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LABDANE DITERPENOIDS FROM *HALIMIUM VISCOSUM* AND *H. VERTICILATUM*

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Key Word Index—*Halimium viscosum*; *H. verticilatum*; Cistaceae; labdane diterpenoid acids.

Abstract—In addition to three known acids, six new diterpenoid acids with a labdane skeleton, were isolated from the acid fraction of *Halimium viscosum*. Four of them were isolated from the acid fraction of *H. verticilatum*, together with another, as their methyl derivatives.

INTRODUCTION

We have previously described the isolation from *H. viscosum* of three new diterpenoid acids with a labdane skeleton containing a C-17 carboxyl group and having two double bonds at C-7 and C-13 [1]. In the present report we describe the isolation from *H. viscosum* of six new diterpenoid acids, two with an unsaturated chain and four of them with a saturated chain. From *H. verticilatum*, Corroios (Portugal) has been isolated five diterpenoid acids, four of them also present in the acid fraction of *H. viscosum*.

RESULTS AND DISCUSSION

The sodium hydroxide soluble part of the hexane extract of *H. viscosum*, Valparaiso (Zamora, Spain) was separated by column chromatography into three major fractions, I (24%), II (8%) and III (49%). A portion of fraction I, was treated with diazomethane and the resulting mixture of methyl esters was chromatographed on silica gel, to yield three mixtures (85/15) of two components **1h/8** (5%), **1i/9** (5%) and **1d/4** (85%). Column chromatography of the methyl esters corresponding to fraction II gave **1e** as the principal component (85%) [1]. Fraction III contained a mixture (85/15) of hydroxy acids **1h/3**. This mixture can be obtained from **1h/8**, **1i/9** and **1d/4** by alkaline hydrolysis. Only a small quantity of **2** was isolated from fraction I by column chromatography. Using the same method it was not possible to separate the methyl esters mixtures **1d/4** and **1f/5**. The hydroxyester mixture **1f/5** was resolved by transformation into the tetrahydropyranyl derivatives **1g** and **6** that can be separated by column chromatography. The acid hydrolysis of **6** gave **5**, which by acetylation yields **4**, which can also be obtained by treatment of **2** with CH_2N_2.

Specimens of *H. verticilatum* were collected at Corroios (Portugal). The sodium hydroxide-soluble part of the hexane extract, after methylation, was separated into three fractions from which five methyl esters (**4**, **5**, **7**, **8**, **9**) were obtained by column chromatography.

Compounds **8** and **9** were also isolated from fraction I of *H. viscosum* as mixtures **1h/8** and **1i/9**, which could not be separated by column chromatography. Compounds **1h** and **8** were obtained from **1f** and **5** respectively, by treatment with isobutyryl chloride [2]. Treatment of **5** with formic acid [3] gave **9**.

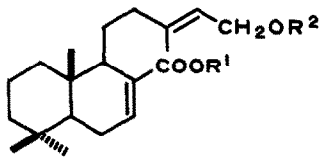
Compound **5** was an α,β-unsaturated hydroxyester (IR: 3420, 1725 and 1640 cm^{-1}). Its ^{13}C NMR spectrum showed signals for 21 carbons: five Me, eight CH_2, four CH and four completely substituted carbons. Its ^1H NMR spectrum showed signals corresponding to the following groups: -CH=C-COOMe (δ6.64, 1H, *m*; 3.70, 3H, *s*), $-CH_2-CH_2OH$ (3.69, 2H, *m*); Me-CH(0.89, 3H, *d*) and three Me-C (0.91, 0.87 and 0.82).

The mass spectrum of **5** exhibited the molecular ion at *m/z* 336 ($C_{21}H_{36}O_3$) corresponding to a bicyclic diterpene with one double bond, a hydroxyl group and a methoxycarbonyl group. The ion of *m/z* 235 was formed by loss of a side chain. The methoxycarbonyl function must be situated at C-8 conjugated with a trisubstituted double bond at C-7 [1]. With respect to the natural compounds **2** and **3** they have the structure of 15-hydroxy-7-labden-17-oic acid and 15-acetoxy-7-labden-17-oic acid, respectively, which is shown upon transforming their carboxylic function into a methyl group, thereby obtaining cativol (**12**) [4].

Treatment of **3** with diacetyl ether–pyridine and diazomethane gave **2** and **5**, respectively. Compound **6** was transformed into **10** by reduction with lithium aluminium hydride. Treatment of **10** with ClMs and reduction of the reaction product gave **11** which on acid hydrolysis produced **12** [5].

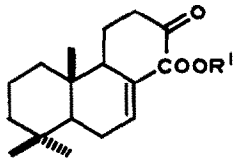
Compound **7** was an α,β-unsaturated diester with an aromatic ring (IR: 1710, 1640 and 1500 cm^{-1}). Its ^{13}C NMR spectrum showed signals for 30 carbons: five Me, eight CH_2, eleven CH (eight sp^2), and six completely substituted carbons. Its ^1H NMR spectrum showed signals corresponding to the following groups: -CH =C-COOMe (δ6.62, 1H, *m*; 3.70, 3H, *s*), $-CH_2-CH_2OOC$ (4.24, 2H, *m*) and -OOC-CH=CH-Ph (7.67, 1H, *d*; 6.44, 1H, *d*).

Alkaline hydrolysis of **7** and treatment with diazomethane of the hydrolysis product yielded **5** and methyl cinnamate (authentic sample); therefore **7** had the structure methyl 15-cinnamoyloxy-7-labden-17-oate.

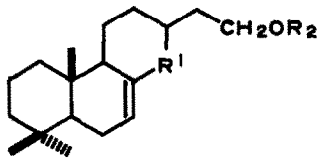


2

	R^1	R^2		R^1	R^2
Ia	H	Ac	**Ig**	Me	THP
Ib	H	H	**Ih**	Me	$OCCH(Me)_2$
Id	Me	Ac	**Il**	Me	OCH
If	Me	H			



	R^1
Ic	H
Ie	Me



	R^1	R^2		R^1	R^2
2	COOH	Ac	**8**	COOMe	$OCCH(Me)_2$
3	COOH	H	**9**	COOMe	OCH
4	COOMe	Ac	**10**	CH_2OH	THP
5	COOMe	H	**11**	Me	THP
6	COOMe	THP	**12**	Me	H
7	COOMe	Cinm			

EXPERIMENTAL

Mps: uncorr, ^1H NMR: 200 MHz, $CDCl_3$, TMS as int. standard; ^{13}C NMR: 50.3 MHz.

Extraction and isolation. The aerial part (0.86 kg) of *H. verticilatum* collected in Corroios (Portugal) was dried and extracted with *n*-hexane in a Soxhlet for 24 hr. The extract (21.2 g) was dewaxed with MeOH (21.1%) and then extracted with 10% Na_2CO_3 (12%) and 4% NaOH (43%). The neutral fraction represented 22% of the original extract. One portion of the NaOH-soluble acid fraction (2.0 g) of the hexane extract of *H. verticilatum*, after treatment with CH_2N_2 was separated by CC into three fractions, I (*n*-hexane–Et_2O, 8:2) (10%), II (*n*-hexane–Et_2O, 1:1) (50%) and III (*n*-hexane–Et_2O, 3:7) (40%).

Fraction I (0.2 g) was resolved by CC. Elution with *n*-hexane–Et_2O (9:1) gave **4** (12 mg), **7** (20 mg) and **8** (10 mg). Fraction II (1.0 g) with the same method gave **5** (400 mg) and **9** (20 mg).

The extraction of the aerial part (1 kg) of *H. viscosum* collected in Valparaiso (Zamora, Spain) has been described elsewhere [1].

15-Acetoxy-7-labden-17-oic acid (**2**). Fraction I (1 g) of *H. viscosum* was resolved by CC. Elution with *n*-hexane–Et_2O (3:2) gave **2** (30 mg). Colourless oil. $[\alpha]_D^{22}$ − 36.6° ($CHCl_3$, *c* 1.10); IR ν_{max}^{film} cm^{-1} : 3500–2500, 1750, 1700, 1650, 1240, 1050,

Table 1. ^{13}C NMR data of compounds **1h** and **2–12*** (50.3 MHz, $CDCl_3$, TMS as internal standard)

C	1h	2	3	4	5	6	7	8	9	10	11	12
1	39.52	39.59	39.61	39.72	39.55	39.70	39.73	39.72	39.52	39.20	39.98	39.81
2	18.60	18.35	18.61	18.62	18.54	18.60	18.63	18.63	18.60	18.87	18.92	18.92
3	42.13	42.05	42.16	42.15	42.08	42.16	42.16	42.16	42.13	42.40	42.48	42.25
4	32.85	32.76	32.84	32.85	32.69	32.78	32.84	32.84	32.85	33.00	33.02	33.02
5	49.56	49.44	49.56	49.62	49.53	49.64	49.63	49.64	49.56	50.09	50.36	50.41
6	24.01	24.11	24.23	23.96	23.88	23.90	23.96	23.97	24.01	23.76	23.92	23.93
7	137.56	139.38	139.20	136.76	136.59	136.25	136.66	136.79	137.05	125.16	122.05	122.17
8	135.40	134.79	134.50	135.62	135.55	135.76	135.63	135.58	135.40	139.63	135.65	135.92
9	50.80	50.94	51.35	51.18	51.10	51.04	51.22	51.22	50.80	52.77	55.55	55.62
10	36.99	36.92	36.99	37.01	36.88	36.98	37.02	37.03	36.99	36.87	37.00	37.04
11	26.89	25.39	25.96	25.60	25.61	25.53	25.62	25.62	26.89	24.37	24.44	24.97
12	41.09	38.17	38.59	38.25	38.33	38.38	38.26	38.22	38.29	38.57	39.31	39.37
13	143.25	30.82	30.68	30.68	30.56	30.80	31.04	30.86	29.71	30.99	31.02	30.62
14	118.36	35.37	39.61	35.41	39.68	36.59	35.57	35.32	35.51	36.53	36.58	40.02
15	62.13	63.04	60.97	63.14	60.62	62.17	63.19	62.60	62.13	62.87	62.32	61.35
16	16.45	19.46	19.94	19.52	19.70	19.71	19.61	19.41	19.55	20.04	20.00	19.84
17	169.70	173.68	173.50	169.82	169.68	169.66	169.73	169.75	169.70	65.72	22.18	22.10
18	33.16	33.08	33.16	33.20	33.09	33.15	33.20	33.19	33.16	33.17	33.23	33.20
19	21.96	21.88	21.26	22.00	21.88	21.95	22.00	21.99	21.96	21.92	21.90	21.87
20	14.46	14.41	14.43	14.47	14.32	14.40	14.48	14.45	14.46	13.68	13.63	13.65
COOMe	51.28			51.25	51.25	51.26	51.22	51.37	51.28			
Me-COO		20.78		21.05								
Me-COO		171.03		171.16								
Me_2CH-COO	51.23								51.23			
Me_2CH-COO	19.04								19.04			
Me_2CH-COO	169.70								169.70			
HCOO								161.11				
1′							169.73					
2′						98.72	136.66			99.40	99.02	
3′						31.06	144.49			33.17	30.88	
4′						25.58	134.66			25.58	25.61	
5′						19.06	128.88			19.79	19.73	
6′						66.02	128.06			65.97	66.13	
7′							130.17					
8′							128.06					
9′							128.88					

*Assignments based on DEPT experiments and, particularly in the case of **5**, on C/H(HCCORR) two dimensional correlations.

870; ^1H NMR: δ6.80 (1H, *m*, H-7), 3.90 (2H, *m*, H-15), 1.90 (3H, *s*) 0.83 (3H, *d*, J = 7.0 Hz, Me-16), 0.85, 0.80, 0.79 (3H, each *s*, Me-19, Me-18 and Me-20, respectively). Treatment of **2** with CH_2N_2 gave **4** and the alkaline hydrolysis (30 mg, 2 ml NaOH–MeOH 10%) yielded **3**.

Methyl 15-acetoxy-7-labden-17-oate (**4**). Colourless oil. $[\alpha]_D^{22}$ − 84.5° ($CHCl_3$, *c* 1.18). IR ν_{max}^{film} 1750, 1730, 1650, 1250; ^1H NMR: δ6.62 (1H, *m*, H-7), 4.09 (2H, *m*, H-14), 3.70 (3H, *s*), 2.04 (3H, *s*), 0.91, 0.87, 0.82 (3H, each *s*, Me-19, Me-18 and Me-20, respectively), 0.88 (3H, *d*, J = 7.7 Hz, Me-16).

15-Hydroxy-7-labden-17-oic acid (**3**). Colourless oil. $[\alpha]_D^{22}$ − 24.1° ($CHCl_3$, *c* 1.31); IR ν_{max}^{film} cm^{-1}: 3500–2600, 1700, 1260; ^1H NMR: δ6.82 (1H, *m*, H-7), 3.67 (2H, *m*, H-15), 0.88 (3H, *d*, J = 7.3 Hz, Me-16), 0.90, 0.87, 0.82 (3H, each *s*, Me-19 Me-18 and Me-20, respectively)

Methyl 15-hydroxy-7-labden-17-oate (**5**). To 5.0 g of methyl esters mixture **1f/5** dissolved in 15 ml dry C_6H_6 were added 2 ml dihydropyrane and 0.18 g TsOH. After shaking the mixture at room temp. 140 mg K_2CO_3 were added and after 30 min the mixture was filtered and evapd to obtain 5.9 g of reaction product which on CC gave 4.2 g (*n*-hexane–Et_2O, 9:1) **1g** and 0.5 g of **6**. Colourless oil. IR ν_{max}^{film} cm^{-1}: 1730, 1655, 1270, 1250, 1080, 980, 910, 870; ^1H NMR: δ6.57 (1H, *m*, H-7), 4.53 (1H, *m*, H-2′), 3.77 (2H, *m*), 3.66 (3H, *s*), 3.40 (2H, *m*), 0.85 (3H, *d*, J = 7.7 Hz, Me-16), 0.87, 0.83, 0.78 (3H, each *s*, Me-19, Me-18 and Me-20, respectively).

Hydrolysis of compound **6**. To 50 mg of **6**, dissolved in 3 ml MeOH, were added 2.5 mg of TsOH and the mixture was shaken at room temp for 1 hr. Following this, the solvent was evapd, Et_2O was added and the product washed with $NaHCO_3$ and H_2O. After evaporating off the solvent, 35 mg of **5** was obtained. Colourless oil. $[\alpha]_D^{22}$ − 51.0° ($CHCl_3$, *c*1.27); UV λ_{max}^{EtOH} nm (log

ε): 2.16 (2.00). IR ν_{max}^{film} cm^{-1}: 3420, 1720, 1640, 1260; ^{1}H NMR: δ6.63 (1H, *m*, H-7), 3.71 (3H, *s*), 3.69 (2H, *m*, H-15), 0.89 (3H, *d*, *J* = 7.5 Hz, Me-16), 0.91, 0.87, 0.83 (3H, each *s*, Me-19, Me-18 and Me-20, respectively). EIMS 70 eV, *m/z* (rel. int): 336 $[M]^+$ (2), 305 (25), 290 (8), 235 (18), 176 (35), 124 (11), 109 (100), 105 (17), 43 (21), 41 (18).

Reduction of **6** with $LiAlH_4$. To 320 mg of **6** dissolved in 3 ml Et_2O, were added 15 mg $LiAlH_4$. The reaction mixture was kept at room temp for 1 hr. The usual procedure gave 283 mg of reduction product which on silica gel CC (*n*-hexane–Et_2O, 9:1) yielded **10** (212 mg). Colourless oil. IR ν_{max}^{film} cm^{-1}: 3420, 1330, 1210, 1140, 1040, 910, 870. ^{1}H NMR: δ5.74 (1H, *m*, H-7), 4.54 (1H, *m*, H-2'), 4.02 (2H, *m*), 3.85 (2H, *m*), 3.50 (2H, *m*), 0.91 (3H, *d*, *J* = 6.8 Hz, Me-16), 0.87, 0.86, 0.72 (3H, each *s*, Me-19, Me-18 and Me-20, respectively).

Preparation and reduction of the mesylate. Et_3N (0.11 ml) was added to 212 mg **10** in 3 ml CH_2Cl_2 and the mixture left to cool between −10° and 0°. $MeSO_2Cl$ (0.06 ml) was added and the product kept at room temp. for 1.5 hr. It was then washed with ice-water, 2 M HCl and then again with H_2O. The reaction product (272 mg) was dissolved in Et_2O (3 ml) and treated with 10 mg $LiAlH_4$. The mixture was shaken for 1 hr. The usual procedure gave 200 mg of reduction production which on silica gel CC (*n*-hexane–Et_2O, 49:1) gave 60 mg **11**. Colourless oil. IR ν_{max}^{film} cm^{-1}: 1330, 1210, 1160, 1140, 1110, 940, 840; ^{1}H NMR: δ5.35 (1H, *m*, H-7), 4.57 (1H, *m*, H-2'), 3.82 (2H, *m*), 3.44 (2H, *m*), 1.66 (3H, *s*, Me-17), 0.92 (3H, *d*, *J* = 6.2 Hz, Me-16), 0.87, 0.85, 0.75 (3H, each *s*, Me-19, Me-18 and Me-20, respectively).

Hydrolysis of compound **11**. *p*-Toluensulphonic acid (2.5 mg) was added to 41 mg **11** dissolved in 2.5 ml MeOH and the mixture shaken for 4 hr at room temp. The usual procedure gave 20 mg **12**. Colourless oil. $[\alpha]_D^{22}$ + 1.4° ($CHCl_3$, *c* 0.85); IR ν_{max}^{film} cm^{-1}: 3360, 1080, 980, 840. ^{1}H NMR: δ5.39 (1H, *m*, H-7), 3.68 (2H, *m*, H-15), 1.67 (3H, *s*, Me-17), 0.93 (3H, *d*, *J* = 6.2 Hz, Me-16), 0.88, 0.85, 0.76 (3H, each *s*, Me-19, Me-18 and Me-20, respectively).

Methyl 15-*cinnamoyloxy*-7-*labden*-17-*oate* (7). Colourless oil. $[\alpha]_D^{22}$ − 33.5° ($CHCl_3$, *c* 1.30); IR ν_{max}^{film} cm^{-1}: 1710, 1640, 1500, 1310, 1240, 1160, 980. ^{1}H NMR: δ7.67 (1H, *d*, *J* = 16 Hz, H-3'), 7.52 (2H, *m*), 7.36 (3H, *m*), 6.40 (1H, *d*, *J* = 16 Hz, H-2'), 6.62 (1H, *m*, H-7), 4.24 (2H, *m*, H-15), 3.70 (3H, *s*), 0.92 (3H, *d*, *J* = 6.4 Hz, Me-16), 0.88, 0.84, 0.80 (3H, each *s*, Me-19, Me-18 and Me-20, respectively).

Methyl 15-*isobutyloxy*-7-*labden*-17-*oate* (**8**). Colourless oil. $[\alpha]_D^{22}$ − 28.4° ($CHCl_3$, *c* 0.74); IR ν_{max}^{film} cm^{-1}: 1750, 1740, 1660, 1250, 1080; ^{1}H NMR: δ6.61 (1H, *m*, H-7), 4.07 (2H, *m*, H-15), 3.67 (3H, *s*), 2.50 (1H, *h*, *J* = 6.8 Hz, H-2'), 1.11 (6H, *d*, *J* = 6.8 Hz, H-3'), 0.92 (3H, *d*, *J* = 6.4 Hz, Me-16), 0.88, 0.84, 0.80 (3H, each *s* Me-19, Me-18 and Me-20, respectively).

Compound **5** (90 mg) dissolved in 0.5 ml dry pyridine were cooled to 0°. Isobutyryl chloride (0.2 ml) was added and after 1 hr the mixture was kept at room temp for 8 hr. Et_2O was added and it was then washed with 2 M HCl, 5% $NaCO_3H$ and H_2O. Evapn of the solvent after drying over Na_2SO_4 gave 110 mg **8**.

Methyl 15-*isobutyloxy*-7,13E-*labdadien*-17-*oate* (**1h**). Treatment of 1.0 g of fraction I of *H. viscosum*, with CH_2N_2, gave 1.0 g of a mixture of Me esters, which was resolved by CC. Elution with *n*-hexane–Et_2O (9:1), gave **1h**/**8** (50 mg), **1i**/**9** (30 mg) and **1d**/**4** (800 mg). By a similar treatment as described before 100 mg of **1f** [1] gave **1h** (100 mg). IR ν_{max}^{film} cm^{-1}: 1750, 1740, 1660,1250, 1080; ^{1}H NMR: δ6.61 (1H, *m*, H-7), 5.29 (1H, *t*, *J* = 7.3 Hz, H-14), 4.54 (2H, *d*, *J* = 7.3 Hz, H-15), 3.68 (3H, *s*), 2.52 (1H, *h*, *J* = 7.1 Hz, H-2'), 1.65 (3H, *s*, Me-16), 1.15 (6H, *d*, *J* = 7.1 Hz, $CH(Me)_2$), 0.88, 0.84, 0.80, (3H, each *s*, Me-19, Me-18 and Me-20, respectively).

Methyl 15-*formyloxy*-7-*labden*-17-*oate* (**9**). Colourless oil. $[\alpha]_D^{22}$ − 56.5° ($CHCl_3$, *c* 1.21); IR ν_{max}^{film} cm^{-1}: 1745, 1650, 1260, 1200, 1090, 800. ^{1}H NMR: δ8.04 (1H, *s*, OOCH), 6.63 (1H, *m*, H-7), 4.19 (2H, *m*, H-15), 3.70 (3H, *s*), 0.85 (3H, *d*, *J* = 6.7 Hz, Me-16), 0.90, 0.86, 0.82 (3H, each *s* Me-19, Me-18 and Me-20, respectively).

Formylation of Compound **5**. Compound **5** (50 mg) was dissolved in HCOOH (0.5 ml) and kept for 1 min, following this it was extracted with Et_2O, then washed with H_2O and dried over Na_2SO_4. Evaporation of the solvent gave **9** (50 mg).

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