Journal of Chromatography, 575 (1992) 295–300
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6235

Short Communication

Identification of new urinary metabolites of trimeprazine in rats by gas chromatography–mass spectrometry

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(First received October 1st, 1991; revised manuscript received December 2nd, 1991)

ABSTRACT

The metabolites of trimeprazine were identified in urine of rats by gas chromatography—mass spectrometry. After the oral administration of trimeprazine, the urinary metabolites were extracted with diethyl ether before or after hydrolysis with β-glucuronidase. The identified metabolites were N-demethyltrimeprazine, 3-hydroxytrimeprazine, N-demethyl-3-hydroxytrimeprazine and trimeprazine sulphoxide.

INTRODUCTION

Trimeprazine tartrate, 10-(3-dimethylamino-2-methylpropyl)phenothiazine tartrate, is an antipruritic and antihistaminic phenothiazine drug. Relatively little research has been reported concerning the metabolism of trimeprazine in animals. Robinson [1] studied the changes undergone by pharmacologically diverse phenothiazine derivatives on incubation with a rat liver preparation. The predominant reactions were dealkylation and hydroxylation of the aromatic ring.

We report here the metabolism of trimeprazine in rats. In this study, five metabolites were identified in the urine after oral administration of trimeprazine to rats, and the major metabolite was identified as 3-hydroxytrimeprazine.

EXPERIMENTAL

Chemicals

Trimeprazine tartrate was obtained from Sigma (St. Louis, MO, USA), β-glucuronidase from Boehringer (Mannheim, Germany) and Amberlite XAD-2 resin (80–150 mesh) from Serva (Westbury, NJ, USA). Hydrogen peroxide, *m*-chloroperbenzoic acid, sodium pyrophosphate decahydrate and ferrous sulphate were purchased from Aldrich (Milwaukee, WI, USA). Diethyl ether, methanol, potassium carbonate and sodium bicarbonate were from Merck (Darmstadt, Germany).

Drug administration and sample collection

Male Wistar rats weighing 250–300 g were

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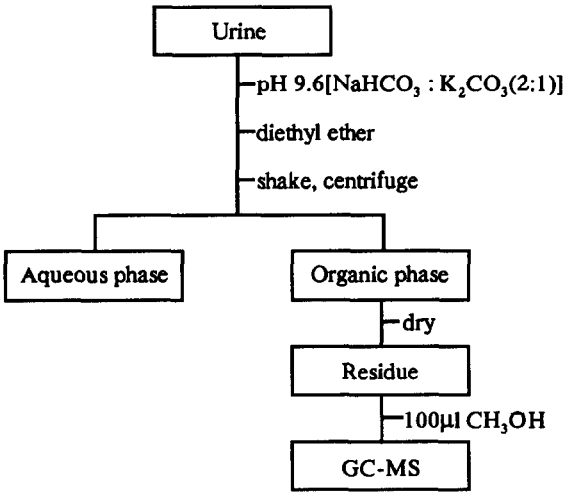

Fig. 1. Analytical procedure for trimeprazine and its metabolites from rat urine.

used. Trimeprazine tartrate was given orally at a dose of 0.5 mg/kg as a 0.5 mg/ml aqueous solution. Urine samples were collected for 24 h and control urine for 12 h before administration.

Isolation of unconjugated metabolites

A schematic outline of this method is shown in Fig. 1.

To 2.0 ml of rat urine, *ca.* 100 mg of sodium bicarbonate–potassium carbonate (2:1) were added, and the metabolites were extracted with 5 ml of diethyl ether. The ether layer was concentrated to dryness in vacuum and dissolved in 100 µl of methanol. The complete methanolic phase was injected into the gas chromatographic–mass spectrometric (GC–MS) apparatus.

Isolation of conjugated metabolites

About 2 ml of urine were transferred to two separate Amberlite XAD-2 columns (Pasteur pipette, 5 mm I.D., closed with a glass pearl, 3 cm bed height of XAD-2). Each Amberlite XAD-2 column was washed with 10 ml of water and eluted with 2 ml of methanol. The methanol was evaporated to dryness in vacuum and hydrolysed with β-glucuronidase (activity 10 U/ml) from *Escherichia coli* in 1 ml of 0.2 *M* phosphate buffer (pH 7.0) for 2 h at 50°C.

After hydrolysis, 200 mg of sodium bicarbonate–potassium carbonate (2:1) were added, and the metabolites were extracted with 5 ml of diethyl ether. The ether layers were evaporated to dryness under vacuum.

Gas chromatography–mass spectrometry

All mass spectra were obtained with a Hewlett-Packard 5890/5970 B instrument. An HP cross-linked 5% phenylmethylsilicone capillary column (SE-54, 16 m × 0.2 mm I.D., film thickness 0.33 µm) was used. Samples were injected in the split mode with a splitting ratio of 1:10. The flow-rate of the helium was 1.2 ml/min. The GC operating temperatures were: injector temperature, 280°C; transfer line temperature, 300°C; oven temperature, programmed from 100°C at 20°C/min to 300°C (held for 2 min).

Syntheses of trimeprazine-related compounds

Trimeprazine sulphoxide was prepared by the oxidation of trimeprazine with 15% hydrogen peroxide [2]. N-Demethyltrimeprazine was obtained by the oxidation of trimeprazine with *m*-chloroperbenzoic acid [3].

3-Hydroxytrimeprazine was synthesized by hydroxylation of trimeprazine with sodium pyrophosphate–ferrous sulphate by the procedure of Ullrich *et al.* [4].

N-Demethyl-3-hydroxytrimeprazine was prepared by the oxidation of 3-hydroxytrimeprazine with *m*-chloroperbenzoic acid [3].

RESULTS AND DISCUSSION

When an aqueous solution of trimeprazine was administered to rats, the parent drug and four metabolites were detected in the urine. Fig. 2 shows a total ion chromatogram of the metabolites. The GC retention times of peaks A, B, C, D and E were 29.18, 29.62, 34.80, 35.39 and 35.61 min, respectively. All five peaks, A–E, were detected in the alkaline urine before hydrolysis with β-glucuronidase and were not observed in the chromatogram of control urine. Determination of the glucuronides showed that the amounts of metabolites present in the urinary extracts after

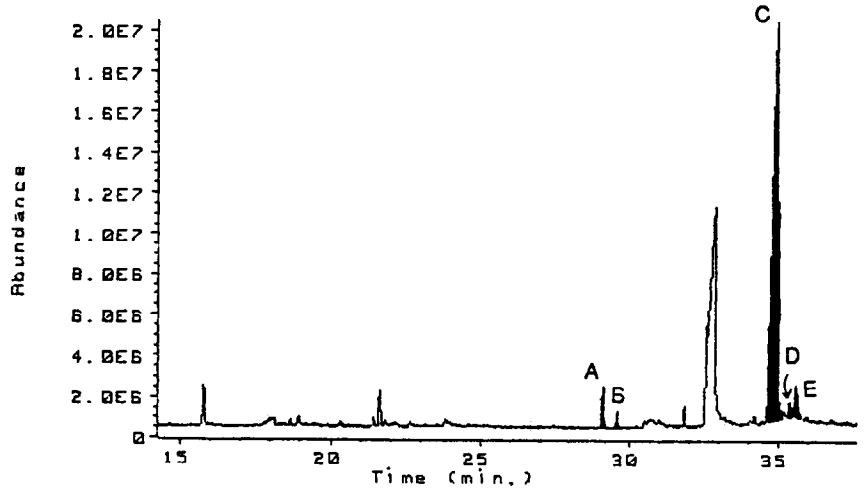

Fig. 2. Total ion chromatogram of extract from rat urine after oral administration of trimeprazine.

hydrolysis with β-glucuronidase were not significantly greater than those present before hydrolysis. It was therefore considered that the metabolites were not excreted as glucuronides in the urine.

The identities of trimeprazine and its metabolites in urinary extracts were confirmed by comparison of their mass spectra and GC retention times with those of authentic standards.

Peak A showed the same retention time as authentic trimeprazine. The mass spectrum of the compound corresponding to peak A was identical with that of authentic trimeprazine. The electron impact (EI) mass spectrum showed a molecular ion at *m/z* 298 and principal fragment ions at *m/z* 58, 198, 180, 100, 252, 154, 238, 84 and 212 (Fig. 3).

Peak B had a longer retention time than trimeprazine. The EI mass spectrum of peak B showed a molecular ion at *m/z* 284 and principal fragment ions at *m/z* 44, 199, 198, 212, 180, 238 and 252 (Fig. 4). The most diagnostic ions were 14 mass units lower than the ions of peak A and indicate demethylation of trimeprazine. The mass spectrum and GC retention time showed compound B to be identical with the reference

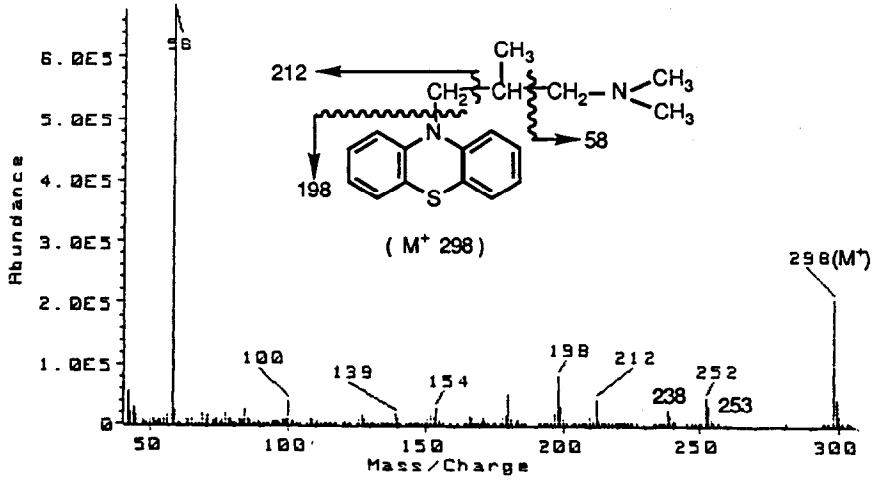

Fig. 3. Postulated mass fragment ions of trimeprazine by EI-MS.

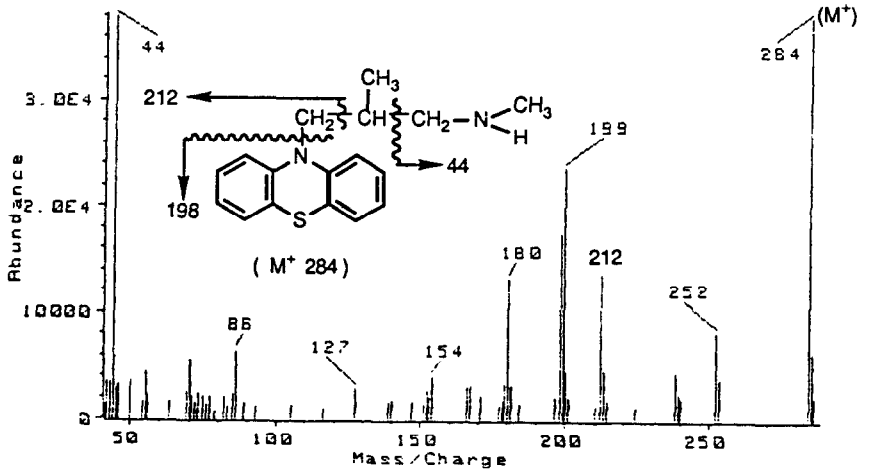

Fig. 4. Postulated mass fragment ions of N-demethyltrimeprazine by EI-MS.

substance, N-demethyltrimeprazine, prepared synthetically.

Peaks C and D had also longer retention times than trimeprazine. The EI mass spectrum of peak C showed a molecular ion at *m/z* 314, the base peak at *m/z* 58 and other diagnostic ions at *m/z* 100, 214, 196, 228, 268, 215, 269, 256, 257 and 84 (Fig. 5). The molecular ion and most of the diagnostic ions were 16 mass units higher than the ions of peak A, and indicate addition of an oxygen atom to trimeprazine. The mass spectrum and GC retention time showed compound C to be identical with the reference substance 3-hydroxytrimeprazine, prepared synthetically.

The EI mass spectrum of peak D showed a molecular ion at *m/z* 300 and principal fragment ions at *m/z* 44, 215, 214, 228, 196, 268, 86, 254, 269, 256 and 257 (Fig. 6). The most diagnostic ions of peak D were 14 mass units lower than the ions of peak C and 16 mass units higher than the ions of peak B, and indicate N-demethylation of 3-hydroxytrimeprazine.

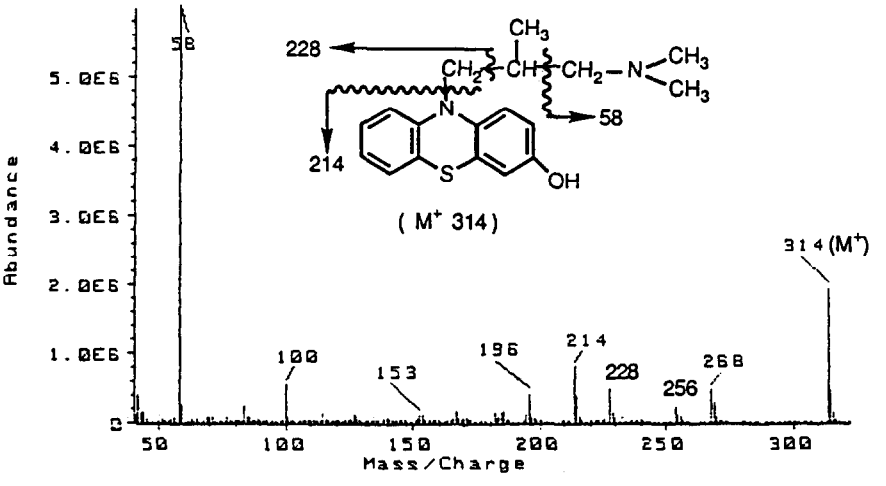

Fig. 5. Postulated mass fragment ions of 3-hydroxytrimeprazine by EI-MS.

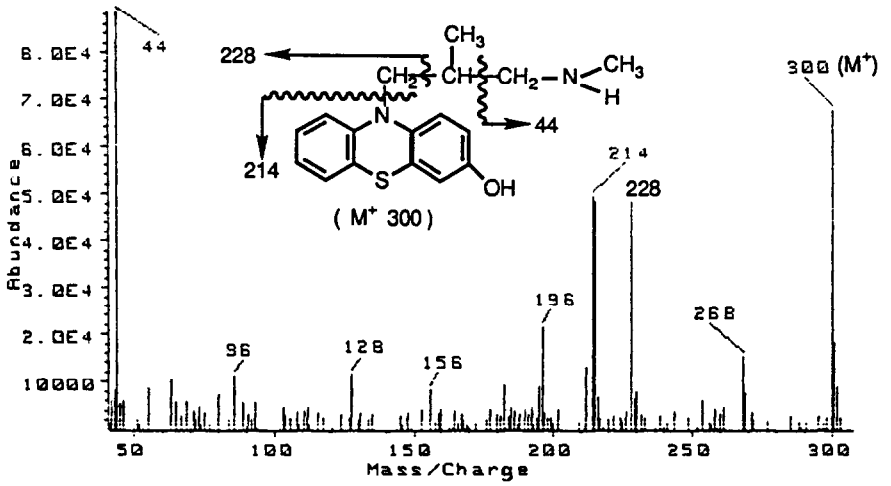

Fig. 6. Postulated mass fragment ions of N-demethyl-3-hydroxytrimeprazine by EI-MS.

The EI spectrum of peak E showed a molecular ion at *m/z* 314, the base peak at *m/z* 58 and other diagnostic ions at *m/z* 212, 298, 198, 199, 180, 252, 84, 140 and 238 (Fig. 7). The molecular ion was 16 mass units higher than the molecular ion of peak A, but other diagnostic ions showed a similar mass spectrum to that of trimeprazine. Also, when the injection port temperature was lowered, peak E increased at the expense of peak A, and this corresponded to reported decomposition phenomena of phenothiazine sulphoxide during GC analysis [5]. The mass spectrum and GC retention time showed compound E to be identical with the reference substance, trimeprazine sulphoxide, prepared synthetically.

The metabolites showed a very characteristic mass fragmentation pattern. Loss of $C_5H_{12}N$ (in case of trimeprazine and its oxidized metabolites) or $C_4H_{10}N$ (in case of demethylated metabolites) was due to a C–C bond cleavage α to the phenothiazine nitrogen. These ion fragments underwent elimination of a sulphur atom from the

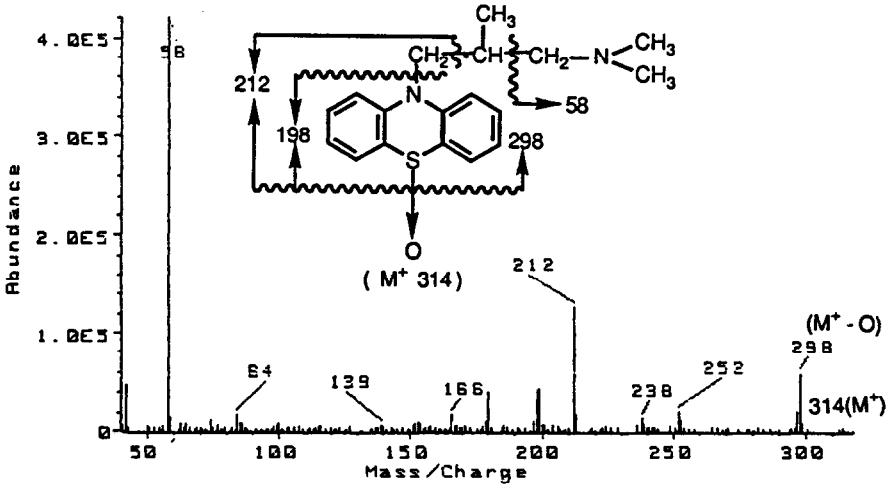

Fig. 7. Postulated mass fragment ions of trimeprazine sulphoxide by EI-MS.

phenothiazine nucleus. Otherwise, the initial process in a sulphoxidic metabolite under EI was the loss of an oxygen atom. High-resolution mass measurements revealed few fragments retaining the sulphoxide oxygen. Most fragment ions were derived from the [M–O] ions, and the loss of oxygen caused the low abundance of the molecular ion.

Thus it appears that the major route of metabolism of trimeprazine in rats is aromatic hydroxylation, and minor routes are sulphoxidation and N-demethylation. The major aromatic hydroxylation takes place at the 3-position in the phenyl group of the phenothiazine moiety.

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