A Novel 2,18-Bridged Biliverdin Derivative

Edward D. Sturrock,*[a] James R. Bull,[b] Ralph E. Kirsch,[a] Ravindra K. Pandey,[c,d] Mathias O. Senge[c] and Kevin M. Smith[c]

[a] *MRC/UCT Liver Research Center, Department of Medicine and* [b] *Department of Chemistry, University of Cape Town, Cape Town 7925, South Africa*

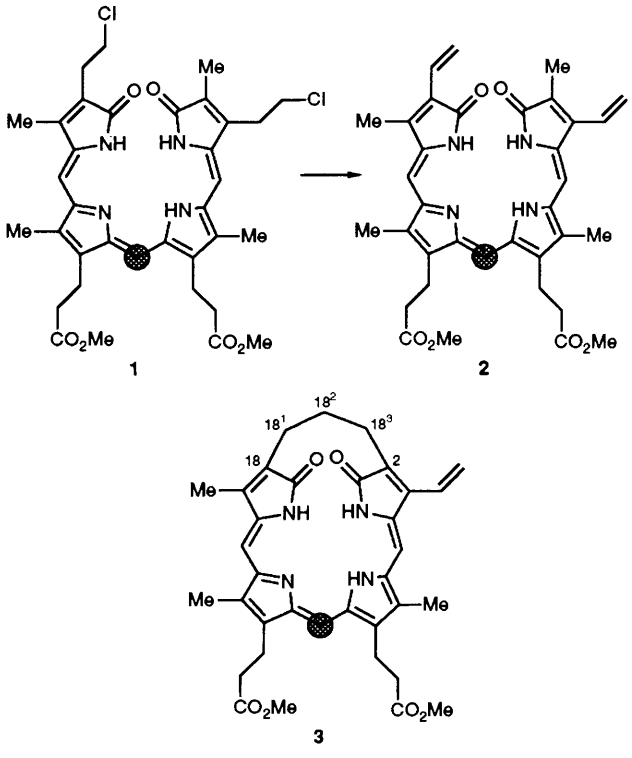
[c] *Department of Chemistry, University of California, Davis, CA 95616, USA*

[d] *Chemistry Division, PDT Center, Department of Radiation Medicine, Roswell Park Cancer Institute, Buffalo, NY 14265, USA*

Base-catalysed dehydrohalogenation of a 3,18-bis(2-chloroethyl)biliverdin **1** affords a novel 3-vinylbiliverdin **3** in which the 2- and 18-positions are bridged with a propano-tether; the structure of **3** has been established using single crystal X-ray and ^{1}H nuclear Overhauser effect studies.

Biliverdins are biologically important products of the oxidative cleavage of haem and are observed in almost all organisms.[1] Derivatives, such as phytochrome, also serve a functional role as the photoreceptor that mediates photomorphogenesis in higher plants and some algae.[2] Biliverdins with a fixed helical geometry have been used to investigate the conformational changes of bilatrienes bound to peptides.[3] The synthesis of such compounds with a four-membered bridge connecting the pyrrolinone rings was achieved by treating biliverdin-IIIα with acidic methanol.[4]

For some time we have been interested in the metabolism of bilirubin in neonates with idiopathic hyperbilirubinaemia. For this purpose we have synthesized [10-^{13}C]biliverdin-IXα[5] employing a previously developed convergent route.[6] Formation of the vinyl groups in the synthesis of bile pigments has usually been accomplished by transformation of urethane groups,[7] by dehydroselenylation of phenylselenylethyl functions,[8,9] or by dehydrohalogenation of 2-chloroethyl substituents.[6,10] 2-Chloroethyl was chosen as the protected vinyl substituent in our synthesis. In this report we describe the novel formation and full characterization of a bridged biliverdin derivative produced as a result of the dehydrohalogenation of bis(2-chloroethyl)biliverdin **1** (Scheme 1).



Scheme 1 Dehydrohalogenation of bis(2-chloroethyl)biliverdin **1** to give biliverdin-IXα dimethyl ester. The hatched circle indicates ^{13}C enrichment at the 10-position.

[10-^{13}C]-Labelled biliverdin **1** was synthesized by following the literature procedure.[6] Treatment of **1** with aqueous potassium hydroxide in refluxing pyridine afforded two major products. The compounds were separated by semi-preparative high-performance liquid chromatography.† The first major fraction was identified as the desired biliverdin-IXα dimethyl ester **2**, identical with the authentic sample. In the NMR spectrum, a doublet at δ 6.78 was observed for the 10-^{13}C proton (*J* 156.9 Hz). The field-desorption mass spectrum (FDMS) showed a molecular ion at *m/z* 611, consistent with the molecular formula $C_{35}H_{38}N_4O_6$ containing one ^{13}C atom.

The other (less polar) compound proved to be more enigmatic. The proton NMR spectrum showed disappearance of the high-field C-2 methyl singlet at δ 1.86. Only one vinyl group was observed suggesting participation of the other chloroethyl in an unexpected side reaction. Three high-field signals (integral 6H) suggested the presence of three methylene groups. The ^{13}C spectrum of the second fraction was very similar to that of biliverdin but lacking one methyl carbon and only one set of vinylic carbons was present. Three signals at δ 23.08, 23.46 and 30.15 provided supporting evidence that the unassigned signals in the proton NMR spectrum were six methylene protons. The high resolution mass spectrum showed a molecular ion at *m/z* 611.‡

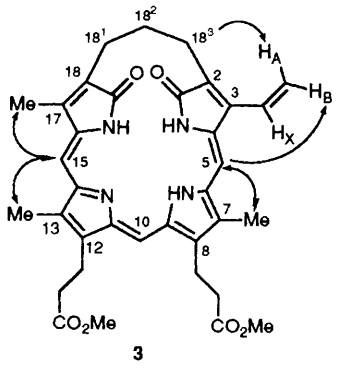
The COSY (correlation spectroscopy) as well as HETCOR NMR spectra showed connectivities which confirmed the presence of three methylenes of the propano bridge. However, no long range coupling between the bridge protons and the substituents of rings A and D was apparent from the spectra. Nuclear Overhauser effect (NOE) experiments were carried out to establish whether any through-space effects could be observed between the propano bridge and its neighbouring substituents. NOE enhancements (3-vinyl to 5-H, 7-Me to 5-H, 13-Me to 15-H, 15-H to 17-Me) confirmed the (5*Z*,15*Z*) conformation of the bilatriene (Fig. 1). Although no bridge NOEs were observed when the 17-Me was

† HPLC conditions: room temp.; 10 mm MicroPorasil (125-Å) column (2.5 × 10 cm); RCM PrePak 25 × 10 cartridge; flow rate 10 ml min^{-1}; absorbance detector set at 375 nm; solvent toluene–acetone–pyridine (95 : 4 : 1).

‡ [10-^{13}C]-8,12-Bis(2-methoxycarbonylethyl)-7,13,17-trimethyl-2,18-propano-3-vinylbilin-1,19(21*H*,24*H*)-dione **3**. Yield 24%, m.p. 227–229 °C. UV–VIS: λ_{max} nm (ε/dm^3 mol^{-1} cm^{-1}): 310 (28 000), 381 (37 000) and 642 (15 300). ^{1}H NMR (300 MHz): δ 2.36, 2.39 and 2.40 (each 3H, s, 7-, 13- and 17-Me), 2.52, 2.78 and 2.93 (6H, m, 18^1, 18^2 and 18^3 CH_2), 3.23 and 3.24 (each 6H, t, CH_2CO_2), 3.69 (6H, s, 2-CO_2Me), 5.11 (1H, s, NH), 5.36 (1H, s, NH), 5.78, 5.74 and 6.88 (3H, ABX, J_{AX} 17.8, J_{BX} 11.6, J_{AB} 1.6 Hz, 3- CH=CH_2), 6.75 (1H, s, 15-H), 6.88 (1H, s, 5-H), 7.54 (1H, d, *J* 156.9 Hz, 10-CH), 9.98 (1H, br s, NH). ^{13}C NMR (75 MHz): δ 9.71, 9.74 and 10.43 (7-, 13- and 17-Me), 20.37, 23.46 and 30.15 (C-18^1, C-18^2, C-18^3), 35.63 (C-8^2 and C-12^2), 51.77 (8^3-and 12^3-OMe), 97.96 (C-5), 99.64 (C-15), 112.15 (C-10), 122.83 (C-3^2), 127.21 (C-3^1), 165.95 (C-1 and C-19) and 166.49 (2 × CO_2Me). HRMS: Found: M$^+$ = 611.284, $C_{35}H_{39}N_4O_6$ requires *M*, 611.287.

saturated, **3** did exhibit an NOE from the C-3 vinyl group to one of the C-18[3] methylene protons.

The UV spectrum of bridged biliverdin **3** showed an additional absorption band (310 nm) flanking the characteristic band at 381 nm and a broad band at 643 nm. Recent studies in extended tetrapyrrole[11] and bridged biliverdin chemistry[12] show that helical conformations such as that conferred by the bridge, give rise to a third low wavelength band. The UV data thus provides supporting evidence for the proposed structure.

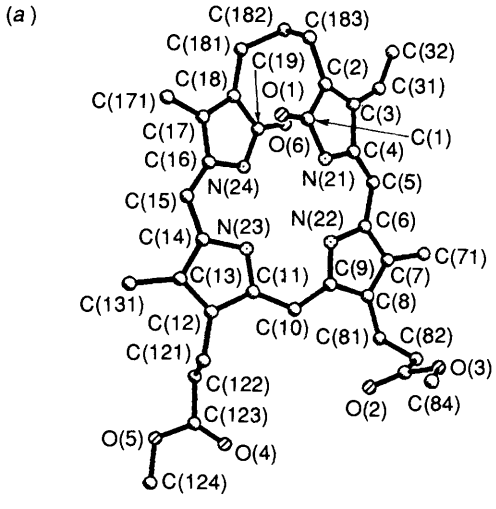


3

Fig. 1 The NOE network of connectivities in bridged biliverdin derivative **3**. Double headed arrows indicate bi-directional connectivities. Vinyl proton nomenclature is also shown.

The X-ray structure of compound **3** is shown in Fig. 2.§ The propano character of the bridging unit is clearly evidenced by the bond lengths of 1.467(9), 1.486(14), 1.556(15) and 1.492(10) Å for the C(18)–C(18[1]), C(18[1])–C(18[2]), C(18[2])–C(18[3]) and C(18[3])–C(2) bonds, respectively. The vinyl character of the substituent at C(3) is confirmed by the short bond length of 1.222(10) Å for the C(3′)–C(3[2]) bond. The molecule has a helical shape [Fig. 2(*b*)] with the bridged pyrrole rings rotated against each other to minimize contacts.

Formation of a bridged biliverdin in the presence of base is unprecedented. In acidic media biliverdin-IIIα undergoes cyclisation to form the unstrained bridged bilatriene.[4] Tetrapyrrole methyl groups flanked by vinyl[13–15] or carbonyl[15,16] groups have been shown to be unusually acidic. In the case of the formation of **3**, we propose (Scheme 2) that a proton abstraction from the 2-Me results in formation of an active methylene species. Attack at the 18[2]-carbon and elimination of the halide ion generates the intramolecular bridge by means of an E2-type mechanism. The competitive nature of this intramolecular elimination reaction depends on both the helical conformation of the bilatriene backbone and the position and orientation of the chloroethyl group.



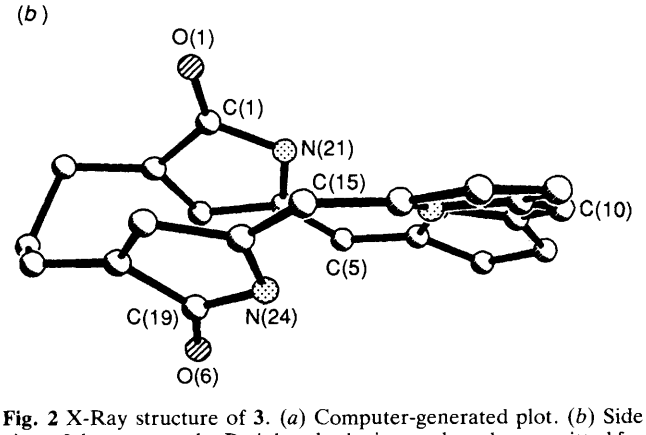
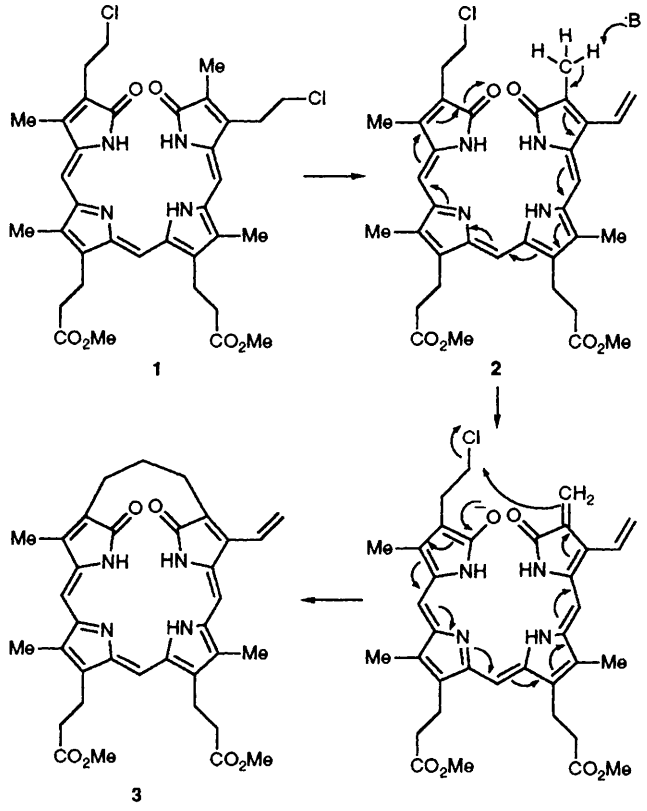


Fig. 2 X-Ray structure of **3**. (*a*) Computer-generated plot. (*b*) Side view of the macrocycle. Peripheral substituents have been omitted for clarity.



Scheme 2 Proposed mechanism for formation of bridged biliverdin **3** from **1**

§ *Crystal data* for **3**: blue parallelepipeds from CH_2Cl_2–hexane; $C_{35}H_{39}N_4O_6$, monoclinic, $P2_1/c$; 130 K, Mo-Kα, a = 15.277(9), b = 20.445(11), c = 10.233(4) Å, β = 100.58(4)°, V = 3142(3) Å^3; 3306 observed reflections with $I > 1.65\sigma(I)$; R = 0.095. The structure suffers from disorder in the propano bridge, the C(3) vinyl group and the propionic ester side chains. Atomic coordinates, bond lengths, and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

We thank H. Spies, who kindly performed the NOE experiments. This research was supported by grants from the National Institutes of Health (HL 22252) and the S. A. Medical Research Council.

Received, 12th February 1993; Com. 3/00869J

References

1 A. F. McDonagh, in *The Porphyrins*, ed. D. Dolphin, Academic Press, New York, 1979, vol. 6.
2 H. Scheer, *Angew Chem., Int. Ed. Engl.*, 1981, **20**, 241; W. Rüdiger and F. Thümmler, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 1216.
3 P. Nesbadba and A. Gossauer, *J. Am. Chem. Soc.*, 1987, **109**, 6545; M. Bois-Choussy and M. Barbier, *Heterocycles*, 1978, **9**, 677.
4 D. Krois and H. Lehner, *J. Chem. Soc., Perkin Trans. 1*, 1989, 2179.
5 E. D. Sturrock, J. R. Bull and R. E. Kirsch, unpublished work.
6 K. M. Smith and R. K. Pandey, *Tetrahedron*, 1984, **40**, 1749.
7 H. Plieninger, F. El-Barkawi, K. Ehl, R. D. Kohler and A. F. McDonagh, *Liebigs Ann. Chem.*, 1972, **758**, 195.
8 J. Weller and A. Gossauer, *Chem. Ber.*, 1980, **113**, 1603.
9 A. Gossauer and J. Weller, *Chem. Ber.*, 1978, **111**, 486.
10 K. M. Smith and D. Kishore, *J. Chem. Soc., Chem. Commun.*, 1982, 888.
11 H. Falk, *The Chemistry of Linear Oligopyrroles and Bile Pigments*, Springer Verlag, New York, 1989.
12 D. Krois, H. Kahlig and H. Lehner, *J. Chem. Soc., Perkin Trans. 1*, 1992, 1043.
13 B. Evans, K. M. Smith, G. N. La Mar and D. B. Viscio, *J. Am. Chem. Soc.*, 1977, **99**, 7070.
14 K. M. Smith, D. W. Parish and W. S. Inouye, *J. Org. Chem.*, 1986, **51**, 666.
15 K. M. Smith, F. Eivazi, K. C. Langry, J. A. P. B. de Almeida and G. W. Kenner, *Bioorg. Chem.*, 1979, **8**, 485.
16 K. M. Smith, H.-K. Leung and D. W. Parish, *J. Chem. Res. (S)*, 1986, 324; *J. Chem. Res. (M)*, 1986, 2743.