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A sesquiterpene lactone glucoside from *Ixeris denticulata* f. pinnatipartita

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Abstract

An extract from the entire *Ixeris denticulata* f. pinnatipartita plant afforded the guaianolide sesquiterpene lactone glucoside, 8β,15-dihydroxy-1(10),3,11(13)-guaiatrien-12,6-olide-15-*O*-glucopyranoside, along with the known flavonoids luteolin-7-*O*-glucoside and luteolin-7-*O*-glucuronide-6′-methyl ester; their structures were determined by spectroscopic methods. Ixerin Y inhibited the growth of human breast cancer MCF7 and MDA468 cell lines.

Keywords: *Ixeris denticulata* f. pinnatipartita Kitag; Compositae; Ixerin Y; Luteolin-7-*O*-glucoside; Luteolin-7-*O*-glucuronide-6′-methyl ester; Cytotoxicity

1. Introduction

Chemical studies of *Ixeris* plants have revealed the existence of sesquiterpene lactones, such as ixerins A–W (Asada, Miyase, & Fukushima, 1984a,b; Asada, Miyase, & Fukushima, 1984c; Nishimura et al., 1985; Seto, Miyase, & Fukushima, 1986), and ixerisosides A–N (Warashina, Ishino, Miyase, & Ueno, 1990). These sesquiterpenes showed a wide-spectrum of biological activities, such as cytotoxicity (Seto et al., 1988), as well as having ant repellant and antifeedant properties (Isman & Rodriguez, 1983; Okunade & Wiemer, 1985; Srivastava, Proksch, & Wray, 1990).

I. denticulata f. pinnatipartita was described by Kitagowa (1939), and is distributed in China, Japan and the former USSR. It is differentiated by pinnately parted leaves from *I. denticulata* f. denticulata which has entire leaves. In this study ixerin Y (**1**) was isolated from *I. denticulata*, together with two known flavonoids, luteolin-7-*O*-glucoside (**2**) and luteolin-7-*O*-glucuronide-6′-methyl ester (**3**). Both flavonoids, from their mass and NMR spectra, were identical to reported data (Wang, Ding, Guo, & Wu, 1980; Yang, 1981; Asada et al., 1984c).

Ixerin Y (**1**), named after the known sesquiterpene lactones ixerins A…W from genus Ixeris, has a molecular formula of $C_{21}H_{28}O_9$ as indicated by its high resolution EI-MS. The presence of an α-methylene-γ-lactone moiety was revealed by its IR absorption bands at 1750 and 1669 cm^{-1}. This was substantiated by a pair of characteristic low field ^1H NMR signals at δ 6.61 (1H, br s, H-13a) and 6.40 (1H, br s, H-13b) (Table 1). An olefinic proton signal at δ 6.19 (1H, br s, H-3) and a vinyl methyl absorption at δ 1.66 (3H, br s, H-14) were also observed. The ^{13}C NMR spectrum of **1** showed the presence of 21 carbons (Table 1), one of which was assigned to a lactone carbonyl at δ 172.0, six were due to olefinic carbons indicating the existence of 3 double bonds, nine were oxygen-bearing carbons including 6 from a glucose moiety; and the

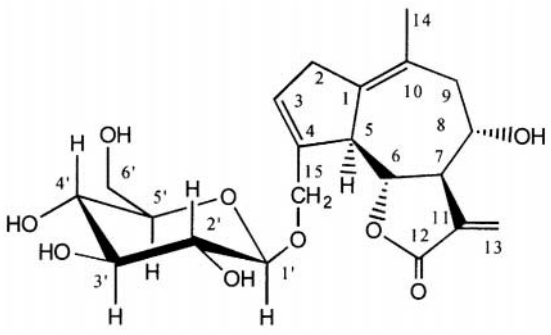

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Table 1. ^{1}H NMR and ^{13}C NMR data of ixerin Y

	^{1}H NMR	^{13}C NMR
Aglycone moiety		
1		137.0 s
2	2.99 (2H, overlapping)	37.1 t
3	6.19 (3H, br s)	129.4 d
4		142.0 s
5	3.84 (1H, d, J = 10.0)	52.3 d
6	3.70 (1H, t, J = 10.0)	82.8 d
7	3.04 (1H, d t, overlapping)	59.0 d
8	3.94 (1H, br t, J = 11.4)	68.8 d
9	α: 2.71 (1H, dd, J = 13.0, 11.4)	46.6 t
	β: 2.46 (1H, dd, J = 13.0, 1.9)	
10		126.8 s
11		140.0 s
12		170.2 s
13	a: 6.61 (1H, br s)	121.4 t
	b: 6.40 (1H, br s)	
14	1.66 (3H, br s)	23.1 q
15	a: 5.06 (1H, br d, J = 13.2)	68.3 t
	b: 4.94 (1H, br d, J = 13.2)	
Glucose moiety		
1′	5.03 (1H, d, J = 7.8)	103.3 d
2′	4.09 (1H, dd, J = 7.8, 8.4)	75.4 d
3′	4.34 (1H, overlapping)	78.5 d
4′	4.34 (1H, overlapping)	71.8 d
5′	4.02 (1H, m)	78.7 d
6′	a: 4.64 (1H, dd, J = 12.0, 2.1)	62.9 t
	b: 4.48 (1H, dd, J = 12.0, 5.2)	

Spectrum was measured in pyridine-d_5. δ values were given in ppm relative to TMS as an internal standard. Numbers in parentheses denote coupling constants in Hz. Signals were assigned by DEPT, ^{1}H–^{1}H COSY, HMQC and HMBC spectra.

others included two methylene, two methine and one methyl carbon at higher field. In the ^{1}H NMR spectrum, a triplet at δ 3.70 (1H, t, J = 10.0 Hz) was attributed to H-6, which was coupled to H-5 at δ 3.84 (1H, d, J = 10.0 Hz) and H-7 at δ 3.04 (1H, overlapped). The latter signal was further coupled with H-8 at δ 3.94 (1H, br t, J = 9.6 Hz). This indicated a *trans*-diaxial relationships between H-5 and H-6, H-6 and H-7, H-7 and H-8, respectively. Since H-7 in all naturally occurring guaianolides from higher plants are α-oriented (Nishimura et al., 1986), so, H-5, H-6 and H-8 should be α, β and β oriented, respectively. This was confirmed by a NOESY experiment on **1**: NOE correlations were observed between H-5, H-7 and H-9α as well as H-6, H-8 and H-9β, respectively. Long range couplings were also observed in the ^{1}H–^{1}H COSY of **1**: H-14 with H-2 and H-5, H-3 with H-15, H-7 with H-13a and H-13b. In the ^{13}C NMR spectrum of **1**, C-15 appeared downfield at δ 68.3 in comparison with other 15-hydroxymethylene guaianolides, indicating that the glucoside was located at C-15. The glucose moiety was deduced form its NMR data as well as TLC analysis after hydrolysis of **1**. Though the absolute configuration of glucopyranose moiety cannot be determined by NMR data, the anomeric structure of **1** was thought to be β from the value of the $J_{1'2'}$ (7.8 Hz).

Based on the above evidence as well as by comparison with data for the known aglycone of crepidiaside E (Adegawa, Miyase, Ueno, Noro, & Kuroyanagi, 1985), ixerin Y was determined to be 8β,15-dihydroxy-1(10),3,11(13)-guaiatrien-12,6-olide-15-*O*-glucopyranoside (**1**).

Ixerin Y **1** showed good inhibitory effects against the growth of human breast cancer MCF7 and MDA468 cell lines, with IC_{50} values of 6.36 μg/ml and 11.87 μg/ml, respectively. IC_{50} values for comparative purposes of the positive control, Etoposide (VP-16), against MCF7 and MDA468 were 3.5 and 6.7 μg/ml, respectively.

2. Experimental

2.1. General

Mps: uncorr; ^{1}H NMR and ^{13}C NMR: 400 and 100 MHz, respectively; 2D-NMR data (^{1}H–^{1}H COSY, HMQC, HMBC, NOESY): 400 MHz using standard pulse sequences on a Bruker-400 instrument. Pyridine-d_5 was used as solvent, with TMS as int. standard; EI-MS: 70 eV, direct int.; FT-IR: KBr; CC: silica gel (coarse silica gel, 100–200 mesh), Diaion HP-20 (16–50 mesh); TLC: precoated silica gel plates (Merck, silica gel 60 F_{254}).

2.2. Plant material

I. denticulata f. pinnatipartita Kitag. (Compositae) was collected in the Dabie Mountains, Luotian county, Hubei province, in October 1992, and identified by Professor Xu, Department of Pharmacognosy, China Pharmaceutical University, where a voucher specimen (No. Ma921028) is deposited.

2.3. Extraction and isolation

The air-dried whole plant material (4 kg) was extracted with hot water (40 l × 3). After filtration, the combined extract was concentrated to 10 l, and precipitated by adding EtOH to a concentration of 60% and the latter removed by filtration after standing overnight. The filtrate was applied to a Diaion HP-20 column after the EtOH was removed under reduced pressure. The column was washed initially with water, then with 20, 40 and 85% EtOH, successively. The fraction from 40% EtOH was chromatographed on a silica gel column and eluted with chloroform–methanol (95:5–8:2). Repeated chromatography afforded compounds **1** (20 mg), **2** (30 mg) and **3** (10 mg).

Ixerin Y (**1**), fine needles (MeOH). Mp 180°C (dec.); UV λ_{max}^{MeCN} nm: 231. $[\alpha]_D^{28}$ −38.9° (MeOH, c 0.11). IR ν_{max}^{KBr} cm^{-1}: 3412 (-OH), 2294, 2880, 1750 (-C=O), 1669, 1448, 1384, 1078, 1029. ^1H NMR (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz): see Table 1. EI-MS *m/z*: 424.17 $[M]^+$ (3), 317 (4), 294 (4), 262 (13), 244 $[M\text{-}C_6H_{12}O_6]^+$ (100), 227 (43), 181 (40), 119 (56).

2.4. Bioassay

Cytotoxicity against human breast tumor MCF7 and MDA468 cell lines, with Etoposide (VP-16) as a positive control, was measured in 7-day MTT tests at the Cancer Research Laboratory, School of Pharmacy, the University of Nottingham.

References

Adegawa, S., Miyase, T., Ueno, A., Noro, T., & Kuroyanagi, M. (1985). *Chemical and Pharmaceutical Bulletin, 33,* 4906.

Asada, H., Miyase, T., & Fukushima, S. (1984a). *Chemical and Pharmaceutical Bulletin, 32,* 3036.

Asada, H., Miyase, T., & Fukushima, S. (1984b). *Chemical and Pharmaceutical Bulletin, 32,* 3403.

Asada, H., Miyase, T., & Fukushima, S. (1984c). *Chemical and Pharmaceutical Bulletin, 32,* 1724.

Isman, M. B., & Rodriguez, E. (1983). *Phytochemistry, 22,* 2709.

Kitagowa, L. (1939). *Flora mashuria* (p. 454). Tokyo: Academic Press.

Nishimura, K., Miyase, T., Ueno, A., Noro, T., Kuroyanagi, M., & Fukushima, S. (1985). *Chemical and Pharmaceutical Bulletin, 33,* 3361.

Nishimura, K., Miyase, T., Uneo, A., Noro, T., Kuroyanagi, M., & Fukushima, S. (1986). *Phytochemistry, 25,* 2375.

Okunade, A. L., & Wiemer, D. F. (1985). *Phytochemistry, 24,* 1199.

Seto, M., Miyase, T., & Fukushima, S. (1986). *Chemical and Pharmaceutical Bulletin, 34,* 4170.

Seto, M., Miyase, T., Umehara, K., Ueno, A., Hirano, Y., & Otani, N. (1988). *Chemical and Pharmaceutical Bulletin, 36,* 2423.

Srivastava, R. P., Proksch, P., & Wray, V. (1990). *Phytochemistry, 29,* 3445.

Wang, C. D., Ding, K., Guo, W. B., & Wu, Y. H. (1980). *Shan-hsi Hsin I Yao, 9,* 49.

Warashina, T., Ishino, M., Miyase, T., & Ueno, A. (1990). *Phytochemistry, 29,* 3217.

Yang, Y. H. (1981). *Yao xue tong bao, 16,* 17.