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Swerilactones C and D, anti-HBV New Lactones from a Traditional Chinese Herb: *Swertia mileensis*

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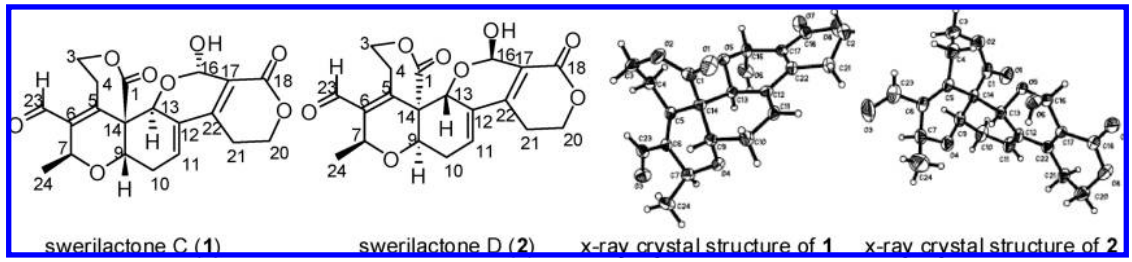
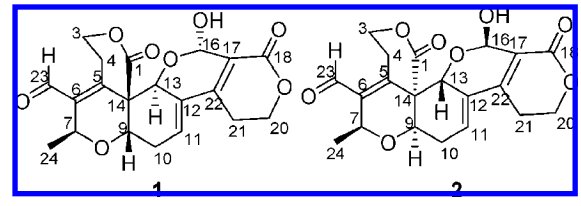
ABSTRACT



Swerilactones C (1) and D (2), two novel diastereomeric lactones with an unprecedented 6/6/6/6/6 pentacyclic ring system, were isolated from the traditional Chinese herb *Swertia mileensis*. Their structures and relative stereochemistry were elucidated on the basis of spectroscopic methods and further confirmed by X-ray single-crystal diffraction analysis. In vitro antihepatitis B virus (HBV) assay on the Hep G 2.2.15 cell line showed that both compounds 1 and 2 exhibited inhibitory activities against the secretion of HBsAg (IC_{50} = 1.24 and 2.96 mM, respectively) and HBeAg (IC_{50} = 0.77 and 1.47 mM, respectively).

As reported in our previous paper, *Swertia mileensis* (= *Swertia leducii*, generally known as "Qing-Ye-Dan" in Chinese) was discovered from the Yi and Hani regions and has been documented in *Chinese Pharmacopoeia* (1977−2005 editions) as a traditional Chinese medicine (TCM) to treat viral hepatitis. Primary anti-HBV assay in vitro showed the 90% and 50% EtOH extracts of the title plant possessed inhibitory activities on HBsAg and HBeAg. In a continuous search for active compounds, our further phytochemical investigation on this plant resulted in the isolation of another pair of novel diastereomeric lactones swerilactones C (**1**) and D (**2**), which were structurally different from the previously reported swerilactones A and B.[1] This paper describes the isolation and structural elucidation of compounds **1** and **2** by extensive spectroscopic and single-crystal X-ray crystallographic analyses, as well as their anti-HBV activities.



The previously isolated fraction A4 (10.2 g)[1] was chromatographed on a silica gel column (150.0 g, 4.0 × 25.0

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Compounds **1** and **2** in Pyridine-d_5 (δ in ppm,J in Hz)

position	**1** δ_H	**1** δ_C	**2** δ_H	**2** δ_C
1		167.2, s		167.2, s
3	4.68 (1H, m)	67.8, t	4.75 (1H, m)	67.4, t
	4.32 (1H, m)		4.28 (1H, m)	
4	3.69 (1H, m)	25.0, t	3.61 (1H, m)	25.4, t
	3.63 (1H, m)		3.55 (1H, m)	
5		154.0, s		153.4, s
6		136.0, s		136.3, s
7	4.93 (1H, q, 6.6)	69.0, d	4.85 (1H, q, 6.3)	71.6, d
9	4.50 (1H, d, 4.3)	66.4, d	3.99 (1H, d, 4.0)	72.1, d
10	3.44 (1H, dd, 20.2, 4.1)	33.4, t	3.38 (1H, dd, 20.1, 4.0)	33.2, t
	2.53 (1H, dd, 20.2, 4.1)		2.58 (1H, dd, 20.1, 4.0)	
11	6.33 (1H, bs)	130.8, d	6.31 (1H, bs)	130.9, d
12		127.9, s		127.8, s
13	6.54 (1H, s)	71.5, d	6.62 (1H, s)	71.8, d
14		50.5, s		51.1, s
16	6.46 (1H, d, 3.8)	88.3, d	6.46 (1H, s)	88.2, d
17		123.0, s		122.9, s
18		164.0, s		164.0, s
20	4.24 (1H, m)	65.6, t	4.22 (1H, m)	65.6, t
	4.14 (1H, m)		4.07 (1H, m)	
21	2.49 (1H, m)	23.2, t	2.48 (1H, m)	23.0, t
	2.32 (1H, m)		2.28 (1H, m)	
22		144.8, s		144.7, s
23	10.26 (1H, s)	189.5, d	10.26 (1H, s)	190.5, d
24	1.50 (3H, d, 6.6)	19.2, q	1.43 (3H, d, 6.4)	21.0, q

cm) eluting with $CHCl_3$/Me_2CO (90:10 → 30:70) to supply subfractions 1−5. The subfraction 2 (1.5 g) was chromatographed on silica gel column (30.0 g, 2.0 × 23.0 cm) with an eluent of CH_3Cl/MeOH (95:5), and further purified with RP-18 column chromatography (120.0 g, 2.8 × 28.0 cm, eluted with MeOH/H_2O, 80:20) to afford swertilactone C (**1**) (30 mg) and swertilactone D (**2**) (10 mg).

Swerilactone C (**1**)[2] was isolated as colorless cubic crystals (Me_2CO), $[\alpha]^{28.2}_D$ +14.66 (*c* 0.20, $CHCl_3$/MeOH v/v = 3:1). Its molecular formula was determined to be $C_{20}H_{20}O_8$ on the basis of (−) HRESIMS (calcd for $[M + Cl]^-$ *m/z* 423.0846, found 423.0843) with 11 degrees of unsaturation. The IR spectrum of compound **1** exhibited OH (3455 cm^{-1}), C═O (1717 cm^{-1}), and double bond (1644 cm^{-1}), respectively. The ^{1}H and ^{13}C NMR spectra of compound **1** (Table 1) showed 20 carbon resonances due to eight quaternary carbons, six tertiary carbons, five methylenes, and one methyl group. Among them, an aldehyde group (δ_C 189.5; δ_H 10.26, s), two lactone carbonyl carbons (δ_C 167.2 and 164.0), six olefinic carbons (δ_C 154.0, 144.8, 136.0, 130.8, 127.9, 123.0), and one dioxygenated carbon (δ_C 88.3; δ_H 6.46, d, J = 3.8 Hz) were deduced. Accordingly, a pentacyclic ring structure was required for compound **1** to fulfill the unsaturation requirement.

(2) Compound **1**: mp 255−256 °C; $[\alpha]^{28.2}_D$ +14.66 (*c* 0.20, $CHCl_3$/MeOH v/v = 3:1); UV (MeOH) λ_{max} (log ε) 377 (3.14), 256 (4.32) nm; IR (KBr) λ'_{max}: 3455, 1717, 1644, 1434, 1394, 1355, 1176, 1089, 1062, 1031, 983, 808 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m/z* 388 ($[M]^+$, 1), 370 (41), 253 (23), 195 (100), 177 (72), 165 (47), 149 (59); ESIMS (−) *m/z* 423 ($[M + Cl]^-$); HRESIMS (−) *m/z* 423.0843 $[M + Cl]^-$ ($C_{20}H_{20}O_8Cl$ calcd 423.0846).

The partial structure **1a** (Figure 1) was established by ^{1}H−^{1}H COSY (H-24/H-7; H-3/H-4) and HMBC (H-23/C-6, C-7; H-7/C-6, C-5 and H-3/C-5, C-1). Similarly, the fragment **1b** [^{1}H−^{1}H COSY (H-9/H-10/ H-11), HMBC (H-10/C-12; H-13/C-11)] and **1c** [^{1}H−^{1}H COSY (H-20/H-21), HMBC (H-16/C-17, C-18, C-22); H-20/C-18, C-22; H-21/C-17] were also determined, respectively. In addition, the linkages of C(7)−O−C(9) and C(13)−O−C(16) were deduced by the detection of the HMBC correlations from H-7 to C-9 and from H-16 to C-13. The other correlations in the HMBC spectrum of compound **1** were revealed as follows: H-13 (δ_H 6.54, s) with C-1 and C-14; H-9 (δ_H 4.50, d, J = 4.3 Hz) with C-1, C-5, C-11, and C-13, which led to the

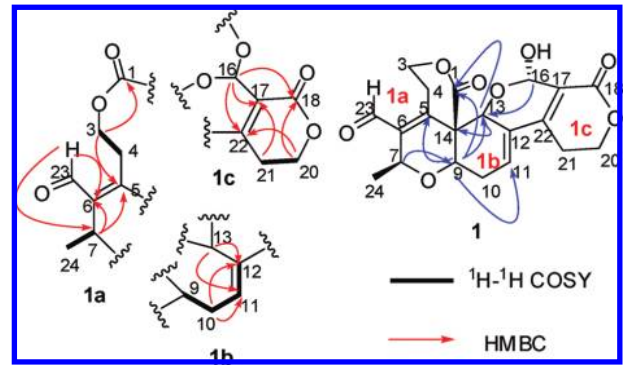

Figure 1. Fragment structures and key COSY and HMBC correlations of compound **1**.

connection of C-1, C-5, C-9, and C-13 with C-14. Thus, the planar structure of compound **1** was determined as shown in Figure 1.

In the ROESY spectrum (Figure 2), the correlations of H-24/H-9, H-11/H-21, and H-23/H-4 were observed. Nevertheless, the above-mentioned evidence could not provide sufficient information to confirm the stereochemistry of compound **1**. Thus, a single X-ray crystallographic analysis was conducted, which not only verified the deduced planar structure of compound **1**, but also determined its relative stereochemistry as shown in Figure 3.[3] Based on the IUPAC nomenclature rule the relative stereocenters of C-7, 9, 13, 14, 16 were deduced as *S**,*S**,*S**,*R**,*S**, respectively.

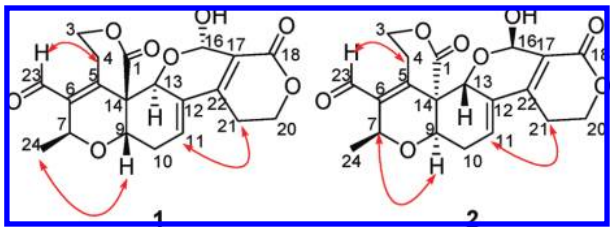

Figure 2. Selected ROESY correlations of **1** and **2**.

Swerilactone D (**2**)[4] had the same molecular formula as that of swerilactone C (**1**) deduced from its (−) HRESIMS [calcd for $[M - H]^-$ *m*/*z* 387.1079, found 387.1079]. Its IR spectrum displayed absorptions of OH (3334 cm^{-1}), C═O (1727, 1701 cm^{-1}), and double bond (1637 cm^{-1}). Detailed comparison of their NMR data indicated that they were a pair of isomers with the main difference of the downfield shift of C-9 from δ_C 66.4 (d) in compound **1** to δ_C 72.1 (d) in compound **2** and the upfield shift of H-9 from δ_H 4.50 (1H, d, J = 4.3 Hz) in compound **1** to δ_H 3.99 (1H, d, J = 4.0 Hz) in compound **2**. With the HMBC spectrum, the planar structure of compound **2** was determined to be identical with that of compound **1**. In its ROESY spectrum (Figure 3), the correlations H-9/H-7, H-11/H-21, H-23/H-4 were observed, instead of the corelation H-9/H-24 observed in compound **1**, and the detected correlation H-9/H-7 indicated the different stereochemistry between their structures. With the above deduction, it is still insufficient to conform the stereochemistry of compound **2**. Therefore, a single X-ray diffraction study was performed and its relative stereochemistry was determined to be 7*S**,9*R**,13*R**, 14*S**,16*R** (Figure 3).[5]

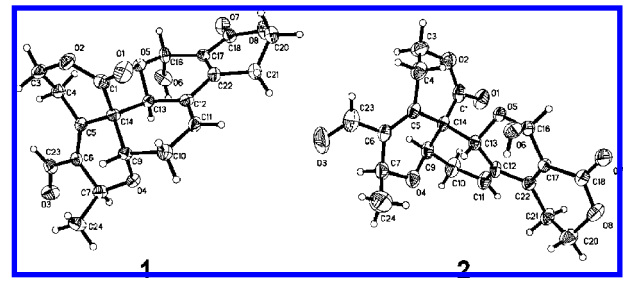

Figure 3. X-ray crystal structures of compounds **1** and **2**.

Swerilactones C (**1**) and D (**2**) were another pair of novel diastereomeric lactones isolated from *S. mileensis*. Compared to swerilactones A and B,[1] swerilactones C (**1**) and D (**2**) also possessed a 6/6/6/6/6 pentacyclic ring system skeleton, but there was little similarity between their chemical structures. Interestingly, swerilactones A−D contained a δ-lactone fragment similar to those of the aglycon part of secoiridoidal glycosides, a family of compounds widely presented in Gentianaceae.[6−8] *S. mileensis* belonging to the *Swertia* genus (Gentianaceae) was reported as being rich in secoiridoids (iridoids).[9,10] Thus, we presumed that swerilactones A−D would be biogenetically generated from secoiridoids. However, this specific biogenetic pathway is still unclear and needs further investigation. Compounds **1** and **2** were tested for their anti-HBV activities in vitro on the HBV-transfected Hep G 2.2.15 cell line as reported previously.[11,12] Both swerilacones **C** and **D** exhibited inhibitory activities against the secretion of HBsAg (IC_{50} = 1.24 and

(3) Crystallographic data of compound **1**: $C_{20}H_{20}O_8$, MW = 388.36; monoclinic, space group $p2_1$; a = 6.547(2) Å, b = 10.892(3) Å, c = 12.418(4) Å, α = 90.00, β = 103.542(4), γ = 90.00, V = 860.9(5) $Å^3$, Z = 2, d = 1.498 g/cm^3, crystal dimensions 0.16 × 0.12 × 0.08 nm^3 were used for measurement on a SHELXL-97 with a graphite monochromater, Mo Kα radiation. The total number of reflections measured was 3852, of which 1635 were observed, $I > 2\sigma(I)$. Final indices: R_1 = 0.0681, wR_2 = 0.1487. The crystal structure of compound **1** was solved by direct method SHLXS-97 (Sheldrick, 1990) and expanded using the difference Fourier technique, refined by the program SHLXL-97 (Sheldrick, 1997), and the full-matrix least-squares calculations. Crystallographic data for the structure of compound **1** have been deposited with the Cambridge Crystallographic Data Centre (deposition no. CCDC 732831). Copies of these data 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).

(4) Compound **2**: mp 240−241 °C; $[\alpha]^{28.9}_D$ −12.90 (*c* 0.22, $CHCl_3$/MeOH v/v = 1:1); UV (MeOH) λ_{max} (log ε) 262 (4.23) nm; IR (KBr) λ'_{max} 3334, 1727, 1701, 1660, 1637, 1449, 1407, 1316, 1177, 1090, 1067, 1030, 982, 809 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS (−) *m*/*z* 387 ($[M - H]^-$); (−) HRESIMS *m*/*z* 387.1079 $[M - H]^-$ ($C_{20}H_{19}O_8$ calcd 387.1079).

(5) Crystallographic data of compound **2**: $C_{20}H_{20}O_8$ (H_2O), MW = 406.38; triclinic, space group $p1$; a = 8.6844(14) Å, b = 14.094(2) Å, c = 17.000(3) Å, α = 73.293(2), β = 87.688(2), γ = 72.958(2), V = 1903.2(5) $Å^3$, Z = 4, d = 1.418 g/cm^3, crystal dimensions 0.20 × 0.14 × 0.08 nm^3 were used for measurement on a SHELXL-97 with a graphite monochromater, Mo Kα radiation. The total number of reflections measured was 8035, of which 3235 were observed, $I > 2\sigma(I)$. Final indices: R_1 = 0.0409, wR_2 = 0.1000. The crystal structure of compound **2** was solved by direct method SHLXS-97 (Sheldrick, 1990) and expanded using the difference Fourier technique, refined by the program SHLXL-97 (Sheldrick, 1997), and the full-matrix least-squares calculations. Crystallographic data for the structure of compound **2** have been deposited with the Cambridge Crystallographic Data Centre (deposition no. CCDC 737797). Copies of these data 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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2.96 mM, respectively) and HBeAg (IC_{50} = 0.77 and 1.47 mM, respectively.[13]

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Supporting Information Available: 1D and 2D NMR, $[\alpha]_D$, UV, IR, MS spectra, and X-ray crystallographic data (CIF) of swerilactones C (**1**) and D (**2**) and experimental procedures. This material is available free of charge via Internet at http://pubs.acs.org.

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(13) 3TC (lamivudine, an antiviral agent) was used as the positive control in our anti-HBV screening. 3TC showed inhibitory activity against HBsAg secretion (IC_{50} = 20.1 mM, SI = 1.3) and against HBeAg secretion (IC_{50} = 30.5 mM, SI < 1).