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Neovitamin B_{12} (cyano-13-epicobalamin)

By R. Bonnett
Department of Chemistry, Queen Mary College, Mile End Road, London, E1 4NS

β-Epimerization in the corrinoid system has recently emerged as a complicating factor in the latter stages of the total synthesis of vitamin B_{12} (Eschenmoser 1971; Woodward 1973). It has also found some application in biosynthetic studies on the origin of the methyl groups in ring C (Scott, Townsend & Cushley 1973; Scott, this Discussion p. 303). This paper sets out to review briefly the β-epimerization of corrinoid polyamides, with particular reference to our work on the neo-series which provided the first established example of this phenomenon.

Early experiments on the chemistry of vitamin B_{12} (figure 1) employed acid conditions with two objects in view – to remove the metal, and to cleave labile peripheral linkages to give a derivative suitable for structural work. The first objective has yet to be achieved – cobalticorrin is a very robust complex – but the second objective was realized. Considerable structural information concerning peripheral groups was obtained and, in Todd's laboratory at Cambridge, crystalline corrinoid polycarboxylic acids were isolated. One of these acids, a hexacarboxylic acid chloride cyanide, proved, in the hands of Dorothy Hodgkin and her colleagues at Oxford,

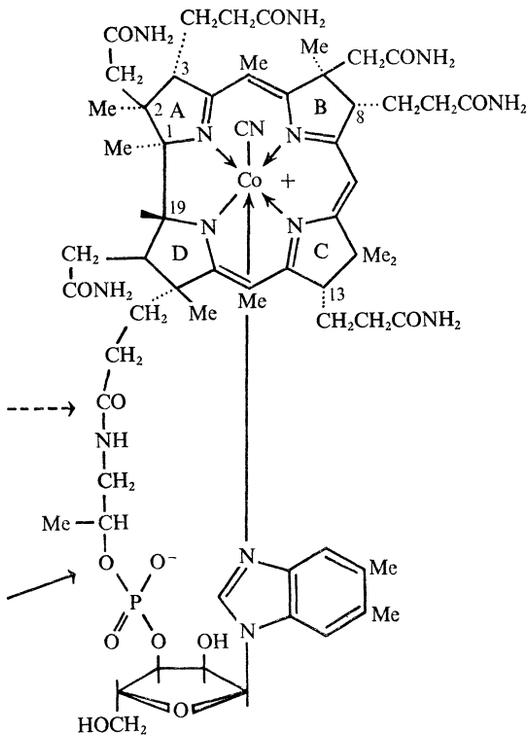

Figure 1. Vitamin B_{12} (cyanocobalamin). Cobinamide is the corrinoid resulting from hydrolysis at the alkly phosphate linkage (⟶) while cobyric acid is the corrinoid resulting from hydrolysis at the secondary amide function (- - →).

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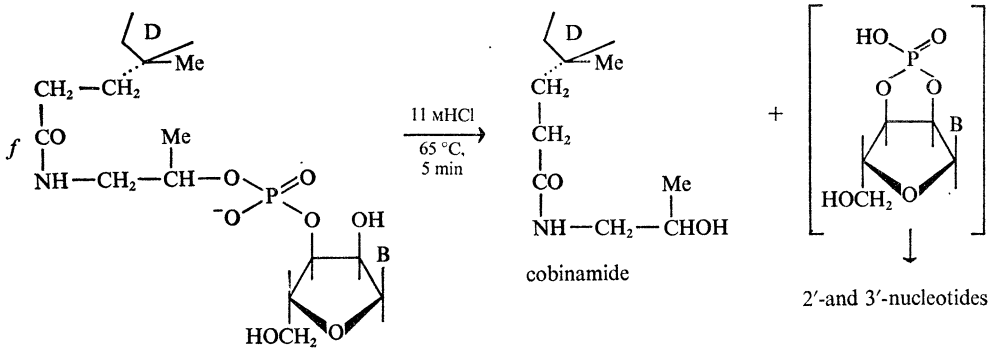
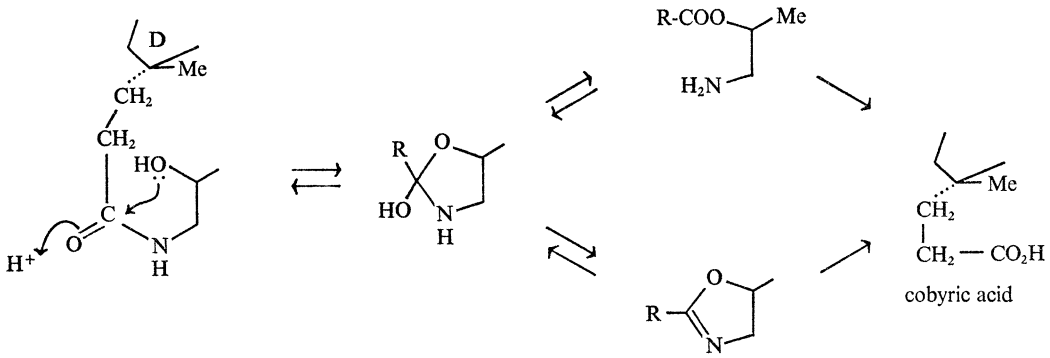
a key substance in the series of X-ray crystal analyses from which the structure of the corrinoid system emerged (reviews: Bonnett 1963; Hodgkin 1965).

Among the various acidic conditions employed were those involving media of high acidity. Thus treating vitamin B_{12} with concentrated hydrochloric acid for a short period (5 min, 65 °C) gave cobinamide (figure 1) (Armitage *et al.* 1953):



B=5,6-dimethylbenziminazol-1-yl

This cleavage appeared to occur (as shown) with the participation of the 2′-hydroxy group of the ribose: it was thought likely that another assisted hydrolysis might occur further along side chain *f* to give cobyric acid:



Indeed cobyric acid could be prepared by prolonging the reaction, but the preparation was made much less attractive than it might otherwise have been by the appearance of a second series of compounds, called the neo series, which on paper chromatograms were slightly darker in appearance than were the normal compounds (Bonnett, Godfrey & Redman 1969). In fact, mention of a darker series of corrinoids had been made earlier (Friedrich & Bernhauer 1954) following a study of the cleavage of factor III (the 5-hydroxybenziminazole analogue of vitamin B_{12}) in perchloric acid.

The neo compounds proved to be formed from a variety of corrinoid polyamides – cobinamide, cobyric acid, a mixture of monopropionic acids ('1.6 m') and a diacid fraction. Various acidic conditions could be employed, e.g. 35 % hydrochloric acid, 60 % perchloric acid, 42 % tetrafluoroboric acid. The reaction occurred in concentrated sulphuric acid, although here it was complicated by the formation of products which were acidic at pH 2 (presumably alkyl hydrogen sulphates derived from a ribose or propanol hydroxy function (Redman 1964)). The most convenient reagent was trifluoroacetic acid.

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Compounds of the neo series resembled their normal analogues very closely (Bonnett, Godfrey & Math 1971). They had the same charge on paper electrophoresis, but the separation on paper chromatograms allowed a small scale preparation to be developed: this was a slow process, however (2 days), and did not work for polyesters (for the separation of which high pressure liquid chromatography has proved effective: Eschenmoser 1971; Woodward 1973).

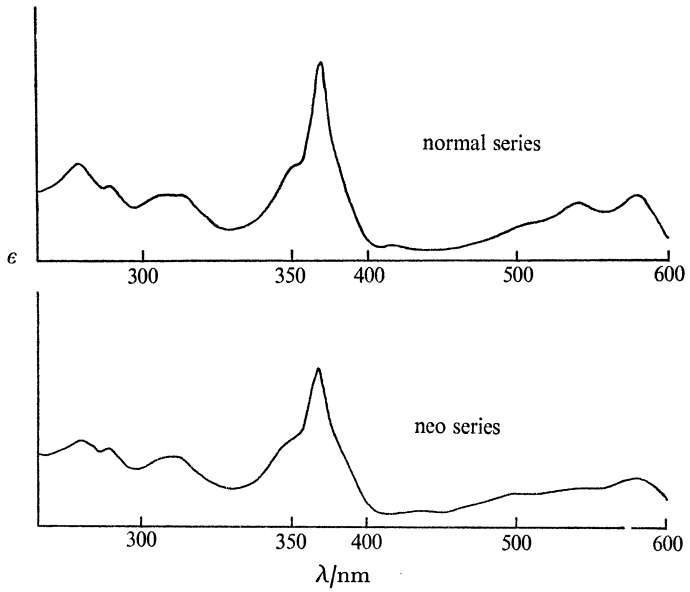

FIGURE 2. Typical electronic spectra of the normal corrinoid system (above) and the neocorrinoid system (below) as the dicyanides in 0.1 M KCN solution.

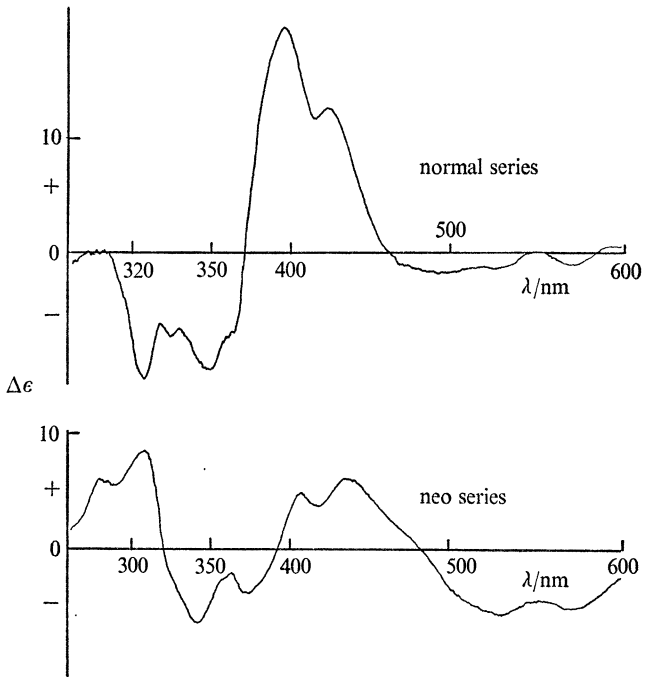

FIGURE 3. Typical circular dichroism spectra of the normal corrinoid system (above) and the neocorrinoid system (below) as the dicyanides in 0.1 M KCN solution. (In figures 2 and 3 vitamin B_{12} and neovitamin B_{12} have been taken as examples.)

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The infrared spectra were essentially indistinguishable: the mass fragmentation and proton n.m.r. spectra of the polyesters formed on methanolysis were distinguishable but very similar. Slight differences were observed in the electronic spectra (figure 2), the β-band for the normal series being more prominent than that for the neo in all spectra studied. The chirooptical spectra (Bonnett *et al.* 1973) displayed the most obvious differences: a typical pair of circular dichroism spectra is shown in figure 3. The most interesting chemical property of the two series was their equilibration under highly acidic conditions, the equilibrium constant being of the order of unity. Some equilibrium constants are given in table 1.

TABLE 1. EQUILIBRIUM CONSTANTS FOR THE CORRINOID $\rightleftharpoons$ NEOCORRINOID INTERCONVERSION

substrate	solvent	$K = \frac{[\text{neo}]}{[\text{normal}]}$
13-epicobinamide	trifluoroacetic acid/20 °C	1.75†
cobyric acid	trifluoroacetic acid/20 °C	1.85† 1.55‡
hexamethyl cobyrinate *f* nitrile	sulphuric acid	2.55§

† Separation by paper chromatography followed by elution and spectroscopic estimation.
‡ By comparison of the c.d. spectra of the equilibrium mixture and the two isomers at 576–8 nm and a 279–285 nm.
§ Reported in Woodward (1973).

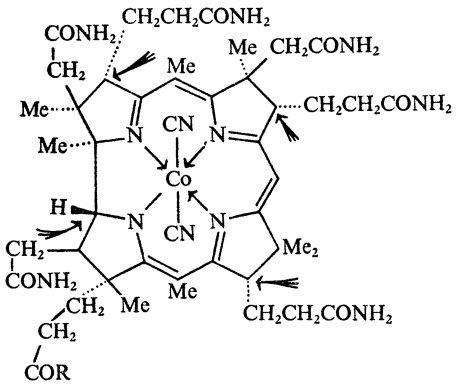

FIGURE 4. Location of potentially epimerizable sites adjacent to the conjugated system of the natural cobalticorrinoid macrocycle.

At this stage the evidence strongly suggested that the normal and neo series were stereoisomeric: the most likely change appeared to be epimerization at C3, C8, C13, and C19, which centres are ψ-allylic with respect to the conjugated system (figure 4). Epimerization at C19 would have led to a sterically unfavourable cisoid arrangement at the direct linkage, which would not tally with the observed equilibrium constant. In the event epimerization at C3 or C13 was thought to be most likely (Godfrey 1970). Although it is possible to examine stereochemistry at C13 by oxidative cleavage (Kuehl, Shunk, Moore & Folkers 1955) there was no chemical method known to us for arriving at the configuration at C3.

None of the neo compounds had been crystalline, but re-examination of the initial stages of the reaction of vitamin B_{12} in trifluoroacetic acid gave the neo analogue of the vitamin itself: its chemical properties are summarized in figure 5. This substance was crystalline, and an X-ray

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crystallographic structure analysis by Dorothy Hodgkin and her colleagues (Stoeckli-Evans, Edmond & Hodgkin 1972) showed that it differed from vitamin B_{12} in two main features – the propionamide at C13 was directed towards the β-face rather than the α, and the tilt of the β–β′ bond of ring C had changed. Thus both configurational and conformational changes had occurred at ring C: they are represented in diagrammatic form in figure 6.

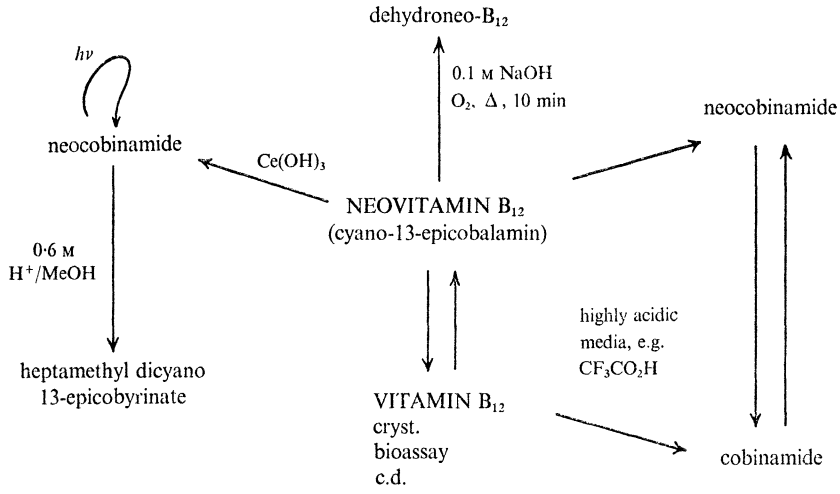
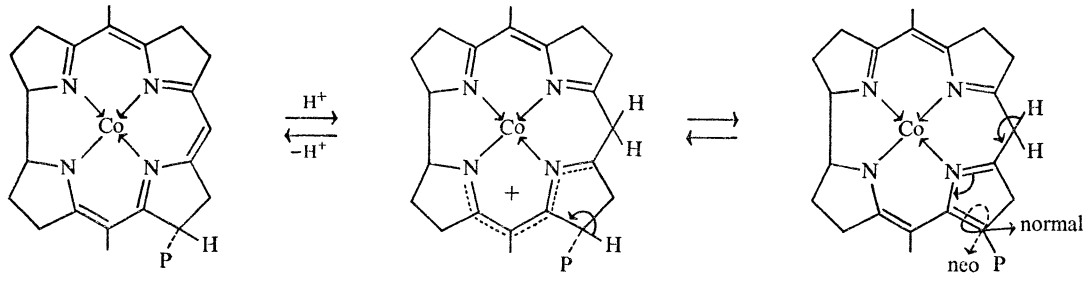


FIGURE 5. Some chemical properties of neovitamin B_{12}.

Thus neovitamin B_{12} was formulated as cyano-13-epicobalamin. The epimerization is regarded as proceeding via the protonated corrinoid chromophore. The position of protonation is not certain, but as deuteriation experiments show (Bonnett & Redman 1964) protonation can occur at C10, and the following scheme can be written:



However, such a scheme can also be written for epimerization at C3 and C8: epimers at these two centres have been encountered in the total synthesis (Eschenmoser 1971; Woodward 1973) but have not been observed in our equilibration experiments, and it is of interest to enquire why this is so. One explanation is that these epimers have been formed, but have not been detected. However, except below 300 nm (where the benziminazole chromophore would be expected to contribute) the circular dichroism spectra of neocobinamide and neocobyric acid in 0.1 M potassium cyanide rather closely resemble the circular dichroism of crystalline neovitamin B_{12} in that solvent, suggesting that the proportion of such epimers must be low. This leads to the view that for the polyamides in acid media the natural configurations for rings A and B are the thermodynamically more stable ones, whereas for ring C the stability of the two epimers is more

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finely balanced. This can be rationalized in the following way. When epimerization occurs at C3 or C8, two large groups (acetamide and propionamide residues) are brought into a *cis* relationship. Moreover, under the strongly acidic conditions of the reaction, the amide groups are expected to be protonated, so that for both steric and electrostatic reasons the natural configuration is likely to be preferred. On ring C, on the other hand, the propionamide faces a methyl group in *either* configuration, and an equilibrium constant of approximately unity is observed. The small preference for the neo isomer may be due to interaction with the propionamido group, necessarily α, at C17.

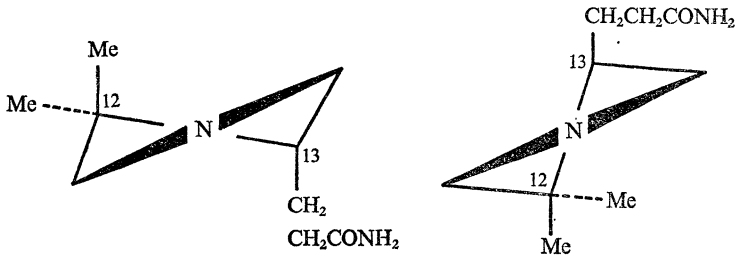

FIGURE 6. Ring C as viewed from the cobalt atom in (i) vitamin B_{12} (cyanocobalamin) and (ii) neovitamin B_{12} (cyano-13-epicobalamin). Diagrammatic, based on Stoeckli-Evans *et al.* (1972).

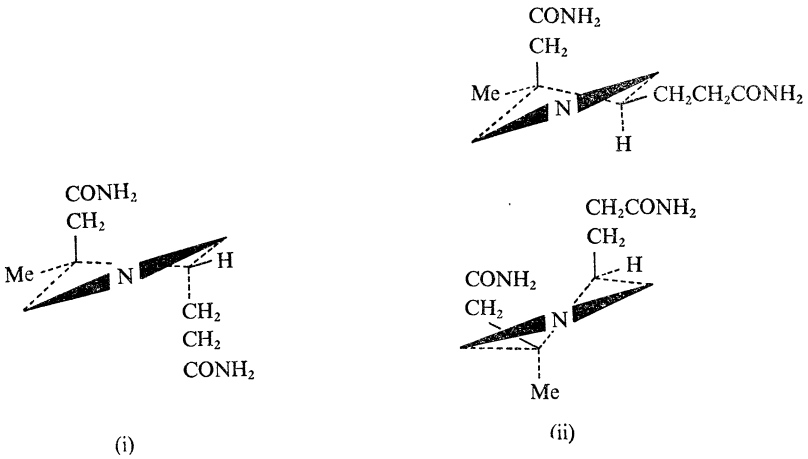

FIGURE 7. Diagrammatic representation of the conformation of ring A as viewed from the cobalt atom in (i) cyanocobalamin and cyano-13-epicobalamin and (ii) the possible extreme conformations for the 3-epi system.

Conformational factors may also be involved, although the arguments here are rather tenuous, and assume that there is little change in conformation on dissolution of the crystal. The X-ray results (Hodgkin 1965; Stoeckli-Evans *et al.* 1972) show that, as expected, the five-membered rings are not planar and, in particular, that the β–β′ bonds tend to adopt a tilt such that the acylamide residues are directed out of the average plane of the macrocycle. This happens for rings A, B and C (but not for the reversed ring D). It has been illustrated for ring C in figure 6 where it is seen that in the 13-epi system the tilt of the β–β′ bond changes to accommodate the redirection of the propionamido group out of the general plane. However, for an epimer at C3 (or at C8), in either of the extreme conformations only *one* of the acylamide residues can be directed out of the general plane of the macrocycle, as illustrated in figure 7.

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It is a pleasure to acknowledge the efforts of David Redman, Joan Godfrey and Veer Math, all of whom contributed to the chemical study of the neo series. It was Joan Godfrey who detected, isolated and crystallized neovitamin B_{12}. I am grateful to Professor W. Klyne and Dr P. M. Scopes (Westfield College) for the circular dichroism measurements; to Dr L. Mervyn (Glaxo) for microbiological assays; and to Professor Dorothy C. Hodgkin, Dr Helen Stoeckli-Evans, and Dr Eric Edmond (Oxford) for the X-ray results which led to a clear solution to this problem.

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