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Two new antiinflammatory elemanolides from *Centaurea chilensis*

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Abstract

Two previously undescribed elemanolide esters, the 2-methylpropanoate and 2-methyl-2-propenoate of 11,13-dehydromelitensin, were isolated in the course of a bioassay-guided fractionation from the aerial parts of *Centaurea chilensis* Hook. et Arn., used traditionally to treat 'gout and rheumatism'. The mixture of both substances exhibits anti-inflammatory activity in the carrageenan-induced paw edema assay.

Key words: Antiinflammatory; Elemanolides; *Centaurea chilensis*; Asteraceae

1. Introduction

The genus *Centaurea* (Asteraceae, tribe Cynareae) is a well-known source of sesquiterpene lactones. As part of a study of species growing in Chile, we have now obtained two new elemanolide esters from *C. chilensis* Hook. et Arn. Earlier chemical studies on this plant had afforded β-sitosterol and stigmasterol (Negrete et al., 1984), the flavonoids apigenin 7-*O*-glucoside, chrysoeriol 7-*O*-glucoside, chrysoeriol, luteolin, kaempferol, quercetin, and hispidulin (Negrete et al., 1988a), as well as the guaianolides dehydrocostus lactone and 8α-hydroxydehydrocostus lactone (Negrete et al., 1984), 8α-acetoxydehydrocostus lactone (Negrete et al., 1988b), and 11β-*H*-11,13-dihydro-desacyl-cynaropicrin 8β-D-glucoside (Negrete et al., 1988a).

No elemanolides have been reported previously from *C. chilensis* Hook. et Arn., but other *Centaurea* species have afforded melitensin, dehydro-melitensin, and several esters of these sesquiterpene lactones: melitensin was obtained originally from *C. melitensis* L. (González et al., 1971) and later from *C. aspera* var. *stenophylla* (Dufour) Nyman (Picher et al., 1984); its structure and stereochemistry have been confirmed by X-ray analysis (Tortajada et al., 1988); this elemanolide has also been synthesized via Cope rearrangement of cnicin to 11,13-dehydro-melitensin (González et al.,1974a), which was first isolated from *C. pullata*

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L. (González et al., 1974b); *C. melitensis* afforded melitensin and 11,13-dehydromelitensin β-hydroxybutyrates (González et al., 1975); *C. arbutifolia* Svent. gave 11,13-dehydromelitensin isovalerate (isoarbutifolin) (González et al., 1981), *C. phrygia* L. yielded the 8α-(5-hydroxy)-angeloyl ester (Tsankova and Ognyanov, 1985) and *C. cineraria* L. ssp. *umbrosa* (Lacaita) Pign. (syn. *C. ucriae* Lacaita ssp. *ucriae*) gave the 2-methyl-3,4-dihydroxybutyrate (Bruno and Herz, 1988).

C. chilensis has been used in folk medicine for 'gout and rheumatism', although the treatment advocated in recent years only involves external use (Muñoz et al., 1981). Several guaianolides are known to possess anti-inflammatory activity, which has been related to the presence of the α-methylene-γ-lactone moiety (Hall et al., 1979, 1980), and this suggested a possible relationship between the chemistry of this plant and its traditional use. In this paper we describe the bioassay-guided isolation, structure elucidation, and anti-inflammatory activity of a mixture of two previously undescribed elemanolide esters: 11,13-dehydromelitensin 2-methylpropanoate (**I**) and 2-methyl-2-propenoate (**II**), from *C. chilensis*.

2. Materials and methods

Aerial parts of *Centaurea chilensis* were collected in the flowering season (December) in Las Tacas, La Serena (near sea level, 30°S latitude), Chile. A voucher specimen (*L. Arancibia 16078*) is on deposit in the Botany Laboratory of the Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago (SQF).

Melting points are uncorrected and were determined on a Kofler hot stage. IR spectra (KBr discs) were recorded utilizing a Leitz IIIG infrared spectrometer. NMR spectra in $CDCl_3$ with TMS as internal standard were recorded at 400 MHz (^1H) and 100.5 MHz (^{13}C) on a Bruker WM-400 spectrometer, using commercially available pulse programs for DEPT and 2D spectra (^1H-^1H and ^1H-^{13}C COSY, COLOC). The HREIMS was recorded on a Kratos MS-30 instrument.

The air-dried, ground plant material (4.0 kg), was extracted successively (at room temperature) with petroleum ether, $CHCl_3$, and MeOH, yielding, respectively 50, 300, and 400 g of residue after removing the solvents in vacuo. All 3 extracts were administered to guinea pigs (see below) to monitor potential antiinflammatory activities. The chloroform extract proved to be the most active in the carageenan-induced paw edema assay, and was therefore chromatographed on a silica gel G column, eluting with petroleum ether-CH_2Cl_2 mixtures of increasing polarity as shown in Table 1.

11,13-Dehydromelitensin 2-methylpropanoate (**I**) and 2-methyl-2-propenoate (**II**) from fractions B, C and D (see Table 1) were purified by further chromatography on silica gel G with petroleum ether-CH_2Cl_2 (3:2) increasing the CH_2Cl_2 proportion up to 100%. The major constituent obtained in this way (300 mg) seemed to be homogeneous on TLC (silica gel; light petrol-EtOAc 7:3; $R_F = 0.35$) and had the appearance of a slightly greenish lacquer, insoluble in petroleum ether, somewhat soluble in $CHCl_3$ and in EtOAc, very soluble in MeOH, giving a purple Liebermann–Burchard reaction. In $CHCl_3$, the mixture apparently polymerizes, giving a white precipitate which cannot be redissolved. EIMS *m/z* data were: (relative abundance) 264.1366 (0.26%; calculated $C_{15}H_{20}O_4$, 264.1361), 246.1265 (7.65%; calculated $C_{15}H_{18}O_3$, 246.1256), 228.1149 (8.43%; calculated $C_{15}H_{16}O_2$, 228.1149). Complete ^1H- and ^{13}C-NMR assignments are shown in Table 2.

Anti-inflammatory activity was tested against carrageenan-induced edema in the guinea pig hindpaw. λ-Carrageenan (1% in saline, 0.1 ml) was injected into the left plantar aponeurosis of groups

Table 1
Fractionation of the chloroform extract of *Centaurea chilensis*

Fraction	Solvent composition	Anti-inflammatory activity
A	100% petroleum ether	none
B	75:25 petroleum ether/CH_2Cl_2	++
C	50:50 petroleum ether/CH_2Cl_2	+++
D	25:75 petroleum ether/CH_2Cl_2	++
E	100% CH_2Cl_2	+
F	100% MeOH	+

of 10–15 guinea pigs of both sexes (Pirbright) weighing 200–300 g, fasted for 24 h, with water ad libitum, and kept at 18–20°C. The volume of the injected paw was determined with a plethysmometer by measuring the volume of water displaced by the paw, immersed up to a mark made previously just above the ankle, before injecting carrageenan, and 3 h later. Drugs or vehicle (propyleneglycol) were administered orally 1 h before injection. The percent inhibition of edema was

Table 2
^{13}C and ^{1}H chemical shifts from the 100 MHz ^{13}C and 400 MHz ^{1}H NMR spectra — assignments based on the 100/400 MHz F1-decoupled CH correlation

C Atoms	$\delta^{13}C$ (ppm)	CH_n	$\delta^{1}H$ (ppm)	$^{3}J_{HH}$ coupling (H)
16[a]	173.72	C		
12	171.40	C		6.03; 5.58; 5.54
16[b]	167.67	C		1.96; 6.14; 5.69
1[a]	147.87	CH	*5.83*	2.49; 5.00; 4.90; 1.71; 1.16
1[b]	147.85	CH	*5.83*	2.51; 5.00; 4.90; 1.68; 1.19
4	145.59	C		[a]2.49; 4.05; 3.94; 5.40
				[b] 2.51; 4.05; 3.94; 5.40
11[a]	139.13	C		2.90; 4.46; 6.03
11[b]	139.08	C		3.05; 4.48; 6.03
17[b]	137.43	C		6.14; 1.96
18[b]	126.95	CH_2	*6.14; 5.69*	1.96
13[b]	119.63	CH_2	*6.03; 5.54*	3.05
13[a]	119.59	CH_2	*6.03; 5.58*	2.90
3[a]	113.28	CH_2	*5.40; 5.00*	4.05; 3.94; 2.49
3[b]	113.25	CH_2	*5.40; 5.00*	4.05; 3.94; 2.51
2	112.97	CH_2	[a]*5.00; 4.90*	
			[b]*5.02; 5.02*	
6[a]	80.03	CH	*4.46*	2.90; 2.49
6[b]	80.00	CH	*4.48*	3.05; 2.51
8[b]	70.96	CH	*5.27*	3.05; 1.88; 1.68
8[a]	70.37	CH	*5.22*	2.90; 1.88; 1.71
15	66.68	CH_2	*4.05; 3.94*	5.40; 5.00
7[a]	53.13	CH	*2.90*	5.22; 6.03; 5.58
7[b]	53.03	CH	*3.05*	4.48; 5.27; 6.03; 5.54
5[a]	51.81	CH	*2.49*	4.46; 1.16; 1.88; 5.00; 5.40
5[b]	51.74	CH	*2.51*	4.48; 1.19; 1.88; 5.00; 5.40
9[a]	46.15	CH_2	*1.88; 1.71*	1.16
9[b]	46.05	CH_2	*1.88; 1.68*	1.19
10[a]	42.95	C		2.49; 5.00; 4.90; 1.16; 1.88; 1.71
10[b]	42.92	C		2.51; 5.02; 5.02; 1.19; 1.88; 1.68
17[a]	35.19	CH	*2.61*	
14[a]	19.31	CH_3	*1.16*	2.49; 1.71
14[b]	19.26	CH_3	*1.19*	2.51; 1.68
18[a]	19.11	CH_3	*1.17*	1.19
19[a]	19.03	CH_3	*1.19*	1.17
19[b]	18.41	CH_3	*1.96*	6.14; 5.69

[a] for compound **I**.
[b] for compound **II**.

calculated for each group with respect to its vehicle-treated control group, and analyzed statistically using Student's *t*-test.

3. Results

A 20% infusion of the aerial parts of this plant, similar to that recommended by folk healers, administered orally to guinea pigs, exhibited weak (20%) but significant ($P < 0.05$) anti-inflammatory activity at 4 ml/kg in a carrageenan-induced paw edema assay (Winter et al., 1962). The residue of the methanol extract of *C. chilensis*, dissolved in propyleneglycol, showed similar activity which attained 29% at 400 mg/kg and 49% at 600 mg/kg ($P < 0.05$). The residue of the chloroform extract was the most potent, giving a significant 35% inhibition of edema at 200 mg/kg, and 65% at 600 mg/kg. Bioassay-guided fractionation by column chromatography of the chloroform extract (Table 1) led to the purification of an active fraction which appeared to be homogeneous on TLC and which gave a significant 41% inhibition of edema at 12 mg/kg, 60% at 33 mg/kg, and 67% at 64 mg/kg (ED_{65} = 18.6 mg/kg). In the same assay, naproxen sodium salt, a commercially available antiinflammatory agent, gave 58% inhibition at 4.3 mg/kg (ED_{50} = 1.65 mg/kg). Consequently, the material from *C. chilensis*, although several times less potent than naproxen, is at least as effective as this synthetic drug in reducing inflammation.

The active fraction displayed an IR spectrum showing the presence of hydroxyl groups, C=C double bonds, γ-lactone and unsaturated ester functions. The HREIMS exhibited an apparent molecular ion at *m/z* 264.1366 (0.26%; calculated $C_{15}H_{20}O_4$, 264.1361) indicative of a sesquiterpene lactone. The ^{1}H-NMR spectrum could be assigned, for the most part, to an elemanolide structure, but the signals due to the ester moieties (doublets at 6.14 and 5.69 ppm assigned to H-18 and -18′ of the methacrylic acid; septuplet at 2.61 ppm attributable to the isopropyl methine of the isobutyric acid) showed that the sample was an approximately 1:1 mixture of two substances, **I** and **II**. This was confirmed by the ^{13}C- NMR spectrum which exhibited characteristic peaks for both esters. The complete ^{1}H and ^{13}C assignments were made on the basis of DEPT, homo- and heteronuclear COSY, and COLOC spectra, and are summarized in Table 2.

4. Discussion and conclusion

The fact that germacradienolides undergo facile thermal isomerization to elemadienolides has led to the suggestion that some of these latter products may be artefacts (Barrero et al., 1989). The drying of the plant material, the extraction and all purification steps relating to compounds **I** and **II**, however, were carried out at room temperature without adding any reagents which might presumably catalyze such a reaction. We therefore conclude that live plants of *C. chilensis* probably contain these elemanolides, regardless of the possibility that they may be produced in vivo, via non-enzymatic Cope rearrangement of salonitenolide or some of its esters. To the best of our knowledge, compounds **I** and **II** have not been described in the literature. Furthermore, the previously unreported anti-inflammatory activity of elemanolides suggests that this may be a common property of α-methylene-γ-lactones with adequate absorption and distribution characteristics, as shown previously for guaianolides (Hall et al., 1979,1980). Finally, the presence of orally active anti-inflammatory sesquiterpene lactones in the aerial parts of *Centaurea chilensis* (at concentrations greater than 0.0075%, w/w) may provide a rationale for the traditional use of this plant in inflammatory diseases.

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