Triterpenoids from *Coluria longifolia*

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**ABSTRACT**

A new triterpenoid named 19-hydroxy swinhoeic acid, together with four known compounds were isolated from the whole plants of *Coluria longifolia* Maxim. Their structures were elucidated on the basis of spectroscopic data analysis and comparison with previously reported data. Cytotoxicities of the five compounds against HepG2 cell lines were also evaluated, unfortunately, no obvious activities were observed.

**1. Introduction**

*Coluria longifolia* Maxim, a member of the genus *Coluria* (Rosaceae), is a plant species unique to China. It is mainly distributed in Qinghai, Xizang and Yunnan provinces. The plants of Rosaceae are rich sources of triterpenoids (Wu et al. 2014), tannins (Feng et al. 1996) and...
flavonoids (Wang et al. 2009) with various activities, such as antitumor (Bilia et al. 1994), anti-hepatitis (Zhang et al. 2004), anti-HIV (Kashiwada et al. 1998) activity, etc. There are four species of genus *Coluria*, however, there are no phytochemical studies on genus *Coluria* to date. During our search for structurally interesting bioactive compounds from *C. longifolia*, a new ursane triterpenoid named 19-hydroxy swinhoeic acid (1), and four known ones (2–5) (see Figure 1) were isolated from 80% ethanol extract of the whole plants of *C. longifolia*. Herein, we describe the isolation, structural elucidation of the new compound and cytotoxicities of the five compounds against HepG2 cell lines.

2. Results and discussion

The 80% ethanol extract of the whole plants of *C. longifolia* (5.0 kg) was suspended in H₂O and partitioned with ethyl acetate. The obtained ethyl acetate-soluble fraction was submitted to repeated column chromatography (CC) over silica gel, reversed-phase C-18, Sephadex LH-20, and finally purified by preparative silica gel TLC, to yield the new compound (1) and four known ones (2–5).

Compound 1 was isolated as a colourless oil. The molecular formula of 1 was established as C₃₀H₄₈O₅ by negative HRESIMS at m/z [M – H]⁻ 487.3424 (Calcd 487.3429), indicating 7° of unsaturation. Its IR spectrum displayed presence of hydroxyl (3421 cm⁻¹), methyl group (2968 cm⁻¹) and carbonyl group (1696 cm⁻¹).

The ¹H NMR spectrum of 1 between δH 0.6 and δH 1.2 showed the presence of seven methyl groups, including two doublets (δH 0.87, J = 6.6 Hz; δH 1.08, J = 6.6 Hz) and five methyl singlets.
$\delta_H$ 0.73, 0.80, 0.97, 0.98, 1.02). $^1$H NMR signals also displayed the presence of three olefinic protons at $\delta_H$ 5.99 (m), 5.66 (d, $J = 12.0$ Hz) (s), 5.44 (s) and three oxygenated protons at $\delta_H$ 3.68 (m), 3.57 (t, $J = 6.0$ Hz), 2.94 (d, $J = 6.0$ Hz). The $^{13}$C and DEPT spectra revealed the presence of 30 carbon resonances, which were divided into seven methyls ($\delta_C$ 15.2, 17.1, 17.2, 19.6, 19.7, 20.4, and 29.1), seven methylenes ($\delta_C$ 19.5, 27.3, 28.3, 28.6, 33.3, 40.2, and 47.8), nine methines ($\delta_C$ 41.9, 55.7, 56.4, 69.6, 72.4, 84.6, 127.9, 129.2, and 131.5), and seven quaternary carbons ($\delta_C$ 39.0, 40.7, 41.8, 42.5, 48.5, 142.8, and 176.0). The above data indicated that 1 is a ursane triterpene with the same skeleton as swinhoeic acid (Zhao et al. 2001). Comparison of the $^1$H and $^{13}$C NMR spectroscopic data of 1 with those of the known compound swinhoeic acid showed great similarity with the exception of the carbonyl group replaced by an oxymethine. The HMBC correlations (see Figure S1) from the methyl at $\delta_H$ 0.87 and 1.08 to C-19 ($\delta_C$ 72.4) confirmed the above deduction.

In the NOESY spectrum (see Figure S2), the correlations between H-2 and H$_2$-24, H-3 with H$_3$-23 displayed that the relative configurations of oH-2 and oH-3 had α, β configurations respectively. Thus, the structure of the compound was elucidated as 18, 19-seco, 2α, 3β, 19-trihydroxy-urs-11, 13 (18) dien-28-oic acid, and named 19-hydroxy swinhoeic acid.

Comparing the physical and spectroscopic data with those reported in the literature, the known compounds were elucidated as swinhoeic acid (2) (Zhao et al. 2001), notohamosin A (3) (Luo et al. 2003), notohamosin B (4) (Luo et al. 2003), and rosamultin (5) (Miao et al. 2008).

*C. longifolia*, known in Tibetan medicine as ‘Regunba’, was traditionally used for its anti-tumor and antihepatitis activity. Therefore, all isolates were tested for their cytotoxicities against HepG2 cell lines using the MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) colorimetric assay. No significant cytotoxicities against HepG2 cell lines were observed for the evaluates (IC$_{50}$ > 100 μg/mL).

### 3. Experimental

#### 3.1. General experimental procedures

Optical rotations were measured on a Perkin-Elmer 341 digital polarimeter (Perkin-Elmer, Norwalk, CT, USA). UV spectra were recorded on a Shimadzu UV-2550 (Shimadzu, Nakagyo Ku, Kyoto, Japan). IR spectra were measured on a Bruker Vector-22 Spectrophotometer (Bruker, Fallanden, Switzerland), whereas NMR spectra were run on Bruker 600 MHz NMR spectrometers (Bruker), with chemical shifts recorded as $\delta$ values. HRESIMS were determined by a Q-TOF micro mass spectrometer (Agilent, Santa Clara, CA, USA). CC was performed using silica gel (200–300 and 100–200 mesh: Huiyou Silica Gel Development Co Ltd, Yantai, China) and Sephadex LH-20 (40–70 μm: Amersham Pharmacia Biotech Ab, Uppsala, Sweden), respectively.

#### 3.2. Plant material

The whole plants of *C. longifolia* were collected in Gongshan Mountain, Nujiang Prefecture, Yunnan province, in China, in August 2011, and identified by Professor Yuan-Chuan Zhou, the director of Nujiang Institute of Medicinal Plant. A voucher specimen is deposited in Research Center of Natural Resources of Chinese Medicinal Materials and Ethnic Medicine, Jiangxi University of Traditional Chinese Medicine (No. 2011-08-16).
3.3. Extraction and isolation

The whole plants of *C. longifolia* (5.0 kg) were powdered and extracted with 80% EtOH at room temperature for three times (each 48 h). The pooled extract (397 g) was suspended in water and partitioned with petroleum ether and ethyl acetate successively. The ethyl acetate-soluble fraction (198 g) was submitted to silica gel CC eluting with a gradient of petroleum ether/EtOAc (20:1, 12:1, 8:1, 5:1, 2:1, 100% MeOH) to give six fractions (Fr. 1–Fr. 6). Fr. 1 (10.0 g) was purified over CC (ODS, MeOH/H₂O 3:1 → 10:1), Sephadex LH-20 (MeOH) and preparative TLC (PTLC) (CHCl₃/MeOH 30:1) to provide compound 1 (12.3 mg) and 3 (8.5 mg). Fr. 3 (76.0 g) was chromatographed on silica gel, eluting with CHCl₃–MeOH (8:1), followed by Sephadex LH-20 chromatography (CHCl₃/MeOH, 1:1) and PTLC (CHCl₃/MeOH, 25:1) to afford compound 2 (43.2 mg) and 4 (7.4 mg).

Compound 5 (9.8 mg) was obtained from Fr. 5 (18.0 g) by Sephadex LH-20 chromatography (MeOH) and PTLC (CHCl₃/MeOH, 10:1).

3.3.1. 19-hydroxy swinhoeic acid (1)

Colourless oil (CH₃OH); [α]-double ²⁰D  −20 (c 0.075, MeOH); IR (KBr) ν_max 3421, 2939, 1696 cm⁻¹; ¹H (CD₂OD, 600 Hz) δ: 5.99 (m, H-12), 5.66 (m, H-11), 5.44 (s, H-18), 3.68 (m, H-2), 3.57 (t, 6.0, 12.0, H-19), 2.94 (d, 6.0, H-3), 1.08 (d, 6.0, H-29), 1.02 (s, H-24), 0.97 (s, H-25), 0.87 (d, 6.0, H-30), 0.80 (s, H-23), 0.73 (s, H-26); ¹³C NMR (CD₂OD, 150 Hz) δ: 47.8 (C-1), 69.6 (C-2), 84.6 (C-3), 40.7 (C-4), 56.4 (C-5), 19.5 (C-6), 32.0 (C-7), 41.8 (C-8), 55.7 (C-9), 39.0 (C-10), 127.9 (C-11), 131.5 (C-12), 143.8 (C-13), 42.5 (C-14), 27.3 (C-15), 28.3 (C-16), 48.5 (C-17), 129.2 (C-18), 72.4 (C-19), 41.9 (C-20), 28.6 (C-21), 40.2 (C-22), 17.2 (C-23), 29.1 (C-24), 19.7 (C-25), 17.1 (C-26), 20.4 (C-27), 179.8 (C-28), 19.6 (C-29), 15.2 (C-20); HR-ESI-MS (negative): m/z 487.3424 [M − H]⁻ (Calcd for C₃₀H₄₇O₅, 487.3429).

4. Conclusions

In this study, five triterpenoids including a new one with rare reported skeleton were obtained from the whole plants of *C. longifolia* Maxim. Their structures were elucidated by spectroscopic analysis and comparison with literature data. All isolated compounds were assayed for cytotoxicities against HepG2 cell lines, however, no obvious effects were detected.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Bilia AR, Palme E, Catalano S, Flamini G, Morelli I. 1994. New triterpenoid saponins from the roots of Potentilla tormentilla. J Nat Prod. 57:333–338.
Feng WS, Xiao XK, Ji Tian LZ, Ao Tian TN. 1996. Five hydrolyzable tannins from Potentilla discolor Bunge. Nat Prod Res Dev. 3:26–30.
Kashiwada Y, Wang HK, Nagao T, Kitanaka S, Yasuda I, Fujioka T, Yamagishi T, Cosentino LM, Kozuka M, Okabe H, et al. 1998. Anti-AIDS agents. 30. Anti-HIV activity of oleanolic acid, pomolic acid, and structurally related triterpenoids. J Nat Prod. 61:1090–1095.
Luo YG, Feng C, Tian YJ, Li BG, Zhang GL. 2003. Three novel nortriterpenoids from Notochaete hamosa Benth. (Labiatae). Tetrahedron. 59:8227–8232.
Miao Q, Bao HY, Pu SJ, Lin HW, Qiu F. 2008. Study on chemical constituents of Duchesnea indica Andr. Fock. Acad J Second Mil Med Univ. 29:1366–1370.
Wang Q, Xu DR, Shi XH, Qin MJ. 2009. Flavones from Potentilla discolor Bunge. Chin J Nat Med. 7:361–364.
Wu XP, Huang XY, Zhang XP, Ma GX, Huang Z, Yuan JQ, Xu XD, Zhong XM. 2014. Triterpenoid components from Rosa cymosa. Chin Tradit Herb Drugs. 5:626–630.
Zhang XQ, Zhao YL, Shan LM, Wei ZM, Cai GM. 2004. Study on protective mechanism of JMS on chemical liver injury. Pharm J Chin People’s Liberation Army. 4:259–261.
Zhao WQ, Ding LS, Zhang Q, Wang MK. 2001. A novel ursane triterpene from Rubus swinhoei. Chin Chem Lett. 12:245–246.