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Swerpunilactones A and B, the first example of xanthone and secoiridoid heterodimers from *Swertia punicea, S. hispidicalyx*, and *S. yunnanensis*

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ABSTRACT

Swerpunilactones A (**1**) and B (**2**), two novel xanthone and secoiridoid heterodimers, together with their presumed biosynthetic precursors (±)-gentiolactone (**3**), bellidifolin (**4**), and norbellidifolin (**5**), were isolated from the whole plants of *Swertia* species. A plausible biogenetic pathway for swerpunilactones A and B was proposed. In vitro anti-hepatitis B virus assay on the Hep G 2.2.15 cell line showed that both compounds **1** and **2** exhibited activities against the secretion of HBsAg (IC_{50} = 0.25 and 0.29 mM), HBeAg (IC_{50} = 0.86 and 0.31 mM), and HBV DNA replication (IC_{50} = 0.18 and 0.19 mM).

Xanthones and secoiridoids are important natural products because of diverse biological activities, which have attracted attention of not only pharmacologists but also synthetic chemists. Xanthones, a class of oxygen heterocyclic compounds with dibenzo-γ-pyrone as the basic skeleton, are mainly distributed in the families of Gentianaceae, Guttiferae, Polygalaceae, and Moraceae, and have activities of diuretic, cardiac, hepatoprotective, and anti-inflammatory.[1–3] Secoiridoids, as a group of monoterpenes with a methylcyclopentane skeleton, principally exist in plants of the families Gentianaceae, Rubiaceae, Verbenaceae, Oleaceae, Loganiaceae, and Caprifoliaceae, and possess many pharmacological properties such as analgesic, hypoglycemic, and hepatoprotective.[4,5] It is well-known that both xanthones and secoiridoids widely coexist in the Gentianaceae family, of which some plants have been used as medicinal plants for the treatment of hepatitis, cholecystitis, and arthritis,[6,7] but a xanthone and secoiridoid heterodimer linked by C–C bond has never been reported.

Plants of the genus *Swertia* (Gentianaceae), are mainly distributed in Asia, Africa, and North America, containing about 170 species, of which 79 species are present in China. Many *Swertia* plants are widely used for the treatment of hepatitis in both traditional Chinese medicine (TCM) and Tibetan medicine systems.[8,9] For example, *S. mileensis*, known as '*Qing-Ye-Dan*', has been documented in Chinese Pharmacopoeia (1977−2010 editions) to cure viral hepatitis clinically.[10] Our previous phytochemical investigations on *S. mileensis* had resulted in a series of new anti-hepatitis B virus (HBV) active lactones: swerilactones A–O and swerilactosides A–C.[11–16] The promising outcome promoted us to investigate other *Swertia* species for active anti-HBV compounds.

Swertia punicea, *S. hispidicalyx*, and *S. yunnanensis*, as congener species of *S. mileensis*, are used for the treatment of hepatitis in the folk of Yunnan Province. Previous studies on the chemical constituents of *S. punicea*, *S. hispidicalyx*, and *S. yunnanensis* revealed several xanthones and secoiridoids.[17–23] Our anti-HBV screening manifested that the EtOAc extract of *S. punicea* showed inhibitory activities on hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) with IC_{50} values of 0.69 and 0.15 mg/mL, respectively. In order to clarify the active components, swerpunilactone A (**1**) and its plausible precursors: (±)-gentiolactone (**3**), bellidifolin (**4**) were isolated from the whole plants of *S. punicea*. Simultaneously, swerpunilactone B (**2**), (±)-gentiolactone (**3**), and norbellidifolin (**5**) were correspondingly obtained from *S. hispidicalyx* and *S. yunnanensis*. Herein, we described the isolation, structure elucidation, possible biogenetic pathway, as well as their anti-HBV activities of swerpunilactones A and B.

The dried and powered plant of *S. punicea* (5.0 kg) was extracted with 90% (v/v) EtOH (20 L) at room temperature for two times, 24 h for each time. The extract was concentrated under vacuum to give a residue, which was suspended in H_2O and partitioned between

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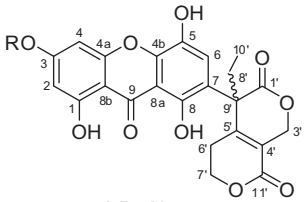
EtOAc and *n*-BuOH, respectively. The EtOAc fraction (450 g) was purified repeatedly by silica gel column and Sephadex LH-20 chromatography to yield swerpunilactone A (**1**), (±)-gentiolactone (**3**), and bellidifolin (**4**). Swerpunilactone B (**2**), (±)-gentiolactone (**3**), and norbellifiifolin (**5**) were isolated from *S. hispidicalyx* and *S. yunnanensis* by the similar separation procedure.



Swerpunilactone A[24] (**1**) was isolated as a yellow powder and had a molecular formula of $C_{24}H_{20}O_{10}$ on the basis of negative ESI-MS (*m/z* 467 $[M-H]^-$) and HR-ESI-MS (*m/z* 467.0980 $[M-H]^-$), indicating fifteen degrees of unsaturation. Its IR spectrum exhibited hydroxyl (3423 cm^{-1}), carbonyl (1722, 1662, 1627 cm^{-1}), and aromatic ring (1588, 1504, 1491 cm^{-1}) groups. The UV spectrum with absorption maxima at 257, 284, and 340 nm indicated a xanthone skeleton.[25,26] In the ^1H NMR spectrum, two hydrogen-bond hydroxyl groups (δ_H 12.14, overlap), located at C-1 and C-8, three aromatic H-atoms [δ_H 7.85 (1H, s), 6.50 (1H, br s), and 6.10 (1H, br s)], and a methoxy group (δ_H 3.64, 3H, s) were observed. The ^{13}C NMR (DEPT) spectrum showed the presence of one conjugated carbonyl carbon (δ_C 184.9, C-9), 12 aromatic carbons, a methoxy carbon, and ten other carbons (Table 1). Comparison of its NMR data with those of bellidifolin[22] indicated they possessed a similar skeleton except for an additional C_{10} moiety (**1b**). From these data, compound **1** was deduced as a xanthone derivative with three hydroxyl groups, one methoxy group, and a C_{10} unit (**1b**). The correlations of δ_H 3.64 and δ_C 98.1 (C-2), 93.0 (C-4) in the HMBC experiment suggested that the methoxy position was located at C-3, which was further confirmed by the corrections of δ_H 3.64 with H-2 and H-4 in ROESY spectrum.

The carbon signals due to the C_{10} unit (**1b**) observed at δ_C 162.4 (C-11′), 121.0 (C-4′), 148.6 (C-5′), 66.7 (C-7′), and 23.7 (C-6′), in combination with proton resonances at δ_H 4.33 (2H, m, H-7′) and 2.21 (1H, m, H-6′a), 2.04 (1H, m, H-6′b), indicated an α, β-unsaturated δ-lactone fragment, which was further confirmed by ^1H–^1H COSY (H-6′/H-7′) and HMBC (H-7′/C-5′, C-11′ and H-6′/C-4′, C-5′, C-7′) experiments. Furthermore, the correlation of H-8′ and H-10′ in the ^1H–^1H COSY spectrum proposed an ethyl group. Comparison of its NMR data with those of (±)-gentiolactone[27,28] demonstrated that they possessed a similar skeleton, and the main difference was that fragment **1b** had one more fragment **1a** at C-9′ instead of the hydroxyl moiety, which was confirmed by the correlations of H-8′/C-7, C-1′, and C-5′ in the HMBC experiment (Fig. 1). In addition, the C_{10} unit (**1b**) was proposed to be linked at C-7 on the basis of the HMBC correlations from H-8′ to C-7, H-6 to C-5, C-8, C-4b (δ_C 144.2), and C-9′[29,30] Thus, the structure of **1** was determined as depicted.

Swerpunilactone B (**2**)[31], a yellow powder, was assigned to have a molecular formula of $C_{23}H_{18}O_{10}$ based on EI-MS (*m/z* 454 $[M]^+$) and HR–EI–MS (*m/z* 454.0905 $[M]^+$). The IR spectrum showed the hydroxyl (3414 cm^{-1}), carbonyl (1712, 1657, 1628 cm^{-1}), and aromatic ring (1590, 1491, 1460 cm^{-1}) groups. Careful analyses of ^1H and ^{13}C NMR spectroscopic data suggested the structure of compound **2** was similar to swerpunilactone A **(1)**, except for the absence of a methoxy moiety. And the down-fielded shift of C-2 (δ_C 99.8) and C-4 (δ_C 95.0) predicated that there was a hydroxyl unit located at C-3. From the above evidence, the structure of compound **2** was established as shown in Figure 1.

In the $[\alpha]_D$ experiments, no or very little rotation values for compounds **1**–**2** were detected, indicating that they were 9′*R*/S racemic mixtures with the ratio close to 1:1, which was further confirmed by the HPLC analyses with a chiral column [Daicel Chemical Industries, Ltd, Japan, 0.46 mm × 25 cm, *n*-hexane–*iso*-PrOH (87:13)].[28]

A plausible biogenetic pathway of compounds **1** and **2** was proposed as shown in Scheme 1 based on the related reference.[30]

In order to evaluate their anti-HBV activities, swerpunilactones A (**1**) and B (**2**), as well as compounds **3–5** were assayed on Hep G 2.2.15 cell line in vitro. The anti-HBV assay was performed according to the previous report[15] by using tenofovir as the positive control (Table 2). The results suggested that compounds **1** and **2** showed moderate activities against HBsAg (IC_{50} = 0.25 and 0.29 mM), HBeAg (IC_{50} = 0.86 and 0.31 mM), and HBV DNA replication (IC_{50} = 0.18 and 0.19 mM). Compared with bellidifolin (**4**)[22] and norbellidifolin (**5**)[32], compounds **1** and **2** exhibited similar activities, but higher cytotoxicity. Compared to (±)-gentiolactone (**3**), compounds **1** and **2** exhibited improved activity against HBV DNA replication. However, none of the assayed compounds showed equivalent activity compared to tenofovir against HBV DNA replication.

Table 1
^1H and ^{13}C NMR data of compounds **1**[a] and **2**[b] in pyridine-d_5 (δ in ppm, *J* in Hz)

No.	**1**		**2**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	
1	—	163.2, C	—	163.7, C
2	6.50, br s	98.1, CH	6.63, br s	99.8, CH
3	—	167.9, C	—	168.5, C
4	6.10, br s	93.0, CH	6.35, br s	95.0, CH
4a	—	157.9, C	—	158.4, C
4b	—	144.2, C	—	144.2, C
5	—	138.9, C	—	138.8, C
6	7.85, s	123.6, CH	7.84, s	123.6, CH
7	—	121.3, C	—	121.1, C
8	—	150.2, C	—	150.3, C
8a	—	108.0, C	—	107.9, C
8b	—	103.0, C	—	102.1, C
9	—	184.9, C	—	184.5, C
1′	—	171.2, C	—	171.2, C
3′	5.60, d (15.9)	68.3, CH_2	5.61, d (16.2)	68.3, CH_2
	5.52, d (15.9)		5.53, d (16.2)	
4′	—	121.0, C	—	120.9, C
5′	—	148.6, C	—	148.7, C
6′a	2.21, m	23.7, CH_2	2.27, m	23.8, CH_2
6′b	2.04, m		2.03, m	
7′	4.33, m	66.7, CH_2	4.34, m	66.7, CH_2
8′a	2.49, m	28.8, CH_2	2.50, m	28.8, CH_2
8′b	2.29, m		2.34, m	
9′	—	50.1, C	—	50.1, C
10′	0.99, t (8.4)	9.1, CH_3	1.00, t (7.2)	9.1, CH_3
11′	—	162.4, C	—	162.4, C
OMe	3.64, s	56.1, CH_3		—
OH	12.14 (overlap)	—	12.30 (overlap)	—
			12.23 (overlap)	

[a] ^1H NMR recorded at 500 MHz; ^{13}C NMR recorded at 100 MHz.
[b] ^1H NMR recorded at 500 MHz; ^{13}C NMR recorded at 125 MHz.

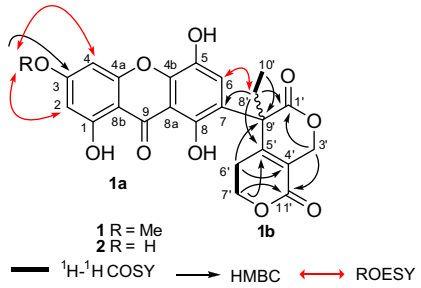
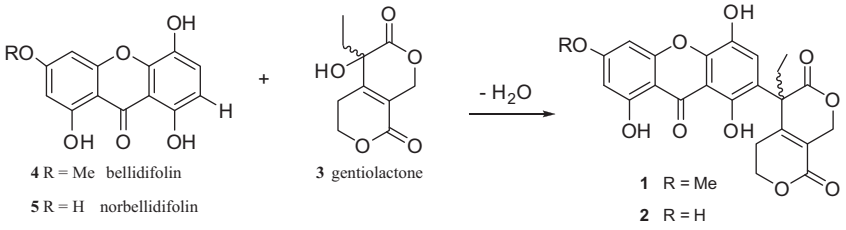


Fig. 1. Selected 2D NMR correlations of compounds **1** and **2**.



Scheme 1. A plausible biogenetic route of compounds **1** and **2**.

Table 2
Anti-HBV data of compounds **1–5**[a]

No.	CC_{50} (mM)[b]	HBsAg[c]		HBeAg[d]		HBV DNA	
		IC_{50} (mM)[b]	SI	IC_{50} (mM)	SI	IC_{50} (mM)	SI
1	0.30 (0.23∼0.37)	0.25 (0.21∼0.29)	1.20	0.86 (0.82∼0.90)	—	0.18 (0.14∼0.22)	>1.67
2	>0.42	0.29 (0.23∼0.35)	>1.45	0.31 (0.27∼0.35)	>1.35	0.19 (0.13∼0.25)	>2.21
3	1.93 (1.86∼2.00)	0.63 (0.58∼0.68)	3.09	1.26 (1.21∼1.31)	1.54	>3.82	—
4[22]	>0.98	>0.98	—	0.35 (0.24∼0.46)	>2.80	0.09 (0.08∼0.10)	>10.89
5[32]	>4.81	0.77 (0.67∼0.87)	>6.25	<0.62	>7.81	0.58 (0.49∼0.67)	>8.29
Tenofovir[e]	>1.83	1.40 (1.36∼1.44)	>1.31	1.14 (1.10∼1.18)	>1.61	0.00052 (0.00042∼0.00062)	>3519.2

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

In conclusion, this is the first report of novel skeleton of xanthone and secoiridoid heterodimers connected with C–C bond even though both xanthone and secoiridoid derivatives widely coexist in the Gentianaceae family, which may be characteristic components of *Swertia* species (Gentianaceae) because we had isolated swerpunilactones A (**1**) and B (**2**) from *S. punicea*, *S. hispidicalyx*, and *S. yunnanensis*. Secondly, the finding of xanthone and secoiridoid heterodimers induces us to infer that the plants of *Swertia* species may contain a particular biological enzyme stimulating the combination of xanthones and secoiridoids. Thirdly, the first example of novel skeleton not only enriches the skeleton types of natural products, but also provides information for the comprehensive understanding of the xanthone and secoiridoid heterodimers.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.03.057.

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24. Swerpunilactone A (1): yellow powder, $[\alpha]_D^{17.6}$ + 0.00 (*c* 0.05, $CHCl_3$:MeOH = 1:1, v/v); UV ($CHCl_3$) λ_{max} (log ε) 340 (3.96), 284 (4.03), 257 (4.22) nm; IR (KBr) λ_{max} 3423, 1722, 1662, 1627, 1588, 1504, 1491 cm^{-1}; (−)ESIMS: *m*/*z* 467 ($[M-H]^-$, 100), 423 ($[M-H-CO_2]^-$, 41), 255 (43), 113 (26); HR–ESIMS: *m*/*z* 467.0980 (467.0978 calcd for $C_{24}H_{20}O_{10}$); ^1H and ^{13}C NMR (C5D5N, 500/100 MHz), see. Table 1.
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31. Swerpunilactone B (**2**): yellow powder, $[\alpha]_D^{15.9}$ −2.35 (*c* 0.14, C_5H_5N); UV ($CHCl_3$) λ_{max} (log ε) 340 (4.06), 282 (4.10), 255 (4.36), 224 (4.33) nm; IR (KBr) λ_{max} 3414, 1712, 1657, 1628, 1590, 1491, 1460 cm^{-1}; EIMS: *m*/*z* 454 $[M]^+$ (100), 425 (42), 381 (34), 337 (37), 273 (50), 260 (67). HR-EIMS: *m*/*z* 454.0905 (454.0900 calcd for $C_{23}H_{18}O_{10}$); ^1H and ^{13}C NMR (C_5D_5N, 500/125 MHz), see. Table 1.
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