Arch Pharm Res Vol 26, No 12, 1014-1017, 2003

Archives of Pharmacal Research
http://apr.psk.or.kr

Two New Phenylpropanoid Glycosides from the Stem Bark of *Acanthopanax trifoliatus*

Phan Van Kiem[1,3], Chau Van Minh[1], Nguyen Tien Dat[2], Xing Fu Cai[2], Jung Joon Lee[3], and Young Ho Kim[2]

[1]Institute of Natural Products Chemistry, NCST, 18 Hoang Quoc Viet, Nghiado, Caugiay, Hanoi, Vietnam, [2]College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea, and [3]Korea Research Institute of Bioscience and Biotechnology, P.O.Box 115, Yusong, Daejeon 305-600, Korea

(Received September 23, 2003)

Two new phenylpropanoid glycosides, 1-β-D-glucopyranosyl-2,6-dimethoxy-4-propenylphenol (**1**) and 1-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-2,6-dimethoxy-4-propenylphenol (**2**) were isolated from the stem bark of *Acanthopanax trifoliatus* along with four known compounds (**3~6**). Their structures were established on the basis of spectral and chemical evidences.

Key words: *Acanthopanax trifoliatus*, Araliaceae, Acantrifoside E, Acantrifoside F

INTRODUCTION

Acanthopanax trifoliatus (L.) Merr., (Araliaceae) is distributed in the Northern Vietnam and used in the folk medicine of SouthEast Asia (Chi, 1997; Loi, 2001) as a drug with ginseng-like activity. Lupane-triterpene carboxylic acids and a lupane-triterpene glycoside were reported from the leaves of *A. trifoliatus* (Ty *et al.*, 1984, 1985; Lischewski *et al.*, 1985; Yook *et al.*, 1998). Herein, we describe the isolation and structures of two new phenylpropanoid glycosides named acantrifoside E (**1**) and acantrifoside F (**2**) along with four known compounds (**3-6**) from the stem bark of *A. trifoliatus*. Based on spectroscopic data, the chemical structures of constituents were determined as 1-β-D-glucopyranosyl-2,6-dimethoxy-4-propenylphenol (**1**), 1-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-2,6-dimethoxy-4-propenylphenol (**2**), syringin (**3**), eleutheroside E (**4**), quercitrin (**5**) and (2*R*,3*R*)-2,3-di-(3,4-methylenedioxybenzyl)-butyrolactone (**6**).

MATERIALS AND METHODS

General experimental procedures

Melting points were determined using a Kofler microhotstage. IR spectra were obtained on a Hitachi 270-30 type spectrometer from KBr discs. Optical rotations were determined on a JASCO DIP-1000 KUY polarimeter. EI-MS was obtained on a Hewlett Packard 5989 B-MS spectrometer and FAB-MS and HR FAB-MS were obtained using a JEOL JMS-DX 300 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker DRX300 and 600 NMR spectrometer. Chemical shifts are referenced to δ using TMS as an internal standard. Column chromatography (CC) was performed on silica gel 60, YMC RP-18 resin or Dianion HP-20 resin.

Plant material

The stem barks of *A. trifoliatus* were collected in Langson province, Vietnam in January 2001 and identified by Prof. Dr. Tran Minh Hoi, Institute of Ecology, Biological Resources, NCST of Vietnam. Voucher specimens (INPC 2539) are deposited at the herbarium of the Institute of Natural Products Chemistry, NCST, Vietnam, and at the herbarium of the College of Pharmacy, Chungnam National University, Korea.

Extraction and isolation

The dried and powdered stem bark (2.0 Kg) was extracted three times with hot MeOH. The combined solutions were evaporated under reduced pressure to give MeOH extract (120.0 g), which was suspended in water and then partitioned with dichloromethane. The residue of dichloromethane fraction (53.0 g) was chromatographed on a silica gel column (ϕ 70 mm×800 mm) using $CHCl_3$-MeOH (from 100:0 to 0:100) as eluent yielding seven fractions (Fr. A - G). Fraction D (2.1 g) was chromato-

Correspondence to: Young Ho Kim, College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea
Tel: 82-42-821-5933, Fax: 82-42-823-6566
E-mail: yhk@cnu.ac.kr

graphed on a silica gel column (ϕ 20 mm×600 mm) using hexane-acetone (4:1) as eluent to yield **6** (10.0 mg). The residue of the water fraction (65.0 g) was adsorbed on highly porous polymer resin (Dianion HP-20, Mitsubishi Chem. Ind. Co. Ltd, Tokyo, Japan; Column ϕ 70 mm×800 mm) and eluted with water containing increasing concentrations of MeOH (100% H_2O, 20% MeOH, 40% MeOH, 60% MeOH, 80% MeOH and 100% MeOH). The 40% MeOH fraction (12.0 g) was chromatographed on a silica gel column (ϕ 40 mm×800 mm) using $CHCl_3$-MeOH-H_2O (70:30: 4) as eluent and gave five fractions (Fr. A1 - A5). Fraction A1 (1.5 g) was purified on a YMC RP-18 column (ϕ 20 mm×600 mm) using MeOH-H_2O (8:2) as eluent yielded **1** (12.3 mg) and **3** (15.4 mg). Fraction A3 (2.7 g) was purified on a YMC RP-18 column (ϕ 20 mm×600 mm) using MeOH-H_2O (7:3) as eluent yielded **2** (8.5 mg), **4** (6.2 mg) and **5** (20.1 mg).

1-β-D-Glucopyranosyl-2,6-dimethoxy-4-propenylphenol (acantrifoside E) (1)

White powder; m.p. 178-180°C; $[\alpha]_D^{25}$ – 28.0° (*c* 0.5, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3400 (br, OH), 2985 (C-H), 1043 (C-O-C); FAB-MS m/z: 379 $[M+Na]^+$, HR FAB-MS found *m/z* 379.1367 $[M+Na]^+$ (cacld 379.1369 for $C_{17}H_{24}O_8Na$). ^{1}H- and ^{13}C-NMR: see Table I.

1-[β-D-Glucopyranosyl-(1→6)-β-D-glucopyranosyl]-2,6-dimethoxy-4-propenylphenol (acantrifoside F) (2)

White powder; m.p. 165-168°C; $[\alpha]_D^{25}$ – 35.0° (*c* 0.5, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3420 (br, OH), 2985 (C-H), 1050 (C-O-C); FAB-MS (positive) *m/z*: 341.1 $[M-glc+H]^+$; ^{1}H- and ^{13}C-NMR: see Table I.

Syringin (3)

White powder; m.p. 190-192°C; $[\alpha]_D^{25}$ – 32° (*c* 0.5, MeOH); ^{1}H-NMR (300 MHz, CD_3OD) δ: 6.77 (2H, s, H-3, 5), 6.54 (1H, d, *J* = 15.8 Hz, H-7), 6.37 (1H, dt, *J* = 15.8, 5.5 Hz, H-8), 4.90 (1H, d, *J* = 7.8 Hz, H-1), 4.23 (2H, dd, *J* = 5.5, 1.4 Hz, H_2-9), 3.88 (6H, s, 2×CH_3O), 3.80 (1H, dd, *J* = 12.0, 2.0 Hz, H_b-6'), 3.67 (1H, dd, *J* = 12.0, 4.0 Hz, H_a-6'), 3.20-3.40 (4H, H-2', 3', 4', 5'); ^{13}C-NMR (75 MHz, CD_3OD) δ: 154.5 (C-2, 6), 136.0 (C-1), 135.3 (C-4), 131.3 (C-7), 130.2 (C-8), 105.6 (C-1'), 105.5 (C-3, 5), 78.5 (C-3'), 78.0 (C-5'), 75.9 (C-2'), 71.5 (C-4'), 63.7 (C-9), 62.8 (C-6'), 57.1 (2×CH_3O).

Eleuthroside E (4)

Needles; m.p. 267-269°C; $[\alpha]_D^{25}$ – 45.0° (*c* 0.5, MeOH); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 6.65 (4H, s, H-2, 6, 2', 6'), 3.80 (12H, s, 4×CH_3O), 4.67 (2H, d, *J* = 6.5 Hz, H-7, 7'), 3.80-4.20 (4H, m, H-9, 9'), 4.90 (2H, d, *J* = 7.8 Hz, glc-H-1, glc-H-1'); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 152.6 (C-3, 3', 5, 5'), 137.1 (C-4, 4'), 133.7 (C-1, 1'), 104.2 (C-2, 2', 6, 6'), 102.7 (C-1-glc, 1'-glc), 85.1 (C-7, 7'), 77.2 (C-3-glc, 3'-glc), 76.5 (C-5-glc, 5'-glc), 74.2 (C-2-glc, 2'-glc), 71.3 (C-9, 9'), 69.9 (C-4-glc, 4'-glc), 61.0 (C-6-glc, 6'-glc), 56.5 (OCH$_3$×4), 53.6 (C-8, 8').

Quercitrin (5)

Yellow powder; m.p. 178-180°C; $[\alpha]_D^{25}$ – 147.0° (*c* 0.5, MeOH); FAB-MS (positive) *m/z*: 449 $[M+H]^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 7.99 (1H, d, *J* = 2.1 Hz, H-2'), 7.68 (1H, dd, *J* = 8.2, 2.1 Hz, H-6'), 7.28 (1H, d, *J* = 8.2 Hz, H-5'), 6.67 (1H, d, *J* = 2.1 Hz, H-6), 6.25 (1H, d, *J* = 7.8 Hz, H-1-rha), 6.24 (1H, d, *J* = 2.1 Hz, H-8), 4.26-5.01 (4H, m, H-2-rha, 3-rha, 4-rha, 5-rha), 1.44 (3H, d, *J* = 5.9 Hz, H-6-rha); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 177.7 (C-4), 164.1 (C-7), 161.2 (C-5), 157.2 (C-9), 156.4 (C-2), 148.4 (C-4'), 145.2 (C-3'), 134.2 (C-3), 121.1 (C-6'), 120.7 (C-1'), 115.6 (C-5'), 115.4 (C-2'), 104.1 (C-10), 101.8 (C-1-rha), 98.6 (C-6), 93.6 (C-8), 71.1 (C-4-rha), 70.5 (C-3-rha), 70.3 (C-2-rha), 70.0 (C-5-rha), 17.6 (C-6-rha).

(2*R*,3*R*)-2,3-Di-(3,4-methylenedioxybenzyl)-butyrolactone (6)

White needles; m.p. 92-95°C; $[\alpha]_D^{25}$ – 27.0° (*c* 0.5, MeOH); EI-MS *m/z*: 354 $[M]^+$; ^{1}H-NMR ($CDCl_3$) δ: 6.73 (1H, d, *J* = 8.0 Hz, H-5"), 6.71 (1H, d, *J* = 8.0 Hz, H-5'), 6.63 (1H, d, *J* = 2.0 Hz, H-2"), 6.47 (2H, dd, *J* = 8.0, 2.0 Hz, H-6', 6"), 5.95 (4H, s, 2×OCH_2O). 4.14 (1H, dd, *J* = 6.0, 3.9 Hz, H_b-4), 3.87 (1H, dd, *J* = 6.0, 3.9 Hz, H_a-4), 3.00 (1H, dd, *J* = 14.3, 6.4 Hz, H_b-α'), 2.80 (1H, dd, *J* = 14.3, 6.4 Hz, H_a-α'), 2.55 (2H, m, H-α"); ^{13}C-NMR ($CDCl_3$) δ: 178.0 (C-1), 147.9 (C-3', 3"), 145.0 (C-4', 4"), 131.6 (C-1'), 131.3 (C-1"), 121.5 (C-6"), 121.2 (C-6'), 109.4 (C-2"), 108.8 (C-2'), 108.4 (C-5"), 108.2 (C-5'), 101.0 (2×OCH_2O), 71.1 (C-4), 46.1 (C-2), 41.3 (C-3), 38.4 (C-α"), 34.8 (C-α').

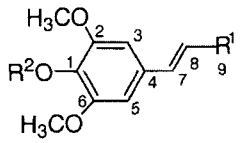
RESULTS AND DISCUSSION

Compound **1** and **2** were obtained as white powder and gave absorption bands at 3400 and 1050 cm^{-1} due to hydroxyl and C-O-C groups in the IR spectrum. Compound **1** showed a $[M+Na]^+$ ion peak at *m/z* 379 in the FAB-MS spectrum. The HR-FAB mass spectrum of **1** produced a molecular ion at *m/z* 379.1367 $[M+Na]^+$ providing the formula $C_{17}H_{24}O_8$ (calcd for $C_{17}H_{24}O_8Na$: 379.1369). The ^{1}H-NMR spectrum of **1** (Table I, in CD_3OD) showed signals due to a methyl group at δ 1.86 (3H, d, *J* = 6.4 Hz), two methoxy groups at δ 3.84 (6H, s), two olefinic protons at δ 6.23 (1H, dq, *J* = 16.8, 6.4 Hz) and 6.34 (1H, d, *J* = 16.8 Hz) in *trans*-configurations, two protons of an aromatic ring at δ 6.67 (2H, s, H-3, 5), two protons for a primary alcohol group at δ 3.53 (1H, dd, *J* = 12.0, 5.4 Hz) and 3.82 (1H, dd, *J* = 12.0, 2.5 Hz), and an anomeric

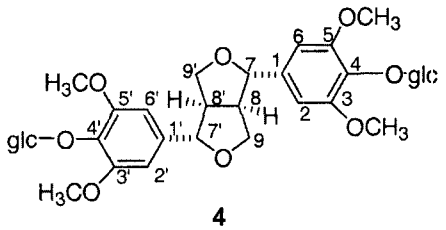
Table I. ^{1}H- and ^{13}C-NMR spectral data of **1** and **2**

Pos.	1		2	
	δ_C[c]	δ_H[d]	δ_C[c]	δ_C[b,d]
1	136.4	–	132.9	–
2	154.4	–	152.7	–
3	105.5	6.67 (1H, s)	103.5	6.65 (1H, s)
4	135.5	–	132.7	–
5	105.0	6.67 (1H, s)	103.5	6.65 (1H, s)
6	154.4	–	152.7	–
7	132.2	6.34 (1H, d, 16.8)	130.2	6.32 (1H, d, 16.8)
8	126.6	6.23 (1H, dq, 16.8, 6.4)	124.3	6.21 (1H, dq, 16.8, 6.4)
9	18.6	1.86 (3H, d, 6.4)	17.5	1.80 (3H, d, 6.4)
10	57.1	3.84 (6H, s)	57.8	3.75 (6H, s)
1-O-glc				
1'	105.6	4.85 (1H, d, 7.8)	102.0	4.87 (1H, d, 7.8)
2'	75.9	3.47*	75.9	3.30*
3'	78.5	3.21*	78.6	3.20*
4'	71.5	3.40*	71.7	3.43*
5'	78.0	3.21*	75.7	3.20*
6'	62.7	3.53 (1H, dd, 12.0, 5.4), 3.82 (1H, dd, 12.0, 2.5)	67.5	3.53 (1H, dd, 12.0, 5.4), 3.82 (1H, dd, 12.0, 2.5)
Glc'-(1°Ê6)-glc				
1"			103.0	4.05 (1H, d, 7.5)
2"			73.4	2.8-3.4*
3"			75.8	2.8-3.4*
4"			68.9	2.8-3.4*
5"			75.5	2.8-3.4*
6"			64.8	3.65 (1H, dd, 12.0, 5.4), 2.90 (1H, dd, 12.0, 2.5)

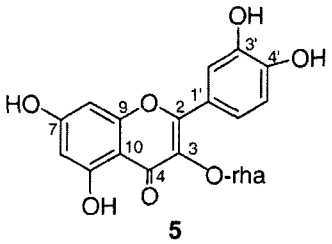
Glc, β-D-glucopyranosyl, [a]in CD_3OD, [b]in DMSO-d_6, [c]150 MHz, [d]600 MHz, *overlap signals.



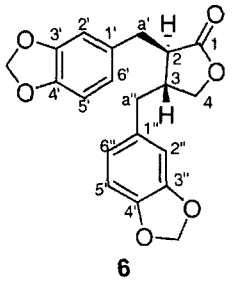
	R^1	R^2
1	CH_3	glc
2	CH_3	glc[1] – [6]glc
3	CH_2OH	glc



4



5



6

Fig. 1. Structures of compounds **1-6**

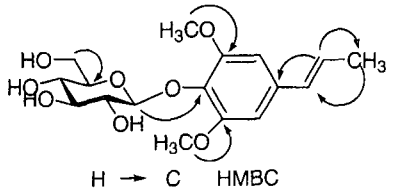


Fig. 2. Selected H-C Long-range Correlations in HMBC Spectrum of **1**

proton at δ 4.85 (1H, d, J = 7.8 Hz). The ^{13}C-NMR and DEPT spectra (Table I, in CD_3OD) revealed 17 carbon signals, including a methyl group at δ 18.6, methoxy groups at δ 57.1, anomeric carbon at δ 105.6. The structure of **1** was determined by comparison with the data of syringin and tracing the connectivities shown in the HMBC spectrum of **1**. Cross peaks (Fig. 2) were observed between the methyl protons at δ 1.86 (CH_3-9) and olefinic carbons at δ 132.2 (C-7)/126.6 (C-8), between olefinic protons at δ 6.34 (H-7)/6.23 (H-8) and methyl carbon at δ 18.6 (C-9) in the HMBC spectrum of **1**. This evidence confirmed that the methyl group was attached to C-8. Moreover, the anomeric proton at δ 4.85 was correlated with C-1 at δ 136.4, confirming that the sugar was attached to C-1 of the aromatic ring. Thus, compound **1** was determined to be 1-β-D-glucopyranosyl-2,6-dimethoxy-4-propenylphenol, which we named acantrifoside E.

Compound **2** showed the following significant FAB-MS fragment peak $[M\text{-glc+H}]^+$ at *m/z* 341 in the positive FAB-MS spectrum, corresponding to a molecular formula of $C_{23}H_{34}O_{13}$. The ^{1}H- and ^{13}C-NMR spectra of **2** (Table I, in DMSO-d_6) were very similar to those of **1** except the additional sugar signals (δ 103.0, 75.8, 75.5, 73.4, 68.9 and 64.8) in the ^{13}C-NMR spectrum, and an anomeric proton (δ 4.05) and protons for a primary alcohol group (δ

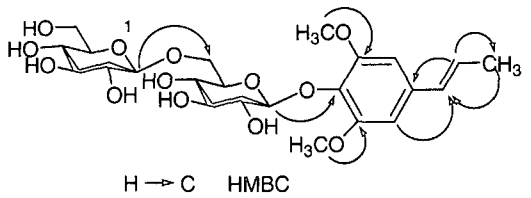


Fig. 3. Selected H-C Long-range Correlations in HMBC Spectrum of **2**

3.65 and 2.90) in the ^{1}H-NMR spectrum of **2**. Furthermore, the C-H long-range correlations between the methyl protons at δ 1.80 (H-9) and olefinic carbons at δ 130.2 (C-7)/124.3 (C-8), between olefinic protons at δ 6.32 (H-7)/6.21 (H-8) and methyl carbon at 17.5 (C-9), between anomeric proton at 4.87 (H-1') and carbon at δ 132.9 (C-1), and between anomeric proton at 4.05 (H-1") and carbon at δ 67.5 (C-6') were observed in the HMBC spectrum of **2**. This evidence confirmed that the methyl group was attached to C-8, one sugar (glc) was attached to C-1 of the aromatic ring and another sugar (glc') was connected to glc at C-6 position. Based on above data and comparison with the data of syringin and **1**, compound **2** was determined to be 1-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-2,6-dimethoxy-4-propenylphenol, which we named acantrifoside F.

Compounds **3-6** were identified as syringin, eleutheroside E, quercitrin and (2*R*,3*R*)-2,3-di-(3,4-methylenedioxybenzyl)-butyrolactone, respectively by comparison of ^{1}H-, ^{13}C-NMR and MS data reported in the literatures (Masateru *et al.*, 1996; Changdong *et al.*, 1999; Kaneko *et al.*, 1998; Choi *et al.*, 1998; Lopes *et al.*, 1983). Compounds **3-5** were already isolated from other *Acanthopanax* species, but compound **6** was isolated for the first time from *Acanthopanax* species.

ACKNOWLEDGEMENTS

This study was supported in part by a grant from the Korea-Vietnam International Cooperation Project and by a grant (PF0320701-00) from Plant Diversity Research Center of 21st Frontier Research Program funded by the Ministry of Science and Technology, in Korea. We are grateful to the KBSI for measuring the NMR and mass spectra, and we thank Prof. Dr. Tran Minh Hoi, Institute of Ecology, Biological Resources, NCST for the plant identification.

REFERENCES

Changdong, J., Ronald, G. M., and Mohsen, D., Phenylpropanoid glycosides from *Stellera chamaejasme*. *Phytochemistry*, 50, 677-680 (1999).

Chi, V. V., Vietnamese Medical Plant Dictionary, Ha Noi Medicine Pub. (1997).

Choi, W. H., Park, W. Y, Hwang, B. Y,. Oh, G. J,. Kang, S. J., Lee, K. S., and Ro, J. S., Phenolic compounds from the stem bark of *Cornus walteri* Wanger. *Kor. J. Pharmacogn.*, 29, 217-224 (1998).

Kaneko, T., Ohtani, K., Kasai, R., Yamasaki, K., and Duc, N. M., *n*-Alkyl glycosides and *p*-hydroxybenzoyloxy glucose from fruits of *Crescentia cujete*. *Phytochemistry*, 47, 259-263 (1998).

Lischewski, M., Ty, P. D., Kutschabsky, L., Pfeiffer, D., Phiet, H. V., Preiss, A., Sung, T. V., and Adam, G., Two 24-nor-triterpenoid carboxylic acids from *Acanthopanax trifoliatus*. *Phytochemistry*, 24, 2355-2357 (1985).

Loi, D. T., Glossary of Vietnamese Medical Plants, Hanoi S&T Pub. (2001).

Lopes, L. M. X., Yoshida, M., and Gottlieb, O. R., Dibenzylbutyrolactone lignans from *Virola sebifera*. *Phytochemistry*, 22, 1516-1518 (1983).

Masateru, O., Yasuyuki, I., Thoru, I., Junichi, K., Yasuko, T., Yujiro, N., and Toshihiro, N., Five new monoterpene glycosides and other compounds from *Foeniculi* Fructus (Fruit of *Foeniculum vulgare* Miller). *Chem. Pharm. Bull.*, 44, 337-342 (1996).

Ty, P. D., Lischewski, M., Phiet, H. V., Preiss, A., Nguyen, Ph. V., and Adam, G., 3α,11α-Dihydroxy-23-oxo-lup-20(29)-en-28-oic acid from *Acanthopanax trifoliatus*. *Phytochemistry*, 24, 867-869 (1985).

Ty, P. D., Lischewski, M., Phiet, H. V., Preiss, A., Sung, T. V., Schmidt, J., and Adam, G., Two triterpenoid carboxylic acids from *Acanthopanax trifoliatus*. *Phytochemistry*, 23, 2889-2891 (1984).

Yook, C. S., Kim, I. H., Hahn, D. R., Nohara, T., and Chang, S. Y., A lupane-triterpene glycoside from leaves of two *Acanthopanax*. *Phytochemistry*, 49, 839-843 (1998).