Biosynthesis of Vitamin B_{12}: Preparation of 12-Methylisobacteriochlorins and Related Systems

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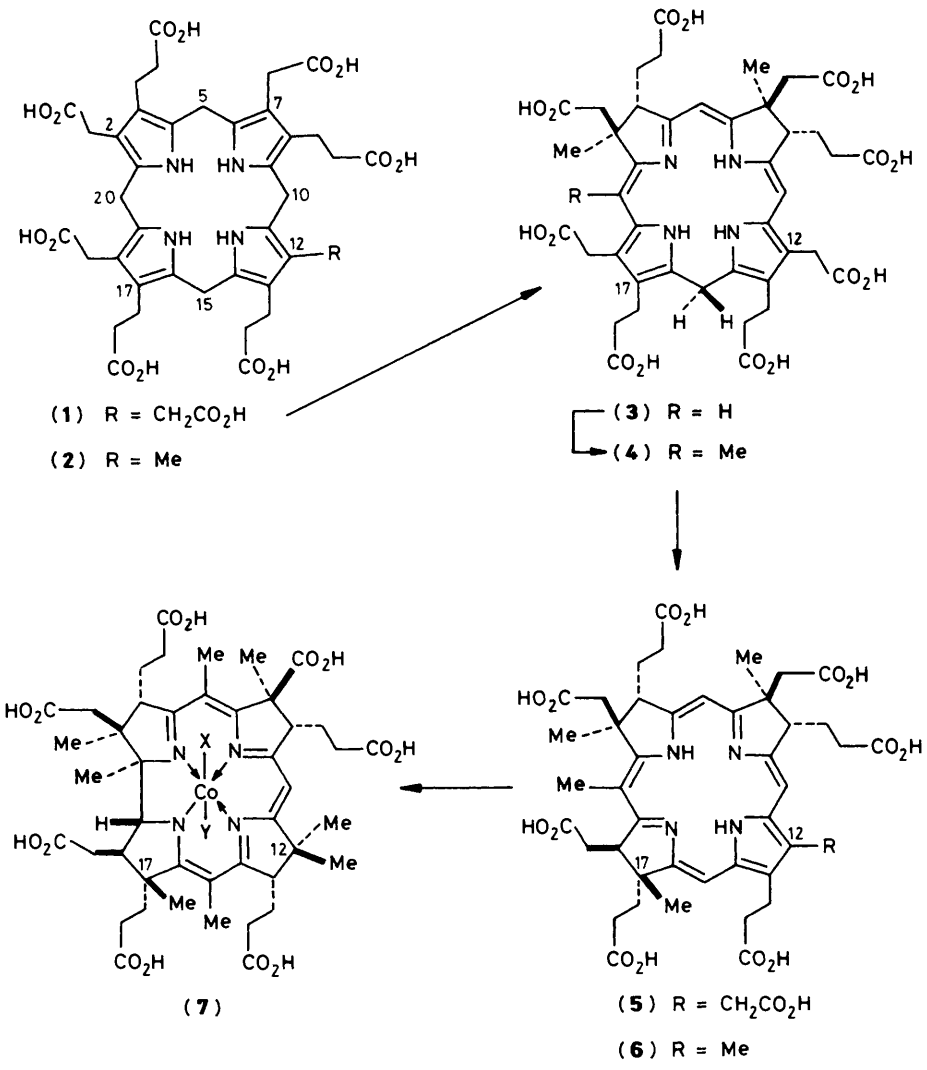
12-Methylisobacteriochlorins, which are important for future research on the biosynthesis of vitamin B_{12}, have been prepared by two methods from isobacteriochlorins carrying a 12-acetic acid residue; enzymic methylation of the 12-methyl analogue of uro'gen-III has yielded the 12-methyl analogue of sirohydrochlorin.

The biosynthetic pathway to vitamin B_{12} goes *via* cobyrinic acid (**7**) and it branches away at an early stage from those leading to the other pigments of life such as protohaem and chlorophyll. This branching towards vitamin B_{12} is initiated by *C*-methylation of uro'gen-III (**1**), the first methylation occurring at C-2, the second at C-7, and the third at C-20.[1] This knowledge of the early methylation sequence came from structure determinations on the isolated aromatised forms of the mono-, di-, and tri-methylated intermediates. The latter two are isobacteriochlorins and their esters have structures (**8**)



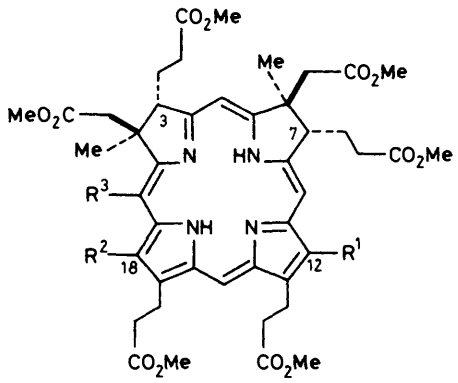
and (**9**), respectively;[1] the dimethylated system (**8**) is usually called sirohydrochlorin octamethyl ester. For the dimethylated system, it has been proved[2] that the true biosynthetic intermediate is the dihydro-isobacteriochlorin (**3**) and it is almost certain that the trimethylated system is also formed and further transformed enzymically at the dihydro oxidation level (**4**). Finally, the acetic acid residue at C-12 of (**3**) and (**4**) is enzymically decarboxylated to form a 12-methyl group at some later stage on the pathway, *cf.* structure (**7**).

The site of attachment of the fourth *C*-methyl group was shown in 1982 to be at C-17 by pulse-labelling experiments[3] and so led to the important knowledge that the next intermediates on the biosynthetic pathway beyond (**4**) are based on the pyrrocorphin ring-system.[4] Attention was thus focussed on the pyrrocorphin structures (**5**) and (**6**) and there has been recent confirmation of the 1982 findings by repetition and inverse pulse-labelling.[5,6] In principle, pyrrocorphins ought to be accessible from isobacteriochlorins either by enzymic or by *in vitro*[7] transformations. So the 12-methylpyrrocorphin structure (**6**) has generated strong interest in 12-methylisobacteriochlorins [*e.g.* (**12**) and (**14**)]; we outline here routes to representative examples.

Two non-enzymic methods have been developed using the synthetic isobacteriochlorin[8] (**15**) as a model and the first depended on thermal decarboxylation. Controlled hydrolysis of (**15**) afforded the separable mono-acids (**16**), 22% and (**17**), 61%. The latter was re-esterified and recycled to yield more of the desired acid (**16**) which was decarboxylated at 200 °C to yield the 12-methyl product (**18**), 34%; this structure was confirmed by synthesis[9] by the general photochemical method.[8,10] Similarly, complete hydrolysis of (**15**) gave the tetra-acid which by heating at 200 °C and esterification of the products afforded the 12,18-dimethyl system (**19**), 16%, and the 12-methyl product (**18**), 23%, identical to that above.

A mild alternative route was opened up by the observation that the mono-acid (**16**) in CH_2Cl_2 under air changes slowly but cleanly into a labile new product having properties expected for the hydroperoxide (**20**). It showed a new signal at δ_H 5.72, it was reduced by $NaBH_4$ to the fully characterised alcohol (**21**), 67%, and by $NaCNBH_3$–HOAc to give the same 12-methyl system (**18**) as above. By using deuteriated or tritiated cyanoborohydride, samples of the 12-methyl material were obtained specifically deuteriated (**22**) or tritiated (**23**) at the new methyl group.

The foregoing methods were then used to prepare the 12-methyl (**12**) and 18-methyl (**13**) analogues of sirohydrochlorin (**8**), as their methyl esters. Controlled hydrolysis of the octa-ester (**8**) was naturally complex but the two mono-acids



(**8**) $R^1 = R^2 = CH_2CO_2Me$, $R^3 = H$

(**9**) $R^1 = R^2 = CH_2CO_2Me$, $R^3 = Me$

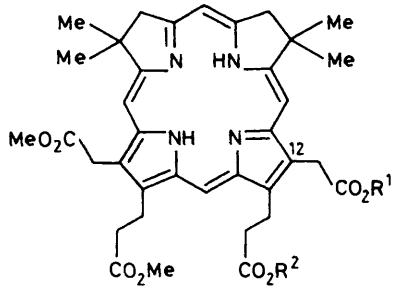
(**10**) $R^1 = CH_2CO_2H$, $R^2 = CH_2CO_2Me$, $R^3 = H$

(**11**) $R^1 = CH_2CO_2Me$, $R^2 = CH_2CO_2H$, $R^3 = H$

(**12**) $R^1 = Me$, $R^2 = CH_2CO_2Me$, $R^3 = H$

(**13**) $R^1 = CH_2CO_2Me$, $R^2 = Me$, $R^3 = H$

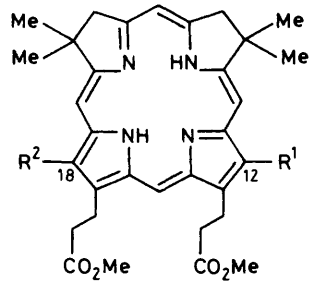
(**14**) $R^1 = R^3 = Me$, $R^2 = CH_2CO_2Me$



(**15**) $R^1 = R^2 = Me$

(**16**) $R^1 = H$, $R^2 = Me$

(**17**) $R^1 = Me$, $R^2 = H$



(**18**) $R^1 = Me$, $R^2 = CH_2CO_2Me$

(**19**) $R^1 = R^2 = Me$

(**20**) $R^1 = CH_2O\text{-}OH$, $R^2 = CH_2CO_2Me$

(**21**) $R^1 = CH_2OH$, $R^2 = CH_2CO_2Me$

(**22**) $R^1 = CH_2D$, $R^2 = CH_2CO_2Me$

(**23**) $R^1 = CH_2T$, $R^2 = CH_2CO_2Me$

(**10**) and (**11**) were isolated chromatographically (*ca.* 8% of each based on unrecovered starting material) whilst the remaining material (mainly the isomeric mono-propionic acids, 57%) by re-esterification and recycling twice provided workable quantities of the acids (**10**) and (**11**).

The 12-acetic acid (**10**) was transformed by the mild oxidative method above into the 12-methyl analogue (**12**) of sirohydrochlorin ester (**8**); found: M^+ 916.4132, $C_{48}H_{60}N_4O_{14}$ requires 916.4106, δ_H 2.84 (in CD_2Cl_2) for the 12-methyl group. The 18-acetic acid (**11**) similarly afforded the 18-methyl analogue (**13**), found M^+ 916.4132, δ_H 2.85 (in CD_2Cl_2) for 18-Me. The proof of orientation for 12- and 18-methyl systems is below.

The foregoing mild method is superior to thermal decarboxylation of (**10**) or (**11**) which though yielding small quantities of the *C*-methyl compounds (**12**) and (**13**) generated a mixture of stereoisomers due to changes at carbons 2 and 7.

The third route to the 12-methyl system (**12**) was enzymic and had the additional importance of discovering whether the cell-free enzyme system from *Propionibacterium shermanii*, which is known to methylate (**1**) to yield (**3**), would accept and transform the 12-methyl analogue (**2**). The porphyrin corresponding to (**2**) was synthesised[11] and reduced to give the porphyrinogen (**2**) which was incubated as usual[11] with the cobalt-free enzyme system and *S*-adenosylmethionine. The 12-methyl analogue of sirohydrochlorin was isolated as its ester (**12**) in up to 3.8% yield. [*cf.* a 'good' enzymic formation of sirohydrochlorin from uro'gen-III (**1**) which on the milligram scale is 5—7%]. The unambiguous structure of the starting material (**2**) leaves no doubt about the position of the *C*-methyl group in this product (**12**). The latter was identical by full spectroscopic and chromatographic comparison with the product prepared above stated to be the 12-methyl isomer (**12**); this correlation settles the orientation of all the foregoing materials (**10**), (**11**), (**12**), and (**13**).

The lack of specificity shown by the methylases of *P. shermanii* is remarkable and useful; in contrast, a cell-free enzyme preparation from *Clostridium tetanormorphum* produced no detectable quantity of the methylated product (**12**) from the porphyrinogen (**2**).

Experiments on B_{12}-biosynthesis which make use of 12-methylisobacteriochlorins will be reported separately.

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