New Bibenzyl Derivatives from the Tubers of *Pleione yunnanensis*

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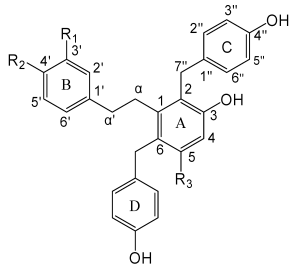
Four new bibenzyl derivatives, shancigusins A—D (1—4) and five known bibenzyls (5—9) were isolated from the tubers of *Pleione yunnanensis* (Orchidaceae). The structures of these compounds were determined by extensive analyses of their spectroscopic data.

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

The tubers of three orchidaceous plants: *Cremastra 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 *Cremastra appendiculata* led to the isolation of many phenanthrenes[2)]; 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**). The new compounds were given the name shancigusins A, B, C, and D, respectively, and their structures were determined by extensive analyses of their spectroscopic data including 1D-, 2D-NMR, and HR-MS. This paper will report the isolation and identification of these new bibenzyl derivatives.

Shancigusin A (**1**) was obtained as yellow syrup. The HR-ESI-MS (*m/z* 441.1718, $[M-H]^-$) and NMR data revealed that the molecular formula of **1** was $C_{28}H_{26}O_5$. 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 ^{1}H-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 A_2B_2 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 ^{13}C-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 ^{13}C–^{1}H 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-EI-MS (*m/z* 426.1814, $[M]^+$) spectrum revealed the molecular formula of **2** was $C_{28}H_{26}O_4$. The ^{1}H-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,

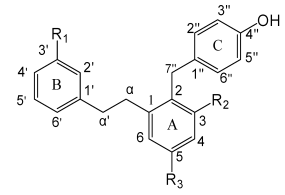


1 R_1 = H R_2 = R_3 = OH

2 R_1 = R_2 = H R_3 = OH

5 R_1 = R_3 = OH R_2 = H

6 R_1 = OH R_2 = H R_3 = OCH_3



3 R_1 = R_2 = R_3 = OH

4 R_1 = H R_2 = R_3 = OH

7 R_1 = OH R_2 = OCH_3 R_3 = OH

8 R_1 = R_2 = OH R_3 = OCH_3

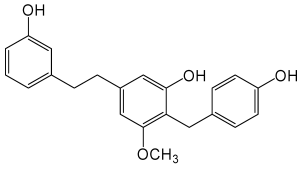


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Fig. 1. Structures of Compounds **1**—**9**

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Table 1. ^{1}H-NMR Data of Compounds **1**—**4** in MeOH-d_4 (**1**, **2**, **4** at 600 MHz, **3** at 500 MHz)

Position	1	2	3	4
4	6.32 (1H, s)	6.34 (1H, s)	6.23 (1H, d, 2.0)	6.17 (1H, d, 2.4)
6			6.19 (1H, d, 2.0)	6.13 (1H, d, 2.4)
2′	6.74 (1H, d, 8.4)	6.92 (1H, d, 7.2)	6.55 (1H, br s)[b]	6.98 (1H, d, 7.2)
3′	6.57 (1H, d, 8.4)[a]	7.13 (1H, t, 7.2)		7.14 (1H, t, 7.2)
4′		7.04 (1H, t, 7.2)	6.57 (1H, br d, 8.0)	7.06 (1H, t, 7.2)
5′	6.57 (1H, d, 8.4)[a]	7.13 (1H, t, 7.2)	7.03 (1H, t, 8.0)	7.14 (1H, t, 7.2)
6′	6.74 (1H, d, 8.4)	6.92 (1H, d, 7.2)	6.54 (1H, br d, 8.0)[b]	6.98 (1H, d, 7.2)
2″, 6″	6.86 (4H, d, 8.4)	6.87 (4H, d, 8.4)	6.94 (2H, d, 8.0)	6.87 (2H, d, 9.0)
3″, 5″	6.57 (4H, d, 8.4)[a]	6.57 (4H, d, 8.4)	6.64 (2H, d, 8.0)	6.58 (2H, d, 9.0)
7″	3.84 (4H, s)	3.86 (4H, s)	3.85 (2H, s)	3.78 (2H, s)
α	2.54—2.57 (2H, m)	2.58—2.62 (2H, m)	2.65—2.69 (2H, m)	2.61—2.64 (2H, m)
α'	2.16—2.20 (2H, m)	2.24—2.28 (2H, m)	2.52—2.55 (2H, m)	2.51—2.55 (2H, m)

Assignments were confirmed by HSQC and HMBC spectra. *J* values in Hz are given in parentheses. *a*) Overlapped signals. *b*) Unresolved signals.

Table 2. ^{13}C-NMR Data of Compounds **1**—**4** at 150 MHz in MeOH-d_4

Position	1	2	3	4
1	142.9	142.7	144.0	143.9
2	118.9	118.9	118.6	118.6
3	155.6	155.6	157.5	157.5
4	101.5	101.6	101.4	101.3
5	155.6	155.6	157.0	157.1
6	118.9	118.9	108.8	108.8
1′	134.7	143.8	145.0	143.4
2′	130.1[a]	129.3	116.2	129.4
3′	116.0	129.2	158.3	129.2
4′	156.3	126.7	113.7	126.7
5′	116.0	129.2	130.2	129.2
6′	130.1[a]	129.3	120.7	129.4
1″	134.6	134.6	134.4	134.4
2″, 6″	130.1[a]	130.1	130.1	130.1
3″, 5″	115.9	115.9	115.9	115.8
4″	155.9	156.0	155.9	156.0
7″	31.2	31.2	30.7	30.7
α	33.8	33.5	36.5	36.6
α'	36.9	37.7	38.6	38.6

Assignments were confirmed by HSQC and HMBC spectra. *a*) Overlapped signals.

H-2′, 6′) due to a 1-subsitituted aromatic ring strongly supported the assumptions above. Taking all these analyses into consideration, the structure of **2** was established 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 ^{1}H-NMR (see Table 1) spectrum of **3** exhibited the resonances of one pair of 4-hydroxybenzyl group, four aliphatic protons due to two benzylic methylenes, and six aromatic protons attributed to the bibenzyl. Among these six aromatic protons, two appeared at δ 6.23 and 6.19 (1H each, both d, J=2.0 Hz, H-4, 6) due to two *m*-coupled protons on A-ring, assignable to H-4 and H-6 from their chemical shifts and splitting patterns; the other four, which appeared at δ 7.03 (1H, t, J=8.0 Hz, H-5′), 6.57 (1H, br d, J=8.0 Hz, H-4′), 6.55 (1H, br s, H-2′) and 6.54 (1H, br d, J=8.0 Hz, H-6′), deduced the presence of a 1,3-disubstituted aromatic ring (B-ring). These data revealed that the structure of **3** was quite similar to that of **7**,[11] except for the absence of the methoxyl group on A-ring. All the information above established the structure of **3** to be 2-(4-hydroxybenzyl)-3,3′,5-trihydroxybibenzyl. The signal assignments and the locations of the functional groups were confirmed by heteronuclear single quantum coherence (HSQC) and HMBC spectra.

Shancigusin D (**4**) was obtained as red syrup. The HR-EI-MS revealed the molecular formula of **4** was $C_{21}H_{20}O_3$. The ^{1}H-NMR (see Table 1) of **4** showed that the structure of **4** was similar to **3**, except for the appearance of one more aromatic proton attached to B-ring. That is to say, there were no substitutions on B-ring in compound **4**. The signals assignable to a 1-substituted aromatic ring at δ 7.14 (2H, t, J=7.2 Hz, H-3′, 5′), 7.06 (1H, t, J=7.2 Hz, H-4′) and 6.98 (2H, d, J=7.2 Hz, H-2′, 6′) were in good agreement with these assumptions. Therefore, the structure of compound **4** was elucidated as 2-(4-hydroxybenzyl)-3,5-dihydroxybibenzyl.

It's noteworthy that the new bibenzyls shancigusins A—D (**1**—**4**) possessed only hydroxyl and 4-hydroxybenzyl substituents, which was not common in bibenzyl derivatives. In addition to the four new bibenzyls, five known bibenzyls were also isolated and characterized as 2,6-bis(4-hydroxybenzyl)-3,3′,5-trihydroxybibenzyl (**5**),[3] 3,3′-dihydroxy-2,6-bis(4-hydroxybenzyl)-5-methoxybibenzyl (**6**),[10] 3′,5-dihydroxy-2-(4-hydroxybenzyl)-3-methoxybibenzyl (**7**),[11] 3,3′-dihydroxy-2-(4-hydroxybenzyl)-5-methoxybibenzyl (**8**)[11] and 3,3′-dihydroxy-4-(4-hydroxybenzyl)-5-methoxybibenzyl (**9**),[11] by comparisons with previously reported physical and spectral data. Among them, compound **5** was isolated previously from *P. bulbocodioides* and compounds **6**—**9** were obtained previously from *Bletilla striata*. All of these compounds were isolated from *P. yunnanensis* for the first time.

Experimental

General ^{1}H-, ^{13}C- and 2D-NMR spectra were measured on a Varian INOVA-600 spectrometer (^{1}H at 600 MHz and ^{13}C at 150 MHz) in MeOH-d_4, except for the ^{1}H-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-EI-MS spectra of **2**—**4** were measured with a Micromass Autospec Ultima-Tof spectrometer and HR-ESI-MS 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 (Pharmazia) was 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^{TM} 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

Medicinal Plant Development, Peking Union Medical College. A voucher specimen (No. YNDSL-2006) was deposited in the herbarium of the Institute of Medicinal Plant Development.

Extraction and Isolation The crushed tubers of *P. yunnanensis* (4.7 kg) were refluxed with 95% EtOH (3×30 l) and 70% EtOH (2×30 l), 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 H_2O and partitioned successively with petroleum ether, $CHCl_3$, EtOAc and *n*-BuOH. The EtOAc fraction (37 g) was first subjected to silica gel column chromatography, eluted with $CHCl_3$–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–H_2O), 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 $C_{28}H_{25}O_5$: 441.1707). ^{1}H-NMR (600 MHz, MeOH-d_4) and ^{13}C-NMR (150 MHz, MeOH-d_4) 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-EI-MS *m*/*z*: 426.1814 $[M]^+$ (Calcd for $C_{28}H_{26}O_4$: 426.1831). ^{1}H-NMR (600 MHz, MeOH-d_4) and ^{13}C-NMR (150 MHz, MeOH-d_4) 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-EI-MS *m*/*z*: 336.1339 $[M]^+$ (Calcd for $C_{21}H_{20}O_4$: 336.1362). ^{1}H-NMR (500 MHz, MeOH-d_4) and ^{13}C-NMR (150 MHz, MeOH-d_4) 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-EI-MS *m*/*z*: 320.1400 $[M]^+$ (Calcd for $C_{21}H_{20}O_3$: 320.1412). ^{1}H-NMR (600 MHz, MeOH-d_4) and ^{13}C-NMR (150 MHz, MeOH-d_4) see Tables 1 and 2.

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