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Phenanthrene and other aromatic constituents of *Bulbophyllum vaginatum*

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Abstract

Two new phenanthrenes (4-methoxyphenanthrene-2,3,7-triol and 4-methoxyphenanthrene-2,3,6,7-tetrol) and two new 9,10-dihydrophenanthrenes (5-methoxy-9,10-dihydrophenanthrene-2,3,7-triol and 4-methoxy-9,10-dihydrophenan-threne-2,3,6,7-tetrol), along with the known 4,6-dimethoxyphenanthrene-2,3,7-triol and its 9,10-dihydro derivative, 3,4′,5-trihydroxy-3′-methoxybibenzyl (tristin), dihydroferulic acid, *p*-coumaric acid, 3,4-dihydroxybenzoic acid and (±)-syringaresinol were isolated from the dichloromethane extract of the orchid *Bulbophyllum vaginatum*. The structures of these compounds were determined through spectroscopic analysis.

Keywords: *Bulbophyllum vaginatum*; Orchidaceae; Phenanthrenes; 9,10-Dihydrophenanthrenes; Isolation; Structure determination

1. Introduction

Bibenzyls, phenanthrenes and 9,10-dihydrophenanthrenes are frequently encountered in extracts of orchids (Majumder, Roychowdhury, & Chakraborty, 1997). We have previously reported the occurrence of a variety of such compounds in the hexane extract of *Bulbophyllum vaginatum* (Lindl.) Reichb.f. (Leong, Kang, Harrison, & Powell, 1997). A subsequent examination of the constituents of the dichloromethane soluble material has afforded four new phenanthrene derivatives along with seven known aromatic compounds.

2. Results and Discussion

Purification of the dichloromethane extract by extensive column chromatography and HPLC afforded four new and seven known compounds. The latter group contained dihydroferulic acid (**1**), 4,6-dimethoxy-9,10-dihydrophenanthrene-2,3,7-triol (**2**), *p*-coumaric acid (**3**), 4,6-dimethoxyphenanthrene-2,3,7-triol (**4**), 3,4-dihydroxybenzoic acid (**5**), 3,4′,5-trihydroxy-3′-methoxybibenzyl (tristin) (**6**) and (±)-syringaresinol (**7**). The known compounds were compared with authentic samples, apart from **6** (Majumder & Pal, 1993) and **7** (Deyama, 1983) which were identified from published physical data. 4,6-Dimethoxy-9,10-dihydrophenanthrene-2,3,7-triol (**2**) and 4,6-dimethoxyphenanthrene-2,3,7-triol (**4**) have already been reported from the hexane extract (Leong et al., 1997).

The first novel compound was 4-methoxyphenanthrene-2,3,7-triol (**8**), $C_{15}H_{12}O_4$. Its UV absorption bands (258, 282 sh, 308 sh, 344 and 362 nm) were characteristic of a phenanthrene derivative (Letcher & Nhamo, 1971), whilst its positive reaction with $FeCl_3$ and IR spectrum (3537 (OH) cm^{-1}) indicated the compound's phenolic nature. The ^1H and ^{13}C NMR spectra showed resonances for a 1,2,4-trisubstituted benzene ring (δ_H 9.27 (1H, d, $J=9.2$ Hz, H-5), 7.24 (1H, d, $J=2.7$ Hz, H-8) and 7.19 (1H, dd, $J=2.7$ and 9.2 Hz, H-6); δ_C 128.7 (C-5), 117.3 (C-8) and 112.3 (C-6)), a pair of *ortho*-coupled aromatic protons (δ_H 7.51 and 7.43 (2H, ABq, $J_{AB}=8.9$ Hz, H-10 and H-9, respectively); δ_C 127.9 (C-9) and 125.3 (C-10)), an isolated aromatic proton (δ_H 7.15 (1H, s, H-1); δ_C 109.5 (C-1)), three hydroxyl groups (δ_H 8.61 (1H, br s, OH) and 8.31 (2H, br s, $2\times$OH), all exchangeable with D_2O), an *ortho*-disubstituted methoxyl group (δ_H 3.88 (3H, s, 4-OCH_3), δ_C 59.8 (q, 4-OCH_3)) and eight fully substituted aromatic carbons (δ_C 155.7 (C-7), 146.1 (C-3), 145.4 (C-4), 140.2 (C-2), 134.7 (C-8a), 126.9 (C-10a), 123.9 (C-4b) and 119.0 (C-4a)). Methylation of **8** afforded a tetramethyl ether (**9**) (δ_H 4.03 (3H, s, 3-OCH_3), 4.01 (3H, s, 4-OCH_3), 4.00 (3H, s, 2-OCH_3) and 3.96 (3H, s, 7-OCH_3)) confirming the presence of three hydroxyl

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groups in the parent compound. The most deshielded aromatic proton signal (δ_H 9.27) was characteristic of H-5 of a phenanthrene and the absence of a second deshielded signal indicated that C-4 was substituted (Majumder, Banerjee, & Sen, 1996). Since saturation of the methoxyl protons resulted in an NOE at H-5 (7%), C-4 was methoxylated. The *ortho*-coupled aromatic protons at δ_H 7.51 and 7.43 were typical of H-9 and H-10 of phenanthrenes (Majumder et al., 1996). Irradiation of the proton at δ_H 7.43 enhanced the signals due to H-8 (14%) and H-10 (9%). When H-10 (δ_H 7.51) was irradiated, it showed the expected NOE at H-9 (9%) as well as enhancing the isolated aromatic proton signal (16%). It followed that C-1 was unsubstituted and that the compound was 4-methoxyphenanthrene-2,3,7-triol (**8**), a new natural product. The tetramethyl ether was therefore 2,3,4,7-tetramethoxyphenanthrene (**9**) which possessed physical properties in good agreement with those reported for the same compound isolated from the orchid *Bletilla striata* (Yamaki, Kato, Bai, Inoue, & Takagi, 1991). Hydrogenation of **8** afforded 4-methoxy-9,10-dihydrophenanthrene-2,3,7-triol (**10**) which has been isolated from the hexane extract of *B. vaginatum* (Leong et al., 1997). The ^{13}C NMR spectrum of **8** was assigned by comparison with previously assigned spectra (Leong et al., 1997).

The second compound, 5-methoxy-9,10-dihydrophenanthrene-2,3,7-triol (**11**) was obtained as a brown gum, $C_{15}H_{14}O_4$. Its UV spectrum showed absorption bands at 270 sh, 278 and 302 nm that were similar to those reported for 9,10-dihydrophenanthrenes (Majumder & Joradar, 1985) whilst the IR spectrum contained hydroxyl (3550 cm^{-1}) and aromatic ring (1608, 1514 and 1464 cm^{-1}) absorptions. The ^1H and ^{13}C NMR spectra (see Section 2) showed resonances for two isolated aromatic protons (δ_H 7.81 (1H, s, H-4) and 6.66 (1H, s, H-1)), a pair of *meta*-coupled aromatic protons (δ_H 6.43 (1H, d, $J=2.4$ Hz, H-6) and 6.36 (1H, d, $J=2.4$ Hz, H-8)), three hydroxyl groups (δ_H 8.29, 7.60 and 7.55 (each 1H, br s, OH), exchangeable with D_2O), a non-*ortho*-disubstituted methoxyl group (δ_H 3.82 (3H, s, 5-OCH_3); δ_C 55.7 (q, 5-OCH_3)), two benzylic methylene groups (δ_H 2.58 (4H, m, H_2-9 and H_2-10); δ_C 31.8 and 29.8 (both t, C-9 and C-10)) and eight fully substituted aromatic carbons. The phenol formed a tetramethyl ether (**12**) (δ_H 3.91, 3.90, 3.89 and 3.84 (each 3H, s, OCH_3)) which agreed with the presence of three phenolic hydroxyl substituents in **11**. Saturation of the two benzylic methylene groups, which were attributed to H_2-9 and H_2-10 of a 9,10-dihydrophenanthrene (Majumder & Pal, 1993), resulted in enhancements of the signals for H-8 (7%) and the more shielded singlet aromatic proton, H-1 (6%). C-1 and C-8 were therefore unsubstituted. The presence of a deshielded aromatic proton (H-4) indicated that C-5 bore an oxygen substituent (Majumder & Pal, 1993; Anton, Kraut, Mues, & Morales, 1997). This was found to be a methoxyl as irradiation of the methoxyl protons enhanced both the H-4 (2%) and H-6 (15%) signals. The three hydroxyl groups were thus located at C-2, C-3 and C-7 and the natural compound was the novel 5-methoxy-9,10-dihydrophenanthrene-2,3,7-triol (**11**). The derived tetramethyl ether was hence 2,3,5,7-tetramethoxy-9,10-dihydrophenanthrene (callosumin) (**12**) which occurs in the orchid *Agrostophyllum callosum*. Its physical properties agreed well with reported values (Majumder et al., 1996). The ^{13}C NMR spectrum of **11** was assigned by comparison with previously assigned spectra (Leong et al., 1997).

The third compound, 4-methoxy-9,10-dihydrophenanthrene-2,3,6,7-tetrol (**13**), was obtained as a brown oil, $C_{15}H_{14}O_5$, with UV and IR spectra that were typical of a 9,10-dihydrophenanthrene. The ^1H and ^{13}C NMR spectra showed resonances for three isolated aromatic protons (δ_H 7.77 (1H, s, H-5), 6.74 (1H, s, H-8) and 6.60 (1H, s, H-1)), four hydroxyl groups (δ_H 5.62 (1H, br s, OH), 5.29, (2H, br s, $2\times$OH) and 5.16 (1H, br s, OH), exchangeable with D_2O), a methoxyl group (δ_H 3.65 (3H, s, 4-OCH_3); 59.9 (q, 4-OCH_3)), two benzylic methylene groups (2.63 (4H, br s, H_2-9 and H_2-10)) and nine substituted aromatic carbons, five of which were oxygenated. The compound was therefore a pentasubstituted 9,10-dihydrophenanthrene with one methoxyl and four hydroxyl substituents. Methylation of the compound resulted in a pentamethyl ether (**14**) (δ_H 3.93, 3.92, 3.91, 3.89 and 3.78 (each 3H, s, OCH_3)). The presence of three singlet aromatic proton resonances was consistent only with the 2,3,4,6,7 oxygenation pattern as was the enhancement of the signals due to H-1 (10%) and H-8 (10%) upon saturation of the benzylic methylene protons. The methoxyl group was shown to be attached to C-4 since its irradiation caused a small enhancement of H-5 (5%). Therefore the compound was 4-methoxy-9,10-dihydrophenanthrene-2,3,6,7-tetrol (**13**), a novel compound. The pentamethyl ether, 2,3,4,6,7-pentamethoxy-9,10-dihydrophenanthrene (**14**), was identical to the compound prepared by the methylation of 4,6-dimethoxy-9,10-dihydrophenanthrene-2,3,7-triol (**2**) (Leong et al., 1997). H-1 and H-8 of **13** were distinguished by comparison of their chemical shifts with those of the corresponding protons of **2** which had previously been assigned using 2D NMR (Leong et al., 1997).

The final compound, 4-methoxyphenanthrene-2,3,6,7-tetrol (**15**), $C_{15}H_{12}O_5$, had UV and IR absorptions which were characteristic of a phenanthrene derivative. The ^1H and ^{13}C NMR spectra showed resonances for three isolated aromatic protons (δ_H 8.91 (1H, s, H-5), 7.23 (1H, s, H-8) and 7.09 (1H, s, H-1); δ_C 112.0 (d, C-5), 112.8 (d, C-8) and 109.4 (d, C-1)), a pair of *ortho*-coupled aromatic protons (δ_H 7.36 and 7.34 (2H, ABq, $J_{AB}=8.8$ Hz, H-9 and H-10); δ_C 125.2 (d, C-9) and 125.0 (d, C-10)), four hydroxyl groups (δ_H 8.43, 8.33, 8.24 and 8.12 (each 1H, br s, OH), all exchangeable with D_2O)), an *ortho*-di-

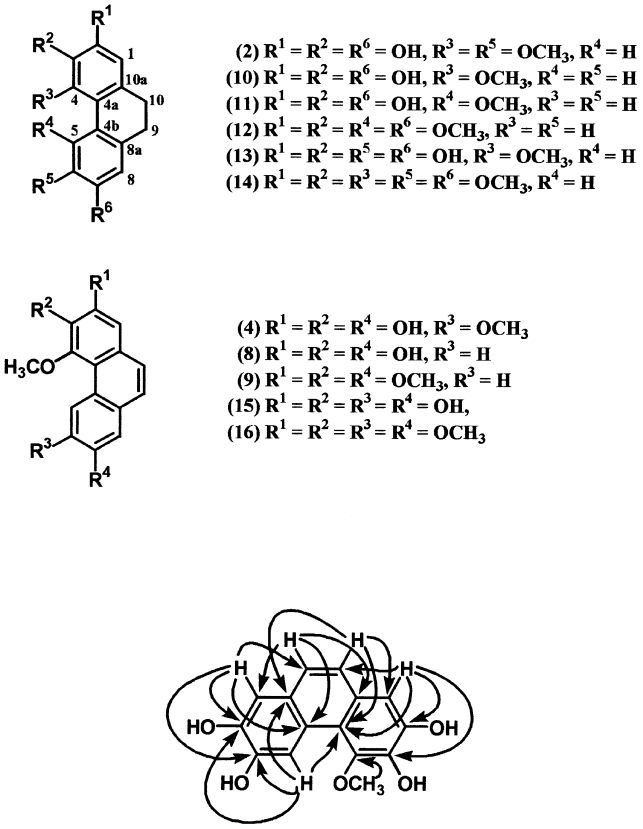

Fig. 1.

substituted methoxyl group (δ_H 3.87 (3H, s, 4-OCH_3); δ_C 59.8 (q, 4-OCH_3)) and nine substituted aromatic carbons. Methylation of **15** gave a pentamethyl ether (**16**) (δ_H 4.04 (6H, s, $2\times OCH_3$), 4.03 (3H, s, OCH_3), 4.01 (3H, s, 2-OCH_3 or 7-OCH_3)). The presence of three uncoupled aromatic protons was again consistent with a 2,3,4,6,7-pentasubstituted phenanthrene with the most deshielded hydrogen at C-5. Irradiation of H-9 and H-10 afforded the expected NOEs at H-1 (5%) and H-8 (5%), respectively. A small NOE at H-5 (9%) upon irradiation of the methoxyl protons established that the methoxyl group was attached to C-4. The compound was therefore 4-methoxyphenanthrene-2,3,6,7-tetrol (**15**) and its permethylated derivative was 2,3,4,6,7-pentamethoxyphenanthrene (**16**) which was identical to a sample prepared previously (Leong et al., 1997). Catalytic hydrogenation of **15** afforded 4-methoxy-9,10-dihydrophenanthrene-2,3,6,7-tetrol (**13**) which was identical to the natural product described above. Confirmation of structure **15** as well as assignment of the ^1H and ^{13}C NMR resonances was performed using HMQC and HMBC spectroscopy (see Fig. 1). The ^{13}C resonances C-6/C-7 and C-2/C-3 could not be distinguished unambiguously due to the heavily substituted nature of the aromatic rings and the absence of correlations to the hydroxyl protons. However, C-2 is expected to be more deshielded than C-3.

3. Experimental

M.p.'s: uncorr. $[\alpha]_D$: $CHCl_3$. IR: $CHCl_3$ unless otherwise specified. UV: EtOH. EIMS: 70 eV. CC: silica gel (Baker, 40 μm); CN (Bakerbond, 40 μm), DIOL (Lichrosorb 40–63 μm); C_{18} (Bakerbond, 40 μm). GPC: Sephadex LH-20 ($CHCl_3$–MeOH 1:1 as eluant). HPLC: Lichrosorb C_{18}, CN or DIOL, 10 μm, 4.5×250 mm or 9.0×250 mm, RI detection.

3.1. NMR spectroscopy

1D: 300 (^1H) and 75 MHz (^{13}C) in $CDCl_3$ (unless specified otherwise) relative to TMS at δ 0.0. 2D and NOE experiments were run at 500 (^1H) and 125 MHz (^{13}C). Difference NOE spectra were run using the NOEMULT programme. The relaxation delay was 2.5 s and the total irradiation time was 3–4 s. Difference NOE results are recorded below in the following manner: H irradiated (H enhanced (% enhancement)). Proton-detected HMQC experiments were optimised for a $^1J_{CH}$ value of 140 Hz. The relaxation delay was 2.5 s. 512 increments, each of 32 scans, were used in t_1 and zero filled to 1K prior to Fourier transformation. In t_2, 2K points were used prior to Fourier transformation. Sine multiplication was used in both dimensions to improve the signal to noise ratio and suppress truncation errors. Proton-detected HMBC experiments were performed under the same conditions as in the HMQC experiment except for modulation tuning which was optimised for $^nJ_{CH}=7$ Hz and a composite pulse which was used to eliminate $^1J_{CH}$.

3.2. Isolation

After exhaustive extraction of the plant material with hot hexane (Leong et al., 1997), the extraction was repeated with CH_2Cl_2. CC of the crude extract (56 g) on silica gel eluting with an EtOAc–hexane gradient gave 17 frs. Frs 1–11 were not investigated further since TLC showed no distinct spots.

Frs 12–15 (859 mg) were subjected to CC (CN, 30–50% EtOAc–hexane) to give three frs A–C. CC of fr. A (199 mg) (CN, 25% EtOAc–hexane) afforded two frs A1 and A2. Fr. A1 (63 mg) yielded dihydroferulic acid (**1**) (6 mg) and 4,6-dimethoxy-9,10-dihydrophenanthrene-2,3,7-triol (**2**) (6 mg) after HPLC (DIOL, 35% EtOAc–hexane). Fr. A2 (100 mg) was also subjected to HPLC (silica gel, 40% EtOAc–hexane) to give *p*-coumaric acid (**3**) (8 mg), *4-methoxyphenanthrene-2,3,7-triol* (**8**) (41 mg) and 4,6-dimethoxyphenanthrene-2,3,7-triol (**4**) (17 mg). Fr. B (360 mg) was chromatographed twice (DIOL, 40% EtOAc–hexane) followed by HPLC (DIOL, 40% EtOAc–hexane) to give, in order of elution, mixture B1, (**8**) (31 mg), *5-methoxy-9,10-dihydrophenanthrene-2,3,7-triol* (**11**) (10 mg), 3,4-dihydroxybenzoic acid (**5**) (15 mg) and *4-methoxy-9,10-dihydrophenanthrene-2,3,6,7-tetrol*

(**13**) (24 mg). Mixture B1 was chromatographed (silica gel, 1% CH_3OH–$CHCl_3$) to give **4** (11 mg) and 3,4′,5-trihydroxy-3′-methoxybibenzyl (tristin) (**6**) (9 mg). Fr. C (152 mg) was chromatographed twice to give mainly *4-methoxyphenanthrene-2,3,6,7-tetrol* (**15**) (14 mg). **1**, **2**, **3**, **4** and **5** were identified by comparison with authentic samples.

Frs 16–17 (1.1 g) were chromatographed twice (C_{18}, 50% acetone–H_2O; CN, 45% acetone–H_2O) to yield two frs, D and E. Fr. D (110 mg) afforded 3,4-dihydroxybenzoic acid (**5**) (5 mg), (**13**) (9 mg) and (**15**) (20 mg) after HPLC (DIOL, 60% EtOAc–hexane). Fr. E (111 mg) yielded (±)-syringaresinol (**7**) as the major component (21 mg) after HPLC purification (CN, 33% EtOAc–hexane).

3.3. 3,4′,5-Trihydroxy-3′-methoxybibenzyl (tristin) (*6*)

Brown gum. UV λ_{max} nm: 226 sh, 282; IR ν_{max} cm^{-1}: 3546, 3328 (OH), 1603, 1514, 1465 (benzene ring); EI-MS *m/z* (rel. int.): 260 $[M]^+$ (56), 137 (100), 123 (15); HREI-MS: *m/z* 260.1057 ($C_{15}H_{16}O_4$ requires *m/z* 260.1049); ^1H NMR (acetone-d_6): δ 8.07 (2H, br s, OH), 7.30 (1H, br s, OH), 6.80 (1H, d, $J=1.7$ Hz, H-2′), 6.72 (1H, d, $J=8.0$ Hz, H-5′), 6.65 (1H, dd, $J=1.7$ and 8.0 Hz, H-6′), 6.22 (2H, m, H-2 and H-6), 6.18 (1H, t, $J=2.1$ Hz, H-4), 3.80 (3H, s, 3-OCH_3), 2.74 (4H, m, CH_2CH_2); ^{13}C NMR (acetone-d_6): δ 159.3 (2×s), 148.1 (s), 145.5 (s), 145.1 (s), 134.2 (s), 121.5 (d), 115.5 (d), 112.9 (d), 107.8 (2×d), 101.1 (d), 56.2 (q), 39.0 (t), 37.9 (t). Difference NOE (acetone-d_6): CH_2CH_2 [H-2′ (3.6), H-6′ (3.2), H-2 and H-6 (7.7)], 3-OCH_3 [H-2 (10.5)].

3.4. (±)-Syringaresinol (*7*)

Needles, m.p. 177–178°C (CH_2Cl_2–hexane), 190–191°C (ethanol) (lit. 170–171°C (Stöcklin, De Silva, & Geissman, 1969)); $[\alpha]_D$ −1.4 (*c* 1.29); UV λ_{max} nm: 232 sh, 272, 304; IR ν_{max} cm^{-1}: 3539 (OH), 1618, 1518, 1464 (benzene ring); EI-MS *m/z* (rel. int.): 481 $[M]^+$ (35), 181 [M-syringoyl moiety]$^+$ (100), 167 [M-(4-hydroxy-3,5-dimethoxybenzyl moiety)]$^+$ (59); HREI-MS: *m/z* 418.1622 ($C_{22}H_{26}O_8$ requires *m/z* 428.1628); ^1H NMR (C_6D_6): δ 6.60 (4H, s, H-2′, H-2″, H-6′ and H-6″), 5.40 (2H, br s, 4′-OH and 4″-OH), 4.75 (2H, br d, $J=4.2$ Hz, H-2 and H-6), 4.14 (2H, dd, $J=6.8$ and 9.0 Hz, H-4 and H-8), 3.86 (2H, dd, $J=3.8$ and 9.0 Hz, H-4 and H-8), 3.38 (12H, s, 4×OCH_3), 2.93 (2H, m, H-1 and H-5); ^1H NMR: δ 6.58 (4H, s, H-2′, H-2″, H-6′ and H-6″), 5.53 (2H, br s, 4′-OH and 4″-OH), 4.73 (2H, br d, $J=4.2$ Hz, H-2 and H-6), 4.28 and (2H, dd, $J=6.9$ and 9.2 Hz, H-4 and H-8), 3.89 (2H, dd, $J=3.8$ and 9.2 Hz, H-4 and H-8), 3.90 (12H, s, 4×OCH_3), 3.09 (2H, m, H-1 and H-5); ^{13}C NMR: δ 147.2 (4×s, C-3′, C-3″, C-5′ and C-5″), 134.4 (2×s, C-4′ and C-4″), 132.1 (2×s, C-1′ and C-1″), 102.8 (4×s, C-2′, C-2″, C-6′ and C-6″), 86.1 (2×s, C-2 and C-6), 71.8 (2×s, C-4 and C-8), 56.4 (4×s, OCH_3), 54.4 (2×s, C-1 and C-5).

3.5. 4-Methoxyphenanthrene-2,3,7-triol (*8*)

M.p. 208–210°C ($CHCl_3$); UV λ_{max} nm: 258, 282 sh, 308 sh, 344, 362; IR ν_{max} cm^{-1}: 3537 (OH), 1613, 1585, 1512, 1475; EI-MS *m/z* (rel. int.): 256 $[M]^+$ (100), 241 (91), 223 (16), 185 (18), 128 (19); HREI-MS: *m/z* 256.0731 ($C_{15}H_{12}O_4$ requires *m/z* 256.0736); ^1H and ^{13}C NMR (acetone-d_6): see text. Difference NOE (acetone-d_6): 4-OCH_3 [H-5 (7.4)], H-9 [H-10 (8.7), H-8 (13.9)], H-10 [H-9 (9.0), H-1 (15.7)].

3.6. Methylation of *8*

Compound **8** (8 mg) was methylated in the usual fashion with CH_3I–K_2CO_3 in Me_2CO. CC of the crude product (silica gel, 8% EtOAc–hexane) afforded 2,3,4,7-tetramethoxyphenanthrene (**9**) (8 mg) as a solid, m.p. 150–151°C; UV λ_{max} nm: 234 sh, 258, 282, 290 sh, 302, 342, 360; IR ν_{max} cm^{-1}: 1614, 1499, 1472 (benzene ring); EI-MS *m/z* (rel. int.): 298 $[M]^+$ (100), 283 (73), 240 (80), 197 (42); HREI-MS: *m/z* 298.1210 ($C_{18}H_{18}O_4$ requires *m/z* 298.1205); ^1H NMR: δ 9.41 (1H, d, $J=9.4$ Hz, H-5), 7.59 (2H, s, H-9 and H-10), 7.25 (1H, dd, $J=2.8$ and 9.2 Hz, H-6), 7.23 (1H, $J=2.8$ Hz, H-8), 7.08 (1H, s, H-1), 4.03 (3H, s, 3-OCH_3), 4.01 (3H, s, 4-OCH_3), 4.00 (3H, s, 2-OCH_3), 3.96 (3H, s, 7-OCH_3); ^{13}C NMR: δ 157.2 (s), 151.84 (s), 151.79 (s), 142.9 (s), 133.4 (s), 129.1 (s), 128.4 (d), 127.1 (d), 126.7 (d), 124.2 (s), 119.2 (s), 116.7 (d), 108.8 (d), 105.3 (d), 61.3 (q), 60.2 (q), 55.9 (q), 55.3 (q). Difference NOE: 4-OCH_3 [H-5 (8.0)], 2-OCH_3 [H-1 (17.7)], 7-OCH_3 [H-6 and H-8 (17.7)].

3.7. Hydrogenation of *8*

The phenanthrene **8** (5 mg) was hydrogenated (H_2-Pd/C). CC (silica gel, 30% EtOAc–hexane) of the crude product gave 4-methoxy-9,10-dihydrophenanthrene-2,3,7-triol (**10**) (2 mg), identical to an authentic sample (Leong et al., 1997).

3.8. 4-Methoxy-9,10-dihydrophenanthrene-2,3,7-triol (*11*)

Brown gum. EI-MS *m/z* (rel. int.): 258 $[M]^+$ (100), 225 (56); HREI-MS: *m/z* 258.0902 ($C_{15}H_{14}O_4$ requires *m/z* 258.0892); UV λ_{max} nm: 270 sh, 278, 302; IR ν_{max} cm^{-1}: 3550 (OH), 1608, 1514, 1464; ^1H NMR (acetone-d_6): δ 8.29 (1H, br s, OH), 7.81 (1H, s, H-4), 7.60 (1H, br s, OH), 7.55 (1H, br s, OH), 6.66 (1H, s, H-1), 6.43 (1H, d, $J=2.4$ Hz, H-6), 6.36 (1H, d, $J=2.4$ Hz, H-8), 3.82 (3H, s, 5-OCH_3), 2.58 (4H, m, CH_2CH_2, H_2-9 and H_2-10); ^{13}C NMR (acetone-d_6): δ 158.6 (C-5), 157.3 (C-7), 143.6 and 143.3 (C-2 and C-3), 141.4 (C-8a), 130.3 (C-4a), 125.8

(C-10a), 116.43 (C-1), 116.38 (C-4b), 115.0 (C-4), 108.3 (C-8), 99.1 (C-6), 55.7 (5-OCH_3), 31.8 and 29.8 (C-9 and C-10). Difference NOE (acetone-d_6): H_2-9 and H_2-10 [H-8 (6.5) and H-1 (6.1)], 5-OCH_3 [H-4 (2.2) and H-6 (15.3)].

3.9. Methylation of **11**

The phenol (7 mg) was methylated as described in Section 2.6 and the crude product was subjected to HPLC (silica gel, 8% acetone–hexane) to give 2,3,5,7-tetramethoxy-9,10-phenanthrene (**12**) (2 mg) as a gum. UV λ_{max} nm: 220, 270 sh, 278, 302, 308 sh; IR ν_{max} cm^{-1}: 1605, 1514, 1464 (benzene ring); EI-MS m/z (rel. int.): 300 $[M]^+$ (100), 285 (50), 257 (13), 226 (18); HREI-MS: m/z 300.1372 ($C_{18}H_{20}O_4$ requires m/z 300.1362); ^{1}H NMR: δ 7.92 (1H, s, H-4), 6.74 (1H, s, H-1), 6.47 (1H, d, $J=2.5$ Hz, H-6 or H-8), 6.44 (1H, d, $J=2.5$ Hz, H-8 or H-6), 3.91, 3.90, 3.89, 3.84 (each 3H, s, $4\times OCH_3$), 2.74 (4H, m, CH_2CH_2).

3.10. 4-Methoxy-9,10-dihydrophenanthrene-2,3,6,7-tetrol (**13**)

Brown oil. UV λ_{max} nm: 234 sh, 282, 318; IR ν_{max} cm^{-1}: 3544, 3277 (OH), 1605, 1515, 1452 (benzene ring); EI-MS m/z (rel. int.): 274 $[M]^+$ (100), 272 (28), 259 (17), 241 (36), 213 (18); HREI-MS: m/z 274.0830 ($C_{15}H_{14}O_5$ requires m/z 274.0841); ^{1}H NMR (acetone-d_6): δ 7.77 (1H, s, H-5), 6.74 (1H, s, H-8), 6.60 (1H, s, H-1), 5.62 (1H, br s, OH), 5.29, (2H, br s, $2\times OH$), 5.16 (1H, br s, OH), 3.65 (3H, s, 4-OCH_3), 2.63 (4H, br s, H_2-9 and H_2-10); ^{1}H NMR (acetone-d_6): δ 7.84 (1H, s, H-5), 7.68 (2H, br s, exchangeable with D_2O, $2\times OH$), 7.52 (1H, br s, exchangeable with D_2O, OH), 6.69 (1H, s, H-8), 6.52 (1H, s, H-1), 3.61 (3H, s, 4-OCH_3), 2.56 (4H, br s, H_2-9 and H_2-10); ^{13}C NMR (acetone-d_6): δ 146.4, (s), 144.9 (s), 144.1 (s), 144.0 (s), 137.6 (s), 130.5 (s), 130.2 (s), 125.4 (s), 119.9 (s), 115.3 (d), 114.8 (d), 111.6 (d), 59.9 (q, 4-OCH_3), 30.7 (t), 30.0 (t). Difference NOE (acetone-d_6): 4-OCH_3 [H-5 (5.3)], H_2-9 and H_2-10 [H-1 (10.1) and H-8 (9.8)].

3.11. Methylation of **13**

Compound **13** (3 mg) was methylated as described in Section 2.6 to afford 2,3,4,6,7-pentamethoxy-9,10-dihydrophenanthrene (**14**) (2.8 mg) (Leong et al., 1997).

3.12. 4-Methoxyphenanthrene-2,3,6,7-tetrol (**15**)

Gum. UV λ_{max} nm: 258, 284, 300 sh, 312, 342, 358; IR ν_{max} cm^{-1}: 3539, 3269, 1600, 1518, 1462; EI-MS m/z (rel. int.): 272 $[M]^+$ (100), 257 (48); HREI-MS: m/z 272.0692 ($C_{15}H_{12}O_5$ requires m/z 272.0685); ^{1}H NMR (acetone-d_6): δ 8.91 (1H, s, H-5), 8.43, 8.33, 8.29, 8.12 (each 1H, br s, exchangeable with D_2O, $4\times OH$), 7.36 (1H, d, $J_{AB}=8.8$ Hz, H-9), 7.34 (1H, d, $J_{AB}=8.8$ Hz, H-10), 7.23 (1H, s, H-8), 7.09 (1H, s, H-1) and 3.87 (3H, s, 4-OCH_3); ^{13}C NMR (acetone-d_6): δ 109.4 (C-1), 146.2 (C-2), 139.8 (C-3), 145.6 (C-4), 118.7 (C-4a), 124.8 (C-4b), 112.0 (C-5), 145.7 (C-6 or C-7), 149.4 (C-7 or C-6), 112.8 (C-8), 128.3 (C-8a), 125.2 (C-9), 125.0 (C-10), 127.4 (C-10a), 60.0 (4-OCH_3). Difference NOE (acetone-d_6): 4-OCH_3 [H-5 (8.5)], H-9 and H-10 [H-1 (5.7) and H-8 (5.2)].

3.13. Methylation of **15**

Compound **15** (3 mg) was methylated as usual to afford 2,3,4,6,7-pentamethoxyphenanthrene (**16**) (3 mg) which was identical (TLC, ^{1}H NMR) to a sample prepared previously (Leong et al., 1997).

3.14. Hydrogenation of **15**

The phenanthrene **15** (5 mg) was subjected to catalytic hydrogenation. CC (DIOL, 50% EtOAc–hexane) of the crude product gave 4-methoxy-9,10-dihydrophenanthrene-2,3,6,7-tetrol (**13**) (1 mg), identical (TLC, ^{1}H NMR) to the natural product.

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References

Anton, H., Kraut, L., Mues, R., & Morales Z, M. I. (1997). *Phytochemistry*, *46*, 1069.
Deyama, T. (1983). *Chem. Pharm. Bull.*, *31*, 2993.
Leong, Y. -W., Kang, C. -C., Harrison, L. J., & Powell, A. D. (1997). *Phytochemistry*, *44*, 157.
Letcher, R. M., & Nhamo, L. R. M. (1971). *J. Chem. Soc. C*, 3070.
Majumder, P. L., Banerjee, S., & Sen, S. (1996). *Phytochemistry*, *42*, 847.
Majumder, P. L., & Joradar, M. (1985). *Ind. J. Chem. B*, *24*, 1192.
Majumder, P. L., & Pal, S. (1993). *Phytochemistry*, *32*, 1561.
Majumder, P. L., Roychowdhury, M., & Chakraborty, S. (1997). *Phytochemistry*, *44*, 167.
Stöcklin, W., De Silva, L. B., & Geissman, T. A. (1969). *Phytochemistry*, *8*, 1565.
Yamaki, M., Kato, T., Bai, L., Inoue, K., & Takagi, S. (1991). *Phytochemistry*, *30*, 2759.