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Phytochemistry 67 (2006) 778–783
PHYTOCHEMISTRY
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Three *ent*-eudesmenones from the liverwort *Plagiochila bifaria*

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Received 30 August 2005; received in revised form 22 December 2005
Available online 17 February 2006

Abstract

The essential oil of the liverwort *Plagiochila bifaria* was analysed by GC and GC–MS. Three eudesmane type sesquiterpenes, *ent*-eudesm-4-en-6-one, *ent*-eudesm-4(15)-en-6-one, *ent*-7-hydroxyeudesm-4-en-6-one were isolated and identified as new natural products. Structure elucidation and the determination of absolute configurations are described.

Keywords: *Plagiochila bifaria*; Liverwort; Sesquiterpenes; *ent*-Eudesmanes

1. Introduction

Sesquiterpenes from lower plants and marine organisms often occur as the optical antipodes to those found in higher plants (Asakawa, 1995). Hence, stereochemical investigations are of special interest, when known sesquiterpenes are isolated from both sources. Enantioselective gas chromatography using modified cyclodextrins as stationary phases has proven to be one of the most powerful methods in the assignment of absolute configurations by chemical correlations and comparison with authentic standards (König and Hochmuth, 2004).

In recent years, the liverwort *Plagiochila bifaria* (Sw.) Lindenb. has been studied intensively by Rycroft and others (Heinrichs et al., 1998a,b, 2004; Rycroft, 1999; Rycroft et al., 1999). For this taxon, a broad morphological species concept has been proposed, based on morphological, genetic and phytochemical investigations. For the European *P. killarniensis* Pears. synonymy with the Neotropical *P. bifaria* was proposed in 1998 and confirmed recently (Heinrichs et al., 1998a,b, 2004). In addition, *P. compressula* (Nees) Lindenb. and *P. centrifuga* Spruce were assigned as new synonyms of *P. bifaria* (Heinrichs et al., 2004).

P. bifaria occurs throughout Atlantic Europe, Macaronesia and the Neotropics and encompasses different morpho- and chemotypes. Both the morphological and chemical variations are more pronounced for the Neotropical populations, while only the "methyl everninate" (**1**) chemotype has been described from Europe so far (including Macaronesia) (Heinrichs et al., 2004). It was mentioned that *P. bifaria* from Madeira does not differ from other European populations (Rycroft, 1999), which is consistent with our results. Nevertheless, a recent study on the volatiles of *P. bifaria* from Madeira also described a methyl everninate depleted population (Figueiredo et al., 2005). In the present work two oxygenated eudesmane derivatives (**2** and **3**), previously unknown from *P. bifaria*, were isolated and identified in addition to a structurally related minor constituent (**4**).

2. Results and discussion

The hydro distillation products and extracts of *Plagiochila bifaria* from two collection sites in Madeira were

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✠ Deceased on 19th November 2004. His scientific achievements keep him among us.

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doi:10.1016/j.phytochem.2006.01.001

investigated by GC and GC–MS. Screening revealed a complex mixture of oxygenated aliphatics and aromatics, monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenoids as well as diterpenes. Known compounds were identified by comparison of their mass spectra and retention indices with a spectral library that was established under identical experimental conditions (Joulain and König, 1998; Hochmuth et al., 2004). Both samples exhibited a similar composition, deviations were observed for minor constituents with a relative concentration of less than 0.5%. The GC profile and GC–MS data showed major resemblance to recently reported data (Figueiredo et al., 2005). The abovementioned chemical marker for European populations, methyl everninate (**1**) (Rycroft et al., 1999), was detected as a major component in all samples in addition to peculiaroxide (**5**) (Wu et al., 1993) and a new oxygenated sesquiterpene (**3**) (Fig. 1). Other constituents of the essential oil are gemacrane-, eudesmane- and cadinane-type sesquiterpenes, e.g., bicyclogermacrene (**6**), eudesma-1,4(15),11-triene (**7**) and frullanolide (**8**), in addition to tricyclic sesquiterpenes of the longipinane-, copaane-, cubebane- or bourbonane- type, e.g., α-longipinene (**9**), α-copaene (**10**) and β-bourbonene (**11**). In contrast to the hydro distillation products, the two 9,10-dihydrophenanthrene derivatives (**12** and **13**) (Rycroft et al., 1999) could only be detected in a CH_2Cl_2 extract. Zierene as reported recently, could not be detected in our samples (Figueiredo et al., 2005).

The essential oil was pre-fractionated by column chromatography. Two fractions containing compounds **2**–**4** were submitted to further isolation procedures. One of the components could be identified as (−)-7-hydroxyeudesm-4-en-6-one (**2**) by comparison of the MS, ^{1}H and ^{13}C NMR data with an authentic sample, isolated from the higher plant *Chloranthus spicatus* (Tesso, 2005). The (+)-enantiomer has previously been described from *C. serrata* and *C. spicatus* (Kawabata et al., 1985; Tesso, 2005). The absolute configuration of (+)-**2** has been established to be (7*R*,10*R*) (Kawabata et al., 1985). Thus, the negative sign of optical rotation of our compound **2** implied (7*S*,10*S*)-configuration. This was confirmed upon enantioselective gas chromatography by co-injection and peak separation of (−)-**2** with an authentic sample of (+)-**2** using 6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl-β-cyclodextrin as the stationary phase. (−)-**2** could be identified for the first time as a natural product. It should further be mentioned that **2** corresponds to the unidentified peak 11 described by Rycroft et al. (1999).

In addition, the abovementioned new major component **3** was isolated from a CC fraction containing **3**, **4** and **5** by

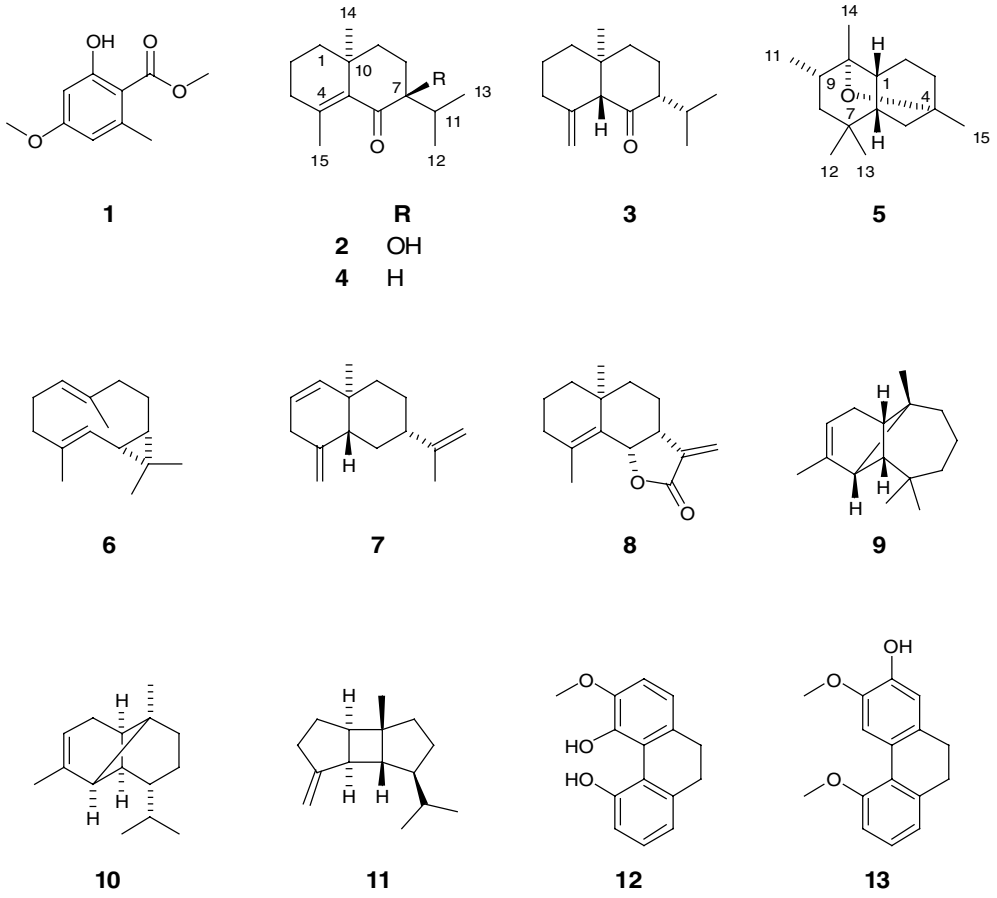

Fig. 1. Selected constituents of *Plagiochila bifaria*.

prep. TLC applying an Et_2O/*n*-hexane solvent system. The ^1H NMR spectrum of **3** indicated the presence of an isopropyl group in agreement with signals for two secondary methyl groups at δ_H 0.86 (3H, *d*) and 0.94 (3H, *d*) and a methine proton at δ_H 2.19 (1H, *m*) (Table 1). In addition, a tertiary methyl group at δ_H 0.71 (3H, *s*) and an exocyclic methylene group at δ_H 5.18 (1H, *m*) and 6.57 (1H, *m*) were observed. From the ^{13}C NMR data the presence of a carbonyl group was deduced from a signal at δ_C 212.0 (*s*), while signals at δ_C 112.6 (*t*) and 142.5 (*s*) confirmed the exocyclic methylene group (Table 2). Interpretation of the H,H-COSY, HMQC and HMBC spectra revealed an eudesmane skeleton with the carbonyl group at C-6 and the double bond located in position C-4/C-15, consistent with an eudesm-4(15)-en-6-one structure. First attempts to isolate **3** by preparative GC resulted in its thermal rearrangement and the isolation of the endocyclic double bond isomer **4**. This compound could also be identified from the essential oil as a minor constituent. The presence of **4** in the CH_2Cl_2 extract confirmed its authenticity as a plant constituent. The NMR data of **4** were very similar to that of **3**, but instead of the exocyclic double bond, an olefinic methyl group at δ_H 1.75 (3H, *s*) was observed. In agreement with the 2D NMR data (H,H-COSY, HMQC and HMBC) and the thermal rearrangement of **3** to **4**, an eudesm-4-en-6-one structure for **4** was concluded. While (+)-**3** and (+)-**4** showing (5*S*,7*S*,10*R*)- and (7*S*,10*R*)-configuration, respectively, were also reported as constituents of *C. serrata* (Kawabata et al., 1985), complete NMR data are presented here for the first time. The observed signs of optical rotation indicated (−)-(5*R*,7*R*,10*S*) and (−)-(7*R*,10*S*) configuration for **3** and **4**. In order to verify its absolute configuration, compound **4** was reduced by treatment with excess $LiAlH_4$ (Fig. 2). With the double bond conjugated to the carbonyl group the reduction resulted in a series of by-products. From the complex mixture of reaction products, (+)-selina-3,5-diene (**14**) and (−)-δ-selinene (**15**) were identified by GC–MS and comparison of the GC retention times with standards of (+)/(−)-selina-3,5-diene und (+)/(−)-δ-selinene by enantioselective gas chromatography using 6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl-β-cyclodextrin as the stationary phase (Fig. 3). This result is in agreement with the assigned configuration reported earlier (Kawabata et al., 1985). In addition to the thermal rearrangement, the absolute configuration of (−)-**3** could be established by chemical conversion to (−)-**4** using deactivated Al_2O_3 as a catalyst (Corano et al., 1975) (Fig. 4). Like (7*S*,10*S*)-7-hydroxyeudesm-4-en-6-one (**2**), (5*R*,7*R*,10*S*)-eudesm-4(15)-en-6-one (**3**) and (7*R*,10*S*)-eudesm-4-en-6-one (**4**) could be identified as new natural

Table 1
^1H NMR spectral assignments of **2**, **3** and **4** (500 MHz, C_6D_6)

H	**2**		**3**		**4**	
	δ_H (ppm)	*m* (*J*/Hz)	δ_H (ppm)	*m* (*J*/Hz)	δ_H (ppm)	*m* (*J*/Hz)
1	1.24	*m*, $H_{(Si)}$	1.17–1.51[a]	*m*	1.26	*m*, $H_{(Re)}$
	1.31	*m*, $H_{(Re)}$			1.35	*m*, $H_{(Si)}$
2	1.33	*m*, $H_{(Re)}$	1.17–1.51[a]	*m*	1.38	*m*
	1.41	*m*, $H_{(Si)}$			1.43	*m*
3	1.77 (2H)	*m*	1.77	*m*, $H_{(Si)}$	1.77 (2H)	*m*
			2.13	*m*, $H_{(Re)}$		
5	–	–	2.54	*bs*	–	–
7	–	–	1.71	*m*	1.91	*ddd* (4.4, 6.5, 12.3)
8	1.59	*m*, $H_{(Re)}$	1.17–1.51[a]	*m*, $H_{(Si)}$	1.55	*m*
	1.74	*m*, $H_{(Si)}$	1.63	*m*, $H_{(Re)}$	1.59	*m*
9	1.23	*m*, $H_{(Re)}$	1.17–1.51[a]	*m*	1.44 (2H)	*m*
	1.82	*m*, $H_{(Si)}$				
11	2.36	*m*	2.19	*m*	2.50	*dqq* (4.4, 6.9, 6.9)
12	0.96	*d* (7.0)	0.86	*d* (6.9)	0.95	*d* (6.9)
13	0.97	*d* (6.3)	0.94	*d* (6.6)	0.97	*d* (6.9)
14	0.83	*s*	0.71	*s*	0.85	*s*
15	1.82	*s*	5.18	*m*, $H_{(E)}$	1.75	*s*
			6.57	*m*, $H_{(Z)}$		

[a] Unresolved multiplet (7H).

Table 2
^{13}C NMR spectral assignments of **2**, **3** and **4** (100 MHz, C_6D_6)

C	**2**		**3**		**4**	
	δ_C (ppm)		δ_C (ppm)		δ_C (ppm)	
1	38.8	*t*	40.8	*t*	39.1	*t*
2	18.9	*t*	23.9	*t*	19.1	*t*
3	33.7	*t*	38.8	*t*	33.2	*t*
4	141.8	*s*	142.5	*s*	135.8	*s*
5	138.2	*s*	60.0	*d*	140.4	*s*
6	202.4	*s*	212.0	*s*	205.5	*s*
7	78.9	*d*	57.8	*d*	58.1	*d*
8	26.7	*t*	26.5	*t*	22.6	*t*
9	35.8	*t*	42.3	*t*	40.8	*t*
10	37.5	*s*	44.1	*s*	38.4	*s*
11	32.8	*d*	26.7	*d*	26.6	*d*
12	16.5	*q*	19.1	*q*	18.9	*q*
13	18.6	*q*	21.8	*q*	21.4[a]	*q*
14	25.4	*q*	17.9	*q*	25.5	*q*
15	22.1	*q*	112.6	*t*	21.4[a]	*q*

[a] Signals coincide.

products. Compound **3** was previously detected by Rycroft et al. (1999) and corresponds to their unidentified peak 7. Though unknown, both compounds, **2** and **3**, were additionally used to characterise the European "methyl evernate" chemotype of *P. bifaria* (Heinrichs et al., 2004).

3. Experimental

3.1. General experimental procedures

3.1.1. Gas chromatography

Carlo Erba Mega 5300 or GC 8000 double column instruments equipped with 25 m silica capillaries with polysiloxane CPSil-5 and polysiloxane CPSil-19 (Chromopack); 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, 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; split ratio ca. 1:30; FID; carrier gas 0.5 bar H_2; injector and detector temperatures were 200 and 250 °C, respectively.

3.1.2. GC–MS and GC–HRMS

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

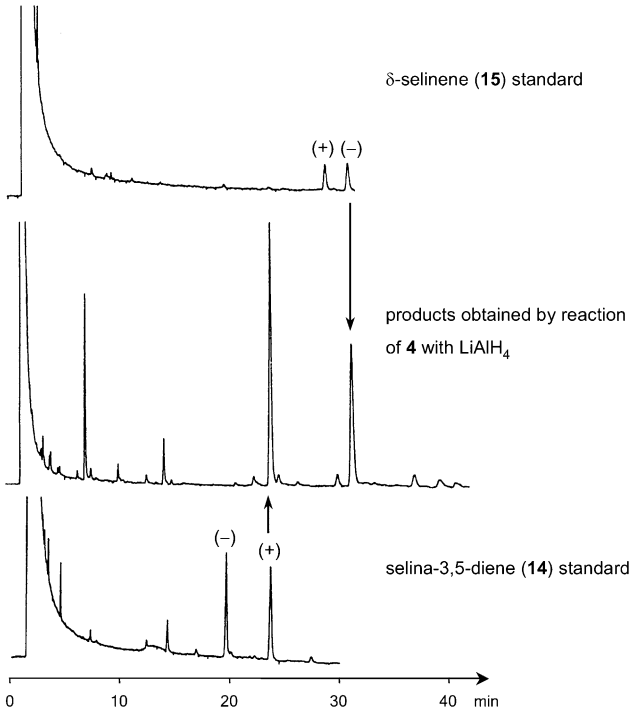

Fig. 3. Enantioselective GC for the assignment of the absolute configuration of (−)-(7*R*,10*S*)-eudesm-4-en-6-one (**4**) (6-*O*-*tert*-butyldimethylsilyl-2,3-*O*-dimethyl-β-cyclodextrin; 120 °C isothermal).

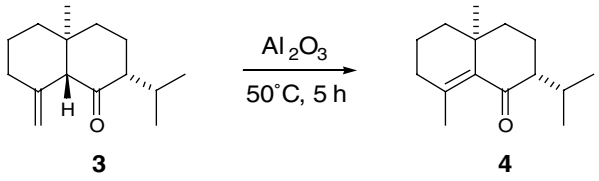

Fig. 4. Catalytic isomerisation of (−)-(5*R*,7*R*,10*S*)-eudesm-4(15)-en-6-one (**3**) to (−)-(7*R*,10*S*)-eudesm-4-en-6-one (**4**).

3.1.3. Preparative GC

Modified Varian 1400 instrument, equipped with stainless steel columns (1.85 m × 4.3 mm) with 10% polydimethylsiloxane SE-30 on Chromosorb W-HP or with 2.5% octakis(2,6-di-*O*-methyl-3-*O*-pentyl)-γ-cyclodextrin in OV-1701 (50%, w/w) on Chromosorb G-HP or with 6% heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin in SE-52 (50%, w/w) on Chromosorb W-HP; FID; He as carrier gas at a flow rate of 120 ml min^{-1}; injector and detector temperatures were 200 and 250 °C, respectively; eluting fractions were trapped in teflon tubes cooled with liquid nitrogen (Hardt and König, 1994).

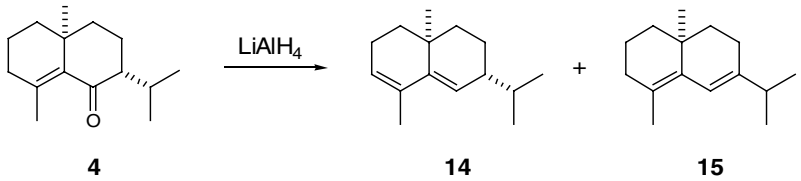

Fig. 2. Reaction of (−)-(7*R*,10*S*)-eudesm-4-en-6-one (**4**) with $LiAlH_4$.

3.1.4. NMR spectroscopy

NMR measurements were carried out with a Bruker WM 400 (400 MHz) or a Bruker DRX 500 (500 MHz) instrument using C_6D_6 as the solvent and TMS as internal standard.

3.1.5. Polarimetry

Measurements were performed with a polarimeter 341 Perkin–Elmer at 589 nm at 20 °C in C_6D_6. To avoid inaccuracies, only the sense of optical rotation was determined due to the small quantity of the isolated compounds.

3.2. Plant material and essential oils

The samples of *P. bifaria* were collected in March 2003 at 'Curral das Freiras' by W.A. König and in April 2003 at 'Pico do Facho' by H. Muhle, Madeira (voucher specimen, HBG2719 and HBG2718, respectively, are stored in the Herbarium Hamburgense). Aqueous homogenates of the fresh plant material were submitted to hydro distillation (2 h) to yield the essential oil, which was collected in 1 ml of *n*-hexane and not further quantified. Because of the largely differing weight the fresh material was not weighed.

3.3. Isolation of single constituents of the essential oil

The essential oil from *P. bifaria* was fractionated by column chromatography over silica gel with increasing proportions of Et_2O in *n*-pentane. Two hydrocarbon fractions and six fractions containing aromatics, oxygenated sesquiterpenoids, and diterpenes were obtained. Each fraction was investigated by GC and GC–MS. Two fractions containing compounds **2**–**5** were submitted to further isolation procedures.

*3.3.1. Isolation of (−)-(7S,10S)-7-hydroxyeudesm-4-en-6-one (**2**)*

(−)-(2*S*,4a*S*)-2-hydroxy-4*a*,8-dimethyl-2-(1-methylethyl)-3,4,4*a*,5,6,7-hexahydro-2*H*-naphthalen-1-one; *ent*-7-hydroxyeudesm-4-en-6-one. **2** was enriched by preparative GC using a column with polysiloxane SE-52 (130–180 °C, 2 °C/min, 1.5 bar He) and later purified by using octakis(2,6-di-*O*-methyl-3-*O*-pentyl)-γ-cyclodextrin as the stationary phase (160 °C isothermal, 1.4 bar He). Colourless oil (ca. 2 mg); $RI_{CPSiL5} = 1712$; sense of optical rotation (C_6D_6): (−); for ^1H and ^{13}C NMR data (see Tables 1 and 2); MS (EI, 70 eV) *m*/*z* (rel. int.): 236 [M^+] (2), 218 (11), 208 (15), 203 (11), 193 (8), 185 (1), 175 (28), 165 (30), 147 (13), 138 (57), 123 (16), 109 (100), 99 (15), 91 (23), 79 (20), 67 (23), 55 (4), 43 (88). HRMS: *m*/*z* = 236.1757 [M^+] (calc. for $C_{15}H_{24}O_2$: 236.1776).

*3.3.2. Isolation of (−)-(5R,7R,10S)-eudesm-4(15)-en-6-one (**3**)*

(−)-(2*R*,4a*S*,7a*R*)-4*a*-methyl-8-methylene-2-(1-methylethyl)-octahydro-2*H*-naphthalen-1-one; *ent*-eudesm-4(15)-en-6-one. **3** was isolated by prep. TLC applying a solvent mixture *n*-hexane/Et_2O 20:1 [R_F (**3**): 0.46; R_F (**4**): 0.26; R_F (**5**): 0.39]. Colourless oil (ca. 0.5 mg); $RI_{CPSiL5} = 1617$; sense of optical rotation (C_6D_6): (−); for ^1H and ^{13}C NMR data (see Tables 1 and 2); MS (EI, 70 eV) *m*/*z* (rel. int.): 220 [M^+] (31), 205 (20), 187 (3), 177 (10), 159 (6), 149 (15), 135 (24), 121 (16), 107 (100), 93 (51), 79 (39), 67 (25), 55 (52), 41 (84). HRMS: *m*/*z* = 220.1825 [M^+] (calc. for $C_{15}H_{24}O$: 220.1827).

*3.3.3. Rearrangement of **3***

A solution of (5*R*,7*R*,10*S*)-eudesm-4(15)-en-6-one (**3**) in 0.5 ml *n*-hexane was treated with 0.2 mg of deactivated alumina and stirred for 5 h at 50 °C. Subsequently the solution was filtered and investigated by GC–MS.

*3.3.4. Isolation of (−)-(7R,10S)-eudesm-4-en-6-one (**4**)*

(−)-(2*R*,4a*S*)-4*a*,8-dimethyl-2-(1-methylethyl)-3,4,4*a*,5,6,7-hexahydro-2*H*-naphthalen-1-one; *ent*-eudesm-4-en-6-one. **4** was purified by preparative GC using heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin as the stationary phase (150 °C isothermal for 20 min, then 160 °C isothermal, 1.5 bar He). Colourless oil (ca. 2 mg); $RI_{CPSiL5} = 1605$; sense of optical rotation (C_6D_6): (−); for ^1H and ^{13}C NMR data, see Tables 1 and 2; MS (EI, 70 eV) *m*/*z* (rel. int.): 220 [M^+] (76), 205 (100), 191 (5), 178 (99), 163 (33), 149 (66), 137 (34), 122 (21), 107 (43), 93 (38), 79 (37), 67 (27), 55 (47), 41 (86); HRMS: *m*/*z* = 220.1824 [M^+] (calc. for $C_{15}H_{24}O$: 220.1827).

*3.3.5. $LiAlH_4$ reduction of **4***

A solution of 0.5 mg (7*R*,10*S*)-eudesm-4-en-6-one (**4**) in 0.5 ml abs. Et_2O was added to a suspension of 4 mg (0.1 mmol) $LiAlH_4$ in 2 ml abs. Et_2O and stirred for 4 h at room temperature. After addition of 5 ml H_2O the resulting solution was extracted three times with each 2 ml of Et_2O. After washing, drying, and concentrating, the reaction products were investigated by GC–MS. (+)-Selina-3,5-diene (**14**) and (−)-δ-selinene (**15**) were identified by comparison of their retention indices and mass spectra, as well as comparison of their GC retention times with data of enantiomerically enriched standards using heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin as the stationary phase.

3.3.6. (+)-(1R,4S*,6S*,9S*,10S*)-Peculiaroxide (**5**)*

^1H NMR (500 MHz, C_6D_6): δ 0.83 (3H, *s*, H-13), 0.87–0.90 (1H, *m*, $H_{(Si)}$-8), 0.91 (3H, *s*, H-12), 1.00 (3H, *d*, *J* = 6.6 Hz, H-11), 1.11 (3H, *s*, H-15), 1.11–1.18 (1H, *m*, $H_{(Si)}$-2), 1.15 (3H, *s*, H-14), 1.24–1.35 (4H, *m*, $H_{(Re)}$-3, $H_{(Si)}$-5, H-6, H-9), 1.38–1.39 (1H, *m*, H-1), 1.43 (1H, *ddd*, *J* = 1.6, 3.2, 11.5 Hz, $H_{(Re)}$-5), 1.52 (1H, *dd*, *J* = 13.2, 13.2 Hz, $H_{(Re)}$-8), 1.57–1.64 (1H, *m*, $H_{(Si)}$-3), 1.95 (1H, *dddd*, *J* = 3.2, 7.3, 11.0, 13.2 Hz, $H_{(Re)}$-2); ^{13}C NMR (100.6 MHz, C_6D_6): δ 15.3 (*q*, C-11), 23.0 (*t*, C-2), 24.3 (*q*, C-14), 26.7 (*q*, C-12), 27.7 (*q*, C-15), 28.3 (*q*, C-13), 32.5 (*t*, C-3), 32.8 (*d*, C-1), 33.1 (*s*, C-7), 35.4 (*t*, C-5), 37.2 (*d*, C-9), 40.1 (*t*, C-8), 44.3 (*d*, C-6), 69.4 (*s*, C-4), 73.7 (*s*, C-10).

Acknowledgements

We thank Professor R. Mues, Universität des Saarlands, and Dr. D.S. Rycroft, University of Glasgow, for their advice. We also thank Dr. V. Sinnwell, University of Hamburg, for his support in the NMR and Mrs. A. Meiners and Mr. M. Preusse for GC–MS and GC–HRMS measurements.

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