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Five new secoiridoid glycosides and one unusual lactonic enol ketone with anti-HBV activity from *Swertia cincta*

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ABSTRACT

Five new secoiridoid glycosides, swericinctosides A and B (**1**–**2**), 9-*epi* swertiamarin (**3**), 2′-*O*-*m*-hydroxybenzoyl swertiamarin (**4**), 4″-*O*-acetyl swertianoside E (**5**), and one unusual lactonic enol ketone, 3-(hydroxymethyl ene) dihydro-*2H*-pyran-2, 4(*3H*)-dione (**6**), together with three known compounds, swertiaside (**7**), swertianoside C (**8**) and decentapicrin B (**9**) were isolated from *Swertia cincta*. The structures of the new compounds were determined by extensive spectroscopic analyses including 1D and 2D NMR, HRESIMS, UV, IR and $[\alpha]_D$ spectra. Anti-HBV assay on HepG 2.2.15 cell line *in vitro* demonstrated that compounds **1**–**9** possessed inhibitory activity on HBV DNA replication with IC_{50} values from 0.05 to 1.83 mM, and compounds **1**, **3**, **5**, **7** and **8** could inhibit the secretion of HBsAg with IC_{50} values from 0.24 to 1.06 mM.

1. Introduction

Plants of genus *Swertia* (Gentianaceae) containing about 170 species are mainly distributed in Asia, Africa, and North America, 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 [1,2]. The main constituents of this genus were revealed as secoiridoids iridoids, xanthones, flavonoids and triterpenoids, which possessed hepatoprotective, anti-hepatotoxic, anti-oxidant, mutagenic, anti-diabetic, anti-ulcer and anti-gastritis activities [3–6]. Our previous investigation on *S. mileensis*, a famous TCM documented in Chinese Pharmacopoeia (1977–2010 editions) to treat viral hepatitis resulted in a series of novel lactones with anti-hepatitis B virus (HBV) activity [7–11]. The promising outcome promoted us to investigate the anti-HBV active constituents from other *Swertia* species.

Swertia cincta, the congener plant of *S. mileensis*, is also used to treat hepatitis in the folk of Yunnan Province [12]. Previous phytochemical studies on *S. cincta* revealed that its main chemical constituents were xanthones, secoiridoids, triterpenoids, and steroids [13]. Our *in vitro* anti-HBV bioassay manifested that the 90% ethanol extract of *S. cincta* could inhibit the secretions of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg), and HBV DNA replication with the IC_{50} value of 151.5 μg/mL (SI > 20.0), 53.7 μg/mL (SI > 40.8) and 21.9 μg/mL (SI > 24.0) respectively. However, the active substances responsible for the anti-HBV property were still unclear. In order to clarify its active constituents, extensive investigation on the ethanol extract of *S. cincta* yielded six new compounds including five secoiridoid glycosides and one unusual lactone enol ketone, as well as three known ones. This paper described the isolation, structural elucidation and anti-HBV activities of the isolates.

2. Experimental

2.1. General experimental procedures

LCMS-IT-TOF (Shimadzu, Kyoto, Japan) provided the mass spectra. UV and IR (KBr) spectra were respectively

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recorded on a Shimadzu UV2401PC spectrophotometer (Shimadzu, Kyoto, Japan) and a Bio-Rad FTS-135 spectrometer (Hercules, California, USA). 1D and 2D NMR were recorded on Bruker AM-400, Bruker DRX-500 or AVANCE III-600 spectrometers (Bruker, Bremerhaven, Germany). Silica gel (200–300 mesh) for column chromatography (CC) and TLC plates (GF_{254}) were purchased from Qingdao Makall Chemical Company (Makall, Qingdao, China). Sephadex LH-20 (20–50 μm) for chromatography was obtained from Pharmacia Fine Chemical Co., Ltd. (Pharmacia, Uppsala, Sweden). Semi-preparative HPLC was carried out on Newstyle™ (pump: NP-7000 serials, detector: NU-3000 serials, Hanbon Sci. & Tech., China) liquid chromatograph with ZORBAX SB-C_{18} (9.4 × 250 mm) column (Agilent, USA). Fractions were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol.

2.2. Plant material

The whole plants of *S. cincta* Burk. were collected in Chuxiong, Yunnan Province, PR China, in November 2008 and identified by Dr. Prof. Li-Gong Lei (Kunming Institute of Botany, CAS). A voucher specimen (No. 20081103) was deposited in the Laboratory of Anti-virus and Natural Medicinal Chemistry, Kunming Institute of Botany, CAS.

2.3. Extraction and isolation

The air-dried whole plants of *S. cincta* (5.0 kg) were powdered and extracted with 90% EtOH under reflux for 2 times, 2 h for each time. After removal of the solvents *in vacuo*, the EtOH extract was suspended in water and partitioned with ethyl acetate (10.0 L × 3). The ethyl acetate extract (155.0 g) was purified by chromatography on a silica gel column chromatography (Si CC) (2.2 kg, 11.0 × 70.0 cm) eluted with MeOH–$CHCl_3$ (0:100, 5:95, 10:90, 20:80, 100:0, *v*/*v*) to furnish ten fractions (Fr. A-J). Fr. H (20 g) was performed on Si CC with Me_2CO-petroleum ether (PE) (10:90, 20:80; 50:50) to yield nine sub-fractions (H1–H9). Compound **3** (5 mg) was isolated from fraction H3 by semi-preparation HPLC (MeOH–H_2O, 10:90). Fr. H4 was further separated to obtain five sub-fractions (H4-1–H4-5). Fr. H4-1 was subjected to Si CC and further purified by semi-preparative HPLC (Agilent ZORBAX SB-C_{18}, 5 μm, 9.4 × 250 mm) with MeOH–H_2O (20:80) to provide compound **2** (25 mg). Fr. H6 was separated by repeated Si CC (MeOH-$CHCl_3$, 10:90; Me_2CO–$CHCl_3$, 25:75, 50:50), and Sephadex LH-20 CC (MeOH) to give compounds **4** (35 mg) and **6** (10 mg). Fr. H9 was chromatographed on a reversed phase C-18 column (300 g, 3.8 × 50 cm) and eluted with MeOH–H_2O (10:90, 30:70, 50:50, 70:30, 100:0) to yield nine sub-fractions (H9-1–H9-9). Fr. H9-3 (674 mg) was purified by a Si CC (60 g, 2.5 × 30 cm) to afford compound **1** (10 mg). Purification of fr. H9-6 on Si CC with MeOH–EtOAc (4:96) yielded eight sub-fractions (H9-6-1 ~ H9-6-8). Fr. H9-6-1 (150 mg) was loaded on Sephadex LH-20 column (50 g, 1.4 × 120 cm, MeOH) to yield compound **5** (21 mg). Fr. H9-6-4 was separated by repeated Si CC [H_2O-MeOH-$CHCl_3$, 0.5:5:95, 1:10:90] and further purified by semi-preparation HPLC with MeOH–H_2O (47:53) as the eluent to afford compounds **8** (20 mg) and **9** (50 mg). Fr. H9-6-8 (2.3 g) was subjected by silica gel column (2.4 × 22 cm) with the eluent MeOH–EtOAc (10:90, 20:80) and further purified by Sephadex LH-20 column (50 g, 1.4 × 120 cm) with MeOH to yield compound **7** (40 mg).

Compound **1**: pale yellow oil; $[a]_D^{17.9} = +52.26$ (*c* 0.016, MeOH); UV (MeOH) λ_{max} (log ε): 204 (3.18) nm; IR (KBr) ν_{max}: 3426, 2926, 2855, 1701, 1630, 1417, 1384, 1279, 1078, 1061, 599, 578 cm^{-1}. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) data, see Tables 1–2; HREIMS *m*/*z* 369.1156 ($[M + Na]^+$, $C_{15}H_{22}O_9Na^+$, calcd for 369.1127).

Compound **2**: white powder; $[a]_D^{17.4} = -104.21$ (*c* 0.29, MeOH); UV (MeOH) λ_{max} (log ε): 200 (3.78), 269 (4.07) nm; IR (KBr) ν_{max}: 3421, 2912, 1702, 1656, 1468, 1421, 1337, 1301, 1246, 1093, 1050, 894, 769 cm^{-1}; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data, see Tables 1–2; HREIMS *m*/*z* 355.1029 ($[M - H]^-$, $C_{16}H_{19}O_9$, calcd for 355.1029).

Compound **3**: white powder; $[a]_D^{20.7} = -140.83$ (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ε): 197 (3.63), 235 (3.94) nm; IR (KBr) ν_{max}: 3414, 2920, 2888, 1694, 1618, 1376, 1273, 1234, 1208, 1157, 1108, 1014, 948, 931, 903, 845, 629 cm^{-1}; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data, see Tables 1–2; HREIMS *m*/*z* 397.1102 ($[M + Na]^+$, $C_{16}H_{22}O_{10}Na^+$, calcd for 397.1105).

Compound **4**: white powder; $[a]_D^{23} = -84.36$ (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε): 210 (4.45), 236 (4.16) nm; IR (KBr) ν_{max}: 3431, 2923, 1716, 1618, 1591, 1456, 1410, 1368, 1309, 1287, 1268, 1231, 1158, 1102, 1062, 1028, 999, 930, 756 cm^{-1}; ^1H NMR (600 MHz) and ^{13}C NMR (125 MHz) data, see Tables 1–2; HREIMS *m*/*z* 493.1273 ($[M - H]^-$, $C_{23}H_{25}O_{12}$, calcd for 493.1352).

Compound **5**: white powder; $[a]_D^{21.9} = -130.96$ (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε): 201 (4.22), 230 (4.28) nm; IR (KBr) ν_{max}: 3439, 1737, 1696, 1620, 1515, 1373, 1237, 1164, 1069, 1037, 930, 904, 837 cm^{-1}; ^1H NMR (600 MHz) and ^{13}C NMR (125 MHz) data, see Tables 1–2; HREIMS *m*/*z* 585.1520 ($[M + Na]^+$, $C_{27}H_{30}O_{13}Na^+$, calcd for 585.1579).

Compound **6**: white powder; UV (DMSO) λ_{max} (log ε): 249 (3.73), 279 (3.97) nm; IR (KBr) ν_{max}: 3386, 1680, 1624, 1535, 1477, 1445, 1377, 1279, 1175, 1090, 1020, 782, 770, 710 cm^{-1}; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data, see Tables 1–2; HREIMS *m*/*z* 141.0194 ($[M - H]^-$, $C_6H_5O_4$, calcd for 141.0193).

2.4. Anti-HBV assay on HepG 2.2.15 cell line in vitro

The anti-HBV assay was performed according to our previous report, with tenofovir (Jiangxi Chenyang Pharmaceutial Co., Ltd, China, purity >97.6%) as the positive control [8].

3. Results and discussion

Swericinctosides A (**1**) had a molecular formula of $C_{15}H_{22}O_9$ by HRESIMS ($[M + Na]^+$ 369.1156; calcd for 369.1127), suggesting five degrees of unsaturation. The absorption bands at 3426, 1701 and 1630 cm^{-1} in IR spectrum indicated the presence of hydroxyl, carbonyl and alkenyl groups. The ^{13}C NMR (DEPT) spectrum of **1** displayed 15 carbons, including one methyl, four methylenes, seven methines and three quaternary carbons, of which a glucosyl group [δ_C 101.6 (d, C-1′), 74.0 (d, C-2′), 74.9 (d, C-3′), 71.2 (d, C-4′), 75.6 (d, C-5′), 62.5 (t, C-6′)] was obviously recognized [14]. The remaining carbon signals were almost identical to swerilactone T [15] except that C-3 was obviously down-field shifted from δ_C 88.3 to δ_C 96.3. The

Table 1
^{1}H NMR data of compounds **1–6**.

No.	1	2	3	4	5	6
1	4.2 , m	6.20, s	5.75, s	5.60, d, 1.8	5.62, d, 4.8	4.19, s
3	5.4, s	5.86, s	7.70. s	7.64, s	7.80, s	2.45, d, 12.4
4	–	–	–	–	–	–
5	–	–	–	–	–	–
6	2.50 , t, 4.0	a: 2.88, m	2.00, t, 7.6	1.72, m	1.94, td, 12.0, 8.0	–
		b: 2.66, m	1.80, td, 12.8, 4.4	1.64, m	1.73, d, 16.0	–
7	4.40, dd, 16.0,4.0	4.51, m	a: 4.68, d, 11.6	4.58, td,12.0, 3.0	4.99, t, 10.0	9.04, s
			b: 4.42, d, 6.8	4.16, m	4.29, dd, 12.0, 5.6	
8	2.30, d, 4.0	6.38, d, 7.2	5.84, m	5.39, m	5.40, m	
9	1.25, d, 8.0	–	2.37, t, 9.6	2.85, m	2.93, d, 1.8	
10	–	2.00, d, 7.2	5.27, m	5.24, m	5.37, m	
1′	5.20, d, 4.0	5.03, d, 7.1	4.81, d, 7.6	5.01, t, 6.4	4.69, d 8.0	
2′	3.36, m	3.36, m	3.26, m	3.34, m	3.63, m	
3′	3.53, t, 9.6	3.57, m	3.39, m	3.56, m	5.03, t, 9.2	
4′	3.34, t, 8.0	3.33, m	3.23, m	3.55, m	3.68, m	
5′	3.49, m	3.38, m	3.33, m	3.56, m	3.65, m	
6′	3.80, dd, 12.0, 4.0	a: 3.88, dd, 12.0, 2.0	3.93, d, 12.0	3.90, m	3.88, d, 12.0	
		b: 3.65, dd, 12.0, 6.0	3.71, d, 8.4	3.74, dd, 12.0, 3.0	3.67, dd, 11.9, 5.6	
2″				7.48, s	7.59, d, 8.7	
3″				–	6.85, d, 8.4	
4″				7.06, d, 8.4	–	
5″				7.27, t, 8.4	6.85, d, 8.4	
6″				7.42, d, 8.4	7.59, d, 8.7	
7″				–	7.67, d, 15.9	
8″				–	6.39, d, 15.9	
Ac					2.06, s	

1, 3 in CD_3OD, **2, 4, 5** in acetone-d_6, **6** in DMSO-d_6, δ in ppm, J in Hz.

above analyses suggested that compound **1** should be the glucoside of swerilactone T, which was further supported by the HMBC correlations of H-7/C-5, C-10; H-6/C-4, C-8; H-8/C-4, C-9; H-3/C-1, C-10 and ^{1}H-^{1}H COSY correlations of H-7/H-6; H-8/H-1; H-1/H-9. The connectivity of C_3-O-$C_{1'}$ was deduced by the HMBC correlations of H-1′/C-3 and H-3/C-1′. Acid hydrolysis of **1** provided glucose which was confirmed by comparison with an authentic sample on Si TLC (Rf = 0.4) and identified to be D-glucose based on its $[\alpha]_D$ value ($[\alpha]_D$ + 46.6, *c* 0.089, MeOH). Based on the above analyses, compound **1** was established as 3-*O*-*β*-D-glucopyranosyl swerilactone T, and named as swericinctosides A (**1**).

Table 2
^{13}C NMR data of compounds **1–6**.

No.	1	2	3	4	5	6
1	64.2 (d)	89.6 (d)	99.3 (d)	98.6 (d)	97.2 (d)	104.4 (s)
2	–	–	–	–	–	168.9 (s)
3	96.3 (d)	92.3 (d)	154.7 (d)	151.7 (d)	150.3 (d)	–
4	123.3(s)	118.6 (s)	108.5 (s)	109.8 (s)	109.9 (s)	62.9 (t)
5	157.4 (s)	151.6 (s)	64.2 (s)	63.8 (s)	62.5 (s)	35.2 (t)
6	29.1 (t)	24.7 (t)	33.7 (t)	33.4 (t)	32.4 (t)	192.3 (s)
7	67.2 (t)	66.7 (t)	66.1 (t)	64.7 (t)	64.2 (t)	187.2 (d)
8	37.2 (t)	134.8 (d)	133.8 (d)	133.6 (d)	132.5 (d)	
9	20.8 (q)	132.4 (s)	52.1 (d)	51.7 (d)	50.9 (d)	
10	165.9 (s)	14.0 (q)	121.2 (t)	121.0 (t)	120.2 (t)	
11	–	165.0 (s)	168.1 (s)	164.3 (s)	165.3 (s)	
1′	101.6 (d)	99.3 (d)	100.1 (d)	98.8 (d)	97.9 (d)	
2′	74.0 (d)	81.7 (d)	74.4 (d)	78.4 (d)	71.7 (d)	
3′	74.9 (d)	76.4 (d)	77.5 (d)	75.3 (d)	75.6 (d)	
4′	71.2 (d)	70.8 (d)	71.2 (d)	71.4 (d)	70.6 (d)	
5′	75.6 (d)	79.1 (d)	78.3 (d)	75.4 (d)	73.7 (d)	
6′	62.5 (t)	62.6 (t)	62.4 (t)	62.5 (t)	61.1 (t)	
1″				132.0 (s)	125.9 (s)	
2″				117.1 (d)	130.3 (d)	
3″				158.3 (s)	115.9 (d)	
4″				121.8 (d)	159.0 (s)	
5″				130.5 (d)	115.9 (d)	
6″				121.3 (d)	130.3 (d)	
7″				167.5 (s)	145.7 (d)	
8″					113.9 (s)	
9″					166.3 (s)	
Ac					171.0 (s)	
					20.1 (q)	

1, 3 in CD_3OD, 2, 4, 5 in acetone-d_6, 6 in DMSO-d_6, δ in ppm.

Swericinctosides B (**2**) showed a molecular formula of $C_{16}H_{20}O_9$ by the negative HRESIMS ($[M-H]^-$ 355.1029, calcd for 355.1029) with seven degrees of unsaturation. The IR spectrum exhibited the absorption of hydroxyl (3421 cm^{-1}), carbonyl (1702 cm^{-1}), alkenyl (1656 cm^{-1}) and glycosyl (1246, 1093, 1050 cm^{-1}) groups. Analysis of the ^{13}C NMR (DEPT) spectrum of compound **2** indicated resonances of 16 carbons composed of four quaternary carbons, eight methines, three methylenes and one methyl (Tables 1 and 2). In the ^{1}H and ^{13}C NMR spectra, the carbon signals at δ_C 99.3 (d, C-1′), 87.1 (d, C-2′), 76.4 (d, C-3′), 70.8 (d, C-4′), 79.1 (d, C-5′), and 62.6 (q, C-6′), in combination with the coupling constant (J = 7.1 HZ) of the anomeric H atom (δ_H 5.03) suggested the *β*-glucosyl moiety [14]. The left 10 carbons assigned for one carbonyl group at δ_C 165.0 (s, C-11), four olefinic carbons at δ_C 151.6 (s, C-5), 134.8 (d, C-8), 132.4 (s, C-9), and 118.6 (s, C-4), two dioxo-oxygenated methines at δ_C 89.6 (d, C-1) and 92.3 (d, C-3), two methylenes (including one oxygenated) at δ_C 66.7 (t, C-7) and 24.7 (t, C-6) and a methyl at δ_C 14.0 (q, C-10), in combination with proton resonances at δ_H 6.38 (1H, q, J = 7.2 HZ, H-8), 4.51 (2H, m, H-7), 2.88 (2H, m, H-6) and δ_H 2.00 (3H, d, J = 7.2 HZ, H-10), indicated a C_{10} skeleton secoiriodid fragment [15], which was confirmed by ^{1}H-^{1}H COSY (H-6/H-7

and H-8/H-10) and HMBC correlations (H-7/C-10, C-5; H-6/C-4, C-9; H-10/C-9; H-1/C-3, C-5 and H-3/C-1, C-5, C-11). Similarly, the connection of C_1-O-$C_{1'}$ and C_3-O-$C_{2'}$ was deduced by the HMBC correlations of H-1′/C-1 and H-3/C-2′. The obviously correlated signal of H-1 with H-10 indicated the α-orientation of H-1, which was consistent with the undetected correlation of H-1 with H-3 [16,17]. Thus, the structure of compound **2** was elucidated to be swericinctoside B (**2**) as shown in Fig. 1.

Compound **3** had the same molecular formula ($C_{16}H_{22}O_{10}$) with that of swertiamarin based on positive HRESIMS ($[M + Na]^+$ 397.1102; calcd for 397.1105), indicating six degrees of unsaturation. The IR and UV spectra of compound **3** were very similar to those of swertiamarin [18]. Detailed comparison of their NMR data indicated that they were a pair of isomers, with the main difference located at C-7, C-8, C-9 and C-1′ positions (Table 1). The TLC comparison indicated the difference between **3** and swertianmarin. With the aid of the HMBC spectrum, it was deduced that compound **3** and swertiamarin possessed the same planar structure. Therefore, their spectroscopic deviation should be due to the stereochemical difference. In the ROESY spectrum, the correlations of H-9 with H-6α and H-1 indicated that H-9 was α-oriented instead of β-orientation in swertiamarin. Therefore, the structure of compound **3** was determined to be 9-*epi* swertiamarin as shown in Fig. 1.

Compound **4** possessed the molecular formula of $C_{23}H_{26}O_{12}$ by negative HRESIMS ($[M-H]^-$ 493.1273; calcd for 493.1352) denoting eleven degrees of unsaturation. The presence of hydroxyl (3431 cm^{-1}) and aromatic ring (1618, 1591, 1456 cm^{-1}) was deduced from the IR spectrum. In the ^1H and ^{13}C NMR spectra, a glucosyl group and a secoiridoid moiety were obviously recognized, in combination with a *m*-hydroxy benzoyl fragment which was identified from the four aromatic proton signals at δ_H 7.48 (1H, s, H-2″), 7.06 (1H, d, J = 8.4 HZ, H-4″), 7.27 (1H, t, J = 8.4 HZ) and 7.42 (1H, d, J = 8.4 HZ). The NMR spectra of compound **4** were almost identical to those of the known compound desacetylcentapicrin [19], with the main difference that the hydrogen at C-5 was changed to be a hydroxy group in compound **4**, together with the obvious downfield shift of C-5 from δ_C 24.2 in desacetylcentapicrin to δ_C 64.2 in **4**. The above deduction was confirmed by the correlations of δ_H 5.60 (H-1, d, J = 1.8 HZ) to δ_C 98.8 (C-1′, d) and δ_H 3.34 (H-2′, m) to δ_C 167.5 (C-7′, s) in the HMBC spectrum (Fig. 2). Hence, compound **4** was assigned to be 2′-*O*-*m*-hydroxybenzoyl swertiamarin.

Compound **5** had a molecular formula of $C_{27}H_{30}O_{13}$ by positive HRESIMS ($[M + Na]^+$ 585.1520; calcd for 585.1579) with 13 degrees of unsaturation. The absorption bands at 3439, 1737, 1696, 1620, and 1515 cm^{-1} in the IR spectrum proposed the presence of hydroxyl, carbonyl and aromatic ring. In the ^1H and ^{13}C NMR spectra, four aromatic proton signals at δ_H 7.59 (2H, d, J = 8.7 HZ, H-2″, 6″), 6.85 (2H, d, J = 8.4 HZ, H-3″, H-5″) and a trans-double bond [δ_H 7.67 (1H, d, J = 15.9 HZ, H-7″), 6.39 (1H, d, J = 15.9 HZ, H-8″); δ_C 145.7 (d, C-7″), 113.9 (s, C-8″)] were obviously identified. The NMR spectral data of compound **5** (Tables 1 and 2) were similar to those of swertianoside E [20], except for the presence of an acetyl group which was confirmed by HMBC connectivity. The connectivity of $C_{3'}$-O-$C_{9''}$ was deduced by the observed signals of H-3′/C-9″. Consequently, the structure of compound **5** was elucidated as 4″-*O*-actyl swertianoside E.

Compound **6** was assigned with a molecular formula of $C_6H_6O_4$ by HRESIMS analysis in negative mode ($[M-H]^-$ 141.0194; calcd for 141.0193), indicating four degrees of unsaturation. The ^{13}C NMR (DEPT) spectrum displayed six carbons, assigned to three methylenes and three quaternary carbons, including one trisubstituted double bond and two carbonyl signals. The NMR spectra of compound **6** were similar to those of gentiocrucine [21,22], and the only difference was that the animo group in gentiocrucine was changed to a

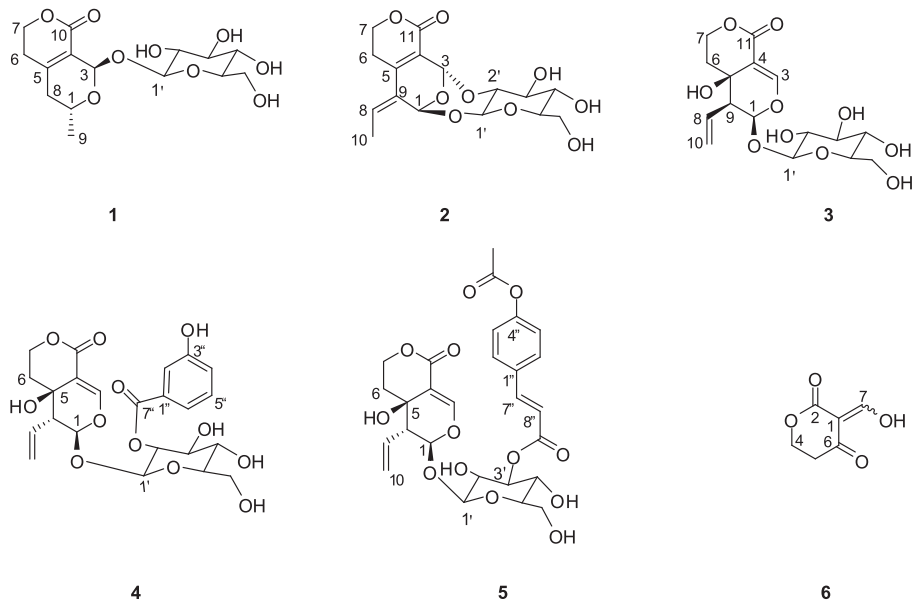

Fig. 1. Structures of compounds 1–6.

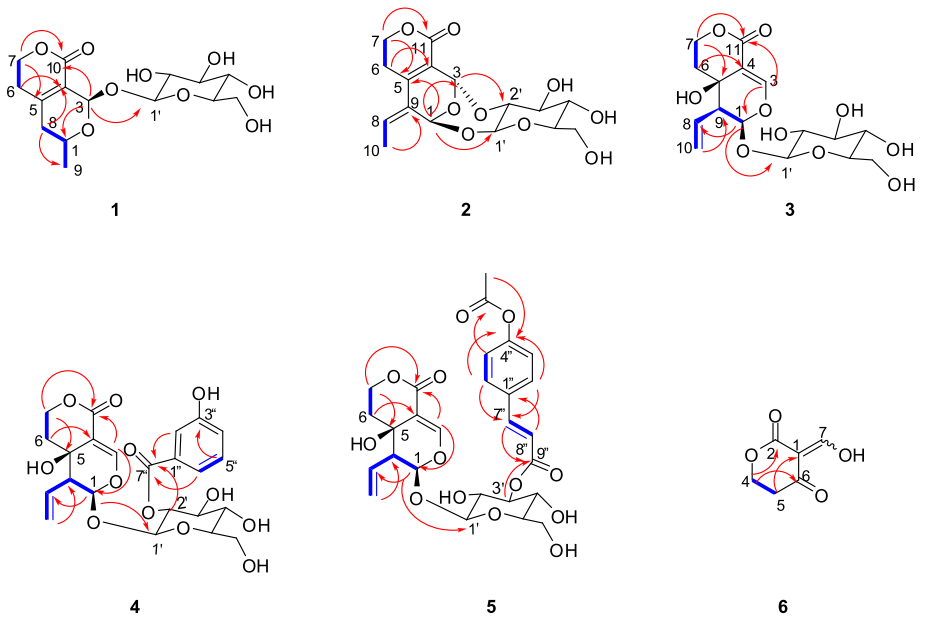

Fig. 2. Selected HMBC and COSY correlations of compounds 1–6.

hydroxyl group in compound **6** which was supported by the HRESIMS and HMBC spectrum. The correlations of δ_H 9.04 (H-7, s) to δ_C 104.4 (C-1, s) in HMBC confirmed the position of alkenyl. From the above evidence, the structure of compound **6** was characterized as shown in Fig. 1.

The other three known compounds were identified as swertiaside (**7**) [23], swertianoside C (**8**) [20] and decentapicrin B (**9**) [24] by comparing their spectroscopic data with the literatures.

Compounds **1**–**9** were evaluated for their anti-HBV activity on HepG 2.2.15 cell line *in vitro*, namely inhibiting the secretions of HBsAg, and HBeAg and HBV DNA replication. As shown in Table 3, compounds **1**, **3**, and **5**–**8** showed moderate activity against HBsAg with IC_{50} values in the range of 0.24–2.46 mM, and compounds **1**, **3**, **7**, and **8** could inhibit HBV DNA replication with IC50 values of 0.30–0.62 mM. Compound **7** exhibited the most promising activity against HBV DNA replication with an IC_{50} value of 0.05 mM (SI = 29.1), as well as moderate activity against the HBsAg secretion (IC_{50} = 0.79 mM).

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Supporting Information

1D and 2D NMR, HRESIMS, IR, UV and $[\alpha]_D$ spectra of compounds **1–6,** and structures of known compounds (**7**–**9**).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2015.02.009.

Table 3
Anti-HBV activities of compounds **1–9**.

Compounds	CC_{50} [mM]	HBsAg		HBeAg		HBV DNA	
		IC_{50} [mM]	SI	IC_{50} [mM]	SI	IC_{50} [mM]	SI
1	1.3	0.32	4.1	2.89	–	0.46	2.8
2	>3.0	>3.00	–	3.00	–	>0.75	–
3	>2.7	1.06	>2.5	2.67	–	0.62	>4.3
4	>2.1	>2.08	–	2.08	–	>0.52	–
5	1.5	0.56	2.8	1.89	–	>0.47	–
6	3.9	2.46	1.6	6.99	0.6	>1.83	–
7	1.6	0.79	2.0	2.14	–	0.05	29.1
8	0.4	0.24	1.8	2.05	–	0.30	1.5
9	>2.2	>2.20	–	2.20	–	>0.55	–
Tenofovir	>1.2	>1.2	–	>1.2	–	0.0023	>521.7

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

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