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Isolation and Identification of Terpenoids from the Fruits of *Acanthopanax chiisanensis*

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Abstract – Phytochemical constituents were isolated from the fruits of *Acanthopanax chiisanensis* by repeated column chromatography. Their structures were identified as β-sitosterol (**1**), daucosterol (**2**), sesamin (**3**), chiisanogenin (**4**), and 22α-hydroxy chiisanogenin (**5**) by spectroscopic analysis (MS, ^{1}H-, and ^{13}C-NMR). Compounds **1** - **5** were isolated for the first time from the fruits of *A. chiisanensis*.
Keywords – *Acanthopanax chiisanensis*, Araliaceae, Column chromatography, Chiisanogenin

Introduction

Acanthopanax species, belonging to the family Araliaceae, is a deciduous perennial herbaceous species which is distributed in Korea, Russia, China and Japan.[1] The dried roots and stem barks of *Acanthopanax* species have been used as a sedative and tonic to treat rheumatism, liver disease and diabetes, induction of apoptosis, immunomodulation, radioprotective effects, chronic bronchitis, antistress, ischemic heart disease, antitumor, antidiabetic activity, antiviral, antihypertension, and gastric ulcer protection.[2-8] The methanol (MeOH) and water (H_2O) extracts of the rhizomes of *A. senticosus* have trophic and beneficial effects, such as neuronal protection, as shown in an *in vitro* assay system for Alzheimer's disease.[9] *Acanthopanax* species have been widely used as health supplements because they have ginseng-like biological activities and are a famous tonic in Korea.[10]

Among *Acanthopanax* species, *A. chiisanensis* Nakai is an indigenous plant self-grown wild in its vertical distribution ranges from 200 to 1400 m altitude stretching from Mt. Jiri across the Korean peninsula. *A. chiisanensis* grows to 3 m in height. There is no thorn and hair in the twigs. The leaves are alternate phyllotaxis and 3 - 5 leaflets are wide obovate, 2.5 - 8.5 cm in length, 0.8 - 3.1 cm in width, the surface is green. The pedicel is 3 - 7 cm in length, hairless but the spiny. The umbel hang at the end of the branches, the pedicel is short and looks like round, have a white hair. The fruits are 6 mm in length, oval, ripens to black.[11]

A. chiisanensis has various biological activities such as anti-inflammatory, neuroprotective antihistaminic anticancer, and antinephrotoxicity activities.[12-18] The investigation of *A. chiisanensis* have been reported various phytochemicals such as eleutheroside E, helioxanthin, taiwanins B, C, sesamin, methyl betulin, chiisanogenin, 24-hydroxy chiisanogenin, 22α-hydroxy chiisanogenin, chiisanoside, isochiisanoside, and hyperin.[19-22]

There have been many reports on the medicinal effects, isolation, and identification of compounds from the roots, stems, and leaves of *A. chiisanensis*. Recently, the studies on the fruits of *A. chiisanensis* have been conducted.[23,24] However, there have been few investigations of the fruits of *A. chiisanensis.* Therefore, this research is focused on the isolation and identification of compounds from *A. chiisanensis* fruits by repeated column chromatography.

Experimental

Plant materials – The fruits of *A. chiisanensis* (Araliaceae) were collected at Gongju and verified by Prof. Seon Haeng Cho, Gongju National University of Education, Korea. A voucher specimen was deposited at the

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General experimental procedures – Electron ionization mass spectrometry (EI-MS) was measured with a Jeol JMS-600W (Tokyo, Japan) mass spectrometer. ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were recorded with a Bruker Avance 300 or 500 NMR (Rheinstetten, Germany) spectrometers in $CDCl_3$, C_5D_5N, DMSO, using tetra methyl silane (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) analysis was conducted with Kiesel gel 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 MeOH. Repeated column chromatography was conducted with a silica gel (200 - 400 mesh ASTM; Merck Co., Germany). All other chemicals and reagents were analytical grade.

Extraction and isolation – The dried fruits of *A. chiisanensis* (1.5 kg) were ground into powder and extracted with methanol (MeOH, 10 L × 3) under reflux. The resultant extracts were combined and concentrated under reduced pressure to afford 594.2 g of the residue. The MeOH extract (524.5 g) was suspended in H_2O and then partitioned successively with equal volumes of *n*-hexane (*n*-Hx, 28.5 g), chloroform ($CHCl_3$, 23.7 g), ethyl acetate (EtOAc, 19.9 g) and *n*-butanol (*n*-BuOH, 82.5 g). In the fruits of *A.chiisanensis*, a portion of the *n*-Hx fraction (7.1 g) was chromatographed on a silica gel column (No. 7734) chromatography column eluted in a gradient *n*-Hx and EtOAc (100% *n*-Hx up to 90% EtOAc) solvent system to yield 7 subfractions. Subfraction 2 (*n*-Hx:EtOAc = 97:3) led to isolation of compound **1** using MeOH recrystallization. Subfraction 3 (*n*-Hx:EtOAc = 95:5) led to isolation of compound **3** using MeOH recrystallization. Subfraction 6 (*n*-Hx:EtOAc = 30:70) led to isolation of compound **2** using MeOH recrystallization. A portion of the $CHCl_3$ fraction (5.4 g) was chromatographed on a silica gel column eluted in a gradients of *n*-Hx and EtOAc (100% *n*-Hx up to 100% EtOAc) to afford 7 subfractions (C_1-C_7). Subfraction C_4 (*n*-Hx:EtOAc = 70:30) led to isolation of compound **4** using MeOH recrystallization. Subfraction C_5 (*n*-Hx:EtOAc = 60:40) was rechromatographed on a silica gel (No. 7729) column eluted in a gradient of *n*-Hx and EtOAc (100% *n*-Hx up to 100% EtOAc) and EtOAc and MeOH (100% EtOAc up to 100% MeOH) solvent system to yield 4 subfractions (C_{51}-$_{54}$). Subfraction C_{51} was recrystallization by MeOH to afford compound **5**.

Compound 1 – white powder; EI-MS *m/z*: 414 $[M]^+$ (100.0), 396 (42.5), 381 (21.8), 329 (25.0), 303 (28.9), 289 (4.0), 273 (25.3), 255 (48.0), 231 (15.9), 213 (25.2), 159 (25.6), 145 (25.8); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 5.35 (1H, m, H-6), 3.53 (H, m, H-3), 2.26 (2H, m, H-4), 1.99 (2H, m, H-11), 1.03 (3H, s, H-19), 0.92 (3H, d, *J*= 6.3 Hz, H-21), 0.86 (3H, d, *J* = 2.1 Hz, H-27), 0.83 (3H, d, *J* = 6.3 Hz, H-26), 0.79 (3H, t, H-29), 0.68 (3H, s, H-18); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 140.9 (C-5), 121.9 (C-6), 72.0 (C-3), 57.0 (C-14), 56.2 (C-17), 50.3 (C-9), 46.0 (C-24), 42.5 (C-4,13), 40.0 (C-12), 37.4 (C-1), 36.7 (C-10), 36.3 (C-20), 34.1 (C-22), 32.1 (C-7,8), 31.8 (C-2), 29.9 (C-25), 29.3 (C-16), 26.2 (C-23), 24.5 (C-15), 23.3 (C-28), 21.3 (C-11), 20.0 (C-27), 19.6 (C-26), 19.2 (C-19), 19.0 (C-21), 12.2 (C-29), 12.0 (C-18).

Compound 2 – white powder; FAB-MS *m/z*: 577 $[M+H]^+$; ^{1}H-NMR (300 MHz, C_5D_5N): δ 5.35 (1H, m, H-6), 5.09 (1H, d, *J* = 6.9 Hz, Glc-1), 1.00 (3H, d, *J* = 6.3 Hz, H-21), 0.94 (3H, s, H-19), 0.94 (3H, d, *J* = 5.4 Hz, H-26), 0.89 (3H, d, *J* = 6.3 Hz, H-27), 0.87 (3H, m, H-29), 0.67 (3H, s, H-18); ^{13}C-NMR (75 MHz, C_5D_5N): δ 141.3 (C-5), 122.3 (C-6), 103.0 (Glc-1), 79.0 (Glc-3), 78.9 (Glc-5), 78.5 (C-3), 75.8 (Glc-2), 72.1 (Glc-4), 63.2 (Glc-6), 56.9 (C-14), 56.6 (C-17), 50.7 (C-9), 46.4 (C-24), 42.7 (C-13), 40.3 (C-12), 38.4 (C-4), 37.3 (C-1), 36.8 (C-10), 34.5 (C-20), 32.6 (C-22), 31.8 (C-7), 30.6 (C-8), 29.4 (C-2), 28.9 (C-25), 26.7 (C-16), 24.9 (C-23), 23.8 (C-15), 21.7 (C-28), 20.4 (C-11), 20.1 (C-27), 19.8 (C-26), 19.6 (C-19), 19.4 (C-21), 12.5 (C-29), 12.4 (C-18).

Compound 3 – colorless crystal; EI-MS *m/z*: 354 $[M]^+$ (100), 323 (12.6), 219 (7.5), 203 (34.7), 161 (64.8), 149 (90.6), 135 (53.8), 103 (8.3); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 6.79 (2H, dd, *J* = 8.0 Hz, H-6'/ 6"), 6.84 (2H, s, H-2'/2"), 6.78 (2H, d, *J* = 8.0 Hz, H-5'/5"), 5.95 (2H, s, -OCH_2O-), 4.71 (2H, d, *J* = 3.5 Hz, H-2,6), 4.23 (2H, dd, *J* = 6.5, 9.0 Hz, H-4eq/8eq), 3.87 (2H, dd, *J* = 3.0, 9.0 Hz, H-4ax/ 8ax), 3.05 (2H, d, *J* = 1.5 Hz, H-1/5).

Compound 4 – white powder; EI-MS *m/z*: 484 $[M]^+$ (21.0), 456 (10.8), 396 (100), 368 (24.8), 161 (60.9), 147 (47.0); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 4.86 (1H, br s, H-23), 4.83 (1H, br s, H-23), 4.76 (1H, br s, H-29), 4.64 (1H, br s, H-29), 4.53 (1H, d, *J* = 9.0 Hz, H-11), 3.57 (1H, d, *J* = 8.1 Hz, H-1), 2.98 (1H, m, H-19), 2.93 (1H, d, *J* = 15 Hz, H-2), 2.74 (1H, dd, *J* = 8.1, 15 Hz, H-2), 1.73 (1H, s, H-24), 1.68 (1H, s, H-30), 1.07 (1H, s, H-27), 1.02 (1H, s, H-26), 0.91 (1H, s, H-25).

Compound 5 – white powder; EI-MS *m/z*: 500 $[M]^+$ (25.9), 413 (46.1), 412 (100), 411 (42.6), 161 (61.6), 147 (48.6); ^{1}H-NMR (500 MHz, C_5D_5N): δ 5.16 (1H, s, H-23), 5.08 (1H, s, H-23,29), 4.83 (1H, d, *J* = 5.3 Hz, H-22),

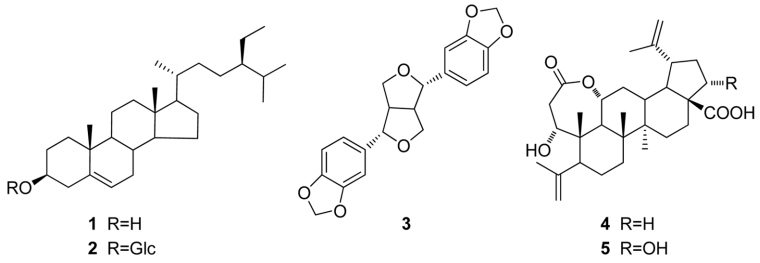

Fig. 1. Structures of compounds **1 - 5**.

4.73 (1H, s, H-29), 4.70 (1H, dd, *J* = 9.0, 17.6 Hz, H-11), 3.77 (1H, d, *J* = 8.0 Hz, H-1), 3.65 (1H, ddd, *J* = 4.8, 11.0, 11.0 Hz, H-19), 3.17 (1H, d, *J* = 14.6 Hz, H-2), 2.88 (1H, dd, *J* = 8.1, 14.6 Hz, H-2), 2.82 (1H, d, *J* = 9.7 Hz, H-9), 2.72 (1H, m, H-21), 2.56 (1H, t, *J* = 11.2 Hz, H-18), 1.99 (3H, s, H-24), 1.87 (3H, s, H-30), 1.83 (1H, m, H-21), 1.22 (3H, s, H-27), 1.09 (3H, s, H-26), 1.04 (3H, s, H-25); ^{13}C-NMR (125 MHz, C_5D_5N): δ 179.1 (C-28), 173.7 (C-3), 151.6 (C-20), 148.3 (C-4), 114.4 (C-23), 111.5 (C-29), 76.1 (C-22), 75.9 (C-11), 71.2 (C-1), 63.1 (C-17), 50.1 (C-5), 48.5 (C-19), 44.8 (C-10), 44.7 (C-9,18), 42.8 (C-14), 42.5 (C-21), 42.3 (C-8), 39.4 (C-2), 35.7 (C-13), 34.3 (C-12), 33.1 (C-7), 29.7 (C-15), 27.5 (C-16), 25.8 (C-6), 24.1 (C-24), 19.6 (C-30), 19.4 (C-25), 18.4 (C-26), 14.3 (C-27).

Results and Discussion

A chromatographic separation of the MeOH extract of *A. chiisanensis* led to the isolation of compounds **1 - 5** (Fig. 1).

Compounds **1** and **2** were obtained as white powders from the *n*-hexane fractions. ^{1}H-NMR spectra of **1** and **2** showed the existence of a sterol skeleton. A molecular ion peak was at *m/z* 414 $[M]^+$ in the EI-MS and *m/z* 577 $[M+H]^+$ in the FAB-MS. The olefinic proton signals (H-6) at δ 5.35 (both compounds **1** and **2**), two angular methyl singlets H-18 at δ 0.68 (compound **1**) and 0.67 (compound **2**) and H-19 at δ 1.03 (compound **1**) and 0.94 (compound **2**), and three doublets H-21 at δ 0.92 (compound **1**) and 1.00 (compound **2**), H-26 at δ 0.83 (compound **1**) and 0.94 (compound **2**), and H-27 at δ 0.86 (compound **1**) and 0.89 (compound **2**) were observed. ^{13}C-NMR spectra of **1** and **2** showed 29 and 35 resonances, respectively. C-5 and -6 signals of **1** and **2** were observed at δ 140.9 - 141.3 and 121.9 - 122.3, respectively. **1** and **2** had similar structural signals. The typical pattern of a glucose moiety was observed in the ^{1}H- and ^{13}C-NMR spectra in **2**. The anomeric proton of **2** produced a peak at δ 5.09 (d, *J* = 6.9 Hz), and the glucose position was at C-3 (β-linkage) of the aglycone according to HMBC correlation. Accordingly, the structures of **1** and **2** were identified as β-sitosterol (stigmast-5-en-3-ol) and daucosterol (β-sitosterol-3-*O*-β-D-glucoside), respectively, by comparison of the spectral data, as described in the literature.[25] In previous papers, β-sitosterol and daucosterol, the most common plant sterol, has anti-inflammatory, antipyretic, antihyperglycemic, antioxidant, immunoregulatory, antitumor, and antimicrobial activities.[26-30]

Compound **3** was obtained as colorless crystals from the *n*-hexane fraction and showed molecular ion peaks at *m/z* 354 $[M]^+$ in the EI-MS. In the ^{1}H-NMR spectrum of **3**, the symmetry methane doublet proton signals of H-1/5 at δ 3.05 and the typical ABX type in aromatic ring signals of H- 2'/2", 5'/5", and 6'/6" at δ 6.84, 6.78 (*J* = 8.0 Hz), and 6.79 (*J* = 8.0 Hz) were observed, respectively. The singlet signals at δ 5.95 indicated the dioxymethylene (-OCH_2O-) in the structure. Accordingly, the structure of **3** was identified as sesamin (eleutheroside B_4) by comparison of the spectral data (^{1}H-NMR) as described in the literature.[31] In previous papers, sesamin decreased fatty acid synthesis in rat liver accompanying the downregulation of sterol regulatory element binding protein-1, induced hypocholesterolemia especially low-density lipoproteins and cholesterol, which are risk factors for human atherosclerosis, suppressed the growth and induced apoptosis in the cells.[5,13,32]

Compounds **4** and **5** were obtained as white powder from the $CHCl_3$ fraction and showed molecular ion peaks at *m/z* 484 $[M]^+$ and 500 $[M]^+$ in the EI-MS, respectively. In the ^{1}H-NMR spectra of **4** and **5**, the two isopropenyl proton signals of H-23a, 23b, 29a, 29b at δ 4.83-5.08, 4.86 - 5.16, 4.64 - 4.73 and 4.76 - 5.08, five methine proton signals of H-24, 25, 26, 27, 30 at δ 1.73 - 1.99, 0.91 - 1.04, 1.02 - 1.09, 1.07 - 1.22, and 1.68 - 1.87, one lactonyloxy proton signals of H-11 at δ 4.53 - 4.70 were observed, respectively. Furthermore, the two proton signals of H-2 at δ 2.74 - 2.88 (dd, *J* = 8.1, 14.6 - 15 Hz) and 2.93 - 3.17 (d, *J* = 14.6-15 Hz) were assigned to methylene protons vicinal to the lactone-carbonyl group. In the ^{13}C-NMR

spectrum of **5**, five methyl carbon signals of C-24, 25, 26, 27 and 30 at δ 24.1, 19.4, 18.4, 14.3, and 19.6 and 145.4, two carbonyl group signal of C-3 and -28 at δ 173.7 and 179.1, two 1,1-di-substituted double bond signal of C-4, 20, 23 and 29 at δ 148.3, 151.6, 114.4 and 111.5 were observed, respectively. Compounds **4** and **5** had similar structure skeleton. However, the downfield shift of C-22 (δ 76.1) of **5** was indicative of a substitution by OH. Accordingly, the structures of **4** and **5** were identified as chiisanogenin and 22α-hydroxy chiisanogenin, respectively, by comparison of the spectral data (EI-MS, ^{1}H- and ^{13}C-NMR, 2D-NMR) as described in the literature.[33,34] In previous papers, chiisanogenin and 22α-hydroxy chiisanogenin exhibited moderate antibacterial, antiinflammatory, and platelet antiaggregating effects. Also, both compounds had effects on mitogen induced proliferation of lymphocytes, lipopolysaccharide-induced nitric oxide, and prostaglandin E_2 production by the RAW 264.7 macrophage cell line.[16,33,35-37]

In conclusion, five compounds, β-sitosterol (**1**), daucosterol (**2**), sesamin (**3**), chiisanogenin (**4**), and 22α-hydroxy chiisanogenin (**5**) were isolated from the fruits of *A. chiisanensis*. The five compounds have been reported from the fruits of *A. senticosus* and *A. sessiliflorus*.[38,39] These study is the first report on the isolation of β-sitosterol (**1**), daucosterol (**2**), sesamin (**3**), chiisanogenin (**4**), and 22α-hydroxy chiisanogenin (**5**) from the fruits of *A. chiisanensis*. The accurate understanding and information about *A. chiisanensis* fruits considered to be helpful in the fruit products and industry.

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