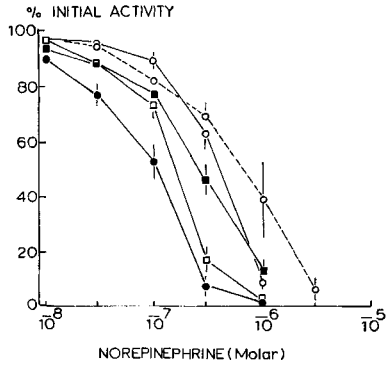

Fig. 2.—Illustrates the cumulative dose-response curves of norepinephrine on rabbit ileum in the presence of 10^{-3} *M* D(−)-ephedrine (○- - -○), L(+)-ephedrine (○——○), L(+)-pseudoephedrine (□——□), D(−)-pseudoephedrine (■——■). Norepinephrine control (●——●). Vertical lines, standard errors of the mean.

produce the same response. It appears that L(+)-pseudoephedrine is < antagonist than D(−)-pseudoephedrine which is ≤ L(+)-ephedrine. The interpretation of the antagonist potency of D(−)-ephedrine is complicated by its intrinsic inhibitory effects. Present results are compatible with the view that sympathomimetic amines may compete for "direct" sites, but the possibility of noncompetitive antagonism must be borne in mind. (Table II.)

TABLE II.—INHIBITION OF NOREPINEPHRINE EFFECTS ON RABBIT ILEUM BY 10^{-3} *M* CONCENTRATION OF EPHEDRINE ISOMERS

	(−)-Norepinephrine −Log ED_{50} with S.E.M.			
Control	D(−)-Ephedrine, 10^{-3} *M*	L(+)-Ephedrine, 10^{-3} *M*	L(+)-Pseudoephedrine, 10^{-3} *M*	D(−)-Pseudoephedrine, 10^{-3} *M*
6.96	6.16	6.41	6.78	6.49
±0.08	±0.13	±0.05	±0.04	±0.08
$n^a = 9^b$	$n = 6^c$	$n = 9^c$	$n = 8^c$	$n = 8^c$
	$P <$ 0.001	$P <$ 0.001	$P <$ 0.1	$P <$ 0.01

[a] *n*, number of observations. [b] Nine animals. [c] Three animals.

REFERENCES

(1) Thienes, C. H., *Proc. Soc. Expil. Biol. Med.*, **26**, 500 (1928–1929).
(2) Thienes, C. H., *Arch. Intern. Pharmacodyn.*, **47**, 453 (1934).
(3) Curtis, F. R., *J. Pharmacol. Expil. Therap.*, **35**, 333 (1929).
(4) Finkleman, B., *J. Physiol.*, **70**, 145 (1930).
(5) Van Rossum, J. M., and Mujic, M., *Arch. Intern. Pharmacodyn.*, **155**, 418(1965).
(6) LaPidus, J. B., Tye, A., Patil, P. N., and Modi, B., *J. Med. Pharm. Chem.*, **6**, 76 (1963).

3-Thenyl Nitrogen Mustards

By W. LEWIS NOBLES and CHARLES M. DARLING

As potential anticancer agents, three new 3-thenyl nitrogen mustards have been prepared from the corresponding diethanolamine derivatives. The NMR data for these compounds are reported.

IN A SERIES of antihistaminic compounds, Clapp and co-workers (1) have suggested that the inclusion of a halogen atom on the thiophene ring improves the therapeutic ratio. This suggestion has not been exploited in thiophene nitrogen mustards. Indeed, besides dimethyl derivatives (2), no nuclear substituted thiophene nitrogen mustards have been reported.

Campaigne's comparative data (3) suggest that in testing thiophene analogs, there is a high probability that the 3-isomer will be at least as active as the 2-isomer, if not more active. Of the six thiophene nitrogen mustards previously reported (2, 4, 5), four are thenyl derivatives (2, 5) and, of these, only two are 3-thenyl isomers (2).

In this investigation, three 3-thenyl nitrogen mustard derivatives have been prepared as potential anticancer agents. These are denoted by structure I in which R = H, 2-bromo, and 2,5-dichloro.

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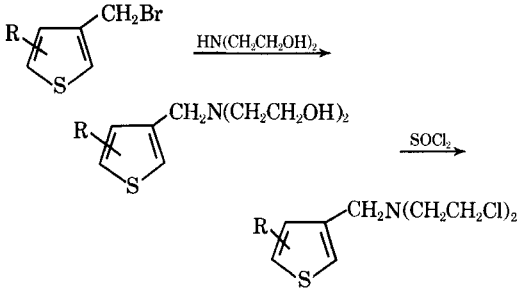
As outlined in Scheme I, the synthetic route employed for the preparation of these compounds is a modification of that of Wilson and Tishler (5).



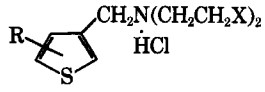
I

Scheme I

The 3-thenyl bromides were obtained by the reaction of *N*-bromosuccinimide with the respective methylthiophenes in the presence of catalytic amounts of benzoyl peroxide (6). The diethanolamine derivatives were prepared by treating the thenyl bromides with diethanolamine.

Preparation of the nitrogen mustard function by halogenation of the appropriate diethanolamine derivative with thionyl chloride (2, 5) or phosphorus oxychloride (4) has been reported in low yields, 12–27%. In each instance, the reaction mixture was cooled during the addition of the halogenating agent, and then it was heated to reflux. In the

TABLE I.—3-THENYLAMINES



Compd.	R	X	Yield, %	M.p., °C.	Formula	Anal., %[a] Calcd.	Found
I	H	OH	26[b]	82.5–83.5	$C_9H_{15}NO_2S\cdot HCl$	C, 45.46	45.75
						H, 6.78	6.88
						N, 5.89	5.51
						S, 13.49	13.03
II	2-Br	OH	16.8[b]	70.5–71.5[c]	$C_{19}H_{14}BrNO_2S$	C, 38.58	38.77
						H, 5.04	5.18
						N, 5.00	5.13
III	2-Cl	OH	24.3[b]	143.5–144.5	$C_9H_{13}Cl_2NO_2S\cdot HCl$	C, 35.25	35.54
						H, 4.60	5.02
						N, 4.57	4.40
						S, 10.46	10.65
IV	H	Cl	78[d]	109–110.5	$C_9H_{13}Cl_2NS\cdot HCl$	C, 39.36	39.27
						H, 5.14	5.39
						N, 5.10	4.77
						S, 11.68	11.71
V	2-Br	Cl	83[d]	130.5–131.5	$C_9H_{12}BrCl_2NS\cdot HCl$	C, 30.57	31.31
						H, 3.71	3.97
						N, 3.96	3.93
						S 9.07	9.16
VI	2-Cl 5-Cl	Cl	61[d]	164–165.5	$C_9H_{11}Cl_4NS\cdot HCl$	C, 31.46	31.50
						H, 3.52	3.78
						N, 4.08	4.10
						S, 9.33	9.26

[a] Analyses by Dr. Alfred Bernhardt, Mulheim, Germany. [b] Yield calculated from corresponding 3-methylthiophene derivative. [c] Melting point of the free base. [d] Yield of halogenation reaction.

present work, the reaction mixture was allowed to reflux during the addition of thionyl chloride, and higher yields of the nitrogen mustard function were obtained consistently. The compounds along with pertinent data are listed in Table I.

EXPERIMENTAL[1]

3-Thenyl Diethanolamines.—The 3-thenyl bromides were prepared by known methods (6, 7) and were used as the crude lachrymatory oils. Each crude 3-thenyl bromide compound was refluxed with a 2 *M* ratio of diethanolamine in chloroform for 16 to 18 hr. After cooling the solution to room temperature, the mixture was poured over ice and the chloroform layer separated. The chloroform was removed with a flash evaporator. The residue of compound II was recrystallized from diethyl ether as the free base. The hydrochloride salt of compound I was prepared by saturating an ethereal solution of I with anhydrous hydrogen chloride and recrystallizing from acetone. The hydrochloride salt of compound III was prepared by the addition of concentrated hydrochloric acid to a solution of III in acetone; the salt was then recrystallized from absolute ethanol.

The NMR spectrum of I in deuterated pyridine was δ = 3.61 (t, probably C—CH_2—O, 4H), 4.33 (t, probably N—CH_2—C, 4H), 4.84 (s, thienyl—CH_2—N, 2H), 7.87 (s, O—H, disappeared in D_2O exchange), and 7.60 (m, undetermined) p.p.m.

[1] All melting points were taken on a Thomas-Hoover Uni-Melt melting point apparatus and are corrected. The NMR spectra were taken using a Varian model A60-A instrument and were measured at 60 Mc. The chemical shifts are reported as δ-values (p.p.m.) from tetramethylsilane (internal standard). The multiplicity is shown by s = singlet, d = doublet, t = triplet, and m = incompletely resolved multiplet. Coupling constants, *J*, are reported in c.p.s. Assignments are shown in parentheses.

The NMR spectrum of II in deuterated acetone was δ = 2.66 (t, probably C.CH_2—O, 4H), 3.57 (t, probably N—CH_2—C, 4H), 3.68 (s, thienyl—CH_2—N, 2H), 3.15 (s, O—H, 2H), 7.05 (d, probably 5-H of ring, 1H), and 7.39 (d, probably 4-H of ring, 1H) p.p.m. (Each J_{4-5} = 5.7 c.p.s. ± 0.2 c.p.s.)

The NMR spectrum of III in deuterated pyridine was δ = 3.48 (t, probably C—CH_2—O, 4H), 4.25 (t, probably N—CH_2—C, 4H), 4.48 (s, thienyl—CH_2—N, 2H), 7.80 (s, 4-H of ring, 1H), and 8.11 (s, O—H, disappeared on D_2 exchange) p.p.m.

2,2′-Dichlorodiethyl-3-thenylamines.—The general procedure for the preparation of the nitrogen mustard function is reported below. The free base of the appropriate diethanolamine derivative was dissolved in chloroform and a large excess (about 5 *M* ratio) of redistilled thionyl chloride was slowly added without cooling. Reflux began spontaneously and was continued for 5 to 7 hr. The reaction mixture was cooled to room temperature, and the chloroform and excess thionyl chloride were removed with a flash evaporator. In each instance, the residue was recrystallized from acetone using charcoal.

The NMR spectrum of IV in deuterated chloroform was δ = 3.52 (t, probably C—CH_2—Cl, 4H), 4.01 (t, probably N—CH_2—C, 4H), 4.50 (s, thienyl—CH_2—N, 2H), 7.36 (d, probably 4,5-H of ring, 2H) (J = 2.2 c.p.s.), and 7.72 (t, probably 2-H of ring, 1H) (J = 2.2 c.p.s.) p.p.m. (all J's ± 0.3 c.p.s.).

The NMR spectrum of V in deuterated chloroform was δ = 3.62 (t, C—CH_2—Cl, 4H), 4.15 (t, N—CH_2—C, 4H), 4.49 (s, thienyl—CH_2—N, 2H), 7.46 (d, probably 5-H of ring, 1H), and 7.78 (d,

probably 4-H of ring, 1H) p.p.m. (each J_{4-5} = 5.7 c.p.s. ± 0.2 c.p.s.).

The NMR spectrum of VI in deuterated chloroform was δ = 3.58 (t, probably C—CH_2—Cl, 4H), 4.08 (t, probably N—CH_2—C, 4H), 4.38 (s, thienyl—CH_2—N, 2H), and 7.54 (s, 4-H of ring, 1H) p.p.m.

REFERENCES

(1) Clapp, R. C., Clark, J. H., Vaughan, J. R., English, J. P., and Anderson, G. W., *J. Am. Chem. Soc.*, **69**, 1549 (1947).

(2) Goldfarb, Y. L., and Kondakova, M. S., *Zh. Obshchei Khim.*, **30**, 102(1960); through *Chem. Abstr.*, **54**, 21037 (1960).

(3) Campaigne, E., *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 129(1957).

(4) Shirley, D. A., and Bell, G. R., *J. Med. Chem.*, **9**, 607(1966).

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

(6) Campaigne, E., and LeSuer, W. M., *ibid.*, **71**, 333 (1949).

(7) Campaigne, E., and Tullar, B. F., *Org. Syn.*, **33**, 96 (1953).

Pemoline and Magnesium Hydroxide *Versus* Pemoline: Enhancement of Learning and Memory of a Conditioned Avoidance Response in Rats

By N. PLOTNIKOFF and P. MEEKMA, JR.

Pemoline and magnesium hydroxide was found to be several times more potent than pemoline in enhancing the acquisition and retention of a conditioned avoidance response in rats.

NUMEROUS STUDIES in Europe indicated that the performance of fatigued humans was enhanced by pemoline under various test conditions (1–3). In previous studies Plotnikoff (4–7) reported that pemoline and magnesium hydroxide (PMH)[1] enhanced the acquisition and retention of a conditioned avoidance response in rats. The present study is a comparative study of PMH and pemoline on the same avoidance response.

EXPERIMENTAL

Methods—The test chamber as well as rates of acquisition and retention used for all conditioning studies were described earlier (4–7). The test equipment consisted of a wood chamber (11 × 12 in.) with a grid flooring. An escape platform was placed 11 in. above the grid floor outside of the test chamber. Male Sprague-Dawley rats (170 to 220 Gm.) were used. Only "slow learners" were used for all drug studies. Suspensions of the test drugs were prepared in 0.3% tragacanth. Acquisition trials consisted of the following 30-sec. sequence: 15 sec. inside the chamber without shock or buzzer, 10 sec. with buzzer, and finally 5 sec. of shock with buzzer. Retention trials consisted of a 30-sec. sequence without buzzer or shock stimulation. Criterion of learning was considered obtained when the mean jump-out time was 15 sec. or less for any given trial and succeeding trials.

Results—The principal difference observed between pemoline and PMH on the jump-out test was one of potency. As the data in Tables I and II illustrate, PMH is more potent in the enhancement of acquisition and retention of the jump-out response. In several studies, significant enhancement was observed with 0.3% tragacanth suspensions of PMH at doses of 1.25, 2.5, and 5.0 mg./Kg. p.o. In contrast, pemoline (0.3% tragacanth suspension) only showed significant enhancement at doses of 10 and 20 mg./Kg. p.o. Lower doses of pemoline (2.5 and 5.0 mg./Kg. p.o.) did not enhance acquisition or retention.

The approximate potency differences on acquisition between the two compounds is at least eightfold. Rats treated with PMH reached criterion of learning by the 9th trial at a dose of 1.25 mg./Kg., whereas pemoline-treated rats reached criterion of learning by the 4th trial at a dose of 10 mg./Kg.

The potency differences between the two compounds on retention were also approximately eightfold (1.25 mg./Kg. *versus* 10.0 mg./Kg.). Significant retention of the jump-out response was observed at a dose of 1.25 mg./Kg. in PMH-treated animals, whereas pemoline-treated animals only showed significant retention at a dose of 10 mg./Kg.

Control studies carried out with $Mg(OH)_2$ indicated there were no significant effects on acquisition or retention.

DISCUSSION

The present study has demonstrated that PMH is a more potent agent in enhancing acquisition and retention than pemoline. A similar difference between pemoline and PMH has been reported by Lange *et al.* (8) on anticonvulsant activity. The principal difference reported was the rate of absorption as determined by onset of anticonvulsant activity. PMH at a dose of 100 mg./Kg. exerted significant activity 15–30 min. after oral administration, whereas pemoline at the same dose exhibited activity only at 60 min. However, both PMH and pemoline have the same LD_{50} values in mice (oral LD_{50} 500 mg./Kg.). The effects of the two compounds are both similar resulting in overt stimulant effects at doses of 5–400 mg./Kg. and paradoxical depression (ataxia followed by coma) at toxic doses (500–1000 mg./Kg.). No convulsions were observed at toxic doses.

In the present study, PMH was demonstrated to be a more potent agent than pemoline in enhancing acquisition and, even more striking, retention of the jump response. Thus, it is possible that magnesium hydroxide component may be enhancing absorption. Clinically (9) PMH has been reported to have a faster onset of action and to have greater potency as an arousal agent than pemoline.

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[1] Marketed as Cylert by Abbott Laboratories, North Chicago, Ill. Abbott-30400; an equimolar combination of 2-imino-5-phenyl-4-oxazolidinone (Abbott-13397) and magnesium hydroxide.