Biosynthesis of Vitamin B_{12}: Derivation of Cobyrinic Acid from Uroporphyrinogen-III

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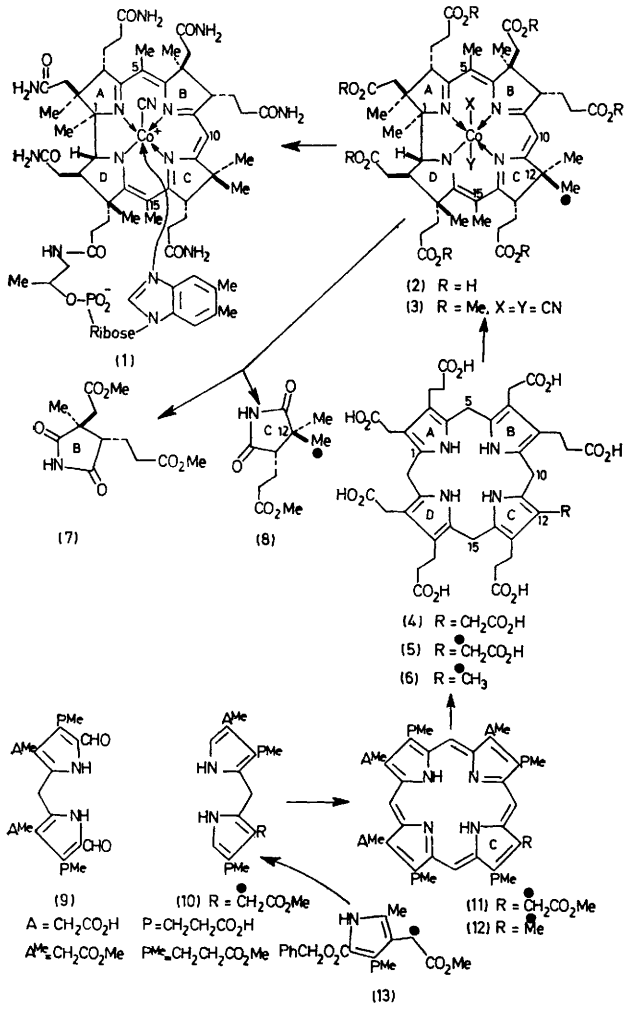
Summary Specifically labelled ^{14}C-uroporphyrin-III is synthesised to allow incorporation experiments with an enzyme system from *Propionibacterium shermanii* which prove rigorously the derivation of corrins (*e.g.* **2**) from uroporphyrinogen-III (**5**).

VITAMIN B_{12} (**1**) and the corresponding coenzyme have been shown[1] to be constructed *in vivo* from cobyrinic acid (**2**). Recent studies[2] largely with ^{13}C-labelled precursors have confirmed earlier reports[3] that the precursors of cobyrinic acid (**2**) are 5-aminolaevulinic acid (ALA) and methionine and they added the important finding that the C-1 methyl group of vitamin B_{12} (**1**) arises from methionine rather than from C-5 of ALA. The early part of the pathway to corrins thus matches the firmly established route[4] to the natural porphyrins and their relatives, *e.g.* to uroporphyrinogen-III (**4**).

The structural similarity of natural corrins (*e.g.* **2**) to uroporphyrinogen-III (**4**), especially the characteristic arrangement in each of the acetic and propionic side-chains, has long led to speculation[5] about the possibility of corrins being formed by biochemical modification of uroporphyrinogen-III (**4**). Experimental tests[6] with unsymmetrically labelled [5,15-^{14}C]uroporphyrinogen-III (as **4**) gave insignificant incorporations into vitamin B_{12} but positive values were obtained when uroporphyrinogen-III plus isomer(s) labelled equivalently in all four propionic side-chains was used.[2b] The possibility was generally recognised that with such equivalent labelling of the four pyrrolic units of (**4**), the same result would be obtained both for intact incorporation and incorporation by breakdown. A single label on ring-c was chosen for uroporphyrinogen-III (**5**) such that intact incorporation would lead only to labelling of ring-c in cobyrinic acid (**2**); ring-c is unambigu-



ously isolable from the ester (**3**) by chemical degradation.[7]

The pyrrole (**13**) ^{14}C-labelled as illustrated[8] was built into the pyrromethane (**10**) (coupling by stannic chloride method[9]) and the dialdehyde[10] (**9**) was synthesised as usual. Combination of (**9**) and (**10**) afforded labelled uroporphyrin-III octamethyl ester (**11**) in 39% overall yield from the pyrrole (**13**). The derived [^{14}C]uroporphyrin-III (*ca.* 2 mg) was reduced to [^{14}C]uroporphyrinogen-III (**5**) and this was incubated for 24 h with a broken cell preparation† of *Propionibacterium shermanii* containing the following co-factors: ATP, NADPH, *S*-adenosylmethionine, cysteine, glutathione, mercaptoethanol, cobalt and magnesium chlorides, and dimethylbenzimidazole. The incubation was completed by the addition of radio-inactive cobyrinic acid (**2**) as carrier and, after work-up, the product was isolated as the crystalline dicyano heptamethyl cobyrinate[7] (**3**; cobester). Chromatography and multiple recrystallisation gave cobester of constant activity corresponding to an incorporation‡ of 5·2%. Radiochemical purity was further checked by preparative high pressure liquid chromatography of the ester and further crystallisation.

Ozonolysis[7] of the labelled cobester (**3**) yielded the crystalline ring-C imide (**8**) and the amorphous ring-B imide (**7**). The molar activity of the ring-C imide (**8**) was 90% of that of cobester (**3**) whereas the ring-B imide (**7**) carried <4%. Rigorous proof is thus provided for the biological derivation of the corrin macrocycle (**2**) from uroporphyrinogen-III (**5**); only very minor randomisation of activity occurs.§

The possibility that the C-12 methyl heptacarboxylic acid (**6**) follows uroporphyrinogen-III (**5**) on the biochemical reaction sequence leading to cobyrinic acid (**2**) is currently being tested with the illustrated labelled form (**6**) derived from totally synthetic porphyrin[9] (**12**).

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† This is an improved form of the enzyme preparation which biosynthesises cobyrinic acid and which was described at the ESBOC Symposium, Gregynog, May 1973 (A. R. Battersby, E. McDonald, and B. Middleton).

‡ The value is an absolute incorporation (total activity of cobyrinic acid formed × 100 divided by total activity of administered uroporphyrinogen-III).

§ Professor A. I. Scott and his co-workers (Yale) have independently demonstrated by a different approach the incorporation of unsymmetrically labelled uroporphyrinogen-III into the corrin macrocycle (personal communication).

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