

THE CHEMISTRY OF PYRROLIC COMPOUNDS

XI.* ACETYLATION OF DEUTEROCHLORIN IX

By P. S. CLEZY† and D. B. MORELL‡

[Manuscript received March 19, 1970]

In connection with an investigation of the prosthetic group of myeloperoxidase¹⁻³ chlorins having electrophilic substituents at β -positions were required so that a spectroscopic comparison could be made between selected haemoprotein derivatives of this type of chlorin and corresponding derivatives of the enzyme. The availability of deuteriochlorin^{4,5} suggested one method by which the required class of compound could be obtained since the electrophilic substitution of the metal complexes of tetrapyrroles is well known.⁶

It has now been shown that treatment of iron(III)-deuteriochlorin IX with acetic anhydride in the presence of stannic bromide, followed by removal of the metal, yields a β -acetyl derivative of deuteriochlorin IX albeit in rather low and variable yield. The acetylchlorin was separated by alumina chromatography from a small quantity of residual deuteriochlorin IX and from other products which possibly arise by further attack at the *meso*-carbon atoms. These positions are known to be susceptible to electrophilic attack in chlorin and porphyrin systems.⁶

Results of the spectroscopic investigation of β -electrophilically substituted chlorins and their derivatives and the relationship of this type of system to myeloperoxidase will be reported elsewhere⁷ but certain aspects of the n.m.r. spectrum of β -acetylideuteriochlorin IX bear on the structure of this chlorin and on the structure of deuteriochlorin IX itself and are discussed here.

* Part X, *Aust. J. Chem.*, 1970, **23**, 603.

† Department of Organic Chemistry, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033.

‡ Institute of Medical Research, Royal North Shore Hospital, Crows Nest, N.S.W. 2065.

¹ Newton, N., Morell, D. B., Clarke, L., and Clezy, P. S., *Biochim. biophys. Acta*, 1965, **96**, 476.

² Morell, D. B., Chang, Y., and Clezy, P. S., *Biochim. biophys. Acta*, 1967, **136**, 121.

³ Morell, D. B., Chang, Y., Hendry, I., Nichol, A. W., and Clezy, P. S., in "Structure and Function of Cytochromes." (Eds. K. Okunuki, M. D. Kamen, and I. Sekuzu.) p. 563. (University of Tokyo Press: 1968.)

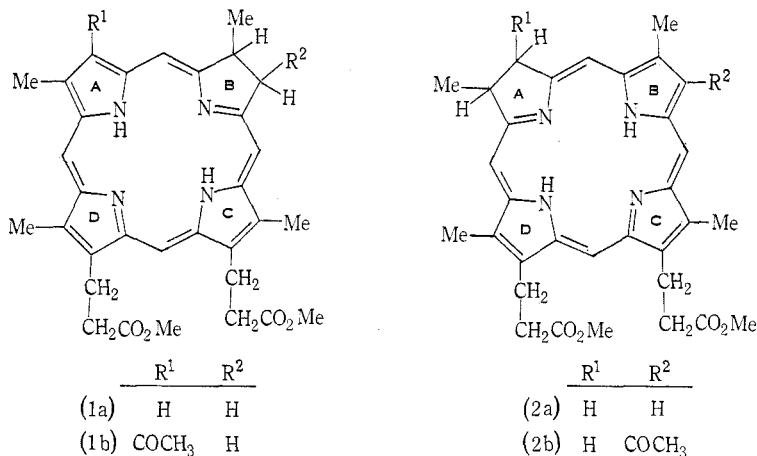
⁴ Fischer, H., and Wecker, G., *Hoppe-Seyler's Z. physiol. Chem.*, 1941, **272**, 1.

⁵ Chang, Y., Clezy, P. S., and Morell, D. B., *Aust. J. Chem.*, 1967, **20**, 959.

⁶ Inhoffen, H. H., Buchler, J. W., and Jager, P., *Fortschr. Chem. org. NatStoffe*, 1968, **26**, 284, and references contained therein.

⁷ Chang, Y., Morell, D. B., Nichol, A. W., and Clezy, P. S., *Biochim. biophys. Acta*, in press.

The n.m.r. spectra of porphyrins are characterized by strong ring current effects, and in consequence the methine protons are found at very low field (*c.* τ 0.00). The reduction of a porphyrin to give a chlorin has a marked effect on the position of these resonances in so far as the two methine protons adjacent to the pyrrole ring which has been reduced are now found at considerably higher field (*c.* τ 1.50) while the other methine signals are not significantly affected. Deuterochlorin⁵ shows this type of spectrum with signals at τ 1.59 (2H), 0.50, and 0.48. However, in β -acetyldeuterochlorin IX one of the low-field protons is further deshielded and appears at τ -0.17, a shift which must result from the substitution of the acetyl group at a β -carbon atom adjacent to this methine proton. Similar n.m.r. changes have been noted by Inhoffen and his colleagues⁸ when some chlorophyll-derived chlorins have been acetylated.



On the basis of earlier n.m.r. studies we have shown that deuterochlorin dimethyl ester⁵ has either structure (1a) or (2a) or perhaps is a mixture of the two isomers although we could find no evidence to suggest that our product was not homogeneous. It follows from the n.m.r. spectrum of acetyldeuterochlorin IX that this derivative has structure (2b) since if (1b) were in fact its structure one of the high-field methine protons would have experienced the deshielding effect of the acetyl group. One can conclude from this that if deuterochlorin IX, as obtained by reduction of deuterohaemin with sodium in isopentyl alcohol, is homogeneous it has structure (2a) and reduction has occurred specifically in ring A.

Experimental

Ferrideuterochlorin IX (700 mg), obtained from deuterochlorin IX by the method of Morell *et al.*,⁹ was treated at room temperature with acetic anhydride (50 ml) and freshly distilled stannic bromide (2 ml). After 10 min, ether (1.5 l.) was added to the mixture, the solution washed well with hydrochloric acid (6 \times 500 ml; 1N) and dried (sodium sulphate). The solvent was removed under reduced pressure, the residue dissolved in glacial acetic acid (1 l.), and the iron removed by the ferrous sulphate procedure.¹⁰ The metal-free products were transferred to

⁸ Inhoffen, H. H., Klotmann, G., and Jeckel, G., *Liebigs Ann.*, 1966, **695**, 112.

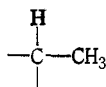
⁹ Morell, D. B., Barrett, J., and Clezy, P. S., *Biochem. J.*, 1961, **78**, 793.

¹⁰ Morell, D. B., and Stewart, M., *Aust. J. exp. Biol. med. Sci.*, 1956, **34**, 211.

ethereal solution which was washed free of acetic acid by repeated extractions with water, treated with ethereal diazomethane to re-esterify any ester groups hydrolysed during the earlier operations, and dried (Na_2SO_4). The tetrapyrroles in this ethereal solution were chromatographed on alumina (Schuchardt, activity I, neutral). Elution with the same solvent gave consecutively three groups of fractions, each series identified by the position of band I: 645-chlorin; 675-chlorin; 665-chlorin. Elution with chloroform: ether (1:1; v/v) gave more of the 665-chlorin and some porphyrin material.

The 665-chlorin could not be obtained crystalline from the usual solvents and was not investigated further. The 645-chlorin was identified as deuteriochlorin IX dimethyl ester by comparison with an authentic sample.⁵

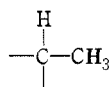
The fractions rich in the 675-chlorin were combined and rechromatographed on alumina in ethereal solution to give *monoacetyldeuteriochlorin IX dimethyl ester* (50 mg) as dark green needles, m.p. 169–172°, from ether (Found: C, 69.9; H, 6.5; N, 9.7. $\text{C}_{34}\text{H}_{38}\text{O}_5\text{N}_4$ requires C, 70.1; H, 6.6; N, 9.7%). λ_{max} in CHCl_3 (log ϵ): 411 (5.13), 507 (4.08), 542 (3.53), 568 (3.26), 620 (3.68), 675 (4.63) nm. λ_{max} in 25% HCl (log ϵ): 417 (5.11), 537 (3.62), 615 (3.86), 667 (4.59) nm. τ (CDCl_3): -0.17 (1H) 0.59 (1H) 1.44 (2H) (methine protons), 6.2–5.2 (7H, m, $\text{CH}_2\text{CH}_2\text{CO}$, structures (A) and (B)), 6.35 (6H, OCH_3), 7.1–6.5 (4H, m, $\text{CH}_2\text{CH}_2\text{CO}$), 6.69 (9H, aromatic CH_3), 6.87 (3H, COCH_3), 8.19 (3H, d, J 6.5 Hz, structure (C)), 12.47 (2H, NH).



(A)



(B)



(C)

Acknowledgments

We are pleased to acknowledge grants from the National Health and Medical Research Council of Australia, the Australian Research Grants Committee, and the National Heart Institute of the U.S. Department of Health (H.E. 07827).