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Pyridoxamine Analogs. Absorption Spectra and Metal Chelate Formation in Methanol[1]

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The absorption spectra of salicylamine (*o*-hydroxybenzylamine) (I), thiosalicylamine (*o*-mercaptobenzylamine) (II), and 3-hydroxy-4-aminomethylpyridine (III), have been measured at various concentrations of added acid or alkali in methanol solution. Assignments of the absorption bands to the various molecular species are made. I existed as a nonpolar species, whereas II did as a dipolar species with a dissociated thiol group, in neutral methanol solutions. This was supported by the p*K*a values (I; 9.2, 10.5: II; 4.7, 9.5) calculated from potentiometric titration curves in aqueous solutions. Both polar and nonpolar species were present in a neutral methanol solution of III. The Al[III], Cd[II], Ni[II] and Zn[II] chelates of II were fairly stable, whereas those of I were not formed appreciably, in methanol. III formed Al[III], Cd[II], Cu[II], Ni[II], and Zn[II] chelates, spectra of which were quite similar to those of metal chelates of pyridoxamine. Spectral change of a solution containing II and Cu[II] ion shows II formed Cu[II] chelate and, then, was converted to benzisothiazol. In the presence of Zn[II] ion, II and sodium pyruvate formed Zn[II] chelate of ketimine Schiff base, which underwent tautomerization to that of aldimine Schiff base very slowly. Possible correlation is noted between p*K*a's of the phenolic group of pyridoxamine and its analogs and their abilities to form metal chelates and to catalyze the nonenzymatic transamination.

Recently we reported a species absorbing in the 500-nmregion in a nonenzymatic transamination system containing pyridoxamine, ethyl pyruvate and aluminum [III] ion in methanol.[3] This species is of special interest, since it is "the key anionoid intermediate in pyridoxal catalysis," first postulated by Metzler, Ikawa and Snell[4] and so termed by Jencks.[5]

In order to study the structural features of pyridoxamine essential for the formation of a similar species, we prepared some analogs of pyridoxamine and analyzed their electronic absorption spectra in methanol solutions. Pyridoxamine analogs studied are salicylamine (*o*-hydroxybenzylamine), (I), thiosalicylamine (*o*-mercaptobenzylamine), (II), and 3-hydroxy-4-aminomethylpyridine (III).

The present paper describes reactions of the pyridoxamine analogs with α-keto acids and metal ions as well as the results of the spectral analyses. This is an essential preliminary to the understanding of the mechanisms of nonenzymatic transamination systems, which is the main subject of this series of works.[3,6]

1) This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education of Japan.

2) Location: *3-1-1, Maidashi, Higshi-ku Fukuoka-shi, 812, Japan*; *a*) Present address: *Tokyo Metropolitan Instituteof Gerontology, 35-2, Sakae-cho, Itabashi-ku, Tokyo, 173, Japan*; *b*) To whom inquiries should be addressed.

3) S. Matsumoto and Y. Matsushima, *J. Amer. Chem. Soc.*, **94**, 7211 (1972).
4) D.E. Metzler, M. Ikawa, and E.E. Snell, *J. Amer. Chem. Soc.*, **76**, 648 (1972).
5) W.P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N.Y., 1969, pp. 133—146.
6) *a*) S. Matsumoto and Y. Matsushima, *J. Amer. Chem. Soc.*, **96**, 5228 (1974); *b*) S. Matsumoto, Y. Karube, and Y. Matsushima, *Chem. Pharm. Bull.*, (Tokyo) "submitted."

Experimental

Materials——Spectrograde methanol and dioxane were used directly as solvents. Inorganic substances were certified reagent grade chemicals and were used without further purification. α-Keto acids were obtained from commercial sources. Other organic substances were prepared as described below.

Salicylamine (I), was prepared by catalytic reduction of salicylaldoxime according to Kanatomi and Murase.[7]

Thiosalicylamine (II)——Reduction with $LiAlH_4$ and followed benzoylation of 2,2′-dithio-bis-benzamide, prepared from thiosalicylic acid by the method of Gialdi, *et al.*,[8a] gave dibenzoylthiosalicylamine, which was hydrolyzed with NaOH.[8b] Thiosalicylamine hydrochloride, purified by sublimation *in vacuo* (120°/1 mmHg), was obtained as white crystalline powder, mp 184°.

2-Methylthiobenzylamine——2-Methylthiobenzoic acid, prepared by treatment of thiosalicylic acid with dimethyl sulfate, was converted to its amide by the method of McClelland and Warren.[9] 2-Methylthiobenzylamine was obtained as colorless crystals, mp 55°, by reduction of 2-methylthiobenzamide with $LiAlH_4$.

Benzisothiazol was separated as colorless crystals, mp 37—38°, by silica gel chromatography of $CHCl_3$ soluble fraction of a reaction mixture of thiosalicylamine and $K_3Fe(CN)_6$.

3-Hydroxy-4-aminomethylpyridine was prepared by reduction of oxime of 3-hydroxy-4-formylpyridine, which was obtained by the method of Heinert and Martell,[10] with a Pd/C catalyst.

Experimental Procedures——Solutions for spectral measurements were prepared so as to contain 1.0×10^{-4} M concentration of the substance under investigation. The desired acid-base species were obtained by adding standard methanol solutions of HCl or KOH, prepared by dissolving gaseous hydrogen chloride or potassium hydroxide in methanol.

For spectral-time study, solutions were mixed in a predetermined order in a volumetric flask and, then, solvent was added to a definite volume. The moment of the addition of the last solution was taken as the initiation of the reaction. An aliquot was transferred to a glass-stoppered 10 mm-silica cell and submitted to absorption measurements. Solutions for the absorption measurements were kept in the dark at room temperature. The absorption spectra were recorded with a Shimadzu Model MPS-50 spectrophotometer with pure methanol as a reference.

The potentiometric titrations were carried out with a Radiometer TTTlc titrator and SBR2 Titrigraph.[11]

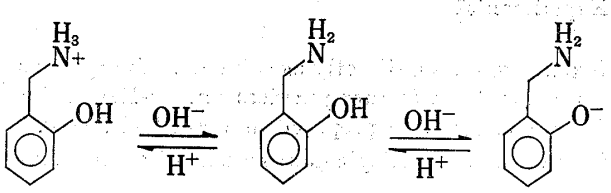
Results and Discussion

Salicylamine (I)

The electronic absorption spectrum of a neutral methanol solution of I had an absorption band with two peaks at 276 nm (ε; 2500) and at 281 nm (ε; 2350) in near ultraviolet and visible regions. This band may be assigned to the ^1L_b band,[12] which is found at 256 nm in benzene and bathochromically shifted by the substituent groups. The wavelength, intensity and shape of the band were almost identical in acidic solutions. Since little spectral change is expected for protonation of the amino group of this compound, the band is assigned to the ^1L_b band of the protonated, I_A, as well as the neutral species, I_B.

In a solution 1×10^{-3} M in KOH, intensity of the band decreased slightly and in a 1×10^{-2} M solution, intense bands were clearly observed at 241 nm and at 294 nm. These bands became more intense with KOH concentration (241 nm, ε; 8600: 291 nm, ε; 3700). These are assigned to the ^1L_a and ^1L_b bands of the phenolate form, I_C, as it is well established that dissociation of phenolic hydrogen causes a large red shift to π bands of substituted benzenes.[13]

7) H. Kanatomi and I. Murase, *Bull. Chem. Soc. Japan*, **43**, 226 (1970).
8) *a*) F. Gialdi, R. Ponci, and A. Baruffini, *Farmaco (Pavia) Ed. Sci.*, **14**, 216 (1959); *b*) R. Boudet and D. Bourgoin-Legay, *Compt. Rend. Ser. C*, **262** (7) 596 (1966).
9) E.W. McClelland and L.A. Warren, *J, Chem. Soc.*, **1929**, 262.
10) D. Heinert and A.E. Martell, *J. Amer. Chem. Soc.*, **81**, 3933 (1959).
11) The potentiometric measurements were performed at the National Institute of Radiological Sciences, Anagawa, Chiba-shi, under the direction of Dr. A. Hanaki, to whom we are very grateful.
12) J.R. Platt, *J. Chem. Phys.*, **17**, 484 (1949).
13) *a*) H.H. Jaffé and M. Orchin, "Theory and Applications of Ultraviolet Spectroscopy," J. Wiley and Sons, Inc., New York, N.Y., 1962; *b*) L. Doub and J.M. Vandenbelt, *J. Amer. Chem. Soc.*, **69**, **2716** (1947).



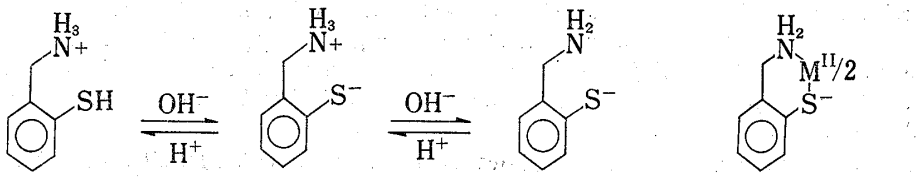
I_A 276 nm, 281 nm, ^1L_b I_B 276 nm, 281 nm, ^1L_b Ic 241 nm, ^1L_a 294 nm, ^1L_b

Chart 1

Addition of Cu [II], Ni [II] or Zn [II] ion to a methanol solution of salicylamine did not produce significant spectral changes. This fact shows that metal chelates of I were not formed in neutral methanol solution. Metal chelates were not detected spectrophotometrically in 20%, 50% and 80% dioxane-methanol solutions.

Thiosalicylamine (II). Molecular Species in Methanol Solutions

Figure 1 shows absorption spectra of thiosalicylamine in methanol. In an acidic solution, an absorption band was observed at 242 nm, which should be ascribed to the fully protonated form, II_A. In a solution containing an equimolar HCl, an absorption appeared at around 273 nm. The 273-nm band became greater and the 242-nm band disappeared in a neutral solution. The spectral change may be caused by dissociation of thiol hydrogen, as dissociation of ammonium hydrogen is not likely to cause red shift of 30 nm. In an alkaline methanol, the 273-nm band showed a blue shift to 271 nm with a slight increase in intensity.



II_A 242 nm, ^1L_a II_B 273 nm, ^1L_a IIc 271 nm, ^1L_a II_D 242–265 nm, ^1L_a

Chart 2

An intense absorption band at 236 nm in the spectrum of thiophenol was assigned to the ^1L_a band and a shoulder on the long wavelength side was interpreted as the ^1L_b band.[14] Therefore, the 242-nm and 273-nm bands of thiosalicylamine may properly be assigned to the ^1L_a band of the undissociated, II_A, and the dissociated thiol species, II_B, respectively. A weak shoulder observed at the long wavelength side may be the partially submerged ^1L_b band.

In neutral methanol, salicylamine was shown to exist as a nonpolar neutral species, I_B. On the other side, only a dipolar neutral species, II_B, was present in a neutral methanol solution of thiosalicylamine.

2-Methylthiobenzylamine showed a strong absorption at 235 nm in neutral methanol. This band did not undergo any spectral changes in acidic or alkaline methanol solutions. This clearly indicates the protonation of the amino group does not produce significant changes in spectra of this series of compounds. A bathochromic shift of 11 nm by methylation of the thiol group corresponds to that of the ^1L_a band of thiophenol to thioanisol (254 nm).[13a]

Acid Dissociation Constants of Salicylamine and Thiosalicylamine

Acid dissociation constants of I and II were measured by potentiometric titration in aqueous 0.1 M KNO_3. The pK_a values calculated from the titration curves are 9.2 and 10.5 for I[15] and 4.7 and 9.5 for II. The following pK_a values have been reported: benzylamine,

14) H.P. Koch, *J. Chem. Soc.*, **1949**, 387.

15) The values 6.27 and 12.87 were reported as pK_a's of I in 75% dioxane in K.E. Jabalpurwala, K.A. Venkatachalam, and M.B. Kabadi, *J. Inorg. Nucl. Chem.*, **26**, 1011 (1964).

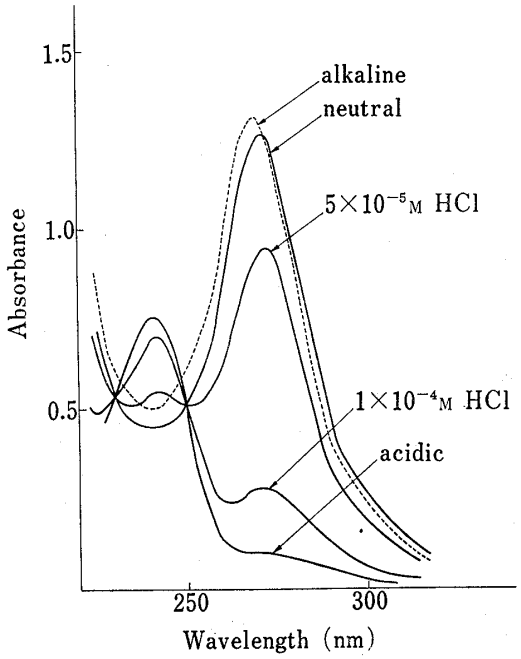


Fig. 1. Absorption Spectra of 1.0×10^{-4}M Thiosalicylamine in Methanol

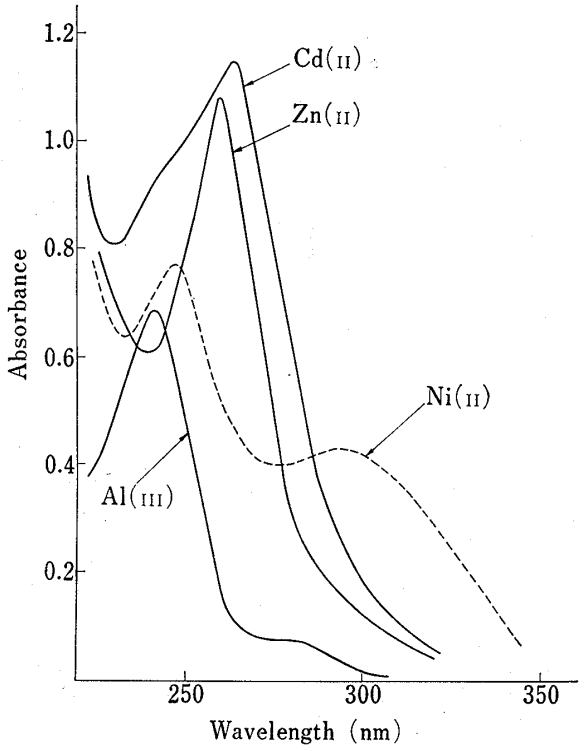


Fig. 2. Absorption Spectra of Metal Chelates of Thiosalicylamine in Methanol

Solutions contained 1.0×10^{-4} M thiosalicylamine and 1.0×10^{-4} M perchlorates of metal indicated besides the curves.

9.62[16a]; phenol, 9.98[16b]; thiophenol, 6.5.[16c] These values clearly indicate that the first dissociation constant of I and the second constant of II correspond to the dissociation of the $-NH_3^+$ group. The pK_a values for the thiol (4.7) and phenol (10.5) groups support the interpretation of the spectral data (Chart 1 and 2).

Thiosalicylamine was also titrated in the presence of metal ions such as Ni [II], Zn [II] and Cd [II]. The metal ions lowered pH values of the titrated solutions. This shows the considerable stability of metal chelates of this compound. Precipitation during the titrations prevented the estimation of the stability constants.

Only a slight difference was detected in the titration curves of I in the presence and in the absence of the metal ions. The fact indicates small stability constants of its metal chelate in agreement with the results of the spectral study.

Spectral Study of Reactions of Thiosalicylamine with Metal Ions

Figure 2 shows the spectra of neutral methanol solutions containing II and metal ions such as Al [III], Cd [II], Ni [II] and Zn (II). When tetrasodium ethylenediaminetetraacetate (EDTA) was added to the solutions, the spectra were converted to that of II. The facts indicate the strong absorption bands between 242 nm and 265 nm are ascribed to the metal chelates, II_D. It is reasonable that π bands of the metal chelates lie between those of the species of the ligand protonated, II_A, (242 nm) and deprotonated, II_C, (271 nm) in the thiol group. The weaker absorption found in the long wavelength side may correspond to the ^1L_b band.

From the method of continuous variation of the concentrations, the ratio of II to metal ion was estimated 2: 1 in the metal chelates, though absorption maxima of the ligand and the metal chelates were too close to avoid some uncertainty in the estimation.

16) *a*) R.J. Bruehlman and F.H. Verhoek, *J. Amer. Chem. Soc.*, **70**, 1401 (1948); *b*) R.M. Milburn, *ibid.*, **77**, 2064 (1955); *c*) M.M. Kreevoy, E.T. Harper, R.E. Duvall, H.S. Wilgus, III, and L.T. Ditsch, *ibid.*, **82**, 4899 (1960).

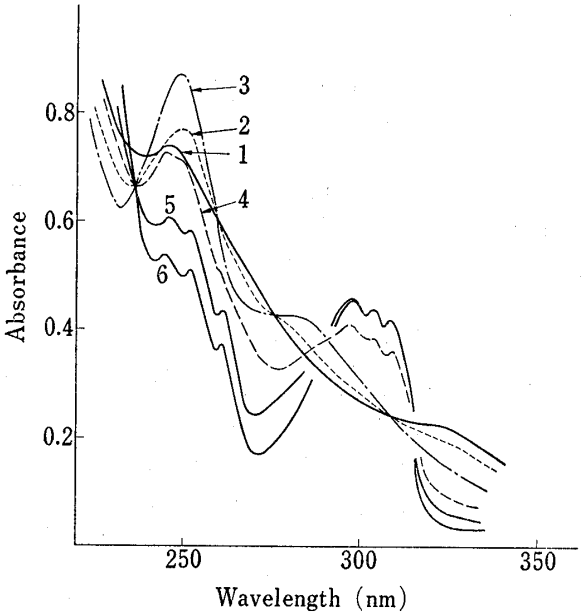


Fig. 3. Spectral-time Study for the Reaction of Thiosalicylamine 1.0×10^{-4}M and Copper Acetate 1.0×10^{-4}M

Times after the initiation of the reaction are, 1, 2 min.: 2, 4 min; 3, 7 min; 4, 13 min; 5, 18 min; 6, 24 min.

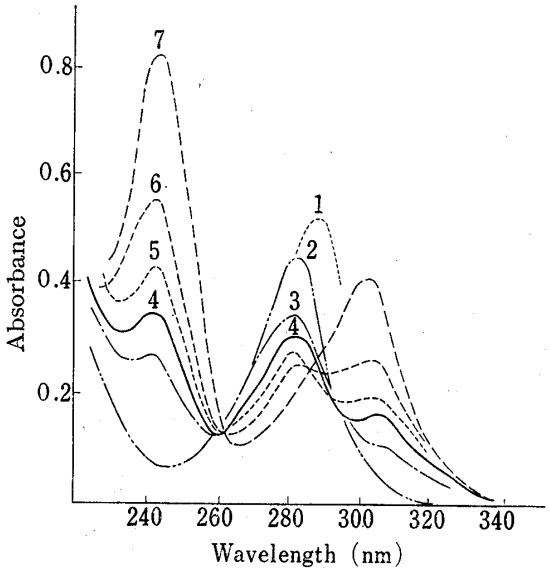


Fig. 4. Absorption Spectra of 1.0×10^{-4}M 3-Hydroxy-4-aminomethylpyridine in Methanol

Solutions were 1, 1.0×10^{-2}M HCl; 2, 2.0×10^{-4} M HCl; 3, 1.0×10^{-4}M HCl; 4, neutral methanol; 5, 2.0×10^{-4}M KOH; 6, 3.0×10^{-4}M KOH; 7, 5.0×10^{-4}M KOH.

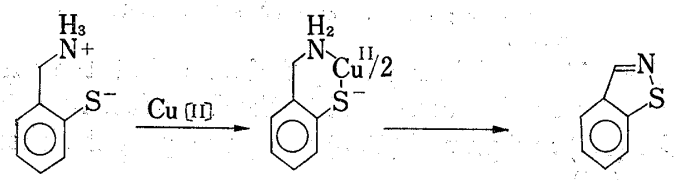


Chart 3

Figure 3 shows the spectral change with time, when copper acetate was added to a neutral methanol solution of thiosalicylamine. An absorption peak appeared at 250 nm with a shoulder near 280-nm. Its intensity reached at its maximum in 7 min and was stable for a few minutes before it decreased gradually. Twenty four minutes after the addition of Cu [II] ion, the spectrum had two bands, which showed a considerable vibrational structure. No further spectral change was observed on standing and on addition of EDTA. The final spectrum was superimposable on that of a methanol solution of benzisothiazol. As the 250-nm band in the intermediary spectrum is ascribable to Cu [II] chelate of II, the whole reaction can be formulated as Chart 3.

Addition of Mn [II] ion to a methanol solution of II produced a similar spectral change, though the reaction was much slower.

3-Hydroxy-4-aminomethylpyridine (III)

Figure 4 shows spectra of 3-hydroxy-4-aminomethylpyridine (III) in methanol containing various concentrations of HCl and KOH. Spectra of this compound in 1N HCl and 1N NaOH aqueous solutions were described by Ayling and Snell.[17] In strongly acidic and alkaline conditions, spectra in methanolic and aqueous media were essentially identical and were analyzed as the presence of the fully protonated, III_A, and the fully deprotonated species, III_F, respectively.

On the other side, spectra in weakly acidic and neutral methanol were somewhat different from those in water. This may be due to different predominant species between these media.

17) J.E. Ayling and E.E. Snell, *Biochemistry*, 7, 1626 (1968).

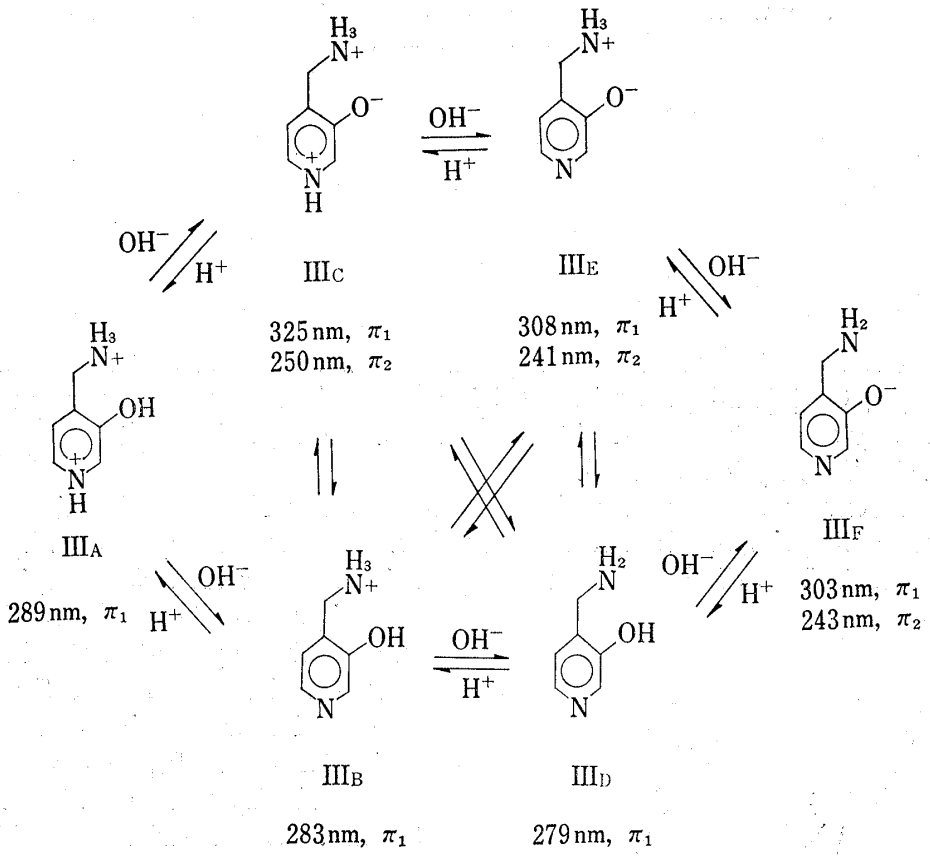


Chart 4[18]

The spectral change shown in Figure 4 were quite similar to those of methanol solutions of pyridoxamine, described by Matsushima and Martell.[19] Accordingly, the assignments of the absorption bands were performed analogously and equilibria of the assigned species are shown in Chart 4.

In dioxane-methanol, the 279-nm band increased, while the bands at 308 nm and 241 nm decreased, with an increase in dioxane content. On the contrary, the 279-nm band decreased and the 308-nm and 241-nm bands increased with an increase of water content in methanol-water. These support the assignment of the 279-nm band to the neutral nonpolar species, III_D, and that of the latter bands to the dipolar species, III_E.

Blue shifts of 5—10 nm in π bands of III from the corresponding species of pyridoxamine are noted.

Absorption spectra obtained by addition of matal salts to methanol solutions of III were characterized by the presence of an intense band between the π bands[18] of the nonpolar neutral form, III_D, and the anionic form, III_F. The fact that the spectra were very similar to those of metal chelates of pyridoxamine[19] indicates the formation of metal chelates of III in methanol. Wavelengths of the bands assigned to the metal chelates of III as well as those of pyridoxamine are shown in Table I.

Reaction of α-Keto Acids with the Pyridoxamine Analogs

No significant spectral change was observed on addition of alkaline salt of α-keto acids such as sodium pyruvate, potassium α-ketobutyrate and potassium α-ketoisovalerate to

18) Of the two π–π^* absorption of pyridine derivatives, the longer wavelength band has been named as π_1, whereas the shorter one as π_2 band. See, for example, K. Nakamoto and A.E. Martell, *J. Amer. Chem. Soc.*, **81**, 5857 (1959).

19) Y. Matsushima and A.E. Martell, *J. Amer. Chem. Soc.*, **89**, 1322 (1967).

Table I. Wavelengths of π Bands of Metal Chelates of 3-Hydroxy-4-aminomethylpyridine and pyridoxamine

Metal Ions	3-Hydroxy-4-aminomethylpyridine (nm)			Pyridoxamine[19] (nm)	
Al [III]	288			296	
Cd [II]	304	286	244	309	248
Cu [II]	294	240		302	244
Ni [II]	302	244		311	248
Zu [II]	296	238		301	240

salicylamine, I, in methanol, dioxane-methanol or dioxane-water. Addition of metal ions such as Al [III] Cu [II], Ni [II] and Zn [II] ion to a mixture of I and sodium pyruvate did not produce any spectral changes. These facts indicate ketimine or its metal chelates were not formed significantly in methanol.

When an excess amount of sodium pyruvate was added to thiosalicylamine (II) in methanol, the absorption band at 273 nm due to II decreased gradually and finally the spectrum of the mixture had no distinct absorption peak in the near ultraviolet region.

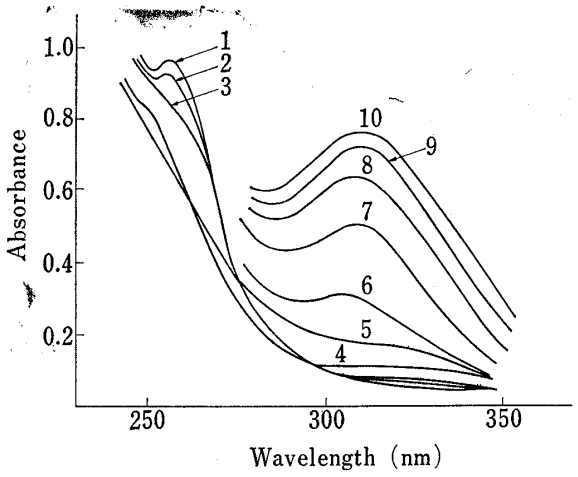


Fig. 5. Spectral-time Study for the Reaction of the Thiosalicylamine 1.0×10^{-4} M with Sodium Pyruvate 1.0×10^{-3}M in the Presence of Zinc Percholorate 1.0×10^{-4}M

Times after the initiation of the reaction are 1, 10 min; 2, 30 min; 3, 1 hour; 4, 3 days; 5, 12 days; 6, 18 days; 7, 32 days; 8, 48 days; 9, 60 days; 10, 70 days.

Figure 5 shows spectral-time study when sodium pyruvate was added to a methanol solution of II and Zn [II] acetate. The band at 257 nm in the initial spectrum assignable to Zn [II] chelate of II decreased very gradually and a new band appeared at 312 nm. When EDTA was added to a solution absorbing at 312 nm, an absorption band appeared at 270 nm instantly with the disappearance of the 312-nm band.

Presence of Ni [II] or Al [III] ion in the place of Zn [II] ion did not produce a similar spectral change. Addition of Cu [II] or Cd [II] ion resulted in formation of benzisothiazol.

The spectral change shown in Figure 5 is quite analogous to that observed in a nonenzymatic transamination system, containing pyridoxamine, α-ketoisovaleric acid and Zn [II] ion.[20] Therefore, it is interpreted as a sequence of reactions as shown in Chart 5. The role of Zn [II] ion in the reactions

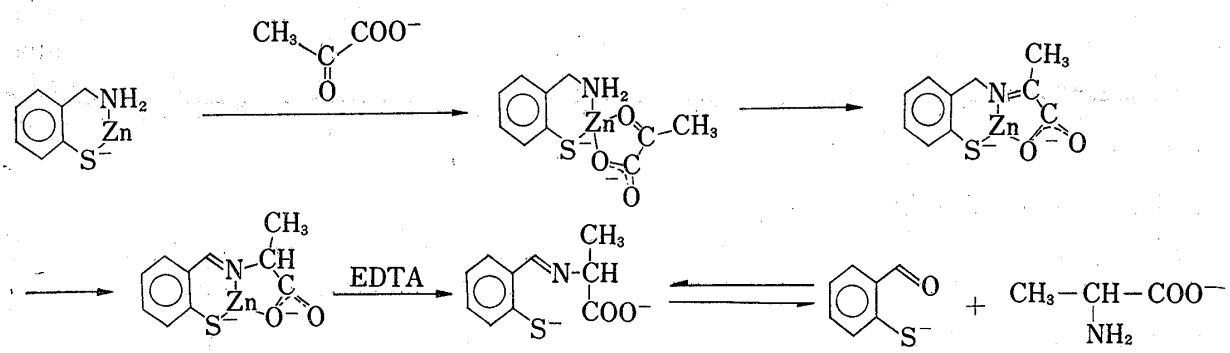


Chart 5

20) Y. Matsushima and A.E. Martell, *J. Amer. Chem. Soc.*, **89**, 1331 (1967).

may be to screen the thiol group from the attack carbonyl group of the keto acid by chelation, to facilitate ketimine formation by the promnastic effect[21] and to promote tautomerization of ketimine and aldimine.[20]

In reactions of III with α-keto acids, the effects of Zn [II] ion were more prominent, which will be reported in the near future.[6b]

Conclusions

Following conclusions may be drawn from the present results. Salicylamine does not form ketimine with α-keto acids nor form metal chelates in neutral solutions. On the other side, its thiol analog, II, forms fairly stable metal chelates in the same conditions. Moreover, thiosalicylamine can catalyze nonenzymatic transamination of an α-keto acid and an α-amino acid in the presence of Zn [II] ion. This catalytic activity has been demonstrated in pyridoxamine[3–6,20,22] and in III.[6b]

Abilities of pyridoxamine and its analogs to form metal chelates and to catalyze the transamination seem to be related to acidity of the phenolic or thiol group *ortho* to the aminomethyl group. It has been emphasized that pyridine nitrogen atom of vitamin B_6 functions as an electron sink in the prototropic shift of ketimine and aldimine.[4,5,22] However, it must be noted that the nitrogen atom plays an important role in lowering the pK_a value of the phenolic group.

21) D. Hopgood and D.L. Leussing, *J. Amer. Chem. Soc.*, **91**, 3740 (1969).
22) 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., 1968. See also literature cited in ref. 5.