for the preparation of 1-(**2-ethyl**)**pyrrolidyl diphenylchlorothiolacetate hydrochloride** may serve as a typical example.

A butanone solution (50 ml.) of 1-(2-mercaptoethyl)pyrrolidine (6.38 g.) was added dropwise with stirring to a butanone solution (50 ml.) of diphenylchloroacetyl chloride (13.0 g.). The mixture was refluxed for 0.5 hr. and concentrated *in vacuo* to 50 ml., anhydrous ether was added, and the precipitated product was filtered and washed with dry ether. The crude product weighed 12.5 g. (65%) and, after recrystallizations from mixtures of acetone, methanol, and ether, had a decomposition temperature of 209.0°.

When the chloro acid chloride was phenylcyclohexylchloroacetyl chloride or phenylcyclopentylchloroacetyl chloride the reflux period was extended to a minimum of 4 hr.

Ester hydrochlorides of substituted hydroxy acids in Table II were prepared from the corresponding α-chloro derivatives (Table I) by a procedure which may be illustrated for the preparation of **1-methyl-4-(2-ethyl)piperazino thiolbenzilate dihydrochloride.** 1-Methyl-4-(2-ethyl)piperazino diphenylchlorothiolacetate dihydrochloride (7.6 g.) dissolved in water (50 ml.) was heated to 60° for 10 min., cooled, made basic with Na_2CO_3, and extracted with $CHCl_3$. The combined extracts were dried ($MgSO_4$) and saturated with HCl gas. The white solid was filtered and recrystallized several times from a mixture of acetone, methanol, and ethyl acetate. The pure product (4 g., 61%) decomposed at 230.5°.

If the α-chloro thiolester salt were derived from phenylcyclohexylchloroacetyl chloride or phenylcyclopentylchloroacetyl chloride, a hydrolysis period of 1–1.5 hr. at the reflux temperature of water was required.

1-Ethyl-3-piperidyl Thiolbenzilate Bifumarate.—An ethereal solution of fumaric acid was added to 1-ethyl-3-piperidyl thiolbenzilate (2.50 g.) in dry ether (50 ml.). On standing at 3° for 24 hr., white needles of the fumarate salt formed. Recrystallization from a mixture of benzene and methanol gave pure product (2.33 g., 70%) melting at 169.5–170.5°.

Acknowledgment.—The authors wish to express their appreciation to Dean Hilton A. Smith for his assistance in the chemical syntheses.

Effect of Organic Compounds on Reproductive Processes. I. Alkylating Agents from Octamethylenediamine and Various Xylylenediamines

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Alkylating agents derived from *m*- and *p*-xylylenediamine and from octamethylenediamine were synthesized and evaluated for their effects on the reproductive processes of the housefly (*Musca domestica* L.) and the Japanese quail (*Coturnix coturnix* japonica). Some of the aziridinyl derivatives were found to interfere with reproduction of the housefly.

A better understanding of the relationship between the chemical structure of organic compounds and their effects on reproductive processes is needed. In order to obtain this kind of needed knowledge, a program was initiated involving the synthesis of chemically related groups of compounds and the evaluation of their effects on the reproductive process in the housefly (*Musca domestica* L.) and the Japanese quail (*Coturnix coturnix* japonica).

A number of alkylating agents are known to inhibit reproduction in mammals, while others are without such effects.[2,3] Recently, aziridinyl derivatives have shown some promise as chemosterilants for insects.[4–6] Chlorambucil[7] has been reported to inhibit the growth of testes and to reduce egg hatch of the Mexican fruit fly when the compound was administered continuously in the food. Many questions remain unanswered regarding the relationship between the type of alkylating moiety or carrier moiety and their influence on the compound's ability to affect reproduction.

We wish to report the results of an initial study in our program. A series of alkylating agents structurally related to two known antispermatogenic agents were synthesized and evaluated in the housefly and Japanese quail. These compounds are related to two other compounds, N,N′-diethyl-N,N′-bis(dichloroacetyl)-1,4-xylylenediamine (I) and N,N′-bis(dichloroacetyl)-1,8-octamethylenediamine (II), which also inhibit spermatogenesis in mammals.[8–13]

Studies by Surrey and Mayer[9] indicated that the octamethylenediamine derivative was more active in inhibiting spermatogenesis than were those compounds derived from diamines of shorter or longer chain length. Structural variations[8] in the xylylenediamine derivatives indicated that the *meta* derivative was inactive in blocking spermatogenesis as was the analog without the N-ethyl group. The dibromoacetyl derivative was also inactive.

The possibility that these compounds were inhibiting spermatogenesis by virtue of their alkylating ability suggested to us the synthesis of alkylating agents having the same carrier moieties as these active compounds but with variation of the alkylating function. It was also of interest to evaluate these active compounds in species other than mammals to ascertain their effects on reproduction.

Chemistry.—The compounds synthesized (**1–22**) are shown in Table I. Our initial interests were concerned with the effect of variation of the alkylating function on

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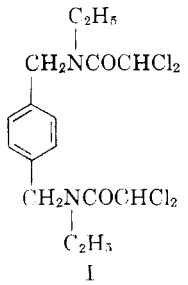
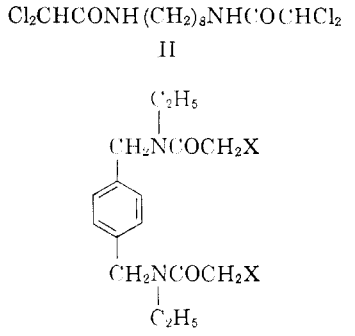
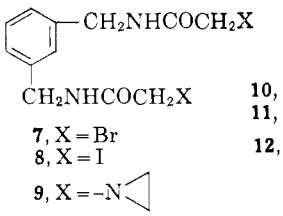


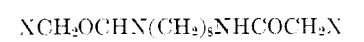
I



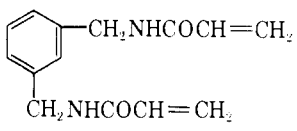
II

1, X = Br
2, X = I
3, X = -N
4, X = N_3
5, X = OCH_3
6, X = $NHCH_2CH_2Cl$





7, X = Br
8, X = I
9, X = -N
10, X = Br
11, X = I
12, X = -N
13, X = N_3
14, X = OCH_3
15, X = $NHCH_2CH_2Cl$



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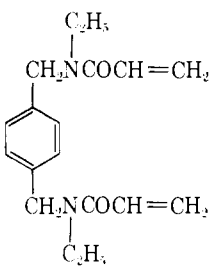

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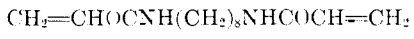

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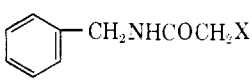

19, X = Br
20, X = -N
21, X = OCH_3
22, X = $NHCH_2CH_2Cl$

antispermatogenic activity of the carrier moiety of N,N′-diethyl-N,N′-bis(dichloroacetyl)-1,4-xylylenediamine (I).

Attempted synthesis of the aziridinyl derivative (**3**) by reaction of aziridine with the bromoacetyl derivative (**1**) in methanol in the presence of anhydrous potassium carbonate repeatedly yielded products whose elementary analysis indicated the presence of excess oxygen. The infrared absorption spectrum showed C–O–C absorption at 8.9 μ, and silica gel G thin layer chromatography showed the presence of two compounds which proved to be inseparable upon repeated crystallizations. Column chromatography on silica gel and elution with ether–acetone (1:1) afforded an analytical sample of the methoxy derivative formed by a competitive reaction of the solvent with **1**. When ethanol was used as the solvent for the reaction, none of the corresponding ethoxy derivative was formed. However, in the case of **3** and **20**, elementary analyses and Beilstein tests indicated the presence of some starting bromo derivative. These syrups were converted to analytically pure chloroethylamines (**6** and **22**) by treatment with dry HCl in methanol.

In the case of the synthesis of the *m*-bromoacetyl derivative (**7**), the use of potassium carbonate as an acid acceptor resulted in considerable production of polymeric materials. A better method for the preparation of **7** involved the use of excess diamine as an acid acceptor.

The octamethylenediamine derivative, N,N′-bis(dichloroacetyl)-1,8-octamethylenediamine (II), had been shown to be very active and selective in its antispermatogenic effects.[9] Variation of the alkylating groups on this carrier moiety led to compounds **10**–**15**. The acrylamido derivative (**18**) was prepared by the method used for the xylylenediamine derivatives.

In order to ascertain the importance of having two alkylating functions per mole, the benzylamine derivatives (**19**–**22**) were synthesized and evaluated.

Experimental

Method A. N,N′-Diethyl-N,N′-bis(bromoacetyl)-1,4-xylylenediamine (**1**).—A solution of 9.6 g. (0.05 mole) of N,N′-diethyl-*p*-xylylenediamine (prepared in 63% yield by reaction of *p*-xylylene bromide with a 10-fold *M* excess of aqueous 70% ethylamine overnight at room temperature after gently warming on a steam bath) in 100 ml. of benzene was added dropwise with vigorous stirring to a suspension of 28 g. (0.2 mole) of anhydrous K_2CO_3 in 100 ml. of benzene containing 20.2 g. (0.1 mole) of bromoacetyl bromide in 100 ml. of benzene at 5–10°. After addition, the thick solution was left standing at room temperature for 1 hr., and then ice water was added. The organic layer was separated, washed with 0.1 *N* HCl and water, dried (Na_2SO_4), and concentrated *in vacuo* to yield 10 g. (46%) of material. An analytical sample was prepared by recrystallization from methanol; m.p. 121.5–122.5°; λ_{max}^{Nujol} absence of NH (3.0 μ), 6.16 μ (C=O, amide).

Method B. N,N′-Bis(bromoacetyl)-1,3-xylylenediamine (**7**).—To 13.6 g. (0.1 mole) of *m*-xylylenediamine in 200 ml. of benzene at 5–10° was added slowly with stirring, 36.4 g. (0.2 mole) of bromoacetyl bromide. After about 1 hr., ice water was added and the solid product was filtered, washed with water, and dried; yield 7.5 g. (20%). An analytical sample was prepared by recrystallization from ethanol-water; m.p. 175–176°; λ_{max}^{Nujol} 2.98, 3.08 (NH), 6.02 (C=O, amide), 6.52 μ (amide II).

Method C. N,N′-Diethyl-N,N′-bis(iodoacetyl)-1,4-xylylenediamine (**2**).—A solution of 4.34 g. (0.01 mole) of bis(bromoacetyl)-N,N′-diethyl-*p*-xylylenediamine in 60 ml. of acetone was added dropwise at room temperature to 12 g. (0.08 mole) of NaI in 125 ml. of acetone. A drop of 0.5 *N* HCl was added and the whole solution was warmed gently on a steam bath for 2 hr. After cooling, cold water was added and the precipitate was filtered; 3.35 g. (63%), m.p. 130–134°. An analytical sample was prepared by two recrystallizations from methanol; m.p. 133–134°; λ_{max}^{Nujol} absence of NH (3.0 μ), 6.18 μ (C=O, amide).

Method D. N,N′-Diethyl-N,N′-bis(acrylyl)-1,4-xylylenediamine (**17**).—A solution of 4.8 g. (0.025 mole) of N,N′-diethyl-*p*-xylylenediamine in 50 ml. of benzene was added dropwise with stirring to a suspension of 138 g. (0.1 mole) of anhydrous K_2CO_3 in 75 ml. of benzene containing 4.56 g. (0.05 mole) of acryloyl chloride. After the addition, the solution was stirred for 3 hr. and then washed, respectively, with 100 ml. of 0.1 *N* HCl, 0.1 *N* NaOH, and water. The organic layer was concentrated *in vacuo* after drying (Na_2SO_4) to yield 4 g. (53%) of product. Recrystallization from benzene–petroleum ether (b.p. 30–60°) and then methanol–water afforded an analytical sample: m.p. 112–112.5°; λ_{max}^{Nujol} 6.06 (C=O, amide), 6.20 μ (C=C, acrylyl).

Method E. N,N′-Diethyl-N,N′-bis(azidoacetyl)-1,4-xylylenediamine (**4**).—A solution of 2.6 g. (0.04 mole) of sodium azide in a minimum amount of water was added dropwise to 4.34 g. (0.01 mole) of bis(bromoacetyl)-N,N′-diethyl-*p*-xylylenediamine in 40 ml. of dimethylformamide. The solution was stirred overnight at 35–45°. The next day water was added and the

TABLE I
CHEMICAL DATA

Compd.	Method	M.p., °C.	Crystn. solvent	Yield, %	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
1	A	121.5–122.5	Methanol	46	$C_{16}H_{22}Br_2N_2O_2$	44.3	5.11	6.45	44.4	5.09	6.48
2	C	133–134	Methanol	63	$C_{16}H_{22}I_2N_2O_2$	36.4	4.20	5.30	36.9	4.43	5.08
3	F			98	$C_{20}H_{30}N_4O_2$	67.0	8.44	15.6	65.4	8.28	15.3
4	E	49–52	Methanol–water	64	$C_{16}H_{22}N_8O_2$	53.6	6.19	31.3	53.4	6.15	30.8
5		58–60		9	$C_{16}H_{28}N_2O_4$	64.3	8.39	8.33	63.9	8.70	8.36
6	G	207–210	Methanol–ether	83	$C_{20}H_{32}Cl_2N_4O_2 \cdot 2HCl \cdot H_2O$	46.0	6.90	10.7	45.4	6.51	10.7
7	B	175–176	Ethanol–water	20	$C_{12}H_{14}Br_2N_2O_2$	38.2	3.74	7.44	38.7	4.04	7.42
8	C	198–199	DMF–water	50	$C_{12}H_{14}I_2N_2O_2$	30.5	3.00	5.95	30.6	3.03	6.08
9	F	153–155	Benzene–hexane	81	$C_{16}H_{22}N_4O_2$	63.5	7.30	18.5	62.8	7.29	18.1
10	A	123–124	Ethanol	81	$C_{12}H_{22}Br_2N_2O_2$	37.4	5.74	7.26	37.5	5.84	7.67
11	C	166–167	Acetone–water	90	$C_{12}H_{22}I_2N_2O_2$	30.0	4.62	5.84	30.5	4.67	5.40
12	F	90	Cyclohexane	64	$C_{16}H_{30}N_4O_2$	61.9	9.74	18.1	62.1	9.60	18.1
13	E	80–81	Methanol–water	82	$C_{12}H_{22}N_8O_2$	46.4	7.14	36.1	46.5	7.18	36.2
14		89–90		12	$C_{14}H_{28}N_2O_4$	58.3	9.79	9.71	58.2	9.86	9.92
15	G	177–180		67	$C_{16}H_{32}Cl_2N_4O_2 \cdot 2HCl$	42.1	7.51	12.3	42.1	7.47	12.1
16	D	145–145.5	Benzene–methanol	50	$C_{14}H_{16}N_2O_2$	68.8	6.60	11.5	68.4	6.53	11.6
17	D	112–112.5	Methanol–water	53	$C_{18}H_{24}N_2O_2$	72.0	8.05	9.33	72.0	7.86	9.47
18	D	143.5–144.5	2-Propanol–water	51	$C_{14}H_{24}N_2O_2$	66.6	9.59	11.1	66.9	9.09	11.0
19	B	109–110		92	$C_9H_{10}BrNO$	47.4	4.42	6.14	47.2	4.30	6.11
20	F			98	$C_{11}H_{14}N_2O$	69.4	7.42	14.7	67.5	7.35	14.1
21		50–51		10	$C_{10}H_{13}NO_2$	67.0	7.31	7.82	66.9	7.31	7.83
22	G	154–157		53	$C_{11}H_{15}ClN_2O \cdot HCl$	50.2	6.12	10.6	50.3	6.15	10.7

gummy product was recrystallized from methanol–water; yield 2.3 g. (64%); m.p. 49–52°; λ_{max}^{Nujol} 4.75 (N_3), 6.05 μ (C=O, amide).

Method F. N,N′-Bis(aziridinylacetyl)-1,8-octamethylenediamine (12).—To a solution of 1.93 g. (0.005 mole) of bis(N,N′-bromoacetyl)octamethylenediamine (**10**) in 100 ml. of ethanol containing 4.2 g. (0.03 mole) of anhydrous K_2CO_3 was added dropwise 1.5 ml. (0.03 mole) of aziridine at room temperature. The solution was stirred several hours, left standing overnight, concentrated *in vacuo* to one-half its original volume, and filtered to remove inorganic salts. The filtrate was concentrated to dryness, extracted with warm benzene, and crystallized by the addition of hexane to yield 1 g. (64%); m.p. 90°; λ_{max}^{Nujol} 3.02 (NH), 6.09 μ (C=O, amide); thin layer chromatography, MN cellulose 300F, ethanol developed, R_f 0.83, iodine detected.

Method G. N,N′-Bis(2-chloroethylaminoacetyl)-1,8-octamethylenediamine Dihydrochloride (15).—A solution of 1 g. (0.0032 mole) of bis(N,N′-aziridinylacetyl)octamethylenediamine in 50 ml. of methanol at 0° was saturated with anhydrous HCl. Then it was left standing at room temperature for 2 hr., evacuated to half its volume, and cooled in an ice bath. Anhydrous ether was added and the precipitated white solid was filtered and washed with anhydrous ether; 1 g. (67%), m.p. 177–180°.

Biological Methods. Effects on Houseflies.—All of the above-mentioned compounds were evaluated as inhibitors of reproduction in our colony of houseflies (*Musca domestica* L.).

Precounted, ready-to-emerge pupae were placed in a fly cage with 10 g. of a dry diet containing 1% of the test compound; an unsupplemented control group was included in each experiment. The dry diet used in this study had been previously described by Labrecque, *et al.*[14] After 48 hr., oviposition diet consisting of 3 parts of water and 1 part of evaporated milk was substituted for the dry diet. Prior to oviposition, a 140-ml. wax-treated cup containing 20–30 ml. of the diluted milk diet and a milk-saturated cotton ball were placed in each cage as an oviposition media.

Eggs were collected twice daily for 1 week after oviposition. Eggs laid overnight were discarded. One-hundred fresh eggs obtained by mid-afternoon were placed on Petri dishes containing 1.5% solidified agar and incubated at 30° for 1 day to determine hatchability. This procedure is only a minor modification of that described by Kilgore and Painter.[15]

The results on compounds showing an effect on the hatchability of the housefly eggs are summarized in Table II. N,N′-Bis(dichloroacetyl)-1,8-octamethylenediamine and all other compounds mentioned in this paper with the exception of those in Table II had no effect on the reproduction of the housefly at 1% concentration in the feed. Results on apholate and 5-fluorouracil were in agreement with the literature.

TABLE II
EFFECTS OF COMPOUNDS ON THE REPRODUCTION OF HOUSEFLIES

Compd.	Concn. (wt. % in feed)	No. of flies	% egg hatch, Days of oviposition 1	2	3	4	5	6	7
Control 1[a]	...	400	94	94	92	96	94	83	..
Control 2[a]	...	250	92	97	90	98	88	91	87
9[a]	1.0	400	31	..	71	..	74	76	..
9[a]	1.0	250	41	56	64	..	76	65	67
9[a]	0.1	250	90	88	87	88	90	98	90
12[a]	1.0	400	19	27	11	30	28	35	..
12[b]	1.0	250	..	2	1	..	0	..	..
12[b]	0.1	250	14	10	18	18	38	23	27
12[b]	0.01	250	..	77	61	72	70	62	72

[a] Acetone used in diet to dissolve compound or as control. [b] Compound mixed in dry diet. Acetone was found to decrease effectiveness of compounds. All were evaluated dry also.

Effects on Japanese Quail.—Several of the compounds were evaluated for their effects on the reproduction of the Japanese quail (*Coturnix coturnix* japonica). Adult Japanese quail from a stock colony maintained at Stanford Research Institute were randomized into groups containing 18 females and 7 males. All birds were housed in commercial chick brooder batteries in an air-conditioned laboratory. During a 3–4 week pretreatment period, a finely ground commercial game bird breeder layer diet was fed *ad libitum*. Eggs were collected daily and stored by groups in a refrigerator at 13°. At weekly intervals noncracked eggs were incubated in a commercial egg incubator–hatcher unit for determination of hatchability and fertility. Body weight and feed consumption were measured weekly.

During a 4–6-week experimental period, the test compounds were added to the ground layer diet at a level of 400 p.p.m. A nonsupplemented group served as controls. During the treatment period eggs were collected daily but set for incubation only at weekly intervals. Body weight and feed consumption were measured weekly and were not significantly different from the controls.

(14) G. C. Labrecque, P. H. Adcock, and C. N. Smith, *J. Econ. Entomol.*, **53**, 802 (1960).

(15) W. W. Kilgore and R. R. Painter, *ibid.*, **55**, 710 (1962).

The following compounds were evaluated for effects on reproduction in the Japanese quail but were without effect at dietary levels as high as 400 p.p.m.: N,N'-diethyl-N,N'-bis(dichloroacetyl)-1,4-xylylenediamine, N,N'-bis(dichloroacetyl)-1,8-octamethylenediamine, **7**, **8**, and **10**.

Discussion of Biological Results

In the series of alkylene and xylylenediamines used as carriers for alkylating groups all active compounds in the fly reproduction experiments had the aziridinylacetyl grouping as the alkylating function. Even with that grouping in the case of the N-ethyl derivative (**3**) no activity was noted. The octamethylene derivative (**12**) was clearly the most effective while the *m*-xylylene derivative (**9**) showed some effect. The lack of activity of the benzyl derivative (**20**) would indicate the need for at least two alkylating groups per mole.

The lack of activity of the N-ethyl derivative (**3**) and the nitrogen mustards (**6** and **15**) emphasizes the specificity of activity toward inhibiting reproduction both with regard to the carrier moiety and the alkylating function. Current work in these laboratories is concerned with defining these parameters in greater detail and, in addition, investigating species specificity in the Japanese quail and in the rat.

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Some Amino and Ammonio Nitrogen Mustard Analogs

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Procedures have been developed for the preparation of nitrogen mustard derivatives containing amino, dimethylamino, and trimethylammonio groups separated from the mustard group by two-, three-, four-, and five-carbon chains. A β-trimethylammonio group diminished the reactivity of an amino group so that it was possible to introduce only one hydroxyethyl group by reaction with ethylene oxide. Biological tests indicated the amino mustards to have toxic and antitumor properties similar to HN-2. The ammonio mustards were devoid of antitumor activity and were much less toxic.

Earlier reports have indicated interesting biological properties for a variety of basic heterocyclic compounds with bis(β-chloroethyl)aminoalkylamino side chains,[2-4] related to nitrogen mustard [HN-2, I, $CH_3N(CH_2CH_2Cl)_2$]. Furthermore, it is likely that analogs of such compounds might be formed *in vivo* through alkylation of primary, secondary, or tertiary nitrogens in proteins, DNA, RNA, or other basic constituents of cells. It, therefore, seemed desirable to study simple amino- and ammonio-substituted mustards, especially since one of the simplest possible analogs, β-aminoethylbis(β-chloroethyl)amine, has shown very promising activity at least comparable to HN-2 in our laboratories and elsewhere.[3]

The compounds selected for study may be represented by the following general structures.

$X\text{-}(CH_2)_2N(CH_2CH_2Cl)_2$ (II) $\quad X\text{-}(CH_2)_3N(CH_2CH_2Cl)_2$ (III)

$X\text{-}(CH_2)_3CH(CH_3)N(CH_2CH_2Cl)_2$ (IV) $\quad X\text{-}(CH_2)_5N(CH_2CH_2Cl)_2$ (V (c only))

a, X = NH_2
b, X = $N(CH_3)_2$
c, X = $N^+(CH_3)_3Cl^-$

Since the ethylenediamine derivatives (II) showed the most interesting biological activities, a more extensive series was prepared for biological testing.

The required diols were prepared by either of two methods: (1) N,N-dialkylethylenediamine, prepared by the method of Turner,[5] was converted to the corresponding diol by reaction with ethylene oxide, or (2) N,N-dialkylaminoethyl chloride was treated with diethanolamine.[6]

Since it may be assumed that nitrogen mustards related to ethylenediamine could cyclize to piperazine derivatives, a related series of N-2-chloroethylpiperazines was also prepared.

The conversion of ethylenediamine to the mustard derivatives IIa and IIb was accomplished by the reactions outlined below.[7]

$$H_2NCH_2CH_2NHAc \xrightarrow[2.\ HCl]{1.\ C_2H_4O} (HOCH_2CH_2)_2NCH_2CH_2NH_2 \quad (II)$$

$$II \xrightarrow[2.\ HCl,\ SOCl_2]{1.\ CH_2O,\ HCOOH} \begin{cases} IIb\cdot 2HCl\ (m.p.\ 157^\circ) \\ IIa\cdot 2HCl\ (m.p.\ 140^\circ) \end{cases} \quad (SOCl_2)$$

The conversion of the methylated diol II to IIb was not successful unless it was first converted to the hydrochloride. Reaction of the free base gave an entirely different product, m.p. 257° dec., which may have been the cyclized piperazinium isomer, although

(1) Supported in part by U. S. Public Health Service Grant No. Cy-2714. Abstracted from the doctoral dissertation of G. Kabas, June 1960.

(2) R. Jones, Jr., C. C. Price, and A. K. Sen, *J. Org. Chem.*, **22**, 783 (1957).

(3) H. J. Creech, R. M. Peck, and R. K. Preston, *J. Am. Chem. Soc.*, **81**, 3984 (1959).

(4) H. H. Lin and C. C. Price, *J. Org. Chem.*, **26**, 108, 264, 266 (1961).

(5) R. Turner, *J. Am. Chem. Soc.*, **68**, 1607 (1946).

(6) G. Drefahl and K. H. König, *Chem. Ber.*, **87**, 1632 (1954).

(7) After we had prepared IIa, it was reported by Creech, Peck, and Preston.[3]