

The *meso*-Reactivity of Porphyrins and Related Compounds. Part VIII.¹ Substitution and Addition Reactions of Octaethyl-21*H*,24*H*-bilin-1,19-dione, a Model Verdin System. X-Ray Analyses of Octaethyl-5-nitro-21*H*,24*H*-bilin-1,19-dione and of 4,5-Diethoxy-octaethyl-4,5-dihydro-21*H*,24*H*-bilin-1,19-dione

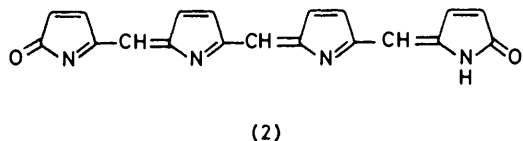
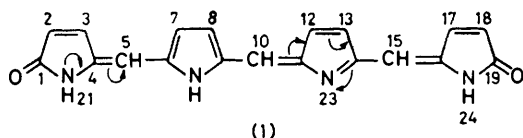
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Nitration ($\text{NaNO}_2\text{--HOAc}$) and deuteration ($\text{CF}_3\text{CO}_2\text{D}$) of octaethylbilindione (4), a model bile pigment, occur preferentially at the C(5) and C(15) positions; in contrast, nucleophilic attack (CN^- , dimethylformamide) occurs at C(10). X-Ray analysis of the 5-nitrobilindione (6) reveals that the stereochemistry of the C(4)–C(5) double bond has changed with respect to that of the starting material: because of steric interactions involving the *meso*-nitro-group, ring A is twisted out of the plane of the other three rings. The resulting steric inhibition of conjugation is attended by a pronounced hypsochromic shift.

Attempts to prepare halogenated bilindiones have been unsuccessful. However, bromine in ethanol (or methanol) produces the 4,5-dialkoxy-adducts [*e.g.* (8)]; these are believed to be formed *via* an oxidative route (attack of solvent on the radical cation). They are also formed by autoxidation of the cadmium complex of the bilindione in an alcoholic solvent. X-Ray analysis of the 4,5-diethoxy-adduct (8) confirms the general structure and reveals that the crystalline product obtained is the (4*RS*,5*SR*) diastereoisomer.

The molecular structures and hydrogen-bonding systems of compounds (6) and (8) are compared and discussed. The bearing of these results on the molecular interpretation of the photoreversibility of phytochrome is considered.

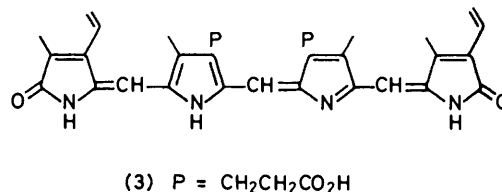
EARLIER parts of this series have been devoted to macrocyclic tetrapyrroles.¹ We now turn our attention to the 'linear' systems, and particularly to those substances of general structure (1) known variously



as verdins, bilatrienes-*abc*, and bilin-1,19-diones.† Although the didehydro-derivative of (1) can be formulated (2), it has not yet been encountered, and structure (1) therefore represents the highest oxidation level available in the bile pigment series. Biologically, structure (1) is an important system.² Thus biliverdin IX α (Fischer nomenclature) (3) occurs as an intermediate in mammalian haem catabolism. The adult human produces *ca.* 300 mg of (3) each day, but it is seldom detected in Man because it is enzymatically reduced to bilirubin with considerable efficiency. However, in some classes of animals (fish, reptiles, insects) biliverdin appears to be a terminal product of haem catabolism, and is not infrequently deposited as a pigment.

† We will use the last-named nomenclature here. See, for example, 'IUPAC-IUB Nomenclature of Tetrapyrroles, TP6 and TP7,' *Pure Appl. Chem.*, 1979, **51**, 2251.

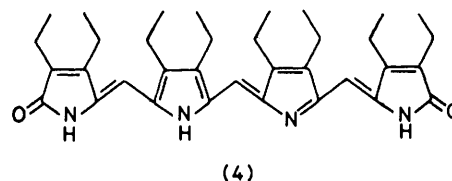
In the plant kingdom, it is the *biliproteins* which are important, and here the key metabolic processes are photochemical ones.³ The phycocyanins and phycoerythrins function as accessory photosynthetic pigments in lower plants, while in phytochrome the bilin chromophore undergoes a reversible photochemical transformation which is thought to provide a trigger for morphological changes.⁴ It has been proposed⁵ that the



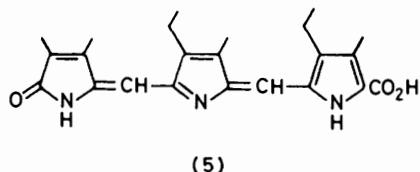
photochemical transformation involves addition at the double bond of a *meso*-bridge (see later).

In the study of the properties of the *meso*-positions in such tetrapyrroles the unsymmetrical substitution pattern of the natural compounds [*e.g.* (3)] introduces complications. As in the porphyrin series⁶ we have sought to avoid these by using the octaethyl substitution pattern: the substrate here is therefore the octaethylbilindione (4).⁷

The reactivity of the bilindione system towards



electrophilic, nucleophilic, and radical reagents is an area of basic chemistry which has been little explored. Nitration was chosen for the initial study, not only because of the wealth of related work with other hetero-aromatic systems, but also because it was considered that the electron-withdrawing effect of the nitro-group would help to stabilise the rather readily oxidised bilindione system.



Nitration. The classical Gmelin test for bile pigments involves treatment with fuming nitric acid and the observation of a play of colours.^{8,*} Prior to the present work⁹ nitro-derivatives of bilindiones had not been reported.

Treatment of the bilindione (4) with the usual nitrating reagents gave a complex mixture, presumably due to a combination of substitution and oxidation reactions as in the Gmelin reaction.⁸ However, with sodium nitrite and acetic acid in aqueous tetrahydrofuran (THF) at

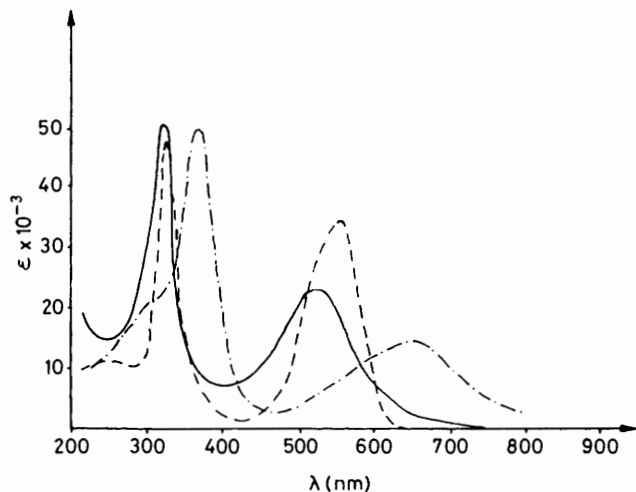


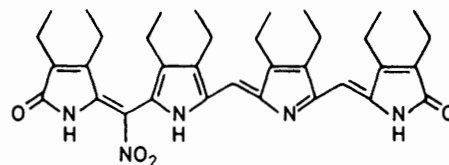
FIGURE 1 Electronic spectra of the octaethylbilindione [(4), — · — · —], its 5-nitro-derivative [(6), —], and its 4,5-diethoxy-adduct [(8), - - -] in chloroform

0 °C, a product which analysed for a mono-nitro-derivative ($C_{35}H_{45}N_5O_4$) was obtained as bright red prisms. The molecular ion appeared in the mass spectrum at m/e 599, but the base peak was at m/e 553 ($M - NO_2$) and was attended by a metastable peak at m/e 510.5. That substitution had occurred at C(5) was evident from the n.m.r. spectrum: the starting material showed two meso-signals in the ratio 1 : 2 [$\delta(CDCl_3)$ 6.64 (s, 10-H) and 5.90 (s, 5-H and 15-H)], while in the nitro-derivative the ratio

* 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.

was 1 : 1 *i.e.* one of the two protons responsible for the signal at higher field in the spectrum of (4) was now missing [$\delta(CDCl_3)$ 6.80 (s, 10-H) and 5.85 (s, 15-H)].

Curiously, the electronic spectrum of the product did not accord with the 5-nitro-bilindione formulation since the absorption had suffered a *hypsochromic* shift to λ_{max} ($CHCl_3$) 323, 506nm, and 533 nm. This absorption rather resembled that of a tripyrrinone [*e.g.* (5); λ_{max} .



($CDCl_3$) 317, 508sh, and 538 nm]¹⁰ and contrasted with that of the parent compound [(4); λ_{max} ($CHCl_3$) 370 and 650 nm] as shown in Figure 1.

The explanation for this anomaly was provided by an X-ray analysis of the 5-nitro-bilindione derivative, which showed that it possessed structure (6). The molecular structure is shown in Figure 2, and geometric parameters in Table 1. Pyrrole rings B, C, and D are approximately co-planar, although there is a significant helical deviation from planarity (Table 2). The geometry of possible N-H ··· N hydrogen bonds is shown in Table 3. Ring A,

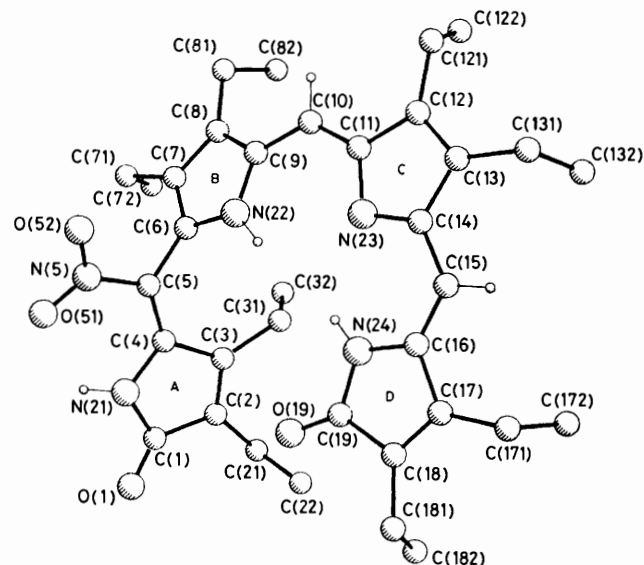


FIGURE 2 Molecular structure and crystallographic atom numbering scheme for the (4Z,10Z,15Z)-octaethyl-5-nitro-bilindione (6)

while itself planar, is tilted at 117° to the mean plane of the other three pyrrole rings (Table 2). The dihedral angle between the plane of the nitro-group and the plane comprising C(5) and ring A is 15.7°. The observed conformation is stabilised by an intramolecular hydrogen bond between one oxygen of the nitro-group [O(51)] and N(21)-H (Table 3). Clearly, this arrangement, which

arises because of steric interactions involving the bulky *meso*-substituent, effectively prevents conjugation through the four rings, and a three-ring (tripyrinone) chromophore involving rings B, C, and D remains.

The geometry of the *meso*-bridges is of particular interest. Firstly there is marked bond localisation: the C(4)–C(5) (1.354 Å), C(10)–C(11) (1.357 Å), and C(15)–C(16) (1.351 Å) bonds have considerable double-bond character compared with the corresponding C(5)–C(6), C(9)–C(10), and C(14)–C(15) bonds (1.465, 1.422, and 1.435 Å respectively). Not surprisingly, the distinction is greatest at the geometrically deconjugated C(5) bridge. For the C(10) and C(15) bridges the *Z*-synperiplanar geometry is found. This geometry has been observed

TABLE 1

Molecular geometry of the octaethyl-5-nitrobilin-dione (6) and the diethoxy-adduct (8)

(a) Bond lengths (Å)

| | (6) | (8) |
|---------------|-----------|-----------|
| C(1)–C(2) | 1.484(7) | 1.483(7) |
| C(1)–O(1) | 1.211(4) | 1.220(6) |
| C(1)–N(21) | 1.380(6) | 1.362(6) |
| C(2)–C(3) | 1.333(5) | 1.323(6) |
| C(3)–C(4) | 1.497(6) | 1.521(6) |
| C(4)–C(5) | 1.354(5) | 1.527(6) |
| C(4)–N(21) | 1.386(4) | 1.443(5) |
| C(5)–C(6) | 1.465(3) | 1.494(6) |
| C(6)–C(7) | 1.393(4) | 1.389(6) |
| C(6)–N(22) | 1.363(3) | 1.361(5) |
| C(7)–C(8) | 1.404(3) | 1.404(5) |
| C(8)–C(9) | 1.388(3) | 1.404(5) |
| C(9)–C(10) | 1.422(3) | 1.423(5) |
| C(9)–N(22) | 1.379(3) | 1.360(5) |
| C(10)–C(11) | 1.357(3) | 1.376(6) |
| C(11)–C(12) | 1.459(3) | 1.444(5) |
| C(11)–N(23) | 1.394(3) | 1.391(5) |
| C(12)–C(13) | 1.346(4) | 1.353(7) |
| C(13)–C(14) | 1.461(3) | 1.445(6) |
| C(14)–C(15) | 1.435(4) | 1.432(7) |
| C(14)–N(23) | 1.322(3) | 1.336(5) |
| C(15)–C(16) | 1.351(4) | 1.363(6) |
| C(16)–C(17) | 1.468(4) | 1.463(7) |
| C(16)–N(24) | 1.369(3) | 1.387(5) |
| C(17)–C(18) | 1.344(4) | 1.335(6) |
| C(18)–C(19) | 1.485(4) | 1.474(7) |
| C(19)–O(19) | 1.221(3) | 1.234(6) |
| C(19)–N(24) | 1.364(4) | 1.373(7) |
| C(4)–O(4) | — | 1.437(5) |
| O(4)–C(41) | — | 1.431(6) |
| C(41)–C(42) | — | 1.460(8) |
| C(5)–O(5) | — | 1.431(5) |
| C(5)–N(5) | 1.453(6) | — |
| O(5)–C(51) | — | 1.426(6) |
| C(51)–C(52) | — | 1.483(8) |
| N(5)–O(51) | 1.214(3) | — |
| N(5)–O(52) | 1.216(5) | — |
| C(2)–C(21) | 1.568(11) | 1.490(7) |
| C(21)–C(22) | 1.367(10) | 1.496(10) |
| C(3)–C(31) | 1.516(6) | 1.505(7) |
| C(31)–C(32) | 1.466(9) | 1.484(10) |
| C(7)–C(71) | 1.502(4) | 1.516(6) |
| C(71)–C(72) | 1.511(8) | 1.494(9) |
| C(8)–C(81) | 1.500(4) | 1.496(6) |
| C(81)–C(82) | 1.533(8) | 1.511(8) |
| C(12)–C(121) | 1.507(4) | 1.509(6) |
| C(121)–C(122) | 1.502(8) | 1.543(8) |
| C(13)–C(131) | 1.504(4) | 1.542(7) |
| C(131)–C(132) | 1.516(8) | 1.473(11) |
| C(17)–C(171) | 1.504(4) | 1.519(7) |
| C(171)–C(172) | 1.506(8) | 1.501(9) |
| C(18)–C(181) | 1.490(4) | 1.507(8) |
| C(181)–C(182) | 1.488(9) | 1.513(8) |

TABLE 1 (continued)

(b) Bond angles (°)

| | (6) | (8) |
|---------------------|----------|----------|
| O(1)–C(1)–C(2) | 129.1(5) | 128.2(4) |
| N(21)–C(1)–C(2) | 106.5(3) | 107.0(4) |
| N(21)–C(1)–O(1) | 124.3(5) | 124.8(5) |
| C(1)–C(2)–C(3) | 108.7(5) | 108.9(4) |
| C(2)–C(3)–C(4) | 108.0(4) | 109.9(4) |
| C(3)–C(4)–C(5) | 128.1(3) | 113.7(4) |
| C(3)–C(4)–N(21) | 106.2(3) | 102.5(3) |
| N(21)–C(4)–C(5) | 125.7(4) | 109.5(3) |
| C(4)–C(5)–C(6) | 127.7(4) | 116.9(3) |
| C(5)–C(6)–C(7) | 127.4(2) | 133.7(3) |
| C(5)–C(6)–N(22) | 123.8(2) | 118.4(4) |
| N(22)–C(6)–C(7) | 108.4(2) | 107.7(3) |
| C(6)–C(7)–C(8) | 107.0(2) | 107.6(3) |
| C(7)–C(8)–C(9) | 107.9(2) | 107.0(4) |
| C(8)–C(9)–C(10) | 128.8(2) | 131.8(4) |
| C(8)–C(9)–N(22) | 107.5(2) | 107.3(3) |
| N(22)–C(9)–C(10) | 123.7(2) | 120.9(4) |
| C(9)–C(10)–C(11) | 128.9(2) | 125.1(4) |
| C(10)–C(11)–C(12) | 126.1(2) | 125.5(4) |
| C(10)–C(11)–N(23) | 123.7(2) | 123.6(3) |
| N(23)–C(11)–C(12) | 110.1(2) | 110.9(3) |
| C(11)–C(12)–C(13) | 106.2(2) | 105.3(4) |
| C(12)–C(13)–C(14) | 106.1(2) | 107.4(4) |
| C(13)–C(14)–C(15) | 123.7(2) | 125.0(4) |
| C(13)–C(14)–N(23) | 112.4(2) | 111.3(4) |
| N(23)–C(14)–C(15) | 123.9(2) | 123.7(4) |
| C(14)–C(15)–C(16) | 125.3(2) | 129.0(4) |
| C(15)–C(16)–C(17) | 128.7(2) | 127.4(4) |
| C(15)–C(16)–N(24) | 124.6(2) | 126.6(4) |
| N(24)–C(16)–C(17) | 106.7(2) | 105.9(4) |
| C(16)–C(17)–C(18) | 107.8(2) | 109.3(4) |
| C(17)–C(18)–C(19) | 108.3(2) | 107.5(5) |
| C(18)–C(19)–O(19) | 129.2(3) | 127.1(5) |
| C(18)–C(19)–N(24) | 105.7(2) | 106.9(4) |
| N(24)–C(19)–O(19) | 125.1(3) | 126.0(5) |
| C(3)–C(4)–O(4) | — | 113.7(3) |
| N(21)–C(4)–O(4) | — | 110.8(3) |
| C(5)–C(4)–O(4) | — | 106.6(3) |
| C(4)–O(4)–C(41) | — | 115.0(3) |
| O(4)–C(41)–C(42) | — | 110.2(5) |
| C(4)–C(5)–O(5) | — | 105.2(3) |
| C(6)–C(5)–O(5) | — | 109.3(3) |
| C(5)–O(5)–C(51) | — | 114.4(3) |
| O(5)–C(51)–C(52) | — | 108.2(5) |
| C(4)–C(5)–N(5) | 119.9(3) | — |
| C(6)–C(5)–N(5) | 112.3(3) | — |
| C(5)–N(5)–O(51) | 120.3(4) | — |
| C(5)–N(5)–O(52) | 119.2(3) | — |
| O(51)–N(5)–O(52) | 121.5(4) | — |
| C(1)–N(21)–C(4) | 110.5(4) | 111.6(4) |
| C(6)–N(22)–C(9) | 109.3(2) | 110.4(3) |
| C(11)–N(23)–C(14) | 105.2(2) | 105.1(3) |
| C(16)–N(24)–C(19) | 111.5(2) | 110.2(4) |
| C(1)–C(2)–C(3) | 120.9(4) | 120.2(4) |
| C(21)–C(2)–C(3) | 130.2(5) | 130.8(5) |
| C(2)–C(21)–C(22) | 113.1(9) | 112.5(5) |
| C(2)–C(3)–C(31) | 125.3(5) | 127.0(4) |
| C(31)–C(3)–C(4) | 126.7(3) | 123.1(4) |
| C(3)–C(31)–C(32) | 113.5(4) | 116.4(6) |
| C(6)–C(7)–C(71) | 125.0(2) | 125.5(4) |
| C(71)–C(7)–C(8) | 128.1(2) | 126.9(4) |
| C(7)–C(71)–C(72) | 113.9(4) | 112.4(5) |
| C(7)–C(8)–C(81) | 127.1(2) | 128.2(4) |
| C(81)–C(8)–C(9) | 125.0(2) | 124.7(3) |
| C(8)–C(81)–C(82) | 121.5(3) | 113.4(4) |
| C(11)–C(12)–C(121) | 124.3(2) | 125.9(4) |
| C(121)–C(12)–C(13) | 129.4(2) | 128.8(4) |
| C(12)–C(121)–C(122) | 113.2(4) | 112.3(4) |
| C(12)–C(13)–C(131) | 128.9(2) | 127.6(4) |
| C(131)–C(13)–C(14) | 124.9(2) | 125.0(4) |
| C(13)–C(131)–C(132) | 112.2(4) | 110.5(6) |
| C(16)–C(17)–C(171) | 122.9(2) | 123.4(4) |
| C(171)–C(17)–C(18) | 129.3(2) | 127.3(5) |
| C(17)–C(171)–C(172) | 112.3(4) | 112.9(4) |
| C(17)–C(18)–C(181) | 130.0(3) | 131.3(4) |
| C(181)–C(18)–C(19) | 121.7(2) | 121.1(4) |
| C(18)–C(181)–C(182) | 112.0(4) | 110.4(5) |

TABLE 2

Least-squares planes and atomic deviations (Å) for the octaethyl-5-nitroindione (6) and the diethoxy-adduct (8). The equations of planes are expressed in the form $Px + Qy + Rz = S$

Compound (6)

Plane 1 Pyrrole rings B, c, and D + C(10) and C(15)

$$9.978x - 10.488y + 9.348z = 3.556$$

[C(6) 0.224, C(7) -0.185, C(8) -0.307, C(9) 0.016, N(22) 0.348, C(10) -0.007, C(11) 0.035, C(12) -0.039, C(13) -0.027, C(14) 0.045, N(23) 0.088, C(15) 0.038, C(16) -0.014, C(17) -0.044, C(18) -0.072, C(19) -0.054, N(24) -0.045]

R.m.s. deviation 0.139 Å

Plane 2 Pyrrole ring A

$$1.684x + 10.457y - 1.341z = 0.901$$

[C(1) -0.004, C(2) 0.012, C(3) -0.015, C(4) 0.012, N(21) -0.005]

R.m.s. deviation 0.010 Å

Plane 3 Pyrrole ring B

$$12.578x - 8.876y + 8.499z = 4.519$$

[C(6) -0.001, C(7) -0.002, C(8) 0.004, C(9) -0.004, N(22) 0.003]

R.m.s. deviation 0.003 Å

Plane 4 Pyrrole ring c

$$10.261x - 10.663y + 8.946z = 3.797$$

[C(11) -0.001, C(12) -0.001, C(13) 0.003, C(14) -0.003, N(23) 0.002]

R.m.s. deviation 0.002 Å

Plane 5 Pyrrole ring D

$$9.809x - 10.451y + 9.480z = 3.515$$

[C(16) 0.007, C(17) -0.001, C(18) -0.004, C(19) 0.009, N(24) -0.010]

R.m.s. deviation 0.007 Å

Interplanar angles (°)

| | |
|--------------|-------|
| 1 \wedge 2 | 117.0 |
| 2 \wedge 3 | 103.3 |
| 3 \wedge 4 | 16.2 |
| 4 \wedge 5 | 4.3 |

Compound (8)

Plane 1 Pyrrole rings B, c, and D + C(10) and C(15)

$$-2.275x + 13.201y + 10.180z = 6.746$$

[C(6) 0.125, C(7) -0.076, C(8) -0.123, C(9) 0.015, N(22) 0.178, C(10) -0.005, C(11) 0.000, C(12) -0.003, C(13) 0.007, C(14) 0.000, N(23) 0.011, C(15) 0.012, C(16) -0.041, C(17) 0.062, C(18) 0.042, C(19) -0.058, N(24) -0.146]

R.m.s. deviation 0.077 Å

Plane 2 Pyrrole ring A

$$-0.909x + 4.855y + 20.605z = 7.477$$

[C(1) -0.007, C(2) 0.000, C(3) 0.007, C(4) -0.010, N(21) 0.011]

R.m.s. deviation 0.008 Å

Plane 3 Pyrrole ring B

$$-1.261x + 13.826y + 7.783z = 7.417$$

[C(6) 0.002, C(7) -0.008, C(8) 0.010, C(9) -0.009, N(22) 0.004]

R.m.s. deviation 0.007 Å

Plane 4 Pyrrole ring c

$$-2.236x + 13.024y + 10.160z = 6.782$$

[C(11) -0.002, C(12) -0.002, C(13) 0.005, C(14) -0.007, N(23) 0.005]

R.m.s. deviation 0.005 Å

TABLE 2 (continued)

Plane 5 Pyrrole ring D

$$-1.933x + 13.568y + 8.511z = 6.348$$

[C(16) 0.010, C(17) 0.000, C(18) -0.009, C(19) 0.015, N(24) -0.016]

R.m.s. deviation 0.012 Å

Interplanar angles (°)

| | |
|--------------|------|
| 1 \wedge 2 | 44.5 |
| 2 \wedge 3 | 50.5 |
| 3 \wedge 4 | 7.8 |
| 4 \wedge 5 | 4.8 |

at all three bridges in biliverdin dimethyl ester,¹¹ and it is thought to hold for octaethylbilindione (4)⁷ (for which a satisfactory X-ray analysis is not yet available). However the configuration at the C(5) bridge has changed: although by the operation of the Cahn-Ingold-Prelog rule it is still designated Z, it has in fact the alternative configuration to that shown at C(5) in structure (4).

TABLE 3

Hydrogen-bonding geometry and possible non-bonded interactions in compounds (6) and (8)

(a) Intramolecular

Compound (6)

N(22) \cdots N(23) 2.93 ÅN(22)-H(22) 1.00 Å; H(22) \cdots N(23) 2.31 Å; \angle N(22)-H(22) \cdots N(23) 119.1°N(23) \cdots N(24) 2.80 ÅN(24)-H(24) 0.83 Å; H(24) \cdots N(23) 2.24 Å; \angle N(24)-H(24) \cdots N(23) 124.8°N(21) \cdots O(51) 2.62 ÅN(21)-H(21) 0.93 Å; H(21) \cdots O(51) 1.97 Å; \angle N(21)-H(21) \cdots O(51) 124.8°

Compound (8)

N(22) \cdots N(23) 2.72 ÅN(22)-H(22) 0.93 Å; H(22) \cdots N(23) 2.19 Å; \angle N(22)-H(22) \cdots N(23) 115.4°N(23) \cdots N(24) 2.99 ÅN(24)-H(24) 0.98 Å; H(24) \cdots N(23) 2.32 Å; \angle N(24)-H(24) \cdots N(23) 124.3°O(19) \cdots N(21) 3.20 ÅN(21)-H(21) 1.00 Å; H(21) \cdots O(19) 2.29 Å; \angle N(21)-H(21) \cdots O(19) 152.5°

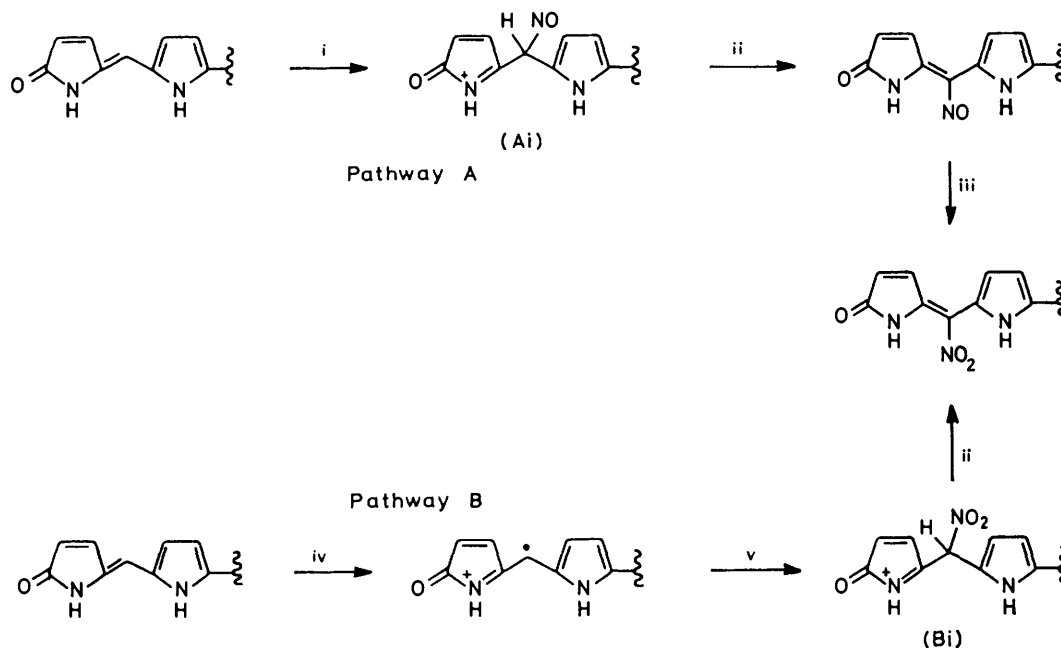
(b) Intermolecular

Compound (6)

O(19)² \cdots N(21)¹ 3.01 ÅN(21)-H(21) 0.93 Å; H(21)¹ \cdots O(19)² 2.20 Å; \angle N(21)-H(21)¹ \cdots O(19)² 144.7°Symmetry code: ¹ = x, y, z; ² = -x, -y, 1 - z

Since the torsion angle N(5)-C(5)-C(6)-N(22) is 99.1°, the geometry at the C(5) bridge can be described as Z-anticlinal.

As in other examples of the nitration of readily oxidised heteroaromatic molecules with acidified nitrite mixtures,¹² at least two mechanistic pathways may be envisaged. One involves a conventional nitrosation, followed by oxidation of the 5-nitroso-compound during work-up (pathway A, Scheme 1). The second possible route requires oxidation of the bilindione to the radical cation (or di-cation) followed by attack of NO₂ or NO₂⁻ (various formulations are possible, e.g. pathway B, Scheme 1). In either event, loss of a proton from the tetrahedral intermediate (e.g. Ai, Bi) can lead to the new, thermo-

SCHEME 1 i, 'NO⁺' source; ii, -H⁺; iii, [O]; iv, -e⁻; v, NO₂

dynamically more stable, configuration at the nitrated C(4)-C(5) double bond.

Deuteriation. Deuteriation of the bilin system has been noted previously. A. W. Johnson and his co-workers observed exchange at C(10) of a synthetic 10,23-dihydrobilin in trifluoroacetic [²H]acid¹³ while Katz *et al.* reported exchange [at C(2), C(3²), and a *meso*-bridge] for both phycocyanobilin¹⁴ and phycoerythrobilin¹⁵ in methanol and/or trifluoroacetic acid.

Deuteriation was followed using the n.m.r. method.¹⁶ A solution of octaethylbilindione (4; 0.03M) in trifluoroacetic [²H]acid was sealed under reduced pressure in an n.m.r. tube and kept at 100 °C: the signals in the *meso* region were observed every 30 min. That at δ 7.54, due to the C(10) proton, scarcely changed, whereas that at δ 6.53, due to the C(5) and C(15) protons, gradually disappeared with a half-life of 59 min (Figure 3). After 17 h the product was isolated; the mass spectrum now showed a molecular ion corresponding to the 5,15-dideuterio-octaethylbilindione.

The conclusion from both the nitration and deuteriation reactions is that the 5- and 15-positions are much more reactive under these conditions than is the 10-position. If these reactions are reasonably to be regarded as electrophilic substitutions, the positional selectivity can be rationalised in terms of π -electron densities which are calculated^{17,18} to be higher at C(5) [C(15)] than at C(10). In a more direct way, the C(5) and C(15) positions can be regarded as part of enamide systems where, as β -positions, they are expected to be electron rich [structure (1), arrows 21 \rightarrow 5]. Because of protonation at N(23), the relative electron deficiency at C(10) with respect to that at C(5) [or C(15)] would be expected to be increased in acidic media [(1), arrows 10 \rightarrow 23].

A corollary of this interpretation is that nucleophilic

attack is expected to occur preferentially at C(10). This is illustrated by the reduction of biliverdin to bilirubin with sodium borohydride¹⁹ and by Falk's observation of the cyanation at C(10) in the presence of cyanide ion.²⁰ This reaction, which presumably involves nucleophilic addition followed by oxidation, has been confirmed for the octaethylbilindione (4), where it leads to the 10-cyano-derivative (7), the electronic spectrum of which

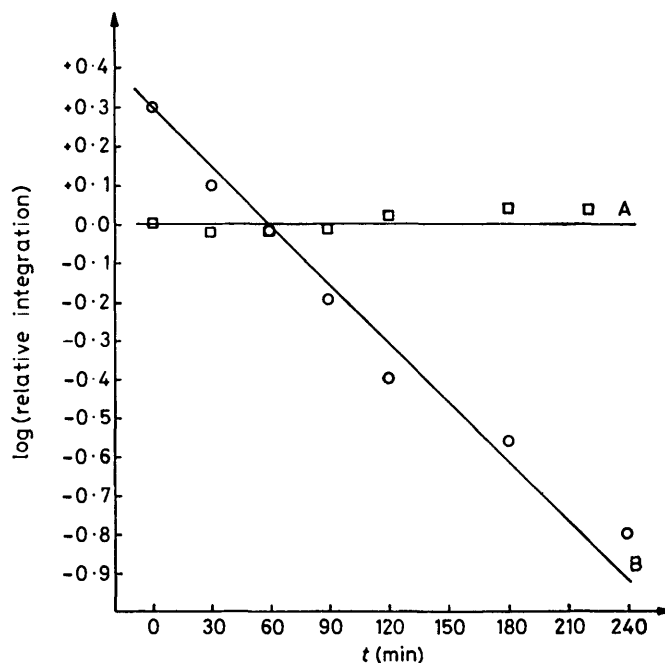
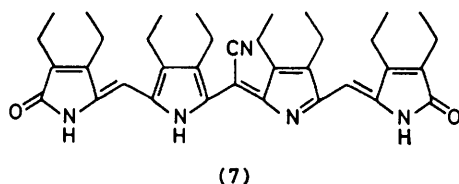


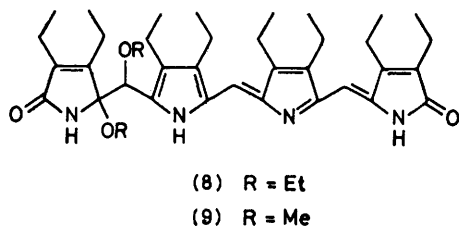
FIGURE 3 Exchange of *meso*-protons of the octaethylbilindione (4) in CF₃CO₂D at 100 ± 0.2 °C. A: 10-H; B: 5- and 15-H

shows a considerable bathochromic shift [λ_{max} (CHCl₃) 379, 680sh, and 730 nm] with respect to that of the parent compound.



Attempted Halogenation. Tiedemann and Gmelin⁸ observed the interaction of halogenating agents with bile pigments. Their observations, unlike those on the fuming nitric acid reaction, were apparently insufficiently spectacular to survive the intervening century-and-a-half, and they receive no mention in recent monographs.

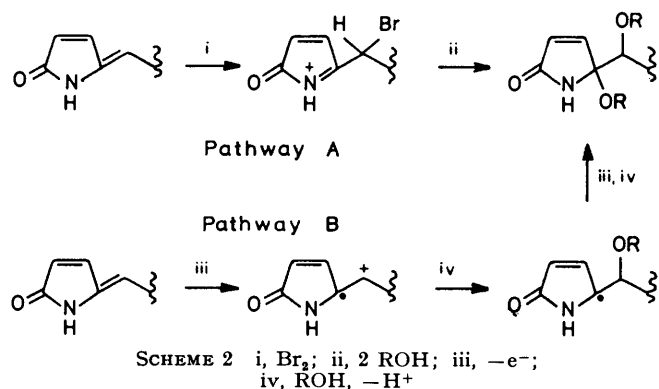
We have made several attempts to prepare brominated and chlorinated derivatives of octaethylbilindione, but without success. Colour changes similar to those of the Gmelin test were observed: the products were generally complex mixtures (t.l.c.) and eventually colourless products were formed. However, when reagent-grade chloroform was used as the solvent, a stable red compound was isolated. This was shown to be the diethoxy-adduct (8), the ethoxy-groups having come from the ethanol present as a stabiliser in the chloroform. von Dobeneck and his colleagues²¹ have described the preparation of dimethoxy- and tetramethoxy-adducts of bilindiones using bromine in methanol as the reagent: our analogous preparations in ethanol or methanol furnished the diethoxy-(8) and the dimethoxy-(9) adducts, respectively, of octaethylbilindione.



The electronic spectra of (8) and (9) resemble one another closely, and are of the tripyrrinone type (Figure 1). As in the nitration reaction, the formation of the adducts (8) and (9) might be rationalised either in terms of electrophilic substitution, followed here by solvolysis and addition of solvent (Pathway A, Scheme 2), or in terms of oxidation of the substrate, followed by attack by solvent molecules on the radical cation (e.g. pathway B, Scheme 2). The generation of these dialkoxy-adducts by anodic oxidation in methanol,²² and by the autoxidation of the cadmium complex in methanol (see Experimental section), leads us to favour the second route.

In order to establish the structure and stereochemistry of the adducts beyond doubt, an X-ray analysis of compound (8) was carried out. The molecular structure is shown in Figure 4 and details of the geometry are given

in Table 1. Again, rings B, C, and D are approximately co-planar, but some helical character is evident (Table 2). The C(10) and C(15) bridges both have the *Z*-synperiplanar geometry. Two pairs of enantiomers of compound (8) are possible: the crystals obtained here have



the (4*RS*, 5*SR*) configuration. Ring A is rotated by 44.5° with respect to the roughly planar portion of the molecule (Table 2) and a short intramolecular contact (3.20 Å, see Table 3) is observed between O(19) and N(21). The gross features of the molecular geometry of compound (8) are very similar to those described²³ for 3,8,12,17-tetraethyl-4,5-dihydro-4,5-dimethoxy-2,7,13,18-tetramethyl-21*H*,24*H*-bilin-1,19-dione, which has been prepared by oxidation of the corresponding bilindione with thallium(III) acetate in methanol.²⁴

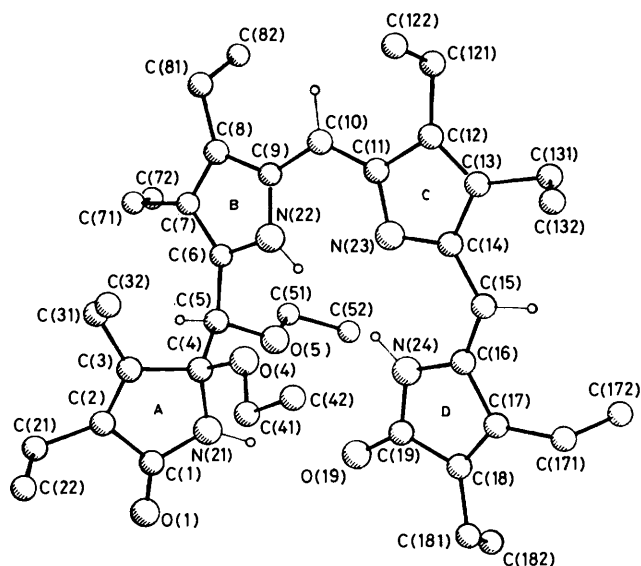


FIGURE 4 Molecular structure and crystallographic atom numbering scheme for the diethoxy-adduct (8)

Comparison of the Structures of (6) and (8). In both structures the imino-hydrogens were located unambiguously from a difference electron density synthesis. Further evidence for the correct assignment of the positions of these hydrogen atoms is provided by the

C-N-C angles. The values of 105.1(3) and 105.2(2)° for ring c (Table 1) are typical for pyrrolenine-type rings (*i.e.* without imino-hydrogens), whereas the values of 109.3(2)—111.6(4) for rings A, B, and D are similar to those found for pyrrole rings (*i.e.* with an N-H bond).²⁵ In adduct (8) the geometry of ring A is modified by the presence of a saturated carbon, C(4), the bonds adjacent to this atom being elongated with respect to the corresponding bonds in ring D. Nevertheless, the five atoms of ring A remain essentially coplanar.

which involve O(19), are the most likely to affect the molecular conformation. The only likely intermolecular hydrogen bond is O(19) ··· H(21)-N(21), although inspection of the geometry (Table 3) suggests that this is an extremely weak interaction compared with those involved in other linear tetrapyrrole structures (see above). Evidently the intramolecular hydrogen bond involving H(21) has far more effect upon the molecular conformation.

Phytochrome. The reactions described above, and

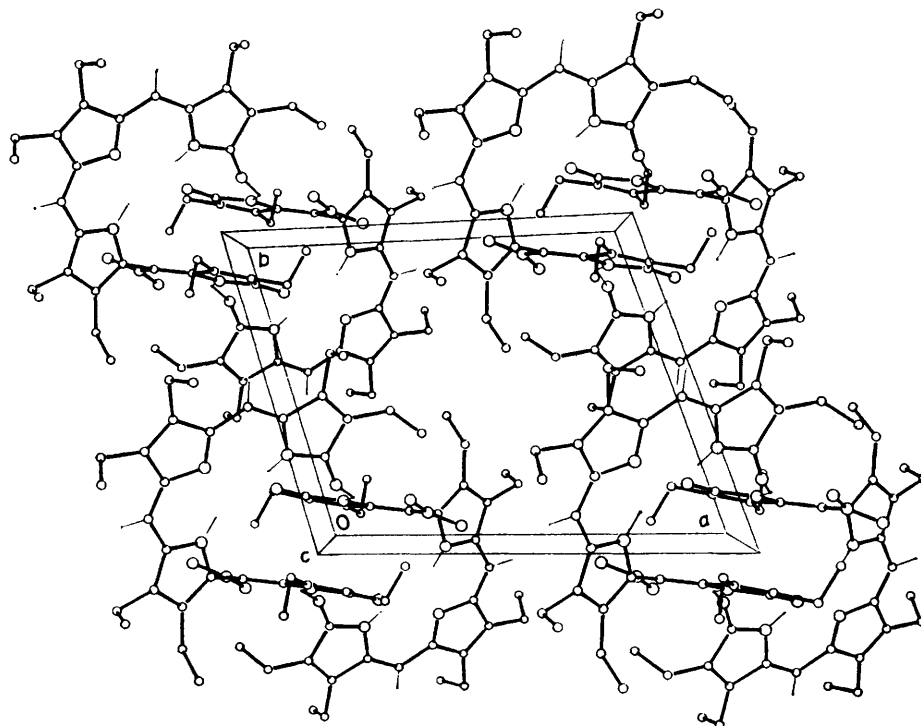


FIGURE 5 Molecular packing in the crystal structure of compound (6) viewed along the crystallographic *c* axis. Centrosymmetrically related molecules are linked in pairs by weak O(19) ··· H(21)-N(21) hydrogen bonds

It has been suggested²⁶ that N-H ··· N intramolecular hydrogen bonding, which leads to relatively small interplanar angles between rings B, C, and D in compounds (6) and (8), may be characteristic of free-base bilin structures. Such hydrogen bonding has been observed both in biliverdin dimethyl ester¹¹ and in 1,19-bis(ethoxycarbonyl)octamethylbilin²⁶ which have the following geometry: N ··· N 2.72—2.85; N-H 0.93—1.06; and N ··· H 1.98—2.29 Å; ∠N-H ··· N 117.2—141.9°. The geometries of the possible N-H ··· N intramolecular hydrogen bonds in compounds (6) and (8) are shown in Table 3. All these bonds are rather weak, with N ··· H distances in the range 2.19—2.32 Å.

The molecular packing in the crystal structures of compounds (6) and (8) is shown in Figures 5 and 6, respectively. In adduct (8) none of the intermolecular distances were shorter than the normal van der Waals contacts. In compound (6) a number of intermolecular contacts <3.5 Å occur (Table 4): the shortest ones,

TABLE 4
Intermolecular contacts <3.5 Å for compound (6).
Distances in Å

| | | | |
|---|------|---|------|
| N(21) ¹ ··· N(21) ² | 3.28 | C(15) ¹ ··· C(17) ³ | 3.48 |
| O(19) ¹ ··· O(51) ² | 2.97 | C(15) ¹ ··· C(18) ³ | 3.48 |
| C(1) ¹ ··· N(21) ² | 3.43 | C(16) ¹ ··· C(16) ³ | 3.45 |

Symmetry code: ¹ = *x*, *y*, *z*; ² = -*x*, -*y*, 1 - *z*; ³ = -*x*, -1 + *y*, -*z*.

particularly structures (6) and (8), have a bearing on the phytochrome problem⁴ which merits a brief comment. While there is, perhaps, still room for some doubt, it is generally considered that the red pigment (P_r, λ_{max} 662 nm)⁵ has structure (10),* possibly with additional

* The bilindione system is often represented (*e.g.* refs. 2, 27, and 28) with the *EEE* configuration. This appears to be a traditional representation, originating from a period when the possibility of geometrical isomerism at bridge double-bonds was not recognised. Where the geometry is unknown it is preferable to use a non-specific representation, as in (10).

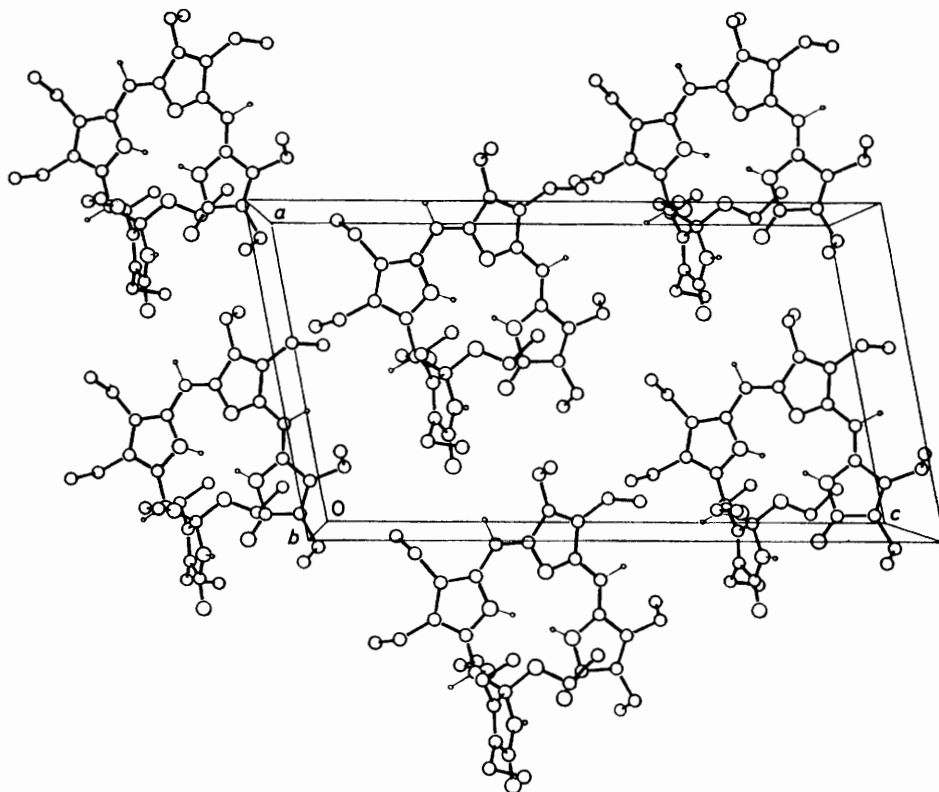
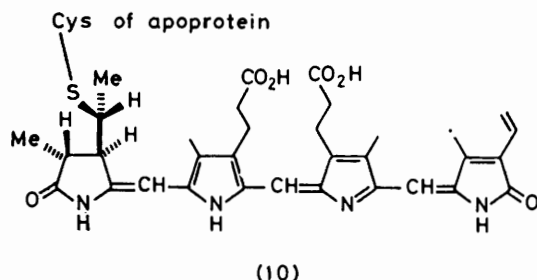


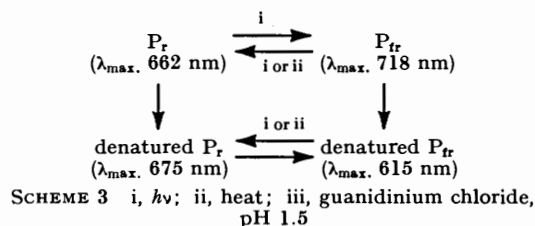
FIGURE 6 Molecular packing in the crystal structure of compound (8) viewed along the crystallographic b axis. Only the section of the structure $0 \leq y \leq 0.5$ is illustrated

binding to the protein *via* one of the propionic acid functions. The structure of the chromophore itself has recently received substantial support from the isolation and characterisation of chromopeptides,²⁷ and from the synthesis of phytochromobilin.²⁸ Considerable uncertainty still exists, however, about the mechanism of the reversible phototransformation to the biologically active far-red pigment (P_{fr} , λ_{max} , 718 nm).⁵ The situation is complicated by the finding that this bathochromic shift apparently obscures a hypsochromic



shift in the bilindione chromophore. Thus, while denaturation of P_r (λ_{max} , 662 nm) with guanidinium chloride at pH 1.5 gives a product with λ_{max} , 675 nm, the analogous reaction with P_{fr} (λ_{max} , 718 nm) gives a denatured P_{fr} with λ_{max} , 615 nm.⁵ The denatured P_{fr} is readily converted (photochemically or thermally) into denatured P_r , but the reaction is no longer reversible

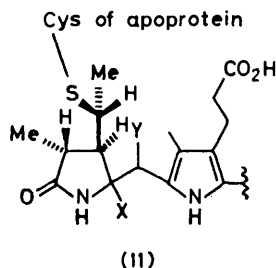
(Scheme 3). If it is safe to assume that other reactions have not taken place under the rather vigorous conditions of the denaturation, then it appears that there has been a photochemically induced change in the chromophore associated with a hypsochromic shift, and that secondary



interactions with protein are responsible for the overall bathochromic shift for the native biliprotein. Working on this assumption, Rüdiger and his colleagues have proposed⁵ that the formation of P_{fr} is due to the photoaddition of unspecified entities to the C(4)-C(5) double bond, as shown in the partial structure (11), leaving a tripyrri- none chromophore. The dimethoxy-compound (8) clearly possesses a chromophore of this general type. [Compound (8) has λ_{max} , ($\text{CHCl}_3\text{-CF}_3\text{CO}_2\text{H}$) *ca.* 595 nm, *cf.* denatured P_{fr} : λ_{max} , 615 nm at pH 1.5.⁵] Since X and Y in structure (11) are not specified, it is difficult to comment on the likelihood of the reversible reaction which is required. Compound (8) does not give appreciable quantities of the octaethylbilindione (4) on mild

thermal or photochemical treatment. A structurally related oxidative photodimerisation of a 2,3-dihydrobilindione has been shown to be thermally reversible, however.²⁹

Of the various alternative explanations for the photo-reversible reaction, photoisomerisation about a bridge double-bond appears to us to be the most plausible on the available evidence. The suggestion was first made in outline by Siegelman and Butler,³⁰ and was later supported by theoretical studies,^{31,32} which, incidentally,

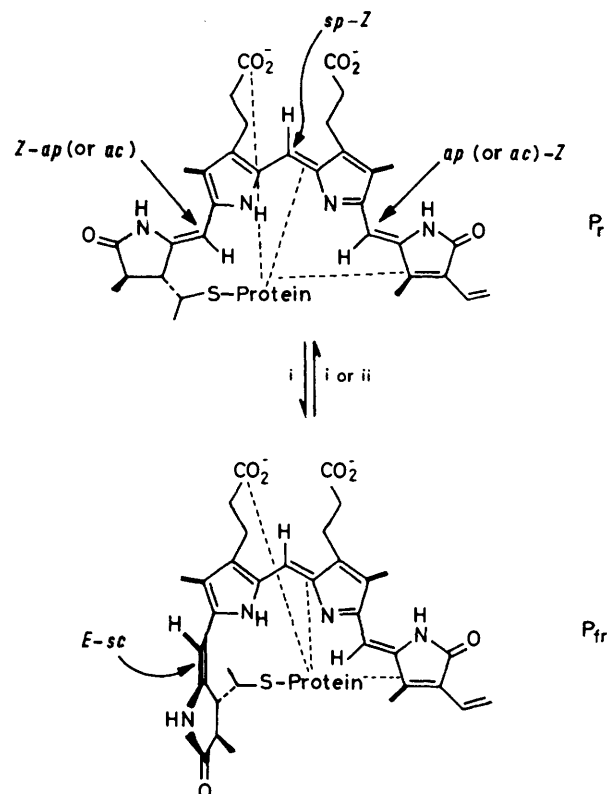


predicted that the relative intensities of the two bands at *ca.* 380 and 650 nm in the electronic spectrum of a bilindione depended on the geometry adopted about the *meso*-bridges, macrocyclic arrangements having a relatively less intense 650 nm band than have more extended arrangements. From an inspection of its electronic spectrum phytochrome would appear to fall into the latter category. The photochemical *Z*→*E* isomerisation at a bridge double-bond is well known in the pyrromethene series,³³ and has recently been encountered in the bilindione series.³⁴ In the present work a geometrical isomerisation at a bridge double-bond of a bilindione has been encountered attendant upon a thermal reaction, and the *X*-ray structure of the product (6) with the unusual geometry has been determined for the first time. The results show that again the chromophore is interrupted, this time for steric reasons, and again a tripyrrinone chromophore results [λ_{max} . (CHCl₃- trace CF₃CO₂H) 590 nm]. In the absence of a *meso*-nitro-group the steric interactions would be less severe. Nonetheless, models show that as long as β -substituents are present, ring A tends to be twisted out-of-plane [around C(5)-C(6)] and in agreement with this a marked hypsochromic shift has been observed in proceeding from a *ZZZ*- to the corresponding *ZZE*-octaalkylbilindione.^{34,35}

A possible formulation of this interpretation of the photoreversible reaction is shown in Scheme 4. The choice of the *4Z*-antiperiplanar, *10Z*-synperiplanar, *15Z*-antiperiplanar geometry for *P_r* is an arbitrary one, but it represents an extended arrangement which, on theoretical grounds, is expected to have a high $\epsilon_{vis.}/\epsilon_{u.v.}$ value.* The photoisomer *P_{fr}* is represented with the

* We have recently observed an arrangement similar to this in the difluoroboron(III) complex of octaethylbilindione, where $\epsilon_{627}/\epsilon_{364} = 1.5$. Here the BF₂ unit controls the observed geometry by a combination of co-ordination and steric effects; in *P_r* the protein must be presumed to exercise this role.

4E-synclinal geometry [equivalent to the *4E*-anticlinal geometry of compound (6)]. Interactions with protein are clearly important, but cannot be specified as yet, and are indicated in a general way. Phytochrome has not yet been crystallised: a crystal structure determination would clarify the stereochemistry.



SCHEME 4 An interpretation of the photoequilibration of phytochrome (*ac* = anticlinal, *ap* = antiperiplanar, *sc* = synclinal, *sp* = synperiplanar). i, *h* ν ; ii, heat

One further refinement involving the protein may be mentioned here. If 2,3-dihydrobilindiones are eventually found to show the same reluctance to photoisomerise at bridge double-bonds that has been found for bilindiones,³⁶ then the protein may also be required to supply a nucleophile which, by reversibly adding to C(10),³⁴ can generate a system of the pyrromethene type which is known to photoisomerise readily.

EXPERIMENTAL

¹H N.m.r. spectra were recorded on either a Bruker WP80 or a Varian HA100 instrument, and were calibrated against internal tetramethylsilane. U.v. spectra were recorded on Pye Unicam SP800, Pye Unicam SP8000 or Perkin-Elmer 552 instruments, the spectra being calibrated with a holmium-glass filter, and i.r. spectra on a Perkin-Elmer 225 Grating spectrophotometer, with polystyrene calibration. Mass spectra were measured on an AEI Ltd. MS902 instrument, with direct insertion (probe temperature as indicated) and an ionising voltage of 70 eV. Accurate mass measurements were made by reference to heptacosafuorotributylamine. Analytical t.l.c. was performed on Merck Kieselgel 60 F₂₅₄, 0.2 mm thick, on plastic sheets. Preparative t.l.c.

was performed on plates coated with Merck Kieselgel 60 HF₂₅₄, 200 × 200 × 1 mm. Light petroleum refers to that fraction with b.p. 60–80 °C.

Crystallographic data and details of intensity data collection are given in Table 5. Fractional atomic co-ordinates are given in Table 6. X-Ray intensity data were measured

TABLE 5
Crystal data for compounds (6) and (8)

| | (6) | (8) |
|---|---|---|
| Formula | C ₃₅ H ₄₅ N ₅ O ₄ | C ₃₉ H ₅₅ N ₄ O ₄ |
| <i>M</i> | 599.8 | 644.9 |
| Crystal system | triclinic | monoclinic |
| Space group | <i>P</i> 1 | <i>P</i> 2 ₁ / <i>n</i> * |
| <i>a</i> (Å) | 14.507 (4) | 12.081(2) |
| <i>b</i> (Å) | 12.405(3) | 14.789(7) |
| <i>c</i> (Å) | 11.855(3) | 21.943(7) |
| α (°) | 116.59(4) | 90.0 |
| β (°) | 67.96(3) | 100.79(2) |
| γ (°) | 114.22(4) | 90.0 |
| <i>U</i> (Å ³) | 1 691.2 | 3 851.3 |
| <i>Z</i> | 2 | 4 |
| <i>D_x</i> (g cm ⁻³) | 1.18 | 1.11 |
| <i>D_m</i> (g cm ⁻³) | 1.16 | — |
| μ(Cu- <i>K</i> _α) (cm ⁻¹) | 5.5 | 5.0 |
| <i>F</i> (000) | 644 | 1 400 |
| Approximate crystal dimensions (mm) | 0.34 × 0.23 × 0.08 | 0.20 × 0.33 × 0.65 |
| θ range (°) | 3–60 | 3–65 |
| Maximum counting time/reflection (s) | 90 | 60 |
| Scan parameters <i>a</i> , <i>b</i> (°) | 0.80, 0.20 | 1.00, 0.15 |
| Aperture parameters <i>A</i> , <i>B</i> (mm) | 3.0, 0.5 | 4.0, 0.0 |
| Reflections measured | <i>h</i> + <i>k</i> ± <i>l</i> | <i>hk</i> ± <i>l</i> |
| No. of reflections measured | 5 030 | 7 312 |
| No. of independent reflections with <i>F</i> ≥ 3σ(<i>F</i>) used in refinement | 3 357 | 3 636 |
| <i>R</i> (= ΣΔ/Σ <i>F</i> _o) | 0.056 | 0.081 |
| <i>R_w</i> (= Σ <i>w</i> ^{1/2} Δ/Σ <i>w</i> ^{1/2} <i>F</i> _o) | 0.064 | 0.081 |
| <i>w</i> ^b | 1/[σ ² (<i>F</i>) + 0.0005 <i>F</i> ²] | 1/[σ ² (<i>F</i>) + 0.0009 <i>F</i> ²] |
| No. of least-squares parameters | 442 | 465 |

* Uniquely determined from systematic absences: *h*0*l* *h* + *l* = 2*n* + 1, 0*h*0 *h* = 2*n* + 1. ^b Chosen to minimise the variance as a function of sinθ and (*F*/*F*_{max})^{1/2}.

on a Nonius CAD4 diffractometer using Ni-filtered Cu-*K*_α radiation and an ω–2θ scan technique. Details of the data collection procedure have been described previously.³⁷ Crystallographic calculations were performed using the SHELX program³⁸ and the diagrams were drawn using the program PLUTO.³⁹ The results for both X-ray analyses in this paper are tabulated together for purposes of comparison as follows: molecular geometry—Table 1; least squares planes—Table 2; hydrogen-bonding geometry—Table 3; intramolecular contacts—Table 4; crystallographic data—Table 5; and atomic co-ordinates—Table 6. Tables of anisotropic thermal parameters, hydrogen-atom co-ordinates, and observed and calculated structure factors for both structures have been deposited as Supplementary Publication No. SUP 23245 (35 pp.).*

(4*Z*, 10*Z*, 15*Z*)-2,3,7,8,12,13,17,18-Octaethyl-5-nitro-21H,-24H-bilin-1,19-dione (6).—(a) *Preparation*. The reaction was carried out under nitrogen, using nitrogen-flushed

solvents, including the quenching solution. A solution of octaethylbilindione (4) (16.6 mg)⁷ in THF (100 ml) and water (30 ml) was treated (ice-bath) with a cold solution of excess of sodium nitrite (550 mg) in water (30 ml). Aqueous acetic acid (30 ml, 67%) was added during 5 min to the stirred ice-cooled mixture, and stirring at *ca.* 0 °C was continued for a further 20 min. The mixture was then poured into chloroform (50 ml) and water (100 ml) and neutralised with aqueous sodium hydrogen carbonate. The organic layer was washed with water, dried, and evaporated to dryness. The residue was subjected to preparative t.l.c. (acetone–light petroleum 8 : 17 v/v) to afford a major purple band (*R_F* *ca.* 0.45) and several minor components. The purple material was extracted with chloroform–hexane (1 : 1) and crystallised from hexane to give red prisms (6.9 mg, 38%) of the octaethyl-5-nitro-21H,24H-bilin-1,19-dione (6), m.p. 193–194 °C (Found: C, 70.0; H, 7.7; N, 12.0%; *M*⁺, 599.346. C₃₅H₄₅N₅O₄ requires C, 70.1; H, 7.55; N, 11.7%; *M*, 599.347; λ_{max} (CHCl₃) (ε) 323 (50 500), 506infr. (22 000), and 533 nm (23 500); λ_{max} (CHCl₃ + trace CF₃CO₂H) 328 (53 500), 574infr. (52 500), and 590 nm (53 000); λ_{max} (KBr) 3 290br, 1 715sh, 1 690, 1 620sh, 1 595, 1 505, 1 375, 1 365, 1 305br, 1 195, 1 100, 1 005, and 945 cm⁻¹; δ (CDCl₃) 6.80 (s, 10-H), 5.85 (s, 15-H), *ca.* 2.27 (m, 8 × CH₂), and *ca.* 1.15

TABLE 6
Fractional atomic co-ordinates (× 10⁴)

| Compound (6) | <i>x/a</i> | <i>y/b</i> | <i>z/c</i> |
|--------------|------------|------------|------------|
| O(1) | –811(2) | 1 796(3) | 5 978(3) |
| C(1) | –183(3) | 1 524(3) | 4 958(4) |
| C(2) | –196(3) | 1 373(4) | 3 649(4) |
| C(3) | 666(3) | 1 101(3) | 2 812(3) |
| C(4) | 1 312(2) | 1 118(3) | 3 565(3) |
| C(5) | 2 264(2) | 939(3) | 3 103(3) |
| N(5) | 2 745(2) | 926(3) | 3 971(2) |
| O(51) | 2 443(2) | 1 378(4) | 5 138(2) |
| O(52) | 3 467(2) | 478(3) | 3 494(2) |
| C(6) | 2 935(2) | 787(3) | 1 794(3) |
| C(7) | 3 826(2) | 1 671(3) | 1 398(3) |
| C(8) | 4 272(2) | 1 057(3) | 101(3) |
| C(9) | 3 647(2) | –181(3) | –274(2) |
| C(10) | 3 756(2) | –1 154(3) | –1 507(3) |
| C(11) | 3 050(2) | –2 271(3) | –1 962(2) |
| C(12) | 3 202(2) | –3 212(3) | –3 259(2) |
| C(13) | 2 293(2) | –4 135(3) | –3 312(3) |
| C(14) | 1 589(2) | –3 745(3) | –2 046(2) |
| C(15) | 524(2) | –4 458(3) | –1 716(3) |
| C(16) | –173(2) | –4 076(3) | –600(3) |
| C(17) | –1 274(2) | –4 736(3) | –197(3) |
| C(18) | –1 654(2) | –4 006(3) | 998(3) |
| C(19) | –801(2) | –2 844(3) | 1 410(3) |
| O(19) | –812(2) | –1 963(3) | 2 440(2) |
| N(21) | 740(2) | 1 358(2) | 4 830(3) |
| N(22) | 2 836(2) | –329(2) | 780(2) |
| N(23) | 2 039(2) | –2 650(2) | –1 251(2) |
| N(24) | 45(2) | –2 954(3) | 393(2) |
| C(21) | –1 083(4) | 1 631(7) | 3 445(6) |
| C(22) | –1 860(6) | 560(9) | 3 044(10) |
| C(31) | 914(3) | 782(4) | 1 358(4) |
| C(32) | 1 344(5) | 1 893(6) | 969(6) |
| C(71) | 4 213(3) | 3 018(3) | 2 236(3) |
| C(72) | 3 983(4) | 3 950(4) | 1 965(5) |
| C(81) | 5 237(2) | 1 620(3) | –758(3) |
| C(82) | 4 996(3) | 1 927(5) | –1 716(4) |
| C(121) | 4 188(3) | –3 078(3) | –4 305(3) |
| C(122) | 4 258(4) | –2 313(5) | –5 036(4) |
| C(131) | 2 008(3) | –5 329(3) | –4 425(3) |
| C(132) | 1 389(4) | –5 224(4) | –5 137(4) |
| C(171) | –1 814(3) | –6 007(3) | –1 083(3) |
| C(172) | –2 147(4) | –5 918(5) | –2 046(5) |
| C(181) | –2 726(3) | –4 211(4) | 1 842(4) |
| C(182) | –3 317(3) | –3 505(5) | 1 788(5) |

* For details, see Notice to Authors No. 7, *J. Chem. Soc., Perkin Trans. 1*, 1981, Index issue.

TABLE 6 (continued)

| Compound (8) | x/a | y/b | z/c |
|--------------|-----------|----------|----------|
| O(1) | 2 035(3) | 5 211(3) | 2 470(2) |
| O(1) | 3 033(4) | 5 369(4) | 2 494(2) |
| C(2) | 3 560(4) | 6 214(3) | 2 322(2) |
| C(3) | 4 663(4) | 6 095(3) | 2 402(2) |
| C(4) | 4 965(3) | 5 140(3) | 2 632(2) |
| O(4) | 5 727(2) | 5 108(2) | 3 219(1) |
| O(41) | 5 272(4) | 5 429(4) | 3 736(2) |
| C(42) | 6 141(6) | 5 430(6) | 4 296(3) |
| C(5) | 5 494(3) | 4 569(3) | 2 181(2) |
| O(5) | 5 416(2) | 3 655(2) | 2 384(1) |
| C(51) | 5 536(4) | 2 990(3) | 1 930(2) |
| C(52) | 5 555(7) | 2 087(4) | 2 225(3) |
| C(6) | 6 688(3) | 4 775(3) | 2 140(2) |
| C(7) | 7 210(3) | 5 082(3) | 1 667(2) |
| C(8) | 8 377(3) | 5 075(3) | 1 891(2) |
| C(9) | 8 544(3) | 4 734(3) | 2 499(2) |
| C(10) | 9 542(3) | 4 549(3) | 2 939(2) |
| C(11) | 9 577(3) | 4 121(3) | 3 498(2) |
| C(12) | 10 584(3) | 3 941(3) | 3 951(2) |
| C(13) | 10 224(4) | 3 515(3) | 4 424(2) |
| C(14) | 9 013(4) | 3 430(3) | 4 255(2) |
| C(15) | 8 305(4) | 3 021(3) | 4 633(2) |
| C(16) | 7 169(4) | 2 883(3) | 4 503(2) |
| C(17) | 6 464(4) | 2 514(3) | 4 918(2) |
| C(18) | 5 386(4) | 2 533(3) | 4 633(2) |
| C(19) | 5 346(4) | 2 934(3) | 4 014(2) |
| O(19) | 4 497(3) | 3 105(3) | 3 622(2) |
| N(21) | 2 882(3) | 4 767(3) | 2 682(2) |
| N(22) | 7 510(3) | 4 572(2) | 2 636(2) |
| N(23) | 8 625(3) | 3 804(2) | 3 702(2) |
| N(24) | 6 440(3) | 3 102(3) | 3 957(2) |
| C(21) | 2 857(5) | 7 032(4) | 2 140(3) |
| C(22) | 2 693(7) | 7 584(5) | 2 688(4) |
| C(31) | 5 529(5) | 6 782(3) | 2 298(3) |
| C(32) | 6 191(6) | 7 212(5) | 2 862(4) |
| C(71) | 6 611(4) | 5 367(4) | 1 026(2) |
| C(72) | 6 417(6) | 4 592(6) | 582(3) |
| C(81) | 9 301(3) | 5 309(3) | 1 551(2) |
| C(82) | 9 791(5) | 4 498(4) | 1 278(3) |
| C(121) | 11 765(3) | 4 222(3) | 3 903(2) |
| C(122) | 12 016(4) | 5 213(4) | 4 103(3) |
| C(131) | 10 947(4) | 3 148(5) | 5 028(3) |
| C(132) | 10 893(6) | 3 762(7) | 5 550(4) |
| C(171) | 6 931(5) | 2 182(4) | 5 570(2) |
| C(172) | 7 448(5) | 1 258(5) | 5 580(3) |
| C(181) | 4 322(4) | 2 276(4) | 4 855(3) |
| C(182) | 3 897(5) | 1 369(4) | 4 589(3) |

(m, 8 × CH₃); m/e (178 °C) 599 (M^+ , 38%), 565 (5), 553 ($M - NO_2$, 100), 537 (4), 523 (5), 510.5 (m^* , 599 → 553), 432 (48), 417 (11), and 403 (27).

(b) X-Ray analysis. The crystal used for data collection was grown from n-hexane. The positions of 40 of the 44 non-hydrogen atoms were determined using an automatic centrosymmetric direct-methods routine. The structure was developed and refined by difference syntheses and full-matrix least-squares procedures. All the heavy atoms were assigned anisotropic thermal parameters. The methyl groups were treated as rigid bodies with hydrogen atoms in calculated positions (C-H 1.08 Å): all other hydrogen atoms were included in positions obtained from a difference map, and were refined without constraint. A common isotropic temperature factor was refined for the methyl hydrogen atoms [0.145(4) Å²]. The final R -factors and weighting scheme are given in Table 5.

5,15-Dideutero-2,3,7,8,12,13,17,18-octaethyl-21H,24H-bilin-1,19-dione.—Octaethylbilindione (4; 5 mg) in trifluoroacetic [²H]acid (0.03M; 0.3 ml) and one drop of tetramethylsilane as internal standard were sealed *in vacuo* in an n.m.r. tube. The tube was heated in a boiling-water bath and n.m.r. spectra were measured at 30 min intervals.

After 17 h the signal at δ 6.53 had virtually disappeared ($t_{1/2}$ 59 min, k $1.95 \times 10^{-4} s^{-1}$). The solution was diluted with chloroform (10 ml) and was washed in turn with 0.2M sodium hydrogen carbonate and water. After drying (Na₂SO₄) the solvent was removed and the residue was submitted to preparative t.l.c. (CHCl₃-MeOH 19 : 1 v/v) to give the 5,15-dideuterobilindione (4 mg) as blue microcrystals, m.p. 258–263 °C (Found: M^+ , 556.374. C₃₅H₄₄²H₂NO₂ requires M , 556.375); ν_{max} (KBr) 3 230, 1 684, 1 608, 1 584, 1 221, 1 007, 947, 729, 681, and 621 cm⁻¹; m/e (182 °C) 556 (M^+ , 49), 541 ($M - CH_3$, 2), 526 (1), and 278 (8).

(4SR,5RS,10Z,15Z)-2,3,7,8,12,13,17,18-Octaethyl-4,5-dihydro-4,5-dimethoxy-21H,24H-bilin-1,19-dione (9).—Method (a). Octaethylbilindione (4; 44.8 mg) in redistilled chloroform (30 ml) was treated dropwise with bromine (12.8 mg, 1 mol equiv.) in methanol (4 ml). The solution was stirred at 30–35 °C for 1 h. The solvent was removed and the residue was subjected to preparative t.l.c. (CHCl₃-MeOH 24 : 1 v/v). Starting material (R_F 0.7; 8.5 mg) was recovered. The major product, a violet band with R_F 0.5 was extracted and crystallised from n-hexane to give the dimethoxy-adduct (9) (20.8 mg, 41%) as brownish red crystals, m.p. 152–153 °C (Found: C, 72.25; H, 8.7; N, 9.15. C₃₇H₅₂N₄O₄ requires C, 72.05; H, 8.5; N, 9.1%); λ_{max} (CHCl₃) (ϵ) 324 (45 000), 520inf. (26 800), and 555 nm (33 500); ν_{max} (KBr) 3 415, 3 040, 1 685br, 1 622, 1 583, 1 380, 1 275, 1 208, 1 079, 1 052, 1 005, and 944 cm⁻¹; δ (CDCl₃) 6.70 (s, 10-H), 6.20 (s, 21-H), 5.83 (s, 15-H), 4.29 (s, 5-H), 3.31 and 2.96 (2 × s, 2 × OCH₃), *ca.* 2.35 (m, CH₂CH₃) and *ca.* 1.12 (m, CH₂CH₃); m/e (296 °C) 584 (29, $M - MeOH$). Precision measurement gave m/e 584.374. C₃₆H₄₈N₄O₃ requires 584.373, 556 (8), 555 (40), and 554 ($M - 2 \times OMe$, 100).

Method (b). Octaethylbilindione (4; 33.2 mg) in methanol (150 ml) was treated with a warm solution of cadmium(II) acetate dihydrate (128 mg) in methanol (100 ml). The mixture was stirred in air at room temperature for 16 d, then diluted with chloroform (100 ml), washed with water (2 × 200 ml), dried (Na₂SO₄), and evaporated to dryness. Preparative t.l.c. as above gave the dimethoxy-adduct (11.5 mg, 31%), identical (mixed m.p., t.l.c., n.m.r.) with the sample prepared using Br₂-MeOH.

(4SR,4RS,10Z,15Z)-4,5-Diethoxy-2,3,7,8,12,13,17,18-octaethyl-4,5-dihydro-21H,24H-bilin-1,19-dione (8).—(a) Preparation. Octaethylbilindione (4; 22.4 mg) in chloroform (15 ml) and ethanol (10 ml) was treated with bromine (64 mg) in ethanol (2 ml) over a period of 2 min. The solution was stirred at 30–35 °C for 2 h before being washed with water (2 × 50 ml) and dried (Na₂SO₄). The solvent was removed and the residue was subjected to preparative t.l.c. (CHCl₃-EtOAc 9 : 1 v/v). Unchanged starting material (R_F 0.57, 9 mg) was recovered. The major, violet-red product (R_F 0.42) was rechromatographed (CHCl₃-MeOH 24 : 1 v/v; R_F 0.6) to give the diethoxy-adduct (8) (10.1 mg, 38%) from n-hexane as brownish red crystals, m.p. 175–177 °C; λ_{max} (CHCl₃) (ϵ) 325 (48 000), 522inf. (27 500), and 556 nm (35 000); ν_{max} (KBr) 3 405, 3 350, 3 025, 1 706, 1 685, 1 612, 1 572, 1 379, 1 269, 1 204, 1 051, 1 001, and 942 cm⁻¹; δ (CDCl₃) 6.68 (s, 10-H), 6.27 (s, 21-H), 5.84 (s, 15-H), 4.42 (s, 5-H), 2.75–3.60 (m, OCH₂CH₃), *ca.* 2.36 (m, 8 × CCH₂-CH₃), and *ca.* 1.03 (m, CCH₂CH₃); m/e (268 °C) 644 (M^+ , 2%), 600 (7), 599 (15), 598 ($M - EtOH$, 22), 582 (7), 570 (8), 556 (23), 554 ($M - 2 \times EtO$, 76), 552 (7), 550 (10), 548 (19), and 546 (13). Accurate mass measurement m/e 598.387. C₃₇H₅₀N₄O₃ ($M - EtOH$) requires 598.388.

(b) *X-Ray analysis*. A large crystal ($1 \times 2 \times 3$ mm) was grown by isothermal distillation from chloroform-*n*-pentane. A fragment of the crystal was used for data collection (crystal data: Table 5). The crystal had a wide reflection profile (peak widths $1.0\text{--}1.7^\circ$) which probably accounts for the fairly high value of the final crystallographic *R*-factor.

The structure was solved by direct methods. *E*-Maps were calculated using 531 reflections with $E \geq 1.6$; the solution with the highest combined figure of merit gave the positions of 40 out of 47 heavy atoms in the asymmetric unit. All the remaining atoms were located on a subsequent difference map. After anisotropic refinement (*R* 0.12) the positions of 42 out of 56 hydrogen atoms, including the imino hydrogens, were found from a difference synthesis. Owing to the restricted amount of data available, hydrogen atoms bonded to carbon were included in calculated positions and refined using a riding model (C-H 1.08 Å): the methyl groups were treated as rigid bodies. The imino-hydrogen atoms were allowed to refine freely with N-H distances constrained to 1.00 ± 0.05 Å. Common isotropic temperature factors were refined for methyl and non-methyl hydrogens [0.203(6) and 0.140(4) Å², respectively]. The final *R*-factors and weighting scheme are given in Table 5 and the molecular geometry and heavy-atom coordinates are presented in Tables 1-4 and 6.

10-Cyano-2,3,7,8,12,13,17,18-octaethyl-21H,24H-bilin-1,19-dione (7).—Octaethylbilindione (4; 55.5 mg) in *N,N*-dimethylformamide (150 ml) was treated with potassium cyanide (6.5 g) and lithium bromide (26 g). Water (30 ml) was added to complete the dissolution, and the mixture was stirred at room temperature for 3 h. Chloroform (100 ml) was added and the solution was washed with water (4×400 ml). The organic layer was dried (Na₂SO₄) and evaporated to dryness. The residue was subjected to preparative t.l.c. (CHCl₃-MeOH 24:1 v/v) to give a major, green product (*R*_F 0.65) which was extracted and crystallised from chloroform-*n*-hexane to give the 10-cyano-derivative (7) (38.9 mg 67%) as tiny green needles, m.p. 226-228 °C (Found: C, 74.5; H, 7.65; N, 12.25%; *M*⁺, 579.359. C₃₆H₄₅N₅O₂ requires C, 74.6; H, 7.8; N, 12.1%; *M*, 579.357; λ_{max} (CDCl₃) (ε) 379 (69 500), 680inf. (15 200), and 730 nm (21 500); λ_{max} (CHCl₃ + trace CF₃CO₂H) (ε) 384 (63 500), 678inf. (13 500) and 722 nm (20 000); ν_{max} (KBr) 3 160br, 2 218, 1 685, 1 612, 1 008, and 944 cm⁻¹; δ (CDCl₃) ca. 8v.br (NH), 6.15 (s, 5- and 15-H), 3.0 [q, *J* 7.5 Hz, C(8)- and C(12)-CH₂], 2.60 [q, *J* 7.5 Hz, C(3)-, C(7)-, C(13)-, and C(17)-CH₂], 2.31 [q, *J* 7.5 Hz, C(2)- and C(18)-CH₂], and 1.19 (m, 8 × CH₃); *m/e* (171 °C) 579 (*M*⁺, 100%), 565 (11), 551.4 (*m*^{*}, 579 → 565), 551 (20), and 524.4 (*m*^{*}, 579 → 551).

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