Anal. Calcd. for $C_{18}H_{23}ClN_2O$: C, 67.80; H, 7.27; N, 8.79. Found: C, 68.01; H, 7.41; N, 9.12.

Acknowledgment.—The help of the following persons who contributed materially to this research is acknowledged: Dr. Robert T. Dillon and his associates for analyses and determination of physical properties; Mr. William Selby and Mr. M. G. Scaros for the running of all catalytic reductions; and Dr. Willard Hoehn and his associates for the preparation of several of the starting materials and intermediates used in these syntheses.

Behavioral and Neuropharmacological Actions of 3-Methyl-4-(1-phenyl-2-propylamino)-2-phenylmorpholine and Associated 4-Aminomorpholines

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Received August 13, 1963

A number of substituted 4-aminomorpholines have been examined for their neuropharmacological and behavioral effects. Methods utilized and results obtained with these materials are presented. One of these compounds, 3-methyl-4-(1-phenyl-2-propylamino)-2-phenylmorpholine, was selected for more intensive biological evaluation. A selective effect of this compound upon conditioned behavior is among the findings presented in detail.

Much laboratory work over the period of the last 20 years has been devoted to the synthesis and biological evaluation of compounds bearing either direct or remote configural resemblances to endogenously occurring psychoactive materials, of which the catecholamines represent a prominent example. Numerous derivatives of epinephrine have been studied[1-3] and several clinically useful materials, notably *d*-amphetamine and methamphetamine, have come out of this research. Of the many lines of investigation which have been opened up by this work, two of importance include phenmetrazine, which may be conceived as a cyclized amphetamine, and the hydrazine derivatives of amphetamine. Although perfectly consistent structure–activity relationships are lacking, it would appear that the central nervous stimulant aspect of drug action will survive either type of structural modification,[4,5] while the hydrazine substitution imparts additionally a monoamine oxidase inhibiting property to chemicals so prepared.[5,6] The possibility that compounds possessing structural features which included both the morpholine and hydrazine substituents might reveal important biological activities led to the investigation of a series of substituted 4-aminomorpholines.

A report covering the chemical preparation of some 41 of these compounds has been presented in the preceding paper.[7] In order to promote effective communication, the compound numbering system utilized therein will be preserved in the present report.

Materials and Methods

A list of several compounds selected for preliminary biological screening is given in Table I. It should be mentioned that for the sake of brevity only structures which were found to be "active" in one or more tests are included therein. A comprehensive list of structures tested, including those which were "inactive," can be found by referring to Table II of the preceding chemical paper.[7] The preliminary screening series included tests 1 through 4 described below. Tests 6 through 13 were considered to represent follow-up work on structures with more interesting activities. All compounds were administered as normotonic saline solutions or as microcrystalline suspensions. Physiological saline (0.9%) was the usual dissolving medium, but in some cases ethylene carbonate, propylene carbonate, or these two in equal parts were utilized. Control tests with the various solvents in appropriate concentrations revealed no measurable biological effects. The methods employed for individual tests were as follows.

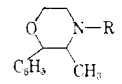
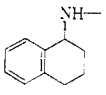
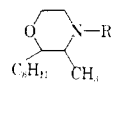
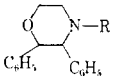
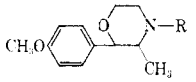
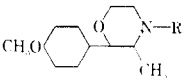
(A) Preliminary Screening Tests. (1) **Four-Hour Mouse Test.**—This test is an adaptation of the mouse screening procedure developed by Irwin.[8] The primary function of this test is to provide as rapidly as possible specific, quantitative data regarding the number and kinds of central and other effects which compounds may induce *in vivo*. To this end, male albino mice are observed and manipulated both before and after intraperitoneal administration of test compounds. A wide variation in dosage is employed, and ratings of both spontaneous and elicited behavior are recorded periodically, in order to provide data relevant to questions of potency and onset, peak, and duration of action. In the present experiments, ratings for dose groups were summed over a 4-hr. period and compared with scores obtained with standard reference materials, such as *d*-amphetamine (stimulant) or thiopropazate (depressant). Compounds achieving scores equal to those produced by the reference materials, whether at doses equal to, 2, or 4 times the minimally active doses of the reference compounds, were considered to be "stimulant" or "depressant." The determination of LD_{50} doses (doses lethal to 50% of mice so treated) was by simple count at the end of the 4-hr. period.

(2) **Four-Hour Cat Test.**—This test combines many of the features of the procedures developed independently by Irwin[8] and S. Norton[9] for the study of the effects of drugs in the cat. In our experiments, testing was carried out in a fully lit laboratory room containing six contiguous wire-mesh observation cages (76.2 × 81.4 × 142.4 cm.), which permitted the cats virtually complete visual access to each other and to the experimenter, as well as providing sufficient floor space to encourage locomotor activity in subjects so inclined. Subjects were of either sex, and no studies involving test drugs were permitted with a given

(1) A. M. Lands, *Pharmacol. Rev.*, **1**, 279 (1949).
(2) C. D. Leake, "The Amphetamines," Charles C Thomas, Springfield, Mass., 1958.
(3) J. W. Schulte, E. C. Reif, J. A. Bacher, Jr., W. S. Laurence, and M. L. Tainter, *J. Pharmacol. Exptl. Therap.*, **71**, 62 (1941).
(4) R. H. Barnes, *J. Am. Med. Assoc.*, **166**, 898 (1958).
(5) J. H. Biel, P. A. Nuhfer, and A. C. Conway, *Ann. N. Y. Acad. Sci.*, **80**, 568 (1959).
(6) L. G. Eltherington and A. Horita, *J. Pharmacol. Exptl. Therap.*, **128**, 7 (1960).
(7) M. J. Kalm, *J. Med. Chem.*, **7**, 427 (1964).
(8) S. Irwin in "Clinical Pharmacology: Animal and Human Techniques," J. Nodine and P. Siegler, Ed., Year Book Medical Publishers, Chicago, Ill., 1964.
(9) S. Norton in "Psychotropic Drugs," S. Garattini and V. Ghetti, Ed., Elsevier Publishing Company, Amsterdam, 1957.

TABLE I

CENTRAL NERVOUS SYSTEM EFFECTS OF SUBSTITUTED 4-AMINOMORPHOLINES[a]

No.	Structure	R	4-Hr. mouse test[b] LD50, mg./kg.	Response	4-Hr. cat test[b] Dose, mg./kg.	Response	2-Hr. rat motility test[b] Dose, mg./kg.	Response	Conditioned avoidance test[c] Dose, mg./kg.	Response
4		—NH_2	>450	O	40	O	25	S	27	O
									81	S
5A		—$NHCH(CH_3)CH_2C_6H_5$	>450	O	30	O	50	O		
					90	D	100	O	27	O
					200	D	200	S	81	S
d-5A		—$NHCH(CH_3)CH_2C_6H_5$	>450	O	40	O	5	D	27	O
									81	S
l-5A		—$NHCH(CH_3)CH_2C_6H_5$	>450	O	5	O	5	D	27	O
									81	O
8		—$NHCH(CH_3)_2$	>450	D	20	O	50	D	81	O
10		—$NHCH(CH_3)C_6H_5$	>450	S	10	O	20	O	27	O
11		—CH_2CH 	>300	S	5	O	10	D	27	O
13		—$NHCH_2CH_2C_6H_5$	>450	S	20	S	40	S	27	O
22		—$NHCH(CH_3)CH_2CH_2C_6H_5$	>300	S	10	O	20	O	27	O
28			>450	O	5	O	20	S	27	O
29		—NH_2	>450	S	40	O	10	O	27	O
34		—$NHCH(CH_3)CH_2C_6H_5$	>450	S	10	O	10	O	9	O
					20	D	20	S	27	S
					40	D			81	S
38		—NH_2	>300	S	...	...	10	O	9	O
							25	O	27	S
41		—NH_2	>320	O	40	O	40	D	...	...
42		—$NHCH(CH_3)CH_2C_6H_5$	>320	O	20	O	25	D	27	O
43		—NH_2	>320	O	10	O	40	D	...	...
44		—$NHCH(CH_3)CH_2C_6H_5$	>320	O	5	O	20	D	...	...

[a] Only compounds which were active in one or more tests are shown. A comprehensive list of structures tested may be found in Table II.[7] [b] Responses are recorded as follows: S = stimulant, D = depressant, O = inactive. [c] Responses are for a 90 min. run, and constitute avoidance data only.

TABLE II

CENTRAL NERVOUS SYSTEM EFFECTS OF PHENMETRAZINE, *d*-AMPHETAMINE, AND PHENIPRAZINE

Compound	4-Hr. mouse test LD50, mg./kg.	Response	4-Hr. cat test Dose, mg./kg.	Response	2-Hr. rat motility test Dose, mg./kg.	Response	Conditioned avoidance test[a] Dose, mg./kg.	Response[b]
Phenmetrazine	165	S	5	O	10	O	10	O
			10	S	20	S	20	S
					40	S	40	S
d-Amphetamine	80	S	0.63	O	0.08	O	0.32	O
			1.25	S	0.16	S	0.63	S
			2.5	S	0.32	S	1.25	S
Pheniprazine	165	S	1.25	O	5	O	3.0	O[c]
			2.5	S	10	S		
			5.0	S	20	S	9.0	S

[a] Responses are recorded as follows: S = stimulant, D = depressant, O = inactive. [b] Responses are for a 90 min. run and constitute conditioned avoidance data only. [c] Pheniprazine is more variable in its effects. Stimulation is sometimes observed with this dose.

subject until a minimum of 10 control observations had been recorded for that cat. Subjects were used on compounds not more than once each week. Test substances were administered intraperitoneally, with concentrations adjusted so that each cat received a volume of 1 ml./kg. of body weight. Doses selected for study are shown in Table I. Pre- and post-injection observations, which were carried out by an experimenter ignorant of the nature of the injections, proceeded as follows.

At periodic intervals, the experimenter observed each rat from a distance, scrutinizing its ongoing behavior for signs of excessive or deficient locomotor-manipulative behavior, and for symptoms of somatic and autonomic involvement. The

features present under these conditions were considered to include spontaneous or unelicited patterns of activity. Immediately following the recording of these observations, the experimenter approached each subject individually, entered its cage and handled it for a short period, completing each of these operations with a brief roughing up of the cat's fur, in order to observe more closely somatic and autonomic features, but particularly to determine the animal's reactivity to social stimuli. Thus, an overtly quiescent cat could show considerable excitement when disturbed in this fashion. Symptoms of excitement, contentment, sociability, and/or hostility were recorded on a checklist, in the manner of Norton.[9] For purposes of evaluating drug activity, the semiquantitative data thus obtained were reduced to a simple qualitative statement indicating only the direction of the activity—that is, stimulation or depression—where such effects were observed. Observations were made routinely on each cat in his home cage for several successive post-drug days, in order to check for possible longer lasting drug effects.

(3) **Two-Hour Rat Mobility Test.**—Adult albino rats were injected intraperitoneally with selected doses of the various materials and then placed immediately into individual, suspended motility cages. Transduction of animal movement was accomplished *via* an electromagnetic circuit linked to integrators and digital counters.[10] Concurrent control groups, injected with solvent, provided comparative data suitable for statistical comparison. Doses of compounds which produced activity counts differing from control groups at the 0.05 level of confidence were considered "active." Duration of test run was 2 hr.

(4) **Conditioned Avoidance Test.**—This test, in contrast to those previously described, measured changes in conditioned or learned behavior. The nondiscriminated avoidance procedure, described earlier by Sidman,[11] was utilized. The experimental paradigm and procedure for assessing drug activity used in these experiments are described in a previous report.[12] Briefly stated, selected doses of compounds were tested for their effects upon the response rates of rats trained to press a small lever in order to avoid periodic electric shocks to the feet. In the preliminary screening series, compound administration was interpolated between two 90-min. test sessions, the first session acting as a statistical control. In follow-up experiments, longer post-injection sessions were employed in some cases, in order to provide data relevant to the question of duration of action.

Evaluation of drug effects upon this type of learned behavior was derived from data pertaining not only to changes in response rates *per se*, but also to changes in the proportion of responses which were specifically avoidance responses. Since in this test shocks occur at regular, periodic intervals in the absence of lever pressing, increased rates of responding tend to increase also the number of shocks avoided. However, this is not an irrevocable relationship. Poor timing of responses, responding immediately after a shock, and otherwise erratic behavior may actually result in a test subject receiving *more* shocks despite an elevated response rate. Statistical evaluation of changes in response rates was straightforward—a comparison of mean response rates under control and drug treatment conditions. Differences between means significant at the 0.05 level of confidence were considered to indicate "active" doses. Avoidance data was converted to probits prior to analysis, however, in order to facilitate a comparison of rates among animals with differing control levels.[13]

(B) **Additional Behavioral and Pharmacological Tests.** (5) **Differential Reinforcement of Low Rate Responding.**—Using a variation of the differential reinforcement of low rates procedure (DRL) described by Sidman,[14] food-deprived rats were trained in a Skinner box to make discrete lever presses approximately 18 sec. apart, for only if these intervals were observed would food pellets be forthcoming. Fully trained rats typically anticipate the reinforcement interval, and the greater percentage of their responses fall in the intervals which just precede the appropriate interval. Even so, some 25% or more of responses will be delayed by 18 sec., resulting in a moderately efficient pattern of responding. In this experimental situation, drugs which enhance lever pressing rates reduced efficiency and decrease the number of pellets issued to the subject. In the present experiments compounds were administered intraperitoneally, and the animal was immediately placed into the test chamber, with the duration of the test period being fixed at 1 hr.

(6) **Hexobarbital Sleep Time.**—Adult, male mice in groups of 10, were injected intraperitoneally with various doses of test compounds. After a predetermined interval, depending upon the known time to peak action of the various substances, hexobarbital sodium[15] was injected intraperitoneally at a dose of 100 mg./kg. At the onset of hypnosis, each mouse was placed on his back on a laboratory bench. When a subject manifested signs of recovery of the righting reflex, he was replaced on his back in order to check his neurologic state more thoroughly. Only when a subject righted himself twice within a 15 sec. interval was the mouse credited with recovery from hexobarbital. The number of minutes during which the righting reflex was absent was taken as the end point in this test. The statistical significance of the results was tested by means of the Wilcoxon rank-sum method.[16]

(7) **Influence on Reserpine Sedation.**—Using a variation of the procedure reported by Burton, *et al.*,[17] groups of mice were given parenteral injections of 10 mg./kg. of reserpine[15] followed, after a latency of 3 to 4 hr., by intraperitoneal injections of various doses of test compounds. The measurement made in this assay was the number of minutes during which treated mice were alert and active, a period designated "reversal time."

(8) **Monoamine Oxidase Inhibition;** *in Vitro.*—*In vitro* testing was by the method proposed by Zeller and co-workers,[18] utilizing rat liver mitochondria.[19] Test compounds were assayed at concentrations of 10^{-4} *M*. The end point employed was the oxygen consumption per mg. of mitochondrial protein during a 40-min. incubation period.

(9) **Monoamine Oxidase Inhibition;** *in Vivo.*—Mice in groups of 4 were used for *in vivo* testing. Test compounds were injected intraperitoneally and, after a latency of 2 hr., were followed by intraperitoneal administration of a 200 mg./kg. dose of dihydroxyphenylalanine. In the present experiments, individual mice were disturbed periodically by pointed but unsharp probes and their response to this treatment was recorded on a three point scale: 0, no response; 1, discernible irritability; and 3, marked irritability. Mean scores were calculated for the various dose groups and compared directly without resort to statistical analysis.

(10) **Blood Pressure in Anesthetized Dogs.**—3-Methyl-4-(1-phenyl-2-propylamino)-2-phenylmorpholine (**5A**), the only material of this series which received experimental examination in this and other cardiovascular studies, was tested for blood pressure effects in 2 normotensive mongrel dogs, each anesthetized to effect with sodium pentobarbital.[15] The compound was administered intravenously as a 1% solution in 50% propylene glycol. Each dog was given successive doses of 0.1, 1.0, and 5 mg./kg. at 15 min. intervals. Femoral arterial pressure was monitored continuously after the initiation of treatment to include a 20-min. period following the last dose.

(11) **Electrocardiogram in Unanesthetized Dogs.**—Compound **5A** was tested in normotensive dogs trained to remain quiescent under conditions of electrocardiographic recording. The compound was administered intravenously to 2 dogs in doses of 5 mg./kg. repeated at 5 min. intervals. One dog received a total dose of 15 mg./kg., the other received 20 mg./kg. Intragastric administration of this substance was made also in two dogs, one receiving a 40 mg./kg. dose, the other being given 60 mg./kg. Lead (II) was used for recording the electrocardiogram, and analysis of the obtained records was by the method described by Arbeit, *et al.*[20]

(12) **Cardiac Output in Dogs.**—Cardiac output was determined using the dye dilution technique described by Mokler and

(10) D. L. Knapp, H. S. Backus, and B. Olson, *Lab. Pract.*, **11**, 138 (1962).

(11) M. J. Sidman, *J. Comp. Psychol.*, **46**, 253 (1953).

(12) D. L. Knapp, G. C. Stone, W. E. Hambourger, and V. A. Drill, *Arch. Intern. Pharmacodyn.*, **135**, 152 (1962).

(13) The author is grateful to Dr. Bernard Bernstein for carrying out and interpreting these experiments.

(14) M. Sidman, *Science*, **122**, 925 (1955).

(15) Hexobarbital sodium (Evipal®); reserpine (Serpasil®); sodium pentobarbital (Somnopentyl®); *d*-amphetamine (Dexedrine®); thiopropazate (Dartal®); phenmetrazine (Preludin®); nialamide (Niamid®).

(16) F. Wilcoxon, *Biometrics Bull.*, **1**, 80 (1945).

(17) R. M. Burton, M. A. Sodd, and A. Goldin, *Arch. Intern. Pharmacodyn.*, **112**, 188 (1957).

(18) E. A. Zeller, J. Barsky, and E. R. Berman, *J. Biol. Chem.*, **214**, 267 (1955).

(19) The author is grateful to Dr. Robert Ranney for carrying out and interpreting these experiments.

(20) S. R. Arbeit, T. L. Rubin, and H. Gross, "Differential Diagnosis of the Electrocardiogram," F. A. Davis and Co., Philadelphia, Pa., 1960.

Van Arman.[21] A single mongrel dog anesthetized with sodium pentobarbital was the subject used. Two intravenous injections of **5A** were made, the first 5 mg./kg. dose being separated from the second by 30 min.[22]

(13) **Appetite Depressant Properties in Rats.**—Possible appetite depressant properties of **5A** were assayed in male Badger rats weighing between 200 and 270 g. at the start of the experiment.[19] Daily, for a period of 11 days, each individually housed rat was given a 20 g. portion of Purina powdered laboratory diet containing either no additional material, or various amounts of compound **5A**, as shown in Table V. Water was available at all times. Daily measurements were made of the food consumed by each rat, careful account being taken of spillage. The rats were weighed 3 times during the experimental period, on the first, fifth, and last experimental day. Phenmetrazine,[15] often used as a standard reference compound in studies of this kind, was also tested. However, in the case of phenmetrazine, the experiment was terminated on the 9th day of compound administration.

Results

Preliminary Screening.—The activities of the various compounds in the 4 preliminary tests are given in Table I. These 17 substances were "stimulant" or "depressant" in one or more tests. Of these, 7 compounds showed only stimulatory effects in 1 or more tests, 6 produced only depression, and the remaining 4 structures—specifically, **5A**, *d*-**5A**, **11**, and **34**—produced mixed effects, depending upon the assay. Toxicity of these materials was uniformly low, no deaths having been observed in mice receiving doses in some cases as high as 300 mg./kg., intraperitoneally, in other cases, doses as high as 450 mg./kg.

Results obtained in this same series of tests with 3 standard reference compounds, phenmetrazine,[15] pheniprazine,[15] and *d*-amphetamine,[15] shown in Table II, provide an interesting comparison. Although differing somewhat in potency, these compounds produced psychomotor stimulation at relatively low doses in all the experimental situations, whether the measurements being made related to spontaneous, unlearned behaviors, as in the 4-hr. observation test and the motility test, or to learned behaviors, as in the conditioned avoidance test. The uniformity of the profiles of activity for phenmetrazine, pheniprazine, and *d*-amphetamine suggest a common mechanism—presumedly sympathomimetic—with these compounds. In contrast, the results shown in Table I for the 4-aminomorpholines—wherein "depression" may be registered in one test and "stimulation" in another (*e.g.*, **5A, 34**)—suggest an element of selectivity. The possibility that compounds affecting learned behaviors more or less specifically might possess other distinctive properties seemed worthy of investigation. In choosing structures for more detailed investigation, therefore, we focused on those which appeared to enhance selectively learned behaviors; that is, those which were active stimulants in the conditioned avoidance situation but which were not stimulatory in the other 3 preliminary tests, all of which presumedly measured changes in unlearned behavior. Of these, 3 materials were of immediate interest, **5A,** *d*-**5A,** and **34**. Follow-up testing was instituted for one of these, **5A,** and it is with this compound that the rest of this report is concerned.

(21) C. M. Mokler and C. G. Van Arman, *J. Pharmacol. Exptl. Therap.*, **136**, 114 (1962).

(22) The author is grateful to Dr. Corwin Mokler for carrying out and interpreting these experiments.

Results with 3-methyl-4-(1-phenyl-2-propylamino)-2-phenylmorpholine Hydrochloride (5A). 1. Four-Hr. Mouse Test.—In the 4-hr. mouse test, **5A**, given intraperitoneally, was essentially inactive over a wide range of doses. As can be seen in Fig. 1, the excitement and depression scores for **5A** are very

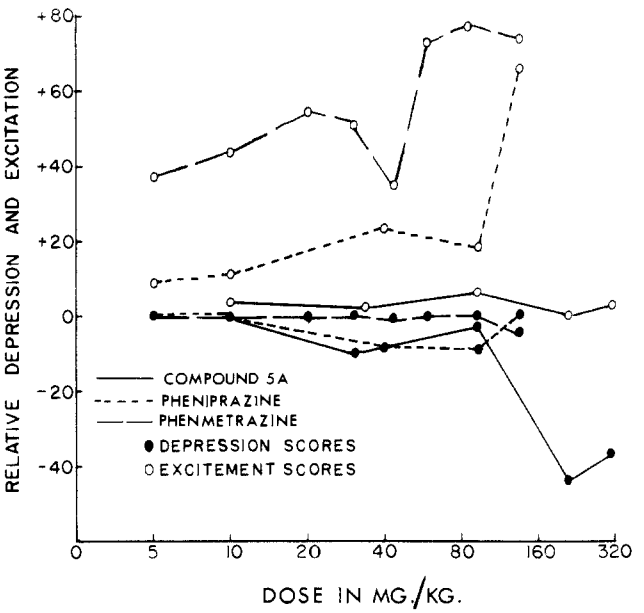


Fig. 1.—Excitory and depressant effects in mice. Injection intraperitoneal.

close to zero, with the exception that doses of 200 and 300 mg./kg. produced moderate depression. Both pheniprazine and phenmetrazine produced higher excitement scores than did **5A**, and both were devoid of significant depressant action. No deaths were observed with **5A** with doses as high as 450 mg./kg. The approximate lethal dose for 50% of animals for both pheniprazine and phenmetrazine was 165 mg./kg. Side effects with **5A** were minimal, consisting of slight ataxia at higher (depressant) doses and occasional Straub-tail reactions.

(2) **Four-Hr. Cat Test.**—Repeated testing with **5A** in doses ranging from 10 through 30 mg./kg. revealed no stimulant or depressant effects in cats. Doses of 90, 100, and 200 mg./kg. produced either no observable effects or mild depression of spontaneous and elicited behavior. Anorexia, lasting in some cases 6 or 7 days, was recorded for this compound. As can be seen in Table II, all of the reference compounds produced observable psychomotor stimulation in the same species.

(3) **Two-Hr. Rat Motility Test.**—Intraperitoneal injections of pheniprazine, phenmetrazine, and *d*-amphetamine, each at a dose of 5 mg./kg., produced motility counts which exceeded those of solvent-injected animals by 95 to 120% in various experiments. All of these results were statistically significant at the 0.05 level of confidence. Compound **5A** was not stimulatory or depressant at doses up to, and including, 100 mg./kg. At 200 mg./kg., however, it produced significant increases in motility counts which varied between 120 and 350%. Given intragastrically, **5A** was inactive in doses as high as 81 mg./kg.

(4) **Conditioned Avoidance Test.**—In preliminary screening tests, in which 90 min. test runs were used, **5A** was found to enhance avoidance responding rates at both 27 and 81 mg./kg. However, the increased rates affected by the lower dose did not reach statistical significance, and this dose is reported as inactive in Table I. As can be seen in Table II, phenmetrazine, *d*-amphetamine, and pheniprazine also increased response rates under similar test conditions. More extensive testing was carried out with **5A** and with pheniprazine, utilizing longer test periods. The time course of the effects of **5A** and of pheniprazine upon conditioned avoidance responding in rats is shown in Fig. 2 and 3. In Fig. 2, the per cent response change for various intraperitoneal doses of the two compounds indicates that both substances are capable of inducing increased rates of responding in rats pressing a small lever to avoid periodic electric shocks to the feet. Compound **5A** (27 mg./kg.) and 3 mg./kg. of pheniprazine produced roughly equivalent increases in response rates (*ca.* 50%), with a suggestion of a delayed effect for phen-

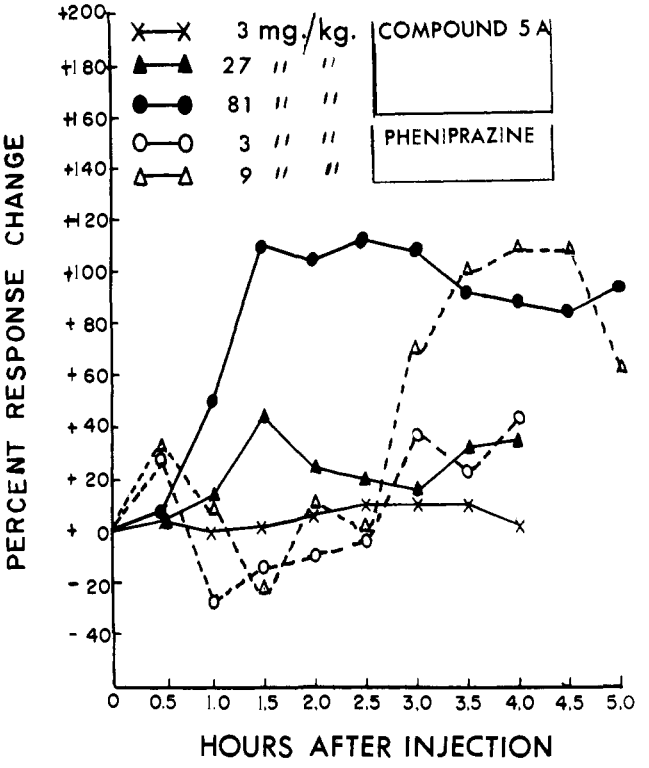


Fig. 2.—Effects on response component of conditioned avoidance behavior in rats. Injections intraperitoneal.

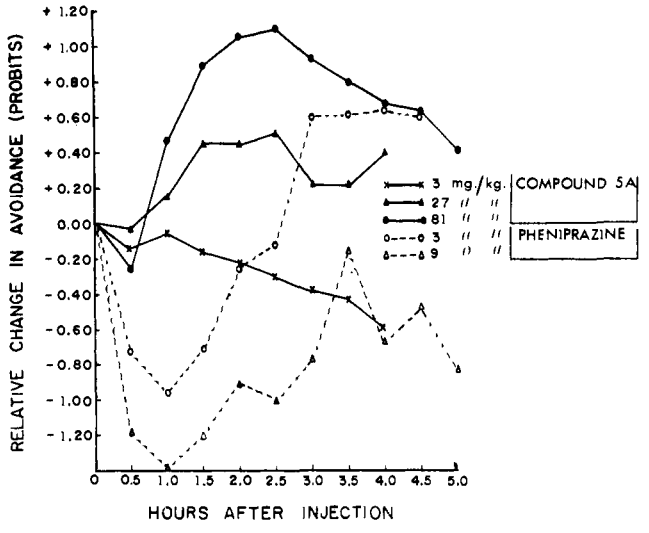


Fig. 3.—Effects on avoidance component of conditioned avoidance behavior in rats. Conversion to probits facilitates comparison of avoidance rates in animals with differing control levels.

iprazine. Increasing these different doses by a factor of 3 led to marked increases in avoidance rates, **5A** producing a response change of 110% at 81 mg./kg. Pheniprazine had a similar quantitative effect, but again, and more clearly than was the case with the lower doses, the peak effect for pheniprazine occurred only after a latency of some 2.5 hr., while **5A** demonstrated peak action at about 1 hr. The effects of **5A** and pheniprazine upon the avoidance component of this behavior is presented in Fig. 3. Compound **5A** increased avoidances at both 27 and 81 mg./kg. Doses of 3 and 9 mg./kg. of pheniprazine actually decreased avoidances, however, over a significant portion of their time of action. Only when response rates had achieved very high levels, such as in the case of the 9 mg./kg. dose after 3 hr., did avoidance rates exceed control means. The apparent decrease in avoidance attributable to the 3 mg./kg. dose of **5A** is well within the limits of expected biological variation and must be considered fortuitous. In the shock avoidance situation, therefore, **5A** was found to increase both responses and avoidances, while pheniprazine enhanced response rates more selectively.

(5) **Differential Reinforcement of Low Rate Responding in Rats (DRL).**—The effects of **5A** on DRL responding are shown in Fig. 4, which is a frequency histogram of interresponse intervals

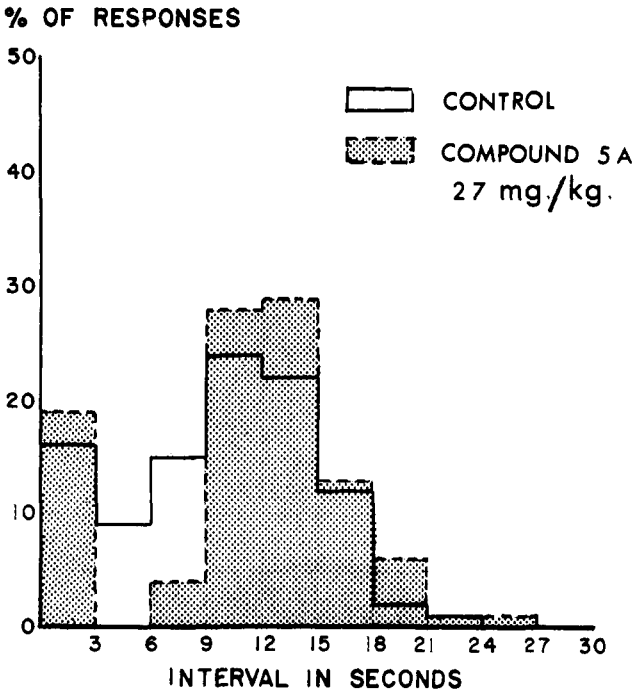


Fig. 4.—Effects on DRL responding in rats. Explanation in text.

recorded for an individual rat under both control and treatment conditions. As is shown there, some 15% of the animal's lever presses under control conditions were spaced by 15-21 sec., the "correct" or reinforced interval. The majority of his responses were spaced more closely together, however, demonstrating clearly the anticipatory feature so characteristic of this behavior. Following an intraperitoneal injection of **5A**, a shift to longer interresponse intervals was manifested by this subject, increasing the efficiency of the behavior. In a similar testing situation, drugs such as *d*-amphetamine have been found to shorten interresponse intervals, thus decreasing the number of pellets issued to the subject.[14] Such a reduction in efficiency has not been observed with **5A**. In several series of tests, **5A** either left the behavior unaltered or lengthened interresponse intervals, thus increasing efficiency. It should be pointed out that this was accomplished at doses (27 mg./kg.) which had enhanced response rates in the conditioned avoidance test, where increased rates were advantageous to the subjects.

(6) **Hexobarbital Sleep Time.**—The effects of **5A** on hexobarbital sleep time are presented in Table III. Under the con-

TABLE III

EFFECT OF 3-METHYL-4-(1-PHENYL-2-PROPYLAMINO)-2-PHENYLMORPHOLINE HYDROCHLORIDE (**5A**) ON HEXOBARBITAL SLEEP TIME

	Dose (i.p.), mg./kg.					
	3	9	27	40	80	160
Potentiation, %[a]	−10.1	+1.4	−3.1	+61.4[b]	+40.2[b]	+106.3[b]

[a] Potentiation was registered as the per cent increase in median sleep time in minutes of treated mice over control mice given only a standard volume of physiological saline plus 100 mg./kg. of hexobarbital (i.p.). [b] Results significant at less than the 0.05 level of confidence by the Wilcoxon rank-sum test.

ditions of testing, **5A** was found to increase the median sleep time of mice given doses of 40 mg./kg. and above, but not those given 27 mg./kg. or less.

(7) **Influence on Reserpine Sedation.**—The effects of **5A**, pheniprazine, phenmetrazine, and *d*-amphetamine in mice pre-

treated with reserpine are given in Table IV. The state of sedation or tonic quiescence induced by reserpine was reversed to one of excitation by all of the compounds tested, except for **5A**.

TABLE IV

EFFECT OF 3-METHYL-4-(1-PHENYL-2-PROPYLAMINO)-2-PHENYLMORPHOLINE HYDROCHLORIDE (**5A**) AND RELATED REFERENCE MATERIALS ON RESERPINE-INDUCED SEDATION IN MICE

Dose, mg./kg.	Compound **5A**	Reversal time, min.[a] Pheniprazine	Phenmetrazine	*d*-Amphetamine
10	0	28	18	132
15	0	39.5	23	>150
30	0	38	37.5	...
50	0	...	>150	...
90	0	...	...	...

[a] Reversal time refers to period during which reserpine-induced sedation was reversed to one of overt motor stimulation.

(8) **Monoamine Oxidase Inhibition;** *in Vitro*.—In two experiments, **5A** produced an inhibition of oxygen consumption of only 10%, a value well within the limits of expected random variation. Nialamide,[15,23] clearly established as an inhibitor of monoamine oxidase, both *in vivo* and *vitro*, affected a pronounced inhibition of oxygen consumption in repeated experiments, typically around 60%.

(9) **Monoamine Oxidase Inhibition;** *in Vivo*.—Compound **5A**, in intraperitoneal doses ranging from 5 through 80 mg./kg., was found to produce no irritability in mice when followed by DOPA after a 2-hr. latency. Nialamide, in repeated experiments under similar conditions, routinely produced a maximum response (+2) when given in doses as low as 10 mg./kg.

(10) **Blood Pressure in Anesthetized Dogs.**—Compound **5A** was found to have no effect upon blood pressure when given intravenously to pentobarbitalized dogs in doses of 0.1, 1.0, and 5 mg./kg., given 15 min. apart. Phenmetrazine and *d*-amphetamine each produced significant pressor effects at doses of 0.1 and 1.0 mg./kg., and depressor effects at 5 mg./kg.

(11) **Electrocardiogram in Unanesthetized Dog.**—Total intravenous doses of 15 and 20 mg./kg. of **5A** were without significant effect on the PR interval, ST segment, or QRS complex in 2 conscious dogs. The P wave showed no significant change in amplitude or duration. Dogs receiving intragastric doses of 40 and 60 mg./kg. were examined at 15 min. intervals from 1.5-6 hr. after administration of the compound. A heart rate decrease from 72/min. to 60/min. was recorded for the dog receiving the 40 mg./kg. dose, while the dog administered 60 mg./kg. showed a decrease in heart rate from 92/min. to 70/min. These changes are within normal limits and could be due to the long period of inactivity.

(12) **Cardiac Output in Dogs.**—An initial 5 mg./kg. dose of **5A** was followed by a 20% decrease in cardiac output. Thereafter, output returned to control levels and was not changed by the subsequent injection of another 5 mg./kg. Heart rate, blood pressure, and electrocardiogram were unchanged from control levels during the experiment.

(13) **Appetite Depressant Properties in Rats.**—Results for these experiments are presented in Table V. These data indicate dose related appetite depressant effects for both **5A** and phenmetrazine. In general, the reduction in food intake produced by **5A** was greater than that attributable to phenmetrazine. Both compounds produced decrements in body weights, although in this respect the data do not permit a clear cut comparison of relative potencies of the two materials.

TABLE V

APPETITE DEPRESSANT EFFECTS OF 3-METHYL-4-(1-PHENYL-2-PROPYLAMINO)-2-PHENYLMORPHOLINE HYDROCHLORIDE (**5A**) AND PHENMETRAZINE

Treatment group	Concentration of compound in daily 20-g. portions[a]	Average daily food consumption, g.[a]	Average daily consumption of compound/kg. wt.[a]	Over-all average change in body weight, g.[b]
Controls	...	14.0	...	+8
Compound **5A**	0.3 mg./g.	10.3	13	0
	0.6 mg./g.	8.6	23	−17
	1.0 mg./g.	5.6	28	−40
		$\bar{x}$ = 8.16	$\bar{x}$ = 21.3	$\bar{x}$ = −19
Controls	...	14.9	...	+12
Phenmetrazine	0.3 mg./g.	11.7	16	−14
	0.6 mg./g.	10.1	28	−21
	1.0 mg./g.	9.8	45	−31
		$\bar{x}$ = 10.5	$\bar{x}$ = 29.6	$\bar{x}$ = −22

[a] Based on data obtained during first 9 days of test. [b] Based on 11 days for **5A**, 9 days for phenmetrazine.

Discussion

Our present search in this series of 4-aminomorpholines for structures which can improve selectively the performance of learned behaviors has met with limited success. Of the 41 structural variations which have been submitted to initial screening evaluation, only a few produced a profile of effects which was considered desirable, *i.e.*, lack of effect or at least lack of stimulating effects upon the spontaneous, unconditioned behavior of mice, rats, and cats, concomitantly with an enhancement of lever pressing rates of rats trained to respond for various reinforcements. One member of this structural series, 3-methyl-4-(1-phenyl-2-propylamino)-2-phenylmorpholine hydrochloride (**5A**), which had produced such a profile, was found in follow-up tests to possess diverse pharmacological and behavioral properties which were of considerable theoretical interest. This material showed itself capable of inducing improved performance in two types of behavioral situations, where the motivational and reinforcement parameters were greatly different and, in a sense, opposed. In the avoidance test, where periodic electric shocks were administered to rats which failed to press an exposed lever at relatively frequent intervals, **5A** enhanced lever pressing rates, increasing the number of shocks avoided. This was accomplished without the sacrifice of accurate timing which was associated with the administration of pheniprazine. When given to rats in an experiment which required lever pressing at a slow rate for food reinforcement, compound **5A** did not shorten interresponse intervals, as would *d*-amphetamine, but rather left the behavior unaffected or lengthened slightly the interresponse interval. Thus **5A**, in rats under two differing motivational states, produced behavioral improvement whether the experimental situation required for greater efficiency either increased

(23) R. P. Rowe, *Diseases Nervous System* (suppl.), **20**, 5 (1959).

or decreased rates of responding. An anorexogenic effect of **5A**, observed initially in cats, was confirmed in experiments with rats, where an appetite depressant action for **5A** was clearly demonstrated. The neurophysiological and biochemical mechanisms which underlie the effects observed with compound **5A** are as yet undetermined. That the mode of action includes a sympathomimetic mechanism, as in the case of *d*-amphetamine, is discounted tentatively on the evidence that the compound does not reverse to excitation the state of behavioral quiescence induced by prior administration of reserpine. That this material potentiates the action of hexobarbital, rather than antagonizing its effects, persuasively supports this interpretation. *In vivo* and *in vitro* tests have revealed no monoamine oxidase inhibiting properties for **5A**. Cardiovascular experiments seem to indicate that **5A** is devoid of the hypertensive and cardiac acceleratory effects associated with *d*-amphetamine. From these considerations we conclude that the behavioral effects observed with 3-methyl-4-(1-phenyl-2-propylamino)-2-phenylmorpholine hydrochloride depend for their manifestation upon other than sympathomimetic or monoamine oxidase inhibiting properties.

Anticonvulsants. II. Spiro Compounds. Dibenzo[*a,d*]cycloheptadiene-5,5′-hydantoins, -5,5′-oxazolidinediones, and -5,2′-succinimides

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Received December 11, 1963

Spiro{dibenzo[*a,d*]cycloheptadiene-5,5'-hydantoin} (IIa), an analog of diphenylhydantoin, was prepared by the rearrangement of dibenzo[*a,e*]cyclooctadiene-5,6-dione (III) with urea and alkali and by heating 5-hydroxydibenzo[*a,d*]cycloheptadiene-5-carboxylic acid (VII, R = H) with urea alone. Substitution of thiourea in the first method gave the corresponding spirothiohydantoin (V) which was then converted to the desthio compound (VI). The spirohydantoins IIb, IIc, and IV were prepared in the normal manner from dibenzo[*a,e*]cycloheptatriene-5-one (Ib), dibenzo[*a,d*]cyclooctadien-5-one (Ic), and dibenzo[*a,e*]cyclooctadiene-5-one *via* interaction with potassium cyanide and ammonium carbonate. This method, however, gave none of the spirohydantoin (IIa) from dibenzo[*a,d*]cycloheptadien-5-one (Ia). The interaction of methyl 5-hydroxydibenzo[*a,d*]cycloheptadiene-5-carboxylate (VII, R = CH_3) and urea in alkali gave a spirooxazolidinedione (VIII). The acid-catalyzed cyclization of 5-cyano-5-carboxyalkyldibenzo[*a,d*]cycloheptadienes (IXa–c) and an analogous cycloheptatriene (IXd) produced the corresponding spirosuccinimide and spiroglutarimide derivatives (Xa–d). A spiropyrrolidone (XIIIa) and a spiropiperidone (XIIIc) were formed by the hydrogenation of the methyl esters of the appropriate cyano acids (IXa,c). Heating the methyl ester of IXc with ammonia and hydrogen sulfide with subsequent cyclization of the thiocarboxamide (XV) by acid gave the spirothioglutarimide (XVI). The spiro{dibenzo[*a,d*]cycloheptadiene-5,2'-succinimide} (Xa) possessed a promising order of anticonvulsant action in mice while the spirohydantoins had, contrary to expectations, only minimal activities.

Our investigation of the dibenzo[*a,d*]cycloheptadiene analogs of anticonvulsants containing the benzhydryl group[1] has now been extended to the synthesis of certain spiro compounds. These include the analogs of diphenylhydantoin, -oxazolidinedione, -succinimide, and -glutarimide.[2] Similar spiro derivatives were also prepared from the dibenzo[*a,e*]cycloheptatriene, dibenzo[*a,d*]cyclooctadiene, and dibenzo[*a,e*]cyclooctadiene ring systems. The spirohydantoin derived from fluorenone has been reported to possess anticonvulsant action in humans.[3]

The interaction of dibenzo[*a,d*]cycloheptadiene-5-one (Ia) with ammonium carbonate and potassium cyanide in acetamide under a variety of conditions[4] failed to give the spirohydantoin IIa. In contrast, dibenzo[*a,e*]cycloheptatriene-5-one (Ib) gave IIb in 40% yield. Low yields of the spirohydantoin derived from xanthone have been reported,[5] although fluorenone forms the hydantoin in good yield.[4,6] It was possible to obtain the desired spirohydantoin IIa by hydrogenation of IIb over palladium or by rearrangement of dibenzo[*a,e*]cyclooctadiene-5,6-dione (III) with urea and alkali, following procedures used for the preparation of diphenylhydantoin from benzil.[7] In the absence of urea, alkali alone or with added copper sulfate[8] did not cause the rearrangement of diketone III to 5-hydroxydibenzo[*a,d*]cycloheptadiene-5-carboxylic acid (VII, R = H). The lower homolog of III, dibenzo[*a,d*]cycloheptadiene-10,11-dione, has been reported to undergo the benzilic acid rearrangement.[9] It was possible to convert the hydroxy acid (VII, R = H), prepared by a different method,[10] into the spirohydantoin IIa by heating it with urea at 135–140°, following a similar preparation for diphenyl-

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