto, or the mature polypeptide thereof.

[0187] The polynucleotide of the invention may be provided by cloning from organisms that naturally produce the polypeptides such as the plant *T. wilfordii* or closely related plants, or it may be provided by chemical synthesis of the polynucleotide sequence based on techniques known in the art. The polynucleotide may have a sequence that is identical to a sequence found in nature, or it may have a sequence that is not found in nature, e.g. the sequence may be codon optimized for the particular selected host cell.

[0188] The polypeptides of the invention may be provided from organisms that naturally produce the polypeptides such

as the plant *T. wilfordii* or related organisms; or they may be provided by inserting and expressing polynucleotides encoding polypeptides into a suitable host cell and recovering the polypeptides from culture broth comprising the host cell transformed with the respective genes. It is preferred to provide the polypeptides of the invention from a suitable selected recombinant host cell.

[0189] In order to transform and express a gene in a suitable host cell, the gene is usually operably connected with suitable regulatory elements and inserted into an expression vector suitable for the particular selected host cell. Selecting suitable regulatory elements, constructing a suitable expression vector and transforming the selected host cell is within the skills of the average practitioner and the invention is not limited by any particular selection of such elements.

[0190] The generated host cells comprising the genes of the invention are suitable grown in a container, e.g. a fermenter or shake flasks; under conditions where the genes are expressed and the oxygenated diterpenoid compounds are formed. When growth ceases or a sufficiently high amount of the oxygenated diterpenoid compounds are accumulated in the culture broth, the oxygenated diterpenoid compounds may be further modified and recovered from the culture broth.

[0191] Sequence identity is understood as a measurement of the similarity between two amino acid or nucleotide sequences. Sequence identity is calculated by first aligning the two sequences, counting the number of positions where the two sequences contain the same amino acid residue or nucleotide and calculating the percent identity as the number of positions with identical amino acid residue or nucleotide with the whole length of the alignment.

[0192] Several algorithms have been developed and are available for the skilled person. In this specification and claim, the sequence identity for amino acid sequences are calculated using the NCBI BLAST+pairwise alignment algorithm, using default parameters (BLOSUM 62 matrix, Gap open penalty 11; gap extend penalty 1, Exp. Thr 10), and the sequence identity for nucleotide sequences are calculated using the NCBI BLAST+pairwise alignment algorithm, using default parameters (Match/mismatch scores 1, -3; gap open penalty 5; gap extend penalty 2; exp. Thr 10). The NCBI BLAST+programs are further described in: Madeira F el al (2019) NAR 47: W636-W641.

EXAMPLES

Materials and Methods

Genetic Engineering of *Nicotiana benthamiana*

[0193] *Tripterygium wilfordii* CYP genes were cloned from plant material and co-expressed in *Nicotiana benthamiana* with the diterpene biosynthesis genes CfDXS (SEQ ID NO: 79) or ScTHMGR (SEQ ID NO: 80), CfGGPPS or SpGGPPS7 (SEQ ID NO: 81), CfTPS1 (SEQ ID NO: 67) or CfTPS1 (SEQ ID NO: 77), and CfTPS3 (SEQ ID NO: 68) or CfTPS3 (SEQ ID NO: 78) using constructs and methods previously described in (1-4). Also coexpressed, were the suppressor of gene silencing, p19. Briefly, binary vectors each containing individual diterpene biosynthesis genes or *Tripterygium wilfordii* CYPs (TwCYPs) were transformed into agrobacteria. Liquid cultures of agrobacteria each containing specific plasmids were mixed for co-expression of specific combinations of TwCYPs.

Genetic Engineering of *Saccharomyces cerevisiae* and Growth Conditions for Engineered *S. cerevisiae* Media [0194] YPD media: 20 g/L Bacto™ Peptone, 10 g/L Bacto™ Yeast extract, 20 g/L glucose. Synthetic complete (SC) media without uracil: 1.92 g/L Yeast Synthetic Drop-out Media Supplements without uracil (Sigma-Aldrich Co. LLC. Catalog number Y1501), 6.7 g/L

[0195] Yeast Nitrogen Base Without Amino Acids (Sigma-Aldrich Co. LLC. Catalog number Y0626), 20 g/L glucose. Feed-In-Time (FIT) was based on EnPump200 (Enpresso GmbH), and made according to protocol enclosed with the product. Agar plates: SC media including agar (15 g/L).

[0196] Uracil auxotrophy in parent strains was introduced by selecting for lack of URA3 function on agar plates of SC medium without uracil containing also 5-Fluoroorotic Acid (5-FOA, 0.74 g/L) and uracil (30 mg/L).

[0197] Yeast transformants were isolated on SC without uracil agar plates.

Feed-Batch Fermentation of Engineering *S. cerevisiae* Strains for Isolation of Miltiradiene Derived Diterpenoids

[0198] All engineered *S. cerevisiae* strains were cultivated in 96-deepwell plates using a Feed-In-Time (FIT; m2p-labs) approach similar to previously described (insert ref Forman et al. 2018). For isolation and purification of key intermediates in the triptonide pathway selected engineered *S. cerevisiae* strain were cultivated in feed batch fermentor using a 2 L Biostat® A bioreactor (Sartorius AG). Fed batch fermentation was initiated by addition of a 100 mL starter culture to the reactor tank (with impellers), which in turn was prepared earlier by autoclavage while containing 200 mL Batch glucose and 300 mL Batch salt mix. Also 5 mL vitamin mix, 5 mL micro elements and 0.5 mL trace elements, were added. Cultivation in the bioreactor was started under the following conditions (monitored and automatically controlled): pH=5, temp.=30° C., dissolved oxygen (DO)=20%. While pH was controlled by feeding of ammonium hydroxide (32%) and sulfuric acid (10%), dissolved oxygen was controlled by air supply combined with stirring. Also foam levels were adjusted by addition of anti-foam emulsion (35119, Serva Electrophoresis GmbH). After 18 hours of initial cultivation in the bioreactor, feeding with Feeding solution at a rate of 1.3% was started. The fermentation process continued for 7 days with daily sampling of the culture.

Extraction of Engineered *S. cerevisiae* for LC-MS Analysis [0199] Genetically engineered *S. cerevisiae* strain was transferred into 0.5 mL media in a 96-well plate and grown for 3 days at 30° C. with orbital shaking at 350 rpm. For extraction 0.1 mL of *S. cerevisiae* culture was transferred to 1.5 mL glass vials. 0.4 mL MeOH uHPLC grade was added. *S. cerevisiae* extract was filtered by using a 0.22 µm 96-well filter plate (Merck Millipore, Darmstadt, Germany) and at stored at 4° C. prior to LC-MS analysis.

Extraction of Diterpenoid Metabolites for LC-MS Analysis

[0200] Samples of yeast cultures for LCMS analysis were prepared in 1.5 mL glass vials by mixing yeast cultures and methanol spiked with 5 ppm andrographolide (internal standard; FA17902, CarboSynth) in a ratio of 1:19 (v/v) for daily bioreactor samples and 1:4 (v/v) for 96-deepwell cultures. Mixing proceeded for 30 min with shaking at room temp. For tobacco samples, 2 leaf discs (ϕ =3 cm) placed in 1.5 mL glass vials, were extracted with 1 mL of the methanol extraction solution, for 1 h with shaking at room tempera-

ture. Before LCMS analysis samples were passed through a 0.22 µM 96-well plate filter (Merck Millipore, Darmstadt, Germany) and stored at 5° C.

LC-MS Analysis:

[0201] Methanol (MeOH) extracts were analysed using an Ultimate 3000 UHPLC+Focused system (Dionex Corporation, Sunnyvale, CA) coupled to a Bruker Compact ESI-QTOFMS (Bruker) system. Samples were separated on a Kinetex XB-C18 column (100×2.1 mm ID, 1:7 µm particle size, 100° A pore size; Phenomenex Inc., Torrance, CA) maintained at 40° C with a flow rate of 0.3 mL min⁻¹ and mobile phase consisting of 0.05% (v/v) formic acid in water (solvent A) and 0.05% (v/v) formic acid in acetonitrile (solvent B).

Two LC Protocols were Used:

[0202] LC method 1:0-0.5 min, 10% B; 0.5-21 min, linear increase from 10 to 80% B; 21-31 min, to 90% B; 31-34 min, to 100% B; 34-39 min 100% B; 39-40 min linear decrease from 100 to 10% B.

[0203] LC method 2:0-0.5 min, 20% B; 0.5-11 min, linear increase from 20 to 80% B; 11-20 min, to 90% B; 20-22 min, to 100% B; 22-27 min 100% B; 27-28 min linear decrease from 100 to 20% B.

[0204] LC method 3:0-0.5 min, 20% B; 0.5-9 min, linear increase from 20 to 100% B; 9-11 min, 100% B; 11-11.5 min, linear decrease from 100 to 20% B; 11.5-15 min, 20% B.

Extraction of Diterpenoid Metabolites for GC-MS Analysis

[0205] Samples of yeast cultures for GCMS analysis were prepared in 1.5 mL glass vials by mixing yeast culture and pure methanol at a ratio of 1:4 (v/v). After brief mixing, apolar constituents, were liquid-liquid extracted into hexane, spiked with 10 ppm 1-eicocene, by mixing at a ratio of 1:1 (v/v) and shaking for 1 h. For tobacco samples, 2 leaf discs (ϕ =3 cm) placed in 1.5 ml glass vials, were extracted with 1 mL of the same hexane solution, via 1 h of shaking. Prior to GCMS analysis hexane layers were transferred to new vials.

Gas Chromatography Mass Spectrometry (GC-MS) Analysis

[0206] GC-MS analysis was carried out on a Shimadzu GCMS-QP2010 Ultra (Shimadzu Corp.) with an Agilent HP-5 MS column (Agilent Technologies) 20 mx0.18 mm i.d., 0.18 µm film thickness). Hydrogen was used as a carrier gas at a constant linear velocity of 50 cm s⁻¹, and the injection volume was 1 µL at 250° C. (splitless mode). The oven program was 80° C. for 2 min, ramp at rate 20° C./min to 180° C., ramp at rate 10° C./min to 300° C., ramp at rate 20° C./min to 310° C., hold for 3 min. Data was stored in .CDF format and processed in MZmine2.

Relative Quantification of Miltiradiene Derived Diterpenoids

[0207] Relative compound quantities in yeast cultures were based on normalized peak areas of characteristic ions (data obtained using targeted feature detection in the MZmine2 software). The signal for the following ions were quantified: 1: miltiradiene m/z 91.1, 2:14-hydroxyabietadiene m/z 189.1, 3: F15P1 m/z 303.2318, 4: F20P2 m/z 283.2059, 5: F15P2 m/z 299.2002, 6: triptophenolide m/z

313.1794, 8: triptonide m/z 359.1481. For LCMS and GCMS data a mass deviation of 5 ppm and 100 ppm, respectively, was tolerated.

[0208] The peak area of the base peak ion (m/z 315.1947) for the internal standard andrographolide was used for normalization.

Absolute Quantifications

[0209] Absolute quantifications of triptophenolide (FT65732, CarboSynth) and triptonide (FT65197, CarboSynth) were done by co-analysis of authentic standards prepared in methanol and a final concentration of 5 ppm internal standard (andrographelide). Quantification was based on normalized peak area and calculated from the slopes of linear extrapolations of the standards response curve (triptophenolide 0.05, 0.5, 1, 2 ppm; triptonide 0.5, 1, 2, 10, 20 ppm).

Isolation and Purification of Miltiradiene Derived Compounds from Engineered *S. Cerevisiae* Strain for NMR Analysis

[0210] Compounds in this invention were isolated from bioreactor cultures yeast strains NVJ8.15, and NVJ3.10, and structurally elucidated by NMR. The combined ethyl acetate extracts of broth and methanol-lysed cells (cells: methanol=1:4, v/v) were initially dried in presence of Celite SR (06858, Sigma-Aldrich) via rotary evaporation. Compounds were subsequently isolated by successive fractionations using a puriFlash® 5.250 (Interchim, Montluçon, France) instrument with detection by UV absorbance and Evaporative Light-Scattering Detection (ELSD). This was equipped with either of columns (C1) PF-15SIHP-F0025 (OV002A, Interchim) and (C2) US5C18HQ-100/300 (SSP750, Interchim) for normal phase- and reverse phase separation, respectively.

[0211] An initial pre-fractionation of the dry mix of Celite SR®/crude extract was achieved using column (ref. 9) with loading from a manually packed dry-loading column. Separation was obtained using mobile phases hexane (A) and ethyl acetate (B), a constant flow rate of 15 mL/min, followed by a final washing step with 100% methanol. Compounds of interest were detected by UV and ELSD and collected. Collected fractions were continuously evaluated by LCMS using LC-MS method 3 and TLC analysis prior to further fractionation or NMR studies. Additional purification of compounds of interest from fraction with multiple compounds was done by an additional normal phase fraction using C1 or a reverse phase column fractionation using C2.

[0212] For reverse phase purification with C2 samples were evaporated using rotor evaporation and resuspended in 2 mL methanol. Sample was injected directly onto the pre conditioned column C2. Mobile phases for C2 consisted of solvent C: deionized water and solvent D acetonitrile each acidified with 0.05% (v/v) formic acid. A constant flow rate of 32 mL/min, was used, with a linear solvent gradient with increasing concentration of solvent D. Compounds of interest were detected by ELSD and UV and collected.

[0213] Additional reverse phase purification was done by multiple injections of 100 µL ontp a semi-prep Phenomenex Luna 5 µm C18 (2) 100 Å 250×10 mm (fully porous) (Phenomenex, Inc., Torrance, CA, USA) column on a Shimadzu HPLC (SPD-M20A diode array detector, FRC-10A fraction collector, DGU-20A5 degasser, LC-20AT pump, CBM-20A System controller, CTO-10AS VP column oven, SIL-10AP autosampler). Mobile phase was a linear gradient

between C and D with an increasing amount of D going from 50-100%. Compounds of interest was detected by UV absorbance at 210 nm and collected.

Mass Spectra

[0214] Mass spectra were acquired in positive ion mode over a scan range of m/z 50-1200 with the following ESI and MS settings: capillary voltage, 4000 V; end plate offset, 500 V; dry gas temperature, 220° C.; dry gas flow of 8 L min⁻¹; nebulizer pressure, 2 bar; in source CID energy, 0 eV; hexapole RF, 50 Vpp; quadrupole ion energy, 4 eV; collision cell energy, 7 eV. Raw chromatogram data was calibrated using an internal sodium formate standard and subsequently exported as mzML format using DataAnalysis 4.3 (Build 110.102.1532) (64-bit), Bruker. MZmine ver 2.53 was used for visualizing the LC-MS chromatograms.

Media Recipes for Bioreactor Starting Media and Feed Media

Batch Glucose:

[0215] Glucose monohydrate 55 g/L

Batch Salt Mix:

[0216] Ammonium sulfate 25 g/L

[0217] Potassium phosphate monobasic 5 g/L

[0218] Magnesium sulfate heptahydrate 1.7 g/L

Feed Glucose:

[0219] Glucose monohydrate 880 g/L

Feed Salt Mix:

[0220] Potassium phosphate monobasic 21.6 g/L

[0221] Magnesium sulfate heptahydrate 24.24 g/L

[0222] Potassium sulfate 8.4 g/L

[0223] Sodium sulfate 0.672 g/L

Preparation Notes:

[0224] Batch- and feed salt mixes as well as batch and feed glucose were prepared in separate BlueCap bottles by dissolving components in Milli-Q water and sterilizing by autoclavation.

Feeding Solution:

[0225] A feeding solution was made by mixing 500 mL of feed glucose with 500 mL of feed salt mix, 10 mL of vitamin mix, 10 mL of micro elements solution and 1 mL of trace elements solution.

Example 1: Expression in *Nicotiana benthamiana*

[0226] Leaf material of *N. benthamiana* co-expressing specific combinations of genes of interest (GOI) was harvested 7 days after agrobacterial infiltration. 1 mL methanol (MeOH) was added to 2 leaf disks (Ø=2 cm). Extraction was done at room temperature at 200 rpm orbital shaking. 200 µL of extract was filtered by using a 0.22 µm 96-well filter plate (Merck Millipore, Darmstadt, Germany) and at stored at 4° C. prior to LC-MS analysis. FIG. 1 shows the obtained LCMS profiles. The results show that the *N. benthamiana* cells transformed with CYP82D274V1 encoding the enzyme having SEQ ID NO: 1, leads to production of

14-OH-dehydroabietadiene; when the cells are further transformed with CYP71BE85 and CYP71BE86 encoding the enzymes having the amino acid sequences SEQ ID NO: 3 and SEQ ID NO: 4, respectively, triptophenolide is formed; and when the cells are further transformed with CYP82D213 encoding the enzyme having the amino acid sequence of SEQ ID NO: 5 triptonide is formed. Further, it can be seen that the enzyme having the sequence of SEQ ID NO: 6 and encoded by the gene CYP82D217 increases the production of triptophenolide and triptonide.

Example 2: Construction of *S. cerevisiae* Strains

Strain Construction

[0227] Parent yeast strain was *S. cerevisiae* S288C (NCYC 3608; National Collection of Yeast Cultures, Norwich, UK).

[0228] Genotypes and source of strains are listed in table 3.

[0229] Constructed yeast strains were made using the lithium acetate transformation method (8). Parent strains without functional URA3 were made competent by the following procedure: Inoculation from a glycerol stock into

5 ml YPD medium and growing at 30° C. O/N. Then, transfer of 3 mL of O/N culture to 50 mL YPD medium and continued growing for 4-5 hours followed by centrifugation at 4000 RPM for 10 minutes then discarding the supernatant. Cells were then ready for transformation after 2 washes in sterile water (1st in 25 mL, 2nd in 1 mL) and resuspension in 0.4 mL of sterile water.

[0230] Transformation of competent yeast cells was done by the following procedure: Mixes of designated NotI digested plasmids (2 µL of each) were each added 10 µL competent yeast cells and mixed with 60 µL PEG 3350 (50% w/v), 9 µL LiAc (1 M) and 12.5 µL preboiled salmon sperm DNA (10 mg/ml). The resulting mixes were next incubated at 42° C. for 40 minutes before cells were collected by centrifugation (3000 RPM for 5 minutes) and removal of supernatant. Cells were then resuspended in 100 µL sterile water and spread on SC without uracil agar plates. Isolated transformants appeared as single colonies after 2 days of incubation at 30° C. Insertion of gene constructs was confirmed by colony PCR, using gene and construct specific primers found in table 1. For colony PCR, yeast colonies were resuspended in 50 µL 20 mM NaOH and incubated at 99° C. for 15 min. 1 µL colony suspension was used for PCR.

TABLE 1

List of primers used				
Name	Sequence	Number in Sequence listing	Target gene	Target vector (Entry and/or Destination)
CO_TwCYP71BE85v1_TEF-F	AGCGATACGNAAAATGGACTTATTGCAATTCTCC	10	CO_TwCYP71BE85v1	pX-3-Ass1-KIURA3
CO_TwCYP71BE85v1_TEF-R	CACCGANTCAGTTAAATGCGGGTGATGG	11		
CO_TwGA3OX1_TEF-F	AGCGATACGNAAAATGGCTCTCCGCCTACAATA	12	CO_TwGA3OX1	pX-3-Ass3
CO_TwGA3OX1_TEF-R	CACCGANTTAAATACCTAAAAGCGAGACGGG	13		
CO_AcoUGT2_TEF-F	AGCGATACGNAAAATGGCTGTTAGCTTAAAAATACCG	14	CO_AcoUGT2	pX-3-Ass3
CO_AcoUGT2_TEF-R	CACCGANTTAACGACTGATATGAGCGACG	15		
CO_TwCYP82D213_PGK-F	ATCAACGGNAAAATGGAATTCTCTGTCA	16	CO_TwCYP82D213	pX-3-Ass3
CO_TwCYP82D213_PGK-R	CGTGCANCTAACCATGTAAAGATGTGATGG	17		
CO_TwCYP71BE86_PGK-F	ATCAACGGNAAAATGGACTTACAATTACCTAGCTTCC	18	CO_TwCYP71BE86	pX-3-Ass1-KIURA3
CO_TwCYP71BE86_PGK-R	CGTGCANCTAACGAGATAAAACTACGATATGGG	19		
TwCYP82D217_pLife-F	GGCTTAANAAAGCATCTCTCTCTAACTAGCTTCTAAAT	20	TwCYP82D217	pLife
TwCYP82D217_pLife-R	GGTTAANCTATTGCAATTCAACCCATGTAGACA	21		
pLifeUP_TEF-F	AGCGATACGNACCTGCAGGCTGAGGCTT	22	TwCYP82D217	pAss2
pLife_TEF-R	CACCGANCCGGGCTGAGGTTAAAT	23		
TwCYP82D274v1_pLife-F	GGCTTAANATGGAGTTCTCTTCACTCCAAAC	24	TwCYP82D274v1	pLife

TABLE 1-continued

List of primers used			
Name	Sequence	Number in Sequence listing	Target vector (Entry and/or Destination)
TwCYP82D274v1_pLife-R	GGTTTAANTCAGCCCATATAGAGATGAGCTGGGA G	25	
pLife_TEF-F	AGCGATACTGCAGGCTGAGGCTTAATATG	26	TwCYP82D2 74v1 pX-4-SI- KIURA3
pLife_TEF-R	CACGCGANCCGGGGCTGAGGTTAAT	27	
TwCPR1_pLife-F	GGCTTAANATGCAATCTTCCTCAAATTCTATGAA GG	28	TwCPR1 pLife
TwCPR1_pLife-R	GGTITAANTTACCACACATCCGGAGATA	29	
pLife_PGK-F	ATCAACGGGNTGCAGGCTGAGGCTTAATATG	30	TwCPR1 pX-4-SI- KIURA3
pLife_PGK-R	CGTGCGANCCGGGGCTGAGGTTAAT	31	
TwB5#1_pLife-F	GGCTTAANATGGCTTCGGATCGGAAGATA	32	TwB5#1 pLife
TwB5#1_pLife-R	GGTTTAANCTATTCTTCTGGTGAAGTGACGTA	33	
pLife_PGK-F	ATCAACGGGNTGCAGGCTGAGGCTTAATATG	34	TwB5#1 pAss2
pLife_PGK-R	CGTGCGANCCGGGGCTGAGGTTAAT	35	
TwB5#2_pLife-F	GGCTTAANATGGGTGGAGACGGAAAGGTT	36	TwB5#2 pLife
TwB5#2_pLife-R	GGTTTAANTTAAGCAGGAGGAGCTGATTGGT	37	
pLife_PGK-F	ATCAACGGGNTGCAGGCTGAGGCTTAATATG	38	TwB5#2 pAss2
pLife_PGK-R	CGTGCGANCCGGGGCTGAGGTTAAT	39	
TwB5#3_pLife-F	GGCTTAANATGGCTGGTCAGAGAGTTTCAC	40	TwB5#3 pLife
TwB5#3_pLife-R	GGTTTAANNTAGAACGATCTGCTCAGGCCCTGTA	41	
pLife_PGK-F	ATCAACGGGNTGCAGGCTGAGGCTTAATATG	42	TwB5#3 pAss2
pLife_PGK-R	CGTGCGANCCGGGGCTGAGGTTAAT	43	
TwB5#4_PGK-F	ATCAACGGGNAAAATGGCTAAACTCTTCATT GCTGAG	44	TwB5#4 pAss2
TwB5#4_PGK-R	CGTGCGANTTAGAAAGGTATCGCAAACCAAATG CC	45	
TwB5#5_PGK-F	ATCAACGGGNAAAATGATTATTGTTGCCGTGGCT CTGA	46	TwB5#5 pAss2
TwB5#5_PGK-R	CGTGCGANTTACTCTAGATCCCCAATGTAAA AATCATCG	47	
TwB5#6_PGK-F	ATCAACGGGXAAAATGCCGACTTAAACGAAGCTG CAC	48	TwB5#6 pAss2
TwB5#6_PGK-R	CGTGCGAXCTACTTCTCCGCAAGTACAGGAGTC	49	
YEA85_UP_Genotyping_Fw	TCTCAGGTATAGCATGAGGTCGCTCAT	50	Genotyping UP_ Genotyping
YEA86_DW_Genotyping_Fw	CCTGCAGGACTAGTGCTGAGGCATTAAT	51	Genotyping DW_ Genotyping
YEA87_X-2_Genotyping_UP	GTTTGTAGTTGGCGGTGGAG	52	Genotyping X-2_ Genotyping
YEA88_X- 2_Genotyping_DW	GAGACAAGATGGGGCAAGAC	53	Genotyping X-2_ Genotyping

TABLE 1-continued

List of primers used			
Name	Sequence	Number in Sequence listing	Target vector (Entry and/or Destination)
YEA89_X-3_Genotyping_UP	TGACGAATCGTTAGGCACAG	54	Genotyping X-3_Genotyping
YEA90_X-3_Genotyping_DW	CCGTGCAATACAAAATCGAG	55	Genotyping X-3_Genotyping
YEA91_X-4_Genotyping_UP	CTCACAAAGGGACGAATTCT	56	Genotyping X-4_Genotyping
YEA92_X-4_Genotyping_DW	GACGGTACGTTGACCAGAG	57	Genotyping X-4_Genotyping
YEA93_XI-1_Genotyping_UP	CTTAATGGGTAGTGCTTGACACG	58	Genotyping XI-1_Genotyping
YEA94_XI-2_Genotyping_UP	GTTTGTAGTTGGCGGTGGAG	59	Genotyping XI-2_Genotyping
YEA95_XI-2_Genotyping_DW	GAGACAAGATGGGGCAAGAC	60	Genotyping XI-2_Genotyping
YEA96_XI-5_Genotyping_UP	CTCAATGATCAAAATCCTGAATGCA	61	Genotyping XI-5_Genotyping
YEA97_XI-5_Genotyping_DW	GCATGGTCACCGCTATCAGC	62	Genotyping XI-5_Genotyping
YEA98_XII-2_Genotyping_UP	CGAAGAAGGCCTGCAATT	63	Genotyping XII-2_Genotyping
YEA99_XII-2_Genotyping_DW	GGCCCTGATAAGGTTGTTG	64	Genotyping XII-2_Genotyping
YEA100_XII-5_Genotyping_UP	CCACCGAAGTTGATTGCTT	65	Genotyping XII-5_Genotyping
YEA101_XII-5_Genotyping_DW	GTGGGAGTAAGGGATCCTGT	66	Genotyping XII-5_Genotyping

Assembly of Genetic Constructs for *S. cerevisiae* Genome Engineering

[0231] Plasmid names and encoded gene constructs are listed in table 2. All plasmids were generated by USER cloning as previously described (5). Also, parent vectors named assembler-1, -2 and -3, for simultaneous genome integration of up to six gene constructs, and harboring AsiSI/Nb.BsmI USER-cassettes, were prepared for USER cloning as previously described (6). Primers used for PCR amplification with USER compatible PfuX7 polymerase (7) are listed in table 1. Vectors used and generated in this work is listed in table 3.

[0232] Codon optimized genes for *S. cerevisiae* were acquired from TWIST Biosciences, USA, San Francisco. All genes denoted with the prefix "CO_" in the below tables were codon-optimized. Codon-optimized genes were amplified using primers identical to those described in Table 1 above, except that the primers were modified to accommodate hybridization to any nucleotide changes in the codon-optimized genes. The primers for amplification of the codon-optimized genes are also disclosed in J. Andersen-Ranberg et al., Expanding the Landscape of Diterpene Structural Diversity through Stereochemically Controlled Combinatorial Biosynthesis. *Angewandte Chemie International Edition*, n/a (2016).

TABLE 2

Vectors and plasmids generated and used		
Vectors for yeast genome-integration		
Name	Description	Source
pCYT183	pX-3-Ass1-KIURA3-PpTDH3::CO_TwCYP71BE86	This study

TABLE 2-continued

Vectors and plasmids generated and used		
Vectors for yeast genome-integration		
Name	Description	Source
pCYT184	pX-3-Ass1-KIURA3- TpCCW12::CO_TwCYP71BE85v1	This study
pCYT185	pX-3-Ass1-KIURA3- PpTDH3::CO_TwCYP71BE86- TpCCW12::CO_TwCYP71BE85v1	This study
pCYT186	pX-3-Ass3-PpENO2::CO_TwCYP82D213	This study
pCYT187	pX-3-Ass3-TpPDC1::CO_TwGAOX1	This study
pCYT188	pX-3-Ass3-PpENO2::CO_TwCYP82D213- TpPDC1::CO_TwGAOX1	This study
p349	pAss2-PpFBA1::TwB5#2- TpSED1::TwCYP82D217	This study
p350	pAss2-PpFBA1::TwB5#3- TpSED1::TwCYP82D217	This study
p351	pAss2-PpFBA1::TwB5#1- TpSED1::TwCYP82D217	This study
p352	pAss2-PpFBA1::TwB5#6- TpSED1::TwCYP82D217	This study
p353	pAss2-PpFBA1::TwB5#5- TpSED1::TwCYP82D217	This study
p354	pAss2-PpFBA1::TwB5#4- TpSED1::TwCYP82D217	This study
p355	pAss2-TpSED1::TwCYP82D217	This study
pJAR1	pX-3-Ass3-TpPDC1::CO_AcUGT2	This study
pJAR2	pX-3-Ass3-PpENO2::CO_TwCYP82D213- TpPDC1::CO_AcUGT2	This study
p320	pX-4-SI-PpTEF2::TwCPR1- TpTDH3::TwCYP82D274v1	This study
pVictor1	pXI-2-Ass1-pICL1::CO_SpGGPPS7	This study
pVictor2	pAss2-pPGK1::CO_CfTPS3	This study
pVictor3	pXI-2-Ass3-pTEF1::CO_CfTPS3	This study
pCYT85	pAss2A-PpPGK1::CO_CfTPS1- TpTEF1::CO_CfTPS3	This study
pTRIP10	pX-3-Ass3-PpSED1::TwCYP82D274v1- TpFBA1::TwB5#1	This study
pTRIP108	pAss2C-PpENO2::CO_TwCYP71BE85	This study
pTRIP110	pX-3-Ass3-PpSED1::TwCYP82D274v1	This study
pTRIP14	pSXI-2-PpPGK1::TwCPR1- TpTP11::TwB5#4	This study
pTRIP4	pAss2B-PpCCW12::CO_TwCYP82D213- TpSED1::CO_TwCYP71BE86	This study
pTRIP5	pAss2C-PpENO2::CO_TwCYP71BE85- TpPDC1::CO_TwCYP82D213	This study
pTRIP50	pXII-2-Ass1-PpPGK1::CO_TwCYP71BE86- TpTP11::CO_TwCYP71BE85	This study
pTRIP52	pAss2A-PpTDH3::CO_TwCYP82D213- TpSED1::CO_TwCYP82D213	This study
pTRIP53	pAss2B-PpPDC1::TwCYP82D274v1- TpENO2::TwCYP82D274v1	This study
pTRIP54	pAss2C-PpTEF1::TwCYP82D213- TpPGK1::TwCYP82D213	This study
pTRIP55	pXII-2-Ass3-PpSED1::TwB5#1- TpFBA1::TwB5#4	This study
pTRIP7	pX-3-Ass1-pTEF2::TwCPR1- TpICL1::CO_SpGG-PPS7	This study
pTRIP8	pX-3-Ass1-pTEF2::TwCPR2- TpICL1::CO_SpGG-PPS7	This study
pTRIP88	pAss2B-TpSED1::CO_TwCYP71BE86	This study
pTRIP92	pX-3-Ass3-PpSED1::TwCYP82D274v3- TpFBA1::TwB5#1	This study
pTRIP95	pX-3-Ass3-PpSED1::TwCYP82D274v4- TpFBA1::TwB5#1	This study
pTRIP89	pAss2C-PpENO2::CO_TwCYP71BE85- TpPDC1::CO_TwCYP82D213v2	This study
pTRIP3	pAss2-PpFBA1::TwB5#1	This study
pCYT185	pX-3-Ass1-PpTDH3::CO_TwCYP71BE86- TpCCW12::CO_TwCYP71BE85	This study
pX-3-Ass3	pX-3-Ass3 empty	This study
pX-3-Ass1	pX-3-Ass1 empty	This study
pAss2A	pAss2A empty	This study
pAss2B	pAss2B empty	This study
pAss2C	pAss2C empty	This study
pVic1	pLIFE-SctHMGR	This study

TABLE 2-continued

Vectors and plasmids generated and used		
Vectors for yeast genome-integration		
Name	Description	Source
pVic2	pLIFE-CfTPS1	This study
pVic3	pLIFE-CfTPS3	This study
pVic4	pLIFE-SpGGPPS7	This study
239.TwCYP756A1	pLIFE-TwCYP82D274v1	This study
59.TwCYP10	pLIFE-TwCYP71BE85	This study
81.TwCYP9		
81.TwCYP9	pLIFE-TwCYP71BE86	This study
297.Twb5#1	pLIFE-Twb5#1	This study
46.TwCYP17	pLIFE-TwCYP82D213	This study
P19	pBin61-p19	Voinnet et al., 2003 (ref.: 10)
pDXS	pLIFE-CfDXS	This study
pCfTPS1	pLIFE-pCfTPS1	This study
pCfTPS2	pLIFE-pCfTPS2	This study
pCfGGPPS	pLIFE-pCfGGPPS	This study

TABLE 3

List of <i>S. cerevisiae</i> strains used and generated.		
Name	Genotype	Source
S288c	MAT α , SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6	National Collection of Yeast Cultures (NCYC)
NVJ0	MAT α , SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, XI-2::(pTEF1-CO_CfTPS1/pPGK1-CO_CfTPS3/pICL1-CO_SpGGPPS7/KIURA3)	This study
NVJ1-3.5	MAT α , SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, XI-2::(pTEF1-CO_CfTPS1/pPGK1-CO_CfTPS3/pICL1-CO_SpGGPPS7), X-4::(pTDH3-TwCYP82D274v1/pTEF2-TwCPR1/KIURA3)	This study
NVJ3.10	MAT α , SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, XI-2::(pTEF1-CO_CfTPS1/pPGK1-CO_CfTPS3/pICL1-CO_SpGGPPS7), X-4::(pTDH3-TwCYP82D274v1/pTEF2-TwCPR1), X-3::(pTDH3-CO_TwCYP71BE86/pCCW12-CO_TwCYP71BE85v1/pFBA1-Twb5#1)	This study
NVJ2-19	MAT α , SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, XI-2::(pTEF1-CO_CfTPS1/pPGK1-CO_CfTPS3/pICL1-CO_SpGGPPS7), X-4::(pTDH3-TwCYP82D274v1/pTEF2-TwCPR1), X-3::(pTDH3-CO_TwCYP71BE86/pCCW12-CO_TwCYP71BE85v1/pENO2-CO_TwCYP82D213/pSED1-TwCYP82D217/pFBA1-Twb5#1/KIURA3)	This study
NVJ11-0	MAT α , SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, X-3::(KIURA3)	This study
NVJ11-1	MAT α , SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, X-3::(pTEF2-TwCPR1/pICL1-CO_SpGGPPS7/pPGK1-CO_CfTPS1/pTEF1-CO_CfTPS3/KIURA3)	This study
NVJ11-2	MAT α , SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, X-3::(pTEF2-TwCPR1/pICL1-CO_SpGGPPS7/pPGK1-CO_CfTPS1/pTEF1-CO_CfTPS3/pSED1-TwCYP82D274V1/KIURA3)	This study
NVJ11-3	MAT α , SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, X-3::(pTEF2-TwCPR1/pICL1-CO_SpGGPPS7/pPGK1-CO_CfTPS1/pTEF1-CO_CfTPS3/pENO2-CO_TwCYP71BE85v1/pSED1-TwCYP82D274V1/KIURA3)	This study

TABLE 3-continued

List of <i>S. cerevisiae</i> strains used and generated.		
Name	Genotype	Source
NVJ11-4	MAT α , SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, X-3::(pTEF2-TwCPR1/pICL1-CO_SpGGPPS7/pPGK1-CO_CfTPS1/pTEF1-CO_CfTPS3/pSED1-CO_TwCYP71BE86/pSED1-TwCYP82D274V1/KIURA3)	This study
NVJ11-5	MAT α , SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, X-3::(pTEF2-TwCPR1/pICL1-CO_SpGGPPS7/pPGK1-CO_CfTPS1/pTEF1-CO_CfTPS3/pSED1-CO_TwCYP71BE86/pENO2-CO_TwCYP71BE85v1/pPDC1-CO_TwCYP82D213/pSED1-TwCYP82D274V1/KIURA3)	This study
NVJ11-6	MAT α , SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, X-3::(pTEF2-TwCPR1/pICL1-CO_SpGGPPS7/pPGK1-CO_CfTPS1/pTEF1-CO_CfTPS3/pSED1-CO_TwCYP71BE86/pENO2-CO_TwCYP71BE85v1/pPDC1-CO_TwCYP82D213/pSED1-TwCYP82D274V1/KIURA3)	This study
NVJ11-7	MAT α , SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, X-3::(pTEF2-TwCPR1/pICL1-CO_SpGGPPS7/pPGK1-CO_CfTPS1/pTEF1-CO_CfTPS3/pSED1-TwCYP82D274V1/pFBA1-Twb5#1/KIURA3)	This study
NVJ11-8	MAT α , SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, X-3::(pTEF2-TwCPR1/pICL1-CO_SpGGPPS7/pPGK1-CO_CfTPS1/pTEF1-CO_CfTPS3/pENO2-CO_TwCYP71BE85v1/pSED1-TwCYP82D274V1/pFBA1-Twb5#1/KIURA3)	This study
NVJ11-9	MAT α , SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, X-3::(pTEF2-TwCPR1/pICL1-CO_SpGGPPS7/pPGK1-CO_CfTPS1/pTEF1-CO_CfTPS3/pSED1-CO_TwCYP71BE86/pSED1-TwCYP82D274V1/pFBA1-Twb5#1/KIURA3)	This study
NVJ11-10	MAT α , SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, X-3::(pTEF2-TwCPR1/pICL1-CO_SpGGPPS7/pPGK1-CO_CfTPS1/pTEF1-CO_CfTPS3/pSED1-CO_TwCYP71BE86/pENO2-CO_TwCYP71BE85v1/pSED1-TwCYP82D274V1/pFBA1-Twb5#1/KIURA3)	This study
NVJ11-11	MAT α , SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, X-3::(pTEF2-TwCPR1/pICL1-CO_SpGGPPS7/pPGK1-CO_CfTPS1/pTEF1-CO_CfTPS3/pSED1-CO_TwCYP71BE86/pENO2-CO_TwCYP71BE85v1/pPDC1-CO_TwCYP82D213/pSED1-TwCYP82D274V1/pFBA1-Twb5#1/KIURA3)	This study
NVJ8-15	MAT α , SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, X-3::(pTEF2-TwCPR2/pICL1-CO_SpGGPPS7/pPGK1-CO_CfTPS1/pTEF1-CO_CfTPS3/pCCW12-CO_TwCYP82D213/pSED1-CO_TwCYP71BE86/pENO2-CO_TwCYP82D213/pPDC1-CO_TwCYP82D213/pSED1-TwCYP82D274V1(XI-2::(pPGK1-TwCPR1/pTPI1-Twb5#4) XII-2::(pPGK1-CO_TwCYP71BE86/pTPI1-TwCYP71BE85v1/pTDH3-CO_TwCYP82D213/pSED1-CO_TwCYP82D213/pPDC1-TwCYP82D274V1/pENO2-TwCYP82D274V1/pTEF1-TwCYP82D213/pPGK1-TwCYP82D213/pSED1-Twb5#1/pFBA1-Twb5#4/KIURA3)	This study
NVJ10-1	MAT α , SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, X-3::(pTEF2-TwCPR1/pICL1-CO_SpGGPPS7/pPGK1-	This study

TABLE 3-continued

List of <i>S. cerevisiae</i> strains used and generated.		
Name	Genotype	Source
NVJ10-3	CO_CfTPS1/pTEF1-CO_CfTPS3/pSED1- CO_TwCYP71BE86/pENO2- CO_TwCYP71BE85v1/pPDC1- CO_TwCYP82D213/pSED1- TwCYP82D274v1/pFBA1-Twb5#1/KIURA3) MATα, SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, X-3::(pTEF2- TwCPR1/pICL1-CO_SpGGPPS7/pPGK1- CO_CfTPS1/pTEF1-CO_CfTPS3/pSED1- CO_TwCYP71BE86/pENO2- CO_TwCYP71BE85v1/pPDC1- CO_TwCYP82D213/pSED1- TwCYP82D274v3/pFBA1-Twb5#1/KIURA3)	This study
NVJ10-6	MATα, SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, X-3::(pTEF2- TwCPR1/pICL1-CO_SpGGPPS7/pPGK1- CO_CfTPS1/pTEF1-CO_CfTPS3/pSED1- CO_TwCYP71BE86/pENO2- CO_TwCYP71BE85v1/pPDC1- CO_TwCYP82D213/pSED1- TwCYP82D274v4/pFBA1-Twb5#1/KIURA3)	This study
NVJ10-8	MATα, SUC2, gal2, mal2, mel, flo1, flo8-1, ho, bio1, bio6, ura3Δ::KanMX, X-3::(pTEF2- TwCPR1/pICL1-CO_SpGGPPS7/pPGK1- CO_CfTPS1/pTEF1-CO_CfTPS3/pSED1- CO_TwCYP71BE86/pENO2- CO_TwCYP71BE85v1/pPDC1- CO_TwCYP82D213v2/pSED1- TwCYP82D274V1/pFBA1-Twb5#1/KIURA3)	This study

**Example 3: Expression in the Yeast,
*Saccharomyces cerevisiae***

Extraction and Metabolite Analysis

[0233] Genetically engineered *S. cerevisiae* strains were transferred into 0.5 mL media in a 96-well plate and grown for 3 days at 30° C. with orbital shaking at 350 rpm. For extraction 0.1 mL of *S. cerevisiae* culture was transferred to 1.5 mL glass vials. 0.4 mL MeOH uHPLC grade was added. *S. cerevisiae* extracts extract was filtered by using a 0:22 μm 96-well filter plate (Merck Millipore, Darmstadt, Germany) and at stored at 4° C. prior to LC-MS analysis.

[0234] LCMS profiles of the extracts can be seen in FIG. 2, where it can be observed that transforming the background strain with TwCYP82D274V1 encoding the enzyme having the amino acid sequence of SEQ ID NO: 1 leads to formation of 14-OH-dehydroabietadiene.

**Example 4: Detection of
14-OH-Dehydroabietadiene by NMR Analysis**

[0235] The compound identified as 14-OH-dehydroabietadiene in example 3 was analyzed by NMR to confirm its identity.

Purification for NMR

Purification of Triptolide Intermediates from Engineered Yeast

[0236] Engineered yeast producing the desired compound of interest was inoculated from SCagar in 10 mL YDP and grown ON at 30° C. 5 mL ON culture was inoculated in 500 mL FIT media and grown for 5 days at 30° C. Compound of interest was extracted from culture with 500 mL EtAc.

Solvent was removed by rotor evaporation and analytes were resuspended in hexane. Extraction was repeated 3 times. Hexane extract was applied on Supelclean™ Florisil®/Na2SO4 SPE Tube (Sigma-Aldrich) and analytes were eluted from column using a step gradient with 1:99-5:95 EtAc: Hexane. Each fraction was analyzed with either LC-MS or GC-MS and the fraction containing the compound of interest was selected for NMR analysis.

NMR Analysis

[0237] NMR data were acquired on a Bruker Avance III HD 600 MHZ NMR spectrometer (1H operating frequency 599.85 MHZ) equipped with a 5 mm cryogenically cooled DCH probe optimized for 13C and 1H (Bruker Biospin, Karlsruhe, Germany). NMR data was recorded in 5 mm tubes in CDCl₃ (Euriso-top, 99.8 atom % D) with temperature equilibration to 300 K, optimization of lock parameters, gradient shimming, and setting of receiver gain, all automatically controlled by Topspin ver. 3.2 and IconNMR ver. 4.7.5 (Bruker Biospin, Karlsruhe, Germany). 1H and 13C chemical shifts were referenced to the residual solvent signals of at respectively pH 7.26 ppm and pC 77.16 ppm. 1D 1H and 13C NMR spectra were acquired with 30° pulses and 64k data points and zero-filled to 256k data points, 1H spectra were acquired with a spectral width of 12 kHz, a relaxation delay of 1 s and an acquisition time of 2.7 s. 13C spectra were 1H-decoupled using the Waltz-16 composite pulse decoupling scheme. 2D homo- and heteronuclear experiments were acquired with 4096 (HMBC), 2048 (DQF-COSY and ROESY), or 1024 (multiplicity edited HSQC) data points in the direct dimension and 256 (DQF-COSY, HMBC and ROESY) or 128 (multiplicity edited HSQC)

data points in the indirect dimension. 2D NMR data was zero-filled to 1k in F1 and zero-filled to twice the number of points in F2, employing forward linear prediction in F1 (LPBIN=0). Processing of NMR data was done using Topspin ver. 4.0.9 (Bruker Biospin, Karlsruhe, Germany).

[0238] The NMR spectroscopic data for 14-OH-dehydroabietadiene is shown in table 4.

TABLE 4

¹ H and ¹³ C NMR spectroscopic data of 1 (14-OH-dehydroabietadiene)			
14-OH-dehydroabietadiene (1)			
Pos	δ_H , nH, δ_C ^a multiplicity (J in Hz) ^{a, b}	HMBC	ROESY
1	39.1 A: 2.28, 1H, br d (12.8) B: 1.40, 1H, td (12.8, 3.4)	2, 3, 5, 10, 20 2, 3, 9, 10, 20 (11)	2A, 2B, 11, (20)
2	19.5 A: 1.75, 1H, m B: 1.62, 1H, m	1, 3, 4 1, 3, 4, 10 1B	1B
3	41.8 A: 1.49, 1H, br d (13.2) B: 1.22, 1H, m	1, 2, 4, 5, 18, 19 2, 4, 18, 19	18
4	33.5 —		
5	49.9 1.34, 1H, dd, (12.7, 2.1)	4, 6, 9, 10, 19, 20	18
6	18.6 A: 1.99, 1H, br dd (13.1, 7.9) B: 1.72, 1H, m	4, 5, 7, 8, 10 5, 7, 10	7B, 18 7A, 19
7	24.5 A: 2.82, 1H, dd (16.5, 6.7) B: 2.62, 1H, ddd (16.5, 11.4, 7.9)	5, 6, 8, 9, (14) 6, 8, 9	6B, 14-OH 6A, 14-OH
8	120.8 —		
9	149.2 —		
10	37.7 —		
11	116.5 6.87, 1H, d (8.2)	8, 10, (12), 13, (14)	1A, (1B), (20)
12	123.4 7.02, 1H, d (8.2)	9, (11), 14, 15	16, 17
13	130.1 —		
14	150.4 —		
14-OH	— 4.63, 1H, s 27.0 3.16, 1H, sep (6.9) 22.9 1.24, 3H, d (6.9) 22.7 1.26, 3H, d (6.9) 33.4 0.97, 3H, s 21.8 0.94, 3H, s 25.0 1.20, 3H, s	8, 13, 14 12, 13, 14, 16, 17 13, 15, 17 13, 15, 16 3, 4, 5, 19 3, 4, 5, 18 1, 5, 9, 10	7A, 7B, 15 14-OH, 16, 17 12, 15 12, 15 3B, 5, 6A 6B, 20 (1B), (11), 19

^a¹H NMR (599.85 MHz) and ¹³C NMR (150.83 MHz) data obtained in CDCl₃.

^bnH = number of hydrogens. Multiplicities reported as apparent splittings: s = singlet, d = doublet, t = triplet, sep = septet, m = multiplet (also in case of overlap), br = broad. 'A' denotes the highest chemical shift value and 'B' denotes the lowest chemical shift value.

[0239] The ¹H NMR spectrum of 14-OH-dehydroabietadiene in CDCl₃ at 599.85 MHz is shown in FIG. 3 and the ¹³C NMR spectrum of 14-OH-dehydroabietadiene in CDCl₃ at 150.83 MHz is shown in FIG. 4 confirming the identity of the compound.

Example 5: Expression in *S. cerevisiae* of Genes Leading to Production of Triptophenolide and Triptonide

[0240] This was a preliminary study to assess the effects of expression of genes leading to production of triptophenolide and triptonide in *S. cerevisiae*.

[0241] The background yeast strain generated in example 2, was further transformed with vectors each containing individual diterpene biosynthesis genes or *Tripterygium wilfordii* CYPs (TwCYPs)

[0242] LCMS profiles of the extracts can be seen in FIG. 5, where it can be seen that transforming the background strain with TwCYP82D274V1, TwCYP71BE85 and TwCYP71BE86 encoding the enzymes having the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 3 and SEQ ID NO: 4, respectively; leads to formation of triptophenolide; and further transformation with CYP82D213 encoding

the enzyme having the amino acid sequence of SEQ ID NO: 5, leads to the formation of triptonide.

[0243] FIG. 6 shows an overview of the content of oxygenated diterpenoid compounds detected in the extracts of the transformants generated.

[0244] The left panel shows the content of triptophenolide and triptonide, and the right panel shows the content of

14-OH-dehydroabietadiene. Expressing the gene TwB5 #1 encoding the enzyme having the amino acid sequence of SEQ ID NO: 8 resulted in a significantly higher production of triptophenolide and triptonide.

[0245] The genes TwB5 #2-6 are other *T. wilfordii* cytochrome B5 genes (sequences not provided) that do not increase the production of triptophenolide or triptonide.

Example 6: Production of Oxygenated Diterpenoid Compounds in *S. cerevisiae* and *N. benthamiana*

[0246] All engineered *S. cerevisiae* strains and *N. benthamiana* were cultured as described herein above in the section "Materials and Methods". Similarly, diterpenoid metabolites were extracted, analyzed by LC-MS, GC-MS and NMR, and quantified as also described herein above.

[0247] It is preferred that the experimental organism is yeast and that the heterologous genes have been stably transfected in the organism as this gives the most precise and reproducible results.

[0248] The results are shown in FIGS. 7-9. As can be clearly seen from the figures, organisms as different as yeast

cells and tobacco plants are both capable of producing the claimed key intermediates in the proposed biosynthetic pathway of triptonide at high titers according to the methods of the invention.

[0249] NMR spectra of other key compounds also produced are shown in FIGS. 10-26. The NMR spectroscopic data for the produced compounds are shown in Tables 5-21, below.

TABLE 5

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F1-14				
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY
1	36.8, CH ₂	α : 1.49 (1H, m) β : 2.27 (1H, dt, 13.3, 3.1)	C-2, C-3, C-9, C-10, C-20 C-2, C-3, C-5, C-10, C-20	H-1 β , H-3 H-1 α , H-2, H-11, H-20
2	27.3, CH ₂	1.79 (2H, m)	C-1, C-3, C-4, C-10	H-1 β , H-19, H-20
3	75.9, CH	3.69 (1H, t, 7.5)	C-2, C-4, C-18, C-19	H-1 α , H-5
4	42.0, C	—		
5	43.5, CH	1.45 (1H, br d, 11.8)	C-1, C-4, C-6, C-7, C-9, C-10, C-18, C-20	H-3, H-7A, H-18A
6	18.2, CH ₂	A: 1.75 (1H, m) B: 1.80 (1H, m)	C-5, C-7, C-10 C-8, C-10	
7	24.3, CH ₂	A: 2.62 (1H, m) B: 2.82 (1H, dd, 16.2, 5.1)	C-6, C-8, C-9, C-14 C-5, C-6, C-8, C-9, C-14	H-5
8	120.7, C	—		
9	147.9, C	—		
10	37.1, C	—		
11	116.4, CH	6.82 (1H, d, 8.2)	C-8, C-10, C-13	H-1 β , H-20
12	123.3, CH	7.01 (1H, d, 8.2)	C-9, C-14, C-15	H-15, H-16, H-17
13	130.4, C	—		
14	150.2, C	—		
15	26.7, CH	3.14 (1H, sep, 6.9)	C-12, C-13, C-14, C-16, C-17	H-12
16	22.7, CH ₃	1.22 (3H, d, 6.9)	C-13, C-15, C-17	H-12
17	22.6, CH ₃	1.24 (3H, d, 6.9)	C-13, C-15, C-16	H-12
18	71.0, CH ₂	A: 3.46 (1H, d, 8.9) B: 3.76 (1H, d, 8.9)	C-3, C-4, C-5, C-19 C-3, C-4, C-5	H-5
19	11.2, CH ₃	0.96 (3H, s)	C-3, C-4, C-5, C-18	H-2, H-20
20	25.2, CH ₃	1.22 (3H, s)	C-1, C-5, C-9, C-10	H-1 β , H-2, H-11, H-19

TABLE 6

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F1-15				
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY
1	32.7, CH ₂	α : 1.69 (1H, m) β : 2.46 (1H, m)	C-2, C-10, C-20 C-2, C-5, C-9, C-20	H-1 β , H-2B, H-5 H-1 α , H-11, H-20
2	22.5, CH ₂	A: 2.45 (1H, m) B: 2.52 (1H, m)	C-1, C-3, C-4, C-10 C-1, C-3, C-4	H-19B H-1 α , H-19A
3	132.0, C	—		
4	150.7, C	—		
5	39.2, CH	2.60 (1H, m)		
6	17.5, CH ₂	α : 3.28 (1H, m) β : 1.73 (1H, m)	C-8, C-10 C-5, C-7, C-10	H-1 α , H-6 α , H-7B H-5, H-7B H-20
7	22.5, CH ₂	A: 2.46 (1H, m) B: 2.80 (1H, td, 9.5, 7.6)	C-5, C-6, C-8, C-9, C-14	H-6 α , H5
8	120.8, C	—		
9	144.5, C	—		
10	36.1, C	—		
11	115.7, CH	6.91 (1H, d, 8.1)	C-8, C-10, C-12, C-13	H-1 β , H-20
12	123.0, CH	7.04 (1H, d, 8.1)	C-9, C-11, C-14, C-15	H-16, H-17
13	131.2, C	—		
14	150.9, C	—		
15	26.9, CH	3.18 (1H, sep, 7.0)	C-12, C-13, C-14, C-16, C-17	
16	22.6, CH ₃	1.24 (3H, d, 7.0)	C-13, C-15, C-17	H-12
17	22.6, CH ₃	1.26 (3H, d, 7.0)	C-13, C-15, C-16	H-12
18	173.5, C	—		
19	53.9, CH ₂	A: 3.90 (1H, dd, 18.7, 2.1) B: 3.94 (1H, dd, 18.7, 2.7)	C-3, C-4, C-18 C-3, C-4, C-18	H-2B, H-1' H-2A, H-1'
20	22.4, CH ₃	1.03 (3H, s)	C-1, C-5, C-9, C-10	H-1 β , H-6 β , H-11
1'	46.1, CH ₂	A: 3.56 (1H, m) B: 3.63 (1H, m)	C-18, C-19, C-2' C-18, C-19, C-2'	H-19 H-19
2'	62.2, CH ₂	3.82 (2H, m)	C-1'	H-19

TABLE 7

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F1-18				
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY
1	34.0, CH ₂	α : 1.23 (1H, m) β : 2.79 (1H, dt, 13.5, 3.3)	C-2, C-10, C-20 C-3, C-5, C-10	H-1 β , H-3 H-1 α , H-2, H-20
2	27.1, CH ₂	1.76 (2H, m)	C-1, C-3, C-4, C-10	H-1 β , H-20
3	75.1, CH	3.68 (1H, dd, 10.3, 5.8)	C-2, C-4, C-18, C-19	H-1 α , H-5, H-18A
4	42.0, C	—		
5	45.2, CH	1.29 (1H, d, 12.5)	C-4, C-6, C-7, C-10, C-18, C-19, C-20	H-3, H-7B, H-18A
6	17.1, CH ₂	α : 1.73 (1H, m) β : 1.48 (1H, qd, 12.5, 5.7)	C-5, C-7, C-8, C-10 C-5, C-7, C-10	H-7B, H-18A, H-18B H-7B, H-19
7	25.8, CH ₂	A: 2.32 (1H, ddd, 20.2, 11.6, 7.4) B: 2.69 (1H, dd, 20.2, 5.5)	C-6, C-8, C-9, C-14 C-5, C-6, C-8, C-9, C-14	H-7B, H-6 α H-5, H-6 α , H-6 β , H-7A
8	142.6, C	—		
9	149.9, C	—		
10	38.0, C	—		
11	187.7, C	—		
12	131.8, CH	6.31 (1H, s)	C-9, C-11, C-14, C-15	H-15, H-16/17
13	152.9, C	—		
14	187.6, C	—		
15	26.2, CH	2.97 (1H, sep, 6.9)	C-12, C-13, C-14, C-16, C-17	H-12
16	21.2, CH ₃	1.08 (3H, d, 6.9)	C-13, C-15, C-17	H-12
17	21.2, CH ₃	1.09 (3H, d, 6.9)	C-13, C-15, C-16	H-12
18	70.4, CH ₂	A: 3.42 (1H, d, 10.3) B: 3.74 (1H, d, 10.3)	C-3, C-4, C-5, C-19 C-3, C-4, C-5, C-19	H-3, H-5, H-6 α , H-19 H-6 α , H-19
19	11.6, CH ₃	0.92 (3H, s)	C-3, C-4, C-5, C-18	H-6 β , H-18A, H-18B, H-20
20	20.4, CH ₃	1.31 (3H, s)	C-1, C-5, C-9, C-10	H-1 β , H-2, H-19

TABLE 8

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F1-23 (F15P1)				
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY
1	37.1, CH ₂	α : 1.55 (1H, td, 13.2, 4.2) β : 2.31 (1H, dt, 13.2, 3.5)	C-2, C-3, C-9, C-10, C-20 C-2, C-3, C-5, C-10, C-20	H-3 H-2, H-11, H-20
2	27.9, CH ₂	1.80 (2H, m)	C-1, C-3, C-4, C-10	H-1 β
3	78.7, CH	3.31 (1H, dd, 11.5, 4.7)	C-2, C-4, C-18, C-19	H-1 α , H-5, H-18
4	38.9, C	—		
5	49.2, CH	1.32 (1H, dd, 12.5, 2.0)	C-3, C-4, C-6, C-7, C-9, C-10, C-18, C-19, C-20	H-1 α , H-3, H-7 α , H-18
6	18.2, CH ₂	α : 2.00 (1H, ddt, 13.3, 7.9, 2.0) β : 1.77 (1H, m)	C-4, C-5, C-7, C-8, C-10 C-5, C-7, C-10	H-7 α , H-7 β , H-18, H-19 H-7 β , H-19
7	24.6, CH ₂	α : 2.62 (1H, ddd, 16.7, 11.6, 7.9) β : 2.86 (1H, dd, 16.7, 6.5)	C-6, C-8, C-9, C-14 C-5, C-6, C-8, C-9, C-14	H-5, H-6 α H-6 α , H-6 β
8	120.6, C	—		
9	148.2, C	—		
10	37.3, C	—		
11	116.4, CH	6.84 (1H, d, 8.3)	C-8, C-10, C-13	H-1 β
12	123.3, CH	7.02 (1H, d, 8.3)	C-9, C-14, C-15	H-16, H-17
13	130.2, C	—		
14	150.2, C	—		
15	26.7, CH	3.15 (1H, sep, 6.9)	C-12, C-13, C-14, C-16, C-17	
16	22.7, CH ₃	1.24 (3H, d, 6.9)	C-13, C-15, C-17	H-12
17	22.5, CH ₃	1.26 (3H, d, 6.9)	C-13, C-15, C-16	H-12
18	28.1, CH ₃	1.09 (3H, s)	C-3, C-4, C-5, C-19	H-3, H-5, H-6 α
19	15.3, CH ₃	0.91 (3H, s)	C-3, C-4, C-5, C-18	H-6 α , H-6 β , H-20
20	24.8, CH ₃	1.21 (3H, s)	C-1, C-5, C-9, C-10	H-1 β , H-19

TABLE 9

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F1-31				
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY
1	34.4, CH ₂	α : 1.22 (1H, m) β : 2.78 (1H, dt, 13.5, 3.6)	C-2, C-3, C-9, C-10, C-20 C-3, C-5, C-10	H-3 H-2, H-20
2	27.7, CH ₂	1.72 (2H, m)	C-1, C-3, C-4, C-10	H-1 β , H-19
3	78.3, CH	3.24 (1H, m)	C-2, C-4, C-18, C-19	H-1 α , H-18
4	39.0, C	—		
5	51.0, CH	1.06 (1H, m)	C-4, C-6, C-10, C-19, C-20	H-3, H-6 α , H-7B, H-7A

TABLE 9-continued

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F1-31				
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY
6	17.1, CH ₂	α : 1.87 (1H, br dd, 13.5, 7.5) β : 1.45 (1H, dt, 13.5, 11.7, 5.7)	C-4, C-5, C-7, C-8, C-10 C-5, C-7, C-10	H-5, H-7B, H-18 H-7B, H-19
7	26.1, CH ₂	A: 2.30 (1H, ddd, 20.2, 11.7, 7.5) B: 2.71 (1H, br dd, 20.2, 5.7)	C-6, C-8, C-9, C-14 C-5, C-6, C-8, C-9, C-14	H-5, H-6 α H-5, H-6 α , H-6 β
8	142.7, C	—		
9	150.0, C	—		
10	38.1, C	—		
11	187.78, C	—		
12	131.8, CH	6.30 (1H, d, 1.0)	C-9, C-11/C-14, C-15	H-16/17
13	152.9, C	—		
14	187.83, C	—		
15	26.2, CH	2.96 (1H, sep d, 6.9, 1.0)	C-12, C-13, C-14, C-16, C-17	
16	21.3, CH ₃	1.07 (3H, d, 6.9)	C-13, C-15, C-17	H-12
17	21.3, CH ₃	1.08 (3H, d, 6.9)	C-13, C-15, C-16	H-12
18	28.2, CH ₃	1.03 (3H, s)	C-3, C-4, C-5, C-19	H-3, H-6 α
19	15.7, CH ₃	0.85 (3H, s)	C-3, C-4, C-5, C-18	H-2, H-6 β , H-20
20	20.1, CH ₃	1.26 (3H, s)	C-1, C-5, C-9, C-10	H-1 β , H-19

TABLE 10

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F2-X				
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY
1	33.5, CH ₂	α : 1.64 (1H, ddd, 13.2, 11.5, 6.5) β : 2.54 (1H, dd, 13.2, 6.2)	C-2, C-5, C-9, C-10, C-20 C-2, C-3, C-5, C-9, C-10, (C-18), C-20	H-1 β , H-2B, H-5 H-1 α , H-11, H-20
2	18.6, CH ₂	A: 2.34 (1H, m) B: 2.43 (1H, dd, 18.1, 5.4)	C-1, C-3, C-4 C-1, C-3, C-4, C-10	H-20 H-1 α , H-20
3	128.6, C	—		
4	164.0, C	—		
5	41.4, CH	2.67 (1H, br s)		H-1 α , H-6 α
6	19.8, CH ₂	α : 2.30 (1H, m) β : 1.91 (1H, m)		H-5, H-6 β , H-7A, H-7B, H-19
7	23.8, CH ₂	A: 2.83 (1H, m) B: 2.91 (1H, dd, 17.8, 7.5)	C-5, C-6, C-8, C-9, C-14	H-6 α , H-6 β
8	123.2, C	—		
9	144.7, C	—		
10	37.0, C	—		
11	116.8, CH	6.91 (1H, d, 8.2)	C-8, C-10, C-13	H-1 β
12	123.7, CH	6.99 (1H, d, 8.2)	C-9, C-14, C-15	H-16, H-17
13	133.7, C	—		
14	152.4, C	—		
15	27.4, CH	3.27 (1H, sep, 6.9) 1.18 (3H, d, 6.9)	C-12, C-13, C-14, C-16, C-17 C-13, C-15, C-17	
16	23.1, CH ₃	1.20 (3H, d, 6.9)	C-13, C-15, C-16	H-12
17	23.0, CH ₃	—		H-12
18	173.4, C	—		
19	99.4, CH	6.10 (1H, br s)		H-6 α , H-6 β
20	22.6, CH ₃	1.02 (3H, br s)	C-1, C-5, C-9, C-10	H-1 β , H-2A, H-2B, H-6 β

TABLE 11

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F2-10				
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY
1	34.8, CH ₂	α : 1.87 (1H, td, 12.7, 5.2) β : 2.10 (1H, dt, 12.7, 3.6)	C-2, C-10, C-20 C-2, C-3, C-5	H-5, H-18 H-2, H-11, H-20
2	32.4, CH ₂	1.80 (2H, m)	C-1, C-3, C-4, C-10	H-1 β , H-5, H-18, H-20
3	73.4, C	—		
4	152.5, C	—		
5	43.4, CH	2.76 (1H, br d, 12.3)	C-4, C-6, C-10, C-19	H-1 α , H-2, H-7 α
6	21.7, CH ₂	α : 1.84 (1H, m) β : 1.71 (1H, m)	C-5, C-7, C-8, C-10 C-5, C-7, C-10	H-7 α , H-7 β , H-19A, H-20
7	25.2, CH ₂	α : 2.60 (1H, m) β : 2.94 (1H, dd, 17.2, 5.8)	C-6, C-8, C-9, C-14 C-5, C-6, C-8, C-9, C-14	H-5, H-6 α , H-6 β , H-7B H-6 α , H-6 β , H-7 α
8	123.5, C	—		
9	146.4, C	—		
10	39.8, C	—		

TABLE 11-continued

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F2-10				
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY
11	118.2, CH	6.85 (1H, d, 8.2)	C-8, C-10, C-13	H-1 β
12	123.6, CH	6.96 (1H, d, 8.2)	C-9, C-14, C-15	H-16/17
13	132.9, C			
14	151.8, C			
15	27.3, CH	3.27 (1H, sep, 6.9)	C-12, C-13, C-14, C-16/17	
16	23.0, CH ₃	1.19 (3H, d, 6.9)	C-13, C-15, C-17	H-12
17	23.0, CH ₃	1.19 (3H, d, 6.9)	C-13, C-15, C-16	H-12
18	68.6, CH ₂	3.71 (2H, s)	C-2, C-3, C-4	H-1 α , H-2, H-19B
19	107.5, CH ₂	A: 4.83 (1H, br s) B: 5.10 (1H, br s)	C-3, C-4, C-5 C-3, C-4, C-5	H-6 α , H-6 β H-18
20	21.8, CH ₃	0.96 (3H, s)	C-1, C-5, C-9, C-10	H-1 β , H-2, H-6 β

TABLE 12

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F20P1				
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY
1	33.1, CH ₂	A: 1.35 (1H, m) B: 1.427 (1H, m)	C-2, C-3, C-5, C-10, C-20	H-2B H-3, H-17, H-18
2	28.9, CH ₂	A: 1.60 (1H, m) B: 1.69 (1H, m)	C-1, C-3	H-1A
3	79.0, CH	3.22 (1H, dd, 11.8, 5.0)	C-2, C-4, C-18, C-19	H-1B, H-5, H-18
4	38.9, C	—		
5	46.0, CH	1.415 (1H, m)	C-3, C-4, C-6, C-7, C-10, C-18, C-19, C-20	H-3, H-18
6	17.5, CH ₂	A: 1.407 (1H, m) B: 1.62 (1H, m)	C-5 C-4, C-5, C-7, C-8, C-10	H-19 H-19
7	38.6, CH ₂	A: 1.62 (1H, m) B: 1.80 (1H, dd, 11.5, 7.6)	C-6, C-9 C-5, C-6, C-8, C-14, C-17	H-19
8	37.9, C	—		
9	53.6, CH	1.24 (1H, dd, 12.9, 3.0)	C-1, C-8, C-10, C-11, C-12, C-17, C-20	H-14, H-20
10	36.9, C	—		
11	24.5, CH ₂	A: 1.32 (1H, qd, 12.9, 4.0) B: 1.64 (1H, m)	C-9, C-12 C-9	H-12A, H-12B, H-17 H-12A, H-12B, H-20
12	37.8, CH ₂	A: 1.94 (1H, tdt, 12.8, 4.6, ~1) B: 2.41 (1H, ddd, 12.8, 4.0, 2.6)	C-11, C-13, C-15 C-9, C-11, C-13, C-14, C-15	H-11A, H-11B H-11A, H-11B, H-15B
13	147.5, C	—		
14	60.8, CH	1.89 (1H, t, 6.5)	C-8, C-9, C-13, C-15, C-16, C-17	H-9
15	107.0, CH ₂	A: 4.69 (1H, dt, 1.4, 1.2) B: 4.96 (1H, dt, 1.4, 1.2)	C-12, C-13, C-14 C-12, C-13, C-14	H-15B, H-16 H-12B, H-15A
16	59.0, CH ₂	3.76 (2H, d, 6.5)	C-8, C-13, C-14	H-7A, H-7B, H-15A, H-17
17	21.0, CH ₃	0.85 (3H, s)	C-7, C-8, C-9, C-14	H-1B, H-11A, H-16
18	29.0, CH ₃	0.98 (3H, s)	C-3, C-4, C-5, C-19	H-1B, H-3, H-5, H-6B
19	15.8, CH ₃	0.79 (3H, s)	C-3, C-4, C-5, C-18	H-6A, H-6B
20	22.3, CH ₃	0.95 (3H, s)	C-1, C-5, C-9, C-10	H-9, H-11B

TABLE 13

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F20P2				
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY
1	33.4, CH ₂	α : 1.60 (1H, m) β : 2.35 (1H, m)	C-5, C-10	H-1 β , H-5
2	26.0, CH ₂	2.35 (2H, m)	C-3, C-4	H-1 α , H-11, H-20 H-18A, H-18B
3	131.8, C	—		
4	129.7, C	—		
5	44.5, CH	2.27 (1H, m)		H-1 α
6	20.0, CH ₂	α : 2.28 (1H, m) β : 1.64 (1H, m)	C-8, C-10 (C-5)	H-7A, H-7B, H-19 H-7B, H-20
7	23.3, CH ₂	A: 2.71 (1H, ddd, 16.8, 10.9, 8.4) B: 2.84 (1H, dd, 16.8, 7.1)	C-6, C-8 C-5, C-6, C-8, C-9, C-14	H-6 α , 14-OH H-6 α , H-6 β , 14-OH
8	120.6, C	—		
9	146.0, C	—		
10	35.6, C	—		
11	116.2, CH	6.93 (1H, d, 8.1)	C-8, C-10, C-13	H-1 β
12	123.1, CH	7.03 (1H, d, 8.1)	C-9, C-14, C-15	H-16, H-17

TABLE 13-continued

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F20P2				
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY
13	130.3, C	—		
14	150.4, C	—		
14-OH	—	4.65 (1H, br s)		H-7 α , H-7 β , H-15
15	26.9, CH	3.14 (1H, sep, 6.9)	C-12, C-13, C-14, C-16, C-17	14-OH
16	22.6, CH ₃	1.25 (3H, d, 6.9)	C-13, C-15, C-17	H-12
17	22.6, CH ₃	1.27 (3H, d, 6.9)	C-13, C-15, C-16	H-12
18	63.4, CH ₂	A: 4.09 (1H, d, 11.6) B: 4.26 (1H, d, 11.6)	C-2, C-3, C-4 C-2, C-3, C-4	H-2, H-19 H-2, H-19
19	15.6, CH ₃	1.78 (3H, br s)	C-3, C-4, C-5	H-6 α , H-18A, H-18B
20	22.4, CH ₃	1.02 (3H, s)	C-1, C-5, C-9, C-10	H-1 β , H-6 β

TABLE 14

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F20P3				
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY
1	38.5, CH ₂	α : 1.46 (1H, m) β : 2.30 (1H, m)	C-2, C-10, C-20 C-2, C-3, C-5, C-10	H1 β , H-3 H1 α , H-2 β , H-11, H-20 H-18A, H-20
2	20.7, CH ₂	α : 1.60 (1H, m) β : 1.47 (1H, m)	C-1, C-3	H1 β , H-18B, H-19
3	44.4, CH	1.76 (1H, m)		H1 α , H-4, H-18A, H-18B
4	35.0, CH	2.07 (1H, m)	C-2, C-3, C-5, C-10, C-19	H-3, H-5, H-6 α , H-18B
5	44.7, CH	1.68 (1H, ddd, 12.8, 4.4, 2.1)	C-4, C-6, C-7, C-10, C-19, C-20	H-4, H-7 α
6	23.7, CH ₂	α : 1.65 (1H, br dd, 12.8, 8.2) β : 2.03 (1H, m)	C-4, C-5, C-7, C-8, C-10 C-5, C-7, C-10	H-4, H-7 α , H-7B H-7 β , 14-OH, H-19, H-20
7	24.1, CH ₂	α : 2.65 (1H, ddd, 16.4, 11.5, 7.8) β : 2.82 (1H, dd, 16.4, 6.6)	C-6, C-8, C-9, C-14 C-5, C-6, C-8, C-9, C-14	H-5, H-6 α , 14-OH H-6 α , H-6 β
8	120.7, C	—		
9	148.2, C	—		
10	37.5, C	—		
11	116.6, CH	6.84 (1H, d, 8.2)	C-8, C-10, C-13	H-1 β , H-20
12	123.4, CH	7.02 (1H, d, 8.2)	C-9, C-14, C-15	H-16, H-17
13	130.0, C	—		
14	150.2, C	—		
14-OH	—	4.64 (1H, br s)	H-8, H-13, H-14	H-7 α , H-7 β , H-15
15	26.9, CH	3.14 (1H, sep, 6.9)	C-12, C-13, C-14, C-16, C-17	14-OH
16	22.7, CH ₃	1.24 (3H, d, 6.9)	C-13, C-15, C-17	H-12
17	22.6, CH ₃	1.25 (3H, d, 6.9)	C-13, C-15, C-16	H-12
18	65.8, CH ₂	A: 3.54 (1H, dd, 10.4, 6.8) B: 3.60 (1H, dd, 10.4, 8.0)	C-3, C-4, C-5 C-3, C-4, C-5	H-2 α , H-2 β , H-3, H-19 H-2 β , H-3, H-4, H-19
19	9.7, CH ₃	0.89 (3H, d, 7.6)	C-3, C-4, C-5	H-2 β , H-6 β , H-18B, H-20
20	25.4, CH ₃	1.16 (3H, s)	C-1, C-5, C-9, C-10	H-1 β , H-2 α , H-6 β , H-11, H-19

TABLE 15

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F20P4				
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY
1	33.3, CH ₂	α : 1.49 (1H, m) β : 2.04 (1H, m)	C-10, C-20 C-3, C-5	H1 β , H-18 H1 α , H-11, H-20
2	19.5, CH ₂	α : 1.66 (1H, m) β : 2.00 (1H, m)		H-18 H-19, H-20
3	42.9, CH	1.72 (1H, m)	C-1, C-2, C-4, C-18, C-19	
4	34.7, CH	1.91 (1H, m)	C-2, C-3, C-5, C-10, C-18, C-19	H-6 α , H-18
5	38.7, CH	1.74 (1H, ddd, 12.8, 5.1, 2.2)	C-4, C-6, C-7, C-10, C-19	
6	23.4, CH ₂	α : 1.59 (1H, br dd, 12.8, 7.8) β : 1.98 (1H, m)	C-4, C-5, C-7, C-8, C-10 C-5, C-7, C-10	H-4, H-7 α , H-7 β H-7 β , 14-OH, H-19, H-20
7	24.0, CH ₂	α : 2.62 (1H, ddd, 16.5, 11.4, 7.8) β : 2.81 (1H, dd, 16.5, 6.7)	C-6, C-8, C-9 C-5, C-6, C-8, C-9, C-14	H-6 α , 14-OH H-6 α , H-6 β
8	120.7, C	—		
9	148.2, C	—		
10	37.4, C	—		
11	116.4, CH	6.82 (1H, d, 8.2)	C-8, C-10, C-13	H-1 β
12	123.3, CH	7.01 (1H, d, 8.2)	C-9, C-14, C-15	H-16, H-17

TABLE 15-continued

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F20P4					
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY	
13	130.0, C	—			
14	150.2, C	—			
14-OH	—	4.63 (1H, br s)	H-8, H-13	H-7 α , H-7 β , H-15	
15	26.8, CH	3.14 (1H, sep, 6.9)	C-12, C-13, C-14, C-16, C-17	14-OH	
16	22.7, CH ₃	1.24 (3H, d, 6.9)	C-13, C-15, C-17	H-12	
17	22.6, CH ₃	1.25 (3H, d, 6.9)	C-13, C-15, C-16	H-12	
18	64.7, CH ₂	3.66 (2H, m)	C-2, C-4	H-1 α , H-2 α , H-4	
19	16.7, CH ₃	1.09 (3H, d, 7.6)	C-3, C-4, C-5	H-2 β , H-6 β , H-20	
20	25.0, CH ₃	1.21 (3H, s)	C-1, C-5, C-9, C-10	H-1 β , H-2 β , H-6 β , H-19	

TABLE 16

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F15P2					
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY	
1	32.7, CH ₂	α : 1.56 (1H, m) β : 2.42 (1H, m)	C-2, C-5, C-10, C-20 C-2, C-3, C-5, C-10, C-20	H-2 α H-11, H-20	
2	20.7, CH ₂	α : 2.53 (1H, m) β : 2.33 (1H, m)	C-1, C-3, C-4, C-10	H-1 α , H-19 H-20	
3	133.5, C	—			
4	156.1, C	—			
5	46.7, CH	2.44 (1H, m)		H-7 α	
6	19.4, CH ₂	α : 2.38 (1H, m) β : 1.76 (1H, tdd, 13.2, 10.9, 7.2)	C-5, C-8, C-10 C-5, C-7, C-10	H-7 α H-7 β , H-20	
7	23.4, CH ₂	α : 2.77 (1H, ddd, 17.0, 10.9, 8.2) β : 2.93 (1H, dd, 17.0, 7.2)	C-6, C-8, C-9, C-14 C-5, C-6, C-8, C-9, C-14	H-5, H-6 α , 14-OH H-6 β , 14-OH	
8	120.6, C	—			
9	145.1, C	—			
10	35.8, C	—			
11	116.4, CH	6.94 (1H, d, 8.2)	C-8, C-10, C-13	H-1 β	
12	123.3, CH	7.05 (1H, d, 8.2)	C-9, C-14, C-15	H-16, H-17	
13	130.6, C	—			
14	150.4, C	—			
14-OH	—	4.68 (1H, s)	C-8, C-13, C-14	H-7 α , H-7 β , H-15	
15	26.9, CH	3.12 (1H, sep, 6.9)	C-12, C-13, C-14, C-16, C-17	14-OH	
16	22.6, CH ₃	1.26 (3H, d, 6.9)	C-13, C-15, C-17	H-12	
17	22.5, CH ₃	1.27 (3H, d, 6.9)	C-13, C-15, C-16	H-12	
18	191.3, CH	10.24 (1H, s)	C-2, C-3	H-19	
19	15.0, CH ₃	2.22 (3H, ddd, 1.8, 1.6, 1)	C-3, C-4, C-5	H-2 α , H-18	
20	22.7, CH ₃	1.02 (3H, s)	C-1, C-5, C-9, C-10	H-1 β , H-2 β , H-6 β	

TABLE 17

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F55P2					
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY	
1	28.5, CH ₂	α : 1.48 (1H, m) β : 2.67 (1H, ddd, 13.1, 5.2, 2.9)		H-1 β , H-5	
2	25.7, CH ₂	2.36 (2H, m)		H-1 α , H-2, H-11, H-20B	
3	130.6 ^d , C	—		H-1 β , H-18A, H-18B, H-20B	
4	131.0 ^d , C	—			
5	43.6, CH	2.41 (1H, br d, 14.2)		H-1 α	
6	20.1, CH ₂	α : 2.29 (1H, dddd, 13.6, 8.4, 3.3, ~1) β : 1.70 (1H, tdd, 13.6, 10.4, 8.0)		H-7 α , H-7 β , H-19	
7	23.0, CH ₂	A: 2.78 (1H, m) B: 2.88 (1H, m)	C-6, C-8 C-5, C-6, C-8, C-9	H-7B, H-20A, H-20B H-6 α , 14-OH	
8	121.7, C	—		H-6 α , H-6 β , 14-OH	
9	140.8, C	—			
10	40.3, C	—			
11	117.2, CH	6.98 (1H, d, 8.2)	C-8, C-10, C-13	H-1 β	
12	123.0, CH	7.07 (1H, d, 8.2)	C-9, C-14, C-15	H-16/17	
13	131.5, C	—			
14	150.9, C	—			
14-OH	—	4.72 (1H, br s)	C-8, C-13	H-7A, H-7B, H-15	
15	27.0, CH	3.15 (1H, sep, 6.9)	C-12, C-13, C-14, C-16, C-17	14-OH	
16	22.6, CH ₃	1.27 (3H, d, 6.9)	C-13, C-15, C-17	H-12	

TABLE 17-continued

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F55P2				
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY
17	22.6, CH ₃	1.27 (3H, d, 6.9)	C-13, C-15, C-16	H-12
18	63.2, CH ₂	A: 4.09 (1H, d, 11.6) B: 4.28 (1H, d, 11.6)	C-2, C-4 C-2, C-4	H-2, H-19 H-2, H-19
19	15.6, CH ₃	1.79 (3H, q, 1.9)	C-3, C-4, C-5	H-6 α , H-18A, H-18B
20	64.7, CH ₂	A: 3.56 (1H, dd, 10.8, 3.0) B: 3.68 (1H, dd, 10.8, 7.7)	C-9	H-6 β H-1 β , H-2, H-6 β
20-OH	—	1.03 (1H, m)		

TABLE 18

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F55P3				
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY
1	38.4, CH ₂	α : 1.45 (1H, m) β : 2.28 (1H, m)	C-2, C-20 C-3, C-5, C-10, C-20	H1 β , H-3 H1 α , H-11, H-20
2	21.3, CH ₂	1.46 (1H, m)	C-1, C-3, C-4, C-10	H-18A, H-18B, H-19B, H-20
3	42.8, CH	1.89 (1H, m)		H1 α , H-4, H-18B
4	44.6, CH	2.21 (1H, dt, 9.0, 4.6)		H-3, H-5, H-6 α , H-19A
5	44.4, CH	1.79 (1H, m)	C-6, C-7, C-19	H-4, H-7 α
6	23.8, CH ₂	α : 1.80 (1H, m) β : 1.98 (1H, tdd, 13.3, 11.6, 6.2)	C-4, C-5, C-7, C-8, C-10 C-5, C-7, C-10	H-4, H-7 α , H-7 β , H-19A H-7 β , 14-OH, H-19A, H-20
7	24.4, CH ₂	α : 2.66 (1H, ddd, 16.4, 11.6, 7.4) β : 2.85 (1H, dd, 16.4, 6.2)	C-6, C-8 C-5, C-6, C-8, C-9, C-14	H-5, H-6 α , 14-OH H-6 α , H-6 β
8	120.5, C	—		
9	146.9, C	—		
10	37.1, C	—		
11	116.9, CH	6.83 (1H, d, 8.3)	C-8, C-10, C-13	H-1 β
12	123.4, CH	7.02 (1H, d, 8.3)	C-9, C-14, C-15	H-16, H-17
13	130.1, C	—		
14	150.2, C	—		
14-OH	—	4.64 (1H, s)	H-8, H-13, H-14	H-7 α , H-7 β , H-15
15	26.8, CH	3.13 (1H, sep, 6.9)	C-12, C-13, C-14, C-16, C-17	14-OH
16	22.7, CH ₃	1.24 (3H, d, 6.9)	C-13, C-15, C-17	H-12
17	22.5, CH ₃	1.25 (3H, d, 6.9)	C-13, C-15, C-16	H-12
18	65.4, CH ₂	α : 3.63 (1H, m) β : 3.65 (1H, m)	C-3, C-4	H-2, H-19B H-2, H-3, H-19B
19	59.5, CH ₂	α : 3.71 (1H, d, 10.4) β : 3.88 (1H, dd, 10.4, 9.2)	C-3, C-4 C-3, C-4	H-4, H-6 α , H-6 β , H-20 H-2, H-18A/B, H-20
20	24.6, CH ₃	0.98 (3H, s)	C-1, C-5, C-9, C-10	H-1 β , H-2, H-6 β , H-19A, H-19B

TABLE 19

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F15P4				
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY
1	37.0, CH ₂	α : 1.16 (1H, td, 13.2, 3.7) β : 1.79 (1H, dt, 13.2, 3.5)	C-2, C-5, C-10, C-20 C-3, C-5	H-3 H-20
2	27.9, CH ₂	A: 1.60 (1H, m) B: 1.71 (1H, m)	C-1, C-3 C-3	H-19
3	78.8, CH	3.25 (1H, dd, 11.8, 4.3)	C-4, C-18, C-19	H-1 α , H-5, H-18
4	39.1, C	—		
5	54.6, CH	1.08 (1H, dd, 12.5, 2.7)	C-4, C-6, C-7, C-9, C-10, C-18, C-19, C-20	H-3, H-7 α , H-9, H-18
6	24.0, CH ₂	α : 1.74 (1H, dddd, 13.0, 5.0, 2.7, 2.5) β : 1.39 (1H, tdd, 13.0, 12.5, 4.2)	C-5, C-7, C-8, C-10 C-5, C-7, C-10	H-7 β , H-18 H-7 β , H-19
7	38.1, CH ₂	α : 1.96 (1H, ddd, 13.0, 12.8, 5.0) β : 2.40 (1H, ddd, 12.8, 4.2, 2.5)	C-6, C-8, C-17 C-5, C-6, C-8, C-9, C-17	H-5 H-6 α , H-6 β , H-17B
8	147.8, C	—		
9	55.9, CH	1.54 (1H, m)	C-7, C-8, C-10, C-11, C-12, C-17, C-20	H-5, H-12B, H-14, H-17A, H-20
10	39.2, C	—		
11	21.8, CH ₂	α : 1.47 (1H, m) β : 1.59 (1H, m)	C-8, C-9, C-12, C-13 C-8, C-9, C-12	H-12B, H-14, H-17A, H-20
12	38.3, CH ₂	α : 1.83 (1H, dddd, 14.0, 9.4, 6.6) β : 2.17 (1H, ddd, 14.0, 10.0, 3.8)	C-9, C-11, C-13, C-14, C-15 C-11, C-13, C-14	H-14 H-9, H-11A, H-14, H-15, H-17A
13	142.7, C	—		

TABLE 19-continued

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F15P4				
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY
14	118.0, CH	5.31 (1H, t sext, 7.1, 1.2)	C-12, C-15, C-16	H-9, H-11A, H-12A, H-12B
15	16.5, CH ₃	1.69 (3H, br s)	C-12, C-13, C-14	H-12B, H-16
16	61.4 CH ₂	4.58 (2H, d, 7.1)	C-13, C-14, C-1	H-15
17	106.0, CH ₂	A: 4.53 (1H, q, ~1) B: 4.85 (1H, q, ~1.4)	C-7, C-8, C-9 C-7, C-9	H-9, H-11A, H-12A, H-12B, H-17B H-7 β , H-17A
18	28.3, CH ₃	1.00 (3H, s)	C-3, C-4, C-5, C-19	H-3, H-5, H-6 α
19	15.3, CH ₃	0.78 (3H, s)	C-3, C-4, C-5, C-18	H-2A, H-6 β
20	14.5, CH ₃	0.69 (3H, s)	C-1, C-5, C-9, C-10	H-9, H-11A
1'	171.0, C	—	—	—
2'	21.1, CH ₃	2.06 (3H, s)	C-1'	—

TABLE 20

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F20P5				
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY
1	59.4, CH ₂	4.16 (2H, d, 6.9)	C-2, C-3	H-3-Me
2	123.4, CH	5.42 (1H, t sext, 6.9, 1.3)	C-3-Me, C-4	H-4
3	139.6, C	—	—	—
3-Me	16.3, CH ₃	1.68 (3H, br s)	C-2, C-3, C-4	H-1
4	39.5, CH ₂	2.04 (2H, m)	C-2, C-3, C-3-Me, C-5	H-2
5	26.3, CH ₂	2.11 (2H, m)	C-4, C-6, C-7	—
6	123.9, CH	5.11 (1H, t sext, 6.9, 1.2)	C-5, C-7-Me, C-8	—
7	135.1, C	—	—	—
7-Me	15.9, CH ₃	1.60 (3H, br s)	C-6, C-7, C-8	—
8	39.6, CH ₂	1.99 (2H, m)	C-6, C-7, C-7-Me, C-9, C-10	—
9	26.5, CH ₂	2.08 (2H, m)	C-7, C-8, C-10, C-11	—
10	124.8, CH	5.16 (1H, t sext, 6.9, 1.2)	C-9, C-11-Me, C-12	H-12B, H-13
11	134.0, C	—	—	—
11-Me	16.0, CH ₃	1.62 (3H, br s)	C-10, C-11, C-12	—
12	36.3, CH ₂	A: 2.08 (1H, m) B: 2.16 (1H, m)	C-10, C-11, C-11-Me, C-13, C-14	H-14
13	27.4, CH ₂	1.63 (2H, m)	C-10, C-11, C-11-Me, C-13, C-14	H-10
14	64.1, CH	2.70 (1H, t, 6.3)	C-11, C-12, C-14, C-15	—
			C-12, C-13, C-15, C-15-Me'	H-12A, H-12B, H-15-Me, H-15-Me'
15	58.2, C	—	—	—
15-Me	18.7, CH ₃	1.26 (3H, s)	C-14, C-15, C-15-Me'	H-14
15-Me'	24.8, CH ₃	1.30 (3H, s)	C-14, C-15, C-15-Me	H-14

TABLE 21

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F60P1				
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY
1	59.3, CH ₂	4.15 (2H, br d, 6.9)	C-2, C-3	H-3-Me
2	123.7, CH	5.42 (1H, t sext, 6.9, 1.2)	C-3-Me, C-4	H-4
3	139.3, C	—	—	—
3-Me	16.2, CH ₃	1.68 (3H, br s)	C-2, C-3, C-4	H-1, H-4
4	39.3, CH ₂	2.07 (2H, m)	—	H-2, H-3-Me
5	25.9, CH ₂	2.14 (2H, m)	C-4, C-6, C-7	—
6	124.3, CH	5.13 (1H, t sext, 6.8, 1.2)	C-4, C-5, C-7-Me, C-8	—
7	136.2, C	—	—	—
7-Me	16.0, CH ₃	1.64 (3H, br s)	C-6, C-7, C-8	H-8
8	42.5, CH ₂	2.10 (2H, m)	C-6, C-7, C-7-Me, C-9, C-10	H-7-Me, H-10, H-15- α Me
9	24.2, CH ₂	A: 1.48 (1H, m) B: 1.55 (1H, m)	C-8, C-10, C-15	—
10	55.3, CH	1.10 (1H, t, 4.3)	C-8, C-9, C-11, C-11-Me, C-15	H-11-Me
11	73.4, C	—	—	—
11-Me	23.0, CH ₃	1.16 (3H, s)	C-10, C-11, C-12	H-9B, H-12B, H-13A, H-15- β Me
12	40.9, CH ₂	A: 1.45 (1H, m) B: 1.78 (1H, m)	C-13	—
13	28.9, CH ₂	A: 1.49 (1H, m) B: 1.75 (1H, m)	C-10, C-11, C-14	H-11-Me
14	78.3, CH	3.31 (1H, dd, 10.6, 2.8)	C-14	H-14
15	40.4, C	—	C-10, C-11, C-14	H-10, H-13B, H-15- α Me

TABLE 21-continued

¹ H and ¹³ C NMR data and 2D HMBC and ROESY correlations for F60P1					
Pos.	δ_C , type ^{a, b}	δ_H , nH, multiplicity (J in Hz) ^{a, c}	HMBC	ROESY	
15- α Me	28.0, CH3	1.04 (3H, s)	C-10, C-14, C-15, C-15- β Me	H-8, H-14	
15- β Me	14.8, CH3	0.80 (3H, s)	C-10, C-14, C-15, C-15- α Me	H-11-Me	

^a¹H NMR (600.13) and ¹³C NMR (150.90 MHz) data obtained with samples in CDCl₃.^bAssignments based on HSQC and HMBC experiments.^cMultiplicities reported as apparent splittings: s = singlet, d = doublet, t = triplet, sext = sextet, m = multiplet (incl. overlapping resonances), br = broad. α denotes Me pointing into the plane and β denotes Me pointing out of the plane. A denotes the lowest chemical shift value and B denotes the highest chemical shift value

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- Items
- [0260] 1. A recombinant host cell, capable of producing oxygenated diterpenoid compound, wherein the host cell is capable of producing miltiradiene and/or dehydroabietadiene and has been transformed with a first gene encoding an enzyme having cytochrome P450 activity and which enzyme is capable of converting miltiradiene and/or dehydroabietadiene into 14-OH-dehydroabietadiene.
- [0261] 2. The recombinant host cell of item 1, wherein the first gene encodes a polypeptide comprising an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity to SEQ ID NO: 1 (TwCYP82D274V1) or the mature polypeptide thereof.
- [0262] 3. The recombinant host cell of item 1 or 2, wherein the recombinant host cell further comprises:
- [0263] a second gene encoding a second enzyme having cytochrome P450 activity and a third gene encoding a third enzyme having cytochrome P450 activity, wherein:
- [0264] the second gene encoding an enzyme having cytochrome P450 activity encodes a polypeptide comprising an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity to SEQ ID NO: 4 (TwCYP71BE86) or the mature polypeptide thereof;
- [0265] the third gene encoding an enzyme having cytochrome P450 activity encodes a polypeptide comprising an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity to SEQ ID NO: 3 (TwCYP71BE85) or the mature polypeptide thereof.
- [0266] 4. The recombinant host cell of item 3, wherein the host cell further comprises a gene encoding a polypeptide having cytochrome B5 activity and comprising an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity to SEQ ID NO: 8 (TwB5 #1) or the mature polypeptide thereof.
- [0267] 5. The recombinant host cell of item 3 or 4, wherein the host cell further comprises a fourth gene encoding a fourth enzyme having cytochrome P450 activity; wherein:
- [0268] the fourth gene encoding a fourth enzyme having cytochrome P450 activity encodes a polypeptide comprising an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence

identity, preferably at least 95% sequence identity more preferred at least 98% sequence identity to SEQ ID NO: 5 (TwCYP82D213) or the mature polypeptide thereof.

[0269] 6. The recombinant host cell of item 5, wherein the host cell further comprises a fifth gene encoding a fifth enzyme having cytochrome P450 activity and/or a sixth gene encoding a sixth enzyme having cytochrome P450 activity, wherein:

[0270] the fifth gene encoding a fifth enzyme having cytochrome P450 activity encodes a polypeptide comprising an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity to SEQ ID NO: 6 (TwCYP82D217) or the mature polypeptide thereof; and the sixth gene encoding a sixth enzyme having cytochrome P450 activity encodes a polypeptide comprising an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity to SEQ ID NO: 7 (TwCYP82D275) or the mature polypeptide thereof.

[0271] 7. The recombinant host cell according to any of the preceding items, wherein the host cell capable of producing miltiradiene and/or dehydroabietadiene is a recombinant cell that has been transformed with one or more gene(s) encoding:

[0272] a. a geranylgeranyl diphosphate synthase;
 [0273] b. a diterpene synthase capable of converting GGPP into miltiradiene;
 [0274] c. a combination of two or more diterpene synthases that in combination is capable of converting GGPP into miltiradiene; or
 [0275] d. a copalyl diphosphate synthase and a miltiradiene synthase.

[0276] 8. The recombinant host cell of item 7, wherein the geranylgeranyl diphosphate synthase is a polypeptide comprising the amino acid sequence of SEQ ID NO: 73 or SEQ ID NO: 81.

[0277] 9. The recombinant host cell of item 7, wherein the combination of two or more diterpene synthases, that is capable of converting GGPP into miltiradiene, is the combination of a polypeptide comprising the amino acid sequence of SEQ ID NO: 67 and a polypeptide comprising the amino acid sequence of SEQ ID NO: 68; or is the combination of a polypeptide comprising

the amino acid sequence of SEQ ID NO: 69 and a polypeptide comprising the amino acid sequence of SEQ ID NO: 70.

[0278] 10. The recombinant host cell of item 7, wherein the combination of a copalyl diphosphate synthase and a miltiradiene synthase is a combination of a polypeptide comprising the amino acid sequence of SEQ ID NO: 71 and a polypeptide comprising the polypeptide of SEQ ID NO: 72.

[0279] 11. The recombinant host cell according to any of the previous items wherein the host cell is selected among prokaryotic and eukaryotic cells.

[0280] 12. The recombinant host cell according to item 11, being a prokaryotic cell selected among *Escherichia*, *Bacillus*, *Lactobacillus* and *Corynebacterium* species.

[0281] 13. The recombinant host cell of item 11, being a eukaryotic cell selected among *Saccharomyces*, *Scizosaccharomyces*, *Klyveromyces*, *Pichia*, *Candida* and *Yarrowia* species.

[0282] 14. The recombinant host cell of item 11, where the cell is a *S. cerevisiae* cell.

[0283] 15. Use of a recombinant host cell according to any of the preceding items for the production of an oxygenated diterpenoid compound.

[0284] 16. The use of item 15, wherein the oxygenated diterpenoid compound is selected among 14-OH-dehydroabietadiene, triptophenolide and triptonide.

[0285] 17. The use of item 16, wherein the oxygenated diterpenoid compound is triptonide, which triptonide is further converted into triptolide.

[0286] 18. The use according to one of the items 15-17, wherein the oxygenated diterpenoid compound is recovered using one or more separation and/or chromatographic steps.

[0287] 19. A polypeptide having cytochrome P450 enzyme activity and comprising an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, preferably at least 96% sequence identity, preferably at least 97% sequence identity, preferably at least 98% sequence identity, or even 100% sequence identity to one of the sequences SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 or the mature polypeptide thereof.

[0288] 20. A polynucleotide encoding the polypeptide of item 19.

[0289] 21. A plasmid, expression vector, expression construct or recombinant host cell comprising a polynucleotide of item 20.

[0290] 22. The compound 14-OH-dehydroabietadiene.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 81

<210> SEQ ID NO 1

<211> LENGTH: 533

<212> TYPE: PRT

<213> ORGANISM: *Tripterygium wilfordii*

<400> SEQUENCE: 1

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-continued

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20	25	30	
Leu Lys Pro Lys Lys Ser Lys Thr Ser Pro Pro Gln Ala Ser Ala Ala			
35	40	45	
Trp Pro Leu Ile Gly His Leu Leu His Leu Arg Gly Pro Gln Ala Pro			
50	55	60	
His Ile Thr Leu Gly Lys Met Ala Asp Lys Tyr Gly Pro Ile Phe Lys			
65	70	75	80
Ile Lys Leu Gly Val His Pro Thr Leu Val Ile Ser Asp Ser Glu Val			
85	90	95	
Ala Lys Glu Cys Leu Thr Thr His Asp Ile Ala Leu Ala Gly Arg Pro			
100	105	110	
Ala Thr Val Ala Met Glu Ile Met Gly Tyr Asn His Ala Met Phe Ala			
115	120	125	
Phe Ser Pro Tyr Gly Pro Tyr Trp Arg His Met Arg Lys Leu Ala Thr			
130	135	140	
Val Glu Leu Leu Ser Ala Gln Arg Leu Glu Thr Phe Lys His Ile Arg			
145	150	155	160
Glu Ser Glu Leu Lys Arg Ser Met Lys Glu Met Tyr Gln Ser Trp Val			
165	170	175	
His Asn Lys Ser Gly Ser Gly Asp Ser Asn His Val Thr Val Asp Met			
180	185	190	
Thr Arg Ile Leu Gly Asp Ile Ile Ala Asn Val Ile Tyr Arg Met Val			
195	200	205	
Val Gly Lys Val Tyr Ala Ser Lys Gly Glu Glu Asp Ala Arg Trp Lys			
210	215	220	
Gln Val Val Trp Glu Tyr Ile Lys Leu Leu Ser His Phe Gly Val Gly			
225	230	235	240
Asp Ala Leu Pro Phe Leu Arg Trp Leu Asp Leu Gly Gly Val Glu Lys			
245	250	255	
Ser Met Lys Ala Ala Lys Glu Leu Asp Ile Tyr Val Glu Glu Trp			
260	265	270	
Leu Glu His Lys Lys Arg Ser Glu Arg Lys Ser Asp Asn Gly			
275	280	285	
Ile Val Glu Glu Asp Phe Met Asp Val Met Leu Ser Val Phe Asp Asp			
290	295	300	
Asp Asp Gln Leu Glu Asn Phe Ala His His Ser Ala His Thr Ile Asn			
305	310	315	320
Lys Ala Met Cys Leu Ala Ile Ile Leu Ala Ala Ser Asp Thr Thr Lys			
325	330	335	
Thr Thr Leu Thr Trp Ala Leu Ser Leu Leu Leu Asn His Pro Asp Val			
340	345	350	
Met Lys Lys Val Gln Gln Glu Leu Ala Ala His Ile Gly Pro Asp Lys			
355	360	365	
Pro Val Lys Glu Ser Asp Val Lys Ser Leu Val Tyr Leu Glu Ala Val			
370	375	380	
Val Lys Glu Thr Leu Arg Leu Tyr Pro Pro Gly Pro Leu Gly Leu Pro			
385	390	395	400
His Glu Ser Met Glu Asp Cys Thr Val Ala Gly Tyr His Val Pro Ser			
405	410	415	

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Gly Thr Arg Ile Leu Tyr Asn Leu Trp Lys Ile Gln Gln Asp Pro Gln
420 425 430

Val Trp Glu Asn Pro Ser Glu Phe Lys Pro Asp Arg Phe Leu Thr Thr
435 440 445

His Lys Asp Val Asp Val Arg Gly Arg Asn Phe Glu Tyr Leu Pro Phe
450 455 460

Gly Ser Gly Arg Arg Met Cys Pro Gly Met Ser Phe Ala Leu Gln Val
465 470 475 480

Met Glu Val Ser Leu Ala Asn Met Leu His Gly Phe Asp Phe Ala Thr
485 490 495

Pro Asn Gly Lys Pro Val Asp Met Thr Glu Val Asn Gly Leu Val Thr
500 505 510

Asp Arg Ala Thr Pro Leu Glu Ala Leu Ile Thr Pro Arg Leu Pro Ala
515 520 525

His Leu Tyr Met Gly
530

<210> SEQ_ID NO 2

<211> LENGTH: 533

<212> TYPE: PRT

<213> ORGANISM: Tripterygium wilfordii

<400> SEQUENCE: 2

Met Glu Phe Leu Leu Ser Leu Pro Thr Asn Thr Ile Ala Pro Lys Ile
1 5 10 15

Phe Ala Val Leu Leu Leu Phe Ile Cys Leu Arg Ile Leu Thr Asn Val
20 25 30

Leu Lys Pro Lys Lys Ser Lys Thr Ser Pro Pro Gln Ala Ser Gly Ala
35 40 45

Trp Pro Leu Ile Gly His Leu Leu His Leu Arg Gly Pro Gln Ala Pro
50 55 60

His Ile Thr Leu Gly Lys Met Ala Asp Lys Tyr Gly Pro Ile Phe Lys
65 70 75 80

Ile Lys Leu Gly Val His Pro Thr Leu Val Ile Ser Asp Ser Glu Phe
85 90 95

Ala Lys Glu Cys Leu Thr Thr His Asp Ile Ala Leu Ala Gly Arg Pro
100 105 110

Ala Thr Val Ala Met Glu Ile Met Gly Tyr Asn His Ala Met Phe Ala
115 120 125

Phe Ser Pro Tyr Gly Pro Tyr Cys Arg His Met Arg Lys Leu Ala Thr
130 135 140

Val Glu Leu Leu Ser Ala Gln Arg Leu Glu Thr Phe Lys His Ile Arg
145 150 155 160

Glu Ser Glu Leu Lys Arg Ser Met Lys Glu Met Tyr Gln Ser Trp Val
165 170 175

His Asn Lys Ser Gly Ser Gly Asp Ser Asn His Val Thr Val Asp Met
180 185 190

Thr Arg Ile Leu Gly Asp Ile Ile Ala Asn Val Ile Tyr Arg Met Val
195 200 205

Val Gly Lys Val Tyr Ala Ser Lys Gly Glu Glu Asp Ala Arg Trp Lys
210 215 220

Gln Val Val Trp Glu Tyr Ile Lys Leu Leu Ser His Phe Gly Val Gly

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225	230	235	240
Asp Ala Leu Pro Phe Leu Arg Trp Leu Asp Leu Gly Gly Val Glu Lys			
245	250	255	
Ser Met Lys Lys Ala Ala Lys Glu Leu Asp Ile Tyr Val Glu Glu Trp			
260	265	270	
Leu Glu Glu His Lys Lys Arg Ser Glu Arg Lys Ser Asp Asn Gly			
275	280	285	
Ile Val Glu Glu Asp Phe Met Asp Val Met Leu Ser Val Phe Asp Asp			
290	295	300	
Asp Asp Gln Leu Glu Asn Phe Ala His His Ser Ala His Thr Ile Asn			
305	310	315	320
Lys Ala Met Cys Leu Ala Ile Ile Leu Ala Ala Ser Asp Thr Thr Lys			
325	330	335	
Thr Thr Leu Thr Trp Ala Leu Ser Leu Leu Leu Asn His Pro Asp Val			
340	345	350	
Met Lys Lys Val Gln Gln Glu Leu Ala Ala His Ile Gly Pro Asp Lys			
355	360	365	
Pro Val Lys Glu Ser Asp Val Lys Ser Leu Val Tyr Leu Glu Ala Val			
370	375	380	
Val Lys Glu Thr Leu Arg Leu Tyr Pro Pro Gly Pro Leu Gly Leu Pro			
385	390	395	400
His Glu Ser Met Glu Asp Cys Thr Val Ala Gly Tyr His Val Pro Ser			
405	410	415	
Gly Thr Arg Ile Leu Tyr Asn Leu Trp Lys Ile Gln Gln Asp Pro Gln			
420	425	430	
Val Trp Glu Asn Pro Ser Glu Phe Lys Pro Asp Arg Phe Leu Thr Thr			
435	440	445	
His Lys Asp Val Asp Val Arg Gly Arg Asn Phe Glu Tyr Leu Pro Phe			
450	455	460	
Gly Ser Gly Arg Arg Met Cys Pro Gly Met Ser Phe Ala Leu Gln Val			
465	470	475	480
Met Glu Val Ser Leu Ala Asn Met Leu His Gly Phe Asp Phe Ala Thr			
485	490	495	
Pro Asn Gly Lys Pro Val Asp Met Thr Glu Val Asn Gly Leu Val Thr			
500	505	510	
Asp Arg Ala Thr Pro Leu Glu Ala Leu Ile Thr Pro Arg Leu Pro Ala			
515	520	525	
His Leu Tyr Met Gly			
530			

<210> SEQ_ID NO 3
<211> LENGTH: 508
<212> TYPE: PRT
<213> ORGANISM: *Tripterygium wilfordii*

<400> SEQUENCE: 3

Met Asp Leu Leu Gln Phe Pro Ser Val Ser Ile Leu Leu Gly Phe Val			
1	5	10	15
Phe Phe Met Phe Met Val Leu Lys Val Trp Lys Arg Phe Glu Ala Asn			
20	25	30	
Gly Ser Thr Ser Asn Leu Pro Pro Gly Pro Trp Lys Leu Pro Ile Ile			
35	40	45	

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Gly	Asn	Leu	His	Gln	Leu	Gly	Gly	Ser	Asp	Pro	Pro	His	Arg	Ala	Leu
50					55					60					
Gly	Glu	Leu	Ala	Lys	Lys	Tyr	Gly	Pro	Leu	Met	Phe	Leu	Gln	Leu	Gly
65					70				75				80		
Glu	Ile	Gln	Thr	Leu	Val	Val	Ser	Ser	Ala	Glu	Tyr	Ala	Glu	Glu	Val
	85					90				95					
Leu	Lys	Thr	His	Asp	Thr	Val	Phe	Ala	Ser	Arg	Pro	Gln	Met	His	Ser
	100					105				110					
Leu	Glu	Ile	Met	Ser	Tyr	Asp	Tyr	Lys	Asp	Ile	Thr	Phe	Ser	Pro	Ser
	115					120				125					
Asp	Gly	Ser	Trp	Arg	Arg	Arg	Lys	Ile	Cys	Val	Gln	Glu	Leu	Leu	
	130					135				140					
Ser	Ala	Lys	Arg	Val	Gln	Ser	Phe	Arg	Ser	Thr	Arg	Glu	Lys	Glu	Leu
	145					150				155			160		
Ser	Lys	Leu	Ile	Gln	Trp	Ile	Phe	Ser	Gln	Ala	Gly	Thr	Ser	Ile	Asn
		165					170				175				
Leu	Thr	Thr	Lys	Ile	Tyr	Ser	Ser	Thr	Cys	Thr	Leu	Ser	Ser	Arg	Met
	180					185				190					
Ala	Phe	Ser	Asp	Glu	Cys	Lys	Tyr	Gln	Glu	Glu	Phe	Ile	Ser	Ile	Leu
	195					200				205					
Lys	Asp	Leu	Leu	Lys	Ile	Ala	Ser	Gly	Phe	Asn	Ile	Glu	Asp	Met	Phe
	210					215				220					
Pro	Ser	Met	Lys	Phe	Leu	His	Leu	Ile	Ser	Gly	Ala	Ser	Ser	Lys	Ile
	225					230				235			240		
Glu	Lys	Leu	His	Lys	Gln	Leu	Asp	Arg	Ile	Val	Gly	Ser	Ile	Ile	Asp
		245					250				255				
Glu	His	Ile	Asn	Leu	Asn	Thr	Arg	Lys	Ser	Glu	Gly	Asn	Glu	Asp	Leu
		260					265				270				
Val	Asp	Val	Leu	Leu	Lys	Tyr	His	Glu	Gln	Gly	Asp	Ser	Glu	Phe	Ser
		275					280				285				
Leu	Ser	Met	Glu	Glu	Ile	Lys	Ala	Ile	Ile	Cys	Asp	Ile	Tyr	Leu	Ala
		290				295				300					
Gly	Thr	Glu	Thr	Ser	Ser	Thr	Thr	Val	Asp	Trp	Thr	Met	Ala	Glu	Leu
	305					310				315			320		
Ile	Lys	Asn	Pro	Arg	Val	Met	Lys	Lys	Ala	Gln	Ala	Glu	Val	Arg	Gln
		325					330				335				
Val	Phe	Asp	Ser	Arg	Gly	Ser	Val	Asp	Glu	Thr	Gly	Ile	Pro	Glu	Leu
		340				345				350					
Lys	Tyr	Leu	Lys	Leu	Val	Ile	Lys	Glu	Thr	Leu	Arg	Leu	His	Pro	Pro
		355				360				365					
Gly	Pro	Leu	Leu	Leu	Pro	Arg	Glu	Asn	Ala	Lys	Ser	Cys	Glu	Ile	Asn
		370				375				380					
Glu	Tyr	Val	Ile	Pro	Ala	Lys	Thr	Arg	Val	Met	Val	Asn	Gly	Trp	Ala
	385					390				395			400		
Ile	Gly	Arg	Asp	Pro	Lys	Tyr	Trp	Pro	Lys	Glu	Pro	Glu	Lys	Phe	Tyr
		405					410				415				
Pro	Glu	Arg	Phe	Ile	Asp	Asn	Pro	Ile	Asp	Tyr	Lys	Gly	Thr	Asn	Phe
		420				425				430					
Glu	Tyr	Ile	Pro	Phe	Gly	Ala	Gly	Arg	Arg	Met	Cys	Pro	Gly	Met	Ala
		435				440				445					
Phe	Gly	Leu	Ala	Asn	Val	Glu	Leu	Pro	Leu	Ser	Gln	Phe	Leu	Tyr	Tyr

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450	455	460
Phe Asp Trp Lys Leu Ala Asp Gly Met Val Pro Glu Asn Leu Asn Met		
465	470	475
		480
Ala Glu Ala Phe Ala Ala Thr Val Cys Arg Lys Asp Asp Leu Tyr Leu		
485	490	495
Ile Pro Thr Pro Tyr Cys Pro Ser Pro Ala Phe Asn		
500	505	
<210> SEQ ID NO 4		
<211> LENGTH: 499		
<212> TYPE: PRT		
<213> ORGANISM: Tripterygium wilfordii		
<400> SEQUENCE: 4		
Met Asp Leu Gln Leu Pro Ser Phe Pro Ile Leu Ser Ser Ile Ile Leu		
1	5	10
		15
Leu Ile Leu Val Val Leu Lys Ser Val Leu Arg Pro Ser Lys Leu Pro		
20	25	30
Pro Gly Pro Trp Lys Leu Pro Leu Ile Gly Asn Leu His Gln Leu Ala		
35	40	45
Gln Asp Leu Pro His Arg Ala Leu Gln Lys Leu Ala Lys Lys His Gly		
50	55	60
Pro Leu Met His Leu His Phe Gly Glu Val Pro Thr Leu Val Val Thr		
65	70	75
		80
Ser Pro Glu Tyr Ala Lys Glu Val Met Lys Thr His Asp Ile Thr Phe		
85	90	95
Ala Ser Arg Pro Leu Leu Asn Ala Met Lys Val Met Thr Tyr Asp His		
100	105	110
Thr Asp Ile Ala Phe Ala Pro Tyr Gly Glu Tyr Trp Arg Gln Leu Arg		
115	120	125
Lys Ile Cys Thr Ile Glu Leu Leu Ser Val Lys Arg Val Gln Ser Phe		
130	135	140
Arg Pro Ile Arg Glu Gln Glu Thr Ser Asn Val Ile Glu Trp Ile Gly		
145	150	155
		160
Ser Asn Ala Gly Ser Ser Ile Asn Leu Thr Glu Arg Leu Tyr Thr Thr		
165	170	175
Ile Tyr Ala Leu Val Ser Lys Val Ala Phe Gly Arg Thr Cys Gly Arg		
180	185	190
Gly Glu His Glu Glu Phe Ile Glu Tyr Ser Lys Ala Ser Gln Asn Arg		
195	200	205
Ala Ser Gly Phe Asn Ile Val Asp Val Phe Pro Ser Leu Lys Leu Val		
210	215	220
His Trp Ile Met Gly Glu Gly Lys Lys Thr Glu Arg Leu His Lys Gln		
225	230	235
		240
Gly Asp Met Leu Leu Gly Asn Ile Ile Asn Gln His Val Lys Lys Pro		
245	250	255
Val Thr Gly Lys Gly Asp Asp Glu His Glu Asp Leu Val Asp Val Leu		
260	265	270
Leu Lys Phe His Glu Glu Gly Asp Phe Pro Leu Thr Ile Asn Asn Ile		
275	280	285
Lys Ser Val Ile Gln Asp Ile Phe Val Ala Gly Glu Thr Ser Ala		
290	295	300

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Thr Thr Ile Asp Trp Ala Met Arg Glu Met Met Lys Asn Pro Arg Val
 305 310 315 320

 Met Lys Lys Ala Gln Ala Glu Val Arg Gln Val Phe Asp Ser Arg Gly
 325 330 335

 Arg Val Asp Glu Thr Ala Val Pro Glu Leu Lys Tyr Leu Lys Leu Val
 340 345 350

 Leu Lys Glu Thr Leu Arg Leu His Pro Pro Leu Pro Phe Leu Leu Pro
 355 360 365

 Arg Ile Asn Trp Glu Arg Cys Glu Ile Asn Gly Tyr Glu Ile Ala Ala
 370 375 380

 Asn Thr Lys Val Ile Val Asn Ala Trp Ala Ile Gly Arg Asp Pro Asn
 385 390 395 400

 Tyr Trp Thr Glu Ala Glu Arg Phe Tyr Pro Glu Arg Phe Leu Glu Lys
 405 410 415

 Ser Ala Asp Tyr Lys Gly Thr Ser Phe Glu Tyr Thr Pro Phe Gly Ala
 420 425 430

 Gly Arg Arg Leu Cys Pro Gly Met Ser Phe Gly Leu Ala Asn Val Glu
 435 440 445

 Phe Pro Leu Ser Gln Leu Leu Tyr His Phe Asp Trp Asn Leu Thr Gly
 450 455 460

 Gly Met Lys Pro Glu Asp Leu Asn Met Ile Glu Ser Phe Asp Val Thr
 465 470 475 480

 Met Arg Ala Lys Asp Asp Leu His Leu Val Pro Thr Pro Tyr Arg Ser
 485 490 495

Leu Ser Gly

<210> SEQ ID NO 5
 <211> LENGTH: 530
 <212> TYPE: PRT
 <213> ORGANISM: Tripterygium wilfordii

<400> SEQUENCE: 5

Met Glu Phe Leu Leu Ser Leu Pro Thr Asn Thr Ile Ala Thr Lys Ile
 1 5 10 15

 Phe Ala Val Leu Leu Tyr Leu Phe Leu Arg Ile Phe Thr Asn Val
 20 25 30

 Leu Lys Pro Lys Lys Ser Lys Thr Ser Pro Pro Gln Ala Gly Gly Ala
 35 40 45

 Trp Pro Leu Ile Gly His Leu His Leu Ile Gly Pro Gln Ala Ser
 50 55 60

 Tyr Ile Thr Leu Ser Lys Met Ala Asp Lys Tyr Gly Pro Ile Phe Lys
 65 70 75 80

 Ile Lys Leu Gly Val His Pro Thr Leu Val Ile Ser Asn Ser Glu Val
 85 90 95

 Ala Lys Glu Cys Leu Thr Thr His Asp Lys Val Leu Ala Asn Arg Pro
 100 105 110

 Ala Thr Val Ala Met Glu Ile Met Gly Tyr Asn His Ala Met Phe Gly
 115 120 125

 Trp Ser Pro Tyr Gly Pro Tyr Trp Arg Gln Leu Arg Lys Leu Val Thr
 130 135 140

 Val Glu Leu Leu Ser Asn Gln Arg Leu Lys Thr Phe Lys His Ile Arg
 145 150 155 160

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Glu	Ser	Glu	Val	Lys	Asn	Ser	Leu	Lys	Glu	Met	Tyr	Gln	Ser	Trp	Val
165							170							175	
His Asn Lys Ser Gly Asp Ser Asn His Val Ser Val Asp Met Thr Arg															
180							185							190	
Ile	Phe	Gly	Asp	Ile	Thr	Gly	Asn	Leu	Ile	Tyr	Arg	Ile	Val	Val	Gly
195							200							205	
Lys	Val	Tyr	Ala	Arg	Lys	Gly	Glu	Gly	Val	Val	Arg	Trp	Lys	Gln	Val
210							215							220	
Val	Gly	Asp	Tyr	Met	Lys	Leu	Leu	Thr	His	Phe	Asn	Val	Gly	Asp	Ala
225							230							240	
Met	Pro	Phe	Met	Arg	Trp	Phe	Asp	Leu	Gly	Gly	Leu	Glu	Lys	Ala	Met
245							250							255	
Lys	Ile	Thr	Phe	Lys	Glu	Leu	Asp	Gly	Tyr	Val	Glu	Glu	Trp	Leu	Glu
260							265							270	
Glu	His	Lys	Lys	Arg	Ser	Asn	Ser	Gly	Gly	His	Gly	Ile	Val	Glu	
275							280							285	
Glu	Asp	Phe	Met	Asp	Val	Met	Leu	Ser	Ile	Phe	Asp	Asp	Gly	Gly	Gln
290							295							300	
Gln	Glu	Tyr	Cys	Thr	Asp	Asn	Ser	Thr	His	Thr	Thr	Asn	Lys	Ala	Met
305							310							320	
Cys	Met	Ala	Leu	Ile	Leu	Gly	Ala	Ser	Glu	Thr	Thr	Lys	Thr	Thr	Leu
325							330							335	
Thr	Trp	Ser	Leu	Ser	Leu	Leu	Asn	Asn	Leu	Asp	Val	Leu	Lys	Lys	
340							345							350	
Val	Lys	Gln	Glu	Leu	Ala	Ala	His	Ile	Gly	Pro	Glu	Thr	Leu	Val	Thr
355							360							365	
Glu	Ser	Asp	Val	Asn	Ser	Leu	Val	Tyr	Leu	Asp	Ala	Val	Ile	Thr	Glu
370							375							380	
Thr	Leu	Arg	Leu	Tyr	Pro	Leu	Gly	Pro	Leu	Gly	Leu	Pro	His	Glu	Ser
385							390							400	
Ile	Glu	Asp	Cys	Thr	Ile	Ala	Gly	Tyr	His	Val	Pro	Ala	Arg	Thr	Arg
405							410							415	
Ile	Leu	Phe	Asn	Leu	Trp	Lys	Ile	His	Gln	Asp	Pro	Arg	Val	Trp	Glu
420							425							430	
Asn	Pro	Leu	Glu	Phe	Lys	Pro	Glu	Arg	Phe	Leu	Lys	Glu	His	Asn	Asn
435							440							445	
Ile	Asp	Val	Arg	Gly	Gly	His	Phe	Glu	Leu	Leu	Pro	Phe	Gly	Ser	Gly
450							455							460	
Arg	Arg	Met	Cys	Pro	Gly	Val	Ser	Phe	Ala	Leu	Gln	Val	Leu	Lys	Leu
465							470							480	
Thr	Leu	Ala	Asn	Met	Leu	His	Gly	Phe	Asp	Phe	Ala	Thr	Pro	Asn	Asp
485							490							495	
Glu	Pro	Val	Asp	Met	Thr	Glu	Val	Asn	His	Met	Ala	Thr	Thr	Arg	Ala
500							505							510	
Thr	Pro	Leu	Glu	Thr	Leu	Ile	Ser	Pro	Arg	Leu	Pro	Ser	His	Leu	Tyr
515							520							525	
Met	Gly														
															530

<210> SEQ ID NO 6
<211> LENGTH: 533
<212> TYPE: PRT

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<213> ORGANISM: Tripterugium wilfordii

<400> SEQUENCE: 6

Met Glu Phe Leu Leu Ser Leu Pro Thr Asn Thr Ile Ala Thr Thr Ser
1 5 10 15

Phe Ala Val Leu Leu Leu Tyr Leu Cys Leu Arg Ile Phe Thr Asn Val
20 25 30

Leu Lys Pro Asn Lys Ser Lys Thr Ser Pro Pro Gln Ala Gly Gly Ala
35 40 45

Trp Pro Leu Ile Gly His Leu His Leu Phe Arg Gly Pro Gln Pro Pro
50 55 60

His Ile Thr Leu Gly Lys Met Ala Asp Lys His Gly Pro Ile Phe Lys
65 70 75 80

Ile Lys Leu Gly Val Ser Pro Thr Leu Val Ile Ser Asp Ser Gln Ile
85 90 95

Ala Lys Glu Cys Phe Thr Thr His Asp Lys Ile Leu Ala Gly Arg Pro
100 105 110

Ala Tyr Val Ala Leu Glu Ile Met Gly Tyr Asn Asn Ala Met Phe Gly
115 120 125

Phe Ser Pro Tyr Gly Pro Tyr Trp Arg Tyr Ile Arg Lys Leu Ala Thr
130 135 140

Ile Glu Leu Leu Ser Asn Lys Arg Leu Glu Thr Phe Lys His Ile Arg
145 150 155 160

Glu Ser Glu Val Lys Asn Ala Met Lys Glu Met Tyr Gln Ser Trp Val
165 170 175

Val His Asn Lys Ser Ala Ser Gly His Ser Asn His Val Ser Val Asp
180 185 190

Met Ser Lys Ile Leu Gly Asp Ile Ser Ser Asn Val Thr Tyr Arg Ala
195 200 205

Met Val Gly Lys Val Tyr Ala Ser Lys Gly Glu Asp Val Arg Trp
210 215 220

Lys Gln Val Leu Ser Glu Tyr Met Lys Leu Leu Ser Asn Phe Ser Ser
225 230 235 240

Cys Asp Ala Leu Pro Phe Leu Arg Trp Phe Asp Phe Gly Leu Glu
245 250 255

Lys Ser Met Lys Arg Thr Phe Lys Glu Leu Asp Asn Tyr Val Glu Glu
260 265 270

Trp Leu Gln Glu His Arg Lys Lys Arg Ser Ser Ser Gly Asp Gly Gly
275 280 285

Ile Val Val Glu Asp Phe Met Asp Val Met Leu Ser Ile Phe Asp Asn
290 295 300

Val Gly Glu His Glu Asn Phe Thr Asp Tyr Ser Pro His Thr Ile Asn
305 310 315 320

Lys Ala Thr Cys Met Ser Leu Leu Leu Gly Ala Ser Asp Thr Thr Lys
325 330 335

Ser Thr Met Ile Trp Ser Leu Ser Leu Leu Leu Asn His Pro Asp Val
340 345 350

Leu Lys Lys Val Gln Gln Glu Leu Asp Ala His Ile Gly Pro Glu Thr
355 360 365

Leu Val Asn Glu Ser Asp Val Lys Ser Phe Val Tyr Leu Asp Ala Val
370 375 380

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Ile	Lys	Glu	Thr	Leu	Arg	Leu	Tyr	Ser	Pro	Gly	Pro	Leu	Gly	Leu	Pro
385				390				395				400			
<hr/>															
His	Glu	Ala	Met	Glu	Asp	Cys	Thr	Val	Ala	Gly	Tyr	His	Val	Pro	Ala
				405				410				415			
<hr/>															
Gly	Thr	Gln	Leu	Leu	Phe	Asn	Gln	Trp	Lys	Met	His	Gln	Asp	Pro	Asn
				420				425				430			
<hr/>															
Val	Trp	Glu	Asp	Pro	Ser	Glu	Phe	Lys	Pro	Glu	Arg	Phe	Leu	Thr	Thr
				435				440				445			
<hr/>															
His	Lys	Asp	Ile	Asp	Phe	Arg	Gly	Arg	His	Phe	Glu	Tyr	Leu	Pro	Phe
				450				455				460			
<hr/>															
Ala	Ser	Gly	Arg	Arg	Ile	Cys	Pro	Gly	Ile	Ser	Phe	Ala	His	Gln	Ile
465					470				475				480		
<hr/>															
Leu	Met	Leu	Ser	Leu	Ala	Asn	Met	Leu	His	Gly	Phe	Asp	Phe	Thr	Thr
				485				490				495			
<hr/>															
Pro	Asn	Gly	Glu	Pro	Val	Asp	Met	Ala	Gln	Val	Ser	Gly	Gly	Thr	Leu
				500				505				510			
<hr/>															
Ile	Arg	Ala	Thr	Pro	Leu	Glu	Ala	Leu	Ile	Ser	Pro	Arg	Leu	Pro	Gly
				515				520				525			
<hr/>															
His	Val	Tyr	Met	Gly											
				530											

<210> SEQ ID NO 7
<211> LENGTH: 530
<212> TYPE: PRT
<213> ORGANISM: Tripterygium wilfordii

<400> SEQUENCE: 7

Met	Glu	Phe	Leu	Leu	Ser	Ile	Pro	Ala	Asn	Thr	Ile	Ala	Thr	Gln	Ile
1				5				10				15			
<hr/>															
Phe	Ala	Leu	Leu	Leu	Tyr	Leu	Cys	Phe	Arg	Lys	Phe	Thr	Asp	Val	
				20				25				30			
<hr/>															
Leu	Lys	Pro	Lys	Gln	Ser	Lys	Thr	Ser	Pro	Pro	Gln	Val	Gly	Gly	Ala
				35				40				45			
<hr/>															
Trp	Pro	Leu	Ile	Gly	His	Leu	His	Arg	Leu	Arg	Gly	Pro	Pro	Ala	Pro
				50				55				60			
<hr/>															
His	Ile	Thr	Leu	Gly	Lys	Met	Ala	Asp	Lys	Tyr	Gly	Pro	Ile	Phe	Lys
				65				70				75			80
<hr/>															
Ile	Lys	Leu	Gly	Leu	His	Pro	Thr	Leu	Val	Ile	Ser	Asn	Ser	Glu	Ile
				85				90				95			
<hr/>															
Ala	Lys	Glu	Cys	Leu	Thr	Thr	His	Asp	Lys	Val	Leu	Ala	Gly	Arg	Pro
				100				105				110			
<hr/>															
Ala	Thr	Val	Ala	Thr	Glu	Ile	Met	Ser	Tyr	Asn	His	Ala	Met	Phe	Thr
				115				120				125			
<hr/>															
Phe	Ser	Ser	Tyr	Gly	Pro	Tyr	Trp	Ser	His	Thr	Arg	Lys	Leu	Val	Thr
				130				135				140			
<hr/>															
Val	Glu	Leu	Leu	Ser	Asn	Lys	Arg	Leu	Glu	Thr	Phe	Lys	His	Ile	Arg
				145				150				155			160
<hr/>															
Glu	Ser	Glu	Val	Lys	Asn	Ser	Val	Lys	Glu	Met	Tyr	Gln	Ser	Trp	Val
				165				170				175			
<hr/>															
His	Asn	Lys	Thr	Gly	Asp	Ser	Asn	Gln	Val	Leu	Val	Asp	Met	Thr	Arg
				180				185				190			
<hr/>															
Ile	Phe	Gly	Asp	Ile	Ile	Ala	Asn	Val	Ile	Tyr	Arg	Ile	Val	Val	Gly
				195				200				205			

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Lys Val Tyr Ala Ser Lys Gly Glu Gly His Val Arg Trp Lys Gln Val
210 215 220

Val Ser Glu Tyr Val Asn Leu Leu Ser His Phe Gly Val Gly Asp Ala
225 230 235 240

Leu Pro Phe Leu Arg Trp Leu Asp Leu Gly Gly Lys Glu Lys Ala Met
245 250 255

Lys Lys Thr Ala Lys Glu Leu Asp Asn Tyr Val Glu Glu Trp Leu Gln
260 265 270

Glu His Lys Lys Arg Ser Ser Ala Gly Asp His Gly Ile Val Glu
275 280 285

Glu Asp Phe Met Asp Val Met Leu Ser Ile Phe Tyr Asp Asp Asp Gln
290 295 300

Glu Glu Ser Phe Ala Asp His Ser Ala His Thr Ile Asn Lys Ala Leu
305 310 315 320

Cys Leu Ser Leu Ile Leu Ala Ala Ser Asp Thr Thr Lys Thr Thr Leu
325 330 335

Thr Trp Val Leu Ser Leu Leu Asn His Arg Asp Ile Leu Asn Lys
340 345 350

Val Gln Gln Glu Leu Ile Ala His Ile Gly Pro Glu Thr Pro Val Asn
355 360 365

Glu Ser Asp Ile Lys Ser Phe Val Tyr Leu Glu Ala Val Ile Lys Glu
370 375 380

Thr Leu Arg Leu Tyr Pro Pro Gly Pro Leu Gly Leu Pro His Glu Ser
385 390 395 400

Met Glu Asp Cys Thr Ile Ala Gly Tyr His Val Pro Ala Gly Thr Arg
405 410 415

Val Leu Phe Asn Gln Trp Lys Ile His His Asp Pro Gln Val Trp Glu
420 425 430

Asn Pro Ser Glu Phe Lys Pro Glu Arg Phe Leu Arg Thr His Lys Glu
435 440 445

Val Asp Val Arg Gly Arg His Phe Glu Leu Leu Pro Phe Gly Ser Gly
450 455 460

Arg Arg Met Cys Pro Gly Ile Ser Phe Ala Leu Gln Val Met Glu Leu
465 470 475 480

Ala Leu Ala Asn Met Leu His Gly Phe Asp Phe Ala Thr Pro Asn Gly
485 490 495

Glu Pro Val Asp Met Thr Glu Asp Asn Gly Phe Val Thr Leu Arg Ala
500 505 510

Thr Pro Leu Glu Ala Leu Ile Ser Pro Arg Leu Pro Gly His Val Tyr
515 520 525

Met Gly
530

<210> SEQ ID NO 8
<211> LENGTH: 134
<212> TYPE: PRT
<213> ORGANISM: Tripterygium wilfordii

<400> SEQUENCE: 8

Met Ala Ser Asp Arg Lys Ile Tyr Met Phe Lys Glu Val Glu Thr His
1 5 10 15

Asn Lys Thr Lys Asp Cys Trp Leu Ile Ile Ser Gly Lys Val Tyr Asp

- continued

20	25	30	
Val Thr Pro Phe Met Glu Asp His	Pro Gly Gly Asp Glu Val Leu	Leu	
35	40	45	
Ser Ser Thr Gly Lys Asp Ala Thr Asn Asp	Phe Glu Asp Val Gly His		
50	55	60	
Ser Asp Asn Ala Arg Asp Met Met Asp Gln	Tyr Cys Ile Gly Glu Ile		
65	70	75	80
Asp Gly Lys Thr Val Pro Glu Lys Arg Asn	Tyr Ile Pro Ala Gln Thr		
85	90	95	
Pro Ala Tyr Asn Gln Asp Lys Thr Pro Glu	Phe Val Val Lys Val Leu		
100	105	110	
Gln Phe Leu Val Pro Leu Leu Ile Leu Gly	Leu Ala Phe Ala Val Arg		
115	120	125	
His Phe Thr Lys Lys Glu			
130			

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<210> SEQ ID NO 9
<211> LENGTH: 708
<212> TYPE: PRT
<213> ORGANISM: Tripterygium wilfordii

<400> SEQUENCE: 9

Met Gln Ser Ser Ser Asn Ser Met Lys Ala Ser Pro Leu Asp Leu Met
1 5 10 15

Ser Ala Ile Ile Lys Gly Lys Val Asp Pro Ser Asn Val Ser Ser Glu
20 25 30

Val Ser Gly Glu Val Thr Ser Ile Ile Phe Glu Asn Arg Glu Phe Val
35 40 45

Met Ile Leu Thr Thr Ser Ile Ala Val Leu Ile Gly Cys Val Val Val
50 55 60

Leu Ile Trp Arg Arg Ser Gly Ala Gln Lys Ser Lys Ala Leu Val Pro
65 70 75 80

Pro Lys Pro Leu Ala Val Lys Leu Pro Glu Pro Glu Val Asp Asp Gly
85 90 95

Lys Ser Lys Ile Thr Val Phe Tyr Gly Thr Gln Thr Gly Thr Ala Glu
100 105 110

Gly Phe Ala Lys Ala Leu Val Glu Glu Ala Lys Ala Arg Tyr Glu Lys
115 120 125

Ala Val Phe Lys Ile Val Asp Leu Asp Asp Tyr Ala Glu Asp Asp Asp
130 135 140

Glu Tyr Glu Glu Lys Leu Lys Lys Glu Ala Ile Phe Phe Leu
145 150 155 160

Ala Thr Tyr Gly Asp Gly Glu Pro Thr Asp Asn Ala Ala Arg Phe Tyr
165 170 175

Lys Trp Phe Leu Glu Gly Lys Glu Arg Gly Glu Cys Phe Gln Asn Met
180 185 190

Lys Phe Ala Val Phe Gly Leu Gly Asn Arg Gln Tyr Glu His Phe Asn
195 200 205

Lys Val Ala Lys Glu Val Asp Gln Ile Leu Ser Glu Gln Gly Ala Thr
210 215 220

Arg Leu Val Pro Val Gly Leu Gly Asp Asp Asp Gln Cys Leu Glu Asp
225 230 235 240

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Asp	Phe	Thr	Ala	Trp	Arg	Glu	Leu	Val	Trp	Pro	Glu	Leu	Asp	Gln	Leu
245						250						255			
Leu	Arg	Asp	Lys	Asp	Gly	Ala	Thr	Thr	Val	Ser	Thr	Pro	Tyr	Thr	Ala
260						265						270			
Thr	Ile	Pro	Glu	Tyr	Arg	Val	Lys	Cys	Tyr	Asp	Thr	Ser	Asp	Ala	Ser
275						280						285			
Val	Glu	Glu	Lys	Ser	Trp	Ser	Asn	Ala	Asn	Gly	His	Ala	Val	Val	Asp
290						295					300				
Ala	Gln	His	Pro	Cys	Arg	Ser	Asn	Val	Ala	Val	Lys	Arg	Glu	Leu	His
305						310					315				320
Thr	Pro	Ala	Ser	Asp	Arg	Ser	Cys	Thr	His	Leu	Glu	Phe	Asp	Ile	Ala
325						330						335			
Gly	Thr	Gly	Leu	Ser	Tyr	Glu	Thr	Gly	Asp	His	Val	Gly	Val	Tyr	Cys
340						345						350			
Glu	Asn	Leu	Thr	Glu	Thr	Val	Glu	Glu	Ala	Val	Arg	Leu	Leu	Gly	Leu
355						360						365			
Ser	Pro	Asp	Thr	Tyr	Phe	Ser	Leu	His	Ser	Asp	Lys	Glu	Asp	Gly	Thr
370						375					380				
Pro	Leu	Ser	Ala	Ser	Ser	Leu	Pro	Pro	Thr	Phe	Pro	Pro	Cys	Ser	Leu
385						390					395				400
Lys	Thr	Ala	Leu	Ala	Arg	Tyr	Ala	Asp	Leu	Leu	Asn	Ser	Pro	Lys	Lys
405						410						415			
Ser	Ala	Leu	Leu	Ala	Leu	Ala	Ala	His	Ala	Ser	Asp	Pro	Thr	Glu	Ala
420						425						430			
Asp	Arg	Leu	Arg	His	Leu	Ala	Ser	Pro	Ala	Gly	Lys	Asp	Glu	Tyr	Ala
435						440						445			
Gln	Trp	Val	Ile	Ala	Ser	Gln	Arg	Ser	Leu	Leu	Glu	Ile	Met	Ala	Glu
450						455					460				
Phe	Pro	Ser	Ala	Arg	Pro	Pro	Leu	Gly	Val	Phe	Phe	Ala	Ala	Val	Ala
465						470					475				480
Pro	Arg	Leu	Gln	Pro	Arg	Tyr	Tyr	Ser	Ile	Ser	Ser	Ser	Pro	Arg	Met
485						490						495			
Ala	Pro	Ser	Arg	Ile	His	Val	Thr	Cys	Ala	Leu	Val	Tyr	Glu	Lys	Thr
500						505						510			
Pro	Ala	Gly	Arg	Ile	His	Lys	Gly	Val	Cys	Ser	Thr	Trp	Met	Lys	Asn
515						520						525			
Ala	Val	Pro	Leu	Glu	Lys	Ser	His	Glu	Ser	Cys	Trp	Ala	Pro	Ile	Phe
530						535						540			
Val	Arg	Gln	Ser	Asn	Phe	Lys	Leu	Pro	Val	Asp	Thr	Lys	Val	Pro	Ile
545						550					555				560
Ile	Met	Ile	Gly	Pro	Gly	Thr	Gly	Phe	Ala	Pro	Phe	Arg	Gly	Phe	Leu
565						570						575			
Gln	Glu	Arg	Leu	Ala	Leu	Lys	Glu	Ser	Gly	Ala	Glu	Leu	Gly	Ser	Ser
580						585						590			
Ile	Leu	Phe	Phe	Gly	Cys	Arg	Asn	Arg	Arg	Leu	Asp	Tyr	Ile	Tyr	Glu
595						600						605			
Glu	Glu	Leu	Asn	Asn	Phe	Val	Glu	Ser	Ala	Ala	Leu	Ser	Glu	Leu	Ile
610						615						620			
Val	Ala	Phe	Ser	Arg	Glu	Gly	Pro	Thr	Lys	Glu	Tyr	Val	Gln	His	Lys
625						630					635				640
Met	Met	Glu	Lys	Ala	Ser	Asp	Ile	Trp	Asn	Met	Ile	Asn	Gln	Gly	Ala

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645	650	655
Tyr Ile Tyr Val Cys Gly Asp Ala Lys Gly Met Ala Arg Asp Val His		
660	665	670
Arg Thr Leu His Thr Ile Val Gln Glu Gln Gly Ser Leu Asp Ser Ser		
675	680	685
Lys Ala Glu Ser Met Val Lys Asn Leu Gln Thr Ser Gly Arg Tyr Leu		
690	695	700
Arg Asp Val Trp		
705		

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<210> SEQ ID NO 10
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer CO_TwCYP71BE85v1_TEF-F
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 10

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agcgatacgn aaaatggact tattgcaatt tccatctg 38

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<210> SEQ ID NO 11
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer CO_TwCYP71BE85v1_TEF-R
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 11

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cacgcgantc agttaaatgc gggtgatgg 29

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<210> SEQ ID NO 12
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer CO_TwGA3OX1_TEF-F
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 12

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agcgatacgn aaaatgagtc ctccgcctac aata 34

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<210> SEQ ID NO 13
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer CO_TwGA3OX1_TEF-R
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 13

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cacgcgantt aaatacctaa aagcgagacg gg	32
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<210> SEQ ID NO 14
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer CO_AcoUGT2_TEF-F
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 14

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agcgatacgn aaaatggctg ttagctaaa aaataccg	38
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<210> SEQ ID NO 15
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer CO_AcoUGT2_TEF-R
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 15

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cacgcgantt aacgactgtat atgagcgacg	30
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<210> SEQ ID NO 16
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer CO_TwCYP82D213_PGK-F
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 16

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atcaacgggn aaaatggaat tccttctgtc attgc	35
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<210> SEQ ID NO 17
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer CO_TwCYP82D213_PGK-R
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 17

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cgtgcganct aaccatgtta aagatgttat gg	32
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<210> SEQ ID NO 18
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer CO_TwCYP71BE86_PGK-F
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<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)

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<223> OTHER INFORMATION: A, C, G or T	
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atcaacgggn aaaatggact tacaattacc tagttcc	38
<210> SEQ ID NO 19	
<211> LENGTH: 33	
<212> TYPE: DNA	
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<220> FEATURE:	
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<220> FEATURE:	
<221> NAME/KEY: misc_feature	
<222> LOCATION: (8)..(8)	
<223> OTHER INFORMATION: A, C, G or T	
<400> SEQUENCE: 19	
cgtgcgantt aaccagataa actacgatat ggg	33
<210> SEQ ID NO 20	
<211> LENGTH: 40	
<212> TYPE: DNA	
<213> ORGANISM: Artificial	
<220> FEATURE:	
<223> OTHER INFORMATION: Primer TwCYP82D217_pLife-F	
<220> FEATURE:	
<221> NAME/KEY: misc_feature	
<222> LOCATION: (8)..(8)	
<223> OTHER INFORMATION: A, C, G or T	
<400> SEQUENCE: 20	
ggcttaanaa gcatttctc tcctaactag ctttctaaat	40
<210> SEQ ID NO 21	
<211> LENGTH: 35	
<212> TYPE: DNA	
<213> ORGANISM: Artificial	
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<223> OTHER INFORMATION: Primer TwCYP82D217_pLife-R	
<220> FEATURE:	
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<223> OTHER INFORMATION: A, C, G or T	
<400> SEQUENCE: 21	
ggtttaanct attgcaattc accccatgta gacaa	35
<210> SEQ ID NO 22	
<211> LENGTH: 29	
<212> TYPE: DNA	
<213> ORGANISM: Artificial	
<220> FEATURE:	
<223> OTHER INFORMATION: Primer pLifeUP_TEF-F	
<220> FEATURE:	
<221> NAME/KEY: misc_feature	
<222> LOCATION: (10)..(10)	
<223> OTHER INFORMATION: A, C, G or T	
<400> SEQUENCE: 22	
agcgatacgn gacctgcagg ctgaggctt	29
<210> SEQ ID NO 23	
<211> LENGTH: 27	
<212> TYPE: DNA	
<213> ORGANISM: Artificial	
<220> FEATURE:	

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<223> OTHER INFORMATION: Primer pLife_TEF-R
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 23

cacgcgancc cggggctgag gtttaat

27

<210> SEQ ID NO 24
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer TwCYP82D274v1_pLife-F
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: A, C, G, or T

<400> SEQUENCE: 24

ggcttaatggatgttctt ctttcactcc caaca

35

<210> SEQ ID NO 25
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer TwCYP82D274v1_pLife-R
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 25

ggtttaantc agcccatata gagatgagct gggag

35

<210> SEQ ID NO 26
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer pLife_TEF-F
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 26

agcgatacgn tgcaggctga ggcttaatat g

31

<210> SEQ ID NO 27
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer pLife_TEF-R
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<221> NAME/KEY: misc_feature
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 27

cacgcgancc cggggctgag gtttaat

27

<210> SEQ ID NO 28

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<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
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<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 28
ggcttaaat gcaatttct tcaaattcta tgaagg                                36

<210> SEQ ID NO 29
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer TwCPR1_pLife-R
<220> FEATURE:
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<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 29
ggtttaantt accacacatc ccggagata                                29

<210> SEQ ID NO 30
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
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<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 30
atcaacgggn tgcaggctga ggcttaatat g                                31

<210> SEQ ID NO 31
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial
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<220> FEATURE:
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<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 31
cgtgcgancc cggggctgag gtttaat                                27

<210> SEQ ID NO 32
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer Twb5#1_pLife-F
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 32

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ggcttaanat ggcttcggat cggaagata

29

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<210> SEQ ID NO 33
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
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<220> FEATURE:
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<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 33

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ggtttaanct attcttctt ggtgaagtga cgta

34

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<210> SEQ ID NO 34
<211> LENGTH: 31
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer pLife_PGK-F
<220> FEATURE:
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<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 34

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atcaacgggn tgcaggctga ggcttaatat g

31

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<210> SEQ ID NO 35
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer pLife_PGK-R
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 35

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cgtgcgancc cggggctgag gtttaat

27

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<210> SEQ ID NO 36
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 36

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ggcttaanat gggtgagac ggaaagggtt

29

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<210> SEQ ID NO 37
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer Twb5#2_pLife-R
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<222> LOCATION: (8)..(8)

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<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 37

ggtttaantt aagcaggagg agctgatttg gt

32

<210> SEQ ID NO 38

<211> LENGTH: 31

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Primer pLife_PGK-F

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (10)..(10)

<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 38

atcaacgggn tgcaggctga ggcttaatat g

31

<210> SEQ ID NO 39

<211> LENGTH: 27

<212> TYPE: DNA

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<223> OTHER INFORMATION: Primer pLife_PGK-R

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (8)..(8)

<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 39

cgtgcgancc cggggctgag gtttaat

27

<210> SEQ ID NO 40

<211> LENGTH: 31

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Primer Twb5#3_pLife-F

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (8)..(8)

<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 40

ggcttaanat ggctggatcg agagtttca c

31

<210> SEQ ID NO 41

<211> LENGTH: 33

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Primer Twb5#3_pLife-R

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (8)..(8)

<223> OTHER INFORMATION: A, C, G, or T

<400> SEQUENCE: 41

ggtttaantt agaagatctg ctcaggcctt gta

33

<210> SEQ ID NO 42

<211> LENGTH: 31

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

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<223> OTHER INFORMATION: Primer pLife_PGK-F
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<223> OTHER INFORMATION: A, C, G or T
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atcaacgggn tgcaggctga ggcttaatat g

31

<210> SEQ ID NO 43
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer pLife_PGK-R
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 43

cgtgcgancc cggggctgag gtttaat

27

<210> SEQ ID NO 44
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer Twb5#4_PGK-F
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 44

atcaacgggn aaaatggcta aacttcttc atttgctgag

40

<210> SEQ ID NO 45
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer Twb5#4_PGK-R
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 45

cgtgcgantt agaaaaggta tcgcaaacc aatgcc

36

<210> SEQ ID NO 46
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer Twb5#5_PGK-F
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 46

atcaacgggn aaaatgatta ttgttgcggt ggctctga

38

<210> SEQ ID NO 47

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<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer Twb5#5_PGK-R
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: A, C, G or T

<400> SEQUENCE: 47

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cgtgcgantt acttctctag atccccatg taaaaatcat cg

42

```

<210> SEQ ID NO 48
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer Twb5#6_PGK-F
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: A, C, G or T
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 48

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atcaacgggn aaaatgccga cttaacgaa gctgcac

37

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<210> SEQ ID NO 49
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer Twb5#6_PGK-R
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: A, C, G or T
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 49

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cgtgcganct acttcttccg caagtacagg agtc

34

```

<210> SEQ ID NO 50
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer YEA85_UP_Genotyping_Fw

<400> SEQUENCE: 50

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tctcaggat agcatgaggt cgctcat

27

```

<210> SEQ ID NO 51
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer YEA86_DW_Genotyping_Fw

<400> SEQUENCE: 51

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cctgcaggac tagtgctgag gcattaat 28

<210> SEQ ID NO 52
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer YEA87_X-2_Genotyping_UP

<400> SEQUENCE: 52

gtttgtatggccggtag 20

<210> SEQ ID NO 53
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer YEA88_X-2_Genotyping_DW

<400> SEQUENCE: 53

gagacaagat gggcaagac 20

<210> SEQ ID NO 54
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer YEA89_X-3_Genotyping_UP

<400> SEQUENCE: 54

tgacgaatcg ttaggcacag 20

<210> SEQ ID NO 55
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer YEA90_X-3_Genotyping_DW

<400> SEQUENCE: 55

ccgtgcaata ccaaaatcga g 21

<210> SEQ ID NO 56
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer YEA91_X-4_Genotyping_UP

<400> SEQUENCE: 56

ctcacaaaagg gacgaatcct 20

<210> SEQ ID NO 57
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer YEA92_X-4_Genotyping_DW

<400> SEQUENCE: 57

gacggtaacgt tgaccagag 19

<210> SEQ ID NO 58

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<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer YEA93_XI-1_Genotyping_UP

<400> SEQUENCE: 58

cttaatgggt agtgcttgac acg 23

<210> SEQ ID NO 59
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer YEA94_XI-2_Genotyping_UP

<400> SEQUENCE: 59

gttttagatt ggccggggag 20

<210> SEQ ID NO 60
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer YEA95_XI-2_Genotyping_DW

<400> SEQUENCE: 60

gagacaagat gggcaagac 20

<210> SEQ ID NO 61
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer YEA96_XI-5_Genotyping_UP

<400> SEQUENCE: 61

ctcaatgatc aaaatcctga atgca 25

<210> SEQ ID NO 62
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer YEA97_XI-5_Genotyping_DW

<400> SEQUENCE: 62

gcatggtcac cgctatcagc 20

<210> SEQ ID NO 63
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer YEA98_XII-2_Genotyping_UP

<400> SEQUENCE: 63

cgagaaggc ctgcaattc 19

<210> SEQ ID NO 64
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:

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<223> OTHER INFORMATION: primer YEA99_XII-2_Genotyping_DW

<400> SEQUENCE: 64

ggccctgata aggttgg

19

<210> SEQ ID NO 65

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Primer YEA100_XII-5_Genotyping_UP

<400> SEQUENCE: 65

ccaccgaagt tgatttgctt

20

<210> SEQ ID NO 66

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Primer YEA101_XII-5_Genotyping_DW

<400> SEQUENCE: 66

gtgggagtaa gggatcctgt

20

<210> SEQ ID NO 67

<211> LENGTH: 786

<212> TYPE: PRT

<213> ORGANISM: Plectranthus barbatus

<400> SEQUENCE: 67

Met Gly Ser Leu Ser Thr Met Asn Leu Asn His Ser Pro Met Ser Tyr
1 5 10 15

Ser Gly Ile Leu Pro Ser Ser Ala Lys Ala Lys Leu Leu Pro
20 25 30

Gly Cys Phe Ser Ile Ser Ala Trp Met Asn Asn Gly Lys Asn Leu Asn
35 40 45

Cys Gln Leu Thr His Lys Ile Ser Lys Val Ala Glu Ile Arg Val
50 55 60

Ala Thr Val Asn Ala Pro Pro Val His Asp Gln Asp Asp Ser Thr Glu
65 70 75 80

Asn Gln Cys His Asp Ala Val Asn Ile Glu Asp Pro Ile Glu Tyr
85 90 95

Ile Arg Thr Leu Leu Arg Thr Thr Gly Asp Gly Arg Ile Ser Val Ser
100 105 110

Pro Tyr Asp Thr Ala Trp Val Ala Leu Ile Lys Asp Leu Gln Gly Arg
115 120 125

Asp Ala Pro Glu Phe Pro Ser Ser Leu Glu Trp Ile Ile Gln Asn Gln
130 135 140

Leu Ala Asp Gly Ser Trp Gly Asp Ala Lys Phe Phe Cys Val Tyr Asp
145 150 155 160

Arg Leu Val Asn Thr Ile Ala Cys Val Val Ala Leu Arg Ser Trp Asp
165 170 175

Val His Ala Glu Lys Val Glu Arg Gly Val Arg Tyr Ile Asn Glu Asn
180 185 190

Val Glu Lys Leu Arg Asp Gly Asn Glu Glu His Met Thr Cys Gly Phe
195 200 205

-continued

Glu Val Val Phe Pro Ala Leu Leu Gln Arg Ala Lys Ser Leu Gly Ile
 210 215 220
 Gln Asp Leu Pro Tyr Asp Ala Pro Val Ile Gln Glu Ile Tyr His Ser
 225 230 235 240
 Arg Glu Gln Lys Ser Lys Arg Ile Pro Leu Glu Met Met His Lys Val
 245 250 255
 Pro Thr Ser Leu Leu Phe Ser Leu Glu Gly Leu Glu Asn Leu Glu Trp
 260 265 270
 Asp Lys Leu Leu Lys Leu Gln Ser Ala Asp Gly Ser Phe Leu Thr Ser
 275 280 285
 Pro Ser Ser Thr Ala Phe Ala Phe Met Gln Thr Arg Asp Pro Lys Cys
 290 295 300
 Tyr Gln Phe Ile Lys Asn Thr Ile Gln Thr Phe Asn Gly Gly Ala Pro
 305 310 315 320
 His Thr Tyr Pro Val Asp Val Phe Gly Arg Leu Trp Ala Ile Asp Arg
 325 330 335
 Leu Gln Arg Leu Gly Ile Ser Arg Phe Phe Glu Ser Glu Ile Ala Asp
 340 345 350
 Cys Ile Ala His Ile His Arg Phe Trp Thr Glu Lys Gly Val Phe Ser
 355 360 365
 Gly Arg Glu Ser Glu Phe Cys Asp Ile Asp Asp Thr Ser Met Gly Val
 370 375 380
 Arg Leu Met Arg Met His Gly Tyr Asp Val Asp Pro Asn Val Leu Lys
 385 390 395 400
 Asn Phe Lys Asp Asp Lys Phe Ser Cys Tyr Gly Gly Gln Met Ile
 405 410 415
 Glu Ser Pro Ser Pro Ile Tyr Asn Leu Tyr Arg Ala Ser Gln Leu Arg
 420 425 430
 Phe Pro Gly Glu Gln Ile Leu Glu Asp Ala Asn Lys Phe Ala Tyr Asp
 435 440 445
 Phe Leu Gln Glu Lys Leu Ala His Asn Gln Ile Leu Asp Lys Trp Val
 450 455 460
 Ile Ser Lys His Leu Pro Asp Glu Ile Lys Leu Gly Leu Glu Met Pro
 465 470 475 480
 Trp Tyr Ala Thr Leu Pro Arg Val Glu Ala Arg Tyr Tyr Ile Gln Tyr
 485 490 495
 Tyr Ala Gly Ser Gly Asp Val Trp Ile Gly Lys Thr Leu Tyr Arg Met
 500 505 510
 Pro Glu Ile Ser Asn Asp Thr Tyr His Glu Leu Ala Lys Thr Asp Phe
 515 520 525
 Lys Arg Cys Gln Ala Gln His Gln Phe Glu Trp Ile Tyr Met Gln Glu
 530 535 540
 Trp Tyr Glu Ser Cys Asn Met Glu Glu Phe Gly Ile Ser Arg Lys Glu
 545 550 555 560
 Leu Leu Val Ala Tyr Phe Leu Ala Thr Ala Ser Ile Phe Glu Leu Glu
 565 570 575
 Arg Ala Asn Glu Arg Ile Ala Trp Ala Lys Ser Gln Ile Ile Ser Thr
 580 585 590
 Ile Ile Ala Ser Phe Phe Asn Asn Gln Asn Thr Ser Pro Glu Asp Lys
 595 600 605

-continued

Leu	Ala	Phe	Leu	Thr	Asp	Phe	Lys	Asn	Gly	Asn	Ser	Thr	Asn	Met	Ala
610						615									620
Leu	Val	Thr	Leu	Thr	Gln	Phe	Leu	Glu	Gly	Phe	Asp	Arg	Tyr	Thr	Ser
625						630									640
His	Gln	Leu	Lys	Asn	Ala	Trp	Ser	Val	Trp	Leu	Arg	Lys	Leu	Gln	Gln
							645			650				655	
Gly	Glu	Gly	Asn	Gly	Gly	Ala	Asp	Ala	Glu	Leu	Leu	Val	Asn	Thr	Leu
						660			665					670	
Asn	Ile	Cys	Ala	Gly	His	Ile	Ala	Phe	Arg	Glu	Glu	Ile	Leu	Ala	His
						675			680					685	
Asn	Asp	Tyr	Lys	Thr	Leu	Ser	Asn	Leu	Thr	Ser	Lys	Ile	Cys	Arg	Gln
						690			695					700	
Leu	Ser	Gln	Ile	Gln	Asn	Glu	Lys	Glu	Leu	Glu	Thr	Glu	Gly	Gln	Lys
						705			710			715			720
Thr	Ser	Ile	Lys	Asn	Lys	Glu	Leu	Glu	Glu	Asp	Met	Gln	Arg	Leu	Val
						725			730					735	
Lys	Leu	Val	Leu	Glu	Lys	Ser	Arg	Val	Gly	Ile	Asn	Arg	Asp	Met	Lys
						740			745					750	
Lys	Thr	Phe	Leu	Ala	Val	Val	Lys	Thr	Tyr	Tyr	Tyr	Lys	Ala	Tyr	His
						755			760					765	
Ser	Ala	Gln	Ala	Ile	Asp	Asn	His	Met	Phe	Lys	Val	Leu	Phe	Glu	Pro
						770			775					780	
Val	Ala														
	785														

<210> SEQ_ID NO 68															
<211> LENGTH: 598															
<212> TYPE: PRT															
<213> ORGANISM: Plectranthus barbatus															
<400> SEQUENCE: 68															
Met	Ser	Ser	Leu	Ala	Gly	Asn	Leu	Arg	Val	Ile	Pro	Phe	Ser	Gly	Asn
1							5			10				15	
Arg	Val	Gln	Thr	Arg	Thr	Gly	Ile	Leu	Pro	Val	His	Gln	Thr	Pro	Met
							20			25				30	
Ile	Thr	Ser	Ser	Ser	Ala	Ala	Val	Lys	Cys	Ser	Leu	Thr	Thr	Pro	
							35			40				45	
Thr	Asp	Leu	Met	Gly	Lys	Ile	Lys	Glu	Val	Phe	Asn	Arg	Glu	Val	Asp
						50			55					60	
Thr	Ser	Pro	Ala	Ala	Met	Thr	Thr	His	Ser	Thr	Asp	Ile	Pro	Ser	Asn
						65			70			75			80
Leu	Cys	Ile	Ile	Asp	Thr	Leu	Gln	Arg	Leu	Gly	Ile	Asp	Gln	Tyr	Phe
						85			90					95	
Gln	Ser	Glu	Ile	Asp	Ala	Val	Leu	His	Asp	Thr	Tyr	Arg	Leu	Trp	Gln
						100			105					110	
Leu	Lys	Lys	Asp	Ile	Phe	Ser	Asp	Ile	Thr	Thr	His	Ala	Met	Ala	
						115			120					125	
Phe	Arg	Leu	Leu	Arg	Val	Lys	Gly	Tyr	Glu	Val	Ala	Ser	Asp	Glu	Leu
						130			135					140	
Ala	Pro	Tyr	Ala	Asp	Gln	Glu	Arg	Ile	Asn	Leu	Gln	Thr	Ile	Asp	Val
						145			150					160	
Pro	Thr	Val	Val	Glu	Leu	Tyr	Arg	Ala	Ala	Gln	Glu	Arg	Leu	Thr	Glu
						165			170					175	

-continued

Glu Asp Ser Thr Leu Glu Lys Leu Tyr Val Trp Thr Ser Ala Phe Leu
 180 185 190
 Lys Gln Gln Leu Leu Thr Asp Ala Ile Pro Asp Lys Lys Leu His Lys
 195 200 205
 Gln Val Glu Tyr Tyr Leu Lys Asn Tyr His Gly Ile Leu Asp Arg Met
 210 215 220
 Gly Val Arg Arg Asn Leu Asp Leu Tyr Asp Ile Ser His Tyr Lys Ser
 225 230 235 240
 Leu Lys Ala Ala His Arg Phe Tyr Asn Leu Ser Asn Glu Asp Ile Leu
 245 250 255
 Ala Phe Ala Arg Gln Asp Phe Asn Ile Ser Gln Ala Gln His Gln Lys
 260 265 270
 Glu Leu Gln Gln Leu Gln Arg Trp Tyr Ala Asp Cys Arg Leu Asp Thr
 275 280 285
 Leu Lys Phe Gly Arg Asp Val Val Arg Ile Gly Asn Phe Leu Thr Ser
 290 295 300
 Ala Met Ile Gly Asp Pro Glu Leu Ser Asp Leu Arg Leu Ala Phe Ala
 305 310 315 320
 Lys His Ile Val Leu Val Thr Arg Ile Asp Asp Phe Phe Asp His Gly
 325 330 335
 Gly Pro Lys Glu Glu Ser Tyr Glu Ile Leu Glu Leu Val Lys Glu Trp
 340 345 350
 Lys Glu Lys Pro Ala Gly Glu Tyr Val Ser Glu Glu Val Glu Ile Leu
 355 360 365
 Phe Thr Ala Val Tyr Asn Thr Val Asn Glu Leu Ala Glu Met Ala His
 370 375 380
 Ile Glu Gln Gly Arg Ser Val Lys Asp Leu Leu Val Lys Leu Trp Val
 385 390 395 400
 Glu Ile Leu Ser Val Phe Arg Ile Glu Leu Asp Thr Trp Thr Asn Asp
 405 410 415
 Thr Ala Leu Thr Leu Glu Glu Tyr Leu Ser Gln Ser Trp Val Ser Ile
 420 425 430
 Gly Cys Arg Ile Cys Ile Leu Ile Ser Met Gln Phe Gln Gly Val Lys
 435 440 445
 Leu Ser Asp Glu Met Leu Gln Ser Glu Glu Cys Thr Asp Leu Cys Arg
 450 455 460
 Tyr Val Ser Met Val Asp Arg Leu Leu Asn Asp Val Gln Thr Phe Glu
 465 470 475 480
 Lys Glu Arg Lys Glu Asn Thr Gly Asn Ser Val Ser Leu Leu Gln Ala
 485 490 495
 Ala His Lys Asp Glu Arg Val Ile Asn Glu Glu Ala Cys Ile Lys
 500 505 510
 Val Lys Glu Leu Ala Glu Tyr Asn Arg Arg Lys Leu Met Gln Ile Val
 515 520 525
 Tyr Lys Thr Gly Thr Ile Phe Pro Arg Lys Cys Lys Asp Leu Phe Leu
 530 535 540
 Lys Ala Cys Arg Ile Gly Cys Tyr Leu Tyr Ser Ser Gly Asp Glu Phe
 545 550 555 560
 Thr Ser Pro Gln Gln Met Met Glu Asp Met Lys Ser Leu Val Tyr Glu
 565 570 575

-continued

Pro Leu Pro Ile Ser Pro Pro Glu Ala Asn Asn Ala Ser Gly Glu Lys
580 585 590

Met Ser Cys Val Ser Asn
595

<210> SEQ ID NO 69
<211> LENGTH: 807
<212> TYPE: PRT
<213> ORGANISM: Tripterygium wilfordii

<400> SEQUENCE: 69

Met His Ser Leu Leu Met Lys Lys Val Ile Met Tyr Ser Ser Gln Thr
1 5 10 15

Thr His Val Phe Pro Ser Pro Leu His Cys Thr Ile Pro Lys Ser Ser
20 25 30

Ser Phe Phe Leu Asp Ala Pro Val Ala Arg Leu His Cys Leu Ser Gly
35 40 45

His Gly Ala Lys Lys Lys Arg Leu His Phe Asp Ile Gln Gln Gly Arg
50 55 60

Asn Ala Val Ser Lys Thr His Thr Pro Asp Asp Leu Tyr Ala Lys Gln
65 70 75 80

Glu Tyr Ser Val Pro Glu Ile Val Lys Asp Asp Asp Lys Glu Glu Glu
85 90 95

Val Val Lys Ile Lys Glu His Val Asp Ile Ile Lys Ser Met Leu Ser
100 105 110

Ser Met Glu Asp Gly Glu Ile Ser Ile Ser Ala Tyr Asp Thr Ala Trp
115 120 125

Val Ala Leu Ile Gln Asp Ile His Asn Asn Gly Ala Pro Gln Phe Pro
130 135 140

Ser Ser Leu Leu Trp Ile Ala Glu Asn Gln Leu Pro Asp Gly Ser Trp
145 150 155 160

Gly Asp Ser Arg Val Phe Leu Ala Phe Asp Arg Ile Ile Asn Thr Leu
165 170 175

Ala Cys Val Val Ala Leu Lys Ser Trp Asn Val His Pro Asp Lys Cys
180 185 190

Glu Arg Gly Ile Ser Phe Leu Lys Glu Asn Ile Ser Met Leu Glu Lys
195 200 205

Asp Asp Ser Glu His Met Leu Val Gly Phe Glu Phe Gly Phe Pro Val
210 215 220

Leu Leu Asp Met Ala Arg Arg Leu Gly Ile Asp Val Pro Asp Asp Ser
225 230 235 240

Pro Phe Leu Gln Glu Ile Tyr Val Gln Arg Asp Leu Lys Leu Lys Arg
245 250 255

Ile Pro Lys Asp Ile Leu His Asn Val Pro Thr Thr Leu Leu His Ser
260 265 270

Leu Glu Ala Ile Pro Asp Leu Asp Trp Thr Lys Leu Leu Lys Leu Gln
275 280 285

Cys Gln Asp Gly Ser Leu Leu Phe Ser Pro Ser Ser Thr Ala Met Ala
290 295 300

Phe Ile Asn Thr Lys Asp Glu Asn Cys Leu Arg Tyr Leu Asn Tyr Val
305 310 315 320

Val Gln Arg Phe Asn Gly Gly Ala Pro Thr Val Tyr Pro Tyr Asp Leu
325 330 335

-continued

Phe Glu His Asn Trp Ala Val Asp Arg Leu Gln Arg Leu Gly Ile Ser
340 345 350

Arg Phe Phe Gln Pro Glu Ile Arg Glu Cys Met Ser Tyr Val Tyr Arg
355 360 365

Tyr Trp Thr Lys Asp Gly Ile Phe Cys Thr Arg Asn Ser Arg Val His
370 375 380

Asp Val Asp Asp Thr Ala Met Gly Phe Arg Leu Leu Arg Leu His Gly
385 390 395 400

Tyr Glu Val His Pro Asp Ala Phe Arg Gln Phe Lys Lys Gly Cys Glu
405 410 415

Phe Ile Cys Tyr Glu Gly Gln Ser His Pro Thr Val Thr Val Met Tyr
420 425 430

Asn Leu Tyr Arg Ala Ser Gln Leu Met Phe Pro Glu Glu Lys Ile Leu
435 440 445

Asp Glu Ala Lys Gln Phe Thr Glu Lys Phe Leu Gly Glu Lys Arg Ser
450 455 460

Ala Asn Lys Leu Leu Asp Lys Trp Ile Ile Thr Lys Asp Leu Pro Gly
465 470 475 480

Glu Val Gly Phe Ala Leu Asp Val Pro Trp Tyr Ala Ser Leu Pro Arg
485 490 495

Val Glu Ala Arg Phe Phe Ile Gln His Tyr Gly Gly Glu Asp Asp Val
500 505 510

Trp Leu Asp Lys Ala Leu Tyr Arg Met Pro Tyr Val Asn Asn Asn Val
515 520 525

Tyr Leu Glu Leu Ala Lys Leu Asp Tyr Asn Tyr Cys Gln Ala Leu His
530 535 540

Arg Thr Glu Trp Gly Arg Ile Gln Lys Trp Tyr Glu Glu Cys Lys Pro
545 550 555 560

Arg Asp Phe Gly Ile Ser Arg Glu Cys Leu Leu Arg Ala Tyr Phe Met
565 570 575

Ala Ala Ala Ser Ile Phe Glu Pro Glu Arg Ser Met Glu Arg Leu Ala
580 585 590

Trp Ala Lys Thr Ala Ile Leu Leu Glu Ile Ile Val Ser Tyr Phe Ser
595 600 605

Glu Val Gly Asn Ser Thr Glu Gln Arg Ile Ala Phe Thr Thr Glu Phe
610 615 620

Ser Ile Arg Ala Ser Pro Met Gly Gly Tyr Ile Asn Gly Arg Lys Leu
625 630 635 640

Asp Lys Ile Gly Thr Thr Gln Glu Leu Ile Gln Met Leu Leu Ala Thr
645 650 655

Ile Asp Gln Phe Ser Gln Asp Ala Phe Ala Ala Tyr Gly His Asp Ile
660 665 670

Thr Arg His Leu His Asn Ser Trp Lys Met Trp Leu Leu Lys Trp Gln
675 680 685

Glu Glu Gly Asp Arg Trp Leu Gly Glu Ala Glu Leu Leu Ile Gln Thr
690 695 700

Ile Asn Leu Met Ala Asp His Lys Ile Ala Glu Lys Leu Phe Met Gly
705 710 715 720

His Thr Asn Tyr Glu Gln Leu Phe Ser Leu Thr Asn Lys Val Cys Tyr
725 730 735

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Ser Leu Gly His His Glu Leu Gln Asn Asn Arg Glu Leu Glu His Asp
740 745 750

Met Gln Arg Leu Val Gln Leu Val Leu Thr Asn Ser Ser Asp Gly Ile
755 760 765

Asp Ser Asp Ile Lys Lys Thr Phe Leu Ala Val Ala Lys Arg Phe Tyr
770 775 780

Tyr Thr Ala Phe Val Asp Pro Glu Thr Val Asn Val His Ile Ala Lys
785 790 795 800

Val Leu Phe Glu Arg Val Asp
805

<210> SEQ_ID NO 70

<211> LENGTH: 589

<212> TYPE: PRT

<213> ORGANISM: Tripterygium wilfordii

<400> SEQUENCE: 70

Met Ala Pro Leu Val Val Ser Leu Thr Ile Ser His Phe Val Ile Gln
1 5 10 15

Thr Gly Ser Thr Ala Leu His Tyr Ser Ala Leu Pro Glu Thr Arg Thr
20 25 30

Lys His Cys His Ser Ser Arg Pro Phe Ala Ser Ile Asn Ser Asn Ser
35 40 45

Leu Gln Met Asn Gln Arg Pro Leu Thr Asp Tyr Arg Pro Ala Ile Trp
50 55 60

Asn Pro Glu Leu Ile Asp Ser Leu Asn Thr Pro Tyr Ser Tyr Gln Ser
65 70 75 80

His Gly Thr Gln Leu Asp Lys Leu Arg Gln Asp Ala Lys Arg Leu Leu
85 90 95

Ser Ser Thr Ser Asp Pro Cys Leu Leu Asn His Val Glu Ser Met
100 105 110

Gln Arg Leu Gly Ile Ala Tyr His Phe Gln Glu Glu Ile Asp Tyr Leu
115 120 125

Leu Asn Thr Arg Ile Gln Pro Tyr Ser Pro Asp Asp His Asp Leu His
130 135 140

Thr Thr Ala Leu Arg Phe Arg Ile Leu Arg Asp Asn Asn Phe Pro Ile
145 150 155 160

Ser Ser Asp Val Phe Gly Lys Phe Met Ser Arg Glu Gly Lys Phe Leu
165 170 175

Asp Ser Leu Ser Arg Asp Val Lys Gly Leu Leu Ser Leu Tyr Glu Ala
180 185 190

Ser Phe Leu Gly Val Asp Gly Glu Val Ile Leu Asp Glu Ala Lys Glu
195 200 205

Phe Ser Ser Lys Asn Leu Arg Ala Leu Leu Gly Arg Leu Glu Ser Thr
210 215 220

Ser Ile Asp Val Ala Glu Gln Val Lys Gln Ser Leu Gln Ile Pro Leu
225 230 235 240

Phe Trp Arg Met Pro Arg Val Glu Ala Arg Asn Phe Ile Asp Phe Tyr
245 250 255

Gln Lys Lys Asp Ala Lys Ser Ser Thr Leu Leu Glu Leu Ala Lys Leu
260 265 270

Asp Phe Asn Leu Val Gln Ser Thr Tyr Gln Gln Glu Leu Lys Glu Leu
275 280 285

-continued

Ser Lys Trp Trp Glu Asn Leu Gly Phe Lys Gln Lys Leu Ser Phe Thr
 290 295 300
 Arg Asp Arg Leu Met Gln Ser Tyr Phe Ser Thr Thr Gly Ile Thr Phe
 305 310 315 320
 Lys Pro Gln Phe Ser Lys Ala Arg Ile Ala Ala Thr Lys Phe Ile Asn
 325 330 335
 Ile Val Asn Thr Ile Asp Asp Ile His Asp Tyr Tyr Gly Ser Gln Asp
 340 345 350
 Asp Leu Lys Leu Phe Asp Ser Ala Val Lys Arg Trp Asp Leu Ala Ala
 355 360 365
 Met Glu Glu Leu Pro Asp Tyr Met Lys Ile Cys Tyr Phe Ala Met Tyr
 370 375 380
 Asn Leu Val Asn Glu Leu Ala Tyr Asp Val Leu Ile Asn Gln Gly Ile
 385 390 395 400
 Asp Val Leu Pro Cys Leu Arg Glu Ala Trp Thr Lys Phe Cys Gly Ala
 405 410 415
 Ala Phe Val Glu Ser Gln Trp Cys Tyr Thr Gly Tyr Thr Pro Ser Met
 420 425 430
 Asp Asp Tyr Leu Lys Asn Cys Trp Ile Ser Ile Gly Val His Gly Ser
 435 440 445
 Leu Asn Phe Ala Arg Ala His Gln Gln Gly Ser Arg Ser Pro Ile Ala
 450 455 460
 Asn Thr Pro Leu His Cys Leu Glu Asp Pro Leu Leu Tyr Trp Ser Ser
 465 470 475 480
 Val Ile Cys Arg Leu Asn Asn Asp Leu Ala Thr Phe Gln His Glu Ser
 485 490 495
 Lys Thr Gly Glu Val Val Ser Phe Val Lys Cys Tyr Met Val Glu Lys
 500 505 510
 Gly Val Ser Gln Glu Gln Ala Cys Asp Glu Ile Arg Glu Leu Ile Lys
 515 520 525
 His Ala Trp Lys Met Leu Asn Thr Glu Arg Arg Ser Asp Leu Pro
 530 535 540
 Pro Leu Met Val Glu Met Cys Met Asp Thr Pro Lys Leu Ser Gln Cys
 545 550 555 560
 Leu Tyr Gln His Gly Asp Gly Phe Gly Val Ala Ile Asp Leu Thr Lys
 565 570 575
 Asp Val Met Ser Ser Leu Ile Phe Arg Gln Ile Pro Ile
 580 585

<210> SEQ ID NO 71
 <211> LENGTH: 793
 <212> TYPE: PRT
 <213> ORGANISM: Salvia miltiorrhiza

<400> SEQUENCE: 71

Met Ala Ser Leu Ser Ser Thr Ile Leu Ser Arg Ser Pro Ala Ala Arg
 1 5 10 15
 Arg Arg Ile Thr Pro Ala Ser Ala Lys Leu His Arg Pro Glu Cys Phe
 20 25 30
 Ala Thr Ser Ala Trp Met Gly Ser Ser Ser Lys Asn Leu Ser Leu Ser
 35 40 45
 Tyr Gln Leu Asn His Lys Lys Ile Ser Val Ala Thr Val Asp Ala Pro

-continued

50	55	60
Gln Val His Asp His Asp Gly Thr Thr Val His Gln Gly His Asp Ala		
65	70	75
80		
Val Lys Asn Ile Glu Asp Pro Ile Glu Tyr Ile Arg Thr Leu Leu Arg		
85	90	95
Thr Thr Gly Asp Gly Arg Ile Ser Val Ser Pro Tyr Asp Thr Ala Trp		
100	105	110
Val Ala Met Ile Lys Asp Val Glu Gly Arg Asp Gly Pro Gln Phe Pro		
115	120	125
Ser Ser Leu Glu Trp Ile Val Gln Asn Gln Leu Glu Asp Gly Ser Trp		
130	135	140
Gly Asp Gln Lys Leu Phe Cys Val Tyr Asp Arg Leu Val Asn Thr Ile		
145	150	155
160		
Ala Cys Val Val Ala Leu Arg Ser Trp Asn Val His Ala His Lys Val		
165	170	175
Lys Arg Gly Val Thr Tyr Ile Lys Glu Asn Val Asp Lys Leu Met Glu		
180	185	190
Gly Asn Glu Glu His Met Thr Cys Gly Phe Glu Val Val Phe Pro Ala		
195	200	205
Leu Leu Gln Lys Ala Lys Ser Leu Gly Ile Glu Asp Leu Pro Tyr Asp		
210	215	220
Ser Pro Ala Val Gln Glu Val Tyr His Val Arg Glu Gln Lys Leu Lys		
225	230	235
240		
Arg Ile Pro Leu Glu Ile Met His Lys Ile Pro Thr Ser Leu Leu Phe		
245	250	255
Ser Leu Glu Gly Leu Glu Asn Leu Asp Trp Asp Lys Leu Leu Lys Leu		
260	265	270
Gln Ser Ala Asp Gly Ser Phe Leu Thr Ser Pro Ser Ser Thr Ala Phe		
275	280	285
Ala Phe Met Gln Thr Lys Asp Glu Lys Cys Tyr Gln Phe Ile Lys Asn		
290	295	300
Thr Ile Asp Thr Phe Asn Gly Gly Ala Pro His Thr Tyr Pro Val Asp		
305	310	315
320		
Val Phe Gly Arg Leu Trp Ala Ile Asp Arg Leu Gln Arg Leu Gly Ile		
325	330	335
Ser Arg Phe Phe Glu Pro Glu Ile Ala Asp Cys Leu Ser His Ile His		
340	345	350
Lys Phe Trp Thr Asp Lys Gly Val Phe Ser Gly Arg Glu Ser Glu Phe		
355	360	365
Cys Asp Ile Asp Asp Thr Ser Met Gly Met Arg Leu Met Arg Met His		
370	375	380
Gly Tyr Asp Val Asp Pro Asn Val Leu Arg Asn Phe Lys Gln Lys Asp		
385	390	395
400		
Gly Lys Phe Ser Cys Tyr Gly Gly Gln Met Ile Glu Ser Pro Ser Pro		
405	410	415
Ile Tyr Asn Leu Tyr Arg Ala Ser Gln Leu Arg Phe Pro Gly Glu Glu		
420	425	430
Ile Leu Glu Asp Ala Lys Arg Phe Ala Tyr Asp Phe Leu Lys Glu Lys		
435	440	445
Leu Ala Asn Asn Gln Ile Leu Asp Lys Trp Val Ile Ser Lys His Leu		
450	455	460

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Pro Asp Glu Ile Lys Leu Gly Leu Glu Met Pro Trp Leu Ala Thr Leu
 465 470 475 480
 Pro Arg Val Glu Ala Lys Tyr Tyr Ile Gln Tyr Tyr Ala Gly Ser Gly
 485 490 495
 Asp Val Trp Ile Gly Lys Thr Leu Tyr Arg Met Pro Glu Ile Ser Asn
 500 505 510
 Asp Thr Tyr His Asp Leu Ala Lys Thr Asp Phe Lys Arg Cys Gln Ala
 515 520 525
 Lys His Gln Phe Glu Trp Leu Tyr Met Gln Glu Trp Tyr Glu Ser Cys
 530 535 540
 Gly Ile Glu Glu Phe Gly Ile Ser Arg Lys Asp Leu Leu Leu Ser Tyr
 545 550 555 560
 Phe Leu Ala Thr Ala Ser Ile Phe Glu Leu Glu Arg Thr Asn Glu Arg
 565 570 575
 Ile Ala Trp Ala Lys Ser Gln Ile Ile Ala Lys Met Ile Thr Ser Phe
 580 585 590
 Phe Asn Lys Glu Thr Thr Ser Glu Glu Asp Lys Arg Ala Leu Leu Asn
 595 600 605
 Glu Leu Gly Asn Ile Asn Gly Leu Asn Asp Thr Asn Gly Ala Gly Arg
 610 615 620
 Glu Gly Gly Ala Gly Ser Ile Ala Leu Ala Thr Leu Thr Gln Phe Leu
 625 630 635 640
 Glu Gly Phe Asp Arg Tyr Thr Arg His Gln Leu Lys Asn Ala Trp Ser
 645 650 655
 Val Trp Leu Thr Gln Leu Gln His Gly Glu Ala Asp Asp Ala Glu Leu
 660 665 670
 Leu Thr Asn Thr Leu Asn Ile Cys Ala Gly His Ile Ala Phe Arg Glu
 675 680 685
 Glu Ile Leu Ala His Asn Glu Tyr Lys Ala Leu Ser Asn Leu Thr Ser
 690 695 700
 Lys Ile Cys Arg Gln Leu Ser Phe Ile Gln Ser Glu Lys Glu Met Gly
 705 710 715 720
 Val Glu Gly Glu Ile Ala Ala Lys Ser Ser Ile Lys Asn Lys Glu Leu
 725 730 735
 Glu Glu Asp Met Gln Met Leu Val Lys Leu Val Leu Glu Lys Tyr Gly
 740 745 750
 Gly Ile Asp Arg Asn Ile Lys Lys Ala Phe Leu Ala Val Ala Lys Thr
 755 760 765
 Tyr Tyr Tyr Arg Ala Tyr His Ala Ala Asp Thr Ile Asp Thr His Met
 770 775 780
 Phe Lys Val Leu Phe Glu Pro Val Ala
 785 790

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<210> SEQ ID NO 72
<211> LENGTH: 595
<212> TYPE: PRT
<213> ORGANISM: Salvia miltiorrhiza
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (221)..(221)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 72

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-continued

Met	Ser	Leu	Ala	Phe	Asn	Pro	Ala	Ala	Thr	Ala	Phe	Ser	Gly	Asn	Gly
1				5				10					15		
Ala	Arg	Ser	Arg	Arg	Glu	Asn	Phe	Pro	Val	Lys	His	Val	Thr	Val	Arg
	20				25							30			
Gly	Phe	Pro	Met	Ile	Thr	Asn	Lys	Ser	Ser	Phe	Ala	Val	Lys	Cys	Asn
	35					40						45			
Leu	Thr	Thr	Thr	Asp	Leu	Met	Gly	Lys	Ile	Ala	Glu	Lys	Phe	Lys	Gly
	50				55						60				
Glu	Asp	Ser	Asn	Phe	Pro	Ala	Ala	Ala	Val	Gln	Pro	Ala	Ala	Asp	
65					70				75			80			
Met	Pro	Ser	Asn	Leu	Cys	Ile	Ile	Asp	Thr	Leu	Gln	Arg	Leu	Gly	Val
	85					90						95			
Asp	Arg	Tyr	Phe	Arg	Ser	Glu	Ile	Asp	Thr	Ile	Leu	Glu	Asp	Thr	Tyr
	100					105						110			
Arg	Leu	Trp	Gln	Arg	Lys	Glu	Arg	Ala	Ile	Phe	Ser	Asp	Thr	Ala	Ile
	115					120						125			
His	Ala	Met	Ala	Phe	Arg	Leu	Leu	Arg	Val	Lys	Gly	Tyr	Glu	Val	Ser
	130					135					140				
Ser	Glu	Glu	Leu	Ala	Pro	Tyr	Ala	Asp	Gln	Glu	His	Val	Asp	Leu	Gln
145					150				155			160			
Thr	Ile	Glu	Val	Ala	Thr	Val	Ile	Glu	Leu	Tyr	Arg	Ala	Ala	Gln	Glu
	165					170					175				
Arg	Thr	Gly	Glu	Asp	Glu	Ser	Ser	Leu	Lys	Lys	Leu	His	Ala	Trp	Thr
	180					185					190				
Thr	Thr	Phe	Leu	Lys	Gln	Lys	Leu	Leu	Thr	Asn	Ser	Ile	Pro	Asp	Lys
	195					200						205			
Lys	Leu	His	Lys	Leu	Val	Glu	Tyr	Tyr	Leu	Lys	Asn	Xaa	His	Gly	Ile
	210					215					220				
Leu	Asp	Arg	Met	Gly	Val	Arg	Gln	Asn	Leu	Asp	Leu	Tyr	Asp	Ile	Ser
225					230				235			240			
Tyr	Tyr	Arg	Thr	Ser	Lys	Ala	Ala	Asn	Arg	Phe	Ser	Asn	Leu	Cys	Ser
	245					250					255				
Glu	Asp	Phe	Leu	Ala	Phe	Ala	Arg	Gln	Asp	Phe	Asn	Ile	Cys	Gln	Ala
	260					265					270				
Gln	His	Gln	Lys	Glu	Leu	Gln	Gln	Leu	Gln	Arg	Trp	Tyr	Ala	Asp	Cys
	275					280					285				
Lys	Leu	Asp	Thr	Leu	Lys	Tyr	Gly	Arg	Asp	Val	Val	Arg	Val	Ala	Asn
	290				295						300				
Phe	Leu	Thr	Ser	Ala	Ile	Ile	Gly	Asp	Pro	Glu	Leu	Ser	Asp	Val	Arg
305					310				315			320			
Ile	Val	Phe	Ala	Gln	His	Ile	Val	Leu	Val	Thr	Arg	Ile	Asp	Asp	Phe
	325					330					335				
Phe	Asp	His	Arg	Gly	Ser	Arg	Glu	Glu	Ser	Tyr	Lys	Ile	Glu	Leu	
	340					345					350				
Ile	Lys	Glu	Trp	Lys	Glu	Lys	Pro	Ala	Ala	Glu	Tyr	Gly	Ser	Glu	Glu
	355					360					365				
Val	Glu	Ile	Leu	Phe	Thr	Ala	Val	Tyr	Asn	Thr	Val	Asn	Glu	Leu	Ala
	370					375					380				
Glu	Arg	Ala	His	Val	Glu	Gln	Gly	Arg	Ser	Val	Lys	Asp	Phe	Leu	Ile
	385					390				395			400		
Lys	Leu	Trp	Val	Gln	Ile	Leu	Ser	Ile	Phe	Lys	Arg	Glu	Leu	Asp	Thr

-continued

405	410	415
Trp Ser Asp Asp Thr Ala Leu Thr Leu Asp Asp Tyr Leu Ser Ala Ser		
420	425	430
Trp Val Ser Ile Gly Cys Arg Ile Cys Ile Leu Met Ser Met Gln Phe		
435	440	445
Ile Gly Ile Lys Leu Ser Asp Glu Met Leu Leu Ser Glu Glu Cys Ile		
450	455	460
Asp Leu Cys Arg His Val Ser Met Val Asp Arg Leu Leu Asn Asp Val		
465	470	475
Gln Thr Phe Glu Lys Glu Arg Lys Glu Asn Thr Gly Asn Ser Val Thr		
485	490	495
Leu Leu Leu Ala Ala Asn Lys Asp Asp Ser Ser Phe Thr Glu Glu Glu		
500	505	510
Ala Ile Arg Ile Ala Lys Glu Met Ala Glu Cys Asn Arg Arg Gln Leu		
515	520	525
Met Gln Ile Val Tyr Lys Thr Gly Thr Ile Phe Pro Arg Gln Cys Lys		
530	535	540
Asp Met Phe Leu Lys Val Cys Arg Ile Gly Cys Tyr Leu Tyr Ala Ser		
545	550	555
Gly Asp Glu Phe Thr Ser Pro Gln Gln Met Met Glu Asp Met Lys Ser		
565	570	575
Leu Val Tyr Glu Pro Leu Thr Ile His Pro Leu Val Ala Asn Asn Val		
580	585	590
Arg Gly Lys		
595		

<210> SEQ ID NO 73
<211> LENGTH: 300
<212> TYPE: PRT
<213> ORGANISM: Synechococcus sp

<400> SEQUENCE: 73

Met Val Val Ala Asp Ala His Thr Gln Gly Phe Ser Leu Ala Gln Tyr			
1	5	10	15
Leu Gln Glu Gln Lys Thr Ile Val Glu Thr Ala Leu Asp Gln Ser Leu			
20	25	30	
Val Ile Thr Glu Pro Val Thr Ile Tyr Glu Ala Met Arg Tyr Ser Leu			
35	40	45	
Leu Ala Gly Gly Lys Arg Leu Arg Pro Ile Leu Cys Leu Ala Ala Cys			
50	55	60	
Glu Met Leu Gly Gly Thr Ala Ala Met Ala Met Asn Thr Ala Cys Ala			
65	70	75	80
Leu Glu Met Ile His Thr Met Ser Leu Ile His Asp Asp Leu Pro Ala			
85	90	95	
Met Asp Asn Asp Asp Leu Arg Arg Gly Lys Pro Thr Asn His Lys Val			
100	105	110	
Tyr Gly Glu Asp Ile Ala Ile Leu Ala Gly Asp Ala Leu Leu Ser Tyr			
115	120	125	
Ala Phe Glu Tyr Val Ala Arg Thr Pro Asp Val Pro Ala Glu Arg Leu			
130	135	140	
Leu Gln Val Ile Val Arg Leu Gly Gln Ala Val Gly Ala Glu Gly Leu			
145	150	155	160

-continued

Val	Gly	Gly	Gln	Val	Val	Asp	Leu	Glu	Ser	Glu	Gly	Lys	Thr	Asp	Val
165							170					175			
Ala	Val	Glu	Thr	Leu	Asn	Phe	Ile	His	Thr	His	Lys	Thr	Gly	Ala	Leu
180							185				190				
Leu	Glu	Val	Cys	Val	Thr	Ala	Gly	Ala	Ile	Leu	Ala	Gly	Ala	Lys	Pro
195							200				205				
Glu	Glu	Val	Gln	Leu	Leu	Ser	Arg	Tyr	Ala	Gln	Asn	Ile	Gly	Leu	Ala
210							215				220				
Phe	Gln	Ile	Val	Asp	Asp	Ile	Leu	Asp	Ile	Thr	Ala	Thr	Ala	Glu	Glu
225							230				235			240	
Leu	Gly	Lys	Thr	Ala	Gly	Lys	Asp	Leu	Glu	Ala	Gln	Lys	Val	Thr	Tyr
245							250				255			255	
Pro	Ser	Leu	Trp	Gly	Ile	Glu	Lys	Ser	Gln	Ala	Glu	Ala	Gln	Lys	Leu
260							265				270				
Val	Ala	Glu	Ala	Ile	Ala	Ser	Leu	Glu	Pro	Tyr	Gly	Glu	Lys	Ala	Asn
275							280				285				
Pro	Leu	Lys	Ala	Leu	Ala	Glu	Tyr	Ile	Val	Asn	Arg				
290							295				300				

<210> SEQ_ID NO 74
<211> LENGTH: 533
<212> TYPE: PRT
<213> ORGANISM: Tripterygium wilfordii
<400> SEQUENCE: 74

Met	Glu	Phe	Leu	Leu	Ser	Leu	Pro	Thr	Asn	Thr	Ile	Ala	Pro	Lys	Ile	
1							5				10			15		
Phe	Ala	Val	Leu	Leu	Leu	Phe	Ile	Cys	Leu	Arg	Ile	Leu	Thr	Asn	Val	
							20				25			30		
Leu	Lys	Pro	Lys	Lys	Ser	Lys	Thr	Ser	Pro	Pro	Gln	Ala	Ser	Gly	Ala	
							35				40			45		
Trp	Pro	Leu	Ile	Gly	His	Leu	Leu	His	Leu	Arg	Gly	Pro	Gln	Ala	Pro	
							50				55			60		
His	Ile	Thr	Leu	Gly	Lys	Met	Ala	Asp	Lys	Tyr	Gly	Pro	Ile	Phe	Lys	
							65				70			75		80
Ile	Lys	Leu	Gly	Val	His	Pro	Thr	Leu	Val	Ile	Ser	Asp	Ser	Glu	Val	
							85				90			95		
Ala	Lys	Glu	Cys	Leu	Thr	Thr	His	Asp	Ile	Ala	Leu	Ala	Gly	Arg	Pro	
							100				105			110		
Ala	Thr	Val	Ala	Met	Glu	Ile	Met	Gly	Tyr	Asn	His	Ala	Met	Phe	Ala	
							115				120			125		
Phe	Ser	Pro	Tyr	Gly	Pro	Tyr	Trp	Arg	His	Met	Arg	Lys	Leu	Ala	Thr	
							130				135			140		
Val	Glu	Leu	Leu	Ser	Ala	Gln	Arg	Leu	Glu	Thr	Phe	Lys	His	Ile	Arg	
							145				150			155		160
Glu	Ser	Glu	Leu	Lys	Arg	Ser	Met	Lys	Glu	Met	Tyr	Gln	Ser	Trp	Val	
							165				170			175		
His	Asn	Lys	Ser	Gly	Ser	Gly	Asp	Ser	Asn	His	Val	Thr	Val	Asp	Met	
							180				185			190		
Thr	Arg	Ile	Leu	Gly	Asp	Ile	Ile	Ala	Asn	Val	Ile	Tyr	Arg	Met	Val	
							195				200			205		
Val	Gly	Lys	Val	Tyr	Ala	Ser	Lys	Gly	Glu	Glu	Asp	Ala	Arg	Trp	Lys	
							210				215			220		

-continued

Gln Val Val Trp Glu Tyr Ile Lys Leu Leu Ser His Phe Gly Val Gly
225 230 235 240

Asp Ala Leu Pro Phe Leu Arg Trp Leu Asp Leu Gly Gly Val Glu Lys
245 250 255

Ser Met Lys Lys Ala Ala Lys Glu Leu Asp Ile Tyr Val Glu Glu Trp
260 265 270

Leu Glu Glu His Lys Lys Arg Ser Glu Arg Lys Ser Asp Asn Gly
275 280 285

Ile Val Glu Glu Asp Phe Met Asp Val Met Leu Ser Val Phe Asp Asp
290 295 300

Asp Asp Gln Leu Glu Asn Phe Ala His His Ser Ala His Thr Ile Asn
305 310 315 320

Lys Ala Met Cys Leu Ala Ile Ile Leu Ala Ala Ser Asp Thr Thr Lys
325 330 335

Thr Thr Leu Thr Trp Ala Leu Ser Leu Leu Leu Asn His Pro Asp Val
340 345 350

Met Lys Lys Val Gln Gln Glu Leu Ala Ala His Ile Gly Pro Asp Lys
355 360 365

Pro Val Lys Glu Ser Asp Val Lys Ser Leu Val Tyr Leu Glu Ala Val
370 375 380

Val Lys Glu Thr Leu Arg Leu Tyr Pro Pro Gly Pro Leu Gly Leu Pro
385 390 395 400

His Glu Ser Met Glu Asp Cys Thr Val Ala Gly Tyr His Val Pro Ser
405 410 415

Gly Thr Arg Ile Leu Tyr Asn Leu Trp Lys Ile Gln Gln Asp Pro Gln
420 425 430

Val Trp Glu Asn Pro Ser Glu Phe Lys Pro Asp Arg Phe Leu Thr Thr
435 440 445

His Lys Asp Val Asp Val Arg Gly Arg Asn Phe Glu Tyr Leu Pro Phe
450 455 460

Gly Ser Gly Arg Arg Met Cys Pro Gly Met Ser Phe Ala Leu Gln Val
465 470 475 480

Met Glu Val Ser Leu Ala Asn Met Leu His Gly Phe Asp Phe Ala Thr
485 490 495

Pro Asn Gly Lys Pro Val Asp Met Thr Glu Val Asn Gly Leu Val Thr
500 505 510

Asp Arg Ala Thr Pro Leu Glu Ala Leu Ile Thr Pro Arg Leu Pro Ala
515 520 525

His Leu Tyr Met Gly
530

<210> SEQ ID NO 75
<211> LENGTH: 533
<212> TYPE: PRT
<213> ORGANISM: Tripterygium wilfordii

<400> SEQUENCE: 75

Met Glu Phe Leu Leu Ser Leu Pro Thr Asn Thr Ile Ala Thr Thr Ser
1 5 10 15

Phe Ala Val Leu Leu Leu Tyr Leu Cys Leu Arg Ile Phe Thr Asn Val
20 25 30

Leu Lys Pro Lys Lys Ser Lys Thr Ser Pro Pro Gln Ala Gly Gly Ala

-continued

35	40	45
Trp Pro Leu Ile Gly His	Leu His Leu Leu Ile	Gly Pro Gln Ala Ser
50	55	60
Tyr Ile Thr Leu Ser Lys	Met Ala Asp Lys Tyr	Gly Pro Ile Phe Lys
65	70	75
Ile Lys Leu Gly Val His	Pro Thr Leu Val Ile	Ser Asn Ser Glu Val
85	90	95
Ala Lys Glu Cys Leu Thr Thr His	Asp Lys Val Leu Ala	Asn Arg Pro
100	105	110
Ala Thr Val Ala Met Glu	Ile Met Gly Tyr Asn His	Ala Met Phe Gly
115	120	125
Trp Ser Pro Tyr Gly Pro	Tyr Trp Arg His Met Arg	Lys Leu Ala Thr
130	135	140
Val Glu Leu Leu Ser Ala	Gln Arg Leu Glu Thr	Phe Lys His Ile Arg
145	150	155
Glu Ser Glu Leu Lys Arg Ser Met Lys	Glu Met Tyr Gln Ser	Trp Val
165	170	175
His Asn Lys Ser Gly Ser Gly Asp	Ser Asn His Val Thr	Val Asp Met
180	185	190
Thr Arg Ile Leu Gly Asp Ile	Ile Ala Asn Val Ile	Tyr Arg Met Val
195	200	205
Val Gly Lys Val Tyr Ala Ser Lys	Gly Glu Glu Asp Ala	Arg Trp Lys
210	215	220
Gln Val Val Trp Glu Tyr	Ile Lys Leu Leu Ser His	Phe Gly Val Gly
225	230	235
Asp Ala Leu Pro Phe Leu Arg	Trp Leu Asp Leu Gly	Gly Val Glu Lys
245	250	255
Ser Met Lys Ala Ala Lys Glu	Leu Asp Ile Tyr Val	Glu Glu Trp
260	265	270
Leu Glu Glu His Lys Lys	Arg Ser Glu Arg Lys	Ser Asp Asn Gly
275	280	285
Ile Val Glu Glu Asp Phe	Met Asp Val Met Leu	Ser Val Phe Asp Asp
290	295	300
Asp Asp Gln Leu Glu Asn	Phe Ala His His Ser	Ala His Thr Ile Asn
305	310	315
Lys Ala Met Cys Leu Ala	Ile Ile Leu Ala Ala	Ser Asp Thr Thr Lys
325	330	335
Thr Thr Leu Thr Trp Ala	Leu Ser Leu Leu Leu	Asn His Pro Asp Val
340	345	350
Met Lys Lys Val Gln Gln	Glu Leu Ala Ala His	Ile Gly Pro Asp Lys
355	360	365
Pro Val Lys Glu Ser Asp	Val Lys Ser Leu Val	Tyr Leu Glu Ala Val
370	375	380
Val Lys Glu Thr Leu Arg	Leu Tyr Pro Pro Gly	Pro Leu Gly Leu Pro
385	390	395
His Glu Ser Met Glu Asp	Cys Thr Val Ala	Gly Tyr His Val Pro Ser
405	410	415
Gly Thr Arg Ile Leu Tyr	Asn Leu Trp Lys Ile	Gln Gln Asp Pro Gln
420	425	430
Val Trp Glu Asn Pro Ser	Glu Phe Lys Pro Asp	Arg Phe Leu Thr Thr
435	440	445

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His Lys Asp Val Asp Val Arg Gly Arg Asn Phe Glu Tyr Leu Pro Phe
450          455          460

Gly Ser Gly Arg Arg Met Cys Pro Gly Met Ser Phe Ala Leu Gln Val
465          470          475          480

Met Glu Val Ser Leu Ala Asn Met Leu His Gly Phe Asp Phe Ala Thr
485          490          495

Pro Asn Gly Lys Pro Val Asp Met Thr Glu Val Asn Gly Leu Val Thr
500          505          510

Asp Arg Ala Thr Pro Leu Glu Ala Leu Ile Thr Pro Arg Leu Pro Ala
515          520          525

His Leu Tyr Met Gly
530

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<210> SEQ ID NO 76
<211> LENGTH: 530
<212> TYPE: PRT
<213> ORGANISM: Tripterygium wilfordii

<400> SEQUENCE: 76

Met Glu Phe Leu Leu Ser Leu Pro Thr Asn Thr Ile Ala Thr Lys Ile
1           5           10          15

Phe Ala Val Leu Leu Leu Tyr Leu Phe Leu Arg Ile Phe Thr Asn Val
20          25          30

Leu Lys Pro Lys Lys Ser Lys Thr Ser Pro Pro Gln Ala Gly Gly Ala
35          40          45

Trp Pro Leu Ile Gly His Leu His Leu Ile Gly Pro Gln Ala Ser
50          55          60

Tyr Ile Thr Leu Ser Lys Met Ala Asp Lys Tyr Gly Pro Ile Phe Lys
65          70          75          80

Ile Lys Leu Gly Val His Pro Thr Leu Val Ile Ser Asn Ser Glu Val
85          90          95

Ala Lys Glu Cys Leu Thr Thr His Asp Lys Val Leu Ala Asn Arg Pro
100         105         110

Ala Thr Val Ala Met Glu Ile Met Gly Tyr Asn His Ala Met Phe Gly
115         120         125

Trp Ser Pro Tyr Gly Pro Tyr Trp Arg Gln Leu Arg Lys Leu Val Thr
130         135         140

Val Glu Leu Leu Ser Asn Gln Arg Leu Lys Thr Phe Lys His Ile Arg
145         150         155         160

Glu Ser Glu Val Lys Asn Ser Leu Lys Glu Met Tyr Gln Ser Trp Val
165         170         175

His Asn Lys Ser Gly Asp Ser Asn His Val Ser Val Asp Met Thr Arg
180         185         190

Ile Phe Gly Asp Ile Thr Gly Asn Leu Ile Tyr Arg Ile Val Val Gly
195         200         205

Lys Val Tyr Ala Arg Lys Gly Glu Gly Val Val Arg Trp Lys Gln Val
210         215         220

Val Gly Asp Tyr Met Lys Leu Leu Thr His Phe Asn Val Gly Asp Ala
225         230         235         240

Met Pro Phe Met Arg Trp Phe Asp Leu Gly Gly Leu Glu Lys Ala Met
245         250         255

Lys Ile Thr Phe Lys Glu Leu Asp Gly Tyr Val Glu Glu Trp Leu Glu

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260	265	270	
Glu His Lys Lys Lys Arg Ser Asn Ser Gly Gly His Gly Ile Val Glu			
275	280	285	
Glu Asp Phe Met Asp Val Met Leu Ser Ile Phe Asp Asp Gly Gly Gln			
290	295	300	
Gln Glu Tyr Cys Thr Asp Asn Ser Thr His Thr Thr Asn Lys Ala Met			
305	310	315	320
Cys Met Ala Leu Ile Leu Gly Ala Ser Glu Thr Thr Lys Thr Thr Leu			
325	330	335	
Thr Trp Ser Leu Ser Leu Leu Asn Asn Leu Asp Val Leu Lys Lys			
340	345	350	
Val Lys Gln Glu Leu Ala Ala His Ile Gly Pro Glu Thr Leu Val Thr			
355	360	365	
Glu Ser Asp Val Asn Ser Leu Val Tyr Leu Asp Ala Val Ile Thr Glu			
370	375	380	
Thr Leu Arg Leu Tyr Pro Leu Gly Pro Leu Gly Leu Pro His Glu Ser			
385	390	395	400
Ile Glu Asp Cys Thr Ile Ala Gly Tyr His Val Pro Ala Arg Thr Arg			
405	410	415	
Ile Leu Phe Asn Leu Trp Lys Ile His Gln Asp Pro Arg Val Trp Glu			
420	425	430	
Asn Pro Leu Glu Phe Lys Pro Glu Arg Phe Leu Lys Glu His Asn Asn			
435	440	445	
Ile Asp Val Arg Gly Gly His Phe Glu Leu Leu Pro Phe Gly Ser Gly			
450	455	460	
Arg Arg Met Cys Pro Gly Val Ser Phe Ala Leu Gln Val Leu Lys Leu			
465	470	475	480
Ile Leu Ala Asn Met Leu His Gly Phe Asp Phe Ala Thr Pro Asn Asp			
485	490	495	
Glu Pro Val Asp Met Thr Glu Val Asn His Met Ala Thr Thr Arg Ala			
500	505	510	
Thr Pro Leu Glu Thr Leu Ile Ser Pro Arg Leu Pro Ser His Leu Tyr			
515	520	525	
Met Gly			
530			

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<210> SEQ ID NO 77
<211> LENGTH: 749
<212> TYPE: PRT
<213> ORGANISM: Plectranthus barbatus

<400> SEQUENCE: 77

Met Ala Trp Met Asn Asn Gly Lys Asn Leu Asn Cys Gln Leu Thr His
1          5           10          15

Lys Lys Ile Ser Lys Val Ala Glu Ile Arg Val Ala Thr Val Asn Ala
20         25           30

Pro Pro Val His Asp Gln Asp Asp Ser Thr Glu Asn Gln Cys His Asp
35         40           45

Ala Val Asn Asn Ile Glu Asp Pro Ile Glu Tyr Ile Arg Thr Leu Leu
50         55           60

Arg Thr Thr Gly Asp Gly Arg Ile Ser Val Ser Pro Tyr Asp Thr Ala
65         70           75           80

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Trp	Val	Ala	Leu	Ile	Lys	Asp	Leu	Gln	Gly	Arg	Asp	Ala	Pro	Glu	Phe
85							90							95	
Pro	Ser	Ser	Leu	Glu	Trp	Ile	Ile	Gln	Asn	Gln	Leu	Ala	Asp	Gly	Ser
100							105							110	
Trp	Gly	Asp	Ala	Lys	Phe	Phe	Cys	Val	Tyr	Asp	Arg	Leu	Val	Asn	Thr
115							120							125	
Ile	Ala	Cys	Val	Val	Ala	Leu	Arg	Ser	Trp	Asp	Val	His	Ala	Glu	Lys
130							135							140	
Val	Glu	Arg	Gly	Val	Arg	Tyr	Ile	Asn	Glu	Asn	Val	Glu	Lys	Leu	Arg
145							150							160	
Asp	Gly	Asn	Glu	Glu	His	Met	Thr	Cys	Gly	Phe	Glu	Val	Val	Phe	Pro
165							170							175	
Ala	Leu	Leu	Gln	Arg	Ala	Lys	Ser	Leu	Gly	Ile	Gln	Asp	Leu	Pro	Tyr
180							185							190	
Asp	Ala	Pro	Val	Ile	Gln	Glu	Ile	Tyr	His	Ser	Arg	Glu	Gln	Lys	Ser
195							200							205	
Lys	Arg	Ile	Pro	Leu	Glu	Met	Met	His	Lys	Val	Pro	Thr	Ser	Leu	Leu
210							215							220	
Phe	Ser	Leu	Glu	Gly	Leu	Glu	Asn	Leu	Glu	Trp	Asp	Lys	Leu	Leu	Lys
225							230							240	
Leu	Gln	Ser	Ala	Asp	Gly	Ser	Phe	Leu	Thr	Ser	Pro	Ser	Ser	Thr	Ala
245							250							255	
Phe	Ala	Phe	Met	Gln	Thr	Arg	Asp	Pro	Lys	Cys	Tyr	Gln	Phe	Ile	Lys
260							265							270	
Asn	Thr	Ile	Gln	Thr	Phe	Asn	Gly	Gly	Ala	Pro	His	Thr	Tyr	Pro	Val
275							280							285	
Asp	Val	Phe	Gly	Arg	Leu	Trp	Ala	Ile	Asp	Arg	Leu	Gln	Arg	Leu	Gly
290							295							300	
Ile	Ser	Arg	Phe	Phe	Glu	Ser	Glu	Ile	Ala	Asp	Cys	Ile	Ala	His	Ile
305							310							320	
His	Arg	Phe	Trp	Thr	Glu	Lys	Gly	Val	Phe	Ser	Gly	Arg	Glu	Ser	Glu
325							330							335	
Phe	Cys	Asp	Ile	Asp	Asp	Thr	Ser	Met	Gly	Val	Arg	Leu	Met	Arg	Met
340							345							350	
His	Gly	Tyr	Asp	Val	Asp	Pro	Asn	Val	Leu	Lys	Asn	Phe	Lys	Lys	Asp
355							360							365	
Asp	Lys	Phe	Ser	Cys	Tyr	Gly	Gly	Gln	Met	Ile	Glu	Ser	Pro	Ser	Pro
370							375							380	
Ile	Tyr	Asn	Leu	Tyr	Arg	Ala	Ser	Gln	Leu	Arg	Phe	Pro	Gly	Glu	Gln
385							390							400	
Ile	Leu	Glu	Asp	Ala	Asn	Lys	Phe	Ala	Tyr	Asp	Phe	Leu	Gln	Glu	Lys
405							410							415	
Leu	Ala	His	Asn	Gln	Ile	Leu	Asp	Lys	Trp	Val	Ile	Ser	Lys	His	Leu
420							425							430	
Pro	Asp	Glu	Ile	Lys	Leu	Gly	Leu	Glu	Met	Pro	Trp	Tyr	Ala	Thr	Leu
435							440							445	
Pro	Arg	Val	Glu	Ala	Arg	Tyr	Tyr	Ile	Gln	Tyr	Tyr	Ala	Gly	Ser	Gly
450							455							460	
Asp	Val	Trp	Ile	Gly	Lys	Thr	Leu	Tyr	Arg	Met	Pro	Glu	Ile	Ser	Asn
465							470							475	
Asp	Thr	Tyr	His	Glu	Leu	Ala	Lys	Thr	Asp	Phe	Lys	Arg	Cys	Gln	Ala

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485	490	495
Gln His Gln Phe Glu Trp Ile Tyr Met Gln Glu Trp Tyr Glu Ser Cys		
500	505	510
Asn Met Glu Glu Phe Gly Ile Ser Arg Lys Glu Leu Leu Val Ala Tyr		
515	520	525
Phe Leu Ala Thr Ala Ser Ile Phe Glu Leu Glu Arg Ala Asn Glu Arg		
530	535	540
Ile Ala Trp Ala Lys Ser Gln Ile Ile Ser Thr Ile Ile Ala Ser Phe		
545	550	555
Phe Asn Asn Gln Asn Thr Ser Pro Glu Asp Lys Leu Ala Phe Leu Thr		
565	570	575
Asp Phe Lys Asn Gly Asn Ser Thr Asn Met Ala Leu Val Thr Leu Thr		
580	585	590
Gln Phe Leu Glu Gly Phe Asp Arg Tyr Thr Ser His Gln Leu Lys Asn		
595	600	605
Ala Trp Ser Val Trp Leu Arg Lys Leu Gln Gln Gly Glu Gly Asn Gly		
610	615	620
Gly Ala Asp Ala Glu Leu Leu Val Asn Thr Leu Asn Ile Cys Ala Gly		
625	630	635
His Ile Ala Phe Arg Glu Glu Ile Leu Ala His Asn Asp Tyr Lys Thr		
645	650	655
Leu Ser Asn Leu Thr Ser Lys Ile Cys Arg Gln Leu Ser Gln Ile Gln		
660	665	670
Asn Glu Lys Glu Leu Glu Thr Glu Gly Gln Lys Thr Ser Ile Lys Asn		
675	680	685
Lys Glu Leu Glu Glu Asp Met Gln Arg Leu Val Lys Leu Val Leu Glu		
690	695	700
Lys Ser Arg Val Gly Ile Asn Arg Asp Met Lys Lys Thr Phe Leu Ala		
705	710	715
720		
Val Val Lys Thr Tyr Tyr Lys Ala Tyr His Ser Ala Gln Ala Ile		
725	730	735
Asp Asn His Met Phe Lys Val Leu Phe Glu Pro Val Ala		
740	745	

<210> SEQ ID NO 78

<211> LENGTH: 567

<212> TYPE: PRT

<213> ORGANISM: Plectranthus barbatus

<400> SEQUENCE: 78

Met Ile Thr Ser Lys Ser Ser Ala Ala Val Lys Cys Ser Leu Thr Thr		
1	5	10
15		
Pro Thr Asp Leu Met Gly Lys Ile Lys Glu Val Phe Asn Arg Glu Val		
20	25	30
Asp Thr Ser Pro Ala Ala Met Thr Thr His Ser Thr Asp Ile Pro Ser		
35	40	45
Asn Leu Cys Ile Ile Asp Thr Leu Gln Arg Leu Gly Ile Asp Gln Tyr		
50	55	60
Phe Gln Ser Glu Ile Asp Ala Val Leu His Asp Thr Tyr Arg Leu Trp		
65	70	75
80		
Gln Leu Lys Lys Asp Ile Phe Ser Asp Ile Thr Thr His Ala Met		
85	90	95

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Ala	Phe	Arg	Leu	Leu	Arg	Val	Lys	Gly	Tyr	Glu	Val	Ala	Ser	Asp	Glu
100							105					110			
<hr/>															
Leu	Ala	Pro	Tyr	Ala	Asp	Gln	Glu	Arg	Ile	Asn	Leu	Gln	Thr	Ile	Asp
115							120				125				
<hr/>															
Val	Pro	Thr	Val	Val	Glu	Leu	Tyr	Arg	Ala	Ala	Gln	Glu	Arg	Leu	Thr
130						135				140					
<hr/>															
Glu	Glu	Asp	Ser	Thr	Leu	Glu	Lys	Leu	Tyr	Val	Trp	Thr	Ser	Ala	Phe
145						150				155				160	
<hr/>															
Leu	Lys	Gln	Gln	Leu	Leu	Thr	Asp	Ala	Ile	Pro	Asp	Lys	Lys	Leu	His
165						170				175					
<hr/>															
Lys	Gln	Val	Glu	Tyr	Tyr	Leu	Lys	Asn	Tyr	His	Gly	Ile	Leu	Asp	Arg
180						185				190					
<hr/>															
Met	Gly	Val	Arg	Arg	Asn	Leu	Asp	Leu	Tyr	Asp	Ile	Ser	His	Tyr	Lys
195						200				205					
<hr/>															
Ser	Leu	Lys	Ala	Ala	His	Arg	Phe	Tyr	Asn	Leu	Ser	Asn	Glu	Asp	Ile
210						215				220					
<hr/>															
Leu	Ala	Phe	Ala	Arg	Gln	Asp	Phe	Asn	Ile	Ser	Gln	Ala	Gln	His	Gln
225						230				235				240	
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Lys	Glu	Leu	Gln	Gln	Leu	Gln	Arg	Trp	Tyr	Ala	Asp	Cys	Arg	Leu	Asp
245						250				255					
<hr/>															
Thr	Leu	Lys	Phe	Gly	Arg	Asp	Val	Val	Arg	Ile	Gly	Asn	Phe	Leu	Thr
260						265				270					
<hr/>															
Ser	Ala	Met	Ile	Gly	Asp	Pro	Glu	Leu	Ser	Asp	Leu	Arg	Leu	Ala	Phe
275						280				285					
<hr/>															
Ala	Lys	His	Ile	Val	Leu	Val	Thr	Arg	Ile	Asp	Asp	Phe	Phe	Asp	His
290						295				300					
<hr/>															
Gly	Gly	Pro	Lys	Glu	Glu	Ser	Tyr	Glu	Ile	Leu	Glu	Leu	Val	Lys	Glu
305						310				315				320	
<hr/>															
Trp	Lys	Glu	Lys	Pro	Ala	Gly	Glu	Tyr	Val	Ser	Glu	Glu	Val	Glu	Ile
325						330				335					
<hr/>															
Leu	Phe	Thr	Ala	Val	Tyr	Asn	Thr	Val	Asn	Glu	Leu	Ala	Glu	Met	Ala
340						345				350					
<hr/>															
His	Ile	Glu	Gln	Gly	Arg	Ser	Val	Lys	Asp	Leu	Leu	Val	Lys	Leu	Trp
355						360				365					
<hr/>															
Val	Glu	Ile	Leu	Ser	Val	Phe	Arg	Ile	Glu	Leu	Asp	Thr	Trp	Thr	Asn
370						375				380					
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Asp	Thr	Ala	Leu	Thr	Leu	Glu	Glu	Tyr	Leu	Ser	Gln	Ser	Trp	Val	Ser
385						390				395				400	
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Ile	Gly	Cys	Arg	Ile	Cys	Ile	Leu	Ile	Ser	Met	Gln	Phe	Gln	Gly	Val
405						410				415					
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Lys	Leu	Ser	Asp	Glu	Met	Leu	Gln	Ser	Glu	Glu	Cys	Thr	Asp	Leu	Cys
420						425				430					
<hr/>															
Arg	Tyr	Val	Ser	Met	Val	Asp	Arg	Leu	Leu	Asn	Asp	Val	Gln	Thr	Phe
435						440				445					
<hr/>															
Glu	Lys	Glu	Arg	Lys	Glu	Asn	Thr	Gly	Asn	Ser	Val	Ser	Leu	Leu	Gln
450						455				460					
<hr/>															
Ala	Ala	His	Lys	Asp	Glu	Arg	Val	Ile	Asn	Glu	Glu	Ala	Cys	Ile	
465						470				475				480	
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Lys	Val	Lys	Glu	Leu	Ala	Glu	Tyr	Asn	Arg	Arg	Lys	Leu	Met	Gln	Ile
485						490				495					
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Val	Tyr	Lys	Thr	Gly	Thr	Ile	Phe	Pro	Arg	Lys	Cys	Lys	Asp	Leu	Phe

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500	505	510
Leu Lys Ala Cys Arg Ile Gly Cys Tyr Leu Tyr Ser Ser Gly Asp Glu		
515	520	525
Phe Thr Ser Pro Gln Gln Met Met Glu Asp Met Lys Ser Leu Val Tyr		
530	535	540
Glu Pro Leu Pro Ile Ser Pro Pro Glu Ala Asn Asn Ala Ser Gly Glu		
545	550	555
Lys Met Ser Cys Val Ser Asn		
565		
 <210> SEQ ID NO 79		
<211> LENGTH: 722		
<212> TYPE: PRT		
<213> ORGANISM: Plectranthus barbatus		
 <400> SEQUENCE: 79		
Met Ala Ser Cys Gly Ala Ile Gly Ser Ser Phe Leu Pro Leu Leu His		
1	5	10
Ser Asp Glu Ser Ser Leu Leu Ser Arg Pro Thr Ala Ala Leu His Ile		
20	25	30
Lys Lys Gln Lys Phe Ser Val Gly Ala Ala Leu Tyr Gln Asp Asn Thr		
35	40	45
Asn Asp Val Val Pro Ser Gly Glu Gly Leu Thr Arg Gln Lys Pro Arg		
50	55	60
Thr Leu Ser Phe Thr Gly Glu Lys Pro Ser Thr Pro Ile Leu Asp Thr		
65	70	75
Ile Asn Tyr Pro Ile His Met Lys Asn Leu Ser Val Glu Glu Leu Glu		
85	90	95
Ile Leu Ala Asp Glu Leu Arg Glu Glu Ile Val Tyr Thr Val Ser Lys		
100	105	110
Thr Gly Gly His Leu Ser Ser Ser Leu Gly Val Ser Glu Leu Thr Val		
115	120	125
Ala Leu His His Val Phe Asn Thr Pro Asp Asp Lys Ile Ile Trp Asp		
130	135	140
Val Gly His Gln Ala Tyr Pro His Lys Ile Leu Thr Gly Arg Arg Ser		
145	150	155
Arg Met His Thr Ile Arg Gln Thr Phe Gly Leu Ala Gly Phe Pro Lys		
165	170	175
Arg Asp Glu Ser Pro His Asp Ala Phe Gly Ala Gly His Ser Ser Thr		
180	185	190
Ser Ile Ser Ala Gly Leu Gly Met Ala Val Gly Arg Asp Leu Leu Gln		
195	200	205
Lys Asn Asn His Val Ile Ser Val Ile Gly Asp Gly Ala Met Thr Ala		
210	215	220
Gly Gln Ala Tyr Glu Ala Met Asn Asn Ala Gly Phe Leu Asp Ser Asn		
225	230	235
Leu Ile Ile Val Leu Asn Asp Asn Lys Gln Val Ser Leu Pro Thr Ala		
245	250	255
Thr Val Asp Gly Pro Ala Pro Pro Val Gly Ala Leu Ser Lys Ala Leu		
260	265	270
Thr Lys Leu Gln Ala Ser Arg Lys Phe Arg Gln Leu Arg Glu Ala Ala		
275	280	285

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Lys	Gly	Met	Thr	Lys	Gln	Met	Gly	Asn	Gln	Ala	His	Glu	Ile	Ala	Ser
290				295						300					
<hr/>															
Lys	Val	Asp	Thr	Tyr	Val	Lys	Gly	Met	Met	Gly	Lys	Pro	Gly	Ala	Ser
305				310				315							320
<hr/>															
Leu	Phe	Glu	Glu	Leu	Gly	Ile	Tyr	Tyr	Ile	Gly	Pro	Val	Asp	Gly	His
						325			330						335
<hr/>															
Asn	Ile	Glu	Asp	Leu	Val	Tyr	Ile	Phe	Lys	Lys	Val	Lys	Glu	Met	Pro
						340			345						350
<hr/>															
Ala	Pro	Gly	Pro	Val	Leu	Ile	His	Ile	Ile	Thr	Glu	Lys	Gly	Lys	Gly
						355			360						365
<hr/>															
Tyr	Pro	Pro	Ala	Glu	Val	Ala	Ala	Asp	Lys	Met	His	Gly	Val	Val	Lys
						370			375						380
<hr/>															
Phe	Asp	Pro	Thr	Thr	Gly	Lys	Gln	Met	Lys	Val	Lys	Thr	Lys	Thr	Gln
						385			390						400
<hr/>															
Ser	Tyr	Thr	Gln	Tyr	Phe	Ala	Glu	Ser	Leu	Val	Ala	Glu	Ala	Glu	Gln
						405			410						415
<hr/>															
Asp	Glu	Lys	Val	Val	Ala	Ile	His	Ala	Ala	Met	Gly	Gly	Thr	Gly	
						420			425						430
<hr/>															
Leu	Asn	Ile	Phe	Gln	Lys	Arg	Phe	Pro	Asp	Arg	Cys	Phe	Asp	Val	Gly
						435			440						445
<hr/>															
Ile	Ala	Glu	Gln	His	Ala	Val	Thr	Phe	Ala	Ala	Gly	Leu	Ala	Thr	Glu
						450			455						460
<hr/>															
Gly	Leu	Lys	Pro	Phe	Cys	Thr	Ile	Tyr	Ser	Ser	Phe	Leu	Gln	Arg	Gly
						465			470						480
<hr/>															
Tyr	Asp	Gln	Val	Val	His	Asp	Val	Asp	Leu	Gln	Lys	Leu	Pro	Val	Arg
						485			490						495
<hr/>															
Phe	Met	Met	Asp	Arg	Ala	Gly	Leu	Val	Gly	Ala	Asp	Gly	Pro	Thr	His
						500			505						510
<hr/>															
Cys	Gly	Ala	Phe	Asp	Thr	Thr	Tyr	Met	Ala	Cys	Leu	Pro	Asn	Met	Val
						515			520						525
<hr/>															
Val	Met	Ala	Pro	Ser	Asp	Glu	Ala	Glu	Leu	Met	His	Met	Val	Ala	Thr
						530			535						540
<hr/>															
Ala	Ala	Val	Ile	Asp	Asp	Arg	Pro	Ser	Cys	Val	Arg	Tyr	Pro	Arg	Gly
						545			550						560
<hr/>															
Asn	Gly	Ile	Gly	Val	Pro	Leu	Pro	Pro	Asn	Asn	Lys	Gly	Ile	Pro	Leu
						565			570						575
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Glu	Val	Gly	Lys	Gly	Arg	Ile	Leu	Lys	Glu	Gly	Asn	Arg	Val	Ala	Ile
						580			585						590
<hr/>															
Leu	Gly	Phe	Gly	Thr	Ile	Val	Gln	Asn	Cys	Leu	Ala	Ala	Gln	Leu	
						595			600						605
<hr/>															
Leu	Gln	Glu	His	Gly	Ile	Ser	Val	Ser	Val	Ala	Asp	Ala	Arg	Phe	Cys
						610			615						620
<hr/>															
Lys	Pro	Leu	Asp	Gly	Asp	Leu	Ile	Lys	Asn	Leu	Val	Lys	Glu	His	Glu
						625			630						640
<hr/>															
Val	Leu	Ile	Thr	Val	Glu	Glu	Gly	Ser	Ile	Gly	Gly	Phe	Ser	Ala	His
						645			650						655
<hr/>															
Val	Ser	His	Phe	Leu	Ser	Leu	Asn	Gly	Leu	Leu	Asp	Gly	Asn	Leu	Lys
						660			665						670
<hr/>															
Trp	Arg	Pro	Met	Val	Leu	Pro	Asp	Arg	Tyr	Ile	Asp	His	Gly	Ala	Tyr
						675			680						685
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Pro	Asp	Gln	Ile	Glu	Glu	Ala	Gly	Leu	Ser	Ser	Lys	His	Ile	Ala	Gly

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690	695	700
Thr Val Leu Ser Leu Ile Gly Gly Lys Asp Ser Leu His Leu Ile		
705	710	715
720		
Asn Met		
<210> SEQ ID NO: 80		
<211> LENGTH: 525		
<212> TYPE: PRT		
<213> ORGANISM: <i>Saccharomyces cerevisiae</i>		
<400> SEQUENCE: 80		
Met Asp Gln Leu Val Lys Thr Glu Val Thr Lys Lys Ser Phe Thr Ala		
1	5	10
15		
Pro Val Gln Lys Ala Ser Thr Pro Val Leu Thr Asn Lys Thr Val Ile		
20	25	30
Ser Gly Ser Lys Val Lys Ser Leu Ser Ser Ala Gln Ser Ser Ser Ser		
35	40	45
Gly Pro Ser Ser Ser Ser Glu Glu Asp Asp Ser Arg Asp Ile Glu Ser		
50	55	60
Leu Asp Lys Lys Ile Arg Pro Leu Glu Glu Leu Glu Ala Leu Leu Ser		
65	70	75
80		
Ser Gly Asn Thr Lys Gln Leu Lys Asn Lys Glu Val Ala Ala Leu Val		
85	90	95
Ile His Gly Lys Leu Pro Leu Tyr Ala Leu Glu Lys Lys Leu Gly Asp		
100	105	110
Thr Thr Arg Ala Val Ala Val Arg Arg Lys Ala Leu Ser Ile Leu Ala		
115	120	125
Glu Ala Pro Val Leu Ala Ser Asp Arg Leu Pro Tyr Lys Asn Tyr Asp		
130	135	140
Tyr Asp Arg Val Phe Gly Ala Cys Cys Glu Asn Val Ile Gly Tyr Met		
145	150	155
160		
Pro Leu Pro Val Gly Val Ile Gly Pro Leu Val Ile Asp Gly Thr Ser		
165	170	175
Tyr His Ile Pro Met Ala Thr Thr Glu Gly Cys Leu Val Ala Ser Ala		
180	185	190
Met Arg Gly Cys Lys Ala Ile Asn Ala Gly Gly Ala Thr Thr Val		
195	200	205
Leu Thr Lys Asp Gly Met Thr Arg Gly Pro Val Val Arg Phe Pro Thr		
210	215	220
Leu Lys Arg Ser Gly Ala Cys Lys Ile Trp Leu Asp Ser Glu Glu Gly		
225	230	235
240		
Gln Asn Ala Ile Lys Lys Ala Phe Asn Ser Thr Ser Arg Phe Ala Arg		
245	250	255
Leu Gln His Ile Gln Thr Cys Leu Ala Gly Asp Leu Leu Phe Met Arg		
260	265	270
Phe Arg Thr Thr Thr Gly Asp Ala Met Gly Met Asn Met Ile Ser Lys		
275	280	285
Gly Val Glu Tyr Ser Leu Lys Gln Met Val Glu Glu Tyr Gly Trp Glu		
290	295	300
Asp Met Glu Val Val Ser Val Ser Gly Asn Tyr Cys Thr Asp Lys Lys		
305	310	315
320		
Pro Ala Ala Ile Asn Trp Ile Glu Gly Arg Gly Lys Ser Val Val Ala		

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325	330	335
Glu Ala Thr Ile Pro Gly Asp Val Val Arg Lys Val Leu Lys Ser Asp		
340	345	350
Val Ser Ala Leu Val Glu Leu Asn Ile Ala Lys Asn Leu Val Gly Ser		
355	360	365
Ala Met Ala Gly Ser Val Gly Gly Phe Asn Ala His Ala Ala Asn Leu		
370	375	380
Val Thr Ala Val Phe Leu Ala Leu Gly Gln Asp Pro Ala Gln Asn Val		
385	390	395
Glu Ser Ser Asn Cys Ile Thr Leu Met Lys Glu Val Asp Gly Asp Leu		
405	410	415
Arg Ile Ser Val Ser Met Pro Ser Ile Glu Val Gly Thr Ile Gly Gly		
420	425	430
Gly Thr Val Leu Glu Pro Gln Gly Ala Met Leu Asp Leu Leu Gly Val		
435	440	445
Arg Gly Pro His Ala Thr Ala Pro Gly Thr Asn Ala Arg Gln Leu Ala		
450	455	460
Arg Ile Val Ala Cys Ala Val Leu Ala Gly Glu Leu Ser Leu Cys Ala		
465	470	475
Ala Leu Ala Ala Gly His Leu Val Gln Ser His Met Thr His Asn Arg		
485	490	495
Lys Pro Ala Glu Pro Thr Lys Pro Asn Asn Leu Asp Ala Thr Asp Ile		
500	505	510
Asn Arg Leu Lys Asp Gly Ser Val Thr Cys Ile Lys Ser		
515	520	525

<210> SEQ ID NO 81

<211> LENGTH: 297

<212> TYPE: PRT

<213> ORGANISM: Synechococcus sp

<400> SEQUENCE: 81

Met Val Ala Gln Thr Phe Asn Leu Asp Thr Tyr Leu Ser Gln Arg Gln		
1	5	10
15		
Gln Gln Val Glu Ala Leu Ser Ala Ala Leu Val Pro Ala Tyr Pro		
20	25	30
Glu Arg Ile Tyr Glu Ala Met Arg Tyr Ser Leu Leu Ala Gly Gly Lys		
35	40	45
Arg Leu Arg Pro Ile Leu Cys Leu Ala Ala Cys Glu Leu Ala Gly Gly		
50	55	60
Ser Val Glu Gln Ala Met Pro Thr Ala Cys Ala Leu Glu Met Ile His		
65	70	75
80		
Thr Met Ser Leu Ile His Asp Asp Leu Pro Ala Met Asp Asn Asp Asp		
85	90	95
Phe Arg Arg Gly Lys Pro Thr Asn His Lys Val Phe Gly Glu Asp Ile		
100	105	110
Ala Ile Leu Ala Gly Asp Ala Leu Leu Ala Tyr Ala Phe Glu His Ile		
115	120	125
Ala Ser Gln Thr Arg Gly Val Pro Pro Gln Leu Val Leu Gln Val Ile		
130	135	140
Ala Arg Ile Gly His Ala Val Ala Ala Thr Gly Leu Val Gly Gly Gln		
145	150	155
160		

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Val	Val	Asp	Leu	Glu	Ser	Glu	Gly	Lys	Ala	Ile	Ser	Leu	Glu	Thr	Leu
165							170							175	
Glu	Tyr	Ile	His	Ser	His	Lys	Thr	Gly	Ala	Leu	Leu	Glu	Ala	Ser	Val
180							185							190	
Val	Ser	Gly	Gly	Ile	Leu	Ala	Gly	Ala	Asp	Glu	Glu	Leu	Leu	Ala	Arg
195							200							205	
Leu	Ser	His	Tyr	Ala	Arg	Asp	Ile	Gly	Leu	Ala	Phe	Gln	Ile	Val	Asp
210							215							220	
Asp	Ile	Leu	Asp	Val	Thr	Ala	Thr	Ser	Glu	Gln	Leu	Gly	Lys	Thr	Ala
225							230							235	
Gly	Lys	Asp	Gln	Ala	Ala	Ala	Lys	Ala	Thr	Tyr	Pro	Ser	Leu	Leu	Gly
245							250							255	
Leu	Glu	Ala	Ser	Arg	Gln	Lys	Ala	Glu	Glu	Leu	Ile	Gln	Ser	Ala	Lys
260							265							270	
Glu	Ala	Leu	Arg	Pro	Tyr	Gly	Ser	Gln	Ala	Glu	Pro	Leu	Leu	Ala	Leu
275							280							285	
Ala	Asp	Phe	Ile	Thr	Arg	Arg	Gln	His							
290							295								

1. A recombinant host cell capable of producing oxygenated diterpenoid compounds, wherein the host cell

- i. is capable of producing miltiradiene and/or dehydroabietadiene; and
- ii. comprises a first heterologous nucleic acid encoding a first enzyme having cytochrome P450 activity, wherein the first enzyme having cytochrome P450 activity is the cytochrome P450 enzyme TwCYP82D274 as set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 74 or SEQ ID NO: 75, or a functional homologue thereof having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity thereto, or the mature polypeptide thereof, whereby the host cell is capable of converting miltiradiene and/or dehydroabietadiene into 14-hydroxydehydroabietadiene.

2. The recombinant host cell according to claim 1, wherein the recombinant host cell further comprises a second heterologous nucleic acid encoding a second enzyme having cytochrome P450 activity, wherein the second enzyme having cytochrome P450 activity is the cytochrome P450 enzyme TwCYP71BE86 as set forth in SEQ ID NO: 4, or a functional homologue thereof having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity thereto, or the mature polypeptide thereof.

3. The recombinant host cell according to claim 2, wherein the recombinant host cell comprises and expresses said first heterologous nucleic acid and said second heterologous nucleic acid, whereby the cell is capable of producing 14-hydroxydehydroabietadiene, 3,14-dihydroxydehydroabietadiene, 3,14-dihydroxyabeodiene and 14-hydroxy-18-aldo-abeodiene.

4. The recombinant host cell according to claim 1, wherein the recombinant host cell further comprises a third

heterologous nucleic acid encoding a third enzyme having cytochrome P450 activity, wherein the third enzyme having cytochrome P450 activity is the cytochrome P450 enzyme TwCYP71BE85 as set forth in SEQ ID NO: 3, or a functional homologue thereof having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity thereto, or the mature polypeptide thereof.

5. The recombinant host cell according to claim 4, wherein the recombinant host cell comprises and expresses said first heterologous nucleic acid and said third heterologous nucleic acid, whereby the cell is capable of producing 14-hydroxydehydroabietadiene.

6. The recombinant host cell according to claim 4, wherein the recombinant host cell comprises and expresses said first heterologous nucleic acid, said second heterologous nucleic acid and said third heterologous nucleic acid, whereby the cell is capable of producing 14-hydroxydehydroabietadiene, 3,14-dihydroxydehydroabietadiene, 3,14-dihydroxyabeodiene, 14-hydroxy-18-aldo-abeodiene and triptophenolide.

7. The recombinant host cell according to claim 1, wherein the recombinant host cell further comprises a fourth heterologous nucleic acid encoding a fourth enzyme having cytochrome P450 activity, wherein the fourth enzyme having cytochrome P450 activity is the cytochrome P450 enzyme TwCYP82D213 as set forth in SEQ ID NO: 5 or SEQ ID NO: 76, or a functional homologue thereof having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity more preferred at least 98% sequence identity thereto, or the mature polypeptide thereof.

8. The recombinant host cell according to claim 7, wherein the recombinant host cell comprises and expresses said first heterologous nucleic acid, said second heterologous nucleic acid, said third heterologous nucleic acid and said fourth heterologous nucleic acid, whereby the cell is

capable of producing 14-hydroxydehydroabietadiene, 3,14-dihydroxydehydroabietadiene, 3,14-dihydroxyabeodiene, 14-hydroxy-18-aldo-abeoediene, triptophenolide and triptonide.

9. The recombinant host cell according to claim 1, wherein the host cell further comprises a fifth heterologous nucleic acid encoding a fifth enzyme having cytochrome P450 activity, wherein the fifth enzyme having cytochrome P450 activity is the cytochrome P450 enzyme TwCYP82D217 as set forth in SEQ ID NO: 6, or a functional homologue thereof having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity thereto, or the mature polypeptide thereof.

10. The recombinant host cell according to claim 1, wherein the host cell further comprises a sixth heterologous nucleic acid encoding a sixth enzyme having cytochrome P450 activity, wherein the sixth enzyme having cytochrome P450 activity is the cytochrome P450 enzyme TwCYP82D275 as set forth in SEQ ID NO: 7, or a functional homologue thereof having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity thereto, or the mature polypeptide thereof.

11. The recombinant host cell according to claim 1, wherein the host cell further comprises a seventh heterologous nucleic acid encoding an enzyme having cytochrome B5 activity, wherein the enzyme having cytochrome B5 activity is the cytochrome B5 TwB5 #1 as set forth in SEQ ID NO: 8, or a functional homologue thereof having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity thereto, or the mature polypeptide thereof.

12. The recombinant host cell according to claim 1, wherein the recombinant host cell expresses one or more of:

- a geranylgeranyl diphosphate synthase;
- a diterpene synthase capable of converting geranylgeranyl-diphosphate (GGPP) into miltiradiene;
- a combination of two or more diterpene synthases that in combination are capable of converting GGPP into miltiradiene; or
- a copalyl diphosphate synthase and a miltiradiene synthase

whereby the cell is capable of producing miltiradiene and/or dehydroabietadiene.

13. The recombinant host cell of claim 12, wherein the geranylgeranyl diphosphate synthase is a polypeptide comprising the amino acid sequence of SEQ ID NO: 73 or SEQ ID NO: 81, or a functional homologue thereof having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity thereto.

14. The recombinant host cell according to claim 12, wherein the combination of two or more diterpene synthases, that is capable of converting GGPP into miltiradiene, is the combination of CfTPS1 as set forth in SEQ ID NO: 67 and CfTPS3 as set forth in SEQ ID NO: 68, or CfTPS1 as set forth in SEQ ID NO: 77 and CfTPS3 as set forth in SEQ ID NO: 78, or a combination of the respective functional

homologues thereof having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity thereto; or is the combination of TwTPS9 as set forth in SEQ ID NO: 69 and TwTPS27 as set forth in SEQ ID NO: 70, or a combination of the respective functional homologues thereof having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity thereto.

15. The recombinant host cell according to claim 12, wherein the combination of a copalyl diphosphate synthase and a miltiradiene synthase is the combination of SmCPS as set forth in SEQ ID NO: 71 and SmKSL as set forth in SEQ ID NO: 72, or a combination of the respective functional homologues thereof having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, more preferred at least 98% sequence identity thereto.

16. The recombinant host cell according to claim 1, wherein the recombinant host cell is a prokaryotic or a eukaryotic cell.

17. The recombinant host cell according to claim 1, wherein the recombinant host cell is a eukaryotic cell of a species selected from the group consisting of *Saccharomyces*, *Schizosaccharomyces*, *Klyveromyces*, *Pichia*, *Candida* and *Yarrowia*.

18. The recombinant host cell according to claim 1, wherein the recombinant host cell is a *S. cerevisiae* cell.

19. The recombinant host cell according to claim 1, wherein the recombinant host cell is a prokaryotic cell of a species selected from the list consisting of *Escherichia*, *Bacillus*, *Lactobacillus* and *Corynebacterium*.

20. The recombinant host cell according to claim 1, wherein the recombinant host cell is a plant cell or comprised in a plant, wherein the plant may be *Nicotiana tabacum*, and/or the host cell is a cell from another multicellular host.

21. A method for production of an oxygenated diterpenoid compound, such as triptonide, said method comprising the steps of

- providing a recombinant host cell according to claim 1;
- culturing said recombinant host cell under conditions suitable for production of said oxygenated diterpenoid compound.

22. The method according to claim 21, wherein the oxygenated diterpenoid compound is selected from the list consisting of 14-OH-dehydroabietadiene, triptophenolide and triptonide.

23. The method according to claim 21, wherein the oxygenated diterpenoid compound is triptonide.

24. The method according to claim 22, further comprising a step of recovering and, optionally, purifying the triptonide.

25. A method of producing triptolide said method comprising

- producing triptonide according to the method of claim 22, and
- converting the triptonide into triptolide and,
- optionally, recovering and/or purifying the triptolide.

26. (canceled)

27. (canceled)

28. (canceled)

29. (canceled)

30. A polypeptide having cytochrome P450 enzyme activity and comprising an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, preferably at least 96% sequence identity, preferably at least 97% sequence identity, preferably at least 98% sequence identity, or even 100% sequence identity to one of the sequences SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or the mature polypeptide thereof or a polypeptide having cytochrome B5 enzyme activity and comprising an amino acid sequence having at least 80% sequence identity, preferably at least 85% sequence identity, preferably at least 90% sequence identity, preferably at least 95% sequence identity, preferably at least 96% sequence identity, preferably at least 97% sequence identity, preferably at least 98% sequence identity, or even 100% sequence identity to SEQ ID NO:8.

31. A polynucleotide encoding the polypeptide of claim 30.

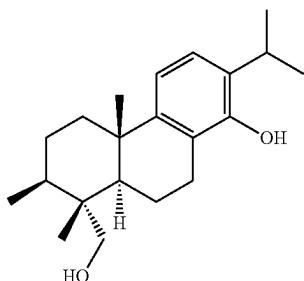
32. A plasmid, expression vector, expression construct or recombinant host cell comprising the polynucleotide of claim 31.

33. The compound 14-OH-dehydroabietadiene.

34. A compound selected from the group consisting of the following formulas (1) to (17):

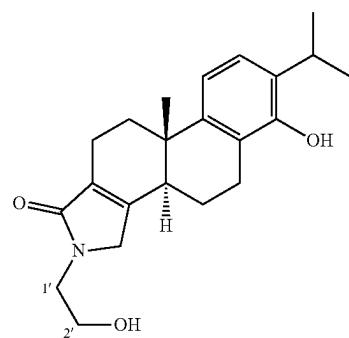
F1-14

(1)



F1-15

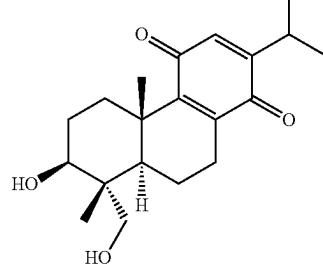
(2)



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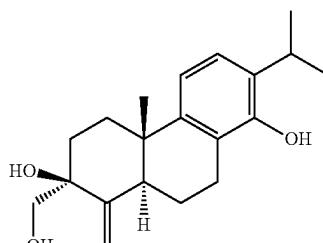
F1-18

(3)



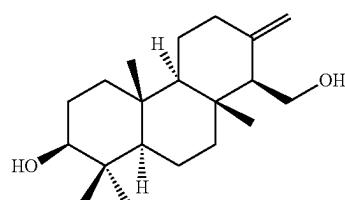
F2-10

(4)



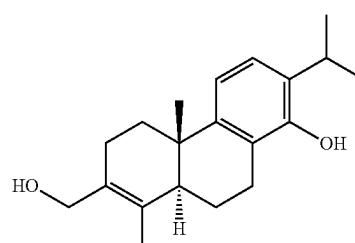
F20P1

(5)



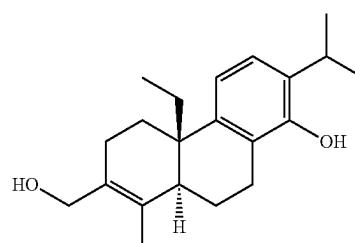
F20P2

(6)



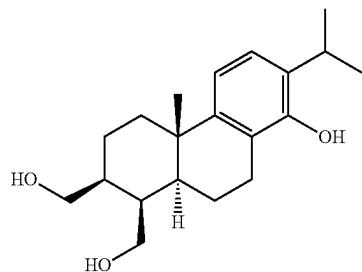
F55P2

(7)

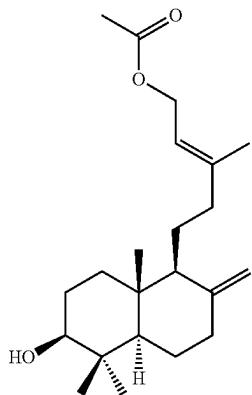


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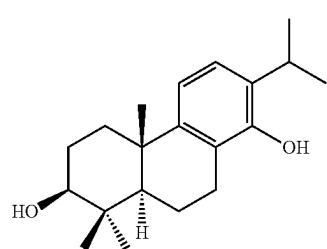
F55P3



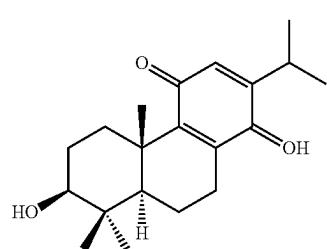
F15P4



F15P1



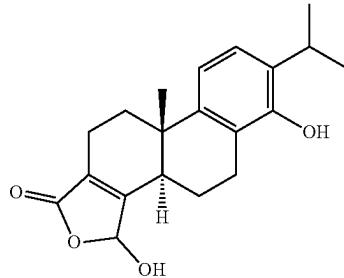
F1-31 (F2-35)



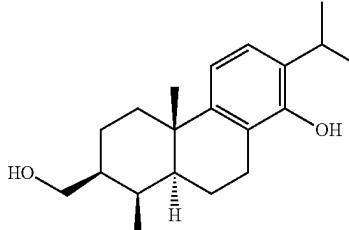
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F2-X

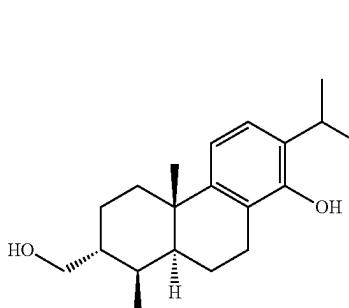
(8)



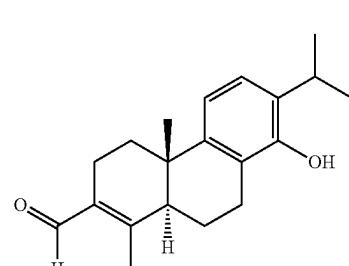
(12)



(13)



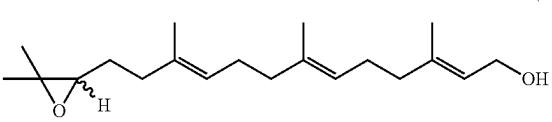
(14)



(15)

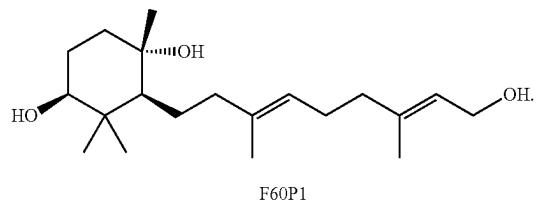
F15P2

F20P5 (F15P3)



(16)

(17)



F60P1

35. The compound according to claim **34**, wherein said compound is the compound according to formula (6) (F20P2).

36. The compound according to claim **34**, wherein said compound is the compound according to formula (10) (F15P1).

37. The compound according to claim **34**, wherein said compound is the compound according to formula (15) (F15P2).

* * * *