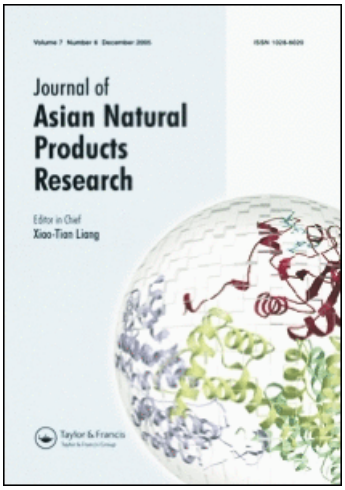

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

A new sesquiterpene lactone glucoside with inhibitory effect on K_{562} cells from *Ixeris sonchifolia* (Bge) Hance

W. -F. He[a]; B. -B. Xu[b]; J. -C. Pan[a]; J. -C. Lu[b]; S. -J. Song[b]; S. -X. Xu[b]
[a] School of Pharmacy, Wenzhou Medical College, Wenzhou, China [b] School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, China

To cite this Article He, W. -F. , Xu, B. -B. , Pan, J. -C. , Lu, J. -C. , Song, S. -J. and Xu, S. -X.(2006) 'A new sesquiterpene lactone glucoside with inhibitory effect on K_{562} cells from *Ixeris sonchifolia* (Bge) Hance', Journal of Asian Natural Products Research, 8: 6, 481 — 484
To link to this Article: DOI: 10.1080/10286020500532140
URL: http://dx.doi.org/10.1080/10286020500532140

Journal of Asian Natural Products Research, Vol. 8, No. 6, September 2006, 481–484

A new sesquiterpene lactone glucoside with inhibitory effect on K_{562} cells from *Ixeris sonchifolia* (Bge) Hance

W.-F. HE†, B.-B. XU‡, J.-C. PAN†, J.-C. LU‡, S.-J. SONG‡ and S.-X. XU‡*

†School of Pharmacy, Wenzhou Medical College, Wenzhou 325035, China
‡School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China

(Received 8 October 2004; revised 5 April 2005; in final form 8 December 2005)

A new minor sesquiterpene lactone glucoside, ixerin Z_A (**1**), together with 16 known compounds, were isolated from the whole plants of *Ixeris sonchifolia* (Bge) Hance. The structure of **1** was elucidated as 1(10),3,11(13)-guaiatriene-12,6-olide-2-one-3-*O*-[6′-(*p*-metheoxyphenylacetyl)]-β-glucopyranoside on the basis of spectroscopic and chemical evidence. Compound **1** exhibited an inhibitory effect on K_{562} cells.

Keywords: *Ixeris sonchifolia*; Compositae; Ixerin Z_A; K_{562} cells

1. Introduction

Ixeris sonchifolia (Bge) Hance, belonging to the Compositae family, is widely distributed in the north east of China. The whole plant has been used by the local population for many years to invigorate circulation and relieve pain [1,2]. Sesquiterpene lactone was reported from other species of this genus. In our previously study, ixerin Z_1 (**2**) was isolated from the titled plant [3]. This paper describes the isolation and structure determination of a new sesquiterpene lactone glucoside, ixerin Z_A (**1**), along with the bioactivities of **1** and **2**.

2. Results and discussion

Compound **1** was obtained as white needles. The molecular formula $C_{30}H_{34}O_{11}$ was deduced from HRFAB-MS at *m*/*z* 593.1843 $[M + Na]^+$. The IR spectrum showed absorption bands due to hydroxyl moiety (3400 cm^{-1}), two different type of carbonyl groups (1770, 1672 cm^{-1}), double bond (1640 cm^{-1}) and aromatic ring (1615, 1516 cm^{-1}).

*Corresponding author. Email: suixuxu@yahoo.com.cn

Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2006 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020500532140

Table 1. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz) data of **1** (pyridine-d_6).

Position	$\delta_H{}^{*,\dagger}$	$\delta_C{}^{\dagger,\ddagger}$	*Position*	$\delta_H{}^{*,\dagger}$	$\delta_C{}^{\dagger,\ddagger}$
1		152.8	1′	6.13 d (7.5)	101.6
2		188.6	2′	4.22 dd (7.5, 8.0)	74.8
3		153.4	3′	4.28 dd (8.0, 8.0)	77.8
4		146.8	4′	4.12 dd (8.0, 8.0)	70.8
5	3.28 d (10.3)	47.8	5′	4.10 m	75.2
6	3.25 t (10.3, 12.3)	84.6	6′	4.98 brd (11.4) 4.72 dd (11.4, 6.0)	64.2
7	2.75 brt (12.3)	51.9	1″		171.8
8	1.86 brd (11.9) 1.07 dd (12.3, 11.9)	23.8	2″	3.68 s	39.8
9	2.28 overlapped 2.07 m	36.6	3″		124.2
10		129.2	4″	7.40 d (8.5)	130.8
11		139.2	5″	7.05 d (8.5)	115.2
12		168.8	6″		158.6
13		117.0	7″	7.05 d (8.5)	115.2
14	2.46 brs	21.4	8″	7.40 d (8.5)	130.8
15	2.30 brs	14.6	9″	3.60 s	55.60

* J in Hz.
† Signals were assigned by HMQC, HMBC experiment, δ in ppm.
‡ Multiplicity determined by DEPT.

On acid hydrolysis, compound **1** released glucose by comparison with authentic samples on TLC and PC. The ^{1}H NMR spectrum (table 1) displayed the characteristic signals of an α-methylene-γ-lactone moiety located at δ 6.13 (1H, d, $J = 3.0$ Hz, H-13a) and 5.32 (1H, d, $J = 3.0$ Hz, H-13b). Signals for two vinyl methyls at δ 2.30 (3H, brs, H-15), 2.46 (3H, brs, H-14), a methoxyl at δ 4.30 (3H, s, H-9) and an anomeric proton of a sugar moiety at δ 6.13 (1H, d, $J = 7.5$ Hz) were also observed. A double doublet at δ 3.25 (1H, $J = 10.3$, 12.3 Hz) was assigned for H-6, which was coupled with the signal at δ 3.30 (1H, d, $J = 10.3$ Hz, H-5) and 2.75 (1H, brt, $J = 12.3$ Hz, H-7). The coupling constants indicated a *trans*-diaxial relationship of the vicinal protons. Because H-7 in naturally occurring guaianolides was α-oriented [4], the configuration of H-5 and H-6 should be α and β, respectively. The signals at δ 7.40 (2H, d, $J = 8.5$ Hz) and 7.05 (2H, d, $J = 8.5$ Hz) showed the presence of a 1,4-disubstituted aromatic ring. The ^{13}C NMR spectrum (table 1) showed the presence of 30 carbons, among which three were attributed to carbonyl moieties, six were olefinic carbons, another six were aromatic carbons and 15 were alkyl carbons. The characteristics of these signals were similar to those of ixerin Z_1.

In the HMBC spectrum of **1** (figure 1), cross peaks between H-6′a and H-6′b with C-1 confirmed that the *p*-methoxyl phenylacetic acid unit was linked at C-6′ of the glucose moiety (figure 1). Accordingly, the glucose was proved to be connected on position 3 of the aglycone by the long-range correlation from H-1′ to C-3. The large coupling constant of H-1′ ($J = 7.5$ Hz) suggested a β-glucopyranosyl moiety [5].

On the above evidence, compound **1** was elucidated as 1(10),3,11(13)-guaiatriene-12,6-olide-2-one-3-*O*-[6′-(*p*-metheoxyphenylacetyl)]-β-glucopyranoside.

Sesquiterpene lactone glucoside was proved to be cytotoxic [6]. Bioassays of **1** and **2** were performed. The results revealed that both of these compounds exhibited an inhibitory effect on the K_{562} cell line (table 2). The activity of compound **1** is more remarkable than that of **2**. It seemed the moiety linked on the glucose unit could be related to the activity. This was only a primary study. Further tests on analogues are needed before drawing conclusions.

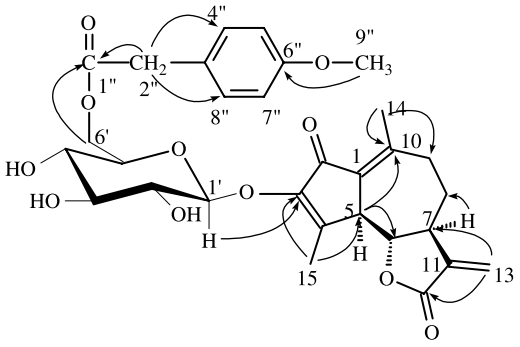

Figure 1. Structure and key HMBC correlations of compound **1**.

Table 2. Inhibition of compounds **1** and **2** on the growth of K_{562} cell lines.

Sample	*mmol/L*	*OD ($x \pm s$)*	*Inhibitory rate (%)*	*p*
Contro1	Saline	3.092 ± 0.18		
1	0.058	1.258 ± 0.41	56.6	< 0.01
	0.116	0.660 ± 0.09	76.5	< 0.01
	0.232	0.352 ± 0.18	86.4	< 0.01
2	0.041	2.288 ± 0.08	28	< 0.05
	0.082	1.770 ± 0.28	40	< 0.01
	0.164	1.256 ± 0.14	57.2	< 0.01

3. Experimental

3.1 General experimental procedures

Melting points were measured on an XT4-100X micromelting point apparatus and are uncorrected. IR spectra were recorded on a Bruker IFS 55 spectrometer with KBr disks. UV spectra were obtained on a Hitachi UV 2201 spectrophotometer. NMR experiments were performed on a Bruker AM 300 FT NMR with TMS as internal standard. Mass spectra were recorded on Finnigan LCQ LC/ESI-MS and VG-7070E.

3.2 Plant material

The whole plant of *Ixeris sonchifolia* (Bge) Hance was gathered from Liaoning, China, in June 2002 and was identified by Professor Jin-Cai Lu (Department of Chinese Traditional Medicines, Shengyang Pharmaceutical University). A voucher specimen is deposited in the Department of Chinese Traditional Medicines, Shengyang Pharmaceutical University.

3.3 Bioassays

K_{562} cells were maintained in PMRI 640 medium supplemented with 5% foetal bovine serum. The cultures were incubated at 37°C in a 5% CO_2 humidified incubator and subcultured every 2 days to maintain them in a logarithmic growth state. Test samples 1 and 2 were dissolved in ethanol. Addition of the samples to cell cultures was performed in such

a way that the final concentration of EtOH did not exceed 0.5% (v/v) (0.34%) K_{562} cells (1×10^5 cells/ml).

3.4 Extraction and isolation

The shade-dried whole plant (10 kg) was extracted with 75% hot EtOH three times. The combined alcohol extract were concentrated *in vacuo* to yield a residue (1850 g), which was separated successively with petroleum ether (60–90°C), $CHCl_3$ and MeOH. The MeOH extract (180 g) was subjected to silica gel (200–300 mesh, 1600 g) column chromatography using $CHCl_3$/MeOH (in gradient) as eluent to obtain 12 fractions. Compound **1** (12 mg) was obtained from the third fraction (8 g) after being repeated chromatographed on silica gel (160H) eluting with $CHCl_3$/EtOAc (8:2, v/v).

3.4.1 Ixerin Z_A(1). $C_{30}H_{34}O_{11}$, white needles, mp 264–266°C; IR (KBr) cm^{-1} 3400, 1770, 1672, 1640, 1615, 1516, 1380, 1253, 1070; HRFAB-MS *m/z*: 593.1843 $[M^+ + Na]$ (calcd for $C_{30}H_{34}O_{11}Na$, 593.1840); ESI-MS *m/z*: 571 $[M + 1]^+$; EI-MS *m/z*: 556, 260 $[M + 1 - (C_6H_{11}O_5 + C_9H_8O_2)]^+$(8), 242 (2), 189 (3), 149 (12), 121 (80), 107 (100); NMR data: see table 1.

3.5 Acid hydrolysis of 1

Compound **1** was refluxed with 2.0 mol/L HCl for 2 h. After cooling to room temperature, the reaction mixture was neutralized with $AgNO_3$ and centrifuged, and then the supernatant was evaporated on a water bath and subjected to TLC analysis on GF_{254} [using $CHCl_3$/MeOH/H_2O (6:4:1)] and paper chromatography [using n-BuOH/HOAc/H_2O (4:1:5)] by comparison with an authentic sample of glucose.

Acknowledgements

We are grateful for the use of HRFAB-MS facilities at the Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Japan.

References

[1] Jiangsu New Medical College. *Dictionary of Chinese Herbal Medicine*, p. 2248, Shanghai people's publishing house, Shanghai (1977).
[2] H. Asada, T. Miyase, S. Fukushima. *Chem. Pharm. Bull.*, **32**, 3036 (1984).
[3] X.Z. Feng, S.X. Xu, M. Dong. *J. Asian Nat. Prod. Res.*, **3**, 247 (2001).
[4] M. Seto, T. Miyase, K. Umehara, A. Ueno, Y. Hirano, N. Otani. *Chem. Pharm. Bull.*, **36**, 2423 (1988).
[5] S. Adagawa, T. Miyase, A. Ueno, T. Noro, M. Kuroyanagi. *Chem. Pharm. Bull.*, **33**, 4906 (1985).
[6] J.Y. Ma, Z.T. Wang, L.S. Xu, G.J. Xu. *Phytochemistry*, **50**, 113 (1999).