

Preparation and Spectroscopic Properties of Co^{III}-Isobacteriochlorins: Relationship to the Cobalt-containing Proteins from *Desulphovibrio gigas* and *D. desulphuricans*

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It is concluded that the prosthetic groups of the cobalt-containing proteins from *Desulphovibrio gigas* and *D. desulphuricans* are cobalt isobacteriochlorins because their absorption spectra exactly match those of authentic cobalt isobacteriochlorins now prepared and characterised.

Desulphovibrio gigas and *D. desulphuricans* are sulphite reducing organisms and, in common with the other members of this family, are considered to be primitive on the evolutionary scale. Recently cobalt-containing proteins have been obtained from *D. gigas*¹ and *D. desulphuricans*² and the prosthetic group, isolated from the former with acidified acetone was shown to be a macrocyclic cobalt complex,¹ possibly a Co^{III}-porphyrin. However, though the published absorption spectrum of the prosthetic group showed general similarities to the spectra of Co^{III}-porphyrins there were some differences. Since extraction of *D. gigas* under appropriate conditions yields³ the isobacteriochlorins sirohydrochlorin (1) and its lactone (5), it seemed possible that the foregoing prosthetic group could in fact be of a novel type, *viz.* the cobalt complex of an isobacteriochlorin. Accordingly, authentic representatives of this class of complexes have been prepared together with Co^{III}-coproporphyrin-III ester (7) for comparison.

Cobalt was inserted into coproporphyrin-III ester (6) using hydrated cobalt acetate in methanol-chloroform at 50 °C. The product was oxidised to the Co^{III}-state with ferricyanide and purified chromatographically as its cyanide complex (7). Spectroscopic data: field desorption-mass spectrometry (F.D.-m.s.) *m/z* 793.2532, C₄₁H₄₃N₅O₈Co requires 793.2521; the ion lacking CN was also observed at *m/z* 767. The u.v.-

visible absorption⁴ is shown in Figure 1; Table 1 gives the main n.m.r. values.

Different conditions were necessary to give a satisfactory rate of cobalt insertion into the isobacteriochlorins. The synthetic system⁵ (8) was heated at reflux with hydrated cobalt acetate for 2.5 h in dry pyridine, the product was oxidised as above, and the Co^{III}-derivative was isolated chromatographically as its CN-complex (9). F.D.-m.s. showed *m/z* 743.2506; C₃₈H₄₄N₄O₈Co, corresponding to the complex (9) minus CN, requires 743.2490. The Co-CN bond of the isobacteriochlorin (9) clearly undergoes readier cleavage in the mass spectrometer than that of the porphyrin (7). Figure 2 shows the u.v.-visible spectrum of the Co^{III}-isobacteriochlorin (9) and Table 1 gives the n.m.r. data. Confirmation of the close proximity of the hydrogens giving the signal at δ 7.68 (H-10, H-20) to those giving signals at δ 1.65, 1.42 (C-methyl groups) and at δ 4.51, 4.46 (ArCH₂CO₂Me) was established when irradiation at δ 7.68 caused clear nuclear Overhauser enhancement of the latter four signals.

Finally, sirohydrochlorin ester⁶ (3) was converted into its Co^{III}-complex (4) as for substance (8) save that strictly anhydrous conditions were necessary. The chromatographically homogeneous product (4) showed in F.D.-m.s. *m/z* 1031.331, C₅₀H₆₀N₄O₁₆Co requires 1031.333 corresponding to (4) minus CN; its u.v.-visible spectrum was almost identical to that shown in Figure 2 for complex (9). The 3-epi- and 8-epi-stereoisomers of complex (4) were similarly prepared from the isomers of sirohydrochlorin ester [as (3) but epimeric

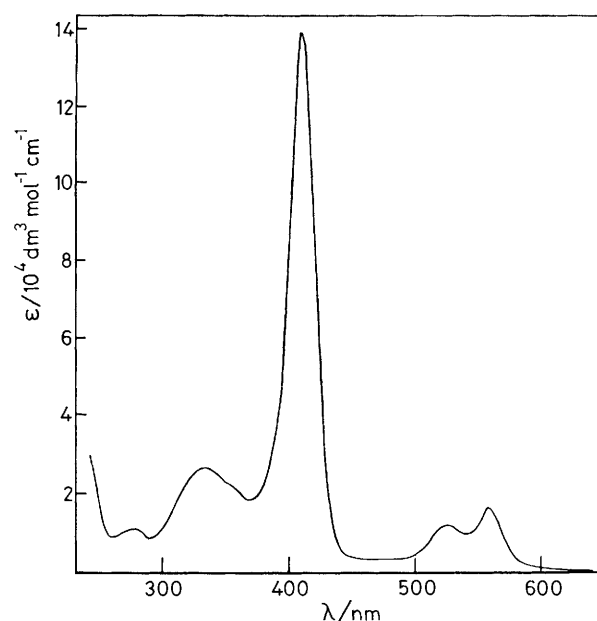
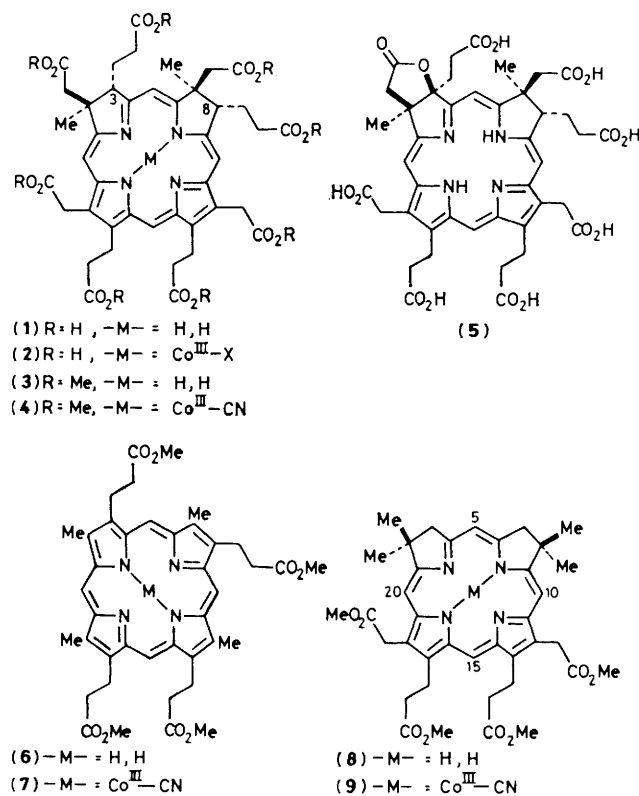
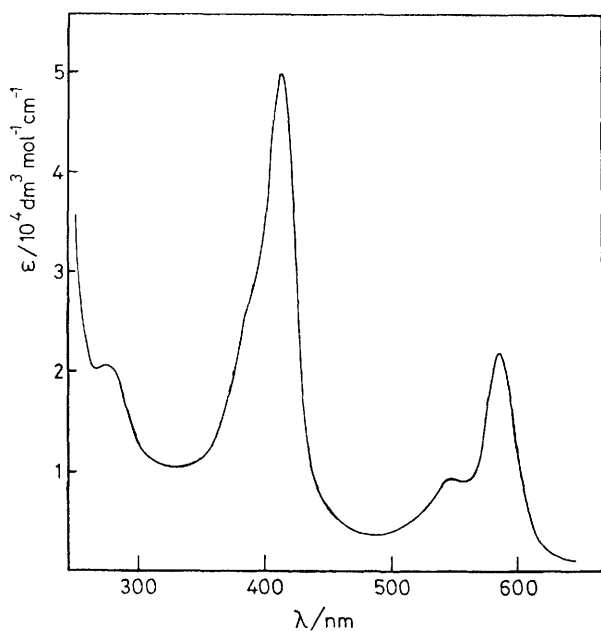


Figure 1. U.v.-visible absorption spectrum of the coproporphyrin-III Co^{III}-complex (7) in chloroform.

Table 1. ^1H N.m.r. signals from the complexes (9) and (7) in $\text{C}_5\text{D}_5\text{N}$, δ -values (multiplicity).

| Compound | H-15 | H-10 H-20 | H-5 | $\text{ArCH}_2\text{CO}_2\text{Me}$ | CH_2 at C-3, C-7 | OMe | $\text{ArCH}_2\text{CH}_2\text{CO}_2\text{Me}$ | C-Me |
|----------|--|--------------|----------|-------------------------------------|------------------------------|----------------------|--|----------------------|
| (9) | 9.18 (s) | 7.68 (s) | 7.09 (s) | 4.51 (d) 4.56 (d) | 3.73 (d) 3.78 (d) | 3.60 (s) 3.53 (s) | 4.02 (t) 4.00 (t) 3.18 (t) | 1.65 (s) 1.42 (s) |
| (7) | 10.59, 10.46, 10.45, 10.29 (each s) | | | — | — | 3.58 (s) 3.51 (s) | 4.52 (m) 3.38 (m) | 3.50 (s) 3.49 (s) |

**Figure 2.** U.v.-visible absorption spectrum of the isobacteriochlorin Co^{III} -complex (9) in chloroform.

respectively at C-3 or C-8].^{7,8} F.D.-m.s. showed m/z 1031 for both products and the u.v.-visible spectra of these isomers were not significantly different from that of Co^{III} -sirohydrochlorin ester (4).

The u.v.-visible spectra of these thoroughly characterised products were then compared with the published spectra^{1,2} of

the cobalt complexes isolated from *D. gigas* and *D. desulphuricans*. The spectrum of the porphyrin (7) differed considerably from, whereas the spectra of the two isobacteriochlorin complexes (4) and (9) were essentially superimposable on, those of the natural materials. We conclude that the Co-containing proteins from these organisms have cobalt isobacteriochlorins as their prosthetic groups. Further, it seems probable, in view of earlier work^{3,9} that the oxidised form of these prosthetic groups is Co^{III} -sirohydrochlorin (2) or a derivative of that macrocycle.

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