



DITERPENOIDS FROM *CALCEOLARIA GLANDULIFERA**

M. CRISTINA CHAMY, MARISA PIOVANO, JUAN A. GARBARINO† and M. PAZ AMÉSTICA

Depto de Química, Universidad Técnica Federico Santa María, Casilla 110-V, Valparaíso, Chile

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Key Word Index—*Calceolaria glandulifera*; scrophulariaceae; diterpenes; 9-*epi-ent*-isopimarane diterpenes; 19-malonyloxy-9-*epi-ent*-7,15-isopimaradiene; 19-hydroxy-9-*epi-ent*-7,15-isopimaradiene.

Abstract—Two new diterpenes and three known diterpenes have been isolated from the aerial parts of *C. glandulifera*. The structures of the new compounds were established by spectroscopic evidence as 19-malonyloxy-9-*epi-ent*-7,15-isopimaradiene and 19-hydroxy-9-*epi-ent*-7,15-isopimaradiene. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Several diterpenes have been isolated from *Calceolaria* species in recent years [1–4]. Continuing our work on terpenoids from this genus, we have examined the aerial parts of *C. glandulifera*, a species that grows on San Felipe, V Región, Chile. From this plant we have isolated two new diterpenes: 19-malonyloxy-9-*epi-ent*-7,15-isopimaradiene, 19-hydroxy-9-*epi-ent*-7,15-isopimaradiene, together with the known diterpenes, 18-hydroxy-9-*epi-ent*-7,15 isopimaradiene [5], 17-malonyloxy-9-*epi-ent*-7,15 isopimaradiene [6], 17-hydroxy-9-*epi-ent*-7,15 isopimaradiene [6].

RESULTS AND DISCUSSION

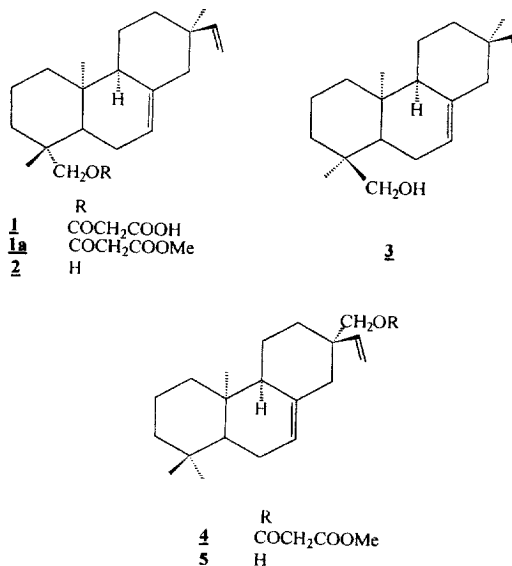
The petrol and methylene dichloride extracts of the fresh aerial part of *C. glandulifera* were subjected separately to CC on silica gel using increasing proportions of ethyl acetate in petrol as solvent affording compounds 1–5.

The acidic nature of compound 1 was evident from characteristic behaviour on TLC and because, by reaction with ethereal diazomethane, the methyl ester **1a** was easily obtained.

The IR spectrum of **1a** showed absorptions for ester and olefinic groups. The ¹H NMR spectrum of **1a** showed the presence of a vinyl groups δ 5.79 (*dd*, H-15), 4.88 (*dd*, H-16c), 4.85 (*dd*, H-16t) and

three tertiary methyl groups. In addition, a methine signal at δ 5.18, (*brd*, H-7) indicated a trisubstituted double bond. In the ¹³C NMR the values at δ 145.7 and δ 111.3 ppm were assigned, to C-15 and C-16, respectively. The multiplicities observed for H-15, besides the values attributed to C-15, C-16 and C-17 (δ_c 27.22), are characteristic for a equatorial methyl group at C-13 [7].

Compound **1a** also showed in its ¹H NMR spectrum a couple of doublets arising at δ 4.34 and δ 3.95 (*J* = 10.9 Hz) indicating the existence of a primary ester group axially orientated at C-4 (C-19 at δ 68.3) [8–10]. This stereochemistry caused a



*Part 19 in the series "Diterpenoids from *Calceolaria* species." For part 18, see Ref. [1].

†Author to whom correspondence should be sent.

deshielding on C-5 (δ 44.4) of *ca.* 7.0 ppm related to the observed value for this carbon in compound having an equatorial primary ester group [11,12]. This is in accordance with the signals observed for similar structures [1,4]. The value for C-5 was also consistent with an uncommon orientation of C-11 [4].

The remaining ^{13}C NMR absorptions were consistent with the structural features of an 7,15 isopimaradiene type skeleton with the primary ester group axially orientated.

That the ester side chain of **1a** was a malonate residue was deduced by a singlet at δ 3.30 in its ^1H NMR spectrum accounting for two hydrogen atoms [13, 14]. The ^{13}C NMR spectrum confirmed the presence of this residue by signals at δ 166.6, 41.5 and 167.0. Therefore, compound **1a** was shown to be **methyl-19 malonyloxy-9 epi-ent 7,15 isopimaradiene**.

The IR spectrum of **2** ($\text{C}_{20}\text{H}_{32}\text{O}$, M^+ at m/z 288) indicated the presence of double bond and hydroxy group. Comparison of the ^1H NMR spectrum of **2** with that of **1a** showed only minor differences for the skeletal proton signals. In particular, the ^1H NMR spectrum of **2** lacked resonances for the malonate protons and the H-19 and H-19' signals shifted upfield from δ 4.34 and 3.95 to δ 3.81 and 3.46, respectively. These differences indicated that **2** must be the demalonyl derivative of **1**. In agreement with this, alkaline hydrolysis of **1a** yielded **2**.

The ^{13}C NMR spectrum of **2** confirmed all the above results and defined the structure as **19-hydroxy-9-epi-ent-7,15 isopimaradiene**.

Based on the evidence of the ^1H NMR and mass spectra, as well as a comparison of physical properties, the diterpenes **3**, **4** and **5** were shown to be identical to 18 hydroxy-9 epi-ent-7,15 isopimaradiene [5], 17 malonyloxy-9-epi-ent-7,15 isopimaradiene [6] and 17-hydroxy-9-epi-ent-7,15 isopimaradiene [6], respectively.

EXPERIMENTAL

General

Mps: uncorr. IR: film on NaCl with CHCl_3 solns. ^1H NMR and ^{13}C NMR (400 MHz): CDCl_3 with TMS an internal standard. Chemical shifts are expressed in δ (ppm); MS: direct inlet system, 70 eV. TLC Merck Kieselgel 60 F_{254} ; CC: Merk Kieselgel 60, Kieselgel 60 GF_{254} . Na_2SO_4 was used for drying organic solvent and the petrol used had bp 60–80°.

Plant material

Calceolaria glandulifera was collected in San Felipe, V Región, Chile, during October (1994). A voucher specimen is on deposit in the herbarium of

the Natural Product Laboratory, Universidad Técnica Federico Santa María, Valparaíso, Chile.

Extraction and isolation

The aerial parts (2 kg) of *C. glandulifera* were powdered and extracted successively with petrol and CH_2Cl_2 for 3 days each, at room temp. to give 10 and 15 g of a syrup, respectively. These crude extracts were applied separately to CC (Kieselgel 60, 500 g) and eluted with mixture of petrol and EtOAc of increasing polarity (1–100% EtOAc) Fractions of 125 ml were taken and combined based upon TLC and ^1H NMR (60 MHz) monitoring; fractions 9–11 of the petrol extract were treated with ethereal CH_2N_2 and subjected to CC on silica gel impregnated with AgNO_3 10% using petrol–EtOAc (8:2) to provide **1a** and **4a**.

The middle fr. were subjected to further CC on Kieselgel impregnated with AgNO_3 (10%) (elution with petrol–EtOAc 8:2) affording compounds **2** and **5**.

The CH_2Cl_2 extract was subjected to CC (Kieselgel 60, elution with petrol EtOAc of increasing polarity affording after purification **3**.

The known compounds **3**, **4** and **5** were identified by comparison of their ^1H NMR, ^{13}C NMR, mass spectra with those previously reported in the literature [5, 6].

Methyl-19 malonyloxy-9-epi-ent-7,15 isopimaradiene (1)

After addition of CH_2N_2 in Et_2O , **1** (200 mg) was transformed to **1a** gummy. IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 3075, 2960–2840, 1740, 1710, 1450, 1430, 1375, 1280, 1150, 1020, 910. ^1H NMR (400 MHz) δ ppm: 5.79 (1H, *dd*, $J = 10.3$, 16.5 Hz, H-15) 5.18 (1H, *brd*, $J = 5.7$ Hz, H-7), 4.88 (1H, *dd*, $J = 1.5$, 10.3 Hz, H-16c), 4.85 (1H, *dd*, $J = 15$, 16.5 Hz, H-16t), 4.34 (1H, *d*, $J = 10.9$ Hz, H-19), 3.95 (1H, *d*, $J = 10.9$ Hz, H-19'), 3.68 (3H, *s*, OMe), 3.30 (2H, *s*, $\text{CH}_2\text{-Mal}$), 0.92, 0.88 and 0.85 (3 H each, *s*, Me-17, Me-18 and Me-20). ^{13}C NMR: see Table 1. MS m/z (rel. int): 388 [$\text{C}_{24}\text{H}_{36}\text{O}_4$, M] $^+$ (3), 370 (6), 270 [$\text{M-HO}_2\text{CCH}_2\text{CO}_2\text{Me}$] (69), 255 [270-Me] $^+$ (100), 251 (24), 187 (40), 148 (35), 133 (50), 119 (75), 109 (90), 105 (75), 81 (65), 55 (75).

19-hydroxy-9-epi-ent-7,15 isopimaradiene (2)

IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 3350, 3075, 2970, 2840, 1630, 1460, 1450, 1410, 1380, 1150, 1070, 1050, 1030, 1010, 910. ^1H NMR (400 MHz) δ (ppm): 5.85 (1H, *dd*, $J = 11$, 16.5 Hz, H-15), 5.23 (1H, *brd*, $J = 5.2$ Hz, H-7), 4.93 (1H, *dd*, $J = 1.1$, 11 Hz, H-16c), 4.91 (1H, *dd*, $J = 1.1$, 16.5 Hz, H-16t), 3.81 (1H, *d*, $J = 11$ Hz, H-19), 3.46 (1H, *dd*, $J = 1.1$, 11 Hz, H-19'), 0.98, 0.94, 0.85 (3 H each, *s*, Me-17, Me-18 and Me-20). ^{13}C NMR: see Table 1. MS: m/z (rel int): 288 [$\text{C}_{20}\text{H}_{32}\text{O}$] $^+$ (4), 270 [$\text{M-H}_2\text{O}$] $^+$ (42), 257 (19), 256 (16), 255 [270-Me] $^+$ (89), 241 (29),

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

C	1a *	2	3 †
1	36.5	35.8	36.5
2	18.3	18.4	18.3
3	36.5	36.6	37.1
4	36.5	37.7	36.5
5	44.4	44.3	38.2
6	23.2	23.2	24.0
7	119.1	119.4	119.1
8	137.1	136.9	137.0
9	53.0	53.0	53.2
10	35.0	35.1	35.1
11	25.5	25.4	25.5
12	39.4	39.4	39.5
13	38.9	38.8	39.1
14	49.5	49.5	49.7
15	145.7	145.8	145.7
16	111.3	111.2	111.4
17	29.7	29.6	30.0
18	27.2	26.9	74.5
19	68.3	65.4	18.2
20	23.2	23.0	22.7

Spectra were recorded using CDCl_3 solutions; chemical shifts are reported in ppm relative to TMS.

*Malonate signals at 167.0, 41.5, 166.5 and 53.3.

†Data taken from Ref. [5].

228 (26), 213 (24), 199 (40), 187 (26), 185 (27), 175 (30), 161 (30), 159 (26), 157 (18), 148 (18), 147 (23), 145 (44), 134 (11), 133 (52), 131 (48), 129 (20), 119 (69), 117 (40), 115 (21), 109 (53), 107 (51), 105 (100), 95 (43), 93 (43), 91 (58), 81 (92), 79 (59), 67 (52), 65 (11), 55 (61).

UNLINKED REFERENCES

[14]

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