Studies on the Structures of Udosaponins A, B, C, D, E and F from *Aralia cordata* THUNB.

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Six new triterpenoid saponins, named udosaponins A, B, C, D, E and F, were isolated as methyl esters, 2, 6, 7, 8, 9 and 11, respectively, along with five known saponins as methyl esters, 1, 3, 4, 5 and 10, from the aerial parts of *Aralia cordata* THUNB. (Araliaceae). On the basis of ^{13}C-nuclear magnetic resonance spectra and chemical evidence, these saponins were established to be oleanane-type saponins having a glucuronopyranosyl residue at the C-3 position of oleanolic acid and hederagenin.

Keywords *Aralia cordata*; Araliaceae; saponin; udosaponin; oleanolic acid; hederagenin; ^{13}C-NMR; *A*-value

Roots of *Aralia* (*A.*) *cordata* THUNB. (Araliaceae) are used as a traditional Chinese medicine (as Dokkatsu)[1] and young stems of this plant are popular edible plants in Japan. From the roots of this plant, several kinds of diterpenes having pimarane and kaurane skeletons, have been isolated,[2] but there have been only a few reports on the constituents of the aerial parts of the plant. Recently we studied the antibacterial constituents of the aerial parts of the plant and isolated farcarindiol, dehydrofarcarindiol and (−)-pimara-8(14),15-dien-19-oic acid as potent antibacterial constituents.[3] In the course of the studies, the presence of saponins in the polar fraction of the methanol (MeOH) extract of the plant was suggested. From other plants of the same family, *Panax* (*P.*) *ginseng*[4] and *P. japonica*,[5] many kinds of saponins have been isolated. We therefore carried out a study of the saponins of the title plant. This paper deals with the isolation and structural elucidation of the saponins from the aerial parts of the plant.

The dried aerial parts of the title plant collected in Tsukuba-shi were extracted with hot MeOH to give an MeOH extract, which was treated as summarized in Fig. 1 to give six new saponins, named udosaponins A, B, C, D, E and F, as their methyl esters, **2**, **6**, **7**, **8**, **9**, **11**, respectively, along with several known saponins as their methyl esters, **1**, **3**, **4**, **5** and **10**. The main saponins, **5′** and **10′**, were isolated as free carboxyl derivatives. The structures of these saponins were elucidated on the basis of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) and circular dichroism (CD) spectra, and chemical evidence.

The structures of the methyl esters of the saponins, **1**,[6] **3**,[6] **4**,[7] **5**[8] and **10**,[8] were elucidated from the ^{13}C-NMR spectra and by methanolysis followed by analysis of sugar composition by means of the exciton chirality method[9,10] with the per-*p*-bromobenzoates; the aglycones were identified as oleanolic acid and hederagenin by comparison with authentic samples by thin layer chromatography (TLC). The structures were confirmed by comparisons of various data with reported values. The methyl esters of the saponins **5** and **10** were identical with methyl esters of salsoloside D[8] and C,[8] respectively.

From the ^{13}C-NMR spectrum (Table I) and the elemental analysis of udosaponin A methyl ester (**2**), the molecular formula of **2** was supposed to be $C_{43}H_{68}O_{13}$. The ^{13}C-NMR spectrum of **2** showed the presence of the methyl oleanolate moiety (δ 177.8, C-28) having a sugar moiety at C-3 (δ 89.2), a 6-*O*-methylglucuronopyranosyl moiety (δ 169.7, C-6) having a glycosylation shift[11-13] at C-4 (δ 81.1) and a terminal xylopyranosyl moiety. The methanolysis of **2** gave an aglycone which was identical with oleanolic acid, and the methyl sugars gave a spot identical with methyl 6-*O*-methylglucuronopyranoside on TLC. Per-*p*-bromobenzo-

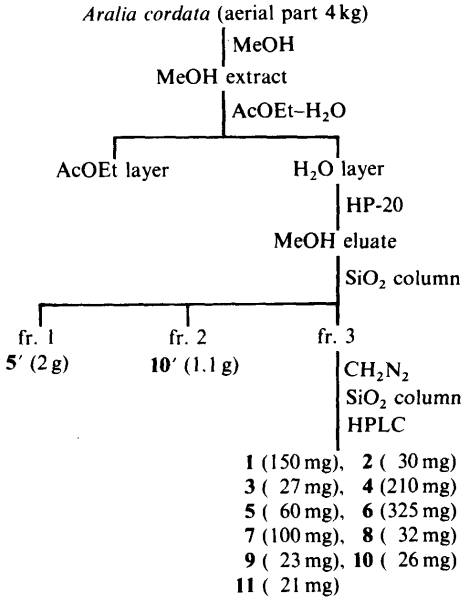
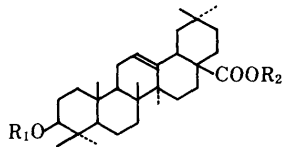
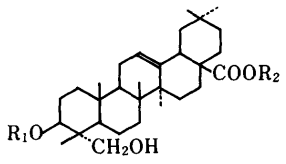


Fig. 1. Isolation of the Saponins



	R_1	R_2
1:	glcA	Me
2:	glcA^{4}xyl	Me
3:	glcA	glc
4:	glcA^{2}gal	Me
5:	glcA^{4}xyl	glc
6:	glcA^{2}gal	glc
7:	glcA^{2}gal (xyl^4)	glc



	R_1	R_2
8:	glcA^{4}xyl	Me
9:	glcA^{2}gal	Me
10:	glcA^{4}xyl	glc
11:	glcA^{2}gal	glc

glcA; 6-*O*-Me-*β*-D-glucuronopyranosyl
glc; *β*-D-glucopyranosyl
gal; *β*-D-galactopyranosyl
xyl; *β*-D-xylopyranosyl

Chart 1

ates of the methyl sugars were analyzed by high performance liquid chromatography (HPLC) and identified as methyl 6-*O*-methylglucuronopyranoside and methylxylopyranoside derivatives; these identifications were confirmed by the *A*-values[14] (Table II) obtained from the CD spectra, which coincided with the *A*-values of authentic derivatives of the methyl sugars. Thus, the structure of the new saponin, udosaponin A, was concluded to be 3-*O*-[β-D-xylopyranosyl-(1→4)-β-D-glucuronopyranosyl]-oleanolic acid.

Udosaponin B methyl ester (**6**) gave the molecular formula, $C_{49}H_{78}O_{19}$ from the fast atom bombardment mass spectrum (FAB-MS) and the elemental analysis. The methanolysis of **6** gave oleanolic acid and methyl sugars, whose per-*p*-bromobenzoate derivative were identical with the authentic per-*p*-bromobenzoate derivatives of 6-*O*-methylglucuronopyranoside, α-methyl- and β-methylglucopyranosides, and α-methyl- and β-methylgalactopyranosides by HPLC. The *A*-value of the HPLC fractions corresponding to the benzoates of the methyl sugars were identical with those of authentic samples (Table II). The ^{13}C-NMR spectrum of **6** showed the presence of the oleanolic acid moiety, the 6-*O*-methylglucuronopyranosyl moiety having a glycosylation shift at C-2 (δ 83.5), a terminal galactopyranosyl moiety and a terminal glucopyranosyl moiety. One of the sugar moieties was linked to the C-28 carboxyl group (δ 176.3); the anomeric carbon appeared at characteristic field (δ 95.6). Alkaline hydrolysis of **6** gave a prosapogenin, which was identical with the saponin **4** after methylation. From these data, the structure of udosaponin B was concluded to be 3-*O*-[β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl]-28-*O*-β-D-glucopyranosyloleanolic acid.

The FAB-MS and the elemental analysis of udosaponin C methyl ester (**7**) gave the molecular formula, $C_{54}H_{86}O_{23}$, which was supported by the ^{13}C-NMR spectrum. Saponin **7** was methanolized to give oleanolic acid and methyl sugars, which were identified as methyl 6-*O*-methylglucuronopyranoside, methylgalactopyranoside, methyl glucopyranoside and methyl xylopyranoside, in the same way as in the case of udosaponin B. Partial acid hydrolysis of **7** gave two prosapogenins, which were identical with **5** and **6**. The ^{13}C-NMR spectrum of **7** showed the presence of glycosylation shifts at C-3 (δ 89.3) and C-28 (δ 176.2) of the oleanolic acid moiety, and at C-2 (δ 81.9) and C-4 (δ 80.2) of 6-*O*-methylglucuronopyranosyl moiety, and the presence of the glucopyranosyl moiety linked to C-28 as an ester (δ 95.5) and a terminal xylopyranosyl moiety. From these data, the structure of udosaponin C was concluded to be 3-*O*-[β-D-galactopyranosyl-(1→2)-{β-D-xylopyranosyl-(1→4)}-β-D-glucuronopyranosyl]-28-*O*-β-D-glucopyranosyloleanolic acid.

The ^{13}C-NMR spectrum of udosaponin D methyl ester (**8**) showed the presence of a 28-*O*-methylhederagenin residue having glucosylation shifts at C-3 (δ 82.5) and the same sugar moiety as those of **2**. Udosaponin D methyl ester was methanolized to give an aglycone and methyl sugars. The aglycone was identified as hederagenin and the methyl sugars were identified as methyl 6-*O*-methylglucuronopyranoside and methyl xylopyranoside in the same way as in the case of **2**. Thus, the structure of this new saponin, udosaponin D, was concluded to be 3-*O*-[β-D-xylopyranosyl-(1→4)-β-D-glucuronopyranosyl]hederagenin.

Table I. ^{13}C-NMR Chemical Shifts of the Saponins (in Pyridine-d_5)

	1	2	3	4	5	6	7	8	9	10	11
C-3	89.1	89.2	89.1	89.1	89.1	89.2	89.3	82.5	82.0	82.3	82.5
C-28	177.9	177.8	176.3	177.8	176.2	176.3	176.2	177.8	177.8	176.2	176.7
Me	51.5	51.5		51.6				51.4	51.4		
C-3											
glcA-1	107.1	106.7	107.1	105.2	106.5	105.1	104.8	105.1	104.2	104.8	104.2
glcA-2	75.3	73.9	75.3	83.5	73.8	83.5	81.9	73.8	82.9	73.7	83.2
glcA-3	77.8[a]	74.9[a]	77.8[a]	76.7[a]	74.8	76.7[a]	73.9[a]	74.8	76.7[a]	74.8	76.8[a]
glcA-4	73.0	81.1	73.0	74.4	80.9	74.0[b]	80.2	81.0	74.2	80.8	74.1[b]
glcA-5	77.0[a]	75.0[a]	77.1[a]	77.3[a]	74.8	77.3[a]	73.9[a]	74.8	77.3[a]	74.8	77.6[a]
glcA-6	170.6	169.7	170.7	170.2	169.6	170.2	169.4	169.6	170.2	169.5	170.5
glcA-Me	51.9	52.3	51.9	51.9	52.2	52.0	52.3	52.2	51.7	52.1	52.2
xyl-1		105.1			104.9		104.8	105.9		105.7	
xyl-2		75.7			75.5		75.2	75.6		75.4	
xyl-3		77.8			77.6		77.7	77.7		77.5	
xyl-4		70.5			70.9[a]		70.5[b]	70.5		70.8[a]	
xyl-5		67.2			67.0		67.1	67.1		66.9	
gal-1				106.9		106.9	106.2		106.4		106.5
gal-2				72.6		72.7	73.9[a]		72.4		72.8
gal-3				74.7		74.8	74.6		74.7		75.0
gal-4				69.4		69.4	69.3		69.4		69.7
gal-5				76.6[a]		76.7[a]	76.7		76.5[a]		77.0[a]
gal-6				61.2		61.2	61.2		61.3		61.5
C-28											
glc-1			95.6		95.4	95.6	95.5			95.4	95.8
glc-2			74.0		73.8	74.4[b]	74.2[a]			73.7	74.3[b]
glc-3			78.8		78.5[b]	79.1[c]	78.6[c]			78.5[b]	79.2[c]
glc-4			70.7		70.4[a]	71.0	70.9[b]			70.3[a]	71.2
glc-5			79.1		78.9[b]	78.7[c]	79.0[c]			78.8[b]	78.8[c]
glc-6			62.1		62.0	62.1	62.1			61.9	62.4

a—*c*) Assignments may be interchangeable within the same column.

Table II. Some Examples of the *A*-Values[a] of the Sugar Benzoates Derived from the Saponins

Peaks corresponding to	2	5	6	7	11
α-Me-xyl-Bz	−1	0		−8	
β-Me-xyl-Bz	0			+7	
α-Me-glc-Bz		+20	+19	+21	+24
β-Me-glc-Bz		+31	+31	+32	+32
α-Me-gal-Bz			+95	+96	+99
β-Me-gal-Bz			+89	+94	+95
α-Me-6-*O*-Me-glcA-Bz	−4	−5	−8	−4	−7

a) These *A*-values showed good agreement with those of authentic materials.[14]

The ^{13}C-NMR spectrum of udosaponin E methyl ester (**9**) showed the presence of the hederagenin methyl ester moiety having a glycosylation shift at C-3 (δ 82.0) and the same sugar moieties as those of **4** (Table I). The methanolysis of **9** gave hederagenin as the aglycone and methyl sugars, which were identified as methyl 6-*O*-methylglucuronopyranoside and methylgalactopyranoside. Thus, the structure of udosaponin E was concluded to be 3-*O*-[β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl]hederagenin.

The FAB-MS and elemental analysis of udosaponin F methyl ester (**11**) gave the molecular formula, $C_{49}H_{78}O_{20}$. Saponin **11** was methanolized to give hederagenin and methyl sugars, which were identified as methyl 6-*O*-methylglucuronopyranoside, methyl glucopyranoside and methylgalactopyranoside in the same way as udosaponin B. The ^{13}C-NMR spectrum of **11** showed the presence of a terminal glucopyranosyl moiety linked to C-28 (δ 95.8) as an ester (δ 176.7), and a terminal galactopyranosyl moiety and 6-*O*-methylglucuronopyranosyl moiety having a glycosylation shift at C-2′ (δ 83.2). Alkaline hydrolysis of **11** gave a prosapogenin, whose methyl ester was identical with **9**. From these data, the structure of udosaponin E was concluded to be 3-*O*-[β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl]-28-*O*-β-D-glucopyranosylhederagenin.

Experimental

^{1}H- and ^{13}C-NMR spectra were measured on a JEOL JNM-FX-90 NMR spectrometer at 89.55 and 22.5 MHz, respectively, in pyridine-d_5; chemical shifts are given in ppm with tetramethylsilane as an internal standard. FAB-MS were recorded on a JEOL JMS-PX303 mass spectrometer. Ultraviolet (UV) spectra were recorded on a Shimadzu UV-360 spectrometer. CD spectra were recorded on a JASCO J-20A automatic recording spectropolarimeter. Optical rotations were determined on a JASCO DIP-140 digital polarimeter. Column chromatography was carried out on Silica gel type 60 (Merck).

Isolation of Saponins Dried aerial parts of *A. cordata* (4.0 kg), collected in September, 1985, at Tsukuba-shi, were extracted with boiling MeOH to give an MeOH extract. The MeOH extract was concentrated and fractionated between AcOEt and water. The resultant water layer was passed through Diaion HP-20 (Mitsubishi Chemical Industry Ltd.) and eluted with water, 50% MeOH and MeOH, successively. The MeOH eluate showed the presence of saponins on TLC and was repeatedly chromatographed on a silica gel (SiO_2) column using two kinds of solvent systems [$CHCl_3$–MeOH–H_2O (65:35:1, lower phase) and $CHCl_3$–MeOH–H_2O (7:13:8, lower phase)], to give salsolosides D (**5′**) (2 g) and C (**10′**) (1.1 g). The residual crude fraction was methylated with diazomethane, then repeatedly chromatographed and further purified by HPLC (Deverosil C-8 column) using various acetonitrile–water solvent systems to give methyl esters of saponins, **1** (150 mg), **2** (30 mg), **3** (27 mg), **4** (210 mg), **5** (60 mg), **6** (325 mg), **7** (100 mg), **8** (32 mg), **9** (23 mg), **10** (26 mg) and **11** (21 mg), as shown in Fig. 1.

Udosaponin A Methyl Ester (**2**): Colorless needles, mp 135—137°C (MeOH), $[\alpha]_D$ −11.2 (c=1.2, MeOH). *Anal.* Calcd for $C_{43}H_{68}O_{13}\cdot 2H_2O$: C, 62.30; H, 8.75. Found: C, 62.35; H, 8.33. The ^{13}C-NMR data are given in Table I.

Udosaponin B Methyl Ester (**6**): White powder, $[\alpha]_D$ −2.4 (c=1.1, MeOH). FAB-MS m/z: 969 $(M-H)^+$, $C_{49}H_{77}O_{19}$. *Anal.* Calcd for $C_{49}H_{78}O_{19}\cdot 5/2H_2O$: C, 57.91; H, 8.23. Found: C, 58.08; H, 7.92. The ^{13}C-NMR data are given in Table I.

Udosaponin C Methyl Ester (**7**): White powder, $[\alpha]_D$ −8.2° (c=0.8, MeOH). FAB-MS m/z: 1101 $(M-H)^+$, $C_{54}H_{85}O_{23}$. *Anal.* Calcd for $C_{54}H_{86}O_{23}\cdot 2H_2O$: C, 56.93; H, 7.96. Found: C, 56.76; H, 7.77. The ^{13}C-NMR data are given in Table I.

Udosaponin D Methyl Ester (**8**): White powder, $[\alpha]_D$ −4.7° (c=1.0, MeOH). The ^{13}C-NMR data are given in Table I. Elemental analysis was not done because the amount of sample was too small, and the compound was hygroscopic.

Udosaponin E Methyl Ester (**9**): White powder, $[\alpha]_D$ −12.3° (c=1.0, MeOH). The ^{13}C-NMR data are given in Table I. Elemental analysis was not done because the amount of sample was too small, and the compound was hygroscopic.

Udosaponin F Methyl Ester (**11**): White powder, $[\alpha]_D$ +1.5°(c=1.2, MeOH). FAB-MS m/z: 987 $(M+H)^+$, $C_{49}H_{79}O_{20}$. *Anal.* Calcd for $C_{49}H_{78}O_{20}\cdot 3H_2O$: C, 56.52; H, 8.13. Found: C, 56.76; H, 7.89. The ^{13}C-NMR data are given in Table I.

Saponin 1 Methyl Ester (**1**): Colorless needles, mp 230—233°C (MeOH), $[\alpha]_D$ +9.4° (c=0.2, MeOH). *Anal.* Calcd for $C_{38}H_{60}O_9\cdot 1/2H_2$: C, 68.13; H, 9.18. Found: C, 68.44; H, 9.06. The ^{13}C-NMR data are given in Table I.

Saponin 3 Methyl Ester (**3**): White powder. The ^{13}C-NMR data are given in Table I. Elemental analysis was not done because of the small amount of sample available, and because the compound was hygroscopic.

Saponin 4 Methyl Ester (**4**): White powder, $[\alpha]_D$ −23.7° (c=0.9, MeOH). The ^{13}C-NMR data are given in Table I. Elemental analysis was not done because the amount of sample was too small, and the compound was hygroscopic.

Saponin 5 Methyl Ester (**5**): Colorless prisms, mp 228—230°C (MeOH), $[\alpha]_D$ −28.3° (c=0.5, MeOH). The ^{13}C-NMR data are given in Table I. FAB-MS of the free carboxyl derivative (**5′**) showed m/z 1003 $[(C_{46}H_{73}O_{16}COOK)+K]^+$.

Saponin 10 Methyl Ester (**10**): Colorless prisms, mp 233—236°C (MeOH), $[\alpha]_D$ −30.8° (c=0.5, MeOH). The ^{13}C-NMR data are given in Table I. FAB-MS of the free carboxy derivative (**10′**) showed m/z 1019 $[(C_{46}H_{73}O_{17}COOK)+K]^+$.

Methanolysis of Saponin Methyl Esters A solution of each sample (*ca.* 1 mg) in 5% HCl–MeOH (3 ml) was refluxed for 2 h. The reaction solution was neutralized with silver carbonate. The neutralized solution was filtered and analyzed by TLC ($CHCl_3$: MeOH=20:1) and HPLC (column, TSK gel 80 Tm, using 90% CH_3CN) with authentic oleanolic acid and hederagenin as standards.

Analysis of the Methyl Sugars The neutralized solution was evaporated to dryness and benzoylated with 1 ml of pyridine and excess *p*-bromobenzoyl chloride at 90°C overnight. After addition of water, the mixture was extracted with hexane. The hexane solution was evaporated. The residue was taken up in 0.5 ml of MeOH, and subjected to TLC and HPLC (Shiseido Capcell Pak C18, 95% CH_3CN, 1 ml/min, 245 nm detection) in comparison with authentic *p*-bromobenzoate derivatives. These derivatives gave peaks as follows: α-Me-glcA perbenzoate at 7.7 min, α-Me-xyl perbenzoate at 10.0 min, β-Me-gal perbenzoate at 13.2 min, β-Me-glc perbenzoate at 14.0 min, α-Me-gal perbenzoate at 19.6 min and α-Me-glc perbenzoate at 20.1 min. Appropriate fractions were collected and evaporated to dryness. Each residue was taken up in a few milliliters of acetonitrile. The UV and CD spectra were measured, and the *A*-value was calculated as described in the previous paper.[10] The *A*-values of sugar benzoates derived from the saponins are shown in Table II.

Alkaline Hydrolysis of Saponins 5, 6, 10 and 11 Saponins (1 mg) in 10 ml of 0.5 N KOH were refluxed for 1 h. The reaction solution was passed through an HP-20 gel column. The MeOH eluates gave prosapogenins. These were methylated with diazomethane and identified by comparison with saponin methyl esters, **2**, **4**, **8** and **9**, respectively, by TLC.

Partial Hydrolysis of Udosaponin C Methyl Ester (7) An aqueous solution of **7** (1 mg) was passed through HP-20 gel (1 g) and the sample was adsorbed on the resin. The resin was heated with 10% HCl (3 ml) at 80°C for 1 h, then washed with water and eluted with MeOH, successively. The MeOH eluate was concentrated and methylated. The resulting prosapogenins were analyzed by TLC, revealing the presence of **5** and **6**.

Acknowledgement The authors wish to thank Mrs. H. Kitamura of the Analysis Center of this University for elemental analysis, and JEOL

Ltd. for FAB-MS measurement.

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