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DITERPENES FROM *IN VITRO* CULTURES OF THE LIVERWORT *JAMESONIELLA AUTUMNALIS**

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Key Word Index—*Jamesoniella autumnalis*; Hepaticae; furanoditerpenoids; jamesoniellides; *ent*-labdanes.

Abstract—Nine new furanoditerpenoids, jamesoniellide D–J, and two new labdane-type diterpenoids together with 19-acetoxy-ent-labda-8 (17),12*E*,14-trien-3α-ol have been isolated from *in vitro* cultures of the liverwort *Jamesoniella autumnalis*. The structures were established by spectroscopic methods.

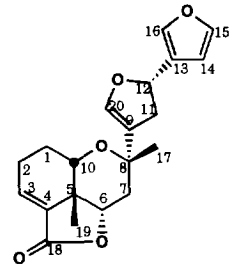
INTRODUCTION

Liverworts are known to be a rich source of sesquiterpenoids and diterpenoids [1–3]. In the course of our investigations, we have previously reported on the isolation and structure determination of six *ent*-labdanes, one *cis*-clerodane, jamesoniellides A, B and C from the liverwort *Jamesoniella autumnalis* (DC) Steph. (Jungermanniaceae) [4, 5]. Although, this species is distributed over the northern hemisphere in Europe, Asia, and America [6], the diterpenoids isolated in our studies were not the same as those isolated from *J. autumnalis* collected in Japan [7]. This fact led us to undertake a further investigation involving the use of larger amounts of an *in vitro* culture of *J. autumnalis*. In this paper, we discuss the structure of nine new diterpenoids from *J. autumnalis*.

RESULTS AND DISCUSSION

A combination of column chromatography on silica gel and Sephadex LH-20 of an ether extract of *J. autumnalis* resulted in the isolation of 12 new diterpenoids (**1**–**12**) together with the five known compounds (**13**–**17**) we reported previously [4, 5].

The IR spectrum of jamesoniellide D (**1**), $C_{20}H_{22}O_5$ (*m/z* 342 $[M]^+$), established the presence of a lactone conjugated to a double bond (1765 and 1685 cm^{-1}). The ^{13}C NMR spectrum contained the signals of two methyls, four methylenes, eight methines and six quaternary carbons indicating four double bonds (δ_C 143.5, 139.7,



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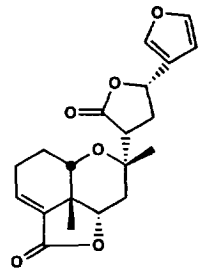

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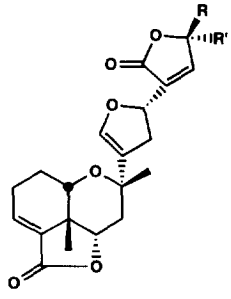
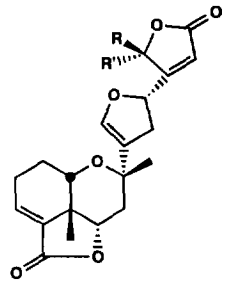
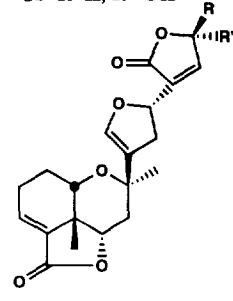


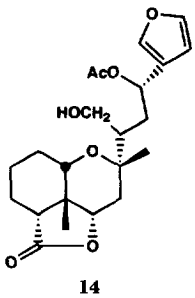
I {**3a** R=OH, R'=H; **3b** R=H, R'=OH



II {**4a** R=OH, R'=H; **4b** R=H, R'=OH



III {**5a** R=OH, R'=H; **5b** R=H, R'=OH



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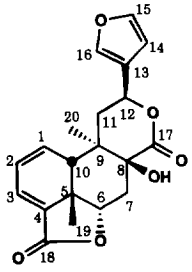

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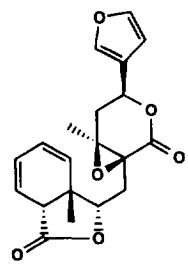

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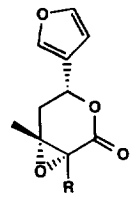

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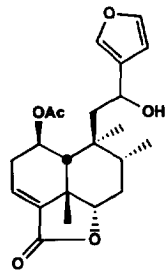

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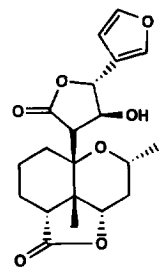

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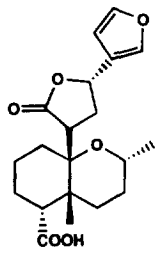

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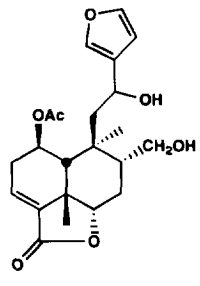

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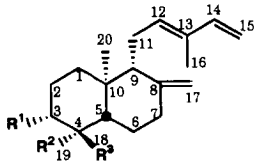
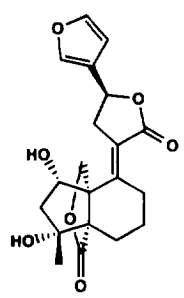


10 : R^1=OAc, R^2=CH_2OH, R^3=CH_3
11 : R^1=OAc, R^2=R^3=CH_3
12 : R^1=OH, R^2=CH_3, R^3=CH_2OH
16 : R^1=OH, R^2=CH_2OAc, R^3=CH_3



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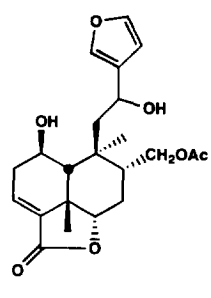

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139.3, 136.7, 130.3, 126.6, 120.3 and 108.4), one conjugated carbonyl group (δ_C 169.7) and four oxygenated aliphatic carbons (δ_C 80.3, 75.9, 71.2 and 68.3). The ^{1}H NMR spectrum (Table 1) contained the signals for two tertiary methyl groups (δ_H 1.31 and 1.20 both *s*) and a β-substituted furan moiety (δ_H 7.34, 7.26 and 6.36, each *brs*) together with five other low-field shifted methine protons, which belonged to double bonds or oxygen-bearing carbons. A ^{1}H COSY experiment revealed three sequences C(10)H-C(1)H_2-C(2)H_2-C(3)H, C(6)H-C(7)H_2 and C(20)H-C(11)H_2-C(12)H, which fitted the secoclerodane skeleton of jamesoniellide B (**14**) we reported previously [4]. The combination of the above partial structures and the remaining quaternary carbons was achieved by ^{1}H–^{13}C COSY and COLOC experiments. Extensive NOE difference measurements allowed the combination of all sequences and led to the relative configurations (Fig. 1). H-17 gave effects with H-6, H-7β, H-10 and H-20. Irradiation of H-19 enhanced the signals of H-10 and H-6, saturation of H-12 revealed a NOE upon H-11β. Thus, the structure of jamesoniellide D was established as **1** with H-6, H-10, H-12, Me-17 and Me-19 in the β-position.

The spectral data of jamesoniellide E (**2**), $C_{20}H_{22}O_6$ (*m/z* 358 $[M]^+$) was close to those of **1**. As followed from the ^{13}C NMR spectrum of **2**, this compound differed from **1** by the presence of an additional carbonyl function (δ_C 174.8) and the absence of one double bond. The resonance of the olefinic proton H-20 found in the ^{1}H NMR spectrum of **2** was replaced by a new signal at δ_H 2.62 in **2** which showed vicinal couplings with H-11α and H-11β (^{1}H COSY). These data established that C-20 was carboxylated and part of a γ-lactone ring. Thus jamesoniellide E (**2**) was formulated as a derivative of **1**.

Jamesoniella autumnalis also contained three mixtures (**I**–**III**) of epimers that could not be separated further even by HPLC. The EI-mass spectrum of mixture **I** did not show a molecular ion but a weak $[M - 15]^+$ peak (*m/z* 359). The ^{1}H and ^{13}C NMR spectra indicated the presence of analogues of **1** in which the furan ring was replaced by a 16,15-γ-butenolide moiety which was substituted at C-15 with either an α- or β-oriented hydroxyl group. Therefore, the structures of the epimers were most probably as depicted in **3a** and **b**. The ^{1}H COSY spectrum supported the structures, further NOE experiments (Fig. 2) indicated the same stereochemistry for both at the other stereocentres as found in **1**.

The ^{1}H and ^{13}C NMR spectra of **4a** and **b** (mixture **II**) were very similar to those of **3a** and **b**, except that the H-14 resonance was moved to higher field (δ_H 6.02). This suggested the presence of a 16-hydroxy-13-ene-15,16-olide moiety in **4a** and **b** instead of the 15-hydroxy-13-ene-16,15-olide moiety as found in **3a** and **b**. Compounds **5a** and **b** (mixture **III**) were found to be stereoisomers of **3a** and **b**. The ^{1}H and ^{13}C NMR spectra of both mixtures showed no major differences, but NOE difference measurements (Fig. 3) required an α-orientation of the Me-17 of **5a** and **b** (**3a** and **b**: β-orientation of Me-17).

The molecular formula of **6** ($C_{20}H_{20}O_6$, *m/z* 356 $[M]^+$) required 11 double bond equivalents. Its UV spectrum

Table 1. H NMR spectral data of compounds **1**–**5** (400 MHz)

H	**1**	**2**	**3**	**4**	**5**
1α	1.84 *m*	1.80 *m*[a]	1.88 *m*[c]	1.85 *m*[e]	1.85 *m*
1β	1.79 *m*	1.80 *m*[a]	1.88 *m*[c]	1.85 *m*[e]	1.79 *m*
2α	2.28 *m*	2.23 *m*[b]	2.31 *m*[d]	2.29 *m*[f]	2.35 *m*
2β	2.22 *m*	2.23 *m*[b]	2.31 *m*[d]	2.29 *m*[f]	2.28 *m*
3	6.85 *dd* (3.4, 3.5)	6.85 *dd* (3.2, 3.3)	6.89 *dd* (3.4, 3.5)	6.90 *t* (3.4)	6.88 *dd* (2.9, 3.2)
6	4.50 *dd* (6.7, 10.1)	4.52 *dd* (6.9, 10.0)	4.49 *dd* (5.9, 7.9)	4.52 *dd* (6.6, 10.0)	4.48 *dd* (7.0, 10.2)
7α	1.35 *dd* (10.1, 13.2)	1.80 *m*[a]	1.41 *dd* (7.7, 14.2) 1.62 *dd* (9.6, 13.5)	1.39 *m*	2.36 *dd* (7.0, 14.1)
7β	2.05 *dd* (6.7, 13.2)	1.96 *m*	2.01 *m* (6.0, 6.6, 13.5, 14.2)	2.06 *dd* (6.4, 13.4)	1.31 *dd* (10.2, 14.1)
9	–	2.62 *m*[b]	—	—	—
10	3.93 *dd* (2.9, 3.0)	3.92 *br s*	3.94 *dd* (2.8, 2.9)	3.96 *dd* (2.9, 3.2)	3.54 *dd* (2.8, 3.0)
11α	2.53 *ddd* (2.1, 8.4,14.4)	2.23 *m*	2.51 *m*	2.66 *dd* (2.0, 7.9)	3.05 *ddd* (1.8, 11.2, 15.4)
11β	2.84 *ddd* (2.1, 10.5, 14.4)	2.58 *m*	2.98 *m*	2.19 *m*	2.48 *ddd* (2.0, 7.2, 15.4)
12	5.41 *dd* (8.4, 10.5)	5.39 *dd* (6.5, 6.7)	5.25 *m*	5.29 *dd* (7.9, 11.0), (7.9, 11.0)	5.40 *dd* 5.34 *m*
14	6.36 *br s*	6.33 *s*	7.03 *m*	6.02 *s*	7.05 *d* (8.2)
15	7.34 *br s*	7.38 *br s*	6.10 *m*	—	6.17 *dd* (7.0, 8.2)
OH-15	—	—	3.85 *m*, 4.62 *m*	—	3.80 *d* (7.0)
16	7.36 *br s*	7.38 *br s*	–	6.10 *d* (6.5), 6.15 *d* (6.5)	—
OH-16	—	—	—	3.98 *d* (6.5), 4.19 *d* (6.5)	—
17	1.31 *s*	1.35 *s*	1.29 *s*, 1.30 *s*	1.33 *s*, 1.34 *s*	1.24 *s*
19	1.20 *s*	1.24 *s*	1.18 *s*, 1.30 *s*	1.22 *s*	1.12 *s*
20	6.17 *s*	—	6.15 *m*	6.19 *dd* (1.9, 2.0), 6.23 *dd* (1.9, 2.0)	6.28 *dd* (1.8, 2.0)

[a–f] Signals partly overlapping.

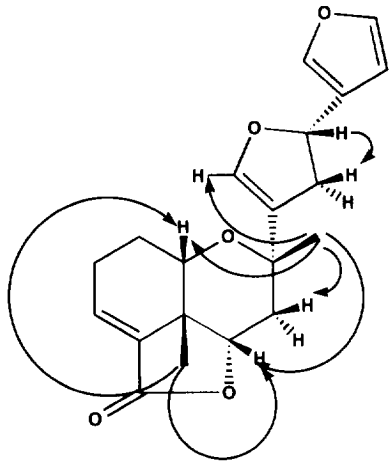


Fig. 1. NOE interactions of jamesoniellide D (1).

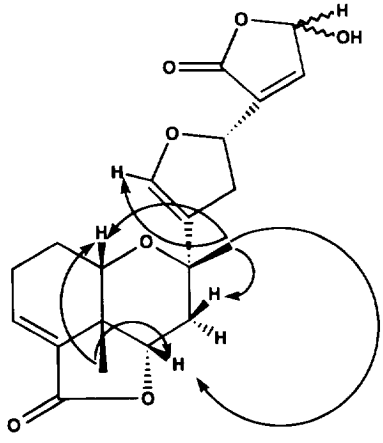


Fig. 2. NOE interactions of 3.

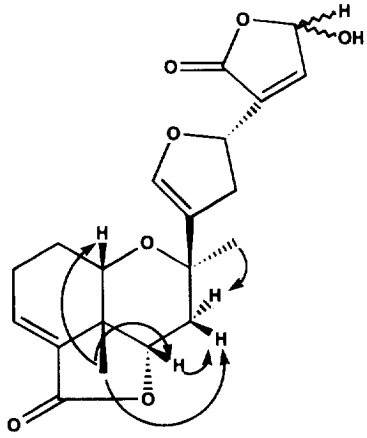


Fig. 3. NOE interactions of 5.

showed an absorption maximum at 305 nm suggesting a diene conjugated system to a γ-lactone ring. The IR spectrum contained peaks for a hydroxyl group (3418 cm^{-1}) and a carbonyl group at (1733 and 1668 cm^{-1}). The ^{13}C NMR spectrum contained the signals of two methyls, two methylenes, nine methines and seven quaternary carbons, indicating the presence of four double bonds (δ_C 143.8, 140.0, 132.6, 129.7, 128.5, 125.8, 124.5 and 108.6) and two carbonyl groups (δ_C 171.0, 170.3) in the molecule. Together with the molecular formula of **6**, these observations suggested **6** was a pentacyclic diterpenoid. The ^1H NMR spectrum of **6** showed the presence of a β-substituted furan ring (δ_H 7.45, 7.39 and 6.44, 1H each). A hydrogen bond led to a sharp singlet for the hydroxyl group (δ_H 3.39) which disappeared on exchange with D_2O. A ^1H COSY experiment established the three sequences C(10)H-C(1)H-C(2)H-C(3)H, C(6)H-C(7)H_2-C(8)OH and C(11)H_2-C(12)H. These sequences fitted well to the skeleton of **15** and indicated that **6** had a conjugated diene system in ring A. The stereochemistry followed from the observed NOEs, which also allowed the combination of each sequence (Fig. 4). H-6β gave an effect with OH-8; H-11β gave effects with H-10, H-14, H-16, OH-8; H-12 gave an effect with H-11α; H-20 gave effects with H-7α, H-11α and H-12, and H-19 gave effects with H-6β and H-10. The observed NOEs were in agreement with H-6, H-10, Me-19 and OH-8 each occupying the β-position. Thus the structure **6** is 15,16-epoxy-8-hydroxy-1,3,13 (16), 14-clerodatetraene-17,12 : 18,6-diolide.

The IR spectrum of jamesoniellide I (7) $C_{20}H_{20}O_6$ (*m/z* 356 $[M]^+$), showed the presence of carbonyl groups at (1770 and 1735 cm^{-1}). The ^{13}C NMR spectrum showed the signals of two methyls, two methylenes, 10 methines and six quaternary carbons, indicating four double bonds (δ_H 143.6, 139.9, 137.0, 134.0, 132.3, 124.9, 123.9 and 108.7) and two carbonyl groups (δ_C 175.2 and 170.4) in the molecule. These observations and the 11 double bond equivalents suggested **7** was a pentacyclic diterpenoid. A ^1H COSY experiment revealed the sequences C(10)H-C(1)H-C(2)H-C(3)H-C(4)H, C(6)H-C(7)H_2 and C(11)H_2-C(12)H (Table 2). The combination of these was achieved by ^1H–^{13}C COSY and COLOC experiments. A set of two singlets at δ_C 90.6 and 89.6 was assigned to a 8,9-epoxide. Furthermore, the stereochemistry followed from the observed NOEs (Fig. 5). H-6 gave effects with both H-7α and

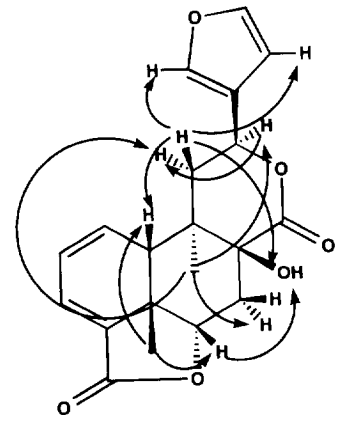


Fig. 4. NOE interactions of 6.

Table 2. ^{1}H NMR spectral data of compounds **6–9** (400 MHz)

H	**6**	**7**	**8**	**9**
1α				1.72 *dd* (4.7, 12.0)
	5.94 *dd* (5.7, 9.6)	6.36 *ddd* (2.0, 4.8, 13.0)	5.43 *d* (7.2)	
1β				1.72 *dd* (4.7, 12.0)
2α			2.79 *ddd* (4.1, 7.2, 21.8)	1.65 *m*
	6.39 *dd* (5.2, 9.6)	5.94 *ddd* (1.5, 4.8, 10.8)		
2β			2.50 *dd* (3.3, 21.8)	1.54 *dd* (1.7, 12.5)
3α				1.38 *ddd* (1.7, 11.9, 12.4)
	6.90 *d* (5.2)	5.68 *ddd* (2,0, 8.6, 10.8)	6.72 *dd* (3.3, 4.1)	
3β				1.88 *dd* (3.0, 12.4)
4	—	3.41 *d* (8.9)	—	3.00 *dd* (3.0, 11.9)
6	4.74 *dd* (7.7, 10.1)	4.92 *dd* (1.1, 8.6)	4.31 *dd* (6.8, 11.0)	4.55 *dd* (8.4, 8.5)
7α	1.55 *dd* (10.1, 14.7)	2.13 *dd* (1.1, 15.4)	1.20 *m*	1.65 *m*
7β	2.97 *dd* (7.7, 14.7)	3.53 *dd* (8.6, 15.4)	2.50 *m*	2.15 *ddd* (3.9, 7.9, 14.2)
8	—	—	1.65 *m*	4.08 *m* (6.0)
9	—	—	—	3.28 *d* (7.5)
10	2.74 *d* (5.7)	5.42 *dd*q (1.2, 13.6)	3.02 *br s*	—
11α	1.84 *dd* (12.6, 13.3)	3.13 *dd* (13.6, 15.4)	2.31 *dd* (10.4, 15.7)	
				4.60 *dd* (6.2, 12.9)
11β	2.56 *dd* (12.6, 13.3)	3.13 *dd* (13.6, 15.4)	2.31 *dd* (10.4, 15.7)	
12	5.35 *dd* (4.5, 12.6)	5.38 *dd* (13.6, 15.4)	4.72 *dd* (1.9, 10.4)	5.05 *d* (6.2)
14	6.44 *dd* (0.7, 0.8), 6.41*br s*	6.41 *br s*	6.42 *br s*	6.41 *d* (1.0)
15	7.39 *s*	7.37 *dd* (1.6, 1.7)	7.34 *br s*	7.46 *dd* (0.7, 1.0)
16	7.45 *s*	7.42 *br s*	7.34 *br s*	7.51 *d* (0.7)
17	—	—	0.81 *d* (6.8)	1.19 *d* (6.0)
19	1.22 *s*	1.45 *s*	1.32 *s*	1.26 *s*
20	1.03 *s*	1.17 *s*	0.61 *s*	—
others	OH-8 3.39 *s*		2.02 *s* (OAc)	OH-11 2.72 *d*

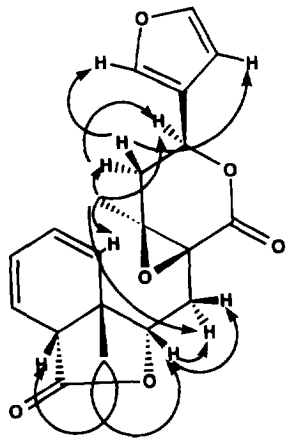


Fig. 5. NOE interactions of jamesoniellide F (7).

H-7β; H-11α gave an effect with H-12; H-11β gave effects with H-14 and H-16; H-19 gave effects with H-6 and H-4, and H-20 gave effects with H-7α, H-10, H-11α and H-12. The coupling constant between H-11β and H-12 was 14.2 Hz, showing that they had a *trans*-axial relationship. This was confirmed by W-type long-range coupling between H-11β and H-20. Thus, inspection of models in the light of the above results showed that the proposed stereochemistry of jamesoniellide F was represented by **7** or **7'**. Though the configuration of the epoxide at C-8-C-9 could not be determined with certainty by NMR, *seco*-clerodane **7** was the most likely, as a biogenetic product of *cis*-clerodane **6**.

Compound **8**, $C_{22}H_{28}O_6$ (*m/z* 388 $[M]^+$), required nine double bond equivalents. Its IR showed an absorption maximum at 3500 and 1760 cm^{-1}. The ^{13}C NMR spectrum of **8** indicated the presence of four methyls, three methylenes, nine methines and six quaternary carbons. The ^1H NMR spectrum of **8** was quite similar to that of **15** [4], except for the absence of one hydroxylmethyl group, which was replaced by a doublet methyl signal at δ_H 0.81. The complete structure of **8** was achieved by consideration of the ^1H (Table 2) and ^{13}C NMR assignments and ^1H–^1H COSY, ^1H–^{13}C COSY, COLOC and NOESY experiments. The observed low field shift of the H-1 resonance in the spectra of **8** and **15** clearly indicated that the acetyl group was positioned at C-1. Therefore, the structure of **8** was that of 1β-acetoxy-12-hydroxy-15,16-epoxy-*cis*-cleroda-3,13(16),14-triene-18,16-olide, and it follows that the published structure of **18** [4] must be revised to **15**.

The IR spectrum of jamesoniellide G (**9**), $C_{20}H_{22}O_6$ (*m/z* 358 $[M]^+$) showed the presence of a hydroxyl group (3442 cm^{-1}) and a carbonyl group (1765 and 1685 cm^{-1}). The ^{13}C NMR spectrum showed the signals of two methyls, four methylenes, nine methines and five quaternary carbons indicating the presence of two double bonds (δ_C 144.4, 140.4, 122.1 and 108.0) and two carbonyl groups (δ_C 175.9 and 173.0) in the molecule. Together with the molecular formula of **9**, these observations suggested **9** was a pentacyclic diterpenoid. The ^1H NMR spectrum contained the signals of one methyl singlet (δ_H 1.26), one methyl doublet (δ_H 1.19) and a furan ring (δ_H 7.51, 7.46 and 6.41). A ^1H COSY experiment revealed the sequences $C(1)H_2$-C(2)(3)H_2-C(4)H, C(6)H-C(7)H_2-C (8) H and C (9)H-C(11)H-C(12) H (Table 2). These sequences were combined by means of the ^1H and ^{13}C assignments and ^1H–^{13}C COSY, COLOC and NOESY experiments. Furthermore, the streochemistry followed from the observed NOEs (Fig. 6). The structure of jamesoniellide G was thus established as **9**.

The absolute configurations of above diterpenoids (**1**–**9**) remain to be clarified.

The ^1H and ^{13}C NMR spectra of **10**–**12** were close to those of the labdanes previously reported [4]. Comparison of the data of **10**–**12** with those of 19-acetoxy-ent-labda-8(17),12*E*,14-trien-3α-ol (**16**) revealed their structures. The observed differences in the chemical shifts in the spectra of **10**, $C_{22}H_{34}O_3$ (*m/z* 346 $[M]^+$), and **16** indicated that the acetyl group in **10** was positioned at C-3. The similar splitting of the H-3 signal of **10** compared to that of **16** suggested β-orientation of H-3.

The spectral data of **11**, $C_{22}H_{34}O_2$ (*m/z* 330 $[M]^+$) were identical with those of 3α-acetoxy-labda-8(17),12*E*,14-triene isolated from *Palatoxia rosea* [18], except for the optical rotation.

As already followed from the molecular formula of **12**, this compound differed from **16** by the absence of the acetyl group. The ^1H NMR spectrum of **12** agreed well with that of 3α-hydroxy-*ent*-labda-8(17), 12*E*,14-trien-19-ol previously isolated from *Mikania alvimii* [9]. However, the chemical shifts of H-18 and H-19 of **12** differed from those given in the literature [9]. Therefore, it is suggested that **12** is in fact 3α-hydroxy-ent-labda-8 (17),12,14-trien-18-ol. The observed NOE between H-19 and H-2*a* supported this structure.

Biogenetic considerations

The diterpenoids in the field collected samples of *J. autumnalis* are almost identical to those found in the *in*

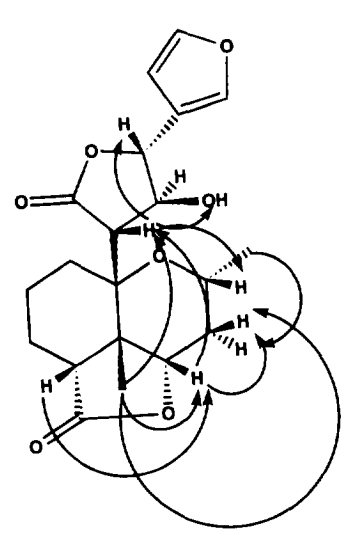
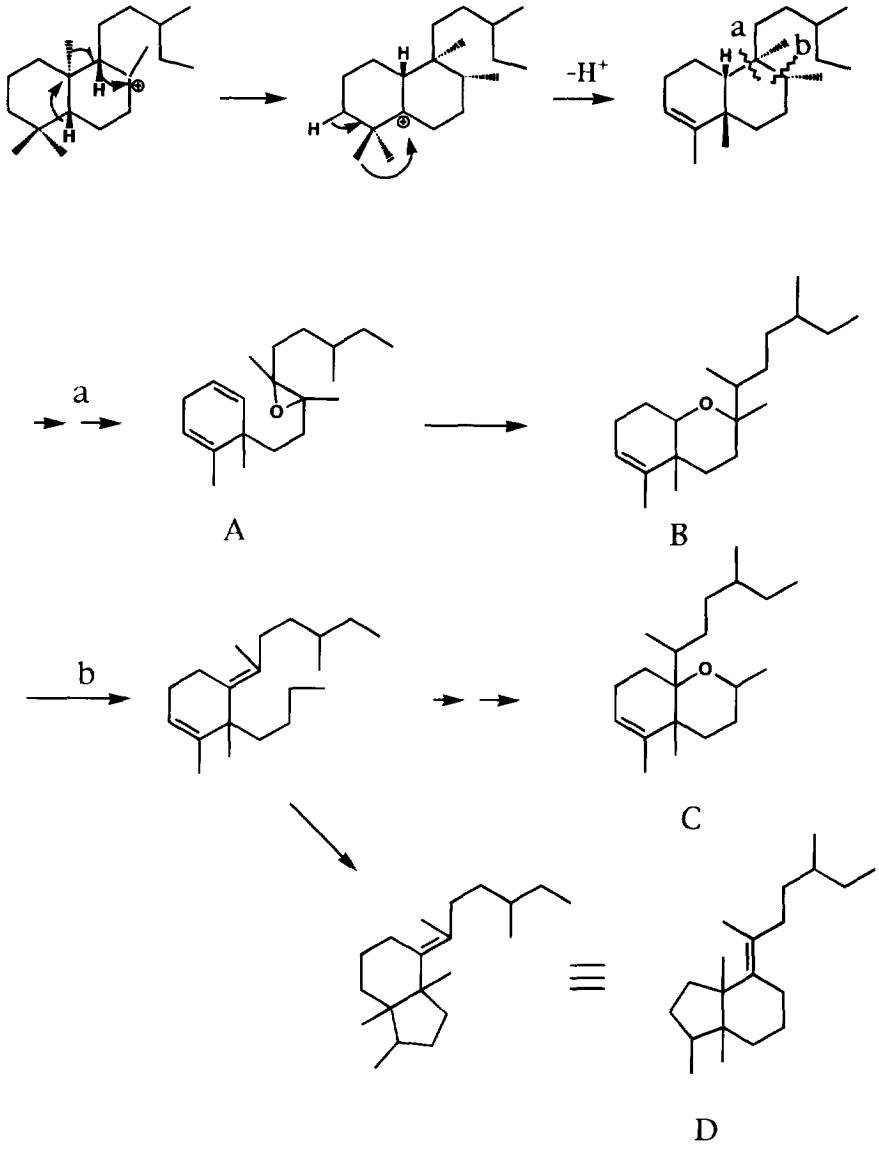


Fig. 6. NOE interactions of jamesoniellide G (**9**).

vitro cultures. Several labdane and clerodane types of diterpenoids have been isolated from various liverwort species [10–20]. The cultures of *J. autumnalis* produced a variety of diterpenoids which could be divided into five classes represented by labdane, clerodane and the three secoclerodanes jamesoniellide A (**13**), B (**14**), C (**17**). On the basis of the compounds isolated from *J. autumnalis*, plausible biogenetic pathways leading to these five different types of diterpenoids can be proposed (Scheme 1). Skeleton **B**, represented by **1**–**5** and **14**, can be envisaged as being formed from the corresponding epoxide type diterpenoid, jamesoniellide F (**7**), which is first transformed by a ring opening to the epoxide followed by formation of an ether linkage at C-8/C-10. The inversion of configuration at C-8 of **5** could be explained by this mechanism. As in the case of skeleton **B**, skeleton **C**, represented by **9** and **13**, could result from a cleavage at C-8/C-9 followed by a formation of the ether linkage at C-8/C-10. The isolation of jamesoniellide C (**17**) is very interesting, because its unusual skeleton has no relationship to the isoprene unit. The structure of jamesoniellide A (**13**) helps to explain the biogenesis of jamesoniellide C (**17**). The pathway to skeleton **D**, represented by jamesoniellide C (**17**), could be proceeded by cleavage of the *cis*-clerodane type diterpenoid at C-8/C-9 followed by a recyclization at C-4/C-8.

EXPERIMENTAL

Optical rotations: $CHCl_3$; UV; EtOH; NMR: ^1H 400 MHz, ^{13}C 100.5 MHz, $CDCl_3$ soln, relative to $CHCl_3$ (δ_H 7.25) and $CDCl_3$ (δ_C 77.0). ^{13}C multiplicities were determined using the DEPT pulse sequence.



Scheme 1. Possible routes for the biogenesis of jamesoniellides.

Jamesoniella autumnalis was collected in December 1988 near Orscholz Saar (Germany) and identified by Professor Mues (Universität des Sarrlandes, Germany). Voucher specimens were deposited at the institute of Pharmakognosie und Analytische Phytochemie der Universität des Saarlandes, Saarbrücken. An axenic culture of *J. autumnalis* was established from the surface-sterilized gametophytes of field material. The cultures were grown in 200 ml flasks with 70 ml solid modified B5 (pH 6.0) medium [21], containing 20 g l^{-1} sucrose. The flasks were kept under constant illumination (2000 lux) at 22°.

Extraction and isolation. Powdered, dry plant material (815 g) was extracted successively with Et_2O and MeOH at room temp.

The Et_2O extracts (37.03 g) were subjected to VLC on silica gel eluted with *n*-hexane containing various concns of EtOAc to give nine fractions: A (*n*-hexane) (4.12 g), B (*n*-hexane–EtOAc, 19:1) (0.11 g), C (*n*-hexane–EtOAc, 9:1) (0.36 g), D (*n*-hexane–EtOAc, 4:1) (2.77 g), E (*n*-hexane–EtOAc, 7:3) (0.66 g), F (*n*-hexane–EtOAc, 1:1) (4.33 g), G (*n*-hexane–EtOAC, 2:3) (2.13 g), H (EtOAc) (1.27 g), I (MeOH) (5.12 g). Sepn of fr. C by HPLC (*n*-hexane–EtOAc, 1:1) yielded **11** (63.4 mg), and sepn of fr. F by HPLC (n-hexane–EtOAc, 9:11) gave **10** (45.8 mg), **13** (34.0 mg) and **16** (278.1 mg). HPLC (DIOL then silica gel Si 60) (*n*-hexane–EtOAc, 7:13) of fr. G resulted in the isolation of **1** (180.0 mg), **6** (301.4 mg), **7** (97.8 mg), **8** (94.3 mg) and **12** (135.3 mg). Sepn of fr. H in the same manner as fr. G yielded **2** (60.7 mg), **9** (101.7 mg), **14** (30.5 mg), **15** (1.8 mg) and **17** (20.3 mg). A combination of VLC on silica gel ($CHCl_3$–MeOH) and HPLC (Diol then silica gel Si 60) (*n*-hexane–EtOAc, 7:13) of fr. I resulted in the isolation of **3** (12.5 mg), **4** (28.0 mg), **5** (5.9 mg), **14** (88.6 mg) and **15** (1491 mg).

Jamesoniellide D (**1**). $[\alpha]_D^{20}$ − 47.88° (*c* 1.36); HR-MS: found $[M]^+$ 342.1471; $C_{20}H_{22}O_5$ required 342.1467. UV λ_{max} nm (log ε): 212 (3.92); IR ν_{max}^{KBr} cm^{-1}: 1765, 1685, 1660, 1510, 1170, 1075, 875, 760; ^1H NMR: Table 1; ^{13}C NMR: δ 169.7 (C-18), 143.5 (C-15), 139.7 (C-20), 139.3 (C-16), 136.7 (C-3), 130.3 (C-4), 126.6 (C-13), 120.3 (C-9), 108.4 (C-14), 80.3 (C-6), 75.9 (C-12), 71.2 (C-8), 68.3 (C-10), 38.5 (C-5), 38.3 (C-7), 35.4 (C11), 24.3 (C-19), 23.2 (C-1), 21.5 (C-2), 20.1 (C-17); EI-MS *m/z* (rel. int.): 342 $[M]^+$ (13), 327 (84), 309 (18), 298 (26), 267 (18), 207 (32), 187 (45), 176 (24), 161 (31), 149 (56), 95 (45), 77 (34), 43 (60).

Jamesoniellide E (**2**). $[\alpha]_D^{20}$ − 77.60° (*c* 1.17); HR-MS: found $[M]^+$ 358.1420; $C_{20}H_{22}O_6$ requires 358.1416. UV λ_{max} nm (log ε): 213 (3.83); IR ν_{max}^{KBr} cm^{-1}; 1765, 1690, 1450, 875, 670; ^1H NMR: Table 1; ^{13}C NMR: δ 174.8 (C-20), 169.3 (C-18), 144.0 (C-15), 139.5 (C-16), 136.5 (C-3), 130.0 (C-4), 124.9 (C-13), 108.2 (C-14), 80.1 (C-6), 73.8 (C-8), 72.8 (C-12), 68.5 (C-10), 49.7 (C-9), 38.5 (C-5), 34.4 (C-7), 30.8 (C-11), 24.2 (C-19), 23.3 (C-1), 21.5 (C-2), 20.2 (C-17); EI-MS *m/z* (rel. int.): 358 ($[M]^+$ 2), 340 (3), 325 (0.2), 299 (0.5), 270 (1), 209 (10), 191 (17), 149 (13), 95 (37), 55 (41), 43 (100).

Mixture **I** (**3a**, **b**). $[\alpha]_D^{20}$ + 5.37° (*c* 0.24); HR-MS: found $[M - 15]^+$ 359.1147; $C_{19}H_{19}O_7$ requires 359.1131. UV λ_{max} nm (log ε): 214 (3.86); IR ν_{max}^{KBr} cm^{-1}: 3412, 1762, 1683, 1656, 1637, 1457, 760; ^1H NMR: Table 1; EI-MS *m/z* (rel. int.): 359 $[M - 15]^+$ (15), 356 (11), 338 (12), 329 (5), 310 (3), 219 (14), 207 (7), 195 (21), 192 (9), 165 (66), 149 (47), 147 (30), 137 (14), 123 (15), 121 (21), 119 (29), 105 (32), 95 (21), 93 (32), 91 (45), 79 (31), 77 (49), 67 (23), 65 (22), 55 (24), 53 (25), 43 (100).

Mixture **II** (**4a**, **b**). $[\alpha]_D^{20}$ + 64.86° (*c* 0.56); HR-MS: found $[M - 15]^+$ 359.1125; $C_{10}H_{19}O_7$ requires 359.1131. UV λ_{max} nm (log ε): 214 (4.07); IR ν_{max}^{KBr} cm^{-1}: 3376, 1761, 1682, 1654, 1456, 893, 761; ^1H NMR: Table 1; ^{13}C NMR: δ 169.9 (*s*), 166.5 (*s*), 140.1 (*d*), 139.6 (*d*), 137.3 (*d*), 130.3 (*s*), 121.0 (*s*), 120.6 (*s*), 118.3 (*d*), 118.1 (*d*), 97.5 (*d*), 80.4 (*d* × 2), 76.6 (*d*), 76.5 (*d*), 71.2 (*s*), 68.6 (*d*), 38.7 (*s*), 38.4 (*t*), 38.2 (*t*), 33.7 (*t*), 33.6 (*t*), 24.4 (*q* × 2), 23.2 (*t*), 21.6 (*t*), 20.6 (*q*), 20.4 (*q*); EI-MS *m/z* (rel. int.): 359 $[M - 15]^+$ (15), 356 (4), 219 (4), 208 (7), 195 (10), 173 (4), 165 (15), 161 (14), 149 (10), 137 (12), 133 (11), 123 (12), 121 (29), 119 (24), 105 (56), 95 (32), 93 (45), 91 (100), 81 (32), 79 (64), 77 (97), 67 (45), 65 (47), 55 (37), 53 (54), 43 (95).

Mixture **III** (**5a**, **b**). $[\alpha]_D^{20}$ − 6.69° (*c* 0.15); HR-MS: found $[M - 15]^+$ 359.1143; $C_{19}H_{19}O_7$ requires 359.1131. UV λ_{max} nm (log ε): 214 (4.05); IR ν_{max}^{KBr} cm^{-1}: 3423, 1761, 1683, 1653, 1456, 762; ^1H NMR: Table 1; ^{13}C NMR: δ 180.2 (*s*), 170.3 (*s*), 168.6 (*s*), 168.5 (*s*), 143.9 (*d*), 143.6 (*d*), 142.0 (*d*), 141.9 (*d*), 138.5 (*s*), 136.8 (*d* × 2), 130.5 (*s*), 130.4 (*s*), 116.4 (*s*), 116.3 (*s*), 96.7 (*d*), 96.4 (*d*), 80.2 (*d*), 80.1 (*d*), 77.0 (*d*), 75.6 (*d*), 75.5 (*d*), 71.1 (*s*), 71.0 (*s*), 69.3 (*d*), 69.2 (*d*), 38.9 (*t*), 38.3 (*s*), 34.5 (*t*), 34.3 (*t*), 29.1 (*q*), 28.9 (*q*), 24.6 (*q* × 2), 23.2 (*t*), 23.2 (*t*), 21.7 (*t* × 2); EI-MS *m/z* (rel. int.): 359 $[M - 15]^+$ (7), 356 (2), 219 (4), 208 (4), 208 (6), 195 (3), 190 (3), 165 (1), 149 (11), 135 (3), 123 (4), 121 (5), 119 (5), 105 (7), 95 (8), 91 (10), 77 (11), 55 (8), 45 (120), 43 (100).

15,16-*Epoxy*-8-*hydroxy*-1,3,13(16),14-*clerodateraene*-17,12:18,6-*diolide* (**6**). $[\alpha]_D^{20}$ − 38.87° (*c* 1.16); HR-MS: found $[M]^+$ 356.1273; $C_{20}H_{20}O_6$ requires 356.1260. UV λ_{max} nm (log ε): 213 (3.94), 305 (3.81); IR ν_{max}^{KBr} cm^{-1}: 3418, 1733, 1668, 1571, 1506, 1449, 876; ^1H NMR: Table 2; ^{13}C NMR: δ 171.0 (C-17), 170.3 (C-18), 143.8 (C-15), 140.0 (C-16), 132.6 (C-4), 129.7 (C-1), 128.5 (C-3), 125.8 (C-2), 124.6 (C-13), 108.6 (C-14), 83.1 (C-6), 75.4 (C-8), 72.2 (C-12), 45.2 (C-10), 39.0 (C-9), 37.2 (C-5), 35.7 (C-11), 32.8 (C-7), 26.2 (C-19), 16.8 (C-20); EI-MS *m/z* (rel. int.): 356 $[M]^+$ (1), 311 (2), 297 (2), 295 (2), 261 (3), 218 (28), 203 (9), 190 (30), 174 (8), 159 (17), 149 (33), 145 (16), 133 (27), 131 (12), 119 (100), 91 (63), 77 (23), 65 (23), 43 (34).

Jamesoniellide F (**7**). $[\alpha]_D^{20}$ + 32.75° (*c* 0.18); HR-MS: found $[M]^+$ 356.1251; $C_{20}H_{20}O_6$ requires 356.1260. UV λ_{max} nm (log ε): 211 (3.80); IR ν_{max}^{KBr} cm^{-1}: 1770, 1735, 1505, 1370, 1100, 875, 755; ^1H NMR: Table 2; ^{13}C NMR: 175.2 (C-17), 170.4 (C-18), 143.6 (C-15), 139.9 (C-16), 137.0 (C-10), 134.0 (C-2), 132.3 (C-1), 124.9 (C-13), 123.9 (C-3), 108.7 (C-14), 90.6 (C-8), 89.6 (C-9), 88.8 (C-6), 72.3 (C-12), 52.4 (C-4), 41.4 (C-5), 36.6 (C-7), 36.5 (C-11), 26.6 (C-19), 23.0 (C-20); EI-MS *m/z* (rel. int.): 356 $[M]^+$ (66), 328 (35), 260 (33), 232 (11), 218 (56), 173 (39), 159 (61), 147 (67), 134 (100), 121 (65), 105 (91), 91 (60), 85 (42), 77 (29), 43 (48).

1β-*Acetoxy*-12-*hydroxy*-15,16-*epoxy*-cis-*cleroda*-3,13(16),14-*triene*-18,16-*olide* (**8**). $[\alpha]_D^{20}$ − 23.20° (*c* 1.21); HR-MS; found $[M]^+$ 388.1894; $C_{22}H_{28}O_6$ requires 388.1886. UV λ_{max} nm (log ε): 215 (4.28); IR ν_{max}^{KBr} cm^{-1}: 3500, 1760,

Table 3. ^{1}H NMR spectral data of compounds **10–12** (400 MHz)

H	**10**	**11**	**12**
1α	1.80 *m*[a]	1.80 *m*	1.81 *m*
1β	1.32 *m*[b]	1.30 *m*	1.21 *m*[e]
2α	1.80 *m*[a]	1.59 *m*	1.66 *m*[f]
2β	1.95 *m*[c]	1.69 *m*[d]	1.77 *m*
3	4.64 *dd* (3.3, 11.7)	4.50 *dd* (4.4, 11.7)	3.41 *dd* (7.1, 8.5)
5	1.32 *m*[b]	1.19 *m*	1.21 *m*[e]
6α	1.32 *m*[b]	1.38 *ddd* (4.3, 12.9, 25.7)	1.21 *m*[e]
6β	1.80 *m*[a]	1.67 *m*[d]	1.74 *m*
7α	2.37 *m*	2.37 *m*	2.34 *dd* (4.9, 13.0)
7β	1.95 *m*[c]	1.98 *m*	1.92 *ddd* (2.4, 4.3, 13.0)
9	1.71 *m*	1.67 *m*[d]	1.66 *m*[f]
11	2.13 *m*	2.13 *m*	2.11 *m*
11′	2.30 *m*	2.28 *m*	2.29 *m*
12	5.36 *t* (6.3)	5.38 *t* (6.6)	5.33 *t* (6.6)
14	6.28 *dd* (10.7, 17.3)	7.30 *dd* (10.7, 17.3)	6.27 *dd* (10.7, 17.3)
15 *cis*	4.85 *d* (10.7)	4.86 *d* (10.7)	4.84 *d* (10.7)
15 *trans*	5.02 *d* (17.3)	5.02 *d* (17.3)	5.00 *d* (17.3)
16	1.71 *s*	1.72 *s*	1.70 *s*
17	4.46 *s*	4.46 *s*	4.42 *s*
17′	4.81 *s*	4.81 *s*	4.78 *s*
18	1.05 *s*	0.86 *s*	3.26 *d*, 4.15 *d* (11.0)
19	3.35 *d*, 4.12 *d* (11.7)	0.84 *s*	1.18 *s*
20	0.67 *s*	0.73 *s*	0.63 *s*
others	2.05 *s* (OAc)	2.03 *s* (OAc)	—

[a–f] Signals partly overlapping.

1505, 857, 800, 760; ^{1}H NMR: Table 2; ^{13}C NMR: 171.4 (MeCO), 169.0 (C-8), 143.4 (C-15), 138.1 (C-16), 134.2 (C-4), 131.6 (C-3), 130.7 (C-13), 108.4 (C-14), 85.5 (C-6), 68.7 (C-1), 63.9 (C-12), 47.9 (C-10), 44.6 (C-11), 39.0 (C-5), 38.3 (C-9), 34.6 (C-7), 32.2 (C-2), 31.4 (C-8), 30.0 (C-19), 21.6 (Ac), 16.7 (C-20), 15.5 (C-17); EI-MS *m/z* (rel. int.): 387 $[M-1]^+$ (29), 328 (96), 292 (7), 232 (23), 217 (31), 200 (31), 173 (24), 163 (14), 149 (23), 137 (24), 120 (100), 105 (54), 97 (88), 69 (39), 43 (54).

Jamesoniellide G (**9**). $[\alpha]_D^{20}$ − 78.44° (*c* 1.13); HR-MS: found $[M-18]^+$ 358.1403; $C_{20}H_{22}O_6$ requires 358.1416. UV λ_{max} nm (log ε): 212 (3.76); IV ν_{max}^{KBr} cm^{-1}: 3442, 1770, 1506, 1450, 876, 756; ^{1}H NMR: Table 2; ^{13}C NMR: 175.9 (C-18), 173.0 (C-20), 144.4 (C-15), 140.4 (C-16), 122.1 (C-13), 108.0 (C-14), 79.6 (C-6), 79.3 (C-10), 78.2 (C-12), 76.7 (C-11), 65.0 (C-8), 55.1 (C-9), 44.6 (C-4), 44.1 (C-5), 34.6 (C-7), 30.3 (C-1), 22.0 (C-17), 21.7 (C-2), 18.4 (C-3), 17.4 (C-19); EI-MS *m/z* (rel. int.): 358 $[M-18]^+$ (14), 340 (8), 298 (2), 294 (3), 270 (7), 242 (9), 225 (11), 209 (100), 181 (8), 167 (28), 152 (12), 139 (22), 134 (16), 110 (47), 95 (60), 81 (24).

3α-*Acetoxy-ent-labda*-8(17),12E,14-*trien*-19-*ol* (**10**). $[\alpha]_D^{20}$ − 3.93° (*c* 0.70); HR-MS: found $[M]^+$ 346.2475; $C_{22}H_{34}O_3$ requires 346.2510. IR ν_{max}^{KBr} cm^{-1}: 3440, 1740, 1640, 1440, 1245, 1030, 890, ^{1}H NMR: Table 3; ^{13}C NMR: 169.8 (Ac), 147.1 (C-8), 141.4 (C-14), 133.5 (C-13), 133.2 (C-12), 110.1 (C-15), 108.4 (C-17), 82.7 (C-3), 63.6 (C-19), 56.6 (C-9), 55.6 (C-5), 42.6 (C-4), 38.9 (C-10), 37.9 (C-7), 36.9 (C-1), 24.3 (C-6), 24.0 (C-2), 23.4 (C-11), 22.4 (C-18), 21.3 (Ac), 15.0 (C-20), 11.8 (C-16); EI-MS *m/z* (rel. int.): 346 $[M]^+$ (0.3), 255 (1), 187 (1), 162 (2), 159 (2), 147 (4), 119 (12), 93 (10), 81 (13), 67 (8), 55 (16), 43 (100).

3α-*Acetoxy-ent-labda*-8 (17),12E,14-*triene* (**11**) $[\alpha]_D^{20}$ − 29.40° (*c* 0.43); HR-MS: found $[M]^+$ 330.2612; $C_{22}H_{34}O_2$ requires 330.2579. IR ν_{max}^{KBr} cm^{-1}: 1740, 1645, 1370, 1240, 1030, 890; ^{1}H NMR: Table 3; ^{13}C NMR: 170.9 (Ac), 147.8 (C-8), 141.6 (C-14), 133.5 (C-13), 133.5 (C-12), 109.9 (C-15), 108.0 (C-17), 80.8 (C-3), 56.8 (C-9), 54.8 (C-5), 39.2 (C-4), 38.1 (C-10), 37.8 (C-7), 37.0 (C-1), 28.3 (C-2), 24.4 (C-6), 23.7 (C-11), 23.4 (C-18), 21.2 (MeCO), 16.6 (C-19), 14.5 (C-20), 11.8 (C-16); EI-MS *m/z* (rel. int.): 330 $[M]^+$, (0.3), 255 (0.8), 218 (1), 203 (1), 189 (2), 175 (2), 149 (5), 135 (12), 119 (8), 107 (8), 95 (10), 81 (10), 69 (11), 55 (17), 43 (100).

3α-*Hydroxy-ent-labda*-8(17),12E,14-*trien*-18-*ol* (**12**). $[\alpha]_D^{20}$ − 16.2° (*c* 0.62); HR-MS: found $[M-18]^+$ 286.2296; $C_{20}H_{30}O$ requires 286.2296. IR ν_{max}^{KBr} cm^{-1} 3370, 1740, 1645, 1610, 1450, 1390, 1080, 1035, 895, 760; ^{1}H NMR: Table 3; ^{13}C NMR: 147.3 (C-8), 141.5 (C-14), 133.5 (C-13), 133.3 (C-12), 109.9 (C-15), 108.1 (C-17), 80.5 (C-3), 64.2 (C-18), 56.8 (C-9), 55.3 (C-5), 42.8 (C-4), 38.9 (C-10), 38.0 (C-7), 37.0 (C-1), 28.2 (C-2), 23.8 (C-6), 23.4 (C-11), 22.7 (C-19), 15.2 (C-20), 11.8 (C-16); EI-MS *m/z* (rel. int.): 286 $[M-18]^+$ (2), 255 (2), 218 (4), 205 (4), 189 (5), 175 (7), 161 (8), 147 (12), 133 (9), 119 (15), 84 (90), 56 (92), 43 (100).

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REFERENCES

1. Zinsmeister, H. D., Becker, H. and Eicher, T. (1991) *Angew. Chem. Int. Ed. Engl.* **30**, 130.
2. Asakawa, Y. (1982) in *Progress in the Chemistry of Organic Natural Products*, Vol. 42, p. 1. Springer, Wien, New York.
3. Huneck, S. (1983) *New Manual of Bryology* Vol. 1, (Schuster, R. M., ed.). p. 1, The Hattori Botanical Laboratory, Japan.
4. Blechschmidt, M. and Becker, H. (1992) *J. Nat. Prod.* **55**, 111.
5. Tazaki, H., Blechschmidt, M., Huch, V., Veith, M. and Becker, H. (1994) *Phytochemsitry* **37**, 491.
6. Schuster, R. M. (1983) *New Manual of Bryology* Vol. 1 (Schuster, R. M., ed.) p. 463. The Hattori Botanical Laboratory, Japan.
7. Nagashima, F., Toyota, M. and Asakawa, Y. (1990) *Phytochemistry* **29**, 2169.
8. Bohlmann, F. and Czerson, H. (1979) *Phytochemistry* **18**, 115.
9. Bohlmann, F., Adler, A., King, R. M. and Robinson, H. (1982) *Phytochemistry* **21**, 173.
10. Matsuo, A., Nakayama, M., Hayashi, S., Seki, T. and Amakawa, T. (1978) *Bryophytorum Bibl.* **13**, 321.
11. Matsuo, A., Nakamoto, T., Nakayama, M. and Hayashi, S. (1976) *Experientia* **32**, 966.
12. Asakawa, Y., Lin, X., Tori, M. and Kondo, K. (1990) *Phytochemistry* **29**, 2597.
13. Asakawa, Y., Tokunaga, N., Toyota, M., Hattori, S. and Mizutani, A. (1979) *J. Hattori Bot. Lab.* **46**, 67.
14. Huneck, S., Connolly, J. D., Harrison, L. J., Joseph, R., Phillips, W. R., Rycroft, D. S., Ferguson, G. and Parvez, M. (1986) *J. Chem. Res. Synop.* 162.
15. Wu, C.-L., Wey, F. F. and Hsieh, C. C. (1982) *Chemistry (Taipei Taiwan)* **40**, 121.
16. Connolly, J. D. (1981) *Rev. Latinoam. Quim.* **12**, 121.
17. Huneck, S., Asakawa, Y., Taira, Z., Cameron, A. F., Connolly, J. D. and Rycroft, D. S. (1983) *Tetrahedron Letters* **24**, 1 15.
18. Wu, C.-L. and Asakawa, Y. (1988) *Phytochemistry* **27**, 940.
19. Matsuo, A., Atsumi, K. and Nakayama, M. (1984) *Z. Naturforsch.* **B39**, 1281.
20. Tori, M., Masuya, T. and Asakawa, Y. (1993) *Phytochemistry* **32**, 1229.
21. Gamborg, O. L., Miller, R. A. and Ojima, K. (1968) *Exp. Cell. Res.* **50**, 151.