BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 46, 863—871 (1973)

The Crystal and Molecular Structure of Vitamin B_6 Derivatives. I. Pyridoxal Phosphate Hydrate and Pyridoxal Phosphate Methyl Hemiacetal

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(Received June 2, 1972)

Pyridoxal phosphate hydrate (PLPHYD) was crystallized from an aqueous solution in the form of monoclinic needles; space group $P2_1/c$ with $a=10.503$, $b=13.608$, $c=7.488$ Å, $\beta=93.71°$ and four molecules in the unit cell. Prism-shaped crystal of pyridoxal phosphate methyl hemiacetal (PLPMHA) was obtained by dissolving the pyridoxal phosphate hydrate crystals into 90% methanol; space group $P\bar{1}$, with cell dimensions, $a=9.42$, $b=10.61$, $c=6.20$ Å, $\alpha=105.6°$, $\beta=95.7°$, and $\gamma=99.7°$, and two molecules per cell. Intensity data were collected on an automated diffractometer for PLPHYD and photographically for PLPMHA. Crystal structures have been determined by the symbolic addition method. Refinements were done by the block-diagonal least-squares procedures, the final R value being 0.093 for PLPHYD and 0.112 for PLPMHA. The free aldehyde group of pyridoxal phosphate interacts with water molecule to form *gem-diol* in the crystalline state of PLPHYD and the *gem-diol* group of PLPHYD with methanol to form methyl hemiacetal. One of the two hydrogen atoms on a phosphate group is liberated and protonated to the pyridine ring nitrogen atom, resulting in the zwitterionic structure for both PLPHYD and PLPMHA molecules. All hydrogen bonds in PLPHYD are intermolecular ones, but in PLPMHA there is one OH–O intramolecular hydrogen bond.

Vitamin B_6 is found in natural foods in various forms as pyridoxine, pyridoxamine, pyridoxal, pyridoxamine-5′-phosphate and pyridoxal-5′-phosphate (PLP). Of these, PLP is the most important coenzyme form of vitamin B_6; that is, as the form of 'co-decarboxylase' or 'co-transaminase', it participates in a vast number of enzyme systems associated with nitrogen metabolisms. A great many PLP-dependent enzymes have been isolated in a crystalline state and a number of mechanisms have been proposed as regards substrate-enzyme interactions, but many substantial points are still obscure.[1–2] In order to elucidate the relationship between the structure and function of PLP-dependent enzymes, it is necessary to determine the molecular structure of the coenzyme PLP.

This paper deals with a detailed X-ray diffraction analysis of two forms of PLP; pyridoxal phosphate hydrate (PLPHYD) and pyridoxal phosphate methyl hemiacetal (PLPMHA); a preliminary report has been published.[3]

Experimental

The yellowish needle crystals of PLPHYD were obtained by dissolving the sample (labelled 'Pyridoxal Phosphate Monohydrate', Sigma Chemicals) into ion-exchanged distilled water (pH=6.5) at room temperature, and then by keeping the solution at 7°C in the dark.

PLPMHA was obtained in the form of transparent yellow prism-shaped crystals by dissolving PLPHYD into about 90% methanol aqueous solution by heating up to boiling point, cooling at room temperature and then standing the solution at 5°C.

Unit cell parameters of PLPHYD were obtained with a

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[1] Y. Morino and E. E. Snell, *J. Biol. Chem.*, **242**, 2800 (1967).

[2] P. Fasella, *Ann. Rev. Biochem.*, **36**, 185 (1967).

[3] T. Fujiwara and K. Tomita, *Tetrahedron Lett.*, **1969**, 2819.

Rigaku Denki four-circle diffractometer and those of PLPMHA from zero-layer Weissenberg photographs about *b* and *c*, calibrated with superimposed aluminum powder pattern and precession photographs of the reciprocal a^*c^* and b^*c^* planes. The density was measured by flotation in a benzene and ethylene dibromide mixture for PLPHYD and in a benzene and carbon tetrachloride mixture for PLPMHA. The crystal data are listed in Table 1.

TABLE 1. CRYSTAL DATA OF PYRIDOXAL PHOSPHATE HYDRATE (PLPHYD) AND PYRIDOXAL PHOSPHATE METHYL HEMIACETAL (PLPMHA)

Compounds	PLPHYD	PLPMHA
Chemical formula	$C_8H_{12}O_7NP$	$C_9H_{14}O_7NP$
Mol. wt.	265.2	279.0
a(Å)	10.503±0.006	9.42±0.01
b(Å)	13.608±0.009	10.61±0.01
c(Å)	7.488±0.003	6.20±0.02
α(°)	90	105.6±0.2
β(°)	93.71±0.04	95.7±0.2
γ(°)	90	99.7±0.2
V(Å^2)	1067.97	580.7
Space group	$P2_1/c$	$P\bar{1}$
Z	4	2
D_m(g·cm^{-3})	1.635±0.003	1.587±0.004
D_c(g·cm^{-3})	1.649	1.595
μ(cm^{-1}) for Cu Kα	26.75	24.95

Intensity data of PLPHYD were collected with a crystal of dimensions 0.04×0.18×0.07 mm^3, on a Rigaku Denki four-circle computer-controlled diffractometer using nickel-filtered Cu$K\alpha$ radiation and ω-2θ scanning technique. Scanning speed (2θ) was 2°/min and scanning width was taken as $\Delta\omega=1°+0.015 \tan\theta$. Background was counted for ten seconds. 1912 independent reflections with $\sin\theta/\lambda$ less than 0.600 Å^{-1} were measured.

Three-dimensional intensity data for PLPMHA were collected from equi-inclination Weissenberg photographs with nickel-filtered Cu$K\alpha$ radiation. X-ray photographs for layers (*hk*0) to (*hk*5), and (*h*0*l*) to (*h*8*l*) were taken with two crystals, 0.31×0.40×0.31 mm^3 and 0.24×0.31×0.38 mm^3, respectively. The intensity was estimated visually by comparison with a standard scale, and corrected for spot size, and Lorentz and polarization factors. Independent 2526 reflections were measured.

The observed structure factors for both compounds were put on an absolute scale by Wilson statistics, and normalized structure factors ($|E_h|$) were computed.$^{4)}$

Phase Determination

Both PLPHYD and PLPMHA structures were solved by the symbolic addition method$^{5)}$ for the centrosymmetric system (program DPD).

With the normalized structure factors for both compounds, the $\sum_2$ listings$^{4)}$ were made for reflections with $|E_h|\geqq 1.5$ and with probability larger than 97%, using a computer program SIGMA.

The starting set for PLPHYD is shown in Table 2, where the sign of (002) reflection is uniquely determined by the $\sum_2$ listing. After three cycles of phase determination, the following relations were found to be predominant; A=−, AC=+, AD=+, BC=+, BD=+, CD=+, and ABC=−, which were reduced to A=B=C=D=−. Out of 261 reflections with $|E_h|\geqq 1.5$, phases of 140 reflections were determined, and a subsequent E map revealed the appropriate peak height for all seventeen non-hydrogen atoms at proper positions. Two large spurious peaks were thought to come from insufficient number of E_h per atom, when it was compared with that of the empirical criterion by Karle.$^{4)}$

TABLE 2. STARTING SET FOR THE APPLICATION OF $\sum_2$ FORMULA TO PLPHYD

h	k	l	E_h	Sign	NINT$^{a)}$
5	10	−1	3.228	+	26
7	1	−4	3.040	+	36
10	8	−3	3.225	+	26
6	9	3	3.120	A	28
9	1	0	3.224	B	32
4	8	1	3.408	C	41
4	10	0	3.151	D	31
0	0	2	2.260	—	18

a) Number of $\sum_2$ interaction pairs.

TABLE 3. STARTING SET FOR THE APPLICATION OF $\sum_2$ FORMULA TO PLPMHA

h	k	l	Sign	E_h	NINT$^{a)}$
6	−10	5	+	2.547	24
−7	4	2	+	3.176	37
−1	1	5	+	3.281	34
0	−6	2	+	3.263	38
6	−2	3	A	3.091	39
4	2	5	B	2.175	8
5	−6	5	C	2.496	12
7	6	0	D	3.928	42

a) Number of $\sum_2$ interaction pairs.

Direct phase determination of PLPMHA was initiated by the starting set of eight reflections as listed in Table 3, where a positive sign of reflection (0−62) was uniquely determined from the $\sum_1$ formula$^{5)}$ with a probability of 0.99. After three cycles of the symbolic addition procedure, the signs of 253 reflections were determined out of 328 reflections with $|E_h|\geqq 1.5$. From relationship occurring between the letters A to D, it seemed probable that A=−, B=+, C=−, and D=−. Three dimensional E map calculated by this combination clearly elucidated the molecular structure of PLPMHA.

Refinement of the Structure

Refinement was done by the successive Fourier synthesis and then by a block-diagonal least-squares method (program BLLS).

PLPHYD. The molecular structure derived from the E map was quite different from the expected one which consists of one pyridoxal phosphate molecule

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4) J. Karle and I. L. Karle, *Acta Crystallogr.*, **21**, 849 (1966).

5) H. Hauptman and J. Karle, "Solution of the Phase Problem" I. The Centrosymmetric Crystal. A.C.A. Monography No. 3. Polycrystal Book Service, Pittsburgh (1953).

and one water molecule of crystallization. The peculiar *gem-diol* structure was confirmed by the two Fourier syntheses, one using the phases except for two oxygen atoms of *gem-diol*, and the other the phases of all non-hydrogen atoms (R=0.28). Further refinement of the positional and thermal parameters was carried out by the least-squares calculation wihch minimized $\sum\omega_i(k|F_o|-|F_c|)^2$, where ω_i, the weight of ith reflection, was zero for $|F_o|=0$ and unity for $|F_o|\neq 0$. Several cycles of refinement employing anisotropic temperature factors for all non-hydrogen atoms reduced the R index to 0.11. At this stage, a difference Fourier synthesis was computed, and all of twelve hydrogen atoms were found to occupy the appropriate positions. Final three cycles of refinement including the hydrogen atoms with isotropic thermal factors reduced the R index to 0.106 (R=0.093 with non-zero reflections). The final atomic positional and thermal parameters are listed in Tables 4 and 5, respectively.

PLPMHA. Refinement of the structure by the least-squares method with isotropic thermal parameters brought the R value from 0.21 to 0.16 after five cycles of refinement. A difference Fourier synthesis was calculated at this stage. Out of 14 hydrogen atoms, thirteen could be located at the peak positions in the difference map. Five cycles of refinement were done

TABLE 4. FINAL FRACTIONAL COORDINATES AND THE ESTIMATED DEVIATIONS (IN Å) OF PLPHYD

Atom[a]	x/a	$\sigma(x)$	y/b	$\sigma(y)$	z/c	$\sigma(z)$	Atom[a]	x/a	$\sigma(x)$	y/b	$\sigma(y)$	z/c	$\sigma(z)$
P	0.2447	0.001	0.1549	0.001	0.2186	0.001	C7	0.9394	0.006	0.3795	0.006	0.2009	0.007
O1	0.6133	0.004	0.0173	0.004	0.1367	0.004	C8	0.7101	0.006	0.0534	0.005	0.2565	0.006
O2	0.6879	0.005	0.0261	0.004	0.4326	0.004							
O3	0.9202	0.004	0.1641	0.004	0.1716	0.005	O6H	0.180	0.07	0.191	0.08	0.483	0.07
O4	0.3745	0.004	0.2147	0.004	0.2394	0.004	C1H1	0.486	0.08	0.101	0.08	0.312	0.08
O5	0.1523	0.004	0.2212	0.004	0.1149	0.004	C1H2	0.462	0.08	0.179	0.08	0.479	0.08
O6	0.2023	0.004	0.1381	0.004	0.4128	0.004	C6H	0.531	0.07	0.362	0.08	0.348	0.07
O7	0.2664	0.004	0.0554	0.004	0.1397	0.004	NH	0.723	0.08	0.432	0.08	0.289	0.08
N	0.7172	0.005	0.3648	0.004	0.2769	0.005	O3H	0.999	0.08	0.194	0.08	0.135	0.08
C1	0.4799	0.005	0.1689	0.006	0.3457	0.006	C8H	0.798	0.07	0.027	0.07	0.221	0.07
C2	0.8241	0.005	0.3192	0.005	0.2315	0.006	O1H	0.665	0.08	−0.016	0.09	0.026	0.08
C3	0.8194	0.006	0.2155	0.006	0.2197	0.006	O2H	0.725	0.09	−0.034	0.09	0.479	0.08
C4	0.7080	0.005	0.1663	0.005	0.2571	0.005	C7H1	0.928	0.09	0.417	0.09	0.095	0.09
C5	0.6012	0.005	0.2187	0.005	0.3003	0.005	C7H2	1.020	0.09	0.337	0.10	0.174	0.09
C6	0.6081	0.006	0.3201	0.006	0.3096	0.006	C7H3	0.957	0.09	0.429	0.09	0.286	0.09

a) Name of the hydrogen atom; for example, C7H3 shows the third hydrogen atom attached to the C7 atom.

TABLE 5. FINAL THERMAL PARAMETERS OF PLPHYD. ANISOTROPIC TEMPERATURE FACTORS ARE IN THE FORM OF EXP $\{-(B_{11}h^2+B_{22}k^2+B_{33}l^2+B_{12}hk+B_{13}hl+B_{23}kl)\}$ AND THEIR STANDARD DEVIATIONS ($\times 10^5$)

Atom	B_{11}	$\sigma(B_{11})$	B_{22}	$\sigma(B_{22})$	B_{33}	$\sigma(B_{33})$	B_{12}	$\sigma(B_{12})$	B_{13}	$\sigma(B_{13})$	B_{23}	$\sigma(B_{23})$
P	284	11	142	7	796	24	37	15	189	26	59	22
O1	624	43	387	27	1207	86	−287	56	−167	97	−416	77
O2	1061	55	283	26	887	79	293	59	46	105	409	72
O3	370	37	238	24	2252	108	22	49	725	101	−145	82
O4	375	35	187	22	1208	79	−69	44	−101	85	−1	67
O5	328	34	227	22	1239	80	108	44	140	84	415	68
O6	612	39	156	21	821	71	100	46	457	85	41	61
O7	618	41	131	20	1046	76	92	46	277	89	−291	63
N	481	44	175	26	980	89	−84	54	13	102	30	75
C1	284	46	297	34	1104	110	54	65	30	114	395	100
C2	351	49	173	30	1272	116	69	60	178	120	175	91
C3	328	49	225	32	1275	117	24	63	155	122	19	97
C4	339	47	170	29	933	100	−112	61	−7	110	−61	88
C5	421	50	146	28	840	98	−71	60	−219	112	−1	84
C6	385	51	261	33	969	109	27	65	147	119	−47	93
C7	533	59	188	33	2117	156	−204	72	387	154	−12	113
C8	441	52	118	28	1539	125	−120	63	155	129	−106	95

Atom	B(isotropic)	Atom	B(isotropic)
O6H	1.6	C8H	1.4
C1H1	2.0	O1H	3.2
C1H2	2.3	O2H	3.8
C6H	1.8	C7H1	4.6
NH	3.4	C7H2	5.2
O3H	2.8	C7H3	3.8

Table 6. Final fractional coordinates and the estimated standard deviations (in Å) of PLPMHA

Atom	x/a	$\sigma(x)$	y/b	$\sigma(y)$	z/c	$\sigma(z)$	Atom	x/a	$\sigma(x)$	y/b	$\sigma(y)$	z/c	$\sigma(z)$
P	0.7538	0.001	1.0394	0.001	0.9790	0.001	C9	1.0730	0.007	0.6582	0.009	0.4407	0.009
O1	0.9232	0.004	0.6245	0.004	0.3580	0.005							
O2	0.9497	0.004	0.6839	0.005	0.0062	0.004	NH	0.361	0.10	0.776	0.11	0.119	0.11
O3	0.6919	0.004	0.5871	0.005	−0.1998	0.005	O3H	0.812	0.12	0.627	0.12	−0.157	0.12
O4	0.6879	0.004	0.9206	0.005	0.7523	0.004	O2H	1.039	0.10	0.744	0.11	0.008	0.11
O5	0.6281	0.003	1.0373	0.004	1.1143	0.004	C6H	0.496	0.07	0.856	0.08	0.477	0.08
O6	0.7922	0.004	1.1663	0.004	0.9190	0.005	C1H1	0.841	0.08	0.824	0.08	0.635	0.08
O7	0.8848	0.004	1.0042	0.004	1.0959	0.004	C1H2	0.850	0.10	0.966	0.10	0.576	0.10
N	0.4535	0.004	0.7422	0.005	0.1416	0.005	C8H	0.969	0.07	0.825	0.07	0.287	0.07
C1	0.7788	0.005	0.8787	0.007	0.5901	0.006	C7H1	0.300	0.09	0.564	0.09	−0.186	0.09
C2	0.5023	0.005	0.6722	0.006	−0.0331	0.006	C7H2	0.467	0.11	0.533	0.11	−0.350	0.10
C3	0.6479	0.005	0.6608	0.006	−0.0157	0.006	C7H3	0.350	0.11	0.683	0.11	−0.325	0.11
C4	0.7403	0.005	0.7272	0.005	0.1891	0.005	C9H1	1.106	0.09	0.629	0.09	0.301	0.09
C5	0.6827	0.005	0.8007	0.006	0.3690	0.006	C9H2	1.110	0.10	0.742	0.10	0.512	0.10
C6	0.5361	0.005	0.8066	0.006	0.3431	0.006	C9H3	1.074	0.09	0.603	0.09	0.570	0.09
C7	0.3975	0.007	0.6090	0.008	−0.2530	0.007	O5H	0.5		1.0		1.0	
C8	0.9027	0.005	0.7251	0.006	0.2133	0.006	O7H	1.0		1.0		1.0	

Table 7. Final thermal parameters of PLPHA. Anisotropic temperature factors are in the form of $\exp\{-(B_{11}h^2+B_{22}k^2+B_{33}l^2+B_{12}hk+B_{13}hl+B_{23}kl)\}$ and their standard deviations ($\times 10^5$)

Atom	B_{11}	$\sigma(B_{11})$	B_{22}	$\sigma(B_{22})$	B_{33}	$\sigma(B_{33})$	B_{12}	$\sigma(B_{12})$	B_{13}	$\sigma(B_{13})$	B_{23}	$\sigma(B_{23})$
P	323	12	723	14	1482	34	337	21	12	32	606	35
O1	630	45	853	48	3815	164	385	74	90	136	1836	146
O2	703	48	1029	53	2635	138	241	80	485	130	238	135
O3	935	55	1185	59	2360	136	508	90	223	137	−89	140
O4	576	44	1229	57	2280	128	401	80	147	120	120	135
O5	419	38	956	47	2140	116	326	67	322	105	933	118
O6	526	42	871	47	3375	149	363	70	242	125	1661	138
O7	501	41	996	49	2698	132	497	72	−313	116	1407	132
N	477	46	705	49	2333	141	392	75	15	127	1116	135
C1	473	57	984	72	2605	188	281	101	40	163	255	183
C2	702	61	725	59	1862	152	294	95	−243	152	897	153
C3	603	58	694	59	2381	173	330	93	−10	159	697	162
C4	504	52	585	52	2046	152	405	84	126	142	808	144
C5	487	53	702	57	2065	156	297	86	186	144	959	153
C6	594	57	695	57	2145	161	450	92	151	152	688	154
C7	915	77	1193	84	2214	187	319	128	−376	189	642	199
C8	557	57	739	60	2416	173	479	93	188	157	813	164
C9	820	77	1423	99	4506	294	693	140	−120	238	2776	286

Atom	B(isotropic)	Atom	B(isotropic)	Atom	B(isotropic)
NH	6.3	C1H2	5.6	C9H1	4.8
O3H	7.5	C8H	2.3	C9H2	5.3
O2H	6.3	C7H1	3.0	C9H3	5.0
C6H	2.5	C7H2	2.6	O5H	2.9
C1H1	3.1	C7H3	4.9	O7H	2.6

with anisotropic temperature factors for non-hydrogen atoms and with isotropic for 13 hydrogen atoms. A subsequent difference Fourier synthesis revealed no plausible peak for the one remaining hydrogen atom attached to the phosphate oxygen atom. This might be due to uncertainty of the hydrogen position; the two P–O bond lengths (P–O5=1.519 Å and P–O7=1.520 Å) were equal within the estimated standard deviation (0.005 Å) and the oxygen-oxygen distances related by a center of symmetry (O5–O5=2.555 Å and O7–O7=2.580 Å) indicated the existence of the symmetrical hydrogen bonding. The final five cycles on the least-squares refinement were carried out by keeping the one hydrogen atom at two centers of symmetry with a half weight, and the R index dropped to 0.112. The final positional and thermal parameters are listed in Tables 6 and 7, respectively.

A list of the observed and calculated structure factors for both crystals is kept as Document No. 7308 at the office of the Chemical Society of Japan.

All the numerical computations were done on an NEAC 2200—500 of the Computing Center of this University, a FACOM 230—60 of the Data Processing Center, Kyoto University and HITAC 5020E of the Computer Centre, the University of Tokyo. Computer programs SIGMA, BLLS, and DAPH were written by

Dr. Tamaichi Ashida and others by the author. The atomic scattering factors were taken from 'International Tables for X-ray Crystallography'.[6]

Discussion

The bond lengths and angles were calculated using the computer program DAPH and are shown in Fig. 1 for PLPHYD and Fig. 2 for PLPMHA.

Phosphate Group. Four P–O bond lengths of 1.587, 1.565, 1.503, and 1.501 Å in PLPHYD are in good agreement with the following average values[7] found in the other known monoanionic phsophates:[7–10] P–OC=1.602, P–OH=1.554, P–O$^-$=1.502, and P=O=1.490 Å, respectively. The P–O4 bond length of 1.600 Å and the P–O6 bond length of 1.487 Å in PLPMHA are very close to the above P–OC and P=O distances, respectively, whereas the P–O5 (1.519 Å) and the P–O7 (1.520 Å) bond lengths associated with the symmetrical hydrogen atom are intermediate to the normal P–OH and P=O bond lengths, similar P–O bond lengths of 1.522 and 1.530 Å being found in DL-*O*-serine phosphate monohydrate.[11] The correla-

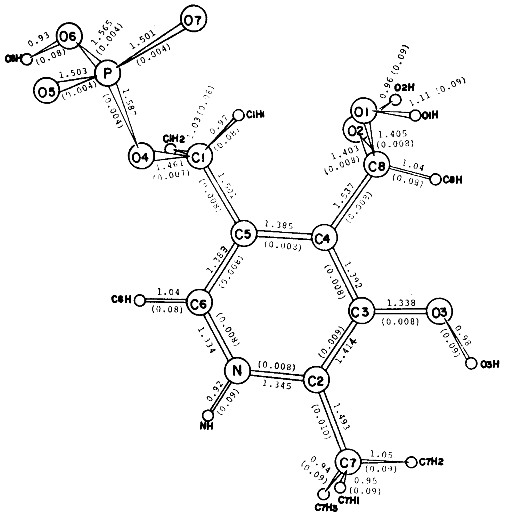

Fig. 1(a). Bond lengths and their standard deviations in parentheses (in Å) of PLPHYD. The molecule is projected on the plane of the pyridine ring.

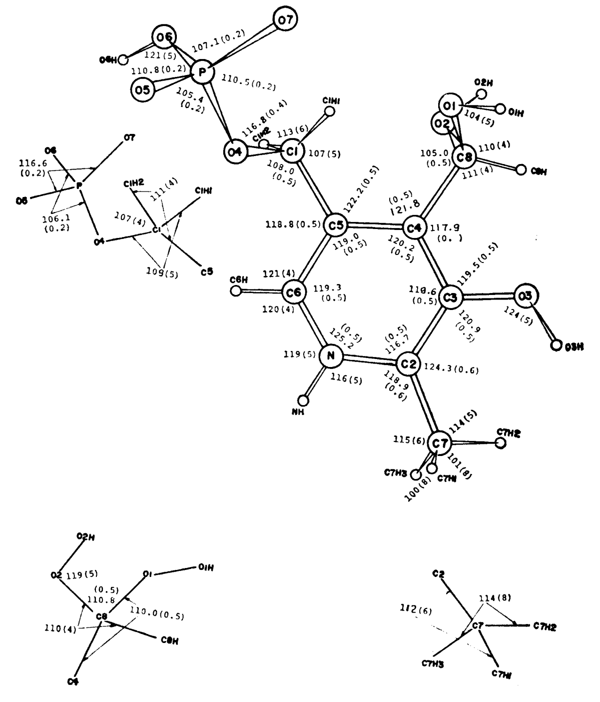

Fig. 1(b). Bond angles and their standard deviations in parentheses (in degree) of PLPHYD.

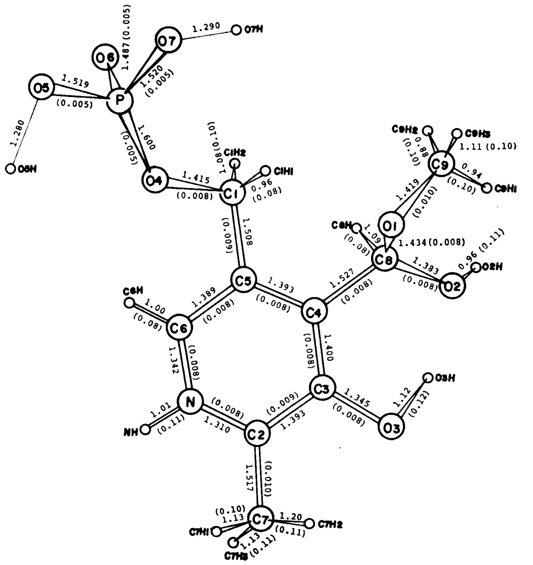

Fig. 2(a). Bond lengths and their standard deviations in parentheses (in Å) of PLPMHA. The molecule is projected on the plane of the pyridine ring.

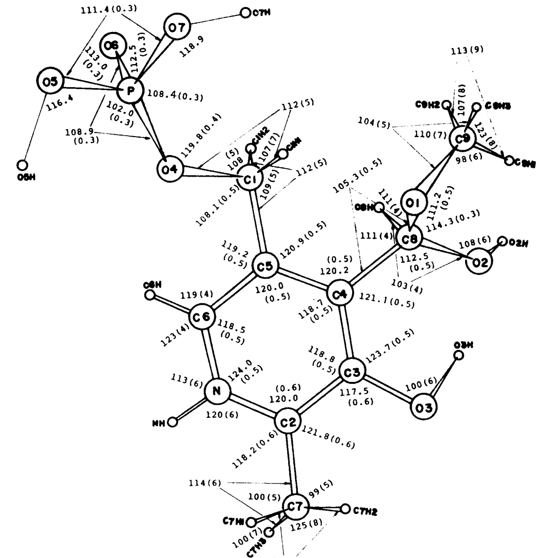

Fig. 2(b). Bond angles and their standard deviations in parentheses (in degree) of PLPMHA.

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[6] "International Tables for X-ray Crystallography," Vol. III, Kynoch Press, Birmingham (1962), p. 202.

[7] M. Sundaralingam and E. F. Putkey, *Acta Crystallogr.*, **B26**, 790 (1970).

[8] J. Kraut, *ibid.*, **14**, 1146 (1961).

[9] J. Kraut and L. H. Jensen, *ibid.*, **16**, 79 (1963).

[10] M. Sundaralingam, *ibid.*, **21**, 495 (1966).

[11] E. Putkey and M. Sundaralingam, *ibid.*, **B26**, 782 (1970).

tion of each four O–P–O angles of PLPHYD and PLPMHA is explainable by considering the repulsive force between two adjacent oxygen atoms;[9] the larger bond angle is found between the two shorter bonds.

TABLE 8. DEVIATIONS OF THE ATOMS FROM THE LEAST-SQUARES PLANE (IN Å)

Atom	PLPHYD	PLPMHA	PINHCL[a]	PLPOXM[b]
The atoms involved in the calculation of the least-squares plane				
C2	0.000	−0.005	−0.009	−0.019
C3	0.008	0.007	0.009	0.003
C4	−0.010	−0.002	−0.001	0.014
C5	0.004	−0.005	−0.007	−0.017
C6	0.006	0.007	0.007	0.003
N	−0.008	−0.002	0.001	0.016
The atoms excluded in the calculation of the least-quares plane				
C1	−0.047	−0.072	−0.015	−0.068
C7	−0.031	−0.067	−0.037	−0.082
C8	−0.107	−0.075	−0.033	0.020
O3	0.046	0.011	0.015	−0.050
C6H	−0.03	0.06		
NH	−0.05	−0.31		
C8H	−0.11	−0.85		
O3H	0.13	−0.36		
O4	1.021	0.204	−0.029	−0.000

a) Pyridoxine hydrochloride[12]
b) Pyridoxal phosphate oxime[13]

Pyridine Ring. Figures 1 and 2 show the molecular projection on the plane of the pyridine ring for PLPHYD and PLPMHA, respectively. The deviations of the individual atoms from the least-squares plane, together with the corresponding values of similar compounds, pyridoxine hydrochloride[12] and pyridoxal phosphate oxime[13] are listed in Table 8. The pyridine ring is exactly planar and C1, C7, C8, O3, and two hydrogen atoms (C6H and NH) are nearly in the plane of the ring, both in PLPHYD and PLPMHA. The equations of the best plane passing through the ring atoms are as follows:

for PLPHYD, $-0.24677X+0.06189Y-0.96709Z+3.51217=0$,

for PLPMHA, $-0.11690X-0.94425Y+0.30779Z+7.16397=0$.

The bond lengths and the angles in the pyridine ring are listed in Table 9, together with those of the related compounds. In the pyridine ring of PLPHYD and PLPMHA, the average C–C bond length is 1.394 Å and the C–N bond length is 1.330 Å. Three C(sp^2)–C(sp^3) bond lengths have a mean value of 1.515 ± 0.009 Å, which is comparable to a standard value of 1.50 Å for a C(sp^2)–C(sp^3) single bond. The bond angles, C2–N–C6 of 125.2 and 124.0° for PLPHYD and PLPMHA indicate that an extra-annular hydrogen atom attaches to the ring nitrogen as suggested by Singh[14] and the position of the attached hydrogen atom is confirmed by the difference Fourier synthesis.

TABLE 9. BOND LENGTHS AND ANGLES OF THE PYRIDINE SKELETON

	PLP-HYD	PLP-MHA	PIN-HCL[a]	PLP-OXM[b]
BOND LENGTHS (IN Å)				
C2–C3	1.414	1.393	1.368	1.41
C3–C4	1.392	1.400	1.379	1.38
C4–C5	1.385	1.393	1.409	1.41
C5–C6	1.383	1.389	1.358	1.36
C6–N	1.334	1.342	1.325	1.34
N –C2	1.345	1.310	1.345	1.35
C2–C7	1.493	1.517	1.483	1.51
C4–C8	1.537	1.527	1.490	1.44
C5–C1	1.501	1.508	1.497	1.55
C3–O3	1.338	1.345	1.354	1.35
e. s. d.	0.009	0.009	0.005	0.02
BOND ANGLES (IN degree)				
N –C2–C3	116.7	120.0	118.1	117
N –C2–C7	118.9	118.2	119.4	119
C7–C2–C3	124.3	121.8	122.5	124
C2–C3–C4	119.6	118.8	120.1	121
C2–C3–O3	120.9	117.5	115.8	116
O3–C3–C4	119.5	123.7	124.1	123
C3–C4–C5	120.2	118.7	118.8	117
C3–C4–C8	117.9	121.1	123.0	122
C8–C4–C5	121.8	120.2	118.2	121
C4–C5–C6	119.0	120.0	119.6	122
C4–C5–C1	122.2	120.9	121.4	119
C1–C5–C6	118.8	119.2	119.0	120
C5–C6–N	119.3	118.5	118.9	119
C6–N –C2	125.2	124.0	124.5	124
e. s. d.	0.5	0.5	0.3	1

a)- b) See footnete a—b, Table 8.

Molecular Conformation. The conformational angles of PLPHYD and PLPMHA molecule are shown in Fig. 3. The torsion angle around the C1–O4 bond (162.2° for PLPHYD and 198.0° for PLPMHA) indicates that the P–O4 bond of the two compounds is almost symmetrical with each other with respect to the plane passing through C5, C1, and O4. The torsion angle around the C1–C5 bond (52.1° for PLPHYD and 13.5° for PLPMHA) means that the O4 atom of PLPHYD deviates appreciably from the plane of the pyridine ring (1.02 Å) but the O4 atom of PLPMHA is roughly in the plane (0.20 Å). As for the O4 atom, it is found that the C1–O4 bond distance of PLPHYD is significantly greater than that of PLPMHA. A large conformational change between PLPHYD and PLPMHA around the C8–C4 bond is probably due to the difference in the type of hydrogen bonding. Both two oxygen atoms (O1 and O2) in the *gem-diol* of PLPHYD are situated symmetrically at both sides of the pyridine ring plane as donors of the intermolecular hydrogen bonds, and the two C–O bonds with nearly equal length of 1.403 and 1.405 Å are in the *gauche-gauche* conformations with regard to the C4–C5 bond. On the other hand, of two oxygen atoms attached to the C8 atom of PLPMHA, O1 is of a methyl hemiacetal group and O2 is of a hydroxyl group, and the formation

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12) F. Hanic, *Acta Crystallogr.*, **21**, 332 (1966).
13) A. N. Barret and R. A. Palmer, *ibid.*, **B25**, 688 (1969).
14) C. Singh, *Acta Crystallogr.*, **19**, 861 (1965).

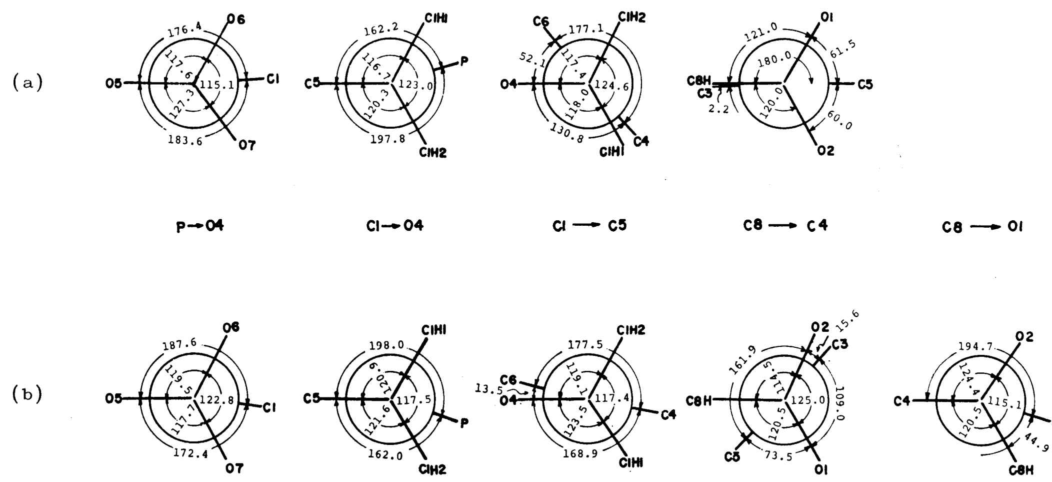

Fig. 3. Molecular conformations of PLPHYD (a) and PLPMHA (b). Torsion angles are in degree.

of the intramolecular hydrogen bond between O2 and O3 restricts the torsion angle between the C8–O2 and C4–C3 bonds to be 15.6°.

Pyridoxal phosphate has been determined to form a stable *gem-diol* type crystalline hydrate in PLPHYD. The reason for the *gem-diol* formation of the pyridoxal phosphate is ascribable to the electron attracting character of the protonated pyridine ring; the withdrawal of electron from the carbon atom of the carbonyl group causes this atom to have a relatively positive charge and hence to be attacked by hydroxide ion, as in the formation of chloral hydrate[15] by chloral. It is also indicated that the reaction from PLPHYD to PLPMHA is not the addition reaction between an aldehyde and a molecule of methyl alcohol but the dehydration reaction between a *gem-diol* and a molecule of methyl alcohol. On the other hand, from NMR spectra measurement of pyridoxal phosphate in D_2O solution at pD=7.8,[16] the ratio of the hydrated form (*gem-diol*) to the free aldehyde form has been calculated to be 0.86, and a similar ratio of 0.66 was estimated from the ultraviolet absorption measurement of 5-deoxypyridoxal at pH=6.88.[17]

Cystal Structure. The projections of the crystal structure and the hydrogen bondings in PLPHYD viewed along the *c* and *b* axes, respectively, are shown in Figs. 4 and 5, and the crystal structure of PLPMHA projected along the *c* axis in Fig. 6. The hydrogen bond distances and angles are listed in Tables 10 and 11 for PLPHYD and PLPMHA, respectively. All the

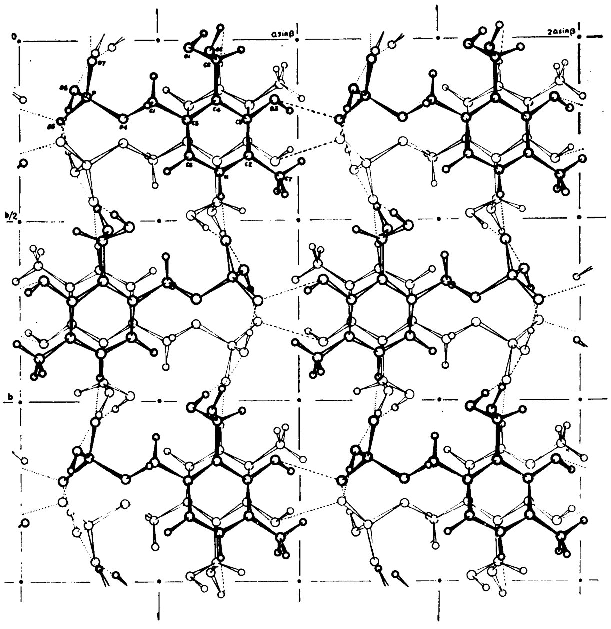

Fig. 4. A projection of PLPHYD structure along the *c* axis. Hydrogen bonds are indicated by the broken lines.

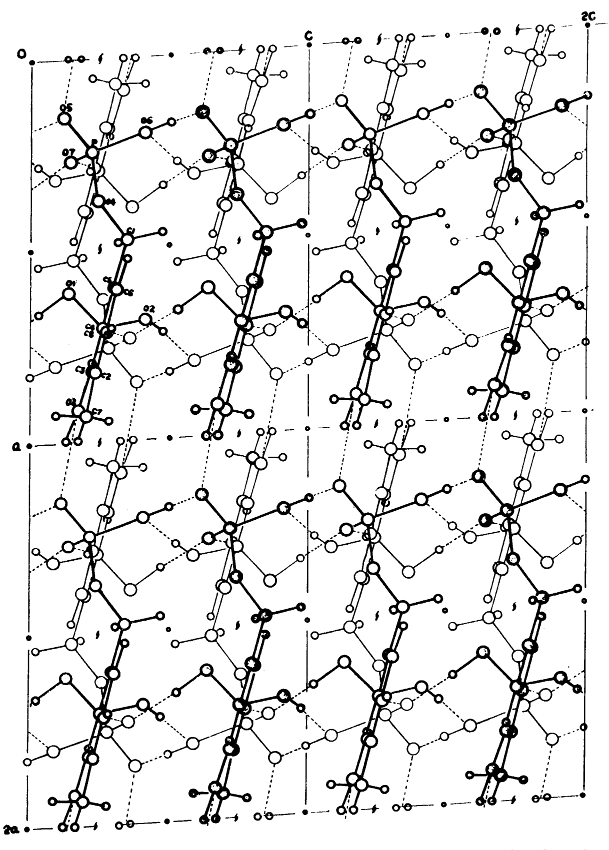

Fig. 5. A projection of PLPHYD structure along the *b* axis. Hydrogen bonds are indicated by the broken lines.

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[16] W. Korytnyk and R. P. Singh, *J. Amer. Chem. Soc.*, **85**, 2813 (1963).

[17] D. E. Metzler and E. E. Snell, *ibid.*, **77**, 2431 (1955).

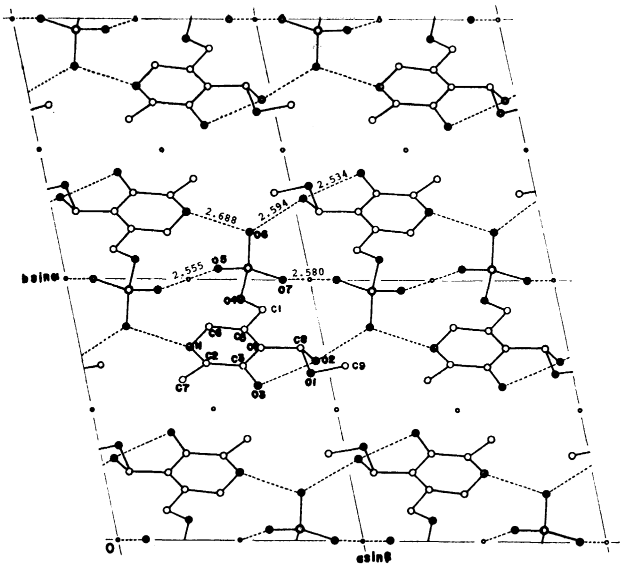

Fig. 6. A projection of PLPMHA structure along the *c* axis. Hydrogen bonds (in Å) are indicated by the broken lines.

hydrogen bonds in PLPHYD are intermolecular ones, whereas in PLPMHA there is one intramolecular hydrogen bond of 2.534 Å between O2 and O3. Phosphate oxygen atoms of both compounds participate in hydrogen bond formation. It is of interest that O5 and O7 atoms of PLPMHA form the symmetrical hydrogen bonds of 2.555 and 2.580 Å, respectively. The shortest OH···O hydrogen bond distance of 2.517 Å found on P–OH···O=P of PLPHYD is comparable to 2.492 Å of L-serine phosphate.⁷⁾ The ester oxygen atom O4 in both compounds and O1 in PLPMHA are not involved in the hydrogen bonding. The protonated nitrogen atom in the pyridine ring also takes part in the hydrogen bonding of NH···O type in both compounds. PLPHYD molecules are fixed by three dimensional networks of hydrogen bonding and the large overlaps of the pyridine rings with spacing of 3.7 Å are found among the molecules related by the *c* glide plane symmetry. Molecular arrangement in the PLPMHA crystal can be described as a layered structure parallel to the *ac* plane. The L and D antipodes

TABLE 10. HYDROGEN BOND DISTANCES AND ANGLES IN PLPHYD

Distances (Å) Donor Acceptor		Angles (degree)	
O3(S2)—O5(S1)	2.619(0.006)[a]	C3–O3(S2)—O5(S1)	130.5(0.4)[a]
		P –O5(S1)—O3(S2)	108.3(0.2)
O6(S1)—O5(S3)	2.517(0.006)	P –O6(S1)—O5(S3)	122.1(0.2)
		P –O5(S3)—O6(S1)	127.9(0.2)
O2(S4)—O6(S1)	2.737(0.006)	C8–O2(S4)—O6(S1)	122.0(0.3)
		P –O6(S1)—O2(S4)	112.0(0.2)
O1(S5)—O7(S1)	2.677(0.006)	C8–O1(S5)—O7(S1)	105.5(0.4)
		P –O7(S1)—O1(S5)	136.7(0.3)
N (S1)—O7(S6)	2.671(0.006)	C2–N (S6)—O7(S1)	117.7(0.4)
		C6–N (S6)—O7(S1)	116.3(0.4)
		P –O7(S1)—N (S6)	142.5(0.3)

S1	x	y	z
S2	$x-1$	y	z
S3	x	$1/2-y$	$1/2+z$
S4	$1-x$	$-y$	$1-z$
S5	$1-x$	$-y$	$-z$
S6	$1-x$	$-1/2+y$	$1/2-z$

a) Estimated standard deviations are in parentheses.

TABLE 11. HYDROGEN BOND DISTANCES AND ANGLES IN PLPMHA

Distances (Å) Donor Acceptor		Angles (degree)	
O3(S1)—O2(S1)	2.534(0.007)[a]	C3–O3(S1)—O2(S1)	86.7(0.4)[a]
		C8–O2(S1)—O3(S1)	92.5(0.4)
O2(S2)—O6(S1)	2.594(0.007)	P –O6(S1)—O2(S2)	125.9(0.3)
		C8–O2(S2)—O6(S1)	101.9(0.4)
N (S3)—O6(S1)	2.688(0.007)	P –O6(S1)—N (S3)	108.0(0.3)
		C6–N (S3)—O6(S1)	113.0(0.4)
		C2–N (S3)—O6(S1)	120.1(0.4)
O5(S1)—O5(S4)	2.555(0.009)	P –O5(S1)—O5(S4)	116.4(0.3)
O7(S1)—O7(S5)	2.580(0.009)	P –O7(S1)—O7(S5)	118.8(0.3)

S1	x	y	z
S2	$2-x$	$2-y$	$1-z$
S3	$1-x$	$2-y$	$1-z$
S4	$1-x$	$2-y$	$2-z$
S5	$2-x$	$2-y$	$2-z$

a) Estimated standard deviations are given in parentheses.

of PLPMHA related by a center of symmetry are held together by the network of hydrogen bonds in each layer, whereas between the adjacent layers the methyl group acts mainly as a spacer. As the number of intermolecular short contacts is greater in PLPHYD than in PLPMHA, it can be said that PLPHYD molecules are in closer packing arrangement than PLPMHA, which explains well the larger observed density of the former (1.635 $g \cdot cm^{-3}$) than that of the latter (1.587 $g \cdot cm^{-3}$).

The author wishes to express his sincere thanks to Professor Masao Kakudo and Dr. Ken-ichi Tomita for their encouragement and helpful discussions in the course of this study. Thanks are also due to Dr. Tamaichi Ashida for the use of his computer programs.