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CLERODANE-TYPE DITERPENOIDS FROM AXENIC CULTURES OF THE LIVERWORT *JAMESONIELLA AUTUMNALIS*

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

Abstract—Six new clerodane-type diterpenoids have been isolated from axenic cultures of the liverwort *Jamesoniella autumnalis*. Their structures were elucidated on the basis of spectroscopic evidence. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Liverworts are known to be a rich source of sesquiterpenoids and diterpenoids [1–3]. In the course of our investigation of these compounds, we have previously reported the isolation and structure determination of eight *ent*-labdanes [4, 5], 13 furanoditerpenes [4–6], including jamesoniellides A–G, together with five lignan derivatives [7] from field collected or *in vitro* cultured *Jamesoniella autumnalis* (DC) Steph. [8] (Jungermanniaceae). In our continuing search for terpenes from the cultured gametophytes of *J. autumnalis*, we report herein the isolation and the structural elucidation of six new diterpenoids from this source.

RESULT AND DISCUSSION

Six diterpenoids, **1–6**, were isolated from the Et_2O extract [5] of *in vitro* cultured *J. autumnalis* by combined CC on silica gel and Sephadex LH-20, and HPLC on Diol and silica gel columns.

Compound **1** was assigned the molecular formula $C_{20}H_{20}O_5$ by EI-HRMS (*m/z* 340.1334, $[M]^+$, calcd 340.1266). Its UV spectrum showed an absorption maximum at 298 nm suggesting a conjugated system to a γ-lactone ring. The IR spectrum exhibited the presence of a carbonyl group at 1735 and 1700 cm^{-1}. As already followed from the ^{13}C NMR spectrum of **1**, this compound differed from **7** [5] by the presence of an additional aliphatic carbon (δ_C 45.6) and the absence of an oxygenated quaternary carbon. In comparison to **7**, the resonance of the hydroxy group at C-8 was absent in the ^1H NMR spectrum of **1**. A new signal at δ_H 2.18 showed vicinal couplings with H-7α and H-7β in ^1H–^1H COSY. Thus the structure of **1** was 15,16-epoxy-1,3,13(16),14-clerodatetraene-17,12:18,6-diolide. The complete ^1H (Table 1) and ^{13}C NMR assignment (Table 2) of **1** was achieved by measuring ^1H–^1H COSY, ^1H–^{13}C COSY, DEPT, NOESY, difference NOE and COLOC experiments. The observed NOEs between H-6β and H-8, between H-7β and H-8, and between H-8 and H-11β in **1** were in agreement with H-8 being in the β-position.

Compound **2** had the molecular formula $C_{20}H_{22}O_8$ (EI-HRMS *m/z* 390.1334, $[M]^+$, calcd 390.1315). The IR spectrum showed the presence of a hydroxyl group (3400 cm^{-1}). The ^1H and ^{13}C NMR spectra of **2** were similar to those of **7** except for the absence of signals corresponding to the furan ring. New signals at δ_H 2.93, (1H, *d*, *J* = 19.1) 3.01 (1H, *d*, *J* = 19.1), and 3.63 (*brs* D_2O exchangeable) were assignable to a hydroxy methyl group. These results and the molecular formula, $C_{20}H_{22}O_8$, suggested that **2** was an analogue of **7** where the furan ring was replaced by a 15-carboxy-16-hydroxy-13-ene moiety. The assignment of the ^1H (Table 1) and ^{13}C (Table 2) NMR data was completed by ^1H–^1H COSY, ^1H–^{13}C COSY and NOESY experiments. The ^1H and ^{13}C NMR spectra of **3** were very similar to those of **2**, except that the H-12, H-14, H-16, and H-16′ resonance moved to lower field (δ_H 4.73, 5.67, 2.75, 2.91). These differences between **2** and **3** suggested that they are geometric isomers at the C-13/C-14 double bond. The observation of the NOE cross-peaks (H-12)–(H-14) in **2** and (H-14)–(H-16) in **3** indicated the stereochemistry

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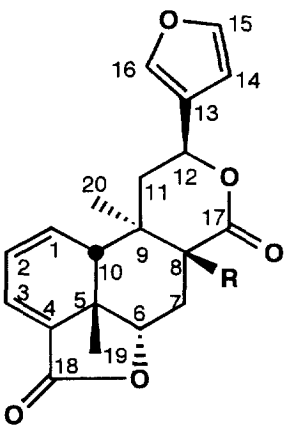

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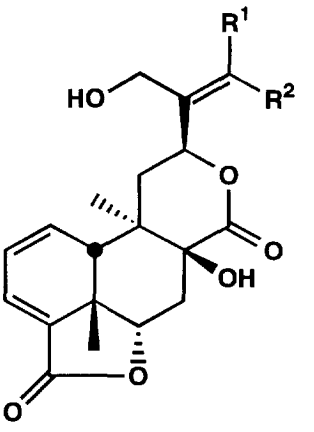
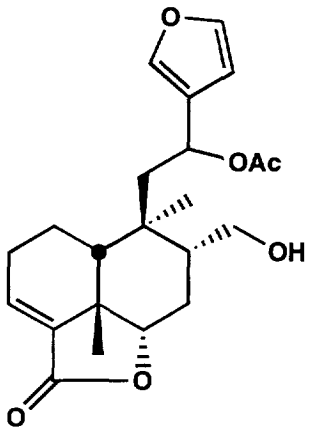
7 : R = OH



2 : R^1 = COOH, R^2 = H
3 : R^1 = H, R^2 = COOH



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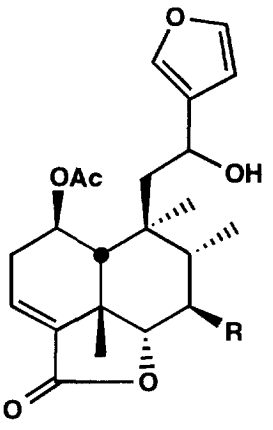
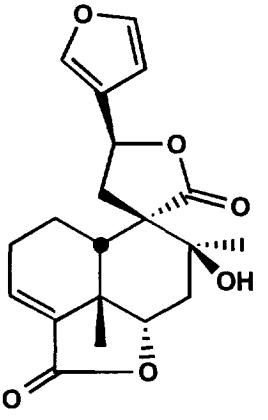


5 : R = OH
8 : R = H



6

of this double bond as 13*E* in **2** and 13*Z* in **3**, respectively.

Compound **4**, $C_{22}H_{28}O_6$ (*m/z* 388.1895 $[M]^+$, calcd 388.1886), required nine double bond equivalents. The IR spectrum exhibited the presence of a hydroxy group and a carbonyl group at 3450 and 1740 cm^{-1}, respectively. The ^{13}C NMR spectrum of **4** indicated the presence of three methyls, five methylenes, eight methines and six quaternary carbons. The carbonyl carbon (δ_C 171.0) together with an acetoxy methyl (δ_C 21.8) confirmed the presence of an acetyl group. The ^1H NMR spectrum of **4** was similar to that of **8** [5] except for the absence of a doublet methyl signal, which was replaced by one hydroxymethyl group at δ_H 3.65, and the upfield shift of the H-1 resonance (δ_H 1.90). The observed low field shift of the H-12 resonance (δ_H 5.77) in the spectra of **4** clearly indicated that the acetyl group was positioned at C-12. Therefore, the structure of compound **4** was 12-acetoxy-15,16-epoxy-17-hydroxymethyl-*cis*-cleroda-3,13(16),14-triene-18,6-olide. The complete ^1H (Table 1) and ^{13}C NMR assignments of **4** were achieved by the ^1H–^1H COSY, ^1H–^{13}C COSY, COLOC and NOESY experiments.

Compound **5** was assigned the molecular formula $C_{22}H_{28}O_7$ (*m/z* 404.1851 $[M]^+$, calcd 404.1835). The IR spectrum exhibited the presence of a hydroxy group (3450 cm^{-1}), and carbonyl groups (1780, 1750 cm^{-1}). The ^{13}C NMR spectrum of **5** showed the presence of four methyls, two methylenes, ten methines and six quaternary carbons including one acetyl group. The ^1H NMR spectrum of **5** was also similar to that of **8**, except for the absence of one methylene signal in **8**, which was replaced by one methine signal bonded to oxygen at δ_H 3.25. The complete structure of **5** was achieved by ^1H (Table 1) and ^{13}C NMR (Table 2) analysis with ^1H–^1H COSY, ^1H–^{13}C COSY, and NOESY experiments. The observed low field shift

Table 1. ^{1}H NMR data of compounds **1–6** (270 MHz)

H	1	2	3	4	5	6
1α	6.03 *dd* (5.8, 9.2)	5.94 *dd* (5.7, 9.6)	5.94 *dd* (5.6, 9.6)	1.90 *m*	5.46 *d* (7.2)	1.86 *m*
1β	—	—	—	1.90 *m*	—	1.71 *m*
2α	6.43 *ddd* (1.3, 5.3, 9.2)	6.43 *ddd* (1.0, 5.2, 9.6)	6.43 *dd* (5.3, 9.6)	2.34 *m*	2.79 *ddd* (4.1, 7.2, 21.8)	2.31 *m*
2β	—	—	—	2.25 *m*	2.53 *dd* (3.2, 21.8)	2.31 *m*
3	6.93 *d* (5.3)	6.90 *d* (5.2)	6.91 *d* (5.3)	6.77 *dd* (3.1, 3.6)	6.72 *dd* (3.2, 4.1)	6.80 *dd* (3.4, 3.6)
6	4.50 *dd* (7.4, 10.6)	4.73 *dd* (7.7, 10.1)	4.69 *d* (7.7, 10.1)	4.62 *dd* (6.5, 10.0)	4.11 *d* (8.2)	4.68 *dd* (6.6, 10.1)
7α	1.36 *ddd* (10.6, 13.2, 14.1)	1.54 *dd* (10.1, 14.7)	1.52 *dd* (10.1, 14.9)	1.33 *dd* (10.0, 12.5)	3.25 *dd* (8.2, 11.4)	2.04 *dd* (10.1, 13.5)
7β	2.90 *ddd* (2.3, 7.4, 14.1)	2.93 *m*	2.91 *dd* (7.7, 14.9)	1.97 *dd*, (6.5, 12.5)	—	2.16 *dd* (6.6, 13.5)
8	2.18 *dd* (2.3, 13.2)	—	—	1.90 *m*	1.91 *m*	—
10	2.27 *dd* (1.3, 5.8)	2.73 *d* (5.7)	2.72 *d* (5.6)	2.29 *s*	3.10 *s*	2.17 *s*
11α	2.28 *dd* (4.1, 13.2)	1.70 *dd* (4.8, 13.5)	1.72 *dd* (5.1, 13.0)	1.79 *dd* (9.1, 13.8)	2.35 *dd* (10.8, 15.9)	2.56 *dd* (7.2, 15.7)
11β	1.88 *dd* (12.4, 13.2)	2.38 *dd* (12.6, 13.5)	2.36 *dd* (12.4, 13.0)	1.69 *dd* (5.8, 13.8)	1.55 *dd* (8.6, 15.9)	2.32 *dd* (6.1, 15.7)
12	5.38 *dd* (4.1, 12.4)	4.44 *dd* (4.8, 12.6)	4.73 *dd* (5.1, 12.4)	5.77 *dd* (5.5, 9.1)	4.74 *dd* (8.6, 10.8)	5.42 *dd* (6.1, 7.2)
14	6.42 *dd* (0.8, 1.7)	5.54 *s*	5.67 *s*	6.38 *brs*	6.45 *s*	6.34 *s*
15	7.43 *dd* (1.7, 1.7)			7.38 *brs*	7.45 *s*	7.41 *s*
16	7.46 *dd* (0.8, 1.7)	3.01 *d*, 2.93 *d* (19.1)	2.91 *d*, 2.75 *d* (22.3)	7.44 *brs*	7.45 *s*	7.41 *s*
17	—			3.65 *m*	0.93 *d* (6.6)	1.50 *s*
19	1.21 *s*	1.23 *s*	1.23 *s*	1.34 *s*	1.38 *s*	1.34 *s*
20	1.00 *s*	0.95 *s*	0.99 *s*	1.32 *s*	0.64 *s*	—
Others		8-OH 3.63 *brs*	8-OH 2.87 *s*	CH_3CO 2.02 *s*	CH_3CO 2.05 *s*	

Table 2. ^{13}C NMR spectral data of compound **1–6** (67.5 MHz, $CDCl_3$)

C	1	2	3	4	5	6
1	128.9	129.0	128.9	31.9	68.4	31.1
2	125.6	126.4	126.3	25.3	32.2	24.9
3	128.4	128.7	128.6	135.2	131.4	135.2
4	132.5	132.0	131.9	133.0	134.0	133.3
5	37.9	37.3	37.1	37.5	39.7	43.2
6	83.8	81.3	82.4	79.5	92.0	97.4
7	27.3	32.6	32.7	36.4	73.4	33.6
8	45.6	76.7	76.7	48.4	37.5	74.7
9	36.3	38.5	38.5	43.8	39.5	43.1
10	50.9	44.8	44.8	46.0	47.2	49.7
11	45.0	35.9	33.4	34.0	45.0	34.6
12	71.5	73.5	72.6	97.7	63.9	72.5
13	124.6	164.6	164.0	124.4	130.5	130.4
14	108.3	128.7	128.9	108.9	108.4	108.2
15	143.9	169.2	169.5	144.0	143.5	144.1
16	139.6	58.5	61.9	141.6	138.2	139.4
17	170.0	170.9	171.0	62.3	9.7	22.4
18	168.6	170.4	170.3	169.5	171.3	171.4
19	26.8	26.2	26.1	24.6	30.3	25.6
20	15.0	16.7	16.8	22.9	17.7	174.5
Others				171.0	169.0	
				21.8	21.6	

of the H-7 resonance in the spectra of **5** clearly indicated that the hydroxyl group was positioned at C-7. The coupling constants between H-6 and H-7 (J = 8.2 Hz) and between H-7α and H-8 (J = 11.4 Hz) showed that these hydrogens have a *trans*-diaxial relationship. Thus the hydroxy group at C-7 and 17-Me had to be equatorial. NOEs between H-6 and H-8, between H-6 and H-10, and between H-8 and H-10 also confirmed that these three protons were axial, and H-6, the hydroxy group at C-7 and H-8 were each in β-positions. To understand better the NOE correlations and the couplings, a stereoscopic view of the conformation of **5** is shown in Fig. 1.

Compound **6** had the molecular formula $C_{20}H_{22}O_6$ (HR-MS m/z 358.1452, $[M]^+$, calcd 358.1416). Its IR spectrum was consistent with the presence of two lactone rings (1750, 1720 cm^{-1}) and a hydroxy group (3450 cm^{-1}). The ^{1}H NMR spectrum displayed the signals for a β-substituted furan ring (δ_H 6.34, 7.41, and 7.41) and two quaternary methyl groups (δ_H 1.34 and 1.50). The ^{13}C NMR spectrum showed the signals of two methyls, four methylenes, seven methines and seven quaternary carbons indicating two carbonyl groups (δ_C 171.4 and 174.5) and three oxygenated aliphatic carbons (δ_C 72.5, 74.7, and 97.4) in the molecule. Therefore, the oxygens in the molecule cor-

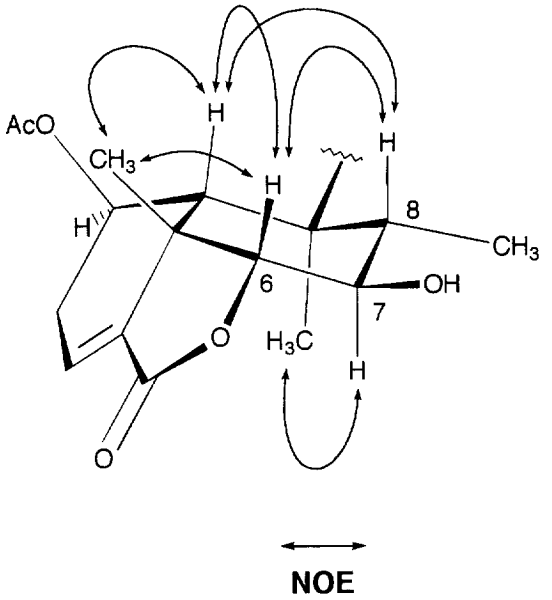


Fig. 1. NOESY correlations used to establish the structure of **5**.

responded to those in one furan ring, one hydroxy group, and two lactone rings. The ^1H and ^{13}C NMR assignments with ^1H–^1H COSY, ^1H–^{13}C COSY, DEPT, NOESY and COLOC experiments revealed the complete structure of **6**. The ^1H–^1H COSY experiment revealed the sequences C(10 H–C(1) H_2–C(2) H_2–C(3) H, C(6) H–C(7) H_2, and C(11) H_2–C(12) H. The long-range correlations observed by COLOC revealed the connectivities of all segments as shown in Fig. 8. Extensive NOE difference measurements supported this result and led to the relative configurations. A segment of C(10) H–C(1) H_2–C(2) H_2–C(3) H in ring A was supported by the observed NOEs between H-10 and 19-Me. A C(6) H–C(7) H_2 unit in the ring was confirmed by the observed NOEs between H-6 and 19-Me and between H-7β and 17-Me. The remaining ABX spin system, C(11) H_2–C(12) H, could be attributed to the γ-lactone ring by the observed NOE among H-12 and H-13 and H-16. Additional NOEs between H-11α and 17-Me, between H-11α and H-12, established the stereochemistry of **6** as indicated in Fig. 2.

EXPERIMENTAL

Optical rotations: $CHCl_3$; UV: EtOH; NMR: 270 MHz (^1H) and 67.5 MHz (^{13}C), $CDCl_3$, relative to $CHCl_3$ at δ_H 7.25 and $CDCl_3$ at δ_C 77.0, respectively. ^{13}C multiplicities were determined using the DEPT pulse sequence.

The origin and the axenic culture of *J. autumnalis* have been previously reported [5].

Extraction and isolation

Powdered dry plant material (815 g) was extracted with Et_2O. The Et_2O extracts (37.03 g) were subjected to VLC on silica gel and eluted with *n*-hexane containing various concns of EtOAc to give nine fractions [A to I (MeOH)] as previously reported [6]. HPLC (DIOL and Kieselgel Si 60) (*n*-hexane–EtOAc, 7:13) of fr. G resulted in the isolation of **2** (7.5 mg) and **3** (5.1 mg). Sepn of fr. H in the same manner as fr. G yielded **1** (15.0 mg). A combination of VLC on silica gel ($CHCl_3$–MeOH) and HPLC (DIOL and Kieselgel

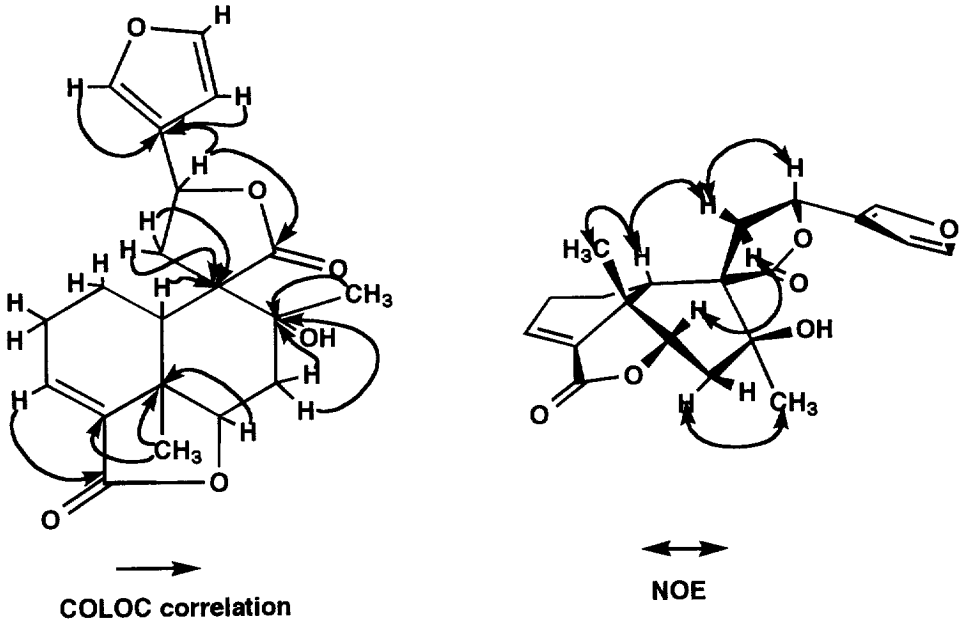


Fig. 2. COLOC and NOESY correlations used to establish the structure of **6**.

Si 60) (*n*-hexane–EtOAc, 7:13) of fr. I resulted in the isolation of **4** (19.8 mg), **5** (6.0 mg), and **6** (12.8 mg).

15,16-*Epoxy*,1,3,13(16),14-*clerodatetraene*-17,12:18,6-*diolide* (**1**). Mp. 253–255°; $[\alpha]_D = -131.0$ (*c* 1.15); HRMS Found $[M]^+$ 340.1334; $C_{20}H_{20}O_5$ requires 340.1266. UV λ_{max} nm (log ε): 225 (3.45), 298 (3.58); IR ν_{max}^{KBr} cm^{-1} 2920, 1735, 1700, 1565, 1430, 1260, 1170, 880; ^1H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): 340 (0.6), 325 (4), 307 (4), 261 (5), 233 (5), 203 (10), 176 (9), 147 (11), 133 (39), 119 (89), 105 (14), 91 (55), 81 (23), 65 (17), 51 (13), 44 (100).

15-*Carboxy*-8β,16-*dihydroxy*-1,3,13*E*-*clerodatriene*-17,12:18,6-*diolide* (**2**). $[\alpha]_D = -218.7$ (*c* 0.75); HRMS Found $[M]^+$ 390.1334; $C_{20}H_{22}O_8$ requires 390.1315. UV λ_{max} nm (log ε): 217 (3.54), 302 (3.47); IR ν_{max}^{KBr} cm^{-1} 3400, 1790, 1740, 1660, 1560, 1440, 1300, 1260, 1040, 960, 900, 730; ^1H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): 390 (1), 373 (0.5), 359 (1), 341 (2), 323 (3), 255 (5), 209 (6), 181 (5), 147 (18), 133 (58), 119 (100), 105 (13), 91 (47), 43 (9).

15-*Carboxy*-8β,16-*dihydroxy*-1,3,13*Z*-*clerodatriene*-17,12:18,6-*diolide* (**3**). $[\alpha]_D = -223.9$ (*c* 0.51); HRMS Found $[M]^+$ 390.1334; $C_{20}H_{22}O_8$ requires 390.1315. UV λ_{max} nm (log ε): 216 (3.48), 302 (3.49); IR ν_{max}^{KBr} cm^{-1} 3400, 1790, 1740, 1660, 1560, 1440, 1360, 1300, 1260, 1160, 1040, 960, 900, 730; ^1H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): 390 (3), 373 (0.5), 360 (1), 359 (1), 323 (2), 283 (1), 255 (3), 209 (4), 183 (4), 147 (17), 133 (60), 119 (100), 105 (11), 91 (43), 43 (96).

12-*Acetoxy*-15,16-*epoxy*-17-*hydroxymethyl*-*cis*-*cleroda*-3,13(16),14-*triene*-18,6-*olide* (**4**). $[\alpha]_D = -22.8$ (*c* 0.44); HRMS Found $[M]^+$ 388.1895; $C_{22}H_{28}O_6$ requires 388.1886. UV λ_{max} nm (log ε): 248 (2.70); IR ν_{max}^{KBr} cm^{-1} 3450, 1740, 1360, 1080, 1050, 1020, 960, 870, 760; ^1H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): 388 (0.5), 360 (2), 342 (0.3), 312 (0.3), 205 (2), 181 (3), 163 (6), 135 (8), 95 (18), 81 (17), 79 (14), 43 (100).

1β-*Acetoxy*-7,12-*dihydroxy*-15,16-*epoxy*-*cis*-*cleroda*-3,13(16),14-*triene*-18,6-*olide* (**5**). $[\alpha]_D = -13.2$ (*c* 0.41); HRMS Found $[M]^+$ 404.1851; $C_{22}H_{28}O_7$ requires 404.1835. UV λ_{max} nm (log ε): 216 (4.15); IR ν_{max}^{KBr} cm^{-1} 3450, 1780, 1750, 1700, 1645, 1505, 1380, 1025, 880; ^1H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): 404 (0.3), 387 (0.3), 359 (1), 344 (4), 297 (2), 233 (3), 217 (5), 179 (11), 119 (20), 105 (16), 97 (30), 81 (19), 69 (18), 55 (14), 43 (100).

8-*Hydroxy*-15,16-*epoxy*-*cis*-*cleroda*-3,13(16),14-*triene*-18,6,20,12-*diolide* (**6**). $[\alpha]_D = -66.7$ (*c* 0.06); HRMS Found $[M]^+$ 358.1452; $C_{20}H_{22}O_6$ requires 358.1416. UV λ_{max} nm (log ε): 221 (3.96); IR ν_{max}^{KBr} cm^{-1} 3450, 1750, 1720, 1690, 1600, 1510, 1465, 1160, 1040, 970, 880; ^1H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): 358 ($[M]^+$, 0.3), 253 (1), 251 (1), 181 (2), 161 (2), 149 (12), 123 (12), 109 (15), 95 (32), 81 (45), 71 (29), 69 (35), 55 (52), 43 (100).

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