Further Refinement of the Crystal Structure of Neovitamin B_{12}

By **Helen Stoeckli-Evans,***† **E. Edmond,** and **Dorothy Crowfoot Hodgkin,** Chemical Crystallography Department, South Parks Road, Oxford

The molecular structure of neovitamin B_{12} has been elucidated by *X*-ray crystallographic analysis from diffractometer data. The crystal structure was solved by means of the anomalous dispersion technique and shown to be cyano-13-epicobalamin. an isomer of cyanocobalamin. vitamin B_{12}. Both vitamin B_{12} and neovitamin B_{12} are parent compounds of two isomeric series. The compound crystallises in space group $P2_12_12_1$ with $Z = 4$ in a unit cell of dimensions: $a = 24{\cdot}98$, $b = 22{\cdot}06$, $c = 15{\cdot}74$ Å. The structure was refined by least-squares techniques to R 0·159 for 2986 independent reflections.

THE prolonged treatment of vitamin B_{12} with a highly acidic reagent produces a number of cleavage products of which two are the light red cobinamide and the closely related darker red compound, neocobinamide.[1,2] It was observed[1,3] that if the same reaction were interrupted after only 2 h a third red component with a lower R_F value than that of vitamin B_{12} was also present. The new compound was isolated by preparative paper chromatography and shown to crystallize as dark red prisms from acetone–water in the presence of a trace of hydrogen cyanide. This compound was shown[3] to be closely related to vitamin B_{12} and named neovitamin B_{12}. The reverse reaction also proved to proceed quite readily in a highly acidic medium yielding mainly neocobinamide, and also cobinamide and vitamin B_{12}. Hence the equilibrium observed between the simpler systems such as cobyric acid $\rightleftharpoons$ neocobyric acid also occurs in the vitamin. It was therefore concluded that vitamin B_{12} and neovitamin B_{12} are isomers and parent compounds of two isomeric series.

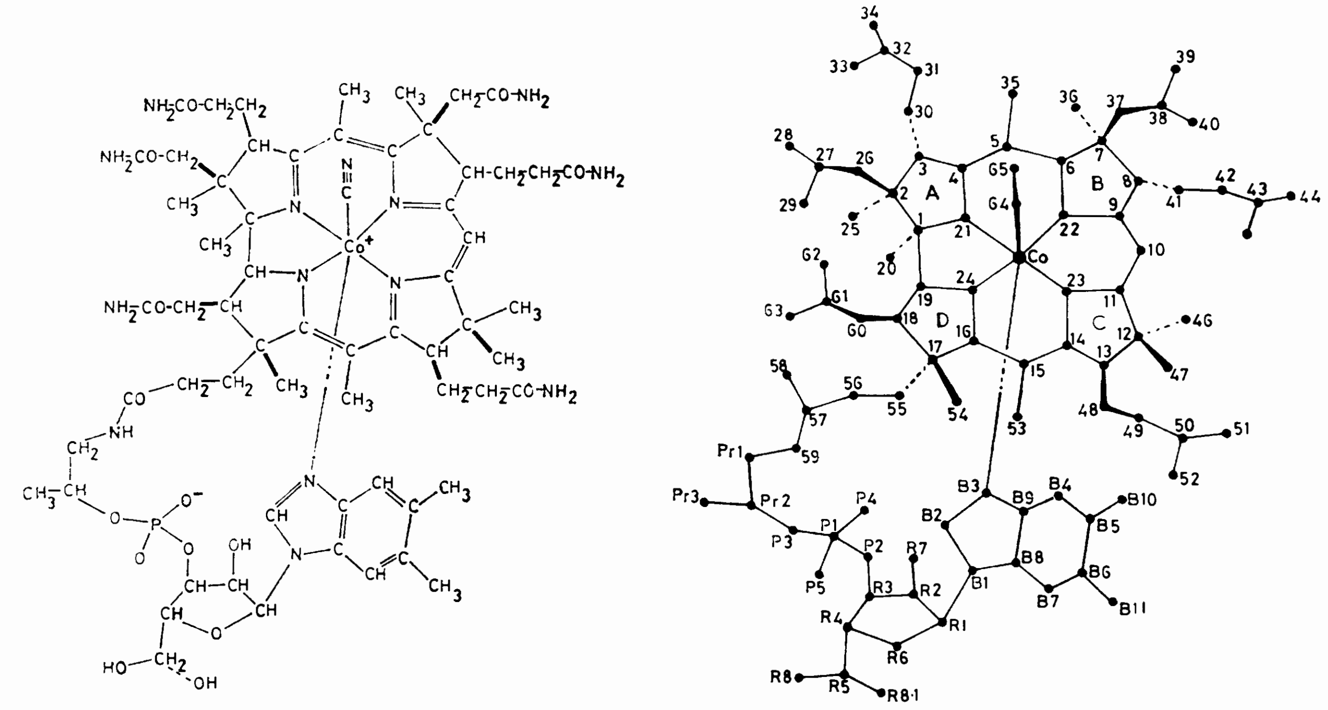


FIGURE 1 Formula and numbering system used for neovitamin B_{12}

On the evidence of the different chiroptical properties of the two series it was suggested[3,4] that the interconversion neo $\rightleftharpoons$ normal series involved a stereochemical and/or a conformational change.

The initial results[4] of the *X*-ray crystallographic analysis of neovitamin B_{12} showed it to be cyano-13-epicobalamin (Figure 1). Here we report briefly the procedures used in the analysis and in more detail the results arising from further refinement of the data for neovitamin B_{12}.

---

† *Present address:* Chemistry Department, University of Neuchâtel, Switzerland.

[1] R. Bonnett, J. M. Godfrey, and D. G. Redman, *J. Chem. Soc.* (*C*), 1969, 1163.

[2] W. Friedrich and K. Bernhauer, *Z. Naturforsch.*, 1954, **9***b*, 685.

[3] J. M. Godfrey, Ph.D. Thesis, University of London, 1969.

[4] R. Bonnett, J. M. Godfrey, V. W. Math, E. Edmond, H. Evans, and O. J. R. Hodder, *Nature*, 1971, **229**, 473.

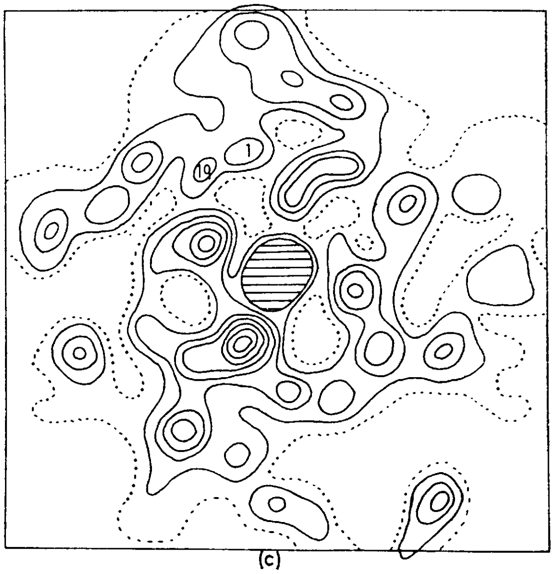
EXPERIMENTAL

During data collection large differences were observed between the h,k,l and h,k,l reflections which suggested the possibility of using the anomalous dispersion phasing technique to solve the crystal structure.

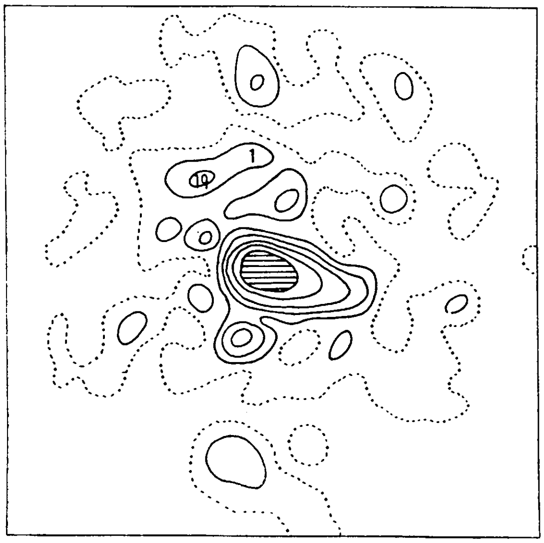
Crystal Data.—$C_{63}H_{88}CoO_{14}N_{14}P$,(probably)$20H_2O$, $M = 1716$, Orthorhombic, $a = 24{\cdot}98$, $b = 22{\cdot}06$, $c = 15{\cdot}74$ Å, $U = 8674$ Å^3, $D_m = 1{\cdot}33 \pm 0{\cdot}01$, $Z = 4$, $D_c = 1{\cdot}314$, $F(000) = 3752$. Space group $P2_12_12_1$. Cu-K_α radiation, $\lambda = 1{\cdot}5418$ Å; μ(Cu-K_α) = $27{\cdot}43$ cm^{-1}.

Some of the larger crystals were mounted in thin-walled glass capillaries as described previously.[4]

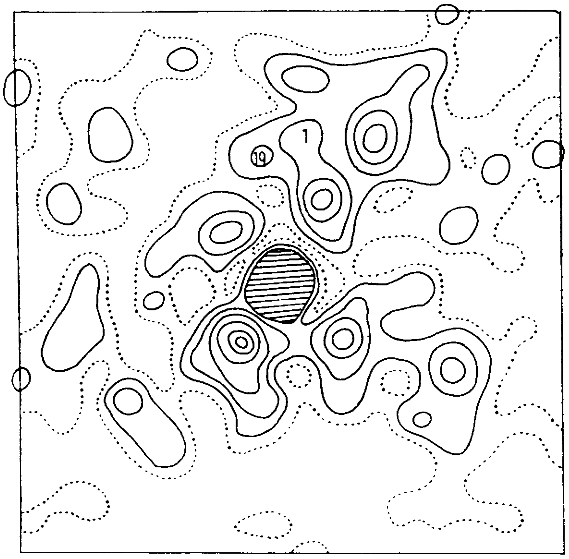
2986 Independent reflections, including 2801 Bijvoet pairs, were measured using a Hilger and Watts four-circle diffractometer, with nickel-filtered Cu-K_α radiation. The ordinate analysis technique [5] was used to collect the data, using ω scans of 40 steps of 0·03°. Data were collected for reflections in the region of reciprocal space given by $h \geqslant 0$, $k \geqslant 0$, l unrestricted. Resolution was *ca.* 1 Å. Lorentz, polarization, and absorption [6] corrections were applied. A Wilson plot on the resultant data gave an overall temperature parameter and scale factor, application of which enabled the data to be put on an absolute scale.



(c)



(a)



(b)

FIGURE 2 Slant Fourier through the four inner nitrogens of (a) heavy-atom synthesis, enantiomorph (+ + +); (b) anomalous phasing synthesis, enantiomorph (+ + +); and (c) anomalous phasing synthesis, enantiomorph (− + +). Contours from 0 e Å^{-3} (dotted line) at intervals of 2 e Å^{-3}.

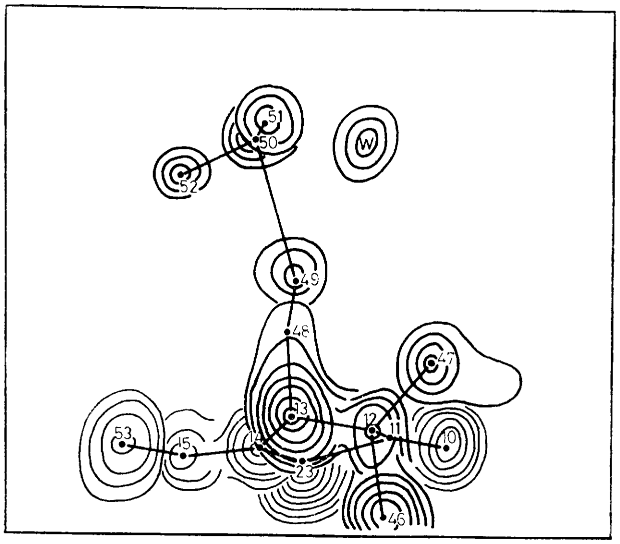


FIGURE 3 A composite projection on the (001) plane of the electron density in the correct anomalously phased Fourier synthesis shown in Figure 2c

The cobalt atom co-ordinates were obtained by examination of the three Harker sections of a three-dimensional Patterson synthesis sharpened to ' point-atoms-at-rest '. A structural enantiomorph was chosen with all the co-ordinates positive and an alternative enantiomorph with the x co-ordinate negative. Figure 2a illustrates

---

[5] H. C. Watson, D. M. Shotton, J. M. Cox, and H. Muirhead, *Nature*, 1970, **225**, 806.

[6] A. C. T. North, D. C. Phillips, and F. S. Mathews, *Acta Cryst.*, 1968, **A24**, 351.

the heavy-atom Fourier synthesis calculated using the cobalt atom co-ordinates (+ + +). It is of poor resolution and exhibits hints of pseudo-symmetry, because the cobalt atom lies close to the *yz* plane.

Structure-factor phase determination by anomalous dispersion methods for the case of a single acentrically placed heavy atom as described in ref. 7 was implemented in a phase-determining program written by O. J. R. Hodder of this laboratory for use on the Oxford KDF 9 computer. Two phase solutions are obtained and the ambiguity is resolved by choosing that closest to the one calculated for the heavy atom alone, since the anomalous scatterer usually represents a high proportion of the scattering power.

TABLE 1

(*a*) Final atomic parameters (× 10⁴) and isotropic temperature parameters (× 10³)

Atom	x/a	y/b	z/c	$\bar{U}^2$
C(1)	297	680	−979	55
C(2)	214	537	−1968	70
C(3)	212	1267	−2351	90
C(4)	2	1564	−1561	61
C(5)	−318	2159	−1620	60
C(6)	−380	2487	−957	44
C(7)	−607	3116	−872	101
C(8)	−418	3336	24	74
C(9)	−352	2739	458	55
C(10)	−445	2692	1297	70
C(11)	−430	2132	1776	39
C(12)	−498	2098	2666	61
C(13)	−590	1428	2801	90
C(14)	−353	1163	1995	50
C(15)	−247	558	1910	52
C(16)	−92	326	1143	60
C(17)	−19	−358	835	65
C(18)	259	−316	2	66
C(19)	20	286	−382	42
C(20)	937	689	−772	68
N(21)	64	1264	−877	42
N(22)	−267	2313	−117	46
N(23)	−315	1611	1411	48
N(24)	−13	657	419	59
C(25)	652	126	−2387	89
C(26)	−331	281	−2137	94
C(27)	−521	228	−3031	97
O(28)	−725	−299	−3225	163
N(29)	−358	590	−3708	148
C(30)	738	1534	−2609	101
C(31)	846	1346	−3514	105
C(32)	1407	1670	−3739	164
N(33)	1838	1345	−3838	163
O(34)	1516	2216	−3876	212
C(35)	−438	2376	−2528	92
C(36)	−499	3580	−1643	104
C(37)	−1264	3042	−746	125
C(38)	−1582	2642	−1406	195
O(39)	−1739	2130	−1369	195
N(40)	−1774	3157	−1847	359
C(41)	117	3676	−79	69
C(42)	429	3770	790	92
C(43)	940	4089	668	86
N(44)	1030	4429	−1	111
O(45)	1286	4079	1225	164
C(46)	70	2252	3110	97
C(47)	−876	2540	3078	110
C(48)	−1167	1275	2850	140
C(49)	−1439	1330	2270	134
C(50)	−2150	970	2467	235
O(51)	−4217	1170	2895	211
N(52)	−2086	429	2669	485
C(53)	−304	165	2686	89
C(54)	−588	−627	846	85
C(55)	355	−751	1501	75
C(56)	906	−487	1706	86
C(57)	1120	−798	2500	80
O(58)	877	−804	3164	104
N(59)	1552	−1103	2430	105
C(60)	60	−888	−621	93
C(61)	452	−1525	−400	123
N(62)	114	−1951	−329	139
O(63)	902	−1406	−133	214
C(64)	−797	1315	−20	55
N(65)	−1218	1163	−157	83
N(B1)	1346	1786	1419	72
C(B2)	850	1522	1278	55
N(B3)	628	1734	582	43
C(B4)	959	2402	−626	61
C(B5)	1465	2792	−802	71
C(B6)	1873	2837	−263	69
C(B7)	1884	2539	514	63
C(B8)	1421	2188	696	45
C(B9)	987	2112	156	50
C(B10)	1451	3160	−1674	93
C(B11)	2320	3262	−431	90
C(R1)	1724	1634	2029	58
C(R2)	2019	1043	1799	69
C(R3)	2116	799	2692	99
C(R4)	1679	930	3089	111
C(R5)	1728	1083	4139	143
O(R6)	1442	1554	2810	111
O(R7)	1641	658	1327	88
O(R8)	2169	1399	4135	196
O(R8·1)	1234	1094	4378	148
O(P2)	2196	117	2597	107
O(P3)	2578	−854	2978	95
O(P4)	3096	−161	1998	148
O(P5)	2912	96	3603	160
Pr(1)	1848	−1414	3075	115
Pr(2)	2191	−1054	3574	78
Pr(3)	2538	−1408	4196	99
Co	−133	1485	231	
P	2735	−163	2805	

Anisotropic temperature parameters

Atom	U_{11}	U_{22}	U_{33}	$2U_{13}$	$2U_{23}$	$2U_{12}$
Co	0·097	0·043	0·048	0·008	−0·034	0·031
P	0·120	0·131	0·144	0·116	−0·029	0·057

(*b*) Water molecule positions (× 10⁴) and temperature parameters (× 10³) *

Atom	x/a	y/b	z/c	$\bar{U}^2$	Occupancy
W(1)	−1188	−1750	2250	383	1
W(2)	767	−144	4586	198	1
W(3)	3277	310	597	226	1
W(4)	2237	−67	258	164	1
W(5)	1561	−1160	−1399	202	½
W(10)	2072	−1275	744	239	1
W(12)	−847	−1845	−1292	236	1
W(15)	−1885	76	−513	221	1
W(A)	−73	652	4754	275	1
W(B)	−2375	−375	3375	410	½
W(C)	−1455	−850	−1457	338	1
W(D)	−674	1858	−4739	228	1
W(E)	−2135	2632	−3428	375	1
W(F)	−1704	2558	−4827	328	1
W(G)	−1865	435	−4447	398	1
W(H)	−2375	2310	3875	238	½
W(J)	−1625	1250	−3125	711	½

* Only isotropic temperature parameters were refined.

The absolute configuration of the molecule was determined by calculating two Fourier syntheses using the *F′* and α values calculated for both the enantiomorphous arrangements (+ + +) and (− + +) of the cobalt atom co-ordinates where α is the phase of the structure-factor corrected for the anomalous dispersion and *F′* the total structure factor without the imaginary component.[7]

---

[7] S. R. Hall and E. N. Maslen, *Acta Cryst.*, 1965, **18**, 265.

The correct enantiomorph will give the right phase solution and hence one Fourier synthesis should show the atomic positions more clearly than the other. Enantiomorph (−++) proved to be correct as synthesis (2c) showed atomic positions much more clearly than synthesis (2b), phased on enantiomorph (+++). Figure 2 shows slant Fourier syntheses through the least-squares plane of the four inner nitrogen atoms.

At this stage it was possible to see that the differences in the structure of neo-B_{12} as compared to vitamin B_{12} were a stereochemical change at C(13) and a change in the tilt of the ββ′ bond C(12)–C(13). Figure 3 is a composite projection on the (001) plane of the electron density in the region of C(13) in the correct anomalously phased Fourier synthesis and clearly shows these structural differences. A second Fourier synthesis based on phases generated by the 85 atoms found in synthesis (2c), yielded a further 9 atomic positions, including two alternative positions for hydroxy-oxygen O(R8). A third structure-factor calculation based on phases generated by all 94 atoms of the neo-B_{12} molecule gave R 0·28.

Refinement was initially performed by full-matrix isotropic least-squares of both temperature parameters and atomic co-ordinates, together with a unit weighting scheme. The F' values were recalculated[7] at various stages during refinement using the latest refined scale-factor and cobalt atom co-ordinates. In the final stages of refinement both the cobalt and phosphorus atoms were given anisotropic temperature parameters and the empirical weighting scheme of Mills and Rollett[8] was adopted. A total of 17 solvent water molecules were found from difference syntheses calculated at various stages during the refinement. Five of these were assigned occupancies of ½ owing to a rapid rise in their temperature parameters during refinement. Two water molecules were removed for this reason and a subsequent difference synthesis

---

[8] O. S. Mills and J. S. Rollett, ‘Computing Methods and the Phase Problem in X-ray Analysis,’ Pergamon, London, 1960.

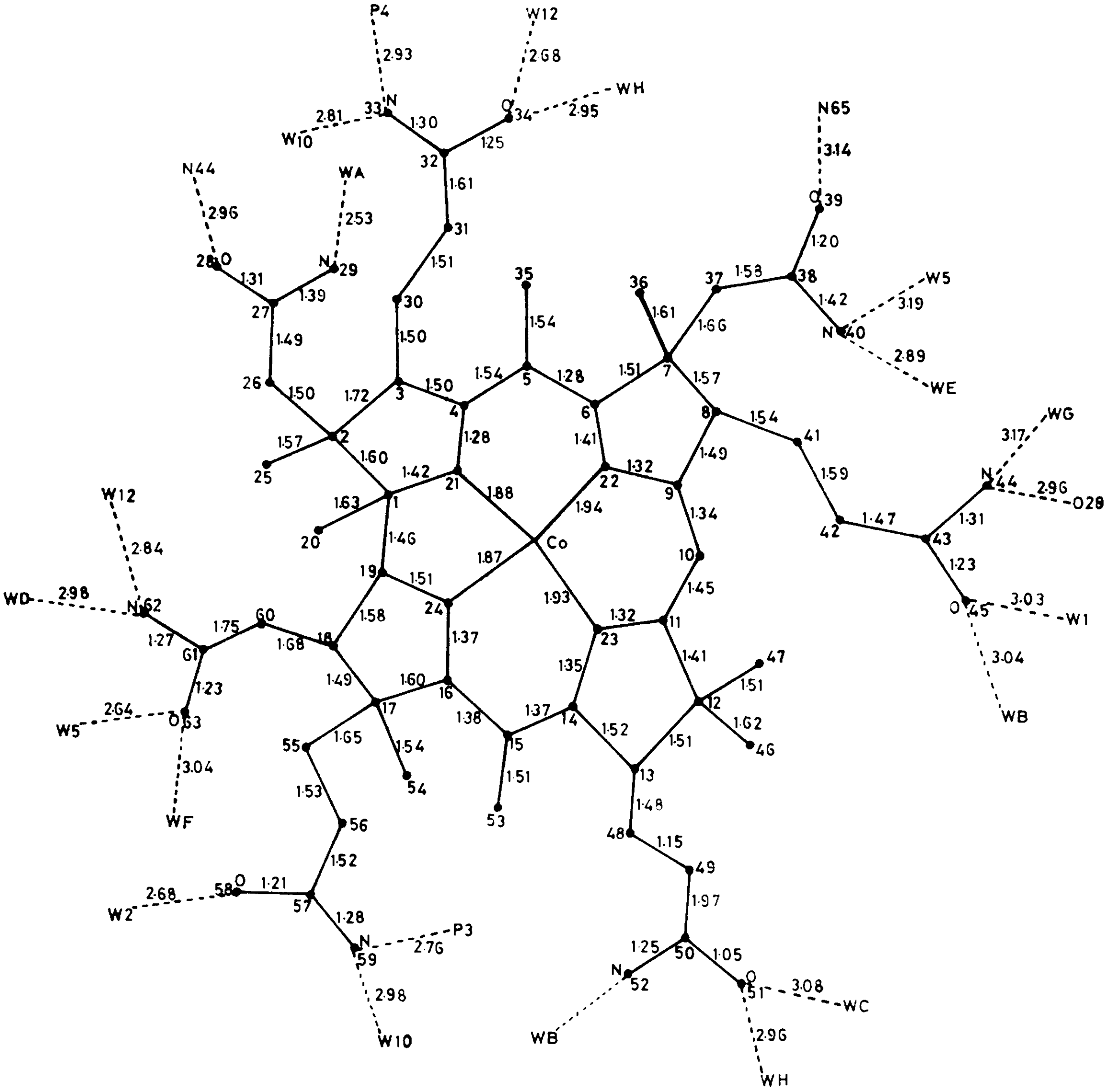


FIGURE 4

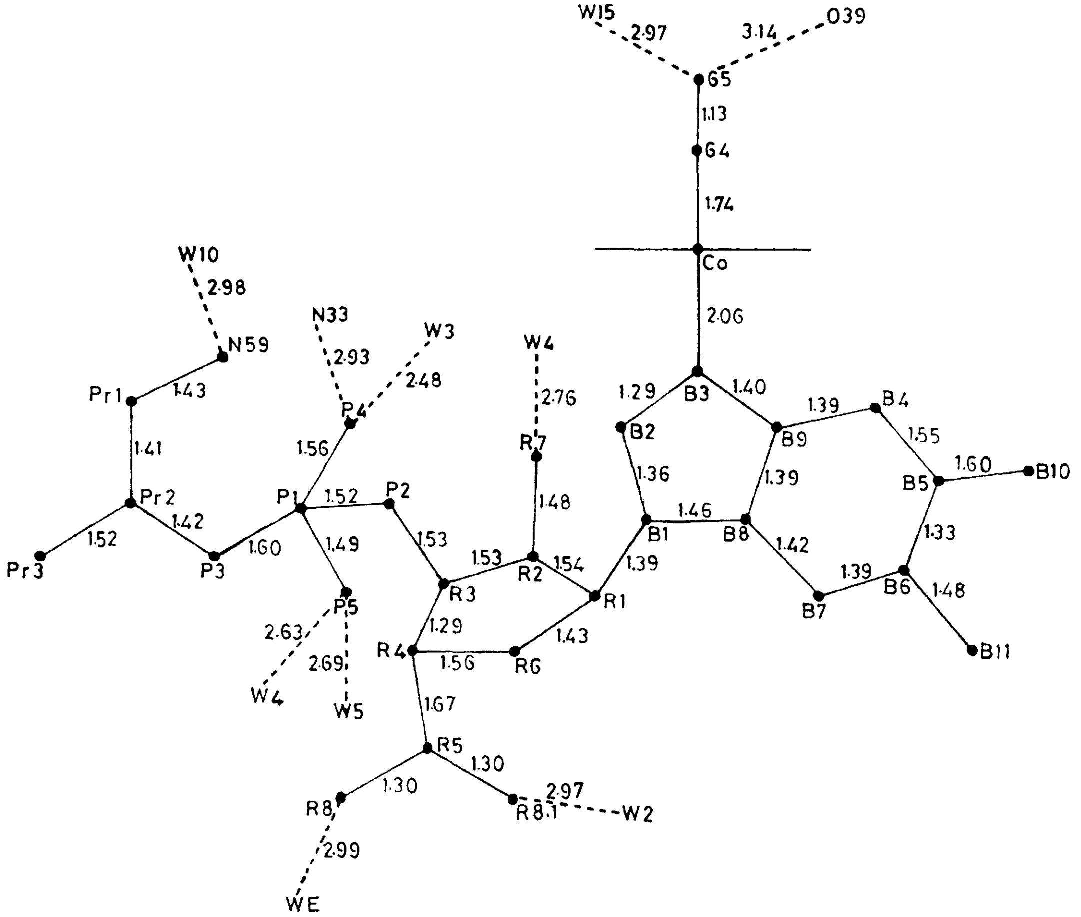


FIGURE 4 Final bond distances

showed them to be of low and diffuse density. One final water molecule was found from this difference synthesis and given an occupancy of ½ and a high temperature parameter. During the final round of refinement shifts in positional parameters were on average much less than one standard deviation and convergence was achieved.

Refinement was terminated at R 0·159 and a difference synthesis calculated at this stage showed no further significant atomic positions. The final atomic co-ordinates and individual isotropic temperature parameters are included in Table 1.*

The accuracy of the intensity measurements made in this structure determination is much better than in the earlier B_{12} analyses and so is the extent of refinement of the atomic parameters. Both still leave much to be desired from the point of view of precise definition of atomic positions. The limitation is due to the data collected and to the large size of the molecule and also to disorder in the crystal, particularly in relation to the water molecules and amide groups present. As a consequence, the standard deviations in atomic positions, and therefore for the bond lengths, are quite large. Various bond-length statistics are summarized in Table 2. Standard deviations for bond lengths vary from 0·017, towards the centre of the molecule, to 0·145 Å in its periphery. The structural analysis accordingly gives reliable evidence on the general stereochemical form of the molecule and of its surroundings in the crystal rather than precise values of bond lengths and bond angles. Final bond lengths and bond angles are illustrated in Figures 4 and 5.

TABLE 2
Bond length statistics

Bond type		No. of bonds	Mean distance/Å	Mean σ/ Å	Max. σ/ Å
Co–N	Rings A, D	2	1·875	0·018	0·019
Co–N	Rings B, C	2	1·934	0·017	0·017
$N-C_{sp^2}$	Inner	6	1·342	0·029	0·031
$C_{sp^2}-C_{sp^2}$	corrin ring	6	1·393	0·034	0·036
$C_{sp^2}-C_{sp^3}$	Corrin	8	1·508	0·032	0·040
$C_{sp^3}-C_{sp^3}$	nucleus	20	1·558	0·039	0·056
$C_{sp^3}-C_{sp^3}$	Side-chains	30 *	1·501	0·046	0·056
		32	1·505	0·048	0·092
C=O	Amide	6	1·210	0·067	0·095
$C-NH_2$	groups	6	1·323	0·092	0·145
$C_{sp^2}-C_{sp^2}$	Benzene	6	1·411	0·035	0·038
C–O	Ribose	6	1·431	0·055	0·095

* Excluding C(48)–C(49) and C(49)–C(50).

RESULTS AND DISCUSSION

The principal structural differences in neovitamin B_{12} compared to vitamin B_{12} are found to be a change

---

* Observed and calculated structure factors are listed in Supplementary Publication No. SUP 20,302 (16 pp., 1 microfiche). For details of Supplementary Publications see *J. Chem. Soc.* (*A*), 1970, Issue No. 20.

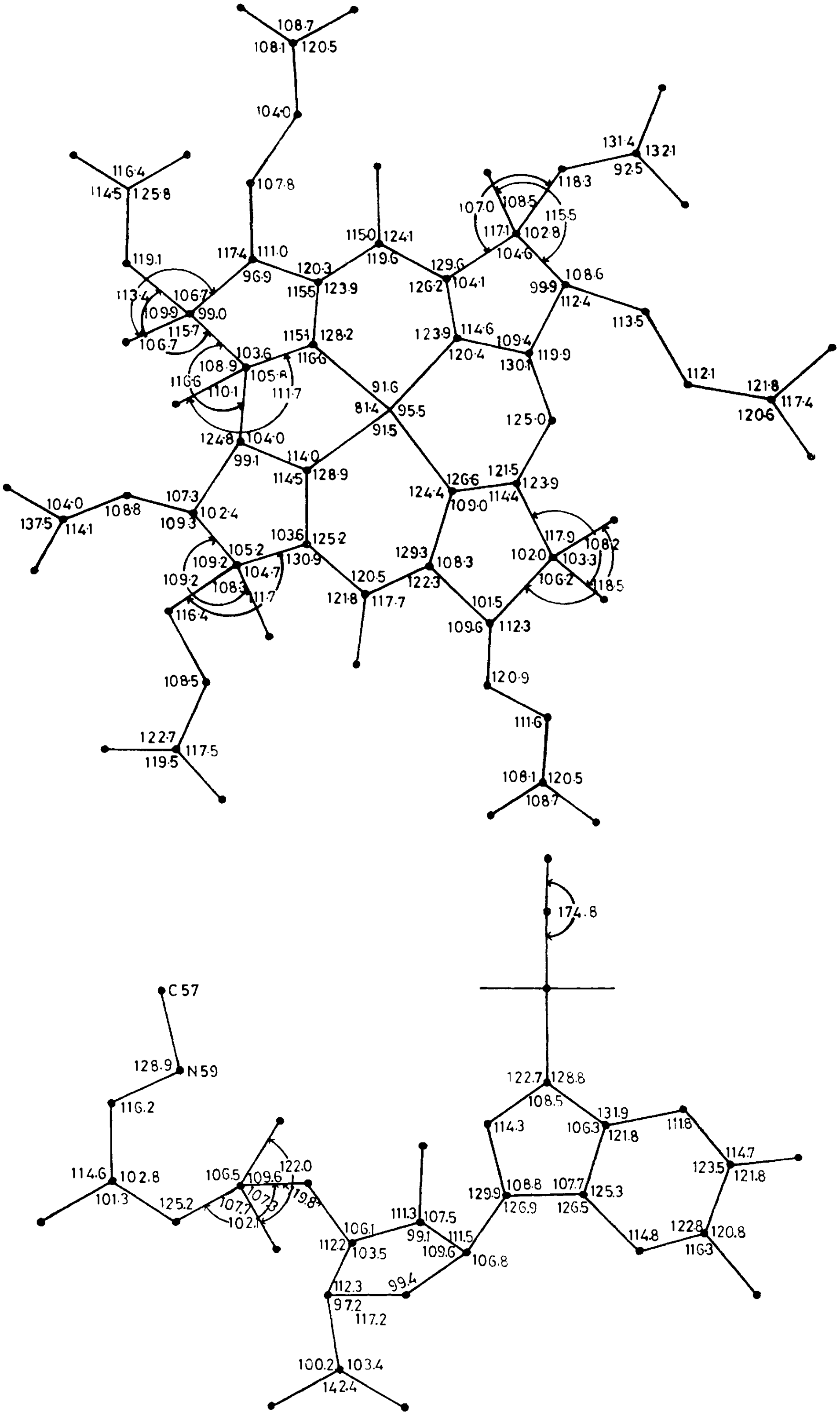


FIGURE 5 Final bond angles

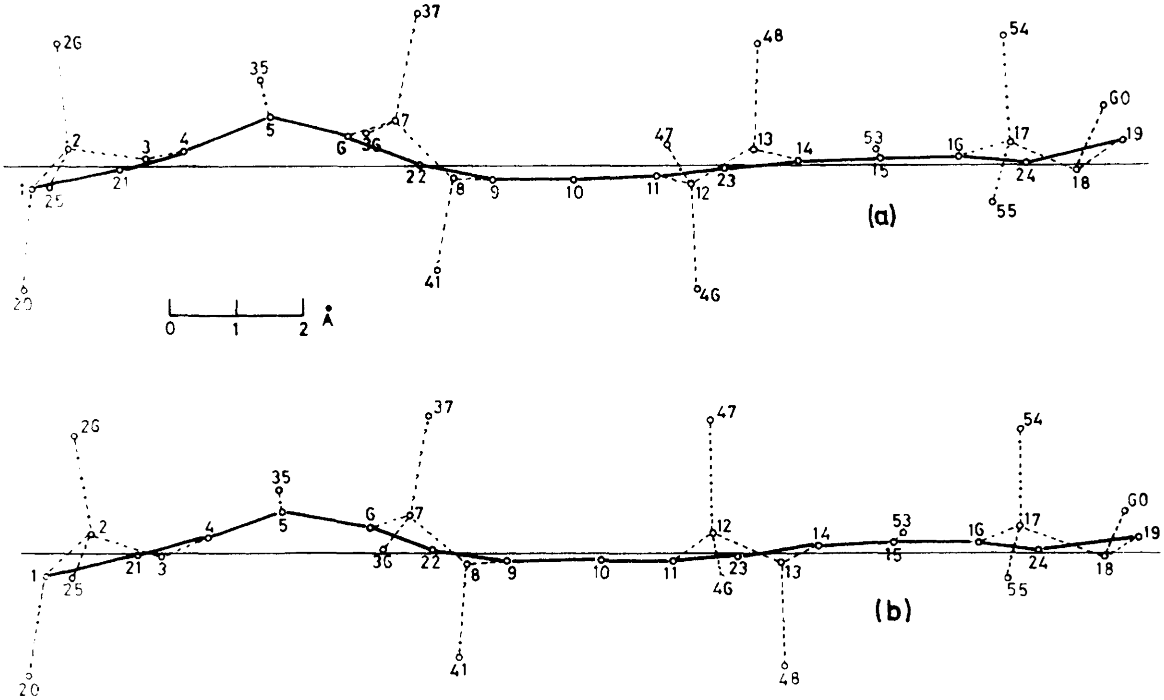


FIGURE 6 A cylindrical projection: the molecules are shown as they would be seen when viewed from the central metal atom outwards. The atoms are projected on a cylinder of radius 2·8 Å. Vertical displacement of each atom corresponds to the distance from the least-squares plane through the four nitrogen atoms (21)—(24); (a) neovitamin B_{12}, (b) vitamin B_{12}

in configuration at C(13) and a change in conformation in the tilt of the ββ′ bond, C(12)–C(13).

The propionamide side-chain attached to C(13) now rises vertically at right angles to the plane of the corrin nucleus in the same direction as the cyanide group attached to the cobalt atom. In B_{12}, this chain is also directed perpendicularly to the corrin nucleus but in the opposite direction. It seems likely that here the conformation adopted at the ββ′ bond is controlled by the preferred packing of the long propionamide chain. So far, such a change in conformation found in neovitamin B_{12} has only been observed among synthetic corrins. For example, in the synthetic pentamethylnickel corrin,[9] the pyrroline rings seem quite flexible in solution while in the crystal determination the tilt of the ββ′ bond of ring B was found to be different from that expected.

The differences in vitamin B_{12} and neovitamin B_{12} are illustrated in Figure 6 which shows a cylindrical projection of the corrin rings as seen from the central metal atom outwards. Apart from the single propionamide chain at C(13) the position of the atoms in the neo-B_{12} molecule and many of the water molecules surrounding it are close to those found in the B_{12} crystal structures, rather nearer to those in air-dry than wet B_{12}.

Cobalt Atom Co-ordination.—The cobalt atom co-ordination, a distorted octahedron, is similar to that found other cobalamins. The cobalt atom lies in the mean plane of the four inner nitrogen atoms. The cobalt–nitrogen bonds involving rings A and D are shorter by 0·06 Å than those involving rings B and C. The angle N(21)–Co–N(24) is 81°, that opposite to it 96°, and the remaining angles are close to 90°.

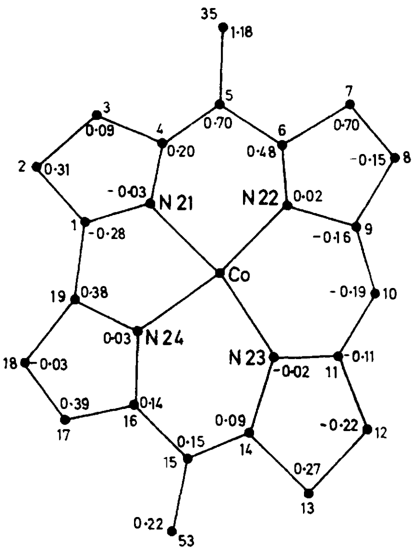


FIGURE 7 Distances of the corrin atoms from the least-squares plane through the four inner nitrogens

Corrin Nucleus.—Figure 7, shows the distances of the atoms from the least-squares plane passing through the four inner nitrogen atoms of the nucleus. The inner ring of atoms is nearly planar; the deviations from planarity being similar to those observed in other

---

[9] J. D. Dunitz and E. F. Meyer, *Helv. Chim. Acta*, 1971, **54**, 77.

cobalamins. As might be expected, the inner pattern of interatomic distances observed is rather closer to those found in the best-defined corrins than are the corresponding distances observed in wet and air-dry B_{12}. The conformation of the four pyrroline rings (Table 3 and Figure 6), clearly shows the puckering of the ββ′ bonds in the outer ring of the corrin nucleus.

TABLE 3

Least-squares planes of pyrrolene rings: deviations (Å) of atoms from the planes

Ring A: N(21) 0, C(1) 0, C(4) 0, C(2) 0·49, C(3) −0·08
Ring B: N(22) 0, C(6) 0, C(9) 0, C(7) 0·11, C(8) −0·34
Ring C: N(23) 0, C(11) 0, C(14) 0, C(12) −0·15, C(13) 0·20
Ring D: N(24) 0, C(16) 0, C(16) 0, C(17) −0·25, C(18) −0·77

TABLE 4

Least-squares planes; deviations (Å) of atoms from the planes

Benzimidazole: N(B1) −0·05, C(B2) −0·02, N(B3) 0·04, C(B4) −0·02, C(B5) −0·02, C(B6) 0·00, C(B7) 0·02, C(B8) 0·05, C(B9) 0·00, C(B10) 0·01 *, C(B11) 0·13 *, C(R1) −0·29 *

Ribose: (*a*) Plane (I): C(R1) −0·06, C(R2) 0·04, C(R3) −0·53 *, C(R4) −0·04, O(R6) 0·06, C(R5) −0·97 *, O(R7) 1·47 *.
(*b*) Plane (II): C(R1) 0·05, C(R2) 0·54 *, C(R3) −0·06, C(R4) 0·09, O(R6) −0·08, C(R5) −1·07 *, O(R7) 2·02 *

* Not included in the calculation.

Nucleotide, Phosphate, and Propanolamine Groups.—The distances and angles in the nucleotide, phosphate amd propanolamine groups (Figures 4 and 5) show little differences from those in B_{12} itself.[11,12] The pronounced bend in the α-glysoside C(R1)–N(B1) bond familiar in other cobalamins is again present, the dihedral angle between the best plane through the ribose and the best plane through the benzimidazole being 83°. As noted in 5′-phosphate B_{12}[7] the methyl group B(11) is significantly out of the plane of the benzimidazole ring (Table 4). Table 4 includes data on the best least-squares planes through atoms in the ribose ring.

TABLE 5

Terminal amide atom identification

Atom number	Density in map 2c/e Å^{-3}	*B*	C–(N, O) distance/Å	Intermolecular contacts	Distance/Å	Suggested identity	B_{12} identity
28	3·8	11·7	1·31	N(44)	2·96	O	N
29	4·4	12·9	1·39	W(A)	2·53	N	O
33	1·2	12·9	1·30	O(P4)	2·93	N	O
				W(10)	2·81		
34	2·9	16·8	1·25	W(12)	2·68	O	N
				W(H)	2·96		
39	3·7	15·4	1·20	N(65)	3·14	O	O
40	3·1	28·3	1·42	W(5)	3·19	N	N
				W(E)	2·89		
44	3·3	8·8	1·31	O(28)	2·96	N	O
				W(G)	3·17		
45	5·1	12·9	1·23	W(1)	3·03	O	N
				W(B)	3·04		
51	2·8	16·6	1·05	W(C)	3·08	O	O
				W(H)	2·96		
52	1·4	38·2	1·25	W(B)	2·21	N	N
62	3·9	10·9	1·27	W(D)	2·98	N	?
				W(12)	2·84		
63	3·7	16·9	1·23	W(5)	2·64	O	N
				W(F)	3·04		

Amide Side-chains.—Table 5 summarizes data on the identification of nitrogen and oxygen atoms of the terminal amide groups. High-temperature parameters were obtained for the majority of the terminal groups particularly those attached to C(7) and C(13). On terminating refinement a difference map was calculated based on phases generated by all the atomic positions except those atoms in the C(7) and C(13) side-chains and the water molecules associated with them. Resolution was poor and there were large areas of density spreading out towards O(51) and N(40). In the case of O(51) this area was intense enough to be a solvent water molecule as previously derived but this was not the case for the region of density near N(40). It was concluded that there was disorder in these regions of the crystal structure and further refinement was not attempted.

Crystal Packing and Molecular Contacts.—The molecules appear to pack in a manner very similar to that of air-dry B_{12}. There is a small lateral displacement of the origin in the *c* axis direction of neo-B_{12} as compared to air-dry and wet B_{12}. In the *a* axis projection, the molecular layers appear to overlap more closely in neo-B_{12}. This is clearly shown in Figure 8, where the benzimidazole groups of molecules in two layers are seen to overlap more closely in neo-B_{12} than in wet B_{12}.

The various molecular contacts between different layers of molecules and within a single layer of molecules are very similar to those in air-dry B_{12}. The epimerization at C(13) is the main cause of any differences. For example, the absence of the O(52) to N(62) contact in neo-B_{12} where N(62) is now hydrogen-bonded to atom (33) by water 12, as in wet B_{12}. O(58) is no longer bridged to N(52) but to O(R8·1) by water (4).

---

[10] S. W. Hawkinson, C. L. Coulter, and M. L. Graves, *Proc. Roy. Soc.*, 1970, *A*, **318**, 143.

[11] C. B. Shoemaker, D. W. J. Cruickshank, D. C. Hodgkin, M. J. Kamper, and D. C. Pilling, *Proc. Roy. Soc.*, 1964, *A*, **278**, 1.

[12] D. C. Hodgkin, J. Lindsay, R. A. Sparks, K. N. Trueblood, and J. G. White, *Proc. Roy. Soc.*, 1962, *A*, **266**, 494.

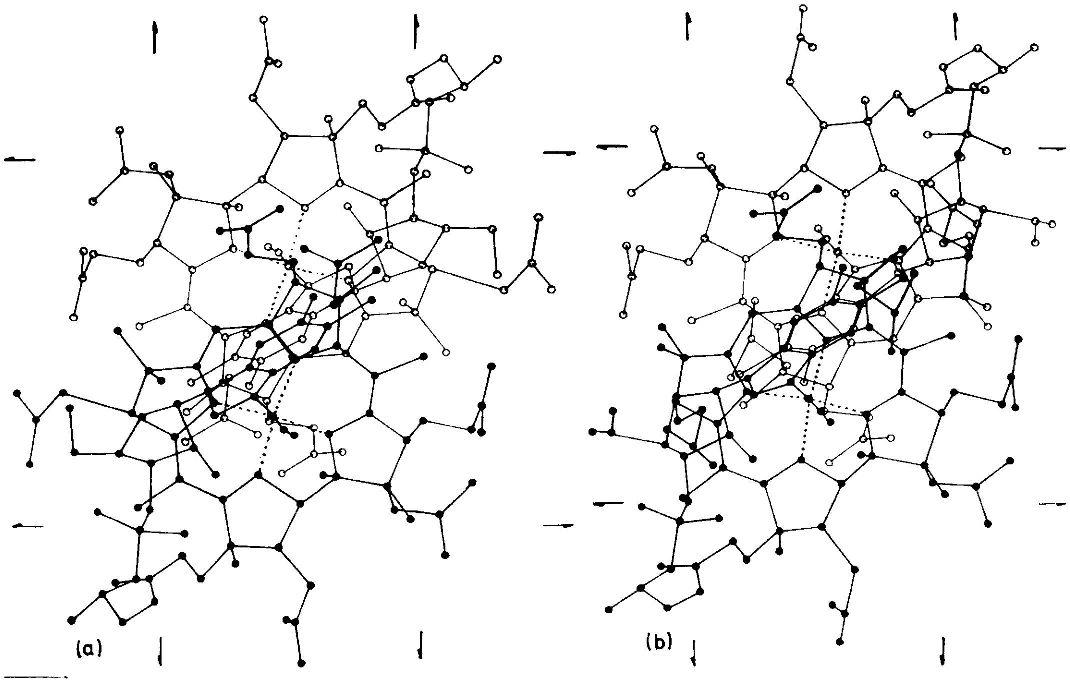


FIGURE 8 Projection of two screw-related molecules along the *a* axis: (a) in wet B_{12}, and (b) in neo-B_{12}

Epimerization at C(13) has caused the displacement of water (6) and O(51) is now bridged to N(33) of a screw-related molecule by water W(H). N(29) and O(39) are involved in an intermolecular bridge by a diffuse water molecule, W(J). The disordered water, W(L), is in contact with O(45). Our identification of the atoms in the terminal amide groups differs from that in air-dry B_{12} (Table 5), since atom (45) is identified as a nitrogen in air-dry B_{12}.

The familiar hydrogen-bonded chain of water molecules W(3) and W(4) link the phosphate groups of screw-related molecules and as in air dry B_{12} waters (2)—(5) and (10) are all associated with the phosphate groups. Waters (12) and (15) occupy similar positions as in air-dry B_{12} while waters (A)—(J) are close but do not exactly correspond to water positions in B_{12}. Figure 9, a projection of the structure along the *c* axis, may be compared with Figure 12 of ref. 13 and Figure 5 of ref. 14.

Inter- and intra-molecular contacts are included in Table 6 together with water–molecule and water–water contacts. Of the 17 solvent water molecules found, at least ten are convincing not only in their appearance in the electron-density difference syntheses but also by the behaviour of their isotropic temperature parameters during refinement and finally by their molecular contacts. The remaining seven water molecules are more clearly affected by disorder. Although density measurements suggested the presence of at least twenty water molecules only a possible seventeen were located during the structural analysis. A difference synthesis calculated after terminating refinement suggested no further convincing solvent positions.

TABLE 6

Possible hydrogen bonds

(*a*) Intermolecular distances/Å		(*b*) Intramolecular distances/Å	
O(39) · · · N(65)	3·14	O(28) · · · N(44)	2·96
O(P3) · · · N(59)	2·76	O(P4) · · · N(33)	2·93
O(P2) · · · N(59)	3·15		
(*c*) Molecule–water distances/Å			
N(65) · · · W(15)	2·97	N(44) · · · W(G)	3·17
N(29) · · · W(A)	2·53	O(45) · · · W(1)	3·03
N(59) · · · W(10)	2·98	O(45) · · · W(B)	3·04
O(58) · · · W(2)	2·68	O(34) · · · W(12)	2·68
O(P4) · · · W(3)	2·48	O(34) · · · W(H)	2·95
O(P5) · · · W(4)	2·63	N(33) · · · W(10)	2·81
O(P5) · · · W(5)	2·69	N(62) · · · W(D)	2·98
O(R7) · · · W(4)	2·76	N(62) · · · W(12)	2·84
O(R8) · · · W(10)	3·18	O(63) · · · W(5)	2·64
O(R8) · · · W(E)	2·97	O(63) · · · W(F)	3·04
O(R8·1) · · · W(2)	2·99	O(51) · · · W(C)	3·08
N(40) · · · W(5)	3·19	O(51) · · · W(H)	2·96
N(40) · · · W(E)	2·89	N(52) · · · W(B)	2·21
(*d*) Water–water distances/Å			
W(3) · · · W(4)	2·78	W(C) · · · W(12)	2·69
W(3) · · · W(2)	2·89	W(C) · · · W(15)	2·75
W(4) · · · W(10)	2·80	W(D) · · · W(F)	3·00
W(10) · · · W(F)	3·09	W(E) · · · W(F)	2·46
W(5) · · · W(E)	3·04	W(F) · · · W(H)	2·69
W(2) · · · W(A)	2·75	W(G) · · · W(J)	2·82
W(A) · · · W(D)	3·16	W(15) · · · W(B)	2·63

Biological Activity.—The biological activity of neo-B_{12} in an *E. coli* mutant system has been shown [1,3] to be approximately one seventh that of B_{12} itself. If, as is likely, the vitamin is acting through the coenzyme form, complete loss of activity might be expected. In the

---

[13] D. C. Hodgkin, J. Kamper, J. Lindsey, M. Mackay, J. Pickworth, J. H. Robertson, C. B. Shoemaker, J. G. White, R. J. Prosen, and K. N. Trueblood, *Proc. Roy. Soc.*, 1957, *A*, **242**, 228.

[14] D. C. Hodgkin, J. Pickworth, J. H. Robertson, R. J. Prosen, R. A. Sparks, and K. N. Trueblood, *Proc. Roy. Soc.*, 1959, **251**, 306.

coenzyme[15] 5′-deoxyadenosine is attached at the site occupied by the cyanide group in B_{12} and lies directly above C(13), a position it could not occupy in neo-B_{12}. The appearance of biological activity might be due either to some transformation of neo-B_{12} back into B_{12} or to action through some other active modifications. It would, for example, still be sterically possible to obtain a methyl neocobalamin which might be biologically active.

Hexacarboxylic Acid.—One interesting suggestion that follows from the structure assigned to neo-B_{12} is that almost certainly the hexacarboxylic acid degradation product of B_{12}, studied much earlier,[13,14] was a mixture of epimers. The side-chain at C(13) in the normal B_{12} position was the most difficult part of the molecule to define in the original electron-density maps. The maps also showed confused density at the position of a possible epimeric chain which would pass from C(13) into the region originally assigned to acetone of crystallization. Since hydrochloric acid was used at one stage in the preparation of the hexacarboxylic acid one would expect some epimer to be formed. There was originally evidence that the hexacarboxylic acid preparation as a whole was inhomogeneous and it now seems likely that the two epimeric molecules were actually accommodated within the single crystal studied by X-ray diffraction. This idea is being tested by a further attempt to refine the hexacarboxylic acid crystal structure.

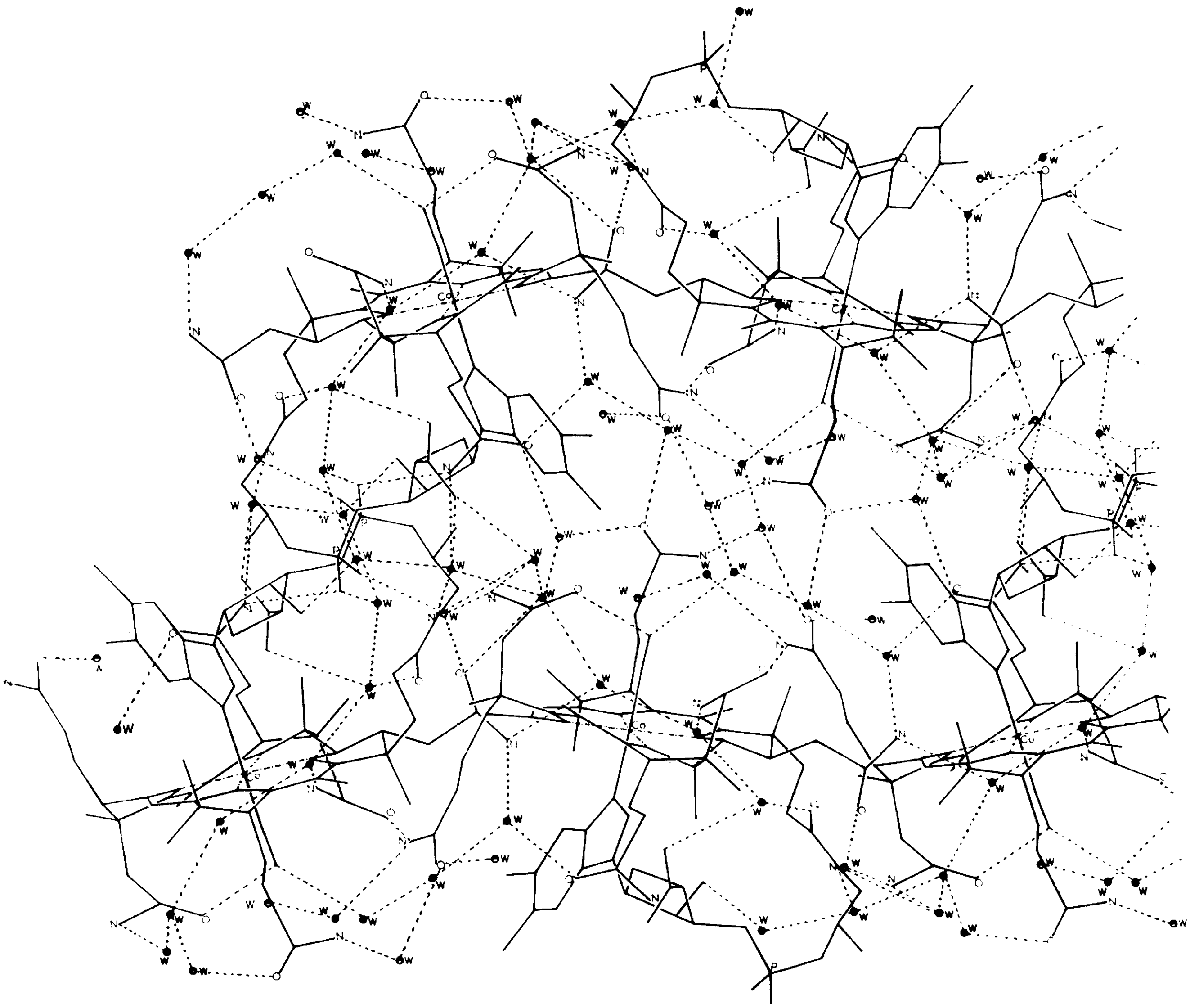


FIGURE 9 Projection of the crystal structure along the c axis; dashed lines are proposed hydrogen bonds

We thank the S.R.C. (for H. S. E.) and the Royal Society (for E. E.) for financial support, and Dr. R. Bonnett for supplying the crystals used.

[1/1900 *Received*, 15*th October*, 1971]

---

[15] P. G. Lenhert, *Proc. Roy. Soc.*, 1968, *A*, **303**, 45.