Article

Two New Chiratane-Type Triterpenoids from *Swertia kouitchensis*

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Abstract: Two rare new chiratane-type triterpenoids, kouitchenoids A and B (**1**, **2**), together with oleanolic acid (**3**) and ursolic acid (**4**), were isolated from ethanol extract of *Swertia kouitchensis*. The new structures were determined by the analysis of MS and NMR data. In addition, compounds **1**–**4** showed moderate inhibitory activity against the α-glucosidase (with IC_{50} values ranging from 1,812 to 2,027 μM).

Keywords: *Swertia kouitchensis*; diabetes; chiratane; triterpenoid; α-glucosidase

1. Introduction

Swertia kouitchensis Franch. (Gentianaceae), widely distributed in China, has been used for the treatment of hepatitis and diabetes [1,2]. Previous study revealed that the ethanol extract of *S. kouitchensis* showed *α*-glucosidase inhibitory effect [3]. Thus, we initiated a study on the subject. As a result, two rare chiratane-type triterpenoids, kouitchenoids A and B (**1** and **2**), along with oleanolic acid (**3**) [4] and ursolic acid (**4**) (Figure 1) [5] were isolated and identified. All of these compounds were evaluated for their inhibitory activities against *α*-glucosidase. Described herein are the isolation, structure elucidation, and biological activities of these compounds.

Figure 1. Structures of compounds **1–4**.
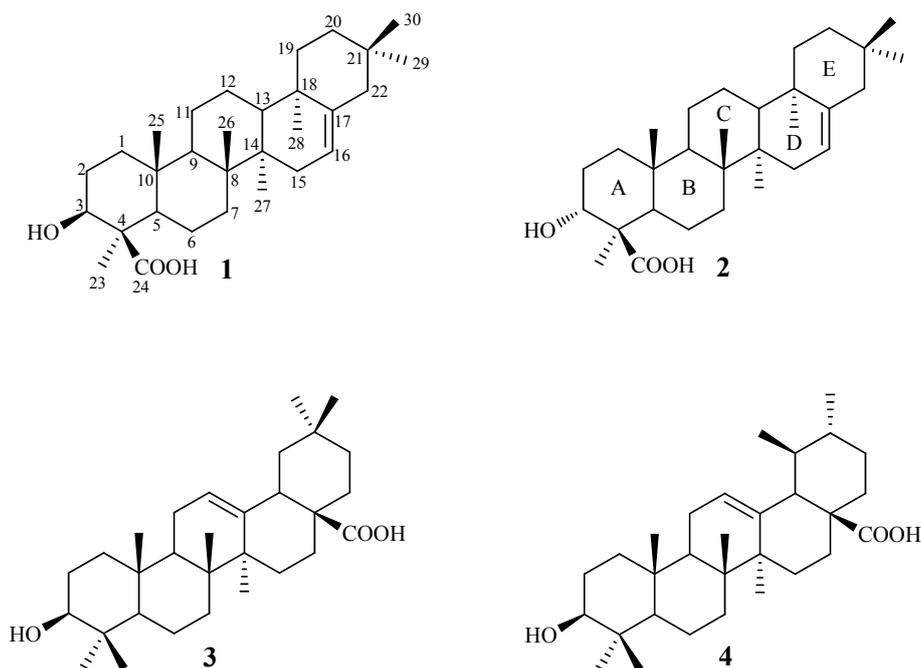
2. Results and Discussion

The 95% ethanol extract of *S. kouitchensis* whole plants was suspended in water and successively partitioned with petroleum ether, CH_2Cl_2, EtOAc and *n*-butanol. The CH_2Cl_2 fraction was subjected to column chromatography and partitioned as described in the Experimental section to afford two new triterpenoids **1** and **2**, along with two known compounds **3** and **4**.

Compound **1** was obtained as white amorphous powder, gave a molecular formula of $C_{30}H_{48}O_3$ by HRESIMS (*m/z* 455.3525 [M−H]⁻, calcd. for $C_{30}H_{47}O_3$, 455.3531). Its IR spectrum exhibited absorptions at 3,453 and 1,696 cm⁻¹, assignable to hydroxyl and carboxyl groups, respectively. The ¹H- and ¹³C-NMR spectra of **1** (Table 1) closely resembled those of chiratenol [6,7], except for the appearance of a carboxyl carbon signal at δ_C 181.0 instead of a methyl (C-24) signal of chiratenol, and the downfield shift (+10.8 ppm) for C-4. The position of this carboxyl group was confirmed by HMBC spectrum (Figure 2), in which cross-peaks were observed between H-3 (δ_H 3.41) and C-2, C-4, C-23, and C-24, so that the carboxyl group is assigned to C-24. And also, H-3 was assigned as *α*-orientation by the NOESY correlation (Figure 2) between H-3 and H-5. Therefore, **1** was deduced to be 3*β*-hydroxy-chirat-16-en-24-oic acid, and named kouitchenoid A.

Compound **2** was obtained as white amorphous powder. Its molecular formula was assigned to be $C_{30}H_{48}O_3$ based on the HRESIMS spectrum at *m/z* 455.3525 [M−H]⁻ (calcd. for $C_{30}H_{47}O_3$, 455.3531). IR spectrum of **2** exhibited absorptions at 3,448 and 1,701 cm⁻¹, assignable to hydroxyl and carboxyl functions, respectively. Its ¹H-NMR and ¹³C-NMR shifts for the B, C, D, and E rings were almost the same as those of compound **1**, while those of the A ring differed. The main differences in the A ring between these two compounds were that the oxygenated proton H-3 at δ_H 3.41 (1H, *dd*, *J* = 12.0, 4.4 Hz) in **1** was changed into δ_H 4.75 (1H, *brs*, $W_{1/2}$ = 5.7Hz) in **2** and the oxygenated C-3 carbon at δ_C 78.7 in **1** was shifted upfield to δ_C 71.1 in **2**, suggesting that the H-3 in a *β*-orientation in **2**. This assignment could be further supported by the missing correlation between H-3 and H-5 in the NOESY

spectrum of **2**. Together with its ^{1}H-^{1}H COSY and HMBC spectra, which also were similar to those of **1**, compound **2** was determined to be 3α-hydroxy-chirat-16-en-24-oic acid and named kouitchenoid B.

Table 1. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) Spectral Data of Compounds **1** and **2** in C_5D_5N (δ in ppm, *J* in Hz).

Position	1		2	
	δ_C	δ_H	δ_C	δ_H
1	40.2	1.87 (*dt, J* = 3.2, 12.8), 1.09 (*m*)	35.3	1.91 (*dt, J* = 3.2, 12.1), 1.73 (*m*)
2	29.7	2.51 (*m*), 2.02 (*m*)	28.1	2.80 (*m*), 2.05 (*m*)
3	78.7	3.41 (*dd, J* = 12.0, 4.4)	71.1	4.75 (*brs, W$_{1/2}$ = 5.7*)
4	49.6		48.8	
5	57.1	1.05 (*m*)	49.7	2.08 (*m*)
6	21.0	2.22 (*m*), 2.06 (*m*)	20.9	2.38 (*m*), 2.07 (*m*)
7	34.6	1.51 (*m*), 1.37 (*m*)	34.7	1.61 (*m*), 1.40 (*m*)
8	41.5		41.8	
9	50.8	1.29 (*m*)	50.8	1.53 (*m*)
10	37.5		38.7	
11	22.4	1.59 (*m*), 1.29 (*m*)	22.3	1.69 (*m*), 1.32 (*m*)
12	24.1	1.59 (*m*), 1.45 (*m*)	24.1	1.58 (*m*), 1.43 (*m*)
13	46.0	1.58 (*m*)	46.0	1.59 (*m*)
14	41.0		41.1	
15	32.8	2.18 (*m*), 1.53 (*m*)	32.7	2.18 (*m*), 1.51 (*m*)
16	120.9	5.34 (*d, J* = 5.1)	121.0	5.33 (*d, J* = 5.0)
17	139.7		139.7	
18	37.5		37.4	
19	38.9	1.61 (*m*), 1.18 (*m*)	38.9	1.60 (*m*), 1.18 (*m*)
20	35.8	1.53 (*m*), 1.18 (*m*)	35.8	1.51 (*m*), 1.16 (*m*)
21	33.0		33.0	
22	46.8	2.28 (*m*), 1.65 (*m*)	46.8	2.27 (*m*), 1.63 (*m*)
23	25.0	1.74 (*m*)	25.7	1.80 (*m*)
24	180.1		181.0	
25	15.1	1.11 (*s*)	14.9	1.21 (*s*)
26	17.3	1.03 (*s*)	17.5	1.09 (*s*)
27	16.7	1.06 (*s*)	16.7	0.97 (*s*)
28	18.0	1.00 (*s*)	18.0	0.97 (*s*)
29	25.0	0.84 (*s*)	25.1	0.83 (*s*)
30	32.8	0.96 (*s*)	32.8	0.95 (*s*)

Figure 2. Key HMBC, and NOESY correlations of compound **1**.
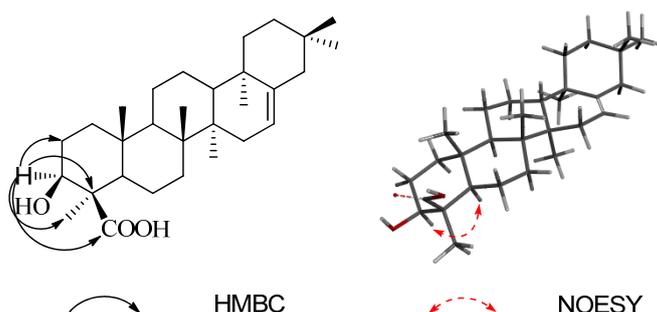
Compounds **1**–**4** were evaluated fort their α-glucosiade inhibitory activity using *p*-nitrophenyl-α-D-glucopyranoside (PNPG) as the substrate [8]. Although, not stronger than the activity of the reference drug acarbose, these compounds still exerted mild inhibitory activity against *α*-glucosidase (Table 2).

Table 2. Inhibitory effects of compounds **1**–**4** and acarbose against *α*-glucosidase [a,b].

Compound	IC$_{50}$ (μM)	Compound	IC$_{50}$ (μM)
1	1932 ± 97	**4**	2017 ± 101
2	1812 ± 85	acarbose	627 ± 28
3	1858 ± 76		

[a] IC$_{50}$, the concentration that inhibits cell growth by 50%; [b] Each value represents the mean ± S.D. (n = 3).

3. Experimental

3.1. General Procedures

Optical rotations were measured on an AA10R digital polarimeter. IR Spectra were detected on Avater-360 spectrophotometer with KBr pellets, and are reported in cm^{-1}. 1D and 2D NMR spectra (all in C$_5$D$_5$N) were recorded on a Bruker AV-400 spectrometer, and chemical shifts are expressed in δ (ppm) and referenced to the solvent peaks at δ$_H$ (8.74, 7.59, 7.22) and δ$_C$ (150.3, 135.9, 123.9) for C$_5$D$_5$N, respectively, and coupling constants are in Hz. HR-ESI-MS were determined on a Agilent 6520 Q-TOF LC-MS mass spectrometer. Semi-Preparative HPLC was performed on a Hitachi Spectra Series HPLC system equipped with an L-2130 pump and a UV L-2400 detector in a YMC-ODS column (10 mm × 250 mm, 5 μm; flow rate at 2.0 mL/min; wavelength detection at 208 nm; retention time 34.2 min for **1**, 38.0 min for **2**, 18.7 min for **3**, and 20.1 min for **4**). Column chromatography (CC) was performed on SiO$_2$ (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China) and Toyopearl HW-40C (Tosoh Bioscience Shanghai Co., Ltd., Shanghai, China). Analytical TLCs were run on silica gel plates (GF$_{254}$, Yantai Institute of Chemical Technology, Yantai, China). Fractions were monitored by TLC, and spots were visualized by heating TLC sprayed with 10% H$_2$SO$_4$.

3.2. Plant Material

The whole plant of *S. kouitchensis* was collected in Enshi, Hubei province, China, in October 2010, and identified by Prof. Jiachun Chen (Tongji Pharmaceutical School of HUST, Wuhan, China). A voucher specimen (*S.k*-2010-1010) has been deposited in the University herbarium for future reference.

3.3. Extraction and Isolation

The chopped, dried whole plants of *S. kouitchensis* (15 kg) were refluxed twice with 120 L of 95% (v/v) EtOH–H$_2$O, two hours each time. After filtration, the filtrate was concentrated under reduced pressure to yield a brownish residue (3.0 kg). Part of the residue (2.5 kg) were suspended in water and partitioned successively with petroleum ether, CH$_2$Cl$_2$, EtOAc, and *n*-butanol to afford five fractions. The CH$_2$Cl$_2$-soluble part (about 400 g) was subjected to CC (SiO$_2$, 200–300 mesh, 3.0 kg, 12 × 100 cm, petroleum ether/acetone 100:0→0:100) to yield five fractions A–E. Fraction B (87.4 g) was subjected to HW 40C (CHCl$_3$/MeOH 1:1) to give four subfractions B$_{1-4}$. B$_2$ was subjected to CC

(SiO$_2$, CHCl$_3$/ EtoAc 20:1→10:1) to give two subfractions B$_{2a}$ and B$_{2b}$. B$_{2a}$ was purified by semi-preparative HPLC (MeOH/H$_2$O 90:10) to yield compound **3** (78.5 mg) and compound **4** (13.0 mg). B$_{2b}$ was purified by semi-preparative HPLC (MeOH/H$_2$O 95:5) to yield compound **1** (4.1 mg) and **2** (5.7 mg).

3β-Hydroxy-chirat-16-en-24-oic acid (**1**). White amorphous powder. $[\alpha]_D^{25}$ +67.2° (*c* = 0.3, pyridine); IR (KBr) ν_{max} 3453, 2940, 1696, 1461, 1379, 1035 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR see Table 1; HRESIMS *m/z* 455.3525 [M−H]$^-$ (calcd. for C$_{30}$H$_{47}$O$_3$, 455.3531).

3α-Hydroxy-chirat-16-en-24-oic acid (**2**). White amorphous powder; $[\alpha]_D^{25}$ +35.3° (*c* = 0.2, pyridine); IR (KBr) ν_{max} 3448, 2934, 1701, 1456, 1378, 1036 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR see Table 1; HRESIMS *m/z* 455.3525 [M−H]$^-$ (calcd. for C$_{30}$H$_{47}$O$_3$, 455.3531).

3.4. In Vitro *Inhibitory Activity against α-Glucosidase*

α-Glucosidase (from *Saccharomyces cerevisiae*, Sigma-Aldrich, St. Louis, MO, USA) inhibitory activities were determined by using *p*-nitrophenyl-α-D-glucopyranoside (PNPG) as the substrate, according to the reported method [8]. Briefly, 20 µL of enzyme solution [0.6 U/mL α-glucosidase in 0.1 M potassium phosphate buffer (pH 6.8)] and 120 µL of the test compound in water containing 0.5% DMSO were mixed, and was preincubated for 15 min at 37 °C prior to initiation of the reaction by adding the substrate. After preincubation, PNPG solution 20 µL [5.0 mM PNPG in 0.1 M potassium phosphate buffer (pH 6.8)] was added and then incubated together at 37 °C for incubation. After the incubation, 80 µL 0.2 M Na$_2$CO$_3$ in 0.1 M potassium phosphate buffer was added to the test tube to stop the reaction. The amount of PNP released was quantified using a UVmax Kinetic Microplate Reader (Bio Tek, Synergy 2, Winooski, VT, USA) at 405 nm.

4. Conclusions

Phytochemical investigation of CH$_2$Cl$_2$-soluble part of *S. kouitchensis* afforded two new chiratane-type triterpenoids, kouitchenoids A (**1**) and B (**2**), together with two known triterpenoids, oleanolic acid (**3**) and ursolic acid (**4**). Their structures were elucidated on the basis of spectral analysis and literature comparisons. All isolated compounds **1**–**4** exhibited moderate inhibitory activities against α-glucosidase *in vitro*, comparable with that of acarbose.

Acknowledgments

Conflict of Interest

The authors declare no conflict of interest.

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Sample Availability: Samples of the compounds **1**, **3** and **4** are available from the authors.