Xanthones with Anti-Hepatitis B Virus Activity from *Swertia mussotii*

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Abstract

Two new xanthones, 8-*O*-*β*-D-glucopyranosyl-1-hydroxy-2,3,5-trimethoxyxanthone (**1**) and 8-*O*-[*β*-D-xylopyranosyl-(1 → 6)-*β*-D-glucopyranosyl]-1-hydroxy-2,3,5-trimethoxyxanthone (**2**), along with eighteen known xanthones (**3**–**20**) were isolated from *Swertia mussotii*. Their structures were elucidated on the basis of extensive spectroscopic analyses (1D- and 2D-NMR, HRESIMS, UV, IR, $[\alpha]_D$). All compounds were evaluated for their anti-hepatitis B virus activities on HepG 2.2.15 cells line *in vitro*, and compounds **3**–**10** exhibited significant activity inhibiting hepatitis B virus DNA replication with IC_{50} values from 0.01 mM to 0.13 mM. Compounds **3**–**5** showed remarkable activity with IC_{50} values of 0.77, > 0.98, and 0.21 mM for HBsAg, and < 0.62, 0.35, and 0.04 mM for HBeAg, respectively. Meanwhile, the effects of different substitutions on the anti-hepatitis B virus activity of xanthones from *S. mussotii* were discussed.

Key words

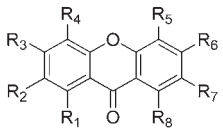
Swertia mussotii · Gentianaceae · xanthones · anti-HBV activity

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

Swertia mussotii, belonging to the *Swertia* genus of the family Gentianaceae, is a well-known Tibetan folk medicine (zangyinchen) to treat hepatitis [1,2]. Presently, many anti-hepatitis agents from this plant have been widely applied in clinics [3,4]. According to our preliminary *in vitro* bioassay, the ethanol extract of *S. mussotii* showed significant inhibitory activity on the secretions of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) with IC_{50} values of 0.55 mg/mL (SI = 2.13) and 0.19 mg/mL (SI = 6.2), respectively, and hepatitis B virus (HBV) DNA replication with an IC_{50} value of 0.043 mg/mL (SI = 27.2). Previous studies on *S. mussotii* demonstrated that secoiridoids and xanthones were its major constituents, however, their anti-HBV activity has been rarely reported [2]. Therefore, this investigation was focused on the xanthones constituents of *S. mussotii* and their anti-HBV activity. As a result, twenty xanthones, including two new ones, were isolated from the active part of the title plant (**Fig. 1**). Herein, we report the isolation, structure elucidation, and the anti-HBV properties of the obtained xanthones.

Compound **1** was obtained as a yellow powder. Its HRESIMS exhibited the quasimolecular ion at *m*/*z* 503.1159 $[M + Na]^+$, corresponding to the molecular formula of $C_{22}H_{24}O_{12}$. The fragment ion at *m*/*z* 319.0788 $[M + H\text{-}162]^+$ indicated the presence of a hexose moiety, which was determined to be D-glucose by acid hydrolysis and comparison with an authentic sample on TLC as well as by an $[\alpha]_D$ experiment ($[\alpha]_D^{25}$: + 80.7, *c* 0.14, H_2O) [5]. Its UV spectrum in MeOH displayed the characteristic absorption at λ_{max} 247, 278, and 315 nm for xanthones [6]. The IR spectrum of compound **1** suggested the presence of hydroxy groups (3345 cm^{-1}), hydrogen bonded ketone (1655 cm^{-1}), and aromatic rings (1613, 1580, 1496 cm^{-1}). The ^1H NMR spectrum displayed signals for three methoxy groups (δ_H 3.93, 3.90, 3.74) and three aromatic protons, including two *ortho*-coupled doublets (δ_H 7.17, 7.44, *J* = 9.0 Hz), and a singlet (δ_H 6.76) (**Table 1**). The characteristic peak at δ_H 12.9 permitted the assignment of a hydroxy group at C-1 or C-8. The proton at δ_H 6.76 was assigned to H-4 due to the long-range coupling correlation with C-3 (δ_C 159.9), C-2 (δ_C 131.3), C-4a (δ_C 152.0), and C-8b (δ_C 103.9). Two *o*-coupled protons at H-6 (δ_H 7.44, *J* = 9.0 Hz) and H-7 (δ_H 7.17, *J* = 9.0 Hz) were confirmed unambiguously through an HMBC experiment. The glucosyl group was proposed at C-8 by an HMBC correlation from H-1′ (δ_H 4.86, d, *J* = 7.5) to C-8 (δ_C 150.2). The anomeric proton displayed a doublet peak with the coupling constant (*J*) of 7.5 Hz indicating the *β* configuration. The positions of the three methoxy groups were confirmed through ROESY and HMBC experiments. In the ROESY spectrum, the methoxy groups at *δ* 3.93 and 3.90 correlated with H-4 and H-6; in the HMBC spectrum, *δ* 3.93, 3.90, and 3.74 correlated with C-3, C-5, and C-2, respectively (**Fig. 2**). Thus, compound **1** was determined to be 8-*O*-*β*-D-glucopyranosyl-1-hydroxy-2,3,5-trimethoxyxanthone.

Compound **2** was obtained as a yellow powder, and its molecular formula was deduced to be $C_{27}H_{32}O_{16}$ from HRESIMS at *m*/*z* 635.1559 $[M + Na]^+$ (calcd. for 635.1583). The fragment ion at *m*/



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
1	OH	OMe	OMe	H	OMe	H	H	OGlc
2	OH	OMe	OMe	H	OMe	H	H	OGlc-Xyl
3	OH	H	OH	H	OH	H	H	OH
4	OH	H	OMe	H	OH	H	H	OH
5	OH	Glc	OH	H	H	H	OH	H
6	OGlc	H	OH	H	OH	H	H	OH
7	OGlc	H	OH	H	H	H	OH	OH
8	OH	H	OMe	H	H	H	OH	OMe
9	OH	H	OMe	H	H	H	OXyl-Xyl	OH
10	OH	Glc	OH	H	H	OH	OH	H
11	OH	H	OMe	H	H	H	ORha-Xyl	OH
12	OH	H	OMe	H	H	H	OMe	OMe
13	OH	H	OMe	H	OH	H	H	OGlc
14	OH	H	OMe	H	OMe	H	H	OGlc
15	OGlc	H	OMe	H	H	H	OMe	OH
16	OGlc	H	OMe	H	H	H	OMe	OMe
17	OGlc-Xyl	H	OMe	H	H	H	OH	OMe
18	OGlc-Glc	H	OMe	H	H	H	OMe	OH
19	OGlc-Xyl	H	OMe	H	H	H	OMe	OMe
20	OH	H	OMe	OMe	H	H	OH	OMe

Fig. 1 Structures of compounds **1**–**20**.

Table 1 ^{1}H-NMR and ^{13}C (400 MHz) NMR data of compounds **1** and **2** in DMSO-d_6 (δ in ppm, J in Hz in parentheses).

H/C	1		2	
	δ_H	δ_C	δ_H	δ_C
1	12.9, s	153.5	12.9, s	153.5
2		131.3		131.3
3		159.9		159.9
4	6.76, s	91.0	6.76, s	91.0
4a		152.0		152.0
4b		146.1		146.0
5		142.9		143.0
6	7.44, d (9.0)	117.5	7.44, d (9.1)	117.8
7	7.17, d (9.0)	111.5	7.27, d (9.1)	111.4
8		150.2		150.1
8a		111.2		111.7
8b		103.9		103.9
9		181.1		181.2
1′	4.86, d (7.5)	102.7	4.82, d (7.6)	102.6
2′	3.38, m	73.5	3.40, m	73.4
3′	3.30, m	76.2	3.30, m	76.0
4′	3.19, m	69.7	3.27, m	69.8
5′	3.34, m	77.4	3.06, dd (12.7, 8.5)	76.7
6′	(a) 3.71, m; (b) 3.49, m	60.8	(a) 4.00, d (9.4) (b) 3.54, d (8.9)	68.4
1′′			4.20, d (7.4)	104.0
2′′			3.00, m	73.4
3′′			3.54, d (8.9)	76.2
4′′			3.17, dd (13.2, 5.5)	69.6
5′′			(a) 3.67, dd (11.2, 5.2) (a) 2.96, m	65.7
2-OMe	3.74, s	60.1	3.70, s	60.1
3-OMe	3.93, s	56.7	3.93, s	56.7
5-OMe	3.90, s	56.4	3.91, s	56.4

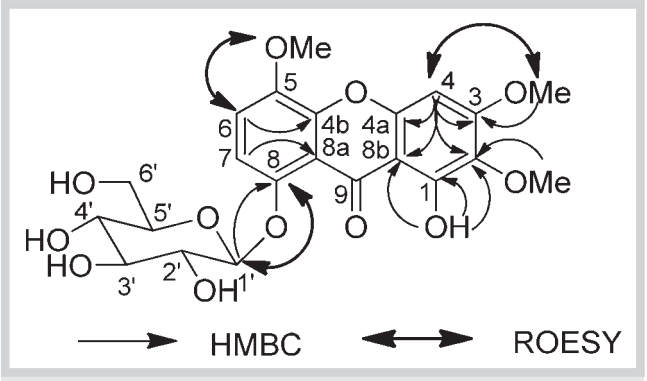


Fig. 2 HMBC correlations of compound **1**.

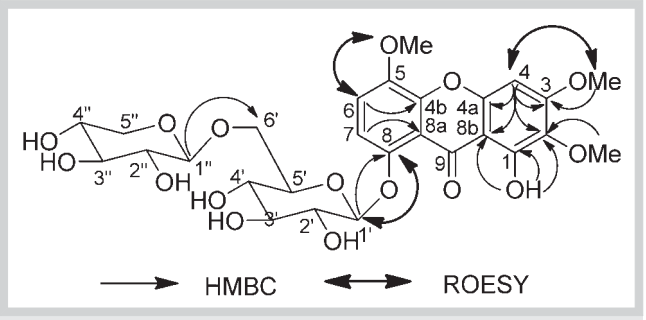


Fig. 3 HMBC correlations of compound **2**.

z 319.0790 [M + H-162-132]$^+$ indicated the presence of a hexose and a pentose moiety. Acid hydrolysis of compound **2** with 2 N aqueous HCl yielded D-glucose and D-xylose, which were identified by comparison with authentic samples on TLC in combination with ($[\alpha]_D^{25}$: +55.9, c 0.13, H_2O) and ($[\alpha]_D^{24}$: +16.8, c 0.05, H_2O) $[\alpha]_D$ experiments, respectively [5, 7]. The ^{1}H NMR spectrum was almost identical to that of compound **1** except for one additional anomeric proton at δ_H 4.20 (J = 7.4) and a group of glycosyl protons between δ_H 3.67 and 2.96 ppm. The ^{13}C NMR spectrum displayed five carbon signals (δ_C 104.0, 73.4, 76.2, 69.6, 65.7) attributed to a xylose moiety, and C-6′ of the glucosyl group shifted from δ_C 60.8 to 68.4. In the HMBC spectrum, the correlation of H-1′′ (δ_H 4.20) with C-6′ (δ_C 68.4) suggested that the xylosyl moiety was attached to C-6′ of the glucosyl group (**Fig. 3**). The coupling constants (J) of 7.6 and 7.4 Hz indicated a β-D-glucose and β-D-xylose. Consequently, the structure of compound **2** was elucidated to be 8-O-[β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl]-1-hydroxy-2,3,5-trimethoxy-xanthone.

By comparison of their spectral and physical data with those published previously, the eighteen known compounds were determined as norbellidifolin (**3**) [8], 1,5,8-trihydroxy-3-methoxyxanthone (**4**) [9], 2-C-β-D-glucopyranosyl-1,3,7-trihydroxyxanthone (**5**) [10], norswertianolin (**6**) [8], norswertianin-1-O-β-D-glucoside (**7**) [11], 1,7-dihydroxy-3,8-dimethoxyxanthone (**8**) [12], 7-O-[β-D-xylopyranosyl-(1 → 2)-β-D-xylopyranosyl]-1,8-dihydroxy-3-methoxyxanthone (**9**) [13], mangiferin (**10**) [14], 7-O-[α-L-rhamnopyranosyl-(1 → 2)-β-D-xylopyranosyl]-1,8-dihydroxy-3-methoxyxanthone (**11**) [15], 1-hydroxy-3,7,8-trimethoxyxanthone (**12**) [8], swertianolin (**13**) [8], 1-hydroxy-8-glucosyloxy-3,5-dimethoxyxanthone (**14**) [16], 1-O-β-D-glucopy-

Table 2 Anti-HBV activities of compounds **3**–**10**[a].

Compounds	CC_{50} [mM]	HBsAg[b]		HBeAg[c]		DNA[d]	
		IC_{50} [mM]	SI[e]	IC_{50} [mM]	SI	IC_{50} [mM]	SI
3	> 4.80	0.77	> 6.2	< 0.62	7.8	0.02	> 1.5
4	> 0.98	> 0.98	–	0.35	2.8	> 0.09	> 10.9
5	> 2.04	0.21	> 9.7	0.04	47.8	0.09	> 21.7
6	> 2.69	–	–	–	–	0.01	> 256.1
7	> 2.07	–	–	–	–	0.06	> 30.0
8	> 3.88	–	–	–	–	0.13	> 30.0
9	> 1.82	–	–	–	–	0.10	> 18.0
10	> 2.07	–	–	1.11	> 1.9	0.01	> 145.2
Tenofovir[f]	> 1.39	1.25	> 1.1	1.21	> 1.2	0.00046	> 3021.7

[a] All values are the mean of two independent experiments; [b] HBsAg: HBV surface antigen; [c] HBeAg: HBV e antigen; [d] DNA: HBV DNA replication; [e] CC_{50} = 50 % cytotoxic concentration, IC_{50} = 50 % inhibition concentration, SI (selectivity index) = CC_{50}/IC_{50}; [f] Tenofovir, an antiviral agent used as a positive control

ranosyl-8-hydroxy-3,7-dimethoxyxanthone (**15**) [17], 1-*O*-*β*-D-glucopyranosyl-3,7,8-trimethoxyxanthone (**16**) [18], 1-*O*-[*β*-D-xylopyranosyl-(1 → 6)-*β*-D-glucopyranosyl]-7-hydroxy-3,8-dimethoxyxanthone (**17**) [12], 1-*O*-gentiobiosyl-3,7-dimethoxy-8-hydroxyxanthone (**18**) [19], 1-*O*-[*β*-D-xylopyranosyl-(1 → 6)-*β*-D-glucopyranosyl]-3,7,8-trimethoxyxanthone (**19**) [12], and 1,7-dihydroxy-3,4,8-trimethoxyxanthone (**20**) [20].

All of the isolated xanthones were evaluated for their anti-HBV activities, namely inhibiting the secretion of HBsAg and HBeAg, and HBV DNA replication in HepG 2.2.15 cells, as reported previously (tenofovir was used as the positive control) [21]. The results of their activity and cytotoxicity evaluations are listed in **Table 2**.

As shown in **Table 2**, eight compounds (**3**–**10**) exhibited significant anti-HBV activity. However, others showed no anti-HBV activity at the maximal testing concentration. Compounds **3**–**5** with three or more hydroxy groups showed significant inhibitory activity with IC_{50} values of 0.77, > 0.98, and 0.21 mM for HBsAg, and < 0.62, 0.35, and 0.04 mM for HBeAg, respectively. From the anti-HBV data of these compounds, it was deduced that hydroxy groups in the xanthones structure were essential for maintaining the inhibitory effects on the secretion of HBsAg and HBeAg. Glycosidation of hydroxy groups led to activity decreasing against HBsAg and HBeAg by comparing the activity of compounds **4, 6**, and **7**. Compounds **3**–**10** showed remarkable inhibition on HBV DNA replication with IC_{50} values from 0.01 mM to 0.13 mM. It was concluded that two or more hydroxy groups were essential for inhibiting HBV DNA replication, and methylation of hydroxy groups decreased or abolished anti-HBV activity. In addition, the position of the hydroxy groups of the isolated xanthones did not significantly affect the inhibition on HBV DNA replication.

The preliminary structure-activity relationships were deduced as: 1) the anti-HBV activity of xanthones depends on the structure and substitution pattern of the hydroxy groups; 2) the hydroxy groups play very important roles in the anti-HBV activity; 3) the anti-HBV activity will be decreased after methylation or glycosidation.

Materials and Methods

The whole plants of *S. mussotii* were collected in Yushu, Qinghai province, PR China, in November 2008 and identified by Prof. Dr. Yan-Duo Tao, Northwest Institute of Plateau Biology, Chinese Academy of Sciences. A voucher specimen (No. 20101128) was deposited at the Laboratory of Antivirus and Natural Medicinal Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences.

The air-dried and powdered whole plants of *S. mussotii* (6 kg) were used for phytochemical investigation, and from them twenty xanthones were obtained by diverse column chromatography methods. The extensive procedure of isolation is provided as Supporting Information.

Isolates

8-O-β-D-glucopyranosyl-1-hydroxy-2,3,5-trimethoxyxanthone (**1**): yellowish powder; $[\alpha]_D^{24}$: − 99.8 (*c* 0.12, H_2O); UV (MeOH): λ_{max} (log ε) = 372 (3.6), 315 (4.1), 278 (4.3), 247 (4.4), 221 (4.4), 205 (4.4) nm; IR (KBr): ν_{max} = 3345, 2918, 1655, 1613, 1580, 1496, 1457, 1429, 1318, 1297, 1267, 1216, 1201, 1144, 1092, 1057, 1042, 987 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR data, see **Table 1**; positive HRESIMS: *m/z* = 503.1159 $[M + Na]^+$ (calcd. for $C_{22}H_{24}O_{12}$, 503.1160), 319.0788 $[M + H\text{-}162]^+$.

8-O-[β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl]-1-hydroxy-2,3,5-trimethoxyxanthone (**2**): yellowish powder; $[\alpha]_D^{24}$: − 120.6 (*c* 0.12, H_2O); UV (MeOH): λ_{max} (log ε) = 373 (3.6), 315 (4.1), 278 (4.3), 247 (4.4), 221 (4.3), 205 (4.4) nm; IR (KBr): ν_{max} = 3424, 1652, 1614, 1581, 1500, 1454, 1427, 1316, 1292, 1270, 1214, 1200, 1147, 1106, 1090, 1058, 1040, 992 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR data, see **Table 1**; positive HRESIMS: *m/z* = 635.1559 $[M + Na]^+$ (calcd. for $C_{27}H_{32}O_{16}$, 635.1583), 319.0790 $[M + H\text{-}162\text{-}132]^+$.

The anti-HBV assay was performed according to our previous report [7]. Tenofovir, purchased from Jiangxi Chenyang Pharmaceutial Co. Ltd. (purity > 97.6 %), was used as the positive control.

Supporting information

1D- and 2D-NMR, HRESIMS, IR, and UV spectra of compounds **1** and **2**, general experimental procedure, extraction and isolation, acid hydrolysis of compounds **1** and **2,** and the procedure for the anti-HBV assay are available as Supporting Information.

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Conflict of Interest

The authors declared no potential conflicts of interest.

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