In summary, the distribution in rats of two radiolabelled derivatives of the potent pepsin inhibitor, pepstatin A, has been monitored by whole-body autoradiography. There was a very poor absorption of pepstatinyl-[^{14}C]glycine and *N*-[^{3}H]acetylstatine across the gastric and intestinal mucosae after oral administration. Both inhibitors were rapidly cleared from the blood by the liver and kidneys after intravenous administration. Neither inhibitor was localised in the gastric mucosa at a concentration that could be expected to be effective in inhibiting intracellular pepsin activity.

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A new specific inhibitor of monoamine oxidase A

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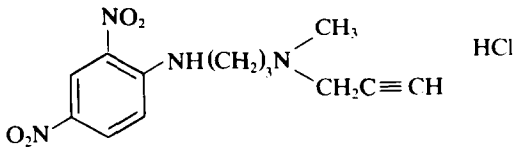
It is now generally agreed that monoamine oxidase [amine–oxygen oxidoreductase (deaminating, flavin-containing), EC 1.4.3.4] (MAO) occurs in two forms differing in substrate specificity and in inhibitor sensitivity. Johnston [1] first showed their existence using the inhibitor *N*-3-(2,4-dichlorophenoxy)propyl-*N*-methylprop-2-ynylamine (clorgyline), which preferentially inhibits oxidation of 5-hydroxytryptamine (5-HT). He called this form MAO A. MAO B, for which preferred substrates include benzylamine (BZA) and 2-phenylethylamine, was shown to be less sensitive to the inhibitor.Tyramine is a good substrate for both forms. Knoll and Magyar [2] described a similar inhibitor, (−)-*N*-methyl-*N*-phenylisopropyl prop-2-ynylamine (deprenyl), with a selectivity opposite to that of clorgyline. Each of these compounds show only partial specificity since either will inhibit their 'non-preferred' form of MAO at higher concentration or on more prolonged time of contact with the enzyme. Earlier studies in this laboratory have produced a number of propynylamines which were for the most part non-selective inhibitors of MAO [3, 4]. In attempts to improve selectivity it has been shown that *N*-desmethyl deprenyl discriminates somewhat better between MAO A and MAO B than does deprenyl, though it is not as potent an inhibitor as the latter [5, 6]. This study reports that the compound N^1-(2,4-dinitrophenyl)-N^2-prop-2-ynyl-1,3-diaminopropane (dinitranyl) is a good irreversible inhibitor of MAO A, as judged by inhibition of 5-HT oxidation, but is apparently without effect on MAO B. The structure of dinitranyl is shown below. Its purity and identity were established by infrared spectroscopy and elemental analysis.



Experimental. Mitochondria from rat liver were prepared by manual homogenization in 0.25 M sucrose (9 vol.) using a Teflon/glass homogenizer, with 6–8 passes of the pestle. Debris was sedimented by centrifuging at 2000 *g* for 10 min. The supernatant was centrifuged at 9000 *g* for 7 min and the pellet was washed twice by resuspension and centrifugation, using first one-half, then one-quarter, of the original volume of sucrose. The pellet was stored frozen in 0.25 M sucrose at a protein concentration of about 30 mg/ml. Protein was measured by the method of Lowry *et al.* [7]. For MAO assay the suspension was diluted with 0.05 M Na/K phosphate buffer, pH 7.5. The assay mixture consisted of 0.85 ml of diluted mitochondrial suspension (approx. 2 mg of protein) and 0.1 ml of buffer. When inhibitor was used the 0.1 ml of buffer was replaced by the same volume of inhibitor solution. Assay tubes were shaken in air at 30° and the reaction was started by adding substrate (0.05 ml),

except when zero-time inhibition was being measured. In this case the enzyme was added to a mixture of inhibitor and substrate. ^{14}C-Labelled substrates (Radiochemical Centre, Amersham, U.K.) were BZA–HCl, TYR–HCl and 5-HT–creatinine sulphate. These were diluted with the corresponding unlabelled amine salts to specific activities and final concentrations in the assay as follows: BZA, 0.01 mCi/mmole, 5 mM; TYR and 5HT, 0.05 mCi/mmole, 1 mM. The reaction was stopped after 15 min by adding 2 M HCl (0.3 ml). Metabolites were extracted by shaking with ethyl acetate (4 ml) for 1 min. After brief centrifuging, duplicate 1-ml aliquots of the upper phase were removed for liquid scintillation counting. All experiments were carried out in duplicate with appropriate blanks. Under these conditions the assay was linear over the period of assay, so that initial velocities were measured.

Results and discussion. Figure 1 shows the effects of different concentrations of dinitranyl incubated with MAO for a fixed time before assay. Figure 2 shows the time-dependent nature of the inhibition at a fixed concentration of inhibitor. Dialysis at 4° for 24 hr against 3 × 1500 ml changes of assay buffer failed to restore enzyme activity to inhibited samples, whilst producing a slight increase in activity in uninhibited samples. It is clear that dinitranyl discriminates well between MAO A and MAO B, failing at 10^{-3} M to show any inhibition of BZA oxidation after a 25 min pre-incubation period. In contrast, at 5×10^{-5} M the time for 50% inhibition of 5HT oxidation is about 3 min. As expected for a propynylamine, the inhibition appears to be irreversible which suggests that the compound inhibits MAO by the same mechanism proposed for other propynylamines [8].

Although 5-HT is usually regarded as a substrate for MAO A, it has been shown by Tipton *et al.* (K. F. Tipton, C. J. Fowler and M. D. Houslay, personal communication) that it can also be oxidized by MAO B. Using the enzyme from rat liver at pH 7.2 and 30°, they found respective K_m values for 5-HT oxidation by the A and B enzymes to be 0.16 and 2 mM, with corresponding V_{max} values of 1.96 and

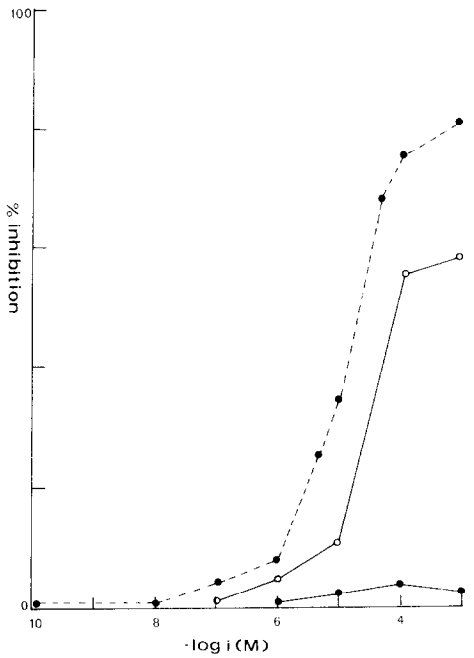


Fig. 1. Effect of increasing concentrations of dinitranyl on the oxidation of 5-HT (●----●), BZA (●——●) or TYR (○——○) by MAO. Mitochondria from rat liver were preincubated for 25 min with inhibitor before assaying residual enzyme activity as described in text.

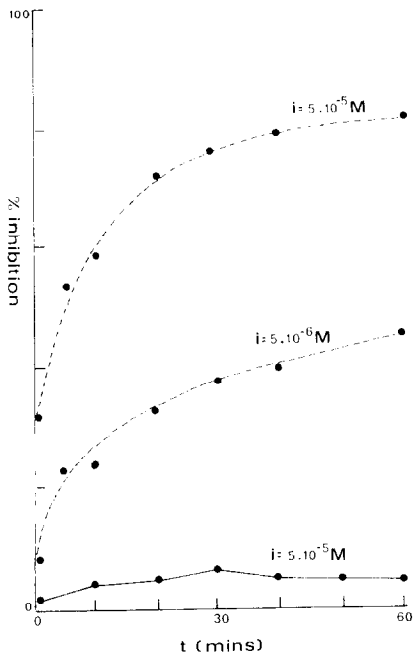


Fig. 2. Time-dependent nature of the inhibition of MAO by dinitranyl, showing its selective activity towards the A enzyme. Mitochondria from rat liver were incubated with the indicated concentrations of inhibitor before assaying for residual enzyme activity with 5-HT (●----●) or BZA (●——●) as substrate. For conditions see text.

0.22 nmoles/min/mg protein. The apparent failure of dinitranyl to fully block 5-HT oxidation is therefore almost certainly because at the concentration of 5-HT of 1 mM used in the present study there is a contribution to the enzymic process from MAO B, which, as the experiments with BZA show, is completely unaffected by the inhibitor. The nature of the biochemical differences between the two forms of MAO and their physiological significance is currently of great interest [9–11]. It is variously suggested that they may be two distinct proteins [12, 13], a single protein with two active sites [14] or with a single site capable of various orientations, depending upon the substrate [15]. Another view is that the sub-mitochondrial environment may contribute to the differences between the two forms [16, 17]. So far, none of these views has gained universal support. The A-specific inhibitor now described may help in answering some of these questions, if not of itself, then by helping to delineate the structural features required for absolute A or B specificity. This in turn could lead to an understanding of the possible differences in topography of the binding sites of the two enzymes. Specific inhibitors may also offer advantages over currently available drugs in treatment of psychiatric and neurological illnesses [18, 19].

Inhibition of MAO by propynylamines occurs in two kinetically distinguishable phases. First, a reversible binding phase at the active site which is competitive with respect to substrate; this is followed by an irreversible step in which the inhibitor forms a covalent bond with the flavin. Tipton and Mantle have recently studied the kinetics of these reactions [20]. Design of potent, specific inhibitors of MAO A and MAO B will need to take account of both these steps, in order to achieve both high specific affinity and a rapid rate of inactivation of the enzyme. Although dinitranyl is loosely based upon the structure of clorgyline, it is not as active an inhibitor. Further studies will need to be carried out to determine which of the structural changes is responsible for the inability of the compound to inhibit

MAO B and whether steric or electronic factors are of greater importance. Thus the real value of dinitranyl in structure/activity studies of propynylamines is that it perhaps points to the features which preclude binding to MAO B and hence confer specificity for MAO A. Studies by Knoll *et al.* [21] and more recently by Kalir *et al.* [22] have produced some structural criteria for MAO B selectivity in propynylamines, but so far the compounds do not seem to show a better selectivity than deprenyl.

In summary, a new propynylamine has been tested as an inhibitor of MAO. Loosely based upon the structure of clorgyline, it is an irreversible inhibitor of MAO A but is apparently indifferent towards MAO B. This compound, N^1-(2,4-dinitrophenyl)-N^2-prop-2-ynyl 1,3-diaminopropane, may be useful as a model for the design of more potent but equally specific inhibitors of MAO A.

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Effects of anticonvulsants on aldehyde reductase and acyl-CoA reductase: implications for the biosynthesis of ether-linked glycerolipids in brain

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Plasmalogens (alk-1-enyl glycerolipids), which are particularly abundant in the membranes of nerve and muscle cells, are formed by desaturation of alkyl glycerolipids [1]. Metabolic and structural studies have established that the alkyl chains of these ether-linked glycerolipids are derived from long-chain aliphatic alcohols [2]. Their enzymic synthesis in brain is maximal during the period of active myelination [3].

Long-chain aliphatic alcohols appear to be synthesized by reduction of the corresponding fatty acids [2,4–6]. The overall reaction, which predominantly occurs in the microsomal fraction of nerve cells, may be dissected into two discrete steps. The first stage requires ATP, Mg^{2+} and coenzyme A and leads to the formation of an aliphatic aldehyde which, in the second step, is converted to an alcohol by NADPH-dependent aldehyde reductase [6]. However, the intermediate formation of the aldehyde in microsomal preparations has not been conclusively shown and the nature of the reductase has not been established.

A suggested candidate [1, 4, 6] for reduction of long-chain aliphatic aldehydes is the non-specific aldehyde reductase (alcohol:$NADP^+$ oxidoreductase, EC 1.1.1.2) present in many mammalian tissues which can reduce a variety of aromatic and aliphatic aldehydes [7–10]. This enzyme is cytosolic in location [8] and is sensitive to inhibition by anticonvulsant drugs including sodium valproate