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149. Seigoro Hayashi, Mitsuru Furukawa, Junko Yamamoto,*1 and Kunihiro Niigata*2 : Studies on Antitumor Substances. V. Reaction of Thiosulfonates with Active Methylene Compounds.

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The reactions of benzyl benzene or *p*-toluenethiosulfonate with active methylene compounds were studied to elucidate the mechanism of anti-tumor activity of the analogous pentamethylene bismethanethiosulfonate with the expectation that any alkylation might occur. It was found that benzene or *p*-toluenethiosulfonate did not benzylate the active methylene compounds but one or two benzylthio groups were introduced. In the cases of less active methylene compounds, the reaction of thiosulfonates themselves occurred.

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Various thiosulfonates were prepared by Hayashi, *et al.*[1] and it was found[2] that several members of them inhibited the growth of solid tumor produced by Ehrlich's ascites cells in mice and that the activities of several enzymes in Ehrlich's ascites cells were significantly inhibited by them. The solid tumors and the activities of these enzymes were unaffected by Myleran, the isosters of thiosulfonates.

Prior to investigate the mechanism of the anti-tumor and the anti-enzyme activities, their chemical behaviors were studied *in vitro* with the expectation that thiosulfonate would alkylate the functional group of amino acids or the related compounds such as -OH, -SH, -COOH or $-NH_2$ in the same way as Myleran. Because, Myleran has been reported to react with cysteine to give the tetrahydrothiophene derivative as shown in Chart 1.

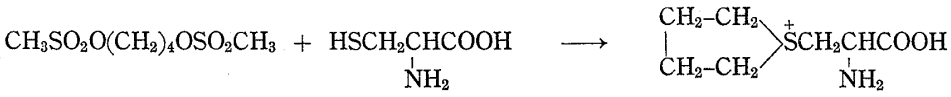

Chart 1.

First, active methylene compounds were treated with benzyl benzenethiosulfonate and benzyl *p*-toluenethiosulfonate under mild conditions. Contrary to our expectation, active methylene compounds were not benzylated but the benzylmercapto group was introduced.

The reactions might be divided into two types as shown in Chart 2. The first was the formation of monobenzylmercapto derivatives, for examples, cyanoacetate, cyanoacetamide and benzoylacetone, from which bisbenzylmercapto derivatives were not formed. The second was the formation of bisbenzylmercapto derivatives, for examples, diethyl malonate, malondiamide, ethyl acetoacetate and acetylacetone, from

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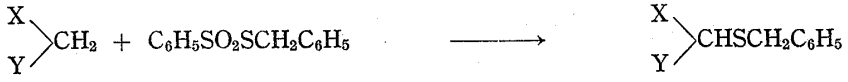
*3 Part Ⅳ : Gann, **55**, 289 (1964).

1) S. Hayashi, H. Ueki, S. Harano, J. Komiya, S. Iyama, K. Harano, K. Miyata, K. Niigata, Y. Yonemura: This Bulletin, **12**, 1271 (1964).
2) S. Hayashi, H. Ueki, J. Komiya : Gann, **55**, 289 (1964).
3) G. M. Timmis, R. F. Hudson : Ann. N. Y. Acad. Sci., **68**, 727 (1958).

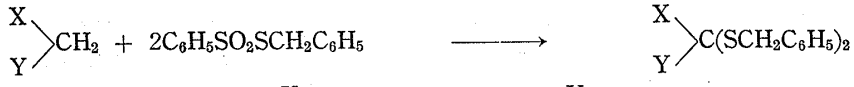
which monobenzylmercapto derivatives were together formed. Progress of the reaction depends upon the reactivity of the methylene group.

Type 1 reaction



X	Y
CN	$COOC_2H_5$
CN	$CONH_2$
CH_3CO	C_6H_5CO

Type 2 reaction



X	Y
$CONH_2$	$CONH_2$
CH_3CO	$COOC_2H_5$
CH_3CO	CH_3CO
$COOC_2H_5$	$COOC_2H_5$

Chart 2.

In the case of the reaction of malondiamide with thiosulfonates, it was necessary to determine either of the two reactive groups of malondiamide, $-CH_2-$ and $-NH_2$, reacted with thiosulfonates. To clarify this problem, the IR absorption spectra of the products and those of barbital and barbituric acid were compared. The IR absorption spectra of these compounds were shown in Fig. 1.

As shown in Fig. 1, the infrared spectra of barbituric acid and malondiamide showed a broad band due to the carbonyl groups having a methylene group between both carbonyl groups. On the other hand, barbital in which the two hydrogens of the methylene group were substituted with ethyl groups showed a comparatively sharp band due to the carbonyl groups. This fact suggests, therefore, that the $-CH_2-$group of malondiamide was participated in the reaction.

The compounds obtained were listed in Table I.

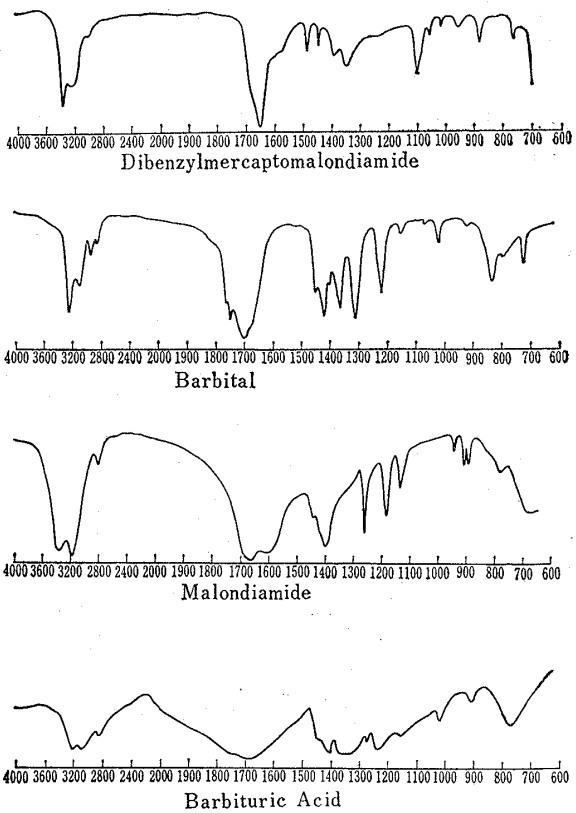
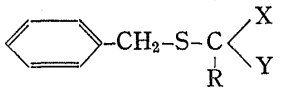
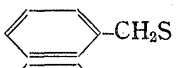
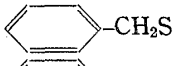
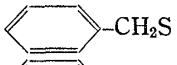
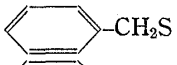
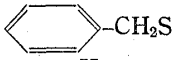
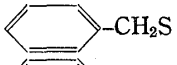
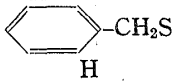


Fig. 1. Infrared Absorption Spectra

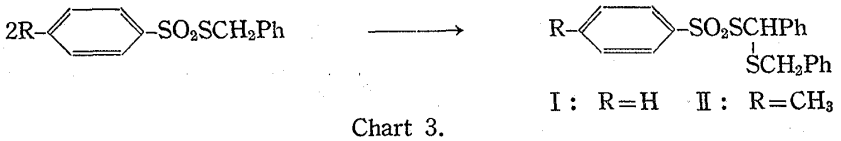
When benzyl benzene and *p*-toluenethiosulfonate were allowed to react with the less active methylene compounds such as malondinitrile, phenylacetonitrile and phenylbenzylsulfone in absolute ether, the reaction between thiosulfonates themselves occurred in every case yielding the products melted at 133~134° (I) and 147.5~148.5° (II) in a fair yield respectively, besides the formation of the corresponding mercapto compounds. It was confimed that the same compounds were obtained by the treatment of benzyl benzene or *p*-toluenethiosulfonate alone with sodium ethoxide under the same conditions.

TABLE I. 

Active methylene compound	Method	Temp. (°C)	Time (hr.)	Ratio[a]	Yield (%)	Product X	Product Y	Product R
Diethyl malonate	I	10~20	2.5	1	58.0	$COOC_2H_5$	$COOC_2H_5$	H
	II	15~25	2.0	1	86.7	$COOC_2H_5$	$COOC_2H_5$	H
Ethyl acetoacetate	I	20~30	3.5	1	69.0	$COCH_3$	$COOC_2H_5$	H
	II	10~20	3.0	1	74.1	$COCH_3$	$COOC_2H_5$	H
Ethyl cyanoacetate	I	10~20	3.0	1	56.2	CN	$COOC_2H_5$	H
	II	15~20	3.0	1	63.1	CN	$COOC_2H_5$	H
Acetyl acetone	I	20~25	2.5	1	94.2	$COCH_3$	$COCH_3$	H
	II	10~20	2.5	1	92.1	$COCH_3$	$COCH_3$	H
Cyanoacetamide	I	20~30	3.0	1	69.9	CN	$CONH_2$	H
	II	20~30	4.0	1	74.2	CN	$CONH_2$	H
Malondiamide	I	20~30	4.0	1	74.0	$CONH_2$	$CONH_2$	
	II	20~30	4.5	1	70.0	$CONH_2$	$CONH_2$	
Diethyl benzylmercaptomalonate	I	40~50	2.5	1	72.3	$COOC_2H_5$	$COOC_2H_5$	
Ethyl acetoacetate	I	40~50	2.5	1/2	56.7	$COCH_3$	$COOC_2H_5$	
Benzylmercaptoacetylacetone	I	50~60	2.0	1	85.4	$COCH_3$	$COCH_3$	
Benzoylacetone	I	20~30	3.0	1	61.9	COC_6H_5	H	H
Malondiamide	III	64	5.0	1	55.9	$CONH_2$	H	
Malondiamide	I	70~78	5.0	1/3	42.8	$CONH_2$	H	
Benzoylacetone	IV	10~20	3.0	1	68.3	COC_6H_5	$COCH_3$	H

a) Active methylene compound/Thiosulfonate

The infrared spectrum of (I) showed a sulfonyl absorption at 1309 cm^{-1} and 1142 cm^{-1}. The nuclear magnetic resonance spectrum of this compound exhibited the following proton signals: >CH- at 4.60τ (singlet), $-CH_2-$ at 3.90τ (singlet) and the three phenyl groups (ten proton signals were singlet at 2.74τ, and five proton signals were multiplet at 2.2~2.8τ). Moreover, the molecular weight was measured as 314 by Rast method. Therefore, the structure of (I) may be presumed as α-benzylmercaptobenzyl benzenethiosulfonate (Chart 3). Analogously, the structure of (II) may be assumed to be α-benzylmercaptobenzyl p-toluenethiosulfonate.



I: R=H II: R=CH_3

Chart 3.

These facts suggest that the methylene groups of benzyl arylthiosulfonates may behave as the moderately active methylene.

Experimental

Sodium Benzenethiosulfonate——In a three necked 100 ml. flask equipped with reflux condenser, stirrer and thermometer, 24 g. (0.1 mole) of $Na_2S \cdot 9H_2O$ was dissolved in 20 ml. of H_2O. The solution was cooled

and 17.7 g. (0.1 mole) of benzenesulfonyl chloide was added maintaining the temperature at 10~15°. After addition, the mixture was warmed to 85° until sulfur which deposited again dissolved. The reaction mixture was evaporated under reduced pressure and the powdered residue was washed with 50 ml. of ether and extracted with 50 ml. of hot abs. EtOH. The colorless crystalline powder which deposited on cooling was dried over P_2O_5 at 120° under reduced pressure. Yield 16.5 g. (84.3%).

Benzyl Benzenethiosulfonate——A mixture of 9.6 g. (0.05 mole) of anhyd. sodium benzenethiosulfonate and 6.3 g. (0.05 mole) of benzyl chloride in 150 ml. of abs. EtOH was refluxed for 7 hr. excluding the moisture. On cooling, deposited crystalline powder which increased by careful dilution with H_2O was filtered by suction and recrystallized from EtOH and H_2O. Plates, m.p. 43°. Yield 9.4 g. (71.2%).

Sodium *p*-Toluenethiosulfonate——A solution of 19.6 g. (0.1 mole) of *p*-toluenesulfonyl chloride in 40 ml. of EtOH was added to a cooled solution of 24.0 g. (0.1 mole) of crystalline Na_2S in 10 ml. of EtOH and 20 ml. of H_2O maintaining the temperature at 10~15°. After addition, EtOH was removed and the mixture was heated to 90° until the deposited sulfur again dissolved. Anhyd. sodium *p*-toluenethiosulfonate (16.3 g., 77.6%) was obtained by the similar procedure to that of sodium benzenethiosulfonate.

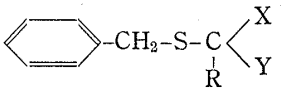
Benzyl *p*-Toluenethiosulfonate——A mixture of 10.5 g. (0.05 mole) of anhyd. sodium *p*-toluenethiosulfonate and 6.3 g. (0.05 mole) of benzyl chloride was refluxed for 15 hr. in 50 ml. of abs. EtOH and treated similarly to the case of benzyl benzenethiosulfonate. Colorless crystals, m.p. 59°. Yield 10.9 g. (78.4%). *Anal.* Calcd. for $C_{14}H_{14}O_2S_2$: C, 60.40; H, 5.07. Found: C, 60.38; H, 5.16.

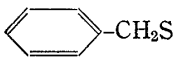
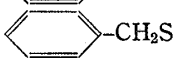
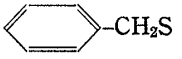
Reactions of Thiosulfonate with Active Methylene Compounds. Method I——Equivalent amounts of active methylene compound, benzyl benzenethiosulfonate and NaOEt dissolved in necessary volume of abs. EtOH were mixed and stirred for 2~5 hr. at room temperature. After completion of the reaction, the residues obtained by the removal of EtOH were treated with ether and H_2O. Benzylmercapto derivatives of active methylene compounds were obtained by the evaporation of ether and purified by recrystallization or distillation. In aqueous phase, existnce of sodium benzenesulfinate was proved by the reaction with benzoquinone to yield 2,5-dihydroxydiphenylsulfone.[4] In the case where diethyl malonate was used as acitve methylene compound, the yield of 2,5-dihydroxydiphenylsulfone was 97.3%.

Method II——Benzyl *p*-toluenethiosulfonate was used instead of benzyl benzenethiosulfonate. The reaction products were the same to those of Method I. In this case, *p*-toluenesulfinic acid was obtiained by acidification of the aqueous layer after washing 2~3 times with ether. The yield of sulfinic acid amounted to 86.7% in the case of diethyl malonate (as active methylene compound).

Method III. Bisbenzylmercaptomalondiamide——Malondiamide (1.02 g., 0.01 mole) was dissolved in a hot solution of 0.23 g. (0.01 mole) of Na in 500 ml. of abs. MeOH and a solution of 2.64 g. (0.01 mole) of benzyl benzenethiosulfonate in 200 ml. of abs. MeOH was added during 1 hr. and the mixture was concentrated to about 100 ml. after reflux for 5 hr. On cooling to room temperature, feathery crystals deposited were recrystallized from MeOH. Bisbenzylmercaptomalondiamide, m.p. 223~225°.

The filtrate was cooled again in refrigerator, needle crystals deposited were recrystallized from MeOH yielding 0.85 g. (55.9%) of a product melting point of which was 147~148°. It was confirmed to be bisbenzylmercaptoacetamide by comparison of melting point and IR spectrum with the synthetic sample prepared by hydrolysis and decarbonation of bisbenzylmercaptomalondiamide.

Table II. 

No.	X	Y	R	m.p. or b.p. (°C)	Appearance
1	$COOC_2H_5$	$COOC_2H_5$	H	162/6 mm.	oil
2	$COCH_3$	$COOC_2H_5$	H	117~118/1.7 mm.	oil
3	CN	$COOC_2H_5$	H	98/1.2 mm.	oil
4	$COCH_3$	$COCH_3$	H	55~56	prisms
5	CN	$CONH_2$	H	169~170	scales
6	COC_6H_5	$COCH_3$	H	56	pillars
7	COC_6H_5	H	H	89~90	pillars
8	$CONH_2$	$CONH_2$		225	needles
9	$CONH_2$	H		139~140	needles
10	$COCH_3$	$COCH_3$		88~89	prisms

4) Hinsberg: Ber., **27**, 3259 (1894).

No.	Analysis (%) Calcd. C	H	N	Found C	H	N	IR (KBr) cm^{-1} $\nu_{C=O}$	ν ⬡		
1	59.54	6.42	—	59.53	6.35	—	1733	768	702	
2	61.88	6.39	—	61.66	6.36	—	1742	769	702	
3	61.25	5.57	5.96	61.73	5.87	5.62	1748	769	702	$\nu_{C\equiv N}$ 2379
4	64.83	6.35	—	64.86	6.30	—	—	769	696	
5	58.23	4.89	13.58	58.32	5.15	13.61	1673	756	693	$\nu_{C\equiv N}$ 2366
6	71.79	5.67	—	71.94	5.66	—	—	770	697	
7	74.34	5.84	—	74.14	5.78	—	1664	750	697	
8	58.93	5.24	8.09	59.16	5.34	7.98	1651	769	688	ν_{NH_2} 3250, 3372
9	63.32	5.66	4.62	63.33	5.67	4.51	1632	751	695	ν_{NH_2} 3187, 3418
10	66.23	5.85	—	66.38	5.90	—	1685	—	704	

Method IV. Benzylmercaptobenzoylacetone——A mixture of 0.34 g. (0.05 mole) of alcohol-free NaOEt and 0.81 g. (0.05 mole) of benzoylacetone in 30 ml. of anhyd. ether was stirred for about 0.5 hr. Pale yellow solids deposited were redissolved by addition of a solution of 1.32 g. (0.05 mole) of benzyl benzenethiosulfonate in 50 ml. of anhyd. ether and another white crystals of sodium benzenesulfinate precipitated. After stirring for 3 hr., the mixture was shaked with 20 ml. of H_2O and the ether layer was seperated to remove ether. The crude product obtained was recrystallized from alcohol yielding 0.97 g. (68.3%) of benzylmercaptobenzoylacetone, m.p. 55~56°.

The results of elemental analysis were shown in Table II.

α-Benzylmercaptobenzyl Benzenethiosulfonate——A mixture of 0.53 g. (0.002 mole) of benzyl benzenethiosulfonate and 0.069 g. (0.001 mole) of NaOEt was stirred in 30 ml. of abs. EtOH at room temperature. After stirring for 3.5 hr., 0.21 g. (54%) of the raw product melted at 128~130° was deposited from the reaction solution. Recrystallization from EtOH gave plates of α-benzylmercaptobenzyl benzenethiosulfonate in 54.4% yield: m.p. 133~134°. *Anal.* Calcd. for $C_{20}H_{18}O_2S_3$: C, 62.14; H, 4.69. Found: C, 62.30; H, 4.70.

α-Benzylmercaptobenzyl *p*-Toluenethiosulfonate——Benzylmercaptobenzyl *p*-toluenethiosulfonate was obtained from 2.78 g. (0.01 mole) of benzyl *p*-toluenethiosulfonate and 0.34 g. (0.005 mole) of NaOEt in 40.0% yield in accordance with the same procedure for α-benzylmercaptobenzyl benzenethiosulfonate, m.p. 147.5~148.5°. *Anal.* Calcd. for $C_{21}H_{20}O_2S_3$: C, 62.97; H, 5.04. Found: C, 63.03; H, 4.96.

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