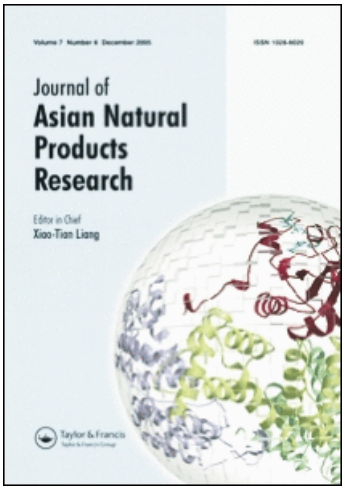

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Sesquiterpene lactones from *Ixeris sonchifolia* Hance and their cytotoxicities on A549 human non-small cell lung cancer cells

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Sesquiterpene lactones from *Ixeris sonchifolia* Hance and their cytotoxicities on A549 human non-small cell lung cancer cells

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A new sesquiterpene lactone glucoside, 11,13-dihydroixerinoside (**1**), together with the five known sesquiterpene lactones, ixerinoside (**2**), ixerin Z (**3**), 11,13α-dihydroixerin Z (**4**), ixerin Z1 (**5**), and 3-hydroxydehydroleucodin (**6**), respectively, were isolated from the whole plants of *Ixeris sonchifolia* Hance. The compounds were identified by spectral analysis and comparison with spectroscopic data reported in the literatures. When the *in vitro* cytotoxic activities of compounds **1**–**6** were evaluated against A549 human non-small cell lung cancer cells, all six compounds exhibited cytotoxic activity against A549 cells, with compounds **2**, **3**, and **6** showing good activities (inhibitory concentration (IC_{50} values $< 30\ \mu g/ml$) that are comparable with well-established chemotherapeutic drug, 5-fluorouracil.

Keywords: *Ixeris sonchifolia* Hance; sesquiterpene lactones; cytotoxicity; A549; 11,13-dihydroixerinoside

1. Introduction

Ixeris sonchifolia Hance, which belongs to the Compositae family, is a small and bitter perennial herb widely distributed in the north-eastern part of China [1–3]. *I. sonchifolia* Hance is a well-known folk medicine and its whole plant has been used for many years in China to invigorate blood circulation, normalize menstruation, and eliminate blood stasis to relieve pain [1–3]. Modern pharmacological studies on *I. sonchifolia* Hance have recently demonstrated biological activity against various human cancer cell lines [4,5].

Sesquiterpene lactones are the active constituents of a variety of medicinal plants used in traditional Chinese medicine [2,6,7]. Extensive research has been carried out to characterize the anti cancer activity, molecular mechanisms, and potential chemopreventive and chemotherapeutic application of sesquiterpene lactones [7,8]. Studies on the chemical constituents of *I. sonchifolia* Hance have revealed several sesquiterpene lactone glucosides, including ixerins H, Z, Z1, macrocliniside A, and 11,13-dihydroixerin [1,3,9,10]. In this work, we carried out extraction experiment on *I. sonchifolia* in order to isolate new sesquiterpene constituents with potential anti cancer activity. We report here the structural elucidation of a new sesquiterpene lactone glucoside and five known sesquiterpene lactones from *I. sonchifolia*, and their cytotoxicities against A549 non-small cell lung cancer (NSCLC) cells.

2. Results and discussion

A new sesquiterpene lactone glucoside, 11,13-dihydroixerinoside (**1**), together with the five known sesquiterpene lactones, ixerinoside (**2**), ixerin Z (**3**), 11,13α-dihydroixerin Z (**4**), ixerin Z1 (**5**), and 3-hydroxydehydroleucodin (**6**), respectively, were

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isolated from the whole plants of *I. sonchifolia* Hance. The structures of these compounds (Figure 1) were identified by spectral analysis and comparison with those reported in the literature [1–3,11,12].

Compound **1** was obtained as a white powder and had a positive Molish reaction. The molecular formula for compound **1** was deduced as $C_{21}H_{30}O_9$ on the basis of its ESI-TOF-MS ($[M - H]^-$, *m/z* 425.1812) in tandem with the ^{13}C and ^1H NMR spectra. The IR spectrum of compound **1** showed absorption bands of a hydroxyl moiety (3400 cm^{-1}), a γ-lactone (1760 cm^{-1}), and an α,β-unsaturated five-member ring carbonyl (1678 and 1640 cm^{-1}). On acid hydrolysis, compound **1** gave glucose that was identified by comparison with authentic glucose samples on co-TLC and by ^{13}C NMR signals [δ 103.9 (C-1′), 75.1 (C-2′), 79.7 (C-3′), 71.6 (C-4′), 79.0 (C-5′), and 63.1 (C-6′)]. The ^{13}C NMR spectrum displayed signals of an α,β-unsaturated ketone at δ 210.8 (C-2), 132.3 (C-3), and 179.9 (C-4). Furthermore, the ^{13}C NMR spectrum reveals a lactone carbonyl at δ 178.3 (C-12), and two carbons bearing oxygen at δ 81.6 (C-6) and 79.7 (C-9). The ^1H NMR spectrum displayed the characteristic signals of three methyl groups at δ 0.67 (3H, d, $J = 6.0$ Hz, H-14), 1.22 (3H, d, $J = 6.0$ Hz, H-13), and 2.11 (3H, s, H-15), of which the latter one is clearly a vinyl methyl group. The spectrum also reveals an anomeric proton of a sugar moiety at δ 4.96 (1H, d, $J = 6.0$ Hz, H-1′), whose coupling constant affirmed a β-glycosidic linkage. The linkage position of the sugar moiety to the aglycone was determined and confirmed by the HMBC experiment (Figure 2). The ^1H NMR spectral data of **1** and **2** showed close similarities, which suggested that the configurations at the C-5, C-6, C-7, and C-9 positions are the same for the two compounds. However, there were also a few significant differences between the two spectra. The ^1H NMR spectrum of compound **1** revealed a methyl proton ($CH_3$13) at δ 1.22 (3H, d, $J = 6.0$ Hz, H-13), which was missing in compound **2**. By contrast, the methylene proton of a terminal olefinic double bond at δ 5.38 and 6.22 (each 1H, d, $J = 3.0$ Hz) in the spectrum of compound **2** was clearly missing in the spectrum of compound **1**. In addition, the ^{13}C NMR signal due to C-13 showed a significant upfield shift from δ 119.3 (compound **2**) to 12.8 (compound **1**). These shifts could be explained by the hydrogenation at C-13 of the α-methylene-γ-lactone unit in compound **2**. The stereochemistry of the aglycone part was determined by NOESY experiments, and the results are shown in Figure 2. Based on these spectral analyses, the structure of **1** was elucidated to be 11,13-dihydroxerinoside.

The cytotoxic activity of compounds **1–6** on A549 cells was examined as a preliminary measure of their potentials to inhibit cancer cell

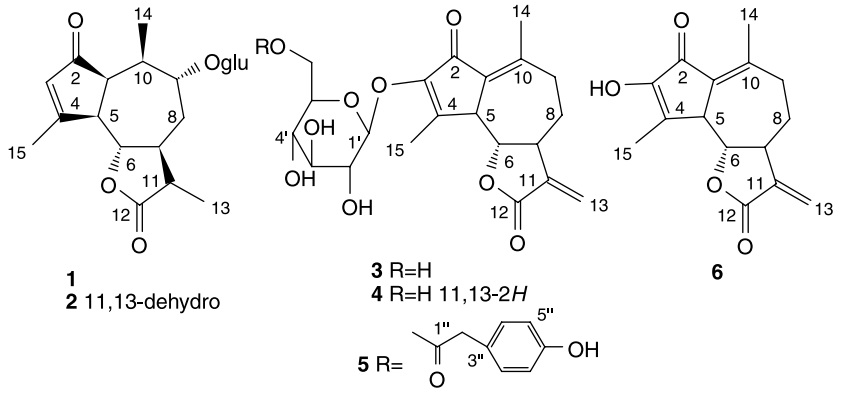

Figure 1. Chemical structures of compounds **1–6**.

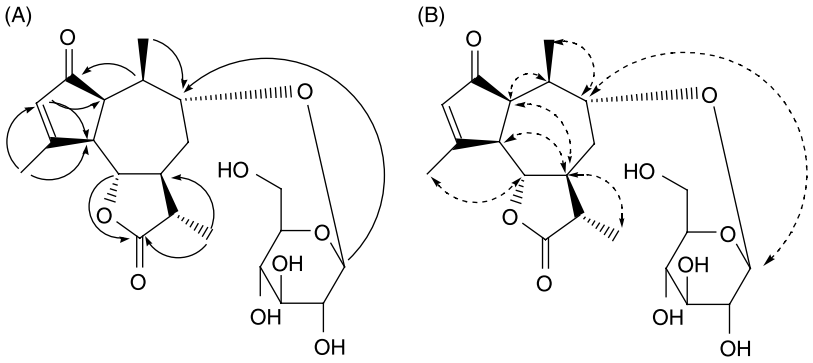

Figure 2. (A) HMBC and (B) NOESY correlations of **1**.

growth. Our results indicated that all compounds showed cytotoxic effects towards A549 cells. When compared with positive control 5-fluorouracil (IC_{50}, 28.6 μg/ml), compounds **1**, **4**, and **5** showed relatively weak cytotoxicities ($IC_{50} > 60$ μg/ml). By contrast, good cytotoxic activities were observed for compounds **2**, **3**, and **6** (IC_{50} values = 29, 25, and 15 μg/ml, respectively). The present results suggest that this class of compounds may be developed as potential agents to treat and/or prevent lung and other cancers.

3. Experimental

3.1 General experimental procedures

Melting points were determined on apparatus of Gallenkamp product (Cat. No. MPD035.BM2.5) and are uncorrected. The IR spectra were recorded on Perkin-Elmer 983G spectrometer. All NMR spectra were obtained on a Bruker ACF300 spectrometer (Karlsruhe, Germany) operating at 300 MHz for proton and 75 MHz for carbon. Mass spectra and ESI-TOF-MS were obtained on QTrap® 2000 (Applied Biosystems, Inc., Foster City, CA, USA) ESI mass spectrometer and a waters Q-TOF premiers™ mass spectrometer, respectively. Data were acquired in continuum mode until acceptable averaged data were obtained. Chromolith® SemiPrep 100-10 mm HPLC column (No. 1.512016.001) was purchased from Merck KGaA (Darmstadt, Germany). All chemicals were purchased from Tedia Company, Inc. (Fairfield, OH, USA) unless otherwise noted. The deuterated solvents for NMR measurement (pyridine-d_5 and dimethyl sulfoxide-d_6) were purchased from Sigma-Aldrich, Inc. (St Louis, MO, USA). The sorbents for chromatographic isolation, such as silica gel 60 (40–63 μm) and precoated silica gel 60 TLC plates, were purchased from Merck KGaA. Sephadex LH-20 was from Pharmacia (Uppsala, Sweden). Ham's F12K dry powder medium was purchased from Sigma-Aldrich. To make the complete growth medium, the following components were added and adjusted to the final concentration: 4.5 g/l D-glucose, 1.5 g/l sodium bicarbonate, 2.38 g/l HEPES, 1 mM sodium pyruvate, 10% fetal bovine serum (Hyclone Laboratories, Logan, UT, USA), and antibiotics (100 μg/ml streptomycin and 100 U/ml penicillin G). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl (MTT) and 5-fluorouracil were obtained from Sigma-Aldrich at AR grade.

3.2 Plant material

The whole plants of *I. sonchifolia* Hance were purchased from Beijing DDLD Medicinal Technology Development Co. Ltd (Beijing, China) in September 2006. The plants were examined and confirmed to be *I. sonchifolia* Hance by Dr Zhou Liang who has multiple years of experiences in natural

product research. The voucher specimen (No. 060902) has been deposited in Department of Pharmacy National University of Singapore, Singapore 117543, Singapore.

3.3 Extraction and isolation

The whole plants of *I. sonchifolia* (5 kg) were cut into small pieces (3 cm) and extracted thrice with 95% C_2H_5OH at 60°C for 3 h. The extracts were evaporated to dryness under reduced pressure to yield a dried residue. The dried crude extract (420 g) was dissolved in 800 ml of water and subjected to liquid–liquid extraction with equal volume of *n*-hexane, chloroform, and ethyl acetate successively to yield four fractions (fractions 1–4). The ethyl acetate fraction (fraction 3, 50 g) was then separated by a Sephadex LH-20 column with $CHCl_3$–MeOH (1:1) system to give eight fractions (fractions 3.1–3.8). Fraction 3.2 was separated by silica gel column chromatography with solvents of increasing polarity ($CHCl_3$/MeOH, 95:5 → 85:15 → 80:20, with methanol added at 1% gradient) to give four fractions (fractions 3.2.1–3.2.4). Fraction 3.2.3 was subjected to further separation with semi-preparative reversed phase HPLC using MeOH–H_2O [flow rate: 4 ml/min, MeOH: H_2O 12:88 (0 min) → 60:40 (25 min)] as eluting solvent to give compounds **5** (R_t = 10.6 min, 10 mg) and **3** (R_t = 14.0 min, 25 mg). Using the same procedure, fraction 3.4 was subjected to further separation to give compound **1** (R_t = 9.8 min, 5 mg). Similarly, fraction 3.5 gives compounds **2** (R_t = 12.8 min, 12 mg) and **4** (R_t = 14.2 min, 10 mg) and fraction 3.7 gives compound **6** (R_t = 15.1 min, 12 mg).

3.3.1 Compound **1** (11,13-dihydroixerinoside)

White amorphous powder; mp 187–189°C; UV (MeOH) λ_{max}: 226 nm; IR (KBr) ν_{max} (cm^{-1}): 3400 (OH), 1760, 1678, 1640; ^1H NMR (300 MHz, pyridine-d_5) δ 0.67 (3H, d, J = 6.0 Hz, H-14), 1.22 (3H, d, J = 6.0 Hz, H-13), 2.11 (3H, s, H-15), 2.41 (1H, m, H-10), 3.60 (1H, overlapped with H-6′, H-9), 4.96 (1H, d, J = 6.0 Hz, H-1′), 6.08 (1H, s, H-3); ^{13}C NMR (75 MHz, pyridine-d_5): see Table 1; negative ESI-MS: (m/z) 425 $[M-H]^-$; negative ESI-TOF-MS (m/z): 425.1805 $[M-H]^-$ (calcd for $C_{21}H_{29}O_9$, 425.1812).

3.3.2 Acid hydrolysis of compound **1**

Compound **1** was refluxed with 2 M HCl for 2 h. After cooling to room temperature, the reaction mixture was neutralized with $AgNO_3$ and centrifuged. The supernatant was evaporated to dryness on a water bath and subjected to TLC analysis on precoated silica gel 60 TLC plates [using *n*-BuOH–HOAc–H_2O (4:1:5)] using an authentic sample of glucose for comparison [3].

3.4 Cell culture

A549 human NSCLC cell line was purchased from the American Type Culture Collection (Rockville, MD, USA) and grown in complete growth Ham's F12K medium at 37°C in

Table 1. ^{13}C NMR spectral data for compounds **1** and **2** in C_5D_5N (75 MHz; δ in ppm).

Position	**1**	**2**
1	47.5	47.4
2	210.8	210.8
3	132.2	132.5
4	179.9	179.7
5	53.2	53.0
6	81.6	81.8
7	44.2	41.0
8	30.2	29.9
9	79.7	80.1
10	40.0	40.2
11	41.1	139.8
12	178.3	169.8
13	12.8	119.3
14	13.7	13.6
15	18.6	18.8
1′	103.9	104.6
2′	75.1	75.1
3′	79.7	79.1
4′	71.6	71.7
5′	79.0	78.4
6′	63.1	63.2

a humidified 5% CO_2 atmosphere. Cell growth was quantified using MTT. For cytotoxicity assay of the compounds, A549 cells were seeded in 96-well (5000 cells per well) tissue culture plates and cultured for 48 h. Cells were treated with media containing pure tested compounds or DMSO vehicle in triplicate for each concentration. After treatment, cell growth was analyzed by addition of MTT following the manufacturers' protocol. The absorbance of MTT was determined at 590 nm using a Tecan Spectra Fluor spectrophotometer (MTX Lab Systems, Inc., Vienna, VA, USA). Absorbance values were normalized to relevant media and vehicle controls [8,13]. The cytotoxicity of compounds **1**–**6** on A549 cells was expressed as half maximal IC_{50}, which represents the concentration of the compounds that is required for 50% inhibition of the cell viability.

Acknowledgements

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