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Dimeric phenanthrenes from the orchid *Bulbophyllum reptans*

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Abstract

Reptanthrin and isoreptanthrin, two new dimeric phenanthrenes, were isolated from the orchid *Bulbophyllum reptans* which also afforded nine known stilbenoids, viz. gymnopusin (**1a**) [2,7-dihydroxy-3,4,9-trimethoxyphenanthrene], confusarin (**1c**) [2,7-dihydroxy-3,4,8-trimethoxyphenanthrene], 2,7-dihydroxy-3,4,6-trimethoxyphenanthrene (**1d**) and its 9,10-dihydro derivative, flavanthrinin (**1e**) [2,7-dihydroxy-4-methoxyphenanthrene] and its 9,10-dihydro derivative (coelonin), 3,3′-dihydroxy-5-methoxybibenzyl (batatasin-III), cirrhopetalanthrin (**2a**) [2,2′,7,7′-tetrahydroxy-4,4′-dimethoxy-1,1′-biphenanthryl] and its 9,9′,10,10′-tetrahydro derivative (flavanthrin). The structures of reptanthrin and isoreptanthrin were established as 2,2′,7,7′-tetrahydroxy-3,3′,4,4′,9,9′-hexamethoxy-1,1′-biphenanthryl (**2c**) and 2,2′,7,7′-tetrahydroxy-3,3′,4,4′,9,9′-hexamethoxy-1,8′-biphenanthryl (**3a**), respectively, from spectral and chemical evidence including their biomimetic synthesis from the congener **1a** with phosphomolybdic acid (PMA) on silica gel.

Keywords: *Bulbophyllum reptans*; Orchidaceae; Reptanthrin; Isoreptanthrin; Dimeric phenanthrenes

1. Introduction

We reported earlier the isolation of a fairly large number of compounds from a series of Indian orchids. These compounds encompass a wide variety of stilbenoids, viz. bibenzyls (Majumder & Pal, 1993; Majumder & Ghosal, 1994; Majumder, Roychowdhury, & Chakraborty, 1997), phenanthrenes (Majumder & Kar, 1987; Majumder & Banerjee, 1988a, 1989, 1990; Majumder & Basak, 1990; Majumder & Lahiri, 1990a; Majumder & Sen, 1991) and 9,10-dihydrophenanthrenes (Majumder, Laha, & Datta, 1982; Majumder & Pal, 1992) and their dimers (Majumder & Banerjee, 1988b; Majumder, Pal, & Joardar, 1990; Majumder & Lahiri, 1990b), phenanthropyrans and pyrones (Majumder & Maiti, 1991; Majumder & Sabzabadi, 1988) and their 9,10-dihydro derivatives (Majumder & Sarkar, 1982; Majumder, Bandyopadhyay, & Joardar, 1982; Majumder, Sarkar, & Chakraborti, 1982; Majumder, Datta, Sarkar, & Chakraborti, 1982; Majumder & Maiti, 1988), fluorenones (Majumder & Chakraborti, 1989) and a few other polyphenolics (Majumder, Lahiri, & Pal, 1994; Majumder, Lahiri, & Mukhoti, 1995), several triterpenoids (Majumder & Ghosal, 1991) and steroids (Majumder & Pal, 1990) of biogenetic importance and some simple aromatic compounds (Majumder & Lahiri, 1989). As part of this general programme of research we have chemically investigated yet another orchid, *Bulbophyllum reptans* which has afforded two new dimeric phenanthrene derivatives, designated reptanthrin and isoreptanthrin, besides nine known stilbenoids. While the known stilbenoids were characterized as gymnopusin (**1a**) (Majumder & Banerjee, 1988a, 1989), confusarin (**1c**) (Majumder & Kar, 1987), 2.7-dihydroxy-3,4,6-trimethoxyphenanthrene (**1d**) (Letcher & Nhamo, 1972; Stermitz, Sucess, Schaver, & Andersoni, 1983) and its 9,10-dihydro derivative (Letcher & Nhamo, 1972; Stermitz et al., 1983), flavanthrinin (**1e**) (Majumder & Banerjee, 1990) and its 9,10-dihydro derivative (coelonin) (Majumder et al., 1982), batatasin-III (3,3′-dihydroxy-5-methoxybibenzyl) (Sachdev & Kulshreshtha, 1986; Juneja, Sharma, & Tandon, 1987), cirrhopetalanthrin (**2a**) (Majumder et al., 1990) and its 9,9′,10,10′-tetrahydro derivative (flavanthrin) (Majumder & Banerjee, 1988b), the structures of reptanthrin and isoreptanthrin were established as **2c** and **3a**, respectively, from the following spectral and chemical evidence.

2. Results and discussion

Both reptanthrin (**2c**) and isoreptanthrin (**3a**) were shown to have the same molecular formula $C_{34}H_{30}O_{10}$ from elemental analysis and their mass spectrometrically derived molecular weight 598. Both the compounds were

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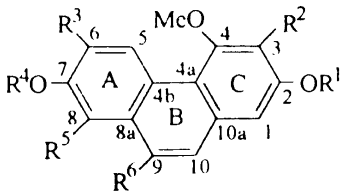
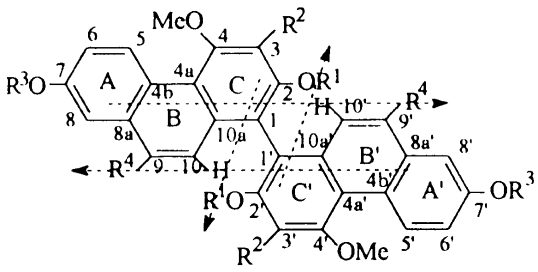
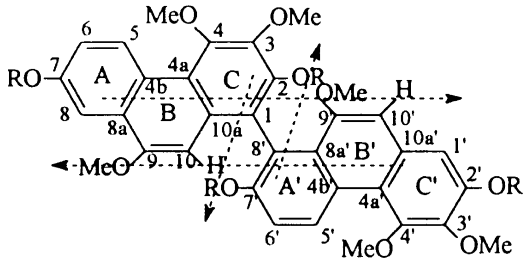


1a : $R^1 = R^3 = R^4 = R^5 = H$, $R^2 = R^6 = OMe$
1b : $R^1 = R^4 = Ac$, $R^2 = R^6 = OMe$, $R^3 = R^5 = H$
1c : $R^1 = R^3 = R^4 = R^6 = H$, $R^2 = R^5 = OMe$
1d : $R^1 = R^4 = R^5 = R^6 = H$, $R^2 = R^3 = OMe$
1e : $R^1 = R^2 = R^3 = R^4 = R^5 = R^6 = H$



2a : $R^1 = R^2 = R^3 = R^4 = H$
2b : $R^1 = R^3 = Ac$, $R^2 = R^4 = H$
2c : $R^1 = R^3 = H$, $R^2 = R^4 = OMe$
2d : $R^1 = R^3 = Ac$, $R^2 = R^4 = OMe$
2e : $R^1 = R^3 = Ac$, $R^2 = OMe$, $R^4 = H$



3a : R = H
3b : R = Ac

found to be optically active [**2c**: $[\alpha]_D$ +9.1° (MeOH); **3a**: $[\alpha]_D$ +9.7° (MeOH)]. Both **2c** and **3a** showed UV absorptions [**2c**: λ_{max} 224, 265.5, 344 and 362 nm (log ε 4.23, 3.21, 3.23 and 3.26); **3a**: λ_{max} 217, 261.5, 342 and 360 nm (log ε 4.39, 4.45, 3.71 and 3.63)] which are similar to those of other phenanthrene derivatives (Majumder & Kar, 1987; Majumder & Banerjee, 1988a, 1989, 1990; Majumder & Basak, 1990; Majumder & Lahiri, 1990a, 1990b; Majumder et al., 1990; Majumder & Sen, 1991). The phenolic nature of the compounds were indicated by their characteristic colour reactions with $FeCl_3$ (violet) and phosphomolybdic acid reagent (deep blue), alkali-induced bathochromic shifts of their UV maxima and by their IR absorption bands [**2c**: ν_{max} 3150–3500 cm^{-1} (OH); **3a**: ν_{max} 3200–3500 cm^{-1} (OH)]. The presence of four phenolic hydroxyl groups in each of **2c** and **3a** were confirmed by the formation of their respective tetraacetyl derivatives **2d** and **3b** [both having the same molecular formula $C_{42}H_{38}O_{14}$ (M^+ at m/z 766)].

The ^1H NMR spectrum of **2c** showed nine sets of signals at δ 3.48 (s), 4.06 (s), 4.10 (s) and 5.27 and 5.77 (each s; disappeared on deuterium exchange), 6.34 (s), 7.16 (dd, $J_1 = 9.3$ Hz and $J_2 = 2.83$ Hz), 7.56 (d, $J = 2.83$ Hz) and 9.42 (d, $J = 9.3$ Hz) in the integral ratio of 3:3:3:1:1:1:1:1:1:1. Since **2a** contains thirty protons, each of these signals thus corresponds to double the number of protons given by their respective integral ratio. This, in turn, also implied that **2c** was a symmetrical dimer of two monomeric units each having an elemental composition of $C_{17}H_{15}O_5$. The ^1H NMR spectrum of **3a**, on the other hand, showed eighteen sets of signals at δ 3.15 (s), 3.46 (s), 3.95 (s), 4.00 (s), 4.03 (s) and 4.06 (s) and 5.17, 5.29, 5.73 and 5.94 (each s; disappeared on deuterium exchange), 6.33 (s), 6.62 (s), 6.93 (s), 7.16 (dd, $J_1 = 9.3$ Hz and $J_2 = 2.75$ Hz), 7.39 (d, $J = 9.2$ Hz), 7.56 (d, $J = 2.75$ Hz), 9.41 (d, $J = 9.3$ Hz) and 9.62 (d, $J = 9.2$ Hz) in the integral ratio of 3:3:3:3:3:3:1:1:1:1:1:1:1:1:1:1:1:1. Since **3a** also contains thirty protons, each of these signals corresponds to the same number of protons given by their integral ratio. Thus, while **2c** was assumed to be a symmetrical dimer, **3a** also appeared to have a dimeric formulation composed of the same monomeric units, as in **2c**, but linked in an unsymmetrical manner. The signals at δ 5.27 and 5.77 in the ^1H NMR spectrum of **2c** cor-

responded to two paris of phenolic hydroxyl protons in two different environments, while those at δ 3.48, 4.06 and 4.10 (each 6H, s) indicated the presence of six aromatic methoxyl groups, the protons of two such groups being shifted upfield (δ 3.48) and those of the remaining four groups appearing at the normal region. The presence of four phenolic hydroxyl groups also in **3a**, all in different environments, was indicated by the presence of the signals at δ 5.16, 5.29, 5.72 and 5.93 (each 1H, s) in the ^{1}H NMR spectrum of the compound. Like **2c**, **3a** also contained six aromatic methoxyl functions as evident from the signals at δ 3.15, 3.46, 3.95, 4.00, 4.03 and 4.06 (each 3H, s) in its ^{1}H NMR spectrum. The protons of two of these methoxyl groups exhibited considerable upfield shifts (δ 3.15 and 3.46), while those of the remaining four methoxyl functions appeared at the normal region.

The exact nature of the monomeric units, their mode of linkage and the relative positions of the hydroxyl and methoxyl functions in **2c** and **3a** were ascertained from the chemical shifts and the splitting patterns of the signals of the aromatic protons of the compounds and their respective tetraacetyl derivatives **2d** and **3b**, as well as from the proton resonances of the methoxyl groups of **2c**, **3a**, **2d** and **3b** and those of the acetate methyls of **2d** and **3b**. Thus, the most downfield aromatic proton signal at δ 9.42 and those at δ 9.41 and 9.62 in the ^{1}H NMR spectra of **2c** and **3a**, respectively, are typical of H-4 and H-5 of a phenanthrene derivative (Majumder & Kar, 1987; Majumder & Banerjee, 1988a, 1989, 1990; Majumder & Basak, 1990; Majumder & Lahiri, 1990a, 1990b; Majumder et al., 1990; Majumder & Sen, 1991) indicating that both **2c** and **3a** are phenanthrene dimers. If these signals are assigned to H-5 and H-5′ of **2c** and **3a**, C-4 and C-4′ of both compounds must be substituted by methoxyl or hydroxyl functions. In the light of the well-documented observation (Letcher & Nhamo, 1971; Letcher & Wong, 1978; Majumder & Lahiri, 1990a) that H-5 of a 4-hydroxy-phenanthrene derivative is shifted upfield by ca. 0.5–0.6 ppm on acetylation, the downfield shift of H-5 and H-5′ of **2d** and **3b** by 0.22 and 0.28 and 0.17 ppm, respectively, compared to the corresponding protons of **2c** and **3a** implied that C-4 and C-4′ of both **2c** and **3a** must contain methoxyl rather than hydroxyl functions. Further, the appearance of these signals in the ^{1}H NMR spectra of both **2c** and **3a** as clear doublets having $J \cong 9$ Hz indicated that C-6 and C-6′ of both compounds must be unsubstituted. The signal at δ 7.16 (2H, dd) of **2c** could then be assigned to H-6 and H-6′, which was split by H-5 and H-5′ ($J_1 = 9.3$ Hz) and also by H-8 and H-8′ ($J_2 = 2.83$ Hz), which appeared at δ 7.56 (2H, d; $J = 2.83$ Hz). The relatively downfield shift of H-8 and H-8′ of **2c** was attributed to the presence of methoxyl groups at C-9 and C-9′ in the compound. That H-6 and H-8 and H-6′ and H-8′ are flanked by hydroxyl functions at C-7 and C-7′ in **2c** was supported by the downfield shifts of these protons by 0.19 and 0.38 ppm, respectively, in the ^{1}H NMR spectrum of **2d**. This was corroborated by the ^{1}H NMR spectra of both **2c** and **2d**, which were similar to those of gymnopusin (**1a**) [δ 9.33 (1H, d, $J = 9.3$ Hz), H-5; 7.21 (1H, dd, $J_1 = 9.3$ Hz and $J_2 = 2.8$ Hz), H-6; 7.69 (1H, d, $J = 2.8$ Hz), H-8; 7.08 (1H, s), H-1; 6.94 (1H, s), H-10; 3.96 (6H, s) and 4.02 (3H, s) and 8.12 and 8.55 (each 1H, s); OH] and its diacetate (**1b**) [δ 9.58 (1H, d, $J = 9.3$ Hz), H-5; 7.41 (1H, dd, $J_1 = 9.3$ Hz and $J_2 = 2.8$ Hz), H-6; 8.07 (1H, d, $J = 2.8$ Hz), H-8; 7.26 (1H, s), H-1; 6.86 (1H, s), H-10; 4.00 (6H, s) and 4.02 (3H, s); 2.40 and 2.37 (each 3H, s)], respectively, except that (i) the signals corresponding to H-1 of **1a** (δ 7.08) and **1b** (δ 7.26) were absent in the spectra of **2c** and **2d**; (ii) the signals corresponding to H-10 of **1a** (δ 6.94) and **1b** (δ 6.86) were shifted upfield in the spectra of **2c** (δ 6.34, 2H, s) and **2d** (δ 6.31, 2H, s); (iii) while the protons of the methoxyl groups of **1a** and **1b** appeared at the normal region, those for the two methoxyl functions of **2c** and **2d** showed considerable upfield shifts [**2c**: δ 3.48 (6H, s); **2d**: δ 3.47 (6H, s)] and (iv) the protons of two of the acetate methyls of **2d** exhibited a substantial upfield shift [δ 1.82 (6H, s)] compared to those of the other two acetate methyls of **2d** (δ 2.29 (6H, s) and those of **1b** (δ 2.40 and 2.37). The above observations, thus, ruled out the possibility of any linkage of the two monomeric units in **2c** involving C-6, C-6′, C-8, C-8′, C-10 and C-10′ and indicated **2c** to be a 1,1′-dimer of its congener gymnopusin (**1a**). More convincing evidence in support of the structure **2c** for reptanthrin was provided by the observed differences in the chemical shifts of some of the protons of **2c** and **2d** from those of the corresponding protons of **1a** and **1b** considered in the light of the most preferred conformations of **2c** and **2d**. Construction of the Dreiding models of **2c** and **2d** shows that in the most preferred conformations of the compounds, the two monomeric units remain almost perpendicular to each other. In these conformations, H-10 and H-10′ and the protons of the methoxyl groups at C-9 and C-9′ of **2c** and **2d** fall in the shielding zones of the neighbouring aromatic rings of the compounds. Accordingly, these protons of **2c** and **2d** were shifted upfield [**2c**: δ 6.34 (H-10, H-10′) and 3.48 (OMe at C-9 and C-9′); **2d**: δ 6.31 (H-10, H-10′) and 3.47 (OMe at C-9 and C-9′)] compared to H-10 and the protons of the methoxyl group at C-9 of **1a** and **1b**, which appeared at the normal regions. Again, in the light of the reported observations that protons of methyl, methoxyl and acetoxyl groups *ortho* to the site of dimerization in similar biaryls, which fall in the shielding zones of neighbouring aromatic rings resonate at higher fields than such protons at other parts of the molecules (Davis & Hodge, 1974; Majumder, Pal, & Shoolery, 1985; Majumder & Lahiri, 1990b; Majumder et al., 1990; Majumder & Basak, 1991), the appearance of the protons of two acetate methyls of **2d** at a much higher field (δ 1.82) compared to those of the other two acetate methyls appearing at the normal region (δ 2.30) required the

highfield acetate methyls to be associated with acetoxy functions at C-2 and C-2′ of **2d**. This, in turn, affirmed the placement of two hydroxyl functions at C-2 and C-2′ in **2c**, the remaining two hydroxyl functions of **2c** being already placed at C-7 and C-7′. The remaining four methoxyl groups of **2c** were thus placed at C-3, C-3′, C-4 and C-4′.

In the case of isoreptanthrin (**3a**), the signals at δ 7.16 (dd, $J_1 = 9.3$ Hz and $J_2 = 2.75$ Hz) and 7.39 (d, $J = 9.2$ Hz) were assigned to H-6 and H-6′, respectively, on the basis of their coupling with H-5 and H-5′. But while the signal for H-6 of **3a** was split by both H-5 (δ 9.41) and H-8 appearing at δ 7.56 (d, $J = 2.75$ Hz), that for H-6′ was split only by H-5′ (δ 9.62). The absence of coupling between H-6′ and H-8′ would imply that while C-8 of **3a** is unsubstituted, as in **2c**, C-8′ of the compound must be involved as one of the sites of dimerization. Again, if the one-proton singlet at δ 6.93 of **3a**, which is strikingly similar to that of H-1 of gymnopusin (**1a**) is assigned to H-1′ of **3a**, C-1 of **3a** must be involved as the other site of dimerization. Isoreptanthrin (**3a**) was, therefore, assumed to be a 1,8′-dimer of **1a** having four hydroxyl functions at C-2, C-2′, C-7 and C-7′ and six methoxyl groups at C-3, C-3′, C-4, C-4′, C-9 and C-9′. The downfield shifts of H-6, H-8, H-1′ and H-6′ of **3a** by 0.18, 0.36, 0.20 and 0.11 ppm, respectively, in the ^{1}H NMR spectrum of **3b** further affirmed the presence of hydroxyl groups at C-7, C-7′, C-2 and C-2′ of **3a**. As in the case of **2c** and **2d**, in the most preferred conformations of **3a** and **3b**, the two monomeric halves in each of these compounds are almost perpendicular to each other. Accordingly, H-10 and H-10′ and the protons of the methoxyl groups at C-9 and C-9′ of **3a** and **3b** fall in the shielding zones of the neighbouring phenanthrene moieties and were thus shifted upfields [**3a**: δ 3.46 (OMe at C-9), 3.15 (OMe at C-9′), 6.33 (H-10) and 6.62 (H-10′); **3b**: δ 3.46 (OMe at C-9), 3.10 (OMe at C-9′), 6.32 (H-10) and 6.63 (H-10′)] compared to the corresponding protons of **1a** and **1b**. The greater shielding of H-10 and the protons of the methoxyl group at C-9′ in **3a** and **3b** compared to H-10′ and the methoxyl protons at C-9 was attributed to the greater proximity of H-10 and the 9′-methoxyl group than H-10′ and the 9-methoxyl group to the shielding zones of the adjacent phenanthrene moieties. Again, based on the same consideration as in **2d**, the upfield acetate methyl signals at δ 1.68 and 1.75 of **3b** were assigned to the acetoxy groups at C-2 and C-7′, which fall in the shielding zones of the neighbouring aromatic rings A′ and C. The methyl of the remaining acetoxy functions at C-2′ and C-7 of **3b** appeared at the normal region (δ 2.30 and 2.33). The other four methoxyl groups of **3a** and **3b**, which appeared at the normal region were placed at C-3, C-3′, C-4 and C-4′.

The structures of reptanthrin (**2c**) and isoreptanthrin (**3a**) were also supported by the ^{13}C NMR spectral data of their more soluble tetraacetyl derivatives **2d** and **3b**. The degree of protonation of each carbon atom was determined by DEPT experiments. The chemical shifts of the carbon atoms of **2d** and **3b** (Table 1) were assigned by comparison with the δ_C values of structurally related compounds **1b** (Majumder & Banerjee, 1989), **2b** (Majumder et al., 1990) and **2e** (Majumder & Basak, 1991). The ^{13}C NMR spectrum of **2d** showed 21 carbon resonances, each for two equivalent carbon atoms, which further affirmed the symmetrical dimeric formulation of **2d** and hence of **2c**. The appearance of 37 carbon signals in the ^{13}C NMR spectrum of **3b**, on the other hand, indicated **3b** and hence **3a** to be unsymmetrical dimers as evident from their ^{1}H NMR spectra. The δ_C values of the carbon atoms of **2d** were virtually identical with those of **1b** except that the protonated C-1 resonance (δ_C 115.8) of the latter was replaced by a relatively lowfield quaternary carbon signal at δ_C 120.8 in the former and that C-10 of **1b** was shifted upfield by 1.4 ppm in **3b**. Such downfield shifts of C-1 (C-1′) and upfield shifts of C-10 (C-10′) of structurally similar phenanthrene dimers compared to the corresponding carbon atoms of their respective monomers are typical of 1,1′-biphenanthryl derivatives like **2b** and **2e** (Majumder et al., 1990; Majumder & Basak, 1991). Again, the δ_C values of the rings C and C′ carbon atoms and C-4b (C-4b′), C-5 (C-5′), C-6 (C-6′) and C-7 (C-7′) of **2d** were similar to those of the corresponding carbon atoms of **2e** indicating the presence of identical substituents at C-2 (C-2′), C-3 (C-3′), C-4 (C-4′) and C-7 (C-7′) in the two compounds. The similarities of the δ_C values of C-4b (C-4b′), C-5 (C-5′), C-6 (C-6′) and C-7 (C-7′) of **2d** with those of the corresponding carbon atoms of **2b** also supported the presence of acetoxy groups at C-7 and C-7′ in both the compounds. The observed differences in the δ_C values of C-8 (C-8′), C-8a (C-8a′), C-9 (C-9′) and C-10 (C-10′) of **2d** from those of **2b** and **2e** were attributed to the presence of methoxyl groups at C-9 and C-9′ in **2d**. The ^{13}C NMR spectrum of **3b** also showed similarities with that of **1b** exhibiting signals corresponding to carbon atoms of two units of **1b** coupled unsymmetrically. Thus, the carbon resonances of rings A, B and C of **3b** were identical with those of the corresponding carbon atoms of **1b** except that the signal for the protonated C-1 of the latter (δ_C 115.8) was replaced by a quaternary carbon signal at δ_C 120.1 in the former as in **2d** and that C-10 of **2d** showed an upfield shift of 1.0 ppm compared to the corresponding carbon of **1b**. This would imply that C-1 of **3b** must be involved as one of the sites of dimerization. The remaining signals of **3b** representing the carbon atoms of rings A′, B′ and C′ were similar to those of the corresponding carbon atoms of **1b** except that the signal for the protonated C-8 of the latter (δ_C 114.0) was replaced by a quaternary carbon signal at δ_C 119.5 and that C-9′ of **2d** showed a similar upfield shift (2.1 ppm) compared to the corresponding carbon of **1b** indicating that C-8′ of **3b** must be involved as the other site of dimerization. This was

Table 1
^{13}C NMR spectral data of **1b**, **2b**, **2d**, **2e** and **3b**

C	Chemical shift (δ, ppm)[j] **2d**	**2b**	**2e**	C	Chemical shift (δ, ppm)[j] **1b**	**3b**	C	Chemical shift (δ, ppm)[j] **3b**
1,1′	120.8	119.2	121.9	1	115.8	120.1	1′	115.2
2,2′	143.1	147.8	145.2	2	143.4	143.5[a]	2′	143.8[a]
3,3′	142.6	104.1	142.5	3	142.9	142.5[b]	3′	142.0[b]
4,4′	152.2[a]	159.2	152.4	4	152.5	152.2[c]	4′	152.5[c]
4a,4a′	118.8	117.5	118.9	4a	118.9	118.7[d]	4a′	118.5[d]
4b,4b′	128.5[b]	128.2	129.2	4b	128.5	128.4[e]	4b′	128.6[e]
5,5′	128.9	129.9	129.2	5	128.7	129.0[f]	5′	128.8[f]
6,6′	121.2	120.8	121.0	6	121.4	121.8[g]	6′	121.1[g]
7,7′	149.0	148.5	149.0	7	148.8	148.7[h]	7′	148.4[h]
8,8′	114.0	119.5	119.6	8	114.0	114.1	8′	119.5
8a,8a′	128.2[b]	133.5	133.8	8a	128.1	128.2[e]	8a′	128.9[e]
9,9′	152.8[a]	128.5	127.0	9	152.9	152.5[c]	9′	150.8
10,10′	101.1	125.7	125.4	10	102.5	101.5	10′	103.0
10a,10a′	129.9	134.4	127.6	10a	130.2	130.5[i]	10a′	130.0[i]
OMe	61.0	56.1	61.0	OMe	61.1			61.0
	60.0		60.0		60.0			60.1
	55.1				55.5			56.3
								56.0
$OCOCH_3$	169.4	169.7	169.8	$OCOCH_3$	169.6			168.9
	168.0	169.3	168.5		169.2			168.7
	21.0	21.4	20.9		21.2			21.0
	20.1	20.8	19.9		20.8			20.6
								20.1

[a–i]Values are interchangeable for each compound.
[j]Spectra were run in $CDCl_3$ and the chemical shifts were measured with δ (TMS) = δ ($CDCl_3$) + 76.9 ppm.

corroborated by the appearance of only one set of protonated carbon signals in **3b** at δ_C 114.1 and 115.2, which could be attributed to C-8 and C-1′, respectively, of **3b**.

The structures of reptanthrin (**2c**) and isoreptanthrin (**3a**) were confirmed by their partial synthesis in the ratio of 3:2 from gymnopusin (**1a**) and by the action of phosphomolybdic acid (PMA) on silica gel surface, a reagent developed in our laboratory for regioselective oxidative phenol-coupling reaction (Majumder & Basak, 1991).

Reptanthrin (**2c**) and isoreptanthrin (**3a**) are, thus, two new additions to the growing list of naturally occurring dimeric phenanthrene derivatives and their synthesis from and cooccurrence with gymnopusin (**1a**) have provided strong circumstantial evidence in favour of the proposed biogenesis (Majumder & Lahiri, 1990b; Majumder et al., 1990) of the natural phenanthrene dimers through oxidative coupling of their respective phenolic monomers.

3. Experimental

M.p.'s: uncorr. CC: silica gel (100–200 mesh). MPLC: silica gel (230–400 mesh). TLC: silica gel G. UV: 95% aldehyde-free EtOH. IR: KBr discs. ^1H and ^{13}C NMR: 300 and 75 MHz, respectively, in $CDCl_3$ using TMS as an int. standard. Chemical shifts are expressed in $\delta_{(ppm)}$. MS: direct inlet system, 70 eV. All analyt samples were routinely dried over P_2O_5 for 24 h in vacuo and were tested for purity by TLC and MS. The petrol used had b.p. 60–80°C.

3.1. Plant materials

Bulbophyllum reptans Lindl. were collected from Kalimpong (Darjeeling), India in September 1993. A voucher specimen was deposited in the Herbarium of the Department of Botany, University of Calcutta (CUH).

3.2. Isolation of reptanthrin (**2c**), isoreptanthrin (**3a**), **1a**, **1c**, **1d**, **1e**, **2a**, 2,7-dihydroxy-3,4,6-trimethoxy-9,10-dihydrophenanthrene, 2,7-dihydroxy-4-methoxy-9,10-dihydrophenanthrene (coelonin), 2,2′7,7′-tetrahydroxy-4,4′-dimethoxy-9,9′,10,10′-tetrahydro-1,1′-biphenanthryl (flavanthrin) and 3,3′-dihydroxy-5-methoxybibenzyl (batatasin-III)

Air-dried powdered whole plant of *B. reptans* (3 kg) was soaked in MeOH (7.5 l) for 3 weeks. The MeOH extract was concd to ca. 100 ml, diluted with H_2O (750 ml) and exhaustively extracted with Et_2O. The Et_2O extract was fractionated into acidic and nonacidic frs with 2 M NaOH. The aq. alkaline soln was acidified in

the cold with concd HCl and the liberated solids were extracted with Et_2O, washed with H_2O, dried and the solvent removed. The residue was chromatographed. The early frs of petrol–EtOAc (30:1) eluate gave **1c** (0.06 g), crystallized from petrol–EtOAc, m.p. 185°C. The frs immediately following the elution of **1c** gave a mixture of **1a**, **1d** and 2,7-dihydroxy-3,4,6-trimethoxy-9,10-dihydrophenanthrene. The above mixture was then subjected to medium pressure liquid chromatography (MPLC) using petrol–EtOAc (3:1) as the solvent. The early frs in the above MPLC gave a semi-solid mass containing mostly 2,7-dihydroxy-3,4,6-trimethoxy-9,10-dihydrophenanthrene, which on further CC gave 0.03 g of the compound in the pure state, crystallized from petrol–EtOAc, m.p. 113°C. The later frs in the above MPLC afforded a mixture of **1a** and **1d**. Repeated CC of this mixture gave pure **1a** (0.08 g), crystallized from petrol–EtOAc, m.p. 192°C, and **1d** (0.20 g), also crystallized from the same solvent mixture, m.p. 138°C. Elution of the main column (CC) with petrol–EtOAc (20:1) yielded a gummy residue containing a mixture of **1e** and coelonin. MPLC of this mixture using petrol–EtOAc (2:1) as the solvent gave pure coelonin (0.02 g) in the early frs and **1e** (0.03 g) in the later frs, both as semi-solid masses. The petrol–EtOAc (10:1) eluate from the main CC afforded pure batatasin-III (0.05 g), also as a semi-solid mass. Washing the main chromatographic column with petrol–EtOAc (5:1) gave a gummy residue containing a mixture of **2c** and **3a**, which on repeated CC using the same solvent mixture finally afforded pure **2c** (0.05 g) in the early frs and **3a** (0.025 g) in the later frs, both as amorphous powders. **2c** (found: C, 68.17; H, 4.99; $C_{34}H_{30}O_{10}$ requires: C, 68.22; H, 5.02%). **3a** (found: C, 68.15; H, 4.97; $C_{34}H_{30}O_{10}$ requires: C, 68.22; H, 5.02%). **2c** UV $\lambda_{max}^{EtOH-0.1\,M\,NaOH}$ nm: 220, 270.5 and 356 ($\log\varepsilon$ 4.36, 4.43 and 3.80); IR: ν_{max} cm^{-1} 3150–3500 (OH), 1620, 1520, 1470, 1410, 1395, 880, 830, 730 and 680 (aromatic nucleus); MS *m/z* (rel. int.): 598 [M^+] (100), 583 (20), 568 (10), 553 (6), 538 (5), 299 (30), 284 (7), 269 (5) and 241 (8). **3a** UV $\lambda_{max}^{EtOH-0.1\,M\,NaOH}$ nm: 223.5 and 273 ($\log\varepsilon$ 4.23 and 4.28); IR ν_{max} cm^{-1} 3200–3500 (OH), 1605, 1565, 1455, 870, 840, 750 and 650 (aromatic nucleus); MS *m/z* (rel. int.): 598 [M^+] (100), 583 (25), 568 (8), 553 (5), 538 (5), 299 (35), 284 (5), 269 (3) and 241 (5). Both **2c** and **3a** were acetylated with Ac_2O and pyridine to give **2d** and **3b**, respectively, also as amorphous powders. **2d** (found: C, 65.75; H, 4.93; $C_{42}H_{38}O_{14}$ requires: C, 65.79; H, 4.96%). UV λ_{max} nm: 224.5, 261.5, 308.5 and 360.5 ($\log\varepsilon$ 4.37, 4.43, 3.84 and 3.16); IR ν_{max} cm^{-1}: 1770 and 1245 (OAc), 1630, 1500, 1470, 1375, 885, 840 and 665 (aromatic nucleus); ^1H NMR: δ 1.82 (6H, s; OAc at C-2 and C-2′), 2.30 (6H, s; OAc at C-7 and C-7′), 3.47 (6H, s; OMe at C-9 and C-9′), 3.98 and 4.02 (each 6H, s; OMe at C-3, C-3′, C-4 and C-4′), 6.31 (2H, s; H-10 and H-10′), 7.36 (2H, dd, $J_1=9.36$ Hz and $J_2=2.71$ Hz; H-6 and H-6′), 7.94 (2H, d, $J=2.71$ Hz; H-8 and H-8′) and 9.64 (2H, d, $J=9.36$ Hz; H-5 and H-5′); MS *m/z* (rel. int.): 766 [M^+] (5), 724 (8), 682 (10), 640 (15), 598 (100), 583 (25), 568 (15) and 299 (25). **3b** (found: C, 65.73; H, 4.92; $C_{42}H_{38}O_{14}$ requires: C, 65.79; H, 4.96%). UV λ_{max} nm: 221, 261.5, 305.0, 342.0 and 359.5 ($\log\varepsilon$ 4.15, 4.38, 3.78, 3.19 and 3.17); IR ν_{max} cm^{-1}: 1765 and 1240 (OAc), 1610, 1530, 1460, 1370, 890, 850, 760 and 675 (aromatic nucleus); ^1H NMR: δ 1.68 and 1.75 (each 3H, s; OAc at C-2 and C-7′), 2.30 and 2.33 (each 3H, s; OAc at C-7 and C-2′), 3.10 (3H, s; OMe at C-9′), 3.46 (3H, s; OMe at C-9), 3.95, 3.97, 3.98 and 3.99 (each 3H, s; OMe at C-3, C-3′, C-4 and C-4′), 6.32 (1H, s; H-10), 6.63 (1H, s; H-10′), 7.13 (1H, s; H-1′), 7.34 (1H, dd, $J_1=9.35$ Hz and $J_2=2.56$ Hz; H-6), 7.50 (1H, d, $J=9.32$ Hz; H-6′), 7.92 (1H, d; $J=2.56$ Hz; H-8), 9.64 (1H, d, $J=9.32$ Hz, H-5′) and 9.79 (1H, d, $J=9.35$ Hz, H-5); MS *m/z* (rel. int.): 766 [M^+] (3), 724 (10), 682 (15), 640 (10), 598 (100), 583 (20), 568 (15) and 299 (30).

Washing the main column (CC) with petrol–EtOAc (2:1) afforded a mixture of **2a** and flavanthrin. Repeated CC of this mixture using the same solvent finally gave pure **2a** (0.03 g), crystallized from petrol–EtOAc, m.p. 296°C, and flavanthrin (0.025 g), crystallized from the same solvent mixture, m.p. 285°C.

*3.3. Conversion of gymnopusin (**1a**) to reptanthrin (**2c**) and isoreptanthrin (**3a**) with PMA on silica gel surface*

0.05 g of gymnopusin (**1a**) in Me_2CO (15 ml) was adsorbed on 10 g of silica gel (100–200 mesh) uniformly impregnated with an aq. Me_2CO soln of 0.4 g of PMA (silica gel after being impregnated with PMA was dried in vacuum desiccator for 30 min). The solvent was removed by vacuum desiccation for 30 min. The silica gel containing **1a** and PMA was placed on fresh silica gel (20 g) in a small chromatographic column and soaked with petrol for 6 h. The silica gel containing **1a** and PMA gradually turned intense blue in colour. The column was eluted with petrol–EtOAc (3:1) and the eluate (containing only the organic constituents) was washed with H_2O, dried and the solvent removed. The residue was subjected to repeated CC using petrol–EtOAc (5:1) to give **2c** (0.024 g) and **3a** (0.016 g).

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