

NOTE

SYNTHESIS OF ISOTOPICALLY-LABELLED ETHYLENETHIOUREA

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SUMMARY

¹³C-, ¹⁴C- and ¹⁵N-labelled ethylenethiourea was synthesized for NMR and radiotracer applications in metabolism and environmental fate studies. The products contained stable isotopes at 99% enrichment and the radiolabelled product had specific activity of 2.0 mCi/mmole.

Key Words: Ethylenethiourea, Thyroid toxicity, NMR, EBDC fungicides.

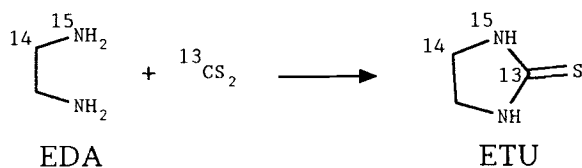
INTRODUCTION

Ethylenethiourea (ETU) is a decomposition product and metabolite from ethylene-bis-dithiocarbamates, the most widely used class of fungicides in the world. ETU is toxic to the thyroid gland, causing goiter and thyroid cancer in experimental animals (1). ETU labelled with stable and radioisotopes is essential to studies of its metabolism and environmental fate. This study describes the synthesis of ETU from commercially available precursors labelled with ¹⁵N and ¹³C as NMR reporter groups and ¹⁴C for radiotracer applications.

RESULTS AND DISCUSSION

The synthetic routes to isotopically-labelled ETU are shown in Scheme 1.

SCHEME 1. SYNTHESIS OF ISOTOPICALLY-LABELLED ETU



1,3-¹⁵N-ETU was synthesized from ¹⁵N₂-ethylenediamine

dihydrochloride (EDA), 4,5-¹⁴C-ETU from 1,2-¹⁴C-EDA and 2-¹³C-ETU from ¹³CS₂ by modifications of the method of Van Allan *et al.* (2). The isotopically-labelled ETU was isolated in yields, purity and specific enrichment/activity sufficient for use in metabolism and environmental fate studies. Table 1 lists the NMR chemical shifts for ETU and a variety of products formed by chemical and metabolic oxidation of ETU (3).

Table 1. ¹³C and ¹⁵N Chemical Shifts for ETU and Metabolites.

2- ¹³ C:	181 ppm	167	157	---
¹⁵ N ₂ :	266	300	265, 350	361

EXPERIMENTAL

Synthesis of 1,3-¹⁵N-ETU. To a solution of 250 mg ¹⁵N₂-ethylenediamine (99% enrichment, Isotec Inc., Miamisburg, OH, 1.9 mmole) in 0.3 ml water was added 210 mg (2 equivalents) potassium hydroxide in 0.3 ml water. The mixture was heated under reflux for 20 min and 0.6 ml ethanol plus 380 mg carbon disulfide (5 mmole). The mixture was refluxed at 60°C for 45 min and the temperature was raised to 100°C for an additional 30 min. Concentrated HCl (0.3 ml) was added and the solution

refluxed at 100°C for 7 hr. The mixture was cooled in ice, filtered and washed with cold acetone to yield crystalline ETU (78%). The product purity, confirmed by TLC and HPLC, was not enhanced by recrystallization and the melting point, NMR and UV spectra were consistent with authentic ETU. ^{15}N -NMR spectra were observed with a Nicolet NT300 spectrometer at 30.5 MHz using broad-band proton decoupling in 30% $\text{D}_2\text{O}/\text{H}_2\text{O}$ as solvent with ^{15}N -acetamide as an external reference to set chemical shifts to 0 ppm for nitromethane.

Synthesis of 4,5- ^{14}C -ETU. To 10 μmole of ^{14}C -EDA diHCl (Amersham Co., Arlington Heights, IL, 4.84 mCi/mmole) in a break-seal tube was added 0.1 ml of 0.88 M potassium hydroxide. This solution was added to 4 mg (30 μmoles) of unlabelled EDA diHCl in a 1 ml reactival. The break-seal tube was rinsed with 0.1 ml ethanol and this added to the reactival along with 5 μl (83 μmole) carbon disulfide. The reactival was sealed and heated 3 hr at 60°C. After cooling to room temperature, 1 drop concentrated HCl was added and the sealed vial heated at 90°C for 8 hr. The cooled mixture was applied to a Uniplate 1000 micron preparative silica TLC plate (Analtech, Newark, DL) and developed with 10% methanol in chloroform. The band containing ^{14}C -ETU was eluted with methanol and the chemical and radiochemical purity determined by TLC and HPLC (Novapak silica, 10 micron, 12.5% acetonitrile/water). The specific activity was determined by HPLC and liquid scintillation counting to be 1.95 mCi/mmole, the yield was 10.2 μmole (40%) with radiochemical yield of 20 μCi .

Synthesis of 2- ^{13}C -ETU. EDA (429 mg, 7.2 mmole) was dissolved in 4 ml of 50% aqueous ethanol and after cooling the round bottom flask, 500 mg (6.6 mmole) ^{13}C -carbon disulfide (99%, Cambridge Isotopes, Cambridge, MA) added. The solution was heated at reflux for 1 hr at 60°C and 3 drops concentrated HCl added after

cooling. The solution was then refluxed for 10 hr at 100°C for 10 hr. The solution was cooled in ice to yield 333 mg (56%) crystalline ETU. Product purity was not increased by recrystallization and the melting point, NMR and UV spectra were consistent with authentic ETU. ¹³C-NMR spectra were observed with a Nicolet NT300 spectrometer at 76 MHz using D₂O as solvent and dioxane as an internal reference ($\delta = 66.5$ ppm) and broad band proton decoupling.

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