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DITERPENOID AND OTHER COMPONENTS OF *CISTUS LAURIFOLIUS*

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Key Word Index –*Cistus laurifolius*; Cistaceae; diterpene; glucosides; inositol.

Abstract –From the ethanol extract of *C. laurifolius* was isolated a new diterpene, 6β,8-dihydroxy-*ent*-13*E*-labden-15-oic acid, which we called laurifolic acid. Four glucosides were separated in the form of their acetyl derivatives: β-D-glucopyranosiloxyethane, 4-β-D-glucopyranosiloxyacetophenone, roseoside and 1,3-dihydroxy-5-β-D-glucopyranosiloxybenzene. An inositol, 1-*O*-methyl-epi-inositol, was also identified.

INTRODUCTION

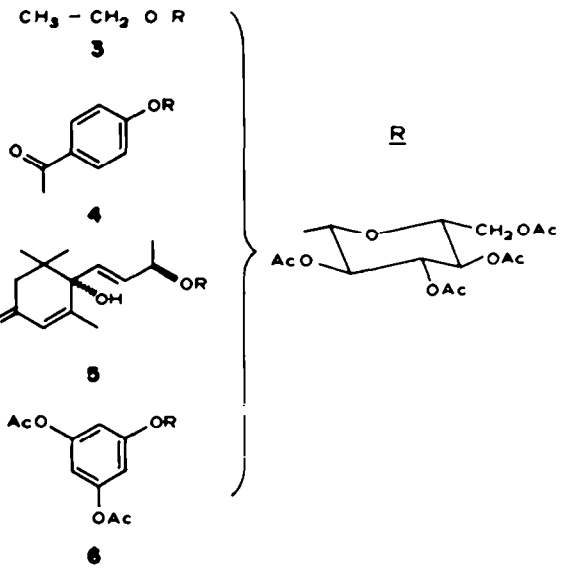
Previous work on *C. laurifolius* [1–4] was performed starting out from the *n*-hexane extract. In those studies, reports were made of the existence in the plant of diterpenes with a labdane skeleton of the normal and antipodes series, *ent*-5-epi-clerodane and rearranged *ent*-labdane with a double bond at $\Delta^{5(10)}$. We now report a study of the polar components of *C. laurifolius*.

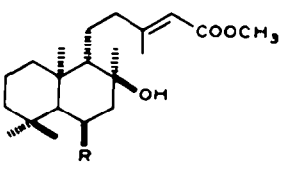
RESULTS AND DISCUSSION

The ethanol extract (8.1%) of *C. laurifolius* suspended in water was fractionated by liquid–liquid extraction with ethyl acetate and the aqueous part was washed with *n*-butanol.

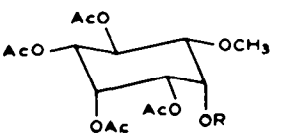
From the methylated ethyl acetate-soluble fraction compound **1** was separated by column chromatography. Compound **1** had spectral absorptions of an unsaturated α,β-hydroxy acid (3440, 1730, 1650 cm^{-1}; 240 nm). The ^1H NMR spectrum of **1** showed signals of the following groups: =CH–COOMe (δ 5.69, *br s*), CH–CHOH–CH_2 (3.86, *dt*), Me–C=C–COOMe (2.17, *s*), Me–C–OH (1.21, *s*) and three methyl groups (1.14, 0.98 and 0.83). The shift of the methyl group on a double bond points to the configuration of the double bond as being *E* [5]. The coupling constants of the δ 3.86 signal (*dt*, J = 9.90 and 3.60 Hz) correspond to the hydrogen geminal to a secondary hydroxyl group at the equatorial position on C-6 of a diterpene with a labdane skeleton.

The ^{13}C NMR spectrum of **1** showed signals of 21 carbons: six Me, six CH_2, four CH and five completely substituted carbons. The shift of C-16 was also in agreement with the *E*-configuration for the double bond of the molecule. Acetylation of **1** yielded **2** [1].





R	
1	OH
2	OAc



R	
7	Ac
8	H

From column chromatography of the *n*-butanol extract two major fractions I and II were separated which following acetylation yielded 3 [6], 4 [7], 5 [8], 6, 7 and 8. However, it is most probable that 3 is an artefact formed during the extraction process.

The ^{1}H NMR spectrum of 6 also exhibited a wide singlet at δ6.66 corresponding to a 1,3,5-trisubstituted aromatic ring and a singlet at 2.27 for two $-O_2CMe$ groups attached to an aromatic ring.

The ^{13}C NMR spectrum of 6 showed signals corresponding to 24 carbons; 14 of them corresponded to a tetra-*O*-acetylglucopyranose so that the aglucone must contain 10 carbons. Four of the carbons corresponded to two acetoxyl groups and the remaining six corresponded to a 1,3,5 trisubstituted aromatic ring, these latter signals appeared at 157.69 (2), 157.61, 110.44 and 108.27 (2). According to the spectroscopic properties of 6 we propose its structure to be 1,3-diacetoxy-5-(tetra-*O*-acetyl-β-D-glucopyranosiloxy)benzene.

Acetylation at room temperature of fraction II permitted the separation of 7 and 8 which are total and partial acetylation products of a single inositol. Compound 8 was only transformed into 7 when treatment with acetic anhydride and pyridine was carried out at 50°.

The ^{13}C NMR spectrum of 8 showed signals of 15 carbons: eight corresponding to four acetoxyl groups (4 $-O_2CMe$), six CH and one Me, such that 8 must be a methyl inositol. The signals of its ^{1}H NMR spectrum (Table 1) together with the results of the double radiation experiment permitted the elucidation of the structure and relative stereochemistry of compound 8.

The existence of a long range coupling (J = 0.98 Hz) between hydrogens over C-2 and C-4 means that they must be situated at the equatorial position. The presence of two functions in a 1,3-*cis*-diaxial relationship in 8 (a hydroxyl at C-2 and an acetoxyl at C-4) implies the existence of two hydroxyl groups in the same situation in the natural inositol. This arrangement accounts for the difficulty in obtaining the peracetylated derivative 7, since acetylation of the hydroxyl of C-2 is prevented due to the presence of the methoxyl at C-1.

From the J values for H-1, H-3, H-5 and H-6 (Table 1), it is possible to deduce the equatorial position for the methoxyl and the acetoxyls groups at C-1, C-3, C-5 and C-6, respectively. As may be inferred from the above, compound 8 must have the structure 1-*O*-methyl-3,4,5,6-tetracetyl-epi-inositol.

EXPERIMENTAL

Mps (Kofler hot stage apparatus) uncorr. ^{1}H NMR spectra were recorded on Bruker WP 200SY (200 MHz) spectrometers using $CDCl_3$ soln and TMS as internal standard. ^{13}C NMR were recorded at 50.3 MHz.

Extraction and isolation. The aerial parts of *C. laurifolius* collected at Valparaíso (Zamora, Spain) was dried and extracted with *n*-hexane (5.8%). Following this, 2 kg of plant were extracted with EtOH at room temp. for 15 days, yielding 161.6 g of extract (8.1%).

The EtOH extract (115 g) was suspended in H_2O and refluxed with EtOAc for 72 hr to yield 48.9 g of EtOAc soluble compound (42.3%). The remaining aq. soln was fractionated by separating with *n*-BuOH (19.3 g, 16.7%).

By CC on silica gel of the EtOAc-fraction, previously esterified with CH_2N_2, elution with $CHCl_3$–MeOH (9:1) separated compound 1 in addition to the acids already isolated in the *n*-hexane extract.

Methyl 6β,8-dihydroxy-ent-13E-*labden*-15-*oate* (1). Colourless oil. $[\alpha]_D^{22}$ −30.77° ($CHCl_3$; *c* 0.87). UV λ_{max}^{EtOH} nm (log ε): 240 (4.20). IR ν_{max}^{film} cm^{-1}: 3440, 1730, 1650, 1230, 1150. ^{1}H NMR: δ5.69 (1H, *s*), 3.86 (1H, *dt*, J = 9.90, 3.60 Hz), 3.69 (3H, *s*), 2.17 (3H, *s*), 1.21, 1.14, 0.98 and 0.83 (3H each, *s*). ^{13}C NMR: δ16.54 (C-20), 18.31 (C-2), 19.04 (C-16), 22.09 (C-18), 23.61 (C-11), 25.72 (C-17), 33.82 (C-4), 36.07 (C-19), 39.39 (C-10), 40.19 (C-1), 43.53 (C-3), 43.78 (C-12), 50.68 (COOMe), 54.29 (C-7), 60.86 (C-5), 61.59 (C-9), 69.06 (C-6), 73.69 (C-8), 115.12 (C-14), 160.70 (C-13), 167.27 (C-15).

Two major fractions were separated from the *n*-butanol soluble part by silica gel CC: I ($CHCl_3$–MeOH–H_2O, 6:3:1) and II ($CHCl_3$–MeOH–H_2O, 6:3:1 + 15% MeOH). Silica gel CC of the acetyl derivatives of fraction I yielded 3 (*n*-hexane–EtOAc, 9:1), 4 (*n*-hexane–EtOAc, 7:3) and 5 (*n*-hexane–EtOAc, 1:1). Fraction II was treated with Ac_2O and C_5H_5N at room temp. over 12 hr. The mixture of acetyl derivatives was first extracted with Et_2O (61.1%) and then by $CHCl_3$ (38.5%). Silica gel CC of the former yielded 6 ($CHCl_3$–Et_2O, 4:1), and 7 ($CHCl_3$–Et_2O, 7:3) and from the $CHCl_3$-soluble derivatives by the same procedure 8 was obtained.

1,3-*Diacetoxy*-5-(*tetra*-O-*acetyl*-β-D-*glucopyranosiloxy*)-*benzene* (6). Mp 154–155°. $[\alpha]_D^{22}$ −10.7° ($CHCl_3$; *c* 0.71). IR ν_{max}^{KBr} cm^{-1}: 1790, 1770, 1635, 1615, 1480, 1400, 1240, 1190, 1150, 1105, 1090, 1060, 910. ^{1}H NMR: δ6.66 (3H, *br s*), 4.27 (1H, *dd*, J_{AB} = 12.25, $J_{A,5'}$ = 5.35 Hz, H_A-6'), 4.18 (1H, *dd*, J_{AB} = 12.25, $J_{B,5'}$ = 2.43 Hz, H_B-6'), 2.27 (6H, *s*), 2.08, 2.06, 2.05, 2.03 (3H, *s* each). ^{13}C NMR: δ151.69 (C-1), 110.44 (C-2), 151.69 (C-3),

Table 1. ^{1}H NMR chemical shifts (δ), multiplicities, J (Hz) and spin-decoupling study of 8 (200 MHz, $CHCl_3$)

H	δ	multipl.	J (Hz)	Spin-decoupling studies
1	3.81	*dd*	8.79, 3.91	
2	3.53	*ddd*	4.39, 3.91, 0.98	
3	5.32	*t*	4.39	
4	5.20	*ddd*	4.39, 3.42, 0.98	
5	5.15	*dd*	8.79, 3.42	
6	5.35	*t*	8.79	
OMe	3.43	*s*		
O_2CMe	2.06	*s*		
	2.05	*s*		
	2.04	*s*		
	1.97	*s*		

108.27 (C-4), 157.61 (C-5), 108.27 (C-6), 99.07 (C-1'), 71.27 (C-2'), 72.36 (C-3'), 68.50 (C-4'), 72.81 (C-5'), 62.10 (C-6'), MeCOO: 20.98 (2 Me), 20.49 (4 Me), MeCOO: 170.41, 170.04, 169.29 (2-COO), 169.15, 168.46.

1-O-*Methyl-pentaacetyl-epi-inositol* (7). Mp 109–110°. $[\alpha]_D^{22}$ −2.6° ($CHCl_3$; *c* 1.12). IR ν_{max}^{KBr} cm^{-1}: 1770, 1750, 1380, 1240, 1070, 1060, 920. ^{13}C NMR: δ169.87, 169.75, 169.53, 169.31, 168.91 (MeCOO, each), 77.70 (C-1), 69.17 (C-2), 69.11 (C-4), 68.96 (C-5), 67.70 (C-3), 67.70 (C-6), 58.81 (MeO), 20.81, 20.76, 20.68, 20.62 and 20.57 (MeCOO, each).

1-O-*methyl*-3,4,5,6-*tetraacetyl-epi-inositol* (8). $[\alpha]_D^{22}$ +21.3 ($CHCl_3$; *c* 1.91). IR ν_{max}^{KBr} cm^{-1}: 3440, 1780, 1380, 1240, 1180, 1050. ^{13}C NMR: δ170.21, 169.73 (2C), 168.98 (MeCOO, each), 79.45 (C-1), 70.12 (C-2), 69.80 (C-4), 69.08 (C-5), 69.08 (C-6), 66.60 (C-3), 58.27 (MeO), 20.72, 20.65 and 20.49 (2C) (MeCOO, each). Treatment of **8** (10 mg) with Ac_2O–C_5H_5N for 10 hr at 50° yielded **7** (10 mg).

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