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Formation of the Dimethylbenzimidazole Ligand of Coenzyme B_{12} under Physiological Conditions by a Facile Oxidative Cascade

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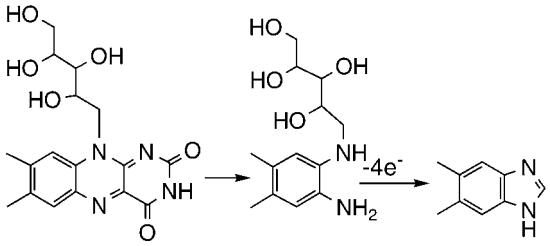
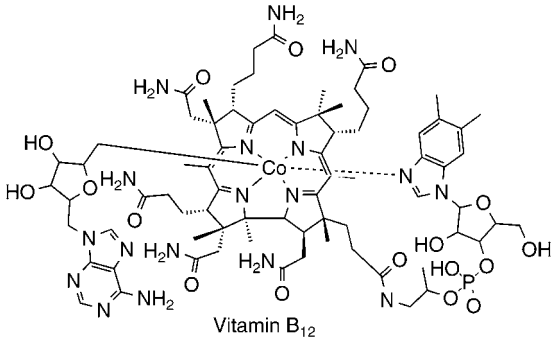
ABSTRACT



Dimethylbezimidazole, the axial ligand of vitamin B_{12}, is synthesized from riboflavin by a two-electron oxidation, a retro-aldol condensation, and a second two-electron oxidation. This oxidative cascade readily takes place nonenzymatically under physiological conditions.

The formation of the 5,6-dimethylbenzimidazole ligand (DMB, **4**) is an unsolved problem in vitamin B_{12} biosynthesis, and no gene or enzyme involved in this process has been identified. In some bacteria (e.g., *Salmonella enterica* and *Propionibacterium freundenreichii*), DMB is formed from riboflavin (**2**), and labeling studies have demonstrated that the C-2 carbon of DMB (**4**) is derived from the C-1 carbon of the ribose moiety (**2**, Scheme 1).[1−3] This remarkable transformation is likely to require oxygen because *S. enterica* appears to synthesize DMB only under aerobic growth conditions.[4,5]



Vitamin B_{12}

While there is no precedent for this complex chemistry, the observations that riboflavin can be converted, in very low yield, to a mixture of diaminobenzene (**3**) and DMB (**4**) under strongly basic conditions[6] and that diaminobenzenes can react readily with aldehydes to give the corresponding substituted benzimidazoles[7] suggest that **3** and its

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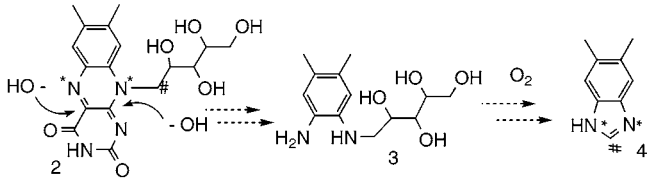
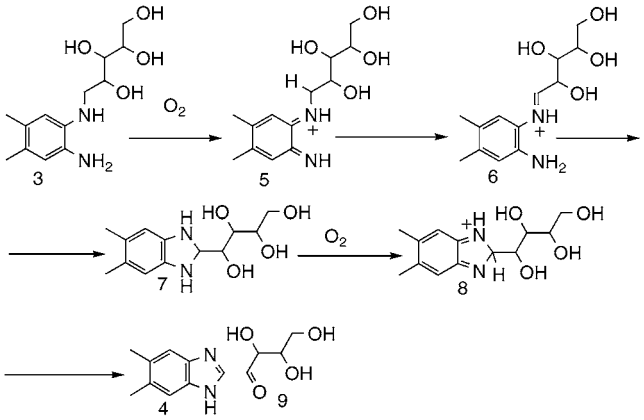
Scheme 1



imine **5** may be intermediates and that it may be possible to find relevant nonenzymatic chemistry for the conversion of **3** to **4**. Here, we demonstrate the validity of this analysis and propose that the conversion of **3** to DMB can occur to a large extent by a facile nonenzymatic oxidative cascade (Scheme 2).

Scheme 2

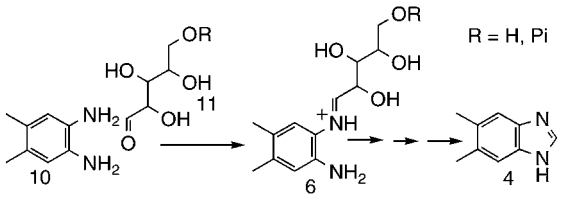


DMB (**4**) was formed when a synthesized sample of **3** was treated with catalytic amounts of potassium ferricyanide, a mild oxidizing agent known to oxidize amino phenols to the corresponding quinone imines. This supports the proposal that DMB can be formed by the nonenzymatic oxidation of **3**.[8] Aerobic oxidation of **3** under physiological conditions (pH 7, 37 °C) also gave DMB. However, the reaction was approximately 30 times slower than the ferricyanide-mediated oxidation. DMB was not formed after 24 h in argon-purged buffer, demonstrating that its formation requires oxygen.

Having established the feasibility of converting **3** to **4**, we next turned our attention to the mechanism of this complex oxidation process. Our current mechanistic proposal is outlined in Scheme 2. In this proposal, oxidation of the electron-rich diamine **3** to the bisimine **5** followed by a tautomerization gives **6**. Cyclization to give **7** followed by a second two-electron oxidation gives **8**. Aromatization of **8** by extrusion of **9** gives **4**. Analogous oxidative cascades, in which electron-rich benzenes undergo complex reactions, have been proposed in the biosynthesis of actinomycin,[8] pyoverdin,[9] melanin,[10] and styelsamine[11] and in the hydroxykynurenine-mediated cross-linking of proteins in the mammalian eye lens.[12]

Several experiments were carried out that support this mechanism and differentiate it from alternatives. When diaminobenzene **10** is treated with ribose phosphate under the same conditions used to oxidize **3**, DMB (yields of 6−10%) is also formed in an oxygen-dependent reaction. This supports the intermediacy of **6** (Scheme 3). In addition,

Scheme 3



when this reaction is carried out with D-[^{13}C-1]ribose, NMR analysis of the resulting DMB demonstrated that the ^{13}C was localized at the C-2 position. This labeling pattern is identical to the biosynthetic labeling pattern [1] suggesting that the biomimetic and the biosynthetic conversion of **6** to DMB are occurring by the same mechanism.

HPLC analysis of the **10** + **11** reaction mixture (Scheme 3) identified DMB and an additional compound as the major reaction products (Figure 1). Structure **12** was assigned to

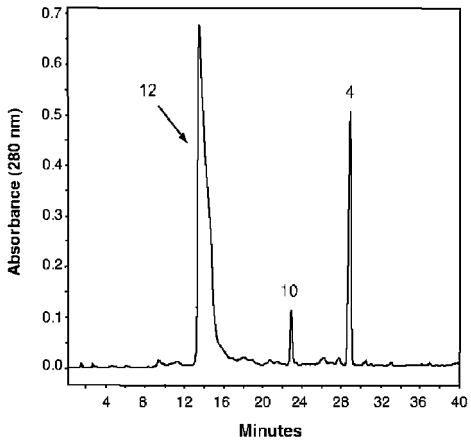


Figure 1. HPLC analysis of the reaction shown in Scheme 3.

this compound on the basis of its MS, UV, and NMR spectra. Compound **12** was stable and did not undergo conversion to DMB (Scheme 4). The detection of **12** provides support

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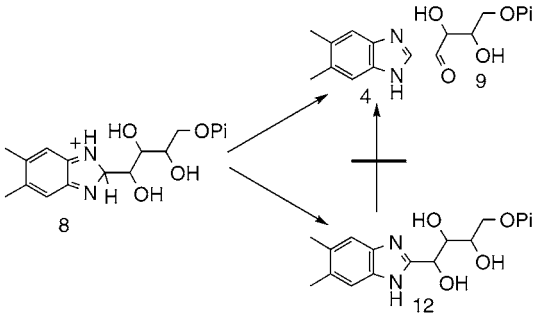
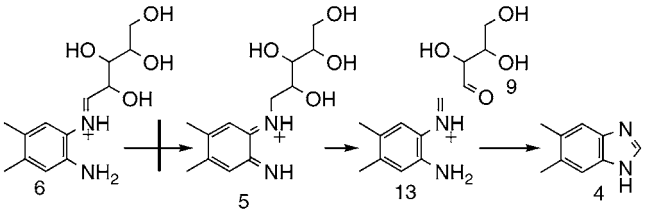
Scheme 4



for the intermediacy of **8** because **12** is likely to be formed by the aromatization of **8**. When the C-2 proton is replaced with a methyl group, as occurs in the condensation of **10** with 3-hydroxy 2-butanone, 2-methyl-DMB is the only isolated product.

We also considered an alternative pathway for the formation of DMB in which **9** is lost from **5** rather than from **8** (Scheme 5). The detection of **12** as a byproduct argues

Scheme 5



against this mechanism. In addition, when the reaction of ribose with **10** is run in D_2O, there is little incorporation of deuterium into **4** (incorporation rate of 0.016 ± 0.001 h^{-1}**),** and when the reaction is run using 1-[^{2}H]-D-ribose in H_2O, there is little incorporation of protium into **4**. The absence of a high level of exchange with solvent in these two experiments demonstrates that **6** is not converted to **5** and therefore that the reaction in Scheme 5 is not the major pathway to **4**. The low level of H/D exchange detected in **4** can be explained by the observation that this compound undergoes slow H/D exchange with water under the reaction conditions (with a rate of 0.013 ± 0.001 h^{-1}).

The observation of a facile conversion of **3** to DMB does not exclude the possibility that this conversion is enzyme catalyzed in vivo, and a previous stereochemical study supports this. In that study, treatment of a 75:25 mixture of 1′-*R*/1′-*S* riboflavin with a cell free extract from *Propionibacterium shermanii* gave DMB with 27% retention of deuterium. In the complementary experiment, a 25:75 mixture of 1′-*R*/1′-*S* riboflavin gave 46% retention of deuterium.[13] While it is unclear as to why the latter reaction shows only 46% retention of label, these experiments suggest that the *pro-S* hydrogen at C1′ of riboflavin is preferentially retained in biosynthesized DMB.[13] However, the stereoselectivity of this reaction does not prove that the formation of DMB is enzyme catalyzed because the stereochemistry of the deprotonation of **5** may be controlled by the adjacent chiral centers on the ribose. To determine if such asymmetric induction is occurring, we have determined the stereochemical outcome of the nonenzymatic reaction described here. Aerobic oxidation of a 72:28 mixture of 1′-*R*/1′-*S* [^{2}H]-**3** gave DMB with 64% retention of deuterium after 5 h, and oxidation of a 28:72 mixture of 1′-*R*/1′-*S* [^{2}H]-**3** gave DMB with 26% retention of deuterium. These experiments suggest that the *pro-R* hydrogen at C1 of **3** is preferentially retained in DMB generated in the model reaction. This is the opposite stereochemical preference to that observed for the biosynthesis of DMB and suggests that the in vivo conversion of **3** to DMB, while facile, is likely to be enzyme catalyzed. One possibility is that the oxidative cascade that converts **3** to **4** takes place in the active site of the putative hydrolase that converts **2** to **3** (Scheme 1).

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Supporting Information Available: Synthesis, spectra, and methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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