606. *Reactions of the Alkylcobalamins.*

By D. Dolphin, A. W. Johnson, and R. Rodrigo.

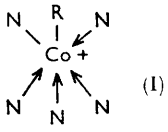
Products obtained from photolyses of the alkylcobalamins under various conditions are described. The reactions of alkylcobalamins with cyanide, acid, hydrogen, and chloramine T have also been studied.

Since the determination of the structure of the vitamin B_{12} coenzyme (5′-deoxyadenosylcobalamin) (I; R = 5′-deoxyadenosyl) by *X*-ray crystallography,[1] a partial synthesis of the coenzyme from vitamin B_{12b} (hydroxocobalamin) (I; R = H_2O) has been developed [2,3] which involves reduction to the so-called vitamin B_{12s}, now thought to be a cobalt hydride [2–5] (hydridocobalamin) (I; R = H) and reaction with 2′,3′-*O*-isopropylidene-5′-tosyladenosine with a final hydrolytic removal of the isopropylidene group. The use of simple alkyl halides in this reaction has made a wide variety of alkylcobalamins (I; R = alkyl) available for the study of their chemical and biological reactions.

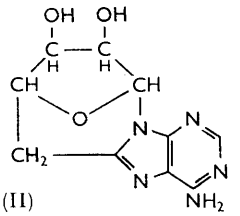
Alternative partial syntheses of alkylcobalamins involve the addition of acetylenes or activated olefines to hydridocobalamin [2] or the reaction of alkyl halides with the products formed from the reaction of hydroxocobalamin with thiols,[6] a reaction which is probably related to the biochemical synthesis of the coenzyme.[7]

[1] Lenhert and Hodgkin, *Nature*, 1961, **192**, 937; "Vitamin B_{12} und Intrinsic Faktor," Enke, Stuttgart, 1962, p. 105.
[2] Smith, Mervyn, Johnson, and Shaw, *Nature*, 1961, **192**, 1175; Johnson, Mervyn, Shaw, and Smith, *J.*, 1963, 4146.
[3] Bernhauer, Müller, and Müller, *Biochem. Z.*, 1962, **336**, 102; Müller and Müller, *ibid.*, p. 299.
[4] Smith and Mervyn, *Biochem. J.*, 1962, **86**, 2P.
[5] Hill, Pratt, and Williams, *J. Theoret. Biol.*, 1962, **3**, 423.
[6] Dolphin and Johnson, *Proc. Chem. Soc.*, 1963, 311.
[7] Brady, Castanera, and Barker, *J. Biol. Chem.*, 1962, **237**, 2325; Weissbach, Redfield, and Peterkovsky, *ibid.*, p. 3217.

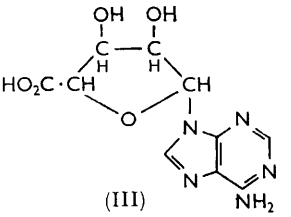
One of the most striking reactions of the coenzyme is the ease with which it undergoes photolysis,[8,9] a process which involves a homolytic cleavage of the cobalt–carbon bond, although evidence for the mechanism of the reaction rests solely on the nature of the products obtained. In the absence of oxygen, photolysis of an aqueous solution of the coenzyme yields vitamin B_{12r}, containing bivalent cobalt,[10] and the 5′-deoxyadenosyl



(I)



(II)



(III)

* The cobalamin chromophore is abbreviated to the five nitrogen atoms adjacent to cobalt. For full structure see refs. 1 and 2.

radical which undergoes cyclisation to the cycloadenosine (II).[11] In the presence of oxygen, photolysis of the coenzyme yields a mixture of (II) and its oxidation product (III) [9] or the corresponding aldehyde,[12] together with hydroxocobalamin, vitamin B_{12b} (spectra: Fig. 1). In the presence of thiols, photolysis of the coenzyme again gives the adenosyl radical, but this is trapped by the thiol and forms the corresponding *S*-adenosyl derivative.[13] It has also been shown recently that photolysis of methylcobalamine in presence of 1,4-naphthaquinone yields 2-methyl-1,4-naphthaquinone.

It has now been found that the amount of oxygen present during the photolysis of the alkylcobalamins also determines the fate of the alkyl radical. When special care was taken to exclude all oxygen, by reducing the pressure above the frozen solution to 10^{-6} mm., then irradiation of ethylcobalamin (100w tungsten bulb 20 cm. from the mixture) did not cause any change, even after 12 hours, although a solution of the coenzyme itself or of the 5′-deoxyuridinyl analogue [2] was decomposed under these conditions. At slightly higher pressures (10^{-3} mm.), however, photolysis of the pure ethyl analogue occurred and yielded vitamin B_{12r} [10] and a gaseous product containing 92% of ethylene, traces of ethane and butane, but no detectable hydrogen. The ethylene could have been formed from ethyl radicals through ethyl hydroperoxide [14] with subsequent elimination of hydrogen peroxide. Aerobic photolysis of a neutral aqueous solution of ethylcobalamin resulted in the formation of hydroxocobalamin, acetaldehyde, and acetic acid.

In the case of methylcobalamin, photolysis at 10^{-3} mm. was shown to yield vitamin B_{12r} and a mixture of ethane (61%), methane (34·5%), and ethylene (4·4%), and aerobic photolysis to yield vitamin B_{12b} and formaldehyde (Fig. 2). It is known [15] that methyl radicals can abstract hydrogen from hydroxylic solvents to yield methane, although the main reaction was the dimerisation of the radicals forming ethane. The production of some methane by photolysis of methylcobalamin, under conditions where ethylcobalamin yields ethylene, is paralleled by the formation of acetic acid [2] by anaerobic photolysis of an aqueous solution of carboxymethylcobalamin (I; R = $CH_2{\cdot}CO_2H$), although ethyl acrylate is formed in a similar reaction with 2-ethoxycarbonylethylcobalamin [2] (I; R = $CH_2{\cdot}CH_2{\cdot}CO_2Et$). In the presence of thiols, the alkyl radicals liberated on photolysis are converted into *S*-alkyl derivatives.[13]

[8] Ladd, Hogenkamp, and Barker, *Biochem. Biophys. Res. Comm.*, 1960, **2**, 143.
[9] Johnson and Shaw, *J.*, 1962, 4608.
[10] Hogenkamp, Barker, and Mason, *Arch. Biochem. Biophys.*, 1963, **100**, 353.
[11] Hogenkamp, *J. Biol. Chem.*, 1963, **238**, 477.
[12] Hogenkamp, Ladd, and Barker, *J. Biol. Chem.*, 1962, **237**, 1950.
[13] Johnson, Shaw, and Wagner, *Biochem. Biophys. Acta*, 1963, **72**, 107.
[14] Baldwin and Simmons, *Trans. Faraday Soc.*, 1957, **53**, 964.
[15] Kharasch, Rowe, and Urry, *J. Org. Chem.*, 1951, **16**, 905.

The action of aqueous potassium cyanide on the coenzyme yields adenine, D-*erythro*-2,3-dihydroxypent-4-enal,[9,16] and dicyanocobalamin,[17] and the reaction has been rationalised [9] as an extended elimination of the nucleoside group, initiated by attack by the cyanide ion. Aqueous solutions of methyl- or ethyl-cobalamin, however, have been found to be stable for up to 24 hours in the presence of potassium cyanide solutions in the absence of light, but subsequent exposure to light for ½ hour resulted in the production of dicyanocobalamin, recognised by its characteristic spectrum (Fig. 2). A solution of methylcobalamin in M-hydrogen cyanide decomposed slowly in air, even in the absence of light, although it was stable in the absence of both oxygen and light. This decomposition

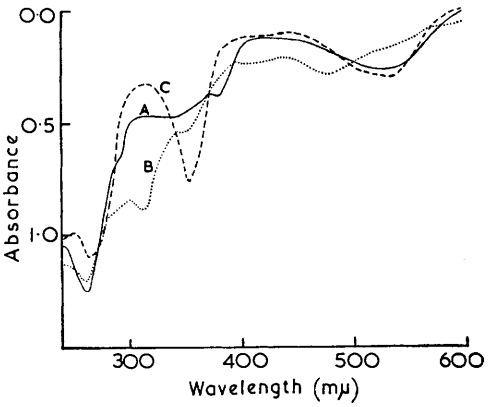


FIG. 1. Absorption spectra. A, Vitamin B_{12} coenzyme; B, vitamin B_{12r} formed by anaerobic photolysis of the coenzyme; C, hydroxocobalamin formed by oxidation of vitamin B_{12r}.

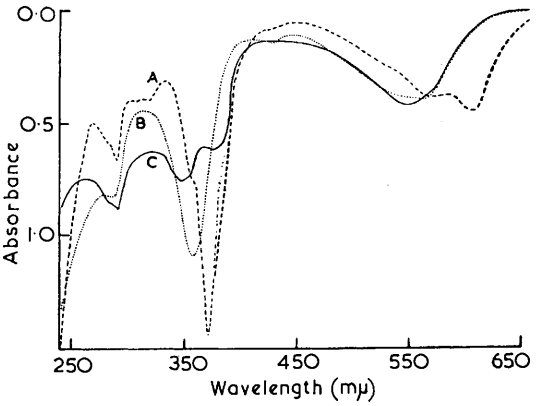


FIG. 3. Absorption spectra. A, The lactone of chloro-dicyanocobalamin; B, the lactone of chloro-hydroxocobalamin; C, the lactone of chloro-methylcobalamin.

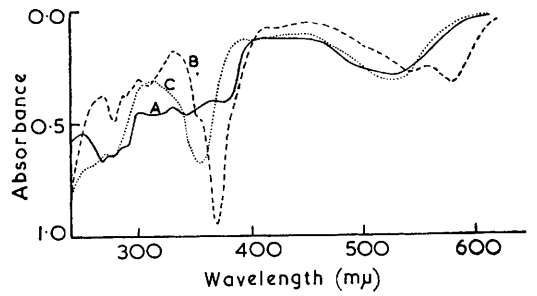


FIG. 2. Absorption spectra. A, Methylcobalamin; B, dicyanocobalamin; C, hydroxocobalamin.

is possibly to be associated with the protonation (brought about by hydrogen cyanide) of the co-ordinated benziminazole grouping " below " the chromophore, consequent fission of the cobalt–benziminazole linkage, and formation of a cobalt–cyano-grouping. The reaction of ethanolic solutions of methylcobalamin with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone [18] in the absence of light yielded up to 30% of cyanocobalamin after 30 minutes but this reaction, which involves a powerful oxidising agent, is probably not just a simple displacement. 2-Ethoxycarbonylethylcobalamin was readily decomposed by aqueous potassium cyanide, even in the absence of light, with the formation of ethyl acrylate and dicyanocobalamin. However the analogous decomposition was caused by N-aqueous

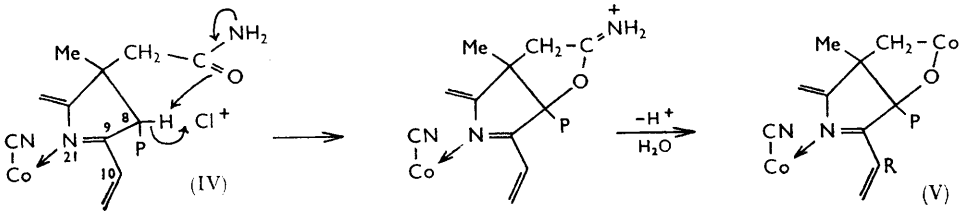
[16] Barker, Smyth, Weissbach, Toohey, Ladd, and Volcani, *J. Biol. Chem.*, 1960, **235**, 1462; 1961, **236**, 3097.
[17] Smith, Ball, and Ireland, *Biochem. J.*, 1952, **52**, 395.
[18] Braude, Brook, and Linstead, *J.*, 1954, 3569.

sodium hydroxide at room temperature, and even by water at 50°. This facile reaction is doubtless a consequence of the activating effect of the ester group.

Hydrogenation of methylcobalamin in the presence of platinum gave methane and vitamin B_{12r}, again identified by its spectrum, although it was unaffected by reducing agents such as sodium borohydride or zinc and acetic acid. The hydrogenolysis involved the uptake of one atom of hydrogen for each molecule of the methylcobalamin, and the simultaneous production of a molecule of methane, *i.e.*, an increase in volume of 0·5 mole. The 5,6-dimethylbenzimidazolyl coenzyme, on the other hand, was unchanged under these conditions. In connection with the hydrogenolysis of methylcobalamin, it is of interest that *Methanisarcina barkeri* has been observed [19] to produce methane from methylcobalamin in the presence of a reducing agent such as pyruvate and, by using methylcobalamin containing a ^{14}C-methyl group, the methane was shown to arise from the cobalt methyl group.

The ready red-to-yellow colour shift which is shown by the coenzyme [5,20] on protonation also occurs with the alkylcobalamins. The ionisation constants give a measure of the ease of this colour change and these have been determined by a spectrophotometric method.[21] The following pK_a values of alkylcobalamins have been determined; methyl, 2·70; n-butyl, 3·93; n-heptyl, 4·01; carboxymethyl, 1·50, and deoxyadenosyl (coenzyme), 3·52. There is thus a qualitative correlation between the ionisation constant and the inductive effect of the alkyl substituent, suggesting that reactivity at the site of protonation is to some extent controlled by the nature of the substituent. According to Hill, Pratt, and Williams,[5] position 10 on the chromophore [see (IV)] is a likely site (as well as the nucleotide base) for this protonation to occur, but this suggestion has still to be proved.

It has been suggested [22] that the chromophore of the coenzymes and the alkylcobalamines contains two hydrogen atoms [*e.g.*, at C-9 and N-21, see (IV)] more than the cobalamin chromophore, but this theory has now been withdrawn.[23] Our own reasons for rejecting the hypothesis were based on the results of quantitative hydrogenations (above), on the failure to obtain evidence for the liberation of hydrogen during anaerobic photolyses, and on a reinterpretation of the results [22] of chlorination of the alkylcobalamins. It is known [24] that the action of chloramine T on cyanocobalamin (IV) first causes cyclisation of the acetamide side-chain of ring B to form a fused lactone (V; R = H) and that the further action of chloramine T causes substitution of chlorine into the chromophore, possibly at C-10, to give the chloro-lactone (V; R = Cl).

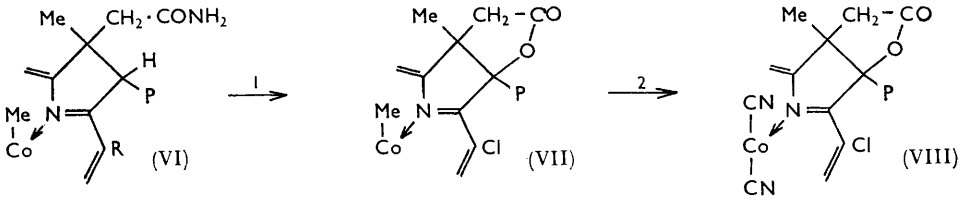


P = $CH_2 \cdot CH_2 \cdot CONH_2$.

The reaction of methylcobalamin (VI; R = H) took a different course and the first equivalent of chloramine T caused substitution without cyclisation and the formation of a monochloro-methylcobalamin (VI; R = Cl). However, use of an excess of chloramine T caused the formation of a monochloro-lactone (VII) of the cobalt-methyl derivative, revealed by its characteristic infrared absorption at 1778 $cm.^{-1}$ and by its electrophoretic behaviour. Photolysis of the product in presence of air and cyanide caused the

[19] Blaycock and Stadtman, *Biochem. Biophys. Res. Comm.*, 1963, **11**, 34; Wolin, Wolin, and Wolfe, *ibid.*, **12**, 464.
[20] Barker, Smyth, Weissbach, Toohey, Ladd, and Volcani, *J. Biol. Chem.*, 1960, **235**, 480.
[21] Paul and Long, *Chem. Rev.*, 1957, **57**, 1.
[22] Wagner and Renz, *Tetrahedron Letters*, 1963, 259.
[23] Wagner and Bernhauer, *Ann. New York Acad. Sci.*, 1964, **112**, 580.
[24] Bonnett, Cannon, Clark, Johnson, Parker, Smith, and Todd, *J.*, 1957, 1158.

formation of the same chloro-lactone (VIII) as had been obtained previously [24] by treatment of cyanocobalamin with an excess of chlorine and then cyanide (Fig. 3).



Reagents: 1, Chloramine T; 2, Cyanide in presence of light and air.

There is, therefore, a series of cobalamins from vitamin B_{12} (I; R = CN) to the alkylcobalamins (I; R = alkyl), including the coenzyme itself, and the properties of a particular cobalamin depend markedly on the nature of the substituent, R. Other partially synthetic cobalamins are known which have properties resembling those of the alkylcobalamins, *e.g.*, sulphitocobalamin (I; R = SO_3^-),[5,25] which is converted into hydroxocobalamin by aerobic photolysis, is readily protonated by dilute acids, and which behaves like methylcobalamin on chlorination with chloramine T, *i.e.*, substitution into the chromophore occurs before the cyclisation of the acetamide side-chain.

Experimental

Ultraviolet and visible spectra were determined on aqueous solutions. Infrared spectra were determined on potassium bromide discs. The principal chromatographic solvent systems used were: solvent A, butan-2-ol–water–25% ammonium hydroxide (50 : 36 : 14); solvent B, n-butanol–ethanol–water (50 : 15 : 35); and solvent C, n-butanol–propan-2-ol–water (10 : 7 : 10).

Anaerobic Photolyses of Alkylcobalamins.—(*a*) *Ethylcobalamin.* The cobalamin (200 mg.; prepared from ethyl iodide purified by gas chromatography) was dissolved in water (10 ml.) in a blackened Pyrex vessel, and cooled in liquid nitrogen. The pressure above the frozen solution was reduced to 10^{-6} mm., and the vessel was isolated from the pumping system and allowed to warm to room temperature. The freezing and degassing were repeated until the pressure above the solution at room temperature was 10^{-3} mm. The solution was then irradiated overnight with a 100w tungsten bulb at 20 cm. The aqueous solution (of vitamin B_{12r}: $\lambda_{max.}$ 313 and 475 mμ) was cooled to −40° and the gas above the frozen solution collected and analysed by gas–liquid chromatography (g.l.c.) (2 m. × 4 mm. silica gel-packed column, 128°, Perkin-Elmer 451 chromatography apparatus, N_2, 50 ml./min.). A flame ionisation detector was used, the flame being maintained by the addition of hydrogen to the nitrogen at the column outlet. The analysis of the product was: ethylene 93·5%, ethane 3·3%, and methane 2·5%, with traces of propane, propene, and n-butane. The presence of ethane and methane may have been caused by small amounts of methyl iodide impurity in the ethyl iodide.

(*b*) *Methylcobalamin.* (i) A similar experiment gave a gaseous product containing 61·1% ethane, 34·5% methane, and 4·4% ethylene. (ii) A solution of methylcobalamin (0·55 mg.) in distilled water (3 ml.) was frozen in a spectrophotometer cell and the pressure in the cell reduced to 10^{-6} mm. The spectrum was measured and shown to be unchanged after photolysis for 24 hr. under vacuum. When the sealed-off cell was opened to the atmosphere, photolysis of the solution gave entirely hydroxocobalamin (spectrum) after 1 hr.

(*c*) *2-Ethoxycarbonylethylcobalamin.*—An aqueous solution (40 ml.) of the cobalamin [2] (200 mg.) was covered with ether (20 ml.) and photolysed in daylight for 1 hr., while nitrogen was bubbled through the solution. The ether was separated and the aqueous layer extracted with more ether (3 × 10 ml.). The combined ether extracts were washed with water, dried, and concentrated to 1 ml. The product was then examined by g.l.c. (12 ft. × ⅛ in. column, GC 22 support, 80—100 mesh, 15% DC 710 silicone oil, 87°, Perkin-Elmer model 800 gas chromatography apparatus, N_2, 33 ml./min.). Under these conditions the retention time, 4·7 min., of the product was identical with that of authentic ethyl acrylate.

In all these experiments the cobalamin fragment was obtained as vitamin B_{12r} (spectrum).

Aerobic Photolysis of Ethylcobalamin.—A solution of ethylcobalamin (125 mg.) in water

[25] Dolphin, Johnson, and Shaw, *Nature*, 1963, **199**, 170.

(25 ml.) was photolysed (daylight) in a stoppered silica tube until completely converted into hydroxocobalamin (no further increase in extinction at 351 mμ). The volatile products were transferred directly into an aqueous solution of 2,4-dinitrophenylhydrazine by means of a stream of pure nitrogen, and the precipitated 2,4-dinitrophenylhydrazone was separated, washed, dried, dissolved in chloroform, and chromatographed on a small column of Spence type H alumina. The main band was collected and the solvent removed to yield yellow needles (8 mg.) of acetaldehyde 2,4-dinitrophenylhydrazone, m. p. and mixed m. p. 162—163° (from aqueous ethenol). The infrared spectra (KBr disc) of the two samples of the 2,4-dinitrophenylhydrazone were identical.

The residual aqueous solution of the photolysed cobalamin was adjusted to pH 10 with dilute ammonium hydroxide and evaporated to dryness under reduced pressure. The residue was dissolved in water (3 drops) and examined by paper chromatography using the following solvents: (i) ethanol–water–ammonium hydroxide (20 : 4 : 1) and (ii) n-butanol–ethanol–water–ammonium hydroxide (10 : 10 : 4 : 1). Acetic, glycolic, succinic, and oxalic acids were used as standards, and the chromatograms were developed by means of B.D.H. Universal Indicator. The following R_F values were observed: photolysis product (i) 0·67, (ii) 0·41; acetic acid (i) 0·68, (ii) 0·43; glycollic acid (i) 0·57, (ii) 0·33; succinic acid (i) 0·37, (ii) 0·15; and oxalic acid (i) streak at origin, (ii) 0·08.

By similar aerobic photolyses, methylcobalamin gave a mixture of formaldehyde (2,4-dinitrophenylhydrazone, m. p. 166—167°) and formic acid [R_F (i) 0·55, (ii) 0·35] and n-propylcobalamin gave propionaldehyde (2,4-dinitrophenylhydrazone, m. p. 155—156°) and propionic acid [R_F (i) 0·61, (ii) 0·39].

Reaction of Alkylcobalamins with Cyanide Ions in the Absence of Light.—(*a*) Ethylcobalamin (300 mg.) in distilled water (30 ml.) and a solution of potassium cyanide (65 mg.) in 0·01N-potassium hydroxide (10 ml.) were simultaneously degassed to 10^{-6} mm. The air-free solutions were mixed under a vacuum and kept overnight in the absence of light. Determination of the spectrum of a sample of the resulting solution in an atmosphere of nitrogen showed that the ethylcobalamin was unchanged.

(*b*) Methylcobalamin (1·9 mg.) was dissolved in N-aqueous potassium cyanide (10 ml.) in the absence of light but without exclusion of air. The spectrum of the solution was determined every hour for 6 hr., but even after 24 hr. it was unchanged ($\lambda_{max.}$ 342, 374, and 522 mμ). On exposure to light, the methylcobalamin was rapidly converted (complete after 30 min.) into dicyanocobalamin ($\lambda_{max.}$ 367, 540, and 580 mμ).

(*c*) A portion (3 ml.) of a solution of methylcobalamin (2·1 mg.) in distilled water (10 ml.) was cooled and degassed to 10^{-6} mm. in a glass vessel to which was attached a 5-mm. silica cell and a smaller vessel in which N-aqueous hydrogen cyanide (0·5 ml.) was degassed simultaneously. The whole assembly was then separated from the high-vacuum frame by sealing it off under a vacuum. The frozen solutions were carefully melted and the spectrum of the methylcobalamin solution determined (in the silica cell) to confirm that it was unchanged. The solutions were then mixed and the spectrum of the mixture ($\lambda_{max.}$ 342, 362, and 522 mμ; relative extinctions 1 : 1·11 : 0·63; possibly indicating the replacement of the nucleotide by cyanide) was unchanged after several days in the absence of light and air. After photolysis of the solution by a 100w tungsten lamp overnight, the spectrum was found to have changed to that of cyanocobalamin ($\lambda_{max.}$ 361 and 548 mμ; relative extinctions 1 : 0·32).

(*d*) A solution of methylcobalamin (0·855 mg.) in N-aqueous hydrogen cyanide was prepared with exclusion of light and the spectrum determined every hour. Cyanocobalamin was gradually formed, *e.g.*, after 4 hr. the spectrum of the solution showed $\lambda_{max.}$ 361 and 510—540br mμ (relative extinctions, 1 : 0·38) and after 24 hr. a solution was obtained, the spectrum of which was unchanged after subsequent photolysis ($\lambda_{max.}$ 361, 545, and 580sh mμ, caused by the presence of some dicyanocobalamin; relative extinctions, 1 : 0·32 : 0·26).

(*e*) 2-Ethoxycarbonylethylcobalamin (100 mg.) was dissolved in N-aqueous potassium cyanide solution (30 ml.) and kept overnight with exclusion of light. The solution was extracted rapidly with ether (3 × 20 ml.) and the combined extract washed, dried, and most of the solvent removed. The residue was examined by g.l.c., as described above, and ethyl acrylate was identified as a sharp peak with a retention time identical with that of an authentic specimen. Ethyl acrylate was also identified in the products when 2-ethoxycarbonylethylcobalamin was (i) dissolved in N-aqueous hydrogen cyanide and kept for 2 days in the absence of light, (ii) dissolved in water and heated at 50° for 6 hr.

(*f*) A solution of methylcobalamin (1·2 mg.) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone [18] in ethanol (10 ml.) was kept in the dark for 30 min. at room temperature. A portion was concentrated at room temperature under reduced pressure and chromatographed on paper separately in solvents A, B, and C, together with several standards. A product (*ca.* 30%) with R_F 0·20, 0·25, and 0·37, respectively, in the three solvents, was shown to be cyanocobalamin. It was separated from methylcobalamin by electrophoresis on paper (pH 2·5; cyanocobalamin stationary), eluted, and its ultraviolet and visible spectra shown to be identical with those of cyanocobalamin.

Hydrogenation of Methylcobalamin.—Adams catalyst (10 mg.) was suspended in distilled water (5 ml.) and hydrogenated until there was no further uptake of hydrogen. Methylcobalamin (52 mg.) was added and the hydrogenation conducted in the absence of light for 2½ hr. until the reaction was complete. The increase in gas volume was noted and the spectrum of the resulting yellow-brown solution was measured, $\lambda_{max.}$ 313 and 475 mμ (vitamin B_{12r}). The solution was separated from the catalyst and allowed to oxidise in the absence of light. The spectrum of the oxidised solution was measured both before, $\lambda_{max.}$ 351 and 526 mμ (hydroxocobalamin), and after the addition of cyanide $\lambda_{max.}$ 367, 540, and 580 mμ (mixture of cyano- and dicyano-cobalamins). In typical experiments using 52 and 30 mg., respectively (corrected for water content), of methylcobalamin, an increase in volume of 0·281 and 0·153 ml. was observed (corrected to s.t.p.) (theoretical, 0·369 and 0·213 ml., respectively).

A sample of the gaseous reduction product was examined by g.l.c. (silica gel, 60 mesh; 2 m. × 4 mm., 100°, N_2, 55 ml./min.), using a flame ionisation detector. Under these conditions a fraction was observed with a retention time of 1·5 min. identical with that of an authentic sample of a 3% methane–nitrogen mixture. No other hydrocarbons were detected in the product.

Chloro-methylcobalamin.—Chloramine T (80 mg.) and glacial acetic acid (0·1 ml.) were added to a solution of methylcobalamin (400 mg.) in water (50 ml.). The mixture was kept in the dark for 1 hr., the product was purified using phenol and chloroform in the usual manner,[2] and the final aqueous solution (30 ml.) reduced to 5 ml. and chromatographed on a column (50 × 3 cm.) of carboxymethylcellulose. After a small fast-running fraction had been eluted, the main fraction was collected, reduced to 1 ml., and treated with acetone until a faint turbidity was observed. An amorphous *product* separated on keeping, which could not be crystallised. It was washed with acetone and dried (360 mg., 89%) (Found: Cl, 2·55. $C_{63}H_{90}ClCoN_{13}O_{14}P$ requires Cl, 2·57%), $\lambda_{max.}$ 347, 377, and 548 mμ (ε × 10^{-4} 1·20, 1·12, and 0·73), R_F (solvent A) chloro-methyl compound, 0·58; cyanocobalamin, 0·43; (solvent B) chloro-methyl compound, 0·45; cyanocobalamin, 0·28; (solvent C) chloro-methyl compound, 0·60; cyanocobalamin 0·45.

Lactone of Chloro-methylcobalamin.—Methylcobalamin (50 mg.) was dissolved in water (10 ml.) and the solution treated with chloroamine T (30 mg.) followed by 0·1N-hydrochloric acid (0·2 ml.). The solution was kept in the dark for 12 hr. and purified using phenol and chloroform.[2] The resulting aqueous solution was concentrated to 0·5 ml. and purified by chromatography on carboxymethylcellulose (50 × 3 cm.), which removed a small amount of hydroxocobalamin. The derivatives of methylcobalamin were subjected to electrophoresis on Whatman 3MM paper at pH 11 using a potential gradient of 10v/cm. After 4 hr. the lactone had moved 15 mm. towards the anode, methylcobalamin 2 cm. towards the anode, and the lactone of cyanocobalamin (used as markers) 16 mm. towards the anode. In another experiment at pH 2·5 (0·5N-acetic acid) for 3 hr., the lactone of chloro-methylcobalamin moved 27·8 mm. towards the cathode and methylcobalamin 21 mm. towards the cathode. The appropriate strip from the alkaline electrophoresis experiment was cut out, eluted, and purified with phenol. The resulting aqueous solution was evaporated in the dark to give the lactone of chloro-methylcobalamin as a red powder (7 mg.), $\lambda_{max.}$ 348, 375, and 556 mμ (ε × 10^{-4} 1·31, 1·06, and 0·76), R_F (solvent A) 0·58, (solvent B) 0·47, (solvent C) 0·62, $\nu_{max.}$ 1778 $cm.^{-1}$ (5-membered lactone).

Lactone of Chloro-cyanocobalamin.—An aqueous solution (10 ml.) of the lactone of chloro-methylcobalamin (20 mg.) was photolysed in daylight for 3 hr. Potassium cyanide (3 mg.) was added to the solution, which was purified using phenol and chloroform.[2] The resulting aqueous solution was evaporated to a small volume and acetone added until the solution became turbid. An amorphous solid (18 mg., 90%) separated, $\lambda_{max.}$ (in M-potassium cyanide solution) 372, 570, and 606 mμ (ε × 10^{-4} 2·56, 0·67, and 0·79) $\lambda_{infl.}$ 353 and 389 mμ (ε × 10^{-4} 1·24 and 0·83), $\nu_{max.}$ 1778 $cm.^{-1}$ (5-membered lactone), R_F (solvent A) 0·43, (solvent B) 0·41, (solvent C) 0·55.

Electrophoretic properties (Whatman No. 1 paper at 10v/cm.): at pH 2·5 for 3 hr.: moved 2·5 mm. towards cathode (cyanocobalamin marker, 1·0 mm. towards cathode) and at pH 11 for 4 hr.: 16 mm. towards the anode (cyanocobalamin marker, 2·0 mm. towards the anode).

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