ORGANIC LETTERS

2009
Vol. 11, No. 21
4838−4841

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

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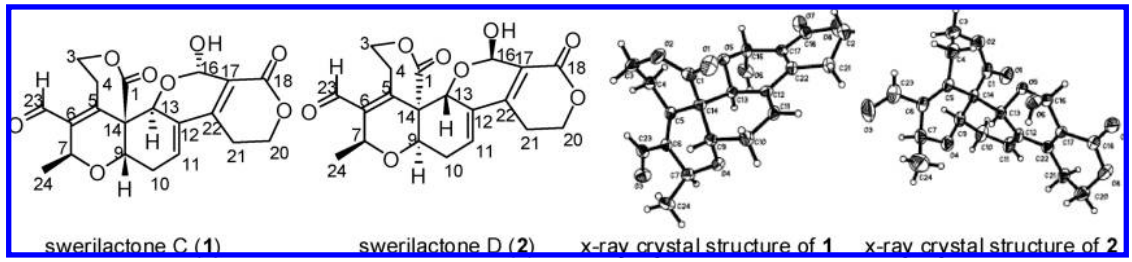
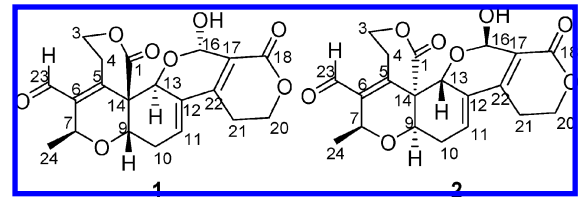
Received August 13, 2009

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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10.1021/ol901881w CCC: $40.75
Published on Web 10/02/2009

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

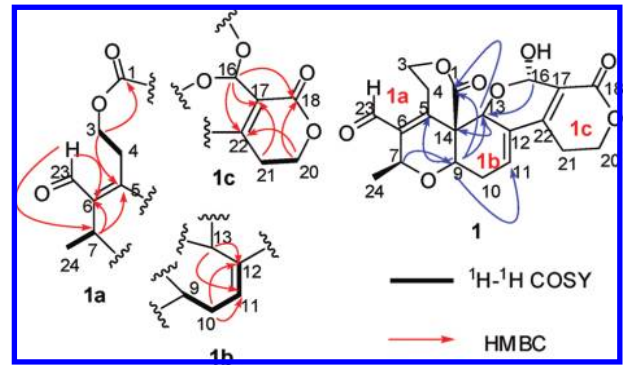

Figure 1. Fragment structures and key COSY and HMBC correlations of compound **1**.

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.

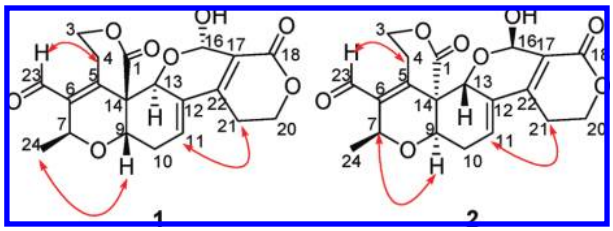

Figure 2. Selected ROESY correlations of **1** and **2**.

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]

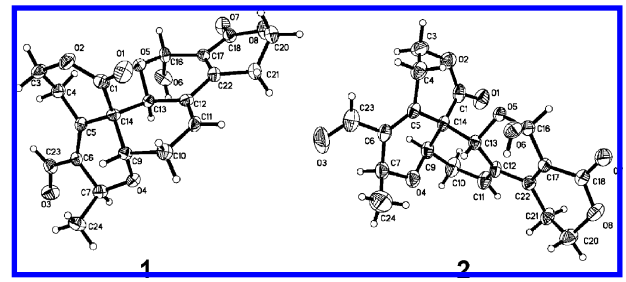

Figure 3. X-ray crystal structures of compounds **1** and **2**.

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]

Acknowledgment. The work was supported by National Natural Science Foundation of China and Yunnan Province (Grant No. U0832603), Xibu Zhiguang Joint-Scholarship. We are grateful to the staff of the analytical group of the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, for measurements of all spectra. We also express thanks to Prof. Ming-Jin Xie (Yunnan University, mjxie@ynu.edu.cn) for his professional measurement of the X-ray diffraction.

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).