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THREE NEW IRIDOID COMPOUNDS FROM *SWERTIA CINCTA* BURKILL

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Abstract – Three new iridoid compounds, 3,4,5,6-tetrahydro-8-hydroxy-6-(hydroxymethyl)pyrano[3,4-*c*]pyran-1(8*H*)-one (**1**), 3,4,5,6-tetrahydro-8-hydroxy-6-methylpyrano[3,4-*c*]pyran-1(8*H*)-one (**2**), 5,6-dihydro-4-(1,3-dihydroxybutan-2-yl)-3-(hydroxymethyl)pyran-2-one (**3**), named swercinctolides A, B and C, were isolated from the 80% methanol extract of the whole plant of *Swertia cincta* Burkill, which is a traditional medicine in China. Their structures were defined on the basis of spectral evidences. Compounds **1**, **2** and **3** showed TMV (Tobacco Mosaic Virus) inhibitory activity.

Swertia cincta Burkill, a plant of the genus *Swertia* (Gentianaceae), is mainly distributed in Sichuan, Guizhou and Yunnan of China.[1,2] It has been used as traditional medicinal plant to remedy jaundice hepatitis.[3] Previous research[4-6] suggests that iridoid, flavone, triterpenoid, and xanthone are main constituents of this plant. Our phytochemical investigation on the constituents of this plant has resulted in the isolation of three new iridoid compounds, named swercinctolides A, B and C. Their structures were elucidated by spectroscopic methods and shown in Figure 1. Compounds **1**, **2** and **3** showed TMV (Tobacco Mosaic Virus) inhibitory activity compared with the positive control spot.

Compound **1** was obtained as colorless gum. Its molecular formula was deduced to be $C_9H_{12}O_5$ by ^{13}C NMR spectra and HRESI-MS, with quasi-molecular ion peaks at *m/z* 199.0606 ($C_9H_{11}O_5$, $[M-H]^-$). The IR spectrum showed the presence of hydroxyl group (3434 cm^{-1}) and C = O bond (1638 cm^{-1}) of *α, β*-unsaturated ester.

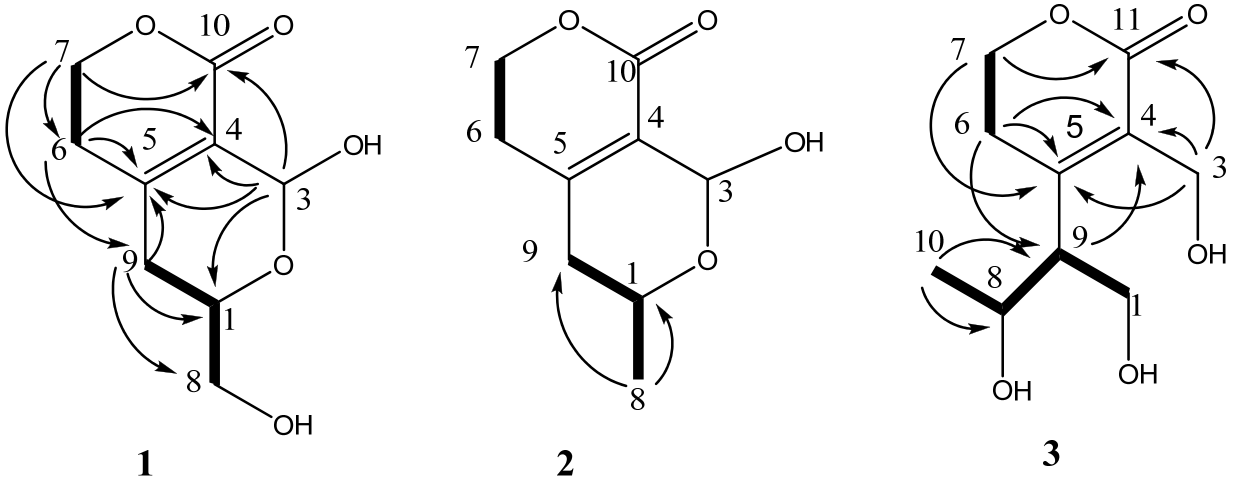

Figure 1. Key HMBC (full-line arrows) and H-H COSY (bold line) correlations of **1**, **2** and **3**

^{13}C and DEPT NMR experiments differentiated the skeleton carbons of **1** as four methylenes (δ_C: 66.9, 65.2, 32.1, 29.5), two methines (δ_C: 88.0, 67.2), and three quaternary (δ_C: 165.2, 156.0, 125.3) carbons. The three quaternary carbons showed the presence of *α, β*-unsaturated ester. The HMQC spectra of **1** showed the position assignment of H-atoms, given in Table 1. In the HMBC spectra of **1**, H-7 (δ_H 4.41) exhibited cross-peaks with C-5, C-6, C-10 at δ_C 156.0, 29.5 and 165.2, and H-6 (δ_H 2.60 and 2.40) showed correlations with C-4, C-5, C-7, and C-9 at δ_C 125.3, 156.0, 66.9, and 32.1, respectively. These suggested the presence of 5,6-dihydropyran-2-one. Also, H-3 (δ_H 5.64) exhibited cross-peaks with C-1, C-4, C-5, and C-10 at δ_C 67.2, 125.3, 156.0, and 165.2, and H-9 (δ_H 2.39 and 2.21) showed correlations with C-1, C-4, C-5, C-6 and C-8 at δ_C 67.2, 125.3, 156.0, 29.5 and 65.2, respectively. And H-8 (δ_H 3.64) did correlations with C-1 and C-9 at δ_C 67.2 and 32.1. The above evidences educed the molecule skeleton of **1**. Further, H-H COSY experiment showed correlations between H-6 and H-7, H-1 and H-8, H-1 and H-9. Meanwhile, ROESY experiment showed same correlations. From ^1H NMR spectrum of **1**, the single peak at δ_H 5.64 (C-3, δ_C 88.0, tertiary carbon) suggested a hydroxyl was attached to C-3. The peak at δ_H 3.64 (C-8, δ_C 65.2, secondary carbon) showed a hydroxyl was attached to C-8. Biogenetically, the H-1 of iridoid skeleton is *α*-orientation. No ROESY cross-peak between H-1 and H-3 revealed that the relative configuration of H-3 was *β*-orientation. Thus, the structure of **1** was determined as 3,4,5,6-tetrahydro-8-hydroxy-6-(hydroxymethyl)pyrano[3,4-*c*]pyran-1(8*H*)-one, named swercinctolide A.

Compound **2** was obtained as colorless gum. Its molecular formula was deduced to be $C_9H_{12}O_4$ by ^{13}C NMR spectra and HRESI-MS, with quasi-molecular ion peaks at *m/z* 185.0815 ($[M+H]^+$) and 207.0633 ($[M+Na]^+$). The IR and UV spectra were similar to those of **1**.

The ^1H and ^{13}C NMR spectra of **2**, given in Table 1, resembled those of **1**. The ^{13}C and DEPT NMR spectroscopic data showed the presence of one methyl, three methylenes, two methines, and three quaternary carbons. The linkage of C-8 (CH_3) to C-1 was confirmed by correlations between H-1 and H-8 in H-H COSY experiment and H-8 (δ_H 1.22) with C-1 and C-9 at δ_C 62.6 and 37.7 in HMBC spectra. The ROESY experiment of **2** suggested the stereo configurations of H-1 and H-3 of **2** resembled those of **1**. Thus, the structure of **2** was determined as 3,4,5,6-tetrahydro-8-hydroxy-6-methylpyrano[3,4-*c*]pyran-1(8*H*)-one, named swercinctolide B.

Compound **3** was obtained as colorless gum. Its molecular formula was deduced to be $C_{10}H_{16}O_5$ by ^{13}C NMR spectra and HRESI-MS, with quasi-molecular ion peaks at *m/z* 215.0917 ($[M-H]^-$). The IR spectrum showed the presence of hydroxyl group (3433 cm^{-1}) and C = O bond (1652 cm^{-1}) of *α, β*-unsaturated ester.

^{13}C and DEPT NMR experiments differentiated the skeleton carbons of **3** as one methyl (δ_C: 19.3), four methylenes (δ_C: 67.5, 63.5, 60.2, 27.0), two methines (δ_C: 71.7, 48.4), and three quaternary (δ_C: 165.9, 153.5, 125.4) carbons. The three quaternary carbons showed the presence of *α, β*-unsaturated ester. The HMQC spectrum of **3** showed the position assignment of H-atoms, given in Table 1. In the HMBC spectrum of **3**, H-7 (δ_H 4.45) exhibited cross-peaks with C-5, C-6, C-11 at δ_C 153.5, 27.0 and 165.9, and H-6 (δ_H 2.75 and 2.35) showed correlations with C-4, C-5, C-7, and C-9 at δ_C 125.4, 153.5, 67.5, and 48.4, respectively. These suggested the presence of 5, 6-dihydropyran-2-one. Also, H-10 (δ_H 1.32) exhibited cross-peaks with C-8 and C-9 at δ_C 71.7 and 48.4, and H-9 (δ_H 2.21) showed correlations with C-1, C-4, C-5, C-6, C-8 and C-10 at δ_C 63.5, 125.4, 153.5, 27.0, 71.7 and 19.3, respectively. And H-3 (δ_H 3.84, 3.74) did correlations with C-4, C-5 and C-11 at δ_C 125.4, 153.5 and 165.9. The above information educed the molecule skeleton of **3**. Further, H-H COSY experiment showed correlations between H-6 and H-7, H-8 and H-10, H-8 and H-9, H-1 and H-9. From ^1H NMR spectrum of **3**, the peak at δ_H 3.83 (C-8, δ_C 71.7, tertiary carbon) showed a hydroxyl was attached to C-8. The peak at δ_H 3.84, 3.74 (C-3, δ_C 60.2, secondary carbon) showed a hydroxyl was attached to C-3. The peak at δ_H 4.31, 4.21 (C-1, δ_C 63.5, secondary carbon) showed a hydroxyl was attached to C-1. Thus, the structure of **3** was determined as 5,6-dihydro-4-(1,3-dihydroxybutan-2-yl)-3-(hydroxymethyl)pyran-2-one, named swercinctolide C.

In the leaves of tobacco, the TMV inhibitory activity of **1**, **2** and **3** was performed. The solution concentrations of three compounds were all 10 μg/mL. The right part of a leaf was sprayed with 100 μL solution of **1** and the left part was done with the same concentration and volume solution of DMSO. Two

hours later, the whole leaf was inoculated with 200 μL solution of TMV (10 μg/mL). After 5 days, the spot numbers of right and left part on leaf were counted up and then the spot inhibitory rate of **1** was calculated according to the spot number [the negative control DMSO gave an inhibitory rate of 0.00%]. In the same way, the spot inhibitory rates of **2** and **3** were calculated. The spot inhibitory rates of **1**, **2** and **3** were 65.41%, 46.01% and 42.59%, respectively.

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1, 2** and **3** [a]

position	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	4.23 m	67.2	4.30 m	62.6	4.31 dd (14.0, 4.0) 4.21 dd (14.0, 4.0)	63.5
3	5.64 s	88.0	5.61 s	88.1	3.84 m 3.74 d (14.0)	60.2
4		125.3		125.1		125.4
5		156.0		156.3		153.5
6	2.60 m 2.40 m	29.5	2.59 m 2.41 m	29.4	2.75 m 2.35 d (16.0)	27.0
7	4.41 m	66.9	4.42 m	67.0	4.45 m	67.5
8	3.64 m	65.2	1.22 d (4.0)	21.1	3.83 m	71.7
9	2.39 m 2.21 dd (18.8, 4.0)	32.1	2.35 m 2.22 dd (18.8, 4.0)	37.7	2.21 m	48.4
10		165.2		165.3	1.32 d (4.0)	19.3
11						165.9

[a] ^{1}H NMR spectral data measured at 400 MHz; ^{13}C NMR spectral data measured at 100 MHz; Proton coupling constants (*J*) in Hz given in parentheses; Methanol-d_4 as solvent.

EXPERIMENTAL

General experimental procedures. Optical rotations and UV data were measured on a JASCO-20 polarimeter and UV-2401PC spectrometer. MS were measured on a VG Auto Spec-3000 spectrometer. HR-ESI-MS data were measured using a JMS-T100CS AccuTOF LC/MS spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker AM-400 instruments with TMS as internal standard. IR

spectra were measured on a Bruker Tensor 27 spectrometer with KBr pellets. Column chromatography was performed with ZORBAX Eclips XDB-C_{18} (10 μm, Agilent, America) and Lichroprep RP-18 gel (40-63 μm; Merk, Darmstadt, Germany), Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd. Uppsala, Sweden), Macroporous resin and MCI gel (75-150 μm; Mitsubishi Chemical Corporation, Japan).

Plant material. Whole herb of *Swertia cincta* Burkill was collected from Xinping County, Yunnan Province, People's Republic of China, in October 2009. The plant was identified by Dr. Lipang Yang (Yunnan University of Traditional Chinese Medicine). A voucher specimen (NO. 0601007) is deposited at the Herbarium of Key Laboratory of Ethnic Medicine Resource Chemistry, Yunnan University of Nationalities.

Extraction and Isolation. The dried whole plants of *Swertia cincta* Burkill (4.5 kg) were extracted four times with MeOH (80%) under ultrasound calefaction (4 h each time). After filtering and evaporating the solvents in vacuo a crude extract (880 g) was obtained. The extract was suspended in water and extracted six times with CH_2Cl_2. The solvents were evaporated to afford CH_2Cl_2 extract (368 g) and a residue (510 g) from water. After eliminating coloring matter in the residue with MCI gel column chromatography (CC), the left residue (485 g) was performed on macroporous resin column, and then eluted with water, water-MeOH (3:7) and pure MeOH. The residue of water elution was 156 g, after evaporating water. The residue was chromatographed on a reverse silica gel column (Agilent Eclips XDB-C_{18}, 21.2×250 mm) and eluted with MeOH-water (3:7) to afford four fractions. Fraction 3 was purified with Sephadex LH-20 CC (MeOH) and Lichroprep RP-18 gel (MeCN-H_2O 1:9), and **1** (6.2 mg) and **2** (7.0 mg) were obtained. Fraction 2 was chromatographed over Lichroprep RP-18 gel column and eluted with MeCN-H_2O (1:9) to yield **3** (6.2 mg).

Compound 1: $C_9H_{12}O_5$, colorless gum; $[\alpha]^{19.1}_D$ +65.1 (*c* 0.025, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.69) nm; IR (KBr): ν_{max} 3434, 2925, 2359, 1638, 1095, 536 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectral data see Table 1; negative-mode ESI-MS *m/z* 199 $[M-H]^-$; HR-ESI-MS *m/z* 199.0606 $[M-H]^-$ (calcd for $C_9H_{11}O_5$, 199.0603).

Compound 2: $C_9H_{12}O_4$, colorless gum; $[\alpha]^{19.1}_D$ +16.0 (*c* 0.020, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.69) nm; IR (KBr): ν_{max} 3429, 2924, 2364, 1628, 1394, 1085, 533 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectral data see Table 1; positive-mode HR-ESI-MS *m/z* 185.0815 $[M+H]^+$ (calcd for $C_9H_{13}O_4$, 185.0814), 207.0633 $[M+Na]^+$ (calcd for $C_9H_{12}O_4Na$, 207.0636).

Compound 3: $C_{10}H_{16}O_5$, colorless gum; $[\alpha]^{19.1}_D$ +60.7 (*c* 0.017, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.67) nm; IR (KBr): ν_{max} 3433, 2926, 2357, 1652, 1093, 672, 538 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectral data see Table 1; negative-mode ESI-MS *m/z* 215 ($[M-H]^-$); HR-ESI-MS *m/z* 215.0917 $[M-H]^-$ (calcd for $C_{10}H_{15}O_5$, 215.0915).

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