

VOLUCRIN, A NEW DIMERIC PHENANTHRENE DERIVATIVE
FROM THE ORCHID *LUSIA VOLUCRIS*

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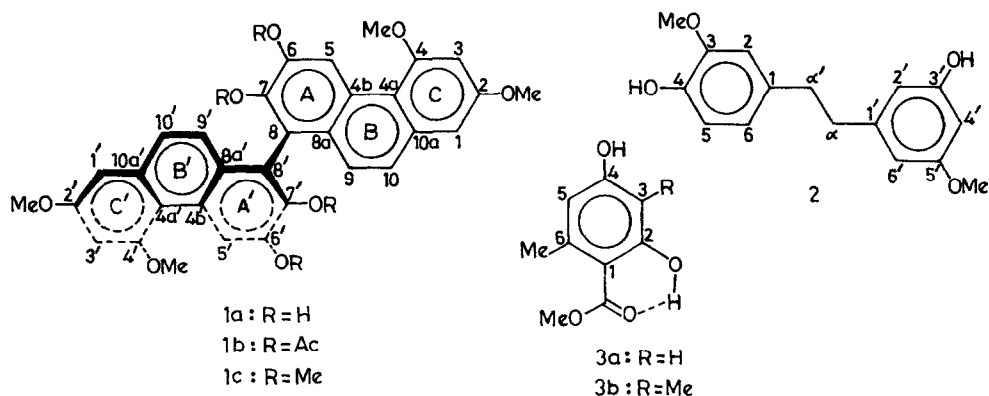
Volucrin, a new dimeric phenanthrene derivative, was isolated from the orchid *Lusia volucris* which also afforded 3',4-dihydroxy-3,5'-dimethoxy-bibenzyl (2) and the perfumery constituents methyl 2,4-dihydroxy-6-methyl benzoate (3a) and methyl 2,4-dihydroxy-3,6-dimethyl benzoate (3b). Volucrin was shown to have the structure 1a mainly from spectral evidence.

We reported^{1,2,3a-h,4-9} earlier the isolation of a fairly large number of compounds from a series of Indian orchids. These compounds represent several structural types, viz. simple benzenoid compounds,¹ bibenzyls,² phenanthrenes,³ phenanthropyrans^{4a} and pyrones,^{4b} 9,10-dihydrophenanthrenes,⁵ 9,10-dihydrophenanthropyrans^{6a-f} and pyrones,^{4b,6a-c,g} coumarins,⁷ fluorenones,⁷ triterpenoids⁸ and steroids⁹. As part of this general programme of research we report in this paper the isolation of a new dimeric phenanthrene derivative, designated as volucrin, from the orchid *Lusia volucris* which also afforded 3',4-dihydroxy-3,5'-dimethoxy-bibenzyl (2)¹⁰ and the perfumery constituents methyl 2,4-dihydroxy-6-methyl benzoate (3a),^{11a-c} and methyl 2,4-dihydroxy-3,6-dimethyl benzoate (3b)^{11a,d} of previously known structures. The structure of volucrin was established as 1a from the following evidence.

Volucrin, m.p. 280°, was found to have the molecular formula C₃₂H₂₆O₈ from elemental analysis and electron impact and chemical ionisation mass spectral measurements of the compound, its tetraacetyl and tetramethyl ether derivatives. The appearance of an intense doubly charged molecular ion peak at m/z 269 in its EI mass spectrum indicated it to be composed of two monomeric halves of the same elemental composition C₁₆H₁₃O₄. Volucrin showed UV absorptions, λ_{max} 215, 265 and 313 nm (logε 4.26, 4.83 and 4.09) which strikingly resemble those of phenanthrene derivatives.^{12a,3} The phenolic nature of the compound was indicated by its characteristic colour reactions, alkali-induced bathochromic shifts of its UV maxima [λ_{max}(EtOH-0.1M NaOH) 246 and 277 nm (logε 4.43 and 4.44)] and by its IR spectrum showing bands at 3340 and 3475 cm⁻¹. The presence of four such hydroxyl groups in volucrin was confirmed by the formation of a tetraacetate, C₄₀H₃₄O₁₂ (M⁺ 706), m.p. 192°, with Ac₂O/pyridine and a tetramethyl ether derivative, C₃₆H₃₄O₈ (M⁺ 594), m.p. 224°, with CH₂N₂.

The ^1H NMR spectrum of volucrin showed 8 sets of signals at δ 3.88, 4.14, 6.78, 6.90, 6.97, 7.27, 7.34 and 9.27 in an integral ratio of 3:3:1:1:1:1:2:1. Since the compound contains a total number of 26 protons, each of these signals corresponds to double the number of protons given by their respective integral ratio. This, in turn, also indicates it to be a symmetrical dimer. Thus, the singlets at δ 3.88 and 4.14 correspond to four methoxyl groups, while that at δ 7.34 (disappeared on deuterium exchange) confirms the presence of an equal number of phenolic hydroxyl functions in the compound. The most downfield signal at δ 9.27(2H,s) is typical^{12b,3a,d-i} of H-5 or H-4 of a phenanthrene derivative. The assignment of this signal to H-5 and H-5' of volucrin implies that H-4 and H-4' of the compound must be substituted, and the appearance of this signal as a sharp singlet further indicates that C-6, C-6' and C-7, C-7' of volucrin are also substituted. The pair of doublets at δ 6.90(J=2.7Hz) and 6.78(J=2.7Hz), each representing two equivalent *meta*-coupled aromatic protons, may, therefore, be assigned to H-1, H-1' and H-3, H-3' requiring C-2 and C-2' of the compound to be also substituted. In the ^1H NMR spectrum of volucrin tetraacetate (**1b**) all the aromatic proton resonances of volucrin remain practically unchanged except that for H-5 and H-5', which is shifted downfield by 0.34 ppm. This implies that except H-5 and H-5' no other aromatic proton of volucrin is *ortho* to a hydroxyl group. Moreover, the downfield shift of the signal for H-5 and H-5' of **1b** rules out the placement of hydroxyl group at C-4 and C-4' of volucrin in view of the fact that the H-5 resonance of a 4-hydroxyphenanthrene derivative is, instead, shifted upfield by $\sim 0.5 - 0.6$ ppm on acetylation.^{12,3h} The remaining pair of the doublet signals of volucrin at δ 6.97(J=9Hz) and 7.27(J=9Hz), each representing two equivalent *ortho*-coupled aromatic protons, are assigned to H-9, H-9' and H-10, H-10' respectively, although these signals, particularly the one at δ 6.97, showed considerable upfield shifts compared to those for H-9 and H-10 ($\sim \delta$ 7.50) of monomeric phenanthrene derivatives.^{12,3} Such upfield shifts of the signals for H-9, H-9' and H-10, H-10', however, speak strongly in support of a 8,8'-coupling of the two monomeric halves in volucrin. Construction of the Dreiding model of **1a** shows that in its most preferred conformation the two monomeric halves remain perpendicular to each other so that H-9 and H-9' fall in the shielding zones of the neighbouring aromatic rings A' and A respectively, and this accounts for the observed upfield shift of the signal for the above protons. H-10 and H-10' of **1a** being at larger distance than H-9 and H-9' from the rings A' and A experience only a long range shielding effects of these rings, and so resonate at a relatively lower field than their 9 and 9' counterparts. This type of differential shieldings of H-9, H-9' and H-10, H-10' by the neighbouring aromatic rings find strong analogy in cirrhoptetalantrin,^{3g} the 1,1'-dimer of 2, 7-dihydroxy-4-methoxy-phenanthrene (recently isolated from the orchid *Cirrhopetalum maculosum* in our laboratory), in which H-10, H-10' are more shielded than H-9, H-9' by the aromatic rings C' and C. The 8,8'-coupling in volucrin with two of the four hydroxyl groups at C-7 and C-7' was further confirmed by the proton chemical shifts of the acetoxyl and methoxyl groups of its tetraacetate (**1b**) and those of the methoxyl functions of its tetramethyl ether

derivative (1c). Thus, two of the four acetate methyls of 1b resonated at δ 1.91 which is significantly upfield, while those of its other two acetoxy groups (δ 2.36) and all the four methoxyl groups (δ 4.12 and 3.91) appeared at the normal positions. The spectrum of 1c, likewise, showed signals for two of its methoxyl groups shifted to an upfield position (δ 3.65) compared to those of its remaining six methoxyl groups which appeared almost at the normal positions (δ 4.16, 4.15 and 3.95). The upfield shifts of the signal for the two acetoxy groups of 1b and that for the two methoxyl groups of 1c are intelligible only in terms of the placement of these groups at C-7 and C-7' in the respective compounds with the site of dimerisation at the 8,8'-position. When placed at the above positions these groups fall in the shielding zones of the neighbouring aromatic rings A' and A of the respective compounds, and so resonate at the observed upfield positions. This type of diamagnetic anisotropic effect of the neighbouring aromatic rings on the acetoxy and methoxyl groups attached to the carbon atoms *ortho* to the site of dimerisation



is a well-documented feature in bianthraquinone derivatives,¹³ and has also been observed in cirrhopetalanthrin tetraacetate^{3g} and its 9,9',10,10'-tetrahydro derivative (flavanthrin tetraacetate^{5c}). The ¹H NMR spectral data of 1a and 1b are also in conformity with those of their respective monomers¹⁴ 6,7-dihydroxy-2,4-dimethoxy-phenanthrene and its diacetate, if they are assumed to have undergone 8,8'-coupling.

More convincing evidence in support of the structure 1a for volucrin was provided by the ¹³C NMR spectral data of its more soluble tetraacetyl (1b) and tetramethyl ether (1c) derivatives (Table 1.). The degree of protonation of each carbon atom was determined by DEPT experiments and the assignments of the carbon chemical shifts were made by comparison with the δ_c values of structurally related compounds^{3g,h,5c} taking into consideration the additive parameters of the functional groups. Thus, the δ_c values of C-1, C-1', C-2, C-2', C-3, C-3', C-4, C-4', C-4a, C-4a', C-10a and C-10a' of both 1b and 1c correspond closely to those calculated for the ring-C carbon atoms of a phenanthrene derivative bearing methoxyl group at C-2 and C-4, and the virtual identity of

Table 1. ^{13}C NMR spectral data of volucrin tetraacetate (1b) and volucrin tetramethyl ether (1c)

Carbon atoms	δ (ppm) ^a		Carbon atoms	δ (ppm) ^a	
	1b	1c		1b	1c
C-1, C-1'	101.49	100.94	C-8, C-8'	125.79	127.01 ^d
C-2, C-2'	158.60 ^b	157.66 ^c	C-8a, C-8a'	129.91	127.59 ^d
C-3, C-3'	99.77	99.06	C-9, C-9'	125.53	124.76
C-4, C-4'	159.68 ^b	159.39 ^c	C-10, C-10'	127.67	125.77
C-4a, C-4a'	115.32	115.40	C-10a, C-10a'	135.23	135.04
C-4b, C-4b'	128.84	126.56 ^d	OMe	55.81(2xOMe)	55.20(4xOMe)
C-5, C-5'	122.15	109.35		55.31(2xOMe)	55.70, 60.25 (each 2xOMe)
C-6, C-6'	141.08	151.51	OCOMe	19.84, 20.74	-
C-7, C-7'	138.79	145.65		167.89, 169.44	-

^aValues are in ppm downfield from TMS: $\delta_{(\text{TMS})} = \delta_{(\text{CDCl}_3)} + 76.9$ ppm.

^{b-d}Values are interchangeable.

the δ_c values of the above carbon atoms of both the compounds ruled out the placement of a hydroxyl group at any carbon atom of ring C and C' of volucrin. Further, the appearance of the four methoxyl carbon signals of 1b at the normal positions (δ_c 55.81 and 55.31) indicates that each of these methoxyl groups must have at least one *ortho* hydrogen atom. In the light of our earlier observations^{3d, g} that the presence of a substituent at C-1 and C-8 in phenanthrene derivatives causes an upfield shift of the signals for C-10 and C-9 respectively by ~ 2 -4 ppm (C-9 and C-10 of phenanthrenes³ normally appear at δ_c 127.5-128.5), the observed upfield shifts of C-9, C-9' resonances of both 1b and 1c affirm the site of dimerisation to be at 8,8'-position in both the compounds, and hence also in volucrin itself. This was further corroborated by the fact that the carbon atoms of two of the four additional methoxyl groups of 1c resonated at a relatively downfield position (δ_c 60.25).^{3a, b, d-f, 6a, d} The two methoxyl groups corresponding to the above signal must, therefore, be placed at C-7 and C-7' flanked by two *ortho* substituents at C-8, C-8' in the form of monomeric units on one side and methoxyl groups at C-6, C-6' on the other. The carbon atoms of the methoxyl groups at C-6 and C-6' having *ortho* hydrogen atoms at C-5 and C-5', as expected, resonated at the normal position ($\sim \delta_c$ 55). An alternative formulation for volucrin having 6,6'-coupling with hydroxyl groups at C-7, C-7', C-8, C-8' and methoxyl groups at C-2, C-2', C-4, C-4' would have caused all the four additional methoxyl carbons of 1c to resonate at δ_c 60-61. The absence of the desired shielding effects of the 7- and 7'-methoxyl groups of 1c may be presumably due to the fact that these groups are forced out of plane by the bulky *ortho* substituents on either sides.

Volucrin (1a) is thus the third^{3g,15} naturally occurring dimeric phenanthrene derivative, and biogenetically it may be assumed to be formed by oxidative coupling¹⁶ of the corresponding phenolic monomer.

EXPERIMENTAL

M.ps are uncorrected. UV spectra were measured in 95% aldehyde-free EtOH and IR spectra in KBr discs. ¹H NMR spectra were recorded with a Bruker 300MHz Supercon instrument in d₆-acetone (1a) and in CDCl₃ (1b and 1c) using TMS as the internal standard. ¹³C NMR spectra were run at 75MHz in the same instrument in CDCl₃ with the same internal standard. Chemical shifts were measured in δ_{ppm}. Mass spectra were recorded with a direct inlet system at 70eV and the figures in the first bracket represent relative intensities of the peaks. Only peaks having rel. int. above 15% in the region of m/z 200-250, and above 20% below m/z 200 are reported. Silica gel (100-200 mesh) was used for CC and silica gel G for TLC. All analytical samples were routinely dried over P₂O₅ for 24 hr *in vacuo* and were tested for purity by TLC and MS. Dry Na₂SO₄ was used for drying organic solvents and petrol used had b.p. 60-80°.

Isolation of volucrin (1a), 2, 3a and 3b. Air-dried whole plant material of *L. volucris* (3kg) was soaked in MeOH (10l) for 3 weeks. The methanolic extract was then drained out, concentrated under reduced pressure to ~150ml, diluted with H₂O (750ml) and then exhaustively extracted with Et₂O. The Et₂O layer was extracted with 2M aq. NaOH. The aq. alkaline soln was acidified with conc. HCl in the cold and the liberated solid was extracted with Et₂O, washed with H₂O, dried and the solvent was removed. The residue was chromatographed. The early fractions of petrol-EtOAc (80:1) elute gave 3b (0.09g), crystallised from petrol-EtOAc, m.p. 139°. The later fractions of the same elute afforded 3a (0.02g), crystallised from the same solvent mixture, m.p. 138°. Washing the column with petrol-EtOAc (10:1) gave 2 as a semi-solid mass (2g). Further elution of the column with petrol-EtOAc (2:1) afforded 1a (1g), crystallised from the same solvent mixture, m.p. 280°; produced blue colouration with phosphomolybdic acid reagent (Found: C, 71.31; H, 4.85. C₂₂H₂₀O₈ requires: C, 71.37; H, 4.83%). δ_{max} (cm⁻¹): 3340 and 3475(OH), 1600, 1580, 1460, 1320, 870, 850, 820, 800 and 735 (aromatic nucleus); MS:EI m/z 538 [M⁺](8), 269(67), 253(19), 241(16), 160(20), 152(22), 151(20), 146(29), 145(23), 144(27), 143(25), 138(62), 137(43), 132(41), 131(63), 126(28), 125(27), 91(29), 73(70), 69(33), 60(100), and 59(89); CI m/z 539[M⁺+1](93).

1a was acetylated with Ac₂O and pyridine in the usual manner to give 1b, crystallised from petrol-EtOAc, m.p. 192°. (Found: C, 67.95; H, 4.83. C₂₄H₂₂O₁₂ requires: C, 67.98; H, 4.81%). λ_{max} nm 227 and 264 (log ε 4.78 and 4.95); δ_{max} (cm⁻¹): 1770 and 1250(OAc), 1615, 900 and 840(aromatic nucleus); ¹H NMR: δ 9.61(2H, s, H-5, H-5'), 7.38(2H, d, J=9Hz, H-10, H-10'), 7.07(2H, d, J=9Hz, H-9, H-9'), 6.81(2H, br. s, H-1, H-1'), 6.79(2H, br. s, H-3, H-3'), 4.12(6H, s, 2xOMe), 3.91(6H, s, 2xOMe), 2.36(6H, s, OAc at C-6 and C-6') and 1.91(6H, s, OAc at C-7 and C-7'); MS:EI m/z 664[M⁺-42](0.8), 622(4), 580(9), 538(21), 269(100), 253(20), and 241(16); CI m/z 707[M⁺+1](3).

1a in MeOH was methylated with an ethereal soln of CH₃N₂ to give 1c, crystallised from petrol-EtOAc, m.p. 224°. (Found: C, 72.69; H, 5.77. C₂₆H₂₄O₈ requires: C, 72.72; H, 5.72%). δ_{max} (cm⁻¹): 1600, 1450, 845, 830 and 745(aromatic nucleus); ¹H NMR: δ 9.37(2H, s, H-5, H-5'), 7.27(2H, d, J=9Hz, H-10, H-10'), 7.05(2H, d, J=9Hz, H-9, H-9'), 6.80(2H, d, J=2Hz, H-1, H-1'), 6.78(2H, d, J=2Hz, H-3, H-3'), 4.16(6H, s, 2xOMe), 4.15(6H, s, 2xOMe), 3.95(6H, s, 2xOMe) and 3.65(6H, s, OMe at C-7 and C-7'); MS:EI m/z 594[M⁺](100), 548(21), 297(52), 282(43), 274(61), 267(18), 253(21), 245(16), 237(16), 224(17), 208(18), 201(16), 194(20), 188(20), 173(21), 167(21), 165(24), 159(25), 158(28), 152(23), 151(27), 150(24), 145(44), 144(27), 143(24), 138(49), 132(25) and 131(22); CI m/z 595[M⁺+1](100).

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