The Effect of Piperidinecarboxamide Derivatives on Isolated Human Plasma Cholinesterase. III. Variation in the N[1]-Hydrocarbon Substituent[1]

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A series of eleven piperidinecarboxamide derivatives containing alkenyl, alkynyl, and aralkyl substituents on the ring nitrogen has been evaluated manometrically in isolated human plasma cholinesterase systems. 1,1'-Xylylene bis(N,N-diethylnipecotamides) were found to be better inhibitors of cholinesterase than the 1,1'-alkenyl and 1,1'-alkynyl derivatives studied.

In an investigation designed to probe the anionic site[2,3] of human plasma cholinesterase (PChE; acylcholine acylhydrolase, EC 3.1.1.8), Lasslo, *et al.*,[4] showed that the inhibitory potencies of unbranched alkanes substituted with a N,N-diethyl-3-piperidinecarboxamide group in the α or α and ω positions vary with the chain length of the alkane substituent. Bergmann, *et al.*,[5] Wilson and Bergmann,[6] Blaschko, *et al.*,[7] Coleman and Eley,[8] Kellett and Hite,[9] and Long and Schueler,[10] among others, have demonstrated that variations in the molecular constitution of quaternary ammonium ions can alter the cholinesterase[11]-inhibitory properties of such entities. Thomas and Marlow[14] found that the presence of an aromatic group in quaternary ammonium inhibitors of AChE influences the inhibitor–enzyme binding characteristics. Coleman and Eley,[8] with AChE, and Long and Schueler,[10] with PChE, have proved the effectiveness of bisquaternary ammonium compounds as anticholinesterase agents and have considered the activity of such inhibitors in relation to the structure of the molecular segment joining the two nitrogen atoms.

The ability of the organic moieties which contain an >NCCCON< grouping to perform effectively as cholinesterase inhibitors has been demonstrated in several previous studies.[1b,4,15] Analogs of nipecotamide (3-piperidinecarboxamide) contain this aminopropionamide segment and have afforded convenient homologous series for use in studying interactions between cholinesterases and inhibitor molecules. We have elected to incorporate this basic structure in a series of N[1]-alkynyl-, N[1]-alkenyl-, and N[1]-aralkyl-substituted N,N-diethylnipecotamides designed for evaluating the effects of structural rigidity and of unsaturation on PChE inhibition.

Experimental Section

Materials.—The inhibitors for this study were prepared by Quintana and co-workers[16] and were used in the form of their hydrohalide salts. All of the compounds employed were of analytically pure grade or the equivalent. Human plasma cholinesterase (Cholase, Cutter Laboratories, Berkeley, Calif.) was the enzyme preparation utilized in our evaluation procedure.

Biochemical Evaluation.—Inhibitors were evaluated manometrically on a GME-Lardy RWB-3 Warburg instrument using a procedure described previously.[1b] The enzyme solutions were prepared by dissolving the lyophilized crystalline powder in 0.9% saline solution and were stored in the refrigerator when not in use. No loss in activity was observed during the period in which activity measurements were conducted. I_{50} values (molarity of compound effecting 50% inhibition) were obtained from least-squares lines. Buffer, inhibitor, and substrate solutions were prepared fresh each day using redistilled water. The response ($I_{50} \pm SE$) of our enzyme preparation against a reference reagent, physostigmine sulfate (Nutritional Biochemical Co.), was checked and found to be $(5.24 \pm 0.19) \times 10^{-8}$ M.

Results and Discussion

Our results have been examined in the light of several factors previously invoked in rationalizing cholinesterase inhibitor interactions. These factors are summarized here: (1) a positively charged center in an inhibitor moiety may react with a negatively charged anionic site[2,3] on an enzyme surface;[6b] (2) the steric requirements of the anionic site of PChE are more restricted than for AChE;[6b] (3) the ionic volume of the cationic portion of inhibitors may govern the stereochemical fit at the anionic site;[9] (4) the hydrocarbon segment of an inhibitor may bind to the enzyme surface through van der Waals forces[5,17] and/or through hydrophobic interactions;[18] (5) an increased lipophilic–lipo-

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(9) J. C. Kellett and C. W. Hite, *J. Pharm. Sci.*, **54**, 883 (1965).

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(11) Research reported in these references applies both to acetylcholinesterase (AChE; acetylcholine hydrolase, EC 3.1.1.7) and to plasma or pseudocholinesterase (PChE). Although the existence of esteratic sites[12,13] in both enzymes is well accepted, the presence of an anionic site[2,3] in PChE is controversial. Nevertheless, we side with Bergmann[5d] in assuming such a site to be present and contend that one may, with due caution, apply the broad concepts derived from these reports to either enzyme system.

(12) Reference 3, pp 18–26.

(13) J. A. Cohen and R. A. Oosterbaan, ref 2, p 145.

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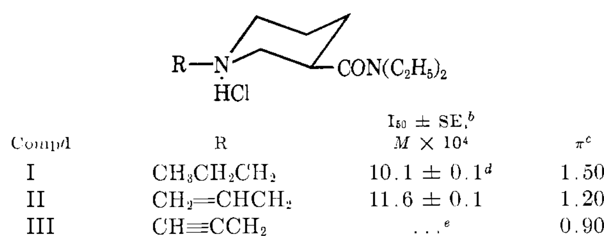
TABLE I
THE INFLUENCE OF UNSATURATION IN THE N[1]-HYDROCARBON SUBSTITUENT UPON THE CHOLINESTERASE INHIBITION ($I_{50} \pm SE$) OF N,N-DIETHYLNIPECOTAMIDES[a]



Compd	R	$I_{50} \pm SE$,[b] $M \times 10^4$	π[c]
I	$CH_3CH_2CH_2$	10.1 ± 0.1[d]	1.50
II	$CH_2{=}CHCH_2$	11.6 ± 0.1	1.20
III	$CH{\equiv}CCH_2$	. . .[e]	0.90

[a] These derivatives may also be named as 3-(N,N-diethylcarbamoyl)piperidines. [b] Standard error calculations are based on the method of G. W. Snedecor and W. G. Cochran ("Statistical Methods," 5th ed, Iowa State College Press, Ames, Iowa, 1956, pp 42–45) and utilize data from two to five independent determinations. [c] Hansch's values[21] (0.50 for CH_3 and CH_2, 0.70 for $CH_2{=}CH$, and 0.40 for $HC{\equiv}C$) are additive to give the substituent constants (π) listed; larger π values may be considered to represent more lipophilic moieties. [d] The biochemical evaluation of this compound has been previously reported.[4] [e] Inhibition not significant at 1×10^{-3} M.

phobic ratio usually enhances inhibitor effectiveness.[14a,19] In this study, special emphasis has been directed toward elucidating characteristics affecting interactions in the region of the anionic site. All of our derivatives possess a positive nitrogen substituted with a hydrocarbon function incorporating varying degrees of unsaturation and structural rigidity.

The effect on inhibition induced in compounds II and III (Table I) by unsaturation is rather unusual. Using the N[1]-propyl analog (I) for comparison purposes, we see that the introduction of a double bond (II) causes no appreciable activity change while insertion of a triple bond (III) is accompanied by a considerable loss in activity. Two factors, electronic and lipophilic, may be considered in regards to this change in activity. Should an electronic factor[19] be involved here, one might expect interactions of the π electrons (1) with the positively charged nitrogen[20] and (2) with the negatively charged anionic site.[20] The probable significance of lipophilic properties may be inferred if one uses Hansch's π values[21] (Table I) as a basis for comparing compounds I, II, and III, respectively. These π values represent a decrease in lipophilic character and, therefore, one would expect a corresponding decrease in hydrophobic binding to the enzyme. However, as the lipophilic properties decrease, the electronic factor appears to become more important and even dominant in the N[1]-propargyl analog (III). Here, both factors may contribute to the decrease in binding energy and provide an explanation for its lessened inhibitory activity.

In light of the finding of Coleman and Eley[8] and Long and Schueler,[10] as well as our own results,[4] a study of a series of bis(N,N-diethylnipecotamide) hydrocarbons (Table II) appeared attractive. These will be considered first with respect to the N[1]-alkyl, N[1]-alkenyl, and N[1]-alkynyl derivatives (IV–VIII) and secondly with respect to the N[1]-xylylene analogs (IX–XI).

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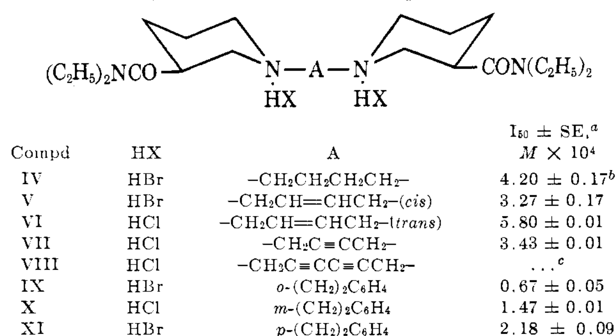
TABLE II
THE INFLUENCE OF UNSATURATION IN THE HYDROCARBON LINKAGE UPON THE CHOLINESTERASE INHIBITION ($I_{50} \pm SE$) OF BIS[N,N-DIETHYLNIPECOTAMIDE] HYDROCARBONS



Compd	HX	A	$I_{50} \pm SE$,[a] $M \times 10^4$
IV	HBr	$-CH_2CH_2CH_2CH_2-$	4.20 ± 0.17[b]
V	HBr	$-CH_2CH{=}CHCH_2-$(*cis*)	3.27 ± 0.17
VI	HCl	$-CH_2CH{=}CHCH_2-$(*trans*)	5.80 ± 0.01
VII	HCl	$-CH_2C{\equiv}CCH_2-$	3.43 ± 0.01
VIII	HCl	$-CH_2C{\equiv}CC{\equiv}CCH_2-$	. . .[c]
IX	HBr	*o*-$(CH_2)_2C_6H_4$	0.67 ± 0.05
X	HCl	*m*-$(CH_2)_2C_6H_4$	1.47 ± 0.01
XI	HBr	*p*-$(CH_2)_2C_6H_4$	2.18 ± 0.09

[a] See Table I, footnote *a*. [b] The biochemical evaluation of this compound has been previously reported.[4] [c] Inhibition was 40% at 1×10^{-3} M.

A different spectrum of activities is found for derivatives IV–VIII. Here, the effect of a single double bond may be related to the geometric configuration with the *cis* derivative (V) being more active than the saturated analog (IV) and the *trans* derivative (VI) less active. A further increase in unsaturation, to the triple bond level, yields a product (VII) with essentially the same activity as the *cis* form (V). This result is in striking contrast to the monosubstituted series (Table I) where the change from single to double bond (I → II) produced no significant change in activity while the change from double to triple bond (II → III) was accompanied by a sharp decrease in activity. In the bis series, however, the insertion of a second triple bond (VIII) does induce a noticeable loss in inhibitory potency. It is possible that in the bis series, the second N,N-diethylnipecotamide function may anchor the molecule to the enzyme surface by means of ion-induced dipole forces, as suggested by Coleman and Eley[8] for their diquaternary compounds, and that the rigidity of the hydrocarbon linkage may affect the extent of such nonspecific binding. The stereochemistry of the inhibitor moiety, therefore, could direct the conformational perturbation of the enzyme surface as suggested by Belleau[18a] and, consequently, control the degree of inhibition. The rigidity of the hydrocarbon linkage in VIII may also indicate the influence of a spatial factor (*e.g.*, nitrogen–nitrogen distance) in binding of inhibitor to enzyme.

Thomas and Marlow[14a] have shown that trimethylbenzylammonium iodide is a fair AChE inhibitor. This prompted us to try an aralkyl group as a source of structural rigidity and unsaturation in our bis-substituted inhibitors and led to the synthesis[16] and biochemical evaluation of compounds IX–XI (Table II).

The order of decreasing inhibitory activity found for these products was *ortho* (IX) > *meta* (X) > *para* (XI). Differences in the stereochemistry and lipophilic characteristics of these molecules would be significant and, we believe, more influential in determining inhibitory properties than the electronic and ionic volume factors which should be similar for each derivative. Quintana[22] has found appreciable differences in the benzene/water partition coefficients of nipecotamide and isonipecotamide (4-piperidinecarboxamide) analogs. The former was more lipophilic and more potent as a ChE

(22) R. P. Quintana, *J. Pharm. Sci.*, **54**, 462 (1965).

inhibitor. Quintana points out, however, that such relationships do not necessarily hold outside a particular homologous or isomeric series and should be used with caution. Molecular models of IX–XI show that sufficient free rotation exists about the bond linking the benzene ring and the methylene group to allow the ring to orient in a plane parallel to the enzyme surface. Such an orientation would permit a greater degree of hydrophobic interaction than is possible for the nonaromatic analogs shown in Tables I and II. It is interesting to note that the most active xylylene analog (IX) has a configuration analogous to that of the more active *cis*-ethenylene isomer (V), a fact which is in agreement with the possible existence of a spatial factor.

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Mammalian Antifertility Agents. IV. Basic 3,4-Dihydronaphthalenes and 1,2,3,4-Tetrahydro-1-naphthols[1,2]

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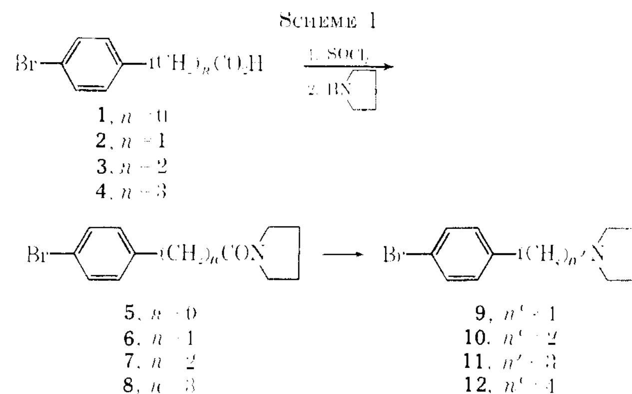
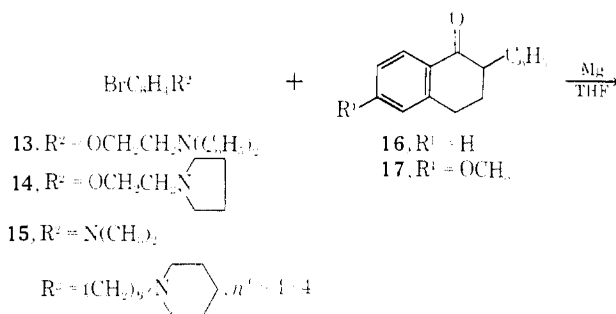
The preparation of basic ethers of 1,2-diphenyl-3,4-dihydronaphthalenes, 1-(pyridyl)-2-phenyl-3,4-dihydronaphthalenes, and 2-(3-pyridyl)-1-aryloxy-3,4-dihydronaphthalenes is described. Further transformations of some of these products are recorded. Many of the compounds prepared were found to be highly potent antifertility agents in rats; some of the active compounds were also potent uterotropic agents while others antagonized the effect of concurrently administered estrogens on uterine weight.

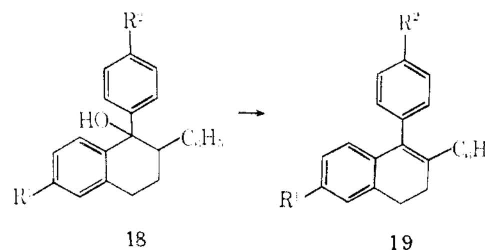
Appropriately substituted derivatives of the 2-phenyl-3,4-dihydronaphthalene systems have previously been shown to exhibit uterotropic activity.[3] More recently[1,4] compounds related to 1,2-diphenyl-3,4-dihydronaphthalene were also found to elicit a uterotropic response. In the continuing search for an orally effective nonsteroidal contraceptive, basic derivatives of the 1,2-diaryl-3,4-dihydronaphthalene system were investigated since the inclusion of basic groups into inherently estrogenic molecules has occasionally been found to lead to estrogen antagonists, which in turn exhibit antifertility activity.[5,6]

Basic Derivatives of 1,2-Diphenyl-3,4-dihydronaphthalenes.—In one of the preferred methods of synthesis, a substituted 2-phenyl-1-tetralone was allowed to react with the Grignard reagent from a basic derivative of bromobenzene. The derivatives of *p*-bromophenol were prepared as described previously.[6] In order to prepare the pyrrolidinoalkylbromobenzenes, the appropriate ω-bromoalkanoic acid[7] was converted to its acid chloride and treated with an excess of pyrrolidine (Table I). Reduction of the amide thus obtained with lithium aluminum hydride afforded the desired bases (Table II). These were carefully purified by distillation and used in the ensuing step (see Scheme I).

SCHEME I







Reaction of the Grignard reagents prepared from basic ethers of *p*-bromophenols[8] **13** and **14** with 6-me-

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(2) Parts of this paper have been published: D. Lednicer, S. C. Lyster, and G. W. Duncan, *Chem. Ind.*, 408 (1963); presented in part at the Symposium on Nonsteroidal Antifertility Agents, 144th National Meeting of the American Chemical Society, Los Angeles, Calif., April 1963.

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(6) D. Lednicer, J. C. Babcock, S. C. Lyster, J. C. Stucki, and G. W. Duncan, *Chem. Ind.*, 2098 (1961).

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