BIOORGANIC CHEMISTRY 9, 227–230 (1980)

Vitamin B_{12}: Catalyst for a Nonenzymic Carbon-Skeleton Model Rearrangement

A. IAN SCOTT[1] AND JEMO KANG[2]

Sterling Chemistry Laboratory, Yale University, New Haven, Connecticut 06520

AND

PAUL DOWD AND B. K. TRIVEDI

Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

Received February 22, 1979

Reaction of a limited amount (0.11 mmol) of vitamin B_{12a} (hydroxocobalamin) with sodium borohydride (3.96 mmol) and the thioethyl ester of bromomethylmethylmalonate I (0.52 mmol) yields the carbon-skeleton rearranged thioethyl ester of methylsuccinate III (0.14 mmol) together with the unrearranged thioethyl ester of dimethylmalonate IV (0.087 mmol). The yield, based on the amount of vitamin B_{12a} used, of the carbon-skeleton rearrangement product III is 131% (average of six runs). This is the first coenzyme B_{12} rearrangement model system sufficiently facile to function in a catalytic fashion.

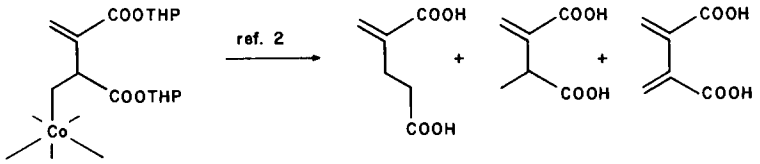
Vitamin B_{12}, in the form of its coenzyme, is an obligatory cofactor in a series of 11 enzyme-catalyzed rearrangement reactions. Of these, three are carbon-skeleton rearrangements. There having been no directly analogous transformations in the organic chemical armamentarium, the coenzyme B_{12}-dependent carbon-skeleton rearrangements have been cause for continuing and wide-ranging mechanistic speculation (*1*).

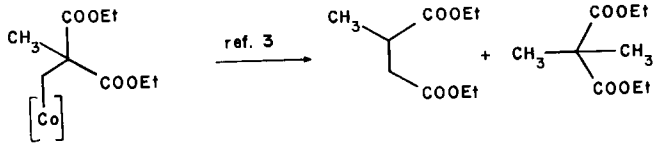
The character of the problem has now been changed with the recent discovery of several nonenzymatic carbon-skeleton rearrangements originating from synthetic coenzyme B_{12} analogs having substrate attached to cobalt in place of 5-deoxyadenosine (Eqs. [1–5]) (*2–7*). The rearrangements starting from the cobalamin-substituted analogs (Eqs. [1], [3], and [5]) are noteworthy for their faithfulness to biologically acceptable conditions; the reactions take place at ambient temperature, in the dark, in aqueous solution and at physiological pH. In the rearrangement of Eq. [1] only the acrylate group migrates (*6*). In the reaction shown in Eq. [5], it is exclusively the thioester group which migrates (*7*). This result is in exact parallel with the coenzyme B_{12}-dependent enzyme-catalyzed rearrangement of methylmalonyl-SCoA to succinyl-SCoA, in which the thioester is the migrating group (*8*).

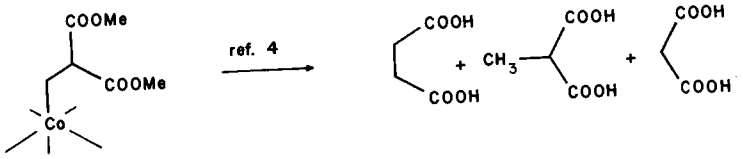
[1] Present address: Texas A & M University, College Station, Texas 77843.

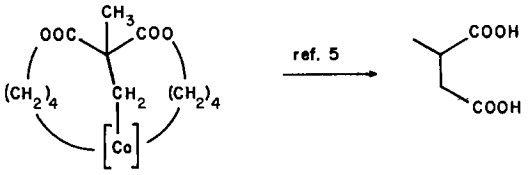
[2] Present address: Tarrytown Technical Center, Union Carbide Corporation, Tarrytown, New York 10591.

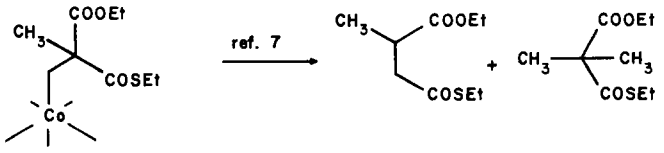
0045-2068/80/020227-04$02.00/0



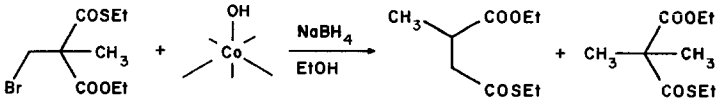








It has now been found that when the model rearrangement of Eq. [5] is carried out in ethyl alcohol as solvent, the reaction is so facile and rapid that the yield of rearrangement product III (based on the quantity of vitamin B_{12} employed) can be induced to exceed 100%.



I II III, 131 % IV, 54 %

A gray-green solution containing less than 1 *M* equivalent of vitamin B_{12s} is

prepared by reaction of hydroxocobalamin with excess sodium borohydride under an atmosphere of nitrogen. In a darkened room a portion of the thioethyl bromomethylmalonate (7) I was added. The reaction mixture became red, indicative of the presence of alkylcobalamin. This is the expected first visible sign in the rearrangement reaction sequence. In the past, in the models of Eqs. [1], [3], and [5], the red color has persisted, red alkylcobalamin being converted to red hydroxocobalamin over a period of time. The present reaction differed from all previous ones in rapidly (1 hr) turning from red back to the gray-green color diagnostic of the presence of vitamin B_{12s}. This behavior suggested that rearrangement had already occurred, yielding hydroxocobalamin which was reduced to vitamin B_{12s} by the excess sodium borohydride present in the reaction mixture. Accordingly, an additional portion of thioethyl bromomethylmalonate I was added, whereupon the same sequence of events transpired. Four more such additions completed this sequence. The reaction was worked up in the normal fashion and the products isolated by thin-layer chromatography on silica gel. In this way the yield of rearranged product methylsuccinate was determined to be 130% based on hydroxocobalamin (average of six runs).

This experimental result constitutes the first model rearrangement reaction catalytic in vitamin B_{12}. A variety of possible synthetic and mechanistic applications may be envisioned.

EXPERIMENTAL

A three-neck flask is equipped with a closed, bent, side-arm tube for sodium borohydride, a nitrogen inlet connected to a nitrogen bubbler-aspirator, and a serum stopper. In the flask vitamin B_{12a} (150 mg, 0.111 mmol, Merck) is stirred in absolute ethanol (5 ml) and cooled in an acetone–ice bath (ca. $-10°C$). The flask is evacuated and flushed with nitrogen seven times. The cooling bath is then removed and solid sodium borohydride (150 mg) is added by rotating the bent closed side-arm. After stirring 15–30 min, the grey-green color characteristic of vitamin B_{12s} appears. Bromomethylthio ester (7) I (40 μl) is added, with stirring, in the dark. The reaction mixture immediately becomes red, turns gradually brown, then becomes grey-green after 60–80 min. An additional amount of bromomethylthio ester I (20 μl) is added. After stirring for 1 hr, the color again becomes grey-green; and a further portion of bromomethylthio ester I (20 μl) is added. The addition of bromomethylthio ester I is repeated two more times (15 μl each) at 1-hr intervals. The total amount of bromomethylthio ester added is 110 μl (0.148 g, 0.523 mmol).

After the mixture becomes grey-green once again, it is extracted with pentane (6×30 ml), dried over anhydrous sodium sulfate, and concentrated to 0.102 g of a yellow oil. The oil is resolved into its components by chromatography on a 2-mm silica gel plate, being eluted with an 8:2 benzene–hexane mixture. The band positions are determined with the aid of a uv lamp. The first band (R_f 0.3) yields 35 mg (154% based on vitamin B_{12a}, 33% based on bromothio ester I) of rearranged

product III (7).[3] the second band (R_f 0.4) gives 12.3 mg (54% based on vitamin B_{12a}, 11.5% based on thio ester I) of reduction product IV (7).[3] The average yield of rearranged product III from six experiments was 131% (range: 115 to 220%).[3]

ACKNOWLEDGMENTS

This investigation was supported by National Institutes of Health Grants AM 17014 and GM 19906, National Science Foundation Grant CHE 76-18975, and The Robert A. Welch Foundation Grant A-713.

REFERENCES

1. For a recent cogent summary see, R. H. Abeles, "Biological Aspects of Inorganic Chemistry" (A. W. Addison, W. R. Cullen, D. Dolphin, and B. R. James, Ed.), p. 245. John Wiley, New York, 1977.
2. P. DOWD, M. SHAPIRO, AND K. KANG, *J. Amer. Chem. Soc.* **97**, 4754 (1975).
3. G. BIDLINGMAIER, H. FLOHR, U. M. KEMPE, T. KREBS, AND J. RETEY, *Angew. Chem.* **87**, 877 (1975).
4. P. DOWD AND M. SHAPIRO, *J. Amer. Chem. Soc.* **98**, 3724 (1976).
5. H. FLOHR, W. PANNHORST, AND J. RETEY, *Angew. Chem.* **88**, 613 (1976).
6. P. DOWD, B. K. TRIVEDI, M. SHAPIRO, AND L. K. MARWAHA, *J. Amer. Chem. Soc.* **98**, 7875 (1976).
7. A. I. SCOTT AND K. KANG, *J. Amer. Chem. Soc.* **99**, 1997 (1977).
8. H. EGGERER, E. R. STADTMAN, P. OVERATH, AND F. LYNEN, *Biochem. Z.* **333**, 1 (1960); E. F. PHARES, M. V. LONG, AND S. F. CARSON, *Biochem. Biophys. Res. Commun.* **8**, 142 (1962); R. W. KELLERMEYER AND H. G. WOOD, *Biochemistry* **1**, 1124 (1962).

[3] All products were identified by direct spectral comparison (nmr and ir) with authentic samples (5). In some runs a third band (R_f 0.43) was observed. This proved on isolation to be unreacted bromothio ester I.