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# REDUCTIVE METABOLISM OF THE CARCINOGEN 4-(5-NITRO-2-FURYL)THIAZOLE TO 1-(4-THIAZOLYL)-3-CYANO-1-PROPANONE BY RAT LIVER SUBCELLULAR FRACTIONS*

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**Abstract**—The reductive metabolism of 4-(5-nitro-2-furyl)thiazole (NFT), a rat mammary gland and forestomach carcinogen, was examined *in vitro* using rat liver tissues. NFT was reduced by rat liver cytosol or microsomes on anaerobic incubation with NADPH. The stoichiometry of microsomal reduction revealed that about 3 moles of NADPH were used per mole of NFT. Gas chromatographic analysis of the reaction mixture showed a major peak with a retention time of about 4.0 min in contrast to NFT with a retention time of about 6.5 min. Catalytic hydrogenation of NFT with palladium and activated carbon yielded a product with a retention time of 4.0 min. The component corresponding with the above metabolite was isolated from chemically reduced NFT and identified as 1-(4-thiazolyl)-3-cyano-1-propanone. The metabolite derived from enzymatic reduction had chromatographic and spectral properties and a mass spectral fragmentation pattern similar to those obtained chemically. These data establish that the enzymatically derived product is identical to that obtained by chemical reduction and that it corresponds to 1-(4-thiazolyl)-3-cyano-1-propanone.

The 5-nitrofurans have been widely used as human and veterinary medicinals, and as food preservatives and feed additives [1]. A large number of these compounds were shown to be carcinogenic [2] and mutagenic [3]. Certain typical members of this class whose carcinogenic activity has been well-documented include *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]-formamide (FANFT) [4–6], 2-amino-4-(5-nitro-2-furyl)thiazole (ANFT) [7, 8], *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide (NFTA) [6, 9], formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide (FNT) [10], 2-hydrazino-4-(5-nitro-2-furyl)thiazole (HNT) [7, 11], and 4-(5-nitro-2-furyl)thiazole (NFT) [2, 12]. Structure–activity oncogenesis relationship studies using a number of 5-nitrofuryl-2-thiazole analogs demonstrated that the substituent at the 2-position of the thiazole ring influenced the site of tumorigenesis [2, 13], since even minor structural modification at that locus drastically altered tissue specificity. Furthermore, the 5-nitro group was found essential for biological activity, since deletion of this group resulted in loss of bactericidal, mutagenic and carcinogenic activities [11, 13]. The 5-nitro group *per se* may not be the biologically active functional group. All biological effects seem to require the reduction of the 5-nitro group to a metabolically reactive intermediate [2, 3]. Earlier *in vitro* metabolic studies of FANFT [14], ANFT [15, 16], NFTA [17], and 2-methyl-4-(5-nitro-2-furyl)thiazole [18] demonstrated that the 5-nitro group of these compounds was easily reduced by rat and mouse liver homogenates, microsomes, and cytosols. The chemical identities of the reduction products, however, have not been conclusively established. Utilizing NFT, structurally the least complex of these analogs, and rat liver subcellular fractions, the reductive metabolism of NFT was examined *in vitro*. This report presents evidence that the reduced metabolite of NFT is 1-(4-thiazolyl)-3-cyano-1-propanone.

## MATERIALS AND METHODS

*Sources of materials.* NFT, purchased from Saber Laboratories Inc., Morton Grove, IL, was recrystallized in ethanol; the crystalline preparations were > 99 per cent pure as evaluated by chromatographic methods. Palladium (5%) on activated carbon, deutero chloroform, tetramethyl silane (TMS), and methanol were obtained from the Aldrich Chemical Co., Inc., Milwaukee, WI. NADPH and Tris were purchased from the Sigma Chemical Co., St. Louis, MO. Dichloromethane and isopropanol for high pressure liquid chromatography were obtained from Burdick and Jackson Laboratories Inc., Muskegon, MI. Male Sprague–Dawley rats were purchased from Sprague–Dawley, Madison, WI.

*Preparation of rat liver subcellular fractions.* Rats, weighing 150–200 g, were decapitated. The livers were immediately removed, perfused and rinsed with ice-cold 0.25 M sucrose, and then homogenized in

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a Potter–Elvehjem homogenizer with a teflon pestle using 5 ml of 0.25 M sucrose/g wet weight of liver. The homogenate was centrifuged at 10,000 *g* for 15 min in a Beckman model J-21 centrifuge. To obtain microsomes and cytosol, the 10,000 *g* supernatant fraction was further spun at 105,000 *g* for 90 min in a Beckman model L-2 ultracentrifuge. The cytosol was decanted, and the microsomal pellet was rinsed a few times with small volumes of 0.25 M sucrose and then resuspended in 2.5 volumes of sucrose/g wet weight of liver.

*Assay for NFT reduction.* NFT was dissolved in polyethylene glycol USP 400, and then it was subsequently diluted with an equal volume of water to give a final concentration of 1.0 mM. The incubation mixture contained 1 μmole NFT, 3 μmoles NADPH, 1.0 ml of 0.1 M Tris buffer, pH 7.4, and 1.0 ml of rat liver subcellular fraction. The reaction, conducted in a Thunberg tube at 37° under nitrogen unless otherwise specified, was terminated by the addition of 1 ml of cold 40% trichloroacetic acid (TCA) in instances where the reduction was monitored spectrophotometrically. The spectrophotometric measurements were made in a Beckman 25 spectrophotometer after centrifugation of the TCA precipitate and dilution of the supernatant fraction with 2 volumes of water. Appropriately treated blanks were used in the reference beam to correct for absorbance from extraneous components.

In experiments involving isolation and characterization of the reduction product, the reaction was terminated by immersion in a boiling water bath for 10 min. The reaction mixture was then extracted five times with an equal volume of chloroform–ether (1:1, v/v). The extracts were pooled, evaporated to dryness, and used for chromatographic analyses.

*Chemical reduction of NFT.* NFT (2 mmoles) was dissolved in 300 ml of methanol to which 1 g of 5% palladium on activated carbon was carefully added. Catalytic hydrogenation was performed in a Parr pressure reaction apparatus (Parr Instrument Co., Inc., Moline, IL) for 1 hr at a pressure of about 60 lb/in$^2$. At the end of the reaction, the catalyst was removed by filtration under vacuum, and the filtrate was evaporated to dryness, dispersed in water, and filtered. The aqueous portion was extracted three times with chloroform. The organic phase was separated, pooled, evaporated to dryness, and chromatographed on a column of silica gel, using ethylacetate and ethylacetate–methanol (95:5, v/v) as eluting solvents in a stepwise gradient. The early eluting fractions with ethylacetate were pooled, evaporated to dryness, and subjected to spectroscopic analysis.

*Chromatography and spectroscopy.* Gas–liquid chromatography was performed in a Hewlett Packard model 5710A chromatograph equipped with an electron capture detector. Samples were injected onto a 2 mm i.d., 6 ft long glass column containing 10% OV-1 on 80/100 chromosorb W. Argon–methane (95:5, v/v) mixture was used as the carrier gas at a flow rate of about 30 cc/min. Isothermal runs were made at 200°.

A high pressure liquid chromatograph (h.p.l.c.), Micromeritics 7000 B, equipped with model 730-universal injection valve and a fixed wavelength (254 nm) detector, was used under constant flow mode. The instrument was fitted with a 25 cm, partisil 10 column. A concave gradient, programmed for 0–100% strong solvent in 30 min, was run using dichloromethane as weak solvent and isopropanol as strong solvent. The chromatograph was operated at a flow rate of 2.0 ml/min at ambient temperature. Preparative runs were made using Whatman-Magnum 9 columns and similar solvents.

Infrared (i.r.) spectra were taken in a Beckman Accu Lab 4 double beam IR-spectrometer using 0.107 cm wide sodium chloride cells. Samples were dissolved in purified chloroform, and spectra were taken against chloroform blanks.

The proton nuclear magnetic resonance (n.m.r.) spectra were measured with a Bruker H × 90E, Fourier transform NMR spectrometer. Samples of about 5 mg were taken in 0.25 ml of deuterochloroform containing about 0.1% TMS, as internal standard. Data from 100 sweeps were used for Fourier transform.

Using a Varian XL-100/15 spectrometer with Varian Fourier transform accessories, $^{13}$C-n.m.r. spectra were obtained. Samples of about 10-mg were dissolved in 0.50 ml deuterochloroform containing a trace amount of TMS. Chromium (III) acetylacetonate was added to the sample to saturation (*ca.* 0.07 M); the spectrum was taken in 5 mm tubes. Data from 37,536 transients were computed for the Fourier transform.

Gas chromatography/mass spectrometry (g.c./m.s.) was carried out in a DuPont 21-491B mass spectrometer, interfaced with a Varian 2740 GC, at an ionizing voltage of 70 eV. The gas chromatograph was fitted with a coiled glass column 180 × 0.2 cm, containing 3% OV-101 on 100/120 mesh Aeropak 30. Chromatograms were run isothermally at 150°, with helium as carrier gas, at a flow rate of 30 ml/min. Samples not requiring g.c.-interfacing were analyzed by direct injection of the sample into the ion source, at an initial inlet temperature of 150°, in an AEI-MS-9 mass spectrometer.

## RESULTS

*Enzymatic reduction of 5-nitro-2-furylthiazole.* The *in vitro* biotransformation of NFT was monitored spectrophotometrically, after TCA precipitation and centrifugation. Absorption spectra obtained after incubation of NFT with rat liver 10,000 *g* supernatant fraction for 1 h at 37° revealed a hypochromic effect and a hypsochromic shift, with the disappearance of the absorption peak at 368 nm and the appearance of a new absorption peak at 268 nm. Under the above experimental conditions, NFT was completely reduced. On deletion of NADPH, however, the reaction proceeded slowly, suggesting the requirement of NADPH for reduction. The observed partial reduction in the absence of NADPH could be due to endogenous cofactor(s) present in the homogenate. The reaction proceeded slowly under aerobic conditions indicating the inhibitory effect of oxygen on the enzyme system.

Table 1 shows the subcellular localization of the enzyme activity in rat liver tissues. These data were obtained both with and without NADPH in the

Table 1. Subcellular localization of enzyme activity in rat liver tissues

| Enzyme source | ($\mu$moles NFT reduced (mean ± S.E.)·$g^{-1}$·$hr^{-1}$) Minus NADPH* | Plus NADPH† |
|---|---|---|
| Whole homogenate | 4.40 ± 0.07 | 28.37 ± 0.24 |
| Boiled homogenate | 0.74 ± 0.04 | 1.64 ± 1.2 |
| 10,000 *g* Supernatant fraction | 2.59 ± 0.11 | 19.58 ± 0.67 |
| Microsomal fraction | <0.1 (<0.1)‡ | 10.02 ± 1.70 |
| 105,000 *g* Supernatant fraction | 2.52 ± 0.24 (7.1 ± 1.06)‡ | 8.11 ± 0.11 |
| Microsomal plus 105,000 *g* supernatant fraction | 1.01 ± 0.12 | 21.24 ± 0.26 |

* The components and the conditions of the reaction were the same as those described in Fig. 1. The data are the results of duplicate assays.

† Preparations equivalent to 100 mg of tissue were incubated for 15 min at 37° under nitrogen. Other components were used in the same quantities as those described in Fig. 1.

‡ Results obtained with 3 $\mu$moles NADH in the incubation mixture.

reaction mixture. In both instances, boiling abolished the enzyme activity. Most of the activity present in the homogenate was recovered in the 10,000 *g* supernatant fraction. However, in the absence of NADPH, very little of the activity was detected in the microsomal preparations. On inclusion of NADPH in the incubation mixture, the activity was substantially enhanced; under these conditions a major portion of the total activity was recovered in the microsomal preparations. These data suggest that the microsomal reductive enzyme utilizes NADPH as a cofactor. Substitution of NADH, in place of NADPH, did not enhance the microsomal enzyme activity, but it did enhance the enzyme activity in the cytosol preparations, as evidenced by the value given in parentheses. These results show that both the microsomal and cytosolic fractions contained enzyme systems capable of reducing NFT, but that the microsomal reductive enzyme was NADPH-specific whereas the cytosol enzyme was nonspecific and could use either NADH or NADPH as a cofactor.

*Stoichiometry of reduction.* Quantitative information on the extent of NADPH consumed per mole of substrate utilized could provide some clue to the mechanism of reduction; therefore, the stoichiometric relationship between NADPH consumption and NFT reduction was examined. The spectrophotometric procedures used in the present assay for the determination of unreduced NFT preclude the direct quantitation of NADPH utilization by conventional methods of measurement of absorbance at 340 nm. Hence, the reduction was carried out under limiting, known, amounts of NADPH with an excess of enzyme source. The reaction was allowed to proceed for about 3 h, so that the reaction might proceed to completion and all of the added NADPH might be utilized. Absorbance at 368 nm was measured in the supernatant fraction, and the disappearance of NFT was determined based on the extinction coefficient of NFT as 10.9 $mM^{-1}cm^{-1}$. Results of these experiments are presented in Table 2. They demonstrate that the extent of reduction of NFT was directly dependent upon the availability of NADPH. The ratio of added NADPH to the reduced NFT was about 3 in all cases, suggesting that about 3 moles of NADPH were utilized per mole of NFT reduced. This conforms with the possibility that the reduction of the 5-nitrofuran may proceed to the aminofuran through nitrosofuran and *N*-hydroxylaminofuran intermediates, with each step requiring a mole of NADPH.

*Chemical characterization of enzymatic reduction product.* Chemical characterization of the reduction product was carried out after enzymatic reduction and extraction with chloroform–ether. The extract was evaporated to dryness, the sample was dissolved in a small volume of methanol, and an aliquot was

Table 2. Stoichiometric relationship between NADPH availability and reduction of NFT*

| NADPH added ($\mu$moles) | NFT reduced ($\mu$moles) | NADPH added/NFT reduced |
|---|---|---|
| 1.0 | 0.28 | 3.58 ± 0.06 |
| 1.5 | 0.43 | 3.48 ± 0.06 |
| 2.0 | 0.63 | 3.18 ± 0.03 |
| 2.5 | 0.75 | 3.32 ± 0.04 |
| 2.75 | 0.95 | 2.90 ± 0.01 |
| 3.0 | 0.99 | 3.04 ± 0.02 |

* Microsomal preparations equivalent to 400 mg of liver tissue were incubated at 37° for 3 hr, under nitrogen, in the presence of 1 $\mu$mole NFT and 100 $\mu$moles Tris (pH 7.4) with various limiting amounts of NADPH in a 3-ml volume of reaction mixture. The assays were run in duplicate; the data are means ± S.E.

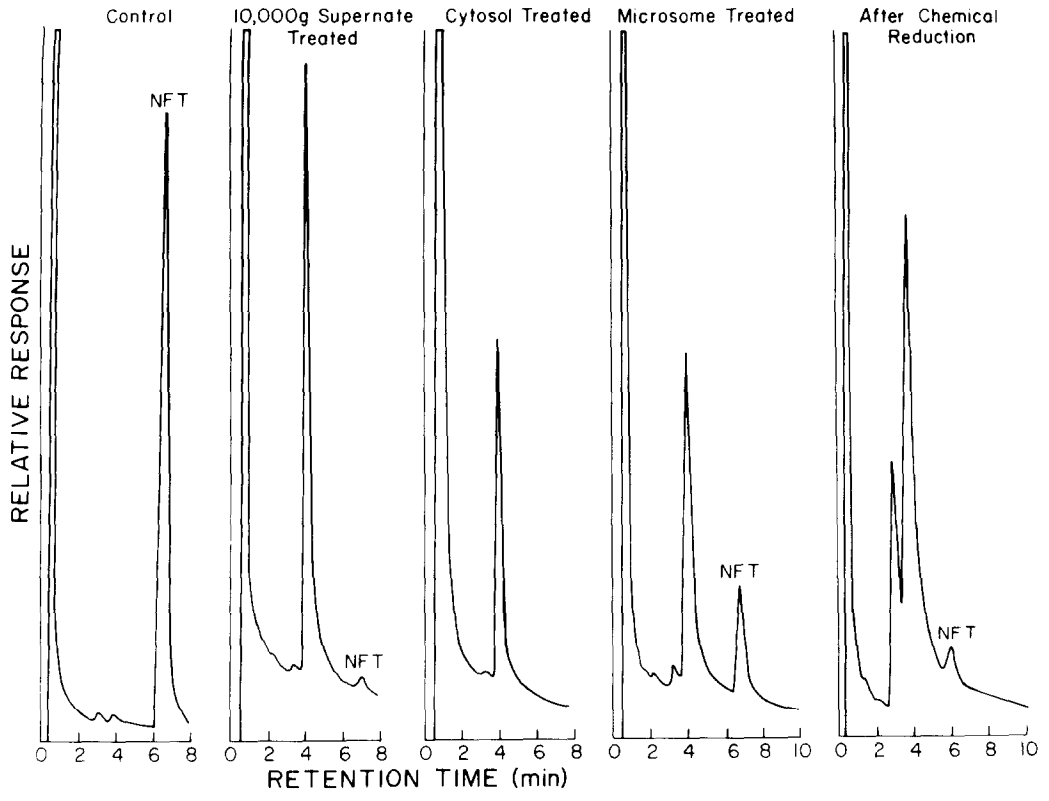


Fig. 1. Gas chromatographic elution profiles of the products of NFT formed by rat liver subcellular fractions and by chemical reduction. NFT was incubated for 1 hr with 200 mg wet weight of liver tissue at 37° under anaerobic conditions. The reaction mixture contained 1 μmole NFT, 3 μmoles NADPH, and 100 μmoles Tris (pH 7.4) in a total volume of 3 ml. At the end of incubation, the mixture was extracted with chloroform–ether, and the organic phase was evaporated to dryness. The dried material was dissolved in 2 ml methanol, and about 1 μl was injected into g.c., after 1 × 10 dilution, at an attenuation of 256. The procedure for chemical reduction and other g.c. parameters are described in Materials and Methods.

injected into the gas chromatograph under the conditions described in Materials and Methods. The g.c. profiles of NFT and those of the metabolite extracted after incubation with different subcellular fractions are presented in Fig. 1. The retention time of NFT was about 6.5 min, whereas that of the reduced product was 4 min. Furthermore, the reduction product appeared to be the same whether derived from the 10,000 *g* supernatant fraction, cytosol, or microsomal preparations.

Analysis by h.p.l.c. of the enzymatically reduced material showed a major peak at about 14 min. The elution time for the reduced product was the same irrespective of the type of subcellular fraction used as enzyme source. Since the establishment of the chemical identity of the reduced product required sufficiently large quantities, and the amounts of products that could be obtained by enzymatic reduction were limited, the feasibility of obtaining the necessary large amounts by chemical reduction was examined, using chemical reduction by catalytic hydrogenation of NFT with palladium on activated carbon.

*Chemical reduction of NFT and characterization of the product.* Chemical reduction of NFT was carried out under pressure by catalytic hydrogenation, using 5% palladium on activated carbon for 1 hr as described in Materials and Methods. The samples, before and after reduction, were subjected to g.c. under the same conditions used for the analysis of enzymatic reduction product. The g.c. profiles obtained are presented in Fig. 1. The chemically reduced material yielded two peaks with retention times of about 3.5 and 4 min, the latter being the same as that obtained by enzymatic reduction. The product was purified on silica gel column chromatography and then subjected to preparative h.p.l.c. Catalytically reduced NFT eluted by h.p.l.c. at the same time of 14 min as that of the sample obtained from enzymatic reduction. The purified material was beige in color, melted at 116–118°, and had no extinction coefficient of 7.24 $mM^{-1}cm^{-1}$, at 249 nm in methanol. The compound was quite stable at room temperature and could be stored for months in a freezer. The structural identity of the metabolite was based upon further spectroscopic analysis of chemically derived material.

*Spectroscopic analysis of the product obtained by catalytic hydrogenation.* The i.r. spectra of the starting material, NFT, and of the purified reduced product are presented in Fig. 2. The spectrum of chemically reduced NFT showed an absence of the bands at 1520 $cm^{-1}$ and 1360 $cm^{-1}$, characteristic of the

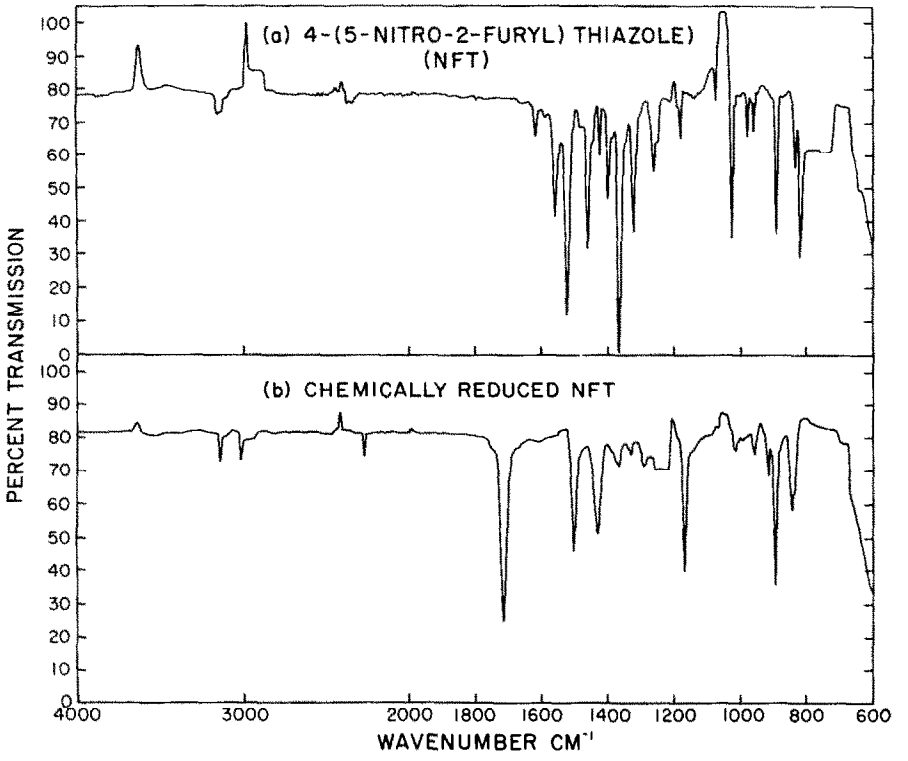


Fig. 2. Infrared spectra of NFT and of the purified product obtained from chemical reduction.

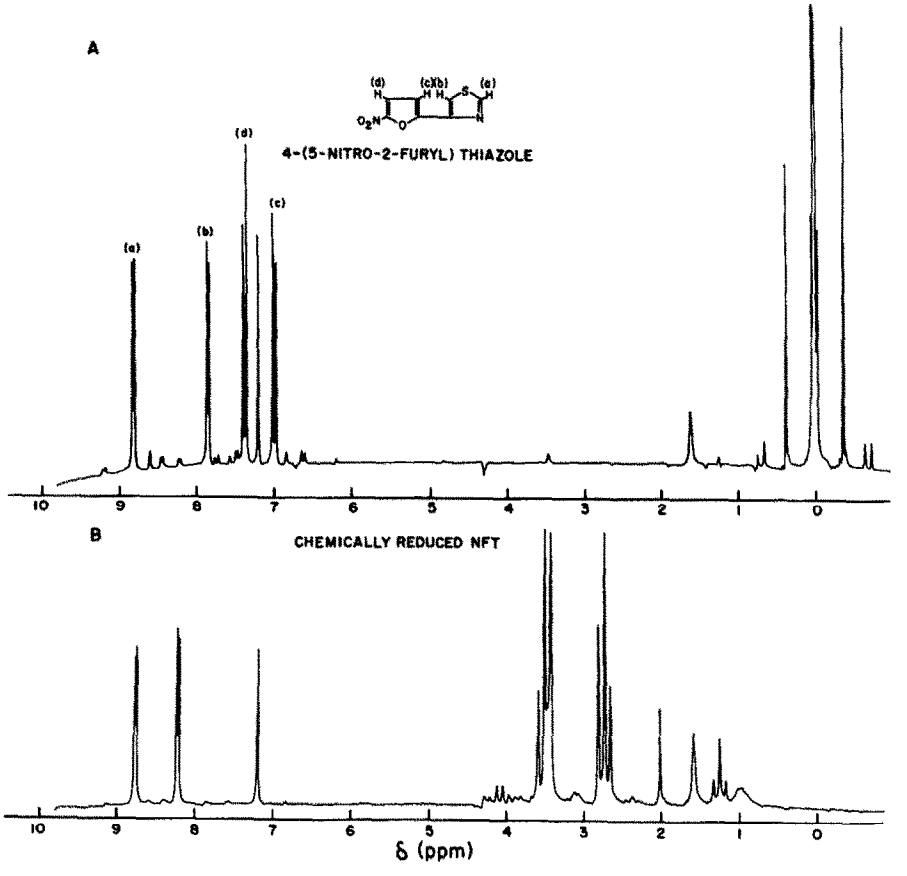


Fig. 3. Panel A: Proton-n.m.r. spectrum of NFT. Panel B: Proton-n.m.r. spectrum of the product obtained from chemical reduction.

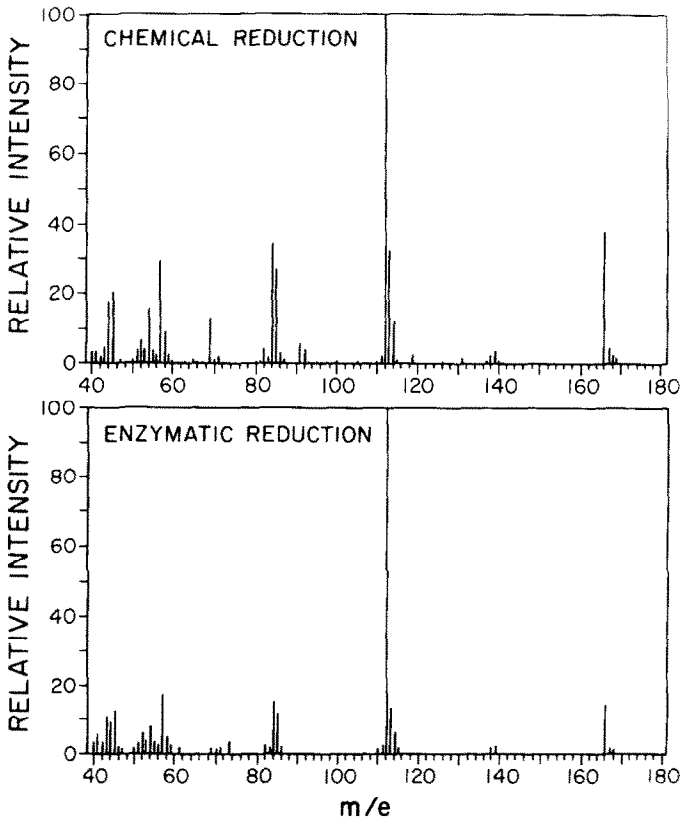


Fig. 4. Low resolution mass spectra of chemical and enzymatic reduction products. The mass spectra of the chemically reduced material were obtained by direct injection of the sample into the ion source in an AEI-MS9 mass spectrometer. The enzymatic reduction product spectrum was obtained from a DuPont 21-491B mass spectrometer, interfaced with a Varian 2740 GC.

nitro group, which were observable with NFT. Furthermore, new bands appeared at 1700 $cm^{-1}$ and 2250 $cm^{-1}$, which were absent in the starting material, the former being very characteristic of a carbonyl group, and the latter of a nitrile group.

The proton n.m.r. spectra of NFT and of the reduction product are shown in Fig. 3 (A and B). There are four protons in the NFT molecule, two in the thiazole ring, and two in the furan ring. The spectral assignments are shown in the structure presented at the top of Fig. 3A. The observed chemical shifts at 8.9 and 7.9 ppm correspond to the thiazole proton, and those at 7.4 and 7.0 ppm correspond to the furan ring. In the chemically reduced material, the peaks associated with the thiazole protons were still present (Fig. 3B), as evidenced by the peaks at $\delta = 8.9$ and 8.3 ppm, whereas the peaks in the region of the furan protons had disappeared, suggesting that upon reduction there is some modification in the furan ring. Further, in the reduced sample, four additional protons could be observed at $\delta = 3.5$ and 2.7 ppm. In much cleaner samples, these appeared as two sets of triplets, suggesting the presence of two methylene protons adjacent to one another.

The mass spectra of the chemically reduced and of the enzymatically reduced material are presented in Fig. 4. The molecular ion appeared at *m/e* 166 in both cases, and the fragmentation patterns were quite similar, with characteristic fragments at *m/e* 139, 112, 84 and 57. On the basis of the mass spectral data alone, the structure of the product could not be unambiguously ascertained, since the following possible reduction products—5-amino-2-furylthiazole (I), 1-(4-thiazolyl)-3-cyano-1-propanone (II), 5-(4-thiazolyl)-1,5-dehydro-2-pyrrolidinone (III) and 4-(5-oxo-2-pyrrolin-2-yl)thiazole (IV)—are isomeric with a molecular weight of 166. The i.r. and proton n.m.r. data, however, suggesting the presence of a carbonyl group and the absence of furan ring protons, are not in conformity with structure I. The proton n.m.r. spectrum suggested that two methylene protons were adjacent to one another, which is compatible with structures II and III, but not with I and IV. Furthermore, i.r. data showed a weak nitrile band, favoring structure II, but the intensity of the peak was very weak.

To differentiate between II and III, $^{13}C$-n.m.r. was performed and the spectrum showed seven different chemical shifts in conformity with the total number of carbon atoms in the molecule. The presence of a carbonyl carbon was strongly suggested by the presence of a peak at $\delta = 190.30 \pm 0.05$ ppm for, in the literature, the aliphatic carbonyl ketones have been reported to absorb the farthest downfield, around 190 ppm [19]. Similarly, a band was observed at $\delta = 118.59 \pm 0.05$ ppm, which is very characteristic of the nitrile carbon. The spectral assignments for the rest of the carbons are tentatively assigned based on the values reported in the literature for related model compounds [20]; the observed chem-

ical shifts were at $\delta = 35.8$, 11.2 (methylene carbons 2 and 3), 153.2, 153.9 and 125.8 (carbons 2, 4 and 5 of the thiazole ring respectively). Thus, the $^{13}C$-n.m.r. spectrum and other spectral data (Figs. 2–4) establish the structural identity of the chemically reduced metabolite to be 1-(4-thiazolyl)-3-cyano-1-propanone (II). Since the enzymatically obtained metabolite had chromatographic properties and mass spectra identical to those of the chemically obtained product, structurally they must be identical.

## DISCUSSION

The spectral changes associated with the transformation of NFT show that the reactions proceed favorably under anaerobic conditions and in the presence of NADPH, suggesting the reductive nature of the metabolic transformation. The functional group subject to reduction appeared to be the 5-nitro group, as evidenced by i.r. spectra (Fig. 2). The starting material was found to have characteristic bands at frequencies corresponding to that of the 5-nitro group, whereas with the reduced material these characteristic bands disappeared. Furthermore, on reduction there was a gradual loss of absorption bands at wavelengths around 370 nm, a region associated with the main chromophore, the 5-nitro group. Correspondingly, there was a concomitant appearance of new bands at lower wavelengths. Similar hypsochromic shifts in the absorption spectra have been reported to accompany nitro reduction in the case of a number of other 5-nitrofurans, such as 5-nitro-2-furaldehyde semicarbazone derivatives [21, 22], 1-[(5-nitrofurfurylidene)amino]hydantoin (nitrofurantoin) [22], and 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) [23].

Examination of rat subcellular fractions (Table 1) showed that the nitroreductase activity was localized in both the cytosolic and the microsomal fractions. There were apparently two enzyme systems differing in their cofactor requirements and exhibiting variable inhibiton in the presence of oxygen. The microsomal fraction was NADPH-specific, whereas the cytosol preparation was NADPH-nonspecific and could utilize either NADH or NADPH as cofactor. A number of carcinogenic 5-nitrofurylthiazole derivatives were shown earlier to be reduced by rat liver homogenates as well as by xanthine oxidase (EC 1.2.3.2), aldehyde oxidase (EC 1.2.3.1), and NADPH-cytochrome *c* reductase (EC 1.6.99.1), with enzyme activities being localized in cytosolic and microsomal fractions [14, 23–28], as observed for NFT in the present studies. The rat cytosol reductase was reported to be dependent on NADH and inhibited by allopurinol; hence it was attributed to xanthine oxidase [25, 26]. In contrast, the microsomal reductase was shown to be dependent on NADPH, and attributed to NADPH-cytochrome *c* reductase [26]. In view of the broad substrate specificity of these nitroreductases and the close structural similarity between the substrates used, it is quite likely that the same enzymes are involved in the reduction of NFT as well.

Examination of the stoichiometric relationship between NADPH availability and that of NFT reduction by microsomes indicated that about 3 moles of NADPH were consumed per mole of NFT

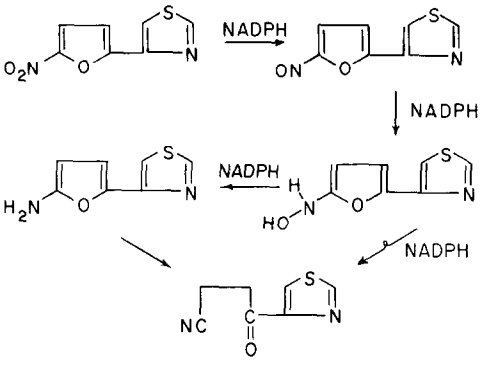


Fig. 5. Postulated metabolic pathway for NFT reduction.

reduced (Table 2). Similarly, in the reduction of nitrofurazone and AF-2 by xanthine oxidase with xanthine as proton donor, uric acid was formed at the rate of about 2.8 moles/mole of 5-nitrofuran reduced [23]. The above stoichiometric observations indicate that reduction may proceed to the aminofuran via the corresponding nitroso and *N*-hydroxylamino intermediates, with each step requiring a mole of NADPH for reduction.

The results of the spectroscopic and chromatographic analyses of chemically and enzymatically reduced NFT clearly demonstrate the structural identity of the reduction product to be 1-(4-thiazolyl)-3-cyano-1-propanone. Although earlier *in vitro* metabolic studies on the carcinogenic 5-nitrofurylthiazoles suggested the involvement of a 5-nitro group in reduction, this is the first time a product of reduction has been isolated and its structural identity established. The postulated metabolic pathway for the reduction of NFT is presented in Fig. 5, wherein the 5-nitro group has been suggested as the primary site of reduction, with reduction proceeding to the amino analog through the nitroso and *N*-hydroxylamino intermediates. Since the aminofurans are generally unstable, they might undergo furan ring fission to yield the open chain nitrile derivative.

Formation of open chain nitrile derivatives from 5-nitrofurans was first proposed by Beckett and Robinson [21, 22], based on their studies of the ultraviolet absorption spectra and polarography of bacterial reduction products of certain 5-nitrofurans, such as nitrofurazone and nitrofurantoin. Subsequently, Gavin *et al.* [29] reported that *Escherichia coli* metabolized 1-[(5-nitrofurfurylidene)amino]-2-imidazolidinone to a product whose melting point and i.r. spectrum resembled that of chemically derived 3-(4-cyano-2-oxobutylideneamino)-2-imidazolidinone. Chatfield [30] reported that after oral administration of 2-(5-nitro-2-furyl)-4-(thiomorpholino-iminomethyl)thiazole-1′,1′-dioxide, a urinary metabolite was isolated whose i.r. and m.s. characteristics were compatible with the corresponding open chain nitrile derivative. Recently, Aufrere *et al.* [31] demonstrated that nitrofurantoin was metabolized by a number of rat tissues to give a major metabolite that was isolated and characterized by spectroscopic methods as 1-{[(3-cyano-1-oxopropyl)methylene]amino}-2,4-imidazolidinedione.

Recently, Tatsumi *et al.* [23] observed that milk and rat liver xanthine oxidase and microsomes reduced nitrofurazone and AF-2. The nitrile compound appeared to be the main product of reduction in the case of nitrofurazone while, in contrast to the nitrile, 2-(2-furyl)-3-(5-oxo-2-pyrroline-2-yl)acrylamide was tentatively identified as the product of AF-2.

Although there is definitive evidence for the formation of open chain nitrile derivatives, the exact sequence and the mechanism of nitroreduction are not established. The fission of the furan ring to yield the nitrile derivative could occur at the stage of the aminofurans, as has been proposed by Beckett and Robinson [21, 22], or at the stage of the *N*-hydroxylaminofuran through a *trans*-oxime derivative as proposed by Gavin *et al.* [29]. For bactericidal, mutagenic, and carcinogenic activities [2, 3, 11, 13, 32], however, as well as for macromolecular binding [15, 16], the 5-nitro group is vital, since analogs lacking the 5-nitro group were devoid of these activities. In this, the 5-nitro group *per se* does not appear to be the active functional group, but requires reduction to chemically reactive intermediates. In view of the reactivity of the intermediates of nitroreduction—namely, the nitroso and *N*-hydroxylamino intermediates—it is quite likely that they represent the biologically active forms involved in bactericidal, mutagenic, or carcinogenic activity.

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