Crystal Structure of *N*-(5-*O*-Phosphopyridoxyl)-L-tyrosine Heptahydrate

By **Alessandro Mangia, Mario Nardelli,*** and **Giancarlo Pelizzi,** Istituto di Chimica Generale ed Inorganica, Centro di Studio per la Strutturistica Diffrattometrica del C.N.R., Parma, Italy
Carla Borri Voltattorni and **Aldo Orlacchio,** Istituto di Biochimica, Facoltà di Farmacia, Università di Perugia, Perugia, Italy
Carlo Turano, Istituto di Biochimica, Facoltà di Farmacia, Università di Roma, Roma, Italy

The structure of the title compound has been solved from diffractometer data by direct methods and refined by least-squares techniques to *R* 0·082 for 1734 observed reflexions. Crystals are monoclinic, space group $P2_1$, with unit-cell dimensions a = 10·575(5), b = 8·985(5), c = 14·930(7) Å, β = 115·9(2)°. The two aromatic rings are nearly parallel, the dihedral angle between their mean planes being 178·4°. A strong intermolecular hydrogen bond [O(5) ··· O(6^{I}) 2·52 Å] joins two molecules related by a 2_1 axis. The packing is mainly built from a three-dimensional network of hydrogen bonds, involving in particular the seven water molecules.

A POWERFUL tool for the understanding of the enzymic catalysis consists in the study of the interaction of enzymes with compounds which are analogues of the intermediates formed in the catalytic process, or even analogues of the postulated transition states.[1]

A promising series of compounds of this sort is constituted by the pyridoxyl-amino-acids, which are the reduction products of the Schiff bases formed between pyridoxal 5-phosphate and amino-acids; they are analogues of coenzyme–substrate complexes, which originate in the reactions catalysed by pyridoxal 5-phosphate-dependent enzymes.[2-4] In fact the pyridoxyl-amino-acids are known to bind with a great affinity to the active centre of many enzymes of this class.[2-6] Particular attention has been recently given to the interaction of *O*-phosphopyridoxyl-L-tyrosine with tyrosine aminotransferase from rat liver and tyrosine decarboxylase from *Streptococcus faecalis*.[5-7] Such a comparison of the binding of the analogues to enzymes provided with different reaction specificity may disclose structural differences at the enzymic active centres correlated with differences in the catalytic action. Moreover, a comparison of the binding of *O*-phosphopyridoxyl-L-tyrosine and its derivatives lacking one or another of the functional groups may provide information on the energetic contributions of the different parts of the analogue molecule. In order to gather this information, however, a knowledge of the three-dimensional conformation of the analogues is needed. In fact, differences in the binding of various analogues to the enzymes may derive not only from structural factors, but also from different preferential conformations. For phosphopyridoxyltyrosine the relative position of the pyridine ring of the coenzyme, the carboxy-group, and the phenolic ring of the substrate appears particularly important. According to Dunathan[8] the relative position of the pyridine ring and the carboxy-group have a direct bearing on the problem of the reaction specificity of the enzymes dependent on pyridoxal 5-phosphate.

While no method can by itself give a detailed description of the conformation in solution of small molecules, a combination of different methods may allow a reasonably safe conclusion to be reached. Raso and Stollar[9] have recently used n.m.r., c.d., and fluorescence to investigate the conformation of *N*-(5-*O*-phosphopyridoxyl)-3′-amino-L-tyrosine in solution.

We now report the crystal structure of *N*-(5-*O*-phosphopyridoxyl)-L-tyrosine, and confirm the general structural features suggested by Raso and Stollar. These results have been applied in the investigation of the constitution of the active site of tyrosine aminotransferase from rat liver.[7]

EXPERIMENTAL

Preparation.—The compound was prepared following ref. 10 and crystallized from water and absolute ethanol as rectangularly shaped crystals, elongated along [010].

Crystal Data.—$C_{17}H_{21}N_2O_8P,7H_2O$, M = 538·5, Monoclinic, a = 10·575(5), b = 8·985(5), c = 14·930(7) Å, β = 115·9(2)°, U = 1275·8 Å^3, Z = 2, D_c = 1·40, $F(000)$ = 572. Cu-K_α radiation, λ = 1·5418 Å; μ(Cu-K_α) = 16·2 cm^{-1}. Space group $P2_1$.

Unit-cell dimensions were determined from a least-squares fit of the values of eight $(\theta,\chi,\phi)_{hkl}$ measurements taken on an automated single-crystal Siemens AED diffractometer.

Intensity Data Collection.—Intensity data were measured by single-crystal diffractometry, and the intensities of 1970 independent reflexions were recorded by use of the ω—2θ scanning technique with Ni-filtered Cu radiation to 2θ(max.) 120°; only 236 reflexions, having $I < 2\sigma(I)$, were considered unobserved and excluded from the analysis.

There was some evidence for decomposition of the sample during *X*-ray exposure, shown by the decreasing intensity of a standard reflexion monitored every 20 reflexions and by the slow yellowing of the crystal. A correction for this crystal decay was applied by means of a computer program taking the intensity of the standard

---

[1] R. Wolfenden, *Nature*, 1969, **223**, 704.

[2] W. B. Dempsey and E. E. Snell, *Biochemistry*, 1963, **2**, 1414.

[3] E. S. Severin, N. N. Gulyaev, E. N. Khurs, and R. M. Khomutov, *Biochem. Biophys. Res. Comm.*, 1969, **35**, 318.

[4] C. Turano, C. Borri Voltattorni, A. Orlacchio, and F. Bossa, FEBS Symposium, 1970, **18**, 123.

[5] C. Turano, C. Borri Voltattorni, A. Orlacchio, and A. Giartosio, *Proc. 1st European Biophys. Congr.*, 1971, **1**, 45.

[6] J. H. Fellman and E. S. Roth, *Biochemistry*, 1971, **10**, 408.

[7] C. Borri Voltattorni, A. Orlacchio, A. Giartosio, F. Conti, and C. Turano, in preparation.

[8] H. C. Dunathan, *Proc. Nat. Acad. Sci. U.S.A.*, 1969, **55**, 712.

[9] V. Raso and B. D. Stollar, *J. Amer. Chem. Soc.*, 1973, **95**, 1621.

[10] M. Ikawa, *Arch. Biochem. Biophys.*, 1967, **118**, 497.

TABLE 1

Final atomic fractional co-ordinates (× 10^4) and thermal parameters (Å^2),* with estimated standard deviations, for non-hydrogen atoms

	x/a	y/b	z/c	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
P	8425(2)	4184(—)	5777(1)	2·64(6)	2·93(8)	3·96(7)	−0·01(6)	1·55(5)	−0·69(6)
O(1)	9860(5)	3699(7)	5953(4)	3·2(2)	4·6(3)	6·1(3)	0·0(2)	2·1(2)	−1·2(2)
O(2)	7636(6)	3003(7)	6133(5)	5·2(3)	3·5(3)	6·4(3)	−0·1(2)	3·5(2)	0·4(2)
O(3)	8324(5)	5651(6)	6228(4)	4·3(2)	3·8(3)	4·7(2)	0·1(2)	2·4(2)	−1·5(2)
O(4)	7566(5)	4264(7)	4619(4)	3·8(2)	3·8(2)	5·0(2)	−0·3(2)	2·8(2)	−0·9(2)
O(5)	4220(4)	2108(6)	1012(3)	2·9(2)	3·5(2)	3·3(2)	−0·5(2)	1·7(2)	−0·8(2)
O(6)	7574(5)	5950(6)	569(4)	4·1(2)	2·3(2)	4·3(2)	−0·1(2)	1·9(2)	1·0(2)
O(7)	6141(5)	3981(6)	83(3)	3·7(2)	2·7(2)	3·5(2)	−0·2(2)	1·3(2)	0·3(2)
O(8)	13149(5)	6082(7)	813(4)	3·5(2)	4·8(3)	5·2(2)	−1·0(2)	2·5(2)	0·6(2)
O(9)	6542(5)	270(6)	816(4)	3·4(2)	2·9(2)	5·4(2)	−0·2(2)	2·3(2)	−0·1(2)
O(10)	742(10)	3410(10)	4336(8)	11·6(6)	4·2(4)	12·8(6)	0·4(4)	8·6(5)	0·5(4)
O(11)	5442(7)	7832(11)	2305(5)	6·0(3)	8·7(5)	6·6(3)	−1·0(3)	3·4(3)	−1·8(3)
O(12)	8192(8)	1078(10)	3543(5)	9·7(4)	7·2(4)	7·0(4)	4·4(4)	5·6(4)	3·9(3)
O(13)	7266(7)	478(8)	5089(5)	6·8(3)	3·6(3)	7·4(3)	−0·4(3)	3·6(3)	−0·4(3)
O(14)	5023(7)	8725(8)	3878(5)	6·8(3)	5·1(3)	6·5(3)	0·0(3)	3·6(3)	−0·1(3)
O(15)	8044(8)	8026(10)	2084(6)	6·8(4)	5·3(4)	7·7(4)	1·5(3)	1·1(3)	−1·7(3)
N(1)	3344(5)	1808(6)	3060(4)	2·9(2)	2·4(2)	3·0(2)	0·1(2)	1·3(2)	0·3(2)
N(2)	7052(5)	3049(6)	1980(4)	2·5(2)	2·1(2)	3·7(2)	−0·3(2)	2·0(2)	0·1(2)
C(1)	6108(7)	4758(9)	4170(5)	3·0(3)	3·7(3)	3·3(3)	−0·4(2)	1·5(2)	−1·0(2)
C(2)	5165(6)	3583(7)	3464(4)	2·3(2)	2·1(3)	2·8(2)	−0·2(2)	1·1(2)	−0·1(2)
C(3)	4217(7)	2816(8)	3713(5)	3·0(3)	3·4(3)	3·0(3)	0·3(2)	1·5(2)	0·3(2)
C(4)	3302(6)	1492(7)	2174(5)	2·5(2)	2·1(3)	3·6(3)	0·6(2)	1·4(2)	0·1(2)
C(5)	2300(7)	311(8)	1563(5)	3·2(3)	2·6(3)	4·5(3)	−0·7(2)	1·3(2)	−0·8(3)
C(6)	4216(6)	2287(7)	1884(4)	2·6(2)	2·2(3)	2·7(2)	0·3(2)	1·4(2)	−0·2(2)
C(7)	5172(6)	3286(7)	2566(4)	2·1(2)	2·4(3)	3·1(3)	−0·1(2)	1·2(2)	0·2(2)
C(8)	6140(6)	4094(8)	2227(4)	3·1(2)	1·8(3)	3·8(2)	0·3(2)	2·2(2)	0·0(2)
C(9)	8041(6)	3923(8)	1687(4)	2·6(2)	2·3(3)	3·6(2)	−0·3(2)	1·7(2)	0·5(2)
C(10)	7156(6)	4653(7)	702(5)	2·2(2)	2·4(3)	3·6(3)	0·5(2)	1·4(2)	0·5(2)
C(11)	9150(6)	2896(8)	1647(5)	2·3(2)	2·6(3)	4·6(3)	0·1(2)	1·7(2)	0·9(2)
C(12)	10203(6)	3741(8)	1403(5)	2·0(2)	2·6(3)	4·6(3)	0·2(2)	1·8(2)	0·5(2)
C(13)	10288(7)	3504(9)	522(5)	2·6(3)	3·0(3)	4·5(3)	−0·4(2)	1·8(2)	−0·3(2)
C(14)	11275(7)	4291(9)	304(5)	3·0(3)	3·4(3)	4·0(3)	0·4(2)	1·7(2)	0·1(3)
C(15)	12145(6)	5315(8)	985(5)	2·3(2)	3·0(3)	4·5(3)	−0·3(2)	1·6(2)	0·3(2)
C(16)	12074(7)	5562(9)	1865(5)	3·1(3)	3·2(3)	4·6(3)	−0·4(2)	1·8(2)	0·0(3)
C(17)	11105(7)	4791(9)	2074(6)	3·1(3)	3·3(4)	5·0(4)	−0·2(2)	1·7(3)	0·0(3)

* Anisotropic thermal parameters are in the form: $\exp[-\frac{1}{4}(B_{11}h^2a^{*2} + B_{22}k^2b^{*2} + B_{33}l^2c^{*2} + 2B_{12}hka^*b^* + 2B_{13}hla^*c^* + 2B_{23}klb^*c^*)]$.

reflexion as internal scaling for the data set. After corrections for Lorentz and polarization effects, the structure amplitudes were put on absolute scale by comparison with the calculated values. Absorption corrections were deemed unnecessary in view of the low absorbance of the sample.

Structure Determination and Refinement.—All attempts to determine the co-ordinates of phosphorus by Patterson methods failed owing to the high overlapping of the peaks. The structure was therefore solved by means of direct methods, using the MULTAN program.[11] The 444 reflexions with $|E| \geqslant 1·10$ and the most significant 2000 phase relationships were considered. The starting reflexions used in connection with the weighted tangent formula, given automatically by the CONVERGE section of the program, were the three origin-determining reflexions: $10,0,\bar{1}$, 1,3,0, 1,2,2, and four additional ones: 0,4,4, $9,2,\bar{9}$, 5,6,2, $6,4,\bar{1}$. The enantiomorph was fixed by 1,2,2. Of the 64 phase combinations, given by the FASTAN section of the program, five sets, having the highest absolute figures of merit in the range 1·05—1·07 and residuals in the range 38·3—39·8, all led to a correct solution. An E map, computed by use of the phases obtained by one of these sets, clearly showed the positions of all the non-hydrogen atoms in the organic molecule. The structure analysis was completed by a Fourier synthesis, phased on the atoms previously found, which was valuable to locate seven water molecules, not expected on the basis of the analytical data. The refinement was carried out by

TABLE 2

Final atomic fractional co-ordinates (× 10^3) and isotropic thermal parameters (Å^2), with estimated standard deviations, for hydrogen atoms

	x/a	y/b	z/c	B
H(1)	719(15)	264(24)	577(11)	13(5)
H(2)	582(11)	522(18)	358(8)	9(3)
H(3)	594(9)	490(13)	467(6)	6(2)
H(4)	429(7)	278(9)	435(5)	3(1)
H(5)	365(10)	211(15)	58(8)	7(3)
H(6)	563(9)	450(12)	167(6)	5(2)
H(7)	677(9)	458(13)	272(6)	6(2)
H(8)	755(9)	286(12)	266(6)	5(2)
H(9)	655(9)	280(13)	133(6)	6(2)
H(10)	853(9)	444(13)	215(6)	6(2)
H(11)	694(10)	612(16)	−26(7)	8(3)
H(12)	888(8)	211(11)	109(6)	4(2)
H(13)	962(9)	238(13)	232(7)	6(2)
H(14)	948(10)	305(16)	−4(8)	8(3)
H(15)	1121(5)	421(8)	−36(4)	2(1)
H(16)	1296(9)	616(14)	248(7)	6(2)
H(17)	1122(8)	483(12)	279(6)	5(2)
H(18)	1302(9)	619(14)	15(6)	6(2)
H[O(9)]	571(8)	13(12)	58(6)	6(2)
H[O(9)]	682(10)	28(15)	155(7)	9(2)
H[O(10)]	74(9)	305(12)	364(6)	6(2)
H[O(10)]	38(9)	412(12)	420(6)	6(2)
H[O(11)]	486(8)	795(12)	159(6)	6(2)
H[O(11)]	614(11)	780(15)	233(8)	8(3)
H[O(12)]	775(10)	58(15)	375(7)	7(2)
H[O(12)]	900(11)	113(16)	425(7)	9(3)
H[O(13)]	759(9)	28(14)	580(6)	7(2)
H[O(13)]	652(9)	20(13)	456(7)	7(2)
H[O(14)]	479(9)	849(13)	310(6)	6(2)
H[O(14)]	452(11)	823(16)	396(8)	9(3)
H[O(15)]	770(10)	845(16)	131(7)	9(3)
H[O(15)]	854(10)	852(14)	274(7)	7(2)

---

[11] G. Germain, P. Main, and M. M. Woolfson, *Acta Cryst.*, 1971, **A27**, 368.

block-diagonal least-squares with anisotropic thermal parameters, minimizing $\Sigma w(\Delta|F|)^2$, down to a value of 0·092 for the conventional R index. In the first stages unit weights, then the scheme $1/w = A + B|F_o| + C|F_o|^2$ were used, with the coefficients A, B, and C obtained by plotting ΔF *vs.* $\bar{F}_o$. Of the 35 hydrogen atoms, 32 were located by a Fourier difference map and refined isotropically. The final R is 0·082. The unlocated hydrogen atoms are those belonging to the methyl group.

Final positional and thermal parameters are given in Tables 1 and 2 together with their standard deviations. Observed and calculated structure factors are listed in Supplementary Publication No. SUP 21121 (9 pp.).* Atomic scattering factors were taken from ref. 12 for non-hydrogen atoms, and from ref. 13 for hydrogen atoms. All calculations were performed on the CDC 6600 computer of the Centro di Calcolo Elettronico Interuniversitario dell'Italia Nord-Orientale.

RESULTS AND DISCUSSION

The structure is shown in Figure 1. The two aromatic rings are both planar and nearly parallel, the dihedral angle between their planes being 178·4°. The substituent atoms of the pyridine ring are displaced from its plane by: C(1) 0·08, C(5) −0·07, C(8) 0, O(5) 0·07 Å; O(8) and C(11) lie almost in the tyrosine plane.

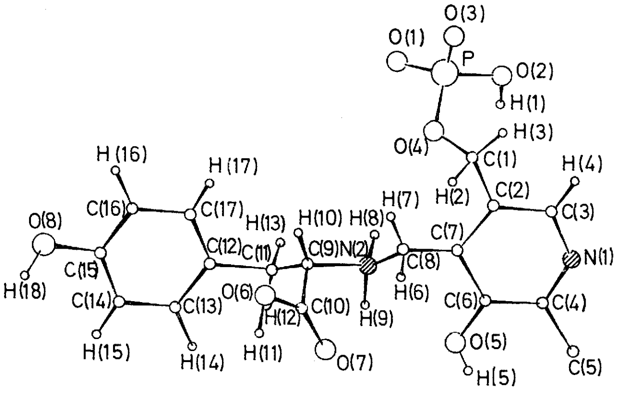

FIGURE 1 Clinographic projection of the molecule showing the atom numbering system used in the analysis

TABLE 3

Bond distances (Å) and angles (°) *

(*a*) Distances			
P–O(1)	1·488	C(8)–N(2)	1·50
P–O(2)	1·579	N(2)–C(9)	1·52
P–O(3)	1·505	C(9)–C(10)	1·51
P–O(4)	1·564	C(10)–O(6)	1·29
O(4)–C(1)	1·46	C(10)–O(7)	1·23
C(1)–C(2)	1·52	C(9)–C(11)	1·51
C(2)–C(3)	1·39	C(11)–C(12)	1·52
C(3)–N(1)	1·36	C(12)–C(13)	1·37
N(1)–C(4)	1·33	C(13)–C(14)	1·41
C(4)–C(5)	1·50	C(14)–C(15)	1·38
C(4)–C(6)	1·41	C(15)–O(8)	1·38
C(6)–O(5)	1·31	C(15)–C(16)	1·37
C(6)–C(7)	1·40	C(16)–C(17)	1·38
C(7)–C(2)	1·37	C(17)–C(12)	1·40
C(7)–C(8)	1·51		
(*b*) Angles			
O(1)–P–O(2)	113·1	C(2)–C(7)–C(8)	122
O(1)–P–O(3)	116·3	C(7)–C(8)–N(2)	112
O(1)–P–O(4)	105·3	C(8)–N(2)–C(9)	110
O(2)–P–O(3)	106·7	N(2)–C(9)–C(10)	107
O(2)–P–O(4)	105·4	N(2)–C(9)–C(11)	110
O(3)–P–O(4)	109·5	C(10)–C(9)–C(11)	113
P–O(4)–C(1)	121	C(9)–C(10)–O(7)	119
O(4)–C(1)–C(2)	110	O(6)–C(10)–O(7)	125
C(1)–C(2)–C(3)	119	C(9)–C(10)–O(6)	116
C(1)–C(2)–C(7)	122	C(9)–C(11)–C(12)	112
C(3)–C(2)–C(7)	118	C(11)–C(12)–C(13)	121
C(2)–C(3)–N(1)	119	C(11)–C(12)–C(17)	120
C(3)–N(1)–C(4)	125	C(13)–C(12)–C(17)	119
N(1)–C(4)–C(5)	118	C(12)–C(13)–C(14)	120
N(1)–C(4)–C(6)	118	C(13)–C(14)–C(15)	119
C(5)–C(4)–C(6)	125	C(14)–C(15)–C(16)	121
C(4)–C(6)–C(7)	118	C(14)–C(15)–O(8)	121
C(4)–C(6)–O(5)	123	C(16)–C(15)–O(8)	118
C(7)–C(6)–O(5)	119	C(15)–C(16)–C(17)	120
C(6)–C(7)–C(2)	122	C(16)–C(17)–C(12)	121
C(6)–C(7)–C(8)	116		

* σ for bond lengths is 0·006—0·011 Å, and for angles 0·7—1·6°.

Table 3 lists bond distances and angles. The two aromatic rings are connected by the chain C(7), C(8), N(2), C(9), C(11), and C(12) in which all distances correspond to single bonds. A usual way of describing the tyrosine moiety is by use of conformational angles, and the nomenclature and conventions used are those of Edsall *et al.*[14] The conformation of the C^{α}–C^{β} bond is described by the torsional angle χ_1, N(2)C(9)C(11)–C(9)C(11)C(12) 182·6°. This angle is usually close to 60, 180, and 300°, corresponding to the three staggered positions of C^{γ}; more precisely χ_1 is close to 60° (*syn*) in tyrosine–metal complexes, to 180° (*anti*) in tyrosine derivatives in the absence of metal ions, and to 300° when there are in the ring other substituents besides the *para* ones. The parameter χ_2, which specifies the torsional angle between the plane of the phenyl ring and the plane C^{α}–C^{β}–C^{γ}, assumes the values: χ_{21} C(11)C(12)C(17)–C(9)C(11)C(12) 66·0° and χ_{22} C(11)C(12)C(13)–C(9)C(11)-C(12) 246·4°, so they are both in the range of values found in a number of thyroid compounds and related amino-acids.[15] The valence angle at C^{β} is a little larger [C(9)–C(11)–C(12) 111·5°] than the tetrahedral, as usually observed in amino-acids. Other bond distances and angles are as expected. The backbone conformation of the amino-acid is described by the parameters ψ_1 and ψ_2 which give the orientation of the carboxy-group with respect to the N–C^{α}–C′ plane. The values observed in the present work [ψ_1 O(6)C(10)C(9)–C(10)-C(9)N(2) 145·4, and ψ_2 O(7)C(10)C(9)–C(10)C(9)N(2) 323·2°] are consistent with those found in tyrosine derivatives.[15,16] The conformation of the junction between pyridoxal and tyrosine moieties is defined by

---

* See Notice to Authors No. 7 in *J.C.S. Perkin II*, 1973, Index issue.

[12] D. T. Cromer and J. B. Mann, *Acta Cryst.*, 1968, **A24**, 321.

[13] R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, 1965, **42**, 3175.

[14] J. T. Edsall, P. J. Flory, J. C. Kendrew, A. M. Liquori, G. Némethy, G. N. Ramachandran, and H. A. Scheraga, *J. Mol. Biol.*, 1966, **15**, 399.

[15] V. Cody, W. L. Duax, and D. A. Norton, *Acta Cryst.*, 1972, **B28**, 2244.

[16] M. Sundaralingam and E. F. Putkey, *Acta Cryst.*, 1970, **B26**, 790.

the torsion angles C(8)–N(2) and N(2)–C(9) (177·6 and 168·4°); *i.e.* the bridging system is nearly planar.

The orientation of the phosphate group is mainly defined by the torsion angle (124°) about the O(4)–C(1) bond. The bond distances in it are not all equal and can be classified in two groups: the longer ones (1·564 and 1·579 Å) which involve oxygen atoms bound to carbon, O(4), or to hydrogen, O(2), and the shorter ones (1·488 and 1·505 Å) which involve O(1) and O(3). This indicates that the double bond is delocalized along these two bonds and the phosphate group, as a whole, bears a negative charge delocalized between O(1) and O(3). The proton is transferred to N(2), which assumes a tetrahedral configuration. The O–P–O angles range from **105·3** to **116·3°**, with the largest value involving unsubstituted oxygen atoms O(1) and O(3).

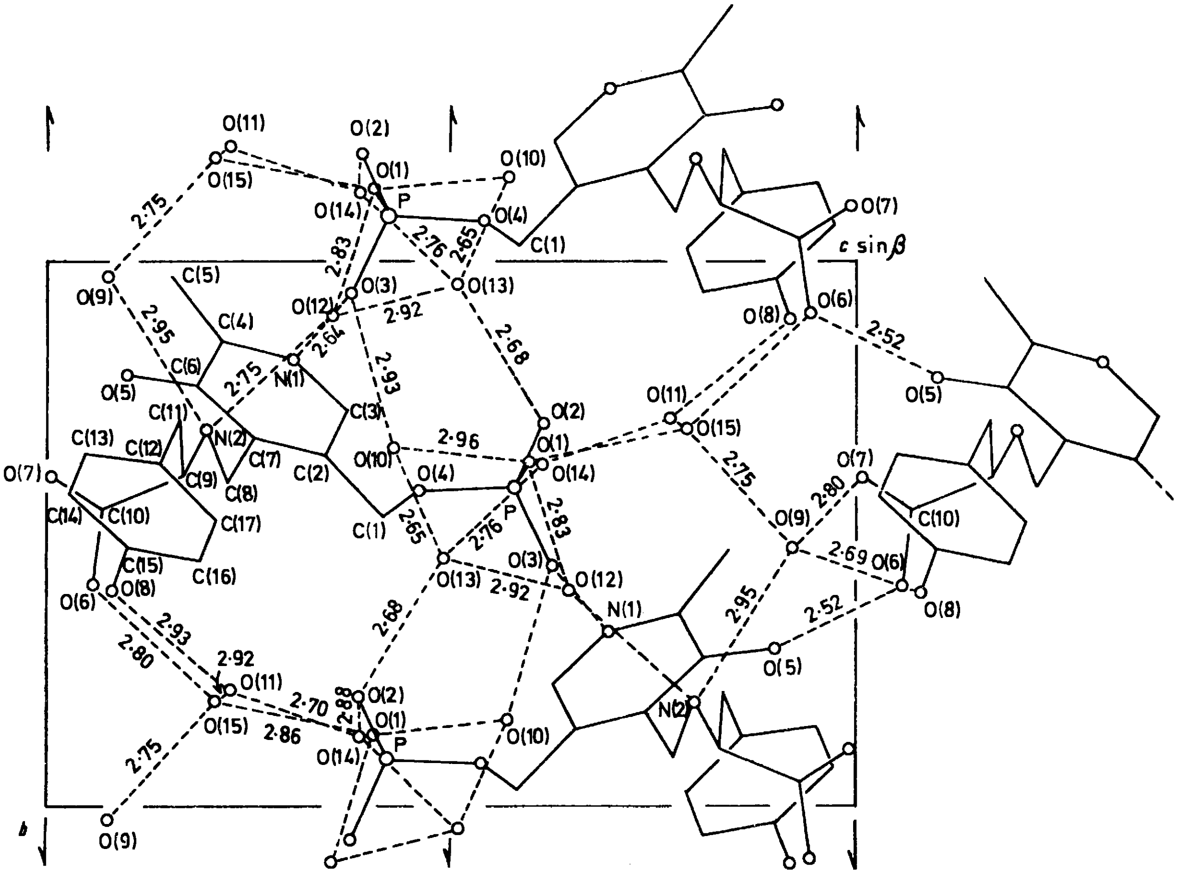

FIGURE 2 Diagrammatic projection of the structure, on a plane perpendicular to [100], showing crystal packing and hydrogen bonding. Hydrogen atoms have been omitted for the sake of clarity

Two organic molecules, related by a 2_1 axis, are linked by a strong intermolecular hydrogen bond O–H · · · O between hydroxy- and carboxy-groups: O(5) · · · O(6^{I}) **2·52** Å. Figure **2** shows that the whole crystal structure is stabilized by an intricate three-dimensional network of hydrogen bonds. These involve especially the seven water molecules located by the *X*-ray analysis, which are thus shown to be essential to the structure.

[4/527 *Received*, 18*th March*, 1974]