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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 November 2011 (24.11.2011)

PCT

(10) International Publication Number
WO 2011/146873 A1

(51) International Patent Classification:
C12C 3/08 (2006.01) *C12C 3/12* (2006.01)

(21) International Application Number:
PCT/US2011/037398

(22) International Filing Date:
20 May 2011 (20.05.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/347,201 21 May 2010 (21.05.2010) US

(71) Applicant *(for all designated States except US)*: **MILLER BREWING INTERNATIONAL, INC.** [US/US]; 3939 West Highland Blvd., Milwaukee, WI 53208 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **TING, Patrick, L.** [US/US]; 230 Bunker Hill Dr., Brookfield, WI 53005 (US). **PRATT, Jason** [US/US]; 1110 E. Odgen Ave., Apt.214, Milwaukee, WI 53202 (US). **RYDER, David, S.** [US/US]; 10727 North Gazebo Hills Parkway, Mequon, WI 53092 (US).

(74) Agent: **ROCHE, Ricahrd, T.**; Quarles & Brady LLP, 411 E. Wisconsin Ave., Milwaukee, WI 53202 (US).

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

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

Published:

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

(54) Title: PREPARATION OF HOP ACIDS AND THEIR DERIVATIVES

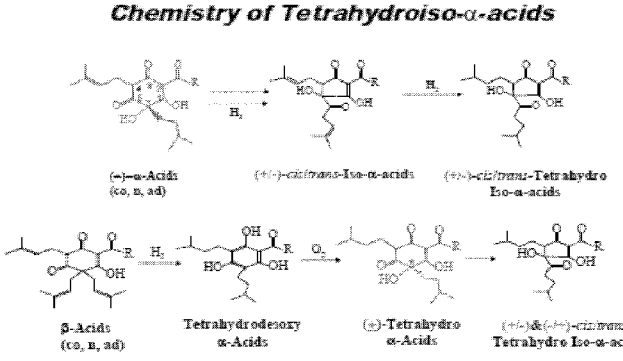


Figure 1

(57) Abstract: A method for preparing a hop acid mixture having an enantiomeric excess of a (+)-tetrahydro-α-acid is disclosed. In the method, a racemate of a tetrahydro- a-acid is contacted with an amine to form a precipitate having an enantiomeric excess of the (+)-tetrahydro-α-acid. A method for preparing a hop acid is also disclosed. In the method, a racemate of a tetrahydro-α-acid is contacted with an amine to form a precipitate comprising a (+)-tetrahydro-α-acid, and the (+)- tetrahydro-α-acid is isomerized to a hop acid selected from the group consisting of (+)-*trans*-tetrahydro-iso-α-acids, (-)-*cis*-tetrahydro-iso-α-acids, and mixtures thereof, and reduced to (+)-*trans*-hexahydroiso-α-acids and (-)-*cis*- hexahydroiso- α-acids. An additive for flavoring a malt beverage is also disclosed. The additive includes a bittering agent selected from the group consisting of (+)-*trans*- tetrahydro-iso-α-acids, (-)-*cis*-tetrahydro-iso-α-acids, (+)-*trans*-hexahydroiso- α-acids, (-)-*cis*-hexahydroiso-α-acids, and mixtures thereof.

WO 2011/146873 A1

Preparation of Hop Acids and their Derivatives

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims priority from U.S. Patent Application No. 61/347,201 filed May 21, 2010.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] Not Applicable.

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0003] This invention relates to novel hop acid compounds that provide improved flavor, foam, and antimicrobial contributions in malt beverages such as beer and active ingredients for health supplements. In particular, the invention relates to methods for preparing a hop acid mixture having an enantiomeric excess of a (+)-tetrahydro-α-acid, methods for preparing (+)-tetrahydro-α-acids that can be isomerized to (+)-*trans*-tetrahydro-iso-α-acids and (-)-*cis*-tetrahydro-iso-α-acids, and reduced to (+)-*trans*-hexahydroiso-α-acids and (-)-*cis*-hexahydroiso-α-acids and malt beverage bittering agents including the (+)-*trans*-tetrahydro-iso-α-acids, (-)-*cis*-tetrahydro-iso-α-acids, (+)-*trans*-hexahydroiso-α-acids, (-)-*cis*-hexahydroiso-α-acids, or mixtures thereof.

2. Description of the Related Art

[0004] Chiral recognition of substances, i.e. the ability to distinguish a molecular structure from its mirror image, is one of the most important and widespread principles of biological activity. The first molecular event in odor perception is the interaction of an odorant with a receptor. As olfactory receptors have been identified as proteins, i.e. chiral molecules, this interaction should also be enantioselective, meaning that odor receptors should react differently with the two enantiomeric forms of a chiral odorant, leading to differences in odor strength and/or quality. Discrepant enantiomer effects are well-established, with numerous examples in taste perception. For example, limonene is present in both orange and lemon peels and responsible for their different odor characteristics because orange contains the right-handed (+) molecule while lemon contains the left-handed (-) molecule; (S)-(+)-carvone is a molecule with caraway-like odor while its mirror image molecule (R)-(-)-carvone has a spearmint odor. Linalool is one of main key hop flavor components in beer, which optical isomers have great

impact on the character of hoppy flavor. (-)-Linalool is perceived with woody, lavender-like aroma, while its mirror image molecule, (+)-linalool, has sweet and citrus-like aroma.

[0005] Tetrahydroiso-α-acids (including three major analogs of tetrahydroisocohumulone, tetrahydrohumulone, and tetrahydroadhumulone) have shown more benefits in brewing than their analogous of iso-α-acids, ρ-iso-α-acids, and hexahydroiso-α-acids. Tetrahydroiso-α-acids impart the most bitter intensity, provide more light stability and flavor stability, enhance more foam, and exhibit stronger antimicrobial activity than the other hop bittering compounds in beer. Tetrahydroiso-α-acids are prepared from either α-acids (including three major analogs of cohumulone, n-humulone, and adhumulone) or β-acids (including three major analogs of colupulone, n-lupulone, and adlupulone) (See, P. Ting & H. Goldstein, J. Am. Soc. Brew. Chem. 54(2):103-109, 1996). From the α-acids (humulones), sequential hydrogenation and isomerization reactions or reversed isomerization and hydrogenation reactions of α-acids are involved as shown in Figure 1, wherein R = $CH_2CH(CH_3)_2$ for n-humulone, R = $CH(CH_3)_2$ for cohumulone, and R = $CH(CH_3)CH_2CH_3$ for adhumulone. From the β-acids (lupulones), multiple reactions are involved including a sequential hydrogenolysis/hydrogenation reaction of β-acids, oxidation reaction of the hydrogenated desoxy-α-acids and then an isomerization reaction of tetrahydro-α-acids as shown in Figure 1, wherein R = $CH_2CH(CH_3)_2$ for n-lupulone, R = $CH(CH_3)_2$ for colupulone, and R = $CH(CH_3)CH_2CH_3$ for adlupulone.

[0006] Both methods produce identical molecules of tetrahydroiso-α-acids, but only different from their stereoisomers. Tetrahydroiso-α-acids prepared from α-acids are optically active compounds, or enantiomers, due to the natural structure of α-acids (asymmetry molecules) (see, D. De Keukeleire and M. Verzele, J. Inst. Brewing, 76:265, 1970). However, tetrahydroiso-α-acids prepared from β-acids (no asymmetry molecules) are a racemic mixture (containing pairs of mirror image molecules or equal opposite enantiomers) with no optical activity (see, Patrick L. Ting and Henry Goldstein, J. Am. Soc. Brew. Chem. 54(2):103-109, 1996).

[0007] The molecular perception of stereochemistry of tetrahydroiso-α-acids and hexahydroiso-α-acids prepared from either α-acids or β-acids is very

important because of their potential flavor, foam, antimicrobial contributions in beer as well as important ingredients for nutraceuticals and functional food (see U.S. Patent No. 7,270,835). However, the stereochemistry and physiological properties (chiral recognition) have not been investigated and reported for tetrahydroiso-α-acids prepared from β-acids.

[0008] Therefore, there still exists a need for tetrahydroiso-α-acid compounds that improve flavor, foam, and antimicrobial contributions in malt beverages such as beer.

SUMMARY OF THE INVENTION

[0009] In one aspect, the invention provides a method for preparing a hop acid mixture having an enantiomeric excess of a (+)-tetrahydro-α-acid. In the method, a racemate of a tetrahydro-α-acid is contacted with an amine to form a precipitate having an enantiomeric excess of the (+)-tetrahydro-α-acid. The precipitate can be treated to prepare a solid having an enantiomeric excess of the (+)-tetrahydro-α-acid of greater than 50%, or more preferably greater than 80%. The amine can be a chiral amine such as (1S, 2R)-(-)-*cis*-1-amino-2-indanol. The racemate of the tetrahydro-α-acid can be prepared by hydrogenating a β-acid to prepare a desoxy-α-acid, and oxidizing and isomerizing the hydrogenated desoxy-α-acid to prepare the racemate of the tetrahydro-α-acid. In one version of the method, the β-acid is colupulone, and the desoxy-α-acid is tetrahydrodesoxycohumulone. The tetrahydro-α-acid can be selected from tetrahydrohumulone, tetrahydrocohumulone, and tetrahydroadhumulone. In one version of the method, the precipitate is separated from a filtrate, and the precipitate is treated such that the precipitate has an enantiomeric excess of the (+)-tetrahydro-α-acid of greater than 80%, and the filtrate is treated such that a solid recovered from the filtrate has an enantiomeric excess of a (-)-tetrahydro-α-acid of greater than 80%. A reversed solid-liquid process is possible using a different amine.

[0010] In another aspect, the invention provides a method for preparing a hop acid. In the method, a racemate of a tetrahydro-α-acid is contacted with an amine to form a precipitate comprising a (+)-tetrahydro-α-acid; and the (+)-tetrahydro-α-acid is isomerized to a hop acid selected from the group consisting of (+)-*trans*-tetrahydro-iso-α-acids, (-)-*cis*-tetrahydro-iso-α-acids, and mixtures thereof. The (+)-*trans*-tetrahydro-iso-α-acid can be selected from (+)-*trans*-tetrahydro-iso-

humulone, (+)-*trans*-tetrahydro-iso-cohumulone, and (+)-*trans*-tetrahydro-iso-adhumulone, and the (-)-*cis*-tetrahydro-iso-α-acid can be selected from (-)-*cis*-tetrahydro-iso-humulone, (-)-*cis*-tetrahydro-iso-cohumulone, and (-)-*cis*-tetrahydro-iso-adhumulone. The amine can be a chiral amine such as (1S,2R)-(-)-*cis*-1-amino-2-indanol. In one version of the method, the racemate of the tetrahydro-α-acid can be prepared by hydrogenating a β-acid to prepare a desoxy-α-acid, and oxidizing and isomerizing the hydrogenated desoxy-α-acid to prepare the racemate of the tetrahydro-α-acid.

[0011] In another aspect, the invention provides a method for preparing group of novel hop acids, (+)-tetrahydro-α-acids isomerized and reduced to a group selected from the group consisting of (+)-hexahydroiso-α-acids, (-)-hexahydroiso-α-acids, and mixtures thereof.

[0012] In yet another aspect, the invention provides an additive for flavoring a malt beverage, wherein the additive includes a bittering agent selected from the group consisting of (+)-*trans*-tetrahydro-iso-α-acids, a (-)-*cis*-tetrahydro-iso-α-acids, and mixtures thereof. The (+)-*trans*-tetrahydro-iso-α-acid can be selected from the group consisting of (+)-*trans*-tetrahydro-iso-humulone, (+)-*trans*-tetrahydro-iso-cohumulone, and (+)-*trans*-tetrahydro-iso-adhumulone, and the (-)-*cis*-tetrahydro-iso-α-acid can be selected from the group consisting of (-)-*cis*-tetrahydro-iso-humulone, (-)-*cis*-tetrahydro-iso-cohumulone, and (-)-*cis*-tetrahydro-iso-adhumulone.

[0013] In still another aspect, the invention provides novel ingredients for nutraceutical and functional foods, wherein the active ingredients includes a bittering agent selected from the group consisting of (+)-tetrahydro-α-acids, (+)-*trans*-tetrahydroiso-α-acids, (-)-*cis*-tetrahydroiso-α-acids, (+)-*trans*-hexahydroiso-α-acids, (-)-*cis*-hexahydroiso-α-acids, and mixtures thereof.

[0014] In yet another aspect, the invention provides a method for preparing a hop acid mixture. The method comprises contacting a racemate of tetrahydroiso-α-acids with a chiral amine to form a hop acid complex as a precipitate or in solution such that the hop acid complex has an enantiomeric excess of (+)-tetrahydroiso-α-acids. The enantiomeric excess of the resolved (+)-tetrahydroiso-α-acids can be greater than 50%, preferably greater than 60%, preferably greater than 70%, preferably greater than 80%, and preferably greater than 90%. The

resolved tetrahydroiso-α-acids can be enantiomerically pure. The resolved (+)-tetrahydroiso-α-acids can be reduced to a hop acid selected from the group consisting of (+)-trans-hexahydro-iso-α-acids, (-)-cis-hexahydro-iso-α-acids, and mixtures thereof.

[0015] In still another aspect, the invention provides a method for preparing a hop acid mixture. The method comprises resolving a racemate of tetrahydroiso-α-acids with a chiral column chromatography to separate an enantiomeric excess of (+)-tetrahydroiso-α-acids. The enantiomeric excess of the resolved (+)-tetrahydroiso-α-acids can be greater than 50%, preferably greater than 60%, preferably greater than 70%, preferably greater than 80%, and preferably greater than 90%. The resolved tetrahydroiso-α-acids can be enantiomerically pure. The resolved (+)-tetrahydroiso-α-acids can be reduced to a hop acid selected from the group consisting of (+)-trans-hexahydro-iso-α-acids, (-)-cis-hexahydro-iso-α-acids, and mixtures thereof.

[0016] In yet another aspect, the invention provides an additive for flavoring a malt beverage wherein the additive comprises a bittering agent selected from the group consisting of (+)-trans-tetrahydroiso-α-acids, (-)-cis-tetrahydroiso-α-acids, (+)-trans-hexahydroiso-α-acids, (-)-cis-hexahydroiso-α-acids, and mixtures thereof. In still another aspect, the invention provides a malt beverage including the additive wherein the bittering agent is present in the malt beverage at a level of 1 ppm to 100 ppm.

[0017] In still another aspect, the invention provides an active ingredient for a health supplement wherein the ingredient comprises a hop acid selected from the group consisting of (+)-tetrahydro-α-acids, (+)-trans-tetrahydro-iso-α-acids, (-)-cis-tetrahydro-iso-α-acids, (+)-trans-hexahydro-iso-α-acids, (-)-cis-hexahydroiso-α-acids, and mixtures thereof.

[0018] These and other features, aspects, and advantages of the present invention will become better understood upon consideration of the following detailed description, drawings and appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] Figure 1 shows a scheme of tetrahydroiso-α-acids preparation from either α-acids or β-acids.

[0020] Figure 2 shows the analytical HPLC resolution of (±)-tetrahydrocohumulone by a 250x4.6mm β–Cyclobond column with 75% CH_3CN + 25% of 1% acetic acid in 20% CH_3OH/H_2O) at 280 nm and 1.2 ml/min.

[0021] Figure 3 is a diagram of the dynamic resolution of (±)-THCO.

[0022] Figure 4 is a plot of circular dichroism of (±),(+), and (-)-THCO.

[0023] Figure 5 shows the chiral HPLC separation of isomerized (+) and (-)-THCO (top) and two CD spectra of (+) vs. (-)-*trans*-THICO (bottom).

DETAILED DESCRIPTION OF THE INVENTION

[0024] In one example method of invention, dynamic resolution of a racemic tetrahydro-α-acid mixture has been achieved. Suitable solvents for the resolution can be selected such that at least some of the desired diastereomeric solid precipitates in the solvent and the other member of the pair remains dissolved in the solution. Non-limiting example solvents include substituted or unsubstituted aliphatic or alicyclic hydrocarbons. Some preferred solvents are hexane, cyclohexane and toluene. The solvents and the temperature of the dynamic resolution will vary with the particular hop acid subjected to the resolution.

[0025] Precipitation of the desired diastereomeric solid can be achieved with a chiral amine. A preferred amine is one that allows formation of a pair of diastereomeric solids, one member of the pair of diastereomers being at least partially insoluble in the solvent system of the process. A preferred amine is one that allows formation of a pair of diastereomers, one member of the pair being preferentially a precipitate under the reaction conditions. The precipitate can be crystalline or non-crystalline. The chiral amine can be, for example, (1S,2R)-(+)-*cis*-1-amino-2-indanol. Other chiral amines can be expected to be useful in effecting the resolution of the hop acid.

[0026] The less soluble diastereomeric salt from the reaction can be isolated, for example, by filtration, centrifugation, or decantation. For instance, the reaction mixture is cooled to room temperature and the resulting precipitate is recovered by filtration. The filter cake containing the product can be washed with a washing solvent such as an aliphatic hydrocarbon (e.g., hexane). Once isolated, the precipitated diastereomeric salt can be liberated from its complexed chiral amine by reaction with a suitably strong acid. Non-limiting example acids include sulfuric acid, phosphoric acid and hydrochloric acid. The diastereomeric compound that remains in solution in the filtrate can also be isolated with an acid.

[0027] Dynamic resolution of the racemate of a tetrahydro-α-acid or racemate of a tetrahydroiso-α-acid in accordance with the invention can produce an enantiomeric excess of the one of the resolved tetrahydro-α-acids or tetrahydroiso-α-acids of greater than 50%, preferably greater than 60%, preferably greater than 70%, preferably greater than 80%, and preferably greater than 90%. The resolved tetrahydro-α-acid or (+)-tetrahydroiso-α-acid can be enantiomerically pure. In one form, the resolved tetrahydro-α-acid is (+)-tetrahydrocohumulone.

[0028] The resolved tetrahydro-α-acids can be isomerized to tetrahydro-iso-α-acids by boiling in a suitable solvent such as an ethanol-water mixture, optionally in the presence of a catalyst such as a calcium or magnesium salt. Other isomerization techniques can be used. The tetrahydroiso-α-acid compounds can provide improved flavor, foam, and antimicrobial contributions when added to malt beverages such as beer. In one form, the tetrahydro-iso-α-acid is (+)-*trans*-tetrahydro-iso-cohumulone or (-)-*cis*-tetrahydro-iso-cohumulone. The resolved tetrahydro-α-acids can be reduced to (+)-*trans*-hexahydroiso-a-acids and (-)-*cis*- hexahydroiso-a-acids.

[0029] A variety of optical isomers have been described as having different odor qualities and/or different odor intensities. Such considerations prompted the following experimental study, which aimed to resolve gram quantities of each enantiomer of (±)-tetrahydrocohumulone and to assess their isomerized enantiomers for bitterness, foam quality, and antimicrobial activity. The following Examples are presented for purposes of illustration and not of limitation.

Examples

HPLC of (±)-tetrahydrocohumulone (THCO) and (+/-) and (-/+)-*cis/trans*-tetrahydroisocohumulones (THICO)

[0030] A 5 µm 250 x 4.6 mm Cyclobond I 2000 column (Advanced Separation Technologies Inc.) was used. To resolve (±)-THCO, an isocratic mixture of 25% A (CH_3CN) and 75% B (1% acetic acid +20% methanol/H_2O) was used as the mobile phase at a flow rate 1.2 mL/min and detection at 280 nm. Two enantiomers, (-) and (+)-THCO, were eluted, respectively in Figure 2. (-)-THCO was identified by the hydrogenated α-acids standard. To resolve (±)-*cis* and (±)-*trans*-THICO, an isocratic mixture of 35% A (CH_3CN) and 65% B (0.01M sodium citrate +20% methanol/H_2O) was used as the mobile phase at a flow rate

1.2 mL/min and detection at 254 nm. The elution order was (-)-*trans*, (+)-*trans*, (-)-*cis*, and (+)-*cis*-THICO identified by the retention times of (+)-*cis*-THICO and (-)-*trans*-THICO standards as shown in Figure 5.

Preparation of tetrahydrodesoxycohumulone

[0031] To a solution of 50 g hexane-crystallized colupulone (0.125 moles) in 250 mL of ethanol was added 10 mL of concentrated sulfuric acid and 5 g of 5% Pd/C catalyst. The mixture was stirred and hydrogenated under 10 psig hydrogen gas in an autoclave. The hydrogenation reaction was completed after 30 min. at 35-50°C. The vessel was purged with nitrogen and the mixture was filtered to give a clear yellow solution of tetrahydrodesoxycohumulone, used directly in the next step.

Oxidation with peracetic acid of tetrahydrodesoxycohumulone to (±)-tetrahydrocohumulone (THCO)

[0032] To the above solution was added 23.75 g of 40% peracetic acid (0.125 moles) slowly in a three-neck round bottom flask which was equipped with a thermometer, a condenser, and additional funnel. After addition, the reaction was heated to 50-60°C for 1 hour and allowed to cool to room temperature. About 100 mL tap water was added and stirred for 1 hour. The ethanol was recovered under vacuum and 200 mL hexane was added to solubilize the THCO in aqueous solution. After a phase separation, the hexane solution was washed with tap water twice to afford 35 g of THCO.

Resolution of (±)-tetrahydrocohumulone (THCO)

[0033] To a solution of 11 g of (±)-tetrahydrocohumulone (THCO) (0.031 moles), 20 mL toluene and 300 mL cyclohexane was added 5.5 g of (1S, 2R)-(-)-*cis*-1-amino-2-indanol (AI) (0.037 moles) in 50 mL cyclohexane. The solution was boiled for 15 min. and allowed to cool at room temperature. A yellow solid was crystallized and filtered from the solution. The yellow solid was mixed with 100 mL hexane and 100 mL of 2N HCl. The hexane phase was washed three times with water. After drying with anhydrous magnesium sulfate, the solvent was removed under vacuum to yield 4.65 g of (+)-THCO and confirmed by a chiral HPLC (β-Cyclobond column) with 90% enantiomeric excess (e.e.). The filtrate was acidified with 100 mL of 2N HCl and washed with water three times. After drying with anhydrous magnesium sulfate, the solvent was removed by

vacuum to afford 5.3 g of 86% e.e. (-)-THCO and confirmed by β-Cyclobond HPLC.

Isomerization of (+), (-), and (±)-tetrahydrocohumulone (THCO) to *cis/trans*-tetrahydroisocohumulone

[0034] To 2.5 g of THCO (7.1 mmoles) and 50 mL ethanol was stirred and heated with 0.3 g NaOH and 43 mg of magnesium sulfate. The isomerization reaction was refluxed for 2 hours and allowed to cool at room temperature. The resulted solution was acidified by 2N HCl and ethanol was recovered by vacuum. The resulted oil was extracted by 50 mL hexane and two phases were separated. The hexane phase was dried by anhydrous magnesium sulfate and the solvent was removed by vacuum to yield enantiomers and a racemate of *cis/trans*-tetrahydroisocohumulone (THICO).

Conclusion

[0035] (1S, 2R)-(-)-*cis*-1-Amino-2-indano is an unequivocal chiral reagent for resolving racemic hop acids. It reacts with (±)-tetrahydrocohumulones to selectively produce a crystal form of (+)-tetrahydrocohumulone (THCO)/(1S, 2R)-(-)-*cis*-1-amino-2-indano from the solution of (-)-THCO and (1S, 2R)-(-)-*cis*-1-amino-2-indano. It is a dynamic process that can easily process several grams of (±)-tetrahydro-a-acids for any application. The (+)-THCO is a novel bittering precursor as well as its derivatives of (-)-*cis*/(+)-*trans*-tetrahydroisocohumulone (THICO). On the other hand, its counterpart, (-)-THCO, is identical to the hydrogenated (-)-cohumulone (a nature α-acid). Three THICO molecules can be enantioselectively distinguished by our receptors, leading to different odor intensities and odor qualities. THICO II from its (+)-THCO have the most bitterness and longest lingering and (±)-THICO III have milder and smooth bitterness than THICO I from (-)-THICO. The foam situation is more complicated by findings of enantioselective effects of each chiral isomer with damaged conformation of LTP (a foam protein) during the kettle boiling. Three THICO I, II, and III molecules demonstrate the same antibacterial effectiveness using the minimum inhibitory concentration (MIC) and the bacterial zone of inhibition (BZI) tests on *Pediococcus damnosus* and *Lactobacillus brevis*. It clearly indicates that the enantioselectively antibacterial interactions between THICO I, II, III and the microbial do not occur.

Results and Discussion

[0036] The molecular perception of stereochemistry of tetrahydroiso-α-acids prepared from either α-acids or β-acids is very important because of their potential flavor, foam, antimicrobial contributions in beer. Verzele and De Keukeleire (D. De Keukeleire and M. Verzele, J. Inst. Brewing, 76:265, 1970; "Chemistry and analysis of Hop and Beer Bitter Acids", M. Verzele and D. De Keukeleire, Elsevier, 1991) have established the R-configuration and (-)-optical rotation of α-acids with an asymmetric center at C-6 and also determined the optical properties of their isomerized derivatives denoted as (+)-*cis*-iso-α-acids and (-)-*trans*-iso-α-acids. The hydrogenation of either α-acids or isomerized-α-acids retains the chirality, in other words, no optical property is changed. On the other hand, the β-acids prepared tetrahydroiso-α-acids are a racemic mixture consisting of two pairs of (+/-)-*cis*/*trans*- and (-/+)-*cis*/*trans*-isomers from the isomerization of a racemic mixture of (±)-tetrahydro-α-acids. Because the β-acids are dissymmetric or achiral compounds, the hydrogenolysis/hydrogenation of β-acids produce planar molecules, tetrahydro desoxy-α-acids with a C2 symmetry. At the oxidation step, a chiral center at C-6 is introduced to generate a pair of (±)-tetrahydro-α-acids as shown in Figure 1.

Resolution by Liquid Chromatography

[0037] Ting and Goldstein confirmed the optical rotations of the hydrogenated iso-α-acids as (+)-*cis*- and (-)-*trans*-tetrahydroiso-α-acids. (Patrick L. Ting and Henry Goldstein, J. Am. Soc. Brew. Chem. 54(2):103-109, 1996), while a zero value of optical rotations of (±)-*cis*- and (±)-*trans*-tetrahydroiso-α-acids is obtained from the β-acids preparation. Ting and Goldstein successfully resolved and assigned (±)-tetrahydro-α-acids using a combination of a semi-preparative C-18 column and an analytical Cyclobond HPLC column (β-Cyclodextrin bonded on 5μ silica gel, a chiral phase column). The column was also used to resolve most of (+) and (-)-enantiomers and diastereomers of total (±)-tetrahydroiso-α-acids.

[0038] To evaluate the properties of each enantiomer of tetrahydroiso-α-acids in beer, a gram quantity of substances is needed. Due to the complex compositions of hop bittering compounds (containing at least of 12 compounds with 3 major analogs and 2 diastereoisomers and 2 enantiomers), a strategy of

simplifying the resolution process is starting with colupulone, one component of β-acids, to produce (±)-tetrahydrocohumulone. Resolution of (±)-tetrahydrocohumulone (THCO), bittering precursors, should be less complicated than their isomerized (±)-*cis*- and (±)-*trans*-tetrahydroisocohumulones (THICO).

[0039] An analytical chiral HPLC (high pressure liquid chromatography) column (β-Cyclobond) was used to analyze and identify the resolved compounds as shown in Figure 2. In Figure 2, (±)-THCO is well-resolved into (-)-THCO identified by authentic (-)-tetrahydro-α-acids and eluted before (+)-THCO. Since a chiral liquid chromatography (LC) was a prevalent technique of resolving enantiomers, two β-Cyclobond 10 x 2" and 20 x 2" columns simulated to the analytical conditions were used to separate the racemic mixture of (±)-THCO at milligrams to gram quantities. The resolution of (±)-THCO was poor and ineffective.

Resolution by Dynamic Crystallization

[0040] Alternatively, using an (-)-alkaloid, (1S, 2R)-(-)-*cis*-1-amino-2-indanol (AI) to react with (±)-THCO becomes a dynamic resolution technique (Chemical & Engineering News, September 9, 2002). Two diastereomeric salts were formed; one, (+)-THCO/(-)-alkaloid, was crystallized out and left one, (-)-THCO/(-)-alkaloid in the solution as shown in Figure 3. After acidification, it regenerated high optically pure (+) and (-)-THCO, separately, as well as more than gram quantities yield sufficient to perform various tests. A chiral HPLC and CD (Circular Dichroism) confirmed the optical purity and optical spectrum of resolved (+) and (-)-THCO vs. (±)-THCO (Figure 4). The identity of (-)-THCO were confirmed by comparison of the retention time of chiral HPLC and CD spectrum with the hydrogenated α-acids. The (+)-THCO is a novel bittering precursor having an opposite CD spectrum as (-)-THCO which is a hydrogenated natural α-acid. The isomerization of (+)-THCO produced two novel (-)-*cis*/(+)-*trans*-tetrahydroisocohumulone (THICO) in opposite to (-)-THCO produced (+)-*cis*/(-)-*trans*-THICO identical to the hydrogenated natural iso-α-acids. Figure 5 shows a chiral HPLC separation/resolution of (+/-) and (-/+)-*cis*- and *trans*-THICO and two CD spectra of (+)- and (-)-*trans*-THICO. The bitter perception, foam quality, and antimicrobial activity of three molecules and their derivatives of (+/-)-*cis*/*trans*-THICO (THICO I), (-/+)-*cis*/*trans*-THICO (THICO II),

and (±)-*cis*/*trans*-THICO (THICO III) were investigated, respectively.

Bitterness Perception

[0041] An aqueous 5% v/v ethanol/H_20 solution was spiked with 6 ppm of THICO I, II, and III. The bitterness of three molecular perceptions is summarized in Table 1. It indicates that the bitterness intensity is II>III>I and the bitter perception of III is smooth and milder than the others.

Table 1. Bitterness of three THICO I, II, and III in 5% ethanol/H_2O

THICO I (natural)	THICO II (novel)	THICO III (racemic)
Less bitter at back of tongue, harsh, slight lingering	Most bitter and lingering, astringent, bitter in whole mouth	Stronger than I, but similar, smooth, milder, clean bitterness

[0042] Two sets of unhopped lagers (A and B) were spiked with 6 ppm and 13 ppm of THICO I, II, and III, respectively. A C-18 reversed phase HPLC analysis of *cis*/*trans*-THICO present in each beer is shown in Table 2.

Table 2. HPLC analysis of *cis*/*trans*-tetrahydroisocohumulones (THICO) in Beer

	THICO I (ppm)	THICO II (ppm)	THICO III (ppm)
A	5.6	6.0	6.0
B	13.7	12.2	13.8

[0043] Sensory evaluation indicated that beer with THCO II was noted as having the strongest initial bitterness and lingered the longest. The other two beers were noted as being similar with the THICO I having a little more initial bitterness and the bitterness in the THICO III beer diminished quickly. In set B, THICO II beer had a strong initial bitterness that increased (described as late bitterness) and also lingered. The other two beers were noted as being similar with initial intense bitterness that diminished slowly with slight lingering bitterness. It indicates that our odor receptors can differentiate two enantiomeric THICO I and II, leading to differences in bitter strength and quality.

Foam

[0044] One major factor of beer foam is an interaction of a lipid transfer protein (LTP) from barley with the hop bittering compounds. (see, L. Lusk, H. Goldstein, D. Ryder, J. Amer. Soc. Brew. Chem. 53(3):93-103, 1995). Tetrahydroiso-α-acids interact preferentially with LTP due to their greater hydrophobicity. (see, K.

Takeshi and T. Shellhammer, *J. Agric. Food Chem.*, 2008, *56* (18), pp 8629–8634). The Nibem and half-life foam test of three bittering molecules in beers in set A do not show any significant differences (see Table 3). Discrepancy of the enantioselective effects between enantiomers of THICO and LPT is not clear in the beer foam formation. It might be due to disruption of the conformation of LPT which has been known to be damaged after long kettle boiling (Sandra N. E. Van Nierop, David E. Evans, Barry C. Axcell, Ian C. Cantrell, and Marina Rautenbach, J. Agric. Food Chem., 2004, 52 (10), pp 3120–3129; E. N. Clare Mill, Chunli Gao, Peter J. Wilde, Neil M. Rigby, Ramani Wijesinha—Bettonis, Victoria E. Johnson, Lorna J. Smith and Alan R. Mackie, Biochemistry, 2009, 48 (51), pp 1208-12088).

Table 3. Results of beer foam and bittering molecules

	Nibem 30 sec.	Half-Life
THICO I	255	4.7
THICO II	259	5.0
THICO III	246	5.3

Antimicrobial activity of THICO I, II, III and minimum inhibitory concentration (MIC) and bacterial zone of inhibition (BZI)

[0045] The antimicrobial effect of three molecules was tested on *Pediococcus damnosus* and *Lactobacillus brevis* with two methods (MIC and BZI). MIC was determined based on the concentration at which no bacteria were detected in the modified BMB without Tween 80 culture medium. The result is summarized in Table 4 and the MIC is 16 ppm for both THICO I and II. The average diameters of the zones of bacterial inhibition in Universal Beer Agar (UBA) produced by the filter paper disks immersed in 4000 ppm of THICO I, II, and III in 70% ethanol/water are shown in Table 5. Zone diameters increased with the same rates for three molecules on two different organisms (*Pediococcus damnosus* and *Lactobacillus brevis*) indicate that all molecules have the same antibacterial effectiveness. No enantioselective antibacterial interactions between THICO I, II, III and the microbial occur.

Table 4. Minimum inhibitory concentration of THICO I and II

Pediococcus damnosus										
Minimum Inhibitory Concentration of Hop Acids in 70% Ethanol										
ppm -->	128	64	32	16	8	4	2	1	0.5	0
THICO I	-	-	-	+/-	+	+	+	+	+	+
THICO II	-	-	-	+/-	+	+	+	+	+	+

+ = Growth of beer spoilage bacteria
- = No growth of beer spoilage bacteria
+/- = Partial inhibition of bacterial growth

Table 5. Antimicrobial effect of THICO I, II, and III on bacterial

Diameter of Bacterial Zone of Inhibition (mm)		
	Pediococcus damnosus	*Lactobacillus brevis*
Control	0	0
THICO I	11.5	26
THICO II	12	26.5
THICO III	12	22.5

[0046] Thus, in the present invention, resolution of a racemic (±)-tetrahydrocohumulone (or tetrahydro-α-acid) and their isomerized tetrahydroisocohumulones (or tetrahydroiso-α-acid) has been achieved in gram quantity by a dynamic crystallization with (1S, 2R)-(-)-1-amino-2-indanol. The resolved (+)-tetrahydrocohumulone (THCO) is a novel bittering precursor while the (-)-THCO is identical to the hydrogenated (-)-cohumulone (a natural α-acid). Both enantiomers are isomerized to the same molecular structures, but with opposite optical rotations. The (+)-THCO is converted into two novel bittering diastereomers, (-)-*cis*- and (+)-*trans*- tetrahydro isocohumulone (THICO II) while (-)-THCO is converted into (+)-*cis*- and (-)-*trans*-THICO (THICO I) identical to the hydrogenated *cis* and *trans*-isocohumulone (a natural iso-α-acid).

[0047] Sensory indicates that the bitter intensity of three molecules is THICO II > (±)-THICO III > THICO I. The perception of (±)-THICO III is smooth, clean and milder than I and II. In the foam situation, it seems no apparent foam quality differences among three molecular beers. In other words, no clear discrepancy of enantioselective effects among three molecules and lipid transfer protein (LTP) is found. It may be due to destruction of LTP conformation during long kettle boiling.

[0048] The minimum inhibitory concentration (MIC) of THICO I and II is similar at 16 ppm against *Pediococcus damnosus*. Zone diameters increased with the same rates for three THICO I, II, and III molecules on two different organisms

(*Pediococcus damnosus* and *Lactobacillus brevis*) indicate that all exhibit the same antibacterial effectiveness or no enantioselective antibacterial effect among THICO I, II, III and the microbial.

[0049] Although the invention has been described in considerable detail with reference to certain embodiments, one skilled in the art will appreciate that the present invention can be practiced by other than the described embodiments, which have been presented for purposes of illustration and not of limitation. Therefore, the scope of the appended claims should not be limited to the description of the embodiments contained herein.

CLAIMS

What Is Claimed Is:

1. A method for preparing a hop acid mixture, the method comprising:

contacting a racemate of tetrahydro-α-acids with a chiral amine to form a hop acid complex as a precipitate or in solution, the hop acid complex having an enantiomeric excess of (+)-tetrahydro-α-acids.

2. The method of claim 1 further comprising:

isomerizing the (+)-tetrahydro-α-acids to hop acids selected from the group consisting of (+)-*trans*-tetrahydro-iso-α-acids, (-)-*cis*-tetrahydro-iso-α-acids, and mixtures thereof.

3. The method of claim 1 further comprising:

isomerizing and reducing the (+)-tetrahydro-α-acids to hop acids selected from the group consisting of (+)-*trans*-hexahydro-iso-α-acids, (-)-*cis*-hexahydro-iso-α-acids, and mixtures thereof.

4. The method of claim 2 further comprising:

reducing the (+)-tetrahydroiso-α-acids to hop acids selected from the group consisting of (+)-*trans*-hexahydro-iso-α-acids, (-)-*cis*-hexahydro-iso-α-acids, and mixtures thereof.

5. The method of claim 1 further comprising:

treating the hop acid complex to prepare a solid having an enantiomeric excess of the (+)-tetrahydro-α-acids of greater than 50%.

6. The method of claim 1 further comprising:

treating the hop acid complex to prepare a solid having an enantiomeric excess of the (+)-tetrahydro-α-acids of greater than 80%.

7. The method of claim 1 wherein:

the amine is (1S, 2R)-(-)-*cis*-1-amino-2-indanol.

8. The method of claim 1 wherein:

the racemate of the tetrahydro-α-acid is prepared by hydrogenating a β-acid to prepare a desoxy-α-acid, and oxidizing and isomerizing the hydrogenated desoxy-α-acid to prepare the racemate of the tetrahydro-α-acid.

9. The method of claim 8 wherein:

the β-acid is colupulone, and

the desoxy-α-acid is tetrahydrodesoxycohumulone.

10. The method of claim 1 wherein:

the tetrahydro-α-acid is selected from tetrahydrohumulone, tetrahydrocohumulone, and tetrahydroadhumulone.

11. A method for preparing a hop acid mixture, the method comprising:

resolving a racemate of tetrahydro-α-acids with a chiral column chromatography to separate an enantiomeric excess of (+)-tetrahydro-α-acids.

12. The method of claim 11 further comprising:

isomerizing the (+)-tetrahydro-α-acids to hop acids selected from the group consisting of (+)-*trans*-tetrahydro-iso-α-acids, (-)-*cis*-tetrahydro-iso-α-acids, and mixtures thereof.

13. The method of claim 12 wherein:

the (+)-*trans*-tetrahydro-iso-α-acid is selected from (+)-*trans*-tetrahydro-iso-humulone, (+)-*trans*-tetrahydro-iso-cohumulone, and (+)-*trans*-tetrahydro-iso-adhumulone, and

the (-)-*cis*-tetrahydro-iso-α-acid is selected from (-)-*cis*-tetrahydro-iso-humulone, (-)-*cis*-tetrahydro-iso-cohumulone, and (-)-*cis*-tetrahydro-iso-adhumulone.

14. The method of claim 11 further comprising:

isomerizing and reducing the (+)-tetrahydro-α-acids to hop acids selected from the group consisting of (+)-*trans*-hexahydro-iso-α-acids, (-)-*cis*-hexahydro-iso-α-acids, and mixtures thereof.

15. The method of claim 14 wherein:

the (+)-*trans*-hexahydro-iso-α-acid is selected from (+)-*trans*-hexahydro-iso-humulone, (+)-*trans*-hexahydro-iso-cohumulone, and (+)-*trans*-hexahydro-iso-adhumulone, and

the (-)-*cis*-hexahydro-iso-α-acid is selected from (-)-*cis*-hexahydro-iso-humulone, (-)-*cis*-hexahydro-iso-cohumulone, and (-)-*cis*-hexahydro-iso-adhumulone.

16. The method of claim 13 further comprising:

reducing the (+)-tetrahydroiso-α-acids to hop acids selected from the group consisting of (+)-*trans*-hexahydro-iso-α-acids, (-)-*cis*-hexahydro-iso-α-acids, and mixtures thereof.

17. The method of claim 16 wherein:

the (+)-*trans*-hexahydro-iso-α-acid is selected from (+)-*trans*-hexahydro-iso-humulone, (+)-*trans*-hexahydro-iso-cohumulone, and (+)-*trans*-hexahydro-iso-adhumulone, and

the (-)-*cis*-hexahydro-iso-α-acid is selected from (-)-*cis*-hexahydro-iso-humulone, (-)-*cis*-hexahydro-iso-cohumulone, and (-)-*cis*-hexahydro-iso-adhumulone.

18. A method for preparing a hop acid mixture, the method comprising:

contacting a racemate of tetrahydroiso-α-acids with a chiral amine to form a hop acid complex as a precipitate or in solution, the hop acid complex having an enantiomeric excess of (+)-tetrahydroiso-α-acids.

19. The method of claim 18 further comprising:

reducing the (+)-tetrahydroiso-α-acids to hop acids selected from the group consisting of (+)-*trans*-hexahydro-iso-α-acids, (-)-*cis*-hexahydro-iso-α-acids, and mixtures thereof.

20. The method of claim 18 further comprising:

treating the hop acid complex to prepare a solid having an enantiomeric excess of the (+)-tetrahydroiso-α-acids of greater than 80%.

21. A method for preparing a hop acid mixture, the method comprising:

resolving a racemate of tetrahydroiso-α-acids with a chiral column chromatography to separate an enantiomeric excess of (+)-tetrahydroiso-α-acids.

22. The method of claim 21 further comprising:

reducing the (+)-tetrahydroiso-α-acids to hop acids selected from the group consisting of (+)-*trans*-hexahydro-iso-α-acids, (-)-*cis*-hexahydro-iso-α-acids, and mixtures thereof.

23. An additive for flavoring a malt beverage, the additive comprising:

a bittering agent selected from the group consisting of (+)-*trans*-tetrahydroiso-α-acids, (-)-*cis*-tetrahydroiso-α-acids, (+)-*trans*-hexahydroiso-α-acids, (-)-*cis*-hexahydroiso-α-acids, and mixtures thereof.

24. A malt beverage including the additive of claim 23, wherein the bittering agent is present in the malt beverage at a level of 1 ppm to 100 ppm.

25. An active ingredient for a health supplement, the ingredient comprising:

a hop acid selected from the group consisting of (+)-tetrahydro-α-acids, (+)-*trans*-tetrahydro-iso-α-acids, (-)-*cis*-tetrahydro-iso-α-acids, (+)-*trans*-hexahydro-iso-α-acids, (-)-*cis*-hexahydroiso-α-acids, and mixtures thereof.

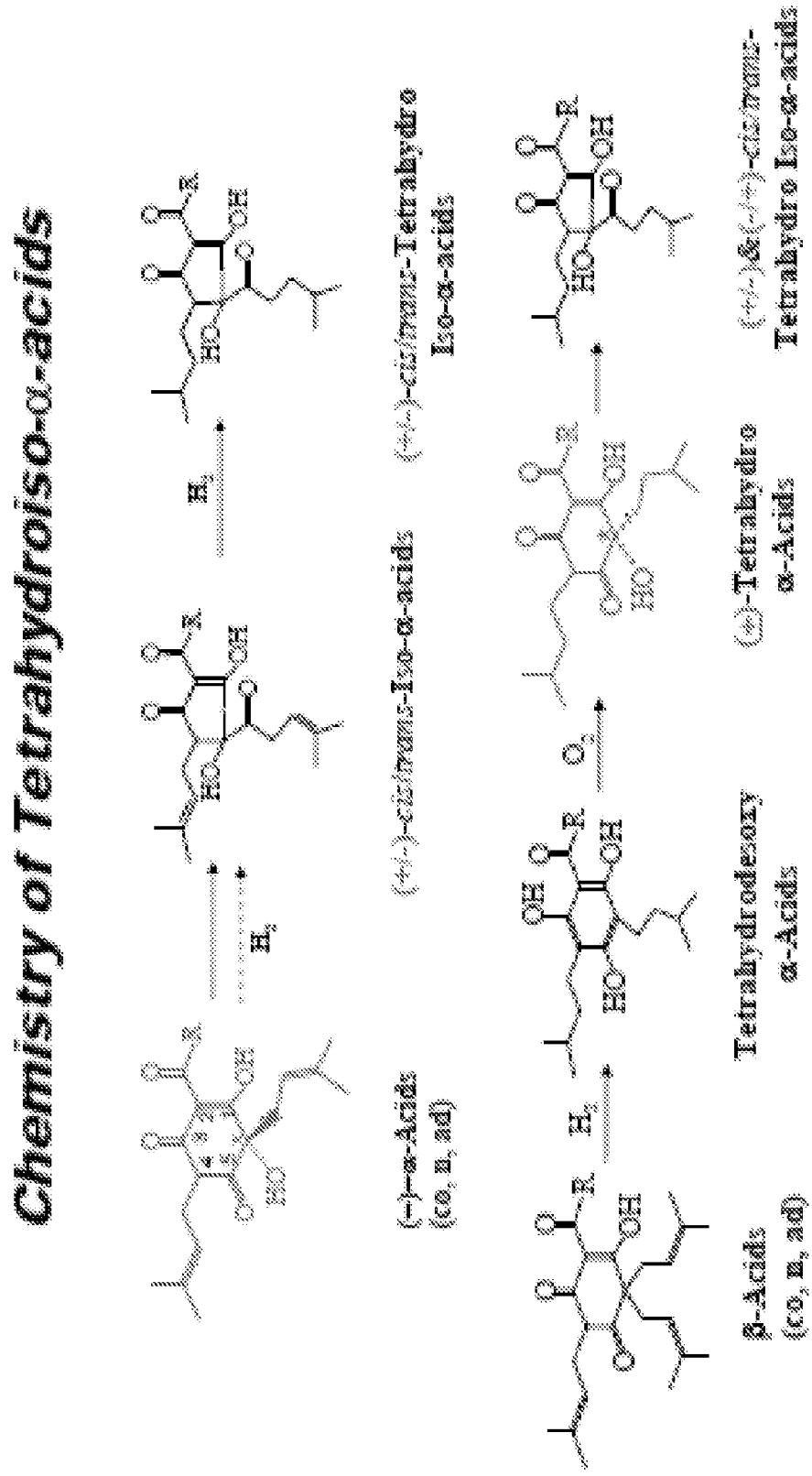

Chemistry of Tetrahydroiso-α-acids
(−)-α-Acids (co, n, ad)
(+/−)-cis/trans-Iso-α-acids
(+/−)-cis/trans-Tetrahydro Iso-α-acids
β-Acids (co, n, ad)
Tetrahydrodeoxy α-Acids
(+)-Tetrahydro α-Acids
(+/−)&(+/−)-cis/trans-Tetrahydro Iso-α-acids

Figure 1

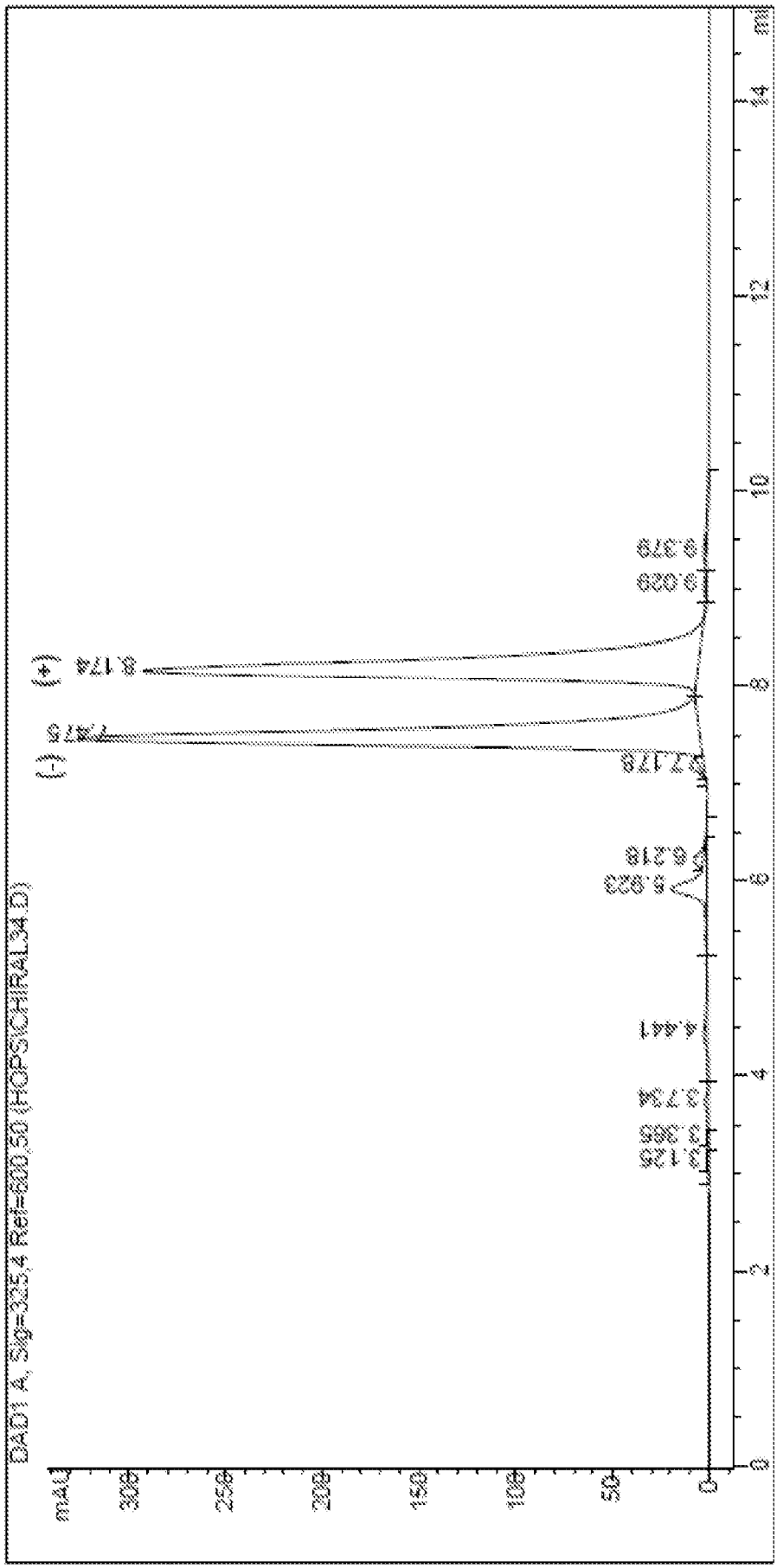

DAD1 A, Sig=325,4 Ref=600,50 (HOPS\CHIRALCD.D)
mAU
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
min
3.125
3.355
3.734
4.441
5.823
6.218
7.175
7.875
8.174
9.029
9.329
(-)
(+)

Figure 2

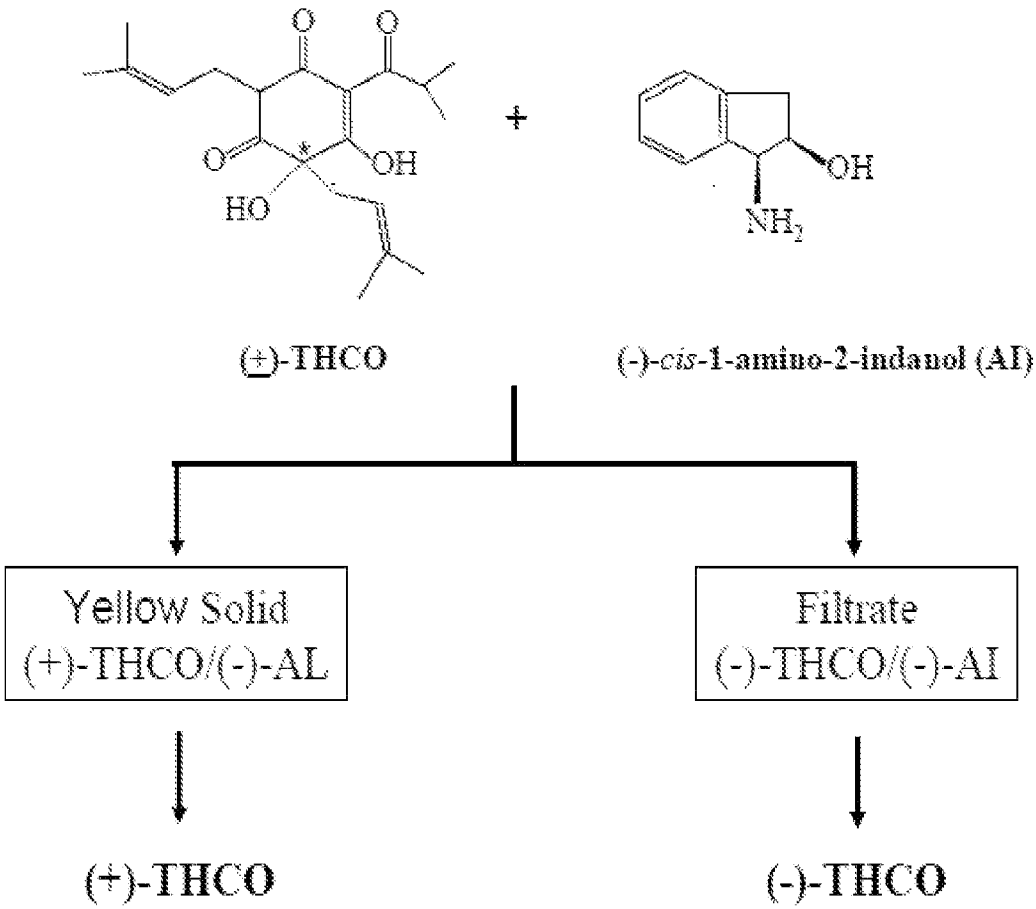

O
O
O
OH
HO
*
+
OH
NH2
(±)-THCO
(-)-cis-1-amino-2-indanol (AI)
Yellow Solid
(+)-THCO/(-)-AL
Filtrate
(-)-THCO/(-)-AI
(+)-THCO
(-)-THCO

Figure 3

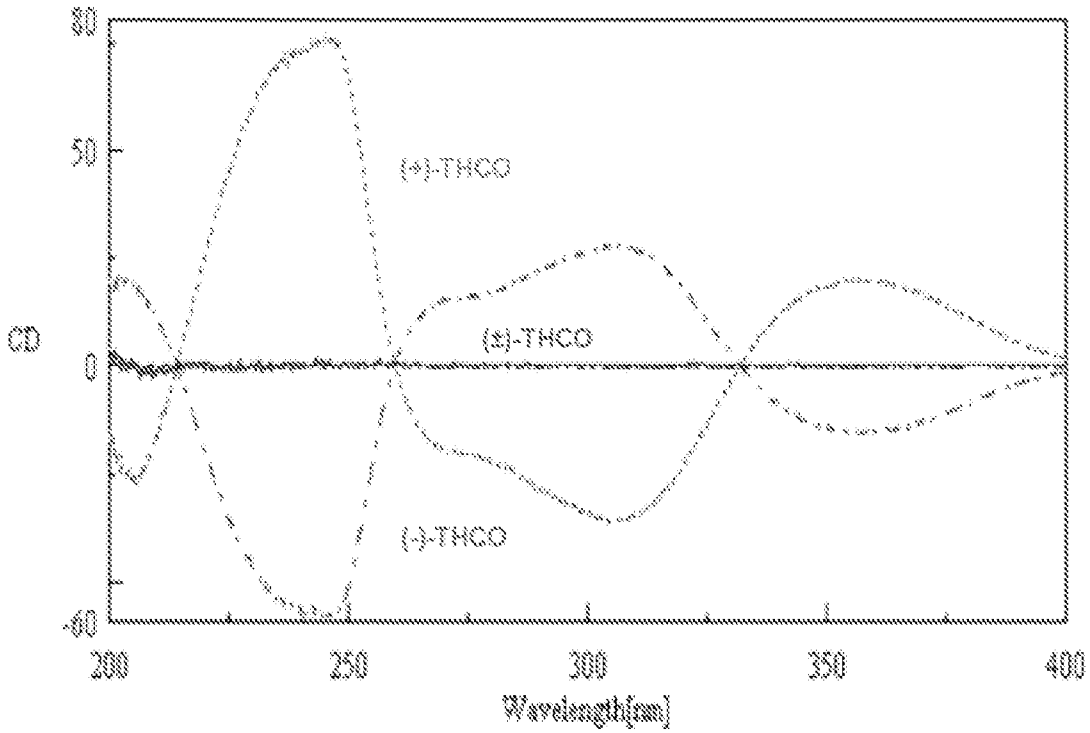
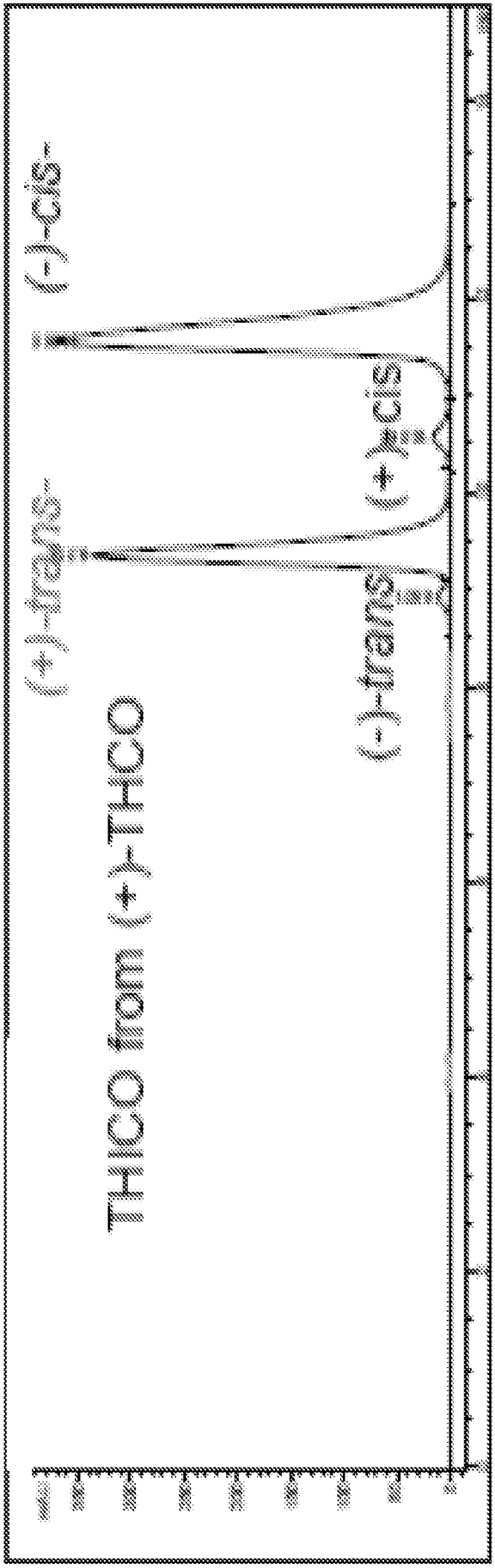
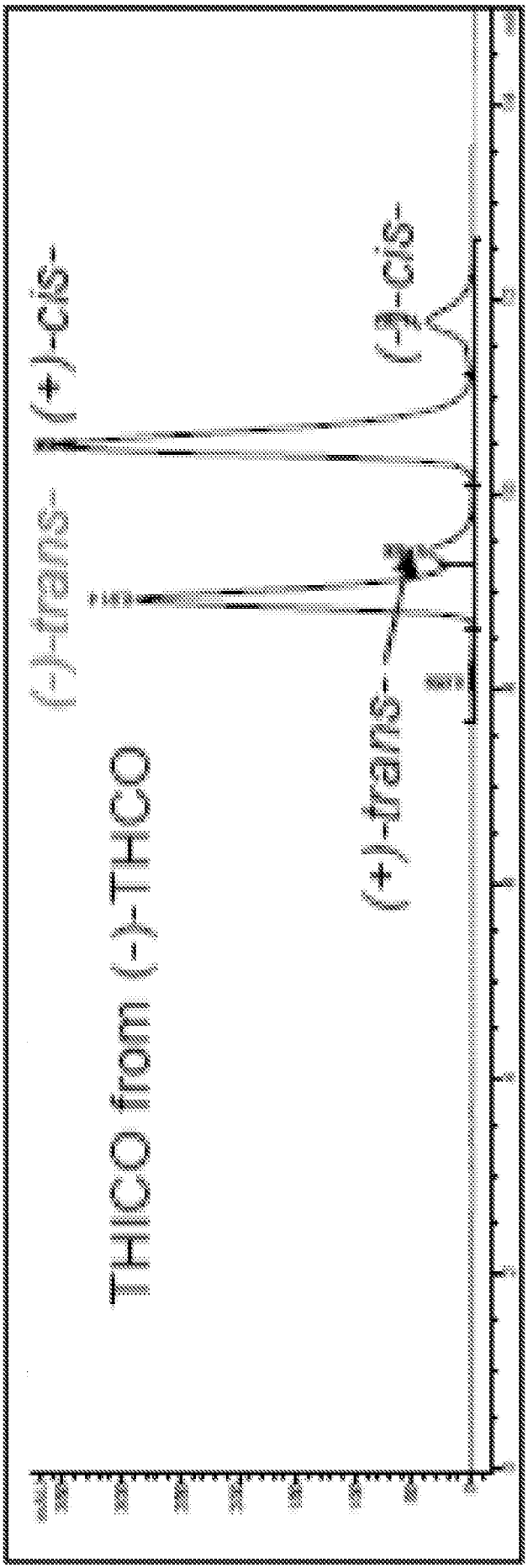


Figure 4



Analytical HPLC Resolution of *cis/trans*-THICO from (+/-)-THCO by β-Cyclobond column

Figure 5



Analytical HPLC Resolution of *cis/trans*-THCO from (+/-)-THCO by β-Cyclobond column

Figure 5

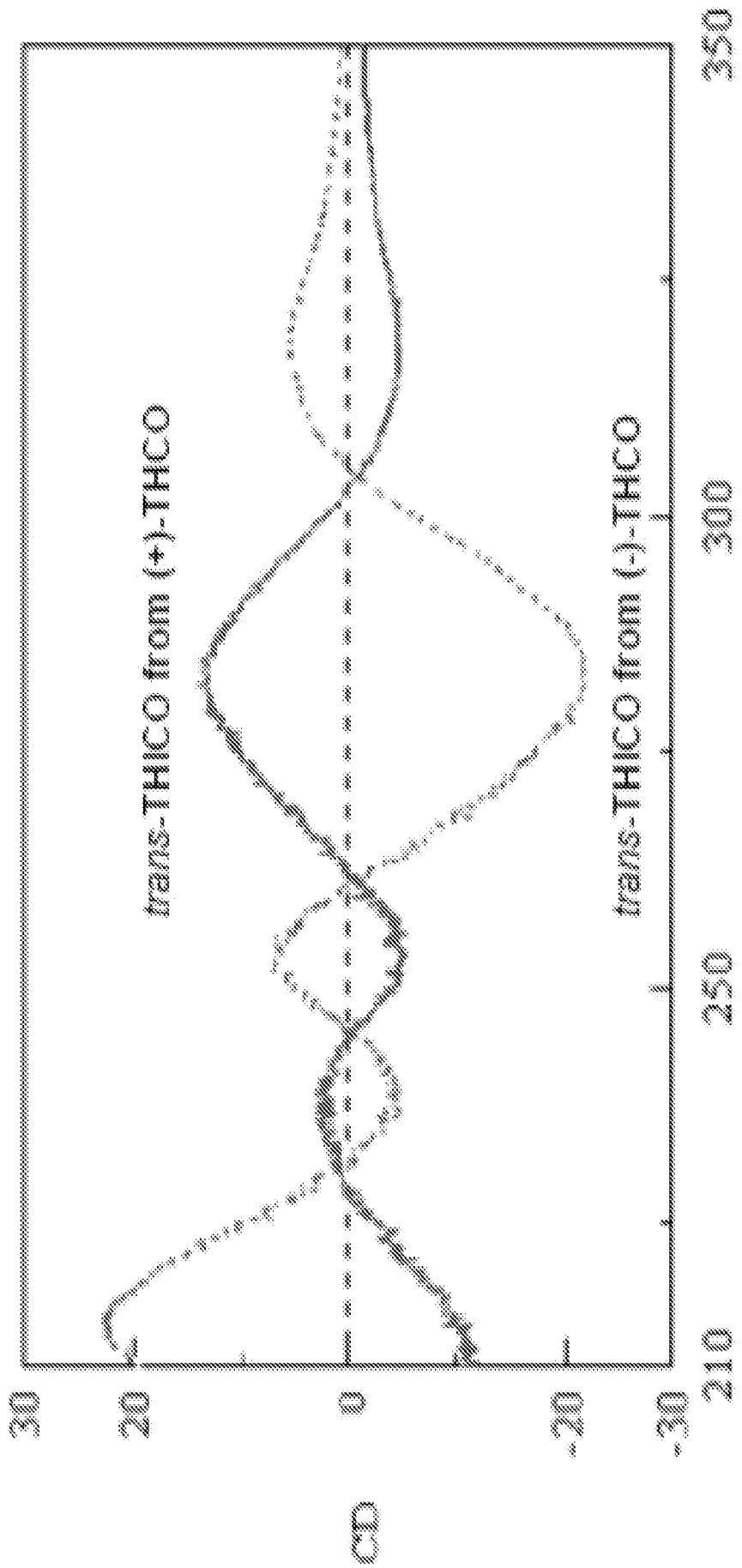

trans-THICO from (+)-THCO
trans-THICO from (-)-THCO
CD
30
20
0
-20
-30
210
250
300
350
Wavelength[nm]

Figure 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/037398

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12C3/08 C12C3/12
ADD.

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)
C12C

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 practical, search terms used)
EPO-Internal, BIOSIS, COMPENDEX, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TING PATRICK LU-PING ET AL: "Preparation and purification of hop acids and their derivatives", JOURNAL OF THE AMERICAN SOCIETY OF BREWING CHEMISTS, AMERICAN SOCIETY OF BREWING CHEMISTS, ST PAUL, MN, US, vol. 54, no. 2, 1 January 1996 (1996-01-01), pages 103-109, XP009150694, ISSN: 0361-0470 cited in the application	11-17, 21-25
Y	page 107, paragraph 2 - page 108, last paragraph; figures 11, 12	1-10, 18-20
X	US 4 644 084 A (COWLES JOHN M [US] ET AL) 17 February 1987 (1987-02-17) column 1, line 15 - line 20; claims; examples	23-25

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 July 2011	04/08/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Smeets, Dieter

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/037398

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 1 266 716 A (P. SHANNON AND W. DONNELLY) 15 March 1972 (1972-03-15) page 1, left-hand column, line 1 - line 24	23-25
Y	WO 96/36584 A1 (SEPRACOR INC [US]) 21 November 1996 (1996-11-21) page 1, line 9 - line 10; claims; examples	1-10, 18-20
Y	KINBARA K ET AL: "CHIRAL DISCRIMINATION OF 2-ARYLALKANOIC ACIDS BY (1S,2R)-1-AMINOINDAN-2-OL THROUGH THE FORMATION OF A CONSISTENT COLUMNAR SUPRAMOLECULAR HYDROGEN-BOND NETWORK", JOURNAL OF THE CHEMICAL SOCIETY, PERKIN TRANSACTIONS 2, CHEMICAL SOCIETY. LETCHWORTH, GB, vol. 1, 1 January 2000 (2000-01-01), pages 111-119, XP001121365, ISSN: 1472-779X, DOI: DOI:10.1039/A905566E the whole document	1-10, 18-20
A	WO 2010/008299 A1 (PRONOVA BIOPHARMA NORGE AS [NO]; HOLMEIDE ANNE KRISTIN [NO]; HOVLAND R) 21 January 2010 (2010-01-21) page 28, line 17 - line 20	1-25

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2011/037398

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4644084 A	17-02-1987	NONE	
GB 1266716 A	15-03-1972	IE 34530 B1	11-06-1975
WO 9636584 A1	21-11-1996	AT 195930 T	15-09-2000
		AU 700193 B2	24-12-1998
		AU 5793296 A	29-11-1996
		CA 2221375 A1	21-11-1996
		DE 69610089 D1	05-10-2000
		DE 69610089 T2	03-05-2001
		DK 828702 T3	02-01-2001
		EP 0828702 A1	18-03-1998
		ES 2152024 T3	16-01-2001
		GR 3034975 T3	28-02-2001
		JP 11511742 T	12-10-1999
		PT 828702 E	31-01-2001
		US 5677469 A	14-10-1997
WO 2010008299 A1	21-01-2010	AU 2009271791 A1	21-01-2010
		CA 2730958 A1	21-01-2010
		EP 2147910 A1	27-01-2010
		EP 2313090 A1	27-04-2011
		KR 20110031497 A	28-03-2011