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Anti-acetylcholinesterase activity of some stereoisomeric aminobornanes

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Bornyl analogues of choline and acetylcholine with eclipsed *cis*- and *trans*-configurations displayed low inhibitory activity towards acetylcholinesterase catalysed hydrolysis of acetylcholine; *cis*-isomers were slightly better inhibitors than their *trans*-counterparts. (+)-Bornan-2-one-3-*endo*-yltrimethylammonium bromide with its oxygen atom and the quaternary ammonium group fixed rigidly in the gauche configuration is much more potent inhibitor than its enantiomorph.

Semi-rigid analogues of acetylcholine have been used to investigate its active conformation in its relation to acetylcholinesterase (AChE); the *cis*-alcohols of (±)-2-dimethylaminocyclohexanol methiodide and (±)-dimethylaminocyclopentanol methiodide were stated to have a slightly better fit to the catalytic surface than their *trans*- counterparts, and similarly, the *cis*-acetates were better substrates (Baldridge, MacCarville & Friess, 1955; Friess & Baldridge, 1956). However, Kay, Robinson & others (1970) recently reported that the *cis*- enantiomers of the acetate of 2-dimethylaminocyclohexanol methiodide were inactive as substrates, and the *trans*-enantiomers were hydrolysed at a much slower rate than previously reported. This appears to agree with the observations of Smissman, Nelson & others (1966) that the *trans*-isomer of 3-dimethylamino-2-acetoxydecalin methiodide is a substrate of AChE, while the *cis*-isomers are not. As these analogues of acetylcholine are only semi-rigid, and in the absence of conformational data, conclusions from these studies require cautious appraisal.

We have reported previously (Beckett, Ngiam & McDonough, 1969a, b) the syntheses of three bornyl analogues of choline, acetylcholine, and their corresponding tertiary amino-esters. Unlike the acetylcholine analogues cited above, the N^+–C–C–O segments of these compounds are *rigidly* held in *cis*-(eclipsed), *trans*-(eclipsed) or gauche configurations. Their absolute configurations, based on the absolute configuration of (+)-camphor (Northolt & Palm, 1966) are known (see Table 1). The *cis*-(eclipsed) analogues have a torsion angle τN^+–C–C–O of about 0°, and that of the *trans*-(eclipsed) analogues is about +120°; some slight deviation from these values would be expected because of steric interference between adjacent groups. The spatial relations of the nitrogen and oxygen atoms of (+)-bornan-2-one-3-*endo*-yltrimethylammonium bromide and its enantiomer are gauche, the former having N^+–C–C–O angle of +60° and the latter, −60°. Enzyme studies with AChE of these compounds is now reported.

METHODS

The syntheses of the three diastereoisomers of 2-acetoxy-bornan-3-yltrimethylammonium bromide (I, II and III); 2-hydroxybornan-3-yltrimethylammonium bromide (IV, V and VI), and 2-acetoxy-3-dimethylaminobornane hydrochloride

(VII, VIII and IX) has been reported (Beckett, Ngiam & McDonough, 1969a, b). (+)-Bornan-2-one-3-*endo*-yltrimethylammonium bromide (X), m.p. 218·5° (sealed tube), and its enantiomer XI, m.p. 218° (sealed tube) were synthesized by quaternization of the corresponding 3-*endo*-dimethylaminobornan-2-one with CH_3Br in methanol.

The procedure of Kay & Robinson (1969) for enzyme kinetic studies was followed. Bovine erythrocyte acetylcholinesterase (Sigma Chemicals) was used. Acetylcholine iodide was used as substrate and the rates of hydrolysis were measured manually employing a PYE Dynacap pH meter, glass electrode and calomel half-cell and an Agla micrometer syringe for delivering volumes of 0·02N NaOH solution to the incubation solution *via* a fine plastic tubing. The titration vessel was a glass beaker with a plastic cover and an inlet for nitrogen just above the liquid level. Because of these precautions, the background acid production from absorption of CO_2 and acid fumes was negligible. The reaction mixture was stirred with a magnetic stirrer, and maintained at $25 \pm 0{\cdot}1°$. All incubations were with 20 ml of enzyme solution previously made 0·04M in $MgCl_2$ and 0·05M in NaCl. With the addition of measured volumes of inhibitor and substrate, the total titration volume was 25 ml. All inhibitors were pre-incubated with the enzyme for 3 min and the reaction was started by the addition of acetylcholine solution. The pH was maintained just above 7·4 with 0·02N NaOH. The time, accurate to the nearest second, was recorded when the pH fell to 7·4. A titration curve of micrometer readings against time (s) was drawn. The velocity of the reaction was calculated from the average slope of the curve during the second and third minutes of the incubation and expressed as mol/min. The velocities were corrected for aqueous hydrolysis. The K_i values for the inhibitors were calculated from Lineweaver-Burk plots using a four-fold range of substrate concentrations and approximately two-fold range of inhibitor concentrations. The K_m value for acetylcholine was determined each time and was found to be consistently $4{\cdot}45 \times 10^{-4}$ throughout the investigation, in agreement with Kay & Robinson (1969).

RESULTS AND DISCUSSION

The three diastereoisomers of 2-acetoxybornan-3-yltrimethylammonium bromide (I, II and III), the acetylcholine analogues, were found either not to be substrates of bovine AChE or alternatively the rate of enzymatic hydrolysis was too slow to be detected by the apparatus over 30 min. The inability of the enzyme to hydrolyse these acetylcholine analogues shows either (*a*) the steric requirements for such a reaction to take place were not fulfilled or (*b*) if the mechanism of such a reaction involves conformational changes of the substrate molecule the rigidity of the N^+–C–C–O segment of these compounds prohibited enzyme hydrolysis.

For optimum hydrolysis, Chothia & Pauling (1969a) proposed that the substrate molecule should assume an ideal conformation (see Fig. 1) in relation to the enzyme receptor, the essential feature of which is that τN^+–C–C–O = +150°. The corresponding torsion angle for the *trans*-analogue II in the present series is +120°, which is near to this ideal value, while the torsion angle for the *cis*-analogues I, and III, being 0°, is widely different. The failure of II to be hydrolysed by AChE may be due only in part to the eclipsed configuration of its N^+–C–C–O segment; the acetyl group, which is conformationally labile, may, because of steric interaction with the C(10) methyl group (IIa), move into a position (IIb) unfavourable for enzyme hydrolysis (compare IIb with Fig. 1).

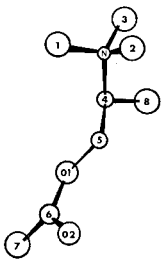
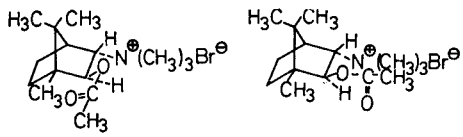


FIG. 1. The conformation of L(−)-acetyl-α-methylcholine in crystals of iodide according to Chothia & Pauling (1969a). τN^{+}-C4-C5-01 = +148° and τC6-01-C5-C4 = 180°.



IIa IIb

From their K_i values (Table 1), these acetylcholine and choline analogues (I, II, III, IV, V and VI) appear to be weak competitive inhibitors of the AChE catalysed hydrolysis of acetylcholine, and their affinity for the free enzyme would appear to be not much greater than that of the trimethylammonium ion ($K_i = 4{\cdot}0 \times 10^{-3}$; Wilson & Alexander, 1962). Comparison within each group of compounds, however, shows a difference between the *cis*- and the *trans*-isomers in their affinity for the enzyme receptor. The *cis*-alcohols (IV, VI) offer a better fit to the catalytic surface than their *trans*-counterpart (V), and this is also true with the *cis*- and *trans*-esters (I and II respectively). The only exception is ester III. The presence of the bridge C(8) methyl group probably prevents the onium and acetoxy group from acquiring a better fit on the receptor. The overall low inhibitory activities of these compounds, however, do not at this stage justify any inference about the topographical feature of the enzyme receptor, except perhaps that an eclipsed arrangement of the N^{+}–C–C–O segment of cholinergics is probably not ideal for optimum interaction with the AChE receptor.

Another interesting observation is that the tertiary amino-esters (VII, VIII and IX) exhibit non-competitive inhibition of the AChE-catalysed hydrolysis of acetylcholine—a deviation from the behaviour of their quaternary ammonium analogues. But with such high K_i values, especially those of VIII and IX, the borderline between non-competitive and mixed inhibition as shown by the Lineweaver-Burk plots is often unclear.

The optical antipodes of bornan-2-one-3-*endo*-yltrimethylammonium bromide, the only two compounds in this series with the nitrogen and oxygen atoms gauche to each other, are of interest. The (+)-isomer (X) has a K_i value of $1{\cdot}7 \times 10^{-4}$ showing a better fit to the enzyme receptor than choline and acetylcholine ($K_i = 4{\cdot}5 \times 10^{-4}$, $K_m = 4{\cdot}45 \times 10^{-4}$ respectively). The (−)-isomer (XI) on the other hand, is a weak AChE inhibitor ($K_i = 2{\cdot}5 \times 10^{-3}$). The stereoselectivity in their inhibitory action is remarkable when the absolute configuration of their N^{+}–C–C–O segments, compared with the absolute conformation of the N^{+}–C–C–O segments of

Table 1. *Enzyme-inhibitor dissociation constants (K_i) of some aminobornanes.*

	Compounds	Absolute configurations	N^+-C(3)-C(2)-O	K_i	Nature of inhibition
I	1(*R*),2(*R*),3(*S*),4(*S*)-2-*endo*-acetoxybornan-3-*endo*-yltrimethylammonium bromide	A	*ca* 0°	$1{\cdot}5 \times 10^{-3}$	Competitive
II	1(*R*),2(*S*),3(*S*),4(*S*)-2-*exo*-acetoxybornan-3-*endo*-yltrimethylammonium bromide	B	*ca* +120°	$2{\cdot}0 \times 10^{-3}$	Competitive
III	1(*R*),2(*S*),3(*R*),4(*S*)-2-*exo*-acetoxybornan-3-*exo*-yltrimethylammonium bromide	C	*ca* 0°	$2{\cdot}7 \times 10^{-3}$	Competitive
IV	1(*R*),2(*R*),3(*S*),4(*S*)-2-*endo*-hydroxybornan-3-*endo*-yltrimethylammonium bromide	D	*ca* 0°	$1{\cdot}4 \times 10^{-3}$	Competitive
V	1(*R*),2(*S*),3(*S*),4(*S*)-2-*exo*-hydroxybornan-3-*endo*-yltrimethylammonium bromide	E	*ca* +120°	$2{\cdot}6 \times 10^{-3}$	Competitive
VI	1(*R*),2(*S*),3(*R*),4(*S*)-2-*exo*-hydroxybornan-3-*exo*-yltrimethylammonium bromide	F	*ca* 0°	$2{\cdot}35 \times 10^{-3}$	Competitive
VII	1(*R*),2(*R*),3(*S*),4(*S*)-2-*endo*-acetoxy-3-*endo*-dimethylaminobornane hydrochloride	G	*ca* 0°	$2{\cdot}15 \times 10^{-3}$	Non-competitive
VIII	1(*R*),2(*S*),3(*S*),4(*S*)-2-*exo*-acetoxy-3-*endo*-dimethylaminobornane hydrochloride	H	*ca* 120°	$5{\cdot}3 \times 10^{-3}$	Non-competitive
IX	1(*R*),2(*S*),3(*R*),4(*S*)-2-*exo*-acetoxy-3-*exo*-dimethylaminobornane hydrochloride	I	*ca* 0°	$8{\cdot}8 \times 10^{-3}$	Non-competitive
X	1(*R*),3(*S*),4(*S*)-bornan-2-one-3-*endo*-yltrimethyl ammonium bromide ..	J	*ca* +60°	$1{\cdot}7 \times 10^{-4}$	Competitive
XI	1(*S*),3(*R*),4(*R*)-bornan-2-one-3-*endo*-yltrimethyl-ammonium bromide ..	K	*ca* −60°	$2{\cdot}5 \times 10^{-3}$	Competitive

[I] = 4·0 − 4·8 × 10^{-4}M.

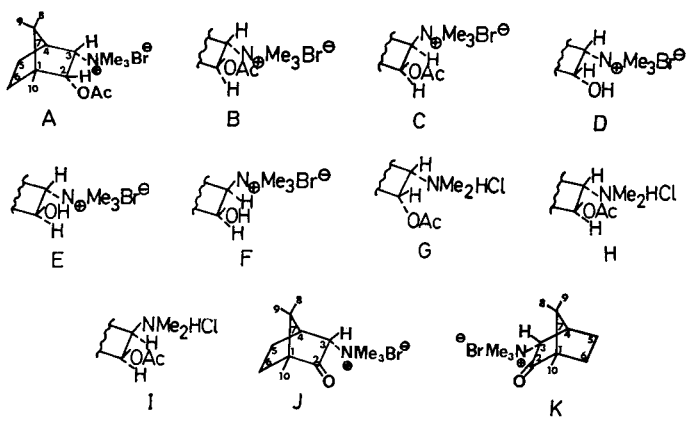


Table 2. *Relation between conformations and muscarinic activities of some cholinergic compounds.*

Compounds	τN^+-C-C-O	K_i	*Muscarinic activities
Acetylcholine	77°		1
X	+60°	$1{\cdot}7 \times 10^{-4}$	
XI	−60°	$2{\cdot}5 \times 10^{-3}$	
L(+)-Muscarine	+73°		0·4
D-(−)-Muscarine	−73°		330
L(+)-Acetyl-β-methylcholine	+85°		1
D(−)-Acetyl-β-methylcholine	−85°		240

* Number of molecules equivalent to 1 molecule of acetylcholine.

L(+)- and D(−)-muscarine, L(+)- and D-(−)-acetyl-β-methylcholine in the solid state (Canepa, Pauling & Sörum, 1966; Chothia & Pauling, 1969b) (see Table 2). The enantiomers of muscarine and acetyl-β-methylcholine are known to be stereoselective in their muscarinic activities (Waser, 1961; Beckett, Harper & Clitherow, 1963). The spatial arrangement of the N^+–C–C–O segment of the potent AChE inhibitor X resembles that of the potent muscarinics L(+)-muscarine and L(+)-acetyl-β-methylcholine in that their N^+–C–C–O torsions angles are all positive and vary between +60° to +85°. Their enantiomers, which have negative N^+–C–C–O torsion angles, are weak AChE inhibitors (XI) or weak muscarinics [D(−)-muscarine and D(−)-acetyl-β-methyl choline], suggesting incorrect fit on the enzyme or tissue receptors.

Previous comparative studies of optically active substrates or inhibitors of the enzyme AChE with their cholinomimetic or cholinolytic activity at the muscarinic receptor have suggested that a close structural similarity exists between the reactive site of the enzyme and of the muscarinic receptor (see references cited in Kay & others, 1970). The present work is an example in which stereoselective activities are shown by a pair of enantiomers with a rigid N^+–C–C–O segment, thus affording unambiguous interpretation of the topographical features of part of the enzyme receptor, and by inference, the muscarinic receptor.

If substrates for hydrolysis by AChE have to assume a *trans*-conformation, with τN^+–C–C–O = +150°, as postulated by Chothia & Pauling (1969), the fact that the AChE enzyme receptor can bind compound X well suggests that it has also the necessary structural features to accommodate cholinergics in the gauche (*cis*)-conformation, a conformation associated with potent muscarinics (Chothia, 1970). This may well explain the parallelism between the muscarinic receptors and AChE receptors in their identical pattern of absolute and relative stereoselectivities towards the dioxolane and muscarine series of stimulants (Belleau & Lacasse, 1964) and which has given rise to the contention that AChE and muscarinic receptor are structurally very similar if not the same.

A single type of enzyme receptor can accommodate the gauche- and *trans*-conformations of acetylcholine either (*a*) by being conformationally labile, and assuming two conformation arrangements corresponding to the two conformations of acetylcholine, or (*b*) by being conformationally non-labile, in which case duplicate sets of receptor sites sharing a common anionic site would be necessary.

To account for the stereoselectivity of these drug-receptor reactions, there must be more than two points of interaction between the drug and the receptors. Compound X and XI have only two recognizable points of attachment with the AChE enzyme receptor, i.e. the onium head and the carbonyl oxygen (corresponding to the ether oxygen of acetylcholine), and yet they also show stereoselectivity in their affinity to the enzyme receptor. There can be only one of two explanations:

(*a*) beside the N^+–C–C–O segment, there is a third point of interaction between the bornane molecule and the receptor, or (*b*) the N^+–C–C–O segment has more than two points of attachment with the receptor, a possibility hitherto unexplored.

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