13. M. Toporek, *Cancer Res.* **31**, 1962 (1971).
14. N. Brock and H. J. Hohorst, *Arzneimittel-Forsch.* **13**, 1021 (1963).
15. J. Květina and A. Guaitani, *Pharmacology (Basel)* **2**, 65 (1969).
16. I. Bartošek, A. Guaitani and S. Garattini, *Pharmacology (Basel)*, **8**, 244 (1972).
17. O. M. Friedman and E. Boger, *Analyt. Chem.* **33**, 906 (1961).
18. J. M. Finkel, *Analyt. Biochem.* **21**, 362 (1967).

Biochemical Pharmacology, Vol. 24, pp. 291–293. Pergamon Press, 1975. Printed in Great Britain.

SHORT COMMUNICATIONS

Identification of 2-methyl-4-(5-amino-2-furyl)thiazole as the reduced metabolite of 2-methyl-4-(5-nitro-2-furyl)thiazole

(*Received* 6 *April* 1974; *accepted* 7 *June* 1974)

The reduction of a number of 5-nitrofurans by mammalian tissues and bacteria has been investigated. Due to the lability of the reduced products, only a few metabolites have been identified. 5-Nitro-2-furaldehyde semicarbazone solution incubated with mammalian tissues [1], xanthine oxidase [1, 2] or aldehyde oxidase [3] in the presence of electron donors resulted in a metabolite suggested to be the corresponding hydroxylamine. Incubation of 5-nitro-2-furaldehyde semicarbazone with *Aerobacter aerogenes* provided a metabolite identified as an aminofuran [4]. When 5-nitro-2-furaldehyde acetylhydrazone was fed to rabbits, 5-acetamidofuraldehyde acetylhydrazone was isolated as a urinary metabolite [5].

A number of 5-nitrofurans have been reduced to the corresponding amines by H_2 with palladium on charcoal as catalyst [6]. After hydrogenation of 5-nitro-2-furaldehyde semicarbazone with Raney nickel, glyoxylpropionitrile was isolated, and was believed to be formed by ring opening of 5-amino-2-furaldehyde semicarbazone [7].

Certain 4-(5-nitro-2-furyl)thiazole derivatives were carcinogenic for experimental animals [8]. The nitro group was implicated in the carcinogenicity [8–11]. Unlike those tested [8–12], 2-methyl-4-(5-nitro-2-furyl)thiazole (MNFT) does not possess an amino substitute at the 2-position of the thiazole ring. It is more soluble in organic and inorganic solvents than the others. These properties make it a potentially useful compound for metabolic investigations of the structurally related carcinogenic 4-(5-nitro-2-furyl)thiazole deriva-

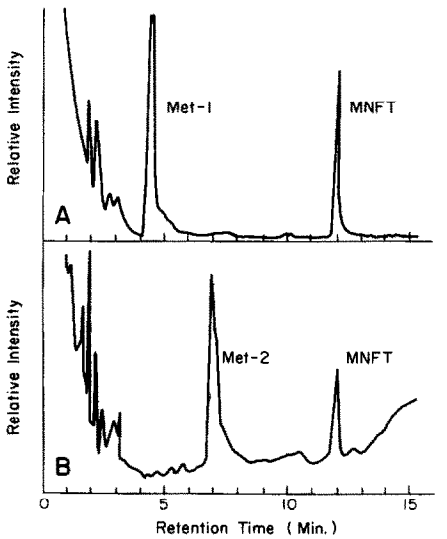

Fig. 1. GLC of methylenechloride extract of MNFT incubated with NADPH and NADPH–cyt. *c* reductase (A), and that of MNFT incubated with NADPH and NADPH–cyt. *c* reductase followed by incubation with mouse liver cytosol (B).

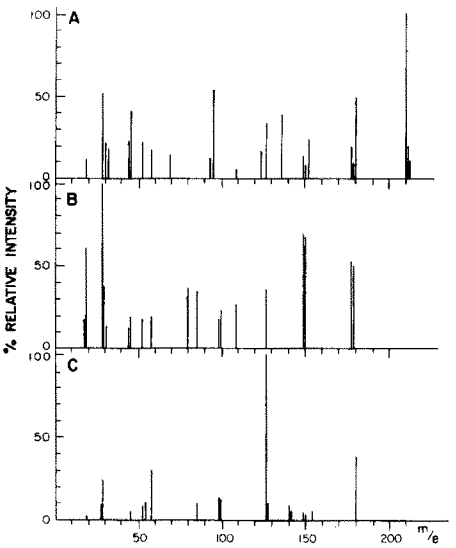

Fig. 2. MS of MNFT (A), Met-1 (B) and Met-2 (C). MS of MNFT was obtained from direct insertion; the other two were from GLC–MS.

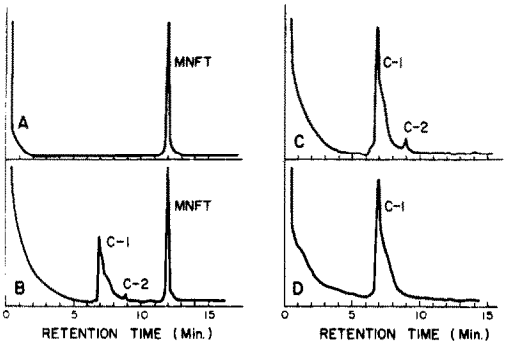

Fig. 3. GLC of MNFT hydrogenated solution: 0 time (A), 2 (B), 5 (C), and 15 (D) min after hydrogenation.

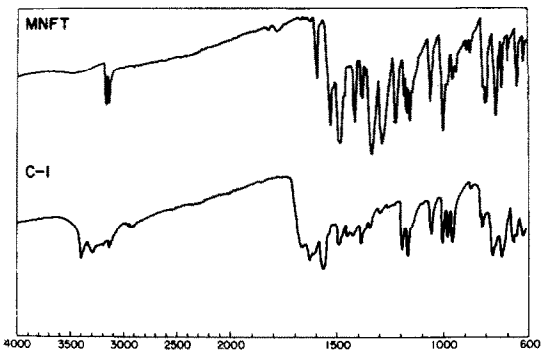

Fig. 4. Infrared spectra of MNFT and the hydrogenated product of MNFT (C-1). A KBr pellet was prepared for the measurement of the infrared spectra.

tives. The carcinogenicity of MNFT is currently being tested in this laboratory. The reduction of MNFT by xanthine oxidase and NADPH–cytochrome *c* reductase has been reported [13]. The present communication deals with the identification of the reduced metabolites of MNFT.

Chemicals. MNFT was a gift from ABIC Ltd., Israel. It was recrystallized from methanol, and the purity and identity were ascertained by melting point, infrared spectrophotometry, gas–liquid chromatography (GLC) and mass spectrometry. NADPH was purchased from Sigma Chemical, St. Louis, Mo.

Enzymatic reduction of MNFT. Partially purified rat liver NADPH–cyt. *c* reductase and mouse liver cytosol, from 20 g female Swiss albino mice (Rolfsmeyer Co., Madison, Wis.), were prepared as previously described [13].

Nitroreduction of MNFT was performed in a Thunberg tube. In a total volume of 3 ml, the incubation mixture contained 1 μmole MNFT (in 0·1 ml absolute ethanol), 60 μmoles potassium phosphate buffer, pH 7·4, 5 μmoles NADPH and about 0·5 mg protein of NADPH–cyt. *c* reductase (sp. act. 0·6 μmole MNFT/mg/min) at 25°. The enzyme and NADPH were placed in the side arm, and the MNFT solution was placed in the main compartment. The reaction vessel was evacuated with a water aspirator for 2 min, and the reaction was started by pouring the enzyme solution into the main compartment. The mixture was incubated for 30 min at 37° and the yellow color disappeared. The reaction mixture was extracted with 3 ml methylenechloride. The organic extract was dried under a stream of N_2 gas, dissolved in 0·1 ml absolute ethanol and immediately subjected to GLC analysis.

Gas–liquid chromatography. The prepared sample was injected onto a 6-ft long glass column containing 10% OV-1 on 80/100 Chromosorb W coupled to a Hewlett Packard model 5700 A gas chromatograph with a hydrogen flame ionization detector. The column temperature was programmed at 170° isotherm for 8 min, with a 4°/min increase from 170° to 250°, and then maintained at 250° for 8 min. The carrier gas was helium with a flow rate of 22 ml/min.

Mass spectrometry (MS). Mass spectra were obtained from a Hewlett Packard mass spectrometer, model 5930A. When combined GLC–MS was obtained, this mass spectrometer was interfaced to the gas chromatograph. The ion source temperature was 230° and the operating voltage was 70 eV.

Chemical reduction of MNFT. H_2 gas was bubbled into 2 ml ethanol solution containing 10 mg MNFT and 5 mg of 5% palladium on charcoal. Aliquots of the solution were withdrawn at 0, 2, 5 and 15 min after hydrogenation, filtered under N_2 gas, and immediately subjected to GLC analysis. The remaining solution was filtered, dried completely under a stream of N_2 gas, and used for infrared measurements with a Beckman IR-8 infrared spectrophotometer.

MNFT was anaerobically reduced by NADPH–cyt. *c* reductase and lost its yellow color within 30 min of incubation. Gas chromatography of the methylenechloride extract of the incubation mixture (Fig. 1A) revealed one metabolite (Met-1) with a retention time of 5 min, and MNFT at 12 min. Mass spectra of MNFT and Met-1 are shown in Fig. 2, A and B respectively. The structural assignment of Met-1 will be discussed later.

MNFT was incubated with NADPH and NADPH–cyt. *c* reductase for 30 min, followed by anaerobic incubation with 100 mg equivalent of mouse liver cytosol for another 30 min. GLC of the organic extract showed only one metabolite, Met-2, with retention time of 7 min (Fig. 1B), suggesting that Met-1 was further reduced to Met-2 by the cytosol enzymes. The MS of Met-2 is shown in Fig. 2 C. The *m/e* 180 was assigned as the molecular ion of 2-methyl-4-(5-amino-2-furyl)thiazole. The structure was further confirmed by chemical synthesis (discussed below).

As shown in Fig. 3 B, after 2 min of hydrogenation of MNFT, two compounds with a retention time of 7 min (C-1) and 9 min (C-2) appeared together with the parent compound. After 5 min, only the reduced products, C-1 and C-2, were observed (Fig. 3C). Complete conversion to C-1 was achieved after 15 min of hydrogenation (Fig. 3D).

The MS of C-1 was identical to that of Met-2 (Fig. 2C). Comparing the infrared spectra of C-1 and MNFT (Fig. 4), C-1 has absorptions at 3400 and 3300 cm^{-1} and does not have absorptions at 1500 and 1350 cm^{-1}, suggesting that MNFT was reduced to 2-methyl-4-(5-amino-2-furyl)thiazole. The interpretation of the infrared spectra is compatible with that of the MS. The same retention time of C-1 and Met-2 in GLC also supported the interpretation that both compounds are identical.

The MS of C-2 was closely similar to that of Met-1 (Fig. 2B), except for a slight difference in their relative intensities. However, their different retention time in GLC clearly showed that they were two different substances. Aromatic nitro groups may be reduced stepwise to nitroso, hydroxylamine and amines [14]. Since Met-1 and C-2 can be further reduced to 2-methyl-4-(5-amino-2-furyl)thiazole, they may be the corresponding nitroso and hydroxylamine deriva-

tives. Apparently the *m/e* 178 was not the parent ion of Met-1 and C-2, but of the fragment $(M-H_2O)^+$ and $(M-O)^+$. These interpretations remain tentative, however, until the metabolites are synthesized and the spectra are analyzed.

2-Nitrosonaphthalene [15] and niridazole [16] are reduced by NADPH in the presence of NADPH–cyt. *c* reductase to the corresponding hydroxylamines. The hydroxylamine of niridazole can be trimethylsilylated by *bis*(trimethylsilyl)acetamide (BSA) and identified by GLC–MS [16]; however, attempts to trimethylsilylate Met-1 with BSA were unsuccessful.

5-Nitrofurans are reduced by several enzyme systems. The cytosol contains xanthine oxidase, aldehyde oxidase, DT diaphorase and lipoyl dehydrogenase, which are capable of reducing 5-nitrofurans [3]. Mouse liver cytosol enzymes, which reduce MNFT or Met-1 to the corresponding amine, remain to be characterized.

Acknowledgement—This research was supported in part by Public Health Service Research Grant CA 11946 from the National Cancer Institute.

Division of Clinical Oncology, University of Wisconsin School for Health Sciences, Madison, Wis. 53706, U.S.A.

CHING-YUNG WANG
CHUNG-WAI CHIU
BERNARD KAIMAN
GEORGE T. BRYAN

REFERENCES

1. H. E. Paul, V. R. Ells, F. Kopko and R. C. Bender, *J. med. Pharmac. Chem.* **2,** 563 (1960).
2. J. D. Taylor, H. E. Paul and M. F. Paul, *J. biol. Chem.* **191,** 223 (1951).
3. M. K. Wolpert, J. R. Althaus and D. G. Johns, *J. Pharmac. exp. Ther.* **185,** 202 (1973).
4. A. H. Beckett and A. E. Robinson, *Chemy Ind.* 523 (1957).
5. J. Olivard, S. Valenti and J. A. Buzard, *J. med. Pharmac. Chem.* **5,** 524 (1962).
6. F. F. Ebetino, J. J. Carroll and G. Gever, *J. med. Pharmac. Chem.* **5,** 513 (1962).
7. F. L. Austin, *Chemy Ind.* 523 (1957).
8. S. M. Cohen and G. T. Bryan, *Proc. Fifth Int. Congr. Pharmacology*, Vol. 2, p. 164. (1973).
9. 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).
10. J. E. Morris, J. M. Price, J. J. Lalich and R. J. Stein, *Cancer Res.* **29,** 2145 (1969).
11. S. M. Cohen, E. Ertürk, J. M. Price and G. T. Bryan, *Cancer Res.* **30,** 897 (1970).
12. S. M. Cohen, G. M. Lower, Jr., E. Ertürk and G. T. Bryan, *Cancer Res.* **33,** 1593 (1973).
13. C. Y. Wang, B. C. Behrens, M. Ichikawa and G. T. Bryan, *Biochem. Pharmac.*, (in press).
14. J. R. Fouts and B. B. Brodie, *J. Pharmac. exp. Ther.* **119,** 197 (1957).
15. D. M. Ziegler, E. M. McKee and L. L. Poulsen, *Drug. Metab. Dispos.* **1,** 314 (1973).
16. D. R. Feller, M. Morita and J. R. Gillette, *Biochem. Pharmac.* **20,** 203 (1971).

Biochemical Pharmacology, Vol. 24, pp. 293–295. Pergamon Press, 1975. Printed in Great Britain.

Influence of esterase inhibitors on platelet aggregation and release induced by phorbol myristate acetate*

(*Received* 19 *April* 1974; *accepted* 2 *July* 1974)

A tumor-promoting principle derived from croton oil, phorbol myristate acetate (PMA), is a powerful platelet-aggregating agent [1]. Minute amounts of PMA labilize platelet granules, convert the organelles to swollen vacuoles, stimulate the release of serotonin and adenine nucleotides, induce clumping of discoid platelets, and cause irreversible aggregation [2, 3]. Platelets washed and resuspended in Hank's balanced salt solution (AWP) are more sensitive to PMA and less variable in response than platelets in citrate-platelet-rich plasma (C-PRP) [1, 4] (Fig. 1). This observation suggested that some factor or factors present in plasma might interfere with the action of PMA on platelets in C-PRP. Since PMA (12-0-tetradecanyol phorbol-13-acetate) is a fatty acid ester, it seemed possible that esterases might modify or inhibit the agent. Acetyl esterases and pseudocholinesterases are known to be present in variable amounts in human plasma [5], and their activity can be blocked by esterase inhibitors [6]. The present investigation was instituted to determine if inhibitors of plasma esterase activity could modify the influence of PMA on platelets. C-PRP and AWP were prepared from the same samples of normal blood in each experiment. The methods used to obtain blood, mix the samples immediately with 3·8% trisodium citrate in a ratio of 9 parts blood to 1 part anticoagulant, separate C-PRP by centrifugation at room temperature [7], prepare AWP after sedimentation on albumin by a modification [8] of the technique of Walsh [9], and study the response of C-PRP and AWP to aggregating agents by recording nephelometry have been described in recent pub-

* Supported by grants from the United States Public Health Service.