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CLEONIOIC ACID, A NEW DITERPENOID FROM *CLEONIA LUSITANICA*

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Key Word Index—*Cleonia lusitanica*; Labiatae; diterpenoids; 11α-acetoxy-7,15-isopimaradien-18-oic acid; ^{13}C NMR data.

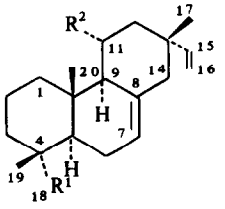
Abstract—From the aerial parts of *Cleonia lusitanica* four previously known diterpenoids have been isolated. In addition, a new isopimarane derivative, cleonioic acid, has been obtained and the structure 11α-acetoxy-7,15-isopimaradien-18-oic acid established by chemical and spectroscopic means and by correlation with 7,15-isopimaradien-18-ol.

INTRODUCTION

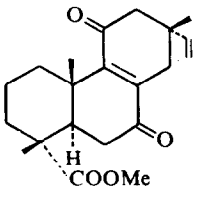
In our search for new natural diterpenoids in the Labiatae species endemic in the Iberian Peninsula [1, 2], we have examined the aerial parts of *Cleonia lusitanica* from which a complex mixture of diterpenic acids has been isolated. After treatment with ethereal diazomethane and chromatographic separation, the previously known 8,11,13-abietatrien-18-oic acid methyl ester (methyl dehydroabietate) [3–5], 8,15-isopimaradien-18-oic acid methyl ester [6, 7], 7,15-isopimaradien-18-oic acid methyl ester (methyl isopimarate) [8, 9] and 9,13-peroxido-8(14)-abieten-18-oic acid methyl ester (probably arising from air oxidation of palustric acid methyl ester) [10] have been isolated, besides a new natural isopimarane derivative, cleonioic acid methyl ester, whose structure of methyl 11α-acetoxy-7,15-isopimaradien-18-oate (**1**) was established.

RESULTS AND DISCUSSION

Cleonioic acid methyl ester (**1**) had a molecular formula $C_{23}H_{34}O_4$ and its ^1H NMR spectrum showed characteristic signals for a vinyl group (ABX system: δ_A 4.83, δ_B 4.87, δ_X 5.71; J_{AB} = 1.5 Hz, J_{AX} = 10.5 Hz, J_{BX} = 17.5 Hz), an olefinic proton (1H, *m*, $W_{\frac{1}{2}}$ = 10 Hz at δ 5.44), a carbomethoxyl group (3H, *s*, at δ 3.62), three C-Me singlets (δ 1.24, 0.95 and 0.92) and an equatorial acetoxyl group (3H, *s*, at δ 2.00) attached to a secondary carbon atom (axial geminal proton appeared as a sextet, $J_{aa'} = J_{aa''}$ = 10 Hz, $J_{ae'}$ = 5 Hz, at δ 5.04). This ^1H NMR spectrum was almost identical to that of 7,15-isopimaradien-18-oic acid methyl ester (**2**) [11], the only differences being the absence in compound **2** of the signals assigned to the acetoxyl group and a paramagnetic shift ($\Delta\delta$ +0.08) shown by the C-20 methyl protons of **1**. All the above data pointed toward a structure for compound **1** based on the 7,15-isopimaradiene skeleton with a carbomethoxyl group on C-18 and an equatorial acetoxyl group at C-11 position. The ^{13}C NMR spectrum of **1** (see Experimental) confirmed this structure with the carbon resonances being in agreement with a 7,15-isopimaradiene skeleton [12] possessing a carbomethoxyl group at the C-18 equatorial position (C-18, C-19 and —OMe at 178.7, 17.5 and 51.8 ppm, respectively) and an acetoxyl group (singlet at 169.8 and quartet at 22.1 ppm) equatorially attached to the C-11 position [C-11 at 70.9 ppm, paramagnetic shifts on C-1, C-9 and C-12 (1.0, 3.3 and 6.1 ppm, respectively) and diamagnetic shifts on C-8 and C-13 carbon atoms (−3.0 and −0.8 ppm, respectively) with respect to compound **2**].



	R^1	R^2
1	COOMe	OAc
2	COOMe	H
3	COOMe	OH
5	COOMe	OTs
6	CH_2OH	H



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On the other hand, alkaline hydrolysis of compound **1** under mild conditions (0.5 N KOH, in EtOH solution, 6 hr at room temperature) yielded the hydroxy ester **3** ($C_{21}H_{32}O_3$, ν_{OH} 3430 cm^{-1}, $\delta_{H\text{-}11}$ 3.87, no MeCOO-signal), which was treated with CrO_3–pyridine for 48 hr at room temperature to give a $C_{21}H_{28}O_4$ compound which had two keto groups at C-7 and C-11 and a double bond between the C-8 and C-9 carbon atoms of the 15-isopimarene skeleton [**4**, λ_{max} 266 nm, ε 7800; carbon resonances: 200.3 and 200.2 (two ketones, C-7 and C-11), 154.2 (*s*, C-9) and 141.9 ppm (*s*, C-8)]. Thus, the hydroxyl group of compound **3** must be placed at the C-7 or C-11 position. Application of Horeau's method of partial resolution [13] to **3** established the absolute configuration of the equatorial alcohol as *R*, in complete agreement with an 11α-OH on the isopimarane skeleton but not with a 7β (equatorial) alcohol in the same hydrocarbon skeleton.

Final proof of the carboxylic function on C-18, of 7,15-isopimaradiene skeleton and hence of the OAc group on C-11 of the molecule of cleonioic acid was established as follows.

Tosyl chloride–pyridine treatment of **3** gave the derivative **5** which was reduced with $LiAlH_4$ yielding 7,15-isopimaradien-18-ol (**6**) [14, 15], identical in all respects (mp, mmp, $[\alpha]_D$, IR, ^1H NMR, MS) with an authentic sample obtained from **2**.

This correlation firmly established the structure and absolute stereochemistry of cleonioic acid methyl ester as methyl 11α-acetoxy-7,15-isopimaradien-18-oate (**1**).

EXPERIMENTAL

Mps were determined in a Kofler apparatus and are uncorr. ^1H and ^{13}C NMR spectra were measured at 100 and 25.2 MHz, respectively, in $CDCl_3$ soln with TMS as internal standard. Assignments of ^{13}C chemical shifts were made with the aid of off-resonance and noise-decoupled ^{13}C NMR spectra. Elemental analyses were carried out in this laboratory (Madrid) with the help of an automatic analyser. Plant materials were collected in June 1977 near Molina de Aragón (Guadalajara, Spain), identified by Dr. J. Borja, Department of Botany, Faculty of Pharmacy (Madrid) and voucher specimens were deposited in the Herbarium of this Faculty.

Extraction and isolation of the diterpenoids. Dried and finely powdered *C. lusitanica* aerial parts (1 kg) were extracted twice with Me_2CO (8 l.) at room temp. for 3 days. The extracts were evapd to dryness under red. pres. and low temp. (30°), dissolved in EtOAc and washed with H_2O. After evapn of the solvent, the residue (32 g) was chromatographed on a Si gel (Merck, 7734, deactivated with 15% H_2O) column (1.5 kg). Elution with petrol and petrol–EtOAc (9:1 and 4:1) gave a mixture of diterpenic acids (10.2 g). This mixture was treated with ethereal CH_2N_2 yielding 10.6 g of the methyl esters, which were repeatedly chromatographed on Si gel and Si gel plus $AgNO_3$ (8%) columns with petrol and petrol–EtOAc mixtures as eluents, yielding the following compounds in order of elution: methyl dehydroabietate (2.7 g), methyl isopimarate (**2**, 1.96 g), methyl 8,15-isopimaradien-18-oate (1.2 g), methyl 9,13-peroxido-8(14)-abieten-18-oate (2.2 g) and cleonioic acid methyl ester (**1**, 1.3 g). The previously known diterpenoids were identified by their physical (mp, $[\alpha]_D$) and spectroscopic (IR, ^1H NMR, ^{13}C NMR, MS) data and by comparison (mmp, TLC) with authentic samples.

Cleonioic acid methyl ester (**1**). Mp 128–130° (aq. MeOH): $[\alpha]_D^{18}$ −16.9° (*c* 0.92, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3100, 3070, 3000, 2980, 2960, 2940, 2930, 2895, 2880, 2850, 1725, 1640, 1465, 1435, 1390, 1380, 1365, 1255, 1190, 1150, 1055, 1030, 1005, 965, 923, 880, 860, 840, 785, 675. ^1H NMR: see Discussion. ^{13}C NMR: carbon atom (chemical shift ppm): 1 (40.2), 2 (18.2), 3 (36.7), 4 (46.8), 5 (44.7), 6 (25.2), 7 (124.4), 8 (133.0), 9 (55.7), 10 (36.2)*, 11 (70.9), 12 (42.1), 13 (36.7)*, 14 (46.4), 15 (148.3), 16 (109.9), 17 (21.8)†, 18 (178.7), 19 (17.5), 20 (15.3), —OMe (51.8), CH$_3$COO- (22.1)†, MeCOO- (169.8). (Assignments marked* or † could be reversed, but those given here are considered to be most likely.) MS (70 eV, direct inlet) *m/e* (rel. int.): 374 (M^+ 0.2), 314 (100), 299 (50), 273 (45), 255 (55), 254 (45), 239 (95), 211 (40), 199 (45), 185 (45), 146 (40), 143 (45), 131 (55), 109 (50), 105 (55), 101 (20), 91 (60). (Found: C, 73.58; H, 9.36. $C_{23}H_{34}O_4$ requires: C, 73.76; H, 9.15%).

Methyl 11α-hydroxy-7,15-isopimaradien-18-oate (**3**). A soln of compound **1** (500 mg) in 0.5 N ethanolic KOH was left 6 hr at room temp. The soln was then diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was dried, filtered and concd *in vacuo* to leave a residue (410 mg) of pure **3**; mp 112–115° (aq. EtOH); $[\alpha]_D^{18}$ −15.0° (*c* 0.89, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3430, 3090, 1725, 1640, 1250, 920. ^1H NMR (δ): ABX system (δ_X = 5.80, δ_A = 4.89, δ_B = 4.94; J_{AB} = 1.5 Hz, J_{AX} = 10.5 Hz, J_{BX} = 17.5 Hz, H-15 and 2H-16 protons), 5.48 (1H, *d(br)*, *J* = 5 Hz, $W_{\frac{1}{2}}$ = 10 Hz, H-7), 3.87 (1H, *sextet*, $J_{aa'}$,J_{ad} = 10 Hz, $J_{ae'}$ = 4 Hz, axial H-11), 3.64 (3H, *s*, —COOMe), C-Me singlets at 1.27 (3H-19), 1.05 (3H-20) and 0.88 (3H-17). MS (70 eV, direct inlet) *m/e* (rel. int.): 332 (M^+ 9), 317 (8), 314 (24), 299 (36), 288 (36), 272 (49), 257 (36), 255 (56), 239 (76), 175 (60), 159 (36), 146 (12), 121 (64), 109 (100), 105 (64), 101 (12), 91 (56). (Found: C, 75.67; H, 9.63. $C_{21}H_{32}O_3$ requires: C, 75.86; H, 9.70%).

Application of Horeau's method [13] *to* **3**. A mixture of (±)-α-phenylbutyric anhydride (0.42 mmol) and **3** (0.173 mmol) in Py soln (2 ml) was kept at room temp. for 20 hr. α_1 = −0.552; α_2 = −0.772; $\alpha_1 - 1.1\alpha_2$ = +0.297. Configuration 11*R*.

Methyl 7,11-dioxo-8,15-isopimaradien-18-oate (**4**). To a suspension of CrO_3 (500 mg) in Py (5 ml) was added **3** (300 mg) in Py soln (5 ml). The mixture was left 48 hr at room temp. The soln was diluted with H_2O and extracted with Et_2O. The Et_2O extract was dried and evapd and the residue was purified on PLC (petrol–EtOAc, 7:3) yielding 230 mg of pure **4**; mp 121–123° (aq. EtOH); $[\alpha]_D^{27}$ +15.2° (*c* 1.25, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3090, 1720, 1670, 1640, 1255, 935, 930, 918, 830, 755, 720, 645. UV λ_{max}^{EtOH} nm: 266 (ε 7800). ^1H NMR (δ): ABX system (δ_X = 5.77, δ_A = 5.03, δ_B = 4.98; J_{AB} = 1 Hz, J_{AX} = 11 Hz, J_{BX} = 17.5 Hz, H-15 and 2H-16 protons), 3.66 (3H, *s*, —COOMe), C-Me singlets at 1.34 (3H-17), 1.26 (3H-19) and 1.04 (3H-20). ^{13}C NMR carbon atom (chemical shift ppm): 1 (37.2), 2 (17.9), 3 (36.2), 4 (46.5), 5 (44.1), 6 (34.4), 7 (200.3)*, 8 (141.9), 9 (154.2), 10 (38.6)†, 11 (200.2)*, 12 (50.7), 13 (37.2)†, 14 (36.8), 15 (144.6), 16 (112.7), 17 (26.3), 18 (177.5), 19 (17.9), 20 (16.3), —OMe (52.2). (Assignments marked * or † could be reversed.) MS (70 eV, direct inlet) *m/e* (rel. int.): 344 (M^+ 100), 329 (4), 316 (8), 312 (12), 302 (12), 301 (11), 285 (36), 284 (52), 201 (32), 101 (8), 91 (32). (Found: C, 73.15; H, 8.25. $C_{21}H_{28}O_4$ requires: C, 73.22; H, 8.19%).

7,15-Isopimaradien-18-ol (**6**) *from* **3**. Compound **3** (250 mg) was dissolved in dry Py (5 ml). To this soln, cooled to 0°, was added *p*-toluenesulfonyl chloride (0.5 g) and the mixture left at room temp. for 3 days, diluted with H_2O and extracted with $CHCl_3$. Evapn of the solvent gave a residue (260 mg) which was purified on PLC (Si gel, petrol–EtOAc, 9:1) yielding 250 mg of the tosylate **5**, a syrup; IR ν_{max}^{NaCl} cm^{-1}: 3080, 1725, 1640, 1600, 1250, 1195, 1180, 920, 765; ^1H NMR (δ): 7.82 and 7.34 (A_2B_2 system, *J* = 8 Hz, 4 aromatic protons), ABX system (part X: 6 lines at 5.86, 5.77, 5.75, 5.70, 5.68 and 5.58, H-15; δ_X = 4.86, δ_B = 4.83; J_{AB} = 1 Hz, J_{AX} = 10.5 Hz, J_{BX} = 17.5 Hz, 2H-16), 5.52

(1H, *d(br)*, $J = 6$ Hz, $W_{\frac{1}{2}} = 11$ Hz, H-7), 4.98 (1H, *sextet*, $J_{aa'} = J_{aa''} = 10$ Hz, $J_{ae'} = 4$ Hz, H-11), 3.64 (3H, *s*, —COOMe), 2.44 (3H, *s*, Ph-Me), C-Me singlets at 1.24 (3H-19), 1.02 (3H-20) and 0.88 (3H-17). $LiAlH_4$ reduction of **5** (200 mg) in Et_2O soln 24 hr at room temp. gave 7,15-isopimaradien-18-ol (**6**, 95 mg after PLC purification), mp 86–87° (aq. MeOH); $[\alpha]_D^{19}$ −24.2° (*c* 0.47, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3310, 3085, 3005, 2920, 2870, 2855, 1640, 1445, 1385, 1300, 1200, 1155, 1145, 1070, 1050, 1005, 980, 915, 865, 840, 825. ^1H NMR (δ): ABX system ($\delta_X = 5.91$, $\delta_B = 4.97$, $\delta_A = 4.93$; $J_{AB} = 1.5$ Hz, $J_{AX} = 10.5$ Hz, $J_{BX} = 17.5$ Hz, H-15 and 2H-17 protons), 5.44 (1H, *m*, $W_{\frac{1}{2}} = 8$ Hz, H-7), 3.45 and 3.15 (AB system, $J = 12$ Hz, 2H-18), C-Me singlets at 0.92, 0.89 and 0.88. MS (70 eV, direct inlet) *m/e* (rel. int.): 288 (M^+ 60), 273 (30), 270 (9), 257 (100), 255 (37), 241 (20), 201 (20), 187 (28), 161 (23), 148 (30), 133 (35), 119 (44), 109 (62), 105 (48), 91 (37). (Found: C, 83.17; H, 11.21. Calc. for $C_{20}H_{32}O$: C, 83.27; H, 11.18%). Identical in all respects with the previously reported compound [14, 15]: mp 85–87°; $[\alpha]_D^{22}$ −24.6°, and also with an authentic sample obtained by $LiAlH_4$ reduction of compound **2** (mp, mmp, $[\alpha]_D$, TLC, IR, ^1H NMR, and MS).

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