Carbon-skeleton Rearrangement of the Alkyl Ligand in a Vitamin B_{12} Derivative

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Summary Cyclopropylmethylcobalamin is isomerised to but-3-enylcobalamin in solution at 60 °C and in the presence of oxygen at a rate which is virtually independent of the dielectric constant of the solvent.

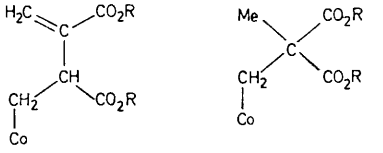
ENZYMES containing the so-called 'coenzyme' derivatives of vitamin B_{12} (*i.e.* 5′-deoxyadenosylcorrinoids) can catalyse a number of rearrangements, of which the most interesting and unusual involve a rearrangement of the carbon skeleton of the substrate; a typical example is the reversible interconversion of certain succinate and methylmalonate derivatives.[1] In spite of considerable effort, no real evidence has yet been obtained to indicate whether the substrate undergoes rearrangement in the form of the carbonium ion, the free radical, or when co-ordinated to the cobalt. Advance has been hampered by the lack of suitable model reactions; however, two such reactions have recently been reported, and the availability of such model reactions could lead to a rapid increase in our knowledge of the isomerase reactions. It has been found that photolysis, or slow decomposition at room temperature, of the cobalamin possessing the ligand (II)[2] and photolysis of the cobaloxime containing the ligand (II)[3] all gave some products involving rearrangement of the carbon skeleton. These reactions involve irreversible cleavage of the Co–C bond and proceed in an inert atmosphere. We report here the first example of a carbon-skeleton rearrangement of a group co-ordinated to cobalt in a vitamin B_{12} derivative, which does not involve irreversible cleavage of the Co–C bond and which, like the enzymatic reactions, is not appreciably inhibited by the presence of oxygen.

We have prepared and studied the reactions of the

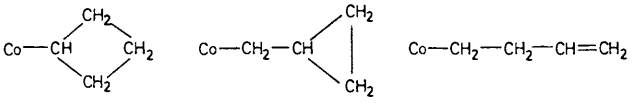
organocobalamins where the ligand is cyclobutyl (III), cyclopropymethyl (IV), and but-3-enyl (V). These alkyl groups were selected for study because (i) there are no heteroatoms to complicate the reactions and their interpretation, and (ii) the reactions of the corresponding carbonium ions and free radicals have been fairly extensively studied. It is well known that all three carbonium ions are interconvertible, as are also the cyclopropylmethyl and but-3-enyl radicals;[4] however, evidence for the rearrangement of the cyclobutyl radical to give but-3-enyl derivatives has only recently been obtained.[5] We have found that (III) is far more stable than (IV) or (V) and we could detect no conversion either of (III) into (IV) or (V) under conditions where the latter are not rapidly and irreversibly decomposed, or of (IV) or (V) into (III). Compound (III) is, therefore, omitted from further discussion.

Compounds (IV) and (V) were prepared by standard methods[6] involving the reduction of aqueous solutions of aquocobalamin with sodium borohydride under nitrogen, treatment with an excess of the alkyl bromide,[7] purification of the product by extraction with a phenol–chloroform mixture, and column chromatography using CM cellulose, followed by freeze-drying to obtain the solids. Both products were fairly typical organocobalamins. The red solids dissolve to give red solutions, which reversibly turn yellow on acidification. The solutions are very light-sensitive and must be handled in the dark or in subdued red light. The absorption spectra of solutions of (IV) and (V) both show a maximum at 500 nm in neutral solution and at 443 nm in acid solution, and are virtually indistinguishable. (IV) and (V) can, however, be distinguished by t.l.c. Use of a 9·5:4 mixture of Bu^{s}OH and water gave well defined spots with the following R_{B12} values (ratio of R_f of given corrinoid to that of vitamin B_{12} itself): (IV), 1·54; (V), 1·59.

Possible reactions of (IV) and (V) were studied in unbuffered aqueous solutions in the presence of air at room temperature, 60 °C (both with *ca.* $1{\cdot}5 \times 10^{-2}$M solutions), and 95 °C (*ca.* 2×10^{-5}M solutions). The course of the reaction at 95 °C was followed by u.v.–visible spectrophotometry, while the products of the reaction at lower temperatures were identified by t.l.c. (V) decomposes slowly, even at room temperature, to give aquocobalamin and an unidentified yellow product with a lower R_{B12}, but no (IV). (IV) decomposes even more slowly at room temperature (<10% per week) to give (V) and then the other products already observed with (V). The conversion of (IV) into (V) was studied in more detail at 60 °C by withdrawing samples at 30—60 min intervals and following the change in composition of the solution by t.l.c. The results showed that (IV) was gradually converted into (V) with complete disappearance after 5 h; visual comparison of the relative intensities of the two spots indicated a half-life of 105 min. Relatively small amounts of aquocobalamin and other products became detectable after 3 h, presumably owing to further decomposition of the initially formed (V). A comparable experiment carried out under nitrogen gave a half-life of *ca.* 2 h; *i.e.* the presence of O_2 has no significant effect on the rate.



(I) (II)



(III) (IV) (V)

In order to establish whether the isomerisation of (IV) to (V) involves a polar or non-polar transition state, the reaction was studied in several other solvents (dielectric constant[8] at 25 °C in parentheses) and the following approximate half-times of reaction obtained: glacial acetic acid (6·2), 120 min; methanol (32·7), 150 min; $HCONMe_2$ (36·7), 180 min; *cf.* water (78·3), 105 min. Formamide (109·5) was also tested, but this solvent interfered with the t.l.c. There is clearly no marked and systematic variation of rate with dielectric constant.

The above results provide a third example of a carbon-skeleton rearrangement occurring in association with the cobalt atom in corrinoid or related complexes. It is also the first example of such an isomerisation occurring without irreversible cleavage of the Co–C bond and in the presence of oxygen. It is worth noting that Dowd *et al.*[2] speculate on the possible role of cyclopropane and but-3-enyl intermediates in the decomposition of (I).

One can distinguish three pathways for the isomerisation of (IV) to (V), depending on the fate of the Co–C bond and the nature of the species undergoing rearrangement, *viz.*: (A) heterolytic fission of Co–C to give Co^I and the carbonium ion, (B) homolytic fission of Co–C to give Co^{II} and the free radical, and (C) no cleavage of the Co–C bond. The intermediates in (A) and (B) cannot be 'free' Co^I or Co^{II} which would be irreversibly oxidised by O_2,[9] but could represent an ion-pair or radical-pair. The absence of any marked effect of the solvent on the rate appears to exclude the polar intermediates required by (A). Further experiments will be carried out to attempt to distinguish between (B) and (C).

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[1] H. Barker, *Ann. Rev. Biochem.*, 1972, **41**, 55.
[2] P. Dowd, M. Shapiro, and K. Kang, *J. Amer. Chem. Soc.*, 1975, **97**, 4754.
[3] G. Bidlingmaier, H. Flohr, U. M. Kempe, T. Krebs, and J. Rétey, *Angew. Chem. Internat. Edn.*, 1975, **14**, 822.
[4] R. Breslow, in 'Molecular Rearrangements,' Vol. 1, ed. P. de Mayo, Interscience, New York, 1963, p. 233; J. W. Wilt, in 'Free Radicals,' Vol. 1, ed. J. K. Kochi, Wiley-Interscience, New York, 1973, p. 333; J. K. Kochi, *ibid.*, p. 591.
[5] T. N. Shatkina and O. A. Reutov, *J. Org. Chem. U.S.S.R.*, 1974, **10**, 876; T. N. Shatkina, A. N. Lovtsova, and Ye. V. Leont'eva, *Doklady Akad. Nauk S.S.S.R.*, 1972, **207**, 1373.
[6] A. W. Johnson, L. Mervyn, N. Shaw, and E. L. Smith, *J. Chem. Soc.*, 1963, 4146.
[7] J. D. Roberts and R. H. Mazur, *J. Amer. Chem. Soc.*, 1951, **73**, 2509.
[8] 'Physical Chemistry of Organic Solvent System,' eds. A. K. Covington and T. Dickinson, Plenum Press, London, 1973; J. Timmermans 'Physico-Chemical Constants of Pure Organic Compounds,' Elsevier, New York, 1950.
[9] J. M. Pratt, 'Inorganic Chemistry of Vitamin B_{12},' Academic Press, London, 1972, p. 202.