Phytochemistry, Vol 28, No 10, pp 2844 2845, 1989
Printed in Great Britain

0031 9422/89 $3 00+0 00

9-*EPI*-*ENT*-7,15-ISOPIMARADIENE DERIVATIVES FROM *CALCEOLARIA GLANDULOSA**

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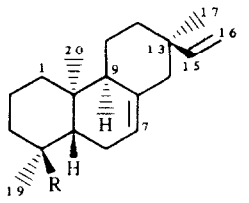
(*Received* 10 *January* 1989)

Key Word Index—*Calceolaria glandulosa*, Scrophulariaceae, bis-diterpene, 9-epimeric pimarane diterpenes

Abstract—In addition to two new 9-*epi*-pimarane diterpenes, the aerial parts of *Calceolaria glandulosa* yielded a new bis-diterpene, glandulosate, derived from the esterification of malonic acid by two 18-hydroxy-*ent*-9-*epi*-7,15-isopimaradiene units The structures of the compounds were elucidated by spectroscopic evidence and chemical transformations

INTRODUCTION

In a previous paper [2], the characterization of *ent*-18-malonyloxy-9-*epi*-isopimarol (**4**), the major diterpene from the chloroform extract of *Calceolaria glandulosa* was described Further examination of the less polar eluates from the chromatography of this extract resulted in the isolation of three additional 9-*epi*-pimarane diterpenoids Two of these terpenoids were identified as *ent*-18-formyloxy-9-*epi*-isopimarol (**1**) and *ent*-9-*epi*-isopimarol (**3**) The other compound, bis (*ent*-9-*epi*-7, 15-isopimaradiene-18-yl) malonate which has been given the trivial name glandulosate, is a further example of a bis-diterpene

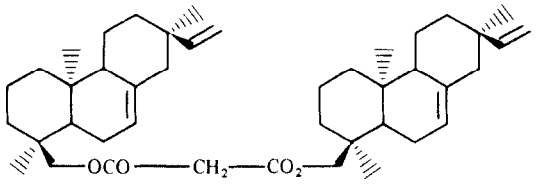


	R
1	CH_2OCOH
3	CH_2OH
4	CH_2OCOCH_2COOH

RESULTS AND DISCUSSION

The chloroform extract of the fresh aerial parts of *C glandulosa* was subjected to column chromatography on silica gel, using increasing proportions of ethyl acetate in petrol as solvent, to afford some diterpene-enriched fractions [2] Repeated chromatography of the petrol-ethylacetate fraction (14 1) led to the purification of compounds **1** and **2**, whereas the next fraction (9 1) led to the isolation of **3**

The less polar diterpene (**1**) had a molecular formula $C_{21}H_{32}O_2$ ([M]$^+$ at *m/z* 316) and its ^{1}H NMR spectrum exhibited the characteristic signals of a 7,15-isopimaradiene-type structure (δ 5 21, *br s*, H-7, 5 82, *dd*, H-15, 4 91 *dd*, H-16c, 4 89, *dd*, H-16t) with a primary ester group (δ 3.86, *d*, H-18, 3 76 *d*, H-18') The nature of the ester side chain of **1** was evident from spectroscopic data. Two strong absorptions, one at 1720 cm^{-1} and the other at 1170 cm^{-1}, in the IR, together with a singlet at δ 8 05, accounting for one proton, in the ^{1}H NMR clearly indicated that the ester side chain of **1** is a formyl unit [3,4]. The ^{13}C NMR spectrum of **1** (Table 1) confirmed the presence of these features and established that the terpenic moiety of the compound closely corresponded to those of *ent*-18-malonyloxy-9-*epi*-isopimarol (**4**) [2] Therefore, **1** is shown to be *ent*-18-formyloxy-9-*epi*-isopimarol As confirmation of the proposed structure, formylation of *ent*-9-*epi*-isopimarol (**3**) [2] afforded **1**, whose spectral and physical data where in full agreement with those of the natural product



2

The structure of glandulosate, **2** ($C_{43}H_{64}O_4$, [M]$^+$ at *m/z* 644) was deduced by comparing its spectral data with that of *ent*-18-malonyloxy-9-*epi*-isopimarol (**4**) [2]. In fact, the ^{1}H NMR spectrum of **2** was superimposable on that of **4**, but the integral due to the terpenic moiety was twice that of the malonate protons (δ 3 36), which suggested that two *ent*-9-*epi*-isopimaryl units must be linked by malonic acid in a similar fashion as found in foliosate [1] Accordingly, the ^{13}C NMR spectrum of **2** (Table 1) exhi-

*Part 4 in the series 'Diterpenoids from *Calceolaria* species' For Part 3 see ref [1]

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

C	1*	2†
1	36 3	36 5
2	18 1	18 1
3	37 0	37.1
4	36 4	36.5
5	38 2	38 5
6	24 0	24.0
7	118 6	119.1
8	136.6	137 2
9	53 1	53 2
10	35 1	35.1
11	25 5	25.5
12	39.5	39.5
13	39 0	39 1
14	49 6	49.8
15	145 2	145.9
16	111.1	111 5
17	29 9	29 9
18	72.8	74.9
19	18 6	18.4
20	22.8	22 7

*Formyl carbon at 160.8 (*s*)
†Malonate carbons at 166 8 (*s*), 2 *CO*; 41 8 (*t*), CH_2

bited 22 signals which agreed well with those of **4** [2]. The observed evidence are completely in accordance with structure **2** and glandulosate corresponded thus to bis-(*ent*-9-*epi*-7,15 isopimaradiene-18-yl) malonate.

Finally, the IR spectrum of compound **3**, $C_{20}H_{32}O$ ($[M]^+$ at *m/z* 288) indicated the presence of olefinic and hydroxyl groups. Comparison of the ^1H NMR spectrum of **3** with that of **1** showed only minor differences for the skeletal proton signals. In particular, the formyl proton singlet was missing and the H-18 and H-18′ signals shifted upfield from δ 3.86 and 3.76 to 3.35 and 3.14, respectively. These differences indicated that **3** must be the deformyl derivative of **1**. The ^{13}C NMR spectrum of **3** confirmed all the above results and defined the proposed structure as *ent*-9-*epi*-isopimarol This is the first report of natural occurrence of **3**, which had been previously produced as a synthetic substance [2].

The accumulation of 9-epimeric diterpenoids, some of them, esterificated by malonic acid in members of the *Calceolaria* genus is of systematic value

EXPERIMENTAL

Mps uncorr ^1H NMR· 400 and 500 MHz in $CDCl_3$ with TMS. as int std; ^{13}C NMR· 100 and 125 MHz, $CDCl_3$ with TMS as int. std. Assignments of ^{13}C NMR chemical shifts were made with the aid of APT and SFORD, IR· film on NaCl or KBr pellets; MS: direct inlet, 70 eV. *Calceolaria glandulosa* Poepp ex Benth., collected in Cuesta Zapata, V-Region, Chile in November 1985, was identified at the Universidad Federico Santa Maria, where a voucher specimen is deposited General details of extn and chromatographic sepn of the $CHCl_3$ extract of the plant have been described previously [2]

ent-18-*Formyloxy*-9-epi-*isopimarol* (**1**). Viscous colourless oil, $[\alpha]_D^{25} -108.1$ ($CHCl_3$, *c* 1 5). IR ν_{max}^{film} cm^{-1} 3060, 2980–2840, 1720, 1635, 1460, 1450, 1410, 1380, 1370, 1170, 915, 850, 820 ^1H NMR (400 MHz): δ 8 05 (1H, *s*, formyl), 5 82 (1H, *dd*, *J* = 11.3, 17.15 Hz, H-15), 5 21 (1H, *br s*, H-7), 4 91 (1H, *dd*, *J* = 11.3, 1.5 Hz, H-16c), 4.89 (1H, *dd*, *J* = 17 5, 1.5 Hz, H-16t), 3.86 (1H, *d*, *J* = 10 7 Hz, H-18), 3 76 (1H, *d*, *J* = 10.7 Hz, H-18′), 0 96 (3H, *s*, Me-17), 0.94 (3H, *s*, Me-20), 0 92 (3H, *s*, Me-19) ^{13}C NMR· see Table 1; MS *m/z* (rel int.): 316 $[C_{21}H_{32}O_2, M]^+$ (73); 301 $[M-Me]^+$ (81), 273 (62), 270 (57), 255 (73), 187 (84), 133 (94), 119 (94), 105 (100), 95 (93), 67 (79), 55 (82), 43 (68), 41 (62)

Formylation of ent-9-epi-*isopimarol*. **3** (200 mg) was treated with 35 mg of HCO_2H (p.a grade). The reagents were refluxed for 30 min and after evapn under vacuum, the mixt was chromatographed on silica gel (20 g) and eluted with petrol–EtOAc (14 1) yielding pure *ent*-18-formyloxy-9-*epi*-isopimarol (155 mg) 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**.

Bis-(ent-9-epi-7,15-*isopimaradien*-18-*yl*) *malonate* (*glandulosate*, **2**). Viscous colourless oil, $[\alpha]_D^{25} -134.0°$ ($CHCl_3$; *c* 1 0). IR ν_{max}^{film} cm^{-1} 3060, 2960–2840, 1730, 1640, 1460, 1450, 1410, 1380, 1330, 1270, 1150, 1030, 1010, 915, 850, 820. ^1H NMR (500 MHz): δ 5 87 (2H, *dd*, *J* = 11 2, 17.5 Hz, H-15 and H-15′), 5 25 (2H, *br s*, H-7 and H-7′), 4.95 (2H, *dd*, *J* = 17 5, 1 5 Hz, H-16t and H-16t′), 3 87 (2H, *d*, J = 10 7 Hz, H_2-18), 3 80 (2H, *d*, *J* = 10.7 Hz, H_2-18′), 3 36 (2H, *s*, H_2-malonyl), 1.00 (6H, *s*, Me-17 and Me-17′), 0 97 (6H, *s*, Me-20 and Me-20′), 0.95 (6H, *s* Me-19 and Me-19′). ^{13}C NMR. see Table 1, MS *m/z* (rel. int) 644 $[C_{43}H_{64}O_4, M]^+$ (10), 629 $[M-Me]^+$ (5), 288 (5), 270 (52), 255 (38), 139 (83), 105 (100), 81 (85), 55 (70).

ent-9-epi-*Isopimarol* (**3**) The compound was identified by direct comparison of its physical (TLC, mp, $[\alpha]_D^{25}$) and spectral (^1H NMR, ^{13}C NMR, MS) properties with those previously reported by us [2].

Acknowledgements—We are grateful to Professor E. G Gros (Universidad de Buenos Aires, Argentina) for MS and Professor M. Nicoletti (Universitá La Sapienza, Italia) for recording the ^1H and ^{13}C NMR. This research was supported by grants # 881303 DGDCYT, Universidad Federico Santa Maria, and CONICYT 1988

REFERENCES

1. Chamy, M. C., Piovano, M, Garbarino, J. A, Gambaro, V and Miranda, C. (1988) *Phytochemistry* (in press)
2 Piovano, M., Gambaro, V, Chamy, M. C, Garbarino, J. A,, Nicoletti, M, Guilhem, J and Pascard, C (1988) *Phytochemistry* **27**, 1145
3. Silverstein, R M and Bassler, G. C (1963) *Spectroscopic Identification of Organic Compound*, p 63 Wiley, New York
4 Abraham, R J and Loftus, P (1978) *Proton and Carbon*-13 *Nuclear Magnetic Resonance Spectroscopy· An Integrated Approach*, p 17 Heyden, Bristol