Derivatization of Ethylenethiourea with *m*-Trifluoromethylbenzyl Chloride for Analysis by Electron-Capture Gas Chromatography

Russell R. King

m-Trifluoromethylbenzyl chloride reacts with ethylenethiourea in the same manner as benzyl chloride. The resultant *S*-(*m*-trifluoromethylbenzyl) derivative is stable, has good GLC characteristics, and can readily be detected at 0.01 ppm by electron-capture detector. The N-trifluoroacetylated analogue was detectable by flame photometric detector (S mode) and electron-capture detector at levels of 0.01 and 0.002 ppm, respectively. Recoveries of ethylenethiourea from apples, green beans, potatoes, and tomatoes at fortification levels of 0.01 to 1.0 ppm averaged 92.5 ± 4.6%.

Ethylenethiourea (ETU) is a degradation product of a widely used group of fungicides, the ethylenebisdithiocarbamates. It has been detected in formulated materials (Bontoyan et al., 1972; Czegledi-Janko and Hollo, 1967; Fishbein and Fawkes, 1965; Petrosini et al., 1963; Ludwig et al., 1954; Lopatecki and Newton, 1952) and as residues in certain food crops (Pecka et al., 1975). Toxicological investigations have demonstrated that at high concentrations ETU is goitrogenic (Graham et al., 1973; Graham and Hansen, 1972; Ulland et al., 1972; Innes et al., 1969), carcinogenic (Meland et al., 1972), and teratogenic (Khera, 1973). Because of the potential health hazard, sensitive and reliable methods are required for determining the residue on or in foods.

When direct analysis of ETU by GLC was found unsatisfactory Onley and Yip (1971) formed the *S*-butyl derivative by reaction with *n*-butyl bromide in the presence of base. Although the procedure required extensive cleanup it was highly sensitive and ETU levels as low as 0.02 ppm could be detected with a thermionic detector. Haines and Adler (1973) modified the derivatization procedure and used a flame photometric detector. Newsome (1972) reacted ETU with benzyl chloride, followed by acidification with HCl to form the S-benzylated hydrochloride which is water soluble. This permitted a preliminary extraction of interfering substances without recourse to lengthy cleanup procedures. After neutralization with alkali the free base was quantitatively extracted with an organic solvent. Formation of the N-trifluoroacetylated derivative with trifluoroacetic anhydride made possible detection of ETU at 0.01 ppm with an electron-capture detector. Nash (1974) substituted pentafluorobenzoyl chloride for trifluoroacetic anhydride to form the more stable *N*-pentafluorobenzoyl analogue but column chromatography to eliminate interfering GLC peaks was required.

We have established that reaction of ETU with *m*-trifluoromethylbenzyl chloride yields a stable *S*-(trifluoromethylbenzyl) derivative which after extractive cleanup can be determined on GLC at levels similar to Newsome's *N*-trifluoroacetyl derivative.

EXPERIMENTAL SECTION

Chemicals. 2-Imidazolidinethione (ETU) was purchased from Eastman Organic Chemicals, Rochester, N.Y., and recrystallized once from 95% ethanol. The recrystallized ETU was dissolved in water and added to the plant material in 0.5-ml volumes per 10 g. *m*-Trifluoromethylbenzyl chloride was obtained from K&K Laboratories, Plainview, N.Y., and used as received with the exception of one batch which required purification by chromatography on a silica gel column (elution with hexane). Trifluoroacetic anhydride was purchased from J. T. Baker Chemical Co., Phillipsburg, N.J., and used as received.

Equipment. NMR spectra were obtained in $CDCl_3$ solution with Me_4Si as an internal standard on a Varian T-60 NMR spectrometer. The MS were determined by a Finnigan 3100 GS/MS coupled to a D 600 data acquisition system.

Gas–Liquid Chromatography. A Tracor 222 gas chromatograph equipped with electron-capture ^{63}Ni detector (with linearizer) and a 1.8 m × 4 mm i.d. glass column packed with 3% OV-275 on 80–100 mesh H.P. Chromosorb W was used. The operating parameters were: injection port, 220 °C; column, 210 °C; detector, 300 °C; and 5% methane–argon flow rate, 60 ml/min. Under these conditions *S*-(*m*-trifluoromethylbenzyl)-ETU had a retention time of 3 min, and the N-trifluoroacetylated *S*-(*m*-trifluoromethylbenzyl)-ETU had a retention time of 3.5 min. A Tracor flame photometric detector operating in the sulfur mode was used to confirm the electron-capture detector results.

Derivatization of ETU (Figure 1). A reference sample was prepared by refluxing ETU (500 mg) with *m*-trifluoromethylbenzyl chloride (2 ml) in 95% ethanol (25 ml) on a water bath for 30 min. The solution was diluted with water (150 ml), acidified with 6 N HCl, and extracted with chloroform (100 ml). The aqueous phase was made alkaline with 10% NaOH and the free base extracted with chloroform (60 ml). Removal of the chloroform on a rotary evaporator and crystallization of the residue from hexane gave *S*-(*m*-trifluoromethylbenzyl)-ETU: mp 57–58 °C; NMR signals at τ 2.53 (4 H, multiplet, aromatic), 5.62 (2 H, singlet, benzylic), and 6.28 (4 H, singlet, ethylenic); MS data, *m/e* 260 (61%), 159 (100%), 109 (60%), 72 (91%), and 70 (75%). Standard solutions of *S*-(*m*-trifluoromethylbenzyl)-ETU (1 μg/ml) in benzene were stable over a period of several months at room temperature. Reference standards of N-trifluoroacetylated *S*-(*m*-trifluoromethylbenzyl)-ETU were most conveniently prepared by dissolving *S*-(*m*-trifluoromethylbenzyl)-ETU in 10% (v/v) trifluoroacetic anhydride in benzene and diluting to the desired concentrations with benzene containing 1% trifluoroacetic anhydride. A sample crystallized from anhydrous ether–hexane had mp 127–129 °C and MS data, *m/e* 356 (10%), 287 (32%), 159 (100%), 109 (87%), and 72 (73%).

Analytical Procedures. Samples of plant material (10 g) were homogenized with 95% ethanol (50 ml) in a Waring blender and the homogenate filtered through Whatman No. 1 filter paper. A 5.0-ml aliquot (equivalent to 1.0 g

Research Station, Research Branch, Agriculture Canada, Fredericton, New Brunswick, Canada E3B 4Z7.

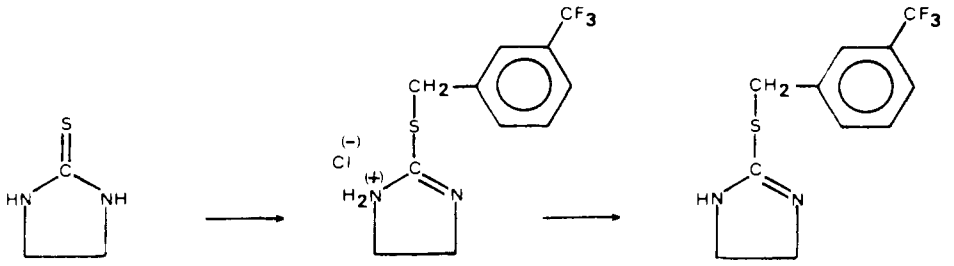

Figure 1. Reaction scheme for derivitization of ETU with *m*-trifluoromethylbenzyl chloride.

of sample) was placed in a 50-ml round-bottomed flask containing a boiling bead. *m*-Trifluoromethylbenzyl chloride (4 drops) was added and the contents refluxed on a water bath for 30 min. After cooling, the condenser was washed with 1–2 ml of 95% ethanol and 1 drop of 6 N HCl was added to the reaction flask. Removal of the ethanol on a rotary evaporator (water bath at 50 °C) left an *aqueous residue* which was diluted with ca. 0.5 ml of H_2O. This was transferred by pasteur pipet to a 5-ml centrifuge tube (with pennyhead stopper). The reaction flask was rinsed with another portion of H_2O (ca. 0.5 ml) which was added to the centrifuge tube. Diethyl ether (ca. 1 ml) was added to the contents in the centrifuge tube, it was shaken, and the top (ether) layer removed by pasteur pipet. The ether extraction was repeated; then the centrifuge tube was placed in hot water (50 °C) for several minutes to assure removal of any remaining ether. After cooling, exactly 0.5 ml of benzene was added followed by 3 drops of 10% NaOH. The tube was shaken immediately and the two layers allowed to separate. Subsequently, 5-μl aliquots were drawn off in a chromatographic syringe for injection into the GLC. For confirmatory purposes 0.2 ml of the benzene layer was removed; then a few grains of anhydrous sodium sulfate and 1 drop of 20% trifluoroacetic anhydride in benzene were added. The reaction was allowed to proceed for 5 min at room temperature and a 10-μl aliquot was injected into a GLC fitted with a Tracor flame photometric detector operating in the S-mode. Levels of 0.01 ppm of ETU were confirmed by this procedure. For the electron-capture detector, the solvent was evaporated just to dryness under a steam of *dry* nitrogen and a known volume of benzene was added for injection into the GLC. Levels of 0.002 ppm of ETU could be detected by this procedure.

RESULTS AND DISCUSSION

In an initial assessment of published ETU analytical procedures, Newsome's method proved the most viable. However, consistent difficulties with quantitative workup convinced us that alternate methods of analysis should be investigated. The simplest procedure visualized was substitution of benzyl chloride with some equally reactive multihalogenated alkyl or aromatic halide and then electron capture GLC of the resultant ETU derivative. An extensive literature survey of commercially available reagents eventually provided one outstanding candidate, *m*-trifluoromethylbenzyl chloride. It reacts with ETU in the same manner as benzyl chloride and *m*-trifluoromethylbenzylation of ETU is complete after 30 min of refluxing in 95% ethanol. Ethanol was favored over methanol as derivatization proceeded more rapidly and the reaction went to completion more consistently.

Once formed, the *S*-(*m*-trifluoromethylbenzyl) hydrochloride proved quite acid stable. This facilitated removal of ethanol, unreacted *m*-trifluoromethylbenzyl chloride, and other extraneous volatiles on a rotary evaporator. After ether extraction of nonvolatiles in the remaining aqueous phase, treatment with alkali liberated the free

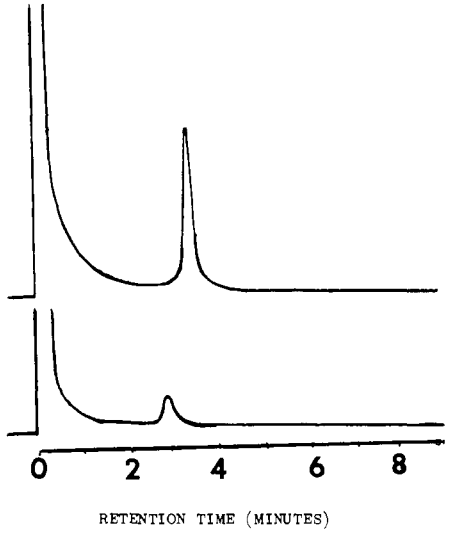

Figure 2. Comparative gas-liquid chromatograms of *S*-(*m*-trifluoromethylbenzyl)-ETU (bottom) and N-trifluoroacetylated *S*-(*m*-trifluoromethylbenzyl)-ETU (top); 0.1-ng injections.

base which was extracted into benzene. Since no drying of the benzene layer was required, aliquots could be removed by syringe for injection directly into the gas chromatograph. Our workup circumvents the potential loss of sample by volatilization during solvent removal that is inherent in other procedures (Newsome, 1972; Nash, 1974). It also eliminates the use of chloroform, small traces of which adversely affect the electron-capture detector response.

Use of the *S*-(*m*-trifluoromethylbenzyl) derivative avoids another reaction sequence and all the attendant possibilities for loss of sample therein. Moreover, background complications arising from derivatization of contaminants in an intermediate preparation are no longer encountered.

Since one can quantitatively monitor *S*-(*m*-trifluoromethylbenzyl)-ETU it is also possible to confirm identity of the compound by further derivatization. For example, N-trifluoroacetylation of *S*-(*m*-trifluoromethylbenzyl)-ETU increased the electron-capture response sixfold and lengthened the retention time (Figure 2). This is more advantageous than determination of two different derivatives for confirmation of ETU in a single plant extract (Nash, 1975).

The lower limit of sensitivity for *S*-(*m*-trifluoromethylbenzyl)-ETU varied slightly with the crop but was always found satisfactory at levels down to 0.01 ppm of ETU. Samples of tomatoes, apples, potatoes, and green beans were fortified with ETU in the 0.01 to 1.0 ppm range. Fortification recovery values ranged from 81 to 97% with an average recovery of 92.5 ± 4.6%. Figure 3 illustrates typical gas chromatograms of samples fortified at 0.05 ppm. GLC–mass spectrometry was used to confirm that the peak occurring on GLC was due to the ETU

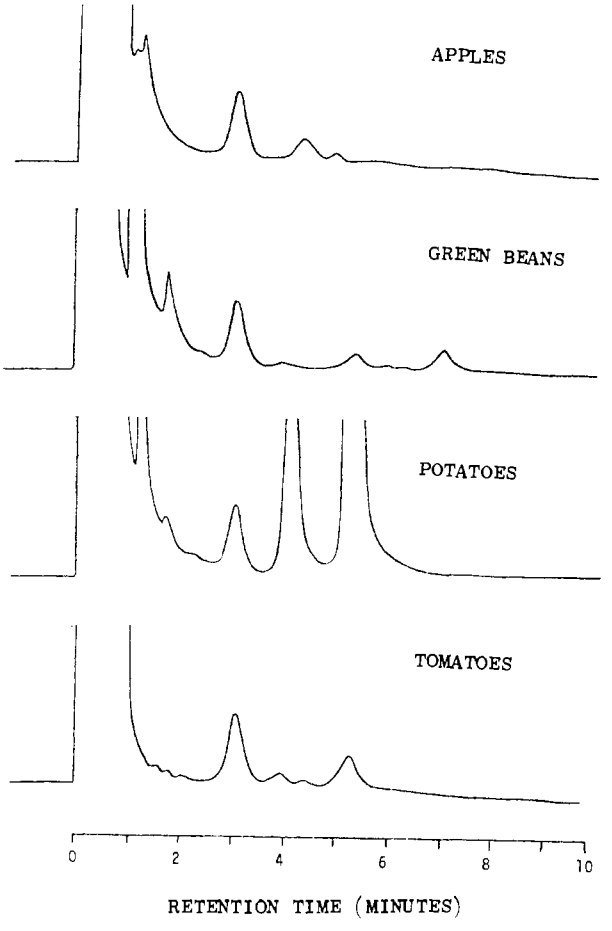

Figure 3. Gas-liquid chromatograms of derivitized apple, green bean, potato, and tomato extracts fortified with ETU at 0.05 ppm.

derivative. It was further evident from an abundant parent ion peak at m/e 260 that the *S*-(*m*-trifluoromethylbenzyl) derivative survived GLC without decomposition.

Because the electron-capture detector is nonspecific, confirmation of positive ETU determinations using an element sensitive detector was also investigated. Consequently, it was observed that the N-trifluoroacetylated *S*-(*m*-trifluoromethylbenzyl)-ETU flame photometric detector (S-mode) response was comparable to that of the *S*-(*m*-trifluoromethylbenzyl)-ETU electron-capture detector response and positive electron-capture detection of the latter could be confirmed by flame photometric (S-mode) analysis of the former. GLC–mass spectrometry confirmed the N-trifluoroacetylated *S*-(*m*-trifluoromethylbenzyl)-ETU peak and indicated a breakdown pattern analogous to Newsome's N-trifluoroacetylated *S*-benzyl ETU derivative.

ACKNOWLEDGMENT

The author wishes to thank Floyd Wood, Agriculture Canada, Fredericton, New Brunswick, Canada, for helpful discussions and also S. M. Skinner and Roy Greenhalgh of the Chemistry and Biology Research Institute, Agriculture Canada, Ottawa, Ontario, Canada, for the NMR and GLC–mass spectral determinations.

LITERATURE CITED

Bontoyan, W. R., Looker, J. D., Kaiser, T. E., Giang, P., Oliver, B. M., *J. Assoc. Off. Anal. Chem.* **55**, 923 (1972).
Czegledi-Janko, G., Hollo, J., *J. Chromatogr.* **31**, 89 (1967).
Fishbein, L., Fawkes, J., *J. Chromatogr.* **19**, 364 (1965).
Graham, S. L., Hansen, W. H., *Bull. Environ. Contam. Toxicol.* **7**, 19 (1972).
Graham, S. L., Hansen, W. H., Davis, K. J., Perry, C. H., *J. Agric. Food Chem.* **21**, 324 (1973).
Haines, L. D., Adler, I. L., *J. Assoc. Off. Anal. Chem.* **56**, 333 (1973).
Innes, J. R. M., Ulland, B. M., Valerio, M. G., Petrucelli, L., Hart, E. R., Pallotta, A. J., Bates, R. R., Falk, H. L., Gart, J. J., Klein, M., Mitchell, I., Peters, J., *J. Natl. Cancer Inst.* **42**, 1101 (1969).
Khera, K. S., *Teratology* **7**, 243 (1973).
Lopatecki, L. E., Newton, W., *Can. J. Bot.* **30**, 131 (1952).
Ludwig, R., Thorn, G. D., Miller, D. M., *Can. J. Bot.* **32**, 48 (1954).
Meland, B. M., Weisburger, J. H., Weisburger, E. K., Rice, J. M., Cypher, R., *J. Natl. Cancer Inst.* **99**, 583 (1972).
Nash, R. G., *J. Assoc. Off. Anal. Chem.* **58**, 566 (1975).
Nash, R. G., *J. Assoc. Off. Anal. Chem.* **57**, 1015 (1974).
Newsome, W. H., *J. Agric. Food Chem.* **20**, 967 (1972).
Onley, J. H., Yip, G., *J. Assoc. Off. Anal. Chem.* **54**, 165 (1971).
Pecka, Z., Baulu, P., Newsome, H., *Pestic. Monit. J.* **8**, 232 (1975).
Petrosini, G., Tafuri, F., Businelli, M., *Not. Mal. Piante* **65**, 9 (1963).
Ulland, B. M., Weisburger, J. H., Weisburger, E. K., Rice, J. M., Cypher, R., *J. Natl. Cancer Inst.* **49**, 583 (1972).

Received for review May 27, 1976. Accepted August 30, 1976.

Extraction and Recovery of Organophosphorus Metabolites from Urine Using an Anion Exchange Resin

Emile M. Lores* and Diane E. Bradway

Urinary metabolites of organophosphorus pesticides are extracted quantitatively from urine by using an ion exchange resin. The metabolites were subsequently derivatized on the resin for FP–GC analysis. Several ion exchange resins and a variety of methods for the recovery of the metabolites from the resin were investigated. When used in coordination with a previously developed method, higher recoveries and decreased gas chromatographic interferences were obtained. Recovery data, limits of detectability, and analysis of urine samples from individuals exposed to organophosphorus pesticides are reported.

With the increasing demand for degradable pesticides, there has been an increase in the use and variety of organophosphorus pesticides. To the chemist, trying to monitor the metabolites of these pesticides, this situation causes a twofold need for: a faster method because of the increased work load and a new method to monitor the new metabolites for which the older methods were not intended. The work to develop a method of analysis for the

Analytical Chemistry Branch, Environmental Toxicology Division, Health Effects Research Laboratory, Research Triangle Park, North Carolina 27711.