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METABOLIC DEGRADATIONS OF NITROFURANS BY RAT LIVER HOMOGENATE

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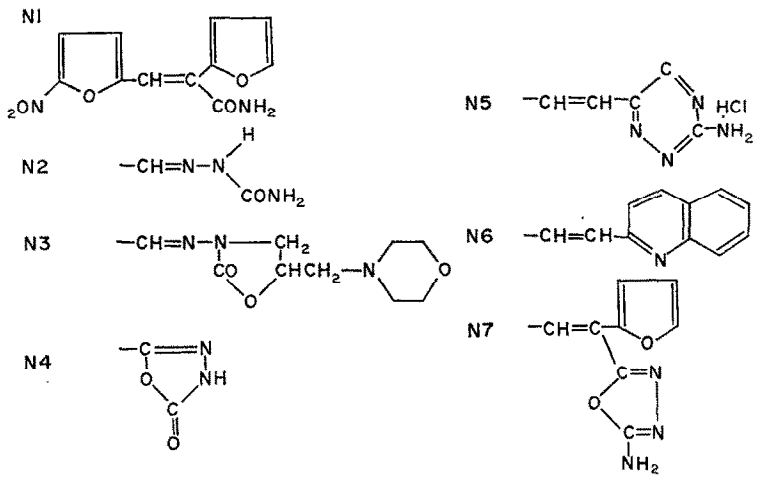
Abstract—The evidence of the metabolic degradations of some nitrofurans by rat liver homogenate was shown in the present study. Nitrofurans were incubated with 8500 *g* supernatant of rat liver homogenate with added $NADPH_2$ as a hydrogen donor and metabolic degradations of nitrofurans were followed by the changes of their characteristic optical absorbances and the loss of their nitro groups. These two properties of most of the tested nitrofurans changed slowly and independently in aerobic condition. In anaerobic condition, they disappeared much more rapidly and nearly to the same extents.

NITROFURANS have strong anti-biotic activities and have been utilized as chemotherapeutic agents and food preservatives. However, some nitrofurans have undesirable biological activities such as inhibition of spermatogenesis in male rats[1,2] and induction of tumors, especially in the mammary glands of female rats.[3,4] As for the metabolism of nitrofurans in animals, the reduction of their nitro groups to amino groups were indicated by Paul *et al.*[5] However, the findings on the metabolic degradations of nitrofurans by rat liver enzymes were few and obscure[6,7] as compared with those on nitrophenyl compounds, e.g. *p*-nitrobenzoic acid and chloramphenicol that are reduced by nitro-reductase, and those on azobenzenes that are reductively cleaved by azo-reductase.[7–11] In view of (i) the possibility that nitrofurans would be metabolized by the enzymes of rat liver homogenate that reduce nitro groups of nitrophenyl compounds and reductively cleave the azo linkage of azobenzene, (ii) the importance of nitro groups in the contributions to the presences of the characteristic optical absorbances and the biological activities of nitrofurans, (iii) the adoption of the recent device by Kanno *et al.* for the analysis of nitro groups of nitrofurans,[12] the metabolic degradations of some nitrofurans were studied in the experiment to follow the changes of their characteristic optical absorbances and the losses of their nitro groups in the mitochondrial supernatant of rat liver homogenate.

MATERIALS AND METHODS

Nitrofurans used in the present experiment were 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide (N1), 5-nitro-2-furaldehyde semicarbazone (N2), 5-morpholinomethyl-3-(5-nitrofurfurylideneamino)oxazolidone (N3), 5-(5-nitro-2-furyl)-1,3,4-oxadiazol-2-one (N4), 2-amino-5-[2-(5-nitro-2-furyl)vinyl-1-]-1,2,3-triazine HCl (N5), 2-[2-(5-nitro-2-furyl)vinyl-1-]quinoline (N6), and 2-amino-5-[2-(5-nitro-2-furyl)-1-(2-furyl)-vinyl-1-]-1,3,4-oxadiazol (N7) which were kindly donated by Ueno Pharmaceutical Co., Ltd. (Osaka). They showed maximum optical absorptions in ethanol solutions

at the wavelengths, 396 mμ (N1), 370 mμ (N2), 355 mμ (N3), 334 mμ (N4), 400 mμ (N5), 390 mμ (N6) and 410 mμ (N7). On the thin layer chromatography, in which the thin layer plates of silicic gel had been activated by heating at 110° for 30–45 min and $CHCl_3$–MeOH(95 : 5) and BuOH–AcOH–H_2O(4 : 1 : 2) were used as the developing solvents, they showed one spots except some residues on the original points.



Chemical structures of nitrofurans.

Tissue preparation

Male rats (Donryu strain, Nippon Rat Co., Ltd., Tokyo) were maintained on the basal diet, CE-2 (CLEA Japan Inc, Tokyo), and sacrificed at 6–10 weeks of age by decapitation. Liver was perfused with 1·15% KCl solution and chilled by immersion in ice cold KCl solution. The liver was homogenized in Potter-type glass-teflon homogenizer with 9 vol. of 0·25 M sucrose solution containing 25 mM KCl, 5 mM $MgCl_2$ and 50 mM Tris HCl buffer (pH7·6). The homogenate was centrifuged at 8500 *g* for 15 min in Hitachi PR18 refrigerated centrifuge, and the supernatant was used for the enzyme assay. Microsomal and soluble fractions were obtained by further centrifugation of 8500 *g* supernatant at 105,000 *g* for 60 min in Hitachi 40P refrigerated ultracentrifuge.

Enzyme activity

The basic incubation mixture consisted of 1·5 ml of 1·15% KCl, 0·1 ml of 0·1 mM $MgCl_2$, 1·0 ml of 0·2M Tris HCl (pH 7·6), 0·1 ml of 1·0M nicotinamide, 0·4 ml of 0·02M glucose-6-phosphate, 0·1 ml of $NADP^+$ (200 μg), 0·1 ml of a nitrofuran ethanol solution (0·2 μmole) and 0·5 ml of 8500 *g* supernatant, microsomal suspension or 105,000 *g* supernatant that was equivalent to 0·05 g of liver. The volume of the incubation mixture was made to 4 ml with distilled water.

After incubation for a specified period at 37°, the reaction was stopped by the addition of 10 ml of methanol. After centrifugation, the supernatant were diluted with methanol and used for the analysis of the optical absorbances and the quantitative analysis of nitro groups. Optical absorbances of the methanol solutions were traced at the wavelengths 300–450 mμ by Hitachi EPS-3 recording spectrophotometer,

and the quantitative analysis of nitro groups were made by the method of Kanno *et al.*[12] except that the treatment with alkali for the release of nitro group of a nitrofuran as nitrous acid was made for 60 min. For the studies on the metabolism of nitrofurans under anaerobic condition, Thunberg tube was used and evacuated before the start of the incubation. Silicic gel was obtained from E Merk AG Darmastadt (Germany), $NADP^+$ and NAD^+ from Sigma Chemical Co., Ltd. (U.S.A.), glucose-6-phosphate and glucose-6-phosphate dehydrogenase from Boehringer and Söhne GmbH (Germany), and FAD from Daiichi Kagaku Co., Ltd. (Tokyo).

RESULTS

Metabolic degradation under aerobic condition

Each nitrofuran was incubated, in an open centrifuge tube, with the basic incubation mixture of 8500 *g* supernatant of the liver homogenate for 15 or 60 min. The optical absorbances of five of seven tested nitrofurans decreased slightly and that of N1 rather increased at the wavelengths near 396 mμ, the maximum absorption wave length of N1 (Fig. 1 top). On the contrary, considerable losses of nitro groups were

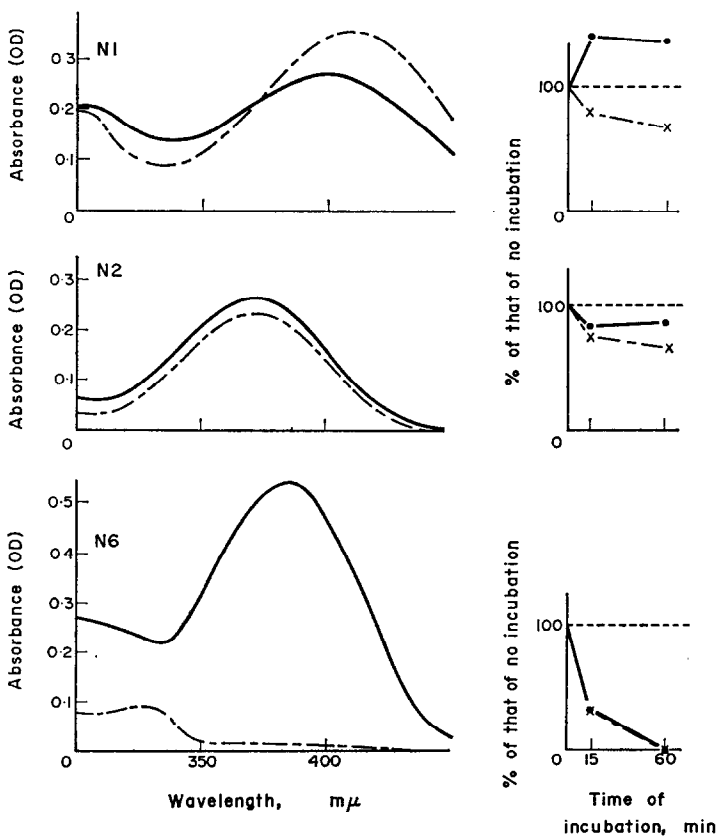


FIG. 1. The change of the optical absorbance and the decrease of nitro group of N1, N2, or N6 by the incubation under aerobic condition with 8500 *g* supernatant of rat liver homogenate. Left graph; the optical absorbance at 300–450 mμ (time of incubation = 0 min, ———; 60 min, —— - ——). Right graph; the change of the optical absorbance at the maximum absorption wavelength, 396 mμ (N1), 370 mμ (N2) or 390 mμ (N6), (———) and the decrease of nitro group (—— - ——).

seen in all nitrofurans. N3, N4 and N5 showed almost the same changes as N2 (Fig. 1 middle). It was only in N6 and N7 that the optical absorbance at the maximum absorption wavelength and the nitro group were seen to decrease almost to the same extents (Fig. 1 bottom). The following study was made on N5 and N6 whose optical absorbances were found to decrease considerably.

The enzyme activity, which reduces the optical absorbances at the maximum absorption wavelengths (400 mμ for N5, 390 mμ for N6), was found in both microsomal and 105,000 *g* supernatant fraction, although glucose-6-phosphate dehydrogenase must be added to the basic incubation mixture of the former. A study on the requirements and the effects of co-factors on the enzyme activity was made on the microsomal fraction. $NADP^+$ was necessary for the enzyme activity and NAD^+ could not replace $NADP^+$. A further addition of NAD^+ (200 μg) or FAD (100 μg) showed no stimulatory effect on the enzyme activity and FAD rather showed an inhibitory effect on it. It was also noted that the metabolic degradations of nitrofurans proceeded rapidly in anaerobic condition.

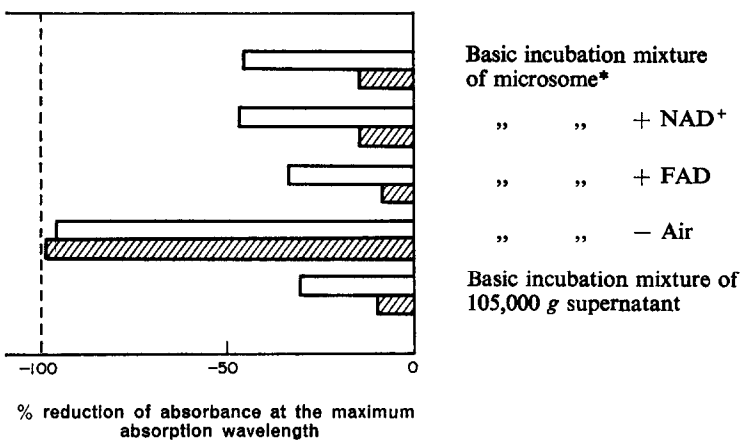


FIG. 2. The effect of NAD^+, FAD or air on the activity of microsomal fraction of rat liver homogenate to reduce the optical absorbance of N5 ▨, and N6 □. Each nitrofuran was incubated for 15 min. The activity of 105,000 *g* supernatant is also indicated at the bottom of the graph.

* 2 units[13] of glucose-6-phosphate dehydrogenase was further added to the basic incubation mixture of the microsomal fraction.

Metabolic degradation under anaerobic condition

Each nitrofuran was incubated, in an evacuated Thunberg tube, with the basic incubation mixture of 8500 *g* supernatant of the liver homogenate. Both the optical absorbance at the maximum absorption wavelength and the quantity of nitro group of any tested nitrofuran except N5 decreased rapidly nearly to the same extents (exemplified with the case of N1 and N2 in Fig. 3). It was noted on N2 that as the absorbances at the wavelength near 370 mμ (the maximum absorption wavelength

of N2) decreased, there increased those near 335 mμ (the maximum absorption wave length of 5-amino-2-furaldehyde semicarbazone[5]). The similar but less clear tendencies were also seen on N4, N5 and N6. A striking difference between the extent of the decrease of absorbance at the maximum absorption wavelength and that of the quantity of nitro group was found in N5; the optical absorbance disappeared almost completely within 10 min of incubation, while a considerable amount of nitro group was found in the incubation mixture of N5 (Fig. 3 bottom). In the present metabolic system, no free nitrous acid was metabolized and no nitrous acid was released from the nitrofurans.

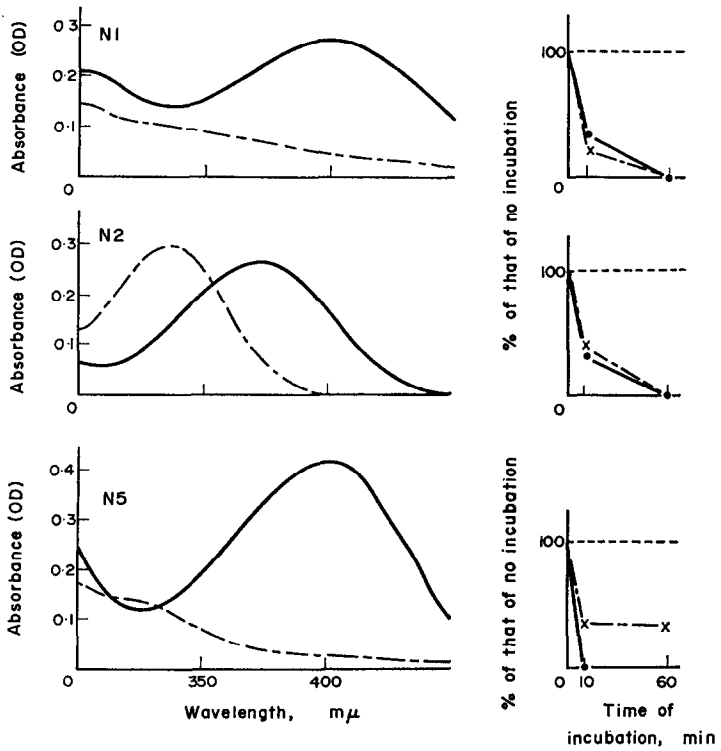


FIG. 3. The change of the optical absorbance and the decrease of nitro group of N1, N2 or N5 by the incubation under anaerobic condition with 8500 *g* supernatant of rat liver homogenate. Left graph; the optical absorbance at 300–450 mμ (time of incubation = 0 min, ———; 60 min,—— – ——). Right graph; the change of the optical absorbance at the maximum absorption wavelength, 396 mμ (N1), 370 mμ (N2) or 400 mμ (N5), (———), and the decrease of nitro group (—— – ——).

DISCUSSION

The present study indicates that nitrofurans are metabolized by the microsomal and supernatant fractions of the liver homogenate which also reduce foreign substances such as nitrophenyl compounds and azobenzenes.[7–11] The main metabolic changes of nitrofurans are considered to be reductive ones, for the changes of

nitrofurans proceeded more rapidly under anaerobic condition, $NADPH_2$ was necessary for the metabolic degradation of nitrofurans. It has been noted that the presence of a nitrofuran inhibits the enzyme activity to metabolize nitrophenyl compounds[7] and azobenzenes.[14] These facts would indicate that nitrofurans are metabolized by the same enzyme systems that reduce nitrophenyl compounds and azobenzenes. However, there are some peculiarities in the metabolic degradations of nitrofurans. In contrast with nitrophenyl compounds, nitrofurans were metabolized, to some extent, under aerobic condition. NAD^+ and FAD, the presences of which promote the reductive cleavage of azobenzenes, showed no such promoting effects on the metabolic degradation of nitrofurans and FAD showed rather an inhibitory effect on it.

It is considered that the presence of nitro group contributes to the presence of the characteristic optical absorbance of a nitrofuran. In consistent with this consideration, when a nitrofuran was metabolized under anaerobic condition, its characteristic optical obsorbance and its nitro group disappeared nearly to the same extents. However, considerable discrepancies were noted between the changes of the optical absorbances and the decreases of nitro groups in the metabolism of nitrofurans, N1–N5 under aerobic condition, and N5 under anaerobic condition. Chemical structures other than nitro groups, which also contribute to the presences of the characteristic optical absorbances of nitrofurans, would be expected to be also changed by the rat liver enzymes, especially under aerobic condition. For the elucidation of more precise aspects of the metabolic degradations of nitrofurans, identifications of the metabolites and studies on the purified enzymes or enzyme systems are necessary.

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