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BIPHENANTHRENES FROM *BLETILLA STRIATA*

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Key Word Index—*Bletilla striata*; Orchidaceae; antibacterial activity; biphenanthrene; bidihydrophenanthrene; blestriarenes A–C; batatasin III; 3′-O-methylbatatasin III.

Abstract—From tubers of *Bletilla striata*, three new biphenanthrenes were isolated, together with batatasin III and 3′-O-methylbatatasin III. The structures were elucidated from their spectroscopic data.

INTRODUCTION

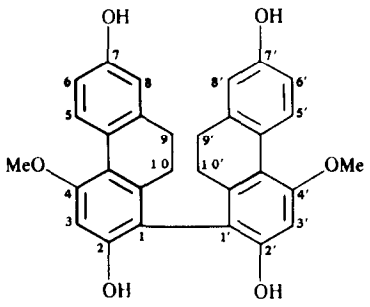
The tubers of *Bletilla striata* Reichb. fil (crude drug name, 'Bai Ji') have been used in the treatment of pneumonorrhagia and pneumonophthisis [1]. In a previous paper, we reported the structural determination of five new antibacterial compounds, two dihydrophenanthrenes and three bibenzyls from the ethyl acetate extract of this plant [2]. Further investigations of the ethyl acetate extract have now led to the isolation of three new biphenanthrenes, blestriarenes A–C, together with the known compounds, batatasin III and 3′-O-methyl batatasin III. In this paper, we report the isolation and characterization of the three new biphenanthrenes **1**–**3**.

RESULTS AND DISCUSSION

The ethyl acetate soluble portion of the methanolic extract [2] of *B. striata* was repeatedly chromatographed over silica gel and LH-20 to give blestriarene A (**1**), B (**2**), C (**3**) and two known compounds.

Blestriarene A **1** was obtained as colourless needles, mp 194–195°, $[\alpha]_D$ −5.1° and gave a positive ferric chloride test and a greenish-blue colour with sulphuric acid. The UV spectrum showed absorption maxima at 273, 283 and 300 nm suggesting that the compound was a dihydrophenanthrene [3]. The IR spectrum showed absorptions at 3200 (OH), 1570 and 1440 cm^{-1} (benzenoids). Acetylation of **1** afforded a tetraacetate ($[M]^+$ *m/z* 650) indicating the presence of four hydroxyl groups. The mass spectrum of **1** exhibited a $[M]^+$ at *m/z* 482 and a significant peak at *m/z* 241 ($C_{15}H_{13}O_3$) corresponding to the fragment $[M/2]^+$, suggesting **1** to be a bis dihydrophenanthrene $C_{30}H_{26}O_6$. The ^1H NMR spectrum confirmed the dimeric structure with a symmetrical coupling of dihydrophenanthrenes since there was no doubling up of signals from the two moieties present; a singlet at δ6.57 due to H-3, H-3′, a doublet at δ8.05 (J = 8.8 Hz) due to H-5, H-5′, a doublet of doublets at δ6.63 (J = 8.8 and 2.8 Hz) due to H-6, H-6′ and a doublet at δ6.59 (J = 2.8 Hz) due to H-8, H-8′. Additionally, the ^1H NMR spectrum of **1** showed signals for two methoxyl groups at δ3.88 and multiplets at δ2.21–2.59 assignable to two ethylene groups. NOE enhancement was used to determine the assignment of signals and the position of the functional groups. Irradiation of the methoxyl group at δ3.88, caused NOE with H-3, H-3′ (19%) and H-5, H-5′ (5%), confirming that the methoxyl groups are at C-4 and C-4′. The ^{13}C NMR spectrum of **1** also suggested the dimeric structure since there were signals due to 15 types of carbon atom (Table 1). Thus, the structure of blestriarene A was established as 4,4′-dimethoxy-9,9′,10,10′-tetrahydro-[1,1′-biphenanthrene]-2,2′,7,7′-tetrol.

Blestriarene B (**2**) was obtained as a colourless powder, mp 313–316°, $[\alpha]_D$ −3.2° and gave a positive ferric chloride test and a greenish-blue colour with sulphuric acid. The UV spectrum showed absorption maxima at 266, 290, and 312 nm similar to that of phenanthrene derivatives [3]. The IR spectrum exhibited absorptions at 3280 (OH), 1570 and 1440 cm^{-1} (benzenoids). The mass



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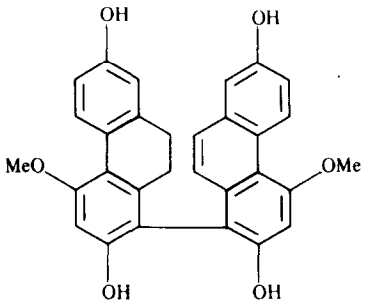

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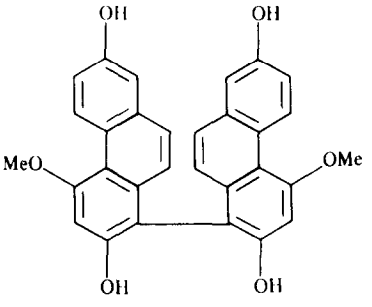

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Table 1. ^{13}C NMR* data of 1–3

C		1	3	C	2
1	1′	115.7	111.0	1	115.0
2	2′	158.6	157.8	2	160.3
3	3′	99.4	99.6	3	100.3
4	4′	155.5	153.2	4	155.4
5	5′	130.3	128.7	5	130.5
6	6′	113.6	110.7	6	113.6
7	7′	156.1	154.0	7	155.4
8	8′	114.8	116.6	8	114.8
9	9′	31.0	126.7	9	30.9
10	10′	28.3	124.7	10	28.3
1a	1a′	140.8	132.4	1a	140.8
4a	4a′	117.6	114.2	4a	117.8
5a	5a′	126.6	123.5	5a	126.6
8a	8a′	141.7	133.4	8a	142.4
OMe		56.1	56.1	1′	112.3
				2′	158.9
				3′	99.5
				4′	154.0
				5′	130.4
				6′	112.1
				7′	154.0
				8′	117.4
				9′	128.5
				10′	125.6
				1a′	134.6
				4a′	116.9
				5a′	125.8
				8a′	134.9
				OMe	55.6

* 125 MHz in MeOH-d_4, TMS as int. standard.

spectrum of **2** showed a $[M]^+$ at m/z 480 ($C_{30}H_{24}O_6$) and a significant fragment at m/z 240 arising from cleavage of the 1–1′ bond followed by the disproponation of a proton. The presence of four hydroxyl groups was confirmed by the formation of a tetraacetate ($[M]^+$ m/z 648). The ^{1}H NMR spectrum showed signals for 10 aromatic protons; a singlet at δ6.65 due to H-3, a singlet at δ6.92 due to H-3′, a doublet at δ8.09 (J = 8.8 Hz) due to H-5, a doublet at δ9.43 (J = 9.0 Hz) due to H-5′, a doublet of doublets at δ6.64 (J = 8.8 and 2.8 Hz) due to H-6, a doublet of doublets at δ7.08 (J = 9.0 and 2.8 Hz) due to H-6′, a doublet at δ6.56 (J = 2.8 Hz) due to H-8, a doublet at δ7.10 (J = 2.8 Hz) due to H-8′, and doublets at δ7.40 (J = 9.2 Hz) and δ7.18 (J = 9.2 Hz) assignable to H-9′ and H-10′ of phenanthrene [3]. Furthermore, signals for an ethylene group and two methoxyl groups were observed in the ^{1}H NMR spectrum. It was, therefore, suspected that blestriarene B may be composed of dihydrophenanthrene and phenanthrene moieties. The signal assignments were confirmed by NOE enhancement and comparison with those of blestriarene A **1**. Selective irradiation of the methoxyl group at δ3.93 gave rise to NOE enhancement of the H-3 (19%) and H-5 (3%); irradiation of the other methoxyl group at δ4.13 caused enhancement of signals from both H-3′ (20%) and H-5′ (5%). Thus, two methoxyl groups were located at C-4 and C-4′. On the basis of the above observations, the structure of blestriarene B was assigned as 4,4′-dimethoxy-9,10-dihydro-[1,1′-biphenanthrene]-2,2′,7,7′-tetrol. The ^{13}C NMR spectral data (Table 2) also supported the structure of **2**.

Blestriarene C **3** was obtained as yellow needles, mp 331–334°, $[\alpha]_D$ −16.7, and gave a positive ferric chloride test and a violet colour with sulphuric acid. The IR spectrum showed the presence of hydroxyl groups and benzenoids; the UV spectrum was very similar to that of blestriarene B. The mass spectrum gave a $[M]^+$ at m/z 478 corresponding to a formula $C_{30}H_{22}O_6$ suggesting a biphenanthrene. The ^{1}H NMR showed signals based on a symmetrically coupled dimer of phenanthrenes; a singlet at δ6.93 due to H-3, H-3′, a doublet at δ9.40 (J = 9.2 Hz) due to H-5, H-5′, a double of doublets at δ7.03 (J = 9.2 and 2.8 Hz) due to H-6, H-6′, a doublet at δ7.00 (J = 2.8 Hz) due to H-8, H-8′, a doublet at δ7.23 (J = 9.2 Hz) due to H-9, H-9′ and a doublet at δ 6.92 (J = 9.2 Hz) due to H-10, H-10′. NOE enhancements were observed for H-3, H-3′ (21%), and H-5, H-5′ (4%) on irradiation of C-4 and C-4′ methoxyl groups, providing evidence for the substitution pattern present in blestriarene C. Thus, the structure of blestriarene C was established as 4,4′-dimethoxy-[1,1′-biphenanthrene]-2,2′,7,7′-tetrol. This structure was further supported by ^{13}C NMR spectral data (Table 1). The known compounds, batatasin III and 3′-*O*-methylbatatasin III were identified by comparison of spectral data with reported values [4].

The *in vitro* antimicrobial activity of the five compounds isolated are given in Table 2. The compounds were active against the Gram positive bacteria, *Staphylococcus aureus* and *Streptococcus mutans* which cause dental caries. Blestriarene B showed the most potent activity against the test organisms.

Table 2. Antibacterial activity *in vitro* (MIC; μg/ml)

		Streptococcus mutans						
Test compds	*Staphylococcus aureus*	HS1* (a)†	FA1 (b)	Ingritt (c)	C67-1 (c)	OMZ176 (d)	OMZ175 (f)	6715 (g)
Blestriarene A	25	12.5	50	25	6.25	25	50	25
Blestriarene B	12.5	6.25	25	6.25	6.25	6.25	12.5	25
Blestriarene C	50	25	100	50	50	50	100	50
Batatasin III	—	—	—	100	50	—	—	—
3-*O*-Methylbatatasin III	—	50	—	—	50	50	—	100

* Strains of *S. mutans*.
† Serotype.

EXPERIMENTAL

Mps: uncorr.; UV: MeOH; ^{1}H and ^{13}C NMR: 500 and 125 MHz, respectively, MeOH-d_3 with TMS. MS: EIMS, 70 eV. CC and TLC were performed using Merck silica gel unless otherwise stated.

Plant material. See ref. [2].

Extraction and isolation. The crushed drug (20 kg) was extd with MeOH at room temp. After evapn of solvents, the residue was dil. with H_2O and partitioned successively with EtOAc and *n*-BuOH. The EtOAc ext. (190 g) was subjected to CC on silica gel using CH_2Cl_2–EtOAc with increasing amounts of EtOAc to give 10 frns. Frs 2 and 4 were repeatedly chromatographed over silica gel to give 3′-O-methylbatatasin III (50 mg) and batatasin III (60 mg), respectively. A part of fr. 7 (1 g) was subjected to CC over LH-20 using MeOH to give blestriarenes A (66 mg), B (44 mg) and C (90 mg).

*Blestriarene A** 1. Colourless needles from $CHCl_3$–MeOH, mp 194–195°, $[\alpha]_D^{20°}$ −5.1°. IR ν_{max}^{KBr} cm^{-1}: 3200, 1570, 1440. UV λ_{max}^{MeOH} nm (log ε): 273 sh (4.60), 283 (4.69), 300 (4.56). ^{1}H NMR: δ 2.21–2.59 (8H, *m*, $-CH_2-CH_2-$ × 2), 3.88 (6H, *s*, OMe × 2), 6.57 (2H, *s*, H-3, 3′), 6.59 (2H, *d*, *J* = 2.8 Hz, H-8,8′), 6.63 (2H, *dd*, *J* = 8.8, 2.8 Hz, H-6,6′), 8.05 (2H, *d*, *J* = 8.8 Hz, H-5,5′). MS *m/z* (rel. int.%): 482 $[M]^+$ (100), 241 (28). Tetraacetate: colourless needles from $CHCl_3$–MeOH, mp 122–125°. ^{1}H NMR ($CDCl_3$): δ 1.93 (6H, *s*, OCOMe × 2), 2.30 (6H, *s*, OCOMe × 2), 2.40 (4H, *s*, H-10,10′), 2.60 (4H, *s*, H-9,9′), 3.93 (6H, *s*, OMe × 2), 6.74 (2H, *s*, H-3, 3′), 6.93 (2H, *d*, *J* = 2.2 Hz, H-8,8′), 6.98 (2H, *dd*, *J* = 2.2, 8.5 Hz, H-6,6′), 8.29 (2H, *d*, *J* = 8.5 Hz, H-5,5′). MS *m/z* (rel. int.%): 650 (22), 608 (100), 566 (85), 524 (72), 482 (67), 241 (54).

Blestriarene B 2. Colourless powder, mp 313–316°, $[\alpha]_D^{20°}$ −3.2°. IR ν_{max}^{KBr} cm^{-1}: 3280, 1570, 1440. UV λ_{max}^{MeOH} nm (log ε): 257 sh (4.82), 266 (4.89), 290 sh (4.60), 300 sh (4.52), 312 (4.44), 360 (3.82), 379 (3.85). ^{1}H NMR: δ 2.21 (2H, *dm*, H-10), 2.45 (2H, *dm*, H-9), 3.93 (3H, *s*, C-4-OMe), 4.13 (3H, *s*, C-4′-OMe), 6.56 (1H, *d*, *J* = 2.8 Hz, H-8), 6.64 (1H, *dd*, *J* = 2.8, 8.8 Hz, H-6), 6.65 (1H, *s*, H-3), 6.92 (1H, *s*, H-3′), 7.08 (1H, *dd*, *J* = 9.0, 2.8 Hz, H-6′), 7.10 (1H, *d*, *J* = 2.8 Hz, H-8′), 7.18 (1H, *d*, *J* = 9.2 Hz, H-10′), 7.40 (1H, *d*, *J* = 9.2 Hz, H-9′), 8.09 (1H, *d*, *J* = 8.8 Hz, H-5), 9.43 (1H, *d*, *J* = 9.0 Hz, H-5′). MS *m/z* (rel. int.%): 480 $[M]^+$ (12), 354 (2), 296 (3), 241 (2), 240 (5), 239 (1). Tetraacetate: colourless needles from $CHCl_3$–MeOH, mp 215–217°. ^{1}H NMR ($CDCl_3$): δ 1.79 (3H, *s*, OCOMe), 2.01 (3H, *s*, OCOMe), 2.28 (3H, *s*, OCOMe), 2.36 (3H, *s*, OCOMe), 2.36 (2H, *m*, H-9 or 10), 2.53 (2H, *m*, H-9 or 10), 3.96 (3H, *s*, C-4-OMe), 4.17 (3H, *s*, C-4′-OMe), 6.84 (1H, *s*, H-3), 6.89 (1H, *d*, *J* = 2.6 Hz, H-8), 7.00 (1H, *dd*, *J* = 8.6, 2.6 Hz, H-6), 7.02 (1H, *s*, H-3′), 7.34 (1H, *d*, *J* = 9.2 Hz, H-10′), 7.38 (1H, *dd*, *J* = 9.4, 2.7 Hz, H-6′), 7.56 (1H, *d*, *J* = 2.7 Hz, H-8′), 7.57 (1H, *d*, *J* = 9.2 Hz, H-9′), 8.34 (1H, *d*, *J* = 8.6 Hz, H-5), 9.69 (1H, *d*, *J* = 9.4 Hz, H-5′). MS *m/z* (rel. int.%): 648 $[M]^+$ (35), 606 (100), 564 (75), 522 (51), 480 (38),240 (39).

Blestriarene C 3. Yellow needles from Me_2CO–MeOH, mp 331–334°, $[\alpha]_D^{20°}$ −16.7°. IR ν_{max}^{KBr} cm^{-1}: 3200, 1560, 1430. UV λ_{max}^{MeOH} nm (log ε): 255 sh (5.02), 265 (5.10), 289 sh (4.67), 299 sh (4.55), 312 (4.50), 362 (3.91), 380 (4.02). ^{1}H NMR: δ 4.14 (6H, *s*, OMe × 2), 6.92 (2H, *d*, *J* = 9.2 Hz, H-10, 10′), 6.93 (2H, *s*, H-3, 3′), 7.00 (2H, *d*, *J* = 2.8 Hz, H-8,8′), 7.03 (2H, *dd*, *J* = 9.2, 2.8 Hz, H-6,6′), 7.23 (2H, *d*, *J* = 9.2 Hz, H-9, 9′), 9.40 (2H, *d*, *J* = 9.2 Hz, H-5,5′). MS *m/z* (rel. int. %): 478 $[M]^+$ (1), 354 (3), 296 (2), 239 (0.3). Tetraacetate: colourless needles from MeOH, mp 267–270°. ^{1}H NMR ($CDCl_3$): δ 1.87 (6H, *s*, OCOMe), 2.38 (6H, *s*, OCOMe), 4.21 (6H, *s*, OMe), 7.12 (2H, *s*, H-3,3′), 7.14 (2H, *d*, *J* = 9.2 Hz, H-10,10′), 7.40 (2H, *dd*, *J* = 9.4, 2.6 Hz, H-6,6′), 7.47 (2H, *d*, *J* = 9.2 Hz, H-9,9′), 7.53 (2H, *d*, *J* = 2.6 Hz, H-8,8′), 9.74 (2H, *d*, *J* = 9.4 Hz, H-5,5′). MS *m/z* 646 $[M]^+$ (44), 604 (97), 562 (100), 520 (61), 478 (56), 239 (21).

*After submission of this paper, **1** was isolated from *Eria flava*: Majumder, P. L. and Banerjee, S. (1988) *Tetrahedron* **44** 7303.

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