Pergamon S0031-9422(95)00068-4 *Phytochemistry*, Vol. 42, No. 3, pp. 853–856, 1996

0031-9422/96 $15.00 + 0.00

STILBENOIDS FROM *PLEIONE BULBOCODIOIDES*

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(*Received* 1 *November* 1995)

Key Word Index—*Pleione bulbocodioides*; Orchidaceae; tubers; (bibenzyldihydrophenanthrene) ether; benzyldihydrophenanthrene; bibenzyl.

Abstract—From tubers of *Pleione bulbocodioides*, three novel stilbenoids, one a (bibenzyldihydrophenanthrene) ether, one a dihydrophenanthrene and one a bibenzyl, were isolated together with lusianthridin, coelonin, and 2,7-dihydroxy-1-(*p*-hydroxybenzyl)-4-methoxy-9,10-dihydrophenanthrene. The novel compounds, named shancilin, shancidin and shanciguol, were shown to be 7-*O*-[4′-(3′,3″-dihydroxy-5′-methoxybibenzyl)]-4-hydroxy-2-methoxy-9,10-dihydrophenanthrene,4,7-dihydroxy-1-(*p*-hydroxybenzyl)-2-methoxy-9,10-dihydrophenanthrene and 2,6-bis(*p*-hydroxybenzyl)-3,3′, 5-trihydroxybibenzyl, respectively, by their spectroscopic data. One of them, shancilin, having an ether linkage between the dihydrophenanthrene and bibenzyl is the first member of a novel group of stilbenoids.

INTRODUCTION

In a previous paper, we reported on the isolation and characterization of dihydrophenanthropyrans [1] from *Pleione bulbocodioides* (Chinese name 'Shan ci-gu'). Further investigation of the same source has resulted in the isolation of three new stilbenoids, shancilin (**1**), shancidin (**2**) and shanciguol (**3**) together with lusianthridin [2], coleonin [3] and 2,7-dihydroxy-1-(f-hydroxybenzyl)-4-methoxy-9,10-dihydrophenanthrene [4]. The structures were determined on the basis of spectral data.

RESULTS AND DISCUSSION

Shancilin (**1**) showed UV maxima at 206, 220 and 280 nm. The IR spectrum exhibited absorptions at 3300(OH), 1595 and 1460 cm^{-1} (benzenoids). The mass spectrum exhibited a $[M]^+$ at *m/z* 484 ($C_{30}H_{28}O_6$) and significant peaks at *m/z* 377 resulting from the loss of a hydroxybenzyl moiety (C_7H_7O) from the $[M]^+$, and at *m/z* 107 $[C_7H_7O]^+$ due to a hydroxybenzyl moiety. The ^{13}C NMR spectrum showed signals for two ethylenes, two methoxyls and 24 aromatic carbons, of which 11 were protonated, six were quaternary and seven bore oxygen; all of which were completely assigned by assistance of the HMQC spectrum. Acetylation of **1** with acetic anhydride and pyridine afforded a triacetate ($[M]^+$ *m/z* 610), indicating the presence of three hydroxyl groups, whose signals were located at δ 4.59, 4.61 and 5.25 in the ^1H NMR spectrum. As the total number of oxygens for hydroxyl and methoxyl groups was five and no carbonyl absorption was observed in the IR spectrum, the remaining oxygen was likely to be an ether. In the ^1H NMR spectrum, the presence of the dihydrophenanthrene moiety was confirmed by the appearance of the typical signals due to an ABX system of H-6, H-8 and H-5 found in dihydrophenanthrene derivatives [5] at δ 6.71 (*dd*, J = 8.6 and 2.6 Hz), 6.74 (*d*, J = 2.6 Hz) and 7.92 (*d*, J = 8.6 Hz), a pair of doublets at δ 6.34 (*d*, J = 2.3 Hz) and 6.42 (*d*, J = 2.3 Hz) due to H-1 and H-3, and a further multiplet at δ 2.68–2.70 due to H-9 and H-10, which on irradiation led to enhancement of the signals for H-1 and H-8. Furthermore, the ^1H NMR spectrum of **1** revealed the presence of a bibenzyl moiety as shown by a singlet at δ 2.73 of four protons characteristic of an ethylene linkage of bibenzyl, and two isolated spin systems of six aromatic protons, of which two protons appeared as a pair of doublets at δ 6.29 (*d*, J = 2.8 Hz) and 6.41 (*d*, J = 2.8 Hz) due to the H-2′ and H-6′, and the remaining four protons appeared as a set of signals for the 1″, 3″-disubstituted pattern at δ 6.39 (*t*, J = 2.3 and 1.5 Hz), 6.60 (*dd*, J = 8.1 and 2.3 Hz), 6.65 (*dd*, J = 7.7 and 1.5 Hz) and 7.07 (*t*, J = 8.1 and 7.7 Hz) due to H-2″, H-4″, H-6″ and H-5″. Signals for two methoxyl groups appeared at δ 3.76 and 3.78. All signal assignments were performed by ^1H–^1H COSY and NOE. Irradiation of the ethylene signal at δ 2.73 caused enhancement of H-2′, H-6′, H-2″ and H-6″. The positions of the methoxyl groups were established by analysing the NOE results. On irradiation of one methoxyl group at δ 3.78, NOEs for H-1 and H-3 were observed. While on irradiation of the other at δ 3.76, only a NOE for H-6′ was observed. These results confirmed that **1** has methoxyl groups at

C-2 on the dihydrophenanthrene moiety and at C-5′ on the bibenzyl moiety. Additionally, the location of three hydroxyl groups were determined by the acetylation shifts; in the ^{1}H NMR spectrum of **1** acetate, the signal of H-3 was shifted downfield by 0.23 ppm and of H-5 to high field by the shielding effect of the acetyl group on C-4 for the dihydrophenanthrene moiety, while the signals for H-2′, H-2″ and H-4″ were shifted strikingly downfield by 0.23, 0.39 and 0.27 ppm for the bibenzyl moiety, compared with those for the corresponding protons of **1** (see Experimental). That is to say, the locations of hydroxyl groups were at C-3′, C-3″ and C-4. The connection of the dihydrophenanthrene to the bibenzyl was at C-7–C-4′.

From the above results, the structure of **1** was determined to be 7 - *O* - [4′ - (3′,3″ - dihydroxy - 5′ - methoxybibenzy)] - 4 - hydroxy - 2 - methoxy - 9,10 - dihydrophenanthrene.

It is noteworthy that **1** consisted of dihydrophenanthrene and bibenzyl moieties connected by an ether linkage in the molecule. Thus, although bidihydrophenenthrene ethers and bisbibenzyl ethers had been isolated from *Bletilla striata* [6, 7], *Marchantia polymorpha* [8] and *Pellia epiphylla* [9], etc., respectively, no dihydrophenanthrene-bibnenzyl ether had ever been isolated. It is the first member of a novel group of stilbenoids, and **1** may be regarded as a chemotaxonomic marker of *Pleione* species.

Shancidin (**2**) was obtained as colourless needles, mp 188–189°. The UV spectrum showed absorption maxima characteristic of the dihydrophenanthrenene series at 253, 279 and 299 nm. The IR spectrum had absorptions at 3250 (OH), 1580 and 1425 cm^{-1} (benzenoids). The mass spectrum exhibited peaks at *m/z* 348 ($[M]^+$, $C_{22}H_{20}O_4$) and at *m/z* 107 $[C_7H_7O]^+$. Acetylation of **2** afforded a triacetate ($[M]^+$ *m/z* 474), indicating the presence of three hydroxyl groups. The ^{1}H NMR spectrum showed that **2** consisted of a methoxyl group (δ 3.76); a *p*-hydroxybenzyl unit (δ 6.64, *d*, J = 8.5 Hz and 6.88, *d*, J = 8.5 Hz due to H-3′, 5′ and H-2′, 6′, and δ 3.90, *s*, due to a benzylic methylene) and a dihydrophenanthrene moiety, whose signals were similar to those of **1**: i.e. a multiplet at δ 2.13–2.61 due to H-9 and H-10, two doublets and a multiplet at δ 6.60 (*d*, J = 3.0 Hz), 8.14 (*d*, J = 8.6 Hz) and 6.62* due to H-6, H-5 and H-8, and additionally a singlet at δ 6.47 instead of a pair of doublets due to H-1 and H-3 of **1**, which indicated that the *p*-hydroxybenzyl group was located at either C-1 or C-3. This assumption was supported by NOE enhancements. In the NOE experiments, an appreciable NOE between H-10 and benzylic methylene was observed, indicating a *p*-hydroxybenzyl group at C-1, and the signal at δ 6.47 could be assigned to the H-3. On the other hand, on irradiation at the methoxyl signal, the NOEs were observed for H-3 and the benzylic methylene, indicating the methoxyl group at C-2 and hence two hydroxyls at C-4 and C-7. The location of the hydroxyl groups was also supported by the acetylation shifts (see Experimental). Therefore, **2** is assigned to be 4,7-dihydroxy-1-(*p*-hydroxybenzyl)-2-methoxy-9,10-dihydrophenanthrene.

At the same time, a structural isomer of shancidin, already known as 2,7-dihydroxy-1-(*p*-hydroxybenzyl)-4-methoxy-9,10-dihydrophenanthrene, was obtained and directly identified with the authentic sample [4]. It is noteworthy that the dihydrophenanthrenes from this plant possess methoxyl groups at C-2 or 4, while only the dihydrophenanthrenes having a methoxyl group at C-4 were obtained from *B. striata*.

Shanciguol (**3**) showed UV maxima at 206, 254sh and 281 nm suggesting **3** to be a bibenzyl [10]. The IR spectrum exhibited absorptions at 3200 (OH), 1590 and 1495 cm^{-1} (benzenoids). The mass spectrum exhibited a $[M]^+$ at *m/z* 442 ($C_{28}H_{26}O_5$) and significant peaks at *m/z* 336 and 230 due to sequential cleavages of two hydroxybenzyl moieties. Acetylation of **3** gave a pentaacetate ($[M]^+$ *m/z* 652) indicating the presence of five hydroxyl groups. The ^{1}H NMR spectrum showed two doublets at δ 6.63 (4H, J = 8.6 Hz) and 6.93 (4H, J = 8.6 Hz) due to two pairs of an A_2B_2 system characteristic of a *p*-substituted aromatic ring, and one singlet at δ 3.95 (4H) due to two benzylic methylenes, supporting the presence of two *p*-hydroxybenzyl groups with a symmetrical structure. In addition, the ^{1}H NMR spectrum exhibited the signals of five aromatic protons of a bibenzyl. Among them, four appeared at δ 6.47 (*d*, J = 7.7 Hz), 6.51 (*t*, J = 2.1 and 1.9 Hz), 6.56 (*ddd*, J = 7.7, 2.6 and 1.9 Hz) and 7.01 (1H, *t*, J = 7.7 Hz), assignable to H-6′, H-2′, H-4′ and H-5′ on one aromatic ring from their chemical shifts and splitting patterns; the remaining one appeared as a singlet at δ 6.39 due to a proton on the other aromatic ring. Furthermore, the signals assignable to two methylenes of the bibenzyl appeared at δ 2.24–2.30 (2H) and 2.62–2.68 (2H) as two multiplets. Usually, as the methylene groups (ϕ–CH_2–CH_2–ϕ) are observed as one multiplet for four protons [10], the splitting to multiplets may be explained by the influence of large substituents at C-2 and C-6. Finally, considering the symmetry of two *p*-hydroxybenzyl units and the marked downfield shift of H-4 (Δ0.56 ppm) in the **2** acetate, the two *p*-hydroxybenzyls had to be located at C-2 and C-6, and the remaining hydroxyls at C-3 and C-5 for both sides of H-4 and at C-3′. This conclusion was further supported by NOE enhancements (Fig. 1) and was consistent with the ^{13}C NMR spectra. On the basis of the above findings, the structure of **3** is established as 2,6-bis(*p*-hydroxybenzyl)-3,3′,5-trihydroxybibenzyl. The known compounds, lusianthridin and coelonin, were identified by comparison of their spectra data with reported values [2, 3].

EXPERIMENTAL

Mps: uncorr.; IR: KBr; UV: MeOH; ^{1}H NMR and ^{13}C NMR: 500 and 125 MHz, respectively, MeOH-d_3 with TMS as int. standard. * peak are overlapped and

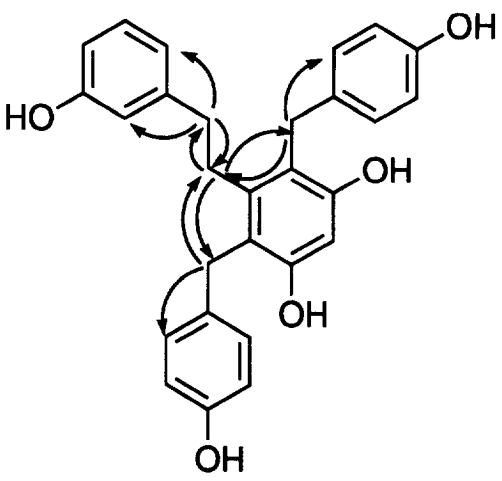


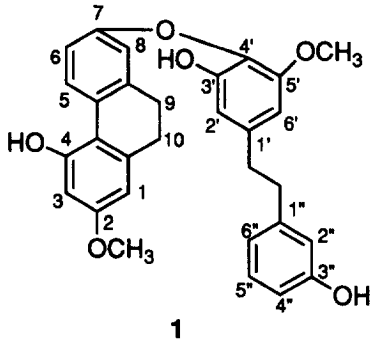
Fig. 1. Compound **3**; observed NOEs are indicated by arrows.

not resolved. MS: EIMS, 70 eV. CC and TLC were performed using Merck silica gel.

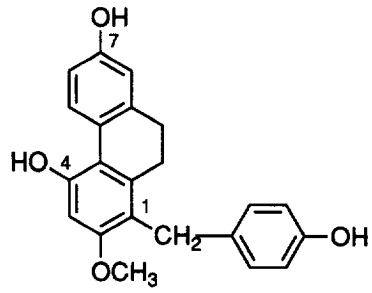
Plant materials. See ref. [1].

Extraction and isolation. See ref. [1]; fr. 3 was rechromatographed over silica gel, LH-20 and Cosmosil C_{18} to give **1** (3 mg) and **2** (44 mg). Fr. 6 was rechromatographed over HP-20, silica gel, LH-20 and Cosmosil C_{18} to give **3** (19 mg).

Compound **1**. Powder. IR ν_{max} cm^{-1}: 3300, 1595, 1460; UV λ_{max} nm (log ε): 206 (4.65), 220 (4.43), 280 (4.18); MS *m/z* (rel. int.): 484 (100), 377 (6), 242 (21), 107 (5); ^{1}H NMR ($CDCl_3$): δ 2.68–2.70 (4H, *m*, $-CH_2-CH_2-$, H-9, 10), 2.73 (4H, *s*, $\phi-CH_2-CH_2-\phi$), 3.76 (3H, *s*, 5′-OMe), 3.78 (3H, *s*, 2-OMe), 4.59 (1H, *s*, OH), 4.61 (1H, *s*, OH), 5.25 (1H, *s*, OH), 6.29 (1H, *d*, J = 2.8 Hz, H-2′), 6.34 (1H, *d*, J = 2.3 Hz, H-1), 6.39 (1H, *t*, J = 2.3, 1.5 Hz, H-2″), 6.41 (1H, *d*, J = 2.8 Hz, H-6′), 6.42 (1H, *d*, J = 2.3 Hz, H-3), 6.60 (1H, *dd*, J = 8.1, 2.3 Hz, H-4″), 6.65 (1H, *dd*, J = 7.7, 1.5 Hz, H-6″), 6.71 (1H, *dd*, J = 8.6, 2.6 Hz, H-6), 6.74 (1H, *d*, J = 2.6 Hz, H-8), 7.07 (1H, *t*, J = 8.1, 7.7 Hz, H-5″), 7.92 (1H, *d*, J = 8.6 Hz, H-5); ^{13}C NMR: δ 30.0 (*t*, C-9 or C-10), 30.5 (*t*, C-9 or C-10), 32.1 (*t*, $\phi-\underline{C}H_2-CH_2-\phi$), 36.1 (*t*, $\phi-CH_2-\underline{C}H_2-\phi$), 55.3 (*q*, 2-OMe), 56.1 (*q*, 5′-OMe), 98.9 (*d*, C-3), 100.9 (*d*, C-1), 106.7 (*d*, C-6′), 108.0 (*d*, C-2′), 110.6 (*d*, C-8), 112.7 (*d*, C-6), 112.9 (*d*, C-4″), 114.8 (*s*, C-4a), 115.4 (*d*, C-2″), 120.9 (*d*, C-6″), 126.0 (*s*, C-4′), 126.9 (*d*, C-5), 129.4 (*d*, C-5″), 135.2 (*s*, C-8a), 136.9 (*s*, C-1a), 140.0 (*s*, C-5a), 141.3 (*s*, C-1′), 143.6 (*s*, C-1″), 153.0 (*s*, C-3′), 153.2 (*s*, C-5′), 153.6 (*s*, C-7), 155.5 (*s*, C-3″), 157.1 (*s*, C-4), 158.8 (*s*, C-2). Triacetate: powder. ^{1}H NMR ($CDCl_3$): δ



1



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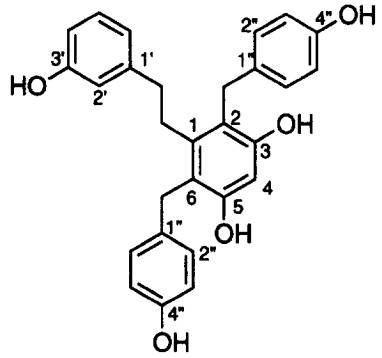

3

2.23 (3H, *s*, OAc), 2.26 (3H, *s*, OAc), 2.31 (3H, *s*, OAc), 2.71–2.75 (4H, *m*, $-CH_2-CH_2-$, H-9, 10), 2.81 (4H, *s*, $\phi-CH_2-CH_2-\phi$), 3.75 (3H, *s*, 5′-OMe), 3.80 (3H, *s*, 2-OMe), 6.52 (1H, *d*, $J = 2.8$ Hz, H-2′), 6.60 (1H, *d*, $J = 2.6$ Hz, H-1), 6.65 (1H, *d*, $J = 2.6$ Hz, H-3), 6.67 (1H, *d*, $J = 2.8$ Hz, H-6′), 6.68 (1H, *dd*, $J = 8.2$, 2.6 Hz, H-6), 6.71 (1H, *d*, $J = 2.6$ Hz, H-8), 6.78 (1H, *m*, H-2″), 6.87 (1H, *dd*, $J = 8.0$, 2.1 Hz, H-4″), 6.91 (1H, *d*, $J = 7.7$ Hz, H-6″), 7.21 (1H, *t*, $J = 8.0$, 7.7 Hz, H-5″), 7.76 (1H, *d*, $J = 8.2$ Hz, H-5). MS *m/z* (rel. int.): 610 $[M]^+$ (100), 568 (98), 526 (48), 484 (13), 241 (13).

Compound **2**. Needles, mp 188–189° (Me_2CO). IR ν_{max} cm^{-1}: 3250, 1580, 1425; UV λ_{max} nm (log ε): 253 (3.87), 279 (4.35), 294 (4.12), 299 (4.13); MS *m/z* (rel. int.): 348 (100), 253 (6), 107 (16); ^1H NMR: δ 2.13–2.61 (4H, *m*, $-CH_2-CH_2-$, H-9, 10), 3.76 (3H, *s*, 2-OMe), 3.90 (2H, *s*, $\phi-CH_2-\phi$), 6.47 (1H, *s*, H-3), 6.60 (1H, *d*, $J = 3.0$ Hz, H-8), 6.62* (1H, *m*, H-6), 6.64 (2H, *d*, $J = 8.5$ Hz, H-3′,5′), 6.88 (2H, *d*, $J = 8.5$ Hz, H-2′,6′), 8.14 (1H, *d*, $J = 8.6$ Hz, H-5). Triacetate: powder. ^1H NMR ($CDCl_3$): δ 2.26 (3H, *s*, OAc), 2.28 (3H, *s*, OAc), 2.29 (3H, *s*, OAc), 2.68 (4H, *s*, $-CH_2-CH_2-$, H-9, 10), 3.81 (3H, *s*, 2-OMe), 4.09 (2H, *s*, $\phi-CH_2-\phi$), 6.59 (1H, *s*, H-3), 6.93 (2H, *d*, $J = 8.1$ Hz, H-3′,5′), 6.94 (1H, *d*, $J = 2.8$ Hz, H-8), 6.95 (1H, *dd*, $J = 8.8$, 2.8 Hz, H-6), 7.09 (2H, *d*, $J = 8.1$ Hz, H-2′,6′), 7.86 (1H, *d*, $J = 8.8$ Hz, H-5); MS *m/z* (rel. int.): 474 $[M]^+$ (61), 432 (74), 390 (100), 348 (32), 254 (86), 107 (12).

Compound **3**. Yellow powder. IR ν_{max} cm^{-1}: 3200, 1590, 1495; UV λ_{max} nm (log ε): 206 (4.76), 254 sh (3.52), 281 (3.96); MS *m/z* (rel. int.): 442 (10), 336 (94), 230 (30), 137 (50), 107 (100); ^1H NMR: δ 2.24–2.30 (2H, *m*, $-\underline{CH_2}-CH_2-$), 2.62–2.68 (2H, *m*, $-CH_2-\underline{CH_2}-$), 3.95 (4H, *s*, $\phi-CH_2-\phi$), 6.39 (1H, *s*, H-4), 6.47 (1H, *d*, $J = 7.7$ Hz, H-6′), 6.51 (1H, *t*, $J = 2.1$, 1.9 Hz, H-2′), 6.56 (1H, *ddd*, $J = 7.7$, 2.6, 1.9 Hz, H-4′), 6.63 (4H, *d*, $J = 8.6$ Hz, H-3″, 5″), 6.93 (4H, *d*, $J = 8.6$ Hz, H-2″, 6″), 7.01 (1H, *t*, $J = 7.7$ Hz, H-5′); ^{13}C NMR: δ31.3 (*t*, $\phi-\underline{CH_2}-\underline{CH_2}-\phi$), 33.4 (*t*, $\phi-CH_2-\phi$), 37.7 (*t*, $\phi-CH_2-\phi$), 101.8 (*d*, C-4), 113.8 (*d*, C-4′), 115.9 (*d*, C-3″, 5″), 116.1 (*d*, C-2′), 119.1 (*s*, C-2, 6), 120.6 (*d*, C-6′), 130.1 (*d*, C-2″, 6″), 130.2 (*d*, C-5′), 134.6 (*s*, C-1″), 142.8 (*s*, C-1′), 145.4 (*s*, C-1), 155.6 (*s*, C-4″), 155.7 (*s*, C-3, 5), 158.3 (*s*, C-3′). Pentaacetate: powder. ^1H NMR ($CDCl_3$): δ 2.13 (6H, *s*, OAc × 2), 2.24 (3H, *s*, OAc × 2), 2.26 (3H, *s*, OAc), 2.46–2.49 (2H, *m*, $-\underline{CH_2}-CH_2-$), 2.85–2.87 (2H, *m*, $-CH_2-\underline{CH_2}-$), 3.99 (4H, *s*, $\phi-CH_2-\phi$), 6.67 (1H, *t*, $J = 2.1$, 1.7 Hz, H-2′), 6.84 (1H, *d*, $J = 7.5$ Hz, H-4′), 6.87 (1H, *d*, $J = 8.0$ Hz, H-6′), 6.95 (1H, *s*, H-4), 6.97 (4H, *d*, $J = 8.6$ Hz, H-3″, 5″), 7.08 (4H, *d*, $J = 8.6$ Hz, H-2″, 6″), 7.22 (1H, *t*, $J = 7.5$, 8.0 Hz, H-5′); MS *m/z* (rel. int.): 652 $[M]^+$ (60), 610 (73), 568 (100), 526 (70), 484 (19), 466 (4), 446 (4), 241 (40), 107 (93).

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