Physiologically Active Compounds. VI. Cyclic Amino Thiolesters of Substituted Chloroacetic, Benzilic, and Glycolic Acids[1,2]

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Thirty-four amino thiolesters of chloroacetic, benzilic, and glycolic acids have been synthesized, usually as salts. The α-hydroxy esters, which were of greatest interest, were obtained *via* the α-chloroamino thiolesters. The compounds were examined for their effect on animal behavior and for their anticholinergic potency. Three devices were used to determine the degree of behavioral disturbance produced by the drugs in animals: a hyperactivity cage, a swim maze, and a "peek" test. For a given drug good correlation was found to exist between the effect on performance in all three tests and the psychotomimetic efficacy in humans. In general the structure–activity relationships of the thioglycolate esters agreed with those of the regular esters, although the thioesters were less potent and of shorter duration of action.

This paper is a continuation of the synthesis and physiological testing of aminothiol esters of the type represented by RR′C(X)COSR″, where R,R′ are combinations of phenyl, *p*-tolyl, cyclopentyl, and cyclohexyl; X is Cl or OH; and R″ is a tertiary amino function, almost always cyclic in nature.

Synthesis of Compounds in Table I.—The α-chloro acid chlorides were obtained from substituted glycolic acids by the method of King and Holmes,[3] or from the chlorination of substituted acetic acids by a modified procedure of Schwenk and Papa.[4] The synthesis of essential aminomercaptans is given in the Experimental. It was found that the conversion of thiolacetates to mercaptans is accomplished best by the use of sodium in methanol, a method mentioned, but not described in great detail, by Reid.[5] The α-chloro acid chlorides and aminomercaptans in a suitable solvent produced the α-chloroamino thiolester hydrochlorides.

Synthesis of Compounds in Table II.—The amino thiolglycolates in Table II were prepared most satisfactorily from the α-chloro thiolester salt as described previously.[2] It was not possible to replace the chlorine atom in 2-N,N-diethylaminoethyl dicyclohexylchlorothiolacetate hydrochloride by this procedure or by those of Bachmann and Ramirez,[6] and McCloskey and Coleman.[7] Thus it would appear that the resonance supplied by at least one aromatic ring is essential for success in this hydrolysis. At the other extreme, compound **124** could not be hydrolyzed successfully to the corresponding glycolate since the free glycolic acid was recovered.

A previous study[2] showed that the CNS activity of amino thiolesters of benzilic and glycolic acids increased as the number of carbon atoms between the carbonyl and basic nitrogen groups decreased. For this reason the glycolic ester corresponding to **124** was of particular interest. Other attempts to obtain this compound by the procedure of McCloskey and Coleman[7] failed. The possibility of utilizing transesterification was ruled out when the procedures of Sasin, *et al.*,[8] and Bowman, *et al.*,[9] were unsuccessful. These methods were applicable to the synthesis of 2-N,N-diethylaminoethyl thiolbenzilate hydrochloride when the reactants were ethyl thiolbenzilate and 2-N,N-diethylaminoethanethiol. Another failure resulted in an attempt to effect an ester interchange with an oxygen ester (methyl cyclohexyl glycolate) and a mercaptan (2-N,N-diethylaminoethanethiol) using sodium methoxide as a catalyst. From these few experiments it appears that (a) a mixed ester interchange between oxygen esters and aminomercaptans will not occur, but (b) an interchange between a thiolester and an aminomercaptan will take place, at least when the sulfur atom in the final product is separated from the nitrogen atom by two carbon atoms.

The purification of the amino thiolglycolates was at times difficult. Crystallization, distillation *in vacuo*, and adsorption chromatography were the methods employed. In one case the compound **145** could be obtained crystalline neither as the free base nor as the hydrochloride.

Pharmacological Studies.—The tests used for the evaluation of the behavioral effects produced by the agents in animals have been described in detail else-

(1) (a) The chemical portion of this study was supported by Grant B652 from the National Institutes of Health, U. S. Public Health Service. (b) Inquiries should be addressed to the senior author.

(2) For paper V, see C. A. Buehler, H. A. Smith, A. C. Kryger, R. L. Wells, and S. F. Thames, *J. Med. Chem.*, **6**, 230 (1963).

(3) F. E. King and D. Holmes, *J. Chem. Soc.*, 164 (1947).

(4) E. Schwenk and D. Papa, *J. Am. Chem. Soc.*, **70**, 3626 (1948).

(5) E. E. Reid, "Organic Chemistry of Bivalent Sulfur," Vol. 1, Chemical Publishing Co., Inc., New York, N. Y., 1958, p. 30.

(6) W. E. Bachmann and F. Ramirez, *J. Am. Chem. Soc.*, **72**, 2525 (1950).

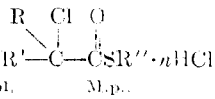
(7) C. M. McCloskey and G. H. Coleman, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 434.

(8) G. S. Sasin, P. R. Schaeffer, and R. Sasin, *J. Org. Chem.*, **22**, 1183 (1957).

(9) R. E. Bowman, J. F. Cavalla, and J. Davoll, British Patent 821,436 (Feb. 22, 1956); *Chem. Abstr.*, **56**, 2427 (1962).

TABLE I

α-CHLORO THIOLESTERS OF SUBSTITUTED ACETIC ACIDS



No.	R	R′	R″	n	Yield, %	M.p., °C. (cor.)	Formula	Calcd., % C	Calcd., % H	Found, % C	Found, % H
122	C_6H_5	C_5H_9	*a*	1	75	102.0–105.0	$C_{15}H_{19}Cl_2NOS$	*e*			
123	C_6H_5	C_5H_9	*b*	1	90	134.0–135.0	$C_{17}H_{22}Cl_2N_2OS$	54.98	5.43	54.64	5.67
124	C_6H_5	C_6H_{11}	*b*	1	96	158.0–159.0	$C_{18}H_{22}Cl_2N_2OS$	56.10	5.75	55.86	6.01
125	C_6H_{11}	C_6H_{11}	*b*	1	93	194.5–195.5	$C_{18}H_{28}Cl_2N_2OS$	55.23	7.12	54.96	7.07
126	C_6H_5	C_6H_{11}	*c*	1	81	162.5–164.0	$C_{20}H_{29}Cl_2NOS$	59.69	7.26	59.37	7.28
127	C_6H_5	C_6H_5	*c*	1	65	209 dec.	$C_{20}H_{23}Cl_2NOS$	60.60	5.85	60.66	5.62
128	4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	*c*	1	78	181 dec.	$C_{22}H_{27}Cl_2NOS$	62.25	6.41	62.02	6.32
129	C_6H_5	C_6H_{11}	*d*	2	95	217 dec.	$C_{21}H_{33}Cl_3N_2OS$	53.90	7.11	53.75	7.10
130	C_6H_5	C_5H_9	*d*	2	70	196.5 dec.	$C_{20}H_{31}Cl_3N_2OS$	52.92	6.88	52.71	6.84
131	C_6H_5	C_6H_5	*d*	2	..	230.5 dec.	$C_{21}H_2Cl_3N_2OS$	*e*			
132	C_6H_5	C_6H_{11}	*f*	1	88	175.5–178.0	$C_{20}H_{29}Cl_2NOS$	59.69	7.26	59.46	7.30
133	C_6H_5	C_6H_{11}	*g*	0	87	*h*	$C_{22}H_{32}ClNOS$	67.06	8.18	67.17	8.37
134	C_6H_5	C_6H_5	*g*	1	90	145.0	$C_{22}H_{27}Cl_2NOS$	62.25	6.41	62.25	6.42
135	C_6H_5	C_5H_9	*i*	1	20	163.5–165.0	$C_{21}H_{29}Cl_2NOS$	59.69	7.26	59.37	7.40
136	C_6H_5	C_6H_{11}	*i*	1	..	169.5–171.0	$C_{21}H_{32}Cl_2NOS$	*e*			
137	C_6H_5	C_5H_9	*c*	1	95	155.5–156.0	$C_{19}H_{27}Cl_2NOS$	58.75	7.00	58.37	7.17

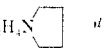
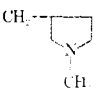
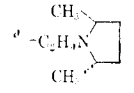
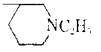
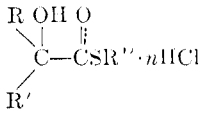
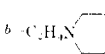
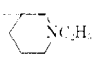
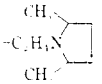
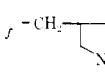
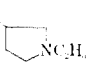
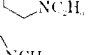
[a] $-C_2H_4N(C_2H_5)_2$ [b]  [c] $-C_2H_4$  [d] $-C_2H_4$  [e] These salts were so hygroscopic that correct elemental analyses were not obtained. They were converted satisfactorily into pure aminothiol glycolates. [f]  [g] $-C_2H_4N$  [h] Free base liquid purified by chromatographing over Florisil. [i] 

TABLE II

THIOLBENZILATES AND SUBSTITUTED THIOLESTERS OF GLYCOLIC ACIDS



No.	R	R′	R″	n	Yield, %	M.p., °C. (cor.)	Formula	Calcd., % C	Calcd., % H	Found, % C	Found, % H
138	C_6H_5	C_6H_5	*a*	1	73	213.0–214.0	$C_{20}H_{24}ClNO_2S$	63.55	6.40	63.67	6.22
139	C_6H_5	C_6H_{11}	*a*	1	77	206.0–207.0	$C_{20}H_{30}ClNO_2S$	62.55	7.87	62.79	7.75
140	C_6H_5	C_5H_9	*a*	1	35	184.0–185.0	$C_{19}H_{28}ClNO_2S$	61.87	7.63	61.53	7.54
141	4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	*a*	1	80	188.0–189.0	$C_{22}H_{28}ClNO_2S$	65.10	6.96	65.04	6.94
142	C_6H_5	C_6H_5	*b*	2	61	230.5 dec.	$C_{21}H_{18}Cl_2N_2O_2S$	56.88	6.36	56.70	6.50
143	C_6H_5	C_6H_{11}	*b*	2	80	213 dec.	$C_{21}H_{34}Cl_2N_2O_2S$	56.11	7.62	55.92	7.72
144	C_6H_5	C_5H_9	*b*	2	50	211 dec.	$C_{20}H_{32}Cl_2N_2O_2S$	55.16	7.40	55.35	7.46
145	C_6H_5	C_5H_9	*c*	0	50	*d*	$C_{20}H_{29}NO_2S$	69.12	8.41	68.91	8.21
146	C_6H_5	C_6H_5	*e*	0	81	91.0	$C_{22}H_{27}NO_2S$	71.50	7.36	71.62	7.54
147	C_6H_5	C_6H_{11}	*f*	1	..	162.5–167.5	$C_{20}H_{30}ClNO_2S$	62.55	7.87	62.36	7.61
148	C_6H_5	C_6H_{11}	*c*	1	30	151.0–152.0	$C_{21}H_{32}ClNO_2S$	63.37	8.10	63.49	7.91
149	C_6H_5	C_6H_5	*f*	1	..	223.0–226.0	$C_{20}H_{24}ClNO_2S$	63.55	6.40	63.31	6.30
150	C_6H_5	C_2H_5	*c*	*g*	70	169.5–170.5	$C_{26}H_{29}NO_6S$	63.67	6.20	63.49	6.08
151	C_6H_5	C_6H_5	*h*	0	52	123.0–125.0	$C_{20}H_{23}NO_2S$	70.34	6.78	70.59	6.86
152	C_6H_5	C_6H_{11}	*e*	1	52	*i*	$C_{22}H_{34}ClNO_2S$	64.13	8.32	64.61	8.52
153	C_6H_5	C_5H_9	*e*	0	27	*j*	$C_{21}H_{31}NO_2S$	69.76	8.65	69.54	8.42
154	C_6H_5	C_6H_5	*k*	1	56	211.0–212.0	$C_{20}H_{24}ClNO_2S$	63.56	6.40	63.33	6.18
155	C_6H_5	C_6H_{11}	*k*	0	27	145.0–147.0	$C_{20}H_{29}NO_2S$	69.12	8.41	68.90	8.20

[a] $-C_2H_4$ [b] $-C_2H_4$  [c]  [d] Free base liquid purified by chromatographing over Florisil; $n^{25}D$ 1.5605 and strong thiolester absorption at 5.95 μ. [e] $-C_2H_4N$  [f] $-CH_2$  [g] Bifumarate; free base melted at 118–120°. [h]  [i] Free base ester distilled at 220° (0.25 mm.) (strong thiolester absorption at 5.92 μ). [j] B.p. 168° (0.07 mm.). [k] 

where.[10–14] Three behavioral tests were employed in the present study: the activity cage, swim maze, and "peek" test; and they will only be described briefly. Anticholinergic activity was evaluated in terms of mydriatic and spasmogenic responsiveness (see Table III).

(10) L. G. Abood and J. H. Biel, *Intern. Rev. Neurobiol.*, **4**, 217 (1962).
(11) V. C. Lipman, P. S. Shurrager, and L. G. Abood, *Arch. intern. pharmacodyn.*, **146**, 174 (1963).
(12) M. E. Kosman, *Proc. Soc. Expll. Biol. Med.*, **115**, 728 (1964).
(13) M. E. Kosman, *ibid.*, in press.
(14) H. C. Chang and J. H. Gaddum, *J. Physiol.*, **79**, 225 (1933).

TABLE III
EFFECT OF THIOLESTERS ON MOTOR ACTIVITY, BEHAVIOR, AND PERIPHERAL CHOLINERGIC SYSTEMS

No.	For 1 mg./kg. Activity cages[a]	Swim maze[b]	Peek test[c]	Mydriasis[d] Concn., %	Time, min.	ED_{50} vs. ACh[e] (1:10^{-5})
125	670	3.0	2.6	0.1+	..	1
130	1500	2.7	2.8	0.1+	..	1
139	1650	3.0	2.7	0.02	40	10
140	4039	3.9	2.8	0.002	60	100
144	667	3.8	2.5	0.1+	..	...
145	2659	4.0	2.5	0.01	150	...
147	4153	3.9	3.2	0.005	200+	50
148	2000	3.0	2.6	0.002	100	130
149	3060	4.2	2.9	0.01	200+	...
150	3256	3.5	2.8	0.1	46	...
151	2289	2.9	2.6	0.1	38	...
153	1147	3.2	2.6	0.1	35	2
154	3649	4.4	3.3	0.005	170	150
155	3500	4.2	3.4	0.005	250	100
3-NPB[f]	4200	4.5	3.5	0.001	220	300
4-NPB[g]	5200	6.0	5.0	0.001	300	200
3-NPyB[h]	4500	4.8	4.0	0.001	220	250
Saline	1300	2.6	2.6	..	..	...

[a] Expressed as oscillations/30 min. [b] In terms of errors on 4th trial. [c] In terms of total number of peeks. [d] Minimal % solution necessary for complete mydriasis and the time (in minutes) necessary for the pupil to return to normal. [e] Dilution of drug (parts per million) capable of inhibiting ACh-induced spasms on the rabbit ileum by 50%, *e.g.*, a value of 300 indicates the extent of dilution of a 1-γ/ml. solution. [f] 3-NPB = N-methyl-3-piperidyl benzilate. [g] 4-NPB = N-methyl-4-piperidyl benzilate. [h] 3-NPyB = N-methyl-3-pyrrolidyl benzilate.

Activity Cage.—The activity cage[10,11] apparatus consists of a circular wire-mesh cage (12.5 cm. in diameter, 1.325 m. in height) suspended from a cantilever beam of spring steel. Any movement of the animal results in vibration of the cantilever which is electronically connected to a pulse counter or automatic ink recorder. Male albino rats weighing 150 ± 25 g. were injected intraperitoneally with 1 mg./kg. of the agent and the total activity was recorded for a period of 30 min. A total of 6 rats was tested with each drug.

Swim Maze.—The swim maze[12] is an I-shaped maze constructed of galvanized iron and has the over-all dimensions of 100 × 75 × 20 cm. (height). The water level is 10 cm. and the temperature is maintained at 27° with a circulation pump and temperature regulator. An adult albino mouse injected intraperitoneally with 1 mg./kg. of the agent is placed in the water 5 min. later, and both the time to swim the maze and the number of errors are recorded. Fresh mice are injected in groups of 6, and 4 training trials are given at intervals of 10 min. Time scores are converted to the log x and the errors to $x^{1/2} + (x + 1)^{1/2}$; only the error scores obtained in the fourth trial were used for the evaluation. The swimming time was generally not affected by the drugs, and their values were not included.

Peek Test.—The apparatus[13] consists of a brass pipe 85 cm. long and 3.75 cm. in diameter. After a mouse is inserted into one end of the pipe, the end is closed with a rubber stopper. Both the time to traverse the pipe and the number of "peeks" made at the opposite end are recorded. A "peek" is defined as an emergence of the mouse's head or forelimbs from the tube. A mouse would generally emerge in and out after reaching the opposite end before exiting completely from the tube. Five minutes was the maximum time allowed, and the square root of the number of peeks was tabulated.

Anticholinergic Potency.—Mydriatic efficacy was found to be a suitable method for the evaluation of the anticholinergic action of the agents.[10] A drop of an aqueous solution of the agent was applied directly to each eye of a rat, and the time of onset of complete mydriasis as well as the duration of mydriasis was recorded. The minimal concentration of the agent necessary to produce complete mydriasis was employed, and 2–3 animals were used at this drug concentration.

The spasmolytic action of some of the agents was determined on the isolated rabbit ileum according to the technique of Chang and Gaddum.[14]

A fundamental objective of the drug screening program has been to develop and examine behavioral techniques which can screen anticholinergic agents rapidly and effectively for central nervous system activity. As has been discussed in detail elsewhere,[10,11] the measurement of hyperactivity in rats is a good indication of CNS stimulation. Although within a given series of glycolate homologs (*e.g.*, the piperidyl and pyrrolidyl series) hyperactivity appears to be correlated with psychotomimetic potency, the relationship does not always hold. In general it can be stated that with respect to the degree of hyperactivity the thioesters are 25–50% less potent than the corresponding O-esters.

The swim maze and peek test have been shown to be quite effective in evaluating the drug effects on certain behavioral patterns associated with higher central nervous function.[12] It can be inferred that the effect of the glycolate esters on the mouse's performance in the swim maze is related, in a general way, to the ability of the esters to impair such functions as concentration, memory, and learning in monkeys and man.[10] It should be pointed out that the glycolate esters do not impair the motor ability of the animal to swim, and, actually, their swimming rate may be slightly increased over the normal value. Nor does mydriasis seem to be a factor, since anticholinergic agents such as atropine which have less CNS action are without effect on the swim maze test.

The significance of the peek test in terms of the CNS action of the glycolate esters is somewhat more difficult to determine. To begin with only the most potent centrally active glycolate esters significantly increase the number of peeks in the test situation, and increasing the dose of the less active compounds does not necessarily alter the test performance. This test was developed by Kosman[13] as a possible quantitative measure of the peculiar head-swaying and head-bobbing effect produced by this class of agents in rodents.[10] Some factors as the natural tendency of the mouse to exhibit exploratory behavior when emerging through a hole from dark to light and his possible sensitivity to light and other ambient changes undoubtedly enter into the test situation. Despite the ultimate interpretation of the test in terms of over-all behavior, it does appear to be useful in screening the centrally active glycolate esters. The most potent thiolesters from the standpoint of this test are the 4-position ring-substi-

nted piperidyl glycolates (**154** and **155**), but, again, they are less potent than the corresponding O-esters.

The mydriatic effect of the drug applied directly to the rat's eye is not only a simple, rapid method for determining the anticholinergic of a drug, but it appears to be better correlated with the CNS action of the agents than is the spasmogenic action on smooth muscle.[10] In general, the mydriatic potency as well as the duration of action of the thiolesters is about 20–50% that of the O-esters.

In general the spasmolytic potency of the thiolesters was 25–50% of that of their corresponding O-ester derivatives (see ref. 10 for additional data). For the most part the spasmolytic activity agreed favorably with the mydriatic potency.

Experimental[15]

Substituted Acetic Acids.—All substituted acetic acids were commercially available with the exception of dicyclohexylacetic acid which was obtained from diphenylacetic acid according to literature directions.[16]

Substituted Chloro Acid Chlorides.—Those obtained by treatment of the acid with thionyl and sulfuryl chlorides were: cyclopentylphenylchloroacetyl chloride,[2] yield 78%, b.p. 124° (0.5 mm.); cyclohexylphenylchloroacetyl chloride,[2] yield 91%, b.p. 134° (0.4 mm.); dicyclohexylchloroacetyl chloride,[2] yield 75%, m.p. 52–54°. Diphenylchloroacetyl chloride[3] and di-*p*-tolylchloroacetyl chloride[4] were obtained by treatment of the α-hydroxy acids with PCl_5.

Mercaptans not available commercially were prepared as described.

1-Methyl-4-(2-mercaptoethyl)piperazine, 1-(2-Mercaptoethyl)-pyrrolidine, and 1-(2-Mercapto)-2,5-dimethylpyrrolidine. A.—Ethylene sulfide was prepared according to a combined procedure of Braz[17] and Schönberg.[18] Ethylene oxide (33 ml.) was added to a mixture of potassium thiocyanate (45 g.) in water (45 g.) at acetone–Dry Ice temperature. The solid mixture was transferred to an ice–salt bath and treated with 2 ml. of ethanethiol as a stabilizer. Gentle stirring was employed while the reaction mixture was held at 0° for 24 hr. The ethylene sulfide was removed by means of a pipet, treated with 0.5 ml. of ethanethiol, and fractionally distilled at 52–56° (760 mm.) to give 33.1 g. (70%) of the desired product.

B. 1-Methyl-4-(2-mercaptoethyl)piperazine was prepared from freshly distilled ethylene sulfide according to the procedure of Haefele and Broge.[19] The pure product (80%) boiled at 102° (20 mm.), $n^{20}D$ 1.5054 [lit.[19] b.p. 95° (10 mm.), $n^{20}D$ 1.5045].

In a similar manner were prepared **1-(2-mercaptoethyl)pyrrolidine,** yield 74%, b.p. 64° (15 mm.), $n^{20}D$ 1.5004 [lit.[19] b.p. 81° (22 mm.), $n^{20}D$ 1.5004], and **1-(2-mercaptoethyl)-2,5-dimethylpyrrolidine,** yield 73%, b.p. 63° (4.5 mm.).

Anal. Calcd. for $C_8H_{17}NS$: C, 60.32; H, 10.75. Found: C, 60.31; H, 10.87.

1-Ethyl-3-mercaptopyrrolidine. A. 1,4-Dibromo-2-butanol was prepared from 1,2,4-butanetriol according to the procedure of Lunsford, *et al.*,[20] to give the product (90%) boiling at 150° (35 mm.), $n^{20}D$ 1.5426 [lit.[20] b.p. 150–155° (40 mm.), $n^{20}D$ 1.5382].

B. 1-Ethyl-3-pyrrolidinol was prepared essentially by the procedure of Lunsford.[20] 1,4-Dibromo-2-butanol (231 g.) and ethylamine (90 g.) afforded 36.7 g. (32%) of the desired product boiling at 98–100° (32 mm.), $n^{20}D$ 1.4655 [lit.[20] b.p. 107–110° (35 mm.), $n^{20}D$ 1.4650].

C. 1-Ethyl-3-chloropyrrolidine.—1-Ethyl-3-pyrrolidinol (36.3 g.) was added with cooling to a large excess of $SOCl_2$, after which the dark solution was refluxed gently for 1 hr. and concentrated *in vacuo*, and the residue was cooled and made basic with Na_2CO_3 solution. Extractions with ether, solvent removal, and distillation of the residue *in vacuo* gave 23 g. (55%) of the desired product, b.p. 72° (48 mm.), $n^{20}D$ 1.4558.

Anal. Calcd. for $C_6H_{12}ClN$: C, 53.93; H, 9.05. Found: C, 54.08; H, 9.16.

D. 1-Ethyl-3-pyrrolidyl Thiolacetate.—The modified procedure of Biel, *et al.*,[2c] was employed. A mixture of 1-ethyl-3-chloropyrrolidine (15 g.), thiolacetic acid (8.6 g.), and anhydrous isopropyl alcohol was refluxed for 8 hr. at which time additional thiolacetic acid (8.6 g.) was added. At the conclusion of an additional 8-hr. reflux period, the mixture was concentrated *in vacuo*, and the residue was dissolved in water, cooled, made basic, and repeatedly extracted with ether. The combined extracts were dried and concentrated, and the residue was distilled to give 14 g. (72%) of the desired product at 73° (3 mm.).

Anal. Calcd. for $C_8H_{15}NOS$: C, 55.45; H, 8.72. Found: C, 55.19; H, 8.69.

E. 1-Ethyl-3-mercaptopyrrolidine.—1-Ethyl-3-pyrrolidyl thiolacetate (6 g.) was dissolved in anhydrous methanol (50 ml.) and sodium (0.03 g.) was added. The resulting solution was refluxed through a 30-cm. Vigreux column while methyl acetate was slowly withdrawn. When the boiling point of methanol was reached, the reaction mixture was concentrated, and the residue was fractionally distilled to give 2.9 g. (65%) of the pure product, b.p. 42° (3 mm.), $n^{25}D$ 1.4884.

Anal. Calcd. for $C_6H_{13}NS$: C, 54.91; H, 9.98. Found: C, 54.74; H, 9.84.

1-Methyl-(3-methylmercapto)pyrrolidine.—1-Methyl-3-hydroxymethylpyrrolidine was supplied by W. T. Sumerford of Mead Johnson Research Center, Evansville, Ind.

A. 1-Methyl-(3-chloromethyl)pyrrolidine was prepared in 60% yield according to the procedure described above for the production of 3-chloro-1-ethylpyrrolidine. The pure product had b.p. 54° (25 mm.) [lit.[23] b.p. 56–57° (15 mm.)].

B. 1-Methyl-3-methylenepyrrolidyl thiolacetate was prepared in 90% yield according to the procedure described above for the production of 1-ethyl-3-pyrrolidyl thiolacetate. The product had b.p. 78° (3 mm.).

Anal. Calcd. for $C_8H_{13}NOS$: C, 55.45; H, 8.72. Found: C, 55.34; H, 8.65.

C. 1-Methyl(3-methylmercapto)pyrrolidine.—1-Methyl-3-methylene]pyrrolidyl thiolacetate (10 g.) was added to anhydrous methanol (24 ml.) dried by distillation over CaO. The solution was treated with metallic sodium (0.2 g.) followed by heating. The slowly refluxing solution was allowed to equilibrate, and the methyl acetate was slowly withdrawn between temperatures of 47 and 57°. When the boiling point of methyl alcohol was reached, the reaction mixture was cooled and treated with glacial acetic acid (0.5 ml.) and dry ether (30 ml.), and the precipitated sodium acetate was removed by filtration. The solvent was removed and the residue was fractionally distilled *in vacuo* through a 30-cm. Vigreux column to give 5.0 g. (65%) of the desired product boiling at 92–94° (35 mm.).

Anal. Calcd. for $C_6H_{13}NS$: C, 54.88; H, 9.22. Found: C, 54.86; H, 9.57.

1-Ethyl-3-mercaptopiperidine was prepared according to the literature[21] directions (yield 48%, b.p. 42–44° (0.9 mm.)] and by hydrolysis of the thiolacetate as described under 1-methyl(3-methylmercapto)pyrrolidine [yield 75%, lit. b.p. 57.5° (1.7 mm.)].

1-Methyl-4-mercaptopiperidine was prepared according to literature[23] directions.

A. 1-Methyl-4,4-dimercaptopiperidine melting at 47–49° was prepared (62 g., 68%) from 1-methyl-4-piperidone (58 g.) [lit.[23] m.p. 48–50°].

B. 1-Methyl-4-mercaptopiperidine was prepared (23.9 g., 53%) from 1-methyl-4,4-dimercaptopiperidine (62 g.) and $NaBH_4$ (22 g.). The pure product distilled at 64° (1 mm.) [lit.[23] b.p. 62° (0.8 mm.)].

Ester hydrochlorides of chloro acid chlorides in Table I were prepared by the method of Mehta.[24] The following procedure

(15) Melting points were taken on a Mel-Temp apparatus and are corrected. Boiling points are uncorrected.

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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°.

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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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