Biochemical Pharmacology, Vol. 26, pp. 62–63. Pergamon Press, 1977. Printed in Great Britain.

# Effect of β-propiolactone and β-nitropropionic acid on rat brain monoamine oxidase

(*Received* 26 *April* 1976; *accepted* 13 *July* 1976)

The chemical and biological uses of β-propiolactone have gained wide interest and the compound is now considered to be a carcinogen [1]. The lactone was found to be mutagenic to *N. crossa*, to be an effective alkylating agent [2], and to react with RNA and various guanine derivatives as well as with DNA and mouse skin chromatin [1]. The compound β-nitropropionic acid is easily derived from β-propiolactone by reaction with sodium nitrite [3]. It was found to be responsible for the toxic symptoms produced in dairy cattle [4], it is also fatal to adult rats at a dose of 100 mg/kg within 1 hr [5]. β-Nitropropionic acid was isolated from four higher plants, *Hiptage madablota* Gertn., *Corynacarpus laevigate* Forst., *Indigofera endecaphylla* Jcap. and *Viola odorata*; and three fungi, *Aspergillus flavus*, *Aspergillus orysae* and *Penicillium atroventum* [6]. The present study describes the effect of β-propiolactone and β-nitropropionic acid on rat brain monoamine oxidase.

## MATERIALS AND METHODS

Chemicals were purchased as follows: 5-hydroxytryptamine creatinine sulphate (containing 43.5% of 5-hydroxytryptamine), May and Baker Ltd., Dagenham, England; Antemovis ampoules (each containing 5 mg of 5-hydroxytryptamine creatinine double sulphate), Vister, Casatenovo, Italy; β-propiolactone, BDH Chemicals Ltd. Poole England. β-nitropropionic acid was prepared from β-propiolactone according to Gresham *et al.* [3]. β-Nitropropionic acid was repeatedly crystallized from chloroform, m.p. 66°.

Brain MAO was prepared according to the method described by Roth and Gillis [7]. Albino rat brains (20 g), were homogenized in 2 vol of potassium phosphate buffer (w/v; pH 7.4, 0.1 M) containing sucrose (0.25 M) in a Waring blender (5 sec, two times) and then in a motor-driven Teflon–glass homogenizer. The resulting homogenate was centrifuged twice at 600 *g* for 10 min to remove cellular debris. The supernatant solution from the second centrifugation was centrifuged at 10,000 *g* for 20 min and the resulting mitochondrial precepitate was resuspended by homogenization in the phosphate buffer (10 ml; pH 7.4; 0.1 M). The protein content was determined by the method of Lowry *et al.* [8]. The enzyme activity was assayed chemically by the method of Udenfriend *et al.* [9], using 5-hydroxytryptamine as substrate.

In this method the assay mixture contained 5-HT, 0.124 μmole/ml; $Na_2HPO_4$: $NaH_2PO_4$, pH 7.4, 375 μmole/ml, and an amount of enzyme equivalent to 200 mg brain tissue. The incubation time was continued for 5 min (the rate of deamination of 5-HT by MAO was found to be linear up to 8 min, Mohammed *et al.* [10]) at 37°, after which the residual substrate was estimated.

Different concentrations of β-propiolactone (50, 100, 150 and 200 μM) as well as β-nitropropionic acid (50, 100, 150 and 200 μM; these amounts made no significant change in the pH of the incubation mixture) were added to the above assay mixture to evaluate the 50 per cent inhibition.

For the determination of the type of inhibition and the enzyme inhibitor dissociation constant ($K_i$), the substrate concentration was varied (62, 94, 124, 185 and 247 μM). The inhibitors β-propiolactone as well as β-nitropropionic acid were kept at constant concentration for each experiment (50, or 100 or 150 μM for β-propiolactone and 25 or 50 or 100 μM for β-nitropropionic acid) and the mixture was then incubated for 5 minutes at 37°.

*Dialysis.* The enzyme (0.1 ml), with inhibitor concentrations of 150 μM for β-propiolactone and 100 μM for β-nitropropionic acid, was dialyzed overnight against phosphate buffer at 4° with occasional change of buffer. Controls of enzyme without inhibitors dialyzed and undialyzed were also taken.

## RESULTS AND DISCUSSION

Using 5-hydroxytryptamine as substrate, the inhibition of rat brain monoamine oxidase by increasing concentrations of β-propiolactone or β-nitropropionic acid was found to give a sigmoid curve (Fig. 1). This behaviour of rat brain monoamine oxidase inhibition is comparable to the results obtained by Mantle and Wilson [11] in their study on the effect of 5-phenyl-3-(*N*-chloropropyl)ethylamine 1,2,4-oxidiazole derivatives on rat liver monoamine oxidase. It is noted that at a critical inhibitor concentration, a further slight increase in its concentration gives an abrupt increase in the inhibition of the enzyme activity (from 20 to 80 per cent inhibition), Fig. 1, with both β-propiolactone ($1 \times 10^{-4}$M) and β-nitropropionic acid ($0.5 \times 10^{-4}$M).

By dialysis of the inhibited enzyme it was found that with β-propiolactone the inhibition was irreversible and the enzyme did not recover its original activity. This is expected since β-propiolactone is known to be an effective alkylating agent. In the case of β-nitropropionic acid the enzyme recovered its original activity after dialysis suggesting that its inhibitory effect is reversible.

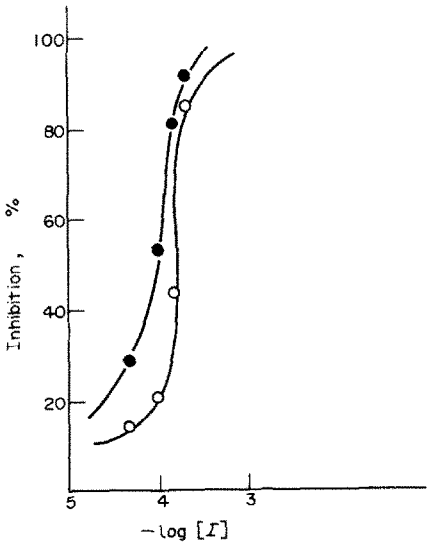


Fig. 1. Effect of β-propiolactone (○) and β-nitropropionic acid (●) on the activity of brain monoamine oxidase. Enzyme was incubated for 5 min at 37° with concentrations of 50, 100, 150 and 200 μM for both β-propiolactone and β-nitropropionic acid, final concentration of the substrate (5-hydroxytryptamine) was 124 μM.

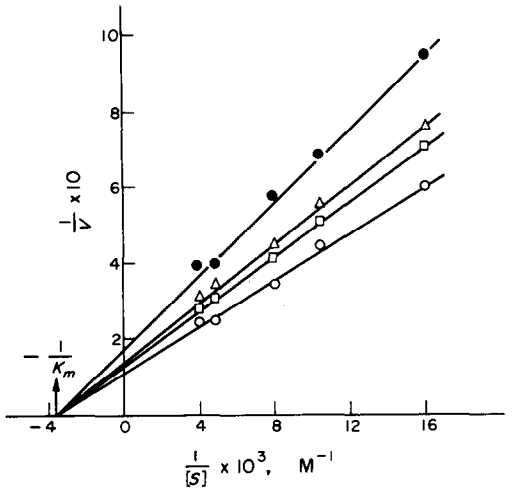


Fig. 2. Instantaneous inhibition of brain monoamine oxidase by β-propiolactone using 5-hydroxytryptamine as substrate. A double reciprocal plot of the initial rate of 5-hydroxytryptamine deamination against substrate concentrations in the presence of 50 μM (□), 100 μM (△) and 150 μM (●) of β-propiolactone, and control (○).

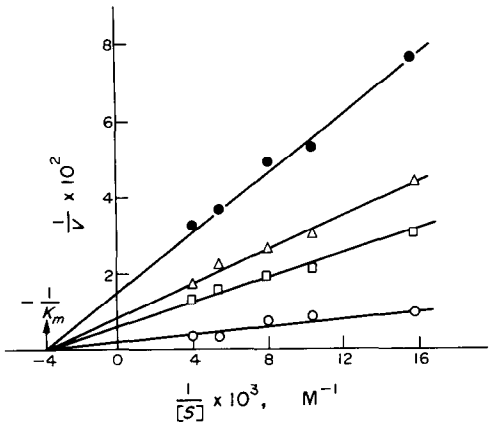


Fig. 3. Instantaneous inhibition of brain monoamine oxidase by β-nitropropionic acid using 5-hydroxytryptamine as substrate. A double reciprocal plot of the initial rate of 5-hydroxytryptamine deamination against substrate concentrations in the presence of 25 μM (□), 50 μM (△) and 100 μM (●), and control (○).

Table 1. The enzyme–inhibitor dissociation constant ($K_i$) for brain monoamine oxidase in the presence of β-propiolactone and β-nitropropionic acid.

| Inhibitor | $K_i$ | $K_m$ |
|---|---|---|
| β-Propiolactone | $1.86 (\pm 0.16) \times 10^{-4}$ M | $2.65 \times 10^{-4}$ M |
| β-Nitropropionic acid | $7.8 (\pm 0.07) \times 10^{-6}$ M | $2.65 \times 10^{-4}$ M |

$K_m$ is the Michaelis constant.

As regards the type of inhibition exerted by β-propiolactone on rat brain monoamine oxidase and using 5-hydroxytryptamine as substrate, the double reciprocal curves of 1/v plotted against 1/[s], keeping the inhibitor at constant concentration in each experiment and changing the substrate concentration, were in accordance with those mentioned by Dixon [12] for the non-competitive type of inhibition (Fig. 2). This would suggest that although β-propiolactone alkylates or slightly modifies the enzyme molecule, its effect does not invlove the enzyme active centre and Michaelis constant remained unchanged. With β-nitropropionic acid and under the analogous condition, the plots resulted a non-competitive type of inhibition (Fig. 3) and similarly to β-propiolactone, the effect of this acid does not involve the enzyme active centre. The values of the enzyme-inhibitor dissociation constant ($K_i$) and Michaelis constant ($K_m$) are given in Table 1.

*Department of Biochemistry, Medical Research Institute, Hadara, Alexandria University, Egypt*

YOUSSEF S. MOHAMMED, MOHAMEDAIN M. MAHFOUZ MOHAMED Y. OSMAN

REFERENCES

1. A. Segal, M. Schroeder, B. Philip and B. L. Van Duuren, *Biochem. Pharmacol.* **23,** 937 (1974).
2. F. Dickens and H. E. Jones, *Br. J. Cancer* **15,** 85 (1961).
3. T. L. Gresham, J. E. Jansen, F. W. Shaver, M. R. Frederick, F. T. Fiedorek, R. A. Bankert, J. T. Gregory and W. L. Beears, *J. Am. chem. Soc.* **74,** 1323 (1952).
4. H. Raistrick and A. Stossl, *Biochem. J.* **68,** 647 (1958).
5. Y. S. Mohamed and M. Y. Osman, unpublished data.
6. J. H. Birkenshaw and A. M. L. Dryland, *Biochem. J.* **93,** 478 (1964).
7. J. Roth and C. Gillis, *Biochem. Pharmac.* **23,** 18 (1974).
8. O. H. Lowry, H. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
9. S. Udenfriend, H. Weissbach and B. B. Brodie, in *Methods of Biochemical Analysis*, (Ed. David Glick). Vol. VI, p. 96. Interscience, London (1958)
10. Y. S. Mohammed, M. Y. Osman and M. M. Mahfouz, *Biochem. Pharmac.* **25,** 1665 (1976).
11. T. J. Mantle, K. Wilson and Robert F. Long, *Biochem. Pharmac.* **24,** 2031 (1975).
12. M. Dixon, *Biochem. J.* **55,** 170 (1953).