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Reactions of Pyridoxal, Amino Acids and Metal Ions in Methanol

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Reactions of pyridoxal and related substances, including reaction products of pyridoxal and amino acids or amines reported in the previous paper,[4)] with divalent transition metal ions in methanol were examined by electronic absorption spectra. Spectral properties of metal chelates of pyridoxylidene amino acids or amines, pyridoxal and pyridoxamine were described and the shifts of π bands with chelation were discussed. Pyridoxine, carbinolamine from pyridoxal and sarcosine, and imidazotetrahydropyridine derivative from pyridoxal and histidine formed very weak complexes, which could exist only in solutions and under large excess of metal ions. Intramolecular cyclization of pyridoxylidenehistidine was prevented by chelation with Cu(II). Thiazolidine derivative from pyridoxal and cystamine was oxidized by Cu(II) to give Cu(II) chelate of pyridoxylidenecystamine.

Metzler and Snell[2)] found that reactions catalyzed by pyridoxal enzymes in biological systems such as transamination, racemization and oxidative deamination could be carried out nonenzymatically in aqueous solution with metal ions as catalysts. Since then many works have been reported on the nonenzymatic reactions and revealed that the reactions proceed through metal chelates of Schiff bases formed from pyridoxal and α-amino acids. In the former work,[3)] Zn (II) chelate catalyzed transamination in methanol were studied kinetically by the changes of electronic absorption spectra. Methanol was proved to be suitable as a solvent for this kind of studies.

In the previous paper,[4)] reactions of pyridoxal with various amino acids and amines in methanol were reported. Most amino acids and amines formed Schiff base, though some of them gave other products. It has been shown,[5)] that every species of pyridoxal and related substances have two characteristic intense bands in ultaviolet region. They are originated from π-π^* transitions of pyridine ring and the extended conjugate systems. The longer wavelength band was called as π_1 band, whereas the shorter one as π_2 band.

When pyridoxal and related substances, including reaction products of pyridoxal and amino acids, were mixed with metal ions in methanol, electronic absorption spectra underwent rapid changes. This showed the formation of metal chelates. It is the purpose of the present paper to discuss about the reactions of pyridoxal and related substances with metal ions, wavelength shifts of π bands with chelate formation and the other spectral properties of the metal chelates.

Experimental

Materials——Pyridoxal hydrochloride, amino acids, amines and methanol used were the same as described in the previous paper.[4)] Pyridoxine hydrochloride was obtained from Wako Pure Chemicals Ltd.

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

[3)] Y. Matsushima and A.E. Martell, *J. Am. Chem. Soc.*, **89**, 1331 (1967).

[4)] Y. Matsushima, *Chem. Pharm. Bull.* (Tokyo), **16**, 2050 (1968).

[5)] Y. Matsushima and A.E. Martell, *J. Am. Chem. Soc.*, **89**, 1322 (1967).

Metal perchlorates were from G.F. Frederic Smith Chemicals Co. These were certified reagent grade chemicals and did not require further purification.

Preparation of Solutions——Methanol solutions of organic materials were prepared as the same manner as described in the previous paper.[4] Inorganic perchlorates were dissolved directly in pure methanol. Solutions were mixed in a predetermined order in a volumetric flask and adjusted to a definite volume by the addition of methanol. A part of the solution was transferred to silica cells and submitted to spectral studies. Methanol solutions for spectral measurements were so prepared to contain 1×10^{-4} M concentration of substances under investigation.

Measurements——The electronic absorption spectra were recorded with a Cary Model 14 spectrophotometer. The temperature of the cell compartment was kept at 25° throughout the measurements.

Results and Discussion

Metal Chelates of Schiff Bases

On addition of transition metal ions such as Ni(II), Cu(II), Co(II), Zn(II) and Mn(II) to methanol solution of Schiff base of pyridoxal and glycine, the solution immediately gave the spectra shown in Fig. 1. These were very similar to the spectra of metal chelates of pyridoxylidenevaline reported before[5] and were supposed to indicate the formation of metal chelates of the Schiff base as I. The spectra were characterized by strong π_1 bands at around 390 mμ and π_2 bands at around 270 mμ. Schiff bases of other amino acids and amines formed metal chelates having the almost same spectra, although slight differences were seen by their constituents. Wavelengths of the assigned bands are listed in Table I and II.

In most Schiff bases, Ni(II) chelates had π_1 band at the longest wavelength among the five divalent transition metal chelates, but π_2 bands at 270 mμ were generally very weak absorption. In Ni(II) chelates of some Schiff bases, π_2 bands were not observable. Ni(II) chelate of pyridoxylideneglycine had an absorption peak at 407 mμ, overlapped by the strong π_1 band at 390 mμ. In Ni(II) chelates of pyridoxylidenevaline and other Schiff bases, corresponding absorption were observed as a shoulder. The origin of this absorption is not clear.

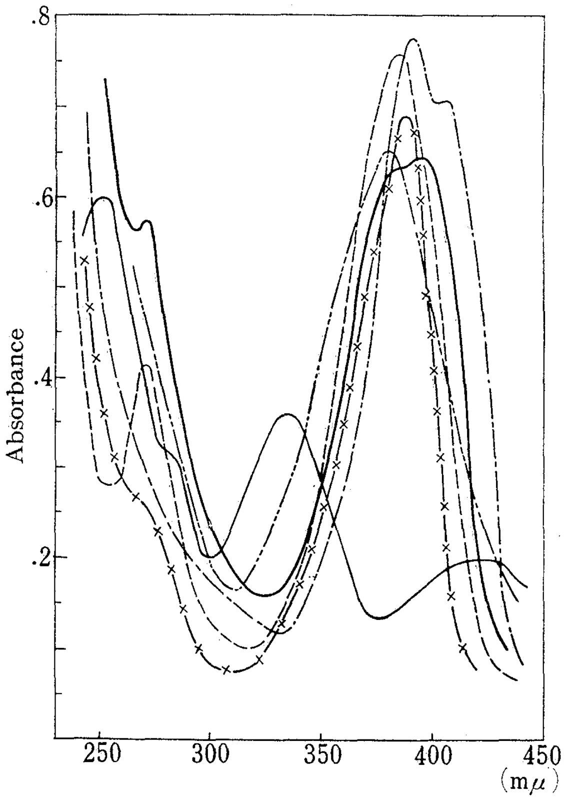
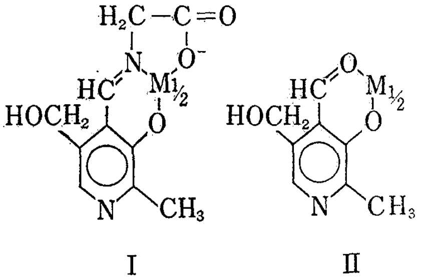


Fig. 1. Electronic Absorption Spectra of Pyridoxylidene glycine and Its Metal Chelates

———— pyridoxylideneglycine
– – – – – Ni(II) chelate
━━━━ Cu(II) chelate
·– – – – – Zn(II) chelate
–·–·–·– Co(II) chelate
— × — × Mn(II) chelate



I II

Chart 1

Cu(II) chelates of Schiff bases of α-amino acids had a broad absorption at around 390 mμ, which were, however, less intense than π_1 bands of other metal chelates. Broadness of this

Table I. $\pi-\pi^*$ Bands of Metal Chelates of Schiff Bases Formed from Pyridoxal and Amino Acids or Amines

Amino acids or amines	Wavelength (mμ)					
	Cu(II) chelate		Ni(II) chelate		Zn(II) chelate	
	π_1	π_2	π_1	π_2	π_1	π_2
α-Alanine	390	270	392	270	385	270
S-Methylcysteine	390	271	392	—	386	273
Homocysteine	391	272	393	—	390	—
Lysine					385	271
Butylamine	375	—			381	268
Cystamine	382	273	395	270	387	268
Benzylamine	373	—			386	267
Glycinamide	385—395				389	271
Glycylglycine	380—390				389	271
Methyl glycylglycinate	380—395				388	271

Table II. Electronic Absorption Bands of Metal Chelates of Pyridoxal and Related Compounds

Ligands	Wavelengths in mμ of the bands of metal chelates					
	Bands	Ni(II)	Cu(II)	Zn(II)	Co(II)	Mn(II)
Pyridoxal	π_1	412	408	400	402	400
	unassigned	277	283	278	270	278
Pyridoxylidenevaline[a]	π_1	393	390	385	383	382
	π_2	270	271	271	270	271
Pyridoxylideneglycine	π_1	390	395	385	380	383
	π_2	—	270	271	—	—
Pyridoxamine[a]	π_1	311	302	301	302	291
	π_2	248	244	240	246	245

a) reference 5

absorption caused the suspect that this might be π_1 band overlapped with other unknown absorption. This was more clearly shown in Cu(II) chelates of Schiff bases from glycinamide, glycylglycine and methyl glycylglycinate, in which very broad and low intensity absorption were observed at 380—395 mμ. On the other hand, in Cu(II) chelates of Schiff bases of simple amines such as butylamine, benzylamine and cystamine, π_1 bands were observed as a single strong peak at somewhat shorter wavelength region. From these fact, absorption which overlapped with π_1 band might have some connection with the coordination of a carboxylate or other functional groups on Cu(II).

Most Zn(II) chelates of Schiff bases of amino acids and amines showed two strong bands at around 385 mμ and 271 mμ, which were assigned to π_1 and π_2 bands, respectively. Wavelengths of these bands of Zn(II) chelates were less affected by structure of amino acids and amines than in Cu(II) chelates.

Co(II) and Mn(II) chelates of the Schiff bases had π_1 bands at 380—390 mμ. Spectra of these chelates in methanol solution, however, were generally unstable and underwent gradual changes to give obscured spectra, presumably because ligands were oxidized catalytically by these metal ions.⁶⁾

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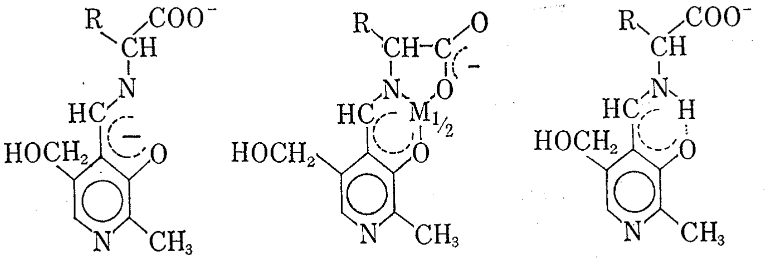
6) A.E. Martell, private communication.

All the π_1 bands of divalent transition metal chelates of the Schiff bases were found between 375 mμ and 400 mμ. These were intermediate between π_1 band of anionic form (370—375 mμ) and that of N-monoprotonated form *i.e.* keto-enamine form (418—426 mμ) of the Schiff bases.[4] The wavelengths of π bands are assumed to be a function of the degree of delocalization of π orbitals. Chelation with metal which attracts electron from phenolate oxygen and from lone pair orbital of imine nitrogen by coordination would extend the delocalization of π orbitals in the molecule and cause the red shift of π bands. The stronger the coordination, the red shift would be larger. When ligand was common, the red shift was larger in Ni(II) and Cu(II) chelates than in Zn(II) and Mn(II) chelates. This was supposed to inflect the interaction of metal and ligand.[7] Large delocalization of π orbitals in N-protonated form was discussed by Heinert and Martell.[8]



Anionic form
370—375mμ

Metal Chelate
375—400mμ

Monoprotonated form
418—426mμ

Chart 2. Wavelenghts of π_1 Bands

Metal Chelates of Pyridoxal

Addition of metal ions to methanol solution of pyridoxal arose two bands at 400—412 mμ and 270—280 mμ regions. Wavelengths of these bands are listed in Table II.

The longer wavelength bands can be assigned to π_1 bands of pyridoxal metal chelates as II. The wavelengths of the bands were found in the order of Ni(II), Cu(II), Co(II), Zn(II), Mn(II) chelates from the longer wavelength side. It can be interpreted as the stronger interaction with ligand makes the larger delocalization of π electrons in the chelate ring and causes the larger red shifts. Absorption peaks at 270—280 mμ had an anormalous band shift with variation of metal ions and cannot be interpreted by the empirical rules of π bands of 3-hydroxypyridine derivatives established by Martell and coworkers.[5,8,9] They are left unassigned.

Metal Chelates of Pyridoxamine

Absorption bands of metal chelates of pyridoxamine were already reported.[5] As seen in Table II, they have π_1 and π_2 bands at around 300 mμ and 245 mμ, respectively. Ni(II) chelate had π_1 band at the longest wavelength, which was almost the same as π_1 band of anionic form (IV). π_1 bands of other metal chelates were at slightly shorter wavelength. They were intermediate between that of anionic form and neutral nonpolar form (III). Unlike metal chelates of Schiff bases and of pyridoxal, pyridoxamine chelates do not have delocalized π orbital in chelate ring. Aminomethyl group is supposed to have little influence on π bands, whether it is free or protonated or even coordinated to metal ions. Large red shift from III to IV is caused by dissociation of phenolic proton. It is reasonable that red shifts caused by the coordination of phenolate to metal ions can not exceed that of deprotonation.

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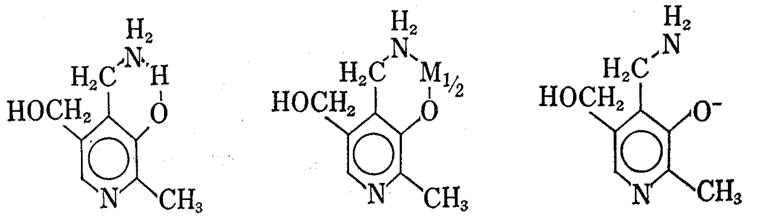
[7] A.E. Martell and Y. Matsushima, "Pyridoxal Catalysis: Enzyme and Model Systems," eds. by E.E. Snell, A.E. Braunstein, E.S. Sevein and Yu.M. Torchinsky, Interscience Publishers, Inc., New York, N.Y., 1968, pp. 33-48.

[8] D. Heinert and A.E. Martell, *J. Am. Chem. Soc.*, **85**, 183 (1963).

[9] K. Nakamoto and A.E. Martell, *J. Am. Chem. Soc.*, **81**, 5857, 5863 (1959).



III neutral nonpolar form 289mμ

metal chelate 291−311mμ

IV anionic form 311mμ

Chart 3. Wavelengths of π_1 Bands of Pyridoxamine

Metal Complexes of Pyridoxine

Metal chelates of pyridoxine have never been reported, as there is little hope that pyridoxine could form stable metal chelates. Addition of an equimolar metal perchlorates to methanol solution of pyridoxine did not bring any spectral changes. However, in the presence of large excess of metal ions, new absorption bands characteristic to each metal ions were formed at 320—330 mμ and 250—260 mμ regions. Wavelengths of these bands are shown in Table III.

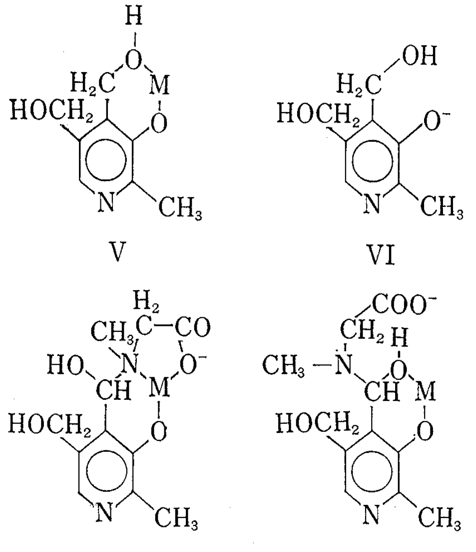
Table III. Absorption Maxima of Complexes

Metal ions	Pyridoxine complexes (mμ)		Carbinolamine complexes (mμ)	
K(I) (anionic form)	308	246	303	
Ni(II)	329	258	324	258
Cu(II)	317	—	315	—
Co(II)	325	258	319	250
Zn(II)	322	250	316	248
Mn(II)	326	255	314	247
Cd(II)	324	256	311	247
Fe(II)	360		315	253
Fe(III)			281	
Al(III)	304		298	

These indicate the formation of metal pyridoxine complexes though stabilities are quite low. One of the possible structures of the complexes is metal chelates as V. However, to assume that π_1 band of metal chelates as V are in longer wavelength region than anionic form (VI) might be unreasonable from the empirical rules of band shifts. Another possible structure is to assume pyridine nitrogen of pyridoxine also coordinate to metal ion. As protonation caused red shift, coordination of pyridine nitrogen is expected to result in red shift of π bands.

Metal Complexes of Carbinolamine

As reported previously,[4] pyridoxal and sarcosine formed carbinolamine derivative in methanol. By adding large excess of transition metal perchlorates to the solution, new intense absorption bands were observed at around 320 mμ and 250 mμ, which are listed in Table III. These spectra



V VI VII VIII

Chart 4

showed close similarity with that of pyridoxine metal complexes in the wavelength shift from anionic form with variation of metal ions and in the shapes and intensities of the bands. That they were observable only in the large excess of metal ions indicates complexes formed were of low stablity and could exist only in solution. The structure of the complexes can not be determined. Two of the possible structures are shown as VII, VIII. Coordination of pyridine nitrogen to metal ions is also possible.

Among metal complexes examined, Al(III) complex showed somewhat different properties. π_1 band of Al(III) complex was at shorter wavelength than anionic form and stability was larger than other complexes. These properties of Al(III) complex was also seen in pyridoxine complex.

Reaction of Pyridoxal, Histidine and Metal Ions

As reported in the previous paper,[4] reaction of pyridoxal and histidine proceeded in two steps, *i.e.* formation of Schiff base in the first step and the followed intramolecular cyclization in the second step. Addition of Cu(II) perchlorate in the beginning of the second step produced Cu(II) chelate of the Schiff base, which, however, did not show any sign of intramolecular cyclization. This suggests that methine of the Schiff base was so stabilized by chelation with Cu(II) to be resistant against the attack of 5 position of imidazole. Ni(II) and Zn(II) chelates of the Schiff base were also formed on addition of metal perchlorates to the methanol solution, but these chelates showed very gradual intramolecular cyclization.

Spectra of final product from pyridoxal and histidine did not change on addition of an equimolar metal perchlorate. However, with large excess of metal perchlorate new intense absorption bands were appeared in the following wavelength; Ni(II) 318 mμ, 255 mμ; Cu(II) 303 mμ; Zn(II) 316 mμ, 255 mμ. Wavelengths, intensities and shapes of these bands were similar to that of metal complexes of pyridoxine and carbinolamine. Weak complexes were supposed to be formed, which were probably metal chelates of the structure as IX or IX with pyridine nitrogen protonated or coordinated to metal ion.

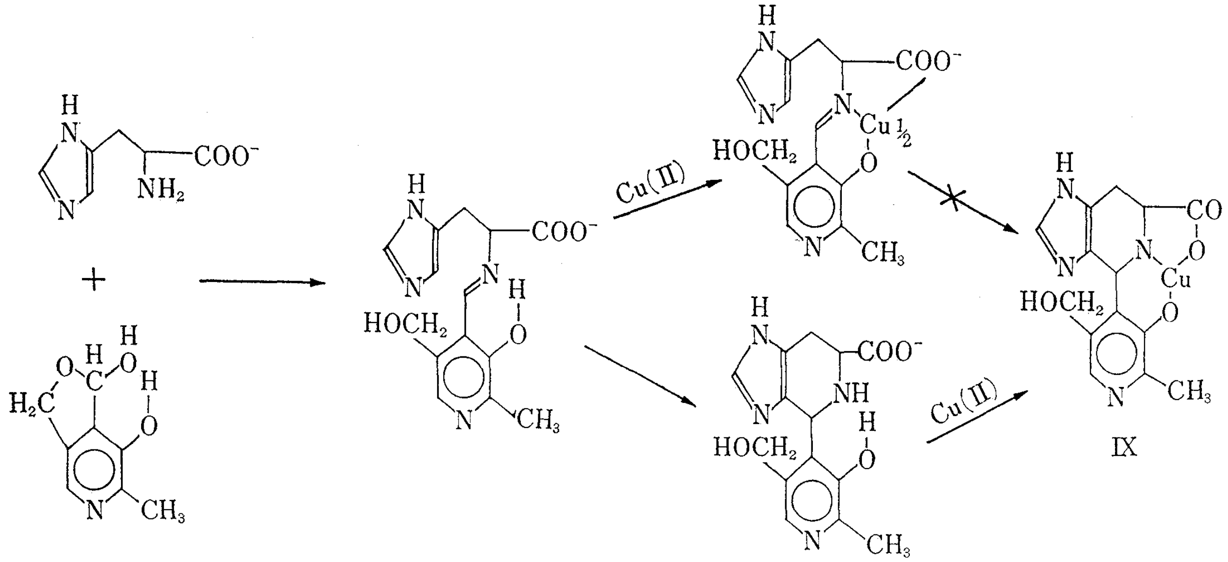


Chart 5

Reaction of Metal Ions with Thiazolidine Derivatives

As reported previously,[4] when reacted with pyridoxal in methanol, cysteine and cysteamine formed thiazolidine derivatives having π_1 bands of neutral nonpolar form (X) at 295 mμ. When Cu(II)perchlorate was added to the methanol solution of thiazolidine derivatives, spectra of the solution showed rapid changes.

In Fig. 2, spectral change is shown when Cu(II) perchlorate was added to thiazolidine from cysteine. Band at 292 mμ in the initial spectrum decreased rapidly and a new absorption band appeared at 360 mμ. The 360 mμ band, however, decreased in the next step and the final spectrum had an absorption peak at 395 mμ.

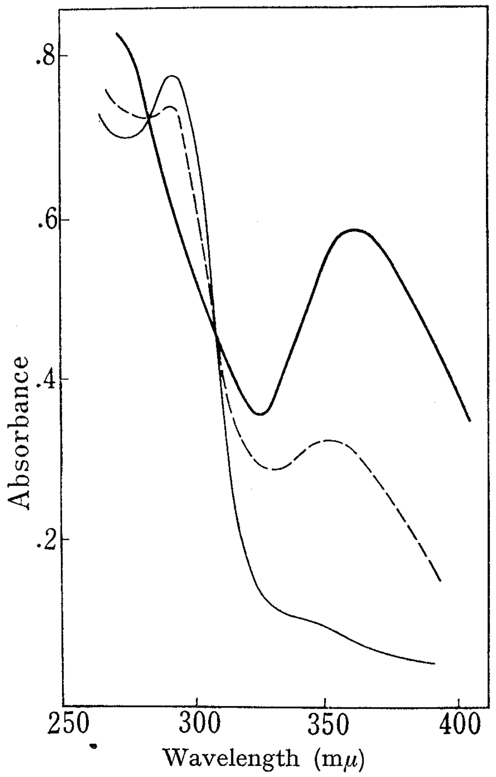


Fig. 2. Reaction of Cu(II) with Thiazolidine Derivative in Methanol (1)

The solution contained 1×10^{-4}M pyridoxal, 1×10^{-3}M cysteine and 1×10^{-4}M $Cu(ClO_4)_2$ Spectra were taken ——— 0.5 min, – – – – – 1.5 min and ━━━ 5 min after the addition of $Cu(ClO_4)_2$.

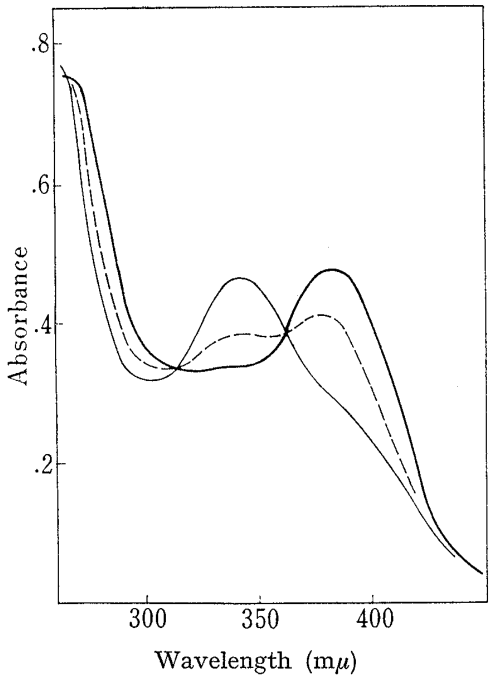
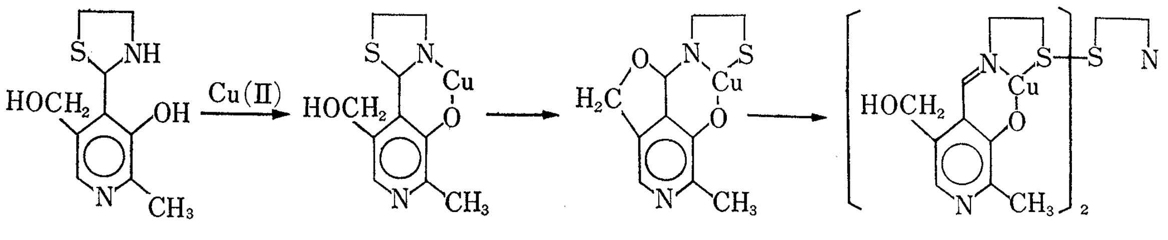


Fig. 3. Reaction of Cu(II) with Thiazolidine Derivative in Methanol (2)

The solution contained 1×10^{-4}M pyridoxal, 1×10^{-3}M cysteamine and 1×10^{-4}M $Cu(ClO_4)_2$. Spectra were taken ——— 0.5 min – – – – – 7 min and ━━━ 30 min after the addition of $Cu(ClO_4)_2$.

Almost the same but more rapid spectral changes were observed in the case of thiazolidine from cysteamine. The first step was too rapid to make detailed analysis. The intermediate spectrum had an absorption peak at 342 mμ, which rapidly decreased in the second step forming a band at 380 mμ. Spectral changes in the second step is shown in Fig. 3. The final spectrum was superimposable to that of Cu(II) chelate of pyridoxylidenecystamine. By adding tetrasodium ethylenediaminetetraacetate to the methanol solution, spectrum was converted to that of the Schiff base from pyridoxal and cystamine. Thus, it was proved that thiazolidine derivatives were converted to Schiff bases, in which thiol group of original amines were oxidized to disulfide form. Intermediate compounds having an absorption at 342 mμ or 360 mμ were presumably N,S,O coordinated complexes. Overall Cu(II) catalyzed oxidation might be



X

Chart 6

shown as Chart 6. Ni(II) and Zn(II) perchlorates also showed analogous oxidation, although by slower reaction rates and possibly by side reactions only obscured spectra of the intermediates were obtainable.

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