Kaufman, D. D., Kearney, P. C., *Residue Rev.* **32**, 235 (1970).
McCloskey, J. A., Stillwell, R. N., Lawson, A. M., *Anal. Chem.* **40**, 233 (1968).
Montgomery, M. L., Botsford, D. L., Freed, V. H., *J. Agric. Food Chem.* **17**, 1241 (1969).
Ross, J. A., Tweedy, B. G., *Org. Mass Spectrom.* **3**, 219 (1970).
von Rumker, R., Lawless, E. W., Meiners, A. F., "Production, Distribution, Use and Environmental Impact Potential of Selected Pesticides"; EPA 540/1-74-001, 1974.

Received for review August 29, 1977. Accepted January 17, 1978.

A Method for the Determination of 2-Imidazoline Residues in Food Crops

William H. Newsome* and Luz G. Panopio

A method was developed for the determination of 2-imidazoline in food crops at levels of from 0.1 to 1 ppm. The procedure involves adsorption on a cation-exchange resin, derivatization with *p*-nitrobenzoyl chloride, purification of the derivative on silica gel, and quantitation by ultraviolet absorption after high-pressure liquid chromatography. Mean recoveries ranged from 87.3 to 101% and were not affected by the presence of zineb, ethylenediamine, or ethylenethiuram monosulfide (DIDT).

2-Imidazoline has been identified in plant extracts as a degradation product of ethylenethiourea, an intermediate in the decomposition of ethylenebis(dithiocarbamate) fungicides (Vonk and Kaars Sijpesteijn, 1971). Plants grown outdoors and treated with labeled mancozeb have been reported to accumulate imidazoline in an amount equivalent to 8% of the applied radioactivity 2 weeks after treatment (Lyman, 1971). More recently, Vonk (1976) has examined the fate of [^{14}C]zineb on lettuce grown in a greenhouse and found a similar accumulation 3 weeks after application. The transformation of ethylenethiourea to 2-imidazoline can be carried out photochemically in the presence of a sensitizer (Vonk, 1975), although it has been concluded that while plant constituents are involved, the reaction in plants is probably not photochemical and may be nonenzymic. In view of the probable occurrence of 2-imidazoline in crops treated with ethylenebis(dithiocarbamate) fungicides, the following method was developed to determine such levels of this compound as may exist in foods.

EXPERIMENTAL SECTION

Materials. 2-Imidazoline was synthesized by the oxidation of ethylenethiourea with hydrogen peroxide as described by Vonk (1975). A solution of 30% hydrogen peroxide (32 g) was added dropwise to a stirred solution of ethylenethiourea (10 g) in water (200 mL). The temperature was maintained at 20–30 °C by cooling in an ice bath. After the addition of the peroxide, the solution was stirred for 2 h, then concentrated to approximately 50 mL on a rotary evaporator. The imidazoline was adsorbed on a 3.3 × 10 cm column of Dowex 50W × 8 (50–100 mesh) and the column washed with distilled water (150 mL). Imidazoline was eluted with 4 M HCl (100 ml) and the eluate taken to dryness on a rotary evaporator. The resulting syrup was placed in dessicator over NaOH pellets and evacuated. Crystallization occurred within 1–2 days.

The picrate of 2-imidazoline hydrochloride was prepared and melted at 165–166 °C (lit. 164–165 °C; Jentzsch and Seefelder, 1965). Thin-layer chromatography on cellulose with acetonitrile–water–ammonium hydroxide (80:18:2) as developing solvent gave a single purple spot (R_f 0.50) when sprayed with ferricyanide–nitroprusside reagent. A mass spectrum of the solid introduced on a probe at 85 °C showed a strong (base peak) molecular ion at m/e 70.

Solutions of imidazoline hydrochloride used for fortifying samples were prepared in distilled water and added to the samples in volumes of 0.5 mL or less before extraction.

Nitrobenzoylimidazoline standard was prepared by adding *p*-nitrobenzoyl chloride (1.86 g, 10 mmol) in acetone (10 mL) to 2-imidazoline hydrochloride (214 mg, 2 mmol) in 1 M aqueous sodium carbonate (15 mL). After stirring for 15 min, further 1 M sodium carbonate (25 mL) was added and the mixture was extracted with dichloromethane (25 mL). The crude product obtained by evaporation of the solvent was purified by chromatography on a 1.5 × 15 cm column of silicic acid (Woelm, activity I, 100–200 μm) in dichloromethane using 2% isopropyl alcohol in dichloromethane as eluting solvent. The purified material ran as a single spot on thin-layer chromatography using silica gel plates (Eastman) impregnated with fluorescent indicator and 2% isopropyl alcohol in dichloromethane as developing solvent. The compound had a melting point of 86–88 °C and gave a weak molecular ion at m/e 386 by electron impact mass spectrometry. Chemical ionization resulted in a molecular ion at m/e 387.

Cation-exchange resin (Dowex 50W × 8, 100–200 mesh) resin was purchased from Sigma Chemical Co., St. Louis, Mo., and was washed in bulk as described previously (Newsome, 1974). Ion-exchange columns containing 3.0 mL settled volume of resin were prepared exactly as described earlier (Newsome, 1974). The columns were eluted before use with 1 M NaOH (20 mL), 5 M NaCl (20 mL), 1 M HCl (20 mL), and finally distilled water until the effluent was neutral.

Silica gel for adsorption chromatography, 100–200 μm particle size, was manufactured by Woelm and was used without washing or deactivation.

p-Nitrobenzoyl chloride, 98% pure, was purchased from Aldrich Chemical Co. (Canada) Ltd., Montreal, Quebec. The reagent for nitrobenzoylation consisted of a 0.1% solution in dichloromethane and was prepared afresh before use.

Analytical Procedure. Samples (10.0 g) of previously blended crop were extracted with 0.1 M HCl (25 mL) by

Food Research Laboratories, Bureau of Chemical Safety, Foods Directorate, Health Protection Branch, Health and Welfare Canada, Tunney's Pasture, Ottawa, K1A 0L2, Ontario, Canada.

0021-8561/78/1426-0638$01.00/0

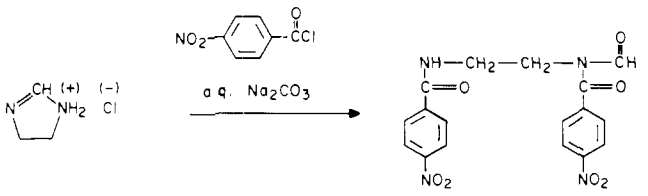

Figure 1. Reaction scheme for the derivatization of 2-imidazoline.

homogenizing at high speed for 1 min in a Sorvall Omni-Mixer. Celite 545, acid washed (approximately 0.7 g), was added and the mixture poured into a 4.25-cm Buchner funnel containing two layers of Whatman No. 1 filter paper. After permitting the suspension to settle for 10 min, the liquid was filtered under vacuum. The funnel was rinsed with a further portion of 0.1 M HCl (5 mL) and the filtrate transferred to an ion-exchange column.

The sample was permitted to flow through the column at a rate of 30 mL/h. The column was then washed with 1 M NaCl (15 mL), and the washings were discarded. 2-Imidazoline was eluted with 3.0 M NaCl (10 mL).

A 0.1% solution *p*-nitrobenzoyl chloride in dichloromethane (0.5 mL) was added to the eluate in a stoppered tube. After the addition of anhydrous sodium carbonate (100 mg), the tube was shaken for 5 min. The derivative was extracted with a further volume of dichloromethane (2 mL) and the extract dried with anhydrous sodium sulfate.

The dried extract was purified by chromatography on a silica gel column (Woelm, activity I, 2.0 g) prepared in dichloromethane. After adsorption of the sample, the column was washed first with dichloromethane (25 mL) and then with 2% isopropyl alcohol in dichloromethane (15 mL). Both fractions were discarded, and the derivative was eluted with a further 10 mL of 2% isopropyl alcohol in dichloromethane. The final eluate was evaporated to dryness with a stream of nitrogen, and the residue was dissolved in dichloromethane for analysis by high-pressure liquid chromatography.

High-Pressure Liquid Chromatography. Samples were quantitated by absorption at 254 nm after chromatography on a 2.2 mm × 50 cm Aerograph Micro Pak Si-10 column fitted with a Valco loop injector (50 μL) and an Isco Model UA-5 absorbance monitor. The detector was equipped with 254-nm source filters and was connected to a 100-mV Sargent-Welch recorder. The eluting solvent was 1.6% isopropyl alcohol in dichloromethane and was delivered to the column by an Aerograph Model 4000 constant pressure pump at a flow rate of 0.5 mL/min. 2-Imidazoline was quantitated by comparison of the peak height of the *p*-nitrobenzoyl derivative to that obtained from a standard of 2-imidazoline carried through the entire procedure simultaneously.

RESULTS AND DISCUSSION

The reaction scheme for the derivatization of 2-imidazoline is shown in Figure 1. Ring opening during the treatment of 2-imidazoline with *p*-nitrobenzoyl chloride in sodium carbonate is typical of these compounds under alkaline conditions in the presence of carboxylic or sulfonic acid chlorides (Aspinall, 1941). As indicated by the data presented in Figure 2, the reaction is rapid, maximum yield (74% of theoretical) being obtained within 1 min. Thereafter, hydrolysis appears to occur as indicated by the decrease in yield with further reaction time. For the analytical procedure, the reaction was carried out for 5 min which resulted in more reproducible recoveries. Under neutral conditions, in 3 M NaCl no derivative was formed. In the presence of sodium bicarbonate (100 mg) or sodium hydroxide (0.1 M), the yield was approximately 5% of that in sodium carbonate.

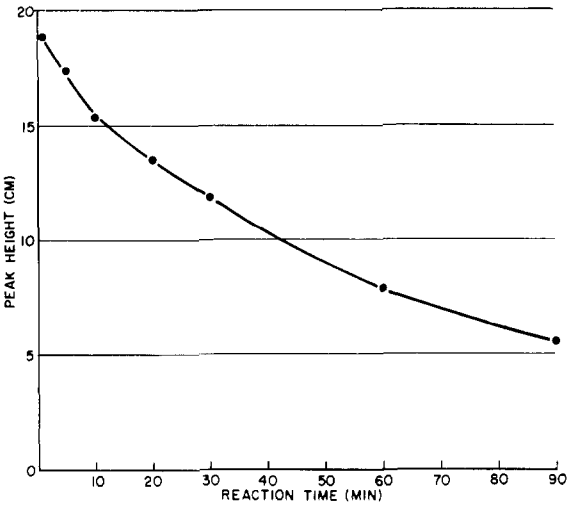

Figure 2. The effect of reaction time on the yield of para-nitrobenzoylated imidazoline.

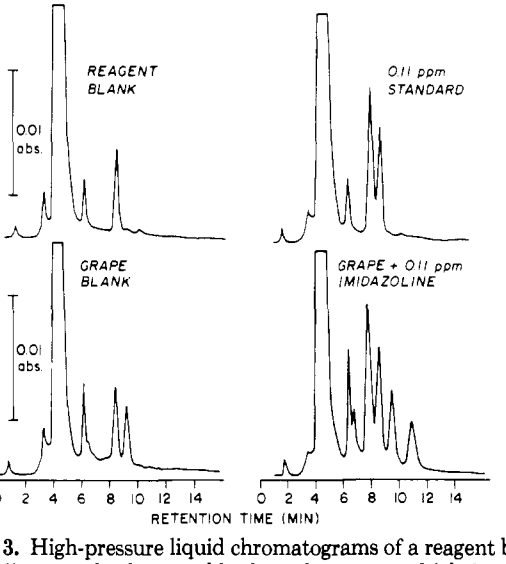

Figure 3. High-pressure liquid chromatograms of a reagent blank, imidazoline standard, grape blank, and grape to which 0.11 ppm 2-imidazoline hydrochloride had been added. A 50-μL aliquot of the final solution was injected, representing the equivalent of 500 mg of sample. The retention time of para-nitrobenzoylated imidazoline is 7.8 min.

Table I. Recovery of 2-Imidazoline from Various Commodities

2-Imidazoline hydrochloride added, ppm	2-Imidazoline hydrochloride recovered, %[a]			
	Tomato	Apple	Lettuce	Grape
0.11	95	94	84	95
0.22	99	87	104	98
0.55	108	84	85	108
1.1	97	94	93	103
Mean recovery ± SE	100 ± 3	87 ± 2	91 ± 5	101 ± 3

[a] Values are the means of duplicate determinations and are relative to a 1:1 μg standard of 2-imidazoline hydrochloride carried through the procedure.

Chromatograms of grape samples analyzed with and without the addition of 2-imidazoline are shown in Figure 3. Similar patterns were obtained with other commodities. Some background peaks were evident when reagents alone were carried through the procedure, but none interfered with the *p*-nitrobenzoylimidazoline peak and no attempt

Table II. Recovery of 2-Imidazoline from Lettuce in the Presence of 7 ppm Zineb, 0.1 ppm Ethylenediamine, or 0.1 ppm DIDT[a]

2-Imidazoline hydrochloride added, ppm	2-Imidazoline hydrochloride found, ppm		
	Zineb	Ethylene-diamine	DIDT
0	0.005	0.009	0.009
0.110	0.089	0.095	0.101
0.550	0.485	0.562	0.483

[a] DIDT = 5,6-dihydro-3*H*-imidazo[2,1-*c*]-1,2,4-dithiazole-3-thione.

was made to remove them. A minimum detectable limit of at least 0.02 ppm is suggested from the peak heights of fortified samples compared to the background of controls.

As shown by the data in Table I, the recoveries of 2-imidazoline obtained from four commodities were constant over a tenfold range of concentration and varied from a mean of 87.3% in apples to 101% in grapes. The values are calculated relative to a standard of 2-imidazoline hydrochloride (1.1 μg) added to a blank extraction solvent and carried through the analytical procedure. The absolute recovery of the standard was 55% of the theoretical yield. A 80% yield of derivative was formed when the standard was reacted and determined without prior column chromatography, indicating a 25% loss on the columns. Attempts to improve the recovery by increasing the concentration of reagent or strength of eluting solvents resulted in unacceptably high background.

The effects of zineb and two of its degradation products which could be adsorbed on the ion-exchange resin and possibly interfere with the determination of imidazoline were examined. The results of this experiment are given in Table II and show that, at the levels studied, there is no reduction or enhancement of the recovery of imidazoline.

ACKNOWLEDGMENT

The authors wish to thank Walter Miles for mass spectral determinations.

LITERATURE CITED

Aspinall, S. R., *J. Org. Chem.* **6**, 895 (1941).
Jentzsch, W., Seefelder, M., *Chem. Ber.* **98**, 1342 (1965).
Lyman, W. R., *Pure Appl. Chem., Suppl.*, 243 (1971).
Newsome, W. H., *J. Agric. Food Chem.* **22**, 886 (1974).
Vonk, J. W., Kaars Sijpesteijn, A., *Pestic. Biochem. Physiol.* **1**, 163 (1971).
Vonk, J. W., Dissertation, State University, Utrecht, 1975.
Vonk, J. W., *Meded. Fac. Landbouwwet., Rijksuniv. Gent* **41**, 1383 (1976).

Received for review October 4, 1977. Accepted January 11, 1978.

Rapid, Simple Procedures for the Simultaneous Gas Chromatographic Analysis of Four Chlorophenoxy Herbicides in Water and Soil Samples

Blythe A. Olson,* Thomas C. Sneath, and Naresh C. Jain

Water and soil samples are acidified and extracted into organic solvent for the determination of 2,4-D, 2,4-DP (dichlorprop), 2,4,5-T, and 2,4,5-TP (silvex). A similar but not generally used compound, 2,3,4-trichlorophenoxyacetic acid (2,3,4-T), is added to the samples prior to extraction for use as an internal standard. Water samples require no further cleanup whereas soil samples are cleaned up by back-extraction into alkali, a single chloroform wash, acidification, and final ether extraction. Samples are derivatized with BF_3–methanol and analyzed by electron-capture gas chromatography. The simultaneous extraction of fortified and blank control samples provides a means for recovery correction for quantitation and reduces the risk of false positives from glassware or reagent contamination. Minimum quantitation level for all four herbicides in both media is 0.001 ppm.

Although numerous gas chromatographic methods for the simultaneous analysis of chlorophenoxy herbicides in soil or water have appeared in the literature, they have, in general, been more time consuming, less sensitive, and/or less easily quantitated (Devine and Zweig, 1969; Glas, 1976; Goerlitz and Lamar, 1967; Purkayastha, 1974) than the procedures described herein. Methods reported for the analysis of a single chlorophenoxy herbicide in soil or water, while useful for their intended applications, have had some of the same relative drawbacks (Gutenmann and Lisk, 1964; McKone and Hance, 1972; Schultz and Harman, 1974; Schultz and Whitney, 1974; Woodham et al., 1971). Considerable additional work has been reported on the analysis of these compounds in a variety of environmental media, including plant and animal tissue.

Several of the chlorinated phenoxyalkanoic acid herbicides have been used routinely in Los Angeles County weed abatement programs for many years. They have proved extremely useful in brush control for firebreaks and control of broadleaf weeds in recreational turf areas. They are also used for weed control by private operators in the county.

Because of their frequent application, a monitoring program was set up in 1973 to test environmental samples from various treated and untreated sites throughout Los Angeles County for these herbicide residues. It was necessary to devise sensitive, rapid, and consistently accurate methods of analysis for the many specimens collected under this program. Resultant procedures for the analysis of water and soil samples for 2,4-D, 2,4-DP (di-

Toxicology Service, Rancho Los Amigos Hospital, Downey, California 90242 (B.A.O., T.C.S., N.C.J.) and the Departments of Pharmacology and Nutrition and of Community and Family Medicine, University of Southern California School of Medicine, Los Angeles, California 90033 (N.C.J.).

0021-8561/78/1426-0640$01.00/0