New Bibenzyl Derivatives from the Tubers of *Pleione yunnanensis*

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Four new bibenzyl derivatives, shancigusins A—D (1—4) and five known bibenzyls (5—9) were isolated from the tubers of *Pleione yunnanensis* (Orchidaceae). The structures of these compounds were determined by extensive analyses of their spectroscopic data.

Key words *Pleione yunnanensis*; bibenzyl; shancigusin; structure determination

The tubers of three orchidaceous plants: *Cremastrum appendiculata* (D. DON) MAKINO, *Pleione bulbocodioides* (FRANCH.) ROLFE and *Pleione yunnanensis* ROLFE, could be used as *Pseudobulbus Cremastrae seu Pleiones* (Chinese name: “Shan-Ci-Gu”), a traditional Chinese medicine used for the treatment of tumours, burns and frostbite.1) Studies on the tubers of *Cremastrum appendiculata* led to the isolation of many phenanthrenes2); chemical researches on the tubers of *Pleione bulbocodioides* also resulted in the isolation of several bibenzyls 3—6) and dihydrophenanthrenes. 7—9) However, no detailed chemical study on *P. yunnanensis* has been reported. Our investigation on the tubers of *P. yunnanensis* resulted in the isolation of four new (1—4) and five known bibenzyls (5—9).

Shancigusin A (1) was obtained as yellow syrup. The HR-ESI-MS (m/z 441.1718, [M]/H11001) spectrum revealed the molecular formula of 1 was C28H26O5. The IR spectrum showed absorption bands at 3235 (hydroxyl), 1587 and 1512 cm−1 (benzenoids), and the UV spectrum exhibited maxima absorption at 278 nm, which were suggestive of a bibenzyl derivative.10) The 1H-NMR spectrum of 1 (see Table 1) exhibited the signals of four methylenes and thirteen aromatic protons corresponding to four aromatic rings (rings A—D, see Fig. 1). Among the thirteen aromatic protons, one singlet at δ 6.32 (1H, s, H-4) belonged to A-ring; the signals assignable to B-ring appeared at δ 6.74 (2H, d, J=8.4 Hz, H-2’, 6’) and 6.57 (2H, d, J=8.4 Hz, H-3’, 5’) due to a pair of an A2B2 system characteristic of a 1,4-disubstituted aromatic ring, which suggested that H-4’ on B-ring was substituted by a hydroxyl group; two doublets at δ 6.86 and 6.57 (4H each, both d, J=8.4 Hz), together with one singlet at δ 3.84 (4H, s, H-7’), due to two benzylic methylenes, supported the presence of two 4-hydroxybenzyl groups with a symmetrical structure. The 13C-NMR (see Table 2) spectrum also suggested that the structure of 1 was symmetrical. All these information above clearly implied that the structure of 1 was very similar to that of the reported shanciguol (5), except for the positions of the hydroxyl group on B-ring. In the heteronuclear multiple bond correlation (HMBC) spectrum, the 13C−1H long range correlation peaks between H-7’ and C-1, C-2, C-3 as well as between H-4 and C-2, C-6 were observed. These data unambiguously established the structure of 1 to be 2,6-bis-(4-hydroxybenzyl)-3,4’,5-trihydroxybibenzyl.

Shancigusin B (2) was obtained as yellow syrup. The HR-ESI-MS (m/z 426.1814, [M]/H11001) spectrum revealed the molecular formula of 2 was C28H26O4. The 1H-NMR spectrum of 2 (see Table 1) was similar to that of 1, except for the appearance of one more aromatic proton attributed to H-4’ on B-ring. The signals appeared at δ 7.13 (2H, t, J=7.2 Hz, H-3’, 5’), 7.04 (1H, t, J=7.2 Hz, H-4’), and 6.92 (2H, d, J=7.2 Hz,
The tubers of *P. bulbocodioides* and compounds 2—6) were isolated and characterized as 2,6-bis(4-hydroxybenzyl)-3,5-dihydroxybibenzyl.

Shancigusin C (3) was obtained as red syrup. Its molecular formula was established as C_{21}H_{20}O_{4} by HR-EI-MS. The 1H-NMR (see Table 1) spectrum of compound 3 showed that the structure of 3 was similar to 2 except for the appearance of one more aromatic proton at A-ring. This proton was assigned as a 1-substituted aromatic ring at 6.57 (1H, d, 8.4 Hz, H-56), due to two benzylic methylenes, and six aromatic protons attributed to the bibenzyl. Among these six aromatic protons, one pair of 4-hydroxybenzyl group, four aliphatic protons attributable to the bibenzyl, and the remaining aromatic proton was assigned to a 1-substituted aromatic ring at 6.34 (1H, d, 2.0 Hz, H-46).

**Experimental**

**General** 1H-, 13C- and 2D-NMR spectra were measured on a Varian INOVA-600 spectrometer (1H at 600 MHz and 13C at 150 MHz) in MeOH-d_{4}, except for the 1H-NMR spectrum of compound 3 on a Varian INOVA-500 spectrometer at 500 MHz. Chemical shifts are given in δ values (ppm) relative to tetramethylsilane (TMS) as an internal standard. HR-ESI-MS spectra of 2—4 were measured with a Micromass Autospec Ultima-Tof spectrometer and HR-ESI-MS-MSSP spectrum of 1 was measured on Jeol JMS-T100CS AccuTOF CS spectrometer. UV spectra were measured with a Shimadzu UV-2550 UV–VIS recording spectrometer. IR spectra were recorded with a Shimadzu FTIR-8400S infrared spectrometer. Silica gel (300—400 mesh, Qingdao Marine Chemical Factory) and Sephadex LH-20 (Pharmacia) were used for column chromatography, and silica gel GF_{254} plates (Yantai Marine Chemical Co., Ltd.) were used for thin-layer chromatography. Preparative HPLC was carried out on a Waters SymmetryPrep Prep C_{18} column (7.8×300 mm) with a Waters HPLC system (pump: DSC; detector: DUAL λ ABSORBANCE detector).

**Plant Materials** The tubers of *P. yunnanensis* were collected in Guizhou Province and identified by Prof. Shun-Xing Guo of the Institute of
Extraction and Isolation  The crushed tubers of *P. yunnanensis* (4.7 kg) were refluxed with 95% EtOH (3×30) and 70% EtOH (2×30), 3 h each time, respectively. The combined EtOH extract was concentrated under reduced pressure at 60 °C to afford a dark-brown residue (600 g). The residue was diluted with H2O and partitioned successively with petroleum ether, CHCl3, CH3COAc and n-BuOH. The CH3COAc fraction (37 g) was first subjected to silica gel column chromatography, eluted with CHCl3–MeOH (100:0→0:100, v/v) gradient to afford several subfractions. The subfractions were further purified by Sephadex LH-20, followed by preparative HPLC (MeOH–H2O), to give compounds 1 (2 mg), 2 (2 mg), 3 (3 mg), 4 (5 mg), 5 (40 mg), 6 (30 mg), 7 (12 mg), 8 (65 mg), and 9 (70 mg).

Shancigusin A (1): Yellow syrup (MeOH). IR (KBr) cm⁻¹: 3235, 1587, 1512. UV λ max (MeOH) nm (log ε): 278 (4.04). HR-ESI-MS m/z: 441.1718 [M–H]⁻ (Calcd for C28H25O5: 441.1707). ¹H-NMR (600 MHz, MeOH-d₄) and ¹³C-NMR (150 MHz, MeOH-d₄) see Tables 1 and 2.

Shancigusin B (2): Yellow syrup (MeOH). IR (KBr) cm⁻¹: 3251, 1597, 1512. UV λ max (MeOH) nm (log ε): 281 (4.09). HR-ESI-MS m/z: 426.1814 [M]⁺ (Calcd for C28H26O4: 426.1831). ¹H-NMR (600 MHz, MeOH-d₄) and ¹³C-NMR (150 MHz, MeOH-d₄) see Tables 1 and 2.

Shancigusin C (3): Red syrup (MeOH). IR (KBr) cm⁻¹: 3203, 1591, 1512. UV λ max (MeOH) nm (log ε): 279 (3.77). HR-ESI-MS m/z: 336.1339 [M]⁺ (Calcd for C21H20O4: 336.1362). ¹H-NMR (500 MHz, MeOH-d₄) and ¹³C-NMR (150 MHz, MeOH-d₄) see Tables 1 and 2.

Shancigusin D (4): Red syrup (MeOH). IR (KBr) cm⁻¹: 3198, 1599, 1512. UV λ max (MeOH) nm (log ε): 280 (3.86). HR-ESI-MS m/z: 320.1400 [M]⁺ (Calcd for C21H20O₃: 320.1412). ¹H-NMR (600 MHz, MeOH-d₄) and ¹³C-NMR (150 MHz, MeOH-d₄) see Tables 1 and 2.

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References