TABLE II

PHARMACOLOGICAL SCREENING RESULTS

Compd	Approx LD_{50} (mouse), mg/kg ip	Analgesic act. (mouse) Increase of reaction time, %[a]	mg/kg ip	Antiinflammatory activity (rat) Inhibition of edema, %[b]	mg/kg ip	Antipyretic act. (rat) Max temp decrease, °C[c]	mg/kg ip	Diuretic activity (rat) Test vol[c]/control vol	mg/kg po	Choleretic activity (rat) Increase of bile flow, %[d] 1 hr	2 hr	mg/kg[e] id	Hypoglycemic activity (rat) Blood sugar decrease, %[f]	mg/kg po
I	1120–1230	14	200	30	200	2.2	200	Inactive	50	32	18	73	Inactive	100
II	560–630	21	100	13	100	1.4	100	1.15	50					
III	1090–1210	37	100	13	100	2.2	100	Inactive	50				14	50
IV	>800	59	200	25	200	2.2	200	1.29	50					
V	540–610	54	200	17	200	2.4	200	1.19	50				Inactive	100
VI	580–620	8	100	22	100	1.4	100	1.15	50				13	100
VII	510–590	38	200	47	200	3.2	200	1.12	50				35	50
VIII	290–320	58	200	37	200	3.1	200	Inactive	50	Inactive	Inactive	80		
IX	550–620	10	200	Inactive	200	3.4	200	Inactive	50				38	50
X	45–70	28	25	26	25	0.8	25	1.30	50	Inactive	36	83	Inactive	100
XI	1100–1260	12	100	50	100	2.9	100	Inactive	50					
XII	570–640	23	200	Inactive	200	2.4	200	1.27	50					
XIII	780–820	34	200	37	200	2.7	200	1.13	50				Inactive	50
XIV	590–650	50	200	30	200	3.3	200	1.14	50				Inactive	100
XV	280–310	14	50	Inactive	50	0.9	50	Inactive	50				Inactive	100
XVI	1140–1220	16	200	45	200	2.1	200	Inactive	50	97	39	83	11	100
XVII	410–450	34	100	Inactive	100	1.3	50	1.18	50	134	131	87		
XVIII	390–410	36	50	38	50	0.7	100	Inactive	50	92	78	90		
XIX	1170–1250	13	100	Inactive	100	1.6	100	Inactive	50					
XX	590–630	28	100	18	100	2.7	100	Inactive	50				12	100
XXI	>1600	9	100	Inactive	100	1.7	100	Inactive	50	28	43	120	Inactive	100
Morphine·HCl		67	5											
Phenylbutazone		61	100	18	100	1.6	100							
Hydrochlorothiazide								1.56	6.25					
Dehydrocholic acid										92	42	100		
Chlorpropamide													37	50

[a] Hot plate test, 1 hr after treatment. [b] Formalin-induced edema, 2 hr after treatment. [c] 5 hr of observation. [d] Values at 1 and 2 hr after treatment. [e] Equimolar doses. [f] Values referred to basal glycemia, 2 hr after treatment.

acetamide (40 g, 0.134 mole) in glacial acetic acid (200 ml). Freshly distilled isoamyl nitrite (50 ml) was then added over 2 hr with stirring. The bright red solution was kept at room temperature for additional 2 hr, and then heated at 100° for 8 hr. The solvent was distilled from the reaction mixture at 50° under reduced pressure, and then ether was added to the residue, giving a solid product which, on crystallization from ethanol–ligroin (bp 75–120°), gave colorless crystals, mp 227–228°.

Pharmacology.—The acute toxicity, and antiinflammatory, analgesic, and diuretic activities were investigated according to the techniques previously described.[1a] The antipyretic action was studied in rats made pyretic by brewer's yeast, according to Smith and Hambourger.[4] The activity on the choleresis was investigated in rats, using the biliary fistula technique of Marazzi-Uberti and Turba.[5] The hypoglycemic action was measured in rats, according to the procedure described by Ceriotti.[6] The antibacterial and antifungal activities were measured against *Micrococcus pyogenes var. aureus* ATCC 6538 P, *Bacillus subtilis* ATCC 6633, *Escherichia coli* McLeod ATCC 10,536, *Salmonella typhi* T 30 Roma M 507, and *Candida albicans* ATCC 10,231, using the serial dilution technique.[7] All compounds were administered as the hydrochlorides in aqueous solution. Morphine, phenylbutazone, hydrochlorothiazide, dehydrocholic acid, and chlorpropamide were used as standards for comparing the analgesic, antiinflammatory–antipyretic, diuretic, choleretic, and hypoglycemic activities, respectively.

Acknowledgments.—The authors thank Mr. O. Boniardi for his assistance in preparing the compounds, Dr. G. Sekules for the microanalyses, and Mrs. G. Pizzamiglio and Mr. E. Pavesi for their cooperation in carrying out the pharmacological tests.

(4) P. K. Smith and W. E. Hambourger, *J. Pharmacol. Exptl. Therap.*, **54**, 346 (1935).

(5) E. Marazzi-Uberti and C. Turba, *Arch. Intern. Pharmacodyn.*, **154**, 297 (1965).

(6) G. Ceriotti, *Clin. Chim. Acta*, **8**, 157 (1963).

(7) G. Coppi, A. Maselli, and C. Ciani-Bonardi, *Farmaco* (Pavia), *Ed. Sci.*, **20**, 203 (1965).

Effect of Organic Compounds on Reproductive Processes. II. Alkylating Agents Derived from Various α,ω-Alkylenediols

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Received January 10, 1966

A program of synthesis of alkylating agents having related carrier moieties but with a variety of alkylating functions has been under way in our laboratories. These compounds are being evaluated for their effect on reproduction in the housefly (*Musca domestia* L.), mice, and Japanese quail. Previous studies have shown that N,N′-bis(aziridinylacetyl)-1,8-octamethylenediamine[1] inhibits reproduction in the housefly at 1 and 0.1% concentration when added to their feed. It was of interest to see whether similar compounds derived from α,ω-alkanediols would also affect their reproduction.

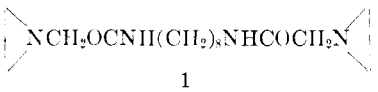
The compounds synthesized (**2**–**21**) are shown in Table I. The bisbromoacetyl esters (**2**, **6**, **10**, and **14**) were best prepared by the addition of diol to chilled bromoacetyl bromide. The iodo derivatives (**3**, **7**, **11**, and **15**) were prepared from the bromo compounds using NaI in acetone. The aziridinyl derivatives (**4**, **8**, and **12**) were derived from the bromo compounds by the addi-

(1) W. A. Skinner, H. C. Tong, T. E. Shellenberger, and G. W. Newell, *J. Med. Chem.*, **8**, 647 (1965).

TABLE I
CHEMICAL DATA

Compd	Method	Method of Purification	Mp or bp (mm), °C	Yield, %	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
2	A	...[a]	Oil	80	$C_{10}H_{16}Br_2O_4$	33.4	4.50		33.6	4.39	
3	B	...[a]	*Ca.* 15	89	$C_{10}H_{16}I_2O_4$	26.5	3.56		26.3	3.64	
4	C	Petroleum ether–ether	58–62	11	$C_{14}H_{24}N_2O_4$						
4·HCl	C	Ethanol–ether	170–174	...	$C_{14}H_{26}Cl_2N_2O_4 \cdot 2HCl$	39.1	6.52	6.52	39.1	6.60	6.64
5	E	Pentane	34–36	88	$C_{16}H_{28}N_2O_4$	61.5	9.03	8.97	61.3	9.13	8.90
6	A	...[a]	33–35	77	$C_{12}H_{20}Br_2O_4$	37.1	5.19		37.2	5.21	
7	B	...[a]	26–27	87	$C_{12}H_{20}I_2O_4$	29.9	4.17		30.1	4.16	
8	C	Hexane	45–55	32	$C_{16}H_{28}N_2O_4$	61.5	9.03	8.97	61.0	9.19	8.81
9	E	...[a]	Oil	99	$C_{15}H_{32}N_2O_4$						
9·HCl	C	Ethanol–ether	155–156	...	$C_{18}H_{34}Cl_2N_2O_4 \cdot 2HCl$	44.5	7.53	5.78	44.2	7.55	5.88
10	A	Chromatography[b]	Oil	66	$C_{14}H_{24}Br_2O_4$	40.4	5.81		40.7	5.96	
11	B	Charcoal	Oil	88	$C_{14}H_{24}I_2O_4$	33.0	4.74		33.1	4.63	
12	C	Petroleum ether–ether	55.5–56.5	24	$C_{18}H_{32}N_2O_4$	63.5	9.47	8.23	63.1	9.82	7.94
13	E	...[a]	Oil	53	$C_{20}H_{36}N_2O_4$						
13·HCl	C	Ethanol–ether	160–163	...	$C_{20}H_{38}Cl_2N_2O_4 \cdot 2HCl$	46.7	7.79	5.45	46.5	7.82	5.36
14[c]	A	Chromatography[d]	54 (0.2)	84	$C_8H_{15}BrO_2$	43.1	6.77		42.9	6.81	
15	B	Charcoal	Oil	70	$C_8H_{15}IO_2$	35.6	5.59		35.5	5.58	
16	F	Distillation	71–80 (0.15)	60	$C_{10}H_{19}NO_2$	64.8	10.3	7.56	64.6	10.4	7.50
17	D	Chromatography[b]	Liquid	27	...[e]						
18	E	Distillation	*Ca.* 100–120(0.3)	51	$C_{11}H_{21}NO_2$	66.3	10.6	7.03	66.3	10.7	6.84
19	D	Chromatography[f]	Oil	27	$C_{12}H_{18}O_4$	63.7	8.02		63.6	8.02	
20	D	Chromatography[f]	Oil	26	$C_{14}H_{22}O_4$	66.1	8.72		66.1	8.83	
21	D	Chromatography[g]	Oil	21	$C_{16}H_{26}O_4$	68.0	9.28		67.9	9.28	

[a] These compounds were obtained in pure state after the standard work-up. Purity was checked by thin layer chromatography, the infrared spectrum, and usually the nmr spectrum. [b] Silica gel; $CHCl_3$ elution. [c] S. I. Gertler, J. Feldmesser, and R. V. Rebois, *J. Agr. Food Chem.*, **6**, 843 (1958). [d] Silica gel; $CHCl_3$ elution. Compound **14** may also be purified by distillation. [e] C. R. Rehberg and C. H. Fisher, *J. Am. Chem. Soc.*, **66**, 1203 (1944). [f] Silica gel; 4:1 petroleum ether–ether elution. [g] Silica gel; $CHCl_3$ elution, or basic alumina; 9:1 petroleum ether–ether elution.



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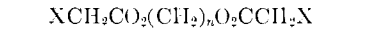
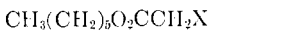
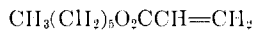


2, $n = 6$; X = Br
3, $n = 6$; X = I
4, $n = 6$; X = 
5, $n = 6$; X = CH_2N 
6, $n = 8$; X = Br
7, $n = 8$; X = I
8, $n = 8$; X = 
9, $n = 8$; X = CH_2N 
10, $n = 10$; X = Br
11, $n = 10$; X = I
12, $n = 10$; X = 
13, $n = 10$; X = CH_2N 



14, X = Br
15, X = I
16, X = 



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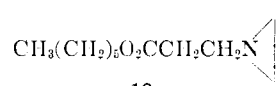

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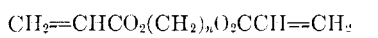

19, $n = 6$
20, $n = 8$
21, $n = 10$

tion of aziridine in tetrahydrofuran to the bromo derivative in the same solvent using potassium carbonate as an acid acceptor. The iodo derivatives gave results in this displacement reaction similar to those obtained with the bromo compounds. The reported yields (Table I) reflect the difficulty encountered in purifying the aziridinyl compounds. The crude yields were generally good (*ca.* 80%), but a good chromatographic procedure was not discovered and considerable loss was encountered owing to the formation of polymer during the recrystallization process. All of these bisaziridinyl derivatives were unstable in solution above 40°. The procedure of Bestian[2] worked well for the monoaziridinyl derivative (**16**), but proved less advantageous in the case of the bisaziridinyl derivatives. The aziridinyl propionates (**5**, **9**, **13**, and **18**) were prepared by the addition of aziridine to the acrylyl esters of the diols in tetrahydrofuran solution and stirring the mixture for several days at room temperature.

Since the completion of this work, the facile addition of aziridine to some monoacrylates, including decyl acrylate, has been reported.[3] We did not attempt this procedure but consider it inapplicable to our compounds as purification by distillation was not possible.

All of the compounds listed in Table I were evaluated as inhibitors of reproduction in our colony of houseflies (*Musca domestica* L.). The method used has previously been described.[1] None of the compounds had a significant effect on either egg production or fertility in the houseflies. In contrast to the N,N′-bis(aziridinylacetyl)-α,ω-methylenediamines previously studied, the O,O′-bis(aziridinylacetyl)-α,ω-methylenediols were not effective in inhibiting the reproduction of houseflies. Three of the compounds tested (**3**, **7**, and **11**) were rather toxic to houseflies at 1 wt % concentration in their feed.

Experimental Section[4]

Method A. O,O′-Bis(bromoacetyl)-1,8-octamethylenediol (6).—To a dried flask cooled to 0° and containing 23.2 g (0.115

(2) H. Bestian, *Ann.*, **566**, 210 (1950).
(3) D. Rosenthal, G. Brandrup, K. H. Davis, Jr., and M. E. Wall, *J. Org. Chem.*, **30**, 3689 (1965).
(4) Melting points were determined by using a Fisher-Johns melting point block and were uncorrected. The nmr spectra were run in $CDCl_3$ on a Varian A-60 spectrometer.

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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Received November 17, 1965

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

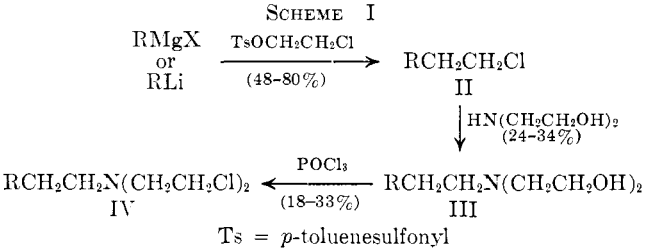
$$\underset{\text{I}}{RCH_2CH_2N(CH_2CH_2Cl)_2}$$

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).