Pergamon

Phytochemistry, Vol. 48, No. 6, pp. 1019–1023, 1998
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Printed in Great Britain
0031–9422/98 $19.00+0.00

PII: S0031–9422(97)00484–6

SESQUITERPENE HYDROCARBONS IN THE LIVERWORT *DUMORTIERA HIRSUTA*

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(*Received in revised form* 6 *May* 1997)

Key Word Index—*Dumortiera hirsuta*; Liverwort; Hepaticae; sesquiterpene hydrocarbons; stereochemistry; 2-dimensional gas chromatography; cyclodextrin derivatives.

Abstract—From a sample of the liverwort *Dumortiera hirsuta* (Sw.) Nees collected near Sao Francisco de Paula, Rio Grande do Sul (Brazil) a series of sesquiterpene hydrocarbons was isolated by preparative gas chromatography (GC) and investigated by spectroscopic methods and 2-dimensional GC (2D-GC) using a combination of a conventional polysiloxane capillary column and a capillary with a cyclodextrin derivative. Some rare sesquiterpenes such as (−)-cyclosativene, (+)-β-copaene, (−)-aristolochene, (+)-aciphyllene and (−)-isoguaiene were identified as well as the unusual enantiomers (+)-α-copaene, (+)-*cis*-α-bergamotene, (+)-*trans*-α-bergamotene, (+)-α-guaiene, (+)-guaia-6,9-diene and (+)-β-caryophyllene.

INTRODUCTION

Liverworts are known to produce a large variety of sesquiterpenoids. In a Japanese sample of *Dumortiera hirsuta* a number of known sesquiterpene hydrocarbons was identified by GC-mass spectrometric investigations by Matsuo *et al.* [1]. More recently Asakawa *et al.* [2] isolated and identified a new sesquiterpene and 13 known oxygenated compounds from the ether extract of the same species. However, the occurrence of at least three chemical races of *D. hirsuta* in Japan [3] indicates a large natural variability. In a sample of the liverwort collected in Brazil only sesquiterpene hydrocarbons were detected by GC-mass spectrometry of a hydrodistillate. Most of these constituents were identified by their typical mass spectra, some others with mass spectra which could not easily be assigned were isolated and investigated by NMR and by enantioselective GC.

RESULTS AND DISCUSSION

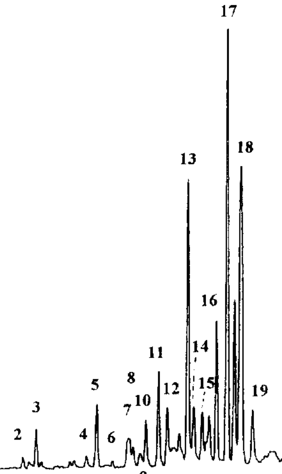

Fig. 1. Gas chromatographic separation of the hydrodistillation products of the liverwort *Dumortiera hirsuta*. 25 m fused-silica capillary column with polysiloxane CpSil 5. Column temp. 50°, temp. Programme 3° min^{-1} to 230°. Peak assignment see Table 1.

As illustrated by Fig. 1, the hydrodistillate of *D. hirsuta* consists of a complex mixture of closely eluting isomeric sesquiterpene hydrocarbons, which could not be easily isolated. In a first separation step the bulk of the main constituents (−)-isoguaiene (**17**) and *Z*-γ-bisabolene (**18**) was removed by preparative GC [4] and further purified by preparative TLC for NMR investigations. The ^{1}H NMR spectrum of **17** was identical with that published by Bohlmann *et al.* [5]. The negative optical rotation and 2D-GC analysis, however, revealed that the unusual (−)-enantiomer was present.

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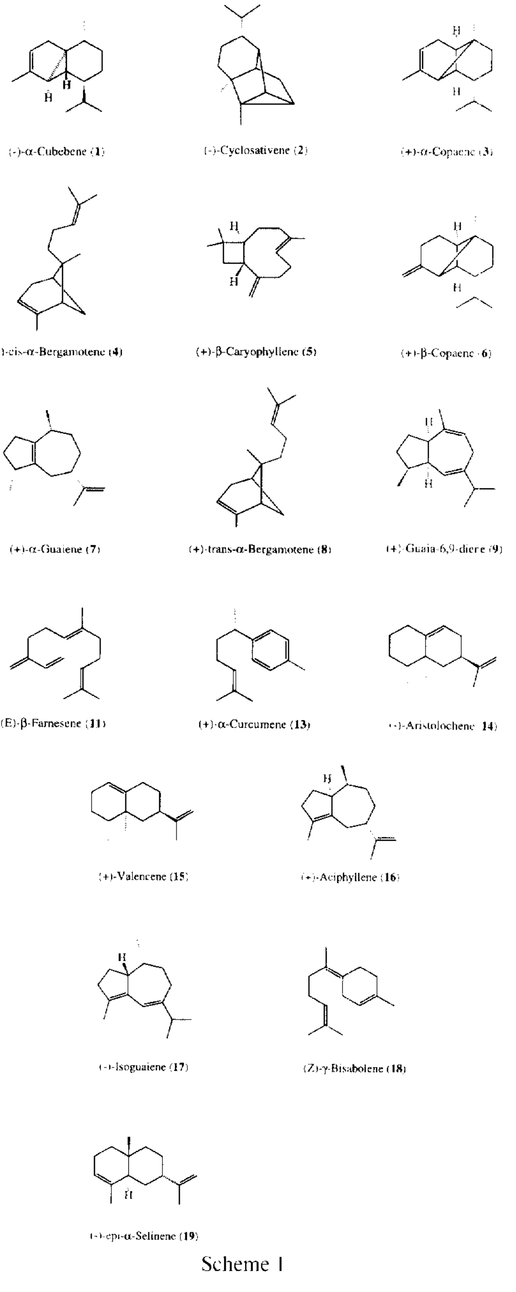
The occurrence of the unusual enantiomers of sesquiterpenes in liverworts has been observed by Huneck and Klein [6] for the first time and was confirmed by recent investigations of hydrodistillation products of several liverworts by enantioselective GC [7, 8]. Stereochemical assignments were achieved by comparison with reference compounds of known configuration and by 2D-GC. In the case of γ-bisabolene complete ^{1}H NMR data of the *E*-isomer were also published by Bohlmann *et al.* [9]. Our assignment of **18** as the *Z*-isomer is based on 2D NMR data and a diagnostic NOE correlation between the C-14 methyl protons and the C-1 methylene protons and their allylic position to an olefinic double bond.

The remaining fraction of the essential oil of *D. hirsuta* (Fraction 1, Fig. 2) was further investigated by GC-mass spectrometry. The compounds listed in Scheme 1 and Table 1 were identified by comparison of their electron impact mass spectra and retention indices on several capillary columns with polysiloxane phases (CpSil 5 and CpSil 19) and cyclodextrin derivatives with previously isolated reference compounds. For stereochemical analysis 2D-GC was applied using a capillary column with a conventional stationary phase for preseparation and a second capillary column with heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin for the determination of enantiomeric compositions as demonstrated in Fig. 2 for *cis*-α-bergamotene (**4**) and aristolochene (**14**). Reference compounds; (−)-*cis*-α-bergamotene, isolated previously by preparative GC from opoponax oil, a sample of (−)-aristolochene which was prepared by synthesis from (+)-valencene according to Cane *et al.* [10] and a sample of (+)-aristolochene, isolated before from the essential oil of the liverwort *Porella arboris-vitae* [11]. As shown in Table 1, β-caryophyllene (**5**), β-copaene (**6**), α-guaiene (**7**) and α-curcumene (**13**) are present as enantiomeric mixtures with the 'unusual' enantiomer in large excess. Although unusual enantiomers are common in liverworts the *Marchantiales* usually contain (−)-β-caryophyllene and (−)-β-caryophylleneoxide [3]. In the case of cyclosativene (**2**), α-copaene (**3**), *cis*-α-bergamotene (**4**), *trans*-α-bergamotene (**8**), guaia-6,9-diene (**9**) and aristolochene (**14**) only the unusual enantiomers were detected. For the bergamotenes this is the first natural occurrence of the (+)-enantiomer. Aciphyllene (**16**), a rare sesquiterpene hydrocarbon with the guaiane skeleton, was first reported as a constituent of *Lindera glauca*

Table 1. Enantiomeric composition of the sesquiterpene hydrocarbons of *Dumortiera hirsuta* as determined by two-dimensional enantioselective gas chromatography

Sesquiterpene	Percentage (+)-Enantiomer	Percentage (−)-Enantiomer
1. α-Cubebene	25	75
2. Cyclosativene	—	100
3. α-Copaene	100	—
4. *cis*-α-Bergamotene	100	—
5. β-Caryophyllene	95	5
6. β-Copaene	73	27
7. α-Guaiene	84	16
8. *trans*-α-Bergamotene	100	—
9. Guaia-6,9-diene	100	—
10. Unknown	—	—
11. (*E*)-β-Farnesene	—	—
12. Unknown		—
13. α-Curcumene	87	13
14. Aristolochene	—	100
15. Valencene	100	—
16. Aciphyllene	100	—
17. Isoguaiene	—	100
18. (*Z*)-γ-Bisabolene	—	—
19. *epi*-α-Selinene	—	100



Scheme 1

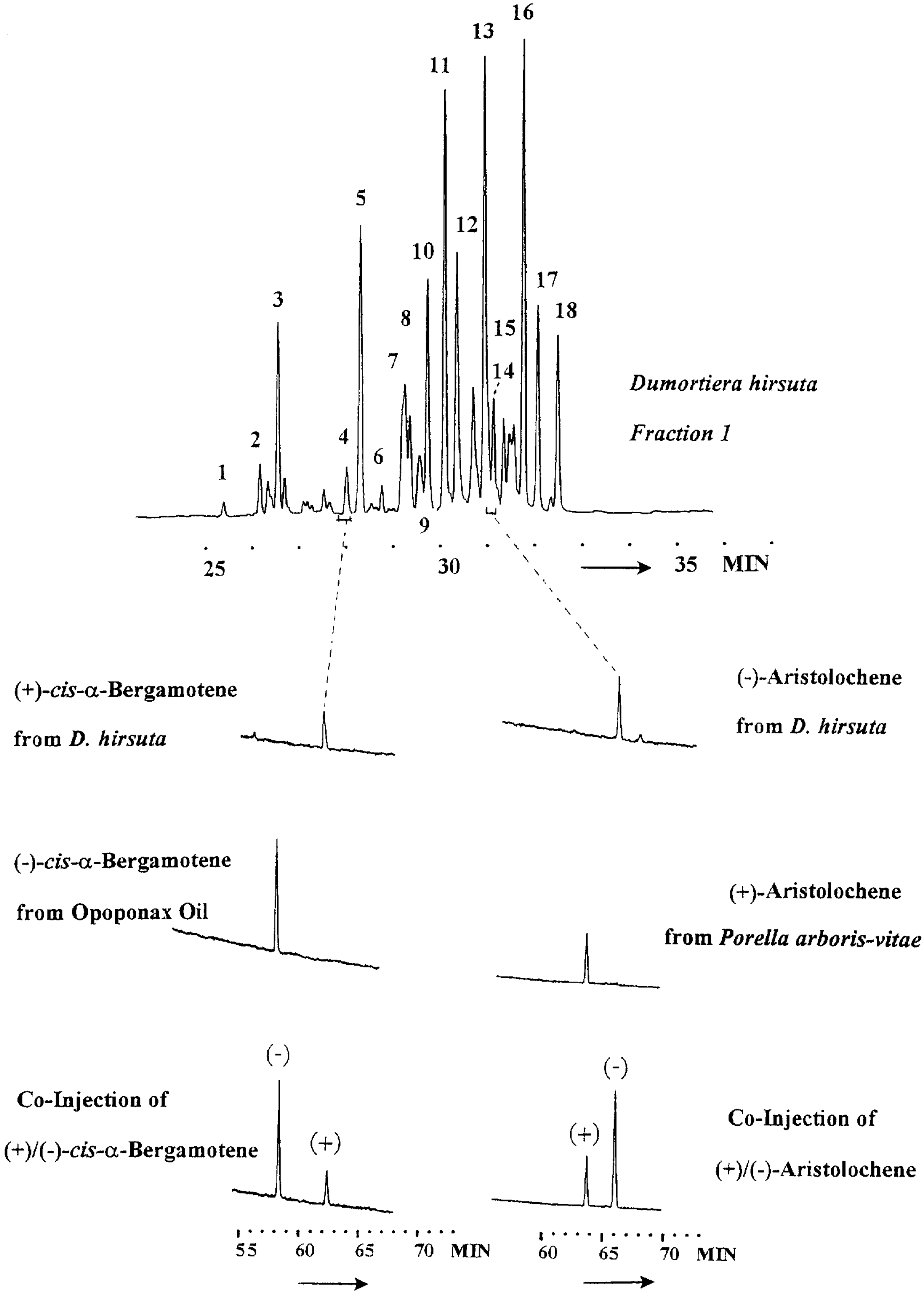


Fig. 2. 2-Dimensional gas chromatographic investigation of fraction 1 of the hydrodistillate of *Dumortiera hirsuta*. Upper part: preseparation on a 25 m capillary column with CpSil 5, column temp. 60°, temp. Programme 3° min^{-1} to 200°. Lower part: transferred fractions of *cis*-α-bergamotene (left) and aristolochene (right) to a 25 m capillary column with heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin at 100° (left) and 110° (right) and comparison with reference compounds under identical conditions.

(Sieb. et Zucc.) by Kubota *et al.* [12]. We have recently isolated aciphyllene as a minor constituent from patchouli oil (unpublished observation). In all cases the enantiomer with positive optical rotation was found. Finally, the (−)-enantiomer of 7-*epi*-α-selinene (**19**) was identified. We have found this compound before in the liverwort *Pellia epiphylla*, and in the higher plants *Nardostachys jatamanii* and *Bixa orellana*. The (+)-enantiomer of this compound was described as a constituent of *Dipterocarpus alatus* Roxb. (gurjun balsam oil) [13] and is also present in the liverwort *Porella arboris-vitae* [11]. α-Cubebene (**1**) with the (−)-enantiomer in excess, (+)-valencene (**15**) and *E*-β-farnesene (**11**) were the only constituents which are also common in higher plants. Two minor components eluting in front and behind **11** could not be identified.

EXPERIMENTAL

Plant material. *Dumortiera hirsuta* was collected near Sao Francisco de Paula, Rio Grande do Sul, Brazil, and identified by H. Muhle [14]. The collected liverwort is deposited in the Abteilung für Spezielle Botanik, Universität Ulm (voucher no. 140496-1-15).

Hydrodistillation. The essential oil of *D. hirsuta* was prepd by hydrodistillation (2 hr) of aq. homogenates of fresh and green plants using *n*-hexane as collection solvent. Because of the greatly differing weight, the fresh material was not weighed.

Enantioselective capillary GC. Capillary columns with cyclodextrin derivatives were prepd as described in ref. [15].

Two-dimensional GC. 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 200°. 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) which was isothermal at 95, 100, 105 or 110°, depending on the solute. The chromatograms from 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. NMR measurements were performed with WM 400 (400 MHz) instrument (Bruker) using TMS as int. standard.

GC-MS. Electron impact (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.

Isolation of sesquiterpene constituents. Isoguaiene (**17**) and *Z*-γ-bisabolene (**18**) were collected in one fr. by prep. GC on a Varian 1400 instrument, equipped with a stainless steel column (1.8 m × 4.3 mm i.d.) with 5% heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin/OV-1701 (1:1) on Chromosorb W-HP. He was used as carrier gas at a flow rate of 240 ml min^{-1}. Compounds **17** and **18** were sepd and purified by prep. TLC on silica gel 60 F$_{254}$ (Macherey–Nagel) using *n*-hexane as the mobile phase. Known compounds were identified by comparison of their R_t and MS with those of authentic materials. The R_t were measured on a two channel gas chromatograph (Orion) equipped with 25 m CpSil 5 and CpSil 19 (Chrompack) capillary columns.

β-Copaene (**6**). MS (EI, 70 eV), *m/z* (rel. int.): 204 (9), 161 (100), 133 (16), 120 (19), 119 (25), 107 (10), 105 (40), 93 (23), 91 (34), 81 (21), 79 (20), 77 (15), 69 (11), 67 (11), 55 (16), 41 (28).

Aristolochene (**14**). MS (EI, 70 eV), *m/z* (rel. int.): 204 (14), 189 (64), 162 (10), 161 (31), 147 (21), 136 (18), 133 (38), 121 (48), 119 (44), 107 (55), 105 (100), 93 (61), 81 (33), 79 (41), 77 (28), 69 (21), 67 (24), 55 (38), 43 (13), 41 (63).

Aciphyllene (**16**). ^{1}H NMR (CDCl$_3$): δ 4.66 (1H, *s*), 4.59 (1H, *s*), 2.95 (1H, *m*), 2.40 (1H, *br d*, $J = 13.3$ Hz), 2.13–2.30 (2H, *m*), 2.00–2.13 (3H, *m*), 1.95 (1H, *ddt*, $J_{d1} = 13.0$ Hz, $J_{d2} = 5.0$ Hz, $J_t = 8.8$ Hz), 1.80–1.90 (1H, *m*), 1.72 (3H, *s*), 1.50–1.73 (3H, *m*), 1.57 (3H, *s*), 1.45 (1H, *dddd*, $J_1 = 13$ Hz, $J_2 = 9.5$ Hz, $J_3 = 6.5$ Hz, $J_4 = 6.3$ Hz), 0.75 (3H, *d*, $J = 7.0$ Hz). ^{13}C NMR (CDCl$_3$): δ 12.94 (*q*), 14.14 (*q*), 20.23 (*q*), 28.52 (*t*), 31.96 (*t*), 35.14 (*t*), 36.96 (*d*), 37.10 (*t*), 37.44 (*t*), 45.72 (*d*), 53.28 (*d*), 107.55 (*t*), 132.54 (*s*), 135.17 (*s*), 152.83 (*s*). MS (EI, 70 eV), *m/z* (rel. int.): 204 (39), 189 (52), 175 (13), 161 (28), 147 (49), 133 (35), 121 (22), 119 (33), 108 (23), 107 (38), 105 (57), 95 (100), 93 (55), 91 (50), 81 (32), 79 (66), 77 (35), 69 (16), 67 (35), 65 (13), 55 (41), 53 (23), 43 (14), 41 (62).

Isoguaiene (**17**). MS (EI, 70 eV), *m/z* (rel. int.): 204 (92), 189 (81), 161 (100), 147 (33), 133 (40), 131 (14), 121 (19), 119 (71), 117 (15), 109 (14), 107 (31), 105 (76), 95 (21), 93 (36), 91 (61), 81 (31), 79 (26), 77 (27), 69 (21), 67 (19), 65 (12), 55 (36), 53 (16), 43 (19), 41 (57).

Z-γ-*Bisabolene* (**18**). ^{1}H NMR (C$_6$D$_6$): δ 5.40 (1H, *br s*), 5.22 (1H, *br s*), 2.82 (2H, *br s*), 2.31 (2H, *t*, $J = 6.3$ Hz), 2.13 (4H, *m*), 1.94 (2H, *t*, $J = 6.3$ Hz), 1.70 (3H, *s*), 1.66 (3H, *s*), 1.63 (3H, *s*), 1.54 (3H, *s*). ^{13}C NMR (C$_6$D$_6$): δ 133.97 (*s*), 131.21 (*s*), 125.67 (*s*), 125.30 (*d*), 121.37 (*d*), 34.83 (*t*), 31.83 (*t*), 29.81, 27.33 (*t*), 27.24 (*t*), 25.86, 23.57, 18.00, 17.66. MS (EI, 70 eV), *m/z* (rel. int.): 204 (27), 189 (4), 161 (11), 147 (8), 135 (23), 134 (26), 133 (14), 121 (24), 119 (62), 107 (68), 105 (29), 93 (100), 91 (40), 79 (37), 77 (26), 69 (27), 67 (12), 55 (39), 53 (15), 43 (35), 41 (78).

7-epi-α-*Selinene* (**19**). MS (EI, 70 eV), *m/z* (rel. int.): 204 (15), 189 (10), 161 (99), 147 (6), 133 (14), 122 (100), 119 (18), 107 (58), 105 (28), 95 (13), 93 (32), 91 (30), 81 (32), 79 (24), 77 (18), 69 (149), 67 (20), 55 (30), 53 (20), 43 (10), 41 (51).

Acknowledgements—We thank Dr V. Sinnwell, University of Hamburg, for his help in the interpretation of NMR spectra and the Fonds der Chemischen Industrie for financial support.

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