PERGAMON

Phytochemistry 51 (1999) 679–682

PHYTOCHEMISTRY

(+)-Bisabola-2,10-diene[1,9]oxide, a constituent of the liverwort *Calypogeia suecica*

Ute Warmers[a], Angela Rieck[a], Wilfried A. König[a,*], Hermann Muhle[b]

[a]*Institut für Organische Chemie, Universität Hamburg, Martin-Luther-King-Platz 6, 20146 Hamburg, Germany*
[b]*Abteilung Spezielle Botanik, Universität Ulm, 89091 Ulm, Germany*

Received 8 January 1999; received in revised form 8 February 1999; accepted 8 February 1999

Abstract

The sesquiterpene constituents of the liverwort *Calypogeia suecica* (Arn. and Perss.) K. Müll. were investigated. In addition to many known compounds a new sesquiterpene oxide, (+)-bisabola-2,10-diene[1,9]oxide, could be isolated and identified. The structure elucidation was carried out by NMR spectroscopic techniques and chemical conversions.

Keywords: *Calypogeia suecica*; Jungermanniales; Liverwort; Sesquiterpene; (+)-Bisabola-2,10-diene[1,9]oxide; Enantioselective gas chromatography

1. Introduction

Calypogeia suecica is a leafy liverwort (*Hepaticae*) of the order Jungermanniales (Frahm & Frey, 1992). Essential oil samples were obtained by hydrodistillation and analysed by gas chromatography (GC) and GC–mass spectrometry (GC–MS). 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.

2. Results and discussion

The following compounds were identified as constituents of the essential oil of *C. suecica*: 7-*epi*-sesquithujene (Joulain & König, 1998), (+)-γ-curcumene (Andersen & Syrdal, 1970), (+)-*ar*-curcumene (Meyers & Stoianova, 1997), (*Z*)-γ-bisabolene (Saritas, Bülow, Fricke, König, & Muhle, 1998), β-sesquiphellandrene (Connell & Sutherland, 1966), (+)-bisabola-2,10-diene[1,9]oxide, bicyclogermacrene (McMurry & Bosch, 1987), anastreptene (Andersen, Ohta, Moore, & Tseng, 1978), 4,5-dehydroviridiflorol (Warmers, Wihstutz, Bülow, Fricke, & König, 1998), 9-aristolene (Wu & Asakawa, 1988), 1(10),8-aristoladiene (Joulain & König, 1998), calarene (Joulain & König, 1998), β-barbatene (König, Rieck, Saritas, Hardt, & Kubeczka, 1996) and italicene (Leimner, Marschall, Meier, & Weyerstahl, 1984).

The major component is the new sesquiterpene oxide, (+)-bisabola-2,10-diene[1,9]oxide {(1*R*,6*S*,7*S*,9*S*)-2*H*-3,4,4a,5,6,8a-hexahydro-4,7-dimethyl-2(2-methylpropylidene)chromene} (**1**) (Scheme 1).

The mass spectrum exhibits a molecular ion signal at *m*/*z* 220 and an elemental composition of $C_{15}H_{24}O$.

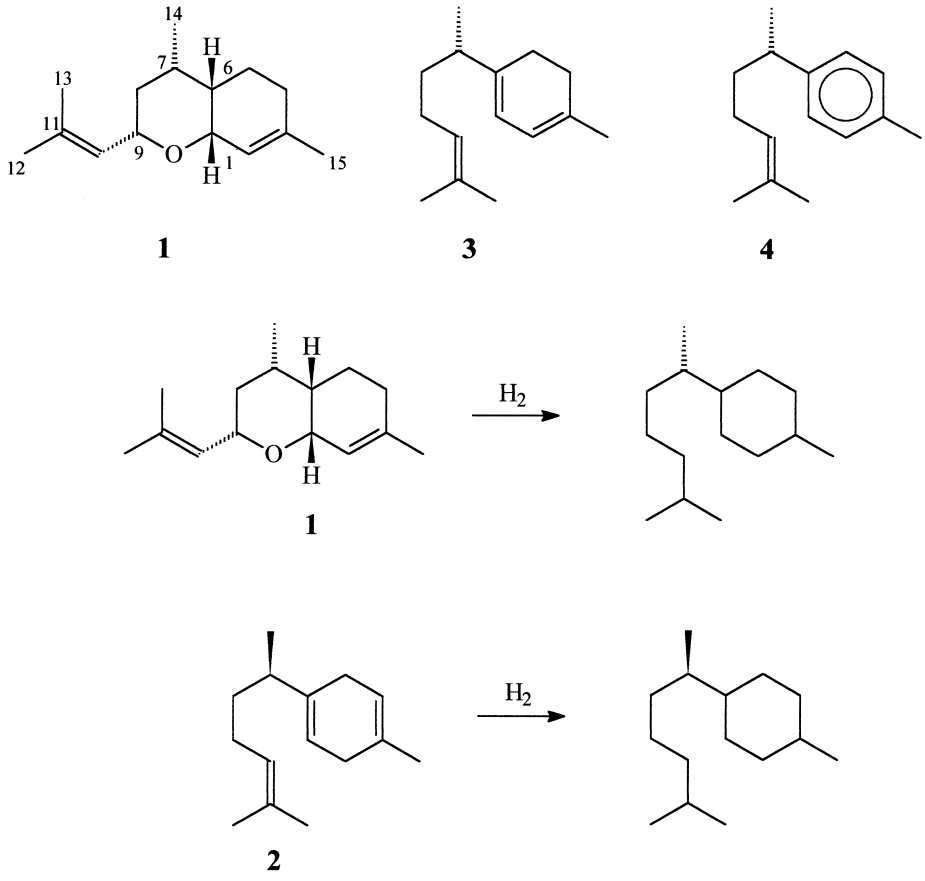
The ^{1}H NMR spectrum shows a doublet for a secondary methyl group at δ 0.96, singlets for three methyl groups on double bonds at δ 1.67, 1.67 and 1.69, two signals for allylic protons adjacent to an oxygen atom at δ 3.78 and 4.07, respectively, and two signals for olefinic protons at δ 5.21 and 5.57.

In the ^{13}C DEPT spectrum signals of four primary

* Corresponding author. Tel.: +49-40-4123-2824; fax: +49-40-4123-2893.

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

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00081-3



Scheme 1

carbon atoms (δ 18.76, 19.35, 23.95, 26.07), three secondary carbons (δ 16.52, 31.62, 36.10), six tertiary carbons (δ 33.44, 38.73, 73.86, 75.34, 121.99, 127.03) and two quaternary carbons (δ 135.04, 140.93) were observed. The slightly low field shifted signals at δ 73.86 and 75.34 were assigned to two tertiary carbons bonded to an oxygen. The strongly low field shifted signals at δ 121.99, 127.03, 135.04 and 140.93 indicate the presence of two double bonds.

The HMQC spectrum shows the correlation of the ^{1}H- and the ^{13}C NMR spectrum. The connection of the groups could be derived from the ^{1}H^{1}H-COSY- and HMBC spectrum. The olefinic methine group (δ 5.21, 127.03, CH-10) shows a coupling correlation with the methine group next to oxygen (δ 4.07, 75.34, CH-9) and with the olefinic quaternary carbon (δ 135.04, C-11) connected to two methyl groups (δ 1.67, 18.67 CH_3-12; 1.69, 26.07, CH_3-13).

The other olefinic methine group (δ 5.57, 121.99, CH-2) couples with the other methine group next to oxygen (δ 3.78, 73.86, CH-1) and with the olefinic quaternary carbon (δ 140.93, C-3), which is connected to a methyl group (δ 1.67, 23.95, CH_3-15) and to a methylene group (δ 1.88–2.00, 2.03, 31.62, CH_2-4). This methylene group is connected to another methylene group (δ 1.38–1.51, 1.54–1.66, 16.52, CH_2-5) coupling with a methine group (δ 1.38–1.51, 38.73, CH-6). This methine group is bonded to the above mentioned methine group next to oxygen (δ 3.78, 73.86, CH-1).

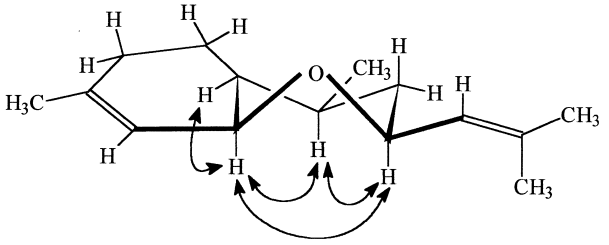
The C-6 methine group further couples with a methine group (δ 1.88–2.00, 33.44, CH-7) which is bonded to a methyl group (δ 0.96, 19.35, CH_3-14) and to a methylene group (δ 1.20, 1.29, 36.10, CH_2-8). This methylene group is connected to the C-9 methine group. Combination of all these data leads to structure **1**.

The relative configuration of the stereogenic centres at C-1, C-6, C-7 and C-9 could be derived from a NOESY spectrum. The saturation of the resonance of H-9 results in an increase of the signal intensities of H-1 and H-7, while H-1 interacts with H-9, H-7 and H-6. Therefore, the protons H-1, H-6, H-7 and H-9 have to be on the same side of the molecule (Fig. 1).

The absolute configuration was obtained by a correlation reaction. **1** was hydrogenated stepwise and the progress of the hydrogenation was followed by GC–MS analysis. First the oxide was opened. Further hydrogenation resulted in a simultaneous dehydration to fully saturated bisabolanes. Enantioselective GC using a cyclodextrin phase unambiguously proved that

these bisabolanes and the corresponding hydrogenation products of (−)-β-curcumene (**2**) (Andersen & Syrdal, 1970) have the opposite configuration (Scheme 1). **2** was isolated from the essential oil of *Curcuma javanica* (Rieck, 1993).

As expected, **1** and the other constituents with bisabolane skeleton from *C. suecica*, (+)-γ-curcumene **3** and (+)-*ar*-curcumene **4**, have the same absolute configuration (Scheme 1).



1

Fig. 1. NOE effects of bisabola-2,10-diene[1,9]oxide (**1**).

3. Experimental

3.1. Plant material

Calypogeia suecica was collected in the Carpathian Mountains (Ukraine) by H. Muhle. It was identified by R. Mues, Universität des Saarlandes, Saarbrücken, 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.

3.3. 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 column instruments 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%, w/w); split injection; flame ionization detection; carrier gas 0.5 bar H_2.

3.4. Isolation

The isolation was carried out using preparative GC.

3.5. 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).

3.6. NMR spectroscopy

NMR measurements were carried out with a Bruker WM 400- or a Bruker WM 500 instrument using TMS as internal standard.

3.7. Mass spectrometry

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

3.8. (+)-Bisabola-2,10-diene[1,9]oxide (1)

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (3H, d, J=7.1 Hz, H-14), 1.20 (1H, m, H-8α), 1.29 (1H, ddd, J=3.6, 3.6, 12.7 Hz, H-8β), 1.38-1.51 (2H, m, H-5β, H-6), 1.54–1.66 (1H, m, H-5α), 1.67 (6H, s, H-12, H-15), 1.69 (3H, s, H-13), 1.88–2.00 (2H, m, H-4α/β, H-7), 2.03 (1H, dd, J=5.6, 17.8 Hz, H-4α/β), 3.78 (1H, bs, H-1), 4.07 (1H, ddd, J=3.6, 8.2, 10.9 Hz, H-9), 5.21 (1H, bd, J=8.2 Hz, H-10), 5.57 (1H, d, J=5.6 Hz, H-2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.52 (t, C-5), 18.76 (q, C-12), 19.35 (q, C-14), 23.95 (q, C-15), 26.07 (q, C-13), 31.62 (t, C-4), 33.44 (d, C-7), 36.10 (t, C-8), 38.73 (d, C-6), 73.86 (d, C-1), 75.34 (d, C-9), 121.99 (d, C-2), 127.03 (d, C-10), 135.04 (s, C-11), 140.93 (s, C-3); ^{1}H NMR (400 MHz, C_6D_6): δ 0.85 (3H, d, J=7.1 Hz, H-14), 1.20–1.28 (2H, m, H-8α, H-8β), 1.25–1.37 (2H, m, H-5α/β, H-6), 1.58 (3H, s, H-15), 1.60 (3H, s, H-12), 1.62 (3H, s, H-13), 1.66–1.84 (3H, m, H-4α/β, H-7, H-5α/β), 1.88 (1H, dd, J=5.6, 17.8 Hz, H-4α/β), 3.76 (1H, s, H-1), 4.10 (1H, dd, J=8.2, 10.9 Hz, H-9), 5.46 (1H, bd, J=8.2 Hz, H-10), 5.74 (1H, d, J=5.6, 1.0 Hz, H-2); ^{13}C NMR (125 MHz, C_6D_6): δ 18.01 (t, C-5), 19.95 (q, C-12), 20.72 (q, C-14), 25.04 (q, C-15), 27.24 (q, C-13), 33.06 (t, C-4), 34.90 (d, C-7), 37.78 (t, C-8), 40.39 (d, C-6), 75.27 (d, C-1), 76.84 (d, C-9), 124.46 (d, C-2), 129.36 (d, C-10), 134.52 (s, C-11), 140.50 (s, C-3); MS (EI, 70 eV): m/z (rel.int.) 220 (2) [M^+], 202 (3) [M^+–H_2O], 164 (16), 131 (13), 121 (16), 109 (12), 105 (11), 94 (100), 79 (36), 67 (16), 55 (17), 41 (24).

3.9. Hydrogenation of (+)-bisabola-2,10-diene[1,9]oxide (1)

To a soln of 1 mg of **1** in 1 ml *n*-hexane, 0.5 mg Pd/C were added. The suspension was treated with H_2

and stirred under H_2 at room temp. for 1 h. This procedure was repeated three times. The reaction mixture was filtered and the reaction products were analysed by GC–MS and by GC on various capillary columns with polysiloxane and cyclodextrin phases.

3.10. Hydrogenation of (−)-β-curcumene (2)

The hydrogenation of **2** was performed analogously to the hydrogenation of **1**. The reaction products were analysed by GC–MS and by GC on various capillary columns with polysiloxane and cyclodextrin phases and compared with the hydrogenation products of **1**. The hydrogenated bisabolanes have the same retention times on the polysiloxane phases, but they could be separated on a column with heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin as stationary phase. The saturated bisabolanes obtained from **1** and **2** have the opposite configuration.

References

Andersen, N. H., & Syrdal, D. D. (1970). *Phytochemistry*, *9*, 1325.
Andersen, N. H., Ohta, Y., Moore, A., & Tseng, C.-L. W. (1978). *Tetrahedron*, *34*, 41.
Connell, D. W., & Sutherland, M. D. (1966). *Aust. J. Chem.*, *19*, 283.
Frahm, J.-P., & Frey, W. (1992). *Moosflora*. Stuttgart: Ulmer-Verlag.
Hardt, I., & König, W. A. (1994). *J. Chromatogr. A*, *666*, 611.
Joulain, D., & König, W. A. (1998). *The atlas of spectral data of sesquiterpenen hydrocarbons*. Hamburg: E.B.-Verlag.
König, W. A., Rieck, A., Saritas, Y., Hardt, I. H., & Kubeczka, K.-H. (1996). *Phytochemistry*, *42*, 461.
Leimner, J., Marschall, H., Meier, N., & Weyerstahl P. (1984). *Chem. Lett.*, 1769.
McMurry, J. E., & Bosch, G. K. (1987). *J. Org. Chem.*, *52*, 4885.
Meyers, A. I., & Stoianova, D. (1997). *J. Org. Chem.*, *62*, 5219.
Rieck, A. (1993). Diploma thesis, Universität Hamburg.
Saritas, Y., Bülow, N., Fricke, C., König, W. A., & Muhle, H. (1998). *Phytochemistry*, *48*, 1019.
Warmers, U., Wihstutz, K., Bülow, N., Fricke, C., & König, W. A. (1998). *Phytochemistry*, *49*, 1723.
Wu, C.-L., & Asakawa, Y. (1988). *Phytochemistry*, *27*, 940.