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SHANCIOL, A DIHYDROPHENANTHROPYRAN FROM *PLEIONE BULBOCODIOIDES*

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Key Word Index—*Pleione bulbocodioides*; Orchidaceae; tubers; shanciol; dihydrophenanthropyrans; dihydrophenanthrene; absolute configurations.

Abstract—A novel dihydrophenanthropyran, shanciol was isolated from tubers of *Pleione bulbocodioides*, together with the known compound bletilol B. The structure of the new compound was elucidated as 3-hydroxy-11-methoxy-2-(4′-hydroxy-3′-methoxy-phenyl)-3,4,5,6-tetrahydro-4H-phenanthro[2, 1-*b*]pyran-8-ol on the basis of spectroscopic data and X-ray analysis. Furthermore, the absolute configurations of bletilol B were determined to be 11*S* and 12*S* by means of Horeau's partial resolution method and chemical correlations.

INTRODUCTION

In the course of our systematic chemical examination of the chemical constituents of Orchidaceae, we have demonstrated the occurrence of biphenanthrenes [1, 2], bis(phenanthrene)ethers [3, 4], phenanthrenes [2, 5], dihydrophenanthropyrans [6] and phenanthrenylspirolactone [7] in *Bletilla striata*. Investigation of the the tubers of *Pleione bulbocodioides* (crude drug name, Shan ci-gu), which have been used in traditional Chinese medicine to treat tumours [8], has resulted in the isolation of one new dihydrophenanthropyran, named shanciol, along with the known compound, bletilol B [6]. In this paper we report on the structural elucidation of shanciol and the determination of the absolute configuration of bletilol B as 11*S* and 12*S*, the relative stereochemistry has previously been elucidated by spectroscopic as data [6].

RESULTS AND DISCUSSION

Shanciol (**1**) was obtained as plates. Its UV spectrum showed absorption maxima at 250, 280 and 300 nm, indicative of a dihydrophenanthrene [9]. The infrared spectrum exhibited absorptions at 3250 (OH), 1590 and 1490 cm^{-1} (benzenoids). The mass spectrum exhibited a $[M]^+$ at *m/z* 420 ($C_{25}H_{24}O_6$) and a significant peak at *m/z* 402 $[M - OH]^+$. The ^{13}C NMR spectrum displayed signals for all 25 carbons in the molecule: one ethylene, one methylene, two methoxyls and two methines bearing oxygen, along with 18 aromatic carbons, of which seven were protonated, six quaternary and five bearing oxygen. Acetylation of **1** yielded a triacetate $[M]^+$ *m/z* 546) whose ^1H NMR spectrum contained two signals at δ 1.98 (3H) and δ 2.30 (6H), suggesting the presence of one secondary and two phenolic hydroxyl groups. As the total number of oxygen atoms needed for the hydroxyl and methoxyl groups was five, this suggested that the remaining oxygen was in an ether group. The ^1H NMR spectrum of **1** showed the signals for four protons as one multiplet at δ2.62, typical of the H-9 and H-10 of dihydrophenanthrene derivatives and seven aromatic protons including two sets of an ABX system and one singlet. The signals forming an ABX system at δ6.62 (*dd*, J = 8.5, 2.5 Hz), δ6.64 (*d*, J = 2.5 Hz) and δ7.99 (*d*, J = 8.5 Hz) due to H-6, H-8, H-5 and a singlet at δ6.49(*s*) due to H-3 were similar to those of corresponding dihydrophenanthrenes, in particular those of H-5 which were characteristically downfield [9]. The signals at δ6.82 (*d*, J = 8.1 Hz), δ6.89 (*d*, J = 8.1, 1.7 Hz) and δ7.01 (*d*, J = 1.7 Hz) due to H-6′, H-5′ and H-2′ formed the second ABX system, suggesting the presence of a phenyl group with a 1′,3′,4′-trisubstitution pattern. The signal assignments and the location of the functional groups were confirmed by NOE enhancements and acetylation shifts. Irradiation of the methoxyl group at δ3.81 gave NOE enhancement of H-3 and H-5. Irradiation of the other at δ3.86 caused enhancement of the H-2′ signal. These results revealed that the methoxyl groups were located at C-4 on the dihydrophenanthrene moiety and C-3′ on the phenyl group, respectively, and hence that there was a phenolic hydroxyl group at C-7 on the former and at C-4′ on the latter. This substitution pattern was consistent with the observations that the signals due to H-6,

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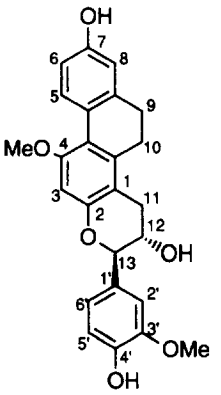

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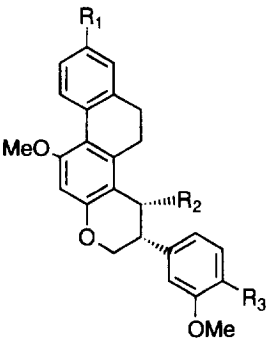

	R_1	R_2	R_3
2	OH	OAc	OH
2a	OH	OH	OH
3	OMe	OAc	OMe
4	OMe	OH	OMe

Table 1. The COLOC correlations of compound **1**

C	H
1, 2	3
7	5
10a	11
13	2′
4′	2′, 6′

H-8 and H-5′ were shifted largely to lower fields in the ^{1}H NMR spectrum of the triacetate.

In addition, the ^{1}H NMR spectrum showed signals for two methines bearing oxygen at δ4.66 (*d*, J = 7.8 Hz, H-13ax), and 4.10 (*ddd*, J = 8.4, 7.8, 5.5 Hz, H-12ax), together with those for one methylene coupled with H-12 at δ2.67 (*dd*, J = 15.7, 8.4 Hz, H-11ax), and δ2.96 (*dd*, J = 15.7, 5.5 Hz, H-11eq). This methylene appeared to be adjacent to C-1, because an enhancement of H-10 was observed on irradiation of H-11ax in the NOE experiment. In the ^{13}C NMR spectrum, signals at δ32.5 (*t*), 69.3 (*d*) and 82.9 (*d*) corresponding to C-11, C-12 and C-13 further proved the presence of these moieties. Therefore, this unit was considered to be a benzopyran involving C-1 and C-2, to which the secondary hydroxyl and 4′-hydroxy-3′-methoxyphenyl groups were attached at the C-12 and C-13 positions, respectively, based on analysis of the long-range COLOL correlations (Table 1).

The relative stereochemistry of the C-12 and C-13 substituents was supposed to be *trans* from the coupling constant (J = 7.8 Hz) between H-12 and H-13 [10], and this was clearly confirmed by X-ray analysis. An ORTEP drawing of compound **1** is shown in Fig. 1. In the crystal used for X-ray analysis, compound **1** existed as a racemate. The crystal was found in the upper side of a small test tube. In the bottom of the small test tube, microcrystals with a different shape from the racemic crystals

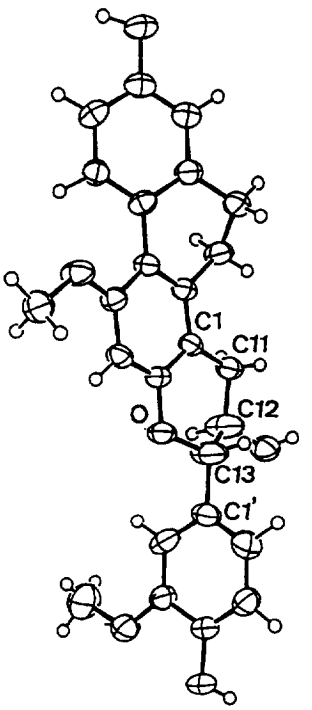

Fig. 1. ORTEP drawing of shanciol.

appeared. Compound **1** has an optical rotation of − 21.8. It is suggested that first the racemic crystals grew and then the optically active molecules crystallized as they became predominant. The molecule of **1** takes the form of a 9,10-dihydrophenanthrene ring, distorted from the plane, with a dihedral angle of 26.4(3)° between the benzene rings. This distortion is consistent with the estimation from the crystal structures in the series of 4,5-disubstituted 9,10-dihydrophenanthrenes [11]. The plane of the *p*-hydroxy-*m*-methoxyphenyl group is perpendicularly linked to the chroman moiety with a dihedral angle of 72.9(3)° between the benzene rings.

More details of the X-ray analysis are given in the Experimental.

On the basis of these findings, shanciol was shown to have structure **1**. In order to compare the spectral data with those of other phenanthrenes, we used the phenanthrene numbering system instead of the systematic nomenclature. Thus shanciol should be called 3-hydroxy-11-methoxy-2-(4′-hydroxy-3′-methoxy-phenyl)-3,4,5,6-tetrahydro-4H-phenanthro[2,1-*b*]pyran-8-ol.

Compound **2**, $C_{27}H_{26}O_7$, was concluded to be dihydrophenanthrene from its UV spectrum. The ^{1}H NMR spectrum of **2** showed the presence of a dihydrophenanthropyran skeleton with one acetyl and one 1′,3′,4′-trisubstituted phenyl group in the molecule. In the ^{1}H NMR spectrum, the signal patterns and chemical shifts closely resembled those of **1**, except for the difference in the chemical shifts of the pyran ring protons. The downfield shift of H-11 and the upfield shift of the neighbouring H-12 suggested that the acetyl and phenyl groups were located at C-11 and C-12, respectively. An alternative structure in which the acetyl and phenyl groups in the pyran ring were placed at C-12 and C-13 was rejected following comparison of the deacetylate **2a** with that of **1**. Compound **2** was finally shown to be identical with bletilol B by direct comparison with an authentic sample, which was isolated previously in our laboratory from *Bletilla striata* [6]. However, only the relative stereochemistry at C-11 and C-12 of **2** has been elucidated to be *cis* by spectroscopic data, its absolute configurations remained unclarified. Therefore, it was necessary to elucidate its absolute configurations. Because bletilol B (**2**) was isolated in larger amounts (83 mg), it was possible to determine the absolute configurations at C-11 and C-12 positions by Horeau's partial resolution method [12–13].

Compound **2** afforded the dimethyl ether **3** on methylation, which was further converted to compound **4** by deacetylation in the presence of 2% NaOH. Compound **4** was then treated with two equivalents of racemic 2-phenylbutanoic anhydride in pyridine. The 2-phenylbutanoic acid formed was found to be laevorotatory. Consequently, application of Horeau's rules shows that the C-11 bearing the hydroxyl group has *S* configuration. The absolute stereochemistry at C-11 and C-12 of compound **4** is thus 11*S*,12*S*. Therefore, bletilol B (**2**) is (11*S*,12*S*)4-acetoxy-11-methoxy-3-(4′-hydroxy-3′-methoxy-phenyl)-3,4,5,6-tetrahydro-2H-phenanthro[2,1-*b*]pyran-8-ol.

This is the first report of the isolation of a dihydrophenanthropyran with a 4H-[2,1-*b*]pyran system.

EXPERIMENTAL

Mps; uncorr.; IR: KBr; UV: MeOH; ^{1}H NMR and ^{13}C NMR: 500 and 125 MHz, respectively, MeOH-d_3 with TMS. The peaks marked with an asterisk are overlapped and not resolved. MS; EIMS, 70eV; CC and TLC. Merck silica gel.

Plant materials. Tubers of *P. bulbocodioides* were obtained as a crude drug in China and identified by Prof. J. S., Li, Faculty of Pharmaceutical Sciences, China Traditional University. A voucher specimen is kept in our laboratory.

Extraction and isolation. The crushed drug (20 kg) was extracted with MeOH at room temp. After evapn of the solvent, the residue was diluted with H_2O and partitioned successively with EtOAc and *n*-BuOH. The EtOAc extract (99 g) was subjected to CC on silica gel using CH_2Cl_2–EtOAc to EtOAc–MeOH with increasing amounts of EtOAc and MeOH, respectively, to give 10 frs. Fr. 4 was rechromatographed over silica gel, LH-20 and Cosmosil C_{18} to give **1** (8 mg) and **2** (83 mg).

Compound **1**. Plates from MeOH, mp 263–265°. $[\alpha]_D$ −20.8. IR ν_{max} cm^{-1}: 3250, 1595, 1440; UV λ_{max} nm (log ε): 205 (4.71), 250 (3.73), 280 (4.35), 300 (4.18); MS *m/z* (rel. int.): 420 (72), 402 (6), 255 (100); ^{1}H NMR δ2.62 (4H, *m*, $-CH_2-CH_2-$, H-9, 10), 2.67 (1H, *dd*, J = 15.7, 8.4 Hz, H-11ax), 2.96 (1H, *dd*, J = 15.7, 5.5 Hz, H-11eq), 3.81 (3H, *s*, 4-OMe), 3.86 (3H, *s*, 3′-OMe), 4.10 (1H, *ddd*, J = 8.4, 7.8, 5.5 Hz, H-12ax), 4.66 (1H, *d*, J = 7.8 Hz, H-13ax), 6.49 (1H, *s*, H-3), 6.62 (1H, *dd*, J = 8.5, 2.5 Hz, H-6), 6.64 (1H, *d*, J = 2.5 Hz, H-8), 6.82 (1H, *d*, J = 8.1 Hz, H-5′), 6.89 (1H, *dd*, J = 8.1, 1.7 Hz, H-6′), 7.01 (1H, *d*, J = 1.7 Hz, H-2′), 7.99 (1H, *d*, J = 8.5 Hz, H-5); ^{13}C NMR: δ26.5 (*t*, C-9), 30.7 (*t*, C-10), 32.5 (*t*, C-11), 56.0 (*q*, 4-OMe), 56.5 (*q*, 3′-OMe), 69.3 (*d*, C-12), 82.9 (*d*, C-13), 99.8 (*d*, C-3), 111.4 (*s*, C-1′), 112.1 (*d*, C-2), 113.7 (*d*, C-8), 114.8 (*s*, C-6), 116.1 (*d*, C-5′), 118.8 (*s*, C-4a), 121.5 (*d*, C-6′), 126.1 (*s*, C-5a), 130.4 (*d*, C-5), 132.0 (*s*, C-1), 139.9 (*s*, C-10a), 140.4 (*s*, C-8a), 147.7 (*s*, C-4′), 149.0 (*s*, C-3′), 154.8 (*s*, C-2), 156.4 (*s*, C-7), 157.6 (*s*, C-4).

Triacetate. Needles from MeOH, mp 90–91°. ^{1}H NMR ($CDCl_3$): δ1.93 (3H, *s*, OAc), 2.30 (6H, *s*, OAc × 2), 2.62 (2H, *m*, $-CH_2-$), 2.72 (2H, *m*, $-CH_2-$), 2.75 (1H, *dd*, J = 15.8, 6.4 Hz, H-11), 3.00 (1H, *dd*, J = 15.8, 5.6 Hz, H-11), 3.81 (3H, *s*, 4-OCH_3), 3.86 (3H, *s*, 3′-OCH_3), 5.11 (1H, *d*, J = 6.6 Hz, H-13), 5.40 (1H, *dd*, J = 6.6, 6.4 Hz, H-12), 6.56 (1H, *s*, H-3), 6.94* (2H, *m*, H-6, 6′), 6.96 (1H, *d*, J = 2.1 Hz, H-8), 7.01 (1H, *d*, J = 2.9 Hz, H-2′), 7.02 (1H, *d*, J = 8.6 Hz, H-5′), 8.22 (1H, *d*, J = 9.4 Hz, H-5); MS *m/z* (rel. int.): 546 $[M]^+$ (28), 504 (18), 462 (6), 402(18).

Methylation of **2**. A mixture of **2** (30 mg), MeI (20 mg) and anhydrous K_2CO_3 (15 mg) in dry Me_2CO (8 ml) was refluxed for 4 hr with stirring. After filtration, the soln was concd to dryness, and the residue chromatographed over silica gel using $CHCl_3$ as solvent to afford dimethyl ether **3** (25 mg) as a colourless oil. ^{1}H NMR: δ2.07 (3H, *s*, OAc), 2.71 (4H, *m*, $-CH_2-CH_2-$), 3.71 (1H, *ddd*, J = 9.5, 4.3, 3.6 Hz, H-12), 3.82 (3H, *s*, 3′-OCH_3), 3.84 (3H, *s*, 4-OCH_3), 3.86 (3H, *s*, OCH_3), 3.87 (3H, *s*, OCH_3), 4.12 (1H, *dd*, J = 11.0, 9.5 Hz, H-13), 4.49 (1H, *dd*, J = 11.0, 4.3 Hz, H-13), 5.58 (1H, *d*, J = 3.6 Hz, H-11), 6.53 (1H, *s*, H-3), 6.77 (1H, *d*, J = 2.7 Hz, H-8), 6.80 (1H, *dd*, J = 2.7, 8.9 Hz, H-6), 6.83 (1H, *d*, J = 8.6 Hz, H-5′), 6.84 (1H, *d*, J = 2.1 Hz, H-2′), 6.89 (1H, *dd*, J = 2.1, 8.6 Hz, H-6′), 8.14 (1H, *d*, J = 8.9 Hz, H-5); MS *m/z* (rel. int.): 490 (100), 430 (63).

Alkaline hydrolysis of **3**. NaOH 2% (2 ml) was added to a soln of **3** (25 mg) in Me_2CO (4 ml) and stirred at

room temp. for 16 hr. After removal of the Me_2CO, the reaction mixture was neutralized with 1 M HCl and extracted with EtOAc. Removal of the solvent gave a residue which was purified by chromatography over a silica gel column with $CHCl_3$ to yield **4** (17 mg) as an oil, $[\alpha]_D$ − 8.3 (benzene; *c* 0.72). ^{1}H NMR: δ2.66 (4H, *m*, $-CH_2-CH_2-$), 3.46 (1H, *ddd*, *J* = 9.0, 3.8, 3.2 Hz, H-12), 3.60 (1H, *dd*, *J* = 11.2, 9.0 Hz, H-13), 3.78 (3H, *s*, 4′-OCH_3), 3.79 (3H, *s*, 7-OCH_3), 3.80 (3H, *s*, 3′-OCH_3), 3.85 (3H, *s*, 4-OCH_3), 3.87 (1H, *dd*, *J* = 3.8, 11.2 Hz, H-13), 5.67 (1H, *d*, *J* = 3.2 Hz, H-11), 6.57 (1H, *s*, H-3), 6.73 (1H, *d*, *J* = 2.5 Hz, H-8), 6.75 (1H, *dd*, *J* = 2.5, 9.0 Hz, H-6), 6.90–6.91* (2H, H-2′, 5′), 6.93 (1H, *s*, H-6′), 8.08 (1H, *d*, *J* = 9.0 Hz, H-5); MS *m/z* (rel. int.): 448 (100), 430 (56), 417 (27), 215 (13).

Determination of the absolute configurations by Horeau's partial resolution method. Racemic 2-phenylbutanoic anhydride (26 mg) was added to a soln of **4** (16 mg) in pyridine (1 ml) and the mixture was left to stand at room temp. for 16 hr. H_2O (1 ml) was then added and the mixture stirred for a further 1 hr. The reaction mixture was then diluted with H_2O (3 ml), extracted with benzene (4 ml × 3), and the benzene extract extracted with 5% Na_2CO_3 aq. (4 ml × 3). The free acid (10.6 mg) was obtained after acidification of the alkaline extract followed by extraction with benzene (4 ml × 3). The residual acid had a specific rotation of − 8.8 (*c* 0.48, benzene).

X-Ray analysis of shanciol. Shanciol was crystallized from MeOH. The crystal data are as follows; $C_{25}H_{24}O_6$, M = 420.46, monoclinic space group C2/C, *a* = 24.818 (4), *b* = 11.668(1), *c* = 15.386(2) Å, β = 112.50(1)°, V = 4114(1) Å^3, *z* = 4, Dc = 1.358 g cm^{-3}, μ (CuK_α = 7.54 cm^{-1}. 3418 Reflections with $2\theta < 126°$ were collected on a Rigaku AFC-5R (40 kv, 200 mA), 3334 of which were unique (R_{int} = 0.034). The structure was solved by direct methods and refined anisotropically by full-matrix least-squares. All hydrogen atoms were located on the difference Fourier map. The final refinement including hydrogen atoms with isotropic temperature factors reduced the *R* value to 0.062 (unit weight) for 1997 reflections with Fo > 2σ(Fo). The final atomic parameters, bond lengths and angles are deposited in the Cambridge Crystallographic Data Centre.

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