

COMMUNICATIONS

Small Scale Preparation of Carbon-14-Labeled 2,6-Dichloro-4-nitroaniline (Dichloran)

A procedure is described for the preparation of carbon-14-labeled dichloran from 10 mg. of

labeled aniline.

Lemin (1965) reported the preparation of carbon-14-labeled dichloran from labeled aniline sulfate, outlining the method but giving no details. A method for the small scale preparation of labeled dichloran was desired, using 10 to 20 mg. of labeled aniline, so that preparations of reasonable activity could be prepared with small amounts of labeled aniline. This was accomplished by the development of reactions suitable for small scale synthesis, by reducing the number of transfers and purifications of intermediates, and by the effective purification of the final product by gas chromatography.

Experimental

Apparatus. A reaction tube (Figure 1) was made from a 24/40 $\frac{3}{8}$ joint. The outer joint tube was closed and a 10-mm. o.d. closed tube 2 cm. long sealed to the bottom forming a reaction well. The inner joint was made into a stopper with a short piece of 10-mm. tubing sealed to it to make room for an enclosed stirring rod. The length of the outer joint section was about 15 cm. and the over-all length with the stopper inserted was about 19 cm. A stirring rod, 5 mm. in diameter and 17 cm. long, was used for mixing the tube contents.

Preparation of Benzalaniline (Cumming *et al.* 1950; Gilman and Blatt, 1941). Both aniline sulfate and hydrochloride were used as labeled starting materials. The sulfate (Nuclear-Chicago Co.) was obtained as a lyophilized powder in a sealed ampoule, and after loosening with a small spatula, the powdered salt could be poured into the reaction tube. The aniline hydrochloride (New England Nuclear Co.) was transferred to the reaction tube by dissolving in 300 to 400 μ l. of alcohol, and transferring with a cemented-needle Hamilton syringe. The alcohol was evaporated from the well of the reaction tube by means of a slow stream of nitrogen. A capillary tube mounted in a notched rubber stopper so that the lower end was above the solution surface carried the nitrogen. The rate of flow was adjusted so that a small dimple formed on the liquid surface. If the flow was too rapid, the aniline chloride spread outside the reaction well. After the alcohol was removed, the reaction tube was placed in a vacuum desiccator and pumped to about 12 mm. for a few minutes, to remove the last traces of alcohol. Sufficient pure cold aniline hydrochloride was added to the reaction tube well to make the total about 14 mg., containing about 10 mg. of aniline. The strength of the

ammonium hydroxide was determined by titration, and the amount necessary to free the aniline from the salt was added to the reaction tube well by means of a 50- μ l. Hamilton syringe, allowing for the holdup in the syringe. The ammonium hydroxide was mixed thoroughly with the aniline chloride with the round end of the stirring rod, rubbing the mixture against the walls of the well. The stopper was inserted into the tube, the stirring rod remaining in the tube. After standing 5 minutes, the calculated amount of redistilled benzaldehyde (1 to 1 mole) was added to the well with the 50- μ l. syringe, and again the contents mixed thoroughly. Fourteen milligrams of aniline hydrochloride (0.108 mmole) required 8.3 μ l. of 13N NH_4OH and 11 μ l. of benzaldehyde. The unstoppered tube containing the stirring rod, supported in a beaker, was placed in an oven at 125° C. It was baked at this temperature for 90 minutes.

Preparation of *p*-Nitroaniline. The reaction tube containing the benzalaniline was cooled and 110 μ l. of concentrated sulfuric acid added with a glass microliter

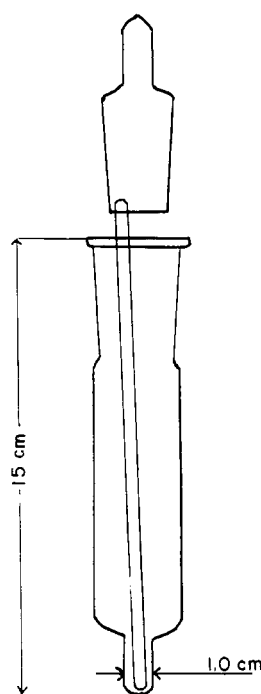


Figure 1. Reaction tube

pipet. The benzaniline was dissolved by rubbing with the stirring rod and warming, not above 50° C. A 600-ml. beaker was nearly filled with ice and water so that the ice filled the upper half of the mixture. The reaction tube was placed in the beaker, the well being below the ice level, and at that point the temperature remained at 5° to 7° C. After the well was cooled, 40 μ l. of concentrated nitric acid was added with a glass pipet. The well contents were mixed, the tube was stoppered, and the system let stand 30 minutes. At the end of this period, 5 to 7 ml. of water was added to the reaction tube, and steam was passed through the mixture rapidly, by means of a capillary tube, but at a rate short of splashing. The benzaldehyde was distilled sufficiently in 3 to 5 minutes. The tube was cooled, and the contents were transferred to a 125-ml. separatory funnel having a Teflon stopcock. The mixture was extracted three times with volumes of benzene equal to that of the aqueous mixture. The benzene layers were transferred to a clean 50-ml. beaker, and after a few minutes to a weighed 50-ml. beaker. The benzene extracts were evaporated on a hot plate with a stream of dry air. The beaker was removed as the last portion of the benzene disappeared, since *p*-nitroaniline is appreciably volatile. The beaker containing the *p*-nitroaniline was dried in a vacuum desiccator for 10 minutes at 12-mm. pressure and weighed.

Chlorination of *p*-Nitroaniline (Witt, 1875 and 1903). A solution of chlorine in acetic acid was prepared by passing chlorine from a cylinder into 99.8% acetic acid in a glass-stoppered Erlenmeyer flask until the solution was yellow and nearly saturated. One milliliter of the chlorine solution was titrated with standard thiosulfate in 150 ml. of water containing 5 ml. of concentrated hydrochloric acid and about 0.5 gram of potassium iodide. The chlorine solution must be standardized and used immediately after preparation. From the normality of the chlorine solution, the amount necessary to chlorinate the *p*-nitroaniline was calculated, 4 equivalents of chlorine being required for each mole of *p*-nitroaniline. The *p*-nitroaniline in the beaker was dissolved in 0.5 ml. of glacial acetic acid. An excess of the chlorine solution, 2.5 times the amount required, was added to the beaker and the contents mixed. The beaker was placed in a vacuum desiccator containing magnesium perchlorate as desiccant and a beaker of sodium hydroxide pellets. The desiccator was pumped down to about 10 mm. and let stand until the acetic acid was evaporated and the dichloran dry. The residue was weighed as crude dichloran.

Gas Chromatographic Purification. The crude labeled dichloran was dissolved in about 1 ml. of acetone, and injected on the column of a gas chromatograph. A glass 122-cm. \times 4-mm. i.d. column was used, filled with Carbowax 20M, 5% on Anakrom ABS (Analabs, Inc.), at a column temperature of

195° C., and a helium flow rate of 50 to 60 ml. per minute, using a thermal conductivity detector. Injections of 50 μ l. each were made with a Hamilton syringe. The dichloran had an elution time of 12 to 16 minutes, and was preceded by a small chlorinated *o*-nitroaniline peak at 4 to 5 minutes, well separated from the dichloran peak. In case of inadequate chlorination, a 2-chloro-4-nitroaniline peak will appear at about twice the elution time of dichloran. This compound does not occur if the chlorination is carried out as described above. The dichloran was collected in a bent 3-mm. glass tube, starting at the beginning of the eluted peak and continuing the collection for 10 to 12 minutes. After each collection, the dichloran was rinsed into a weighed bottle. Another collection tube was inserted into the gas chromatograph outlet before and after the collection of the dichloran peak in order to determine active impurities.

Discussion

In addition to many cold preparations of dichloran, nine preparations have been made using labeled materials. Yields of pure dichloran varied from 50 to 64%, melting at 195° C., and having specific activities of about 10,000 d.p.m. per microgram. The material showed no radioactive peaks other than dichloran when chromatographed on Eastman Chromagram silica gel sheets (Eastman Kodak Type K 301R2) developed with benzene saturated with formamide. The chromatograms were scanned on an Actigraph II (Nuclear-Chicago Co.). The crude dichloran was successfully purified by thin-layer chromatography on silica gel, but the gas chromatography purification was the method of choice.

Literature Cited

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KERMIT GROVES

Washington State University,
Pullman, Wash.

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