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Sesquiterpene constituents of the liverwort *Lophozia ventricosa*

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Abstract—The essential oil of *Lophozia ventricosa* was investigated and yielded the two new sesquiterpenoids ventricos-7(13)-ene and 6,7-epoxyeudesm-3-ene. The first has a new skeleton, which is named ventricosane and is numbered after the structurally closest known sesquiterpene skeleton pentalenane. The absolute configuration of the latter compound was established by conversion to known compounds. The isolated compounds and reaction products were investigated using enantioselective GC and extensive NMR measurements (^{1}H; ^{1}H–^{1}H COSY; HSQC; HMBC and NOE experiments).

1. Introduction

The essential oil of the liverwort *Lophozia ventricosa* belonging to the family of Lophoziaceae was investigated by means of GC–MS, which yielded two unknown mass spectra for compounds (−)-ventricos-7(13)-ene **1** and (+)-6,7-epoxyeudesm-3-ene **2** that were hence isolated and their structures assigned.

Earlier investigations[1–6] of the said liverwort extracts yielded sesquiterpene hydrocarbons and oxygenated sesquiterpenes γ- and δ-elemene, β-caryophyllene, β-chamigrene, humulene, γ-cuparenene, bicyclogermacrene, trinoranastreptene, eudesma-4(15),11-dien-8-one, ventricosenediolide, eudesma-4(15),7(11)-dien-8-one (= ventricosin A), *ent*-maalioxide (= ventricosin B), eudesm-3-ene-6α,7β-diol as well as the phytosterols brassicasterol, campesterol, sitosterol and cycloartenol.

2. Results and discussion

2.1. Composition of the essential oil of *Lophozia ventricosa*

The hydrodistillation product investigated herein consisted of the following compounds (sorted in the order of their relative amounts; if the signs of the specific rotations are given, they have been proven by coinjections with authentical samples on enantioselective phases or isolated and directly measured): (−)-Maalioxide (45.6%), (−)-eudesma-4(15),7(11)-dien-8-one (28.9%), (+)-1(10)-spirovetiven-7β-ol (5.7 %), eudesma-4(15),11-dien-8-one (5.7%), 1(10)-valencen-7β-ol (1.6%), β-barbatene (1.5%), (+)-eudesma-3,7(11)-dien-8-one (1.4%), (+)-6,7-epoxyeudesm-3-ene **2** (0.8%), selina-4(15),11-dien-8-ol (0.8%), (−)-ventricos-7(13)-ene **1** (0.3%), germacrene B (0.3%), lepidozenal (0.3%), anastreptene (0.2%), (−)-maaliol (0.1%), δ-cadinene (0.1%), striatol (0.1%), viridiflorol (0.1%), globulol (0.1%), and traces of fusioccadiene, α-pinene, 1-octene-3-ol, 1-oct-3-enylacetate, trinoranastreptene, δ-elemene, β-caryophyllene, guaia-6,9-diene-4β-ol, rosifoliol, eudesm-4-ene-7-ol, and traces of other unidentified constituents.

2.2. Structure elucidation of (−)-ventricos-7(13)-ene 1

The mass spectrum of **1** exhibits a molecular ion signal at *m*/*z* 204 and an elemental composition of $C_{15}H_{24}$ with four double bond equivalents. The ^{13}C NMR and DEPT spectra of **1** show signals for three primary carbons (δ 15.15, 30.1, 30.4), six secondary carbons (δ 32.8, 34.1, 41.0, 49.6, 57.4, 103.9), three tertiary carbons (δ 45.8, 50.8, 52.5) and three quaternary carbons (δ 40.1, 65.0, 150.1), thus suggesting, that three rings and one double bond are present. The ^{1}H NMR chemical shifts of the methyl groups appear at δ 0.93 as doublet, at 1.00 and 1.02 as singlets. The latter two are thus bonded to a quaternary carbon. The olefinic carbon signals at δ 103.9 (t) and 150.1 (s) suggest an *exo*-methylene

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✠ Passed away November 19th 2004. His scientific achievements keep him among us.

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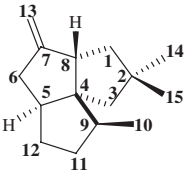
double bond, which is confirmed by a broad singlet in the ^{1}H NMR spectrum at δ 4.84 integrating to two protons. The connectivity of the two singlet methyl groups (C-14, C-15) is established through HMBC: They are bound to the same C-2 atom, which is neighboured by two methylene groups C-1 and C-3. From the COSY experiment, methylene group C-1 must be adjacent to methine C-8, which itself shows signals of proton coupling with the exomethylene protons H-13a and b, which couple to one of the methylene protons attached to C-6, which itself couples to the methine proton H-5. H-5 shows couplings to H-12a and b. As H-12b couples to H-11a and H-11a is coupled to H-9 which itself is connected to C-10 the methyl-shifted pentalenane skeleton ventricosane is established. The relative configuration of **1** is derived from the NOESY spectrum: Protons H-8, H-10 and H-15 are on one side of the plane and H-5 and H-14 occupy the other.



ventricos-7(13)-ene 1

2.3. Absolute configuration of (+)-6,7-epoxyeudesm-3-ene 2

The mass spectrum exhibits a molecular ion signal at *m*/*z* 220, corresponding to an elemental composition of $C_{15}H_{24}O$ with four double bond equivalents. An eudesmane skeleton was deduced from its MS and NMR data: The ^{1}H and ^{13}C NMR spectra of **2** show the presence of an *endo*-cyclic double bond (δ 124.5, 133.7) and four methyl groups, two of which are bound to a methine forming an isopropyl group; one linked to a quaternary aliphatic carbon and one attached to an olefinic quaternary carbon at δ 0.82 (d, 6.94 Hz), 0.92 (d, 6.94 Hz), 1.06 (s) and 1.73 (s), respectively. An ether bridge was observed due to two downfield shifted carbons at δ 72.20 and 74.94. The connectivity of the atoms was established through COSY and HMBC correlations and the relative configuration was derived from the NOESY spectrum (Table 2). To gain information about the absolute configuration, **2** was treated with Amberlyst and further hydrogenated. These derivatives of **2** were compared to total hydrogenation products of (+)-α-selinene. Coinjections on enantioselective GC columns showed coelution and thus the absolute configuration of **2** at positions of 5, 7 and 10 proved the same as in (+)-α-selinene (Figs. 1 and 2).

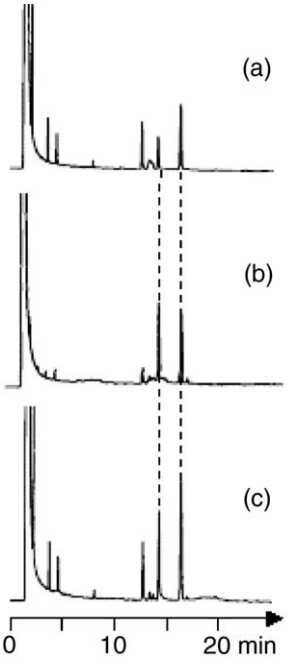


Figure 2. Elucidation of the absolute configuration of (+)-**2**: Comparison of reaction products of the chemical correlations [GC phase: heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin; 110 °C isothermal]: (a) deoxygenated and total hydrogenation products of (+)-**2**; (b) total hydrogenation products of (+)-α-selinene; (c) coinjection of (a) and (b).

3. Experimental

3.1. General experimental procedures

3.1.1. GC–MS. Electron impact (70 eV) GC–MS was carried out with a Hewlett Packard HP 5890 gas chromatograph coupled to a VG Analytical 70-250S mass spectrometer. All compounds were identified by comparison of their mass spectra and gas chromatographic

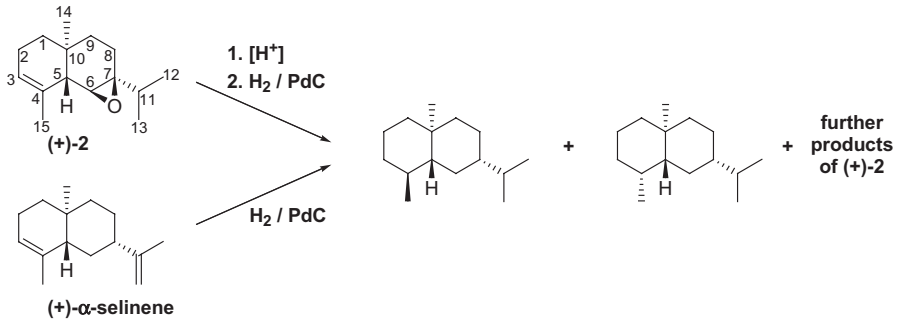


Figure 1. Chemical correlation of (+)-**2** and (+)-α-selinene.

retention indices with a spectral library established under identical experimental conditions.[7,8]

3.1.2. NMR spectroscopy. NMR measurements were carried out with a Bruker WM 500 (500 MHz) instrument in C_6D_6 using TMS as the internal standard. NMR-experiment acquisition for ^1H–^1H COSY was with 4096 (F2) and 256 (F1) data points. Delay was 1.1 s. F2 and F1 were each processed as Qsine with a sinus shift of 0. HSQC was recorded as a phase sensitive experiment with TD = 4096 (F2) and TD = 128 (F1) data points and with a delay of 1/4*145 Hz. Processing was done as an exponential multiplication in F2 with line broadening of 4.00 Hz and with QSINE in F1 with a sinus shift of 2. HMBC was recorded as a J filtered experiment with TD = 4096 (F2) and TD = 256 (F1). Delay was 0.065 s. F2 and F1 were each processed as Qsine with a sinus shift of 4. NOESY was recorded as phase sensitive with TD = 4096 (F2) and TD = 400 (F1) data points, respectively. Mixing time was 600 ms. F2 and F1 were each processed as Qsine with a sinus shift of 2.

3.1.3. 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 or 4160 gas chromatographs with 25 m fused silica capillaries with octakis(2,6-di-*O*-methyl-3-*O*-pentyl)-γ-cyclodextrin[9], heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin[9] or heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin[10] in OV 1701 (50%, w/w), split injection; split ratio approx. 1:30; FID; carrier gas 0.5 bar H_2; injector and detector temperatures were 200 and 250 °C, respectively.

3.1.4. Preparative GC. For the isolation of **1**: Agilent Technologies 6890N Network GC system with autosampler and automatic fraction collector (−30 °C), equipped with a 30 m wide bore 530 μm i.d. column and 5 μm DB-1 film as phase. The temperature was kept at 150 °C for 65 min and then raised to 200 °C for 5 min. Helium as carrier gas was kept at 60 mL/min.

For the isolation of **2**: Modified Varian 1400 and 2800 instruments, equipped with stainless steel columns (1.85 m × 4.3 mm) with 15% SE-52 on Chromosorb W-HP; helium as carrier gas at a flow rate of 240 mL/min.; injector and detector temperatures were 200 and 250 °C, respectively. The temperature was kept at 100 °C for 50 min and then raised to 150 °C for 20 min.

3.1.5. Polarimetry. Measurements were performed with a polarimeter 341 (Perkin–Elmer) at 589 nm at 20 °C. Due to very small amounts of isolated compounds, only the sign of the specific rotation is given to avoid inaccuracies.

3.1.6. Reactions. Acidic treatment of a hexane solution of **2** was performed using a few granules of Amberlyst®

Table 1. 1D and 2D NMR data of compound **1** (in C_6D_6)

Atom no.	^{13}C (ppm)	^1H (ppm)	COSY	HMBC	NOESY
1	49.61 (t)	1.53–1.66 (m, 6H, H-1a,b/3b/9/11b/12b)	m→3a, 5, 8, 10, 11a, 12a	3a, 14, 15	m→5
2	40.12 (s)	—	—	1a,b/3b/9/11b/12b, 3a, 14, 15	—
3	57.37 (t)	a: 1.48 (d, 1H, 13.2 Hz) b: 1.53–1.66 (m, 6H, H-3b/1a,b/9/11b/12b)	1a,b/3b/9/11b/12b m→3a, 5, 8, 10, 11a, 12a	14, 15	10, 14, 15 m→5
4	65.01 (s)	—	—	1a,b/3b/9/11b/12b, 3a, 10	—
5	52.52 (d)	2.14–2.19 (m, 1H)	1a,b/3b/9/11b/12b, 6a, 6b, 12a	1a,b/3b/9/11b/12b, 3a	1a,b/3b/9/11b/12b, 14
6	41.03 (t)	a: 1.93–1.99 (m, 1H) b: 2.74–2.81 (m, 1H)	5, 6b, 13a,b 5, 6a, 13a,b	13a,b	—
7	150.07 (s)	—	—	1a,b/3b/9/11b/12b	—
8	50.77 (d)	2.79–2.84 (m, 1H)	1a,b/3b/9/11b/12b, 13a,b	1a,b/3b/9/11b/12b	10, 15
9	45.79 (d)	1.53–1.66 (m, 6H H-9/1a,b/3b/11b/12b)	m→3a, 5, 8, 10, 11a, 12a	3a, 10	m→5
10	15.15 (q)	0.93 (d, 6.9 Hz, 3H)	1a,b/3b/9/11b/12b	—	3a, 8
11	34.13 (t)	a: 1.15–1.21 (m, 1H) b: 1.53–1.66 (m, 6H, H-11b/1a,b/3b/9/12b)	1a,b/3b/9/11b/12b, 12a m→3a, 5, 8, 10, 11a, 12a	10	— m→5
12	32.81 (t)	1.29–1.34 (m, 1H) 1.53–1.66 (m, 6H, H-12b/1a,b/3b/9/11b)	1a,b/3b/9/11b/12b, 5, 11a m→3a, 5, 8, 10, 11a, 12a	—	5 m→5
13	103.87 (t)	4.84 (br s, 2H)	6a, 6b, 8	—	—
14	30.06 (q)	1.02 (s, 3H)	—	1a,b/3b/9/11b/12b, 3a, 15	1a,b/3b/9/11b/12b, 5
15	30.39 (q)	1.00 (s, 3H)	—	1a,b/3b/9/11b/12b, 3a, 14	1a,b/3b/9/11b/12b, 3a, 8

Table 2. 1D and 2D NMR data of compound **2**

Atom no.	^{13}C (ppm)	^{1}H (ppm)	COSY	HMBC	NOESY
1	36.06 (t)	a: 1.15–1.22 (m, 2H, H-1a/H-8a) b: 1.31–1.47 (m, 3H, H-1b/H-8b/H-9a)	1b/8b/9a 1a/8a, 14	1a/8a, 1b/8b/9a, 9b, 14	—
2	23.64 (t)	a: 1.87–1.99 (m, 2H, H-2a/H-11) b: 2.05–2.15 (m, 1H)	3 —	1a/8a, 1b/8b/9a	—
3	124.46 (d)	5.45 (br s, 1H)	2a/11, 15	1a/8a, 15	2a/11, 2b, 15
4	133.69 (s)	—	—	1a/8a, 15	—
5	45.84 (d)	2.53 (s, 1H)	6, 15	1a/8a, 1b/8b/9a, 14, 15	1b/8b/9a, 6
6	72.20 (d)	3.89 (s, 1H)	5	1a/8a, 1b/8b/9a	5, 13, 15
7	74.94 (d)	—	—	1a/8a, 1b/8b/9a, 2a/11, 6, 9b, 12, 13	—
8	40.05 (t)	a: 1.15–1.22 (m, 2H, H-8a/H-1a) b: 1.31–1.47 (m, 3H, H-8b/H-1b/H-9a)	1b/8b, 9b 1a/8a, 9b	1a/8a, 1b/8b/9a, 13	—
9	28.38 (t)	a: 1.31–1.47 (m, 3H, H-9a/H-1b/H-8b) b: 1.69 (d, 4.4 Hz, 1H)	1a/8a, 1b/8b/9a, 9b 1a/8a, 1b/8b/9a	1a/8a, 1b/8b/9a, 2a/11	b: 2a/11
10	32.05 (s)	—	—	1a/8a, 1b/8b/9a, 6, 9b, 14	—
11	33.45 (d)	1.87–1.99 (m, 2H, H-11/H-2a)	12, 13	9b, 12, 13	—
12	16.37 (q)	0.82 (d, 6.9 Hz, 3H)	11	11, 13	—
13	16.37 (q)	0.92 (d, 6.9 Hz, 3H)	11	11, 12	6
14	18.60 (q)	1.06 (s, 3H)	1b/8b/9a	1a/8a, 1b/8b/9a	15
15	20.94 (q)	1.73 (s, 3H)	2a/11, 2b, 3, 5	—	3, 6, 14

15 at room temperature until the dehydration products were the major compounds in the reaction mixture monitored by GC. This mixture was filtered and then submitted to hydrogenation.

Hydrogenation of the above prepared mixture and (+)-α-selinene was performed by bubbling hydrogen gas through a stirred solution of ca. 1 mg of sample in 1 mL *n*-hexane and 0.5 mg Pd/C at room temperature for 30 min. The reaction mixture was filtered and the reaction products were analysed by GC–MS and GC on several capillary columns with cyclodextrin derivatives.

3.1.7. Origin of *Lophozia ventricosa*. *L. ventricosa* was collected in the Harz mountains near Altenau, Germany on humid rocks in August 2001 and May 2002.

3.1.8. Isolation of compounds 1 and 2. The hydrodistillate of *Lophozia ventricosa* was submitted to flash column chromatography (column packed dry with silica gel; elution with 100 mL *n*-hexane to yield the hydrocarbon fraction and 100 mL 10% ethyl acetate in hexane to yield the oxygenated fraction). The hydrocarbon fraction was further partitioned using the 6890N wide bore preparative GC to yield **1** and the oxygenated fraction was further separated into six fractions by means of preparative GC Varian 1400 and 2800 on a packed column with SE-52. The sixth fraction yielded **2**.

3.1.9. Characterisation of (−)-1. Colourless oil; RI_{CPSIL5} = 1355; sign of specific rotation (C_6D_6): (−); ^{1}H NMR (500 MHz, C_6D_6) and ^{13}C NMR (125.7 MHz, C_6D_6) see Table 1. MS (EI, 70 eV) *m/z* (rel. int.): 204 [M^+](29), 189(25), 175(7), 163(95), 147(62), 133(35), 119(30), 105(58), 91(100), 77(46), 65(23), 55(41), 41(96).

3.1.10. Characterisation of (+)-2. Colourless oil; RI_{CPSIL5} = 1787; sign of specific rotation (benzene): (+); ^{1}HNMR (500 MHz, C_6D_6) and ^{13}C NMR (127.5 MHz, C_6D_6) see Table 2. MS (EI, 70 eV), *m/z* (rel. int.): 220 [M^+] (8), 205(8), 177(100), 159(5), 149(3), 135(4), 121(10), 107(10), 91(14), 77(9), 71(10), 55(9), 43(37).

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