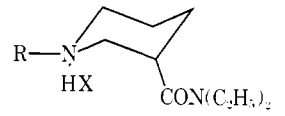
TABLE III[a]

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



R	% inhib at 1×10^{-3} 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]

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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).

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(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).

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(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).

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TABLE I
MUSCARINIC ACTIVITIES OF DIOXOLANES

Compd	ED_{50}, mol/l. ± SD	No. of molecules	Intrinsic act.[c,d]
IV	$1.56 \pm 0.41 \times 10^{-7}$ (10)[b]	1[a]	1
I	$1.57 \pm 0.49 \times 10^{-6}$ (5)	9.9	1.01 ± 0.13 (5)
V	$4.8 \pm 0.75 \times 10^{-5}$ (5)	308	0.56 ± 0.14 (5)

[a] The dioxolane IV is equipotent or more potent than acetylcholine. [b] Number of experiments. [c] Maximum height of contraction of agonist/maximum height of contraction of IV. [d] These compounds have no nicotinic activity and their activity in this preparation is unaffected by hexamethonium.

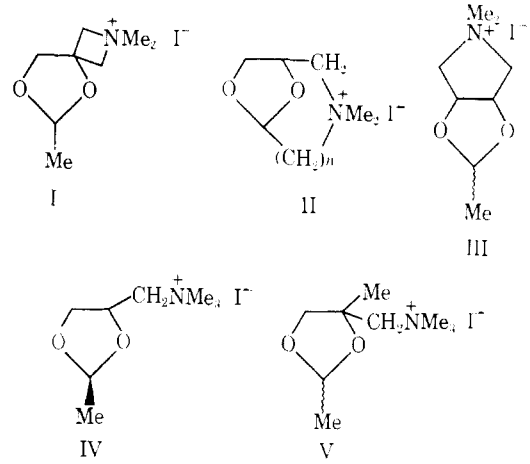
trimethylammonium series, some members of which are well known to have powerful cholinomimetic properties.

Further considerations suggest also the improbability that all cholinomimetic agents will share a common mode of binding and that, in fact, a number of modes of binding or binding sites will exist. Recent work from this laboratory[15] favors the assumption that acetylcholine and the alkytrimethylammonium agents occupy different binding sites with a common anionic area. Belleau has reached[16] a similar conclusion from studies on the binding of quaternary ammonium ligands to ACH-esterase. Such observations are not unexpected for they are paralleled by recent studies on enzyme–substrate interactions including carboxypeptidase,[17] trypsin,[18] and lysozyme.[19]

An alternative approach to the analysis of structure–activity relationships is the design of rigid analogs of active molecules in which the possibilities of conformational variation are minimized. This approach has limitations also in that the very structural changes necessary to impose predetermined conformational features may also alter the mode of binding. By careful choice of compounds this possibility can be minimized, though not entirely eliminated.

In continuation of our examination[1,20] of rigid and semirigid analogs of acetylcholine we have synthesized the title compound I. Previous studies on conformationally restricted dioxolanes II and III have suggested that their relative inactivity might be attributed to an inability to reproduce a conformation of the parent dioxolane IV in which the quaternary head is maximally extended from the O_1 and O_3 atoms of the dioxolane ring. 3,7-Dimethyl-2,4-dioxo-7-azaspiro[3.4]octane methiodide (I) provides an approximation, within a rigid molecule, to this conformation and also satisfies our previously discussed stipulation[20] that the production of conformation restriction should be unaccompanied by significant additional molecular structure.

The results (Table I) demonstrate that I is some ten times less active than *cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide (IV) as a muscarinic agent. Effective comparison of I and IV requires, however, that an estimate be provided of the possible effect on activity of the additional 4 substituent (azetidine CH_2) in I that is absent in IV. For this purpose, 2,4-dimethyl-4-dimethylaminomethyl-1,3-dioxolane methiodide (V) was prepared and evaluated as



a *cis,trans* mixture (*cis,trans* isomerism is not possible with I). The 300-fold reduction in activity observed with V is probably due to unfavorable binding by the 4-Me substituent and to the hindrance offered by this substituent to the correct orientation of the 4-CH_2-N^+Me_3 group. The relative importance of these two factors is unknown (and interpretation may be complicated by a comparison of agents with differing intrinsic activities), but if the former is at all significant, then it is clear that the conformation represented by I is indeed a good approximation to that attained by the flexible analog IV bound at the muscarinic receptor. However, in view of the probable existence of binding subsites of the cholinergic receptor an extension of this conclusion to other cholinomimetic molecules is not warranted.

Experimental Section

Chemical Section.—Melting points were determined on a Thomas–Kofler hot stage and are corrected. Nmr spectra were recorded with a Varian A-60; glpc analyses were carried out with a 10% Carbowax column using an F and M research chromatograph (Model 5750). Mass spectra were run by Sadtler Laboratories, Philadelphia, Pa. Elemental analyses were by Dr. A. Bernhardt, Mulheim, West Germany, and, where indicated only by symbols of the elements, are within ±0.4% of the theoretical values.

1-Chloro-2-chloromethyl-2,3-dihydroxypropane.—Performic acid oxidation[21,22] of 3-chloro-2-chloromethylpropene (5 g, 0.04 mole) afforded 4 g (63%) of product with bp 80–82° (2 mm). *Anal.* ($C_4H_8Cl_2O_2$) C, H, Cl.

4,4-Bischloromethyl-2-methyl-1,3-dioxolane was prepared in 80% yield from the above diol and paraldehyde in refluxing PhH with azeotropic removal of H_2O[20] and had bp 81–82° (15 mm); nmr (neat, Me_4Si as internal reference), 2-H τ 4.83 (quartet), 2-CH_3 8.67 (doublet), multiplet at 5.83–6.50. *Anal.* ($C_6H_{10}Cl_2O_2$) C, H, Cl.

3,7-Dimethyl-2,4-dioxo-7-azaspiro[3.4]octane methiodide (I) was prepared in 15% yield from 4,4-bischloromethyl-2-methyl-1,3-dioxolane and $MeNH_2$ and subsequent quaternization with

(15) J. F. Moran and D. J. Triggle in "Fundamental Concepts in Drug-Receptor Interactions," J. F. Danielli, J. F. Moran, and D. J. Triggle, Ed., Academic Press, New York, N. Y., in press.
(16) B. Belleau, ref 15.
(17) B. L. Vallee, J. F. Riordan, J. L. Bethune, T. L. Cooms, D. S. Auld, and M. Sokulovsky, *Biochemistry*, **7**, 3547 (1968).
(18) B. M. Sanborn and G. E. Hein, *ibid.*, **7**, 3616 (1968).
(19) M. A. Raftery, F. W. Dahlquist, S. M. Parsons, and R. G. Wolcott, *Proc. Natl. Acad. Sci. U. S.*, **62**, 44 (1969).
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MeI according to the procedure of Gaj and Moore[23] and had mp 195–198° (EtOH); mass spectrum (70 eV) *m/e* 285 (M), 284 (M − 1, $C_8H_{15}INO_2$), 228 (M − 57, $C_5H_{11}INO$), 224 (M − 61, $C_6H_{11}IN$), 183 (M − 102, C_3H_4IO), 158 (M − 127, $C_8H_{16}NO_2$), 155 (M − 100, C_3H_4IO), 142 (M − 143, CH_3I), 141 (M − 144, CH_2I), 85 (M − 200, $C_4H_5O_2$). *Anal.* ($C_8H_{10}INO_2$) C, H, I, N.

1-Chloro-2,3-dihydroxy-2-methylpropane.—Performic acid oxidation[21,22] of 3-chloro-2-methylpropene afforded 35% of product with bp 53–55° (0.75 mm). *Anal.* ($C_4H_9ClO_2$) C, H, Cl.

***cis,trans*-2,4-Dimethyl-4-chloromethyl-1,3-dioxolane** was prepared in 44% yield from 1-chloro-2,3-dihydroxy-2-methylpropane and paraldehyde in refluxing C_6H_6 with azeotropic removal of H_2O and had bp 29–32° (0.5 mm), lit.[24] 148–151° (760 mm). Glpc (10% Carbowax column, 110° isothermal, He 30 ml/min) revealed two major peaks with retention times of 9 and 10 min; nmr (neat, Me_4Si as internal reference), 2-H τ 4.88 and 4.93 (two overlapping quartets), 2-CH_3 and 4-CH_3 8.65–8.70 (overlapping), remaining multiplet at 5.82–6.60. *Anal.* ($C_6H_{11}ClO_2$) C, H, Cl.

***cis,trans*-2,4-Dimethyl-4-dimethylaminomethyl-1,3-dioxolane Methiodide (V)** was obtained in 37% yield from *cis,trans*-2,4-dimethyl-4-chloromethyl-1,3-dioxolane and Me_2NH in C_6H_6 followed by quaternization with MeI[1] and had mp 185–187° (EtOH), lit.[24] 139–140°; nmr (CD_3CN, Me_4Si as internal reference), $N^+(CH_3)_3$ τ 6.74 (singlet), 4-CH_3 8.47, 2-CH_3 8.67 (doublet).

Biological Section.—Muscarinic activities were determined using the rat ileum preparation as described previously.[1,20]

(23) B. J. Gaj and D. R. Moore, *Tetrahedron Lett.*, 2155 (1967).

(24) E. Gryszkiewicz-Trochimowski, O. Gryszkiewicz-Trochinowski, and R. Levy, *Bull. Soc. Chim. France*, 610 (1958), have reported this melting point for material derived from the dioxolane obtained through the $SnCl_4$-catalyzed reaction of MeCHO and 2-chloromethyl-2-methylethylene oxide.

Derivatives of 2-Azabicyclo[2.2.2]octane. III. Substituted Phenylsulfonylcarbamoyl Derivatives

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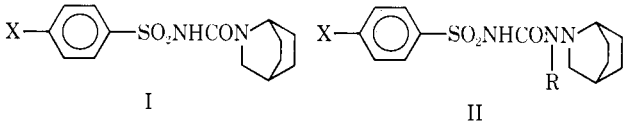
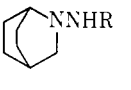
Continuing our studies[1] on the replacement of simple amine functions in clinically effective drugs by the bicyclic 2-azabicyclo[2.2.2]octane moiety[2] we have prepared the title compounds as potential hypoglycemic agents. Compounds of formulas I and II (Table I)



I II

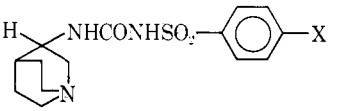


IIIa, R = H
b, R = $C_6H_5CH_2$
c, R = *p*-$ClC_6H_4CH_2$
d, R = Me

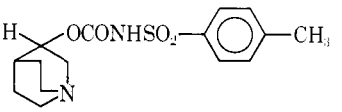
were prepared by the condensation of isoquinuclidine or a 2-aminoisoquinuclidine with a substituted phenylsulfonyl isocyanate or a substituted phenylsulfonyl carbamate ester according to known procedures. 2-Aminoisoquinuclidine (IIIa) was prepared by LAH reduction of the 2-nitroso compound. Complete conversion of the latter and maximum yield of IIIa was obtained if the reduction was carried out for prolonged periods of time in the presence of a large excess of LAH. Catalytic hydrogenation of the 2-nitroso compound using PtO_2, 5% Pd–C, or Raney Ni catalysts in a variety of solvents invariably gave excellent yields of isoquinuclidine.

Condensation of IIIa with benzaldehyde or *p*-chlorobenzaldehyde gave the benzylidene derivatives which were reduced (LAH in THF)[3] to the substituted benzyl compounds IIIb and IIIc, respectively. The 2-methylamino derivative IIId was prepared from the 2-formyl compound by reduction (LAH).[3a]

In addition, the sulfonylurea derivatives IV, pre-

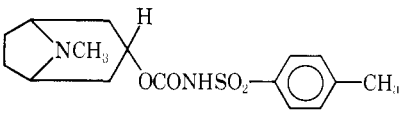


IVa, X = CH_3
b, X = Cl



V

pared from 3-aminoquinuclidine, the sulfonyl carbamates derived from 3-quinuclidinol (V), 3-tropinol (VI), and 3-tropinone oxime (VII) were prepared.



VI

VII

These compounds, listed in Table II, were inactive in the hypoglycemic screen.

The hypoglycemic potency[4] of these compounds was determined in normal mice and in diazoxide-induced hyperglycemic mice.[5] The activities of compounds of formulas I and II were compared with known hypoglycemic agents. At the screening dose of 160 mg/kg orally in normal mice, **2** (Table I) and 1-(*p*-chlorophenylsulfonyl)-3-propylurea were equally potent for periods of 1–3 hr after treatment. Compound **1** and 1-(*p*-tolylsulfonyl)-3-butylurea were equally effective at the 1-hr bleeding period but **1** was of lower potency at the 3-hr period.

Compound **4** (Table I) was the most potent compound in this series. On a milligram basis, **4** was

(1) For preceding papers in this series see F. J. Villani, and C. A. Ellis, *J. Med. Chem.*, **9**, 185, 264 (1966).

(2) Throughout this work the common name, isoquinuclidine, is used.

(3) Contrary to the results from other laboratories on the reduction of similar types of compounds, the benzylidene derivatives of this ring system resisted catalytic hydrogenation or $NaBH_4$ reduction; LAH in Et_2O gave variable results. See, for example, (a) M. J. Kalm, *J. Med. Chem.*, **7**, 427 (1964); (b) J. H. Biel, A. E. Drukker, T. F. Mitchell, E. P. Sprengeler, P. A. Nuhfer, A. C. Conway, and A. Horita, *J. Amer. Chem. Soc.*, **81**, 2805 (1959).

(4) The biological data herein reported were obtained by Miss A. Gulbenkian and Dr. I. Tabachnick of the Biological Research Division of the Schering Corporation.

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