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(−)-7-*epi*-Isojunenol and (+)-7-*epi*-junenol, constituents of the liverwort *Tritomaria quinquedentata*

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Abstract

The sesquiterpene constituents of the liverwort *Tritomaria quinquedentata* (Huds.) Buch were investigated. In addition to many known compounds a new sesquiterpene alcohol, (−)-7-*epi*-isojunenol, has been isolated and identified. (+)-7-*epi*-Junenol, another sesquiterpene alcohol, has been found in nature for the first time.

Keywords: *Tritomaria quinquedentata*; Jungermanniales; Liverwort; Sesquiterpene; (−)-7-*epi*-Isojunenol; (+)-7-*epi*-Junenol; Enantioselective gas chromatography

1. Introduction

Tritomaria quinquedentata is a leafy liverwort (*Hepaticae*) of the order Jungermanniales (Frahm & Frey, 1992). The essential oil of *T. quinquedentata* has been investigated before (Tori, Nagai, Asakawa, Huneck & Ogawa, 1993). (−)-Dihydrodiplophyllolide, (−)-β-spathulenol, (−)-diplophyllolide and stigmasterol were described as constituents of *T. quinquedentata*.

2. Results and discussion

We reinvestigated the sesquiterpene constituents of *T. quinquedentata*. The essential oil was 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. 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 *T. quinquedentata*: anastreptene (Andersen, Ohta, Moore & Tseng, 1978), isoledene (Joulain & König, 1998), β-elemene (Joulain & König, 1998), tritomarene (Warmers, Wihstutz, Bülow, Fricke & König, 1998), 11-bourbonene (Warmers et al., 1998), calarene (Joulain & König, 1998), β-barbatene (Connolly, Harding & Thornton, 1974, 1972), *allo*-aromadendrene (Joulain & König, 1998), β-acoradiene (Joulain & König, 1998), α-selinene (Williams et al., 1995), bicyclogermacrene (McMurry & Bosch, 1987), maaliol (Asakawa, Toyota & Takemoto, 1980), β-spathulenol (Asakawa et al., 1980), 4,5-dehydroviridiflorol (Warmers et al., 1998), globulol (Asakawa et al., 1980), β-cedrol (Breitholle & Fallis, 1978), guai-5-en-11-ol (Rücker & Hefendehl, 1978), (−)-7-*epi*-isojunenol (**1**) (+)-7-*epi*-junenol (**4**), isoalantolactone (Marshall & Cohen, 1964), diplophyllolide A (Kaur & Kalsi, 1985) (Scheme 1) (Fig. 1). Except for the new compounds **1** and **4** the absolute configurations were not determined and remain to be clarified.

(−)-7-*epi*-Isojunenol {(1*S*,2*R*,4a*S*,8a*R*)-1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethyl)-naphthalen-1-ol} (**1**) is a new sesquiterpene alcohol. The mass spectrum exhibits a molecular ion signal at *m*/*z* 222 and an elemental composition of $C_{15}H_{26}O$. The ^1H NMR spectrum shows a signal for an olefinic proton

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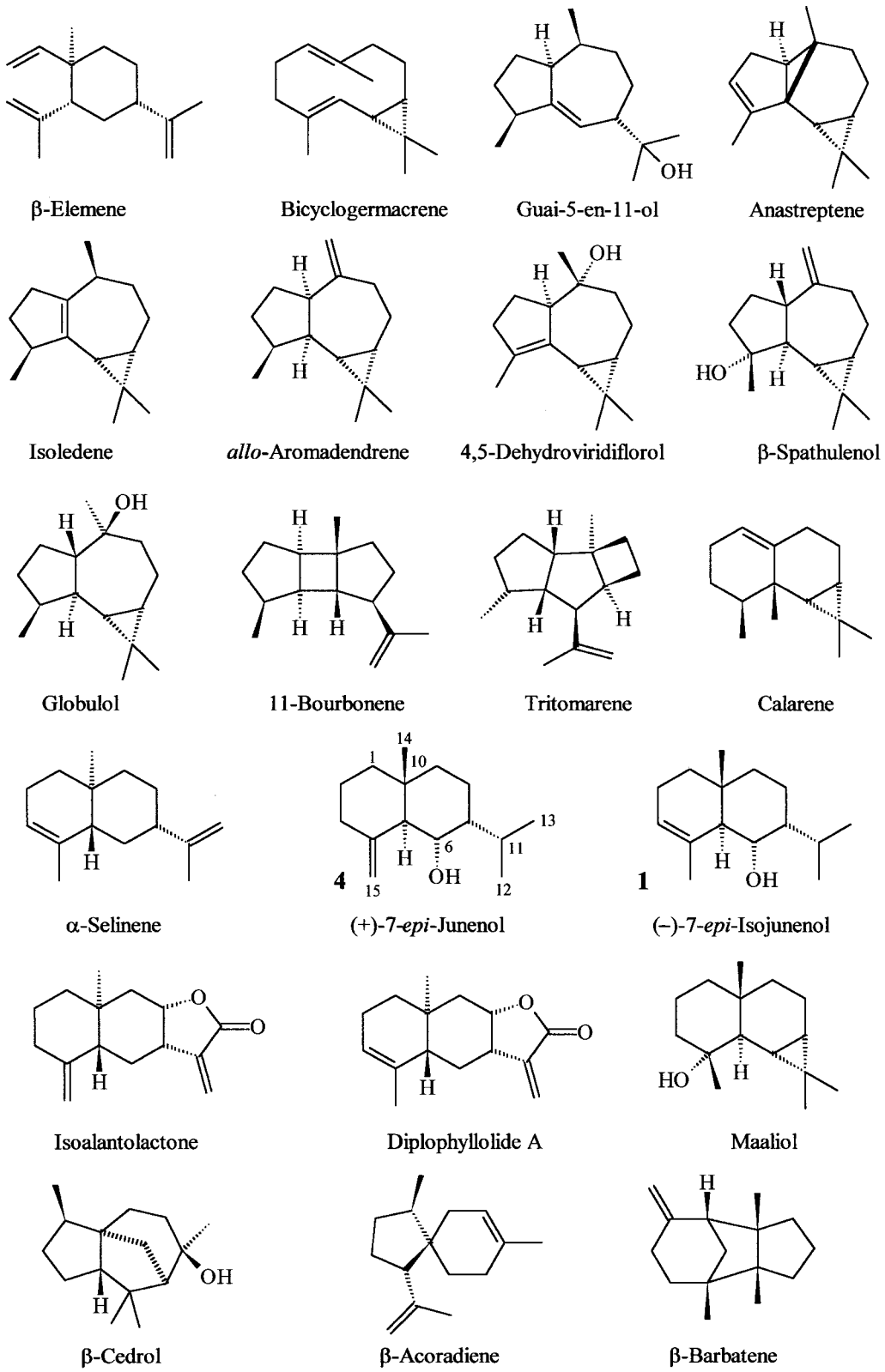
β-Elemene
Bicyclogermacrene
Guai-5-en-11-ol
Anastreptene
Isoledene
allo-Aromadendrene
4,5-Dehydroviridiflorol
β-Spathulenol
Globulol
11-Bourbonene
Tritomarene
Calarene
α-Selinene
(+)-7-epi-Junenol
(−)-7-epi-Isojunenol
Isoalantolactone
Diplophyllolide A
Maaliol
β-Cedrol
β-Acoradiene
β-Barbatene

Scheme 1.

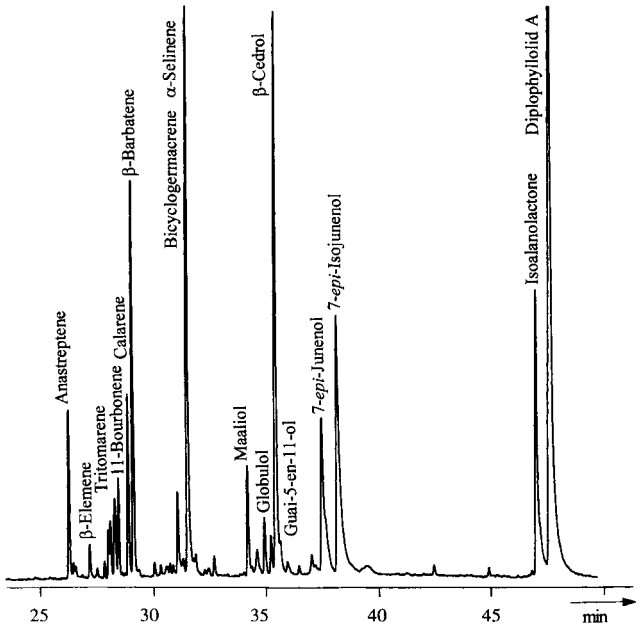

Fig. 1. Gas chromatogram of the essential oil of *Tritomaria quinquedentata* on a 25 m fused silica capillary with polysiloxane CpSil5; column temp. 50°, temp. program 3°/min to 230°; carrier gas 0.5 bar H_2.

at δ 5.36, a signal for a proton adjacent to an oxygen atom at δ 3.95, doublets for two methyl groups at tertiary carbon atoms at δ 0.95 and 1.04 and singlets for two methyl groups at quaternary carbons at δ 0.90 and 1.87; the low field shifted signal indicates an allylic methyl group.

The ^{13}C- and the DEPT spectra show signals for four primary carbon atoms (δ 21.53, 21.58, 22.46, 22.69), four secondary carbons (δ 20.29, 23.25, 37.53, 39.78), five tertiary carbons (δ 27.26, 45.70, 50.39, 70.09, 122.62) and two quaternary carbons (δ 31.75, 134.94). The slightly low field shifted signal at δ 70.09 is assigned to a tertiary carbon bonded to an oxygen. The strongly low field shifted signals at δ 122.62 and 134.94 indicate the presence of one double bond.

In the HMQC spectrum the correlation of the ^1H and the ^{13}C NMR absorptions is verified. The connectivity of partial structures was derived from the COSY GS and the HMBC spectrum.

The olefinic methine group (δ 5.36, 122.62, CH-3) shows a coupling correlation with the olefinic quaternary carbon (δ 134.94, C-4) connected to a methyl group (δ 1.87, 22.69, CH_3-15) and with a methylene group (δ 1.89–1.99, 1.99–2.10, 23.25, CH_2-2). This methylene group couples to another methylene group (δ 1.33–1.40, 1.33–1.40, 39.78, CH_2-1), which is bonded to the quaternary carbon (δ 31.75, C-10). This quaternary carbon further couples with a methyl group (δ 0.90, 21.53, CH_3-14), with a methine group (δ 1.99–2.10, 50.39, CH-5) and with a chain of two additional methylene groups (δ 1.39–1.43, 1.39–1.43, 37.53, CH_2-9; 1.43–1.52, 1.53–1.69, 20.29, CH_2-8). The latter methylene group is bonded to a methine group (δ 1.53–1.69, 45.70, CH-7), which is connected to an isopropyl group (δ 1.71–1.83, 27.26, CH-11; 1.04, 22.46, CH_3-12; 0.95, 21.58, CH_3-13) and to the methine group next to oxygen (δ 3.95, 70.09, CH-6). This methine group is bonded to the CH-5 methine group, which further couples to the atoms of the double bond system. Combination of all these data leads to the structure **1**.

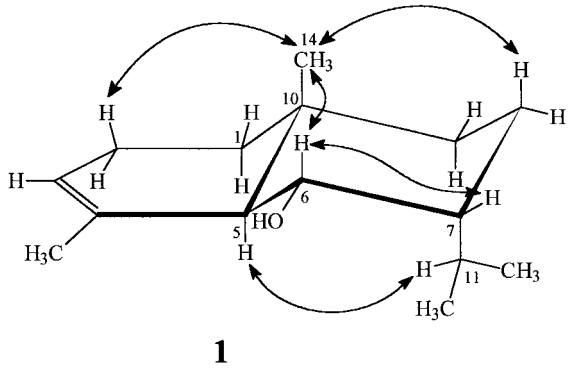

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Fig. 2. NOE effects of 7-*epi*-isojunenol (**1**).

The relative configuration of the stereogenic centres at C-5, C-6, C-7 and C-10 was obtained from the NOESY spectrum. The saturation of the resonance of H-6 results in an increase of the signal intensities of H-7 and H-14. H-5 interacts with H-11. Hence the protons H-6 and H-7 and the methyl group at C-10 have to be on the same side of the molecule, while the proton H-5 and the isopropyl group at C-7 have to be on the other side (Fig. 2).

The structure of **1** was verified by a chemical conversion. The dehydration of **1** yields (−)-*trans*-eudesma-3,5-diene (**2**) (Jakupovic et al., 1992) and (−)-*trans*-eudesma-3,7-diene (**3**) (Joulain & König, 1998) (Fig. 3).

(+)-7-*epi*-Junenol (**4**) has been found in nature for the first time. Compound **4** was known as a reaction product (Brennan & Erickson, 1982; Toyota & Asakawa, 1990).

The dehydration of **4** yields *trans*-eudesma-4(15),6-diene (**5**) (Joulain & König, 1998; König et al., 1996) and *trans*-eudesma-4(15),7-diene (vetiselinene) (**6**) (Joulain & König, 1998) (Fig. 3).

The absolute configuration of both **1** and **4** was obtained by a correlation reaction. The dehydration products **2**, **5** and **6** were hydrogenated and the hydrogenation products were compared with the hydrogenation products of (+)-δ-selinene (**7**). Hydrogenation of **5**, **6** and **7** yields mainly two identical saturated eudesmanes; the addition of hydrogen at C-4 (and C-5 in

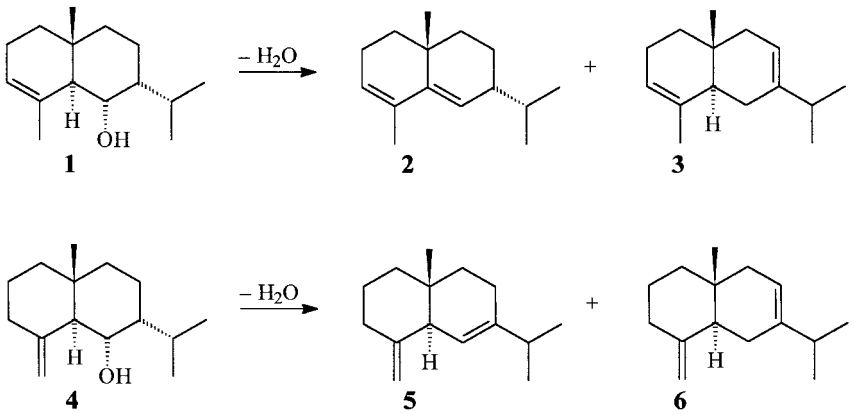

Fig. 3. Dehydration of (−)-7-*epi*-isojunenol (**1**) to (−)-*trans*-eudesma-3,5-diene (**2**) and (−)-*trans*-eudesma-3,7-diene (**3**) and dehydration of (+)-7-*epi*-junenol (**4**) to *trans*-eudesma-4(15),6-diene (**5**) and *trans*-eudesma-4(15),7-diene (**6**).

the case of **7**) occurs only from the back side of the molecule. Hydrogenation of **2** yields four saturated eudesmanes. One of them is identical with a hydrogenation product of **5**, **6** and **7**. These eudesmanes have the same MS and retention times on achiral and chiral GC phases. Hence the relative and absolute configuration have to be identical (Fig. 4).

3. Experimental

3.1. Plant material

Tritomaria quinquedentata was collected in the Alps, Genova-Valley (Italy). It was identified by R. Mues, Universität des Saarlandes, Saarbrücken, Germany.

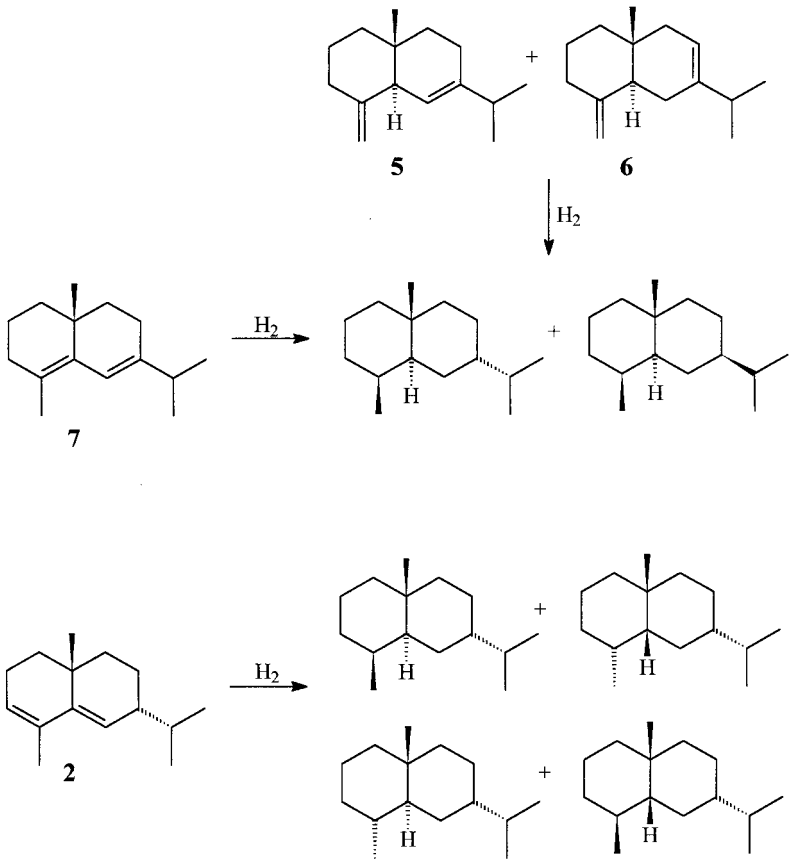

Fig. 4. Hydrogenation of (−)-*trans*-eudesma-3,5-diene (**2**), *trans*-eudesma-4(15),6-diene (**5**), *trans*-eudesma-4(15),7-diene (**6**) and (+)-δ-selinene (**7**).

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 states the fresh plant material was not weighed. The essential oil was further processed from the *n*-hexane solution without isolation. Therefore it was not possible to define the exact yield of essential oil.

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 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 wt%); split injection; flame ionisation 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 ionisation 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. Polarimetry

Only the sense of optical rotation was determined. The amounts of isolated compounds were too small for an accurate determination of the specific optical rotation.

3.8. 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.

3.9. (−)-7-epi-Isojunenol (*1*)

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (3H, s, H-14), 0.95 (3H, d, J = 6.6 Hz, H-13), 1.04 (3H, d, J = 6.6 Hz, H-12), 1.33–1.40 (2H, m, H-1α, H-1β), 1.39–1.43 (2H, m, H-9α, H-9β), 1.43–1.52 (1H, m, H-8α), 1.53–1.69 (2H, m, H-7, H-8β), 1.71–1.83 (1H, m, H-11), 1.87 (3H, s, H-15), 1.89–1.99 (1H, m, H-2α), 1.99–2.10 (2H, m, H-2β, H-5), 3.95 (1H, dd, J = 5.6, 9.2 Hz, H-6), 5.36 (1H, bs, H-3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.29 (t, C-8), 21.53 (q, C-14), 21.58 (q, C-13), 22.46 (q, C-12), 22.69 (q, C-15), 23.25 (t, C-2), 27.26 (d, C-11), 31.75 (s, C-10), 37.53 (t, C-9), 39.78 (t, C-1), 45.70 (d, C-7), 50.39 (d, C-5), 70.09 (d, C-6), 122.62 (d, C-3), 134.94 (s, C-4); MS (EI, 70 eV): m/z (rel. int.) 222 (3) [M^+], 207 (13), 204 (22) [M^+-H_2O], 189 (22), 161 (70), 133 (15), 121 (20), 109 (100), 108 (34), 107 (66), 105 (40), 97 (20), 95 (32), 93 (52), 91 (36), 81 (39), 79 (27), 77 (24), 69 (28), 67 (28), 55 (38), 53 (21), 43 (39), 41 (79), 39 (24).

3.10. Dehydration of (−)-7-epi-isojunenol (*1*)

To a soln. of 1 mg of **1** in 1 ml pyridine, 0.1 ml $SOCl_2$ was added and the rection mixture was stirred at 0°C for 12 min. *n*-Hexane and H_2O were added. The organic phase was washed three times with H_2O. The reaction products were isolated and identified as (−)-*trans*-eudesma-3,5-diene (**2**) and (−)-*trans*-eudesma-3,7-diene (**3**). **2**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.90 (3H, d, J = 6.9 Hz, H-12/13), 0.94 (3H, d, J = 6.9 Hz, H-12/13), 0.96 (3H, s, H-14), 1.30–1.40 (3H, m, H-1α/β, H-9α, H-9β), 1.44 (1H, dd, J = 5.7, 12.6 Hz, H-1α/β), 1.56–1.66 (2H, m, H-8α/β, H-11), 1.79 (3H, bs, H-15), 1.79–1.87 (1H, m, H-8α/β), 1.96 (1H, m, H-7), 2.04 (1H, dt, J = 5.6, 17.0 Hz, H-2α/β), 2.25 (1H, t, J = 17.0 Hz, H-2α/β), 5.51 (1H, d, J = 4.4 Hz, H-3), 5.56 (1H, d, J = 4.4 Hz, H-6); MS (EI, 70 eV): m/z (rel. int.) 204 (43) [M^+], 189 (10), 162 (39), 161 (100), 145 (11), 133 (16), 131 (17), 119 (39), 105 (64), 95 (31), 93 (26), 91 (45), 81 (76), 79 (19), 77 (21), 67 (34), 55 (23), 41 (47), 39 (21). **3**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.75 (3H, s, H-14), 1.01 (6H, d, J = 6.6 Hz, H-12, H-13), 1.65 (3H, bs, H-15), 5.34 (1H, bs, H-3/8), 5.37 (1H, bs, H-3/8); MS (EI, 70 eV): m/z (rel. int.) 204 (86) [M^+], 189 (63), 175 (15), 161 (100), 147 (39), 133 (45), 121 (35), 119 (39), 108 (70), 107 (41), 105 (88), 95 (41), 93 (86), 91 (79), 81 (41), 79 (37), 77 (41), 67 (30), 55 (40), 53 (33), 43 (36), 41 (85), 39 (39).

3.11. (+)-7-epi-Junenol (*4*)

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.80 (3H, s, H-14), 0.95 (3H, d, J = 7.1 Hz, H-13), 1.11 (3H, d, J = 6.6 Hz, H-12), 1.14–1.21 (1H, dt, J = 3.6, 13.7 Hz, H-9β),

1.26–1.36 (2H, m, H-1α, H-9α), 1.36–1.43 (1H, m, H-1β), 1.51–1.61 (1H, m, H-8β), 1.55–1.71 (2H, m, H-2α, H-2β), 1.61–1.73 (1H, m, H-8α), 1.69–1.78 (1H, m, H-7), 1.87 (1H, bs, OH), 1.94–2.07 (2H, m, H-3α/β, H-11), 2.17 (1H, d, J = 10.7 Hz, H-5), 2.32 (1H, bd, J = 12.2 Hz, H-3), 4.06 (1H, dd, 5.1, 10.7 Hz, H-6), 4.66 (1H, d, J = 1.0 Hz, H-15α/β), 4.97 (1H, d, J = 1.0 Hz, H-15α/β); ^{13}C NMR (125 MHz, $CDCl_3$): 17.92 (q, C-14), 22.12 (q, C-13), 22.47 (t, C-8), 24.25 (t, C-2), 25.02 (q, C-12), 25.15 (d, C-11), 36.08 (t, C-9), 37.64 (s, C-10), 38.18 (t, C-3), 42.14 (d, C-1), 44.84 (d, C-7), 52.85 (d, C-5), 70.12 (d, C-6), 106.40 (t, C-15), 148.11 (s, C-4); MS (EI, 70 eV): m/z (rel. int.) 222 (2) [M^+], 207 (5), 204 (21) [M^+-H_2O], 189 (10), 179 (11), 161 (30), 137 (21), 121 (23), 109 (100), 105 (21), 95 (37), 93 (33), 91 (24), 81 (39), 79 (30), 77 (18), 69 (20), 67 (28), 55 (34), 43 (27), 41 (64), 39 (21).

3.12. Dehydration of (+)-7-epi-junenol (4)

The dehydration was performed analogously to the dehydration of **1**. The reaction products were isolated and identified as *trans*-eudesma-4(15),6-diene (**5**) and *trans*-eudesma-4(15),7-diene (**6**). **5**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.63 (3H, s, H-14), 1.02 (6H, d, J = 7.0 Hz, H-12, H-13), 2.53 (1H, bs, H-5), 4.56 (1H, bs, H-15α/β), 4.75 (1H, bs, H-15α/β), 5.41 (1H, s, H-6); MS (EI, 70 eV): m/z (rel. int.) 204 (41) [M^+], 189 (22), 161 (100), 133 (43), 119 (30), 105 (54), 95 (23), 93 (24), 91 (54), 81 (27), 79 (25), 77 (23), 67 (25), 55 (24), 41 (52). **6**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.67 (3H, s, H-14), 1.01 (3H, d, J = 7.0 Hz, H-12), 1.02 (3H, d, J = 7.0 Hz, H-13), 4.57 (1H, bs, H-15α/β), 4.78 (1H, bs, H-15α/β), 5.29 (1H, d, J = 5.7 Hz, H-8); MS (EI, 70 eV): m/z (rel. int.) 204 (68) [M^+], 189 (38), 161 (80), 147 (33), 133 (70), 121 (24), 119 (42), 108 (42), 107 (31), 105 (100), 95 (35), 93 (84), 91 (80), 81 (33), 79 (50), 77 (39), 67 (36), 55 (36), 53 (29), 43 (29), 41 (68), 39 (30).

3.13. Hydrogenation of (+)-δ-selinene (7)

To a soln. of 1 mg of **7** 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. 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.

3.14. Hydrogenation of 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 **7**. One of the hydrogenated eudesmanes from both reactions has identical retention times on polysiloxane- and on cyclodextrin phases.

3.15. Hydrogenation of 5 and 6

The hydrogenation of **5** and **6** 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 **7**. The hydrogenated eudesmanes obtained from the different reactions have all identical retention times on polysiloxane- and on cyclodextrin phases.

Acknowledgements

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