Biosynthesis of Vitamin B_{12}: Evidence from Double-labelling Studies ($^{13}CD_3$) for Intact Incorporation of Seven Methyl Groups

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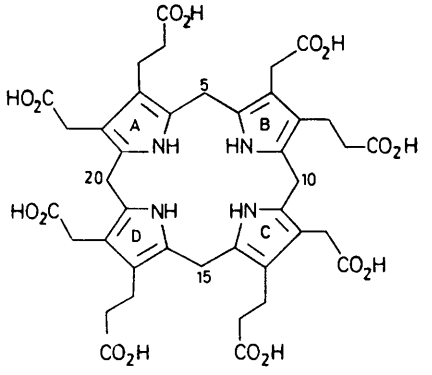
Summary The deuterium noise-decoupled ^{13}C n.m.r. spectrum of dicyanocobalamin (**5**) biosynthesised from [*methyl*-$^{13}CD_3$]methionine shows intact incorporation of seven methyl groups into the macrocycle of vitamin B_{12} without exchange of D with H of the medium.

EXPERIMENTS with labelled uro'gen-III (**1**) have shown[1-4] its enzymic conversion into cobyrinic acid (**2**), the corrin precursor[5] of vitamin B_{12} (**3**). Current interest centres on the intermediates between uro'gen-III (**1**) and the corrin

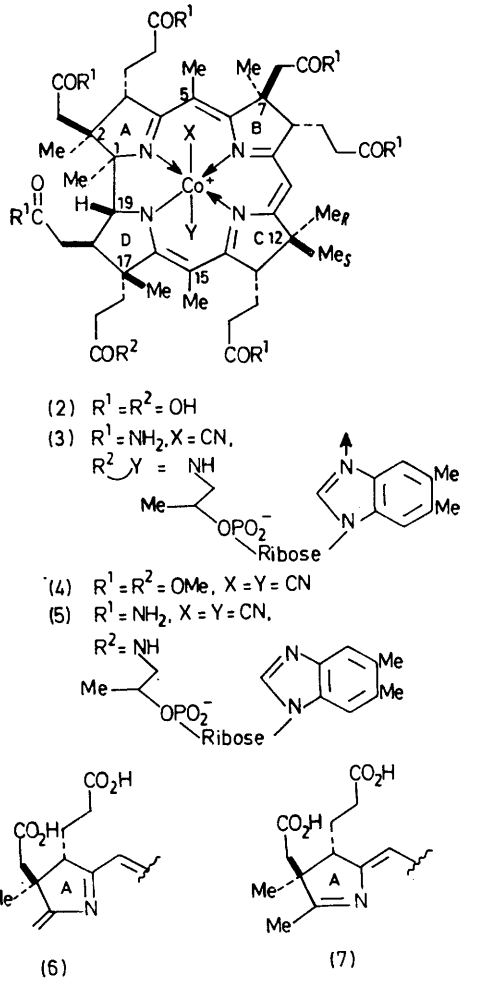


(1)

(**2**) during which the following operations are needed: (a) introduction of seven methyl groups, one each at carbons 1, 2, 5, 7, 12 (*pro-R*), 15, and 17, all derived from methionine[4] and those at C-7 and C-12 (*pro-R*) are transferred intact;[6] (b) loss of C-20 from (**1**); (c) decarboxylation of the ring-c acetic acid side chain to give the *pro-S* methyl group at C-12 of (**2**); (d) redox changes; (e) insertion of cobalt.

The number of possible sequences between uro'gen-III (**1**) and cobyrinic acid (**2**) is thus enormous and it would be helpful to distinguish between processes for C-1 to C-19 bond formation in which the C-1 methyl group has temporarily been a methylidene residue having ring-A as *e.g.* (**6**) and those where it remains unaffected, ring-A *e.g.* as (**7**); for an elegant reductive corrin ring-closure *in vitro* involving a methylidene pyrrolenine (as **6**) see ref. 7. It was not possible to examine the history of the C-1 methyl group by an earlier method[6] because of the impracticability of isolating ring-A by degradation of cobester (**4**). However, the synthesis from $^{13}CD_3I$ and homocysteine of [*methyl*-$^{13}CD_3$]-methionine (90 atom % ^{13}C; 98 atom % D) and its use for the biosynthesis of vitamin B_{12} by *Propionibacterium shermanii* gave the desired information.

The vitamin B_{12} (**3**) isolated from this experiment was examined by ^{13}C-n.m.r. spectroscopy as dicyanocobalamin (**5**) using deuterium noise-decoupling and ^1H-lock. Under these conditions, $^{13}CD_3$ groups will give rise to singlets, $^{13}CD_2H$ to doublets (*J ca.* 130 Hz), $^{13}CDH_2$ to triplets, and $^{13}CH_3$ to quartets. The observed spectrum (Figure) shows no splitting of any of the signals from ^{13}C-enriched methyl groups by protium directly bonded to ^{13}C. Long-range



(2) $R^1 = R^2 = OH$
(3) $R^1 = NH_2$, X = CN, R^2–Y = NH
(4) $R^1 = R^2 = OMe$, X = Y = CN
(5) $R^1 = NH_2$, X = Y = CN, R^2 = NH

(6) (7)

^1H—^{13}C couplings broaden the ^{13}C resonances, however, so two signals merge together at *ca.* 18·7 p.p.m.† It should be noted that the two signals at high field from the methyl groups at C-5 and C-15 are very sharp, the nearest ^1H-atom being four bonds away.

The ^{13}C n.m.r. spectrum of cobester[6] (**4**) derived from the ^{13}C-enriched vitamin B_{12} was recorded in benzene under

† The deuterium substitution causes a small upfield shift of the ^{13}C-signals.

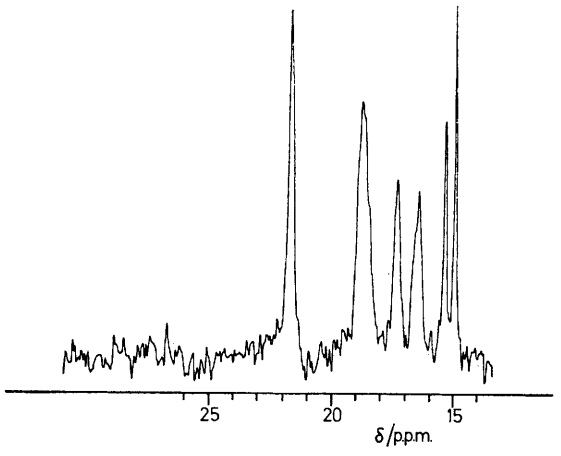


FIGURE. Deuterium noise-decoupled ^{13}C-Fourier transform n.m.r. spectrum (methyl region) of ^{13}C-enriched dicyanocobalamin (**5**) biosynthesised from [*methyl*-$^{13}CD_3$]methionine. (0·20M in 0·1M KCN–H_2O, 84, 785 transients); chemical shifts downfield from Me_4Si.

the same decoupling conditions; it was in agreement with that above, though for computing reasons the quality was somewhat less.

The combined results show that (a) the seven methyl groups of vitamin B_{12} (**3**) derived from methionine are transferred intact and (b) none of them undergoes significant exchange of hydrogen with the medium during the biosynthesis of vitamin B_{12} (**3**). The intermediacy of a system with the C-1 methyl group temporarily as a methylidene residue (*e.g.* as **6**) is thus unlikely since to satisfy the spectroscopic findings, deuterium removed from the C-1 methyl group would need to be returned to the same carbon atom without exchange with the medium.

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