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BIOSYNTHESIS OF VITAMIN B_{12}: STRUCTURAL STUDIES ON THE CORRIPHRYINS FROM PROPIONIBACTERIUM SHERMANII AND THE LINK WITH SIROHYDROCHLORIN

Alan R. Battersby *, Edward McDonald, Howard R. Morris +, Mervyn Thompson, and D. Clive Williams
University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, U.K., and + Department of Biochemistry, Imperial College, London, U.K.
and Vladimir Ya. Bykhovsky *, Natalia I. Zaitseva and Vasilii N. Bukin
A.N. Bach Institute of Biochemistry of Academy of Sciences, Leninskii prospect 33, Moscow 117071, U.S.S.R.

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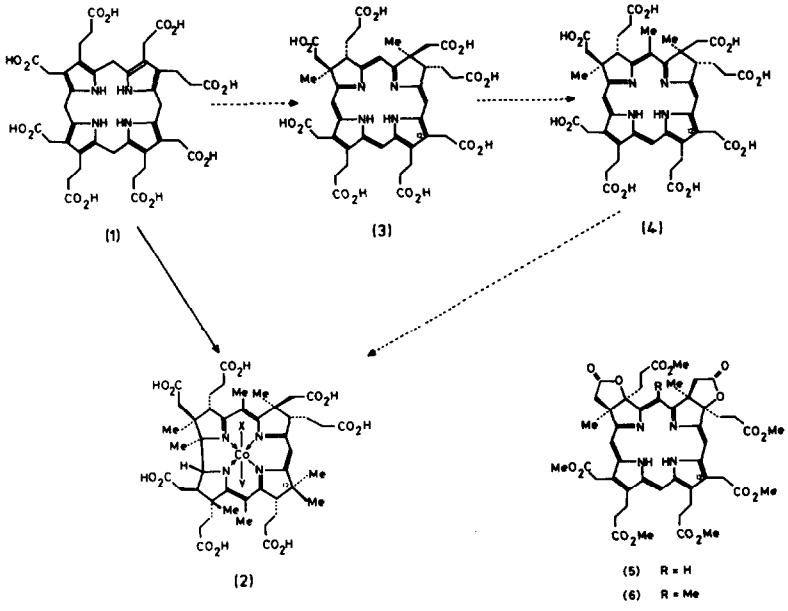
Cobyrinic acid (2), the precursor [1] of vitamin B_{12}, is biosynthesised [2-5] from uro'gen-III (1) by many steps. Discovery of their natural sequence requires the trapping for structural study of intermediates (as such or modified) between (1) and (2). Such substances, the partly purified corriphyrins, were first obtained in 1975 from the B_{12}-producer P.shermanii [6]. Spectroscopic data [6,7] and labelling studies with ^{14}C-methionine and ^{3}H-aminolaevulinic acid [8] supported their being methylated tetrapyrroles. Joint work on these materials and others is now reported.



The methyl esters of the total corriphyrin fraction from P.shermanii [6,7] were fractionated by h.p.l.c. to yield the methyl esters of corriphyrin-4 and corriphyrin-3, together with several

other pure components (see later) . Evidence supporting structure (5) for corriphyrin-4 and (6) for corriphyrin-3 is as follows.

Both corriphyrin esters were found to be isobacteriochlorins (cf. previous paper): (a) by their u.v.-visible spectra (b) corriphyrin-4 ester by n.m.r. showed four well-spread [9] singlets from the meso-positions, that at high field being rapidly lost by exchange [9] of the corresponding proton with CF_3CO_2D; long exchange caused decomposition (c) corriphyrin-3 ester, in contrast, showed only three meso-H signals, that at lowest field being unaffected by CF_3CO_2D (after 290 h) but the other two meso-protons exchanged slowly (complete after 290 h). The two exchangeable protons are therefore assigned [9] to the meso-positions adjacent to the reduced rings and the third proton to the carbon between the non-reduced rings; note that a rapidly exchanging proton between reduced rings is lacking from corriphyrin-3 ester.

F.D. mass spectrometry showed M^+ 942 for corriphyrin-4 ester and 956 for corriphyrin-3 ester; the former was found to have the same accurate mass as uroporphyrin-III octamethyl ester by direct comparison so corresponding to $C_{48}H_{54}N_4O_{16}$. The CD_3 esters of corriphyrin-4 and corriphyrin-3 showed by F.D. in each case an increase of 18 units relative to the protio series, to M^+ 960 and 974, respectively, proving each to be a hexamethyl ester. Strong i.r. absorptions at 1775 and 1728 cm^{-1} support γ-lactone ester structures for both corriphyrins.

A singlet at δ3.38 for corriphyrin-3 ester, still present in the corresponding CD_3 ester, was absent from the spectrum of corriphyrin-4 ester. This signal is assigned to a C-methyl group on the meso-position between the reduced rings and this also accounts for the rest of the above data. Both esters show two singlets at high field corresponding to two quaternary C-methyl groups. The foregoing n.m.r. assignments were supported by n.m.r. of the CD_3 esters and by T_1-measurements on corriphyrin-3 ester.

All the evidence so far supports (5) for corriphyrin-4 ester and (6) for corriphyrin-3 ester but isomers would also fit. Rings A-B and A-D are considered for the methylated pair on grounds of structural relations to cobyrinic acid (2); the decarboxylation at C-12 required at some stage to form the natural corrins, on mechanistic grounds must occur before C-12 methylation. Rings A-B are chosen because this allows the meso-C-methyl group of (6) to be between the reduced rings and correctly placed for cobyrinic acid.

Interlocking evidence came from proof that corriphyrin-4 ester (5) and the dilactone ester, isolated in the preceeding paper as a "trapped" form of sirohydrochlorin, are identical (h.p.l.c., n.m.r., F.D.m.s., u.v.-vis.). Independent evidence was given there that sirohydrochlorin has rings A-B reduced. Further, one of the pure components reported at the outset of this paper as accompanying the corriphyrins from P.shermanii has been shown to be identical (proof as above) with the monolactone ester (2, preceeding paper) from sirohydrochlorin. The links between sirohydrochlorin (3) and the B_{12}-organism P.shermanii are thus secure. Finally, modification of the extraction process largely avoided lactone formation, allowing sirohydrochlorin ester (ester of 3, n.m.r. in Figure) to be isolated; it was identical with the ester from D.gigas (preceeding paper).

The above work shows that lactone formation occurs readily in this series and clearly corriphyrin-4 (5), corriphyrin-3 (6) and the monolactone (2, preceeding paper) represent "trapped" forms of the original isobacteriochlorins in P.shermanii; one of these, sirohydrochlorin (3) has been isolated in quantity. Importantly, these substances point out the

early methylation sequence on the pathway [§] to vitamin B_{12} as (1)→(3)→(4)→(2).

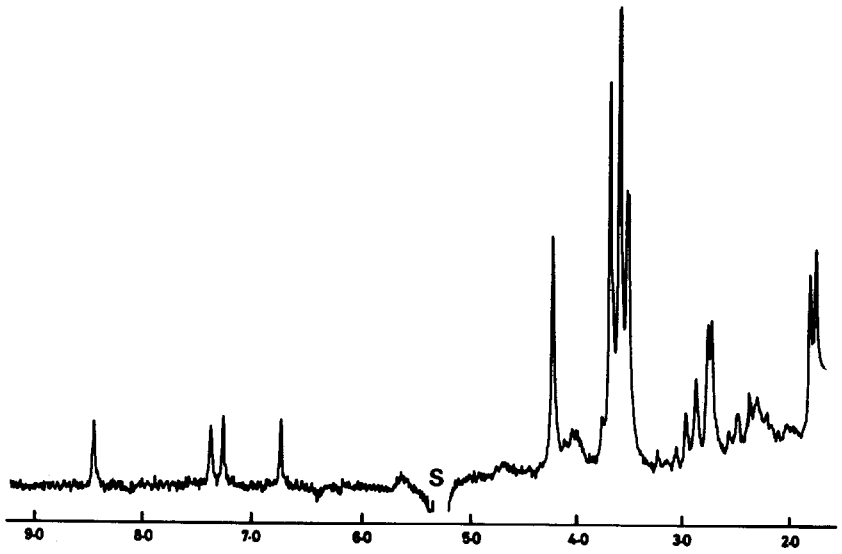


Figure. ^{1}H-n.m.r. spectrum of sirohydrochlorin octamethyl ester (ester of 3) in CD_2Cl_2 at 80 M Hz; S marks solvent.

During the preparation of these manuscripts, Müller et al described the isolation of "Faktor I" and "Faktor II" from the B_{12}-producer Clostridium tetanomorphum and from P.shermanii [10]; labelled "Faktor II" was well incorporated into cobyrinic acid (2). The reported properties of "Faktor II" leave no doubt that it is sirohydrochlorin (3); this work and ours thus interlock perfectly and provide complementary strength.

Acknowledgements.
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[§] It is possible that the biosynthetic intermediates may be dihydro derivatives of (3) and (4).

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