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Some new urinary metabolites of famprofazone and morazone in man

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Abstract: The human urinary metabolism of two pyrazolone derivatives, morazone and famprofazone, has been investigated. After administration of morazone, the metabolites *p*-hydroxymorazone and phenazone-4-carboxylic acid were excreted in addition to the known metabolite, phenmetrazine, and unchanged morazone. Metabolism of famprofazone led to the formation of methamphetamine; the pyrazolone moiety was excreted as 3-hydroxymethyl-propyphenazone.

Keywords: *Metabolism; gas–liquid chromatography; mass spectrometry; morazone; famprofazone; phenmetrazine; methamphetamine; pyrazolone derivatives.*

Introduction

Morazone (2,3-dimethyl-4-(3-methyl-2-phenyl-morpholinomethyl)-1-phenyl-3-pyrazolin-5-one)(Fig. 1) and famprofazone (4-isopropyl-2-methyl-3-[*N*-methyl-*N*-(α-methylphenylethyl)-aminomethyl]-1-phenyl-3-pyrazolin-5-one) (Fig. 3) are moderately strong analgesics which are available in a number of compound preparations (morazone in Rosimon-Neu®, FRG and Delimon®, UK; famprofazone in Gewodin®, FRG).

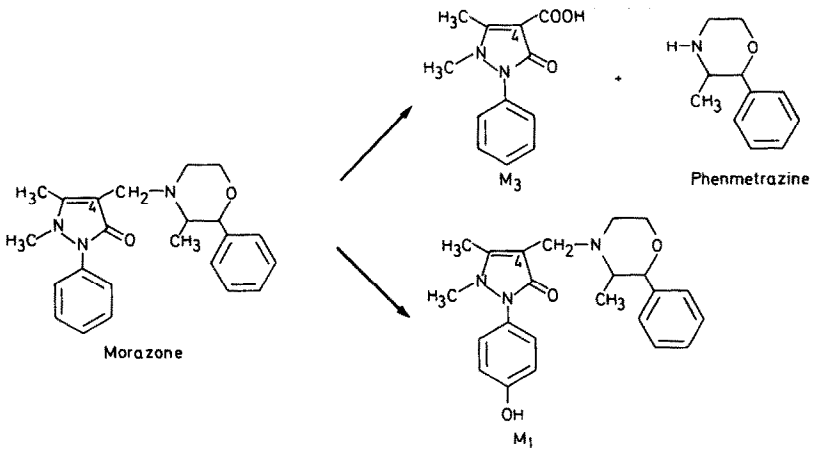


Figure 1
Urinary metabolites of morazone in man. M_1, *p*-hydroxymorazone; M_3, phenazone-4-carboxylic acid.

Morazone and other C_4-phenylethylamine-substituted pyrazolones are hydrolysed *in vitro* by dilute hydrochloric acid to the free phenylethylamines and 4-hydroxymethylpyrazolones via a retro-Mannich reaction [1, 2]. The stability depends on the basicity of the amine substituent [2]. In contrast, C_3-phenylethylamine-substituted pyrazolones, such as famprofazone, are very stable in dilute hydrochloric acid [2].

Phenmetrazine has been found as a human urinary metabolite of morazone [3], which may explain the non-medical use of morazone [4]. Some of the drug was excreted unchanged [3], but there is no information about the excretion of the corresponding pyrazolone part of the molecule.

For famprofazone it has been assumed that the intact molecule is active in man and that no methamphetamine is released in the body [5]. However, no investigations on metabolism in man have been published. In mice, methamphetamine and 3-hydroxymethyl-2-methyl-4-isopropyl-1-phenyl-3-pyrazolin-5-one (3-hydroxymethyl-propyphenazone) have been identified as urinary metabolites [6].

The general aims of the present work were to compare the human metabolism of morazone and famprofazone, and to relate the results to the stability of these drugs *in vitro*. Specific objectives were to identify the metabolites of the pyrazolone moieties of morazone and famprofazone, and to look for free psychostimulating amines in urine after administration of famprofazone.

Experimental

Gas–liquid chromatography (GLC)

GLC was performed on a Packard model 428 gas chromatograph equipped with a thermo-ionic nitrogen–phosphorus selective detector (for morazone) or a flame-ionization detector (for famprofazone). The 2 m × 2 mm i.d. glass column was packed with 3.8% SE30 on Chromosorb W AW-DMCS 80–100 mesh. The carrier gas (nitrogen) flow rate was 25 ml/min. For morazone, the detector temperature was 280°C and the detector ionizing current was 430 mA; the injection port temperature was 250°C and the column oven temperature was programmed from 100°C (1 min) to 270°C (5 min) at a rate of 10°C/min. For famprofazone, the flame-ionization detector temperature was 290°C; the injection port temperature was 270°C and the column oven temperature was programmed from 60°C (1 min) to 270°C (13 min) at a rate of 10°C/min.

Gas chromatography–mass spectrometry (GC–MS)

For morazone, GC–MS was performed on a Finnigan/MAT 1020 instrument. The 30 m × 0.25 mm i.d. fused silica column was coated with DB5 (J & W Scientific Inc., Rancho Cordova, USA). The carrier gas (helium, 13 psi) flow rate was 3 ml/min; the injection split was 1:20. The column oven temperature was programmed from 100°C (2 min) to 300°C (5 min) at a rate of 15°C/min. The ion source temperature for MS was 180°C. The electron energy was 70 eV and the multiplier voltage was 1.8 kV.

For famprofazone, GC–MS was performed on a 3700 (Varian)/44S (Varian/MAT) instrument fitted with Spectrosystem 188 (Varian/MAT). The carrier gas (helium, 14 psi) flow rate was 3 ml/min; the injection split was 1:10. The column oven temperature was programmed from 60°C (1 min) to 270°C (10 min) at a rate of 10°C/min. The ion source temperature for MS was 230°C. The electron energy was 80 eV and the multiplier voltage was 1.7 kV.

Nuclear magnetic resonance spectrometry (NMR)

^{1}H NMR spectra were obtained on a Brüker–Physik WH-90 spectrometer. The compounds were dissolved in $CdCl_3$.

Mass spectrometry

Mass spectra were obtained on a MS50 mass spectrometer combined with a Kratos Data-System 50. The inlet temperature was 80°C and the ionization energy was 70 eV.

Thin-layer chromatography (TLC)

TLC silica gel F_{254} plates (E. Merck, Darmstadt, FRG), thickness 0.25 mm, were used. Solvent systems were: (I) methylethylketone–ethanol–diethylamine (7:3:1, v/v/v); (II) chloroform–ethanol (9:1, v/v); (III) methanol–strong ammonia solution (99:1, v/v); and (IV) ethyl acetate. The spray solution was Dragendorff's reagent [7].

Column chromatography

For column chromatography, a Lobar® column filled with Si 60, size B (E. Merck, Darmstadt, FRG) was used. The solvent system used was (I).

Materials

Famprofazone (Ed. Geistlich Chemie, Wolhusen, CH); methamphetamine (Knoll AG, Ludwigshafen, FRG); phenmetrazine (Ravensberg GmbH, Konstanz, FRG). Morazone [8], phenazone-4-carboxylic-acid-methylester [9, 10], *p*-hydroxy-morazone and 3-hydroxymethyl-propyphenazone [2] were synthesized in this laboratory according to cited literature.

Phenazone-4-carboxylic-acid-methylester [9, 10]. MS data: *m/z* (%): 246 (100), 216 (13), 215 (75), 214 (23), 199 (19), 188 (44), 154 (17), 121 (26), 77 (36).

2,3-*Dimethyl*-4-(3-*methyl*-2-*phenyl-morpholinomethyl*)-1-(p-*hydroxy-phenyl*)-3-*pyrazolin*-5-*one* (p-*hydroxymorazone*). 824 mg (4 mmol) *p*-hydroxy-phenazone (synthesized from *p*-ethoxy-phenylhydrazine [11] according to [12]) was dissolved in 15 ml of methanol and stirred for 24 h at 40°C with 1.6 ml (4 mmol) of formaldehyde solution 10%, 710 mg (4 mmol) of phenmetrazine and 4.5 ml of 1.0 M hydrochloric acid. The solution was acidified with hydrochloric acid to pH 1 and extracted with ether. The aqueous solution was made alkaline with ammonia and extracted with chloroform–ethanol (9:1, v/v). The organic layer was dried over anhydrous sodium sulphate, evaporated and, after addition of some ether, the oily residue crystallized.

Yield: 0.52 g (33%); Mp: 208–209°C; ^{1}H-NMR: cf. Table 1; MS data: *m/z* (%): 337 (2), 306 (3), 258 (9), 257 (32), 218 (35), 217 (100), 177 (11), 176 (37), 71 (72).

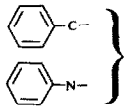
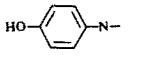
Molecular formula $C_{23}H_{27}N_3O_3$ (mol wt = 393.49): elemental analysis data found (calculated values in parentheses) — C, 70.17 (70.20); H, 7.17 (6.92); N, 10.50 (10.68).

3-*Hydroxymethyl*-4-*isopropyl*-2-*methyl*-1-*phenyl*-3-*pyrazolin*-5-*one* (3-*hydroxymethyl-propyphenazone*) [2]. MS data: *m/z* (%): 246 (75), 232 (17), 231 (100), 215 (6), 154 (11), 126 (7), 93 (9), 82 (7), 77 (22), 66 (8), 42 (14).

Drug administration to volunteers

Urine was collected from five healthy male and female volunteers, aged from 25 to 50

Table 1.
^{1}H-NMR chemical shift and multiplicity of signals of morazone, metabolite M_1 and synthesized *p*-hydroxy-morazone

Structure	Morazone (δ, ppm)	M_1 and *p*-hydroxymorazone (δ, ppm)
–OH	—	9.58 (s, broad)
	7.49–7.29 (m)	7.40–7.27 (m*) —
	—	6.90, 621 (AA'–BB')
CH and CH_2 of morpholine, N–CH_2	4.26–2.52 (m)	4.20–2.41 (m)
N–CH_3	3.07 (s†)	3.10 (s)
C–CH_3 (pyrazolone)	2.30 (s)	2.33 (s)
C–CH_3 (morpholine)	1.02 (d‡)	1.06 (d)

* m = multiplet.
† s = singlet.
‡ d = doublet.

years who had not taken any drugs for at least 1 week. The volunteers were advised to avoid nicotine, canned food and drinks containing caffeine. Control urine was collected over 24 hours. The next day the volunteers had a normal breakfast and the drugs were given orally, dissolved or suspended in water. The quantities of drugs administered (morazone HCl: 100 mg, famprofazone: 37 mg) were determined by the content and dosage of the commercial dosage forms, i.e. 1 tablet of Rosimon–Neu® or 1.5 tablets of Gewodin®. Urine was collected over 24 h and stored at −20°C until extraction.

Enzymatic hydrolysis

The following quantities are given for 1 l of urine; urine was adjusted to pH 5.5 with 100 ml of acetate-buffer (2 mol/l). Samples were mixed with 2.5 ml β-glucuronidase (12 units/ml)/arylsulphatase (60 units/ml) (E. Merck, Darmstadt) and incubated for 20 h at 40°C.

Metabolism of morazone

Urine samples were hydrolysed enzymatically and extracted with methylene chloride, first at pH 9, followed by extraction with methylene chloride at pH 2. The organic layers were dried over anhydrous sodium sulphate and concentrated. Control urine was treated in the same way.

Extracts of alkaline urine

TLC of the extracts of alkaline urine (solvent I) showed phenmetrazine (R_f = 0.61) in addition to unchanged morazone (R_f = 0.81) and two further spots with a positive reaction to Dragendorff's reagent (M_1, R_f = 0.30; M_2, R_f = 0.15). M_1 and M_2 from the urine extract of one volunteer were isolated by column chromatography (solvent I) to yield 4 mg of M_1 and 2.5 mg of M_2.

Extracts from acidic urine

The extracts of acidic urine were dissolved in methanol and treated with diazomethane in diethyl ether.

TLC (solvent II, R_f = 0.46) and GLC (nitrogen–phosphorus detector, retention time = 17.3 min) of all samples revealed a metabolite (M_3) which was not present in the control urine.

Metabolism of famprofazone

Urine samples were adjusted to pH 9 and extracted with methylene chloride, followed by enzymatic hydrolysis and a further extraction with methylene chloride at pH 9. The organic layers were dried over sodium sulphate and concentrated after addition of 3 ml of diethyl ether saturated with hydrochloric acid. The residue was shaken with 5 ml of 0.5 M sodium hydroxide. The organic layer was separated and dried again. Control urine was treated in the same way.

Extracts from alkaline urine before hydrolysis

TLC (solvent III, R_f = 0.27, Dragendorff positive) and GLC (retention time = 7.1 min) of all samples revealed a metabolite (F_1), which was not present in control urine.

Extracts from alkaline urine after hydrolysis

TLC (solvent IV, R_f = 0.54) and GLC (retention time = 18.1 min) of all samples revealed a metabolite (F_2), which was not present in control urine.

Results

After administration of morazone, phenmetrazine and two other basic metabolites (M_1 and M_2) were found in human urine (Fig. 1) together with an acidic metabolite (M_3), in addition to unchanged morazone.

In the ^{1}H-NMR spectrum (Table 1), M_1 displayed one monosubstituted phenyl and another *p*-disubstituted aromatic ring (AA′–BB′ system, 6.90 and 6.21 ppm, J = 8 Hz). In addition to the unchanged signals of morazone, there was a broad OH-proton singlet, which was exchangeable with D_2O. This evidence suggested that M_1 was *p*-hydroxylated morazone. Using high-resolution mass spectrometry, it was deduced that the OH-group was in the *p*-position on the phenyl ring, which is located at N_1 of the pyrazolone ring (Fig. 2). M_1 was found to be identical in its chromatographic and spectroscopic properties with synthesized *p*-hydroxy-morazone. Metabolite M_2 could not be further characterized because of decomposition during column chromatography.

The acidic metabolite (M_3) was identified as phenazone-4-carboxylic acid by GC–MS after methylation with diazomethane; M_3 was confirmed in all urine samples by co-chromatography with synthesized phenazone-4-carboxylic acid-methylester. The mass spectra of M_3 and the reference compound were identical. Neither 4-hydroxymethyl-phenazone nor 4-formyl-phenazone were detected. M_1, M_2 and M_3 were found in larger amounts after enzymatic hydrolysis.

Famprofazone was metabolized to two metabolites, F_1 and F_2 (Fig. 3). Both metabolites were extracted from alkaline urine. F_1 was identified as methamphetamine from unhydrolysed urine by GC–MS and TLC, and was confirmed in all samples by co-chromatography with the reference compound. The mass spectra of F_1 and methamphetamine were identical. The pyrazolone part of the molecule was excreted as 3-

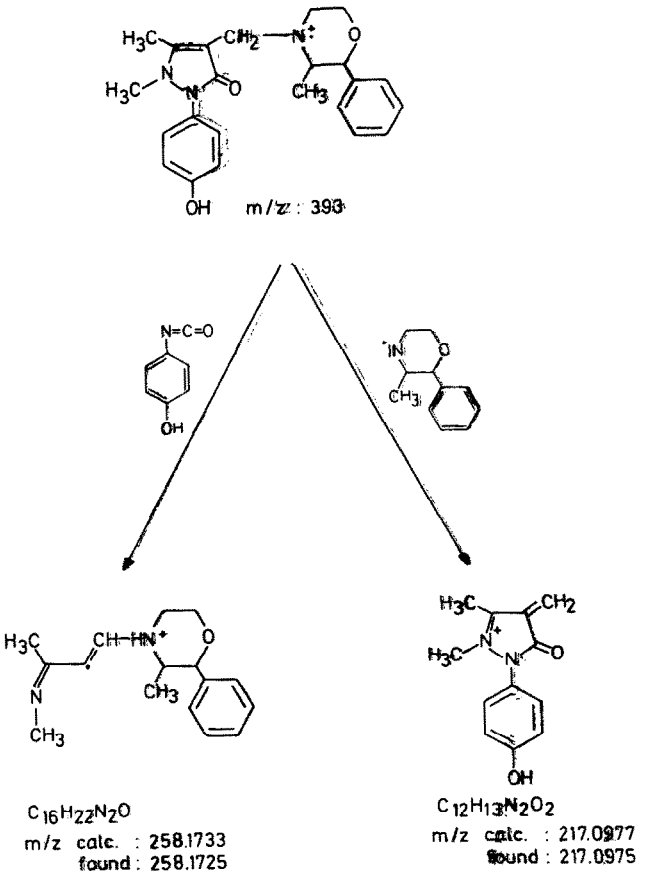


Figure 2
Part of the mass-spectrometric fragmentation of metabolite M_1 (*p*-hydroxy-morazone). $C_{16}H_{22}N_2O$: *m/z* (calculated) = 258.1733; *m/z* (found) = 258.1725. $C_{12}H_{13}N_2O_2$: *m/z* (calculated) = 217.0977; *m/z* (found) = 217.0975.

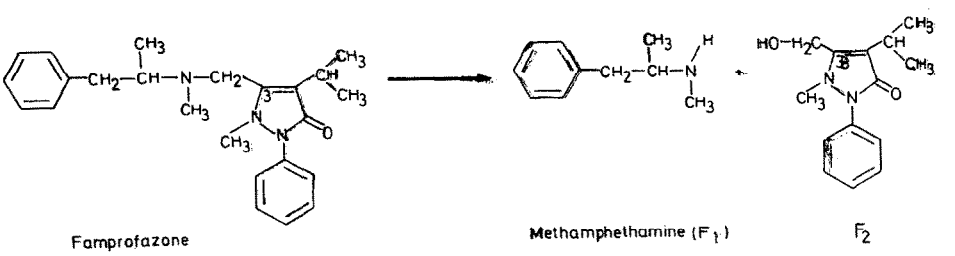


Figure 3
Urinary metabolites of famprofazone in man. F_2 = 3-Hydroxymethylpropyphenazone.

hydroxymethyl-propyphenazone (F_2). This metabolite was identified by GC–MS and confirmed by TLC and GLC in the urine of all volunteers by co-chromatography with the authentic substance [2]. F_2 was completely conjugated and could be detected only after hydrolysis of the urine samples. 3-Formyl-propyphenazone, unchanged famprofazone, hydroxylated famprofazone, and 3-carboxyl-propyphenazone were not detected.

Discussion

In this study of the human metabolism of the analgesic drugs, morazone and famprofazone, it was found that cleavage of the molecules led to the formation of free phenmetrazine [3] and methamphetamine, respectively, together with the corresponding pyrazolone derivatives. It is remarkable that morazone is partly excreted as the intact molecule together with the hydroxylated and other metabolites, since morazone is unstable in dilute hydrochloric acid [2]. Aromatic *p*-hydroxylation of pyrazolones has recently been observed also for propyphenazone [13, 14] and phenazone (antipyrine) [12, 15].

In contrast, after administration of famprofazone, no intact or hydroxylated famprofazone could be found although the drug is very stable in dilute hydrochloric acid [2]. All the famprofazone metabolites resulted from cleavage of a C–N bond in the molecule. The pyrazolone moieties of morazone and famprofazone were excreted at different stages of oxidation; morazone was excreted as pyrazolone-4-carboxylic acid and famprofazone as 3-hydroxymethyl-pyrazolone. The formation of pyrazolone-4-carboxylic acid can be explained by the well-known reaction, oxidative amine desalkylation. Pyrazolone-carboxylic acids have also been found in the metabolism of phenazone (phenazone-3-carboxylic acid [16]) and propyphenazone (oxidation of the isopropyl-group at C_4 [13, 14]).

In the case of famprofazone, the pyrazolone part of the molecule was excreted as 3-hydroxymethyl-pyrazolone, which is known to be formed in the biotransformation of phenazone [17]. This metabolite could be formed by hydrolysis of the amine, a pathway which is seldom observed. The more common α-hydroxylation pathway [18] would result in the formation of the pyrazolone-3-aldehyde which was not detected, probably due to its immediate reduction to the alcohol. It is possible that nucleophilic displacement of an N-oxidized species occurs, as suggested by Hawkins and Zacchei *et al.* [19, 20].

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References

[1] G. Bohn, G. Rücker and H. Kröger, *Arch. Toxicol.* **35**, 213–220 (1976).
[2] G. Rücker, R. Mrongovius and M. Neugebauer, *Arch. Pharm. (Weinheim)* **315**, 839–846 (1982).
[3] G. P. Cartoni, A. Cavalli, A. Giarusso and F. Rosati, *J. Chromatogr.* **84**, 419–422 (1973).
[4] E. Klug, *Z. Rechtsmed.* **71**, 27–36 (1972).
[5] S. Ebel, *Synthetische Arzneimittel*, p. 55. Verlag Chemie, Weinheim (1979).
[6] R. Mrongovius, M. Neugebauer and G. Rücker, *Eur. J. Med. Chem.* **13**, 161–166 (1984).
[7] *Anfärbereagenzien für Dünnschicht- und Papier-chromatographie*, p. 39. Merck, Darmstadt (1970).
[8] Ravensberg G.m.b.H., *Chemische Fabrik* (H. Siemer and A. Doppstadt), Brit. 830577 (16.3.1960); through *Chem. Abstr.* **54**, 18561 (1960).

[9] H. P. Kaufmann and L. S. Huang, *Ber. Dtsch. Chem. Ges.* **75**, 1214-1236 (1942).
[10] K. Bodendorf, J. Mildner and T. Lehmann, *Liebigs Ann. Chem.* **563**, 1–11 (1949).
[11] J. Altschul. *Ber. Dtsch. Chem. Ges.* **25**, 1842–1854 (1892).
[12] J. Böttcher, H. Bässmann and R. Schüppel, *J. Pharm. Pharmacol.* **34**, 168–175 (1982).
[13] W. Ehrenthal, Dissertation, Universität des Saarlandes (1979).
[14] M. Tateishi and H. Shimizu, *Xenobiotica* **6**, 207–215 (1976).
[15] T. Inaba, H. Uchino and W. Kalow, *Res. Comm. Chem. Pathol. Pharmacol.* **33**, 3–8 (1981).
[16] H. Yoshimura, H. Shimeno and H. Tsukamoto, *Chem. Pharm. Bull.* **19**, 41–45 (1971).
[17] H. Yoshimura, H. Shimeno and H. Tsukamoto, *Biochem. Pharmacol.* **17**, 1511–1516 (1968).
[18] B. Testa and P. Jenner, in *Drug Metabolism*, pp. 82–83. Marcel Dekker, New York (1976).
[19] D. R. Hawkins, *Progress in Drug Metabolism*, Part 6 (J. W. Bridges and L. F. Chasseaud, Eds), pp. 117–118. John Wiley, London (1981).
[20] A. G. Zacchei, L. L. Weidner, G. H. Bendaar and E. B. Reftery, *Drug Metab. Dispos.* **4**, 387–401 (1976).

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