The Biosynthesis of 5,6-Dimethylbenzimidazole from 6,7-Di[^{14}C]methyl-8-ribityl-lumazine

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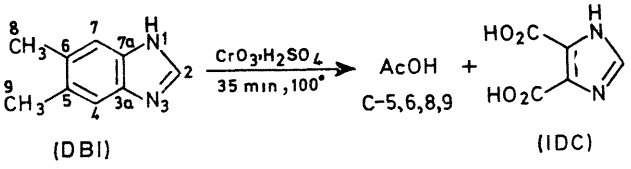
Summary Degradation of [^{14}C]-5,6-dimethylbenzimidazole isolated from biosynthetic vitamin B_{12} indicated that the 4,5-dimethyl-1,2-phenylene unit was formed by a bimolecular condensation of added 6,7-di[^{14}C]methyl-8-ribityl-lumazine precursor

RECENT experimental results indicate that the biosyntheses of riboflavin and of the 5,6-dimethylbenzimidazole (DBI) moiety of vitamin B_{12} are connected Labelled compounds tested as precursors of DBI[1-3] led to incorporation efficiencies and patterns that parallelled the results of investigations into the origin of ring A of riboflavin [4] Renz has also reported that [^{14}C]riboflavin was efficiently converted into [^{14}C]DBI in cell preparations of *P shermanii* [5] We report that 6,7-di[^{14}C]methyl-8-ribityl-lumazine is an effective precursor of the DBI moiety of vitamin B_{12}, and that the labelling pattern within the resulting [^{14}C]DBI is consistent with its biosynthesis *via* a bimolecular 6,7-di[^{14}C]methyl-8-ribityl-lumazine condensation

6,7-Dimethyl-8-ribityl-lumazine labelled with ^{14}C in the methyl carbons was synthesized according to Plaut's procedure [6] It was purified by column chromatography and crystallized from 80% EtOH, m p 280—283° (decomp) The identity and purity were checked by elemental analysis, u v spectroscopy, and paper chromatography [7]

100 2 mg (2 07 × 10^6 d p m) of 6,7-di[^{14}C]methyl-8-ribityl lumazine was added to a *P shermanii* culture that had been incubated anaerobically for 4 days After 5 more days of aerobic growth, the cells were harvested, and the vitamin B_{12} was purified (36 mg, 1 9 × 10^5 d p m) The growth conditions and the procedures used to purify the vitamin B_{12} have been described [2,8] The [^{14}C]DBI sample obtained by hydrolysis of the vitamin B_{12} was diluted with unlabelled DBI, purified, and the specific activity determined The incorporation data were then corrected to take into account the observed yield of DBI (59%) from hydrolysis of the vitamin B_{12}

The labelling pattern within the DBI was established by determining the specific activities of chemical degradation products in a liquid scintillation counter The conversion of DBI into 1,2-dibenzamido-4,5-dimethylbenzene with release of the C-2 carbon as CO_2 has been described [8] Determination of the labelling pattern within the remainder of the DBI molecule was based upon Kuhn–Roth oxidation



Reaction 1

(reaction 1) Distribution of the label within the AcOH was established by Roseman's procedure [9] Roseman's degradation yields benzimidazole containing C-1 of AcOH [C-5(6) of DBI] and releases C-2 of AcOH [C-8(9) of DBI] as CO_2 The distribution of label within the imidazole-4,5-dicarboxylic acid (IDC) was determined by decarboxylating the IDC to imidazole [C-2,3a(7a) of DBI] and CO_2 [C-4(7) of DBI] This degradative scheme has been reported in greater detail [3]

About 9% of the ^{14}C added to the culture was recovered as radioactive vitamin B_{12} The specific activity of the purified DBI indicated that about 75% of the total vitamin B_{12} activity was present in the DBI moiety The specific activities of the degradation products established that the label within the biosynthetic DBI was completely confined to C-4(7) and to C-8(9) 44% of the DBI label was located at the equivalent C-4 and C-7 positions, and 38% of the label was located at the equivalent C-8 and C-9 positions Degradation products representing the other carbon atoms

were each found to contain less than 1% of the total DBI label. The methyl carbons of 6,7-di[^{14}C]methyl-8-ribityl-lumazine are therefore specific biosynthetic precursors of carbon atoms C-4(7) and C-8(9) of DBI.

Plaut established that 6,7-di[^{14}C]methyl-8-ribityl-lumazine is converted into [^{14}C]riboflavin specifically labelled in the methyl carbons and at C-5 and C-8.[6] The results reported here therefore indicate that the 4,5-dimethyl-1,2-phenylene unit of DBI is derived by the same type of bimolecular 6,7-dimethyl-8-ribityl-lumazine condensation as is involved in the biosynthesis of riboflavin.[10] This observation, when combined with the experimental results cited earlier,[1-3,5] establishes that Woolley was correct when he suggested that the biosyntheses of riboflavin and of DBI were connected.[11] It remains, however, to be definitively established that riboflavin is an obligatory intermediate in the biosynthesis of DBI as suggested by Renz.[5] Possibly related but branching pathways lead to the formation of both riboflavin and DBI directly from the common 6,7-dimethyl-8-ribityl-lumazine precursor.

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