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AGROSTOPHYLLIN, A NATURALLY OCCURRING PHENANTHROPYRAN DERIVATIVE FROM *AGROSTOPHYLLUM KHASIYANUM*

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Kew Word Index—*Agrostophyllum Khasiyanum*; Orchidaceae; agrostophyllin; phenanthropyran derivative.

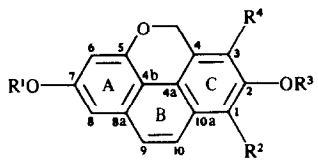
Abstract—Agrostophyllin, the first naturally occurring phenanthropyran derivative, was isolated from the orchid *Agrostophyllum khasiyanum*. It was shown to be 2,6-dimethoxy-7-hydroxy-5H-phenanthro[4,5-*bcd*]pyran (**1a**) mainly from spectroscopic evidence.

INTRODUCTION

As part of our general programme of chemical investigation of Indian orchids we reported [1–18] earlier the isolation of about 24 new compounds from a series of Himalayan orchids. These compounds represent several structural types like bibenzyls [1, 2], phenanthrenes [3–7, 19], 9,10-dihydrophenanthrenes [8], 9,10-dihydrophenanthropyrans [9–14] and pyrones [9–11, 15], triterpenoids [16, 17] and steroids [18]. Our continued search for phytochemicals from the same source has resulted in the isolation of yet another new compound, designated as agrostophyllin, from the orchid *Agrostophyllum khasiyanum*. The structure of agrostophyllin was established as **1a** from the following evidence.

RESULTS AND DISCUSSION

Agrostophyllin, $C_{17}H_{14}O_4$ ($M^{+\cdot}$ 282), mp 86°, was isolated from the methanolic extract of the whole plant of *A. khasiyanum*. The UV spectrum of the compound, λ_{max} 226, 268 and 300 nm (log ε 4.60, 4.69 and 4.13) showed a striking resemblance to those of oxygenated phenanthrene derivatives [20]. The phenolic nature of agrostophyllin was indicated by its characteristic colour reactions, alkali-induced bathochromic shifts of its UV maxima [$\lambda_{max}^{0.1(N)NaOH-EtOH}$ 223, 275 and 306 nm (log ε 4.47, 4.72 and 4.21)], and by its IR spectrum showing strong absorption band at 3380 cm^{-1}. The presence of a single phenolic hydroxyl group in agrostophyllin was confirmed by its PMR spectrum exhibiting a one-proton singlet at δ5.74 (disappeared on deuterium exchange), and by the formation of a monoacetyl derivative, $C_{19}H_{16}O_5$ ($M^{+\cdot}$ 324), mp 125°, with acetic anhydride and pyridine. The ^1H NMR spectrum of agrostophyllin also confirmed the presence of two aromatic methoxyl groups (δ3.88, 6H, *s*) in the compound. The two-proton singlet at δ5.64 is reminiscent of the two oxymethylene protons of the 9,10-dihydrophenanthropyrans like coelogin (**1c**) [9], flavidin (**1d**) [13], flavidinin (**1f**) [10], isoflavidinin (**1h**) [11], imbricatin (**1j**) [12] and flaccidin (**1l**) [14]. The downfield shift of this signal by ~0.4 ppm of agrostophyllin compared with those of the above compounds may be attributed to the stronger aromatic phenanthrene moiety in agrostophyllin. The ^1H NMR spectrum of agrostophyllin also displayed signals for five aromatic protons, none of which appeared below δ8.5. This ruled out the presence of any proton at C-4 and C-5 of agrostophyllin and suggested the placement of the oxymethylene bridge between these two carbon atoms. Two of the aromatic protons of agrostophyllin appeared at δ7.56 (*s*) which corresponded to the 9- and 10-protons of a phenanthrene system [21]. The two one-proton *meta*-coupled doublets at δ6.72 (J = 2.5 Hz) and 6.86 (J = 2.5 Hz) were assigned to H-6 and H-8 flanked by a methoxyl group at C-7, and the one-proton singlet at δ7.26 was attributed to



1a R¹ = Me; R² = R³ = H; R⁴ = OMe
1b R¹ = Me; R² = H; R³ = Ac; R⁴ = OMe
1c R¹ = H; R² = OMe; R³ = Me; R⁴ = OH, 9,10-Dihydro
1d R¹ = R² = R³ = R⁴ = H, 9,10-Dihydro
1e R¹ = R³ = Ac; R² = R⁴ = H, 9,10-Dihydro
1f R¹ = R² = R⁴ = H; R³ = Me, 9,10-Dihydro
1g R¹ = Ac; R² = R⁴ = H; R³ = Me, 9,10-Dihydro
1h R¹ = Me; R² = R³ = R⁴ = H, 9,10-Dihydro
1i R¹ = Me; R² = R⁴ = H; R³ = Ac, 9,10-Dihydro
1j R¹ = R² = R³ = H; R⁴ = OMe, 9,10-Dihydro
1k R¹ = R³ = Ac; R² = H; R⁴ = OMe, 9,10-Dihydro
1l R¹ = R² = H; R³ = Me; R⁴ = OH, 9,10-Dihydro
1m R¹ = Ac; R² = H; R³ = Me; R⁴ = OAc, 9,10-Dihydro

H-1. The placement of the hydroxyl group at C-2 in agrostophyllin was suppported by the downfield shift of the signal at δ 7.26 by 0.18 ppm in the ^{1}H NMR spectrum of its acetyl derivative which has its other aromatic protons appearing essentially at the same positions as those of agrostophyllin.

More compelling evidence in support of the structure **1a** for agrostophyllin was provided by the ^{13}C NMR spectral data of the compound and its acetyl derivative (**1b**). The degree of protonation of each carbon atom was determined by APT experiments [22]. The chemical shifts of the carbon atoms of **1a** and **1b** (Table 1) were assigned by comparison with the δ_c values of structurally similar compounds taking into consideration the additive parameters of the functional groups on the benzenoid system [23]. Thus the oxymethylene carbon of agrostophyllin appeared essentially at the same position (δ_c63.46) as the corresponding carbon atoms of coelogin (**1c**) [9], imbricatin diacetate (**1k**) [12] and flaccidin diacetate (**1m** [14], all having a substituent at their C-3 position. Absence of a substituent at C-3 in agrostophyllin would have caused its oxymethylene carbon appearing at the normal position (δ_c67–68) as in flavidin diacetate (**1e**) [13], flavidinin acetate (**1g**) [10] and isoflavidinin acetate (**1i**) [11]. That this substituent at C-3 in agrostophyllin is a methoxyl rather than a hydroxyl group was supported by the carbon chemical shifts of its aromatic methoxyl groups, one of which was shifted downfield to δ_c61.16, while the other appeared at the normal position (δ_c54.90). In the light of our earlier observations [1–12, 14, 24] that carbon atoms of aromatic methoxyl groups having at least one *ortho*-hydrogen atom appear at the normal position (δ_c54–56), while those of such groups flanked by two *ortho*-substituents are shifted downfield by ~5–6 ppm, the signal at δ_c61.16 of agrostophyllin corresponded to a methoxyl group at its C-3 position, and the other signal at δ_c54.90 was due to its C-7 methoxyl function. This was further corroborated by the expected upfield shift of its C-10a (δ_c125.21), which would have normally appeared at ~δ_c 131 without any oxygen substituent at C-3 and C-1. The placement of the hydroxyl group at C-2 of agrostophyllin was in conformity with the observed downfield shifts of C-1, C-3 and C-4a of its acetyl derivative. C-9 and C-10 appeared at the normal positions of the corresponding carbon atoms of a phenanthrene system [3–6]. Placement of a methoxyl group at C-7 is consistent with the highly upfield position (δ_c100.82) of C-6 which is flanked by two *ortho*-oxygen substituent, as well as with the expected shielding of C-4b compared with corresponding carbon atoms of phenanthrene derivatives having a 7-acetoxy function [3]. An interesting feature of agrostophyllin is the upfield shift of its C-8 compared with the corresponding carbon atom of the 9,10-dihydrophenanthropyran analogue isoflavidinin acetate (**1i**) [11]. Such difference may be presumably due to a greater shielding at C-8 by the oxymethylene function involving a more stable *para*-quinonoid canonical form in the fully aromatic phenanthrene system than that in the 9,10-dihydro analogue.

Agrostophyllin is thus the first naturally occurring phenanthropyran. In terms of systematic nomenclature it may be designated as 2,6-dimethoxy-7-hydroxy-5H-phenanthro [4, 5-*bcd*] pyran (**1a**), although the phenanthrene numbering system has been followed in this paper for convenience of comparison of spectral data.

Table 1. The carbon chemical shifts of agrostophyllin (**1a**) and agrostophyllin acetate (**1b**)

	Chemical shifts (δ values)*			Chemical shifts (δ values)*	
C	**1a**	**1b**	C	**1a**	**1b**
1	110.03	119.33	8	101.30[b]	101.49[d]
2	147.31	142.14	8a	130.43	131.60
3	141.65	145.08	9	125.36[c]	125.60[c]
4	118.77[a]	119.95	10	125.26[c]	125.44[c]
4a	117.92[a]	122.39	10a	125.21	124.23
4b	111.78	111.42	–O–CH_2–	63.46	63.71
5	151.95	152.85	–OMe(C-3)	61.16	60.95
6	100.82[b]	101.11[d]	–OMe(C-7)	54.90	54.98
7	158.49	159.62	–OAc		168.54, 20.24

* Values are in ppm downfield from TMS: $\delta_{(TMS)} = \delta_{(CDCl_3)} + 76.9$ ppm. [a–e] Values are interchangeable.

EXPERIMENTAL

Mps: uncorr. CC: silica gel (60–120 mesh). TLC: silica gel G; UV; 95% aldehyde-free EtOH. IR: KBr. ^{1}H NMR: 100 and 300 MHz, $CDCl_3$, TMS as int. standard. ^{13}C NMR: 75 MHz, $CDCl_3$, TMS; MS: direct inlet, 70 eV. All the analytical samples were routinely dried over P_2O_5 for 24 hr *in vacuo* and were tested for purity by TLC and mass spectrometry. Na_2SO_4 was used for drying organic solvents and the petrol used had bp 60–80°.

Isolation of agrostophyllin (**1a**). Air-dried powdered whole plant of *A. khasiyanum* (2 kg) was soaked in MeOH (5 l) for 3 weeks. The MeOH extract was then drained out and concd under red. pres. to about 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. The aq. alkaline soln was then acidified with conc HCl in the cold and the liberated solid extracted with Et_2O, washed with H_2O, dried and the solvent removed. The residue was chromatographed. The petrol–EtOAc (20:1) eluate gave a gummy solid which on rechromatography gave **1a** (0.1 g), crystallized from petrol–EtOAc mixture, mp 86°. (Found: C, 72.31; H, 4.94; $C_{17}H_{14}O_4$ requires: C, 72.34; H, 4.96%) **1a** gives intense blue colouration with phosphomolybdic acid reagent. IR ν_{max} cm^{-1}: 3380 (OH), 1630, 1605, 1500, 850, 820 and 780 (aromatic nucleus); MS *m/z* (rel. int.): 282 (M^+, 100), 281 (27), 267 (25), 266 (20), 251 (11), 239 (14), 224 (6), 223 (7), 211 (8), 196 (4), 179 (3), 168 (5), 152 (7), 141 (6), 139 (12) and 111 (12).

Agrostophyllin acetate (**1b**). Agrostophyllin (0.05 g) was acetylated with Ac_2O and pyridine in the usual manner to give **1b** (0.048 g), crystallized from petrol–EtOAc mixture, mp 125°. (Found: C, 70.31; H, 4.90; $C_{19}H_{16}O_5$ requires: C, 70.37; H, 4.93%). UV λ_{max} nm: 231, 268 and 299 (log ε 4.71, 4.62 and 4.20); IR ν_{max} cm^{-1}: 1755, 1280 and 1255 (OAc), 1630, 1600, 830 and 790 (aromatic nucleus); ^{1}H NMR: δ 2.37 (3H, *s*; OCOMe), 3.86

and 3.89 (each 3H, *s*, 2Ar-OMe), 5.65 (2H, *s*, Ar–O–CH$_2$–Ar), 6.72 (1H, *d*, $J = 2.5$ Hz, H-6), 6.87 (1H, *d*, $J = 2.5$ Hz, H-8), 7.44 (1H, *s*, H-1) and 7.58 (2H, *s*, H-9 and H-10).

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