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Swerilactones E–G, three unusual lactones from *Swertia mileensis*

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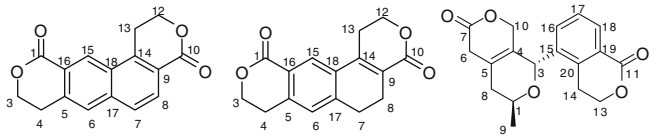
ABSTRACT

Swerilactones E (**1**), F (**2**), and G (**3**), three unusual lactones with a phenyl group, were isolated from the traditional Chinese herb of *Swertia mileensis*. Their structures were determined based on extensive spectroscopic analysis and X-ray single crystal crystallography. Our anti-HBV assay on the Hep G 2.2.15 cell line in vitro showed that compounds **1** and **2** exhibited significant inhibitory activities against the secretion of HBsAg with IC_{50} of 0.22 and 0.70 mM and HBeAg with IC_{50} of 0.52 and >6.78 mM, respectively.

Swertia mileensis (= *Swertia leducii*, namely 'Qing-Ye-Dan' in Chinese), belonging to the *Swertia* genus of the family *Gentianaceae*, is mainly distributed in Mile and Kaiyuan counties, Honghe prefecture, Yunnan, China.[1] It has been documented in *Chinese Pharmacopoeia* (1977–2005 editions) as a traditional Chinese medicine (TCM) to treat viral hepatitis.[2] The previous phytochemical investigation revealed that the main constituents of *Swertia* species were secoiridoid glucosides, xanthones, flavones, and triterpenoids.[3] However, non-glycosidic secoiridoids have been rarely reported except for two minor lactones erythrocentaurin and swermirin.[4] Our anti-hepatitis B virus (HBV) screening in vitro manifested that the 50% and 90% ethanol extracts of *S. mileensis* possessed high inhibitory activities on the secretion of HBsAg and HBeAg.[5a] In order to clarify the active components, the previous bioassay-guided fractionation has led to the isolation of four novel lactones: swerilactones A–D.[5] As a continuous search for active anti-HBV compounds from natural products, our further investigation yielded other three lactones, swerilactones E (**1**), F (**2**), and G (**3**) which could be classified as secoiridoids. Different from the previously reported swerilactones A–D, swerilactones E–G contained the unusual phenyl group (even a naphthyl group in swerilactone E) which was infrequent in secoiridoids. Herein, we describe the isolation and structure elucidation of swerilactones E–G based on extensive spectroscopic analysis and X-ray single crystal crystallography, together with their anti-HBV activity.

In connection with our previous isolation,[5] the fraction B (8.5 g) was subjected to a silica gel column (100.0 g, 3.0 × 30.0 cm) with a gradient elution of $CHCl_3/Me_2CO$ (90:10→50:50, v/v) to afford four fractions B1–B4. The fraction B1 (1.5 g) was further chromatographed on silica gel column (30.0 g, 1.7 × 25.0 cm) eluted with $CHCl_3$/MeOH (90:10→80:20, v/v) to furnish three sub-fractions B1-1 to B1-3. The sub-fraction B1-1 (500 mg) was subsequently purified using silica gel chromatography (15.0 g, 1.5 × 20.0 cm, PE/Me_2CO, 90:10→50:50, v/v) and Sephadex LH-20 (50.0 g, 1.4 × 145.0 cm, MeOH) to yield swerilactones E (**1**, 30 mg) and F (**2**, 22 mg). The sub-fraction B1-2 (300 mg) was purified using silica gel chromatography (15.0 g, 1.5 × 20.0 cm, $CHCl_3/Me_2CO$ = 85:15, v/v), which after recrystallization gave swerilactone G (**3**, 20 mg).



Structures of compounds **1**–**3**.

Swerilactone E (**1**),[6] colorless needles (C_6H_6), exhibited the molecular formula $C_{16}H_{12}O_4$ by positive HRESIMS (*m/z* 269.0807 $[M+H]^+$, calcd for $C_{16}H_{13}O_4$ *m/z* 269.0813), indicating 11 degrees of unsaturation. The IR spectrum of compound **1** showed the absorptions for carbonyl group (1720 cm^{-1}) and aromatic ring (1628, 1577, and 1472 cm^{-1}). The ^1H and ^{13}C NMR displayed 16 carbon resonances due to eight quaternary carbons (including two lactones, six aromatic ones), four methines, and four

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methylenes (including two oxygenated ones). The HSQC spectrum allowed the assignments of all the protons to their bonding carbons. In the ^{1}H NMR spectrum, four down-field-shifted aromatic protons at δ_H 8.88 (1H, s, H-15), 8.20 (1H, d, J = 8.7 Hz, H-8), 7.82 (1H, d, J = 8.7 Hz, H-7), and 7.77 (1H, s, H-6) were observed, together with the ten aromatic carbons (in the range of δ_C 165.0 to 123.1, Table 1) detected in ^{13}C NMR, which led to the deduction of a tetra-substituted naphthalene. In addition, two **1a** units (Fig. 1) were characterized from the ^{1}H NMR [δ_H 4.71 (2H, t, J = 6.0 Hz, H-12), 4.63 (2H, t, J = 5.9 Hz, H-3), 3.28 (2H, t, J = 5.6 Hz, H-4), 3.50 (2H, t, J = 6.1 Hz, H-13)] and ^{13}C NMR [δ_H 165.0 (s), 164.8 (s), 67.5 (t), 28.2 (t), 66.6 (t), 24.2 (t)], which were also supported by ^{1}H–^{1}H COSY (H-3/H-4 and H-12/H-13) and HMBC (H-3/C-1 and H-13/C-10) spectra. The connections among the two **1a** units and naphthyl ring were determined based on the correlations from H-15 to C-1; from H-4 to C-5; from H-8 to C-10, and from H-12 to C-14 in the HMBC spectrum, and the ROESY correlations: H-4 with H-6; H-15 with H-13. Thus, the structure of swerilactone E (**1**) was depicted as shown in Figure 1. Furthermore, an X-ray single crystal diffraction analysis was carried out, which confirmed the structure (Fig. 2).[7]

Swerilactone F (**2**)[8] was isolated as a white powder and had a molecular formula of $C_{16}H_{14}O_4$ by positive HRESIMS (m/z 293.0799 [M+Na]$^+$, calcd for 293.0789), indicating 10 degrees of unsaturation. The IR spectrum displayed absorption bands at 1719, 1700, 1612, 1562, and 1466 cm^{-1}, which were indicative of carbonyl groups and aromatic ring moieties. The ^{13}C NMR spectrum showed 16 carbon signals ascribed to eight quaternary carbons, two methines, and six methylenes. The NMR data of compound **2** were very similar to those of compound **1** (Table 1), except for the following changes: δ_H 7.82 (1H, d, J = 8.7 Hz, H-7), 8.20 (1H, d, J = 8.7 Hz, H-8) in compound **1** up-field shifted to δ_H 2.87 (2H, m, H-7), 2.63 (2H, m, H-8) in compound **2**, and δ_C 127.0 (d, C-7), 128.2 (d, C-8) in compound **1** up-field shifted to δ_C 27.8 (t, C-7), 21.4 (t, C-8) in compound **2**, respectively, which suggested that the double bond between C-7 and C-8 in **1** was hydrogenized in compound **2**. The above deduction could be supported by the detected ROSEY (H-6/H-7), ^{1}H–^{1}H COSY (H-7/H-8) and HMBC (H-7/C-9, 18 and H-8/C-10, 14) correlations. Thus, the structure of compound **2** was assigned as shown in Figure 1.

Swerilactone G (**3**),[9] colorless cubic crystals (MeOH), possessed the molecular formula $C_{18}H_{18}O_5$ from positive HRESIMS (m/z 337.1044 [M+Na]$^+$, calcd for $C_{18}H_{18}O_5Na$ m/z 337.1051), indicating 10 degrees of unsaturation. The IR spectrum suggested the presence of carbonyl groups (1745 and 1713 cm^{-1}) and aromatic rings (1595, 1472, and 1458 cm^{-1}). The ^{13}C (DEPT) NMR spectrum displayed 18 carbon signals containing seven quaternary carbons (two carbonyl groups and five olefinic carbons), five methines (three olefinic carbons and two oxygenated ones), five methylenes (including two oxygenated ones), and one methyl group. In the ^{1}H–^{1}H COSY spectrum, the following correlations H-13/H-14 and H-16/H-17/H-18 were detected, together with the HMBC correlations: from H-13 to C-11 and C-20; from H-14 to C-15 and C-19; from H-18 to C-11 and C-20; and from H-17 to C-15 and C-19, which fully supported the establishment of partial structure **3b**. Considering the ^{1}H–^{1}H COSY correlations: H-8/H-1/H-9 and the HMBC correlations: from H-1 to C-3 and C-5; from H-3 to C-5 and C-10; from H-6 to C-4 and C-8; and from H-10 to C-7, another partial fragment **3a** was constructed. The connection between **3a** and **3b** was deduced at C-3 and C-15 based on the HMBC correlation from H-3 to C-16 and C-20. Thus, the planar structure of compound **3** was established by spectroscopic analyses. However, the ROESY spectrum showed the correlation of neither H-3/H-1 nor H-3/H-9, which could not provide sufficient information to determine the stereochemistry of compound **3**. Therefore, an X-ray single crystal diffraction analysis[10] was conducted, which not only verified the planar structure but also clarified the stereochemistry of compound **3** as shown in Figure 2. According to the IUPAC

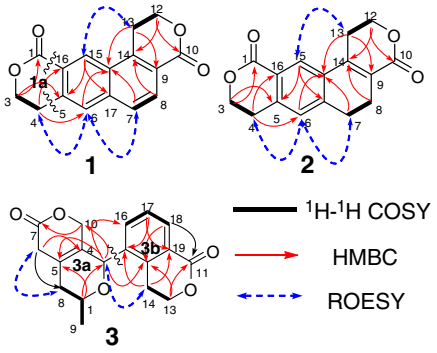

Figure 1. Selected 2D NMR correlations for compounds **1**–**3**.

Table 1
^{1}H and ^{13}C NMR data for swerilacones E (**1**), F (**2**), and G (**3**)[a]

No.	1		2		3	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	–	165.0, s		164.8, s	3.58, m	63.4, d
3	4.63, t, 5.9	67.5, t	4.54, t, 6.0	67.1, t	5.29, s	70.8, d
4	3.28, t, 5.6	28.2, t	3.06, t, 6.0	27.7, t		124.4, s
5	–	137.4, s		141.4, s		128.1, s
6	7.77, s	126.8, d	7.15, s	127.2, d	3.19, bs	34.3, t
7	7.82, d, 8.7	127.0, d	2.87, m, overlapped	27.8, t		168.9, s
8	8.20, d, 8.7	128.2, d	2.63, m	21.4, t	1.84, bs	35.3, t
9	–	137.8, s	–	124.7, s	1.14, d, 6.2	21.0, q
10	–	164.8, s	–	165.6, s	a: 4.78, d, 15.4 b: 4.62, d, 15.4	68.7, t
11	–	–	–	–	–	164.9, s
12	4.71, t, 6.0	66.6, t	4.52, t, 6.0	65.6, t	–	–
13	3.50, t, 6.1	24.2, t	2.91, t, 7.6, overlapped	24.4, t	4.57, m	66.8, t
14	–	140.3, s		144.4, s	a: 3.37, m b: 3.16, m	24.4, t
15	8.88, s	128.9, d	8.03, s	125.9, d	–	134.9, s
16	–	124.2, s	–	124.0, s	7.35, dd, 7.6, 1.4	133.1, d
17	–	137.8, s	–	143.6, s	7.40, t, 7.6	126.8, d
18	–	123.1, s	–	132.0, s	8.13, dd, 7.6, 1.3	130.8, d
19	–	–	–	–	–	126.8, s
20	–	–	–	–	–	140.6, s

[a] Data were recorded in $CDCl_3$, δ in ppm, ^{1}H NMR at 400 MHz, ^{13}C NMR at 100 MHz.

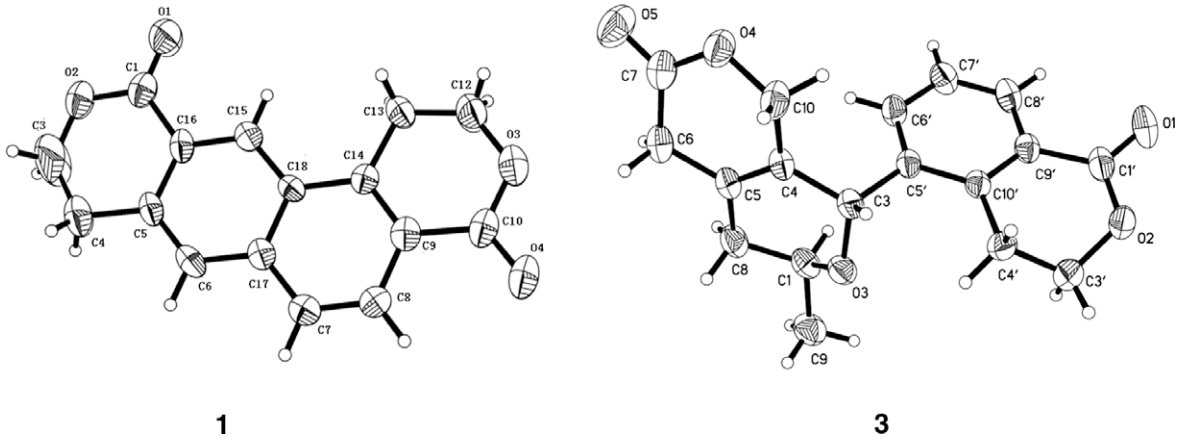

Figure 2. X-ray crystal structures of compounds **1** and **3**.

nomenclature rule, the relative configurations of C-1 and C-3 were both deduced as *S**.

Compounds **1**–**3** were three unusual lactones possessing the characteristic δ-lactone fragment of secoiridoid glycosides, which indicated that compounds **1**–**3** could be ascribed as secoiridoid analogs.[11] Compared to compound **2**, compound **1** had a corresponding but completely unsaturated system, namely a naphthyl ring system which was rare in natural products. Compound **3** could be regarded as a dimer of one dihydroisocoumarin moiety (**3a**) and one secoiridoid moiety (**3b**). To the best of our knowledge, compounds **1**–**2** were the first examples of secoiridoid aglycone with a naphthyl (or dihydronaphthyl) ring, and compound **3** was the first example of secoiridoid aglycone dimer by C–C connectivity.

Compounds **1**–**3** were tested for their anti-HBV activity in vitro on the HBV-transfected Hep G 2.2.15 cell line as reported previously,[12] (3TC, lamivudine was used as the positive control[13]). Compound **1** showed significant anti-HBV activity against the secretion of HBsAg with IC_{50} of 0.22 mM (SI = 9.84) and HBeAg with IC_{50} of 0.52 mM (SI = 4.16), which was more potent than its dihydrogenized derivative compound **2** [$IC_{50HBsAg}$ = 0.70 mM, SI_{HBsAg} = 2.11; $IC_{50HBeAg}$ > 6.78 mM, SI_{HBeAg} < 1], however, compound **3** showed no anti-HBV activity at the tested (highest) concentration of 3.22 mM.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.02.156.

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6. *Swerilactone E* (**1**): colorless needles (C_6H_6), mp 232–233 °C; UV (MeOH) λ_{max} (log ε) 217 (4.23), 257 (4.62), 296 (3.81), 349 (3.35) nm; IR (KBr) ν_{max} 2904, 1720, 1628, 1577, 1472, 1423, 1400, 1317, 1259, 1246, 1099, 1030, 779, 744 cm^{-1}; NMR data found in Table 1; EIMS *m*/*z* 268 (100, M^+), 238 (79), 210 (67), 180 (23), 152 (55); HR-ESIMS *m*/*z* found 269.0807 $[M+H]^+$ ($C_{16}H_{13}O_4$ calcd 269.0813).
7. Crystallographic data for compound **1**, provided in Supplementary data (Deposition number: CCDC 738731), can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.htm. (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, U.K.; fax: (+44) 1223-336-033; or desposit@ccdc.cam.ac.uk).
8. *Swerilactone F* (**2**): white powder, mp 207–208 °C; UV (MeOH) λ_{max} (log ε) 250 (4.32), 298 (3.94) nm; IR (KBr) ν_{max} 2958, 2904, 1719, 1700, 1625, 1612, 1562, 1466, 1426, 1409, 1298, 1247, 1212, 1196, 1129, 1096, 767 cm^{-1}; NMR data found in Table 1; EIMS *m*/*z* 270 (100, M^+), 251 (27), 225 (83), 181 (73), 165 (63), 152 (78); HR-ESIMS *m*/*z* 293.0799 $[M+Na]^+$ ($C_{16}H_{14}O_4Na$ calcd 293.0789).
9. *Swerilactone G* (**3**): colorless cubic crystals (MeOH), mp 178–179 °C; $[\alpha]_D^{28.0}$ −8.64 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 205 (4.59), 235 (4.01), 283 (3.34) nm. IR (KBr) ν_{max}: 2972, 2922, 2887, 1745, 1713, 1595, 1472, 1458, 1417, 1403, 1302, 1236, 1122, 1014, 835, 758 cm^{-1}. EIMS *m*/*z* 314 (M^+, 26), 272 (73), 256 (75), 227 (25), 175 (100), 167 (37), 153 (32). HRESIMS *m*/*z* 337.1044 $[M+Na]^+$ ($C_{18}H_{18}O_5Na$ calcd for 337.1051).
10. Crystallographic data for compound **3**, provided in Supplementary data (Deposition number: CCDC 737918), can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.htm. (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, U.K.; fax: (+44) 1223-336-033; or desposit@ccdc.cam.ac.uk).
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13. 3TC (lamivudine, an antiviral agent) was used as the positive control in our anti-HBV screening and showed inhibitory activity against HBsAg secretion (IC_{50} = 15.3 mM, SI = 1.9) and HBeAg secretion (IC_{50} = 36.1 mM, SI < 1).