Structure of a Monocarboxylic Acid Derivative of Vitamin B₁₂

One particular monocarboxylic acid derived from cyanocobalamin can be grown in very large crystals on which both X-ray and neutron diffraction measurements have been made. Calculations lead to the placing of some 205 atoms in the crystal asymmetric unit, including 98 hydrogen atoms, and thus to the detailed definition of the chemical structure of the molecule.

Crystal and Molecular Structure from X-ray Analysis

THE mild acid hydrolysis of cyanocobalamin gives rise to a mixture of mono- and di-carboxylic acids and one tricarboxylic acid, a finding consistent with the view that the propionamide side chains on the molecule are the first objects of attack. The exact structure of one of these acids, which has particularly interesting properties, is the subject of the present investigation. This acid, first called E_1, constitutes more than 90 per cent of the monocarboxylic acid fraction of the B_{12} hydrolysate[1]: it crystallizes in large monoclinic crystals, easily separated by fractional crystallization from the isomeric acids. It also occurs as a natural product in the fermentation liquors of *Propionibacterium shermanii* (CMS_1^3, CMS-e^3; Zone 1[4]) where it behaves as an intermediate in the biosynthesis of the B_{12} vitamins[3]. It has antimetabolite activity compared with B_{12} in *Escherichia coli* and *Ochromonas malhamensis*[5].

The very large size of the crystals of the acid prompted an attack on its structure by a combination of X-ray and neutron diffraction. It was hoped by such measurements to define the crystal structure of the acid itself, to place the acid group on the molecule and to add the details of the arrangement of the hydrogen atoms to the structure of vitamin B_{12}. The X-ray analysis began on the air dried crystals because particularly perfect specimens of these, several mm on edge, had already been given to us by Dr. E. Lester Smith. Later, as experience with neutron diffraction indicated that the more stable crystals in their mother liquor should be the main subject of attack, wet crystals were also investigated by X-rays. Preliminary data on the crystals are given in Table 1.

easily obtained from an electron density distribution calculated with 2,567 terms phased from the Bijvoet differences, with α chosen as the alternative nearest the cobalt atom contribution. The structure was, in fact, solved first from a less favourable approximation and the atomic positions improved in successive electron density distributions and least squares calculations. The present reliability index is 0·142; the average standard deviation in the carbon–carbon single bonds is 0·04. Fig. 1 shows the agreement at this stage between α_c, calculated on the present atomic parameters, and α_o, the phase angles derived from the Bijvoet effect. The curve shows that for 72 per cent of the reflexions, the first phase angle chosen was within 45° of the final value.

Once the structure of the air dried crystals was known, it was easy to derive that of the wet crystals, starting from a cobalt atom phased map alone, and sorting out the correct mirror image atoms by inspection of the three dimensional distribution. This route was followed at Harwell (F. M. M.). An alternative route using Bijvoet pairs of reflexions measured on oscillation photographs was taken at Auckland (T. N. M. W., J. M. W.). This alternative has some general interest as a possible method for deriving rapidly the general atomic distribution within a molecule in the correct absolute configuration.

The crystal structure of the air dried monoacid is illustrated in Fig. 2. It shows no resemblances to that of cyanocobalamin, wet or dry[1,6], nor to that of 5′-deoxyadenosylcobalamin[6]. The molecules are arranged in broad layers within which the planes of the corrin rings are inclined at about 80° to one another. Between the layers there is a marked cleavage; contact is made across the
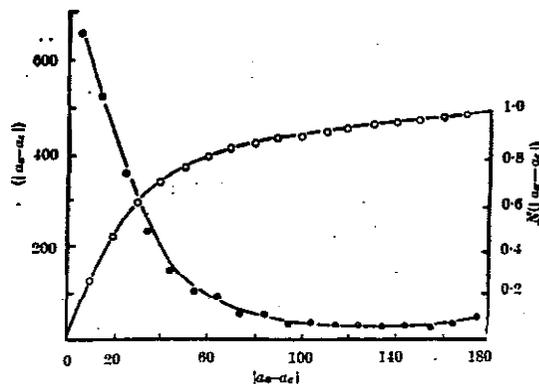
Table 1. PRELIMINARY DATA ON THE B₁₂ MONOACID, C₆₃H₈₇O₁₄N₁₃PCo·

	Unit cell dimensions				Space group	Density	Probable No. of water molecules
	a	b	c	β			
Air dried	14·61	17·09	16·35	103°	P2₁	1·335	13
Wet	14·915	17·486	16·409	104·11°	P2₁	1·339	16
	0·009	0·009	0·007	0·047			

The preferred values for the wet crystals were derived by neutron diffraction.

The air dried crystals of the acid gave very good X-ray data, extending to the limit of the copper sphere and showing marked Bijvoet differences due to anomalous dispersion at the cobalt atom. A total of 6,323 individual hkl and $\overline{hkl}$ reflexions were measured visually. Average F^2 values were derived and used for the initial three-dimensional Patterson calculations to place the cobalt atom. Two alternative phase angles were then calculated by the expressions given by Ramachandran and Raman[2]. Trials of a number of functions and variations of their expressions were made which will be described elsewhere. Evidence for the correct atomic arrangement was most

Fig. 1. Phase analysis. Here n is the number of reflexions in each 10° range and $N(|\alpha-\alpha_c|)$ is the normalized cumulative function for each value of $|\alpha-\alpha_c|$ where α_c is the phase from the Bijvoet effect, α_c the final phase calculated.

gap through hydrogen bonds between water molecules and the active groups at the ends of the side chains. The terminating atoms of two of the acetamide groups occupy alternative disordered sites and many of the water molecule positions also show disorder. There does not appear to be any obvious feature of the molecular arrangement that would suggest the position of the carboxylic acid groups. among the amides. Direct observation of the electron density maps favours a definite orientation of each of the three acetamide groups in agreement with the neutron diffraction data below; the longer side chains, however, are less well defined in position and conclusions from the peak heights or interatomic distances are unreliable here.

The general atomic distribution in the molecule is closely similar to that in cyanocobalamin with one quite interestng difference. This is illustrated by Fig. 3, which shows a projection of the atomic positions on to a calculated least squares plane passing through the cobalt and four inner nitrogen atoms. When the corresponding projection of cyanocobalamin is compared with this, it is clear that there is a variation in the position of the nucleotide-like side chains in the two molecules. The phosphate and sugar
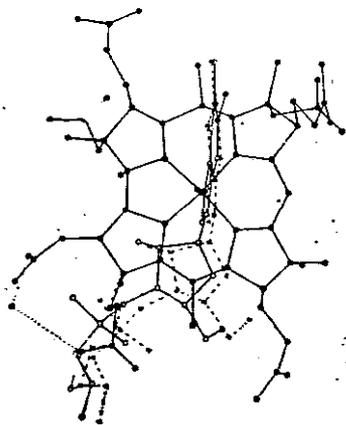

Fig. 3. Projection of atomic positions on the least squares plane through cobalt, N21, N22, N23 and N24. Solid line, B₁₂ monoacid; dotted line, cyanocobalamin (both air dried).

group particularly have moved about 1 Å relative to the positions they occupy in cyanocobalamin'. The benziminazole, sugar, phosphate, and propanolamine groups are rather flexible and the new positions may represent no more than an adjustment to new packing conditions within the present crystal structure. They do not seem to be connected specifically with the position of the acid group.

We thank Dr. E. Lester Smith, Professor Bernhauer, Dr. Beisbarth and Dr. Wagner for crystals used in this research and for interesting discussions. Computing was carried out on the KDF9 computer, University of Oxford. One of us (S. R.) thanks the Science Research Council for the award of a senior research fellowship.

C. K. NOCKOLDS   T. N. M. WATERS
S. RAMASESHAN   J. M. WATERS
DOROTHY CROWFOOT HODGKIN
Chemical Crystallography Laboratory,
University of Oxford.

Fig. 2. Projection of the atomic positions found in air dried crystals of the B₁₂ monoacid: *a*, along [010]; *b*, along [100]. Filled circles represent atoms at *y*, open circles at *y + ½*.

Crystal and Molecular Structure from Neutron Diffraction Analysis

The distinction between an amide and a carboxylic acid can be made more easily by neutron diffraction than by X-ray diffraction. In contrast to X-ray scattering. the coherent neutron scattering lengths of nitrogen ($b = 9.4f$*) and of oxygen ($b = 5.8f$) are widely different and in addition the coherent neutron scattering length of hydrogen ($b = 3.8f$), although negative, has a magnitude approximately equal to that of other atoms. Consequently it has been possible by neutron diffraction to define in detail the structure of the monocarboxylic acid derivative of cyanocobalamin.

The neutron diffraction data for this purpose were measured using a wet crystal of weight 11 mg and density 1·339 g/c.c. All unique reflexions from planes with interplanar spacings, $d > 1.3$ Å, were investigated and 1,531 reflexions were classed as observable. The mean peak (including background) to background ratio for the observable reflexions was 1·3 : 1, but the count rate was sufficient to give a fractional standard deviation $\sigma (f)/F \sim 8$ per cent for the structure amplitudes after counting for 30 min equally divided between peak and background.

The structure analysis is summarized in the flow sheet. The neutron Patterson synthesis first calculated proved, as expected, to be uninterpretable because there are many atoms present in the unit cell, scattering with both
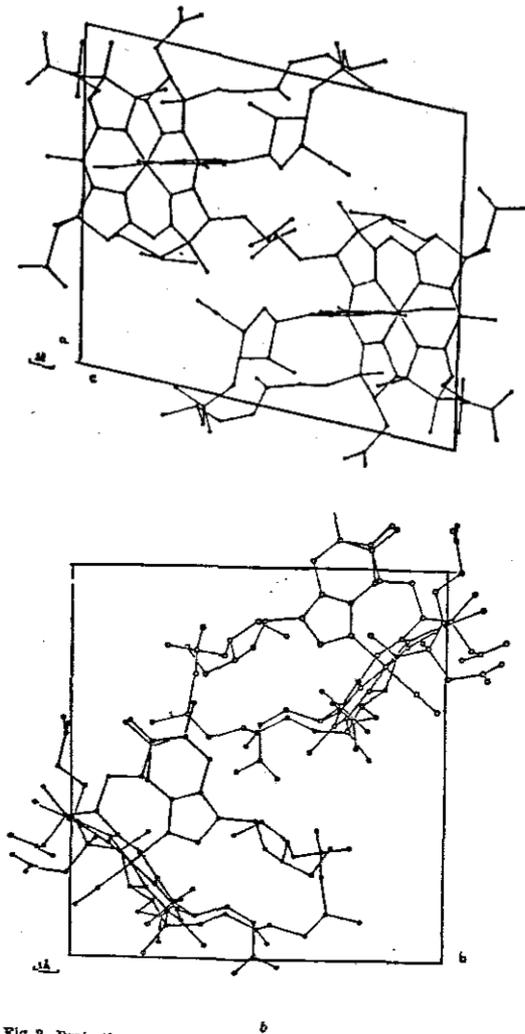
* $f = 10^{-13}$ cm (fermi unit).