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SESQUITERPENE LACTONES FROM *CENTAUREA ALBA* AND *C. CONIFERA*

ISABEL FERNÁNDEZ, JOSÉ R. PEDRO* and ESTHER POLO

Departament de Química, Orgánica, Facultat de Química, Universitat de València, 46100-Burjassot (València), Spain

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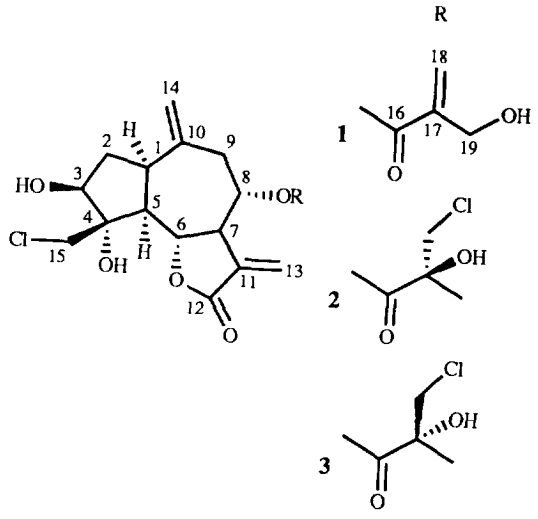
Key Word Index—*Centaurea alba*, *C. conifera*; *Leuzea*; Compositae; sesquiterpenes; germacranolides; guianolides; eudesmane; loliolide.

Abstract—The aerial parts of *Centaurea alba* yielded five know germacranolides: salonitenolide, 11β,13-dihydrosalonitenolide, salonitenolide 8-*O*-(4′-acetoxy-5′-hydroxy)-angelate, cnicin 4′-O-acetate and cnicin. The aerial parts of *C. conifera* yielded loliolide, 1β,6α-dihydroxy-4(15)-eudesmene, chlorojanerin, chlorohyssopifolin A (centaurepensin) and its C-17 epimer. The latter compound is described for the first time.

INTRODUCTION

In continuation of our work on Spanish Compositae [1–3], we have now investigated specimens of *Centaurea alba* L. and *C. conifera* L. [= Leuzea conifera (L.) DC.]. Previous work is limited to the mention that salonitenolide and cnicin were present in *C. alba* [4, 5]. Our own work on the aerial parts of this plant resulted in the isolation of salonitenolide [6, 7], 11β,13-dihydrosalonitenolide [8], salonitenolide 8-*O*-(4′-acetoxy-5′-hydroxy)-angelate [9], cnicin 4′-O-acetate [10] and cnicin [7]. With regards the species *C. conifera*, on which no chemical studies have been reported, we have found loliolide [1], 1β,6α-dihydroxy-4(15)-eudesmene [11], chlorojanerin (**1**) [12, 13], chlorohyssopifolin A (centaurepensin) (**2**) [14, 15] and its C-17 epimer (**3**). This latter compound is described for the first time in this paper.



RESULTS AND DISCUSSION

Lactones **2** and **3** were obtained as a mixture and could not be separated. The CI-mass spectrum showed protonated molecular ion peaks at *m/z* 435, 437 and 439 indicating the presence of two chlorine atoms and the common molecular formula $C_{19}H_{24}O_7Cl_2$. The ^{1}H NMR of this mixture was very similar to that of chlorojanerin (**1**), a guaianolide frequently found in *Centaurea* spp. [12, 13], except for the signals of the ester side-chain. Hydroxymethacrylate signals (two singlets at δ6.33 and 5.95 of CH_2 =C and a broad singlet at 4.38 of CH_2OH) in the ^{1}H NMR of chlorojanerin (**1**) were replaced by those of a 2-chloromethyl-2-hydroxy-propionate residue [two doublets at δ3.87 and 3.63 (J = 11.6 Hz) of CH_2Cl and a singlet at 1.55 of Me] in the ^{1}H NMR spectra of **2** and **3**. The chemical shifts of the other protons were unchanged. Consequently, the structure of chlorohyssopifolin A (**2**) [14, 15] was assigned to the major component of the mixture of lactones. The chemical shifts in the ^{1}H NMR spectrum of the minor component were virtually identical to the chlorohyssopifolin A, with the exception of the signals of H-13 (δ5.83 vs 5.57), H-14 (δ4.82 vs 5.01), H-8 (δ5.16 vs 5.21) and H-9 (δ2.46 vs 2.48). These differences could be explained, as in related cases [16], by assuming that **3** was the C-17 epimer of chlorohyssopifolin A (**2**). Indeed, Dreiding models indicated that a change of stereochemistry at C-17 would have the greatest steric and anisotropic effects at H-13, H-14, H-8 and H-9, as indicated by Merill and Stevens [16].

Further characterization of **1**–**3** were obtained by ^{13}C NMR spectra and heteronuclear multiple quantum correlation ^{1}H–^{13}C (HMQC), which allowed us to assign unambiguously the signals of all the carbons. Thus, for **2**,

*Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR spectral data of compounds 1–3 (400 MHz, $CDCl_3$, δ values, J in Hz).

H	1	2	3
1	3.61 *ddd* (10.8, 9.0. 8.5)	3.62 *ddd* (10.8, 9.0, 8.5)*	3.62 *ddd* (10.8; 9.0, 8.5)*
2	1.62 *dd* (15.0, 8.0)	1.59 *dd* (15.0; 7.2)	1.59 *dd* (15.0; 7.2)
2′	2.53 *ddd* (14.8, 11.2, 6.8)	2.55 *ddd* (15.0; 11.0; 6.4)	2.55 *ddd* (15.0; 11.0; 6.4)
3	4.17 *br d* (6.8)	4.17 *br d* (6.4)	4.17 *br d* (6.4)
5	2.32 *br t* (9.2)	2.31 *br t* (10.4)	2.31 *br t* (10.4)
6	4.73 *dd* (11.2, 9.2)	4.73 *t* (10.8)	4.73 *t* (10.8)
7	3.16 *tt* (9.4, 3.3)	3.12 *tt* (9.6, 3.2)	3.12 *tt* (9.6; 3.2)
8	5.15 *ddd* (9.6, 5.2, 1.6)	5.16 *m*†	5.21 *ddd* (9.6; 4.0; 1.0)
9	2.65 *dd* (15.2, 5.2)	2.65 *dd* (15.6; 5.2)	2.65 *dd* (15.6; 5.2)
9′	2.44 *d* (15.2)	2.46 *d* (15.2)	2.48 *d* (16.0)
13	6.20 *d* (3.6)	6.24 *d* (3.2)	6.22 *d* (3.2)
13′	5.61 *d* (2.8)	5.83 *d* (3.2)	5.57 *d* (3.2)
14	5.14 *br s*	5.14 *br s*	5.16 *br s*
14′	4.82 *br s*	4.82 *br s*	5.01 *br s*
15	4.33 *d* (11.6)	4.34 *d* (12.0)	4.33 *d* (11.6)
15′	3.95 *d* (12.0)	3.96 *d* (12.0)	3.96 *d* (11.6)
18	6.33 *s*	3.87 *d* (11.6)	3.87 *d* (11.6)
18′	5.95 *s*	3.63 *d* (11.6)	3.64 *d* (11.6)
19	4.38 *br s*	1.55 *s*	1.55 *s*

*A part of this signal is overlapped with H-18′.
†Overlapped signal with H-14.

Table 2. ^{13}C NMR spectral data of compounds 1–3 (100 MHz, $CDCl_3$, δ values)

C	1	2	3
1	47.1	47.1	47.3
2	37.7	38.0	38.0
3	77.2	77.2	77.2
4	84.5	84.7	84.7
5	57.5	57.6	57.5
6	76.2	76.1	76.1
7	46.4	46.6	46.5
8	74.1	76.0	76.0
9	35.1	34.7	34.6
10	142.1	142.0	141.9
11	168.6	168.4	168.4
12	136.7	136.5	136.9
13	122.8	123.2	122.3
14	118.0	118.2	118.5
15	49.9	50.0	50.0
16	165.3	173.0	173.0
17	139.2	76.0	75.9
18	126.8	51.2	51.0
19	62.3	23.9	23.4

the signals at δ77.2, 76.1 and 76.0 were assigned to C-3, C-6 and C-8 by their correlation with the doublet of H-3 (δ4.17), the triplet of H-6 (δ4.73) and the multiplet of H-8 (δ5.16), respectively. In the same way, the signals at δ47.1, 57.6 and 46.6 were assigned to C-1, C-5 and C-7 by their correlation with the double triplet at δ3.62 for H-1, the triplet at δ2.31 for H-5 and the triple triplet at δ3.12 for H-7. Similarly, the signals at δ38.1 and 34.7 were assigned at C-2 and C-9 as they are correlated with the signals at δ1.59 and 2.55 for H-2 and δ2.65 and 2.46 for H-9, respectively. Finally, the signals at δ50.0 (C-15) and 51.2 (C-18) correlated with a pair of doublets at δ4.34 and 3.96 (H-15) and with another pair of doublets at δ3.87 and 3.63 (H-18), respectively, and the signals at δ123.2 (C-13) and δ118.2 (C-14) correlated with two doublets at δ6.24 and 5.83 (H-13) and two singlets at δ5.14 and 4.82 (H-14), respectively.

The chlorinated sesquiterpene lactones isolated in this work from *C. conifera* are metabolites very characteristic of *C. solstitialis* [16, 17], a neurotoxic weed that causes a neurodegenerative disorder in horses, which is comparable to Parkinson's disease with respect to symptoms and pathology.

EXPERIMENTAL

HPLC: Lichrospher RP-18 (250 × 8 mm), elution MeOH–H_2O mixtures; NMR: Varian Unity 400 (inverse probehead); CI-MS (CH_4): Hewlett-Packard 5988A.

Plant material. Aerial parts of *C. alba* (1.0 kg) were collected in Saldes (Barcelona), Spain. *C. conifera* (1.7 kg) was collected in Altura (Castellón), Spain. Voucher specimens are deposited in the herbarium of the Botany Department (Faculty of Biological Sciences) of Valencia University.

Extraction and chromatography. The aerial parts were finely ground and extracted at room temp. with hexane–Et_2O–MeOH (1:1:1). The extracts were evapd *in vacuo* and the respective residues fractionated by CC on silica gel with hexane–(EtOAc or Et_2O)–MeOH mixtures of increasing polarity. The residue from the *C. alba* fr. eluted with EtOAc–MeOH (4:1) was further sepd by flash chromatography on silica gel and HPLC to yield salonitenolide (4 mg), 11β,13-dihydrosalonitenolide

(8 mg), salonitenolide-8-*O*-(4′-acetoxy-5′-hydroxy)-angelate (3 mg), cnicin 4′-*O*-acetate (16 mg) and cnicin (30 mg). In the same way, the residue from the *C. conifera* fr. eluted with Et_2O–MeOH (19:1) yielded loliolide (5 mg), 1β,6α-dihydroxy-4(15)-eudesmene (3 mg), chlorojanerin (**1**) (24 mg), chlorohyssopifolin A (**2**) and its C-17 epimer (**3**) (12 mg).

Chlorojanerin (**1**). Oil, IR ν_{max} cm^{-1}: 3480, 1750, 1720, 1635; CI-MS (CH_4) *m/z* (rel. int): 441 (4) and 439 (12) isotopic peaks for $[M + C_3H_5]^+$, 429 (5) and 427 (13) $[M + C_2H_5]^+$, 401 (18) and 399 (49) $[M + 1]^+$, 299 (38) and 297 (100) $[M + 1 - C_4H_6O_3]^+$, 281 (29) and 279 (83) $[M + 1 - C_4H_6O_3 - H_2O]^+$, 261 (19), 243 (11) $[M + 1 - C_4H_6O_3 - H_2O - HCl]^+$ and 201 (26).

Chlorohyssopifolin A (**2**) *and 17-epichlorohyssopifolin A* (**3**). Oil, IR ν_{max} cm^{-1}: 3460, 1745, 1720, 1650; CI-MS (CH_4) *m/z* (rel. int): 479 (4), 477 (11) and 475 (13) isotopic peaks for $[M + C_3H_5]^+$, 467 (4), 465 (15) and 463 (20) $[M + C_2H_5]^+$, 439 (16), 437 (50) and 435 (71) $[M + 1]^+$, 301 (4), 299 (40) and 297 (96) $[M + 1 - C_4H_7O_3Cl]^+$, 283 (3), 281 (31) and 279 (100) $[M + 1 - C_4H_7O_3Cl - H_2O]^+$, 261 (50), 243 (27) and 201 (43).

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