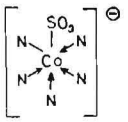
Sulphitocobalamin

A WIDE range of cobalamins exist where the cyano group of the original vitamin B_{12} is replaced, for example, by water (vitamin B_{12b}), halides, nitrite, sulphur-containing groups, and alkyl groups (the coenzymes and analogues) and the nature of the ligand attached to the cobalt has a profound influence on the chemical and physical properties of the cobalamin.

Preliminary experiments directed towards the synthesis of the vitamin B_{12} coenzyme[1] consisted of a re-investigation of the action of certain sulphur-containing reducing agents on hydroxocobalamin following the report of Pawelkiewicz[2], who claimed a synthesis of the coenzyme form of Factor *B* by the sodium dithionite reduction of cobinamide, Factor *B*, in the presence of adenine. We have repeated this experiment with hydroxocobalamin and failed to obtain any product which contained adenine linked to the cobalt. However, a new light-sensitive product was obtained from the reaction, the same product also being obtained in the absence of adenine. The reaction of sodium bisulphite with hydroxocobalamin also gave the same crystalline product, the spectrum of which was somewhat similar to that of vitamin B_{12} coenzyme[3]. Electrophoresis at *p*H 8 indicated that the compound contained an additional acidic group compared with vitamin B_{12} and that it was stable both in solution and in the solid state in the absence of light. The product contained sulphur and the presence of the monoacidic sulphite ligand was suggested by the electrophoretic behaviour and by the presence of two strong bands at 983 and 1,150 cm^{-1} in the infra-red spectrum; these bands have been associated with the cobalt-sulphur linked sulphito group by Baldwin[4] in another series of cobalt-containing complexes. On this basis the sulphito compound is formulated as in the partial structure (I), the remainder of the molecule being the normal cobalamin skeleton.



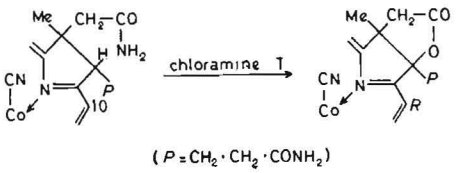
(I)

Sulphitocobalamin has been recorded in the earlier literature[5], but thought to exist only in solutions containing excess sulphite ions. More recently, Hill, Pratt and Williams[6] have described the properties of solutions of sulphitocobalamin and proposed a structure essentially similar to (I); both these workers and Bernhauer, Renz and Wagner[7] have described similar sulphito complexes from cobinamide (Factor *B*).

Like the coenzyme and its alkyl analogues[1,8], sulphitocobalamin readily reacts with acid (*p*H 2) to form a yellow protonated form (see ref. 6), but the acid solution is unstable and rapidly reverts to hydroxocobalamin. Cyanocobalamin (vitamin B_{12}) and hydroxocobalamin (vitamin B_{12b}) require treatment with strong mineral acid to yield the yellow protonated forms.

Aerobic photolysis of an aqueous solution of the sulphito complex gave hydroxocobalamin although under anaerobic conditions (10^{-6} mm) the complex was stable to photolysis. In this respect it resembled the simple alkyl analogues of the coenzyme[9] although the coenzyme itself undergoes fission when subjected to anaerobic photolysis[10]. Treatment of the sulphito complex with either potassium cyanide or hydrogen cyanide gives dicyanocobalamin. In this reaction the substitution at the cobalt atom occurs much easier, especially with potassium cyanide, than the similar reaction with the coenzyme and its alkyl analogues.

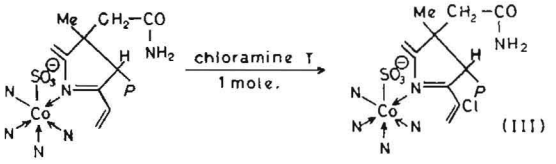
The course of the reaction of chloramine *T* with sulphitocobalamin also resembles the corresponding reactions with the coenzyme and the alkyl cobalamins, and differs in certain respects from the reaction of chloramine *T* with cyano- and hydroxo-cobalamins. In an earlier communication[11] it was shown that the action of the first equivalent of chloramine *T* with cyano- or hydroxo-cobalamin yielded a lactone (II; R = H) fused to ring *B*; further reaction caused substitution probably at C(10) of the chromophore to give the chloro-lactone (II; R = Cl).



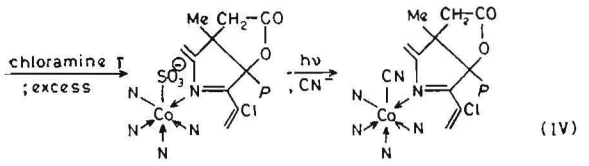
(II)

($P = CH_2 \cdot CH_2 \cdot CONH_2$)

The reaction of the sulphito complex with one mole of chloramine *T* caused substitution without cyclization and the product was the 10(?)-monochloro derivative (III). Reaction with excess chloramine *T* caused the formation of a monochloro-lactone (III) of the sulphito compound revealed by the appearance of the characteristic lactone carbonyl band at 1,778 cm^{-1}. Photolysis of this chloro-lactone (IV) in presence of air caused the formation of the corresponding hydroxocobalamin derivative and this with cyanide gave the same chloro-lactone (II; R = Cl) as had been obtained previously[11] by treatment of cyano-cobalamin with excess chloramine *T*.



(III)



(IV)

The course of the chlorination reaction is thus similar to that observed[9] with methylcobalamin, but the order of reaction at different points of the chromophore differs in the chlorination of cyano- and hydroxo-cobalamins.

D. H. DOLPHIN
A. W. JOHNSON
N. SHAW

Department of Chemistry,
University of Nottingham,
Nottingham.

[1] Smith, E. L., Mervyn, L., Johnson, A. W., and Shaw, N., *Nature*, **194**, 1175 (1962); *J. Chem. Soc.* (in the press).
[2] Pawelkiewicz, J., Bartosinski, B., and Walerych, W., *Bull. Acad. Polon. Sci.*, Ser. Sci. Biol., **8**, 123 (1960).
[3] Barker, H. A., Smyth, R. D., Weissbach, H., Munch-Petersen, A., Toohey, J. I., Ladd, J. N., Volcani, B. E., and Wilson, R. M., *J. Biol. Chem.*, **235**, 181 (1960).
[4] Baldwin, M. E., *J. Chem. Soc.*, 3123 (1961).
[5] Smith, E. L., Ball, S., and Ireland, D. M., *Biochem. J.*, **52**, 395 (1952). Fricke, H. H., U.S. Patent, 2,721,162; *Chem. Absl.*, **50**, 2129 (1956).
[6] Hill, J. A., Pratt, J. M., and Williams, R. J. P., *J. Theor. Biol.*, **3**, 423 (1962).
[7] Bernhauer, K., Renz, P., and Wagner, F., *Biochem. Z.*, **335**, 443 (1962).
[8] Bernhauer, K., Müller, O., and Müller, G., *Biochem. Z.*, **336**, 102 (1962). Müller, O., and Müller, G., *ibid.*, **336**, 299 (1962).
[9] Dolphin, D., Johnson, A. W., and Rodrigo, R., *Annal. New York Acad. Sci.* (in the press).
[10] Hogenkamp, H. P. C., and Barker, H. A., *Fed. Proc.*, **21**, 470 (1962). Johnson, A. W., and Shaw, N., *J. Chem. Soc.*, 4608 (1962).
[11] Bonnett, R., Cannon, J. R., Clark, V. M., Johnson, A. W., Parker, L. F. J., Smith, E. L., and Todd, A. R., *J. Chem. Soc.*, 1158 (1957).