Sesquiterpene Lactones from *Inula montana* L.

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Z. Naturforsch. **55 c,** 697–700 (2000); received April 10/May 29, 2000

Asteraceae, *Inula montana* L., Sesquiterpene Lactones

Aerial parts of *Inula montana* were investigated for its sesquiterpenoid composition. Five sesquiterpene lactones, isoinuviscolide, gaillardin, 1β-hydroxy-3β-acetoxy-eudesm-4(15), 11(13)-dien-12–8β-olide, pulchellin-C and pulchellin-E were identified for the first time in this plant. One of them, 1β-hydroxy-3β-acetoxy-eudesm-4(15),11(13)-dien-12–8β-olide, is a novel natural product. The structures of this compounds were established by 1D and 2D-NMR spectroscopy.

Introduction

Inula montana L. (tribe Inuleae, fam. Asteraceae) is a perennial species occurring in the east of Spain on alkaline and dry soils, extending to west of France and east of Italy (Tutin, 1976). Several species from the genus *Inula* are traditionally used in folk medicine as antipyretic (Muzaffer *et al.,* 1992), antiinflammatory and hepatoprotective drugs (Kurma and Mishra, 1997). However, there are no data in the literature on the chemical constituents and the possible pharmacological effects of *Inula montana.*

The secondary plant metabolites that mediate these pharmacological effects are mainly sesquiterpene lactones. This class of compounds includes over 3000 naturally occurring substances, one of the largest group of plant products described (Fischer, 1991).

Sesquiterpene lactones occur in many plant families, but are most widely distributed within the Asteraceae. They include a wide variety of biological and pharmacological activities. Antitumor, antimicrobial, antifeedant, cytotoxic, antibacterial, antifungal, allergenic contact dermatitic and plant growth regulatory activity of several sesquiterpene lactones has been previously reported (Beckman *et al.,* 1998; Picman and Towers, 1983; Picman,1984; Warshaw and Zug, 1996). In a previous paper we have described the antileishmanial activity of a chloroform extract from the aerial parts of *Inula montana* against *Leishmania infantum* (promastigote forms) (Martín *et al.,* 1998).

In the present paper, we report the isolation and identification of five sesquiterpene lactones, isoinuviscolide, gaillardin, pulchellin-E, pulchellin-C and the new natural product 1β-hydroxy-3β-acetoxy-eudesm-4(15),11(13)-dien-12–8β-olide, for the first time from *Inula montana.*

Results and Discussion

In the chloroform extract of the leaves of *Inula montana* L. the following known sesquiterpene lactones were identified: isoinuviscolide (**1**), gaillardin (**2**), pulchellin E (**4**) and pulchellin-C (**5**). In addition, we have isolated and identified one new natural product, 1β-hydroxy-3β-acetoxy-eudesm-4(15),11(13)-dien-12–8β-olide (**3**). The structures of the known compounds (Fig. 1) were determined by comparison of their spectroscopic features with those reported in the literature (Yoshioka *et al.,* 1970; Sanz *et al.,* 1991; Ito and Lida, 1981; Pired, 1977).

Compound **3** was obtained as a yellow syrup and had the molecular formula $C_{17}H_{22}O_5$ (*m/z* 306) as established by EIMS. The IR spectrum confirmed the presence of the OH group (3600 cm^{-1}) and the acetyl carbonyl (1760 cm^{-1}). The ^{1}H-NMR spectrum (Table I) showed four multiplets between 6.5 and 4.5 ppm corresponding to two exocyclic methylene groups. Two broadened singlets at δ4.97 and 4.66 were assigned to the methylene protons at C-4 and the presence of a pair of doublets at δ6.15 and 5.61 (1H each) is character-

0939–5075/2000/0900–0697 $ 06.00

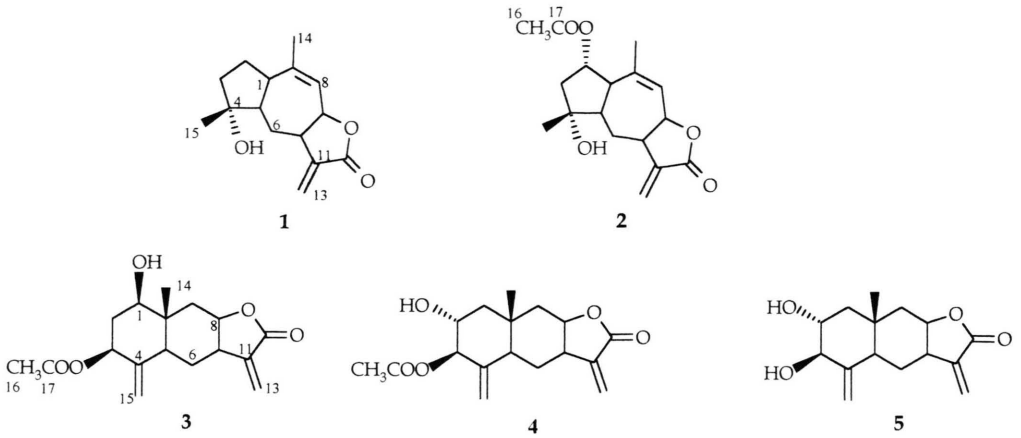

Fig. 1. Structures for sesquiterpene lactones **1–5**.

Table I. ^{1}H NMR chemical shift assignments for the isolated sesquiterpene lactones.

H	**1**	**2**	**3**	**4**	**5**
1	2.57	2.60	3.49 (J=11.8/4.3)	1.32 and 1.94	1.6 and 1.82
2	1.72 and 1.93	5.20	1.62 and 2.16 (J=11.8)	3.71	3.43
3	1.58 and 1.78	1.97 and 1.97	5.17 (J=1.5)	4.99	3.74
4	–	–	–	–	–
5	1.48	2.20	1.75 (J=1.5)	1.93	1.97
6	1.45 and 2.50	1.49 and 2.54	1.50 and 1.78 (J=11.6/6.2)	1.35 and 1.76	1.05 and 1.82
7	2.51	2.59	2.96 (J=6.2/1.5)	2.96	3.10
8	4.81	4.57	4.54 (J=4.9/2.0)	4.48	4.54
9	5.80	5.89	1.45 and 2.63 (J=15.7)	1.50 and 2.26	1.60 and 2.20
10	–	–	–	–	–
11	–	–	–	–	–
12	–	–	–	–	–
13	5.90 and 6.10	6.14 and 5.64	6.15 and 5.61	5.59	6.07 and 5.71
14	1.82	1.84	0.82	0.82	0.80
15	1.19	1.22	4.97 and 4.66	4.90 and 4.63	5.26 and 4.70
16	–	2.05	2.13	2.15	–
17	–	–	–	–	–

istic of a methylenic group (C-11) conjugated with a lactonic carbonyl group. The region 2.2–0-8 ppm showed two methyl singlets at 2.13 and 0.82 ppm. The chemical shift of the methyl singlet at δ2.13 indicated that this methyl group should be adjacent to an oxygen function (acetate methyl).

The ^{13}C-NMR spectrum (Table II) showed two signals (δ170.2 and 169.9) of carbonyl groups of the quaternary carbons, one at δ170.2 was clearly due to the lactone carbonyl, while the other at δ169.9 could be assigned to an acetyl group. The multiplicity of each carbon was achieved by the DEPT experiment, which revealed the presence of two methyl, five methylene, five methine and five quaternary carbons. The assignment of proton and carbon signals was achieved from DQCOSY and 2D-TOCSY Homonuclear Hartmann-Hahn Spectroscopy (HOHAHA) (mixing time = 80 ms) experiments and also from ^{1}H-^{13}C heteronuclear multiple quantum (HMQC) and multiple bond correlations (HMBC).

The compounds **1**, **2**, **4** and **5** were obtained as white crystalline solids. The ^{1}H NMR spectra of **1** and **2** suggested two guaianolides with an α-meth-

Table II. ^{13}C NMR chemical shift assignments for the isolated sesquiterpene lactones.

	1	2	3	4	5
1	47.2	51.9	76.9	47.9	49.3
2	25.9	77.5	36.9	69.6	73.1
3	42.0	48.5	71.0	80.0	79.5
4	80.4	79.5	141.7	141.5	150.4
5	53.5	51.5	41.7	44.3	45.2
6	32.0	30.4	26.3	27.1	28.7
7	47.9	47.2	40.2	40.8	41.3
8	83.7	84.4	76.0	76.2	78.7
9	126.7	128.7	37.8	40.5	41.6
10	143.1	138.0	29.7	33.5	34.6
11	141.6	141.4	144.6	143.5	144.0
12	172.3	172.1	169.9	170.5	172.6
13	119.5	119.9	120.6	120.7	121.0
14	21.4	22.8	11.2	18.5	19.1
15	23.8	26.7	105.6	106.4	106.3
16	–	21.5	21.0	20.9	–
17	–	172.6	170.2	170.2	–

ylene-γ-lactone group. The difference between compounds **1** and **2** was the presence of the acetyl group at C-2 in **2**. As expected the H-2 signal was shifted downfield (δ5.20) and an acetate methyl singlet was visible (δ2.05).

The ^1H NMR spectra of **4** and **5** suggested two eudesmanolides: 2α-hydroxy-3β-acetoxy-isoalantolactone and 2α,3β-dihydroxy-isoalantolactone, respectively. Their structures were determined by comparison of their spectroscopic features with those reported in the literature (Yoshioka *et al.*, 1970).

Experimental

MS spectra were obtained in a Hewlett Packard 5988 A mass spectrometer. Analytical TLC was performed on precoated Si gel plates (Kieselgel G-60, F-254, 0.25 mm, Merck) using mixtures of chloroform-methanol. Visualization of the TLC plates was achieved with a long wavelength UV lamp and sulfuric acid spray reagent.

All 1D and 2D NMR spectra were recorded by a Varian Unity Plus spectrometer in $CDCl_3$ or CD_3OD solutions (^1H at 500 MHz, ^{13}C at 125 MHz). Multiplicities were assigned through DEPT experiments. The standard pulse sequences from the Varian software were used for homonuclear and heteronuclear correlation experiments (DQCOSY, TOCSY, HMQC and HMBC).

Collection, extraction and isolation

The aerial parts of *Inula montana* were collected in San Andrés del Congosto (Guadalajara, Spain) in July 1996 and identified by Professor Carmen Bartolomé Esteban. A voucher specimen is kept in the Department of Vegetal Biology, Faculty of Sciences, University of Alcalá (Madrid, Spain).

Air-dried plant material (820 g) was extracted with chloroform. The dry chloroform extract (23.43 g) was subjected to solvent partitioning between n-hexane and H_2O/MeOH (5/95 v/v). The H_2O/MeOH portion (12.84 g) was column chromatographed over silicagel, eluted with chloroform and mixtures chloroform-methanol of increasing polarity, giving fractions A_1-A_{20}. Fractions A_1 to A_{10} were eluted with pure chloroform. Fraction A_2 was submitted to sucesive flash column chromatography (CC) over silicagel (chloroform-methanol and toluene-diethyl ether) to give isoinuviscolide (**1**,14.4 mg). Fraction A_5 was rechromatographed over silicagel (hexane-acetone) to give gaillardin (**2**, 302.7 mg). Fraction A_8 was submitted to CC over silicagel (toluene-diethyl ether) to give the sesquiterpene lactones 1β-hydroxy-3β-acetoxy-eudesm-4(15),11(13)-dien-12–8β-olide (**3**, 13.6 mg) and pulchellin-E (**4**, 29.5 mg). Finally, fraction A_9 was rechromatographed over silicagel (toluene-diethyl ether) to give pulchellin-C (**5**, 10.7 mg).

Acknowledgements

This work was realized with the financial support of the following institutions: PICASSO (Acciones Integradas Hispano-Francesas, Refs. H-211 and 98-B), FIS (Ministerio de Sanidad y Consumo, Ref. 94/1671). CAM (Comunidad Autónoma de Madrid, Ref. C101/91), Universidad de Alcalá (E001/99), and Consejería de Educación y Cultura de la Comunidad Autónoma de Madrid (Convocatoria 1998).

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