REFERENCES

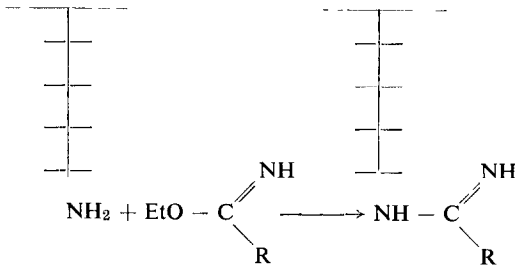
1. M. T. HAKALA, S. F. ZAKRZEWSKI and C. A. NICHOL, *J. biol. Chem.* **236**, 952 (1961).
2. G. A. FISCHER, *Biochem. Pharmac.* **7**, 75 (1961).
3. M. FRIEDKIN, E. CRAWFORD, S. R. HUMPHREYS and A. GOLDIN, *Cancer Res.* **22**, 600 (1962).
4. S. F. ZAKRZEWSKI, *J. biol. Chem.* **235**, 1780 (1960).
5. L. GOODMAN, J. DEGRAW, R. L. KISLIUK, M. FRIEDKIN, E. J. PASTORE, E. J. CRAWFORD, L. T. PLANTE, A. AL-NAHAS, J. F. MORNINGSTAR, JR., G. KWOK, L. WILSON, E. F. DONOVAN and J. RATZAN, *J. Am. chem. Soc.* **86**, 308 (1964).
6. K. SLAVIK, *Fedn. Proc.* **25**, 278 (1966).
7. E. R. KASHKET, E. J. CRAWFORD, M. FRIEDKIN, S. R. HUMPHREYS and A. GOLDIN, *Biochemistry* **3**, 1928 (1964).
8. M. T. HAKALA and T. ISHIHARA, *Cancer Res.* **22**, 987 (1962).
9. V. I. OYAMA and H. EAGLE, *Proc. Soc. exp. Biol. med.* **91**, 305 (1956).
10. J. I. DEGRAW, J. P. MARSH, JR., E. M. ACTON, O. P. CREWS, C. MOSHER, A. N. FUJOWARA and L. GOODMAN, *J. org. Chem.* **30**, 3404 (1965).
11. L. DELMONTE and T. H. JUKES, *Pharmac. Rev.* **14**, 91 (1962).
12. M. T. HAKALA, *Biochim. biophys. Acta* **102**, 198 (1965).

Biochemical Pharmacology, 1966, Vol. 15, pp. 1623–1625. Pergamon Press Ltd., Printed in Great Britain.

Retention of enzymatic activity by egg white lysozyme bearing alkylamidino substituents

(*Received* 8 *June* 1966; *accepted* 14 *June* 1966)

FAVOURABLE modification of biological activity sometimes follows the incorporation of alkyl chains into drug molecules. The principle has been applied to lysozyme (E.C. 3.2.1. 17; N-acetylmuramide glycanohydrolase) while seeking either to modify its limited antibacterial spectrum by imparting a degree of lipid affinity, or conferring a degree of resistance to peptic hydrolysis by steric hindrance. Most chemical reagents attack the free amino groups and cause inactivation, probably by reducing the basic character of the enzyme.[1] Converting the lysine residues to arginine residues, however, leaves activity unimpaired.[2] By reaction with imino esters, the epsilon amino groups of lysine residues in peptides may be converted to alkylamidino groups[3] permitting retention of basic character while introducing alkyl chains (I; R = alkyl).



(I)

EXPERIMENTAL

Amidination of lysozyme

The appropriate nitriles were converted to imino ester hydrochlorides, by the Pinner method and reacted with commercial crystallized egg white lysozyme as described by Wofsy and Singer,[4] using their conditions for both partial and exhaustive amidination. The reaction mixtures were dialysed against water, aliphatic esters removed by filtration and the product freeze-dried. The extent of amidination was determined by condensation of residual free amino groups with fluoro-dinitrobenzene, hydrolysis, and estimation of dinitro-phenyl-lysines as described by Levy.[5] The N-terminal

lysine, converted to α-ε-bis-dinitrophenyl-lysine, gave poor recoveries (approx 50 per cent) and the extent of amidination of this particular lysine residue could only be estimated approximately. Products with >40 per cent substitution gave one band, separable from lysozyme, by electrophoresis on cellulose acetate in bicarbonate buffer, pH 10·5.

Enzyme assay

The amidinated products were compared with equi-molar amounts of the original sample of lysozyme (0·1 mg/ml) in 0·066M phosphate buffer, pH 7·1, for ability to lyse a suspension of spray dried Micrococcus lysodeikticus using spectrophotometry at 450 mμ. The rate at 15 sec after mixing was taken as a measure of enzymatic activity. A similar comparison was made for lysis of Bacillus megaterium (kindly grown on Hartley digest broth with 1·5% agar by Dr R. A. Cowan), but using a 20-fold concentration of enzyme.

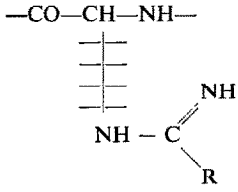
Peptic hydrolysis

The enzyme (7 mg) in 0·1 N HCl (7 ml) was digested at 30° with a solution of pepsin (0·2 mg/ml) in 0·1 N HCl (2·1 ml). Aliquots (1·3 ml) were taken at 4 min intervals into 4% trichloroacetic acid (2·5 ml), centrifuged, and solubilized protein measured as the optical density at 280 mμ.

RESULTS

Results and analyses are summarized in Table 1. The degree of amidination, especially of the terminal lysine, decreased as the alkyl chain lengthened. For short chain lengths the enzymatic

TABLE 1. AMIDINATED LYSOZYME



R	Substitution		Lytic activity (lysozyme = 1)		Peptic hydrolysis (lysozyme = 1)
	Non-terminal lysines %	Terminal lysine % approx	*M. lysodeikticus*	*B. megaterium*	
CH_3	80	100	0·8	1·0	5·9
	72	100	1·1	1·0	2·2
C_2H_5	77	100	1·1	1·0	2·6
	66	100	1·2	1·0	1·7
C_3H_7	77	50	1·0	0·7	—
	66	<50	—	0·9	1·7
C_4H_9	73	<50	1·0	0·8	1·5
	68	<50	1·1	0·8	1·5
C_5H_{11}	67	<50	0·8	0·7	1·4
	53	<50	1·0	0·7	1·3
C_6H_{13}*	66	<50	0·6	0·6	—
	45	<50	0·9	0·7	1·3
$Me_2N(CH_2)_2$	71	100	1·1	0·9	3·6
	43	0	1·1	0·9	1·2
C_6H_5	43	0	1·0	0·7	1·0

* No substitution occurred by $C_{15}H_{31}$ or $C_{17}H_{35}$.

activity was fully retained, even possibly increased, but the rate of peptic hydrolysis was also markedly increased. Activity and rate of peptic hydrolysis fell as the chain length increased. The products showed no other enzymatic activity when presented with a variety of ester substrates and they had no chitinase activity.

Biological Research Laboratories,
Messrs Reckitt & Sons Ltd,
Dansom Lane,
Hull

A. McCoubrey
M. H. Smith

REFERENCES

1. P. Jollès, *Angew. Chem. internat. Edit.* **3**, 28 (1964).
2. I. I. Geschwind and C. H. Li, *Biochim. biophys. Acta* **25**, 171 (1957).
3. M. J. Hunter and M. L. Ludwig, *J. Am. chem. Soc.* **84**, 3491 (1962).
4. L. Wofsy and S. J. Singer, *Biochemistry* **2**, 104 (1963).
5. A. L. Levy, *Meth. biochem. Analysis*, **2**, 359 (1955).

Biochemical Pharmacology, 1966, Vol. 15, pp. 1625–1627. Pergamon Press Ltd., Printed in Great Britain.

Structure–action relationship of β-carbolines as monoamine oxidase inhibitors*

(*Received* 18 *March* 1966; *accepted* 6 *June* 1966)

The hypothesis that monoamine oxidase (MAO) inhibitory activity could account for the clinical effects of the antidepressant drugs, and the subsequent observations that many of the hydrazine drugs proved to be toxic, has focused attention on the nonhydrazine MAO inhibitors.

Harmaline and related β-carbolines have been shown to be potent MAO inhibitors,[1] and numerous studies utilizing assays of potency both *in vitro* and *in vivo* have been comprehensively reviewed.[2]

The majority of the compounds examined so far, however, have been closely related to harmaline, i.e. 7-substituted. The ease with which tryptamines, related to the neurohormone serotonin, cyclize to form 6-substituted β-carbolines has been reported.[3] These compounds, which it has been postulated might arise endogenously,[4] have been found to be potent serotonin antagonists and to affect conditioned behavior.[5, 6] To further elucidate the structure–action relationship of these compounds, a series of β-carbolines has been assayed as MAO inhibitors

METHODS

Mitochondrial monoamine oxidase was prepared from fresh calf liver by differential centrifugation. Inhibition was measured by a modification of the method described by Otsuka and Kobayashi.[7] Tyramine-^{14}C was used as substrate and the product *p*-hydroxyphenyl acetaldehyde extracted into ethyl acetate, a single extraction giving a 93 per cent recovery, and assayed by liquid scintillation. Confirmation of the identity of the product was obtained by chromatography and scanning.

RESULTS AND DISCUSSION

The β-carbolines studied were found to be competitive inhibitors and did not require preincubation. The results in Table 1 express the inhibitory potency as I_{50} values, i.e. concentration at which 50 per cent inhibition occurs, and as pI_{50} values, i.e. negative logarithm of the I_{50} values.

The effect of substitution in the aromatic ring of the β-carboline nucleus indicated that the position of substitution has little effect on potency. 6-Methoxy- and 7-methoxy-β-carboline analogues were found to be equipotent. The nature of the substituent did however affect potency; a hydroxyl group in the 6- or 7-position reduced activity, whereas corresponding methoxylated compounds were equipotent with the unsubstituted β-carboline. These findings are in agreement with previous reports.[8]

* Supported by Grant MH-11168 and the Britton Fund.