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METABOLISM AND PHARMACOKINETIC STUDIES OF PROPIONYLPROMAZINE IN HORSES

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SUMMARY

The propionylpromazine concentrations in plasma after intramuscular administration to horses were determined using gas chromatography with nitrogen–phosphorus detection After hydrolysis by β-glucuronidase/arylsulphatase, the parent drug and three metabolites were detected in urine. The metabolites were identified as 2-(1-hydroxypropyl)promazine, 2-(1-propenyl)promazine and 7-hydroxypropionylpromazine by gas chromatography–mass spectrometry No N-demethylated or sulphoxidated metabolites of propionylpromazine were observed in the horse urine.

INTRODUCTION

Propionylpromazine (Combelene) is an effective tranquillizer mainly used for veterinary purposes. Even though the U.S. FDA has withdrawn approval for the use of propionylpromazine in horses, it is still widely used and readily available. Because this compound is often abused in horseracing to alter the performance of horses, the detection and identification of propionylpromazine and its metabolites are critical for drug test laboratories. However, relatively little research has been reported concerning the metabolism of propionylpromazine in horses. Only 2-(1-hydroxypropyl)promazine sulphoxide has been reported as a metabolite of propionylpromazine [1].

We report here the metabolism and single-dose kinetics of propionylpromazine in horses.

EXPERIMENTAL

Chemicals

All the chemicals were of analytical-reagent grade. Propionylpromazine was obtained from Sigma (St. Louis, MO, U.S.A.), β-glucuronidase/arylsulphatase

0378-4347/89/$03.50

(from *Escherichia coli*) from Boehringer (Mannheim, F.R.G.) and Amberlite XAD-2 resin (80–150 mesh) from Serva (Westbury, NJ, U.S.A.). Lithium aluminum hydride, *m*-chloroperbenzoic acid, methyl iodide, hydrogen peroxide, sodium pyrophosphate decahydrate and iron(II) sulphate were purchased from Aldrich (Milwaukee, WI, U.S.A.) and used without purification.

Drug administration

A commercial veterinary injectable preparation of propionylpromazine hydrochloride 50 mg (1% solution, 5 ml) was given intramuscularly (i.m.).

Extraction procedure for plasma

The extraction procedure (Fig. 1) for plasma developed by Javaid et al. [2] was adopted. In a 20-ml glass-stoppered test-tube, 2.0 ml of the plasma sample and 15 ng of chlorpromazine as an internal standard were placed and the pH was adjusted to 9.6 ± 0.1 with 5 *M* potassium hydroxide solution containing ascorbic

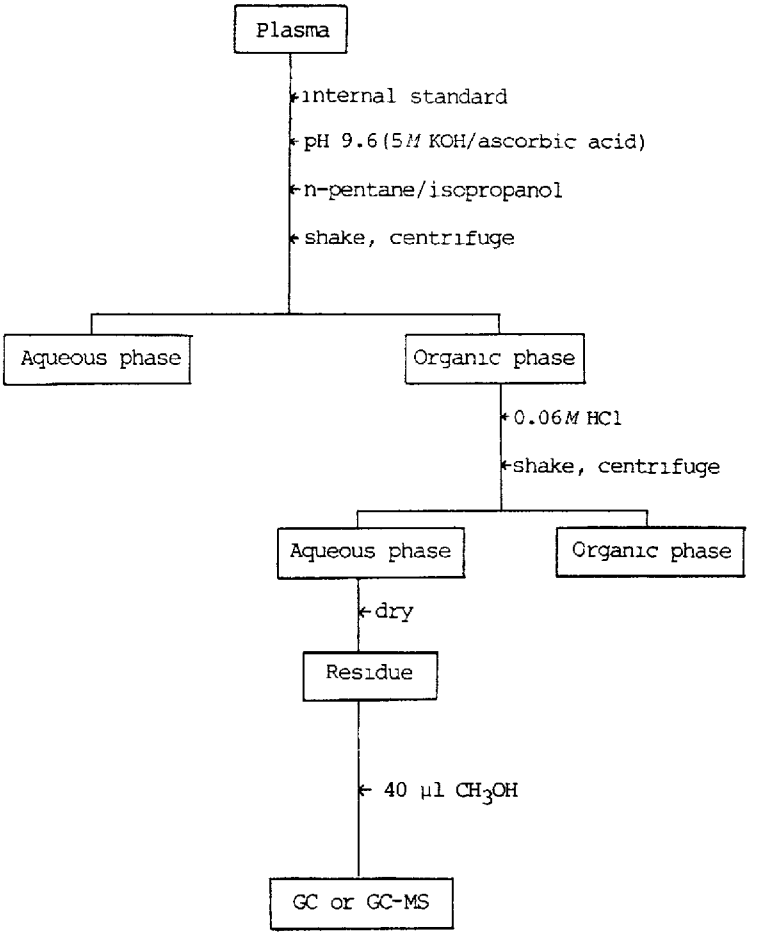

Fig. 1. Procedure for the extraction of propionylpromazine and its metabolites from equine plasma.

acid (5 ml=0.5 g mixture). The sample was extracted with 8 ml of *n*-pentane–isopropanol (97:3) by mechanical shaking for 20 min. The two phases were separated by centrifugation (5 min at 1500 *g*) and the organic phase was transferred into a 20-ml glass-stoppered tube. To this sample, 0.4 ml of 0.06 *M* hydrochloric acid was added and the tube shaken mechanically for 20 min. The two phases were separated by centrifugation (5 min at 1500 *g*) and the organic phase was removed by suction and discarded. The aqueous layer was dried in a desiccator over phosphorus pentoxide–potassium hydroxide, the dried residue was dissolved in 40 μl of methanol and 3-μl portions of the solution were injected into a gas chromatographic (GC) or GC–mass spectrometric (GC–MS) instrument.

Hydrolysis and extraction procedure for urine [3]

An XAD-2 slurry, previously washed with acetone, methanol and water, was filled into a Pasteur pipette until a bed 25 mm high was obtained, then 5 ml of urine were added and the column washed with an equal volume of water. The adsorbed lipid fraction was eluted with 3 ml of methanol. The methanolic solution was evaporated to dryness using a vacuum rotary evaporator. A 1-ml volume of potassium phosphate buffer (0.2 *M*, pH 7.0) was added to the residue, then 25 μl of the enzyme preparation (β-glucuronidase; activity 10 U/ml, from *E. coli*) were added. Hydrolysis was performed at 50°C for 1 h. After cooling, the solution was adjusted to pH 9.6 ± 0.1. The procedure for the extraction of phenothiazines from this basic solution is identical with that for plasma samples.

Gas chromatography

A Hewlett-Packard 5890 gas chromatograph with nitrogen–phosphorus detection (NPD) was used for the determination of drug concentrations in plasma. A cross-linked 5% phenylmethylsilicone capillary column (16 m × 0.2 mm I.D., film thickness 0.33 μm) was used. Helium at a flow-rate of 1.57 ml/min was used as the carrier gas. Samples were injected in the split mode with a splitting ratio of 1:10. The GC operating conditions were as follows: detector temperature, 300°C; injector temperature, 280°C; oven temperature, programmed from 100°C at 20°C/min to 300°C (held for 3 min).

Gas chromatography–mass spectrometry

Most of the mass spectra were obtained with a Hewlett-Packard 5890/5970B instrument. The same capillary column as described above for GC was also used for GC–MS. The flow-rate of the helium was 0.698 ml/min. Chemical ionization (CI) mass spectra were obtained with a Hewlett-Packard 5890/5988 GC–MS instrument. The GC operating temperature was as follows: injector temperature, 280°C; transfer line temperature, 300°C; oven temperature, programmed from 100°C at 20°C/min to 300°C (held for 3 min).

Syntheses of propionylpromazine-related compounds

The possible metabolites of propionylpromazine shown in Fig. 2 were synthesized by the following methods. Propionylpromazine was reduced with lithium aluminum hydride to give 2-(1-hydroxypropyl)promazine [4], which was con-

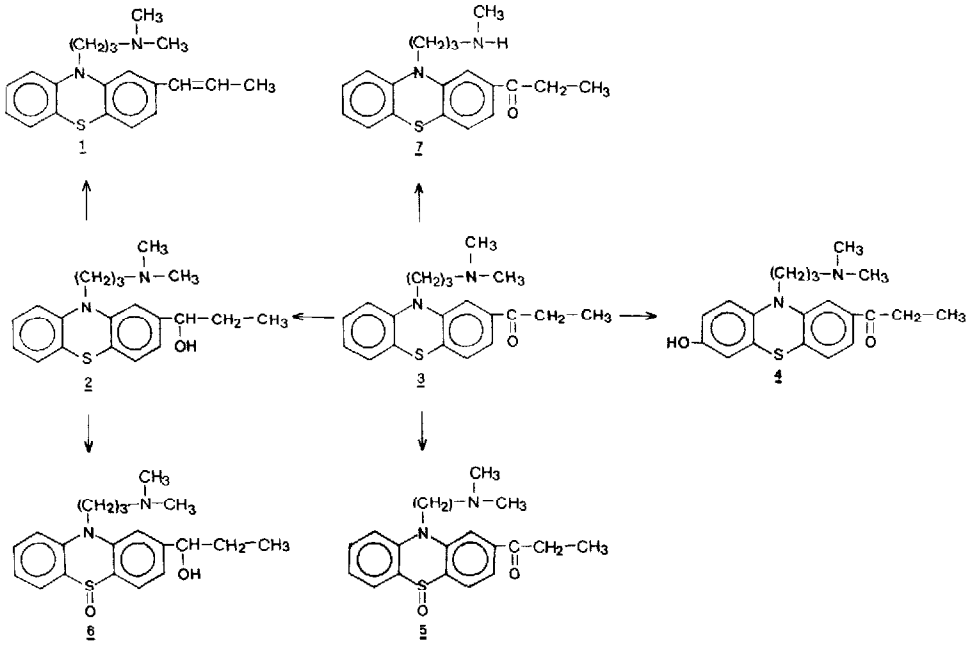

Fig 2. Possible metabolites of propionylpromazine

verted into 2-(1-hydroxypropyl)promazine sulphoxide with 15% hydrogen peroxide [5]. Dehydration of the latter to 2-(1-propenyl)promazine was achieved by the action of concentrated sulphuric acid. Propionylpromazine sulphoxide was also prepared by oxidation of propionylpromazine with 15% hydrogen peroxide. 7-Hydroxypropionylpromazine was obtained from propionylpromazine by the procedure of Dewey et al. [6].

RESULTS AND DISCUSSION

Calibration graph and extraction yield

Two methanolic stock solutions were prepared, one containing propionylpromazine and the other chlorpromazine (both 0.5 μg/ml). Portions of these solutions were then added to five 2.0-ml portions of blank plasma to obtain standard plasma samples with the propionylpromazine concentrations of 1.0, 2.5, 5.0, 10 and 15 ng/ml and a chlorpromazine concentration of 7.5 ng/ml. The samples were then extracted and examined by GC to construct a calibration graph. When the peak-area ratio was plotted against the amount of propionylpromazine, the calibration graph showed good linearity with $r=0.998$ [relative standard deviation (R.S.D.) = 2.2–5.4%; $n=3$]. To determine the extraction recovery of drugs from plasma, 7.5 ng of propionylpromazine were added to 2.0 ml of plasma. When seven samples were tested, the mean recovery yield was 71% (R.S.D. = 7%).

Pharmacokinetics

A gas chromatogram of an extract of equine plasma taken 0.5 h after i.m. administration is shown in Fig. 3. The peak-area ratios of propionylpromazine at

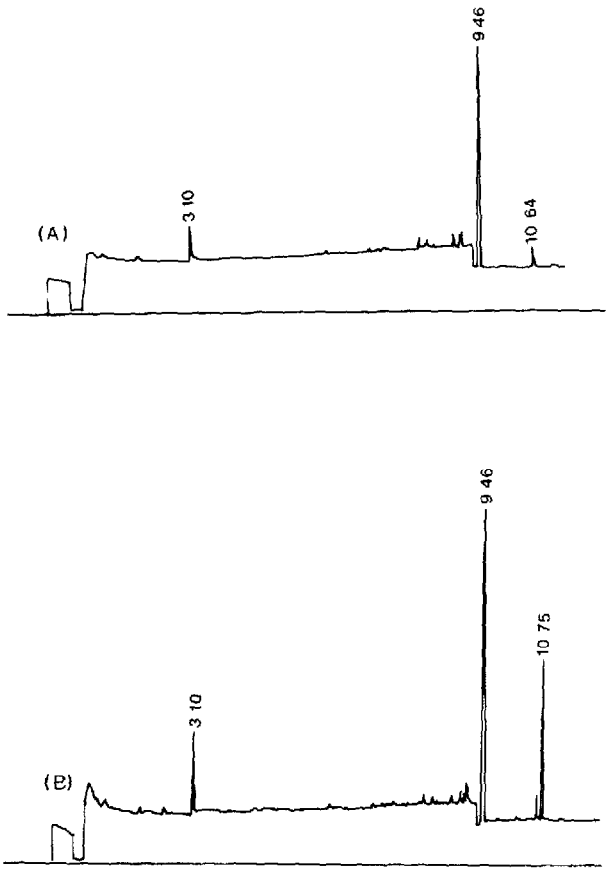

Fig. 3. Gas chromatograms of extracts from 2.0 ml of plasma. (A) Pre-dose sample; (B) plasma sample 30 min post-dose (50 mg, i.m).

TABLE I

PLASMA CONCENTRATIONS OF PROPIONYLPROMAZINE AFTER i.m. ADMINISTRATION OF 50 mg

Time after drug administration (h)	Propionylpromazine[a] (ng/ml)	R.S.D. (%)
0.25	4.17	2 40
0.5	5.21	2.69
1	4.33	0.69
3	4.00	3.75
5	3.22	1.55
7	2.72	5.88
9	1.36	5.88
11	1 26	11.11

[a]Mean values of three analyses.

10.74 min to the internal standard at 9.46 min were compared with the calibration graph. Plasma concentrations of propionylpromazine in horses after i.m. administration of the drug are shown in Table I. When the decrease in propionylpromazine concentration in plasma versus time was plotted as a semi-logarithmic graph, the half-life of propionylpromazine was 5.12 h and peak plasma level was reached after 0.5 h. The detection limit was 0.2 ng/ml.

Urinary metabolites

Extracts from equine urine after enzyme hydrolysis were analysed by GC–MS. Three metabolites of propionylpromazine were found. Figs. 4 and 5 show ion chromatograms and individual mass spectra, respectively, of the metabolites. Peak I in Fig. 4 was identified as 2-(1-propenyl)promazine. The mass spectrum (Fig. 5A) showed a molecular ion peak at *m/z* 324, which is 18 a.m.u. less than 2-(1-hydroxypropyl)promazine, and therefore it could be formed through dehydration of the corresponding alcohol. The fragmentation pattern of this metabolite was identical with that of independently synthesized 2-(1-propenyl)promazine, which had been easily prepared by acid-catalysed dehydration of 2-(1-hydroxypropyl)promazine in tetrahydrofuran. However, when the same reaction was carried out in methanolic solution, the major product identified was 2-(1-methoxypropyl)promazine. These results suggest that under the reaction conditions 2-(1-hydroxypropyl)promazine forms a stable carbocation at C-1 which either loses a proton to form 2-(1-propenyl)promazine (in a non-nucleophilic solvent) or combines with methanol (in a nucleophilic solvent) to give 2-(1-methoxypropyl)promazine. The mass spectrum of the peak II in Fig. 4 was identical with that of synthesized 2-(1-hydroxypropyl)promazine. Dewey and Maylin [1] reported that not 2-(1-hydroxypropyl)promazine but its sulphoxide was the metabolite of propionylpromazine. However, no trace of 2-(1-hydroxypropyl)promazine sulphoxide was detected in this work. Because the sulphoxides were reported to be decomposed during GC analysis [7], 2-(1-hydroxypropyl)promazine sulphox-

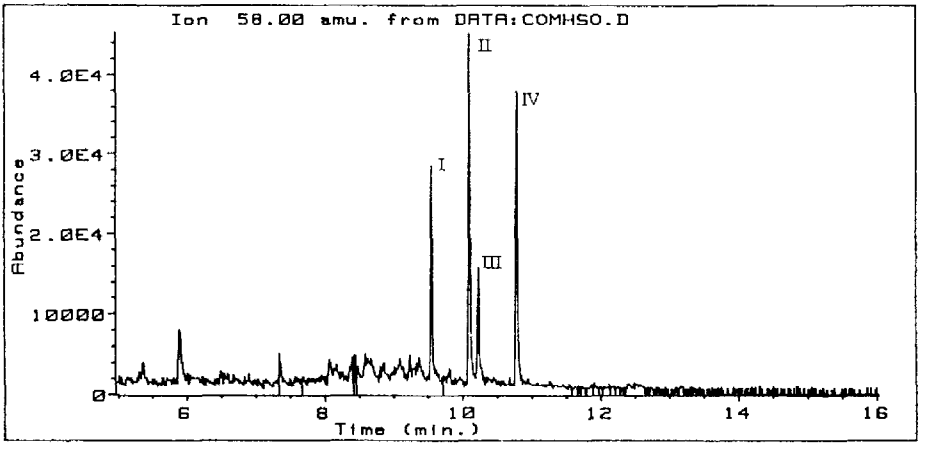

Fig. 4. Extracted ion chromatograms of extracts from enzyme-hydrolysed urine after i.m. administration of propionylpromazine.

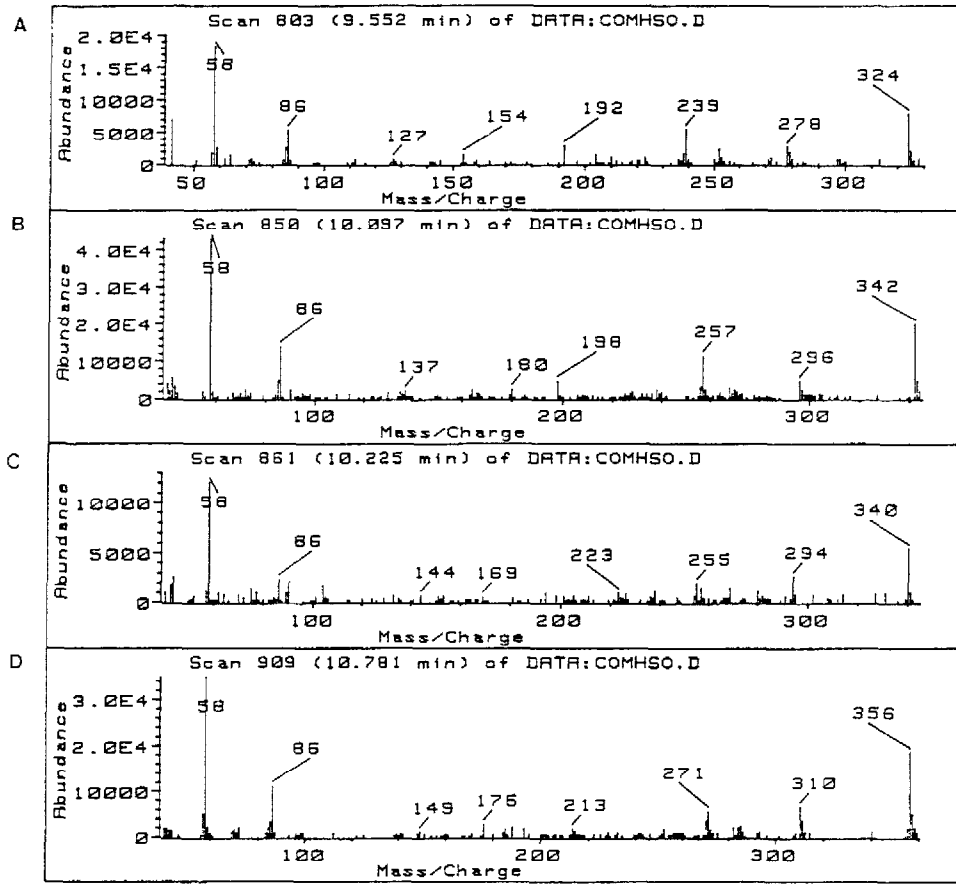

Fig. 5. Electron-impact mass spectra of four compounds found in the extracts of equine urine. (A) 2-(1-Propenyl)promazine; (B) 2-(1-hydroxypropyl)promazine; (C) propionylpromazine; (D) 7-hydroxypropionylpromazine.

ide was prepared (Fig. 6B) by oxidation of 2-(1-hydroxypropyl)promazine with hydrogen peroxide and its GC behaviour was carefully monitored. When this sulphoxide was injected into the GC–MS system with an injector temperature as high as 310°C, the compound was clearly detected. Peak III in Fig. 4 was identified as unchanged propionylpromazine by its mass spectrum. The mass spectrum of peak IV in Fig. 4 was identical with that of the compound obtained by the hydroxylation of propionylpromazine with $Na_2P_2O_7$–$FeSO_4$ as described by Dewey et al. [6] and it was therefore assigned as 7-hydroxypropionylpromazine. Dewey et al. synthesized 7-hydroxyacetylpromazine from acepromazine by the same method and concluded that 7-hydroxyacetylpromazine was the metabolite of acepromazine in horses. Phenolic hydroxylation at the 7-position was also observed in the biotransformation of chlorpromazine [7]. 2-(1-Hydroxypropyl)promazine and 7-hydroxypropionylpromazine were detected only after enzyme hydrolysis. With urine samples with no enzymatic hydrolysis, only 2-(1-propenyl)promazine and unchanged propionylpromazine were detected. However, the relative amounts of the three metabolites were different from sample to sample.

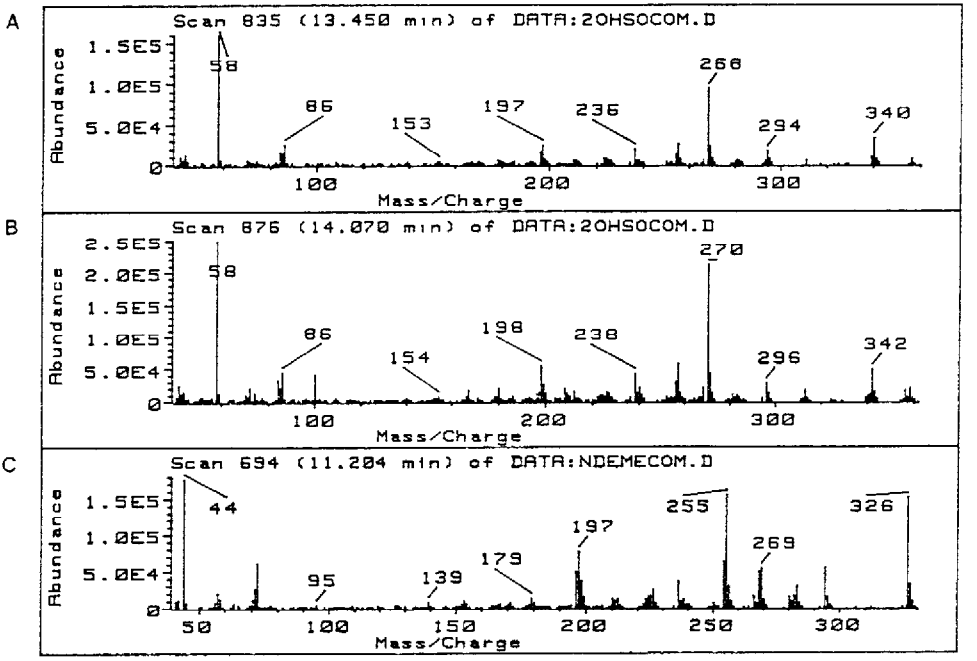

Fig. 6. Electron-impact mass spectra of three compounds prepared chemically. (A) Propionylpromazine sulphoxide; (B) 2-(1-hydroxypropyl)promazine sulphoxide; (C) N-desmethylpropionylpromazine.

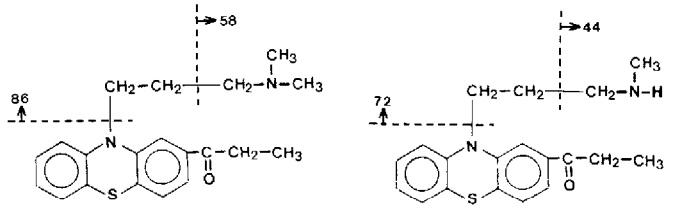

Fig. 7. Fragmentation patterns of propionylpromazine and N-desmethylpropionylpromazine

As N-oxidation at the terminal dimethylamino group was known to be one of the metabolic pathways of phenothiazines [6], N-oxidation was attempted on propionylpromazine with *m*-chloroperbenzoic acid. The reactant remained mostly unchanged after the reaction. However, unexpectedly, a small amount of N-desmethylpropionylpromazine was formed. The mass spectrum (Fig. 6C) of this compound showed peaks at *m/z* 44 and 72 in addition to the molecular ion peak. Fragmented ions with *m/z* 44 and 72 are known to be the characteristic ions of N-demethylated metabolites of acepromazine [6]. The fragmentation pattern of propionylpromazine and N-desmethylpropionylpromazine are compared in Fig. 7. The structure of the synthesized N-desmethylpropionylpromazine was further confirmed after derivatization. When N-desmethylpropionylpromazine was derivatized to its N-trifluoroacetyl derivative and subjected to GC–MS, its mass spectrum showed a molecular ion at *m/z* 422. However, no N-demethylated metabolite was observed in horse urine.

TABLE II

RELATIVE RETENTION TIMES AND CHARACTERISTIC IONS OF PROPIONYLPROMAZINE-RELATED COMPOUNDS

Compound No [a]	Name	Metabolite of propionylpromazine	Relative retention time [b]	Characteristic ions						
				EI						CI ($M^+ + H$)
1	2-(1-Propenyl)promazine	Yes	0.934	58	86	239	278	324		325
2	2-(1-Hydroxypropyl)promazine	Yes	0.987	58	86	257	296	342		343
3	Propionylpromazine	Yes	1	58	86	255	294	340		341
4	7-Hydroxypropionylpromazine	Yes	1.054	58	86	271	310	356		357
5	Propionylpromazine sulphoxide	No	1.315	58	84	86	268	340	356	357
6	2-(1-Hydroxypropyl)promazine sulphoxide	No	1.376	58	84	86	270	342	358	359
7	N-Desmethylpropionylpromazine	No	1.096	44	72	255	294	326		327

[a] See Fig. 2.
[b] Relative to propionylpromazine.

The relative retention times and characteristic ions (EI and CI mass spectral data) of propionylpromazine-related compounds are given in Table II.

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