Phytochemistry, Vol. 20, No. 9, pp. 2255–2258, 1981.
Printed in Great Britain.

0031–9422/81/092255–04 $02.00/0

LABDANE DITERPENES FROM *CISTUS SYMPHYTIFOLIUS*

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(*Received* 23 *January* 1981)

Key Word Index—*Cistus symphytifolius*; Cistaceae; jara; sitosterol; trimethoxykaempferol; labdane diterpenes; cativic acid; labdenic acid; labdanolic acid; cistadienic acid; cistenolic acid; labd-13(*E*)-ene-8α,15-diol; labdan-8α,15-diol.

Abstract—The investigation of the aerial part of *Cistus symphytifolius* afforded, in addition to sitosterol, trimethoxykaempferol, cativic acid, labdenic acid, labdanolic acid and labdan-8α,15-diol, three new bicyclic diterpenes: cistadienic acid, cistenolic acid and labd-13(*E*)-ene-8α,15-diol. The structures of these were determined by spectral studies and correlations. CD spectral studies showed that cistenolic acid and salvic acid are enantiomeric compounds, so the stereochemistry of salvic acid (7α-hydroxy-labd-8(17)-ene-15-oic acid) should be changed to 7β-hydroxy-eperu-8(17)-ene-15-oic acid (7β-hydroxy-*ent*-labd-8(17)-ene-15-oic acid)*.

INTRODUCTION

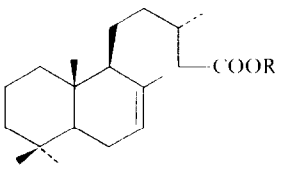
In our investigations of Canary Island flora, *Cistus symphytifolius* has been studied. Previous work on species of this genus has resulted in the isolation of labdane diterpenes [1–3]. In addition to sitosterol, trimethoxykaempferol, cativic acid (**1a**), labdenic acid (**2a**), labdanolic acid (**3a**) and labdan-8α,15-diol (**3c**), we isolated three new diterpenes with the labdane skeleton: cistadienic acid (**4a**), cistenolic acid (**2c**) and labd-13(*E*)-ene-8α,15-diol (**5a**). The structures proposed are based on spectroscopic data and chemical transformations. Cistadienic acid methyl ester (**4b**) was prepared from cistenolic acid methyl ester (**2d**) by dehydration of the hydroxyl group at C-6. Cistadienic acid (**4a**) was obtained from **4b** by saponification. CD spectral studies showed that cistenolic acid (**2c**) and salvic acid are enantiomeric products [4]. On the other hand, cistenolic acid methyl ester (**2d**) was prepared from cativic acid methyl ester (**1b**) by epoxidation to **6** and further acid treatment. In view of this it is advisable to reconsider the stereochemistry proposed for the salvic acid isolated from *Eupatorium salvia* [4]. Labd-13(*E*)-ene-8α,15-diol (**5a**) has been previously prepared [5].

RESULTS AND DISCUSSION

Cistadienic acid (**4a**) was studied as its methyl ester (**4b**) which is a diterpene, $C_{21}H_{34}O_2$ (microanalysis and mass spectrum). It has IR absorptions at 3090, 3020, 1600 and 890 cm^{-1} corresponding to the $-CH{=}CH{-}C{=}CH_2$ grouping (UV absorption at λ_{max} 235 nm) and ester (1740 cm^{-1}). The ^1H NMR spectrum shows signals for three tertiary methyl groups (δ 0.66, 0.82, 0.93) and one secondary methyl group at 0.95 (*d*, $J = 6$ Hz) and the system $CH{-}CH{=}CH{-}C{=}CH_2$ (vinyl methylenic protons at 4.85 (*m*, $W_{1/2} = 9$ Hz) and two olefinic protons at 5.73 (*d*, $J = 10$ Hz) and 6.18 (*dd*, $J = 10$ Hz and $J = 3$ Hz). The mass spectrum showed considerable similarities to mass spectral fragmentation patterns of compounds in the labdane series [6–8]; a peak at *m/z* 189 corresponds to the fragment [a_1] while peaks at 59 (COOMe) and 74 (McLafferty rearrangement) belong to the carboxylic acid methyl ester moiety. Formula and spectral data are characteristic of a bicyclic diterpene of the labdane series with a conjugate system at carbons 6, 7, 8, 17 and the carboxylic acid group at C-14. The cistadienic acid methyl ester was obtained from cistenolic acid methyl ester (**2d**) by dehydration of the hydroxyl group at C-6. Cistadienic acid (**4a**) was prepared from **4b** by saponification. The structure of cistadienic acid (**4a**) therefore corresponds to labd-6,8(17)-diene-15-oic acid.

Cistenolic acid (**2c**) ($C_{20}H_{34}O_3$, microanalysis and mass spectrum) has a carboxylic acid group (absorptions at 3300–2400 and 1700 cm^{-1}) which was confirmed by formation of the methyl ester **2d**, a secondary hydroxyl group (ν_{max} 3500 cm^{-1}) which can be acetylated under mild conditions to give the corresponding monoacetate **2e** and an exocyclic methylene group (ν_{max} 3080, 1650 and 910 cm^{-1}). The ^1H NMR spectrum of **2c** shows signals for a single hydroxyl group (geminal proton at δ 4.38) with axial configuration (*m*, $W_{1/2} = 6$ Hz) which is deshielded to 5.40 in the acetate methyl ester **2e**, signals for three angular methyl groups, singlets at 0.64, 0.78 and 0.86, and for one secondary methyl group at 0.95 (*d*, $J = 6$ Hz), while the vinylic methylene protons are observed at 4.62 and 5.02 (*m*, $W_{1/2} = 4$ Hz and *m*, $W_{1/2} = 4$ Hz). The mass spectrum is characteristic of a bicyclic labdanic diterpene carrying the carboxylic group on the side chain [6–8]; it shows a peak at *m/z* 207 (fragment [a_2]) which corresponds to the loss of the side chain and confirms the existence of the hydroxyl and methylene groups in the bicyclic moiety. Hence cistenolic acid has the structure 7α-hydroxy-labd-8(17)-ene-15-oic acid (**2c**). Cistenolic acid methyl ester (**2d**) was prepared from cativic acid methyl ester (**1b**) by α-epoxidation to **6** and further acid treatment.

* Nomenclature is based on (1969) *The Common and Systematic Nomenclature of Cyclic Diterpenes*, 3rd revision.



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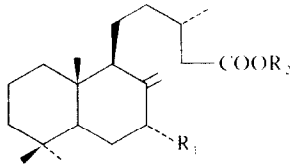
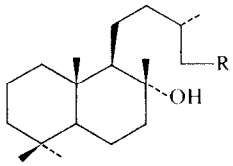
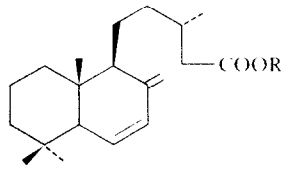
1b R = Me



2a $R_1 = R_2 = H$
2b $R_1 = H$, $R_2 = Me$
2c $R_1 = OH$, $R_2 = H$
2d $R_1 = OH$, $R_2 = Me$
2e $R_1 = OAc$, $R_2 = Me$



3a R = COOH
3b R = COOMe
3c $R = CH_2OH$



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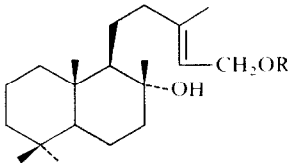

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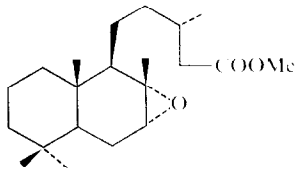
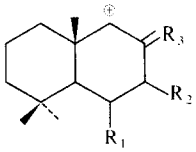
5b R = Ac



6



$[a_1]$ $R_1 = R_2 = \Delta^6$, $R_3 = CH_2$
$[a_2]$ $R_1 = H$, $R_2 = OH$, $R_3 = CH_2$
$[a_3]$ $R_1 = R_2 = H$, R_3 = 

Recently this structure has been assigned to salvic acid [4]. However, cistenolic acid ($[\alpha]_D$ −21.5°, methyl ester $[\alpha]_D$ −17°) and salvic acid ($[\alpha]_D$ +21.5°, methyl ester $[\alpha]_D$ +18.8°) are enantiomeric compounds as shown by their CD spectra which show at λ_{max} 194 nm negative ($[\theta]$ − 36 900) and positive ($[\theta]$ +42 900) Cotton effects, respectively. Thus, the stereochemistry of salvic acid should be changed to 7β-hydroxy-eperu-8(17)-ene-15-oic acid.

Compound **5a** has been characterized as labd-13(*E*)-ene-8α,15-diol by spectral data. Its microanalysis and mass spectrum are in agreement with the formula $C_{20}H_{36}O_2$. Its IR spectrum shows the presence of hydroxyl groups (3600 cm^{-1}), one of them tertiary or hindered secondary (acetate 3600 cm^{-1}). The ^1H NMR spectrum shows signals for five tertiary methyl groups: singlets at δ 0.80 (double), 0.88, 1.14 (Me–C–O) and a broad singlet at 1.70 ($W_{1/2}$ = 4 Hz) indicating a vinylic methyl group. An olefinic proton at 5.40 (triplet) indicates that the double bond is trisubstituted. The presence of a primary allylic alcohol is confirmed by a doublet at 4.15 that is deshielded to 4.58 in the monoacetate **5b**. The ^{13}C NMR spectrum of **5b** agreed with these assignments: two olefinic carbons at 143.4 (singlet in off-resonance) and 118.2 (*d*) and a triplet at 61.45 corresponding to the primary carbinol; a singlet at 73.9 corresponds to a quaternary carbon geminal to an oxygenated function. The mass spectrum shows the fragmentation pattern characteristic of the labdane diterpene series. A peak at *m/z* 191 corresponds to the fragment $[a_3] - H_2O$. Hence **5a** is an enantiomeric compound of eperu-13(*E*)-ene-8β,15-diol [9]. However, all efforts to confirm this fact

(CD in vacuum, UV and ORD region 215–350 nm) were unsuccessful. Thus, the stereochemistry for **5a** is based on the presence of labdane diterpenes in *Cistus symphytifolius*.

EXPERIMENTAL

Mps were determined on a Kofler block and are uncorr. Optical rotations were measured in $CHCl_3$ and ^1H NMR and ^{13}C NMR spectra in $CDCl_3$ with TMS as internal reference. Column and dry column chromatography were performed on Si gel 0.2–0.5 and 0.063–0.2 mm respectively. The methyl esters were obtained from acids by treatment with excess CH_2N_2 in Et_2O.

Isolation of the products. The aerial part of the plant (30 kg) collected in May in Icod, Tenerife, Canary Islands, was finely cut, air-dried, extracted in a Soxhlet with EtOH and concd *in vacuo*. The syrupy residue was dissolved in H_2O and thoroughly extracted with EtOAc. The concd extracts were chromatographed on Si gel (C_6H_6, C_6H_6–EtOAc) giving: sitosterol (2 g), trimethoxykaempferol (90 mg) and two diterpene mixtures: A (76 g) and B (26 g). A was methylated and first chromatographed on Si gel (C_6H_6) and then separated by dry column chromatography on $AgNO_3$–Si gel (1:5; petrol–C_6H_6, 4:1) to give cativic acid methyl ester (**1b**; 6 g), labdenic acid methyl ester (**2b**; 1 g) and cistadienic acid methyl ester (**4b**; 0.5 g). The mixture B was methylated and chromatographed (C_6H_6, C_6H_6–EtOAc) to give cistenolic acid methyl ester (**2d**; 0.6 g), labdanolic acid methyl ester (**3b**; 2 g), labd-13(*E*)-ene-8α,15-diol (**5a**; 0.6 g) and labdan-8α,15-diol (**3c**; 0.8 g).

Cistadienic acid methyl ester (**4b**). Oil $[\alpha]_D$ +5.2° (*c*, 0.24). (Found: C, 78.88; H, 10.92; $C_{21}H_{34}O_2$ requires: C, 79.19; H, 10.76%). MS (probe) 70 eV *m/z* (rel. int.): 318 $[M]^+$ (4), 287 (1), 189 $[a_1]^+$ (6), 119 (21), 105 (17), 74 (McLafferty, 72), 59 $[COOMe]^+$ (100). IR ν_{max}^{film} cm^{-1}: 3090, 3020, 1600, 890 (–CH=CH–C=CH_2), 1740 (ester); UV λ_{max}^{EtOH} (log ε) nm: 235 (4.14); ^1H NMR: δ 0.66 (3 H, *s*), 0.82 (3 H, *s*), 0.93 (3 H, *s*), 0.95 (3 H, *d*, *J* = 6 Hz, H-16), 3.65 (3 H, *s*, –COOMe), 4.85 (2 H, *m*, $W_{1/2}$ = 9 Hz, H-17), 5.73 (1 H, *d*, $J_{7,6}$ = 10 Hz, H-7), 6.18 (1H, *dd*, $J_{6,7}$ = 10 Hz, $J_{6,5}$ = 3 Hz. H-6). By treatment of **4b** with 5% KOH–MeOH cistadienic acid (**4a**) was obtained, oil, $[\alpha]_D$ −6° (*c*, 0.182). IR ν_{max}^{film} cm^{-1}: 3500–2300 (br), 1710 (COOH), 3080, 3020, 1600, 890 (–CH=CH–C=CH_2); ^1H NMR: δ 0.67 (3 H, *s*), 0.82 (3 H, *s*), 0.93 (3 H, *s*), 0.99 (3 H, *d*, *J* = 6 Hz, H-16), 4.86 (2 H, *m*, $W_{1/2}$ = 9 Hz, H-17), 5.74 (1 H, *d*, $J_{7,6}$ = 10 Hz, H-7), 6.20 (1 H, *dd*, $J_{6,7}$ = 10 Hz, $J_{6,5}$ = 3 Hz, H-6), 9.70 (1 H, *m*, $W_{1/2}$ = 6 Hz, –COOH).

Cistenolic acid methyl ester (**2d**). Oil, $[\alpha]_D$ −17° (*c*, 0.2). MS (probe) 70 eV *m/z* (rel. int.): 336 $[M]^+$ (2), 318 (32), 303 (18), 275 (4), 207 $[a_2]^+$ (16), 192 (5), 189 (15), 138 (18), 123 (100), 74 (McLafferty, 9), 59 $[COOMe]^+$ (18). IR ν_{max}^{film} cm^{-1}: 3500 (OH), 3070, 1650, 910 (C=CH_2), 1720 (ester); ^1H NMR: δ 0.64 (3 H, *s*), 0.78 (3 H, *s*), 0.92 (3 H, *d*, *J* = 6 Hz, H-16), 3.64 (3 H, *s*, –COOMe), 4.36 (1 H, *m*, $W_{1/2}$ = 6 Hz, H-7), 4.62, 502 (2 H, *m*, $W_{1/2}$ = 4 Hz, *m*, $W_{1/2}$ = 4 Hz, H-17).

Cistenolic acid methyl ester acetate (**2e**). Oil, $[\alpha]_D$ −1.3° (*c*, 0.22). IR ν_{max}^{film} cm^{-1}: 3080, 1650, 910 (C=CH_2), 1735 (C=O). ^1H NMR: δ 0.65 (3 H, *s*), 0.77 (3 H, *s*), 0.80 (3 H, *s*), 0.92 (3 H, *d*, *J* = 6 Hz, H-16), 2.02 (3 H, *s*, MeCOO–), 3.64 (3 H, *s*, –COOMe), 4.72, 5.14 (2 H, *m*, $W_{1/2}$ = 4 Hz, *m*, $W_{1/2}$ = 4 Hz, H-17), 5.40 (1 H, *m*, $W_{1/2}$ = 6 Hz, H-7).

Cistenolic acid (**2c**). Obtained from **2d**. Treatment of **2d** with 5% KOH–MeOH gave **2c**, mp 139–141° (MeOH–Me_2CO), $[\alpha]_D$ −21.5° (*c*, 0.232). (Found: C, 74.25; H, 10.42. $C_{20}H_{34}O_3$ requires: C, 74.49; H, 10.63%). MS (probe) 70 eV *m/z* (rel. int.): 322 $[M]^+$ (2), 304 (34), 289 (29), 271 (8), 261 (7), 207 $[a_2]^+$ (21), 192 (17), 189 (22), 138 (21), 123 (100). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (OH), 3300–2400 (br), 1700 (COOH), 3080, 1650, 910 (C=CH_2). UV λ_{max}^{MeCN} (log ε) nm: 194 (3.892); CD (MeCN) $[\theta]_{215}$ 0, $[\theta]_{194}$ 36 900. ^1H NMR: δ 0.64 (3 H, *s*), 0.78 (3 H, *s*), 0.86 (3 H, *s*), 0.95 (3 H, *d*, *J* = 6 Hz, H-16), 4.38 (1 H, *m*, $W_{1/2}$ = 6 Hz, H-7), 4.62, 5.02 (2 H, *m*, $W_{1/2}$ = 4 Hz, *m*, $W_{1/2}$ = 4 Hz, H-17), 6.46 (1 H, *m*, $W_{1/2}$ = 12 Hz, –COOH).

Labd-13(E)-*ene*-8α,15-*diol* (**5a**). Mp 131–134° (C_6H_6), $[\alpha]_D$ 0° (*c*, 2.53), MS (probe) 70 eV *m/z* (rel. int.): 290 $[M-H_2O]^+$ (4), 275 (6), 272 (7), 257 (3), 205 (5), 192 (89), 191 $[a_3-H_2O]^+$ (50), 177 (100), 138 (4), 137 (28), 123 (42). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH). UV λ_{max}^{MeCN} (log ε) nm: 196 (4.02). ^1H NMR: δ 0.80 (6 H, *s*, H-18, H-19), 0.88 (3 H, *s*, H-20), 1.12 (3 H, *s*, H-17), 1.70 (3 H, *br s*, $W_{1/2}$ = 4 Hz, H-16), 4.15 (2 H, *d*, *J* = 8 Hz, H-15), 5.40 (1 H, *t*, *J* = 8 Hz, H-14).

Labd-13(E)-*ene*-8α,15-*diol*-15-*yl acetate* (**5b**). Prepared as usual, oil, MS (probe) 70 eV *m/z* (rel. int.): 290 $[M-HOAc]^+$ (4), 275 (4), 272 (7), 257 (8), 205 (5), 192 (72), 191 $[a_3-H_2O]^+$ (53), 177 (100), 138 (5), 137 (31), 123 (46). IR ν_{max}^{film} cm^{-1}: 3480 (OH), 1745 (CO). ^1H NMR: δ 0.80 (6 H, *s*, H-18, H-19), 0.87 (3 H, *s*, H-20), 1.13 (3 H, *s*, H-17), 1.71 (3 H, *br s*, $W_{1/2}$ = 4 Hz, H-16), 2.05 (3 H, *s*, MeCOO–), 4.58 (2 H, *d*, *J* = 8 Hz, H-15), 5.36 (1 H, *t*, *J* = 8 Hz, H-14). ^{13}C NMR, ppm (TMS = 0), (carbon number): 39.8 (1), 18.5 (2), 42.05 (3), 33.4 (4), 56.2 (5), 20.6 (6), 42.9 (7), 73.9 (8), 61.3 (9), 39.3 (10), 23.9 (11), 44.7 (12), 143.4 (13), 118.2 (14), 61.45 (15), 16.6 (16), 23.5 (17), 33.4 (18), 21.5 (19), 15.5 (20), 171.0 (C=O), 21.0 (MeC=O).

Cistadienic acid methyl ester (**4b**) *from cistenolic acid methyl ester* (**2d**). To a soln of **2d** (91 mg) in C_6H_5N was added methanesulfonyl chloride at 0° and the soln was kept at this temp. for 12 hr. It was poured into H_2O and worked up with Et_2O and the ethereal layer was washed with satd $NaHCO_3$ and H_2O. The residue (50 mg) without further purification was refluxed in DMF (10 ml) for 4 hr. Standard work-up followed by chromatography (petrol–C_6H_5, 4:1) gave **4b** (40 mg), oil, $[\alpha]_D$ +6° (*c*, 0.33) identical with an authentic sample (TLC. UV, IR, ^1H NMR).

7α,8α-*Epoxy-labdanolic acid methyl ester* (**6**). To a soln of cativic acid methyl ester (**1b**) (0.5 g) in CH_2Cl_2 (20 ml) was added *m*-chloroperbenzoic acid (0.5 g) and an aq. soln of $NaHCO_3$ (20 ml) was added. The mixture was stirred at room temp. for 24 hr. The organic layer was washed with satd $NaHCO_3$ and H_2O. Chromatography (C_6H_6) yielded **6** (460 mg), oil; IR ν_{max}^{film} cm^{-1}: 1720 (ester). ^1H NMR: δ 0.70 (3 H, *s*), 0.81 (6 H, *s*), 0.91 (3 H, *d*, *J* = 6 Hz, H-16), 1.26 (3 H, *s*, H-17), 2.91 (1 H, *m*, $W_{1/2}$ = 6 H, H-7), 3.62 (3 H, *s*, COOMe).

Cistenolic acid methyl ester (**2d**) *from* **6**. A soln of **6** (450 mg) in dry C_6H_6 (40 ml) was added to *p*-toluene sulfonic acid (10 mg) while stirring and the mixture was refluxed 24 hr. Work-up as for **6** and purification by dry chromatography gave **2d** (200 mg), oil, $[\alpha]_D$ −16° (*c*, 0.21), which was identical with an authentic sample (TLC, IR, ^1H NMR).

Acknowledgements—Part of this work was subsidized by the Comisión Asesora de Investigación Científica y Técnica. M.C. wishes to thank the Centro Iberoamericano de Cooperación de Spain for a fellowship. Dr. D. Wegmann kindly sent the UV and CD spectra and Prof. K. Schreiber the ORD spectra. Dr. M. Silva gave us a sample and the spectra of salvic acid and Prof. J. de Pascual Teresa a sample of labdanolic acid.

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