benzene in a three-necked flask equipped with a thermometer, dropping funnel, and reflux condenser connected through a drying tube to a hydrogen halide absorber. The reaction flask was placed in a constant-temperature bath at 25 ± 0.5°. With vigorous stirring (magnetic stirrer), 0.05 mol of propionyl chloride or propionic anhydride dissolved in 30 g of CH_3NO_2 was added dropwise over a period of 10 min. The reaction was allowed to proceed an additional 10 min. The solution was then washed with water three times with approximately 200 ml of a 5% solution of NaOH (to remove CH_3NO_2) and again with water. The organic layer was separated, dried over $CaCl_2$, and analyzed by gas–liquid partition chromatography.

Determination of Isotope Effect. (a) Benzene-d_6 (0.025 mol) and toluene (0.025 mol) were dissolved in 5 g of tetramethylene sulfone and acetylated with 0.01 mol of $RCO^+SbX_6^-$ as previously described. The products were analyzed by gas–liquid partition chromatography. (b) Benzene-d_6 (0.025 mol) and benzene (0.025 mol) were dissolved in 5 g of tetramethylene sulfone and acetylated with 0.01 mol of $RCO^+SbX_6^-$ as previously described. The products were analyzed by mass spectroscopy. (c) Benzene (0.025 mol) and toluene-d_8 (0.025 mol) were dissolved in 5 g of tetramethylene sulfone and acetylated with 0.01 mol of $RCO^+SbF_6^-$ as previously. The products were analyzed by gas–liquid partition chromatography. (d) Benzene-d_6 (0.025 mol) and toluene-d_8 (0.025 mol) were dissolved in 5 g of tetramethylene sulfone and acetylated with 0.01 mol of $RCO^+SbF_6^-$ as previously described. The products were analyzed by gas–liquid partition chromatography. (e) Benzene (0.025 mol) and toluene-α,α,α-d_3 (0.025 mol) were dissolved in 5 g of tetramethylene sulfone and acetylated with 0.01 mol of $R^+SbF_6^-$ as described in a. The products were analyzed by gas–liquid partition chromatography. (f) Benzene (0.025 mol) and toluene-d_5 (0.025 mol) were acylated as in e. (g) Benzene (0.025 mol) and *m*-xylene-α-d_6 (0.025 mol) were acetylated as in e.

Gas–Liquid Partition Chromatography Analyses. The analyses were carried out on a Perkin-Elmer Model F 11 gas chromatography, using a Disc integrator or on a Perkin-Elmer 226 chromatograph with electronic integrator. On both instruments, capillary polypropylene glycol columns of 150 and 100 ft length, respectively, were used. The products were identified by comparison with authentic samples. When these were not available, they were prepared by standard methods. Molar response factors were determined by standard methods.

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A Conformationally Defined Imine Derivative of Pyridoxal: 7,8-Dihydro-3-methyl-2,6-naphthyridin-4-ol[1]

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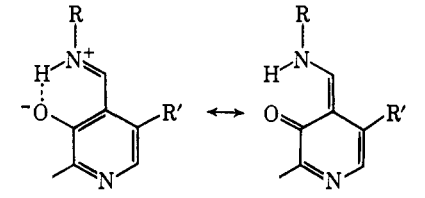
Contribution from the Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa 50010. *Received April 28, 1969*

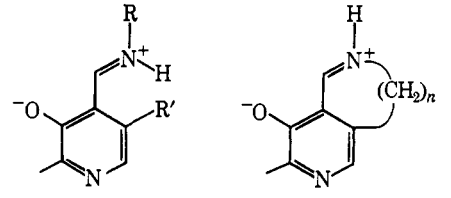
Abstract: 7,8-Dihydro-3-methyl-2,6-naphthyridin-4-ol (**9**), a cyclic imine derivative of pyridoxal, has been synthesized. The C=N group of this compound lies in a fixed position corresponding to one of the possible conformations of the imine group in pyridoxal phosphate dependent enzymes and opposite to the conformation of hydrogen-bonded imines of pyridoxal previously studied. Although the cyclic imine is catalytically inactive, due to the stability of the six-membered ring which includes the imine group, its electronic absorption spectrum is strikingly similar to those of some pyridoxal phosphate containing enzymes. Derivatives of the cyclic imine have been prepared by oxidation, reduction, and reaction with hydroxylamine.

Studies on several B_6-dependent enzymes have revealed that an imine bond links the aldehyde function of the coenzyme to the ϵ-amino group of a lysine residue on the apoenzyme.[2] The imine group is thought to have a central function in the catalytic mechanism of these enzymes. Thus, a thorough understanding of the chemistry involved is of fundamental importance.

Imines of pyridoxal and related substances with amino acids and amines have been studied.[3–5] At low pH most of these substances exist as internally hydrogen-bonded structures, **1**. Resonance form **1a** depicts the compound as a protonated imine, emphasizing its direct relationship to the parent aldehyde and amine, while resonance form **1b** shows more clearly the keto–enamine nature of structure **1**, which Heinert and Martell have shown to predominate in aqueous solution.[4] The hydrogen bonding is assumed to hold the molecule in conformation **1**. However, a second conformation, **2**, in which the imine double bond and the pyridine ring are again coplanar, is also possible, and doubtless exists at high pH where the chelated hydrogen has dissociated.



1a **1b**



2 **3**

(1) This investigation was supported by a grant from the U. S. Public Health Service (AM-01549). Use of the Varian HA-100 nmr spectrometer was made possible by an equipment grant to Iowa State University from the National Science Foundation.

(2) B. M. Guirard and E. E. Snell in "Comprehensive Biochemistry," Vol. 15, M. Florkin and E. H. Stotz, Ed., American Elsevier Publishing Co., New York, N. Y., 1964, pp 138–174.

(3) D. E. Metzler, *J. Am. Chem. Soc.*, **79**, 485 (1957).

(4) D. Heinert and A. E. Martell, *ibid.*, **85**, 183 (1963).

(5) T. C. French, D. S. Auld, and T. C. Bruice, *Biochemistry*, **4**, 77 (1965).

However, it could also exist in enzymes in the N-protonated form as shown. (In addition, other conformations in which the imine group is not coplanar with the pyridine ring are possible.)

The possibility that the aldimine linkage in pyridoxal-dependent enzymes could exist in conformation **2** prompted us to a consideration of compounds of type **3**. Here, for small values of n, the imine is locked in conformation **2**, permitting a study of the chemical and spectral characteristics of this conformation. This investigation examined the compound for which $n = 2$.

Addition of substrate to the imine double bond is the first step in the action of a pyridoxal-containing enzyme, and Cordes and Jencks have argued that the preexisting imine group of the enzyme reacts faster than would a free aldehyde group to yield the substrate-containing imines which are intermediates in further steps of the catalysis.[6]

Experimental Section

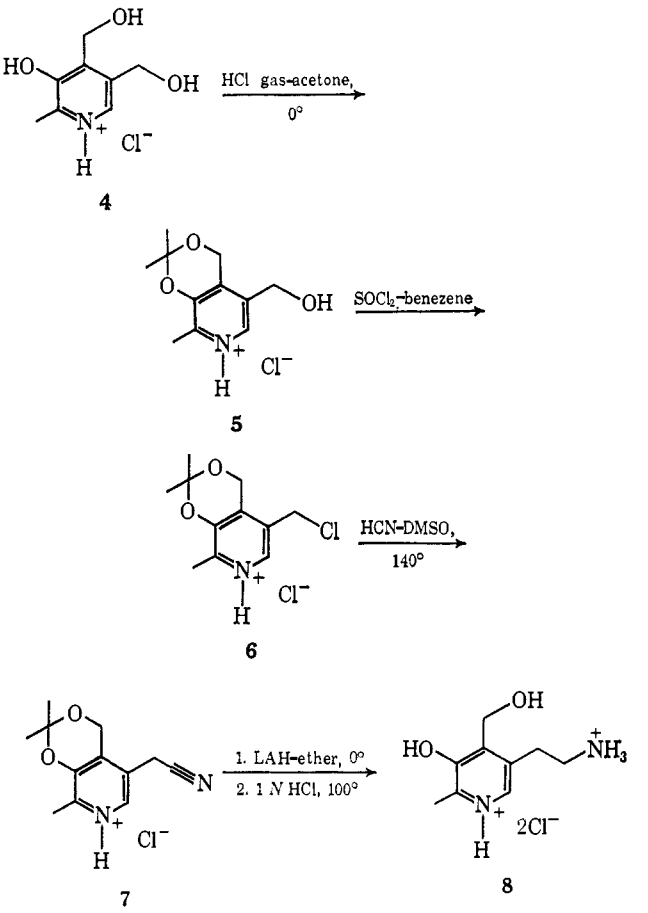
The following instrumentation and techniques were employed: Varian A-60 and HA-100 nuclear magnetic resonance spectrometers, probe at ambient temperature; Perkin-Elmer 270 mass spectrometer, molecular ions identified by observing the change in relative intensities as the ionizing voltage was reduced; Perkin-Elmer 21 infrared spectrophotometer, KBr pellet, absorption maxima reported in kaysers (K), 1 K = 1 cm^{-1}.

A Cary 15 spectrophotometer equipped with a Datex digital output and a Corning 12 pH meter were used to determine the pH dependence of the ultraviolet–visible spectra. The apparent pK's and the spectra of the pure ionic forms of the compounds were then calculated by computer.[7] Absorption maxima are reported in kilokaysers (kK); wave number (kK) = 10^4/wavelength (mμ). Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

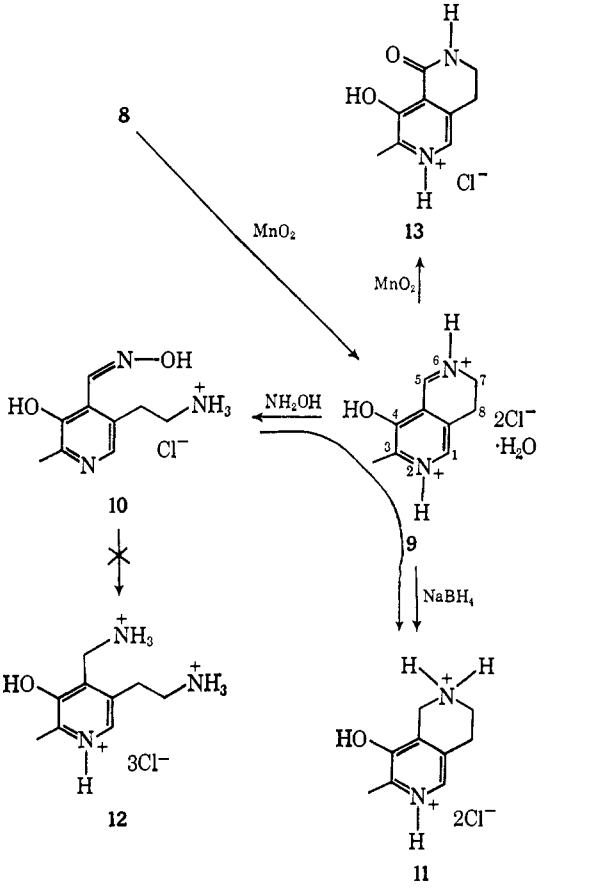
5-(2′-Aminoethyl)-3-hydroxy-2-methyl-4-pyridinemethanol dihydrochloride (8) was prepared from vitamin B_6 (pyridoxol hydrochloride) (**4**) by the reaction sequence shown in Scheme I. Compounds **5**, **6**, **7**, and **8** were synthesized by the methods of Iwata,[8] Tomita and others,[9] Korytnyk and others,[10] and Tomita and others,[9] respectively.

7,8-Dihydro-3-methyl-2,6-naphthyridin-4-ol Dihydrochloride Monohydrate (9, Scheme II). 5-(2′-Aminoethyl)-3-hydroxy-2-methyl-4-pyridinemethanol dihydrochloride (**8**) (0.51 g, 2 mmol) was dissolved in 50 ml of water and vigorously stirred by a magnetic stirrer. Freshly pulverized manganese dioxide (1.0 g), prepared according to the method of Iwata,[8] was added all at once. The progress of the reaction was monitored by withdrawing 0.1 ml of the reaction suspension and diluting this aliquot to 50 ml with 0.1 M acetate buffer, pH 5.0. The sample was then filtered through Whatman no. 1 filter paper after which absorbance at 23.3 kK was measured. When the 23.3-kK peak reached its maximum (~15 min), the reaction was complete. Subsequent operations were performed in the dark. The reaction suspension was filtered through Whatman no. 1 filter paper, and the residue of manganese dioxide was washed with two 25-ml portions of water. The clear, yellow-orange solution was then evaporated under reduced pressure in a 50° water bath to a volume of about 3 ml. The solution was applied to an 8.9 × 2.75 cm column of Dowex AG 50W-X8 and eluted at a rate of 2 ml/min with 2 N hydrochloric acid to remove the divalent manganese and starting material. The fluorescent yellow band was collected and was evaporated to dryness under reduced pressure (50° water bath). The residue was dried over magnesium sulfate, under vacuum, with protection from light: yield, 0.43 g (85%) of yellow-white solid; nmr (D_2O, pD 3.7, DSS) δ 2.57 (s, 3, 3-CH_3), ~3.17 (t, 2, splitting = 7.8 Hz, 8-CH_2), ~4.03 (t, 2, splitting = 7.8 Hz, 7-CH_2), 7.37 (broad s, 1, 1-H), 9.07 ppm (broad

Scheme I



Scheme II



(6) E. H. Cordes and W. P. Jencks, *J. Am. Chem. Soc.*, **84**, 826 (1962).
(7) K. Nagano and D. E. Metzler, *ibid.*, **89**, 2891 (1967).
(8) C. Iwata in "Biochemical Preparations," Vol. 12, W. E. M. Lands, Ed., John Wiley & Sons, Inc., N. Y., 1968, p 117.
(9) I. Tomita, H. G. Brooks, and D. E. Metzler, *J. Heterocycl. Chem.*, **3**, 178 (1966).
(10) W. Korytnyk, B. Paul, A. Bloch, and C. A. Nichol, *J. Med. Chem.*, **10**, 345 (1967).

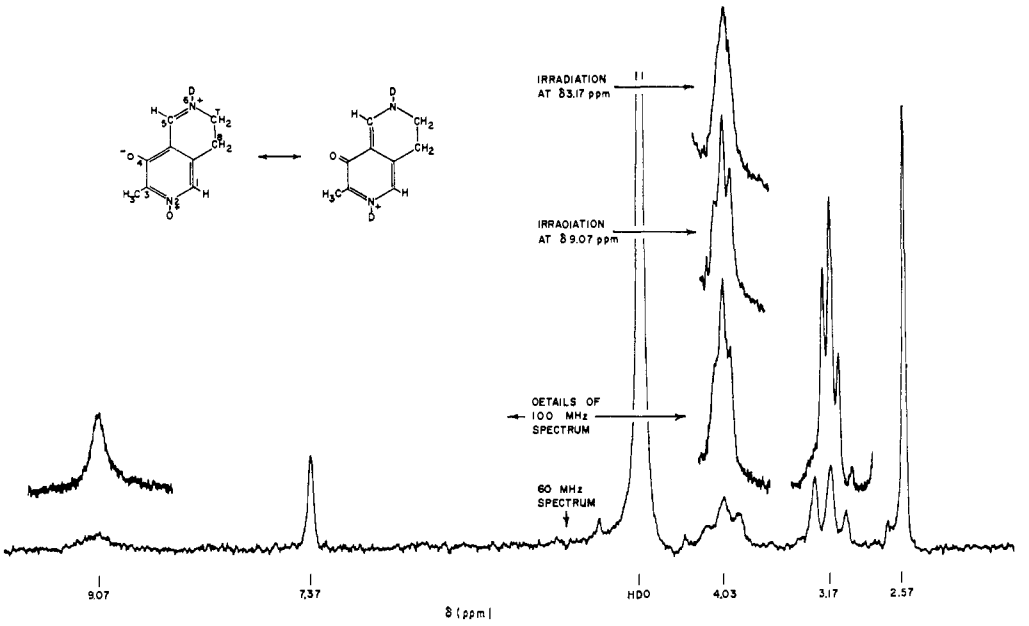


Figure 1. Nmr spectrum of cyclic imine **9** in D_2O, pD 3.7.

s, 1, 5-H); mass spectrum, *m/e* 162 (M^+); ir 3300, 2550 (b), 1669, 1543, 1319 and 1297, 1245, 927, 717 K; uv (0.1 *M* formate, pH 3.70) ν_{max} 23.3 (ϵ 10,200), 33.8 (2090), 41.3 kK (11,700). See also Figures 2 and 3. For other properties, see Table I. Note: detectable decomposition occurs on exposure of this compound to temperatures above 50° or to room lighting for more than 1 hr.

Table I. Properties of Synthesized Compounds

Compd	Mp,[a] °C	p*K*[b]	Tlc, R_f[c]	Hve, cm[d]
9	~200	1.35, 5.99, 8.86	0.47[e,f]	18.1
10	227–229	*g*	*g*	*g*
11	239–241	3.74, 8.48, 10.0	0.45[f,h]	20.3
13		4.53, 10.1	0.47[f]	14.1

[a] Hot stage microscope, uncorrected. [b] Determined by computer, see text. [c] 250 μ × 20 cm × 20 cm coating of silica gel H, 30% acetic acid solvent. [d] Hve = high voltage electrophoresis, Whatman no. 3 paper, 2000 V for 1.0 hr, pyridine–acetic acid–water (1.0:3.4:409, pH 4.0) buffer. [e] Intense yellow fluorescence. [f] Gibbs positive (phenol *para* to aromatic hydrogen present). [g] Unstable in solution. [h] Ninhydrin positive (amine present).

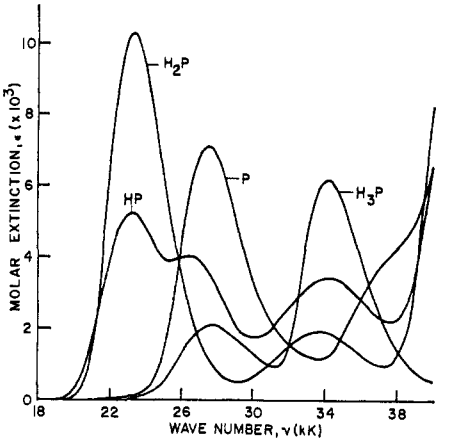


Figure 2. Molar extinction coefficients of four ionic forms of cyclic imine **9** plotted against wave number. H_3P represents the most highly protonated form and P the completely deprotonated form. See Scheme III.

Anal. Calcd for $C_9H_{14}Cl_2N_2O_2$: C, 42.70; H, 5.58; Cl, 28.01; N, 11.07; O, 12.64. Found: C, 42.80, 42.51; H, 5.27, 5.62; Cl, 27.53; N, 11.04; O, 12.60.

Equilibrium constants for the reactions of **9** with hydroxylamine and with sodium bisulfite at pH 5.0 (0.05 *M* acetate buffer) were evaluated spectrophotometrically by observing the intensity of the 23.3-kK peak in ~10^{-4} *M* solutions of **9**. The carbonyl reagent concentration was varied from 10^{-1} to 10^{-5} *M*.

The equilibrium constant for the reaction of 2,5-dimethyl-3-hydroxy-4-pyridinecarboxaldehyde (5-deoxypyridoxal) was determined in a similar manner.

5-(2′-Aminoethyl)-3-hydroxy-2-methyl-4-pyridinecarboxaldehyde Oxime Hydrochloride (10). Hydroxylamine hydrochloride (0.04 g, 0.6 mmol) was added to a stirred solution of **9** (0.12 g, 0.5 mmol) in 2 ml of water. The solution was passed through a Millipore filter, the pH was adjusted to 5.0 with concentrated sodium hydroxide, and the preparation was stored overnight at 4°. The beige crystals were isolated by filtration and dried under vacuum, over magnesium sulfate; yield, 0.073 g (67%).

Addition of another 0.04 g of hydroxylamine hydrochloride to the supernatant resulted in only 0.003 g of additional material: nmr (DMSO-d_6, TMS) δ 2.38 (s, 3, CH_3), ~2.52 (t, 2, splitting = 2 Hz, $-CH_2-{}^+ND_3$), ~2.98 (t, 2, splitting = 2 Hz, $ArCH_2-$), 7.90 (s, 1, aromatic H), 8.78 ppm (s, 1, aldoxime H); mass spectrum, no M^+, *m/e* 162 ($-NH_2OH$); ir 3420, 2970 (b), 1618, 1460, 1395, 1263, 1144, 1042, 945, 876, 732 K (b); uv (saturated hydroxylamine hydrochloride solution, pH 1.38) ν_{max} 30.6 (ϵ 7020), 36.1 kK (9160); for other properties, see Table I.

Anal. Calcd for $C_9H_{14}ClN_3O_2$: C, 46.66; H, 6.09; Cl, 15.30; N, 18.14. Found: C, 46.78; H, 6.14; Cl, 15.15; N, 18.02.

3-Methyl-5,6,7,8-tetrahydro-2,6-naphthyridin-4-ol Dihydrochloride (11). Compound **9** (0.12 g, 0.5 mmol) was dissolved in 2 ml of 1.0 *M* formate buffer, previously adjusted to pH 3.7. Sodium borohydride (0.06 g, 1.6 mmol) was cautiously added to the vigorously stirred solution in ~0.01-g portions. The original yellow color of the solution eventually disappeared. The reaction solution was then applied directly to a 26 × 1.2 cm column of Dowex AG 50W-X8. Approximately 500 ml of 2 *N* hydrochloric acid was passed through the column at a rate of 0.3 ml/min. The eluent was then changed to 4 *N* hydrochloric acid, and 5-ml fractions were collected for the next 200 ml. The larger of the peaks absorbing at 35.7 kK was isolated and evaporated to dryness under reduced pressure, at which time crystals formed. The sample (0.092 g) was kept overnight under vacuum over magnesium sulfate; yield 82%.

The analytical sample was recrystallized from methanol–ether: nmr (D_2O, DSS) δ 2.70 (s, 3, 3-CH_3), ~3.28 (t, 2, splitting = 5.3 Hz, 8-CH_2), ~3.65 (t, 2, splitting = 5.3 Hz, 7-CH_2), 4.60 (s, 2, 5-CH_2), 8.22 ppm (s, 1, 1-H); mass spectrum, *m/e* 164 (M^+); ir

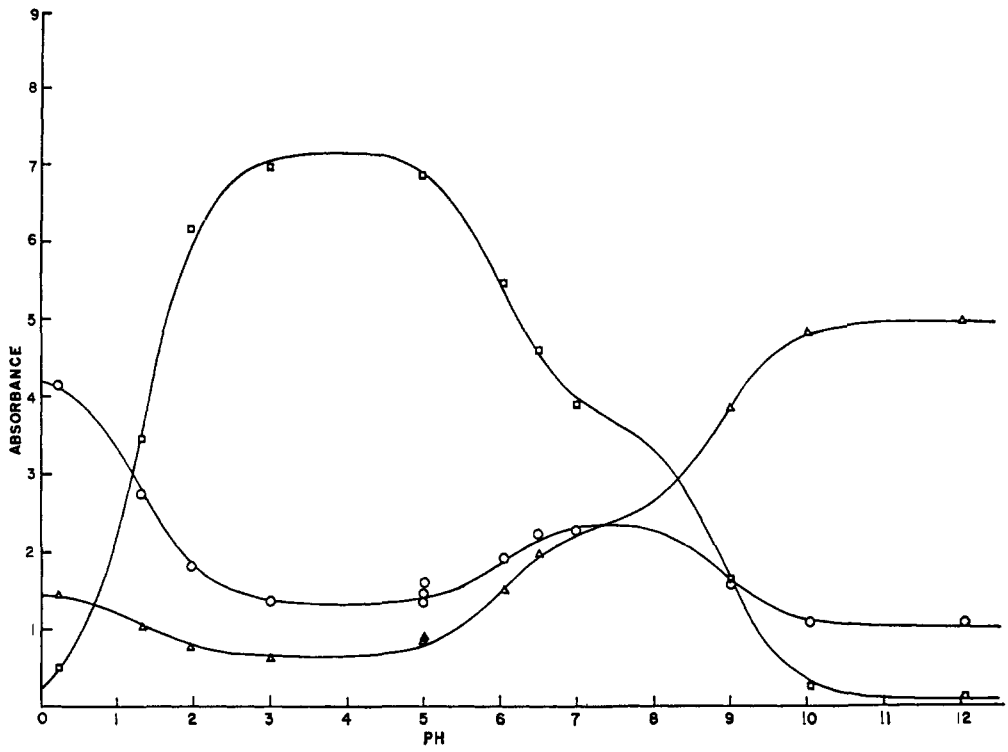


Figure 3. Absorbance of **9** at 23.3 (○), 27.8 (△), and 34.5 kK (□) plotted aginst pH. The calculated curves (———) are based on the spectra in Figure 2 and pH values of 1.35, 5.99, and 8.86.

3410, 2670 (b), 1541, 1198, 983, 908, 902 K; uv (0.1 *M* NaOH, pH 12.46) ν_{max} 33.5 (ϵ 7560), 40.7 kK (6940). See also Figure 4. For other properties, see Table I.

Anal. Calcd for $C_9H_{14}Cl_2N_2O$: C, 45.59; H, 5.95; Cl, 29.90; N, 11.81. Found: C, 45.33; H, 5.90; Cl, 29.77; N, 11.69.

Synthesis of 5-(2′-aminoethyl)-4-aminomethyl-2-methyl-3-pyridinol trihydrochloride (**12**) was attempted. Sodium borohydride (0.05 g, 1.3 mmol) was dissolved in 2 ml of water to which 1 drop of ~1 *N* sodium hydroxide had been added. Finely powdered **10** (0.11 g, 0.5 mmol) was added to the vigorously stirred borohydride solution, and the reaction solution was stirred for 1 hr. Concentrated hydrochloric acid was added dropwise until the bubbling ceased. The solution was then applied to a Dowex column, and a procedure similar to that used for the isolation of **11** was followed. Thin layer chromatography, mass spectrometry, and mixture melting point determination indicated that the product was **11** rather than the anticipated **12**.

4-Hydroxy-3-methyl-5,6,7,8-tetrahydro-2,6-naphthyridin-5-one Hydrochloride (13). The procedure for the preparation of **11** was followed except that the very small peak (the peak area was about $^1/_{100}$ that of **11** at 35.7 kK) which eluted immediately before **11** was isolated. The yield of **13** was estimated by spectrophotometry to be 1.5 mg. The solution was evaporated to dryness under reduced pressure and dried under vacuum, over $CaCl_2$. The material was sufficient for measurement of the mass spectrum and electronic spectra at several values of pH.

Attempts to increase the yield of **13** by a longer oxidation with MnO_2 led only to a complex mixture of products containing a small amount of **13**; mass spectrum, *m/e* 178 (M^+).

From electronic spectra at 15 values of pH, the spectra of the three ionic forms were evaluated: H_2P, ν_{max} 31.2; HP, 31.4; P, 28.5 kK. For other properties, see Table I.

Results

Proof of Structure. The central compound in this study, **9**, has not been recrystallized despite repeated efforts. It exists as an amorphous solid. Its unusual structure is supported by detailed analysis of the electronic absorption spectra, chemical reactions, and the following instrumental data.

To obtain an interpretable nmr spectrum (Figure 1), it was necessary to adjust the pD to 3.7. At that pD only one ionic form is present in appreciable concentration; the peaks, particularly those at δ ~3.17 and ~4.03 ppm, became much more distinct.[11]

Three of the absorptions are readily interpreted. The sharp peak at δ 2.57 ppm falls at approximately the same place as the methyl group of most other vitamin B_6 derivatives. Similar correlations identify the δ 7.37 ppm peak with the one-aromatic proton. The extremely low-field, very broad absorption represents the aldimine proton. Double irradiation at 100 MHz made possible the assignment of the two apparent triplets. Irradiation of the aldimine proton sharpened the triplet at δ 4.03 ppm but had no effect upon the δ 3.17 ppm peak. The δ 4.03 ppm peak must represent the 7-methylene protons. Irradiation of the aromatic proton had negligible effect on the triplet at δ 3.17 ppm, even though the aromatic proton would be expected to be broadened somewhat by allylic coupling to the 8-methylene protons. On the other hand, the coupling between the aldimine proton and the 7-methylene protons is quite apparent when the triplet is collapsed to a broad singlet by decoupling the other triplet at δ 3.17 ppm.

In addition to the broadening effects on the two triplets by the downfield singlets, there is an additional amount of broadening caused either by ring puckering of the partially saturated ring or deuteron exchange, or both. Inasmuch as the two methylene peaks form an A_2B_2 system, the chemical shifts are approximate.

Under suitable conditions the mass spectrum of the compound gives a molecular ion of *m/e* 162. The mo-

(11) It was thought initially that the indistinct spectrum might be caused by a small amount of Mn(II) remaining after purification. However, three types of analyses yielded a mole ratio of Mn(II) to **9** of considerably less than 1:1000.

lecular ion is increased by one mass unit if the compound is isolated from D_2O. Pyrolysis products become prevalent if the probe temperature exceeds 50°.

The infrared spectrum shows no carbonyl absorption band.

The electronic absorption spectrum, discussed in detail later, is consistent with an aromatic system possessing one exocyclic imine double bond.

The aldimine yields a positive Gibbs test, thus confirming the presence of a phenolic function *para* to an aromatic hydrogen.

Derivatives. Compound **9** is reduced by either sodium borohydride or hydrogen on 10% palladium–charcoal to yield **11**. If the compound existed as a free aldehyde, it would be expected to revert, upon reduction, to **8**. A careful search for this product following reduction of **10** revealed only one component other than the expected **11**. This minor component was found to be the lactam **13**, which was subsequently shown to be the only detectable impurity associated with **9**. Lactam **13** cannot be separated from **9** chromatographically, but it is resistant to reduction, thus making its isolation feasible (Experimental Section).

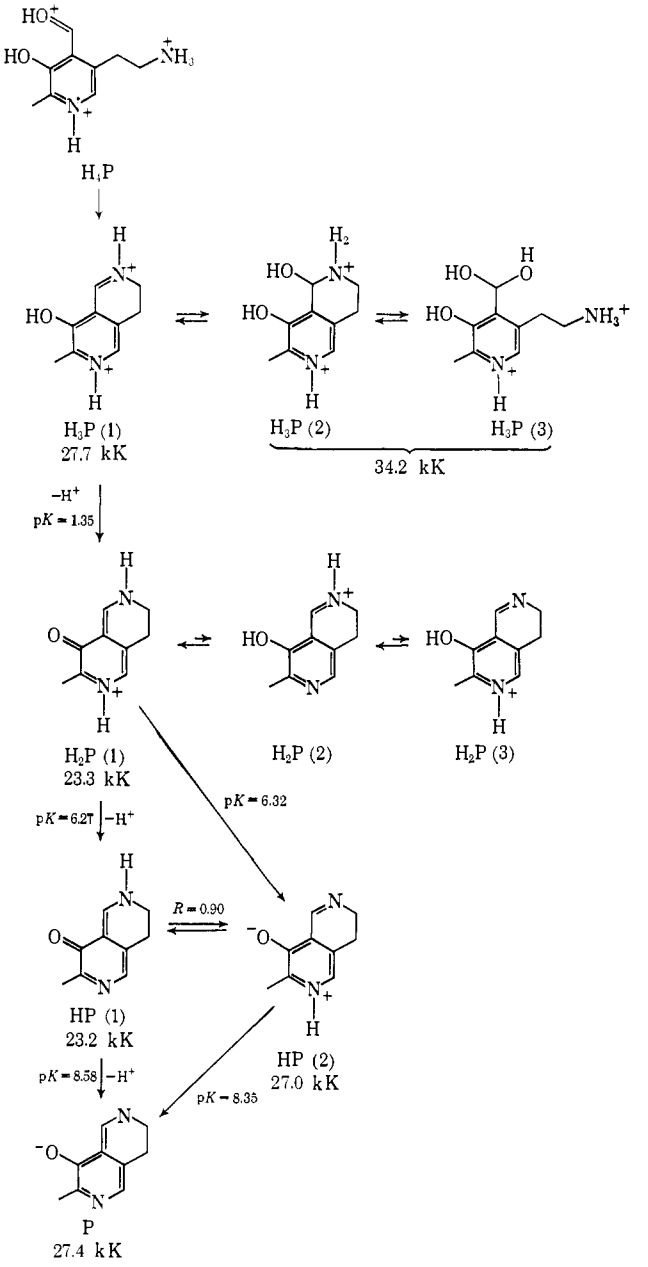
Attempts to synthesize 5-(2′-aminoethyl)-4-aminomethyl-2-methyl-3-pyridinol trihydrochloride (**12**) from the oxime resulted in formation of **11**. This is due to the instability of the oxime. It readily loses hydroxylamine in aqueous solution or under high vacuum to give **9**. If a reducing agent is present, the imine is then reduced in the expected manner.

The imine **9** (10^{-4} *M*) showed no tendency to participate in a transamination reaction with alanine (10^{-2} *M*) when incubated for up to 40 hr within a pH range of 1–9, or for up to 20 hr under the same conditions but with 10^{-3} *M* $KAlSO_4$ present as a catalyst.[12] Absence of transamination was inferred from the lack of change in the electronic spectrum.

Electronic Absorption Spectra. The pH dependence of the electronic absorption spectrum of **9** was subjected to extensive computer analysis. The p*K*'s for the compound were found to be 1.35, 5.99, and 8.86. Thus four ionic forms, each bearing one less proton than the preceding one, are clearly distinguished; they are designated as H_3P, H_2P, HP, and P (Figure 2, Figure 3). A further spectral alteration at very low pH's (~4 *N* HCl) suggests the existence of still another form, H_4P. Each of the ionic forms can consist of several subforms, the most important of which are indicated in Scheme III.

A possible structure for the ionic form, H_4P, is the ring-opened, protonated carbonyl (Scheme III). Under conditions in which form H_3P predominates, a major fraction of **9** must exist as hydrates, such as H_3P (2) or H_3P (3), which absorb at about 34.2 kK, while H_3P (1) absorbs at 27.7 kK (Figure 2). The H_2P form, which exists in weakly acidic solutions, has been assigned the structure H_2P (1) on the basis of spectral studies of similar systems by Heinert and Martell.[4] Form H_2P (1) predominates in water–dioxane solutions in which the dioxane concentration is as high as 50%, presumably because of the nonpolar character of this subform. The spectra in Figure 2 have been resolved by fitting log normal distribution curves.[13] It is clear that the

Scheme III



half-width (3.55 kK) and skewness (ρ = 1.377) of the 23.3-kK band in the spectrum of H_2P, Figure 2, are within the normal range observed for single ionic species of related substances. However, the peak at 33.7 kK (half-width = 5.70 kK, skewness = 1.094) is abnormally broad and less skewed than usual, a strong indication that this peak represents a mixture. Structures H_2P (2) and H_2P (3), their hydrates, a second weak absorption band belonging to structure H_2P (1), and a small amount of **13** (a known impurity in **9**) all contribute to the 33.7-kK peak. Similar considerations apply to the higher energy band of the spectrum of HP.

Form HP, which predominates in the pH range 7–8, appears to possess two components in approximately equal amounts which, by comparison with spectra of other imines, are assigned structures HP (1) and HP (2). By assuming the extinction coefficient of HP (1) is the same as that of H_2P (1) and that of HP (2) the same as that of P, and resolving the curve as log normal distri-

(12) D. E. Metzler and E. E. Snell, *J. Am. Chem. Soc.*, 74, 979 (1952).

(13) D. B. Siano and D. E. Metzler, *J. Chem. Phys.*, in press.

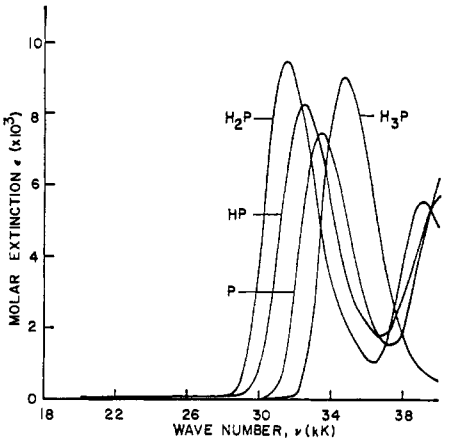
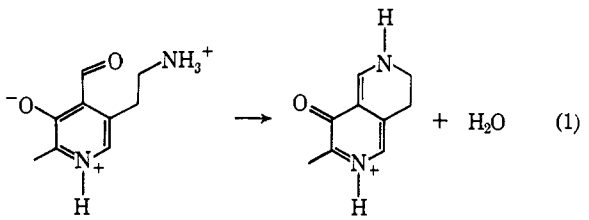


Figure 4. Molar extinction coefficients of four ionic forms of cyclic amine **11**. H_3P represents the most highly protonated form and P the completely unprotonated amine.

butions, the tautomeric ratio, R = HP (2)/HP (1) is evaluated as 0.90. If it is assumed that H_2P and P consist of single species, the intrinsic pK's shown in Scheme III can be estimated for the individual groups in structures H_2P (1), HP (1), and HP (2) from pK_2, pK_3, and the tautomeric ratio, R. Similar analysis was done on the spectrum of **11** (Figure 4).

Discussion

The inactivity of **9** in the model nonenzymic transamination is probably a consequence of the rather high stability of the cycloaldimine ring. This hypothesis is supported by the observed difference in equilibrium constants for the reaction with hydroxylamine of **9** and of 5-deoxypyridoxal. The constants at pH 5 are 1.2×10^3 and 38×10^3 l. mole^{-1}, respectively. If all other differences between the two compounds are ignored, the standard free energy change for ring formation (eq 1) can be calculated from the ratio of these equilibrium constants as $\Delta G^\circ = -2.0 \pm 0.2$ kcal/mole.



8

Although the imine is apparently resistant to transaldimination, it does undergo reactions in nonaqueous conditions. The nature of these reactions is currently under study.

It is not surprising that **9** does not bind specifically to the apoenzyme forms of muscle phosphorylase b (rabbit), aspartate aminotransferase (soluble, α-subform, pig heart), or glutamic decarboxylase (*E. coli*). The lack of binding may be, in part, a consequence of the stability of the cycloaldimine ring. However, even if **9** did react with the ϵ-amino group of the lysine at the binding site for pyridoxal phosphate, the ammonium group of **9**, which would be released by this transaldimination reaction, would doubtless be repelled by any positively charged group which might be present at the phosphate binding site, thus inhibiting binding.

The electronic spectrum of **9** is strikingly similar to that of the pyridoxal phosphate dependent enzyme, aspartate aminotransferase (pig heart, soluble, α-subform). The absorption maximum of the enzyme is at 23.3 kK at low pH and shifts to 27.5 kK upon dissociation of a single proton with a pK of 6.3. From Scheme III we see that form H_2P (1) also absorbs at 23.3 kK and shifts to 27.0 kK with dissociation of a single proton of pK 6.3. This suggests that structures of type **2** must be considered for vitamin B_6 containing enzymes. However, the identity of the pK's is probably fortuitous, because it is hard to believe that the presence of the phosphate group in the coenzyme will not have a substantial effect on the pK. It is also noteworthy that structure HP (1) (Scheme III) absorbs at 23.2 kK with a peak shift to 27.4 kK, even closer to that of the enzyme at high pH and a pK of 8.6. Thus, rather similar spectra are obtained independently of the state of protonation of the pyridine ring nitrogen. Care must be exercised in assuming that a particular state of protonation of the ring of the coenzyme occurs in the enzymes.

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