PERGAMON

Phytochemistry 52 (1999) 1501–1505

PHYTOCHEMISTRY

Gymnomitrane-type sesquiterpenes of the liverworts *Gymnomitrion obtusum* and *Reboulia hemisphaerica*

Ute Warmers, Wilfried A. König*

Institut für Organische Chemie, Universität Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany

Received 27 January 1999; received in revised form 10 March 1999; accepted 22 March 1999

Abstract

The sesquiterpene constituents of the liverworts *Gymnomitrion obtusum* Lindb. and *Reboulia hemisphaerica* (L.) Raddi were investigated. In addition to many known compounds, a new sesquiterpene hydrocarbon, (−)-gymnomitra-3(15),4-diene was isolated and identified. Gymnomitrone, a sesquiterpene ketone, was found in nature for the first time. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Gymnomitrion obtusum*; Jungermanniales; *Reboulia hemisphaerica*; Marchantiales; Liverwort; Sesquiterpene; (−)-Gymnomitra-3(15),4-diene; Gymnomitrone; Enantioselective gas chromatography

1. Introduction

Gymnomitrion obtusum is a leafy liverwort (*Hepaticae*) of the order Jungermanniales and *Reboulia hemisphaerica* a thalloid liverwort of the order Marchantiales (Frahm & Frey, 1992). *G. obtusum* and *R. hemisphaerica* are rich sources of sesquiterpenes with the gymnomitrane skeleton, which are typical constituents of liverworts. (−)-β-Barbatene, (+)-gymnomitrol and (+)-gymnomitrol acetate are known as sesquiterpene constituents of *G. obtusum* (Connolly, Harding, & Thornton, 1972, 1974), (+)-gymnomitr-3(15)-en-4α-ol, (+)-3β-hydroxygymnomitran-4-one, *R*-(−)-1,4-dihydro-α-cuparenone, *R*-(−)-α-cuparenone as sesquiterpene constituents of *R. hemisphaerica* (Morais, Harrison & Becker, 1988, 1991; Morais & Becker, 1991). (Here the farnesane numbering system was used). Three chemotypes of *R. hemisphaerica* are known: an European chemotype containing several sesquiterpenes with gymnomitrane skeleton (as described above) and two Asian chemotypes with different constituents (Asakawa & Matsuda, 1982; Wei & Wu, 1995).

2. Results and discussion

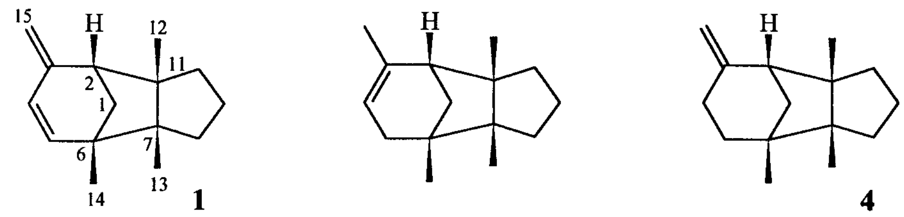
We have reinvestigated the sesquiterpene constituents of *G. obtusum* and *R. hemisphaerica* collected in Europe. Samples of plant volatiles were obtained by hydrodistillation or solvent extraction (in the case of *R. hemisphaerica*) and analyzed by gas chromatography (GC) and GC–mass spectrometry (GC–MS). There were only small differences in the relative percentages of single constituents in the hydrodistillation and in the solvent extraction products. Individual components were isolated by preparative GC and investigated by NMR (^{1}H, ^{13}C, ^{1}H^{1}H-COSY, HMQC, HMBC, NOESY). The absolute configuration was derived by polarimetric measurements and enantioselective GC using cyclodextrin phases. The following compounds were identified as constituents of the essential oil of *R. hemisphaerica*: (−)-gymnomitra-3(15),4-diene (**1**), α-barbatene, (−)-β-barbatene, gymnomitr-3(15)-en-4-one, gymnomitran-4-one, (+)-gymnomitr-3(15)-en-4α-ol, gymnomitrol, isobazzanene, β-bazza-

---

* Corresponding author.

E-mail address: wkoenig@chemie.uni-hamburg.de (W.A. König).

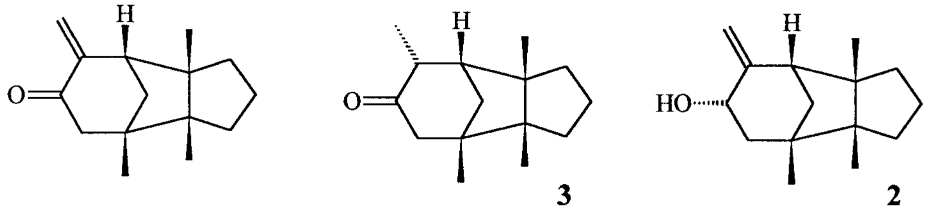
PII: S0031-9422(99)00205-8



1 (–)-Gymnomitra-3(15),4-diene

(+)- α-Barbatene

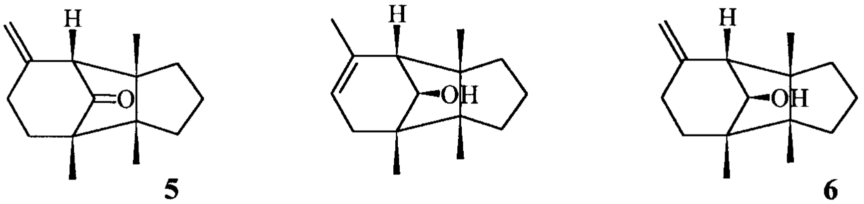
4 (–)-β-Barbatene



(+)-Gymnomitr-3(15)-en-4-one

3 (+)-Gymnomitran-4-one

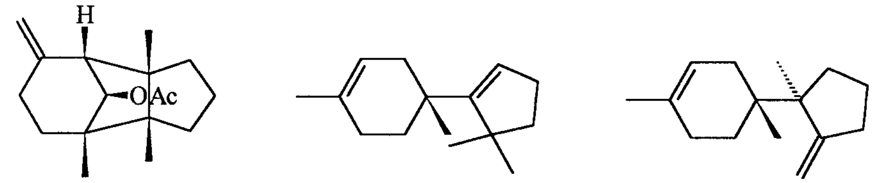
2 (+)-Gymnomitr-3(15)-en-4-ol



5 Gymnomitrone

(+)-Isogymnomitrol

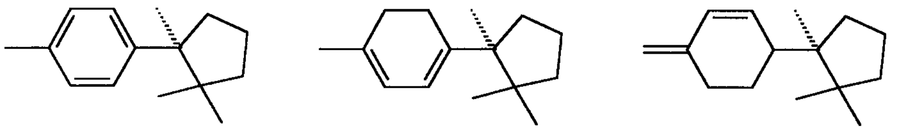
6 (+)-Gymnomitrol



(+)-Gymnomitrol acetate

Isobazzanene

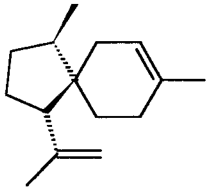
(+)-β-Bazzanene



(–)-Cuparene

(–)-α-Cuprenene

(–)-δ-Cuprenene



β-Acoradiene

Scheme 1.

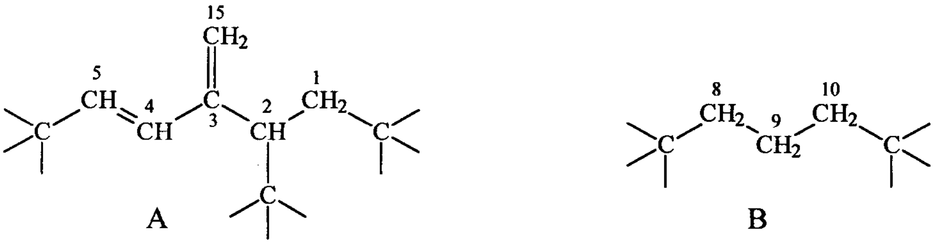


Fig. 1. Substructures A and B of gymnomitra-3(15),4-diene (**1**).

nene, cuparene, α-cuprenene, δ-cuprenene and β-acoradiene (Scheme 1).

(−)-Gymnomitra-3(15),4-diene (**1**) is a new sesquiterpene hydrocarbon. **1** is also a constituent of the liverworts *Plagiochasma rupestre* and *Gymnomitrion corallioides*. The mass spectrum exhibits a molecular ion signal at *m*/*z* 202 and an elemental composition of $C_{15}H_{22}$. The ^1H NMR spectrum shows singlets for three methyl groups at quaternary carbon atoms at δ 0.84, 0.91 and 1.03, broad singlets for two geminal olefinic protons at δ 4.72 and 4.85, doublets for two vicinal olefinic protons at δ 5.59 and 6.02 and a broad doublet for an allylic proton at δ 2.32.

The connection of the groups could be derived from the $^1H^1H$-COSY NMR spectrum. The two vicinal olefinic protons at δ 5.59 (CH-5) and 6.02 (CH-4) couple with each other and with the two geminal olefinic protons at δ 4.72 and 4.85 (CH_2-15). The allylic proton at δ 2.32 (CH-2) has to be bonded to the quaternary carbon atom of the exocyclic double bond (C-3). This proton couples to the protons of a methylene group at δ 1.62 and 1.83–1.93 (CH_2-1). This suggests substructure A (Fig. 1).

The protons of three methylene groups at δ 1.04–1.14, 1.20–1.28, 1.54–1.70, 1.65–1.81, 1.83–1.93 and 1.86–1.99 (CH_2-8, CH_2-9, CH_2-10) couple with each other. This suggests the existence of a chain of three methylene groups as shown in substructure B (Fig. 1).

The three quaternary methyl groups at δ 0.84, 0.91 and 1.03 (CH_3-12, CH_3-13, CH_3-14) have to be located at the ends of these two substructures A and B, so that they could be combined to a tricyclic molecule with the structure **1**.

The structure of **1** was verified by a chemical correlation. The major compound of *R. hemisphaerica* is (+)-gymnomitr-3(15)-en-4α-ol (**2**). Dehydration of **2** yields the rearrangement product gymnomitran-4-one (**3**) (Morais et al., 1988) and the hydrocarbon (−)-gymnomitra-3(15),4-diene (**1**) (Fig. 2). So the relative and absolute configuration of **1** has to be identical with **2**. The dehydration product and the natural product **1** were compared by GC–MS and enantioselective GC using cyclodextrin phases.

Futhermore, **1** was correlated with (−)-β-barbatene (**4**), another constituent of *R. hemisphaerica*. Hydrogenation of **1** and **4** yields the same products (Fig. 3).

The following sesquiterpenes were identified as constituents of the essential oil of *G. obtusum*: α-barbatene, (−)-β-barbatene, gymnomitrone (**5**), isogymnomitrol, (+)-gymnomitrol, (+)-gymnomitrol acetate, isobazzanene and cuparene (Scheme 1).

Gymnomitrone (**5**) has not been found before in nature and was only known as a synthetic product (Han & Paquette, 1979; Coates, Shah, & Mason, 1979, 1982; Paquette & Han, 1981; O'Neil, Quickley, & Snider, 1997). The spectral data are identical to those reported for the synthetic product. The absolute configuration was obtained by a correlation reaction. Reduction of **5** yields (+)-gymnomitrol (**6**) (O'Neil et al., 1997), the major constituent of *G. obtusum* (Fig. 4). So the absolute configuration of **5** at C-2, C-6, C-7 and C-11 must be identical to **6**. The reduction product and the natural product **6** were compared by GC–MS and enantioselective GC using cyclodextrin phases.

The identification of **1** and **5** as new constituents of liverworts complements the already know variety of

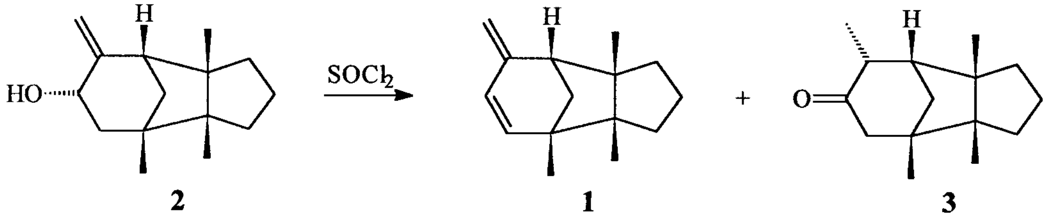


Fig. 2. Dehydration and rearrangement of (+)-gymnomitr-3(15)-en-4α-ol (**2**) to (−)-gymnomitra-3(15),4-diene (**1**) and gymnomitran-4-one (**3**).

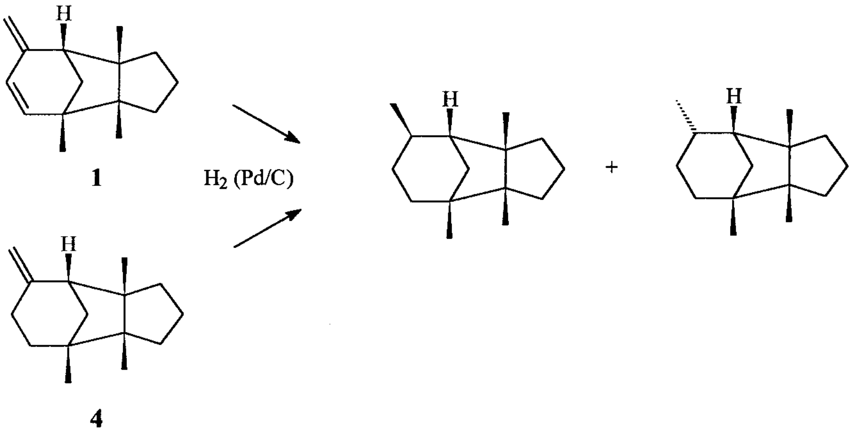


Fig. 3. Hydrogenation of (−)-gymnomitra-3(15),4-diene (**1**) and (−)-β-barbatene (**4**) to saturated gymnomitranes.

structures with gymnomitrane skeleton as typical constituents of the *Hepaticae*.

3. Experimental

3.1. Plant material

Fresh plant material of *R. hemisphaerica* was collected at the river Argout near Burlats and Les Salvages (France) by U. Heseler, St. Ingbert. A tissue culture of *R. hemisphaerica* was obtained from Professor Dr. H. Becker, Universität des Saarlandes, Saarbrücken, Germany. *R. hemisphaerica* was identified by Professor Dr. R. Mues, Universität des Saarlandes, Saarbrücken, Germany. In the natural and cultured plant material of *R. hemisphaerica* identical constituents are found. *G. obtusum* was collected at the mount Meißner (Germany) and identified by Dr. H. Muhle, Universität Ulm, Germany.

3.2. Hydrodistillation

The essential oil was prepared by hydrodistillation (2 h) of aqueous homogenates of fresh and green plants using *n*-hexane as collection solvent. Because of the different drying state of the plants the material was not weighed.

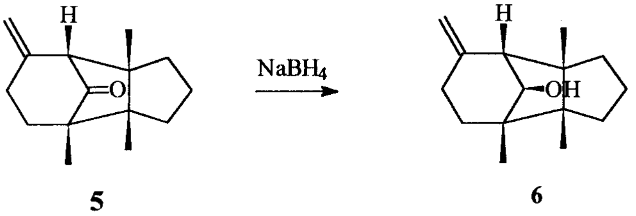


Fig. 4. Reduction of gymnomitrone (**5**) to (+)-gymnomitrol (**6**).

3.3. Extraction

Samples of plant volatiles were prepared by extraction (7 days, 20°C) of fresh and green plants with diethyl ether.

3.4. Gas chromatography

Orion Micromat 412 double column instrument with 25 m fused silica capillaries with polysiloxane CpSil 5 and polysiloxane CpSil 19 (Chrompack); Carlo Erba Fractovap 2150, 4160 instrument with 25 m fused silica capillaries with heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin or heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin in OV 1701 (50 wt%); split injection; flame ionization detection; carrier gas 0.5 bar H_2.

3.5. Isolation

The isolation was carried out by preparative GC.

3.6. Preparative GC

Modified Varian 1400 instrument, equipped with a stainless steel column (1.85 m × 4.3 mm) with 10% polydimethylsiloxane SE 30 on Chromosorb W-HP; flame ionization detection; helium as carrier gas at a flow rate of 240 ml/min (Hardt & König, 1994).

3.7. NMR spectroscopy

NMR measurements were carried out with a Bruker WM 500 instrument.

3.8. Mass spectrometry

GC–MS measurements were run on a HP 5890 gas chromatograph coupled to a VG Analytical 70-250S mass spectrometer.

*3.9. (−)-Gymnomitra-3(15),4-diene (**1**)*

^{1}H NMR (500 MHz, C_6D_6): δ 0.84 (3H, s, H-12/13/14), 0.91 (3H, s, H-12/13/14), 1.03 (3H, s, H-12/13/14), 1.04–1.14 (1H, m, H-8/10), 1.20–1.28 (1H, m, H-8/10), 1.54–1.70 (1H, m, H-9), 1.62 (1H, d, J = 11.1 Hz, H-1), 1.65–1.81 (1H, m, H-9), 1.83–1.93 (2H, m, H-1, H-8/10), 1.86–1.99 (1H, m, H-8/10), 2.32 (1H, d, J = 4.4 Hz, H-2), 4.72 (1H, bs, H-15), 4.85 (1H, bs, H-15), 5.59 (1H, d, J = 9.5 Hz, H-5), 6.02 (1H, d, J = 9.5 Hz, H-4); MS (EI, 70 eV): m/z (rel. int.) 202 (4) [M^+], 107 (19), 106 (94), 105 (17), 96 (21), 95 (22), 91 (100), 81 (17), 79 (13), 77 (14), 65 (9), 55 (15), 41 (19).

*3.10. Dehydration of (+)-gymnomitr-3(15)-en-4α-ol (**2**)*

To a sol. of 1 mg of **2** in 1 ml pyridine 0.1 ml $SOCl_2$ was added and the reaction mixture was stirred at 0°C for 10 min. *n*-Hexane and H_2O were added. The organic phase was washed three times with H_2O. The reaction product was analyzed by GC–MS and by GC on various capillary columns with cyclodextrin phases. It was identical with **1**.

*3.11. Hydrogenation of (−)-gymnomitra-3(15),4-diene (**1**)*

To a sol. of 1 mg of **1** in 1 ml *n*-hexane, 0.5 mg Pd/C was added. The suspension was treated with H_2 and stirred under H_2 at room temp. for 1 h. The reaction mixture was filtered and the reaction products were analyzed by GC–MS and by GC on various capillary columns.

*3.12. Hydrogenation of (−)-β-barbatene (**4**)*

The hydrogenation of **4** was performed analogously to the hydrogenation of **1**. The reaction products were analyzed by GC–MS and by GC on several capillary columns. The hydrogenated compounds were identical with the reaction products of the hydrogenation of **1**.

Acknowledgements

We thank Professor Dr. H. Becker, Professor Dr. R. Mues, U. Heseler and Dr. H. Muhle for the plant material and Dr. V. Sinnwell, Universität Hamburg and his team for the NMR measurements. The financial support of the *Fonds der Chemischen Industrie* is gratefully acknowledged.

References

Asakawa, Y., & Matsuda, R. (1982). *Phytochemistry*, *21*, 2143.

Frahm, J.-P., & Frey, W. (1992). *Moosflora.* Stuttgart: Ulmer-Verlag.

Coates, R. M., Shah, S. K., & Mason, R. W. (1979). *Journal of the American Chemical Society*, *101*, 6765.

Coates, R. M., Shah, S. K., & Mason, R. W. (1982). *Journal of the American Chemical Society*, *104*, 2198.

Connolly, J. D., Harding, A. E., Thornton, I. M. S. (1972). *Journal of the Chemical Society Chemical Communications*, 1321.

Connolly, J. D., Harding, A. E., Thornton, I. M. S. (1974). *Journal of the Chemical Society Perkin Transactions 1*, 2487.

Han, Y. K., & Paquette, L. A. (1979). *Journal of Organic Chemistry*, *44*, 3731.

Hardt, I., & König, W. A. (1994). *Journal of Chromatography A*, *666*, 611.

Morais, R. M. S. C., & Becker, H. (1991). *Zeitschrift für Naturforschung*, *46c*, 28.

Morais, R. M. S. C., Harrison, L. J., & Becker, H. (1988). *Journal of Chemical Research Synopses*, 380.

Morais, R. M. S. C., Harrison, L. J., & Becker, H. (1991). *Phytochemistry*, *30*, 1013.

O'Neil, S. V., Quickley, C. A., & Snider, B. B. (1997). *Journal of Organic Chemistry*, *62*, 1970.

Paquette, L. A., & Han, Y. K. (1981). *Journal of the American Chemical Society*, *103*, 1831.

Wei, H. C., & Wu, C. L. (1995). *Phytochemistry*, *39*, 91.