A Method for the Determination of Ethyleneurea in Foods as the Pentafluorobenzamide

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A method was developed for the determination of ethyleneurea in foods at levels of from 0.01 to 5 ppm. Residues were extracted with acetone and the extract alkylated to remove ethylenethiourea (ETU). After partial cleanup on alumina, ethyleneurea was pentafluorobenzoylated and the product purified on a column of silicic acid. Quantitation of the pentafluorobenzamide was carried out by high-pressure liquid chromatography with confirmation by GLC and mass spectrometry. Recoveries from five commodities were generally greater than 80%. The method was unaffected by zineb, whereas 3% of added ETU was converted to ethyleneurea. A preliminary survey of three commodities indicated that approximately 33% of the samples contained ethyleneurea.

Ethyleneurea (2-imidazolidinone) is a reaction product of the ethylenebis(dithiocarbamates) (EBDCs) and represents a major metabolite of these fungicides. It has been identified in plants treated in the greenhouse with [^{14}C]zineb (Vonk, 1976) or outdoors with [^{3}H]mancozeb (Lyman, 1971) or [^{14}C]maneb, [^{14}C]zineb, [^{14}C]nabam, and [^{14}C]ETU (Nash, 1976). Ethylenethiourea (ETU), a transient metabolite of the EBDCs, is a precursor of ethyleneurea in vitro in the presence of light and photosensitizers (Ross and Crosby, 1973; Cruickshank and Jarrow, 1973) and in vivo in plants (Hoagland and Frear, 1976; Nash, 1976).

When fed with nitrite, ethyleneurea has been found carcinogenic in rats (Sander and Bürkle, 1971). Although nitrosoethyleneurea is relatively unstable in aqueous solution (Lee et al., 1977) and thus unlikely to be found in foods, nitrosation of ethyleneurea has been reported to occur readily (Sander, 1972). Nitrosoethyleneurea is mutagenic in bacteria (Lee et al., 1977) and carcinogenic in rats (Druckrey et al., 1967). Since ethyleneurea constitutes a potential hazard to health and residue methods have not been described in the literature, the present method was developed to permit its determination in foods.

The analytical procedure involves extraction and preliminary cleanup on alumina, followed by derivatization to form the pentafluorobenzamide. The derivative combines good absorption at 254 nm for the detection of residues by high-pressure liquid chromatography (LC), with sufficient volatility and electron-capture response to permit its subsequent confirmation by gas–liquid chromatography (GLC). In addition, the derivative may be confirmed by high-resolution mass spectrometry after isolation by LC.

EXPERIMENTAL SECTION

Materials. Ethyleneurea was purchased from Eastman Organic Chemicals, Rochester, N.Y., and was crystallized from acetone to give white needles, mp 131–132 °C (lit. 131–132 °C, Duschinsky and Dolan, 1946). Solutions for fortification were prepared in methanol and added to samples in volumes of 1.0 mL or less before extraction. Pentafluorobenzoyl chloride was obtained from PCR Research Chemicals, Inc., Gainesville, Fla. Pentafluorobenzoylethyleneurea standard was prepared by refluxing ethyleneurea (215 mg, 2.5 mmol) with pentafluorobenzoyl chloride (1.15 g, 5 mmol) in benzene (10 mL) for 2 h. The reaction mixture was concentrated to 2–3 mL and applied to a 1.5 × 9 cm column of silicic acid (Woelm, 100–200 μm) in benzene. After washing with benzene (5 mL), the column was eluted with 10% ethyl acetate in benzene and the 55–135-mL fraction collected. The solvent was removed and the derivative crystallized from benzene–hexane. The crystals melted at 165–167 °C and gave a mass spectrum as shown in Figure 1.

Alumina used for adsorption chromatography (Neutral Alumina AG-7, 100–200 mesh) was purchased from Bio-Rad Laboratories, Richmond, Calif., and was deactivated to activity III by the addition of 6% water. Silicic acid (Woelm, Activity I, 100–200 μm) was supplied by ICN Pharmaceuticals Inc., Cleveland, Ohio, and was used without deactivation.

Analytical Procedure. Samples of blended crop (5.0 g) were homogenized with acetone (50 mL) in a Sorvall Omni Mixer. The homogenate was filtered through Whatman No. 1 paper in a Buchner funnel using slight negative pressure. The filtrate was transferred to a 250-mL round-bottomed flask and methyl iodide (0.1 mL) added. After 30 min at room temperature the solvent was evaporated to slightly less than 1 mL on a rotary evaporator. The residue was transferred to a 5-mL volumetric flask with methanol (4.0 mL) and made to the mark with water. Ethyleneurea was dissolved in 20% aqueous methanol to give a solution containing 1 μg/mL and an aliquot (1.0 mL) was carried through the remainder of the procedure as reference standard.

Alumina adsorption columns were prepared by adding neutral alumina, activity III (4.0 g) to 50% methanol in acetone (15 mL) in a chromatography tube. After settling the adsorbent, fines and supernatant were aspirated. An aliquot (1.0 mL) of extract or standard was added to the column which, after adsorption of the sample, was eluted with 50% methanol in acetone (10 mL). The eluate was collected in a 15-mL centrifuge tube and evaporated to dryness under a stream of nitrogen in a 50 °C water bath. A freshly prepared solution of pentafluorobenzoyl chloride (20 μL) in acetone (0.5 mL) was added, an air condenser fitted, and the tube heated at 75 °C for 1 h. After removal of the solvent with a stream of nitrogen, the samples were taken up in dichloromethane (2 mL).

Silicic acid adsorption columns were prepared by adding silicic acid (2.0 g) to dichloromethane (15 mL) in a glass column. The excess solvent was run through the column and the sample added. After adsorption of the sample, the column was washed with dichloromethane (20 mL) and elution begun with 15% acetonitrile in dichloromethane (20 mL). The last 10 mL of this eluate was collected and evaporated under a stream of nitrogen to dryness. The sample was then taken up in an appropriate volume of

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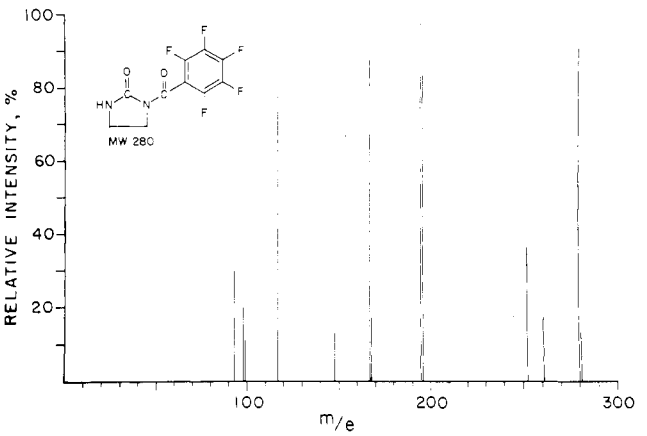

Figure 1. Mass spectrum of 1-pentafluorobenzoyl-2-imidazolidinone; ionization voltage 70 eV.

15% acetonitrile in dichloromethane and an aliquot (50 μL) injected onto the liquid chromatograph.

High-Pressure Liquid Chromatography (LC). Samples were injected onto a 2.2 mm × 50 cm Aerograph Micro Pak Si-10 column by a Valco loop injector fitted with a 50-μL sample loop. The column was eluted with 3% isopropyl alcohol in dichloromethane delivered at a flow rate of 0.5 mL min^{-1} by an Aerograph Model 4000 constant pressure pump. The pentafluorobenzamide was detected and quantitated by absorption at 254 nm on a Waters Model 440 absorbance detector coupled to a 1-mV Honeywell recorder.

For confirmation, the fraction with a retention time of the pentafluorobenzamide was collected in a 6 × 50 mm tube and evaporated to dryness with a stream of nitrogen. The residue was taken up in benzene and an aliquot (5 μL) analyzed by gas–liquid chromatography. For mass spectral confirmation the residue was dissolved in dichloromethane (30 μL) and an aliquot (2 μL) placed on the probe.

Gas–Liquid Chromatography (GLC). Determinations were carried out on a Hewlett Packard Model 5700 A fitted with a ^{63}Ni electron-capture detector and a 6 ft × 4 mm i.d. glass column packed with 6% QF-1 and 4% SE-30 on 80–100 mesh Supelcoport. Argon–methane (95:5) carrier gas was supplied to the column at a flow rate of 35 mL min^{-1}. The column was operated at 205 °C, while the injection port and detector were maintained at 200 and 300 °C, respectively. Under these conditions, and with routine working attenuation, approximately 0.2 ng of pentafluorobenzamide produced 50% full-scale deflection.

Mass Spectrometry. Eluate from the LC was analyzed on a Varian Mat 311A double-focusing spectrometer using single ion monitoring of the molecular ion (*m/e* 280.03) with a resolution of 5000. The sample was introduced at 25 °C and heated to 200 °C within 20 s. By this procedure, 0.1 ng of pentafluorobenzamide corresponding to a sample containing 0.01 ppm ethyleneurea produced an ion current 70% of full-scale deflection.

Samples determined by LC, GLC, or mass spectrometry were quantitated by comparison of the peak heights to that produced by a standard of ethyleneurea carried through the procedure.

RESULTS AND DISCUSSION

Derivatization of ethyleneurea was considered a necessary step in its determination, since it is insufficiently volatile for GLC and is devoid of chromophores which would permit its detection after LC. Previous work (Greenhalgh and Weinberger, 1965) had shown ethyleneurea amenable to acylation with various reagents although yields were often low. This approach was investigated with *p*-nitrobenzoyl chloride and pentafluorobenzoyl chloride, the latter being selected because of its favorable physical characteristics. The reaction with pentafluorobenzoyl chloride occurs as shown in Figure 2, giving the monoacylated product. A yield of approximately 60% was obtained after 1 h of reaction as shown in Figure 3. The yield could not be increased by longer reaction times, increases in reagent concentration, or change in solvent to dichloromethane, benzene, acetonitrile, or dimethylformamide. The addition of powdered anhydrous sodium carbonate or triethylamine was without affect. Because of the incomplete reaction, it was necessary to carry a standard of ethyleneurea through the procedure for quantitation purposes.

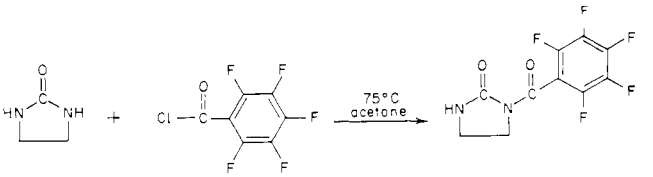

Figure 2. Reaction scheme for the derivatization of ethyleneurea with pentafluorobenzoyl chloride.

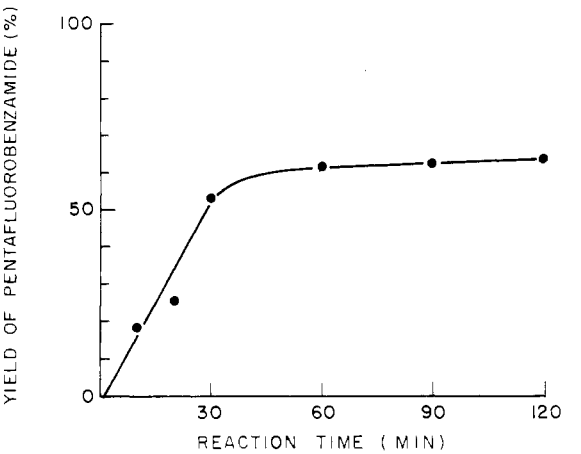

Figure 3. Time course of reaction of pentafluorobenzoyl chloride with ethyleneurea in acetone at 75 °C. Ethyleneurea (1 μg) was heated for various time intervals with pentafluorobenzoyl chloride (20 μL) in acetone (0.5 mL). Yield of pentafluorobenzamide was determined by LC.

Table I. Effect of Methyl Iodide on the Conversion of Various Levels of ETU to Ethyleneurea[a]

ETU added, ppm	ethyleneurea found, ppm	
	without CH_3I	with CH_3I
0.10	0.016	0.004
0.50	0.067	0.010
1.0	0.113	0.025

[a] ETU was added to tomato homogenate and the homogenate analyzed for ethyleneurea.

A preliminary cleanup on alumina was found necessary to avoid decreases in yields of derivative during the reaction step. To obtain quantitative recovery of ethyleneurea from the alumina, it was important to control the amount of water in the sample and to deactivate the alumina by the addition of 6% water to give activity III (Brockmann).

Ethylenethiourea (ETU), an EBDC metabolite which may occur concomitantly with ethyleneurea, was found to be partially converted to ethyleneurea by the analytical method. As shown by the data in Table I, alkylation reduced the conversion from an average of 13% to an

Table II. Effect of 5.7 ppm Zineb or 0.1 ppm ETU on the Recovery of Ethyleneurea from Tomato

ethyleneurea added, ppm	ethyleneurea found, ppm	
	zineb	ETU
0	0	0
0.103	0.102	0.105
0.513	0.505	0.458

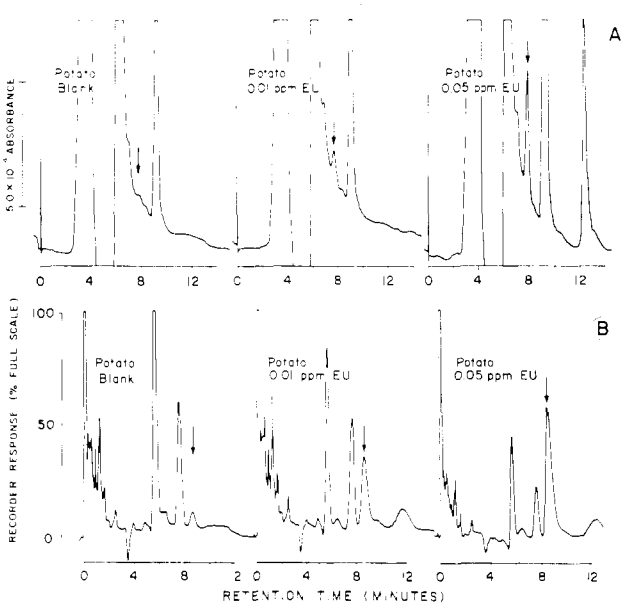

Figure 4. Chromatograms obtained from derivatized extracts of potato fortified with various amounts of ethyleneurea. Chromatograms in A resulted from LC with detection at 254 nm. Eluate from the silicic acid cleanup was evaporated to dryness, the residue taken up in 1.0 mL of solvent, and a 50-μL aliquot representing the equivalent of 100 mg of sample injected. Fractions from samples chromatographed in A were collected, evaporated, and dissolved in benzene (100 μL for blank and 0.01 ppm, and 200 μL for 0.05 ppm). A 5-μL aliquot was injected into a GLC with electron-capture detector to give the tracings shown in B.

average of 3% by weight. The data in Table II show that neither ETU nor zineb affected the recovery of ethyleneurea, nor was any degradation of zineb to ethyleneurea evident. In practice, levels of ETU found on raw foods are generally less than 0.1 ppm (Newsome, 1976; Pease and Holt, 1977; Ripley et al., 1978) and such amounts would result in ethyleneurea values less than 0.01 ppm. However, cooked foods may contain ETU at levels considerably in excess of 0.1 ppm (Newsome, 1976; Onley et al., 1977; Report of the IUPAC Commission on Pesticide Terminal Residues, 1977). In the latter instance, significant amounts of ethyleneurea would be generated by the method, the magnitude of which may be determined by analysis for ETU.

The recoveries of ethyleneurea added to various commodities are given in Table III and are generally greater than 80% from 0.01 to 5.1 ppm. Typical chromatograms are shown in Figure 4. Somewhat lower recoveries were obtained from apple of levels below 0.1 ppm. This was attributed to syrupy impurities present after chromatography on alumina which may have reduced the derivatization. Although rigorous recovery studies were not carried out at levels below 0.01 ppm, 0.005 ppm has been readily detected by GLC and the identity confirmed by mass spectrometry. With some commodities at concentrations below 0.1 ppm, accurate quantitation was possible only by GLC, since the rapidly declining baseline obtained on LC precluded an accurate measurement of peak heights.

Table III. Recoveries of Ethyleneurea Added to Various Commodities

ethyleneurea added, ppm	ethyleneurea recovered,[a] %				
	tomato	potato	carrot	apple	lettuce
0.01	93	97	97[b]	46[b]	91[b]
0.05	79	87	81[b]	62[b]	72[b]
0.10	93	86	80	81	95[b]
0.51	82	85	80	94	87
1.0	84	103	83	96	92
5.1	99	90	85	86	92

[a] Recoveries are calculated relative to a standard of ethyleneurea carried through the procedure simultaneously. Results are the means of duplicate determinations. [b] Values determined by gas-liquid chromatography.

Table IV. Ethyleneurea Content of Three Commercial Commodities

commodity		positive samples	mean of positive samples, ppm	range of positive samples, ppm
spinach	fresh	13/23	0.124	0.026-0.796
	processed	0/10		
tomato	fresh	3/10	0.020	0.011-0.037
	processed	8/15	0.018	0.010-0.025
potato		4/15	tr	[0.004-0.007][a]

[a] Tentative assignment.

A preliminary survey of spinach, tomatoes, and potatoes purchased in the Ottawa area was carried out to obtain an indication of the presence of ethyleneurea in foods of commerce. As shown by the data in Table IV approximately 33% of the 73 samples contained ethyleneurea. Representative positive samples of each commodity were confirmed by mass spectrometry. The levels of ethyleneurea found in potatoes are considered tentative, since recovery data was not obtained below 0.01 ppm.

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LITERATURE CITED

Cruickshank, P. A., Jarrow, H. C., *J. Agric. Food Chem.* **21**, 333 (1973).
Druckrey, H., Preussmann, R., Ivankovic, S., Schmähl, D., *Z. Krebsforsch.* **69**, 103 (1967).
Duschinsky, R., Dolan, L. A., *J. Am. Chem. Soc.* **68**, 2350 (1946).
Greenhalgh, R., Weinberger, M. A., *Can. J. Chem.* **43**, 3340 (1965).
Hoagland, R. E., Frear, D. S., *J. Agric. Food Chem.* **24**, 129 (1976).
Lee, K., Gold, B., Mirvish, S. S., *Mutation Res.* **48**, 131 (1977).
Lyman, W. R., *Pure Appl. Chem., Suppl.*, 243 (1971).
Nash, R. G., *J. Agric. Food Chem.* **24**, 596 (1976).
Newsome, W. H., *J. Agric. Food Chem.* **24**, 999 (1976).
Onley, J. H., Giuffrida, L., Ives, N. F., Watts, R. R., Storherr, R. W., *J. Assoc. Off. Anal. Chem.* **60**, 1105 (1977).
Pease, H. L., Holt, R. F., *J. Agric. Food Chem.* **25**, 561 (1977).
Report of the IUPAC Commission on Pesticide Terminal Residues, *Pure Appl. Chem.* **49**, 675 (1977).
Ripley, B. D., Cox, D. F., Wiebe, J., Frank, R., *J. Agric. Food Chem.* **26**, 134 (1978).
Ross, R. D., Crosby, D. G., *J. Agric. Food Chem.* **21**, 335 (1973).
Sander, J., Bürkle, G., *Z. Krebsforsch.* **75**, 301 (1971).
Sander, J., in "*N*-Nitroso Compounds Analysis and Formation", IARC Scientific Publication No. 3, 1972 p 97.
Vonk, J. W., *Med. Fac. Landbouww. Rijksuniv. Gent*, **41**, 1383 (1976).

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