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Detection and identification of famprofazone and its metabolite in human urine

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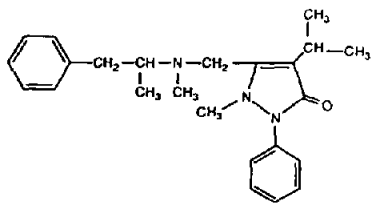
Abstract

Detection and identification of famprofazone and its metabolite, *p*-hydroxydesmethylfamprofazone, were described. The identity of the new metabolite was confirmed by comparing its mass spectrum and gas chromatographic retention time with that of the synthetic standard and its derivatives. Unchanged famprofazone was detected up to 6 h and the metabolite was detected up to 32 h in human urine after administration of famprofazone. The sum of the two compounds excreted in urine was ca. 1.5% of the dose.

1. Introduction

Famprofazone, 4-isopropyl-2-methyl-3-[N-methyl-N-(α-methylphenylethyl)aminomethyl]-1-phenyl-3-pyrazolin-5-one (I, Fig. 1) is an analgesic drug, which is now available in combination preparations in several countries.



(I)

Fig. 1. Structure of famprofazone (I).

After administration of famprofazone in mice, Mrongovius et al. [1] found two urinary metabolites: methamphetamine and 3-hydroxymethylpropyphenazone. Also, in the additional study of famprofazone metabolism in humans Neugebauer [2] found that cleavage of the molecule led to the formation of free methamphetamine together with the corresponding pyrazolone derivatives. Oh et al. [3] described plasma and urinary concentrations of methamphetamine after oral administration of famprofazone to man.

Analysis of famprofazone in human urine to date has been limited by the fact that the concentration of the parent drug in urine is too

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low to be detected by the until today available analytical methods. The present study was undertaken to identify and to detect the parent drug and one metabolite in human urine after oral administration of famprofazone.

2. Experimental

2.1. Materials

Isopropylantipyrine was obtained from Sam Jin Pharmaceutical (Seoul, Korea) and *p*-hydroxyamphetamine was purchased from Eli Lilly (Indianopolis, IN, USA). N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) methyl iodide and ephedrine hydrochloride were purchased from Sigma (St. Louis, MO, USA). All other chemicals and reagents were special grade.

3-(Bromomethyl)-4-isopropyl-2-methyl-1-phenyl-3-pyrazolin-5-one was prepared as follows [4]. A solution of 0.5 g of isopropylantipyrine with 0.4 g of N-bromosuccinimide (NBS) in 10 ml of CCl_4 was refluxed for 2 h. The reaction solution was washed with water, dried, and recrystalized from *n*-hexane.

p-Hydroxydesmethylfamprofazone was prepared as follows [2,5]: 0.28 g of *p*-hydroxyamphetamine was refluxed with 1.1 ml of MSTFA in 10 ml of chloroform, and 0.58 g of 3-(bromomethyl)-4-isopropyl-2-methyl-1-phenyl-3-pyrazolin-5-one was added slowly. The solution was refluxed for 4 h. To the reaction mixture, 10 ml of water was added, the solution was made alkaline with K_2CO_3–$NaHCO_3$ (2:1, w/w) and extracted. The organic layer was transferred into a glass beaker and dried with Na_2SO_4. The solution was evaporated at reduced pressure, dissolved in methanol, then recrystalized.

α-Hydroxyfamprofazone (internal standard) was synthesized as follows: 0.26 g of ephedrine hydrochloride and 0.27 ml of triethylamine were heated in 10 ml of acetonitrile and 0.4 g of 3-(bromomethyl)-4-isopropyl-2-methyl-1-phenyl-3-pyrazolin-5-one were added slowly. The solution was refluxed for 4 h and filtered. The precipitate was washed with acetonitrile and recrystalized from methanol.

2.2. Drug administration and sample collection

Famprofazone (30 mg, two tablets of Gewodin, Ed. Geistlich Sohne, Wolhusen, Switzerland) were orally administered to three male volunteers. They all were free from other medication whilst the experiment lasted. Urine collection was made before drug administration and at intervals of 2, 4, 6, 8, 10, 17, 24, 32, 40, 48, 56, 66 and 72 h after administration. Samples were stored at 4°C.

2.3. Isolation of metabolites

A 5 ml volume of urine was adjusted to pH 9.6 with sodium bicarbonate–potassium carbonate (2:1, w/w) and 50 ng of internal standard (50 μl of 1.0 μg/ml in methanol) were added. The mixture was extracted with diethyl ether. The organic layer was evaporated in vacuo and dried in a desiccator over phosphorus pentoxide–potassium hydroxide.

For enzymatic hydrolysis samples were adjusted to pH 5.2 with 1 ml of 0.1 *M* phosphate buffer and incubated with 50 μl of β-glucuronidase/arylsulphatase (*Escherichia coli*, Boehringer, Mannheim, Germany) for 4 h. After cooling, the solution was adjusted to pH 9.6 with 200 mg of sodium bicarbonate–potassium carbonate (2:1, w/w). The procedure for the extraction of the hydrolyzed metabolites from urine was identical with that for the extraction of unconjugated metabolites.

2.4. Quantitation of famprofazone and its metabolite

A calibration curve of peak-area ratios of standard (famprofazone or *p*-hydroxydesmethylfamprofazone) to internal standard (α-hydroxyfamprofazone) against concentrations of standard (ng/ml) was constructed over the range 1.0–5000 ng/ml of urine. Samples of drug-free human urine (5 ml) and urine spiked with 500 ng/ml standards and 50 ng of internal standard

were taken through the extraction procedure described earlier and analyzed by GC with nitrogen–phosphorus detection (NPD). Calibration standards were chromatographed each day along with the unknown samples. The concentrations of the unknown samples were determined by comparison to peak-area ratios from the standard curve obtained that day.

2.5. Derivatization procedures

Trimethylsilylation

The trimethylsilyl derivatives were prepared by dissolving the dry residue in 100 μl of MSTFA and allowing the reaction to proceed for 10 min at 25°C.

Methylation

The dry residue was dissolved in 200 μl of dry acetone and 20 μl of methyliodide was added. A 100-mg amount of K_2CO_3 was then added and the solution heated for 30 min at 60°C.

2.6. Gas chromatography–mass spectrometry (GC–MS)

All mass spectra were obtained with a Hewlett–Packard (Avondale, PA, USA) 5890/5971A instrument including an HP 9144 disc drive and an HP Think Jet printer. Separation was achieved with an HP fused-silica capillary column with crossed-linked 5% phenylmethylsilicone (SE-54), ca. 17 m length, 0.2 mm I.D., 0.33 μm film thickness. Operating temperatures were as follows: injector, 280°C; oven, from 140 to 310°C at 20°C/min; interface, 300°C; source, 150°C. The carrier gas was helium, at a flow-rate of ca. 1 ml/min. The mass spectra were obtained at 70 eV and scanned from 50 to 500 amu. The volume injected was 2 μl.

2.7. Gas chromatography with nitrogen–phosphorus detection (GC-NPD)

All GC experiments were performed with a HP 5890 A gas chromatography equipped with a NPD connected to a HP 3392 A integrator. All injections were made by a HP 7673 A autosampler. GC temperature and column conditions were identical with those for GC–MS above.

2.8. Nuclear magnetic resonance (NMR) spectrometer

All spectra were recorded on an ^1H NMR spectrometer Gemini-300 BB (Varian, Palo Alto, CA, USA) ($CDCl_3$ as internal standard).

3. Results and discussion

3.1. Identification of famprofazone and its metabolite in urine

When two tablets of Gewodin were administered, the parent drug (peak A) and a new metabolite (peak C) from the basic urine before and after hydrolysis were detected by GC (Fig. 2).

The retention time of famprofazone was 8.29 min. Fig. 3 shows the fragmentation pattern of this drug. The EI mass spectrum showed a molecular ion at m/z 377 and diagnostic ions at m/z 286 ($M^+ - CH_2C_6H_5$), 229 ($M^+ - NCH_3CHCH_3CH_2C_6H_5$), 215 (pyrazolone ring) and 91 ($CH_2C_6H_5^+$).

The metabolite (peak C) was first identified with the mass spectra of free form and its

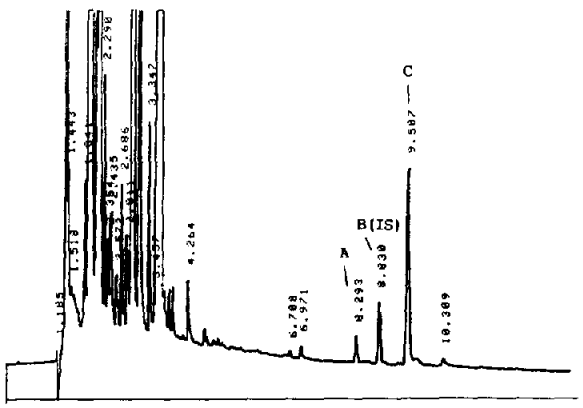


Fig. 2. Gas chromatogram of human urine extract. (A) famprofazone; (B) internal standard; (C) metabolite (*p*-hydroxydesmethylfamprofazone).

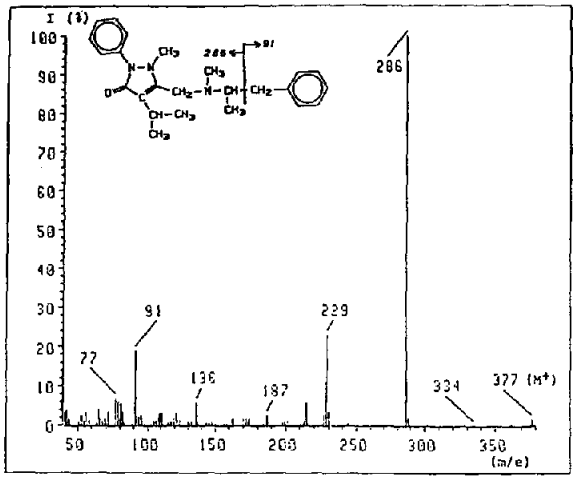


Fig. 3. Electron-ionization mass spectrum of famprofazone showing the structure of molecule and major ion fragments.

dimethylated, and trimethylsilylated derivatives. These spectra (Figs. 4–6) were compared with that of famprofazone. Peak C had a retention time longer than that of famprofazone. The mass spectrum of the peak showed a molecular ion at m/z 379 and diagnostic ions at m/z 272, 229, 215 and 107 (Fig. 4). The ion m/z 272 may result from the loss of an -N-methyl group of ion m/z 286 from famprofazone. This was supported by detection of the same fragment ions at m/z 229 and 215 of the metabolite and famprofazone. Another ion at m/z 107 may result from hydroxylation on the phenylethyl group, but that does not indicate whether the hydroxy group is in a phenylring or in an α-position of the phenylring.

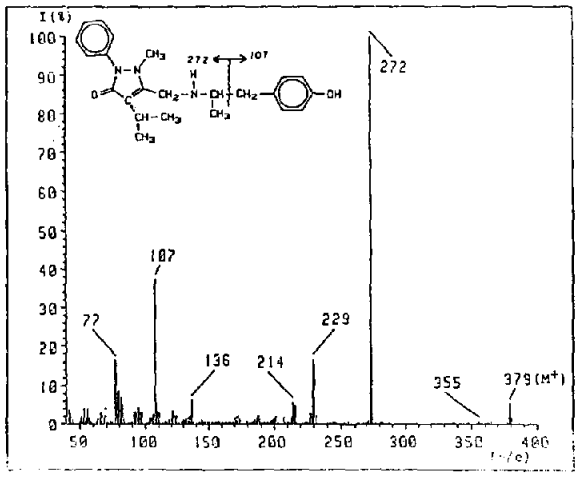


Fig. 4. Electron-ionization mass spectrum of p-hydroxydesmethylfamprofazone showing the structure of molecule and major ion fragments.

The main fragments of the methylated metabolite derivatives are shown in Fig. 5. The fragmentation showed the dimethylation of the metabolite. This suggests the presence of a phenolic-type function in the structure of the metabolite, because the hydroxyl group at the α-position of the phenylring can not be methylated under the derivatization conditions employed.

Fig. 6 shows the various fragmentation patterns of the trimethylsilyated metabolite derivatives. The molecular ion at m/z 451 and a fragment ion at m/z 179 were formed from the fragmentations containing the trimethylsilylated phenol group.

The identity of the metabolite was finally confirmed by comparison of the EI mass spectrum and the gas chromatographic retention time of the synthetic standard. MSTFA was used as a catalytic base and protecting agent in this synthesis [5]. The spectrum of the synthetic compound revealed the base peak of m/z 272, the molecular ion peak at m/z 379 and significant fragment ions m/z 107, 229, 77 and 214. All diagnostic ions of the synthetic compound corresponded with those of the new metabolite. The ^{1}H NMR spectrum of the synthetic compound was similar to that of famprofazone. In addition to the signals of famprofazone there was a signal of a p-hydroxylated aromatic ring (δ = 6.89 and 6.69 ppm, 4H). The signal at $\delta = 2.25$ ppm disappeared. From the data, it was apparent that the new metabolite was p-hydroxydesmethylfamprofazone (II, Fig. 7).

α-Hydroxyfamprofazone (III, Fig. 7) was synthesized by the method described in Experimental. From the spectrum of the synthetic substance, we found the base peak m/z 286, the molecular ion peak m/z 393, and significant fragment ions m/z 107, 229, 214 and 136. The crude residue was crystallized from methanol. In the ^{1}H NMR spectrum the signals of this com-

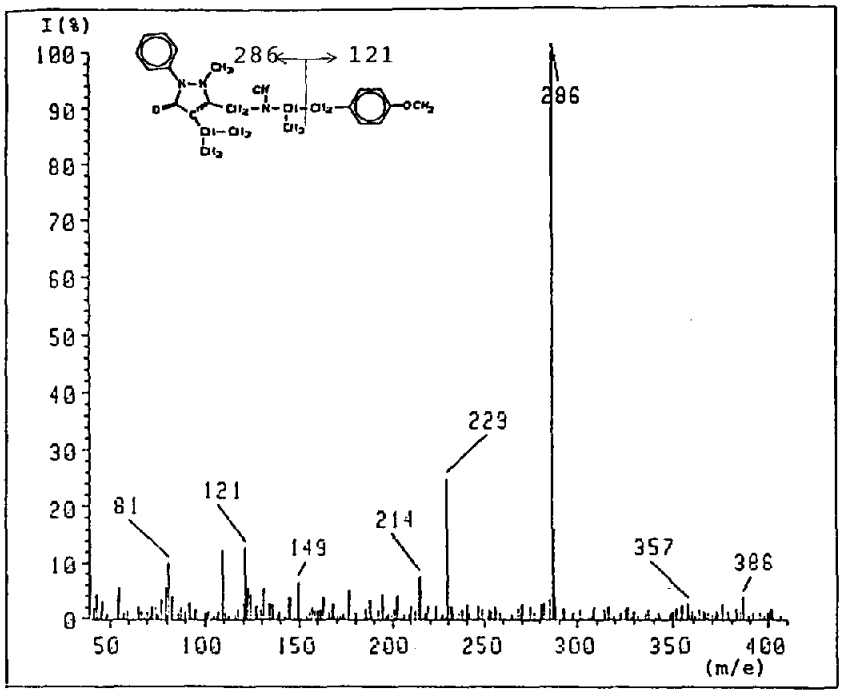


Fig. 5. Electron-ionization mass spectrum of *p*-hydroxydesmethylfamprofazone showing the methylated structure of molecule and major ion fragments.

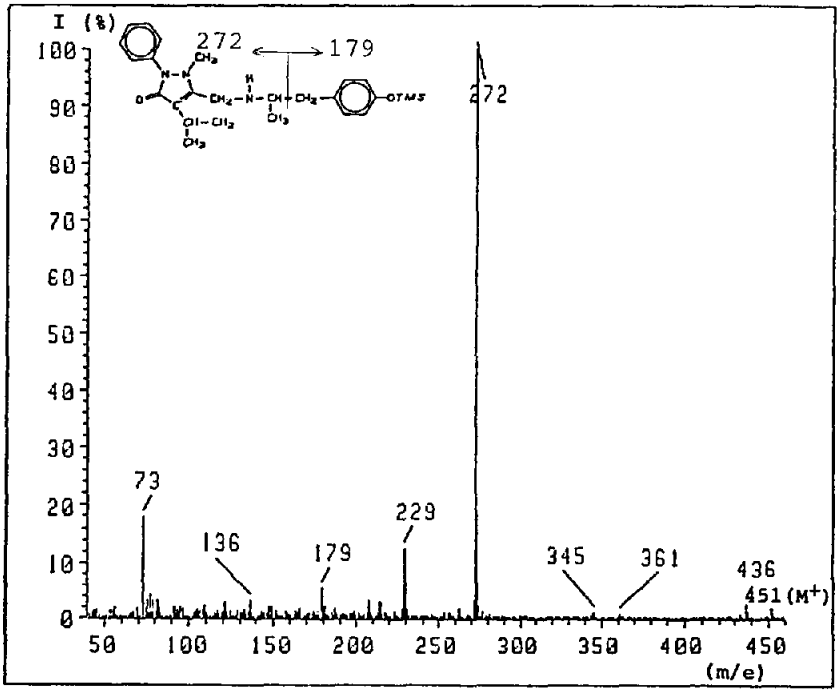
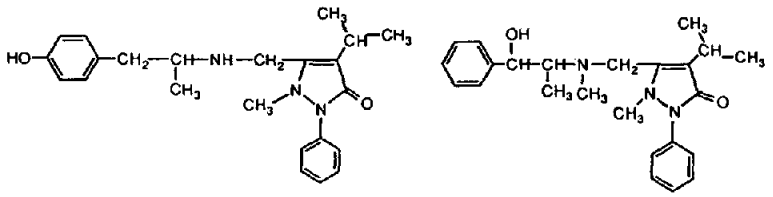


Fig. 6. Electron-ionization mass spectrum of *p*-hydroxydesmethylfamprofazone showing the trimethylsilylated structure of molecule and major ion fragments.



(II) (III)

Fig. 7. Structures of *p*-hydroxydesmethylfamprofazone (II) and α-hydroxyfamprofazone (III).

pound were also similar to those of famprofazone, but integration of the signal at δ = 4.77 and 4.79 ppm gave only one proton, indicating the substitution of an hydroxyl group at α-position of phenyl ring.

3.2. Urinary elimination of famprofazone and p-hydroxydesmethylfamprofazone

Elimination of the two products in human urine was followed quantitatively by GC-NPD. As shown in Fig. 1, no interfering peaks from endogenous substance are present near the peaks of famprofazone, *p*-hydroxydesmethylfamprofazone and internal standard. The α-hydroxylated famprofazone (III) was used as internal standard.

The calibration curves for famprofazone and its metabolite from 1 to 5000 ng/ml were linear. The lower limits of detection in urine for the two drugs were approx. 1 ng/ml (signal-noise ratio 3) and the detectability was approx. 3 pg. The within-run precisions evaluated at a drug concentration of 0.5 μg/ml were 2.9% for famprofazone and 3.5% for the metabolite and the day-to-day precisions determined by analysing prepared urine samples at a drug concentration of 0.5 μg/ml on three different days were 5.3% for famprofazone and 6.3% for the metabolite.

Determination of the conjugated form of these compounds showed that the amounts of the parent compound in the urinary extracts after hydrolysis were not higher than those present before hydrolysis. It was therefore considered that famprofazone was not excreted in its conjugated form in urine. Instead, the hydroxylated metabolite was excreted in conjugated form (approx. 90%) in urine. The conjugated *p*-hydroxylated metabolite was hydrolysed for 4 h with arylsulfatase–β-glucuronidase from *Helix Pomatia* (Boehringer). Overnight hydroxylation yielded the same amount of hydrolyzed metabolite indicating that hydrolysis was complete within 4 h.

The urinary excretion of famprofazone and the metabolite after oral administration of famprofazone is shown in Table 1. The metabolite, *p*-hydroxydesmethylfamprofazone, excreted in human urine accounted for approx. 1.4% of the dose administered. The parent drug could be detected up to 6 h (excretion represented approx. 0.03% of the dose) and the metabolite up to 32 h in the urine.

Table 1
Urinary excretion of famprofazone and *p*-hydroxydesmethylfamprofazone after oral administration of 50 mg of famprofazone

Time (h)	Famprofazone		*p*-Hydroxydesmethylfamprofazone	
	μg/ml	μg/h	μg/ml	μg/h
0	–	–	–	–
2:00	0.082	1.58	3.74	72.0
4:00	0.179	3.18	8.87	157.5
6:00	0.08	2.05	1.84	50.6
8:00	–	–	0.28	14.7
10:00	–	–	0.33	12.5
17:00	–	–	0.30	9.1
24:00	–	–	0.03	1.4
32:00	–	–	0.03	1.3
40:00	–	–	–	–
48:00	–	–	–	–

References

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