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Studies on Antioxidant Activities of Chemical Constituents of *Swertia mussotii* Franch

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To study the chemical constituents in *Swertia mussotii* Franch and their scavenging effects of active oxygens species. A new xanthone named 1,7-dihydroxy-2,3,8-trimethoxyxanthone (**1**), together with 1,8-dihydroxy-2,6-dimethoxyxanthone (**2**) and sweroside 2'-O-(3,3',5-trihydroxy-2-biphenylcarboxylate (**3**), were isolated from *Swertia mussotii* Franch. Their structures were elucidated by spectroscopic methods, including one and two-dimensional NMR (COSY, HMQC, HMBC and ROESY). Scavenging effects of them on active oxygen species ·OH and O_2^- generated by Fenton reaction and riboflavine photosensitization *in vitro* were investigated by the means of spectrophotometry for the first time and the results showed that three compounds could scavenge active oxygen species efficiently.

Key Words: *Swertia mussotii* Franch, Fenton reaction, Riboflavine, Active oxygen species.

INTRODUCTION

Swertia mussotii Franch is a plant of genus *Swertia*, belongs to the family Gentianaceae. It is manily distributed in Qinghai, Xizang, Sichuan and Yunnan of China. The character of the plant is cold and the taste is bitter[1,2]. It is frequently used drug for acute epioephysitis, soprethroat, tonsillitis, icterichepatitis, cholecystitis, gastroenteritis bacillary dysentery and urinary tract infection[3]. Primary studies have shown that triterpenoid, flavones, iridoid and xanthone are main constituments of this plant. Our continuing phytochemical investigation on the constituents of this plant has resulted in the isolation of a new xanthone named 1,7-dihydroxy-2,3,8-trimethoxyxanthone (**1**), together with 1,8-dihydroxy-2,6-dimethoxyxanthone (**2**) and sweroside 2'-O-(3,3',5-trihydroxy-2-biphenylcarboxylate (**3**). Their structure elucidation and the antioxidant activities by active oxygen species ·OH and O_2^- are reported in this paper.

Compound **1** was obtained as yellow crystales, m.p. 166-169 ºC. It has the molecular formula $C_{16}H_{14}O_7$ determined by HRESI-MS (m/z: 341.0634 $[M + Na]^+$, calcd. (%): 341.0637); IR (KBr, ν_{max}, cm^{-1}): 3432 (hydroxyl), 2942, 1464 (methyl), 1662 (carbonyl), 1634, 1605, 1518 (aryl), the data of IR spectrum was in accordance with the same kind of compound and indicated that it was a xanthone. The ^{13}C NMR spectrum showed signals of one carbonyl (δ = 181.8) and three methoxy (δ = 56.4, 61.4, 61.7). Other signals were found almost the same with compounds of xanthone. The ^1H NMR spectrum showed that δ 11.90 and 12.07 were two hydroxyl substitutions, δ 7.18 and δ 7.55 were the chemical shifts of two *ortho* substitutions and that δ 6.63 was the singlet of an aromatic proton whose chemical shifts indicated that it was located between the C-4a and a methoxy group. On the basis of these studies and biogenetic considerations we proposed it was a xanthone of quintuple substituent. The HMQC spectrum showed that three aromatic protons at δ 6.63, 7.18 and 7.55 connected with the carbons δ 95.2, 114.9 and 126.1, respectively. The HMBC spectrum of compound **1** illustrated in Fig. 1, showed connectivities between hydrogens and carbons in the range of two or three bonds, demonstrated that the proton appearing at δ 6.63 (H-4) was correlated with carbons δ 160.0 (C-3) and δ 115.7 (C-8a), the proton appearing at δ 7.33 (H-5) was correlated with carbons δ 125.4 (C-6) and δ 104.0 (C-8b). The proton appearing at δ 7.61 (H-6) was correlated with carbons δ 148.6 (C-7) and δ 149.5 (C-4b). The proton appearing at δ 3.97 (H-10) was correlated with carbons δ 159.9 (C-1), δ 125.4 (C-2) and δ 160.0 (C-3). The proton appearing at δ 4.08 (H-11) was correlated with carbon δ 160.0 (C-3) and the proton appearing at δ 4.08 (H-12) was correlated with carbon δ 146.5 (C-8). The HMBC spectrum also showed that two hydroxyl were connected with carbons C-1 and C-7 and that three methoxy groups were connected with carbons C-2, C-3 and C-8, respectively. Hence, in comparison with the spectrum data of the compound of the same kind, compound **1** was identified as 1,7-dihydroxy-2,3,8-

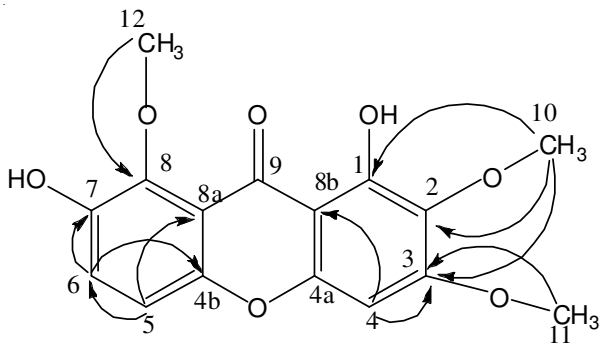


Fig. 1. Selected HMBC correlations of compound **1**

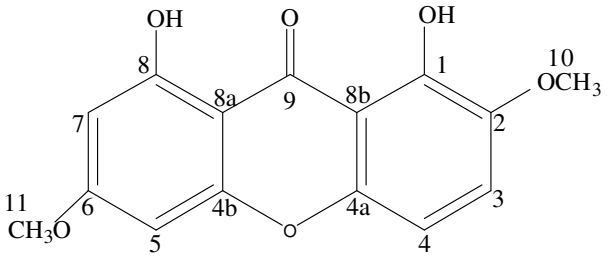
trimethoxyxanthone and it was a new xanthone. Fig. 1 shows the selective correlations of the HMBC of compound **1**. Table-1 gives the ^{1}H NMR, ^{13}C NMR and HMBC spectral data.

TABLE-1
^{1}H NMR, ^{13}C NMR AND HMBC SPECTRAL DATA OF COMPOUND **1** IN $CDCl_3$

C atom	δ_C (ppt)	δ_H (*J*/Hz)	HMBC
1	159.9 (C)	8.71 (1H, s)	H-10
2	125.4 (C)	7.61(1H, d, = 9.0)	H-10
3	160.1 (C)	7.33(1H, d, *J* = 9.1)	H-4, 10, 11
4	95.2 (CH)	6.63 (1H, d)	–
4a	150.6 (C)	4.08 (3H, s)	–
4b	149.4 (C)	3.97 (3H, s)	H-6
5	114.1 (CH)	3.82 (3H, s)	–
6	125.0 (CH)	–	H-5
7	148.6 (C)	–	H-6
8	146.5 (C)	–	H-12
8a	115.7 (C)	–	H-5
8b	104.0 (C)	–	H-4
9	181.8 (C)	–	–
10	61.7 (CH_3)	–	–
11	61.4 (CH_3)	–	–
12	56.4 (CH_3).	–	–

Compound **2** was obtained as fine yellow powder and the formula was determined to be $C_{15}H_{12}O_6$ based on EI-MS (m/z = 288 [M^+]); IR (KBr, ν_{max}, cm^{-1}): 3428 (hydroxyl), 2938, 1468 (methyl), 1667 (carbonyl), 1630, 1606, 1520 (aryl); ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 3.80 (3H, s, 2-OMe), 3.84 (3H, s, 6-OMe), 6.22 (1H, d, *J* = 2.2 Hz, H-7), 6.28 (1H, d, *J* = 2.2 Hz, H-5), 6.73 (1H, d, *J* = 9.0 Hz, H-4), 7.18 (1H, d, *J* = 9.0 Hz, H-3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 142.8 (s, C-1), 149.8 (s, C-2), 121.3 (d, C-3), 105.6 (d, C-4), 150.1 (s, C-4a), 158.0 (s, C-4b), 92.8 (d, C-5), 167.4 (s, C-6), 97.1 (d, C-7), 62.6 (s, C-8), 102.2 (s, C-8a), 107.7 (s, C-8b), 184.8 (s, C-9). The data of ^{1}H and ^{13}C NMR correlated well with the reported data[4,5], compound **2** was identified as 1,8-dihydroxy-2,6-dimethoxyxanthone.

Compound **3** was obtained as yellow powder and the formula was determined to be $C_{29}H_{30}O_{13}$ based on FAB-MS (m/z = 587 [(M + H)$^+$, 391, 229]). m.p. 229-230 ºC (MeOH), ^{1}H NMR (CD_3OD, 500 MHz) δ :7.43(1H, d, *J* = 2.6 Hz, H-3), 5.41 (1H, dt, *J* = 17.1, 9.9 Hz, H-8), 7.17 (1H, t, *J* = 7.8 Hz, H-5'''), 6.73 (1H, d, *J* = 6.72 Hz, H-6'''), 5.23 (2H, m, H-10), 6.70 (1H, m, H-2'''), 6.76 (1H, dd, *J* = 8.0, 2.6 Hz, H-4'''), 6.16 (1H, d, *J* = 2.6 Hz, H-6''), 6.29 (1H, d, *J* = 2.6 Hz, H-4''), 5.39 (1H, d, *J* = 1.6 Hz, H-1), 4.28 (1H, d, *J* = 7.9 Hz, H-1'), 3.07 (1H, m, H-5'), 2.81 (1H, t, *J* = 9.2 Hz, H-3'), 4.72 (1H, dd, *J* = 9.5, 7.9 Hz, H-2'), 3.22 (1H, t, *J* = 9.3 Hz, H-4'), 4.28 (1H, m, H-7a), 4.49 (1H, m, H-7b), 3.60 (1H, dd, *J* = 12.0, 6.0 Hz, H-6'a), 3.84 (1H, dd, *J* = 12.0, 2.1 Hz, H-6'b), 2.57 (1H, ddd, *J* = 9.4, 5.4, 1.4 Hz, H-9), 2.79 (1H, m, H-5), 1.68 (1H, m, H-6a), 1.58 (1H, qd, *J* = 12.8, 4.3 Hz, H-6b); ^{13}C NMR (CD_3OD, 125MHz) δ: 171.5 (s, C-7''), 167.6 (s, C-10), 166.0 (s, C-3''), 163.8 (s, C-5''), 157.4 (s, C-3'''), 153.7 (d, C-2), 148.6 (s, C-1''), 146.5 (s, C-1'''), 132.8 (d, C-7), 129.3 (d, C-5'''), 121.2 (d, C-6'''), 121.0 (t, C-9), 116.5 (d, C-2'''), 114.5 (d, C-4'''), 112.8 (d, C-6''), 105.6 (s, C-3), 104.1 (s, C-2''), 103.1 (d, C-4''), 97.2 (d, C-1), 96.8 (d, C-1'), 78.3 (d, C-5'), 74.8 (d, C-3'), 74.6 (d, C-2'), 71.6 (d, C-4'), 69.5 (t, C-6), 62.4 (t, C-6'), 43.4 (d, C-8), 28.7 (d, C-4), 25.8 (t, C-5). The data of ^{1}H and ^{13}C NMR correlated well with the reported data[6], compound **3** was identified as Sweroside 2'-O-(3,3',5-trihydroxy-2-biphenylcarboxy late).



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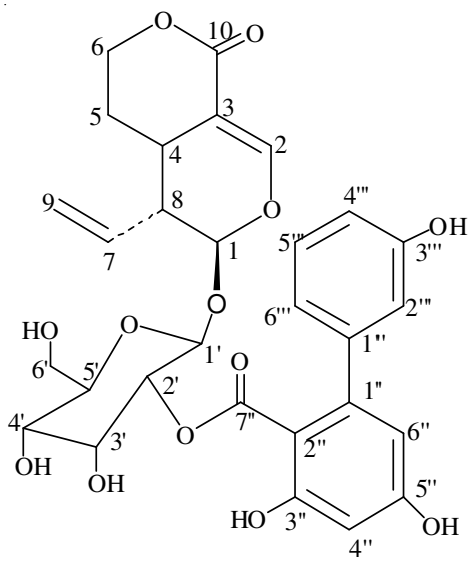

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EXPERIMENTAL

MS were measured on a VG Auto Spec-3000 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AM-400 and DRX-500 instruments with TMS as internal standard. IR were measured on Bio-Rad FTS-135 spectrometer. Column chromatography was performed on 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). Ultraviolet-visible spectrophotometer (WFJ 7200, UNICO Shanghai Instruments Co., Ltd.).

Ferrous sulfate ($FeSO_4$), methionine (MET), ethylene diaminetetraacectic acid (EDTA), nitroblue tetrazolium (NBT), riboflavin, safranine T, hydrogen peroxide (H_2O_2), phosphor buffer sodium (PBS), all chemical reagents used in this research were AG grade.

Swertia mussotii Franch was collected from Xianggelila, Yunnan Province, People's Republic of China, in July 2006. The plant was identified by Physician Zhaxiduoze.

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 EtOAc extract (168 g) was chromatographed on a silica gel (200-300 mesh) column and eluted with gradient mixtures of petroleum ether/EtOAc from 20:1 (v/v) to 1:1 (v/v) to afford eight fractions fraction 2 was further subjected to repeated silica gel column chromatography using $CHCl_3$-MeOH (15:1, v/v) as eluent and passed over RP-18 eluted with methanol/water (4:6, v/v) to afford compound **1** (21 mg). Fraction 1 was further subjected to silica gel (200-300 mesh) column chromatography using a mixture of petroleum ether/EtOAc (8:1, v/v) as eluent to yield pure **2** (18 mg) and **3** (26 mg).

Superoxide anion scavenging activity test: The measurement of superoxide anion scavenging activity of the sample was based on the method of riboflavin photosensitization[7]. Riboflavin (1.67×10^{-5} mol/L), methionine (0.01 mol/L) and nitroblue tetrazolium (4.6×10^{-5} mol/L) were confected with phosphor buffer sodium (0.05 mol/L pH = 7.4). 2 mL of three kinds of solution above and 1 mL of the sample were added into the tubes, respectively. In the control group, sample was replaced by phosphor buffer sodium. The mixture was illuminated for 15 min. The absorbance was determined at 560 nm. The superoxide anion scavenging ability was calculated using the following formula:

$$\text{Inhibiting ratio (\%)} = \left[\frac{(A_0 - A_s)}{A_0}\right] \times 100$$

A_s = absorbance of sample, A_0 = absorbance of the solution without sample.

Hydroxyl radical scavenging activity test: Hydroxyl radical resulted from Fenton reaction may react safranine T, which could reduced the absorption value of the measuring system. So the scavenging activities of hydroxyl radical could be assayed by the changing rate of absorption value of the measuring system[8]. The assay system consisted of 1 mL of phosphate buffer solution (PBS) (pH 7.4, 0.15 mol/ L), 1 mL of safranine T solution (40 μg/mL), 1 mL of EDTA-Fe(II) solution (0.945 mmol/L), 0.5 mL of sample solution and 1 mL of H_2O_2 (3 %) were added into the tubes. In the control group, sample was replaced by phosphor buffer sodium. The solution was incubated in water bath at 37 ºC for 0.5 h. The absorbance was determined at 520 nm. All tests were carried out in triplicate and the results were averaged. The hydroxyl radical scavenging ability was calculated by the following formula:

$$\text{Inhibiting ratio (\%) D} = \left[\frac{(A_s - A_0)}{(A - A_0)}\right] \times 100$$

A_s = absorbance of sample, A = absorbance of control and A_0 is the absorbance of the solution without sample and H_2O_2.

RESULTS AND DISCUSSION

Results are summarized in Table-2. IC_{50} values were the sample concentration which could scavenge 50 % of the superoxide anion and hydroxyl radical, rutin was used as controls. Three compounds could scavenge active oxygen efficiently, among them, the antioxidation function of these compounds showed higher activity on scavenging ·OH than O_2^-. Compond **1** and rutin almost had the same antioxidation ability.

TABLE-2
ANTIOXIDANT ACTIVITIES (IC_{50} VALUES AND Sr_{max}/%) OF COMPOUNDS **1-3** AND RUTIN

Samples	Superoxide anion		Hydroxyl radical	
	IC_{50} (mg/mL)	Sr_{max} (%)	IC_{50} (mg/mL)	Sr_{max} (%)
Rutin	0.050	88.7	0.0025	99.2
Compound **1**	0.047	89.3	0.0038	98.7
Compound **2**	0.055	84.2	0.0080	88.4
Compound **3**	0.075	76.9	0.0370	82.2

*Sr_{max} was the maximum rate of scavenging ·OH and $O_2^{\cdot -}$.

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