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TWO NEW LIGNAN GLYCOSIDES FROM *SCHISANDRA RUBRIFLORA*

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Abstract - Two new lignan glycosides, rubriflosides A (**1**) and B (**2**), were isolated from the aerial parts of *Schisandra rubriflora*. Their structures were determined on the basis of spectroscopic evidences.

Schisandra rubriflora (Schisandraceae) is distributed in the southwestern provinces of the People's Republic of China. Different parts of the plant are used in the folklore and traditional systems of medicine for treatment of various problems such as stomach pain and neurasthenia.[1] A number of species of genus *Schisandra* have been investigated, describing the isolation of lignans and triterpenoids.[2-7] The present study deals with the isolation and structure elucidation of two new lignan glycosides, rubriflosides A (**1**) and B (**2**), from the ethanol extract of the aerial parts of *Schisandra rubriflora*.

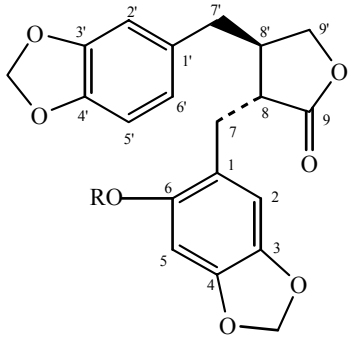

Figure 1

1: R=Glu
2: R=Glu^{6}-Ara(f)

Compound (**1**) was obtained as an amorphous solid. The molecular formula was determined by HRESI-MS: found 531.1512 for $C_{26}H_{17}O_{12}$ $[M-H]^-$, calcd 531.1502. Its negative FABMS spectrum showed a peak at *m/z* 531 $[M-H]^-$ and a significant peak at *m/z* 369 for aglycon, indicating the lost unit to be 162 mu for the existence of glucose ($C_6H_{12}O_6$). The UV spectrum showed absorption maxima at λ_{max}

234 and 290 nm for an aromatic ring, and the IR spectrum showed absorptions for carbonyl (1752 cm^{-1}), aromatic ring (1637, 1503, 1487 cm^{-1}) and hydroxy groups (3421 cm^{-1}).

The ^{1}H- and ^{13}C-NMR spectra of **1** showed signals for two methylenedioxies, two substituted benzyl groups, a butyrolactone ring and a glucose, suggesting that **1** is a diarylbutyrolactone-type lignan. The signals in the ^{1}H and ^{13}C NMR spectra (Table 1) were assigned by HMQC and HMBC experiments. The signals assigned to the aglycon moiety were in good agreement with those of hinokinin.[8]

The COSY spectrum of **1** showed correlations between H-8 (δ 2.77) and H-7 protons (3.04 and 2.85). H-8 and H-8′ (2.62), H-8′ and H-7′ (2.44), H-8′ and H-9′ (4.25 and 3.95), H-5′ (6.63) and H-6′ (6.47). This spectrum also showed correlations of the signals of glucose. The ^{1}H-NMR signals assigned to glucose were confirmed by HMQC-TOCSY.

Acid hydrolysis of **1** afforded sugar component identified by TLC and PC analysis as D-glucose. Coupling constant of the anomeric proton of D-glucose at δ 4.69 (7.5 Hz) indicates the anomeric configuration was *β*. The position of the sugar unit was unambiguously defined by HMBC experiment. A cross peak due to long-range coupling between C-6 (δ_C 151.8) of the aglycon and H-G1 (4.69), indicated that D-glucose unit was linked to C-6 of the aglycon. Moreover, the ROESY spectrum of **1** showed NOE contours between H-5 (6.85) and H-G1 (4.69).

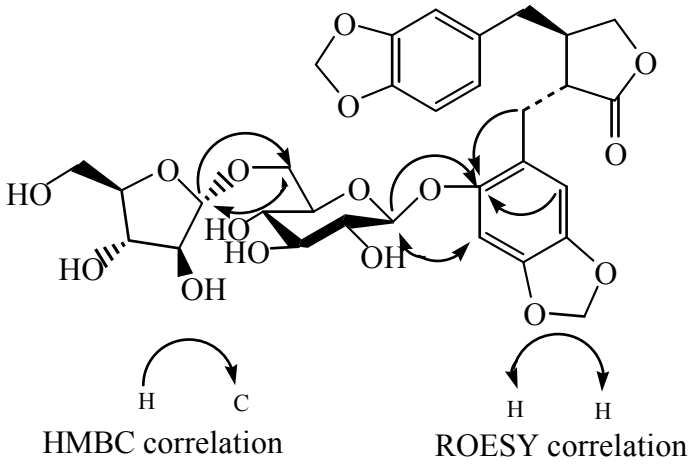

Figure 2 Key HMBC and ROESY correlations of **2**.

As regards the absolute configuration of the lactone junctions, negative Cotton effects appeared at 246 nm and 276 nm in circular dichroism (CD) spectrum, as in case of analogous compounds,[9,10] suggesting that the configurations of C-8 and C-8′ are 8R and 8′R.

Thus, compound (**1**) was assigned as 6-*O*-*β*-D-glucopyranosylhinokinin and has been named rubrifloside A.

Compound (**2**) was obtained as an amorphous solid. The molecular formula was determined by HRESI-MS: found 663.1914 for $C_{31}H_{35}O_{16}$ $[M\text{-}H]^{-}$, calcd 663.1925. Its negative FABMS spectrum

showed a peak at *m/z* 663 $[M-H]^-$ and a significant peak at m/z 369 for aglycon. The IR, UV and CD spectra of **2** resembled with those of **1**. The aglycon part of **2** was found to be the same as that of **1** as deduced by extensive NMR spectroscopy. Acid hydrolysis of **2** afforded L-arabinose and D-glucose. The ^1H and ^{13}C NMR spectral data of **2** were closely related to those of **1**, except for downfield shift of C-G6 (δ_C 68.5) and the appearance of signals due to a α-L-arabinofuranose moiety. The structure of the second sugar was determined as α-L-arabinofuranose by comparison with NMR spectral data reported in the literature.[11,12] The additional α-L-arabinofuranose moiety was shown to be linked to the glucose unit though a 1→6 linkage, as indicated by a downfield shift (δ_C 5.8 ppm) of C-G6 (δ_C 68.5) relative to the corresponding carbon in **1** (δ_C 62.7). This was confirmed from HMBC and NOSEY, as shown in Figure 2. On the basis of the above results, the structure of **2** was determined as 6-*O*-[α-L-arabinofuranosyl (1→6)]-β-D-glucopyranosyl hinokinin and has been named rubrifloside B.

EXPERIMENTAL

General experimental procedures--IR spectra were taken on Nicolet AVATAR-360. The UV spectra were recorded on Shimadzu-2401PC spectrophotometer. CD spectra were measured on JASCO-20C spectropolarimeter. Optical rotations were taken on Perkin-Elmer-341 polarimeter. The 1D and 2D NMR spectra were recorded on Bruker DRX-500 spectrometer. FAB-MS was performed on VG-Autospec-3000 spectrometer. HRESI-MS was performed on API Qstar Pulsar spectrometer. Column chromatography: silica gel (200-300 mesh, Qingdao, PR China), Sephadex LH-20 (Amersham Pharmacia Biotech). TLC: silica gel (GF_{254}, Qingdao, PR China).

Plant material--The plant material was collected in lincang county, Yunnan province, PR China, in September 2001. The identification of the plant was confirmed by Prof. Zhihao Hu, Department of Botany, Yunnan University. A voucher specimen was deposited in School of Pharmacy, Yunnan University.

Extraction and Isolation--The air-dried and powdered plant (6.0 kg) was extracted with 95% EtOH (4×10 L) at rt for 48 h each time. The residue (986 g) was suspended in water, and then extracted with petrol, EtOAc and n-BuOH, successively. The n-BuOH extract (66 g) was chromatographied on silica gel (1.2 kg, 200-300 mesh) and eluted with $CHCl_3$ containing increasing amounts of MeOH ($CHCl_3$-MeOH, 95:5-50:50). Fraction B (obtained with $CHCl_3$-MeOH 90:10) was chromatographied over Sephadex LH-20 with MeOH to afford compound (**1**) (41 mg) and compound (**2**) (13 mg).

Rubrifloside A (**1**). $[\alpha]^{24}_{D}$ +5.1° (MeOH, *c* = 0.45); UV λ_{max}^{MeOH}nm (log ε): 204 (4.71), 234 (4.07), 290 (4.00); CD (99% MeOH) Δε (nm) : –2.16 (246), –1.29 (276); IR (KBr): 3421, 2922, 1752, 1637, 1503, 1487, 1444, 1387, 1246, 1188, 1073, 1038, 928, 862, 811, 771 cm^{-1}; ^{1}H- and ^{13}C- NMR (see Table 1).

Table 1. ^{1}H- and ^{13}C-NMR spectral data (500 and 125 MHz, CD_3OD) for compounds (**1**) and (**2**).

Attribution	**1**		**2**	
	δ ^{13}C	δ ^{1}H (*J* in Hz)	δ ^{13}C	δ ^{1}H (*J* in Hz)
1	121.8	—	121.8	—
2	110.7	6.61 s	110.7	6.60 s
3	148.4	—	148.4	—
4	144.2	—	144.2	—
5	100.4	6.85 s	100.4	6.88 s
6	151.8	—	151.7	—
7	31.4	2.85 dd (9.8, 13.3)	31.3	2.86 dd (10.0, 13.4)
		3.04 dd (4.7, 13.4)		3.04 dd (4.7, 13.6)
8	46.5	2.77 m	46.6	2.75 m
9	182.2	—	182.1	—
1’	133.8	—	133.8	—
2’	110.0	6.50 d (1.6)	110.0	6.51 d (1.6)
3’	149.1	—	149.1	—
4’	147.5	—	147.5	—
5’	109.0	6.63 d (7.9)	109.0	6.63 d (8.1)
6’	122.8	6.47 dd (7.9, 1.6)	122.8	6.47 dd (8.1, 1.6)
7’	39.4	2.44 d (7.6)	39.5	2.44 d (7.6)
8’	43.4	2.62 m	43.3	2.62 m
9’	73.2	3.95 dd (7.0, 9.0)	73.2	3.96 t (7.7)
		4.25 dd (7.5, 9.0)		4.26 t (7.7)
$3OCH_2O4$	102.6	5.89 d (1.2); 5.90 d (1.2)	102.6	5.89 d (1.0); 5.90 d (1.0)
$3’OCH_2O4’$	102.2	5.87 d (1.2); 5.88 d (1.2)	102.2	5.87 d (1.0); 5.88 d (1.0)
Glc-1	104.1	4.69 d (7.5)	103.8	4.68 d (7.2)
Glc-2	75.1	3.45 m*	75.1	3.47 m*
Glc-3	78.3	3.44 m*	78.3	3.45 m*
Glc-4	71.5	3.39 m	72.0	3.36 m
Glc-5	78.3	3.38 m	76.9	3.59 m
Glc-6	62.7	3.70 dd (5.5, 12.0)	68.5	3.62 m
		3.90 dd (1.7, 12.0)		4.08 dd (14.3, 4.0)
Ara-1			110.1	4.92 d (1.2)
Ara-2			83.2	4.04 m
Ara-3			78.8	3.85 m
Ara-4			85.9	3.97 m
Ara-5			63.0	3.63 m
				3.73 d (11.9, 2.9)

Chemical shift values are in ppm from TMS. Glc = *β*-D-glucopyranosyl, Ara = *α*-L-arabinofuranosyl. *Assignments may be interchangeable.

Rubrifloside B (**2**). $[\alpha]^{24}_{D}$ –22.0° (MeOH, *c* = 0.45); UVλ_{max}^{MeOH}nm (log ε): 202 (4.33), 230 (3.66), 288 (3.51); CD (99% MeOH) Δε (nm) : –4.53 (237), –11.94 (288); IR (KBr): 3406, 2912, 1752, 1629, 1503, 1487, 1444, 1364, 1247, 1189, 1168, 1038, 928, 861, 811, 770 cm^{-1}; ^{1}H- and ^{13}C- NMR (see Table 1).

Acid hydrolysis of 1 and 2. A solution of **1** and **2** (4 mg each) in 2N HCl (5 ml) was heated for 2 h. After removing HCl by evaporation in a vacuum, the mixture was diluted with H_2O and extracted with EtOAc. The aqueous layer was neutralized with 1N NaOH and subjected to TLC analysis [using $CHCl_3$-acetone-MeOH-H_2O, 3:3:3:1] and PC [using n-BuOH-HOAc-H_2O, 4:1:5] with standard D-glucose or L-arabinose, in which the presence of sugar was established.

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