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174. Seigoro Hayashi, Hiroshi Ueki, Seiko Harano, Junko Komiya, Susumu Iyama, Kazunobu Harano, Katsuaki Miyata, Kunihiro Niigata, and Yoshiro Yonemura : Studies on Antitumor Substances. III.*1 Syntheses of Bis(methanesulphonylthio)alkanes.

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Various types of compounds have been synthesized seeking the chemotherapeutic agents for the malignant tumors. One of them, methanesulphonate, especially those of glycols (I) were prepared[1] and found to be promising as carcinostatic agents.[2]

$$CH_3SO_2O(CH_2)_nOSO_2CH_3$$

I

Myleran, bismethanesulphonate of tetramethyleneglycol (I, $n=4$), was highly effective in the treatment of patients suffering chronic myelocytic leukemia.[3]

The carcinostatic effect of this compound was attributed to the reaction with SH group of cysteine, peptide or proteins.[4] In fact, Roberts and Warwick[5] isolated 3-hydroxytetrahydrothiophene 1,1-dioxide (Ⅶ) from the urine of rat, rabbit and mouse which were treated with Myleran. They concluded that this compound was resulted from the reaction between Myleran and cysteine or cysteinyl moiety (Ⅲ) through the formation of a cyclic sulphonium ion (Ⅳ) which in turn converted to tetrahydrothiophene (Ⅴ) and its 1,1-dioxide (Ⅵ) (Chart 1).

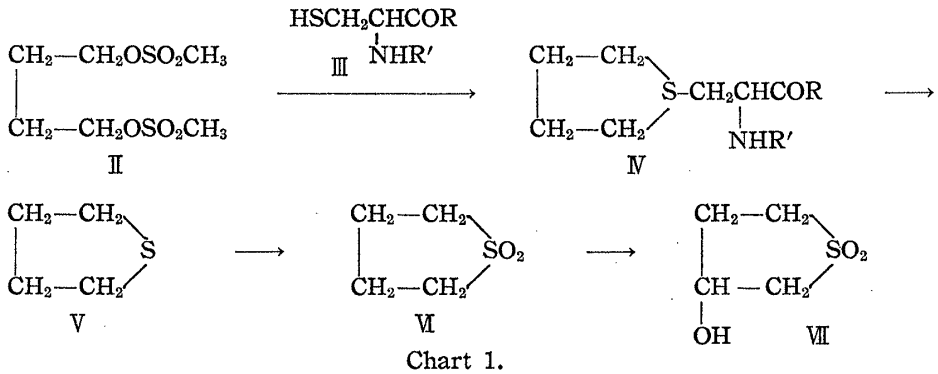

Chart 1.

*1 Part Ⅱ: Gann, 55, 1 (1964).

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1) G.M. Timmis : Annual Reports of the British Empire Cancer Campaign, **27**, 43 (1949).
2) A. Haddow, G.M. Timmis : Lancet, **264**, 208 (1953).
3) D.A. Galton : *Ibid.*, **264**, 207 (1953).
4) G.M. Timmis, R.F. Hudson : Ann. N.Y. Acad. Sci., **68**, 727 (1958).
5) J.J. Roberts, G.P. Warwick : Biochem. Pharmacol., **6**, 217 (1961).

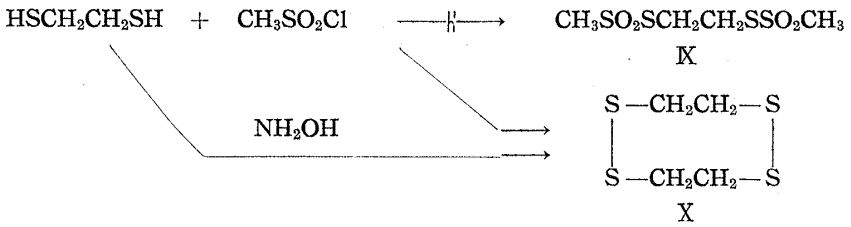
There seems to exist a relationship between the facts that the maximum biological activity in this series occurred when the chain length was 4 of 5 carbon atoms (I, $n=4$ or 5) and that the formation of a cyclic sulphonium ion was easy on the same conditions.

Thus, alkyl methanesulphonate react with fission of C-O bonds, what may happen if this C-O bonds were replaced by C-S bonds because of the decrease in bond energy from 70.0 kcal./mol. for C-O to 54.5 kcal./mol. for C-S.

Authors attempted to prepare polymethylene bisalkylthiosulphonate (Ⅷ) using dithioglycol in place of glycol.

$$RSO_2S\text{-}(CH_2)_n\text{-}SSO_2R$$
Ⅷ

This type of compounds was first reported by Otto.[6] He prepared ethyl ethanethiosulphonate from potassium ethanethiosulphonate and ethylbromide and ethylene 1,2-bis(*p*-toluenethiosulphonate) from potassium *p*-toluenethiosulphonate and ethylenedibromide. Ethylenedithioglycol and mesylchloride were reacted for the purpose of preparing 1,2-bis(methanesulphonylthio)ethane (Ⅸ) but unexpectedly tetramethylene tetrasulfide (X) was formed which was identical with those obtained from ethylenedithioglycol by the action of hydroxylamine[8] in respects of their properties and infrared spectra.



Thus, 1,2-bis(methanesulphonylthio)alkanes were prepared by Otto's method using sodium methanethiosulphonate instead of potassium salt, according to the schema :

$$Br(CH_2)_nBr + 2CH_3SO_2SNa \longrightarrow CH_3SO_2S(CH_2)_nSSO_2CH_3$$

But this procedure was found to produce unexpected disulfide in certain circumstances, for example, dibenzyldisulfide from sodium methanethiosulphonate and benzylchloride.

Twenty-five compounds which were synthesized by this procedure were tested their carcinostatic activity preliminary by CAP method. Some of them were found to have pronounced carcinostatic activity.

Experimental

Reaction of Mesylchloride with Dithioethyleneglycol——12 g. (0.1 mole) of mesylchloride was added to a solution of 4.7 g. (0.05 mole) of dithioethyleneglycol in 8 g. of pyridine with vigorous stirring and maintaining the temperature at 0°. The precipitations were collected by suction and washed with H_2O. Although it was insoluble in all organic solvents, it was able to recrystallize from phenol resulting white crystalline powder, m.p. 150~152°. The analysis showed that this substance was not the expected 1,2-bis(methanesulphonylthio)ethane but rather seemed to be tetramethylenetetrasulfide. The use of triethylamine in place of pyridine, the use of benzene as solvent or the use of the sodium salt of dithioglycol also resulted the same compound. The identity of this compound with tetramethylenetetrasulfide was confirmed by the sameness of melting point, chemical properties and IR spectra.

Sodium Methanethiosulphonate——5.75 g. (0.05 mole) of mesylchloride was added gradually to a solution of 15 g. (0.065 mole) of crystalline sodium sulfide in 10 ml. of H_2O. On every addition, an appreciable amount of sulphur precipitated but disappeared again in solution by stirring and warming the

6) R. Otto : Ber., **15**, 129 (1882).
7) *Idem* : *Ibid.*, **25**, 1478 (1892).
8) H. Fasbender : Ber., **21**, 1470 (1888).

mixture on the water bath for a while. After evaporating in vacuum to dryness, pure sodium methanethiosulphonate was obtained by recrystallization of the residue from hot alcohol. White crystalline powder. Yield 5.0 g. (75%).

1,2-Bis(methanesulphonylthio)ethane——A solution of 6.7 g. (0.05 mole) of sodium methanethiosulphonate and 4.7 g. (0.025 mole) of ethylenedibromide in 100 ml. of alcohol was refluxed for 3 hr. White crystals, which precipitated by cooling the reaction mixture, were collected by suction and recrystallized from benzene. Colorless plates, m.p. 119°. Yield 3.0 g. (65%). This compound is soluble in almost all organic solvents and insoluble in H_2O. It was reduced to dithioethyleneglycol by Zn and HCl and other reducing agents. Tetramethylenetetrasulfide, above mentioned, was not able to subject this reduction.

In a similar manner, the higher analogues were prepared as shown in Table I. But as the number of methylene groups increased, it was required to prolong the reaction time or to raise the reflux temperature and to thicken the concentration of the reactants. The IR spectrum showed that all of these compounds have characteristic peaks at 753, 958, 1132, 1312, 1431, 2820, and 3030 cm^{-1}.

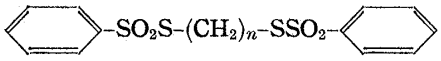
Table I. $CH_3SO_2S(CH_2)_nSSO_2CH_3$

n	m.p. (°C)	Form of crystal	Yield (%)	Analysis (%) Calcd. C	Calcd. H	Found C	Found H
2	119	plates	64	19.18	4.03	19.12	3.78
3	55	needles	10	22.71	4.58	22.58	4.43
4	87	leaflets	43	25.88	5.01	26.02	4.93
5	76	needles	9	28.44	5.51	28.43	5.35
6	85.5	leaflets	7	32.35	5.92	33.01	5.91
10	80	needles	8	39.74	7.23	40.16	6.85

Sodium Benzenethiosulphonate——50 g. (0.28 mole) of benzenesulphonylchloride was added within 2 hr. to the solution of 67 g. (0.22 mole) of crystalline sodium sulfide in the mixture of 80 ml. of alcohol and 80 ml. of H_2O with continuous stirring and maintaining the temperature at 20°. After addition, deposited sulphur was dissolved in the solution by warming the mixture on the water bath. Then the solution was evaporated in vacuum to dryness and the residue was recrystallized from alcohol. White prisms, readily soluble in H_2O resulting neutral solution. Yield 45 g. (80.3%).

1,2-Bis(benzenesulphonylthio)ethane——A solution of 3.5 g. (0.018 mole) of sodium benzenethiosulphonate and 1.7 g. (0.009 mole) of ethylenedibromide in 40 ml. of alcohol was refluxed for 5 hr. Sodium bromide was filtered off and the filtrate was allowed to cool to room temperature, amorphous solids which separated were recrystallized from alcohol. Colorless needles, m.p. 84~85°. Yield 0.8 g. (22.9%).

In a similar manner, the higher analogues were prepared as shown in Table II.

Table II. 

n	m.p. (°C)	Form of crystal	Yield (%)	Analysis (%) Calcd. C	Calcd. H	Found C	Found H
2	84~85	needles	32	44.89	3.77	44.99	3.66
4	93~94	prisms	74	47.73	4.51	47.98	4.57
5	66~67	needles	20	49.00	4.84	49.12	4.92
6	82~83	plates	58	50.20	5.15	50.27	4.97
10	63.5	prisms	23	54.29	6.19	54.45	6.17

Sodium *p*-Toluenethiosulphonate——3.8 g. (0.02 mole) of *p*-toluenesulphonylchloride was added gradually to a solution of 4.8 g. (0.02 mole) of crystalline sodium sulfide in 2 ml. of H_2O with continuous stirring. An exothermic reaction occurred and the solution became yellow and began to reflux. After the reaction ceased, the mixture was evaporated in vacuum to dryness and the residue was recrystallized from alcohol. White crystalline powder which readily soluble in H_2O, soluble in hot alcohol and insoluble in ether and benzene. Yield 3.1 g. (73.8%).

1,2-Bis(*p*-toluenesulphonylthio)ethane——A solution of 4.2 g. (0.02 mole) of sodium *p*-toluenethiosulphonate and 1.88 g. (0.01 mole) of ethylenedibromide in 100 ml. of hot alcohol was refluxed for 4 hr.

After the removal of alcohol by distillation in vacuum, the residual amorphous solids were washed with H_2O and recrystallized from alcohol. Colorless needles, m.p. 76°. Yield 1.0 g. (26%).

In a similar manner, the higher analogues were prepared as shown in Table III.

Table III. CH_3-⟨⟩-SO_2S-$(CH_2)_n$-SSO_2-⟨⟩-CH_3

n	m.p. (°C)	Form of crystal	Yield (%)	Analysis (%) Calcd. C	Calcd. H	Found C	Found H
2	76	needles	26	44.74	4.50	47.74	4.52
3	69	〃	29	49.02	4.84	48.86	4.83
4	119.5	platelets	50	50.20	5.16	50.10	5.19
6	88.5	prisms	55	52.35	5.71	51.93	5.63
10	91	platelets	56	56.00	6.65	56.07	6.69

1,6-Bis(benzylsulphonylthio)hexane——A solution of 2.4 g. (0.01 mole) of sodium sulfide in 20 ml. of alcohol and a solution of 1.9 g. (0.01 mole) of benzylsulphonylchloride in 20 ml. of alcohol were mixed, an exothermic reaction occurred and the solids precipitated. After refluxing 30 min., the solids were filtered off and the filtrate, mixed with 1.2 g. (0.005 mole) of hexamethylenedibromide, were refluxed for 3 hr. On cooling the reaction mixture, amorphous solids deposited were recrystallized from acetone yielding 1.2 g. of colorless needles, m.p. 115°. Yield 52% based on hexamethylenedibromide.

The lower analogues were prepared similarly as shown in Table IV.

Table IV. ⟨⟩-CH_2SO_2S-$(CH_2)_n$-SSO_2CH_2-⟨⟩

n	m.p. (°C)	Form of crystal	Yield (%)	Analysis (%) Calcd. C	Calcd. H	Found C	Found H
3	121	prisms	33	49.01	4.84	49.36	4.90
4	141	〃	42	50.20	5.15	50.56	5.20
6	115	〃	39	52.33	5.71	52.45	5.72

Sodium 2-Naphthalenethiosulphonate——A solution of 70 g. (0.31 mole) of 2-naphthalenesulphochloride in 100 ml. of acetone was added in 1 hr. to a solution of 74 g. (0.31 mole) of crystalline sodium sulfide in a mixture of 74 ml. of H_2O and 100 ml. of alcohol maintaining the temperature under 30°. After warming the mixture on the water bath for 30 min., acetone and alcohol were evaporated and the residue was recrystallized from alcohol. Colorless leaflets. Yield 42 g. (58.2%).

1,6-Bis(2-naphthalenesulphonylthio)hexane——A solution of 4.3 g. (0.017 mole) of sodium 2-naphthalenethiosulphonate and 2.0 g. (0.008 mole) of hexamethylenedibromide in 50 ml. of acetone was refluxed for 5 hr. After cooling, any solids were filtered off and the filtrate was evaporated to dryness under reduced pressure. The residue was recrystallized from MeOH resulting colorless needles, m.p. 122~123°. Yield 1.7 g. (40%).

In a similar manner, lower analogues were prepared as shown in Table V.

Table V. ⟨⟩-SO_2S-$(CH_2)_n$-SSO_2-⟨⟩

n	m.p. (°C)	Form of crystal	Yield (%)	Analysis (%) Calcd. C	Calcd. H	Found C	Found H
2	142~143	needles	10	55.69	3.83	55.83	4.01
4	107~109	〃	64	57.22	4.40	56.69	4.31
6	122~123	〃	40	58.83	4.93	58.83	4.98

α,α′-Bis(methanesulphonylthio)-*p*-xylene——A solution of sodium methanethiosulphonate prepared from 2.3 g. (0.02 mole) of mesylchloride and 4.8 g. (0.02 mole) of sodium sulfide and extracted with 150 ml. of alcohol was mixed with 2.64 g. (0.01 mole) of *p*-xylylenedibromide and refluxed for 5 hr. Crude crystals which separated on cooling the concentrated reaction mixture were recrystallized from alcohol. Colorless plates, m.p. 185°. Yield 2.0 g., 60% of the theory. *Anal.* Calcd. for $C_{10}H_{14}O_4S_4$: C, 36.78; H, 4.33. Found : C, 36.75; H, 4.39.

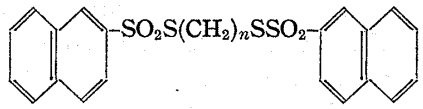
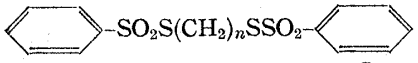
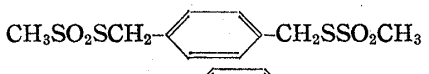
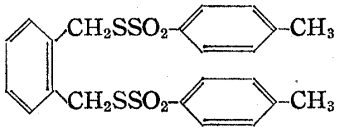
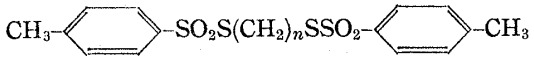
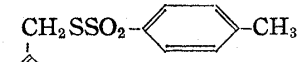
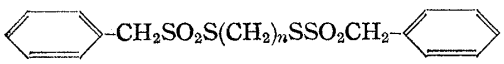
α,α′-Bis (*p*-toluenesulphonylthio)-*o*-xylene——3.8 g. (0.02 mole) of *p*-toluenesulphonylchloride was added in portions to a solution of 4.8 g. (0.02 mole) of crystalline sodium sulfide in a little H_2O. An exothermic reaction occurred and a mass of sulphur deposited which then disappeared again in solution by warming the mixture on the water bath. After filtration, the reaction mixture was evaporated to dryness under reduced pressure. The colorless crystalline residue was recrystallized from abs. alcohol, yielding 3.2 g. (75%) of sodium *p*-toluenethiosulphonate.

2.64 g. (0.01 mole) of *p*-xylylenedibromide was added to a solution of 4.1 g. (0.02 mole) of this sodium salt in 50 ml. of alcohol. After an exothermic reaction subsided, the mixture was refluxed for 9 hr. Colorless solids were deposited by cooling the concentrated filtrate of the reaction mixture. They were recrystallized from alcohol, yielding 4.3 g. (95%) of products. Colorless needles, m.p. 75°. *Anal.* Calcd. for $C_{22}H_{22}O_4S_4$: C, 55.20; H, 4.63. Found : C, 55.33; H, 4.54.

α,α′-Bis(*p*-toluenesulphonylthio)-*p*-xylene——In the previous method, *p*-xylylenedibromide was used in place of *o*-isomer. Colorless needles, m.p. 150°. Readily soluble in acetone, soluble in hot alcohol and insoluble in benzene, ether and H_2O. *Anal.* Calcd. for $C_{22}H_{22}O_4S_4$: C, 55.20; H, 4.63. Found : C, 55.61; H, 4.76.

Carcinostatic Activity——Carcinostatic activity of these compounds was tested by Cylinder Agar Plate (CAP) method[9] using Ehrlich ascites carcinoma cell. The activity was expressed by the diameter in mm. of the zone which did not show the dehydrogenase activity on 2,6-dichlorophenol-indophenol. The results were shown in Table Ⅵ. Some of these compounds showed pronounced carcinostatic activity in the test of solid tumors produced by the subcutaneous transplantation of Ehrlich ascites carcinoma cells to the mouse. The results will be published in other papers.

Table Ⅵ.

$CH_3SO_2S(CH_2)_nSSO_2CH_3$			
n	mm.[a]	*n*	mm.[a]
2	C[b]	4	C
3	14	6	C
4	17		
5	15		
6	30		
10	C	2	—
		4	0
		6	C
2	C		30
4	C		
5	10		0
6	C		
10	C		
			
2	C		
3	C		0
4	0[c]		
6	20		
10	20		
			
3	33		

a) Diameter of inhibition zone in mm.
b) Inhibition only in cylinder.
c) No inhibition even in cylinder.

9) S. Yamazaki, *et al.* : J. Antibiotics, **9A**, 135 (1956).

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Summary

Dimethanesulphonylthioalkanes and analogous compounds were synthesized by the reaction of corresponding sodium thiosulphonate and appropriate dibromide to test the carcinostatic activity compared with Myleran. It was found that some of these compounds inhibited pronouncedly the growth of the solid tumor produced by Ehrlich ascites carcinoma cells.

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UDC 616-074 : 612.398.19

175. Masahiro Nakadate, Takatsune Maki, and Michiya Kimura : Fundamental Studies on Clinical Chemistry. VIII.*1 A New Method for the Colorimetric Determination of Urinary Creatinine with 2,2′,4,4′-Tetranitrobiphenyl.

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The Folin-Wu's procedure,[1] that is based upon the Jaffé's reaction[2] using picric acid and alkali, is one of the most commonly employed methods for the determination of urinary creatinine at present. Although this procedure is characteristic with regard to its higher sensitivity, it seems that reproducible results are difficult to obtain for several reasons as follows : First, the absorbances should be measured inevitably at the wave lengths of between 510 mμ and 520 mμ where the absorption curve shows a remarkably steep inclination, in order to avoid a extremely higher blank value at 480 mμ of maximum absorption in the reaction mixture; secondly, since picric acid can be reduced in an alkaline solution into picramic acid[3] which shows higher light absorption at the wave lengths around 515 mμ, the presence of reducing substances such as, for instance, glucose and ascorbic acid in the urine sample can affect some errors on the determination of creatinine; thirdly, the color produced is sensitive for the temperature of reaction mixture as well as even at the moment of absorbance measurement within spectrometer.[4] Although some improvements have been made on this method,[5~7] most of them seemed to be scarcely satisfactory in respects to some of the sensitivity, specificity, and stability.

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*1 Part Ⅶ : This Bulletin, **12**, 1138 (1964).

*2 Kita-12-Jo, Nishi-5-Chome, Sapporo (中舘正弘, 真木孝常, 木村道也).

1) O. Folin, H. Wu : J. Biol. Chem., **38**, 81 (1919).
2) M. Jaffé : Z. physiol. Chem., Hoppe-Seyler's **10**, 399 (1886).
3) J. F. Van Pilsum, R. P. Martin, E. Kito, J. Hess : J. Biol. Chem., **222**, 225 (1956).
4) H. Koishi : Seikagaku, **28**, 477 (1956).
5) *Idem* : *Ibid.*, **28**, 706 (1956).
6) T. Momose, Y. Mukai : Rinsho Kensa, **5**, 451 (1961).
7) J. A. Owen, B. Iggo, F. J. Scandrett, C. P. Stewart : Biochem. J., **58**, 426 (1954).