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.

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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

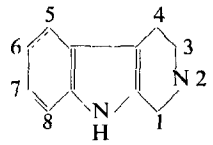
The β-carbolines studied were found to be competitive inhibitors and did not require preincubation. The results in Table 1 express the inhibitory potency as I_{50} values, i.e. concentration at which 50 per cent inhibition occurs, and as pI_{50} values, i.e. negative logarithm of the I_{50} values.

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]

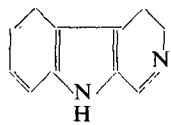
* Supported by Grant MH-11168 and the Britton Fund.

The effect of degree of saturation of the pyridine rings was found to be similar in the unsubstituted and in both the 6- and 7-substituted β-carbolines. In any triad the tetrahydro was least active, and the aromatic compound most active, with the dihydro compound being intermediate. These findings are in agreement with previous reports in which harmine and harmaline were found to be more active than tetrahydroharmine *in vitro*.[1, 8] Also Pletscher *et al.*[8] found that serotonin levels *in vivo* were increased to a greater extent by harmine and harmaline than by tetrahydroharmine.

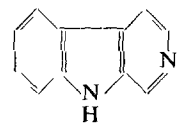
TABLE 1. INHIBITION OF MAO BY β-CARBOLINES AND RELATED COMPOUNDS



(1,2,3,4-tetrahydro-β-carboline)



(3,4-dihydro-β-carboline)



(β-carboline)

Inhibitors	I_{50}	pI_{50}
Non-active control compound		
Benzoic acid		0
No nitrogen in position 2		
Carbazole*		0
Iproniazid	$4{\cdot}75 \times 10^{-10}$	9·32
Nitrogen blocked: 2-acetyl-		
1-Methyl-2-acetyl-1,2,3,4-tetrahydro-β-carboline		0
1-Methyl-2-acetyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline		0
No methyl group in position 1		
Norharmane (β-carboline)	$7{\cdot}5 \times 10^{-10}$	9·12
1-Methyl-		
1-Methyl-1,2,3,4-tetrahydro-β-carboline	$4{\cdot}25 \times 10^{-8}$	7·37
1-Methyl-3,4-dihydro-β-carboline	(not tried)	
1-Methyl-β-carboline	5×10^{-9}	8·3
1-Methyl-6-methoxy-		
1-Methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline	$5{\cdot}0 \times 10^{-5}$	4·3
1-Methyl-6-methoxy-3,4-dihydro-β-carboline	$4{\cdot}5 \times 10^{-7}$	6·35
1-Methyl-6-methoxy-β-carboline	$1{\cdot}5 \times 10^{-8}$	7·82
1-Methyl-7-methoxy-		
1-methyl-7-methoxy-1,2,3,4-tetrahydro-β-carboline	(not tried)	
1-Methyl-7-methoxy-3,4-dihydro-β-carboline (Harmaline)	$1{\cdot}0 \times 10^{-6}$	6·00
1-Methyl-7-methoxy-β-carboline (Harmine)	$1{\cdot}5 \times 10^{-8}$	7·82
1-Methyl-6-hydroxy-		
1-Methyl-6-hydroxy-1,2,3,4-tetrahydro-β-carboline	$7{\cdot}5 \times 10^{-3}$	2·12
1-Methyl-6-hydroxy-3,4-dihydro-β-carboline	(not tried)	
1-Methyl-6-hydroxy-β-carboline	(not tried)	
1-Methyl-7-hydroxy-		
1-Methyl-7-hydroxy-1,2,3,4-tetrahydro-β-carboline	(not tried)	
1-Methyl-7-hydroxy-3,4-dihydro-β-carboline (Harmalol)	$2{\cdot}5 \times 10^{-8}$	7·6
1-Methyl-7-hydroxy-β-carboline (Harmol)	$2{\cdot}75 \times 10^{-8}$	7·56
Corynanthine	$2{\cdot}45 \times 10^{-2}$	0–1·6
Yohimbine	$4{\cdot}25 \times 10^{-8}$	7·37

* In propyleneglycol.

Although it has been reported that N-methylation does not cause inactivation,[9] the importance of an unsubstituted pyridine nitrogen is emphasized by the fact that carbazole had no action and that acetylation of the pyridine nitrogen of potent β-carbolines reduced monoamine oxidase inhibitory potency to zero. It is worth noting in this context that serotonin antagonism potency[5] and monoamine oxidase inhibitory potency appear to parallel each other, indicating possibly a close relationship between the serotonin receptor site and active site of the associated metabolic enzyme. Further studies of the binding of the β-carbolines are in progress.

Stereospecificity of monoamine oxidase is indicated by the fact that yohimbine proved to be a potent inhibitor while corynanthine, a stereoisomer, was completely inactive.

Although the exact nature of the specificity cannot be determined from this observation, both compounds containing more than one asymmetric carbon, it may explain the relatively greater toxicity of yohimbine, which is approximately four times more toxic than corynanthine.

Of greater significance is the fact that corresponding β-carbolines derived from serotonin, 5-methoxytryptamine, and melatonin are all potent MAO inhibitors as well as being serotonin antagonists and capable of disrupting conditioned behavior.[5, 6]

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Biochemical Pharmacology, 1966, Vol. 15, pp. 1627–1629. Pergamon Press Ltd., Printed in Great Britain.

Increased sensitivity in a specific fluorometric method for brain histamine

(*Received* 9 *May* 1966; *accepted* 2 *June* 1966)

THE FLUOROMETRIC determination of tissue histamine by the method of Shore *et al.*[1] has been reported to be inadequate for the analysis of brain histamine because of the presence of interfering substances[2, 3] that are not removed by butanol extraction or by the use of certain ion-exchange columns.[4] Recently, Kremzner and Pfeiffer[5] have shown that the major interfering substance, spermidine, may be separated from histamine by the use of a phosphorylated cellulose column. Their procedure requires elution of histamine from the column with 20–25 ml of 0·03 N HCl. As the concentration of histamine in rat brain is reportedly in the range of 50–76 ng/g,[2, 5] the use of this large elution volume limits the sensitivity of the method, particularly if it is desired to perform regional analysis of histamine in brain. We have modified the Kremzner and Pfeiffer procedure, therefore, so as to allow elution with only 5 ml fluid, thus increasing sensitivity sufficiently so that 0·5 g brain or less may be analyzed, instead of the 3 g required by Kremzner and Pfeiffer.[5]

Phosphorylated cellulose (Cellex-P, Bio Rad Laboratories) was purified as described by Kremzner and Wilson[6] and suspended in 0·03 M phosphate buffer, pH 6·0. The cellulose was then transferred into a chromatographic column (Scientific Glass no. JT-7390, 200 mm length, 6 mm bore) to a height of 40 mm and washed with 10 ml of 0·03 M phosphate buffer, pH 6·0. Histamine (0·1 μg), spermidine (100 μg), or both were applied to the columns in 10 ml of 0·03 M phosphate buffer, pH 6·0. The columns were then washed with 5 ml water and the histamine eluted with 5 ml of 0·2 M NaCl. The eluates were analyzed for histamine and spermidine fluorometrically after condensation with *o*-phthalaldehyde.[1] Recovery of histamine added to the column was 101 per cent. Spermidine could not be detected in the eluates from the columns containing spermidine.

Male Sprague-Dawley rats weighing 200–250 g were used for the determination of brain histamine. Brains were homogenized in 10 volumes of 0·4 N $HClO_4$, centrifuged, and a 5-ml aliquot of the