One new berberine from the branches and leaves of Polyalthia obliqua Hook.f. & Thomson

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ABSTRACT
One new alkaloid consanguine B (1), together with 10 known alkaloids (2–11), were isolated from ethanol extract of the branches and leaves of Polyalthia obliqua Hook.f. & Thomson collected in the Hainan Province, China. Their structures were elucidated by the detailed analysis of comprehensive spectroscopic data. All compounds were evaluated for their cytotoxic activities. Compound 1 showed weak cytotoxic activities against Hela and MCF-7 human cancer cell lines.

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1. Introduction
There are about 120 species of Polyalthia in the world, of which only 17 species are distributed in China and Polyalthia obliqua Hook.f. & Thomson is one of the seven Polyalthia species growing on Hainan Province, P.R. China. The genus is known for its folk medicine applications against a number of ailments such as stomach ache, dysmenorrhoea and pharynx neurosis (Jiang & Li 1979). Various parts of the genus have been used in traditional medicine to treat ailments such as stomach ache, helminthiasis, dysmenorrhoea, pharynx neurosis, fever, skin disease, diabetes and hypertension(Sari et al. 2013; Wang et al. 2014; Bhattacharya et al. 2015). There were a series of interesting compounds isolated from the genus Polyalthia, such
as alkaloids, diterpenoids, triterpenoids, sesquiterpenoids, flavonoids, styllactones furans, polyacetylenes, acetogenins and essential oils from plants of the genus (Kanokmedhakul et al. 2007; Sashidhara et al. 2010; Wang et al. 2012, 2014; Dai et al. 2014; Liu et al. 2014; Wu et al. 2014; Shuai et al. 2015; Yang et al. 2015).

In our previous work on this genus, 10 compounds were isolated from the roots of P. obliqua Hook.f. & Thomson like suberosol, marcanine A and so on (Wang et al. 2012). Two new lanostane triterpenoids were isolated from the branches and leaves of P. obliqua Hook.f. & Thomson. (Wang et al. 2014). In order to search for new bioactive compounds from this genus, bioassay-guided fractionation of the bioactive extract led to the isolation of one new alkaloid derivative consanguine B (1), together with ten known compounds (2–11) (Figure 1). This paper mainly deals with the isolation, structural characterisation and in vitro cytotoxic evaluation of isolated compound against MCF-7 and Hela cancer cell lines.

2. Results and discussion

Compound 1 was isolated as a brown, amorphous powder with $[\alpha]_D^{27} = -30.9^\circ$ (c = 0.15, CHCl$_3$). The molecular formula was determined as C$_{18}$H$_{17}$NO$_5$ (11° of unsaturation) based on high-resolution–electrospray ionisation–mass spectrometry (HR–ESI–MS), which was in agreement with the $^1$H and $^{13}$C nuclear magnetic resonance (NMR) data. The infrared (IR) spectrum display characteristic absorption attributable to hydroxyl group (3360 cm$^{-1}$) and aromatic group (1616 and 1592 cm$^{-1}$). In the $^1$H NMR spectrum, the presence of one singlet resonance for a methoxy group at $\delta_H 3.76$ (3H, s), an AB-coupling system $[\delta_H 6.61$ (1H, d, $J = 8.0$ Hz) and 6.89 (1H, d, $J = 8.0$ Hz] and two singlet protons at $\delta_H 6.77$ (1H, s) and 6.75 (1H, s) indicated four aromatic substitutions on rings A and D of the oxoprotoberberine nucleus of 1 (Figure S3). In addition, an important feature for this oxoprotoberberine alkaloid in the $^1$H NMR spectrum was a downfield-shifted proton at $\delta_H 4.64$ (H-6$\alpha$), caused by the deshielding effect of the amide and the anisotropic effect of the C-8 carbonyl group, whereas H-6$\beta$ appeared at $\delta_H 2.92$ (Hou & Hui 1991). The $^{13}$C NMR spectrum displayed 18 signals, containing 1 carbonyl, 12 aromatic ring carbon signals, 3 methylene carbon signals, a methane carbon signal, a methoxy group. All the above data indicated that 1 had a berberine skeleton (Figure S4). Comparison of the $^1$H and $^{13}$C NMR data of 1 with those of the reported

![Figure 1. Structures of compounds 1–11.](image-url)
(−)-8-oxo-10-hydroxy-2,3,9-trimethoxyberberine. (Lee et al. 2009), revealed that the structures of these two compounds were very similar except the presence of two hydroxyl δ_c (C-2 and C-9) in 1. Furthermore, hydrogen bond networks between the carbonyl group (C-8) and hydroxy (C_9–OH 12.76) was described. The HMBC correlation from the methoxy at δ_H 3.76 (s) to C-3 (δ_C 145.1, C) indicated that the methoxy group was attached to C-3 in 1 (Figure S1), instead of three methoxy groups in 8-oxo-2,9,10-trihydroxy-3-methoxyberberine. In the HMBC spectrum, the correlations from H-4 (δ_H 6.75) to C-2 (δ_C 146.5) and C-5 (δ_C 28.2), H-11 (δ_H 6.89) to C-9 (δ_C 149.4) indicated that the two hydroxyl groups at C-2 and C-9. The relative configuration of H-14 was confirmed by the NOESY spectrum, NOESY correlations assisted in the determination of the orientations of H_2-5, H_2-6 and H_2-13 as well as the positions of all the substituents (Lee et al. 2009). Cross peaks from H-14 (δ_H 4.80) to H_{13-α} (δ 3.19) in ROESY spectra of 1 suggested the H-14 was determined to have an α-orientation (Figure S2). The negative optical rotation as well as a typical 1H NMR signal at δ_H 4.80 (1H, dd, H-14) revealed that 1 adopts a 14S-configuration (α-orientation), which is in agreement with the literature reports (Pinho et al. 1992). Therefore, 1 was characterised as (−)-8-oxo-2,9,10-trihydroxy-3-methoxyberberine.

Ten known compounds were identified as pendulamine (2) (Faizi et al. 2003), marcanine A (3) (Hou & Hui 1991), 8-azabenzanthrone (4) (Tang et al. 2009), lycicamine (5) (Costa et al. 2010), liriodenine (6) (Li et al. 2009), hydroxyonychine (7) (Zhang et al. 1987), 7-hydroxy-1-4-azafluoren-9-one (8) (Tadic et al. 1988), oxylopinine (9) (Zhang et al. 1987), 6-Methoxyonychine (10) (Irie et al. 1988), isooncodine (11) (Wu et al. 1990), were identified.

Cytotoxic activities of compounds 1–11 were evaluated against Hela and MCF-7 (Table S1) cell lines using the MTT method. The results showed that 1 possessed inhibitory activities against Hela and MCF-7 cell lines with IC_{50} values of 24.1 and 33.5 μM, respectively.

3. Experimental
3.1. General
Optical rotations were measured on a JASCO P-1020 digital polarimeter. IR spectra were recorded on a Thermo Nicolet 6700 (using KBr disks) spectrophotometer (Thermo Scientific, Madison, WI, USA). 1D and 2D NMR spectra were measured on a Bruker AV-400 (Bruker Corporation, Switzerland) instrument with tetramethylsilane as the internal standard. HR–ESI–MS spectra were made on the Bruker Daltonics Apex-Ultra 7.0 T (Bruker Corporation, Billerica, MA, USA). Preperative high-performance liquid chromatography (HPLC) was used for Agilent 1260 prep-HPLC system with an Agilent C18 anal. HPLC column (4.6 × 250 mm, 5 μm) and semipreperative column (9.4 × 250 mm, 7 μm). Sephadex LH-20 (Pharmacia Co Ltd, Sandwich, UK) and Silica gel (200–300 mesh, 300–400 mesh Qingdao Marine Chemical Factory, Qingdao, China) were used for column chromatography (CC). All solvents were purchased from Xilong Chemical Reagent Factory (Guangzhou, China).

3.2. Plant materials
The branches and leaves of P. obliqua Hook.f. & Thomson were collected in Changjiang County, Hainan Province, China, in June 2012 the fruiting season and authenticated by associate Professor Qiong-Xin Zhong, College of Life Science, Hainan Normal University.
The air-dried and powdered branches and leaves of *P. obliqua* Hook.f. & Thomson (20 kg) were extracted with 75% EtOH at 50 °C (3 × 6 h). The extracts were then suspended in 2 L water and then partitioned successively with petroleum ether and ethyl acetate. The column was packed with silica gel (200–300 mesh size) and eluted in petroleum ether and ethyl acetate in a gradient manner (from 100:0 to 0:100) to generate seven fractions (Fr. 1–Fr. 7).

Fraction 3 was subjected to a silica gel CC (300–400 mesh) with petroleum ether/EtOAc (1:3) and then subjected to Sephadex LH-20 CC eluting with MeOH to obtain compounds 3 (13.0 mg), 4 (13.0 mg), 5 (6.0 mg) and 6 (15.0 mg). Fr. 4 was subjected to a silica gel CC (300–400 mesh) with petroleum ether/EtOAc (1:3), followed by HPLC column eluted with 75% MeOH/H$_2$O to give 1 (7.1 mg in $R_t$ 17.0 min), 2 (16.5 mg in $R_t$ 26.0 min). Fr. 5 was subjected to a silica gel CC (300–400 mesh) with petroleum ether/EtOAc (1:3) and then subjected to Sephadex LH-20 CC eluting with MeOH to obtain compounds 3 (13.0 mg), 4 (13.0 mg), 5 (6.0 mg) and 6 (15.0 mg). Fr. 4 was subjected to a silica gel CC (300–400 mesh) and eluted in petroleum ether and chloroform in a gradient manner (from 100:0 to 0:100). Each fraction of 50 mL was collected, affording 160 fractions to give 7 (5.6 mg), 8 (3.5 mg), 9 (6.5 mg), 10 (11.3 mg), 12 (17.2 mg).

3.3.1. **(−)-8-oxo-2,9,10-trihydroxy-3-methoxyberberine (1)**

Brown amorphous powder; IR (KBr) $v_{max}$: 3360, 1616, 1592 cm$^{-1}$; HR–ESI–MS m/z 350.1002 [M + Na]$^+$ (C$_{18}$H$_{17}$NO$_5$; calcd for 327.1008); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$: 6.77 (1H, s, H-1), 6.61 (1H, d, $J = 8.0$ Hz, H-12), 6.75 (1H, s, H-4), 6.89 (1H, d, $J = 8.0$ Hz, H-11), 4.80 (1H, dd, $J = 13.0$, 3.6 Hz, H-13), 4.64(1H, m, H-6$\alpha$), 3.76 (3H, s, –OCH$_3$), 3.19 (1H, dd, $J = 15.0$, 3.6 Hz, H-13$\alpha$), 2.92 (1H, m, H-6$\beta$), 2.76 (1H, m, H-5$\alpha$), 2.68 (1H, dd, $J = 15.0$, 13.0 Hz, H-13$\beta$), 2.76 (1H, m, H-5$\beta$); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$: 168.7 (C-8), 149.4 (C-9), 144.9 (C-3), 145.5 (C-2), 143.9 (C-10), 126.0 (C-4a), 128.8 (C-12a), 128.8 (C-14a), 116.7 (C-12), 119.0 (C-11), 112.5 (C-4), 112.1 (C-1), 111.3 (C-8a), 56.0 (3-OMe), 54.2 (C-14), 38.1 (C-6), 35.7 (C-13), 28.2 (C-5).

3.4. **Cytotoxicity assays**

Cytotoxic activity was assessed using the MTT assay against human cell line Hela and MCF-7 according to the methodology previously described in the literature. Positive control of adriamycin. The experiments were repeated three times, and the cytotoxicity was expressed as the IC$_{50}$ value, which reduces the number of viable cells by 50%.

4. **Conclusion**

One new alkaloid named consanguine B (1) together with 10 known alkaloids (2–11) were isolated from ethanol extract of the branches and leaves of *P. obliqua* Hook.f. & Thomson collected in the Hainan Island, China. The result showed that compound 1 possessed inhibitory activity against Hela and MCF-7 cell lines with IC$_{50}$ values of 24.1 and 33.5 μM, respectively.
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