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A new triterpenoidal saponin from *Ixeris sonchifolia* and its cytotoxic activity

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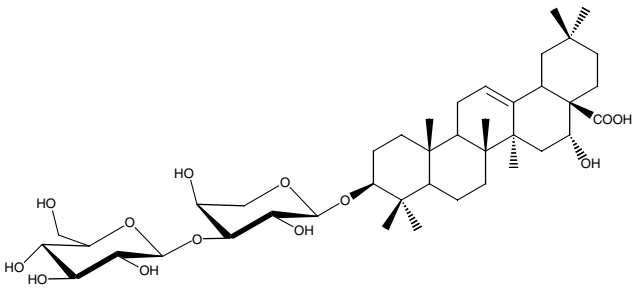
A new triterpenoidal saponin, echinocystic acid 3-O-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranoside (**1**), was isolated from *Ixeris sonchifolia*. Its structure was elucidated on the basis of full spectral data analysis and chemical methods. The cytotoxic activity of compound **1** was evaluated using the tumor cell lines of A375, Hela, and L929 *in vitro*.

1. Introduction

Ixeris sonchifolia (Bge.) Hance (Compositae) is abundantly distributed throughout northeastern China. It has been used as a folk medicine in China for invigorating the circulation of blood, normalizing menstruation and eliminating blood stasis to relieve pain [1]. Previous phytochemical studies on other species of this genus revealed the presence of sesquiterpene lactones [2–4]. When clarifying active compounds, further isolation resulted in a new saponin. In the present communication, we report the isolation and structural determination of this new saponin. Furthermore, we report tests for cytotoxic activity against the tumor cell lines of A375, Hela, and L929 *in vitro*.

2. Investigations, results and discussion

Compound **1** had a molecular formula of $C_{41}H_{66}O_{13}$ determined from its positive ion ESI-MS spectrum at m/z 789 $(M + Na)^+$ as well as ^{13}C-, DEPT NMR data. Its spectral features and physicochemical properties suggested **1** to be a triterpenoidal saponin. Among the 41 carbons in the ^{13}C NMR spectrum, 30 were assigned to the aglycone (see Table); the remaining 11 signals were indicative of the presence of one hexose and one pentose, in good agreement with the two anomeric signals appearing at δ 4.74 (d, J = 7.2 Hz) and 5.37 (d, J = 7.5 Hz) in the ^1H NMR spectrum and the two anomeric carbons observed at δ 107.5 and 106.4 in the ^{13}C NMR spectrum. The seven sp^3 hybrid carbons at δ 15.7, 17.1, 17.6, 24.9, 27.3, 28.2, and 33.3 and the two sp^2 hybrid carbons at δ 122.4 (d) and 145.3 (s) coupled with the information from ^1H NMR (seven methyl proton singlets and a broad triplet vinyl proton at δ 5.63) indicated that the aglycone possessed an olean-12-ene skeleton. After an extensive 2D NMR study, the aglycone was identified as echinocystic acid (Table), a common aglycone of the triterpenoidal glucosides isolated from many sources [5, 6]. The chemical shifts of C-3 (δ 88.8) and C-28 (δ 180.3) indicated that **1** was a monodesmosidic glycoside and the disaccharide chain was attached to the C-3 position of the aglycone. Acid hydrolysis yielded monosaccharide components, identified as arabinose and glucose by paper chromatography in comparison with authentic samples.



Compound **1**

Table: ^{13}C and ^1H NMR data of the new triterpenoidal glycoside 1

position	Carbon-13 Chemical shifts (C_5D_5N)	Proto chemical shifts (coupling constants) (C_5D_5N)
Aglycone part		
1	39.0	1.53; 1.07
2	26.8	1.75, 2.20
3	88.8	3.36 dd (9.9, 3.0)
4	39.7	–
5	56.0	0.83
6	18.6	1.50 m
7	33.6	1.35, 1.70
8	40.0	–
9	47.3	1.78 m
10	37.2	–
11	23.9	1.96
12	122.4	5.63 (br t)
13	145.3	–
14	42.2	–
15	36.2	1.37, 2.48
16	74.9	5.24
17	49.0	–
18	41.6	3.61 dd (10.8, 3.0)
19	47.4	1.40 m, 2.81 bt
20	31.1	–
21	36.0	1.73, 2.37
22	32.9	2.26, 2.51
23	28.2	1.28 s
24	17.1	0.96 s
25	15.7	0.86 s
26	26.8	1.17 s
27	27.3	1.85 s
28	180.3	–
29	33.5	1.01 s
30	24.9	1.05 s
Saccharide part		
Ara: 1′	107.5	4.74 d (7.2)
2′	72.0	4.58
3′	84.2	4.21
4′	69.4	4.43
5′	67.1	3.74, 4.18
Glc: 1″	106.4	5.37 d (7.5)
2″	75.8	4.03
3″	78.4	4.23
4″	71.6	4.16
5″	78.7	3.95
6″	62.7	4.35, 4.53

The identification and the full assignments of the proton and carbon signals for the sugar moieties were accomplished by a combination of DEPT, ^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY studies. The ^{13}C NMR spectral data for the sugar moieties indicated these two monosaccharides were in pyranose forms. The glucose was found to have the β-configuration, and the arabinose to have the α-configuration from the chemical shift and the coupling constant of each of the anomeric protons as well as from NOE correlations of H-1 with H-3 and H-1 with H-5 for each sugar unit. In the HMBC spectrum of **1**, the sequence of the disaccharide chain at the C-3 position was indicated by the following long-range coupling: H-1 (δ 5.37) of Glc with C-3 (δ 84.2) of Ara; H-1 (δ 4.74) of Ara with C-3 (δ 88.8) of aglycone. On the basis of the foregoing evidence, compound **1** was identified as echinosystic acid 3-O-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranoside.

The cytotoxicity of compound **1** was tested against A375, Hela, and L929 cultured tumor cell lines. Compound **1** showed moderate cytotoxic activity against the L929 cell line with an IC_{50} value of 43.2 μg/ml, and was inactive against the other two tumor cell lines of A375 and Hela (the IC_{50} values were >100 μg/ml).

3. Experimental

3.1. Equipment

ESI-MS was recorded on a Finnigan LCQ LC/ESI-MS spectrometer. IR spectra were recorded on a Bruker IFS 55. ^{1}H- and ^{13}C NMR, along with 2D NMR (^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY) spectra were taken on a Bruker ARX-300 FT NMR spectrometer (^{1}H at 300 MHz and ^{13}C at 75 MHz) in C_5D_5N. CC was performed on silica gel H (200–300 mesh, Qingdao Haiyang Chemical Factory). TLC analysis was carried out on silica gel GF_{254} (10–40 μm, Qingdao Haiyang Chemical Factory) with $CHCl_3$: MeOH : H_2O (60 : 40 : 10) as developing solvent. Preparative HPLC was carried out on a LC-10A Liquid Chromatograph (Japan Analytical Industry Co., Ltd.) equipped with a RI detector, using an ODS column (22 mm × 250 mm, 10 μm, Alltech); mobile phase: MeOH : H_2O (45 : 55, v/v, 300 ml for each injection); flow rate: 4.0 ml/min; sample solution: 8.0 mg/ml; injection volume: 500 μl; range of detector: 16.

3.2. Plant material

The plant material was collected from Liaoning Province, China, in June 1996, and authenticated as *Ixeris sonchifolia* (Bge.) Hance by Prof. Qi-shi Sun (Department of Pharmacognosy, Shenyang Pharmaceutical University). A voucher specimen is deposited at the herbarium of Shenyang Pharmaceutical University (No. 5208), Liaoning, P.R. China.

3.3. Extraction and isolation

The material was shade-dried and after grinding, 7.5 kg were extracted with hot 70% EtOH three times. The extracted solutions were evaporated at 50 °C under reduced pressure. The EtOH extract (1000 g) was resuspended in more than 10 volumes of water, and extracted several times with $CHCl_3$ until no colored constituents were transferred to the $CHCl_3$ layer. The resulting aqueous layer was sequentially extracted with EtOAc and then n-BuOH in the same way. A portion (80 g) of n-BuOH extract was subjected to column chromatography on silica gel H (1200 g, 200–300 mesh) with $CHCl_3$–MeOH gradients (50 : 1–1 : 1) as elute to give fractions 1–11. Part (300 mg) of fraction 4 (2.0 g) was subjected to preparative HPLC (ODS, 10 μm, 22 mm × 250 mm) eluted by MeOH : H_2O (45 : 55) to give compound **1** (50 mg).

3.4. Echinocystic acid 3-O-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranoside (**1**)

White, amorphous power, $C_{41}H_{66}O_{13}$, IR ν_{max} (KBr) cm^{-1}: 3407, 2935, 1045. ESI-MS: *m/z*: 789 $(M + Na)^+$, 765 $(M-H)^-$, 603 $(M-H-162)^-$, 471 $(M-H-162-132)^-$. For ^{1}H and ^{13}C NMR data see Table.

3.5. Cytotoxicity test

The tumor cell lines A375 (human melanoma), Hela (human cervicoma) and L929 (murine pneumoepithelia carcinoma) were obtained from the American Tissue Culture Collection (ATCC, USA) and cultured with RPMI 1640 (Gibco, USA) supplemented with 10% heated-inactivated fetal calf serum (FCS, Gibco), 1% L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. The cell lines were maintained in an incubator at 37 °C in humidified air containing 5% CO_2.

The cytotoxic activity of compound **1** against A375, Hela, and L929 was measured with an MTT assay [6, 7]. 100 μl culture of each cell line was established at 1500 cells/well in 96-well tissue culture plates. Compound **1** was dispensed to established cultures at eight concentrations in triplicate. A375, Hela and L929 cells were enumerated using MTT after five days of incubation, respectively. 20 μl of 5 mg/ml MTT were added to each well, and plates were incubated at 37 °C in 5% CO_2/air for a further 5 h, then the supernatant was removed from every well, and 150 μl DMSO was added to dissolve the formazan crystals.

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