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Extractive Acylation of Ethylenethiourea from Water

J. Singh, W. P. Cochrane, and J. Scott

Laboratory Services Division, Food Production and Marketing Branch, Agriculture Canada, Ottawa, Ontario, Canada, K1A OC5

Various techniques have been derived for the determination of ethylenethiourea (ETU) residues (IUPAC 1977). Derivatisation of ETU is usually necessary prior to gas chromatography to obtain increased sensitivity, improved separation characteristics and greater specificity. In every case so far, derivatisation involves alkylation of the thiocarbonyl group (ONLEY & YIP 1971, NEWSOME 1972) and in some methods a second derivatisation involving the NH-group is also used (KING 1977, NASH 1974, 1975).

However, these procedures invariably require lengthy reaction times at reflux temperatures to achieve acceptable yields of the derivative. Also, one major problem is that under these conditions any ethylenebisdithiocarbamate fungicide residues present is converted to ETU to a certain extent (PEASE & HOLT 1977, KELLAR & OTTO 1978). We report here an alternative derivatisation procedure which involves the extractive derivatisation of ETU from water at room temperature. In this one-step extraction and derivatisation method the ETU is partitioned from water into the organic phase, which contains the reagent, using acetonitrile as phase transfer agent.

EXPERIMENTAL

Apparatus and Reagents

(a) Gas chromatograph equipped with a linearised ^{63}Ni electron capture detector and 1.8 m x 2 mm id glass column filled with either 3% OV-330 or 3% OV-17 on 80-100 mesh Chromosorb 750 was used. Operating conditions: injector 200°C, column, 180-200°C, argon-methane (1:9) carrier gas flow rate, 45 mL/min.

(b) Mass spectral data were obtained using a Finnigan 300 GC/MS coupled to a D6000 data acquisition system. Column, 1.5 m x 2 mm id glass containing 3% SE-30 Ultra-phase at 165°C with a helium flow rate of 35 mL/min.

(c) Infrared spectra were determined using KBr pellets.

(d) Nuclear Magnetic Resonance was measured using a 10% Solution of the ETU derivative in deuterated dimethylsulphoxide with tetramethylsilane as internal standard.

(e) Dichloroacetic Anhydride (DCAA) redistilled. A 0.01% stock solution was prepared containing 20 mg reagent in 200 mL CH_2Cl_2.

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Procedure

(a) Microscale extractive acylation. Dissolve 2.5 ug ETU to 50 mL CH_3CN water mixture (1:10) in 125-mL separatory funnel. Add 15 mL of 0.01% DCAA in CH_2Cl_2, shake, let stand until layers separate (1-3 min). Draw off lower CH_2Cl_2 layer and pass through plug of Na_2SO_4 into 125-mL r.b. flask. Wash Na_2SO_4 twice with 5 mL portions of CH_2Cl_2. Combine and evaporate the CH_2Cl_2 to 1-2 mL on a rotary evaporator, and then to dryness using a gentle stream of dry nitrogen. Dissolve residue in 5 mL benzene and analyse via GC-ECD.

(b) Preparative-scale reaction. ETU (1.0 g) was dissolved in 100 mL CH_3CN in a 250-mL flask. DCAA (1.6 mL) was added, the mixture shaken and allowed to stand at room temperature for 30 min. Evaporate, on a rotary evaporator to about 1 mL and complete using stream of nitrogen. Dissolve product in minimum amount of hot benzene (ca 5mL), allow to crystallize in an ice/water bath. Second crop of crystals can be obtained by adding cold hexane to the solution. Filter and was crystals with cold hexane. Recrystallize from 85% ethanol m.p. 123-125°C (decomp). Calculated for $C_5H_6Cl_2N_2OS$: C,28.2; H, 2.84; N, 13.2; Cl, 33.3; S, 15.2. Found: C, 28.8; H, 2.99; N, 12.7; Cl, 32.4; S, 15.1. Infrared bands (KBr) 3305 (NH), 1675 (C=O), 1525, 1380 and 1215 (C=S) cm^{-1}.

RESULTS AND DISCUSSION

Extractive alkylation has been proven to be a convenient simple and quantitative procedure for the derivatisation of acidic organic compounds such as carboxylic acids and phenols (DAVIS 1977, ROSENFELD & CROCCO 1978). In these studies pentafluorobenzyl bromide was used for extractive alkylation from serum and environmental substrates prior to ECD-GC. Similarly, the phase-transfer-catalysed alkylation of reactive NH-compounds in a two-phase system has also been reported (BARCO et al.1976). However, using various alkyl bromides and iodides as reagents, several hours reaction time were required to give yields in the 80-98% range. In the case of ETU, reaction with alkyl halides results in derivatisation at the thiocarbonyl group, especially under rigorous reaction conditions. This reaction has, in fact, been utilized for the characterisation of alkyl halides (BOYD & MEADOW 1960). Therefore, in preliminary investigations into an extractive derivatisation procedure for ETU, the effectiveness of haloacetic anhydrides as either N-acylation or S-alkylation reagents, was studied (CAMPAIGNE & WANI 1964).

Dichloroacetic anhydride was found to give a single product on extractive derivatisation from water and subsequent ECD-GC analysis. In a survey of seven different GC columns (Table 1) for this derivative, OV-330 and OV-17 proved superior in that they gave acceptable GC peaks without excessive tailing and it required less frequent priming to maintain reproducibility. When benzene:water or CH_2Cl_2: water mixtures were tried, no extractive derivatisation of ETU was observed indicating that a phase transfer agent was necessary. Acetonitrile is extensively used in the partitioning of pesticide residues and when added to the above mixtures, derivatisation was obtained.

TABLE 1

Peak characteristics of mono-dichloroacetyl ETU on various stationary phases[a]/

Column	Temperature °C	Retention Time (min)	Remarks
3% SE-30 Ultraphase	150	4.88	Tailing with 20 ng. No peak at 200 pg.
3% OV-17 [b,c]/	200	5.2	Sharp peak with 1 ng.
3% Silar 5-CP	180	4.35	Tailing with 20 ng. No peak at 200 pg after priming.
3% OV-330[c]/	200 180	2.54 5.5	Sharp peak with 1 ng.
3% OV-275	160-220	--	No peak with 0.5 ng.
3% Versamid	215	--	No peak
11% OV-17/QF-1	180	8.35 and 9.76	2 peaks observed.
4% OV-101/6% OV-210	180	6.10	Tailing with 20 ng. No peaks at 200 pg after priming.

a/ Using electron capture detection unless state otherwise.
b/ Using an alkali flame ionisation detector.
c/ See Figure 1.

Varying the acetonitrile content in water from 5-30%, the highest percent conversion was obtained with the 10% CH_3CN:water combination. For example, extractive acylation of 2.5 ug ETU (0.05 ppm) in 10% CH_3CN: water (50 ml) with 15 ml CH_2Cl_2 containing 0.01% DCAA reagent a 80% yield was obtained.

Elemental analysis of the isolated product gave an empirical formula of $C_5H_6Cl_2N_2OS$ which is consistent with mono-dichloroacetyl derivative of ETU (Structure 1). However, GC/MS displayed a parent ion peak at m/e 176 (containing 1 chlorine) together with fragments at m/e 141, 97 and 70 base peak). Therefore, it was postulated that the ETU derivative, which had a m.p. of 123-125°C, with decomposition, was losing HCl on-column to give 2,3,5,6-tetrahydro-6-chloro-imidazo (2,1-b) thiazol-3-one (Structure 2). Since this latter compound could theoretically be derived from its own HCl salt or loss of HCl from the monodichloracetyl derivative of ETU, the nature of the micro-scale ETU derivative was further investigated. Infra-red analysis of the isolated product showed major absorptions at 3320 (NH), 1675 (C=O) and 1215 cm^{-1}, while nmr displayed a singlet at 1.52 (1H) and an almost symmetrical multiplet

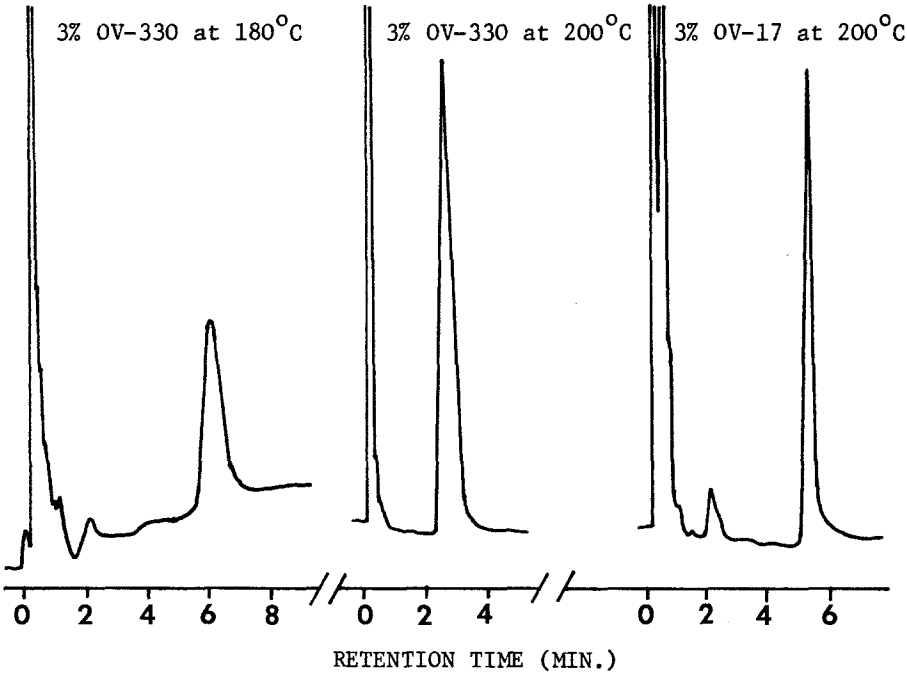

Figure 1: Chromatographic characteristics of the ETU derivative on different stationary phases.

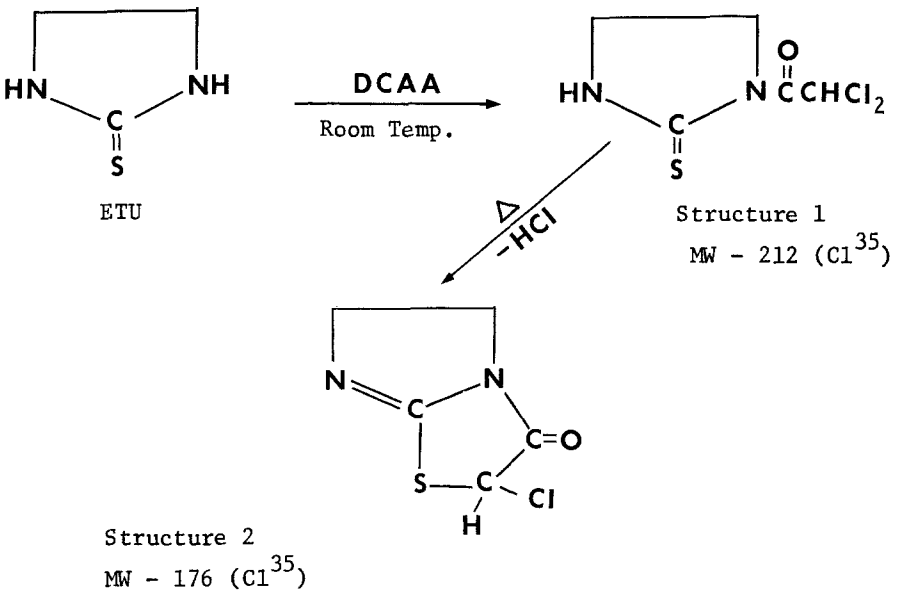

Figure 2: Derivatisation scheme for ETU.

at 6.0-6.9 (5 Hs). To conclusively prove that mono-acetylation had occurred the isolated product was further derivatised to the di-dichloroacetyl product (m.p. 113-114°C) using dichloroacetyl chloride as reagent at room temperature. Interestingly, the nmr displayed only 2 singlets at 4.67 (2H) and 5.8 (4H). This collapse of the methylene proton multiplet in the mono-derivative to a singlet on further derivatisation is consistent with acylation of both nitrogens to give a symmetrical compound (GREENHALGH & WEINBERGER 1965). A proposed reaction scheme is shown in Figure 2.

Since ETU is very soluble in water nearly all current extraction procedures involve organic solvents which are miscible with water. Therefore, our current use of the above method has been as a rapid screening procedure for the presence of ETU in water samples at the 0.01-0.05 ppm level. Further studies with alternate reagents and solvent systems are continuing in an effort to devise a quantitative procedure for ETU in biological substrates.

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