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DITERPENOIDS FROM *CALCEOLARIA DENTATA**

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IN HONOUR OF PROFESSOR ANTONIO G. GONZALEZ

Key Word Index—*Calceolaria dentata*; Scrophulariaceae; diterpenes.

Abstract—Five diterpenoids were isolated from the aerial parts of *Calceolaria dentata*. Their structures were elucidated by spectroscopic methods. Three of them are esterified with isovaleric acid.

INTRODUCTION

The large genus *Calceolaria* has been the subject of several chemical studies [1–3]. In the course of our current research on the chemistry of this genus, we have now investigated the aerial parts *Calceolaria dentata* and shown them to contain the new thyrsiflorane derivatives **1–3**, the new stemarane derivatives **4** and **5**, and the known 17-hydroxy-9-epi-ent-isopimaradiene [4].

RESULTS AND DISCUSSION

Compound **1** was purified and characterized as its methyl ester derivative **1a** ($C_{26}H_{40}O_6$, $[M]^+$ at m/z 448). The ^{1}H NMR spectrum of **1a** showed the presence of four quaternary methyl groups (δ0.81, 0.85, 0.93 and 1.02), a singlet at δ3.38 corresponding to the methylene group of a malonate moiety, a singlet at δ3.76 (3H, *s*, OMe) and a singlet at δ2.03 corresponding to an acetoxyl group. Based on the molecular formula and the ^{1}H NMR and ^{13}C NMR data, it was concluded that **1a** was a tetracyclic diterpene of the thyrsiflorane-type with a malonate and an acetyl group.

Treatment of **1a** with $LiAlH_4$ in dry diethyl ether gave **3b**, whose spectral and physical data were in full accord with those of thyrsiflorin C [5], while treatment of **1a** with K_2CO_3–methanol gave methyl thyrsiflorin B (**1b**). Therefore, **1a** was shown to be methyl thyrsiflorin B acetate [5].

The spectral data for **2**, together with the molecular formula $C_{27}H_{44}O_4$ (M^+ at m/z 432), suggested that **2** could be a diterpenoid of the same type as **1**. Comparison of the ^{1}H NMR spectrum of **2** with that of **1a** showed minor differences in the skeletal proton signals. In particular, the malonyl proton singlet was replaced by a doublet at δ0.95 corresponding to two methyl groups and a doublet corresponding to a methylene of an acyl moiety. The ester substituent was assigned to an isovaleryl group based on the typical ^{1}H NMR spectrum (see Experimental), together with the loss of 57 (C_4H_9) and 102 ($C_5H_{10}O_2$) amu in the mass spectra. The ^{13}C NMR spectrum confirmed the presence of this residue (Table 1).

Compound **2** on treatment with K_2CO_3–methanol gave **2a**, whose spectral data were very similar to those of **1b**, except that the signals due to the malonate moiety were replaced by those of an isovaleroyl group. Furthermore, treatment of **2** with $LiAlH_4$ in dry ether gave **3b**. Therefore, **2** was shown to be 13-isovaleroyl-7-acetoxy-thyrsiflorane.

Compound **3** was purified and characterized as its methyl ester derivative **3a** ($C_{29}H_{46}O_6$, $[M]^+$ at m/z 490). Its ^{1}H NMR and ^{13}C NMR spectra were very similar to those of **2** for the skeletal protons, but the acetoxyl ester signals were replaced by those of a malonate ester group. Placement of the malonate group at C-7 was established by means of the ^{13}C NMR data. Therefore, compound **3** was shown to be 13-isovaleryl-7-malonyloxy-thyrsiflorane.

The methyl ester **4a** of the new diterpenoid **4** was assigned the molecular formula $C_{24}H_{36}O_4$ ($[M]^+$ at m/z 388). It showed IR absorptions for an ester carbonyl at 1710 and 1730 cm^{-1} and an exomethylene group at 1630 and 880 cm^{-1}. Its ^{1}H NMR spectrum showed a singlet at δ3.38 characteristic of a malonate group attached to a carbocyclic secondary carbon at δ4.8 (1H, *dt*, J = 5.7, 10.8 Hz, H-7). The ^{13}C NMR and DEPT spectra showed the presence of three methyl, nine methylene, four methine and four quaternary groups (Table 1) besides the signals of the malonate moeity. From these data, we deduced that **4** was a tetracyclic diterpene with a stemodane or stemarane skeleton, with a malonate group at C-7. However, the presence of three quaternary methyl groups and the malonate attached to a secondary carbon and the exocyclic methylene are only compatible with the stemarane skeleton. This assumption was also supported by the ^{13}C NMR data [6–8].

*Part 17 in the series "Diterpenoids from *Calceolaria* Species. For part 16 see ref. [1].

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Table 1. ^{13}C NMR spectra data for compounds **1a**, **2**, **2a**, **3a**, **3b**, **4a**, **4b**, **5** and **5a**

C	1a	2	2a	3a	3b	4a	4b	5	5a
1	32.3	32.4	32.4	32.4	32.5	32.1	32.3	47.1	51.5
2	18.6	18.8	18.7	18.8	18.7	18.7	18.7	69.7	65.6
3	41.9	42.0	42.0	42.1	42.0	42.0	42.0	38.4	42.4
4	33.2	33.3	33.1	33.3	33.1	33.2	33.1	34.7	34.9
5	45.9	46.0	46.2	46.1	46.2	36.4	46.6	48.2	48.2
6	27.8	28.1	30.9	28.0	30.7	27.9	30.9	21.7	21.8
7	77.7	76.0	73.7	77.0	73.7	78.5	74.3	39.9	39.9
8	41.7	41.9	45.1	41.8	45.2	43.8	47.1	39.4	39.3
9	53.3	53.4	53.2	53.5	53.1	53.2	53.0	52.6	52.6
10	38.4	38.5	38.6	38.9	38.5	38.6	38.8	40.6	40.8
11	44.5	44.7	44.6	44.7	44.7	39.7	39.7	37.0	37.1
12	43.0	46.2	43.1	43.2	44.2	44.1	44.3	44.4	44.5
13	77.7	77.8	77.9	77.8	76.2	152.3	152.9	153.4	153.6
14	30.4	32.2	32.0	30.6	34.4	30.8	33.3	30.4	30.5
15	32.0	30.7	32.1	32.2	31.8	33.0	32.1	30.7	30.9
16	25.9	26.0	25.9	26.0	26.0	24.7	24.6	23.4	23.4
17	23.0	23.3	23.2	23.1	23.3	104.3	104.0	103.4	103.3
18	22.0	22.6	22.0	22.2	22.1	22.0	22.1	22.6	23.1
19	33.5	33.6	33.5	33.7	33.6	33.5	33.6	33.6	33.7
20	17.5	17.7	17.6	17.7	17.6	17.7	17.8	18.3	18.6

Acetate at δ21.20 and 170.8.
Malonate at δ53.3, 166.4, 41.9 and 167.
Isovaleroyl at δ172.8, 43.9, 25.5, 22.5 and 20.1.

Reduction of **4a** with $LiAlH_4$ gave the alcohol **4b**. As expected, the ^{1}H NMR spectrum of **4b** lacked the resonances for the malonate protons and the H-7 were signals shifted upfield from δ4.80 to 3.48. On the basis of the above data, and by comparison with the spectral data for similar stemarane diterpenes [7–10], **4b** had to be 13-methylene-7-hydroxy-stemarene and, therefore, **4a** was methyl-7-malonyloxy-13-methylene-stemarane.

The ^{1}H and ^{13}C NMR spectra of compound **5** showed characteristic signals of a tetracyclic diterpene with a stemarane skeleton [8–10]. The ^{1}H NMR spectrum showed a signal for an acetyl group (δ2.03, *s*) three methyl groups [δ0.94 (6H) and 1.03 (3H)]. Two doublets at δ4.40 and 4.44 with a very small coupling constant were attributable to an exocyclic methylene group. The splitting pattern of a signal at δ5.05 (1H, *tt*, J = 4.1, 11.7 Hz), indicated that the ^{1}H geminal to the acetoxyl group must be between two methylene groups, which meant that it could only be located at C-2, and from the J values this hydrogen must be axial, and in consequence, the acetoxyl must be equatorially oriented. The ^{13}C NMR data were in agreement with the proposed structure (Table 1).

Treatment of **5** with K_2CO_3–methanol gave **5a**. As expected, the ^{1}H NMR spectrum of **5a** lacked the signals for the acetyl protons and the H-2 signal was shifted upfield at δ3.90. Therefore, **5a** was shown to be 2β-hydroxy-13-methylene-stemarane, and the natural product **5** is 2β-acetoxy-13-methylene-stemarane.

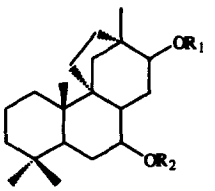
EXPERIMENTAL

Mps: uncorr.; ^{1}H NMR: 60, 100 and 360 MHz in $CDCl_3$ with TMS; ^{13}C NMR: 100 MHz. Assignments of ^{13}C NMR chemical shifts were made with the aid of APT and SFORD. IR: film; MS: direct inlet, 70 eV.

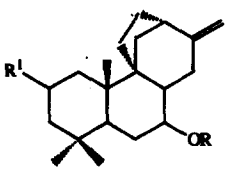
Calceolaria dentata Ret. Pav, collected in Malleco, IX Región, Chile, in November 1992, was identified by Dr. Otto Zoellner, Universidad Católica de Valparaíso. A voucher specimen is deposited at the Universidad Técnica Federico Santa María. The aerial parts of *C. dentata* (1500 g) were extracted at room temp. successively with petrol and CH_2Cl_2 for 48 hr each, affording 80 g syrup. The crude material was chromatographed on a silica gel column (400 g HF_{254} for TLC), and eluted with mixts of petrol and EtOAc of increasing polarity. Frs (125 ml) were combined based on TLC and ^{1}H NMR (60 MHz) monitoring, and the combined frs. purified by repeated CC on silica gel or silica gel impregnated with $AgNO_3$ (10%).

The molecular formulae were deduced by a combination of low resolution MS and the ^{13}C NMR spectra.

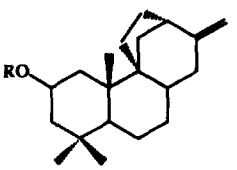
Methyl thyrsiflorin B acetate (**1a**). Oil, $[\alpha]_D^{25}$ − 76.5° (*c*, 1.0 $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 2910–2760, 1720–1700, 1430, 1270, 1190, 1140, 1020, 980; ^{1}H NMR (300 MHz) δ: 4.77 (1H, *dt*, J = 5.7, 10.9 Hz, H-7), 4.60 (1H, *dd*, J = 6.0, 10.5 Hz, H-13), 3.76 (3H, *s*, OMe), 3.38 (2H, *s*, CH_2 malonyl), 2.03 (3H, *s*, $OCOCH_3$), 1.02 (3H, *s*, Me-17), 0.93 (3H, *s*, Me-20), 0.85 (3H, *s*, Me-18), 0.81 (3H, *s*, Me-19); ^{13}C NMR: Table 1; MS *m/z* (rel. int.): 448 ($C_{26}H_{40}O_6$) $[M]^+$ (2.0), 331 $[M - C_4H_5O_4]^+$ (1.0), 330



	R_1	R_2
1	$COCH_2COOH$	COMe
1a	$COCH_2COOMe$	COMe
1b	$COCH_2COOMe$	H
2	$COCH_2CH(Me)_2$	COMe
2a	$COCH_2CH(Me)_2$	H
3	$COCH_2CH(Me)_2$	$COCH_2COOH$
3a	$COCH_2CH(Me)_2$	$COCH_2COOMe$
3b	H	H



	R
4	$COCH_2COOH$
4a	$COCH_2COOMe$
4b	H



	R
5	COMe
5a	H

$[M - C_4H_6O_4]^+$ (3.0), 272 $[M - C_4H_5O_4 - C_2H_3O_2]^+$ (2.0), 270 $[M - C_4H_6O_4 - C_2H_4O_2]^+$ (3.0), 175 (3.0), 173 (4.0), 171 (4.0), 162 (4.0), 159 (7.0), 157 (6.0), 147 (4.0), 145 (7.0), 133 (6.0), 131 (8.0), 123 (5.0), 118 (9.0), 104 (10.0), 95 (9.0), 93 (10.0), 81 (13), 69 (15), 59 (12), 57 (10), 55 (15), 43 (100), 41 (20).

Treatment of **1a** with K_2CO_3–MeOH gave **1b**, whose physical and spectral characteristics were identical to methyl thyrsiflorin B [5]. Treatment of **1a** with $LiAlH_4$ in dry Et_2O gave thyrsiflorin C (**3b**).

13-*Isovaleroyl-7-acetoxy-thyrsiflorane* (**2**). Oil, $[\alpha]_D^{25}$ + 22.35 (*c* 1.2, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2920–2780, 1710, 1700, 1455, 1380–1360, 1250, 1200, 1150, 1120, 1040–1020, 1000, 930, 800–730; ^1H NMR (300 MHz) δ: 4.71 (1H, *dt*, *J* = 5.5, 11 Hz, H-7), 4.60 (1H, *dd*, *J* = 5.9, 10.5 Hz, H-13), 2.15 (2H, *d*, *J* = 6.0 Hz, H-2′), 2.09 (3H, *s*, $OCOCH_3$), 1.02 (3H, *s*, Me-17), 0.95 (6H, *d*, *J* = 6.4 Hz, Me-4′ and Me-5′), 0.92 (3H, *s*, Me-20), 0.84 and 0.82 (3H each, *s*, Me-18 and Me-19); ^{13}C NMR: Table 1.

13-*Isovaleroyl-7-hydroxy-thyrsiflorane* (**2a**). Compound **2** (100 mg) was treated with K_2CO_3–MeOH. After usual work, **2a** was obtained as an oil, $[\alpha]_D^{22}$ = − 14.13° (*c*, 5.0, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520–3460, 2900–2780, 1705, 1450, 1380–1360, 1290, 1260–1200, 1040, 1010, 1000, 980, 930–910, 720; ^1H NMR (300 MHz) δ: 4.65 (1H, *dd*, *J* = 6.0, 10 Hz, H-13) 3.39 (1H, *dt*, *J* = 5, 10.5 Hz, H-7), 2.16 (2H, *d*, *J* = 6.1 Hz, H-2′), 1.42 (1H, *m*, H-3′), 1.02 (3H, *s*, Me-17), 0.95 (6H, *d*, *J* = 6.4 Hz, Me-4′ and Me-5′), 0.92 (3H, *s*, Me-20), 0.84 and 0.82 (3H each, *s*, Me-18 and Me-19); ^{13}C NMR: Table 1; MS *m/z* (rel. int.): 390 ($C_{25}H_{42}O_3$) $[M]^+$ (1.0), 288 $[M - C_5H_{10}O_2]^+$ (92.5), 270 $[288 - H_2O]^+$ (52.2), 246 (18.9), 203 (13.7), 260 (17.4), 255 (21.1), 231 (12.6), 175 (10.6), 165 (19.5), 164 (59.4), 149 (13.0), 123 (20.3), 109 (18.9), 107 (18.0), 104 (16.3), 95 (21.1), 93 (21.6), 81 (35.5), 79 (20.9), 69 (38.4), 67 (23.9), 57 (96.1), 55 (47.6), 41 (100.0).

Treatment of **2** with $LiAlH_4$ in dry Et_2O gave **3b**.

13-*Isovaleroyl-7-malonyloxy-thyrsiflorane* (**3**). After addition of ethereal CH_2N_2, **3** was transformed into **3a**, crystals, mp 67–69° (MeOH), $[\alpha]_D^{25}$ = − 10.7° (*c* 1.3, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 2920–2830, 1680, 1720, 1250, 1190, 1140, 1120, 1040–1020, 980, 870, 800; ^1H NMR (300 MHz) δ: 4.77 (1H, *dt*, *J* = 5.6, 10.9 Hz, H-7), 4.60 (1H, *dd*, *J* = 5.9, 10 Hz, H-13), 3.73 (3H, *s*, OMe), 3.35 (2H, *s*, CH_2 malonate), 2.16 (2H, *d*, *J* = 6.2 Hz, H-2′), 1.01 (3H, *s*, Me-17), 0.94 (6H, *d*, *J* = 6.5 Hz, Me-4′ and Me-5′), 0.91 (3H, *s*, Me-20), 0.83 and 0.80 (3H each, *s*, Me-18 and Me-19); ^{13}C NMR: Table 1; MS *m/z* (rel. int.): 490 ($C_{29}H_{46}O_6$) $[M]^+$ (2), 388 $[M - C_5H_{10}O_2]^+$ (2), 373 $[M - OCOCH_2 - COOCH_3]^+$ (6), 372 $[M - C_4H_6O_4]^+$ (10), 357 $[372 - Me]^+$ (8), 287 $[M - C_5H_{10}O_2 - C_4H_5O_3]^+$ (3), 272 $[M - C_5H_9O_2 - C_4H_5O_4]^+$ (5), 270 $[M - C_5H_{10}O_2 - C_4H_6O_4]^+$ (100), 255 $[270 - Me]^+$ (27.5), 242 (16), 159 (11.4), 149 (11.7), 145 (11.8), 133 (13), 118 (17), 109 (15), 107 (12.8), 105 (17.8), 101 $[C_5H_9O_2]$ (8.5), 95 (14.2), 91 (13.9), 85 (15), 81 (16.2), 69 (13.5), 57 (18.4).

Treatment of **3a** with $LiAlH_4$ in dry Et_2O gave **3b** whose physical and spectroscopic characteristics were in full agreement with those of thyrsiflorin C [5].

Methyl-13-methylene-7-malonyloxy-stemarane (**4a**). Crystals, mp: 75–77° (M), $[\alpha]_D^{25} = 0°$ (*c*, 2.0, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 2900–2820, 1730–1710, 1620, 1410, 1370, 1360, 1240, 1140, 1100, 1080, 1040, 1010, 980, 900, 850; ^1H NMR (300 MHz) δ: 4.80 (1H, *dt*, $J = 6$, 11 Hz, H-7), 4.49 (1H, *brs*, H-17), 4.41 (1H, *brs*, H-17′), 3.76 (3H, *s*, OMe), 3.39 (2H, *s*, CH_2 malonyl), 2.62 (1H, *t*, $J = 4.9$ Hz, H-12), 1.02 (3H, *s*, Me-20), 0.84 and 0.81 (3H each, *s*, Me-18 and Me-19); ^{13}C NMR: Table 1; MS *m/z* (rel. int.): 388 $[M]^+$ ($C_{24}H_{36}O_4$) (2.3), 271 $[M - C_4H_5O_4]^+$ (22.0), 270 $[M - C_4H_6O_4]^+$ (68.8), 255 $[270 - Me]^+$ (100), 185 (15.7), 159 (18.8), 149 (15.8), 148 (59.5), 147 (288), 131 (22.2), 119 (16.4), 117 (15.1), 107 (15.0), 105 (21.3), 91 (21.5), 79 (11.2).

Treatment of **4a** with $LiAlH_4$ in dry Et_2O gave 7-hydroxy-13-methylene-stemarane (**4b**) crystals, mp 166–168° (MeOH). ^1H NMR (300 MHz) δ: 4.54 (1H, *d*, $J = 1.9$ Hz, H-17), 4.50 (1H, *d*, $J = 1.9$ Hz, H-17′), 3.48 (1H, *dt*, $J = 5.4$, 10.6 Hz, H-7), 2.65 (1H, *dd*, $J = 5.0$, 15 Hz, H-12), 1.05 (3H, *s*, Me-20), 0.89 and 0.88 (3H each, *s*, Me-18 and Me-19); ^{13}C NMR: Table 1.

2-Acetoxy-13-methylene-stemarane (**5**). Oil, $[\alpha]_D^{25} = -38.07$ (*c*, 3.0, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2940–2780, 1710, 1630, 1450, 1350, 1250, 1200, 1150, 1020, 980, 930, 870, 800; ^1H NMR (300 MHz) δ: 5.05 (1H, *tt*, $J = 4.1$, 11.7 Hz, H-2), 4.44 (1H, *d*, $J = 4.5$ Hz, H-17), 4.40 (1H, *d*, $J = 4.5$ Hz, H-17′), 2.62 (1H, *t*, $J = 5.1$ Hz, H-12), 2.02 (3H, *s*, OAc), 1.09 (3H, *s*, Me-20), 0.90 (6H, *s*, Me-18 and Me-19).

Treatment of **5** with K_2CO_3–MeOH gave 2-hydroxy-13-methylene-stemarane (**5a**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3800–3000, 2940–2900, 2840, 2680, 2660, 2440, 1640, 1590, 1520, 1470, 1420, 1230, 1200, 1020, 940; ^1H NMR (300 MHz) δ: 4.46 (1H, *d*, $J = 4.4$ Hz, H-17), 4.39 (1H, *d*, $J = 4.4$ Hz, H-17′), 3.91 (1H, *tt*, $J = 4.0$, 11 Hz, H-2), 1.03 (3H, *s*, Me-20), 0.90 and 0.88 (3H each, *s*, Me-18 and Me-19).

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