Communications to the Editor

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DETECTION OF A NEW SERIES OF MONOAMINE OXIDASE INHIBITORS

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A new series of monoamine oxidase (MAO) inhibitors structurally analogous to tetrazolo[5,1-a]phthalazine (Tetra-P) was detected using rat brain mitochondrial MAO. In the tricyclic group, naphtetrazole (NTE) indicated a marked potency of MAO inhibition almost equal to that of iproniazid, and naphtriazole (NTR) showed similar potency as did Tetra-P. The nonselective and competitive inhibition for both types, MAO-A and MAO-B, was observed in some Tetra-P analogues.

KEYWORDS ———— Tetra-P analogue; MAO inhibitor; rat brain mitochondrial MAO; nonselective inhibition; competitive inhibition; fluorometry

In a previous paper, we reported that tetrazolo[5,1-a]phthalazine (Tetra-P) was formed from hydralazine (HP) in human saliva under acidic conditions.[1] The pharmacological tests indicated that Tetra-P had an activity analogous to that of the monoamine oxidase (MAO) inhibitor nialamide. In order to confirm the characteristics of Tetra-P, its inhibitory effect on MAO was examined by a fluorometric assay using human platelet MAO. The results showed that the ability of Tetra-P as a MAO inhibitor was identical to that of isoniazid but lower than that of iproniazid.[2] Many compounds have been claimed as MAO inhibitors, but Tetra-P, a tricyclic tetrazolophthalazine compound, is quite a new kind of MAO inhibitor in its chemical structure. In the present work, several compounds which are structurally analogous to Tetra-P were prepared and examined for their inhibitory effects on rat brain mitochondrial MAO from the standpoint of the structure-activity relationship.

The following compounds, which were not on the market, were synthesized. Tetra-P, s-triazolo[3,4-a]phthalazine (Tri-P), 3-methyl-s-triazolo[3,4-a]phthalazine (MTP) and 3-ethyl-s-triazolo[3,4-a]phthalazine (ETP) were synthesized by a modified method of Haegele et al.[3] 3-Hydroxymethyl-s-triazolo[3,4-a]phthalazine (HTP) was prepared following the method of Zimmer et al.[4] Naphtetrazole (NTE), naphtriazole (NTR), benzoisotetrazole (BTE) and benzoisotriazole (BTR) were obtained using the method of Marckwald et al.[5] The structures of these compounds were confirmed by examining melting point, elemental analyses, nuclear magnetic resonance, and infrared and mass spectra.

The MAO activities in rat brain mitochondria were determined by fluorometry

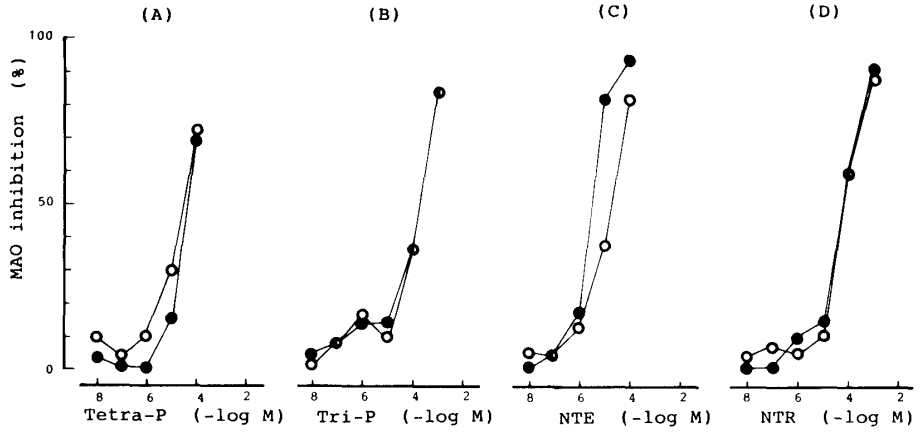


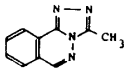
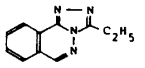
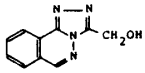
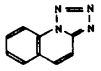
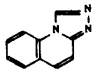
Fig. 1. Effects of the Concentration of (A) Tetra-P, (B) Tri-P, (C) NTE and (D) NTR on the Inhibition of MAO-A and MAO-B

Rat brain mitochondrial fractions were incubated for 15 min at 37°C with various concentrations of each compound. MAO activities were assayed using 19.1 mM* p-sulfamoylbenzylamine (MAO-A) and 0.095 mM* benzylamine (MAO-B) as substrates, and are expressed as percent inhibitions of the activities of the control samples. -O- : MAO-A -●- : MAO-B * : final concn.

using p-sulfamoylbenzylamine and benzylamine as substrates of MAO-A and MAO-B, respectively.[6] A concentration of the test compound required to inhibit 50% of the enzyme activity (I_{50} in Table I) was determined by using a graph and plotting logarithmic concentrations of the compounds (-log M) versus percent inhibition. This is shown in Fig.1 which depicts the inhibition curves for Tetra-P, Tri-P, NTE and NTR. Iproniazid which is well-known as a potent MAO inhibitor was used as a reference standard in this investigation. The I_{50} values of the compounds obtained from the inhibition curves are shown in Table I, which indicates some compounds strongly inhibited MAO activity in rat brain mitochondria. In the tricyclic group, NTE has a strong MAO inhibitory potency (I_{50}: 10^{-5} M for MAO-A, 4.0×10^{-6} M for MAO-B) almost equal to that of iproniazid (I_{50}: 3.2×10^{-6} M for MAO-A, 3.2×10^{-6} M for MAO-B). Both NTR and Tetra-P have a significant but slightly weaker potency than NTE. Tri-P is the mildest inhibitor among the unsubstituted tricyclic compounds. The bicyclic compounds also showed weak but detectable MAO inhibitory effects. All compounds tested indicated a nonselective inhibition for both types of MAO-A and MAO-B. Lineweaver-Burk plots of the kinetic data indicated that the inhibition by Tetra-P, Tri-P, NTE and NTR were competitive for both MAO-A and MAO-B.

In conclusion, we detected some MAO inhibitors structurally analogous to Tetra-P. We are now examining the MAO inhibitory effects and their physico-chemical properties in many other compounds, which are structurally associated with these tricyclic compounds, in order to formulate the quantitative structure-activity relationship of a new series of MAO inhibitors.

Table I. Results of MAO Inhibitory Test of 12 Compounds Using Rat Brain Mitochondrial Fraction

Formula	Name	I_{50} values ($\times 10^{-6}$M) with A or B Substrate (preinc.time=15 min) p-Sulfamoyl-benzylamine	Benzylamine
	Iproniazid	3.2	3.2
	Phthalazine	630	250
	Quinoline	200	320
	BTE	10^3 (30% inhibition)	Inactive
	BTR	Inactive	10^3 (30% inhibition)
	Tetra-P	32	40
	Tri-P	200	100
	MTP	10^3 (40% inhibition)	10^3 (40% inhibition)
	ETP	10^3 (35% inhibition)	10^3 (30% inhibition)
	HTP	Inactive	Inactive
	NTE	10	4.0
	NTR	63	79

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