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35. Morizo Ishidate, Yoshio Sakurai, and Sakahiko Owari : Studies on Cancerocidal Substances. XVI.* Some Sulfonic Acid Esters as Anti-cancer Agents.

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Recently, numerous reports[1] have been published on antimitotic activity of sulfonic acid esters of glycols or amino-glycols, out of which dimethanesulfonic acid ester of 1,4-butanediol has been adopted as a clinical antileukemic.

The sulfonic acid esters are supposed to be the so-called alkylating antimitotic and, were it true, its reactivity on thiosulfate, measured after Bergmann *et al.*,[2] should be a match for that of anti-cancer β-chloroethylamines, of which it has been established that an appearance of anti-cancer effect is observed only when it shows an adequate S-alkylating reactivity on thiosulfate *in vitro*.

The purpose of the present investigation was to discuss similar correlation among the sulfonic acid esters as among the β-chloroethylamines and to find a more effective agent in a series of these compounds.

In order to prepare the compounds, two procedures were adopted. The one is to react sodium alkoxide with sulfonyl chloride in a solvent, mostly in the same alcohol as the alkoxide used, and the other is to react alcohol with sulfonyl chloride under ice-cooling in an appropriate solvent, such as acetonitrile, in the presence of some quantity of pyridine.

Of the esters prepared, the monoesters are shown in Table I, in which MED represents a minimum antimitotic dose against the Yoshida sarcoma cell ascites of living rat.[3]

It is shown in Table I that an alkylating reactivity depended less on a variety of sulfonic acid residue than on a kind of the alcohol, and the esters of secondary alcohols

Table I.

Compounds	Thiosulfate consumption*		
	10 mins.	2 hrs.	24 hrs.
CH_3SO_2OEt	0.07	0.19	0.78
ϕ-SO_2OEt	0.08	0.22	0.72
p-CH_3-ϕ-SO_2OEt	0.11	0.18	0.72
p-NO_2-ϕ-SO_2OEt	0.20	0.71	0.85
CH_3CONH-ϕ-SO_2OEt	0.08	0.14	0.72
ϕ-SO_2OCH=$(CH_3)_2$	—	0.05	0.15
p-CH_3-ϕ-SO_2OCH=$(CH_3)_2$	—	0.02	0.12
p-NO_2-ϕ-SO_2OCH=$(CH_3)_2$	—	0.14	0.15
CH_3CONH-ϕ-SO_2OCH=$(CH_3)_2$	—	0.05	0.14
$CH_3N(C_2H_4Cl)_2$	0.92	1.88	1.88
TEM	—	0.12	0.85
Thia-TEPA	—	0.21	0.76

* Figures show molecular equivalent values of thiosulfate consumption in 50% acetone-water mixture at 37°. Calculated maximum value is 2 in case of bifunctional ester.

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1) A. Haddow, G.M. Timmis : Lancet, **264**, 207(1953); G.M. Timmis : Abstracts of papers of XIIth International Congress of Pure and Applied Chemistry,(1951) 330; Brit. Pat. 700677, 662645, 672691 (C.A., **46**, 11240(1952), **49**, 1773(1955)); J.M. Sprague, E.L. Engelhardt : U.S. Pat., 2,671,105(C.A., **49**, 1776(1955)).

2) M.A. Stahmann, M. Bergmann : J. Org. Chem., **11**, 586(1946).

3) M. Ishidate, T. Yoshida, Y. Sakurai, H. Satoh : Gann, **47**, 372(1956).

Table II.

	Compound	Thiosulfate consumption[a] 2 hrs.	Thiosulfate consumption[a] 24 hrs.	MED[b] mg./kg.	MTD
(I)	$(CH_3SO_2O\cdot C_2H_4-)_2$[c]	0.16	0.78	100	> 200
(II)	$(p\text{-}CH_3\text{-}\phi\text{-}SO_2O\cdot C_2H_4-)_2$	0.08	0.66	100	> 200
(III)	$(p\text{-}NO_2\text{-}\phi\text{-}SO_2O\cdot C_2H_4-)_2$[d]	—	—	400	> 400
(IV)	$(p\text{-}CH_3\text{-}\phi\text{-}SO_2O\cdot \underset{\underset{H}{\vert}}{\overset{\overset{CH_3}{\vert}}{C}}\text{-}CH_2-)_2$	0.02	0.04	inactive	>1000
(V)	$(p\text{-}CH_3\text{-}\phi\text{-}SO_2O\cdot C_2H_4)_2O$	0.04	0.36	inactive	>1500
(VI)	$(p\text{-}CH_3\text{-}\phi\text{-}SO_2O\cdot CH_2-)_2$[e]	0.04	0.16	inactive	> 200
(VII)	$(p\text{-}CH_3COHN\text{-}\phi\text{-}SO_2O\cdot CH_2-)_2$	0.02	0.20	200	>1000

a) Titration same as in Table I.
b) Minimum antimitotic dose on Yoshida sarcoma cells in ascites of living rat.
c) G.M. Timmis: Brit. Pat. 700677 (C.A. 1773(1955)).
d) The sample could not be titrated because of its insolubitity in 50% acetone.
e) F. Drahowzal, D. Klamann: Monatsh., 82, 452(1951).

was proved to be almost nonreactive. In spite of the fact that the alkylating reactivity of ethyl *p*-toluenesulfonate was the same as or greater than that of the biologically active esters of glycol, mentioned later, this monofunctional ester exhibited no antimitotic activity. Cross-linking alkylation seemed therefore to be indispensable in exhibiting an anti-cancer effect for these sulfonic esters same as for nitrogen mustards.

In Table II, the data are given for disulfonic acid esters (I-VII) of simple glycols. Dimethyl ester (I) and ditosyl ester (II) of 1,4-butanediol was found also to be effective even on the Yoshida sarcoma, although (II) seemed to be somewhat better than (I) with respect to the ratio of maximum tolerable dose to MED. A differernce by the kind of sulfonic acid ester residue seemed not to give any essential influence upon efficacy. S-Alkylating reactivity of the two compounds was almost alike.

p-Toluenesulfonic acid ester of a secondary alcohol, viz. 2,5-hexanediol (IV), showed only a slight thiosulfate up-take and no antimitotic activity. Effect of the distance between two hydroxyl groups of glycols on the antimitotic activity of the esters was discussed by Haddow and Timmis.[1] They stated that the ester of glycol, the distance of two hydroxyl groups of which was as short as in ethyleneglycol, was completely inactive to animal tumor and normal myelocyte and a maximum of action was attributed to the glycol having four- to six-carbon chain.

In the present experiment, it was found that ditosylates of ethyleneglycol and diethyleneglycol were inactive, though the latter had enough chain length to be active after Haddow's[1] account. Since the thiosulfate up-take of these inactive compounds were comparatively scant, it was presumed that their biological inactivity seemed to result not only from the unsuitable steric configuration but from the influence of β-substituted oxygen or nitrogen upon alkylating reactivity. Bis(*p*-acetamidobenzenesulfonate) of ethyleneglycol was however found to be exceptionally effective on this ascites tumor.

Sulfonic esters of bis(β-hydroxyethyl)amine are summarized in Table III, which were found to be more reactive on thiosulfate *in vitro* and more effective on the tumor than the afore-mentioned esters of simple glycols.

Out of these, ditosylate of N,N-bis(β-chloroethyl)-*p*-chloroaniline (XII) was claimed by Timmis[4] as a highly active agent, but it was only slightly effective on the Yoshida sarcoma because of its scant solubility in ascites.

Attempts to prepare sulfonic esters of N-methyldiethanolamine or N,N-diethyl-

4) G.M. Timmis: Ann. Rept. Brit. Empire Cancer Campaign, No. 27, 43(1949).

Table III.

	Compound	Thiosulfate consumption 2 hrs.	Thiosulfate consumption 24 hrs.	MED mg./kg.	LD_{50}
(VIII)	ϕ-N(C_2H_4Cl)$_2$[a]	0.08	0.94	(±)[b]	100
(IX)	ϕ-N(C_2H_4Benz.)[a]	1.28	1.54	0.1	150
(X)	ϕ-N(C_2H_4Tos.)$_2$[a]	0.40	1.14	1	300
(XI)	ϕ-N(C_2H_4Nitro.)$_2$	0.00	0.04	0.1	75
(XII)	Cl-ϕ-N(C_2H_4Tos.)$_2$[a]	0.08	0.84	100	—
(XIII)	HO_3S-ϕ-N=N-ϕ-N(C_2H_4Cl)$_2$	0.02	0.08	50	150
(XIV)	HO_3S-ϕ-N=N-ϕ-N(C_2H_4Tos.)$_2$ pyridine salt	0.04	0.54	(±)	> 1000

Tos.=*p*-Toluenesulfonyloxy-, Benz.=Benzenesulfonyloxy- Nitro.=*p*-Nitrobenzenesulfonyloxy-

a) The compounds were already reported by G.M. Timmis.

b) Effectiveness is equivocal.

ethanolamine did not succeed and the dimerisation product alone, viz. 1,4-dimethyl-1,4-(β-hydroxyethyl)piperazinium ditosylate, was obtained.

In conclusion, the thiosulfate consumption *in vitro* and an appearance of antimitotic activity in these compounds were mutually related and an agent which had no alkylating activity was proved to have no antimitotic activity. It could be accepted from this fact that the sulfonic esters acted *in vivo* analogous to the mode of action of nitrogen mustards, the initial action of which has been believed to be its alkylating reaction on constitutents of the proliferating cells.

Esters of N-methyldiethanolamine of strong acids, viz. monochloroacetic, trichloroacetic, and sulfuric acids, were prepared but they were proved inactive as an antimitotic, although their thiocyanate was remarkably toxic on rats (LD_{50}, 10 mg./kg.) and also chemically reactive enough to dimerize into piperazinium compound when stood in the presence of water.

The results of animal experiment involved in the paper were reported by Ishidate, Yoshida, Sakurai, *et al.* at the annual meeting of Japan Cancer Association held at Sapporo on July 15, 1956.

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Experimental

Determination of Thiosulfate Consumption—A sample (1 m.mole for monofunctional ester or 0.5 m.mole for the bifunctional) was dissolved in 25 cc. of acetone, 10 cc. of 0.1*N* $Na_2S_2O_3$ and 2 m.mole of $NaHCO_3$ were added to it, and the whole was made exactly to 50 cc. by addition of water. The solution was kept at 37°. An aliquot of 10 cc. each was taken out after 10 mins., 2 hrs., and 24 hrs. and titrated with 0.02*N* iodine solution under ice-cooling. Equivalent value of thiosulfate consumption (E) was calculated from the following equation: $E=k\cdot\frac{F(A-X)}{10}$, where F is a factor of 0.02*N* iodine solution, A is titration value (cc.) of 0.02*N* iodine in blank titration, X is titration value (cc.) of 0.02*N* iodine with the samples, and k is a constant, which is 1 in the case of bifunctional and 2 in the case of monofunctional compounds.

Ethyl *p*-Nitrobenzenesulfonate—EtOH solution (100 cc.) of *p*-nitrobenzenesulfonyl chloride (42 g.) was added into EtOH solution (100 cc.) of EtONa (from 4.5 g. Na). Reaction proceeded without external heating and the reaction ceased after 1 hr. The reaction mixture was poured into a large volume of water and the precipitate was purified by repeated recrystallization from EtOH or benzene until Cl test (Beilstein's) became negative. Yield, 35 g. Pale yellow plates, m.p. 92°. *Anal.* Calcd. for $C_8H_9O_5NS$: N, 6.06. Found: N, 6.05.

Isopropyl *p*-Nitrobenzenesulfonate—The benzene solution (50 cc.) of *p*-nitrobenzenesulfonyl chloride (11 g.) was dropped into a mixture of *iso*-PrOH and 50 cc. of benzene containing 1.15 g. of Na. The solution obtained was kept at room temperature for 2 hrs. Excess of *iso*-PrOH and benzene were distilled off *in vacuo*, the residue was washed with water, and recrystallized from light petroleum (b.p. 60~80°). Yield, 10 g. Pale yellow needles, m.p. 53~54°. *Anal.* Calcd. for $C_9H_{11}O_5NS$: C, 44.08; H, 4.52; N, 5.71. Found: C, 44.28; H, 4.72; N, 5.87.

Isopropyl *p*-Acetamidobenzenesulfonate—Recrystallized from benzene to colorless plates, m.p. 115~116°. Yield, 85%. *Anal.* Calcd. for $C_{11}H_{15}O_4NS$: C, 51.36; H, 5.88; N, 5.45. Found: C, 51.22; H, 5.91; N, 5,56.

Ditosyl Ester of 1,4-Butanediol (II)—Tosyl chloride (38 g.) was added portionwise at 0° into a mixture of 1,4-butanediol (9 g.) and pyridine (16 g.) and the mixture was left for 5 hrs. The reaction mixture was treated with dil. HCl and the precipitate was recrystallized from acetone-ether mixture to colorless plates, m.p. 82~83°. Yield, 15 g. *Anal.* Calcd. for $C_{18}H_{22}O_6S_2$: C, 54.27; H, 5.57. Found: C, 54.22; H, 5.43.

The following five compounds were prepared by the same method.

Bis(*p*-nitrobenzenesulfonic Acid) Ester of 1,4-Butanediol (III)—Recrystallized from AcOH to pale yellow or colorless prisms, m.p. 182°. Yield, poor. *Anal.* Calcd. for $C_{16}H_{16}O_{10}N_2S_2$: C, 41.74; H, 3.48; N, 6,09. Found: C, 41.84; H, 3.51; N, 6.19.

Ditosyl Ester of 2,5-Hexanediol (IV)—Recrystallized from acetone to colorless prisms, m.p. 116~116.5°. Yield, 30%. *Anal.* Calcd. for $C_{20}H_{21}O_6S_2$: C, 56.33; H, 6.15. Found: C, 56.32; H, 6.04.

Ditosyl Ester of Diethyleneglycol (V)—Recrystallized from acetone to colorless plates, m.p. 88~89°. Yield, 60%. *Anal.* Calcd. for $C_{18}H_{22}O_7S_2$: C, 52.17; H, 5.35. Found: C, 52.06; H, 5.35.

Ditosyl Ester of Ethyleneglycol (VI)—Recrystallized from acetone to colorless prisms, m.p. 128° Yield, 50%. *Anal.* Calcd. for $C_{16}H_{18}O_6S_2$: C, 51.89; H, 4.90. Found: C, 51.71; H, 5.03.

Bis(*p*-acetamidobenzenesulfonic Acid) Ester of Ethyleneglycol (VII)—Recrystallized from AcOH to colorless prisms, m.p. 203~204°. Yield, 50%. *Anal.* Calcd. for $C_{18}H_{20}O_8N_2S_2$: C, 47.37; H, 4.39; N, 6.14. Found: C, 47.55; H, 4.63; N, 5.97.

N,N-Bis[β-(*p*-nitrobenzenesulfonyloxy)ethyl]aniline (XI)—*p*-Nitrobenzenesulfonyl chloride (22 g.) was dissolved in CH_3CN (20 cc.), and into the solution was added N,N-bis(β-hydroxyethyl)aniline (9 g.) and pyridine (8 g.). The reaction mixture was kept at 0° for 2 hrs. and the solidified product was washed first with ether and then with water. The residue was recrystallized from benzene or an acetone-ether mixture. Orange yellow prisms, m.p. 139~140°. Yield, 14 g. *Anal.* Calcd. for $C_{22}H_{21}O_{10}N_3S_2$: C, 47.87; H, 3.83; N, 7.61. Found: C, 47.66; H, 3.87; N, 7.60.

4-[4-Bis(β-hydroxyethyl)aminophenylazo]benzenesulfonic Acid—MeOH solution (50 cc.) of N,N-bis(β-hydroxyethyl)aniline (18 g.) was added into 100 cc. of an aqueous solution of *p*-diazobenzenesulfonic acid (18 g.). The solution colored deep red orange. After 1 hr., the reaction mixture was warmed at 40~50° on a water bath for 30 mins., cooled, and the fine bluish-purple crystals that separated were recrystallized from EtOH or water to plates. Yield, 36 g. *Anal.* Calcd. for $C_{16}H_{19}O_5N_3S$: C, 52.60; H, 5.24; N, 11.50. Found: C, 52.79; H, 5.05; N, 11.20.

4-[4-Bis(β-tosyloxyethyl)aminophenylazo]benzenesulfonic Acid(Pyridine Salt)(XIV)—*p*-Toluenesulfonyl chloride (5 g.), dissolved in 20 cc. of CH_3CN, was dropped at 0° into a solution (5 cc.) of the foregoing hydroxy compound (5 g.) in pyridine. The reaction mixture was then kept at room temperature for 12 hrs. and excess of pyridine and CH_3CN were evaporated *iv vacuo*. The residue was kept in an ice box for 48 hrs. and then washed with a small amount of MeOH. An orange colored crystalline solid was obtained, which was dissolved in acetone and precipitated by addition of ether. Orange-yellow prisms (as pyridine salt). *Anal.* Calcd. for $C_{30}H_{31}O_9N_3S_3 \cdot C_5H_5N$: C, 55.85; H, 4.79; N, 7.45. Found: C, 55.98; H, 4.82; N, 7.40.

Reaction of N,N-Diethylethanolamine with *p*-Toluenesulfonyl Chloride—N,N-Diethylethanolamine (3.3 g.) was dissolved in 30 cc. of acetone and heated with 5.7 g. of *p*-toluenesulfonyl chloride for 1~2 hrs. on a water bath. Colorless plates separated from the solution, which decomposed at about 290°. It was purified by recrystallization from a mixture of acetone and EtOH, m.p. 290~291° (decomp.). It was identified as 1,1,4,4-tetraethylpiperazinium ditosylate. *Anal.* Calcd. for $C_{12}H_{28}N_2 \cdot 2C_7H_7O_3S$: C, 57.93; H, 7.74; N, 5.16. Found: C, 57.74; H, 7.75; N, 5.13.

Picrate: Yellow prisms, m.p. 163°(decomp.). *Anal.* Calcd. for $C_{12}H_{28}N_2 \cdot 2C_6H_2N_3O_7$: C, 43.90; H, 4.87; N, 17.07. Found: C, 43.93; H, 4.72; N, 17.20.

One mole each of N,N-diethylethanolamine, *p*-toluenesulfonyl chloride, and *p*-toluenesulfonic acid were mixed and heated first at 110~120° over night, and then at 220° for 2 hrs. The reaction mixture was dissolved in water and after being made slightly alkaline, extracted with ether. The ether extract was shaken with dil. HCl and the aqueous layer was evaporated to dryness. The residue was recrystallized from acetone, m.p. 205~206°, showing no depression on admixture with an authentic sample of N-(β-chloroethyl)diethylamine hydrochloride. Picrate, m.p. 115~116°.

Reaction of N-Methyldiethanolamine with *p*-Toluenesulfonyl Chloride—One mole of N-methyldiethenolamine and 2 moles of *p*-toluensulfonyl chloride were dissolved in ether containing a small quantity of acetone and kept for several hrs. at room temperature. An oily precipitate was separated and dissolved in water. An aqueous solution of picric acid was added to this solution and the picrate obtained was recrystallized from water. Needles, m.p. 230~233°. Yield, 1 g. from 6 g. of N-methyldiethanolamine. It was identified from analysis to be 1,4-dimethyl-1,4-(β-hydroxyethyl)piperazinium dipicrate. *Anal.* Calcd. for $C_{14}H_{24}O_2N_2 \cdot 2C_6H_2O_7N_3$: C, 40.00; H, 4.24; N, 16.97. Found: C, 40.40;

H, 4.19; N, 17.00.

Methyl-bis(β-monochloroacetoxyethyl)amine—Into a solution of N-methyldiethanolamine (12 g.) in $CHCl_3$(20 cc.), monochloroacetyl chloride (20 cc.) dissolved in $CHCl_3$(20 cc.) was added dropwise under a brine cooling. The mixture was heated on a water bath for 2 hrs. and an excess of $CHCl_3$ and the acid chloride were evaporated under a reduced pressure. The residue crystallized and was recrystallized from acetone to colorless scales (free base), m.p. 102°. Yield, quantitative. *Anal.* Calcd. for $C_9H_{15}O_6NCl_2$: C, 35.01; H, 5.19; N, 4.54. Found: C, 35.32; H, 5.06; N, 4.59.

Methyl-bis(β-trichloroacetoxyethyl)amine—Prepared as above. Very hygroscopic. m,p. 108° (decomp.). *Anal.* Calcd. for $O_9H_{11}O_4Cl_6 \cdot HCl$: C, 24.24; H, 2.71; N, 3.14. Found: C, 24.25; H, 2.76; N, 3.16. This anhydrous compound absorbed moisture from air and liquefied, but solidified again in the presence of water to a crystalline hydrate as colorless prisms, m.p. 50~55°. *Anal.* Calcd. for $C_9H_{11}O_4Cl_6 \cdot HCl \cdot 3H_2O$: C, 23.42; H, 2.43; N, 2.90. Found: C, 23.50; H, 2.41; N, 3.05.

Methyl-bis(β-thiocyanoethyl)amine—Methyl-bis(β-chloroethyl)amine hydrochloride (20 g.) was dissolved in water (200 cc.) with KCNS (80 g.)(pH=3.4). The mixture was heated on a water bath for 40 mins. and, after cooled, was neutralized to pH 7. An oily substance separated and the solution was extracted with ether. After evaporation of ether, 30 g. of the free base was obtained.

Hydrochloride: Colorless prisms (from acetone), m.p. 115~117°. *Anal.* Calcd. for $C_7H_{11}N_3S_2 \cdot HCl$: C, 35.37; H, 5.05; N, 17.69. Found: C, 35.30; H, 5.13; N, 17.48.

Picrate: Yellow prisms (from acetone-EtOH mixture), m.p. 176°.

The free base transformed to a dimer when it was kept with water and was identified as 1,4-dimethyl-1,4-(β-thiocyanoethyl)piperazinium dithiocyanate, m.p. 180°(decomp.). *Anal.* Calcd. for $C_{12}H_{22}N_6S_4$: C, 41.79; H, 5.47; N, 20.90. Found: C, 41.83; H, 5.54; N, 21.01.

N-Methyldiethanolamine *O,O'*-Bis(potassium Sulfonate)—Chlorosulfonic acid (4 moles) was added drop by drop upon dried and powderd N-methyldiethanolamine sulfate under ice cooling. After the addition ended, the mixture was poured into ice water and neutralized with $BaCO_3$. The precipitate ($BaSO_4$) was filtered off and the filtrate was added with K_2CO_3. The precipitate ($BaCO_3$) was again filtered off and the filtrate was dried *in vacuo*. The residue was recrystallized from dil. AcOH. *Anal.* Calcd. for $C_5H_{11}O_8NK_2$: C, 16.89; H, 3.12; N, 3.94. Found: C, 16.68; H, 3.32; N, 3.76.

Monopotassium salt was often obtained by repeated recrystallization from dil. AcOH. *Anal.* Calcd. for $C_5H_{12}O_8NK$: C, 18.92; H, 3.81; N, 4.41. Found: C, 18.95; H, 3.94; N, 4.29.

Summary

A number of sulfonic acid esters were prepared and the relation between their thiosulfate up-take *in vitro* and their antimitotic activity on proliferating cells of the Yoshida sarcoma was discussed. It was found that these esters acted in a similar manner both in chemical and in biological actions as nitrogen mustard.

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