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A NEW LABDANE-TYPE DITERPENE AND OTHER COMPOUNDS FROM THE LEAVES OF *CISTUS INCANUS* SSP. *CRETICUS*

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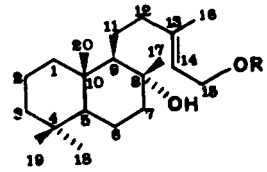
ABSTRACT.—Seven labdane-type diterpenoids, together with quercetin, myricetin, kaempferol, kaempferol-3-methyl ether, apigenin, luteolin, and aesculin, were isolated from the leaves of *Cistus incanus* ssp. *creticus*. Their structures, (5,8,9,10*R*)-labd-13(*E*)-ene-8a,15-diol [**1**], (5,8,9,10*R*)-labd-7,13(*E*)-dien-15-ol [**3**], (5,8,9,10*R*)-labd-7,13(*E*)-dien-15-yl acetate [**4**], 8,13-epoxylabd-14-ene [**5**], 13-*epi*-8,13-epoxylabd-14-ene [**6**], labd-14-ene-8,13-diol [**7**], and 13-*epi*-sclareol [**8**], were established by spectroscopic means. The structure of compound **4** in particular was established by chemical correlation with compound **3** after acetylation.

The Mediterranean region is known to be the natural habitat of the genus *Cistus* L. (Cistaceae) (1,2). This genus comprises 16 species (1) of perennial shrubs. The leaves of all *Cistus* species are covered with glands secreting essential oils and resins. A brownish resin on the surface of the leaves and stems consists mainly of terpenoids, but flavonoid aglycones (3–5) and glycosides (6) are also found in it. The common Greek name for the resin since antiquity is "Ladano." We report the isolation of seven labdane-type diterpenoids from the petroleum ether and Et_2O extracts of *Cistus incanus* ssp. *creticus* L.

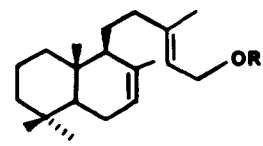
Compound **1** after acetylation afforded (5,8,9,10*R*)-labd-13-ene-8a,15-diol-15-yl acetate [**2**] (7). Compound **1** was characterized as (5,8,9,10*R*)-labd-13-(*E*)-ene-8a,15-diol by spectral data (7,8). The absolute stereochemistry was established by optical rotation, and the presence of a primary allylic alcohol was confirmed by a doublet at δ 4.15 ppm, which was deshielded to δ 4.57 ppm in the monoacetate **2** (8). The configuration at C-8 in **1** can be assigned from consideration of the ^{1}H-nmr spectra.

The ^{1}H-nmr spectrum of compound **3** was similar to that of **1**. The only difference was the presence of a multiplet at δ 5.37 ppm indicating the presence of two protons in double bonds H-14, -7). Compound **3** was characterized as (5,8,9,10*R*)-labd-7,13(*E*)-dien-15-ol by comparison with spectral data (9,10), as well as by eims and cidms spectra. The absolute configuration was determined by optical rotation.

Compound **4** is a new natural product. It was converted to compound **3** after acetylation, and has therefore been characterized as (5,8,9,10*R*)-labd-



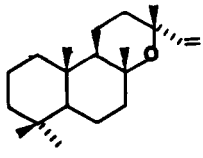
1 R=H
2 R=Ac



3 R=H
4 R=Ac

7,13(*E*)-dien-15-yl acetate. The doublet at δ 4.15 ppm in the ^{1}H-nmr spectrum of **3** was deshielded to δ 4.57 ppm in compound **4**. The absolute configuration of **4** was confirmed by optical rotation.

Compounds **5** and **6** were characterized by spectral data as 8,13-epoxylabd-14-ene and 13-*epi*-8,13-epoxylabd-14-ene, respectively (7,11,12). Similarly, compounds **7** and **8** were characterized as labd-14-ene-8,13-diol and 13-*epi*-sclareol (7, 13–16). The relative stereochemistry of compounds **3–8** is the same as in compound **1**.



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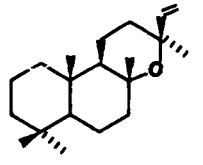

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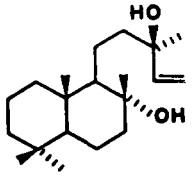

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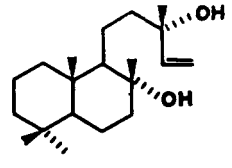

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The confirmation of the structure of these diterpenes led us to re-examine by gc-ms some of the non-identified components in the essential oil of *C. incanus* ssp. *creticus*, which had been examined in previous work (17), in order to identify them by direct comparison with the mass spectra of the above compounds. The components of the essential oil numbered as 50, 54, and 47 (17) proved to be (5,8,9,10*R*)-labd-13(*E*)-ene-8a,15 diol [**1**], (5,8,9,10*R*)-labd-7,13(*E*)-dien-15-ol [**3**], (5,8,9,10*R*)-labd-7,13(*E*)-dien-15-yl acetate [**4**], respectively.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded in Nujol, $CHCl_3$, and KBr disc (Hitachi 100-60 IR spectrometer). The nmr spectra were obtained in $CDCl_3$ (Bruker HX-270 MHz spectrometer) using TMS as an internal standard. Mass spectra (ei and cid) were determined at 70 eV on a VG Micromass, 70 70F.

PLANT MATERIAL.—Plant material (500 g) was collected in July 1986, on the island of Crete (Greece). It was authenticated by Dr. A Yiannitsaros (University of Athens). A voucher specimen is maintained in the Department of Pharmacy, University of Athens.

EXTRACTION AND ISOLATION.—The leaves of *C. incanus* ssp. *creticus* were dried in a cool dark place and coarsely powdered. The powdered plant material (500 g) was successively extracted with petroleum ether and Et_2O. The residue of the petroleum ether extract was chromatographed over an Al_2O_3 column, using as solvent $CHCl_3$-EtOH (9:1). The obtained eluates were fractionated and purified by preparative tlc on Si gel with mobile phase petroleum ether-Et_2O (1:1). Thus compound **1** (20 mg) was obtained. Some fractions of the column were rechromatographed over a Si gel column using $CHCl_3$-Me_2CO (9:1) as eluent to yield compound **3** (5 mg). The residue of the Et_2O extract was chromatographed over a Si gel column. Fractions were eluted with $CHCl_3$/*n*-hexane. This yielded compound **4** (10 mg), compounds **5** and **6** (12 mg) as a mixture of two isomers, and compounds **7** and **8** (15 mg) as a mixture of two isomers.

(5,8,9,10*R*)-LABD-13(*E*)-ENE-8a,15-DIOL [**1**].—Compound **1**: $[\alpha]^{20}_D$ 0.0° ($CHCl_3$, $c=1$); $[\alpha]_{578}$ +0.4° ($CHCl_3$, $c=0.25$); $[\alpha]_{546}$ 0.0° ($CHCl_3$, $c=0.25$); $[\alpha]_{436}$ +0.8° ($CHCl_3$, $c=0.25$); $[\alpha]_{365}$ +2° ($CHCl_3$, $c=0.25$); ir ν max cm^{-1} 3200, 1675, 1270, 1175, 1110, 1075, 1070, 1060, 1000, 980, 965, 930, 890; ^{1}H nmr ($CDCl_3$) δ 5.40 (t, H-14, $J=8$ Hz), 4.15 (d, H-15, $J=8$ Hz), 2.10 (t, H-12, $J=8$ Hz), 1.70 (br s, H-16, $J=1$ Hz), 1.14 (s, H-17), 0.90 (s, H-18), 0.82 (br s, H-19, -20); eims *m/z* (rel. int. %) $[M]^+$ 308 (10), $[M-H_2O]^+$ 290 (45), 275 (70),

245 (100), 205 (10), 192 (65), 177 (97), 123 (28), 109 (35), 81 (78), 69 (100); cidms *m/z* (rel. int. %) $[M+NH_4]^+$ 326 (20), 308 (35), 290 (15), 273 (100), 205 (10), 192 (15), 177 (10), 95 (8), 60 (5). Acetylation of **1** (15 mg) with AC_2O/pyridine (overnight, room temperature) gave the monoacetate compound **2** (12 mg): $[\alpha]^{20}D$ +7 ($CHCl_3$, *c* = 1); ir *ν* max cm^{-1} 3480, 1745; ^1H nmr ($CDCl_3$) δ 5.15 (t, H-14, *J* = 8 Hz), 4.57 (d, H-15, *J* = 8 Hz), 2.10 (t, H-12, *J* = 8 Hz), 2.06 (s, OAc), 1.71 (s, H-16), 1.13 (s, H-17), 0.86 (s, H-18), 0.80 (s, H-19, -20); eims *m/z* (rel. int. %) $[M-HOAc]^+$ 290 (50), 275 (15), 273 (20), 257 (20), 245 (45), 205 (8), 191 (80), 177 (100), 123 (90), 109 (100), 81 (100), 67 (100); cidms *m/z* (rel. int. %) $[M+NH_4]^+$ 368 (10), $[M]^+$ 350 (12), 333 (60), 313 (10), 290 (15), 273 (100), 245 (15), 208 (10), 191 (10), 177 (10), 130 (12), 102 (88), 86 (23), 60 (12).

(5,8,9,10*R*)-LABD-7,13(*E*)-DIEN-15-OL [**3**].—Compound **3**: $[\alpha]^{20}D$ +7° ($CHCl_3$, *c* = 0.1); ir *ν* max cm^{-1} 3350, 1680, 1020; ^1H nmr ($CDCl_3$) δ 5.37 (m, H-14, -7), 4.15 (d, H-15, *J* = 8 Hz), 1.67 (s, H-16, -17), 0.85 (br s, H-18), 0.74 (s, H-19, -20); eims *m/z* (rel. int. %) $[M]^+$ 290 (5), 275 (8), 204 (70), 189 (20), 161 (25), 135 (21), 109 (35), 81 (100), 69 (99); cidms *m/z* (rel. int. %) $[M+NH_4]^+$ 308 (8), 272 (40), 204 (15), 191 (13), 163 (10), 135 (12), 109 (10), 81 (10), 60 (10).

(5,8,9,10*R*)-LABD-7,13(*E*)-DIEN-15-YL ACETATE [**4**].—Compound **4**: $[\alpha]^{20}D$ +6° ($CHCl_3$, *c* = 0.1); ir *ν* max cm^{-1} 2700, 2640, 1600, 1500, 1350, 1270, 1025, 890, 790; ^1H nmr ($CDCl_3$) δ 5.31 (m, H-14, -7), 4.57 (d, H-15, *J* = 8 Hz), 2.06 (s, OAc), 1.71 (s, H-17), 1.69 (s, H-16), 0.86 (s, H-18), 0.77 (s, H-19, -20); eims *m/z* (rel. int. %) $[M]^+$ 332 (5), 317 (10), 272 (5), 257 (10), 204 (100), 189 (23), 161 (24), 133 (55), 123 (23), 109 (100), 81 (90), 69 (70); cidms *m/z* (rel. int. %) $[M+NH_4]^+$ 350 (70), 290 (30), 273 (100), 257 (10), 204 (80), 191 (18), 163 (25), 133 (15), 109 (27), 81 (25), 78 (55), 60 (40).

8,13-EPOXYLABD-14-ENE [**5**].—Compound **5**: ir *ν* max cm^{-1} 3049, 1818, 1629, 1400, 1073, 1121, 987, 923; ^1H-nmr ($CDCl_3$) δ 5.85 (dd, *J* = 17, 10 Hz), 5.13 (dd, *J* = 17, 1 Hz), 4.94 (dd, *J* = 10, 1 Hz), 1.28 (s, H-16), 1.26 (s, H-17), 0.86 (s, H-20), 0.81 (s, H-18, -19); eims *m/z* (rel. int. %) $[M]^+$ 290 (3), 275 (85), 257 (80), 245 (18), 205 (10), 192 (48), 177 (45), 137 (70), 123 (60), 95 (70), 81 (77); cidms *m/z* (rel. int. %) $[M+NH_4]^+$ 308 (100), 290 (90), 273 (100), 257 (100), 192 (95), 177 (45), 137 (45), 123 (30).

13-*epi*-8,13-EPOXYLABD-14-ENE [**6**].—Compound **6**: ^1H nmr ($CDCl_3$) δ 5.98 (dd, *J* = 17, 10 Hz), 4.95 (dd, *J* = 17, 1 Hz), 4.93 (dd, *J* = 10, 1 Hz), 1.23 (s, H-16), 1.12 (s, H-17), 0.86 (s, H-20), 0.78, 0.73 (s, H-18, -19).

LABD-14-ENE-8,13-DIOL [**7**].—Compound **7**: ir *ν* max cm^{-1} 3400, 2940, 1465, 1395, 900; ^1H nmr ($CDCl_3$) δ 5.84 (dd, *J* = 17, 10 Hz), 5.20 (dd, *J* = 17, 1 Hz), 5.01 (dd, *J* = 10, 1 Hz), 1.28 (s, H-16), 1.17 (s, H-17), 0.88 (s, H-20), 0.78 (s, H-18, -19); eims *m/z* (rel. int. %) $[M]^+$ 308 (3), 290 (8), 257 (7), 191 (15), 177 (30), 123 (25), 95 (55); cidms *m/z* (rel. int. %) $[M+NH_4]^+$ 326 (20), 308 (23), 291 (10), 273 (100).

13-*epi*-SCLAREOL [**8**].—Compound **8**: ^1H nmr ($CDCl_3$) δ 5.92 (dd, *J* = 17, 10 Hz), 5.21 (dd, *J* = 17, 1 Hz), 5.05 (dd, *J* = 10, 1 Hz), 1.26 (s, H-16), 1.15 (s, H-17), 0.88 (s, H-20), 0.78 (s, H-18, -19).

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