Phytochemistry, Vol. 24, No. 4, pp. 791–794, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

DITERPENOIDS OF *HALIMIUM VISCOSUM*

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(*Revised received* 10 *July* 1984)

Key Word Index—*Halimium viscosum*; Cistaceae; diterpenes.

Abstract—From the neutral fraction of the hexane extract of *Halimium viscosum* the following components were isolated; 7-labdene-3β,15-diol, 15-acetoxy-7-labden-3β-ol and a new diterpene-lactone with a rearranged *ent*-labdane skeleton, 13*S*-*ent*-9, 1-friedo-labd-1(10)-en-15-acetoxy-2*R*,18-olide. From the non-saponifiable part, beside 7-labdene-3β,15-diol and 7,13*E*-labdadiene-3β,15-diol, the new diterpene 8(17)-labdene-3β,7α,15-triol was extracted. The structures were elucidated by spectroscopic methods, correlations or synthesis.

INTRODUCTION

Halimium viscosum is found in the West of the Iberian Peninsula. It is morphologically very similar to *H. umbellatum*.

The components of both plants are bicyclic diterpenes, principally labdanes of the normal series in the neutral fractions and rearranged *ent*-labdanes in the acid fractions [1–3].

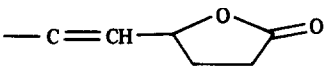
RESULTS AND DISCUSSION

The neutral fraction of the hexane extract of *H. viscosum* (38.5% of the original extract) was fractioned by dry chromatography into four fractions of increasing polarity: I (40%), II (20%), III (21%) and IV (16%). The least polar fraction (I) was composed of a very complex mixture with a predominance in linear chain compounds such that its study was carried out after saponification.

CC of fraction II yielded a compound (**2**) whose IR spectrum showed the presence of a hydroxyl group(s) (3440, 1060 cm^{-1}), an acetoxyl group(s) (1730, 1240 cm^{-1}) and a double bond(s) (1640 cm^{-1}). The ^1H NMR spectrum of **2** showed signals of the following groups: H–C= (δ5.40, 1H, *m*), CH_2–CH_2OAc (δ4.10, 2H, *t*, J = 6 Hz), CH_2–CHOH–C (δ3.25, 1H, *m*, $W_{1/2}$ = 17 Hz), Me–COO (δ2.01, 3H, *s*), Me–C= (1.65, 3H, *s*) and four methyl groups (three Me–C and one Me–CH). The mass spectrum of **2** (M^+ at *m/z* 350, $C_{22}H_{38}O_3$) corresponded to that of a bicyclic diterpene with a hydroxyl group, an acetoxyl group and a double bond.

Alkaline hydrolysis of **2** yielded **1**, isolated from fraction I of the chromatography by crystallization in benzene. Treatment of **1** and **2** with acetic anhydride and pyridine gave rise to the same acetyl derivative **3** [1]. **1** and **3** were identified by comparison with authentic samples.

CC of fraction III yielded an unsaturated acetoxy-γ-lactone **4** (IR 3080, 1650, 1790, 1760, 1250 cm^{-1}) which in its ^1H NMR spectrum showed signals of the following groups: H–C= (δ5.86, 1H, *d*, J = 6 Hz), HC–OOC– (δ4.68, *m*, $W_{1/2}$ = 17 Hz), CH_2–CH_2OAc (δ4.05, 2H, *t*, J = 6 Hz), Me–COO (δ2.01, 3H, *s*) and four methyl groups, two Me–C (at δ1.21 and 0.90) and two Me–CH. On decoupling the ^1H NMR signal centred at δ4.68, the doublet (δ5.86) was changed into a singlet whilst on irradiation at δ5.86 the multiplet at 4.68 was transformed into an apparent triplet, confirming the presence of the grouping:



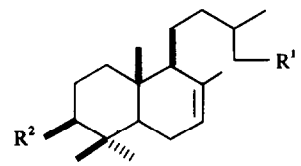
In the mass spectrum of **4** (M^+ at *m/z* 362, $C_{22}H_{34}O_4$) the base peak at *m/z* 219 corresponded to the loss of $C_8H_{15}O_2$, the side chain of a bicyclic diterpene in which the primary acetoxyl and one of the Me–CH were situated.

The structure of **4** was established by reduction with lithium aluminium hydride to yield the triol **5**.

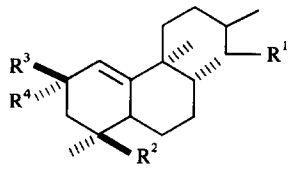
Oxidation of the methyl ester of acetyl hydrohalimic acid (**7**) with sodium chromate yielded an α,β-unsaturated ketone (**8**) which when reduced with lithium aluminium hydride yielded similar amounts of the diol **9** and the triol **5**. Treatment of **5** with acetic anhydride and pyridine at room temperature gave the monoacetate **6**.

Bearing in mind the β configuration of the methoxycarbonyl group of C-4 in **7**, the hydroxymethylene groups of C-4 in **5**, **8** and **9** should show the same configuration. As **5** originates from the reduction of the lactone **4**, necessarily *cis*, the configuration of C-2 in **4** and **5** is *R*.

The structure corresponding to **4** is 13*S*-*ent*-9,1-friedolabd-1(10)-en-15-acetoxy-2*R*,18-olide.



	R^1	R^2	
1	CH_2OH	OH	
2	CH_2OAc	OH	
3	CH_2OAc	OAc	
12	CH_2OAc	OAc	Δ^{13}



	R^1	R^2	R^3	R^4
5	CH_2OH	CH_2OH	OH	H
6	CH_2OAc	CH_2OH	OH	H
7	CH_2OAc	COOMe	H	H
8	CH_2OAc	COOMe	O	
9	CH_2OH	CH_2OH	H	H
10	CH_2OH	COOMe	H	OH
11	CH_2OH	CH_2OH	H	OH

The signal in the ^1H NMR spectrum of **5** corresponding to the hydrogen over C-2 (*dd*, $J_1 = 4$ Hz, $J_2 = 6$ Hz) required that it should form an angle of 90° with one of the hydrogens on C-3. This required a half-boat conformation for ring A, in which the hydroxymethylene group of C-4 appears as pseudo-equatorial.

The determination of the β configuration of the hydroxyl group at C-2 of **5** means that we have to correct the assigned stereochemistry of 2-hydroxyhalimic acid (**10**) [2]. Thus oxidation of **10** with manganese dioxide yielded **8** whilst reduction of **10** with lithium aluminium hydride gave the triol **11** which is the C-2 epimer of **5**. These results meant that the configuration of the hydroxyl group at C-2 of **11** is *S*. This is in agreement with the signals of the olefinic hydrogens in the ^1H NMR spectra of **5** and **11** which appear as a doublet of $J = 6$ Hz and a singlet, respectively.

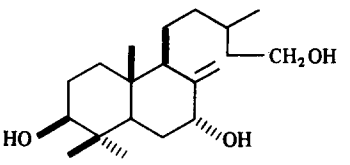
The neutral fraction of the hexane extract contained mainly esters which were not readily separated. It was saponified and the unsaponifiable extract subjected to CC to give **1**, **13** and a mixture from which **12** [1] was separated after treatment with acetic anhydride and pyridine by preparative TLC.

The unsaturated alcohol **13** (IR 3340, 3080, 1640, 900 cm^{-1}) was separated from the more polar fraction. Its ^1H NMR spectrum contained signals for the following groups: $CH_2=$ (δ4.95 and 4.58 each, 1H, *s*), CH_2–CHOH–C= (δ4.30, 1H, *br*, *s*). CH_2–CH_2OH (δ3.58, 2H, *t*, $J = 6$ Hz), CH_2–CHOH–C (δ3.22, 1H, *m*, $W_{1/2} = 17$ Hz), and four methyl groups (three Me–C and one Me–CH). In its mass spectrum the highest peak (*m/z* 306) corresponded to $[M-18]^+$. Thus **13** was an unsaturated bicyclic diterpene triol with the molecular formula $C_{20}H_{36}O_3$. The base peak (*m/z* 83) corresponded to the $[C_6H_{13}O-H_2O]^+$ fragment of the side chain of a labdane [4].

The structure of 8(17)-labdene-3β,7α,15-triol for **13** was confirmed by synthesis from **1**.

The reaction product of **1** with *m*-chloroperbenzoic acid, when crystallized from ethyl acetate, yielded **14** and after acetylation the residue yielded **15** and **16**. The α stereochemistry of the major oxiran **14** and its high yield were in agreement with the ready access of the reagent to the substrate and such a stereochemistry was established on the basis of the hydrogen signal at C-7 of **15** and **16** in the ^1H NMR spectrum.

Treatment of **14** with lithium diethylamide [5] yielded **13**.



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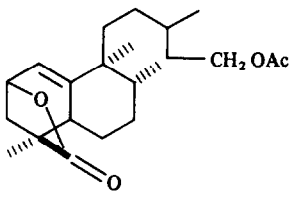

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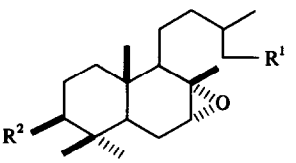
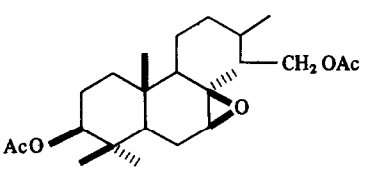


	R^1	R^2
14	CH_2OH	OH
15	CH_2OAc	OAc



16

EXPERIMENTAL

Mps (Kofler hot stage apparatus): uncorr. ^1H NMR: CCl_4, TMS as int. standard; TLC, silica gel G; prep. TLC: silica gel $PF_{254+366}$; and CC: silica gel 60.

Extraction and isolation. The aerial parts of *H. viscosum* (10 Kg) collected in la Fregeneda (Salamanca),* were dried and extracted with *n*-hexane in a Soxhlet for 24 hr to give 673 g of extract. This was dewaxed with MeOH (18%) and then extracted with 6% $NaHCO_3$ (5.3%), 12% Na_2CO_3 (32.4%) and 4% NaOH (13.6%). The neutral fraction remaining represented 38.5% of the original extract.

*The plant material (Halimium viscosum) was identified by Professor B. Casaseca Mena. A herbarium specimen is deposited at the Department of Botany, Fac. Sciences, University of Salamanca, Spain.

A portion (10 g) of the neutral fraction was dry-chromatographed (500 g silica gel, 5 × 100 cm. column, C_6H_6–Et_2O, 1:1) to give four fractions (I–IV).

CC of fraction II on silica gel (C_6H_6–Et_2O, 1:1) gave **2**, whilst CC of fraction III (after removal of β-sitosterol by crystallization) on silica gel (C_6H_6–Et_2O, 9:1) gave **5**. Crystallization in C_6H_6 (40% of the neutral fraction) of fraction IV yielded **1**.

7-Labdene-3β,15-diol (**1**). Mp 111–112° $[\alpha]_D^{22}$ − 5.2° ($CHCl_3$; *c* 1.40); IR ν_{max}^{KBr} cm^{-1}: 3340, 1640, 1060, 1020, 1000, 960, 910, 810. ^1H NMR: δ5.40 (1H, *m*), 3.65 (2H, *t*, *J* = 6 Hz), 3.20 (1H, *m*, $W_{1/2}$ = 17 Hz), 1.67 (3H, *s*), 0.98 (3H, *s*), 0.85 (3H, *s*), 0.75 (3H, *s*); EIMS 70 eV, *m/z* (rel. int.): 310 $[M]^+$ (3), 290 (16), 275 (12), 208 (65), 207 (64), 190 (36), 189 (70), 135 (57), 121 (100), 107 (74), 83 (26), 81 (57), 69 (19).

15-Acetoxy-7-labden-3β-ol (**2**). Colourless oil. $[\alpha]_D^{22}$ − 3.6° ($CHCl_3$; *c* 0.55); IR $\nu_{máx}^{film}$ cm^{-1}: 3440, 1745, 1730, 1640, 1240, 1060, 1050, 1040, 1020, 960; ^1H NMR: δ5.40 (1H, *m*), 4.10 (2H, *t*, *J* = 6 Hz), 3.25 (1H, *m*, $W_{1/2}$ = 17 Hz), 2.01 (3H, *s*), 1.65 (3H, *s*), 0.98 (3H, *s*), 0.85 (3H, *s*), 0.76 (3H, *s*); EIMS 70 eV, *m/z* (rel. int.): 350 $[M]^+$ (2), 207 (4), 189 (5), 150 (12), 149 (100), 140 (2), 121 (15), 107 (7), 83 (8), 81 (10), 69 (9).

Alkaline hydrolysis of **2** (100 mg, 5 ml NaOH–MeOH 10%) yielded **1**.

3β,15-Diacetoxy-7-labdene (**3**). Colourless oil. $[\alpha]_D^{22}$ + 3.5° ($CHCl_3$, *c* 1.14); IR $\nu_{máx}^{film}$ cm^{-1}: 1730, 1640, 1240, 1020, 970, 920; ^1H NMR: δ5.40 (1H, *m*), 4.52 (1H, *m*, $W_{1/2}$ = 17 Hz), 4.10 (2H, *t*, *J* = 7 Hz), 2.05 (6H, *s*), 1.69 (3H, *s*), 0.93 (3H, *s*), 0.87 (3H, *s*), 0.80 (3H, *s*).

(13S)-ent-9,1-Friedolabd-1(10)-en-15-acetoxy-2R,18-olide (**4**). Colourless oil. $[\alpha]_D^{22}$ + 33.3° ($CHCl_3$; *c* 1.95); IR $\nu_{máx}^{film}$ cm^{-1}: 3080, 1790, 1760, 1650, 1250, 1110, 1090, 970, 950, 800; ^1H NMR: δ5.86 (1H, *d*, *J* = 6 Hz), 4.68 (1H, *m*, $W_{1/2}$ = 17 Hz), 4.05 (2H, *t*, *J* = 6 Hz), 2.01 (3H, *s*), 1.21 (3H, *s*), 0.90 (3H, *s*), 0.80 (6H, *d*, *J* = 6 Hz); EIMS 70 eV, *m/z* (rel. int.): 362 $[M]^+$ (1), 328 (3), 219 (15), 175 (100), 173 (72), 161 (17), 159 (22).

Reduction of **4** *with* $LiAlH_4$. $LiAlH_4$ (60 mg) suspended in 2 ml Et_2O was added to 60 mg **4** dissolved in 3 ml dry Et_2O. The mixture was refluxed for 1 hr and then worked up by normal procedures to give 48 mg **5**. Colourless oil. IR $\nu_{máx}^{film}$ cm^{-1}: 3360, 3070, 1640, 1060, 1030, 1020, 990, 900, 880, 845, 790, 750; ^1H NMR: δ5.65 (1H, *d*, *J* = 6 Hz), 4.29 (1H, *dd*, J_1 = 4 Hz, J_2 = 6 Hz), 3.68 (2H, *t*, *J* = 6 Hz), 3.51 (2H, *s*), 1.10 (3H, *s*), 0.90 (3H, *d*, *J* = 6 Hz), 0.88 (3H, *s*), 0.78 (3H, *d*, *J* = 7 Hz).

Acetylation of **5**. **5** (48 mg) was treated with 1 ml Ac_2O and 1 ml C_5H_5N. The acetylated product (50 mg) was recovered and purified by prep. TLC (C_6H_6–Et_2O, 19:1), to give 30 mg **6**. Colourless oil. $[\alpha]_D^{22}$ + 48.8° ($CHCl_3$, *c* 1.03); IR $\nu_{máx}^{film}$ cm^{-1}: 3040, 1740, 1640, 1240, 1040, 1000, 925, 880, 790, 730; ^1H NMR: δ5.60 (1H, *d*, *J* = 6 Hz), 4.30 (1H, *dd*, *J* = 4 Hz, *J* = 6 Hz), 4.00 (2H, *t*, *J* = 6 Hz), 3.50 (2H, *s*), 2.00 (3H, *s*), 1.10 (3H, *s*), 0.91 (3H, *d*, *J* = 6.5 Hz), 0.89 (3H, *s*), 0.75 (3H, *d*, *J* = 7 Hz).

Oxidation of **7** *with* Na_2CrO_4. To 437 mg **7** dissolved in 3 ml C_6H_6 were added 226 mg dry Na_2CrO_4, 3.8 ml Ac_2O and 375 mg dry NaOAc. When the reaction was finished H_2O was added and after 1 hr the mixture was extracted with Et_2O. The ethereal extract was washed with $NaHCO_3$ and H_2O to give 346 mg. Silica gel CC, eluting with C_6H_6–Et_2O (19:1) yielded 240 mg **8**. Colourless oil. $[\alpha]_D^{22}$ + 106.6° ($CHCl_3$; *c* 0.24); UV $\lambda_{máx}^{EtOH}$ nm: 240 (ε: 18 000) (C.D.) $\lambda_{max}^{n\text{-hexane}}$ nm: 345 (Δε + 0.29), 232 (Δε + 16.95), 195 (Δε − 4.62); IR $\nu_{máx}^{film}$ cm^{-1}: 3040, 1735, 1675, 1610, 1240, 1160, 1110, 1040; ^1H NMR: δ5.65 (1H, *s*), 4.03 (2H, *t*, *J* = 6 Hz), 3.60 (3H, *s*), 3.00 (1H, *m*), 2.59 and 2.10 (2H, ABq, J_{gem} = 16 Hz), 1.19 (3H, *s*), 0.98 (3H, *s*), 0.95 (3H, *d*, *J* = 7 Hz), 0.85 (3H, *d*, *J* = 7 Hz).

Reduction of **8** *with* $LiAlH_4$. **8** (240 mg) dissolved in 7 ml Et_2O was added dropwise to a suspension of 248 mg $LiAlH_4$ in 10 ml dry Et_2O. Usual work up afforded 200 mg reduction product which on silica gel CC yielded **9** (40 mg) and **5** (54 mg).

Compound **9**. Colourless oil. IR $\nu_{máx}^{film}$ cm^{-1}: 3320, 3040, 1640, 1050; ^1H NMR: δ5.31 (1H, *br*, *t*), 3.62 (2H, *t*, *J* = 6 Hz), 3.38 and 3.21 (2H, ABq, J_{gem} = 10 Hz), 0.86 (12H, *br*, *s*).

Reduction of **10** *with* $LiAlH_4$. $LiAlH_4$ (10 mg) were added to 20 mg **10** dissolved in 3 ml Et_2O. The reaction was kept at room temp. for 1 hr, yielding 15 mg **11**. Colourless oil. IR $\nu_{máx}^{film}$ cm^{-1}: 3360, 3070, 1640, 1060, 1030, 910, 870; ^1H NMR: δ5.34 (1H, *s*), 4.24 (1H, *m*), 3.65 (2H, *t*, *J* = 6 Hz), 3.40 and 3.24 (2H, ABq, J_{gem} = 10 Hz), 0.92 (3H, *s*), 0.91 (3H, *d*, *J* = 6 Hz), 0.87 (3H, *s*), 0.83 (3H, *d*, *J* = 6 Hz).

Oxidation of **10** *with* MnO_2. Compound **10** (65 mg) and 747 mg MnO_2, previously activated, in 14 ml C_6H_6 were shaken for 2 days at room temp. and for a further 6 days at 65°. The mixture was filtered while still hot and the residue washed several times with C_6H_6. Following evaporation of the solvent, 45 mg of reaction product were obtained, which were then acetylated with 1 ml Ac_2O and 1 ml C_5H_5N. Purification of the acetylation product on silica gel CC (C_6H_6–Et_2O, 19:1) gave 20 mg **8**.

Saponification of the neutral fraction. A portion (152 g) of the neutral fraction was treated with 100 ml KOH in MeOH (10%) for 24 hr at room temp. After evaporation of the MeOH, H_2O and ClH were added, and the mixture extracted with Et_2O. The ethereal soln. was washed with NaOH (4%) and with H_2O, yielding an acid fraction (30 g, 19.7%) and a neutral fraction (120 g, 78.9%). Silica gel CC of the neutral fraction, obtained from saponification, yielded the following results: C_6H_6–Et_2O (1:1) **1** (60%), C_6H_6–Et_2O (3:7), a mixture which after treatment with Ac_2O and C_5H_5N on silica gel CC yielded **3** and **12**; Et_2O, **13** was obtained.

8(17)-Labdene-3β,7α,15-triol (**13**). Colourless oil. $[\alpha]_D^{22}$ − 16.9° ($CHCl_3$; *c* 1.1); IR $\nu_{máx}^{film}$ cm^{-1}: 3340, 3080, 1640, 1060, 1040, 1020, 900, 850; ^1H NMR: δ4.95 (1H, *s*), 4.58 (1H, *s*), 4.30 (1H, *br*, *s*), 3.58 (2H, *t*, *J* = 6 Hz), 3.22 (1H, *m*, $W_{1/2}$ = 17 Hz), 0.96 (3H, *s*), 0.74 (3H, *s*), 0.64 (3H, *s*); EIMS 70 eV, *m/z* (rel. int.): 306 $[M-18]^+$ (8), 149 (4), 125 (3), 101 (8), 87 (13), 85 (67), 83 (100).

Treatment of **1** *with* m-*chloroperbenzoic acid.* m-Chloroperbenzoic acid (1.4 g) dissolved in 8 ml CH_2Cl_2 was added slowly to a soln of 2.6 g **1** in 15 ml CH_2Cl_2. The mixture was then shaken at room temp. for 3 hr, after which work up in the usual fashion yielded 2.55 g of a mixture of two substances. The major component (**14**) was separated by crystallization in EtOAc. Mp 126–127°. $[\alpha]_D^{22}$ + 12.0° ($CHCl_3$; *c* 1.0); IR $\nu_{máx}^{film}$ cm^{-1}: 3440, 1175, 1080, 1060, 1025, 970, 930, 870 cm^{-1}; ^1H NMR: δ4.10 (2H, *t*, *J* = 6 Hz), 3.10 (1H, *m*, $W_{1/2}$ = 17 Hz), 2.95 (1H, *br*, *s*), 1.30 (3H, *s*), 0.91 (3H, *s*), 0.80 (3H, *s*), 0.71 (3H, *s*).

C_5H_5N (2 ml) and Ac_2O (2 ml) were added to 200 mg crystallization residue from **14**, to give 195 mg of a mixture which following prep. TLC (*n*-hexane–Me_2CO, 9:1) yielded 90 mg **15** and 20 mg **16**.

Compound **15**. Colourless oil. IR $\nu_{máx}^{film}$ cm^{-1}: 1750, 1240, 1040, 970, 920, 870; ^1H NMR: δ4.45 (1H, *m*, $W_{1/2}$ = 17 Hz), 4.10 (2H, *t*, *J* = 6 Hz), 2.98 (1H, *m*, $W_{1/2}$ = 5 Hz), 2.10 (6H, *s*), 1.32 (3H, *s*), 0.91 (3H, *s*), 0.84 (3H, *s*), 0.79 (3H, *s*).

Compound **16**. Colourless oil. IR $\nu_{máx}^{film}$ cm^{-1}: 1750, 1250, 1040, 910, 870, 740; ^1H NMR: δ4.40 (1H, *m*), 4.11 (2H, *t*, *J* = 6 Hz), 2.99 (1H, *m*, $W_{1/2}$ = 8 Hz), 2.05 (6H, *s*), 1.28 (3H, *s*), 0.91 (12H, *br*, *s*).

Treatment of **14** *with lithium diethylamide.* BuLi (2.4 ml) was added slowly to ice-cold Et_2NH (0.34 g) in 2.4 ml THF in a N_2 atmosphere. After 10 min of shaking, a soln of 0.61 g **14** in 3 ml THF was added dropwise. After this the mixture was refluxed for 27 hr and then poured onto ice, the THF evaporated and extraction carried out with Et_2O. The ethereal soln was washed with 1 N HCl, $NaHCO_3$ and H_2O yielding 0.60 g of reaction product, which on prep. TLC (*n*-hexane–EtOAc, 1:4) yielded 200 mg **13**.

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