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Cytotoxic Constituents of the Leaves of *Ixeris sonchifolia*

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The ethyl acetate extract of the leaves of *Ixeris sonchifolia* afforded two new and two known sesquiterpene lactone glucosides of the guaiane-type, together with a known alkenol glucoside. The known compounds were identified as ixerin Z (**1**), ixerin Z-6′-*p*-hydroxyphenylacetate (**2**), and (*Z*)-3-hexen-1-ol-β-D-glucopyranoside (**3**), respectively. The structures of the new compounds were elucidated as 11,13a-dihydroixerin Z [**4**, 3-hydroxy-2-oxo-guaia-1(10), 3-dien-5α,6β,7α,11βH-12,6-olide-3-*O*-β-D-glucopyranoside], and 3,10β-dihydroxy-2-oxo-guaia-3,11(13)-dien-1α,5α,6α,7aH-12,6-olide-10-*O*-β-D-glucopyranoside (**5**), respectively. The cytotoxicity of these compounds against human hepatocellular carcinoma cell (HepG2) and human melanoma cell (SK-MEL-2) was evaluated.

Key words: *Ixeris sonchifolia*, Compositae, Sesquiterpene lactone glucoside, Cytotoxicity, MTT assay

INTRODUCTION

The whole herb of *Ixeris sonchifolia* Hance (Compositae) is either an important food source or a folk medicine for digestive, diuretic, and anti-inflammatory activities. Two sesquiterpene lactones, 8-desoxyartelin and 9a-hydroxyzaluzalin C (Ma *et al*, 1998a), and two sesquiterpene lactone glucosides, ixerin Z (Ma *et al*, 1998b) and ixerin Z1 (Feng *et al*, 2001) were reported from *I. sonchifolia*. It was reported that sesquiterpene lactones show diverse biological activities, such as cytotoxicity (Seto *et al*, 1988), as well as having ant-repellent and antifeedant properties (Isman *et al*,1983, Okunade *et al*, 1985 and Srivastava *et al*, 1990). In our study of the bioactive constituent of *I. sonchifolia*, two new sesquiterpene lactone glucosides were isolated. The structure elucidation and cytotoxicity of the compounds are described in this paper.

MATERIALS AND METHODS

Plant material

I. sonchifolia Hance (Compositae) was harvested from the cultivated field in Hadong, Kyungnam in November, 2000, and the voucher specimen is deposited in Natural Product Chemistry laboratory, Pusan National University.

Instruments

Melting points were determined on a Fisher-Johns melting point apparatus and were uncorrected. Optical rotations were determined with a Jasco DIP-370 polarimeter. CD spectra were recorded with a Jasco J-715 spectropolarimeter. IR spectra were taken on a Jasco FT/IR-410 infrared spectrophotometer. NMR spectra were recorded on a Varian Unity Inova AS 500 and Bruker AC 200. Chemical shifts were given on the δ (ppm) scale with tetramethylsilane as an internal standard. FABMS data were obtained using a JEOL JMS-HX110A spectrometer. HPLC was performed with an YMC ODS-H80 (semipreparative, 250 x 10 mm id., 4 μm) column using a Shodex RI-71 detector. Silica gel 60 (0.063-0.200 mm, Merck) was used for column chromatography. TLC was done on precoated silica gel plates (Merck, silica gel 60 F_{254}).

Extraction and isolation

Air-dried leaves of *I. sonchifolia* H. (800 g) was extracted twice with MeOH under reflux. The ext. was suspended in water, and extracted with ether (Et_2O), ethyl acetate (EtOAc), and 1-butanol (*n*-BuOH), successively.

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The EtOAc-soluble fraction (4 g) was chromatographed on a silica gel column with *n*-hexane-ethyl acetate (10:1 → 2:1), and chloroform-MeOH (15:1 → 100% MeOH) as eluents, to afford 22 fractions. Fr. 8 (0.6 g) was subjected to HPLC (H_2O- MeOH, 1 : 1) to afford compound 1 (6.0 mg, 1.02% of Fr. 8), 2 (2.0 mg, 0.34%), 3 (4.0 mg, 0.68%), and 4 (3.5 mg, 0.60%). Fraction 9 (12 mg) afforded compound 5 (1.0 mg, 8.33% of Fr. 9) on HPLC (H_2O-MeOH, 2 : 3) separation.

Compound 1 (ixerin Z): amorphous powder, IR ν_{max}^{KBr} (cm^{-1}): 3419 (OH), 1757, 1642 (γ-lactone carbonyl); ^1H NMR (500 MHz, MeOH-d_4): d 3.10 (1H, d, J = 10.5 Hz, H-5), 4.54 (1H, t, J = 9.7 Hz, H-6), 2.79 (1H, br t, J = 9.0 Hz, H-7), 1.48 (m, H-8a), 1.64 (1H, dt, J = 13.5, 3.2 Hz, H-8b), 2.04 (1H, dd, J = 10.2, 3.2 Hz, H-9a), 2.23 (m, H-9b), 5.55 (1H, d, J = 3.0 Hz, H-13a), 6.09 (1H, d, J = 3.0 Hz, H-13b), 2.43 (3H, s, H-14), 2.25 (3H, s, H-15), 5.23 (1H, d, J = 7.5 Hz, H-1'); ^{13}C NMR (125 MHz, MeOH-d_4) : δ 156.1 (C-1), 191.0 (C-2), 154.4 (C-3), 150.0 (C-4), 53.7 (C-5), 85.3 (C-6), 47.9 (C-7), 25.3 (C-8), 37.9 (C-9), 130.5 (C-10), 140.6 (C-11), 171.3 (C-12), 119.1 (C-13), 22.2 (C-14), 15.2 (C-15), 102.3 (C-1'), 75.3 (C-2'), 78.3 (C-3'), 71.2 (C-4'), 77.9 (C-5'), 62.4 (C-6').

Compound 2 (ixerin Z1, ixerin Z-6'-*p*-hydroxyphenylacetate): yellow oil, IR ν_{max}^{KBr} (cm^{-1}): 3410 (OH), 1770 (g-lactone), 1740 (ester), 1690 (enone), 1622, 1518; $[\alpha]_D^{23}$ -8.6° (*c* 0.087, MeOH); ^1H NMR (500 MHz, MeOH-d_4): δ 3.46 (1H, d, J = 9.0 Hz, H-5), 3.09 (1H, t, J = 9.0 Hz, H-6), 2.81 (1H, dd, J = 14.0, 5.0 Hz, H-7), 1.04 (1H, m, H-8a), 2.51 (1H, t, J = 12.5 Hz, H-8b), 2.10 (1H, dt, J = 13.0, 2.0 Hz, H-9a), 2.26 (1H, m, H-9b), 5.44 (1H, d, J = 3.0 Hz, H-13a), 6.04 (1H, d, J = 3.0 Hz, H-13b), 2.20 (3H, s, H-14), 2.41 (3H, s, H-15), 5.65 (1H, d, J = 8.0 Hz, H-1'), 6.95 (2H, d, J = 9.0 Hz, H-2", H-6"), 6.61 (2H, d, J = 9.0 Hz, H-3", H-5"); ^{13}C NMR (125 MHz, MeOH-d_4): d 155.5 (C-1), 190.4 (C-2), 153.5 (C-3), 149.3 (C-4), 54.9 (C-5), 86.6 (C-6), 47.9 (C-7), 25.0 (C-8), 37.5 (C-9), 130.5 (C-10), 140.4 (C-11), 171.2 (C-12), 118.9 (C-13), 22.3 (C-14), 14.9 (C-15), 100.7 (C-1'), 75.5 (C-2'), 78.6 (C-3'), 71.9 (C-4'), 77.8 (C-5'), 65.3 (C-6'), 175.0 (C-a), 40.8 (C-b), 128.9 (C-1"), 131.9 (C-2"), 116.2 (C-3"), 157.3 (C-4"), 116.2 (C-5"), 131.9 (C-6").

Compound 3 (Z-3-hexen-1-ol-b-D-glucopyranoside): colorless oil, ^1H NMR (500 MHz, MeOH-d_4,): δ 4.25 (1H, d, J = 7.0 Hz, H-1), 2.37 (2H, q, J = 7.0 Hz, H-2), 5.38 (1H, dt, J = 10, 7.0 Hz, H-3), 5.43 (1H, dt, J = 10, 7.0 Hz, H-4), 2.07 (2H, quintet, J = 7.0 Hz, H-5), 0.96 (3H, t, J = 7.5 Hz, H-6); ^{13}C NMR (50 MHz, MeOH-d_4): d 70.5 (C-1), 28.8 (C-2), 134.5 (C-3), 125.9 (C-4), 21.5 (C-5), 14.6 (C-6), 104.4 (C-1'), 75.1 (C-2'), 78.1 (C-3'), 71.7 (C-4'), 77.9 (C-5'), 62.8 (C-6').

Table 1. ^1H NMR Data of Compounds 4 and 5 (500 MHz in MeOH-d_4)

Position	4	5
1		2.13 (d, 7.0)
5	3.47 (d, 9.5)	3.11 (m)
6	3.67 (t, 10.0)	4.49 (dd, 6.5, 3.0)
7	2.09 (qd, 9.5, 2.5)	2.84 (td, 6.5, 3.0)
8	2.02 (dddd, 9.5, 3.0)	1.89 (m)
	1.38 (q, large coupling)	1.73 (quintet, 7.0)
9	2.56 (t, 12.2)	2.57 (t, 7.3)
	2.40 (m)	
11	2.38 (m)	
13	1.23 (d, 7.0)	6.27 (d, 2.0)
		5.78 (d, 2.0)
14	2.45 (s)	2.14 (s)
15	2.24 (s)	2.11 (s)
1'	5.23 (d, 8.0)	5.31 (d, 8.0)

Multiplicity, J in Hz

Table 2. ^{13}C NMR and HMBC Data of Compounds 4 and 5

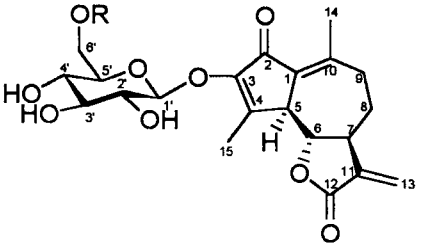
position	4		5	
	δ (ppm)[a]	HMBC[c]	δ (ppm)[b]	HMBC[c]
1	130.5	H-5, 9, 14	29.9	H-6, 9
2	191.3	H-14, 15	212.7	H-1, 9
3	154.5	H-5, 15, 1'	157.0	H-1, 5, 14
4	150.9	H-5, 15	152.7	H-1, 14
5	49.1	H-15	44.1	H-1, 14
6	86.9	H-5, 7, 14, 15	85.1	H-5, 15
7	57.1	H-5, 8, 9, 11, 13	42.9	H-9, 13
8	26.9	H-9, 11	29.5	H-6, 9
9	37.4	H-7, 8, 14	41.3	H-8, 14
10	156.5	H-7, 8, 9, 11, 14	82.1	H-1'
11	42.2	H-13	139.8	H-7, 13
12	180.3	H-11, 13	171.4	H-13
13	12.6	H-8, 14	124.7	H-7
14	22.2	H-9, 11	26.0	H-1, 9
15	15.4	H-7, 11	14.7	H-7
1'	102.6		101.5	
2'	75.2		75.3	
3'	78.4		78.3	
4'	71.4		71.3	
5'	78.0		77.9	
6'	62.6		62.5	

[a] Measured at 50 MHz in MeOH-d_4, [b] Measured at 125 MHz in MeOH-d_4, [c] Measured at 125 MHz in MeOH-d_4

Table 3. Cytotoxicity (IC_{50} values, μg/ml) of Compounds **1-5**

Cell line[a]	1	2	3	4	5
HepG2	8.0	7.5	10.5	8.5	6.4
SK-MEL-2	15.2	14.0	20.6	14.8	15.7

[a] HepG2, hepatocellular carcinoma cells; SK-MEL-2, human melanoma cells



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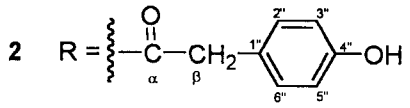
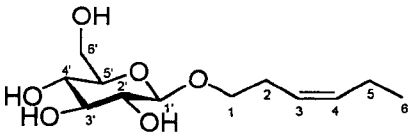
2 R = 



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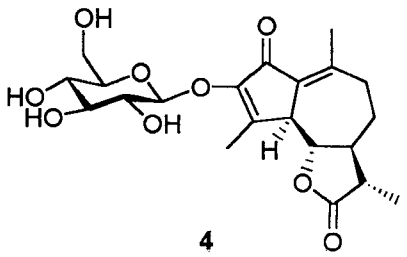

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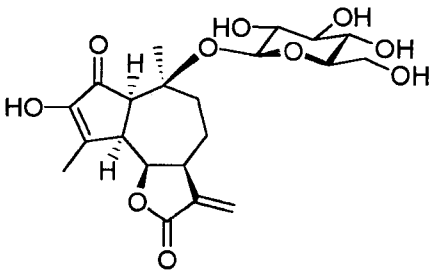

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Compound 4 (11,13α-dihydroixerin Z): colorless crystal, mp 168-169°C; $[\alpha]_D^{23}$ -28.3° (*c* 0.12, MeOH); IR ν_{max}^{KBr} (cm^{-1}) : 3415 (OH), 1757, 1646 (γ-lactone carbonyl); LRFABMS *m/z* 447 [M + Na]$^+$ (calcd for $C_{21}H_{28}O_9$); ^{1}H NMR: see Table 1 ; ^{13}C NMR: see Table 2.

Compound 5 : colorless oil, $[\alpha]_D^{23}$ + 10.00 (*c* 0.04, MeOH); IR ν_{max}^{KBr} (cm^{-1}): 3420 (OH), 1750, 1650 (γ-lactone carbonyl); LRFABMS *m/z* 463 [M + Na]$^+$ (calcd for $C_{21}H_{28}O_{10}$); CD (MeOH) $[\theta]_{250}$ + 3500; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2

Bioassay

Cytotoxicity against human hepatocellular carcinoma cells (HepG2) and human melanoma cells (SK-MEL-2) were measured by MTT method (Mosmann, 1983).

RESULTS AND DISCUSSION

Compound **1**, the major constituent of the extract, was identified as ixerin Z (Ma *et al*, 1998c), and compound **2** as ixerin Z1 (Feng *et al*, 2001), respectively, which were previously isolated from the whole herb of the same plant. Compound **3** was identified as (*Z*)-3-hexen-1-ol-β-D-glucopyranoside (Mizutani *et al*, 1988), previously isolated from the Chinese traditional crude drug *Codonopsis pilosula*, by comparing ^{1}H and ^{13}C NMR data with those reported .

Compound **4** is a β-D-glucopyranoside of 8-deoxyartelin (Ma *et al*, 1998d). The IR spectrum showed absorption bands due to hydroxyl (3415 cm^{-1}) and γ-lactone (1757, 1646 cm^{-1}) groups. The quasimolecular ion peak was observed at *m/z* 447 [M + Na]$^+$, suggesting that **4** is a dihydro derivative of **1**. This was confirmed by the doublet methyl signal at δ 1.23 (3H, d, J = 7.0 Hz) which replaced the exomethylene proton signals [δ 5.55 (1H, d, J = 3.0 Hz, H-13a) and δ 6.09 (1H, d, J = 3.0 Hz, H-13b)] of **1**. Of the three methyl groups, two could be assigned to vinyl methyls [δ 2.45 (3H, s, H-14), δ 2.24 (3H, s, H-15)], and the third one could be assigned to α-methyl group [δ 1.23 (3H, d, J = 7.0 Hz, H-13)] of the γ-lactone ring. The resonance at d 3.67 (1H, t, J = 9.5 Hz) was assigned to H-6, which was coupled to both H-5 [δ 3.47 (1H, d, J = 9.5 Hz)] and H-7 [δ 2.09 (1H, dd, J = 9.5, 2.5 Hz)]. Coupling constant (J = 9.5 Hz) established the trans-diaxial relationship of these three protons (Ma *et al*, 1998e). It suggests that the configurations of these protons are 5α, and 6β, respectively, since the configuration of H-7 of natural guaianolides is always α (Nishimura *et al*, 1986).

Of the twenty-one carbon signals in the ^{13}C NMR spectrum, six were assigned to carbons of the glucose moiety including an anomeric carbon signal at d 102.6, and the two downfield carbon signals were assigned to the γ-lactone carbonyl carbon (δ 180.3) and the carbonyl carbon of the a, b-unsaturated ketone (δ 191.3). The others were consisted of four vinyl carbons, two methylene carbons, three methyl carbons, and four methine carbons. Careful examination of the 2D NMR data (^{1}H-^{1}H-COSY, HMQC, and HMBC) allowed the assignment of all signals. The stereochemistry of C-11 was determined by NOE experiment, NOE was observed

between H-11 and H-6. Accordingly, **4** was defined as 8-desoxyartelin-b-D-glucopyranoside [3-hydroxy-2-oxo-guaia -1(10),3-dien-5a,6b,7a,11bH-12,6-olide-3-*O*-b-D-glucopyranoside].

Compound **5** was obtained as colorless oil. Compound **5** was assigned the molecular formula $C_{21}H_{28}O_{10}$ based on NMR and FABMS data. The molecular formula suggested that **5** possess an additional hydroxyl group compared to **1**. The ^1H NMR spectrum exhibited a-methylene-g-lactone signals at δ 6.27 (1H, d, J = 2.0 Hz, H-13a) and δ 5.78 (1H, d, J = 2.0 Hz, H-13b) as in **1**. In contrast to compound **1**, one (d 2.45) of the two vinyl methyl signals was replaced by a methyl signal at an oxygen-bearing carbon [δ 2.14 (3H, s)], and an additional proton signal [δ 2.13 (1H, d, J = 7.0 Hz, H-1) appeared, indicating that **5** is a 1,10 - dihydro-10-oxy derivative of **1**. This was confirmed by comparision of the NMR data with those of a closely resembled guaianolide, diosphenol (Herz *et al*, 1980). An anomeric proton signal [δ 5.31 (1H, d, J = 8.0 Hz)] and an anomeric carbon signal (δ 101.5) as well as additional signals for a glucose moiety (Table 2) indicated that **5** is a glucoside, of which glucose has a b-configuration. Compared to that of ixerin V (Seto *et al*, 1986), the chemical shift of C-10 was apparently shifted to downfield (ca. 6 ppm), indicating that glucose was attached to C-10 hydroxyl group. This was further confirmed by HMBC experiment. Correlation between the signal at δ 82.1 (C-10) and signal at δ 5.31 (anomeric proton) was observed. The CD spectrum of compound **5** showed positive Cotton effect at 250 nm (nÆp*), suggesting that **5** has 6α, 7α cis-fused g-lactone (Stöcklin *et al.*, 1970). The a-configurations of H-1, 5, 6, and 7 were confirmed by NOESY experiment, in which correlation peaks between these protons were observed. Thus, the structure of compound **5** could be defined as 3,10-dihydroxy-2-oxo-guaia-3,11(13)-dien-1α,5α,6α,7αH-12,6-olide-10-*O*-β-D-glucopyranoside and given the trivial name ixeriside A.

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REFERENCES

Feng, X.-Z., Xu, S.-X., and Dong, M., A New sesquiterpene lactone glucoside from *Ixeris sonchifolia*. *J. Asian Nat. Prod. Res.*, 3, 247-251 (2001).

Herz, W. and Govindan, S. V., Glycosidic Disecoeudesmanolides and Other Secosesquiterpene Lactones from *Picradeniopsis* Species. X-ray Analysis of Bahial, *J. Org. Chem.*, 45, 3163-3172 (1980).

Isman, M. B., and Rodriguez, E., Larval Growth Inhibitors from Species of *Parthenium*. *Phytochemistry*, 22, 2709-2713 (1983).

Ma, J.-Y., Wang, Z.-T., Xu, L.-S., Xu, G.-J, Kadota, S., and Namba, T., Sesquiterpene Lactones From *Ixeris sonchifolia*. *Phytochemistry*, 48, 201-203 (1998).

Mizutani, K., Yuda, M., Tanaka, O., Saruwatari, Y.-I., Fuwa, T., Jia, M.-R., Ling, Y-K., and Pu, X.-F., Chemical Studies on Chinese Traditional Medicine, Dangshen. I. Isolation of (*Z*)-3- and (*E*)-2-Hexenyl-β-D-Glucosides. *Chem. Pharm. Bull.*, 36, 2689-2690 (1988).

Mosmann, T., Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *J. Immunol. Methods*, 65, 55-66 (1983).

Nishimura, K., Miyase, T., Ueno, A., Noro, T., Kuroyanagi, M., and Fukushima, S., Sesquiterpene Lactones From *Lactuca laciniata*. *Phytochemistry*, 25, 2375-2379 (1986).

Okunade, A. L., and Wiemer, D. F., Ant-Repellent Sesquiterpene Lactones from *Eupatorium Quadrangularae*. *Phytochemistry*, 24, 1199-1201 (1985).

Seto, M., Miyase, T., and Fukushima, S., Sesquiterpene Lactones from Ixeris dentata NAKAI. *Chem. Pharm. Bull.*, 34, 4170-4176 (1986)

Seto, M., Miyase, T., Umehara, K., Ueno, A., Hirano, Y., and Otani, N., Sesquiterpene Lactones from *Cichorium endivia* L. and *C. intybus* L., and Cytotoxic Activity. *Chem. Pharm. Bull.*, 36, 2423-2429 (1988).

Srivastava, R., Proksch, P., and Wray, V., Toxicity and Antifeedant Activity of a Sesquiterpene Lactone from *Encelia* against *Spodoptera Littoralis*. *Phytochemistry*, 29, 3445-3448 (1990).

Stöcklin, W., Waddell, T. G., and Geissman, T. A., Circular Dichroism and Optical Rotatory Dispersion of Sesquiterpene Lactones. *Tetrahedron*, 26, 2397-2410 (1970).