Biosynthesis of Vitamin B_{12}: Stereochemistry of the Decarboxylation Step which generates the 12-*si*-Methyl Group

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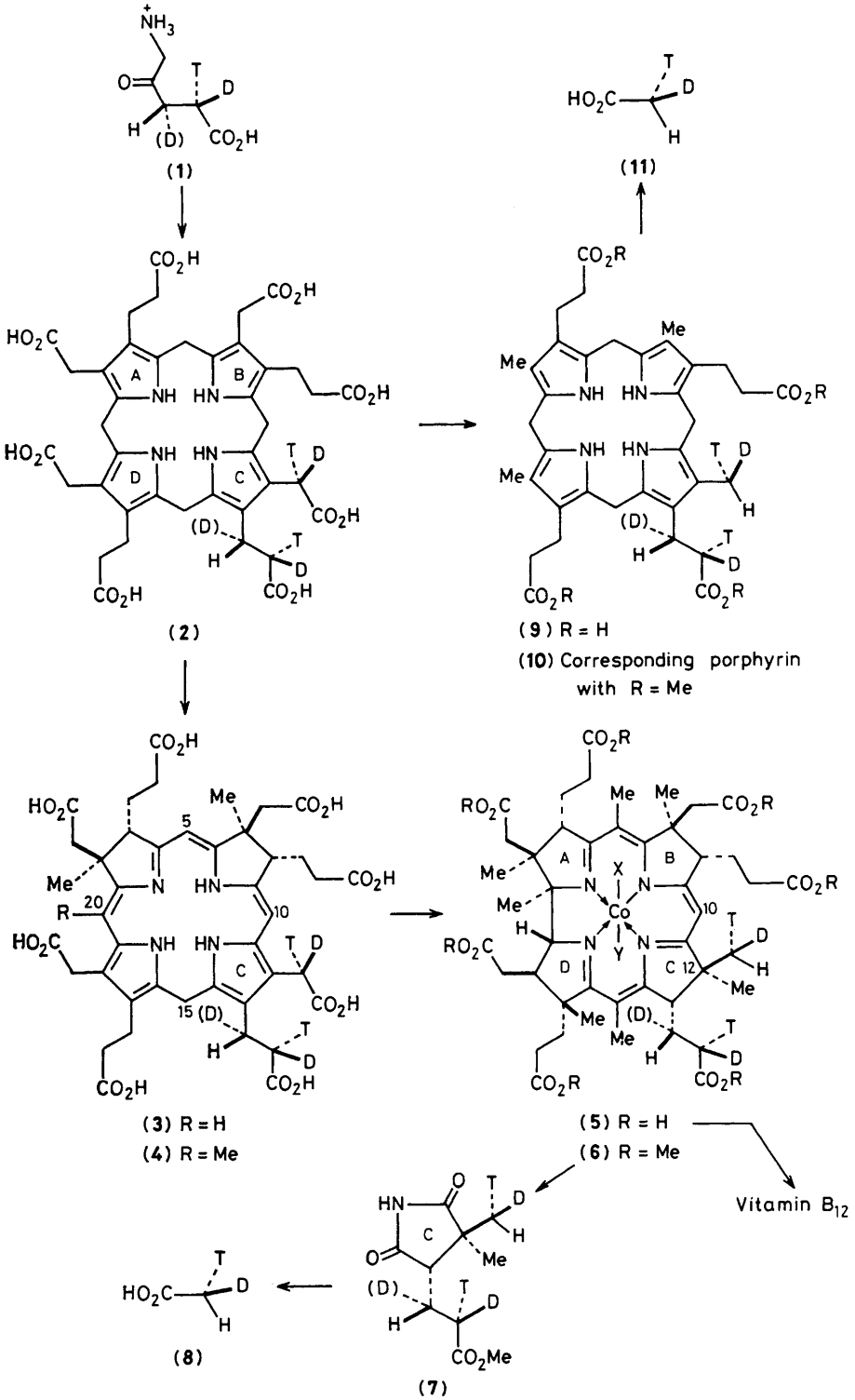
The 12-*si*-methyl group of vitamin B_{12}, unique among the eight *C*-methyl groups of the vitamin in originating from an acetate residue by decarboxylation, is shown to be formed with overall *retention* of configuration.

Cobyrinic acid (**5**), a late intermediate for biosynthesis of vitamin B_{12}, carries two methyl groups at C-12. Of these two, the *si*-methyl on the β-face of the molecule is unique among the eight methyl groups of the corrin system (**5**) in that it is derived from an acetic acid residue by decarboxylation;[1] the 12-*re*-methyl group (α-face) and the others are all introduced by transfer from *S*-adenosyl methionine.[1] The precise stage on the pathway at which this decarboxylation takes place is at present unknown but it certainly occurs after the dihydroisobacteriochlorins (**3**) and (**4**)[2] have been built. Thus, the decarb-



oxylation takes place with the acetic acid residue attached to a novel macrocycle and accordingly, it was important to determine the stereochemistry of this enzymic step.

The synthesis has been described[3] of δ-aminolaevulinic acid† (1) (ALA), made chiral at C-2 (*S*-configuration) by isotopic substitution with ^2H and ^3H. This was fed as earlier[4] to growing cells of *Propionibacterium shermanii* and the crude vitamin B_{12} isolated therefrom was methanolysed to yield the crystalline cobester (**6**); the incorporation levels ranged up to 3.7%. The 12-*si*-methyl group should be chiral at this stage and will have the *R*-configuration if the decarboxylation occurs stereospecifically with overall retention of configuration; conversely, it will have the *S*-configuration if there is overall inversion. For simplicity, only the labels on the side-chains of ring c have been illustrated.

Ozonolysis[5] of the labelled cobester (**6**) gave the crystalline

† The ^2H at C-3 is shown in parentheses because this label, though present at an earlier stage of the synthesis,[3] may not survive the later steps. For the present purposes, this is immaterial.

imide (7) corresponding to ring C; this was purified by sublimation and recrystallisation to constant specific activity. Oxidation of unlabelled samples of the imide [as (7)] by the standard Kuhn–Roth procedure (heating) run in deuteriated solvents gave, under all conditions tested, acetic acid which had undergone significant exchange of the hydrogen atoms in the methyl group (up to 60% $^2H_3$ species present; mass spectroscopy on *p*-bromophenacyl acetate). However, Kuhn–Roth oxidation of (7) at room temperature‡ caused little exchange (84.4 $^2H_0$, 8.4 $^2H_1$, and 7.1% $^2H_2$ in the isolated *p*-bromophenacyl acetate). When this mild procedure was applied to the labelled imide (7), it gave a good chemical yield of acetic acid (45—50%) but the radiochemical yield, which measures the amount of acetic acid derived from the 12-*si*-methyl group, was only 2—4%. Evidently, there had been preferential attack by the chromium trioxide at the 12-*si*-methyl group of the imide (7), unwelcome in the present studies but which worked to the advantage of related experiments on the 12-*re*-methyl group.[6]

Assay of the chirality of the acetic acid (**8**) obtained in the current experiments from two independent degradations of the imide (7) was carried out using malate synthetase and fumarase.[7] The *F*-values of 76 and 65 (both ±5) so obtained proved unambiguously that the acetic acid had the *R*-configuration (**8**). Accordingly, the enzymic decarboxylation which generates the 12-*si*-methyl group occurs with overall *retention* of configuration.

The same stereochemical outcome had been found for the enzymic decarboxylation of the acetic acid residues of uro'gen III (**2**) to form copro'gen III (**9**), in both chicken erythrocytes[8] and in *Rhodopseudomonas spheroides*.[3] The *P. shermanii* organism used in the present work also produces and decarboxylates uro'gen III (**2**), and the copro'gen III (**9**) so formed in the feeding experiments above with chiral ALA (**1**) was isolated, after aromatisation, as the coproporphyrin III ester (**10**). The acetic acid (**11**) isolated from standard hot Kuhn–Roth oxidations of the ester (**10**) gave in the stereochemical assay[7] *F*-values of 70, 72, and 77 (all ±5), corresponding to the *R*-configuration (**11**); suitable blanks with deuteriated solvents showed no detectable exchange of the methyl hydrogens had occurred. Thus, the decarboxylase acting on uro'gen III (**2**) in the anaerobic bacterium *P. shermanii* again converts substrate into product with overall *retention* of configuration.

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‡ The substrate (10 mg) was shaken for 17 h at 20 °C with chromium trioxide (Merck, 2 g) in water (5 ml); then 85% H_3PO_4 (1.5 ml) was added and the mixture was steam distilled.

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