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Enantioselective Synthesis of 3-Hydroxycitronellic Acid Isolated from *Ceratocystis fimbriata sp. platani.*

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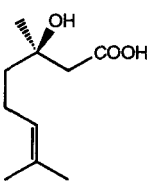
Abstract: (3S)- and (3R)-3-hydroxy-citronellic acid were obtained by Sharpless epoxidation of geraniol, followed by reduction of the epoxide and subsequent oxidation of the primary alohol to a carboxylic acid. This enantioselective approach allowed us to assign absolute configuration 3R to the naturally occuring monoterpene derivative.

INTRODUCTION

Ceratocystis fimbriata[1] is a perithecial ascomycete which infests highly commercial crops and trees such as hevea, sweet potato, coffea, cacao, plane tree, oak tree, aspen and coconut palm[2,3]. This pathogen enters, through wounds, the roots and branches, and causes foliar and fruit withering accompained by trunk canker in adult trees[4,5].

As part of our investigation of biologically active fungal metabolites of *Ceratocystis fimbriata sp. platani*, we obtained a pale yellow oil from a polar and highly phytotoxic fraction. Spectroscopic data were in agreement with the structure of 3-hydroxycitronellic acid, a new natural monoterpene **3b**.



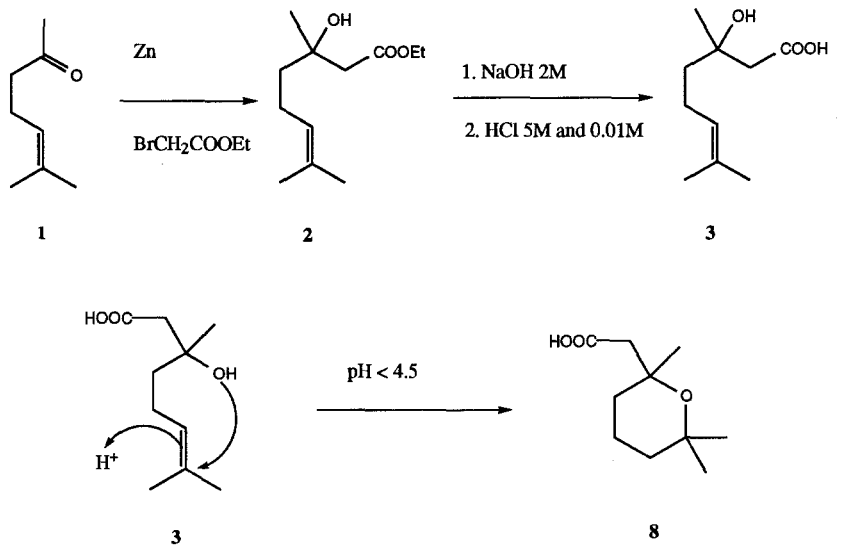
3b

3-Hydroxy-citronellic acid methyl ester has been described without stereochemical details as a derivative of geranyl acetate from the fungus *P. Incognita*'s culture medium[6].

Synthesis of the racemic mixture and the pure enantiomers was necessary to confirm the structure and to establish the absolute configuration of the natural product. Furthermore, production of the corresponding pure synthetic enantiomer in sufficient amounts has allowed us to perform biological activity tests.

RESULTS AND DISCUSSION

Condensation of ethylbromoacetate on 6-methyl-5-hepten-2-one **2** using Reformatsky's reaction[7] yielded pure racemic hydroxy-3-citronellic acid, ethyl ester as a colourless oil (66%). Subsequent saponification under strict pH control yielded the desired carboxylic acid **3** (scheme I). Under less controlled conditions (pH<4), the cyclic compound **8** was formed, as a by-product, in yields up to 20%. Spectroscopic data (IR, ^{1}H-, ^{13}C-NMR and MS) of the synthetic compound (±)- **3** were identical to those of the natural one.



Scheme I: synthetic pathway to racemic hydroxy-3-citronellic acid

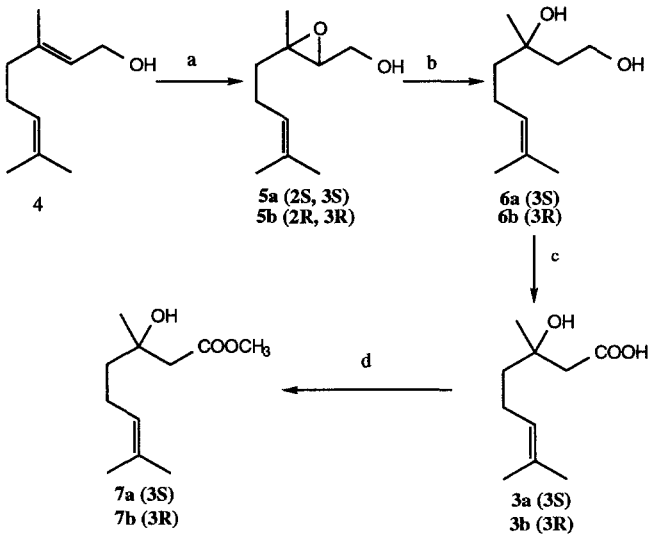
The approach to the enantioselective synthesis of both enantiomers was based on asymmetric epoxidation[10] of geraniol followed by a regioselective reduction of the epoxide by Red-al[11]. The ultimately successful synthesis is shown in scheme II.

The commercialy available geraniol **4** was subjected to the titanium-catalysed asymmetric epoxidation process[1], which yielded the desired (2S,3S)-**5a** and (2R,3R)-**5b** epoxy alcohols with a 50% yield and with an enantiomeric excess of 94% over several runs.

A regioselective reduction of these epoxides with bis (2-methoxyethoxy) aluminium hydride (Red-al) gave the corresponding 1,3-diols **6a** and **6b**[11].

The last step was the direct oxidation of the alcohol **6a** and **6b** to the acid **3a** and **3b**. It proved to be particularly hard. Acidic conditions (PCC) are known to be complicated by cationic cyclization of the intermediate aldehyde as described by Corey for citronellol[12]. Under milder conditions (PDC-DMF)[13], the major product was citral, obtained by dehydration of the hydroxy aldehyde intermediate.

This oxidation was finally accomplished by using a catalytic amount of platinium in an oxygen atmosphere[14]. The product was converted to the methyl ester **7a** (and **7b**) with ethereal diazomethane. Thus the enantiomeric 3-hydroxy-citronellic acid can be readily obtained in stereochemically pure form in only three operations. Spectral data of the synthetic compounds were identical to those of the natural metabolite.



Scheme II: enantioselective pathway to (3R and 3S) methyl 3-hydroxy citronellate (**7a** and **7b**)

a) t-BuOOH, $Ti(O\text{-}iPro)_4$, D(-) DET or L(+) DET, -30^0C.
b) Red-al, THF
c) PtO_2, H_2O, O_2
d) ether, CH_2N_2

The specific rotation of the natural compound $[\alpha]_D^{25}$ = -2.01, c = 0.02, MeOH) is in fair agreement with those of the 3R isomer $[\alpha]_D^{25}$ = - 1.87, c = 0.33 MeOH). Final identification, as far as enantiomeric purity of the natural acid **3b** was as obtained by chiral GC applied to both methyl ester of synthetic and natural compounds[15].

Despite its occurrence in a quite toxic fraction of *Ceratocystis fimbriata sp. platani's* extract, the synthetic enantiomer **3b** showed no biological activity when applied to our specific bioassays.

We would like to thank Dr. S. Claude for his assistance in NMR spectroscoy. Financial support from the Swiss National Science Foundation (grant n° 20-40392 .94) is gratefully acknowledged.

EXPERIMENTAL

General.. All commercially available chemical reagents were used without further purification. All reactions were followed by TLC: aluminium sheets silica gel 60 F 254 (Merck). Prep. column chromatography (CC): silica gel (Merck 60, 0.063-0.200 mm). FT-IR: Perkin -Elmer 1720X, KBr disks, unless otherwise indicated. ^1H- and ^{13}C-NMR: Bruker AMX 400 and Varian Gemini 200, δ in ppm, J in Hz. EI-MS: positive mode, Nermag-R-3010 spectrometer. Optical rotation: Jasco DIP 370.

***Ceratocystis fimbriata sp. platani's* culure medium:**

C.F. was grown as described by S. Witt and T. Pattner[8,9]; 15l of ZMA medium were poured in 250 ml flasks, each being inoculated by 2 ml additions of 4 days-old liquid cultures and shaken at 120 rpm during 20 days.

Aqueous phases were separated from myecelium by filtration, centrifuged (10'000 tpm, 30 min, 4°c) and sterilized by filtration through 1 µm porous membranes.

Isolation of natural 3-hydroxy-citronellic acid 3b:

Ethyl acetate extraction of ZMA medium after 20 days of fungal growth yielded an organic extract (520 mg) that was submitted to reverse phase Lobar chromatography. One of the phytotoxic fractions revealed an intense blue spot by spraying TLC with vanillin. This oily fraction was further purified by column chromatography on silica gel (EtOAc:hexane:AcOH 15:84:1) and on reverse phase Lobar chromatography (EtOH:H_2O 40:60) to give a pale yellow oil (6 mg) identified as (3R) 3-hydroxy-citronellic acid **3b**, $[\alpha]_D^{25}$ = -2.01 (c = 0.02, MeOH).

3,7-Dimethyl-3-hydroxy-oct-6-en, ethyl ester 2:

To a suspension of zinc powder (prealably washed with ether) (8.9 g, 0.136 mol) in dimethoxymethane (10 ml) was added a solution of ethylbromoacetate (11.3 g, 0.68 mol) in dry dimethoxymehtane (40 ml). The solution was heated under reflux for 90 min, then allowed to cool at room temperature. A solution of methyl-6-hepten-5-one-2 **1** (8.55 g, 0.68 mol) in dry dimethoxymethane (40 ml) was added and the mixture was again heated under reflux for 90 min. The resulting solution was cooled at 0°c and hydrlyzed with 15% HCl (25 ml). The organic phase was separated and the aqueous one was extracted with ether (100 ml). The combined organic phases were washed with saturated sodium carbonate and water, concentrated and distilled under reduced pressure to yield the ethyl ester **2** (9.59 g, 66%, bp 78-79°c under 0.38 mmHg) and the excess of methyl-6-hepten-5-one-2. IR (neat, NaCl), 3518, 2975, 2930, 1718, 1451, 1376, 1333, 1203, 1117, 1031, 923, 838, 666 cm^{-1}. ^{1}H-NMR ($CDCl_3$); 5.09 (1H, t x sept, J=6.9, J=0.7 Hz, C_6H), 4.18 (2H, q, J=6.6 Hz, OCH_2), 3.41 (1H, bs, OH), 2.53 (1H, d, J=15.5 Hz, C_2H), 2.41 (1H, d, J=15.5 Hz, C_2H), 2.05 (2H, dd, J=17.5, J=6.9 Hz, C_5H), 1.68 (3H, d, J=1.1 Hz, CH_3), 1.60 (3H, s, CH_3), 1.52 (2H, m, C_4H), 1.27 (3H, t, J=6.6 Hz, CH_3), 1.24 (3H, s, CH_3); ^{13}C-NMR ($CDCl_3$), 177.70 (C-1), 132.44 (C-7), 124.76 (C-6), 71.59 (C-3), 61.28 (OCH_2), 45.55, 42.54, 27.31, 26.33, 23.35, 18.26, 14.85.

3,7-Dimethyl-3-hydroxy-octen-6-oic acid 3 (racemic):

To the ethyl ester **2** (0.5 g, 2.3 mmol) were added NaOH 2M (3.5 ml, 7 mmol); a strong stirring was maintened to enhance the emultion of the two phases. After 3 hours, magnetic stirring was interrupted, the solution was cooled at 0°c and carrefully acidified with HCl 5M up to pH 6. The solution was further acidified with HCl 0.01M up to pH 4.5. After ether extraction (4x5ml, organic phases were washed with water and dried with magnesium sulfate. Evaporation of the solvent gave pur racemic hydroxy-3-citronellic acid (350 mg, 80%). IR (neat, NaCl) : 3307, 1559, 1409 cm^{-1}. MS : m/z (EI, +) 186 (0.3), 126 (9), 111 (33), 108 (100), 93 (35), 83 (24); (DCI, NH_3+) 187 (3), 168 ([M-H_2O]$^+$, 0.6), 144 (26), 127 (100), 109 (39); ^{1}H-NMR (CD_3OD); 5.19 (1H, t, J=7 Hz, C_6H), 2.44 (1H, d, J=15 Hz, C_2H), 2.34 (1H, d, J=15 Hz, C_2H), 2.13 (2H, m, C_5H), 1.76 (3H, s, CH_3), 1.70 (3H, s, CH_3), 1.60 (2H, m, C_4H), 1.29 (3H, s, CH_3); ^{13}C-NMR (D_2O) : 183.3, 136.1, 127.0, 74.5, 50.0, 43.8, 28.2, 27.5, 24.9, 19.4. UV : 207, 239 et 282 nm. Anal. calcd $C_{10}H_{18}O_3$ + 0.3 H_2O: C, 62.67; H, 9.78. Found: C, 62.41; H, 9.83

Tetrahydro-2,6,6-trimethyl-2H-2-pyrane-acetic acid 8:

The ethyl ester **2** (0.4 g, 1.9 mmol) was hydrolyzed as described above and acidified with conc. HC1 at room temperature. After extraction with ether, the solvent was evaporated under reduced pressure and the oily residue was purified by column chromatography RP-C18 (eluent: MeOH-water) to give the acid **3** (250 mg, 72% yield) and the cyclic product **8** as colorless oil (70 mg, 20% yield).

IR(neat, NaCl), 3387, 2973, 2004, 1708, 1467, 1380, 1310, 1261, 1207, 1085, 1047, 933, 910, 837, 801, 764, 701, 620 cm^{-1}. MS: m/z 187 ([M+H]$^+$, 1), 171 ([M-CH_3]$^+$, 9), 153 (16), 128 ([M-CH_2COOH]$^+$, 8), 109 (25), ^{1}H-NMR ($CDCl_3$), 3.2 (1H, bs, OH), 2.6 (1H, d, J=16 Hz, C_2'H), 2.48 (1H, d, J=16 Hz, C_2'H),

1.5 (6H, m, 3 CH_2), 1.3 (3H, s, CH_3), 1.22 (6H, s, 2 CH_3); ^{13}C-NMR; 175.8 (COOH), 72.3 (C_{quat}), 72.2 (C_{quat}), 45.5 (CH_2), 44.4 (CH_2), 42.8 (CH_2), 29.74 (CH_3), 29.71 (CH_3), 27.3 (CH_3), 19.2 (CH_2).

(2S,3S)-Epoxygeraniol 5a:

A 500 ml, one-neck round-bottom flask equipped with a Teflon-coated magnetic stirring bar is oven dried then fitted with a serum cap, and flushed with nitrogen. The flask is charged with dry, reagent-grade dichloromethane (400 ml; distilled from calcium hydride) and cooled by stirring in a -23 °c bath. Then the following liquids are added sequencially via syringe while stirring in the cooling bath: titanium tetraisopropoxide (8.12 g, 28.8 mmol), L-(+)-diethyl tartrate (5.88 g, 28.8 mmol), the mixture is stirred 5 min before the next addition, geraniol (4.44 g, 28.8 mmol), and finally a dichloromethane solution (250 ml) contaning anhydrous *t*-butyl hydroperoxide (18.5 ml, 57 mmol). The resulting homogeneous solution is then stirred for 2 hours and 10 % aqueous tartaric acid solution (65 ml) is added. The aqueous layer solidifies. After 30 min, the cooling bath is removed and stirring is continued at room temperature for until the aqueous layer become clear. After separation of the aqueous layer, the organic layer is washed once with water, dried with magnesium sulphate, and concentrated to afford a colourless oil with an odour revealing contamination by t-butyl hydroperoxyde. This oil is diluted with ether (220 ml). The resulting solution is cooled, and 1N sodium hydroxide solution (90 ml) is added. This produces a two-phase mixture which is stirred at 0 °c for 30 min. The ether phase is washed with brine, dried with magnesium sulphate and concentrated to give a clear oil (6.64 g). Chromatography on silica gel (eluent: AcOEt/hexane 7/3) affords (2S, 3S)-epoxygeraniol; yield: 48%, $[\alpha]_D^{25}$=-6.06 (c 0.76, MeOH). IR (neat, NaCl), 3419, 2972, 1747, 1673, 1451, 1385, 1256, 1198, 1134, 1094, 1032, 865 cm^{-1}. ^{1}H-NMR ($CDCl_3$); 5.07 (1H, t x sept, J=7.1 Hz, J=1.4 Hz, H-6); 3.79 (1H, dd, H-1); 2.96 (1H, dd, H-2); 2.07 (2H, t d, J=7.4 Hz, J=7.9 Hz, H-5); 1.67 (4H, m, CH_3 and H-4); 1.60 (3H, s, CH_3); 1.45 (1H, m, H-4); 1.29 (3H, s, CH_3). ^{13}C-NMR ($CDCl_3$); 132.83 (C-7); 124.00 (C-6); 63.64 (C-2); 62.12 (C-1); 61.86 (C-3); 39.16 (C-4); 26.32 (C-3); 24.34 (C-5); 18.30 (C-8); 17.41 (C-10).

The (2R, 3R) isomer **5b** prepared analogously (55% yield) using D (-)-diethyl tartrate, showed identical properties.

(S)-3,7-Dimethyl-6-octen-1,3-diol (hydroxy-3-citonellol) 6a:

A solution of epoxide **5b** (4.1 g, 26 mmol) in THF (30 ml) (freshly distilled on sodium) was added dropwise at 0°C to a stirred solution of Red-Al (15.5 ml, 78 mmol) in THF (60 ml). After completion of the addition the reaction mixture was stirred at room temperature for 16 h. The resulting solution is cooled in an ice bath, and then water (120 ml) was added dropwise. The precipitate was filtered off, the filtrate was extracted 3 times with ether, and the combined solvent phases were washed with brine, dried with magnesium sulphate, and concentrated. Chromatography on silica gel (eluent: EtOAc:hexane 2:3) affords (S)-3,7-dimethyloct-6-en-1,3-diol (2.98 g); yield 67 %, $[\alpha]_D^{25}$=+1.9 (c 0.54, MeOH). IR (neat, NaCl), 3349, 2970, 2927, 1673, 1451, 1377, 1342, 1300, 1267, 1239, 1163, 1119, 1061, 1029, 970, 935, 891, 835 cm^{-1}. CI-MS: m/z 173 ($[M+H]^+$, 23), 154 (70), 137 (23), 121 (50), 109 (100), 95 (30), 69 (70), 43 (57). ^{1}H-NMR ($CDCl_3$); 5.07 (1H, t x sept, J=7.00 Hz, J=1.02 Hz, H-5); 3.83 (2H, m, H-1); 3.35 (1H, br, OH); 3.23 (1H, br, OH); 1.99 (2H, m, H-5); 1.72 (1H, m, H-2); 1.64 (4H, m, CH_3 and H-2); 1.58 (3H, s, CH_3); 1.50 (2H, m, H-4); 1.20 (3H, d, CH_3). ^{13}C-NMR ($CDCl_3$); 132.34 (C-7); 124.88 (C-6); 74.37 (C-3); 60.17 (C-1); 42.94 (C-4); 42.12 (C-2); 27.10 (C-10); 26.27 (C-8 or C-9); 23.32 (C-5); 18.23 (C-8 or C-9).

The 3R-isomer **6b** prepared analogously (89% yield), showed identical properties, $[\alpha]_D^{25}$=-2.3 (c 0.51, MeOH).

(S) 3,7-Dimethyl-6-octen-3-ol methyl ester (methyl hydroxy-3-citronellate) (3a):

A suspension of platinium oxide catalyst (50 mg) in water (5 ml) was reduced under a hydrogen atmosphere (30 bar) for 1h. The hydrogen was replaced by air, and the diol **6a** (55 mg, 0.32 mmol) was added to the suspension. The mixture was stirred under oxygen atmosphere (30 bar) for 18 h. The platinium was filtered, the solution basified by NaOH 1N and extracted with AcOEt. The aqueous solution was acidified with HCl 5%, extracted with AcOEt and the organic layer was washed with brine, dried with magnesium sulphate and concentrated to give 30 mg of a yellow oil (51% yield of **3a**) $[\alpha]_D^{25}$ = + 1.8 (c 0.08, MeOH).treated for 15 min with excess ethereal diazomethane. Anal. calcd $C_{10}H_{18}O_3$ + 0.3 H_2O: C, 62.67; H, 9.78. Found C, 62.67; H, 9.90.

The 3R-isomer **3b** prepared analogously (68% yield), showed identical spectroscopic properties $[\alpha]_D^{25}$=-1.9 (c 0.33, MeOH).

Both isomers were treated for 15 min with excess ethereal diazomethane to afford **7a** $[\alpha]_D^{25}$=+3.8 (c 0.17, MeOH) and **7b** $[\alpha]_D^{25}$=-1.5 (c 0.07, MeOH).

IR (neat, NaCl), 3510, 2970, 2927, 2858, 1736, 1439, 1377, 1342, 1207, 1178, 1120, 1009, 927, 839, 750, 666 cm^{-1}. ^{1}H-NMR ($CDCl_3$); 5.09 (1H, t x sept, J=6.9 Hz, J=0.7 Hz, H-5), 3.70 (3H, s, OCH_3), 2.53 (1H, d, J=15.5 Hz, H-2), 2.44 (1H, d, J=15.5 Hz, H-2), 2.05 (2H, dd, J=17.5 Hz, J=6.9 Hz, H-5), 1.69 (3H, d, J=1.1 Hz, CH_3), 1.61 (3H, s, CH_3), 1.52 (2H, m, H-4), 1.25 (3H, s, CH_3). ^{13}C-NMR ($CDCl_3$), 174.07 (C-1), 132.50 (C-7), 124.71 (C-6), 71.59 (C-3), 52.31 (CH_3), 45.39, 42.49, 27.30, 26.33, 23.32, 18.25.

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15. The chiral column is 20% 6-TBDMS 2,3-diMe ß-CD, L: 10 m, ID 0.3 mm, df 0.225 µm, vector gas He, $T_{inj.}$ 220°C, $T_{det.}$ 280°C, isotherm 80°C. The enantiomer S as methyl ester appears at 45.3' and the other at 47.9'. S. Claude, R. Tabacchi and A. Saxer, unpublished result.

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