Title: PROCESS FOR PRODUCING OXYGENATED PRODUCTS OF VALENCE

Abstract: The present disclosure relates to methods for producing oxygenated terpenoids, and preparation of compositions and formulations thereof. Polynucleotides, derivative enzymes, and host cells for use in such methods are also provided.

FIG. 1
## INTERNATIONAL SEARCH REPORT

**International application No**

PCT/US2015/04642

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**A. CLASSIFICATION OF SUBJECT MATTER**

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<th>Inv.</th>
<th>C12P7/02</th>
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According to International Patent Classification (IPC) or to both national classification and IPC.

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

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<th>C12P</th>
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronis data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, CHEMABS Data, COMPENDEX, EMBASE, FSTA, IM-TDB, WPI Data

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**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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

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Date of the actual completion of the international search: 21 December 2015

Date of mailing of the international search report: 22/03/2016

Name and mailing address of the ISA/

European Patent Office, P.B. 5618 Patentlaan 2 NL-2280 HJ Rijswijk

Tel. (+31-70) 340-2040, Fax (+31-70) 340-3016

Authorized officer:

Fuchs, Ulrike
### DOCUMENTS CONSIDERED TO BE RELEVANT

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Form PCT/ISA/210 (continuation of second sheet) (April 2009)
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**INTERNATIONAL SEARCH REPORT**

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

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

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

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

3. ☐ Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 64(a).

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

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

see additional sheet

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

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

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers:

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

1-27, 58-61, 64-88, 131-133, 164(completely) ; 34-43, 52-54, 63, 136-156

161-163(partially)

**Remark on Protest**

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

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

☐ No protest accompanied the payment of additional search fees.
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-27, 58-61, 64-88, 131-133, 164 (completely); 34-43, 52-54, 63, 136-156, 161-163 (partly)

   method for making an oxygenated product comprising:
   contacting valencene with Stevia rebaudiana Kaurene Oxidase (SrKO) or an SrKO derivative having valencene oxidizing activity to produce an oxygenated product, wherein the oxygenated product comprises nootkatone and nootkatol,
   method for making a formulation comprising:
   contacting valencene with Stevia rebaudiana Kaurene Oxidase (SrKO) or an SrKO derivative having valencene oxidizing activity to prepare a formulation comprising an oxygenated product, wherein the oxygenated product comprises nootkatone and nootkatol,
   method for making a formulation comprising:
   contacting valencene with Stevia rebaudiana Kaurene Oxidase (SrKO) or an SrKO derivative having valencene oxidizing activity to produce an oxygenated product, wherein the oxygenated product comprises nootkatone and nootkatol and incorporating at least part of the oxygenated product into a formulation on
   modified SrKO polypeptide comprising at least one mutation relative to SEQ ID NO: 37 or SEQ ID NO: 38 that increases valencene oxidase activity or increases production of at least one of nootkatone and nootkatol
   modified SrKO polypeptide comprising at least one mutation relative to SEQ ID NO: 37 or SEQ ID NO: 38 that increases valencene oxidase activity or increases production of at least one of nootkatone and nootkatol
   modified SrKO polypeptide having from 1 to 50 mutations independently selected from substitutions, deletions, or insertions relative to SEQ ID NO: 37 or SEQ ID NO: 38, wherein one or more mutations are selected from Table 2.1, Table 2.2, Table 2.3, and/or Table 6,
   modified SrKO polypeptide comprising an amino acid sequence selected from SEQ ID NO: 38, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111

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2. claims: 28-33 (completely); 34-43, 52-54, 161 (partly)

   method of any of claims 1-27, further comprising reacting the oxygenated product or the formulation with an alcohol dehydrogenase (ADH)

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3. claims: 44-51 (completely); 52-54 (partly)

   method of any of claims 34-43, wherein the host cell further
expresses a valencene synthase (VS) ---

4. claims: 55-57

method for making a composition comprising:
incorporating the oxygenated product or the formulation
prepared by the method of any of claims 1-54 into the
composition ---

5. claims: 62(completely) ; 63, 136-156, 161-163 (partially)
modified SrKO polypeptide comprising an amino acid sequence
having at least 50% sequence identity, at least 60% sequence
identity, at least 70% sequence identity, at least 80%
sequence identity, or at least 90% sequence identity to SEQ
ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 55, SEQ ID NO: 56, SEQ
ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ
ID NO: 61, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106,
SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, or SEQ ID NO: 111 ---

6. claims: 89-101, 107-118, 159, 160(completely) ; 105,
106(partially)
oxygenated product obtainable by the method of any of claims
1-54,
formulation obtainable by the method of any of claims 2-54,
method of making a composition comprising:
providing an oxygenated product obtainable by the method of
any of claims 1-54; and
adjusting the amount of at least one of
hydroxygermacra-i((10)5-diene, murolan-3,9(II)
diene-10-peroxy, alpha-nootkatol, beta-nootkatol, and
nootkatone in the oxygenated product
method of making a composition comprising:
providing a formulation obtainable by the method of any of
claims 2-54; and
adjusting the amount of at least one of valencene,
hydroxygermacra-i((10)5-diene, murolan-3,9(II)
diene-10-peroxy, alpha-nootkatol, beta-nootkatol, and
nootkatone in the formulation,
flavor product, natural flavor product, fragrance product,
cosmetic product, cleaning product, detergent product, soap
product, or pest control product comprising the oxygenated
product according to any of claims 89-92,
flavor product, natural flavor product, fragrance product,
cosmetic product, cleaning product, detergent product, soap
product, or pest control product comprising the formulation on
according to any of claims 93-97,
natural flavor, beverage, chewing gum, candy, artificial
flavor, or flavor additive comprising the oxygenated product
according to any of claims 89-92,
beverage, a chewing gum, a candy, an artificial flavor, or a flavor additive comprising the formulation according to any of claims 93-97, comprising the oxygenated product of any of claims 89-92 and at least one additional ingredient, comprising the formulation of any of claims 93-97 and at least one additional ingredient, method of using the oxygenated product according to any of claims 89-92, comprising incorporating the oxygenated product into a consumer product, an industrial product, a flavor product, a fragrance product, a cosmetic product, a cleaning product, a detergent product, a soap product, a pest control product, a beverage, a chewing gum, a candy, a natural flavor, an artificial flavor, or a flavor additive, method of using the formulation according to any of claims 93-97, comprising incorporating the formulation into a consumer product, an industrial product, a flavor product, a fragrance product, a cosmetic product, a cleaning product, a detergent product, a soap product, a pest control product, a beverage, a chewing gum, a candy, a natural flavor, an artificial flavor, or a flavor additive

7. Claims: 102-104, 158 (completely); 105, 106 (partially)

method of making a composition comprising:
providing an oxygenated product obtainable by the method of any of claims 1-54, and
adding at least one additional ingredient, wherein the at least one additional ingredient comprises a sesquiterpene,
method of making a composition comprising:
providing a formulation obtainable by the method of any of claims 2-54, and
adding at least one additional ingredient, wherein the at least one additional ingredient comprises a sesquiterpene

8. Claims: 119-122

valencene synthase (VS) enzyme comprising an amino acid sequence selected from SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, and SEQ ID NO: 129,
valencene synthase (VS) enzyme comprising one or more mutations relative to SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 12, or SEQ ID NO: 129,
valencene synthase (VS) enzyme, wherein the VS has from 1 to 40 mutations, from 1 to 20 mutations, or from 1 to 10 mutations independently selected from substitutions, deletions, or insertions relative to SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 12, or SEQ ID NO: 129,

9. Claims: 123-130, 165
recombinant nucleic acid molecule comprising:
a nucleotide encoding an SrKO or an SrKO derivative,
a nucleotide encoding a leader sequence, and
a nucleotide encoding an SrCPR or derivative thereof,
recombinant fusion protein encoded by the recombinant
nucleic acid molecule according to any of claims 123-129,
recombinant fusion protein comprising:
an SrKO or an SrKO derivative,
a leader sequence,
a linker sequence, and
SrCPR or derivative thereof

10. claims: 134, 135 (completely) ; 163 (partially)
modified SrKO polypeptide comprising an amino acid sequence
having one or more mutations relative to SEQ ID NO: 37,
wherein the one or more mutations comprise at least one of
H46R, R76K, M94V, T131Q, F231L, H284Q, R383K, I390L, V400Q,
1444A, T468I, T488D, and T499N,
modified SrKO polypeptide comprising an amino acid sequence
having one or more mutations relative to SEQ ID NO: 38,
wherein the one or more mutations comprise at least one of
H34R, R64K, M82V, T119Q, F219L, H272Q, R371K, I378L, V388Q,
1432A, T456I, T476D, and T487N

11. claims: 157, 183-188 (completely) ; 161, 162 (partially)
method of oxidizing valencene comprising:
contacting valencene with Stevia rebaudiana Kaurene Oxidase
(SrKO) or an SrKO derivative having valencene oxidizing activity
to produce an oxygenated product,
wherein the oxygenated product comprises nootkatone,
alpha-nootkatol, and beta-nootkatol.

12. claims: 166-168, 177, 179 (completely) ; 172-176,
180-182 (partially)
formulation comprising valencene, nootkatone,
alpha-nootkatol, and beta-nootkatol.

13. claims: 169-171, 178 (completely) ; 172-176, 180-182 (partially)
formulation comprising nootkatone, alpha-nootkatol, and
beta-nootkatol.
# INTERNATIONAL SEARCH REPORT

**International application No**

PCT/US2015/04642

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**Patent document cited in search report** | **Publication date** | **Patent family member(s)** | **Publication date**
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 |  | EP 2776571 Al | 17-09 -20 14
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 |  | US 2013236942 Al | 12-09-20 13
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