Crystal and Molecular Structure of Biliverdin Dimethyl Ester

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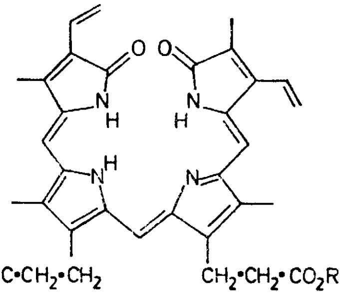
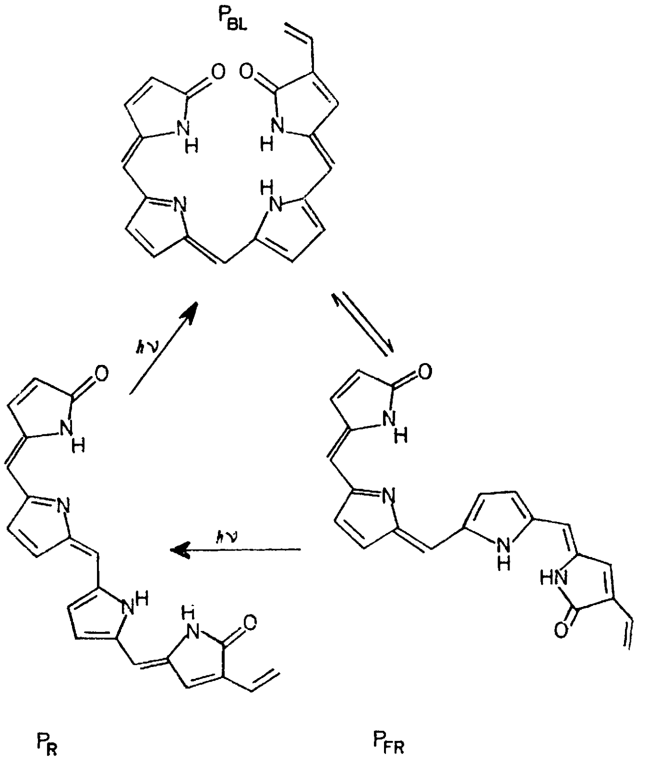
Crystals of the title compound (II) are triclinic, space group $P\bar{1}$, with $a = 12.467(6)$, $b = 14.567(5)$, $c = 9.788(4)$ Å, $\alpha = 111.56(3)$, $\beta = 90.64(4)$, $\gamma = 97.91(3)°$, $Z = 2$. The biliverdin chromophore, which is in the lactam form with three 'pyrrole' N–H protons, takes up a near-planar helical conformation. Two such molecules, related by a crystallographic centre of symmetry are linked into dimers with an extended helical conformation, through two N–H $\cdots$ O hydrogen bonds (N–H $\cdots$ O 2.80, N–H 1.12, H $\cdots$ O 1.74 Å) between symmetry-equivalent pyrrolone rings. A considerable degree of bond fixation is observed within the tetrapyrrole skeleton. The vinyl and one of the methyl ester groups are disordered. The structure was solved by direct methods and refined to R 0.090 for 2 357 diffractometer-measured unique reflections.

BILIVERDIN (I) is the first isolable product of the oxidative breakdown of haem in nature, and a precursor of bilirubin.[1] It is observed, together with the latter in variable ratio, in the bile liquid of the vertebrates, and is, in fact, the major pigment in certain organs or (particularly calcified) tissues of several of these. The chromophore of the plant hormone phytochrome, whose exact structure has not yet been established, is believed to be a related bilatriene bile pigment.[2] This biliprotein regulates many of the light responses in plants, thereby exerting a controlling influence over important aspects of plant growth and development, such as flowering, seed germination, and stem elongation.[3] It occurs in two forms distinguished mainly by their different absorption spectra in the visible range. A 'red form' ($\mathrm{P_R}$) with an intense absorption maximum at 664 nm is converted to a 'far-red form' ($\mathrm{P_{FR}}$) with a broad peak at 730 nm (the extinction coefficient of this band is *ca.* one-third of the 664 nm bond), by irradiation with red light.[1] It has been suggested by Burke *et al.*,[2] on the basis of simple Hückel MO calculations that this spectral shift may be accounted for in terms of a *cis–trans* geometric isomerisation around the methine bridge atoms of the chromophore in an extended form. A bleached intermediate ($\mathrm{P_{BL}}$) formed by a non-reversible photochemical reaction when $\mathrm{P_R}$ is irradiated at —45° was believed,[4] on the basis of these calculations, to have a more folded nature.



(I) R = H
(II) R = Me

E.s.r. studies on biliverdin and related bilatriene metal complexes, which undergo similar reversible spectroscopic changes when carefully titrated with acids and bases,[5,6] have demonstrated that the 'far-red forms' (absorption *ca.* 730 nm) have a cyclic and near-planar conformation in solvents such as chloroform or methanol.[7] Addition of acid to these solutions leads to a shift in the absorption maxima to shorter wave lengths (*ca.* 660 nm). Definite establishment of the conformation of these 'red forms', for which an open-chain form has been suggested,[8] was however hindered by the fact that well-defined e.s.r. signals could not be obtained under these



Possible mechanism for the transformation in phytochrome

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[1] W. Rüdiger, *Fortschr. Chem. org. Naturstoffe*, 1971, **29**, 60.

[2] M. J. Burke, D. C. Pratt, and A. Moscowitz, *Biochemistry*, 1972, **11**, 4025.

[3] H. W. Siegelmann, in 'The Physiology of Plant Growth and Development,' ed. M. B. Wilkins, McGraw-Hill, New York, 1970, p. 487.

[4] D. Cross, H. Linschitz, V. Kasche, and J. Tenenbaum, *Proc. Nat. Acad. Sci. U.S.A.*, 1968, **61**, 1095.

[5] P. Wasser and J.-H. Fuhrhop, *Ann. New York Acad. Sci.*, 1973, **206**, 533.

[6] J.-H. Fuhrhop, P. Wasser, J. Subramanian, and U. Schrader, *Annalen*, 1974, 1450.

[7] J. Subramanian, J.-H. Fuhrhop, A. Salek, and A. Gossauer, *J. Magn. Resonance*, 1974, **15**, 19.

[8] J.-H. Fuhrhop, A. Salek, J. Subramanian, C. Mengersen, and S. Besecke, *Annalen*, 1975, 1131.

conditions. Pariser–Parr–Pople–SCF calculations predicted that a cyclic conformation should absorb at longer wavelengths.[8] Although the phytochrome chromophore does not contain a metal ion, a speculative postulation was made, in view of the similar photochemically induced spectroscopic change, that its 'far-red form' might also have a folded near-planar conformation. Recently, the first direct structural description of a biliverdin-type chromophore has been provided by the *X*-ray analysis of zinc octaethyl formyl biliverdinate and its dehydrated bis-helical dimer.[9] A chloroform solution of the former produces an absorption band in the near-i.r. (830 nm), which shifts to shorter wave lengths (750 nm) on addition of strong acid. The monomer has a cyclic helical conformation, with distorted square-planar co-ordination at zinc, similar to that predicted by the e.s.r. analysis of the corresponding copper complex,[7] in which steric interactions preclude complete planarity. The latter 'acid form' is a wide bis-helical dimer, in which each tetrahedrally co-ordinated (distorted) zinc ion is bound to two nitrogen atoms of each chromophore molecule. These results suggest, therefore, that the spectroscopic shifts observed for the phytochrome chromophore may well be produced not by *cis–trans* isomerism but by a simple stretching of the molecule perpendicular to its molecule plane.

The free ligand biliverdin and its dimethyl ester (II) show similar changes in their electronic spectra. The neutral biliverdins with three N–H protons show a broad band around 650 nm which is intensified on protonation with strong acid.[2] A broader weaker band at 760 nm is obtained on addition of strong base. I.r. and n.m.r. studies [10] of (I) and (II) have suggested that both compounds are in the lactam form. The i.r. spectrum of (I) showed hydrogen-bonded N–H absorption over a broad range around 3 100 cm^{-1}, which could not, however, be observed for the ester, probably on account of extensive broadening. An *X*-ray analysis of a free ligand biliverdin is of more general interest in view of the biological relevance, particularly their possible relationship to the structural phytochrome problem, and the manifold speculation that the 'red form' of the bilatrienes has an open-chain extended conformation.[2] A structural investigation of (II) was undertaken because of the relative ease with which suitable crystals may be grown.

EXPERIMENTAL

Well formed blue prismatic crystals of (II) were obtained upon its recrystallisation from a methanol solution containing a little chloroform. The intensity data from a crystal with dimensions *ca.* 0.22 × 0.16 × 0.56 mm, mounted with the *c* axis parallel to the goniometer axis, were collected on a Syntex $P2_1$ four-circle diffractometer by use of graphite-monochromated Mo-K_α radiation. Measurements were carried out in the θ—2θ mode ($3.0 \leqslant 2\theta \leqslant 50.0°$) at scan speeds varying linearly between 2.93° min^{-1} ($\leqslant$150 c.p.s.) and 8.37° min^{-1} ($\geqslant$5 000 c.p.s.). Scan and total background times were equal. The intensities of three standard reflections monitored regularly during data collection, showed no significant variations. A standard deviation $\sigma(I) = t(N_s + N_b)^{1/2}$ was assigned to each net intensity I, with t being the scan rate, N_s the gross count, and N_b the total background count. With the application of the rejection criterion $I \geqslant 2.5\ \sigma$ (I), 2 357 of the 6 050 reflections recorded (one half of reciprocal space) were considered to be observed. Lorentz and polarisation, but no absorption corrections were applied to the raw intensity data. Accurate unit-cell dimensions were obtained from measurements of 15 reflections ($\pm$ *hkl*) by use of Mo-K_α radiation.

Crystal Data.—$C_{35}H_{38}N_4O_6$, $M = 610.72$. Triclinic, $a = 12.467(6)$, $b = 14.567(5)$, $c = 9.788(4)$ Å, $\alpha = 111.56(3)$, $\beta = 90.64(4)$, $\gamma = 97.91(3)°$, $U = 1\,633.7(11)$Å^3, $Z = 2$, $D_c = 1.24$ g cm^{-3}. Mo-K_α radiation, $\lambda = 0.710\,69$ Å; μ(Mo-K_α) $= 0.50$ cm^{-1}. The centrosymmetric space group $P\bar{1}$ was confirmed by the subsequent refinement.

Structure Solution and Refinement.—The structure was solved by a multisolution technique [11] in which 2^{20} sign permutations were expanded by the Σ_2 formula. All 6 050 reflections measured were included in these calculations, those with $I \leqslant 1.0\ \sigma(I)$ being assigned a value of $0.25\ \sigma(I)$. A permutation is rejected in this method if its internal consistency falls at any stage below a preset value. Additionally, a similarity test is applied to avoid the calculation of too many closely similar *E* maps. In this case 13 *E* maps were computed, of which the second best (in terms of Σ_2 consistency) revealed the positions of 31 of the 45 heavy atoms and of all the members of the bilatriene skeleton.

Refinement was carried out by block-diagonal least-squares with the function $\Sigma w\Delta^2$ being minimised. It soon, however, became apparent that although the bilatriene skeleton was well defined, there was considerable disorder in the vinyl/methyl groups on C(2), C(3), C(7), and C(8) and in the methyl ester side-chain on C(13). Although no completely adequate model to describe these disorders could be constructed, it was eventually found that a best approximation could be obtained in the following manner. (1) The two vinyl and the methyl groups are randomly disordered with fixed site-occupation factors (s.o.f.) of 0.50, about positions 2, 3, 7, and 8. One of these vinyl groups, that at position 7, is further disordered and may best be described by the introduction of two carbon positions [C(72)$^{\text{II}}$ and C(73)$^{\text{II}}$] with fixed s.o.f.s of 0.25. Attempts to refine these positions were however unsuccessful, presumably because their scattering power is similar to that of the hydrogen atoms of the disordered methyl group. They were, therefore, included as fixed parameters with a joint isotropic temperature factor. The two vinyl C=C distances of 1.13 and 1.17 Å are unrealistically short, once again reflecting the presence of disordered methyl C–H bonds. All other vinyl and methyl groups refined anisotropically in a satisfactory manner and led to reasonable C–C and C=C bond lengths. (2) The methyl ester grouping at position 13 is also randomly disordered (fixed s.o.f. 0.50) about a pivot atom C(133). An initial isotropic refinement in which the s.o.f. was also included as a free variable led to a value for it of 0.60(1) [O(134), O(135), C(136)] and 0.40(1) [O(137), O(138), C(139)] respectively. Although reasonable temperature factors were obtained, the bond lengths were unsatisfactory and inspection of the difference Fourier suggested

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[9] G. Struckmeyer, U. Thewalt, and J.-H. Fuhrhop, *J. Amer. Chem. Soc.*, 1976, **98**, 278.

[10] A. W. Nichol and D. B. Morell, *Biochim. Biophys. Acta*, 1969, **177**, 599.

[11] G. M. Sheldrick, 1975, unpublished results.

the presence of a considerable degree of anisotropy in the thermal motions of these atoms. A subsequent anisotropic refinement with a fixed s.o.f. of 0.50 for all these disordered atoms led to a very significant improvement in the generalised index R_G $[\Sigma w\Delta^2/\Sigma wF_o^2]^{1/2}$ from 0.131 to 0.116. As these ester groupings are not involved in any particularly short intra-or inter-molecular contacts, the assumption of a fixed s.o.f. of 0.50 would appear to be physically reasonable. One of the disordered groups [O(137)–C(139)] appears, therefore, to display more thermal motion than the other. Inspection of the bond lengths obtained [*e.g.* C(133)–O (137) 1.05(2) Å] indicates that even this model fails to describe completely the disorder and from the difference Fourier it would appear that the ester group may also assume intermediate positions between these extremes.

TABLE 1

Atom co-ordinates and anisotropic vibrational amplitudes (Å^2 × 10^3),* with estimated standard deviations in parentheses

Atom	x/a	y/b	x/c	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{21}
N(21)	0.482 5(5)	0.022 3(4)	0.329 9(6)	54(4)	44(4)	48(4)	16(3)	0(3)	−8(3)
C(1)	0.452 5(6)	0.062 3(5)	0.228 2(8)	47(5)	55(5)	46(5)	17(4)	6(4)	−1(4)
C(2)	0.362 8(6)	−0.012 1(5)	0.128 6(7)	46(5)	61(5)	40(4)	2(4)	2(4)	−3(4)
C(3)	0.348 0(6)	−0.089 6(5)	0.173 7(8)	55(5)	40(4)	50(5)	7(4)	10(4)	−6(4)
C(4)	0.418 4(6)	−0.067 6(5)	0.304 1(8)	51(5)	47(5)	52(5)	10(4)	6(4)	−2(4)
O(4)	0.424 0(4)	−0.117 1(3)	0.382 1(5)	75(4)	52(3)	53(3)	22(3)	1(3)	−10(3)
C(6)	0.582 3(5)	−0.198 5(4)	0.102 8(7)	107(5)	77(4)	85(5)	19(4)	−26(4)	−13(4)
N(22)	0.690 2(5)	−0.110 2(4)	0.318 6(7)	65(5)	47(4)	62(4)	15(3)	−5(4)	−7(3)
C(6)	0.622 2(7)	−0.190 5(6)	0.221 6(11)	58(6)	72(6)	74(6)	26(5)	−4(5)	6(5)
C(7)	0.611 2(7)	−0.269 6(6)	0.286 4(10)	72(6)	50(5)	89(7)	20(5)	−4(5)	−2(5)
C(8)	0.672 9(7)	−0.232 5(5)	0.415 3(10)	74(6)	38(5)	95(7)	25(5)	23(5)	7(4)
C(9)	0.724 0(6)	−0.128 1(6)	0.436 2(9)	53(5)	59(5)	63(5)	24(4)	16(5)	10(4)
C(10)	0.795 6(7)	−0.065 4(6)	0.546 5(8)	80(6)	62(5)	50(5)	26(4)	12(5)	16(5)
N(23)	0.803 4(5)	0.082 9(4)	0.490 4(6)	53(4)	50(4)	53(4)	11(3)	6(3)	0(3)
C(11)	0.843 4(6)	0.032 2(5)	0.560 2(8)	54(5)	40(4)	54(5)	13(4)	2(4)	6(4)
C(12)	0.939 8(6)	0.874 5(5)	0.657 1(8)	67(6)	53(5)	46(5)	13(4)	4(4)	14(4)
C(13)	0.957 5(6)	0.176 6(6)	0.6371 (8)	51(5)	64(5)	42(5)	0(4)	4(4)	1(4)
C(14)	0.872 6(6)	0.177 1(5)	0.538 1(8)	40(5)	49(5)	49(5)	−1(4)	4(4)	−3(4)
C(15)	0.851 2(6)	0.255 4(5)	0.499 1(8)	62(6)	44(5)	50(5)	8(4)	1(4)	1(4)
N(24)	0.672 2(5)	0.186 1(4)	0.373 4(6)	29(4)	21(3)	32(3)	18(3)	−11(3)	−2(3)
C(16)	0.763 0(6)	0.257 7(5)	0.415 8(8)	64(6)	39(4)	45(4)	6(4)	−9(4)	−1(4)
C(17)	0.744 3(6)	0.331 0(5)	0.360 5(8)	65(6)	36(4)	58(5)	8(4)	−3(4)	−9(4)
C(18)	0.644 1(7)	0.301 6(5)	0.280 4(8)	71(6)	42(4)	47(5)	9(4)	−2(4)	−1(4)
C(19)	0.602 6(7)	0.209 1(5)	0.293 7(8)	70(6)	33(4)	63(5)	13(4)	19(5)	−10(4)
C(20)	0.500 9(6)	0.147 2(5)	0.213 4(7)	64(5)	39(4)	52(5)	14(4)	5(4)	1(4)
C(21)	0.308 7(7)	0.005 2(6)	0.008 0(8)	71(6)	73(5)	56(5)	16(4)	−12(5)	−5(5)
C(22^{I})	0.225 3(13)	−0.045 5(11)	−0.068 4(17)	57(10)	63(10)	68(11)	13(8)	−8(9)	−8(9)
C(31)	0.271 9(7)	−0.185 7(6)	0.103 8(9)	81(6)	58(5)	66(6)	5(4)	3(5)	−17(5)
C(32^{I})	0.269 6(14)	−0.259 8(13)	0.141 6(17)	80(13)	76(12)	59(11)	8(10)	−3(9)	− 5(10)
C(71)	0.542 9(9)	−0.366 9(7)	0.208 4(13)	110(8)	80(7)	171(11)	47(7)	11(8)	−24(6)
C(72^{II})	0.478 1	−0.400 6	0.114 7	157(13)					
C(73^{II})	0.486 1	−0.387 1	0.291 2	157(13)					
C(81)	0.695 3(8)	−0.279 5(7)	0.522 3(11)	126(9)	63(6)	130(9)	56(6)	18(7)	7(6)
C(82^{I})	1.358 1(21)	0.354 9(15)	0.476 4(21)	212(26)	76(14)	88(11)	43(12)	7(16)	−11(16)
C(121)	1.003 9(7)	0.051 8(6)	0.749 6(8)	97(7)	74(6)	69(6)	37(5)	−6(5)	14(5)
C(131)	1.052 5(6)	0.236 0(5)	0.708 4(8)	63(5)	62(5)	56(5)	3(4)	−3(4)	−8(4)
C(132)	1.026 8(7)	0.331 4(6)	0.860 0(9)	79(6)	71(5)	60(5)	10(4)	1(5)	−5(5)
C(133)	1 127 1(9)	0.402 6(6)	0.940 3(12)	100(8)	65(6)	86(7)	−11(5)	−35(7)	7(6)
O(134^{I})	1.200 0(10)	0.441 7(9)	0.876 5(12)	109(10)	93(9)	74(8)	39(7)	20(7)	−77(8)
O(135^{I})	1.140 4(10)	0.415 8(12)	0.075 2(14)	89(10)	212(16)	63(9)	41(10)	4(8)	−65(10)
C(136^{I})	1.221 2(18)	0.512 0(16)	1.163 8(23)	108(17)	138(19)	103(16)	−17(14)	−34(14)	−31(15)
O(137^{I})	1.121 0(15)	0.469 1(12)	0.029 1(26)	146(16)	98(12)	343(27)	−91(14)	−83(17)	27(12)
O(138^{I})	1.187 0(19)	0.362 4(19)	1.004 4(34)	177(21)	214(24)	415(40)	9(24)	−157(25)	39(18)
C(139^{I})	1.259 1(20)	0.439 2(25)	1.143 9(32)	103(19)	288(36)	180(25)	117(25)	−96(19)	−55(22)
C(171	0.823 7(8)	0.426 8(6)	0.379 3(10)	79(7)	42(5)	80(6)	21(5)	−5(5)	−17(4)
C(172)	0.904 5(9)	0.408 5(7)	0.258 3(12)	87(8)	68(6)	104(8)	52(6)	−5(7)	−22(6)
C(173)	0.984 6(8)	0.345 1(7)	0.273 9(10)	78(7)	72(6)	69(6)	17(5)	−8(5)	−34(6)
O(175)	0.973 7(6)	0.254 4(5)	0.165 7(6)	154(6)	77(4)	67(4)	13(4)	−19(4)	−16(4)
O(174)	1.051 2(5)	0.367 3(4)	0.374 5(7)	80(4)	86(4)	87(4)	4(4)	−20(4)	16(4)
C(176)	1.049 4(11)	0.188 4(7)	0.174 6(13)	216(14)	81(7)	130(10)	32(7)	−15(9)	75(9)
C(181)	0.591 7(7)	0.357 1(5)	0.203 6(9)	98(7)	43(4)	100(6)	36(4)	−15(5)	10(4)

* In the form $\exp[-2\pi^2(U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{22}l^2c^{*2} + 2U_{23}klb^*c^* + 2U_{31}lhc^*a^* + 2U_{12}hka^*b^*)]$. Atoms with superscript II have fixed-atom co-ordinates together with a joint isotropic temperature factor for disordered carbon atoms with site-occupation factors of 0.25; those with superscript I are disordered atoms with site-occupation factors of 0.5.

The positions of all hydrogen atoms bonded to non-disordered heavy atoms were clearly revealed by difference-Fourier syntheses and included in the final cycles of refinement. Attempts to refine the positional parameters for H(15), H(N24), H(121)—(123), and H(181)—(183) were however unsatisfactory and these were accordingly fixed. Group isotropic temperature factors were included for equivalent side-chains. Although it was necessary to fix the position of one of the inner ' pyrrole ' N–H protons [H(N24)] there can nevertheless, by reference to the C–N–C angles, be no doubt that this assignment is correct (Figure 1). The value of 105.4(6)° at N(23) is typical for pyrrole rings without imino-hydrogen atoms, as observed in porphyrin macrocycles.[12] A small peak (0.24 eÅ^{-3}) was found at 1.17 Å from N(23) in the final difference Fourier but

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[12] J. W. Lauter and J. A. Ibers, *J. Amer. Chem. Soc.*, 1973, **95**, 5148.

attempts to include this as a hydrogen atom with either fixed or variable positional parameters led immediately to an unreasonably high associated isotropic temperature factor. The terminal value of R_G was 0.116 with a weighted residual R' [$= \Sigma w^{1/2}/\Sigma w^{1/2}F_o$] of 0.094 and a corresponding unweighted R of 0.090. The weights applied were given by expression $w = k/[\sigma^2(F_o) + gF_o^2]$, where k and g refined to 2.455 and 0.001 7, respectively. In the final cycle of refinement, the largest observed shift-to-σ was -0.123. A final difference-Fourier synthesis displayed no peaks or troughs of electron density >0.31 eÅ^{-3}. Complex neutral-atom scattering factors [13,14] were employed for the non-hydrogen atoms. A comparison of the R_G values for the following refinement conditions with that for the final model confirmed that the improvement upon adoption of this latter model is clearly significant at the 0.005 level [15]: (1) With O(134), O(135), C(136), O(137), O(138), and C(139) isotropic and refinement of the s.o.f.: R_G 0.131, R 0.102. (2) Without C(72^{II}) and C(73^{II}): R_G 0.127, R 0.094. (3) Without hydrogen atoms: R_G 0.140, R 0.106. The results from the final least-squares (Tables 1 and 2) were used, together with the full covariance matrix, to calculate the bond lengths and angles and their estimated standard deviations presented in Table 3 and Figure 1.

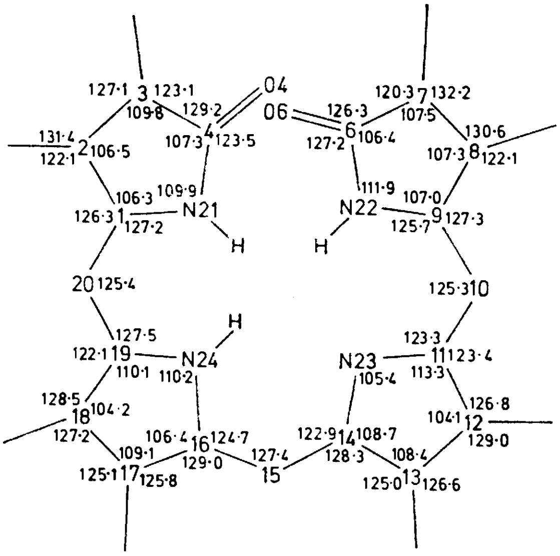

FIGURE 1 Bond angles (°) in the bilatriene skeleton (standard deviations 0.6—0.8°)

TABLE 2

Hydrogen atom co-ordinates and isotropic vibrational amplitudes (Å^2 × 10^3), with estimated standard deviations in parentheses

	x/a	y/b	z/c	U
H(10)	0.815 3(39)	−0.081 3(35)	0.616 3(52)	36(14)
H(15)	0.919 7	0.315 8	0.543 8	213(48)
H(20)	0.470 3(47)	0.162 8(42)	0.119 9(62)	65(19)
H(N21)	0.537 2(42)	0.050 3(37)	0.433 1(55)	45(16)
H(N22)	0.711 0(48)	−0.050 5(43)	0.301 1(65)	69(19)
H(N24)	0.683 2	0.136 3	0.408 3	68(20)
H(131)	1 124 3(56)	0.198 8(50)	0.712 2(76)	98(9)
H(132)	1.081 4(55)	0.296 2(49)	0.626 3(74)	98(9)
H(133)	0.958 4(57)	0.377 0(52)	0.874 9(75)	98(9)
H(134)	1.014 7(58)	0.284 5(50)	0.921 9(75)	98(9)
H(171)	0.777 1(64)	0.476 3(58)	0.360 7(84)	98(9)
H(172)	0.865 9(66)	0.449 3(58)	0.470 6(88)	98(9)
H(173)	0.852 3(63)	0.364 4(56)	0.153 2(67)	98(9)
H(174)	0.949 8(66)	0.463 5(60)	0.274 4(87)	98(9)
H(121)	0.968 9	0.041 8	0.841 2	174(19)
H(122)	1.072 6	0.090 6	0.808 7	174(19)
H(123)	1 005 0	−0.018 5	0.725 9	174(19)
H(181)	0.610 1	0.415 3	0.182 0	174(19)
H(182)	0.532 6	0.335 0	0.132 3	174(19)
H(183)	0.529 4	0.407 7	0.250 4	174(19)

TABLE 3

Interatomic bond distances (Å) with standard deviations in parentheses

N(21)–C(1)	1.396(11)	N(21)–C(4)	1.372(9)
C(1)–C(2)	1.497(9)	C(2)–C(3)	1.347(12)
C(3)–C(4)	1.448(11)	C(4)–O(4)	1.234(11)
N(22)–C(6)	1.372(9)	N(22)–C(9)	1.346(12)
C(6)–O(6)	1.219(13)	C(6)–C(7)	1.497(15)
C(7)–C(8)	1.356(13)	C(8)–C(9)	1.505(11)
C(9)–C(10)	1.351(10)	C(10)–C(11)	1.421(11)
N(23)–C(11)	1.314(11)	N(23)–C(14)	1.428(9)
C(11)–C(12)	1.462(10)	C(12)–C(13)	1.373(12)
C(13)–C(14)	1.429(11)	C(14)–C(15)	1.385(12)
C(15)–C(16)	1.373(12)	N(24)–C(16)	1.374(9)
N(24)–C(19)	1.315(11)	C(46)–C(17)	1.407(12)
C(17)–C(18)	1.394(11)	C(18)–C(19)	1.427(11)
C(19)–C(20)	1.477(10)	C(20)–C(1)	1.357(11)
C(2)–C(21)	1.470(12)	C(21)–C(22^{I})	1.245(16)
C(3)–C(31)	1.499(10)	C(31)–C(32^{I})	1.26(2)
C(7)–C(71)	1.475(11)	C(71)–C(72^{II})	1.13
C(71)–C(73^{II})	1.17	C(8)–C(81)	1.489(16)
C(81)–C(82^{I})	1.207(2)	C(12)–C(121)	1.473(13)
C(13)–C(131)	1.549(9)	C(131)–C(132)	1.519(10)
C(132)–C(133)	1.510(12)	C(133)–O(134^{I})	1.29(2)
C(133)–O(135^{I})	1.27(2)	O(135^{I})–C(136^{I})	1.56(2)
C(133)–O(137^{I})	1.05(2)	C(133)–O(138^{I})	1.29(3)
O(138^{I})–C(139^{I})	1.58(3)	C(171)–C(171)	1.545(11)
C(171)–C(172)	1.536(15)	C(172)–C(173)	1.493(16)
C(173)–O(174)	1.198(11)	C(173)–O(175)	1.344(9)
O(175)–C(176)	1.460(16)	C(18)–C(181)	1.493(13)
C(10)–H(10)	0.84(6)	C(15)–H(15)	1.09
C(20)–H(20)	1.10(7)	N(21)–H(N21)	1.12(5)
N(22)–H(N22)	0.95(7)	N(24)–H(N24)	0.93
C(131)–H(131)	1.39(8)	C(131)–H(132)	1.12(8)
C(132)–H(133)	1.13(8)	C(132)–H(134)	1.07(8)
C(171)–H(171)	1.05(9)	C(171)–H(172)	0.95(8)
C(172)–H(173)	1.13(7)	C(172)–H(174)	0.88(8)
H(121)–H(121)	0.95	C(121)–H(122)	0.94
C(121)–H(123)	1.13	C(181)–H(181)	1.05
C(181)–H(182)	1.00	C(181)–H(183)	0.97
O(4) · · · N(21)	2.799	O(4) · · · H(N21)	1.74

DISCUSSION

The crystal structure determination confirms that biliverdin dimethyl ester (II) and thereby presumably biliverdin (I) is present in the lactam form. Both these compounds show amide carbonyl absorption, at 1 655 and 1 640 cm^{-1}, respectively.[10] The two C=O distances [1.234(11) and 1.219(13) Å] in (II) are typical for this bond and furthermore only three imino-hydrogen atoms could be located, as would be expected for the lactam formulation. Despite the various types of disorder associated with the chromophore side-chains, there is no evidence for any disorder, whether dynamic or static, of the inner 'pyrrole' N–H protons. Further evidence for the correctness of this assignment of the imino-hydrogen atoms is provided, as mentioned previously, by a comparison of the internal C–N–C angles [109.9(6), 111.9(7), 105.4(6), and 110.2(7)°].

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[13] D. T. Cromer and J. T. Waber, *Acta Cryst.*, 1965, **18**, 104.

[14] D. T. Cromer and D. Libermann, *Acta Cryst.*, 1970, **53**, 1891.

[15] W. C. Hamilton, *Acta Cryst.*, 1965, **18**, 502.

The biliverdin chromophore takes up a helical conformation which leads to an intramolecular distance of 3.34 Å between O(4) and O(6). Dihedral angles of 9.2, −20.8, −9.8, and 19.3° are observed between the planes of the pyrrole rings based on N(21)—(24) respectively. A projection of the molecule, including for the sake of clarity only one example of each type of disorder, on a least-squares plane through the four nitrogen atoms [distances N(21)—(24), 0.280, −0.325, 0.396, −0.409 Å] is shown in Figure 2. Consideration of the short intramolecular distances between adjacent pyrrolone rings [O(4) · · · N(22) 3.38, O(4) · · · C(6) 3.05, O(4) · · · C(7) 3.35, O(6) · · · N(21) 3.57, O(6) · · · C(4) 3.18, O(6) · · · C(3) 3.47, C(4) · · · C(6) 3.25, C(4) · · · N(22) 3.54, C(6) · · · N(21) 3.59 Å] leads to the conclusion that the bilatriene skeleton takes up that conformation which involves the best compromise between two mutually exclusive goals: (1) the preservation of a cyclic planar structure similar to that of the porphyrin macrocycle, and (2) minimisation of the intermolecular interactions between adjacent pyrrolone rings. This finding is particularly interesting in view of the fact that e.s.r. studies on biliverdin and related bilatriene metal complexes have demonstrated that the ' far-red forms ' have a cyclic and near-planar conformation in solvents such as chloroform or methanol.[7] As this determination has shown that the shorter wavelength or ' red form ' (II) also has such a conformation, rather than the open-chain form previously suggested,[8] this suggests that the shifts in absorption maxima which accompany addition of strong acid or base to such systems are in fact associated with very small changes in the bilatriene skeleton, involving simple stretching of the molecule perpendicular to its molecular plane. Likewise, by extrapolation, the two forms of the chromophore of the plant hormone phytochrome may also have very similar cyclic conformations rather than being accounted for by *cis–trans* geometric isomerisation as was discussed earlier.

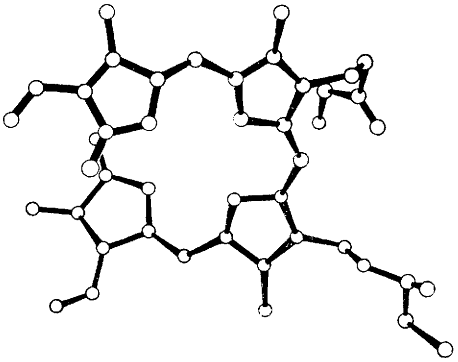

FIGURE 2 The molecule of (II) shown in projection upon the nitrogen atom least-squares plane

Figure 2 shows that there is considerable bond fixation and little evidence for extended π-delocalisation within the bilatriene skeleton of (II). In particular, there are very short distances for the N(23)–C(11) and N(24)–C(19) bonds [1.314(11) and 1.315(11) Å]. Two molecules of (II), related by a crystallographic centre of symmetry, are linked into dimers with an extended helical conformation, through two N–H · · · O hydrogen bonds [N(21)–H · · · O(4) 2.80, N(21)–H 1.12, H · · · O(4) 1.74 Å] between symmetry-equivalent pyrrolone rings. The resultant mode of dimer formation may best be appreciated from Figure 3. (Figure 4 shows a projection of unit-cell contents.) The other pyrrolone oxygen O(6) does not take part in any hydrogen bonding. It is interesting to speculate whether such dimerisation will be observed for biliverdin itself, where protons of the free propionic acid side-chains are also available for hydrogen bonding. A sharp absorption band at 1 680 cm^{-1} in the spectrum of (I), not observed for (II), has been attributed to the propionic acid carbonyl groups. It has been suggested [10] that its sharpness, and the fact that it occurs at relatively low-frequency, may be a result of intramolecular hydrogen bonding between adjacent acid groups. Hydrogen-bonded N–H absorption appears over a broad range around 3 100 cm^{-1} for (I), which could also theoretically involve the propionic acid groups. However such an intra- or inter-molecular interaction must also involve considerable changes in the bilatriene skeleton in (I) as opposed to that in (II). Such changes

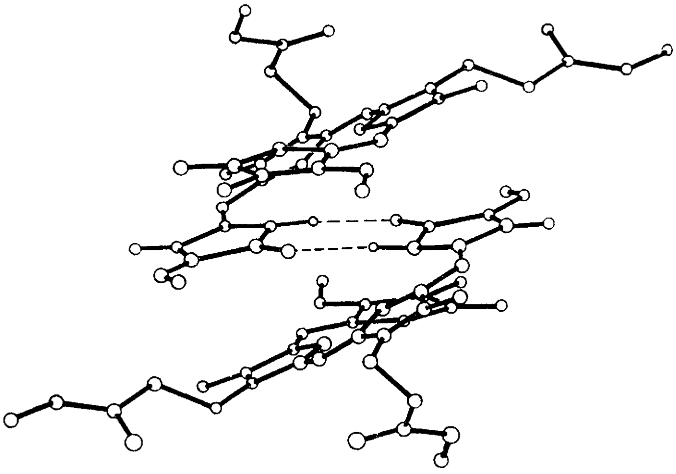

FIGURE 3 The mode of dimerisation of (II)

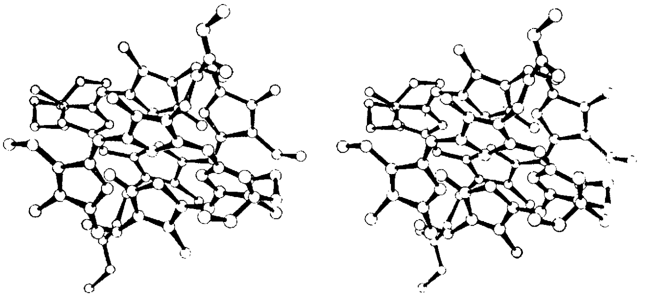

FIGURE 4 Projection of the unit-cell contents perpendicular to [001]

are not easily compatible with the evidence of the e.s.r. studies. There are no particularly short intermolecular contacts involving the ester side-chains in (II).

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