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LC-MS Guided Isolation of (±)-Swerileduginin A, a Pair of Enantiomeric Lactones, from *Swertia leducii*

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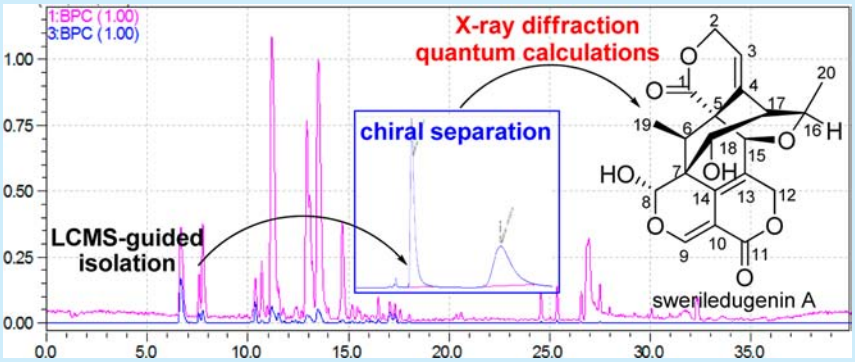
Supporting Information



ABSTRACT: (±)-Swerileduginin A, a pair of novel enantiomeric lactones, were isolated from *Swertia leducii* under the guidance of LC-MS investigation. The enantiomeric separation was achieved by HPLC on a chiral column. Their structures were determined by extensive NMR spectra, X-ray, and quantum calculations. (+)-Swerileduginin A and (−)-swerileduginin A showed activities inhibiting HBV DNA replication with the IC_{50} values of 36.86 and 26.55 μM on the HepG 2.2.15 cell line *in vitro*.

Swertia leducii (Mengzi Zhangyacai in Chinese) is an annual herbaceous plant mainly distributed in Mengzi County of the Yunnan Province. Many traits of *S. leducii* are similar to those of *S. mileensis*, except for petioles and flowers.[1] Therefore, some botanists consider that the two species should be combined. Due to their close morphology, *S. leducii* was always used as the alternative for *S. mileensis* in producing related Qing-Ye-Dan medicines. In our previous investigation, a series of novel secoiridoid dimers and trimers were isolated from *S. mileensis*.[2] However, no chemical investigation has been conducted on *S. leducii*. Presently, liquid chromatography linked with mass spectrometry (LC-MS) has become a routine method in many areas of analytical chemistry.[3] The Shimadzu UFLC-MS-IT-TOF apparatus equipped with an electrospray ionization source coupled to ion-trap and time-of-flight mass analyzers (ESI-IT-TOF) enables high-resolution mass spectra in both positive and negative modes and, thus, is effective for characterizing trace components in a complex mixture of natural products.[4] To clarify the chemical constituents of *S. leducii*, we used LC-MS guided isolation to obtain a pair of novel enantiomers, (±)-swerileduginin A (**1**), with an unprecedented hexacyclic system (Figure 1). Herein, we reported their isolation, structural elucidation, and anti-HBV activities.

The whole plant of *S. leducii* was collected in Mengzi County, Yunnan Province, China, in October 2012, which was identified as *Swertia leducii* Franch. by Prof. Dr. Li-Gong Lei, Kunming Institute of Botany, CAS (voucher No. 2012-10-11-1). The dried and powdered whole plants (3.0 kg) were extracted with EtOH (10 L) under reflux 3 times, and the combined solvent was evaporated *in vacuo*. The residue was dissolved in water and partitioned with EtOAc, to afford aqueous and EtOAc parts. The EtOAc part (98 g) was loaded on a silica gel chromatography column (CC) eluting with a MeOH−$CHCl_3$ system to give six fractions (A−F). Fraction B (5.0 g) was further fractionated by MPLC on an Rp-18 column with MeOH−H_2O as the mobile phase and yielded five subfractions (B1−B5). Subfraction B2 was analyzed by UFLC-MS-IT-TOF to afford a chromatographic peak with the molecular formula $C_{20}H_{20}O_8$ determined from the $[M + H]^+$ ion (m/z 389.1227, −1.03 ppm) in positive mode and the $[M-H]^-$ ion (m/z 387.1084, −0.26 ppm) in negative mode. Consequently, Fr. B2 (100 mg) was purified by HPLC on an Rp-18 column eluted with acetonitrile−H_2O (30:70), and after recrystallization in MeOH, swerileduginin A (**1**, 3 mg) was obtained.

Swerileduginin A (**1**)[5] was isolated as colorless needles with the molecular formula $C_{20}H_{20}O_8$ which was deduced from $[M + H]^+$ (m/z 389.1227) and $[M - H]^-$ (m/z 387.1084) ions in HRESIMS, corresponding to 11 degrees of unsaturation. In

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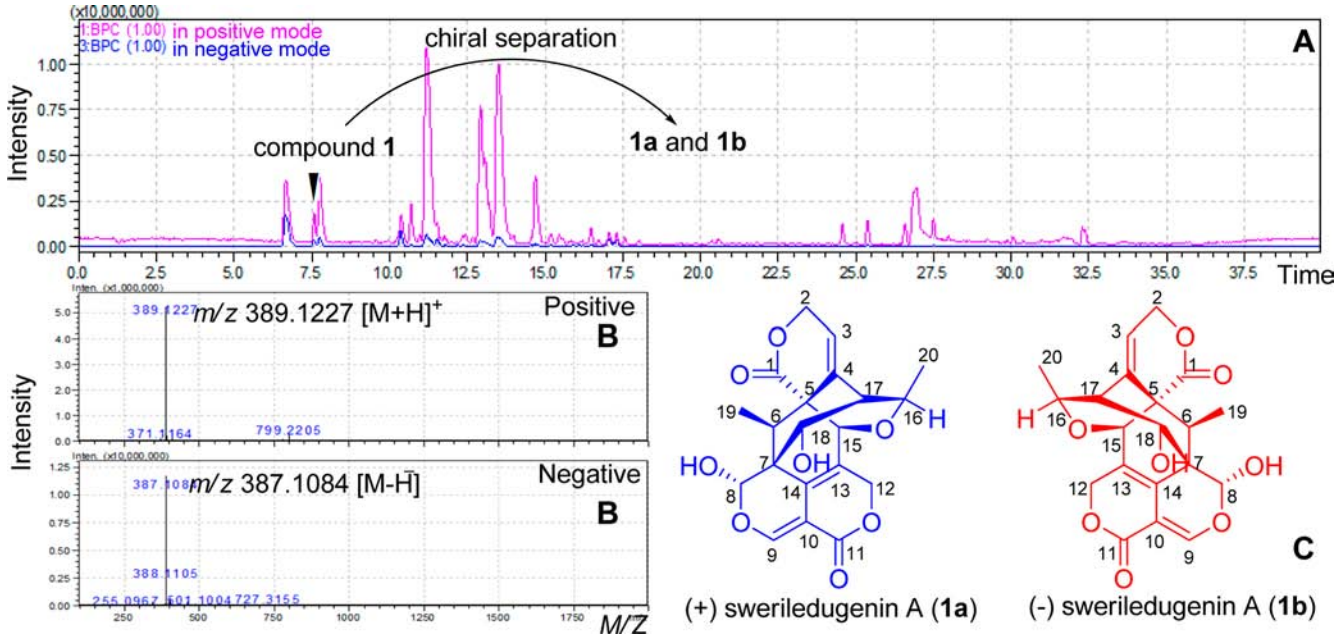

Figure 1. UFLC-MS base peak chromatogram (BPC) of Fr. B2 (A) and HRESIMS (B) as well as structures of **1a** and **1b** (C).

accordance with its molecular formula, all 20 carbons were well resolved in the ^{13}C NMR (DEPT) spectrum, which were recognized as two methyls, two methylenes, eight methines, and eight quaternary carbons. Two ester carbonyl groups (δ_C 174.2 and 162.8) and six olefinic carbons (from δ_C 151.8 to 104.7) were characterized in the downfield region of ^{13}C NMR spectrum, of which one tetrasubstituted and two trisubstituted double bonds were revealed based on two olefinic protons (δ_H 6.03 and 7.39). One doublet at δ_H 5.62 (1H, J = 4.6 Hz) in the ^1H NMR spectrum, along with the carbon signal at δ_C 97.2 (d), indicated a dioxygenated methine, which was further proved to be linked with a hydroxyl goup by ^1H ^1H COSY of H-8/OH-8. Furthermore, two oxygenated methylenes and two secondary methyls were easily recognized according to the carbons at δ_C 70.6 (t), 70.2 (t), 22.5 (q), and 11.4 (q), as well as protons at δ_H 1.08 (3H, d, J = 6.3 Hz) and 1.00 (3H, d, J = 7.0 Hz). Based on the above analyses, 5 out of 11 degrees of unsaturation were assigned, and thus, the residual 6 degrees of unsaturation required sweriledugenin A (**1**) to possess a hexacyclic skeleton.

The connectivity of $CH_3(20)-CH(16)-CH(17)-CH(18)-OH$ can be well interpreted from the correlations of H-20/H-16/H-17/H-18/OH in the ^1H ^1H COSY spectrum. Similarly, the correlations of H-2/H-3 and H-6/H-19 suggested the direct linkage of $C_{(2)}-C_{(3)}$ and $C_{(6)}-C_{(19)}$. However, these partial structures could not be assigned with confidence on the basis of the HMBC data. Therefore, an X-ray diffraction analysis was performed, from which the structure of sweriledugenin A was unambiguously determined (Figure 2).[6] Detailed interpretation of the HMBC (Table 1) and ROESY correlations of H-3/H-17, H-19/H-18 and H-18/H-8 (Figure 3) was well consistent with the structure deduced above.

It is worth noting that the crystal of sweriledugenin A (**1**) had a $p21/c$ space group, indicating a racemic nature, which was in accordance with its $[\alpha]_D$ value (+1.76). Subsequent chiral resolution was performed on a chiral column (Daicel Chiralpak AS-H) to yield (+) sweriledugenin A (**1a**) and (−) sweriledugenin A (**1b**), which were opposite in terms of optical rotation (Supporting Information). The final assignment of **1a**

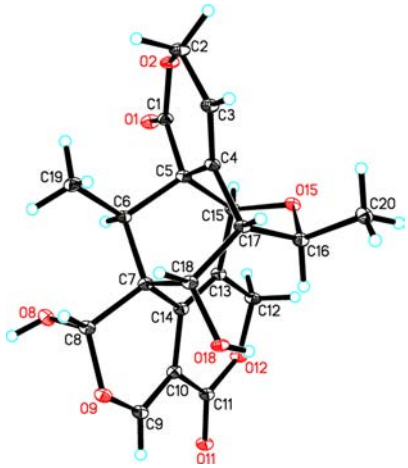

Figure 2. X-ray structure of compound **1**.

and **1b** was achieved using a quantum method by comparing the calculated $[\alpha]_D$ values and electronic circular dichroisms (ECDs) with experimented data.[7] The absolute configurations of **1a** and **1b** were obtained from X-ray data, and $[\alpha]_D$ values were computed at the b3lyp/6-311g(d,p)//b3lyp/6-311g(d,p) and b3lyp/6-311 +g(2d,p)//b3lyp/6-311+g(2d,p) levels. The calculated $[\alpha]_D$ values were +202/+215 for **1a** and −202/−215 for **1b**, which were very close to the experimented ones of +230 (**1a**) and −172 (**1b**). Similarly, the ECDs calculated at the b3lyp/6-311++g(2d,p) level showed high agreement with the experimented spectra (Figure 4).[8] From the above evidence, the absolute stereochemistry for **1a** (5*R*, 6*R*, 7*R*, 8*S*, 15*S*, 16*S*, 17*S*, 18*S*) and **1b** (5*S*, 6*S*, 7*S*, 8*R*, 15*R*, 16*R*, 17*R*, 18*R*) were unambiguously determined as shown in Figure 1.

According to the anti-HBV assay on the HepG 2.2.15 cell line *in vitro*,[2] compounds **1a** and **1b** both showed moderate activity inhibiting HBV DNA replication with the IC_{50} values of 36.86 μM (SI = 10.5) and 26.55 μM (SI = 31.6), respectively.

Sweriledugenin A with a complicated hexacyclic skeleton was isolated under the guidance of the LC-MS method, which was

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Table 1. 1D and 2D NMR Data of 1 in Acetone-d_6 (δ in ppm, J in Hz)[a]

no.	^{1}H NMR[a]	^{13}C NMR[b]	^{1}H ^{1}H COSY	HMBC
1	–	174.2, s	–	–
2	5.06, 1H, d, 16.9 4.96, 1H, d, 16.9	70.6, t	H-3	C-1, 4
3	6.03, 1H, t, 2.3	118.1, d	H-2	C-5, 17
4	–	131.1, s	–	–
5	–	49.1, s	–	–
6	2.48, 1H, q, 7.0	45.0, d	H-19	C-1, 4, 8, 14, 18, 19
7	–	44.3, s		
8	5.62, 1H, d, 4.6	97.2, d	HO-8	C-6, 9, 14, 18
9	7.39, 1H, s	151.8, d	–	C-8, 11, 14
10	–	104.7, s	–	–
11	–	162.8, s	–	–
12	4.98, 2H, brs	70.2, t	–	C-11, 14, 15
13	–	118.3, s	–	–
14	–	130.8, s	–	–
15	4.61, 1H, s	75.0, d	–	C-4, 6, 12, 14, 16
16	4.27, 1H, q, 6.3	65.5, d	H-17, H-20	C-4, 15, 18, 20
17	2.57, 1H, d, 5.7	52.5, d	H-16, H-18	C-3, 5, 7, 20
18	4.34, 1H, dd, 5.7, 5.2	73.1, d	H-17	C-4, 6, 8, 14, 16
19	1.00, 3H, d, 7.0	11.4, q	H-6	C-5, 6, 7
20	1.08, 3H, d, 6.3	22.5, q	H-16	C-16, 17
HO-8	6.60, d, 4.6	–	H-8	C-7, 8
HO-18	4.11, 1H, d, 5.2	–	H-18	C-7, 17, 18

[a]^{1}H NMR recorded in 600 MHz. [b]^{13}C NMR recorded in 150 MHz.

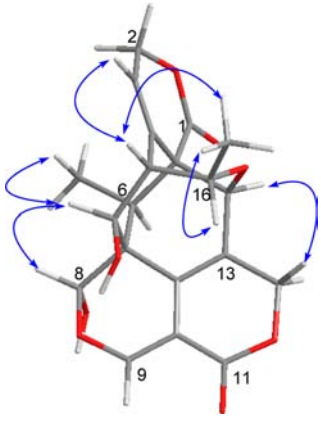

Figure 3. Key ROESY correlations of compound 1.

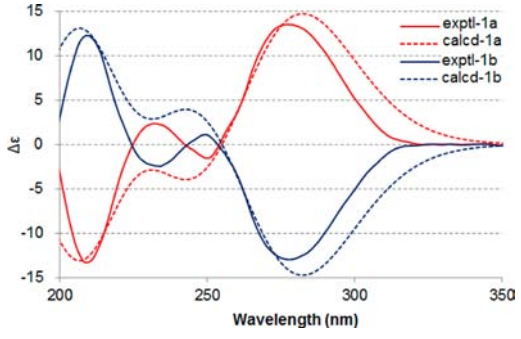

Figure 4. Experimental and calculated ECDs of 1a and 1b.

further proven to be a pair of enantiomers by chiral separation and quantum calculations. This investigation is a valuable attempt for guided isolation from a completed natural complex.

■ ASSOCIATED CONTENT

Supporting Information

NMR, HRESIMS, $[\alpha]_D$, CD, UV and IR spectra, X-ray data, and computational methods of compound 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(5) Swerilediugenin A (1): colorless crystals (MeOH); mp 266–267 °C; UV λ_{max} (acetonitrile) (log ε) 277 (4.08) nm; IR (KBr) ν_{max} 3485, 3396, 2929, 1716, 1614, 1456, 1406, 1230, 1096 cm^{-1}; (+) HRESIMS m/z 389.1227 (calcd for $C_{20}H_{21}O_8$, −0.4 mDa); (−) HRESIMS m/z 387.1084 (calcd for $C_{20}H_{19}O_8$, −0.1 mDa).

(6) Crystal data for 1: $C_{20}H_{20}O_8{\cdot}CH_3OH$, M = 420.40, monoclinic, a = 12.7431(4) Å, b = 11.7643(4) Å, c = 12.0525(4) Å, α = 90.00°, β = 90.7990(10)°, γ = 90.00°, V = 1806.66(10) Å^3, T = 100(2) K, space group $P21/c$, Z = 4, μ(Cu Kα) = 1.027 mm^{-1}, 11 636 reflections measured, 3069 independent reflections (R_{int} = 0.0500). The final R_1 value was 0.1048 ($I > 2\sigma(I)$). The final $wR(F^2)$ value was 0.2894 ($I > 2\sigma(I)$). The final R_1 value was 0.1056 (all data). The final $wR(F^2)$ value was 0.2912 (all data). The goodness of fit on F^2 was 1.445. Crystallographic data have been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 957147).

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