BIOSYNTHESIS OF THE 4,5-DIMETHYL-1,2-PHENYLENE MOIETY OF VITAMIN B_{12}

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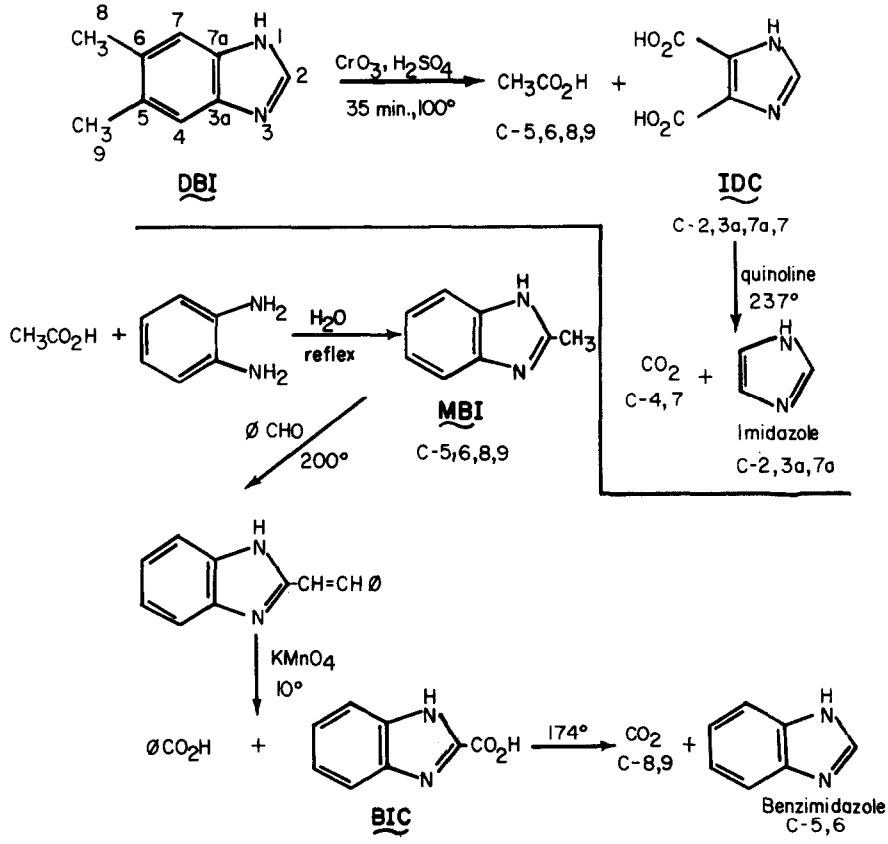
SUMMARY

The pattern of ribose-1-^{14}C incorporation into the 5,6-dimethylbenzimidazole unit of vitamin B_{12} has been determined. The labeling pattern within the 4,5-dimethyl-1,2-phenylene moiety is totally consistent with the involvement of the established riboflavin precursor, 6,7-dimethyl-8-ribityllumazine, in 5,6-dimethylbenzimidazole biosynthesis. In addition, the ribose-1-^{14}C incorporation pattern, when compared with other precursor incorporations, suggests that ribose is a close but not immediate precursor of the four carbon unit involved in the 6,7-dimethyl-8-ribityl-lumazine biosynthesis.

Recently we reported experiments bearing upon the biosynthetic origin of the 5,6-dimethylbenzimidazole (DBI) unit of vitamin B_{12} (Alworth *et al.*, 1969). The relative incorporations of a series of radioactive potential precursors led to the conclusion that DBI and riboflavin were biosynthetically related. It was also concluded that the 4,5-dimethyl-1,2-phenylene moiety of DBI was derived from a pentose or tetrose intermediate, rather than from acetate or pyruvate (*Cf.*, Renz and Reinhold, 1967). In order to test these conclusions, a complete carbon-by-carbon degradation of the DBI-^{14}C obtained from *Propionibacterium shermanii* cultures supplied with ribose-1-^{14}C has been performed.

MATERIALS AND METHODS

The isolation of the DBI-^{14}C from P. shermanii cultures supplied with ribose-1-^{14}C has been previously described (Alworth, et al., 1969). The experimental procedures used to determine the label at the C-2 position of DBI have also been previously reported (Alworth and Baker, 1968). The procedures used to determine the labeling pattern in the 4,5-dimethylphenylene moiety of DBI are outlined schematically below.



The oxidative cleavage of DBI to acetic acid and 4,5-imidazoledicarboxylic acid (IDC) was carried out under modified Kuhn-Roth conditions. After removal of the acetic acid by steam distillation, the solution was cooled and the solid IDC collected by centrifugation. The IDC was then

purified by several precipitations from aqueous solution followed by ion exchange chromatography (yield 43%). The acetic acid (yield 51%) was degraded via 2-methylbenzimidazole according to the method of Roseman (1953).

RESULTS AND DISCUSSION

The results of the degradation of DBI-^{14}C formed biosynthetically from ribose-1-^{14}C are summarized in Table I.

TABLE I: Distribution of ^{14}C in DBI labeled biosynthetically from ribose-1-^{14}C.

Compound counted	Specific activity[a] (dpm/mM)	Carbon atoms represented	Percent of total DBI activity
DBI	6374	all	100
IDC	4342	C-2,3a,4,7a,7	68
$BaCO_3$	698[b]	C-4(7)	11[b]
Imidazole	2792	C-2,3a,7a	44
		C-2	40[c]
		C-3a(7a)	2[b,d]
MBI	969[b]	C-5,9(6,8)	15[b]
$BaCO_3$	619[b]	C-8(9)	10[b]
Benzimidazole	137[b]	C-5(6)	2[b]

[a] The specific activities of all samples were determined by liquid scintillation counting in a Beckman Series 200 instrument using external standardization. Carbon dioxide, precipitated as $BaCO_3$, was analyzed according to the procedure of Woeller (1961). The organic compounds were routinely dissolved in 1 ml of dimethylformamide and counted in a toluene solution of PPO.
[b] Values determined and reported are for one of the equivalent atoms, or groups, of the total DBI molecule.
[c] Previously determined, Alworth et al., 1969.
[d] Value determined by difference (C-2,3a,7a minus C-2)/2.

Many years ago D. W. Woolley (1951), found that 1,2-diamino-4,5-dichlorobenzene was selectively toxic towards

organisms capable of forming riboflavin and vitamin B_{12}. Woolley proposed that the 1,2-diamino-4,5-dichlorobenzene was functioning as a selective antimetabolite towards a biosynthetic unit which was common to both riboflavin and the DBI moiety of B_{12}. Subsequent investigations, however, established that the riboflavin molecule was derived from two 6,7-dimethyl-8-ribityllumazine molecules in a condensation reaction (Harvey and Plaut, 1966). It was also established that the lumazine molecule was derived from a purine through loss of the C-8 carbon (McNutt, 1961), and incorporation of a new four carbon biosynthetic unit. Since there seemed no obvious way to derive DBI from the purine ⟶ lumazine ⟶ riboflavin pathway, and since 1,2-diamino-4,5-dimethylbenzene itself functioned as a precursor of DBI (Perlman and Barrett, 1958), Woolley's proposal was largely forgotten. Recent studies of DBI formation in P. shermanii, however, have indicated that DBI and riboflavin biosyntheses are intimately connected (Renz and Reinhold, 1967; Alworth et al., 1969; Renz, 1970).

The labeling pattern in the DBI-^{14}C resulting from ribose-1-^{14}C metabolism reported in Table I adds to the growing experimental evidence that DBI biosynthesis proceeds through a 6,7-dimethyl-8-ribityllumazine intermediate. A 4,5-dimethyl-1,2-phenylene unit formed by the condensation between two lumazine molecules will be composed of two biosynthetically equivalent four carbon units (Harvey and Plaut, 1966). The labeling pattern reported above, with C-3a(7a) = C-5(6) and C-4(7) = C-8(9), is consistent with the formation of the dimethylphenylene moiety of DBI via such a lumazine condensation.

The relative incorporation data from a series of labeled compounds into the dimethylphenylene portion of DBI was previously interpreted in terms of a tetrose or pentose precursor (Alworth et al., 1969). A similar proposal has also been made with respect to the analogous dimethylphenylene moiety of riboflavin (Ali and Al-Khalidi, 1966). The specific labeling of the 4,5-dimethyl-1,2-phenylene unit by ribose-1-^{14}C reported here supports these interpretations--up to a point. It is clear that the ribose incorporation is occurring in a specific manner without extensive randomization of the added ribose-1-^{14}C label. The pattern reported in Table I is analogous to the patterns determined by Plaut (1954) in the dimethylphenylene unit of riboflavin following glucose-1-^{14}C or glucose-6-^{14}C metabolism by Ashbya gossipii. The ribose-1-^{14}C pattern is the converse of the pattern resulting from lactate-2-^{14}C metabolism in P. shermanii (Renz and Reinhold, 1967) or acetate-1-^{14}C metabolism in A. gossippi (Plaut, 1954).

It should also be noted that, of 14 labeled compounds tested, erythritol-U-^{14}C was found to be the most specific precursor of the dimethylphenylene moiety of B_{12} (Alworth et al., 1969).

It is not yet clear how all of these observations pertaining to dimethylphenylene biosynthesis should be interpreted. The results obtained in studies of DBI formation in P. shermanii cultures lead us to propose that the four carbon biosynthetic unit involved in 6,7-dimethyl-8-ribityllumazine formation is derived from a tetrose, formed by pentose cycle activity. According to this interpretation, the labeling pattern within the dimethylphenylene moiety

from ribose-1-^{14}C incorporation would be due to the involvement of a tetrose-1-^{14}C unit in 6,7-dimethyl-8-ribityllumazine biosynthesis; the tetrose-1-^{14}C molecule being derived from the ribose-1-^{14}C $\longrightarrow$ sedoheptulose-3-^{14}C $\longrightarrow$ fructose-3-^{14}C $\longrightarrow$ erythrose-1-^{14}C interconversions permitted by non-oxidative pentose cycle reactions (Cf. Katz and Rognstad, 1967). Experiments to test this interpretation are currently underway.

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