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Triterpenoid Saponins from the Root Barks of *Aralia elata*

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From the root barks of *Aralia elata* Seem.(Araliaceae) three known saponins together with oleanolic acid and β-sitosterol 3-O-β-D-glucoside were isolated. The saponins were identified as oleanolic acid 28-O-β-D-glucopyranosyl ester, oleanolic acid 3-O-β-D-glucuronopyranoside and oleanolic acid 3-O-α-L-arabinofuranoysyl-(1→4)-β-D-glucuronopyranoside(narcissiflorine) on the basis of chemical and spectra data. The latter two saponins were isolated as their dimethyl-esters as well as monomethylesters.

Key words: *Aralia elata*, Araliaceae, Root bark, Saponin, Oleanolic acid glycoside

INTRODUCTION

The root barks of *Aralia elata* Seem. [*A. chinensis* var. *glabrescens* Matsum. (Araliaceae)] has long been used in Korean folk medicine to cure cough, cancer, and diabetes (Perry, 1980). It has been said to be an efficacious remedy for diabetes mellitus and stomach disease in Japan (Perry, 1980; Hsu *et al.*, 1986). Despite of its known uses in Southeast Asian folk medicine chemical studies on this plant have only recently been reported. A number of saponins and flavonoid glycosides from the leaves of *A. elata* have been isolated, among which two saponins showed improvement of CCl_4-induced damage in liver (Saito *et al.*, 1990; Nishida *et al.*, 1991). From the tender shoots in the spring of *A. elata* saponins and adenosine have also been isolated (Lee *et al.*, 1989; Kim *et al.*, 1990; Park *et al.*, 1991). However, only protocatechuic acid (Kuwata, 1929) and oleanolic acid as well as β-sitosterol and stigmasterol (Murakami *et al.*, 1966) were isolated from the root barks of *A. elata*. Very recently, isolation and structure elucidation of triterpenoid saponins such as aralosides A~C as well as a new one, araloside G from BuOH fraction were reported (Jiang *et al.*, 1992). This paper describes the isolation of triterpenoid saponins from the EtOAc soluble fraction of MeOH extract responsible for the hypoglycemic activity of the crude plant extract (Kim and Lee, 1992).

MATERIALS AND METHODS

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General experimental procedures

Melting points were determined on a Mitamura-Riken apparatus and are uncorrected. Optical rotations were measured on a Rudolph Autopol III automatic polarimeter. IR spectra were recorded on a Perkin-Elmer 283B spectrophotometer. ^{1}H-NMR spectra were obtained on either a Varian FT-80A (80 MHz), a Varian VXR-5200 (200 MHz), or a Bruker AM-300 (300 MHz) spectrometer using TMS as an internal standard. ^{13}C-NMR spectra were recorded with a Varian XVR-5200 (50.3 MHz) or a Bruker AM-300 (75.5 MHz) instrument. EI mass were determined on a Hewlett-Packard 5985B GC/MS system equipped with direct inlet system. For TLC, Kieselgel 60 F_{254} glass plates (Merck) were used.

Plant material

The root barks of *A. elata* were collected in early spring 1991 near Cheonan, Chungcheongnam-do province.

Extraction and isolation

The dried and chopped root barks (*ca.* 10 kg) were refluxed with MeOH for 3 hr 5 times. The extracts were concentrated in vacuo to give a residue, which was partitioned successively with $CHCl_3$, EtOAc and then BuOH. A portion of the EtOAc soluble fraction (65 g) which showed positive activity in alloxan-induced hyperglycemic rats was subjected to SiO_2 column chromatography with $CHCl_3$-MeOH-H_2O (7 : 3 : 0.5) to give 17 subfractions (E01~E17). Subfraction E03 was rechromatographed over SiO_2 with hexane-EtOAc (gradient) to afford compound **I**.

Table I. ^{1}H-NMR spectral data for compounds II, V~VIII in pyridine-d_5[a,b]

Proton	II	V	VI	VII	VIII
CH_3	0.99 s	0.82 s	0.80 s	0.81 s	0.82 s
	1.01 s	0.85 s	0.95 s (×2)	0.84 s	0.96 s (×2)
	1.03 s	0.92 s	0.97 s	0.91 s	0.99 s
	1.04 s	0.94 s	1.00 s	0.93 s	1.02 s
	1.08 s	0.98 s	1.29 s (×2)	0.96 s	1.29 s
	1.24 s	1.24 s		1.23 s	1.31 s
	1.30 s	1.31 s		1.28 s	
H-18	3.15 dd	3.09 brdd	3.27 brdd	3.08 dd	3.29 brdd
	(4.3, 12.0)	(4.0, 14.1)	(3.8, 13.5)	(3.9, 13.6)	(3.5, 13.6)
H-3	3.44 dd	3.37 dd	3.36 dd	3.32 dd	3.35 dd
	(4.2, 9.8)	(4.3, 11.8)	(4.3, 11.7)	(4.2, 11.6)	(4.0, 11.5)
H-12	5.49 brs	5.37*	5.45*	5.36*	5.47 brs
Anomeric H	6.08 d	4.97 d	4.96 d	4.92 d	4.73 d
	(7.9)	(7.7)	(7.8)	(7.9)	(7.8)
				5.75 s	5.77 brs
$COOCH_3$		3.69 s	3.72 s	3.72 s	3.73 s
		3.73 s		3.77 s	

[a] Data are δ(ppm), multiplicity, and *J* (in parentheses) in Hz, [b] Measured with a Bruker AM-300 instrument.
*t-like.

Compound I—mp. 303-306°, which was identified as oleanolic acid by direct comparison with an authentic sample.

Subfraction E09 was purified with a SiO_2 column by elution with hexane-EtOAc (8 : 5) to give compound **II**.

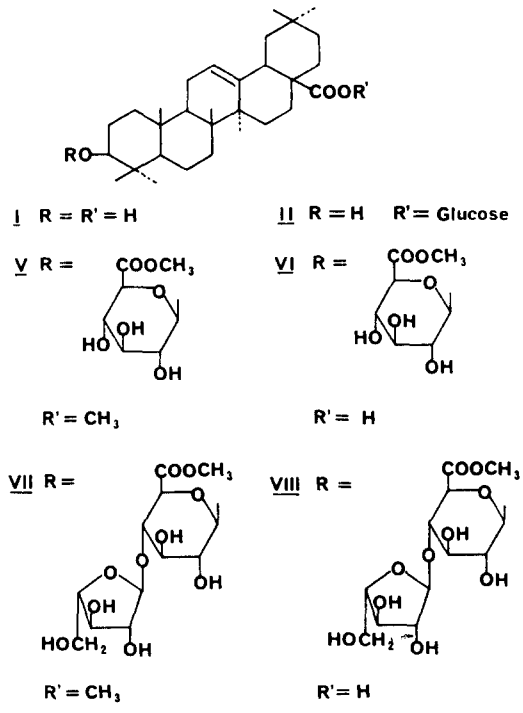
Compound II—crystallized from MeOH as colorless needles. mp. 226-9℃; $[\alpha]_D^{20}$ +50.1° (c, 0.38, pyridine); IR ν_{max}(KBr) cm^{-1} 3420, 1735, 1640, 1070, 1028, 1009, 825, 816 and 800; ^{1}H- and ^{13}C-NMR: see Tables I and II.

Subfractions E10 and E11 were separately recrystallized from MeOH to yield compounds **III** and **IV**, respectively, as amorphous powder.

Compound III—mp. 273-6℃, $[\alpha]_D^{20}$ −41.2° (c, 0.3, pyridine); IR ν_{max}(KBr) cm^{-1} 3400, 1650, 1080, 1028, 840 and 800; ^{1}H-NMR (200 MHz, pyridine-d_5) δ: 0.67 (3H, s, 18-CH_3), 0.95 (3H, s, 19-CH_3), 5.08 (1H, d, *J*=7.6 Hz, Glc H-1), 5.36 (1H, br d, *J*=4.4 Hz, H-6); ^{13}C-NMR: see Table II.

Compound IV—mp. 218-9℃.

Subfraction E17 was methylated with CH_2N_2 at room temperature to afford a methylated product which was rechromatographed by a SiO_2 column eluted with EtOAc saturated with H_2O to give subfractions E001 and E002. Subfractions E001 and E002 were separately purified by SiO_2 column chromatography eluted with



EtOAc to yield compounds **V** and **VI** from subfraction E001 and compounds **VI** and **VII** from subfraction E002, respectively.

Compound V—crystallized from MeOH to yield pure **V** as colorless needles. mp. 238-41℃, $[\alpha]_D^{20}$ + 14.6° (c, 0.2, pyridine); IR ν_{max}(KBr) cm^{-1} 3400, 1735, 1714, 1165, 1060, 1020 and 810; ^{1}H- and ^{13}C-NMR: see Tables I and II.

Table II. ^{13}C-NMR chemical shifts of *Aralia* saponins in pyridine-d_5

Carbon No.		II	II	V	VI	VII	VIII
C-1		38.97	37.50	38.70	38.60	38.63	38.64
C-2		28.24	30.28	26.61	26.56	26.51	26.52
C-3		78.08	78.09	89.21	89.13	89.30	89.33
C-4		39.36	39.36	39.56	39.50	39.50	39.51
C-5		55.80	140.88	55.84	55.76	55.78	55.82
C-6		18.80	121.90	18.50	18.44	18.46	18.49
C-7		33.14	32.20	33.16	33.26	33.06	33.24
C-8		39.90	32.08	39.73	39.72	39.69	39.78
C-9		48.13	50.37	47.97	47.98	47.93	48.04
C-10		37.35	36.95	37.01	36.95	36.97	37.00
C-11		23.66	21.31	23.48	23.68	23.44	23.73
C-12		122.78	39.97	122.89	122.50	122.85	122.54
C-13		144.13	42.51	144.20	144.82	144.19	144.88
C-14		42.12	56.84	42.02	42.14	41.98	42.20
C-15		28.07	24.55	28.14	28.30	28.10	28.35
C-16		23.81	28.57	23.76	23.68	23.73	23.73
C-17		46.98	56.26	47.01	46.65	46.97	46.71
C-18		41.74	12.01	41.87	41.97	41.84	42.04
C-19		46.20	19.25	46.15	46.46	46.12	46.53
C-20		30.77	36.42	30.84	30.94	30.82	30.99
C-21		33.99	19.04	34.04	34.23	34.00	34.29
C-22		32.50	34.24	32.86	33.18	32.82	33.24
C-23		28.76	26.41	28.23	28.16	28.17	28.18
C-24		16.53	46.06	16.96	16.90	16.92	16.93
C-25		15.64	29.49	15.48	15.41	15.44	15.44
C-26		17.49	19.45	17.20	17.36	17.16	17.41
C-27		26.09	20.01	26.17	26.16	26.14	26.20
C-28		176.42	23.42	177.99	180.16	177.98	180.18
C-29		33.13	12.19	33.16	33.26	33.15	33.30
C-30		23.65		23.70	23.75	23.67	23.79
OCH_3				51.56		51.56	
GluA	C-1			107.29	107.26	106.97	106.97
	C-2			75.46	75.39	75.14	75.14
	C-3			77.98	77.89	76.00	76.01
	C-4			73.19	73.16	78.54	78.55
	C-5			77.27	77.19	76.95	76.98
	C-6			170.78	170.80	170.35	170.38
	OCH_3			51.99	51.99	52.30	52.32
Ara(*f*)	C-1					108.72	108.73
	C-2					82.62	82.65
	C-3					78.54	78.55
	C-4					87.56	87.54
	C-5					62.57	62.59
Glc	C-1	95.72	102.57				
	C-2	74.08	75.35				
	C-3	79.90	78.50				
	C-4	71.04	71.70				
	C-5	78.87	78.62				
	C-6	62.15	62.85				

Compound VI—recrystallized from MeOH to afford pure **VI** as amorphous powder. mp. 194-6℃, $[\alpha]_D^{20}$ −20° (c, 0.3, pyridine); IR ν_{max}(KBr) cm^{-1} 3500, 1700, 1070, 1050 and 810; ^{1}H- and ^{13}C-NMR: see Tables I and II.

Compound VII—crystallized from MeOH to yield pure **VII** as colorless needles. mp. 169-174℃, $[\alpha]_D^{20}$ +14.6° (c, 0.2, pyridine); IR ν_{max}(KBr) cm^{-1} 3400, 1735, 1165, 1060, 1020 and 810; ^{1}H- and ^{13}C-NMR: see Tables I and II.

Compound VIII—recrystallized from MeOH to afford pure **VIII** as amorphous powder. mp. 224-6℃, $[\alpha]_D^{20}$ −20° (c, 0.3, pyridine); IR ν_{max}(KBr) cm^{-1} 3500, 1700, 1070, 1050 and 810; ^{1}H- and ^{13}C-NMR: see Tables I and II.

Acid hydrolysis of compounds II, V~VIII

A solution of each saponin (about 5 mg) in 10% HCl-dioxane (1 : 1) was heated at 95° for 3 hr. The reaction mixture was blowed to dryness with a N_2 stream. The residue was identified with TLC by comparison with authentic samples, using $CHCl_3$ as development. Saponins **II**, **VI** and **VIII** gave oleanolic acid, while saponins **V** and **VII** gave oleanolic acid methylester. Sugars were checked with TLC (precoated cellulose plate, pyridine-EtOAc-HOAc-H_2O=36 : 36 : 7 : 21). D-glucose from saponin **II**, D-glucuronic acid from saponins **V** and **VI**, and D-glucuronic acid and L-arabinose from saponins **VII** and **VIII** were detected.

Methylation of compounds VI and VIII

Compounds **VI** and **VIII** were individually dissolved in MeOH and treated with ethereal CH_2N_2 at room temperature overnight. Workup in the usual manner gave the methylated products which were separately purified by SiO_2 column chromatography in the same manner as described above and then recrystallized from MeOH to yield pure methylesters (**V** and **VII**) as colorless needles. Identification of each methylester was made by comparing with those of authentic compounds.

RESULTS AND DISCUSSION

The EtOAc soluble fraction of *A. elata* which showed significant inhibition of blood glucose in alloxan-induced diabetic rats was chromatographed over SiO_2 to afford subfractions. Non-polar subfractions yielded after chromatographic separation followed by recrystallization oleanolic acid (**I**), compound **II**, and β-sitosterol 3-O-β-D-glucoside (**III**), among which **I** and **III** were identified by comparison of their physical and NMR data with literature values (Kim *et al.*, 1989; Lee *et al.*, 1992). Compound **II** show positive results in Molisch and Liebermann-Burchard tests. Its IR spectrum showed absorptions of hydroxyl functions (3420 cm^{-1}) and ester group (1735 cm^{-1}). Acid hydrolysis of compound **II** afforded D-glucose as the sugar and an aglycone, mp. 300-302℃, which was identified as oleanolic acid by direct comparison with compound **I**. The ^{1}H-NMR spectrum showed signals for oleanolic acid and glucose. The presence of a typical anomeric proton doublet (J=7.9 Hz) at δ 6.08 ppm supported that compound **II** is a β-glucopyranosyl ester of oleanolic acid. This was corroborated by the corresponding signals in the ^{13}C-NMR spectra. Thus the structure of **II** was proved to be oleanolic acid 28-O-β-D-glucopyranosyl ester which has already been isolated from other *Aralia* species (Cai *et al.*, 1982) and *Hemsleya* species (Nie *et al.*, 1984; Kasai *et al.*, 1990). The physical properties of polar subfraction (E017) suggested to be a mixture of carboxylated saponins (Kang *et al.*, 1988). Therefore this fraction was methylated with CH_2N_2 and then chromatographed over SiO_2 to give compounds **V-VIII**. Acid hydrolysis of compounds **V-VIII** afforded oleanolic acid methylester, mp. 200-2℃, from compounds **V** and **VIII** and oleanolic acid (**I**) from compounds **VI** and **VIII** as an aglycone together with glucuronic acid from compounds **V** and **VI** and glucuronic acid and arabinose from compounds **VII** and **VIII** as the sugar components. As shown in Table II, ^{13}C-NMR data of compounds **V** and **VII** were almost superimposable to those compounds **VI** and **VII**, respectively, except for the chemical shifts of C-17, 22 and 28 due to the esterification shifts (Kang, 1987). Complete methylation of **VI** and **VIII** with CH_2N_2 gave the same products to **V** and **VIII**, respectively. Thus compounds **VI** and **VIII** seem to be partially desmethylated products of the respective compounds **V** and **VII**. These results led to the conclusion that all the saponins **V-VIII** are monodesmosides of oleanolic acid (**I**). The ^{1}H-NMR spectrum of **V** showed an anomeric proton doublet at δ 4.97 ppm with relatively large coupling constant (J=7.7 Hz) ascribable to β-glucuronopyranoside at C-3. Based on these results, the structures of compounds **V** and **VI** were determined to be 3-O-[6′-O-methyl-β-D-glucuronopyranosyl] oleanolic acid methyl ester and oleanolic acid 3-O-[6′-methyl-β-D-glucuronopyranoside], respectively.

The ^{1}H-NMR spectra of **VII** showed two anomeric proton signals at δ 4.92 (d, J=7.9 Hz) and 5.75(s) attributed to one mole each of glucuronic acid and arabinose, respectively at C-3. These assignments were supported by the ^{13}C-NMR spectra of **VII**, which showed two anomeric carbon signals at δ 106.97 and 108.72 ppm together with a set of signals corresponding to a α-L-arabinofuranosyl unit in the molecule (Gunzinger *et al.*, 1986). The interglycosidic linkage was determined by the observed downfield shift (Δδ 5.35 ppm) for C-4 of the inner 6-O-methyl-β-D-glucuronopyranose, comparing with the compound **V**. From these observations the structures were elucidated as 3-O-α-L-arabinofuranosyl-(1→4)-6′-O-methyl-β-D-glucuronopyranosyl oleanolic acid methylester (narcissiflorine dimethylester) (Massod *et al.*, 1981) for **VII** and 3-O-α-L-arabinofuranosyl-(1→4)-6′-O-methyl-β-D-glucuronopyranosyl oleanolic acid for **VIII**. The isolation of oleanolic acid 3-O-β-D-glucuronopyranoside and narcissiflorine has been reported from a number of plant sources (Gunzinger *et al.*, 1986; Masood *et al.*, 1981; Takabe *et al.*, 1980; Sun *et al.*, 1991; Kawai *et al.*, 1989).

Very recently, Jiang *et al.* reported the isolation of narciflorine and other saponins from this plant (1992). However, such glucuronide saponins of oleanolic acid have so far not been encounted in the leaf parts of *A. elata* which are of interest.

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