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TRITERPENOID SAPONINS FROM THE ROOTS OF *ARALIA SPINIFOLIA*

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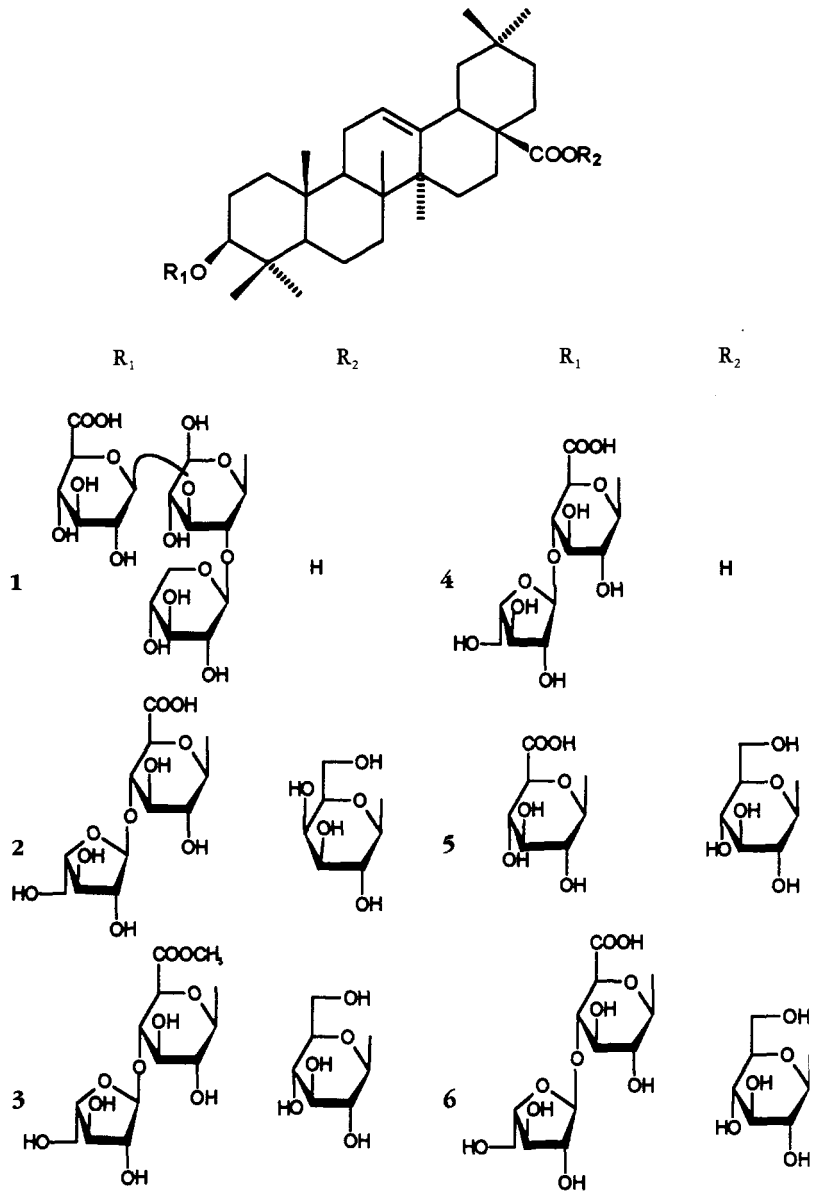
ABSTRACT.—Two new triterpenoid saponins named aralosides H [**1**] and J [**2**], along with four known triterpenoid saponins **3–6** were isolated from the roots of *Aralia spinifolia*. Their structures were determined as 3-*O*-β-D-xylopyranosyl-(1→2)-[β-D-glucuronopyranosyl(1→3)]-β-D-glucopyranosyl-oleanolic acid [**1**], 3-*O*-α-L-arabinofuranosyl-(1→4)-β-D-glucuronopyranosyl-oleanolic acid 28-*O*-β-D-galactopyranosyl ester [**2**], araloside A methyl ester [**3**], chikusetusaponin Ib [**4**], chikusetusaponin IV [**5**], and araloside A [**6**] on the basis of spectral and chemical data.

Aralia spinifolia Merrill (Araliaceae) is used as a medicinal plant, and is a member of a genus whose representatives have been employed in traditional medicine for their tonic and stimulant activities and in the treatment of neurosis (1). The chemical constituents of *Aralia spinifolia* have not been reported previously. In this paper, we describe the isolation and structure elucidation of six saponins.

Saponin **1** was obtained as a white powder with the molecular formula of $C_{47}H_{74}O_{18}$ as determined from fabms and elemental analysis. On acidic hydrolysis, saponin **1** liberated oleanolic acid which was identified by nmr, ms, and tlc comparison with an authentic sample. The sugars, D-glucose, D-xylose, and D-glucuronic acid were detected in the hydrolysate by hptlc (2) and paper chromatography. In the ^{13}C-nmr spectrum (Table 1), three anomeric carbon signals were observed at δ 104.9, 104.5, and 104.5. A glycosylation shift was observed for C-3 of the aglycone indicating that the saccharide unit was attached at this location (δ 89.8, representing a downfield shift by 11.9 ppm compared with the analogous signal of oleanolic acid). The fabms showed an $[M+Na]^+$ ion at *m/z* 949 and an ion at *m/z* 641 $[M-xyl-glcA+Na]^+$. This led to the conclusion that xylose or glucuronic acid was not directly attached to the aglycone. The mass spectrum of the peracetate of **1** gave ions at *m/z* 259 [(xyl)Ac_3, terminal xylose] and 303 [(glcA)Ac_3, terminal glucuronic acid], indicating that xylose and glucuronic acid were in the terminal positions. The linkage positions of xylose and glucuronic acid were deduced as follows.

In the ^{13}C-nmr spectrum of **1**, the C-2 and C-3 signals of glucose were shifted downfield by 4.6 ppm and 8.7 ppm compared with reference data (3). This indicated that xylose and glucuronic acid were attached to C-2 and C-3 of the glucose unit. By comparison of the ^{1}H-nmr spectrum with reference data (4), the anomeric proton of glucose in the sugar chain was located at δ 4.33 ppm. The H-2 signal of glucose was assigned at δ 3.60 ppm by a decoupling experiment, wherein on irradiation of the signal at δ 3.60 the anomeric proton of glucose was decoupled. The H-3 signal of glucose was assigned at δ 4.65 ppm by analysis of the ^{13}C-^{1}H COSY nmr spectrum of **1** which showed a correlation with the carbon signal at δ 87.2 ppm. Assignments of the carbon and proton signals of xylose and glucuronic acid were achieved by analysis of ^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY spectra. These assignments suggested that the anomeric protons of xylose and glucuronic acid were at δ 4.68 ppm and δ 4.54 ppm, respectively. The positions of xylose and glucuronic acid were determined by nOe difference experiments. By irradiating the anomeric proton signal of xylose at δ 4.68 ppm, an nOe enhance-



ment was observed on the signal at δ 3.60 ppm (H-2-glc), and by irradiating the anomeric glucuronic acid signal at δ 4.54, an nOe enhancement was observed on the signal at δ 4.65 (H-3-glc).

In the ^{1}H-nmr spectrum of **1**, anomeric proton signals at δ 4.33 (1H, d, J=7.5 Hz, H-1 of β-glc), 4.54 (1H, d, J=7.8 Hz, H-1 of β-glcA), 4.68 (1H, d, J=7.6 Hz, H-1 of β-xyl) led to assignments of anomeric configurations of glucose, glucuronic acid, and xylose units as β; these assignments were supported by their ^{13}C-nmr signals (Table 1). The accumulated evidence described above indicated that the structure of **1** is 3-*O*-β-D-xylopyranosyl-(1→2)-[β-D-glucuronopyranosyl(1→3)]-β-D-glucopyranosyl-oleanolic acid (araloside H).

Saponin **2** was also a white powder with the molecular formula determined as $C_{47}H_{74}O_{18}$ from fabms and elemental analysis. Acid hydrolysis yielded D-galactose, L-arabinose, D-glucuronic acid,

TABLE 1. ^{13}C-Nmr Chemical Shifts of the Sugar Moieties (125 MHz, in C_5D_5N) of Compounds **1–6**.

	3-*O*-Sugars					
	1	**2**	**3**	**4**	**5**	**6**
GlcA-1	104.5	106.7	106.9	106.9	107.7	107.0
GlcA-2	75.2	75.3	74.0	75.5	74.1	74.2
GlcA-3	78.7	78.6	75.0	76.5	78.8	77.0
GlcA-4	71.3	78.9	79.2	78.7	71.1	79.3
GlcA-5	76.1	77.2	75.8	77.5	78.0	76.5
GlcA-6	174.0	174.1	170.3	175.0	173.8	174.1
-OCH_3			52.0			
Ara-1		108.5	108.5	109.1		109.1
Ara-2		82.2	82.5	82.8		82.6
Ara-3		78.7	78.3	78.6		78.8
Ara-4		87.4	87.4	87.1		87.3
Ara-5		62.3	62.0	63.0		63.0
Xyl-1	104.5					
Xyl-2	76.1					
Xyl-3	78.3					
Xyl-4	71.3					
Xyl-5	67.2					
Glc-1	104.9					
Glc-2	79.3					
Glc-3	87.2					
Glc-4	71.9					
Glc-5	78.5					
Glc-6	62.1					
	28-*O*-Sugars					
Glc-1			95.6		95.7	96.0
Glc-2			75.0		75.1	75.4
Glc-3			78.8		78.9	78.9
Glc-4			70.9		71.1	71.4
Glc-5			76.7		75.2	78.3
Glc-6			62.4		62.5	62.5
Gal-1		95.6				
Gal-2		71.2				
Gal-3		74.0				
Gal-4		70.3				
Gal-5		76.2				
Gal-6		62.0				

and oleanolic acid. The fabms of **2** showed an $[M+Na]^+$ ion at *m/z* 949. The ^{13}C-nmr spectrum of **2** showed three anomeric signals (δ 108.5, 106.5, and 95.6 ppm). Glycosylation shifts were observed at C-3 and C-28 of the aglycone (δ 89.1 ppm and δ 176.2 ppm, representing a downfield shift by 11.2 ppm and an upfield shift by 3.9 ppm, respectively, compared with those of oleanolic acid). The eims of the peracetate of **2** exhibited fragments at *m/z* 259 [terminal (ara)Ac_3] and *m/z* 331 [terminal (gal)Ac_4], indicating that arabinose and galactose are in the terminal positions.

Alkaline hydrolysis of **2** gave prosaponin **4** based on ^{1}H-nmr, ^{13}C-nmr, and hptlc data. In the ^{1}H-nmr spectrum, the anomeric proton signals at δ 4.85 (1H, d, J=7.6 Hz, H-1 of β-glcA), 6.08 (1H, s, H-1 of α-ara), and 6.23 (1H, d, J=8.0 Hz, H-1 of β-gal) led to assignments of β-anomeric configurations for the glucuronic acid and galactose units

and α- for the arabinose unit; these assignments were supported by their ^{13}C-nmr signals (Table 1). Otherwise, because C-2 and C-4 of the arabinose were located, respectively, at δ 82.2 and 87.4 ppm, and its anomeric proton signal was observed at δ 6.08 (1H, s), it was determined that the arabinose was in the furanose form rather than the pyranose form. Hence, **2** was characterized as 3-*O*-α-L-arabinofuranosyl-(1→4)-β-D-glucuronopyranosyl-oleanolic acid 28-*O*-β-D-galactopyranosyl ester (araloside J).

Compounds **3**–**6** were isolated from the EtOH extract. Acid hydrolysis of these compounds yielded the same aglycone (oleanolic acid) as **1** and **2**. Their structures were elucidated as araloside A methyl ester (6), chikusetusaponin Ib (7), chikusetusaponin IV (8), and araloside A (5), respectively, by comparison of ir, ms, ^{1}H-nmr, ^{13}C-nmr, and tlc data with those of authentic saponins.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a micro melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 automatic digital polarimeter. Nmr spectra were taken on a Bruker AM-500 (^{1}H nmr at 500 MHz and ^{13}C nmr at 125 MHz) spectrometer in C_5D_5N with TMS as an internal standard. Ms were recorded on JEOL JMS-DX 300 and JMS-DX 300 mass spectrometers. The ir spectra were recorded on a Perkin-Elmer 683 infrared spectrometer.

PLANT MATERIAL.—*Aralia spinifolia* (Araliaceae) was collected from Sichuan Province (People's Republic of China). The plant was identified by Prof. Ja-Lin Wu, Sichuan School of Chinese Traditional Medicine. A voucher specimen is deposited at the Institute of Materia Medica, Chinese Academy of Medical Sciences.

EXTRACTION AND ISOLATION.—The roots of the plant (3.5 kg) were extracted with 70% EtOH under reflux. The extract was concentrated to dryness and the residue was subjected to cc on a highly porous polymer (RA, Seventh Chemical and Industrial Factory, Beijing) with H_2O, 30% EtOH, and 70% EtOH. The first 2000 ml of H_2O were extracted with *n*-BuOH saturated with H_2O. The *n*-BuOH solution was concentrated to give 36 g of a residue, which was then chromatographed on a Si gel column with $CHCl_3$-MeOH-H_2O (7:3:1) to give three fractions (a–c). The 70% EtOH (20 g) eluate was chromatographed on Si gel with $CHCl_3$-MeOH-H_2O (75:25:10, 70:30:10, and 65:35:10) to give four additional fractions (d–g).

Fraction b (1.5 g) was chromatographed on Si gel eluted with $CHCl_3$-MeOH-H_2O (70:30:10) and mplc (RP-18 reversed-phase 10–20 μm, eluted with MeOH-H_2O, 65:35), respectively, to give fraction 2′. Chromatography of fraction 2′ on Sephadex LH-20 with MeOH afforded **1** (60 mg).

Fraction f was chromatographed on Si gel with $CHCl_3$-MeOH-H_2O (70:30:10) to give fraction 3′. The chromatography of fraction 3′ by RP-18 reversed-phase (10–20 μm) mplc with MeOH-H_2O (63:33) gave **2** (60 mg) and **6** (110 mg).

Fraction d was chromatographed on Si gel with $CHCl_3$-MeOH-H_2O (75:25:10) to afford **3** (80 mg) and **4** (50 mg).

The chromatography of fraction e on Si gel with $CHCl_3$-MeOH-H_2O (75:25:10) gave **5** (50 mg).

CHARACTERIZATION OF **1**.—White powder, mp 238–241° (dec); $[\alpha]^{18}_D$ −2.7° (*c*=0.157, MeOH). *Anal.* calcd for $C_{47}H_{74}O_{18}\cdot 10H_2O$, C 50.99, H 6.69, found C 51.11, H 6.97; ir ν max (KBr) 3400 (OH), 2923 (C-H), 1680 (O=C-OH), 1640 (C=C) cm^{-1}; ^{1}H nmr (C_5D_5N) δ 0.78, 0.95, 0.97, 0.99, 1.07, 1.20, 1.34 (3H each, s, Me), 4.33 (1H, d, *J*=7.5 Hz, H-1 of β-glc), 4.54 (1H, d, *J*=7.8 Hz, H-1 of β-glcA), 4.68 (1H, d, *J*=7.6 Hz, H-1 of β-xyl), 5.28 (1H, m, H-12); ^{13}C nmr, see Table 1; fabms *m/z* $(M+Na)^+$ 949, $(M-xyl-glcA+Na)^+$ 641; Liebermann-Burchard reaction, reddish purple.

CHARACTERIZATION OF **2**.—White powder, mp 208–210° (dec), $[\alpha]^{20}_D$ −31.1° (*c*=0.1, MeOH). *Anal.* calcd for $C_{47}H_{74}O_{18}\cdot 3H_2O$, C 57.55, H 7.55, found C 57.65, H 7.60; ir ν max (KBr) 3400 (OH), 2920, 2918 (C-H), 1740 (C=O, ester), 1700 (O=C-OH), 1640 (C=C) cm^{-1}; ^{1}H nmr (C_5D_5N) δ 0.80, 0.88, 0.93, 0.95, 1.07, 1.23, 1.26 (3H each, s, Me), 4.85 (1H, d, *J*=7.6 Hz, H-1 of β-glcA), 5.38 (1H, m, H-12), 6.08 (1H, s, H-1 of α-ara), 6.23 (1H, d, *J*=8.0 Hz, H-1 of β-gal); ^{13}C nmr, see Table 1; fabms *m/z* $[M+Na]^+$ 949, $[M+K]^+$ 965; Liebermann-Burchard reaction, reddish purple.

CHARACTERIZATION OF **3**.—Colorless needles, mp 212–214° (dec). Ir ν max (KBr) 3400 (OH), 2950 (C-H), 1740 (C=O ester), 1728 (C=O, ester), 1640 (C=C) cm^{-1}; ^{1}H nmr (C_5D_5N) δ 0.79, 0.85, 0.88, 0.93, 1.06, 1.23, 1.25 (3H each, s, Me), 3.84 (3H, s, OMe), 4.91 (1H, d, *J*=7.8 Hz, H-1 of β-glcA), 5.39 (1H, m, H-12), 5.76 (1H, s, H-1 of α-ara), 6.31 (1H, d, *J*=8.0 Hz, H-1 of β-glc); ^{13}C nmr, see Table 1; fabms *m/z* $[M+Na]^+$ 963; Liebermann-Burchard reaction, reddish purple.

CHARACTERIZATION OF **4**.—White powder, mp 237–240°, $[\alpha]^{20}_D$ −20.0° (*c*=0.1, MeOH). Ir

ν max (KBr) 3400 (OH), 2950 (C-H), 1681 (O=C-OH), 1641 (C=C) cm^{-1}; ^1H nmr (C_5D_5N) δ 0.81, 0.94, 0.97, 1.08, 1.23, 1.34 (3H each, s, Me), 4.90 (1H, d, J=7.5 Hz, H-1 of β-glcA), 5.49 (1H, m, H-12), 6.10 (1H, s, H-1 of α-ara); ^{13}C nmr, see Table 1; fabms m/z $[M+2Na]^+$ 810, $[M-ara+2Na)^+$ 678; Liebermann-Burchard reaction, reddish purple.

CHARACTERIZATION OF **5**.—White powder, mp 228–229° (dec), $[\alpha]^{24}D$ +21.1° ($c=0.1\times10^{-2}$, MeOH); ir ν max (KBr) 3400 (OH), 2948 (C-H), 1740 (C=O, ester), 1701 (O=C-OH), 1640 (C=C) cm^{-1}; ^1H nmr (C_5D_5N) δ 0.83, 0.89, 0.92, 0.96, 1.08 (3H each, s, Me), 1.27 (6H, s, Me), 4.46 (1H, d, J=8.3 Hz, H-1 of β-glcA), 5.42 (1H, m, H-12), 6.31 (1H, d, J=8.0 Hz, H-1 of β-glc); ^{13}C nmr, see Table 1; Liebermann-Burchard reaction, reddish purple.

CHARACTERIZATION OF **6**.—White powder, mp 221–223° (dec), $[\alpha]^{12}D$ −17.0° (c=0.1, MeOH). Ir ν max (KBr) 3400 (OH), 2945 (C-H), 1725 (C=O, ester), 1645 (C=C) cm^{-1}; ^1H nmr (C_5D_5N) δ 0.82, 0.90, 0.93, 0.98, 1.07, 1.28, 1.29 (3H each, s, Me), 4.93 (1H, d, J=7.6 Hz, H-1 of β-glcA), 5.44 (1H, m, H-12), 6.09 (1H, s, H-1 of α-ara), 6.24 (1H, d, J=8.0 Hz, H-1 of β-glc); ^{13}C nmr, see Table 1; Liebermann-Burchard reaction, reddish purple.

ACID HYDROLYSIS OF **1–6**.—A solution of each sample (e.g., **1**) and 7% HCl-EtOH (3:7) was refluxed for 4 h. The mixture was diluted with H_2O and extracted with Et_2O. The Et_2O layer was evaporated to dryness. The residue was recrystallized in EtOH to afford oleanolic acid, which was compared with an authentic sample (mmp, co-tlc, ir). The aqueous layer was then neutralized with 1 N NaOH, concentrated, and subjected to hptlc analysis on Kieselgel 60 F_{254} (Merck) [using $CHCl_3$-MeOH-H_2O (30:12:4), 9 ml, and HOAc, 1 ml] and paper chromatography [using n-BuOH-HOAc-H_2O (4:1:5); phenol-H_2O (4:1); n-BuOH-C_5H_5N-C_6H_6-H_2O (5:3:1:3)], which showed glc, glcA and xyl in **1**; glcA, ara and gal in **2**; ara, glcA, and glc in **3** and **6**; glcA and glc in **4**; ara and glcA in **5**.

ALKALINE HYDROLYSIS OF **2**, **3** AND **6**.—A mixture of each sample (20 mg) and 2% KOH in 70% EtOH (7 ml) was refluxed for 6 h. After slow neutralization with 0.1 N HCl, the reaction mixture was extracted with n-BuOH saturated with H_2O. The n-BuOH solution was concentrated *in vacuo* and the residue showing a zone on tlc [solvent: $CHCl_3$-MeOH-H_2O (75:25:10)] was recrystallized to give **4** (identified by co-tlc, ir, ^1H nmr and ^{13}C nmr).

ACETYLATION OF **1–3** AND **6**.—A solution of each sample (10 mg) in a mixture of Ac_2O (0.4 ml) and pyridine (0.4 ml) was allowed to stand at room temperature, and the mixture was worked up as usual to give the peracetate of **1** (6 mg), a white powder (EtOH); mp 120–122° (dec); eims m/z 437, 393, 303 [(glcA)Ac_3], 259 [(xyl)Ac_3]. Peracetate of **2** (7 mg), white powder (EtOH); mp 109–111° (dec); eims m/z 437, 393, 331 [(gal)Ac_4], 259 [(ara)Ac_3]. Peracetate of **3**, white powder; mp 115–117° (dec); eims m/z 437, 393, 331 [(glc)Ac_4], 259 [(ara)Ac_3]. Peracetate of **6**, white powder; mp 113–115° (dec); eims m/z 437, 393, 331 [(glc)Ac_4], 259 [(ara)Ac_3].

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LITERATURE CITED

1. S.Y. Sokolov, *Farmakol. Toksikol.*, **27**, 396 (1964).
2. P.P. Zhao, B.M. Li, and L.Y. He, *Acta Pharm. Sin.*, **22**, 70 (1987).
3. F. Abe and T. Yamauchi, *Chem. Pharm. Bull.*, **35**, 1751 (1987).
4. S.X. Chen and J.K. Snyder, *J. Org. Chem.*, **54**, 3679 (1989).
5. W.J. Sun, D.K. Zhang, Z.F. Sha, H.L. Zhang, and X.L. Zhang, *Acta. Pharm. Sin.*, **26**, 197 (1991).
6. Y.T. Jiang, S.X. Xu, X.H. Gu, L. Ren, Y.J. Chen, X.S. Yao, and Z.C. Miao, *Acta Pharm. Sin.*, **27**, 533 (1992).
7. Z.H. Jia and Z.Y. Xiao, *Chin. Trad. Herbal Drugs*, **21**, 2 (1990).
8. T.D. Lin, N. Kondo, and J. Shoji, *Chem. Pharm. Bull.*, **34**, 253 (1976).

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