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Trimethylammonium Phenyl Ketones. Actions on the Cholinergic Receptor and Acetylcholinesterase†

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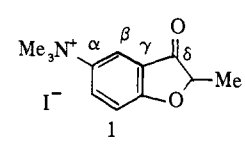
The syntheses of models for a study of acetylcholinesterase (AChE) binding are described. 6-(*N,N,N*-Trimethylammonium)indan-1-one iodide (**4**) as a muscarinic agent possessed $^1/_{100}$ the activity of acetylcholine. As an inhibitor of AChE (eel) the calculated K_i for **4** was $1.6 \times 10^{-7} M$. 4-(*N,N,N*-Trimethylammonium)indan-1-one iodide (**5**), 3-(*N,N,N*-trimethylammonium)propiophenone iodide (**6**), and 3-(*N,N,N*-trimethylammonium)butyrophenone iodide (**7**) were weakly muscarinic ($<^1/_{1000}$ ACh) and had K_i's ranging from 2 to $5 \times 10^{-6} M$ as inhibitors of AChE.

Pfeiffer,[1] in one of the earliest studies relating the acetylcholine (ACh) structure to biological actions, described the interatomic distances in ACh between the proposed binding sites. Further refinements in understanding the diverse biological properties of ACh have recently been directed at a description of the relative positions of the carbon, oxygen, and quaternary nitrogen of ACh that elicit a particular response. Excluding the "nicotinic action" two receptor responses related to ACh structure that are of current interest are the "muscarinic structure" and the "esterase structure."[2]

Archer and coworkers[3] advanced the trans relationship of the O-C-C-N portion of ACh as the muscarinic structure. Related in terms of the torsional angle, the O and N viewed along the C-C bond approaches 180° or is antiperiplanar. Additional support for the 140-180° muscarinic structure comes from the potent cyclopropyl analog study of Chiou and coworkers[4] (137° torsional angle[5]), dimethylacetylcholines,[6] decalins,[7,8] decahydroquinolines,[9] and bicyclo[2.2.2]octane[10] analogs. These interpretations contrast with those investigators favoring a 60-90° torsional angle for muscarinic action.[11-24]

Conclusions regarding the esterase structure, the optimal torsional angle for substrate activity using acetylcholinesterase (AChE), are not as divergent. Values ranging from 150 to 180° as derived from physical measurements and analog activities are generally quoted.[6-9,19-25]

Biological testing of a series of benzo analogs of muscarine revealed potent inhibition of AChE by **1**. A K_i of $2.5 \times 10^{-8} M$ calculated for both the butyryl (BChE) and acetylcholinesterase revealed that **1** was bound 10,000 times more effectively than the substrate ACh ($K_m = 4 \times 10^{-4} M$ for BChE and $1 \times 10^{-4} M$ for AChE). In contrast, the 7-isomer **2** was much less potent: $K_i = 2.5\text{-}3.8 \times 10^{-4}$ for BChE and



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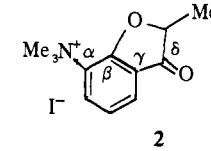

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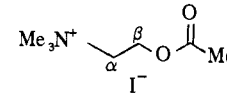

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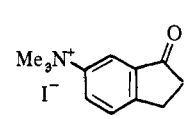
AChE. Additional analogs were desired to evaluate the requirement for a rigid 180° torsional angle imposed on the N-C_α-C_β-C_γ and C_α-C_β-C_γ-C_δ chains in **1** and **2**, those portions of the inhibitor simulating the analogous N-C_α-C_β-O and C_α-C_β-O-C fragments in ACh (**3**). Furthermore, the ether oxygen and the 2-methyl group were questionable requirements for binding of **1** and **2** to AChE. The carbonyl and the quaternary ammonium groups considered to be the two most important binding sites for AChE were retained in the analogs **4-7** synthesized.



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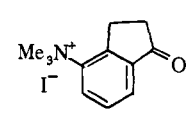

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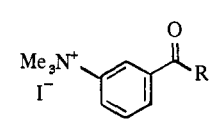

6, R = Et
7, R = *n*-Pr

Nitration of 1-indanone gave a 7:1 mixture of **6** and 4-nitroindan-1-one (**8**, **9**) separated by silica gel chromatography.[26] Nmr evidence in support of these assignments is derived from the downfield shift of the benzylic protons ($\Delta \sim \delta$ 0.24) of **9** compared to **8** attributed to the diamagnetic anisotropic effect[27] of the ortho nitro in **9**. Further, double irradiation of the H-5 or H-7 proton signals of **9** collapses the H-6 triplet (δ 7.62, $J = 8$ cps) to a doublet ($J_{6,7} = J_{5,6} = 8$ cps). Reductive methylation[28] of **8** and **9**

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followed by treatment with methyl iodide gave **4** and **5**.

Propiophenone and butyrophenone were nitrated at −20° using the method of Zenitz and Hartung[29] to give the required 3-nitrophenyl ketones. Reductive methylation and quaternization with methyl iodide gave **6** and **7**.

Biological Results. The muscarinic activity was tested on five preparations of guinea pig ileum at five concentrations of inhibitor and evaluated by the cumulative dose-response method using ACh^+Cl^- as the reference. The most effective agent was **4** ($1 \times 10^{-6} M$) giving the same response as ACh ($1 \times 10^{-8} M$) at 100 times the concentration. The 4-isomer **5** and the open chain analog **6** at $1 \times 10^{-5} M$ showed only 10 and 30% of the maximum ACh contraction (ACh = 1×10^{-8} *M*). Compound **7** was inactive at $1 \times 10^{-4} M$.

Highest muscarinic activity resided in structure **4** which was 1/100 as potent as ACh and had the same activity as **1**. These results are difficult to rationalize in keeping with the proposed 60-90° torsional angle as defined as the muscarinic structure for ACh analogs. If we can consider the quaternary nitrogen and the carbonyl oxygen as binding sites to the receptor, the analogy to ACh is the fully extended 180-180° torsional model wherein the N and O reside in the same plane. Muscarone also can be represented in a manner analogous to **4** and **5**; however, when this is examined the prediction is that **5** should be muscarinic.

The results on inhibition of AChE are easier to reconcile. As noted in Table I, with the exception of **4**, none of the compounds approached the activity of **1**. Structure **4**, differing from **1** in the absence of the methyl and the carbocyclic ring instead of the cyclic ether, was 1/5 as active as **1**. A further tenfold drop in inhibitory potency occurred when the ring was opened (**6**).

While minor structural-activity relationships can be made concerning these activities, in our view, the only prominent difference is that relating to the rigid (**1**, **4**) and nonrigid (**6**) carbonyl group. The absence of the constraining ring (**6**) drops the activity at least tenfold. Model **6** was designed with the presumption that it can adapt the carbonyl to the optimal structure (C_β-C_γ-C_δ-O angle) for esterase binding by rotation of the carbonyl out of plane with the ring. Since the inhibition is 10-15 less than **1** and **4** then the latter, being fixed planar N-O models, are optimal for esterase binding. Additionally, considering the N-C_α-C_βO, C_α-C_β-O-C, and C_β-O-C-O torsional angles in ACh, binding to the esterase occurs in the fully extended 180-180-180° orientation which approximates a planar chain for the N-C_α-C_β-O-C-O fragment of ACh.

Table I. Inhibition of Cholinesterase

Compd	K_i, *M*, of AChE (eel)[a]	K_i, *M*, of BChE (horse serum)[b]
1[c]	3.0×10^{-8}	2.5×10^{-8}
2[c]	3.8×10^{-5}	2.5×10^{-5}
4	1.6×10^{-7}	6.5×10^{-6}
5	5.1×10^{-6}	1.0×10^{-5}
6	1.9×10^{-6}	4.6×10^{-6}
7	2.2×10^{-6}	1.1×10^{-5}

[a] Sigma eel enzyme Type III, ACh $K_m = 1 \times 10^{-4} M$. [b] Sigma horse serum Type IV, ACh $K_m = 4 \times 10^{-4} M$. [c] See ref 25.

Experimental Section[§]

Nitration of Indan-1-one. Indan-1-one (13.2 g, 100 mmol) was added slowly to a cooled (−15°) solution of fuming HNO_3 (sp gr 1.5) maintaining a constant temperature while stirring vigorously. After the addition was completed, the solution was stirred for an additional 10 min at −10 to +10° and poured into 300 g of ice and the precipitate was filtered. The filtrate was extracted with C_6H_6 and the precipitate dissolved in the C_6H_6 extract. The C_6H_6 solution was washed with 20% NaOH solution until the washings were almost colorless and then with water until the washings were neutral to litmus and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure gave 11.3 g (70%) of the nitroindan-1-ones.

The mixture (8 g) was chromatographed in a 600-g silica gel column and eluted with a C_6H_6-EtOAc (4:1) mixture. After a forerun of 250 ml, 20-ml fractions were collected. Evaporation of fractions 55-66 gave 0.7 g of 4-nitroindan-1-one (**9**), mp 100-101° (lit.[26] 101-102°). Evaporation of fractions 71-100 gave 3.6 g of 6-nitroindan-1-one (**8**), mp 72-73°.

6-(*N,N,N*-Trimethylammonium)indan-1-one Iodide (4). 6-Nitroindan-1-one (8, 3.2 g, 18 mmol) dissolved in a minimum amount of EtOAc was hydrogenated at atmospheric pressure and room temperature in MeOH containing 3.2 ml (36 mmol) of 37% CH_2O solution and about 2 g of 5% Pd/C as a catalyst. The solution was filtered and the solvent evaporated to give 2.8 g (89% yield) of 6-(*N,N*-dimethylamino)indan-1-one. This (1.0 g, 5.8 mmol) was dissolved in about 50 ml of anhydrous Et_2O and refluxed with 3 ml of MeI for 5 min. The solution was stirred overnight at room temperature and the precipitate was filtered, washed with anhydrous Et_2O, and dried to give 0.8 g (43% yield) of 4, mp 220-222° dec. *Anal.* ($C_{12}H_{16}INO$) C, H, N.

4-(*N,N,N*-Trimethylammonium)indan-1-one Iodide (5). 4-Nitroindan-1-one (9, 0.88 g, 5 mmol) dissolved in the minimum amount of EtOAc was hydrogenated at atmospheric pressure and room temperature in MeOH containing 0.8 ml of 37% CH_2O solution and about 2 g of 5% Pd/C. The solution was filtered and the solvent evaporated to give 0.45 g (50% yield) of 4-(*N,N*-dimethylamino)indan-1-one. This (0.9 g, 5.2 mmol) was dissolved in about 50 ml of anhydrous Et_2O. The solution was refluxed with 3 ml of MeI for 3 days. The precipitate was filtered, washed with Et_2O, and dried at room temperature. The unreacted starting material was precipitated from the mother liquor by cooling at 0° and was again refluxed with 2 ml of MeI for 4 days to give 0.7 g of **5** (24% yield), mp 170-171° dec. *Anal.* ($C_{12}H_{16}INO$) C, H, N.

3-(*N,N,N*-Trimethylammonium)propiophenone Iodide (6). 3-Nitropropiophenone[29] (9.0 g, 50 mmol) was hydrogenated at atmospheric pressure and room temperature using an excess of 10% Pd/C (3.0 g) in THF and 8.5 ml of 37% CH_2O solution (100 mmol). The catalyst was filtered and the solvent evaporated to give 3-(*N,N*-dimethylamino)propiophenone as a yellow oil (3.54 g, 20%).

A solution of the amino ketone (3.54 g 20 mmol) in about 50 ml of absolute EtOH was treated with 3.6 g (26 mmol) of MeI. The solution was stirred for 12 hr at room temperature. The precipitate was filtered, washed with several portions of anhydrous Et_2O, and dried to give 2.2 g (35% yield) of **6**, mp 199-200° dec. *Anal.* ($C_{12}H_{18}INO$) C, H, N.

3-(*N,N,N*-Trimethylammonium)butyrophenone Iodide (7). A solution of 7.72 g (40 mmol) of 3-nitrobutyrophenone[29] in about 50 ml of THF was hydrogenated at atmospheric pressure and room temperature using 6.5 ml of 37% CH_2O solution (80 mmol) and about 4 g of 10% Pd/C as a catalyst. The catalyst was filtered, the solvent evaporated, and the residue distilled under reduced pressure to give 3.35 g (48%) of 3-(*N,N*-dimethylamino)butyrophenone.

This amino ketone (3.0 g, 15.7 mmol) in about 50 ml of absolute EtOH was stirred for 12 hr with 2.84 g (20 mmol) of MeI at room temperature. The precipitate was filtered, washed with anhydrous Et_2O, and dried to give 2.5 g of 7 (48% yield), mp 162-163° dec. *Anal.* ($C_{13}H_{20}INO$) C, H, N.

Enzyme Inhibition. The kinetic measurements were performed as described previously.[23] The concentration of AChE (Sigma Chemical Co. electric eel Type III) was 0.094 μM units/ml of salt solution The concentration of BChE (Sigma Chemical Co., from horse serum Type IV) was 0.41 μM units/ml. One micromolar unit hydrolyzes 1 μmol of ACh/min at pH 8.0 and 37°. Measurements were performed by titrating for liberated AcOH under a stream of N_2 using an automatic pH Stat assembly at pH 7.2 ± 0.1 and 24.90 ± 0.05°. Titrant was 0.0100 *M* NaOH. Deionized H_2O was used throughout. Raw data, consisting of a chart trace of per cent of full buret *vs.* time, were fitted by an iterative least-squares technique directly to the Michaelis-Menten equation.

[§] Melting points were obtained on a calibrated Thomas-Hoover Unimelt and are corrected. Infrared data were recorded on Beckman IF 8 and IR 10 spectrophotometers. Nuclear magnetic resonance spectra were recorded on Varian Associates Model A-60A and T-60 spectrometers in $CDCl_3$ using tetramethylsilane as internal standard and were as expected. Microanalyses were conducted on an F & M Model 185 CHN analyzer at the University of Kansas and, where reported, are within ±0.4% of theoretical values.

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2,3-Disubstituted 1,6-Naphthyridines as Potential Diuretic Agents

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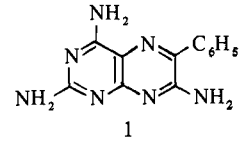
A series of 2,3-disubstituted 1,6-naphthyridines was synthesized either directly from 4-aminonicotinaldehyde or by subsequent reaction of the bicyclic products. The title compounds were prepared because of their relationship to triamterene (**1**). Most were active in a saline-loaded rat screen at 15 mg/kg but inactive at 2 mg/kg (ip). 2-Methylamino-3-(3-pyridyl)- (**9**), 2-methylamino-3-cyano- (**12**), and 2-amino-3-cyano- (**21**) 1,6-naphthyridines displayed diuretic activity comparable to **1** but markedly less than 2-amino-1,8-naphthyridine-3-carboxamide hydrochloride monohydrate (**3**). Structure-activity relationships of the title compounds are discussed and comparisons are made to **1** and **3**.

The clinical need for a potassium-sparing diuretic has resulted in series of papers appearing on the synthesis and preliminary screening of various nitrogen heterocyclic systems,[1] especially pteridines[2,3] and pyrazines.[4] Of two series of pteridines widely investigated, 2,4,7-triamino-6-phenylpteridine (**1**) and 4,7-diamino-2-phenylpteridine-6-carboxamide (**2**) were the most potent diuretics found.[2] The former (**1**), triamterene, also has potent potassium-sparing properties.[5] It was concluded that the major site for drug-receptor interaction in **1** was N-1 or N-8 or both and that the phenyl ring may enhance hydrophobic binding.[2] Since **1** contains many electron-donating amino groups and electron-withdrawing aza atoms, which are probably not essential for drug-receptor interactions, some were deleted in the molecules being investigated in the present work. A series of 1,8-naphthyridines, of which 2-amino-1,8-naphthyridine-3-carboxamide hydrochloride monohydrate (**3**) is the most potent, has been shown to possess diuretic and antikaliuretic activity in rats.† Since in the naphthyridine systems the electronic effects in the ground state of N-6 and N-8 atoms at N-1 are approximately the same,[6] it came of interest to screen a series of 2,3-disubstituted 1,6-naphthyridines to gain further insight into which aza atoms are essential for activity.



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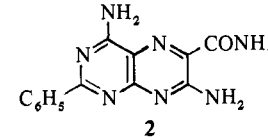

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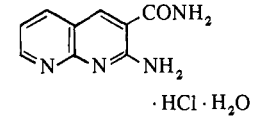

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Synthesis. The methods for preparing several of the 2-amino compounds appearing in Table I have been described previously; these are **21** and **28–39**.[7] The procedure involved an application of the classical Friedlander method:[8] condensation of substituted acetonitriles with 4-aminonicotinaldehyde in boiling alcohol with an appropriate base catalyst. Similar procedures were employed in preparing **4**, **5**, **40**, and **41**, in which 4-aminonicotinaldehyde was condensed with ethyl cyanoacetate, ethyl 3-pyridylacetate, methyl ethyl ketone, and benzoylacetonitrile, respectively. In the case of ethyl cyanoacetate and benzoylacetonitrile the products did not result from cyclization into the nitrile group which was anticipated by analogy to classical Friedländer reactions with *o*-aminobenzaldehyde.[9,10]

Since in preliminary screening **21**, **28**, and **33** showed

†E. M. Hawes, D. K. J. Gorecki, and D. D. Johnson, unpublished results.