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DEHYDROABIETANE DITERPENOIDS FROM *CALCEOLARIA ASCENDENS*

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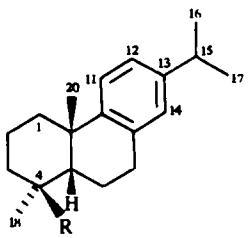
Abstract—Two new dehydroabietane diterpenes, dehydroabietinol and 19-malonyloxy-dehydroabietinol, and the known compound 4-epi-dehydroabietic acid were isolated from the aerial parts of *Calceolaria ascendens*. The structures of the new compounds were elucidated by spectroscopic methods.

INTRODUCTION

The genus *Calceolaria* is represented in Chile by *ca* 50 species [1]. Some of these plants, commonly known as 'topa-topa' or 'zapatito', are used in folk medicine as a stomach tonic, as antibacterial agents and as sweeteners [1, 2]. Very little is known about the secondary metabolites of these species [3, 4]. This paper deals with the isolation and structure elucidation of two new dehydroabietane derivatives, dehydroabietinol (**1**) and 19-malonyloxy-dehydroabietinol (**3**), present in *Calceolaria ascendens*, a medium size herb that grows in the coastal hills of central Chile.

RESULTS AND DISCUSSION

The petrol extract of the aerial parts of *C. ascendens* was subjected to column chromatography on silica gel, using increasing proportions of ethyl acetate in petrol, to afford dehydroabietinol (**1**), 4-epi-dehydroabietic acid (**2**) [5] and 19-malonyloxy-dehydroabietinol (**3**). The first diterpene (**1**) had a molecular formula $C_{20}H_{30}O$ ($[M]^+$ at *m/z* 286) and its IR spectrum showed hydroxyl and aromatic group absorptions. In the ^1H NMR spectrum signals at δ7.08 (*d*, J = 8.3 Hz), 6.93 (*dd*, J = 8.3, 2.0 Hz) and 6.80 (*d*, J = 2.0 Hz) accounted for the presence of a 1,2,4-trisubstituted benzene nucleus, while a one-proton quartet (J = 6.7 Hz) centred at δ 2.73 and a two-methyl doublet (J = 6.7 Hz) at δ1.15 established the presence of an isopropyl group linked to the aromatic ring. Characteristic adsorptions due to a primary hydroxy group [δ 3.78 (*d*, J = 11.2 Hz) and 3.47 (*d*, J = 11.2 Hz)], tentatively assigned to C-19, and two three-proton singlets at δ1.10 and 0.98, suggested that compound **1** contained a dehydroabietane skeleton. The assignments of the ^{13}C NMR spectral signals of **1** (Table 1), made on the basis of the observed multiplicities (SFORD) and by comparison with reported ^{13}C NMR spectral data of similar compounds [6], confirmed all the above results, including the β-axial dis-



	R
1	CH_2OH
1a	CH_2OAc
2	COOH
2a	COOMe
3	CH_2OCOCH_2COOH
3a	CH_2OCOCH_2COOMe

Table 1. ^{13}C NMR spectral data of compounds **1**, **3a** and **2a**

C	1	3a*	2a†
1	39.0	38.0	39.6
2	19.0	18.9	20.1
3	35.3	35.9	37.9
4	38.7	37.2	44.1
5	51.4	51.3	53.1
6	19.3	19.2	21.2
7	31.0	30.9	32.2
8	134.6	134.4	135.1
9	147.2	146.8	145.7
10	37.5	37.4	38.3
11	124.4	124.4	125.6
12	123.9	124.0	124.1
13	145.6	145.7	145.6
14	126.8	126.8	126.9
15	33.4	33.4	33.5
16	24.0	23.9	24.1
17	24.0	23.9	24.1
18	26.8	27.1	28.7
19	65.3	68.2	178.0
20	25.7	25.6	23.1

*Malonate carbons at 166.3 (*s*) 41.5 (*t*) and 166.6 ppm (*s*). Methyl ester carbon at 52.3 ppm (*q*).

†Methyl ester carbon at 51.3 ppm (*q*).

position of the CH_2OH at C-4. Therefore, placing the hydroxyl group at C-19, **1** is shown to be dehydroabietinol. This is the first report of the natural occurrence of **1**, which had been previously produced as a synthetic product [5]. As confirmation of the proposed structure, acetylation of **1** afforded **1a**, whose spectral and physical data were in full agreement with those of dehydroabietinol acetate, isolated from *Pinus silvestris* [7].

Compound **2** was purified as its methyl ester derivative, **2a**. The spectral properties of **2a**, $C_{21}H_{30}O_2$ ($[M]^+$ at *m/z* 314), showed it to be methyl 4-epi-dehydroabietate. The presence of 4-epi-dehydroabietic acid (**2**) free acid was suggested in *Callistris columellaris*, since its methyl ester was found in a fraction which had been treated with diazomethane [5]. Further confirmation of the identity of **2a** was obtained by comparison of its ^1H and ^{13}C NMR (Table 1) with that of **1** and other related diterpenoids [6].

Compound **3**, also purified as its methyl ester, **3a** ($C_{24}H_{34}O_4$, $[M]^+$ at *m/z* 386), showed saturated ester and aromatic groups in the IR. Comparison of the ^1H NMR spectrum of **3a** with that of **1** showed only minor differences for the skeletal proton signals. In particular, the H-19 and H-19′ signals were shifted downfield from δ3.78 and 3.47 to 4.37 and 4.00 and differences between the ^1H NMR spectra indicated that the new compound, **3a**, must be an acyl derivative of **1**. In agreement with this treatment of **3a** with lithium aluminium hydride afforded a product identical in all respects to dehydroabietinol (**1**). The nature of the ester side chain of **3a** was evident from the chemical and spectroscopic data. Since **3** gave a methyl ester (**3a**) after treatment with diazomethane, the new carbomethoxyl group must be part of the acyl moiety, thus indicating the dicarboxylic nature of this residue. In addition, a two-proton singlet centred at δ3.35, in the ^1H NMR spectrum of **3a**, suggested that the ester side chain of **3a** was a malonate unit [8–10]. The ^{13}C NMR spectral data of **3a** (Table 1) were in agreement with previous assignments. In particular, the esterification at position 19 was confirmed by the deshielding α-effect on C-19 ($+2.9$ ppm) and the shielding β-effect on C-4 (-1.5 ppm) when compared with the corresponding signals of **1**, whereas the signals of the methyl ester side chain were assigned as follows: δ166.3, C-1′; 41.5, C-2′; 166.6, C-3′; and 52.3 ppm, OMe. The other carbon resonances remained almost unchanged when compared to those of **1**, leading to the assignments to **3** and **3a** of the structures 19-malonyloxy-dehydroabietinol and its methyl derivative, respectively.

To the best of our knowledge, only three diterpenes esterified by malonic acid have been found in nature prior to the present work. They are 3β,19-dimalonyloxy-isopimara-9(11),15-diene from *Newcastlia viscida* [8], bacchalineolmalonate from *Baccharis tricuneata* var. *lineata* [9], and barticulidiolmalonate from *B. articulata* [10].

EXPERIMENTAL

Mps: uncorr; ^1H NMR: 60 and 400 MHz in $CDCl_3$ with TMS; ^{13}C NMR: 22.15 MHz, $CDCl_3$ with TMS. Assignments of ^{13}C NMR chemical shifts were made with the aid of SFORD. IR: film on NaCl or KBr pellets; MS: direct inlet, 70 eV.

Calceolaria ascendens Lind. collected in Cuesta Zapata, V Región, Chile, in November 1985, was identified at the Universidad Federico Santa María, where a voucher specimen is deposited.

The aerial parts of *C. ascendens* (2 kg) were extracted at room temp. with petrol for 24 hr, affording 30 g of a syrup. This crude material (10 g) was chromatographed on a silica gel column (400 g) and eluted with mixtures of petrol and EtOAc of increasing polarity. Fractions of 100 ml were taken and combined to give in order of elution: dehydroabietinol (**1**, 900 mg), a mixture containing 4-epi-dehydroabietic acid (**2**) and a mixture containing 19-malonyloxy-dehydroabietinol (**3**). Both mixtures, named fractions B and C, were treated separately with ethereal C_2H_2N.

Fraction B (200 mg) was rechromatographed on a silica gel column (10 g) and eluted with petrol–EtOAc (30:1) yielding pure **2a** (92 mg); whereas fraction C (155 mg) was rechromatographed on a silica gel column (10 g) and eluted with petrol–EtOAc (20:1) yielding pure **3a** (35 mg).

Dehydroabietinol (**1**). Viscous colourless oil, $[\alpha]_D^{25}$ +43.4° (*c* 2.0, $CHCl_3$). IR ν_{max}^{film} cm^{-1}: 3320, 2980–2860, 1600, 1490, 1460, 1370, 1030, 970, 890, 825; ^1H NMR (400 MHz): δ7.08 (1H, *d*, *J* = 8.3 Hz, H-11), 6.93 (1H, *dd*, *J* = 8.3, 2.0 Hz, H-12), 6.80 (1H, *d*, *J* = 2.0 Hz, H-14), 3.78 (1H, *d*, *J* = 11.2 Hz, H-19), 3.47 (1H, *d*, *J* = 11.2 Hz, H-19′), 2.82 (1H, *ddd*, *J* = 16.2, 7.0, 2.0 Hz, H-7β), 2.78 (1H, *m*, H-7α), 2.73 (1H, *q*, *J* = 6.7 Hz, H-15), 1.44 (1H, *dd*, *J* = 12.5, 2.0 Hz, H-5), 1.15 (6H, *d*, *J* = 6.7 Hz, H-16 and H-17), 1.10 (3H, *s*, H-18), 0.98 (3H, *s*, H-20); ^{13}C NMR: see Table 1; MS *m/z* (rel. int.): 286 [$C_{20}H_{30}O$, M]$^+$ (68), 271 [M − Me]$^+$ (74), 253 [271 − H_2O]$^+$ (70), 211 (55), 173 (59), 159 (77), 131 (46), 117 (60), 86 (92), 84 (100), 81 (69), 69 (59), 51 (67), 49 (86), 43 (58), 41 (64).

Dehydroabietinol acetate (**1a**). **1** (100 mg) was treated with Ac_2O (2.0 ml) and C_5H_5N (0.5 ml) at room temp. for 6 hr. After addition of EtOH, the mixture was evapd to dryness and yielded pure **1a**. Mp 60–61°, $[\alpha]_D^{25}$ +54.3° (*c* 1.1, $CHCl_3$) (ref. [6] mp 61°, $[\alpha]_D$ +62.2, $CHCl_3$). IR ν_{max}^{film} cm^{-1}: 2980–2840, 1740, 1610, 1495, 1455, 1390, 1370, 1240, 1035, 990, 825; ^1H NMR (60 MHz): δ7.07 (1H, *d*, *J* = 8.0 Hz, H-11), 6.95 (1H, *dd*, *J* = 8.0, 2.0 Hz, H-12), 6.85 (1H, *br s* H-14), 4.37 (1H, *d*, *J* = 11.0 Hz, H-19), 3.98 (1H, *d*, *J* = 11.0 Hz, H-19′), 2.80 (3H, *m*, H-7β, H-7α and H-15), 2.05 (3H, *s*, $COCH_3$), 1.18 (6H, *d*, *J* = 7.0 Hz, H-16 and H-17), 1.15 (3H, *s*, H-18), 1.00 (3H, *s*, H-20); MS *m/z* (rel. int.) 328 [$C_{22}H_{32}O_2$,M]$^+$ (54), 313 [M − Me]$^+$ (57), 271 [M − Me − CH_2=C=O]$^+$ (62), 255 (67), 254 (72), 253 (80), 225 (55), 211 (94), 185 (65), 183 (82), 171 (62), 159 (91), 155 (74), 129 (77), 95 (62), 83 (64), 81 (57), 55 (60), 43 (100), 41 (73).

Methyl 4-epi-dehydroabietate (**2a**). Mp 78.5–79.0°, $[\alpha]_D^{25}$ +128.3° (*c* 1.0, $CHCl_3$) (ref. [5] mp 79.9, $[\alpha]_D$ +137°, EtOH). IR ν_{max}^{KBr} cm^{-1}: 2980–2850, 1720, 1490, 1770, 1380, 1240, 1260, 1035, 890, 840; ^1H NMR (400 MHz): δ7.10 (1H, *d* 8.2 Hz, H-11) 6.92 (1H, *dd*, *J* = 8.2, 2.0 Hz, H-12), 6.81 (1H, *d*, *J* = 2.0 Hz, H-14), 3.58 (3H, *s*, –COOMe), 2.82 (1H, *ddd*, *J* = 16.3, 7.1, 2.0 Hz, H-7β), 2.79 (1H, *m*, H-7α), 2.75 (1H, *q*, *J* = 6.8 Hz, H-15), 1.48 (1H, *dd*, *J* = 12.5, 2.0 Hz, H-5), 1.21 (3H, *s*, H-18), 1.17 (6H, *d*, *J* = 6.8 Hz, H-16 and H-17), 0.97 (3H, *s*, H-20); ^{13}C NMR: see Table 1; MS *m/z* (rel. int.): 314 [$C_{21}H_{30}O_2$, M]$^+$ (65), 299 [M − Me]$^+$ (88), 298 (100), 266 (64), 254 (42), 239 (96), 238 (68), 196 (61), 159 (77), 140 (69), 129 (70), 43 (74), 41 (53).

Methyl 19-malonyloxy-dehydroabietinol (**3a**). Colourless oil. $[\alpha]_D^{25}$ +32.5° (*c* 0.6, $CHCl_3$). IR ν_{max}^{film} cm^{-1}: 2980–2850, 1740, 1610, 1495, 1430, 1330, 1270, 1150, 1020, 820; ^1H NMR (400 MHz): δ7.10 (1H, *d*, *J* = 8.3, 2.0 Hz, H-11), 6.92 (1H, *dd*, *J* = 8.3, 2.0 Hz, H-12), 6.81 (1H, *d*, *J* = 2.0 Hz, H-14), 4.37 (1H, *d*, *J* = 11.2 Hz, H-19), 4.00 (1H, *d*, *J* = 11.2 Hz, H-19′), 3.69 (3H, *s*, –COOMe), 3.35 (2H, *s*, $OCOCH_2COO$), 2.85 (1H, *ddd*, *J* = 16.2, 7.0, 2.0 Hz, H-7β), 2.80 (1H, *m*, H-7α), 2.74 (1H, *q*, *J* = 6.8 Hz, H-15), 1.45 (1H, *dd*, *J* = 12.5, 2.0 Hz, H-5), 1.16 (6H, *d*, *J* = 6.8 Hz, H-16 and H-17), 1.14 (3H, *s*, H-18), 0.98 (3H, *s*, H-20); ^{13}C NMR: see Table 1; MS *m/z* (rel. int.): 386 [$C_{24}H_{34}O_4$,M]$^+$, (31), 371 [M − 15]$^+$ (40), 255 [M − $C_5H_7O_4$]$^+$ (23), 254 [M − $C_5H_8O_4$]$^+$

(100), 211 $[254-C_3H_7]^+$ (36), 185 (18), 159 (18), 101 $[C_4H_5O_3]^+$ (8), 83 (12).

Reduction of methyl 19-malonyloxy-dehydroabietinol. **3a** (20 mg) was treated with $LiAlH_4$ in dry Et_2O. After usual work-up, dehydroxyabietinol (**1**) was obtained. The spectral and physical properties (TLC, IR, ^1H NMR and MS) of this compound were in full agreement with those of the natural product **1**.

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