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LABDANE DITERPENES FROM *CALCEOLARIA DENSIFOLIA*<sup>1</sup>

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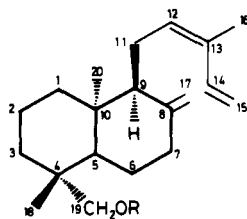
ABSTRACT.—The aerial parts of *Calceolaria densifolia* afforded two new labdane-type diterpenes, *ent*-9-*epi*-labda-8(17), (12Z), 14-trien-19-ol [**1**] and a bisditerpene **2** derived from the esterification of malonic acid by two units of the above diterpene. The structures of the new compounds have been established by spectroscopic means.

We have been studying the diterpenes present in the *Calceolaria* genus (1,2). We now report the isolation and structure elucidation of two new labdatriene diterpenes from *Calceolaria densifolia* Phil. (Scrophulariaceae), a species that grows on the hills of northern Chile (3). This is the first report of labdane-type diterpenes isolated from *Calceolaria* species.

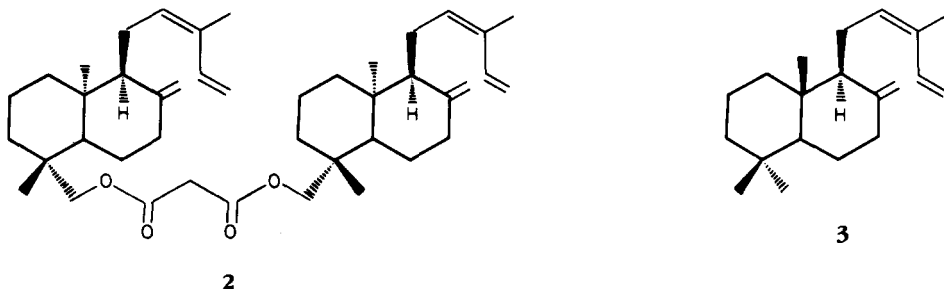
## RESULTS AND DISCUSSION

The petroleum ether extract of the aerial parts of *C. densifolia*, after successive cc purifications, afforded the compounds **1** and **2**.

Compound **1** was purified and characterized after acetylation as **1** acetate, C<sub>22</sub>H<sub>34</sub>O<sub>2</sub> ([M]<sup>+</sup> at *m/z* 330). It was an oil whose ir spectrum showed absorption bands characteristic of acetate and double bonds. Its <sup>1</sup>H- and <sup>13</sup>C-nmr spectra displayed characteristic signals of a diterpene having a labdatriene skeleton (4–7).



**1** R = H  
**1** acetate R = Ac



<sup>1</sup>Part 9 in the series "Diterpenoids from *Calceolaria* Species." For Part 8 see Chamy *et al.* (2).

TABLE 1.  $^{13}\text{C}$ -nmr Spectral Data of Compounds 1 acetate, 2, and 3 (in  $\text{CDCl}_3$  with TMS as internal standard).

Carbon	DEPT	Compound		
		1 acetate	2	3
C-1	$\text{CH}_2$	36.50	36.47 <sup>a</sup>	39.3
C-2	$\text{CH}_2$	18.65	18.64	19.5
C-3	$\text{CH}_2$	36.50	36.42 <sup>a</sup>	42.2
C-4	C	36.76 <sup>a</sup>	36.93 <sup>b</sup>	33.5
C-5	CH	46.63	46.68	55.5
C-6	$\text{CH}_2$	24.53	24.56	24.3
C-7	$\text{CH}_2$	31.71	31.71	38.2
C-8	C	147.96	147.89	148.2
C-9	CH	58.22	58.24	57.4
C-10	C	37.82 <sup>a</sup>	37.85 <sup>b</sup>	39.6
C-11	$\text{CH}_2$	23.41	23.42	22.1
C-12	CH	133.84	133.87	133.9
C-13	C	131.64	131.93	131.4
C-14	CH	130.35	130.35	131.6
C-15	$\text{CH}_2$	113.16	113.23	113.0
C-16	Me	19.70	19.78	19.7
C-17	$\text{CH}_2$	109.96	110.08	107.7
C-18	Me	27.39	27.33	33.6
C-19	$\text{CH}_2$	66.88	68.09	21.8
C-20	Me	23.05	23.05	14.5
OAc	C	171.22	—	—
OAc	Me	20.93	—	—
$\text{COCH}_2\text{CO}$	C	—	166.67	—
$\text{COCH}_2\text{CO}$	C	—	166.67	—
$\text{COCH}_2\text{CO}$	$\text{CH}_2$	—	41.70	—

<sup>a,b</sup>These values may be interchanged within the same column.

The  $^1\text{H}$ -nmr spectrum showed signals of two quaternary methyls at  $\delta$  0.90 and 0.96. An AB system was present corresponding to an acetoxymethylene group. From the chemical shifts of  $-\text{CH}_2\text{OAc}$  ( $^1\text{H}$  nmr, two doublets at  $\delta$  3.85 and 4.23;  $^{13}\text{C}$  nmr, 66.88), a C-19 axial orientation was deduced for this grouping (8,9).

The  $^{13}\text{C}$ -nmr spectrum showed signals at  $\delta$  147.96 and 109.96 attributed, respectively, to C-8 and C-17 of the exocyclic methylene group; these were consistent with  $^1\text{H}$ -nmr absorptions at  $\delta$  4.48 (1H, d, H-17) and 4.65 (1H, d, H-17').

The  $^1\text{H}$ -nmr spectrum also exhibited signals at  $\delta$  5.25 (1H, t, H-12), 6.72 (1H, dd, H-14), 5.15 (1H, dd, H-15 trans), 5.05 (1H, dd, H-15 cis), and 1.75 (3H, br s, H-16), showing the presence of a Z-type conjugated diene system (4,5,10). The  $^{13}\text{C}$ -nmr signals at  $\delta$  133.84, 131.64, 130.35, and 113.16, which correspond to C-12, C-13, C-14, and C-15, respectively, were in agreement with the proposed structure for the side chain (6,7). Table 1 shows a comparison of  $^{13}\text{C}$ -nmr data with those of a known related labdane 3 (6).

The chemical shifts assigned to C-1 ( $\delta$  36.5) and to C-5 ( $\delta$  46.63) occur upfield; on the other hand, the signal attributed to C-20 ( $\delta$  23.05) appeared downfield compared with the values observed in similar compounds (6). These effects, which are also observed in other diterpenoids obtained from the *Calceolaria* genus (11–13), are explained by the interaction imposed by a different configuration of C-11 (14) observed in the 9-epimeric diterpenoids, which cause a shielding effect on C-1 and C-5 and a deshielding effect on C-20. This can be clearly demonstrated with molecular models. Therefore, the

spectral data of compound **1** acetate correspond to *ent*-9-*epi*-labda-8(17),(12Z),14-trien-19-yl acetate, and thereby the natural substance **1** is *ent*-9-*epi*-labda-8(17),(12Z),14-trien-19-ol.

The structure of compound **2** was deduced by comparing its spectral data with those of the compound **1** acetate. In fact, the  $^1\text{H}$ -nmr spectrum of **2** was very similar to that of **1** acetate and exhibited characteristics of the same 8(17),(12Z),14-labdatriene nucleus but with a malonate unit ( $\delta$  3.38, 2H, s) instead of an acetoxy group at C-19. However, integration due to the terpene moiety was twice that of the malonate residue, which suggested that two diterpene units must be linked by malonic acid. The  $^{13}\text{C}$ -nmr spectrum exhibited 22 signals that agreed well with those of **1** acetate. Therefore, on the basis of these data, **2** is shown to be bis-[*ent*-9-*epi*-labda-8(17),(12Z),14-trien-19-yl] malonate.

Assignments of structures and configurations for both compounds were made by comparison with spectral data of similar diterpenes (4–7) and also from biogenetic considerations (11–13).

This is the first report of the presence of labdane metabolites in the genus *Calceolaria*. These 9-epimeric diterpenes correspond to a modification of the normal diterpenoid cyclization of geranylgeranyl-pyrophosphate, which occurs in other members of the genus. The formation of these compounds might involve a chair-boat cyclization to form a bicyclic intermediate possessing an H-9 $\alpha$  and a 10 $\alpha$ -Me group (11). The presence of 9-epimeric diterpenoids and malonyl esters in species of *Calceolaria* may be of chemosystematic value.

## EXPERIMENTAL

**GENERAL EXPERIMENTAL PROCEDURES.**— $^1\text{H}$ -nmr spectra were recorded at 250 MHz in  $\text{CDCl}_3$  with TMS as internal standard; assignments of  $^{13}\text{C}$  nmr chemical shifts were made with the aid of DEPT. Mass spectra were obtained by direct inlet, 70 eV.  $\text{CHCl}_3$  solutions were used for ir, and MeOH solutions for uv.

**PLANT MATERIAL.**—*C. densifolia* was collected in Parque Nacional Fray Jorge, IV Región, Chile, in September 1987. A voucher specimen (# 0138) is deposited at Universidad Técnica Federico Santa María.

**EXTRACTION AND ISOLATION.**—The aerial parts of *C. densifolia* (1.5 kg) were extracted at room temperature with petroleum ether for 72 h, affording 33 g of a syrup. This crude material was chromatographed on a Si gel column and eluted with petroleum ether/EtOAc mixtures of increasing polarity. Fractions of 125 ml were taken and combined based upon tlc and  $^1\text{H}$ -nmr (60 MHz) monitoring. The intermediate fractions 13–16 provided a mixture that contained compound **2**. Fractions 26–28 gave a mixture containing compound **1**. This mixture was treated with  $\text{Ac}_2\text{O}$ /pyridine for 3 h at room temperature; the acetylated material was subjected to Si gel cc using petroleum ether-EtOAc (40:1), and fractions of 25 ml were taken. Fractions 14–17 were repurified by Si gel cc impregnated with  $\text{AgNO}_3$  (10%), using petroleum ether-EtOAc (8:1), affording **1** acetate (210 mg).

The mixture containing **2** was subjected to Si gel using petroleum ether-EtOAc (50:1), and fractions of 25 ml were taken. Fractions 20–30, which contained **2**, were repurified by Si gel cc impregnated with  $\text{AgNO}_3$  (10%), using petroleum ether-EtOAc (4:1), affording **2** (60 mg).

*ent*-9-*epi*-Labda-8(17),(12Z),14-trien-19-yl acetate [**1** acetate].—Viscous yellow oil:  $[\alpha]_D^{25} + 23.8^\circ$  ( $\text{CHCl}_3$ ,  $c = 1.85$ ); uv  $\lambda$  max nm (log  $\epsilon$ ) 227 (4.03); ir  $\nu$  max ( $\text{CHCl}_3$ )  $\text{cm}^{-1}$  3080, 3020, 2980–2950, 1735, 1650, 1470, 1450, 1400, 1360, 1250, 1210, 1030, 990, 900;  $^1\text{H}$  nmr (250 MHz)  $\delta$  6.72 (1H, dd,  $J = 17.5, 11.0$  Hz, H-14), 5.25 (1H, t,  $J = 7.0$  Hz, H-12), 5.15 (1H, dd,  $J = 17.5, 1.0$  Hz, H-15 trans), 5.05 (1H, dd,  $J = 11.0, 1.0$  Hz, H-15 cis), 4.65 (1H, d,  $J = 2.0$  Hz, H-17), 4.48 (1H, d,  $J = 2.0$  Hz, H-17'), 4.23 (1H, d,  $J = 12.5$  Hz, H-19), 3.85 (1H, d,  $J = 12.5$  Hz, H-19'), 2.00 (3H, s, OAc), 1.75 (3H, br s, H-16), 0.96, 0.90 (3H, each, s, H-18, H-20);  $^{13}\text{C}$  nmr see Table 1; ms  $m/z$  (rel. int.)  $[\text{C}_{22}\text{H}_{34}\text{O}_2]^+$  330 (8),  $[\text{M} - \text{Me}]^+$  315 (3),  $[\text{M} - \text{HOAc}]^+$  270 (7),  $[\text{M} - \text{CH}_2\text{OAc}]^+$  257 (98),  $[\text{M} - \text{HOAc} - \text{C}_6\text{H}_9]^+$  189 (56),  $[\text{C}_6\text{H}_9]^+$  81 (100).

Bis-[*ent*-9-*epi*-labda-8(17),(12Z),14-trien-19-yl] malonate [**2**].—Viscous yellow oil:  $[\alpha]_D^{25} + 68.3^\circ$  ( $\text{CHCl}_3$ ,  $c = 1.20$ ); uv  $\lambda$  max nm (log  $\epsilon$ ) 226 (4.01); ir  $\nu$  max ( $\text{CHCl}_3$ )  $\text{cm}^{-1}$  3080, 3020, 2980–2850, 1730, 1650, 1470, 1450, 1380, 1160, 1010, 900;  $^1\text{H}$  nmr (250 MHz)  $\delta$  6.72 (2H, dd,  $J = 17.5, 11.0$

Hz, H-14 and H-14a), 5.25 (2H, t,  $J = 7.0$  Hz, H-12 and H-12a), 5.15 (2H, dd,  $J = 17.5, 1.0$  Hz, H-15 trans and H-15a trans), 5.05 (2H, dd,  $J = 11.0, 1.0$  Hz, H-15 cis and H-15a cis), 4.66 (2H, d,  $J = 2.0$  Hz, H-17 and H-17a), 4.50 (2H, d,  $J = 2.0$  Hz, H-17' and H-17'a), 4.32 (2H, d,  $J = 12.5$  Hz, H-19 and H-19a), 3.89 (2H, d,  $J = 12.5$  Hz, H-19' and H-19'a), 3.38 (2H, s, malonyl), 1.75 (6H, br s, H-16 and H-16a), 0.98, 0.93 (6H each, s, H-18, H-18a, H-20, and H-20a);  $^{13}\text{C}$  nmr see Table 1; ms  $m/z$  (rel. int.)  $[\text{M}-\text{C}_6\text{H}_9]^+$  563 (2),  $[\text{M}-\text{C}_{20}\text{H}_{32}\text{O}]^+$  356 (2),  $[\text{C}_{20}\text{H}_{32}\text{O}]^+$  288 (3),  $[\text{M}-\text{C}_{23}\text{H}_{34}\text{O}_4]^+$  270 (5),  $[\text{C}_{19}\text{H}_{29}]^+$  257 (14), 189 (13), 175 (20), 160 (28), 145 (100), 135 (26), 119 (32), 109 (42), 95 (48),  $[\text{C}_6\text{H}_9]^+$  81 (57), 79 (35), 67 (32).

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