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SESQUITERPENE HYDROCARBONS IN THE ESSENTIAL OIL OF *MEUM ATHAMANTICUM*

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Key Word Index—*Meum athamanticum*; *Bazzania trilobata*; liverwort; enantioselective two-dimensional capillary GC; (−)-α-barbatene; (+)-β-barbatene; β-bazzanene; isobarbatene; isobazzanene.

Abstract—The sesquiterpene hydrocarbons β-bazzanene and α- and β-barbatene, typical constituents of liverworts (Hepaticae), were identified for the first time as constituents of a higher plant in the roots of *Meum athamanticum* (L.) Jacq. In addition, isobazzanene and isobarbatene, together with a variety of common sesquiterpene hydrocarbons, were identified. For α- and β-barbatene and isobarbatene the opposite configurations from those in liverworts were determined by enantioselective GC using modified cyclodextrins as chiral stationary phases. The configuration of β-bazzanene and isobazzanene could not be assigned. Isobarbatene was found for the first time as a natural compound.

INTRODUCTION

The sesquiterpene skeletons of β-bazzanene (**1**) and of the barbatenes (**2**, **3**) are believed to be unique to the liverworts [1]. β-Barbatene (**3**) has been detected in virtually every leafy liverwort of the Jungermanniales, but has never been found in higher plants. In the course of our investigation of the essential oil from *Meum athamanticum*, a strongly aromatic umbellifer of the mountains of Western and Central Europe, in addition to known constituents [2, 3] some uncommon compounds were found. The hydrocarbon fraction of the hydrodistillate of *M. athamanticum* roots, which contains a large variety of sesquiterpenes was investigated by GC-mass spectrometry. In addition to major peaks of (−)-β-elemene (**6**), (+)-bicyclogermacrene (**7**), (−)-germacrene D (**8**), (−)-α-chamigrene (**9**), (+)-β-bisabolene (**10**) and germacrene B (**11**), the mass spectra indicated the presence of some unusual constituents.

RESULTS AND DISCUSSION

The sesquiterpene hydrocarbons listed in Fig. 1 were identified by their mass spectra and retention indices on an unpolar stationary phase (CpSil 5). Most remarkably, among the many common sesquiterpene hydrocarbons, the members of a series of compounds so far only known as constituents of liverworts [4, 5] were unambiguously identified by their characteristic EI-mass spectra. To confirm their structures α- and β-barbatene were isolated by preparative GC using cyclodextrin derivatives as stationary phases [6]. The ^{1}H NMR spectra of the isolated fractions clearly confirmed the structures derived from the mass spectra. They were identical to those of α- and β-barbatene samples which were isolated by preparative GC from *Bazzania trilobata* and with published data [7]. Capillary GC with modified cyclodextrins as chiral stationary phases [8] indicated the presence of enantiomers with a configuration opposite to those isolated from *B. trilobata*. This was also confirmed by measuring the sense of optical rotation of the isolated fractions. Two-dimensional capillary GC [9] further confirmed these findings. The α- and β-barbatene fractions, respectively, were transferred from a capillary with a nonpolar stationary phase (CpSil 5) to a second capillary with heptakis (2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin [10] and compared by co-injection with the corresponding isomers isolated from *B. trilobata* under identical experimental conditions (Fig. 1).

Much smaller amounts of β-bazzanene (**1**) [11], isobazzanene (**4**) and isobarbatene (**5**) (with a 70% enantiomeric excess of the (−)-enantiomer, as determined by enantioselective capillary gas chromatograpy) could also be identified in the essential oil of *M. athamanticum* (Fig. 1) by comparing their GC retention times and their characteristic mass spectra with those of authentic reference compounds. While β-bazzanene (**1**)

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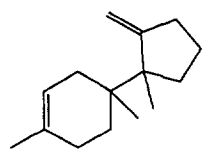

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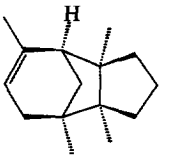

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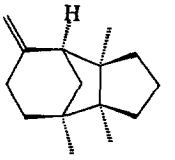

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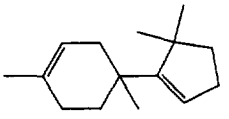

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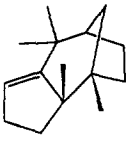

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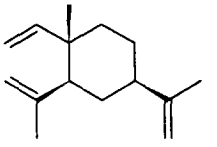

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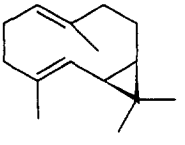

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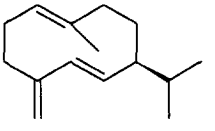

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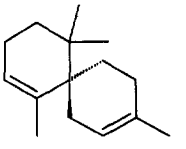

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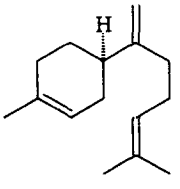

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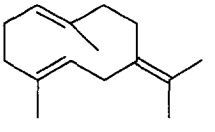

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has been found as a common constituent of liverworts [1], isobazzanene (**4**) was identified as a constituent of *B. fauriana* and *B. angustifolia* [12] and could be prepared from β-bazzanene (**1**) by rearrangement under acidic conditions [13]. Isobarbatene (**5**) has been obtained as a stable trifluoroacetic acid rearrangement product from α- (**2**) or β-barbatene (**3**) but never observed as a natural product [14].

EXPERIMENTAL

Plant material. Bazzania trilobata was collected in Southern Germany (Fichtelgebirge, Bischofsgrün-Karches) in November 1993. *Meum athamanticum* was harvested in July 1994 in the Botanical Garden of the University of Hamburg.

Hydrodistillation. The essential oils of *M. athamanticum* (L.) Jacq. and of *B. trilobata* (L.) S. Gray was prepared by steam distillation (6 and 2 hr, respectively) of aq. homogenates of fresh and green plants using *n*-hexane as collection solvent. Because of the greatly differing weights, the fresh material was not weighed.

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

Two-dimensional GC [16]. 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 at 50° and programmed at a rate of 3° min^{-1} to 200°. Sample transfer was performed after 33.83 min (the R_t of α-barbatene) and after 35.21 min (the R_t of β-barbatene) to a 25-m capillary column containing heptakis (2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin (50% in polysiloxane OV1701, w/w) which was kept isothermally at 100°. 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

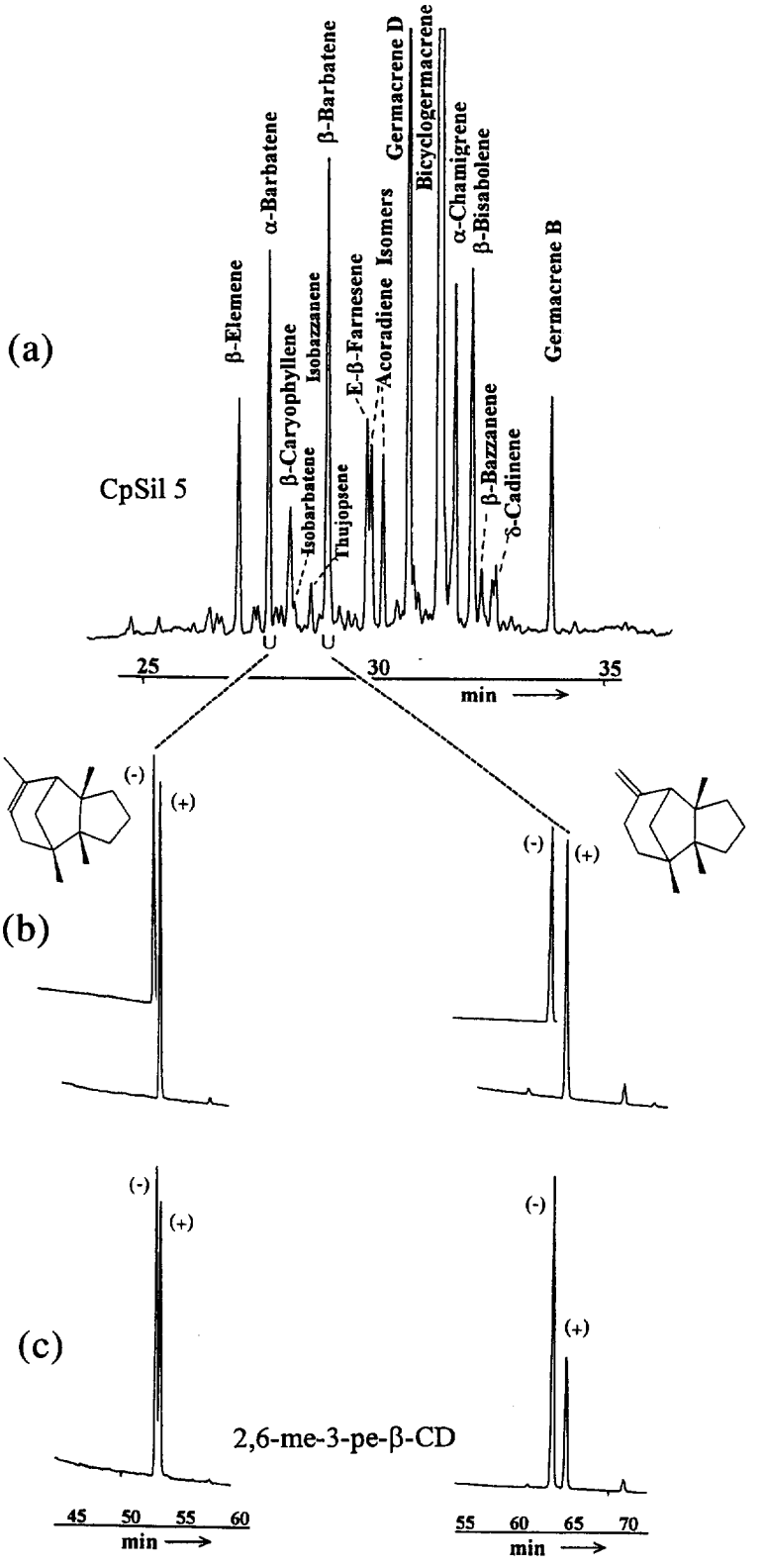

Fig. 1. Gas chromatographic investigation of the essential oil of the roots of *Meum athamanticum* (L.). (A) Partial gas chromatogram of the sesquiterpene portion on a 25 m fused silica capillary with methyl-polysiloxane CpSil 5; (B) gas chromatograms of *M. athamanticum* fractions of α- and β-barbatene transferred from the CpSil 5 capillary to a 25 m fused silica capillary with heptakis (2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin and comparison with α- and β-barbatene from the liverwort *Bazzania trilobata* transferred under identical conditions; (C) co-injection of *M. athamanticum* sample with α- and β-barbatene from *B. trilobata* and transfer of these fractions to the chiral capillary column.

for the CpSil 5 capillary and 65 kPa for the cyclodextrin capillary was used as a carrier gas.

Preparative GC. Isolation of **2** and **3** was performed by prep. GG on a Varian 1400 instrument, equipped with a stainless steel column (1.8 m × 4.3 mm) with 5% heptakis (2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin/OV-1701 (1:1, w/w) on Chromosorb W-HP (phase A) [6]. The rearrangement products isobazzanene (**4**) and isobarbatene (**5**) were isolated using a stainless steel column (2.0 m × 5.3 mm) with 2.5% heptakis (6-*O*-dimethylthexylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin-SE-52 (20:80) on Chromosorb G-HP (phase B). He was used as carrier gas at a flow rate of 240 ml min^{-1}.

NMR spectra (400 MHz) were measured in $CDCl_3$ using TMS as int. standard.

GC–EI-MS (70 eV) measurements were carried out on a HP 5890 gas chromatograph coupled to a VG Analytical VG 70-250S mass spectrometer.

β-Bazzanene (**1**). ^{1}H NMR: δ 5.29 (1H, *m*), 4.94 (1H, *m*), 4.79 (2H, *d*, *J* = 3 Hz), 1.64 (3H, *bs*), 1.02 (3H, *s*), 0.84 (3H, *s*); MS (EI, 70 eV), *m/z* (rel. int.): 204 $[M]^+$ (1), 109 (100), 108 (42), 93 (24), 67 (37), 41 (21).

α-Barbatene (**2**). α-Barbatene isolated from *M. athamanticum* was not completely separated from β-elemene. Therefore, the optical rotation was not measured. $[\alpha]_D^{22}$ +55.3° (*c* 0.001, $CHCl_3$, α-barbatene from *B. trilobata*); ^{1}H NMR: δ 5.19 (=CH, *m*), 1.65 (=C–CH_3, *s*), 1.00 (3H, *s*), 0.89 (3H, *s*), 0.84 (3H, *s*); EI-MS (70 eV), *m/z* (rel. int.): 204 $[M]^+$ (14), 109 (28), 108 (79), 96 (37), 95 (60), 94 (35), 93 (100), 81 (28).

β-Barbatene (**3**). $[\alpha]_D^{22}$ −22.5° (*c* 0.009, $CHCl_3$, β-barbatene from *B. trilobata*), $[\alpha]_D^{22}$ +19.4° (*c* 0.001, $CHCl_3$, β-barbatene from *M. athamanticum*); ^{1}H NMR: δ 4.60 (1H, *m*), 4.57 (1H, *m*), 1.03 (3H, *s*), 0.90 (3H, *s*), 0.84 (3H, *s*); EI-MS (70 eV), *m/z* (rel. int.): 204 $[M]^+$ (3), 111 (36), 108 (79), 96 (100), 95 (73), 94 (50), 93 (91), 81 (66), 79 (38).

Isobazzanene (**4**). EI-MS (70 eV), *m/z* (rel. int.): 204 $[M]^+$ (9), 189 (18), 136 (50), 121 (100), 107 (21), 93 (43), 91 (22), 79 (20), 41 (31).

Isobarbatene (**5**). (−)-β-Barbatene, 3 mg, (from *B. trilobata*) was dissolved in 0.5 ml *n*-hexane and a small amount of strong acidic ion exchange resin Amberlyst 15 was added. The reaction mixt. was stirred for 36 hr at 70°. Isobarbatene was isolated by prep. GC using phase A. Optical rotation measurement yielded a positive value (α = +29° of approx. 1 mg sample in 1.5 ml *n*-hexane). ^{1}H NMR: δ 5.25 (=CH, *dd*, *J* = 31 Hz, *J* = 2.0 Hz), 1.15 (3H, *s*), 1.09 (3H, *s*), 1.01 (3H, *s*), 0.94 (3H, *s*); EI-MS (70 eV), *m/z* (rel. int.): 204 $[M]^+$ (7), 124 (42), 123 (100), 122 (21), 109 (21), 107 (23), 105 (23), 93 (23), 91 (29), 81 (68), 79 (23), 55 (23), 41 (37).

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