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Electrophilic Substitution in Verdins: Deuteriation and Nitration of 2,3,7,8,12,13,17,18-Octaethylbilin-1,19(21*H*,24*H*)-dione (Octaethylbilatriene-*abc*): *X*-Ray Crystal Structure of the 5-Nitro-derivative

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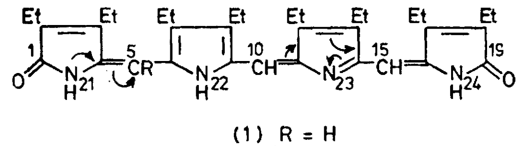
Summary Electrophilic substitution (deuteriation, nitration) of octaethylbilindione occurs preferentially at the outer bridge positions (C-5 and C-15), and there is evidence that *meso*-nitration occurs during the Gmelin reaction; *X*-ray analysis of the 5-nitro derivative shows that the C-4–C-5 bond has the *Z*-configuration, and that ring A is approximately at 90° to ring B, thus accounting for the unexpected absorption spectrum.

ALTHOUGH electrophilic substitution at carbon in macrocyclic tetrapyrroles has been quite extensively studied,[1] there are few reports[2] of such experiments in the linear tetrapyrrole series. We now report that such substitution in octaethylbilindione[3] [octaethylbilatriene-*abc* (**1**), a model system for the natural haem catabolic product, biliverdin] occurs with a striking positional selectivity.

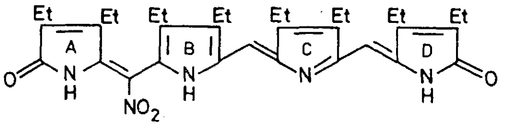


(1) R = H
(2) R = NO_2

The n.m.r. spectrum of (**1**) in CF_3CO_2H shows signals at δ 7·55 (1H, 10-H) and 6·54 (2H, 5- and 15-H). In CF_3CO_2D in the absence of oxygen a solution (0·07 M) of (**1**) presents a similar spectrum, but whereas the signal at δ 7·55 is virtually unaffected, the signal at δ 6·54 gradually disappears ($t_{\frac{1}{2}}$ *ca.* 77 min at 100 ± 0·1 °C). The mass spectrum of the crystalline product shows a molecular ion at *m/e* 556·372 corresponding to dideuterio-(**1**). The reaction is reversed in CF_3CO_2H, and the product has been shown to be identical with the starting material.

In an analogous way treatment of (**1**) with sodium nitrite and acetic acid in aqueous tetrahydrofuran at 0 °C gave 5-nitro-octaethylbilindione (**2**, but see below), as bright red prisms, m.p. 193—194 °C, which analysed (elemental, m.s.) for $C_{35}H_{45}N_5O_4$. The evidence for 5-substitution here again comes from the n.m.r. spectrum, which in $CDCl_3$ shows singlets corresponding to one proton each at δ 6·80 and 5·91 [(**1**) in $CDCl_3$: δ 6·64 (s, 1H) and 5·90 (s, 2H], and also from the mass spectrum (loss of NO_2 to give $C_{35}H_{45}N_4O_2$). It appears that nitration at C-5 and C-15 is one of the reactions occurring during the Gmelin test.[4] Thus after treatment of (1) in chloroform with a trace of fuming nitric acid (the conditions of the Gmelin test)[4] the 5-nitro derivative (**2**) can be detected (t.l.c.) if the reaction is quenched at the red-purple stage.

One piece of evidence does not fit structure (**2**): the electronic absorption of the nitro derivative [λ_{max} ($CHCl_3$) 328, 510 (infl.), and 536 nm] is at shorter wavelengths than that of the parent compound [**1**, λ_{max} ($CHCl_3$) 370 and 650 nm]. An *X*-ray structure determination of the nitro derivative reveals the cause of this. The configuration at the C-4–C-5 bond is as shown in (**3**), and ring A is in a plane approximately at right angles to that in which ring B resides (Figure). Clearly this conformation, which is attributed to steric interaction between the *meso*- and β-substituents,



(3)

will lessen orbital overlap and shorten the chromophore. Such processes may have implications for the mechanism of phytochrome action; there are examples of photochemically induced geometrical isomerisation in this series,[5] and a recent view[6] of the photochemical phytochrome (red) → phytochrome (far red) change involves attack (in an undefined way) at the C-5 *meso*-bridge.

The nitro derivative (**3**) was crystallised from hexane. *Crystal data*: $C_{35}H_{45}N_5O_4$, $M = 599{\cdot}78$; triclinic, $a = 14{\cdot}507$, $b = 12{\cdot}405$, $c = 11{\cdot}855$ Å, $\alpha = 116{\cdot}59$, $\beta = 67{\cdot}96$, $\gamma = 114.22°$, $U = 1691{\cdot}2$ Å^3; $F(000) = 644$; space group $P\bar{1}$; $Z = 2$; $D_c = 1{\cdot}18$ g cm^{-3}; $D_m = 1{\cdot}16$ g cm^{-3}; $\mu(\text{Cu-}K_\alpha) = 5{\cdot}47$ cm^{-1}. The intensities of 5030 independent reflections, in the range $3 < \theta < 60°$ were measured on a Nonius CAD4 diffractometer using Cu-K_α radiation. Of these, 3357 obeyed the condition $|F_0| > 3\sigma(F_0)$ and were used in the subsequent analysis. The structure was solved by application of the automatic centrosymmetric direct-methods routine in the SHELX[7] system (40 out of the 44 non-hydrogen atoms) and developed and refined by difference syntheses and full-matrix least squares. With anisotropic temperature factors for the heavy atoms and isotropic for hydrogen, the final R value is 0·056. Although the methyl groups were treated as rigid bodies, with a common isotropic temperature factor for the hydrogen atoms, all other hydrogen atoms were determined experimentally and individually refined. In this way the potentially tautomeric imino hydrogen atoms were found to be located on atoms N-21, N-22, and N-24 in this compound.†

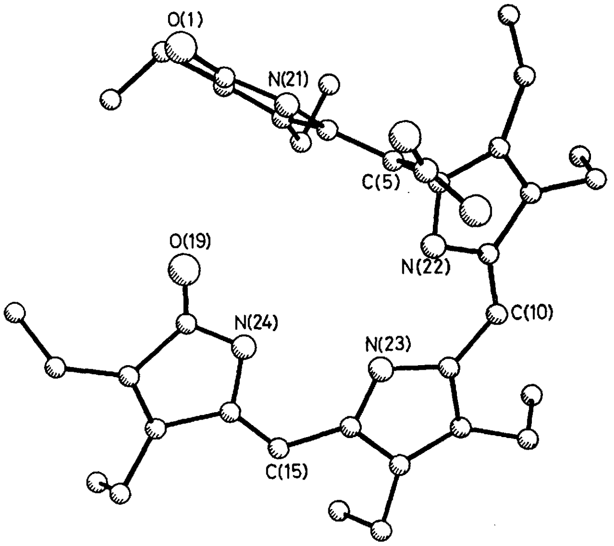


FIGURE. Molecular structure[8] of (4*Z*,10*Z*,15*Z*)-2,3,7,8,12,13,-17,18-octaethyl-5-nitrobilin-1,19(21*H*,24*H*)-dione.

The positional selectivity of these electrophilic substitutions can be rationalised in terms of π-electron densities, which are calculated[9] to be higher at C-5 and C-15 than at C-10. Alternatively the C-5 and C-15 bridges can be regarded as part of enamide systems, where, as β-positions, they are expected to be electron rich (**1**, arrows 21→5) and this effect will be accentuated in acidic media when N-23 is protonated (**1**, arrows 10→23).

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† The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.

[1] Review: H. H. Inhoffen, J. W. Buchler, and P. Jager, *Fortschr. Chem. org. Naturstoffe*, 1968, **26**, 284.

[2] *E.g.* For deuteriation (a) at C-10 of a synthetic 10,23-dihydrobilin: D. Dolphin, A. W. Johnson, J. Leng, and P. van den Broek, *J. Chem. Soc.* (*C*), 1966, 880; (b) at C-2, C-3, and C-15 of phycocyanobilin: H. L. Crespi, U. Smith, and J. J. Katz, *Biochemistry*, 1968, **7**, 2232; (c) at C-2, C-3 and a methine bridge of phycoerythrobilin: H. L. Crespi and J. J. Katz, *Phytochemistry*, 1969, **8**, 759. (There appear to be no reports of nitrobilindiones).

[3] R. Bonnett and M. J. Dimsdale, *J.C.S. Perkin I*, 1972, 2540.

[4] Gmelin test: the rubin in chloroform solution is treated with one drop of fuming nitric acid. The yellow solution becomes green (the verdin stage) and then in rapid succession blue, violet, reddish-orange, and finally pale yellow or colourless; F. Tiedemann and L. Gmelin, 'Die Verdauung nach Versuchen,' Karl Groos, Heidelberg and Leipzig, 1826, 2nd Edn., 1831, p. 80; T. With, 'Bile Pigments,' Academic Press, London and New York, 1968, p. 56.

[5] H. Falk, K. Grubmayr, U. Herzig, and O. Hofer, *Tetrahedron Letters*, 1975, 559. One of us (R. B., Biochem. Soc. Meeting, Leeds, 1976) has suggested that the photosolubilisation of bilirubin may occur by this mechanism.

[6] S. Grombein, W. Rüdiger, and H. Zimmermann, *Z. physiol. Chem.*, 1975, **356**, 1709.

[7] The SHELX System for Crystallographic Calculations, G. M. Sheldrick, University of Cambridge, 1976.

[8] PLUTO, A Molecular Drawing Program, W. D. S. Motherwell, University of Cambridge.

[9] B. Pullman and A. Pullman, 'Quantum Biochemistry,' Interscience, New York, 1963, p. 433; J.-H. Fuhrhop and J. Subramanian, *Phil. Trans. Roy. Soc.*, 1975, B**273**, 335.