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FLACCIDININ AND OXOFLACCIDIN, TWO PHENANTHRENE DERIVATIVES OF THE ORCHID *COELOGYNE FLACCIDA*

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Key Word Index—*Coelogyne flaccida*; Orchidaceae; flaccidinin, phenanthropyrone; oxoflaccidin, 9,10-dihydrophenanthropyrone derivative.

Abstract—Flaccidinin, a new phenanthropyrone derivative, and oxoflaccidin, the corresponding 9,10-dihydro compound, were isolated from the orchid *Coelogyne flaccida* which also yielded the previously reported 9,10-dihydrophenanthropyran derivatives flaccidin and imbricatin. The structures of flaccidinin and oxoflaccidin were established from spectral evidence and by chemical correlation.

INTRODUCTION

As part of our general programme of research on the chemical constituents of Indian orchids we reported earlier [1–20] the isolation of a number of compounds. These compounds represent several structural types, such as, bibenzyls [1, 2], phenanthrenes [3–7, 21], phenanthropyrans [8], 9,10-dihydrophenanthrenes [9, 10], 9,10-dihydrophenanthropyrans [11–15, 17] and pyrones [11–13, 16], triterpenoids [18, 19] and steroids [20]. From one of these orchids, *Coelogyne flaccida*, we previously reported the isolation of a new 9,10-dihydrophenanthropyran derivative, flaccidin (**1e**) [17]. Further investigation of the same orchid has resulted in the isolation of two more new phenanthrenoids, designated as flaccidinin and oxoflaccidin, besides imbrication (**1g**) [14] of known structure. The structures of flaccidinin and oxoflaccidin were established as **1a** and **1c**, respectively, from the following spectral and chemical evidence.

RESULTS AND DISCUSSION

Flaccidinin, $C_{16}H_{10}O_5$ ($M^{+\cdot}$ at *m/z* 282) and oxoflaccidin, $C_{16}H_{12}O_5$ ($M^{+\cdot}$ at *m/z* 284) were obtained as a mixture which could not be separated by conventional chromatography owing to their very close polarity. Fractional crystallization was also of no use for this purpose, since they formed mixed crystals, mp 325° (dec.). However, the mixture of their acetyl derivatives, obtained by acetylation of the above material with acetic anhydride and pyridine, on repeated chromatography and fractional crystallization afforded pure flaccidinin diacetate, $C_{20}H_{14}O_7$ ($M^{+\cdot}$ at *m/z* 366), mp 258°, and oxoflaccidin diacetate which still contained ~ 20% of the former compound. Further purification of oxoflaccidin diacetate was not possible due to paucity of material, and the mixture was studied as such. The spectral data of oxoflaccidin diacetate were readily obtained by comparison of the spectra of this enriched mixture with those of pure flaccidinin diacetate. Pure flaccidinin was obtained by acid hydrolysis of its pure diacetate, mp. 360° (dec.).

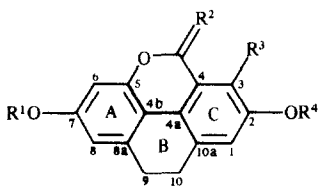
Flaccidinin diacetate showed UV absorptions, λ_{max} 218, 257, 367 and 384 nm ($\log\varepsilon$ 4.28, 4.53, 3.73 and 3.72) resembling those of phenanthrene derivatives [22] with an appreciable bathochromic shift due to the presence of a conjugated carbonyl function, while those of oxoflaccidin diacetate λ_{max} 222, 257 and 285 nm ($\log\varepsilon$ 4.27, 4.21 and 4.00) corresponded to those of 9,10-dihydrophenanthropyrones [11–13, 16]. The IR spectrum of flaccidinin diacetate exhibited an intense band at 1735 cm^{-1} for a δ-lactone function, besides those for an acetoxy group (ν_{max} 1760 and 1270 cm^{-1}). The presence of a similar δ-lactone function in oxoflaccidin diacetate was also indicated by its IR band at 1725 cm^{-1} and the bands for an acetoxy group appeared essentially at the same positions as in the case of flaccidinin diacetate.

The presence of an aromatic methoxyl function and two phenolic hydroxyl groups in each of flaccidinin and oxoflaccidin was indicated by their respective ^1H NMR spectra [flaccidinin: δ 4.0 (3H, *s*) and 7.85 and 7.97 (each 1H, *s*; disappeared on deuterium exchange); oxoflaccidin δ 3.92 (3H, *s*) and 7.70 and 7.88 (each 1H, *s*; disappeared on deuterium exchange)]. However, while the spectrum of oxoflaccidin is characterized by the appearance of a four-proton singlet at δ 3.05 resembling those of 9- and 10-methylene protons of the 9,10-dihydrophenanthrene derivatives [9–17, 22, 23], that of flaccidinin shows a two-proton singlet at δ 7.73 typical of H-9 and H-10 of the phenanthrenes [3–8, 21–23]. This, therefore, suggests that while oxoflaccidin is a 9,10-dihydrophenanthrene derivative, flaccidinin is the corresponding phenanthrene analogue. The absence of any downfield aromatic proton signal at ~ δ 9.0 characteristic of H-5 and H-4 [3–7, 21–23] of a phenanthrene derivative in the spectrum of flaccidinin, and similar signals at ~ δ 8.0 for such protons of the 9,10-dihydrophenanthrenes in the spectrum of oxoflaccidin indicated that C-4 and C-5 of both the compounds were substituted. These two carbon atoms may thus be conceived to be the only possible sites for holding the δ-lactone moiety in both the compounds. The ^1H NMR spectrum of flaccidinin showed the presence of three more aromatic protons which appeared as two

meta-coupled doublets at δ 7.12 (J = 1.8 Hz) and 7.29 (J = 1.8 Hz) and as a singlet at δ 7.70. But while the two *meta*-coupled doublets are shifted downfield by 0.12 and 0.18 ppm, respectively, those at δ 7.70 and 7.73 (H-9 and H-10) remained essentially unchanged in the ^{1}H NMR spectrum of flaccidinin diacetate, which also exhibited signals for two acetate methyls, one resonating at the normal region (δ 2.39) and the other at a relatively downfield position (δ 2.53). These observations thus suggest that while each of the two *meta*-coupled protons of flaccidinin has an *ortho*-hydroxyl group, that at δ 7.70 bears a methoxyl group at its *ortho*-position in **1a**. The signals at δ 7.12 and 7.29 of flaccidinin may be attributed to H-8 and H-6, respectively, both having an *ortho*-hydroxyl group at C-7. The signal at δ 7.70 may be assigned to H-1 with a methoxy group at C-2, and the relatively downfield shift of this signal compared to the corresponding proton of flaccidin (**1e**) may be attributed to the combination of a greater diamagnetic anisotropic effect of the phenanthrene ring of flaccidinin and the inductive effect of the lactone carbonyl group at C-4. The downfield shift of one of the acetate methyls (δ 2.53) of flaccidinin diacetate may be due to the diamagnetic anisotropic effect of the lactone carbonyl at C-4, and this justifies the placement of this acetate function at C-3.

The ^{1}H NMR spectrum of oxoflaccidin showed signals for three aromatic protons at δ 6.66 (1H, *d*, J = 1.9 Hz), 6.76 (1H, *d*, J = 1.9 Hz) and 7.37 (1H, *s*), which exhibited essentially similar splitting patterns as those for H-8, H-6 and H-1 of flaccidinin, but, as expected of a 9,10-dihydrophenanthropyrone derivative, they appeared at relatively upfield positions. As in the case of flaccidinin, upon acetylation of oxoflaccidin only the *meta*-coupled protons are shifted downfield by ~ 0.2 ppm, the other proton remaining unchanged. The two acetate methyls of oxoflaccidin diacetate resonated at δ 2.31 and 2.44. The downfield acetate methyl is thus comparable with the acetoxy group at C-3 of flaccidinin diacetate. The foregoing observations thus indicate that while flaccidinin has the structure **1a**, oxoflaccidin is the corresponding 9,10-dihydro derivative **1c**.

The structures **1a** and **1c** for flaccidinin and oxoflaccidin, respectively, were also supported by the ^{13}C NMR spectra of their respective diacetates **1b** and **1d**. The degree of protonation of each carbon atom of both **1b** and **1d** were determined by DEPT experiments and the



1a R¹ = H, R² = O, R³ = OH, R⁴ = Me, 9,10-Dehydro-
1b R¹ = Ac, R² = O, R³ = OAc, R⁴ = Me, 9,10-Dehydro-
1c R¹ = H, R² = O, R³ = OH, R⁴ = Me
1d R¹ = Ac, R² = O, R³ = OAc, R⁴ = Me
1e R¹ = H, R² = H_2, R³ = OH, R⁴ = Me
1f R¹ = Ac, R² = H_2, R³ = OAc, R⁴ = Me
1g R¹ = R⁴ = H, R² = H_2, R³ = OMe
1h R¹ = R⁴ = Ac, R² = H_2, R³ = OMe
1i R¹ = R⁴ = Ac, R² = O, R³ = H

Table 1. Carbon chemical shifts of flaccidinin diacetate (**1b**) oxoflaccidin diacetate (**1d**) and oxoflavidin diacetate (**1i**)

C	Chemical shifts (δ values)*		
	1b	**1d**	**1i**
1	114.96[a]	118.69	127.3
2	150.19[b]	150.04[e]	150.30[g]
3	151.62[c]	150.84	119.80
4	113.0	114.69	120.0
4a	126.85	125.01	136.0
4b	128.14	128.09	129.0
5	151.88[c]	150.84	151.30
6	107.46	107.93	108.0
7	149.80[b]	149.70[e]	150.40[g]
8	114.73[a]	116.94	116.90
8a	136.0	135.50	135.30
9	126.89[d]	27.30[f]	26.50
10	126.49[d]	27.69[f]	26.50
10a	130.62	132.19	135.30
lactone >C=O	157.17	157.62	160.0
Ar-OMe	56.58	56.52	
ArOCOMe	169.17, 169.0	169.0	168.80, 168.7
	21.19, 20.94	20.81, 20.94	20.70

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

carbon chemical shifts of both the compounds (Table 1) were assigned by comparison with the δ_c values of structurally similar compounds [11, 16]. Thus the δ_c values of C-4b, C-5, C-6, C-7, C-8 and C-8a constituting the ring A of oxoflaccidin diacetate (**1d**) appeared almost at the same positions as the corresponding carbon atoms of oxoflavidin diacetate (**1i**) [16]. This confirmed the structural identity of ring A of the two compounds. The δ_c values of these carbon atoms of flaccidinin diacetate (**1b**) also exhibited a close resemblance to the ring A carbon atoms of both **1d** and **1i** indicating identical substitution patterns of its ring A, the marginal differences in the δ_c values being due to its phenanthrene ring system. This is borne out by the fact that the signals at δ_c 27.30 and 27.69 characteristic of C-9 and C-10, respectively, of the 9,10-dihydrophenanthrene derivatives [11–17] in **1d** are replaced by the signals at δ_c 126.89 and 126.49 typical of the corresponding carbon atoms of a phenanthrene derivative [3–8, 21] in the spectrum of **1b**. The lactone carbonyl carbons of **1b** and **1d** appeared at δ_c 157.17 and 157.62, respectively. The relatively upfield shifts of C-1, C-4, C-4a and C-10a of both **1b** and **1d** compared with the corresponding carbon atoms of oxoflavidin diacetate (**1i**) are consistent with the placement of an acetoxy group at C-3 and a methoxy group at C-2 in both the compounds. The methoxyl carbon atoms in both **1b** and **1d** appeared at the normal region (δ_c 55.5–56.5) indicating the presence of at least one hydrogen atom *ortho* to the methoxyl group. An interchange of the methoxy and acetoxy groups at C-2 and C-3 of **1b** and **1d** would have caused a downfield shift of the methoxyl carbon by ~ 5 ppm as in imbricatin diacetate (**1h**) [14].

The structures of flaccidinin (**1a**) and oxoflaccidin (**1c**) were finally confirmed by the following chemical evidence. Oxidation of flaccidin diacetate (**1f**), the diacetyl

derivative of the congener flaccidin (**1e**), with DDQ [22] in dry benzene afforded flaccidinin diacetate (**1b**) which was also obtained by a similar DDQ oxidation of the 80% pure oxoflaccidin diacetate as the sole product.

The possibility of oxoflaccidin (**1c**) being an artefact of flaccidin (**1e**) was ruled out by the fact that the latter was stable to triplet oxygen even on long exposure. Adsorption of a solution of flaccidin on silica gel surface followed by exposure of the silica gel to air for more than a month yielded not a trace of oxoflaccidin, and flaccidin was mostly converted to an uncharacterized gummy material. Alternatively, treatment of flaccidin diacetate (**1f**) or flaccidin dimethyl ether with *m*-chloroperbenzoic acid in methylene chloride at room temperature for 10 hr afforded an uncharacterized heterogeneous polymeric material, but none of the corresponding oxoflaccidin derivatives.

Flaccidinin (**1a**) and oxoflaccidin (**1c**), the former being the first naturally occurring phenanthropyrone derivative, are thus two new additions to the growing list of phytochemicals isolated from the orchids. In terms of systematic nomenclature **1a** and **1c** may be called 2,6-dihydroxy-7-methoxy-5*H*-phenanthro[4,5-*bcd*] pyran-5-one and 2,6-dihydroxy-7-methoxy-9,10-dihydro-5*H*-phenanthro [4,5-*bcd*] pyran-5-one, respectively, although the phenanthrene numbering system has been used in this paper for convenience of comparison of spectral data.

EXPERIMENTAL

Mps: uncorr. UV spectra were measured in 95% aldehyde-free EtOH and IR spectra in KBr discs. ^{1}H NMR spectra were recorded at 300 MHz in $CDCl_3$ and d_6-acetone soln. using TMS as int. standard. ^{13}C NMR spectra were measured at 62.5 and 75 MHz in $CDCl_3$ soln. 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 at 70 eV using a direct inlet system. Silica gel (100–200 mesh) was 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. Dry Na_2SO_4 was used for drying organic solvents and petrol used had bp 60–80°.

Isolation of flaccidin (**1e**), *flaccidinin* (**1a**), *oxoflaccidin* (**1c**) *and imbricatin* (**1g**). Air-dried powdered whole plant of *C. flaccida* (5 kg) was soaked with MeOH (15 l) for 3 weeks. The MeOH extract was then drained out and concd under red. pres. to 150 ml, diluted with H_2O (1 l) and exhaustively extracted with Et_2O. The Et_2O 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 (15:1) eluate on evapn gave a semisolid mass which on repeated chromatography gave in the early fractions of petrol–EtOAc (15:1) flaccidin (**1e**), (0.25 g), crystallized from petrol–EtOAc, mp 200°. The later fractions of petrol–EtOAc (10:1) eluate from the main column gave, on evapn, a light yellow solid. On warming the above solid with $CHCl_3$ part of it went into solution. The $CHCl_3$ soln was evapd to give a solid which on repeated crystallization afforded imbricatin (**1g**) (0.9 g), mp 145°. The $CHCl_3$-insoluble light yellow residue containing mainly mixture of flaccidinin (**1a**) and oxoflaccidin (**1c**) was dissolved in boiling EtOAc and rechromatographed. The early fractions of petrol–EtOAc (10:1) eluate gave a light yellow solid (0.15 g) which contained a mixture of **1a** and **1c** in the ratio of *ca* 3:2. The above mixture was acetylated with Ac_2O and pyridine in the usual manner. The mixture of the acetylated product was chromatographed. The petrol–EtOAc (15:1) eluate afforded a solid containing a mixture of **1b** and **1d**, which on several crystallizations gave pure **1b**, crystallized from petrol–EtOAc mixture, mp 258°, (Found: C, 65.54; H, 3.90; $C_{20}H_{14}O_7$ requires: C, 65.57; H, 3.82%). IR ν_{max} cm^{-1}: 1270 and 1760 (OAc), 1735 (δ-lactone), 1615, 890, 835, 760 and 730 (aromatic nucleus); ^{1}H NMR: δ 7.75 (2H, *s*; H-9 and H-10), 7.71 (1H, *s*; H-1), 7.47 (1H, *d*, J = 2.1 Hz; H-6), 7.23 (1H, *d*, J = 2.1 Hz; H-8), 4.0 (3H, *s*; ArOMe), 2.53 (3H, *s*; OAc at C-3) and 2.39 (3H, *s*; OAc at C-7); MS *m/z* (rel. int.): 366 [M$^+$·] (4), 324 (37), 282 (100), 267 (10), 264 (5), 253 (5), 239 (18) and 43 (20).

The combined mother liquor after crystallization of **1b** on repeated crystallization afforded a solid which was shown (by ^{1}H NMR) to be a mixture of **1d** and **1b** in the ratio of 4:1. IR ν_{max} cm^{-1}: 1250 and 1760 (OAc) and 1725 (δ-lactone), 1625, 870 and 790 (aromatic nucleus); ^{1}H NMR: δ 7.37 (1H, *s*; H-1), 6.97 (1H, *d*, J = 1.9 Hz; H-6), 6.89 (1H, *d*, J = 1.9 Hz; H-8), 3.92 (3H, *s*; ArOMe), 3.08 (4H, *s*; H_2-9 and H_2-10), 2.44 (3H, *s*; OAc at C-3) and 2.31 (3H, *s*; OAc at C-7); MS *m/z* (rel. int.): 368 [M$^+$·] (5), 326 (45), 284 (100), 266 (5), 255 (8), 241 (36), 139 (20) and 43 (20).

Hydrolysis of **1b** *and* **1d** *with aq. methanolic* HCl. A soln of **1b** (0.05 g) in MeOH (5 ml) was treated with 2 M aq. HCl (5 ml) and the mixture refluxed on a boiling H_2O bath for 3 hr. MeOH was then removed under red. pres. and the solid residue extracted with Et_2O, washed with H_2O, dried and the solvent removed. The residue (0.045 g) on crystallization from petrol–EtOAc mixture, gave pure **1a**, mp 360° (dec.) (Found: C, 68.01; H, 3.50; $C_{16}H_{10}O_5$ requires: C, 68.08; H, 3.54%). IR ν_{max} cm^{-1}: 3320 (OH), 1665 (lactone >C=O), 1630, 870, 845 and 800 (aromatic nucleus); MS *m/z* (rel. int.): 282 [M$^+$·] (100), 267 (14), 239 (74), 211 (9), 184 (4), 155 (22), 139 (8), 126 (16), 77 (12), 75 (11), 69 (12), 63 (22), 51 (19) and 43 (68).

The 80% enriched **1d** was hydrolysed under identical conditions to give **1c** which contained about 20% **1a**. IR ν_{max} cm^{-1}: 3330 (OH) and 1682 (lactone >C=O).

DDQ oxidation of **1f** *and* **1d**. Flaccidin diacetate (**1f**) (0.1 g) in C_6H_6 (10 ml) was treated with DDQ (0.2 g) and the mixture was refluxed for 16 hr. C_6H_6 was removed under red. pres. and the residue extracted with Et_2O. The Et_2O extract was washed with 2 M NaOH, and then with water, dried and the solvent removed. The residue on crystallization from petrol–EtOAc mixture gave pure **1b** (0.03 g), mp 258°. In a similar manner **1d** (0.02 g) was oxidized with DDQ (0.04 g) and the reaction product was worked-up as above to give **1b** (0.015 g).

Attempted oxidation of (**1e**) *with oxygen.* A soln of flaccidin (0.02 g) in EtOAc was adsorbed in silica gel (10 g) and kept exposed to air for 1 month with occasional stirring to ensure better exposure to air. The silica gel was then taken in a CC column and eluted with petrol–EtOAc (5:1). The eluate, on evapn gave a solid. TLC of the solid showed the absence of any iodine-staining spot corresponding to that of oxoflaccidin (**1c**) and revealed the presence of unchanged flaccidin (**1e**) and an uncharacterized heterogeneous material. The solid was then chromatographed. The petrol–EtOAc (15:1) eluate gave **1e** (0.01 g). Exposure of adsorbed **1e** on silica gel to air for a shorter period (10 days) gave mostly unchanged **1e** and not a trace of oxoflaccidin.

Attempted oxidation of **1f** *and flaccidin dimethyl ether with m-chloroperbenzoic acid.* To a soln of **1f** (0.03 g) in CH_2Cl_2 (10 ml) was added *m*-chloroperbenzoic acid (0.04 g) and the mixture was stirred at room temperature for 10 hr. The CH_2Cl_2 layer was extracted with $NaHCO_3$, washed with H_2O, dried and the

solvent removed. TLC of the residue showed the absence of iodine-staining spots corresponding either of **1f** or **1d**. The reaction product was found to be an uncharacterised heterogeneous polymeric material. Similar experiment with flaccidin dimethyl ether also yielded heterogeneous polymeric material.

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