THE *IN VITRO* HEPATIC MICROSOMAL METABOLISM OF METHYL 2-(2(3H)-BENZOXAZOLONE-3-YL)ACETATE IN RATS

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SUMMARY

The *in vitro* hepatic microsomal metabolism of methyl 2-(2(3H)-benzoxazolone-3-yl)acetate (**I**) was studied using hepatic washed rat microsomal preparations fortified with NADPH. The substrate (**I**) and its potential hydrolytic metabolite 2-(2(3H)-benzoxazolone-3-yl)acetic acid (**II**) and 2(3H)-benzoxazolone (**III**), a potential dealkylation metabolite, were separated using a reverse phase HPLC system which consisted of a C_{18} column and a mobile phase of acetonitrile: 0.02 M phosphate buffer (30:70, final pH 7) at a flow rate of 1 ml/min with UV detection at 254 nm. The substrate (**I**) was incubated with rat microsomal preparations, extracted into DCM, and finally evaporated under nitrogen. The results from HPLC studies showed that (**I**) was metabolised to (**II**) and (**III**) by rat microsomes in the presence of NADPH.

KEY WORDS

benzoxazolone, microsomes, rat, *in vitro* metabolism

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INTRODUCTION

Although several substituted benzoxazolone derivatives have previously been synthesised and their antinociceptive and anti-inflammatory activities have been elucidated /1/, studies on their *in vitro* metabolism are lacking. Our literature search revealed that there were only a few reports on the metabolism of similar structures to the benzoxazolones, i.e. the demethylation of the oxazolidine derivative anticonvulsant drugs trimethadione and paramethadione /2/ and the hydrolytic ring scission of benoxazole itself /3/.

We therefore planned to study the *in vitro* metabolism of methyl 2-(2(3H)-benzoxazolone-3-yl)acetate (**I**) using rat microsomes fortified with NADPH to establish the metabolic products of this model benzoxazolone derivative (Figure 1).

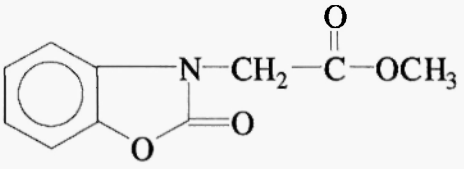

Fig. 1: Structure of methyl 2-(2(3H)-benzoxazolone-3-yl)acetate, compound **I.**

MATERIALS AND METHODS

The substrate, methyl 2-(2(3H)-benzoxazolone-3-yl)acetate (**I**), and its potential hydrolytic metabolite 2-(2(3H)-benzoxazolone-3-yl)acetic acid (**II**) and 2(3H)-benzoxazolone (**III**) were synthesized previously and recrystallized just before use /1/. All chromatographic solvents were obtained from Merck Chemical Company. The separation techniques were based on an isocratic HPLC system (Table 1). The HPLC column, μ-Bondopac C_{18}, (5 μm, 25 cm length x 4.6 mm i.d.) was purchased from Waters Limited. The guard column packing material (Whatman Pellicular ODS) was purchased from Whatman International Ltd., Maidstone, Kent, UK. The HPLC chromatograph consisted of an isocratic system comprising a Gilson Model 302 solvent delivery system, a Rheodyne syringe loading sample injector valve (model 7125) fitted with a 20 μl sample loop, a SpectroMonitor

III Model 1204A LDC variable wavelength UV detector, and a Tekman recorder. The reaction products were eluted under isocratic conditions with a mobile phase of acetonitrile:0.02 M phosphate buffer (30:70) (final pH 7), at a flow rate of 1.5 ml/min. The metabolic products were detected by their absorbance at 254 nm. Retention times of compounds under these conditions are given in Table 1.

TABLE 1

The HPLC retention times of compounds under study

Compound	**HPLC Rt value* (min)**
Methyl 2-(2(3H)-benzoxazolone-3-yl) acetate **(I)**	11.4
2-(2(3H)-Benzoxazolone-3-yl) acetic acid **(II)**	3.5
2(3H)-Benzoxazolone **(III)**	6.4

* For HPLC solvent system see text.

Incubation and extraction procedures

Glucose-6-phosphate dehydrogenase was purchased from the Boehringer Mannheim Corporation (London) Ltd. Nicotinamide adenine dinucleotide phosphate monosodium salt (NADP) and glucose-6-phosphate disodium salt were obtained from Sigma Ltd. All other chemicals used in the experiments were obtained as mentioned earlier. The animals used in the investigations were male albino rats (Wistar). The animals were fed standard diet and water *ad libitum*. Animals were deprived of food overnight and sacrificed the next day by cervical dislocation. The livers were removed and immersed immediately in ice-cold saline solution (0.9% w/v). Hepatic microsomes were prepared at 0°C using the calcium chloride precipitation method of Schenkman and Cinti /4/. Incubations were carried out in a shaking water bath at 37°C using a standard co-factor solution at pH 7.4 Co-factors consisting of NADP (2 μmol), glucose-6-phosphate (10 μmol), glucose-6-phosphate dehydrogenase (1 unit), $MgCl_2$ (20 μmol) prepared in phosphate buffer (2 ml, 0.2 M, pH 7.4) were pre-incubated

for 5 minutes before addition of substrate (2 μmol in 20 μl methanol) and microsomes (0.5 ml equivalent to 0.5 g original liver) or soluble fraction (0.5 ml). Controls which were run simultaneously with normal incubations comprised: (a) substrate, co-factors and inactivated microsomal preparation (previously heated in boiling water for 15 minutes), and (b) substrate plus tissue without cofactors.

The metabolic reactions were terminated at 30 min by placing the flasks on ice. The incubation mixtures were extracted with dichloromethane (2x5 ml). Organic extracts were evaporated to dryness under a stream of nitrogen at 20°C. Dry organic residues were reconstituted in 200 μl of methanol for HPLC.

RESULTS AND DISCUSSION

The substrate (**I**), its potential metabolites, i.e. the corresponding acid (**II**) and 2(3H)-benzoxazolone, the dealkylation product (**III**), were separated by HPLC using the conditions described in the text (Table 1, Figure 2). The results showed that (**I**) was metabolised to the corresponding acid (**II**) by hydrolytic reaction which plays an important role in the metabolism of xenobiotics and several pro-drugs. The hydrolytic potential of the liver is due to its high concentration of carboxylesterases /5/. The dealkylation of (**I**) to benzoxazolone (**III**) was also demonstrated (Figure 2).

In addition to these metabolites, another metabolite which had a retention time of 8.5 min was observed by HPLC, but no attempt has yet been made to identify this product. This uncharacterized metabolite could be either a ring hydroxylated product or a ring scission derivative. A number of heterocyclic compounds are metabolized by hydrolytic scission of the heterocyclic ring. For instance, benoxazole is metabolised in the rabbit by scission of the oxazole ring to give *o*-formamidophenol and then *o*-aminophenol derivatives /3/.

The other possibility is that this unidentified metabolite would be an N-methyl derivative formed by the further decarboxylation of the corresponding acid metabolite (**II**). This proposed reaction was previously reported by Kuntzmann *et al.* for N-*tert*-butylchlorocyclizine /6/. Experiments are in progress to examine these proposals and to identify this product in our laboratories.

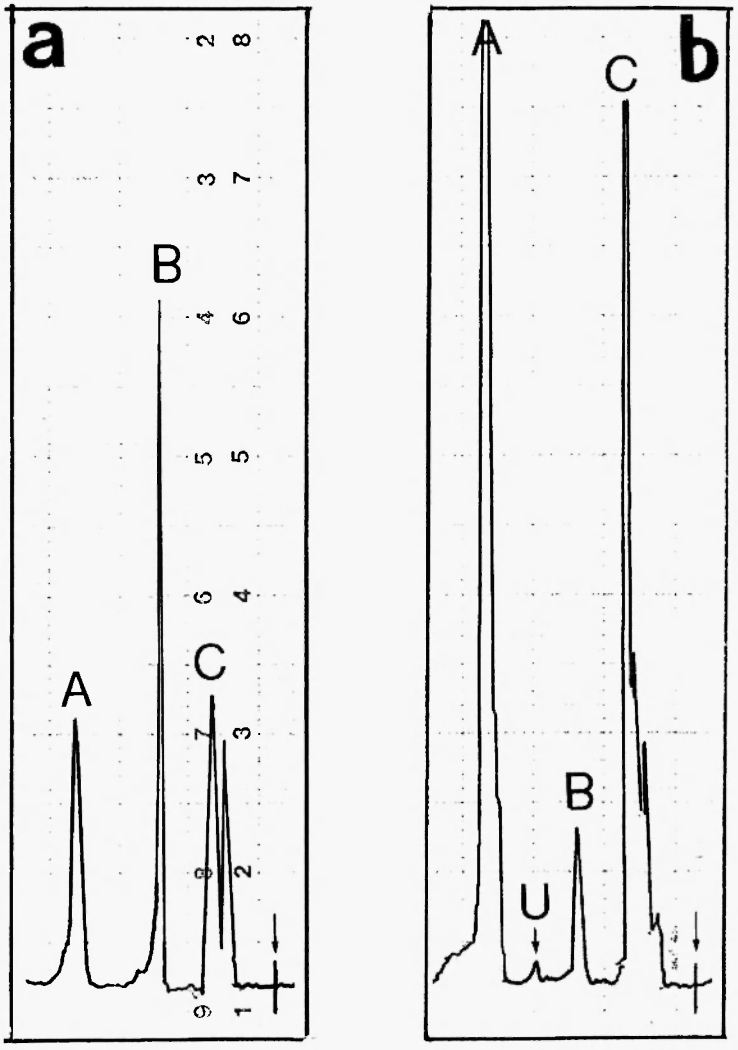

Fig. 2: HPLC chromatogram obtained: (**a**) from standards; (**b**) following extraction of metabolites from a rat microsomal incubation mixture with (**I**) as a substrate. A = ester (**I**); B = benzoxazolone (**III**); C = acid (**II**); U = unidentified metabolite. (See Table 1 for abbreviations and text for HPLC system.)

No dealkylated metabolites were observed in control experiments; this indicates the involvement of microsomal enzymes and NADPH as a cofactor. The established metabolic products of (**I**) are presented in Figure 3.

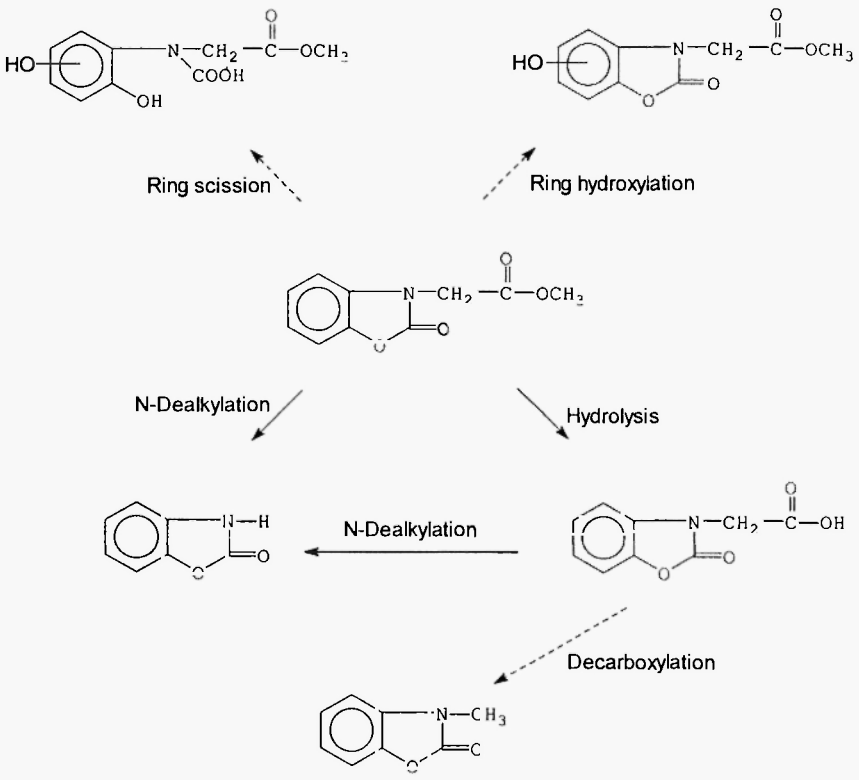

Fig. 3: Potential metabolites of compound **I.**
- - - → suggested and —→ established metabolic pathways.

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