A Biphenanthrene and a Phenanthro[4,3-*b*]furan from the Orchid *Bulbophyllum vaginatum*

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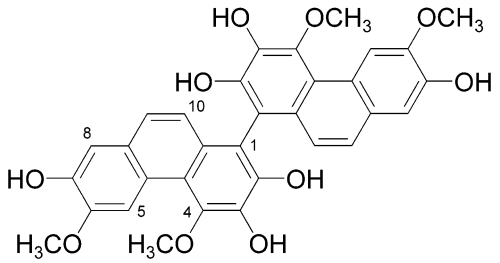
Two minor metabolites of the whole plant of *Bulbophyllum vaginatum* collected in Singapore were identified as the biphenanthrene **1** and the phenanthro[4,3-*b*]furan derivative **2**. Structure determinations were performed using a combination of 1D and 2D NMR techniques.

Members of the Orchidaceae in general are rich sources of isoprenoids and aromatic compounds such as phenanthrenes, 9,10-dihydrophenanthrenes, bibenzyls, pyrones, and fluorenones.[1] Those of the genus *Bulbophyllum* contain mainly phenanthrenes and bibenzyls,[1,2a−d] and, as part of a phytochemical study of Singapore plants, we have previously reported that *Bulbophyllum vaginatum* (Lindl.) Reichb.f. is no exception in this respect.[3,4] A final study of some minor fractions remaining from the hexane extract has yielded the structurally interesting biphenanthrene **1** and phenanthro[4,3-*b*]furan derivative **2**.

Compound **1**, $[\alpha]^{27}_{D}$ +5.8° (*c* 0.26 in MeOH), was obtained as a gum with UV absorption peaks (260, 282, 317 sh, 344, and 360 nm) characteristic of a phenanthrene derivative.[5] The IR spectrum (3536, 1601, and 1513 cm^{-1}) and a positive reaction with $FeCl_3$ indicated that the compound is phenolic. Whereas the EIMS was consistent with the molecular formula $C_{32}H_{26}O_{10}$ (*m*/*z* 570.1517), the ^{13}C NMR spectrum showed only 16 signals and helped establish that the compound was a symmetrical dimer.



1

The ^1H and ^{13}C NMR spectra showed resonances for two pairs of *ortho*-coupled aromatic protons [δ_H 7.23 (2H, d, J = 9.0 Hz, H-9 and H-9′) and 6.91 (2H, d, J = 9.0 Hz, H-10 and H-10′); δ_C 124.8 (d, C-9 and C-9′) and 123.5 (d, C-10 and C-10′)], four isolated aromatic protons [δ_H 9.14 (2H, s, H-5 and H-5′) and 7.19 (2H, s, H-8 and H-8′); δ_C 108.3 (s, C-5 and C-5′) and 112.2 (s, C-8 and C-8′)], six phenolic hydroxyl groups [δ_H 8.25 (2H, br s, OH-3 and OH-3′), 7.95 (2H, br s, OH-7 and OH-7′) and 7.72 (2H, br s, OH-2 and OH-2′), exchangeable with D_2O], four methoxyl groups, two of which were *ortho*-disubstituted [δ_H 4.08 (6H, s, OCH_3-6 and OCH_3-6′) and 4.06 (6H, s, OCH_3-4 and OCH_3-4′); δ_C 56.2 (q, OCH_3-6 and OCH_3-6′) and 60.2 (q, OCH_3-4 and OCH_3-4′)], and 20 fully substituted aromatic carbons.

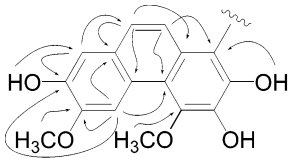


Figure 1. Important HMBC correlations for compound **1**.

The structure of compound **1** and the assignments of the ^1H and ^{13}C NMR spectra were determined by a combination of NOE, HMQC, and HMBC spectroscopy (see Figure 1 and Table S1, Supporting Information). Both of the isolated aromatic protons, H-5 and H-8, correlated to the same two oxygenated aromatic carbons (C-6 and C-7) and must therefore be *para* to each other in the same aromatic ring. These two carbons could be differentiated since the more deshielded methoxyl protons (δ_H 4.08) had ^3J correlations to C-6, while the hydroxyl proton at δ_H 7.95 correlated via a ^3J pathway to C-8. Therefore, C-7 was hydroxylated and C-6 was methoxylated, which was supported by the observation of an NOE at H-5 when the C-6 methoxyl protons were irradiated. H-8 showed a ^3J correlation to C-9, and therefore C-9 was *ortho* to H-8. H-5, H-9, and H-10 all correlated with C-8a, which meant that C-9 was attached to this ring at C-8a and that C-9 and C-10 were bonded. H-9 also had ^3J correlations to C-4b and C-10a, but they could not be distinguished. Correlations to C-4a from both H-5 and H-10 revealed the presence of a bond between C-4a and C-4b. The remaining aromatic ring must bear another identical monomer unit, one methoxyl, and two hydroxyl groups. One of these two hydroxyl protons and H-10 showed ^3J correlations to a substituted aromatic carbon (δ_C 114.7), which must be assigned to C-1. C-2 was therefore hydroxylated. Since the chemical shift of C-1 indicated that it was not oxygenated, it must be the point of attachment of the second phenanthryl unit. The last two carbons of this aromatic ring must be oxygenated. Irradiation of the methoxyl group enhanced the signals for H-5 and a hydroxyl group. It followed that C-3 and C-4 must be hydroxylated and methoxylated, respectively. C-3 was predicted to be more shielded than C-2 since it is *ortho* to two oxygen substituents.

Further evidence for the structure came from comparison of the ^{13}C NMR shifts of **1** with those of its corresponding monomer.[3] The major difference was a deshielding of C-1/C-1′ of **1** by ca. 5 ppm due to the linkage at this position. In addition, H-10 was more shielded than H-9 since it falls in the deshielding zone of the second monomer unit. This is in agreement with the chemical shifts of H-9/H-9′ and H-10/H-10′ of known 1,1′-biphenanthrenes.[6] Compound **1**

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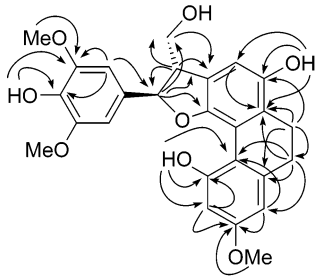
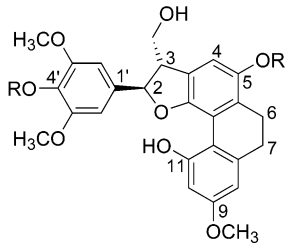


Figure 2. Important HMBC correlations for compound **2**.

is therefore a new compound, 4,4′,6,6′-tetramethoxy-[1,1′-biphenanthrene]-2,2′,3,3′,7,7′-hexol.

Compound **2**, $C_{26}H_{26}O_8$ (*m*/*z* 466.1617), $[\alpha]_D$ −3.5° (*c* 0.85), had UV (λ_{max} 230, 278, 300, and 318 nm) and IR spectra (ν_{max} 3536, 1603, 1514, and 1465 cm^{-1}) that were characteristic of a phenol, as was its positive reaction with $FeCl_3$. The ^1H and ^{13}C NMR spectra showed resonances for four hydroxyl groups [δ_H (500 MHz, acetone-d_6) δ 8.50 (1H, s, OH-11), 8.02 (1H, br s, OH-5), 7.37 (1H, br s, OH-4′) and 4.15 (1H, br s, OH-7′), all exchangeable with D_2O], three methoxyl groups [δ_H 3.80 (6H, s, OCH_3-3′ and OCH_3-5′) and 3.76 (3H, s, OCH_3-9); δ_C 56.7 (2 × q, C-3′ and C-5′) and 55.4 (q, C-9)], a pair of *meta*-coupled aromatic protons [δ_H 6.46 (1H, d, J = 2.7 Hz, H-8) and 6.33 (1H, d, J = 2.7 Hz, H-10); δ_C 107.3 (d, C-8) and 102.9 (d, C-10)], three aromatic proton singlets [δ_H 6.90 (1H, s, H-4) and 6.80 (2H, s, H-2′ and H-6′); δ_C 111.3 (d, C-4) and 104.9 (2 × d, C-2′ and C-6′)], two coupled benzylic methylene groups [δ_H 2.82 (1H, m, H-6α or H-6β), 2.64 (1H, m, H-6β or H-6α), and 2.67 (2H, m, H_2-7); δ_C 22.8 (t, C-6) and 31.4 (t, C-7)], a hydroxymethyl group [δ_H 3.90 (2H, br s, H_2-7′); δ_C 63.8 (t, C-7′)], two methine groups, the first being oxygenated [δ_H 5.65 (1H, d, J = 7.3 Hz, H-2) and 3.69 (1H, br q, J = 6.3 Hz, H-3); δ_C 89.4 (d, C-2) and 54.0 (d, C-3)], and 13 substituted aromatic carbons. The 18 ^{13}C NMR resonances in the deshielded region were consistent with the presence of three benzene rings, thus accounting for 12 units of unsaturation and indicating the existence of two additional rings. The molecule must contain an ether linkage since there were nine oxygenated carbons (seven aromatic and two aliphatic) but only eight oxygens. In the ^{13}C NMR spectrum, the presence of equivalent pairs of carbons (δ_C 148.9), methines (δ_C 104.9), and methoxyls (δ_C 56.7) indicated that there was a symmetrically substituted benzene ring in the molecule.



2 R = H
3 R = CH_3

The molecular structure and NMR assignments were determined using HMQC and HMBC spectroscopy (see Figure 2 and Table S2, Supporting Information) in a manner similar to that of **1**. The symmetrical ring was shown to be a 5-substituted syringol unit which was joined to a dihydrophenanthrene moiety through a dihydrofuran ring to give **2**.

The relative stereochemistry at C-2 and C-3 in **2** was readily established by difference NOE spectroscopy. When the C-7′ methylene protons were saturated, the H-2 resonance was enhanced, indicating that H-2 and the hydroxymethyl group were *cis*. It followed that H-3 and the symmetrically substituted benzene ring must also be *cis* to each other. The observed enhancements of the C-11 hydroxyl proton signal when the protons H-2, H-2′, and H-6′ were irradiated revealed that these three protons and the hydroxyl group were, as expected, in close proximity. The compound was therefore (2*R**,3*S**)-3-hydroxymethyl-9-methoxy-2-(4′-hydroxy-3′,5′-dimethoxyphenyl)-2,3,6,7-tetrahydrophenanthro[4,3-*b*]furan-5,11-diol, which is a new compound. Other NOE enhancements observed were in agreement with the proposed structure. Methylation of **2** with MeI−K_2CO_3 gave a pentamethyl ether (**3**). Difference NOE studies confirmed that it was the hindered C-11 hydroxyl group that remained unmethylated.

The phenanthro[4,3-*b*]furan ring system is uncommon in general and previously known from natural sources only as the degraded diterpenoids such as dihydroisotanshinone II from the roots of *Salvia* species.[7] It is probably formed by a radical coupling of sinapyl alcohol with 1,4,5-trihydroxy-7-methoxy-9,10-dihydrophenanthrene.

Experimental Section

General Experimental Procedures. Flash chromatography was carried out on silica gel (40 μm, Baker) as well as C_{18} (40 μm, Baker), DIOL (Lichroprep 40−63 μm, Merck), and Cyano (40 μm, Baker) bonded phases. For gel permeation column chromatography, Fractogel TSK HW-40 (F) (32−63 μm) with $CHCl_3$−CH_3OH (1:1) as eluent was used. High-performance liquid chromatography (HPLC) was performed on a Shimadzu LC-8A system with RI detection. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. UV spectra were obtained using a Hewlett-Packard 8452A diode array spectrophotometer. IR spectra were measured with a Perkin-Elmer 598 infrared spectrophotometer. NMR spectra were measured using a Bruker AMX 500 [500 MHz (^1H) and 125 MHz (^{13}C)] instrument relative to TMS as internal standard. Electron impact (70 eV) mass spectra were obtained on a VG 7035E double-focusing mass spectrometer.

Plant Material. As reported previously.[3]

Extraction and Isolation. Investigation of the penultimate fraction of a hexane extract of *Bulbophyllum vaginatum* (remaining from a previous study)[3,4] afforded 4,4′,6,6′-tetramethoxy-[1,1′-biphenanthrene]-2,2′,3,3′,7,7′-hexol (**1**) (8.5 mg) after gel permeation chromatography [Fractogel TSK gel HW-40 (F)], flash chromatography (C_{18}, 45% acetone−H_2O), and HPLC (CN, 45% EtOAc−hexane). The final fraction (1.0 g) was subjected to gel permeation chromatography [Fractogel TSK gel HW-40 (F)] and flash chromatographed twice (cyano, 45% EtOAc−hexane; then DIOL, 3% CH_3OH−$CHCl_3$). This was followed by HPLC (DIOL, 1.5% CH_3OH−$CHCl_3$) to give (2*R**,3*S**)-3-hydroxymethyl-9-methoxy-2-(4′-hydroxy-3′,5′-dimethoxyphenyl)-2,3,6,7-tetrahydrophenanthro[4,3-*b*]furan-5,11-diol (**2**) (12.5 mg).

Compound 1: gum; $[\alpha]^{27}_D$ +5.8° (*c* 0.26, CH_3OH); UV (EtOH) λ_{max} (log ϵ) 260 (4.78), 282 (4.05), 317 (3.29, sh), 344 (2.87), 360 (2.56) nm; IR ($CHCl_3$) ν_{max} 3536, 1601, 1513 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) δ 9.14 (1H, s, H-5), 8.25 (1H, br s, exchangeable with D_2O, OH-3), 7.95 (1H, s, exchangeable with D_2O, OH-7), 7.72 (1H, br s, exchangeable with D_2O, OH-2), 7.23 (1H, d, J = 9.0 Hz, H-9), 7.19 (1H, s, H-8), 6.91 (1H, d, J = 9.0 Hz, H-10), 4.08 (3H, s, OCH_3-6), 4.06 (3H, s, OCH_3-4); ^{13}C NMR (acetone-d_6, 125 MHz) δ 148.5 (C, C-6), 146.5 (C, C-7), 145.0 (2C, C-2 and C-4), 139.8 (C, C-3), 128.6 (C, C-8a), 126.9 (C, C-10a), 124.8 (CH, C-9), 124.5 (C, C-4b), 123.5 (CH, C-10), 119.1 (C, C-4a), 114.7 (C, C-1), 112.2 (CH, C-8), 108.3 (CH, C-5), 60.2 (CH_3, OCH_3-4), 56.2 (CH_3, OCH_3-6); EIMS *m*/*z*

57 $[M]^+$ (100), 285 (96); HREIMS *m*/*z* 570.1517 (calcd for $C_{30}H_{26}O_{10}$, 570.1526).

Compound 2: gum; $[\alpha]^{27}_D$ −3.5° (*c* 0.85, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 230 sh (3.12), 278 (4.85), 300 (4.01), 318 (3.77) nm; IR ($CHCl_3$) ν_{max} 3536, 3290 (OH), 1603, 1514, 1465 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) δ 8.50 (1H, s, OH-11), 8.02 (1H, br s, OH-5), 7.37 (1H, br s, OH-4′), 6.90 (1H, s, H-4), 6.80 (2H, s, H-2′ and H-6′), 6.46 (d, J = 2.7 Hz, H-8), 6.33 (d, J = 2.7 Hz, H-10), 5.65 (1H, d, J = 7.3 Hz, H-2), 4.15 (1H, br s, OH-7′), 3.90 (2H, m, H_2-7′), 3.80 (6H, s, OCH_3-3′ and OCH_3-5′), 3.76 (3H, s, OCH_3-9), 3.69 (1H, br q, J = 6.3 Hz, H-3), 2.82 (1H, m, H-6), 2.67 (2H, m, H_2-7), 2.64 (1H, m, H-6); ^{13}C NMR (acetone-d_6, 125 MHz) δ 160.9 (C-9), 156.2 (C-11), 149.3 (C-5), 148.9 (C-3′ and C-5′), 146.8 (C-11c), 142.5 (C-7a), 137.0 (C-4′), 131.1 (C-1′), 127.3 (C-3a), 125.3 (C-5a), 117.3 (C-11b), 113.8 (C-11a), 111.3 (C-4), 107.3 (C-8), 104.9 (C-2′ and C-6′), 102.9 (C-10), 89.4 (C-2), 63.8 (C-7′), 56.7 (OCH_3-3′ and OCH_3-5′), 55.4 (OCH_3-9′), 54.0 (C-3), 31.4 (C-7), 22.8 (C-6); EIMS *m*/*z* 466 $[M]^+$ (9), 448 $[M - H_2O]^+$ (100); HREIMS *m*/*z* 466.1617 (calcd for $C_{26}H_{26}O_8$, 466.1628).

Methylation of 2. The phenol **2** (8 mg) was methylated by refluxing overnight with CH_3I−K_2CO_3 in acetone. The crude product was subjected to HPLC (DIOL, 50% EtOAc−hexane) to afford 3 mg of the pentamethylated compound **3** as a gum: $[\alpha]_D$ −3.5° (*c* 0.25, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 228 (3.67), 278 (4.19), 300 (3.79), 316 (3.67) nm; IR ($CHCl_3$) ν_{max} 3292 (OH), 1595, 1509, 1459 (benzene ring) cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) δ 8.43 (1H, s, OH-11), 7.04 (1H, s, H-4), 6.82 (2H, s, H-2′ and H-6′), 6.47 (d, J = 2.5 Hz, H-8), 6.35 (1H, d, J = 2.5 Hz, H-10), 5.74 (1H, d, J = 7.3 Hz, H-2), 4.19 (1H, t, J = 5.5 Hz, OH− 7′), 3.98 (2H, t, J = 5.5 Hz, H_2-7′), 3.85 (3H, s, OCH_3), 3.80 (6H, s, OCH_3-3′and OCH_3-5′), 3.77 (3H, s, OCH_3), 3.73 (3H, s, OCH_3), 3.73 (1H, m, H-3), 2.64 (4H, m, H_2-6 and H_2-7); ^{13}C NMR (acetone-d_6, 125 MHz) δ 161.9 (C), 157.4 (C), 155.4 (2 C), 153.1 (C), 148.5 (C), 143.2 (C), 140.1 (C), 137.8 (C), 128.4 (C), 127.9 (C), 118.5 (C), 114.3 (C), 108.5 (CH), 108.1 (CH), 105.4 (2 CH), 103.8 (CH), 90.0 (CH), 64.7 (CH_2), 61.2 (CH_3), 57.5 (CH_3), 57.2 (2 CH_3), 56.1 (CH_3), 55.2 (CH), 32.1 (CH_2), 23.4 (CH_2); EIMS *m*/*z* 494 $[M]^+$ (73), 476 $[M - H_2O]^+$ (100); HREIMS *m*/*z* 494.1938 (calcd for $C_{28}H_{30}O_8$, 494.1941).

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Supporting Information Available: Table S1, complete NMR data for compound **1** (HMBC, difference NOE). Table S2, complete NMR data for compound **2** (HMBC, difference NOE). This material is available free of charge via the Internet at http://pubs.acs.org.

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