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PINGUISANE SESQUITERPENES FROM THE LIVERWORT *PORELLA NAVICULARIS*†‡

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Key Word Index—*Porella navicularis*; Hepaticae; liverwort; sesquiterpene pinguisane, drimane, albicanol; *allo*-cedrol.

Abstract—Four new pinguisanes, 5-pinguisen-11-ol, 6α-methoxy-pinguis-5(10)-en-11,6-olide, 6α-methoxy-pinguis-5(10)-en-11,6-olide-15-carboxylic acid and 5α,10α-epoxy-pinguisane-11,6-olide-15-carboxylic acid methyl ester, have been isolated from the North American liverwort *Porella navicularis* along with two known pinguisane-type sesquiterpenes, the drimane albicanol, *allo*-cedrol and the known diterpenes naviculide and *trans*-communic acid. The structures were elucidated by spectroscopic methods. Furthermore, sesquiterpene and diterpene hydrocarbons were identified by GC-MS analysis.

INTRODUCTION

Liverworts of the genus *Porella* are a rich source of sesquiterpenes predominantly with pinguisane, drimane, aromadendrane and striatane skeletons [1, 2]. In a recent study, several pinguisanes, striatenone and the diterpenes perrottetianal and naviculide have been reported from the North American species. *P. navicularis* [4]. The availability of larger amounts of this liverwort led us to reinvestigate the species.

RESULTS AND DISCUSSION

A combination of column chromatography (Sephadex LH-20), vacuum liquid chromatography and HPLC of the diethyl ether extract of *P. navicularis* afforded the four pinguisanes 5-pinguisen-11-ol (**1**), 6α-methoxy-pinguis-5(10)-en-11,6-olide (**2**), 6α-methoxy-pinguis-5(10)-en-11,6-olide-15-carboxylic acid (**3**) and 5α,10α-epoxy-pinguisane-11,6-olide-15-carboxylic acid methyl ester (**4**) along with the known pinguisanes naviculol (**6**) and norpinguisone methyl ester (**7**) [4], the drimane sesquiterpene albicanol (**8**) [5] and the diterpenes naviculide (**9**) and *trans*-communic acid (**10**) [4]. From the mass spectrometric and 1D and 2D NMR spectroscopic data compound **5** was identified as *allo*-cedrol, a sesquiterpene alcohol isolated from *Juniperus rigida* [6]. This is the first report of this rare tricyclic sesquiterpene in a liverwort.

Compound **1**. Colourless oil, was assigned the molecular formula $C_{15}H_{26}O$ (EIMS, m/z 222.25 $[M]^+$, calc. 222.3708). The ^1H NMR spectrum displayed the signals of two tertiary methyl groups (δ_H 0.67 and δ_H 0.74), two secondary methyl groups (δ_H 0.80 and δ_H 0.94, both $J = 7.24$ Hz) and an olefinic proton (δ_H 5.35, *br s*) together with a signal at δ_H 3.60 (*m*) corresponding to a primary hydroxyl group (3450 cm^{-1}). The ^{13}C NMR spectrum showed the presence of 15 carbon atoms. Based on the DEPT spectra the signals could be assigned to four methyls, five sp^3 methylenes, two sp^3 methines, one sp^2 methine and three quaternary carbons. The singlet at δ_C 122.6 and the doublet at δ_C 134.8 indicated a trisubstituted double bond; the triplet at δ_C 60.5 was assigned to a hydroxymethyl group. The spectroscopic data coupled with the molecular formula showed that **1** was a bicyclic sesquiterpene alcohol of the pinguisane series.

The comparison of the NMR spectra of **1** with those of naviculol (**6**), the major sesquiterpene of this liverwort [4], showed much similarity but indicated a cyclic position of the double bond instead of the exocyclic arrangement present in compound **6**. Thus, the structure of **1** could be formulated as 5-pinguisen-11-ol, a tautomer of **6** with all methyl substituents in the β-position, the characteristic stereochemical feature of all pinguisanes. Compound **1** might be an artifact of **6**.

* Author to whom correspondence should be addressed.

† Dedicated to Prof. T. Eicher on the occasion of his 65th birthday.

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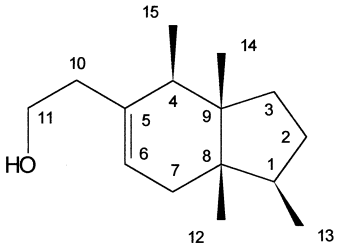

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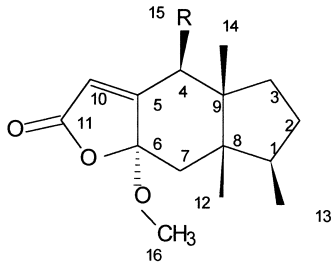

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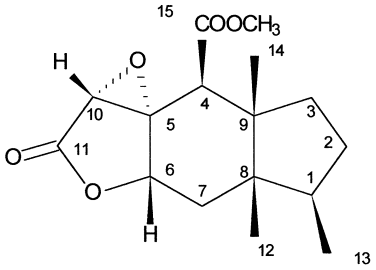

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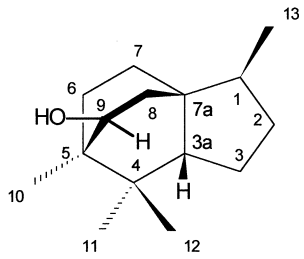

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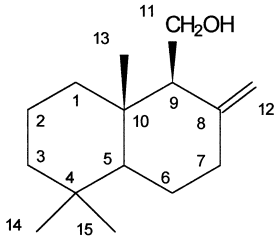

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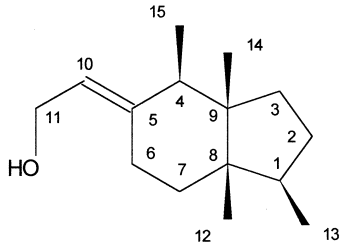
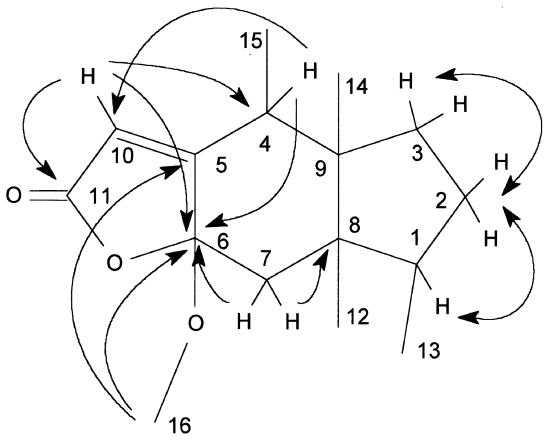


6

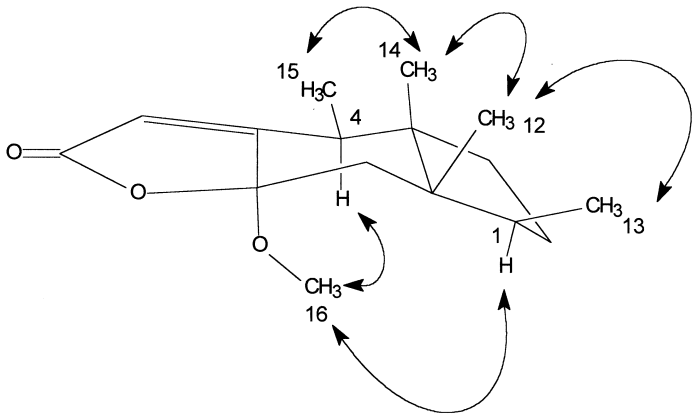
Compound **2**. Colourless oil, was assigned the molecular formula $C_{16}H_{24}O_3$ (EI MS, m/z 264.30 $[M]^+$ calc. 264.3648). The ^1H NMR spectrum displayed the signals of two singlet methyls (δ_H 0.64 and δ_H 0.68), two doublet methyls (δ_H 0.87, $J = 6.70$ Hz and δ_H 1.12, $J = 6.77$ Hz), the singlets of a methoxy group (δ_H 3.11) and of an olefinic proton (δ_H 5.78). From the DEPT spectra, the 16 carbon signals could be assigned to five singlet methyls, three methylenes, three methines and five quaternary carbons with the signals of a conjugated carbonyl-group (δ_C 172.4) and an olefinic carbon (δ_C 170.2), belonging to a γ-lactone ring supported by the absorption at 1750 cm^{-1} in the IR spectrum. The ^1H-^1H-COSY, ^1H-^{13}C-COSY and HMBC spectra revealed the structure of the pinguisane derivative as 6-methoxy-pinguis-5(10)-ene-11,6-olide (**2**) (Fig. 1).

From the NOESY experiment the stereochemistry



2

Fig. 1. Significant HMBC and ^1H-^1H-COSY couplings of **2**.



2

Fig. 2. Significant NOESY couplings of **2**.

of the four methyl groups and the methoxy group could be deduced (Fig. 2). As there were cross-peaks between H-14 and H-15, H-12 and both H-14 and H-13, all methyls groups had to be in the β-position. This was also confirmed by correlations between H-16 and H-4 and between H-16 and H-1. Compound **2** is an analogue of the keto lactone 6α-methoxy-3-oxo-pinguis-5(10)-en-11,6-olide that has been recently isolated from axenic cultures of the liverwort *Aneura pinguis* [7].

Compound **3**. The molecular formula, $C_{16}H_{22}O_5$, of **3** was obtained from the EI mass spectrum (m/z 294.52 $[M]^+$, calc. 294.3477). The ^{1}H NMR and ^{13}C NMR spectral data of **3** were similar to those of **2**. Compound **3** also possessed a methoxy group at the quaternary carbon C-6 (δ_H 3.75, *s*) and an olefinic proton (δ_H 5.20, *s*), belonging to a γ-lactone ring (1750 cm^{-1}). However, the NMR spectra only revealed the presence of three, instead of four methyl groups. The IR, ^{1}H-^{1}H-COSY, ^{1}H-^{13}C-COSY and HMBC data confirmed the structure of a pinguisanolide. Compared to compound **2**, the signal of H-4 in the ^{1}H NMR spectrum was shifted to δ_H 3.76 (*s*) indicating a carboxylic group (IR band at 1720 cm^{-1}) at position 4, that could be assigned to the ^{13}C NMR-signal at δ 170.3 (C-15). The corresponding methyl ester has been described from the liverwort *Porella canariensis* [12].

The NOESY spectrum displayed correlations between the methoxy group H-16 (δ_H 3.75) and H-4 (δ_H 3.76) as well as cross-peaks between the three methyl groups. Interactions between H-4 or H-16 and the methyls were not observed. These facts indicated the β-orientation of the methyl groups and the α-orientation of the methoxy substituent. Thus, compound **3** is 6α-methoxy-pinguis-5(10)-ene-11,6-olide-carboxylic acid.

Compound **4**. Colourless amorphous powder, with the molecular formula $C_{16}H_{22}O_5$ (EI MS, m/z 294.25 $[M]^+$, calc. 294.3477). The IR spectrum showed the presence of a lactone group (1750 cm^{-1}). The chemical shifts and multiplicities of the three methyl group signals in the ^{1}H NMR spectrum (δ_H 0.81, *s*; δ_H 1.08, *s*; δ_H 0.83, *d*, $J = 6.8$ Hz) indicated a structure similar to the pinguisane derivative **3**. The singlet at δ_H 3.66 (3H) was attributed to a methyl ester group (1730 cm^{-1}). The ^{1}H NMR spectrum also showed the well separated signals of a methylene group (δ_H 2.41, *m*) and three methine protons (δ_H 3.79, *s*; δ_H 3.42, *d*, $^4J = 2.17$ Hz; δ_H 5.45, *d*, $^4J = 2.17$ Hz). The ^{13}C NMR spectrum displayed the signals of four methyl, three methylene and four methine groups and five quaternary carbons. The signals at δ_C 171.1 (*s*) and δ_C 51.6 (*q*) confirmed the methyl ester. The lactone group signals appeared at δ_C 77.0 and δ_C 178.1. The signals at δ_C 54.8 and δ_C 57.0 were characteristic of an epoxy group, supported by the IR band at 3080 cm^{-1}. The final structure of this pinguisane derivative was deduced from the ^{1}H-^{1}H-COSY, ^{1}H-^{13}C-COSY and HMBC data (Fig. 3). In the ^{1}H-^{1}H-COSY spectrum no coupling was observed between H-6 and H-7. A possible explanation could be the electronegative substituent at C-6 and the dihedral angle of approximately 90°, both minimising the ^3J-coupling constant. The NOESY experiment allowed

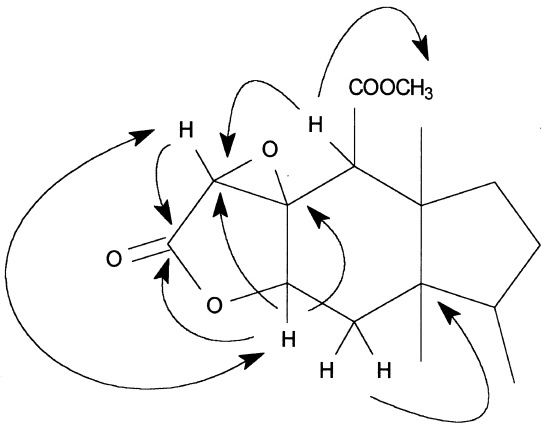


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Fig. 3. Significant HMBC and ^{1}H-^{1}H-COSY couplings of **4**.

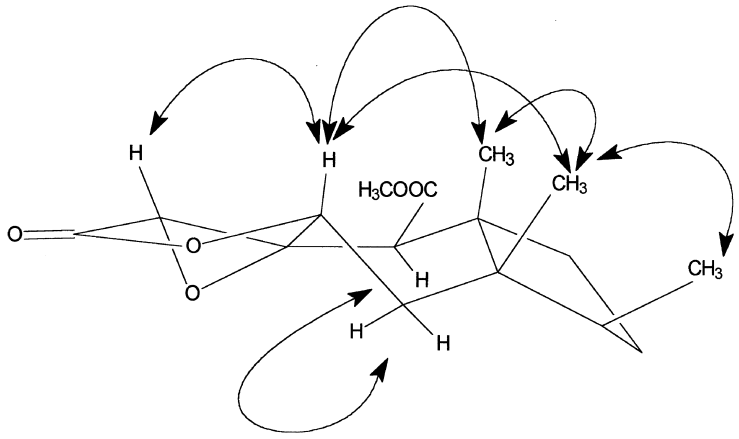

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Fig. 4. Significant NOESY couplings of **4**.

the determination of the stereochemistry of **4** (Fig. 4). The correlation of the methyl groups H-12, H-13 and H-14 indicated the β-position of all three methyl groups. The cross-peaks of H-6 with both methyl groups H-12 and H-14 and with H-10 revealed the stereochemistry in the lactone ring. H-6 and H-10 were in axial positions. Finally, the interaction between H-7 and H-4 showed the β-orientation of the methyl ester group at C-4. Therefore, the structure of **4** was established as 5α,10α-epoxy-pinguisane-11,6-olide-15-carboxylic acid methyl ester.

Compound **5**. The MS, IR, ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, ^{1}H-^{13}C-COSY and HMBC spectra of compound **5** showed it to be the tricyclic sesquiterpene alcohol *allo*-cedrol. Compound **5**, enantiomeric to khusiol from vetiver oil (khus oil) [8], is known from the higher plant *Juniperus rigida* Sieb. et Zucc. [6]. Recently, a total synthesis of this sesquiterpene alcohol has been reported [9]. Comparison with an original ^{1}H NMR spectrum of the synthetic compound proved the identity of **5**.

The hydrocarbon fractions of *P. navicularis* were subjected to GC-MS analysis. The sesquiterpenes α-copaene, α-cedrene, β-santalene, α-patchoulene, *trans*-β-farnesene, β-acoradiene, β-selinene, α-muurolene and β-bisabolene, and the diterpenes rosa-5,15-diene and sandaracopimaradiene were identified as the major terpene hydrocarbons of the liverwort.

EXPERIMENTAL

NMR: $CDCl_3$ (^{1}H NMR: 400 MHz, ^{13}C NMR: 100.5 MHz for 1D spectra, 500 and 125 MHz, for 2D spectra, respectively) relative to $CDCl_3$ at δ_H 7.24, δ_C 77.0. ^{13}C multiplicities were determined using the DEPT pulse sequence. Optical rotations were measured in $CHCl_3$.

Plant material

P. navicularis (Lehm. et Lindenb.) Lindb. was collected by K.-P. A. near Randle, Lewis county, Washington, U.S.A., in February 1995 and identified by Prof. R. Mues. A voucher specimen (Nr. 5071) is deposited in the Herbarium Saar, Universität des Saarlandes, Saarbrücken.

Extraction and isolation

The extraction scheme followed the standard procedures of our group [10, 11]. Powdered air dried plant material (800 g) was extracted with Et_2O. The Et_2O extract (12.8 g) was chromatographed on Sephadex LH-20 (150 × 2.5 cm i.d.) with MeOH–CH_2Cl_2 (1:1) as eluent to give four fractions (I–IV). Fraction III was separated by VLC (Silica gel 15 μm, 60 mm × 35 mm i.d., stepwise with an *n*-hexane-EtOAc gradient) and gave the fractions III-1 (0–3% EtOAc, 805 mg), III-2 (3.5–4% EtOAc, 168 mg), III-3 (4.5–5% EtOAc, 152 mg), III-4 (5.5–8% EtOAc, 188 mg), III-5 (9–11% EtOAc, 180 mg), III-6 (12–19% EtOAc, 100 mg), III-7 (20–35% EtOAc, 140 mg) and III-8 (35–100% EtOAc, 245 mg). Fractions III-2, III-3, III-4, III-5 and III-6 were further purified by HPLC on silica gel LiChrospher Si 60 5 μm, 4 × 250 mm [*n*-hexane-EtOAc (96.5:3.5) for **5** (40.7 mg), **7** (18.1 mg), *n*-hexane-EtOAc (94:6) for **1** (2.4 mg), **2** (6.3 mg) and **8** (11.2 mg), *n*-hexane-EtOAc (93.5:6.5) for **6** (64.5 mg), *n*-hexane-EtOAc (91:9) for **4** (5.2 mg) and *n*-hexane-EtOAc (83:17) to afford **3** (10.0 mg)]. Fraction IV was separated by VLC, conditions as for III, and gave six subfractions (IV-1, 0–3.5% EtOAc; IV-2, 4.0–5.5% EtOAc; IV-3, 6.0–11.0% EtOAc; IV-4, 12.0–20.0% EtOAc; IV-5, 22.0–35.0% EtOAc; IV-6, 40.0–100% EtOAc). Fractions IV-2 and IV-4 were further purified by HPLC on diol LiChrospher 5 μm, 4 × 250 mm [*n*-hexane-EtOAc (46:54) for **10** (10.1 mg) and *n*-hexane-EtOAc (88.5:11.5) for **9** (22.8 mg)].

GC-MS of hydrocarbons. GC-MS was performed on an HP-5 column (30 m × 0.25 mm i.d.), carrier gas He, 1 ml/min, injector 250°, detector 280°, temperature program: 70°:3 min, 70°–120°:5°/min, 120°–250°:15°/min, 250°:5 min. 2 μl of the *n*-hexane-soluble

compounds of fractions II and III-1 were injected (split) at a concentration of 100 μg/ml (in *n*-hexane). Compounds were identified by comparison of their mass spectra with reference spectra (MS data base, Wiley and Sons 1988 (London)).

5-*Pinguisen*-11-*ol* (**1**). $[\alpha]_D^{20}$ +21° ($CHCl_3$; $c = 0.24$); EI-MS *m/z*: 222.25 $[M]^+$; IR ν_{max}^{KBr} cm^{-1}: 3530, 2950, 1470, 1370; ^1H NMR ($CDCl_3$): δ_H 5.35 (1H, *br s*, H-6), 3.60 (2H, *m*, H-11), 2.34 (1H, *m*, H-4), 1.55 (1H, *m*, H-1), 0.94 (3H, *d*, $J = 7.2$ Hz, H-15), 0.80 (3H, *d*, $J = 7.24$ Hz, H-13), 0.74 (3H, *s*, H-12), 0.67 (3H, *s*, H-14).

6α-*Methoxy-pinguis*-5(10)-*en*-11,6-*olide* (**2**). $[\alpha]_D^{20}$ +68.2° ($CHCl_3$; $c = 0.21$); EI-MS *m/z*: 264.30 $[M]^+$; IR ν_{max}^{KBr} cm^{-1}: 3450, 2950, 2900, 1750, 1470, 1380, 1250, 1180, 930; ^1H NMR ($CDCl_3$): δ_H 5.77 (1H, *s*, H-10), 3.11 (3H, *s*, H-16), 2.75 (1H, *m*, H-1), 2.60 (1H, *q*, $J = 1.8$ Hz, H-4), 2.33 (1H, *m*, H-7β), 1.80 (1H, *m*, H-3β), 1.77 (1H, *m*, H-2β), 1.45 (1H, *m*, H-3α), 1.42 (1H, *m*, H-7α), 1.26 (1H, *m*, H-2α), 1.12 (3H, *d*, $J = 6.7$ Hz, H-15), 0.87 (3H, *d*, $J = 6.7$ Hz), 0.68 (3H, *s*, H-14), 0.64 (3H, *s*, H-12).

6α-*Methoxy-pinguis*-5(10)-*ene*-11,6-*olide*-*carboxylic acid* (**3**). $[\alpha]_D^{20}$ −82.1° ($CHCl_3$; $c = 0.40$); EI-MS *m/z*: 294.25 $[M]^+$; IR ν_{max}^{KBr} cm^{-1}: 3320, 2980, 2900, 1750, 1450, 1360, 1250, 1075, 990; ^1H NMR ($CDCl_3$): δ_H 6.18 (1H, *s*, H-10), 3.76 (1H, *s*, H-4), 3.75 (3H, *s*, H-16), 2.80 (1H, *m*, H-1), 2.30 (1H, *s*, H-7β), 2.01 (1H, *m*, H-3β), 2.00 (1H, *m*, H-2β), 1.55 (1H, *m*, H-3α), 1.31 (1H, *s*, H-7α), 1.30 (1H, *m*, H-2α), 0.92 (3H, *d*, $J = 6.70$, H-13), 0.88 (3H, *s*, H-14).

5α,10α-*Epoxy*-*pinguisane*-11,6-*olide*-15-*carboxylic acid methyl ester* (**4**). $[\alpha]_D^{20}$ +48.0° ($CHCl_3$; $c = 0.22$); EI-MS *m/z*: 294.30 $[M]^+$; IR ν_{max}^{KBr} cm^{-1}: 3450, 3080, 2950, 1750, 1730, 1460, 1430, 1200, 1160, 1060, 850, 810; ^1H NMR ($CDCl_3$): δ_H 5.45 (1H, *d*, $J = 2.17$, H-6β), 3.79 (1H, *d*, $J = 2.17$, H-10β), 3.66 (3H, *s*, H-16), 3.42 (1H, *s*, H-4α), 2.41 (1H, *m*, H-1), 2.02 (1H, *m*, H-7β), 1.99 (1H, *m*, H-2β), 1.97 (1H, *m*, H-3β), 1.55 (1H, *m*, H-7α), 1.43 (1H, *m*, H-3α), 1.36 (1H, *m*, H-2α), 1.08 (3H, *s*, H-14), 0.83 (3H, *d*, $J = 6.8$ Hz, H-13), 0.81 (3H, *s*, H-12).

allo-*Cedrol* (**5**). $[\alpha]_D^{20}$ +89° ($CHCl_3$; $c = 1.20$), EI-MS *m/z*: 222.15 $[M]^+$; IR ν_{max}^{KBr} cm^{-1}: 3300, 2950, 2850, 1500, 1460, 1380, 1150, 1050, 1010; ^1H NMR ($CDCl_3$): δ_H 3.95 (1H, *m*, H-9), 2.06 (1H, *m*, H-8β), 1.92 (1H, *m*, H-2β), 1.57 (1H, *m*, H-6β), 1.45 (1H, *m*, H-7β), 1.43 (1H, *m*, H-3β), 1.42 (1H, *m*, H-1), 1.39 (1H, *m*, H-3α), 1.24 (1H, *m*, H-3α), 1.21 (1H, *m*, H-6α), 1.10 (1H, *m*, H-7α), 1.04 (1H, *m*, H-8α), 0.96 (1H, *m*, H-2α), 0.86 (3H, *s*, H-11), 0.82 (3H, *s*, H-12), 0.78 (3H, *d*, $J = 7.20$, H-13), 0.73 (3H, *s*, H-10); ^{13}C NMR ($CDCl_3$): δ_C 71.00 (*s*, C-9), 53.12 (*d*, C-3a), 41.35 (*s*, C-7a), 40.74 (*t*, C-8), 40.02 (*d*, C-1), 39.77 (*s*, C-5), 35.00 (*t*, C-2), 34.47 (*s*, C-4), 30.98 (*t*, C-7), 26.27 (*q*, C-12), 24.24 (*t*, C-3), 24.23 (*t*, C-6), 21.00 (*q*, C-11), 17.64 (*q*, C-13), 16.14 (*q*, C-10).

Table 1. ^{13}C NMR spectral data of compounds **1**–**4** in $CDCl_3$

C	**1**	**2**	**3**	**4**
1	38.0 *d*	36.1 *d*	36.0 *d*	40.8 *d*
2	38.6 *t*	28.4 *t*	28.2 *t*	30.7 *t*
3	34.8 *t*	33.3 *t*	33.5 *t*	35.9 *t*
4	36.9 *d*	35.9 *d*	47.7 *d*	57.8 *d*
5	134.8 *s*	170.3 *s*	164.9 *s*	54.8 *s*
6	122.6 *d*	107.4 *s*	104.1 *s*	77.0 *d*
7	32.3 *t*	41.2 *t*	42.1 *t*	44.7 *t*
8	44.1 *s*	46.8 *s*	47.1 *s*	55.4 *s*
9	46.4 *s*	52.9 *s*	53.9 *s*	57.9 *s*
10	29.2 *t*	115.9 *d*	117.0 *d*	57.0 *d*
11	60.5 *t*	172.4 *s*	170.3 *s*	178.1 *s*
12	18.9 *q*	18.6 *q*	18.4 *q*	16.1 *q*
13	14.6 *q*	14.7 *q*	14.6 *q*	15.0 *q*
14	15.9 *q*	18.6 *q*	16.5 *q*	19.9 *q*
15	13.7 *q*	11.4 *q*	170.3 *s*	171.1 *s*
16		50.4 *q*	52.0 *q*	51.6 *q*

Acknowledgements—The authors thank Prof. R. Mues, Saarbrücken, for identification of the plant material and Prof. Subba Rao, Indian Institute of Science, Bangalore, for the ^1H NMR spectrum of synthetic *allo*-cedrol.

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