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YEMUOSIDE I, A NEW NORTRITERPENOID GLYCOSIDE FROM *STAUNTONIA CHINENSIS*

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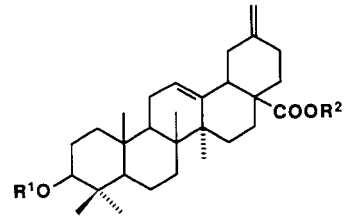
ABSTRACT.—A new nortriterpenoid glycoside, named yemuoside I [**1**], was isolated from *Stauntonia chinensis*. On the basis of chemical and spectral evidence, its structure was determined as 3-*O*-[α-L-arabinopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-30-noroleana-12,20(29)-dien-28-oic acid 28-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester.

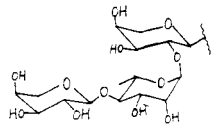
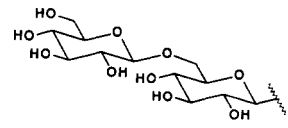
Stauntonia chinensis DC. (Lardizabalaceae), commonly known as "Ye Mu Gua," is used in Chinese traditional medicine (1). The aqueous or EtOH extract of this plant was shown to have analgesic and sedative activities (2). Many glycosides were isolated from this plant (3–7), and, as part of a series of studies, we have further undertaken the investigation of the *n*-BuOH-soluble fraction of the 70% EtOH extract and isolated a new nortriterpenoid glycoside, named yemuoside I [**1**].

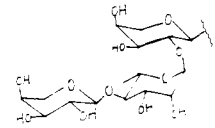
The glycoside mixture obtained from the 70% EtOH extract of the whole plant was purified by Si gel cc to yield **1**. The structure of **1** was determined by negative ion fabms, ^{1}H- and ^{13}C-nmr measurements, acetylation, and sugar analysis. Upon acid hydrolysis of **1**, larreagenin A (8) and 3β-hydrooxy-30-norolean-12,19-dien-28-oic acid (9) as well as three sugars (D-glucose, L-arabinose, and L-rhamnose) were obtained and identified by comparison with authentic samples (4).

Analysis of ^{1}H- and ^{13}C-nmr data of **1** (Table 1) suggested the identity of the aglycone moiety as one of the previously reported nortriterpenoid glycosides (4–6). By means of alkaline degradation (10), we succeeded in the isolation of the aglycone 3β-hydroxy-30-noroleana-12,20(29)-dien-28-oic acid [**3**] (11).

When **1** was refluxed with 7% KOH, it gave a prosapogenin **2**, which was



1 R^1 =  , R^2 = 

2 R^1 =  , R^2 = H

3 $R^1 = R^2 = H$

further hydrolyzed in acidic medium to give L-rhamnose and L-arabinose. Compound **2** showed the molecular ion peak at *m/z* 889 $[M+K]^+$ in the fdms. The eims of the peracetate of **2** had fragment ions at *m/z* 259 $[(Ara)Ac_3$, terminal arabinose$]^+$, 489 $[(Ara-Rha)Ac_5]^+$, and 705 $[(Ara-Rha-Ara)Ac_7]^+$, which indicated the sequence of sugars in **2**. The ^{1}H- and ^{13}C-nmr anomeric signals of two arabinoses [H-1′ (δ 4.86, d, $J=5.1$ Hz), H-1‴ (δ 5.08, d, $J=7.3$ Hz), C-1′ (104.84), C-1‴ (107.56 ppm)] and of a rhamnose [H-1″ (δ 6.23, s), C-1″ (100.71 ppm)] confirmed that three sugars, all in the α-L-pyranosyl form, were present in **2** as a trisaccharide attached to the aglycone through an α-arabinoside linkage. The ^{13}C-nmr sugar signals (Table 1) were in agreement with a terminal arabinose unit (12) linked at C-3 of an inner rhamnose unit, and the inner rhamnose linked with C-2 of an inner arabinose unit. Compared with methyl-α-L-arabinopyranoside and methyl-α-L-rhamnopyranoside (13), the C-2 of the arabinose and the C-3 of the rhamnose were, respectively, shifted downfield by 4.2 ppm (from 72.2 to 76.4 ppm) and 9.4 ppm (from 72.5 to 81.9 ppm), as expected for a glycosidation shift. Furthermore, the invariant signal at δ 88.72 ppm suggested the remaining glycosidation site at C-3 of the aglycone (14). From these data the structure 3-*O*-[α-L-arabinopyranosyl-(1↦3)-α-L-rhamnopyranosyl-(1↦2)-α-L-arabinopyranosyl-(1↦2)-α-L-arabinopyranosyl]-30-norolean-12,20(29)-dien-28-oic acid was assigned to **2**.

The negative ion fabms spectrum of **1** showed a quasi-molecular anion at *m/z* 1173 $[M-H]^-$, indicating a mol wt of 1174, and fragments at *m/z* 1041 $[M-Ara-H]^-$, 849 $[M-Glc-Glc-H]^-$, 717 $[849-Ara]^-$, 571 $[717-Rha]^-$ and 439 $[aglycone-H]^-$ corresponded to the consecutive losses of sugar residues. The eims of the peracetate of **1** displayed fragment ions *m/z* 259 $[(Ara)Ac_3$, terminal arabinose$]^+$, 331 $[(Glc)Ac_4$, terminal glucose$]^+$, and 489 $[(Ara\text{-}Rha)Ac_5]^+$. These spectral data demonstrated the following sequence of sugars. The interglycosidic linkage in the C-28 sugar chain was determined by comparison with ^{13}C-nmr data of methyl-β-D-glucopyranoside (13) and known compounds (6, 12).

The anomeric proton signals of **1** at δ 4.86 (1H, d, $J=5.1$ Hz), 4.98 (1H, d, $J=7.8$ Hz), 5.08 (1H, d, $J=7.3$ Hz), 6.21 (1H, d, $J=7.8$ Hz), and 6.23 (1H, s) indicated the anomeric configuration of arabinoses and rhamnose to be α and of glucoses to be β; these assignments were supported by the corresponding carbon signals (Table 1) (12, 14, 15).

Based on the evidence mentioned above, the structure of **1** was determined as 3-*O*-[α-L-arabinopyranosyl-(1↦3)-α-L-rhamnopyranosyl-(1↦2)-α-L-arabinopyranosyl]-30-norolean-12,20(29)-dien-28-oic acid 28-*O*-[β-D-glucopyranosyl-(1↦6)-β-D-glucopyranosyl] ester.

EXPERIMENTAL

PLANT MATERIAL.—The plants of *S. chinensis* were collected in Jiangxi province of China. The specimen was identified by Prof. W.Z. Song, Institute of Materia Medica, Chinese Academy of Medical Sciences, and deposited in the Herbarium of this institute.

GENERAL EXPERIMENTAL PROCEDURES.—All mp's were determined on a Boetius micromelting apparatus and were uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. ^{1}H-nmr (400 MHz) and ^{13}C-nmr (100 MHz) spectra were recorded on a Jeol GX-400 spectrometer in pyridine-d_5. Chemical shifts (δ) are expressed in ppm using TMS as an internal standard. The fabms and eims were taken on JMS-SX102 gc/ms system and ZAB-2F system, respectively. The fabms was measured with an accelerating potential of 3.0 kV for a Xe beam source with glycerol-thioglycerol (1:1) as matrices.

EXTRACTION AND ISOLATION.—Dry plants (3 kg) were pulverized and extracted with 70% EtOH (6 liters × 4, 1.5 h for each extraction) at 80°. The extracts were combined and concentrated in vacuo to give a brown residue (0.42 kg), which was suspended in H_2O (0.7 liters) and extracted with EtOAc (0.3 liters × 5) and then with

TABLE 1. ^{13}C-nmr Chemical Shifts of **1** and **2** in C_5D_5N.

Carbon	Compound		Carbon	Compound		Sugar	Compound		Sugar	Compound	
	1	**2**		**1**	**2**		**1**	**2**		**1**	**2**
C-1	38.86	38.82	C-16	23.48	23.74	C_{28}-Sugar			C_3-Sugar		
C-2	26.48	26.51	C-17	47.31	46.99	Glc-1	95.74		Ara-1′	104.84	104.95
C-3	88.72	88.70	C-18	47.48	47.85	2	73.77		2′	76.44	76.40
C-4	39.87	39.71	C-19	41.65	42.06	3	78.43		3′	74.75	74.75
C-5	55.88	55.89	C-20	148.35	149.05	4	70.78		4′	69.70	69.66
C-6	18.46	18.47	C-21	30.04	30.37	5	77.83		5′	64.92	65.08
C-7	33.04	33.10	C-22	37.62	38.34	6	69.39		Rha-1″	100.71	100.71
C-8	39.44	39.47	C-23	28.06	28.06	Glc-1′	105.25		2″	72.71	72.72
C-9	47.97	47.94	C-24	16.91	16.94	2′	75.10		3″	81.89	81.89
C-10	36.93	39.95	C-25	15.62	15.47	3′	78.60		4″	73.23	73.25
C-11	23.71	23.74	C-26	17.45	17.29	4′	71.45		5″	69.70	69.72
C-12	123.29	122.94	C-27	26.00	26.14	5′	78.30		6″	18.46	18.57
C-13	143.41	144.12	C-28	175.82	179.40	6′	62.58		Ara-1‴	107.56	107.57
C-14	42.04	41.94	C-29	107.31	107.12				2‴	73.77	73.88
C-15	28.06	28.26							3‴	74.44	74.47
									4‴	69.85	69.97
									5‴	67.41	67.41

n-BuOH saturated with H_2O (0.3 liters × 5). The *n*-BuOH solution was concentrated to give the *n*-BuOH-soluble fraction (95 g), which was chromatographed on a Si gel (100–200 mesh) column with $CHCl_3$-MeOH-H_2O (2.6:1.6:0.3) to give twelve fractions (I–XII).

Fraction VI (7.87 g) was further chromatographed on a Si gel column (100–200 mesh) and eluted with $CHCl_3$-MeOH-H_2O (65:30:3) to afford seventeen crude saponin fractions (fractions 1–17). Fraction 12 (680 mg) was purified further over a Si gel column to give yemuoside I [**1**] (77 mg).

Yemuoside I [**1**].—White powder: mp 204–208° (dec), $[\alpha]^{26}D$ +14.1 (*c* = 0.092, MeOH). *Anal.* calcd for $C_{57}H_{90}O_{25}\cdot 2H_2O$, C 57.81, H 7.85; found C 57.74, H 7.56. Ir λ max (KBr) cm $^{-1}$ 3400 (OH), 2900 (C-H), 1730 (C=O, ester), 1630 (C=C), 1350, 1050 (C-O-C); ^{1}H nmr (C_5D_5N) δ 0.84, 1.03, 1.06, 1.15, 1.20 (each 3H, s, 5 × Me), 1.54 (3H, d, *J* = 5.9 Hz, Me-5 of Rha), 3.09 (1H, dd, *J* = 4.9, 13.6 Hz, H-18), 3.23 (1H, dd, *J* = 4.0, 12.5 Hz, H-3α), 4.67, 4.70 (each 1H, br s, H-29), 4.86 (1H, d, *J* = 5.1 Hz, H-1 of α-Ara), 4.98 (1H, d, *J* = 7.8 Hz, H-1 of β-Glc), 5.08 (1H, d, *J* = 7.3 Hz, H-1 of α-Ara), 5.39 (1H, m, H-12), 6.21 (1H, d, *J* = 7.8 Hz, H-1 of β-Glc), 6.23 (1H, s, H-1 of α-Rha); ^{13}C nmr see Table 1; fabms (negative mode) *m/z* $[M-H]^-$ 1173, $[M-Ara-H]^-$ 1041, $[M-Glc-Glc-H]^-$ 849, $[849-Ara]^-$ 717, $[717-Rha]^-$ 571, $[aglycone-H]^-$ 439. Liebermann-Burchard reaction: reddish purple.

ACID HYDROLYSIS OF **1**.—A sample of **1** in 8% HCl-dioxane (1:1) was refluxed for 4 h. The mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was evaporated to dryness and chromatographed on Si gel using $CHCl_3$-MeOH (20:1) as solvent to give larreagenin A (mmp, co-tlc, ir) and 3β-hydroxy-30-norolean-12,19-dien-28-oic acid (mmp, co-tlc, ir) (8, 9).

IDENTIFICATION OF SUGARS.—The aqueous layer was neutralized with 1 N NaOH, concentrated, and subjected to hptlc analysis on Kiesel gel $60F_{254}$ (Merck) using EtOAc-H_2O-MeOH-HOAc (13:3:3:4), which showed L-arabinose, L-rhamnose, and D-glucose in **1**.

ACETYLATION OF **1**.—A solution of the sample (10 mg) in a mixture of Ac_2O (0.3 ml) and C_5H_5N (0.3 ml) was allowed to stand for 24 h at room temperature, and the reaction mixture was worked up as usual to give the peracetate of **1** (6 mg): white powder (MeOH); ^{1}H-nmr ($CDCl_3$) δ 1.90–2.20 (14 × Ac); eims *m/z* $[(Ara-Rha)Ac_5]^+$ 489, $[aglycone-OH]^+$ 423, $[(Glc)Ac_3]^+$ 331, $[(Ara)Ac_3]^+$ 259.

ALKALINE HYDROLYSIS OF **1**.—Compound **1** (40 mg) was refluxed with 2% KOH in 50% EtOH (5 ml) for 5h. The reaction mixture was carefully neutralized with dilute HCl and concentrated in vacuo. A suspension of the residue in H_2O was chromatographed on Diaion HP-20; elution with H_2O gave a sugar mixture, and subsequent elution with MeOH gave **2** (20 mg): white powder (MeOH): mp 232–237° (dec), $[\alpha]^{26}D$ +40.3 (*c* = 0.122, MeOH); ir λ max (KBr) cm $^{-1}$ 3400 (OH), 2900 (C-H), 1690 (C=O, acid), 1640 (C=C); ^{1}H nmr (C_5D_5N) δ 0.79, 0.94, 1.02, 1.17, 1.25 (each 3H, s, 5 × Me), 1.54 (3H, d, *J* = 6.3 Hz, Me-5 of Rha), 3.22 (2H, dd, *J* = 4.5, 13.0 Hz, H-18β, H-3α), 4.86 (1H, d, *J* = 5.4 Hz, H-1 of α-Ara), 5.08 (1H, d, *J* = 7.3 Hz, H-1 of α-Ara), 5.45 (1H, m, H-12), 6.23 (1H, s, H-1 of α-Rha); ^{13}C nmr see Table 1; fdms *m/z* $[M+K]^+$ 889, $[aglycone-H]^-$ 439.

IDENTIFICATION OF SUGARS IN THE 28-*O*-GLYCOSIDIC ESTER CHAIN.—The aqueous solution that passed throught the Diaion HP-20 column was concentrated to dryness, refluxed with 8% HCl-dioxane (1:1) for 4 h, neutralized with 1 N NaOH, concentrated, and subjected to hptlc analysis on Kiesel gel $60F_{254}$ (Merck) using EtOAc-H_2O-MeOH-HOAc (13:3:3:4). Only D-glucose was obtained.

Peracetate of **2**.—White powder (MeOH): eims *m/z* $[(Ara-Rha-Ara)Ac_7]^+$ 705, $[(Ara-Rha)-Ac_5]^+$ 489, $[aglycone]^+$ 440, $[aglycone-OH]^+$ 423, $[(Ara)Ac_3]^+$ 259.

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