The inoculum size was about 2000. The culture medium used contained : K_2HPO_4 0.7%, KH_2PO_4 0.3%, $(NH_4)_2SO_4$ 0.2%, $MgSO_4$ 0.01%, casamino acid 1.5%, nicotinic acid 0.01%, thiamine 0.01%, and glucose 0.2%, pH 7.0. The compound was taken as effective when complete supression of colony-formation is observed after incubation for 48 hr. and considered as ineffective when one or more colonies were observed.

Summary

N^1-(2-Alkylthio-6-alkoxy-4-pyrimidinyl)sulfanilamide and N^1-[2,6-bis(alkylthio)-4-pyrimidinyl]sulfanilamide were synthesized and were screened for their antibacterial activity using *Escherichia coli* C_{14} and K_{12}, *Aerobactor aerogenes* 1033, *Salmonella enteritidis* No. 11, *Staphylococcus aureus* Terashima.

N^1-(2-Methylthio-6-methoxy(and ethoxy)-4-pyrimidinyl)sulfanilamides were effective on some of these bacteria. The latter compound had an effect comparable to that of sulfadimethoxine.

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UDC 547.569-386

3. **Hisashi Tanaka and Akira Yokoyama :** Studies on Sulfur-containing Chelating Agents. VII.[*1] Syntheses of β-Mercaptothiolic Acid Esters and their Metal Chelates.

(*Faculty of Pharmacy, Kyoto University*[*2])

In the previous papers,[1),*1] synthesis of β-mercapto-acid esters and their metal chelates was reported. The present paper deals with the synthesis of β-mercaptothiolic acid esters and their metal chelates.

As in the case of β-mercapto-acid esters, the chelate-formation of β-mercaptothiolic acid esters would occur through the combination of carbonyl group of the ester and mercapto group. Considering the difference between the character of sulfur in thiolic acid ester and that of oxygen in the ester, that is, sulfur is more polarizable and less electronegative than oxygen, β-mercaptothiolic acid ester would be expected to show different attitude towards chelate-formation from that of β-mercapto-acid ester. The synthesis of β-mercaptothiolic acid esters was therefore planned to compare their chelating ability with that of β-mercapto-acid esters.

S-Alkyl or S-aryl esters of β-mercaptothiohydrocinnamic acid were prepared as β-mercaptothiolic acid esters. Addition of hydrogen sulfide to S-esters of thiocinnamic acid was found to be satisfactory for the preparation of the S-esters of β-mercaptothiohydrocinnamic acid. As shown in Chart 1, hydrogen sulfide was reacted with S-alkyl or S-aryl thiocinnamate in a manner which was found to be the best in the case of β-mercapto-acid esters. S-Alkyl or S-aryl thiocinnamates were prepared by the method described by Bestram.[2)] Separation of β-mercaptothiolic acid esters from the reaction mixture

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[*1] Part VI. H. Tanaka, A. Yokoyama : This Bulletin, **9**, 110 (1961).

[*2] Yoshida, Sakyo-ku, Kyoto (田中 久, 横山 陽).

1) Part V. H. Tanaka, A. Yokoyama : This Bulletin, **9**, 66 (1961).

2) H. J. Bestram : Chem. Ber., **92**, 530 (1959).

was difficult, because it was readily hydrolyzed to β-mercaptohydrocinnamic acid by treatment with alkali. However, β-mercaptothiolic acid esters were separated and purified by repeated fractional distillation or through their lead salts.

In an attempt to investigate the influence of alkyl or aryl group of the ester, S-ethyl, -isopropyl, -butyl, -isopentyl, -dodecyl, -phenyl, and -benzyl esters were prepared. In the case of the addition of hydrogen sulfide to S-isopropyl thiocinnamate and S-benzyl thiocinnamate, a small amount of the corresponding disulfide and monosulfide were respectively obtained, besides the expected β-mercaptothiolic acid esters. As reported in Part V,[1] the preparation of β-mercaptohydrocinnamoyl chloride was unsuccessful and the addition of hydrogen sulfide to S-esters of thiocinnamic acid is considered to be the only method for the preparation of β-mercaptothiolic acid esters.

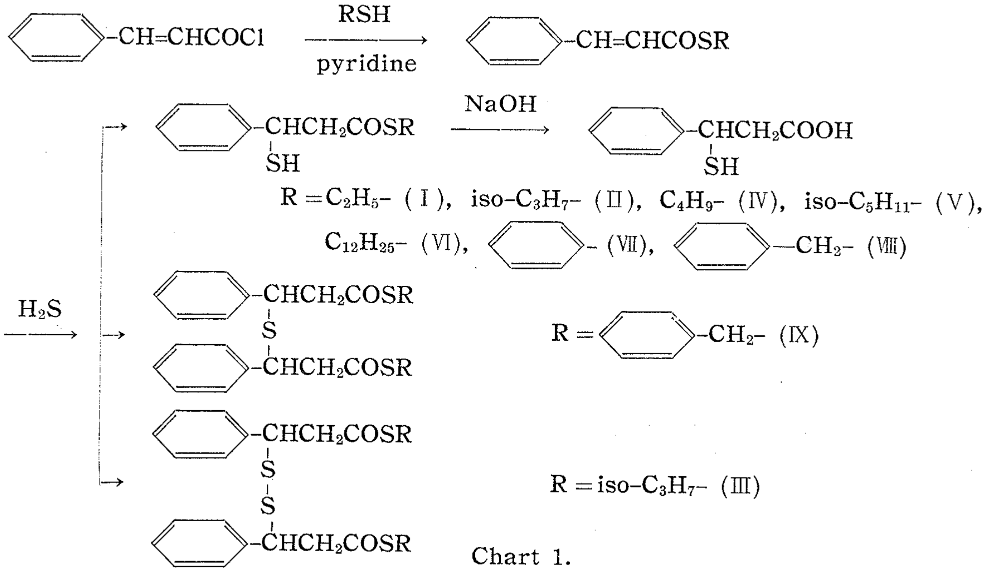
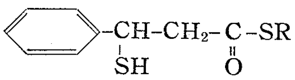


Chart 1.

The reaction of β-mercaptothiolic acid esters with metal ions was examined by the spot test in acid (acetic acid), neutral, and alkaline (ammonium hydroxide) solution, and the result is shown in Table I. No marked difference in selectivity was observed according to the difference in alkyl or aryl group. Among the metal ions listed in Table I, the salts of mercury, silver, and lead that precipitated were not soluble in organic solvents such as chloroform or ethyl acetate. Other metals such as cobalt, nickel, copper, palladium, gold, bismuth, and platinum produced a precipitate or an oily substance, considered to be a chelate compound, which were extractable by organic solvents. Comparing the results obtained from β-mercaptothiolic acid esters with that from β-mercapto-

Table I. Spot Test (in neutral solution)



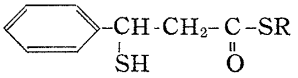
$C_6H_5\text{-}\underset{\large SH}{CH}\text{-}CH_2\text{-}\underset{\large O}{\overset{}{C}}\text{-}SR$

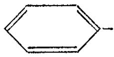
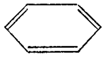
Metal ion	Reaction	Properties
Co^{2+}	Brown oil	sol. in AcOEt
Ni^{2+}	Brick red ppt.	〃
Cu^{2+}	Yellow ppt.	〃
Pd^{2+}	Red ppt.	〃
Ag^{+}	White ppt.	insol. in AcOEt
Pt^{4+}	Pale yellow ppt.	sol. in AcOEt
Au^{3+}	Brown ppt.	〃
Hg^{2+}	White ppt.	insol. in AcOEt
Pb^{2+}	Yellow ppt.	〃
Bi^{3+}	〃	sol. in AcOEt

Reagent : 1% EtOH solution.

acid esters, a few marked differences were observed. β-Mercaptothiolic acid esters reacted with cobalt, while β-mercapto-acid esters did not, and β-mercaptothiolic acid esters reacted with nickel more slowly than β-mercapto-acid esters did. The recognizable limit of nickel, copper, cobalt, palladium, and bismuth was measured and, as shown in Table II, these β-mercaptothiolic acid esters were especially sensitive to nickel, so that they could be used as the analytical reagent for nickel. The sensitivity increased with increasing number of carbon atoms in the ester group until isopentyl, and the sensitivity unexpectedly decreased in the case of S-dodecyl ester.

Tabel II. Recognizable Limit of the Metal Ions by β-Mercaptothiolic Acid Esters



Ligand R	Metal ion (γ/0.05 cc.)					
	Co^{2+}*	Ni^{2+}	Cu^{2+}	Pd^{2+}	Pt^{4+}	Bi^{3+}
C_2H_5-	1	0.1	20	0.2	20	5
iso-C_3H_7-	1	0.1	10	0.25	25	5
C_4H_9-	1	0.05	20	0.3	25	5
iso-C_5H_{11}-	1	0.05	10	0.3	25	5
$C_{12}H_{25}$-	10	1	50	0.1	50	10
 -CH_2-	10	0.3	30	0.5	30	5
 -	10	0.5	50	0.5	30	5

Reagent : 1% EtOH solution of β-mercaptothiolic acid ester.
* Measured in Na_2CO_3 solution.

Copper chelates were separated and purified as a pale yellow crystalline powder, which melted sharply. The ratio of the ligand to the metal was confirmed as 1:1 from microanalytical data. After the copper chelate was separated, β,β′-dithiodihydrocinnamic acid was obtained, when the residual solution was hydrolyzed. Judging from these facts, the process and mechanism of chelate-formation with copper are regarded to be quite similar to those in the case of β-mercaptoketones, reported in Part IV.[3] Nickel chelates were separated and purified as a brick red crystalline powder, which melted sharply with decomposition. The ratio of the ligand to the metal was confirmed as 2:1 from microanalytical data. The structures of these chelates are considered to be as shown in the experimental part, from these results.

The infrared spectra of β-mercaptothiolic acid esters and their copper and nickel chelates were measured in chloroform solution and in hexachlorobutadiene mull. Clear absorption bands based on the mercapto group and ester group were observed at 2580 cm^{-1} and 1670∼1680 cm^{-1}, respectively, in all of the ligands. In copper and nickel chelates, the band at 2580 cm^{-1} disappeared, but no obvious shift of the band of ester group was observed. The absorption spectra in visible and ultraviolet regions of the copper, nickel, and cobalt chelates are shown in Figs. 1, 2, and 3. The ligand showed an absorption maximum at 300 mμ, but a little bathochromic shift was observed in the copper and nickel chelates. The copper chelate did not show a clear maximum in the visible region, while nickl chelate showed two absorption maxima at 460 and 540 mμ. Cobalt chelate had a shoulder at 300 mμ and a considerable absorption was observed in the visible region. From the fact that the type of this absorption curves of these metal chelates is different from each other, it is expected that the type of bonding between the ligand and the metal is different in these three cases. Cobalt chelate could not be separated but the absorption curve confirms its existence. The ratio of the ligand to cobalt in chloroform solution was found to be 3:1, by the continuous method, even when cobalt(II) acetate was used. It

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[3] Part IV. H. Tanaka, A. Yokoyama : This Bulletin, 8, 1012 (1960).

was not possible to obtain cobalt(II) chelate even when the chelate-formation was carried out in a stream of hydrogen or in the presence of a reducing agent. Judging from these results, it was presumed that cobalt(II) chelate changed into cobalt(III) chelate immediately, taking octahedral configuration. In general, cobalt chelate has a tendency to take an octahedral configuration and the present results are not contrary to this tendency.

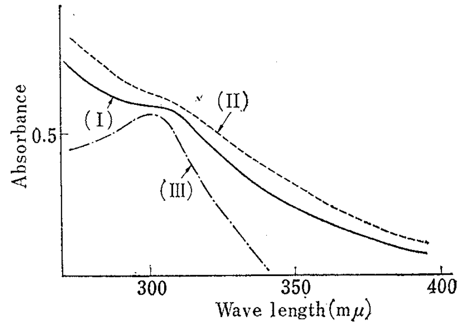
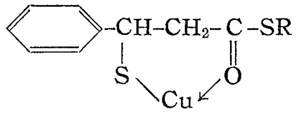


Fig. 1.



1×10^{-4} mole in $CHCl_3$

(I) ——— R = C_2H_5-
(II) -------- R = iso-C_5H_{11}-
(III) ---- R = C_2H_5- (Ligand)

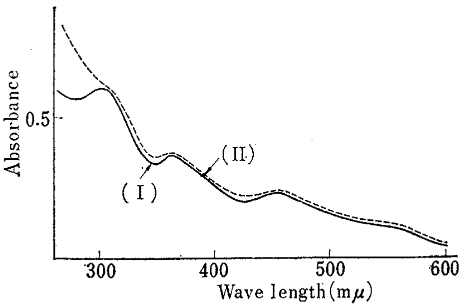
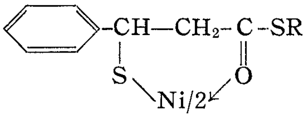


Fig. 2



5×10^{-5} mole in $CHCl_3$
(I) —— R = C_2H_5-
(II) ------ R = iso-C_5H_{11}-

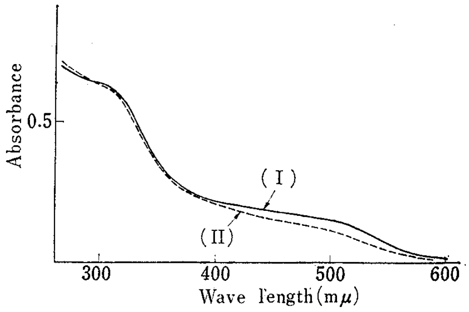
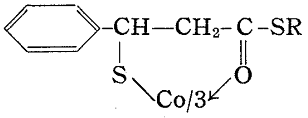


Fig. 3

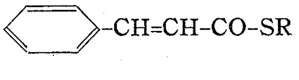


3.3×10^{-5} mole in $CHCl_3$
(I) —— R = C_2H_5-
(II) ------ R = iso-C_5H_{11}-

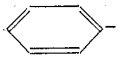
Experimental

S-Ester of Thiocinnamic Acid——To a solution of 0.05 mole of thiol in 50 cc. of dehyd. pyridine, 0.05 mole of cinnamoyl chloride was added in small portions under ice cooling. After standing over night, the reaction mixture was poured into 200 cc. of 20% H_2SO_4 in ice water. The oil or precipitate that separated out was extracted into Et_2O, Et_2O layer was washed successively with H_2O, saturated $NaHCO_3$ solution, and H_2O, and dried over Na_2SO_4. Evaporation of the solvent left a colorless oil or precipitate which was purified in a suitable way. Boiling point, melting point, yield, and microanalytical data are given in Table III.

TABLE III. S-Esters of Thiocinnamic Acid



C_6H_5-CH=CH-CO-SR

R	b.p. (°C/mm. Hg)	Yield (%)	Formula	Analysis (%) Calcd. C	H	S	Found C	H	S
iso-C_3H_7-	135~137/4	72	$C_{12}H_{14}OS$	69.88	6.84		69.84	7.02	
C_4H_9-	143/4	73	$C_{13}H_{16}OS$	70.89	7.32		70.68	7.41	
iso-C_5H_{11}-	166/4	65	$C_{14}H_{18}OS$	71.77	7.74	13.66	71.50	8.03	13.57
$C_{12}H_{25}$-	m.p. 49*	65	$C_{21}H_{32}OS$	75.86	9.70	9.62	75.97	9.88	9.49
	m.p. 91*	74	$C_{15}H_{12}OS$	74.99	5.03	13.32	74.93	5.08	13.44

* Recrystallized from EtOH.

S-Ethyl β-Mercaptothiohydrocinnamate (I)——To a solution of 8 g. of S-ethyl thiocinnamate and 3 g. of Et_3N in 100 cc. of dry $CHCl_3$ placed in a pressurized bottle, dry H_2S gas was introduced and liquified by chilling with dry ice-acetone to $-70°$ to $-80°$ until the increase in volume became 10~12 cc. The bottle was closed and allowed to stand for a week at room temperature under pressure. After removal of excess H_2S, the reaction mixture was washed with dil. HCl and H_2O, and dried over Na_2SO_4. The solvent was evaporated, the residual oil was distilled in a diminished pressure, using a fractionating column, 2 g. of the starting material was recovered, and 4 g. of (I) was collected and purified by redistillation. Pale yellow oil, b.p$_8$ 166°. Rheinboldt test, red. *Anal.* Calcd. for $C_{11}H_{14}OS_2$: C, 58.40; H, 6.24. Found : C, 58.67; H, 6.33.

S-Isopropyl β-Mercaptothiohydrocinnamate (II) and S,S′-Diisopropyl β,β'-Dithiobis(thiohydrocinnamate) (III)——To a solution of S-isopropyl thiocinnamate and 3 g. of Et_3N in 100 cc. of $CHCl_3$, 10 cc. of H_2S was added as described above. The reaction mixture was distilled using a fractionating column, 2 g. of the starting material was recovered, and 3 g. of (II) was collected and purified by redistillation. Pale yellow oil, b.p$_5$ 150~154°. Rheinboldt test, red. *Anal.* Calcd. for $C_{12}H_{16}OS_2$: C, 59.99; H, 6.71; S, 26.64. Found : C, 60.11; H, 6.51; S, 26.45.

After distillation, 0.3 g. of (III) was obtained from the residue and recrystallized from petr. ether to colorless needles, m.p. 125°. Rheinboldt test, negative. *Anal.* Calcd. for $C_{24}H_{30}O_2S_4$: C, 60.24; H, 6.32; S, 26.75. Found : C, 60.61; H, 6.47; S, 26.49.

S-Butyl β-Mercaptothiohydrocinnamate (IV)——To a solution of 8 g. of S-butyl thiocinnamate and 3 g. of Et_3N in 100 cc. of dry $CHCl_3$, 10 cc. of H_2S was added as described above. The reaction mixture was distilled using a fractionating column, 3 g. of the starting material was recovered, and 3 g. of (IV) was collected and purified by redistillation. Pale yellow oil, b.p$_5$ 165~168°. Rheinboldt test, red. *Anal.* Calcd. for $C_{13}H_{18}OS_2$: C, 61.40; H, 7.14; S, 25.17. Found : C, 61.58; H, 7.36; S, 24.96.

S-Isopentyl β-Mercaptothiohydrocinnamate (V)——To a solution of 5 g. of S-isopentyl thiocinnamate and 2 g. of Et_3N in 100 cc. of dry $CHCl_3$, 10 cc. of H_2S was added as described above. The reaction mixture was distilled using a fractionating column, 3 g. of (V) was collected, and purified by redistillation. Pale yellow oil, b.p$_5$ 157~158°. Rheinboldt test, red. *Anal.* Calcd. for $C_{14}H_{20}OS_2$: C, 62.67; H, 7.51; S, 23.86. Found : C, 62.06; H, 7.12; S, 23.01.

S-Dodecyl β-Mercaptothiohydrocinnamate (VI)——To a solution of 7 g. of S-dodecyl thiocinnamate and 2 g. of Et_3N in 100 cc. of dry $CHCl_3$, 13~15 cc. of H_2S was added as described above. After standing for 10 days at room temperature under pressure, the reaction mixture was washed with dil. HCl and H_2O, and dried over Na_2SO_4. The solvent was evaporated and the residual oil was distilled in a diminished pressure. After a small forerun of dodecylmercaptan (b.p$_5$ 105~110°), crude (VI) (b.p$_{0.8}$ 130~150°) was collected and 3 g. of the starting material (m.p. 45°) was recovered from the residue. Crude (VI) was dissolved in EtOH and treated with $(AcO)_2Pb$ in H_2O. The Pb mercaptide that precipitated was collected, washed with EtOH, and (VI) was regenerated by passing H_2S through a suspension of the Pb mercaptide in Et_2O. PbS was removed, the solvent was evaporated, and 1 g. of (VI) was obtained in a pure state as a pale yellow oil. Rheinboldt test, red. *Anal.* Calcd. for $C_{21}H_{34}OS_2$: C, 68.82; H, 9.35. Found : C, 69.14; H, 9.81.

S-Phenyl β-Mercaptothiohydrocinnamate (VII)——To a solution of 7 g. of S-phenyl thiocinnamate and 3 g. of Et_3N in 100 cc. of dry $CHCl_3$, 15 cc. of H_2S was added as described above. After standing for 10 days at room temperature under pressure, the reaction mixture was washed with dil. HCl and H_2O, and dried over Na_2SO_4. The solvent was evaporated and the residual oil was distilled in a diminished pressure. After a small forerun of thiophenol, 1 g. of (VII) was collected and purified by redistillation. Pale yellow oil, b.p$_1$ 155°, solidified after standing for a few hours. Rheinboldt test, red. *Anal.* Calcd. for $C_{15}H_{14}OS_2$: C, 65.69; H, 5.15. Found : C, 65.72; H, 5.32.

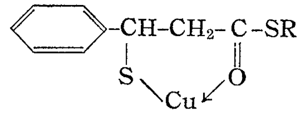
S-Benzyl β-Mercaptothiohydrocinnamate (VIII) and S,S′-Dibenzyl β,β'-Thiobis(thiohydrocinnamate) (IX)——To a solution of 7 g. of S-benzyl thiocinnamate and 4 g. of Et_3N in 100 cc. of $CHCl_3$, H_2S was added as described above. The reaction mixture was washed with dil. HCl and H_2O, and dried over Na_2SO_4. The solvent was evaporated and the residual oil was distilled in a diminished pressure. After a small forerun of $PhCH_2SH$, 0.5 g. of (VIII), b.p$_4$ 160~168°, and 1 g. of (IX), b.p$_{0.2}$ 188°, were collected. (VIII) was purified through Pb mercaptide as described for (VI). Pale yellow oil. Rheinboldt test, red. *Anal.* Calcd. for $C_{16}H_{16}OS_2$: C, 66.66; H, 5.59. Found : C, 65.82; H, 5.24.

(IX) was purified by redistillation. Reddish oil. Rheinboldt test, negative. *Anal.* Calcd. for $C_{32}H_{30}O_2S_3$: C, 70.83; H, 5.57; S, 17.74. Found : C, 70.31; H, 5.44; S, 17.09.

Preparation of Copper Chelate and Separation of β,β'-Dithiodihydrocinnamic Acid——EtOH solution of β-mercaptothiolic acid ester and aqueous solution of excess $(AcO)_2Cu\cdot H_2O$ were mixed and warmed on a water bath with shaking for a few minutes. Yellow viscous oil separated out. The solvent was decanted and the crude chelate, which solidified while standing in the air, was washed with H_2O and purified by reprecipitation from $CHCl_3$-EtOH. To the decanted solution, after the crude chelate was separated, 10% NaOH was added, warmed on a water bath for a few minutes, and filtered. The filtrate was acidified with dil. HCl, extracted with Et_2O, and Et_2O was evaporated.

A small amount of β,β'-dithiodihydrocinnamic acid, m.p. 145°, was obtained. It did not show any depression of melting point with an authentic specimen. Microanalytical data and melting point of Cu chelates are shown in Table IV.

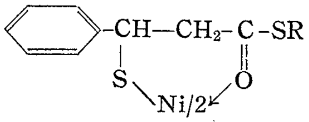
Table IV. Coppr Chelate of β-Mercaptothiolic Acid Esters

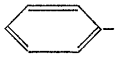


R	m.p. (°C)	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. Cu	Found C	Found H	Found Cu
C_2H_5-	95	$C_{11}H_{13}OCuS_2$	45.75	4.50	22.01	45.54	4.50	22.19
iso-C_3H_7-	98～99	$C_{12}H_{15}OCuS_2$	47.60	4.96	20.99	46.96	5.17	20.11
C_4H_9-	79～82	$C_{13}H_{17}OCuS_2$	49.28	5.37	20.06	49.54	4.92	19.80
iso-C_5H_{11}-	108～109	$C_{14}H_{19}OCuS_2$	50.83	5.74	19.21	51.15	5.18	19.62

Preparation of Nickel Chelate——EtOH solution of β-mercaptothiolic acid ester and aqueous solution of excess $(AcO)_2Ni\cdot 4H_2O$ were mixed and shaken for a few minutes. Brick-red Ni chelate that precipitated was collected and washed with H_2O and EtOH. Microanalytical data and melting point are shown in Table V.

Table V. Nickel Chelate of β-Mercaptothiolic Acid Esters



R	m.p. (decomp.) (°C)	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. Ni	Found C	Found H	Found Ni
C_2H_5-	195～196	$C_{22}H_{26}O_2NiS_4$	51.90	5.11	11.54	52.89	5.42	11.63
iso-C_3H_7-	191	$C_{24}H_{30}O_2NiS_4$	53.66	5.59	10.94	54.25	5.74	10.80
C_4H_9-	175.5	$C_{26}H_{34}O_2NiS_4$	55.25	6.02	10.39	54.95	6.04	10.62
iso-C_5H_{11}-	191～193	$C_{28}H_{38}O_2NiS_4$	56.69	6.41	9.90	56.05	6.10	10.35
-CH_2-	118	$C_{32}H_{30}O_2NiS_4$	60.69	4.74	9.28	59.55	4.66	10.20

The authors express their gratitude to Prof. Toyozo Uno for his helpful advices. They are also indebted to the members of the Microanalytical Center of the Kyoto University for the analytical data. The infrared spectra were measured by Mr. K. Machida and Mrs. I. Hamanaka of Kyoto University, to whom the authors are also grateful.

Summary

S-Esters of β-mercaptothiohydrocinnamic acid were prepared by the addition of hydrogen sulfide to the S-esters of thiocinnamic acid. S-Methyl, -ethyl, -isopropyl, -butyl, -isopentyl, -dodecyl, -phenyl, and -benzyl esters were prepared and their chelating ability with metals was tested. They formed stable chelates with copper and nickel, and the ratio of ligand to the metal was 1:1 in copper chelate and 2:1 in nickel, chelate. Cobalt chelate was stable only in the organic solvent, with the ratio of 3:1. S-Isopentyl β-mercaptothiohydrocinnamate was very sensitive with nickel and recognizable limit of nickel by this compound was 0.05γ/0.05 cc.

(Received June 14, 1961)