[Chem. Pharm. Bull.
32(5)2036—2039(1984)]

Mutagenic Activity, Antibacterial Activity and Enzymatic Reducibility of Geometrical Isomers of Nitrofurans in *Salmonella typhimurium* TA100

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(Received August 5, 1983)

Mutagenic activity, antibacterial activity and enzymatic reducibility of geometrical isomers of several nitrofurans were examined comparatively in *Salmonella typhimurium* TA100. These biological activities were well correlated to each other, not only between *cis* and *trans* isomers of each nitrofuran, but also among all the nitrofurans tested. Furthermore, the reducibility of each pair of *cis* and *trans* isomers by the bacterial enzymes also appeared to be correlated to the above biological activities.

Keywords——nitrofuran derivative; geometrical isomer; *cis* isomer; *trans* isomer; *Salmonella typhimurium* TA100; mutagenic activity; antibacterial activity; enzymatic reducibility

Previously, AF-2 (3-(5-nitro-2-furyl)-2-(2-furyl)acrylamide) and related nitrofurans were comparatively assayed to determine their mutagenic and antibacterial activities against *Salmonella typhimurium* TA100, and the relationship between such biological activities and the reducibility of these chemicals was examined. It was found that when nitrofurans possess structurally similar side chains at the 2-position of the furan ring, the biological activities of these chemicals can be correlated to their enzymatic reducibility, but not when the side chains are structurally different.[1] On the other hand, Tomoeda *et al.*[2] pointed out in a study with *Escherichia coli* K-12 JE 2100 R 100-1$^+$ that the biological activities of some nitrofurans can be well correlated to their metabolic reduction rate in the bacteria.

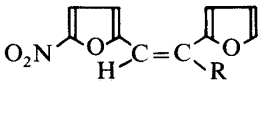
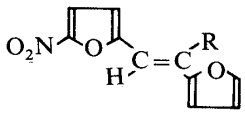
The present report describes for the first time the comparative mutagenic activity, antibacterial activity and enzymatic reducibility of *cis* and *trans* isomers of several nitrofurans in *S. typhimurium* TA100.

Experimental

Materials——The geometrical isomers of nitrofurans used are listed in Table I. Among them, NF-1, -3 and -5 were prepared by the method of Saikachi *et al.*,[3] and NF-2, -4 and -6 through -8 by that of Kato *et al.*[4] Prior to use, the purity of the nitrofurans was examined by thin-layer chromatography (TLC). When trace amounts of impurities were noted, the test chemicals were purified by preparative TLC or by recrystallization. NADPH was purchased from Sigma Chemical Co.

Bacterial Strain and Culture Media——*S. typhimurium* TA100 was used in this study. Culture media used were as follows: N-broth consisting of 25.0 g of Oxoid nutrient broth No. 2 and 1 l of water; penassay broth (Bacto antibiotics medium No. 3) consisting of 17.5 g of Bacto-penassay broth (Difco) and 1 l of water; Davis minimal medium base (M buffer) consisting of 10.5 g of K_2HPO_4, 4.5 g of KH_2PO_4, 1.0 g of $(NH_4)_2SO_4$, 0.5 g of sodium citrate dihydrate,

TABLE I. Geometrical Isomers of Nitrofuran Used

cis	*trans*	R	Name
			
NF-1	NF-2	COOH	3-(5-Nitro-2-furyl)-2-(2-furyl)-acrylic acid
NF-3	NF-4	$COOCH_3$	Methyl 3-(5-nitro-2-furyl)-2-(2-furyl)acrylate
NF-5	NF-6	$CONH_2$	3-(5-Nitro-2-furyl)-2-(2-furyl)-acrylamide
NF-7	NF-8	CN	3-(5-Nitro-2-furyl)-2-(2-furyl)-acrylonitrile

0.05 g of $MgSO_4 \cdot 7H_2O$ and 1 l of water; M agar, which is M buffer containing glucose and agar at concentrations of 0.2 and 1.5%, respectively; soft agar consisting of 6.0 g of NaCl, 12.2 mg of biotin, 9.6 mg of L-histidine hydrochloride, 7 g of agar and 1 l of water.

Mutation Assay——The assay was carried out as described by Ames *et al.*[5] with a slight modification. A nitrofuran dissolved in 0.1 ml of dimethylformamide was preincubated for 30 min at 33 °C with 0.1 ml of overnight culture of the tester strain in N-broth and 0.5 ml of M buffer. Soft agar (2 ml) was melted at 48 °C, added and mixed, and the mixture was poured immediately over an M agar plate. The plate was left to harden for several minutes. After incubation for 2 d at 37 °C, the numbers of colonies that were revertants to the histidine prototroph were counted in both test and control plates. Results are expressed as numbers of revertant colonies per nmol of the nitrofuran after subtraction of control values.

Assay for Antibacterial Activity——An exponential culture (2×10^5 cells) of *S. typhimurium* TA100 in penassay broth was added to 2 ml of the broth containing serial 2- or 5-fold dilutions of a nitrofuran. After incubation for 20 h at 37 °C, the minimum inhibitory concentration (MIC) was calculated from the lowest drug concentration at which no visible growth of bacteria was detected.

Assay for Reducibility——Early stationary-phase cells (10^8 cells/ml) grown in 2 l of N-broth were harvested by centrifugation, resuspended in 2 l of 67 mM potassium phosphate buffer (pH 7.2) and sonicated for 2 periods of 5 min each at full power in a Kubota ultrasonic disruptor (model 200M). After centrifugation at $13000 \times g$ for 15 min, the supernatant was subjected to ammonium sulfate fractionation. The protein precipitated between 30 and 60% saturation was collected and then dissolved in 30 mM potassium phosphate buffer (pH 7.2) at a concentration of 4 mg of protein per ml. All operations were carried out at 4 °C. Each incubation mixture consisted of 0.075 μmol of a nitrofuran, 0.3 μmol of NADPH and the enzyme solution (the 30—60% ammonium sulfate fraction, 0.4 mg of protein) in a final volume of 3 ml of 30 mM phosphate buffer (pH 7.2). Incubation was carried out at 37 °C in an open cell, and the decrease in absorbance at 340 nm (the absorption maximum of NADPH) was in a Hitachi 124 ultraviolet spectrophotometer.

Results and Discussion

Mutagenic and antibacterial activities of *cis* and *trans* isomers of nitrofurans were examined comparatively in *S. typhimurium* TA100. The results are shown in Table II. In each pair of isomers, the one which had stronger antibacterial activity also exhibited stronger mutagenic activity. With the exception of the pair of NF-7 and -8, the *cis* isomer was more biologically active than the corresponding *trans* isomer. In all the nitrofurans tested, the mutagenic activities were also well correlated to the antibacterial activities. The highest activities were observed with NF-3, and the lowest ones with NF-2.

The mutagenic and antibacterial activities of nitrofurans seem to be linked to nitro reduction of the chemicals by enzymes of the tester strain.[6,7] It is known that nitrofurans are mainly reduced by NADPH- and NAD(P)H-dependent, oxygen-insensitive enzymes present in cell-free extracts of bacteria.[8-11] In the present study, therefore, the comparative reducibility of the nitrofuran isomers was examined by using the cell-free extract of *S.*

Table II. Mutagenic and Antibacterial Activities of Geometrical Isomers of Nitrofurans against *Salmonella typhimurium* TA100

Compound	Revertants per nmol of nitrofuran[a)]	MIC[b)] (μM)
NF-1	175	1
NF-2	18	40
NF-3	30700	0.002
NF-4	26500	0.004
NF-5	9220	0.02
NF-6	6090	0.04
NF-7	8600	0.02
NF-8	11500	0.01

Each value is the mean of three experiments.
a) Values obtained from linear-response curves.
b) Minimum inhibitory concentration.

Table III. Reduction of Geometrical Isomers of Nitrofurans by Cell-Free Extract of *Salmonella typhimurium* TA100

Compound	Reduction rate[a)]
NF-1	5.5
NF-2	4.5
NF-3	19.4
NF-4	8.9
NF-5	11.4
NF-6	8.3
NF-7	6.8
NF-8	10.7

Each value is the mean of four experiments.
a) The reduction rate is expressed as nmol of NADPH consumed per min per mg of protein.

typhimurium TA100. In all the pairs except for NF-7 and -8, the *cis* isomer was more readily reduced than the *trans* isomers, whereas with NF-7 and -8, the *trans* isomer was more reducible than the *cis* isomer.

These facts suggested that there is a close correlation between the reducibility and the biological activities of geometrical isomers of each nitrofuran in *S. typhimurium* TA100.

Acknowledgement This work was supported in part by a Meisen 80 Research and Development Grant from the Meisen-kai.

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