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Saponins from the Aerial Parts of *Aralia continentalis*

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Abstract – Seven triterpenoid saponins such as oleanolic acid 28-O-β-D-glucopyranosyl ester, hederagenin 28-O-β-D-glucopyranosyl ester, chikusetsusaponin IVa, udosaponin A, salsoloside C, udosaponins F and C were isolated from the aerial parts of *Aralia continentalis*, among which two 28-O-β-D-glucopyranosyl esters of oleanolic acid and hederagenin are isolated for the first time from this plant. These results suggested that the chemical components of Korean Dokwhal are practically identical to those of Japanese Udo supporting the chemotaxonomical point of view.

Key words – *Aralia continentalis*, Araliaceae, aerial parts, saponins.

Introduction

The roots of *Aralia continentalis* Kitagawa (Araliaceae) have been used as an analgesic and a remedy for labor pains and headache in Korea. On the other hand, the roots of *A. cordata* Thunb. are used to treat rheumatism, lumbago, and lameness in Japan (Perry, 1980). Recently both plants are taxonomically regarded as the synonymous plant (Lee, 1997). A few number of diterpenes from root parts have been isolated as active components for analgesic and antiinflammatory agents (Han *et al.*, 1983; 1985; Okuyama *et al.*, 1991). Eleven saponins from the aerial parts of *A. cordata* and a number of terpenes from the essential oils have also been identified (Kawai *et al.*, 1989; Yoshihara and Hirose, 1973, Ito *et al.*, 1978; Sawamura *et al.*, 1989; Okugawa *et al.*, 1990). We have studied the antioxidant components from the young shoots of *A. continentalis* and isolated flavonoid glycosides as active principles and adenosine (Kim *et al.*, 1995). Further studies on this plant we have led to the isolation and identification of saponins from the aerial parts. The present paper reports the isolation of saponins from the aerial parts of *A. continentalis*.

Experimental

General experimental procedures – Melting points were measured on a Mitamura-Riken apparatus and are uncorrected. The IR spectra were obtained on a JASCO FT/IR-5300 spectrometer. The NMR spectra were measured in pyridine-d_5 either on a Bruker AMX-500 or a Gemini 2000 instrument, and the chemical shifts were referenced to TMS. TLC was performed on silica gel $60F_{254}$ (Merck) and cellulose plates (Art No. 5716, Merck).

Plant material – The aerial parts of *A. continentalis* was collected in May 1993, Corp Experimental Station, RDA, Suwon, Korea.

Extraction and isolation – The dried aerial parts of *A. continentalis* (1.4 kg) was extracted three times with MeOH at room temperature. The MeOH extract was evaporated to dryness, and the dry residue was partitioned in succession between H_2O and *n*-

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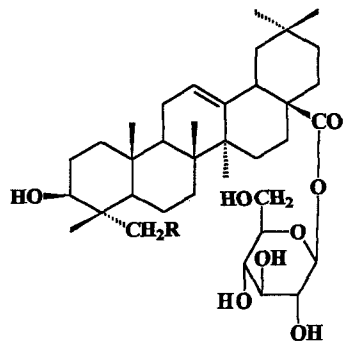
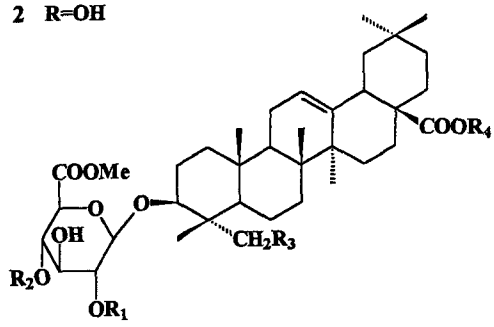
hexane, $CHCl_3$, EtOAc and then *n*-BuOH affording 37.3, 7.8, 10 and 53.5 g of the respective extracts. A portion of the BuOH fraction (20 g) was subjected to SiO_2 column chromatography. Elution with $CHCl_3$-MeOH-H_2O (7:3:1) gave 17 subfractions. Subfraction 14 was purified by a column with SiO_2 eluted with EtOAc saturated with H_2O-MeOH (gradient) to give subfactions. Subfaction 8 and 9 were separately crystallized from MeOH to give compound **1**. Subfraction 11 was crystallized from MeOH to yield compound **2**. A portion of BuOH fraction (8 g) was treated with 0.02N -H_2SO_4 in 60% dioxane solution overnight, filtered and fractionated with BuOH. The BuOH soluble portion was washed with water, concentrated in vacuo to give residue which was methylated with CH_2N_2 to yield methylated saponin fraction. The methylated saponin fraction was subjected to column chromatography on SiO_2 eluted with $CHCl_3$-MeOH-H_2O (10:2:0.5) to give 25 subfractions. Subfractions 4, 10, 14 and 23 were separately chromatographed on a SiO_2 column with EtOAc, EtOAc saturated with H_2O and EtOAc saturated with H_2O-MeOH (gradient) to give compound **3** from subfraction 4, compound **4** from subfraction 10, and compound **5** from subfraction 14, and compounds **6** and **7** from subfraction 23, respectively.



1 R=H

2 R=OH



3 R_1=R_3=H, R_2=Xyl, R_4=Me

4 R_1=R_2=R_3=H, R_4=Glc

5 R_1=R_3=H, R_2=Xyl, R_4=Glc

6 R_1=Gal, R_2=H, R_3=OH, R_4=Glc

7 R_1=Gal, R_2=Xyl, R_3=H, R_4=Glc

Compound **1** was crystallized from MeOH as needles, mp 237~240℃; IR (KBr) ν_{max} 3439, 1735, 1074 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125.8 MHz): see Tables 1 and 2.

Compound **2** was crystallized from MeOH as needles, mp 233~235℃; IR (KBr) ν_{max} 3430, 1736, 1076 cm^{-1}; ^1H NMR (pyridine-d_5, 300 MHz) and ^{13}C NMR (pyridine-d_5, 75.5 MHz): see Tables 1 and 2.

Compound **3** was crystallized from MeOH as needles, mp 168~173℃; IR (KBr) ν_{max} 3447, 1734, 1041 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125.8 MHz): see Tables 1 and 2.

Compound **4** was crystallized from MeOH as stout needles, mp 197~205℃; IR (KBr) ν_{max} 3450, 1741, 1072 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125.8 MHz): see Tables 1 and 2.

Compound **5** was obtained as amorphous powder, mp 229~231℃; IR (KBr) ν_{max} 3430, 1742, 1074 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125.8 MHz): see Tables 1 and 2.

Compound **6** was obtained as amorphous powder, mp 210~215℃; IR (KBr) ν_{max} 3422, 1736, 1074 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125.8 MHz): see Tables 1 and 2.

Compound **7** was obtained as amorphous powder, mp 215~218℃; IR (KBr) ν_{max} 3424,

Table 1. ^{1}H NMR chemical shifts of the saponins in pyridine-d_5

Proton	1	2	3	4	5	6	7
CH_3	0.89 s	0.88 s	0.80 s	0.82 s	0.80 s	0.86 s	0.80 s
	0.91 s	0.88 s	0.82 s	0.88 s	0.87 s	0.87 s	0.87 s
	0.93 s	1.00 s	0.91 s	0.90 s	0.89 s	0.90	0.89 s
	1.02 s	1.06 s	0.92 s	0.96 s	0.95 s	1.02 s	1.03 s
	1.13 s	1.16 s	0.97 s	1.08 s	1.07 s	1.10 s	1.07 s
	1.22 s	1.19 s	1.22 s	1.25 s	1.24 s	1.18 s	1.23 s
	1.24 s		1.28 s	1.29 s	1.26 s		1.24 s
H-3	3.43 dd	4.21 m	3.30 dd	3.35 dd	3.29 dd		
	(5.7, 9.7)		(4.2, 11.7)	(4.2, 11.7)	(3.9, 11.5)		
H-12	5.45	5.46	5.35	5.41	5.40	5.40	5.40
Anomeric H	6.31 d	6.35 d	4.91 d	4.98 d	4.91 d	5.13 d	4.86 d
	(7.7)	(8.1)	(7.6)	(7.7)	(7.6)	(7.6)	(7.6)
			4.95 d	6.31 d	4.95 d	5.28 d	4.91 d
			(7.8)	(8.2)	(7.8)	(7.7)	(6.6)
					6.32 d	6.31 d	5.23 d
					(8.0)	(8.1)	(7.6)
							6.32 d
							(8.1)
$COOCH_3$			3.85 s	3.72 s	3.85 s	3.66 s	3.83 s
			3.69 s				

Figures in parentheses are coupling constants in Hz.

1736, 1076 cm^{-1}; 1H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125.8 MHz): see Tables 1 and 2.

Acid hydrolysis of saponins – Acid hydrolysis of saponin was refluxed with 5% HCl in 60% aqueous dioxane (10 ml) for 3 hr. The resulting solution was evaporated under reduced pressure, and the hydrolysate was extracted with ether. The ether extract was evaporated to yield oleanolic acid from compounds **1, 4, 5** and **7**, oleanolic acid methylester from **3**, and hederagenin from **2** and **6**, which were identified by direct comparison with authentic samples. The H_2O layer was neutralized with Ag_2CO_3, filtered, and the filtrate was concentrated under reduced pressure. The residue was compared with standard sugars by cellulose TLC [pyridine-EtOAc-HOAc-H_2O (36:36:7:21)], which indicated that the sugars to be glucose from compounds **1** and **2**, glucuronic acid and xylose from **3**, glucuronic acid and glucose from **4**, glucuronic acid, xylose and glucose from **5**, glucuronic acid, galactose, and glucose from **6**, and glucuronic acid, galactose, xylose and glucose from **7**.

Alkaline hydrolysis of compound 5 – Compound **5** (20 mg) was refluxed with 5% alcoholic NaOH solution for 30 min, neutralized with dil-HCl and filtered. The residue was methylated with CH_2N_2 followed by column chromatographic purification on SiO_2 eluted with EtOAc, EtOAc saturated with H_2O and EtOAc saturated with H_2O-MeOH (gradient) to give prosapogenin **5a**. The physical properties and NMR spectra of the prosapogenin **5a** were in good agreement with compound **3** by direct comparison.

Partial acid hydrolysis of saponin 7 – Compound **7** was refluxed with 0.5% HCl in MeOH (10 ml) for 0.5 hr. The resulting solution was neutralized with Ag_2CO_3, filtered, and the filtrate was concentrated under reduced pressure. The residue was compared with standard saponins by TLC [$CHCl_3$-MeOH-H_2O (10:2:0.5)], which indicated that one of the prosapogenins to be compound **5**.

Table 2. ^{13}C NMR chemical shifts of the saponins in pyridine-d_5

Carbon No.		1	2	3	4	5	6	7
C- 3		78.2	73.3	89.4	89.4	89.3	82.4	89.2
C-28		176.5	176.2	178.2	176.6	176.4	176.5	176.2
Me				51.8				
C-3								
GlcA	C-1			107.1	107.4	106.9	104.3	104.8
	C-2			74.3	75.6	74.1	83.5	81.9
	C-3			75.2	77.4	75.0	76.9	73.9
	C-4			81.4	73.3	81.3	74.3	80.2
	C-5			75.4	78.1	75.2	77.77	73.9
	C-6			170.1	170.6	169.9	170.5	169.4
	Me			52.6	52.2	52.4	52.1	52.2
Xyl	C-1			105.5		105.3		105.0
	C-2			76.0		75.9		75.2
	C-3			78.2		78.0		77.8
	C-4			70.9		70.7		70.5
	C-5			67.6		67.4		67.1
Gal	C-1						106.8	106.3
	C-2						72.8	73.9
	C-3						75.1	74.6
	C-4						69.8	69.3
	C-5						77.2	76.7
	C-6						61.6	61.2
C-28								
Glc	C-1	95.8	95.2		95.9	95.8	95.8	95.5
	C-2	74.2	73.3		74.3	74.1	74.6	74.3
	C-3	79.3	78.7		79.1	78.9	79.4	78.7
	C-4	71.3	72.6		71.4	71.2	71.2	70.9
	C-5	79.0	78.1		79.5	79.3	79.0	79.1
	C-6	62.4	61.4		62.5	62.3	62.3	62.0

Results and Discussion

The dried aerial parts of *A. continentalis* was extracted with MeOH. The residue left after evaporation of MeOH was successively fractionated with hexane, $CHCl_3$ EtOAc and *n*-BuOH. The *n*-BuOH fraction was subjected to SiO_2 column chromatography to give subfractions, among which two nonpolar acylated saponins **1** and **2** were obtained and identified as 28-O-β-D-glucopyranosyl esters of oleanolic acid and hederagenin, respectively, by spectral data and direct comparison with authentic samples. A portion of BuOH fraction was methylated with CH_2N_2 and subjected to SiO_2 column chromatography to give **3-7** which were assumed to be saponins on the basis of their positive colouration in Liebermann-Burchard and Molisch reagents. Acid hydrolysis of the compounds yielded oleanolic acid except compounds **3** and **6**, which gave oleanolic acid methylester and hederagenin, respectively, as the aglycone and glucuronic acid and xylose from **3**, glucuronic acid and glucose from **4**, glucuronic acid, xylose and glucose from **5**, glucuronic acid, galactose, and glucose from **6**, and glucuronic acid, galactose, xylose and glucose from **7** as sugar components, respectively. These results indicated that compounds **4-7** suggested to be bisdesmosides. Compound **3** showed two anomeric proton resonances at δ4.91 (1H, d, J=7.6 Hz) and 4.95 (1H, d, J=7.8 Hz) in its

^{1}H NMR spectrum, suggesting that **3** contains 1 mol each of glucuronic acid and xylose in the molecule. It showed signals for the typical terminal xylosyl unit as well as a downfield shifted carbon signal for glucuronic acid C-4 at δ81.4 ppm in the ^{13}C NMR spectra suggesting that xylosyl unit was linked at C-4 of glucuronic acid. Therefore the structure of **3** was determined as oleanolic acid 3-O-β-D-xylopyranosyl(1 → 4)-β-D-glucuronopyranoside (udosaponin A) which was isolated from Japanese Dokwhal, Udo (Kawai *et al*, 1989). The assignments of ^{1}H and ^{13}C NMR data of sugar moieties were carried out by using homo COSY, TOCSY and HMQC and supported the above conclusion (Kang, 1996). Compound **4** exhibited two anomeric proton signals at δ4.98 (1H, d, *J*=7.7 Hz) and 6.31 (1H, d, *J*= 8.2 Hz) in its ^{1}H NMR spectrum. Taking into account one set each of β-D-glucopyranose (δ_C 95.9, 74.3, 79.1, 71.4, 79.5, 62.5) and β-D-6-O-methyl glucuronopyranose (δ_C 107.4, 75.6, 77.4, 73.3, 78.1, 170.6, 52.2) in the ^{13}C NMR signals, β-D-6-O-methyl glucuronopyranose was linked at C-3 and β-D-glucopyranose was acylated at C-28 in the molecule. Accordingly, compound **4** was determined to be 3-O-β-D-glucuronopyranosyl oleanolic acid 28-O-β-D-glucopyranosyl ester (silphioside A; chikusetsusaponin IVa). This compound is one of the well-known saponin and has found in a number of plants (Kang, 1996). Compound **5** is one of major components and showed three anomeric signals (δ_H 4.91, d, *J*=7.6 Hz; 4.95, d, *J*=7.8 Hz and 6.32, d, *J*= 8.0 Hz; δ_C 106.9, 105.3 and 95.8) in its NMR spectrum. In the ^{13}C NMR spectrum, it showed signals for udosaponin A moiety as well as a set of the acylated glucose unit. Saponification of 5 with KOH followed by chromatographic purification afforded a prosapogenin(**5a**) which was identified as udosaponin A methylester by direct comparison with an authentic sample. Therefore, the structure of compound **5** was determined to be 3-O-β-D-xylopyranosyl(1 → 4)-β-D-glucuronopyranosyl oleanolic acid 28-O-β-D-glucopyranosyl ester (salsoloside C). Compound **6** showed three anomeric signals (δ_H 5.13, d, *J*= 7.6 Hz; 5.28, d, *J*=7.7 Hz and 6.31, d, *J*=8.1 Hz; δ_C 104.3, 106.8 and 95.8) in its NMR spectrum, which suggested that saponin **6** is a hederagenin bisdesmosidic trisaccharide carrying an acylated glucosyl moiety linked at C-28, and a galactosyl-6-O-methylglucuronosyl unit at C-3 of hederagenin. The HMQC and DEPT experiments permitted assignments of the interglycosidic linkages by comparison of the ^{13}C shifts observed with those of the corresponding methyl pyranosides and taking into account the known effects of glycosidation (Seo *et al.*, 1978). Therefore the structure of compound **6** was deduced to be 3-O-β-D-galactopyranosyl (1 → 2)-β-D-glucuronopyranosyl hederagenin 28-O-β-D-glucopyranosyl ester (udosaponin F) which was isolated from *A. cordata*. Compound **7** suggested an oleanolic acid bisdesmosidic tetrasaccharide. Analysis of NMR data of compound **7** and comparison with those of **6** showed **7** to differ from **6** only in the presence of one additional xylosyl unit. The additional xylosyl unit was located C-4 of glucuronopyranosyl unit on the basis of the analysis of its spectral data. These results were further supported by the partial acid hydrolysis of this compound. Partial acid hydrolysis of compound **7** gave prosapogenins, one of which was identical with salsoloside C(**5**). From the above results, the structure of saponin **7** was determined to be 3-O-β-D-galactopyranosyl(1 → 2)-[β-D-xylopyranosyl(1 → 4)]-β-D-glucuronopyranosyl oleanolic acid 28-O-β-D-glucopyranosyl ester (udosaponin C). In light of the above observations, it is suggested that the chemical components from Korean Dokwhal (*Aralia continentalis*) are practically identical to those of Japanese Udo (*A. cordata*) supporting the taxonomical point of view.

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