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Swerilactones A and B, Anti-HBV New Lactones from a Tradtional Chinese Herb: *Swertia mileensis* as a Treatment for Viral Hepatitis

Chang-An Geng,[†,‡] Zhi-Yong Jiang,[†] Yun-Bao Ma,[†] Jie Luo,[†] Xue-Mei Zhang,[†] Hong-Ling Wang,[†,‡] Yong Shen,[†,‡] Ai-Xue Zuo,[†,‡] Jun Zhou,[†] and Ji-Jun Chen*[,†]

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, PRC, and Graduate School of Chinese Academy of Sciences, Beijing 100039, PRC

chenjj@mail.kib.ac.cn

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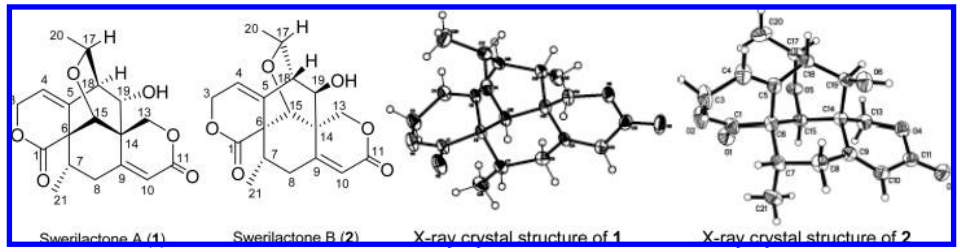
ABSTRACT



Swerilactones A (1) and B (2), two novel lactones with an unprecedented 6/6/6/6/6 pentacyclic ring system, were isolated from the traditional Chinese herb of *Swertia mileensis* with activity against the hepatitis virus. Their structures and relative stereochemistry were elucidated based on spectroscopic methods and further confirmed by X-ray single crystal diffraction analysis. In vitro antihepatitis B virus (HBV) assay on Hep G 2.2.15 cell line showed that compound 1 inhibited HBsAg and HBeAg secretion with IC_{50} values of 3.66 and 3.58 mM, respectively.

HepatitisB virus (HBV) infection is a major global public health problem, especially in Asia. Approximately 350 million people are chronic carriers of HBV worldwide, although effective vaccines against HBV infection have been available since 1982.[1] As the 10th leading cause of death, HBV infection accounts for 500000 to 1.2 million deaths per year.[2] Interferon α and nucleoside analogues are the two major treatment options available for treatment of chronic HBV infection but they are far from satisfactory.[3−5] Therefore, novel compounds with unique modes of mechanism are urgently needed.

Swertia mileensis (= *Swertia leducii*), traditionally known as "Qing-Ye-Dan" in Chinese, belongs to the *Swertia* genus of the family Gentianaceae.[6] It has long been used as a traditional Chinese medicine (TCM) to treat viral hepatitis in Yi and Hani minority regions in Mile and Kaiyuan counties, Yunnan province. In the 1970s, a series of phytochemical, pharmacological, and toxic principles investigation were conducted, which prompted *S. mileensis* to be

* To whom correspondence should be addressed. Phone: (86) 871-5223265. Fax: (86) 871-5223265.
† Kunming Institute of Botany.
‡ Graduate School of Chinese Academy of Sciences.

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1**[a] and **2**[b] in Pyridine-d_5 (δ in ppm,J in Hz)

no.	**1** δ_H	**1** δ_C	**2** δ_H	**2** δ_C
1		172.5, s		170.5, s
3α	4.82, dd, 16.7, 6.2	69.2, t	5.33, dd, 16.0, 2.0	67.6, d
3β	5.09, dd, 17.5, 1.4		4.03, dd, 16.0, 6.2	
4	6.24, d, 5.0	127.3, d	6.18, dd, 6.2, 2.5	124.8, d
5		133.8, s		139.0, s
6		55.2, s		53.8, s
7	2.28, m	41.3, d	2.69, m	30.7, d
8α	3.02, td, 14.2, 1.8	38.3, t	3.41, ddd, 24.6, 6.0, 2.0	37.3, t
8β	2.22, dd, 11.7, 4.8		2.21, dd, 15.3, 1.7	
9		161.3, s		160.8, s
10	6.09, d, 1.7	115.8, d	6.16, d, 2.0	118.2, d
11		165.0, s		164.7, s
13a	4.55, d, 11.5	75.9, t	4.63, d, 11.0	75.8, t
13b	4.63, d, 11.5		4.70, d, 11.2	
14		46.4, s		47.3, s
15	4.33, s	77.0, d	4.53, s	71.5, d
17	4.10, q, 6.0	73.4, d	4.13, q, 6.0	73.1, d
18	2.70, bs	50.6, d	2.69, t, 1.2	49.2, d
19	4.60, bs	72.6, d	4.63, t, 2.8	73.7, d
20	1.00, d, 6.0	20.4, q	1.03, d, 6.3	20.7, q
21	1.08, d, 6.5	17.9, q	1.05, d, 7.2	17.4, q
OH	7.99, bs		8.07, d, 5.0	

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

documented in *Chinese Pharmacopoeia* (1977−2005 editions) as a new TCM source.[7] Previous phytochemical and pharmacological studies on this plant revealed that secoiridoid glucosides, xanthones, flavones, and triterpenoids were the main components,[8−13] of which sweroside, swertisin, and oleanolic acid could reduce the alanine transaminase (ALT) and aspartate transaminase (AST) levels.[14−16] Presently, Qing-Ye-Dan tablets prepared from its water extract have been used to cure acute viral hepatitis with high ALT and AST levels resulting in a curing rate up to 95.3% for 422 patients and 96.8% for 93 patients clinically.[17,18] Our in vitro anti-HBV screening manifested that the 50% and 90% ethanol extracts of *S. mileensis* showed higher inhibitory activities on the HBsAg secretion with 50% inhibitory concentration (IC_{50}) of 0.61 mg/mL [selective index (SI) = 7.6], 0.33 mg/mL (SI > 13.8), and on the HBeAg secretion with IC_{50} of 1.06 mg/mL (SI = 4.4), 0.55 mg/mL (SI > 8.3), respectively, and the H_2O extract showed lower inhibitory activities on the HBsAg secretion (IC_{50} = 1.43 mg/mL, SI = 3.6) and HBeAg secretion (IC_{50} = 2.98 mg/mL, SI = 1.7).[19] As part of our continuous investigation of active anti-HBV leads from natural sources,[20−23] the 50% and 90% EtOH extracts were investigated to yield novel compounds **1** and **2**. 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 whole plant of *S. mileensis* was collected in Mile county, Yunnan province, China, on November 6, 2008, and was identified as *Swertia mileensis* T. N. Ho et W. L. Shi by Prof. Dr. Li-Gong Lei, Kunming Institute of Botany, Chinese Academy of Sciences (voucher No. 2008-11-01). The air-dried and powdered whole plant (5.0 kg) was extracted with 90% and 50% EtOH under reflux successively (each time 2 h, 15 L × 2 times). All the extracts were combined and concentrated under vacuum to give a residue (1.3 kg). The residue was suspended in water and extracted with petroleum ether (1 L × 2), ethyl acetate (1 L × 3) and *n*-butanol (1 L × 3) successively. The organic solvents were evaporated under reduced pressure to provide extracts of

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petroleum ether (51.0 g), ethyl acetate (170.5 g) and *n*-butanol (120.5 g). The ethyl acetate part (170.5 g) was chromatographed on silica gel column (2.0 kg, 11.0 × 50.0 cm) eluted with $CHCl_3$−MeOH (from 100:0 to 0:100, v/v) to furnish 10 fractions A−J. The fraction A (29.0 g) was subjected to a silica gel column (300.0 g, 5.0 × 24.0 cm) with an eluent of $CHCl_3$/Me_2CO (0:100 → 50:50) to afford fractions A1−A5. The fraction A3 (8.5 g) was performed on a silica gel column (100.0 g, 4.0 × 19.0 cm) eluting with petroleum ether/acetone (90:10→50:50) to supply subfractions 1−8. The subfraction 3 (1.0 g) was further subjected to RP-8 column (120.0 g, 2.8 × 33.0 cm) with MeOH−H_2O (10:90) to obtain compounds **1** (30 mg) and **2** (26 mg).

Swerilactone A (**1**) was obtained as colorless cubic crystals (Me_2CO).[24] Its molecular formula was determined as $C_{18}H_{20}O_6$ by the positive HRESIMS at *m/z* 333.1332 $[M + H]^+$ (calcd 333.1338), indicating 9 degrees of unsaturation. The IR spectrum of compound **1** showed the absorptions for hydroxyl group (3402 cm^{-1}), double bond (1637 cm^{-1}), and δ-lactone (1713 cm^{-1}).[25] The ^{13}C NMR exhibited 18 carbon resonances due to two methyls, three methylenes, seven methines, and six quaternary carbons (including two lactone ones and two olefinic ones). The ^1H NMR spectrum displayed two olefinic protons at δ_H 6.24 (1H, d, J = 5.0 Hz, H-4), 6.09 (1H, d, J = 1.7 Hz, H-10) (Table 1).

The partial structure of one δ-lactone unit **1a** (Figure 1) was established by HMBC experiment (H-13/C-9, C-11; H-10/C-14). Similarly, the correlations in $^1H-^1H$ COSY (H-3/H-4) and HMBC spectra (H-3/C-1, C-5; H-4/C-6 and H-15/C-1, C-5, C-14, C-13) clarified another δ-lactone unit **1b** connected with **1a** through C-15 (Figure 1). Furthermore, the correlative signals in $^1H-^1H$ COSY (H-21/H-7; H-7/H-8; H-20/H-17; H-18/H-19; and H-19/OH-19) and HMBC (H-20/C-18; H-17/C-19) revealed the presence of other two fragments: **1c** (C-21/C-7/C-8) and **1d** (C-20/C-17/ C-18/C-19/OH).

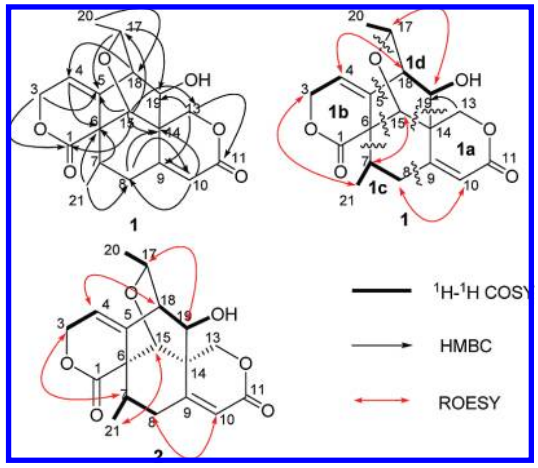

Figure 1. Selected 2D NMR correlations of **1** and **2**.

In the HMBC spectrum, the correlations from H-21 and H-7 to C-6 and from H-8 to C-10, C-14 indicated the linkages of C-6/C-7 and C-8/C-9. Meanwhile, the cross-peaks of H-18/C-4 and H-13/C-19 displayed the linkages of C-5/C-18 and C-14/C-19. Considering its molecular formula and unsaturation degrees, an ether bond between C-17 and C-15 was deduced, which was supported by the correlation signal H-15/C-17 in HMBC. Thus, the planar structure of compound **1** was elucidated as shown in Figure 1.

The correlations in the ROESY spectrum (Figure 1) were detected as follows: H-3β/H-21, H-4/H-18, H-17/H-19, H-7/H-15, and H-8/H-10, which could not provide sufficient information to establish the stereochemistry of compound **1**. Therefore, a single X-ray crystallographic analysis was conducted, which not only verified the planar structure but also clarified the stereochemistry of compound **1** as shown in Figure 2.[26] According to the IUPAC nomenclature rule,

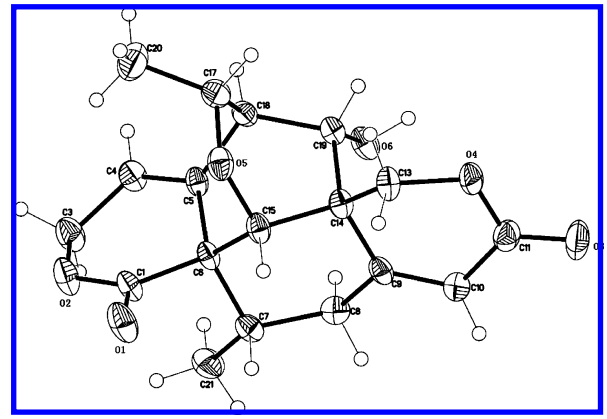

Figure 2. X-ray crystal structure of swerilactone A (**1**).

the relative stereocenters of C-6, 7, 14, 15, 17, 18, 19 were deduced as *S**,*S**,*R**,*R**,*R**,*S**,*S**, respectively.

Swerilactone B (**2**)[27] possessed the same molecular formula $C_{18}H_{20}O_6$ as that of compound **1** by positive HRESIMS at *m/z* 355.1155 $[M + Na]^+$ (calcd 355.1157).

(24) Compound **1**: mp 254−255 °C; $[\alpha]^{29.4}_D$ −9.95 (*c* 0.22, $CHCl_3$/MeOH v/v = 1:1); UV (MeOH) λ_{max} (log ε): 228 (4.15) nm; IR (KBr) λ_{max} 3402, 1713, 1637, 1472, 1451, 1399, 1372, 1303, 1231, 1142, 1039, 867 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 332 (14, M^+), 288 (83), 260 (95), 243 (60), 213 (100); HRESIMS (+) *m/z* 333.1332 $[M + H]^+$ ($C_{18}H_{21}O_6$ calcd 333.1338).

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(26) Crystallographic data of compound **1**: $C_{18}H_{20}O_6$, MW = 332.34; monoclinic, space group $p2_1$; a = 9.1170(18) Å, b = 8.6280(17) Å, c = 19.717(4) Å, α = 90.00, ν = 95.16(3), β = 90.00, V = 1544.7(5) $Å^3$, Z = 4, d = 1.429 g/cm^3, crystal dimensions 0.20 × 0.10 × 0.05 nm^3 were used for measurement on a SHELXL-97 with a graphite monochromater, Mo Kα radiation. The total number of reflections measured was 2795, of which 1468, were observed, $I > 2\sigma(I)$. Final indices: R_1= 0.0412, wR_2 = 0.0912. The crystal structure of compound **1** was solved by direct method SHLXS-97 (Sheldrick, 1990) and expanded using difference Fourier technique, refined by the program SHLXL-97 (Sheldrick, 1997) and the full-matrix least-squares calculations. Crystallographic data for compound **1** have been deposited in 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 deposit@ccdc.cam.ac.uk).

Its UV and IR spectra were similar to those of compound **1**. Detailed comparison of their NMR data indicated that they were a pair of isomers, with the only difference assigned to 4, 5, 7, and 15 positions (Table 1). The HMBC spectrum suggested that its planner structure was indentical to that of compound **1**. In the ROESY spectrum, the following correlations [H-7/H-3β, H-21/H-15] were detected, instead of the correlations [H-21/H-3β, H-7/H-15] in compound **1**. To confirm the stereochemistry of **2**, a single-crystal X-ray diffraction analysis was performed and revealed its relative stereochemistry to be 6*R**,7*S**,14*S**,15*S**, 17*S**,18*R**,19*R** (Figure 3).[28]

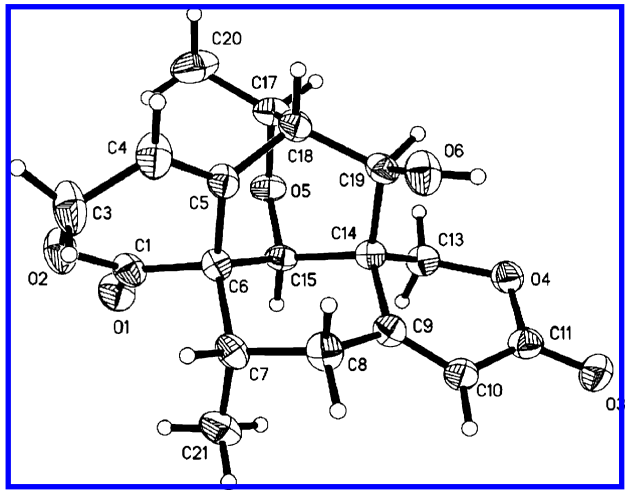

Figure 3. X-ray crystal structure of swerilactone B (**2**).

Swerilactones A (**1**) and B (**2**) were two novel diastereomeric lactones with an unprecedented 6/6/6/6/6 pentacyclic ring system skeleton. In order to evaluate their anti-HBV activities, compounds **1** and **2** were assayed on an HBV-transfected Hep G 2.2.15 cell line in vitro as reported previously.[19,29] Compound **1** showed inhibitory activity against HBsAg secretion with IC_{50} of 3.66 mM, and against HBeAg secretion with IC_{50} of 3.58 mM; whereas compound **2** showed no anti-HBV activity. The 50% cytotoxicity of compounds **1** and **2** were not reached at the tested (highest) concentration of 3.86 and 4.64 mM, respectively. The biogenetic passway for compounds **1** and **2** was still unknown, which needs to be further investigated.

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Supporting Information Available: 1D and 2D NMR, UV, IR, MS spectra, and X-ray crystallographic data in CIF format of swerilactones A (**1**) and B (**2**) and experimental equipment descriptions. This material is available free of charge via the Internet at http://pubs.acs.org.

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(27) Compound **2**: mp 245−246 °C; $[\alpha]^{25.2}_{D}$ −12.26 (*c* 0.12, $CHCl_3$/MeOH *v*/*v* 1:1); UV (MeOH) ν_{max} (log ε) 209 (4.10), 230 (4.12) nm; IR (KBr) λ_{max} 3383, 1730, 1699, 1468, 1397, 1285, 1271, 1138, 1056, 1027, 1009, 845 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS (+) *m*/*z* 355 ($[M + Na]^+$); HRESIMS (+) *m*/*z* 355.1155 $[M + Na]^+$ ($C_{18}H_{20}O_6Na$ calcd 355.1157).

(28) Crystallographic data of compound **2**: $C_{18}H_{20}O_6$, MW = 332.35; monoclinic, space group *pc*; *a* = 7.4578 (12) Å, *b* = 15.834 (3) Å, *c* = 7.0431(12) Å, α = 90.00, β = 111.745(2), γ = 90.00, *V* = 772.5 (2) $Å^3$, *Z* = 2, *d* = 1.433 g/cm^3, crystal dimensions 0.14 × 0.08 × 0.06 nm was used for measurement on a SHELXL-97 with a graphite monochromater, Mo Kα radiation. The total number of reflections measured was 3116, of which 2181, were observed, $I > 2\sigma(I)$. Final indices: R_1= 0.0274, wR_2 = 0.0526. The crystal structure of **2** was solved by direct method SHLXS-97 (Sheldrick, 1990) and expanded using difference Fourier technique, refined by the program SHLXL-97 (Sheldrick, 1997) and the full-matrix least-squares calculations. Crystallographic data for the structure of **2** have been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 737518). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving. html (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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