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A RING-B OXYGENATED PHENANTHRENE DERIVATIVE FROM THE ORCHID *BULBOPHYLLUM GYMNOPUS*

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Key Word Index—*Bulbophyllum gymnopus*; Orchidaceae; gymnopusin, ring-B oxygenated phenanthrene derivative.

Abstract—Gymnopusin, the first naturally occurring phenanthrene derivative having a hydroxyl group at C-9, was isolated from the orchid *Bulbophyllum gymnopus*. It was shown to be 7,9-dihydroxy-2,3,4-trimethoxyphenanthrene (**1a**) mainly from the spectral data of the compound and those of its diacetyl (**1b**) and dimethyl ether (**1c**) derivatives.

INTRODUCTION

From a series of Indian orchids we reported earlier the isolation of a number of compounds representing several structural types, bibenzyls [1, 2], phenanthrenes [3–6], 9,10-dihydrophenanthrenes [7], 9,10-dihydrophenanthropyrans [8–12] and pyrones [8–10, 13], triterpenoids [14, 15] and steroids [16]. Our continued search for phytochemicals from the same source has resulted in the isolation of yet another new phenanthrene derivative, designated as gymnopusin, from the orchid *Bulbphyllum gymnopus* which, in addition, has also yielded 2,7-dihydroxy-3,4,6-trimethoxyphenanthrene (**1g**) [17], its 9,10-dihydroderivative (**1d**) [17, 18] and 2,7-dihydroxy-3,4,8-trimethoxyphenanthrene (**1e**) [6] of previously known structures. The structure of gymnopusin was established as **1a** from the following evidence.

RESULTS AND DISCUSSION

Gymnopusin, $C_{17}H_{16}O_5$ (M^+ 300), mp 192°, showed UV absorptions, λ_{max} 211, 230, 261, 285 and 340 nm ($\log\varepsilon$ 4.28, 4.25, 4.70, 4.15 and 3.14) resembling those of phenanthrene derivatives [19]. The phenolic nature of the compound was indicated by its characteristic colour reactions, alkali-induced bathochromic shifts of its UV maxima [$\lambda_{max}^{EtOH-0.1\ N\ NaOH}$ 214, 275, 305 and 312 nm ($\log\varepsilon$ 4.35, 4.72, 4.12 and 4.13)] and from its IR spectrum showing bands at 3360 cm^{-1}. The presence of two phenolic hydroxyl groups in gymnopusin was confirmed by the formation of a diacetate, $C_{21}H_{20}O_7$ (M^+ 384), mp 113°, with acetic anhydride and pyridine, and a dimethyl ether derivative, $C_{19}H_{20}O_5$ (M^+ 328), mp 122°, with diazomethane.

The ^{1}H NMR spectrum of gymnopusin, which showed two one-proton singlets at δ8.12 and 8.55 (both disappeared on deuterium exchange) further corroborated the presence of two phenolic hydroxyl groups in the compound. The spectrum also exhibited signals for five aromatic protons, besides those for three aromatic methoxyl groups [δ3.96 (6H, *s*) and 4.02 (3H, *s*)]. Of the five aromatic protons, one appearing at a relatively downfield position as an *ortho*-coupled doublet at δ9.33 (J = 9.3 Hz) is typical of phenanthrene H-5 or H-4 having an *ortho*-hydrogen atom. This signal of gymnopusin was assigned to H-5 which coupled with H-6 appearing as an *ortho–meta*-coupled doublet of doublet at δ7.21 (J_1 = 9.3 Hz and J_2 = 2.8 Hz). The chemical shift and the splitting patterns of H-6 further indicated that H-8 of gymnopusin was also unsubstituted, which, accordingly resonated as a *meta*-coupled doublet at δ7.69 (J = 2.8 Hz). H-6 and H-8 of gymnopusin are thus flanked by an oxygen substituent. The relatively downfield shift of H-8 compared with that of H-6 is presumably due to the presence of a hydroxyl group at C-9. Similar downfield shift of an aromatic proton *peri* to a phenolic hydroxyl or phenolic ether group has been observed in 4-hydroxy coumarin or their derivatives [20]. The remaining two aromatic protons appearing at δ6.94 and 7.08, each as singlet, were assigned to H-1 and H-10, respectively. The upfield shift of H-10 compared with those of the usual phenanthrene H-9 and H-10 (normally appearing at ~ δ7.5) justifies the placement of one of the phenolic hydroxyl groups at C-9 in gymnopusin. This was further supported by the ^{1}H NMR spectral data of gymnopusin diacetate, which in addition, also provided evidence for the assignments of the other oxygen substituents of the compound. Thus, while the signal at δ6.94 (H-1) of gymnopusin remains practically unaffected in the ^{1}H NMR spectrum of its diacetate, those at δ9.33 (H-5), 7.21 (H-6), 7.69 (H-8) and 7.08 (H-10) of the former are shifted downfield to δ9.58, 7.41, 8.07 and 7.26 respectively in the latter. The essentially unchanged chemical shift of H-1 of gymnopusin on acetylation implied the absence of a hydroxyl group at C-2 and or C-4. Further, the presence of a hydroxyl group at C-4 of gymnopusin would have caused an upfield shift [21] of its H-5 on acetylation, which, on the otherhand, is shifted downfield by 0.25 ppm in the diacetyl derivative. The deshielding of H-5 in gymnopusin diacetate may be attributed to acetylation of a hydroxyl group at C-9 in the parent compound. The observed downfield shifts of H-6, H-8 and H-10 of gymnopusin diacetate compared with those of the corresponding protons of the parent compound suggest that each of these protons is *ortho* to one of the hydroxyl

groups of gymnopusin, and thus lend support to the placement of the two hydroxyl groups of gymnopusin at C-7 and C-9. This in turn left C-2, C-3 and C-4 as the only possible sites for the three methoxyl groups in gymnopusin. The relatively greater deshielding of H-8 (0.38 ppm) of gymnopusin diacetate compared with those of H-6 and H-10 ($\sim$0.2 ppm) may be due to the diamagnetic anisotropic effect of the acetoxy carbonyl function at C-9. Construction of a Dreiding model of gymnopusin diacetate (**1b**) shows that in a particular conformation of the acetoxyl group at C-9, H-8 falls in the deshielding zone of the 9-acetoxy carbonyl function producing an average deshielding of H-8 in **1b**.

More compelling evidence in support of the structure **1a** for gymnopusin was provided by the ^{13}CNMR spectral data of its more soluble diacetyl (**1b**) and dimethyl ether (**1c**) derivatives. The degree of protonation of each carbon atom was determined by DEPT experiments. The chemical shifts of the carbon atoms of **1b** and **1c** (Table 1) were assigned by comparison with the δ_c values of structurally related compounds taking into consideration of the known additive parameters of the functional groups on benzenoid system [22]. Thus, while the δ_c values of C-4b, C-5, C-6 and C-7 of **1b** are essentially similar to those of the corresponding carbon atoms of nudol diacetate (**1h**) [3], those of C-8 and C-8a of the former are shifted upfield by 6.84 and $\sim$5 ppm respectively compared with C-8 and C-8a of the latter. In the light of the earlier observations that substituent at C-8 in the phenanthrene system causes an upfield shift of C-9 by $\sim$6 ppm as in confusarin (**1e**) or its diacetate (**1f**) [6], the upfield shift of C-8 of gymnopusin diacetate (**1b**) by 6.84 ppm provides the most convincing proof for the placement of one of the acetoxyl group at C-9 in **1b** and hence one of the hydroxyl groups at the same position in gymnopusin. Similar shieldings of carbon atoms by substituents at the *peri* position have also been observed in 9,10-dihydrophenanthrene derivatives, C-10 of coelogin [8] and coeloginin diacetate [8] by methoxyl group at C-1, and oxymethylene carbons of imbricatin diacetate [11] and coelogin [8] by C-3 substituents. The upfield shift of C-8a of **1b** by $\sim$5 ppm compared with that of the corresponding carbon atom of nudol diacetate corresponds to the additive parameter of an acetoxyl function at the *ortho*-position thereby further corroborating the location of one of the acetoxyl group at C-9 of **1b**. This is also in accord with the upfield shift of C-10 of **1b**, compared with that of the corresponding carbon atom of nudol diacetate (**1h**) [3]. The greater shielding of C-10 than is expected for an *ortho*-acetoxy group may be attributed to the additional shielding caused by consecutive substitutions at the carbon atoms of Ring B. In case of such polysubstituted benzenoid systems simple additive parameters do not exactly follow [22, 23]. Such departure has also been observed in the δ_c values of C-1, C-2, C-3, C-4 of **1b**, **1c** and **1h**. The assignments of the above carbon atoms were further verified by functional group manipulation. Thus the change in the δ_c values of the above carbon atoms of gymnopusin dimethyl ether (**1c**) exactly correspond to the changed additive parameters caused by replacement of the acetoxyl groups in **1b** by methoxyl functions in **1c** at C-7 and C-9. The upfield shift of C-1 of **1b** by 14.6 ppm compared with that of C-1 of **1h** is due to the replacement of the acetoxyl group at C-2 by a methoxyl group which together with the 9-acetoxyl group causes the observed upfield shift of C-4a of **1b** by 4.16 ppm compared with that of C-4a of **1h**. C-4a of **1c** is further shielded by 3.93 ppm due to the replacement of the acetoxyl group at C-9 by a methoxyl function. An acetoxy group or a methoxy group at C-10 of gymnopusin diacetate or dimethyl ether would not have caused any such shielding of C-4a, and, on the other hand, would have caused a significant upfield shift of C-10a which shows comparable δ_c values in **1b**, **1c** and **1h**. Besides, a substituent at C-10 of **1b** and **1c** would have caused an upfield shift of C-1 by $\sim$6 ppm. In such a case C-1 of **1b** and **1c** would have resonated below 100 ppm, and C-4b would have also shown some upfield shift, which in **1b**, **1c** and **1h** shows comparable δ_c values. The assignments of the δ_c values of the carbon atoms of the aromatic methoxyl groups in **1b** and **1c** are in conformity with the observation [1, 2, 6, 8, 9] that carbon atoms of such groups having at least one *ortho*-hydrogen atom resonate at the normal region δ_c 55–56, while those flanked by two *ortho*-substituents have their carbon atoms shifted downfield by $\sim$5–6 ppm.

Gymnopusin (**1a**) is thus the first naturally occurring phenanthrene derivative containing a 9-hydroxyl function and is of considerable biogenetic importance.

EXPERIMENTAL

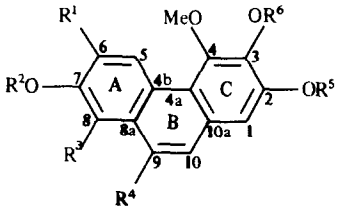
Mps are uncorr. UV spectra were measured in 95% aldehyde-free EtOH and IR spectra in KBr discs. ^{1}H NMR spectra were recorded at 80 MHz in $CDCl_3$ soln. using TMS as int. standard. ^{13}C NMR spectra were measured at 62.5 MHz as above. Chemical shifts were measured in δ ppm and for ^{13}C NMR $\delta_{TMS} = \delta_{CDCl_3} + 76.9$ ppm. MS were recorded at 70 eV. Si gel (60–120 mesh) were used for CC and Si gel G for TLC. All analytical samples were routinely dried over P_2O_5 for 24 hr *in*

Table 1. The carbon chemical shifts of gymnopusin diacetate (**1b**) and gymnopusin dimethyl ether (**1c**)

	Chemical shifts (δ values)*	
	1b	**1c**
1	102.52	102.51[a]
2	152.48[a]	151.97[d]
3	142.86	140.99
4	152.87[a]	152.82[d]
4a	118.85	114.92
4b	130.21	129.59[e]
5	128.66	128.22
6	121.36	117.34
7	148.85	157.25
8	114.03	102.47[c]
8a	128.49[b]	125.21
9	143.43	151.80[d]
10	115.75	104.18
10a	128.12[b]	127.42[e]
-OCOMe	169.19, 169.57 20.75, 21.19	
OMe	55.46 (OMe at C-2), 60.01 and 61.13 (OMe at C-3 and C-4)	55.29, 55.40 and 55.78 (OMe at C-2, C-7 and C-9), 60.19 and 61.26 (OMe at C-3 and C-4)

*Values are in ppm downfield from TMS: $\delta_{TMS} = \delta_{CDCl_3} + 76.9$ ppm.

[a,b,c,d,e] Values are interchangeable.



1a $R^1 = R^2 = R^3 = H$, $R^4 = OH$, $R^5 = R^6 = Me$

1b $R^1 = R^3 = H$, $R^2 = Ac$, $R^4 = OAc$, $R^5 = R^6 = Me$

1c $R^1 = R^3 = H$, $R^2 = R^5 = R^6 = Me$, $R^4 = OMe$

1d $R^1 = OMe$, $R^2 = R^3 = R^4 = R^5 = H$, $R^6 = Me$, 9,10-dihydro

1e $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = OMe$, $R^6 = Me$

1f $R^1 = R^4 = H$, $R^2 = R^5 = Ac$, $R^3 = OMe$, $R^6 = Me$

1g $R^1 = OMe$, $R^2 = R^3 = R^4 = R^5 = H$, $R^6 = Me$

1h $R^1 = R^3 = R^4 = H$, $R^2 = R^5 = Ac$, $R^6 = Me$

vacuo and were tested for purity by TLC and MS. Dry Na_2SO_4 was used for drying organic solvents and petrol used had bp 60–80°.

Isolation of gymnopusin (**1a**), **1d**, **1e** *and* **1g**. Air-dried powdered whole plant of *B. gymnopus* (2 kg) was soaked in MeOH (5 l) for 3 weeks. The MeOH extract was then drained out and concd under red. pres. to 100 ml, diluted with H_2O (750 ml) and exhaustively extracted with Et_2O. The Et_2O layer was then extracted with 2 M aq. NaOH soln. The aq. alkaline soln was then acidified with conc HCl in the cold and the liberated solid was extracted with Et_2O, washed with H_2O, dried and the solvent removed. The residue was chromatographed. The early fractions of petrol–EtOAc eluate (10:1) gave **1d** (0.02 g), crystallized from petrol–EtOAc, mp 111–113°. The fractions immediately following the elution of **1d** gave a mixture of **1e** and **1g**. Repeated rechromatography of this mixture finally yielded pure **1e** (0.04 g), crystallized from petrol–EtOAc, mp 185°, and **1g** (0.05 g), crystallized from the same solvent mixture, mp 135°. The identity of **1d** and **1e** was established by direct comparison (co-TLC, mmp and IR spectra) with authentic samples of 2,7-dihydroxy-3,4,6-trimethoxy-9,10-dihydrophenanthrene [18] and 2,7-dihydroxy-3,4,8-trimethoxy-phenanthrene [6] earlier isolated from the orchids *Coelogyne ovalis* and *Eria confusa*, respectively. The diacetate of **1d** on dehydrogenation with DDQ in C_6H_6 gave a compound identical in all respect with the diacetyl derivative of **1g**.

The later fractions of the petrol–EtOAc (10:1) after elution of **1d**, **1e** and **1g** in the original chromatography afforded **1a** (0.1 g), crystallized from petrol–EtOAc, mp 192° (Found: C, 67.89; H, 5.43; $C_{17}H_{16}O_5$ requires: C, 68.00; H, 5.33%). IR ν_{max} cm^{-1}: 3360 (OH), 1610, 1578, 860 and 828 (aromatic nucleus); MS: *m/z* 300 (M^+, 100), 286 (14), 285 (73), 271 (6), 270 (6), 257 (14), 242 (35), 227 (10), 199 (8), 185 (9), 171 (5), 150 (5), 84 (9), 49 (11) and 43 (9).

Gymnopusin diacetate (**1b**) *and gymnopusin dimethyl ether* (**1c**). Gymnopusin (0.04 g) was acetylated with Ac_2O and pyridine in the usual manner to give **1b** (0.035 g), crystallized from petrol–EtOAc, mp 113°. (Found: C, 65.54; H, 5.12; $C_{21}H_{20}O_7$ requires: C, 65.62; H, 5.21%). UV λ_{max} nm: 218, 258, 301, 310, 339 and 356 (log ε 4.27, 4.52, 3.74, 3.72, 2.97 and 2.98); IR ν_{max} cm^{-1}: 1251 and 1755 (OAc), 1627, 1612, 1492, 860 and 840 (aromatic nucleus); ^{1}H NMR: δ9.58 (1H, *d*, J = 9.3 Hz; H-5), 8.07 (1H, *d*, J = 2.8 Hz; H-8), 7.41 (1H, *dd*, J_1 = 9.3 Hz and J_2 = 2.8 Hz; H-6), 7.26 (1H, *s*; H-10), 6.86 (1H, *s*; H-1), 4.00 (6H, *s*; 2 × Ar-OMe), 4.02 (3H, *s*; Ar-OMe), 2.40 (3H, *s*; –OCOMe) and 2.37 (3H, *s*; –OCOMe).

A soln of **1a** (0.04 g) in MeOH (25 ml) was treated with an excess of ethereal soln of CH_2N_2 in the cold. The reaction mixture was kept overnight. Thereafter the solvent was removed under red. pres. and the residue was chromatographed. The petrol–EtOAc (15:1) eluate gave **1c** (0.035 g), crystallized from the same solvent mixture, mp 122°. (Found: C, 69.40; H, 6.20; $C_{19}H_{20}O_5$ requires: C, 69.51; H, 6.10%). IR ν_{max} cm^{-1}: 1615, 1469, 855, 845 and 827 (aromatic nucleus); ^{1}H NMR: δ9.39 (1H, *d*, J = 9.4 Hz; H-5), 7.72 (1H, *d*, J = 2.9 Hz; H-8), 7.25 (1H, *dd*, J_1 = 9.4 Hz and J_2 = 2.9 Hz; H-6), 6.99 (1H, *s*; H-10), 6.89 (1H, *s*; H-1), 3.98 (6H, *s*; 2 × OMe), 4.06, 3.99 and 3.96 (each 3H, *s*; 3 × OMe).

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