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THE CRYSTAL DATA AND CRYSTAL STRUCTURE OF SOME VITAMIN B_6 DERIVATIVES

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During past forty years, many detailed studies of Vitamin B_6(1) have been done in chemical, physical and biological fields. The authers had especially the deep interest in relation between structure and function of pyridoxal phosphate, a physiologically very important substance correlated to the active center of B_6-dependent enzymes.

This communication presents the crystal data of pyridoxal phosphate monohydrate and the crystal and molecular structure of pyridoxal phosphate methyl hemiacetal.

The crystal data of pyridoxal phosphate monohydrate was shown in Table 1(A). The cell dimensions and X-ray diffraction intensities of the yellowish needle-shaped crystals were measured by the Rigaku computer-controlled automatic four circles diffractometer with CuKα radiation, and the density was measured by the flotation method with benzene and ethylene dibromide mixture. Determination of the crystal and molecular structure of this compound is now in progress in this laboratory.

The crystal and molecular structure of pyridoxal phosphate methyl hemiacetal has now been determined. Transparent yellow prism-shaped crystals were used in this analysis. The crystal data determined by precession and Weissenberg photographs and the density by flotation in benzene carbon tetrachloride mixture, are also listed in Table 1(B). Intensity data of 2524 independent reflections were collected on an equi-inclination Weissenberg photographs about the b- and the c-axes, taken at room temperature using CuKα radiations. The intensities were estimated by visual comparison with a standard scale and applied to the Lorentz-polarization and spot-shape corrections. Calculated statistical averages of the normalized structure factors indicated that the crystal had center of symmetry and the space group was decided to be $P\bar{1}$.

The structure was solved by the symbolic addition method by Karle and Karle(2). Σ_2 lists were calculated with program 'SIGMA' written by Tamaichi Ashida on the HITAC 5020 computer of Tokyo University. Number of reflections of normalized structure factors, E, greater than 1.5 was 326 and that greater than 1.0 was 860, which corresponded to 12.8% and 33.8% of the total observed intensities, respectively. At the first stage, starting set for the application of Σ_2 formula was selected as shown in Table 2.

Table 1

	(A)	(B)
a(Å)	9.42 ± 0.01	10.503 ± 0.006
b(Å)	10.61 ± 0.01	13.608 ± 0.009
c(Å)	6.20 ± 0.02	7.488 ± 0.003
α(°)	105.6 ± 0.2	90
β(°)	95.7 ± 0.2	93.71 ± 0.04
γ(°)	99.7 ± 0.2	90
V(Å^3)	580.7	1067.97
space group	$P\bar{1}$	$P2_1/c$
D_m(g.cm^{-3})	1.587	1.635
D_c(g.cm^{-3})	1.595	1.649
Mol.wt.	279.0	265.2
Formula	$C_9H_{14}O_7N\ P$	$C_8H_{12}O_7N\ P$

(A) ; Pyridoxal phosphate methyl hemiacetal

(B) ; Pyridoxal phosphate monohydrate

Table 2

h	k	l	sign	E
6	-10	5	+	2.53
-7	4	2	+	3.17
-1	1	5	+	3.28
0	-6	2	+	3.27
0	-3	1	A	3.04
4	2	5	B	2.17
5	-6	5	C	2.49
7	6	0	D	3.92
6	-2	3	E	3.09

By the application of Σ_1 formula, the probability that the sign be positive for the (0 -6 2) reflection was 87%, and it was also included in the first starting set. The next procedure based on the single interaction pairs with $E \geqq 1.5$ and probability greater than 97% were carried out by hand calculations and many relations between five symbols, A to E, were found at the end of the procedure. Out of total 32 combinations given by assigning plus or minus signs to these symbols, were searched those which give good agreement with sign relations and have nearly equal number of reflections with plus or minus signs. Three-dimensional E-maps were calculated with these normalized structure factors as coeficients and the preliminary coordinates of the eighteen atoms except hydrogens, were obtained. Refinement of the structure were carried out by using the block diagonal least-squares program 'HBLS' by T.Ashida. After five cycles of the refinement employing isotropic temperature factors, the R index was 0.161. Difference Fourier map at this stage revealed the appropriate positions of hydrogen atoms except one attached to phosphate oxygen atom. The following refinement with anisotropic temperature factors which included contributions from hydrogen atoms reduced the R index to 0.115. Fig.1 shows the superimposed electron density projected along the c-axis. Contours are drawn at intervals of approximately 1 e/Å^3, except in the vicinity of the phosphorous atom, where the interval is 5 e/Å^3. Fig.2 shows the bond distances and angles at this stage where the maximum standard deviations are 0.009Å and 0.56°, respectively.

Fig.1

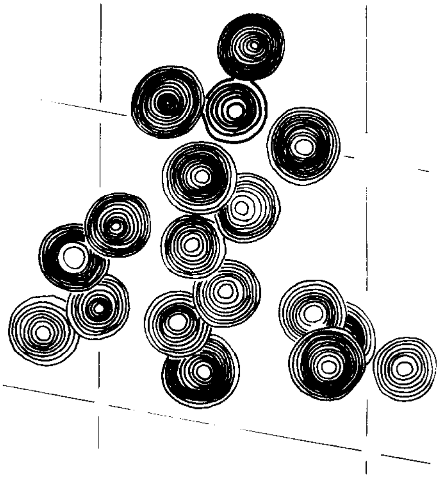
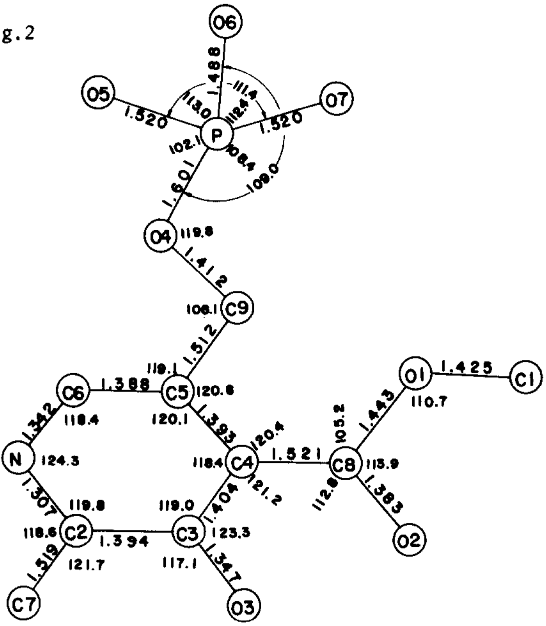


Fig.2



In the pyridine ring, the average C-N bond distance is 1.325Å and C-C is 1.395Å. Three C-C single bond lengths are averaged to 1.517Å but C(3)-O(3) bond length, 1.347Å, is shorter than the average four C-O single bond lengths of 1.416Å and it indicates that the O(3) atom is resonated with pyridine ring system. These shows fairly good agreement with the result of pyridoxine hydrochloride by Hanic(3). It is worthy to note the four P-O bond distances of the phosphate group as proposed by Kraut and Jensen(4), that is, P-O(4)=1.601Å has the single bond character and P-O(6)=1.488Å, double bond character, but the other two P-O(5)=1.520Å and P-O(7)=1.520Å are equal in bond length and both have just intermidiate distance of single and double bond length. Bond angle, C(2)-N-C(6)=124.3° indicates that an extra-annular hydrogen atom attaches to the ring nitrogen as suggested by Singh(5) and this attached hydrogen atom is confirmed by the difference Fourier synthesis.

From the location of hydrogen positions, it is established that pyridoxal phosphate occurs in the zwitterionic form in the crystalline state. Pyridine ring atoms and O(3) atom are coplanar. The view of the structure projected down the c-axis are shown in Fig.3. One intramolecular hydrogen bond of length 2.533Å was found between O(3) and O(2). The crystal structure is mainly stabilized by four kinds of intermolecular hydrogen bond, namely, O(2)-O(6')=2.594Å, N-O(6')=2.691Å, O(5)-O(5')=2.556Å and O(7)-O(7')=2.580Å. The fact that the last two hydrogen bond distances are equal within the standard deviation and two bond lengths, P-O(5) and P-O(7), are equal as above mentioned, seems to restrict the position of one hydrogen to locate at a center of symmetry. Detailed studies will be undertaken about this problem. The normal van der Waals contacts take part in the packing of the molecules. Full details of the structure will be published elsewhere in the near future.

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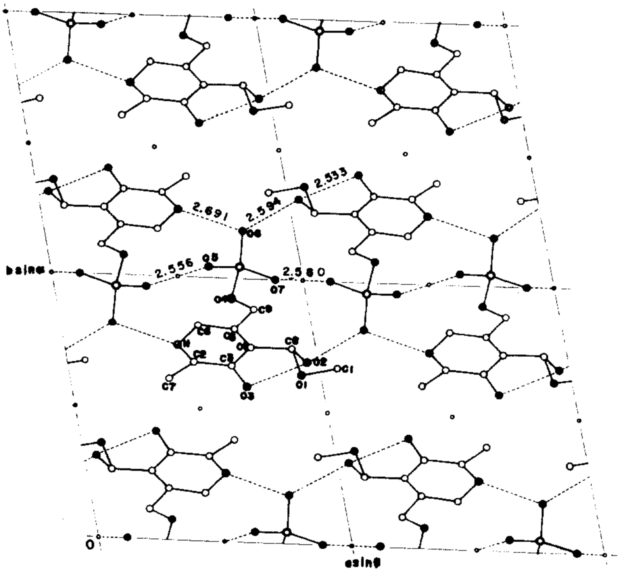
Fig.3



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