

HETEROCYCLES, Vol. 83, No. 8, 2011, pp. 1897 - 1902. © The Japan Institute of Heterocyclic Chemistry
Received, 12th May, 2011, Accepted, 13th June, 2011, Published online, 23rd June, 2011
DOI: 10.3987/COM-11-12257

TWO NEW XANTHONE GLUCOSIDES FROM *SWERTIA MUSSOTII* FRANCH

Lu Gao, Yongfu Zhou, Haiying Yan, Feiyan Huang, Rongrong Wen, and Ganpeng Li *

Key Laboratory of Ethnic Medicine Resource Chemistry, State Ethnic Affairs Commission & Ministry of Education, School of Chemistry and Biotechnology, Yunnan University of Nationalities, Kunming 650031, P. R. China; E-mail address: ganpeng_li@sina.com

Abstract – Two new xanthone glucosides, 1-O- β -D-glucopyranosyl-3,5,6-trimethoxy-xanthone (**1**), 1-O-[β -D-xylopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl]-3,5,6-trimethoxy-xanthone (**2**), were isolated from a EtOH extract of the overground parts of *Swertia mussotii* Franch, which is a traditional Tibet medicine. Their structures were elucidated by spectroscopic methods as well as by chemical studies. Compound **1** showed α -glucosidase inhibitory activity, while compound **2** showed DPP-IV (Dipeptidyl Peptidase IV) inhibitory activity.

Plants of the genus *Swertia* have been used as traditional medicinal plants in various parts of the world. *Swertia mussotii* Franch, known as “Zangyingcheng” (Chinese), is mainly distributed in Qinghai, Xizang, Sichuan, and Yunnan of China. In traditional Chinese medicine, the plant is of cold property and its taste is bitter. It has been used as a traditional Tibetan remedy to treat acute epioephsitis, soprethroat, tonsillitis, icterichepatitis, cholecystitis, gastroenteritis bacillary dysentery and urinary tract infection.¹ Our previous studies have shown that xanthone, triterpenoid, iridoid, and steroid are main constituents of this plant.²⁻⁴ As a continuation of our studies on chemical constituents of *Swertia* plant, from the overground parts of *Swertia mussotii* Franch, two new xanthone glucosides, 1-O- β -D-glucopyranosyl-3,5,6-trimethoxy-xanthone (**1**), 1-O-[β -D-xylopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl]-3,5,6-trimethoxy-xanthone (**2**), were isolated. Their structures were elucidated by spectroscopic methods as well as by chemical studies. In the *in vitro* assays, compound **1** showed weak α -glucosidase inhibitory activity compared with the positive control acarbose, while compound **2** showed weak DPP-IV

(Dipeptidyl Peptidase IV) inhibitory activity compared with the positive control INDPP-2.

Compound **1** was obtained as a colorless gum. Its molecular formula was deduced to be $C_{22}H_{24}O_{11}$ by ^{13}C NMR spectra (Table 1) and ESI-MS, with quasi-molecular ion peaks at m/z 465 ($[M+H]^+$) and 487 ($[M+Na]^+$). The IR spectrum showed the presence of hydroxyl group (3406 cm^{-1}), aromatic ring (1624 , 1588 , 1453 cm^{-1}), and $C=O$ bond (1650 cm^{-1}). The UV spectrum exhibited absorption maxima at λ_{max} 357, 303, and 248 nm, characteristic of a 1,3,5,6-tetraoxygenated xanthone skeleton.⁵

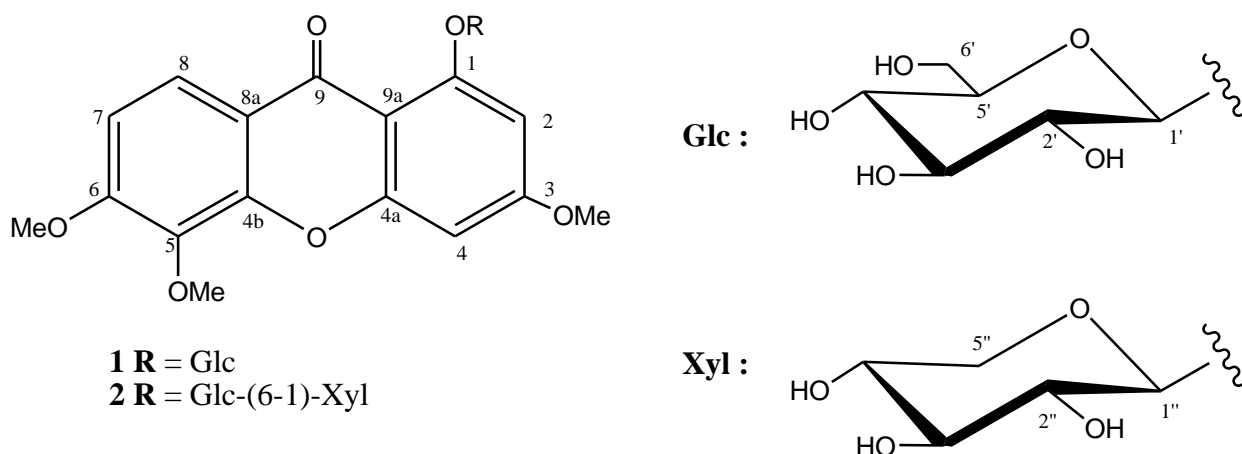


Figure 1. Structures of compounds **1** and **2**

The 1H NMR spectrum of compound **1** showed two meta-coupled H-atoms at δ_H 7.26 (1H, d, $J = 2.4$ Hz) and 6.61 (1H, d, $J = 2.4$ Hz), assigned to H-2 and H-4, and a pair of ortho-coupled H-atoms at δ_H 7.33 (1H, d, $J = 9.2$ Hz) and 7.21 (1H, d, $J = 9.2$ Hz) attributed to H-7 and H-8, respectively. The three signals at δ_H 3.74 (s), 3.76 (s), and 4.08 (s) were determined to three MeO groups. The ^{13}C NMR (DEPT) spectroscopic data further showed the presence of three methyls, one methylenes, nine methines, and nine quaternary carbons, among which carbon signals at δ_C 105.3 (d), 75.1 (d), 79.6 (d), 71.2 (d), 77.8 (d), and 62.5 (t) suggested the presence of one glucose, which was confirmed by its MS fragmentation peak at m/z 303 $[M+1-glc]^+$. Acid hydrolysis of compound **1** also showed the presence of glucose.

In the HMBC spectrum of **1**, H-2 (δ_H 7.26) exhibited cross-peaks with C-1, C-3, C-4, and C-9a at δ_C 160.6, 164.9, 95.4, and 108.8, respectively. Also, H-4 (δ_H 6.61) showed correlations with C-2, C-3, C-4a, and C-9a at δ_C 101.3, 164.9, 158.6, and 108.8, respectively. Further, H-7 (δ_H 7.33) exhibited cross-peaks with C-5, C-6, C-8, and C-8a at δ_C 148.8, 150.3, 112.6, and 118.7, and H-8 (δ_H 7.21) showed correlations with C-4b, C-6, C-7, C-8a, and C-9 at δ_C 149.9, 150.3, 119.5, 118.7, and 175.9, respectively, in agreement with the assignments for 1,3,5,6-tetraoxygenated xanthone skeleton. The linkage of sugar moiety to C-1 was determined by the HMBC correlations from H-1' (δ_H 5.53) to C-1 (δ_C 160.6). The

configuration of anomeric proton of the glucose was determined as β based on J value ($J = 7.6$ Hz) in the ^1H NMR spectrum. The linkages of three OCH_3 to C-3, C-5, and C-6 were confirmed by the HMBC correlations from OCH_3 ($\delta_{\text{H}} 3.74$) to C-3 ($\delta_{\text{C}} 164.9$), OCH_3 ($\delta_{\text{H}} 4.08$) to C-5 ($\delta_{\text{C}} 148.8$), and OCH_3 ($\delta_{\text{H}} 3.76$) to C-6 ($\delta_{\text{C}} 150.3$). These assignments were supported by the presence of ROESY correlations of between 3- OCH_3 with H-2 and H-4, H-2 with H-1', and 6- OCH_3 with H-7. Thus, the structure of **1** was determined as 1- O - β -D-glucopyranosyl-3,5,6-trimethoxy-xanthone.

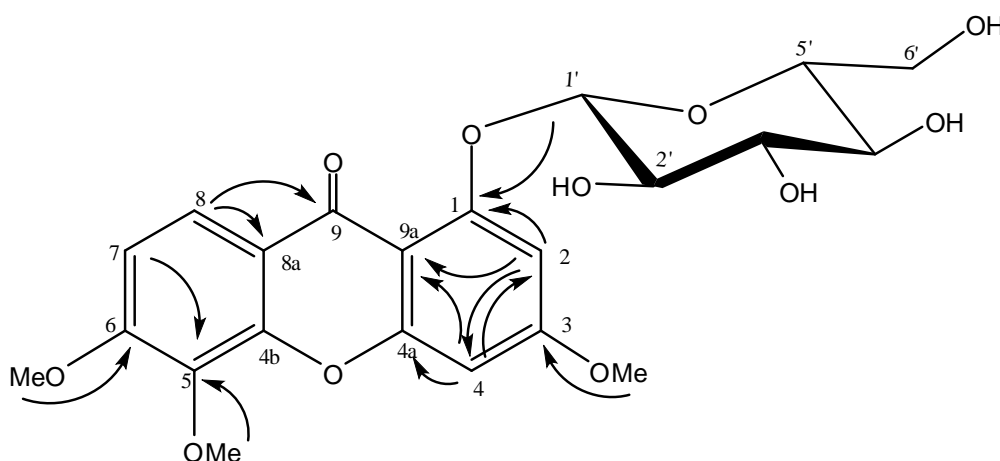


Figure 2. Key HMBC correlations of compound **1**

Compound **2** was deduced to have a molecular formula of $\text{C}_{27}\text{H}_{32}\text{O}_{15}$ from ^{13}C NMR spectra and HR-ESI-MS $[\text{M}+\text{H}]^+$ ion peak at m/z 597.1780, and $[\text{M}+\text{Na}]^+$ ion peak at m/z 619.1595. The IR spectrum showed the presence of hydroxyl group (3425 cm^{-1}), aromatic ring (1624 , 1586 , 1450 cm^{-1}), and $\text{C}=\text{O}$ bond (1645 cm^{-1}). The UV spectrum exhibited absorption maxima at λ_{max} 356, 304, and 245 nm. The IR and UV spectra were similar to those of compound **1**.

The ^1H and ^{13}C NMR spectra of compound **2** closely resembled those of compound **1**, except for the appearance of one more pentosyl unit in **2**. The pentosyl was assigned to be a β -D-xylopyranosyl based on the characteristic ^1H and ^{13}C NMR spectrum signals [δ_{H} 4.95 (1H, d, $J = 7.6$ Hz, anomeric H); δ_{C} 106.1, 74.9, 78.2, 71.0, and 67.1].⁶ Acid hydrolysis of **2** produced glucose and xylose identified on the basis of TLC by comparing with those authentic sugar sample. The obvious downfield shift of glucosyl C-6' by +7.7 ppm suggested that the xylosyl unit was linked at C-6' of glucosyl unit, which was confirmed by HMBC correlation of anomeric proton (δ_{H} 4.95) of the xylosyl with the C-6' (δ_{C} 70.2) of glucosyl. Therefore, the structure of compound **2** was established to be 1- O -[β -D-xylopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl]-3,5,6-trimethoxy-xanthone.

In the *in vitro* assays, the α -glucosidase and DPP-IV (Dipeptidyl Peptidase IV) inhibitory activities of compounds **1** and **2** were performed. Compound **1** showed weak α -glucosidase inhibitory activity with 5.4% inhibition at 40 μ M, while the positive control acarbose gave an inhibitory rate of 98.8% at the same concentration.⁷ Compound **2** showed weak DPP-IV inhibitory activity with 2.1% inhibition at 10⁻⁵ M, while the positive control INDPP-2 gave an inhibitory rate of 96.6% at the same concentration [the negative control DMSO gave an inhibitory rate of 0%]. Compound **1** was inactive for DPP-IV inhibitory activity compared with compound **2**, and compound **2** was inactive for α -glucosidase inhibitory activity.

Table 1. ¹H and ¹³C NMR spectral data of compounds **1** and **2**^a

position	1		2	
	proton	carbon	proton	carbon
1		160.6		160.5
2	7.26 d (2.4)	101.3	7.36 d (2.4)	101.4
3		164.9		165.0
4	6.61 d (2.4)	95.4	6.60 d (2.4)	95.5
4a		158.6		158.4
4b		149.9		149.8
5		148.8		148.7
6		150.3		150.0
7	7.33 d (9.2)	119.5	7.33 d (9.2)	119.4
8	7.21 d (9.2)	112.6	7.20 d (9.2)	112.6
8a		118.7		118.6
9		175.9		175.8
9a		108.8		108.7
1'	5.53 d (7.6)	105.3	5.49 d (7.2)	105.2
2'	4.47 t (8.4)	75.1	4.41 dd (7.2, 8.8)	74.8
3'	4.18 m	79.6	4.36 m	77.7
4'	4.29 t (8.8)	71.2	4.25 m	71.2
5'	4.40 t (8.4)	77.8	4.36 m	77.7
6'	4.62 d (10.8)	62.5	4.37 m	70.2
	4.37 m		4.84 dd (9.2, 5.2)	
1''			4.95 d (7.6)	106.1
2''			4.05 dd (7.6, 8.8)	74.9
3''			4.15 t (8.4)	78.2
4''			4.25 m	71.0
5''			3.66 dd (10.0, 10.8)	67.1
			4.35 m	
3-OCH ₃	3.74 s	55.9	3.77 s	55.9
5-OCH ₃	4.08 s	61.4	4.09 s	61.4
6-OCH ₃	3.76 s	56.7	3.79 s	56.6

^a¹H NMR spectral data measured at 400 MHz; ¹³C NMR spectral data measured at 100 MHz; Proton coupling constants (*J*) in Hz given in parentheses. Pyridine-d₅ as solvent.

EXPERIMENTAL

General experimental procedures. Optical rotations and UV data were measured on a JASCO-20 polarimeter and UV-2401PC spectrometer. MS were measured on a VG Auto Spec-3000 spectrometer. HR-ESI-MS data were measured using a JMS-T100CS AccuTOF LC/MS spectrometer. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra were recorded on Bruker AM-400 instruments with TMS as internal standard. IR spectra were measured on a Bruker Tensor 27 spectrometer with KBr pellets. Column chromatography was performed with silica gel (200-300 mesh; Qingdao Marine Chemical Inc. Qingdao, People's Republic of China) and Lichroprep RP-18 gel (40-63 μm ; Merk, Darmstadt, Germany) and Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd. Uppsala, Sweden).

Plant material. Whole herb of *Swertia mussotii* Franch was collected from Xianggelila, Yunnan Province, People's Republic of China, in July 2006. The plant was identified by Physician Zhaxiduoze (Diqing Tibetan Medicine Hospital). A voucher specimen (NO. 0601005) is deposited at the Herbarium of Key Laboratory of Ethnic Medicine Resource Chemistry, Yunnan University of Nationalities.

Extraction and Isolation. The air-dried leaves and stem of *Swertia mussotii* Franch (9.5 kg) were extracted with EtOH (70%) under reflux at room temperature (3 h each time, total 4 times), After filtering and evaporating the solvents in vacuo a residue was obtained (980 g). Then the residue was extracted successively with petroleum ether, EtOAc, and BuOH. The BuOH extract (250 g) was chromatographed on a silica gel (200-300 mesh) column and eluted with gradient mixtures of EtOAc-MeOH from 50:1 (V/V) to 1:5 (V/V) to afford four fractions. Fraction 3 was purified with silica gel CC (EtOAc-MeOH, 10:1) and then Sephadex LH-20 (MeOH) CC and semipreparative HPLC (MeOH-H₂O, 55:45 and 65:35) afforded **1** (12 mg) and **2** (28 mg).

Compound 1: C₂₂H₂₄O₁₁, colorless gum; $[\alpha]^{19.1}_{\text{D}} -165.1$ (*c* 0.087, MeOH+CHCl₃); UV (MeOH) λ_{max} (log ϵ) 214 (3.85), 248 (4.09), 303 (3.74), 357 (3.30) nm; IR (KBr): ν_{max} 3406, 2924, 2853, 1650, 1624, 1588, 1481, 1453, 1430, 1288, 1201, 1148, 1103, 1074, 978 cm⁻¹; $^1\text{H NMR}$ (C₅D₅N, 400 MHz) and $^{13}\text{C NMR}$ (C₅D₅N, 100 MHz) spectral data see Table 1; positive-mode ESI-MS *m/z* 487 [M+Na]⁺ and 503 [M+K]⁺; HR-ESI-MS *m/z* 487.1169 [M+Na]⁺ (calcd for C₂₂H₂₄O₁₁Na, 487.1216).

Compound 2: C₂₇H₃₂O₁₅, light yellow powder; $[\alpha]^{16.5}_{\text{D}} -116.0$ (*c* 0.037, MeOH+CHCl₃); UV (MeOH) λ_{max} (log ϵ) 215 (3.96), 245 (4.16), 304 (3.82), 356 (3.33) nm; IR (KBr): ν_{max} 3425, 2923, 2853, 1645, 1624, 1586, 1480, 1450, 1429, 1283, 1204, 1170, 1065, 1044, 979 cm⁻¹; $^1\text{H NMR}$ (C₅D₅N, 400 MHz) and $^{13}\text{C NMR}$ (C₅D₅N, 100 MHz) spectral data see Table 1; positive-mode ESI-MS *m/z* 619 [M+Na]⁺; HR-ESI-MS *m/z* 597.1780 [M+H]⁺ (calcd for C₂₇H₃₃O₁₅, 597.1819), 619.1595 [M+Na]⁺ (calcd for C₂₇H₃₂O₁₅Na, 619.1633).

Acid hydrolysis of compounds 1 and 2. Compounds **1** (3 mg) and **2** (3 mg) in 2N HCl (3 mL) were each

heated at 80 °C for 1 h, then cooled for 30 min. The mixture was extracted with EtOAc (5 mL×3). The residue and authentic sugars were dotted to the plated developed with CHCl₃-CH₃OH-H₂O (5:3:0.5), and phenylene diamine-aniline-phosphoric acid used as spray reagent, followed by heating at 80 °C. From compound **1** gulucose was detected; *R_f* glucose 0.45. From compound **2** gulucose and xylose were detected; *R_f* glucose 0.45, xylose 0.58.

ACKNOWLEDGEMENTS

We wish to acknowledge financial support from the Key Project of Chinese Ministry of Education (NO. 208132), and the Young Academic and Technical Leader Rising Foundation of Yunnan Province (2007PY01-27).

REFERENCES

1. M. R. Jia and X. W. Li, 'Zhongguo Minzu Yaozhi Yao', Chinese Medical Science and Technology Press, Beijing, 2005, pp. 591-593.
2. J. S. Zhang, X. M. Wang, X. H. Dong, H. Y. Yang, and G. P. Li, *J. Chin. Med. Mater.*, 2009, **32**, 511.
3. Y. F. Zhou, F. Y. Huang, R. R. Wen, and G. P. Li, *J. Yunnan Univ. Nationalities*, 2011, **20**, 14.
4. J. S. Zhang, X. M. Wang, Y. T. Gao, and G. P. Li, *Asian J. Chem.*, 2011, **5**, 1966.
5. R. Gonda, T. Takeda, and T. Akiyama, *Chem. Pharm. Bull.*, 2000, **48**, 1219.
6. V. S. Rana and M. S. M. Rawat, [Chemistry & Biodiversity, 2005, 2, 1310](#).
7. R. Zhang, Q. Liu, Z. F. Shen, and G. H. Du, *Chin. Pharm. J.*, 2007, **42**, 740.