SYNTHESIS OF 3,5,6-TRICHLOROPYRIDIN-2-OL-2,3,4,5,6-$^{13}C_5$-^{15}N; A METABOLITE OF O,O-DIETHYL-O-(3,5,6-TRICHLORO-2-PYRIDYL)-PHOSPHOROTHIOATE (CHLORPYRIFOS)

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SUMMARY

A stable isotope-labelled analog of 3,5,6-trichloropyridin-2-ol has been synthesized. 3,5,6-Trichloropyridin-2-ol-2,3,4,5,6-$^{13}C_5$-^{15}N was synthesized in a copper catalyzed cyclization from trichloroacetyl chloride-1,2-$^{13}C_2$ and acrylonitrile-1,2,3-$^{13}C_3$-^{15}N.

Key words: Chlorpyrifos, 3,5,6-Trichloropyridin-2-ol, Stable Isotope, Quantitation, Internal Standard

INTRODUCTION

As part of our continuing efforts to establish normative data on the exposure of the U. S. population to environmental toxicants, the stable isotopically-labelled compound 3,5,6-trichloropyridin-2-ol-^{15}N-2,3,4,5,6-$^{13}C_5$ was synthesized for use as an isotope dilution standard in mass spectrometry for the quantitation of 3,5,6-trichloropyridin-2-ol, a human urinary metabolite of O,O-diethyl-O-(3,5,6-trichoro-2-pyridyl)-phosphorothioate (tradename Dursban). The use of a stable isotopically-labelled analog as an internal standard for a target analyte is an essential feature of the isotope dilution method in gas chromatography-mass spectrometry (ID-GC/MS). An estimation of the level of exposure by a sample of the U. S. population to Dursban and other chemicals is currently underway using this ID-GC/MS method for measuring the metabolites or the pesticides in urine.

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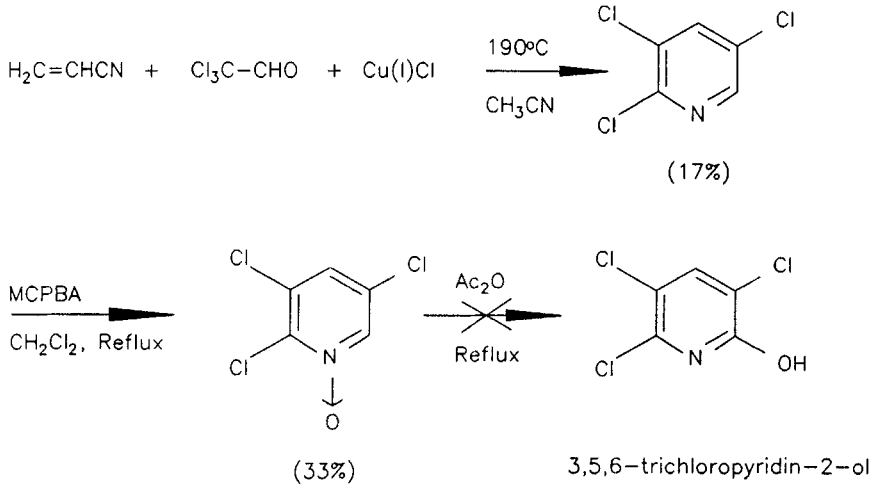
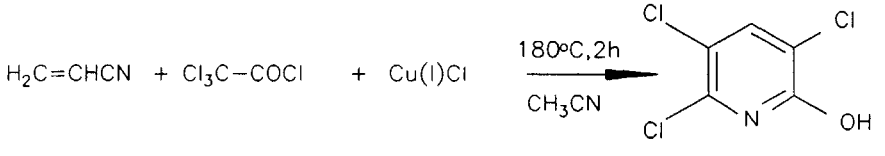
RESULTS AND DISCUSSION

Previous workers had reported the copper catalyzed cyclization of acrylonitrile and chloral to give 2,3,5-trichloropyridine (1). This prompted us to examine this route for preparing the corresponding pyridinol(equation 1).



Equation 1

The cyclization reaction followed by oxidation of the trichloropyridine compound with metachloro perbenzoic acid (MCPBA) afforded the N-oxide product in 33% yield. However, unlike the rearrangement of the N-Oxide of pyridine (2), this chlorinated N-oxide would not rearrange in refluxing Ac_2O and resulted in recovery of the starting material. Elsewhere (3), the same workers had reported a variation of the same cyclization in which trichloroacetyl

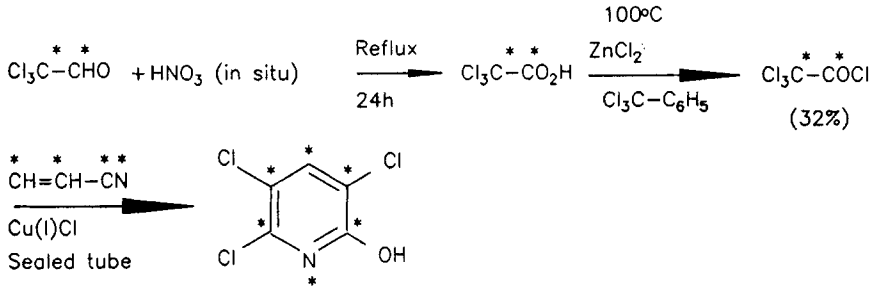


Equation 2

chloride and

acrylonitrile reacted to give 3,5,6-trichloropyridin-2-ol directly (Equation 2). Hence, commercially available chloral-1,2-$^{13}C_2$ was converted into the corresponding acid chloride in

good yield (Equation 3). Copper catalyzed cyclization of the acid chloride with commercially available acrylonitrile-^{15}N-1,2,3-$^{13}C_3$ afforded the desired 2,3,5-trichloro-pyridin-6-ol-^{15}N,2,3,4,5,6-$^{13}C_5$ in a modest yield (238 mg, 11%) in spite of a mechanical loss of about half of the reaction mixture due to a rupture of a pressure seal during heating in the final step (Equation 3). The pyridinol was purified by ion-exchange chromatography on BioRad AG 1-X2 anionic resin in methanol, eluting with glacial acetic acid.



Equation 3

EXPERIMENTAL

Preparation of anhydrous HNO_3 (4). Into a round bottomed flask were weighed 7.0 g (82 mmol) of $NaNO_3$ and 14 g (ca. 138 mmol) of concentrated H_2SO_4. The mixture was heated, and the HNO_3 distilled through a short path condenser to collect about 3 ml or <90% of HNO_3, bp 84°C. For convenience, the HNO_3 was collected at -78°C in a receiver containing the chloral for the next reaction.

Preparation of trichloroacetic acid-1,2-$^{13}C_2$ (4). A mixture of chloral-1,2-$^{13}C_2$ and 3 ml of anhydrous HNO_3 was refluxed with stirring under a dry atmosphere. Copious red fumes evolved early in the heating. After 24 h, the clear dark yellow reaction was fractionally distilled to separate a low boiling fraction, bp 80-120°C, followed by 2.233 g (72.4%) of trichloroacetic acid-1,2-$^{13}C_2$ as a white, crystalline solid (too hygroscopic for an accurate mp), bp 196°C.

Preparation of trichloroacetyl chloride-1,2-$^{13}C_2$ (5). In a flask fitted with a condenser and suitable HCl gas trap was weighed 2.233 g (13.49 mmol) of trichloroacetic acid-1,2-$^{13}C_2$, 2.6423 g (13.52 mmol) of α,α,α-trichlorotoluene, and 23.7 mg (0.174 mmol) of $ZnCl_2$. The mixture was heated by a bath at 100-110°C for 2 h, during which time HCl gas was evolved. The clear pale yellow solution was distilled through an efficient column under a blanket of argon to separate 2.0277 g (81.7%) of trichloroacetyl chloride-1,2-$^{13}C_2$ as a clear, colorless liquid, bp ca. 110°C, n_D^{20} 1.4698.

Preparation of 2,3,5-trichloro-pyridin-6-ol-UL-^{15}N,2,3,4,5,6-$^{13}C_5$ (3). Acetonitrile was dried by distillation from CaH_2 immediately before use. Copper (I) chloride was purified by precipitation from sulfurous acid (6). In a commercial pressure tube (ACE 8648-03) equipped with a Teflon stirring bar and Teflon screw cap was weighed, in the order named, 1.9504 g (10.60 mmol) of trichloroacetyl chloride-1,2-$^{13}C_2$; 0.6046 g (10.61 mmol) of acrylonitrile-^{15}N,1,2,3-$^{13}C_3$; 2 mL of dry acetonitrile; and lastly, 68.1 mg (0.69 mmol) of Cu(I)Cl. The tube was purged briefly with argon, then sealed and immersed in an oil bath at 180°C. [Note: An unfortunate rupture of the O-ring after 90 minutes of heating resulted in the loss of approximately half of the reaction material]. The light brown residue was extracted with hot pentane (6 x 5 mL). The pentane was evaporated under reduced pressure, and the near black residues were dissolved in 5 mL of MeOH. A portion of this solution was passed through a column packed with 10 mL (8 meq) of BioRad AG 1-X2 anionic resin (acetate form) packed in MeOH (7). MeOH was collected until the eluate was clear, followed by elution with glacial acetic acid (ca. 50 mL or 5 column volumes). The acetic acid was evaporated under reduced pressure at 40°C to afford a tan solid. Sublimation of this material at 1.5 mm Hg and 110°C afforded 235.7 mg (11%) of 2,3,5-trichloro-pyridin-6-ol-UL-^{15}N,2,3,4,5,6-$^{13}C_5$ as a white solid, mp 164-168°C, mass spectrum, m/z (relative intensity) 207 (29, M+4), 205 (94, M+2), 203 (100, M^+), 176 (56), 174 (60), 139(28), 112(26), 110(39), 103(24).

Anal. Calcd. for M_r 202.9340061. Found M_r 202.935349.

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