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DITERPENOIDS FROM *CALCEOLARIA HYPERICINA**

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Key Word Index—*Calceolaria hypericina*; Scrophulariaceae; diterpenes; pimarane; abietane.

Abstract—Four new diterpenoids were isolated from the aerial parts of *Calceolaria hypericina*. Their structures were elucidated by spectroscopic methods. One of them has a dehydroabietane skeleton, and the others a pimarane skeleton.

INTRODUCTION

In previous papers we have described the isolation of phenylpropanoid glucosides from *Calceolaria hypericina* L. [2], and of diterpenoids from plants of the genus *Calceolaria* [3–5]. We have now studied the non-polar extracts of *C. hypericina*, a medium sized herb that grows on the hills of Central Chile [6]. This paper deals with the isolation and structure elucidation of four new diterpenes: 2α, 19-diacetoxy-dehydroabietane (**1**), 2α-hydroxy-19-isovaleroyl-9-epi-ent-pimara-7,15-diene (**2**), 2α,19-dyhydroxy-9-epi-ent-pimara-7,15-diene (**3**) and 2α,19-diacetoxy-9-epi-ent-pimara-7,15-diene (**4**).

RESULTS AND DISCUSSION

The petrol and dichloromethane extracts of the aerial parts of *C. hypericina* were subjected respectively to column chromatography on silica gel, using increasing proportions of ethyl acetate in petrol as solvent to afford compounds **1**–**4**, and the known β-sitosterol and ursolic acid.

Diterpene **1** was characterized as its acetate derivative **1a**, $C_{24}H_{34}O_4$ ([M]$^+$ at *m/z* 386). The IR spectrum showed carbonyl, ester, and aromatic group absorptions. In the ^{1}H NMR spectrum signals at δ7.15 (1H, *d*, *J* = 8 Hz), 7.01 (1H, *dd*, *J* = 1.6, 8 Hz) and 6.90 (1H, *d*, *J* = 1.6 Hz) established the presence of a 1,2,4-trisubstituted benzene nucleus while a one-proton septet (*J* = 7.0 Hz) centred at δ2.86 and a two-methyl doublet (*J* = 7.0 Hz) at δ1.21 showed the presence of an isopropyl group linked to the aromatic ring.

These signals, together with two three-proton singlets at δ1.0, 1.25 and 1.28, suggested a dehydroabietane skeleton. The ^{1}H NMR spectrum showed an AB pattern corresponding to an acetoxymethyl group (δ4.05 (1H, *d*, *J*: 11.2 Hz, H-19) and 4.22 (1H, *d*, *J* = 11.2 Hz, H-19′) and the ^{13}C NMR signal (δ67.2) attributed to this group indicated the axial orientation for the acetoxymethyl group in C-4. Furthermore, the signals at δ5.15 and 2.06 indicated that **1a** had a secondary acetoxyl group. Thus, the diterpenoid **1a** was presumed to be related to 2α-acetoxy-abietatriene previously isolated from *C. purpurea* [7]. This presumption was proved to be correct because the ^{13}C NMR chemical shifts of **1a** closely corresponded to those of 2α-acetoxy-abietatriene [7] except for C-3, C-4 and C-5. The differences observed in the chemical shifts can be rationalized by considering the effects of the primary acetoxyl group at C-19. Therefore, **1a** is shown to be 2α,19-diacetoxy-dehydroabietane. The probable precursor of the natural product **1**, dehydroabietinol, was isolated from *C. ascendens* [8].

The mass spectrum of compound **2** revealed a molecular ion at *m/z* 388 corresponding to $C_{25}H_{40}O_3$, and its IR spectrum indicated the presence of hydroxyl, ester and olefinic groups. The ^{1}H NMR spectrum of **2** showed signals for a vinyl group (δ5.82 *dd*, 4.94 *dd* and 4.86 *dd*), a primary ester group (δ4.25 *d*, 4.03 *d*), and three tertiary methyl groups. The ^{13}C NMR spectrum confirmed the presence of these features and suggested that the compound possessed an ent-7,15-pimaradiene-type structure. The multiplicities observed for H-15, apart from the values attributed to C-15, C-16 and C-17 (δ149.96, 109.27 and 21.7), were characteristic for an equatorially orientated vinyl group at C-13 in 7,15-pimaradiene [9, 10]. Compound **2** showed, in addition to the above-mentioned signals, a couple of doublets arising at δ4.25 and 4.03 (*J* = 11.2 Hz) indicating the existence of a primary ester group axially orientated at C-4 (C-19 at δ67.1) [11, 12]. The remaining oxygen atom was part of a secondary hydroxyl group, because the ^{1}H NMR spectrum showed a signal at δ3.97 (C-2, 64.3). The other five carbons of the molecule must be part of the acyl moiety. That the acyl group of **2** was an isovaleroyl residue was deduced from

*Part 15 in the series 'Diterpenoids from *Calceolaria* species'. For part 14, see Ref. [1].

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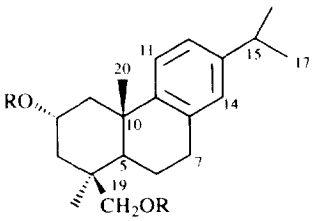
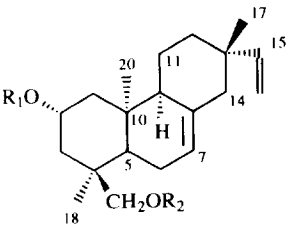
its molecular formula and in the ^{1}H NMR spectrum gave rise to signals at 0.92 (6H, *d*, *J*: 6.5 Hz, Me-4′, Me-5′), 1.27 (1H, *m*, H-3′), and 2.22 (1H, *d*, *J*: 6.5 Hz, H-2′). The ^{13}C NMR spectrum of **2** (Table 1) confirmed the presence of this residue by signals at δ173.32 (C-1′), 43.48 (C-2′), 25.59 (C-3′) and 22.38 (C-4′, C-5′) [13].

To confirm all these features and to establish the configuration of the hydroxyl group, compound **2** was treated with Ac_2O/py to give **2a**. The signal due to the H geminal to the hydroxyl group was shifted downfield from δ3.97 to δ5.32. The splitting pattern of the signal at δ5.32 indicated that the H geminal to the acetoxyl group had to be between two methylenes, which meant that it could only be located at C-2 [14]; thus, the diterpene moiety of **2a** was presumed to be related to 2α-malonyloxy-9-epi-ent-7,15-pimaradiene, isolated from *C. purpurea* [11]. Therefore, **2** must be 2α-hydroxyl-19-isovaleroyl-9-epi-ent-7,15-pimaradiene.

The IR spectrum of **3**, $C_{20}H_{34}O_2$ ($[M]^+$ at *m/z* 304) indicated the presence of hydroxyl and vinyl groups. Comparison of the ^{1}H NMR spectrum of **3** with that of **2**, showed only minor differences for the skeletal proton signals. In particular, the signals due to the isovaleroyl moiety were missing and H-19 and H-19′ were shifted





	R
1	H
1a	Ac

	R_1	R_2
2	H	$COCH_2CH(CH_3)_2$
2a	Ac	$COCH_2CH(CH_3)_2$
3	H	H
4	Ac	Ac

Table 1. ^{13}C NMR spectral data of compounds **1**, **2**, **2a**, **3** and **4** ($CDCl_3$)

C	**1a**	**2**	**2a**	**3**	**4**
1	43.5	42.3	41.2	45.0	41.4
2	68.4	64.3	68.1	64.7	68.4
3	43.7	41.2	41.6	45.9	41.8
4	38.9	38.6	38.5	38.7	38.6
5	50.6	43.7	43.6	43.8	43.8
6	26.7	25.1	25.0	25.3	25.2
7	30.7	119.3	119.2	119.7	119.4
8	134.1	136.6	136.3	136.8	136.6
9	145.7	52.8	52.6	53.2	52.9
10	38.4	38.0	37.8	39.7	38.0
11	126.9	23.0	22.8	23.1	23.0
12	124.2	37.4	37.3	37.7	37.5
13	146.1	36.7	36.5	37.0	36.7
14	124.2	47.6	47.5	47.9	47.7
15	33.4	150.0	149.7	150.3	150.1
16	23.9	109.3	109.3	109.3	109.4
17	23.9	21.7	21.6	21.8	20.8
18	27.6	27.6	27.5	27.1	27.7
19	66.7	67.1	66.6	66.1	67.1
20	22.4	23.9	23.5	23.9	23.6
$OCOCH_3$	173.2; 170.5				171.2; 170.4
$OCOCH_3$	22.9; 21.4				21.7 21.3
C-1′		173.3	172.9		
C-2′		43.5	43.3		
C-3′		25.6	25.4		
C-4′		22.4	22.3		
C-5′		22.4	22.3		

upfield from δ4.86 and 4.03 to δ3.74 and 3.53, respectively. These differences indicated that **3** must be the deisovaleroyl derivative of **2**. The ^{13}C NMR spectrum of **3** (Table 1) confirmed all the above results and defined the proposed structure as 2α, 19-dihydroxy-9-epi-ent-pimara-7,15-diene. In agreement with these, alkaline hydrolysis of **2** yielded **3**.

Compound **4**, $C_{24}H_{36}O_4$ ([M]$^+$ at *m/z* 388), showed signals in its IR spectrum due to ester and vinyl groups. The ^{1}H NMR spectrum of **4** was very similar to that of **2a** except that the signals for the isovaleroyl moiety on C-19 of **2a**, were replaced by those of an acetoxymethylene group in **4** (δ4.11 *d*, 3.99 *d*, H-19 and H-19′, respectively, and 2.02 *s*, $COCH_3$). To confirm this, compound **3** was acetylated to give **4**. Therefore, compound **4** is shown to be 2α, 19-diacetoxy-9-epi-ent-pimara-7,15-diene.

EXPERIMENTAL

Mps: uncorr; ^{1}H NMR: 60, 250 and 300 MHz in $CDCl_3$ with TMS as int. standard; ^{13}C NMR: 62.86 and 75.432 MHz, $CDCl_3$. Assignments were made with the aid of APT and SFORD. IR: film on NaCl or KBr pellets; MS: direct inlet, 70 eV. *Calceolaria hypericina* was collected in Cajón del Maipo at 1800 m a.s.l., Región Metropolitana, Chile, in November 1990. A voucher specimen is deposited at Universidad Técnica Federico Santa María.

The aerial parts of *C. hypericina* (2 kg) were extracted at room temp. successively with petrol and CH_2Cl_2 affording 110 g of a syrup. A portion of the syrup (25 g) was chromatographed on a silica gel column (600 g HF_{254} for TLC) and eluted with mixts of petrol and EtOAC of increasing polarity. Frs (125 ml) were combined, based upon TLC and ^{1}H NMR (60 MHz) monitoring and the combined frs purified by repeated silica gel CC or silica gel impregnated with $AgNO_3$ (10%) CC.

The molecular formulae were deduced by low resolutions MS jointly with hydrogen and carbon counts on the NMR spectra.

2,19-Diacetoxy-dehydroabietane (**1**). $[\alpha]_D^{20}$ + 115.9 ($CHCl_3$, *c* 0.5); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2920, 2890, 2870, 2840, 1730, 1460, 1430, 1360, 1260, 1200, 1030, 940, 820, 740; ^{1}H NMR (250 MHz): δ7.15 (1H, *d*, *J* = 8 Hz, H-11), 7.01 (1H, *dd*, *J* = 1.6, 8 Hz, H-12), 6.90 (1H, *d*, *J* = 1.6 Hz, H-14), 5.15 (1H, *m*, H-2), 4.22 (1H *d*, *J* = 11.2 Hz, H-19), 4.05 (1H, *d*, *J* = 11.2 Hz, H-19′), 2.86 (1H, *sept*, *J* = 7 Hz, H-15), 2.06 (6H, *s*, 2 $OCOCH_3$), 1.28 (3H, *s*, Me-20), 1.21 (6H, *d*, *J* = 7 Hz, Me-16, and Me-17), 1.09 (3H, *s*, Me-18); ^{13}C NMR: Table 1; MS *m/z* (rel. int.): 386 ($C_{24}H_{34}O_4$) [M]$^+$ (1.80), 325 [M − $HCOOCH_3$]$^+$ (2.00), 265 [325 − $HCOOCH_3$]$^+$ (12.44), 222 [265 − C_3H_7]$^+$ (2.37), 207 (12.44), 187 (29.68), 175 (23.52), 133 (27.17), 121 (30.14), 107 (47.95), 81 (56.16), 69 (42.82), 57 (100.00).

2α-Hydroxy-19-isovaleroyl-9-epi-ent-pimara-7,15-diene (**2**). $[\alpha]_D^{20}$ − 76, 25° ($CHCl_3$, *c* 2, 5); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3330, 2930–2910, 2880–2840, 1710, 1460, 1370, 1240, 1200, 1030, 930; ^{1}H NMR (250 MHz): δ5.82 (1H, *dd*, *J* = 11.0; 17.5 Hz, H-15), 5.32 (1H, *m*, H-7), 4.94 (1H, *dd*, *J* = 1.1; 17.5 Hz, H-16*t*), 4.86 (1H, *dd*, *J* = 1.0; 11.0 Hz, H-16e), 4.25 (1H, *d*, *J* = 11.2 Hz, H-19), 4.03 (1H, *d*, *J* = 11.2 Hz, H-19′), 3.97 (1H, *m*, H-2), 2.22 (2H, *d*, *J* = 6.3 Hz, H-2′), 1.27 (1H, *m*, H-3′), 1.04 (6H, *s*, Me), 0.97 (6H, *d*, *J* = 6.5 Hz, H-4′ and H-5′); ^{13}C NMR: Table 1; MS *m/z* (rel. int.): 388 ($C_{25}H_{40}O_3$) [M]$^+$ (1.25), 370 (28.57), 268 (370–$C_5H_{10}O_2$)]$^+$ (43.96), 253 (100.00), 239 (21.97), 213 (38.46), 187 (41.76), 145 (37.91), 131 (44.51), 105 (43.96), 85 (37.36), 57 (45.05), 41 (23.63).

Compound **2** was treated with Ac_2O in Py to afford **2a** $[\alpha]_D^{20}$ − 68.95° ($CHCl_3$; *c* 1.2); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2940–2920, 2880–2860, 1720, 1470–1460, 1360, 1250, 1035, 935; ^{1}H NMR (250 MHz): δ5.82 (1H, *dd*, *J* = 11.0; 17.5 Hz, H-15), 5.32 (1H, *bd*, *J* = 6.0 Hz, H-2), 4.94 (1H, *dd*, *J* = 1.0; 17.5 Hz, H-16*t*), 4.86 (1H, *dd*, *J* = 1.0; 11.0 Hz, H-16e), 4.20, (1H, *d*, *J* = 11.2 Hz, H-19), 4.03 (1H, *d*, *J* = 11.2 Hz, H-19′), 2.22 (2H, *d*, *J* = 6.0 Hz, H-2′), 2.02 (3H, *s*, OAc), 1.27 (1H, *m*, H-3′), 1.04 (6H, *s*, Me), 0.96 (6H, *d*, *J* = 6.4 Hz, H-4′ and H-5′), 0.89 (3H, *s*, Me); ^{13}C NMR: Table 1, MS *m/z* (rel. int.): 430 ($C_{27}H_{47}O_4$) [M]$^+$ (1.15), 370 [M − $HCOOCH_3$]$^+$ (8.13), 269 [370 $C_5H_9O_2$]$^-$ (22.89), 268 (46.84), 253 (100.00), 239 (21.45), 213 (54.74), 185 (40.53), 171 (36.32), 145 (61.05), 131 (73.16), 119 (77.89), 105 (94.21), 85 (70.00), 69 (29.47), 57 (63.16), 43 (98.42).

9-epi-ent-Pimara-7,15-diene-2α,19-diol (**3**). Crystals, mp 164–165° (MeOH); $[\alpha]_D^{20}$ − 198.8 ($CHCl_3$, *c* 1.5); IR ν_{max}^{KBr} cm^{-1}: 3240, 2860–2770, 1460, 1370–1350, 1030, 910; ^{1}H NMR (250 MHz): δ5.83 (1H, *dd*, *J* = 10.7; 17.5 Hz, H-15), 5.33 (1H, *bd*, *J* = 5.5 Hz, H-7), 4.93 (1H, *dd*, *J* = 1.07; 17.5 Hz, H-16t), 4.89 (1H, *dd*, *J* = 1.0; 10.7 Hz, H-16e), 3.90 (1H, *tt*, *J* = 3.8; 11.5 Hz, H-2), 3.74 (1H, *d*, *J* = 10.8 Hz, H-19), 3.53 (1H, *d*, *J* = 10.8 Hz, H-19′), 1.07 (3H, *s*, Me-20), 1.00 (3H, *s*, Me-18), 0.91 (3H, *s*, Me-17); ^{13}C NMR: Table 1; MS *m/z* (rel. int.): 304 ($C_{20}H_{32}O_2$) [M]$^+$ (45.05), 286 [M − H_2O]$^+$ (10.23), 273 [M − CH_2OH]$^+$ (21.42), 255 (100.00), 231 (23.08), 213 (17.03), 187 (30.77), 145 (39.56), 133 (41.21), 105 (65.93), 91 (54.40), 55 (20.33), 41 (18.13).

2α,19-Diacetoxy-9-epi-ent-pimara-7,15-diene (**4**). $[\alpha]_D^{20}$ − 120.6° ($CHCl_3$, *c* 4.0); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2970, 2960–2920, 1730, 1720, 1510, 1460, 1440, 1390, 1360, 1250, 1200, 1150, 1035, 990, 870; ^{1}H NMR (250 MHz) δ5.80 (1H, *dd*, *J* = 11; 17 Hz, H-15), 5.30 (1H, *m*, H-7), 4.92 (1H, *dd*, *J* = 1; 17 Hz, H-16t), 4.86 (1H, *dd*, *J* = 1; 11 Hz, H-16e), 4.11 (1H, *d*, *J* = 11 Hz, H-19), 3.99 (1H, *d*, *J* = 11 Hz, H-19′), 2.07 (3H, *s*, $OCOCH_3$), 2.01 (3H, *s*, $OCOCH_3$), 1.04 (6H, *s*, Me-18 and Me-20), 0.88 (3H, *s*, Me-17); ^{13}C NMR: Table 1; MS *m/z* (rel. int.): 388 ($C_{24}H_{36}O_4$) [M]$^+$ (23.25), 328 [M − $HCOOCH_3$]$^+$ (33.91), 268 [328 − $HCOOCH_3$]$^+$ (41.60), 253 [268 − CH_3]$^+$ (100.00), 239 (22.58), 214 (20.14), 213 (46.20), 211 (23.38), 199 (23.33), 197 (21.29), 187 (22.29), 185 (23.83), 171 (27.02), 169 (16.53), 159 (20.10), 157 (28.30), 145 (37.60), 143 (25.11), 133 (17.03), 131 (34.54), 129 (15.57), 119 (25.83), 117 (16.60), 107 (17.53), 105 (34.31), 90 (28.58), 81 (15.34), 79 (18.05), 43 (74.08).

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