[Chem. Pharm. Bull. 23(8)1852—1855(1975)]

UDC 547.821'233.03

Pyridoxamine Analogs. III.[1] Molecular Species protonated and methylated on the Pyridine Nitrogen[2]

Yoshiharu Karube[3] and Yoshikazu Matsushima[3a]

Faculty of Pharmaceutical Sciences, Kyushu University[3]

(Received January 27, 1975)

To know the absorption spectra associated with species protonated and nonprotonated on the pyridine nitrogen of pyridoxamine and its analogs, spectra of methanol solutions of the following four compounds were examined; pyridoxamine (I), 3-hydroxy-4-aminomethylpyridine (II), 1-methylpyridoxamine chloride (III), and 1-methyl-3-hydroxy-4-aminomethylpyridinium chloride (IV). Assignments of the absorption bands to molecular species present in methanol solutions are summarized in Table I. Similarities of spectral characteristics of N-protonated and N-methylated species were shown by comparison of the spectra of the compounds. On the basis of the absorption bands assigned to Zn(II), Cu(II) and Ni(II) chelate of III and IV (Table II), the presence of N-protonated species of metal chelates of I and II in acidic methanol and their spectra are discussed (Table III).

Reactions catalyzed by vitamin B_6 have been extensively studied both in enzymatic and nonenzymatic systems.[4] In most nonenzymatic reactions, metal chelates play very important roles.[1,5] It has been revealed that one of the structural features of vitamin B_6 essential for the catalysis is the heterocyclic nitrogen.[5a]

However, it is still ambiguous whether the nitrogen bears a proton during the catalysis. To elucidate the problem, it is essential to obtain a detailed knowledge of the absorption spectra associated with the species protonated and nonprotonated on the nitrogen. Since absorption spectra for N-methylated and N-protonated species are expected to be similar, it is worthwhile to investigate spectra of N-methylated derivatives in comparison with those of the parent compounds.

In this paper, we discuss absorption spectra and formation of divalent metal chelates in methanol solution on the following four compounds; pyridoxamine (I), 3-hydroxy-4-aminomethylpyridine (II), 1-methylpyridoxamine chloride (III), and 1-methyl-3-hydroxy-4-aminomethylpyridinium chloride (IV) (Chart 1).

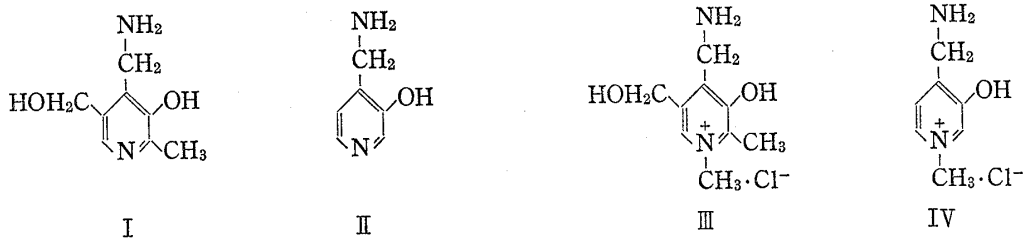


Chart 1

1) Part II: S. Matsumoto, Y. Karube, and Y. Matsushima, *Chem. Pharm. Bull.* (Tokyo), **23**, 1819 (1975).
2) This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education of Japan.
3) Location: *3-1-1, Maidashi, Higashi-ku, Fukuoka-shi, 812, Japan*; *a*) To whom inquiries should be addressed.
4) See, for example, E.E. Snell, A.E. Braunstein, E.S. Severin, and Yu. M. Torchinsky, eds., "Pyridoxal Catalysis: Enzymes and Model Systems" Interscience, New York, N.Y., U.S.A.; Y. Matsushima and S. Matsumoto, *Kagaku Zokan*, **61**, 49 (1974).
5) *a*) D.E. Metzler, M. Ikawa, and E.E. Snell, *J. Amer. Chem. Soc.*, **76**, 648 (1954); *b*) Y. Matsushima and A.E. Martell, *ibid.*, **89**, 1331 (1967).

Solution equilibria in methanol and band assignments for I and II have already been studied in detail.[6] Preparation and spectra in aqueous solution of III were reported by Pocker and Fischer,[7] though the detailed spectral assignments were not given. IV is not previously described in the literature.

Solution Equilibria and Band Assignments

It has been shown[6] that a molecular species of pyridoxamine and its derivatives exhibits two intense absorption bands in the near ultraviolet and visible regions. They are ascribed to the bands due to π—π^* transitions, which are found at 256 and 194 nm in pyridine[8] and bathochromically shifted by the substituent groups. Of the two π—π^* bands, the longer wavelength band has been designated π_1 band, whereas the shorter one π_2 band.[9b]

Figure 1 shows absorption spectra of IV in methanol. In acidic methanol solutions, there is a strong band at 293 nm and a shoulder at 230 nm, which are assigned to the π_1 and π_2 bands of the cationic species, IV_A, shown in Chart 2, respectively.

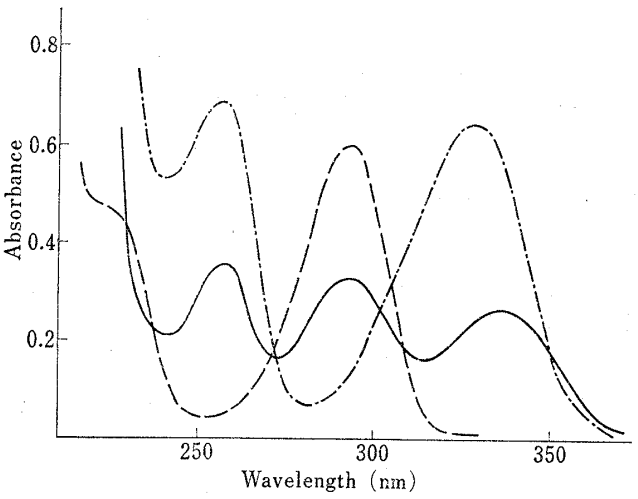


Fig. 1. Absorption Spectra of 1-Methyl-3-hydroxy-4-aminomethylpyridinium Chloride in Methanol (1×10^{-4}M)

– – – : 5×10^{-4}M HCl, ——— : 5×10^{-5}M HCl,
—·— : 5×10^{-4}M KOH

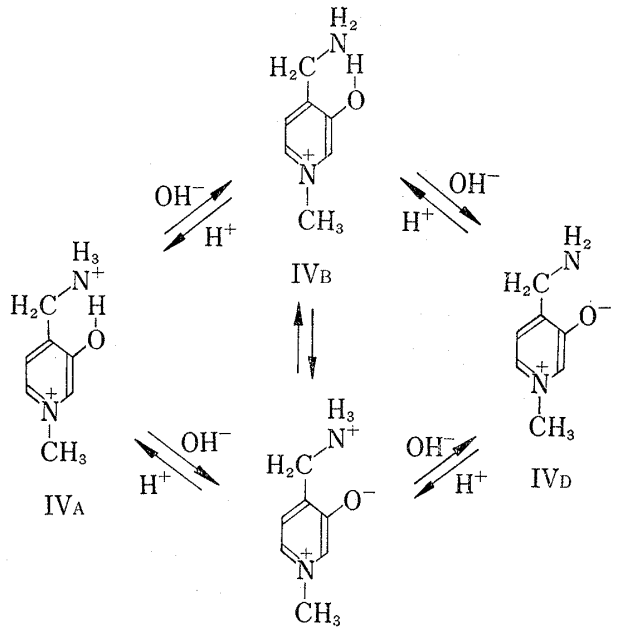


Chart 2

In weakly acidic and neutral solutions, absorption peaks were observed at 336, 294 and 257 nm. This indicates the presence of two species. One of the possible species is the neutral phenol species, IV_B. The 294-nm band is assigned to the π_1 band of this species, as little spectral change is to be expected by dissociation of a proton from the $-NH_3^+$ group of IV_A. The π_2 band of IV_B, which should be at around 230 nm, is hidden by an end absorption. The other possible species is the neutral phenolate species, IV_C. The 336 and 257-nm bands are assigned to the π_1 and π_2 bands of this species, IV_C, respectively. It is well established that a π band is shifted 30—40 nm bathochromically by dissociation of a phenol group.[9]

6) *a*) Y. Matsushima and A.E. Martell, *J. Amer. Chem. Soc.*, **89**, 1322 (1967); *b*) S. Matsumoto and Y. Matsushima, *Chem. Pharm. Bull.* (Tokyo), **23**, 106 (1975).
7) A. Pocker and E.H. Fischer, *Biochemistry*, **8**, 5181 (1969).
8) H.P. Stephenson, *J. Chem. Phys.*, **22**, 1077 (1954).
9) *a*) H.H. Jaffè and M. Orchin, "Theory and Application of Ultraviolet Spectroscopy," J. Wiley and Sons, New York, N.Y., U.S.A., 1962; *b*) K. Nakamoto and A.E. Martell, *J. Amer. Chem. Soc.*, **81**, 5857 (1959); *c*) Y. Matsushima, *Chem. Pharm. Bull.* (Tokyo), **16**, 2046 (1968).

For alkaline methanol solutions, two absorptions observed at 329 and 258 nm are assigned to the π_1 and π_2 bands of the anionic species, IV_D, respectively.

III gave similar absorption spectra in methanol and the band assignments were made analogously. Table I lists the absorption bands thus assigned with those of I and II. The data confirm the band assignments made previously for I and II,[6] and ascertain that the corresponding N-methylated and N-protonated species have almost the same spectral characteristics.

Table I. Absorption Bands of N-Methylated and N-Protonated Species[a]

Species[d]	Compounds							
	III		I[b]		IV		II[c]	
	π_1	π_2	π_1	π_2	π_1	π_2	π_1	π_2
Cationic (A)	302	226	296		293	226	289	
			(287	220)			(283)	
Neutral phenol (B)	303	226	(289)		294		(279)	
Neutral phenolate (C)	342	259	333	260	336	257	325	250
			(310	244)			(308	241)
Anionic (D)	337	260	(311	248)	329	258	(303	243)

a) Wavelength for band is given in nm. Numbers in parentheses are wavelengths of the bands of the corresponding N-deprotonated species.
b) reference 6*a*
c) reference 6*b*
d) Letters in parentheses correspond to subscripts used for the species in Chart 2 and in text.

Metal Chelates

Absorption spectra obtained by addition of perchlorates of divalent metals to neutral methanol solutions of III and IV were characterized by the presence of intense bands at slightly shorter wavelength than the corresponding π bands of the neutral phenolate species. These bands indicate the formation of metal chelates of the N-methylated compounds.[6] Wavelengths of the bands are listed in Table II.

Table II. Absorption Bands of Metal Chelates of N-Methylated Compounds[a]

Compounds	Metal ions					
	Zn(II)		Cu(II)		Ni(II)	
1-Methylpyridoxamine (III)	328	253	327	264	340	262
1-Methyl-3-hydroxy-4-amino-methylpyridinium (IV)	320	251	323		333	258

a) Wavelength for band is given in nm.

Metal chelates of I and II reported previously[6] had absorption bands at around 300 nm. Considerable difference between the π bands of the metal chelates of the N-methylated and the parent compounds indicates that the metal chelates of I and II absorbing at the 300-nm region are the N-nonprotonated species.

With a view to obtaining spectra of N-protonated species of metal chelates of I and II, methanol solutions of I and II containing excess metal ion were acidified with HCl methanol solution. Figure 2 shows spectra of solutions 1×10^{-4}M in I and 5×10^{-4}M in Ni(II) ion. In neutral and slightly alkaline solutions, there are two bands at 311 and 248 nm, which were previously assigned to Ni(II) chelate of I.[6a] The spectrum of a solution 3×10^{-4}M in HCl is identical to that of I in acidic media,[6a] and indicates that I do not complex with Ni(II) ion under the conditions. In a solution 1×10^{-4}M in HCl, however, three absorption peaks were observed at 330, 287 and 262 nm. The 287-nm band may be due to uncomplexed I.

The 330 and 262-nm bands are quite similar to those of Ni(II) chelate of III and, hence, should be assigned to N-protonated species of Ni(II) chelate of I. Similar bands were observed at around 320 nm in the presence of Zn(II) and Cu(II) ions under the similar conditions.

In the cases of II, the absorption peaks in the 320-nm region were lower, especially with Zn(II) ion. This indicates that the N-protonated metal chelates are less stable in II than in I. Wavelengths of the bands assigned to metal chelates of I and II are listed in Table III.

The general rule[9b] that ionization of pyridinium hydrogen causes the blue shift to the π bands is shown to hold also for metal chelates of I and II.

On the basis of results of potentiometric titration in aqueous media, Gustafson and Martell[10] discussed the presence of the N-protonated species of metal chelate of I. We believe this work provides the first spectroscopic evidence for their presence.

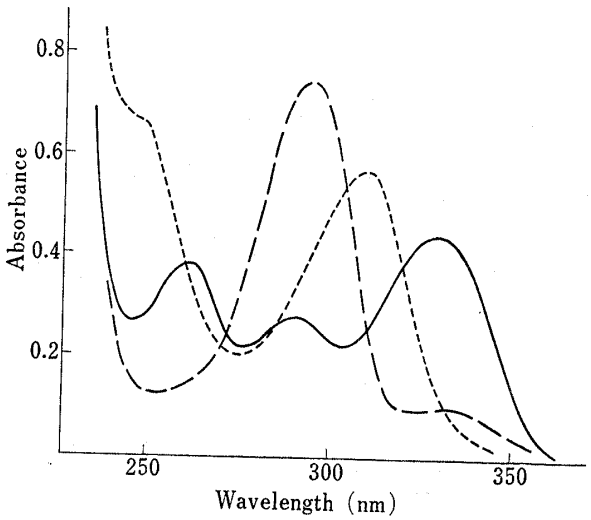
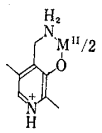
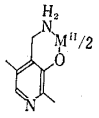


Fig. 2. Absorption Spectra of Methanol Solutions 1×10^{-4}M in Pyridoxamine and 5×10^{-4}M in Ni(II) perchlorate

– – – : 3×10^{-4}M HCl, ——— : 1×10^{-4}M HCl,
--------- : 3×10^{-4}M KOH

Table III. Absorption Bands of N-protonated and N-nonprotonated Species of Metal Chelates of Pyridoxamine and the Analog[a]

Species	Compounds	Metal ions					
		Zn(II)		Cu(II)		Ni(II)	
	I	320	250	322		330	262
	II	(310)		313		324	
	I[b]	301	240	302	244	311	248
	II[c]	296	238	294	240	302	244

a) Wavelength for band is given in nm.
b) reference 6*a*
c) reference 6*b*

Experimental

General experimental procedures for the spectral studies are given in the previous papers.[1,6,9c] The absorption spectra were recorded at room temperature with a Union Giken Model SM-202 spectrophotometer.

Pyridoxamine dihydrochloride, inorganic substances and the solvent for spectral studies were obtained from commercial sources. 3-Hydroxy-4-aminomethylpyridine was prepared as described previously.[6b] 1-Methylpyridoxamine chloride·HCl was obtained according to the method of Pocker and Fischer.[7]

1-Methyl-3-hydroxy-4-aminomethylpyridinium Chloride·HCl——1-Methyl-3-hydroxy-4-formylpyridinium chloride, prepared by the method of Maley and Bruice,[11] was converted to its oxime and, then, reduced with a Pd/C catalyst in methanol. Recrystallization from methanol-ether gave fine colorless needles. mp 240° (decomp.). *Anal.* Calcd. for $C_7H_{12}ON_2Cl_2$: C, 39.82; H, 5.73; N, 13.27. Found: C, 39.73; H, 5.81; N, 13.20. The compound was light sensitive and oxidized readily in air.

10) R.G. Gustafson and A.E. Martell, *Arch. Biochem. Biophys.*, **68**, 485 (1957).
11) J.R. Maley and T.C. Bruice, *Arch. Biochem. Biophys.*, **136**, 187 (1970).