Biochemical Pharmacology, Vol. 32, No. 1, pp. 21–27, 1983.
Printed in Great Britain.

0006-2952/83/010021–07 $03.00/0

DEACETYLATION OF 4-(5-ACETYLAMINO-2-FURYL)THIAZOLE AND FORMATION OF 1-(4-THIAZOLYL)-3-CYANO-1-PROPANONE BY RAT LIVER TISSUES*

SANTHANAM SWAMINATHAN† and GEORGE T. BRYAN
Department of Human Oncology, Wisconsin Clinical Cancer Center, University of Wisconsin, Center for Health Sciences, Madison, WI 53792, U.S.A.

(*Received* 9 *January* 1982; *accepted* 7 *May* 1982)

Abstract—The reductive metabolism of the rat carcinogen 4-(5-nitro-2-furyl)thiazole (NFT) to 1-(4-thiazolyl)-3-cyano-1-propanone (TCP) is reported. Formation of TCP from NFT involved furan ring fission. This could have occurred through involvement of either aminofuran or *N*-hydroxylaminofuran as precursors. To examine if 4-(5-amino-2-furyl)thiazole is a precursor for TCP, a stable model compound, 4-(5-acetylamino-2-furyl)thiazole (AAFT), was prepared and subjected to enzymatic deacetylation, using rat liver tissue homogenates. AAFT was synthesized by catalytic hydrogenation of NFT with 5% palladium on activated carbon, followed by acetylation with acetic anhydride. AAFT, a white crystalline powder, melted at 168–170°, had an extinction coefficient of 17.9 mM^{-1} cm^{-1} at 293 nm in ethyl acetate, and exhibited spectroscopic and mass spectral characteristics consistent with the assigned structure. Incubation with rat liver 10,000 *g* supernatant preparations resulted in the biotransformation of AAFT as evidenced by a decrease in absorption at 290 nm. Incubation of ^{14}C-labeled AAFT followed by extraction with chloroform–diethyl ether (1:1) resulted in the recovery of a major portion (56%) of the radioactivity in the organic phase when the label was at the 2-position of the thiazole ring, while the major amount (82%) of radioactivity was recovered in the aqueous phase when the 1-^{14}C-acetyl group was labeled. The radioactivity from the aqueous phase was extractable into the organic phase following acidification to pH 1, an observation consistent with deacetylation. Furthermore, the deacetylation product exhibited a mass spectrum, and retention times in gas and high pressure liquid chromatography, similar to those of synthetic TCP. These data establish 4-(5-amino-2-furyl)thiazole, derived from AAFT by deacetylation, as a precursor for TCP.

In recent years a large number of 5-nitrofurans were shown to be genotoxic [1] and carcinogenic [2]. These observations stimulated investigations concerning the biotransformation and molecular mechanisms of carcinogenicity of 5-nitrofurans. A variety of biotransformations involving modifications in the side chains [3, 4], hydroxylation of the furan ring [5, 6], co-oxidation by prostaglandin endoperoxide synthetase [7, 8], and nitroreduction [9–14] were reported. Structure–activity relationship studies using FANFT‡, FNT, or NFTA and their corresponding nor-nitro analogs showed that the nor-nitro analogs were devoid of mutagenic and oncogenic activities [15–20]. In addition, 5-nitrofurans exhibited positive responses in nitroreductase-competent bacterial mutagenic assays [1, 21], while they were negative when tested in a nitroreductase-deficient strain derived from the same parent organism [22]. Using rat liver preparations and ^{14}C-labeled NFT, FANFT, ANFT or NFTA, we have demonstrated that reactive electrophiles are generated under reductive conditions as evidenced by their covalent binding with nucleic acids and proteins [13, 23–26]. For these reasons, we focused our attention on the nitroreduction of these compounds. Recently in the case of NFT, we identified a product of nitroreduction as TCP, and postulated a metabolic pathway for its reductive bioconversion [9]. In this pathway, the 5-nitro group was suggested as the primary site of reduction, with postulated nitroso, *N*-hydroxylamine, and amine intermediates. TCP could be generated by fission of the furan ring from the aminofurans or *N*-hydroxylaminofurans through a *trans*-oxime derivative [27]. Extreme lability and reactivity of these intermediates precluded their direct isolation and identification. To examine if 4-(5-amino-2-furyl)thiazole is a precursor for TCP, we synthesized a stable derivative AAFT and subjected it to enzymatic deacetylation procedures under conditions where *N*-oxidation could not occur. The deacetylation product formed

* Preliminary results were published previously in *Proc. Am. Ass. Cancer Res.* **22**, 104 (1981).

† Author to whom all correspondence should be sent: Department of Human Oncology, K4/548, Clinical Science Center, 600 Highland Ave., Madison, WI 53792, U.S.A.

‡ Abbreviations: FANFT, *N*-[4-(5-nitro-2-furyl)-2-thia-zolyl]formamide; ANFT, 2-amino-4-(5-nitro-2-furyl)thiazole; FNT, formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide; NFTA, *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide; NFT, 4-(5-nitro-2-furyl)thiazole; TCP, 1-(4-thiazolyl)-3-cyano-1-propanone; AAFT, 4-(5-acetylamino-2-furyl)thiazole; TMS, tetramethyl silane; TLC, thin-layer chromatography; GC, gas–liquid chromatography; HPLC, high pressure liquid chromatography; u.v., ultraviolet; IR, infrared; NMR, nuclear magnetic resonance; MS, mass spectrum; TCA, trichloroacetic acid; and DMAB, *p*-dimethylaminobenzaldehyde.

under these conditions was isolated and characterized. The results presented here suggest that 4-(5-amino-2-furyl)thiazole could act as a direct precursor for TCP, and that *N*-hydroxylamine and the subsequent *trans*-oxime are not obligatory intermediates for TCP biosynthesis.

MATERIALS AND METHODS

Sources of materials. NFT, purchased from Saber Laboratories Inc., Morton Grove, IL, was recrystallized in ethanol, and the crystalline preparations were >99% pure as evaluated by chromatographic methods. Palladium (5%) on activated carbon, deutero chloroform, TMS, and acetic anhydride were obtained from the Aldrich Chemical Company Inc., Milwaukee, WI. Methanol, ethyl acetate, ethylene dichloride, and chloroform were obtained from Burdick and Jackson Laboratories Inc., Muskegan, MI. [1-^{14}C]Acetic anhydride was bought from the Amersham Corp., Arlington Heights, IL, and [^{14}C]formamide from the California Bionuclear Corp., Sun Valley, CA. Sprague–Dawley, 150–200 g, female rats were purchased from Sprague–Dawley, Madison, WI.

Syntheses of TCP and AAFT. TCP was synthesized by chemical reduction of NFT as described [9]. AAFT synthesis involved two steps. The first step was the nitroreduction of NFT to the amine, and the second step was the acetylation of the amine with acetic anhydride. NFT (1 mmole) was dissolved in 200 ml of ethyl acetate to which 1 g of 5% palladium on activated carbon was carefully added. Catalytic hydrogenation was performed as described [9]. At the end of the reaction, about 5 g of magnesium sulfate was added, mixed well, and filtered under vacuum. Pyridine (0.5 ml) and 5.0 ml of acetic anhydride were immediately added to the filtrate, and it was then concentrated to about 5 ml. The acetylation was allowed to proceed for 4 hr at 25°. At the end of the reaction, 10 ml of water was added, and the solution was evaporated to dryness under vacuum. The residue was redispersed in 10 ml of ice-cold water, filtered, and dried. The light-brown material (73 mg) obtained from filtration was chromatographed on a column of silica gel 60 using a mixture of ethyl acetate–*n*-hexane (3:1) as the eluting solvent. The eluate was monitored at 280 nm. The chromatogram revealed two peaks, an early eluting minor peak, followed by AAFT. The fractions containing AAFT were pooled and evaporated to dryness, and the dried amorphous powder recovered was about 0.3 mmole.

Synthesis of [^{14}C]AAFT labeled at the 1-position of the acetyl group involved the same procedure described above, except that 500 μCi of [1-^{14}C]acetic anhydride was mixed with 1.0 ml of cold acetic anhydride and was used for acetylation. The radiochemical purity of the product was assessed by TLC, followed by measurement of radioactivity in a liquid scintillation counter. The specific activities of the final preparations were about 2 μCi/mmole. Synthesis of [2-^{14}C]AAFT involved first the preparation of [2-^{14}C]NFT as described [10]. This was then further subjected to reduction and acetylation as described above. The specific activities of the final preparations were about 1 μCi/mmole. GC and TLC analyses indicated the radiochemical purity to be >98%.

Chromatography and spectroscopy. GC was performed under the same conditions described earlier [9], except for the temperature of the oven which was maintained at 210° in the present studies.

HPLC was done using a Micromeritics 7000 B equipped with a model 730-universal injection valve and a fixed wavelength (254 nm) detector. The instrument was fitted with a 25 cm, Whatman partisil 5 column, and the chromatograms were run isocratically under constant flow mode using a mixture of methanol–ethylene dichloride (1:49, v/v) as the eluting solvent.

TLC was performed on No. 13181 silica gel plates (Eastman Kodak Co., Rochester, NY), using an ethyl acetate–*n*-hexane (1:1, v/v) mixture as the developing solvent. The R_f values observed for AAFT and TCP were 0.23 and 0.43 respectively.

Ultraviolet absorption measurements were made using a Beckman 25 recording spectrophotometer with 1 cm path length quartz cuvettes. Infrared spectra of the samples were obtained in a Beckman Accu Lab 4 double beam IR-spectrometer using 0.107 cm wide sodium chloride cells. Proton NMR spectra were obtained with a Jeol FX-90 Q, Fourier transform NMR spectrometer. About 5 mg of AAFT was dissolved in 0.4 ml of deutero chloroform containing a trace amount of TMS as internal standard. Data from 100 sweeps were computed for the Fourier transform.

Low resolution MS of AAFT was obtained by direct injection of the sample into the ion source, at an initial inlet temperature of 170°, in an AEI-MS-9 mass spectrometer. GC/MS was carried out in a Hewlett–Packard 5930A mass spectrometer, interfaced with a Hewlett–Packard 5700A GC, at an ionizing voltage of 70 eV. The gas chromatograph was fitted with a coiled glass column 180 × 0.2 cm, containing 6.5% OV-1 on 80/100 mesh chromosorb WHP. Chromatograms were run isothermally at 180° with helium as carrier gas, and at a flow rate of about 20 cc/min. The temperature of the source and filter was 220°.

Assay for AAFT deacetylation. Rat liver subcellular fractions needed for the assays were prepared as described [9]. The total volume of the reaction mixture was 7.0 ml and contained 3.5 μmoles AAFT dissolved in 0.2 ml methanol, 350 μmoles Tris buffer, pH 7.4, 0.5 mmole manganese chloride and rat liver subcellular fractions equivalent to about 500 mg wet weight of tissue. The reactions were conducted at 37° under aerobic conditions, unless otherwise specified. They were terminated by the addition of 1 ml of 30% TCA to 2 ml of the reaction mixture, and the substrate utilization was monitored spectrophotometrically. Absorbance at 290 nm was measured in a Beckman 25 spectrophotometer after centrifugation of the TCA precipitate and dilution of the supernatant fraction with 2 vol. of water. Appropriately treated blanks were used in the reference beam to correct for absorbance from extraneous components. For measurement of the amine, a 2-ml aliquot of the reaction mixture was added to 2.0 ml of DMAB reagent (500 mg DMAB in 50 ml ethanol diluted with 50 ml of 1 M acetate–HCl buffer at pH

1.4). Absorbance at 404 nm was measured after 1 hr following centrifugation at 12,000 *g* for 20 min.

In experiments involving isolation and characterization of the deacetylation product, the reaction mixture was extracted five times with an equal volume of ethyl acetate. The extracts were pooled, evaporated to dryness, and solubilized in appropriate solvents for chromatographic and spectroscopic analyses.

RESULTS

Synthesis and chemical characteristics of AAFT. Purified AAFT was white in color, melted between 168 and 170°, and had an extinction coefficient of 17.9 mM^{-1} cm^{-1}, at 293 nm in ethyl acetate. The compound was quite stable at 25° and could be stored for months at −20°. The authenticity of its structure was established by spectroscopic analysis. The IR spectrum (in chloroform) had bands at 3400, 1710, and 1015 cm^{-1} corresponding with >NH, >C=O, and furan—CH respectively. The proton NMR spectrum of AAFT revealed chemical shifts at 8.8 and 7.3 ppm corresponding with the thiazole protons, and at 6.8 and 6.4 ppm corresponding with the furan ring protons. This showed the intactness of both furan and thiazole nuclei during reduction and acetylation steps. The singlet observed at $\delta =$ 2.2 ppm corresponded to the three protons of the methyl group. The N—H proton resonance was broad and weak, but definite as evidenced by the observed chemical shift at $\delta = 7.9$ ppm.

The MS showed the molecular ion at *m/e* 208 ($C_9H_8N_2O_2S$) with additional fragment ions at *m/e* 166 $(M—C_2H_2O)^+$, 139 $(M—C_3H_3NO)^+$, 122 $(M—C_3H_4NO_2)^+$, 112 $(M—C_5H_6NO)^+$, and 85 $(M—C_6H_5NO_2)^+$. The relative abundance of the ions observed, along with a possible fragmentation pattern, are shown in Fig. 1. The *m/e* of the molecular ion, the fragmentation pattern, and other spectroscopic data are consistent with the structure of AAFT, and thus the chemical identity of AAFT was established.

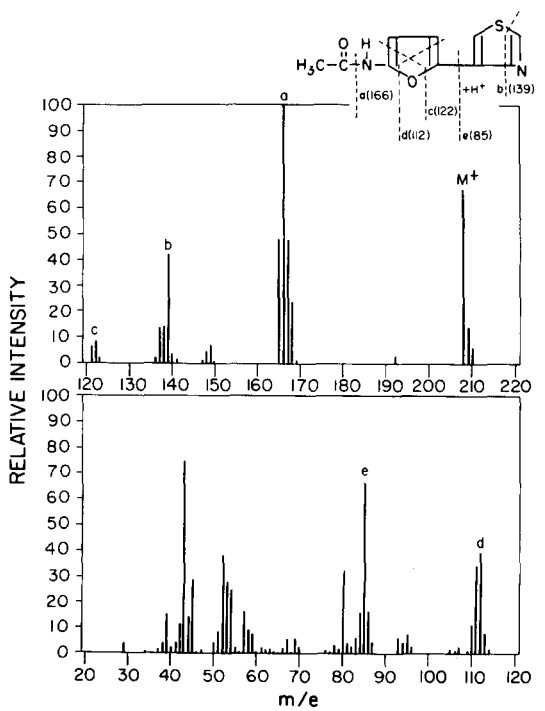

Fig. 1. Low resolution mass spectrum of AAFT obtained by direct injection of the sample into the ion source.

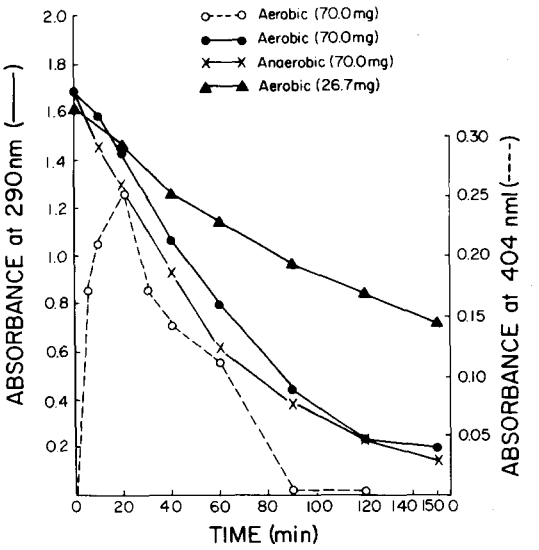

Fig. 2. Time course for the deacetylation of AAFT by rat liver 10,000 *g* supernatant preparations. AAFT (0.53 mM) was incubated for various periods of time at 37° using rat liver 10,000 *g* supernatant preparations. Anaerobic incubation was carried out as described [9]. Absorbance was measured at 290 nm and at 404 nm following precipitation with TCA or reaction with DMAB as described in Materials and Methods. The amount of tissues used per ml of the reaction mixture is given above.

Biotransformation of AAFT in vitro. The *in vitro* biotransformation of AAFT was monitored spectrophotometrically by measuring the disappearance of the substrate as evidenced by the decrease in absorbance at 290 nm. Figure 2 represents the time course of the reaction and, as shown, under the experimental conditions described, most of the AAFT disappeared in about 2 hr. The extent of substrate disappearance was dependent upon the amount of rat liver 10,000 *g* supernatant fraction in the assay mixture. The reaction proceeded favorably under both aerobic and anaerobic conditions. No exogenous addition of cofactors or reducing agents was mandatory, suggesting that the reaction was not a reduction.

Following enzymatic reaction, coupling with DMAB gave a colored complex, suggesting the generation of an amino group during the reaction. The time course of the formation of the colored complex and that of the utilization of substrate measured simultaneously are presented in Fig. 2. As shown, they were not inversely related throughout the time course. While the substrate utilization occurred up to 2 hr, as evidenced by the decrease in absorbance at 290 nm, the concentration of the product peaked within 20 min. This may be due to the instability of

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

Enzyme source	AAFT utilized [μmoles·(g liver)$^{-1}$·hr^{-1}]
Whole homogenate	3.98 ± 0.17
Boiled homogenate	0.14 ± 0.11
10,000 *g* Supernatant fraction	4.20 ± 0.07
Microsomal fraction	0.32 ± 0.16
105,000 *g* Supernatant fraction	3.74 ± 0.08
Microsomal plus 105,000 *g* supernatant fraction	4.92 ± 0.22

* In this experiment, AAFT (1.75 μmoles) was incubated for 1 hr at 37° with the different subcellular fractions equivalent to 200 mg wet weight of liver under the conditions described in Materials and Methods. The assays were run in duplicate; the data are means ± S.E.

the deacetylation product, 4-(5-amino-2-furyl)-thiazole, which might have a very short half-life resulting in the failure to quantitate it accurately during the entire time course of the reaction. This is further substantiated by the following studies on the isolation and characterization of the product, indicating that the final product of the reaction is not the amino analog but a stable keto nitrile, TCP, derived from the amine.

Table 1 shows the subcellular localization of the enzyme activity in rat liver tissues. As shown, boiling abolished the enzyme activity. Most of the activity present in the homogenate was recovered in the 10,000 *g* supernatant fraction. Further fractionation revealed that most of the activity was recovered in the cytosolic preparations. Manganese in the range of about 20–100 mM enhanced the enzyme activity significantly. In its absence, the enzyme activity was about half that of the maximal activity.

Deacetylation of [^{14}C]*AAFT.* The formation of a colored complex with DMAB, following enzymatic reaction of AAFT, suggested that AAFT is possibly deacetylated, resulting in the generation of the free amine. To further examine whether the *in vitro* biotransformation involves a deacetylation step, [^{14}C]AAFT, labeled at the carboxyl group of the *N*-acetyl function or at the 2-position of the thiazole nucleus, was synthesized, and the distribution of radioactivity monitored in the aqueous and organic phases following deacetylation. Following incubation of ^{14}C-labeled AAFT with rat liver 10,000 *g* supernatant fractions, the samples were extracted five times with chloroform–diethyl ether (peroxide-free) (1:1), and the radioactivity was measured in the various phases. Table 2 shows the relative distribution of the radioactivity in the chloroform–diethyl ether phase, the aqueous phase, and the interphase. When AAFT radiolabeled at the acetyl group was used, about 82% of the recovered radioactivity remained in the aqueous phase, after incubation with rat liver 10,000 *g* preparations. With the same incubation system, use of either boiled enzyme or deletion of the enzyme resulted in the recovery of <4% of the radioactivity in the aqueous phase, suggesting that the reaction was enzyme mediated. When the aqueous phase obtained following incubation with the 10,000 *g* supernatant fraction was acidified to pH 1.0, and then extracted with the chloroform–diethyl ether mixture, most of the radioactivity was recovered in the organic phase. This observation is consistent with the biotransformation being a deacetylation reaction. The polar acetate

Table 2. Distribution of radioactivity in various phases following incubation of [^{14}C]AAFT with rat liver 10,000 *g* supernatant preparations*

	Percentage of radioactivity recovered					
	N-[1-^{14}C]AAFT			[2-^{14}C]AAFT		
Incubation system	Organic phase	Aqueous phase	Inter-phase	Organic phase	Aqueous phase	Inter-phase
10,000 *g* Supernatant fraction	12.3	81.6	6.1	56.0	30.1	13.9
Boiled 10,000 *g* supernatant fraction	89.5	1.6	8.9	83.4	5.9	10.7
Absence of 10,000 *g* supernatant fraction	88.4	3.9	7.7	81.7	10.0	8.3

* *N*-[1-^{14}C]AAFT (2.5 μmoles, 2 μCi/mmole) or [2-^{14}C]AAFT (3.5 μmoles, 1 μCi/mmole) was incubated with the rat liver 10,000 *g* supernatant preparations for 2 hr at 37°. Following incubation, they were diluted to 10 ml with water and extracted five times with equal volumes of chloroform–diethyl ether (1:1). The various phases were separated by centrifugation and the organic phase was pooled. The radioactivity measurements were made using a liquid scintillation counter, and relative distribution in various phases was calculated. In the extraction system used, the ratio of distribution of TCP was 3.4:1 (organic phase:aqueous phase).

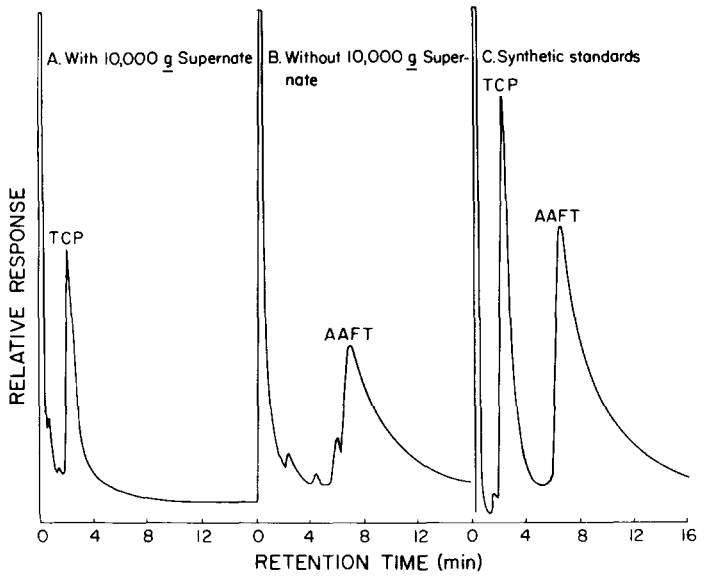

Fig. 3. Gas chromatographic elution profiles of the ethyl acetate extract following incubation of AAFT: (A) with rat liver 10,000 *g* supernatant preparation; (B) without rat liver 10,000 *g* supernatant preparation; and (C) synthetic AAFT and TCP. The ethyl acetate extracts were prepared, as described in Materials and Methods, and evaporated to dryness. The dried material was dissolved in 10 ml for run A, and in 4 ml for run B. About 0.5 and 1.0 μl were respectively injected into the gas chromatogram at an attenuation of 256. A 1-μl mixture containing 20 ng TCP and 500 ng AAFT in ethyl acetate was used for getting the tracings of the synthetic standards, run C.

anion produced during deacetylation remained in the aqueous phase under incubation conditions. Acidification suppressed the ionization and, thus, facilitated partitioning into the organic phase.

When the [^{14}C]AAFT, labeled at the 2-position of the thiazole ring, was incubated under the same conditions, about 56 and 80% of the recovered radioactivity were in the organic phase, in the presence or absence of active enzyme respectively. TLC of the chloroform–diethyl ether extract of the control samples, which either contained boiled enzyme or lacked it, revealed only one radioactive spot with an R_f value of 0.25, corresponding with AAFT. In contrast, the TLC of the test samples showed no radioactivity corresponding with AAFT, but showed activity at two different spots, one at the origin and another with an R_f value of 0.45. The R_f value of the latter spot corresponded with that of synthetic TCP.

Chemical characterization of the product. Chemical characterization of the product was carried out after incubation of AAFT with the 10,000 *g* supernatant fraction followed by extraction with ethyl acetate. The ethyl acetate extract was evaporated to dryness, the sample was dissolved in a small volume of ethyl acetate, and an aliquot was injected into the gas chromatograph under the conditions described in Materials and Methods. The gas chromatographic profiles of the extract obtained after incubation under different conditions are given in Fig. 3. Figure 3A and 3B show the chromatographic profile of the extract prepared after incubation of AAFT with or without 10,000 *g* supernatant fraction. Figure 3C shows the pattern obtained with synthetic TCP and AAFT, and the observed retention times were 2.0 and 7.0 min respectively. In the absence of the 10,000 *g* supernatant fraction (Fig. 3B), the major peak observed was at 7.0 min, corresponding with AAFT. In contrast, incubation with 10,000 *g* supernatant fraction resulted in the disappearance of the peak at about 7.0 min, but instead a new peak appeared at 2.0 min, similar to that seen with synthetic TCP. Further confirmation of the product of deacetylation as TCP was established, based on HPLC and MS studies.

Analysis by HPLC of the enzymatic product showed a major peak at about 7 min, while that of synthetic AAFT eluted at about 15 min (Fig. 4). Synthetic TCP, when run under the same conditions, exhibited a similar retention time (7 min) as that of enzymatic reduction product. Furthermore, the MS of the enzymatic product (Fig. 5) showed a molecular ion at 166 and also exhibited a similar fragmentation pattern as that of synthetic TCP. Since the enzymatically obtained metabolite had chromatographic properties and MS identical to those of the chemically synthesized TCP, structurally they must be identical.

DISCUSSION

To examine whether 4-(5-amino-2-furyl)thiazole is a precursor of TCP, a synthetic model compound, AAFT, was prepared by catalytic hydrogenation of NFT, followed by acetylation with acetic anhydride, and its authenticity was established. AAFT was rapidly metabolized *in vitro* (Fig. 2). Enzyme activity was present primarily in the cytosol fraction (Table 1). The reaction product gave a colored complex

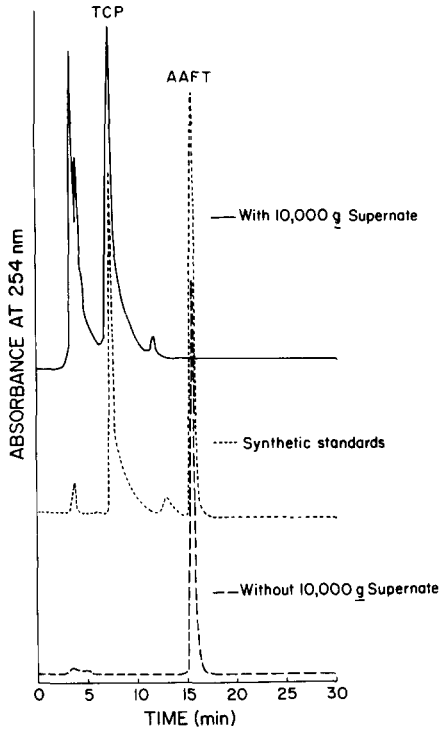

Fig. 4. High pressure liquid chromatographic traces of the deacetylation product of AAFT. The ethyl acetate extracts were dispersed finally in 4.0 ml of methanol, and an aliquot was injected into a Micromeritics 7000 B high pressure liquid chromatograph equipped with a fixed wavelength (254 nm) detector at a sensitivity of 0.1 AUFS. The chromatograms were run isocratically at a flow rate of 1 ml/min using a methanol–ethylene dichloride (1:49, v/v) mixture as the eluting solvent: Key: (——) 30 μl extract after incubation with rat liver 10,000 *g* supernatant preparation; (· · · ·) 10 μl mixture of synthetic TCP (0.4 μg) and AAFT (1.0 μg); and (– – –) 10 μl extract after incubation without rat liver 10,000 *g* supernatant preparation.

with DMAB, suggesting the presence of an amino group and implying deacetylation of AAFT. When [^{14}C]AAFT labeled at the acetyl group was incubated with rat liver preparations, and then extracted with chloroform–diethyl ether, most of the radioactivity remained in the aqueous phase, in contrast to that of the control or when the label was in the thiazole nucleus (Table 2). Attempts to isolate the amine have not been successful, probably due to its instability. This is evidenced from Fig. 2, illustrating the kinetics of the biotransformation reaction. Although the amino analog could not be isolated, a stable product presumably derived from it could be isolated. This product had the same R_f values in TLC, similar retention times in GC and HPLC (Figs. 3 and 4), and MS fragmentation patterns (Fig. 5) identical to those of synthetic TCP.

Although the formation of keto nitrile from 5-nitrofurans on reduction was reported earlier by us [9, 10] and other workers [14, 28–30], the exact sequence and the mechanism of nitroreduction are not established. In the postulated pathway for the biosynthesis of TCP from NFT, the furan ring cleavage to the keto nitrile could occur at the stage of *N*-hydroxylaminofurylthiazole after tautomerisation to a *trans*-oxime and elimination of water. Alternatively, it could occur at the stage of the aminofurylthiazole, involving hydrolysis followed by the keto-enol tautomerisation and subsequent elimination of water to yield the final keto nitrile. The formation of TCP from AAFT on deacetylation implies the occurrence of the latter pathway. The other pathway for the synthesis of TCP from AAFT involves *N*-oxidation and tautomerisation to the *N*-oxime, followed by furan ring cleavage to yield 1-(4-thiazolyl)-3-cyano-2,3-dehydro-1-propanone which is then reduced to TCP (Fig. 6). Under the conditions of incubation, no *N*-oxidation is likely to occur since no exogenous NADPH was added to the

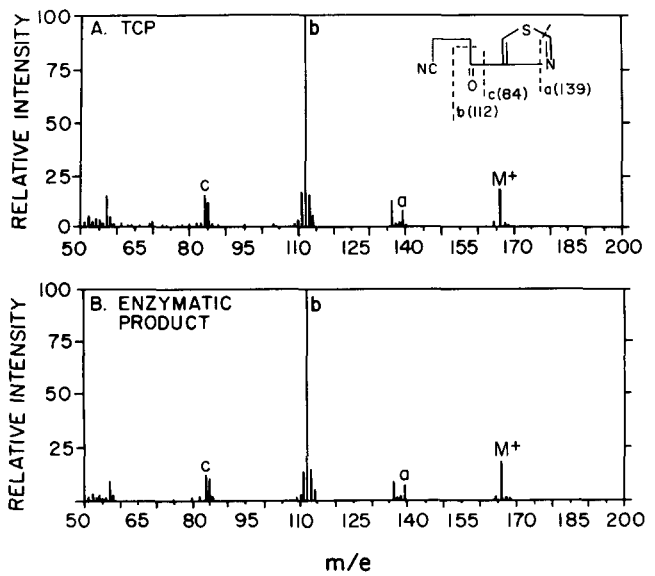

Fig. 5. Low resolution MS of synthetic TCP and that of the product from AAFT. Panel A: The GC/MS spectrum of synthetic TCP (2 μg). Panel B: The GC/MS spectrum of the product derived from AAFT after incubation with rat liver 10,000 *g* supernatant preparation. An aliquot (2 μl) from a total of 50 μl of the extract was used for the above spectrum.

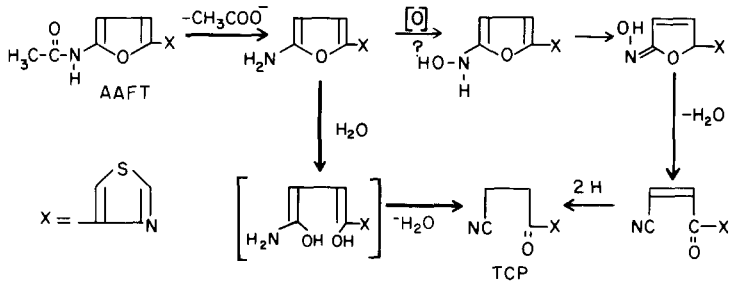

Fig. 6. Postulated metabolic pathway for the formation of TCP from AAFT.

reaction mixture. Second, the deacetylation of AAFT to TCP occurred with cytosolic preparations devoid of microsomal mixed function oxidases, and thus *N*-oxidation of the amine to the *N*-hydroxylamino analog was unlikely to occur. Thus, our present data show that the aminofurylthiazole is a precursor for TCP and that it is not mandatory to go through the *N*-oxime route to generate TCP. However, whether the latter pathway is operative under reductive conditions remains to be established. In this regard, Gavin *et al.* [27], have found that *Escherichia coli* metabolized 1-[(5-nitrofurfurylidene)-amino]-2-imidazolidinone (NF-246) to the open chain nitrile, 3-(4-cyano-2-oxobutylideneamino)-2-imidazolidinone. But the amino derivative, 1-[(5-aminofurfurylidene)amino]-2-imidazolidinone, when subjected to reduction, chemically or enzymatically with whole cells or cell-free extracts, did not produce the corresponding nitrile. Hence, it was argued [27] that the aminofurans are not obligatory intermediates in the formation of the open chain nitrile. Instead they [27] suggested that the *trans*-oxime intermediate may be involved. However, no direct evidence was presented by them [27] for the presence of the oxime or other related intermediates during the reaction. Our results clearly demonstrate that the 4-(5-amino-2-furyl)thiazole could directly act as a precursor for TCP.

Acknowledgements—This work is supported by NCI Grants CA 11946, CA 14520 (WCCC Core), and CA 14524 through the National Bladder Cancer Project. We thank Suzi Pertzborn for editorial assistance and Mary Pankratz for manuscript preparation.

REFERENCES

1. J. M. Klemencic and C. Y. Wang, in *Carcinogenesis, A Comprehensive Survey* (Ed. G. T. Bryan), Vol. 4, pp. 99–130. Raven Press, New York (1978).
2. S. M. Cohen, in *Carcinogenesis, A Comprehensive Survey* (Ed. G. T. Bryan), Vol. 4, pp. 171–231. Raven Press, New York (1978).
3. C. Y. Wang and G. T. Bryan, *Chem. Biol. Interact.* **9**, 423 (1974).
4. D. L. Pugh, J. Olivard, H. R. Snyder and J. P. Heotis, *J. med. Chem.* **15**, 270 (1972).
5. J. Olivard, G. M. Rose, G. M. Klein and J. P. Heotis, *J. med. Chem.* **19**, 729 (1976).
6. H. G. Jonen, F. Oesch and K. L. Platt, *Drug. Metab. Dispos.* **8**, 446 (1980).
7. T. V. Zenser, M. B. Mattammal and B. B. Davis, *Cancer Res.* **40**, 114 (1980).
8. T. V. Zenser, M. B. Mattammal and B. B. Davis, *J. Lab. clin. Med.* **96**, 425 (1980).
9. S. Swaminathan, G. M. Lower, Jr. and G. T. Bryan, *Biochem. Pharmac.* **29**, 3285 (1980).
10. S. Swaminathan, E. Ertürk and G. T. Bryan, *Cancer Res.* **41**, 2648 (1981).
11. K. Tatsumi, S. Kitamura and H. Yoshimura, *Archs Biochem. Biophys.* **175**, 131 (1976).
12. C. Y. Wang, B. C. Behrens, M. Ichikawa and G. T. Bryan, *Biochem. Pharmac.* **23**, 3395 (1974).
13. S. Swaminathan and G. M. Lower, Jr., in *Carcinogenesis, A Comprehensive Survey* (Ed. G. T. Bryan), Vol. 4, pp. 59–97. Raven Press, New York (1978).
14. M. B. Aufrere, B-A. Hoener and M. Vore, *Drug Metab. Dispos.* **6**, 403 (1978).
15. S. M. Cohen and G. T. Bryan, in *Pharmacology and the Future of Man, Proceedings of the Fifth International Congress on Pharmacology* (Ed. G. H. Acheson), Vol. 2, pp. 164–70. Karger, Basel (1973).
16. E. Ertürk, J. E. Morris, S. M. Cohen, A. M. Von Esch, A. J. Crovetti, J. M. Price and G. T. Bryan, *J. natn. Cancer Inst.* **47**, 437 (1971).
17. E. Ertürk, D. B. Headley and G. T. Bryan, *Proc. Am. Ass. Cancer Res.* **21**, 75 (1980).
18. D. B. Headley, R. G. Klopp, P. D. Michie, E. Ertürk and G. T. Bryan, *Cancer Res.* **41**, 1397 (1981).
19. E. Ertürk, D. B. Headley, M. Matsushima, J. F. Hatcher, M. Ichikawa and G. T. Bryan, *Fedn Proc.* **39**, 1022 (1980).
20. S. M. Cohen, G. M. Lower, Jr., E. Ertürk and G. T. Bryan, *Cancer Res.* **33**, 1593 (1973).
21. D. R. McCalla and D. Voutsinos, *Mutation Res.* **26**, 3 (1974).
22. H. S. Rosenkranz and W. T. Speck, *Biochem. biophys. Res. Commun.* **66**, 520 (1975).
23. S. Swaminathan, G. M. Lower, Jr. and G. T. Bryan, *Proc. Am. Ass. Cancer Res.* **19**, 150 (1978).
24. S. Swaminathan, R. C. Wong, G. M. Lower and G. T. Bryan, *Proc. Am. Ass. Cancer Res.* **18**, 187 (1977).
25. G. M. Lower, Jr. and G. T. Bryan, *Proc. Am. Ass. Cancer Res.* **12**, 3 (1971).
26. C. Y. Wang, C. W. Chiu and G. T. Bryan, *Drug Metab. Dispos.* **3**, 89 (1975).
27. J. J. Gavin, F. F. Ebetino, R. Freedman and W. E. Waterbury, *Archs Biochem. Biophys.* **113**, 399 (1966).
28. A. H. Beckett and A. E. Robinson, *J. mednl pharm. Chem.* **1**, 135 (1959).
29. A. H. Beckett and A. E. Robinson, *J. mednl pharm. Chem.* **1**, 155 (1959).
30. D. H. Chatfield, *Xenobiotica* **6**, 509 (1976).