

Radiochemical Preparation of 3, 4, 3', 4',-Tetrachloroazobenzene-¹⁴C

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SUMMARY

Interest for the preparation of ¹⁴C-labeled 3,4,3',4'-tetrachloroazobenzene for metabolism studies was developed after recent reports indicated that soil micro-organisms could transform some chlorinated aniline based herbicides into azobenzene compounds. The preparation of tetrachloroazobenzene was carried out in a three-step synthesis starting with ring-¹⁴C-3-(3,4-dichlorophenyl)-1,1-dimethylurea (Diuron) which was previously prepared in the laboratory. The overall product yield for the synthesis was estimated to be 33.6 %, and the specific activity of the labeled material was 252 μ Ci/mM.

INTRODUCTION.

A herbicide, 3,4-dichloropropionilide, has been demonstrated to yield 3,4,3',4'-tetrachloroazobenzene by microbial transformation in soil ⁽¹⁾. Since tetrachloroazobenzene can be formed under environmental conditions, interest has developed concerning the metabolic fate of this compound in plant ⁽²⁾ and microbial ⁽³⁾ systems. In view of this interest, tetrachloroazobenzene-¹⁴C was prepared for use in metabolism studies.

The radioactive starting material for this synthesis was ¹⁴C-ring labeled 3-(3,4-dichlorophenyl)-1,1-dimethylurea which was previously prepared in the laboratory ⁽⁴⁾. The series of reactions given in Figure 1 were utilized for the preparation of the labeled material.

The experimental reactions for the preparation of the tetrachloroazobenzene were carried out several times employing nonradioactive materials

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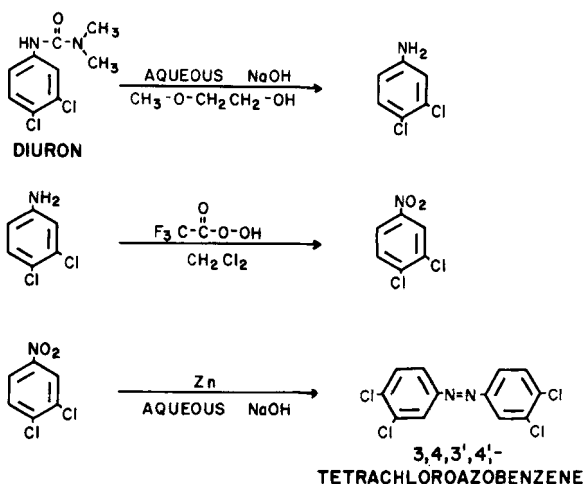


FIG. 1. Synthesis of 3,4,3',4'-tetrachloroazobenzene.

in order to optimize conditions for the synthesis. The final product from the preliminary synthesis was characterized as tetrachloroazobenzene by melting point (156°C), cochromatography (thin-layer and gas chromatography), and infrared spectroscopy.

EXPERIMENTAL.

The radioactivity measurements were performed on a Packard 3375 liquid scintillation counter. Samples were prepared for counting by dissolving the radioactive material in 5 ml of absolute ethanol and diluting to counting volume with 10 ml of fluor solution. The fluor solution contained 2,5-diphenyloxazole (4 g/l) and 1,4-bis-2-(5-phenyloxazolyl)-benzene (30 mg/l) dissolved in toluene. Counter efficiency was determined by the internal standard method.

The radiochemical product was cleaned up by preparative thin-layer chromatography. Thin-layer plates of silica gel HF of 500 micron thickness were developed in benzene : n-hexane : acetone (3 : 7 : 1) for purification of the tetrachloroazobenzene.

The chemical and radiochemical purity of the final product was estimated by means of gas chromatography. A Barber-Coleman series 5000 gas chromatograph equipped with flame ionization detector and radioactivity proportional counter was utilized. Separation was achieved at a column temperature of 210°C on a 6-foot glass column packed with 3% SE-30 coated on 60-80 mesh Chromosorb W. Retention times for the product and trace impurity were approximately 9 and 15 minutes, respectively.

A Thomas-Hoover capillary melting point apparatus was used to measure the melting point of the nonradioactive product prepared during reaction

development. Melting point measurements of the radioactive material were determined on a Kofler micro hot stage. All melting points reported were uncorrected.

The radiochemical purity of the final product was estimated to be greater than 98 % as verified by autoradiography and gas chromatography using the radioactivity monitoring system. The overall yield for the three-step synthesis was determined to be 33.6 %, and the specific activity of the final product was 252 $\mu\text{Ci}/\text{mM}$.

If greater than 98 % purity is desired, the material can be further purified by gradient elution using n-hexane and methanol as solvent on a silicic acid column ⁽²⁾. Purification may also be achieved by preparative gas chromatography. Both methods will afford a homogeneous product upon analytical analysis.

Synthesis of 3,4-dichloroaniline.

A 50 ml pear-shaped flask was charged with 63 mg (270 μCi) of 3-(3,4-dichlorophenyl)-1,1-dimethylurea ring-¹⁴C (specific activity of 1 mCi/mM), 437 mg of the nonradioactive carrier material, 7 ml of ethylene glycol monomethyl ether, and 3 ml of 16 M aqueous sodium hydroxide. This flask was connected to a reflux condenser equipped with a hydrochloric acid trap to capture any volatilized 3,4-dichloroaniline. The mixture was gently refluxed for 2 hours in an oil bath (130° C), cooled to room temperature, and transferred to a beaker containing approximately 25 g of crushed ice. The precipitated 3,4-dichloroaniline was filtered, washed with cold water, and dried without vacuum. The pale-brown product had a melting point range of 73.5-74.0° C (MP of standard 74.0° C; Lit. MP 71.5° C) and the yield of 298 mg of 3,4-dichloroaniline was estimated to be 86 % based on 3-(3,4-dichlorophenyl)-1,1-dimethylurea ring-¹⁴C.

Synthesis of 3,4-dichloronitrobenzene.

This compound was prepared by modification of the procedure reported by Emmons ⁽⁶⁾ for the oxidation of 4-chloroaniline to 4-chloronitrobenzene. A mixture of 10 ml of dichloromethane and 1 ml of 30 % hydrogen peroxide was stirred, while 3 ml of trifluoroacetic acid was pipetted dropwise down the condenser into the stirring mixture. This reaction mixture was refluxed for 1 hour in an oil bath (45° C) and allowed to cool to room temperature. The freshly prepared solution of peroxytrifluoroacetic acid was slowly added to the 3,4-dichloroaniline (298 mg) and allowed to reflux for 1 hour. After reflux the excess solvent and volatile reactants were removed by rotary vacuum evaporation. Then 5 ml of distilled water was added to help destroy the remaining peroxides. The sample was reduced to a crude brown product of 3,4-

dichloronitrobenzene by removal of the water. This material was used without further purification for the coupling reaction in preparation of tetrachloroazobenzene.

Synthesis of 3,4,3',4'-tetrachloroazobenzene.

This procedure was based on the method described by Bigelow and Robinson ⁽⁶⁾ for the preparation of azobenzene. To prepare tetrachloroazobenzene, 8 ml of 2-propanol, 0.5 ml of 10 M sodium hydroxide, 0.5 g of zinc dust, and the 3,4-dichloronitrobenzene (ca. 250-270 mg) were added into a 50 ml round-bottomed flask equipped with reflux condenser and magnetic stirrer. The mixture was vigorously refluxed for 2 hours with continuous stirring. After reflux the reaction mixture was allowed to cool to room temperature and distilled water was added to precipitate the product. Vacuum filtration through a sintered glass funnel achieved separation of the solid material from the solvent. The crude product was then dissolved in hot toluene and filtered to remove the excess zinc and other particulate impurities. The toluene solution of tetrachloroazobenzene was heated at reflux for 1 hour *. Following reflux, the solvent was removed *in vacuo* and the product was purified by preparative thin-layer chromatography. The yield of tetrachloroazobenzene (115 mg) for the combined oxidation and coupling reaction was determined to be 39 % based on 3,4-dichloroaniline-¹⁴C **. The melting point range of the final product was measured to be 157-159° C (MP of the standard 159.5° C; Lit. MP 157° C).

* During the course of reaction development, the product yield was improved by heating the crude product as a toluene solution at reflux. Although the reason for this result was not pursued, the possibility exists that geometrical transformation was taking place. Since tetrachloroazobenzene can exist as either the *cis* or *trans* isomer, heating of the product may have allowed conversion of the product to one geometrical form.

** The product yield rapidly decreased as the quantity of 3,4-dichloroaniline was reduced below 500 mg during trial experiments.

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