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The Synthesis and Antineoplastic Activity of Alkyl Thiosulfates and Diaryl Disulfides*

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Abstract—Seven alkylthiosulfates and three diaryl thiosulfates were synthesized as potential antineoplastic agents, and subjected to antineoplastic activity test against Ehrlich ascites carcinoma, SN-36 Leukemia and Sarcoma 180. Allyl thiosulfate, n-propylthiosulfate and β-hydroxyethylthiosulfate were found as to be active against experimental tumors.

Intensive investigations of garlic constituent were carried out by Sugihara[1], Ominato[2] and Karai[3], who reported that specific garlic odor is based upon organic sulfur compound such as allyl sulfide. Babs[4], Eguchi[5] and Shida[6] first reported the fact that these organic sulfur compounds of garlic exhibit strong bactericidal action. Cavallito[7], and Stool[8] also reported that allin, the main constituent of garlic, does not exhibit bactericidal action but allicin decomposed by allinase exhibits strong bactericidal action.

$$CH_2{=}CH{-}CH_2{-}S{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}{-}COOH \xrightarrow{\text{Allinase}} CH_2{=}CHCH_2{-}S{-}S{-}CH_2CH{=}CH_2$$

Allin (inactive against tumor) — Allicin (active against tumors)

The investigation on antineoplastic activity of garlic constituent is first performed by Weiger[8] who reported that allicin produces antineoplastic activity against ascitic experimental tumor.

Fujiwara and Nakada[9], recently reported immunologic effect of garlic extract against Ehrlich ascitic carcinoma and SN-36 Leukemia, by way of injecting the mixture of ascitic tumor and garlic extract (1 : 1) into the peritoneal cavity of experimental mouse. Hayashi

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[10,11] and his co-workers performed the studies on the synthesis and antineoplastic activity of alkylalkane thiosufonate ($R—SO_2—SR'$).

In view of the results of recent studies on allicin and allicin homologue compounds, authors tried to synthesize sodium alkyl thiosulfonate and diaryl disulfide (Ar-S-S-Ar), and to carry out screening of antineoplastic activities of synthesized compounds.

EXPERIMENTAL

Sodium alkylthiosulfates(Ethyl. N-propyl, N-Autyl, Isobutyl, Allyl, Amyl)—A solution of 0.1 mole of alkyl bromide in 25ml of EtOH was added to 0.1 mole of sodium thiosulfate pentahydrate in 25ml of H_2O and the mixture was heated under reflux for 2-10 hours. The mixture was then evaporated to dryness in vacuum, and the residue was extracted with boiling 90% EtOH, from which the product separated on cooling.

Sodium-β-hydroxyethylthiosulfate—A solution of 62g of sodium thiosulfate in 150ml of H_2O is added to 20.1g of ethylene chlorohydrin in 150ml of EtOH and the mixture was heated for 3 hours under reflux with stirring. The mixture was then evaporated to dryness in vacuum, and the residue was washed with ether and was extracted with boiling 90% EtOH, from which the product separated on cooling.

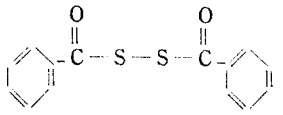
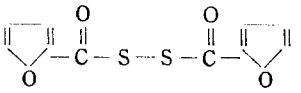
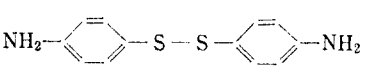
Dibenzoyldisulfide—H_2S gas was introduced with mechanical stirring to the solution of 31.5g of KOH in 315ml of absolute EtOH. The reatcion mixture was cooled to 10-15° and freshly distilled benzoyl chloride was added dropwise while the temperature was maintained between 10-15° during the dropping of benzoyl chloride. Potassium chloride deposited was removed by suction filtration and washed with 20ml of EtOH. Filtrate was concentrated to half volume from which the crude dibenzoyl disulfide seperated on cooling. The crude crystal was filtered by suction and dissolved in ethylene chloride by warming, and 91ml of absolute EtOH was added. The mixture was allowed to stand for overnight in an ice box while the product crystallizes. Colorless needles, Yield 28g, m.p. 129-130°.

Difuroyldisulfide—Difuroyl disulfide was synthesized using absolutely the same procedure as described for dibenzoyl disulfide synthesis.

Bis(*p*-aminophenyl) disulfide—In a 500ml round-bottomed flask equipped with a reflux condenser and a mechanical stirrer were placed 23.6g (0.15 mole) of *p*-chloronitrobenzene, 96g (0.4 mole) of sodium sulfide, and 250ml of H_2O. The mixture was heated to the reflux temperature with rapid agitation. Heating was continued for 20 hours. The mixture was cooled to room temperature and filtered on a large Büchner funnel to remove insoluble material (*p*-chloroaniline).

The filtrate was placed in a 500ml three necked flask, equipped with a dropping funnel, a mechanical stirrer and a downward Liebig condenser, and was concentrated by distillation to a volume of 150ml. The condenser was replaced by a thermometer, the

Table I—Survival effect of tumor transplanted mouse.

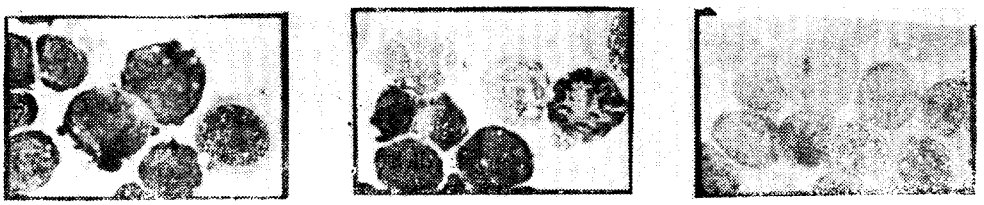
No.	Compound	Tumor	Survival	
			Days	Surv./Treated
S-1	$C_2H_5S\cdot SO_3Na$	Ehrlich	17	1/15
		SN-36	14	0/5
		S-180	15	0/15
S-2	$HOCH_2CH_2S\cdot SO_3Na$	Ehrlich	26	3/5
		SN-36	19	2/5
		S-180	22	2/5
S-3	$CH_3CH_2CH_2S\cdot SO_3Na$	Ehrlich	28	3/5
		SN-36	24	3/5
		S-180	25	4/5
S-4	$CH_3CH_2CH_2CH_2S\cdot SO_3Na$	Ehrlich	16	0/5
		SN-36	16	0/5
		S-180	18	2/5
S-5	$CH_2=CH\text{-}CHS\cdot SO_3Na$	Ehrlich	23	3/5
		SN-36	18	2/5
		S-180	26	2/5
S-6	$(CH_3)_2CH\cdot CH_2S\cdot SO_3Na$	Ehrlich	14	0/5
		SN-36	13	0/5
		S-180	14	0/5
S-7	$CH_3CH_2CH_2CH_2CH_2S\cdot SO_3Na$	Ehrlich	14	0/5
		SN-36	14	0/5
		S-180	15	0/5
S-8		Ehrlich	16	0/5
		SN-36	14	0/5
		S-180	15	0/5
S-9		Ehrlich	13	0/5
		SN-36	14	0/5
		S-180	15	0/5
S-10		Ehrlich	13	0/5
		SN-36	14	0/5
		S-180	14	0/5
	Control	Ehrlich	14	0/5
		SN-36	14	0/5
		S-180	15	0/5

stirring was started, and the dropping funnel was charged. The temperature of the reaction mixture was maintained at 65-70° while the H_2O_2 was added dropwise over a period of 2 hours. The reaction mixture was cooled, and the crude *p*-aminophenyl disulfide was collected on a Büchner funnel. The crystal was recrystallized from hot EtOH. The yield of pure product melting at 75-78° was 10.5g.

Antitumor Test*— Synthetic compounds specified on Table I. Sodium alkylthiosulfates were dissolved in saline water and aryl disulfides were suspended in saline water. Each 0.5ml. of solutions or suspension were dispensed to contain 10mg, 7.5mg, or 5mg of each synthetic compounds respectively. 0.5% of sodium carboxymethylcellulose, was added to each suspension. Female *dd* strain mice, weighing 18-22g, were fed on standard laboratory diet and given water ad libitum. Ehrlich ascites carcinoma, SN-36 leukemia, and Sarcoma 180 were maintained by the intraperitoneal injection of about 0.1 ml. (10^6 tumor cells by hemocytometer count) of ascites fluids every 7-9 days. The test was consisted of intraperitoneal inoculation of 10^6 cells of each tumor cells respectively, and the survival effect was observed on the continued administration of 10mg, 7.5mg, 5mg of samples by way of intraperitoneal injection for 7 days, 24hr. after transplatation of acites tumors.

DISCUSSION

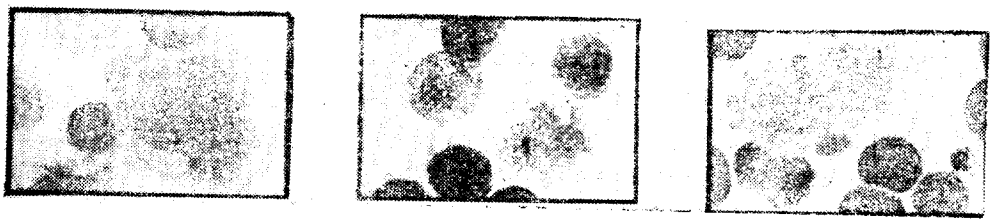
According to the report by Fugiwara, *et al.* that garlic extract exhibit immunologically active antineoplastic action against Ehrlich ascites carcinoma, authors tried to perform screening test on antineoplastic activities against tumor transplanted mice (*dd* strain). In synthesises, physiologically active moiety of garlic is substituted by seven alkylthiosulfates and three aryl disulfides. Interest was feasible between immunological effect of garlic extract against Ehrlic ascites carcinoma and antineoplastic effect of the compounds synthesized by authors against transplanted Ehrlich ascites carcinoma.



Ehrlich SN-36 S-180

Plate 1—Tumor cells at 7 days after transplantation (Wright stain, ×1000).

* Strains were obtained from the Biological Research Institute of the Tanabe Pharmaceutical Company, Saitama, Japan, and Natural Products Research Institute, Seoul National University



Ehrlich SN-36 S-180

Plate 2—Degeneration of tumor cells caused by S-3 (Wright stain, ×100).

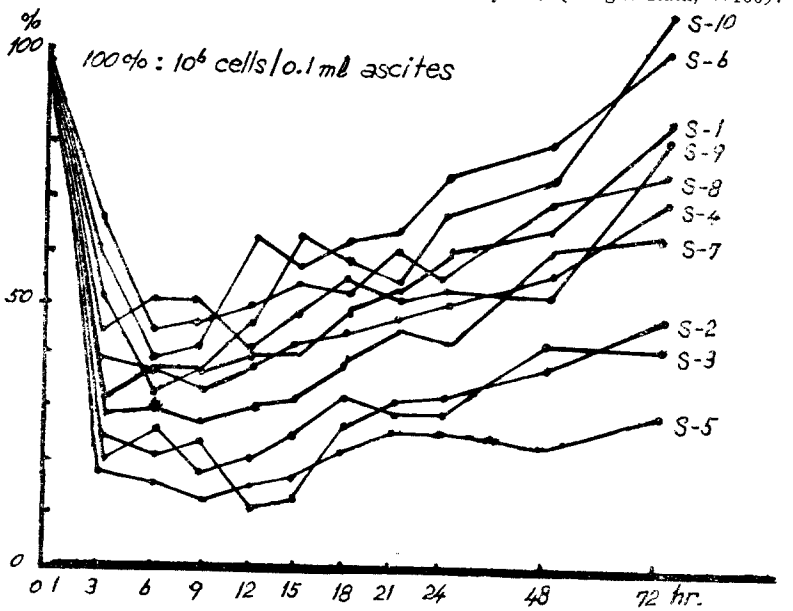


Fig. 1—Changes in number of sarcoma 180.

CONCLUSION

Seven alkylthiosulfates and three diaryl thiosulfates, specified in Table I, were synthesized.

As the result of screening test of survival effect, cytological morphology and number of cells caused to the peritoneal administration of synthesized compounds against Ehrlich carcinoma, SN-36 Leukemia and Sarcoma 180, β-hydroxyethylthiosulfate, n-propyl thiosulfate and allylthiosulfate were found to produce considerably active antineoplastic activities.

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