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Metabolism of Furazolidone in Eels

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The metabolism of an antibacterial nitrofuran, furazolidone (*N*-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone), was investigated in eels. When eels were bathed in a solution of the nitrofuran, ethyl acetate-extractable metabolites and polar, water-soluble metabolite(s) were formed from the nitrofuran. One of the former metabolites was isolated from the bathing solution and unequivocally identified as 3-(4-cyano-2-oxobutylideneamino)-2-oxazolidone by comparison with an authentic sample. This metabolite was also formed from the nitrofuran by an eel liver homogenate-catalyzed reaction.

Keywords——nitrofuran derivative; furazolidone (*N*-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone); eel; metabolism; metabolite; 3-(4-cyano-2-oxobutylideneamino)-2-oxazolidone

Certain antibacterial nitrofurans are used in clinical and veterinary medicine in many countries. One of them, furazolidone (*N*-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone) is commonly added to feed for swine and poultry to prevent intestinal disease. In Japan, this nitrofuran is also used in fish breeding (yellowtails, sea breams, eels, carps, trouts and so on) to stimulate growth or to prevent infection.

On the other hand, many nitrofurans are mutagenic[1,2] and some are carcinogenic.[3,4] Furazolidone was also found to be mutagenic in *Escherichia coli* WP2,[1] *Salmonella typhimurium* TA100[5] and *Drosophila*.[6]

Recently, the metabolic fate of furazolidone was examined in rats of furazolidone. When ^{14}C-furazolidone was administered orally to rats, most of the radioactivity was recovered from urine and feces during the first 24 h. Two metabolites of the nitrofuran were isolated from the urine and identified as 3-(4-cyano-2-oxobutylideneamino)-2-oxazolidone and *N*-(5-acetamido-2-furfurylidene)-3-amino-2-oxazolidone.[7] However, there is virtually no information available concerning the metabolic fate of the nitrofuran in fishes. The present communication describes the *in vivo* and *in vitro* metabolism of furazolidone in eels.

Experimental

Materials——Furazolidone (mp 257 °C (dec.)) (I) and ^{14}C-labeled furazolidone (formyl-^{14}C, 1.03 mCi/mmol) (^{14}C-I), which was radiochemically pure, were kindly donated by Ueno Fine Chemical Industries, Ltd. 3-(4-Cyano-2-oxobutylideneamino)-2-oxazolidone (mp 179—180 °C) (II) was prepared by hydrogenation of I in the presence of Raney nickel (Wako Pure Chemical Industries, Ltd.) as described previously.[5] *N*-(5-Amino-2-furfurylidene)-3-amino-2-oxazolidone (mp 191—192 °C) (III) was prepared by hydrogenation of I in the presence of 5% palladium on charcoal (Wako Pure Chemical Industries, Ltd.) as described previously.[8] *N*-(5-Acetamido-2-furfurylidene)-3-amino-2-oxazolidone (IV) was prepared by acetylation of III according to the method of Ebetino *et al.*[9]

Analytical Procedures——Electron impact mass spectra (MS) were recorded with a Shimadzu 7000 mass spectrometer. Ultraviolet (UV) spectra were obtained with a Hitachi 320 spectrophotometer. Radioactivity was determined on a Packard liquid scintillation spectrometer (model 3255) with automatic external standardization.

Thin-Layer Chromatography (TLC)——TLC was conducted on silica gel plates (Kieselgel 60 GF_{254}, Merck;

0.25 mm thick) using the following solvent systems: (a) benzene–MeOH (9 : 1), (b) dioxane–benzene (1 : 1) and (c) ether–acetone (3 : 2). Chromatograms were visualized under UV light (2536 Å). The distribution of radioactivity on plates was examined by counting the scraped-off silica gel with a liquid scintillation spectrometer.

Silica Gel Column Chromatography——The residue was taken up in a small volume of $CHCl_3$ and placed on a column of silica gel (Kieselgel 60, 70—230 mesh, Merck; 20 g). The column was eluted stepwise with 400 ml each of $CHCl_3$, $CHCl_3$–acetone (9 : 1), $CHCl_3$–acetone (1 : 1) and acetone. Each fraction (50 ml) was examined by TLC developed with solvent system a.

***In Vivo* Experiment**——Eels weighing about 170 g were used in this study. For TLC examination of metabolites, two eels were bathed for 24 h at room temperature in 2 l of a 0.5 ppm solution of ^{14}C-I. For isolation and identification of a metabolite, four eels were also bathed for 4 d in 3 l of a 15 ppm solution of nonradioactive I. Bathing was carried out in the dark. The drug solution was extracted three times with an equal volume of ethyl acetate. The combined ethyl acetate extract was dried over anhydrous Na_2SO_4 and evaporated to dryness *in vacuo*. The radioactive residue was subjected to TLC and the nonradioactive residue to silica gel column chromatography as described above. The radioactive aqueous solution after ethyl acetate extraction was evaporated to dryness *in vacuo* and the residue was also subjected to TLC.

***In Vitro* Experiment**——Eel livers were homogenized in two volumes of 1.15% KCl for 3 periods of 30 s each with a Polytron (PT10ST "OD-S", Kinematica) and then for 3 min with a Teflon-glass homogenizer. The incubation mixture consisted of 20 μmol of I and the liver homogenate (equivalent to 70 g of liver) in a final volume of 340 ml of 1/30 M phosphate buffer (pH 7.4) containing dimethyl sulfoxide at a concentration of 3%. The incubation was carried out for 1 h at 37 °C in an open vessel in the dark. After incubation, the mixture was extracted three times with an equal volume of ethyl acetate. The combined ethyl acetate extract was dried over anhydrous Na_2SO_4 and evaporated to dryness *in vacuo*. The residue was subjected to silica gel column chromatography as described above. A metabolite of I, which was obtained from the $CHCl_3$–acetone (9 : 1) eluate, was further purified by preparative TLC using the solvent systems a and c.

Results and Discussion

In the present study, the *in vivo* metabolism of I in eels was investigated by a bathing method. Eels were bathed in a solution of ^{14}C-I, then the drug solution was extracted with ethyl acetate, and the ethyl acetate extract and the remaining aqueous solution were evaporated to dryness for TLC examination as described in Experimental. When the residue from the ethyl acetate extract was developed in solvent system a, two radioactive peaks due to metabolites 1 and 2 were observed at *Rf* 0.5 and 0.2, together with that due to unreacted I at *Rf* 0.75. When the residue from the remaining aqueous solution was developed in the same solvent system, a single radioactive peak was located at the origin, indicating the formation of polar, water-soluble metabolite(s) from I in eels. The relative ratio of I and its metabolites after bathing of the eels in the solution is shown in Table I.

Our previous study[8] demonstrated that metabolic conversion of I to the open-chain cyano derivative (II), the aminofuran (III) and the dimer-type derivative in a xanthine oxidase-hypoxanthine system. In addition, we recently showed that when I was given orally to rats, the acetamidofuran (IV) was isolated from the urine, together with II.[7] Among the above metabolites of I in eels, metabolite 1 had the same *Rf* value (0.5) as an authentic sample of II in TLC (solvent system a). However, no metabolites corresponding to III (*Rf* 0.3) and IV (*Rf* 0.25) could be detected by TLC examination.

TABLE I. Relative Ratio of Furazolidone and Its Metabolites in the Bathing Solution of Nitrofuran

	Relative ratio (%)
Furazolidone	53.0
Metabolite 1	12.9
Metabolite 2	2.1
Water-soluble metabolite(s)	32.0

Each value represents the mean of four experiments.

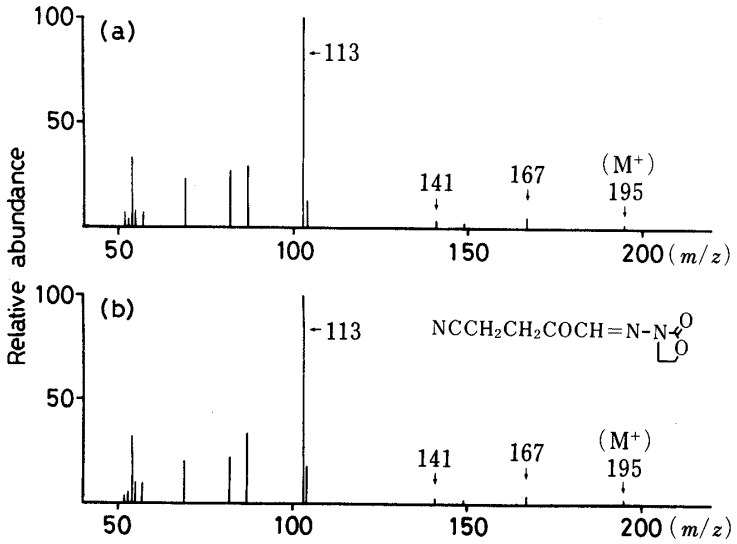

Fig. 1. Mass Spectra of Metabolite 1 (a) and 3-(4-Cyano-2-oxobutylideneamino)-2-oxazolidone (b)

Isolation and identification of metabolite 1, which is the main ethyl acetate-extractable metabolite of I, was carried out on a large scale with bathing solution containing a high concentration of I. The ethyl acetate extract was obtained and chromatographed on a silica gel column as described in Experimental. The metabolite was obtained as a solid material from the chloroform–acetone (9 : 1) eluate and recrystallized from ethyl acetate to give plates, mp 179—180 °C. The MS of the metabolite (Fig. 1) gave a molecular ion at *m/z* 195, with a base peak at *m/z* 113, and the UV spectrum had an absorption maximum at 267 nm. The mass and UV spectra, and the TLC behavior of the metabolite were identical with those of an authentic sample of II. Furthermore, no depression of melting point occurred on admixture of the two compounds. Thus, an *in vivo* metabolite of I in eels was unequivocally identified as the open-chain cyano derivative (II).

Next, the *in vitro* metabolism of I was investigated by using eel liver homogenate. When the ethyl acetate extract from the incubation mixture was chromatographed on a silica gel column, a metabolite of I was obtained from the chloroform–acetone (9 : 1) eluate. The metabolite had *Rf* values of 0.5, 0.42 and 0.62 in TLC developed with solvent systems a, b and c. Its UV spectrum had an absorption maximum at 267 nm. The TLC behavior and the UV spectrum of the metabolite were again identical with those of an authentic sample of II. Thus, the metabolism of I to II in eels was demonstrated *in vitro* in addition to *in vivo*.

When the nitro group of I undergoes chemical or enzymatic reduction, II is formed as one of the reduction products.[5,8] Therefore, nitroreductases seem to play an important role in the metabolism of I in eels, as well as in mammalia. However, our preliminary study showed that the I-reducing activity in eel liver is not inhibited by oxygen, unlike that in rat liver. Eel liver nitroreductases are now under investigation.

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