Solid-state Self-association of the Two-electron Oxidation Product of a Biliverdin Analogue

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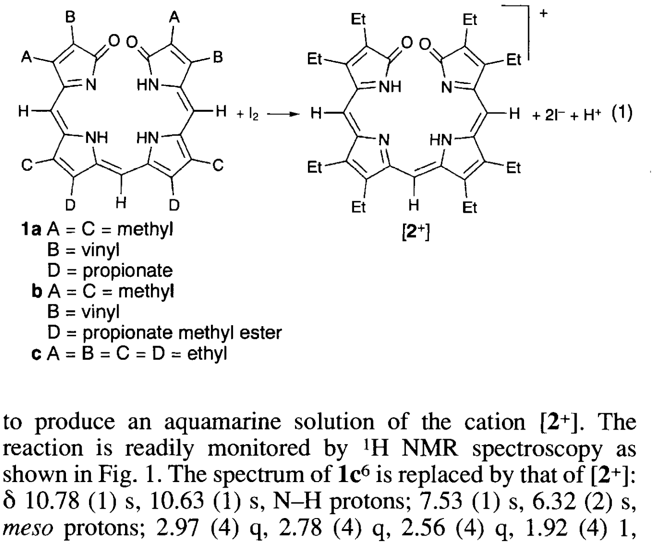
Oxidation of octaethylbilindione with one molar equivalent of iodine produces an open-chain tetraaza cation $[2^+]$ which self-associates in the solid state through a set of hydrogen bonds to form a dimeric entity that consists of two interpenetrating C-shaped units.

Biliverdin 1Xa **1a** is formed by haem oxygenase through oxidative attack upon the α-methine position of haem.[1] During haem catabolism, biliverdin is generally reduced to bilirubin. However, recent studies have suggested that biliverdin itself may have two significant physiological activities: anti-oxidant activity[2] and anti-HIV activity.[3,4] In order to illuminate the molecular basis for these potentially beneficial biological activities, it is important to acquire a thorough knowledge of the chemical behaviour of biliverdin, especially in redox reactions that may be relevant to the antioxidant activity. This laboratory[5] has recently explored the geometric and electronic structures of transition metal complexes of the symmetric biliverdin analogue octaethylbilindione **1c**.[6] During that work we discovered that **1c** undergoes facile 2-electron oxidation. Here we report on the spectroscopic and crystallographic characterization of the oxidation product, the cation $[2^+]$.

Titration of a blue chloroform solution of **1c** with diiodine reveals that **1c** is oxidized by 1 mol equiv. of diiodine [eqn. (1)] to produce an aquamarine solution of the cation $[2^+]$. The reaction is readily monitored by ^{1}H NMR spectroscopy as shown in Fig. 1. The spectrum of **1c**[6] is replaced by that of $[2^+]$: δ 10.78 (1) s, 10.63 (1) s, N–H protons; 7.53 (1) s, 6.32 (2) s, *meso* protons; 2.97 (4) q, 2.78 (4) q, 2.56 (4) q, 1.92 (4) 1, methylene protons; 1.35 (6) t, 1.23 (6) t, 1.11 (6) t, 0.78 (6) q, methyl protons. The two lowest field, equally intense resonances decrease in intensity when exposed to D_2O or when the cation is deprotonated by treatment with sodium methoxide. The electronic absorption spectrum of $[2^+]$ shows strong features in the ultraviolet [λ_{max}/nm (ε/dm^3 cm^{-1} mol^{-1}): 298 (3.5×10^4), 354 (4.6×10^4)sh] and in the visible [656 (1.7×10^4), 706 (1.9×10^4)sh]. The cation has been isolated in 61% yield by precipitation of the salt $[2^+]I_3{\cdot}0.5I_2{\cdot}0.5CH_2Cl_2{\cdot}0.2C_6H_{14}$ through the addition of excess iodine and hexane to a dichloromethane solution of the cation.



1a A = C = methyl
B = vinyl
D = propionate
b A = C = methyl
B = vinyl
D = propionate methyl ester
c A = B = C = D = ethyl

$[2^+]$

The results of an X-ray diffraction study† of $[2^+]I_3{\cdot}0.5I_2{\cdot}0.5CH_2Cl_2{\cdot}0.2C_6H_{14}$ are presented in Figs. 2–4. Fig. 2 shows a view of an individual cation of $[2^+]$, and gives some of its molecular dimensions. In the solid state both the biliverdin dimethyl ester **1b**[7] and $[2^+]$ have approximate C_2 symmetry, but the cation $[2^+]$ is more nearly planar than is **1b** or related biliverdin derivatives.[8] These frequently form helical units in which the two keto groups overlap one another. The helical arrangement of **1b** is shown in Fig. 3(*a*). Fig. 3(*b*) shows the structure of $[2^+]$ from a similar perspective.

The flattening of the cation appears to be a consequence of a tight pairing of the cations through hydrogen bonds. The

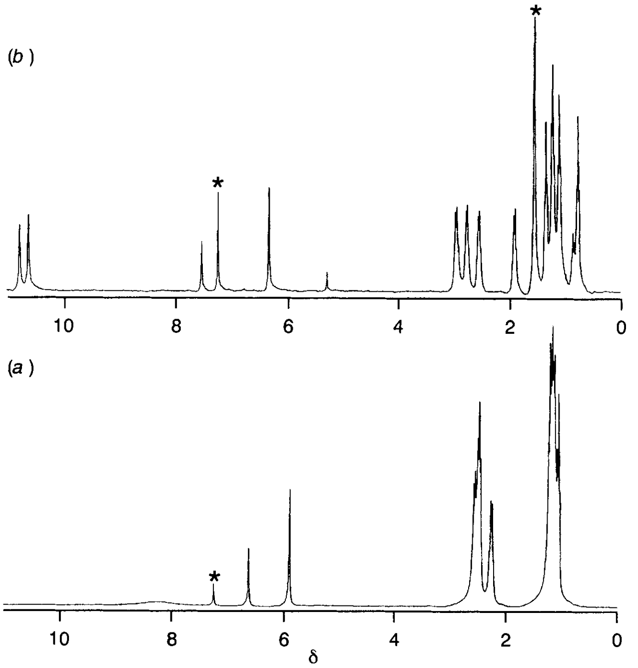


Fig. 1 ^{1}H NMR spectra of (*a*) **1c**, and (*b*) $[2^+]I_3$ in $CDCl_3$ solutions at 25 °C. Resonances due to impurities are denoted by *.

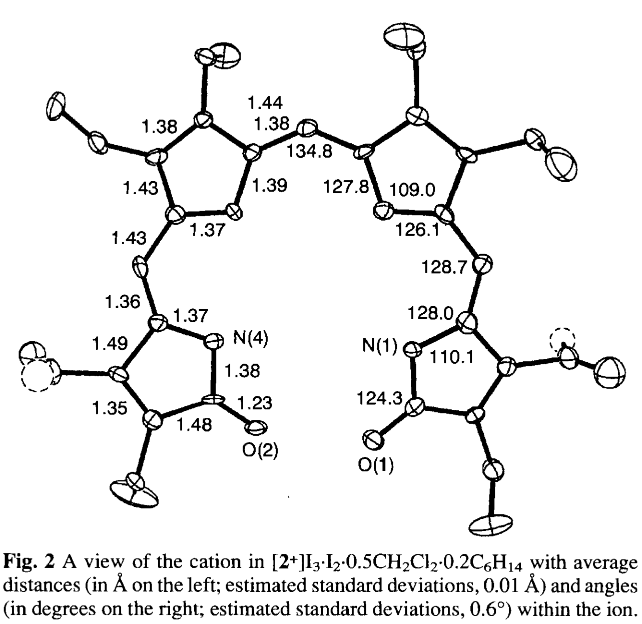


Fig. 2 A view of the cation in $[2^+]I_3{\cdot}I_2{\cdot}0.5CH_2Cl_2{\cdot}0.2C_6H_{14}$ with average distances (in Å on the left; estimated standard deviations, 0.01 Å) and angles (in degrees on the right; estimated standard deviations, 0.6°) within the ion. The alternate positions of the methyl groups of the two disordered ethyl groups are shown as dashed lines.

interconnections between the two individual cations in such a pair are shown in Fig. 4. The two cations pack around a twofold axis and form an interpenetrating pair of C-shaped units. The angle between the planes of the four nitrogen atoms of the two C-shaped entities is 78.1°. There are hydrogen atoms on three of the four nitrogen atoms of the cation, but sites H(3) and H(4) have only half occupancy while site H(1) is fully occupied. Each N–H unit is hydrogen-bonded to a nearby keto group. The hydrogen-bonded pair, N(1)–H(1)···O(2′) and N(4′)–H(4′)···O(1), form a unit that is analogous to the one present in crystalline **1b**. However, in [**2**$^+$] the two cations are coiled so that both ends engage in such a hydrogen-bonding scheme, whereas in **1b** only one end of each molecule is hydrogen-bonded to the adjacent molecule. Distances within these units (with an assumed N–H distance of 0.94 Å) are: N(1)–O(2′) 2.88(1), H(1)–O(2′) 1.96(1), H(4)–O(1′) 1.92(1), N(4)–O(1′) 2.83(1) Å. Additionally, there is weaker hydrogen-bonding from N(3)–H(3) to O(2′) with an H(3)–O(2′) distance of 2.04(1) Å and an N(3)–O(2′) distance of 2.93(1) Å. The dimeric structure seen here does not seem to be sensitive to the anion that accompanies it. We have observed similar dimeric units in the crystalline salts, [**2**$^+$][$FeCl_4$]0.5 (thf) and [**2**$^+$][$FeBr_4$].

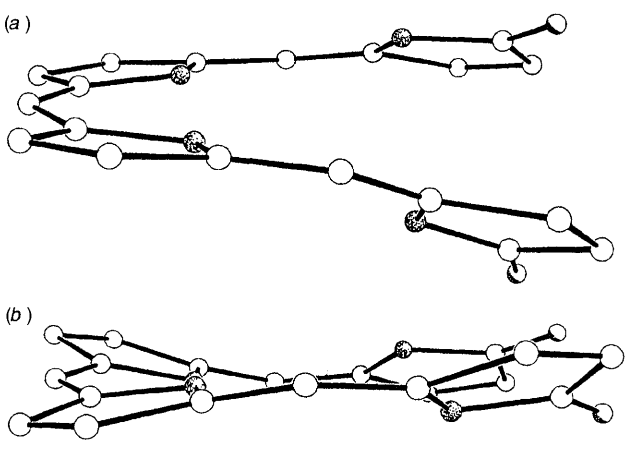


Fig. 3 Drawings of (*a*) biliverdin dimethyl ester **1b**, and (*b*) [**2**$^+$] from related perspectives. For clarity the ethyl groups have been omitted. The nitrogen atoms are stippled and the oxygen atoms are shaded.

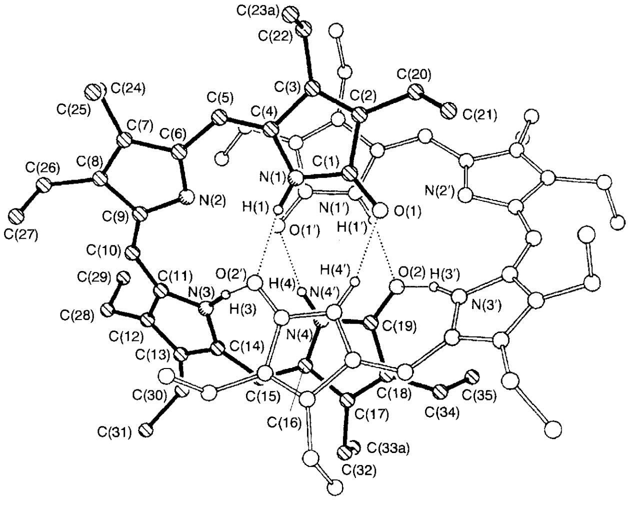


Fig. 4 A diagram which emphasized the relative orientation of, and the hydrogen bonding between, a pair of the cations [**2**$^+$]. The site H(1) is fully occupied while sites H(3) and H(4) have occupancies of 0.5.

In order to accommodate the hydrogen-bonding scheme shown in Fig. 4, the central enclosed portion of the cation [**2**$^+$] has opened relative to that in the helical biliverdin derivatives such as **1b**. In **1b** the N(1)···N(4) separation is 3.42 Å, whereas in [**2**$^+$] it is 4.64 Å. This opening is accomplished by enlargement of the internal angles, especially those at the *meso* carbon atoms as shown in Fig. 2. As a further consequence of these changes, the O···O separation in [**2**$^+$] (2.98 Å) is less than that in helical **1b** (3.34 Å).

Further work to uncover the molecular basis of the antioxidant activity of biliverdin and the possible role of the oxidation described in eqn. (1) is in progress.

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Footnote

† *Crystal data*: aquamarine plates of [$C_{35}H_{45}N_4O_2$]I_3·0.5I_2·0.5CH_2Cl_2·0.2C_6H_{14} that were obtained by diffusion of hexane into a dichloromethane solution of the compound form in the monoclinic space group *P*2/*c* with *a* = 10.241(3), *b* = 15.021(2), *c* = 27.783(6) Å, β = 91.96(2)° at 130 K with *Z* = 4 with the use of graphite-monochromated Mo-Kα (λ = 0.71073 Å) radiation. Refinement of 5102 reflections with $F > 4.0\sigma(F)$ and 420 parameters yielded $R = 0.050$, $R_w = 0.055$. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Information for Authors, Issue No. 1.

References

1 M. D. Maines, *Heme Oxygenase: Clinical Applications and Functions*, CRC Press, Boca Raton, FL, 1992.
2 R. Stocker, Y. Yamamoto, A. F. McDonagh, A. N. Glazer and B. N. Ames, *Science*, 1987, **235**, 1043.
3 T. Nakagami, S. Taji, M. Takahashi and K. Yamanishi, *Microbiol. Immunol.*, 1992, **36**, 381.
4 H. Mori, T. Otake, M. Morimoto, N. Ueba, N. Kunita, T. Nakagami, N. Yamasaki and S. Taji, *Jpn. J. Cancer Res.*, 1991, **82**, 755.
5 A. L. Balch, M. Mazzanti, B. C. Noll and M. M. Olmstead, *J. Am. Chem. Soc.*, 1994, **116**, 9114; A. L. Balch, M. Mazzanti, B. C. Noll and M. M. Olmstead, *J. Am. Chem. Soc.*, 1993, **115**, 12206; A. L. Balch, L. Latos-Grażyński, B. C. Noll, M. M. Olmstead and N. Safari, *J. Am. Chem. Soc.*, 1993, **115**, 9056.
6 R. Bonnett, D. G. Buckley and D. Hamzetash, *J. Chem. Soc., Perkin Trans. 1*, 1981, 322.
7 W. S. Sheldrick, *J. Chem. Soc., Perkin Trans. 2*, 1976, 1457.
8 W. S. Sheldrick, *Isr. J. Chem.*, 1983, **23**, 155.