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STRUCTURES OF TWO NORTRITERPENOID SAPONINS FROM *STAUNTONIA CHINENSIS*

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ABSTRACT.—Two new nortriterpenoid saponins, designated yemuoside YM_8 [**1**] and yemuoside YM_9 [**2**], were isolated from *Stauntonia chinensis*. A new saponin **3** was obtained from the cleavage of the ester-glycoside linkage of **1** or **2**. Structures were elucidated on the basis of chemical and physicochemical evidence.

Stauntonia chinensis Dcne. (Lardizabalaceae) is a medicinal plant frequently used in Chinese traditional medicine (1). In preceding papers (2–4), we reported the isolation and structural elucidation of six nortriterpenoid saponins and two lignan glycosides. As a continuation of studies on this plant, we present here spectral and chemical evidence for two new nortriterpenoid saponins, designated as yemuosides YM_8 [**1**] and YM_9 [**2**].

RESULTS AND DISCUSSION

Dried and pulverized whole plants of *S. chinensis* were extracted with 70% EtOH. The purified extract obtained by the usual method (see Experimental) was subjected to cc to give yemuosides YM_8 [**1**] and YM_9 [**2**], which responded to the Liebermann-Burchard test (5). The ir spectra of **1** and **2** showed ester group absorption (1730 cm^{-1}) together with strong hydroxyl (3400 cm^{-1}) and olefinic (1630 cm^{-1}) absorptions.

Acid hydrolysis of **1** and **2** afforded two compounds, which were identified as larreagenin A and 3β-hydroxy-30-noroleana-12,19-dien-28-oic acid (6) by comparison with authentic samples (mp, tlc, ^1H and ^{13}C nmr). Thus, it appeared that the aglycone moiety of intact **1** and **2** should be 3β-hydroxy-30-noroleana-12,20(29)-dien-28-oic acid [**4**] (7), giving rise to larreagenin A by lactonization at C-20 and the second compound by double-bond migration. The exocyclic double bond in ring E of **4** can be readily discerned from the ^{13}C nmr.

Acid hydrolysis of **1** and **2** also gave arabinose, rhamnose, and glucose, which were identified by hptlc (8). The ^{13}C-nmr spectra indicated the presence of five sugar residues in **1** by its five anomeric carbon signals (δ 95.74, 100.93, 104.96, 105.28, and 107.82 ppm), and six sugar residues were indicated in **2** (δ 95.70, 100.88, 102.67, 104.87, 104.97, 107.88 ppm). The signals at δ 95.74 and 95.70 ppm suggested that **1** and **2** both have a 28-*O*-glycosidic ester linkage (9), which was supported by slight shifts of the signals of C-28 (10) (see Table 1). The simultaneous presence of a 3-*O*-glycosidic linkage was also easily seen by the attendant downfield shifts of C-3 (11). Thus, **1** and **2** are bidesmosidic saponins.

On alkaline hydrolysis, both **1** and **2** gave **3**, which was further hydrolyzed with acid to yield arabinose, rhamnose, glucose, and two isomerized forms of the aglycone mentioned above. Compound **3** showed a molecular ion peak at *m/z* 904 $[M+Na+H]^+$ in the fdms. The eims of the peracetate of **3** had fragment ions at *m/z* 331 [$(Glc)Ac_4$, terminal glucose] and [$(Glc-Rha)Ac_6$]. In the ^1H-nmr spectrum of **3**,

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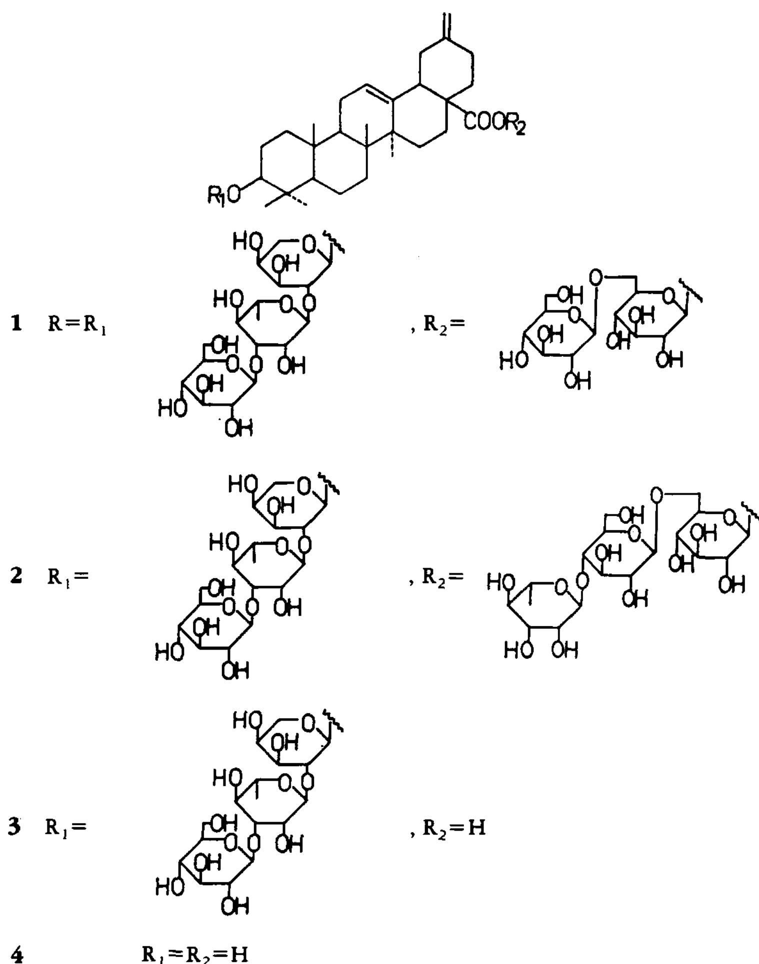

TABLE 1. ^{13}C-nmr Chemical Shifts of Aglycone Moieties in C_5D_5N.

Carbon	Compound			Carbon	Compound		
	1	2	3		1	2	3
C-1	38.88	38.86	38.82	C-16	23.49	24.45	23.76
C-2	26.53	26.51	26.53	C-17	47.31	47.29	46.99
C-3	88.62	88.59	88.62	C-18	47.46	47.45	47.84
C-4	39.87	39.83	39.69	C-19	41.65	41.64	42.06
C-5	55.92	55.91	55.92	C-20	148.34	148.30	149.06
C-6	18.47	18.46	18.47	C-21	30.06	30.06	30.35
C-7	33.04	33.00	33.10	C-22	37.62	37.59	38.32
C-8	39.43	39.44	39.46	C-23	28.02	28.01	28.25
C-9	47.95	47.94	47.93	C-24	16.93	16.91	16.93
C-10	36.92	36.93	36.95	C-25	15.64	15.61	15.50
C-11	23.70	23.70	23.76	C-26	17.45	17.43	17.28
C-12	123.30	122.95	122.92	C-27	26.00	25.96	26.12
C-13	143.41	143.38	144.11	C-28	175.78	175.70	179.37
C-14	42.06	42.03	41.94	C-29	107.30	107.36	107.09
C-15	28.16	28.16	28.02				

TABLE 2. ^{13}C-nmr Chemical Shifts of Sugar Moieties in C_5D_5N.

Sugar	Compound			Reference compound[a]	Sugar	Compound			Reference compound[b]
	1	2	3			1	2	3	
28-sugar					3-sugar				
									(A)
Glc-1	95.74	95.70		95.7	Ara-1	104.96	104.87	104.99	104.4
Glc-2	73.79	73.76[c]		73.9	Ara-2	75.28	75.31[e]	75.34	75.2
Glc-3	78.31[c]	77.90		78.0	Ara-3	74.49[e]	74.44[g]	74.55[c]	74.6
Glc-4	70.81	70.77		70.4	Ara-4	69.41	69.45	69.47	69.3
Glc-5	77.81	77.11[d]		76.6	Ara-5	65.50	65.53	65.53	65.8
Glc-6	69.41	69.25		69.7					(R)
Glc-1′	105.28	104.97		105.0	Rha-1	100.93	100.88	100.94	101.2
Glc-2′	75.10	75.28[e]		75.4	Rha-2	72.50	72.53	72.53	71.7
Glc-3′	78.63[c]	76.43		76.6	Rha-3	82.43	82.47	82.49	82.5
Glc-4′	71.45	78.63		78.8	Rha-4	73.29	73.29	73.29	72.9
Glc-5′	78.43	78.11		77.2	Rha-5	69.03	69.07	69.06	69.3
Glc-6′	62.58[d]	61.24		61.5	Rha-6	18.47	18.45[f]	18.47	18.2
									(G)
Rha-1		102.67		102.7	Glc-1	107.82	107.88	107.88	105.5
Rha-2		72.73		72.5	Glc-2	74.37[e]	74.43[g]	74.43[c]	74.9
Rha-3		72.53		72.2	Glc-3	77.17	77.17[d]	77.20	78.3
Rha-4		73.94[c]		73.9	Glc-4	70.26	70.24	70.29	71.6
Rha-5		70.24		70.4	Glc-5	76.85	76.73	76.82	78.3
Rha-6		18.49[f]		18.8	Glc-6	62.29[d]	62.28	62.32	62.7

[a]Sugar moiety in cussonoside (15).

[b](A) and (R): Arabinose and rhamnose parts in saponin A from *Sapindus mukurossi* (12). (G): Methyl-β-D-glucopyranoside.

[c–g]Assignments may be reversed.

the anomeric proton signals at δ 4.52(1H, d, J = 5.8 Hz), 5.15(1H, d, J = 7.8 Hz), and 6.37(1H, s) led to the assignment of the anomeric configurations of the arabinose and rhamnose units as α and the glucose unit as β. A comparison of the ^{13}C-nmr spectrum of **3** with those of methyl glycopyranosides and allied saponins showed that the signals of arabinose and rhamnose units of **3** were in good agreement with those in saponin A from *Sapindus mukurossi* (Sapindaceae) (12), while the terminal glucose was in good agreement with methyl-β-D-glucopyranoside (Table 2). Thus, **3** was identified as 3-*O*-β-D-glucopyranosyl-(1↦3)-α-L-rhamnopyranosyl-(1↦2)-α-L-arabinopyranosyl-30-noroleana-12,20(29)-dien-28-oic acid.

The negative fabms of **1** and **2** yielded molecular ions at *m/z* 1203 and 1349 $[M-H]^-$, indicating molecular weights of 1204 and 1350, respectively. Signals at *m/z* 1042 $[M-Glc-H]^-$, 895 $[M-Glc-Rha-H]^-$, 879 $[M-Glc-Glc-H]^-$, 717 $[879-Glc]^-$, 571 $[717-Rha]^-$ for **1** and 1203 $[M-Rha-H]^-$, 1187 $[M-Glc-H]^-$, 879 $[M-Glc-Glc-Rha-H]^-$, 717 $[879-Glc]^-$, 571 $[717-Rha]^-$ for **2** correspond to sequential losses of sugar residues. The eims of the peracetate of compound **1** displayed fragment ions at *m/z* $[(Glc-Rha)Ac_6+H]$ and 331 $[(Glc)Ac_4$, terminal glucose], and the peracetate of **2** similarly displayed fragment ions at *m/z* 562 $[(Glc-Rha)Ac_6+H]$, 331 $[(Glc)Ac_4$, terminal glucose], and 273 $[(Rha)Ac_3$, terminal rhamnose]. These data fully established the sequence of sugars. The interglycosidic linkages were established by ^{13}C-nmr spectroscopy (Table 2) (12–15). Close kinship among the saponins was shown by the enzymatic hydrolysis of compound **1** which furnished, among others, yemuoside YM_{12} (3) with a terminal glucose. In a similar manner, **2** furnished yemuoside YM_{10} (3).

In the ^{1}H-nmr spectra, the anomeric proton signals for **1** at δ 4.76 (1H, d, J = 6.5 Hz), 5.06 (1H, d, J = 7.7 Hz), 5.14 (1H, d, J = 8.0 Hz), 6.19 (1H, d, J = 8.0 Hz), and 6.35 (1H, s), for compound **2** at δ 4.82 (1H, d, J = 6.0 Hz), 4.97 (1H, d, J = 8.0 Hz), 5.18 (1H, d, J = 8.0 Hz), 5.86 (1H, s), 6.21 (1H, d, J = 8.1 Hz), and 6.38 (1H, s) led to the assignments of the anomeric configurations of glucose units as β and those of rhamnose and arabinose units as α; these assignments were supported by their carbon signals (Table 2).

Based on the above results, **1** and **2** were established as 3-*O*-β-D-glucopyranosyl-(1↦3)-α-L-rhamnopyranosyl-(1↦2)-α-L-arabinopyranosyl-30-noroleana-12,20(29)-dien-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1↦4)-β-D-glucopyranosyl-(1↦6)-β-D-glucopyranoside and 28-*O*-β-D-glucopyranosyl-(1↦6)-β-D-glucopyranoside, respectively.

EXPERIMENTAL

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 polarmeter. Ir spectra were recorded on a Perkin-Elmer 683 specrophotometer. ^{1}H-nmr and ^{13}C-nmr spectra were obtained on a JEOL GX-400 spectrometer at 400 MHz and 100 MHz, respectively. Chemical shifts are given in δ (ppm) with TMS as an internal standard. The eims were measured on a ZAB-2F spectrometer, and the fabms spectra were taken on a JEOL DX303-HF system and JMS-SX102 GC/MS system spectrometer with an accelerating potential of 3.0 kV and 6.0 kV for an Xe beam source with glycerol and thioglycerol as matrices.

EXTRACTION AND ISOLATION.—The dried whole plants (3 kg) of *S. chinensis* were collected in Jiangxi province of China in the summer of 1985; a specimen was identified by Prof. W.Z. Song, the Institute of Materia Medica, Chinese Academy of Medical Sciences, where a voucher specimen is deposited. The plants 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 l) and extracted with EtOAc (0.3 liters × 5) and then with *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

developed 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 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. Combined fractions 15–17 (522 mg) were further purified by repeated Si gel cc to give yemuoside YM_8 [**1**] (126 mg). Fractions IV and V (8.34 g) were separated into fourteen crude saponin fractions in the same manner as that for fraction VI, and fraction 12 (680 mg) was purified further over a Si gel column to give yemuoside YM_9 [**2**] (150 mg).

CHARACTERIZATION OF **1**.—White powder, mp 208–212° (dec), $[\alpha]^{28}D$ +16.08° (c = 0.227, MeOH). *Anal.* calcd for $C_{58}H_{92}O_{26}\cdot 2H_2O$: C 57.81, H 7.85; found C 57.74, H 7.56; ir ν max (KBr) cm^{-1} 3400 (OH), 2900 (C-H), 1730 (C=O, ester), 1630 (C=C), 1350, 1050 (C-O-C); ^1H nmr (C_5D_5N) δ 0.83, 1.02, 1.05, 1.15, 1.19 (each 3H, s, Me), 1.53 (3H, d, J = 6.2 Hz, Me-5 of Rha), 3.08 (1H, dd, J = 5.0, 14.0 Hz, H-18), 3.23 (1H, dd, J = 4.0, 12.2 Hz, H-3α), 4.63, 4.69 (each 1H, br s, H-29), 4.76 (1H, d, J = 6.5 Hz, H-1 of α-Ara), 5.06 (1H, d, J = 7.7 Hz, H-1 of β-Glc), 5.14 (1H, d, J = 8.0 Hz, H-1 of β-Glc), 5.39 (1H, m, H-12), 6.19 (1H, d, J = 8.0 Hz, H-1 of β-Glc), 6.35 (1H, s, H-1 of Rha); ^{13}C nmr see Tables 1 and 2; fabms m/z $[M-H]^-$ 1203, $[M-Glc-H]^-$ 1041, $[M-Glc-Rha-H]^-$ 895, $[M-Glc-Glc-H]^-$ 879, $[879-Glc]^-$ 717, $[717-Rha]^-$ 571, $[aglycone-H]^-$ 439; Liebermann-Burchard reaction reddish purple.

CHARACTERIZATION OF **2**.—White powder, mp 207–210° (dec), $[\alpha]^{28}D$ +20.6 (c = 0.136, MeOH). *Anal.* calcd for $C_{64}H_{102}O_{30}\cdot 3/2\,H_2O$: C 55.77, H 7.63; found C 55.87, H 7.51; ir ν max (KBr) cm^{-1} 3400 (OH), 2900 (C-H), 1730 (C=O, ester), 1630 (C=C), 1350, 1050 (C-O-C); ^1H nmr (C_5D_5N) δ 0.87, 1.07, 1.08, 1.19, 1.23 (each 3H, s, Me), 1.58 (3H, d, J = 5.8 Hz, Me-5 of Rha), 1.71 (3H, d, J = 6.2 Hz, Me-5 of Rha), 3.11 (1H, dd, J = 4.8, 13.7 Hz, H-18), 3.26 (1H, dd, J = 4.0, 12.0 Hz, H-3α), 4.70, 4.76 (each 1H, br s, H-29), 4.82 (1H, d, J = 6.0 Hz, H-1 of α-Ara), 4.97 (1H, d, J = 8.0 Hz, H-1 of β-Glc), 5.18 (1H, d, J = 8.0 Hz of β-Glc), 5.44 (1H, m, H-12), 5.86 (1H, s, H-1 of α-Rha), 6.21 (1H, d, J = 8.1 Hz, H-1 of β-Glc), 6.38 (1H, s, H-1 of α-Rha); ^{13}C nmr see Tables 1 and 2; fabms m/z $[M-H]^-$ 1349, $(M-Rha-H]^-$ 1203, $[M-Glc-H]^-$ 1187, $[M-Glc-Glc-Rha-H]^-$ 879, $[879-Glc]^-$ 717, $[717-Rha]^-$ 571, $[aglycone-H]^-$ 439, $[aglycone-H_2O-H]^-$ 421; Liebermann-Burchard reaction: reddish purple.

ACID HYDROLYSIS OF **1** AND **2**.—A mixture of sample and 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, ^1H nmr) and 3β-hydroxy-30-noroleana-12,19-dien-28-oic acid (mmp, co-tlc, ir, ^1H nmr) (6).

IDENTIFICATION OF SUGARS.—The aqueous layer was neutralized with 1 N NaOH, concentrated, and subjected to the hptlc analysis on Kieselgel 60 F_{254} (Merck) using EtOAc-H_2O-MeOH-HOAc (13:3:3:4), which showed Ara, Rha, and Glc in **1** and **2**.

ACETYLATION OF **1** AND **2**.—A solution of sample (10 mg) in a mixture of Ac_2O (0.3 ml) and C_5D_5N (0.3 ml) was allowed to stand at room temperature, and the reaction mixture was worked up as usual to give the peracetate of **1** (9 mg): white powder (MeOH), mp 148–151° (dec). *Anal.* calcd for $C_{88}H_{122}O_{41}\cdot 3H_2O$: C 55.93, H 6.78; found C 56.00, H 6.89. ^1H nmr ($CDCl_3$) δ 2.00–2.20 (15 × Ac-); eims m/z $[(Glc\text{-}Rha)Ac_6+H]^+$ 562, $[(Glc)Ac_4]^+$ 331, $[aglycone-OH]^+$ 423. Peracetate of **2** (10 mg): white powder (MeOH); mp 161–164° (dec). *Anal.* calcd for $C_{98}H_{136}O_{47}\cdot 2H_2O$: C 56.00, H 6.67; found C 56.19, H 6.69. ^1H nmr ($CDCl_3$) δ 2.00–2.20 (17 × Ac-); eims m/z $[(Glc\text{-}Rha)Ac_6+H]^+$ 562, $[(Glc)Ac_4]^+$ 331, $[(Rha)Ac_3]^+$ 273.

ALKALINE HYDROLYSIS OF **1** AND **2**.—Compound **1** (60 mg) was refluxed with 2% KOH in 50% EtOH (6 ml) for 5 h. The reaction mixture was slowly neutralized with dilute HCl and concentrated in vacuo. The residue showing a spot on tlc [solvent: $CHCl_3$-MeOH-H_2O (10:5:1)] was recrystallized to give **3** (35 mg): white powder (MeOH), mp 238–242° (dec); $[\alpha]^{28}D$ +21.68° (c = 0.249, MeOH). *Anal.* calcd for $C_{46}H_{72}O_{16}\cdot 2H_2O$: C 60.26, H 8.30; found C 60.04, H 8.44; ir ν max (KBr) cm^{-1} 3400 (OH), 2900 (C-H), 1680 (C=O, acid), 1640 (C=C); ^1H nmr (C_5D_5N) δ 0.78, 0.94, 1.03, 1.17, 1.24 (each 3H, s, Me), 3.23 (2H, dd, J = 4.5, 13.0 Hz, H-18, H-3α), 4.52 (1H, d, J = 5.8 Hz, H-1 of α-Ara), 4.73, 4.78 (each 1H, br s, H-29), 5.15 (1H, d, J = 7.8 Hz, H-1 of β-Glc), 5.45 (1H, m, H-12), 6.37 (1H, s, H-1 of α-Rha); ^{13}C nmr see Tables 1 and 2; fdms m/z $[M+Na+H]^+$ 904, $[aglycone]^+$ 440. Peracetate of **3**: white powder, mp 173–176° (dec); ^1H nmr ($CDCl_3$) δ 1.98–2.20 (8 × Ac-); eims m/z $[(Glc\text{-}Rha)Ac_6]^+$ 561, $[(Glc)Ac_4]^+$ 331.

Compound **2** (60 mg) was hydrolyzed with alkali and worked up in the same manner as that for **1** to provide a white powder (28 mg) which was identical with **3** (mmp, tlc, ir, and ^{13}C nmr).

ENZYMATIC HYDROLYSIS OF **1** AND **2**.—A mixture of sample (3 mg), β-glucosidase (10 mg), and

H_2O (1.0 ml) was left to stand at 37° for 48 h. Control tlc using $CHCl_3$-MeOH-H_2O (2.6:1.6:0.3) as eluent showed that **1** gave yemuoside YM_{12} (3) and **2** gave yemuoside YM_{10} (3).

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