IRON CHELATORS OF THE CLASS OF PYRIDOXAL ACYLHYDRAZONE . PART 5[1]. CRYSTAL STRUCTURE AND PATTERNS OF HYDROGEN BONDING IN PYRIDOXAL ISONICOTINOYL HYDRAZONE (PIH).

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Abstract - The water-free Pyridoxal Isonicotinoyl Hydrazone {PIH} $C_{14}H_{14}N_4O_3$ (mp 268°C) was synthesized, and its crystal structure determined from three-dimensional X-Ray data. The chelator crystallizes in the space group $P2_1/n$ with $Z = 4$ and cell dimensions: $a = 13.072(6)$ Å, $b = 13.06(5)$ Å, $c = 8.186(2)$ Å, $b = 90.21°(2)$. The data produced here confirms that PIH crystallizes in the *non-dipolar form*, assembled as, **3-D** tetramer, composed of a planar 16-membered ring dimer, involving *intermolecular* H-bondings between the acoholic hydroxyl-oxygen (O^2) and the azinic (N^3-*H*) hydrogen., The dimeric macrocycle is attached to two *PIH molecules* in a perpendicular fashion, and in opposite orientations, *to form a* **3-D** tetramer, by involving *intermolecular* H-contacts between the alcoholic hydroxyl-**H** and the pyridinic ring -nitrogen (N^4). The azinic-nitrogen (N^2) is *intramolecularly* H-bonded with the phenolic ***H*** {O^1-*H*}, to form a stable six-membered ring system.

INTRODUCTION

Pyridoxal isonicotinoylhydrazone (PIH) (II) was initially recorded in the literature (1954)[2] as a synthetic tuberculostatic agent, prior to its identification as a true metabolite of the isoniazide drug (in current clinical use against tuberculosis), in 1979,[3] causing "hypochromic microcytic anemia)[4] among patients on chronic use of the drug. Soon after, it was demonstrated that PIH was virtually a non-toxic lipophilic agent endowed with high affinity for iron.[5] On oral administration to hypertransfused rats, labelled with ^{59}Fe, PIH demonstrated high capability

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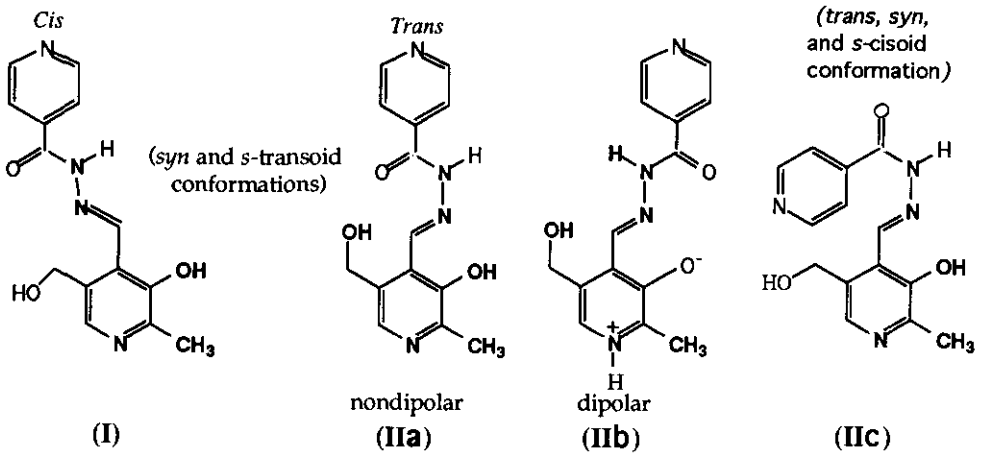
Dedicated to Professor Koji Nakanishi on the occasion of his 75th birthday in appreciation for his excellence in Science and devotion to human values

in removing radioiron from parenchymal iron-pool,[5a] and releasing iron from reticulocytes. Because of its biological properties, PIH was viewed[6] as a promising candidate in chelation therapy, to replace the naturally occurring siderophore, desferrioxamine B,[7] the only drug (desferal, DF) in current clinical use for the removal of toxic accumulation of iron in blood disorders, such as thalassemia,[8] and in transfusional iron overload.[9] Understandably, PIH elicited immense biochemical and pharmacological research activities in fields concerned with metalloenzymes, such as DNA synthesis,[10] cell proliferation and differentiation,[11] cancer chemotherapy,[12] malaria chemtherapy,[13] and immunology.[14]

PIH contains one phenolic group (O^1), three basic sites (N^1, N^2, and N^4), one enolizable site (HN^3-CO^3), and one primary alcoholic group (O^2), allowing it to enter into some tautomeric shifts {Scheme **2**, (IId), (IIIa), (IIIb}, depending on reaction conditions. Infrared spectral data[5b] suggested that in the solid state PIH exist in the non-dipolar forms, (IIa) and (IId) {Schemes **1** & **2**}, but crystallographic study of the hydrated form, 1:1-PIH:H_2O, confirmed[15] the existance of its *dipolar* form. We deemed it interesting to establish whether in contrast to PIH.H_2O, the water-free species tends to crystallize in a *nondipolar* form, and structurally to assume the trans geometry,[16] and whether it prefers any of the tautomeric forms, IId, and/or, IIIa-IIIb ?

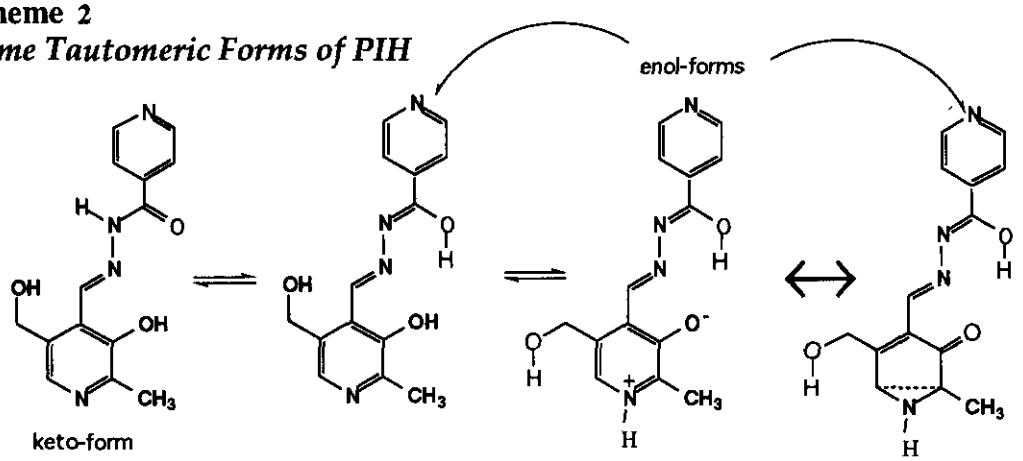
Scheme 1

Some Geometric and Conformational Forms of PIH



Towards this end, we undertook a study of the synthesis and X-Ray analysis of water-free PIH. Study herein presented confirm that free-PIH crystallizes in the *nondipolar* form, and structurally assume the trans geometry, and the s-transoid conformation for the hydrazidic carbonyl-O^3 and N^3-hydrogen tautomeric site. Evidently, the energetically unfavored enol-forms, IIIa-IIIb, which actually observed to occur in crystalline {$[PIH]_2Fe(III).^1/_2\ H_2SO_4$ },[17] are non-exsitent in *absence of metal ions*.

Scheme 2
Some Tautomeric Forms of PIH



EXPERIMENTAL

Material- PIH.2HCl (mp 253°C)[18] was prepared according to literature.[5b] The later was suspended in cold water, and a solution of ammonium hydroxide (5%) added to reach pH ~ 6. The resulting free base was recrystallized from methanol to yield pale-yellow prisms, melting at 268°C.

X-Ray Crystal Structure Analysis. Data (see Table 1) were measured on a PW1100 / 20 Philips Four-Circle Computer-controlled Difractometer. MoKα (λ=0.71069 Å) radiation with a graphite crystal monochromator in the incident team was used. The unit cell dimensions were obtained by a least-square fit of 24 centered reflections in the range of $11° \leq \Theta \leq 14°$. Intensity data were collected using the $\omega - 2\Theta$ technique to a maximum 2Θ of 45°. The scan width, $\Delta\omega$, for each reflection was $1.00 + 0.35 \tan\Theta$ with a scan speed of 3.0 deg/min. Background measurements were made for a total of 20 seconds at both limits of each scan. These standard reflections were monitored every 60 minutes. No systematic variations in intensities were found. Intensities were corrected for Lorenz and polarization effects. All non-hydrogen atoms were found by using the results of the SHELAX-86 direct method analysis.[19] After several cycles of refinements‡ the positions of hydrogen atoms were found and added with a constant isotropictemperature factor of 0.08 Å^{22} to the refinement process. Refinement processes to convergence by minimizing the function $\Sigma\omega(|Fo| - |Fc|)^2$. A final difference Fourier synthesis map showed several peaks less than 0.4 e/Å^3 scattered about the unit cell without a significant feature. The discrepancy indices, $\mathbf{R} = \Sigma \; ||Fo| - |Fc| / \Sigma \; |Fo|$ and $\mathbf{Rw} = \{\Sigma\omega \; (|Fo| - |Fc|)^2 / \Sigma\omega(|Fo|^2\}^{1/2}$ are presented with other pertinent crystallographic data in Table 1.
The equivalent isotropic **B**'s were calculated from the equation $\mathbf{B}_{eq} = (^8/_3)\pi^2(u_{11} + u_{22} + u_{33})$. Tables of atomic coordinates and the isotropic **B** equivalents of thermal parameters are given in Table 2. Additional data are available from the author (S.S.).
Final atomic coordinates for PIH are in Table **2**; bond distances and bond angles in Tables **3** and **4**.

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‡ All crystallographic computing was done on CYBER 855 computer at the Hebrew University of Jerusalem, using the SHELx 1977 Structure Determination Package.

Table 1. *Experimental data for the crystallographic analysis*

Formula	$C_{14}H_{14}N_{14}O_3$
M	**286.29**
Space Group	**P21/n**
a /Å	**13.072(6)**
b/ Å	**13.06(5)**
c /Å	**8.186(2)**
β / °	**90.21(2)**
V / A^3	**1397.3(5)**
Z	**4**
ρ_{calcd}, g cm^{-3}	**1.36**
μ(MoKα), mm^{-1}	**6.10**
no. of unique reflections	**1799**
no. of reflections with I ≥ 26 (I)	**1375**
R	**0.050**
R_w	**0.064**
w^{-1}	**Δ^2_F +0.000629F^2**

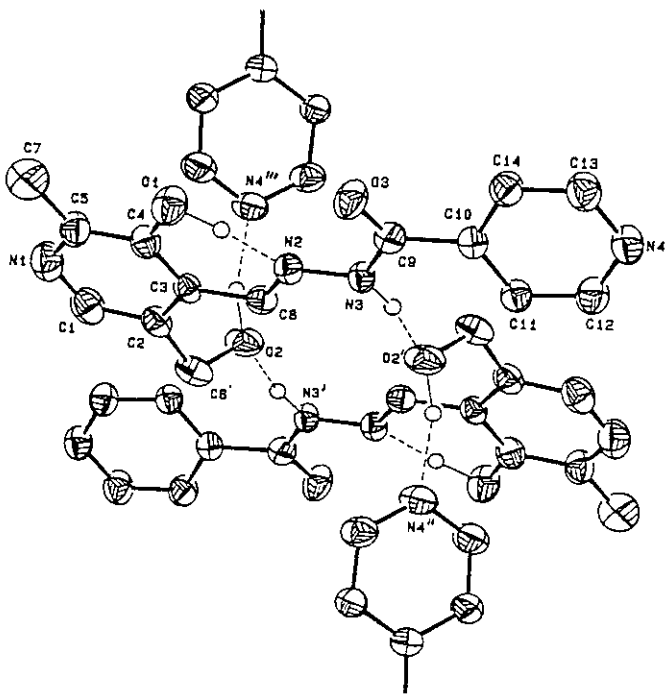

Figure 1. **ORTEP deawing of PIH with the atom-numbering scheme and thermal ellipsoids at the 50% probability**

Table 2. *Positional Parameters ans Isotropic Squivalent B Parameters of PIH (with Estimated Standard Deviations in Parantheses)*

atom	X	Y	Z	B
O(1)	.2622 (2)	.5147 (2)	.5340 (3)	4.10 (1)
O(2)	.3845 (2)	.4007 (2)	-.0833 (3)	3.63 (1)
O(3)	.4479 (2)	.7178 (2)	.6025 (3)	3.92 (1)
N(1)	.1565 (2)	.3001 (2)	.3174 (4)	4.39 (1)
N(2)	.4262 (2)	.5688 (2)	.3801 (3)	2.71 (1)
N(3)	.5055 (2)	.6366 (2)	.3751 (3)	2.55 (1)
N(4)	.7354 (2)	.9496 (2)	.4436 (3)	3.55 (1)
C(1)	.2278 (3)	.2872 (3)	.1996 (5)	3.86 (1)
C(2)	.3142 (2)	.3480 (2)	.1811 (4)	3.02 (1)
C(3)	.3288 (2)	.4291 (2)	.2935 (4)	2.50 (1)
C(4)	.2556 (2)	.4408 (2)	.4171 (4)	3.08 (1)
C(5)	.1702 (3)	.3760 (3)	.4244 (4)	3.60 (1)
C(6)	.3871 (3)	.3263 (3)	.0459 (4)	3.63 (1)
C(7)	.0923 (3)	.0881 (3)	.5573 (5)	
C(8)	.4156 (2)	.4991 (2)	.2797 (4)	2.71 (1)
C(9)	.5083 (2)	.7132 (2)	.4889 (4)	2.76 (1)
C(10)	.5898 (2)	.7930 (2)	.4660 (4)	2.63 (1)
C(11)	.6762 (2)	.7815 (2)	.3702 (4)	2.86 (1)
C(12)	.7472 (3)	.8612 (3)	.3633 (4)	3.42 (1)
C(13)	.6513 (3)	.9604 (3)	.5344 (5)	4.00 (1)
C(14)	.5777 (3)	.8847 (2)	.5502 (4)	3.55 (1)

Table 3 *Bond Distances (Å) in PIH*
(with Estimated Standard Deviations in Parantheses)

Distance	Å	Distance	Å
O(1)--C(4)	1.362 (4)	C(2)--C(6)	1.490 (5)
O(2)--C(6)	1.438 (4)	C(3)--C(4)	1.403 (4)
O(3)--C(9)	1.223 (4)	C(3)--C(8)	1.462 (4)
N(1)--C(1)	1.354 (5)	C(4)--C(5)	1.401 (5)
N(2)--N(3)	1.368 (3)	C(5)--C(7)	1.502 (5)
N(2)--C(8)	1.278 (4)	C(9)--C(10)	1.503 (4)
N(3)--C(9)	1.368 (4)	C(10)--C(11)	1.385 (4)
N(4)--C(12)	1.338 (4)	C(10)--C(14)	1.392 (4)
N(4)--C(13)	1.336 (4)	C(11)--C(12)	1.396 (4)
C(1)--C(2)	1.389 (5)	C(13)--C(14)	1.386 (5)
C(2)--C(3)	1.416 (4)		

Table 4 *Bond Angles (°) in PIH*
(with Estimated Standard Deviations in Parantheses)

Angle	(°)	Angle	(°)
C(1)--N(1)--C(5)	117.9 (3)	N(1)--C(5)--C(7)	117.6 (3)
N(3)--N(2)--C(8)	119.2 (2)	C(4)--C(5)--C(7)	120.7 (3)
N(2)--N(3)--C(9)	116.0 (2)	O(2)--C(6)--C(2)	113.8 (3)
C(12)--N(4)--C(13)	117.4 (3)	N(2)--C(8)--C(3)	118.3 (3)
N(1)--C(1)--C(2)	124.7 (3)	O(3)--C(9)--N(3)	122.5 (3)
C(1)--C(2)--C(3)	117.7 (3)	O(3)--C(9)--C(10)	121.4 (3)
C(1)--C(2)--C(6)	119.7 (3)	N(3)--C(9)--C(10)	116.1 (3)
C(3)--C(2)--C(6)	122.7 (3)	C(9)--C(10)--C(11)	125.1 (1)
C(2)--C(3)--C(4)	117.3 (3)	C(9)--C(10)--C(14)	117.0 (3)
C(2)--C(3)--C(8)	121.4 (3)	C(11)--C(10)--C(14)	117.9 (3)
C(4)--C(3)--C(8)	121.3 (3)	C(10)--C(11)--C(12)	119.1 (3)
O(1)--C(4)--C(3)	122.8 (3)	N(4)--C(12)--C(11)	123.1 (3)
O(1)--C(4)--C(5)	116.5 (3)	N(4)--C(13)--C(14)	123.3 (3)
C(3)--C(4)--C(5)	120.7 (3)	C(10)--C(14)--C(13)	119.2 (3)
N(1)--C(5)--C(4)	121.7 (3)		

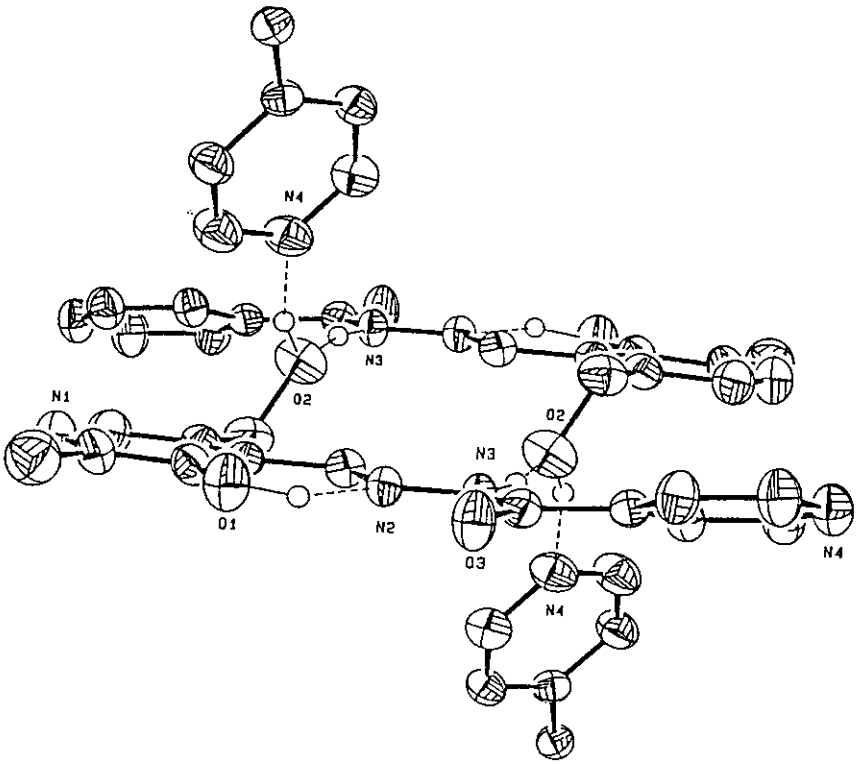

Figure 2. **Perspective view of pyridoxal isonicotinoyl hydrazone with the atom numbering scheme**

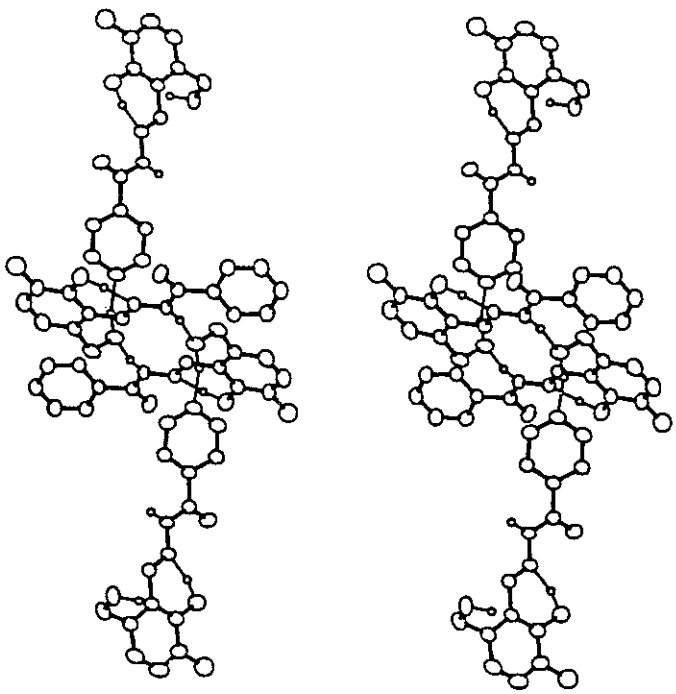

Figure 3. **The stereoscopic view of pyridoxal isonicotinoylhydrazone**

RESULTS AND DISCUSSION

From the crystallographic data assembled in Tables 1 - 4, the *ortep* drawing (Figure 1), the *perspective view* (Fi.gure 2), and the *stereoscopic view* of PIH, shown in (Figure 3), it can be seen that in the crystalline state, PIH exists as a tetramer of its *nondipolar form.* The tetramer comprise a planar 16-membered ring dimer {preferred motif [20] of $\mathbf{R^2(16)}$}, to which two additional units of PIH are H-bonded, each at right angle and in an opposite direction, to the macrocyclic ring plan at the respective alcoholic hydrogens, H-O(2). This affords a three-dimensional supramolecular structure presented by Figures. 2, and 3.

The observed bond-angle of 117.1°, for the pyridoxal-ring C(5)-N(1)-C(1) angle (see, Table 4), is consistent with a *nondipolar* rather than with a *dipolar form* for PIH, since the respective bond-angle for the latter should have been larger by 7°, namely, to be around 124°.[21] The later is indicaive also for the energetically favored trans relationship between the hydrazidic, and the hydroxymethyl side-chains,[22] as delineated by formula (IIb) (see Scheme 1). Moreover, the crystal structure confirms a s-trans conformation for the hydrazide function, namely, that the carbonyl-oxygen, **O**(3), and the hydrazinic-hydrogen, **H**-N(3), are trans oriented.

Analysis of the intramolecular X-**H** ...Y distances in crystalline PIH (see Table 4) indicate that the tetrameric assembly is stabilized by virtue of two distinctly different *intermolecular* modes of H-bondings. One, is of a longer distance (1.850 Å), and of the "non-linear three-centered" type (bond-angle:133.09°, **3-C** bond).[23] This mode allows the formation of a 16-*membered ring dimer* by linking two alcoholic oxygens, **O**(2), with two hydrazidic hydrogens, **H**-N(3). As with H_2O, the **3-C** bond is energetically favored due to "cooperative effect".[24]

The second mode, is of the "linear" ("two center", **2-C**) type, characterized by its *shorter length* (1.726 Å), and its *larger* bond-angle (159.84°). It is favored for attaching two additional PIH units, to the protruding alcoholic hydroxyl groups at the *upper* and at the *lower* sides of the dimeric macrocycle plan. Thus, the alcoholic hydroxyl-hydrogen, H-O(2), function as H-donors, whereas the pyridoxal-ring nitrogens N(1), function as H-acceptors. It is noteworthy that the pyridoxal alcoholic group in H_2O-free PIH plays a pivotal role in aggregating the four molecules in a specific form to yield the observed tetrameric supramolecule (Figure 2).

Unlike H_2O-free PIH, the hydrated form {PIH.H_2O}[25] reportedly[15] crystalizes differently, forming another type of 18-membered ring dimer, in which the water molecules function as links in the cyclic dimer, connecting the two PIH molecules, by use of $\mathbf{HO^1 ... H_2O ...H}$-$N^3$ *intermolecular* **H**-contacts. This indicates that water O competes effectively with hydrazidic-N^2, as H-acceptor for the phenolic H, rendering *intermolecular* H-bonding more favorable than the *intramolecular* H-bonding. The later becomes favorable only in absence of H_2O, since it gives rise to strong O(1)-H ... N(2) H-contacts due to its short length (1.554 Å). [25]

Whereas all potential proton donors in PIH, H-O(1), H-O(2), and H-N(4), are utilized in H-bonding, only three of the potential proton-acceptors (N^1, N^2, and O^2) of pKa higher than 4.52, [27] are actually engaged in H-bonding. It is significant that the pyridinic ring-N^4, of lower basisity (pKa = 2.45),[27] is not utilized for forming H-bonds.

In this context it is worthy of note that the insertion of Fe^{3+} ions renders PIH prone to tautomeric shift, and consequently to changes in patterns of *H*-bonding. The PIH moiety in the $[PIH]_2Fe(III).^1/_2H_2SO_4$ complex was shown[17] to exist in an *enolic-dipolar* form (IIIb)-(IIIc), structured as a 26-membered ring dimer, in which, the pyridinic-**N**(4) and the alcoholic **H**-*O*(2) hydrogen, make *H*-contacts, and the pyridoxal ring-$\mathbf{N}^1$ is protonated. These confer low potential energy[22] to the dipolar form of PIH moiety. Further stabilization of the later atises from H-contacts between sulphate ion-oxygen and N^1-H proton. A very special demonstration of dimer formation involving pyridoxal phenolic hydrogen of pyridoxinium ions, were shown reportedly[28] to give rise to *intermolecular* $O(1)$-H ... $O(2)$ *H*-contacts.

IN CONCLUSION

PIH contains three H-donors: (i) phenolic (O^1-**H**) (pKa =7.44±0.14);[27] (ii) hydrazidic (N^3-**H**) (pKa =9.94±0.50) ;[27] and (iii) alcoholic (O^2-**H**) (pKa =13.35±0.03),[27] capable of forming three distinctly different water-dependent types of H-bondings, namely, two of the "two-centered" (demonstrated by O^1-H and N^3-H), and one of the "three-centered" type (cooperative effect) shown by O^2-*H* group. The latter is observed in absence of water molecules in the crystal lattice. In presence of water, however, the alcoholic O^2-H group shows no tendency of forming H-contacts. The N^3-**H** proton, by contrast, invariably gives rise to *intermolecular* H-bonding to yield macrocyclic dimers. Unlike the latter, the phenolic proton tends to enter into *intermolecular* **H**-contacts only in presence of water molecules, whereas in absence of water it lends itself to *intramolecular* **H**-bonding, giving rise to 6-membered rings (involving O^1-H ··· N^2 bonding). Due to "cooperative effect", the pyridoxal ring-nitrogen (N^1) (pKa =2.45±0.10)[27] in H_2O-free PIH, becomes activated, lending itself to function as H-acceptor to produce three dimensional tetramer by utilizing the N^4-H ··· O^2 bond. Similar activation of pyridoxal ring-N^1 was observed following sequestration of Fe^{3+} ions. The presence of H_2O molecules in the crystal lattice of $[PIH]_2Fe(III).^1/_2H_2SO_4$[17] seemingly renders pyridoxal ring-N^1 inactive as **H**-acceptor.

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25. PIH is polymorphic, tending to crystallize in a variety of forms, differing in their crystal lattice coherence, in the ordering: PIH:H_2O:HCl, mp 246°C[5b] < PIH:$^1/_2$HCl, mp 253°C[5b] < PIH E-Z isomeric mixture, mp 255°C[5b] < PIH:H_2O, mp 257°C[2a] < PIH:$2H_2O$, mp 260-1°C[2b] < PIH, mp 262°C,[18] 268°C.[26] The presence of H_2O, and/or of HCl, in the crystal lattice of PIH appears to weaken its coherence.

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