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.
2. Results and Discussion

The 95% ethanol extract of *S. kouitchensis* whole plants was suspended in water and successively partitioned with petroleum ether, CH$_2$Cl$_2$, EtOAc and n-butanol. The CH$_2$Cl$_2$ 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 $^1$H- and $^{13}$C-NMR spectra of 1 (Table 1) closely resembled those of chiratenol [6,7], except for the appearance of a carboxyl carbon signal at $\delta_C$ 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 ($\delta_H$ 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 $\alpha$-orientation by the NOESY correlation (Figure 2) between H-3 and H-5. Therefore, 1 was deduced to be 3$\beta$-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 $^1$H-NMR and $^{13}$C-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 $\delta_H$ 3.41 (1H, dd, $J = 12.0, 4.4$ Hz) in 1 was changed into $\delta_H$ 4.75 (1H, brs, $W_{1/2} = 5.7$Hz) in 2 and the oxygenated C-3 carbon at $\delta_C$ 78.7 in 1 was shifted upfield to $\delta_C$ 71.1 in 2, suggesting that the H-3 in a $\beta$-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$_5$D$_5$N ($\delta$ in ppm, $J$ in Hz).

| Position | $^1$H  | $^1$C  | $^1$H  | $^1$C  |
|----------|--------|--------|--------|--------|
|          | $\delta$ | $J$    | $\delta$ | $J$    |
| 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 for their α-glucosidase 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
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(SiO2, CHCl3/ EtoAc 20:1→10:1) to give two subfractions B2a and B2b. B2a was purified by semi-preparative HPLC (MeOH/H2O 90:10) to yield compound 3 (78.5 mg) and compound 4 (13.0 mg). B2b was purified by semi-preparative HPLC (MeOH/H2O 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. \([\alpha]_D^{25} +67.2^\circ\) (c = 0.3, pyridine); IR (KBr) \(\nu_{\text{max}}\) 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; \([\alpha]_D^{25} +35.3^\circ\) (c = 0.2, pyridine); IR (KBr) \(\nu_{\text{max}}\) 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

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Conflict of Interest

The authors declare no conflict of interest.

References

1. State Administration of Traditional Chinese Medicine. Chinese Materia Medica; Shanghai Scientific and Technical Publishers: Shanghai, China, 1999; pp. 5573–5574.
2. Pan, X.; Lin, Y.; Li, B. A pharmaceutical composition for treating hepatitis and diabetes. CN Patent 1686141 A, 2005.
3. Wan, L.S.; Chen, C.P.; Xiao, Z.Q.; Wang, Y.O.; Min, Q.X.; Yue, Y.; Chen, J. *In vitro and in vivo* anti-diabetic activity of *Swertia kouitchensis* extract. *J. Ethnopharmacol.* **2013**, *147*, 622–630.
4. Maillard, M.; Adewunmi, C.O.; Hostettmann, K. A triterpene glycoside from the fruits of *Tetrapleura tetraptera*. *Phytochemistry* **1992**, *31*, 1321–1323.
5. Mukherjee, K.S.; Bhattacharya, M.K.; Ghosh, P.K. A triterpene acid constituent of *Salvia lanata*. *Phytochemistry* **1982**, *21*, 2416–2417.
6. Chakravarty, A.K.; Das, B.; Masuda, K.; Ageta, H. Chiratenol, a novel rearranged hopane triterpenoid from *Swertia chirata*. *Tetrahedron Lett.* **1990**, *31*, 7649–7652.
7. Guo, H.; Piao, H. Study on the anti-tumor active constituents of *Gentiana scabra*. *Huaxi Yaoxue Zazhi* **2011**, *26*, 204–207.
8. Feng, J.; Yang, X.W.; Wang, R.F. Bio-assay guided isolation and identification of α-glucosidase inhibitors from the leaves of *Aquilaria sinensis*. *Phytochemistry* **2011**, *72*, 242–247.

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

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