Biosynthesis of Vitamin B_{12}: Proof of A-B Structure for Sirohydrochlorin by its Specific Incorporation into Cobyrinic Acid

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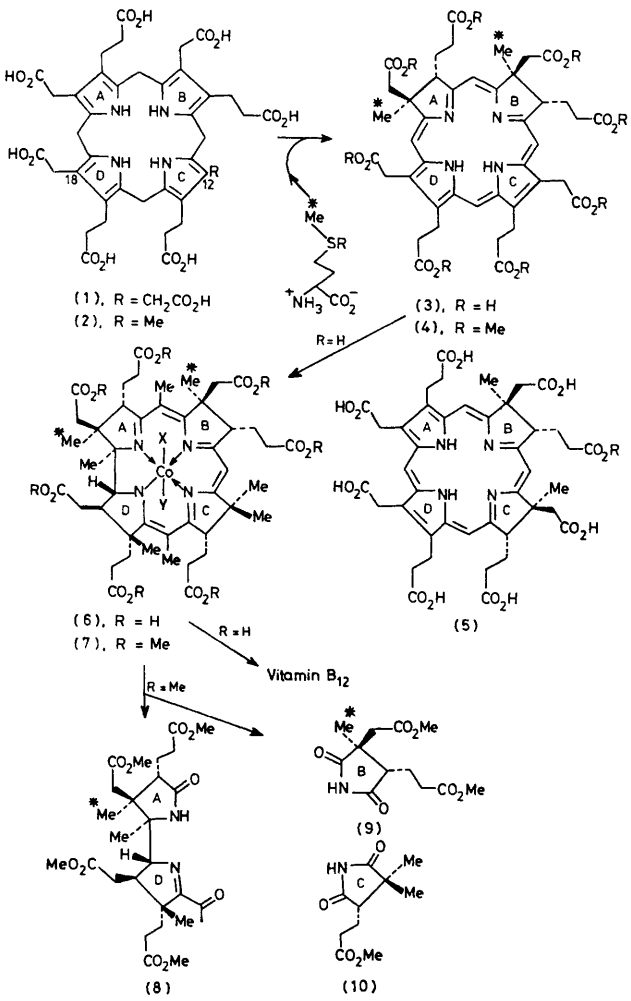
Summary Of the two alternative structures we previously derived for sirohydrochlorin, the preferred one (**3**) is now established by specific incorporation of methyl-labelled sirohydrochlorin into cobyrinic acid (**6**) followed by degradation to determine the labelling pattern.

OUR earlier studies[1] had led to the isolation (among other pigments) of an octacarboxylic isobacteriochlorin from *Propionibacterium shermanii*, an organism which produces vitamin B_{12}. This isobacteriochlorin was rigorously proved[1,2] to be identical with sirohydrochlorin obtained from the sulphite reducing organism *Desulphovibrio gigas*. In addition, the two samples of sirohydrochlorin were shown to have the same absolute stereochemistry by c.d. measurements.[3] Sirohydrochlorin was first isolated as the iron-free form of sirohaem during the important work of Kamin and Siegel[4] on several sulphite reducing bacteria and they showed it was an isobacteriochlorin; it was also tentatively suggested to arise by two *C*-methylations at C-12 and C-18 of uro'gen-III (**1**). Finally, 'Faktor II' isolated[5] from two B_{12}-producing organisms was shown[6] to be identical with sirohydrochlorin.

Chemical and spectroscopic work at Cambridge[1,2] led to two alternatives for sirohydrochlorin, the A-B structure (**3**) and the B-C structure (**5**); the former was preferred. Evidence is now outlined which rigorously establishes the A-B structure (**3**) for sirohydrochlorin; its specific incorporation into cobyrinic acid (**6**) is also demonstrated.



The origin of the two *C*-methyl groups of sirohydrochlorin (**3**) from *S*-adenosylmethionine had been indicated[2,5] and was confirmed by incorporation of (2*S*)-[*methyl*-^{13}C]-methionine, using *D. gigas*, and examination of the product by ^{13}C-n.m.r. spectroscopy.[7] Accordingly, (2*S*)-[*methyl*-^{14}C]methionine (0·88 mCi) was incubated with *P. shermanii* cells under conditions which produce sirohydrochlorin (**3**); the purified ester (**4**), total activity 7·43 × 10^6 dis. min^{-1}, was proved to be radiochemically pure by h.p.l.c.[8] The derived labelled acid (**3**) was then incubated in the broken cell system[9] from *P. shermanii* and the incorporation into cobyrinic acid (**6**), isolated as crystalline cobester (**7**), ranged from 3 to 7%. This proof that sirohydrochlorin† (**3**) is efficiently converted into cobyrinic acid (**6**) confirms the earlier finding[9] that the heptacarboxylic acid (**2**) is not the next intermediate on the pathway beyond uro'gen-III (**1**) *en route* to vitamin B_{12}, contrary to what had been thought.[10]

Ozonolysis[11] of the labelled cobester (**7**) gave the amorphous ring B imide (**9**), the crystalline ring C imide (**10**), and the fragment‡ (**8**) from rings A-D (found: M^+, 552; $C_{27}H_{40}N_2O_{10}$ requires 552). The amorphous fragments were purified to constant activity by repeated preparative layer chromatography and high vacuum distillation. The activities of the three degradation products are set out in the Table.

TABLE. Degradation of labelled cobester (**7**).

	Relative molar activities
Cobester (**7**)	1·00 ± 0·05
Ring B imide (**9**)	0·47 ± 0·03
Ring C imide (**10**)	<0·02
Rings A-D ketone (**8**)	0·52 ± 0·03

The values show that ring C of cobyrinic acid (**6**) is unlabelled and thus the two labelled *C*-methyl groups must be on rings A and B or on rings A and D. But ring B carries *ca.* half the total activity, so rings A and B are the labelled pair as illustrated. The foregoing results, when combined with the earlier ones,[1,2] establish that sirohydrochlorin has the structure and absolute configuration (**3**), sirohaem[4] is the corresponding iron complex, and the middle section of the biosynthetic pathway to vitamin B_{12} can be defined as (**1**) → (**3**)† → (**6**) → vitamin B_{12}.

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† It is also possible that the biosynthetic intermediate may be a dihydro derivative (ref. 6) of (**3**).

‡ This fragment was first identified by Professor D. Arigoni and his colleagues (E.T.H., Zurich); we are indebted to him for kindly informing us of this valuable work.

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