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(+)-1(10)-SPIROVETIVEN-7β-OL FROM THE LIVERWORT *LEPIDOZIA REPTANS*

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Key Word Index—*Lepidozia reptans*; Jungermanniales; Hepaticae; sesquiterpenes; 1(10)-spirovetiven-7β-ol.

Abstract—The structure of a new sesquiterpene alcohol from the liverwort *Lepidozia reptans* has been established as 1(10)-spirovetiven-7β-ol by means of spectroscopic methods and chemical conversion. The configuration of the sesquiterpene was proved by enantioselective gas chromatography. The sesquiterpene alcohol was previously described as a constituent of *Lepidozia reptans* without further investigation of the structure. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

The isolation of sesquiterpenoids has already been reported [1–4] from the liverwort *Lepidozia reptans* (L.) Dum., which belongs to the Lepidoziaceae. The constituents α-barbatene, β-barbatene, bicyclogermacrene, β-cubebene, cuparene, β-elemene, δ-elemene, α-longipinene and eudesm-3-en-6β,7α-diol have been described. Connolly *et al.* [3] have isolated a sesquiterpene alcohol from a certain chemotype of *Lepidozia reptans*, collected in south-west Scotland, but the spectroscopic data were listed without assigning the structure. We report now on the isolation and structural elucidation of (+)-1(10)-spirovetiven-7β-ol (**1**) from *Lepidozia reptans*, collected near Hamburg in northern Germany.

RESULTS AND DISCUSSION

The hydrodistilled fresh plant material of *Lepidozia reptans* was analysed by GC-mass spectrometry. All the above mentioned volatile compounds, except α-longipinene and β-cubebene, were detected. Two-dimensional gas chromatography was employed to investigate the enantiomeric composition of the sesquiterpene hydrocarbons. The essential oil of *Lepidozia reptans* was found to contain (+)-α-barbatene, (−)-β-barbatene, (−)-bicyclogermacrene, (+)-β-bourbonene (72% ee), (−)-cuparene, (−)-α-cuprenene, (+)-β-elemene and racemic δ-elemene (Cope rearrangement from germacrene C [5]). Additionally, germacrene B, (−)-1(10)-valencen-7β-ol (**8**) [6, 7], lepidozenal [8, 9] and the major compound **1**, with the elemental composition $C_{15}H_{26}O$. Compounds **1** and **8** were isolated by preparative GC [10].

The ^{1}H NMR spectrum of **1** indicated signals of three secondary methyl groups (δ 0.95, 0.93, each *d*, $J = 6.9$ Hz, and 0.90, *d*, $J = 6.3$ Hz), one olefinic methyl group (δ 1.80, *d*, $J = 1.3$ Hz) and one olefinic proton (δ 5.32, *m*). The ^{13}C NMR spectrum showed the presence of four methyl groups (δ 16.0, 17.6, 17.6 and 20.6), five methylene groups (δ 23.2, 27.4, 35.9, 38.7 and 45.3), two methine carbons (δ 37.7 and 37.9), two olefinic carbons (δ 121.5 and 139.7) and one oxygenated carbon (δ 85.4). The ^{13}C NMR data were completely identical with those described in ref. [3]. Further NMR techniques (^{1}H–^{1}H and ^{1}H–^{13}C correlated 2D NMR) confirmed the structure of **1**.

To verify the structure of **1** the alcohol and its known isomer (−)-hinesol (**5**) were treated with $SOCl_2$ yielding the same main product **3a** (Scheme 1). The main dehydration products obtained from the alcohols **1** and **5** were found to be identical with respect to spectroscopic (NMR, mass spectra) and chromatographic properties on diverse capillary columns with cyclodextrin derivatives [11]. (−)-Hinesene (**6**) was identified as a further dehydration product of **5**. Dehydration of **1** yielded **2a** and **4** as by-products. Partial hydrogenation of (+)-α-vetispirene (**7**) afforded **2b** and **3b** (Scheme 1). Compounds **2a** and **2b**, and, respectively, **3a** and **3b** showed identical mass spectra and retention indices on capillary columns with different polarity (CpSil 5 and CpSil 19). The enantiomers **2a** and **2b** were resolved on a capillary column with heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin using two-dimensional GC (Fig. 1) [12]. Compounds **3a** and **3b** were isolated by preparative GC and separated by co-injection on a capillary column with

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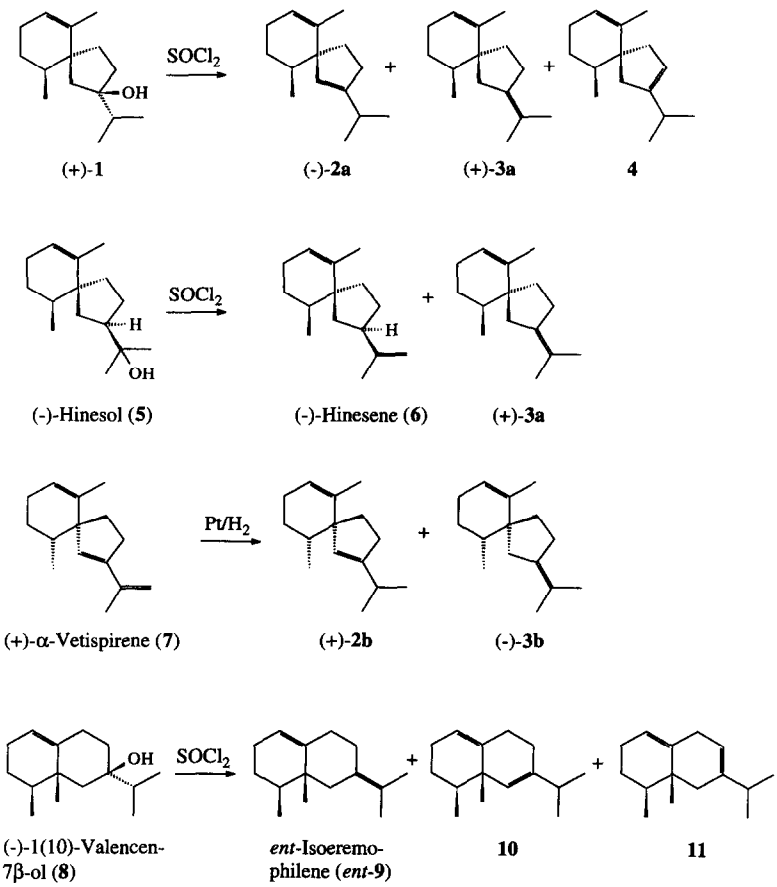

octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin [13], as illustrated in Fig. 2. Traces of **3a** were also present in the essential oil of *Lepidozia reptans*.

The assumption of a 7β-hydroxyl group of **1** was confirmed by the presence of the biogenetically related (−)-1(10)-valencen-7β-ol (**8**) in the essential oil of *Lepidozia reptans* in conformity with β-eudesmol and its congener hinesol (**5**) [14]. Dehydration of **8** yielded, besides **10** and **11**, *ent*-isoeremophilene (*ent*-**9**), which was proved to be the enantiomer of isoeremophilene (**9**) [15] isolated from vetiver oil (Scheme 1) (S. Jung and W. A. König, unpublished results).

EXPERIMENTAL

Plant material. Lepidozia reptans (L.) Dum. was collected in the Sachsenwald near Hamburg (Germany) in March 1994 and identified by Dr H. Muhle. The collected liverwort is deposited in the Institut für Allgemeine Botanik, Universität Hamburg.

Hydrodistillation. The essential oil was prepared by steam distillation (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 prepared as described earlier [11].

Prep. GC. Isolation of **1**, **8** and **3b** was performed by prep. GC on a Varian 1400 instrument, equipped with a stainless steel column (Silcosteel, Amchro) (2.05 m × 5.1 mm) with 6% octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin–PS-086 (1 : 1; w/w) on Chromosorb W-HP. Synthetic products **2a**, **3a**, **4**, **6**, *ent*-**9**, **9**, **10** and **11** were isolated using a stainless steel column (1.85 m × 4.3 mm) with 10% SE-30 on Chromosorb W-HP. He was used as carrier gas at a flow rate of 240 ml min^{-1}.

Two-dimensional GC. The reaction products were injected on a 25 m (0.25 mm i.d.) capillary column with dimethylpolysiloxane CpSil 5 (Chrompack) at 50° and programmed at a rate of 3° min^{-1} to 200°. Sample transfer was performed after 33.87 min (the R_t of **2a** and **2b**) 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 95°. The chromatograms from both columns were recorded with a two-channel 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 spectra were measured in $CDCl_3$ using TMS as int. standard.

GC-MS. Electron impact GC-MS measurements were carried out at 70 eV.

Polarimetry. Optical rotation measurements were performed in $CHCl_3$.

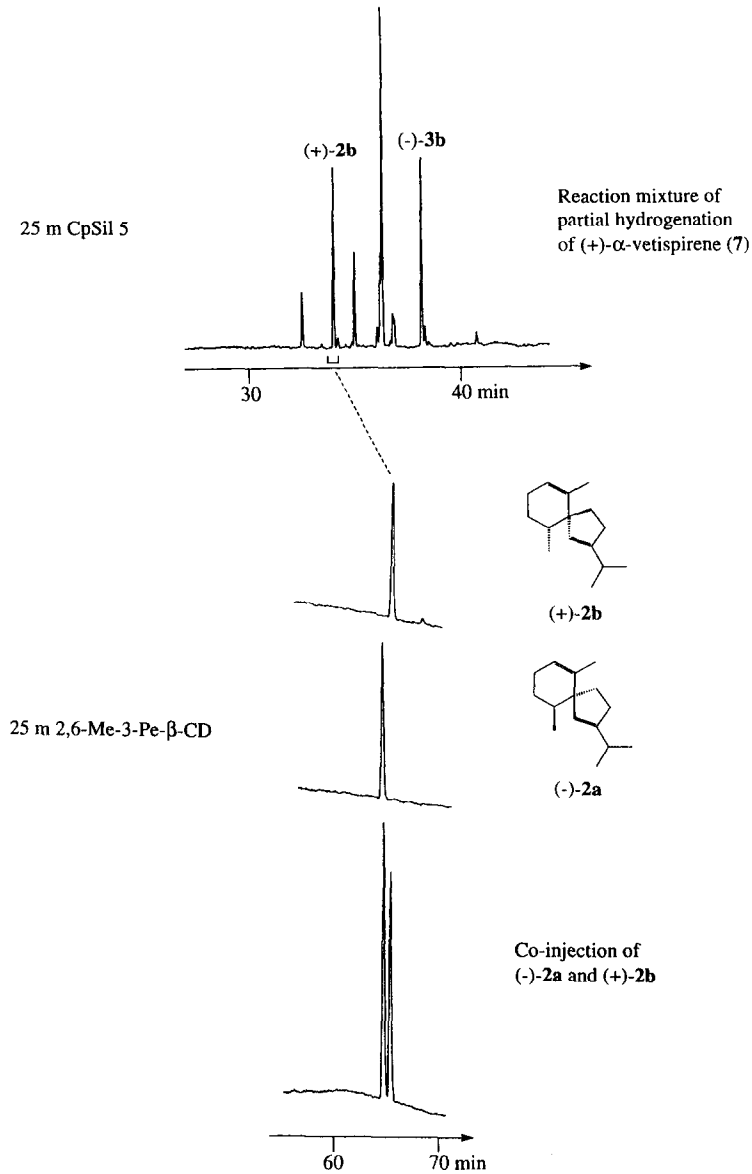

Fig. 1. Two-dimensional GC investigation of **2a** and **2b** (transfer from a 25 m CpSil 5 capillary column, 50°, 3° min^{-1} to 200°, to a 25 m capillary column with 50% heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin in polysiloxane OV 1701 (w/w) at 95°).

(+)-1(10)-*Spirovetiven-7β-ol* (**1**). $[\alpha]_D^{22}$ +21° (*c* 0.33); ^{1}H NMR (500 MHz): δ 5.32 (1H, *m*), 2.15 (1H, *ddd*, *J* = 12.2 Hz, *J* = 9.9 Hz, *J* = 9.9 Hz), 1.92 (2H, *m*), 1.80 (3H, *d*, *J* = 1.3 Hz), 1.73 (1H, *d*, *J* = 14.8 Hz), 1.69 – 1.52 (7H, *m*), 1.36 (1H, *m*), 0.95 (3H, *d*, *J* = 6.9 Hz), 0.93 (3H, *d*, *J* = 6.9 Hz), 0.90 (3H, *d*, *J* = 6.3 Hz); ^{13}C NMR (125 MHz): δ 139.7, 121.5, 85.4, 49.2, 45.3, 38.7, 37.9, 37.7, 35.9, 27.4, 23.2, 20.6, 17.6, 17.6, 16.0; MS (EI, 70 eV), *m/z* (rel. int.): 204 (32), 162 (65), 161 (100), 147 (30), 121 (58), 119 (21), 117 (43), 109 (25), 107 (30), 105 (36), 95 (21), 93 (38), 91 (31), 81 (21), 79 (25), 71 (26), 55 (26), 42 (41) 41 (39); MS (CI, MeOH), *m/z* (rel. int.): 223 $[M + 1]^+$ (0.4%).

Dehydration of **1**. To a soln of **1** (10 mg) in pyridine (1 ml) $SOCl_2$ (0.2 ml) was added and left for 10 min at 0°. To the reaction mixt. 10% $NaHCO_3$ solution was added and extracted with *n*-hexane. The reaction products **2a** (1 mg), **3a** (2 mg) and **4** (1 mg) were isolated by prep. GC. **2a**: $[\alpha]_D^{22}$ −81 (*c* 0.03); ^{1}H NMR (400 MHz): δ 5.35 (1H, *m*), 5.04 (1H, *bs*), 2.38–2.26 (3H, *m*), 1.97 (2H, *m*), 1.90 (1H, *ddd*, *J* = 13.4 Hz, *J* = 8.5 Hz, *J* = 6.3 Hz), 1.74 (1H, *ddd*, *J* = 13.4 Hz, *J* = 8.5 Hz, *J* = 6.3 Hz), 1.66-1.57 (2H, *m*), 1.56 (3H, *bs*), 1.43–1.35 (1H, *m*), 1.04 (6H, 2 *d*, *J* = 6.6 Hz, *J* = 6.6 Hz), 0.83 (3H, *d*, *J* = 6.6 Hz); MS (EI, 70 eV), *m/z* (rel. int.): 204 (4), 162 (100), 147 (37), 119 (42), 105 (18), 91 (19), 41 (20). **3a**: $[a]_D^{22}$ +26 (*c* 0.06); ^{1}H NMR (400 MHz): δ 5.32 (1H, *m*), 2.29 (2H, *m*), 2.20 (2H, *m*), 1.99 (1H, *m*), 1.91 (1H, *m*), 1.84 (1H, *ddd*, *J* = 13.3 Hz, *J* = 8.9 Hz, *J* = 7.7 Hz), 1.78–1.67 (2H, *m*), 1.64 (3H, *bs*), 1.62 (3H, *bs*), 1.61 (1H, *m*), 1.60 (3H, *bs*), 1.41–1.31 (1H, *m*), 0.86 (3H, *d*, *J* = 7.1 Hz); MS (EI, 70 eV),

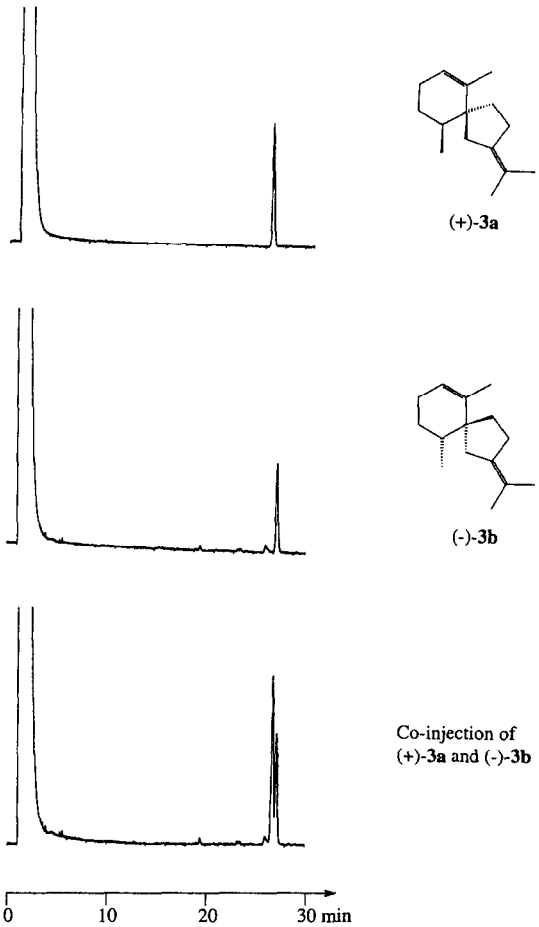

Fig. 2. GC enantiomer separation of **3a** and **3b** on a 25 m capillary column with 50% octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin in polysiloxane OV 1701 (w/w) at 100°; carrier gas H_2 at 50 kPa.

m/z (rel. int.): 204 (40), 161 (21), 146 (20), 147 (27), 122 (26), 121 (100), 120 (26), 119 (21), 107 (26), 105 (38), 93 (40), 91 (33), 79 (22), 67 (30), 55 (28), 41 (43). Compound **4**: Optical rotation measurements of **4** were not performed due to incomplete separation from **10**. ^{1}H NMR (400 MHz): δ 5.27 (1H, *m*), 5.22 (1H, *m*), 1.60 (3H, *bs*), 1.03 (6H, *d*, *J* = 6.9 Hz), 0.85 (3H, *d*, *J* = 6.7 Hz).

Dehydration of (−)-**5**. Dehydration of (−)-**5** (10 mg) was performed analogously to **1**. The reaction products **3a** (2 mg) and **6** (1 mg) were isolated by preparative GC. Compound **6** was identical with hinesene in all spectroscopic data [16]. Compound **3a**, obtained from **5**, was identical with the main dehydration product of **1** in all spectroscopic and chromatographic properties on diverse capillary columns with cyclodextrin derivatives.

Partial hydrogenation of (+)-**7**. A soln of (+)-**7** (1 mg) and Pt(IV)-oxide-hydrate in $CHCl_3$ was treated with H_2 and left for 1 hr at room temp. The reaction mixt. was filtered to give **2b** (0.2 mg) and **3b** (0.2 mg). Compounds **2b** and **3b** were identical with **2a** and **3a**, respectively, in their mass spectra and retention indices on different stationary phases (CpSil 5 and CpSil 19, Chrompack). NMR and optical rotation measurements of **2b** and **3b** failed because of insufficient sample amount.

(−)-1(10)-*Valencen-7β-ol* (**8**). $[\alpha]_D^{22}$ −72 (*c* 0.08). ^{1}H and ^{13}C NMR spectra were identical with published data [6, 7].

Dehydration of (−)-**8**. Dehydration of (−)-**8** (2 mg) was performed analogously to **1**. *ent*-**9** (0.4 mg), **10** (0.8 mg) and **11** (0.8 mg) were isolated by prep. GC. Optical rotation measurements failed because of insufficient sample amount. *ent*-**9**: ^{1}H NMR (400 MHz): δ 5.31 (1H, *m*), 2.79 (1H, *dd*, *J* = 13.4 Hz, *J* = 3.3 Hz), 0.92 (3H, *d*, *J* = 6.6 Hz), 0.80 (3H, *bs*); MS (EI, 70 eV), *m/z* (rel. int.): 204 (78), 189 (43), 162 (35), 161 (100), 146 (24), 147 (60), 134 (28), 133 (36), 121 (28), 119 (60), 107 (38), 105 (66), 93 (43), 91 (70), 81 (30), 79 (38), 77 (34), 67 (33), 55 (43), 53 (24), 41 (75). Compound **10**: ^{1}H NMR (400 MHz): δ 5.41 (1H, *bs*), 5.33 (1H, *m*), 0.98 (6H, 2 *d*, *J* = 6.8 Hz, *J* = 6.8 Hz), 0.94 (3H, *s*), 0.93 (3H, *d*, *J* = 6.6 Hz). Compound **11**: ^{1}H NMR (400 MHz): δ 5.40 (1H, *m*), 5.30 (1H, *m*), 2.90 (1H, *bd*, *J* = 21.3 Hz), 2.60 (1H, *bd*, *J* = 21.3 Hz), 2.17 (1H, *m*), 2.08 (1H, *d*, *J* = 17.2 Hz), 2.05–1.90 (3H, *m*), 1.81 (1H, *bd*, *J* = 17.2 Hz), 1.43 (2H, *m*), 0.99 (3H, *d*, *J* = 6.9 Hz), 0.98 (3H, *d*, *J* = 6.9 Hz), 0.94 (3H, *d*, *J* = 6.6 Hz), 0.85 (3H, *d*, *J* = 0.8 Hz); MS (EI, 70 eV), *m/z* (rel. int.): 204 (25), 161 (100), 119 (35), 105 (62), 91 (49), 41 (35).

Isoeremophilene (**9**). Compound **9** was isolated by prep. GC from vetiver oil (Bourbon) (S. Jung and W. A. König, unpublished results) and was identical with *ent*-**9** in all spectroscopic data [15].

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