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# Carcinostatic Sulfonic Acid Esters of Selected 1,4-Diols

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# CARCINOSTATIC SULFONIC ACID ESTERS OF SELECTED 1,4-DIOLS

by

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Robert A. Earl

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# INTRODUCTION

The purpose of this research was to prepare the bis-arylsulfonic acid esters of 1,4-butanediol and 1,4-but-2-ynediol.

This series of compounds is of interest because of the antitumor activity exhibited by the bismethanesulfonates of 1,4-butanediol and 1,4-but-2-ynediol. The compounds which were prepared were submitted to Cancer Chemotherapy National Service Center for evaluation in their anticancer program.

It was hoped that the compounds synthesized would be of pharmacological value. It was further hoped that the biological test data of these compounds would demonstrate a correlation between types of substituents on the aromatic nucleus and the degree of activity of the compounds. It was also hoped that biological test results would shed some light on the mechanism of action of the bissulfonic acid esters.

# HISTORICAL REVIEW

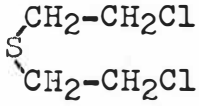
## Alkylating Agents

In 1898 Paul Ehrlich[1] presented a manuscript which seems to be the first recognition of the unique biological properties of ethylene oxide and ethyleneimine. He concluded from the biological evidence that these compounds directly attacked and reacted with the protoplasm of the effected tissue. These conclusions of Ehrlich have since been verified many times. Compounds which exhibit this type of activity have been classified as alkylating agents, because they actually do attach an alkyl function to the center of their attack.

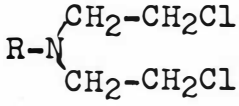
It was not until the years just after World War II that publications began to appear telling of the use of alkylating agents in the treatment of certain neoplasms of lymphoid character. In 1946 Gilman and Phillips[2] presented a summary of the wartime work on the biological actions and therapeutic applications of the β-chloroethyl amines and β-chloroethyl sulfides. The authors pointed out the similarity between the cellular effects caused by the alkylating agents and those brought on by treatment with x-rays. They suggested that by careful alteration of the parent compounds one might be able to produce compounds of much greater therapeutic value.

It was this report which spurred the tremendous

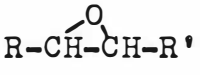
interest in alkylating agents as anticancer compounds. Today a number of the different types of compounds which are being used in cancer chemotherapy are alkylating agents. Some of the compounds which are classified as alkylating agents are: The nitrogen and sulfur mustards, epoxides, ethyleneimines, β-lactones and methanesulfonates.



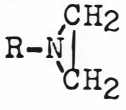
Sulfur Mustard



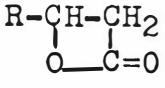
Nitrogen Mustards



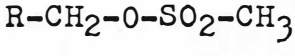
Epoxides



Ethyleneimines



β-lactones



Methanesulfonates

R and R' may be alkyl or aryl

## Carcinostatic Activity of Myleran

Sulfonic acid esters have been found to be important anticancer compounds. The sulfonic acid esters are like the nitrogen and sulfur mustards in that they are alkylating agents.

One sulfonic acid ester in particular has been shown to be an important therapeutic agent. This compound is known by the trivial names of myleran and busulfan. Timmis and Haddow[3] found that myleran could be used ef-

fectively in the management of granulocytic leukemia. The growth inhibiting action of myleran was established by other workers using different tumor systems. This ester

$$H_3C\text{-}SO_2\text{-}O\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}OSO_2\text{-}CH_3$$

MYLERAN
(methane sulfonic acid, tetramethylene ester)

was found to retard the growth of an adenocarcinoma 755, glioma 26, and a Brown-Pierce carcinoma[4]. Koller[5] did extensive work with aliphatic sulfonic acid esters and studied their effects on the Walker carcinoma. He found that myleran was the most active ester of those which he tested.

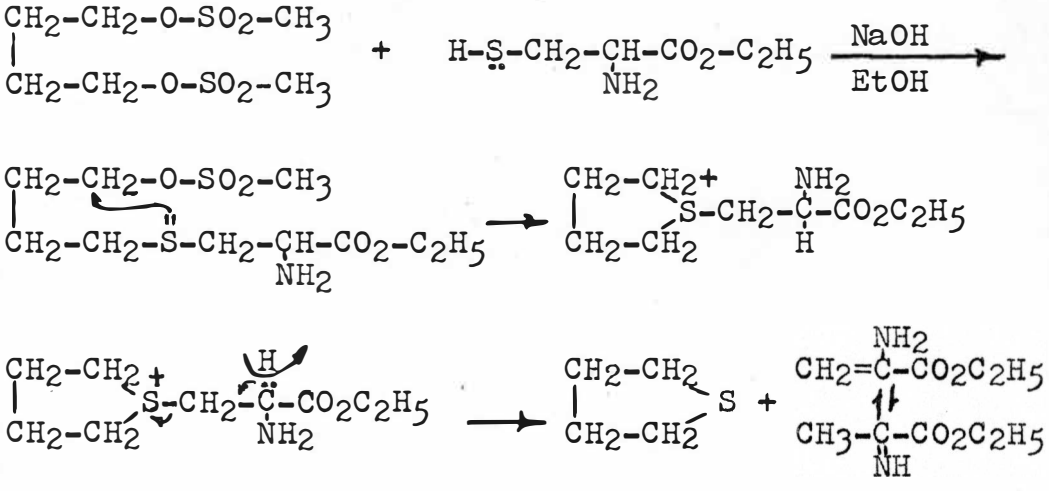
## Mechanism of Action of Bisalkylating Agents

The discovery that myleran was effective as an anti-cancer drug resulted in many investigations of the properties of this compound. One question of particular interest is that of the mechanism of action of the drug.

The chemist is particularily interested in the mechanism of action of a drug, such as myleran. With such knowledge he can attempt to design new compounds. These compounds, though closely related to the original, may exhibit much greater activity in the system of interest.

Parham and Wilbur[6] studied the action of myleran in an <u>in vitro</u> system, and Roberts and Warwick[7] studied the action of myleran in an <u>in vivo</u> system. These studies indicated that the mechanism of action of myleran is the

same in both systems. Parham and Wilbur[6] reacted myleran with the ethyl ester of cysteine and obtained the expected bisalkylated derivative and also some tetrahydrothiophene. They suggested that the tetrahydrothiophene might have been synthesized through the following reaction sequence.



They depicted the nucleophilic attack of myleran by the sulfur of cysteine, to give a sulfide. This sulfide can then undergo rapid intramolecular attack of the sulfur portion of the sulfide to give a sulfonium ion. Extraction of a proton from the carbon bearing the amino group will result in the neutralization of the charge on the sulfur and removal of the side chain to give tetrahydrothiophene.

They suggested that this "sulfur-stripping" reaction may be responsible for the physiological activity of the bisalkylating agents in cancer chemotherapy. More recent work by Parham and Wilbur[8] involving the reaction of myleran with various mercaptans, gave results quite analogous to those obtained from the reaction of myleran with the ethyl

ester of cysteine.

Roberts and Warwick[7] in an independent study involving the reaction of myleran and cysteine, observed this sulfur-elimination reaction. Subsequent studies[9], in which carbon atoms of the alcohol portion of myleran were labeled with carbon 14, have shown that myleran is metabolized in the mouse and excreted as 3-hydroxytetrahydrothiophene-1, 1-dioxide.

Other workers have attempted to determine the center of attack of myleran and other alkylating agents. Ogston[10] determined the relative rate at which certain nucleophiles will react with an alkylating agent. He termed this rate the "competition factor". He went further and determined what he called the effective competition factor for various nucleophiles. The effective competition factor takes into account the relative amount of the nucleophile present in the system. Ross[11] compared the work of Ogston with that of others and concluded that the ionized acids, the free sulfhydryl and free amino groups were the most likely centers for reaction with alkylating agents in biological systems. Ross and Hendry[12] have tested compounds which have higher reactivity toward amino groups than to acidic groups. However, these compounds did not demonstrate appreciable inhibitory action toward the Walker rat carcinoma. Similarly, compounds more reactive toward sulfhydryl groups than acidic groups have shown

low activity against the Walker rat carcinoma. By the process of elimination Ross has suggested that biological alkylating agents produce their antitumor effects by esterifying ionized acid groups. Stacey and co-workers[16] did extensive work which supported Ross's conclusion. They found that the ionized acids are more reactive than amines and thiols toward the alkylating agents.

Other workers[13, 14, 15] have shown that DNA and RNA are likely centers for reaction with the alkylating agents. In vitro experiments of Stacey and co-workers[16] gave strong evidence that DNA in the presence of amines is preferentially attacked by alkylating agents. They also demonstrated that reaction of nitrogen mustard with DNA is confined almost entirely to esterification of the phosphate. They showed experimentally that crosslinking between nucleophilic centers occurs when polyfunctional alkylating agents are used. They found that intramolecular crosslinking occurred when the concentration of macromolecules was small compared to that of the alkylating agent. At high concentrations of the macromolecule intermolecular crosslinking was prevalent. They concluded that the mere formation of esters is not a very effective method of inducing radiometric effects. Polyfunctional alkylating agents produce cross-linking which completely alters the character of the molecule and prevents it from exercising its normal biological function. This effect they concluded, probably explains the much greater biological activity of poly-

functional alkylating agents over that of the monofunctional compounds.

Methods of Preparation of Sulfonic Acid Esters

Sulfonic acid esters were of interest prior to the discovery of their therapeutic value. The sulfonoxy group is easily displaced by inorganic nucleophiles. For this reason the carbohydrate chemist has used the sulfonic acid esters as a means of getting to various derivatives[17].

$$\text{Sugar-OSO}_2\text{-R'} + :\text{N} \rightleftharpoons \text{Sugar:N} + \text{R'-SO}_3\text{H}$$

Numerous experimenters have reported the preparation of sulfonic acid esters. One of the first practical methods was reported by Hinsberg[18]. His method was an adaption of the method used by Schotten[19] for the benzoylation of amines and Baumann[20] for the benzoylation of alcohols. This method involved the shaking of the sulfonyl chloride with a solution or suspension of the alcohol in sufficient concentrated alkali to neutralize the hydrochloric acid formed.

Methods[21, 22] have been reported which do not use a base to remove the hydrogen chloride. These methods have only limited practicality.

The most commonly used procedures involve the use of an organic base to remove the hydogen chloride as it forms. Some of the bases which have been used are pyridine[23, 24], quinoline[25] and diethylaniline[24, 26, 27].

Marvel and Sekera[28] developed a procedure which used pyridine as a base. His method was used for many years by other investigators. In this method one equivalent of the alcohol and the sulfonyl chloride was stirred for four hours at 0°C in the presence of two equivalents of pyridine. The product was isolated by extraction with ethyl ether. This method was used for the preparation of the methanesulfonates of low molecular weight alcohols.

Earlier workers[29] had noted that sulfonic acid esters tend to react with pyridine and form pyridinium salts. Marvel and Sekera[28] tried to minimize quaternary salt formation by using low reaction temperatures.


For polyvinyl sulfonic acid esters[30], the relative rate of quaternary salt formation with pyridine is benzenesulfonate > p-toluenesulfonate > methanesulfonate. If the nucleophilic center of the tertiary base is sterically hindered then the tendency toward quaternary salt formation with sulfonic acid esters is decreased[31]. The tendency to quaternize with sulfonic acid esters decreases in the order 2-methylpyridine > 2,4-dimethylpyridine > 2,6-dimethylpyridine (2,6-lutidine).

Tipson[32] prepared a number of esters of p-toluenesulfonic acid. He had to modify the procedure reported by Marvel and Sekera[28] in order to obtain maximum yields. In order to maximize yields he varied the following con-

ditions: the ratio of sulfonyl chloride to alcohol; the ratio of sulfonyl chloride to pyridine; the temperature and duration of the reaction. He emphasized that the pyridine and alcohol must be dry because sulfonyl chlorides are repidly hydrolyzed by moist pyridine. For compounds which are susceptible to side reactions, temperatures of 0°C or lower were recommended. A constant ratio of 25 grams of sulfonyl chloride to 100 ml. pyridine, and a ten percent excess of sulfonyl chloride to alcohol was used. Short reaction times of fifteen minutes to two hours proved to be most favorable for high yields. In isolating the ester, water was added in small portions with cooling and agitation of the mixture; this was followed by addition of a large volume of water to hydrolyze the sulfonyl chloride. If the ester did not crystallize at this stage it was extracted from the water with chloroform.

Eglington and Whitling[33] prepared a series of esters of monofunctional acetylenic alcohols in high yield. Their procedure was similar to that reported by Tipson[32] except they allowed the reaction mixture to warm up to room temperature and set for 18 hours before isolating the ester. When they tried the same procedure with 1,4-but-2-ynediol they could not isolate the ester. Instead, they obtained a water soluble oil which they suspected to be a quaternary salt of pyridine. They succeeded in preparing the p-toluenesulfonate of 1,4-but-2-ynediol when they followed the procedure of Schlichting and

Klager[34]. In this method the sulfonyl chloride and alcohol was dissolved in acetonitrile at 10°-20°C, then an equivalent of a saturated aqueous solution of potassium hydroxide was added; the temperature was not allowed to rise above 20°C during the addition. After the reaction had subsided the mixture was stirred without cooling for 18 hours. Water was then added and the mixture extracted with ethyl ether to isolate the ester.

Recent workers[35] have demonstrated the importance of the time element in the preparation of sulfonic acid esters using pyridine as a base. If the reaction time was either greater or less than 30 minutes the percent yield dropped off rapidly.

Ross and Davis[36] prepared an ester of 2,4-dinitrobenzenesulfonic acid. This ester hydrolyzed so rapidly that good yields could be obtained only when temperatures of -40°C were used and 2,6-lutidine was used instead of pyridine.

Emmons and Ferris[37] prepared a series of alkyl sulfonates by reacting silver methanesulfonate with alkyl halides in acetonitrile for one hour at room temperature. Elderfield and co-workers[38] prepared a sulfonic acid ester analogue of a nitrogen mustard by means of a silver salt. He heated the silver methanesulfonate and nitrogen mustard in acetonitrile for 67 hours and obtained the ester in a high yield.

## EXPERIMENTAL

Infrared spectra were measured with a Beckman I.R.-8 spectrophotometer. Elemental analyses were performed by Galbraith Microanalytical Laboratories, Knoxville, Tennessee. All analytical samples were dried *in vacuo* over phosphorus pentoxide for two days prior to sending them in for analysis. All melting points and boiling points are expressed in degrees centigrade and are uncorrected. Chemicals used in this investigation were used as they were obtained unless otherwise noted.

Pyridine and 2,6-lutidine were dried by storing over potassium hydroxide pellets.

SILVER 2,5-DICHLOROBENZENESULFONATE (I) was prepared by heating to boiling a mixture of 10.51 g. (0.04 mole) of 2,5-dichlorobenzenesulfonic acid and 5.52 g. (0.02 mole) of silver carbonate in 50 ml. of 95% ethanol. The mixture was heated under reflux for 20 min. The mixture was then allowed to stand overnight at room temperature. The solution was filtered, and the solid remaining on the filter was washed with a small amount of boiling water. The alcohol and water washings were combined and concentrated *in vacuo* to give 10.4 g. (77%) of a white solid.

SILVER 4-NITROBENZENESULFONATE (II) was prepared by the addition of a solution of 8.50 g. (0.05 mole) of silver nitrate in a small amount of water to a stirred

solution of 10.15 g. (0.05 mole) of p-nitrobenzenesulfonic acid in a small amount of 50% ethanol. A yellow precipitate quickly formed which was collected by vacuum filtration. The dried solid weighed 4.19 g. (31%).

2,5-DIBROMOBENZENESULFONYL CHLORIDE (III) was prepared by a previously described method[39]. From 107.8 g. (0.0458 mole) of p-dibromobenzene was obtained an impure mass of brown crystals of 2,5-dibromobenzenesulfonyl chloride with a m.p. of 60-70°. The crude material was decolorized with Norite and recrystallized once from acetone to give 60.7 g. (40%) of white platelets, m.p. 70-72° (lit.[40] m.p. 70-71°).

1,4-DIIODOBUTANE (IV) was prepared from 18 g. (0.025 mole) of tetrahydrofuran by a previously described method[41]. The crude material was distilled to give 75 g. (97%) of product, b.p. 116-116.5° (10 mm.), $n_D^{20°}$ 1.6227, (lit.[41] $n_D^{20°}$ 1.615). The material slowly darkened, but the index of refraction did not change appreciably during one year of storage.

Preparation of the Bisaryl Esters of 1,4-Butanediol

Using pyridine as a base

TETRAMETHYLENE 2,5-DICHLOROBENZENESULFONATE (V) was prepared by adding, dropwise, a solution of 2.25 g. (0.025 mole) of 1,4-butanediol in 50 ml. of dry pyridine to a well stirred solution of 12.25 g. (0.05 mole) of 2,5-

dichlorobenzenesulfonyl chloride in 100 ml. of dry pyridine at -40°. Addition required 30 min. After addition was completed the reaction mixture was transferred to the refrigerator (-20°). After standing overnight in the refrigerator the reaction mixture was poured with stirring over 800 g. of crushed ice, to which 27 ml. of concentrated sulfuric acid had been previously added. The mixture was allowed to warm up to slightly below room temperature during which time a white precipitate formed. The solid was collected by vacuum filtration, washed with three 20 ml. portions of ice-water and dried on the filter. The yield of product was 3.54 g. (27.8%). A sample submitted for analysis was recrystallized twice from acetone and afforded white needles, m.p. 158-159°. $\nu_{max.}$(nujol) 1650, 1620, 1355, 1180, 1140, 1100, 1060, 1040, 1020, 1010, 925, 900, 850 and 825 $cm.^{-1}$

Anal. Calcd. for $C_{16}H_{14}Cl_4O_6S_2$: C, 37.81; H, 2.78. Found: C, 37.96; H, 2.81.

TETRAMETHYLENE 2,4-DIMETHYLBENZENESULFONATE (VI) was prepared by adding, dropwise, a solution of 2.25 g. (0.025 mole) of 1,4-butanediol in 25 ml. of dry pyridine to a mechanically stirred solution of 10.23 g. (0.05 mole) of 2,4-dimethylbenzenesulfonyl chloride in 50 ml. of dry pyridine at -40°. Addition required 30 min. After addition was completed the reaction mixture was allowed to warm up to 0°. The reaction mixture was then poured with stirring over 400 g. of crushed ice, to which 13.5 ml.

of concentrated sulfuric acid had been previously added. The mixture was stirred vigorously and a light yellow precipitate separated out of solution. The solid was collected by vacuum filtration, washed with three 20 ml. portions of ice-water and dried on the filter. The yield of product was 7.79 g. (73.5%). A sample submitted for analysis was recrystallized twice from an acetone-ethyl ether mixture (8:1) and afforded white needles, m.p. 125.2-125.8°. $\nu_{max.}$ (KBr) 1605, 1575, 1345, 1180, 1150, 1060, 1025, 925, 840 and 820 $cm.^{-1}$

Anal. Calcd. for $C_{20}H_{26}O_{16}S_2$: C, 56.31; H, 6.14. Found: C, 56.20; H, 6.22.

TETRAMETHYLENE 2,5-DIMETHYLBENZENESULFONATE (VII) was prepared by adding, dropwise, a solution of 9.01 g. (0.10 mole) of 1,4-butanediol in 50 ml. of dry pyridine to a well stirred solution of 40.9 g. (0.20 mole) of 2,5-dimethylbenzenesulfonyl chloride in 100 ml. of dry pyridine at -40°. Addition required 30 min. The reaction mixture was then stirred for 1 hr. at -40° followed by pouring with stirring over 400 g. of crushed ice, to which 27 ml. of concentrated sulfuric acid had been previously added. A white precipitate quickly formed. The solid was collected by vacuum filtration, washed with three 20 ml. portions of ice-water and dried on the filter. The yield of crude product was 41.7 g. (98%). A sample submitted for analysis was recrystallized twice from acetone and gave white

needles, m.p. 97.5-98.° $\nu_{max.}$ (nujol) 1560, 1340, 1210, 1170, 1060, 1035, 925, 880 and 800 cm.$^{-1}$

Anal. Calcd. for $C_{20}H_{26}O_6S_2$: C, 56.31; H, 6.14. Found: C, 56.45; H, 6.20.

TETRAMETHYLENE 3,4-DICHLOROBENZENESULFONATE (VIII) was prepared by adding, dropwise, a solution of 2.25 g. (0.025 mole)of 1,4-butanediol in 25 ml. of dry pyridine to a well stirred solution of 12.25 g. (0.05 mole) of 3,4-dichlorobenzenesulfonyl chloride in 50 ml. of dry pyridine at -40°. Addition required approximately 30 min. The reaction mixture was then transferred to the refrigerator (-20°). After standing overnight in the refrigerator the reaction mixture was poured with stirring over 400 g. of crushed ice, to which 15 ml. of concentrated sulfuric acid had been previously added. A strawberry colored precipitate quickly formed. The solid was collected by vacuum filtration, washed with three 20 ml. portions of ice-water and dried on the filter. The yield of product was 8 g. (64%). A sample submitted for analysis was recrystallized three times from acetone and gave a white powder, m.p. 136-137°. $\nu_{max.}$ (nujol) 1375, 1360, 1200, 1180, 1100, 1040, 1020, 925, 900 and 825 cm.$^{-1}$

Anal. Calcd. for $C_{16}H_{14}Cl_4O_6S_2$: C, 37.81; H, 2.78. Found: C, 37.77; H, 2.77.

TETRAMETHYLENE 4-NITROBENZENESULFONATE (IX) was prepared by adding, dropwise, a solution of 2.25 g. (0.025

mole) of 1,4-butanediol in 25 ml. of dry pyridine to a well stirred solution of 11.05 g. (0.05 mole) of p-nitrobenzenesulfonyl chloride in 50 ml. of dry pyridine at -40°. Addition required approximately 30 min. After addition was completed the reaction mixture was stirred for an additional hour at -40°. Then the reaction mixture was transferred to the refrigerator (-20°). After standing overnight in the refrigerator the reaction mixture was poured with stirring over 400 g. of crushed ice, to which 15 ml. of concentrated sulfuric acid had been previously added. A white powder precipitated from solution. The solid was collected by vacuum filtration, washed with three 20 ml. portions of ice-water and dried on the filter. The yield of product was 3.14 g. (27%). The solid was recrystallized once from boiling acetone to give a white powder, m.p. 175-176° (dec.).

Using 2,6-lutidine as a base

TETRAMETHYLENE 2,5-DIBROMOBENZENESULFONATE (X) was prepared by adding, dropwise, a solution of 2.25 g. (0.025 mole) of 1,4-butanediol in 25 ml. of dry 2,6-lutidine to a well stirred solution of 16.73 g. (0.05 mole) of 2,5-dibromobenzenesulfonyl chloride (III) in 50 ml. of dry 2,6-lutidine at -15°. Addition required approximately 1 hr. During that time the temperature was adjusted so that the mixture formed a paste. During the addition the reaction mixture developed a yellow color. After

addition was completed the mixture was allowed to warm up to -10° and then was quickly transferred to the refrigerator (-20°). After standing overnight in the refrigerator the reaction mixture was poured with stirring over 200 g. of crushed ice, to which 15 ml. of concentrated sulfuric acid had been previously added. The ester soon precipitated from solution. The ester was collected by vacuum filtration, washed with three 20 ml. portions of ice-water and dried on the filter. The yield of product was 12.1 g. (71%) of a white powder. A sample submitted for analysis was recrystallized three times from an acetone-water mixture (3:1) and afforded white platelets, m.p. 173.5-175°. $\nu_{max.}$ (nujol) 1370, 1220, 1170, 1055, 1025, 935, 880 and 820 $cm.^{-1}$

Anal. Calcd. for $C_{16}H_{14}Br_4O_6S_2$: C, 28.00; H, 2.06. Found: C, 28.10; H, 2.17.

TETRAMETHYLENE 3,4-DICHLOROBENZENESULFONATE (VIII) was obtained from 12.25 g. (0.05 mole) of 3,4-dichlorobenzenesulfonyl chloride and 2.25 g. (0.025 mole) of 1,4-butanediol by a procedure analogous to that used in the preparation of X. The crude product weighed 10.1 g. (79%) and was a pink powder. After one recrystallization from acetone the ester had a m.p. of 136-137° (white needles).

TETRAMETHYLENE 4-NITROBENZENESULFONATE (IX) was obtained from 11.05 g. (0.05 mole) of p-nitrobenzenesulfonyl chloride and 2.25 g. (0.025 mole) of 1,4-butanediol

by a procedure analogous to that used in the preparation of X. A yellow powder weighing 8.9 g. (77%) was recovered from the reaction mixture. A sample submitted for analysis was recrystallized three times from acetone and afforded a white powder, m.p. 184-185°. $\nu_{max.}$ (nujol) 1630, 1620, 1600, 1530, 1400, 1360, 1340, 1230, 1200, 1175, 1115, 1105, 1040, 1030, 1005, 950, 855 and 830 $cm.^{-1}$

Anal. Calcd. for $C_{16}H_{16}N_2O_{10}S_2$: C, 41.51; H, 3.49. Found: C, 41.67; H, 3.62.

From the silver arylsulfonates

TETRAMETHYLENE 4-NITROBENZENESULFONATE (IX) was prepared by heating to reflux temperature a mixture of 7.45 g. (0.024 mole) of silver p-nitrobenzenesulfonate and 3.73 g. (0.012 mole) of 1,4-diiodobutane in 50 ml. of acetonitrile. The reaction mixture was protected from light. After a few minutes of heating a yellow precipitate started to form. The solution was heated under reflux for 2.5 hrs. and then filtered while hot. A white precipitate separated from solution when the acetonitrile was cooled to 0°. The solid from the reaction mixture was extracted four times with 50 ml. portions of hot (80°) acetonitrile, which when cooled yielded more white powder. A total of 3.49 g. (63%) of white powder was collected, m.p. 180-186° (dec.). The infrared spectra of this material was identical with that of a sample of V prepared by reaction of p-nitrobenzenesulfonyl chloride with 1,4-butanediol

in the presence of pyridine.

TETRAMETHYLENE 2,5-DICHLOROBENZENESULFONATE (V) was prepared by heating to reflux temperature a mixture of 8.05 g. (0.025 mole) silver 2,5-dichlorobenzenesulfonate and 3.10 g. (0.01 mole) of 1,4-diiodobutane in 50 ml. of benzene. The reaction mixture was heated under reflux for 2 hrs. during which time a yellow precipitate formed. At the end of the 2 hrs. the reaction mixture was concentrated in vacuo until only a solid remained. The solid was extracted with boiling chloroform. The addition of a small amount of petroleum ether (30-60° boiling range) to the hot chloroform followed by slow cooling to 0° gave 1.81 g. (37%) of light brown crystals, m.p. 141-153°. The infrared spectra of this material was identical with that of a sample of IV prepared by the reaction of 2,5-dichlorobenzenesulfonyl chloride with 1,4-butanediol in the presence of 2,6-lutidine.

Preparation of the Bisaryl Esters of 1,4-But-2-ynediol

Using long reaction times at room temperature

BUT-2-YNE-1,4-DIYL 2,5-DIMETHYLBENZENESULFONATE (XI) was prepared by adding in approximately eight portions, a cooled (0°) solution of 6 g. (0.101 mole) of potassium hydroxide dissolved in 10 ml. water to a stirred solution of 20.4 g. (0.1 mole) of 2,5-dimethylbenzenesulfonyl chloride and 4.3 g. (0.05 mole) of 1,4-but-2-ynediol in 40 ml. of acetonitrile at 10-20°. Addition required ap-

proximately 30 min. After addition was completed, and the reaction had subsided, the reaction mixture was allowed to warm up to room temperature. Stirring was continued at room temperature for 18 hrs. During the course of the reaction a thick precipitate formed which made stirring difficult. The solid was collected by vacuum filtration, washed with three 20 ml. portions of ice-water and dried on the filter. The yield of solid was 12.51 g. Extraction of the solid with boiling acetone yielded 7.3 g. (29%) of impure ester. A sample submitted for analysis was recrystallized three times from an acetone-water mixture (3:2) and afforded white needles, m.p. 104-105°. $\nu_{max.}$ (nujol) 1345, 1205, 1220, 1170, 1133, 1060, 950, 885 and 820 $cm.^{-1}$

Anal. Calcd. for $C_{20}H_{22}O_6S_2$: C, 56.85; H, 5.25. Found: C, 56.91; H, 5.25.

BUT-2-YNE-1,4-DIYL 2,5-DICHLOROBENZENESULFONATE (XII) was obtained from 24.5 g. (0.1 mole) of 2,5-dichlorobenzenesulfonyl chloride and 4.3 g. (0.05 mole) of 1,4-but-2-ynediol by a procedure analogous to that used in the preparation of XI. A white solid weighing 23.3 g. was recovered from the reaction mixture. Extraction of the solid with boiling acetone yielded 10.35 g. (42%) of impure ester. A sample submitted for analysis was recrystallized four times from acetone and afforded white needles, m.p. 136-137.5°. $\nu_{max.}$ (nujol) 1385, 1380,

1180, 1140, 1100, 1040, 950, 830 and 820 cm.$^{-1}$

Anal. Calcd. for $C_{16}H_{10}Cl_4O_6S_2$: C, 38.11; H, 1.999. Found: C, 37.94; H, 2.01.

BUT-2-YNE-1,4-DIYL 4-NITROBENZENESULFONATE (XIII) was obtained from 22.2 g. (0.1 mole) of p-nitrobenzenesulfonyl chloride and 4.3 g. (0.05 mole) of 1,4-but-2-ynediol by a procedure analogous to that used in the preparation of XI. A light yellow solid weighing 19.9 g. was recovered from the reaction mixture. Extraction of the solid with boiling acetone yielded 7.48 g. (33%) of ester. A sample submitted for analysis was recrystallized three times from boiling acetone and afforded white platelets, m.p. 178-180° (dec.). $\nu_{max.}$ (nujol) 1600, 1530, 1375, 1340, 1105, 1010, 995 and 935 cm.$^{-1}$

Anal. Calcd. for $C_{16}H_{12}N_2O_{10}S_2$: C, 42.10; H, 2.65. Found: C, 42.31; H, 2.84.

BUT-2-YNE-1,4-DIYL 2,4-DIMETHYLBENZENESULFONATE (XIV) was obtained from 20.4 g. (0.1 mole) of 2,4-dimethylbenzenesulfonyl chloride and 4.3 g. (0.05 mole) of 1,4-but-2-ynediol by a procedure analogous to that used in the preparation of XI. A white solid weighing 21.05 g. was recovered from the reaction mixture. Extraction of the solid with boiling acetone yielded 7.65 g. (36%) of impure ester. A sample submitted for analysis was recrystallized three times from acetone and afforded white platelets, m.p. 105-107°. $\nu_{max.}$ (nujol) 1600, 1380, 1360, 1230, 1180, 1160, 1130, 1050, 1030, 955 and 825 cm.$^{-1}$

Anal. Calcd. for $C_{20}H_{22}O_6S_2$: C, 56.85; H, 5.25. Found: C, 57.03; H, 5.19.

3-NITRO-4-CHLOROBENZENESULFONYL CHLORIDE, AND 3-NITROBENZENESULFONYL CHLORIDE were reacted with 1,4-but-2-ynediol in the same proportions, and by a procedure analogous to that used in the preparation of XI. The only materials obtained from the reactions were high yields of acetone insoluble salts.

Using short reaction time at 0°

BUT-2-YNE-1,4-DIYL 4-BROMOBENZENESULFONATE (XV) was prepared by adding, dropwise, a cooled solution (0°) of 3 g. (0.055 mole) of potassium hydroxide in 5 ml. of water to a mechanically stirred solution of 12.78 g. (0.05 mole) of p-bromobenzenesulfonyl chloride and 2.15 g. (0.025 mole) of 1,4-but-2-ynediol in 20 ml. of acetonitrile at 0°. Addition required approximately .5 hrs. The reaction mixture was stirred for an additional 1.5 hrs. at 0°. During the reaction the mixture became filled with a solid. The solid was collected by vacuum filtration, washed with three 20 ml. portions of ice-water and dried on the filter. The yield of impure ester was 5.86 g. (45.9%). A sample submitted for analysis was recrystallized three times from acetone and afforded white needles, m.p. 137-138°. $\nu_{max.}$ (nujol) 1560, 1375, 1180, 1160, 1085, 1065, 1005, 955, 830 and 813 $cm.^{-1}$

Anal. Calcd. for $C_{16}H_{12}Br_2O_6S_2$: C, 36.65; H, 2.09.

Found: C, 36.58; H, 2.18.

BUT-2-YNE-1,4-DIYL 3,4-DICHLOROBENZENESULFONATE (XVI) was obtained from 12.25 g. (0.05 mole) of 3,4-dichlorobenzenesulfonyl chloride and 2.15 g. (0.025 mole) of 1,4-but-2-ynediol by a procedure analogous to that used in the preparation of XV. The reaction mixture was stirred for a total of 5 hrs. at 0° and yielded 10.12 g. (81%) of a white solid. A sample submitted for analysis was recrystallized two times from petroleum ether (60-110° boiling range) and afforded white platelets, m.p. 105-107°. $\nu_{max.}$ (nujol) 1550, 1380, 1180, 1140, 995, 945 and 845 $cm.^{-1}$

Anal. Calcd. for $C_{16}H_{10}Cl_4O_6S_2$: C, 38.11; H, 1.999. Found: C, 38.30; H, 2.07.

BUT-2-YNE-1,4-DIYL 2,5-DICHLOROBENZENESULFONATE (XII) was obtained from 24.5 g. (0.1 mole) of 2,5-dichlorobenzenesulfonyl chloride and 4.3 g. (0.05 mole) of 1,4-but-2-ynediol by a procedure analogous to that used in the preparation of XV. The reaction mixture was stirred for a total of 4.5 hrs. at 0° and yielded 20.19 g. (81%) of a white solid, m.p. 136-139°.

BUT-2-YNE-1,4-DIYL 2,5-DIBROMOBENZENESULFONATE (XVII) was obtained from 16.3 g. (0.05 mole) of 2,5-dibromobenzenesulfonyl chloride and 2.15 g. (0.025 mole) of 1,4-but-2-ynediol by a procedure analogous to that used in the preparation of XV except that the total reaction time was 4.5 hrs. A white powder weighing 13.5 g. (81%), m.p. 114-118°, was collected from the reaction mixture. A sample

submitted for analysis was recrystallized three times from acetone and afforded white platelets, m.p. 125.5-127°. $\nu_{max.}$ (nujol) 1365, 1350, 1170, 1135, 1100, 1080, 1020, 950, 885 and 820 cm.$^{-1}$

Anal. Calcd. for $C_{16}H_{10}Br_4O_6S_2$: C, 28.19; H, 1.46. Found: C, 28.33; H, 1.49.

BUT-2-YNE-1,4-DIYL 4-CHLORO-3-NITROBENZENESULFONATE (XVIII) was obtained from 12.8 g. (0.05 mole) of 4-chloro-3-nitrobenzenesulfonyl chloride and 2.15 g. (0.025 mole) of 1,4-but-2-ynediol by a procedure analogous to that used in the preparation of XV except for the following modifications. After addition was completed the reaction was continued for one hour. The mixture was then filtered to give a small quantity of solid which was soluble in water. The filtered reaction mixture was quickly returned to the reaction flask to be stirred for an additional 4 hrs. at 0°. The solid that formed during the 4 hrs. of stirring was collected by vacuum filtration, washed with three 20 ml. portions of ice-water and dried on the filter. The dried solid weighed 5 g. (38.9%). A sample submitted for analysis was recrystallized five times from acetone and afforded a white powder, m.p. 116-117°. $\nu_{max.}$ (nujol) 1580, 1540, 1365, 1360, 1185, 1150, 1050, 995, 940, 890 and 830 cm.$^{-1}$

Anal. Calcd. for $C_{16}H_{10}Cl_2N_2O_{10}S_2$: C, 36.52; H, 2.06. Found: C, 36.70; H, 2.08.

BUT-2-YNE-1,4-DIYL THIOPHENE-2-SULFONATE (XIX) was

obtained from 9.13 g. (0.05 mole) of thiophene-2-sulfonyl chloride and 2.15 g. (0.025 mole) of 1,4-but-2-ynediol by a procedure analogous to that used in the preparation of XV with the following modifications. After 2 hrs. of reaction no solid had formed so the mixture was allowed to warm up to room temperature followed by quick chilling to 0°. The solution was stirred at 0° for an additional 2 hrs. and a solid soon precipitated. The solid was collected in the usual manner and weighed 3.6 g. When the acetonitrile was diluted with ice-water more solid appeared which when dried weighed 2.6 g. The combined material weighed 6.2 g. (66%). A sample submitted for analysis was recrystallized four times from an acetone-water mixture (3:2) and afforded white platelets, m.p. 96-97°. $\nu_{max.}$ (nujol) 1395, 1360, 1340, 1220, 1165, 1140, 1090, 1065, 1020, 950, 940, 850 and 820 $cm.^{-1}$

Anal. Calcd. for $C_{12}H_{10}O_6S_4$: C, 37.10; H, 2.65. Found: C, 37.22; H, 2.73.

BUT-2-YNE-1,4-DIYL 3-NITROBENZENESULFONATE (XX) was obtained from 11.08 g. (0.05 mole) of m-nitrobenzenesulfonyl chloride and 2.15 g. (0.025 mole) of 1,4-but-2-ynediol by a procedure analogous to that used in the preparation of XV. A white powder weighing 2.61 g. (23%) was recovered from the reaction mixture. A sample submitted for analysis was recrystallized four times from acetone and afforded a white powder, m.p. 127-128.5°. $\nu_{max.}$ (nujol) 1600, 1520, 1500, 1375, 1340, 1175, 1150, 1080, 1075, 935,

880 and 780 cm.$^{-1}$

Anal. Calcd. for $C_{16}H_{12}N_2O_{10}S_2$: C, 42.10; H, 2.65. Found: C, 41.93; H, 2.49.

Summary of Anticancer Screening Experiment[42]

Sulfonic acid esters of 1,4-butanediol

NSC No. Compound Name

69929 Tetramethylene 2,5-dimethylbenzenesulfonate

69930 Tetramethylene 2,4-dimethylbenzenesulfonate

70889 Tetramethylene 3,4-dichlorobenzenesulfonate

70890 Tetramethylene 3-nitrobenzenesulfonate

| NSC No. | Dose mg/kg. | No. of Survivors | Animal Wt. dif. T-C | Tumor wt. or survival days test/control | State index or %T/C |
|---|---|---|---|---|---|
| | | | ADENOCARCINOMA 755 | | |
| 69929 | 175 | 10/10 | -1.5 | 1038/1054 | 98 |
| 69930 | 175 | 10/10 | +0.5 | 1416/1054 | 134 |
| | | | LYMPHOID LEUKEMIA L-1210 | | |
| 69929 | 175 | 6/6 | -1.5 | 8.1/8.6 | 94 |
| 69930 | 175 | 6/6 | 0.0 | 8.1/8.5 | 95 |
| 70889 | 400 | 6/6 | -0.6 | 8.5/9.1 | 93 |
| 70890 | 400 | 6/6 | -0.2 | 8.3/9.1 | 91 |
| | | | SARCOMA 180 | | |
| 69929 | 250 | 5/6 | -0.6 | 2320/2019 | 114 |
| 69930 | 250 | 5/6 | -0.8 | 2055/2019 | 101 |
| 70889 | 500 | 6/6 | -0.3 | 1143/913 | 125 |
| 70890 | 500 | 6/6 | -1.8 | 900/913 | 98 |

| NSC No. | Dose mg/kg. | No. of Survivors | Animal wt. dif. T-C | Tumor wt. or Survival days test/control | Stage index or %T/C |
|---|---|---|---|---|---|
| | | | SOLID FRIEND VIRUS LEUKEMIA | | |
| 70889 | 400 | 10/10 | +0.1 | 908/1021 | 88 |
| 70890 | 400 | 10/10 | -1.4 | 987/1021 | 96 |

Sulfonic acid esters of 1,4-but-2-ynediol

| NSC No. | Compound Name |
|---|---|
| 79198 | But-2-yne-1,4-diyl 2,5-dichlorobenzenesulfonate |
| 79199 | But-2-yne-1,4-diyl 2,4-dimethylbenzenesulfonate |
| 79527 | But-2-yne-1,4-diyl 2,5-dimethylbenzenesulfonate |
| 79533 | But-2-yne-1,4-diyl 4-nitrobenzenesulfonate |
| 79537 | But-2-yne-1,4-diyl 3,4-dichlorobenzenesulfonate |
| 84134 | But-2-yne-1,4-diyl 3-nitrobenzenesulfonate |
| 84135 | But-2-yne-1,4-diyl 3-nitro-4-chlorobenzenesulfonate |
| 84136 | But-2-yne-1,4-diyl 4-bromobenzenesulfonate |
| 87444 | But-2-yne-1,4-diyl thiophene-2-sulfonate |
| 87455 | But-2-yne-1,4-diyl 2,5-dibromobenzenesulfonate |

| NSC No. | Dose mg./kg. | No. of Survivors | Animal wt. dif. T-C | Tumor wt. or survival days test/control | Stage index or %T/C |
|---|---|---|---|---|---|
| | | | LEWIS LUNG CANCER | | |
| 79198 | 200 | 6/6 | -2.5 | 446/1235 | 36 |
| 79198 | 200 | 1/6 | -6.2 | /1266 | - |
| 79198 | 100 | 6/6 | -3.3 | 443/652 | 67 |
| 79199 | 400 | 6/6 | -1.4 | 698/927 | 75 |
| | | | LYMPHOID LEUKEMIA L-1210 | | |
| 79198 | 200 | 6/6 | -1.1 | 8.5/8.9 | 95 |
| 79199 | 400 | 6/6 | 0.0 | 9.1/9.1 | 100 |

| NSC No. | Dose mg./kg. | No. of Survivors | Animal wt. dif. T-C | Tumor wt. or survival days test/control | Stage index or %T/C |
|---|---|---|---|---|---|
| | | | LYMPHOID LEUKEMIA L-1210 | | |
| 79527 | 500 | 6/6 | +1.0 | 9.0/9.3 | 96 |
| 79533 | 500 | 6/6 | -3.3 | 9.5/9.7 | 97 |
| 79537 | 500 | 3/6 | -2.7 | 10.7/9.4 | - |
| 79537 | 300 | 5/6 | -0.5 | 8.2/9.3 | 88 |
| | | | SARCOMA 180 | | |
| 79198 | 500 | 5/6 | -1.6 | 576/2237 | 25 |
| 79198 | 250 | 6/6 | -3.7 | 300/1297 | 23 |
| 79198 | 250 | 6/6 | -4.8 | 385/685 | 56 |
| 79198 | 250 | 0/6 | - | - | - |
| 79198 | 250 | 6/6 | -3.8 | 923/1844 | 50 |
| 79198 | 250 | 6/6 | -5.0 | 912/1690 | 53 |
| 79198 | 250 | 6/6 | -6.8 | 535/1441 | 37 |
| 79199 | 500 | 6/6 | -2.9 | 1620/2237 | 72 |
| 79527 | 500 | 5/6 | +1.7 | 1101/1615 | 68 |
| 79537 | 31 | 6/6 | -0.8 | 937/1152 | 81 |
| 79533 | 500 | 5/6 | -0.7 | 1270/1615 | 78 |
| 79537 | 125 | 0/6 | - | - | - |
| | | | WALKER 256 | | |
| 79527 | 500 | 6/6 | -9.0 | 4.7/5.1 | 92 |
| 79533 | 500 | 5/6 | -13.0 | 2.2/5.1 | 43 |
| 79537 | 500 | 5/6 | -23.0 | .6/5.3 | 11 |

## EVALUATION OF RESULTS

The reaction of an arylsulfonyl chloride with 1,4-butanediol in the presence of an organic base proved to be the most useful method for the preparation of the bisaryl-sulfonates of 1,4-butanediol.

Initially the reactions were run at 0° for eighteen hours using pyridine as a base. The esters were isolated by pouring the reaction mixtures over crushed ice. However, only the ester from 2,4-dimethylbenzenesulfonyl chloride could be prepared in this manner. The ester of 2,5-dimethyl-benzenesulfonic acid was successfully prepared when the same procedure was followed using 2,6-lutidine as the base instead of pyridine. It was found that by lowering the temperature of the reaction mixture to -40°, fair yields of various esters could be obtained using pyridine both as catalyst and solvent. It was also found that long reaction times resulted in lowered yields. It was further observed that the base must be neutralized during the isolation of the ester in order to minimize loss of ester through hydrolysis. Esters with electron withdrawing groups on the benzene ring were difficult to prepare.

When 2,6-lutidine was used as the base in place of pyridine the yields were greatly improved, and the reactions proceeded smoothly at temperatures of about -15°. The usual procedure was to add a solution of the alcohol in

2,6-lutidine to a solution of the sulfonyl chloride in 2,6-lutidine which had been previously cooled to its freezing point. During the addition the temperature of the reaction mixture was adjusted so that the mixture had the consistancy of a paste. The mixture was stirred for one hour at this temperature and then was placed in the refrigerator for eighteen hours at -20°. The percent yields of the esters remained high when the reactions were run for eighteen hours. The formation of pyridinium salts probably accounted for the low yields observed when reactions utilizing pyridine as a base were run for this length of time.

The products obtained from these reactions were in the form of acetone soluble, lightly colored powders. Filtration was often difficult due to the fineness of the material. It was found that the material must be washed with ice-water in order to remove any residual acid. Failure to do so in one case resulted in decomposition of the ester during storage. Analytical samples of the esters were easily prepared by recrystalization from acetone or an acetone-water mixture.

Three absorption bands in the infrared region were observed for all of the esters. These absorptions occurred in the following regions: 1340-1370 ($-SO_2-$), 1165-1180 ($-SO_2-$) and 925-955 ($cm^{-1}$.) (R-O-S). The two $-SO_2-$ absorptions were sharp and intense. The band in the region 925-955 was intense but generally quite wide. This absorption showed up when the samples were analyzed both in the

form of a nujol mull or as potassium bromide pellets. This absorption was not assigned to carbon-carbon stretching because the infrared spectra of carbamates of 1,4-butanediol failed to show this absorption. It was further noted that this absorption occurred in the infrared spectra of the bisarylsulfonates of 1,4-but-2-ynediol.

Esters of 1,4-butanediol were also prepared by reaction of the silver salts of arylsulfonic acids with 1,4-diiodobutane. This method of preparation was not very satisfactory because the yields of esters were usually low.

The aryl esters of 1,4-but-2-ynediol were prepared by a procedure similar to that described by Eglington and Whitling[33]. In this procedure a saturated solution of potassium hydroxide was added to a stirred and cooled solution of the sulfonyl chloride and alcohol in acetonitrile. Large quantities of water soluble salts were obtained in addition to the ester, when the reactions were run at room temperature for eighteen hours. In some cases no ester was obtained. Hydrolysis of the ester, once formed, would account for the formation of the salts.

When the reactions were run at $0^{o}$ for two to four hours the yields of the esters were greatly improved, and little if any acetone insoluble material was obtained.

Isolation of the bisarylsulfonates of 1,4-but-2-ynediol was accomplished by vacuum filtration of the reaction mixture. The solid thus obtained was washed with ice-water so

as to remove any residual base. When the reactions had been run at room temperature for eighteen hours it was necessary to isolate the esters from contaminating salts by extraction of the crude material with acetone. The esters were easily prepared for analysis by recrystallization from acetone or an acetone-water solution.

The bismethanesulfonate of 1,4-but-2-ynediol, previously reported by others[5], could not be prepared by this procedure. This bismethanesulfonate was successfully prepared on several occasions in 40-70% yields by reaction of methanesulfonyl chloride with 1,4-but-2-ynediol in the presence of pyridine at -40°. Short reaction time of about two hours gave maximum yields of this ester. An attempt to prepare the ester from 2,5-dimethylbenzenesulfonyl chloride and 1,4-but-2-ynediol, using the same procedure, was unsuccessful. Also the esters from ethane and n-butanesulfonyl chlorides could not be prepared by this procedure. It would be of interest to study these reactions using 2,6-lutidine as a base in place of pyridine.

The infrared spectra of the bisarylsulfonates of 1,4-but-2-ynediol had the same pattern of absorptions as those of 1,4-butanediol. No carbon-carbon triple bond absorption was observed, because of the symmetry of the molecules.

Not all of the biological test data for these compounds had been received at the time of this writing. Some interesting results have been observed, however. None of

the bisaryl esters of 1,4-butanediol have shown activity in the four systems that they were tested in. The bisarylsulfonates of 1,4-but-2-ynediol have shown some antitumor activity in these systems. The esters of 1,4-butanediol might be expected to be active due to the possible existance of a cyclic sulfur elimination reaction as has been reported in the case of myleran[7, 8, 9]. The esters of 1,4-but-2-ynediol could not form a cyclic structure of this sort because of the strain that would be introduced by the triple bond of the molecule. The results of the biological testing indicate that the mechanism of action of the 1,4-bisarylsulfonates is not the same as the mechanism postulated for myleran.

## SUMMARY

Bisarylsulfonates of 1,4-butanediol have been prepared. Three methods of preparation of these esters have been utilized. The reaction between an arylsulfonyl chloride and 1,4-butanediol in the presence of pyridine was favored by low temperatures. The yields of esters were greatly improved when 2,6-lutidine was used as a base instead of pyridine. The best yields were obtained when the initial phase of the reaction was run at a temperature near the freezing point of the 2,6-lutidine. The esters were easily hydrolyzed by aqueous base. In order to minimize the loss of esters due to hydrolysis the bases were neutralized with sulfuric acid during the isolation of the esters.

Reaction of the silver arylsulfonates with 1,4-diiodobutane in refluxing benzene or refluxing acetonitrile gave low yields of the esters.

Bisarylsulfonates of 1,4-but-2-ynediol have been prepared. The esters were prepared by reacting the arylsulfonyl chloride with 1,4-but-2-ynediol in the presence of an equivilent of potassium hydroxide. Slow addition of the base to a well stirred acetonitrile solution of the sulfonyl chloride and alcohol at 0° followed by stirring at 0° for two to four hours gave maximum yields of the esters.

The esters that were prepared during this investigation were sent to the Cancer Chemotherapy National Service Center for evaluation in their anticancer program. A summary of the biological test data for those compounds that have been evaluated is presented in this paper.

The esters of 1,4-butanediol have shown little activity in four test systems. Some of the esters of 1,4-but-2-ynediol have shown activity in each of the four systems that they were tested. The results of the biological testing indicate that the 1,4-bisarylsulfonates do not have the same mechanism of action as that displayed by myleran.

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# VITA

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APPROVAL OF THE EXAMINING COMMITTEE

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(Chairman)

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Date _____