Quaternary Ammonium Compounds. IV. Antiacetylcholinesterase Activity and Ring Size in Aromatic Quaternary Ammonium Compounds

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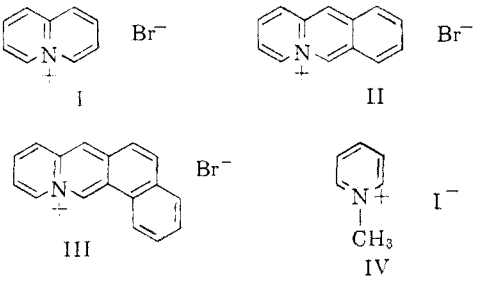
It is generally considered that quaternary ammonium compounds inhibit acetylcholinesterase by competing with acetylcholine for the anionic site of the enzyme.[1] Once the quaternary ammonium inhibitor is adsorbed on the enzyme it prevents hydrolysis of the substrate, acetylcholine, by hindering the approach of substrate molecules either by electrostatic repulsion between the positive charges on the inhibitor and acetylcholine or by the bulk of the inhibitor molecule covering the esteratic site.[2] The total adsorption force between quaternary ammonium inhibitors and acetylcholinesterase is comprised of coulombic interaction between the anionic site and the positive charge of the inhibitor and van der Waals' forces between the hydrocarbon moiety of the quaternary ammonium ion and the enzyme surface.[3,4]

Thomas and Marlow[2] have classified quaternary ammonium compounds, from an antiacetylcholinesterase point of view, into two types, "aliphatic" and "aromatic." They did this after studying the antiacetylcholinesterase activities of a series of trimethylphenylalkylammonium compounds. The factors which were considered characteristic of "aromatic" type compounds are (a) the molecules are flat and (b) the unit positive charge is delocalized around the flat ring. This allows the whole of the unit positive charge to be utilized in coulombic attraction between the quaternary ammonium ion and the anionic site on the enzyme. Heterocyclic aromatic quaternary ammonium compounds with these characteristics are quinolizinium bromide (I), benzo-*b*-quinolizinium bromide (II), and naphtho(2,1-*b*)quinolizinium bromide (III). It was considered of interest to examine these compounds together with N-methylpyridinium iodide (IV) for antiacetylcholinesterase activity in order to determine the effect of ring size of the aromatic ion on its activity. This is the first time that a biological action of the quinolizinium nucleus has been reported.



I II III IV

Experimental

Measurement of Antiacetylcholinesterase Activity.—The antiacetylcholinesterase activities of the compounds were determined by a potentiometric method as described by Thomas and Marlow.[2] The details of the method are given in the legend to Table I. Each I_{50} value reported is the mean of two independent determinations. The difference between each pair of determinations on a particular compound was less than 10%. The values are corrected for non-enzymic hydrolysis. The results are given in Table I.

TABLE I

ANTIACETYLCHOLINESTERASE ACTIVITIES OF AROMATIC QUATERNARY AMMONIUM COMPOUNDS[a]

Compound	I_{50}	Relative activities
N-Methylpyridinium iodide	3.3×10^{-3}	0.00877
Quinolizinium bromide	1.0×10^{-3}	.0296
Benzo-*b*-quinolizinium bromide	3.0×10^{-5}	1.0
Naphtho(2,1-*b*)quinolizinium bromide	1.0×10^{-4}	0.294

[a] I_{50} values in g. moles/l. Substrate concentration 0.012 *M* (acetylcholine perchlorate). Ox erythrocyte stromata used as source of enzyme. Temperature 37°. Sodium chloride 0.1 *M*, magnesium chloride 0.04 *M*.

TABLE II

AROMATIC QUATERNARY AMMONIUM COMPOUNDS

Compound	Solv. for recrystn.	Mol. formula	M.p., °C.[a]	Lit. m.p., °C.	Analyses: Calcd. C	Calcd. H	Calcd. Br	Calcd. I	Found C	Found H	Found Br	Found I
N-Methylpyridinium iodide	Ethanol	C_6H_8IN	117	117[d] 123-125[e]	32.6	3.6		57.5	32.6	3.5		57.4
Quinolizinium bromide	Ethanol/ethyl acetate	C_9H_8BrN	265.5–266.5	262–264[f]	51.4	3.8	38.0		51.7	4.0	37.5	
Benzo-*b*-quinolizinium bromide	Ethanol	$C_{13}H_{10}BrN \cdot H_2O$	238–240 dec.	238–240[g]	56.1	4.3	28.7		56.4	4.3	28.7	
Naphtho(2,1-*b*)quinolizinium bromide	Ethanol	$C_{17}H_{12}BrN$	316.5–317.5 dec.[b]	308–309 dec.[c,h]	65.8	3.9	25.8		65.7	3.8	25.8	

[a] M.p. values determined on a Kofler block, corrected. [b] Capillary tube method, uncorrected. [c] Monohydrate. [d] E. M. Kosower, *J. Am. Chem. Soc.*, **77**, 3883 (1955). [e] E. D. Bergmann, F. E. Crane, and R. M. Fuoss, *ibid.*, **74**, 5979 (1952). [f] E. E. Glover and G. Jones, *J. Chem. Soc.*, 3021, (1958). [g] C. K. Bradsher and L. E. Beavers, *J. Am. Chem. Soc.*, **77**, 4812 (1955). [h] C. K. Bradsher and L. E. Beavers, *ibid.*, **78**, 2459 (1956).

Discussion

Except for the first two, the compounds increase by 4 carbon atoms from one to the next, and there is no change in charge type, all being "aromatic." However the change in activity is not regular. Quinolizinium bromide is 3.4 times more active than N-methylpyridinium iodide but one thirty-third as active as the next higher compound in the series, benzo-*b*-quinolizinium bromide. Activity reaches a peak with the three ring

(1) D. Nachmansohn and I. B. Wilson, *Advan. Enzymol.*, **12**, 259 (1951).
(2) J. Thomas and W. Marlow, *J. Med. Chem.*, **6**, 107 (1963).
(3) D. K. Myers, *Arch. Biochem.*, **27**, 341 (1950).
(4) F. Bergmann, *Discussions Faraday Soc.*, **20**, 126 (1955).

system as the next larger ion, naphtho(2,1-*b*)quinolizinium, is 1/3.4 times as active.

In an aliphatic system, such as the trimethylalkylammonium compounds, the difference in activity between the *n*-propyl ($I_{50}3 \times 10^{-3}$) and *n*-heptyl ($I_{50}6 \times 10^{-4}$) homologs (4 carbon atoms different) was 5 times and between the *n*-butyl ($I_{50}2 \times 10^{-3}$) and *n*-heptyl homologs (3 carbon atoms different), was 3.3 times.[5] The effect on activity of adding three carbon atoms both to the "aliphatic" series and the first two compounds in the "aromatic" series now examined is practically the same. This suggests that the effect of adding 3 extra carbon atoms, to either lengthen an aliphatic chain or to go from a pyridinium system to a quinolizinium one, is non-specific and, in both cases, increases activity by increasing the number of van der Waals' interactions. However, when the increase in activity brought about by the addition of 4 carbon atoms to the "aliphatic" series is compared with the difference in activity between the quinolizinium ion and its benzhomolog the situation is different. The marked increase in activity which occurs in the 'aromatic' series suggests that the adsorption of the benzo-*b*-quinolizinium ion onto acetylcholinesterase is more specific than other quaternary ammonium ions. This conclusion is supported by the fact that naphtho(2,1-*b*) quinolizinium bromide is less active than acridizinium bromide even though it has 4 more carbon atoms.

Acknowledgment.—The quinolizinium compounds were prepared in the Pharmacy Department by J. M. Midgley.

(5) F. Bergmann and A. Shimoni, *Biochem. Biophys. Acta*, **10**, 49 (1953).

Esters of Farnesylacetic Acid

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A series of new alkyl and aryl farnesylacetates, prepared by standard methods described in the Experimental part, have been found to exhibit preventive and curative activity on different gastric ulcers in guinea pigs when administered parenterally in small doses; they had no autonomic action.[2] Table I lists the physical properties of the most interesting compounds prepared, as well as the anti-ulcer activity at the dosage levels used.[3] The anti-ulcer activity of the compounds was measured in terms of inhibition and cure of experimental gastric ulcer produced by histamine in guinea pigs pretreated with the antihistaminic, tripelennamine. The procedural details have been described in previous publications.[4]

The most promising of these esters, geranyl farnesylacetate[5] had an i.v. LD_{50} in mice of 2821 mg./kg. (confidence limits, 95%). The i.p. LD_{50} was higher than 4000 mg./kg., the oral LD_{50} higher than 8000 mg./kg. Chronic administration of this drug to growing young rats (70 mg./kg./day) for 91 days did not interfere with normal development. No blood dyscrasias or macroscopic or microscopic alterations of the vital organs were observed on necropsy.

Anti-ulcer tests with geranyl farnesylacetate revealed no local action on the gastric mucosa. The prophylactic doses in the restrained rat was 50 mg./kg. s.c., in reserpine ulcer (rat) 20 mg./kg. i.p. and in the fasting rat 10 mg./kg. i.p. No activity was found in the Shay ulcer in the rat up to 200 mg./kg. The drug relaxes the isolated guinea pig ileum, apparently unspecifically, at a rate of one-ninth that of papaverine. The compound is practically not anticholinergic (2780 times less than atropine), nor antihistaminic (1305 times less than diphenhydramine), or antinicotinic (126 times less than hexamethonium). No analgetic or anti-inflammatory effects have been noted. The mechanism of the rather specific anti-ulcer activity has not yet been elucidated.

Experimental

Preparations of Esters. Method A.—An alcoholic solution of potassium hydroxide (0.5 mole per mole of ester) was added to an alcoholic solution of alkyl farnesylmalonate[6] and the mixture was allowed to stand at room temperature for about 30 hr. After removal of alcohol, acidification, and extraction with ether, the residue was decarboxylated by heating *in vacuo* for 4 hr. at 150°. It then was dissolved with ether and washed with sodium carbonate or some other weakly alkaline agent. After removal of the ether, it was distilled *in vacuo*.

B.—One mole of an ester of farnesylacetoacetic[7] acid was added to an alcoholic solution of 1 mole of the appropriate sodium alkoxide and refluxed for 2 hr. After removal of the alcohol, the residue was dissolved in water, extracted with ether, dried, and distilled.

C.—Farnesylacetic acid[6b,7b] was esterified by refluxing either with a large excess of the proper alcohol or with a small excess, but in benzene solution. It is advisable to add a little hydrochloric acid or *p*-toluenesulfonic acid as catalyst. After removal of the solvent and washing with a weakly alkaline agent, the ester was distilled *in vacuo*.

D.—Silver farnesylacetate was prepared by adding an aqueous solution of silver nitrate to a solution of farnesylacetic acid in 0.5 *N* ammonium hydroxide. After drying in the dark, the salt was dissolved in boiling benzene and a benzene solution of alkyl iodide was added. After boiling for 9 hr. and removal of the solvent and filtration, the residue was distilled *in vacuo*.

E.—Ketene was passed into ice-cooled farnesylacetic acid until a small excess was absorbed. After heating *in vacuo* for 5 hr. at 160°, a large excess of alcohol (or a small excess of alcohol and benzene as solvent) was added and refluxed for 11 to 15 hr. After dilution with ether and washing with an alkaline agent, the solvent was removed and the residue distilled *in vacuo*.

F.—Farnesylacetyl chloride is prepared by adding without heating a small excess of a benzene solution of thionyl chloride to farnesylacetic acid and refluxing for 2 hr. It distils *in vacuo* with slight decomposition. For the subsequent reactions, the fraction passing at 145–155° (0.7–1 mm.) was used.

Farnesylacetyl chloride was added dropwise to a solution of the alcohol and an excess of dimethylaniline in anhydrous benzene, the mixture was then refluxed for 8 hr. and worked up.

(1) To whom inquiries should be addressed.

(2) E. Adami, *Experientia*, **18**, 461 (1962).

(3) E. Adami, E. Marazzi-Uberti, and C. Turba, *Medicina Experimentalis*, **7**, 171 (1962).

(4) E. Marazzi-Uberti and C. Turba, *ibid.*, **4**, 284 (1961); **5**, 9 (1961).

(5) Gefarnate (International non-proprietary name, proposed to W.H.O.).

(6) For ethyl farnesylmalonate see: (a) P. Dietrich and E. Lederer, *Compt. rend.*, **234**, 637 (1952); *Helv. Chim. Acta*, **35**, 1148 (1952). Dibutyl farnesylmalonate boiled at 137–145° (0.02–0.03 mm.).

(7) Ethyl farnesylacetoacetate has been used as an intermediate by various authors, but has never been isolated in the pure state; *cf.* (a) L. Ruzicka and G. Firmenich, *Helv. Chim. Acta*, **22**, 392 (1939); (b) A. Caliezi and H. Schinz, *ibid.*, **35**, 1649 (1952); (c) A. Mondon, *Chem. Ber.*, **88**, 724 (1955).