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A Nonenzymic Model for the Coenzyme B_{12}-Dependent Isomerization of Methylmalonyl-SCoA to Succinyl-SCoA

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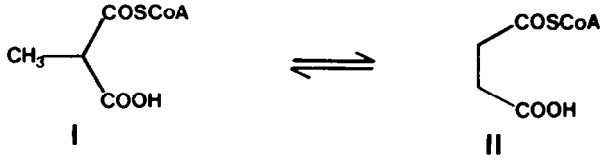
Abstract - Dimethyl bromomethylmalonate (IV) reacts with vitamin B_{12s} in aqueous solution yielding a relatively unstable carbon-cobalt bonded adduct V, which shows visible spectra in good accord with expectation. The adduct V was allowed to decompose in water, in the dark, at room temperature and at physiological pH. Three products: succinic acid (VI), methylmalonic acid (VIII) and malonic acid (VII) were formed in 3, 18, and 13% yields respectively. Isolation of the succinic acid rearrangement product provides support for the intermediacy of the carbon-cobalt bond in the coenzyme B_{12} dependent enzymic carbon-skeleton rearrangement of methylmalonyl-SCoA to succinyl-SCoA.

Introduction

The coenzyme B_{12}-dependent, enzyme-catalyzed rearrangement of methylmalonyl SCoA (I) to succinyl-SCoA (II) (eq. 1) is a critical element of mammalian metabolism.[1] This enzymic transformation effects the return of propionic acid, a product of amino acid and branched-chain



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and odd-membered fatty acid catabolism, to the mainstream tricarboxylic acid cycle metabolic pathway.[2] Deficiency of coenzyme B_{12} or malfunction of methylmalonyl-SCoA mutase, as a consequence of dietary deprivation or genetic disorder, usually is attended by disasterous consequences resulting from bodily excesses of propionic acid and methylmalonic acid. It has been hypothesized that in fatty acid biosynthesis the presence of methylmalonyl-SCoA in higher than normal concentrations may result in incorporation of methylmalonate instead of malonate into the fatty acid chain.[3] This would lead to methyl branches interfering with the proper packing of the fatty acid molecules in nerve membranes. Neural degeneration is one of the consequencies of pernicious anemia, the most notorious vitamin B_{12}-related disorder.

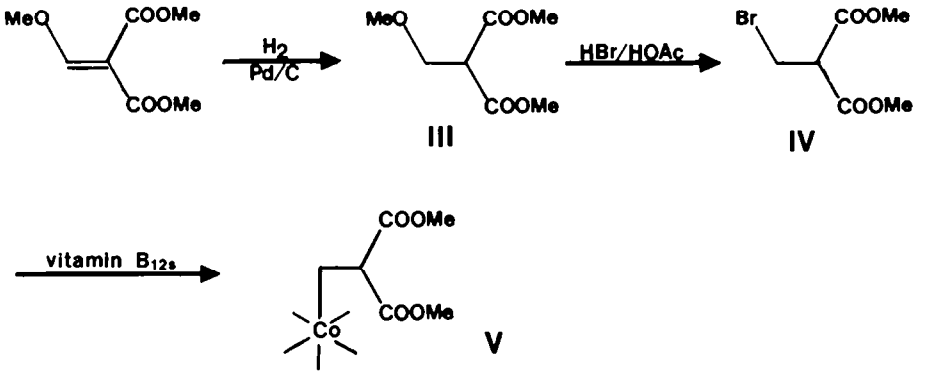
For the rearrangement shown in equation 1, carbon-labelling studies have shown that the carbonyl-SCoA group is the migrating group[4] and that the reaction is intramolecular.[5] The hydrogen migration is an intermolecular reaction in which hydrogen of the substrate is abstracted by the 5'-methylene group of the deoxyadenosine of coenzyme B_{12}. The hydrogen is later returned to the rearranged substrate. As a consequence of this sequence of steps, no exchange exchange between solvent D_2O and substrate protons occurs in the course of the enzymic rearrangement.[6,7] During the rearrangement, configuration is maintained at both termini of the rearranging system.[8]

In spite of the importance of the step which converts methylmalonyl-SCoA to succinyl-SCoA (eq. 1) and the intense effort devoted to it, this reaction is still not well understood. For biologically important reactions, it is advantageous to be able to examine nonenzymic model reactions. Unfortunately, in the present instance, there have been no working model reactions, and exploratory research into the nature of this carbon-skeleton rearrangement has been hampered by this lack.

The challenge of this reaction and of this series of rearrangments is to isolate the catalytic contribution of the enzymes from that of the coenzyme. This is one reason for exploring the reactions by way of reactive intermediates and models. The experiments described here also serve as the first test of the general applicability of the recent model for the methylitaconate ⇄ α-methylene-glutarate interconversion.[9] The principal thesis to be explored is the utility in model building of direct attachment of substrate to cobalt.

Results and Discussion

Dimethyl bromomethylmalonate IV was prepared by a modification of the method of Simonsen[10] treating dimethyl methoxymethylmalonate III with 3 equivalents of 32% HBr in acetic acid. The bromide IV is very sensitive. All purification steps must be carried out at room temperature or below. The most effective means of purification was bulb-to-bulb distillation at 25°C/10^{-5} mm. The spectral properties of IV are described in the experimental section.



A solution of vitamin B_{12s}, prepared by reduction of hydroxocobalamin with sodium borohydride,[11] was reacted with the bromomethylmalonate IV yielding a carbon-cobalt bonded adduct V.

The ultraviolet and visible spectral properties of V are in good accord with expectation (Fig. 1).[11] Exposure of the solution of V to visible light produced the characteristic spectrum of hydroxocobalamin (Figure 1) signalling cleavage of the carbon-cobalt bond.

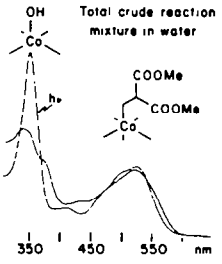
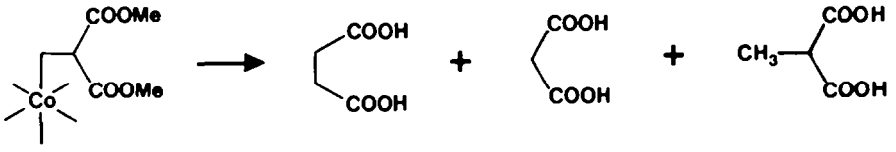


Figure 1. Visible absorption spectrum of the cobalamin methylmalonate before and after exposure to light.

The alkyl cobalamin V is unstable. Attempts to isolate V by precipitation from the aqueous reaction mixture using acetone or tetrahydrofuran as precipitant resulted in loss of the carbon-cobalt bond to the extent of 50-70% as judged by the appearance of the 352 nm hydroxocobalamin band in the visible spectrum. Extraction into phenol also resulted in destruction of the carbon-cobalt bond.

Accordingly, the experiments described below were carried out with freshly prepared solutions of alkylcobalamin V. After extracting the reaction mixtures with ether to remove excess bromomethylmalonate IV, the aqueous solutions were allowed to stand in the dark for 48 hours until conversion of the alkylcobalamin to hydroxocobalamin was complete. Ester hydrolysis



extraction and chromatographic separation yielded the products: succinic acid (VI) (3%), malonic acid (VII) (13%) and methylmalonic acid (VIII) (18%). The spectral properties of the products were in good accord with those of authentic samples.

A control reaction and workup in the absence of cobalamin yielded no succinate.

Conclusion

The model experiments were carried out under conditions close to those which prevail in biological circumstances: at ambient temperature, in the dark, at or near physiological pH and in aqueous solution. The model rearrangement thus establishes that the concept of attaching substrate to the central cobalt atom of vitamin B_{12}, first demonstrated in the rearrangement of the methylitaconate-B_{12} adduct,[9] is a fruitful one. This is also now apparent in the work of others[12,13,14,15] following upon the initial lead.[9]

Experimental Part

Dimethyl methoxymethylenemalonate was prepared according to Parham and Reed.[16]

Dimethyl methoxymethylmalonate (III)

A solution of 17.4 g (0.1 mole) of dimethyl methoxymethylenemalonate and 2 g of 5% palladium on carbon in 100 ml of ethyl acetate was treated with hydrogen under pressure at 20 lb/in^2 and at room temperature for 1 h on a Parr shaker. The ethyl acetate solution was filtered and concentrated to 17.4 g of colorless oil. Distillation yielded 13 g (75%), bp 112-117°C/0.25 mm, of III. The nmr spectrum (CCl_4, 60 MHz) of III showed: δ 3.35 (s, 3H) and δ 3.7 (s, 9H); ir(neat): 2975 (m), 1739 (vs) and 1030 (s) cm^{-1}; mass spectrum (15 eV), m/z (rel. int.): 176 (6.5, M^+), 175 (44, M^+-H), 145 (42, M^+-OMe), 117 (100, M^+-CO_2Me).

Dimethyl bromomethylmalonate (IV)

A mixture of 3.6 g (0.016 moles) of dimethyl methoxymethylmalonate (III) and 12 g (0.054 moles) of 30% HBr in acetic acid was stirred at room temperature for 20 hours. The excess acid was removed under vacuum at 25°C/0.1 mm by bulb-to-bulb distillation. The residue was then distilled bulb-to-bulb at 25°C/5 x 10^{-5} mm yielding 2.7 g (60%) of a colorless oil. The nmr spectrum ($CDCl_3$, 60 MHz) showed δ 3.8 (s); ir (neat): 3030 (m), 1739 (vs), 1274 (m) and 1031 cm^{-1}; mass spectrum (70 eV), m/z (rel. int.): 224, 222 (9, M^+), 195, 193 (14, M^+-OMe), 167, 165 (100, M^+-COOMe), 145 (34, M^+-Br).

Adduct V of Dimethyl Methylmalonate with Vitamin B_{12}.

A solution of 1.346 g (1.0 mmol) of hydroxocobalamin in 85 ml of water was reduced with 0.600 g (15.9 mmol) of sodium borohydride in 5 ml of water under an atmosphere of nitrogen to the grey-green solution characteristic of vitamin B_{12s}. This solution was treated in the dark with 0.700 g (3.1 mmoles) of dimethyl bromomethylmalonate (IV). After 6 min a visible spectrum (Figure 1) showed complete formation of the carbon-cobalt bond with λ_{max}^{water} (ε) 525 (6920), 500

(sh, 6450), 430 (3590), 375 (8000), 336 (11,065), 315 (sh, 10,480), 285 (sh, 14,365), 282 (15,530) and 260 (17,800) nm and with λ_{min}^{water} 410 (3,320) and 445 (3,400) nm. In acid solution (pH 3) the 525 nm peak shifts to 474 nm (ε7740) and the two peaks at 350 (10,500) and 314 nm (11,490) become more sharply defined. This spectral change is reversed upon addition of base, but some decomposition can be observed, by the rise of the peak at 352 nm indicating an increased amount of hydroxocobalamin, a consequence of the brief exposure to acid. Since the alkyl cobalamin V is an unstable substance, the ε values cited above could not be determined from a weighed sample. Instead they were estimated by allowing the sample to decompose with light, then calculating the ε values from those established for hydroxocobalamin.

The aqueous reaction mixture was extracted with three 100 ml portions of ether. The aqueous layer solution was then allowed to stand at room temperature in the dark at pH 8-9 for 48 h. At the end of this time, the visible spectrum showed complete cleavage of the carbon cobalt bond and conversion to hydroxocobalamin. The reaction mixture was made acidic with 10% HCl and extracted continuously overnight with ether for 24 hr. The nmr spectrum of the ether extract showed a mixture of acids and esters. Accordingly, it was stirred overnight with 1 ml of 10% NaOH then reacidified and extracted again continuously for 24 h with ether. The resulting oily product was chromatographed on a 1.1 cm by 28 cm column of silicic acid. The products were eluted with a 55:45 mixture of ether-chloroform collected in 5 ml fractions. Methylmalonic acid was eluted first in fractions 7, 8, and 9. Malonic acid was eluted in fractions 10, 11 and 12 followed finally by succinic acid in fractions 13 and 14. The yields of recrystallized, sharp-melting solids, based on hydroxocobalamin are 16.1 mg (13.5%) of methylmalonic acid (VIII), 18.7 mg (18%) of malonic acid (VII) and 4.4 mg (3.7%) of succinic acid (VI). The yield of the latter varied from 1-3% in several experiments. The spectral properties (ir, nmr and ms) of the products were in good agreement with those of authentic samples.

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