In a run on 1/10 scale, IIa was isolated in 42% yield by means of column chromatography on alumina (60 g) [benzene–ethanol (20:1, v/v)].

9-Phenethyladenine (IId)——To a warm, stirred mixture of I (2.70 g, 20 mmoles), anhyd. K_2CO_3 (2.76 g, 20 mmoles), and DMAC (15 ml) was added dropwise a solution of phenethyl bromide (7.40 g, 40 mmoles) in DMAC (5 ml). The resulting mixture was stirred at 110° for 1 hr and was treated in the same way as described above for IIc. The crude IId thus obtained was dissolved in hot 99% aq. ethanol (60 ml), and conc. hydrochloric acid (4.5 g) and ether (100 ml) were successively added. The colorless needles that formed were filtered off and recrystallized from 99% aq. ethanol to give a pure sample of the hydrochloride of IId. The total amount of the purified salt was then dissolved in hot H_2O (20 ml), and the aq. solution was rendered alkaline with conc. aq. NH_4OH and cooled to produce colorless minute crystals (2.48 g, 52%), mp 178—179° (lit.[13] mp 179—180°), homogeneous by TLC on silica gel. This material was found to be identical to an authentic sample of IId.

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Antitumor Effects of Pentamethylene Bismethanethiosulfonate Hydrolysates and Difunctional Bunte Salt

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Hayashi, *et al.*[2,3] synthesized a series of polymethylene bisalkanethiosulfonate which is a isoster of Myleran (I) and showed that trimethylene bismethanethiosulfonate, tetramethylene bismethanethiosulfonate and pentamethylene bismethanethiosulfonate (II) had antitumor effect on the solid form, but not on the ascites form, of Ehrlich carcinoma. From the relationship between the antitumor effect and the toxicity of these compounds, II was found to be the most effective of the three.[3]

$CH_3SO_2O(CH_2)_4OSO_2CH_3$ (I) $\quad CH_3SO_2S(CH_2)_5SSO_2CH_3$ (II) $\quad NaO_3SS(CH_2)_nSSO_3Na$ (III)

Owing to the structural similarlity, difunctional polymethylene bisthiosulfate (Bunte salt) (III) may be expected to show chemical behaviors and antitumor effects analogous to that of II. Thus, III and the related compounds were prepared by heating the corresponding dihalide with sodium thiosulfate heptahydrate in 50% ethanol under reflux according to the following schema.

It is known that the nucleophilic attack of OH anion to thiosulfonate is achieved on the sulfenyl S atom but not on the sulfinyl S atom to give the corresponding sulfinic acid and

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$$(CH_2)_n\begin{matrix}Br\\Br\end{matrix} + 2\ Na_2S_2O_3 \longrightarrow (CH_2)_n\begin{matrix}SSO_3Na\\SSO_3Na\end{matrix} \qquad n=2,\ 3$$

$$X{=}(CH_2)_n\begin{matrix}Cl\\Cl\end{matrix} + 2\ Na_2S_2O_3 \longrightarrow X{=}(CH_2)_n\begin{matrix}SSO_3Na\\SSO_3Na\end{matrix} \qquad X=O,\ CO,\ CH_3N$$

thiol.[4] The nucleophilic attack to difunctional thiosulfonate[5] is also reported to be carried out on only sulfenyl S atom, probably because of the fielding effect and the steric hindrance of the SO_2 group. Therefore, it is presumed that II may be split by hydrolysis into methanesulfinic acid (IV) and 1,5-pentanedithiol (V) *in vivo*.

In the present study, an attempt was made in the following way to find a clue to clarify the mechanism by which II acts as antitumor agent. First, the antitumor effects of IV,[6] V,[7] III and the related compounds were compared with that of II, and second, *in vitro* treatment of the tumor cells with II, IV,[6] and V was carried out to see whether or not they react directly with the tumor cells.

Table I. Antitumor Effects of various Compounds on Solid and Ascites Forms of Ehrlich Carcinoma

No.	Compound	Dose (mg/kg/day × 7)	Treated/Control (%)	
			Tumor weight[a] (solid form)	Life-span (ascrites form)
II	$(CH_2)_5\begin{matrix}SSO_2CH_3\\SSO_2CH_3\end{matrix}$	5	49.4	92.5
		3	51.4	100.0
IV[6]	CH_3SO_2Na	2	49.7	107.2
		0.7	93.7	97.6
V	$(CH_2)_5\begin{matrix}SH\\SH\end{matrix}$	1.4	42.2	95.2
		0.5	104.1	108.4
VI	CH_2SSO_3Na	100	128.1	69.8
	$\vert$	50	159.9	82.6
	CH_2SSO_3Na			
VII	$CH_2\begin{matrix}CH_2SSO_3Na\\CH_2SSO_3Na\end{matrix}$	100	173.0	130.5
		50	181.6	155.1
VIII	$CO\begin{matrix}CH_2SSO_3Na\\CH_2SSO_3Na\end{matrix}$	100	150.7	70.6
		50	181.7	102.4
IX	$O\begin{matrix}CH_2CH_2SSO_3Na\\CH_2CH_2SSO_3Na\end{matrix}$	100	125.7	90.7
		50	213.7	103.3
X	$CH_3N\begin{matrix}CH_2CH_2SSO_3Na\\CH_2CH_2SSO_3Na\end{matrix}$	100	dead	100.0
		50	92.4	98.4
XI	$N{-}CH_2CH_2SSO_3Na$	100	55.3	123.0
	$\vert$	50	89.4	113.1
	$CH_2CH_2SSO_3Na$			

five mice in group
A daily injection was given intraperitoneally for 7 days from 24 hr after inoculation of tumor cells.
a) Killed 14 days after inoculation of tumor cells.

As shown in Table I, IV,[6] and V were effective against the solid form, but not against the ascites form, of Ehrlich carcinoma. These effects were similar to that of II. In the case of combined injections of 2 mg/kg/day on IV[6] and 1.4 mg/kg/day on V for 7 days, the value of T/C in tumor weight was 56.8%. Appreciable difference in inhibitory effect was not observed among II, IV,[6] V and IV[6]+V. All of VI—XI did not show any inhibitory effect

4) S. Oae, R. Nomura, Y. Yoshikawa and W. Tagaki, *Bull. Chem. Soc. Japan*, **42**, 2903 (1969).
5) S. Hayashi, M. Furukawa, Y. Fujino and H. Matsukura, *Chem. Pharm. Bull.* (Tokyo), **17**, 419, 954 (1969).
6) R. Brown and R.C.G. Moggridge, *J. Chem. Soc.*, **1946**, 818. Sodium methanesulfinate was used in place of methanesulfinic acid, because of the instability of methanesulfinic acid.
7) W.P. Hall and E.E. Reid, *J. Am. Chem. Soc.*, **65**, 1466 (1943). *Org. Synth.*, **30**, 35 (1950).

Table II. Effects of *in Vitro* Treatments with Pentamethylene Bismethanethiosulfonate, Sodium Methanesulfinate and 1,5-Pentanedithiol on Growth of Tumor Cells

Compound	Concentration (mM)	Treated/Control (%)	
		Tumor weight[a] (solid form)	Life-span (ascites form)
II	1.0	1.3	128.4
	0.02	51.9	86.3
IV[6]	2.0	182.0	144.1
	0.04	92.5	80.8
V	1.0	55.8	90.8
	0.02	107.1	93.5

five mice in group.
a) Killed 14 days after inoculation of tumor cells.

on the solid and ascites forms of Ehrlich carcinoma, except that the value of T/C in tumor weight was 55.3% in the case of injection of 100 mg/kg/day on XI for 7 days.

The results of *in vitro* treatments of the tumor cells with II, IV,[6] and V are shown in Table II. In a concentration of 1.0 mM of II, the growth of the solid form of Ehrlich carcinoma was inhibited completely while in 0.02 mM, the value of T/C in tumor weight was 51.9%. IV[6] did not inhibit the growth of the solid form of Ehrlich carcinoma in both concentrations of 2.0 and 0.04 mM. V possessed only weak inhibitory effect on the growth of the solid form of Ehrlich carcinoma in 1 mM. These compounds were not, however, effective against the ascites form.

These results suggest that the inhibitory effect of II is produced by its own action, and not by a co-operative one of IV[6] and V, both of which may be produced from II by *in vivo* degradation. By the *in vitro* treatment of the tumor cells with II, their growth in the tissue of mouse groin was strongly inhibited. The antitumor effect of II would be a direct, not a host-mediated one. II had no effect on the growth of the ascites form of Ehrlich carcinoma, as observed in the life-span test. The difference in the effect of II on growth of the solid and ascites forms may be attributed to the difference in the position, in which the tumor cells were implanted. Further studies, however, will be needed to clarify this matter.

Experimental

General Method for Synthesis of Polymethylene bisthiosulfate[8] and the Related Compound— A solution of 0.01 mole of polymethylenedihalide or the related dihalide in 25 ml of EtOH was added to 0.02 mole of sodium thiosulfate heptahydrate in 25 ml of H_2O and the mixture was heated for 2 hr under reflux. The mixture was then evaporated to dryness *in vacuo*, and the residue was extracted with boiling 90% ethanol, from which the product separated on cooling.

Antitumor Test——For the ascites form of Ehrlich carcinoma, antitumor tests were carried out by the method described in previous paper.[9] After the mice were inoculated with 0.2 ml of the cell suspension (10^7 cells/ml), treatment was started on 24 hr after the transplantation. A dose of the compound to be tested was injected daily into intraperitoneal cavity for 7 days. The effect was evaluated by the difference in life-span between treated and contol mice group.

For the solid form of Ehrlich carcinoma, mice were inoculated subcutaneously with 0.2 ml of the cell suspension (2×10^7 cell/ml) in the right groin. Subsequent treatment was the same as described above. The effect was expressed as the ratio of the mean treated-tumor weight to the mean control-tumor weight (T/C) on the 14th day after transplantation.

In vitro treatment of the tumor cells with the compounds to be tested was carried out to detect their direct effects on the cells. The 7-day-old tumor cells were suspended in Krebs-Ringer phosphate (KRPB),

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9) S. Hayashi, H. Ueki and Y. Ueki, *Gann*, **54**, 381 (1963).

pH 7.4, in a concentration of 5×10^7 cells/ml. To this cell suspension, 1 ml each of KRPB and the sample solution in 5% carboxymethyl cellulose (CMC) was added. The mixture was incubated for 1 hr at 37°. The cells were then washed 3 times with KRPB. In the control group, the sample solution was substituted for 0.5% CMC. The cell suspension was prepared from the above cells in a concentration of 1 or 2×10^7 cells/ml with physiological saline containing streptomycin (100 μg/ml) and penicilline (100 u/ml). For the life-span test, 0.2 ml of the cell suspension (10^7 cells/ml) was inoculated intraperitoneally into mice. For the depression test for the solid form, 0.2 ml of the cell suspension (2×10^7 cells/ml) was inoculated subcutaneously into the right groin. In this test, the mice were sacrificed 14 days after inoculation.