Alkylating Agents Related to 2,2′-Biaziridine. I. Compounds Derived from 1,4-Diamino-2,3-butanediol

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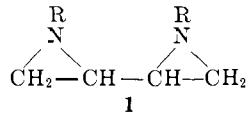
The synthesis of *meso*-, DL-, and (2*S*:3*S*)-1,4-diaminobutane-2,3-bissulfuric acid (**9**), and *meso*-, DL-, and (2*S*:3*S*)-1,4-diamino-2,3-butanediol 2,3-bismethanesulfonate (**11**) is described. The reason for the interest in this type of alkylating agents is discussed, and the anticancer screening data for the compounds are summarized. The structure of *meso*-diaminobutanediol and its derivatives previously described is revised.

Over the past two decades intense interest in aziridines and the related mono- and bifunctional nitrogen mustards as possible anticancer drugs has led to the preparation and evaluation of various analogs and derivatives in the search for enhanced anticancer properties. However, most investigations were made to find carrier molecules for the alkylating groups, which could influence the alkylating properties, the biological transport, or lead to an accumulation in the target tissue, in order to reach increased therapeutical indices. From pharmacological and therapeutical points of view the reported results were rather disappointing.[1] However, we considered it desirable to synthesize alkylating agents which may be considered *in vivo* precursors for the unknown 2,2′-biaziridine (**1**, R = H) or N-substituted biaziridines **1** including the "activated



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type"[2] of aziridines. Furthermore, we wished to investigate some of the corresponding N-substituted biaziridines.

Our initial interest in this type of compounds was based on the antineoplastic activity[3] of (2*S*:3*S*)-threitol 1,4-bismethanesulfonate,[4,5] which might act after *in vivo* transformation to epoxy compounds, since the transformation to (2*S*:3*S*)-1,2:3,4-diepoxybutane [(*S*:*S*)-**4**] is proved to proceed *in vitro* under physiological conditions,[6] and furthermore on the acceptance that nitrogen mustards probably involve aziridine or aziridinium compounds as reactive intermediates *in vivo* as recently reinvestigated *in vitro* by nmr studies.[7]

Compounds of the general formula **1**, and consequently their possible precursors, can exist in three optical isomers. In order to get acceptable information about the biological activities, it appeared important to us to attempt the preparation of the *meso* isomers, one of the optically active isomers, and the racemates of the desired compounds, since optical stereospecificity of anticancer agents has been demonstrated even when alkylating agents are involved.[8]

In the present paper the synthesis of derivatives **9** and **11** of 1,4-diamino-2,3-butanediol as possible *in vivo* precursors of the corresponding **1** (R = H) is reported (Table I). Attempts to isolate the expected biaziridine **1** (R = H) after alkali treatment of both **9** and **11** were unsuccessful.

Chemistry.—Kilmer and McKennis[9] described the reaction of *meso*-2,3-dibromo-1,4-butanediol (*meso*-**2**) with potassium phthalimide in boiling xylene, resulting in a bisphthalimidobutanediol. Hydrolysis gave the corresponding diaminobutanediol which could be transformed to a diaminobutane-bissulfuric acid by heating in concentrated H_2SO_4. The authors assigned the structure of these compounds by placing the amino groups in the 2 and 3 positions, since an identical diaminobutanediol also could be prepared by conversion of *meso*-1,4-diacetoxy-2,3-dibromobutane (*meso*-**6**) to a bisphthalimidodiacetoxybutane followed by hydrolysis. In the present paper the authors' procedure was reinvestigated but the obtainable diaminobutanediol was not identical with *meso*-2,3-diamino-1,4-butanediol prepared by other routes.[10] Because the reaction of an alkyl halogenide with potassium phthalimide is accompanied by Walden inversion, racemization to a DL and *meso* mixture could be excluded in the present case.

Since, from a theoretical point of view, halohydrins should form epoxy compounds intermediately under these conditions, we suggested that the above reaction of *meso*-**2** with potassium phthalimide involves formation of epoxides followed by ring opening to *meso*-1,4-bisphthalimido-2,3-butanediol (*meso*-**5**). This was proved by the fact that both *meso*-1,4-dibromo-2,3-butanediol (*meso*-**3**) by heating with potassium phthalimide and more conveniently *meso*-1,2:3,4-diepoxybutane (*meso*-**4**) by reaction with phthalimide likewise gave the bisphthalimidobutanediol *meso*-**5** (see Scheme I).

On the basis of these results the hydrolysis product of *meso*-**5**, the diaminobutanediol of Kilmer and McKennis, is assigned as *meso*-1,4-diamino-2,3-butanediol

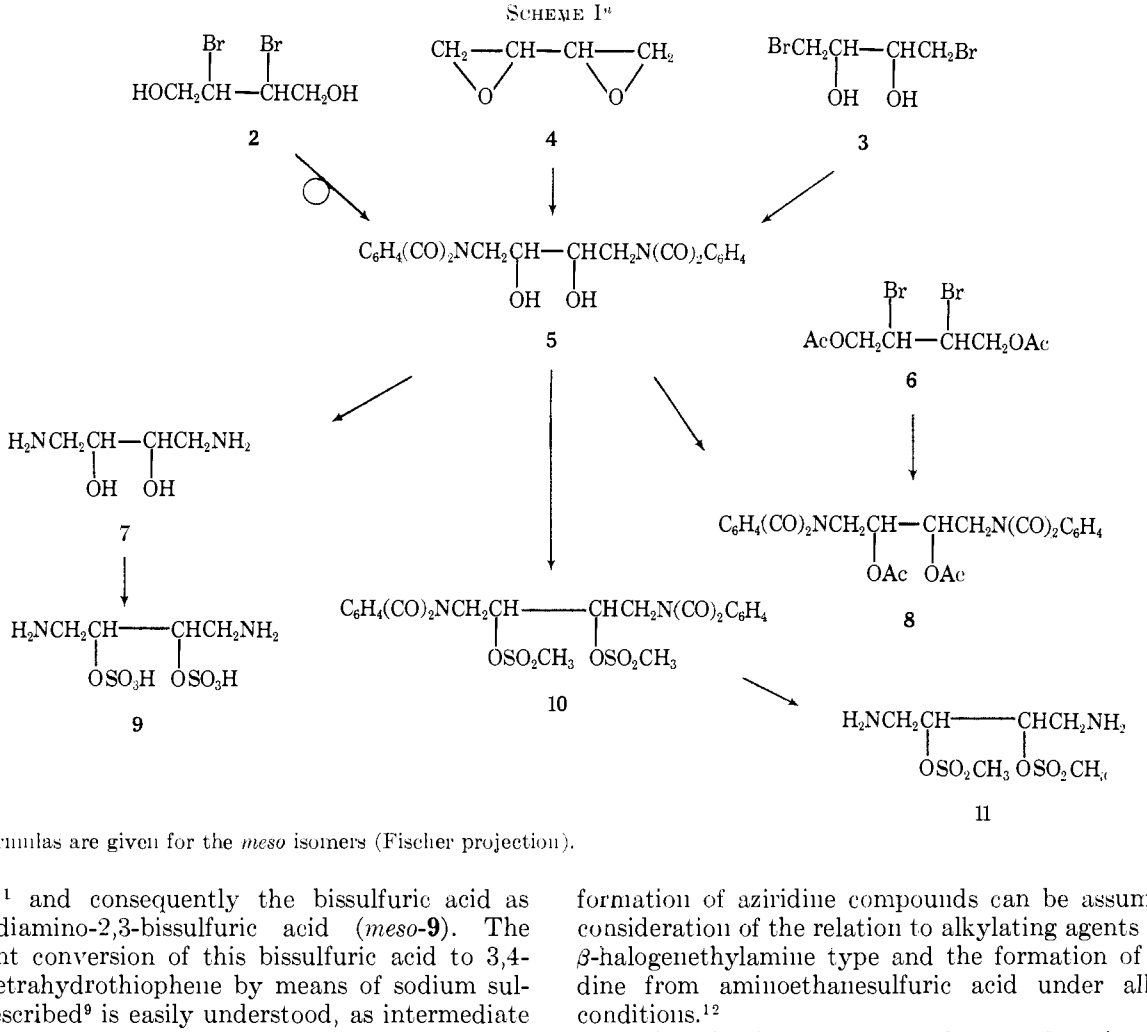
(1) L. H. Schmidt, R. Fradkin, R. Sullivan, and A. Flowers, *Cancer Chemotherapy Rept., Suppl. 2*, 1 (1965).
(2) G. E. Ham, *J. Org. Chem.*, **29**, 3052 (1964).
(3) (a) R. Jones, W. B. Kessler, H. E. Lessner, and L. Rane, *Cancer Chemotherapy Rept.*, **10**, 99 (1960); (b) F. R. White, *ibid.*, **24**, 95 (1962); (c) V. Loeb, Jr., *ibid.*, **42**, 39 (1964).
(4) P. W. Feit, *J. Med. Chem.*, **7**, 14 (1964).
(5) Earlier[4] the configuration of this compound was designed by the prefix L. In this paper the Cahn–Ingold–Prelog system is used for the optically active compounds to avoid confusion since the papers of this series concern compounds which could be based on the configuration of either glycerolaldehyde or serine: R. S. Cahn, C. K. Ingold, and V. Prelog, *Angew. Chem.*, **78**, 413 (1966), and preceding papers.
(6) W. Davis and W. C. J. Ross, *Biochem. Pharmacol.*, **12**, 915 (1963).
(7) P. L. Levins and Z. B. Papanastassiou, *J. Am. Chem. Soc.*, **87**, 826 (1965).
(8) F. Bergel, *Farmaco* (Pavia), *Ed. Sci.*, **19**, 99 (1964).
(9) G. W. Kilmer and H. McKennis, Jr., *J. Biol. Chem.*, **152**, 103 (1944).
(10) P. W. Feit and O. Tvaermose Nielsen, *J. Med. Chem.*, in press.

TABLE I
DERIVATIVES OF 1,4-DIAMINO-2,3-BUTANEDIOL
$RR'NCH_2CHXCHXCH_2NRR'$

No. and config	R	R'	X	Mp, °C	Solvent of recrystn	$[\alpha]^{20}D$, deg	Method[a]
5, *meso*		Phthaloyl	OH	294.5–295.5[b]	Methyl Cellosolve		A(a–d)
DL		Phthaloyl	OH	273–274	Methyl Cellosolve		A(c,d)
(*S*:*S*)		Phthaloyl	OH	263–264.5	DMF	−71.1[c]	A(d)
7·2HBr, *meso*[d]	H	H	OH	317–320 dec[e]	80% ethanol		B
DL[f]	H	H	OH	245–246 dec	Ethanol		B
(*S*:*S*)[g]	H	H	OH	Dec from 265	90% ethanol	−20.1[h]	B
9, *meso*	H	H	OSO_3H	Dec from 300[i]	*j*		D
DL	H	H	OSO_3H	Dec from 300	*j*		D
(*S*:*S*)	H	H	OSO_3H	Dec from 310	*j*	−3.2[k]	D
10, *meso*		Phthaloyl	OSO_2CH_3	237.5–240 dec	Methyl Cellosolve		E
DL		Phthaloyl	OSO_2CH_3	237.5–239.5 dec	Methyl Cellosolve		E
(*S*:*S*)		Phthaloyl	OSO_2CH_3	218.5–220.5 dec	Methyl Cellosolve	−33.7[c]	E
11·$2CH_3SO_3H$, *meso*	H	H	OSO_2CH_3	216.5–219 dec	*l*		F
DL	H	H	OSO_2CH_3	200–201.5 dec	*l*		F
(*S*:*S*)	H	H	OSO_2CH_3	201–202.5 dec	*l*	−25.8[k]	F
12, *meso*	H	COC_6H_5	$OCOC_6H_5$	204–204.5[m]	Ethanol		C
DL	H	COC_6H_5	$OCOC_6H_5$	154–155.5	Ethanol		C
(*S*:*S*)	H	COC_6H_5	$OCOC_6H_5$	157–158	Ethanol	−28.5[c]	C

[a] The letters relate to the general procedures in the Experimental Section. [b] Lit.[9] mp 300–302°. [c] (*c* 2, DMF). [d] The analytically pure bissalicylaldehyde Schiff base had mp 228.5–230°, lit.[11] mp 229–230°. [e] After darkening from about 240°, lit.[9] dec from about 220°. [f] The analytically pure bissalicylaldehyde Schiff base had mp 216.5–217.5°, lit.[11] mp 214–215°. [g] The analytically pure bissalicylaldehyde Schiff base had mp 224–225° dec; M. L. Wolfrom, F. Shafizadeh, J. O. Wehrmüller, and R. K. Armstrong, *J. Org.*

SCHEME I[a]



[a] The formulas are given for the *meso* isomers (Fischer projection).

(*meso*-**7**),[11] and consequently the bissulfuric acid as *meso*-1,4-diamino-2,3-bissulfuric acid (*meso*-**9**). The subsequent conversion of this bissulfuric acid to 3,4-diaminotetrahydrothiophene by means of sodium sulfide as described[9] is easily understood, as intermediate formation of aziridine compounds can be assumed in consideration of the relation to alkylating agents of the β-halogenethylamine type and the formation of aziridine from aminoethanesulfuric acid under alkaline conditions.[12]

On the other hand we were able to confirm the results of the reaction of *meso*-1,4-diacetoxy-2,3-dibromobutane (*meso*-**6**) with potassium phthalimide.[9] The iso-

(11) After completion of this work F. I. Carroll, *J. Org. Chem.*, **31**, 366 (1966), isolated *meso*-**7** prepared by other routes as its bissalicylaldehyde Schiff base with mp 229–230°. We found for this Schiff base obtained from the diaminobutanediol of Kilmer and McKennis,[9] mp 228.5–230°, in contrast to that of *meso*-2,3-diamino-1,4-butanediol with mp 185–185.5°.[10]

(12) H. Wenker, *J. Am. Chem. Soc.*, **57**, 2328 (1935).

Formula	% calcd C	H	N	S	Hal	% found C	H	N	S	Hal
$C_{20}H_{16}N_2O_6$	63.15	4.24	7.37			63.07	4.39	7.11		
						63.08	4.35	7.31		
						63.12	4.40	7.27		
$C_4H_{12}N_2O_2 \cdot 2HBr$	17.04	5.00	9.94		56.68	17.00	5.08	9.76		56.71
						17.04	5.14	10.00		57.04
						16.86	5.03	9.86		56.73
$C_4H_{12}N_2O_8S_2$	17.14	4.32	10.00	22.88		17.15	4.41	10.02	22.60	
						17.16	4.49	10.08	22.64	
$C_4H_{12}N_2O_8S_2 \cdot 0.5H_2O$	16.61	4.53	9.69	22.17		16.64	4.56	9.74	22.11	
$C_{22}H_{20}N_2O_{10}S_2$	49.25	3.76	5.22	11.95		49.21	3.84	5.17	11.78	
						49.16	3.92	5.13	11.76	
						49.04	3.91	5.13	11.80	
$C_6H_{16}N_2O_6S_2 \cdot 2CH_3SO_3H$	20.51	5.16	5.98	27.37		20.38	5.33	5.83	26.92	
						20.65	5.29	5.92	27.09	
						20.54	5.17	5.99	27.41	
$C_{32}H_{28}N_2O_6$	71.63	5.26	5.22			71.60	5.36	5.17		
						71.48	5.33	5.19		
						71.58	5.38	5.17		

Chem., **23**, 571 (1958), report mp 228–231° for the (*R*:*R*) compound. [h] (*c* 2, H_2O). [i] Lit.[9] dec from 280°. [j] Purified by repeated reprecipitations from alkaline solution with dilute HCl. [k] (*c* 2, 0.5 *N* NaOH). [l] Purified by reprecipitation from H_2O acidified with methanesulfonic acid by addition of ethanol. [m] Lit.[9] mp 203–204°.

lated bisphthalimidodiacetoxybutane (*meso*-**8**) was shown to be identical with that obtained after acetylation of *meso*-1,4-bisphthalimido-2,3-butanediol (*meso*-**5**). This apparent disagreement with the revised structures can be explained only if acyl migration from carbons 2 and 3 to **1** and 4 is assumed in the course of the alkylation. The participation of an acetoxy neighbor group in nucleophilic substitution reactions by intermediate formation of an acetoxonium ion as investigated by Winstein and co-workers[13] might explain this migration. Using the above sequence the bissulfuric acids DL-**9** and (*S*:*S*)-**9** were prepared.

To obtain the desired *meso*-, DL-, and (2*S*:3*S*)-1,4-diamino-2,3-butanediol 2,3-bismethanesulfonates (*meso*-**11**, DL-**11**, (*S*:*S*)-**11**) the corresponding 1,4-bisphthalimido-2,3-butanediols **5** were mesylated. Hydrolysis of the phthalimido groups in the resulting bismethanesulfonates **10** without attack at the methanesulfonyloxy groups was, as expected, rather difficult but could be performed by heating in moist methanesulfonic acid. This reaction was sensitive. Prolonged heating or too high temperature caused total hydrolysis accompanied by racemization. Only a mixture of DL- and *meso*-**7** was isolable. On one occasion under changed conditions and starting from (*S*:*S*)-**10** the partial hydrolysis product (2*S*:3*S*)-1-phthalimido-4-amino-2,3-butanediol 2,3-bismethanesulfonate could be isolated. Methanesulfonation of DL-1,4-bis(dimethylamino)-2,3-butanediol prepared from DL-**7** by reductive alkylation gave DL-1,4-bis(dimethylamino)-2,3-butanediol 2,3-bismethanesulfonate in poor yield.

In agreement with reported results[9] for the *meso* isomer attempts to prepare the stereoisomeric 1,4-diamino-2,3-dichlorobutanes from **7** failed.

Anticancer Screening.—The 1,4-diaminobutane-2,3-bissulfuric acids (*meso*-**9**, DL-**9**, (*S*:*S*)-**9**), and the bismethanesulfonates *meso*-**11**, DL-**11**, and (*S*:*S*)-**11** were submitted to anticancer screening at the Cancer Chemotherapy National Service Center, National Institutes of Health. Compounds **9** and **11** were moderately active in the KB cell culture assay (ED_{50} about 20 μg/ml) except (*S*:*S*)-**9** which was inactive up to 100 μg/ml. As far as Dunning leukemia (ascites), L1210 lymphoid leukemia, and P1798 lymphosarcoma test systems are concerned, the bissulfuric acids **9** lacked significant antineoplastic activity. The bismethanesulfonates **11** were evaluated in the Walker carcinosarcoma 256 system. The screening data indicate an expected difference between the stereoisomers *meso*-**11** and (*S*:*S*)-**11** (Table II).

TABLE II

SCREENING DATA IN THE WALKER CARCINOSARCOMA 256 (SUBCUTANEOUS) SYSTEM

Compd[c]	Daily dose, mg/kg[a]	Survivors	Mean tumor wt, %[b] test/control
meso-**11**	100	6/6	34
	50	6/6	29
	25	6/6	75
	12.5	6/6	58
DL-**11**	200	6/6	0
	100	6/6	15
	50	6/6	64
	25	6/6	95
(*S*:*S*)-**11**	200	4/6	0
	100	6/6	4
	50	6/6	12
	25	6/6	21

[a] Administered intraperitoneally once daily, days 1–5 postinoculation. [b] Sacrificed and evaluated 10 days postinoculation. [c] Tested as the dimethanesulfonate salt.

(13) (a) S. Winstein and R. E. Buckles, *J. Am. Chem. Soc.*, **64**, 2780 (1942); (b) S. Winstein, H. V. Hess, and R. E. Buckles, *ibid.*, **64**, 2796 (1942); (c) S. Winstein and R. E. Buckles, *ibid.*, **65**, 613 (1943); (d) S. Winstein, C. Hanson, and E. Grunewald, *ibid.*, **70**, 820 (1948).

Experimental Section[14]

1,4-Bisphthalimido-2,3-butanediols (5). Method A. (a) Reaction of **3** (10 g) with potassium phthalimide (20 g) in boiling dimethylformamide (DMF) (60 ml) and working up as described earlier[9] yielded about 75% of crude **5**. The infrared spectrum (KBr), analysis, and melting point were identical with those of material prepared from **2** in either (**b**) xylene[9] or (**c**) DMF.

(**d**) To a hot (135°) solution of phthalimide (41 g) in DMF (70 ml), **4** (10.7 g) was added dropwise under vigorous stirring, the heat of reaction causing reflux. The reaction mixture was heated for an additional 30 min, and after cooling the precipitate was thoroughly washed with 1 *N* NaOH, H_2O, ethanol, and diethyl ether to give 32–40 g of crude **5**. The physical properties were identical with those of the compounds prepared as in parts a, b, and c.

***meso*-1,4-Bisphthalimido-2,3-diacetoxybutane** (*meso*-**8**).—A suspension of *meso*-**5** (1.9 g) in acetic anhydride (10 ml) and pyridine (12 ml) was stirred at room temperature for 20 hr and then poured into ice–2 *N* HCl (150 ml). The resulting precipitate was washed with water, ethanol, and diethyl ether to give 1.9 g of crude *meso*-**8**. After recrystallization from acetic acid the melting point was 250–250.5°; lit.[9] mp 250°. This material was by its infrared spectrum, (KBr) melting point, and analysis identical with that prepared from *meso*-2,3-dibromo-1,4-diacetoxybutane (*meso*-**6**) by reaction with potassium phthalimide as described.[9]

Anal. Calcd for $C_{24}H_{20}N_2O_8$: C, 62.06; H, 4.34; N, 6.03. Found: C, 61.98; H, 4.37; N, 6.00.

1,4-Diamino-2,3-butanediol Dihydrobromides (7·2HBr). Method B.—Compound **5** was hydrolyzed in 48% HBr as described.[9] Crude **7**·2HBr was obtained after evaporation of the reaction mixture, from which the separated phthalic acid had been removed by filtration.

Method C.—The **tetrabenzoates** (**12**) of **7** were prepared with excess benzoyl chloride in pyridine.

1,4-Diaminobutane-2,3-bissulfuric Acids (9). Method D.—The dihydrobromides of **7** were treated with concentrated H_2SO_4 as described[9] for the corresponding sulfate of the *meso* isomer.

1,4-Bisphthalimido-2,3-butanediol 2,3-Bismethanesulfonates (10). Method E.—To a suspension of **5** (38 g) in pyridine (200 ml), methanesulfonyl chloride (26 ml) was added dropwise while stirring at 0–5° over a period of 30° min. The mixture was stirred at room temperature for an additional 3 hr, and then poured into ice–2 *N* HCl (2 l.). The resulting precipitate was washed with H_2O, ethanol, and diethyl ether to give 50–57 g of crude **10**.

1,4-Diamino-2,3-butanediol 2,3-Bismethanesulfonate Dimethanesulfonates (11·2CH$_3$SO$_3$H). Method F.—A mixture of **10** (50 g), methanesulfonic acid (90 ml), and H_2O (10 ml) was heated in an oil bath to 126–133° while stirring for 3.5–4 hr.[15] After cooling, ethanol (200 ml) and diethyl ether (600 ml) were added, and the mixture was left at room temperature for 20 hr. The precipitated material was washed with ethanol and diethyl ether and extracted with water. The extract was treated with decolorizing carbon, and the resulting colorless solution was evaporated under reduced pressure. The residue was treated with ethanol to give 12–14 g of crude **11**·2CH$_3$SO$_3$H.

By increasing the reaction temperature to 140° and decreasing the reaction time to 1 hr, the only isolable reaction product from (*S*:*S*)-**10** was **(2*S*:3*S*)-1-phthalimido-4-amino-2,3-butanediol 2,3-bismethanesulfonate methanesulfonate** with mp 180–182.5° dec (H_2O-ethanol), $[\alpha]^{20}D$ −43.3° (c 2, H_2O)

Anal. Calcd for $C_{14}H_{18}N_2O_8S_2\cdot CH_3SO_3H$: C, 35.85; H, 4.41; N, 5.58; S, 19.14. Found: C, 35.27; H, 4.45; N, 5.40; S, 19.14.

DL-1,4-Bis(dimethylamino)-2,3-butanediol Dihydrobromide.—A solution of DL-**7**·2HBr (28.2 g) in H_2O (500 ml) and 35% formalin solution (60 g) was hydrogenated in the presence of 20 g of 10% Pd–C under 1.1 atm pressure. The hydrogen uptake was complete in about 1.5 hr. The catalyst was removed by filtration, and the resulting solution was evaporated under reduced pressure. Trituration of the residue with ethanol gave 32.2 g of crude material with mp 175.5–178° dec. Recrystallization from 90% ethanol raised the melting point to 181–183° dec.

Anal. Calcd for $C_8H_{20}N_2O_2\cdot 2HBr$: C, 28.42; H, 6.56; Br, 47.27; N, 8.29. Found: C, 28.50; H, 6.68; Br, 47.34; N, 8.16.

DL-1,4-Bis(dimethylamino)-2,3-butanediol 2,3-Bismethanesulfonate Dihydrochloride.—DL-1,4-Bis(dimethylamino)-2,3-butanediol dihydrobromide (16.9 g) was treated with the theoretical amount of sodium methoxide in methanol (100 ml) and left overnight. Acetone (100 ml) was added and, after filtration from NaBr, the solvents were removed under reduced pressure. The residue was extracted ($CHCl_3$, 50 ml) and filtered. After evaporation, the resulting DL-1,4-bis(dimethylamino)-2,3-butanediol (9.3 g) was dissolved in $CHCl_3$ (50 ml) and added dropwise to a mixture of methanesulfonyl chloride (25 ml) and $CHCl_3$ (25 ml) over a period of 30 min, while stirring at 18–25°. After additional stirring at 25–27° for 2 hr, the precipitated dihydrochloride of the starting material was removed by filtration. To the filtrate, diethyl ether (50 ml) was added, and the resulting precipitate was washed with diethyl ether and dried *in vacuo* over P_2O_5 to give 7.6 g of crude material with mp 115–145°. After two recrystallizations from a mixture methanol–ethanol (1:1) the melting point was raised to 173–175.5° dec.

Anal. Calcd for $C_9H_{24}N_2O_6S_2\cdot 2HCl$: C, 29.63; H, 6.47; Cl, 17.49; N, 6.91; S, 15.82. Found: C, 29.87; H, 6.53; Cl, 17.26; N, 6.69; S, 15.95.

Acknowledgment.—The authors are indebted to the Cancer Chemotherapy National Service Center, National Institutes of Health, Bethesda 14, Md., for the screening of the compounds and for making the results available.

(14) Analyses were performed by G. Cornali and W. Egger of these laboratories. Melting points were taken in open glass capillaries and rounded off to half degrees, using a Hershberg apparatus with thermometers subdivided in 0.1°.

(15) In the case of the *meso* isomer it was necessary to extend the reaction time to 6.5 hr.