ethyl acetate was evaporated and the residue was crystallized from ethyl acetate–ether to give 10 g. (77%) of **11**, m.p. 213–214°.

10-(2-Dimethylaminopropionyl)phenoxazine Hydrochloride (12).—A suspension of 27.3 g. (0.1 mole) of 10-(2-chloropropionyl)phenoxazine and 1 g. of potassium iodide in 600 ml. of ethyl methyl ketone was saturated with 15 g. (0.25 mole) of dimethylamine at 5–10°. The suspension was kept in a pressure bottle 24 hr. at room temperature, then heated to 80° and kept at that temperature for 48 hr. The solution was cooled, filtered, and evaporated under reduced pressure. The residue was shaken with a mixture of 10% hydrochloric acid and ether. The acid solution was separated and made alkaline, and the base was extracted with ether. The ether was evaporated, and the residue was converted to the hydrochloride in acetone–ethyl acetate. The product, crystallized from ethyl acetate, weighed 24 g. (75%).

2-Acetyl-10-(2-dimethylaminopropionyl)phenoxazine Hydrochloride (16).—A mixture of 22.5 g. (0.1 mole) of 2-acetylphenoxazine, 250 ml. of benzene, and 17.8 g. (0.14 mole) of 2-chloropropionyl chloride was refluxed for 24 hr. The solvent and excess 2-chloropropionyl chloride were removed under reduced pressure. The resulting oil was dissolved in 150 ml. of dimethylformamide and 0.75 g. of potassium iodide was added. This solution was saturated with 15 g. of ethylamine at 5–10°. The suspension was kept in a pressure bottle 24 hr. at room temperature and then was heated to 60° and kept at that temperature for 48 hr. After being cooled and filtered, the solution was evaporated under reduced pressure. The residue was shaken with a mixture of 10% hydrochloric acid and ether. The acid solution was separated and made alkaline, and the base was extracted with ether. After evaporation of the ether, the residue was converted to the hydrochloride in acetone–ether. The hydrochloride, crystallized from ethyl acetate–ethanol and then recrystallized from butanol, weighed 9 g. (25%).

Phenoxazine-10-carbonyl Chloride (17). Method A.—To a solution of 36.6 g. (0.2 mole) of phenoxazine in 200 ml. of benzene was added a solution of 24.8 g. (0.25 mole) of phosgene in 100 ml. of toluene. The mixture was kept 3 hr. at room temperature, then heated gradually over a period of 2 hr. to the boiling point, and refluxed for 2 hr. The liquid was evaporated and the residue crystallized from benzene to yield 25.9 g. (52%).

Method B.—A slurry of 36.6 g. (0.2 mole) of phenoxazine, 15.8 g. (0.2 mole) of pyridine, and 80 ml. of toluene was added gradually at 5–10° to a solution of 24.8 g. (0.25 mole) of phosgene in 100 ml. of toluene. The mixture was stirred at room temperature for 72 hr. and then filtered. The precipitate was washed with water and recrystallized from benzene to yield 6 g. of product. The filtrate was washed with water, dried, and distilled under reduced pressure. The residue recrystallized from benzene yielded 20 g. The total yield was 52%.

10-Carbethoxyphenoxazine (18).—A solution of 24.5 g. (0.1 mole) of phenoxazine-10-carbonyl chloride in 300 ml. of ethanol was refluxed for 24 hr. The ethanol was evaporated partially, and the precipitated solid was collected. It weighed 20.5 g. (81%).

(2-Diisopropylaminoethyl)phenoxazine-10-carboxylate Methiodide (19).—To a solution of 9.8 g. (0.04 mole) of phenoxazine-10-carbonyl chloride in 100 ml. of dry benzene, 11.8 g. (0.08 mole) of 2-(diisopropylamine)ethanol was added. The mixture was refluxed for 12 hr., cooled, washed with water, and the benzene layer was separated and dried. After evaporation of the solvent, 40 g. (0.28 mole) of methyl iodide was added and the mixture was refluxed for 2 hr. The excess methyl iodide was evaporated. The residue was crystallized from ethanol to yield 4.5 g. (31%).

Phenoxazine-10-carboxhydrazide (20).[5]—Hydrazine (4.5 g., 0.14 mole, 95%) was added at 0° to a solution of 7.5 g. (0.03 mole) of phenoxazine-10-carbonyl chloride. The mixture was kept at 0° for 20 min., then brought to room temperature and filtered. The residue was washed with water and then crystallized from isopropyl alcohol to yield 5.4 g. (73%) of white plates.

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(5) Prepared by Mr. Walter W. Bennetts, Jr.

The Effect of Piperidinecarboxamide Derivatives on Isolated Human Plasma Cholinesterase[1]

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In two preceding papers[2,3] we suggested that the substituted β-aminopropionamide moiety (>N–C–C–CON<), present in (+)-lysergic acid diethylamide (LSD) as well as in β-(arylalkylamino)propionamide[2,3] and piperidinecarboxamide compounds (carbamoylpiperidine compounds),[4–6] derived from the corresponding components of the parent LSD molecule, might be involved in the inhibitor–enzyme complex formation in human plasma "pseudo"-cholinesterase systems. Since LSD may be looked upon as a derivative of the partially unsaturated 1-methyl-3-(N,N-diethylcarbamoyl)piperidine (N,N-diethyl-1-methyl-3-piperidinecarboxamide) component of its molecule, and since several other piperidinecarboxylic acid derivatives (cocaine,[7a] meperidine,[7b] etc.) are known to effect psychic disturbances, a study of relationships between the molecular constitution, physicochemical characteristics, and biochemical response of piperidinecarboxamides was undertaken.[4,5,8–10]

The member compounds of each series have been designed with gradual changes in their chemical structure or physical properties or both. Furthermore, they have been planned in such a manner that potential differences in the biochemical response effected by structural variation may permit a detailed study of the nature of the interaction between the member compounds of a given synthetic series and a given enzyme. The data reported for these specific compounds reflect the responses effected by (1) the nature and degree of alkyl substitution on the amido function, (2) the mono- and the corresponding bis(carbamoylpiperidino) substitution on the alkane homologs, (3) the number and arrangement of methylene units in the alkane component attached to the ring-nitrogen(s), and (4) unsaturation in the piperidine ring.

The study involving substituent variation on the amido function of 1-methyl-3-carbamoyl-1,2,5,6-tetrahydropyridine was inspired by the report of Bergmann, *et al.*,[11] suggesting relationships between the electrophilic character of the carbonyl carbon in nicotinic

(1) This investigation is being supported by grants from the National Institute of Mental Health (USPHS MY-2072/MH-04379) and the Geschickter Fund for Medical Research, Inc.

(2) A. Lasslo, P. D. Waller, A. L. Meyer, and B. V. Rama Sastry, *J. Med. Pharm. Chem.*, **2**, 617 (1960).

(3) A. Lasslo, P. D. Waller, and G. J. Epperson, *J. Med. Chem.*, **6**, 26 (1963).

(4) A. Lasslo, W. M. Marine, and P. D. Waller, *J. Org. Chem.*, **21**, 958 (1956).

(5) A. Lasslo and P. D. Waller, *ibid.*, **22**, 837 (1957).

(6) 3-Piperidinecarboxamides are also known as nipecotamides.

(7) L. S. Goodman and A. Gilman, "The Pharmacological Basis of Therapeutics," 2nd Ed., The Macmillan Co., New York, N. Y., 1955: (a) p. 363; (b) p. 264.

(8) S. E. Jordan, A. Lasslo, H. L. Livingston, H. Alperin, and A. Gersing, *Arch. Intern. Pharmacodyn.*, **115**, 452 (1958).

(9) A. Lasslo and P. D. Waller, *J. Med. Pharm. Chem.*, **2**, 107 (1960).

(10) R. P. Quintana and W. A. Shrader, *J. Pharm. Sci.*, in press.

(11) F. Bergmann, I. B. Wilson, and D. Nachmansohn, *J. Biol. Chem.*, **186**, 693 (1950); *cf.* I. B. Wilson and F. Bergmann, *ibid.*, **186**, 683 (1950).

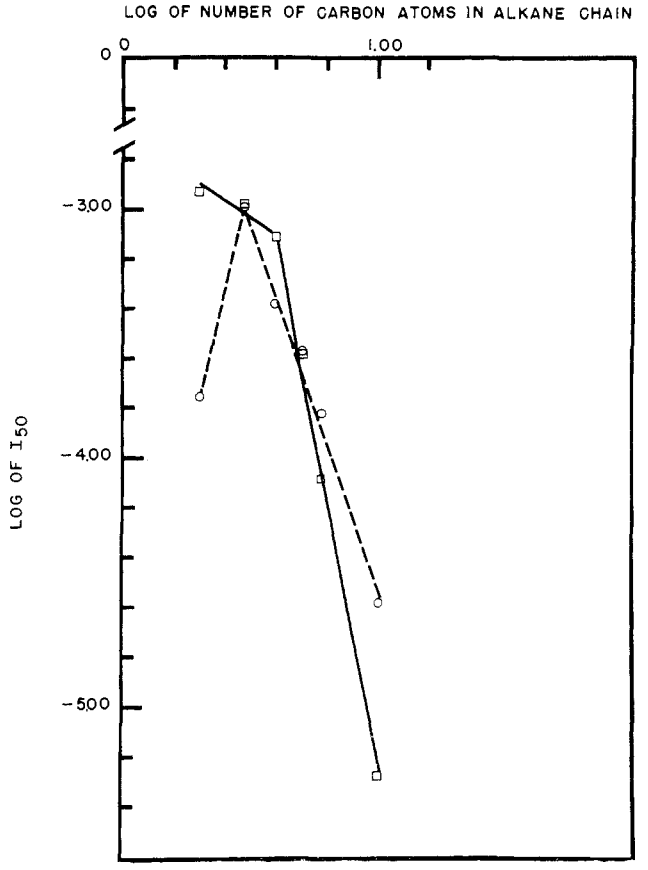
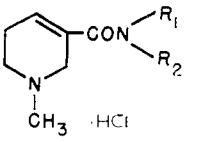


Fig. 1.—Graphic interpretation of the inhibitory characteristics of mono- (□--□) and bis- (O--O) carbamoylpiperidino alkanes in isolated human plasma "pseudo"-cholinesterase systems.

acid derivatives and their inhibition of cholinesterases. It is interesting that of the monomethyl-, dimethyl-, monoethyl-, and diethylcarbamoyl derivatives evaluated (see Table I), only the diethyl compound [1-methyl-3-(N,N-diethylcarbamoyl)-1,2,5,6-tetrahydropyridine] displayed significant inhibitory activity, and that the amide function in the parent LSD molecule is also an N,N-diethylamide. Comparison of I_{50} values for the mono- and bis(carbamoylpiperidino)-alkanes (see Table II) reveals several striking phenomena. Excluding the 1-methyl-3-(N,N-diethylcarbamoyl)piperidine (Table II, $n = 0$), the inhibitory activity increases dramatically with the chain length of the monosubstituted alkanes; *i.e.*, the mono(carbamoylpiperidino)decane (Table II, $n = 9$) is 225 times more potent than the correspondingly substituted ethane (Table II, $n = 1$). The spectrum of activity among the respective bis-substituted alkanes is considerably different; for example, the bis(carbamoylpiperidino)-decane (Table II, $n = 9$) is not quite seven times as active as the bis-substituted ethane (Table II, $n = 1$). While among the decanes the monosubstituted one (Table II, $n = 9$) is the more powerful inhibitor (almost five times), among the ethanes the bis-substituted derivative (Table II, $n = 1$) has the more potent inhibitory action (almost seven times). Although the mono- and bis-substituted propane derivatives overlap in their potency of inhibition, this very striking switch in inhibitory potencies between the bis- and monosubstituted n-alkanes takes place at the pentane level. A graphic interpretation of the comparative inhibitory characteristics of the mono- and bis-carbamoylpiperidino substituted alkanes is depicted in Fig. 1.

Table I

The Influence of N-Alkyl Substitution in the Carbamoyl Function of a Carbamoylpiperidine Moiety upon Isolated Human Plasma "Pseudo"-Cholinesterase

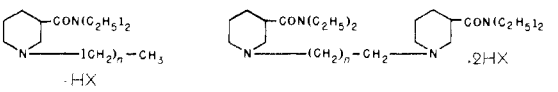


R_1	R_2	$I_{50} \pm$ S.E.
CH_3	H	Inhib. not sig. at 100×10^{-5} *M*
CH_3	CH_3	Inhib. not sig. at 100×10^{-5} *M*
C_2H_5	H	Inhib. not sig. at 100×10^{-5} *M*
C_2H_5	C_2H_5	$(73.0 \pm 0.3) \times 10^{-5}$ *M*

For details on the chemistry of the compounds cited in this Table, see ref. 4 and 5.

Table II

Relationships between the Molecular Constitution of Substituted Carbamoylpiperidines and their Effects upon Isolated Human Plasma "Pseudo"-Cholinesterase



n	$I_{50} \pm$ S.E.					
0	(63.5	± 1.0)	$\times 10^{-5}$ *M*[a]			
1	(118.5	± 0.5)	$\times 10^{-5}$ *M*	(17.5	± 1.0)	$\times 10^{-5}$ *M*
2	(101.0	± 1.0)	$\times 10^{-5}$ *M*	(99.3	± 1.8)	$\times 10^{-5}$ *M*
3	(78.0	± 2.0)	$\times 10^{-5}$ *M*[b]	(42.0	± 1.7)	$\times 10^{-5}$ *M*[c]
4	(26.1	± 1.1)	$\times 10^{-5}$ *M*[c,d]	(27.1	± 1.3)	$\times 10^{-5}$ *M*
5	(8.13	± 0.33)	$\times 10^{-5}$ *M*	(15.0	± 0.3)	$\times 10^{-5}$ *M*
9	(0.527	± 0.011)	$\times 10^{-5}$ *M*[e]	(2.59	± 0.06)	$\times 10^{-5}$ *M*

[a] The inhibitory activity of the corresponding pyridinium analog is not significant at 100×10^{-5} *M* concentrations. [b] The $I_{50} \pm$ S.E. value of the corresponding spiro-derivative (spiro[3-(N,N-diethylcarbamoyl)piperidine-1,1′-pyrrolidinium] bromide) is $(130.0 \pm 6.0) \times 10^{-5}$ *M*. [c] The $I_{50} \pm$ S.E. value of the corresponding N-cyclopentyl analog is $(7.35 \pm 0.23) \times 10^{-5}$ *M*. [d] The $I_{50} \pm$ S.E. value of the corresponding spiro-derivative (1,1′-spiro-3-(N,N-diethylcarbamoyl)bipiperidinium bromide) is $(78.8 \pm 1.3) \times 10^{-5}$ *M*. [e] The $I_{50} \pm$ S.E. value of the corresponding pyridinium analog is $(0.365 \pm 0.000) \times 10^{-5}$ *M*. For details on the chemistry of the compounds cited in this Table, see ref. 4 and 5.

It is interesting to note that of all the carbamoylpyridinium analogs studied, only the decyl derivative (Table II, footnote *e*) has shown significant inhibition; in this instance, however, it was more potent than the corresponding carbamoylpiperidine compound (Table II, $n = 9$). This suggests that the length of the alkyl chain may have a preponderant influence upon the inhibitory action of these moieties.

It is anticipated that the physicochemical studies currently in progress will assist in a more exhaustive interpretation of the present as well as our earlier findings.

Experimental

The manometric determinations were carried out on a GME-Lardy RWB-3 instrument, at 37°. The derivatives discussed in this report were evaluated under exactly the same conditions as those reported in a preceding paper (procedure I).[2] As indicated earlier,[2,3] the human plasma "pseudo"-cholinesterase system used in these experiments was characterized kinetically, and its response was checked against the parent compound (LSD tartrate SANDOZ, $I_{50} \pm$ S.E.: $4.06 \pm 0.16 \times 10^{-7}$ *M*),

and a reference reagent (physostigmine sulfate NBCo., $I_{50} \pm$ S.E.: $5.24 \pm 0.19 \times 10^{-8} M$).

The rate for the reaction is expressed as

$$V = \left(\frac{(\mu l.\ CO_2 \text{ at } 30 \text{ min.}) - (\mu l.\ CO_2 \text{ at } 10 \text{ min.})}{20} \right) \times 60$$

where V signifies $\mu l.\ CO_2$/hr. evolved within the reaction interval of +10 through +30 min., during which time the rate was linear in all instances. The percentages of inhibition were calculated as follows: $I = ((V_c - V_i)/V_c) \times 100$, where V_c represents the control rate and V_i the inhibited rate.

All compounds were first screened for inhibitory properties at $100 \times 10^{-5} M$ concentrations. For purposes of our evaluation compounds exhibiting less than 15% inhibition under these conditions were considered to have insignificant activity; at least two independent duplicate determinations were run to confirm responses of less than 15% inhibitory action. Conversely, an observed inhibition of 15% or higher at $100 \times 10^{-5} M$ concentration was deemed sufficient to warrant further evaluation.

The effect of such compounds was evaluated at four appropriate concentrations, with at least two independent duplicate determinations for each concentration, and the I_{50} (molarity of compound effecting 50% inhibition) was graphically determined. Since it is our intent to subject the data reported in this communication to further mathematical treatment, we have computed the standard error[12] for each I_{50} value; they are included in Tables I and II.[13]

Acknowledgment.—We wish to express our sincere thanks to Mr. John M. Cole and Dr. Leonard B. Achor of Sandoz Pharmaceuticals for furnishing us with (+)-lysergic acid diethylamide tartrate.

(12) G. W. Snedecor and W. G. Cochran, "Statistical Methods," 5th Ed., Iowa State College Press, Ames, Iowa, 1956, pp. 42–45.

(13) Although standard error values have not been included in the related preceding publications,[2,3] in no instance did the deviations in our determinations exceed the ranges reported in this paper.

N-Alicyclic Amphetamines

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The availability of some aminocyclanes suggested to us that it might be worthwhile to prepare N-alicyclic amphetamines and investigate the physiological effect of these compounds. They were readily prepared by reductively alkylating the amines with 1-phenyl-2-propanone (phenylacetone) in the presence of platinum oxide catalyst under a pressure of a few atmospheres of hydrogen. Hydrogenation proceeded at a moderately rapid rate. The yield of distilled bases ranged from 60 to 83%. The hydrochloride salts were also prepared. As the size of the ring increased, water solubility of the salts decreased.

Pharmacology.—The compounds, as hydrochloride salts, were tested as appetite depressants in normal trained rats at two dose levels: at 0.011 and 0.044 mmole/kg. Compounds I, II, and III were administered in aqueous solution. The salts of IV and V, much less soluble, were used in suspension in 0.3% tragacanth solution. The anorectic effect was determined by comparison of the food intake of the treated and untreated animals.[1]

The only active member of the series was the cyclopropyl derivative. The next in the series, the cyclobutyl derivative, had only slight activity; the remainder were inactive.

Experimental

All melting points were taken in a Thomas–Hoover melting point apparatus calibrated against known standards. The amines used in this work are either available commercially[2] or can be prepared by known methods.[3]

The following is an example of the method used to prepare the amines listed in Table I.

2-(N-Cycloheptyl)-1-methylphenethylamine.—A solution of 20.1 g. (0.15 mole) of 1-phenyl-2-propanone and 16.95 g. (0.15 mole) of cycloheptylamine in 150 ml. of absolute alcohol was allowed to stand for about 1 hr. Platinum oxide catalyst (0.6 g.) was added and hydrogenation then carried out under 2 atm. pressure. When the uptake of hydrogen was complete (less than 2 hr.), the solution was filtered and concentrated. The residue was then distilled and the results recorded (see Table I).

To form the hydrochloride salt, an anhydrous ether solution of the amine was treated with an equivalent of alcoholic hydrogen chloride and allowed to stand. In a short period the salt precipitated. It was filtered, washed with anhydrous ether, and analyzed after thorough drying.

Acknowledgment.—The author is indebted to Mr. Francis Fischer and his associates of the preparations group of this Laboratory and to Mr. Bruce Horrom for the cyclobutylamine used in this work, and to Mr. Orville Kolsto and staff for the microanalyses.

TABLE I

$C_6H_5CH_2CH(CH_3)NHR$

	R	Yield, %	B.p., °C. (mm.)	$n^{25}D$	Hydrochloride, m.p., °C.	Formula	% C Calcd.	% C Found	% H Calcd.	% H Found	% N Calcd.	% N Found
I	Cyclopropyl	70.8	123–125 (18)	1.5130		$C_{12}H_{17}N$	82.22	82.12	9.79	10.11	8.01	7.98
					108–109	$C_{12}H_{18}ClN$	68.06	68.12	8.56	8.58	6.61	6.65
II	Cyclobutyl	83	122 (10)	1.5143		$C_{13}H_{19}N$	82.48	82.67	10.11	10.28	7.41	7.56
					126.5	$C_{13}H_{20}ClN$[a]	69.15	68.86	8.93	8.87	6.20	6.02
III	Cyclopentyl	79	126 (5.5–6.0)	1.5148		$C_{14}H_{21}N$	82.69	83.00	10.41	10.57	6.89	7.12
					178	$C_{14}H_{22}ClN$	70.12	69.85	9.37	9.19	5.84	6.03
IV	Cyclohexyl	60	140 (3)	1.5145		$C_{15}H_{23}N$	82.88	83.00	10.66	10.35	6.46	6.88
					184–184.5	$C_{15}H_{24}ClN$	70.98	71.26	9.53	9.48	5.52	5.68
V	Cycloheptyl	72.1	145–147 (4)	1.5193		$C_{16}H_{25}N$	83.04	82.94	10.89	10.84	6.06	6.26
					194	$C_{16}H_{26}ClN$	71.74	71.70	9.78	9.65	5.23	5.04

[a] *Anal.* Calcd.: Cl, 15.74. Found: Cl, 15.84.

(1) A more detailed description of the test method will be published elsewhere by the Pharmacology Department of this Laboratory.

(2) Cyclopropylamine and cyclopentylamine can be purchased from Aldrich Chemical Company, Inc., Milwaukee, Wisconsin; cyclohexylamine is manufactured by Abbott Laboratories.

(3) Cycloheptylamine: M. Freifelder, W. D. Smart, and G. R. Stone, *J. Org. Chem.*, **27**, 2209 (1962). Cyclobutylamine was prepared by a series of reactions described by G. B. Heisig, *J. Am. Chem. Soc.*, **63**, 1698 (1941), starting from cyclobutanecarboxylic acid.