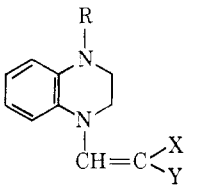
TABLE IV
ANTINEOPLASTIC ACTION[a] OF



X	Y	R	KB cell culture[b] ED_{50}, μg/ml	Slope	% (T/C)/dose, mg/kg SA[c]	LL[d]	LE[e]
CN	CN	H	$M1.0 \times 10^{2}$		70/500	89/400	101/400
CN	$CO_2C_2H_5$	H	$M1.0 \times 10^{2}$		89/500	79/400	90/400
CN	CN	$COCH_2CH_2Cl$	2.9×10^{0}	−1.2	134/500	36/400	101/400
CN	$CO_2C_2H_5$	$COCH_2CH_2Cl$	5.5×10^{0}	−0.5	64/500	71/350	93/350

[a] See footnote *a*, Table III. [b] See footnote *c*, Table III. [c] Sarcoma 180. [d] Lewis lung carcinoma. [e] L1210 lymphoid leukemia.

1,4-bis(3-chloropropionyl)-1,2,3,4-tetrahydroquinoxaline[1a] against KB cell culture since II (R = $COCH_2CH_2Cl$) is also active against this system.

Experimental Section[5]

β,β-Disubstituted N-Vinyltetrahydroquinolines and -isoquinolines.—A solution of 0.05 mole of 1,2,3,4-tetrahydroquinoline or 1,2,3,4-tetrahydroisoquinoline and 0.05 mole of the ethoxymethylene compound (I)[6] in 125 ml of methanol was refluxed for 1 hr and concentrated *in vacuo*. Solids were recrystallized from methanol and oils were distilled to give the compounds listed in Table I.

1-(β,β-Disubstituted vinyl)-1,2,3,4-tetrahydroquinoxaline and 1,4-Bis(β,β-dicarbethoxyvinyl)-1,2,3,4-tetrahydroquinoxaline.—As described above 0.05 mole of 1,2,3,4-tetrahydroquinoxaline and 0.1 mole of the ethoxymethylene compound (I) gave the compounds of the type II as indicated in Table II. In the case of diethyl ethoxymethylenemalonate two products were obtained and separated on acid-washed alumina.

1-(β,β-Disubstituted vinyl)-4-(3-chloropropionyl)-1,2,3,4-tetrahydroquinoxaline.—To a solution of II (R = H; X = CN; Y = CN or $CO_2C_2H_5$) in 125 ml of anhydrous chloroform at 0° was added dropwise, with constant stirring, a solution of 0.05 mole of 3-chloropropionyl chloride in 30 ml of anhydrous $CHCl_3$. The mixture was refluxed for several hours, filtered, and concentrated *in vacuo* to give compounds which are included in Table II.

(5) Analyses by Spang Microanalytical Laboratory, Ann Arbor, Mich. Melting points were taken in capillaries and are corrected.

(6) Initial samples of these compounds were generously donated by the Kay-Fries Chemicals, Inc.

Carcinostatic Sulfonic Acid Esters of Butyne- and Butane-1,4-diols[1a,b]

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The synthesis and evaluation of the anticancer properties of substituted benzenesulfonate esters of 1,4-butanediol and 1,4-butynediol was undertaken since esters of alkanesulfonic acids[2] have been found to be important anticancer compounds and, like nitrogen and sulfur mustards, are alkylating agents.[3] One sulfonic acid ester in particular has been shown to be an important therapeutic agent. This compound, 1,4-butanediol dimethanesulfonate (Ia) (known as Myleran® and Busulfan B®) was shown by Timmis and Haddow[4] to be effective in the management of granulocytic leukemia. It was also found to retard the growth of Adenocarcinoma 755, Glioma 26, and Brown–Pierce carcinoma[5] and was most active in Koller's[6] series on the effects of aliphatic sulfonate esters on the Walker carcinoma.

Although many studies have been made to elucidate the mechanism of action of alkylating agents, the literature is still filled with controversy. The following is a summary of some, although not all, of the studies which have been previously reported. Compound Ia in the *in vitro* studies of Parham and Wilbur[7] as in *in vivo* systems of Roberts and Warwick[8] appears to exhibit the same mechanism of action. The former workers followed the reaction of 1,4-butanediol dimethanesulfonate with the ethyl ester of cysteine in the presence of sodium hydroxide in ethanol and obtained the bisalkylated ethyl ester of cysteine and some tetrahydrothiophene. Parham and Wilbur[7] suggested a cyclic mechanism for the formation of tetrahydrothiophene from Ia and cysteine, and thought that this sulfur-stripping reaction might be responsible for the physiological activity of bifunctional alkylating agents in cancer chemotherapy. More recent work[9] with Ia and a number of mercaptans gave results quite analogous to those obtained from the reaction of Ia with the ethyl ester of cysteine. Meanwhile, Roberts and Warwick,[8] in an independent study of the reaction of Ia and cysteine *in vivo*, observed this same sulfur-stripping reaction. Subsequent studies,[10] in which carbon atoms of the alcohol portion of Ia were labeled with ^{14}C, have shown that it is metabolized in the mouse and excreted as 3-hydroxythiophene 1,1-dioxide.

(1) (a) This work was supported by Research Grant CA-06140 from the National Cancer Institute, Public Health Service. (b) Reported in part before the Medicinal Chemistry Division, 149th National Meeting, American Chemical Society, Detroit, Mich., March 1965. (c) Taken from the thesis of R. A. Earl which was submitted as partial fulfillment of the requirements for the Master of Arts Degree. (d) To whom inquiries should be sent.

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TABLE I

No.	Dose, mg/kg	No. of survivors	Animal wt dif T − C	Tumor wt or survival days T/C	Stage index or % T/C
			Lewis Lung Carcinoma		
VIII	200	6/6	−2.5	446/1235	36
VIII	200	1/6	−6.2	/1266	...
VIII	100	6/6	−3.3	443/652	67
X	400	6/6	−1.4	698/927	75
			Lymphoid Leukemia L1210		
VIII	200	6/6	−1.1	8.5/8.9	95
X	400	6/6	0.0	9.1/9.1	100
VI	500	6/6	+1.0	9.0/9.3	96
IX	500	6/6	−3.3	9.5/9.7	97
XI	500	3/6	−2.7	10.7/9.4	...
XI	300	5/6	−0.5	8.2/9.3	88
			Sarcoma 180		
VIII	500	5/6	−1.6	576/2237	25
VIII	500	6/6	−3.7	300/1297	23
VIII	250	6/6	−4.8	385/685	56
VIII	250	0/6	...	...	...
VIII	250	6/6	−3.8	923/1844	50
VIII	250	6/6	−5.0	912/1690	53
VIII	250	6/6	−6.8	535/1441	37
X	500	6/6	−2.9	1620/2237	72
VII	500	5/6	+1.7	1101/1615	68
XI	31	6/6	−0.8	937/1152	81
IX	500	5/6	−0.7	1270/1615	78
			Walker 256		
VII	500	6/6	−9.0	4.7/5.1	92
IX	500	5/6	−13.0	2.2/5.1	43
XI	500	5/6	−23.0	.6/5.3	11

The diols, 1,4-butanediol and 1,4-butynediol, were chosen because the mechanism of action of the sulfonate esters of these two compounds should be different if the triple bonds in the 1,4-butynediol diarylsulfonates remain intact when displacement takes place. The bisarylsulfonates of 1,4-butanediol showed no anticancer activity regardless of the substituents present, but several of the butynediol bisarylsulfonates showed either confirmed or presumptive anticancer activity in the Cancer Chemotherapy National Service Center anticancer screen. The toxicity and antitumor activity data of a few of the compounds are reported in Table I. Test procedures, methods, and protocol are adequately described elsewhere.[14]

Experimental Section

All melting points are corrected; analyses were done by Galbraith Laboratories, Knoxville, Tenn. The infrared spectra were determined on a Beckman IR-8 spectrophotometer.

The following absorptions in the infrared region were observed for all of the esters: 1340–1370 (SO_2), 1165–1180 (SO_2), and 925–955 cm^{-1} (ROS).[15] The two infrared absorption bands due to the sulfonyl group were sharp and intense. The absorption band in the region 925–955 cm^{-1} was intense, but generally quite wide. This absorption was present when the sulfonates were analyzed as Nujol mulls or as potassium bromide pellets. This band was not assigned to carbon–carbon stretching because the infrared spectra of carbamate esters of 1,4-butanediol fails to show this absorption, but it is present in the spectra of the bisarylsulfonates of but-2-yne-1,4-diol.

Preparation of Arylsulfonic Acid Esters of 1,4-Butanediol. Method A.—The tetramethylene bisarylsulfonates (I–V, Table II) were prepared by the method of Marvel and Sekera.[16] A solution of 0.025 mole of 1,4-butanediol in 50 ml of dry pyridine at −40° was added to a well-stirred solution of 0.050 mole of arylsulfonyl chloride in 100 ml of dry pyridine at −40°. The addition was completed in 30 min. The reaction mixture was allowed to warm to 0° with stirring and poured with stirring over 400 g of crushed ice and 27 ml of concentrated H_2SO_4. Longer reaction times and higher temperatures resulted in lower yields.

Method B.—A modification of the above reaction using 75 ml of 2,6-lutidine at −20° instead of pyridine at −40° and longer reaction times resulted in good yields of sulfonate esters.

Method C.—Sulfonic acid esters containing electron-withdrawing substituents were obtained in poor yield using the above procedures. These esters were prepared by treating 0.012 mole of 1,4-diiodobutane in 50 ml of acetonitrile with 0.024 mole of silver arylsulfonate. The reaction mixture was protected from the light and was heated under reflux for 2.5 hr. The solution was filtered while hot. A white solid separated from the filtrate when cooled to 0°.

TABLE II

$ArSO_2OCH_2CH_2CH_2CH_2OSO_2Ar$

No.	Ar	Method of prepn[a]	Yield, %	Recrystn solvent[b]	Mp, °C	Formula	Calcd, % C	Calcd, % H	Found, % C	Found, % H
I	2,5-Dichlorophenyl	A, C	28, 37	E	158–159	$C_{16}H_{14}Cl_4O_6S_2$	37.81	2.78	37.96	2.81
II	2,4-Dimethylphenyl	A	74	F	125–126	$C_{20}H_{26}O_6S_2$	56.31	6.14	56.20	6.22
III	2,5-Dimethylphenyl	A	98	E	97–98	$C_{20}H_{26}O_6S_2$	56.31	6.14	56.45	6.20
IV	3,4-Dichlorophenyl	A, B	64, 79	E	136–137	$C_{16}H_{24}Cl_4O_6S_2$	37.81	2.78	37.77	2.77
V	*p*-Nitrophenyl	A, B, C	27, 77, 63	E	175–176	$C_{16}H_{16}N_2O_{10}S_2$	41.51	3.49	41.67	3.62
VI	2,5-Dibromophenyl	B	71	F	174–175	$C_{16}H_{14}Br_4O_6S_2$	28.00	2.06	28.10	2.17

[a] See Experimental Section. [b] E = acetone, F = acetone–ether (8:1).

Other workers[11] have attempted to determine the center of attack by Ia and other alkylating agents. Ross[12] concluded on the basis of his observations and those of the other workers that such alkylating agents produce their effects by esterifying ionized acid groups.

Most of the investigations dealing with sulfonate esters previously reported have dealt with synthesis and anticancer evaluation of the esters of alkanesulfonic acids. In the present investigation substituted benzenesulfonate esters of 1,4-butanediol and 1,4-butynediol were prepared. Placing electron-withdrawing substituents on the benzene ring should increase the alkylating power of the sulfonate esters since electronic effects are transmitted across the sulfonyl group.[13] Very few substituted benzenesulfonate esters of 1,4-diols have been investigated for their anticancer activity, and no information of the effect of substituents has been available.

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TABLE III
$ArSO_2OCH_2C{\equiv}CCH_2OSO_2Ar$

No.	Ar	Method of prepn[a]	Yield, %	Recrystn solvent[b]	Mp, °C	Formula	Calcd, % C	Calcd, % H	Found, % C	Found, % H
VII	2,5-Dimethylphenyl	D	29	G	104–105	$C_{20}H_{22}O_6S_2$	56.85	5.25	56.91	5.25
VIII	2,5-Dichlorophenyl	D	81	E	137–139	$C_{16}H_{10}Cl_4O_6S_2$	38.11	2.00	37.94	2.01
IX	*p*-Nitrophenyl	D	33	E	178–180	$C_{16}H_{12}N_2O_{10}S_2$	42.10	2.65	42.31	2.84
X	2,4-Dimethylphenyl	D	36	E	105–107	$C_{20}H_{22}O_6S_2$	56.85	5.25	57.03	5.19
XI	3,4-Dichlorophenyl	D	81	H	105–107	$C_{16}H_{10}Cl_4O_6S_2$	38.11	2.00	38.30	2.07
XII	2,5-Dibromophenyl	D	81	E	114–118	$C_{16}H_{10}Br_4O_6S_2$	28.19	1.46	28.33	1.49
XIII	4-Chloro-3-nitrophenyl	D	39	E	116–117	$C_{16}H_{10}Cl_2N_2O_{10}S_2$	36.52	2.06	36.70	2.08
XIV	Thienyl	D	66	G	96–97	$C_{12}H_{10}O_6S_4$	37.10	2.65	37.22	2.73
XV	*m*-Nitrophenyl	D	23	E	127–128.5	$C_{16}H_{12}N_2O_{10}S_2$	42.10	2.65	41.93	2.49
XVI	*p*-Bromophenyl	D	49	E	135–137	$C_{14}H_{12}Br_2O_6S_2$	36.65	2.09	36.58	2.18

[a] See Experimental Section. [b] E = acetone, G = acetone–H_2O (3:2), H = petroleum ether (bp 60–110°).

Preparation of Arylsulfonic Acid Esters of 1,4-But-2-ynediol. Method D.—The 1,4-but-2-ynediol bis(arylsulfonates) (VII-XVI, Table III) were prepared by a procedure similar to that described by Eglington and Whitling.[17] A solution of 0.101 mole of KOH in 10 ml of water at 0° was added to 40 ml of acetonitrile at 10–20°, containing 0.10 mole of arylsulfonyl chloride and 0.05 mole of 1,4-but-2-ynediol. The addition took 30 min. The solution was then stirred at room temperature for 2–4 hr. The material precipitated was collected by vacuum filtration. The solid was air dried and then extracted with boiling acetone. Good to excellent yields of the esters recrystallize from the acetone solutions upon cooling to 0°.

Acknowledgment.—We wish to thank Dr. Harry B. Wood, Mr. Robert B. Ing, and Dr. Saul Shephartz of the Cancer Chemotherapy National Service Center for the test data we are including in this publication. We also wish to thank Mr. Philip Doyle, a National Science Foundation Undergraduate Research Participant, for preparing some of the sulfonyl chlorides used in this investigation.

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Synthesis of N-Monosubstituted 2-Mercaptoethylamines with Thioureido Substituents[1,2]

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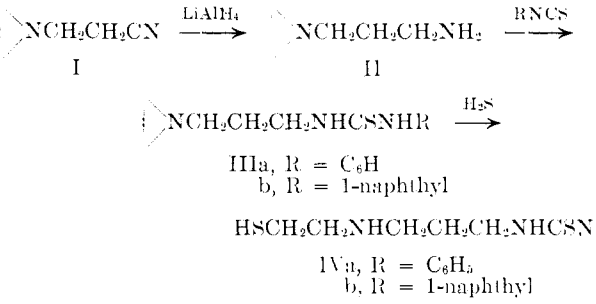
Because of the interest in 2-mercaptoethylamines and their derivatives as potential antiradiation drugs,[3-7] and the fact that certain 1-alkyl-3-(2-mercaptoethyl)-thioureas[8] were found to be effective radiation-protective agents,[9] we have synthesized a few N-monosubstituted 2-mercaptoethylamines, $RNHCH_2CH_2SH$, in which the R group includes thioureido. The method is shown in Scheme I. Data on the compounds with

SCHEME I



structures III and IV are presented in Tables I and II, respectively.

Both compounds were tested at 51–150 mg/kg ip in mice for 30-day survival against lethal radiation of 1000 r.[10] Neither of the amino mercaptans bearing the thioureido group provided mice any protection against lethal doses of radiation.

Experimental Section[11]

N-(3-Aminopropyl)ethylenimine.—A solution of 20.0 g (0.21 mole) of β-ethyleniminopropionitrile[12] in 40 ml of dry ether was added dropwise to a slurry of 7.9 g (0.21 mole) of $LiAlH_4$ in 200 ml of dry ether at 0° with stirring in a nitrogen atmosphere. The resultant reaction mixture was stirred at 0° for 30 min. Then, at 0°, 8 ml of water, 6 ml of 20% NaOH, and 28 ml of water were cautiously added in that order. The granular white solids formed in this way were removed by suction filtration, then washed with ether. The ethereal solutions were combined, dried ($MgSO_4$), and then concentrated by distillation through a Vigreux column. The residue obtained was distilled *in vacuo* and gave 6.3 g (30%) of product, bp 67–69° (27 mm), $n^{23}D$ 1.4567. Bestian[12] gives bp 61–62° (19 mm) for the compound prepared by catalytic hydrogenation.

Anal. Calcd for $C_5H_{12}N_2$: C, 59.95; H, 12.08; N, 27.97. Found: C, 59.75; H, 12.11; N, 27.76.

1-(3-Ethyleniminopropyl)-3-(1-naphthyl)thiourea.—1-Naphthyl isothiocyanate (4.6 g, 0.025 mole) and N-(3-aminopropyl)-ethylenimine (2.5 g, 0.025 mole) were dissolved in reagent grade benzene, heated to boiling, and cooled. A white solid, 4.7 g (66%), mp 128–131°, precipitated and was collected by suction on

(1) This work was supported by the U. S. Army Medical Research and Development Command, Department of the Army, under Contract No. DA-49-193-2174.

(2) This is the second paper dealing with the synthesis of N-monosubstituted 2-mercaptoethylamines. For the preceding paper see A. F. Ferris, O. L. Salerni, and B. A. Schutz, *J. Med. Chem.*, **9**, 391 (1966).

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