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BULBOPHYLLANTHRIN, A PHENANTHRENE OF THE ORCHID *BULBOPHYLLUM LEOPARDIUM*

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Key Word Index—*Bulbophyllum leopardium*; Orchidaceae; bulbophyllanthrin; phenanthrene; two dimensional NMR.

Abstract—Bulbophyllanthrin, a novel phenanthrene derivative from the orchid *Bulbophyllum leopardium*, was shown to have the structure **1a** mainly on the basis of spectral evidence including ^{13}C and 2D NMR spectra.

INTRODUCTION

Systematic chemical investigations of a series of Himalayan orchids in our laboratory earlier afforded five 9,10-dihydrophenanthropyrans [1–5] and four corresponding pyrones [1–3, 6], two 9,10-dihydrophenanthrenes [7, 8], one phenanthrene [9], a bibenzyl derivative [10] and two novel steroidal ketones [11]. Our continued search for phytochemicals in Indian orchids has resulted in the isolation of yet another novel phenanthrene derivative, designated bulbophyllanthrin, from the orchid *Bulbophyllum leopardium*. We report in this paper evidence leading to the structure of this compound.

RESULTS AND DISCUSSION

Bulbophyllanthrin, $C_{16}H_{14}O_4$ ($[M]^+$ 270), mp 175°, showed UV absorptions, λ_{max} 258, 307, 318, 332, 349 and 367 nm (log ε4.64, 3.94, 3.96, 3.43, 3.70 and 3.84), resembling those of phenanthrene derivatives [12]. The phenolic nature of the compound was indicated by its characteristic colour reactions, the alkali-induced bathochromic shift of the UV maxima [$\lambda_{max}^{EtOH-0.1\,N\,NaOH}$ 258, 267, 341 and 380 nm (log ε4.60, 4.65, 4.07 and 4.08)] and its IR spectrum showing bands at 3180 and 3300 cm^{-1}. The higher wavelength band suggests that one of the phenolic hydroxyl groups is involved in intramolecular hydrogen bonding. The presence of two phenolic hydroxyl groups in bulbophyllanthrin was indicated by the formation of a diacetyl derivative, $C_{20}H_{18}O_6$ ($[M]^+$ 354), mp 122°, and by the formation of a mixture of mono- and dimethyl ether derivatives in the ratio of 2:3 on refluxing with dimethyl sulphate in acetone for 8 hr. The monomethyl ether could not be separated from an ethereal solution of the mixture by extraction even with 2 N aq. sodium hydroxide solution. Because of their similar polarity the two compounds also could not be separated by chromatography and were studied as the mixture.

The 300 Mz ^1H NMR spectrum of bulbophyllanthrin showed signals at δ3.85 and 4.07 for two aromatic methoxyl groups and two one-proton singlets at δ5.99 and 10.3 (disappearing on deuterium exchange) for a normal and an intramolecularly hydrogen-bonded phenolic hydroxyl proton, respectively. The spectrum also displayed signals for six aromatic protons, two of which appearing as a pair of one-proton doublets at δ7.50 (J = 8 Hz) and 7.57 (J = 8 Hz) are typical of the 9- and 10-proton of a phenanthrene derivative. The remaining four aromatic protons resonated at δ7.15 (1H, *s*), 7.26 (1H, *dd*, J_1 = 8 Hz and J_2 = 2 Hz), 7.42 (1H, *dd*, J_1 = 8 Hz and J_2 = 2 Hz) and 7.51 (1H, apparent *t*, J = 8 Hz). These spectral data, while suggesting the gross tetraoxygenated phenanthrene structure of bulbophyllanthrin, also provide evidence for ascertaining its substitution pattern. Thus the absence of any signal beyond δ8.0 in the ^1H NMR spectrum of the compound implies [13] that both C-4 and C-5 are substituted. Further, in the absence of a carbonyl function in the compound, the downfield phenolic hydroxyl proton at δ10.3 requires that one of these substituents must be a phenolic hydroxyl and the other is an aromatic methoxyl so that they may be well-positioned for a fairly strong intramolecular hydrogen bonding. This was also supported by the observed reluctance of one of the phenolic hydroxyl groups of the compound to undergo methylation, and also by the properties of its monomethyl ether, the phenolic hydroxyl proton of which appeared at δ10.5. The sharp singlet at δ7.15, which remained essentially unchanged in the ^1H NMR spectrum of bulbophyllanthrin diacetate suggests that one of the aromatic rings of bulbophyllanthrin contains three of the four oxygen substituents and that the substituent *ortho* to the proton corresponding to the above signal is an aromatic methoxyl rather than a phenolic hydroxyl group. If this proton is assigned to C-1, C-2 of the compound should bear an aromatic methoxyl group. The aromatic proton signal at δ7.26, on the other hand, was shifted downfield by 0.5 ppm in the spectrum of the diacetyl derivative. This would then correspond to the C-6 proton making obligatory the C-5 substituent to be a hydroxyl group. The C-4 substituent should therefore be a methoxyl group, leaving C-3 to accommodate the remaining phenolic hydroxyl function. Based on these observations bulbophyllanthrin was assumed to have the structure **1a**. The relative positions of the methoxyl and hydroxyl groups in **1a** were also corroborated by the extraordinary upfield shift of the C-4 methoxyl protons

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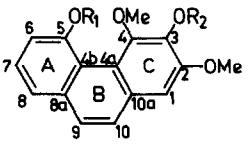
(δ3.39) of its diacetyl derivative (**1b**), compared with that of the parent compound (δ4.07 or 3.85). Sandwiched between the C-2 and C-4 methoxyl groups, the C-3 acetoxy group assumes a relatively rigid conformation. Similar is the situation with the C-4 methoxyl group which is flanked between the C-5 and C-3 acetoxy functions. As a result, the C-4 methoxyl protons fall in the shielding cone of the C-3 acetoxy carbonyl and partly also that of the carbonyl group of the C-5 acetoxy function. This would explain the observed upfield shift of the C-4 methoxyl protons of **1b**, which finds analogy with a similar upfield shift of the oxymethylene protons of coelogin diacetate [1] caused by its C-3 acetoxy carbonyl.

Structure **1a** for bulbophyllanthrin was further supported by its ^{13}C NMR spectral data. The degree of protonation of each carbon atom was determined by APT [14] experiments. The assignments of the carbon chemical shifts were made by comparison with the δ_c values of structurally related compounds taking into consideration of the additive parameters of the functional groups. Thus except for C-2, C-3, C-4a, C-5 and C-10a, the observed chemical shifts of all the carbon atoms of bulbophyllanthrin are in excellent agreement with the values calculated for **1a** using the known additive parameters [15, 16] of hydroxyl and methoxyl groups on the reported δ_c values of the parent phenanthrene [15]. The downfield shifts of C-2 (~ 2.4 ppm), C-3 (~ 16.1 ppm), C-4a (~ 9 ppm), C-5 (~ 4.1 ppm) and C-10a (~ 2.7 ppm) compared to the calculated δ_c values may be primarily due to the polysubstituted nature of ring C of **1a** bearing three consecutive oxygen substituents as in 1,3-O,O-dimethyl pyrogallol [17] and ring C of coelogin [1], where chemical shift values do not follow simple additive parameters of the functional groups. The involvement of the C-4 methoxyl group in intramolecular hydrogen bonding with the C-5 hydroxyl function may also contribute partly to this deshielding effect. The placement of the methoxyl groups at C-2 and C-4 in bulbophyllanthrin is also supported by the chemical shifts of its methoxy carbons (δ_c 56.27 and 62.28). This is in conformity with the general observation [17] that the carbon atom of a methoxyl group having an *ortho* hydrogen atom resonates at ~ 56 ppm, while that of such a group flanked between two *ortho* substituents appear around 60–62 ppm.



1a $R_1 = R_2 = H$
1b $R_1 = R_2 = Ac$
1c $R_1 = H, R_2 = Me$
1d $R_1 = R_2 = Me$

Confirmation of the structure **1a** for bulbophyllanthrin was provided by the results of 2D NMR studies. The homonuclear correlation experiment (COSY) shown in Fig. 1 clearly shows a correlation due to five-bond proton–proton spin coupling between the methoxyl protons at δ4.07 and the singlet aromatic proton at δ7.15. This requires them to be on adjacent carbons and confirms the conclusions drawn earlier from the proton and ^{13}C spectral data. The methoxyl group at δ3.85, on

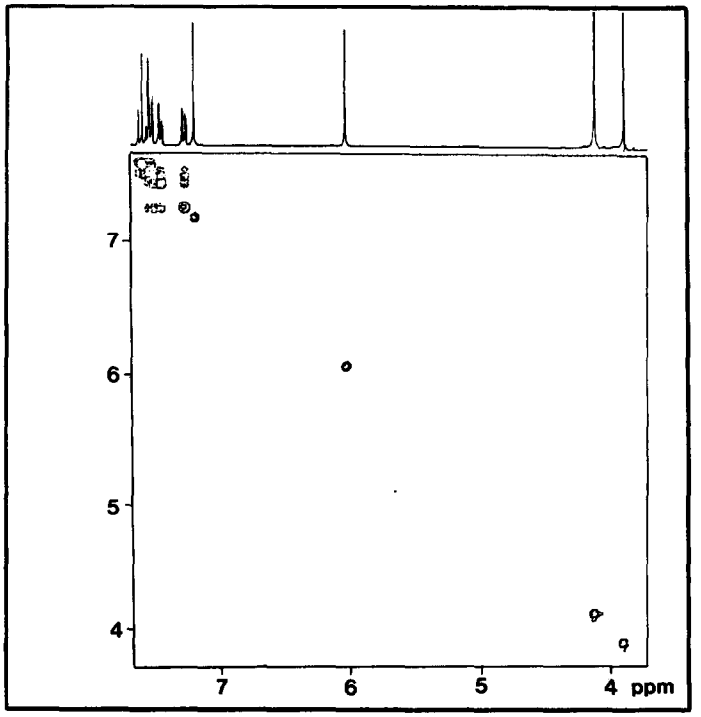


Fig. 1. COSY plot of bulbophyllanthrin (**1a**) showing correlation between methyl protons at δ4.07 and an aromatic singlet proton at δ7.15.

the other hand, shows no correlation to any aromatic proton. The singlet aromatic proton could, however, conceivably be located at C-2 or C-3 instead of at C-1, and in view of the polysubstituted nature of ring C of **1a** these alternatives cannot unequivocally be ruled out on the basis of chemical shift data. The heteronuclear correlation (HETCOR) plots of Figs 2 and 3, showing the one-bond correlations arising from direct C–H spin coupling and the long range correlations arising from two-bond or three-bond C–H couplings, respectively, providing unambiguous spectral assignments and a choice between the alternative structures.

The hydroxyl at C-5 in **1a** is the only substituent on the A-ring. The three protons H-6, H-7 and H-8 show a characteristic three-spin coupling pattern for consecutive aromatic protons. The chemical shift of H-6 is decreased by the effect of the adjacent hydroxyl group with $\Delta\delta$ *ca* −0.3. This permits H-6 to be assigned as δ7.26 (*dd*, 8, 2 Hz). Figure 2 clearly shows that C-6 must then be assigned as the carbon at δ115.74. Unequivocal confirmation of this assignment is provided by the three-bond correlation between C-6 and the hydroxyl proton at C-5 shown in Fig. 3. This leaves the proton at δ7.42 (*dd*, 8, 2 Hz) with no possible assignment other than H-8, and the one-bond correlation to this proton in Fig. 2 confirms the C-8 assignment as δ120.32.

In aromatic systems, 3-bond correlations are strong while 2-bond correlations are nearly non-existent. Consequently, C-8 should show correlations to H-6 and H-9. Again, the data of Fig. 3 confirms the assignment of H-6 and establishes H-9 as δ7.57.

The only possible assignment remaining for H-10 is δ7.50. Figure 2 reveals that the assignment for C-10 must then be δ126.14. Figure 3 also reveals a three-bond correlation between C-10 and H-1, the singlet aromatic proton. The alternative structures are thus ruled out and structure **1a** confirmed.

Other correlations in Figs 2 and 3 allow unequivocal assignments of *all* carbons in **1a**, illustrating the power and utility of this experiment. Both 1-bond and 3-bond correlations are observed for the methoxyl protons in Fig. 3, presumably due to the one-bond coupling being close to an exact multiple of the three-bond coupling.

Bulbophyllanthrin is thus the first phenanthrene bear-

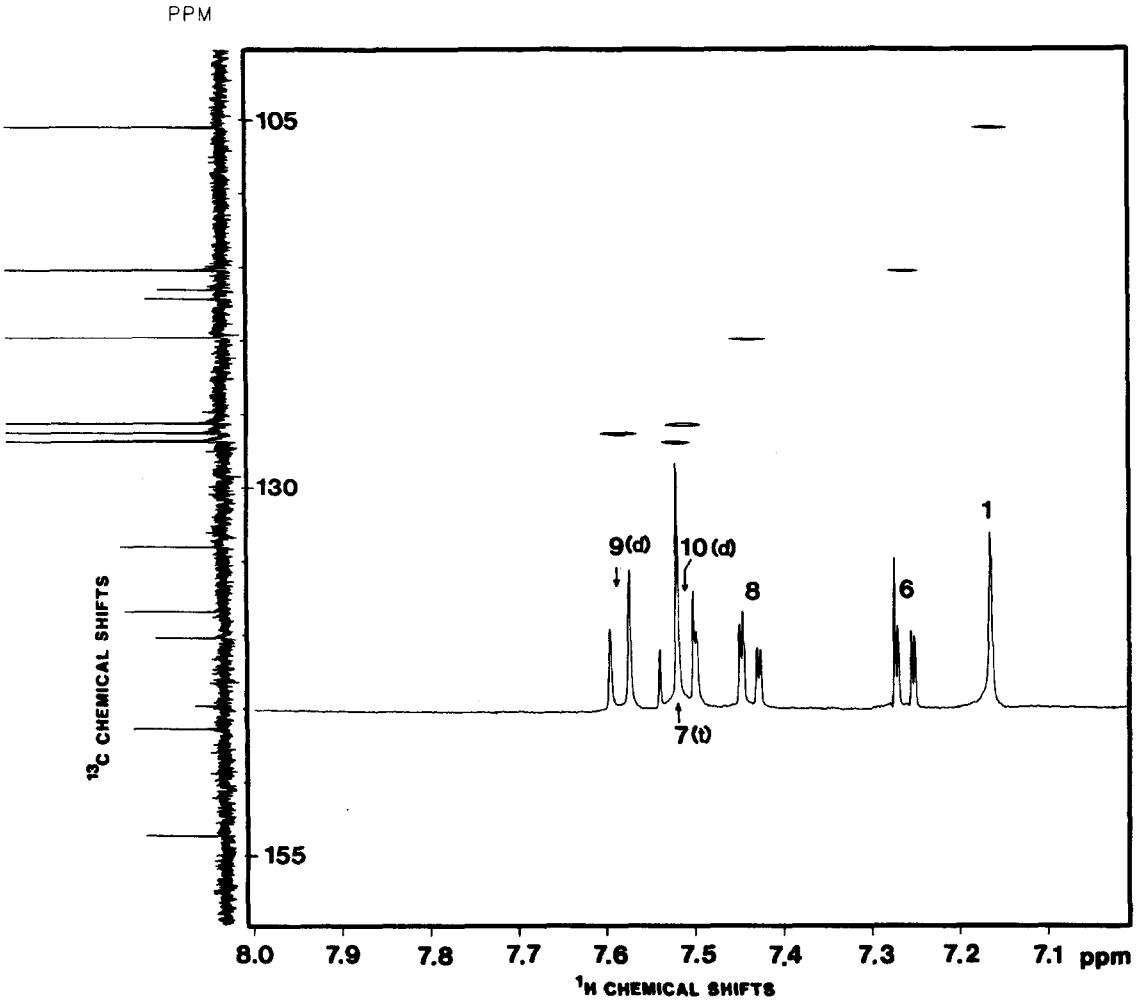


Fig. 2. HETCOR contour plot of bulbophyllanthrin (**1a**) showing one-bond correlations for the protonated aromatic carbons. The J_{CH} parameter was set to 160 Hz.

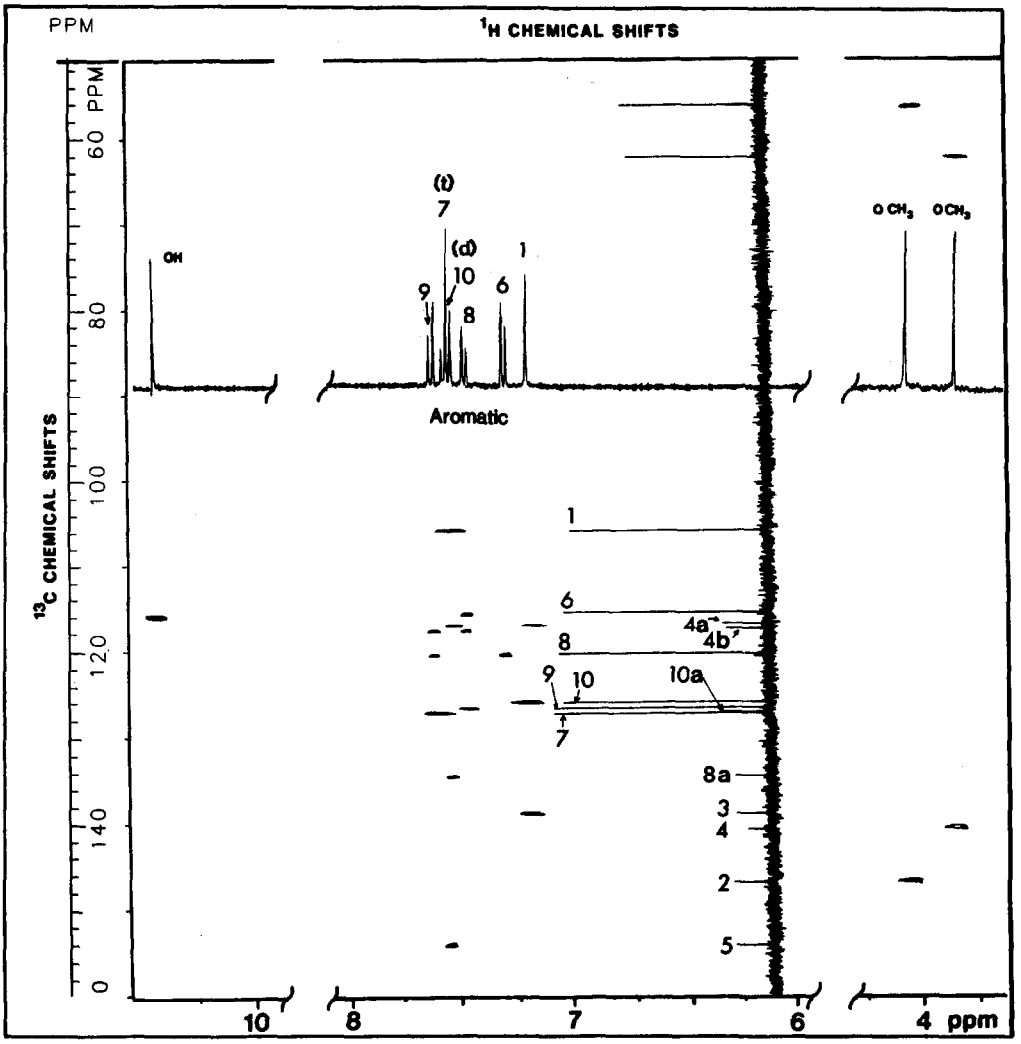


Fig. 3. HETCOR contour plot of bulbophyllanthrin (**1a**) showing three-bond correlations for all carbons. The J_{CH} parameter was set to 7 Hz.

ing oxygen functions at both C-4 and C-5, and the absence of any oxygen substituent at either C-6 or C-7 has invoked considerable interest regarding its possible biogenesis.

EXPERIMENTAL

Mps are uncorr. UV spectra were measured in 95% aldehyde-free EtOH, IR spectra in KBr discs. ^{1}H NMR spectra were recorded at 300 MHz (400 MHz for **1c** and **1d**) in $CDCl_3$ soln using TMS as int. standard. ^{13}C NMR spectra were measured at 75 MHz in the same solvent and using the same int. standard. Chemical shifts were measured in δ ppm and for ^{13}C NMR $\delta_{TMS} = \delta_{CDCl_3} + 76.9$ ppm. MS were recorded in an instrument equipped with a direct inlet system and operating at 70 eV. Figs in the first bracket attached to m/z values represent rel. intensities of peaks. Silica gel (60–100 mesh) were used for CC and silica gel G for TLC. All analytical samples were routinely dried over P_2O_5 for 24 hr *in vacuo* and were tested for purity by TLC and MS. Anhydrous Na_2SO_4 was used for drying organic solvents and petrol used had bp 60–80°.

Isolation of bulbophyllanthrin (**1a**). Air-dried powdered whole plant of *B. leopardium* (3 kg) was kept soaked in MeOH for 3 weeks. The MeOH ext was then drained out and concd under red. pres. to 150 ml, dil with H_2O (750 ml) and exhaustively extracted with Et_2O. The Et_2O layer was then extracted with 2 N aq. NaOH soln. The aq. alkaline soln was acidified with conc HCl in the cold and the liberated solid extracted into Et_2O, washed with H_2O, dried and the solvent removed. The residue was chromatographed. The petrol–EtOAc (10:1) eluate gave **1a** (0.12 g), crystallized from the same solvent mixture, mp 175° (Found: C, 71.01; H, 5.24. $C_{16}H_{14}O_4$ requires: C, 71.11; H, 5.19%). IR ν_{max} cm^{-1}: 3180 (hydrogen bonded OH), 3300 (normal phenolic OH), 1615, 1570, 870, 840 and 815 (aromatic nucleus); ^{13}C NMR: δ_c 154.24 (C-5), 146.90 (C-2), 140.71 (C-4), 138.90 (C-3), 134.53 (C-8a), 127.43 (C-7), 127.34 (C-10a), 126.85 (C-9), 126.14 (C-10), 120.32 (C-8), 117.69 (C-4b), 117.11 (C-4a), 115.74 (C-6), 106.00 (C-1), 62.28 (4-OMe) and 56.27 (2-OMe); MS m/z (rel. int.): 270 $[M]^+$ (100), 255 (29), 237 (8), 227 (19), 212 (20), 195 (32), 184 (18), 155 (9), 139 (15), 127 (7) and 43 (7).

Compound **1a** was acetylated with Ac_2O–pyridine in the usual manner to give **1b**, crystallized from petrol–EtOAc, mp 122° (Found: C, 67.71; H, 5.13. $C_{20}H_{18}O_6$ requires: C, 67.80; H, 5.08%).

UV λ_{max} nm: 206, 264, 297 and 310 (log ε4.59, 4.84, 4.04 and 3.99); ν_{max} (cm^{-1}): 1225 and 1770 (OAc), 1615, 1560, 860 and 840 (aromatic nucleus); ^1H NMR: δ7.72 (1H, *dd*, J_1 = 8 Hz and J_2 = 2 Hz, H-6), 7.65 (1H, apparent *t*, *J* = 8 Hz, H-7), 7.56 and 7.67 (each 1H, *d*, *J* = 8 Hz, H-9 and H-10), 7.42 (1H, *dd*, J_1 = 8 Hz and J_2 = 2 Hz, H-8), 7.13 (1H, *s*, H-1), 4.01 (3H, *s*, OMe at C-2), 3.39 (3H, *s*, OMe at C-4), 2.45 (3H, *s*, OCOMe) and 2.37 (3H, *s*, OCOMe); MS *m/z* (rel. int.): 354 ($[M]^+$, 9), 312 (32), 270 (100), 255 (17), 237 (7), 227 (4), 210 (4), 195 (10) and 43 (14).

Methylation of **1a**. Compound **1a** (0.06 g) in Me_2CO (50 ml) was refluxed for 8 hr with Me_2SO_4 (2 ml) in the presence of dry K_2CO_3 (2 g). The product was filtered hot and washed with Me_2CO. The filtrate was concd, diluted with H_2O and kept overnight. The liberated solid was dissolved in Et_2O and the Et_2O layer extracted with 2N aq. NaOH soln. The aq. alkaline soln was acidified in the cold and extracted with Et_2O, dried and the solvent removed to give practically no residue. The original Et_2O layer after the above treatment was dried and removal of solvent gave a residue (0.05 g) of **1c** and **1d** in 2:3 ratio. ^1H NMR: **1c**: δ3.87, 4.05 and 4.12 (each 3H, *s*, OMe), 7.19 (1H, *s*, H-1), 7.28 (1H, *dd*, J_1 = 8.8 Hz and J_2 = 1.7 Hz, H-6), 7.46 (1H, *dd*, J_1 = 8.8 Hz and J_2 = 1.7 Hz, H-8), 7.53 (1H, *t*, H-7), 7.64 and 7.52 (each 1H, *d*, *J* = 8 Hz, H-9 and H-10) and 10.5 (1H, *s*, hydrogen bonded OH); **1d**: δ3.76 and 4.03 (each 3H, *s*, OMe), 4.02 (6H, *s*, OMe), 7.03 (1H, *s*, H-1), 7.08 (1H, *dd*, J_1 = 8.8 Hz and J_2 = 1.7 Hz, H-6), 7.42 (1H, *dd*, J_1 = 8.8 Hz and J_2 = 1.7 Hz, H-8), 7.48 (1H, *t*, H-7), 7.51 and 7.53 (each 1H, *d*, *J* = 8 Hz, H-9 and H-10).

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