Four New Saponins from the Root Bark of *Aralia elata*

Shao-jiang SONG,[a,b] Norio NAKAMURA,[a] Chao-mei MA,[a] Masao HATTORI,*,[a] and Sui-xu XU[b]

Institute of Natural Medicine, Toyama Medical and Pharmaceutical University,[a] 2630 Sugitani, Toyama 930–0194, Japan and Department of Natural Product, Shenyang Pharmaceutical University,[b] Shenyang 110015, China.

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Four new saponins, 3-*O*-[β-D-glucopyranosyl (1→3)-α-L-arabinopyranosyl]-16α-hydroxyoleanolic acid 28-*O*-β-D-glucopyranosyl ester (called aralia-saponin I), 3-*O*-[β-D-glucopyranosyl(1→3)-α-L-arabinopyranosyl]-16α-hydroxyhederagenin 28-*O*-β-D-glucopyranosyl ester (aralia-saponin II), 3-*O*-[β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl(1→3)-α-L-arabinopyranosyl]-16α-hydroxyoleanolic acid 28-*O*-β-D-glucopyranosyl ester (aralia-saponin III), 3-*O*-[β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl]-16α-hydroxyoleanolic acid 28-*O*-β-D-glucopyranosyl ester (aralia-saponin IV), were isolated from the root bark of *Aralia elata* (Miq.) Seem., together with nineteen known compounds including glycosides of (20*S*)-protopanaxadiol and (20*S*)-protopanaxatriol. Their structures were determined on the basis of chemical and spectroscopy methods.

Key words Araliaceae; *Aralia elata*; 16α-hydroxyhederagenin; 16α-hydroxyoleanolic acid; saponin

Aralia elata (Miq.) Seem. (Japanese name: Taranoki) (Araliaceae) is widely distributed in the northeast of China and Korea. Its root bark has been used as a folk medicine for rheumatism, diabetes and as a tonic in China, Japan and Russia.[1)] Many saponins have been isolated from this plant, and reported to show anti-diabetic activity and a cytoprotective effect on carbon tetrachloride-induced hepatic injury.[2—7)] The present paper deals with the isolation and structure elucidation of four new saponins, called aralia-saponins I—IV (**1**—**4**), and nineteen known compounds, including nine saponins (**14**, **16**—**23**) that were isolated for the first time from this plant.

Results and Discussion

The air-dried root bark of *A. elata* was extracted with methanol. The methanol extract was suspended in water, washed with petroleum ether and chloroform, and then extracted with butanol saturated with water. The butanol soluble part was repeatedly chromatographed over normal and reversed-phase silica gel to give 23 saponins. The structures of isolated saponins are shown in Chart 1. The known compounds were identified as 3-*O*-[β-D-glucopyranosyl (1→3)-α-L-arabinopyranosyl] hederagenin 28-*O*-β-D-glucopyranosyl ester (**5**),[3)] congmuyenoside B (**6**),[4)] 28-*O*-β-D-glucopyranosyloleanolic acid (**7**),[5)] 3-*O*-[β-D-galactopyranosyl (1→6)-β-D-glucopyranosyl]oleanolic acid (**8**),[5)] elatoside F (**9**),[6)] calenduloside E (**10**),[6)] spinasaponin A (**11**),[7)] spinasaponin A 28-*O*-glucoside (**12**),[6)] calenduloside G (**13**),[5)] elatoside A (**14**),[7)] elatoside C (**15**),[7)] 3-*O*-{[β-D-glucopyranosyl (1→2)]-[α-L-arabinopyranosyl (1→3)]-β-D-glucuronopyranosyl}-oleanolic acid (**16**),[5)] (20*S*)-protopanaxadiol 3-*O*-β-D-glucopyranosyl-20-β-D-glucopyranoside (**17**),[8)] (20*S*)-protopanaxadiol 3-*O*-β-D-glucopyranosyl-20-α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside (**18**),[8)] ginsenoside Rb_2 (**19**),[9)] (20*S*)-protopanaxadiol 3-*O*-[β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-20-α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside (**20**),[8)] (20*S*)-protopanaxatriol-20-α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside (**21**),[8)] (20*S*)-protopanaxatriol 6-*O*-[β-D-glucopyranosyl (1→2)-β-D-glucopyranoside (**22**),[10)] ginsenoside Re (**23**),[10)] by comparing their spectral data with those reported previously.

Compound **1** (aralia-saponin I) showed quasi-molecular ion peaks at *m/z* 951 $[M+Na]^+$ in the positive ion electrospray ionization (ESI)-MS, and at *m/z* 927 $[M-H]^-$ in the negative ion ESI-MS, suggesting the molecular formula of $C_{47}H_{76}O_{18}$ [HR-FAB-MS, *m/z*: 951.4910 $[M+Na]^+$ (Calcd for $C_{47}H_{76}O_{18}Na$: 951.4931)]. The IR spectrum showed the presence of an ester carbonyl group at 1740 cm^{-1} and an olefinic group at 1640 cm^{-1}. The ^{1}H-NMR spectrum of **1** exhibited signals characteristic of seven singlet methyls at δ 0.87, 0.96, 0.99, 1.02, 1.12, 1.26 and 1.84, one trisubstituted olefinic proton at δ 5.59, and three anomeric protons at δ 6.33, 5.39 and 4.73. The ^{13}C-NMR spectrum of **1** showed signals of a pair of olefinic carbons at δ 122.7 and 144.4, three anomeric carbons at δ 107.3, 106.2 and 95.7, and a car-

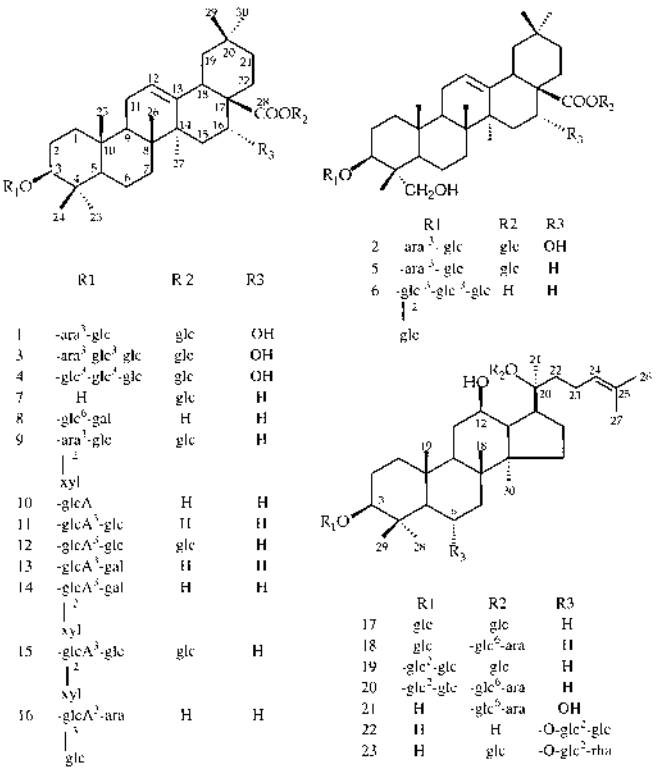

Chart 1. Structures of Compounds Isolated from the Root Bark of *Aralia elata*

* To whom correspondence should be addressed. e-mail: saibo421@ms.toyama-mpu.ac.jp

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Table 1. ^{1}H-NMR Spectrum Data of Compounds **1**—**4** in C_5D_5N (500 MHz)

	1	**2**	**3**	**4**
Aglycon				
3	3.34 (dd, *J*=4.0, 11.5 Hz)	4.25	3.34 (dd, *J*=4.0, 11.5 Hz)	3.35 (dd, *J*=4.0, 11.5 Hz)
12	5.59 (br s)	5.58 (br s)	5.59 (br s)	5.59 (br s)
16	5.31 (br s)	5.26 (br s)	5.31 (br s)	5.31 (br s)
18	3.51 (dd, *J*=4.5, 14.0 Hz)	3.48 (dd, *J*=4.0, 14.0 Hz)	3.51 (dd, *J*=4.0, 14.0 Hz)	3.51 (dd, *J*=4.0, 12.0 Hz)
23	1.26 (s)	3.66 (d, *J*=11 Hz)	1.28 (s)	1.26 (s)
		4.27 (d, *J*=11 Hz)		
24	0.96 (s)	0.91 (s)	0.97 (s)	0.98 (s)
25	0.87 (s)	0.96 (s)	0.88 (s)	0.85 (s)
26	1.12 (s)	1.34 (s)	1.12 (s)	1.12 (s)
27	1.84 (s)	1.75 (s)	1.83 (s)	1.84 (s)
29	0.99 (s)	0.95 (s)	0.98 (s)	0.98 (s)
30	1.02 (s)	1.00 (s)	1.02 (s)	1.02 (s)
3-*O*-Sugar	Ara	Ara	Ara	Glc
1′	4.73 (d, *J*=7.5 Hz)	4.93 (d, *J*=7.5 Hz)	4.70 (d, *J*=7.5 Hz)	4.85 (d, *J*=8.0 Hz)
2′	4.58[a)]	4.56[a)]	4.55[a)]	4.02
3′	4.20[a)]	4.05[a)]	4.20[a)]	4.19
4′	4.42[a)]	4.35[a)]	4.36[a)]	3.92
5′	3.59 (d, *J*=11.5 Hz)	3.59 (d, *J*=11.5 Hz)	3.69 (dd, *J*=11.5, 2.5 Hz)	4.05
	4.13 (d, *J*=11 Hz)	4.13 (d, *J*=11 Hz)		
6′				4.52[a)]
Glc				
1″	5.39 (d, *J*=8.0 Hz)	5.29 (d, *J*=8.0 Hz)	5.39 (d, *J*=8.0 Hz)	5.28 (d, *J*=8.0 Hz)
2″	4.02	4.01	4.14	4.06
3″	3.98	4.25	4.21	4.22
4″’	4.21	4.35	4.11	4.16
5″	3.95	4.26	3.91	4.42
6″	4.45[a)]	4.44[a)]	4.51[a)]	4.43[a)]
Glc				
1‴			5.28 (d, *J*=8.0 Hz)	5.31 (d, *J*=8.0 Hz)
2‴			4.18	4.04
3‴			4.23	4.21
4‴			4.17	3.92
5‴			4.01	4.42
6‴			4.45[a)]	4.52[a)]
C-28-Glc				
1	6.33 (d, *J*=8.0 Hz)	6.31 (d, *J*=8.0 Hz)	6.32 (d, *J*=8.0 Hz)	6.35 (d, *J*=8.0 Hz)
2	4.13	4.12	4.15	4.14
3	4.24	4.25	4.25	4.25
4	4.30	4.31	4.30	4.28
5	4.00	3.99	4.02	4.01
6	4.53[a)]	4.52[a)]	4.32[a)]	4.43[a)]

a) Chemical shifts of these signals were determined by ^{1}H–^{1}H COSY, HOHAHA and HMQC.

bonyl carbon at δ 175.9. On acid hydrolysis, an aglycone of **1** was determined to be 16-hydroxyoleanolic acid by comparing the ^{1}H-NMR and ^{13}C-NMR spectra data with those reported.[11] The configuration of a hydroxyl group at C-16 was determined to be α, for a nuclear Overhauser effect (NOE) correlation was found between proton signals at δ 5.31 (H-16) and δ 1.12 (H_3-26) in the nuclear Overhauser enhancement and exchange spectroscopy (NOESY) spectrum. The monosaccharide units obtained were identified by co-TLC with authentic samples as arabinose and glucose; their absolute configurations were determined as L and D-forms, respectively, by a method developed by Hara *et al.*[12] The spin systems for sugars were assigned on the basis of spectroscopic evidence obtained by ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H-detected multiple quantum coherence (HMQC) and homonuclear Harmann-Hahn (HOHAHA) experiments. The sugar components were identified as one arabinose and two glucose, their protons and carbons being assigned as shown in Tables 1 and 2. The sugar linkages were determined on the basis of the heteronuclear multiple-bond correlation (HMBC) spectrum. Long-range couplings ($^3J_{HCOH}$) were observed between a proton signal at δ 6.33 (glc-H-1) and a carbonyl carbon signal at δ 175.9 (C-28), between a proton signal at δ 4.73 (ara-H-1′) and a carbon signal at δ 88.5 (C-3), and between a proton signal at δ 5.39 (glc-H-1″) and a carbon signal at δ 84.0 (ara-C-3′), suggesting glycosylation at C-3 with a glc (1→3) ara moiety and at C-28 with a glucose moiety. Furthermore, the characteristic ion peaks at *m*/*z* 789 $[M+Na-glc]^+$ in the positive ion ESI-MS, and at *m*/*z* 765 $[M-H-glc]^-$, 603 $[M-H-glc-glc]^-$ and 461 $[M-H-glc-glc-ara]^-$ in the negative ion ESI-MS confirmed the sugar linkages. The anomeric configurations of the sugar moieties were determined to be β for glucose and α for arabinose, on the basis of the J_{H-H} values (8.0 and 7.5 Hz, respectively) . From the above evidence, the structure of **1** was concluded to be 3-*O*-[β-D-glucopyranosyl (1→3)-α-L-arabinopyranosyl]-16α-hydroxyoleanolic acid 28-*O*-β-D-glucopyranosyl ester.

Compound **2** (aralia-saponin II) with HR-FAB-MS *m*/*z*: 967.4868 $[M+Na]^+$ (Calcd for $C_{47}H_{76}O_{19}Na$: 967.4879),

Table 2. ^{13}C-NMR Spectrum Data of Compounds **1**—**4** in C_5D_5N (500 MHz)

C-position	**1**	**2**	**3**	**4**
1	38.9	38.8	38.8	38.8
2	26.7	26.2	27.1	27.2
3	88.5	81.9	88.7	88.9
4	39.6	43.5	39.6	39.5
5	55.9	47.8	55.9	55.8
6	18.5	18.1	18.5	18.5
7	33.4	32.2	33.2	33.2
8	40.1	41.9	40.1	40.0
9	47.2	47.3	49.1	47.1
10	37.1	36.9	37.0	36.9
11	23.8	23.8	23.8	23.8
12	122.7	122.7	122.7	122.7
13	144.4	144.3	144.4	144.4
14	42.1	40.1	42.1	42.0
15	35.9	36.1	36.1	36.1
16	74.1	74.5	74.5	74.5
17	49.1	48.9	49.1	49.1
18	41.3	41.1	41.2	41.3
19	47.2	47.0	47.1	47.5
20	30.8	30.7	30.8	30.8
21	36.1	35.9	33.4	36.9
22	32.2	32.2	32.2	32.1
23	28.1	64.1	28.0	28.1
24	16.9	13.6	16.9	17.0
25	15.7	16.3	15.7	15.6
26	17.6	17.5	17.5	17.5
27	27.2	27.1	27.2	27.2
28	175.9	175.9	175.9	175.9
29	33.2	33.1	33.2	33.2
30	24.6	24.5	24.5	24.5
3-*O*-Sugar	Ara	Ara	Ara	Glc
1′	107.3	106.5	107.4	106.3
2′	71.8	71.9	71.9	74.5
3′	84.0	84.1	83.3	88.2
4′	69.2	69.2	69.4	69.8
5′	66.9	67.0	67.1	78.2
6′				62.2
Glc				
1″	106.2	106.3	105.7	105.8
2″	75.6	75.8	74.3	74.4
3″	78.3	78.8	88.3	88.4
4″	71.5	71.4	69.6	69.8
5″	78.6	78.3	78.2	78.1
6″	62.2	62.1	62.1	62.2
Glc				
1‴			105.9	105.2
2‴			75.6	75.6
3‴			78.1	78.7
4‴			71.5	71.6
5‴’			79.4	77.8
6‴			62.2	62.5
C-28-Glc				
1	95.7	95.7	95.8	95.9
2	74.4	74.1	74.0	74.3
3	78.8	78.6	78.3	78.9
4	71.1	70.1	70.9	71.1
5	79.3	79.3	78.1	79.3
6	62.6	62.6	62.5	62.5

showed quasi-molecular ion peaks at *m/z* 967 $[M+Na]^+$ in the positive ion ESI-MS, and at *m/z* 943 $[M-H]^-$ in the negative ion ESI-MS. The IR spectrum showed the presence of an ester carbonyl group at 1740 cm^{-1} and an olefinic group at 1640 cm^{-1}. The ^{1}H-NMR and ^{13}C-NMR spectra of **2** were similar to those of **1**, especially in the sugar region. The difference in chemical shift for the aglycone of **2** was that a methyl group at C-23 was replaced by a hydroxymethyl group (δ_H 3.66, 4.27, δ_C 64.1). The ^{1}H-NMR spectrum of **2** showed signals [δ 5.26 (br s. 16-H), δ 1.75 (s, 27-H_3)] characteristic of hederagenin bearing a 16-hydroxyl group.[13] The spin systems for sugars were assigned by detailed analysis of the two-dimensional (2D)-NMR (^{1}H–^{1}H COSY, HMQC, HOHAHA) spectra. The sugar linkages were determined on the basis of the HMBC spectrum, correlations were observed between a proton signal at δ 6.31 (glc-H-1) and a carbonyl carbon signal at δ 175.9 (C-28), whereas the proton signals at δ 4.93 (ara-H-1′) and 5.29 (glc-H-1″) showed correlations with the carbon signals at δ 81.9 (C-3) and δ 84.1 (ara-C-3′), respectively. The configuration of a hydroxyl group at C-16 was determined to be α, for an NOE correlation was found between proton signals at δ 5.26 (H-16) and δ 1.34 (H_3-26) in the NOESY spectrum. On acid hydrolysis, **2** yielded L-arabinose and D-glucose. Based on the above data, **2** was determined to be 16α-hydroxyhederagenin glycosylated at C-3 with a glc (1→3) ara moiety and at C-28 with a glucose moiety. Moreover, significant fragment ions observed at *m/z* 781 $[M-H-glc]^-$, 619 $[M-H-glc-glc]^-$ and 487 $[M-H-glc-glc-ara]^-$ in the negative ion ESI-MS confirmed the linkages of sugars. The anomeric configurations of the sugar moieties were determined to be β for glucose and α for arabinose, on the basis of the J_{H-H} values (8.0 and 7.5 Hz, respectively). From the above evidence, the structure of **2** was concluded to be 3-*O*-[β-D-glucopyranosyl(1→3)-α-L-arabinopyranosyl]-16α-hydroxyhederagenin 28-*O*-β-D-glucopyranosyl ester.

Compound **3** (aralia-saponin III), HR-FAB-MS *m/z*: 1113.5437 $[M+Na]^+$ (Calcd for $C_{53}H_{86}O_{23}Na$: 1113.5458), showed quasi-molecular ion peaks $[M+Na]^+$ at *m/z* 1113 in the positive ion ESI-MS, and at *m/z* 1089 $[M-H]^-$ in the negative ion ESI-MS. Compound **3** displayed four anomeric proton signals at δ 6.32, 5.39, 5.28 and 4.70 in the ^{1}H-NMR spectrum, and four anomeric carbon signals at δ 107.4, 105.9, 105.7 and 95.8 in the ^{13}C-NMR spectrum. Comparison of the spectral data between **1** and **3** showed that signals for an aglycone part were superimposable, indicating that the aglycone of **3** was also 16α-hydroxyoleanolic acid and the sugars were linked at both C-3 and C-28 positions. On acid hydrolysis, the monosaccharide units obtained were identified as L-arabinose and D-glucose by the method used for **1**. The spin systems for sugars were assigned on the basis of spectroscopic evidence obtained in ^{1}H–^{1}H-COSY, HMQC and HOHAHA experiments. The sugar components were identified as one arabinose and three glucose, and sugar linkages were determined by HMBC. Long-range coupling ($^3J_{HCOH}$) observed between a proton signal at δ 4.70 (ara-H-1′) and a carbon signal at δ 88.7 (C-3), between a proton signal at δ 5.39 (glc-H-1″) and a carbon signal at δ 83.3 (ara-C-3′), between a proton signal at δ 5.28 (glc-H-1‴) and a carbon signal at δ 88.3 (glc-C-3″), and between a proton signal at δ 6.32 (glc-H-1) and a carbon signal at δ 175.9 (C-28), confirmed glycosylation at C-3 with a glc(1→3)glc(1→3)-ara moiety and at C-28 with a glucose moiety. In addition, the significant fragment ions observed at *m/z* 928 $[M-glc]^-$, 766 $[M-glc-glc]^-$ and 604 $[M-glc-glc-glc]^-$ in the negative ion ESI-MS supported the linkages of sugars. The anomeric configurations of the sugar moieties were determined to be β for glucose and α for arabinose, on the basis

of the J_{H-H} values (8.0 and 7.5 Hz, respectively). From the above evidence, the structure of **3** was concluded to be 3-*O*-[β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl(1→3)-α-L-arabinopyranosyl]-16α-hydroxyoleanolic acid 28-*O*-β-D-glucopyranosyl ester.

Compound **4** (aralia-saponin IV), HR-FAB-MS *m/z*: 1143.5583 $[M+Na]^+$ (Calcd for $C_{54}H_{88}O_{24}Na$: 1143.5564), showed quasi-molecular ion peaks $[M+Na]^+$ at *m/z* 1143 in the positive ion ESI-MS, and at *m/z* 1119 $[M-H]^-$ in the negative ion ESI-MS. Compound **4** displayed four anomeric proton signals at δ 6.35, 5.31, 5.28 and 4.85 in the ^{1}H-NMR spectrum, and four anomeric carbon signals at δ 106.3, 105.8, 105.2 and 95.9 in the ^{13}C-NMR spectrum. Signals for the aglycone part were similar to those of **3**, suggesting that the aglycone of **4** was also 16α-hydroxyoleanolic acid linked with sugars at C-3 and C-28 positions. On acid hydrolysis, the monosaccharide unit was identified to be D-glucose. By detailed analysis of the 2D-NMR (^{1}H–^{1}H COSY, HMQC, HOHAHA) spectra, all signals due to the sugar protons were assigned. In the HMBC spectrum, long-range correlations were observed between a proton signal at δ 4.85 (glc-H-1′) and a carbon signal at δ 88.9 (C-3 of the aglycone), between a proton signal at δ 5.28 (glc-H-1″) and a carbon signal at δ 88.2 (glc-C-3′), between a proton signal at δ 5.31 (glc-H-1‴) and a carbon signal at δ 88.4 (glc-C-3″), and between a proton signal at δ 6.35 (glc-H-1) and a carbon signal at δ 175.9 (C-28), indicating glycosylation at C-3 with a glc (1→3)glc(1→3)glc moiety and at C-28 with a glucose moiety. The anomeric configurations of the sugar moieties were determined to be β on the basis of the J_{H-H} values (8.0 or 7.5 Hz). From this evidence, the structure of **4** was determined to be 3-*O*-[β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl(1→3)-β-D-gluco-pyranosyl]-16α-hydroxyoleanolic acid 28-*O*-β-D-glucopyranosyl ester.

Experimental

General Optical rotations were measured with a JASCO DIP-360 automatic polarimeter. IR spectra were measured with a JASCO FT/IR-230 IR spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured with a JNM-LA400WB Lambda (JEOL) NMR (^{1}H, 400 MHz; ^{13}C, 100 MHz) or a Varian UNITY 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrometer. ESI-MS were measured with a Perkin-Elmer SCIEX API III biomolecular mass analyzer. HR-FAB-MS were measured with a JEOL SX-102A mass spectrometer with a resolution of 5000 and glycerol containing a faint amount of NaCl. GC was performed on a Shimadzu GC-17A instrument.

Column chromatography was carried out on Silica-gel 60 (Merck,70—230 mesh). Medium pressure liquid chromatography (MPLC) was carried out on Lichroprep RP-18 (Merck, size A), Lichroprep Si 60 (Merck, size B). Preparative HPLC was carried out on a Gilson instrument with a 231XL injector, a 119 UV/VIS detector and a TSK-gel ODS-80TM column (21.5×300 mm, Tosoh Co.). Spots were detected after spraying with ethanol–5% H_2SO_4.

Isolation of Compounds 1—23 from the Root Bark of *Aralia elata* The root bark of *A. elata* was collected at Xinbin (Liaoning, China), and its botanical source was confirmed by Professor Qishi Shun, Shenyang Pharmaceutical University (SPU), Shenyang, China. The specimen is deposited in the Department of Natural Products of SPU. Dried bark (3.5 kg) of A. elata was extracted three times with methanol (7 l each time) for 2 h under reflux, and the combined solutions were concentrated in vacuo to a syrup (1.25 kg), then suspended in water. The suspension was extracted with petroleum ether, chloroform and *n*-butanol saturated with water, successively, to give the respective extracts after removal of the solvent. The *n*-butanol-soluble part (100 g) was subjected to silica gel 60 column chromatography (10×100 cm) with $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5) to give six fractions (fr. I—VI). Fraction III (15 g) was further separated by silica gel 60 column chromatography with a solvent system of $CHCl_3$–MeOH–H_2O (7 : 3 : 1) to yield **7** (30 mg), **8** (10 mg), **10** (7 mg) and a mixture. The mixture was then applied to RP-18 MPLC with MeOH–H_2O (7 : 3) and preparative HPLC [MeOH–0.1% trifluoroacetic acid (TFA)/H_2O (8 : 2→10 : 0), 5 ml/min, monitored at 205 nm] to afford **17** (12 mg), **18** (30 mg), **19** (400 mg), **20** (20 mg), **21** (8 mg), **22** (5 mg) and **23** (200 mg). Meanwhile, fraction V (10 g) was further separated by column chromatography on silica gel 60 with $CHCl_3$–MeOH–H_2O (6 : 4 : 1) to give three fractions (Va—Vc). Fraction Va (1.6 g) was applied to Si 60 MPLC with $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower phase) to yield **13** (20 mg) and **16** (23 mg). Fraction Vb (2.5 g) was applied to RP-18 MPLC with MeOH–H_2O (6 : 4) to yield **1** (25 mg), and then the residual mixture was applied to preparative HPLC [MeOH–0.1% TFA/H_2O (7 : 3→10 : 0), 5 ml/min, monitored at 205 nm] to afford **2** (15 mg), **3** (12 mg), **4** (4 mg), **5** (25 mg) and **6** (25 mg). Fraction Vc (1.2 g) was applied to preparative HPLC [MeOH–0.1% TFA/H_2O (7 : 3→10 : 0), 5 ml/min, monitored at 205 nm] to afford **9** (8 mg), **11** (12 mg), **12** (40 mg), **14** (7 mg) and **15** (35 mg).

3-*O*-[β-D-Glucopyranosyl(1→3)-α-L-arabinopyranosyl]-16α-hydroxyoleanolic Acid 28-*O*-β-D-Glucopyranosyl Ester (Aralia-saponin I, 1) Colorless amorphous powder, $[\alpha]_D$ −28.9° (*c*=0.15, pyridine). IR (KBr) cm^{-1}: 3400, 2950, 2880, 1740, 1640, 1464, 1394, 1300, 1278, 1030, 995. ^{1}H-NMR: Table l. ^{13}C-NMR: Table 2. Positive ion ESI-MS *m/z*: 951 $[M+Na]^+$, 789 $[M+Na-glc]^+$. Negative ion ESI-MS *m/z*: 927 $[M-H]^-$, 765 $[M-H-glc]^-$, 603 $[M-H-glc-glc]^-$, 461 $[M-H-glc-glc-ara]^-$. HR-FAB-MS *m/z*: 951.4910 $[M+Na]^+$ (Calcd for $C_{47}H_{76}O_{18}Na$: 951.4931).

Acid Hydrolysis of 1 Compound **1** (5 mg) was refluxed with 7% HCl in H_2O–EtOH (1 : 1, 1 ml) for 3 h. The reaction mixture was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was concentrated to dryness to afford 16-hydroxyoleanolic acid (1.5 mg) as an aglycone. The structure was determined by comparison of its ^{1}H-NMR and ^{13}C-NMR spectroscopic data with those of 16-hydroxyoleanolic acid.[11,13] The water layer was concentrated and checked by cellulose TLC [pyridine–ethyl acetate–acetic acid–water (36 : 36 : 7 : 2l), *Rf*: L-arabinose, 0.61; D-glucose, 0.38]. The residual water layer was used to determine the absolute configuration of sugar as reported by Hara *et al*.[12] After being desalted with Amberlite MB-3 and dried, the residue was dissolved in pyridine (0.1 ml), then 0.1 M L-cysteine methyl ester hydrochloride in pyridine (0.2 ml) was added to the sugar solution. The mixture was kept at 60 °C for 1.5 h, dried *in vacuo*, and trimethylsilylated with hexamethyl disilazane–trimethyl chlorosilane (HMDS–TMCS) (0.1 ml) at 60 °C for 1h. After partition with hexane (0.3 ml) and water (0.3 ml), the hexane layer was analyzed by GC [column, DB-1, J & W Scientific, 0.25 mm i.d.×30 m; column temperature, 50—230 °C, 15 °C/min then 230 °C, 18 min; carrier gas, He]. The sugar derivatives thus obtained showed retention times of l6.4 and 21.4 min, identical to those of authentic L-arabinose and D-glucose derivatives, respectively. Under the same conditions, derivatives of D-arabinose and L-glucose showed retention times of 17.0 and 22.0 min, respectively.

3-*O*-[β-D-Glucopyranosyl(1→3)-α-L-arabinopyranosyl]-16α-hydroxyhederagenin 28-*O*-β-D-Glucopyranosyl Ester (Aralia-saponin II, 2) Colorless amorphous powder, $[\alpha]_D$ −39.4° (*c*=0.22, pyridine). IR (KBr) cm^{-1}: 3400, 2920, 1740, 1640, 1550, 1460, 1394, 1300, 1260, 1030, 1260, 1080, 1035 and 995. ^{1}H-NMR: Table l. ^{13}C-NMR: Table 2. Positive ion ESI-MS *m/z*: 967 $[M+Na]^+$. Negative ion ESI-MS *m/z*: 943 $[M-H]^-$, 781 $[M-H-glc]^-$, 619 $[M-H-glc-glc]^-$ and 487 $[M-H-glc-glc-ara]^-$. HR-FAB-MS *m/z*: 967.4868 $[M+Na]^+$ (Calcd for $C_{47}H_{76}O_{19}Na$: 967.4879).

Acid Hydrolysis of 2 Compound **2** (1 mg) was refluxed with 7% HCl in H_2O–EtOH (1 : 1, 1 ml) for 3 h. The reaction mixture was partitioned between $CHCl_3$ and H_2O. The water layer was concentrated and checked by cellulose TLC [pyridine–ethyl acetate–acetic acid–water (36 : 36 : 7 : 2l), *Rf*: L-arabinose, 0.61; D-glucose, 0.38]. The residual water layer was desalted with Amberlite MB-3, treated with L-cysteine methyl ester hydrochloride and trimethyl-silylated with HMDS–TMCS then analyzed by GC in the same manner as described for **1**. The sugars were confirmed as L-arabinose and D-glucose, respectively.

3-*O*-[β-D-Glucopyranosyl(1→3)-β-D-glucopyranosyl(1→3)-α-L-arabinopyranosyl]-16α-hydroxyoleanolic Acid 28-*O*-β-D-Glucopyranosyl Ester (Aralia-saponin III, 3) Colorless amorphous powder, $[\alpha]_D$ −13.3° (*c*=0.2, pyridine). IR (KBr) cm^{-1}: 3400, 2920, 2850, 1740, 1640, 1550, 1464, 1380, 1310, 1080, 1030 and 890. ^{1}H-NMR: Table l. ^{13}C-NMR: Table 2. Positive ion ESI-MS *m/z*: 1113 $[M+Na]^+$ and 951 $[M+Na-glc]^+$. Negative ion ESI-MS *m/z*: l089 $[M-H]^-$, 927 $[M-H-glc]^-$, 765 $[M-H-glc-glc]^-$ and 603 $[M-H-glc-glc-glc]^-$. HR-FAB-MS *m/z*: 1113.5437 $[M+Na]^+$ (Calcd for $C_{53}H_{86}O_{23}Na$: 1113.5458).

Acid Hydrolysis of 3 Compound **3** (1 mg) was refluxed with 7% HCl in H_2O–EtOH (1 : 1, 1 ml) for 3 h. The reaction mixture was partitioned between $CHCl_3$ and H_2O. The water layer was concentrated and checked by cellulose TLC [pyridine–ethyl acetate–acetic acid–water (36 : 36 : 7 : 2l),

Rf: L-arabinose, 0.61; D-glucose, 0.38] and the residual water layer was analyzed by GC as described above. The sugars were identified as L-arabinose and D-glucose, respectively.

3-*O*-[β-D-Glucopyranosyl(1→3)-α-D-glucopyranosyl(1→3)-β-D-glucopyranosyl]-16α-hydroxyoleanolic Acid 28-*O*-β-D-Glucopyranosyl Ester (Aralia-saponin IV, 4) Colorless amorphous powder, $[\alpha]_D$ −18.8° (*c*= 0.2, pyridine). IR (KBr) cm^{-1}: 3400, 2860, 1738, 1628, 1450, 1394, 1310, 1210, 1040, 890. ^{1}H-NMR: Table l. ^{13}C-NMR: Table 2. Positive ion ESI-MS *m*/*z*: 1143 $[M+Na]^+$, 981 $[M+Na-glc]^+$. Negative ion ESI-MS *m*/*z*: 1119 $[M-H]^-$, 957 $[M-H-glc]^-$, 795 $[M-H-glc-glc]^-$, 633 $[M-H-glc-glc-glc]^-$ and 471 $[M-H-glc-glc-glc-glc]^-$. HR-FAB-MS *m*/*z*: 1143.5583 $[M+Na]^+$ (Calcd for $C_{54}H_{88}O_{24}Na$: 1143.5564).

Acid Hydrolysis of 4 Compound **4** (1 mg) was refluxed with 7% HCl in H_2O–EtOH (1 : 1, 1 ml) for 3 h. The reaction mixture was worked up as described above. The sugar component was identified as D-glucose alone.

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References

1) New Medical College of Jiangsu (ed.), "Dictionary of Chinese Materia Medica," Shanghai Scientific and Technological Publishing Co., Shanghai, 1977, p. 2583.
2) Saito S., Ebashi J., Sumita S., Furumoto T., NagamuraY., Nishida K., Ishiguro I., *Chem. Pharm. Bull*., **41**, 1935—1401 (1993).
3) Yang G., Zhou J., Song C., *Zhongcaoyao.*, **26**, 514—517 (1993).
4) Kuang H., San H., Zhang N., Okada Y., Okuyama T., *Chem. Pharm. Bull*., **44**, 2183—2185 (1996).
5) Shao C., Kasai R., Xu J., Tanaka ., *Chem. Pharm. Bull.*, **37**, 311—314 (1989)
6) Yoshikawa M., Matsuda H., Harada E., *Chem. Pharm. Bull*., **42**, 1354—1356 (1994).
7) Yoshikawa M., Murakami T., Harada E., Murakami N., *Chem. Pharm. Bull*., **44**, 1923—1927 (1996).
8) Koizumi H., Sanada S., Ida Y., *Chem. Pharm. Bull*., **30**, 2393—2398 (1982).
9) Tanaka O., Yahara S., *Phytochemistry*, **17**, 1353—1358 (1978).
10) Yahara S., Kaji K., Tanaka O., *Chem. Pharm. Bull*., **27**, 88—92 (1979).
11) Nagao T., Tanaka R., Shimokawa H., Okabe H., *Chem. Pharm. Bull*., **39**, 1719—1725 (1991).
12) Hara S., Okabe H., Mihashi K., *Chem. Pharm. Bull*., **35**, 501—506 (1987).
13) Kitagawa I., Yoshikawa M., Yosioka I., *Tetrahedron Lett*., **5**, 469—472 (1974).