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THE STRUCTURES AND CHEMISTRY OF ISOBACTERIOCHLORINS FROM DESULPHOVIBRIO GIGAS

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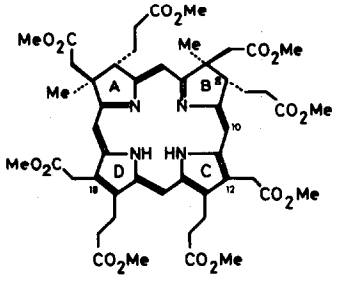
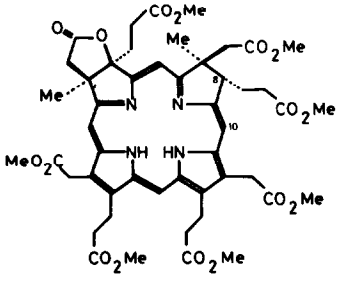
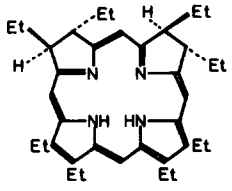
Sirohydrochlorin, the metal-free form of the prosthetic group from several sulphite reducing bacteria [1], was shown by Siegel and Kamin et al [2] to be an octacarboxylic isobacteriochlorin (tetrahydroporphyrin). They suggested (a) a structure for it involving C-methylation at C-12 and C-18 of uroporphyrin-III (but recognised that other isomers would fit) and (b) that sirohydrochlorin (or a relative) might be on the biosynthetic pathway to vitamin B_{12}. Our interest in the biosynthesis of this vitamin [3] led us to isolate the metal-free prosthetic group from Desulphovibrio gigas. Sonication of the cells and chromatography on DEAE cellulose



(1)



(2)



(3)

gave desulphoviridin from which the metal-free prosthetic group was released. Esterification with methanol-H_2SO_4 gave a small quantity of sirohydrochlorin octamethyl ester but mainly a

monolactone; the two esters were separated by h.p.l.c. [4]. All the properties of the former (u.v.-visible spectrum, fluorescence, chromatography) match those reported [1,2] and F.D. mass spectrometry confirmed M^{+} 974.

Further work is needed before the above remarkably specific isolation of monolactone is understood but, importantly, we find that oxidative conversion of sirohydrochlorin and of its ester (1) into the monolactone (2) can be achieved on isolated materials. Structural knowledge derived from the monolactone (2), which is most informative by n.m.r., thus holds good for sirohydrochlorin ester (1). Oxidative formation of lactone and lactam groups is known [5] in the corrin series. The published spectra show the presence of lactone in some earlier preparations of sirohydrochlorin ester [1,2].

Transesterification of the monolactone ester (F.D. M^{+} 958) with CD_3OH-H_2SO_4 gave a product showing two parent peaks in the F.D. spectrum at M^{+} 976 and 979. Further transesterification with CD_3OH-H_2SO_4 greatly increased the 979 peak. Thus, the monolactone has seven ester groups, one of which is hindered. The i.r. spectrum showed absorption at 1775 cm^{-1} (γ-lactone) not present in the spectrum of (1).

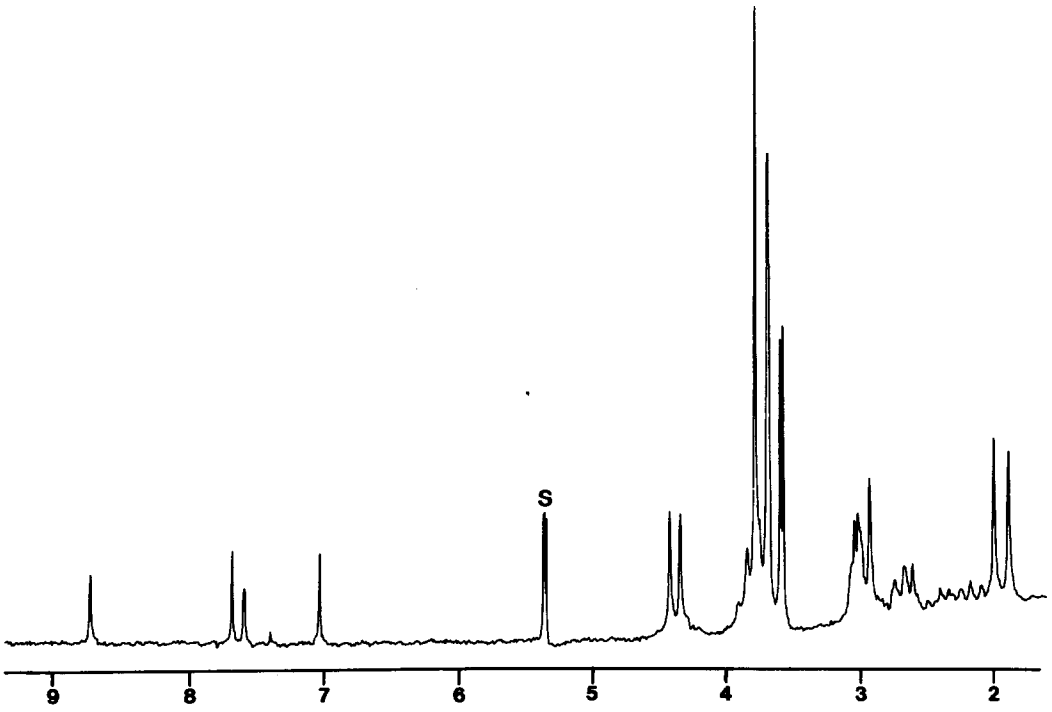


Figure. ^{1}H-n.m.r. spectrum of monolactone ester (2) in CD_2Cl_2 at 100 M Hz; S marks solvent.

The u.v.-visible spectra of the monolactone ester (2) determined in piperidine and in 5% CF_3CO_2H in CH_2Cl_2 correspond to an isobacteriochlorin [6] (adjacent rings reduced). In agreement, the F.T. n.m.r. spectra of (2), see Figure, and of its CD_3 ester showed the characteristic well-spread meso-signals [7], one of which in this case is a doublet (J, 0.9 Hz); see later. In CF_3CO_2D, the high-field meso signal was rapidly lost, followed by the two central meso-signals

whilst that at low field was essentially unchanged after 40 hours. This exchange behaviour matches that of known isobacteriochlorins [7], (e.g. 3) confirmed here under our conditions of measurement; the CH between the reduced rings exchanges fastest and the two CH's adjacent to the reduced rings are slower [7].

Two high-field singlets appear in the n.m.r. spectrum of (2) corresponding to two quaternary C-methyl groups and three methylene singlets arise from CH_2CO_2Me residues, two being at low-field (attached to "porphyrin" part) and one at high-field (attached to "reduced" part).

The foregoing results are in full agreement with structure (2) for the monolactone ester [§] but alternatives involving other pairs of reduced rings need to be excluded. Because of the relationship of sirohydrochlorin and the monolactone to cobyrinic acid demonstrated in the following paper, only reduced rings A-B and reduced A-D need be considered; the stereochemistry for (1) and (2) is also assigned on the corrin relationship.

The reduced A-D system was excluded by a full n.m.r. decoupling study on (2) which showed that the doublet from one of the meso-H adjacent to (not between) the reduced rings arises by coupling to a single proton at ca. δ4.3. Work on octaethylisobacteriochlorin (3, one diastereoisomer illustrated) shows this chemical shift corresponds to C-H at the β-position of a reduced ring. These data can be accommodated by allylic coupling from C-8 to C-10 on (2) but cannot be explained on the reduced A-D structure which also fails on other counts.

Finally, the F.T. n.m.r. spectra of the monolactone ester (2) were examined [8] with pulse delays varying stepwise over the range 0.05 sec to 5 sec; the relaxation rates of the various protons compared with those of uroporphyrin-II octamethyl ester and octaethylisobacteriochlorin (3) added further strength to the above assignments.

Support for the expected origin of the two C-methyl groups of (1) and (2) from S-adenosylmethionine was obtained by the formation of highly radioactive (2) when ^{14}C-L-methionine was included in the growth medium (minimum specific incorporation, 55%). The corresponding ^{13}C-experiment is in progress.

The following paper shows how knowledge of the structures of sirohydrochlorin ester (1) and of the monolactone ester (2) greatly helps research on the biosynthesis of vitamin B_{12}. In addition, a major product isolated in our early extractions of desulphoviridin (and later shown to be a dilactone) is shown there to have similar significance.

Acknowledgements.

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[§] The monolactone (2) and the lactones in the following paper undergo subtle spectroscopic and chromatographic changes with acids and bases. Studies of protonation and possible tautomerism and/or ring-opening are in progress.

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