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Terpenoids from the liverwort *Blepharostoma trichophyllum*

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Dedicated to Professor Dr. R. Mues on the occasion of his 60th birthday

Abstract

Blepharostol, a new sesquiterpenoid alcohol with a rearranged drimane skeleton and five new *ent*-labdane diterpenoids, *ent*-labda-13(16),14-diene-8α-ol, *ent*-labda-13(16),14-diene-1β,8α-diol, *ent*-labda-13(16),14-diene-8α,9β-diol, *ent*-labda-13(16), 14-diene-1β,8α,9β-triol and *ent*-8α,9β-dihydroxylabda-13(16),14-dien-1-one, have been isolated from the liverwort *Blepharostoma trichophyllum*. Their structures have been assigned on the basis of their spectroscopic properties.

Keywords: *Blepharostoma trichophyllum*; Pseudolepicoleaceae; Hepaticae; In vitro culture; Blepharostol; Rearranged drimane; *ent*-Labdanes

1. Introduction

The liverwort *Blepharostoma trichophyllum* (L.) Dumort. grows in northern mountainous parts of Europe, Asia and North America. It is also found in some equatorial mountain ranges. Seldom present in pure tufts, it is usually found creeping amongst other bryophytes in a variety of communities (Hill et al., 1991). Accordingly, collecting sufficient and appropriate plant material from its natural habitat is very difficult which may explain why only very little is known about the chemistry of this tiny plant. An earlier study reported the isolation of five *C*-glycosylflavones from an aqueous methanol extract of 2 g collected material (Mues, 1982). A more detailed phytochemical investigation of *B. trichophyllum* was made possible by using in vitro culture as an alternative way of providing pure plant material (350 g).

2. Results and discussion

In the course of our on-going research on axenic cultures of liverworts (Valcic et al., 1997; Tazaki et al., 1999) we have studied the constituents of the dichloromethane extract of in vitro cultured *B. trichophyllum*. The crude extract was first fractionated by size exclusion chromatography, then a combination of vacuum liquid chromatography and HPLC of the fractions led to the isolation of the sesquiterpenoid blepharostol (**1**) and of five labdane diterpenoids (**2**–**6**), *ent*-labda-13(16),14-diene-8α-ol (**2**), *ent*-labda-13(16),14-diene-1β,8α-diol (**3**), *ent*-labda-13(16),14-diene-8α,9β-diol (**4**), *ent*-labda-13 (16), 14-diene-1β,8α,9β-triol (**5**) and *ent*-8α,9β-dihydroxylabda-13(16),14-dien-1-one (**6**). The absolute stereochemistry of the *ent*-labdanes was determined by analysis of the CD spectrum of **6**. Within this series of *ent*-labdane diterpenoids it is extremely unlikely that the absolute configurations of the compounds **2**–**5** will be different from that of the 1-ketone **6**.

The IR spectrum of blepharostol **1** showed a characteristic hydroxyl band at 3446 cm^{-1}. The GC-mass spectrum gave a peak at *m*/*z* 222 consistent with a molecular formula of $C_{15}H_{26}O$ and hence three double bond equivalents which could be accommodated by a trisubstituted double bond (δ 141.4, 122.7; δ 5.32, *m*)

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and two rings. The ^{13}C NMR spectrum revealed a primary alcohol at δ 69.8 which was in accordance with the resonances of a hydroxylic methylene (δ 3.81 and 3.48, both *d*, $J = 11.0$ Hz each) in the ^{1}H NMR spectrum. The ^{1}H NMR spectrum displayed the signals of four methyls (δ 1.63, 1.12, 1.03, 0.90). The methyl at δ 1.63 was attached to the double bond and appeared as doublet ($J = 2.0$ Hz) due to the allylic coupling with the olefinic proton H-3. The singlet methyl at δ 1.12 was positioned at the ring junction. The HMBC spectrum revealed that the methyl at δ 1.03 was geminal to the hydroxymethylene group. The remaining methyl at δ 0.90 was a doublet ($J = 7.0$ Hz) and therefore bound to a methine. All those features were represented by the sesquiterpenoid skeletons of the drimane or the rearranged drimane type. The protons of the doublet methyl group at δ 0.90 correlated in the HMBC with both the methylene carbon at δ 27.4 and the quaternary carbon that bore the hydroxyl methylene and the singlet methyl. Thus, this doublet methyl had to be at C-8 and hence the molecule was a rearranged drimane. The proton and carbon signals were assigned unambiguously to its sesquiterpenoid framework using a combination of HSQC, HMBC and ^{1}H, ^{1}H COSY experiments (see Table 1). The chemical shift of Me-14 was used to determine the stereochemistry of the ring junction. In similar clerodanes that only differ from blepharostol by their additional side chain the methyl at a *trans* ring junction appears at δ 20.6 and δ 1.05, whereas the methyl at a *cis* ring junction is shifted to δ 27.7 and δ 1.15 (Nogueira et al., 2001). With the signals of Me-14 at δ 28.3 and δ 1.12 the rings had to be *cis*. The relative stereochemistry was further supported by NOE difference experiments. Irradiation of H-11a resulted in an increase of the intensity of H-7β, H-11b and Me-14β while irradiation of H-11b increased the intensities of H-11a and Me-12β. Me-15α showed NOEs with H-1α, H-11b and Me-12β and Me-12β showed NOEs with H-7β and H-11b. Irradiation of Me-14β afforded NOEs at H-6β, H-7β and H-11a. No distinct NOEs could be observed for H-10 due to overlap but its β-orientation was assigned on the basis of the above chemical shift argument. Similar rearranged drimane sesquiterpenoids were firstly reported from the marine sponge *Disidea avara* which yielded the sesquiterpenoid hydroquinone avarol (Minale et al., 1974). Related structures are sesquiterpenoid coumarins such as kamalol that were found in *Ferula* species (Veselovskaya et al., 1979). In contrast to blepharostol all the other rearranged drimanes of this type reported so far represent the terpenoid moiety of compounds of mixed biogenetic origin and have a *trans* ring junction. It is also conceivable that blepharostol is a degraded clerodane. Liverworts are known as sources of both drimanes and clerodanes (Asakawa, 2004).

The ^{1}H and ^{13}C NMR spectra of compound **2** (*m*/*z* 290 $[M]^{+}$, $C_{20}H_{34}O$) showed signals for an olefinic methylene group (δ 5.00, *s*; 4.99, *s*), a vinyl group (δ 6.35, *dd*, $J = 17.5$, 10.5 Hz; 5.22, *d*, $J = 17.5$ Hz; 5.04, *d*, $J = 10.5$ Hz), four singlet methyls (δ 1.15; 0.93; 0.86; 0.81) and a tertiary alcohol (δ 73.2). Due to its downfield shift the methyl at δ 1.15 had to be geminal to the hydroxy group. The molecular formula $C_{20}H_{34}O$ led to

Table 1
NMR spectral data of blepharostol (**1**) in $CDCl_3$

C	δ_C	H	δ_H (multiplicity, *J*)	HMBC correlations (H–C)	^{1}H, ^{1}H COSY correlations
1	19.7	1α	1.54 (*m*)	2, 3, 5, 9, 10	1β
		1β	1.84 (*m*)	2, 3, 5, 9, 10	1α, 2β, 10β
2	25.4	2α	2.00[a](*m*)		13
		2β	1.96[a](*m*)		1β, 3, 13
3	122.7	3	5.32 (*m*)		2α, 2β
4	141.4				
5	37.9				
6	31.8	6α	1.62 (*m*)	4, 5, 7, 8, 10, 14	7β
		6β	1.45 (*m*)	4, 5, 7, 8, 10	
7	27.4	7α	1.47 (*m*)	9, 12	
		7β	1.30 (*m*)	5, 6, 8, 9, 12	6α, 6β, 7α, 8α
8	36.3	8α	1.58 (*m*)	6, 7, 9, 10, 11, 12	7β, 12
9	40.6				
10	42.0	10β	1.55 (*m*)	1, 2, 4, 5, 6, 8, 11, 15, 14	1β
11	69.8	11a	3.81 (*d*, 11.0 Hz)	8, 9, 10, 15	11b, 15
		11b	3.48 (*d*, 11.0 Hz)	8, 9, 10, 15	11a
12	15.4	12	0.90 (*d*, 7.0 Hz)	7, 8, 9	8α
13	19.4	13	1.63 (*d*, 2.0 Hz)	3	2α, 2β, 3
14	28.3	14	1.12 (*s*)	4, 5, 6, 10	
15	23.7	15	1.03 (*s*)	8, 9, 10, 11	

[a] Assignments interchangeable.

four double bond equivalents for the molecule and thus it had to be bicyclic. The two double bonds were positioned in the side chain. The proton and carbon signals were assigned using a combination of ^{1}H ^{1}H COSY, HSQC and HMBC experiments. The spectroscopic data were very close to those of the labdane diterpenoid isoabienol **7** (Toyota et al., 1989). The ^{13}C NMR data of both molecules were in good agreement except for the shifts of C-8 and the carbons close to it, namely C-6, C-7, C-9 and C-17. Me-17 showed the biggest difference (**2**: δ 30.6; **7**: δ 24.1). It was reasonable to assume that the molecules were epimeric at C-8. Since isoabienol has an equatorial tertiary hydroxy group and an axial Me-17 compound **2** should have an axial hydroxy group and an equatorial Me-17. This was in agreement with reported ^{13}C shift differences between axial and equatorial methyl groups of methylcyclohexane. The ^{13}C shift of the axial methyl is 6 ppm to higher field than that of the deshielded equatorial methyl group (Anet et al., 1970) and thus **2** is *ent*-labda-13(16),14-dien-8α-ol.

Compounds **3**–**6**, presented below, belonged to a series of very similar *ent*-labdanes. The characteristic features they all had in common were the four singlet methyl groups, the diene side chain at C-9 and the hydroxy function at C-8 which resulted in a downfield shift of Me-17 in the ^{1}H NMR spectrum (see Section 3). For all the compounds of this series the carbon chemical shift of Me-17 determined its stereochemistry as equatorial. Me-17 of compounds **4**–**6** appeared at about 3 ppm to higher field than in **2** and **3**. This shift was not due to a different configuration at C-8 but caused by the additional hydroxy group at C-9 which had a shielding γ-effect on C-17. The five *ent*-labdanes differed only in the substitution of C-1 and C-9.

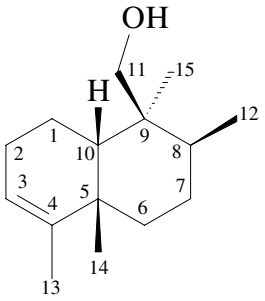
The ^{1}H and ^{13}C NMR spectra of compounds **2** and **3** were quite similar. However, the ^{13}C NMR spectrum revealed that compound **3** was a diol: a tertiary alcohol appeared at δ 73.8 and a secondary alcohol at δ 70.9, which corresponded to the molecular ion peak at *m*/*z* 306 $[M]^+$ ($C_{20}H_{34}O_2$) in the EIMS. The tertiary alcohol was at C-8 since Me-17 appeared as a singlet at δ 1.19. By examination of the HMBC and ^{1}H ^{1}H COSY spectra the position of the secondary alcohol was assigned to C-1. Me-20 and H-3b showed $^3J_{CH}$ correlations to the carbon of the secondary alcohol at δ 70.9. The proton that was attached to the secondary hydroxy function appeared at δ 3.62 as broad singlet. Its couplings with its vicinal protons H-2a and H-2b were very small and about the same size. This indicated that H-1 was equatorial and β-oriented. The correlation of Me-20 with H-1 in the NOESY spectrum provided further proof of structure and led to the assignment of structure **3**, *ent*-labda-13(16),14-diene-1β-,8α-diol, for the compound.

Compound **4** was an isomer of compound **3** and its ^{13}C NMR spectrum had resonances for two tertiary hydroxy groups (δ 76.3, 77.9). The HMBC spectrum showed correlations between H-11a, H-11b, H-12, Me-17 and Me-20 and the carbon at δ 77.9 which readily established its position at C-9. The β-configuration at C-9 was deduced from the NOESY correlations between Me-20 and H-11a and H-11b of the side chain. The most downfield methyl appeared at δ 1.21 and was bound to the remaining oxygen-bearing carbon (δ 76.3) C8. Hence compound **4** was identified as *ent*-labda-13(16),14-diene-8α-,9β-diol.

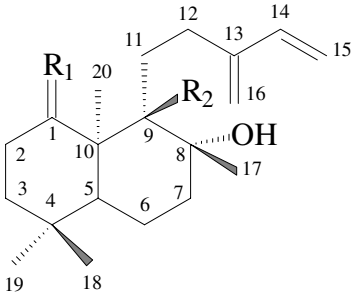
Compound **5** (*m*/*z* 322, $C_{20}H_{34}O_4$) was the major component of the dichloromethane extract. It yielded 470 mg of **5** as colourless crystals. It was a trihydroxylabdadiene derivative. The resonances for the alcohols in the ^{13}C NMR spectrum were at δ 79.5 (C), 76.7 (C) and 74.4 (CH). The two tertiary hydroxy groups at δ 76.7 and 79.5 were assigned to C-8 and C-9, respectively. C-9 had $^3J_{CH}$ correlations from H-12, Me-20, H-7b, Me-17; C-8 had $^3J_{CH}$ correlations from H-6a and $^2J_{CH}$ correlations from H-7b and Me-17. Evidence from HMBC and ^{1}H, ^{1}H COSY spectra determined the position of the secondary hydroxy group at C-1. The downfield proton H-1α (δ 4.04, *brs*) that was attached to the hydroxylated carbon at δ 74.4 showed vicinal couplings to H-2a and H-2b in the COSY. HMBC correlations were observed between the protons H-2b, H-3a, H-5 and Me-20 and C-1 and also between H-1 and C-2, C-3, C-5, C-10 and C-20 and established the structure as *ent*-labda-13(16),14-diene-1β,8α,9β-triol. The NOESY spectrum supported the given relative stereochemistry.

Compound **6**, a colourless oil with $[\alpha]_D^{20}$ +13.6°, had a molecular ion peak at *m*/*z* 320 in the GCMS and hence a molecular formula of $C_{20}H_{32}O_3$. The IR spectrum of **6** revealed prominent absorption bands at ν_{max} 3514 and 1684 cm^{-1}, attributed to hydroxyl and carbonyl functionalities, respectively. The ^{1}H and ^{13}C NMR spectra showed, besides resonances for a 13(16),14-diene side chain, the signals of a ketone (δ 221.5), two tertiary hydroxy groups (δ 76.6; 75.9) and four singlet methyls (δ 1.44; 1.22; 1.06; 0.93). Correlations of H-2a, H-2b, H-3b and Me-20 with the carbonyl function in the HMBC spectrum led to the assignment of the ketone to C-1. Me-20 was deshielded because of its close vicinity to the ketone and was shifted downfield to δ 1.44. Since the HMBC spectrum showed correlations from H-6b, H-7a, H-11a, H-11b and Me-17 to the carbon at δ 75.9 it was assigned as C-8 and was carrying the methyl at δ 1.22. The remaining alcohol (δ 76.6) was at C-9 due to the observed long-range correlations from H-5, H-11a, H-11b, H-12a, H12b, Me-17 and Me-20. The correlation of Me-20 with H-11a in the NOESY spectrum provided evidence for the α-configuration of C-9. Thus the compound was *ent*-8α,9β-dihydroxylabda-13(16),14-dien-1-one **6**. To determine the absolute stereochemistry of **6** a CD spectrum was recorded. The curve showed a positive Cotton effect at 310 nm. According to the octant rule the compound had to be of the *ent*-labdane type. This was

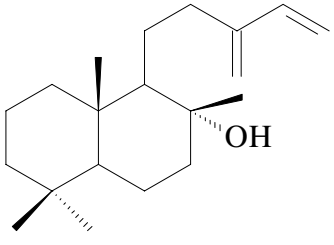
furthermore supported by the negative Cotton effect that was reported for a known 1-oxo-labdane of the normal series (Huneck et al., 1986). Labdanes are widespread in liverworts and they have been found in both enantiomeric series (Asakawa, 1995, 2004).



1



	R_1	R_2
2	H_2	H
3	H, β-OH	H
4	H_2	OH
5	H, β-OH	OH
6	O	OH



7

3. Experimental

3.1. Spectroscopy and spectrometry

Optical rotations were measured in $CHCl_3$. NMR spectra were recorded in $CDCl_3$ (^1H NMR: 500 MHz, ^{13}C NMR: 125 MHz) relative to $CDCl_3$ at δ 7.24, δ_C 77.0. ^{13}C multiplicities were determined using the DEPT pulse sequence. 2D NMR spectra were recorded as ^1H, ^1H COSY, HSQC and HMBC experiments. The IR spectra were recorded on a Bio-Rad FTS-3000 spectrophotometer. For GC-mass spectra a Hewlett Packard 5890 Series II with a HP 5971 Series Mass Selection Detector G1512 A was used. The mass spectra (70 eV) were recorded in the positive EI mode. The UV spectra were recorded on a Shimadzu UV mini-1240 UV–VIS spectrophotometer. The CD spectrum was recorded on a Jasco J-715 instrument.

3.2. Plant material

Cultures of *B. trichophyllum* (L.) Dumort. were obtained from the Collection of Autotrophic Organisms, Department of Hydrobotany, Institute of Botany, Czechoslovakian Academy of Sciences, Trebon, Czech Republic. They were grown in 250 ml Erlenmeyer flasks with 100 ml solid modified B5 medium (pH 6.0) (Gamborg et al., 1968) containing 0.75% sucrose.

3.3. Extraction and isolation

The dried plant material (350 g) was powdered and extracted with CH_2Cl_2. After removal of the solvent the crude extract (9.88 g) was chromatographed by CC on Sephadex LH-20 (150 × 2.5 cm i.d.) with MeOH:CH_2Cl_2 (1:1) as eluent, to give three fractions (0.86, 2.57 and 6.21 g, respectively). The Sephadex fraction 3 was separated using a combination of VLC and HPLC. For VLC silica gel 15 μm was used, for HPLC either a silica gel column (Knauer LiChrospher 100 Si, 5 μm) or a diol silica gel column (Knauer LiChrospher 100 DIOL 5.0 μm) was used. Fraction 3 (6.21 g) was first separated by VLC (silica gel, *n*-hexane–EtOAc gradient) and gave the fractions 3.1 (0–1.5% EtOAc, 484 mg), 3.2 (2% EtOAc, 158 mg), 3.3 (2.5–3.5% EtOAc, 795 mg), 3.4 (4–5% EtOAc, 599 mg), 3.5 (6–8% EtOAc, 982 mg), 3.6 (8–12% EtOAc, 476 mg), 3.7 (12–20% EtOAc, 448 mg), 3.8 (25–35% EtOAc, 641 mg), 3.9 (40–100% EtOAc, 387 mg). Fraction 3.3 was separated by HPLC (silica gel, *n*-hexane–EtOAc 97:3) to yield **2** (15 mg) and **4** (23 mg). HPLC of fraction 3.4 (silica gel, *n*-hexane–TBME 90:10) gave nine fractions (3.41–3.49). Fraction 3.47 yielded **1** (20 mg). HPLC of fraction 3.43 (93 mg; diol silica gel, *n*-hexane–EtOAc 97:3) yielded compounds **3** (8 mg) and **6** (16 mg). Compound **5** (470 mg) crystallized out of fraction 3.5.

3.4. Blepharostol (*1*)

Colourless oil, $[\alpha]_D^{20}$ +27.8 ($CHCl_3$; *c* 0.19); UV λ_{max} nm (logε): 239 (2.9); EIMS: *m/z* (rel. int.) = 222 (13) $[M]^+$, 207 (3), 191 (7), 175 (10), 161 (15), 147 (9), 135

(17), 121 (26), 107 (53), 95 (100), 81 (28), 69 (16), 55 (17); IR ν_{max}^{KBr}: cm^{-1}: 3446, 2929, 1711, 1665, 1454, 1378, 1022, 891; ^1H NMR, ^{13}C NMR ($CDCl_3$): Table 1.

3.5. ent-Labda-13(16),14-dien-8α-ol (2)

Colourless oil, $[\alpha]_D^{20}$ −15.7 ($CHCl_3$; *c* 0.19); UV λ_{max} nm (log ε): 240 (3.1); EIMS: *m/z* (rel. int.) = 290 (1) $[M]^+$, 191 (100), 177 (45), 137 (27), 121 (38), 109 (58), 95 (83), 81 (97), 69 (69), 55 (50); IR ν_{max}^{KBr}: cm^{-1}: 3495, 2924, 2844, 1748, 1678, 1593, 1462, 1387, 1186, 989, 904; ^1H NMR ($CDCl_3$): 6.35 (*dd*, *J* = 17.5, 10.5 Hz, H-14), 5.22 (*d*, *J* = 17.5 Hz, H-15a), 5.04 (*d*, *J* = 10.5 Hz, H-15b), 5.00 (*s*, H-16a), 4.99 (*s*, H-16b), 2.24 (*td*, *J* = 14.5, 5.5 Hz, H-12a), 2.19 (*td*, *J* = 14.5, 5.5 Hz, H-12b), 1.74 (*m*, H-7a), 1.67 (*m*, H-1a), 1.59 (*m*, H-2a), 1.56 (*m*, H-11a), 1.50 (*m*, 2×H-6), 1.48 (*m*, H-7b), 1.46 (*m*, H-11b), 1.39 (*m*, H-2b), 1.38 (*m*, H-3a), 1.15 (*s*, 3×H-17), 1.13 (*m*, H-3b), 0.93 (*s*, 3×H-20), 0.90 (*m*, H-1b), 0.87 (*m*, H-9), 0.86 (*m*, H-5), 0.86 (*s*, 3×H-18), 0.81 (*s*, 3×H-19); ^{13}C NMR: Table 2.

3.6. ent-Labda-13(16),14-diene-1β,8α-diol (3)

Colourless oil, $[\alpha]_D^{20}$ −14.7 ($CHCl_3$; *c* 0.21); UV λ_{max} nm (log ε): 240 (3.5); EIMS: *m/z* (rel. int.) = 306 (1) $[M]^+$, 166 (79), 149 (31), 135 (32), 121 (48), 107 (46), 95 (66), 81 (100), 69 (44), 55 (57); IR ν_{max}^{KBr}: cm^{-1}: 3465, 2946, 2912, 1632, 1593, 1462, 1389, 1042, 905; ^1H NMR: 6.36 (*dd*, *J* = 17.5, 11.0 Hz, H-14), 5.28 (*d*, *J* = 17.5 Hz, H-15a), 5.06 (*d*, *J* = 11.0 Hz, H-15b), 5.03 (*brs*, H-16a), 5.01 (*brs*, H-16b), 3.62 (*brs*, H-1), 2.46 (*m*, H-12a), 2.34 (*m*, H-12b), 2.00 (*tdd*, *J* = 14.5, 4.0, 2.5 Hz, H-2a), 1.70 (*dt*, *J* = 13.0, 3.0 Hz, H-7a), 1.60 (*m*, H-3a), 1.54 (*m*, H-9), 1.53 (*m*, 2×H-11), 1.52 (*m*, 2×H-6), 1.49 (*m*, H-7b), 1.45 (*m*, H-2b), 1.30 (*dd*, *J* = 11.5, 2.5 Hz, H-5), 1.19 (*s*, 3×H-17), 1.14 (*ddd*, *J* = 13.5, 4.0, 3.0 Hz, H-3b), 0.94 (*s*, 3×H-20), 0.89 (*s*, 3×H-18), 0.83 (*s*, 3×H-19); ^{13}C NMR: Table 2.

3.7. ent-Labda-13(16),14-diene-8α,9β-diol (4)

Colourless oil, $[\alpha]_D^{20}$ −2.8 ($CHCl_3$; *c* 0.51); UV λ_{max} nm (log ε): 241 (3.0); EIMS: *m/z* (rel. int.) = 306 (1) $[M]^+$, 197 (18), 177 (63), 137 (36), 123 (58), 109 (70), 95 (68), 81 (81), 69 (100), 55 (60); IR ν_{max}^{KBr}: cm^{-1}: 3478, 2945, 2871, 1636, 1462, 1389, 1053, 990, 914; ^1H NMR: 6.35 (*dd*, *J* = 17.5, 11.0 Hz, H-14), 5.25 (*d*, *J* = 17.5, H-15a), 5.07 (*d*, *J* = 11.0 Hz, H-15b), 5.04 (*s*, H-16a), 5.01 (*s*, H-16b), 2.29 (*m*, H-12a), 2.26 (*m*, H-12b), 1.95 (*ddd*, *J* = 15.0, 10.5, 6.5 Hz, H-11a), 1.86 (*m*, H-7a), 1.79 (*ddd*, *J* = 15.0, 10.5, 6.5 Hz, H-11b), 1.63 (*m*, H-2a), 1.60 (*m*, H-2b), 1.54 (*m*, H-6a), 1.45 (*m*, H-6b), 1.47 (*m*, H-7b), 1.44 (*m*, H-5), 1.38 (*m*, 2×H-1), 1.34 (*m*, H-3a), 1.21 (*s*, 3×H-17), 1.13 (*td*, *J* = 13.5, 4.0 Hz, H-3b), 1.07 (*s*, 3×H-20), 0.88 (*s*, 3×H-18), 0.83 (*s*, 3×H-19); ^{13}C NMR: Table 2.

3.8. ent-Labda-13(16),14-diene-1β,8α,9β-triol (5)

Colourless needles, m.p. 114 °C (*n*-hexane), $[\alpha]_D^{20}$ −58.8 ($CHCl_3$; *c* 0.16); UV λ_{max} nm (log ε): 239 (3.9); EIMS: *m/z* (rel. int.) = 322 (1) $[M]^+$, 195 (20), 177 (100), 136 (45), 121 (64), 109 (43), 95 (58), 81 (81), 69 (45), 55 (40); IR ν_{max}^{KBr}: cm^{-1}: 3480, 3277, 3169, 2951, 1719, 1594, 1464, 1389, 1064, 990, 901; ^1H NMR: 6.41 (*dd*, *J* = 17.5, 11.0 Hz, H-14), 5.35 (*d*, *J* = 17.5 Hz, H-15a), 5.09

Table 2
^{13}C NMR spectral data for compounds **2**–**7** in $CDCl_3$

C	2	3	4	5	6	7
1	39.2	70.9	32.3	74.4	221.5	39.7
2	18.1	25.5	18.1[a]	27.0	36.3	18.4
3	42.0	34.3	41.7	33.9	43.4	41.9
4	33.2	33.1	33.3	33.1	33.4	33.2
5	55.9	48.0	46.7	40.6	50.4	56.1
6	18.3	18.1	18.0[a]	18.2	18.5	20.5
7	42.2	41.9	37.8	37.4	37.4	44.5
8	73.2	73.8	76.3	76.7	75.9	74.1
9	59.0	49.8	77.9	79.5	76.6	62.2
10	38.9	42.5	43.8	45.3	59.5	39.1
11	24.2	23.2	28.1[a]	28.8	32.1	24.5
12	34.8	33.3	28.2[a]	28.1	28.0	35.1
13	146.9	147.5	147.3	147.9	147.6	147.4
14	138.9	138.8	138.7	139.1	138.8	138.8
15	113.0	113.5	113.8	113.3	113.5	113.5
16	115.6	115.7	115.8	115.5	115.4	115.5
17	30.6	31.3	27.1	27.1	26.8	24.0
18	33.4	33.2	33.7	33.5	32.2	33.4
19	21.6	21.6	21.8	21.8	22.0	21.5
20	15.1	15.6	16.4	17.1	15.9	15.4

[a] Assignments interchangeable.

(*d*, $J = 11.0$ Hz, H-15b), 5.07 (*brs*, H-16a), 5.04 (*brs*, H-16b), 4.04 (*brs*, H-1), 2.46 (*m*, H-12a), 2.42 (*m*, H-12b), 2.12 (*tdd*, $J = 15.0$, 4.0, 2.0 Hz, H-2a), 2.04 (*m*, H-5), 1.98 (*m*, H-7a), 1.99 (*ddd*, $J = 15.0$, 12.0, 6.0 Hz, H-11a), 1.87 (*ddd*, $J = 15.0$, 11.5, 6.0 Hz, H-11b), 1.63 (*m*, H-3a), 1.57 (*m*, H-6a), 1.48 (*m*, H-6b), 1.43 (*dt*, $J = 13.5$, 3.0 Hz, H-7b), 1.41 (*dq*, $J = 15.0$, 3.5 Hz, H-2b), 1.23 (*s*, 3×H-17), 1.19 (*dt*, $J = 14.0$, 3.5 Hz, H-3b), 1.08 (*s*, 3×H-20), 0.98 (*s*, 3×H-18), 0.89 (*s*, 3×H-19). ^{13}C NMR: Table 2.

3.9. *ent*-8α,9β-Dihydroxylabda-13(16),14-dien-1-one (**6**)

Colourless oil, $[\alpha]_D^{20}$ +13.6 ($CHCl_3$; *c* 0.21); UV λ_{max} nm (log ε): 240 (3.4); CD (*n*-hexane) λ ($\Delta\varepsilon$) 196 (0), 219 (−3.5), 282 (0), 310 (+1.0) nm; EIMS: *m/z* (rel. int.) = 320 (1) $[M]^+$, 193 (17), 165 (14), 152 (39), 139 (100), 111 (42), 95 (39), 81 (27), 67 (23), 55 (32); IR ν_{max}^{KBr}: cm^{-1}: 3514, 2952, 2876, 1684, 1462, 1368, 1308, 1125, 1090, 988, 919; ^{1}H NMR ($CDCl_3$): 6.34 (*dd*, $J = 17.5$, 11.0 Hz, H-14), 5.34 (*d*, $J = 17.5$ Hz, H-15a), 5.04 (*d*, $J = 11.0$ Hz, H-15b), 5.00 (*s*, H-16a), 4.97 (*s*, H-16b), 2.98 (*ddd*, $J = 14.0$, 13.0, 5.5 Hz, H-2a), 2.56 (*td*, $J = 13.0$, 4.5 Hz, H-12a), 2.30 (*td*, $J = 13.0$, 3.5 Hz, H-12b), 2.09 (*dd*, $J = 12.0$, 2.5 Hz, H-5), 2.03 (*m*, H-2b), 1.99 (*m*, H-11a), 1.88 (*td*, $J = 13.5$, 4.0 Hz, H-7a), 1.78 (*m*, H-11b), 1.77 (*m*, H-6a), 1.76 (*m*, H-3a), 1.62 (*td*, $J = 14.0$, 4.5 Hz, H-3b), 1.44 (*s*, 3×H-20), 1.43 (*m*, H-6b), 1.39 (*m*, H-7b), 1.22 (*s*, 3×H-17), 1.06 (*s*, 3×H-19), 0.93 (*s*, 3×H-18); ^{13}C NMR: Table 2.

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