mole) of bromoacetyl bromide, under nitrogen, was added 5.0 g (0.034 mole) of 1,8-octanediol over 25 min. The mixture was stirred at room temperature 2.5 hr. Ether was then added and the solution was treated with excess solid K_2CO_3. The mixture was then filtered, the filtrate was washed with 5% K_2CO_3 and dried, and the ether was removed *in vacuo* to yield 10.14 g (77%) of solid, mp 33–35°, R_f ($CHCl_3$) 0.49 (silica gel G).

Method B. O,O′-Bis(iodoacetyl)-1,8-octamethylenediol (7).—A solution of 5.0 g (0.013 mole) of **6** in 80 ml of acetone was added to a solution of 15.8 g (0.105 mole) of NaI in 160 ml of acetone. After addition was complete, the reaction mixture was refluxed on a steam bath for 2 hr. The mixture was then cooled, ice was added, and the crystalline product was collected by filtration. An additional portion of product was obtained *via* ether extraction of the filtrate to yield a combined total of 5.52 g (87%) of **7**, mp 25.5–26.5°; R_f ($CHCl_3$) 0.55 (silica gel G).

Method C. O,O′-Bis(aziridinylacetyl)-1,6-hexamethylenediol (4).—In a thoroughly dried flask under a N_2 atmosphere was placed 2.32 g (0.0168 mile) of K_2CO_3 and 1.0 g (0.0028 mole) of **2** in 40 ml of tetrahydrofuran (THF). This mixture was cooled to 0° and treated with a solution of 0.72 g (0.017 mole) of aziridine over a 5-min period. The reaction mixture was stirred at 0° for 3 hr then allowed to warm to room temperature and stirred for 15 hr. Ether was then added, the mixture was filtered, and the solvent was removed from the filtrate *in vacuo*, keeping the temperature below 40°, to yield a liquid which later solidified. Crystallization from petroleum ether (bp 30–60°)–ether gave 0.081 g (11%) of product, mp 58–62, which partly decomposed to a polymeric mass over a period of several days. An analytical sample was prepared by formation of the chloroethylamine hydrochloride.

The aziridine **4** (90 mg) was dissolved in 10 ml of ethanol. The solution was then cooled to 0° and saturated with HCl. After removal of the solvent, the white residue was crystallized from ethanol–ether, mp 170–174°.

Method D. O,O′-Bis(acrylyl)-1,6-hexanediol (19).—In a dried flask under a N_2 atmosphere was placed 5.9 g (0.05 mole) of 1,6-hexanediol dissolved in 200 ml of THF and 27.6 g (0.2 mole) of K_2CO_3. The mixture was cooled to 0° and 13.6 g (0.15 mole) of acrylyl chloride was added. The reaction was allowed to warm to room temperature and stirred for 62 hr. The mixture was then filtered and the solvent was removed from the filtrate. The residue was washed well with ether and filtered again, and the ether was removed from the combined organic solution to yield 6.9 g of crude product. This material was chromatographed on silica gel using 4:1 petroleum ether–ether elution. Three grams (27%) of clear, colorless product was obtained; infrared λ_{max}^{neat} (μ) 5.80 (C=O, ester), 6.1 and 6.2 doublet (C=C, acrylyl).

Method E. O,O′-Bis(aziridinylpropionyl)-1,6-hexanediol (5).—Compound **19** (0.75 g, 0.0032 mole) dissolved in 10 ml of THF was placed in a dry flask under N_2. Aziridine (0.916 g, 0.021 mole) was slowly added and the reaction mixture was stirred for 62 hr. Aliquots were periodically taken and the infrared spectrum was checked for the presence of remaining double-bond absorption. The solvent was then removed *in vacuo*, keeping the temperature below 40°, to yield a solid, 0.88 g (90%), mp 30–34°, R_f ($CHCl_3$) 0.67 (alumina). An analytical sample was prepared by twice recrystallizing from pentane; mp 34–36°. The nmr and infrared spectra were in agreement with the assigned structure.

Method F. O-(Aziridinylacetyl)hexanol (16).—Compound **14** (9.7 g, 0.044 mole) was added over 10 min to a stirred solution of 3.18 g (0.074 mole) of aziridine in 16 ml of triethylamine cooled to 0°. The reaction was held at 0° for 50 hr. The copious precipitate of triethylamine salts was then filtered off and washed with ether. After removal of the ether *in vacuo* the remnant was distilled, bp 71–80° (0.15 mm), to yield 4.8 g (60%) of colorless product. This procedure is similar to that of Bestian.[2]

Tsou, Hoegerle, and Su[5] have discussed the infrared spectral characteristics of some aziridyl derivatives related to those prepared in this paper. We are in agreement with their findings that a weak peak or shoulder from about 3.30–3.35 μ is characteristic of these adducts.

The nmr spectra of the various series were also definitive for structure identification. The triplet from the alcohol methylene next to oxygen at 4.0–4.2 ppm was chosen as the basis for integral determinations. The acetyl methylene was characteristically a singlet at 2.9–3.1 ppm. The two propionyl methylenes from the acrylyl adducts overlapped to form a poorly defined triplet at 2.4–2.5 ppm. All of the aziridinyl adducts possessed sharp multiplets (*ca.* 1.7 and 1.1 ppm) up- and downfield from the remainder of the methylene absorption. Each multiplet contained about half of the integral area required for the four aziridinyl methylene protons.

Acknowledgment.—This work was supported by U. S. Public Health Service Grant GM-11491. We wish to thank V. Tovar for assistance with the biological studies.

(5) K. C Tsou, K. Hoegerle, and H. C. F. Su, *J. Med. Chem.*, **6**, 435 (1963).

Nitrogen Mustard Type Derivatives of Thiophene and Benzo[*b*]thiophene

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This paper reports the synthesis for anticancer evaluation of a series of "nitrogen mustard" derivatives (I) of thiophene and benzo[*b*]thiophene; specific cases

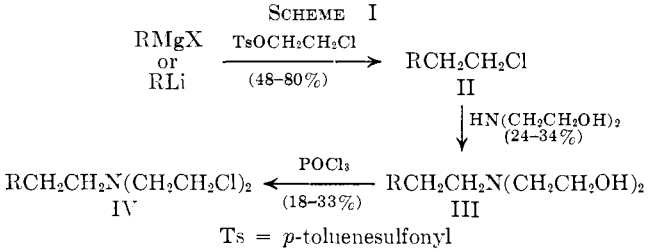
$$\underset{\text{I}}{RCH_2CH_2N(CH_2CH_2Cl)_2}$$

include those in which R = 2-thienyl, 3-thienyl, 2-benzo[*b*]thienyl, and 3-benzo[*b*]thienyl.

The only prior report of nitrogen mustards related to the above types describes[1] the synthesis of N,N-bis(2-chloroethyl)-2-thienylamine. Similar derivatives of furan and tetrahydrofuran were reported by Landing, *et al.*,[2] and of thiazole by Mikhailov, *et al.*[3]

The synthetic route employed for our synthesis is indicated in Scheme I. Yield ranges for the four

SCHEME I



Ts = *p*-toluenesulfonyl

examples of R are given in parentheses along the arrows. Samples of IV for biological testing were converted to the hydrochloride salts in 60–85% yields.

In the formation of II in the cases in which R = 2-thienyl and 2-benzo[*b*]thienyl, it was planned to use the organolithium reagents (RLi) readily formed in high yield by metalation of the parent heterocycle with *n*-butyllithium.[4] This was a satisfactory route for formation of 2-(2-chloroethyl)benzo[*b*]thiophene; however, 2-thienyllithium and 2-chloroethyl *p*-toluenesulfonate gave as the only major product 2-thienyl *p*-tolyl sulfone. No 2-(2-chloroethyl)thiophene could be isolated from this system even though several variations of

(1) E. Wilson and M. Tishler, *J. Am. Chem. Soc.*, **73**, 3635 (1951).

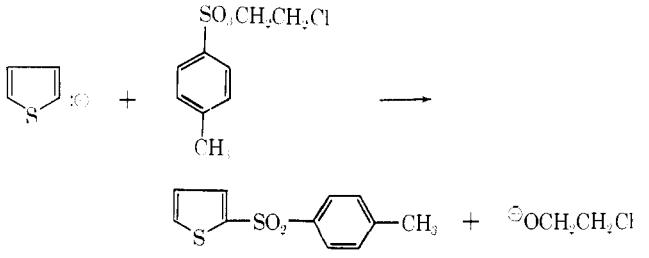
(2) B. H. Landing, A. Goldin, H. A. Noe, B. Goldberg, and D. M. Shapiro, *Cancer*, **2**, 1055 (1949).

(3) B. M. Mikhailov, V. P. Bronovitskaya, and J. K. Platova, *Zh. Obshch. Khim.*, **26**, 3445 (1956); *Chem. Abstr.*, **51**, 9588 (1957).

(4) (a) H. Gilman and D. A. Shirley, *J. Am. Chem. Soc.*, **71**, 1870 (1949); (b) D. A. Shirley and M. D. Cameron, *ibid.*, **72**, 2788 (1950).

reaction conditions were tried. 2-Thienylmagnesium bromide gave the expected 2-(2-chloroethyl)thiophene as did 2-benzo[*b*]thienyllithium. Formation of sulfones RSO_2R' has occasionally been noted from reactions of $R'MgX$ and RSO_2OR''. Kharasch and Reinmuth[5] point out that tendency to sulfone formation appears to be more marked in the alkanesulfonic esters and in the aryl esters of arylsulfonic acids. Apparently only quite low yields of sulfones from alkyl arylsulfonates have been observed.

It seems likely that sulfones arise *via* nucleophilic attack on the sulfur atom of the ester by a carbanion or incipient carbanion from the organometallic agent. The greater tendency of RLi types to provide $R:^-$



compared with RMgX may explain why 2-thienyllithium, but not the Grignard reagent, gave sulfone. Surprising, however, is the failure of the organolithium to yield sulfone in the closely related 2-benzo[*b*]thienyl case.

The nitrogen mustard hydrochloride salts (IV) were submitted to the Cancer Chemotherapy National Service Center for biological evaluation. Toxicity tests were performed in rats by intraperitoneal daily injections in dose levels of 3.0–100 mg/kg. Three animals were used in each of four dose levels and injections were continued for 5 days. All test animals survived for 10 days in the tests with each of the four compounds corresponding to IV. Tests were performed, using standard screening procedures,[6] with the four compounds against Walker carcinosarcoma 256 at dose levels up through 200 mg/kg/day. None of the compounds showed significant activity. The compounds were also inactive in tests against a cell culture of human epidermoid carcinoma of the nasopharynx.

Experimental Section[7]

3-(2-Chloroethyl)thiophene.—2,3,4,5-Tetraiodothiophene was prepared according to Steinkopf and Hanske[8] and this compound was reduced to 3-iodothiophene[9] with zinc amalgam.

3-Thienylmagnesium iodide was prepared in the normal manner from 0.61 g (0.025 g-atom) of Mg turnings, 30 ml of ether, and 5.25 g (0.025 mole) of 3-iodothiophene. To the solution of the Grignard reagent was added 17.6 g (0.075 mole) of 2-chloroethyl *p*-toluenesulfonate in 50 ml of ether over a 25-min period. The mixture was stirred for 3 hr at room temperature, then poured into a slurry of ice and water, and acidified with HCl. The ether layer was separated and the aqueous layer was extracted with ether. The combined extracts were dried and distilled to yield 2.93 g (80%) of colorless liquid, bp 92–93° (7 mm).

Anal. Calcd for C_6H_7ClS: C, 49.30; H, 4.83. Found: C, 49.22, 49.40; H, 5.12, 5.19.

3-{2-[Bis(2-chloroethyl)amino]ethyl}thiophene.—A mixture of 14.7 g (0.1 mole) of 3-(2-chloroethyl)thiophene, 210 g (2.0 moles) of diethanolamine, and 100 ml of 2-butanol was stirred at a temperature of 100–105° for 45 hr. The yellow solution was allowed to cool to room temperature, after which water was added. The product was removed from the aqueous suspension by extracting several times with 50-ml portions of ether. The combined ether extracts were evaporated to remove all ether yielding 7 g (32%) of 3-{2-[bis(2-hydroxyethyl)amino]ethyl}thiophene as a dark brown oil.

$POCl_3$ (25 ml) was added to 10.8 g (0.050 mole) of the hydroxy compound in 30 ml of benzene which had been cooled in an ice bath. After the mixture had become homogeneous, the dark solution was heated on the steam bath for 70 min. After removal of excess $POCl_3$ under reduced pressure, the tarry mass which remained was dissolved in acetone, and the solution was poured over crushed ice. The acetone–water mixture was neutralized with aqueous Na_2CO_3 and then extracted several times with 20-ml portions of chloroform. The combined $CHCl_3$ extracts were washed with water, evaporated to a small volume, and chromatographed on a neutral Woelm alumina column. Benzene eluted the product in the first fraction. The yield was 2.26 g (18% based on the hydroxy compound) of a pale yellow liquid which failed to crystallize.

A solution of 2.00 g (0.0080 mole) of the amine in the minimum quantity of absolute ethanol was treated with dry HCl. The volume of the solution was reduced by evaporation, after which ether was added until a permanent cloudiness was detected. The hydrochloride was allowed to crystallize slowly from the ether–ethanol mixture in the cold room. A yield of 1.66 g (84%) was obtained. The product melted at 200–201°.

Anal. Calcd for $C_{10}H_{16}Cl_3NS$: C, 41.88; H, 5.59; N, 4.86. Found: C, 41.81, 41.94; H, 5.61, 5.57; N, 4.88, 4.90.

2-(2-Chloroethyl)benzo[*b*]thiophene.—Benzo[*b*]thiophene (26.8 g, 0.20 mole) was metalated with 0.20 mole of *n*-butyllithium (Foote Mineral Co. product in pentane–hexane) in 120 ml of ether.[10] To the resulting solution was added (55 min) at ice-bath temperature a solution of 141 g (0.60 mole) of 2-chloroethyl *p*-toluenesulfonate in 100 ml of ether. The reaction mixture was stirred for 3 hr at room temperature, excess water was added, the ether layer was separated, and the water layer was extracted with ether. The combined ether solutions were dried and distilled to yield 18.9 g (48%) of colorless product, bp 113–114° (6 mm). Komppa[10] reported bp 110–112° (4 mm).

2-{2-[Bis(2-chloroethyl)amino]ethyl}benzo[*b*]thiophene.—Reaction of 2-chloroethylbenzo[*b*]thiophene with diethanolamine was in similar fashion as described above. The product, formed in 34% yield, was treated with $POCl_3$ also as described above to give 27% of the nitrogen mustard as a colorless oil. This was converted to the **hydrochloride**, mp 228–229°, in 62% yield.

Anal. Calcd for $C_{14}H_{18}Cl_3NS$: C, 49.63; H, 5.35; N, 4.14. Found: C, 49.61, 49.68; H, 5.21, 5.37; N, 4.07, 4.12.

3-{2-[Bis(2-chloroethyl)amino]ethyl}benzo[*b*]thiophene.—3-Bromobenzo[*b*]thiophene[11] was converted to the Grignard reagent in the usual manner and reaction with 2-chloroethyl *p*-toluenesulfonate was carried out as described above to yield 3-(2-chloroethyl)benzo[*b*]thiophene (67% based on 3-bromobenzo[*b*]thiophene). Conversion to the nitrogen mustard occurred as described above with a 30% yield of the hydroxyethyl intermediate and a 33% yield of the nitrogen mustard, mp 84.5°.

Anal. Calcd for $C_{14}H_{17}Cl_2NS$: C, 55.60; H, 5.67; N, 4.64. Found: C, 55.66, 55.58; H, 5.41, 5.67; N, 4.60, 4.72.

2-{2-[Bis(2-chloroethyl)amino]ethyl}thiophene.—2-(2-Chloroethyl)thiophene was prepared from equimolar amounts of 2-thienylmagnesium bromide and 2-chloroethyl *p*-toluenesulfonate essentially according to Blicke and Leonard.[12] The yield was 47% but was increased to 78% by using a 3:1 *M* ratio of sulfonate ester to Grignard reagent. Reaction with diethanolamine occurred in 24% yield and conversion to the nitrogen mustard in 27% yield. Procedures used were similar to those described in detail above. The **hydrochloride** of the nitrogen mustard melted at 193–194° and was formed in 75% yield.

Anal. Calcd for $C_{10}H_{16}Cl_3NS$: C, 41.88; H, 5.59; N, 4.86. Found: C, 41.76, 41.84; H, 5.43, 5.69; N, 4.76, 4.84.

(5) M. S. Kharasch and O. Reinmuth, "Grignard Reactions of Nonmetallic Substances," Prentice-Hall, Inc., New York, N. Y., 1954, p 1284.

(6) Cancer Chemotherapy National Service Center "Protocol for Screening Chemical Agents and Natural Products Against Animal Tumors and Other Biological Systems," *Cancer Chemotherapy Rept.*, **7**, 11, 17 (1962).

(7) Elemental microanalyses were by Weiler and Strauss, Oxford, England. Melting points were taken on a Mel-Temp apparatus. All reactions involving organometallic reagents were performed in a dry nitrogen atmosphere.

(8) W. Steinkopf and W. Hanske, *Ann.*, **527**, 247 (1937).

(9) J. T. Rinkes, *Rec. Trav. Chim.*, **53**, 648 (1934); **55**, 991 (1936).

(10) G. Komppa, *J. Prakt. Chem.*, **122**, 319 (1929).

(11) J. Szmuszkovicz and E. J. Modest, *J. Am. Chem. Soc.*, **72**, 574 (1950).

(12) F. F. Blicke and F. Leonard, *ibid.*, **68**, 1934 (1946).

2-Thienyl *p*-Tolyl Sulfone.—2-Lithiothiophene was formed by metalation of thiophene with *n*-butyllithium.[4a] The yield has been shown to be essentially quantitative.[13] A solution of 2-lithiothiophene from 8.4 g (0.10 mole) of thiophene in ether–pentane–hexane solvent was maintained at room temperature during the addition (50 min) of 70.4 g (0.30 mole) of 2-chloroethyl *p*-toluenesulfonate in 85 ml of ether. The mixture was stirred for 3 hr at room temperature, hydrolyzed with excess water, and extracted with ether in the usual fashion. Distillation of the extracts gave unreacted 2-chloroethyl *p*-toluenesulfonate and thiophene from unreacted 2-lithiothiophene as the only distillable products. The residual red oil was crystallized from acetone with charcoal treatment to yield 8.1 g (34%) of colorless crystals, mp 120–121°.

Anal. Calcd for $C_{11}H_{10}O_2S_2$: C, 55.40; H, 4.24; S, 26.90. Found: C, 55.36, 55.51; H, 4.20, 4.36; S, 26.71, 26.88.

The infrared spectrum (KBr disk) of the sulfone showed strong bands at approximately 7.6 and 8.7 μ which may be assigned[14] to the sulfone group.

Acknowledgment.—The authors would like to express appreciation to the National Cancer Institute for pharmacological testing and for partial financial support under Public Health Service Research Grant No. CA-04068 from the National Cancer Institute.

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(14) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1958, pp 360–361.

Studies on Methylglyoxal Bis(guanylhydrazone)[1] Analogs. V. Methylglyoxal Guanylhydrazone Thiosemicarbazones and Related Compounds[2]

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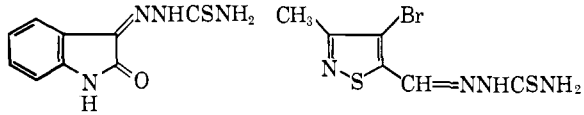
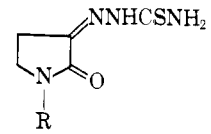
Derivatives of benzaldehyde thiosemicarbazone have been reported to be active tuberculostatic[3] and antiviral[4] agents. Additional work on thiosemicarbazone compounds led to the discovery of antiviral activity of isatin 3-thiosemicarbazone (I) (ITSC) against the pox group of viruses in human and type 2 polio in ERK cells.[5] A number of monocyclic thiosemicarbazones, such as derivatives of nicotinaldehyde, isonicotinaldehyde, and 2- and 3-thenaldehydes, have also shown high antiviral activity.[6] 4-Bromo-3-methylisothiazole-5-carboxaldehyde thiosemicarbazone (II), when given orally, was found to protect mice infected intracerebrally with neurovaccinia.[5] Two thiosemicarbazones derived from substituted pyrrolidine-2,3-diones (III, R = C_2H_5 and CH_2CH_2OH) demonstrated protection against experimental influenza infection in mice.[5]



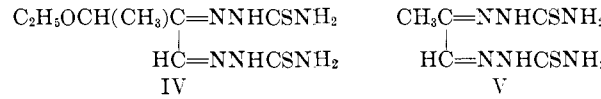
I

II

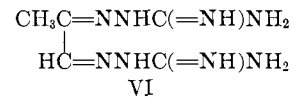


III

2-Keto-3-ethoxybutyraldehyde (kethoxal) has been shown to have antiviral activity in embryonated eggs infected with PR-8 influenza and Newcastle disease.[7] Its bis(thiosemicarbazone) derivative (IV, KTS) was



IV V



VI

found to be very effective when given orally or intraperitoneally to rats bearing the Walker 256 carcinosarcoma and S180.[8] This activity pattern is similar to a closely related compound, methylglyoxal bis(thiosemicarbazone)[9] (V), but *different* from that of the guanylhydrazone analog (VI). The latter compound, the structural modifications of which have been systematically studied in our laboratories for the past 3 years,[10] was found to be active in leukemia L1210 and Ca755 and is considered as one of the few agents clinically effective in the therapy of adult acute myelogenous leukemia.[11]

In view of the antituberculous, antiviral, and antileukemic as well as other antitumor activities demonstrated by the thiosemicarbazone and guanylhydrazone derivatives, and the importance of the methylglyoxal moiety in cell growth,[12] synthesis and biological evaluation of compounds VII and VIII are of pertinent interest.[13]

(1) According to *Chemical Abstracts*, the name for this compound is 1,1'-[(methyl)ethanediylidenedinitrilo]diguanidine.

(2) This investigation was supported by the Cancer Chemotherapy National Service Center (CCNSC), National Cancer Institute, National Institutes of Health, Public Health Service, Contract PH-43-65-94.

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(4) D. Hamre, J. Bernstein, and R. Donovick, *Proc. Soc. Exptl. Biol. Med.*, **73**, 275 (1950).

(5) *Cf.* H. E. Whipple, Ed., *Ann. N. Y. Acad. Sci.*, **130**, 71 (1965).

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(11) (a) B. L. Freedlander and F. A. French, *Cancer Res.*, **18**, 360 (1958); (b) E. J. Freireich and E. Frei, III, *Proc. Am. Assoc. Cancer Res.*, **3**, 319 (1962); (c) E. J. Freireich, E. Frei, III, and M. Karon, *Cancer Chemotherapy Rept.*, **No. 16**, 183 (1962).

(12) A. Szent-Györgyi [*Science*, **149**, 34 (1965)] recently stated that substances which promote ("promine") or retard ("retine") cell growth may provide keys to fundamental problems of cellular biology. Further study with the isolated "retine" indicated that methylglyoxal, combined in some fashion with a nitrogen-containing moiety, is the chief constituent.

(13) According to Thompson, *et al.*,[6] most thiosemicarbazones possessing high antiviral activity are those in which the thiosemicarbazone group, $=NNHCSNH_2$, is separated by *two* carbon atoms from a N or S atom. This statement has since been substantiated by Furst[14] based on chelation studies. Our structure–activity study of the guanylhydrazone derivatives in antileukemic screenings indicated that a similar relationship is also one of the fundamental requirements in the $=NNHC(=NH)NH_2$ series.

(14) A. Furst, "Chemistry of Chelation in Cancer," Charles C Thomas, Springfield, Ill., 1963, p 67.