[Chem. Pharm. Bull. 15(3) 290~295 (1967)]

UDC 547.569-385

35. Akira Yokoyama and Hisashi Tanaka: Studies on the Sulfur-containing Chelating Agents. XV.*1 Thioxo-thioenol Tautomerism in Ethyl β-Mercaptothiocinnamates and β-Mercaptocinnamamides.

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Thioxo-thioenol tautomerism was discussed in ethyl β-mercaptothiocinnamate and β-mercaptocinnamamides, in connection with their chelating abilities, through the ultraviolet absorption spectra in various solvents, and the estimation of the thioenol by the iodometry. Ethyl β-mercaptothiocinnamate and N-phenyl-β-mercaptocinnamamide were confirmed to be present mainly as the *cis*-thioenol form, and the equilibrium should be considered only between the thioxo and the *cis*-thioenol forms. On the contrary, in N-ethyl-β-mercaptocinnamamide, the *trans*-thioenol form should be taken into consideration in its thioxo-thioenol tautomerism.

(Received February 21, 1966)

In the previous papers,*1,1) the syntheses of alkyl β-mercaptothiocinnamates and β-mercaptocinnamamides, as the strong chelating agents, were reported. The thioxo-thioenol tautomerism shown in Chart 1 was also investigated by the infrared and the nuclear magnetic resonance spectroscopy, and the *cis*-thioenol form was found to be predominant. However, it has not been clear whether or not the tautomerism between the thioxo and the *trans*-thioenol forms can be neglected in these equilibria. On the other hand, on ethyl β-mercaptocinnamate, Reyes, *et al.*2) reported that in the similar tautomeric equilibria to that shown in Chart 1, the thioxo, the *cis*-thioenol and the *trans*-thioenol forms were proved to be present by the infrared spectrum, namely a band which could be assigned to the thioenol group of the *trans*-thioenol form was observed, besides a band corresponding to the intramolecularly hydrogen bonded thiol group. In the same way, three bands based on the carbonyl groups of the thioxo, the *cis*-thioenol and the *trans*-thioenol forms, were observed in the infrared spectrum of ethyl β-mercaptocinnamate. As the tautomerism is considered to have close connection with the chelating abilities of alkyl β-mercaptothiocinnamates and β-mercaptocinnamamides, it was investigated further, mainly by the ultraviolet spectroscopy, prior to the studies on their metal chelates.

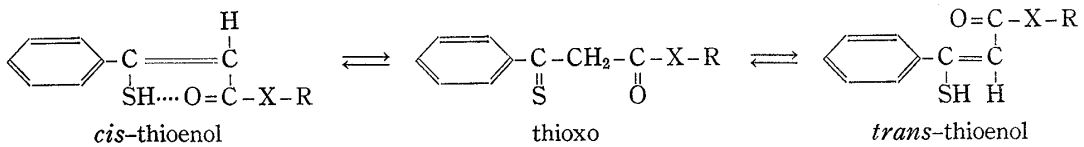

Chart 1. Tautomerism in β-Mercaptothiocinnamates and β-Mercaptocinnamides

Although the literatures contain many references to the keto-enol tautomerism, the investigation of the tautomerism between the thioxo and the thioenol has not been extended beyond on ethyl β-mercaptocinnamates2) and ethyl thioacetoacetates.3)

*1 Part XIV: This Bulletin, **12**, 690 (1964).
*2 Yoshida, Shimoadachi-cho, Sakyo-ku, Kyoto (横山 陽, 田中 久).
1) A. Yokoyama, H. Tanaka: This Bulletin, **12**, 683 (1964).
2) Z. Reyes, R. M. Silverstein: J. Am. Chem. Soc., **80**, 6367 (1958).
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Several sulfur-containing derivatives of β-diketones, such as 4-mercapto-3-penten-2-one and 3-mercapto-1,3-diphenyl-2-propen-1-one, were recently synthesized[4~6] but their tautomerism was not discussed in detail. In the tautomeric equilibria among the keto, the *cis*-enol and the *trans*-enol forms, ethyl acetoacetate,[7] in which only the equilibrium between the keto and the *cis*-enol forms is present, involves a six-membered hydrogen bonded ring which determines its general character or polarity and its solvation in various solvents. In the case of ethyl acetoacetate, Meyer's rule[8] was proved to be applicable, as to the solvent effect on the equilibrium. Meyer's rule is represented by the following equation (1).[9]

$$\frac{\text{Concentration of the } cis\text{-enol form}}{\text{Concentration of the keto form}} = K_{cis(sol)} \qquad (1)$$

In the equation (1), the equilibrium constant, $K_{cis(sol)}$, in the given solvent is estimated as the value which is related to the solvation and the polarity of the solvent, by the way as described in the part of results and discussion. In general, it is known that increased polarity of solvent results in decrease of enolization. On the other hand, in the case that the equilibrium is only between the keto and the *trans*-enol forms, for example, in the compounds, such as dimedone,[10] and α-alkyltetronic acids,[11] the relation between the *trans*-enolization and the solvents is complicated and the simple relation is not derived, unlike in the case of the equilibrium between the keto and the *cis*-enol forms.

As previously reported,*1,1) alkyl β-mercaptothiocinnamates and β-mercaptocinnamamides were presumed to be present mainly as the *cis*-thioenol form, in which the intramolecular hydrogen bonds are involved, by the infrared and the nuclear magnetic resonance spectroscopy. In an attempt to investigate the equilibria of the thioxo and the thioenol in detail and to clarify whether *trans*-thioenolization can be neglected or not, the applicability of Meyer's rule to these compounds was discussed through the solvent effect to the ultraviolet spectra and the determination of the mercapto group by the iodometric titration.

Experimental

Materials——Ethyl β-mercaptothiocinnamate[1] (Compound I), N-phenyl-β-mercaptocinnamamide*1 (Compound II) and N-ethyl-β-mercaptocinnamamide*1 (Compound III) were taken as the examples.

Solvent Effect on the Ultraviolet Spectra——Ultraviolet spectra of 5×10^{-5} mole/L. solution were measured in the various solvents such as *n*-hexane, CCl_4, $CHCl_3$, 99% EtOH and 75% EtOH, by Shimadzu Spectrophotometer QR 50. Each spectrum was measured after keeping the sample solution in thermostat at 25° for 10 hr.

Estimation of the Thioenol by Iodometry——The iodometry was carried out by Mitra's method[3] with a little modification. About 0.1 g. of the sample was taken accurately and 10 ml. of the solvent such as *n*-hexane, CCl_4, $CHCl_3$, 99% EtOH and 75% EtOH, was added to it. The solution was kept at 25° for 10 hr., and 5 ml. of ethanolic iodine solution (1.550 g./100 ml.), which was cooled previously at −7° was added to it. Excess iodine was titrated by *N*/10 $Na_2S_2O_3$ after stirring for 1 min.

Effect of the Dilution in the Nuclear Magnetic Resonance Spectra——Nuclear magnetic resonance spectra in $CDCl_3$ and CCl_4 were measured, by Varian A-60 Spectrometer at 60 Mc.p.s., the concentration of the solution being varied (1, 0.5, 0.25, and 0.1 mole/L.).

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5) D. E. Uhlmann, H. Müller: Angew. Chem., **77**, 172 (1965).
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11) L. A. Dunkanson: J. Chem. Soc., 1207 (1953).

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Results and Discussion

Compound I——The ultraviolet spectra in the various solvents are shown in Fig. 1. Two absorption bands were observed near 315 mμ (band I) and 290 mμ (band II). The intensity of band I decreases in accordance with increase of the polarity of the solvent, namely the order of the effect of the solvent is tetrachloromethane, *n*-hexane, chloroform, 99% ethanol and 75% ethanol. This relationship suggests that band I is attributed to the *cis*-thioenol form. In absorption spectra of ethyl β-(ethylthio)thiocinnamate,[1] which was regarded to be *cis*-thioenol form by its infrared spectrum, in various solutions, absorption bands were always found around 315 mμ. An absorption spectrum in 99% ethanol is shown in Fig. 2 as an example. Similarity between the absorption band in Fig. 2 and the band I in less polar solvents such as *n*-hexane, tetrachloromethane, and chloroform, may support that the band I is based on the *cis*-thioenol form. On the other hand, the relationship between the intensity and the polarity of the solvent in band II was not clearly observed. It was difficult to assign band II, because the contribution of the *trans*-thioenol form as well as the thioxo form may not be neglected to this band. Though the results of the titrations are not considered to be the absolute amount of the thioenol, Mitra's method[3] could be used for the comparative study of the equilibrium,[2] and the values obtained can possibly be used as the relative amount of thioenol. As shown in Table I, the amount of thioenol was higher in the less polar solvent, and the effects of the solvents were similar to those observed in the ultraviolet spectra. These facts suggest that the thioxo and the *cis*-thioenol forms could be considered mainly in this system of the tautomerism. In order to make this point clear, the applicability of Meyer's rule[9] was investigated on the tautomerism of Compound I. In the Compound I, if Meyer's rule can be applicable, the tautomerism should be present only between the thioxo and the *cis*-thioenol forms and the *trans*-thioenol form should be neglected in the tautomerism. According to Meyer's rule, equation (2) is derived from equation (1). Equation (3) is also obtained and equation (4) is derived from equations (2) and (3). In these equations, $\varepsilon_{obs.}$, ε_e and ε_o represents molar absorption coefficient observed, that of the thioenol form, and that of the thioxo form, respectively and C_t, C_e and C_o represents respectively total concentration, concentration of the thioenol form, and that of the thioxo form.

$$K_{cis(sol)}=C_e/C_o=C_e/(C_t-C_e) \qquad (2)$$

$$\varepsilon_{obs.}\cdot C_t=\varepsilon_e\cdot C_e+\varepsilon_o\cdot C_o \qquad (3)$$

$$\varepsilon_{obs.}\left(\frac{1}{K_{cis(sol)}}+1\right)=\varepsilon_e+\varepsilon_o\frac{1}{K_{cis(sol)}} \qquad (4)$$

A linear relation should be obtained between $\varepsilon_{obs.}\left(\frac{1}{K_{cis(sol)}}+1\right)$ and $\frac{1}{K_{cis(sol)}}$, provided that the tautomerism is present only between the thioxo and the *cis*-thioenol forms. The value of $\frac{1}{K_{cis(sol)}}$ can be calculated from the amount of the thioenol obtained by the iodometry by the equation (2). The relationship between the solvent and intensity of absorption is shown in Fig. 3 and the value of $\frac{1}{K_{cis(sol)}}$ calculated are shown in Table I. From the linearity seen in Fig. 3, it is proved that Meyer's rule can be applicable. Judging from these results, it is confirmed that the tautomerism is present between the thioxo and the *cis*-thioenol forms and the contribution of the *trans*-thioenol form is possibly neglected.

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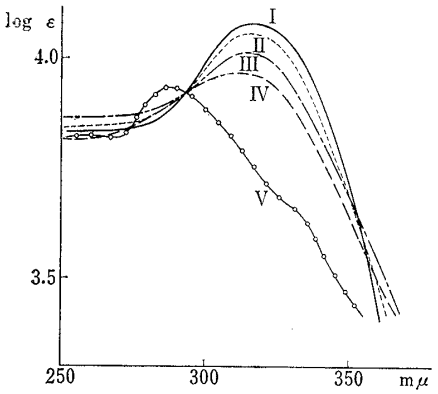

Fig. 1. Ultraviolet Absorption Spectra of Ethyl β-Mercapto-thiocinnamate

I : in CCl_4, II : in n-C_6H_{14}, III : in $CHCl_3$, IV : in 99% EtOH, V : in 75% EtOH, T=25°

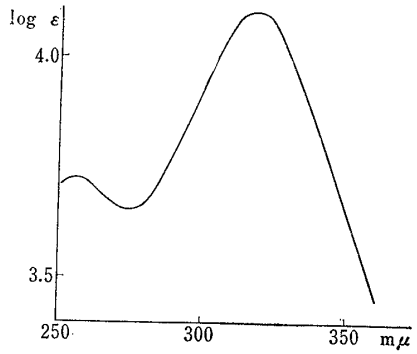

Fig. 2. Ultraviolet Absorption Spectrum of Ethyl β-(Ethylthio)thiocinnamate in EtOH

Table I. Estimation of the Thioenol by the Iodometry and Values of $\frac{1}{K_{cis(sol)}}$ Calculated

Solvent	Compound I (g.)	$N/10$ $Na_2S_2O_3$ (f=1.051) (ml.)	$\frac{1}{K_{cis(sol)}}$	average	Thioenol average (%)
75% EtOH	0.1105	4.42	2.37	2.36	29.8
	0.0895	4.66	2.30		
	0.1155	4.37	2.40		
99% EtOH	0.1060	2.93	0.561	0.574	63.4
	0.1110	2.82	0.568		
	0.1129	2.80	0.592		
$CHCl_3$	0.1330	1.72	0.382	0.388	72.0
	0.1130	2.34	0.383		
	0.1114	2.43	0.399		
CCl_4	0.1010	2.28	0.215	0.215	82.3
	0.1046	2.16	0.217		
	0.1000	2.31	0.214		
n-C_6H_{14}	0.1100	2.35	0.282	0.281	78.0
	0.1080	2.41	0.280		
	0.1107	2.33	0.281		

Compound II and Compound III——As previously reported,*1 Compound II (aromatic amide) and Compound III (aliphatic amide) showed remarkably different character each other on the chelate formations and this difference is considered to be due to the difference in the tautomerism. In this connection, the similar investigations were planned on these compounds. However, the iodometry could not be carried out because the oxidation did not take plase quantitatively and hence the applicability to Meyer's rule could not be investigated. The ultraviolet absorption spectra of Compound II and III in the various solvents are shown in Figs. 4 and 5. In the case of the Compound II, the intensity of the band near 315 mμ (band III) is observed to be a little higher in the less polar solvent. Accordingly, band III was considered to be based on the *cis*-thioenol form, and the absorption spectrum (Fig. 6) of N-phenyl-β-(ethylthio)cinnamamide,*1

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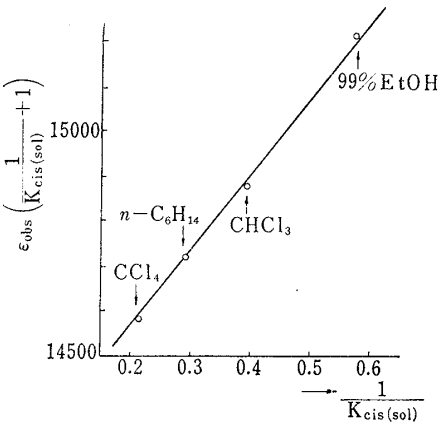

Fig. 3.[a] Relationship between Solvents and Intensity of Absorption in Ethyl β-Mercaptothiocinnamate (at 315 mμ).

a) The values of ε_e and ε_o were obtained from Fig. 3, respectively as 14200 and 1700. The value of $\varepsilon_{obs.}$ in 75% ethanol (5300) coincides with the value of calculated from the equation (4) (5400), although a value of $\frac{1}{K_{cis(sol)}}$ in 75% ethanol was far higher from others, and is not shown in Fig. 3.

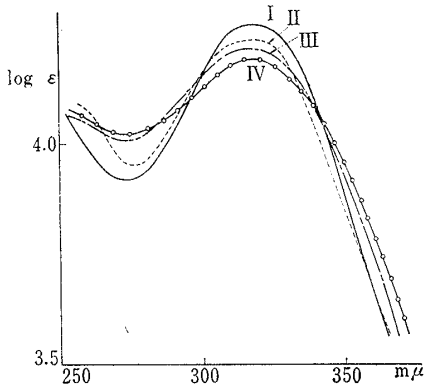

Fig. 4. Ultraviolet Absorption Spectra of N-Phenyl-β-mercaptocinnamamide

I : in CCl_4, II : in $CHCl_3$, III : in 99% EtOH, IV : in 75% EtOH, T=25°

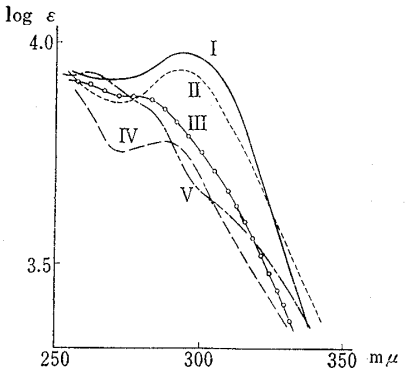

Fig. 5. Ultraviolet Absorption Spectra of N-Ethyl-β-mercaptocinnamamide

I : in n-C_6H_{14}, II : in $CHCl_3$, III : in 75% EtOH, IV : in CCl_4, V : in 99% EtOH. T=25°

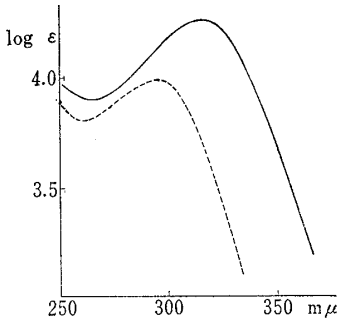

Fig. 6. Ultraviolet Absorption Spectra of N-Phenyl-β-(ethylthio)cinnamamide and N-Ethyl-β-(ethylthio)cinnamamide

——— N-Phenyl-β-(ethylthio)cinnamamide

- - - - - - N-Ethyl-β-(ethylthio)cinnamamide

in EtOH

which was referred for this assignment, as in the case of Compound I. On the contrary, in the Compound III, as shown in Fig. 5, the absorption band was observed near 295 mμ (band IV) in n-hexane, chloroform and tetrachloromethane, and band IV was considered to be based on the thioenol form, the absorption spectrum (Fig. 6) of N-ethyl-β-(ethylthio)cinnamamide*[1] being referred, as in the case of Compound I. However, the polarity of the solvents and the intensity of the band IV did not correlate clearly.

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Therefore, in the Compound Ⅲ, the equilibrium is not simply between the *cis*-thioenol and the thioxo forms, and the *trans*-thioenol form should be taken into consideration. The difference in the tautomerism is considered to reveal the difference in the chelating property as reported previously,*1 between the Compound Ⅱ and Ⅲ.

As previously reported in nuclear magnetic resonance spectrum of Compound I,[1] the signals corresponding to the thioxo form were not observed. In nuclear magnetic resonance spectra, the mercapto group was presumed to be hydrogen bonded with the carbonyl group intramolecularly, because the signal ($\tau=2.02$) based on the mercapto group was not influenced by the dilution. This result also supports the above mentioned conclusions obtained from the investigation by the ultraviolet spectroscopy.

The authors extend their gratitude to Prof. T. Uno of Kyoto University for his helpful advices. They are also indebted to Dr. T. Shingu of Kyoto University for the measurement of the nuclear magnetic resonance spectra.

[Chem. Pharm. Bull. 15(3) 295~302 (1967)] UDC 615.739-03 : 577. 16B

36. Hideyo Shindo, Koichi Okamoto, and Jun-ichi Totsu : Transport of Organic Compounds through Biological Membranes. I. Accumulative Uptake of S-Benzoylthiamine by Human Erythrocytes.

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The accumulative uptake of S-benzoylthiamine (SBT) by human red cells was demonstrated and the detail of the transport mechanism was investigated. From the studies on i) the transport of SBT into the red cells and into the ghosts, ii) the decomposition of SBT in the red cell homogenate and in an aqueous medium, iii) the effect of the concentration gradient on the uptake, and iv) the effect of the extracellular pH on the uptake, it was concluded that the penetration of SBT into the red cells proceeds through two steps; the first, a passive diffusion of SBT through the cell membrane, and the second, a rapid decomposition of SBT to undiffusible thiamine and the resultant its accumulation in the cell. In a series of substituted S-benzoylthiamines, the rate of their decomposition to thiamine in the red cell was found to be a predominant factor determining the rate of the penetration into the red cells, both being a linear function to Hammett's σ-constants of the substituents.

An important role of SBT in the intestinal absorption of S-benzoylthiamine monophosphate and the followed high blood thiamine concentration and its duration was pointed out.

(Received March 25, 1966)

In this laboratories, it has been found[1] that S-benzoylthiamine monophosphate (BTMP),*2 I, is much more readily absorbed from intestine than thiamine, and the oral administration results in higher thiamine and co-carboxylase levels in blood and in organs; moreover these levels last for a longer period of time. It has been well

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*2 The following abbreviations will be used in this paper. BTMP : S-Benzoylthiamine monophosphate, SBT : S-Benzoylthiamine, OBT : O-Benzoylthiamine, TMP : Thiamine monophosphate, TDP : Thiamine diphosphate.

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