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A REARRANGED LABDANE: SALMANTIC ACID FROM *CISTUS LAURIFOLIUS*

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Key Word Index—*Cistus laurifolius*; Cistaceae; rearranged labdane; salmantic acid; salmantidiol.

Abstract—Salmantic acid, its methyl ester and its diol, salmantidiol, all of which have a new rearranged *ent*-labdane skeleton, $\Delta^{5(10)}$, and a shift of the C-10 methyl group to C-9, were isolated as minor components from *Cistus laurifolius*.

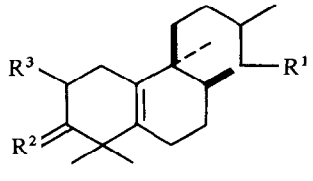
INTRODUCTION

In the *Cistus* species that have been studied, the major components are bicyclic diterpenes, principally labdanes of the normal series. Rearranged labdanes have also been discovered, but they all belong to the antipodes series; this is the case of *Cistus laurifolius* L., from which labdanes, *ent*-labdanes and rearranged *ent*-labdanes (*cis*-clerodanes) have been isolated [1, 2]. Among the *ent*-labdanic compounds isolated from *C. laurifolius* is acetyl-laurifolic acid [3] whose structure was determined by synthesis and which has recently been confirmed by X-ray analysis [4].

The present work describes three minor compounds with a new rearranged *ent*-labdane skeleton: salmantic acid (**1**), its methyl ester (**2**), isolated as a natural compound in the neutral fraction, and the corresponding 3,15-diol (salmantidiol).

RESULTS AND DISCUSSION

The $NaHCO_3$-soluble acid fraction of a hexane extract of the aerial parts of *C. laurifolius* after esterification with diazomethane was chromatographed on silica gel. Elution with benzene–diethyl ether gave a mixture of two unsaturated keto esters, **2** and the methyl ester of 3-oxocativic acid (**7**) (major component) [2]. The less polar keto ester **2** was only isolated in pure form after careful and repeated chromatography on $AgNO_3$–silica gel.



	R¹	R²	R³
1	COOH	O	H, H
2	COOMe	O	H, H
3	COOMe	O	D, D
4	CH_2OH	H αOH	H, H
5	CH_2OAc	H αOAc	H, H
6	COOMe	H, H	H, H

2 had IR absorption bands characteristic of a keto ester (1735 and 1710 cm^{-1}) and its ^1H NMR spectrum showed signals for five methyl groups (three singlets: 1.10 (6H, *s*), 1.02 (3H, *s*) and two doublets) and did not show signals for olefinic hydrogens or for methyls on double bonds. Its UV spectrum had a maximum at 280 nm with an ε value (160) characteristic of an unsaturated β–γ ketone [5]. Its mass spectrum (*m/z* 334 $[M]^+$, $C_{21}H_{34}O_3$) corresponded to that of an unsaturated bicyclic diterpene. Huang–Minlon reduction of **2** yielded **6** whose mass spectrum was identical to the mass spectrum of the enantiomers **8** and **9** obtained from labdanolic and populifolic acids [6, 7]. The base peak of **2** was at *m/z* 205 and corresponded to a decalin with four methyl groups, a carbonyl group and a Δ^5-double bond.

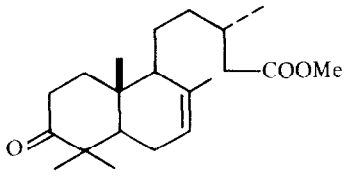
The ^{13}C NMR spectrum showed signals for six methyl groups (*q*), seven methylene groups (*t*), two methine groups (*d*) and six totally substituted carbons (*s*). Singlets at δ134.39 and 135.31 confirmed the presence of the tetrasubstituted double bond on 5.

The position of the carbonyl group was shown by deuteration of **2**, to yield **3** whose mass spectrum had a $[M]^+$ of *m/z* 336, i.e. 2 amu greater than **2**.

The same mixture of keto esters **2** and **7** was separated from the neutral fraction though the diol **4** was isolated from the more polar fractions.

Diol **4** had an IR absorption band indicative of the presence of hydroxyl groups (3320 cm^{-1}) and in its ^1H NMR spectrum a multiplet of three hydrogens geminal to the hydroxyl groups appeared at δ3.58. Its mass spectrum showed an $[M]^+$ of *m/z* 308 ($C_{20}H_{36}O_2$), and a base peak of *m/z* 207 which corresponded, as in the case of **2**, to the loss of the side chain. Oxidation of **4** and later esterification with diazomethane yielded **2**. Reduction of **2** with lithium aluminium hydride yielded a diol identical to **4** whose derived diacetyl **5** showed a double doublet in its ^1H NMR spectrum at δ4.65 of the hydrogen geminal to a secondary acetoxyl group on C-3.

The CD curve of **2** showed a negative Cotton effect such that the carbonyl group must be above the plane of the double bond (the conformation of ring A must be as shown in **10**). The 4α-methyl group must therefore be in a pseudo-axial position, thus determining the entrance of a hydride anion from the β-side (less hindered) and in the reaction product the hydroxyl group is α, as in the natural compound **4**.



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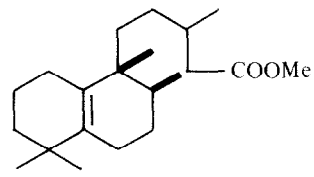

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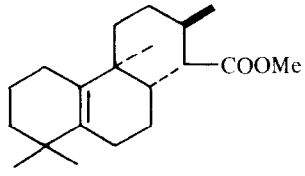

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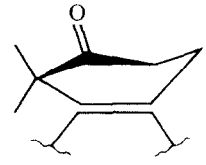

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In order to determine the stereochemistry of these natural compounds, the spectroscopic properties of esters **8** and **9** were compared with those of **6** (their mass spectra were identical). The only appreciable differences were found to be in their ^{1}H NMR spectra: in **6** the three C-methyl groups appeared at δ0.98 while in **8** and **9**, two of the C-methyl groups appeared at δ0.98 and the other was at δ0.78. Bearing in mind the absolute stereochemistry of the rearranged products **8** and **9** (C-8 and C-9 methyl groups *cis*), the methyl groups of **6**, and therefore of **2**, should have a relative configuration of *trans* from the above consideration. Thus, the absolute stereochemistry shown in the figure is proposed for **2**.

EXPERIMENTAL

Mps (Kofler hot stage apparatus): uncorr. ^{1}H 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 *C. laurifolius* (9 kg), collected in Bohoyo (Avila, Spain), were dried and extracted with *n*-hexane in a Soxhlet apparatus for 24 hr yielding 504 g of extract. This was dewaxed with MeOH (16%) and then extracted with 6% $NaHCO_3$ (19%), 12% Na_2CO_3 (41%) and 4% NaOH (6%). The neutral fraction remaining represented 34% of the original extract. The $NaHCO_3$-soluble acid fraction was esterified with CH_2N_2 and the methyl esters were chromatographed on silica gel. The fractions eluted with C_6H_6–Et_2O (9:1) contained a mixture of **2** and **7**.

25 g of the neutral fraction was chromatographed in a dry column [1 kg silica gel (0.5 × 200 cm) developed with *n*-hexane–Et_2O, 1:1] to give 5 fractions. Fraction II was essentially composed of the same mixture of **2** and **7**, which was resolved by repeated $AgNO_3$–silica gel (1:4) CC eluting with C_6H_6. Crystallization of fraction IV from CCl_4 gave **4**.

Methyl ester **2**. Colourless oil, $[\alpha]_D^{22}$ −3.2° ($CHCl_3$; *c* 1.25); UV λ_{max}^{EtOH} nm: 280 (ε: 160); IR ν_{max}^{film} cm^{-1}: 1735, 1710, 1300, 1160, 1100, 1000, 970, 870, 840; ^{1}H NMR: δ3.55 (3H, *s*), 1.10 (6H, *s*), 1.02 (3H, *s*), 0.97 (3H, *d*, *J* = 6 Hz), 0.94 (3H, *d*, *J* = 6 Hz); ^{13}C NMR: 27.65 (*t*, C-1)*, 32.76 (*t*, C-2)†, 216.18 (*s*, C-3), 47.66 (*s*, C-4), 135.31 (*s*, C-5), 25.02 (*t*, C-6)*, 23.56 (*t*, C-7)*, 37.59 (*d*, C-8), 37.13 (*s*, C-9), 134.39 (*s*, C-10), 32.66 (*t*, C-11)†, 41.50 (*t*, C-12), 31.43 (*d*, C-13), 40.16 (*t*, C-14), 173.58 (*s*, C-15), 18.89 (*q*, C-16), 16.00 (*q*, C-17), 23.87 (*q*, C-18)‡, 24.41 (*q*, C-19)‡, 26.51 (*q*, C-20)‡, 51.37 (*q*, C-21) (*, †, ‡, interchangeable); EIMS 70 eV, *m/z* (rel. int.): 334 $[M]^+$ (5), 319 (2), 304 (1), 205 (100), 163 (87), 129 (12), 93 (11), 91 (16).

Deuteration of **2**. To a soln of 40 mg **2** in 5 ml MeOD and 0.7 ml D_2O 7 ml dry Na_2CO_3 was added. The mixture was refluxed for 15 min and then distilled *in vacuo*. Another 5 ml of MeOD and 0.7 ml of D_2O were added and the mixture was refluxed for 20 min. The soln was evapd to dryness and then extracted with Et_2O. 38 mg **3** was obtained. Colourless oil; IR ν_{max}^{film} cm^{-1}: 1740, 1710, 1300, 1100, 1010, 950; ^{1}H NMR: δ3.59 (3H, *s*), 1.10 (6H, *s*), 1.02 (3H, *s*), 0.97 (3H, *d*, *J* = 6 Hz), 0.92 (3H, *d*, *J* = 6 Hz); EIMS 70 eV, *m/z* (rel. int.): 336 $[M]^+$ (3), 207 (100), 165 (97), 129 (20), 93 (10), 91 (11).

Salmantidiol (**4**). Mp 74–75°, $[\alpha]_D^{22}$ −8.4° ($CHCl_3$; *c* 0.76); IR ν_{max}^{film} cm^{-1}: 3320, 1060, 1030, 975, 950; ^{1}H NMR: δ3.60 (2H, *t*, *J* = 6 Hz), 3.55 (1H, *m*), 1.04 (*s*, *br*), 0.99 (*s*, *br*); EIMS 70 eV, *m/z* (rel. int.): 308 $[M]^+$ (3), 290 (2), 275 (1), 207 (100), 189 (67), 147 (26), 55 (25).

26 mg **4** in 0.7 ml C_5H_5N was treated with 1 ml Ac_2O and left overnight. This gave **5** (26 mg) which crystallized in *n*-hexane. Mp 81–83°, $[\alpha]_D^{22}$ 0.0° ($CHCl_3$; *c* 0.86); IR ν_{max}^{film} cm^{-1}: 1735, 1240, 1030, 980, 900; ^{1}H NMR: δ4.65 (1H, *dd*, J_1 = 3.5 Hz, J_2 = 8 Hz), 3.95 (2H, *t*, *J* = 6 Hz), 1.95 (6H, *s*), 0.97 (15H, *s*, *br*); EIMS 70 eV, *m/z* (rel. int.): 392 $[M]^+$ (1), 332 (6), 317 (1), 189 (100), 147 (32), 55 (17), 43 (45).

Reduction of **2** *with* $LiAlH_4$. 50 mg $LiAlH_4$ suspended in 2 ml Et_2O was added to 85 mg **2** dissolved in 3 ml dry Et_2O. This was refluxed for 1 hr. The normal procedure was followed to give 80 mg salmantidiol (**4**).

Oxidation of **4** *with Jones reagent.* 40 mg **4** dissolved in 5 ml Me_2CO were oxidized with Jones reagent in the normal way to yield 35 mg **2** after esterification with CH_2N_2.

Huang–Minlon reduction of **2**. 80 mg KOH and 0.5 ml hydrazine hydrate (85%) were added to 97 mg **2** in 3 ml diethyleneglycol. This was heated to 175° for 20 hr. The reflux condenser was removed for a few min and following this, the mixture was heated to 220° for 3 hr. After this, cold H_2O and 6 M HCl were added and the mixture was extracted with Et_2O. The ethereal soln was evapd and the residue esterified with CH_2N_2, yielding 90 mg esters which were purified by CC to give **6** (45 mg). Colourless oil, $[\alpha]_D^{22}$ −9.9° ($CHCl_3$; *c* 0.91); IR ν_{max}^{film} cm^{-1}: 1735, 1280, 1250, 1180, 1160, 1000, 960, 940, 860; ^{1}H NMR: δ3.60 (3H, *s*), 0.98 (15H, *s*, *br*); EIMS 70 eV, *m/z* (rel. int.): 320 $[M]^+$ (1), 191 (100), 149 (26), 107 (31), 93 (38), 91 (39).

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