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LABDANE DITERPENOIDS FROM *CISTUS LADANIFERUS*

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Key Word Index—*Cistus ladaniferus*; Cistaceae; diterpenic acids.

Abstract—Three new diterpenic acids have been isolated from *Cistus ladaniferus*: 6,8(17) labdadien-15-oic, 7-oxo-8-labden-15-oic and 6β-acetoxy-7-oxo-8-labden-15-oic acids, beside labdanolic, 6-oxocativic, 7α-hydroxy-8(17)-labden-15-oic, 8α-methoxy-labda-15-oic and 8α-hydroxy-13(*E*)-labden-15-oic acids.

INTRODUCTION

Our studies on components of *Cistus ladaniferus* were preformed with a labdanum gum[1–5]. In this paper we report on the minor diterpenic components of *C. ladaniferus*.

RESULTS AND DISCUSSION

The acid fraction of a hexane extract from *C. ladaniferus*, L. was resolved by dry chromatography of the methyl esters.

The less polar fraction I consisted of a mixture of methyl esters of fatty acids. From fraction II, we isolated the methyl esters **1** and **2** Compound **2** had already been isolated from *C. palinhae* and its structure was confirmed by synthesis[6]. Compound **1** was a methyl ester with two conjugated double bonds [IRν cm^{-1}: 3120, 1740, 1600, 880, 800, and UVλ_{max}^{EtOH} nm: 243 (ϵ = 33400)].

The ^{1}H NMR spectrum of **1** showed signals due to four methyl groups (three of them, as singlets and one, as a doublet). The spectrum showed signals due to four olefinic protons, [δ4.82(2H, *br s*, C=CH$_2$), 5.63(1H, *br d*, J = 10 Hz) and 6.13(1H, *dd*, J = 10 and 3 Hz) CH=CH–C=CH$_2$].

The mass spectrum of **1**, showed the molecular ion at m/z 318, which was in agreement with the formula $C_{21}H_{34}O_2$; the base peak at m/z 129 was consistent with the fragment $C_7H_{13}O_2$, and it is accounted for by the loss of the side chain and we proposed for **1**, the structure methyl labda-6,8(17)-dien-15-oate. The stereochemistry proposed for **1** at C-13 is the same as found in the other diterpenoids of this type[7].

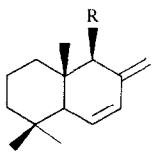
Fraction III consisted mainly of methyl 6-oxo-7-labden-15-oate (methyl oxocativate) (**3**) which is already known[4, 5].

From fraction IV, we isolated three methyl esters, **4**, **9** and **12**. The IR and UV spectra of **4**, showed the presence of one α-β unsaturated C=O group [IRν cm^{-1}: 1670, 1610, and UVλ_{max}^{EtOH} nm: 249 (ϵ = 13.700)] and the ^{1}H NMR spectrum showed signals due to five methyl groups (three of them, Me–C, one, Me–CH and one Me–C=). There were no signals due to olefinic protons and thus the double bond must be a tetrasubstituted one. The mass spectrum of **4**, showed the molecular ion at m/z 334 ($C_{21}H_{34}O_3$). All these data agree with the proposed structure, methyl 7-oxo-8-labden-15-oate. This structure was confirmed by partial synthesis from labdanolic acid, which is the main component of the hexane extract. Dehydration of the methyl ester of labdanolic acid with $POCl_3$–pyridine, yielded a mixture of **6**, **7** and **8** which was resolved by prep. TLC on Si gel–$AgNO_3$. Oxidation of **6** with sodium chromate gave a product identical in all respects with **4**.

The IR and UV spectra of **9** showed the presence of an α-β unsaturated C=O group and an acetyl group (IRν cm^{-1}: 1754, 1678, 1618, 1240, and UVλ_{max}^{EtOH} nm: 251). The ^{1}H NMR spectrum showed signals due to five methyl groups (three Me–C, one Me–CH and one Me–C=) and a doublet centred at δ5.60 (J = 4 Hz, H–C–OAc). The chemical shift and multiplicity of the signal due to the acetoxyl group geminal proton is proof that the axial acetoxyl group on C–6 is in the α-position with the C=O group. The mass spectrum of **9** showed the loss of the fragment $C_7H_{13}O_2$. The C-13 stereochemistry of **9** is not established, but from biogenetic considerations it may be assumed that it is the same as found in the other diterpenoids[7].

Treatment of **4** with lead tetraacetate[8], yielded **10** and **11**. The ^{1}H NMR spectrum of **10** showed the signal due to the acetoxyl group geminal proton, as a doublet, centered at δ5.30 (J = 12 Hz), which agrees with a dihedral angle of 180° between the geminal proton and the C-5 proton. The ester **11**, was identified as methyl 7-oxo-5,8-labdadien-15-oate by comparison with an authentic sample, which was obtained by oxidation of **4** with SeO_2.

Compound **12**, showed IR absorptions, due to a hydroxyl group and a C=CH$_2$ unsaturation (3500, 3090, 1740, 1670, 1040, 904 cm^{-1}). The ^{1}H NMR spectrum showed signals due to four methyl groups (three Me–C and one Me–CH), an equatorial allylic geminal hydroxyl proton at 4.22 ($W_{1/2}$ = 6.5 Hz) and a C=CH$_2$ group at 4.54 and 4.94 (2H, *s*) which are in agreement with the proposed structure as methyl 7-hydroxy-8(17)-labden-15-oate. These data agree with those communicated by Hoeneisen *et al.*[9]. The assigned structure was confirmed by partial synthesis. Oxida-



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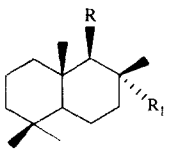
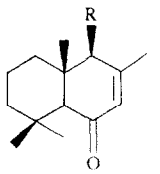


2 R_1 = OMe
5 R_1 = OH



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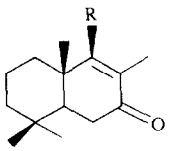

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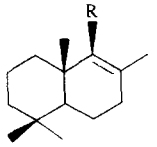

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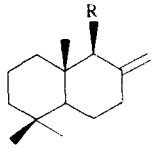

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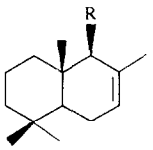

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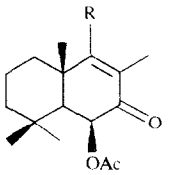

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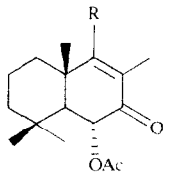

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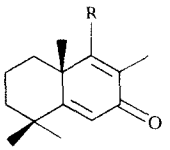

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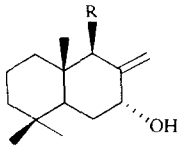

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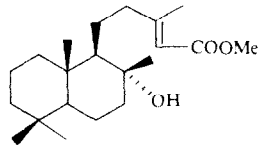

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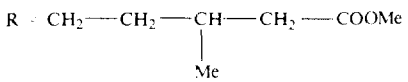

tion of **7** with SeO_2[10], yielded a product identical with **12**.

Crystallization of fractions V and VI, gave **5**[4, 5] and prep. TLC of the mother liquors produced a pure sample of **13**[6].

EXPERIMENTAL

Mps are uncorr. and were determined on a Kofler hot stage apparatus. UV spectra were recorded in EtOH. ^{1}H NMR spectra were recorded using TMS as int. standard, in CCl_4. Analytical TLC was performed on Si gel G, prep. TLC on Si gel $PF_{254+366}$ and CC, on Si gel 60.

Extraction and isolation. The aerial parts of *C. ladaniferus* (12.0 kg), collected at Guimeré (Zamora, W. Spain) were air dried and extracted with hot hexane in a Soxhlet, for 24 hr, giving 1200 g extract. This was dewaxed with MeOH (14.1%) and then steam distilled, giving 2.60% essential oil.

The non-volatile part, was extracted with 6% $NaHCO_3$ (17.20%), 10% $NaCO_3$ (23.70%) and 4% NaOH (4.50%). The residual neutral part was 34.60% of the initial hexane extract.

Treatment of 75.00 g of the bicarbonate soluble acid part with CH_2N_2, yielded 73.00 g of a mixture of methyl esters, which were dry-chromatographed (1700 g of Si gel in a 4 × 130 cm column, eluted with C_6H_6–Et_2O, 7:3), giving seven fractions.

Treatment of fraction II (11.00 g) with a satd methanolic soln of urea, gave 9.40 g methyl esters soluble in MeOH, which were resolved by CC on Si gel and Si gel–$AgNO_3$ (20%), yielding pure samples of **1**, **2** and **3**.

Fraction III (14.49 g), consisted only of methyl 6-oxocativate, and from fraction IV, by CC, followed by TLC, the methyl esters **4**, **9** and **12** were isolated.

The methyl ester **4** was eluted in CC with petrol–Et_2O (4:1); methyl esters **9** and **12** were both eluted with petrol–Et_2O (1:1). Compound **9** was obtained with C_6H_6–Et_2O (19:1) and **12** with C_6H_6–$CHCl_3$–Et_2O (1:3:1) in prep. TLC.

Crystallization of fraction V (17.04 g) and fraction VI (6.11 g) from hexane yielded pure methyl labdanolate, **5** (9.8 g). CC of the residual part gave another 6.2 g **5** and **13**.

Methyl 6,8(17)-*labdadien*-15-*oate* (**1**). Colourless oil. $[\alpha]_D^{22} = +5.8°$ ($CHCl_3$; c 1.21). UV λ_{max}^{EtOH} nm: 234 (ϵ = 33.400). IR ν_{max}^{film} cm^{-1}: 3120, 2960, 1740, 1600, 1150, 1010, 880, 800,

775. ^{1}H NMR: δ0.65(3H, *s*), 0.85(3H, *s*), 0.95(3H, *s*), 0.95(3H, *d*, *J* = 7 Hz), 3.6(3H, *s*), 4.82(2H, *s*), 5.63(1H, *d*, *J* = 10 Hz), 6.13(1H, *dd*, J_1 = 10 Hz, J_2 = 3 Hz). MS (70 eV) *m/z* (rel. int): 318[M]$^+$ (20), 303(6), 271(18), 203(27), 190(95), 189(83), 175(37), 119(100).

Methyl 8α-methoxylabda-15-oate (**2**). Colourless oil. $[\alpha]_D^{22} = -8.6°$ ($CHCl_3$; *c* 0.8). IR ν_{max}^{film} cm^{-1}: 1740, 1450, 1380, 1200, 1155, 1072, 1030. ^{1}H NMR: δ0.90(3H, *d*, *J* = 6.5 Hz), 0.78(3H, *s*), 0.80(3H, *s*), 0.84(3H, *s*), 1.01(3H, *s*), 3.03(3H, *s*), 3.56(3H, *s*). MS(70 eV) *m/z* (rel. int.): 352[M]$^+$ (0.5), 191(5), 177(11), 137(10), 129(12), 123(12), 109(14), 85(100), 81(13).

Methyl 6-oxo-7-labden-15-oate (**3**). Colourless oil. $[\alpha]_D^{22} = +13.3°$ ($CHCl_3$; *c* 1.2). UV λ_{max}^{EtOH} *nm*: 238 (ε = 11.400). IR ν_{max}^{film} cm^{-1}: 1740, 1680, 1635, 1450, 1380, 1220, 1170, 1025, 1000, 890. ^{1}H NMR: δ5.61(1H, *brs*, H-7), 3.59(3H, *s*, –COOMe), 1.91(3H, *d*, *J* = 2 Hz, H_3-17), 1.12 and 1.08(each, 3H, *s*, H_3-18 and H_3-19), 1.05(3H, *d*, *J* = 6 Hz, H_3-16), 0.84(3H, *s*, H_3-20).

Methyl-7-oxo-8-labden-15-oate (**4**). Colourless oil. $[\alpha]_D^{22} = +43.5°$ ($CHCl_3$; *c* 1.38). UV λ_{max}^{EtOH} nm: 249 (ε = 13.700). IR ν_{max}^{film} cm^{-1}: 1740, 1668, 1612, 1465, 1440, 1158, 1010. ^{1}H NMR: δ3.60 (3H, *s*, –COOMe), 1.65(3H, *s*, H_3-17). 1.06(3H, *s*, H_3-20), 0.93 (6H, *s*, H_3-18 and H_3-19). MS (70 eV) *m/z* (rel. int.): 334[M]$^+$ (27), 233(100), 205(90), 135(85), 109(45).

Dehydration of methyl labdanolate. $POCl_3$ (2.8 ml) was added drop-wise to a stirred cold soln of 1.10 g methyl labdanolate (**5**) in 21.8 ml dry pyridine. After addition, the stirring was continued for another 4.5 hr. Ice–water was then added and the mixture extracted with Et_2O.

The Et_2O soln was washed with 1 N HCl, 6% $NaHCO_3$ and H_2O and dried with dry Na_2SO_4. Evaporation of the solvent, gave 900 mg of product, which by CC gave pure samples of methyl 8-labden-15-oate (**6**, 89 mg), methyl-7-labden-15-oate (methyl cativate) (**7**, 90 mg) and methyl-8(17)-labden-15-oate (methyl labdenate) (**8**, 450 mg).

Methyl-8-labden-15-oate (**6**). Colourless oil. $[\alpha]_D^{22} = +63.1°$ ($CHCl_3$; *c* 1.1). IR ν_{max}^{film} cm^{-1}: 1735, 1190, 1150, 1000. ^{1}H NMR: δ3.58(3H, *s*), 1.51(3H, *s*), 0.95(3H, *d*, *J* = 6.5 Hz), 0.90(3H, *s*), 0.87(3H, *s*), 0.82(3H, *s*).

Methyl 7-labden-15-oate (methyl cativate) (**7**). Colourless oil. $[\alpha]_D^{22} = -7.3°$ ($CHCl_3$; *c* 0.88). IR ν_{max}^{film} cm^{-1}: 1740, 1666, 1200, 1070, 1000, 820. ^{1}H NMR: δ5.32(1H, *brs*), 3.60(3H, *s*), 1.63(3H, *s*), 0.94(3H, *d*, *J* = 6.5 Hz), 0.88(6H, *s*), 0.73(3H, *s*).

Methyl 8(17)-labden-15-oate (methyl labdenate) (**8**). Colourless oil. $[\alpha]_D^{22} = +24.3°$ ($CHCl_3$; *c* 1.2). IR ν_{max}^{film}: cm^{-1} 3080, 1740, 1640, 1200, 1150, 890. ^{1}H NMR: δ4.48(1H, *brs*), 4.75(1H, *br s*), 3.61(3H, *s*), 0.93(3H, *d*, *J* = 6.5 Hz), 0.88(3H, *s*), 0.81(3H, *s*), 0.68(3H, *s*).

Oxidation of methyl 8-labden-15-oate. To a soln of 150 mg methyl 8-labden-15-oate (**6**) in 1.5 ml C_6H_6 dry sodium chromate (138 mg) and NaOAc (106 mg) in 0.7 ml HOAc and 1.3 ml Ac_2O were added. The mixture was held at 45° for 8 hr and then poured over ice–water extracted with Et_2O, and the extract washed with $NaHCO_3$ soln and H_2O. Prep. TLC of the reaction product gave 85 mg pure **4**.

Methyl 6β-acetoxy-7-oxo-8-labden-15-oate (**9**). Colourless oil. $[\alpha]_D^{22} = -25°$ ($CHCl_3$; *c* 1.16). UV λ_{max}^{EtOH} nm: 251 (ε = 12.400). IR ν_{max}^{film} cm^{-1}: 1745, 1678, 1240, 1160, 1044, 1022. ^{1}H NMR: δ5.62(1H, *d*, *J* = 4 Hz, H-6), 3.63(3H, *s*, –COOMe), 2.01(3H, *s*, –OCO–Me) 1.73(3H, *s*, H-17), 1.40, 1.05 and 1.02 (each 3H, *s*, H_3-18, H_3-19 and H_3-20). MS (70 eV) *m/z* (rel. int.): 392[M]$^+$ (8), 332(22), 237(80), 231(100), 203(85), 161(84), 109(32).

Methyl 6α-acetoxy-7-oxo-8-labden-15-oate (**10**). To a soln of 85 mg methyl 7-oxo-8-labden-15-oate (**4**) in 6.0 ml toluene, 0.33 g of $Pb(OAc)_4$ was added. The mixture was refluxed for 8 hr and then filtered and extracted with Et_2O. Prep. TLC of the reaction product, gave 39 mg **4**, 25 mg **10** and 15 mg methyl 7-oxo-5,8-labdadien-15-oate (**11**).

Methyl 6α-acetoxy-7-oxo-labden-15-oate (**10**). Colourless oil. $[\alpha]_D^{22} = +33.8°$ ($CHCl_3$; *c* 0.71). IR ν_{max}^{film} cm^{-1}: 1740, 1670, 1610, 1240, 1040, 1020. ^{1}H NMR: δ5.33(1H, *d*, *J* = 12 Hz, H-6), 3.65(3H, *s*, COOMe), 2.12(3H, *s*, –OCOMe), 1.73(3H, *s*), 1.29(3H, *s*), 1.02(9H, *brs*).

Methyl 7-oxo-5,8-labdadien-15-oate (**11**). Colourless oil. $[\alpha]_D^{22} = -30.7°$ ($CHCl_3$; *c* 1.79). UV λ_{max}^{EtOH} nm: 249 (ε = 18.600). IR ν_{max}^{film} cm^{-1}: 1740, 1660, 1630, 1600, 1200, 1160, 1010, 960, 690. ^{1}H NMR: δ6.07(1H, *s*, H-6), 3.60(3H, *s*, COOMe), 1.79(3H, *s*, H_3-17), 1.34(3H, *s*, H_3-18), 1.30(3H, *s*, H_3-19), 1.23(3H, *s*, H_3-20); 0.95(3H, *d*, *J* = 6.5 Hz, H_3-16).

Methyl 7α-hydroxy-8(17)-labden-15-oate (**12**). Colourless oil. $[\alpha]_D^{22} = -18.3°$ ($CHCl_3$; *c* 0.93). IR ν_{max}^{film} cm^{-1}: 3500, 3090, 1740, 1640, 1040, 904. ^{1}H NMR: δ4.94 and 4.54 (each, 1H, *brs*, C=CH_2), 4.22(1H, *m*, H-7), 3.61(3H, *s*, COOMe), 0.95(3H, *d*, *J* = 6 Hz), 0.90(3H, *s*), 0.80(3H, *s*), 0.64(3H, *s*).

Methyl labdenate (**8**) (181 mg) in 15 ml of EtOH was refluxed with 36 mg freshly sublimated SeO_2, for *ca* 2 hr. EtOH was distilled *in vacuo* and the residue extracted with Et_2O. Prep. TLC (C_6H_6–Et_2O, 8:3) yielded 79 mg pure **12**.

Methyl 8α-hydroxylabda-15-oate (methyl labdanolate) (**5**). Mp 73–74°. $[\alpha]_D^{22} = -8.6°$ ($CHCl_3$; *c* 1.06). IR ν_{max}^{film} cm^{-1}: 3500, 1740, 1200, 1160, 1080, 1010, 940, 910. ^{1}H NMR: δ3.60(3H, *s*), 1.08(3H, *s*), 0.94(3H, *d*, *J* = 6.5 Hz), 0.88(3H, *s*), 0.78(6H, *s*).

Methyl 8α-hydroxy-13(E)-labden-15-oate (**13**). Colourless oil. $[\alpha]_D^{22} = +7°$ ($CHCl_3$; *c* 0.7). IR ν_{max}^{film} cm^{-1}: 3470, 1730, 1660, 1230, 1160, 940, 910. ^{1}H NMR: δ5.52(1H, *brs*, H-13), 3.58(3H, *s*, COOMe), 2.11(3H, *d*, *J* = 1.5 Hz), 1.10(3H, *s*, H_3-17), 0.86(3H, *s*), 0.79(6H, *s*).

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