988. *The Chemistry of Vitamin B_{12}. Part II.*[1] *Photochemical Reactions.*

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The photochemistry of several cobalamins has been studied. No photochemical reactions were detected with aquo-, hydroxo-, or thiocyanatocobalamin. Light catalyses the hydrolysis of ammonia- to aquo-cobalamin, and displaces the (" dark ") equilibrium between cyano- and aquo-cobalamin in favour of the latter. Ethynyl-, vinyl-, and methyl-cobalamin, and 5,6-dimethylbenzimidazolylcobamide coenzyme (DBC) yield vitamin B_{12r} on photolysis in nitrogen, and the rate of photolysis of methylcobalamin is accelerated by oxygen; the kinetics of these reactions have been studied. B_{12r} is also produced by the photolysis of B_{12a} in the presence of thiosulphate or cysteine.

Many corrinoids are sensitive to light. Two types of photochemical reaction have been described, leading either to an aquocobaltic complex, as in the case of vitamin B_{12},[2,3] or to the cobaltous complex B_{12r}, as in the case of DBC.[4-6] Other photolabile corrinoids include ammoniacobalamin,[7] other coenzymes,[8] alkyl-cobalamins and -cobinamides,[9-11] and sulphito-cobalamins and -cobinamides.[6,12,13]

The experiments described in this Paper include (1) a qualitative survey to determine the extent of photochemical reactivity amongst the corrinoids, (2) a quantitative study of the photochemistry of cobalamins containing the ligands H_2O, OH^-, NH_3, CN^-, SCN^-, ethynyl, vinyl, methyl C_5'-deoxyadenosyl (DBC), thiosulphate, and cysteine, (3) a comparison of the photolysis of methylcobalamin in nitrogen and in air, and (4) the testing of two other reactions, which probably do not involve the axial ligands, for catalysis by light.

The same nomenclature is used as in Part I.[1] B_{12r} and B_{12s} are used to designate the reduced complexes corresponding to Co(II) and Co(I), respectively.[14,15]

Experimental

Materials.—Samples of corrinoids were given by Dr. E. Lester Smith and Professor D. C. Hodgkin. AnalaR reagents were used wherever possible.

Absorption Spectra.—Spectra were taken with Beckman DK ratio-recording and Unicam S.P. 600 spectrophotometers, in 1-cm. silica cells.

Photolysis.—A 230 v 125 w Osram mercury lamp was used for photolysis. A direct comparison was made between possible thermal and photochemical reactions at the same temperature by passing water, either from a thermostat or from a tap, over the irradiated cells and through a Beckman thermostat block, which housed the " dark " cells. Absorption of light by the coloured corrinoids causes heating of the solution; the temperature of a solution of B_{12a} was 2—3° when the temperature of the water was 0° and the lamp was placed at 15 cm.

Deoxygenation.—Solutions were deoxygenated with a brisk stream of oxygen-free nitrogen for 10—30 min. and poured into the cells which were sealed with a greased stopper. The

[1] Part I, Hill, Pratt, and Williams, preceding Paper.
[2] Veer, Edelhausen, Wijmenga, and Lens, *Biochim. Biophys. Acta*, 1950, **6**, 225.
[3] Baxter, Horsford, Wokes, Norris, and Fernandes, *J. Pharm. Pharmacol.*, 1953, **5**, 723.
[4] Bernhauer and Müller, *Biochem. Z.*, 1961, **334**, 199.
[5] Brady and Barker, *Biochem. Biophys. Res. Comm.*, 1961, **4**, 373.
[6] Hill, Pratt, and Williams, *J. Theoret. Biol.*, 1962, **3**, 423.
[7] Cooley, Ellis, Petrow, Beaven, Holiday, and Johnson, *J. Pharm. Pharmacol.*, 1951, **3**, 271.
[8] Weissbach, Toohey, and Barker, *Proc. Nat. Acad. Sci. U.S.A.*, 1950, **45**, 521.
[9] Müller and Müller, *Biochem. Z.*, 1962, **336**, 299.
[10] Johnson, Mervyn, Shaw, and Smith, *J.*, 1963, 4146.
[11] Dolphin, Johnson, Rodrigo, and Shaw, *Pure Appl Chem.*, 1963, **7**, 539.
[12] Bernhauer, Renz, and Wagner, *Biochem. Z.*, 1962, **335**, 443.
[13] Dolphin, Johnson, and Shaw, *Nature*, 1963, **199**, 170.
[14] Bonnett, *Chem. Rev.*, 1963, **63**, 573.
[15] Hill, Pratt, and Williams, *Chem. and Ind.*, 1964, 197.

maximum concentration of oxygen within the cells could be determined by making use of the fact that it strongly catalyses the photolysis of methylcobalamin and produces B_{12a} (see below). An initial rapid photolysis to B_{12a} was sometimes observed, followed by slow photolysis to B_{12r}; the change in reaction occurred abruptly, suggesting that oxygen was consumed by the first reaction. On the assumption that one mole of oxygen oxidises one mole of methylcobalamin [16,17] the maximum initial concentration of oxygen was found to be $\leqslant 1{\cdot}3 \times 10^{-7}$M.

Determination of B_{12r}.—The yields of B_{12r} from the photolysis of the alkylcobalamins in 0·05M-sodium borate were determined using the molar extinction coefficients previously given for the alkylcobalamins [1] and the values: B_{12r}, $\varepsilon_{311} = 2{\cdot}75 \times 10^4$; B_{12a}, $\varepsilon_{311} = 0{\cdot}92 \times 10^4$.

Kinetics.—For fast photochemical reactions "time" only includes the period during which the solution is being irradiated. For slow photochemical reactions, or where there is a concurrent thermal reaction, "time" is the whole period spent in both the light and the dark (while taking spectra, etc.). $t = 0$ represents the start of photolysis or the admission of air to the solution. In the former case $t = 0$ does not represent the starting-time for any thermal reaction that occurs, since this reaction will have been proceding during deoxygenation.

Experimental Conditions.—The gas present in solution, the temperature, and the distance of the lamp from the cell are given in parentheses for each experiment.

Results

Aquo- and Hydroxo-cobalamin (B_{12a}).—Possible thermal and photochemical reactions of B_{12a} were compared at three different pH's: (1) $5{\cdot}5 \times 10^{-5}$M-B_{12a} in acetate buffer pH 2·0 (N_2, ~9°, 15 cm.); (2) $4{\cdot}9 \times 10^{-5}$M-B_{12a} in borate buffer pH 9·3 (N_2, 20°, 15 cm.); (3) $5{\cdot}5 \times 10^{-5}$M-B_{12a} in N-sodium hydroxide (N_2, ~9°, 15 cm.). The pK of B_{12a} is 7·5; [18] solution (1) contains aquocobalamin, and solutions (2) and (3) hydroxocobalamin. No thermal or photochemical reactions were detected at pH 2·0 or 9·3 during 3 hr. The initial and final optical densities (figures in parentheses for the solutions kept in the dark) were: (1) pH 2; 316 mμ 0·35, 0·33 (0·35, 0·35); 350 mμ 1·36, 1·37 (1·37, 1·37); 470 mμ 0·32, 0·31 (0·32, 0·31); 520 mμ 0·44, 0·45 (0·44, 0·45); (2) pH 9·3; 311 mμ 0·48, 0·47 (0·48, 0·48); 356 mμ 0·94, 0·93 (0·94, 0·93); 470 mμ 0·26, 0·26 (0·26, 0·26); 535 mμ 0·40, 0·40 (0·40, 0·40). But at pH 14 a slow reaction (first order, $t_{\frac{1}{2}}$ ~8 hr.) gave a product with a spectrum very similar to that of B_{12r}. The rate was unaffected by light (less than 1·2% difference in ε_{311} over at least three-quarters of the reaction). At pH 15 (10M-KOH) at room temperature the reaction was more than half complete during ten minutes' deoxygenation, whilst at pH 12 (0·01N-NaOH) an extremely slow reaction was observed, which was not followed beyond one day.

Vitamin B_{12r}.—B_{12r}, prepared by photolysis of the alkylcobalamins at pH 9·3, is stable towards light for a month or more (see below). At pH 0 the spectrum of B_{12r} prepared by photolysis of methylcobalamin in N-sulphuric acid showed no detectable change above 300 mμ ($\leqslant$0·5%) during irradiation for a further 12 hr. (N_2, 20°, 15 cm.) and 12 hr. in the dark.

Ammoniacobalamin.—Ammoniacobalamin has already been reported.[6,7] It is readily formed by treating B_{12a} with ammonium salts in alkaline solution ($t_{\frac{1}{2}} = 2$ min. in 0·033M-ammonium sulphate and 0·1N-sodium hydroxide at room temperature) but is unstable towards hydrolysis to B_{12a} in acid. Ammoniacobalamin can, therefore, be prepared in alkaline solution, and its photochemistry studied after acidification, without interference from excess ligand. Two solutions were prepared as follows: (1) 0·5 ml. 0·2N-NaOH, 4·0 ml. $1{\cdot}1 \times 10^{-4}$M-B_{12a}, 1·0 ml. 2M-$(NH_4)_2SO_4$, left in the dark for 1 hr., then 0·5 ml. 0·2N-HCl and 2·0 ml. acetate buffer pH 2·0; (2) same composition as (1), but B_{12a} added last. The two solutions now contain equal concentrations of ammonia- and aquo-cobalamin, respectively, under identical conditions at pH 2. Each solution was deoxygenated and divided into two portions, one of which was irradiated for 70 hr. (N_2, ~6°, 15 cm.) and the other kept in the dark. Ammoniacobalamin was hydrolysed to aquocobalamin both in the light and in the dark, but the initial rate was 4—5 times greater in the light. No ammoniacobalamin was formed from B_{12a} either in the light or in the dark, and no detectable amount of B_{12r} was formed during the photolysis of ammoniacobalamin.

[16] Hogenkamp, *Ann. New York Acad. Sci.*, 1964, **112**, 552.
[17] Wagner and Bernhauer, *Ann. New York Acad. Sci.*, 1964, **112**, 580.
[18] Smith, Fantes, Ball, Waller, Emergy, Anslow, and Walker, *Biochem. J.*, 1952, **52**, 389.

Thiocyanatocobalamin.—B_{12a} reacts with thiocyanate to give two products. The product at lower thiocyanate concentration shows a γ-band at 356 mμ. The dependence on thiocyanate concentration and the formation constant of this complex were determined spectrophotometrically at 315 mμ, using a solution of $6{\cdot}0 \times 10^{-5}$M-B_{12a} in acetate buffer pH 5·20 (5/6N-sodium acetate, 1/6N-HCl). The complex contains one thiocyanate; ignoring the possible effect of acetate as a ligand, the formation constant, $K = [Co{\cdot}SCN^-]/[Co{\cdot}H_2O][SCN^-]$, was found to be $1{\cdot}0 \times 10^3$. The γ-band is also situated at 356 mμ in 0·1M-potassium thiocyanate at pH 3·5, whilst the inflection due to benzimidazole occurs at 288 mμ; since the pK of the unco-ordinated base is 4·7 [19] and the band of the protonated form occurs at 285 mμ,[20] this shows that the base is still co-ordinated to the cobalt. The two ligands are, therefore, benzimidazole and thiocyanate, and the complex is established as monothiocyanatocobalamin. The second equilibrium, which is detected by the appearance of a shoulder at about 365 mμ when the thiocyanate concentration is increased above 0·1M, presumably involves the formation of dithiocyanatocobalamin. Evidence for the formation of a dithiocyanate has previously been obtained from solvent-partition studies.[21] These equilibria are established "instantaneously." In agreement with this, a sample of crystalline thiocyanatocobalamin gave the spectrum of B_{12a} when dissolved in water. This probably explains the claim [22] that B_{12a} and thiocyanatocobalamin have identical spectra.

Because of the rapid establishment of the equilibrium thiocyanatocobalamin can only be tested for the occurrence of photoreduction. A solution of $3{\cdot}8 \times 10^{-5}$M-B_{12a} in 0·1N-potassium thiocyanate was irradiated for 16 hr. (N_2, 20°, 15 cm.). The α- and γ-bands show a very gradual fall both in the light and in the dark; the cause is not known. There is no detectable formation of B_{12r}.

Vitamin B_{12a} with Thiosulphate, Cysteine, and Sulphite.—B_{12a} reacts "instantaneously" with thiosulphate and cysteine to give products which show more than one absorption band in the γ-region; by analogy with the alkycobalamins, which have been studied in more detail,[1] that at longest wavelength (~370 mμ) is called the γ-band. The identity of the complexes has not been established. The following experiments were carried out merely to test whether sulphur-containing ligands such as thiosulphate and cysteine, in contrast to thiocyanate, will support photoreduction.

A solution containing $5{\cdot}5 \times 10^{-5}$M-B_{12a} and M-sodium thiosulphate was irradiated for 3 hr. (N_2, ~15°, 15 cm.). The rise of bands at 311 and 474 mμ, which disappeared on admittance of air, showed the formation of B_{12r} in the light. The solution kept in the dark showed a slow reaction leading to the appearance of a new band at ~415 mμ, which persisted on shaking in air; there was no detectable formation of B_{12r}. After being shaken in air both solutions showed almost identical spectra, showing that the slow thermal reaction also proceeded in the light.

Many thiols, including cysteine, reduce B_{12a} to B_{12r} by a thermal reaction whose rate increases with pH.[6,23] Fig. 1 compares the rates of reduction of $5{\cdot}5 \times 10^{-5}$M-B_{12a} in the light (N_2, ~15°, 15 cm.) and in the dark in 0·1M-cysteine at pH 7 and in 0·05M-cysteine at pH 5. Light accelerates the reaction at pH 5 (ratio of initial rates ~4/3), but has no detectable effect on the much faster reaction at pH 7.

As already reported,[6] both the red and yellow complexes formed when B_{12a} reacts with sulphite are photolabile. The thermal and photochemical reactions, however, are complicated and the species involved have not all been identified, so no experimental results will be given.

Cyanocobalamin (B_{12}).—A $2{\cdot}6 \times 10^{-5}$M-solution of B_{12} in acetate buffer pH 4·75 was photolysed (air, not thermostatted, 25 cm.). The product was B_{12a}, and the reaction was first-order ($t_{\frac{1}{2}}$ = 10 min.). The photolysed solution was then placed in the dark. After 6 days, over 98% of the B_{12} had been re-formed, as calculated from the optical density at 360 mμ. The reaction followed second-order kinetics ($t_{\frac{1}{2}}$ = 4·5 hr.), as expected for the reaction between equal concentrations of B_{12a} and cyanide.

An attempt was made to photolyse a $4{\cdot}6 \times 10^{-5}$M-solution of B_{12} in borate buffer pH 9·27 (N_2, 20°, 15 and 7 cm.) for comparison with the alkylcobalamins (see below). The γ-band of

[19] Davies, Mamalis, Petrow, and Sturgeon, *J. Pharm. Pharmacol.*, 1951, **3**, 420.
[20] Beaven, Holiday, Johnson, Ellis, Mamalis, Petrow, and Sturgeon, *J. Pharm. Pharmacol.*, 1949, **1**, 957; Beaven, Holiday, Johnson, Ellis, and Petrow, *ibid.*, 1950, **2**, 944.
[21] Smith, Ball, and Ireland, *Biochem. J.*, 1952, **52**, 395.
[22] Buhs, Newstead, and Trenner, *Science*, 1951, **113**, 625.
[23] Peel, *Biochem. J.*, 1963, **88**, 296.

B_{12} showed only a slight fall on irradiation, and rose again rapidly while the spectrum was being examined. It was concluded that the re-formation of B_{12} was too rapid at this pH, and the experiment was discontinued.

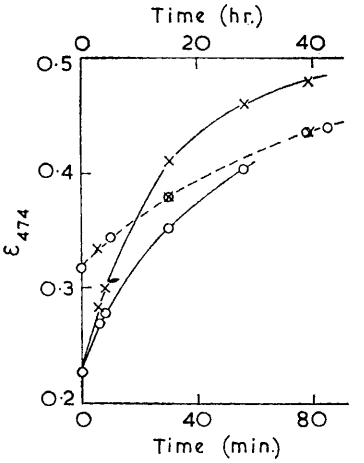

FIG. 1. Comparison of the rates of formation of B_{12r} from B_{12a} in 0·1M-cysteine at pH 7 (broken line, lower time-scale) and 0·05M cysteine at pH 5 (full line, upper time-scale) in the dark (○) and in the light (×).

Alkylcobalamins.—The kinetics and products of photolysis of ethynyl-, vinyl-, ancd methylcobalamin, and DBC in nitrogen, and methylcobalamin in air, in 0·05M-sodium borate pH 9·27 (all 20°, 15 cm.) are summarised in Table 1. In each case identical solutions kept in the dark

TABLE 1.

Photolysis of the alkylcobalamins in borate buffer, pH 9·27, at 20°.

Cobalamin	Concn. (M)	Gas in solution	Kinetics: Order	Kinetics: $t_{\frac{1}{2}}$	Product (%)
Ethynyl	$5·8 \times 10^{-5}$	N_2	First	25 days	B_{12r} (~100)
Vinyl	$4·9 \times 10^{-5}$	N_2	First	2·1 hr.	B_{12r} (100)
Methyl	$5·1 \times 10^{-5}$	N_2	Approx. first	~20 hr.	B_{12r} (97)
DBC	$4·6 \times 10^{-5}$	N_2	First	3·1 min.	B_{12r} (101)
Methyl	$5·1 \times 10^{-5}$	Air	First	1·0 min.	"B_{12a}"

showed negligible change during the period of the photochemical reaction. The first-order kinetic plots based on ε_{311} are shown in Fig. 2.

The photolysis of ethynylcobalamin showed the occurrence of two reactions, both leading to the formation of B_{12r}; a very slow reaction of the bulk of the sample, which because of its inconveniently slow rate was not followed beyond one half-life (25 days), and a more rapid initial reaction, which from its half-time of 2 hr. can be ascribed to vinylcobalamin present as an impurity and which accounted for about 8% of the total reaction. The kinetic plot in curve B was constructed by taking ε_{311} at 12 hr. as the initial value and ε_{311} corresponding to 100% formation of B_{12r} as the final value. The linearity of the kinetic plot is the evidence for the formation of ~100% B_{12r} quoted in Table 1. The possibility that the B_{12r} was produced by the very slow self-reduction of B_{12a} formed by an initial photoaquation of ethynylcobalamin was eliminated by observing the same slow formation of B_{12r} on photolysis of ethynylcobalamin in water.

The photolysis of a $1·3 \times 10^{-4}$M-solution of DBC in water was not reversed on standing in the dark for 48 hr. (<0·2% as determined from ε_{375}, ε_{474}, and ε_{522}).

The photolysis of methylcobalamin in air is rapid and complex, and no isosbestic points are observed; the photochemical reaction is followed and accompanied by one or more thermal reactions. B_{12r} was not detectable as an intermediate from the spectra. The final spectrum was very similar to that of B_{12a} under the same conditions but not identical; the β-band and the inflections around 470 and 320 mμ are less pronounced. There is no trace of any by-product with an absorption band at ~460 mμ (see next Section). The spectra suggest that the initial photolytic step produces a compound with a B_{12a}-type of spectrum and that the subsequent thermal reactions modify the details of the spectrum, while hardly affecting the position or

intensity of the γ-band. This explains why first-order kinetics are observed (Table 1) even in the absence of isosbestic points, when the rate is calculated from the rise of the γ-band at 356·5 mμ.

Oxidation of B_{12r}.—The rate of oxidation of B_{12r} (prepared by the photolysis in nitrogen of vinylcobalamin at pH 9·27) by air was unaffected by light (15 cm., both solutions at 20°; $\leqslant$3% difference in ε_{311} over at least $\frac{7}{8}$ of the reaction). The oxidation is first-order ($t_{\frac{1}{2}}$ = 21 min.). The final spectra in the light and in the dark were identical, but differed slightly from that of B_{12a}, in particular by showing a marked inflection at ~460 mμ. A yellow by-product has been separated from the mixture obtained by oxidising B_{12r} with air;[24] the first main absorption band of a solution of this compound in water occurs at 458 mμ.

The rate of oxidation of B_{12r} during the photolysis of methylcobalamin in air was also studied. A solution of methylcobalamin at pH 9·27, photolysed (N_2, 20°, 15 cm.) until about 35% had been converted into B_{12r}, was shaken in air and divided into two portions; one was

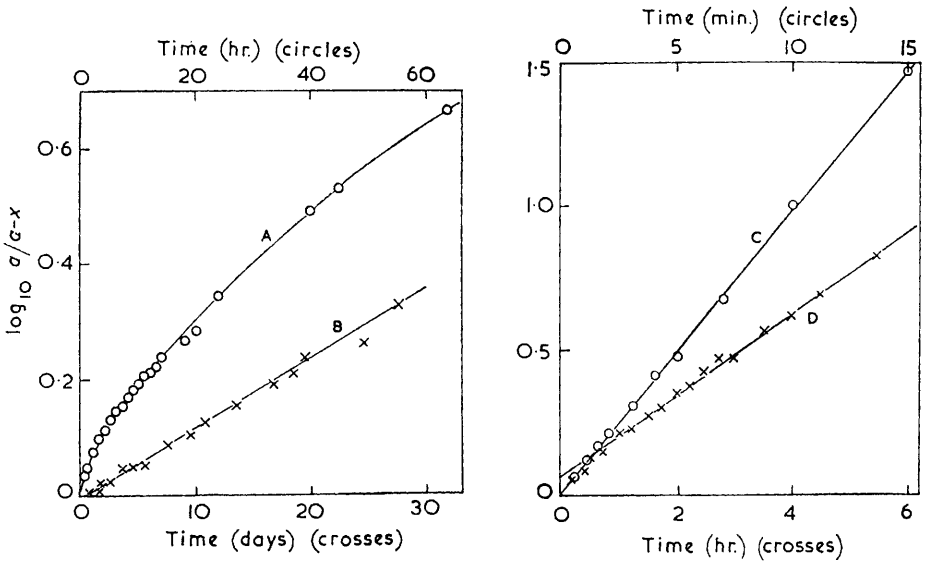

FIG. 2. First-order kinetic plots of photolyses in nitrogen.
A, Methylcobalamin; B, ethynylcobalamin; C, DBC; D, vinylcobalamin.

kept in the dark and the other in the light, and the rates and products of reaction were compared. The final spectrum in the dark was that expected for a mixture of B_{12a} and methylcobalamin; the rate of formation of B_{12a} ($t_{\frac{1}{2}}$ = 21 min.) was that already found for the rate of oxidation of B_{12r} alone by oxygen. The spectrum of the product in light was the same as that obtained when methylcobalamin was photolysed in air throughout; the rate of formation of B_{12a} was considerably faster than in the dark ($t_{\frac{1}{4}}$ = 7 min., $t_{\frac{7}{8}}$ = 14 min.).

Other Results.—Three other groups of corrinoids were tested for the occurrence of photochemical reactions.

(1) *Cobalamins in acid solution.* Methylcobalamin undergoes a reversible change with a pK of 2·5,[25] analogous to that of DBC,[6,26] in which the benzimidazole is displaced from co-ordination to the cobalt by water and becomes protonated. Methylcobalamin in N-sulphuric acid was slowly photolysed in nitrogen to B_{12r} and more rapidly in air to give a product with a spectrum very similar to that of B_{12a}.

(2) *Cobalamins in concentrated sulphuric acid.* The nature of these complexes has not yet been established; for the spectrum of B_{12} in concentrated sulphuric acid see ref. 6. The cobalt–cyanide and cobalt–methyl bonds remain intact, however, as shown by the regeneration of the

[24] E. Lester Smith, personal communication.
[25] Hill, Pratt, and Williams, unpublished results.
[26] Ladd, Hogenkamp, and Barker, *J. Biol. Chem.*, 1961, **236**, 2114.

usual spectra of cyano- and methyl-cobalamin when their solutions in concentrated sulphuric acid are diluted with water. Methylcobalamin is rapidly and cyanocobalamin (B_{12}) very slowly decomposed by irradiation in air of their solutions in concentrated sulphuric acid.

(3) *Cobinamides.* Photolysis of cyanoaquocobinamide (Factor B) in 0·01N-sulphuric acid in air gave diaquocobinamide, the reaction being reversed on standing in the dark. Methylcobinamide was slowly photolysed to cobinamide-B_{12r} in nitrogen and much more rapidly to the aquo-complex in air.

Discussion

The basis of the quantitative study of photoaquation and photoreduction in the cobalamins is the demonstration that both B_{12a} and B_{12r} are stable to light. The only complicating factor is the self-reduction of B_{12a} at high pH; this reaction is probably analogous to a self-reduction of B_{12} observed by Bonnett *et al.*[27] Particular attention has been paid to thermostatting during the photochemical reactions in order to avoid spurious effects due to heating of the solution by irradiation, and to making a direct comparison between the thermal and photochemical reactions; the importance of the latter is shown in the case of ammonia- and cysteine-cobalmin.

No photochemical reactions were detected with aquo-, hydroxo-, or thiocyanato-cobalamin. This eliminates photoreduction, except at an extremely slow rate, but not photoaquation, which cannot be detected spectrophotometrically either in the case of B_{12a}, because it merely involves replacing ligand by solvent water, or in the case of the thiocyanate, because the thermal equilibrium is so rapidly re-established.

Previous work on the photolysis of B_{12} [2,3] has been confirmed. B_{12} is photolysed to B_{12a} and the reaction is reversed in the dark; light, therefore, displaces the ("dark") thermodynamic equilibrium. The photolysis of ammoniacobalamin to B_{12a} has also been previously reported;[7] the present results show that no B_{12r} is formed and that under the conditions used light merely catalyses a slower, thermal reaction.

The photolysis of ethynyl-, vinyl-, and methyl-cobalamin, and DBC, in nitrogen gives virtually stoicheiometric yields of B_{12r}. The photolysis of DBC to B_{12r} has already been reported;[4-6] this reaction was not reversed in the dark. B_{12r} is also produced by the irradiation of B_{12a} in the presence of thiosulphate. Photoreduction of B_{12a} in the presence of cysteine is complicated by the simultaneous occurrence of a thermal reduction. Light had no detectable effect on the fast reduction at pH 7, but accelerated the much slower reduction at pH 5, which suggests that a slow photoreduction does occur, but is only detected when the thermal reaction is sufficiently suppressed.

The dependence of the course of the photochemical reaction and the position of the γ-band on the ligand are shown in Table 2. The thiosulphate and cysteine complexes are enclosed in parentheses because their identity has not been conclusively established.

Table 2.

Influence of the ligand on the course of the photochemical reaction.

Ligand	λ_γ (mμ)	Ligand atom on cobalt	Product of photolysis B_{12a}	Product of photolysis B_{12r}
H_2O	350	O	?	—
NH_3	355·5	N	+	—
SCN^-	356	S	?	—
OH^-	356·5	O	?	—
CN^-	360·5	C	+	—
Ethynyl	365	C	—	+
Vinyl	372	C	—	+
Methyl	374	C	—	+
C′$_5$-Deoxyadenosyl (DBC)	375	C	—	+
($S_2O_3^{2-}$	~370	S	?	+)
(Cysteine	~370	S	?	+)

[27] Bonnett, Cannon, Clark, Johnson, Parker, Smith, and Todd, *J.*, 1957, 1158.

Thiocyanate is bound to the cobalt through the sulphur atom in the solid cobalamin,[28] and the presence of a cobalt–sulphur bond in the cysteine and thiosulphate complexes is suggested by the similar effect of sulphur-containing ligands such as sulphite, thiosulphate, and thiols on the spectrum,[6] and its occurrence in sulphitocobalamin [13] and other cobalt complexes containing sulphite [29] and thiosulphate.[30] B_{12}, B_{12a} (see ref. 31) and DBC [1] are diamagnetic cobaltic complexes; by analogy one can assume the same for the ammonia-, thiocyanato-, and other alkyl-cobalamins. The ability to form B_{12r} from B_{12a} in the presence of excess of cysteine or thiosulphate suggests that the initial cobalamins containing these ligands are also cobaltic complexes.

It is clear that the course of photolysis does not depend on either the nature of the ligand atom (cf. CN^- and C_2H^-) or whether the ligand is saturated or unsaturated (cf. NH_3 and CH_3^-, CN^- and C_2H^-), but it does show a correlation with the position of the γ-band. The occurrence of photoreduction with the sulphur-containing ligands thiosulphate and cysteine ($\lambda_\gamma \sim 370$ mμ), but not thiocyanate ($\lambda_\gamma = 356$ mμ), is in agreement. The position of the γ-band has tentatively been correlated with the effective electronegativity of the ligand atom [1] and the electron density in the corrin ring.[6]

The corrinoids which undergo photoreduction all appear to be cobaltic complexes. In each case the product is the cobaltous complex B_{12r}; no photoreduction to B_{12s} has yet been observed. The scheme of photoreduction,[9,11]

$$\text{Co(III)L}^- + h\nu \longrightarrow \text{Co(II)} + \text{L}\cdot$$

is also supported by the first-order kinetics of photolysis. Very little is known, however, about the immediate fate of the ligand, although an unidentified organic radical has been detected by electron spin resonance during the photolysis of DBC under nitrogen; this may be derived from the ligand.[32]

Two points can be made about the rates of photolysis of the alkylcobalamins (see Table 1). First, the rates vary widely (from $t_{\frac{1}{2}} = 3{\cdot}1$ min. to 25 days) and show no correlation with the wavelength of the γ-band. Secondly, oxygen markedly accelerates the rate of photolysis of the methyl complexes (normal and acid forms of methylcobalmin, methylcobinamide). An effect of oxygen on the rate of photolysis of ethylcobalamin and certain other unspecified alkylcobalamins has already been noted; [11] it was claimed that no photolysis occurred at very low concentrations of oxygen, but unfortunately no experimental details or upper limits to the rate were given. The fact that B_{12r} is oxidised much more rapidly during the photolysis of methylcobalamin in air than in light and air alone, or with methylcobalamin in air in the dark, indicates that B_{12r} is reacting with some oxidising agent produced as a result of the photolysis of methylcobalamin in air, for example, a methylperoxy-radical formed by the reaction of the methyl radical with oxygen.

Photochemical reactions have now been observed in a wide range of corrinoids including the normal and acid forms of cobalamins, cobalamins in concentrated sulphuric acid, and cobinamides (references to previous work have been given in the introduction). The same pattern of reversible photoaquation of the cyano-complex and photoreduction of the methyl complex occurs in both the cobalamins and cobinamides.

The self-reduction of B_{12a} and the oxidation of B_{12r} by oxygen, which do not appear to involve the axial ligands, are not catalysed by light.

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[28] D. C. Hodgkin, personal communication.
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