Pergamon

PII: S0031-9422(98)00152-6

Phytochemistry, Vol. 48, No. 4, pp. 661–664, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

NATURAL OCCURRENCE OF α- AND β-MICROBIOTENE

STEPHANIE MELCHING,† ASTRID BLUME,† WILFRIED A. KÖNIG,†* and HERMANN MUHLE‡

† Institut für Organische Chemie, Universität Hamburg, D-20146 Hamburg, Germany; ‡ Abteilung Spezielle Botanik, Universität Ulm, D-89081 Ulm, Germany

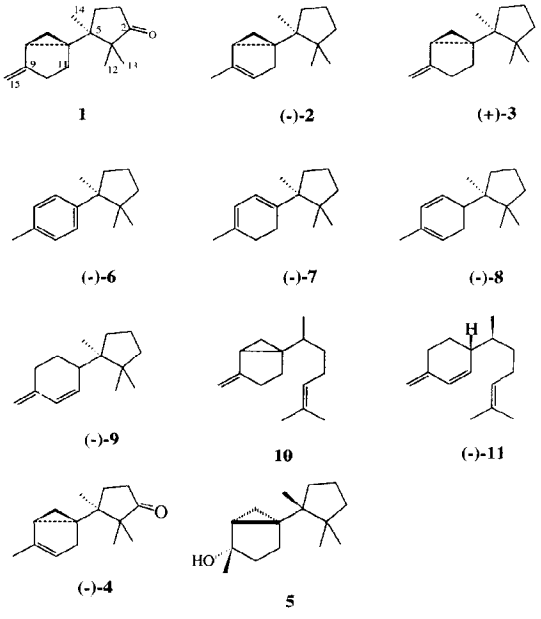
Mannia fragrans; *Microbiota decussata*; Hepaticae; sesquiterpenes; α- and β-microbiotene; cyclocupar-9-en-2-one.

Key Word Index—(Received 2 February 1998)

Abstract—(-)-α-Microbiotene {(-)-(1S,5S)-2-methyl-5[(1S)-1,2,2-trimethylcyclopentyl]-bicyclo[3.1.0]hex-2-ene} (+)-β-microbiotene {(+)-(1S,2S)-2-methyl-5[(1S)-1,2,2-tri-methyl-cyclopentyl]-bicyclo[3.1.0.]hex-2(7)-ene} and (-)-cyclocupar-9-en-2-one have been isolated and identified from the essential oil of the liverwort *Mannia fragrans* and correlated with constituents of opposite configuration from *Microbiota decussata* (Cupressaceae).

INTRODUCTION

Mannia fragrans, a liverwort occasionally found in central Europe [1], has a pleasant and intensive odour, which is associated with its major constituent grimaldone (**1**) [2]. We have collected two specimens of *Mannia fragrans* in the Altmühl valley (southern Germany) and in the Aosta valley (northern Italy) and investigated their hydrodistillation products from which, in addition to **1**, the corresponding sesquiterpene hydrocarbons α-microbiotene (**2**), β-microbiotene (**3**) and an isomer of **1**, cyclocupar-9-en-2-one (**4**), were isolated. The cyclocuparane skeleton is also present in microbiotol (**5**), which was first isolated and identified by Asakawa *et al* [3]. from the liverwort *Marchantia polymorpha* and which is a major constituent of *Microbiota decussata* (Cupressaceae) [4]. Tkachev *et al* [5]. have assigned the absolute configuration to **5** by chemical correlation with (+)-cuparene (**6**) and molecular mechanics calculations in conjunction with conformational analysis by NMR. We have prepared **2** and **3** by dehydration of **5** which was isolated from *M. decussata* by liquid chromatography and compared its structure and configuration with the same compounds from *Mannia fragrans* by spectroscopic methods and enantioselective gas chromatography.



RESULTS AND DISCUSSION

GC-mass spectrometry of the hydrodistillate of *M. fragrans* and comparison with published mass spectral and retention index data, using enantioselective GC with modified cyclodextrins as chiral stationary phases, allowed the identification of grimaldone (**1**), (-)-cuparene (**6**), (-)-α-cuprenene (**7**), (-)-γ-cuprenene (**8**), (-)-δ-cuprenene (**9**) [6, 7, 8], sesquisabinene (**10**) [9] and (-)-sesquiphellandrene (**11**) [10]. The sesquiterpene hydrocarbon (-)-α-microbiotene (**2**) was isolated from the essential oil of *M. fragrans* by prep. GC and its structure identified by 1- and 2D-NMR techniques. The ^{1}H NMR spectrum shows an olefinic proton at $\delta = 4.93$, which couples to the C-11 methylene protons at $\delta = 2.20$ and 2.69 and to the C-15

*Author to whom correspondence should be addressed.

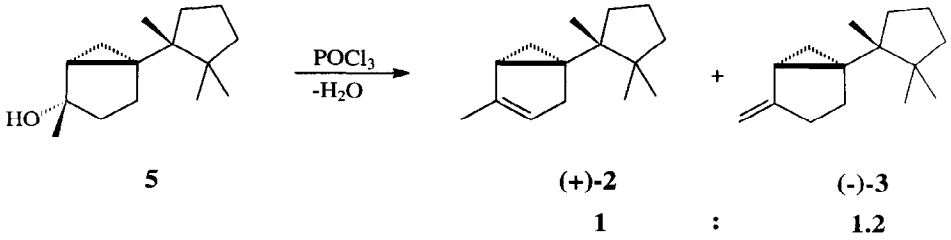


Fig. 1. Dehydration of microbiotol (**5**), isolated from *M. decussata*, to (+)-α-microbiotene and (−)-β-microbiotene.

methyl group at $\delta = 1.76$. The C-11 methylene group also exhibits a homoallyl coupling correlation to the C-15 methyl. A cyclopropyl proton signal appears as a triplet at $\delta = 0.03$, which further couples with two cyclopropyl protons at $\delta = 1.23$ and 1.37, respectively, which again couple with each other.

(+)-β-Microbiotene (**3**) was isolated by silica gel column chromatography from the essential oil of *M. fragrans*. In its ^{1}H NMR spectrum the typical signals of an exocyclic methylene group appear at $\delta = 4.84$ and 5.05. One cyclopropyl proton signal appears at $\delta = 0.60$ as a *dd* system with coupling correlations to two multiplets at $\delta = 1.13$ and 1.45 (two cyclopropyl protons), respectively, which themselves are coupled to each other. Furthermore, a long-range coupling from the signals of the exocyclic methylene group to the signals of the C-10 methylene group ($\delta = 1.94$–2.14) is observed.

To verify the structures of **2** and **3**, a sample of microbiotol (**5**) was isolated from the essential oil of *Microbiota decussata* and dehydrated with phosphoryl chloride (Fig. 1). Two of the compounds formed proved to be identical with **2** and **3** by comparison of their mass spectra and GC retention times. The other compounds formed in the dehydration reaction were identical with compounds **6**–**9**. α-Microbiotene (**2**) is present as a component in the hydrodistillate and is also found in the petrol extract of *M. decussata* but we did not find β-microbiotene (**3**). The formation of the cuparane type sesquiterpenes can be rationalized by an acid catalyzed cyclopropane ring opening reaction (Fig. 2). The facile formation of the cuparane skeleton from the cyclocuparane system is also documented by the almost identical mass spectra of **2** and **3** with the spectrum of δ-cuprenene (**9**).

Measurement of the optical rotation of the microbiotenes isolated from *M. fragrans* indicated a negative rotation for α-microbiotene and a positive rotation for β-microbiotene. Both compounds were compared with the dehydration products of microbiotol (**5**) from *Microbiota decussata* by 2D-GC (Fig. 3). It turned out that **2** and **3** from *M. fragrans* and the dehydration products from microbiotol (**5**) had opposite configuration. In this way, the microbiotenes from *M. fragrans* are correlated with the known absolute configuration of microbiotol (**5**) from *M. decussata*. Microbiotol (**5**) is not present in the hydrodistillate of *M. fragrans*.

Further investigations of the fractions isolated from *M. fragrans* led to another compound with a cyclocuparane skeleton. Inspection of its ^{1}H- and ^{13}C-NMR spectra (1-, 2D-NMR, ^{13}C-NMR and ^{1}H-^{13}C-COSY) resulted in the structure of (-)-cyclocupar-9-en-2-one (**4**). At $\delta = 4.97$ a broad singlet of an olefinic proton is observed, which couples to the C-11 methylene signal (complex multiplet at $\delta = 2.66$ and 2.3), which again couples to the C-15 methyl group at $\delta = 1.80$. A cyclopropane proton at $\delta = 0.15$ of C-7a couples to a complex multiplet of two additional cyclopropyl protons, C-7b and C-8, at $\delta = 1.36$–1.44. The resonances of two additional methylene groups are coupled to each other and appear in the range of $\delta = 2.20$–2.40 (C-3) and, widely split, at $\delta = 1.75$ and 1.20 (C-4). One of the C-4-protons shows a long-range coupling to the methyl group C-14 ($\delta = 0.97$, *s*).

EXPERIMENTAL

Plant material

Microbiota decussata was obtained from the botanical garden of the University of Hamburg (no. AO-

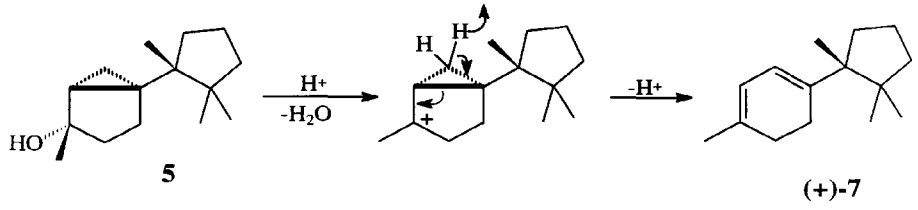


Fig. 2. Acid induced dehydration of microbiotol (**5**) and ring opening reaction leading to the formation of (+)-α-cuprenene (**7**).

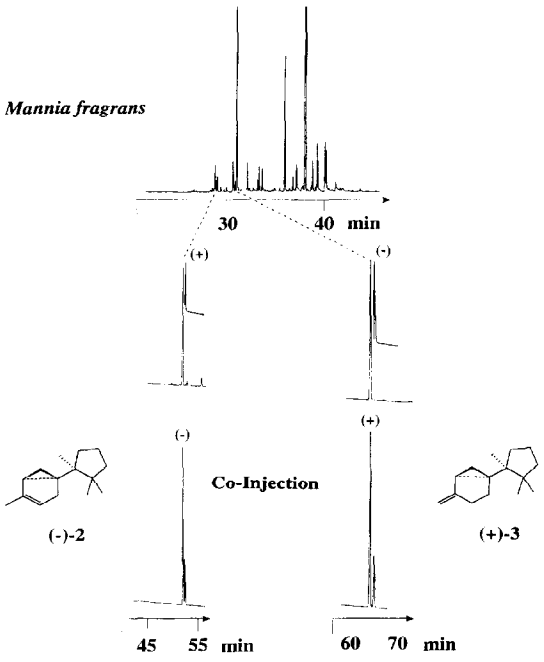


Fig. 3. Determination of the enantiomeric composition of α-microbiotene (**2**) and β-microbiotene (**3**) in the essential oil of *Mannia fragrans* by 2D-GC. Preseparation on a 25 m capillary column with CpSil 5 at 50°, temp. program: 3°/min to 230°. Enantiomer separation on a 25 m fused silica capillary with heptakis(2,6-di-O-methyl-3-O-pentyl)-β-cyclodextrin (50% in OV 1701, w/w) at 110°. Co-injection with the dehydration products of microbiotol (**5**).

F-5995). *Mannia fragrans* was collected in the Altmühl valley (Southern Germany, Bavaria, dry hills with rock outcrops, north of Boehming, no. 1-15.10.95, Herbarium Ulm) in October 1995 and in the Aosta valley (northern Italy, Valle di Cogne, dry grasslands, west of Lillaz, 1600 m, no. 2-17.9.95, Herbarium Ulm) in September 1995.

Hydrodistillation

The volatile constituents were obtained by hydrodistillation (2 h) of aq. homogenates of fresh plant material using n-hexane as collection solvent. Because of the greatly differing weights, the fresh material was not weighed.

Extraction

The constituents were obtained by extraction (over night) of aq. homogenates of fresh plant material with petrol. The raw extract was filtered and analyzed by GC and GC-MS.

Liquid chromatography

Microbiotol (**5**) was isolated by chromatography of the essential oil of *M. decussata* at −18°C on an Al_2O_3 column with an n-pentane/ethyl acetate gradient as eluent. β-Microbiotene from *M. fragrans* was isolated by silica gel cc with n-hexane as eluent.

Dehydration

Two mg of microbiotol (**5**) was stirred in 0.5 ml dry pyridine and 1 drop of $POCl_3$ at room temperature for 1 hr. 1 ml H_2O was added to stop the reaction. The dehydration product were extracted with n-hexane (3 times), washed with water (several times) and dried over $Na_2SO_4 * H_2O$, to give **2** and **3** in a molar ratio of 1: 1.2.

Enantioselective capillary GC

Capillary columns with cyclodextrin derivatives were prepared as described in ref.[11].

Two-dimensional GC [12]

The essential oil samples were injected on a 25 m (0.25 mm i.d.) capillary column containing nonpolar CpSil 5 (Chrompack) in a Siemens Sichromat 2 gas chromatograph (equipped with a T-piece live-switching unit) at 50° and programmed at a rate of 3° min^{-1} to 230°. Sample transfer was performed manually to a 25 m capillary column with heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin (50% in polysiloxane OV 1701, w/w) which was kept isothermal at 110°. The chromatograms of both columns were recorded with a two-channel Merck-Hitachi model 2500 integrator. H_2 at an entrance pressure of 80 kPa for the CpSil 5 capillary and 65 kPa for the cyclodextrin capillary was used as a carrier gas.

NMR spectroscopy

Brucker WM 400 (400 MHz) and WM 500 (500 MHz) using TMS as int. standard.

GC-MS

EI (70 eV) GC-MS measurements were carried out on a Hewlett Packard HP 5890 gas chromatograph equipped with a 25 m CpSil 5 (Chrompack) fused silica capillary column coupled to a VG Analytical VG 70-250S mass spectrometer.

Polarimetry

Optical rotation measurements were performed with a Perkin Elmer 241 polarimeter. Because of the small sample amounts only the sign of optical rotation was determined.

(-)-α-*Microbiotene* (**2**)

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.03 (1H, *t*, *J* = 4 Hz), 0.88–0.97 (1H, *m*), 0.97 (3H, *s*), 1.00 (6H, *s*), 1.21–1.26 (1H, *m*), 1.35–1.69 (6H, *m*), 1.76 (3H, *m*), 2.20 (1H, *m*, *J* = 17 Hz), 2.69 (1H, *m*, *J* = 17 Hz), 4.93 (1H, *br s*). EIMS (70 eV), *m/z* (rel.int.): 204 (12), 161 (30), 119 (32), 111 (42), 93 (56), 91 (32), 69 (100), 55 (54).

(+)-β-*Microbiotene* (**3**)

^{1}H-NMR (400 MHz, C_6D_6): δ = 0.60 (1H, *dd*, J_{d1} = 4 Hz, J_{d2} = 5 Hz), 0.77–0.86 (1H, *m*), 0.96 (3H, *s*), 0.97 (3H, *s*), 1.01 (3H, *s*), 1.13 (1H, *m*), 1.35–1.75 (8H, *m*), 1.94–2.01 (1H, *m*), 2.04–2.14 (1H *m*), 4.84 (1H, *br s*), 5.05 (1H, *br s*); EIMS (70 eV), *m/z* (rel. int.): 204 (2), 111 (66), 93 (32), 91 (32), 69 (100), 55 (54).

(-)-*Cyclocupar*-9-*en*-2-*one* (**4**)

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.15 (1H, *t*, H-7, *J* = 3 Hz), 0.97 (3H, *s*, H-14), 1.05 (3H, *s*, H-12/13), 1.07 (3H, *s*, H-12/13), 1.20 (1H, *m*, H-4, *J* = 3 Hz), 1.36–1.44 (2H, *m*, H-7 and H-8), 1.70–1.78 (1H, *m*, H-4), 1.80 (3H, *br s*, H-15), 2.20–2.40 (3H, *m*, H-3a and H-3b and H-11), 2.66 (1H, *dt*, H-11, *J* = 17 Hz), 4.97 (3H, *br s*, H-10); ^{13}C-NMR (100.62 MHz, $CDCl_3$): δ = 16.19 (*q*, C-15), 19.49 (*q*, C-12/13), 21.22 (*q*, C-12/13), 21.58 (*q*, C-14), 22.38 (*t*, C-7), 27.84 (*t*, C-3/4), 28.51 (*d*, C-8), 33.74 (*t*, C-3/4), 33.79 (*s*, C-6), 38,39 (*t*, C-11), 45.51 (*s*, C-5), 53.55 (*s*, C-1), 121,00 (*d*, C-10), 144.85 (*s*, C-9), 223.40 (*s*, C-2). EIMS (70 eV), *m/z* (rel.int.): 218 (1), 203 (2), 111 (100), 93 (90), 91 (38), 83 (62), 77(30), 55 (38).

Acknowledgements—We thank Dr. V. Sinnwell, Universität Hamburg, for his help in discussing the NMR spectra. The support of the *Hans-Böckler-Stiftung* by a scholarship to S.M. and the financial support of the *Fonds der Chemischen Industrie* is gratefully acknowledged.

REFERENCES

1. Huneck, S., Connolly, J. D., Rycroft, D. S. and Woods, N., *Journal of Chemical Research*, 1988, **78**.
2. Huneck, S., Connolly, J. D., Freer, A. A. and Rycroft, D. S., *Phytochemistry*, 1988, **27**, 1405.
3. Asakawa, Y., Toyota, M., Bischler, H., Campbell, E. O. and Hattori, S., *Journal of the Hattori Botanical Laboratory*, 1984, **57**, 383.
4. Raldugin, V. A., Storozhenko, V. G., Rezvukhin, A. I., Pentegova, V. A., Gorovoi, P. G. and Baranov, V. I., *Chemistry of Natural Compounds*, 1981, **17**, 124, translated from (1981) *Khimiya Prirodnykh Soedinenii* **2**, 163.
5. Tkachev, A. V., Shakirov, M. M. and Raldugin, V. A., *Journal of Natural Products*, 1991, **54**, 849.
6. Dauben, W. G. and Oberhänsli, P., *Journal of Organic Chemistry*, 1966, **31**, 315.
7. Matsuo, A., Nakayama, N. and Nakayama, M., *Phytochemistry*, 1985, **24**, 777.
8. Hardt, I. (1995) PhD Thesis, University of Hamburg.
9. Weyerstahl, P., Wahlburg, H.-C., Splittgerber, U. and Marschall, H., *Flavour and Fragrance Journal*, 1994, **9**, 179.
10. Connell, D. W. and Sutherland, M. D., *Australian Journal of Chemistry*, 1966, **19**, 283.
11. König, W. A., Krüger, A., Icheln, D. and Runge, T., *Journal of High Resolution Chromatography*, 1992, **15**, 184.
12. Hardt, I. H., Rieck, A., Fricke, C. and König, W. A., *Flavour and Fragrance Journal*, 1995, **10**, 165.