2-Amino-6-methylamino-9-β-D-ribofuranosylpurine (X)——i) A solution of 1.0 g. (2.57 mmoles) of V in 7 ml. of 40% $MeNH_2$ was heated in a sealed tube at 130~140° for 20 hr. After cooling, benzylmercaptane was removed by extracting with Et_2O and aqueous layer was concentrated to dryness *in vacuo*. The residual sirup was triturated with Et_2O and yellow amorphous solid, 0.65 g. (86.4%), m.p. 80~90°, $[\alpha]_D^{21}$ −38.8° (c=1.02, 0.1*N* HCl). UV $\lambda_{max}^{H_2O}$ mμ (ε): 265 (shoulder) (10300), 280 (13100).

Attempts to crystallize this solid from a variety of solvents failed. The picrate of this compound showed m.p. 215~218° (decomp.). *Anal.* Calcd. for $C_{17}H_{19}O_{11}N_9$: C, 38.83; H, 3.64; N, 24.00. Found: C, 38.79; H, 3.59; N, 24.34.

ii) A mixture of 0.50 g. (1.6 mmoles) of Ⅷ and 3 ml. of W-7 Raney Ni in 30 ml. of H_2O was refluxed for 1 hr. The mixture was filtered, the catalysts were washed several times with hot H_2O. The filtrate and washings were combined and evaporated to dryness *in vacuo*. The residual sirup was triturated with Et_2O and amorphous solid separated, 0.30 g. (63.3%), UV $\lambda_{max}^{H_2O}$ mμ: 265 (shoulder), 280.

The picrate showed m.p. 216~218° (decomp.). *Anal.* Calcd. for $C_{17}H_{19}O_{11}N_9$: N, 24.00. Found: N, 24.35. A mixed melting point with a sample prepared above showed no depression.

2-Amino-6-dimethylamino-9-β-D-ribofuranosylpurine (XI)——A solution of 1.0 g. (2.57 mmoles) of V in 7 ml. of 40% Me_2NH was heated in a sealed tube at 130~140° for 20 hr. After cooling, benzylmercaptane was removed by extracting with Et_2O and aqueous layer was evaporated to dryness *in vacuo*. A pale yellow amorphous residue was recrystallized from Me_2CO-MeOH to white tiny prisms, 0.5 g. (62.5%), m.p. 202~203° (decomp.), $[\alpha]_D^{21}$ −44.2° (c=1.47, 0.1*N* HCl). UV $\lambda_{max}^{H_2O}$ mμ (ε): 228 (19100), 268 (shoulder) (11500), 284 (15600). *Anal.* Calcd. for $C_{12}H_{18}O_4N_6$: C, 46.44; H, 5.81; N, 27.09. Found: C, 45.85; H, 6.02; N, 27.35.

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Summary

In the 2-amino-6-substituted-9-β-D-ribofuranosylpurines series, 6-hydrazino (Ⅵ), 6-methylhydrazino (Ⅶ), 6-hydroxylamino (Ⅷ), 6-methylamino (X), and 6-dimethylamino (XI) derivatives were synthesized by condensation of 6-alkylthioguanosines with respective amines. Ⅵ and Ⅶ were reduced with Raney nickel to 2,6-diamino derivative (Ⅸ). Similarly, Ⅷ was also reduced with Raney nickel to X.

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134. Yoshio Sakurai*1 and Mahmoud M. El-Merzabani*2: Carcinostatic Methanesulfonic Acid Esters of Some Aminoglycols.

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Numerous reports[1~4] have been published on the antitumor activity of sulfonic acid esters of glycols and aminoglycols, out of which dimethanesulfonic ester of 1,4-butanediol (Myleran) had been used at present as a clinical antileukemic agent of practical value.

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1) A. Haddow, G. M. Timmis: Lancet, **264**, 207 (1953).
2) G. M. Timmis: Abstracts of papers of 12th International Congress of Pure and Applied Chemistry, 330 (1951); Brit. Pat., 700,677, 662,645, 672,691 (C. A., **46**, 11240 (1952); **49**, 1773 (1955)).
3) M. Ishidate, Y. Sakurai, S. Owari: This Bulletin, **5**, 203 (1957).
4) J. M. Sprague, E. L. Engelhardt: U. S. Pat., 2,671, 105 (C. A., **49**, 1776 (1955)).

However, owing to the insolubility of this agent, its use had been usually limited only to the oral administration.

The present paper deals with the synthesis of some water-soluble derivatives of methanesulfonic esters of aminoglycols, which may have a wider application in cancer treatment by parenteral administration than the insoluble derivatives ever known.

Several methods had been tried for the preparation of these compounds. The pyridine and sodium alkoxide method[3] were not successful in obtaining a crystalline product. By the silver salt method reported by Sprague[4] starting with 2,2′-dichloro-N-methyldipropylamine, 1,5-bis(2-hydroxypropyl)-1,5-dimethyl-2,5-dimethylpiperazine was only a product being identified as its dipicrate. The only successful method was the one with methanesulfonic acid anhydride reported by the same author,[4] by which all the listed compounds in Table I, except No. 844, were prepared. However, in case of N,N′-bis(2-hydroxyethyl)-N,N′-dimethylethylenediamine, only dimethanesulfonic acid salt of the aminoglycol was obtained by reaction with methanesulfonic acid anhydride and not the desired ester.

Table I. Screening Data of Methanesulfonic Esters against Yoshida Sarcoma

Compd. No.	Formula	*in vivo*			*in vitro*	
		LD_{50} (mg./kg.)	MTD[c] (mg./kg.)	MED[d] (mg./kg.)	CI[e] (LD_{50}/MED)	MEC[f] (mM)
839[a]	$CH_3N(CH_2CH_2OSO_2CH_3)_2$	7.5	5	0.5	15	5×10^{-5}
840[a]	$CH_3N(CH_2CH(CH_3)OSO_2CH_3)_2$	37.5	25	1	37.5	5×10^{-3}
838[b]	$CH_3N(CH_2CH_2CH_2OSO_2CH_3)_2$	75	50	1	75	2.5×10^{-2}
844[a]	$(CH_3)_2N^{+}(CH_2CH_2CH_2OSO_2CH_3)_2$	37.5	25			1×10^{-1}
851[b]	$CH_3SO_2OCH_2CH_2(CH_3)N(CH_2)_3N(CH_3)CH_2CH_2OSO_2CH_3$	75	50	1	75	
953[b]	$CH_3SO_2OCH_2CH_2(CH_3)N(CH_2)_3N(CH_3)CH_2CH_2OSO_2CH_3$	37.5	25	1	37.5	5×10^{-4}
852[b]	$CH_3SO_2OCH_2CH_2(CH_3)N(CH_2)_6N(CH_3)CH_2CH_2OSO_2CH_3$	37.5	25	0.25	150	2.5×10^{-3}

a) Picrylsulfonate.
b) Hydrochloride.
c) Maximum tolerance dose on rats bearing Yoshida sarcoma.
d) Minimum effective dose determined by the method by Yoshida, *et al.*: Gann, **45**, 489 (1954).
e) Chemotherapeutic index.
f) Minimum effective concentration in tissue culture, determined by the method reported by M. Ishidate, *et al.*: This Bulletin, **7**, 873 (1958).

The screening data so far obtained against Yoshida sarcoma are shown in Table I. All the tested compounds except No. 844 had a strong antitumor activity both *in vivo* and *in vitro*, a brief note on which was already published.[5]

5) M. Ishidate, Y. Sakurai, Z. Tamura, T. Nambara, M. M. El-Merzabani: This Bulletin, **11**, 1218 1218 (1963).

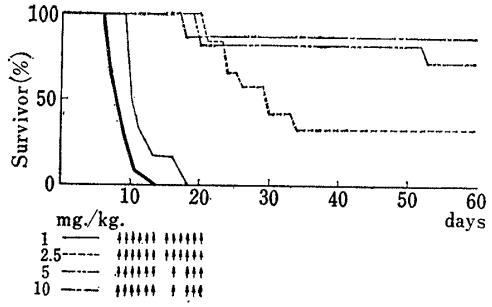

Fig. 1. Life Prolongation Effect of No. 838 on Rats bearing Yoshida Sarcoma (i. p. injections)

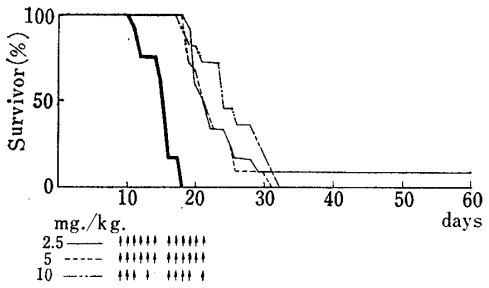

Fig. 2. Life Prolongation Effect of No. 838 on Rats bearing AH : 7974 (i. p. injections)

Among these compounds, No. 838 was of particular interest, since its effect on prolongation of life span of rats bearing Yoshida sarcoma was found to be far better than that of nitrogen mustard N-oxide, especially at relatively high doses as 5 mg./kg., as shown in Fig. 1. Also its toxicity seemed to be comparatively low as seen in body weight change during the period of injections, indicated in Table II. The effect of No. 838 on

Table II. Body Weight Change during Injection Period of No. 838 in Rats bearing Yoshida Sarcoma

Single dose			No. inj.	Total doses	Animal valid	Body weight (g.)				Survivor (%)	
mg./kg.	×MED	×LD_{50}		mg./kg.	No.	0 (day)	3	7	11	1 (month)	2
1	1	1/75	12	12	12	100	108	109	99	0	0
2.5	2.5	1/30	12	30	12	100	105	109	110	42	25
5	5	1/15	10	50	11	100	109	105	103	82	73
10	10	1/7.5	10	100	7	100	101	99	91	86	86

Table III. Body Weight Change during Injection Period of No. 838 in Rats bearing AH : 7974

Single dose			No. inj.	Total doses	Animal valid	Body weight (g.)				Average life span
mg./kg.	×MED[a)]	×LD_{50}		mg./kg.	No.	0 (day)	3	7	10	(day)
	control				12	100	104	122	133	15.3
2.5	2.5	1/30	12	30	12	100	105	116	126	22.2
5	5	1/15	12	60	12	100	100	105	106	20.9
10	10	1/7.5	9	90	11	100	94	102	101	23.2

a) MED to Yoshida sarcoma

life span prolongation of rats bearing ascites hepatoma AH-7974, which has been known to be far less sensitive for alkylating agents than Yoshida sarcoma, was also examined resulting in about one week life-prolongation in average compared with control animals irrespective of the applied doses as shown in Fig. 2. No significant decrease of body weight was observed even with dose as high as 10 mg./kg. (Table III). Now this compound is being subjected to the detailed pharmacological examinations in order to propose for clinical trials.

The thiosulfate uptake, as shown in Table IV, indicates that, the chemical activity is almost similar to the nitrogen mustard analogues in case the mesyloxy group is in the β-position to the nitrogen atom, and when it is in the γ-position, reactivity is fairly suppressed but still remains. It is also to be noted that compound No. 844, which has

Table IV. Thiosulfate Uptake in Sodium Carbonate Buffer at 37°

Compd. No.	Medium	Thiosulfate Uptake			
		10 min.	30 min.	2 hr.	24 hr.
838	H_2O	0.05	0.15	0.42	0.89
838	50% Me_2CO	0.07	0.15	0.40	0.82
844	〃	0.13	0.14	0.39	—a)
853	H_2O	1.31	1.49	1.51	1.53
852	〃	1.33	1.46	1.58	1.58

a) Solution cannot be titrated at 24 hr. because of formation of deep red color.

a quaternary ammonium structure, exhibited chemical reactivity on thiosulfate almost same as compound No. 838, in spite of complete ineffectiveness of the former in inhibition of tumor growth.

It was reported that, effectiveness of the disulfonic esters depends on the distance between the two alkylating groups and they are most effective when the spatial arrangement of their two alkylating groups permit the facile formation of a five or six-membered cyclic ring sulfonium compound with sulfhydryl compound like cysteine. This was assumed by the study of the metabolite *in vivo* of methanesulfonic ester of 1,4-butanediol by Roberts and Warwick.[6] The results obtained by the present authors indicate, however, that the compound having far longer distance between two sulfonic ester groups like compound No. 838 is more effective and so we come to the conclusion that, at least in the case of sulfonic ester analogues of aminoglycols, the distance between the two alkylating groups is not the only factor in the exhibition of antitumor activity.

Experimental

3,3′-Dihydroxy-N-methyldipropylamine——Et_2O solution of 0.1 mole of bis(2-carboethoxyethyl) methylamine[7] was added dropwise with stirring to Et_2O solution of 0.1 mole of $LiAlH_4$, in such a rate that the capacity of the reflux condenser was not exceeded. After more than 3 hr., H_2O was added cautiously with stirring to decompose the excess $LiAlH_4$ and the precipitated $Al(OH)_3$ was filtered off. The filtrate was made strongly alkaline and extracted with $CHCl_3$. Solvent was removed and the residue was fractionated *in vacuo* at 126~128°/3 mm. Hg.

General Method for the Preparation of Methanesulfonic Acid Esters——A solution of 0.05 mole of aminoalcohol in 100 ml. of CH_3CN was added with stirring to a solution of 0.11 mole of methanesulfonic acid anhydride in 150 ml. of CH_3CN at room temperature. Stirring was continued for 24~48 hr. depending on the cases. During this stirring period, small samples were occasionally taken out from the reaction mixture to detect, the two characteristic absorption bands at 1375 cm^{-1} and 1175 cm^{-1} in IR spectrum, which are characteristic for sulfonic ester group in order to check the end of reaction. Disappearance of the two absorption bands of the hydroxyl group at about 3650 cm^{-1} and 1050 cm^{-1} were also used for this purpose. The solvent was then evaporated *in vacuo*, and the residue was dissolved in abs. EtOH containing HCl in 5*N* concentration, added with anhyd. Et_2O and kept overnight at −5°. When the hydrochloride did not crystallized, the product was separated as picrylsulfonate.

2,2′-Dimesyloxy-N-methyldiethylamine (No. 839)——From 2,2′-dihydroxy-N-methyldiethylamine, isolated as picrylsulfonate in poor yield, m.p. 161~162° (from H_2O). *Anal.* Calcd. for $C_{13}H_{20}O_{15}N_4S_3$: C, 27.46; H, 3.52; N, 9.86. Found: C, 27.88; H, 3.55; N, 9.89.

2,2′-Dimesyloxy-N-methyldipropylamine (No. 840)——From 2,2′-dihydroxy-N-methyldipropylamine,[8] isolated as picrylsulfonate in poor yield, m.p. 150° (from Me_2CO-EtOH). *Anal.* Calcd. for $C_{15}H_{24}O_{15}N_4S_3$: C, 30.20; H, 4.03; N, 9.34. Found: C, 30.02; H, 4.02; N, 9.35.

6) J. J. Roberts, G. P. Warwick: Nature, **183**, 1509 (1959); **184**, 1288 (1959).
7) Org. Syntheses, vol. 20, p. 35.
8) W. E. Handy, H. N. Rydon: J. Chem. Soc., **1947**, 513.

3,3′-Dimesyloxy-N-methyldipropylamine (No. 838)——From 3,3′-dihydroxy-N-methyldipropylamine, isolated as a hygroscopic hydrochloride in 65～75% yield, m.p. 95° (in sealed tubes). *Anal.* Calcd. for $C_9H_{22}NO_6S_2Cl$: C, 31.76; H, 6.47; N, 4.12. Found: C, 31.80; H, 6.73; N, 3.99.

N,N′-Bis(2-mesyloxyethyl)-N,N′-dimethyltrimethylenediamine (No. 851)——From N,N′-bis(2-hydroxyethyl)-N,N′-dimethyltrimethylenediamine,[9] isolated as dihydrochloride in good yield, m.p. 111～113° (from MeOH-EtOH). *Anal.* Calcd. for $C_{11}H_{30}O_6N_2S_2Cl_2$: C, 31.50; H, 6.68; N, 6.68. Found: C, 30.90; H, 6.63; N, 6.46.

N,N′-Bis(2-mesyloxyethyl)-N,N′-dimethyltetramethylenediamine (No. 853)——From N,N′-bis(2-hydroxyethyl)-N,N′-dimethyltetramethylenediamine,[9] isolated as a dihydrochloride in good yield, m.p. 139～140° (from EtOH-Et_2O). *Anal.* Calcd. for $C_{12}H_{30}O_6N_2S_2Cl_2$: C, 33.26; H, 6.98; N, 6.47. Found: C, 33.17; H, 7.17; N, 6.56.

N,N′-Bis(2-mesyloxyethyl)-N,N′-dimethylhexamethylenediamine (No. 852)——From N,N′-bis(2-hydroxyethyl)-N,N′-dimethylhexamethylenediamine,[9] isolated as a dihydrochloride in good yield, m.p. 112～113° (from MeOH-Et_2O). *Anal.* Calcd. for $C_{14}H_{34}O_6N_2S_2Cl_2$: C, 36.45; H, 7.43; N, 6.07. Found: C, 36.10; H, 7.95; N, 6.07.

3,3′-Dimesyloxy-N-dimethyldipropyl Ammonium Salt——One gram of compouund No. 838 was dissolved in 1 ml. of H_2O and neutralized with $NaHCO_3$, then extracted with Et_2O. Et_2O extract was dried over anhyd. Na_2SO_4 and mixed with 2 g. of CH_3I and kept overnight with stirring at room temperature. After evaporation of Et_2O, the residue was treated with aqueous picrylsulfonic acid. The precipitated crystals were then filtered and recrystallized from Me_2CO-EtOH, m.p. 65～75° (no sharp melting point could be obtained). *Anal.* Calcd. for $C_{16}H_{26}O_{15}N_4S_3$: C, 31.42; H, 4.42; N, 9.17. Found: C, 31.31; H, 3.93; N, 8.88.

Determination of Thiosulfate Uptake——Determination was carried out at 37° in $NaHCO_3$ buffer by the same experimental procedure as reported previously.[10]

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Summary

Seven methanesulfonic esters analogues of aminoglycols were prepared and their antitumor activity was investigated with two kinds of rat tumors. Of these compounds, 3,3′-dimesyloxy-N-methyldipropylamine (No. 838), was proved to be the most effective and seems to be a promising candidate for clinical trial.

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9) M. Ishidate, W. Sakurai, K. Maruyama: This Bulletin, **5**, 435 (1957).
10) I. Aiko: *Ibid* **9**, 350 (1961).