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DITERPENOIDS AND FLAVONOIDS FROM *CISTUS PALINHAE*

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Key Word Index—*Cistus palinhae*; Cistaceae; diterpenes; flavonoids.

Abstract—Cativic, ladenic, labdanolic, 8α-hydroxy-13(*E*)-labden-15-oic and 3-phenylpropionic acids were isolated from *Cistus palinhae*. In addition two new acids were characterized as 8α-methoxy-labd-15-oic and (5*R*, 8*R*, 9*S*, 10*S*)-2-oxo-3-*cis*-cleroden-15-oic. From the neutral fraction were isolated the known 8,15-labdanediol, 8(17)labden-15-ol, 6-oxo-7-labden-15-ol and 6β-hydroxy-8(17)-labden-15-ol and also identified were the hydroxy derivatives 8α-hydroxy-15-phenylpropionoxy-labdane,8α-hydroxy-15-acetoxy-labdane, 8-labden-15-ol and 8-*epi*-15-labdanediol. The weak acid fraction gave jaranol (5,4′-dihydroxy-3,7-dimethoxyflavone), genkwanin (5,4′-dihydroxy-7-methoxyflavone), 3-methylkampferol and (−)betuligenol.

INTRODUCTION

Cistus palinhae, which is found only in the extreme south west of the Iberian peninsula (Cape St Vincent, Portugal), together with *C. clusii* and *C. heterophylus* are the only members of the *Cistus* genus which grow on chalky soil; the others grow on acid soils.

C. palinhae is morphologically very similar to *C. ladaniferus*, known as 'jara', which is to be found on acid soils in the south and west of Spain. The major component of both plants is labdanolic acid, but in *C. palinhae*, 6-oxo-cativic acid, abundant in *C. ladaniferus* [1], was not isolated.

RESULTS AND DISCUSSION

The acid fraction of a hexane extract of *C. palinhae*, which was soluble in sodium bicarbonate solution, was esterified and separated by dry chromatography of the methyl esters into compounds **1**–**6**. In the ^{1}H NMR spectrum of **3** a three proton singlet of a methyl ether appeared at δ3.03 and another singlet centered at 1.01 was due to a methyl geminal to the methoxyl group. Its mass spectrum (M^+ at *m/z* 352, $C_{22}H_{40}O_3$) corresponded to the methyl ester of a bicyclic diterpene acid with a tertiary methoxyl group. The structure of methyl 8α-methoxy-labd-15-oate (**3**) was confirmed by synthesis from methyl labdanolate by treatment with methyl iodide and sodium hydride [2].

Compound **6** was a ketoester with an unsaturated α,β-carbonyl group (IR 1730, 1660 and 1620 cm^{-1}, UV 234 nm, ε = 14 500). The ^{1}H NMR spectrum showed signals of five methyl groups (two Me–C, two Me–CH and one Me–C= at δ1.82) and a wide singlet of an olefinic hydrogen at 5.52.

The mass spectrum of **6** (M^+ at *m/z* 334, $C_{21}H_{34}O_3$) corresponded to a bicyclic diterpene with a C_7 side chain (*m/z* at 129, $C_7H_{13}O_2$) and it was identical to the mass spectrum of the methyl ester of oxopopulifolic acid [3]. The fragment at *m/z* 205 corresponds [4] to a decalin with four methyl groups, a carbonyl group and a double bond. By double irradiation of a ^{1}H NMR signal centered at δ5.52 the doublet at 1.84 was changed into a singlet and *vice versa*, confirming the presence of the –CO–CH =CMe grouping, which together with the existence of only two methyl singlets indicated a clerodane or cistane skeleton with the carbonyl group at C-2 and the double bond at C-3.

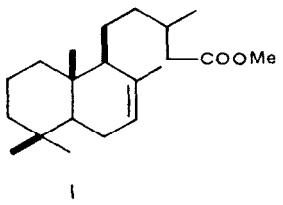
The CD curve of **6** in hexane showed maxima at 342 nm (Δε = +0.94), 229 nm (Δε = −4.45) and 206 nm (Δε = +6.31). Applying the reversed octant law of Snatzke and that of the helicity of transoid enones, the curve is only compatible with possibilities I, II and III.

The signal of the ^{1}H NMR spectrum [C_6D_6, Eu (dpm$_3$)] corresponding to the hydrogen on C-10 (*dd*, J_{AX+BX} = 14 Hz) excludes possibility I. The stereochemistry 5*R*, 8*R*, 9*S*, 10*S* for II was determined [2] by applying the McConnell and Robertson ratio [5]. However, this is still to be confirmed by X-ray diffraction.

The neutral fraction of a hexane extract of *Cistus palinhae* was resolved by dry chromatography. From the less polar fraction, a hydroxyester **7** with an aromatic system was obtained (IR 1610, 1500 cm^{-1}; ^{1}H NMR δ7.09, 5H, *br s*) whose molecular ion in the mass spectrum was at *m/z* 442, which corresponds to the formula $C_{29}H_{46}O_3$. Alkaline hydrolysis of **7** yielded **8** [6] and β-phenylpropionic acid identified as a methyl ester by comparison with an authentic sample.

Compounds **7** and **9** were isolated from fraction II (see Experimental). Compound **9** was a hydroxyester with an acetoxyl group, and had the formula $C_{22}H_{40}O_3$ (M^+ at *m/z* 352). Compound **9** was obtained by acetylation of **8**. The more polar fraction IV contained **8** (8,15-labdanediol).

Saponification of the neutral part yielded the unsaponifiable part which was fractionated by silica gel chromatography. Compounds **10**–**14** and sitosterol were isolated. Compound **10** showed signals in its ^{1}H NMR spectrum of the following groupings: –CH_2–CH_2OH (δ3.53, 2H, *t*, *J* = 6 Hz), Me–C= (1.51, *s*, 3H) and four methyl groups (three Me–C and one Me–CH). Treatment of **8** with formic acid gave **15** which by alkaline hydrolysis yielded **10**.



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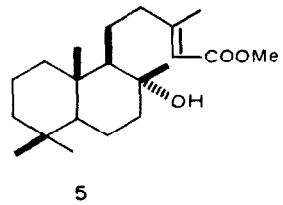

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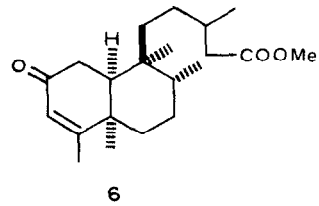

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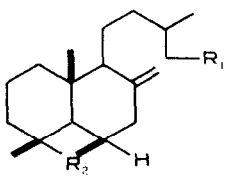
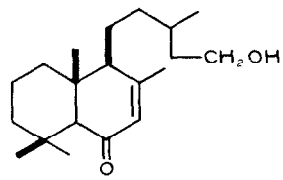


	R_1	R_2
2	COOMe	H
11	CH_2OH	H
13	CH_2OH	OH



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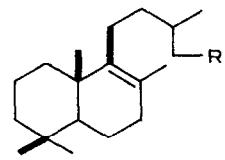
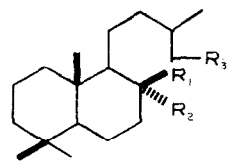
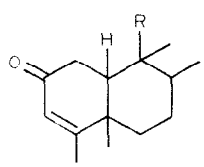


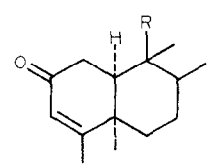
	R
10	CH_2OH
15	CH_2OCHO



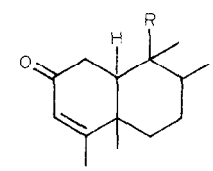
	R_1	R_2	R_3
3	Me	OMe	COOMe
4	Me	OH	COOMe
7	Me	OH	$CH_2OCO(CH_2)_2C_6H_5$
8	Me	OH	CH_2OH
9	Me	OH	CH_2OAc
14	OH	Me	CH_2OH
16	OH	Me	CH_2OAc



I 5*S*,10*R* ($^{19}C_I$)



II 5*R*,10*S* ($^{19}C_{II}$)



III 5*S*,10*S* ($^{7}C_{O}$)

Compounds **11**, **12** and **13** were identified by comparison with authentic samples [7]. Compound **14** of $[\alpha]_D$ + 6.3° was a dihydroxyderivative with signals for the following groupings in its ^{1}H NMR spectrum: $-CH_2OH$ (δ3.60, *t*, 2H, J = 6 Hz), Me–C–OH (δ1.11, *s*, 3H), Me–C (0.97, 0.87 and 0.83, each *s*, 3H) and Me–CH (0.89, *d*, 3H, J = 6.5 Hz).

The mass spectrum showed the $[M]^+$ at m/z 310, corresponding to $C_{20}H_{38}O_2$. In the monoacetyl derivative of **16** the ^{1}H NMR signal of the methyl geminal to the hydroxyl group remained centered at δ1.11. Compound **14** was given the structure of 8β,15-labdanediol, thus accounting for the deshielding of the C-20 protons (δ0.83 in **8** and 0.97 in **14** and **16**). The spectroscopic properties of **14** were identical to those of 8α,15-*ent*-labdanediol, obtained by reduction of the methyl ester of the 8α-*ent*-labda-15-oic acid, isolated from *Trachylobium verrucosum* [8].

Three flavonoids and one phenol were isolated from the weak acid fraction, soluble in sodium hydroxide and identified by comparison with authentic samples [9]. The flavonoids were jaranol (5,4'-dihydroxy-3,7-dimethoxy-

flavone), genkwanin (5,4′-dihydroxy-7-methoxyflavone) and 3-methylkampferol (5,7,4′-trihydroxy-3-methoxyflavone) and the phenol was betuligenol or (−)1(4-hydroxyphenyl)-butan-3-ol [10]. The primary components of the acidic part were β-phenylpropionic and labdanolic acid. No D(−)β-hydroxy acids were detected; these are very important components of *Cistus ladaniferus* [11].

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 in CCl_4 using TMS as internal standard. Analytical TLC was performed on silica gel, prep. TLC was on silica gel $PF_{254+366}$ and CC was on silica gel 60. The CD curves were carried out on a Yobin Yvon Dicrograph III dicrograph using hexane as the solvent. The mass spectra were carried out with a source temperature of 180° and ionization energy of 70 eV.

Extraction and isolation. The aerial parts of *C. palinhae* (10 kg) collected at Cape St Vincent (Portugal) were dried and extracted with *n*-hexane in a Soxhlet for 24 hr giving 840 g of extract. This was dewaxed with MeOH (16%) and later extracted with 6% $NaHCO_3$ (26.2%) and 4% NaOH (0.5%), a neutral fraction remaining which represented 68.7% of the original extract. Treatment of 22.0 g of the bicarbonate-soluble acid part with CH_2N_2, yielded 21.8 g of a mixture of methyl esters, which were dry-chromatographed (770 g of silica gel, eluted with *n*-hexane–Et_2O, 1:1) giving four fractions.

Treatment of fraction I (2.2 g) with a satd methanolic soln of urea, gave 1.2 g of methyl esters soluble in MeOH, which were resolved by CC on $AgNO_3$–silica gel (2:8), yielding pure samples of **1** and **2**. From fraction II (1.9 g) the methyl ester **3** was isolated by CC, followed by prep. TLC (C_6H_6–$CHCl_3$, 9:1). Crystallization of fraction III (7.4 g) from hexane yielded pure methyl labdanolate (**4**). CC of the residual part (in $AgNO_3$–silica gel (2:8) gave **6**, **5** and **4**. Compounds **5** and **6** were isolated by prep. TLC (C_6H_6–$CHCl_3$–Et_2O, 1:3:1).

(5R,8R,9S,10S)-*Methyl*-2-*oxo*-3-cis-*cleroden*-15-*oate* (**6**). Colourless oil. $[\alpha]_D^{22}$ −3° ($CHCl_3$; *c* 0.56). UV λ_{max}^{EtOH} nm: 234 (ε12 800). CD $\lambda_{max}^{n\text{-hexane}}$ nm: 342 (Δε + 0.94), 329 (Δε − 4.45), 206 (Δε + 6.31). IR ν_{max}^{film} cm^{-1}: 1730, 1660, 1610, 1425, 1370, 1260, 1220, 1140, 1000, 840. ^{1}H NMR: δ5.52 (1H, *s*, H-3), 3.67 (3H, *s*), 1.84 (3H, *d*, *J* = 1.5 Hz), 1.12 (3H, *s*), 0.98 (3H, *s*). EIMS 70 eV, *m/z* (rel. int.): 334 $[M]^+$ (6), 205 (39), 125 (38), 121 (95), 109 (100), 95 (90), 69 (40). The neutral fraction (8.2 g) was chromatographed on a dry column eluting with C_6H_6–Et_2O (7:3) to yield four fractions: I (38.5%); II (17.5%); III (8.0%) and IV (22.3%).

The NaOH soluble fraction was saturated with a stream of CO_2 yielding a precipitate which was separated by filtration and from which three flavonoids were isolated by CC on silica gel–NaOAc (92:8): jaranol, genkwanin and 3-methylkampferol. The aq. soln, after separating the flavonoids was acidified and extracted with Et_2O and (−)betuligenol was isolated by silica gel CC.

8-*Hydroxy*-15-β-*phenylpropionoxy*-*labdane* (**7**). Fraction I was composed exclusively of **7**. Colourless oil. $[\alpha]_D^{22}$ −5.8° ($CHCl_3$; *c* 5.70). IR ν_{max}^{film} cm^{-1}: 3510, 1740, 1610, 1500, 1160, 1080, 940, 910, 785, 750, 700. ^{1}H NMR: δ7.09 (5H, *s*), 4.03 (2H, *t*, *J* = 6 Hz), 2.69 (4H, *m*), 1.07 (3H, *s*), 0.85 (3H, *s*), 0.77 (3H, *s*), 0.89 (3H, *d*, *J* = 6.5 Hz). EIMS 70 eV, *m/z* (rel. int.): 442 $[M]^+$ (3), 424 (2), 409 (2), 191 (7), 177 (13), 150 (63), 125 (34), 105 (63), 98 (100), 91 (63), 83 (55), 69 (95), 55 (46). Alkaline hydrolysis of **7** (200 mg, 10 ml NaOH–MeOH 10%) yielded **8** and β-phenylpropionic acid (the methyl ester identical to an authentic sample).

8-*Hydroxy*-15-*acetoxy*-*labdane* (**9**). Compound **9** was isolated from fraction II by prep. TLC eluting with *n*-hexane–EtOAc. Colourless oil. $[\alpha]_D^{22}$ −6.5° ($CHCl_3$; *c* 2.60). IR ν_{max}^{film} cm^{-1}: 3480, 1740, 1250, 1085, 1040, 940, 910. ^{1}H NMR: δ4.02 (2H, *t*, *J* = 6 Hz), 1.96 (3H, *s*), 1.07 (3H, *s*), 0.91 (3H, *d*, *J* = 6.5 Hz), 0.86 (3H, *s*), 0.78 (6H, *s*). EIMS 70 eV, *m/z* (rel. int.): 352 $[M]^+$ (2), 329 (3), 324 (3), 177 (26), 164 (26), 150 (19), 137 (22), 125 (57), 109 (55), 95 (69), 83 (100), 60 (96), 61 (81), 55 (43).

8,15-*Labdanediol* (**8**). By crystallization in C_6H_6, **8** was separated from fraction IV, mp 82–83°. $[\alpha]_D^{22}$ −5.15° ($CHCl_3$; *c* 1.00). IR ν_{max}^{film} cm^{-1}: 3350, 1130, 1080, 1050, 940, 910. ^{1}H NMR: δ3.55 (2H, *t*, *J* = 6 Hz), 1.08 (3H, *s*), 0.90 (3H, *d*, *J* = 6 Hz), 0.87 (3H, *s*), 0.78 (6H, *s*), EIMS 70 eV, *m/z* (rel. int.): 310 $[M]^+$ (3), 292 (3), 277 (3), 177 (13), 157 (12), 137 (13), 123 (25), 109 (37), 95 (48), 83 (57), 81 (62), 60 (100), 55 (47).

A soln of 86 g of the neutral part in 200 ml NaOH–MeOH (10%) was left to stand overnight at room temp. The MeOH was evaporated, H_2O and HCl were added and the mixture was extracted with Et_2O. The ethereal soln was washed with 6% $NaHCO_3$ and 4% NaOH. The neutral part (61.2%) was chromatographed on silica gel, with C_6H_6–Et_2O (9:1). A fraction (12.8%) was eluted which by later silica gel–$AgNO_3$ (8:2) CC and prep. TLC yielded **10**, **11** and sitosterol. Compounds **12** and **13** were separated from the first fraction by CC on silica gel–$AgNO_3$ (8:2) eluted with C_6H_6–Et_2O (1:1) (5.4%). Following this, elution with the same mixture gave a material (3.7%) which by crystallization in *n*-hexane gave **14**. The remainder (76.9%) of the neutral fraction was **8**.

8-*Labden*-15-*ol* (**10**). Colourless oil. $[\alpha]_D^{22}$ +31° ($CHCl_3$; *c* 1.30). IR ν_{max}^{film} cm^{-1}: 3330, 1470, 1380, 1060, 1020, 890. ^{1}H NMR: δ3.53 (2H, *t*, *J* = 6 Hz), 1.51 (3H, *s*), 0.91 (3H, *s*), 0.89 (3H, *d*, *J* = 6.5 Hz), 0.87 (3H, *s*), 0.72 (3H, *s*).

Treatment of 8,15-*labdanediol* (**8**) with HCO_2H. Compound **8** (111 mg) was treated with 1 ml HCO_2H at room temp. for 10 min, adding Et_2O dropwise to facilitate the dissolution of the product which appeared suspended in the soln. Et_2O was added, the soln was washed with 6% $NaHCO_3$ and H_2O. CC of the reaction product (110 mg) gave **15** (83 mg) and **10** (26 mg).

15-*Formyloxy*-8-*labdene* (**15**). Colourless oil. $[\alpha]_D^{22}$ +40.5° ($CHCl_3$; *c* 2.55). IR ν_{max}^{film} cm^{-1}: 1740, 1715, 1460, 1360. ^{1}H NMR: δ7.82 (1H, *s*), 4.10 (2H, *t*, *J* = 6 Hz), 1.50 (3H, *s*), 0.93 (3H, *d*, *J* = 6 Hz), 0.92 (3H, *s*), 0.89 (3H, *s*), 0.85 (3H, *s*). Alkaline hydrolysis of **15** yielded **10**.

8(17)-*Labden*-15-*ol* (**11**). Colourless oil. $[\alpha]_D^{22}$ +25.2° ($CHCl_3$; *c* 1.40). IR ν_{max}^{film} cm^{-1}: 3350, 3050, 1650, 1470, 1215, 1070, 895. ^{1}H NMR: δ4.75 (1H, *s br*), 4.45 (1H, *s br*), 3.56 (2H, *t*, *J* = 6 Hz) 0.89 (3H, *d*, *J* = 6.5 Hz), 0.88 (3H, *s*), 0.81 (3H, *s*), 0.67 (3H, *s*). EIMS 70 eV *m/z* (rel. int.): 292 $[M]^+$ (8), 277 (10), 191 (9), 177 (36), 137 (61), 123 (39), 109 (41), 95 (87), 81 (100), 69 (57), 55 (61).

6-*Oxo*-7-*labden*-15-*ol* (**12**). Colourless oil. $[\alpha]_D^{22}$ +12° ($CHCl_3$; *c* 1.20). IR ν_{max}^{film} cm^{-1}: 3410, 1670, 1240, 1050, 1030, 970, 880. ^{1}H NMR: δ5.62 (1H, *s*), 3.56 (2H, *t*, *J* = 6 Hz), 1.89 (3H, *s*), 1.10 (3H, *s*), 1.07 (3H, *s*), 0.91 (3H, *d*, *J* = 6.5 Hz), 0.81 (3H, *s*).

6β-*Hydroxy*-8(17)-*labden*-15-*ol* (**13**). Colourless oil. $[\alpha]_D^{22}$ +23° ($CHCl_3$; *c* 1.30), IR ν_{max}^{film} cm^{-1}: 3380, 3080, 1650, 1120, 1040, 890, 860. ^{1}H NMR: δ4.93 (1H, *s br*), 4.73 (1H, *s br*), 4.31 (1H, m), 3.57 (2H, *t*, *J* = 6 Hz), 2.28 (2H, *d*, *J* = 3.5 Hz). EIMS 70 eV, *m/z* (rel. int.): 290 $[M-18]^+$ (5), 275 (5), 153 (43), 135 (18), 109 (88), 95 (68), 81 (84), 69 (100), 55 (65).

8-*Epi*-15-*labdanediol* (**14**). Mp 137–140°. $[\alpha]_D^{22}$ +6.3° ($CHCl_3$; *c* 0.97). IR ν_{max}^{KBr} cm^{-1}: 3360, 1190, 1140, 1060, 1015, 910. ^{1}H NMR: δ3.60 (2H, *t*, *J* = 6 Hz), 1.11 (3H, *s*), 0.97 (3H, *s*), 0.89 (3H, *d*, *J* = 6.5 Hz). 0.87 (3H, *s*), 0.83 (3H, *s*). EIMS 70 eV, *m/z* (rel. int.): 310 $[M]^+$ (3), 292 (4), 278 (5), 195 (13), 178 (26), 157 (24), 137 (15), 123 (35), 109 (60), 95 (52), 83 (66), 81 (66), 69 (100), 55 (63), 43 (76).

Compound **14** (20 mg) in 0.6 ml C_5H_5N was treated with 1 ml

of Ac_2O and left overnight to yield 20 mg **16**. Colourless oil. $[\alpha]_D^{22}$ +6.1° ($CHCl_3$; *c* 0.97). IR ν_{max}^{film} cm^{-1}: 3530, 1745, 1250, 1140, 1060, 1040, 910. ^1H NMR: δ4.07 (2H, *t*, *J* = 6 Hz), 2.02 (3H, *s*), 1.11 (3H, *s*), 0.97 (3H, *s*), 0.89 (3H, *d*, *J* = 6.5 Hz), 0.87 (3H, *s*), 0.82 (3H, *s*).

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