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FLACCIDIN, A 9,10-DIHYDROPHENANTHROPYRAN DERIVATIVE FROM THE ORCHID *COELOGYNE FLACCIDA*

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Key Word Index—*Coelogyne flaccida*; Orchidaceae; orchid; 9,10-dihydrophenanthropyran; flaccidin.

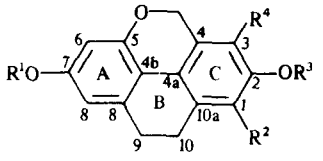
Abstract—Flaccidin, a new 9,10-dihydrophenanthropyran derivative, was isolated from the orchid *Coelogyne flaccida*. It was shown to be 2,6-dihydroxy-7-methoxy-9,10-dihydro-5H-phenanthro[4,5-*bcd*]pyran mainly from spectroscopic evidence.

INTRODUCTION

As part of our chemical investigation of Indian orchids, we reported earlier the isolation of many new compounds from a series of Indian orchids. These compounds comprise several structural types, i.e. 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 phenolic compound, flaccidin, from the orchid *Coelogyne flaccida* Lindl. which, in addition, also yielded imbricatin (**1d**) [11] earlier isolated from a taxonomically related orchid *Pholidota imbricata*. The structure of flaccidin was established as **1a** from the following evidence.

RESULTS AND DISCUSSION

Flaccidin, $C_{16}H_{14}O_4$ (M^+ 270), showed UV absorptions λ_{max} 223, 284, 309 and 321 nm (log ε 4.40, 4.03, 4.06 and 4.02)] which were strikingly similar to those of substituted 9,10-dihydrophenanthrenes [7, 8–12, 17]. The phenolic nature of the compound was indicated by its colour reactions with ferric chloride and cerric ammonium sulphate reagent. This was also corroborated by alkali-induced bathochromic shifts of its UV maxima and by its IR spectrum showing bands at 3420 and 3380 cm^{-1}. The presence of two phenolic hydroxyl groups in flaccidin was confirmed by the formation of a diacetate, $C_{20}H_{18}O_6$ (M^+ 354) with acetic anhydride and pyridine and a dimethyl ether derivative, $C_{18}H_{18}O_4$ (M^+ 298), with diazomethane. The latter, incidentally, was found to be identical in all respects with imbricatin dimethyl ether (**1c**). This implies that flaccidin differs from imbricatin (**1d**) [11] only by their relative positions of the methoxyl and the two hydroxyl groups located at C-2, C-3 and C-7.



	R^1	R^2	R^3	R^4
1a	H	H	Me	OH
1b	Ac	H	Me	OAc
1c	Me	H	Me	OMe
1d	H	H	H	OMe
1e	Ac	H	Ac	OMe
1f	H	OMe	Me	OH
1g	Ac	H	Ac	H
1h	Ac	H	Me	H
1i	Me	H	Ac	H

Evidence for the exact positions for the methoxyl and hydroxyl groups in flaccidin was provided by a comparison of its ^1H NMR spectral data with those of its diacetyl derivative, imbricatin (**1d**) and imbricatin diacetate (**1e**). The spectrum of flaccidin in d_6-acetone showed striking similarity with that of imbricatin by exhibiting two one-proton singlets at δ7.61 and 8.46 (each disappeared on deuterium exchange) for two phenolic hydroxyl groups, a three-proton singlet at δ3.83 for an aromatic methoxyl group, a four-proton singlet at δ2.86 characteristic of the 9- and 10-methyline protons of a 9,10-dihydrophenanthrene system [17–19], a two-proton singlet at δ5.01 reminiscent of the oxymethylene protons of imbricatin [11], coelogin [8], flavidin [12], flavidinin [9] and isoflavidinin [10], and signals for three aromatic protons in the region δ6.25–6.69. Two of these aromatic protons of flaccidin appeared as a pair of *meta*-coupled doublets at δ6.25 (J = 2.2 Hz) and 6.34 (J = 2.2 Hz) essentially similar to those of H-6 and H-8 of imbricatin. By analogy with imbricatin these two signals were assigned to H-6 and H-8 of flaccidin, flanked by a hydroxyl group at C-7. The third aromatic proton of flaccidin resonated at δ6.69 as a sharp singlet which was assigned to H-1 leaving C-2 and C-3 as the only possible sites for the remaining hydroxyl and methoxyl groups in the compound. In the ^1H NMR spectrum of flaccidin diacetate the signals for the *meta*-coupled protons of flaccidin at δ6.25 and 6.34 assigned to H-6 and H-8 were shifted downfield to give a broad signal at δ6.46. Similar downfield shifts of the signals for H-6 and H-8 were also discernible in the spectra of the acetyl derivatives of imbricatin, coelogin, flavidin and flavidinin,

and are assumed to be caused by the acetylation of the hydroxyl group at C-7, which is *ortho* to each of these protons. By analogy, this would suggest the placement of one of the hydroxyl groups in flaccidin also at C-7. But unlike the signal for H-1 of imbricatin, which shows a similar downfield shift in the ^{1}H NMR spectrum of its diacetyl derivative, that at δ6.69 attributed to H-1 of flaccidin remained practically unchanged (δ6.66) in the spectrum of flaccidin diacetate. This implies that while imbricatin contains a hydroxyl group at C-2, the substituent at C-2 in flaccidin is a methoxyl group leaving C-3 as the only possible site for the remaining hydroxyl group in the latter. On the basis of the above observations flaccidin was assigned the structure **1a** which differs from that of imbricatin (**1d**) only by an interchange of a hydroxyl and a methoxyl group at their C-2 and C-3 positions.

Further confirmation of the structure **1a** for flaccidin was provided by the ^{13}C NMR spectral analysis of its more soluble diacetyl derivative (**1b**). The degree of protonation of each carbon atom was determined by DEPT experiments, and the assignments of the carbon chemical shifts (Table 1) were made by comparison with the δ_c values of structurally similar compounds like imbricatin diacetate (**1e**) [11], flavidin diacetate (**1g**) [12], flavidinin acetate (**1h**) [9], isoflavidinin acetate (**1i**) [10] and coelogin (**1f**) [8], taking into consideration the additive parameters of the functional groups on the benzenoid system [20]. Thus the corresponding signals for ring-A carbon atoms (C-4b, C-5, C-6, C-7, C-8 and C-8a) of flaccidin diacetate, **1e**, **1g** and **1h** appear essentially at the same spectral positions confirming the structural identity of the ring-A part of their molecules. A methoxyl group at C-7 in flaccidin diacetate would have caused these carbon atoms to resonate at positions similar to those of the corresponding carbon atoms of isoflavidinin acetate (**1i**). C-9 and C-10 of flaccidin diacetate resonate at the normal positions ($\sim$27 ppm). This excludes the possibility of any substituent being at C-1 and C-8, which would have caused an upfield shift of C-10 and C-9 respectively by $\sim$6–7 ppm as evident by the shielding of C-10 of coelogin (**1f**) by the methoxyl group at C-1 [8]. A similar upfield shift of $\sim$5 ppm has been observed for the oxymethylene carbon by the *peri* effect of a substituent at C-3 in this type of compounds. Thus while the oxymethylene carbon atoms of **1g**, **1h** and **1i** having no substituent at C-3 appear at the normal positions δ_c 67.8, 68.2 and 67.9 respectively, the corresponding carbon atoms of **1e** and **1f** bearing a C-3 substituent are found to resonate at δ_c 63.1 and 63.2 respectively. In the light of this observation the chemical shift of the oxymethylene carbon of flaccidin diacetate (δ_c 63.42) confirms the presence of a substituent (acetoxy or methoxy) at its C-3. It has been further observed that while an aromatic methoxyl group having at least one *ortho* hydrogen has its carbon atom resonating at the normal position δ_c 55–56 (cf. methoxyl carbon resonances of **1h** and **1i**), the carbon resonance of such a group possessing two *ortho* substituents is shifted downfield to δ_c 60–62 as is evident from the δ_c values of the carbon atoms of the methoxyl groups of imbricatin diacetate (**1e**) and coelogin (**1f**). The methoxy carbon resonance of flaccidin diacetate appearing at the normal position (δ_c 56.08) thus provides the most convincing evidence in support of the placement of the methoxyl group in the compound at C-2 leaving C-3 as the only possible site for the remaining acetoxyl group. This has been further corroborated by the observed upfield shifts of C-1 and C-4a and a downfield shift of C-10a of flaccidin

Table 1. ^{13}C NMR spectral data of flaccidin diacetate (**1b**), imbricatin diacetate (**1e**), flavidin diacetate (**1g**), flavidinin acetate (**1h**), isoflavidinin acetate (**1i**) and coelogin (**1f**)

C	Chemical shifts*					
	1b	**1e**	**1g**	**1h**	**1i**	**1f**
1	111.08	121.5	120.2	112.7	120.0	149.1
2	150.22[a]	142.2	149.8	159.4	149.0	136.6
3	137.50	145.2	115.5	108.0	115.3	142.9
4	122.31	122.2	129.9	129.3	128.7	117.3
4a	119.25	124.5	123.7	119.3	124.5	122.9
4b	116.8	116.3	116.6	117.3	112.0	112.2
5	152.46	152.9	153.2	152.5	153.8	156.2
6	108.16	107.8	108.1	107.8	99.1	101.3
7	150.58[a]	150.6	150.6	149.8	160.4	154.9
8	114.3	114.1	114.3	114.1	107.4	108.9
8a	135.07	135.6	134.6	134.9	133.7	138.4
9	27.45[b]	27.1[c]	27.1[d]	27.5	27.5	27.4
10	27.57[b]	26.4[c]	27.2[d]	27.5	27.5	20.6
10a	131.59	128.9	135.7	134.9	135.9	109.8
$-O-CH_2-$	63.42	63.1	67.8	68.2	67.9	63.2
$-OCOMe$	169.42, 168.59	168.7	169.3	169.4	169.4	—
	21.07	168.9				
			20.9	20.9	20.8	
	20.30	20.3				
		20.6				
OMe	56.08	60.98	—	55.3	55.1	60.1, 61.3

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

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

diacetate compared with the corresponding carbon atoms of imbricatin diacetate (**1e**). The virtually identical chemical shift of C-4 of flaccidin diacetate and imbricatin diacetate may be due to substitution on consecutive carbon atoms on either side of C-4. For such carbon atoms in a benzenoid system simple additive parameters of functional groups alone are not adequate to account for the carbon chemical shift [8, 21].

Flaccidin is thus a new addition to the growing list of naturally occurring 9,10-dihydrophenanthropyrans. In terms of systematic nomenclature it may be designated as 2,6-dihydroxy-7-methoxy-9,10-dihydro-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.

EXPERIMENTAL

Mps: uncorr; CC: silica gel (60–120 mesh); TLC: silica gel G; UV: 95% aldehyde-free EtOH; IR: KBr discs; ^{1}H NMR: 80 MHz, $CDCl_3$ and d_6-acetone, TMS as int. standard, and chemical shifts are expressed in δ values; ^{13}C NMR: 62.5 MHz, $CDCl_3$, using TMS as int. standard; MS: direct inlet, 70 eV. All the analytical samples were routinely dried over P_2O_5 for 24 hr *in vaccuo* 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 flaccidin (**1a**) *and imbricatin* (**1d**). Air-dried powdered whole plant of *C. flaccida* (4 kg) was soaked in MeOH (10 L) for 30 days. The MeOH extract was then drained off 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 2M NaOH. The aq. alkaline soln was 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 earlier fractions of petrol–EtOAc (5:1) on evaporation gave a semi-solid mass which contained a mixture of **1a** and **1d**. Repeated chromatography of this semi-solid material over silica gel (100–200 mesh) gave in the earlier fractions of petrol–EtOAc (15:1) pure **1a** (0.2 g), crystallized from $CHCl_3$, mp 200°. Violet colouration with $FeCl_3$ and blue colouration with ammonium ceric sulphate reagent. (Found: C, 70.95; H, 5.21; $C_{16}H_{14}O_4$ requires: C, 71.11; H, 5.18%). UV (EtOH–0.1 M NaOH soln): λ_{max} 225, 297 and 324 nm (logε 4.16, 3.79 and 3.98); IR ν_{max}cm^{-1}: 3420 and 3380 (OH) 1620, 860 and 845 (phenyl nucleus); MS *m/z* (rel. int.): 270 [M]$^+$ (98), 255 (100), 241 (10), 227 (8), 209 (7), 187 (7), 184 (10), 152 (6), 141 (5), 135 (10), 127 (7), 115 (7), 104 (5), 76 (8) and 63 (8).

The later fractions of petrol–EtOAc (15:1) in the above rechromatography afforded pure **1d** (0.4 g), crystallized also from $CHCl_3$, mp 145°. It formed a diacetyl derivative with Ac_2O and pyridine, which crystallized from petrol–EtOAc, mp 116°. The original phenol and the diacetate were identified with imbricatin and its diacetate respectively by comparison (TLC, mmp and IR spectra) with authentic samples.

Flaccidin diacetate (1b) *and flaccidin dimethyl ether* (1c). Flaccidin (0.1 g) was acetylated with Ac_2O and pyridine in the usual manner. The reaction mixture afforded **1b** (0.095 g), crystallized from petrol–EtOAc, mp 162°. (Found: C, 67.71; H, 5.14; $C_{20}H_{18}O_6$ requires: C, 67.80; H, 5.08%). UV λ_{max} nm: 220, 282 and 304 (logε 4.46, 4.14 and 4.15); IR ν_{max} cm^{-1}: 1225, 1280 and 1765 (OAc), 1610, 890, 860 and 845 (phenyl nucleus); ^{1}H NMR: δ2.25 and 2.31 (each 3H, *s*; –OCOMe), 2.86 (4H, *s*; H_2-9 and H_2-10), 3.80 (3H, *s*; Ar–OMe), 5.01 (2H, *s*; Ar–O–CH_2–Ar), 6.46 (2H, *br s*; H-6 and H-8) and 6.66 (1H, *s*; H-1); MS *m/z* (rel. int.): 354 [M]$^+$ (28), 312 (31), 295 (5), 270 (100), 255 (45), 241 (14), 225 (5), 209 (4), 197 (5), 181 (5), 169 (4), 152 (5), 139 (5) and 115 (5).

A soln of **1a** (0.05 g) in MeOH (25 ml) was treated with an excess of ethereal soln of CH_2N_2 in the cold and the reaction mixture was kept overnight. Thereafter the solvent was removed under red. pres. and the residue was crystallized from petrol–EtOAc to give **1c** (0.045 g), mp 101°. IR ν_{max} cm^{-1}: 1620, 870, 850 and 840 (phenyl nucleus). It was found to be identical in all respects (TLC, mmp, IR spectra) with an authentic sample of imbricatin dimethyl ether.

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REFERENCES

1. Majumder, P. L. and Joardar, M. (1984) *Indian J. Chem.* **23B**, 1040.
2. Majumder, P. L. and Sen, R. C. (1987) *Phytochemistry* **26**, 2121.
3. Bhandari, S. R., Kapadi, A. H., Majumder P. L., Joardar, M. and Shoolery, J. N. (1985) *Phytochemistry* **24**, 801.
4. Majumder, P. L., Kar, A. and Shoolery, J. N. (1985) *Phytochemistry* **24**, 2083.
5. Majumder, P. L. and Sen, R. C. (1987) *Indian J. Chem.* **26B**, 18.
6. Majumder, P. L. and Kar, A. (1987) *Phytochemistry* **26**, 1127.
7. Majumder, P. L. and Joardar, M. (1985) *Indian J. Chem.* **24B**, 1192.
8. Majumder, P. L., Bandyopadhyay, D. and Joardar, S. (1982) *J. Chem. Soc. Perkin Trans. I*, 1131.
9. Majumder, P. L. and Datta, N. (1982) *Indian J. Chem.* **21B**, 534.
10. Majumder, P. L., Sarkar, A. K. and Chakraborti, J. (1982) *Phytochemistry* **21**, 2713.
11. Majumder, P. L. and Sarkar, A. K. (1982) *Indian J. Chem.* **21B**, 829.
12. Majumder, P. L., Datta, N., Sarkar, A. K. and Chakraborti, J. (1982) *J. Nat. Prod.* **45**, 730.
13. Majumder, P. L. and Datta, N. (1984) *Phytochemistry* **23**, 671.
14. Majumder, P. L. and Pal, A. (1985) *Phytochemistry* **24**, 2120.
15. Majumder, P. L., Pal, A. and Lahiri, S. (1987) *Indian J. Chem.* **26B**, 297.
16. Majumder, P. L. and Chakraborti, J. (1985) *Tetrahedron* **41**, 4973.
17. Letcher, R. M. and Nhamo, L. R. M. (1973) *J. Chem. Soc. Perkin Trans* 1, 1179.
18. Cross, A. D., Carpio, H. and Crabbe, P. (1963) *J. Chem. Soc.* 5539.
19. Erdtman, H. (1969) *Acta Chem. Scand.* **23**, 249.
20. Stothers, J. B. (1972) C-13 NMR *Spectroscopy*. Academic Press, New York.
21. Wenkert, E., Gottlieb, H. E., Gottlieb, O. R., Pereira Das, M. O. and Formiga, M. D. (1976) *Phytochemistry* **15**, 1547.