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PHENOLIC GLYCOSIDES FROM RHIZOMES OF *SMILAX GLABRA*

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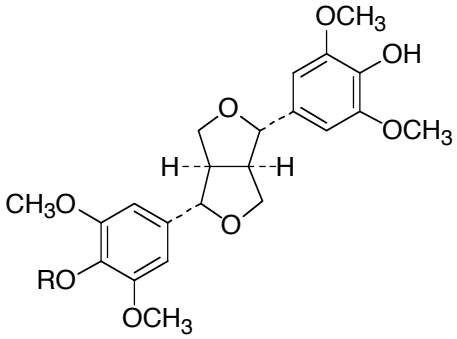
Abstract – A new lignan glycoside, (+)-syringaresinol 4-*O*-β-D-glucopyranosyl-(1→6) -β-D-glucopyranoside (**1**), together with twelve known compounds, were isolated from the rhizomes of *Smilax glabra*. The structure was established on the basis of chemical and spectroscopic methods.

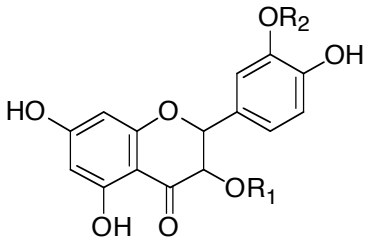
The rhizomes of *Smilax glabra* Roxb. (Liliaceae) have been extensively used in Chinese traditional medicines, called "Tufuling" for the treatment of syphilis, acute bacterial dysentery, acute and chronic nephritis. Also, its methanol extract has been shown to possess hypoglycemic activity.[1] Recently a number of flavonol glycosides and phenylpropanoid glycosides were isolated from the plant.[2, 3] In our phytochemical studies on this species, we have examined the chemical constituents of the rhizomes of *S. glabra*. Herein we report the isolation and structural elucidation of a new lignan glycoside (**1**), along with twelve known compounds from the plant. The known compounds were identified as astilbin (**2**), neoastilbin (**3**), isoastilbin (**4**), neoisoastilbin (**5**),[4,5] 3'-*O*-β-D-glucopyranosyl-(2*R*, 3*R*)-taxifolin (**6**),[6] 3-(β-D-glucopyranosyloxy)-1-(4-hydroxy-3, 5-dimethoxyphenyl)-1-propanone (**7**),[7] 8, 8'-bisdihydrosieingenin glucopyranoside (**8**),[8] 3,4,5-trimethoxyphenyl β-D-glucopyranoside (**9**),[9] kelampayoside A (**10**),[10] osmanthuside F (**11**),[11] *trans*-resveratrol 3-*O*-β-D-glucopyranoside (**12**),[12] and 6-hydroxy-2, 4-di-*O*-(β-D-glucopyranosyl) acetophenone (**13**)[13] by comparison of various spectral and chemical data with those reported in literature.

Compound (**1**) was obtained as an amorphous white powder. The ESI-MS of **1** showed a pseudo molecular ion peak at *m/z* 765.8 $[M+Na]^+$. Its molecular formula, $C_{34}H_{46}O_{18}$, was established by the HR-FAB-MS. The IR spectrum of **1** showed absorption bands due to hydroxyl group (3426 cm^{-1}) and aromatic ring (1628, 1517 cm^{-1}), while the UV spectrum showed absorption band at 271 nm, suggesting the presence of aromatic ring in its structure. The ^{1}H-NMR spectrum of **1** showed signals assignable to



1 R= Glc(1→6)Glc

Glc=β-D-glucopyranosyl
Rha=α-L-rhamnopyranosyl



2 (2*R*, 3*R*), R_1=Rha, R_2=H
3 (2*S*, 3*S*), R_1=Rha, R_2=H
4 (2*S*, 3*R*), R_1=Rha, R_2=H
5 (2*R*, 3*S*), R_1=Rha, R_2=H
6 (2*R*, 3*R*), R_1=H, R_2=Glc

Table 1. ^{1}H- and ^{13}C-NMR spectral data of **1** (DMSO-d_6)

Position	δ_C [a]	δ_H (mult, *J* in Hz) [b]	Position	δ_C [a]	δ_H (mult, *J* in Hz) [b]
Aglycone moiety			Sugar moiety		
1	137.2		Glc-1	102.4	4.90 (d, 7.3)
2 and 6	104.6	6.64 (s)	-2	74.0	3.23 [c]
3 and 5	152.6		-3	76.4	3.20 [c]
4	133.6		-4	69.8	3.18 [c]
7	84.9	4.70 (d, 4.8)	-5	76.3	3.25 [c]
8	53.4	3.10 (m)	-6	67.9	3.57 (dd, 11.6, 5.7)
9	71.3	3.79 (dd, 9.0, 1.6)			3.88 (dd, 11.6, 1.6)
		4.21 (dd, 9.0, 6.8)	Glc-1'	102.7	4.07 (d, 7.6)
1'	131.4		-2'	73.5	2.87 (dd, 8.9, 7.6)
2' and 6'	103.8	6.60 (s)	-3'	76.5	3.02 (t, 8.9)
3' and 5'	147.9		-4'	70.0	3.00 (dd, 9.2, 8.9)
4'	135.0		-5'	76.6	2.90 (ddd, 9.2, 5.7, 2.0)
7'	85.3	4.60 (d, 5.1)	-6'	61.0	3.42 (dd, 11.6, 5.7)
8'	53.6	3.06 (m)			3.62 (dd, 11.6, 2.0)
9'	71.1	3.80 (dd, 9.0, 1.2)			
		4.15 (dd, 9.0, 6.8)			
3, 5-OCH_3	56.5	3.77 (s)			
3', 5'-OCH_3	56.1	3.76 (s)			

[a] Measured at 500 MHz. [b] Measured at 125 MHz. [c] Overlapped signals.

four benzene-ring protons [δ 6.64 (2H, s) and 6.60 (2H, s)], four methoxyl groups on the benzene ring [δ 3.77 (6H, s) and 3.76 (6H, s)], and two anomeric protons [δ 4.90 (d, *J* = 7.3 Hz) and 4.07 (d, *J* = 7.6 Hz)], suggesting that **1** is a diglycoside of a symmetrically substituted benzenoid aglycone. The ^{1}H-NMR spectrum of **1** analysed with the aid of DQF COSY, CHSHF and HMBC experiments was in agreement with reported data for lignans of the 2, 6-diaryltetrahydrofurofuran ring system.[14] The ^{1}H-NMR signals

for H-7 and H-7' which appear at 4.70 and 4.60, and the signals for H-9 and H-9' which appear at 3.79 (H_{ax}-9), 3.80 (H_{ax}-9'), 4.21 (H_{eq}-9) and 4.15 (H_{eq}-9'), suggested that both aryl groups are equatorial oriented.[15,16] The ^{1}H-NMR spectrum also supported the presence of two 1, 3, 4, 5-tetrasubstituted aromatic rings. In the HMBC spectrum, it was evident that the two pairs of methoxyl groups are placed at each 3, 5 and 3', 5' position of the two aromatic rings. Upon enzymatic hydrolysis, **1** liberated D-glucose[17] and (+)-syringaresinol (**1a**).[18] In the ^{13}C-NMR spectrum of **1**, the signal due to glc-C-6 was observed at lower field (67.9), suggested that the sequence in the sugar moiety of **2** is D-glucopyranosyl (1 6)-D-glucopyranose, this was confirmed by HMBC spectrum. Furthermore, the coupling constants (7.3 and 7.6 Hz) of the anomeric proton signals of the two D-glucosyl moieties demonstrated that both sugar moieties have -anomeric configuration. Based on the aforementioned evidence, the structure of **1** was elucidated to be (+)-syringaresinol-4-*O*--D-glucopyranosyl (1 6)--D-glucopyranoside.

EXPERIMENTAL

General Experimental Procedures. The UV spectra were obtained with a Shimadzu UV-160 spectrophotometer, the IR spectra were measured with a JASCO FT/IR-300E (by a KBr disk method) spectrometer. The optical rotations were measured with a JASCO DIP-370 digital polarimeter in a 1.0 dm length cell. The ESI-MS was taken on an LCQ ESI-MS system, and HR-FAB-MS was taken on a JEOL JMS DX-700 MStation. The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL ECP-500 spectrometer and chemical shifts are expressed in (ppm) referring to TMS. For HPLC, JASCO HPLC system was used. Column chromatography was carried out using Chromatorex DM1020T ODS and Kieselgel 60 silica gel. TLC was conducted on Kieselgel 60 F_{254} plates (Merck).

Plant Material. Rhizomes of *Smilax glabra* were collected in Shenyang, China. A voucher specimen is deposited in the herbarium of Shenyang Pharmaceutical University, China.

Extraction and Isolation. The dried rhizomes (6.0 kg) of *S. glabra* were extracted with 80% EtOH (10 L) under reflux for 2 h. The extract (256 g) was suspended in H_2O (2 L) and extracted with petrol ether, EtOAc and *n*-BuOH successively to give the petrol ether-soluble fraction (13.8 g), EtOAc-soluble fraction (15.1 g) and *n*-BuOH-soluble fraction (36.4 g). The *n*-BuOH-soluble fraction was chromatographed on a Diaion HP-20 column using H_2O, 40% MeOH and 100% MeOH, to afford 3 fractions. Fraction 2 (40% MeOH eluate, 5.9 g) was further purified by silica gel ($CHCl_3$-MeOH-H_2O), ODS (H_2O-MeOH) and HPLC to give compounds **7** (2 mg), **9** (14 mg), **10** (11 mg), **11** (11 mg), **13** (8 mg). The fraction 3 (100% MeOH eluate, 12.5 g) was also purified in the same manner to afford compounds **1** (5 mg), **2** (77 mg), **3** (22 mg), **4** (15 mg), **5** (16 mg), **6** (19 mg), **8** (3 mg) and **12** (4 mg).

(+)-Syringaresinol 4-*O*--D-glucopyranosyl(1 6)--D-glucopyranoside (1). Amorphous white powder, $[]_D^{24}$ –10.7° (*c* = 0.55, MeOH). ESI-MS (positive) *m/z*: 765.8 $[M+Na]^+$. HR-FAB-MS *m/z*: 765.2574

([M+Na]$^+$) (Calcd for $C_{34}H_{46}O_{18}Na$, 765.2581). UV λ_{max} (MeOH) nm (log ε): 210 (4.67), 271 (3.44). IR (KBr) cm^{-1}: 3426, 1628, 1517. ^{1}H-NMR (DMSO-d_6, 500MHz) and ^{13}C-NMR (DMSO-d_6, 125 MHz): see Table 1.

Enzymatic Hydrolysis of 1. A solution of **1** (5.0 mg) in 0.1 M acetate buffer (pH 4.0, 1.0 mL) was treated with naringinase (Sigma Chemical Co., 3.0 mg) and then the reaction mixture was stirred at 40 °C for 12 h. The reaction mixture was passed through a Sep-Pak C_{18} cartridge using H_2O and MeOH. The H_2O eluate was concentrated to give sugar fraction. The MeOH elute was evaporated and further separated by HPLC to give the aglycone (**1a**, 1.0 mg). **1a** was identified as (+)-syringaresinol by its MS, ^{1}H-NMR spectral and $[\alpha]_D$ data.

Identification of Sugar. The sugar fraction from enzyme hydrolysis of **1** was dissolved in 1 mL of H_2O, to which (-)-α-methylbenzylamine (5 mg) and $NaBH_3CN$ (3 mg) in EtOH (1 mL) were added. After being set aside at 40 °C for 4 h followed by addition of glacial acetate (0.2 mL) and evaporated to dryness, the resulting solid was acetylated with acetic anhydride (0.3 mL) in pyridine (0.3 mL) for 24 h at rt. The reaction mixture was evaporated 3 times by adding water to remove pyridine, and then the residue was passed through a Sep-Pak C_{18} cartridge (Waters) with 20% and 50% MeCN (each 10 mL) as solvents. The 50% MeCN eluate was further passed through a Toyopak IC-Sp M cartridge (Tosoh, Tokyo, Japan) with EtOH (10 mL) to give mixture of the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides, which was then analyzed by HPLC under following conditions: Column, Inertsil ODS-3 (4.6 x 250 mm); Solvent: 40% MeCN; flow rate, 0.8 mL/min; detection, UV 230 nm. The derivatives of D-glucose were detected with the t_R of 27.7 min.

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