The derivatives (I) were prepared by the reaction of amino acids (0.5 mmole) and *d*-10-camphorsulfonyl chloride[2] (0.5 mmole) in ether (10 ml) and 1N NaOH aqueous solution (10 ml) under vigorous stirring at room temperature for one hour, and then, the reaction mixture was acidified with 1N HCl and extracted with ether. The resulting sulfonylamide was treated with *p*-nitrobenzyl bromide (0.5 mmole) in chloroform solution under reflux for 30 min, and the chloroform solution was washed with H_2O, dried with anhyd. Na_2SO_4, and evaporated.

The experimental conditions of HPLC and the chromatogram of amino acids derivatives[3] were shown in Fig. 1.

In order to allocate the peaks, derivatives were prepared from optically active amino acids with the same reaction condition described above.[4]

The derivatives of D-amino acids had in all cases a longer retention times (Table I).

The application to the preparative resolution and the quantitative treatment are under investigation.

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2) P.D. Bartlett and L.H. Knox, "Organic Syntheses," Vol. 45, John Wiley & Sons, Inc., N. Y., 1965, p. 45.
3) The NMR spectra (100 MHz, in $CDCl_3$) of all compounds are in accordance with the structures.
4) For the derivatives of optically active amino acids have a single peak by HPLC, no racemization proceeds during preparation of the derivatives under the reaction condition employed.

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The Effect of AF2 [2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide] on Hepatic Microsomal Mixed Function Oxidase System in Rats

Male rats were fed for 16 days on the diet containing 0.2% of AF2. Hepatic microsomes were isolated and the activities of mixed function oxidase system were assayed. Cytochrome P-450 was significantly reduced in its content and, on the contrary, cytochrome b_5 was much elevated in the AF2-treated animals. AF2 treatment also depressed the activities of aminopyrine N-demethylase and aniline hydroxylase. The changes in the contents of hepatic constituents were also analyzed.

Furylfuramide [2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, abbreviated to AF2] had been widely used as an antimicrobial preservative for foods in Japan for about ten years. But recently, the use of the compound as a food additive was prohibited by the Japanese Ministry of Health and Welfare because of its possible carcinogenic action when fed to experimental animals. The mutagenicity of AF2 has been also shown in the testing system in microorganisms. Apart from the carcinogenicity, few studies disclosed other important toxicities of AF2 on the experimental animals. Miyaki, *et al.*[1] observed the atrophy of testis in rats fed on AF2, but Miyaji[2] did not find any changes in testis by the administration of the compound. Liver hypertrophy is generally observed in animals treated with AF2 and Miyaji[2] deduced

1) K. Miyaki, M. Akao, K. Terao, and K. Kuroda, *Gann*, **103**, 167 (1969).
2) T. Miyaji, *Tohoku J. Exp. Med.*, **103**, 331 (1971).

that the induced hypertrophy of the liver cells might be the consequence of the enhanced function of the liver required to metabolize the ingested compound, because the liver hypertrophy was reversible and no serious lesions were observed in microscopical, histological and histochemical examinations of the AF2-treated liver. However, the significance of the liver enlargement induced by the compound still remains to be elucidated.

In this regard, we have obtained evidences that AF2 depressed the microsomal mixed function oxidase system which plays an essential role in the function of liver to metabolize ingested chemicals.

Male rats of Wistar strain (Nippon Rat Co., Ltd.) of 5 weeks of age were maintained for 16 days on the diet containing 0.2% of AF2 (Ueno Pharmac. Inc., Osaka). The animals were sacrificed at the end of the experimental period. The livers were excised and subjected to the assay of the microsomal mixed function oxidase system and to the quantitative analyses of the tissue constituents. Hepatic microsomes were isolated according to the method of Cinte, *et al.*[3] The contents of microsomal protein and of cytochromes and the activities of aminopyrine N-demethylase and of aniline hydroxylase were determined.[4] These results are shown in Table I. The hepatic constituents which were analyzed are water, desoxyribonucleic acid (DNA) and ribonucleic acid (RNA)[5,6,7] lipid,[8] glycogen[9] and protein.[10] The results are shown in Table II.

The increase in body weight of the AF2-treated animals was approximately the same as that of the controls. The amounts of food intake of the AF2-treated animals were somewhat lower than those of the controls. The liver and the kidney weights of the AF2-treated animals were significantly greater than those of the controls by 60.0% and 10.5%, respectively. No statistical differences, however, were obtained between those two groups in the weights of other organs such as lung, spleen, testis and thymus.

Table I. Effects of AF2 on Microsomal Cytochromes and Drug-Metabolizing Enzymes

Groups	Microsomal protein	Contents of cytochromes		Enzyme activities	
		P-450	b_5	Aminopyrine N-demethylase	Aniline hydroxylase
		n moles/mg protein		*n* moles/min/mg protein	
Control (5)		0.76±0.09	0.28±0.02	2.63±0.30	0.121±0.025
AF2 (5)		0.46±0.02[b]	0.40±0.03[b]	1.73±0.10[b]	0.088±0.007
	mg/g liver	*n* moles/g liver		*n* moles/min/g liver	
Control	26.3±0.3	20.1±2.3	7.42±0.61	69.6±8.3	3.21±0.66
AF2	25.6±0.5	11.9±0.5[c]	10.35±1.00[a]	44.6±3.3[b]	2.28±0.18
		n moles/100 g body wt.		*n* moles/min/100 g body wt.	
Control		47.1±5.0	17.5±1.4	164±18	7.54±1.55
AF2		45.1±3.1	39.0±3.7[c]	168±13	8.64±0.82

Data represent mean ± standard error. Figures in parentheses indicate the number of animals used in each experiment. Significant differences;
a) $p<0.05$ *vs.* control, *b*) $p<0.025$ *vs.* control, *c*) $p<0.01$ *vs.* control

3) D.L. Cinte, P. Moldeus, and J.B. Schenkman, *Biochem. Pharmacol.*, **21**, 3249 (1972).
4) P. Mazel, "Fundamentals of Drug Metabolism and Drug Dispostion," ed. by N. La Du, H.G. Mandel, and E.L. Way, Williams & Wilkins, Baltimore (1972), pp. 546—578.
5) S. Mizuno and R. Whiteley, *J. Bacteriol.*, **95**, 1221 (1968).
6) K. Burton, *Biochem. J.*, **62**, 315 (1956).
7) W. Mejbaum, *Z. Physiol. Chem.*, **258**, 117 (1939).
8) N.S. Rodin, *Methods in Enzymol.*, **14**, 245 (1969).
9) D.L. Morris, *Science*, **107**, 254 (1948).
10) O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

The treatment with AF2 generally suppressed the activities of the microsomal mixed function oxidase system in liver (Table I). The cytochrome P-450 was significantly reduced in its content per microsomal protein and per unit of wet weight of liver in the AF2-treated animals. However, AF2 treatment did not alter the total amount of P-450 in the liver microsomes when calculated as amount per 100 g body weight. Contrary to it, the concentration and the total amount of cytochrome b_5 were much elevated in the AF2-treated animals. The activities of aminopyrine N-demethylase and of aniline hydroxylase which depend largely on cytochrome P-450 were also depressed approximately to the same extent as cytochrome P-450. Furthermore, the results of our succeeding experiments have shown that the compound did depress the activity of the system both in a short-term administration and in a prolonged administration even in a lower dose than that employed in the present study. All these facts would exclude the possibility that, as Miyaji[2] suggested, the liver hypertrophy induced by AF2 is the expression of the adaptation of liver to the ingestion of AF2 by the body.

TABLE II. Effects of AF2 on the Contents of Hepatic Components

Groups	Liver weight	Water	Glycogen	Lipid	DNA	RNA	Protein
	g/100 g body wt.	Total amount, mg/100 g body weight					
Control (5)	4.29±0.18	3062±145	162±26	168±5	9.56±0.87	46.4±3.4	904±31
AF2 (5)	6.86±0.21[a]	4815±153[a]	388±29[a]	269±32[b]	12.25±0.38[b]	64.7±2.8[a]	1365±50[a]
		content, mg/g liver					
Control (5)		713±6	38.2±5.9	39.6±2.6	2.22±0.13	10.7±0.4	211±2
AF2 (5)		701±1	56.4±3.3[c]	38.7±3.8	1.79±0.07[b]	9.43±0.32[c]	199±4[c]

For explanation and experimental details, see the text and the footnote of Table I.
a) $p<0.01$ *vs.* control, *b*) $p<0.025$ *vs.* control, *c*) $p<0.05$ *vs.* control

Then, what is the significance of the liver enlargement induced by AF2? We have determined the principal constituents of liver, because quantitative changes in components, if any, would give some indications to this problem. The results have shown that AF2 treatment elevated, much or less, the total amount of most hepatic components per 100 g body weight but depressed their concentrations per unit of liver weight, except that of glycogen (Table II). The increase in the total amount of DNA might signify that the number of hepatic cells increased. The reduction in DNA content per unit of liver weight, on the other hand, would indicate that the relative content of some other components increased, which indicates an increase in the volume of a cell. These results coincide well with the histological findings of Miyaji[2] in which the number of hypertrophic cells increased in the AF2-treated liver. The reductions in the contents of RNA and protein and a marked increase in that of glycogen in hepatic cells might represent that the cells are metabolically less active under the AF2 treatment, for the liver synthesizes RNA and protein in greater quantity and utilizes glycogen more in the normal state than in the pathological state.

From the data obtained in the present study, we could not obtain definite evidences as for the toxicological significance of the liver enlargement induced by AF2. Further studies are undergoing from the viewpoint of hepatotoxicity of the compound in relation to the liver functions including the microsomal mixed function oxidase system.

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