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ISOFLAVIDININ AND ISO-OXOFLAVIDININ, TWO 9,10-DIHYDROPHENANTHRENES FROM THE ORCHIDS *PHOLIDOTA ARTICULATA*, *OTOCHILUS PORECTA* AND *OTOCHILUS FUSCA*

PRIYALAL MAJUMDER, ACHINTYA KUMAR SARKAR and JAYATI CHAKRABORTI

Department of Chemistry, University College of Science, Calcutta, 700009, India

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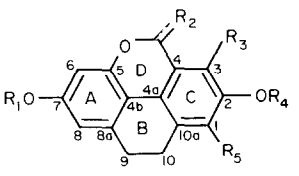
Key Word Index—*Pholidota articulata*; *Otochilus porecta*; *O. fusca*; Orchidaceae; isoflavidinin; iso-oxoflavidinin; modified 9,10-dihydrophenanthrenes.

Abstract—The structures of isoflavidinin and iso-oxoflavidinin, two new modified 9, 10-dihydrophenanthrenes of the orchids *Pholidota articulata*, *Otochilus porecta* and *O. fusca* have been established. ^{13}C NMR spectral analysis of isoflavidinin acetate was made to confirm its structure.

INTRODUCTION

Orchids constitute one of the largest botanical families, yet from a chemical point of view they have remained practically unexplored, despite considerable interest evoked by the isolation of the physiologically active alkaloids, such as dendrobine and some of its structural analogues from a few orchids of the *Dendrobium* genus [1–4]. This situation has prompted us to investigate a number of high altitude Himalayan orchids and has led to the isolation of five new modified 9, 10-dihydrophenanthrenes, coelogin (**1a**) [5], coeloginin (**1b**) [5], flavidin (**1c**) [6], flavidinin (**1e**) [7] and oxoflavidinin (**1g**) [7], in addition to 2, 7-dihydroxy-4-methoxy-9,10-dihydrophenanthrene (coelonin) [8] and 2, 7-dihydroxy-3, 4, 6-trimethoxy-9, 10-dihydrophenanthrene [9]. Our continued interest in this area has been further rewarded by the isolation of two more new phenolic compounds, designated isoflavidinin and iso-oxoflavidinin from the orchids *Pholidota articulata*, *Otochilus porecta* and *O. fusca*, the latter, however, producing only isoflavidinin. All three orchids, in addition, also contain flavidin (**1c**). The present paper deals with the structure elucidation of isoflavidinin and iso-oxoflavidinin.



1a R_1 = H, R_2 = H_2, R_3 = OH, R_4 = Me, R_5 = OMe
1b R_1 = H, R_2 = O, R_3 = OH, R_4 = Me, R_5 = OMe
1c R_1 = R_3 = R_4 = R_5 = H, R_2 = H_2
1d R_1 = R_4 = Ac, R_2 = H_2, R_3 = R_5 = H
1e R_1 = R_3 = R_5 = H, R_2 = H_2, R_4 = Me
1f R_1 = Ac, R_2 = H_2, R_3 = R_5 = H, R_4 = Me
1g R_1 = R_3 = R_5 = H, R_2 = O, R_4 = Me
1h R_1 = Ac, R_2 = O, R_3 = R_5 = H, R_4 = Me
1i R_1 = Me, R_2 = H_2, R_3 = R_4 = R_5 = H
1j R_1 = Me, R_2 = H_2, R_3 = R_5 = H, R_4 = Ac
1k R_1 = R_4 = Me, R_2 = H_2, R_3 = R_5 = H
1l R_1 = Me, R_2 = O, R_3 = R_4 = R_5 = H
1m R_1 = Me, R_2 = O, R_3 = R_5 = H, R_4 = Ac

RESULTS AND DISCUSSION

Isoflavidinin, $C_{16}H_{14}O_3$ ($M^{+\cdot}$ 254), mp 120°, $[\alpha]_D \pm 0°$($CHCl_3$), and iso-oxoflavidinin, $C_{16}H_{12}O_4$ ($M^{+\cdot}$ 268), mp 259°, $[\alpha]_D \pm 0°$ (EtOH), were isolated in low yields from ethanolic extracts of whole plants of the three orchids. The UV spectrum of isoflavidinin, λ_{max} 215, 286 and 303 nm (log ϵ 4.55, 4.26 and 4.18) showed a striking resemblance to those of substituted 9, 10-dihydrophenanthrenes [5–10] and in particular to those of flavidin (**1c**) and flavidinin (**1e**), while that of iso-oxoflavidinin, λ_{max} 207, 221, 246, 287 and 365 nm (log ϵ 4.34, 4.38, 4.34, 4.15 and 3.77) is somewhat different and exhibits a close similarity to those of coeloginin (**1b**) [5] and oxoflavidinin (**1g**) [7] indicating the presence of a conjugated carbonyl function similar to that in **1b** and **1g**. This is supported by the IR spectrum of iso-oxoflavidinin which shows a band at 1695 cm^{-1}, besides one at 3100 cm^{-1} for a hydroxyl group. Isoflavidinin also contains a hydroxyl group as indicated by its IR band at 3410 cm^{-1}. With acetic anhydride–pyridine, isoflavidinin and iso-oxoflavidinin both form monoacetyl derivatives, $C_{18}H_{16}O_4$ ($M^{+\cdot}$ 296), mp 131°, and $C_{18}H_{14}O_5$ ($M^{+\cdot}$ 310), mp 180°, respectively. The ^1H NMR spectrum of isoflavidinin shows a four-proton singlet at δ 2.85 which is typical [5–13] of the 9- and 10-methylene protons of a

9, 10-dihydrophenanthrene, a two-proton singlet at δ 5.07 reminiscent [5–7] of the oxymethylene protons of **1a**, **1c** and **1e**. The spectrum also displays signals for an aromatic methoxyl (δ 3.76), a phenolic hydroxyl group (δ 5.07, obscured in the signal of the oxymethylene protons and exchangeable with D_2O) and four aromatic protons. Two of these appearing as a pair of doublets at δ 6.46 (J = 3 Hz) and 6.60 (J = 3 Hz) are comparable to those of H-1 and H-3, respectively, of flavidin (**1c**) and flavidinin (**1e**), while the other two aromatic protons resonate as a broad singlet at δ 6.40. In isoflavidinin acetate, while the signal at δ 6.40 of isoflavidinin remains practically unchanged, those at δ 6.46 and 6.60 are shifted to δ 6.70 and 6.84, respectively. These values are almost identical to those of H-1 and H-3 of flavidin diacetate (**1d**). The above spectral data thus suggest structure **1i** for isoflavidinin having a hydroxyl group at C-2 like flavidin. The signal at δ 6.40 of isoflavidinin corresponds to H-6 and H-8, which in both flavidin and flavidinin, also appears as a broad singlet at δ 6.25 shifting downfield by *ca* δ 0.3 in the respective acetyl derivatives **1d** and **1f**, the difference being due to the replacement of the C-7 hydroxyl group in **1c** by a methoxyl function in isoflavidinin. The identity of isoflavidinin methyl ether (**1k**), $C_{17}H_{16}O_3$ ($M^{+\cdot}$ 268), mp 138° with flavidinin methyl ether and flavidin dimethyl ether is in conformity with this contention. The proposed structure of isoflavidinin is also in accord with its mass spectral fragmentation which is almost identical to that of flavidinin, the essential difference being in the relative abundance of the ion fragments. Like **1a**, **1c** and **1e**, isoflavidinin is also resistant to hydrogenolysis with 10% Pd–C under normal condition.

Further evidence in support of structure **1i** for isoflavidinin was provided by the ^{13}C NMR spectral analysis of its more soluble acetyl derivative (**1j**). The substitution profile of the carbon centres was determined by off-resonance decoupling techniques. The assignments of the carbon chemical shifts (Table 1) are in good agreement with the calculated values using the known additivity parameters [14] of the functional groups on the reported carbon chemical shifts of the parent 9, 10-dihydrophenanthrene [14], and are further confirmed by comparison with the chemical shifts of the carbon atoms of flavidin diacetate (**1d**) [6] and flavidinin acetate (**1f**) [7]. The C-4a of all the three compounds **1d**, **1f** and **1j**, as well as that of coelogin diacetate shows a general trend of upfield shift by *ca* δ 7–9 compared to the calculated values based on the additivity parameters of the functional groups. In the absence of any such shielding of the corresponding carbon atom in the tricyclic 9, 10-dihydrophenanthrenes, this may be regarded as a diagnostic ^{13}C NMR spectral feature of the above tetracyclic compounds bearing an oxymethylene bridge between C-4 and C-5. The observed upfield shift of C-4a of these compounds may be attributed to the effect of the γ-hetero atom [15] of the oxymethylene bridge. The aromatic carbon atoms of ring C of isoflavidinin acetate (**1j**) have chemical shifts almost identical to the corresponding carbons of flavidin diacetate (**1d**) indicating an identical substitution pattern of this part of the two compounds. The two compounds differ essentially in the chemical shifts of the ring A carbon atoms. The upfield shift of C-6 (δ 99.7) of isoflavidinin acetate demands the placement of the methoxyl group at C-7, which also accounts for the upfield shifts of the C-4b and C-8 compared to the corresponding carbon atoms of **1d**. A comparison of the carbon shifts of isoflavidinin acetate (**1j**) with those of flavidinin acetate (**1f**) lends further support to the structure of the former. The carbon chemical shifts of **1j** and **1f** differ essentially with respect to C-1–C-3, C-4a, C-4b and C-6–C-8, and such differences are expected for an interchange of the methoxyl and acetoxyl functions in the two compounds. This is evident from the observed shielding of C-2, C-4b, C-6 and C-8 and deshielding of C-1, C-3, C-4a and C-7 of **1j** compared to the corresponding carbon atoms of **1f**.

Table 1. The carbon chemical shifts of flavidin diacetate (**1d**), flavidinin acetate (**1f**) and isoflavidinin acetate (**1j**)

Carbon atoms	Chemical shifts (δ values)*		
	1d	**1f**	**1j**
C-1	120.0	112.7	120.0
C-2	149.8	159.4	149.0
C-3	115.5	108.0	115.3
C-4	129.9	129.3	128.7
C-4a	123.7	119.3	124.5
C-4b	116.6	117.3	112.0
C-5	153.2	152.5	153.8
C-6	108.1	107.8	99.7
C-7	150.6	149.8	160.4
C-8	114.3	114.1	107.4
C-8a	134.6	134.9	133.7
C-9	27.1†	27.5	27.5
C-10	27.2†	27.5	27.5
C-10a	135.7	134.9	135.9
–O–$\underline{C}H_2$–	67.8	68.2	67.9
–OCO$\underline{C}H_3$	20.9	20.9	20.8
–O$\underline{C}$OMe	169.3	169.4	169.4
–OC$\underline{H}_3$	—	55.3	55.1

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

†Values are interchangeable.

The ^1H NMR spectrum of iso-oxoflavidinin acetate is essentially similar to that of isoflavidinin acetate (**1j**) except that the signal at δ 5.07 for the oxymethylene protons of the latter is missing in the former. This suggests structure **1l** for iso-oxoflavidinin in which the oxymethylene bridge of isoflavidinin (**1i**) is replaced by a lactone moiety as in coeloginin (**1b**) and oxoflavidinin (**1g**). This is further corroborated by the fact that, as in oxoflavidinin acetate (**1h**), all the aromatic protons of iso-oxoflavidinin acetate are appreciably shifted downfield compared with those of isoflavidinin acetate (**1j**), the maximum downfield shift (δ 7.82) being observed for H-3. This is evidently due to the lactone carbonyl in iso-oxoflavidinin acetate and the additional deshielding of the H-3 is due to the diamagnetic anisotropic effect of this lactone carbonyl at the *ortho*-position (C-4). The mass spectral fragmentation of iso-oxoflavidinin is almost identical to that of oxoflavidinin (**1g**), the

essential difference being in the relative abundance of the ion-fragments as in the case of the flavidinin (**1e**)–isoflavidinin (**1i**) pair.

The structure of iso-oxoflavidinin was also confirmed by its conversion from isoflavidinin (**1i**). The latter adsorbed on a Si gel surface, when kept exposed to air, is slowly converted (less than 5% in 10 days) to the former. Similarly, isoflavidinin acetate (**1j**) on treatment with *m*-chloroperbenzoic acid in methylene chloride at room temperature is partially converted to iso-oxoflavidinin acetate (**1m**) in greater yield.

Like **1(a–c)**, **1e** and **1g**, isoflavidinin and iso-oxoflavidinin are optically inactive. This may be explained by the fact that the energy barrier between the two possible conformers of each compound formed by flipping of rings B and D is quite low (as indicated by Dreiding models), and that for each compound the flipped conformer is the optical antipode of the other. Thus at room temperature rapid interconversion of one conformer into the other renders each compound optically inactive. This view is in conformity with the appearance of the oxymethylene protons of isoflavidinin as a singlet instead of an AB quartet.

Isoflavidinin (**1i**) and iso-oxoflavidinin (**1l**) are thus two new additions to the growing list of naturally occurring modified 9, 10-dihydrophenanthrenes bearing an oxymethylene bridge or a lactone moiety flanked between C-4 and C-5.

EXPERIMENTAL

Mps are uncorr. Si gel (60–100 mesh) was used for CC and Si 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 60 MHz in $CDCl_3$ using TMS as int. standard and chemical shifts are expressed as δ values. ^{13}C NMR spectra were run in the same solvent with the same int. standard. MS were recorded with a direct inlet system operating at 70 eV, 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 mass spectrometry. Na_2SO_4 was used for drying organic solvents and the petrol used had bp 60–80°.

Isolation of isoflavidinin (**1i**) *and iso-oxoflavidinin* (**1l**). Air-dried, powdered whole plant of *P. articulata* Lindl. (1 kg) was extracted with EtOH in a Soxhlet for 40 hr. The EtOH extract was concd under red. pres., diluted with H_2O and exhaustively extracted with Et_2O. The total Et_2O-soluble material was extracted with 2 N aq. NaOH soln. The aq. alkaline soln was acidified in the cold with conc. HCl and the liberated solids extracted with Et_2O, washed, dried and the solvent removed. The residue was chromatographed. The petrol–EtOAc (10:1) eluate gave isoflavidinin (0.1 g) crystallized from petrol–EtOAc, mp 120°. (Found: C, 75.48; H, 5.42. $C_{16}H_{14}O_3$ requires C, 75.59; H, 5.51%.) MS *m/z* 254 ($M^{+\cdot}$, 100), 253 (48), 252 (4), 240 (4), 239 (20), 181 (5.4), 165 (3.6), 152 (4.2) and 127 (18). Isoflavidinin acetate (**1j**) (prepared by treatment of **1i** with Ac_2O–C_6H_5N in the cold) crystallized from petrol–EtOAc, mp 131°. (Found: C, 72.85; H, 5.36. $C_{18}H_{16}O_4$ requires: C, 72.97; H, 5.41%.) UV λ_{max} nm: 216, 284–285 and 304 (log ϵ 4.52, 4.16 and 4.16); IR ν_{max} cm^{-1}: 1250, 1274 and 1762 (OAc); ^{1}H NMR: δ 2.28 (3H, *s*, $OCOCH_3$), 2.89 (4H, *s*, H_2-9 and H_2-10), 3.76 (3H, *s*, Ar–OCH_3), 5.1 (2H, *s*, Ar–O–CH_2–Ar), 6.38 (2H, *br s*, H-6 and H-8), 6.70 (1H, *d*, J = 3 Hz; H-1) and 6.84 (1H, *d*, J = 3 Hz; H-3); MS *m/z* 268 ($M^{+\cdot}$, 100), 267 (22.6), 254 (10.3), 253 (54.9), 239 (5.6), 165 (4.7), 153 (4.0), 152 (4.3). Isoflavidinin methyl ether (**1k**) (prepared by treatment of a methanolic soln of **1i** with CH_2N_2–Et_2O) crystallized from petrol–EtOAc, mp 138°. (Found: C, 76.25; H, 5.89. $C_{17}H_{16}O_3$ requires: C, 76.12; H, 5.97%.) UV λ_{max} nm: 215, 286 and 303 (log ϵ 4.50, 4.21 and 4.15); ^{1}H NMR: 2.85 (4H, *s*, H_2-9 and H_2-10), 3.76 (6H, *s*, $ArOCH_3$), 5.06 (2H, *s*, Ar–O–CH_2–Ar), 6.35 (2H, *s*, H-6 and H-8), 6.45 (1H, *d*, J = 3 Hz; H-1) and 6.63 (1H, *d*, J = 3 Hz; H-3).

Further elution of the chromatogram with petrol–EtOAc (5:1) gave in the early fractions iso-oxoflavidinin (**1l**) (0.03 g) crystallized from petrol–EtOAc, mp 259°. (Found: C, 71.52; H, 4.39. $C_{16}H_{12}O_4$ requires: C, 71.64; H, 4.48%.) MS *m/z* 268 ($M^{+\cdot}$, 100), 267 (10), 254 (4), 253 (31.8), 225 (6.2), 197 (5.1), 168 (8.4), 151 (5.2), 149 (4.5), 139 (8.2) and 115 (6.5). Iso-oxoflavidinin acetate (**1m**) crystallized from petrol–EtOAc, mp 180°. (Found: C, 69.59; H, 4.45. Calc. for $C_{18}H_{14}O_5$: C, 69.68; H, 4.52%.) IR ν_{max} cm^{-1}: 1295, 1762 (OAc) and 1726 (lactone $\diagdown$C=O); ^{1}H NMR: δ 2.32 (3H, *s*, $OCOCH_3$), 3.08 (4H, *s*, H_2-9 and H_2-10), 3.92 (3H, *s*, Ar–OCH_3), 6.68 (2H, *br s*, H-6 and H-8), 7.30 (1H, *d*, J = 3 Hz; H-1) and 7.82 (1H, *d*, J = 3 Hz; H-3); MS *m/z* 310 ($M^{+\cdot}$, 17.4), 269 (17.7), 268 (100), 267 (11.9), 264 (6), 253 (19), 168 (5.2), 152 (4.1) and 139 (7.6).

The latter fractions of the petrol–EtOAc (5:1) eluate gave flavidin (**1c**) (0.2 g), mp 210°.

The same isolation procedure was employed with *O. porecta* Lindl. and *O. fusca* Lindl., the former giving **1i** (yield 0.008%), **1l** (yield 0.002%) and **1c** (yield 0.02%) and the latter only **1i** (yield 0.009%) and **1c** (yield 0.02%).

Conversion of isoflavidinin (**1i**) *to iso-oxoflavidinin* (**1l**) *and of isoflavidinin acetate* (**1j**) *to iso-oxoflavidinin acetate* (**1m**). Isoflavidinin (20 mg) in $CHCl_3$ was adsorbed on Si gel (5 g) and the material kept exposed to air in a column for 10 days. The total material was then eluted with petrol–EtOAc (3:1). Evapn of solvent gave a residue which on TLC showed an additional I_2-staining spot having an R_f value the same as that of **1l**. Chromatography of this residue gave unchanged **1i** (0.017 g) and only *ca* 1 mg of **1l**.

A soln of **1j** (20 mg) in dry CH_2Cl_2 (3 ml) was treated with *m*-chloroperbenzoic acid (20 mg) in CH_2Cl_2 (3 ml). The mixture was kept 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 (20:1) eluate gave unchanged **1j** (0.014 g). Further elution of the column with petrol–EtOAc (15:1) gave **1m** (0.003 g).

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