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# Qualitative and Quantitative Determination of Drotaverine Metabolites in Rat Bile

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SUMMARY

After oral and intravenous administration of drotaverin-$^{14}$C its metabolites were determined in rat bile. Three major metabolites were identified by tlc. All the metabolites apeared in conjugated form. No unchanged drotaverine was detectable in the bile, except after treatment with doses much in excess of the therapeutic range. The ratio of major metabolites to unchanged product was determined by two-dimensional densitometry using a Telechrom Video Densitometer.

## INTRODUCTION

Although drotaverine (No-Spa$^{R}$) (6,7,3',4'-tetraethoxy-1-benzyl-3,4-dihydro-isoquinoline hydrochloride) is chemically similar to papaverine, has a more potent spasmolytic effect (1,2,3,4).

It has been shown in animal experiments that the liver plays a major role in the metabolism of papaverine (5,6,7) and drotaverine (8,9), and that a considerable amount of both compounds is eliminated in the bile (10,11). Belpaire (1975) identified four metabolites of papaverine in rat bile (1-(3-methoxy-4-hydroxybenzyl)-6,7-dimethoxyisoquinoline; 1-(3,4-dimethoxybenzyl)-6-methoxy-7-hydroxyisoquinoline; 1-(3,4-dimethoxybenzyl)-6-hydroxy-7-methoxyisoquinoline; 1-(3-methoxy-4-hydroxybenzyl)-6-hydroxy-7-methoxyisoquinoline). The present paper reports the identification and quantitative determination of drotaverine metabolites in the bile of rats treated with the compounds at different dose levels and by different routes.

## MATERIALS AND METHODS

Drotaverine-1-$^{14}$C was synthetized by Koltai and coworkers (12).

Position of the labelling : 1-carbon atom in the isoquinoline ring.

Specific activity : 5.79 mCi/mmole.

The purity of the labelled compound was checked by thin layer chromatography. The radiochemical purity of the administered samples was never less than 95%. Kieselgel $GF_{254}$ (Merck) was used for thin layer chromatography, β-glucuronidase (Serva) and β-glucuronidase/arylsulphatase (Serva) were employed for the enzymic cleavage of the conjugated metabolites.

### Animals and experimental procedures

Male albino rats (110-260 g) of the CFY strain were used. The animals were fasted for 16 hrs prior to the experiment. The common bile duct was catheterized by a polyethylene cannula under Nembutal anasthesia to collect bile fractions for the radioactivity assay and tlc. Drotaverine-1-$^{14}$C was administered to the rats by three different routes, i.e. :

a. intravenous bolus
b. intravenous perfusion
c. oral

For oral treatment, the labelled compound was administered in aqueous solution, through a gastric tube.

Short- and long-term tests were performed with a., and c., administration, as follows :

I. collection of bile started immediatly after administration of drotaverine-$^{14}$C (acute test).

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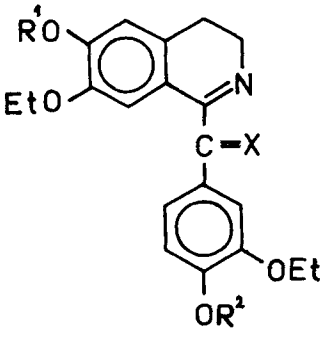


1. $R^1 = C_2H_5$ $R^2 = C_2H_5$ $X = H_2$
2. $R^1 = C_2H_5$ $R^2 = H$ $X = H_2$
3. $R^1 = H$ $R^2 = C_2H_5$ $X = H_2$
4. $R^1 = C_2H_5$ $R^2 = C_2H_5$ $X = O$
5. $R^1 = C_2H_5$ $R^2 = H$ $X = O$

1. drotaverine
2. 4'-desethyl-drotaverine
3. 6-desethyl-drotaverine
4. drotaveraldine
5. 4'-desethyl-drotaveraldine

*Fig. 1 :* Inactive reference standards for identification of metabolites.

| | | $R_F$ values in solvent system | | | | |
|---|---|---|---|---|---|---|
| | | I. | II. | III. | IV. | V. |
| drotaverine | 1. | 0.38 | 0.72 | 0.43 | 0.88 | 0.64 |
| metabolite | 2. | 0.28 | 0.61 | 0.61 | 0.80 | 0.36 |
| — " — | 3. | 0.12 | 0.54 | 0.62 | 0.66 | 0.14 |
| — " — | 4. | 0.80 | 0.82 | 0.52 | 0.79 | 0.73 |
| — " — | 5. | 0.71 | 0.79 | 0.57 | 0.87 | 0.72 |

*Table I :* $R_F$ values of the reference standards.

II. collection of bile started 24 hrs after treatment, until which time the rats were kept in metabolic cages (chronic test).

## Measurement of radioactivity

The measurement was carried out in a liquid scintillation spectrometer of the type LKB Wallace 8100. Quench correction was made by external standardizing. 10 ml of Bray scintillation cocktail (PPO 4 g, POPOP 0.2 g, naphtalene 60 g, ethyleneglycol 20 ml, methanol 100 ml, dioxane ad 1 l) were added to 5-50 $\mu$l of bile samples or to 100 $\mu$l of the extraction residue.

## Thin layer chromatography

20 x 20 cm chromatographic plates were coated with 0.25 mm Kieselgel $GF_{254}$. After activation (60 min. 105°C) the chromatograms were developed in the following solvent systems :

I. Chloroform : methanol 98:6 v/v
II. Chloroform : methanol 80:20 v/v
III. n-Buthanol : acetic acid : water 4:1:1 v/v
IV. i-Propanol : cc.$NH_4OH$ : water 7:1:2 v/v
V. Ethylacetate : methanol : cc.$NH_4OH$ 95:5:5 v/v

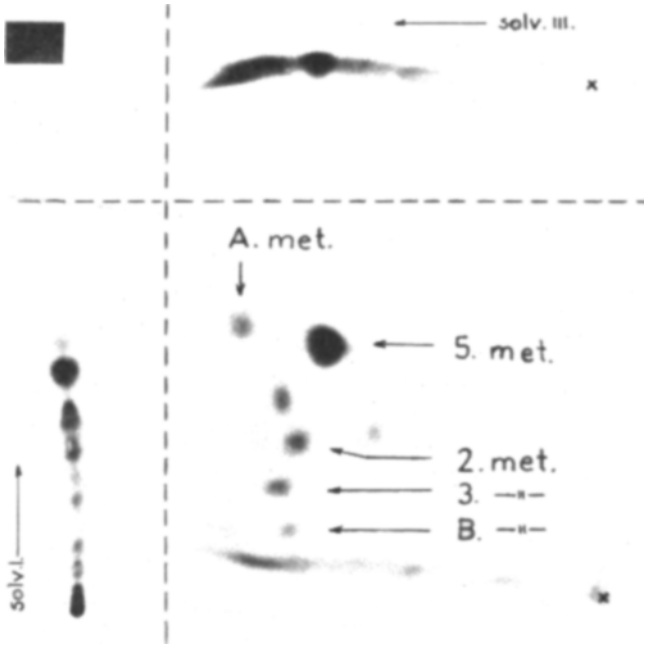


*Fig. 2 :* Radiochromatogram of a bile fraction. The structures of 2.,3. and 5.met. can be seen in Figure 1.

Of the supposed metabolites of drotaverine the following ones were available in synthetic (unlabelled) form for use as reference standards (Figure 1).

Table I shows the $R_F$ values of drotaverine and its metabolites on Kieselgel $GF_{254}$ in various solvent systems. Solvent systems I., III. and IV. were used for the separation of metabolites by two-dimensional chromatography. Purity of drotaverine was checked by systems I., III. and V.

## Treatment of bile samples

Bile samples were examined by tlc after the following treatments :

a. 0.8 ml acetate buffer (pH 5) and 2000 FU β-glucuronidase were added to 200 µl bile and incubated for 24 hours at 37°C.

b. 0.8 ml acetate buffer (pH 5) and 2000 FU β-glucuronidase/arylsulphatase were added to 200 µl bile and incubated for 24 hours at 37°C.

The enzymic hydrolysis was followed by extraction with 3 x 3 volumes of chloroform. The extracts were used for tlc and the determination of conjugation degree by measuring their radioactivity. The organic layer was evaporated under reduced pressure at room temperature.

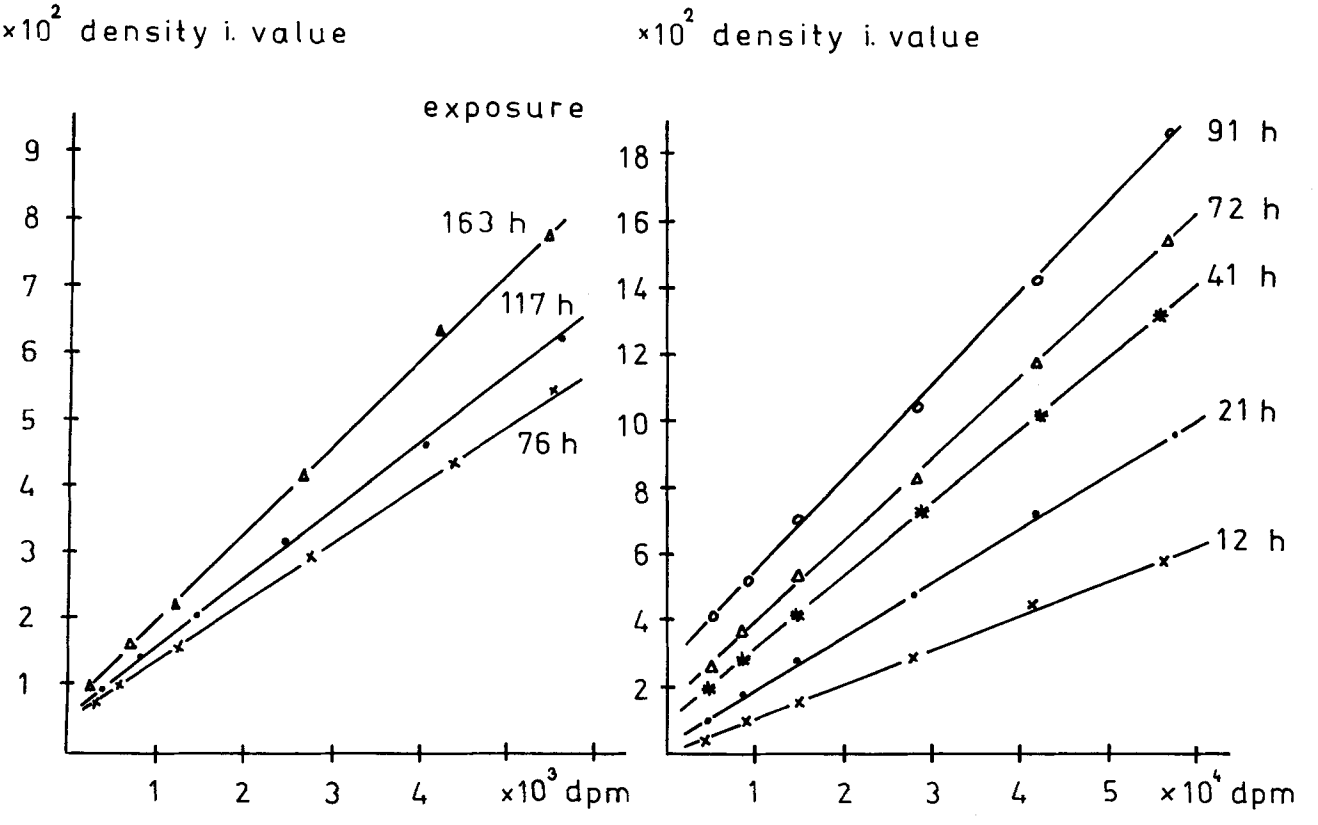


*Fig. 3 :* Calibration curves for densitometry of autoradiograms.

| route | dose mg | n | 1. | 2. | 3. | 5. | A. | B. |
|---|---|---|---|---|---|---|---|---|
| i.v. | 0.5 | 3 | — | 21 – 32 | 0 – 5 | 58 – 80 | 0 – 12 | 0 – 5 |
| i.v.[24h] | 0.5 | 3 | — | 0 – 15 | 10 – 35 | 31 – 41 | — | — |
| i.v. perf | ~ 5 | 2 | 0 – 10 | 5 – 15 | 5 – 15 | 26 – 41 | 10 – 19 | 8 – 19 |
| oral | 0.5 | 3 | — | 5 – 15 | 5 – 15 | 40 – 60 | 5 – 15 | 5 – 15 |
| oral [24h] | 0.5 | 3 | — | 10 – 21 | 11 – 23 | 35 – 52 | 5 – 25 | 4 – 12 |
| oral | 2.5 | 5 | 0 – 5 | 5 – 15 | 0 – 10 | 41 – 58 | 21 – 38 | 10 – 22 |

*Table II :* The ratio of major metabolites of drotaverine in the bile.

A.,B. unknown metabolites
the structure of metabolites 1.,2.,3.,5. can be seen in Figure 1.

The residue was redissolved in 0.5 ml methanol and an aliquot was used for two-dimensional tlc.

## RESULTS

### Identification of biliary metabolites

The enzyme treated bile samples were run together with the synthetic inactive reference standards on the chromatographic plate. The radiochromatograms were evaluated by contact autoradiography, using X-ray film.

The inactive reference standards were evaluated with a 1 : 1 mixture of the following reagents :

1. 2 g potassium iodide in 50 ml cc.HCl.
2. 2 g iodine dissolved in 50 ml methanol.

Identification of the metabolites was based on the complete congruence of the radioactive spots with the reagent visualized spots of the inactive reference standards on the two-dimensional chromatograms.

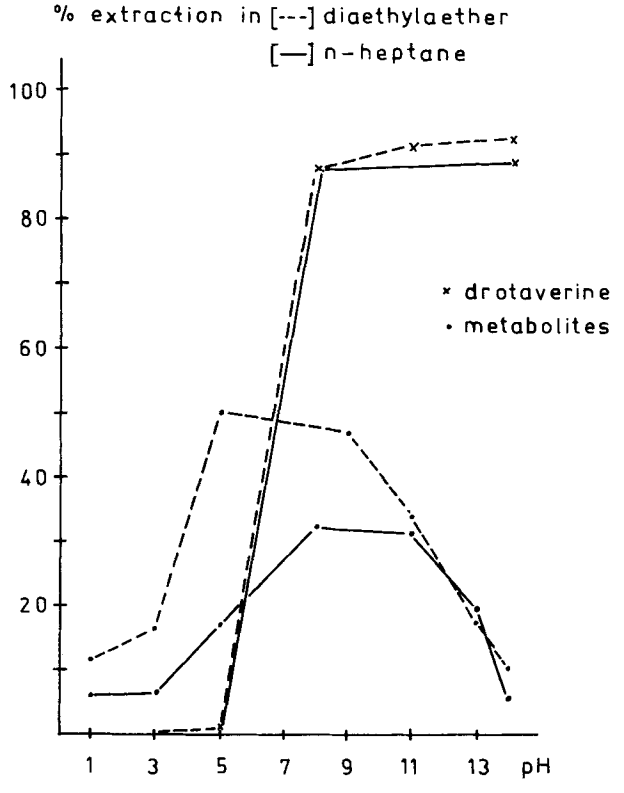


*Fig. 4 :* Extraction of unchanged drotaverine and its metabolites from enzyme treated bile samples at different pH-s.

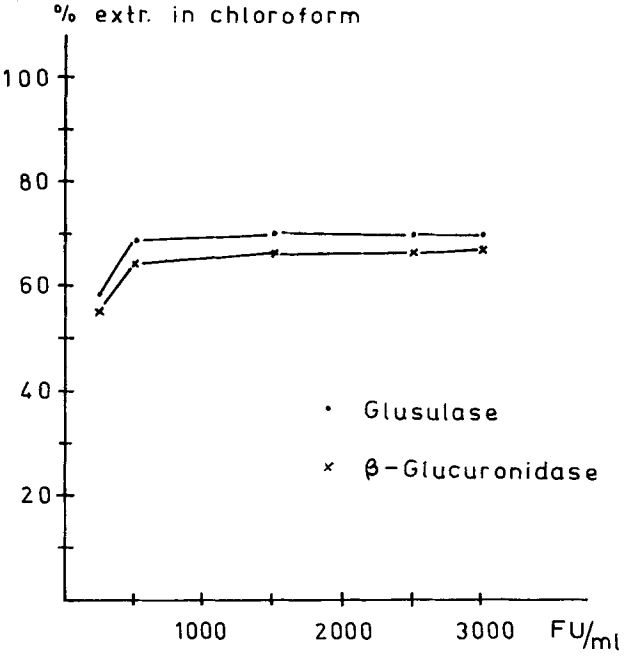


*Fig. 5 :* Changes of extractable part of the enzyme treated bile samples at different enzyme activity.
(FU = Fishman unit).

Figure 2, shows an X-ray copy of a two-dimensional radiochromatogram. The following radioactive spots were identical with the inactive reference standards.

(2.met. = 4'-desethyl-drotaverine
3.met. = 6-desethyl-drotaverine
5.met. = 4'-desethyl-drotaveraldine).

## Metabolite ratios

A two-dimensional densitometric procedure (Telechrom Automatic Video Densitometer, typ. OE-976) was developed for the quantitative evaluation of radioactive spots by using X-ray film contact autoradiograms.

The calibration curves (Figure 3) show that the density values are linear in wide ranges (350-6,000 dpm, 4,000-60,000 dpm) and linearity is independent of the radioactivity and exposure time. The sensitivity of the method is 300 dpm/radioactive spot, and its accuracy is : ± 7%. The ratios for the major metabolites are shown in Table II.

## Determination of unchanged drotaverine

Unchanged drotaverine and its metabolites were extracted from the bile samples at different pH-s (Figure 4). n-Heptane proved to be the most suitable solvent because no conjugated metabolite was extractable at pH 14.0 while 92 ± 2.2% of unchanged drotaverine was extracted. After enzymic digestion, "free" metabolites were extractable only at low proportion (3-5%) at the same pH. The radioactivity of the extraction residues was measured by liquid scintillation. This simple extraction procedure (Axelrod and Belpaire used nearly the same extraction procedure for the determination of unchanged papaverine) made possible the quantitative determination of unchanged drotaverine eliminated in the bile. It was found that no unchanged drotaverine was eliminated in the bile, except after treatment with doses much in excess of the therapeutic range, in which case 0-10% of the compound was detected in unchanged form.

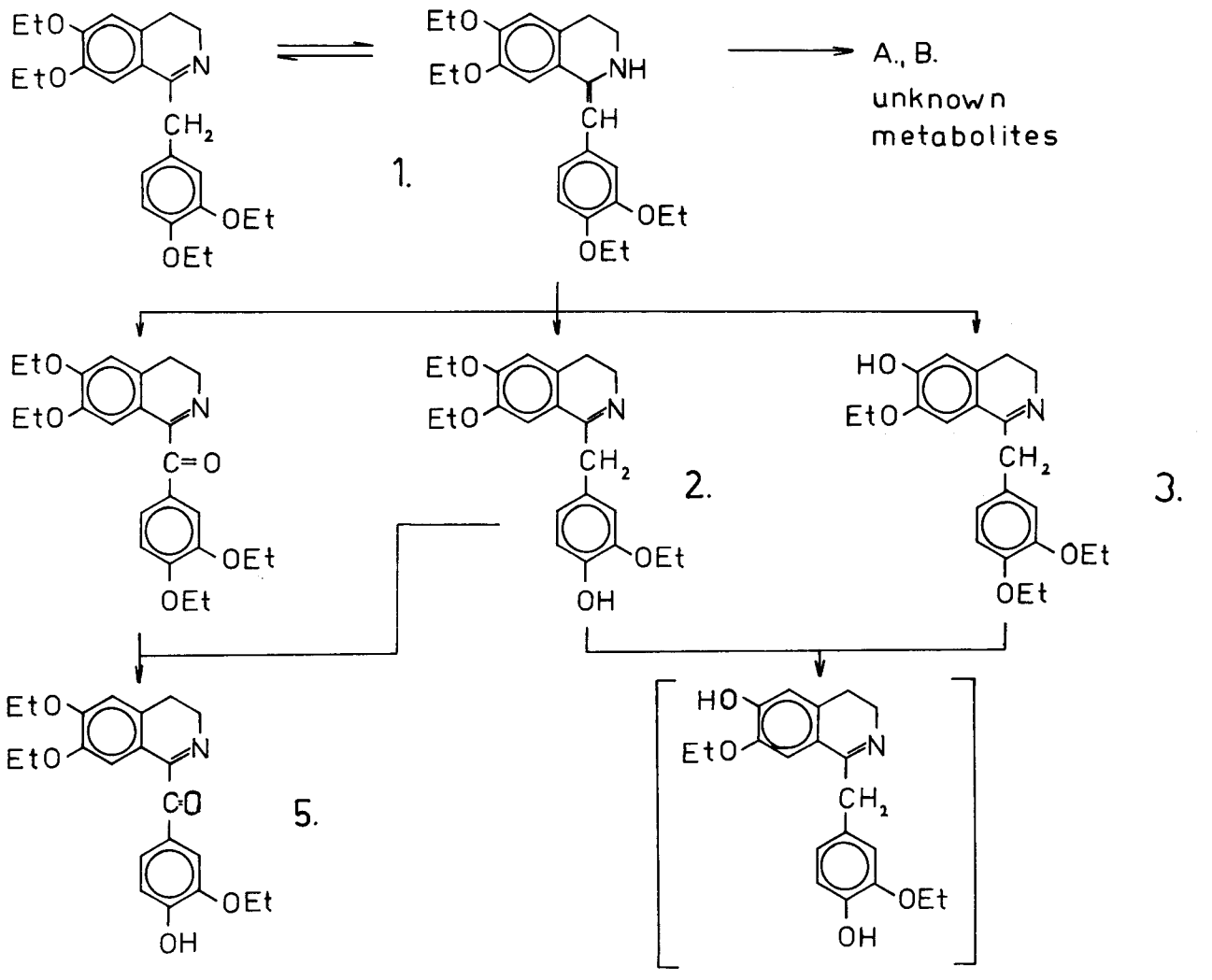


*Fig. 6 :* The pathway of the drotaverine metabolites eliminated in the bile.

## DISCUSSION

Results of the present study demonstrate that the metabolic pattern of drotaverine (Figure 6) is very similar to that of papaverine (10). The biliary excretion of radioactivity has been found to be considerable after both i.v. and oral administration of drotaverine-1-14C (Simon et al., unpublished), indicating that the liver plays a very important role in the metabolism of this compound. Drotaverine is almost completely metabolized by O-desethylation to monophenolic compounds. These metabolites appear in the bile in conjugated form, almost 100% as glucuronide while the sulphate conjugate represents a negligibly low percentage (Figure 5). After hydrolysis of conjugated metabolites three major metabolites were identified by tlc in rat bile (4'-desethyl-drotaverine, 6-desethyl-drotaverine and 4'-desethyl-drotaveraldine). The experiments have unequivocally shown that unchanged drotaverine and drotaveraldine, when administered in the therapeutic dose range, are not eliminated by the bile. A two-dimensional densitometry technique was developed for determination of the ratio of drotaverine metabolites. (This technique can be generally applied to the quantitative evaluation of autoradiograms).

Among the metabolites eliminated into the bile, 4'-desethyl-drotaveraldine was predominant. In vitro testing with liver microsomes (Vargay unpublished results) has shown that drotaverine is very easily oxidized. The possibility that the metabolites containing the keto-group might be artefacts occuring during the isolation and identification procedures can be definitely ruled out. Direct oxidation of drotaverine, and desethylation of the oxidation product seem to play a major part in the metabolic degradation of the compound.

The dose and route of application of drotaverine had no notable effect on the relative proportion of metabolites (Table II).

The pathway of the drotaverine metabolites eliminated in the bile can be characterized by the following diagram shown in Figure 6.

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