Ethylenethiourea Degradation

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Ultraviolet irradiation (above 285 nm) of ethylene-thiourea (ETU) on a solid substrate such as silica gel affords 2-imidazolidone as the major identifiable product. Several other products are formed, of which only bis(imidazolin-2-yl) sulfide has been identified. The 2-imidazolidone does not appear to undergo further degradation under these conditions. Decomposition of ETU is rapid, particularly in the presence of photosensitizers. Aqueous solutions of ETU undergo a very slow photolysis, which is markedly accelerated by sensitizers. ETU is stable to hydrolysis over the pH range 5.0 to 9.0 at 90°.

The ethylenebisdithiocarbamates constitute an important class of fungicides widely used in current agricultural practice. Ethylenethiourea (ETU), recently described as tumorigenic in mice (Innes et al., 1969), has been found to be a decomposition product of and contaminant in these fungicides (Czegledi-Janko and Hollo, 1967; Fishbein and Fawkes, 1965; Lopatecki and Newton, 1952; Petrosini et al., 1962). Because of its tumorigenic properties, it is important to know the fate of any ETU which may reach the environment.

Field experiments with maneb (manganese ethylenebisdithiocarbamate) indicated that ETU initially present in the formulation rapidly decreased to undetectable amounts (Yip et al., 1971). In order to determine the effects of physical factors on this disappearance, a laboratory study on the photolysis and hydrolysis of ETU was undertaken. Results presented here include photolysis on a solid substrate (silica gel), photolysis in aqueous solution, and hydrolysis over a 5.0–9.0 pH range.

EXPERIMENTAL SECTION

Chemicals. 2-Imidazolidinethione (ETU) was purchased from Eastman Organic Chemicals, Rochester, N. Y., and was recrystallized from 95% ethanol; the material, mp 202–203°, was homogeneous by thin-layer chromatographic analysis. Stock solutions containing 1% ETU in methanol and in distilled water were prepared. The ^{14}C-labeled ETU (Niagara Chemical Division, Middleport, N. Y.) with a specific activity of 6.59 mCi/mmol was dissolved in methanol at a 0.01% concentration. Bis(imidazolin-2-yl) sulfide was prepared by the method of Johnson and Edens (1941). Photosensitizers were purchased from J. T. Baker Chemical Co., Phillipsburg, N. J.

ETU   2-Imidazolidone

Bis(imidazolin-2-yl) sulfide
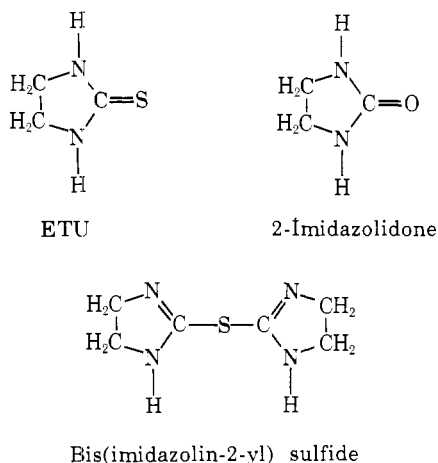
Determination of ETU. ETU was determined by thin-layer chromatography (tlc) on Brinkmann Silica Gel F-254

Niagara Chemical Division, FMC Corporation, Princeton, New Jersey 08540.

precoated plates using ether-methanol (9:1, v/v); under these conditions ETU has R_f = 0.5. Quantitation for amounts between 30 and 100 μg was effected with a chromatogram scanner (Nester/Faust, Newark, Del.) in the fluorescence quench mode. Although a linear response was obtained over this range (Figure 1), the absolute value for a given quantity of ETU varied from plate to plate, and the slope of the response could vary with different lots of tlc plates. For these reasons ETU standards were run at two different levels on each analytical plate in order to establish the appropriate baseline.

For determination of ETU at levels <10 μg, material labeled with ^{14}C at the thione carbon was used. The ETU spots were precisely located by autoradiography, and the amounts were determined by scintillation counting of radioactivity in the recovered spots.

Hydrolysis. Aqueous solutions containing 1% ETU were buffered at pH 5 (phosphate), pH 7 (phosphate), and pH 9 (phosphate-borate). Portions of these solutions, as well as a 1% solution of ETU in distilled water, were held in vials sealed with rubber septums at room temperature and at 90° for 3 months. Aliquots were removed periodically by means of a syringe for determination of ETU.

Irradiation. Silica gel plates were exposed to a General Electric 275-W sunlamp (essentially no emission below 280 nm) at a distance to afford 3300 μW/cm², or to a pair of uv fluorescent lamps (one 300 nm phosphor and one 350 nm phosphor) through a Pyrex filter, and adjusted to give 300 μW/cm². Pyrex is essentially opaque to irradiation below 285 nm, and transmits only a small amount of the incident irradiation between 285 and 300 nm. Aqueous solutions contained in Pyrex tubes were exposed in a Rayonet "Merry-go-Round" Reactor (Southern New England Ultraviolet Co., Middletown, Conn.) containing eight 300 nm phosphor lamps and eight 350 nm phosphor lamps; the intensity at the center of this reactor was 1900 μW/cm². Ultraviolet light intensity was measured in microwatts per square centimeter with a Black-Ray Ultraviolet Intensity Meter (Ultra-Violet Products, Inc., Model J-227 metering unit, J-222 long wavelength cell).

Photolysis on silica gel tlc plates was carried out with 7 μl (70 μg of ETU) of the methanolic stock solution. The size of the spot was adjusted to afford approximately 100 μg of ETU per cm² of surface area. For photosensitized experiments, an equal weight (1% w/v) of the sensitizer was dissolved in the methanolic stock solution. Experiments also were carried out with [^{14}C]ETU at a loading of 1 μg/cm².

Aqueous photolysis was carried out on 5-ml aliquots of the stock solution in Pyrex tubes. Photosensitized experiments were carried out with 0.1% w/v added sensitizer; methanol was added as a cosolvent when required, always maintaining the initial ETU concentration at 1%.

Photolysis Products. The products of ETU photolysis on silica gel can be partially separated by tlc using butanol-acetic acid-water (4:1:1). Components were detected by fluorescence quench, ninhydrin, Ehrlich's reagent,
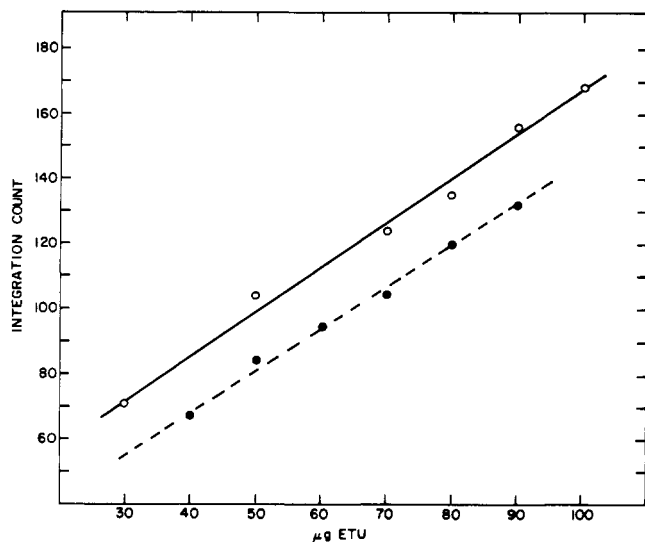
Figure 1. Fluorescence quench analysis of ETU on silica gel thin-layer plates containing a phosphor activated by 254-nm uv radiation. Plates represent duplicate runs (see Experimental Section); 90% confidence limits for the slope = 1.28–1.42.

or by autoradiography. Product identification was confirmed by tlc using 2-butanone–pyridine–water–acetic acid (70:15:15:2) or benzene–ethyl acetate–diethylamine (5:4:1) plus 8% methanol. Relative amounts of photolysis products were determined by scintillation counting of radioactivity in recovered tlc zones.

RESULTS AND DISCUSSION

Ethylenethiourea (ETU) was found to be extremely stable toward hydrolysis. No changes in concentration were observed in aqueous solutions held at 90° for 3 months. Simple hydrolysis therefore cannot contribute to the disappearance of ETU from the environment.

Photolysis, however, does appear to be an important pathway by which ETU can be destroyed. Exposure of ETU adsorbed on silica gel (100 μg/cm^2) to near ultraviolet irradiation (above 285 nm) affords a slow but significant loss (Figure 2). Addition of 1-acetonaphthone as a photosensitizer markedly enhances the initial rate of ETU decomposition; subsequent decrease in the rate presumably is due to inactivation of the sensitizer. Exposure of the ETU-sensitizer combination to a more intense source (GE sunlamp) resulted in decomposition of 97% of the ETU in 20 hr. At a loading of 1 μg/cm^2, less than 1% of the ETU remained after 20 hr.
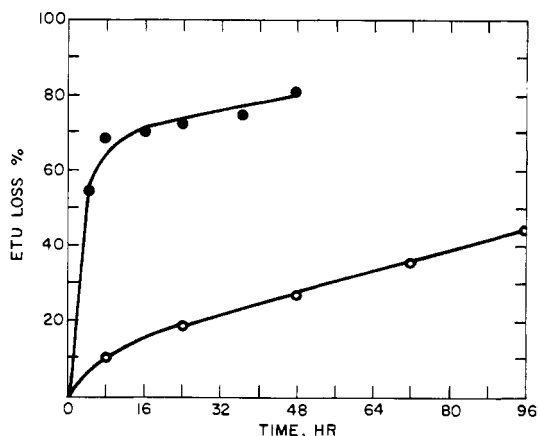

Figure 2. Photodecomposition of ETU (100 μg/cm^2) on silica gel. Irradiation source intensity, 300 μW/cm^2; -O-, without sensitizer; -●-, with equal quantity of 1-acetonaphthone.

Table I. Sensitized Photodecomposition of ETU on Silica Gel; 20-hr Exposure at 300 μW/cm^2

Photosensitizer	% loss	Photosensitizer	% loss
	20	Methylene blue	40
1-Acetonaphthone	70	Flavone	30
1-Naphthaldehyde	70	Benzophenone	30
Eosin	60	Benzil	20
trans-Stilbene	40	Carbazole	10

Several sensitizers in addition to 1-acetonaphthone were found to enhance the rate of ETU photodecomposition. Results with selected materials are shown in Table I.

The possibility of a dark reaction on silica gel between ETU and added sensitizer was excluded by holding samples in the dark for several days; there was no loss of ETU. There do not appear to be any products formed by direct photochemical reaction between ETU and the added compounds. The composition of the photodegradation products as determined by thin-layer chromatography was not changed by the addition of photosensitizers.

Impurities in the ETU could conceivably act as quenchers or photosensitizers. This possibility was minimized by carrying out an experiment in which 1 μg of [^{14}C]ETU was chromatographed in one dimension of a 20 cm × 20 cm silica gel tlc plate prior to exposure, and chromatographed in the second dimension after 20 hr of irradiation. The loss of ETU and the composition of the photodegradation products was identical to a sample exposed concurrently on the same plate without prior chromatography.

Chromatography of an irradiated ETU spot with a polar solvent system affords the pattern shown in Figure 3. Nine components in addition to unchanged ETU were detected by a combination of fluorescence quench, ninhydrin and Ehrlich's reagent stains, and autoradiography

Table II. Products of ETU Photolysis on Silica Gel

Spot no.	Detection				Zone	Quan-tity,[b] %
	FQ[a]	Nin-hydrin	Ehrlich's	Auto-radiog-raphy		
1	–	–	–	+ }	A	6
2	+	–	–	+		
3	–	+	–	+		
4	+	?[c]	–	+ }	B	43
5	–	+	–	+		
6	+	–	–	+		
7	–	–	–	+	C	10
8	–	–	+	+	D	38
9	+	–	+	+	E	2
10	–	–	–	+	F	1

[a] Fluorescence quench. [b] Determined by scintillation counting of radioactivity in recovered zones. [c] Possible ninhydrin response masked by components 3 and 5.

Table III. Identification of Photolysis Components

Spot no.[a]	R_f in solvent system[b]				Identity
	I	II	III	IV	
6	0	0.28	0.16	0.46	Bis(imidazolin-2-yl) sulfide
8	0.17	0.58	0.69	0.41	2-Imidazolidone
9	0.50	0.71	0.85	0.57	2-Imidazolidinethione

[a] From Figure 3. [b] I = ether–methanol (9:1); II = butanol–acetic acid–water (4:1:1); III = 2-butanone–pyridine–water–acetic acid (70:15:15:2); IV = benzene–ethyl acetate–diethylamine (5:4:1) plus 8% methanol.
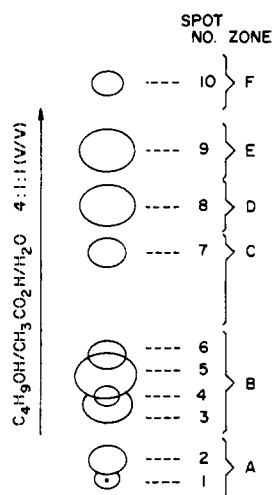
Figure 3. Autoradiogram of ETU photolysis on silica gel.

Table IV. Sensitized Photodecomposition of ETU in Aqueous Solution; 24-hr Exposure at 1900 μW/cm^2

Photosensitizer	% loss	Photosensitizer	% loss
	< 5	Flavone	10
1-Acetonaphthone	30	Eosin	10
1-Naphthaldehyde	30	Benzophenone	10
Methylene blue	30	Acetophenone	<10
2-Acetonaphthone	20	Crystal violet	<10

(Table II). Of these, only 2-imidazolidone and bis(imidazolin-2-yl) sulfide have been identified by comparison of R_f values in four different solvent systems (Table III). Table II also gives the amount of the various components following 20 hr of exposure to the GE sunlamp.

2-Imidazolidone has been reported to undergo photooxidation to hydantoin (Ingamells, 1963). However, hydantoin has not been detected among the ETU photodecomposition products, nor did irradiation of 2-imidazolidone on silica gel afford any of the other products of ETU photolysis.

The ninhydrin positive components of ETU photolysis remain unidentified. Although component no. 3 (Figure 3) has an R_f value identical to glycine, attempts to detect a dinitrophenyl derivative (Levy and Chung, 1955) or the volatile N-trifluoroacetylglycine methyl ester (Cruickshank and Sheehan, 1964) were unsuccessful.

ETU is much more stable toward photolysis in aqueous solution. Irradiation at an intensity of 1900 μW/cm^2 for 24 hr gave an insignificant loss (<5%); however, irradiation at 3300 μW/cm^2 for 15 days resulted in a 33% loss of

ETU. Addition of photosensitizers to the aqueous solution again enhanced the rate of photolysis; results are summarized in Table IV. The aqueous solution experiments were discontinued upon publication of work by Ross and Crosby (1972).

From the results of this work, it is apparent that photochemical degradation can play an important role in the removal of ETU from the environment. Still to be determined, however, is the nature and fate of the ninhydrin positive components (Zone B, Figure 3) which constitute a major portion of the photolysis products on silica gel.

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Photolysis of Ethylenethiourea

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Ethylenethiourea (ETU) in aqueous solution (0.5–50 ppm) was stable to sunlight. However, in the presence of dissolved oxygen and sensitizers such as acetone or riboflavin, it was rapidly photooxidized via ethyleneurea and glycine sulfate. ETU decomposition also occurred in boiled samples of agricultural drainage waters in sunlight but not in the dark, indicating that natural photosensitizers may play an important part in the environmental transformations of xenobiotics.

Ethylenethiourea (2-imidazolidinethione, ETU) occurs as an impurity in technical ethylene bisdithiocarbamate fungicides as well as from both their metabolic (Engst and Schnaak, 1967; Seidler et al., 1970; Vonk, 1971) and nonbiological (Klöpping and Van der Kerk, 1951; Petrosi-

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ni et al., 1963; Viel and Chancogne, 1971) alteration. It dissolves in water to the extent of 2 g/l. at 30° (Stecher, 1960) but, with the exception of methanol and ethanol, it has low to negligible solubility in common organic solvents. This water solubility helps to explain its lack of persistence as a foliage residue (Onley and Yip, 1971) and suggests that it might be found as an environmental contaminant in agricultural runoff waters. Because of the