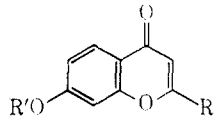
TABLE I



Comp no.	R	R′	Recrystn solvent	Yield, %	Mp, °C	Formula	Analyses	Pharmacological data: Dose, mg/kg	PO_2 % change	Duration, min
III	2-Fu	$CH_2CO_2C_2H_5$	DMF–MeOH	50.5	155–157	$C_{24}H_{18}O_9$	C, H, O	10	−8	5
IV	2-Fu	$(CH_2)_2N(C_2H_5)_2$	EtOH–Me_2CO	42.5	218–220	$C_{23}H_{22}ClNO_4$	C, H, Cl, N, O	10	−4	4
V	2-Fu	2-COFu	Me_2CO	60.0	168–170	$C_{19}H_{10}O_6$	C, H, O	2	0	0
VI[a]	2-Fu	2-CH_2Fu-5-$CO_2C_2H_5$	DMF–MeOH	40.8	189	$C_{23}H_{16}O_7$	C, H, O	2	+11	3
VII[a]	C_6H_5	2-CH_2Fu-5-$CO_2C_2H_5$	DMF–MeOH	45.0	168	$C_{25}H_{18}O_5$	C, H, O	10	−5	8
VIII	C_6H_5	2-COFu	DMF–EtOH	60.5	167–168	$C_{20}H_{12}O_5$	C, H, O	10	0	0
IX	C_6H_5	$(CH_2)_2OC_6H_5$	EtOH	50.8	162	$C_{25}H_{18}O_4$	C, H, O	10	−40	3
X	4-$C_6H_4OCH_3$	2-COFu	DMF–MeOH	70.3	195	$C_{28}H_{18}O_6$	C, H, O	10	−9	11
XI[a]	4-$C_6H_4OCH_3$	2-CH_2Fu-5-$CO_2C_2H_5$	DMF–MeOH	50.3	165	$C_{28}H_{20}O_7$	C, H, O	2	−9	10
XII	2-Fu	$OCC_6H_4(OCH_3)_3$-3,4,5	DMF–MeOH	70.0	215	$C_{28}H_{18}O_8$	C, H, O	10	+19	8

[a] The intermediate ethyl 5-(chloromethyl)-2-furoate ($ClCH_2$—furan—$CO_2C_2H_5$) used in the synthesis of these compounds was prepared according to a known procedure: A. L. Mndzhoian, "Synthesis of Heterocyclic Compounds", Vol. 1 and 2, Consultants Bureau, Inc., New York, N. Y., 1959, p 29.

Pharmacology.—It was presumed that coronary vasodilating activity of III–XII will cause an increase in PO_2 as measured in the coronary sinus. The compounds were injected into the jugular vein of anesthetized dogs at doses of 2 and 10 mg/kg and the effect on the oxygen tension of the coronary sinus blood (PO_2) was measured by essentially the same method as described by Schoepke, *et al.*[6] The data described in Table I show that only VI and XII showed a slight increase in PO_2, but it was not sufficient to be of any further interest.

Experimental Section[7]

Synthesis of 2-(2-Furyl)-7-hydroxychromone (II, R = 2-Fu). 2′,4′-Dihydroxyacetophenone Difuroate.—2-Furoyl chloride (26.0 g, 0.20 mole) in dry PhH (80 ml) was added gradually to a well-stirred ice-cold solution of 2,4-dihydroxyacetophenone (15.20 g, 0.1 mole) in pyridine (70 ml). After 24 hr the mixture was added to excess dilute HCl. The product, which separated, crystallized from EtOH; yield 39.10 g (70%), mp 115–116°. *Anal.* ($C_{18}H_{12}O_7$) C, H, O.

1-(2,4-Dihydroxyphenyl)-3-(2-furyl)-1,3-propanedione-4-(2-furoate).—Powdered KOH (2.0 g) was added to a solution of 2′,4′-dihydroxyacetophenone difuroate (5.0 g) in dry pyridine (75 ml). The mixture was shaken vigorously for 15 min and set aside for 12 hr. The crude product, liberated by the addition of cold dilute AcOH, was washed with H_2O. It crystallized (Me_2CO) in yellow needles, yield 1.3 g (25%), mp 154°. *Anal.* ($C_{18}H_{12}O_7$) C, H, O.

2-(2-Furyl)-7-hydroxychromone (II, R = 2-Fu).—1-(2,4-Dihydroxyphenyl)-3-(2-furyl)-1,3-propanedione-4-(2-furoate) (1.0 g) was dissolved in concentrated H_2SO_4 (5 ml) and set aside for 4 hr at room temperature. It was poured onto crushed ice and neutralized with 10% NaOH. The product which separated crystallized from DMF and MeOH mixture; yield 0.2 g (25%), mp 320–325°. *Anal.* ($C_{13}H_8O_4$) C, H, O.

{[2-(2-Furyl)-4-oxo-4H-1-benzopyran-7-yl]oxy}acetic Acid Ethyl Ester (IX).—A mixture of 4.6 g (0.02 mole) of II (R = 2-Fu) and 4.0 g of K_2CO_3 in 100 ml of MeCOEt was refluxed with stirring at 70° for 1 hr. Then 2.44 g (0.02 mole) of ethyl chloroacetate was added dropwise. The reaction mixture was stirred under reflux for 9 hr. The hot reaction mixture was filtered with suction and the filtrate was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 and the solution was washed several times with dilute NaOH and finally with H_2O. The CH_2Cl_2 solution was concentrated *in vacuo* to dryness. The residue was recrystallized from a mixture of DMF–MeOH; mp 155–157° (see Table I, III for physical data). Compounds IV, VI, VII, IX, and XI were synthesized by the same method as III from various 2-substituted 7-OH chromones and chloromethyl intermediates.

2-(2-Furyl)-7-hydroxychromone 2-Furoate (Table I, V).—To a solution of 4.6 g (0.02 mole) of II (R = 2-Fu) in 50 ml of dry C_5H_5N was added dropwise 2.6 g (0.02 mole) of 2-furoyl chloride. The mixture was stirred at room temperature for 24 hr. The crude product, liberated by the addition of dilute HCl, was recrystallized from Me_2CO; mp 168–170° (see Table I, V for physical data). VIII, X, and XII were synthesized by the same procedure as V.

Acknowledgments.—The author wishes to express his appreciation to Dr. Thomas Darby, Mr. Leo Wiemeler, and Mr. Charles Shannon of the Pharmacology Department of Abbott Laboratories, North Chicago, Illinois, for pharmacological investigations and permission to use their data.

(6) H. A. Schoepke, T. D. Darby, and H. D. Brandyk, *Pharmacologist*, **8**, 204 (1966).

(7) Melting points were determined with the Thomas–Hoover capillary melting point apparatus. Microanalyses were prepared at the Microanalytical Laboratories of Abbott Laboratories, North Chicago, Ill.

N-[3-(1-Alkylpiperidyl)]acetamides and N,N-Dimethyl-N′-[3-(1-alkylpiperidyl)]ureas as Cholinesterase Inhibitors. I

IAN W. MATHISON, JAMES G. BEASLEY, KATHLEEN C. FOWLER, AND ELIZABETH R. PETERS

Department of Medicinal Chemistry, University of Tennessee Medical Units, College of Pharmacy, Memphis, Tennessee 38103

Received April 4, 1969

Previous investigations by Lasslo,[1] Beasley,[2] Quintana,[3] Purcell[4] and their coworkers have involved studies of the effects of 3-(1-alkylpiperidyl)carbox-

(1) (a) A. Lasslo, P. D. Waller, A. L. Meyer, and B. V. Rama Sastry, *J. Med. Chem.*, **2**, 617 (1960); (b) A. Lasslo, P. D. Waller, and G. J. Epperson, *ibid.*, **6**, 26 (1963); (c) A. Lasslo, J. G. Beasley, G. G. Nelms, and G. J. Epperson, *ibid.*, **6**, 811 (1963).

(2) J. G. Beasley, R. P. Quintana, and G. G. Nelms, *ibid.*, **7**, 698 (1964).

(3) R. P. Quintana and W. A. Schrader, *J. Pharm. Sci.*, **52**, 1186 (1963).

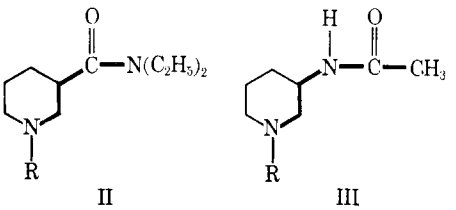
(4) (a) W. P. Purcell, J. G. Beasley, and R. P. Quintana, *Biochim. Biophys. Acta*, **88**, 233 (1964); (b) W. P. Purcell, J. G. Beasley, R. P. Quintana, and J. A. Singer, *J. Med. Chem.*, **9**, 297 (1966); (c) W. P. Purcell and J. G. Beasley, *Mol. Pharmacol.*, **4**, 404 (1968).

amides (II) as cholinesterase (ChE) inhibitors. The compounds studied incorporated the >NCCCON< (aminopropionamide) moiety which is well known to be an effective entity in ChE inhibition.[1] The effects of increasing the length of the 1-alkyl chain[1c] and variations in the amide substituent[2,4a] were examined and correlations were made in regard to enzyme inhibition, partition coefficients, electric moments, and electronic structures.[4b] As an extension of this work, a homologous series of piperidylacetamide derivatives (III) containing the acetylcholine skeleton (I), in which the ester oxygen has been isosterically replaced by the NH grouping, has been synthesized and evaluated for ChE inhibitory activity.

$$\overset{+}{\text{N}}\text{-C-C-O-CO-CH}_3$$

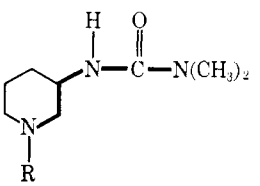
I

In view of the presence of the carbamate function in some classical ChE inhibitors (*e.g.*, physostigmine, neostigmine[5]) we considered it an interesting approach to evaluate some ureas of general formula IV for inhibitory activity since the urea function is isosteric with the carbamate moiety. We rationalized that the ChE inhibitory activities of the above compounds coupled with data obtained from compounds synthesized in earlier studies[1,4] (II) would yield some interesting structure–activity relationships in regard to ChE inhibition. The relationship between the ureas (IV), acetamides (III), acetylcholine (I), and the previously reported 3-piperidylcarboxamides (II) is emphasized by the dark lines in the illustrations.



II

III



IV

Our synthetic studies on these derivatives initially centered around attempts to form the urea function by treating 3-aminopyridine with N,N-dimethylcarbamoyl chloride. The extreme lability of the reaction product [expected to be N,N-dimethyl-N′-(3-pyridyl)urea] led us to attack the synthesis by an alternative route. Alkylation of 3-acetamidopyridine under standard conditions yielded the expected 1-alkyl-3-acetamidopyridinium salt which was then hydrogenated to the N-[3-(1-alkylpiperidyl)]acetamide. Acid hydrolysis of these piperidylacetamides followed by treatment of the resulting 3-aminopiperidines with N,N-dimethylcarbamoyl chloride yielded the desired ureas. In view of the structural similarity of the piperidylacetamides (-(R)NC(O)-) to the N-alkylpiperidylcarboxamides (RCON<) previously investigated[1c] (*i.e.*, inverted amides), it was decided to evaluate these also for ChE inhibitory properties. Homologous series of both the piperidylacetamides and piperidylureas, substituted on the heterocyclic nitrogen with normal alkyl groupings ranging in composition from C_1 through C_{10} have been prepared and evaluated in an isolated butyrylcholinesterase system.

Experimental Section

Synthetic Work.—All melting points were determined using a Swissco melting point apparatus and are corrected. Elemental analyses were carried out by Galbraith Laboratories, Inc., Knoxville, Tenn. Ir spectra were recorded on a Perkin-Elmer Model 137B Infracord spectrophotometer and examination of the ir spectra of all compounds showed them to be consistent with the proposed structures. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.3% of the theoretical values.

N-[3-(1-Alkylpiperidyl)]acetamides.—3-Acetamidopyridine (1.0 mole), prepared essentially by the method of Farley and Eliel,[6,7] and *n*-alkyl bromide (1.1 moles) were refluxed in absolute EtOH for 16 hr. EtOH was removed *in vacuo* leaving the crude N-alkyl-3-acetamidopyridinium bromide as a brown, viscous oil. The crude oil was dissolved in absolute EtOH and hydrogenated at 3.16 kg/cm^2 over PtO_2 (5–10% ratio of catalyst to compound). After 18 hr the hydrogenation was stopped and the EtOH was removed *in vacuo* leaving a viscous, amber oil. The oil was suspended in H_2O, made strongly alkaline with NaOH pellets, and extracted with Et_2O. The dried Et_2O extract was concentrated to yield a viscous, yellow oil which was purified by vacuum distillation to yield the product in all cases as a white, wax-like solid. The physical data for these acetamides can be found in Table I.

N,N-Dimethyl-N′-[3-(1-alkylpiperidyl)]ureas.—N-[3-(1-Alkylpiperidyl)]acetamide (0.05 mole) was refluxed in H_2O (138 ml) containing concentrated H_2SO_4 (11 ml) for 24 hr. The solution was concentrated, then made strongly alkaline with NaOH, and extracted with Et_2O. The Et_2O was dried (Na_2SO_4) and distilled off leaving the N-alkyl-3-aminopiperidine as a viscous, yellow oil. The oil was weighed and immediately dissolved in dry Et_2O to prevent formation of a carbonate on exposure to air. The N-alkyl-3-aminopiperidine (1.0 mole) in Et_2O was cooled in an ice bath and dimethylcarbamoyl chloride (Aldrich Chemical Co.) (4.0 moles) was added rapidly. The mixture was stirred until it reached room temperature and allowed to stand overnight. It was then refluxed for 1.5 hr and Et_2O was removed *in vacuo* leaving a dark yellow oil. This was made alkaline by adding 10% Na_2CO_3 and extracted with Et_2O. The dried Et_2O extract was distilled off leaving a light yellow oil which crystallized to a yellow solid on standing at room temperature. The solid was purified either by vacuum distillation followed by recrystallization from *i*-Pr_2O or simply by recrystallization from *i*-Pr_2O. The products were white or off-white wax-like crystals. The physical properties are listed in Table II.

The above procedures were used for the synthesis of all the compounds outlined in Tables I and II with the exception of N-[3-(1-methylpiperidyl)]acetamide (1).

N-[3-(1-Methylpiperidyl)]acetamide (1).—3-Acetamidopyridine (15.0 g, 0.11 mole) was dissolved in absolute EtOH (225 ml) and the solution was cooled in an acetone–Dry Ice bath. Gaseous bromomethane was bubbled into this solution for 1 hr and the solution was allowed to warm to room temperature and to stand for 48 hr with constant stirring. A white powdery precipitate formed during this time; this was purified by recrystallization from EtOH to yield 23.2 g (91%) of colorless needles which gave satisfactory analyses for 1-methyl-3-acetamidopyridinium bromide, mp 257–259°. *Anal.* ($C_8H_{11}BrN_2O$) C, H, N, Br.

This salt (23.0 g, 0.1 mole) was dissolved in H_2O (200 ml) and hydrogenated over PtO_2 (2.0 g) for 18 hr. The catalyst was removed by filtration and the colorless aqueous solution was made strongly alkaline with NaOH and extracted with Et_2O. The dried Et_2O extract was distilled off leaving a pale yellow oil

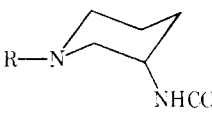
(5) G. B. Koelle in "The Pharmacological Basis of Therapeutics," L. S. Goodman and A. Gilman, Ed., The Macmillan Co., New York, N. Y., 1965, p 441.

(6) C. P. Farley and E. L. Eliel, *J. Am. Chem. Soc.*, **78**, 3477 (1956).

(7) F. K. Beilstein, "Handbuch der Organischen Chemie," Vol. XXII, B. Prager and P. Jacobsen, Ed., Julius Springer, Berlin, 1935, p 432.

TABLE I

INHIBITION OF HORSE SERUM CHOLINESTERASE BY N-[3-(1-ALKYLPIPERIDYL)]ACETAMIDES[a]

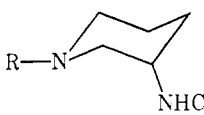


No.	R	Bp, °C (mm)	Mp, °C	Yield, %[c]	Formula[c]	% inhib at 1×10^{-3} M[b]
1	CH_3		85–86[c]	54	$C_8H_{16}N_2O$	7[d]
2	C_2H_5	95 (0.2)	73.5–76	73	$C_9H_{18}N_2O$	14[d]
3	C_3H_7	90 (0.15)	31.5–34.5	58	$C_{10}H_{20}N_2O$	20
4	C_4H_9	124 (0.34)	57.5–59	77	$C_{11}H_{22}N_2O$	16
5	C_5H_{11}	100 (0.15)	41.5–48.5	64	$C_{12}H_{24}N_2O$	35
6	C_6H_{13}	112 (0.3)	66.5–67.5	54	$C_{13}H_{26}N_2O$	45
7	C_7H_{15}	86 (0.1)	58.5–60	65	$C_{14}H_{28}N_2O$	67
8	C_8H_{17}	116 (0.1)	70–72	64	$C_{15}H_{30}N_2O$	84
9	C_9H_{19}	128 (0.75)	64–67	77	$C_{16}H_{32}N_2O$	87
10	$C_{10}H_{21}$	166 (0.5)	81.5–83	48	$C_{17}H_{34}N_2O$	89

[a] Sufficient evidence is available on the conformation of piperidines to justify depiction of these derivatives in the chair conformation: W. Barbieri and L. Bernardi, *Tetrahedron*, **21**, 2453 (1961); E. L. Eliel, "Stereochemistry of the Carbon Compounds," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 246; W. P. Purcell and J. A. Singer, *J. Chem. Soc.*, 1431 (1966). [b] The per cent inhibition values recorded here represent the average of triplicate determinations conducted at 3.28×10^{-3} *M* substrate concentrations. Similar results were also obtained at 1.64×10^{-3} *M*. [c] Compound 1 was recrystallized from *i*-Pr_2O. [d] The data for compounds inhibiting less than 20% represent an average of triplicate evaluations conducted on 2 separate days. [e] All compounds analyzed satisfactorily for C, H, N.

TABLE II

INHIBITION OF HORSE SERUM CHOLINESTERASE BY N,N-DIMETHYL-N′-[3-(1-ALKYLPIPERIDYL)]UREAS[a]



No.	R	Mp, °C	Yield, %	Formula[d]	% inhib at 1×10^{-3} M[b]
11	CH_3	111.5–114[c]	28	$C_9H_{19}N_3O$	84
12	C_2H_5	103.5–104.5	31	$C_{10}H_{21}N_3O$	86
13	C_3H_7	114–115.5	58	$C_{11}H_{23}N_3O$	85
14	C_4H_9	103–104	58	$C_{12}H_{25}N_3O$	87
15	C_5H_{11}	92–93.5[e]	43	$C_{13}H_{27}N_3O$	90
16	C_6H_{13}	95.5–97	51	$C_{14}H_{29}N_3O$	90
17	C_7H_{15}	92.5–93.5	63	$C_{15}H_{31}N_3O$	90
18	C_8H_{17}	95.5–96.5	67	$C_{16}H_{33}N_3O$	89
19	C_9H_{19}	93.5–95	72	$C_{17}H_{35}N_3O$	94
20	$C_{10}H_{21}$	95–96	53	$C_{18}H_{37}N_3O$	93

[a,b] See corresponding footnotes in Table I. [c] Y. Deguchi, *Iyaku Shigen Kenkyusho Nempo*, 17 (1956); *Chem. Abstr.*, **54**, 2325*b*,*c* (1960). [d] See footnote *e* in Table I. [e] Bp 158° (2.8 mm).

which solidified on standing at room temperature. The solid was recrystallized from *i*-Pr_2O to yield 1 as white needles (see Table I).

Biochemical Evaluation. Procedure.—The subject compounds have been evaluated as inhibitors of horse plasma ChE (acylcholine acylhydrolase, EC 3.1.1.8; Worthington Biochemical Corp.) at 26.0 ± 0.05° and pH 7.40 ± 0.05 using essentially the potentiometric procedure and equipment previously described in detail.[8] All compounds were evaluated at 1×10^{-3} *M* concentration using at least triplicate samples with acetylcholine chloride (Sigma Chemical Co.) in 1.64×10^{-3} *M* and 3.28×10^{-3} *M* solutions as substrate. The rates were linear during the reaction period, as shown on the graphical display, and were actually calculated from figures obtained from a numerical digital readout attachment.[9] The per cent inhibition, *I*, is given by the expression $I = [(V_c - V_i)/V_c]100$, where V_c is the control rate and V_i the inhibited rate.

Results and Discussion

A number of interesting observations may be drawn from examination of the results outlined in Tables I and II.

In both series of compounds it is apparent that as the length of the alkyl chain increases so does the binding of the inhibitor to the enzyme. However, it is equally well demonstated that in the piperidylacetamide series (Table I), there is a much greater variation in inhibitory potency between the lower and higher homologs of the series than in the piperidylureas (Table II). These data support the conclusion that hydrophobic interactions are playing a significant, although somewhat different, role in the inhibitor enzyme reaction for both series. One may infer that in the case of the acetamide series the hydrophobic interaction may exert an orienting effect in regard to the positioning of the acetamide function at the hydrolytic site allowing for a greater inhibitory potency as the alkyl chain length increases. The much smaller variation (~10%) in inhibitory activity as the alkyl chain length is increased from C_1 through C_{10} in the piperidylureas (Table II) suggests that the urea moiety ($-NHCONMe_2$) provides a better "fit" in the vicinity of the esteratic site[10,11] than either the acetamide or carboxamide (Table III below) portions of the other series reported here; thus, the piperidylurea homologs are less dependent on hydrophobic interactions of the alkyl chain for their inhibitory potencies. Additionally, some interesting observations emerged upon comparison of the ChE inhibitory potencies of the piperidylacetamides[12] (Table I) with

(8) J. G. Beasley, S. T. Christian, W. R. Smithfield, and L. L. Williford, *J. Med. Chem.*, **10**, 1003 (1967); S. T. Christian and J. G. Beasley, *J. Pharm. Sci.*, **57**, 1025 (1968).

(9) J. G. Beasley, A. C. York, S. T. Christian, and W. A. Frase, *Med. Biol. Eng.*, **6**, 181 (1968).

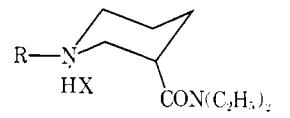
(10) This is not an unanticipated result in view of the known cholinesterase inhibitory potencies of several known carbamates.

(11) I. B. Wilson and F. Bergmann, *J. Biol. Chem.*, **185**, 479 (1950); I. B. Wilson, *ibid.*, **208**, 123 (1954).

(12) These derivatives may be considered to be reversed amides of the piperidylcarboxamides.

TABLE III[a]

INHIBITION OF HUMAN PLASMA PSEUDOCHOLINESTERASE BY N,N-DIETHYL[3-(1-ALKYLPIPERIDYL)]CARBOXAMIDE HYDROHALIDES[b]



R	% inhib at 1×10^{-5} M
CH_3	54
C_2H_5	45
C_3H_7	47
C_4H_9	48
C_5H_{11}	76
C_6H_{13}	82
$C_{10}H_{21}$	91

[a] The data reported here, previously not published as such, were kindly provided by Dr. Andrew Lasslo. The values were obtained manometrically, at 37°, and are averages from two or more flasks; human plasma "pseudo"-cholinesterase (Cholase, Cutter Laboratories, Berkeley, Calif.) was used as the enzyme and acetylcholine iodide as the substrate. Details of the specific procedure appeared in earlier papers.[1a,c] [b] All of the compounds were hydrobromides except for the Me derivative which was a hydrochloride.

those of the piperidylcarboxamides (Table III).[13] The data in Table III show a range of inhibition from 45% for the C_2 compound to 91% for the C_{10} homolog; while the range is not as great as for the acetamides (7–88%), it is greater than for the ureas (83–93%). It would appear that the reversal of the amide function from –C–N–C(O)– to –C–C(O)N< slightly improves the "fit" at the esteratic site yet does not negate the need for the orienting effect of a hydrophobic interaction.

While the importance of the partitioning of inhibitors between aqueous and lipoid components is appreciated,[4b] it appears highly unlikely that this factor alone can account for the variations in inhibition observed in these three series of ChE inhibitors. Subsequent studies are planned, however, to investigate partition phenomena for both the acetamide and urea series as well as a more detailed analysis of their enzymodynamic behavior.

In conclusion, the results reported in this study support the proposal of Augustinsson[14] of the presence of a hydrophobic binding area in plasma cholinesterase. Urea components of the general type $RNHCONMe_2$ appear to be more effective in blocking the esteratic site of ChE than does the acetamide ($RNHCOCH_3$) or carboxamide ($RCONEt_2$) moieties.

Acknowledgment.—The authors wish to thank Mrs. Marjorie O. Clark for her technical assistance in the evaluation of the cholinesterase inhibitory properties, the National Science Foundation for their continued support (GB-4453, GB-7383) of this research project, and Dr. Andrew Lasslo for kindly permitting us to report the data given in Table III.

(13) Although the experimental circumstances involved in obtaining the data for Tables I and III were somewhat different, we have previously demonstrated in unpublished results from our laboratory that results obtained from evaluation of a series of derivatives under similar conditions (those used for obtaining the data in Tables I and III) were comparable. Therefore, we feel justified in correlating the trends indicated in the cited data.

(14) K. B. Augustinsson, *Biochim. Biophys. Acta*, **128**, 351 (1966).

Studies on the Cholinergic Receptor. IV.[1] The Synthesis and Muscarinic Activity of 3,7-Dimethyl-2,4-dioxo-7-azaspiro[3.4]octane Methiodide[2]

H. F. RIDLEY, S. S. CHATTERJEE,[3a] J. F. MORAN,[3b] AND D. J. TRIGGLE[3c]

Faculty of Health Sciences, State University of New York at Buffalo, Buffalo, New York 14214

Received March 17, 1969

A basic assumption in the analysis of structure–activity relationships is that the ligand molecule exhibits a complementary structural relationship to the macromolecular receptive surface. This relationship may be obscured by at least two factors, (a) lack of knowledge of the conformation of receptor-bound ligand and (b) the existence of multiple modes of ligand binding.

Elucidation of the binding mode of acetylcholine and related compounds at the cholinergic receptor is complicated by just these factors. Despite the evidence from X-ray,[4–7] pmr,[8] and MO[9,10] methods that the $^+$N–C–C–O grouping in acetylcholine, muscarine, lactoylcholine, etc., assumes the *gauche* conformation, there can be, by virtue of the undoubtedly low rotation barriers existing in these compounds, no *necessary* relationship between the conformations of such molecules in the solid or solution state and when bound at the receptor where the ligand environment is likely to be very different from the crystal lattice or aqueous solution. In any case exceptions exist to any attempted correlation between this *gauche* conformation and biological activity. Only the *trans* isomer (*trans* $^+$N–C–C–O) of 2-acetoxycyclopropyltrimethylammonium iodide has muscarinic activity;[11,12] acetylthiocholine and acetylselenocholine both have *trans* $^+$N–C–C–X arrangements in the crystalline state[13] yet differ very markedly in their depolarizing ability on the electroplax preparation.[14] For those molecules in which a *gauche* $^+$N–C–C–O arrangement has been observed it is proposed[7] that electrostatic interactions between the O and N^+ groups serve to stabilize this interaction. However, such interactions cannot exist in the alkyl-

(1) Part II of this series: D. R. Garrison, M. May, H. F. Ridley, and D. J. Triggle, *J. Med. Chem.*, **12**, 130 (1969); part III: H. F. Ridley, M. May, and D. J. Triggle, *ibid.*, **12**, 320 (1969).

(2) This work was supported by grants from the National Institutes of Health, U. S. Public Health Service (GM 11603) and NASA (NGR-33-015-016).

(3) (a) Department of Biochemical Pharmacology, School of Pharmacy; (b) Department of Biochemistry, Schools of Medicine, Dentistry, and Pharmacy; (c) Department of Biochemical Pharmacology and Center for Theoretical Biology.

(4) F. Jellinek, *Acta Cryst.*, **10**, 277 (1955).

(5) M. E. Senko and D. H. Templeton, *ibid.*, **15**, 281 (1960).

(6) F. P. Canepa, P. Pauling, and H. Sorum, *Nature*, **210**, 907 (1966).

(7) C. Chothia and P. Pauling, *ibid.*, **219**, 1156 (1968).

(8) C. C. J. Culvenor and N. S. Ham, *Chem. Commun.*, 537 (1966).

(9) L. B. Kier, *Mol. Pharmacol.*, **3**, 487 (1967).

(10) A. M. Liquori, A. Damiani, and J. L. DeCoen, *J. Mol. Biol.*, **33**, 445 (1968).

(11) P. D. Armstrong, J. G. Cannon, and J. P. Long, *Nature*, **220**, 65 (1968).

(12) C. Y. Chiou, J. P. Long, J. G. Cannon, and P. D. Armstrong, *J. Pharmacol. Exptl. Therap.*, **166**, 243 (1969).

(13) E. Shefter and O. Kennard, *Science*, **153**, 1389 (1966).

(14) H. G. Mautner, E. Bartels, and G. D. Webb, *Biochem. Pharmacol.*, **15**, 187 (1966).