³⁵S Labeling of Thiourea Derivatives by Isotope Exchange Reaction

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The exchange reactions of thiourea derivatives having —NH—C=S group with sodium hydrosulfide-³⁵S or with elementary sulfur-³⁵S were investigated. In all systems the exchange reaction occurred under the more drastic condition than that on dithiocarbamates. Thiol form as tautomer of these thiourea derivatives appeared to inhibit the exchange reaction. ³⁵S labeled thiourea derivatives were isolated from the reaction mixtures.

1. Introduction

Dithiocarbamates¹⁾ and xanthates²⁾ easily exchange their sulfur atoms with sodium hydrosulfide-³⁵S as well as elementary sulfur-³⁵S. If the polarization of thiocarbonyl group contributes to these exchange reactions as has been assumed, the similar exchange reaction would take place on thiourea derivatives. A few informations on the exchange reaction of thiourea³⁾ and its derivatives⁴⁾ with elementary sulfur-³⁵S are found in literatures. However, the studies on the exchange reaction with any other radioactive sulfur compounds have not yet been reported.

This report deals with the isotope exchange reactions of three thiourea derivatives, methyl thiourea, trimethyl thiourea and ethylene thiourea, with elementary sulfur-³⁵S or sodium hydrosulfide-³⁵S. The application of the exchange reactions to preparation of ³⁵S labeled thiourea derivatives was also described.

2. Experimental

2.1 Materials

Commercially available thiourea derivatives were purified by recrystallization before use. Methyl thiourea, mp 120–121°C; trimethyl thiourea, mp 88°C; ethylene thiourea, mp 198–199°C.

Sulfur-³⁵S and sodium hydrosulfide-³⁵S were prepared from sulfuric acid-³⁵S by the method described in the previous paper¹⁾.

2.2 Determination of sulfur and measurement of radioactivity

The solution which contained elementary sulfur, sodium hydrosulfide or thiourea derivative was treated with alkaline hydrogen peroxide, and the resulting sulfate was precipitated by adding excess barium chloride after acidifying the solution with hydrochloric acid. Excess barium was titrated with EDTA, and the amount of sulfur in the sample solution was estimated from the titer.

The barium sulfate was collected on a small disk of filter paper, and then its radioactivity was measured under the end window GM tube. Measurement was carried out for the sample with infinite thickness and the counting time was limited within a counting error of 5 per cent.

2.3 Exchange reaction with elementary sulfur-³⁵S

Two ml of methanol solutions containing 0.3 mmol of a thiourea derivative were transferred into glass tubes. After carefully distilling off methanol under reduced pressure, 2 ml of toluene solutions containing a known amount of elementary sulfur-³⁵S

were added, then tubes were sealed and heated at 130°C in an air bath. After 5, 25 and 35 hours, the tubes were successively taken out of heat, cooled and opened. Thiourea derivatives were extracted from the reactants with three 1 ml portions of water, and toluene layers were subjected to measurement of radioactivity of elementary sulfur-³⁵S.

2.4 Exchange reaction with sodium hydrosulfide-³⁵S

A weighed amount (ca. 7.5 mmoles) of a thiourea derivative was dissolved in 50 ml of the aqueous solution of 0.3 M sodium hydrosulfide-³⁵S. Two ml portions of the solution were heated at 150°C in the sealed tubes. After the given reaction periods, the tubes were cooled and opened. The solution was transferred into a flask of "acid decomposition apparatus"¹¹⁾ which was connected to a gas absorption tube containing 20 ml of 1 N sodium hydroxide solution. Six normal hydrochloric acid was added to the reactant in the flask, and air was passed through the apparatus for 5 minutes at a rate of 100 ml/min. The solution in the flask was boiled under further aeration for 20 minutes. After complete removal of hydrogen sulfide, the residual solution in the flask was filtered and the filtrate was subjected to measurement of the radioactivity of thiourea derivative portion.

2.5 The isolation of ³⁵S labeled thiourea derivatives from the exchange reaction systems

Reaction mixtures were evaporated to dryness under reduced pressure, and methanol solution of the corresponding thiourea derivative was added to the residue. Sulfur-³⁵S or sodium hydrosulfide-³⁵S was removed by means of filtration, and the filtrate was concentrated under reduced pressure to give crystal of the thiourea derivative. The radioactivities of the labeled products were measured after four or five recrystallizations.

3. Results and Discussion

Trimethyl thiourea gave a homogeneous reaction mixture with elementary sulfur-³⁵S at 130°C in toluene, while methyl thiourea and ethylene thiourea, which did not dissolve in toluene even at 130°C, made heterogeneous reaction mixtures.

Aqueous extracts from exchange reaction systems of elementary sulfur-³⁵S should contain only thiourea derivative. In aqueous layer from methyl thiourea system, however, another unexpected material was found by means of paper chromatography. While, in all toluene layers only elementary sulfur was identified. Therefore, in this experiment the exchange reactions between thiourea derivatives and elementary sulfur-³⁵S were traced by radioactivities of elementary sulfur-³⁵S in toluene layers. The experimental results are shown in Table 1.

Table 1 The exchange reaction of thiourea derivatives with elementary sulfur-³⁵S

Thiourea deriv.		Sulfur conc. (mmol/ml)	Reaction time (hr)	Ex-change per cent (%)
Compd.	Amounts (mmol)			
Methyl thiourea	0.30	0.18	0	0.0
			5	43.7
			25	81.1
			35	100.0
Trimethyl thiourea	0.29	0.15	0	0.0
			5	25.9
			25	73.6
			35	93.4
Ethylene thiourea	0.31	0.15	0	0.0
			5	21.4
			25	50.5
			35	67.0

In the case of methyl thiourea and trimethyl thiourea exchange equilibrium was completely established within 35 hours at 130°C. In the systems of ethylene thiourea the exchange attained to only 67% after 35 hours.

Results on the exchange reaction between

thiourea derivatives and sodium hydrosulfide- ^{35}S are shown in Table 2.

Table 2 The exchange reaction of thiourea derivatives with sodium hydrosulfide- ^{35}S

Thiourea deriv.		Hydro-sulfide conc. (mmol/ml)	Reaction time (hr)	Ex-change per cent (%)
Compd.	Conc. (mmol/ml)			
Methyl thiourea	0.13	0.15	0	0.0
			5	39.2
			20	51.6
			30	90.3
			40	90.3
Trimethyl thiourea	0.17	0.15	0	0.0
			20	99.4
			30	99.4
Ethylene thiourea	0.16	0.15	0	0.0
			20	18.5
			30	26.4
			40	31.5

Under the given set of operating conditions in this experiment there is no detectable zero time exchange, and no chemical change was observed on paper chromatogram during 30 hours reaction at 150°C . In the case of ethylene thiourea the exchange reaction proceeded only to an extent of 30

per cent even after 40 hours. While the exchange on the other two were completed within 30 hours.

Although the isotope exchange reactions of thiourea derivatives with sodium hydrosulfide- ^{35}S as well as with elementary sulfur- ^{35}S proceeded as expected under appropriate conditions, the reaction rates were rather slow at high temperature in comparison with the case of dithiocarbamates previously reported¹¹.

The thiourea derivatives used in the present study involve $-\text{NH}-\text{C}(=\text{S})-\text{NH}_2$ group. This group may have the interconvertible thiol form as tautomer as follows:

Miklukhin, et al.⁵⁾ observed no exchange reaction in thiol compounds under drastic conditions. Therefore, contribution of thiol tautomer in these thiourea derivatives appeared to make the exchange reaction slower, and less exchange in ethylene thiourea may be explained on the basis of increased contribution of thiol tautomer by stabilization as follows:

Table 3 The ^{35}S labeled thiourea derivatives

Reactants					Temp.	Time	Products		
Thiourea deriv.		³⁵ S material					Amounts (mmol)	Apparent radio- activity (10 ³ cpm)	Radio- chem. yield (%)
Compd.	Amounts (mmol)	Material	Amounts (mmol)	Apparent radio- activity (10 ³ cpm)					
Methyl thiourea	0.28	³⁵ S	0.30	4.30	130	35	0.42	1.14	37
Trimethyl thiourea	0.24	NaH ³⁵ S	0.30	4.30	130	35	0.78	0.68	41
Ethylene thiourea	0.30		0.30	4.30	130	35	0.72	0.34	19
Methyl thiourea	0.23		0.30	7.14	150	30	0.52	1.23	30
Trimethyl thiourea	0.26		0.30	7.14	150	30	0.52	1.15	28
Ethylene thiourea	0.30		0.30	4.18	150	40	0.43	0.56	19

A further investigation on the mechanism of these isotope exchange reactions is in progress.

Finally, with expectation that the ^{35}S labeled thiourea derivatives may be obtained, isolation of those compounds from the exchange reaction mixtures was examined. Since the direct isolation of radiochemical pure labeled compounds from the reaction mixtures in this study was difficult, carriers were used for each thiourea derivate. The ^{35}S labeled compounds isolated in this paper are shown in Table 3.

References

- 1) T. Sato: *Nippon Kagaku Zasshi*, **85**, 215, 220 (1964)
- 2) M.N. Foka, L.E. Burkser: *Doklady Acad. Nauk S.S.S.R.*, **99**, 1011 (1954)
- 3) I.I. Kukhtenko, G.P. Miklukhin: *Doklady Akad. Nauk S.S.S.R.*, **100**, 931 (1955)
- 4) E.N. Gur'yanova: *Zuhr. Fiz. Khim.*, **28**, 67 (1954)
- 5) G.P. Miklukhin, et al.: "Proc. Conf. Peaceful Use of Atomic Energy in Moscow, Chem. Sec." (Transl. into Japanese), p. 253 (1957)

要 旨

同位体交換反応によるチオ尿素誘導体の ^{35}S 標識

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メチルチオ尿素, トリメチルチオ尿素およびエチレンチオ尿素について, 元素イオウ- ^{35}S または水硫化ナトリウム- ^{35}S との同位体交換反応を検討し, ジチオカルバミン酸類の場合にくらべて高温かつ長時間という条件で, 全反応系につき交換反応が認められた。本実験のチオ尿素誘導体は

$\text{—NH—}\overset{\text{I}}{\underset{\text{I}}{\text{C}}}=\text{S}$ の構造をもち, これには互変異性体として $\text{—N}=\overset{\text{I}}{\underset{\text{I}}{\text{C}}}\text{—SH}$ なるチオール形がある。その寄与の程度が同位体交換反応の難易に関連するものと考えられる。さらに, これらの交換反応系から ^{35}S 標識チオ尿素誘導体を単離することができた。