ENZYME INHIBITING ACTION OF TETRAHYDROAMINOACRIDINE AND ITS STRUCTURAL FRAGMENTS

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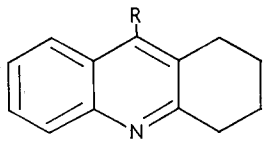
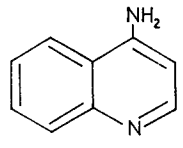
Received December 15, 1961

Tetrahydro-5-aminoacridine and four compounds representing its structural fragments have been compared as inhibitors of acetylcholinesterase and of monoamine oxidase. The entire structure of tetrahydro-5-aminoacridine appears to be essential for optimal inhibition of the esterase, less than 10^{-6} M concentration showing a 50 per cent inhibition of the enzyme, with the inhibition constant K_i as 1×10^{-4}. For optimum inhibition of monoamine oxidase, the 4-aminoquinoline part of the acridine molecule appears to be a structural requirement. 4-Aminoquinoline shows a stronger monoamine oxidase inhibition than any known therapeutically used inhibitor. It gives a 50 per cent inhibition of the oxidase in 10^{-6} M concentration, with K_i as $1{\cdot}1 \times 10^{-5}$.

1,2,3,4-TETRAHYDRO-5-AMINOACRIDINE (THA) has been developed in this laboratory as a partial antagonist of morphine (Shaw and Bentley, 1949). It has been used clinically with morphine for the treatment of intractable pain of terminal carcinoma (Stone, Moon and Shaw, 1961). Shaw and Bentley (1953) have demonstrated that THA strongly inhibits cholinesterase. Based on this observation, Gershon and Shaw (1958) showed that THA acts as a decurarizing agent.



I



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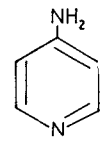

III

I, R=NH_2=Tetrahydroaminoacridine
I, R=NHC_4H_9=*N*-Butylaminotetrahydroacridine
I, R=H=Tetrahydroacridine
II=4-Aminoquinoline
III=4-Aminopyridine

A consideration of the molecular structure of THA (I) suggested that a fractionation of the total structure into simpler available units retaining one or more features of the original molecule, might yield structures with optimum inhibition on acetylcholinesterase (AChE) and on monoamine oxidase. With this in mind, THA, tetrahydroacridine, 4-aminoquinoline, 4-aminopyridine and *N*-butylaminotetrahydroacridine were tested for acetylcholinesterase inhibitory action and the first four compounds were also tested for monoamine oxidase inhibition. Procaine hydrochloride provided a control inhibitor of AChE.

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EXPERIMENTAL

Materials

THA (Monsanto), *N*-butylaminotetrahydroacridine, tetrahydroacridine, 4-aminoquinoline, 4-aminopyridine and procaine hydrochloride were all recrystallised twice and used as buffered solutions; acetylcholinesterase, crystalline (Nutritional Biochemical Corporation); sodium bicarbonate, A.R.; buffer-indicator mixture, 0·001 per cent phenol red in 0·05 M phosphate buffer, pH 8, as described by Caraway (1956); acetylcholine chloride solutions, freshly prepared before use or overnight solutions stored at 0°; 5-hydroxytryptamine (5-HT) creatinine sulphate; various reagents for colorimetric estimation of 5-HT, prepared as described by Udenfriend, Weissbach and Clark (1955). As a source of monoamine oxidase, the liver was removed from decapitated rats and instantly homogenised at 0° in pH 7·4 phosphate buffer (U.S.P.), maintaining a dilution of 1 in 20.

Methods

Determination of esterase activity. The rate of hydrolysis of acetylcholine was used as an index of enzyme activity. The photometric method of Caraway (1956) was employed with modifications. To the enzyme solution (0·1 ml., 340 units/ml.) in a corex cuvette were added an inhibitor solution (0·5 ml.) and the buffer-indicator mixture (2 ml.). After allowing to stand for 2 min. acetylcholine chloride solution was added (0·4 ml.). The contents were mixed instantly and the zero time reading of absorption at 558 mμ was immediately recorded. Subsequent absorptions were measured at definite time intervals to obtain the rate data. The decrease in the initial extinction gives a measure of the hydrolysis.

Determination of oxidase activity. The rate of oxidation of 5-HT in an incubated enzyme-substrate system was measured as an index of the enzyme activity. The unoxidised 5-HT remaining in the incubated mixture was estimated by the method of Udenfriend and others (1955) with modifications.

To the enzyme solution (1·5 ml.) in each of a series of 50 ml. glass-stoppered centrifuge tubes was added the inhibitor solution (1·5 ml.), and the tubes were placed in a water bath at 37·5 ± 0·5°. After incubation for 15 min. the 5-HT solution (1 ml.) was added to each tube at a noted time. At definite time intervals, the oxidation reactions were arrested by adding 3 drops of octanol to the incubating mixture, followed by an immediate addition of butanol-saturated borate buffer (pH 10, 2 ml.), butanol (15 ml.) saturated with sodium chloride and the borate buffer, and sodium chloride (2 g.). The tubes were stoppered, shaken for 7 min. and centrifuged. The lower aqueous layer was removed by aspiration with a suitable syringe. The butanol extract was washed with 5 ml. of pH 10 borate buffer, diluted with 10 ml. of heptane and shaken for 5 min. with 3 ml. of 0·2 N hydrochloric acid. The contents were centrifuged.

A 2 ml. aliquot of the acid layer was treated at 50° for 7 min. with nitrosonaphthol reagent (1 ml.) and nitrous acid reagent (1 ml.). The coloured solution was washed with ethyl acetate (10 ml.) to remove excess of any unreacted reagents. The extinction of the coloured aqueous phase

was determined at 540 mμ against the reagent blank run under identical conditions but with no 5-HT added.

Use of fixed-volume stop-syringes facilitated the transfers of organic solvents and the single extractions conserved time.

The reactions were run in the presence of varying inhibitor concentrations at two different substrate concentrations. Each series was run at a constant temperature (±1°).

RESULTS

Acetylcholinesterase Inhibition

Optimum substrate concentration. Fig. 1 shows the substrate concentration-enzyme activity curve. A concentration of $2{\cdot}8 \times 10^{-3}$ M acetylcholine appears to be the optimum and this agrees with the value cited in literature (Augustinsson, 1948). In the inhibition experiments the two substrate concentrations used were $5{\cdot}56 \times 10^{-3}$ M [s_1] and $2{\cdot}78 \times 10^{-3}$ M [s_2].

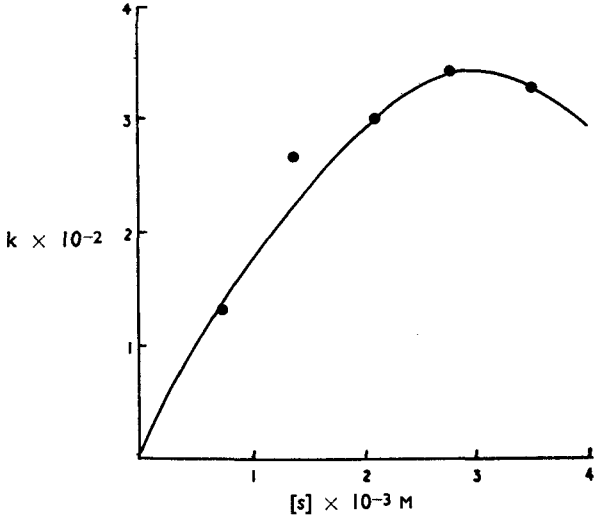

FIG. 1. Effect of the concentration of acetylcholine chloride on the activity of acetylcholinesterase.

Inhibition studies. Rate constants of hydrolysis of acetylcholine at two substrate concentrations in the presence of varying concentrations of inhibitors are shown in Table I. The respective inhibition constants derived by the method of Dixon (1953) are also included.

Table II gives the relative concentrations of the various compounds required to produce a 50 per cent inhibition of AChE and also their pKa values obtained from the literature.

Monoamine Oxidase Inhibition

Optimum substrate concentration. Fig. 2 shows that the rat liver monoamine oxidase has two optimum substrate concentrations. The one at

TABLE I

RATES OF HYDROLYSES OF ACETYLCHOLINE IN THE PRESENCE OF VARYING CONCENTRATIONS OF INHIBITORS

Inhibitor compound	Inhib. conc. (M)	Velocity const. k(x 10^{-2} min.$^{-1}$ at $[S_1]$	Velocity const. k(x 10^{-2} min.$^{-1}$ at $[S_2]$	K_i (Dixon)
Tetrahydroaminoacridine	1×10^{-6}	0·75	1·00	
	2×10^{-6}	0·41	0·54	
	3×10^{-6}	0·28	0·41	
	5×10^{-6}	0·20	—	
	8×10^{-6}	—	0·15	1×10^{-4}
Tetrahydroacridine	1×10^{-6}	2·00	2·20	
	2×10^{-6}	1·50	1·70	
	3×10^{-6}	1·00	1·30	
	5×10^{-6}	0·73	0·80	4×10^{-4}
N-Butylaminotetrahydroacridine ..	1×10^{-6}	2·60	2·80	
	2×10^{-6}	1·70	1·80	
	3×10^{-6}	1·60	1·50	
	5×10^{-6}	1·30	1·20	$1·7 \times 10^{-3}$
4-Aminoquinoline	2×10^{-5}	2·20	2·30	
	4×10^{-5}	1·40	1·40	
	6×10^{-5}	1·00	1·00	
	10×10^{-5}	0·68	0·68	14×10^{-2}
4-Aminopyridine	1×10^{-4}	2·90	3·00	
	2×10^{-4}	2·60	2·90	
	3×10^{-4}	—	2·50	
	5×10^{-4}	2·10	2·10	5×10^{-1}
Procaine hydrochloride	2×10^{-5}		4·60	
	4×10^{-5}		4·20	
	6×10^{-5}		4·10	
	10×10^{-5}		3·80	

TABLE II

INHIBITION OF AChE BY VARIOUS COMPOUNDS

Substance	Concentration required to produce 50 per cent inhibition (M)	pKa
Tetrahydroaminoacridine ..	$<1 \times 10^{-6}$	9·4
Tetrahydroacridine	2×10^{-6}	5·0 (approx.)
Butylaminotetrahydroacridine	3×10^{-6}	9·4 (approx.)
4-Aminoquinoline	4×10^{-5}	9·17
4-Aminopyridine	$>5 \times 10^{-4}$	9·17
Procaine	$>1 \times 10^{-4}$	—

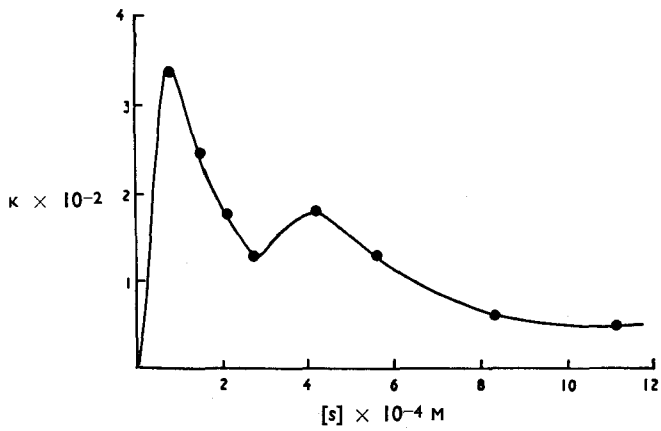

FIG. 2. Effect of the concentration of 5-HT on the activity of monoamine oxidase.

7×10^{-5} M is too low to work with in the present method of assay. Therefore the second concentration, $4{\cdot}2 \times 10^{-4}$ M, was used as the optimum for these studies.

Stability of the oxidase. A 1 in 20 homogenate of the rat liver in pH 7·4 phosphate buffer, when stored overnight at 5°, lost only 5 per cent of its monoamine oxidase activity. It is safe to work with the same liver preparation for one day, though a homogenate maintained overnight in a frozen condition can be used the following day. Fresh liver chilled at 0° also maintains its monoamine oxidase activity for several days.

TABLE III

PER CENT INHIBITION OF MONOAMINE OXIDASE AT 20 MIN.

Inhibitor conc. (M)	Per cent inhibition caused by			
	THA	TACR*	4-AQ†	4-AP‡
$1{\cdot}5 \times 10^{-4}$	54–57	89–94	100	7·5–10
$1{\cdot}0 \times 10^{-4}$	18·4	85	91·8	5·4
$3{\cdot}3 \times 10^{-5}$	0·0	42·8	87·8	0·0

* Tetrahydroacridine. † 4-Aminoquinoline. ‡ 4-Aminopyridine.

Inhibition of the oxidase. Table III shows inhibition per cent of the enzyme caused by various concentrations of the inhibitors at 20 min.

Inhibition of the enzyme by varying concentrations of THA and 4-aminoquinoline at two different substrate concentrations were studied to obtain rate data for the estimation of the inhibition constant (K_i). The K_i values as determined by the method of Dixon (1953) were found to be 2×10^{-4} and $1{\cdot}1 \times 10^{-5}$ for THA and 4-aminoquinoline, respectively.

DISCUSSION

From the data in Table I and II it appears that THA is the most potent inhibitor of AChE in the related small series of compounds. *N*-Alkyl-aminotetrahydroacridine is more basic than THA, though in the *N*-butyl derivative the steric hindrance arising from the bulky group may counteract the basicity increasing effect of the alkyl group. Tetrahydroacridine itself is a much weaker base than its 5-amino derivative (THA), and therefore also weaker than the *N*-butylamino derivative; yet it shows an activity nearly as great as THA and certainly much greater than the *N*-butyl compound. It is possible that such a decreased activity of the *N*-butyl derivative of THA is due to the steric hindrance of the butyl group which may produce a poor-enzyme-inhibitor interaction.

4-Aminoquinoline is as basic as 4-aminopyridine, but the two compounds show widely different potencies of inhibition. Again, both these compounds are nearly as basic as THA, but they both show very weak AChE inhibiting potency compared to that of THA. All these facts suggest that the maximum inhibition of AChE requires an optimum structure, in this case THA, and not the basicity alone. Indeed some basicity is necessary, because at least one nitrogen needs to be ionized to ensure interaction of the inhibitor with the ionic site of the enzyme.

The modified procedure of assay for monoamine oxidase activity described above enables 36 to 48 analyses to be made each working day. Of the compounds examined, 4-aminoquinoline is a structure required for maximum inhibition of the oxidase. It is possible that THA owes its oxidase inhibiting potency mainly to the 4-aminoquinoline part of the molecule. However, the significance of the reduced ring cannot be overlooked. This is obvious from the inhibiting potency shown by the tetrahydroacridine.

Detailed toxicological studies of 4-aminoquinoline are needed to evaluate the usefulness of this agent as a psychomotor stimulant like other monoamine oxidase inhibitors already in use.

Acknowledgements. I am grateful to Professor F. H. Shaw for making available the funds and facilities for this work and to Professor Adrien Albert, F.R.S., for kindly sparing some of the compounds used in these studies. My thanks are also due to Mr. D. W. Bruce for his co-operation and to Mr. D. Lamb and Mr. W. Gay for their technical help.

REFERENCES

Augustinsson, K. B. (1948). *Acta. physiol. scand.*, **15** Suppl., 52–53.
Caraway, W. T. (1956). *Amer. J. clin. Path.*, **26**, 945–955.
Dixon, M. (1953). *Biochem. J.*, **55**, 170–171.
Gershon, S. and Shaw, F. H. (1958). *J. Pharm. Pharmacol.*, **10**, 638–641.
Shaw, F. H. and Bentley, G. (1949). *Med. J. Aust.*, **2**, 868–874.
Shaw, F. H. and Bentley, G. (1953). *Aust. J. exp. Biol. med. Sci.*, **31**, 573–576.
Stone, V., Moon, W. and Shaw, F. H. (1961). *Brit. med. J.*, **1**, 471–473.
Udenfriend, S., Weissbach, H. and Clark, C. T. (1955). *J. biol. Chem.*, **215**, 337–344.