Biochemical Pharmacology, Vol. 32, No. 23, pp. 3661–3664, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + 0.00

DIFFERENTIAL INACTIVATION OF MITOCHONDRIAL MONOAMINE OXIDASE BY STEREOISOMERS OF ALLENIC AMINES*

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(Received 12 September 1983; accepted 15 September 1983)

Mitochondrial monoamine oxidase (EC 1.4.3.4, MAO) has been a prime pharmacological target since its inhibition was correlated with the relief of depression nearly three decades ago [for reviews, see Refs. 1-3]. Recent interest has focussed on the selective inhibition of the multiple forms of MAO (4), termed A and B, structure–activity relationships of inhibitors to define the active site of these forms (5), and the design of "safe" inhibitors which do not exhibit the "cheese effect" and other untoward properties (4).

Both reversible and irreversible stereoselective inhibitors of MAO have been developed (6-10). Of particular interest, the enantiomers of 2,3-dichloro-α-methyl benzylamine (6) and 4-dimethylamino-2,α-dimethyl-phenethylamine (7) exhibit opposite selectivity for the reversible inhibition of the A and B forms of MAO. α-Allenic amines, which are known inhibitors of MAO (11-14), provide a unique opportunity for the study of stereoselective irreversible inhibition, as a penta-2,3-dienamine possesses chirality which lies within the latent reactive functionality rather than in an asymmetric aralkyl substituent [such as in deprenyl (10)]. Krantz *et al.* (12,13) have demonstrated that MAO inhibition by 1^{o}, 2^{o} and 3^{o} amines containing a 2,3-butadienyl moiety is both time dependent and irreversible. (±)-N-Benzyl-N-methyl-penta-2,3-dienamine is less active than the corresponding butadienyl system, but this racemic mixture still functions as a MAO inactivator, thus providing the basis for the present study. The ability of allenic amines to function as effective inhibitors is dependent on their binding ability, the ease of abstraction of a hydrogen alpha to the allene group, and the ease of capture of a putative allenic iminium ion. We now wish to report our findings, which represent the first systematic study of the effect of the chirality of allenic amines on the inhibition of beef liver MAO (B).

The allenic amines were synthesized by condensation of the mesylates of (R)- and (S)-penta-2,3-dien-1-ol with the appropriate secondary amine; this method is based on the work of Claesson and co-workers (14). On the basis of optical rotation and ^{1}H-NMR/Eu(dcm)$_3$ measurements, these allenic amines were estimated to be greater than 96% isomerically pure. Inhibition of bovine liver MAO, which was isolated and purified according to published procedures (15,16), by each allenic amine was carried out in phosphate buffer (pH 7.4) at 25^{o}, and residual enzyme activity was monitored spectrophotometrically (17). The parameters K_i and k_2 reported in Table 1 were calculated by the method of Kitz and Wilson (18).

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* Contribution No. 178 from the Institute of Bio-Organic Chemistry, Syntex Research.

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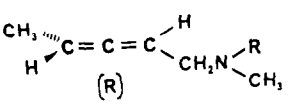
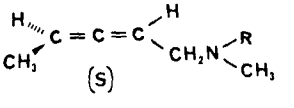
Table 1. Kinetic constants for the time-dependent inhibition of beef liver mitochondrial MAO by allenic amines*





R	Compound No.	K_i (mM)	k_2 (min^{-1})	Compound No.	K_i (mM)	k_2 † (min^{-1})	$k_2(R)/k_2(S)$
CH_3	1	0.06	0.22	2	0.10	0.29	0.8
CH_2Ph ‡	3	0.20	1.1	4	0.82	0.16	7
(R)-$CHCH_3Ph$	5	0.73	0.61	6	2.6 §	0.012 ‖	51
(S)-$CHCH_3Ph$	7	1.8	0.44	8	1.1 §	0.007 ‖	63
CH_2CH_2Ph	9	0.43	0.99	10	0.65	0.013	76
(R)-$CHCH_3CH_2Ph$	11	1.3	0.15	12	0.69 §	0.003 ‖	50
(S)-$CHCH_3CH_2Ph$	13	0.18	0.009	14	0.72 §	0.015 ‖	0.6
(R)-1,2,3,4-Tetrahydro-1-naphthyl	15	0.23	0.79	16	0.15 §	0.012 ‖	66
(S)-1,2,3,4-Tetrahydro-1-naphthyl	17	0.25	0.13	18	0.15 §	0.0006 ‖	220

* The time-dependent inhibition is represented (18) by $k_{obs} = k_2/(1 + K_i/[I])$, in accordance with $E + I \rightleftharpoons E\text{-}I \xrightarrow{k_2} E\text{-}I_{inact}$. The first-order rate constants (k_{obs}) for the exponential decay of MAO activity were determined by an iterative least-squares matrix technique (19,20), and linear regression analysis was used to obtain K_i and k_2 from secondary reciprocal plots.

† Values of k_2 for all (S)-allenic amines other than 2 and 14 represent upper limits, as the low values observed for these isomers may be accounted for in whole or in part by a few percent (calc'd 0.6 - 4.5%) of diastereomeric (R)-allenic amine impurities.

‡ Samples of 3 and 4 were provided by Prof. A. Claesson (14).

§ Obtained from experiments in which the allenic amine was treated as a reversible competitive inhibitor.

‖ Calculated from measured rates of inactivation at one allenic amine concentration and K_i.

Our results clearly demonstrate that the stereochemistry of the allene group has a great effect on the efficacy of time-dependent inhibition. In general, the k_2 values indicate that the (R)-allenic amines were much more effective than their (S)-allenic counterparts, with the notable exception of amines 1-2 and 13-14. In these two cases, the rate constants (k_2) differed by a factor of less than 2. The most dramatic demonstrations of a preference by MAO-B were with respect to the enantiomers (R,R)-15 and (S,S)-18 [$k_2(RR)/k_2(SS) \sim 1300$] and the diastereomers (R,S)-17 and (S,S)-18 [$k_2(RS)/k_2(SS) \sim 220$].

For the enantiomeric pairs 1-2, 3-4 and 9-10, an increasing preference for the (R)-allene results from increasing the size of the aralkyl group. Addition of an α-methyl group to the phenethyl substituent has a much more profound effect on k_2 (compounds 9-14) than does the addition of an α-methyl to the benzyl substituent (compounds 3-8). In fact, the α-methyl-phenethyl compounds (11-14) are all rather poor inactivators, indicating that a limit in structural shape and size for facile inhibition of MAO-B by aralkyl allenic amines may have been reached.

Thus, our study has demonstrated emphatically that a chiral allenic moiety can be used as a sensitive probe of active site geometry. The inactivation of MAO-B by aralkyl allenic amines is intimately related to the configuration of the allene group, which places severe active-site geometric/steric constraints on either the formation or the nucleophilic capture of the allenic imminium ion. Investigations directed toward establishing the MAO A/B selectivity of chiral allenic amines, and toward the development of ultra-specific inhibitors of multiple forms of MAO, are in progress.

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