METHOD OF SYNTHESIS OF MONOCAFFEYOYLQUINIC OR DICAFFEOYLQUINIC ACID

Process for the synthesis of an isomer of monocaffeoylquinic acid, wherein an ester of O, O-di-acetylcaffeic acid being an acyl donor, is subjected to enzymatic transesterification in the presence of quinic acid or quinic acid ester in a solvent selected from polar solvents.
Method of synthesis of monoaacafeoylquinic or dicaaffeoylquinic acid.

The present invention relates to a method of synthesis of monoaacafeoylquinic or dicaaffeoylquinic acid isomer.

The methods of obtaining of monoaacafeoyl and dicaaffeoyl acid reported to date base on their enzymatic extraction from plant material, as reported in CN1687435 or solvent extraction, as reported in CN103214371 and US201 1054022. The required monoaacafeoylquinic or dicaaffeoylquinic acid isomer is isolated via chromatography from a mixture of isomers of monoaacafeoylquinic and dicaaffeoylquinic acids, as reported in US201 1054022. Such a nonspecific method of obtaining an isomer of monoaacafeoylquinic or dicaaffeoylquinic acid is burdened with a need of simultaneous production of other isomers of monoaacafeoylquinic and dicaaffeoylquinic acid, therefore leading to low yields. The aim of the invention was to develop a method of synthesis of an isomer of monoaacafeoylquinic or dicaaffeoylquinic acid, without the need to isolate them from a mixture.

The method for the synthesis of isomer of monoaacafeoylquinic or dicaaffeoylquinic acid according to the invention is that the vinyl ester of 0,0-diacetylcffeic acid, which is the acyl donor, is subjected to enzymatic transesterification in the presence of quinic acid or a quinic acid methyl ester in a solvent selected from polar solvents. Preferably, the solvents are selected from solvents with ether characteristics, in particular from MTBE, tert-butanol, THF, acetonitrile, hexane, dichloroethane, or mixtures thereof.
The enzyme used as a catalyst is a lipase, and is selected from Lipozyme RM IM, that is, lipase from Mucor miehei immobilized on an ion exchange resin, Lipozyme TL IM, that is lipase from Thermomyces lanuginosus immobilized on an acrylic resin, Novozym 435, that is Lipase B from Candida antarctica immobilized on acrylic resin, PPL, that is porcine pancreatic lipase, CCL, that is Candida rugosa lipase, Amano AK, that is Pseudomonas fluorescens lipase and Amano PS, that is Burkholderia cepacia lipase.

The names Lipozyme RM IM, Lipozyme TL IM, Novozym 435 are trademarks of Novozymes A/S, PPL is the trademark of Lee Biosolutions Inc., CCL is a trademark of Sigma-Aldrich Co., Amano AK and Amano PS are trademarks of Amano Enzyme Inc.

Lipozyme RM IM and Lipozyme TL IM catalyze the acylation reaction at the 3' position of the benzene ring of quinic acid or quinic acid methyl ester, wherein the preferred enzyme is Lipozyme RM IM. Novozym 435 catalyzes the acylation reaction at the 4' position of the benzene ring of quinic acid or quinic acid methyl ester. PPL, CCL, Amano AK and Amano PS catalyze the acylation reaction in the 5' position of the benzene ring of quinic acid or quinic acid methyl ester, wherein the preferred enzymes are CCL and Amano PS.

3,4-Isomer of dicafeoylquinic acid is obtained in a reaction with Novozym 435, and then, after filtration, a reaction with Lipozyme RM IM or Lipozyme TL IM, wherein the order of preparation is not critical, but the reactions are carried out separately. 3,5-Isomer of dicafeoylquinic acid is obtained by reaction with Lipozyme RM IM or Lipozyme TL IM, and then, after filtration, a reaction with PPL or CCL.
or Amano AK or Amano PS. 4,5-Isomer of dicaffeoylquinic acid is obtained by reaction with Novozym 435, and then, after filtration, PPL or CCL or Amano AK or Amano PS.

Preferably the acylation is carried out in a molar ratio of substrate to the acyl group donor contained in the range of 2 to 10. For each 1 mmol of substrate from 100 to 1000 mg of enzyme and 5 ml of solvent is used. Acylation process is conducted at a temperature from 10 to 60° Celsius, preferably from 30 to 40° Celsius. The isolated product is subjected to deacetylation and possible demethylation, where the acyl group donor is a methyl ester of quinic acid.

An advantage of the invention is obtaining of only a selected isomer of monocaffeoylquinic or dicaffeoylquinic acid because of high specificity of the enzyme used in the reaction and reduction of the cost of production of enantiomerically pure material due to the lack of necessary separation of the mixture of isomers of monocaffeoylquinic and dicaffeoylquinic acids.

The invention is illustrated in the embodiments.

Example 1.

Synthesis of 3-caffeoylquinic acid

100 mM of caffeic acid was dissolved in 25 mL of pyridine and treated with 120 mM acetic anhydride. The reaction was left on a magnetic stirrer at room temperature for 24 hours. To the mixture was added 50 mL of water and extracted with 6x50 mL of ethyl acetate. The organic layers were combined and washed with saturated sodium bisulfate until the dissapearance of odor of pyridine, then with saturated sodium
bicarbonate solution until disappearance of foam and with water. The organic layer was dried over anhydrous sodium sulfate and then evaporated.

The resulting solid was air dried until constant weight. The obtained white solid was dissolved in 100 mL of dry THF, 150 mL of vinyl acetate and 1 g of mercury (II) acetate or 1.5 g of palladium (II) acetate were added. The suspension was stirred for 30 minutes, after which 3 mL of an ethereal solution of BF₃ was added, and heated to 40° Celsius. After 4 hours and TLC control, sodium acetate was added to the solution to neutralize, the mixture was filtered, THF and excess vinyl acetate were evaporated, the residue was dissolved in 200 mL of methylene chloride and washed with saturated sodium bicarbonate until disappearance of foaming, saturated sodium bisulfate to a pH of about 6 and water. The organic layer was dried over anhydrous sodium sulfate and evaporated to give 92 mM of vinyl ester of 0,0-diacetylcaffeic acid. Palladium acetate was recovered by dissolving the solid isolated after vinylation reaction in water and evaporating the solvent.

1 mM of quinic acid methyl ester was suspended in 5 mL of MTBE in a conical flask. To the suspension 8 mM of vinyl ester of O, O-diacetylcaffeic acid and 200 mg Lipozyme RM IM were added. The reaction was performed at 30° degrees Celsius until the disappearance of quinic acid methyl ester signal in HPLC. To isolate the product the solvent was evaporated and the residue was suspended in methylene chloride and then filtered. The remaining product residue was the desired product, and the unused vinyl ester of O,O-diacetylcaffeic acid was recovered quantitatively from the solvent. The isolated product was
deacetylated and demethylated by treatment with 3N HCl in water under TLC control. The mixture was then neutralized with solid sodium bicarbonate, evaporated and purified using an ion exchange column. Obtained was \((\text{IR},3\text{R},4\text{S},5\text{R})-3-\{(2\text{E})-3-(3,4\text{-dihydroxyphenyl})\text{prop-2-enoyl}\text{oxy}\}-1,4,5\text{-tri\-hydroxy}cyclohexanecarboxylic\) acid of a structural formula:

![Chemical Structure](image)

The product was identified via Chromatography and spectra analysis.

**HPLC conditions:** DIONEX system, P580 pump, ASI-100 Automatic Sample Injector, STH 585 thermostatic oven, UVD 340S detector, controlled by Chromeleon 6.20 software, Thermo Scientific BDS Hypersil C18 column, mobile phase water-acetonitrile-THF-acetic acid (85:10:2:3), flow 1.5 mL/min, detection at 300 nm wavelength, injection volume 1 µL. Product retention time 2.2 min

**H NMR conditions:** Bruker DPX 250 Advance apparatus, samples dissolved in CDC13 with TMS as an internal standard. Signals represent the protons of quinic acid.

Product spectra data: 1.47 (m, 2H), 1.98 (m, 2H), 3.74 (m, 2H), 5.75 (m, 1H)

Example 2.

**Synthesis of 5-caffeoylquinic acid**
Vinyl ester of 0,0-diacetylcaffeic acid was obtained according to Example 1.

1 mM of quinic acid was suspended in a conical flask in 5 mL of MTBE:THF with a volume ratio 7:3. To the suspension 10 mM of vinyl ester of O, O-diacetylcaffeic acid and 500 mg Amano PS were added. The reaction was performed at 30° degrees Celsius until the disappearance of quinic acid signal in HPLC. To isolate the product the solvent was evaporated and the residue was suspended in methylene chloride and then filtered. The remaining product residue was the desired product, and the unused vinyl ester of O,O-diacetylcaffeic acid was recovered quantitatively from the solvent. The isolated product was deacetylated and demethylated by treatment with 3N HCl in water under TLC control. The mixture was then neutralized with solid sodium bicarbonate, evaporated and purified using an ion exchange column. Obtained was (1S,3R,4R,5R)-3-\{[(2E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy\} -1,4,5-trihydroxycyclohexanecarboxylic acid of a structural formula:

Product retention time 3,1 min

Product spectral data: 1.38 (m, 2H), 2.00 (m, 2H), 3.71 (m, 1H), 3.95 (m, 1H), 5.44 (m, 1H)
Example 3  

Synthesis of 4-cafeoylquinic acid

Vinyl ester of O,O-diacetylcaffeic acid was obtained according to Example 1.

1 mM of quinic acid methyl ester was suspended in 5 mL of MTBE in a conical flask. To the suspension 8 mM of vinyl ester of O,O-diacetylcaffeic acid and 500 mg Novozym 435 were added. The reaction was performed at 40° degrees Celsius until the dissapearance of quinic acid methyl ester signal in HPLC. To isolate the product the solvent was evaporated and the residue was suspended in methylene chloride and then filtered. The remaining product residue was the desired product, and the unused vinyl ester of O,O-diacetylcaffeic acid was recovered quantitatively from the solvent. The isolated product was deacetylated and demethylated by treatment with 3N HCl in water under TLC control. The mixture was then neutralized with solid sodium bicarbonate, evaporated and purified using an ion exchange column. Obtained was (1S,3R,4S,5R)-4-{[(2E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}-1,3,5-trihydroxycyclohexanecarboxylic acid of a structural formula:

![Structural formula](image)

Product retention time 2.7 min

Product spectral data: 1.43 (m, 2H), 1.58 (m, 2H), 3.92 (m, 1H), 4.03
Example 4

Synthesis of 3,4-dicaffeoylquinic acid

Vinyl ester of O,O-diacylcaffeic acid was obtained according to Example 1.

1 mM of quinic acid methyl ester was suspended in 5 mL of MTBE in a conical flask. To the suspension 8 mM of vinyl ester of O, O-diacylcaffeic acid and 500 mg Novozym 435 were added. The reaction was performed at 40° degrees Celsius until the disappearance of quinic acid methyl ester signal in HPLC. To isolate the 4-caffeoylquinic acid the solvent was evaporated and the residue was suspended in methylene chloride and then filtered.

1 mM of 4-caffeoylquinic acid was suspended in 5 mL of MTBE in a conical flask. To the suspension 10 mM of vinyl ester of O, O-diacylcaffeic acid and 200 mG Lipozyme RM IM were added. The reaction was performed at 40° degrees Celsius until the disappearance of 4-caffeoylquinic acid signal in HPLC. To isolate the product the solvent was evaporated and the residue was suspended in methylene chloride and then filtered. The remaining product residue was the desired product, and the unused vinyl ester of O,O-diacylcaffeic acid was recovered quantitatively from the solvent. The isolated product was deacylated and demethylated by treatment with 3N HCl in water under TLC control. The mixture was then neutralized with solid sodium bicarbonate, evaporated and purified using an ion exchange column. Obtained was

$$\text{(1Pv,3Pv,4S,5Pv)-3,4-di \{(2E)-3-(3,4-dihydroxyphenyl)prop-2-}$$
enoyl]oxy]-1,4-dihydroxycyclohexanecarboxylic acid of a structural formula:

Product retention time 10.1 min

Product spectra data: 1.61 (m, 2H), 2.05 (m, 2H), 4.02 (m, 1H), 5.02 (m, 1H), 5.87 (m, 1H)

Example 5

Synthesis of 4,5-dicaffeoylquinic acid

Vinyl ester of O,O-diacetylcaffeic acid was obtained according to Example 1.

1 mM of quinic acid methyl ester was suspended in 5 mL of MTBE in a conical flask. To the suspension 8 mM of vinyl ester of O, O-diacetylcaffeic acid and 500 mg Novozym 435 were added. The reaction was performed at 40° degrees Celsius until the dissapearance of quinic acid methyl ester signal in HPLC. To isolate the 4-caffeeoylquinic acid the solvent was evaporated and the residue was suspended in methylene chloride and then filtered.
1 mM of 4-caffeoylquinic acid was suspended in 5 mL of MTBE in a conical flask. To the suspension 10 mM of vinyl ester of O, O-diacetylcaffeic acid and 800 mg CCL were added. The reaction was performed at 40° degrees Celsius until the disappearance of 4-caffeoylquinic acid signal in HPLC. To isolate the product the solvent was evaporated and the residue was suspended in methylene chloride and then filtered. The remaining product residue was the desired product, and the unused vinyl ester of O,O-diacetylcaffeic acid was recovered quantitatively from the solvent. The isolated product was deacetylated and demethylated by treatment with 3N HCl in water under TLC control. The mixture was then neutralized with solid sodium bicarbonate, evaporated and purified using an ion exchange column. Obtained was (1S,3R,4R,5R)-3,4-di-[(2E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,5-dihydroxy cyclohexanecarboxylic acid of a structural formula:
Product retention time 14.2 min

Product spectral data: 1.43 (m, 2H), 2.03 (m, 2H), 4.12 (m, 1H), 5.00 (m, 1H), 5.54 (m, 1H)

Example 6

Synthesis of 3,5-dicaffeoylquinic acid

Vinyl ester of 0,0-diacetylcaffeic acid was obtained according to Example 1.

1 mM of quinic acid methyl ester was suspended in 5 mL of MTBE in a conical flask. To the suspension 8 mM of vinyl ester of O, O-diacetylcaffeic acid and 200 mg Lipozyme RM IM were added. The reaction was performed at 30° degrees Celsius until the disappearance of quinic acid methyl ester signal in HPLC. To isolate the 3-cafeoylquinic acid the solvent was evaporated and the residue was suspended in methylene chloride and then filtered.

1 mM of 3-cafeoylquinic acid was suspended in a conical flask in 5 mL of MTBE:THF with a volume ratio 1:1. To the suspension 10 mM of vinyl ester of O,O-diacetylcaffeic acid and 800 mg CCL were added. The reaction was performed at 40° degrees Celsius until the disappearance of 3-cafeoylquinic acid signal in HPLC. To isolate the product the solvent was evaporated and the residue was suspended in methylene chloride and then filtered. The remaining product residue was the desired product, and the unused vinyl ester of O,O-diacetylcaffeic acid was recovered quantitatively from the solvent. The isolated product was deacetylated and demethylated by treatment with 3N HCl in water under TLC control. The mixture was then neutralized with solid sodium
bicarbonate, evaporated and purified using an ion exchange column. Obtained was (lR,3R,4S,5R)-3,5-di\{[(2E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl] oxy }-1,4-dihydroxycyclohexanecarboxylic acid of a structural formula:

![Structural formula](image)

Product retention time 22.0 min

Product spectral data: 1.99 (m, 4H), 3.70 (m, 1H), 5.51 (m, 1H), 5.83 (m, 1H)

Applications of the products obtained using the method according to the invention are anti-high blood pressure agents, potential laxative substances, psychostimulant compounds, weight loss supporting supplements, substances supporting regulation of liver and pancreas, and antioxidants.
Patent claims

1. Method of synthesis of isomer of monocaffeoylquinic or dicaffeoylquinic acid, wherein the ester of O,O-diacetylcaffeic acid, being the acyl donor, is subjected to enzymatic transesterification in the presence of quinic acid, or quinic acid ester in a solvent selected from polar solvents.

2. Method according to claim 1 wherein the O,O-diacetylcaffeic acid ester is the vinyl ester of O,O-diacetylcaffeic acid.

3. Method according to claim 1 albo 2 wherein the ester of quinic acid is quinic acid methyl ester.

4. Method according to claim 1 albo 2 albo 3 wherein the solvents were chosen from solvents with an ether characteristics.

5. Method according to claim 4 wherein the ether solvents are MTBE, tert-butanol, THF, acetonitrile, hexane, dichloroethane or mixtures thereof.

6. Method according to claim 5 wherein the ether solvent is a MTBE:THF mixture with a volume ratio of 1:1.

7. Method according to claim 5 wherein the ether solvent is a MTBE:THF mixture with a volume ratio of 7:3.

8. Method according to any of the claims 1-7 wherein the enzyme used as a catalyst belongs to lipases.

9. Method according to claim 8 wherein the enzyme used as a catalyst belongs to a group consisting of Lipozyme RM IM, Lipozyme TL IM, Novozym 435, PPL, CCL, Amano AK and Amano PS.

10. Method according to any of the claims 1 - 9 wherein the acylation is conducted using a molar ratio of substrate to acyl group donor in a range from 1 to 2 and 1 to 10.

11. Method according to any of the claims 1 - 10 wherein per every 1 mmol of substrate used is from 100 to 1000 mg of enzyme.

12. Method according to any of the claims 1 - 11 wherein per every 1 mmol of substrate used is 5 mL of solvent.

13. Method according to any of the claims 1 - 12 wherein the acylation process is conducted in a temperature from 10 to 60 °Celsius.
14. Method according to claim 13 wherein the acylation process is conducted in a temperature from 30 to 40 ° Celsius.
**A. CLASSIFICATION OF SUBJECT MATTER**

INV. C12P7/62  C12P7/42  C12P7/22

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)

C12P

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

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

EPO-Internal, FSTA, BIOSIS, EMBASE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>A</td>
<td>BONG-GYU KIM ET AL: &quot;Producti on of hydroxyci nnamoyl-shi kimates and chl orogeni c aci d i n Escheri chi a col i : producti on of hydroxyci nnamici aci d conjugates&quot;, MICROBIAL CELL FACTORIES, vol. 12, 2013, pages 1-11, XP021146867, See page 7 (Figure 5) *</td>
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<td>A</td>
<td>ZHULIANG TAN ET AL: &quot;A novel chemoenzymati c synthesi s of phytosteryl caffeates and assessment of thei r anti oxidant acti vity&quot;, FOOD CHEMISTRY, vol. 133, 2012, pages 1427-1434, XP002745023, See page 1430 (Figure 1) *</td>
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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**

25 September 2015

**Date of mailing of the international search report**

05/10/2015

**Name and mailing address of the ISA**

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<td>A</td>
<td>LALLEMAND ET AL: &quot;Puri fi cati on, crystal l i zati on on and prel iminary X-ray di ffracti on analysi s of a hydroxy cyanamoyl -CoA shi kimate/qui nate hydroxy cyanamoyl transferase (HCT) from Coffea canephora invol ved i n chl orogeni c aci d bi osynthesis s&quot;, ACTA CRYSTALLOGRAPHICA SECTION F, vol. F68, 2012, pages 824-828, XP002745002, * See page 825 (Figure 1) *</td>
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<td>A</td>
<td>GUYOT ET AL: &quot;Esteri f cati on of phenolic aci ds from green coffee with an immobil i zed l i pase from Candida antarctica i n sol vent-free medi um&quot;, BIOTECHNOLOGY LETTERS, vol. 19, 1997, pages 529-532, XP002958839, * See page 530 (Figure) and pages 531-532 (Concl usi ons) *</td>
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<td>SEFK0W: &quot;First eff i cient synthesi s of chl orogeni c aci d&quot;, EUROPEAN JOURNAL OF ORGANIC CHEMISTRY, 2001, pages 1137-1141, XP002744848, * See page 1137 (Abstract and Introd ucti on) *</td>
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<td>A</td>
<td>SEFK0W: &quot;First eff i cient syntheses of 1-, 4-, and 5-caffeoyl qui nic aci d&quot;, EUROPEAN JOURNAL OF ORGANIC CHEMISTRY, 2001, pages 2735-2742, XP002744849, * See page 2735 (Introd ucti on) *</td>
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<td>A, P</td>
<td>MOGLIA ET AL: &quot;Dual catalytic activity of hydroxycinnamoyl Coenzyme A quinonate transferase from tomato allows it to moonlight in the synthesis of both mono- and dicaffeoyl quinic acids&quot;, PLANT PHYSIOLOGY, vol. 166, 9 October 2014 (2014-10-09), pages 1777-1787, XP002744831, * See page 1783 (Figure 7); early online publication *</td>
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