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STRUCTURE OF OXOFLAVIDIN, A 9,10-DIHYDROPHENANTHROPYRONE FROM *COELOGYNE ELATA*

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Key Word Index—*Coelogyne elata*; Orchidaceae; 9,10-dihydrophenanthropyrane; 2,7-dihydroxy-9,10-dihydro-5H-phenanthro[4,5-bcd]pyran-5-one; structural determination.

Abstract—Oxoflavidin, a new phenolic compound isolated from the Himalayan orchid *Coelogyne elata* was shown to be 2,7-dihydroxy-9,10-dihydro-5H-phenanthro[4,5-bcd]pyran-5-one (**2d**) by spectral and chemical evidence.

INTRODUCTION

We reported earlier the isolation of a number of 9,10-dihydrophenanthropyrans [1–5] (**1a–1e**) and pyrones [1–3] (**2a–2c**), besides 2,7-dihydroxy-3,4,6-trimethoxy-9,10-dihydrophenanthrene [6] and 2,7-dihydroxy-4-methoxy-9,10-dihydrophenanthrene (coelonin) [7], from a series of Himalayan orchids. Further chemical investigation of one of these orchids, *Coelogyne elata*, which was earlier shown to contain coelonin, has now resulted in the isolation of yet another new phenolic compound, designated oxoflavidin. The present communication deals with the structure elucidation of this compound.

RESULTS AND DISCUSSION

Oxoflavidin, $C_{15}H_{10}O_4$ $[M]^+$ *m/z* 254, mp > 300°, $[\alpha]_D \pm 0°$ (EtOH) shows UV spectrum, λ_{max} 220, 246, 288, 368–373 nm (log ε4.32), 4.15, 4.04 and 3.66) resembling those of coeloginin [1] (**2a**), oxoflavidinin [2] (**2b**) and isooxoflavidinin [3] (**2c**) indicating similarity in their chromophoric system. This is also supported by the IR spectrum of oxoflavidin showing bands at 1727 and 3350 cm^{-1} for lactone carbonyl and phenolic hydroxyl groups, respectively. The characteristic colour reactions of oxoflavidin and the alkali-induced bathochromic shift of the UV maximal positions of the compound also indicate its phenolic nature.

The 80 MHz ^1H NMR spectrum of oxoflavidin in d_6-acetone shows a four-proton singlet at δ2.97 which is typical [1–9] of the four equivalent protons of the 9- and 10-methylene groups of the 9,10-dihydrophenanthrenes. The spectrum also displays signals for four aromatic protons [δ6.54 (1H, *d*, *J* = 2.5 Hz), 6.60 (1H, *d*, *J* = 2.5 Hz), 7.09 (1H, *d*, *J* = 3 Hz) and 7.37 (1H, *d*, *J* = 3 Hz)] each appearing as a *meta*-coupled doublet, and a two-proton broad signal at δ8.99 (disappearing on deuterium exchange) for two phenolic hydroxyl groups. The chemical shift of the downfield aromatic proton indicates that it is *ortho* to a carbonyl group.

The presence of two phenolic hydroxyl groups in oxoflavidin was confirmed by the formation of a dimethyl diether, $C_{17}H_{14}O_4$ $[M]^+$ *m/z* 282, mp 205°, and a diacetyl derivative, $C_{19}H_{14}O_6$ $[M]^+$ *m/z* 338, mp 200°. The spectral features of these derivatives are essentially similar to those of the parent compound except those expected for the change in functionalities. In the ^1H NMR spectrum of diacetyl oxoflavidin (in $CDCl_3$) all the four aromatic protons are shifted downfield by ~ 0.15–0.3 ppm compared to those in the parent compound. This is indicative of the fact that each aromatic proton in oxoflavidin is *ortho* to a phenolic hydroxyl group.

On the basis of the foregoing observations oxoflavidin was assumed to have the 2,7-dihydroxy-9,10-dihydro-5H-phenanthro[4,5-bcd]pyran-5-one structure* (**2d**) which is also in accord with its mass spectral fragmentation. The structure of oxoflavidin was further confirmed by the following chemical evidence. Reduction of oxoflavidin dimethyl diether with lithium aluminium hydride in THF afforded compound A, $C_{17}H_{18}O_4$ $[M]^+$ *m/z* 186, mp 180° as the major product along with a trace of another compound which on TLC comparison corresponded to flavidin dimethyl diether (**1f**). Flavidin (**1d**) was earlier isolated [5] from the orchids *Coelogyne flavida*, *Pholidota articulata* and *Otochilus fusca*. The UV spectrum of compound A, λ_{max} 217, 274 and 300–302 nm (log ε4.55, 4.29 and 4.10) is similar to those of 9,10-dihydrophenanthrenes. Its IR spectrum lacks the carbonyl absorption of oxoflavidin dimethyl diether, and instead shows band at 3500 cm^{-1} for a hydroxyl group. The ^1H NMR spectrum of compound A exhibits two one-proton broad signals at δ8.55 and 3.98 (each disappears on deuterium exchange) for a phenolic and an alcoholic proton, respectively, and a two-proton ill-resolved AB quartet at δ4.56 characteristic of the hydroxymethyl protons of a benzylic alcohol function. The spectrum also displays signals for two aromatic methoxyl groups (δ3.76 and 3.80), the 9- and 10-methylene protons (δ2.57) of the 9,10-dihydrophenanthrene system, and four aromatic protons at δ7.10 (1H, *d*, *J* = 3 Hz), 6.74 (1H, *d*, *J* = 3 Hz) and 6.44 (2H, *br s*). Compound A forms a diacetyl derivative, $C_{21}H_{22}O_6$ $[M]^+$ *m/z* 370, mp 170°. The

*For convenience of comparison of spectral results the phenanthrene numbering system as shown in the structural formulae is followed in this paper.

^{1}H NMR spectrum of the latter is essentially similar to that of the former except that in the spectrum of the latter (i) the signals for the phenolic and alcoholic hydroxyl protons of compound A are replaced by two acetylmethyl singlets at δ2.04 and 2.11, (ii) the hydroxymethyl signal of compound A is shifted downfield by 0.60 ppm and appears as a clear AB quartet (J = 13 Hz), (iii) the signal for one of the aromatic protons of compound A appearing at δ6.44 is shifted downfield by 0.32 ppm, and (iv) the signal for another aromatic proton of compound A appearing at δ7.10 is shifted upfield by 0.25 ppm. These spectral data of compound A and its diacetyl derivative suggest structures **3a** and **3b** for the two compounds, respectively, and are intelligible in terms of the reduction of the lactone moiety of oxoflavidin dimethyl diether (**2e**) to a phenolic and a primary benzyl alcoholic function in **3a**. The proton at C-6 of **3a** resonating at δ6.44 because of its being *ortho* to the newly generated phenolic hydroxyl group shows the observed downfield shift in the ^{1}H NMR spectrum of **3b** which has its methylene protons of the acetoxymethyl function also shifted downfield. The proton at C-3 of **3b** is shifted upfield compared to the corresponding proton of **3a** appearing at δ7.10 presumably because of the restriction imposed by the 5-acetoxyl group on the free rotation of the acetoxymethyl function which is forced to assume such a preferred conformation in which H-3 falls within the shielding zone of its carbonyl group. This finds analogy [1] in the shielding of the oxymethylene protons by the 3-acetoxyl group of diacetylcoelogin (**1h**).

A more convincing proof of the structure of oxoflavidin was provided by the derivation of oxoflavidin dimethyl diether (**2e**) from flavidin dimethyl diether (**1f**). The latter on treatment with *m*-chloroperbenzoic acid in CH_2Cl_2 at ambient temperature was slowly converted to **2e**.

The structure of oxoflavidin is also supported by ^{13}C NMR spectral analysis of its diacetate (**2f**) and its dimethyl diether (**2e**). The degree of protonation of each carbon atom in **2f** and **2e** was determined by off-resonance decoupling. The assignments of carbon chemical shifts of **2f** and **2e** (Table 1) are in fairly good agreement with the calculated values using known additive parameters of the functional groups on the reported carbon chemical shifts of the parent 9,10-dihydrophenanthrene [10]. This was further confirmed by comparison of the δ_c values of **2f** and **2e** between themselves and with those of coeloginin diacetate [1] (**2g**), flavidin diacetate [5] (**1g**) and other structurally related compounds [2–4, 6, 7]. The difference in δ_c values are in conformity with the changed additive parameters attendant with the change in the peripheral functional groups. With the exception of C-5 the δ_c values of the ring A carbon atoms of **2f** compare excellently with those of the corresponding carbon atoms of **2g** indicating similarity of this part of their molecules. The observed downfield shift of C-5 and the upfield shift of the lactone carbonyl carbon of **2g** compared to the corresponding carbon atoms of **2f** (and also **2e**) may be due to the acetoxyl group at C-3 in **2g** exerting a steric compression on the lactone carbonyl. It is interesting to note that in coeloginin diacetate (**2g**) and also in coelogin diacetate (**1h**) C-10 is shifted upfield by 6–7 ppm compared to C-9 which appears at the normal position (δ26–27). Such upfield shift of C-10 and C-9 has been observed to be a diagnostic feature [1] for the presence of an oxygen substituent at C-1 and C-8, respectively, in this series of compounds. The appearance of both C-9 and C-10 of **2f** and **2e** at the normal position thus rules out the possibility of a hydroxyl group being at C-1 or C-8 in oxoflavidin.

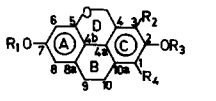
Table 1. Carbon chemical shifts of oxoflavidin diacetate (**2f**), oxoflavidin dimethyl diether (**2e**), coeloginin diacetate (**2g**) and flavidin diacetate (**1g**)

Carbon atoms	Chemical shifts (δ values)*			
	2f	**2e**	**2g**	**1g**
C-1	127.3	122.6	150.3	120.2
C-2	150.3[a]	155.9[b]	145.6	149.8
C-3	119.8	110.6	144.4	115.5
C-4	120.0	119.2	108.3	129.9
C-4a	136.0	131.5	124.2	123.7
C-4b	129.0	125.5	128.7	116.6
C-5	151.3	150.9	156.1	153.2
C-6	108.0	99.0	107.5	108.1
C-7	150.4[a]	156.3[b]	151.3	150.6
C-8	116.9	108.3	116.5	114.3
C-8a	135.3	135.6[c]	136.2	134.6[e]
C-9	26.5	27.5[d]	26.1	27.1[f]
C-10	26.5	27.1[d]	20.2	27.2[f]
C-10a	135.3	134.8[c]	112.3	135.7[e]
–OCOAr	160.0	160.4	157.0	—
ArOMe	—	55.6	60.5, 61.1	—
Ar–OCOMe	168.8, 168.7	—	168.8, 168.5	169.3
Ar–OCOMe	20.7	—	20.5, 20.7	20.9
–OCH_2–	—	—	—	67.8

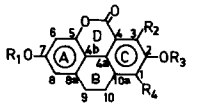
*δ values are in ppm downfield from TMS: $\delta_{(TMS)} = \delta_{(CDCl_3)} + 76.9$ ppm.

[a–f], Values are interchangeable.

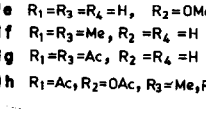
Like other members of this series, oxoflavidin is optically inactive. This may be explained, as in other cases [1–5], by the assumption that the energy barrier between the two possible conformers **4a** and **4b** of oxoflavidin obtained by flipping of rings B and D is quite low, and that the two conformers bear a mirror-image relationship. Thus at ordinary temperature oxoflavidin becomes optically inactive due to rapid interconversion of **4a** and **4b**.



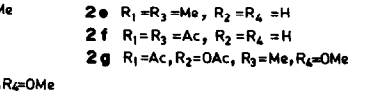
1a R_1=H, R_2=OH, R_3=Me, R_4=OMe
1b R_1=R_2=R_4=H, R_3=Me
1c R_1=Me, R_2=R_3=R_4=H
1d R_1=R_2=R_3=R_4=H
1e R_1=R_3=R_4=H, R_2=OMe
1f R_1=R_3=Me, R_2=R_4=H
1g R_1=R_3=Ac, R_2=R_4=H
1h R_1=Ac, R_2=OAc, R_3=Me, R_4=OMe



2a R_1=H, R_2=OH, R_3=Me, R_4=OMe
2b R_1=R_2=R_4=H, R_3=Me
2c R_1=Me, R_2=R_3=R_4=H
2d R_1=R_2=R_3=R_4=H
2e R_1=R_3=Me, R_2=R_4=H
2f R_1=R_3=Ac, R_2=R_4=H
2g R_1=Ac, R_2=OAc, R_3=Me, R_4=OMe



R
3a H
3b Ac



4a R_1=R_2=R_3=R_4=H **4b** R_1=R_2=R_3=R_4=H

Oxoflavidin is thus a new addition to the growing list of the naturally occurring 9,10-dihydrophenanthropyrones. The possibility of its being an artefact of flavidin (**1d**) is ruled out by the complete absence of **1d** even in a fresh sample of *C. elata*, and more importantly by the total absence of oxoflavidin in none of the orchids *C. flavida*, *P. articulata* and *O. fusca* producing flavidin.

EXPERIMENTAL

Mps are uncorr. Silica gel (60–100 mesh) was used for CC and silica gel G for TLC. 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 $CDCl_3$ (unless otherwise stated) using TMS as int. standard, and ^{13}C NMR spectra were run on the same instrument at 20 MHz in the same solvent and with the same int. standard. Chemical shifts are expressed as δ values. MS were recorded with a direct inlet system operating at 70 eV, and figures in the first bracket attached to m/z values represent rel. int. of peaks. All the analytical samples were routinely dried over P_2O_5 at 80° for 24 hr *in vacuo* and were tested for purity by TLC and MS. Na_2SO_4 was used for drying organic solvents and petrol used had bp 60–80°.

Isolation of oxoflavidin (**2d**). Air-dried, powdered whole plant of *C. elata* (1 kg) was successively extracted with $CHCl_3$ and MeOH in a Soxhlet for 50 hr. After removal of solvents the combined residue was taken up in EtOAc and chromatographed. The petrol–EtOAc (5:1) eluate gave coelonin (200 mg). Further elution of the column with petrol–EtOAc (3:1) gave, on evapn of the solvent, a crude mass of phenolic residue which on repeated chromatography afforded oxoflavidin (200 mg), amorphous, mp > 300°. (Found: C, 71.05; H, 3.75. $C_{15}H_{10}O_4$ requires: C, 70.87; H, 3.93%.) UV λ_{max} nm: 220, 246, 288 and 368–373 (log ε4.32, 4.15, 4.04 and 3.66); λ_{max} nm (0.1 N NaOH–EtOH): 214, 230 sh, 258 and 309 (log ε4.29, 4.19, 4.19 and 4.16); IR ν_{max} cm^{-1}: 3350 (OH), 1727 (δ-lactone), 1650, 1629, 1484, 860 and 760 (phenyl nucleus); ^{1}H NMR: δ2.97 (4H, *s*, H_2-9 and H_2-10), 6.54 (1H, *d*, J = 2.5 Hz, H-8), 6.60 (1H, *d*, J = 2.5 Hz, H-6), 7.09 (1H, *d*, J = 3 Hz, H-1), 7.37 (1H, *d*, J = 3 Hz, H-3), 8.99 (2H, *s*, Ar–OH); MS: m/z 254 $[M]^+$ (100), 253 (47.3), 252 (16.0), 237 (5.1), 197 (10.7), 181 (19.0), 168 (8.5), 141 (7.6), 139 (13.1) and 115 (10.2). Oxoflavidin diacetate (**2f**) (prepared by treatment of **2d** with Ac_2O–C_5H_5N in the cold), crystallized from petrol–EtOAc, mp 200°. (Found: C, 67.08; H, 4.30. $C_{19}H_{14}O_6$ requires: C, 67.46; H, 4.14%.) UV λ_{max} nm: 220, 226, 232 sh, 240 sh, 279 and 332 (log ε4.49, 4.51, 4.46, 4.29, 4.45 and 3.81); IR ν_{max} cm^{-1}: 1780, 1297 (OAc), 1745 (δ-lactone), 1620, 1470, 785 (phenyl nucleus); ^{1}H NMR: δ2.25 (6H, *s*, –OCOMe), 3.05 (4H, *s*, H_2-9 and H_2-10), 6.80 (1H, *d*, J = 2.5 Hz, H-8), 6.88 (1H, *d*, J = 2.5 Hz, H-6), 7.26 (1H, *d*, J = 3 Hz, H-1), 7.74 (1H, *d*, J = 3 Hz, H-3); MS m/z (rel. int.): 338 $[M]^+$ (7.1), 296 (16.4), 255 (17.1), 254 (100), 253 (16.8), 252 (9.4) and 43 (40.4). Oxoflavidin diMe diether (**2e**) (prepared by refluxing **2d** with Me_2SO_4 in dry Me_2CO in presence of dry K_2CO_3 for 8 hr, followed by usual work up), crystallized from petrol–EtOAc, mp 205°. (Found: C, 72.80; H, 4.76. $C_{17}H_{14}O_4$ requires: C, 72.34; H, 4.96%.) UV λ_{max} nm: 222, 234 sh, 246, 287 and 363 (log ε4.43, 4.39, 4.27, 4.19 and 3.86); IR ν_{max} cm^{-1}: 1745 (δ-lactone), 1650, 1622, 1565, 1493 (phenyl nucleus); ^{1}H NMR: δ3.06 (4H, *s*, H_2-9 and H_2-10), 3.84 and 3.88 (each 3H, *s*, Ar–OMe), 6.68 (2H, *br s*, H-6 and H-8), 7.12 (1H, *d*, J = 3 Hz, H-1), 7.52 (1H, *d*, J = 3 Hz, H-3).

Conversion of oxoflavidin diMe diether (**2e**) *to flavidin diMe diether* (**1f**) *and* **3a**. Oxoflavidin diMe diether (150 mg) in dry THF soln (50 ml) was added to a suspension of $LiAlH_4$ (15 mg) in dry Et_2O (20 ml) and the mixture refluxed under anhydrous conditions for 4 hr. After usual work-up the product was chromatographed. The petrol–EtOAc (15:1) eluate gave traces of **1f**, identified by TLC, R_f 0.3 in petrol–EtOAc (5:1) as developer. Further elution of the column with petrol–EtOAc (2:1) afforded **3a** (120 mg), crystallized from petrol–EtOAc, mp 180°. (Found: C, 70.99; H, 6.48. $C_{17}H_{18}O_4$ requires: C, 71.33; H, 6.29%.) UV λ_{max} nm: 217, 274 and 300–302 (log ε4.55, 4.29 and 4.10); UV λ_{max} nm (0.1 N NaOH–EtOH): 222, 243 sh, 272, 278, 322–323 (log ε4.44, 4.24, 3.97, 3.99 and 4.02); IR ν_{max} cm^{-1}: 3500 (OH), 1612, 1520, 1470, 850 (phenyl nucleus); ^{1}H NMR in d_6-acetone: δ2.57 (4H, *s*, H_2-9 and H_2-10), 3.76 and 3.80 (each 3H, *s*, Ar–OMe), 3.98 (1H, *br*, –CH_2–OH), 4.56 (2H, ill-resolved AB quartet, Ar–CH_2–OH), 6.44 (2H, *br s*, H-6 and H-8), 6.74 (1H, *d*, J = 3 Hz, H-1), 7.10 (1H, *d*, J = 3 Hz, H-3), 8.55 (1H, *br s*, Ar–OH). Diacetyl derivative of **3a** (**3b**) (prepared by treatment of **3a** with Ac_2O–C_5H_5N in the cold), crystallized from petrol–EtOAc, mp 170°. (Found: C, 68.02; H, 5.99. $C_{21}H_{22}O_6$ requires: C, 68.11; H, 5.95%.) UV λ_{max} nm: 213 and 276 (log ε4.44 and 4.27); IR ν_{max} cm^{-1}: 1785, 1750, 1620, 1575, 1250, 865; ^{1}H NMR: δ2.04 and 2.11 (each 3H, *s*, –OCOMe), 2.67 (4H, *s*, H_2-9 and H_2-10), 3.81 and 3.82 (each 3H, *s*, ArOMe), 5.16 (2H, AB quartet, J = 13 Hz, Ar–CH_2OAc) 6.54 (1H, *d*, J = 2.5 Hz, H-8), 6.75 (1H, *d*, J = 2.5 Hz, H-1), 6.76 (1H, *d*, J = 2.5 Hz, H-6), 6.85 (1H, *d*, J = 2.5 Hz, H-3).

Conversion of flavidin diMe diether (**1f**) *to oxoflavidin diMe diether* (**2e**). A soln of **1f** (40 mg) in dry CH_2Cl_2 (10 ml) was treated with *m*-chloroperbenzoic acid (40 mg). The mixture was stirred at room temp for 2 days. The CH_2Cl_2 soln was then washed with aq. $NaHCO_3$, dried and then chromatographed. The petrol–EtOAc (15:1) eluate gave unchanged **1f** (28 mg). Further elution of the column with petrol–EtOAc (6:1) afforded **2e** (5 mg).

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