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LABDANE DITERPENOIDS FROM *HALIMIUM VISCOSUM*

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

Abstract—Three new diterpenoid acids isolated from the aerial parts of *Halimium viscosum* were identified as 15-acetoxy-7,13*E*-labdadien-17-oic acid, 15-hydroxy-7,13*E*-labdadien-17-oic acid and 14,15-dinor-13-oxo-7-labden-17-oic acid by spectroscopic and chemical correlation methods. They share the novel feature of containing a C-17 carboxyl group.

INTRODUCTION

The aerial parts of *Halimium viscosum* collected at La Fregenda on the SW border of the Salamanca province (200 km from the capital) with Portugal contain diterpenoid acids with a rearranged *ent*-labdane skeleton [1, 2]. We have now shown, somewhat to our surprise, that the aerial parts of this plant collected at Valparaíso (40 km to the north of Salamanca in the province of Zamora, Spain), where it grows abundantly, contain diterpenoid acids with a labdane skeleton. These are the first labdanic acids described in plants of the genus *Halimium* [1–3]. [A herbarium sample of both plants is available from the Dept. of Botany of the Faculty of Biology of the University of Salamanca, Spain. The chromosome number of both plants is the same ($2n = 18$).]

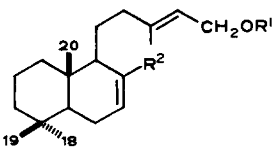
RESULTS AND DISCUSSION

The sodium hydroxide-soluble part of the hexane extract of *H. viscosum* was separated by column chromatography into three major fractions I (24 %), II (8 %) and III (49 %). Fraction III contained **1** as the principal component (85 %). Separation of **1** was not possible by chromatography either as the free acid or in the form of its methyl ester **4**. It was only possible to purify **1** by hydrolysis of its cyclohexylamine salt. Column chromatography of the methyl esters corresponding to fraction II gave **2**. Fraction I was composed of a mixture in which the major component (85 %) was **3**. Spectroscopic study of fraction I showed that it contained the mixture of acetates corresponding to the components of fraction III [IR: 1750, 1240; ^{1}H NMR: 4.56 (2H, *d*), 2.04 (3H, *s*)]. Alkaline hydrolysis of fraction I yielded a mixture of similar characteristics to that separated from fraction III.

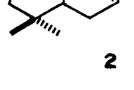
Compound **4** is an α,β-unsaturated hydroxyester (IR: 3420, 1720 and 1650 cm^{-1}). Its ^{13}C NMR spectrum shows signals for 21 carbons: five Me, seven CH$_2$, four CH and five completely substituted carbons. Its ^{1}H NMR spectrum shows signals corresponding to the following groups: –CH=C–COOMe (δ6.64, 1H, *m*; 3.71, 3H, *s*), –C(Me)=CH– (δ5.36, 1H, *tq*; 1.65, 3H, *d*), =CH–CH$_2$OH (δ4.13, 2H, *d*) and three Me–C (δ0.90, 0.86 and 0.82). On double radiation of the signal centred at δ5.36, the signals are simplified to δ1.65 and 4.13 such that the presence of the following group is inferred: –C(Me)=CH–CH$_2$OH. The appearance of this methyl group at δ16.40 in the ^{13}C NMR spectrum indicates the double bond configuration is *E* [4]. As has been mentioned, the natural compounds **1** and **3**, or their methyl esters **4** and **5**, are related as an alcohol and its acetyl derivative, respectively.

The mass spectrum of **5** exhibits the molecular ion at *m/z* 376 ($C_{23}H_{36}O_4$) corresponding to a bicyclic diterpene with two double bonds, an acetoxyl group and a methoxycarbonyl group. The ion of *m/z* 235 is formed by loss of a side chain, such as that of labdanes and clerodanes, containing the above mentioned allylic grouping. It corresponds to a decalin containing three methyls and a methoxycarbonyl conjugated with a trisubstituted double bond.

The presence of three methyl singlets in the decalin suggests a labdane skeleton for these compounds. The base peak at *m/z* 109 is typical of compounds with this



	R^1	R^2		R^1	R^2
1	H	COOH	**6**	THP	COOMe
3	Ac	COOH	**7**	THP	CH$_2$OH
4	H	COOMe	**8**	THP	Me
5	Ac	COOMe	**9**	H	Me



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skeleton unfunctionalized at ring A [5]. The methoxycarbonyl function must therefore be situated at C-8 conjugated with a trisubstituted double bond at C-7. With respect to the natural compounds **1** and **3**, they have the structure of 15-hydroxy-7,13*E*-labdadien-17-oic acid and 15-acetoxy-7,13*E*-labdadien-17-oic acid, respectively, which is shown upon transforming their carboxylic function into a methyl group, thereby obtaining the well known compound **9** [4].

Dihydropyrane treatment of **4** yields **6** which is transformed into **7** by reduction with lithium aluminium hydride. Treatment of **7** with ClMs and reduction of the reaction product yields **8** which on acid hydrolysis gives **9**.

The IR spectrum of **2**, apart from the band due to the α,β-unsaturated ester (1720 cm^{-1}), also shows another band corresponding to a carbonyl group (1730 cm^{-1}). The ^{1}H NMR spectrum of **2** shows a signal of a methyl group corresponding to Me–CO (δ2.11, 3H, *s*) besides the signals of the grouping –CH=C–COOMe (δ6.72, 1H, *m* and 3.69, 3H, *s*). The ^{13}C NMR spectrum of **2** reveals signals for 19 carbons: five Me, six CH$_2$, three CH and five completely substituted carbons of which three are sp^2 hybridized: –COMe (δ208.89), COOMe (169.21) and –C=CH (134.72).

The most outstanding differences between the ^{13}C NMR spectra of **2** and **4** or **5** is due to the displacements of the side chain carbons (Table 1).

Compound **2** is a dinorditerpene with its side chain partially degraded; its structure corresponds to methyl 14,15-dinor-13-oxo-7-labden-15-oic acid and this is confirmed upon obtaining **2** by oxidation of **4** with Jones reagent.

EXPERIMENTAL

Mps (Kofler hot stage apparatus) uncorr; ^{1}H NMR: 200 MHz, CDCl$_3$, TMS as int. standard; ^{13}C NMR: 50.3 MHz.

Extraction and isolation. The aerial part (1 kg) of *H. viscosum*, collected in Valparaíso (Zamora, Spain), was dried and extracted with *n*-hexane in a Soxhlet for 24 hr. The extract (76 g) was dewaxed with MeOH (15.6 %) and then extracted with 12 % Na$_2$CO$_3$ (33.6 %) and 4 % NaOH (39.1 %). The neutral fraction represented 21 % of the original extract.

CC of 21.5 g of the NaOH-soluble acid part gave three fractions: I (*n*-hexane–Et$_2$O, 1:1) (24 %), II (*n*-hexane–Et$_2$O, 3:7) (8 %), and III (*n*-hexane–Et$_2$O, 1:4) (49 %).

Cyclohexylamine salt of **1**. To 493 mg of fraction III dissolved in Et$_2$O, were added 152 mg of cyclohexylamine dropwise. The salt which formed was filtered off and recrystallized from CH$_2$Cl$_2$–Et$_2$O several times until colourless crystals were obtained with a mp 117–120°; $[\alpha]_D^{22}$ −29.2° (CHCl$_3$; *c* 1.24).

15-*Hydroxy*-7,13E-*labdadien*-17-*oic acid* (**1**). A soln of 140 mg of the cyclohexylamine salt of **1** in 3 ml of H$_2$O was acidified with 0.5 M HCl, and extracted with Et$_2$O to obtain 121 mg **1**. Colourless oil; $[\alpha]_D^{22}$ −46.6° (CHCl$_3$; *c* 0.82); IR ν_{max}^{film} cm^{-1}: 3400–2700, 1700, 1640, 1020, 840; ^{1}H NMR: δ6.86 (1H, *m*, H-7),

Table 1. ^{13}C NMR data of compounds **1–9*** (50.3 MHz, CDCl$_3$, TMS as internal standard)

C	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	39.53	39.33	39.49	39.56	39.53	39.49	39.13	39.30	39.92
2	18.60	18.48	18.57	18.60	18.57	18.57	18.82	18.90	18.88
3	42.10	42.08	42.10	42.14	42.12	42.12	42.32	42.47	42.44
4	32.80	32.76	32.84	32.86	32.85	32.79	32.98	33.01	33.01
5	49.44	49.39	49.47	49.58	49.50	49.55	50.05	50.36	50.33
6	24.20	23.98	24.30	24.01	24.22	23.96	23.72	23.93	23.91
7	139.56	137.97	140.43	137.05	137.96	136.73	125.08	122.32	123.56
8	134.82	134.72	134.50	135.45	134.58	135.50	139.47	135.36	135.25
9	50.64	50.33	50.64	50.82	50.91	50.81	51.73	54.61	54.68
10	37.02	36.99	37.07	37.04	37.08	36.96	36.76	36.92	36.94
11	26.85	21.91	26.90	26.08	26.99	26.94	25.14	25.61	25.67
12	41.09	45.49	41.25	41.09	41.12	41.12	41.28	42.15	42.11
13	140.66	208.89	143.24	140.68	143.26	140.76	140.76	140.42	140.33
14	122.83	—	118.02	123.15	118.04	120.53	120.98	121.09	122.41
15	59.13	—	61.51	59.47	61.50	62.18	62.21	62.24	59.45
16	16.17	29.73	16.39	16.29	16.40	16.37	16.55	16.51	16.37
17	173.78	169.21	173.91	169.70	169.31	169.57	65.74	22.14	22.12
18	33.17	33.10	33.15	33.16	33.15	33.13	33.13	33.18	33.18
19	21.98	21.91	21.96	21.97	21.96	21.93	21.87	21.86	21.86
20	14.47	14.08	14.40	14.38	14.39	14.32	13.67	13.64	13.63
COOMe		51.32		51.30	51.31	51.18			
Me–COO			21.03		21.03				
Me–COO			171.12		171.15				
2′						97.83	97.85	97.86	
3′						30.76	30.76	30.82	
4′						25.57	25.57	25.61	
5′						19.63	19.60	19.66	
6′						63.74	63.75	63.78	

*Assignments based on DEPT experiments and, particularly in the case of **4**, on C/H (HCCORR) two dimensional correlations.

5.37 (1H, *t*, J = 6.9 Hz, H-14), 4.10 (2H, *d*, J = 6.9 Hz, H-15), 1.64 (3H, *br s*, Me-16), 0.90, 0.86 and 0.82 (3H, each *s*, Me-19, Me-18 and Me-20 respectively).

Treatment of **1** with Ac_2O–C_5H_5N and CH_2N_2 gave **3** and **4** respectively.

Methyl 15-hydroxy-7,13E-labdadien-17-oate (**4**). Colourless oil. $[\alpha]_D^{22}$ −42.3° ($CHCl_3$; *c* 0.39); IR ν_{max}^{film} cm^{-1}: 3420, 1730, 1650, 1260, 1020, 840, 800, 760; ^{1}H NMR: δ6.64 (1H, *m*, H-7), 5.36 (1H, *tq*, J = 6.9, 1.2 Hz, H-14), 4.13 (2H, *d*, J = 6.9 Hz, H-15), 3.71 (3H, *s*, COOMe), 1.65 (3H, *d*, J = 1.2 Hz, Me-16), 0.90, 0.86, 0.82 (3H, each *s*, Me-19, Me-18 and Me-20 respectively).

15-Acetoxy-7,13E-labdadien-17-oic acid (**3**). Colourless oil. $[\alpha]_D^{22}$ −53.4° ($CHCl_3$; *c* 1.48); IR ν_{max}^{film} cm^{-1}: 3400–2700, 1750, 1700, 1650, 1240, 1040, 860; ^{1}H NMR: δ6.91 (1H, *m*, H-7), 5.31 (1H, *tq*, J = 7.1, 0.6 Hz, H-14), 4.56 (2H, *d*, J = 7.1 Hz), 2.04 (3H, *s*), 1.67 (3H, *d*, J = 0.6 Hz, Me-16), 0.90, 0.87, 0.82 (3H, each *s*, Me-19, Me-18 and Me-20 respectively).

Alkaline hydrolysis of **3** (24 mg, 2 ml, NaOH–MeOH 10%) yielded **1**.

Methyl 15-acetoxy-7,13E-labdadien-17-oate (**5**). Colourless oil. $[\alpha]_D^{22}$ −53.37° ($CHCl_3$; *c* 0.30); IR ν_{max}^{film} cm^{-1}: 1750, 1720, 1650, 1250, 1150, 860, 830, 790, 760; ^{1}H NMR: δ6.64 (1H, *m*, H-7), 5.33 (1H, *tq*, J = 7.0, 1.5 Hz, H-14), 4.56 (2H, *d*, J = 7.0 Hz, H-14), 3.71 (3H, *s*), 2.05 (3H, *s*), 1.68 (3H, *d*, J = 1.4 Hz, Me-16), 0.90, 0.86, 0.82 (3H, each *s*, Me-19, Me-18, Me-20 respectively); EIMS 70 eV, *m/z* (rel. int.): 376 $[M]^+$ (2), 316 (35), 285 (38), 270 (10), 249 (30), 235 (25), 176 (40), 153 (30), 125 (45), 109 (100), 105 (40), 92 (42), 43 (50), 41 (40).

Methyl 14,15-dinor-13-oxo-7-labden-15-oate (**2**). Treatment of 0.4 g of fraction II with CH_2N_2 gave 0.4 g of a mixture of Me esters, which was resolved by CC. Elution with *n*-hexane–Et_2O (4:1) gave **2** (274 mg); colourless oil, $[\alpha]_D^{22}$ −52.0° ($CHCl_3$; *c* 0.70). UV λ_{max}^{EtOH} nm (log ε): 215 (3.65). IR ν_{max}^{film} cm^{-1}: 1730, 1720, 1640, 1250, 1170, 1110, 980, 850, 820. ^{1}H NMR: δ6.72 (1H, *m*, H-7), 3.69 (3H, *s*), 2.81 (1H, *ddd*, J = 17.1, 11.2, 4.6 Hz, H_A-12), 2.33 (1H, *ddd*, J = 17.1, 11.2, 4.6 Hz, H_B-12), 2.11 (3H, *s*, Me-16), 0.90, 0.86, 0.81 (3H, each s, Me-19, Me-18, Me-20 respectively).

Oxidation of **4** *with Jones reagent.* Compound **4** (202 mg) was dissolved in 10 ml Me_2CO (previously distilled over $KMnO_4$). Jones reagent was added with vigorous shaking until the colour of the reagent persisted. The reaction mixture was then shaken for 7 hr at room temp., after which drops of MeOH were added slowly and the mixture was diluted with H_2O. The Me_2CO was evaporated off and the mixture extracted with Et_2O; it was washed with Na_2CO_3 and H_2O. After evaporating off the solvent, 120 mg of reaction product were obtained. Silica gel CC yielded 84 mg **2**.

Preparation of **6**. To 5 g of **4** dissolved in 15 ml dry C_6H_6 were added 2 ml dihydropyrane (3,4-dihydro-2H-pyrane) and 0.18 g *p*-toluenesulphonic acid. After shaking the mixture at room temp., 140 mg K_2CO_3 were then added and after 30 min the mixture was filtered and evaporated to obtain 6 g of reaction product, which on CC gave 4.3 g (*n*-hexane–Et_2O, 9:1) **6**. Colourless oil. IR ν_{max}^{film} cm^{-1}: 1730, 1660, 1260, 1090, 1050, 1040, 920, 880, 820; ^{1}H NMR: δ6.56 (1H, *m*, H-7), 5.26 (1H, *t*, J = 6.5 Hz, H-14), 4.54 (1H, *m*, H′-2), 4.13 (1H, *dd*, J_{AB} = 12.2, J = 6.5 Hz, H_A-15), 3.93 (1H, *dd*, J_{AB} = 12.2, J = 6.5 Hz, H_B-15), 3.63 (3H, *s*), 1.59 (3H, *s*, Me-16), 0.83, 0.79, 0.75 (3H, each *s*, Me-19, Me-18, Me-20 respectively).

Reduction of **6** *with* $LiAlH_4$. $LiAlH_4$ (26 mg, 50% excess) was added to 624 mg **6** dissolved in 5 ml Et_2O. The reaction mixture was kept at room temp. for 1 hr. Usual work up gave 520 mg of reduction product which on silica gel CC (*n*-hexane–Et_2O, 4:1) yielded **7** (421 mg). Colourless oil. IR ν_{max}^{film} cm^{-1}: 3420, 1670, 1210, 1150, 1140, 1040, 920, 880, 825; ^{1}H NMR: δ5.74 (1H, *m*, H-7), 5.37 (1H, *t*, J = 6.4 Hz, H-14), 4.61 (1H, *m*), 1.68 (3H, *s*, Me-16), 0.87, 0.85, 0.74 (3H, each *s*, Me-19, Me-18, Me-20 respectively).

Preparation and reduction of the mesylate. Et_3N (0.15 ml) was added to 290 mg **7** in 3 ml CH_2Cl_2 and the mixture left to cool to between −10° and 0°. $MeSO_2Cl$ (0.06 ml) was added and the product kept at room temp. for 4 hr. It was then washed with ice-water, 2 M HCl and then again with H_2O. The reaction product (222 mg) was dissolved in Et_2O (2 ml) and treated with 6 mg $LiAlH_4$. The mixture was shaken for 4 hr to yield 198 mg of reaction product which on silica gel CC (*n*-hexane–Et_2O, 9:1) gave 51 mg **8**. Colourless oil. IR ν_{max}^{film} cm^{-1}: 1200, 1150, 1120, 1030, 920, 880, 820; ^{1}H NMR: δ5.36 (1H, *t*, J = 6.3 Hz, H-14), 4.62 (1H, *m*), 4.23 (1H, *dd*, J_{AB} = 12.2, J = 6.3 Hz, H_A-15), 4.00 (1H, *dd*, J_{AB} = 12.2, J = 6.3 Hz, H_B-15), 3.87 (1H, *m*, H_A-6′), 3.50 (1H, *m*, H_B-6′), 1.68 (6H, *s*, H-16 and H-17), 0.86, 0.84y, 0.74 (3H, each *s*, Me-19, Me-18, Me-20 respectively).

Hydrolysis of **8**. *p*-Toluenesulphonic acid (2.5 g) was added to 35 mg **8** dissolved in 3 ml MeOH and the mixture shaken for 1 hr at room temp. Following this, Et_2O was added and the product washed with $NaHCO_3$ and H_2O. After evaporating off the solvent, 30 mg of reaction product were obtained. Silica gel CC (*n*-hexane–Et_2O, 9:1) gave 28 mg **9**. Colourless oil. $[\alpha]_D^{22}$ + 5° ($CHCl_3$; *c* 0.98); IR ν_{max}^{film} cm^{-1}: 3360, 1670, 1010; ^{1}H NMR: δ5.42 (1H, *t*, J = 6.8 Hz, H-14), 5.40 (1H, *m*, H-7), 4.15 (2H, *d*, J = 6.8 Hz, H-15), 1.69 (3H, *s*, H-16), 1.68 (3H, *s*, H-17), 0.87, 0.84, 0.74 (3H, each *s*, Me-19, Me-18, Me-20 respectively).

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