Organocobalamin Reactions Relevant to the Mechanism of the α-Methyleneglutarate Mutase Enzyme [1]

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The organocobalamin with methylitaconic acid attached to cobalt via its methyl group has been synthesized for the first time. This compound is believed to be an intermediate in the interconversion of methylitaconic acid and α-methyleneglutaric acid, catalysed by the coenzyme B_{12}-dependent α-methyleneglutarate mutase enzyme. Reactions of this organocobalamin and the corresponding dimethyl ester demonstrate that cleavage of the Co—C bond leads to the rearranged α-methyleneglutarate structure under conditions where intermediate carbanions are formed.

In a recent paper [2] we described reactions of several substituted organocobalamins which simulate key steps of the coenzyme B_{12}-Dependent enzymatic rearrangement of methylmalonyl-CoA to succinyl-CoA [3]. These studies demonstrated that carbanions are the critical intermediates in the rearrangement step, as was first proposed by Ingraham in 1964 [4]. We have now extended our investigations to the rearrangement of methylitaconic acid to α-methyleneglutaric acid (eq. (1)), another coenzyme B_{12}-dependent mutase reaction [3].

Model studies for this rearrangement have already been initiated by Dowd; he synthesized organocobalamins 1 and 2 and showed that 2 undergoes spontaneous decomposition with Co—C bond cleavage to yield mixtures of butadiene-2,3-dicarboxylic acid, methylitaconic acid, and α-methyleneglutaric acid (4—6 respectively, shown in eq. (2)) [5, 6].

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Because of its instability, cobalamin 2 was only isolated in crude form by precipitation from aqueous synthesis solutions with acid acetone [5]. This behavior was in contrast to that of cobalamin 1, which is quite stable and could easily be purified by conventional methods [5]. This compound, however, yielded only 4 and 5 upon photolysis [6]. The sizes of the ester groups of cobalamins 1 and 2 were therefore suggested to play an important role in both the stabilities of 1 and 2, and in their tendencies to yield the rearranged product (6) [6].

We now report the first synthesis of organocobalamin 3, in which methylitaconic acid is attached to cobalt without prior esterification of its carboxyl groups. This compound has not been previously prepared because (bromomethyl)itaconic acid undergoes rapid lactonization in the alkaline solutions typically used for organocobalamin synthesis [6]. We have avoided this problem by reacting vitamin B₁₂ with (bromomethyl)itaconic acid in methanol containing 10% (w/v) NH₄I, and organocobalamin 3 is formed in high yields [7]. Cobalamin 1 can also be conveniently prepared by the same method using dimethyl (bromomethyl)itaconate as the alkylating agent.

Both cobalamins 1 and 3 are stable in neutral and acidic aqueous solutions, where they exist in the base-on and base-off, forms, respectively. Phenol extracts them from aqueous solution without inducing decomposition, and they are easily purified by column chromatography on CM-cellulose with 10 mM pH 7 phosphate buffer. Spectral data are given in Table 1.

Photolysis reactions of cobalamins 1 and 3 were investigated in both neutral solution and in 0.12 M NaCN solution [8]. The latter conditions were chosen because coordination of cyanide to cobalt is known to induce the formation of carbanions in the photolysis of methylcobalamin (Scheme 1) [9] and because we believed that the generation of intermediate carbanions would be necessary to obtain high yields of rearranged products from the cleavage of the Co–C bonds of cobalamins 1 and 3. This hypothesis was confirmed by the ratios of the organic products obtained from the photolyses of 1 and 3 in the presence and absence of cyanide.

Table 1. Spectral data for cobalamins 1 and 3.

<table>
<thead>
<tr>
<th>Cobalamin</th>
<th>( \lambda_{\text{max}} \text{nm}(\varepsilon) )</th>
<th>( \lambda_{\text{min}} \text{nm}(\varepsilon) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>331(12600) 374(9830) 428(4660) 518(7900) 409(4260)</td>
<td>332(13900) 378sh(9060) 445(5170) 507(7870) 415(4480)</td>
</tr>
<tr>
<td>3</td>
<td>320(13900) 378(9060) 445(5170) 507(7870) 415(4480)</td>
<td>332(17000) 459(8020) 422(6830)</td>
</tr>
</tbody>
</table>

In anaerobic pH 7 phosphate buffer 1 and 3 photolyzed to produce mixtures of 4, 5, and 6 (see eq. (2)). The \( \beta \)-elimination product (4) was predominant (60%), as is typical of the photolytic behavior of organocobalamins containing a \( \beta \)-hydrogen [9]. In anaerobic 0.12 M NaCN solution, the same products were observed, but the rearranged 6 was by far the main product, accounting for 80–90% of the product mixture. The formation of intermediate carbanions under the latter conditions was confirmed by the concomitant formation of dicyanocob(III)alamin, identified by its characteristic violet color and absorption spectrum [10].

Accordingly, the mechanism for the rearrangement of the methylitaconate structure is formulated as shown in Scheme 2.
The intermediacy of these anions is supported by the reaction chemistry above, and by previous labelling studies. When Dowd allowed cobalamin 2 to decompose in D₂O, deuterium was incorporated into 5 and 6 at the positions indicated by anions 7 and 9 [11, 12]. In addition, the relative stabilities of these anions (9 > 7) is consistent with the position of the enzymatic equilibrium; α-methylene glutaric acid (6) is favored over methylitaconic acid (5) by a factor of approximately 4 [13].

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[7] Cobalamins 1 and 3 were prepared by first reducing 350 mg of hydroxocobalamin with zinc dust in 10 ml of anaerobic methanol containing 10% (w/v) ammonium iodide in a 15 ml capacity centrifuge tube. 0.1 ml of neat dimethyl (bromomethyl)itaconate was injected to prepare cobalamin 1, or 0.25 g of (bromomethyl)itaconic acid dissolved in 1 ml of anaerobic methanol was injected to prepare cobalamin 3. The reaction solutions were worked up according to procedures in ref. [2], except that acetone for precipitation need not be anaerobic.
[8] Photolyses were carried out in 160 ml capacity bottles sealed with silicon septa and deaerated with argon (purified by two chromous scrubbers in series) for 1–2 h. After photolyzing the cobalamins for 2–5 h with a tungsten lamp, the organic products were recovered from the reaction solutions by first acidifying them with HCl and then extracting continuously with ether for two days. (Reaction solutions from cobalamin 1 were first made basic with 1 N NaOH and stored overnight to hydrolyse the esters.) The ether extracts were dried and evaporated. The residue was weighed, dissolved in acetone-d₆, and the 360 MHz proton NMR spectrum was measured to determine the products and their relative ratios. Photolyses in 0.12 M NaCN were carried out by deaerating a sample of the organocobalamin in 75 ml of water while 500 mg of NaCN and 10 ml of water were deaerated in separate 25 ml capacity serum vials. Then the 10 ml of water were transferred under argon pressure by catheter into the vial containing the NaCN, which quickly dissolved. This NaCN solution was transferred by catheter into the large vial containing the cobalamin solution. Caution: Solutions of NaCN give off toxic HCN gas! After photolysis, the reaction vial was opened in the hood, the solution was cautiously acidified with HCl, and argon was bubbled through it overnight to remove HCN. The solution was made basic and worked up as above. Residues from the photolysis reactions weighed from 20–29 mg (65 bis 95% yields).
[10] Attempts to produce the proper carbanions from cobalamins 1 and 3 by reductive cleavage reactions were not fruitful. NaBH₄ did not effectively cleave the Co–C bond of these stable base-on cobalamins. Zinc dust in methanol containing 5% (w/v) ammonium bromide did cleave the Co–C bond, but the double bond of the methylitaconate structure was also reduced, and 2,3-dimethylsuccinic acid was formed.
[12] Protonation of 8 would lead to methylcyclopropane-1,2-dicarboxylic acid. This product was not observed in the model reactions, and is not a substrate of the α-methylene glutarate mutase enzyme [13]. The cyclic carbanion is unstable because it is primary, and may be further destabilized by ring strain.