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NOTE

Chiisanenside, a new triterpene glycoside from the fruits of *Acanthopanax chiisanensis*

Jeong Min Lee[1] · Jaemin Lee[1,2] · Seon Haeng Cho[3] · Sang-Won Lee[4] · Young-Ock Kim[4] · Ik-Hyun Cho[5] · Hak-Jae Kim[6] · Sanghyun Lee[2]

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Abstract A new triterpene glycoside was isolated from the fruits of *Acanthopanax chiisanensis* and identified as 3-*O*-β-D-[(6-carboxymethyl)-glucopyranosyl-(1 → 3)-β-D-glucuronopyranosyl]-olean-12-en-28-oic acid by spectral analyses. This compound was isolated for the first time from nature, and named chiisanenside.

Keywords *Acanthopanax chiisanensis* · Chiisanenside · Triterpene glycoside

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✉ Sanghyun Lee
slee@cau.ac.kr

[1] Natural Products Research Team, National Marine Biodiversity Institute of Korea, Seocheon 33662, Korea

[2] Department of Integrative Plant Science, Chung-Ang University, Anseong 17546, Korea

[3] Gongju National University of Education, Gongju 32553, Korea

[4] Department of Medicinal Crop Research Institute, National Institute of Horticultural & Herbal Science, Rural Development Administration, Eumseong 27709, Korea

[5] Department of Convergence Medical Science, Brain Korea 21 Plus Program, and Institute of Korean Medicine, College of Oriental Medicine, Kyung Hee University, Seoul 02447, Korea

[6] Department of Clinical Pharmacology, College of Medicine, Soonchunhyang University, Cheonan 31538, Korea

Introduction

Acanthopanax species, belonging to the family Araliaceae, are deciduous perennial herbaceous species in the family Araliaceae (Soejarto and Farnsworth 1978). *Acanthopanax* species have been widely used as health supplements because they have ginseng-like biological activities and are a famous tonic in Korea (Gaffeny et al. 2001). *A. chiisanensis* is an indigenous plant in the wild (Lee 2003). Many phytochemicals, such as eleutheroside E, helioxanthin, taiwanins B and C, sesamin, methyl betulin, chiisanogenin, 24-hydroxychiisanogenin, 22α-hydroxychiisanogenin, chiisanoside, isochiisanoside, and hyperin, have been isolated from *A. chiisanensis* (Kasai et al. 1986; Shin et al. 1992). Among them, chiisanogenin and chiisanoside, the principal active components of *A. chiisanensis*, have been reported to have anti-hepatotoxic, anti-diabetic, and anti-inflammatory effects on the proliferation of lymphocytes and to have prevented oxidative damage due to ROS overproduction in the rheumatoid arthritis response (Jung et al. 2005; Won et al. 2005). The purpose of this research is to isolate and identify phytochemicals from the fruits of *A. chiisanensis*.

Materials and methods

Plant materials

The fruits of *A. chiisanensis* (Araliaceae) were collected from Gongju and verified by Prof. Seon Haeng Cho, Gongju National University of Education, Korea. A voucher specimen (No. LEE 2008-01) was deposited at the Herbarium of Department of Integrative Plant Science, Chung-Ang University, Korea.

General experimental procedures

Electron ionization mass spectrometry was measured using a Jeol JMS-600 W (Japan) mass spectrometer. ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were recorded with a Bruker Avance 500 NMR (Germany) spectrometer in pyridine (C_5D_5N) using tetramethylsilane (TMS) as an internal standard. Chemical shifts were reported in parts per million (δ), and coupling constants (J) were expressed in Hertz (Hz). Thin-layer chromatography (TLC) was conducted with Kieselgel 60 F254 (Art. 5715, Merck Co., Germany) plates (silica gel, 0.25 mm layer thickness), with compounds visualized by spraying with 10 % H_2SO_4 in methanol (MeOH). Repeated column chromatography was conducted with a silica gel (200–400 mesh ASTM; Merck Co.). All the other chemicals and reagents were of analytical grade.

Extraction and isolation

The dried fruits of *A. chiisanensis* (1.5 kg) were ground into powder and extracted with MeOH (10 L × 3) under reflux, followed by in vacuo evaporation. The MeOH extract (524.5 g) was suspended in water (H_2O) and then partitioned successively with equal volumes of *n*-hexane (28.5 g), chloroform (23.7 g), ethyl acetate (EtOAc, 19.9 g), and *n*-butanol (82.5 g). A portion of the EtOAc fraction (5.0 g) was chromatographed on a silica gel chromatography column (No. 7734) and eluted in a gradient $CHCl_3$ and MeOH (100 % $CHCl_3$ and up to 90 % MeOH) solvent system to yield 7 subfractions (E_1–E_7). Subfraction E_5 ($CHCl_3$: MeOH = 95:5) was rechromatographed on a silica gel (No. 7729) column and eluted in a gradient $CHCl_3$ and MeOH (100 % $CHCl_3$ and up to 100 % MeOH) to yield compound **1** (2.3 mg).

Acid hydrolysis of compound 1

Compound **1** (1 mg) was refluxed with 5 % HCl in 60 % aqueous dioxane (10 mL) for 2 h. The reaction solution was concentrated and then the hydrolysate was extracted with ether. The ether extract was concentrated to yield aglycone oleanolic acid, which was identified by direct comparison with an authentic sample. The H_2O layer was neutralized with Ag_2CO_3 and filtered, and the filtrate was concentrated. The residue was compared with standard sugars by cellulose TLC [pyridine-EtOAc-HOAc-H_2O (36:36:7:21)], which showed the sugars to be D-glucose.

Determination of the absolute configuration of sugars in compound 1

Compound **1** (1 mg) was treated as previously described. The dried sugar mixture was dissolved in pyridine (0.1 mL) and then added to a pyridine solution (0.1 mL) of L-cysteine methyl ester hydrochloride (2 mg) followed by warming to 60 °C for 1 h. The solvent was concentrated under an N_2 stream and the residue was dried in vacuo. The residue was trimethylsilylated with TMS-HT (0.1 mL) at 60 °C for 30 min. After addition of *n*-hexane and water to the trimethylsilylated residue, the *n*-hexane layer was separated and analyzed by GC. The retention time (t_R) of the peak was 22.03 min (D-glucose).

Results and discussion

Compound **1** was yielded as a white powder with a molecular formula of $C_{44}H_{68}O_{16}$ based on the analysis of FAB-MS data. In the ^{1}H-NMR spectrum of compound **1**, one olefinic proton (H-12) signal of typical oleane type at δ 5.47 (*t* like) and one oxygen-bearing methine double doublet proton signals of H-3 at δ 3.31 (br. d, J = 10.8 Hz) were observed. The seven tertiary methyl group singlet signals of H-23, -24, -25, -26, -27, -29, and -30 were observed at δ 0.78–1.33. Two monosaccharides units were manifested in the ^{1}H-NMR spectrum of compound **1**. The two monosaccharide units were confirmed as glucose through GC and acid hydrolysis of compound **1**. The anomeric proton of the two glucose units of compound **1** was observed at δ 5.02 (d, J = 8.0 Hz) and 5.21 (overlap). In the ^{13}C-NMR spectrum of compound **1**, two sp^2 carbon signals of C-12 and C-13 at δ 123.1 and 145.4 and one ester carboxyl group signal of C-28 at δ 180.8 were observed. The chemical shift of the oxygen-bearing carbon signal of C-3 was observed at δ 89.8, suggesting that the sugar moieties were attached. The chemical shift of the oxygen-bearing carbon signal of C-6 of glucose was observed at δ 171.1, suggesting that the carboxyl group was attached. One of the glucose units was confirmed as glucuronic acid. In the HMBC spectroscopic data, the correlations between δ 5.02 (H-1 of GlcA) and 89.8 (C-3), between δ 5.21 (H-1 of Glc) and 83.9 (GlcA-3), and between δ 4.62 (H-6 of Glc) and 173.3 (–CH_2–COOH-) were linked to C-3 of the aglycone of compound **1** (Table 1). It was confirmed that –CH_2–COOH– was attached to the C-6 of Glc, which has a glycosidation sequence similar to that of betavulgaroside (Yoshikawa et al. 1995).

In previous studies, the Glc–Glc glycosidation sequence and the GlcA–GlcA glycosidation sequence of C-3 were

Table 1 ^{1}H- and ^{13}C-NMR spectral data of compound **1** (C_5D_5N, 500 MHz)

No.	δ_H	δ_C	HMBC	No.	δ_H	δ_C	HMBC
1		39.3		23	1.33 (s)	28.5	C-24
2		27.2		24	0.97 (s)	17.0	C-23
3	3.31 (br d, *J* 10.8)	89.8	C-1 of GlcA	25	1.02 (s)	17.9	C-5
4		40.1		26	0.78 (s)	16.0	C-7
5	1.63 (t, *J* 8.5)	56.4	C-23,24,25	27	1.33 (s)	26.8	
6		19.0		28		180.8	
7	0.76 (d, *J* 11.5)	33.8		29	0.99 (s)	33.8	
8		40.3		30	1.02 (s)	24.3	
9		48.5		GlcA-1	5.02 (d, *J* 8.0)	105.9	C-3
10		37.5		2		74.3	
11		24.3		3		83.9	C-1 of Glc
12	5.47 (*t* like)	123.1		4		73.4	
13		145.4	C-27	5		77.3	
14		42.6	C-12	6		171.1	C-4 of GlcA
15		28.9		Glc-1	5.21 (overlap)	107.2	C-3 of GlcA
16		24.3		2		74.8	
17		47.0		3		78.2	
18		42.7		4		69.7	–CH_2–COOH
19		47.2		5		77.9	
20		31.5		6	4.62 (m)	73.8	
21		34.8		–CH_2–COOH	4.63 (m)	67.6	
22		33.8		–CH_2–COOH		173.3	C-6 of Glc

Chemical shifts were reported in parts per million (δ), and coupling constants (J) were expressed in Hertz

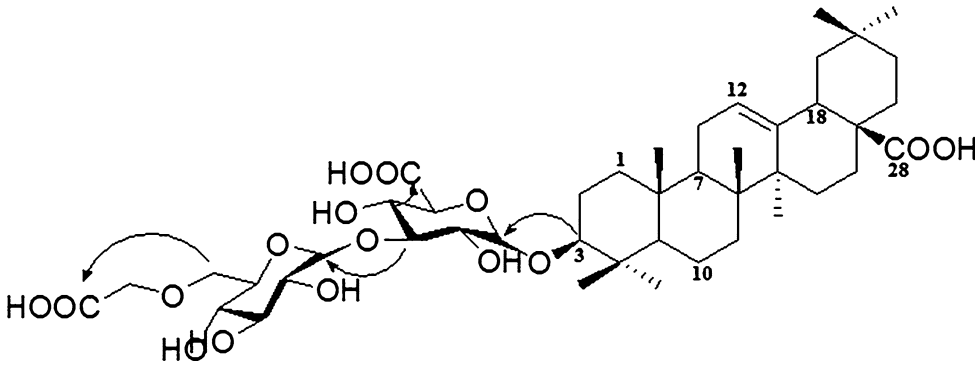

Fig. 1 The structure of compound **1**

described and characterized by ^{13}C-NMR spectroscopic analyses (Kitagawa et al. 1983; Seto et al. 1986; Kitagawa et al. 1988; Hernández-Carlos et al. 2011). The ^{13}C-NMR of the glycosidation sequence of compound **1** was similar to that found in previous studies. Glycosidation sequence of compound **1** was GlcA–Glc–COOH. The GlcA–Glc–COOH glycosidation sequence has not been reported but a similar glycosidation sequence has been reported in the roots and leaves of sugar beet (Yoshikawa et al. 1995).

Accordingly, the structure of compound **1** was identified as 3-*O*-β-D-[(6-carboxymethyl)-glucopyranosyl-(1 → 3)-β-D-glucuronopyranosyl]-olean-12-en-28-oic acid by analyses of the spectral data (FAB-MS, ^{1}H-, ^{13}C–, and 2D-NMR). Compound **1** was therefore isolated for the first time from nature and named chiisanenside (Fig. 1).

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