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METABOLIC FATE OF FURAZOLIDONE IN RATS

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The metabolic fate of the widely used veterinary drug, furazolidone (N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone) was examined in rats. When ^{14}C-furazolidone was administered orally to rats, most of the radioactivity was recovered from urine and feces during the first 24 hr, indicating rapid clearance of the nitrofuran from the animal bodies. The radioactivity in feces appears to be partly due to its biliary excretion and partly due to its exsorption. Two metabolites of the nitrofuran were isolated from the urine and unequivocally identified as 3-(4-cyano-2-oxobutylideneamino)-2-oxazolidone and N-(5-acetamido-2-furfurylidene)-3-amino-2-oxazolidone by comparison with authentic specimens.

KEYWORDS —— nitrofuran derivative; furazolidone (N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone); rats; metabolic fate; excretion into urine, feces and bile; urinary metabolites; 3-(4-cyano-2-oxobutylideneamino)-2-oxazolidone; N-(5-acetamido-2-furfurylidene)-3-amino-2-oxazolidone.

Furazolidone (N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone) is an antimicrobial feed additive as well as a growth promoter for swine, poultry and other animals, which is widely used in many country. In Japan, the nitrofuran has been also used as a feed additive since 1961. But, the nitrofuran has been found to be mutagenic in *Escherichia coli* WP_2,[1] *Salmonella typhimurium* TA100[2] and Drosophila.[3] Recently, controversy regarding its carcinogenicity has arisen especially in the United States. Under these conditions information about the behavior and metabolism of the nitrofuran in animal bodies is urgently needed to evaluate its safety. We wish to describe here the metabolic fate of furazolidone in rats.

^{14}C-Furazolidone was administered orally to rats in a single dose of 10 mg/kg. As shown in Table I, the major excretory pathways were by way of both urine and feces, and most of the radioactivity given was recovered during the first 24 hr. The ^{14}C-nitrofuran was similarly administered to rats with biliary fistulae. As shown in Table II, the radioactivity was partly excreted in bile as well as urine. The radioactivity in feces and gastrointestinal contents seems to be partly due to its exsorption, for about 5% of radioactivity appeared in these samples following intraperitoneal dosage of the ^{14}C-nitrofuran (1 mg/kg) into the rats with biliary fistulae. Recovery of unchanged furazolidone from the 48 hr samples were only 0.05% in urine, 0.50% in feces and 0.05% in bile, respectively, indicating that the nitrofuran undergoes almost complete metabolism in rats.

TABLE I. Excretion of Radioactivity into Urine and Feces following Oral Administration of ^{14}C-Furazolidone in Rats

	Sampling time (day)				
	1	2	3	4-7	Total
	% of dose				
Urine	47.7	1.7	0.6	1.1	51.1
Feces	38.6	5.0	0.5	0.6	44.7

Each value represents mean of four rats.

TABLE II. Excretion of Radioactivity into Bile, Urine and Feces following Oral Administration of ^{14}C-Furazolidone in Rats with Biliary Fistulae

	Sampling time (day)		
	1	2	Total
	% of dose		
Bile	13.3	1.5	14.8
Urine	55.3	4.4	59.7
Feces[a)]	—	19.0	19.0

Each value represents mean of four rats.

a) The 48 hr feces was combined with gastrointestinal contents at 48 hr after medication prior to measurement of radioactivity.

In the present study, two metabolites of furazolidone were isolated from rat urine. Metabolite 1 showed by mass spectrometry a molecular ion at m/z 195, accompanying fragment ions at m/z 167, 141 and 113 (the base peak). The UV spectrum had an absorption maximum at 266 nm in ethanol, and the IR spectrum had absorption bands at 2260 (νCN), 1770 (νoxazolidone, C=O) and 1680 (να,β-unsaturated ketone) cm^{-1}. These mass, UV and IR spectra, and the Rf value in thin-layer chromatography (TLC) were identical with those of an authentic specimen of 3-(4-cyano-2-oxobutylideneamino)-2-oxazolidone, which had been found to be a product of furazolidone in other biological system.[2,4)] As shown in Fig.1, the mass spectrum of metabolite 2 showed a molecular ion at m/z 237, suggesting that it is the monoacetyl derivative of the corresponding aminofuran. In fact, the fragment ion (the base peak) at m/z 195 resulted from loss of the acetyl group to form the aminofuran. The UV absorption maximum was observed at 323 nm in ethanol and shifted to 371 nm in alkaline ethanol. These mass and UV spectra, and the Rf value in TLC were all identical with those of an authentic specimen of N-(5-acetamido-2-furfurylidene)-3-amino-2-oxazolidone. This is the first example of acetaminofuran formation in furazolidone metabolism. Thus, the two urinary metabolites of furazolidone (Fig.2) were unequivocally identified by comparison with authentic specimens.

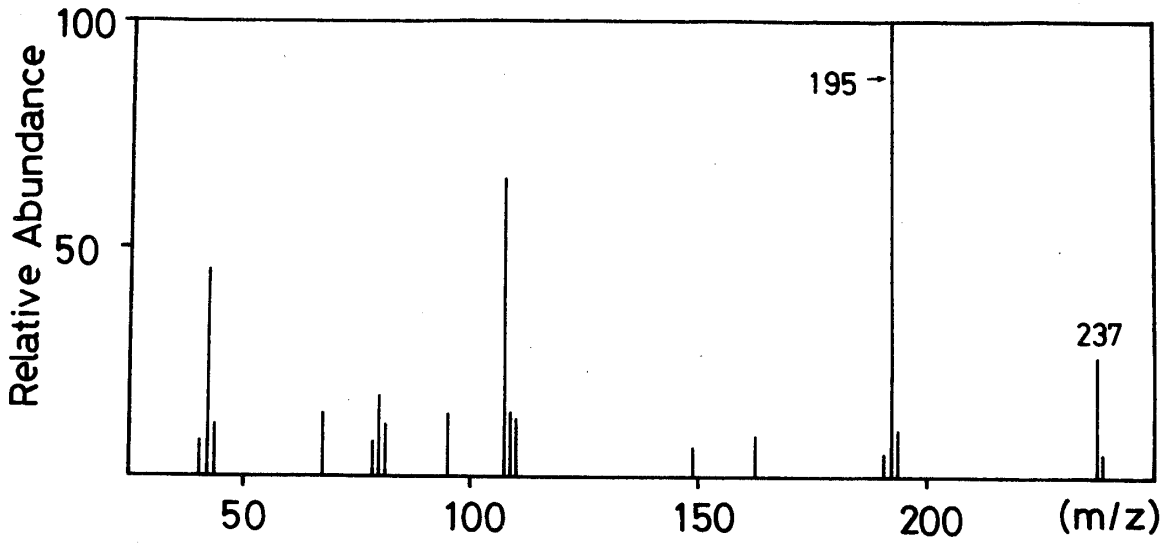

Fig. 1. Mass Spectrum of Metabolite 2 (N-(5-Acetamido-2-Furfurylidene)-3-Amino-2-Oxazolidone)

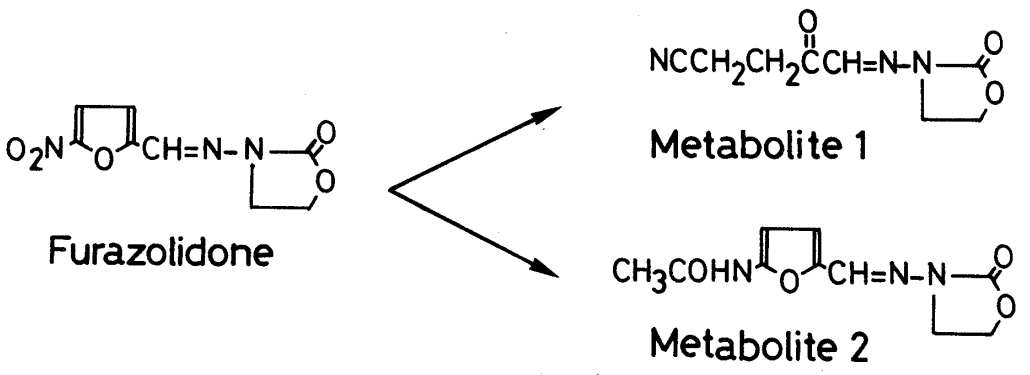

Fig. 2. Urinary Metabolites of Furazolidone in Rats

Furthermore, when the aminofuran (N-(5-amino-2-furfurylidene)-3-amino-2-oxazolidone), a suggested intermediate in furazolidone metabolism, was administered orally to rats, the cyano derivative and the acetaminofuran described above were again isolated and identified as urinary metabolites of the aminofuran. This indicates that the aminofuran is an intermediate in the metabolism of furazolidone to metabolites 1 and 2.

Preliminary study showed that these two metabolites accounted for almost all of the lipophilic metabolites of furazolidone in urine. However, about 90% of radioactivity excreted into urine still remained unidentified water-soluble metabolites. These polar metabolites are now under investigation in our laboratory.

EXPERIMENTAL

Furazolidone (mp 275°C (dec.)) and its radioactive compound (formyl-^{14}C, 1.03 mCi/mmol) were gifts from Ueno Fine Chemical Industries,Ltd. N-(5-Amino-2-furfury-

lidene)-3-amino-2-oxazolidone (mp 191-192°C) and N-(5-acetamido-2-furfurylidene)-3-amino-2-oxazolidone (mp 231-232°C) were prepared by the method of Ebetino et al.[5] 3-(4-Cyano-2-oxobutylideneamino)-2-oxazolidone (mp 179-180°C) was prepared as described previously.[2]

Male Wistar-strain rats weighing 250-300 g were used in this study. Animals were not fed overnight prior to use. Rats with biliary fistulae were prepared by the method of Abou-El-Makarem et al.[6]

For isolation of the urinary metabolites of furazolidone, the nitrofuran was given orally to 6 animals in doses of 100 mg/kg/day for 4 days. The collected urine was passed through an Amberlite XAD-4 column (Rohm & Haas Co., 3x60 cm), and the column was washed with 1 liter of water and then eluted with 500 ml of MeOH. The eluate was evaporated to dryness in vacuo and the residue was dissolved in an appropriate volume of water. The aqueous solution was extracted three times with an equal volume of ethyl acetate. The extract, after removal of the solvent, was redissolved in a small volume of $CHCl_3$, placed on a column of silica gel (40 g, Kiesel gel 60, Merck) and eluted stepwise with 300 ml each of $CHCl_3$, $CHCl_3$-acetone (9:1) and $CHCl_3$-acetone (1:1). Each fraction (50 ml) was examined by TLC (0.25 mm thick silica gel GF, Analtech) developed in the solvent system benzene-acetone-MeOH (7:2:1). Spots were visualized under UV light. Metabolite 1 (Rf 0.39) was obtained from the $CHCl_3$-acetone (9:1) eluate, while metabolite 2 (Rf 0.25) from the $CHCl_3$-acetone (1:1) as a mixture with metabolite 1. Metabolite 1 was purified by TLC using the above solvent system to give a colorless solid material. The mixture of metabolites 1 and 2 was separated by rechromatography on a silica gel column (10 g) eluted stepwise with 100 ml each of $CHCl_3$, $CHCl_3$-acetone (9:1) and $CHCl_3$-acetone (1:1). Metabolite 2, which was obtained from the $CHCl_3$-acetone (1:1) eluate, was also purified by TLC to give a light-brown solid material.

Radioactivity was determined on a Packard liquid scintillation spectrometer (Model 3255) with automatic external standardization. Feces were treated on a Packard automatic sample combustion system (Model 306). The unchanged furazolidone was determined by reversed dilution analysis. UV spectra were recorded with a Hitachi 320 spectrophotometer. Electron impact mass spectra were obtained with a Shimadzu 7000 mass spectrometer. IR spectra were taken with a Shimadzu 402 IR spectrophotometer.

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