SUPPLEMENTARY MATERIAL

A new 3,4-Seco-lupane Triterpenene Glycosyl Ester from the leaves of *Eleutherococcus sessiliflorus*

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Abstract

A new minor 3,4-seco-lupane triterpenene glycosyl ester, named sessiloside-A1 (**1**), along with three known 3,4-seco-lupane triterpenenes were isolated from the which alcohol extract of the leaves of *Eleutherococcus sessiliflorus* by silica gel column chromatography, and their structures were determined by using spectroscopic methods (1D and 2D NMR, HRMS). Compound **1** was elucidated to be *β*-D-glucopyranosyl ester of chiisanogenin. At the same time, a new efficient two-step enzymatic hydrolysis method was established to transform chiisanoside (**2**)→ divaroside (**3**)→**1**.

Keywords: *Eleutherococcus sessiliflorus*; 3,4-seco-lupane triterpenene; sessiloside-A1; chiisanoside; divaroside; chiisanogenin; enzymatic hydrolysis

Experimental

General experimental procedures

Optical rotation was measured on a JASCO P1020 digital polarimeter (Maryland, USA). The melting points were measured on an X-4 digital melting point apparatus without correction (Chengdu YiKe Instrument Co. Ltd., China). UV absorption spectra were recorded on a UV-6100 double beam spectrophotometer (Shanghai Mapada Instrument Co. Ltd., China). IR spectrum was recorded from KBr disks on a WGH-30A double-beam infrared spectrophotometer (Gangdong Sci & Tech. development Co., Ltd. Tianjin, China). HR-ESI-MS data were taken on a Qstar spectrometer (Florida, USA). NMR spectra were taken in $CDCl_3$, CD_3OD or C_5D_5N on a Bruker Avance-500 spectrometer (Germany) and the chemical shift (δ) values were given in ppm with TMS as internal standard. For column chromatography, D101 macroporous resin was from Langfang Miyang Chemical Co., Ltd., China, and silica gel (200 ~ 300 mesh) was produced by Qingdao Ocean Chemical Group Co., Ltd., China. For enzymatic hydrolysis, rhamnosidase and β-glycosidase were from Suzhou Kunlan Biotechnology Co., Ltd., China.

Plant material

The air-dried leaves of *Eleutherococcus sessiliflorus* (Rupr. & Maxim.) S.Y.Hu (synonym of *Acanthopanax senticosus*) were collected from the Changbai Mountain area in China. A voucher specimen (VS-TCM1708) has been deposited in the College of Chinese Medicinal Materials of Jilin Agricultural University.

Extraction and isolation

The air-dried leaves (2 kg, 20 mesh) were extracted three times with 95% ethanol in an ultrasonic generator (3000 W, 40 kHz, KQ-3000B, Kunshan, China), each time 30 min. The extract was filtered, combined and concentrated under reduced pressure to no alcohol odor, and then passed through a D101 macroporous resin column, washed with water to the distillate without color, then eluted with 95% ethanol, collected the distillate and concentrated to dryness to give total triterpenoid saponins (385 g). Part of total triterpenoid saponins (20 g) was chromatographed on a silica gel column with chloroform – methanol (100 : 1 ~ 3 : 1) to successively afford **4** (0.27 g), **1** (3.5 mg), **3** (28 mg), and **2** (1.15 g). Comparison of the NMR data with reported values led to identification of known compounds as chiisanoside (**2**) (Shirasuna, Miyakoshi et al. 1997), divaroside (**3**) (Matsumoto, Kasai, Kanamaru, Kohda, & Tanaka, 1987),

chiisanogenin (**4**) (Shirasuna et al., 1997) The structure of new compound **1** was elucidated by UV, IR, NMR and HRMS.

Sessiloside-A1 (**1**): white needles (ethanol); $[\alpha]_D^{20}$+187.5(c 0.20, DMSO); m.p = 206-208 ℃; UV(MeOH) λ_{max}(logH) 203(4.72), 279(3.14); IR (KBr) ν_{max} 3412, 2956, 2984, 1749, 1713, 1641, 1377, 1068 cm^{-1}; HR-ESI-MS (*m/z*): 647.37028 $[M+H]^+$, Calcd 647.37170 for $C_{36}H_{55}O_{10}$; ^{1}H-NMR (300 MHz, C_5D_5N) and ^{13}C-NMR (75 MHz, C_5D_5N): shown in Table S1.

Enzymatic hydrolysis

Taking **2** with high content in the leaves of *E. sessiliflorus* as the substrate for enzymatic hydrolysis, the effects of enzymes on the enzymatic hydrolysis of substrate were investigated from the influence factors of the solubility of solvents to substrates, pH, reaction temperature and reaction time, and HPLC was used to monitor the results of enzymatic hydrolysis. The aim is to establish a preliminary enzymatic hydrolysis process for the product of **1** and **3** which are the trace and rare secondary metabolites in *E. sessiliflorus*.

HPLC analysis

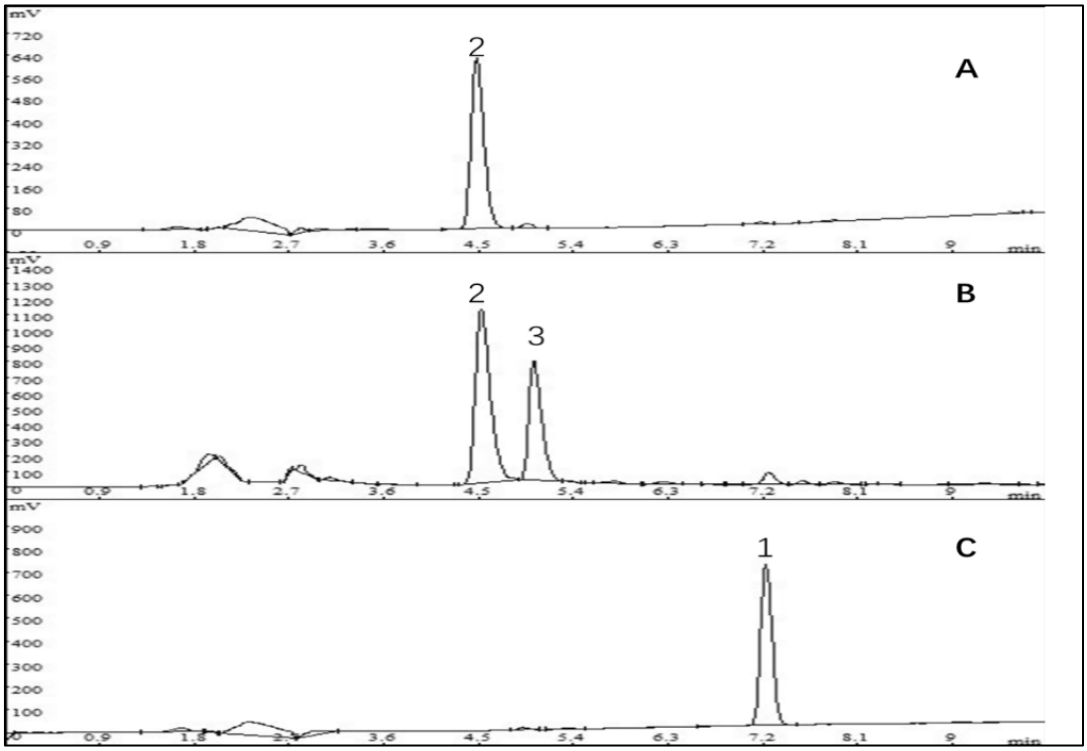
Analytical procedure was performed using CXTH-3000 HPLC chromatographic system. The column was Odyssll C_{18} column (200 mm×4.6 mm, 5 μm), and the column temperature is 40 ℃, with detection wavelength of 205nm. The mobile phase was a gradient elution with acetonitrile-water (0-10 min, 35 : 65 – 90 : 10; 10 - 15 min, 90 : 10; 15 - 17 min, 90 : 10 – 35 : 65; 17-20 min, 35 : 65) at a constant flow rate of 1.0 mL/min and the sampling amount was 10 μL.

Figures and table captions:

Figure S1. HPLC analysed the products of **2** by enzymatic hydrolysis. (A) HPLC spectra of compound **2**, (B) HPLC spectra of the product of compound **2** enzymatic hydrolysed by rhamnosidase, (C) HPLC spectra of the product of compound **3** enzymatic hydrolysed by *β*-glucosidase.

Table S1. NMR Spectroscopic Data of Compound 1

Figure S1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C

Table S1. NMR Spectroscopic Data of Compound **1**

Position	δ_C: ppm	δ_H: ppm	Position	δ_C: ppm	δ_H: ppm
1	70.54	3.74(*dd*, 3.0, 8.1)	19	47.65	3.45(*dt*, 4.2, 10.8)
2	38.83	3.13(*dd*, 8.1, 14.7)	20	150.16	
		2.82(*dd*, 3.0, 14.7)	21	30.82	2.12(*ddd*, 7.8, 10.8, 18.3)
3	173.05				1.42(*dd*, 4.2, 10.8, 16.5)
4	147.75		22	36.73	2.22(*dd*, 7.8, 16.5)
5	49.62	2.92(*dd*, 5.1, 12.0)			1.51(*m*)
6	25.22	1.81(*m*)	23	113.93	5.14 (*d*, 1.8)
		1.41(*m*)			5.04 (brs)
7	32.39	1.37(*dd*, 6.6,10.8)	24	23.56	1.88(*s*)
		1.17(*dd*, 4.2, 10.8)	25	19.05	0.99(*s*)
8	41.78		26	17.91	1.12(*s*)
9	44.15	2.75(*d*, 9.9)	27	13.83	1.05(*s*)
10	44.12		28	175.00	
11	75.30	4.55(*ddd*, 2.1, 4.8, 9.9)	29	110.76	4.90 (*d*, 2.1)
12	33.53	1.74(*dd*, 4.8, 14.1)			4.63 (brs)
		2.55(*ddd*, 2.1, 7.8, 14.1)	30	18.95	1.67(*s*)
13	35.27	2.87(*d*, 7.8)			
14	42.23		G1	95.55	6.44(*d*, 8.1)
15	29.58	1.96(*dd*, 8.1, 13.2)	G2	74.36	4.22(*t*, 8.1)
		1.15(*m*)	G3	79.56	4.11(*m*)
16	32.17	2.69(*dd*, 8.1, 12.6)	G4	71.11	4.38(*dd*, 6.6, 8.7)
		1.52(*m*)	G5	78.95	4.32(*ddd*, 2.7, 4.8, 8.7)
17	56.81		G6	62.20	4.49(*dd*, 2.7, 12.0)
18	49.78	1.70(*dd*, 7.8, 10.8)			4.41(*dd*, 4.8, 12.0)

The coupling constants (*J* in Hz) were given in parentheses. The assignments based on HMQC and HMBC experiments.

References:

Matsumoto, K., Kasai, R., Kanamaru, F., Kohda, H., & Tanaka, O. (1987). 3,4-Secolupane-Type Triterpene Glycosyl Esters from Leaves of Acanthopanax divaricatus SEEM(Pharmacognosy,Chemical). *Chem.pharm.bull*, *35*(1), 413-415.

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