ISOLATION AND IDENTIFICATION OF THE METABOLITE OF N-(5-NITRO-2-FURFURYLIDENE)-3-AMINO-2-OXAZOLIDONE (FURAZOLIDONE)

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The metabolism of a widely used veterinary drug, N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone (furazolidone) was investigated *in vitro* and *in vivo*. When the nitrofuran was incubated with milk xanthine oxidase supplemented with hypoxanthine, two reduction products were isolated as metabolites from the reaction mixture. One (mp 179—180°) was identified to be 3-(4-cyano-2-oxobutylideneamino)-2-oxazolidone by mass, ultraviolet and nuclear magnetic resonance spectrometries, from its formation of 2,4-dinitrophenylhydrazone and by comparison with an authentic specimen. The metabolite was also isolated from the urine of rabbits given orally with fufurazolidone. However, the structure of another metabolite (mp 198 ° (dec.)) remains to be clarified. The mutagenicity test showed that the nitrofuran was strongly mutagenic against *Salmonella typhimurium* TA 100, while above cyano derivative was completely inactive.

Keywords—furazolidone; nitrofuran; milk xanthine oxidase; *Salmonella typhimurium* TA 100; urinary metabolite; mutagenicity

Certain nitrofurans have been used in clinical and veterinary medicine and as a preservative for animal feeds. One of them, N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone (furazolidone) (Fig.1), has been widely used against salmonellosis, coccidiosis and aerosacculitis in poultry, and against bacterial enteritis in swine, and so on. On the other hand, many nitrofurans have been found to be mutagenic^{1,2)} and some are carcinogenic.^{3,4)} Furazolidone was also demonstrated to be mutagenic in *Escherichia coli* WP₂ (*trp*⁻) and its substrain (*uvrA*⁻*trp*⁻).¹⁾ Recent works⁵⁻⁷⁾ indicate that the formation of active metabolites from nitrofurans may be responsible for their biological activities such as antibacterial, mutagenic and carcinogenic activities. Therefore, we considered that the information about the metabolism of furazolidone is

very important for a safety reevaluation of the veterinary drug. However, there is virtually no information available concerning this matter.

The present paper describes the isolation and identification of a metabolite of furazolidone, which is produced *in vitro* and *in vivo*, and describes the mutagenicity tests of the nitrofuran and its metabolite against *Salmonella typhimurium* TA 100.

FIG. 1. *Structure of Furazolidone*

EXPERIMENTAL

Materials—Furazolidone (mp 275° (dec.)) was kindly donated by Ueno Pharmaceutical Co., Ltd. Hypoxanthine and 2,4-dinitrophenylhydrazine were purchased from Nakarai Chemical, Ltd. Raney nickel was obtained from Wako Pure Chemical Industries, Ltd. and activated by the method of Mozingo.⁸⁾ Silica gel (Kieselgel 60, 70—230 mesh) was purchased from Merck. Thin-layer silica gel plates were prepared by coating with 0.25 mm thick Wako gel B-5 FM which was obtained from Wako Pure Chemical Industries, Ltd. Milk xanthine oxidase was partially purified from fresh raw cream until step 3 by the method of Horecker and Heppel.⁹⁾ Polypeptone and yeast extract were purchased from Daigo-Eiyo Co., Ltd.

Analytical Procedures—Infrared (IR) spectra were taken with a Japan Spectroscopic DS-701 G IR spectrophotometer. Nuclear magnetic resonance (NMR) spectra were obtained with a Japan Electron Optics (JEOL) JNM PS-100 spectrometer using tetramethylsilane as internal standard. High and low resolution mass spectra (MS) were taken with a Japan Electron Optics (JEOL) JMS-01SG mass spectrometer. Ultraviolet (UV) spectra were recorded with a Hitachi 124 spectrophotometer.

Enzymatic Reduction of Furazolidone—The incubation mixture consisted of furazolidone (300 μ mol, 67.6 mg), hypoxanthine (1200 μ mol) and partially purified xanthine oxidase (400 units) in a final volume of 6 liter of 1/30 M phosphate buffer (pH 7.4) containing dimethyl sulfoxide (DMSO) at a concentration of 3.5 %. The incubation was carried out at 37° for 1 hr under an atmosphere of nitrogen in the dark. After incubation, the incubation mixture was extracted three times with an equal volume of ethyl acetate. The pooled ethyl acetate extract was once washed with an equal volume of water, dried over anhydrous Na₂SO₄ and evaporated to dryness *in vacuo*. The residue (1.2 g) was taken up in a small volume of CHCl₃ and transferred to a column containing silica gel (20 g). The column was eluted stepwise with 400 ml each of CHCl₃, CHCl₃-acetone (9:1), CHCl₃-acetone (1:1) and acetone. Each fraction (50 ml)

was examined by thin-layer chromatography (TLC) developed in a solvent system, benzene-acetone-MeOH (7:2:1). Spots were visualized under UV light (2536 Å) or by exposing to iodine vapor. Unreacted furazolidone (*R_f* 0.4) and metabolite 1 (*R_f* 0.33) were obtained as a mixture from the CHCl₃-acetone (9:1) eluate, and metabolite 2 (*R_f* 0.28) from the CHCl₃-acetone (1:1) eluate, respectively. When the mixture was rechromatographed through a column of silica gel (20 g), furazolidone was first eluted with CHCl₃ and then metabolite 1 eluted with CHCl₃-acetone (9:1), separately. Metabolite 1 was recrystallized from ethyl acetate to give colorless plates (23 mg), mp 179—180°, and metabolite 2 from ethanol to give colorless plates (14 mg), mp 198° (dec.), respectively.

Chemical Reduction of Furazolidone—A suspension of furazolidone (1 g) in water (25 ml) was hydrogenated in the presence of Raney nickel (0.5 g) under normal atmosphere and room temperature. Reduction was arbitrarily stopped after three molar equivalents of hydrogen had been absorbed, the catalyst was removed by filtration, and the filtrate was allowed to stand overnight at pH 9.0. The solution was extracted three times with an equal volume of ethyl acetate. The pooled ethyl acetate was dried over anhydrous Na₂SO₄ and evaporated to dryness *in vacuo*. The residue (1 g) was redissolved in a small volume of CHCl₃, placed on a column of silica gel (40 g) and eluted stepwise with 400 ml each of CHCl₃, CHCl₃-acetone (9:1) and CHCl₃-acetone (2:1). Each fraction (50 ml) was examined by TLC as described above. The CHCl₃-acetone (9:1) eluate, which contained a reduction product with *R_f* value of 0.33, was evaporated to dryness *in vacuo*. The residue was recrystallized from ethyl acetate to give colorless plates (150 mg), mp 179—180°. High resolution MS : Calcd. for C₈H₁₀N₃O₃ (*M*⁺+1, *m/e*), 196.0722; Found: 196.0709. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ : 2260, 1773, 1685. NMR (DMSO-*d*₆) : 2.69 (2H, t), 3.18 (2H, t), 3.68 (2H, t), 4.56 (2H, t), 7.16 (1H, s). UV $\lambda_{\text{max}}^{\text{ethanol}}$ nm (log ϵ) : 268 (4.02).

Isolation of *in Vivo* Metabolite of Furazolidone—Four male albino rabbits

weighing about 3 kg were fasted overnight prior to administration of furazolidone. The drug was suspended in 5 % gum arabic and administered orally at a dose of 100 mg/kg. After medication, the animals were then placed in the metabolic cages, and 48-hr urine was collected. The urine was extracted three times with an equal volume of ethyl acetate. The pooled ethyl acetate extract was dried over anhydrous Na_2SO_4 and evaporated to dryness *in vacuo*. The residue (0.26 g) was placed on a column of silica gel (60 g) and eluted stepwise with 300 ml each of CHCl_3 , CHCl_3 -acetone (9:1), CHCl_3 -acetone (4:1) and CHCl_3 -acetone (1:1) as described above. The CHCl_3 -acetone (9:1) and CHCl_3 -acetone (4:1) eluates were combined and then evaporated to dryness. The residue (14 mg), a crude metabolite of furazolidone, was recrystallized from ethyl acetate to colorless plates, mp 179–180°. The crystals yielded a single spot (R_f 0.33) on TLC developed in benzene-acetone-methanol (7:2:1).

Preparation of Dinitrophenylhydrazone of Metabolite 1—To a solution of 20 mg of metabolite 1 in 1 ml of diethylene glycol dimethyl ether were added 1 ml of 2,4-dinitrophenylhydrazine solution (2,4-dinitrophenylhydrazine 4 g in diethylene glycol dimethyl ether 100 ml) and three drops of conc. HCl, and the solution was allowed to stand overnight at room temperature. After dilution with 20 ml of water, the solution was extracted three times with 20 ml each of ethyl acetate. The combined extract was washed once with an equal volume of water, dried over anhydrous Na_2SO_4 and evaporated to dryness *in vacuo*. The residue was submitted to preparative TLC developed in benzene-acetone (9:1) and the main spot with R_f value of 0.46 was scraped and eluted with ethyl acetate. The eluate was evaporated to dryness *in vacuo* to leave light brown crystals, mp 210–212° (dec.).

Mutation Assay—*Salmonella typhimurium* TA100 was generously supplied by Dr.M.

Ishizawa, Cancer Research Institute, Kyushu University. The assay using TA100 was carried out as described by Ames *et al.*¹⁰⁾ with a slight modification¹¹⁾. Chemicals dissolved in 0.1 ml of DMSO were pre-incubated for 20 min at 37° with 0.5 ml of 0.1 M sodium phosphate buffer (pH 7.4) and 0.1 ml of overnight culture of TA 100. Two ml of 0.5% soft agar* molten at 48° were added and mixed, poured immediately over 1.2% agar in Vogel-Bonner minimal medium E**, and the plate was left to harden for several minutes. After incubation for 2 days at 37°, the numbers of colonies which are revertants to histidine prototroph were counted in both test and control plates.

RESULTS AND DISCUSSION

It is known that nitrofurans are metabolized by nitroreducing enzymes such as xanthine oxidase (EC 1.2.3.2),¹³⁻¹⁶⁾ aldehyde oxidase (EC 1.2.3.1)¹⁷⁾ and liver microsomes.^{15,16)} In the present experiment, milk xanthine oxidase was used as a model enzyme for reduction of furazolidone. Two reduction products of furazolidone, metabolite 1 and 2, formed by this enzyme were isolated from the incubation mixture.

The UV spectrum of metabolite 1 (mp 179–180°) revealed an absorption maximum at 268 nm ($\log \epsilon$ 4.02, in ethanol) and no bathochromic shift was observed in acidic or alkaline ethanol. Low resolution mass spectrometry of the metabolite demonstrated its molecular ion at m/e 196 ($M^+ + 1$). In this case, the following facts suggest that an ion peak at m/e 196 is $M^+ + 1$: 1) the molecule of this metabolite contains probably odd-numbered (three) nitrogen atoms, considering from the structure of its parent compound and it disagrees with an even-numbered molecular weight; 2) previously, we demonstrated that nitrofurans such as 5-nitro-2-furfural semicarbazone (nitrofurazone) and 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2) are reduced by xanthine

* 0.5% Soft agar consists of NaCl 6 g, L-histidine·HCl 9.58 mg (final concentration, 50 μM), biotin 12.22 mg (final concentration, 50 μM) and water 1 liter.

** 1.2% Agar in Vogel-Bonner minimal medium E (pH 7.2) consists of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 g, citric acid 2 g, K_2HPO_4 10 g, $\text{NaNH}_4\text{HPO}_4 \cdot 4\text{H}_2\text{O}$ 3.5 g, glucose 20 g and water 1 liter.¹²⁾

FIG. 2. NMR Spectrum of Metabolite 1 of Furazolidone

oxidase to 5-amino-2-furfural semicarbazone and 2-(2-furyl)-3-(5-oxo-2-pyrrolin-2-yl)acrylamide, respectively¹⁶; 3) the molecular weight of the compound corresponding to the amine derivative of furazolidone is 195. This indicated that metabolite 1 has the same molecular weight as the amine derivative of furazolidone, N-(5-amino-2-furfurylidene)-3-amino-2-oxazolidone. However, the melting point and UV spectrum of the metabolite mentioned above, were different from those (mp 202—203°, $\text{UV}\lambda_{\text{max}}^{\text{H}_2\text{O}}$ nm (log ϵ): 340(4.35)) of the amine derivative reported by Ebetino *et al.*¹⁸ Furthermore, its IR spectrum (Fig. 3) had absorption bands at 2260 ($\nu\text{C}\equiv\text{N}$), 1773 (ν oxazolidone, $\text{C}=\text{O}$) and 1685 (ν α , β -unsaturated ketone) cm^{-1} . From these results, its structure appears to be 3-(4-cyano-2-oxobutylideneamino)-2-oxazolidone (Fig. 2). Mass fragment ions $\text{M}^+ - \text{CO}$ (m/e 167), $\text{M}^+ - \text{CH}_2\text{CH}_2\text{CN}$ (m/e 141) and $\text{M}^+ - \text{COCH}_2\text{CH}_2\text{CN}$ (m/e 113, base peak) can be explained as being derived from this structure. The high resolution mass spectrum showed the following:

	m/e Found	Calcd. for
Molecular ion ($\text{M}^+ + 1$)	196.0709	$\text{C}_8\text{H}_{10}\text{N}_3\text{O}_3$ 196.0722
$\text{M}^+ - 82$ ($\text{NCCH}_2\text{CH}_2\text{CO}$)	113.0346	$\text{C}_4\text{H}_5\text{N}_2\text{O}_2$ 113.0351

Its NMR spectrum shown in Fig. 2 also supports the structure of metabolite 1 as the cyano derivative. The singlet at 7.16 (1H) is attributed to

the olefinic proton. The triplets at 4.56 (2H) and 3.86 (2H) must be resulted from the protons of oxazolidone ring, and the triplets at 3.18 (2H) and 2.69 (2H) from the protons of 4-cyano-2-oxobutylidene moiety, respectively.

For further confirmation of the structure, metabolite 1 was treated with 2,4-dinitrophenylhydrazine. The mass spectrum of the reaction product (mp 210—212° (dec.)) gave a molecular ion at m/e 472 ($\text{M}^+ + 1$), accompanying fragment ions at m/e 289 ($\text{M}^+ - 182$, $\text{C}_6\text{H}_4\text{N}_3\text{O}_4$) and 107 ($\text{M}^+ - 364$, $\text{C}_{12}\text{H}_8\text{N}_6\text{O}_8$). This result indicates the formation of 4-cyano-2-oxobutyr-aldehyde di-2,4-dinitrophenylhydrazone which is the expected product from metabolite 1.

So far it is known that the hydrogenation of nitrofurazone in the presence of Raney nickel results in the formation of a cyano derivative, 4-cyano-2-oxobutyr-aldehyde semicarbazone. Its structure has been established by alternative synthesis of its di-semicarbazone.¹⁹ Therefore, such chemical reduction of furazolidone was carried out in order to firmly prove the structure of metabolite 1 as described in Experimental. As a result, the IR (Fig. 3), UV, NMR and mass spectra of the chemical reduction product was completely identical with those of metabolite 1. Furthermore, no depression of melting point occurred on admixture of both compounds. Thus an *in vitro* metabolite of furazolidone was identified as 3-(4-cyano-2-oxobutylideneamino)-2-oxazolidone.

FIG. 3. Infrared Spectra of Metabolite 1 and Chemical Reduction Product of Furazolidone (KBr Disk)

Two possible pathways are postulated for the formation of such cyano compounds from nitrofurans. In the first pathway, nitrofurans are reduced to the corresponding aminofuran derivatives which undergo ring cleavage to yield the cyano compounds.^{20,21)} In the second pathway, they are reduced to the corresponding hydroxylaminofuran derivatives which can tautomerize to the *cis* and *trans*-oximes, respectively. The *trans*-oxime would be a preferred configuration for *trans* elimination of water and ring opening to the cyano compounds.²²⁾

In addition to metabolite 1 described above, metabolite 2 (mp 198° (dec.)) was also isolated as another reduction product of furazolidone from the incubation mixture. The UV spectrum of this metabolite revealed an absorption maximum at 274 nm (log ϵ 4.01, in ethanol). Its IR spectrum had absorption bands at 3300, 2300 and 1740 cm^{-1} , suggesting that hydroxyl, nitrile and carboxyl groups may be present in the molecule. However, the metabolite, unlike metabolite 1, revealed no ion peaks in the electron impact mass spectrometry, indicating its non-volatile properties. Its structure remains unknown and is under investigation.

In further experiment, a metabolite (mp 179—180°) was isolated from the urine of rabbits given orally with furazolidone. Its mass and UV spectra, and its thin-layer chromatographic properties were identical with those of metabolite 1 described above. Furthermore, no depression of melting point was observed on admixture of both

compounds. From these results, it was concluded that 3-(4-cyano-2-oxobutylideneamino)-2-oxazolidone was formed from furazolidone *in vivo* as well as *in vitro* and excreted in urine as a metabolite of the nitrofuran. Previously, Paul *et al.*²³⁾ suggested the excretion of 4-cyano-2-oxobutylaldehyde semicarbazone in the urine of nitrofurazone-fed rats by spectrophotometric study, but could not isolate it. The present study provided the first evidence for *in vivo* formation of the open-chain cyano derivative from a nitrofuran.

Recent works^{1,2)} demonstrated mutagenic activities of numerous nitrofurans in microorganisms. However, only few information²⁴⁻²⁶⁾ is available about mutagenicity tests for the metabolites of nitrofurans. In this study, furazolidone and metabolite 1 were assayed in comparison for their mutagenic activities against *Salmonella typhimurium* TA 100 as described in Experimental. As a result, furazolidone was strongly mutagenic, but metabolite 1 was nonmutagenic against this organism under the same conditions (Table I), suggesting that the latter is not an active metabolite of furazolidone.

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REFERENCES

- 1) D.R.McCalla and D.Voutsinos: On the mutagenicity of

Table I. *Mutagenicity Tests of Furazolidone and Metabolite 1 in Salmonella typhimurium* TA 100

Compound	$\mu\text{g}/\text{plate}$	Revertant colonies/plate
Furazolidone	0.03	250
	0.1	985
	0.3	1510
	1	2000
Metabolite 1	1	123
	3	96
	10	133
	100	94

The number of spontaneous revertants per plate was 90—120.

- nitrofurans, *Mutation Res.*, **26**, 3—16 (1974).
- 2) Y.Tazima, T.Kada and A.Murakami: Mutagenicity of nitrofurans derivatives, including furylfuramide, a food preservative, *Mutation Res.*, **32**, 55—80 (1975).
 - 3) J.E.Morris, J.M.Price, J.J.Lalich and R.J.Stein: The carcinogenic activity of some 5-nitrofurans derivatives in the rat, *Cancer Res.*, **29**, 2145—2156 (1969).
 - 4) S.M.Cohen, E.Ertürk, A.M.von Esch, A.J.Crovetti and G.T.Bryan: Carcinogenicity of 5-nitrofurans, 5-nitroimidazoles, 4-nitrobenzenes, and related compounds, *J.Natl.Cancer Inst.*, **51**, 403—417 (1973).
 - 5) D.R.McCalla, A.Reuvers and C.Kaiser: Mode of action of nitrofurazone, *J.Bacteriol.*, **104**, 1126—1134 (1970).
 - 6) T.Kada: Metabolic activation and *Escherichia coli* mutagenesis of furylfuramide, a nitrofurans food additive, *Ann.Rep.Natl.Inst.Genet.Jpn.*, **24**, 39—41 (1974).
 - 7) H.S.Rosenkranz and W.T.Speck: Activation of nitrofurantoin to a mutagen by rat liver nitroreductase, *Biochem.Pharmacol.*, **25**, 1555—1556 (1976).
 - 8) R.Mozingo: Catalyst, Raney nickel, W-2, "Organic Synthesis," Coll.Vol.III, ed. by E.C.Horning, Wiley, New York, 1955, pp.181—183.
 - 9) B.L.Horecker and L.A.Heppel: Xanthine oxidase from milk, "Method in Enzymology," Voll.II, ed. by S.P.Colowick and N.O.Kaplan, Academic Press Inc., New York, 1955, pp.482—485.
 - 10) B.N.Ames, F.D.Lee and W.E.Durston: An improved bacterial test system for the detection and classification of mutagens and carcinogens, *Proc. Nat. Acad. Sci. U.S.A.*, **70**, 782—786 (1973).
 - 11) T.Yahagi, M.Nagao, Y.Seino, T.Matsushima, T.Sugimura and M.Okada: Mutagenicities of N-nitrosamines on *Salmonella*, *Mutation Res.*, **48**, 121—129 (1977).
 - 12) H.J.Vogel and D.M.Bonner: Acetylornithinase of *E.coli*: Partial purification and some properties, *J. Biol. Chem.*, **218**, 97—106 (1956).
 - 13) J.D.Taylor, H.E.Paul and M.F.Paul: Metabolism of the nitrofurans.III. Studies with xanthine oxidase *in vitro*, *J.Biol.Chem.*, **191**, 223—231 (1951).
 - 14) M.Morita, D.R.Feller and J.R.Gillette: Reduction of niridazole by rat liver xanthine oxidase, *Biochem.Pharmacol.*, **20**, 217—226 (1971).
 - 15) C.Y.Wang, B.C.Behrens, M.Ichikawa and G.T.Bryan: Nitroreduction of 5-nitrofurans derivatives by rat liver xanthine oxidase and reduced nicotinamide adenine dinucleotide phosphate-cytochrome c reductase, *Biochem.Pharmacol.*, **23**, 3395—3404 (1974).
 - 16) K.Tatsumi, S.Kitamura and H.Yoshimura: Reduction of nitrofurans derivatives by xanthine oxidase and microsomes. Isolation and identification of reduction products, *Arch.Biochem.Biophys.*, **175**, 131—137 (1976).
 - 17) M.K.Wolpert, J.R.Althaus and D.G.Johns: Nitroreductase activity of mammalian liver aldehyde oxidase, *J.Pharmacol.Exp.Ther.*, **185**, 202—213 (1973).
 - 18) F.F.Ebetino, J.J.Carroll and G.Gever: Reduction of nitrofurans.I. Aminofurans, *J.Med.Pharm.Chem.*, **5**, 513—524 (1962).
 - 19) F.L.Austin: Reduction of nitrofurazone with Raney nickel, *Chem.Ind. (London)*, 1957, 523.
 - 20) A.H.Beckett and Ann E.Robinson: The reactions of nitrofurans with bacteria—II. Reduction of a series of antibacterial nitrofurans by *Aerobacter aerogenes*, *J.Med.Pharm.Chem.*, **1**, 135—154 (1959).
 - 21) A.H.Beckett and Ann E.Robinson: The reaction of nitrofurans with bacteria—III. Reduction of a series of antibacterial nitrofurans (type B compounds) by *Aerobacter aerogenes*, *J.Med.Pharm.Chem.*, **1**, 155—164 (1959).
 - 22) J.J.Gavin, F.F.Ebetino, R.Freedman and W.E.Waterbury: The aerobic degradation of 1-(5-nitrofur-furylideneamino)-2-imidazolidinone (NF-246) by *Escherichia coli*, *Arch. Biochem. Biophys.*, **113**, 399—404 (1966).
 - 23) H.E.Paul, V.R.Ells, F.Kopko and R.C.Bender: Metabolic degradation of the nitrofurans, *J.Med.Pharm.Chem.*, **2**, 563—584 (1960).
 - 24) T.Sugimura, M.Nagao, M.Takeuchi, T.Yahagi, K.Hara and T.Matsushima: Carcinogenic mechanisms of 4-nitroquinoline 1-oxide and related compounds, Excerpta Medica International Congress Series No.350, Chemical and Viral Oncogenesis, Proceedings of the XI International Cancer Congress, Florence, Italy, 1974.
 - 25) T.Ou, K.Tatsumi and H.Yoshimura: Isolation and identification of urinary metabolites of AF-2 (3-(5-nitro-2-furyl)-2-(2-furyl)acrylamide) in rabbits, *Biochem. Biophys. Res. Commun.*, **75**, 401—405 (1977).
 - 26) K.Tatsumi, S.Kitamura, T.Ou, H.Yamada and H.Yoshimura: Studies on metabolic activation of nitrofurans: Formation of active metabolites and their binding to cysteine and glutathione, *J.Pharm.Dyn. (Tokyo)*, **1**, 105—113 (1978).