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LABDANE DITERPENES FROM *HALIMIUM VISCOSUM*

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Key Word Index—*Halimium viscosum*; Cistaceae; labdanes; diterpenes; 7-labden-3β,15-diol; X-ray.

Abstract—Three labdane diterpenes have been isolated from *Halimium viscosum*, and their structures determined by spectroscopic methods and chemical correlations. The structure for the major compound 7-labden-3β,15-diol has been confirmed by X-ray studies and its absolute stereochemistry determined by CD of its 3-oxo derivative.

INTRODUCTION

In previous papers [1–4] we have reported on the phytochemical study of three chemotypes of *Halimium viscosum*. The chemotype from Villarino de los Aires afforded *ent*-halimanic acids [5, 6] with an unsaturated or degraded side-chain and a series of valparenes and one valparolane [7]. Now we report on the isolation of three labdanes (**1**, **3** and **5**). Their structures have been determined by spectroscopic methods and chemical correlations. The structure for the major compound (**5**) has been confirmed by X-ray studies.

RESULTS AND DISCUSSION

Compound **1** showed absorptions of hydroxyl and carbonyl groups in the IR spectra. The ^{13}C NMR spectrum showed signals corresponding to 20 carbon atoms, five quaternary (two olefinic and one carbonyl group) and 15 protonated sorted by DEPT multiplicity into five methyl groups, six methylenes and four methines (two of them olefinic). In the ^{1}H NMR spectrum, in addition to the signals corresponding to three aliphatic methyl singlets (δ 1.08, 1.04 and 0.98), there were signals corresponding to the following groupings: Me–C=CH- (δ 1.68, 3H, *s*; 5.38, 1H, *m*) and Me–C=CH–CH_2OH (δ 1.71, 3H, *s*; 5.38, 1H, *m*; 4.12, 2H, *d*, J = 6.8 Hz). When **1** was acetylated affording **2**, the *gem*-hydrogens of the acetoxyl group suffered a deshielding and moved to δ4.58. All these spectroscopic properties are in agreement with a labdane skeleton with a double bond in the side chain and another one at C-7. The stereochemistry of the side-chain double bond was established as *E* by virtue of the Me-16 chemical shifts (δ_H 1.71; δ_C 16.4) [8]. The carbonyl group at C-3 was confirmed by reduction of **1** with LiAlH affording 7,13*E*-labdadien-3β,15-diol (**6**) previously isolated from *H. umbellatum* [9]. Therefore, **1** possessed the structure 3-oxo-7,13*E*-labdadien-15-ol.

Compound **3** was isolated as its acetate **4** by acetylation of the fractions containing **3**. Compound **4** showed in its ^{1}H NMR spectrum, in addition to the signals corresponding to three aliphatic methyl singlets (δ 0.92, 0.84 and 0.79), the signals corresponding to the following groupings: AcO–CH– (δ2.04, 3H, *s*: 4.49, 1H, *m*), Me–CO– (δ2.12, 3H, *s*) and Me–C=CH– (δ1.65, 3H, *s*; 5.41, 1H, *m*). The ^{13}C NMR spectrum showed only the signals of 20 carbon atoms (six methyl groups, five methylenes, four methines and five quaternary carbon atoms), hence **3** must be a di*nor* derivative.

The structure and stereochemistry of 14,15-di*nor*-3β-acetoxy-7-labden-13-one for **3** was established by chemical correlation with **6**. Sharpless expoxidation of **6** [L (+)DET] [10] gave epoxide **7** which after treatment with H_6IO_5 and subsequent acetylation afforded **3**.

The major component was isolated by crystallization and identified as 7-labden-3β,15-diol (**5**) by comparison with an authentic sample previously isolated from *H.*

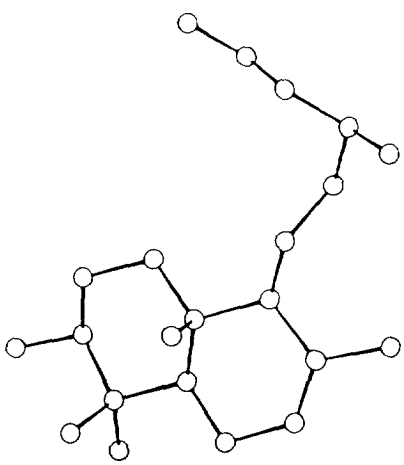
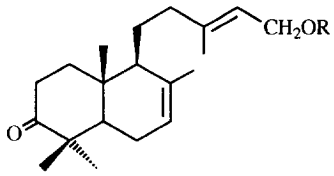


Fig. 1. Structure of **5**.



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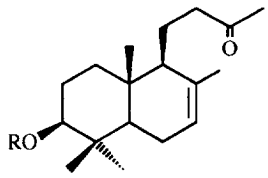

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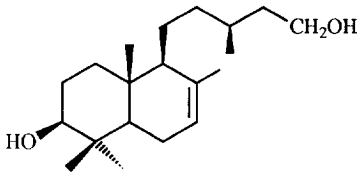
4 R= Ac



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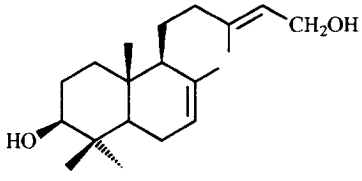

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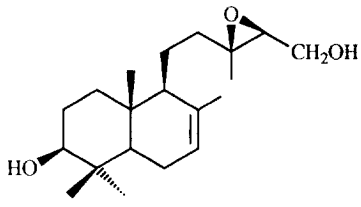

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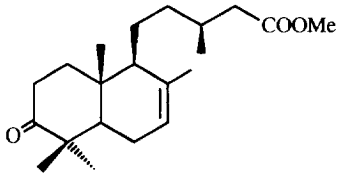

8

umbellatum [9]. Because, some time ago, it was argued that this compound does not belong to the normal labdane series [11] and also because the C-13 stereochemistry was not determined, an X-ray diffraction study was carried out to confirm the structure. The results are shown in Fig. 1.

The absolute stereochemistry of **5** was confirmed by a CD study of methyl 3-oxo-7-labden-15-oate (methyl-3-oxo-cativate) (**8**) [12, 13] obtained by oxidation of **5** with Jones reagent and subsequent esterification with CH_2N_2. A negative Cotton effect ($\Delta\varepsilon_{294} = -0.67$) confirmed that **8** and **5** belong to the normal labdane series.

EXPERIMENTAL

Spectral analysis. NMR: 200 MHz (^{1}H) and 50.3 MHz (^{13}C), chemical shifts are given in ppm and are referenced in $CDCl_3$ to the residual $CHCl_3$, 7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C, respectively, unless otherwise stated; Mp: uncorr.; EI-MS: 70 eV.

Extraction and isolation. The extraction of the aerial parts of *Halimium viscosum* (Villarino) and fractionation of the neutral part was performed as in an earlier study [7]. The more polar fraction E (50%) is the one studied now. Compound **5** (2 g) was isolated from fraction E by crystallization in C_6H_6. The mother liquor afforded **1** (130 mg) by CC on silica gel–$AgNO_3$ (10%) eluting with hexane–EtOAc (7:3). Further elution with hexane–EtOAc (1:1) gave 105 mg of a mixture that by acetylation with Ac_2O (0.5 ml) and pyridine (0.5 ml) and subsequent CC (hexane–EtOAc, 4:1) afforded **4**.

3-Oxo-7,13E-labdadien-15-ol (**1**). Oil. $[\alpha]_D$ −13.9° ($CHCl_3$; *c* 0.82). IR ν_{max}^{film} cm^{-1}: 3400, 1720, 1650, 1100 and 910; ^{1}H NMR: δ5.38 (2H, *m*, H-7, H-14), 4.12 (2H, *d*, *J* = 6.8 Hz, H-15), 1.71 (3H, *s*, Me-16), 1.68 (3H, *s*, Me-17), 1.08, 1.04 and 0.98 (3H, *s*, ea); ^{13}C NMR: Table 1. Acetate **2**: oil, IR ν_{max}^{film} cm^{-1}: 1720 and 1460; ^{1}H NMR: δ5.38 (2H, *m*, H-7 and H-14), 4.58 (2H, *d*, *J* = 6.8 Hz, H-15). 2.04 (3H, *s*, -OCOMe), 1.70 (6H, *s*, ea, Me-16 and Me-17), 1.08 (3H, *s*, Me-18), 1.04 (3H, *s*, Me-19), 0.98 (3H, *s*, Me-20); ^{13}C NMR: Table 1.

Reduction of compound **1**. $LiAlH_4$ (22 mg) was added to a stirred soln of **1** (110 mg) in dry Et_2O (8 ml) and the mixture was stirred for 1 hr at room temp. under N_2. Then Et_2O (40 ml), some drops of water and Na_2SO_4 (200 mg) were added, and the mixture left for 15 min. Then, it was filtered and the solvent evapd off to give **6**: 7,13*E*-labdadien-3β,15-diol (105 mg). Oil. IR ν_{max}^{film} cm^{-1}: 3400, 1650, 1230, 1110 and 1020; ^{1}H NMR: δ5.38 (2H, *m*, H-7 and H-14), 4.12 (2H, *d*, *J* = 6.8 Hz, H-15), 3.21 (1H, *m*, H-3), 1.66 (6H, *s*, Me-16 and Me-17), 0.94, 0.82 and 0.73 (each 3H, *s*); ^{13}C NMR: Table 1.

14,15-Dinor-3β-acetoxy-7-labden-13-one (**4**). Obtained by acetylation of the fraction containing **3**. Oil. $[\alpha]_D$

Table 1. ^{13}C NMR data of compounds **1**, **2**, **4**–**6** and **8**

C	1	4	5	2	6	8
1	38.0	37.2	36.8	38.0	37.4	38.0
2	34.6	23.9	27.5	34.5	27.5	34.6
3	216.0	81.0	79.2	216.0	79.2	218.4
4	47.4	37.6	38.7	47.4	38.7	47.3
5	51.5	49.9	49.8	51.5	49.8	51.3
6	24.1	23.3	23.5	24.1	23.5	24.1
7	122.1	122.6	122.0	122.1	122.3	121.9
8	135.3	134.5	135.3	135.3	135.1	135.4
9	53.7	54.3	55.4	53.5	54.5	54.4
10	36.6	36.7	37.4	36.6	36.8	36.7
11	25.7	20.9	24.5	25.5	25.6	24.8
12	41.8	45.7	39.7	41.7	42.0	41.3
13	139.6	208.9	30.6	142.1	139.9	31.2
14	124.0	—	40.0	119.0	123.8	39.1
15	59.4	—	61.2	61.3	59.3	173.4
16	16.4	29.8	19.8	16.6	16.4	19.9
17	21.9	21.9	21.9	21.9	21.9	21.8
18	25.2	27.9	27.9	25.1	27.9	25.1
19	22.1	16.2	15.1	22.1	15.1	22.1
20	13.4	13.7	13.7	13.4	13.7	13.4
Me-CO_2		170.8		171.9		
Me-CO_2		21.2		20.9		
CO_2Me						51.4

+ 25.5° ($CHCl_3$, *c* 0.54). IR ν_{max}^{film} cm^{-1}: 1740, 1720, 1640, 1240, 1160, 1090, 1030, 920, 910 and 830; ^{1}H NMR: δ5.41 (1H, *m*, H-7), 4.49 (1H, *m*, H-3), 2.12 (3H, *s*, H-16), 2.04 (3H, *s*, OCOMe), 1.65 (3H, *s*, Me-17), 0.92, 0.84 and 0.79 (each 3H, *s*); ^{13}C NMR: Table 1.

Sharpless epoxidation of compound **6**. A 50 ml flask equipped with a Teflon-coated magnetic stirring bar was oven-dried, then flushed with N_2. The flask was charged with dry CH_2Cl_2 (3 ml) and cooled with stirring in a − 23° bath. The following liquids were then added sequentially via syringe while stirring in the cooling bath: titanium tetraisopropoxide (0.09 ml, 0.3 mmol), L-(+)-diethyltartrate [L-(+)-DET, 0.05 ml, 0.3 mmol]: after 10 min **6** (91 mg) in dry CH_2Cl_2 (4 ml) was added, the mixture left for 10 min and, finally, 3 M *t*-butyl hydroperoxide (0.21 ml) was added. The resulting homogeneous soln was stored in a sealed reaction vessel in the freezer (14 hr, − 20°). The flask was then placed in a − 23° bath and 10% aq. tartaric acid soln (0.75 ml) was added with stirring; the aq. layer solidified. After 30 min, the cooling bath was removed and stirring was continued at room temp. until the aq. layer became clear. After separation of the aq. layer, the organic layer was washed once with H_2O, dried with Na_2SO_4 and concd to give **7** (68 mg). Oil ^{1}H NMR: δ5.30 (1H, *m*, H-7), 3.80 and 3.60 (2H, *m*, H-15), 3.20 (1H, *m*, H-3), 2.90 (1H, *m*, H-14), 1.60 (3H, *s*, Me-17), 1.27 (3H, *s*, Me-16), 0.93, 0.81 and 0.73 (each 3H, *s*).

Oxidation of **7** *with* H_5IO_6 *and subsequent acetylation.* Compound **7** (68 mg) was dissolved in dry THF (0.4 ml), then a soln of H_5IO_6 (70 mg), in THF (0.80 ml) and H_2O (0.55 ml), was added dropwise. The reaction mixture was stirred for 6 hr then extracted with Et_2O. The Et_2O extract was washed with 10% $NaHCO_3$ and H_2O, dried (Na_2SO_4) and concd *in vacuo* to give 58 mg of crude product which was acetylated in the usual way affording **4** (54 mg).

7-Labden-3β,15-diol (**5**). Crystals, mp 111–112°. $[\alpha]_D$ − 5.2° ($CHCl_3$, *c* 1.40). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300, 1640, 1480, 1230 and 1050; ^{1}H NMR: δ5.35 (1H, *m*, H-17), 3.63 (2H, *m*, H-15), 3.20 (1H, *m*, H-3), 1.64 (3H, *s*, Me-17), 0.93 (3H, *s*, Me-18), 0.91 (3H, *d*, J = 6.8 Hz, Me-16), 0.82 and 0.72 (each 3H, *s*); ^{13}C NMR: Table 1; EI-MS *m/z* (rel. int.): 308 $[M]^+$ (15), 290 (16), 275 (12), 208 (65), 207 (64), 190 (36), 189 (70), 135 (57), 121 (100), 107 (74), 83 (26), 81 (57), 69 (19).

X-Ray data for compound (**5**). $C_{20}H_{36}O_2$, M = 308.5, monoclinic, a = 11.103 (1), b = 12.603 (1), c = 27.701 (2) Å, β = 97.323 (8)°, U = 3845 Å [12] (by least squares refinement of the diffractometer angles for 25 accurately centred reflections, λ = 1.5418 Å), space group C2, Z = 8, D_x = 1.07 g cm^{-3}, crystal dimensions = 1.00 × 0.50 × 0.70 mm, μ (CuK_α) = 4.77 cm^{-1}.

Data were collected on a CAD4 diffractometer using graphite-monochromated CuK_α and an ω-2θ scan mode. 4809 reflections were measured ($0 \leq \theta \leq 72°$, $-13 \leq h \leq 15$, $-1 \leq k \leq 15$, $-1 \leq l \leq 34$) of which 3959 were unique [merging R = 0.02 after an empirical absorption correction based on azimuthal scan data (min., max. correction − 1.17, 1.46)] and 3271 were observed [$I \geq 3\sigma(I)$].

The structure was solved by direct methods [14] and Fourier syntheses. Refinement was by full matrix least squares with anisotropic thermal parameters for all non-hydrogen atoms. The hydrogen atoms were included in the model at geometrically idealized positions and were

not refined. A 3 term Chebychev polynomial weighting scheme [15] was employed and yielded a satisfactory agreement analysis. The refinement converged with $R = 0.052$ and $R_w = 0.063$ for 397 parameters. All calculations were performed on the Chemical Crystallography Laboratory VAX computer using the Oxford CRYSTALS program package [16].

Oxidation of **5** *with Jones reagent and subsequent esterification.* Compound **5** (50 mg) was dissolved in Me_2CO (6 ml) then 12 drops of Jones reagent were added. After 5 min the soln was cooled to 0° and some drops of i-PrOH and H_2O added. The soln was then concd *in vacuo* and extracted with Et_2O. The organic phase was washed with Na_2CO_3 and H_2O, dried (Na_2SO_4) and concd *in vacuo* to give 48 mg. The crude product obtained was esterified with a saturated soln of CH_2N_2 in Et_2O and the mixture left for 12 hr. The solvent was removed to give **8** (50 mg), Mp 60–61° (hexane). $[\alpha]_D$ −42.7° ($CHCl_3$; *c* 1.10). IR ν_{max}^{film} cm^{-1}: 1740, 1710, 1640, 1200, 1160, 1040, 1000 and 840; ^1H NMR: δ5.39 (1H, *m*, H-7), 3.62 (3H, *s*, OMe), 1.62 (3H, *s*, Me-17), 1.06 (3H, *s*, Me-19), 1.01 (3H, *s*, Me-20), 0.96 (3H, *s*, Me-18), 0.94 (3H, *d*, $J = 8.7$ Hz, Me-16); ^{13}C NMR: Table 1. EI-MS *m/z* (rel. int.): 334 $[M]^+$ (8), 319 (4), 303 (3), 249 (70), 205 (35) 121 (100), 55 (95). CD: $\Delta\varepsilon_{294}$ − 0.67.

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