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SHORT COMMUNICATIONS

The metabolic fate of 4-acetyl-2-[2-(5-nitro-2-furyl)vinyl]-Δ^2-1,3,4-oxadiazoline-5-one in man

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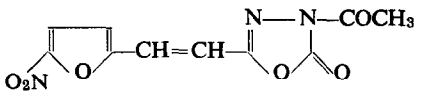
4-ACETYL-2[2-(5-nitro-2-furyl)vinyl]-Δ^2-1,3,4-oxadiazoline-5-one (hereafter referred to as NF-124) is a synthetic chemical substance with a considerably high antimicrobial activity *in vitro* against gram-positive and gram-negative bacteria, and protozoa.[1]

The chemical structure of NF-124 is shown below:



The present paper deals with identification of urinary metabolites after oral administration of NF-124 to human subjects.

NF-124 in an oral dose of 500 mg was administered to three healthy male adults. Blood samples were collected at 1·5, 3·5, and 7 hr after administration, and antimicrobial activity in the serum was determined.

Seven-hour urine samples, collected from the same subjects (averaging 1500 ml each), were concentrated to 50 ml *in vacuo*, and the concentrate was extracted with 150 ml ethylacetate. The ethylacetate layer was washed with water and concentrated to 10 ml *in vacuo*. The extracts were used as samples for determination of activity and also for thin-layer chromatography.

The extracts were again concentrated to about 0·5 ml *in vacuo* and allowed to stand in the refrigerator to obtain yellowish-brown precipitates, which were used as samples for determining infrared absorption.

Antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli*, and *Trichomonas vaginalis* were determined by the conventional tube dilution method.

Thin-layer chromatography with silica gel G (Merck) was carried out by the one-dimensional ascending technique with various solvents. To estimate the residual activity of NF-124, various tissue homogenates of rats were prepared and incubated with NF-124 (0·5 ml of 0·1% solution in dimethylformamide) in a Krebs–Ringer phosphate buffer (pH 7·4) at 37° for a given period of time. Antibacterial activities in the samples (serum, urine, and tissue homogenates) were determined by the turbidimetric method, with *E. coli* as test organism.[2]

Serum level and urinary excretion of antimicrobial activities after oral administration of NF-124. As shown in Table 1, effective activity level was maintained in the serum for a period from 1·5 to 7 hr,

TABLE 1. SERUM CONCENTRATION AND URINARY EXCRETION OF ANTIMICROBIAL ACTIVITY IN MAN AFTER A SINGLE DOSE (500 MG) OF NF-124

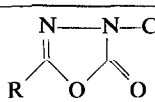
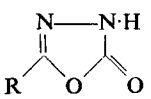
Volunteer	Serum concentration* (μg/ml)				Antibacterial activity in 7-hr urine	
	1·5 hr	3 hr	5 hr	7 hr	(mg)	(%)
60 kg ♂	1·9	2·2	1·7	0	1·05	0·21
55 kg ♂	1·7	4·6	2·8	1·3	2·60	0·52
52 kg ♂	3·2	7·4	4·4	5·2	3·20	0·64
Mean	2·3	4·7	3·0	2·2	3·28	0·46

* Values are in terms of equivalent amount of NF-124 calculated from antimicrobial activity determined by tube dilution method.

and the activity detected in 7-hr urine corresponded to from 0·21 to 0·64 per cent of the amount of NF-124 administered.

Identification of urinary metabolites of NF-124. Each thin-layer chromatogram of the ethylacetate extracts of the urines with various solvents gave only one spot showing antimicrobial activity. As indicated in Table 2, the R_f value of this spot was consistent with that of the authentic sample of 2-[2-(5-nitro-2-furyl)vinyl]-Δ^2-1,3,4-oxadiazoline-5-one (hereafter referred to as NF-44).

TABLE 2. R_f VALUES OF THIN-LAYER CHROMATOGRAPHY OF NF-124, NF-44 AND URINARY EXTRACT

	Solvents			
	$CHCl_3$	Et-Ac	Et-OH	Acetone
NF-124 	0·48	1	0·90	1
NF-44 	0	0·91	0·83	0·96
Urinary extract	0·05	0·86	0·80	0·91

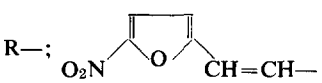
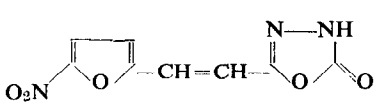


TABLE 3. ANTIMICROBIAL ACTIVITY OF NF-44 AND NF-124*

Test organisms	MIC	
	NF-44 (μg/ml)	NF-124
Staphylococcus aureus 209-P	1	2·5
Escherichia coli NIHJ	1	5
Trichomonas vaginalis 4F	1	1·5

* Antimicrobial activity was determined by tube dilution method.

NF-44 is a known substance, having higher antimicrobial activity than NF-124 (Table 3). The chemical structure of this substance is as follows:



The LD_{50} value of NF-44 in mice was 440 mg/kg by the oral route; that of NF-124 was 2400 mg/kg.

On the infrared absorption spectrum, the precipitates obtained from the ethylacetate extracts gave no absorbance of the carbonyl group at 1770 cm^{-1} but showed that of the imino group at 3440 cm^{-1}, which was consistent with that of NF-44 (Fig. 1).

NF-124, orally administered, was deacetylated to NF-44 in the body, which was the only antimicrobial substance excreted in the urine (Table 3).

Incubation of NF-124 with rat tissue homogenates. As indicated in Table 4, the activity of NF-124

decreased by 20–40 per cent after 60-min incubation with homogenates of the liver, kidneys, or heart. However, incubation with homogenates of intestine or muscle under similar conditions did not decrease NF-124 activity.

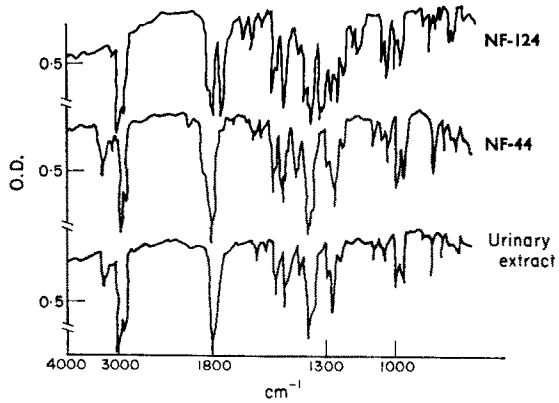


FIG. 1. Infrared spectra of authentic samples of NF-124, NF-44, and urinary extract.

TABLE 4. RESIDUAL ANTIMICROBIAL ACTIVITY OF NF-124 AFTER INCUBATION WITH VARIOUS TISSUE HOMOGENATES OF RAT

Tissue	Residual activity* (%) at			
	0 min	15 min	30 min	60 min
Liver	100	92	76	62
Kidney	100	87	59	62
Heart	100	81	74	81
Small intestine	100	100	102	104
Muscle	100	106	95	100

* Residual activity of incubation mixture (pH 7·4) was determined by turbidimetric method. Incubation temperature, 37°.

The authors confirmed in these experiments that the antimicrobial substance excreted in the urine after oral administration of NF-124 is not NF-124 itself, but NF-44, a deacetylated metabolite of the starting material. It was interesting that NF-44 showed both antimicrobial activity and toxicity higher than those of NF-124, even though NF-44 is not the main metabolite of NF-124. The metabolism of NF-44 itself in mammals has not yet been studied, and it will be of interest to clarify to what degree NF-44 is acetylated in the human body.

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