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STRUCTURE OF FLAVANTHRIN, THE FIRST DIMERIC 9,10-DIHYDROPHENANTHRENE DERIVATIVE FROM THE ORCHID ERIA FLAVA

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Abstract - Flavanthrin, the first dimeric 9,10-dihydrophenanthrene derivative, was isolated from the orchid Eria flava which also yielded the previously reported 9,10-dihydrophenanthrene coelonin (2a). The structure of flavanthrin was established as the 1,1'-dimer (1a) of its congener coelonin from spectral and chemical evidence.

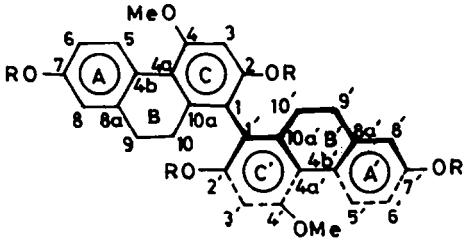
As part of our general programme of research on the chemical constituents of Indian orchids we reported earlier[1,2a,2c-f,3-7] the isolation of a number of compounds from a series of such orchids. These compounds represent several structural types, viz., bibenzyls[1], phenanthrenes[2], phenanthropyrans[3a] and pyrones,[3b] 9,10-dihydrophenanthrenes[4], 9,10-dihydrophenanthropyrans[5a-e,5g] and pyrones,[5a-c,5f] triterpenoids[6] and steroids[7]. Our continued search for new phytochemicals from the same source has resulted in the isolation, for the first time, of a dimeric 9,10-dihydrophenanthrene derivative, designated as flavanthrin, from the orchid Eria flava which also yielded the previously reported 9,10-dihydrophenanthrene coelonin[4a] (2a). The structure of flavanthrin was established as 1a from the following spectral and chemical evidence.

The molecular formula of flavanthrin, m.p.285°, was established as $C_{30}H_{26}O_6$ from elemental analysis and from its mass spectrometrically derived molecular weight of 482. That flavanthrin is built-up of two monomeric halves of the same elemental composition $C_{15}H_{13}O_3$ was indicated by the appearance of an intense doubly charged molecular ion at m/z 241 in its mass spectrum. The compound showed UV absorptions, λ_{max} 217 and 281 nm (log ε 4.66 and 4.50), which strikingly resemble those of 9,10-dihydrophenanthrene derivatives[4,5a-e,10]. The phenolic nature of flavanthrin was indicated by characteristic colour reactions, alkali-induced bathochromic shift of its UV maxima [$\lambda_{max}^{EtOH-0.1N\ NaOH}$ 222 and 304 nm (log ε 4.54 and 4.53)] and by its IR spectrum showing bands at 3440 cm^{-1}. The presence of four such phenolic hydroxyl groups in flavanthrin was confirmed by the formation of a tetraacetyl derivative, $C_{38}H_{34}O_{10}$ ($M^{+\cdot}$ 650), m.p.180°, with Ac_2O and pyridine.

The ^1H NMR spectrum of flavanthrin showed nine set of signals at δ 2.36, 2.50, 3.92, 6.59, 6.66, 6.67, 7.54, 8.07 and 8.30 in an integral ratio of 2:2:3:1:1:1:1:1:1. Each of these signals when considered in the light of the

molecular formula of flavanthrin corresponded to exactly double the number of protons given by their respective integral ratio. This, in turn, also suggested a symmetrical dimeric structure for flavanthrin. Thus, the two two-proton singlets at δ 7.54 and 8.30 (each disappeared on deuterium exchange) in the ^{1}H NMR spectrum of flavanthrin indicated the presence of four phenolic hydroxyl groups in the compound, while the six-proton singlet at δ 3.92 corresponded to two aromatic methoxyl functions in identical environment. The spectrum of flavanthrin showed four different set of aromatic proton signals, of which the two-proton ortho-coupled doublet at δ 8.07 (J = 8.3 Hz) is typical of H-5 or H-4 of a 9,10-dihydrophenanthrene derivative[4,11]. The assignment of this signal to H-5 and H-5' of flavanthrin would require its C-6 and C-6' to be unsubstituted and C-7 and C-7' to contain an identical substituent. The ill-resolved ortho-meta coupled signal at δ 6.67 would then correspond to H-6 and H-6' of flavanthrin, and the splitting pattern of this signal also indicated the unsubstituted nature of its C-8 and C-8'. Accordingly, H-8 and H-8' of flavanthrin appeared at δ 6.66 as an ill-resolved meta-coupled doublet. That the substituent at C-7 and C-7' of flavanthrin is a hydroxyl group was borne out by the fact that the signals at δ 6.67 and 6.66 of flavanthrin were shifted downfield by 0.3 and 0.27 ppm in the ^{1}H NMR spectrum of its tetraacetyl derivative. The striking similarities of the chemical shifts and splitting patterns of the above aromatic protons of flavanthrin and its tetraacetate with the corresponding protons of coelonin[4a] (2a) and its diacetate[4a] (2b) not only established the structural identity of rings A and A' of flavanthrin with ring A of coelonin, but also ruled out the possibility of any linkage of the two monomeric halves through any carbon atom of this part of their molecules. The absence of any downfield proton signal around δ 8.0 other than that for H-5 and H-5' (δ 8.07) implied the substituted nature of C-4 and C-4' of flavanthrin. In the light of the observation[8,11] that H-5 of a 4-hydroxy-9,10-dihydrophenanthrene shows an upfield shift in the ^{1}H NMR spectrum of its acetyl derivative, the chemical shift of H-5 and H-5' of flavanthrin tetraacetate which, instead, showed a downfield shift (δ 8.65) ruled out the placement of a hydroxyl group at C-4 and C-4' of flavanthrin. The remaining aromatic proton signal of flavanthrin at δ 6.59 appeared essentially at the same position as the H-3 of coelonin (2a). This permits the assignment of this signal to H-3 and H-3' of flavanthrin with methoxyl group at C-4 and C-4' and hydroxyl group at C-2 and C-2' of the compound. The downfield shift of this signal by 0.14 ppm in the ^{1}H NMR spectrum of flavanthrin tetraacetate also suggests that these protons bear an ortho-hydroxyl group. The appearance of this signal as a sharp singlet compared to H-3 of coelonin as a meta-coupled doublet (δ 6.38, J = 2.3 Hz) leaves C-1 and C-1' as the only possible site for dimerisation in flavanthrin. The most convincing evidence for 1,1'-coupling in flavanthrin was provided by the non-equivalence of the hydrogen atoms at C-9, C-10 and C-9', C-10' of both flavanthrin and its tetraacetate, and the relatively upfield shift of two of the four acetate methyl functions in flavanthrin tetraacetate. The 9- and 10-methylene protons in all monomeric 9,10-dihydrodrophenanthrene derivatives have been found to appear as a sharp singlet for four equivalent protons as in coelonin[4a] (δ 2.63) and its diacetate (δ 2.69), while in the ^{1}H NMR spectra of flavanthrin and its tetraacetate these protons resonated as two four-proton ill-resolved triplets at δ 2.36 and 2.50 (flavanthrin) and 2.43 and 2.58 (flavanthrin tetraacetate). Further, the methyl protons of two of the four acetoxy functions in flavanthrin tetraacetate resonated at a relatively upfield

position (δ 1.92) compared to the other two acetate methyls which appeared at the normal position (δ 2.29) as in coelonin diacetate (δ 2.23). These observations can only be rationalised in terms of a 1,1'-coupling of two coelonin units in flavanthrin as in 1a and a similar formulation (1b) for its tetraacetate. Construction of Dreiding models of 1a and 1b shows that in the most preferred conformation of the two molecules the two monomeric halves remain perpendicular to each other so that 10-, 10'-methylene protons fall in the shielding zone of the neighbouring aromatic rings C' and C respectively and this accounts for the observed upfield shift of these protons (δ 2.36 in 1a and 2.43 in 1b). The rings C' and C also exert a long range shielding effect on the 9- and 9'-methylene protons which appeared at slightly upfield positions in flavanthrin (δ 2.50) and its tetraacetate (δ 2.58) compared to the corresponding protons of coelonin and its diacetate. The two acetate methyls at C-2 and C-2' in flavanthrin tetraacetate likewise fall in the shielding zone of rings C' and C respectively, and as a result they are shifted upfield compared to the other two acetate methyls. This type of diamagnetic anisotropic effect of the neighbouring aromatic rings on the methyl, acetoxyl and methoxyl groups attached to the carbon atoms ortho to the site of coupling is a well-documented feature in the bianthraquinone derivatives[9,10].

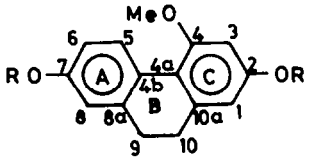


1a : R = H

1b: R = Ac

1c: R = Ac, 9,10,9',10'-Dehydro

1d: R = H, 9,10,9',10'-Dehydro



2a: R = H

2b: R = Ac

The structure 1a for flavanthrin was further corroborated by the ^{13}C NMR spectral data of the compound and its tetraacetate (1b) (Table 1). The degree of protonation of each carbon atom was determined by DEPT experiments and the assignment of the carbon chemical shifts of 1a and 1b were made by comparison of the δ_c values with those of coelonin (2a) and coelonin diacetate[4a] (2b) and and other structurally related compounds[4b,5a-g].

The ^{13}C NMR spectra of both flavanthrin (1a) and its tetraacetate (1b) showed signals which corresponded to just half the number of carbon atoms present in their respective molecular formulae. This is again in conformity with their symmetrical dimeric formulation. Further, except for C-1, C-1' and C-10, and C-10', the signals for all the carbon atoms of 1a and 1b appeared essentially at the same positions as those for the corresponding carbon atoms of coelonin (2a) and its diacetate (2b), respectively. The signal for the protonated C-1 of 2a (δ_c 108.04) and that of 2b (δ_c 113.47) are replaced by the relatively downfield non-protonated carbon signals at δ_c 113.15 and 121.09, in the spectra of 1a and 1b, respectively. This is another convincing evidence that flavanthrin is a symmetrical dimer of its congener coelonin (2a) linked at its C-1 position. The upfield shift of C-10 and C-10' of 1a and 1b by ~3-4 ppm compared to their

C-9 and C-9', which finds analogy with similar upfield shifts of C-10 in 1-substituted 9,10-dihydrophenanthrene derivatives like coelogin[5a] and coeloginin[5a] provides further evidence for the 1,1'-dimeric formulation 1a for flavanthrin.

Table 1. ^{13}C NMR spectral data of flavanthrin (1a), flavanthrin tetraacetate (1b), coelonin (2a) and coelonin diacetate (2b)

Carbon atoms	δ_c(ppm)		Carbon atoms	δ_c(ppm)	
	1a[a]	1b[b]		2a[a]	2b[b]†
C-1,C-1'	113.15	121.09	C-1	108.04	113.47
C-2,C-2'	155.27[c]	149.16[f]	C-2	155.77[i]	149.90[m]
C-3,C-3'	98.76	104.49	C-3	99.05	103.96
C-4,C-4'	157.96	156.91	C-4	158.50	157.56
C-4a,C-4a'	116.77	119.74	C-4a	116.11	120.67
C-4b,C-4b'	125.78	130.01	C-4b	125.47	129.88
C-5,C-5'	129.80	129.65	C-5	129.55	129.43
C-6,C-6'	114.44[d]	120.0[g]	C-6	114.74[j]	120.25[n]
C-7,C-7'	155.84[c]	148.50[f]	C-7	157.06[i]	148.96[m]
C-8,C-8'	114.24[d]	118.74[g]	C-8	113.28[j]	118.91[n]
C-8a,C-8a'	140.88[e]	141.36[h]	C-8a	141.01[k]	141.12[o]
C-9,C-9'	30.04	29.37	C-9	30.51[l]	29.46[p]
C-10,C-10'	27.62	26.72	C-10	31.10[l]	30.08[p]
C-10a,C-10a'	139.89[e]	140.22[h]	C-10a	139.69[k]	139.78[o]
OMe	55.42	55.62	OMe	55.48	55.69
$OCOCH_3$	-	20.47	$OCOCH_3$	-	21.17
		21.03			169.47
		168.95			169.57
		169.36			

[a]Spectra were run in d_6-acetone and chemical shifts were measured with $\delta_{(TMS)} = \delta_{(d_6\text{-acetone})} + 29.6$ ppm.

[b]Spectra were run in $CDCl_3$ and the chemical shifts were measured with $\delta_{(TMS)} = \delta_{CDCl_3} + 76.9$ ppm.

[c-p]Values are interchangeable.

† In our earlier paper[4a] some of the nonprotonated carbon resonances were confused with noise signals, as the spectrum was run in a low resolution instrument with poor sample size. These have since been revised here by fresh measurement of the spectra of both 2a and 2b.

The structure 1a for flavanthrin was finally confirmed by the conversion of its tetraacetate (1b) to the corresponding phenanthrene derivative, $C_{38}H_{30}O_{10}$ ($M^{+\cdot}$ 646), m.p.282° by DDQ[11]. The latter from its various spectral data was shown to have the structure 1c and was identical in all respects with the tetraacetyl derivative of cirrhopetalanthrin[12] (1d), the first dimeric phenanthrene derivative isolated in our laboratory in the recent past from a taxonomically related orchid Cirrhopetalum maculosum. The structure of 1d has been established from an extensive 2D NMR correlation studies of its tetraacetate (1c), the details of which have been described in a separate communication[12]. The above conversion of 1b to 1c served as complementary evidence for the confirmation of the structures of both the compounds.

Flavanthrin (1a) is thus the first dimeric 9,10-dihydrophenanthrene derivative and its co-occurrence with coelonin (2a) in the same orchid Eria flava provides a strong circumstantial evidence for the role of oxidative coupling[13,14,15] in the biogenesis of naturally occurring phenolic dimers from the corresponding monomeric phenols, although it is difficult to predict at which stage of biogenesis the dimerisation has taken place.

EXPERIMENTAL

M.ps were determined in a Kofler Block and were uncorrected. UV spectra were measured in 95% aldehyde-free EtOH and IR spectra were run in KBr discs. ^{1}H NMR spectra were recorded in a Bruker 300 MHz supercon instrument in d_6-acetone (1a and 2a) and in $CDCl_3$ (1b and 2b) using TMS as internal standard. ^{13}C NMR were run at 75 MHz in the same instrument in the same solvents and with the same internal standard. Chemical shifts were measured in δ ppm. Mass spectra were recorded at 70 eV and the figures in the first bracket attached to m/z values represent relative intensities of peaks. Silica gel (100-200 mesh) was used for chromatography 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. Anhyd. Na_2SO_4 was used for drying organic solvents and petrol used had b.p. 60-80°.

Isolation of flavanthrin (1a) and coelonin (2a). Air-dried powdered whole plant of Eria flava (2 kg) was soaked in MeOH for 3 weeks. The methanolic extract was then drained out and concentrated under reduced pressure to 100 ml, diluted with water (750 ml) and exhaustively extracted with Et_2O. The ether layer was then extracted with 2N aqueous NaOH solution. The aqueous alkaline solution was then acidified with conc. HCl in the cold and the liberated solid was extracted with Et_2O, washed with water, dried and the solvent removed. The residue was chromatographed. The petrol-EtOAc (15:1) eluate gave coelonin (2a) (0.1 g), crystallised from petrol-EtOAc mixture, m.p.84°; Diacetate, m.p.130°.

Further elution of the column with petrol-EtOAc (2:1) afforded a gummy residue consisting mainly of flavanthrin (1a), which on repeated chromatography finally gave pure flavanthrin (0.3 g) in the petrol-EtOAc (5:1) eluate, crystallised from petrol-EtOAc mixture, m.p.285°, produced blue colouration with phosphomolybdic acid reagent. (Found : C, 74.61; H, 5.45. $C_{30}H_{26}O_6$ requires : C, 74.69; H, 5.39%) ν_{max} (cm^{-1}) : 3440 (OH), 1600, 1580 and 820 (aromatic nucleus); MS : m/z 482 (M^+·, 100), 272 (9), 248 (16), 241 (M^{++}/2, 26), 225 (7), 220(6), 211(7), 197(10), 181(5), 169(5), 157(4), 115(4) and 75(5).

Flavanthrin (1a) (0.05 g) was acetylated with Ac_2O and pyridine in the usual manner and the crude acetyl derivative was chromatographed. The petrol-EtOAc (7:1) eluate gave flavanthrin tetraacetate (1b) (0.045 g), crystallised from petrol-EtOAc mixture, m.p.180° (Found : C, 69.99; H, 5.28. $C_{38}H_{34}O_{10}$ requires : C, 70.15; H, 5.23%). λ_{max} nm : 217, 243, 273, 294 and 307 (log ε 4.76, 4.44, 4.57, 4.36 and 4.20); ν_{max} (cm^{-1}) : 1288 and 1760 (OAc), 1592, 1570, 890 and 822 (aromatic nucleus); ^{1}H NMR : δ8.28 (2H, d, J = 8.6 Hz; H-5 and H-5'), 6.97 (2H, dd, J_1 = 8.6 Hz, J_2 = 2.4 Hz; H-6 and H-6'), 6.92 (2H, d, J = 2.4 Hz; H-8 and H-8'), 6.73 (2H, s; H-3 and H-3'), 3.91 (6H, s; 2x ArOMe), 2.58 (4H, ill-resolved triplet, J = 5.6 Hz; H_2-9 and H_2-9'), 2.42 (4H, ill-resolved triplet, J = 5.6 Hz; H_2-10 and H_2-10'), 2.29 (6H, s; OAc at C-7 and C-7') and 1.92 (6H,s; OAc at C-2 and C-2').

Oxidation of flavanthrin tetraacetate (1b) to cirrhopetalanthrin tetraacetate (1c) by DDQ. A solution of 1b (0.04 g) in dry benzene (5 ml) was heated under reflux with DDQ (0.08 g) for 18 hr. Benzene was then removed under reduced pressure and residue was taken in Et_2O (50 ml). The Et_2O extract was repeatedly washed with 15% NaOH solution, and then washed with water till free from alkali, dried and the solvent removed. The residue was chromatographed. The petrol-EtOAc (2:1) eluate gave a solid (0.025 g), crystallised from the same solvent mixture, 282°, which was found to be identical in all respects (m.p., IR, ^{1}H and ^{13}C NMR and mass spectra) with cirrhopetalanthrin tetraacetate (1c).

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