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Triterpenoids from *Acanthopanax koreanum* Root and Their Inhibitory Activities on NFAT Transcription

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Two triterpenoids (**1**,**4**) and two triterpenoid glycosides (**2**,**3**) were isolated from the root of *Acanthopanax koreanum* (Araliaceae). Their structures were identified as impressic acid (**1**), acankoreoside A (**2**), 3-*epi*-betulinic acid 28-*O*-[α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)]-β-D-glucopyranosyl] ester (**3**), and ursolic acid (**4**) by physicochemical and spectroscopic methods. Of these compounds, impressic acid (**1**) exhibited a potent inhibitory activity against NFAT transcription factor (IC_{50}: 12.65 μM).

Key words: *Acanthopanax koreanum*, Araliaceae, Triterpenoid, NFAT transcription factor, Inhibitory effect

INTRODUCTION

Acanthopanax koreanum (Araliaceae) is a medicinal plant indigenous to Korea (Lee, 1996), and is used as a tonic, a sedative, and for the treatment of rheumatism and diabetes in traditional Oriental medicine (Bensky and Gamble, 1986). It is further known that the root of this plant contain diterpenoids, lignans, triterpenoids, polyacetylenes, phenylpropanoids, flavonoids (Shin and Lee, 2002), and polysaccharides (Han *et al.*, 2003). Previous pharmacological study on *A. koreanum* reported anti-inflammatory (Cai *et al.*, 2003a), immunostimulatory (Han *et al.*, 2003), and antioxidant activity (Kim and Yang, 2003). As a result of our continued study of *A. koreanum* (Kim *et al.*, 1988; Kim *et al.*, 1990; Cai *et al.*, 2003b), we isolated four known triterpenoids from its MeOH extract, and tested these compounds for transcription factor ($NFAT^{TF}$) inhibitory activity. Of these compounds, impressic acid (**1**) was found to exhibit a potent inhibitory activity.

MATERIALS AND METHODS

General

Melting points were measured using a Yanaco micro melting point apparatus, optical rotation using a Jasco DIP-370 automatic polarimeter, UV spectra with a Beckman Du-650 UV-VIS recording spectrophotometer, and FT-IR spectra with a Jasco Report-100 infrared spectrometer. Preparative HPLC was carried out using a Waters HPLC system (600 pump, 600 controller, and a 996 photodiode array detector). NMR spectra were recorded using a Bruker DRX 300 spectrometer (^1H, 300 MHz; ^{13}C, 75 MHz), and FAB-MS using a JEOL JMS-HX/HX110A tandem mass spectrometer. Column chromatography was performed using a silica-gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck), and thin layer chromatography (TLC) using a pre-coated silica-gel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254S} plates (0.25 mm, Merck).

Plant material

The root of *A. koreanum* was donated by Susin Ogapi Co. and identified by Prof. Young Ho Kim, College of Pharmacy, Chungnam National University. Voucher specimens (CNU 96076) were deposited at the Herbarium of College of Pharmacy, Chungnam National University, Korea.

Extraction and isolation

The root of *A. koreanum* (10 kg) was extracted with MeOH three times under reflux for 15 h to yield 960 g of a dark solid extract, 950 g of which was then suspended in H_2O and extracted with CH_2Cl_2. The CH_2Cl_2 soluble fraction (200 g) was chromatographed on a silica gel column, and eluted with a gradient of hexane-EtOAc, to give 5 fractions (fr. A~E). Fr. D (13.0 g) was subjected to a silica gel

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column chromatography and eluted with hexane-EtOAc to give nine subfractions (subfr. DA~DI). Subfr. DH (1.0 g) was subjected to a silica gel ($CHCl_3$-acetone, 8:1) and a YMC gel (MeOH-H_2O, 3:1) column chromatography to afford **1** (31.3 mg), and subfr. DD (2.9 g) was subjected to a silica gel ($CHCl_3$-acetone, 20:1) and a YMC gel (MeOH-H_2O, 9:2) column chromatography to afford **4** (36.5 mg). The H_2O layer was concentrated *in vacuo* to yield a H_2O-soluble fraction (300 g), and a portion of this (100 g) was chromatographed on a MCI gel (Mitsubishi Chemical Corporation) eluted with a gradient of H_2O-MeOH to give six fractions (fr. a~f). Fr. e (1.1 g) was subjected to a silica gel column chromatography and eluted with $CHCl_3$-MeOH-H_2O to give five subfractions (subfr. ea~ee). Subfr. ed (0.08 g) was subjected to a YMC gel column chromatography and was eluted with MeOH-H_2O (3:2) to afford **2** (18.9 mg). Subfr. eb (0.06 g) was subjected to a YMC gel column chromatography and eluted with MeOH-H_2O (3:2) to afford **3** (38.0 mg).

Impressic acid (1)

White crystal, mp 210~213°C, $[\alpha]_D^{20}$: +4.2° (*c* 0.1, MeOH); FAB-MS *m/z*: 495.2 $[M+Na]^+$; ^1H-NMR (300 MHz, CD_3OD) δ: 0.86 (3H, s, H-23), 0.93 (3H, s, H-24), 0.97 (3H, s, H-25), 1.07 (6H, s, H-26 and 27), 1.73 (3H, s, H-30), 3.30 (1H, t, $|J_{AX} + J_{BX}|$ = 5.4 Hz, H-3β), 3.86 (1H, ddd, *J* = 10.8, 10.8, 5.1Hz, H-11β); ^{13}C-NMR (75 MHz, CD_3OD) δ: 35.5 (t, C-1), 25.4 (t, C-2), 75.7 (d, C-3), 37.8 (s, C-4), 49.0 (d, C-5), 18.3 (t, C-6), 35.4 (t, C-7), 42.4 (s, C-8), 55.4 (d, C-9), 39.2 (s, C-10), 70.0 (d, C-11), 37.3 (t, C-12), 37.5 (d, C-13), 42.7 (s, C-14), 29.6 (t, C-15), 32.3 (t, C-16), 56.4 (s, C-17), 49.0 (d, C-18), 47.2 (d, C-19), 150.5 (s, C-20), 30.7 (t, C-21), 36.9 (t, C-22), 28.4 (q, C-23), 21.9 (q, C-24), 16.7 (q, C-25), 15.9 (q, C-26), 13.9 (q, C-27), 178.9 (s, C-28), 109.5 (t, C-29), 18.6 (q, C-30).

Acankoreoside A (2)

White powder, mp 223~225°C, $[\alpha]_D^{20}$: -43° (*c* 0.5, EtOH); FAB-MS *m/z*: 979.4 $[M+Na]^+$; ^1H-NMR (300 MHz, CD_3OD) δ: 0.94 (3H, s, H-26), 1.00 (3H, s, H-24), 1.09 (3H, s, H-27), 1.18 (3H, s, H-25), 1.29 (3H, d, *J* = 6.5 Hz, H-6 of Rha), 1.74 (3H, s, H-30), 4.41 (1H, d, *J* = 7.8 Hz, H-1 of Glc II), 5.50 (1H, d, *J* = 8.0 Hz, H-1 of Glc I); ^{13}C-NMR (75 MHz, CD_3OD) δ: 33.1 (t, C-1), 26.2 (t, C-2), 73.0 (d, C-3), 52.8 (s, C-4), 45.0 (d, C-5), 21.8 (t, C-6), 34.6 (t, C-7), 42.8 (s, C-8), 51.2 (d, C-9), 37.6 (s, C-10), 21.2 (t, C-11), 26.0 (t, C-12), 38.4 (d, C-13), 43.0 (s, C-14), 30.1 (t, C-15), 32.4 (t, C-16), 57.7 (s, C-17), 49.7 (d, C-18), 47.5 (d, C-19), 151.0 (s, C-20), 30.9 (t, C-21), 37.2 (t, C-22), 179.0 (s, C-23), 18.0 (q, C-24), 16.8 (q, C-25), 16.6 (q, C-26), 14.8 (q, C-27), 175.9 (s, C-28), 110.0 (t, C-29), 19.3 (q, C-30), Glc I 95.7 (d, C-1), 74.1 (d, C-2), 78.7 (d, C-3), 70.9 (d, C-4), 78.0 (d, C-5), 69.4 (t, C-6), Glc II 105.4 (d, C-1'), 75.3 (d, C-2'), 76.5 (d, C-3'), 78.2 (d, C-4'), 77.2 (d, C-5'), 61.5 (t, C-6'), Rha 102.7 (d, C-1), 72.5 (d, C-2), 72.7 (d, C-3), 73.9 (d, C-4), 70.3 (d, C-5), 18.5 (q, C-6).

3-*Epi*-betulinic acid 28-*O*-[α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)]-β-D-glucopyranosyl] ester (3)

White crystal, mp 180~182°C, $[\alpha]_D^{20}$: -40° (*c* 0.5, EtOH); FAB-MS *m/z*: 925.5 $[M-H]^+$; ^1H-NMR (300 MHz, CD_3OD) δ: 0.86 (3H, s, H-26), 0.92 (3H, s, H-24), 0. 96 (3H, s, H-27), 1.06 (3H, s, H-25), 1.07 (3H, s, H-23), 1.27 (3H, d, *J* = 6.5 Hz, H-6 of Rha), 1.74 (3H, s, H-30), 4.41 (1H, d, *J* = 8.0 Hz, H-1 of Glc II), 5.47 (1H, d, *J* = 8.0 Hz, H-1 of Glc I). ^{13}C-NMR (75 MHz, CD_3OD) d: 36.5 (t, C-1), 26.6 (t, C-2), 75.3 (d, C-3), 40.3 (s, C-4), 49.9 (d, C-5), 19.2 (t, C-6), 36.3 (t, C-7), 43.4 (s, C-8), 50.2 (d, C-9), 38.3 (s, C-10), 24.2 (t, C-11), 26.5 (t, C-12), 38.2 (d, C-13), 43.8 (s, C-14), 31.6 (t, C-15), 32.8 (t, C-16), 57.9 (s, C-17), 50.1 (d, C-18), 48.2 (d, C-19), 151.3 (s, C-20), 30.6 (t, C-21), 37.5 (t, C-22), 29.5 (q, C-23), 23.0 (q, C-24), 17.8 (q, C-25), 17.0 (q, C-26), 14.9 (q, C-27), 176.2 (s, C-28), 110.8 (t, C-29), 19.6 (q, C-30), Glc I 95.3 (d, C-1), 73.7 (d, C-2), 79.6 (d, C-3), 70.9 (d, C-4), 78.1 (d, C-5), 69.4 (t, C-6), Glc II 104.4 (d, C-1'), 76.7 (d, C-2'), 76.8 (d, C-3'), 78.2 (d, C-4'), 76.9 (d, C-5'), 61.9 (t, C-6'), Rha 102.9 (d, C-1), 72.2 (d, C-2), 72.4 (d, C-3), 74.0 (d, C-4), 70.7 (d, C-5), 17.8 (q, C-6).

Ursolic acid (4)

White powder, mp 259~261°C, $[\alpha]_D^{20}$: + 60° (*c* 1, EtOH); FAB-MS *m/z*: 479.2 $[M+Na]^+$; ^1H-NMR (300 MHz, CD_3OD) δ: 0.70 (3H, s, H-25), 0.76 (3H, d, *J* = 9.3 Hz, H-29), 0.81 (3H, d, *J* = 6.3 Hz, H-30), 0.88 (3H, s, H-24), 0.90 (6H,s, H-26, H-27), 1.04 (3H, s, H-23), 3.08 (1H, dd, *J* = 10.5, 4.8 Hz, H-3), 5.15 (1H, t, *J* = 3.6 Hz, H-12). ^{13}C-NMR (75 MHz, CD_3OD) δ: 37.5 (t, C-1), 25.4 (t, C-2), 77.2 (d, C-3), 37.4 (s, C-4), 54.3 (d, C-5), 17.0 (t, C-6), 31.9 (t, C-7), 38.3 (s, C-8), 46.5 (d, C-9), 35.6 (s, C-10), 21.9 (t, C-11), 124.4 (d, C-12), 137.2 (s, C-13), 40.8 (s, C-14), 26.7 (t, C-15), 22.8 (t, C-16), 46.5 (s, C-17), 51.9 (d, C-18), 37.9 (d, C-19), 37.9 (d, C-20), 29.3 (t, C-21), 35.6 (t, C-22), 26.7 (q, C-23), 13.5 (q, C-24), 13.9 (q, C-25), 15.3 (q, C-26), 21.6 (q, C-27), 179.1 (s, C-28), 15.2 (q, C-29), 19.1 (q, C-30).

Inhibitory activity of triterpenoids on NFAT transcription

The inhibitory activities of isolated triterpenoids on $NFAT^{TF}$ were investigated using a modified SEAP assay (Yang *et al.*, 1997), as described previously (Lee *et al.*, 2002; Lee *et al.*, 2003). For this assay, 100 μL of cells ($1{\times}10^4$ cells/well) were incubated with 50 μL of sample and 50 μL of stimulator (phorbol-12-myristate 13-acetate and ionomycin) at 37°C for 18 h, and centrifuged. 100 μL of the supernatant obtained was then heated at 65°C for

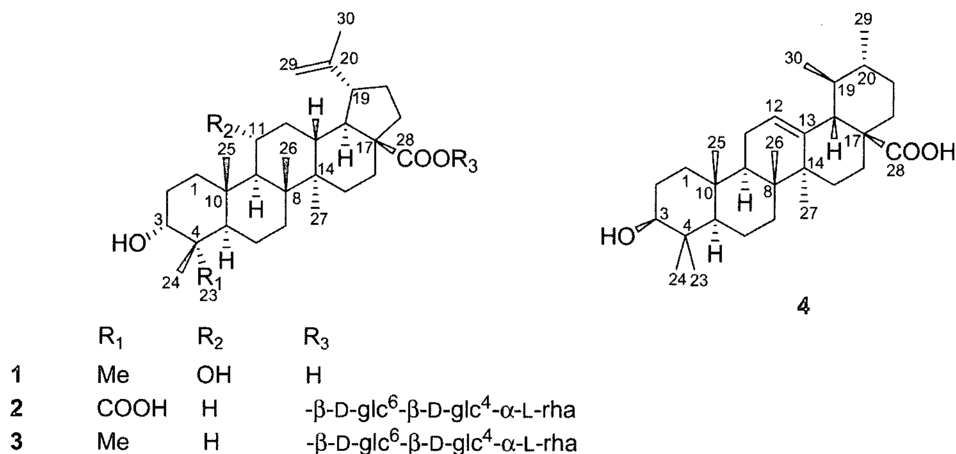

Fig. 1. Structures of compounds **1-4**

1 h, and incubated with 50 μL of SEAP buffer and 50 μL of the substrate (*p*-nitrophenylphosphate) at 37°C for 4 h. Optical density was then measured at 405 nm. The inhibitory activities of triterpenoids on $NFAT^{TF}$ were expressed as percentages of the uninhibited control. Cyclosporin A was used as a positive control, because it is known to block the phosphatase activity of calcineurin, thereby preventing the subsequent dephosphorylation and translocation of NFAT to the nucleus (Jain *et al.*, 1995). Cell viability was determined using an MTT cell proliferation Kit (1465007, Roche).

RESULTS AND DISCUSSION

The structures of isolated compounds were identified as impressic acid (**1**), acankoreoside A (**2**), 3-*epi*-betulinic acid 28-*O*-[α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)]-β-D-glucopyranosyl] ester (**3**), and ursolic acid (**4**) by comparing physicochemical and spectroscopic data with previously reported data (Ty *et al.*, 1984; Chang *et al.*, 1998; Sung *et al.*, 1991; Min *et al.*, 2000). The present study is the first report on the isolation of these compounds from the root of *A. koreanum*.

Four triterpenoids (**1**, **2**, **3**, and **4**) were examined for their $NFAT^{TF}$ inhibitory activities. Of these compounds, impressic acid (**1**) exhibited a potent inhibition (IC_{50}: 12.65 μM), though it was less active than cyclosporin A (IC_{50}: 0.29 μM). However, compounds **2** and **3** (lupane-type triterpene glycosides at C-28 carboxylic acid) showed no activity (IC_{50}: > 100 μM). In addition, ursolic acid (**4**) was found to be cytotoxic at inhibitory concentrations, although it has been previously reported to have low toxicity (Jeong *et al.*, 1999).

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