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A HIGH YIELD PHOTOCHEMICAL PREPARATION OF 4 - NITROCATECHOL - UL -^{14}C.

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SUMMARY

4-Nitrocatechol -UL- ^{14}C was synthesized in 48,9% yield by the photolysis of 4-nitrophenol -UL- ^{14}C in aqueous hydrogen peroxide.

INTRODUCTION.

4-Nitrocatechol has been detected as a product of the catabolism of some herbicides by soil microflora (1). Radioactive 4-nitrocatechol was desired so that the metabolic fate of this compound could be determined in soil and higher plants.

Radiolabelled 4-nitrocatechol has been synthesized in a multistep synthesis from ethyl acetoacetate and nitromalondialdehyde, in which a given carbon atom may be specifically labelled (2). However, our study required uniformly labelled 4-nitrocatechol-^{14}C.

A number of reagents have been used to effect the direct hydroxylation of phenols. They include Fenton's reagent, the

Udenfriend system, organic peracids, inorganic peroxides, radiolysis of water and photolysis of hydrogen peroxide (3). The yield of dihydroxybenzenes is generally low due to further oxidation and side reactions such as the formation of Pummerer's ketone (3,4).

The present synthesis is based on the study of the photolysis of different phenols in the presence of hydrogen peroxide by Omura and Matsuura (5). Their results demonstrated that acetonitrile was the solvent of choice over water as it increased the solubility of phenols but was not affected by the reaction conditions. The major product from their photolyses of phenols were the corresponding catechols.

The experimental conditions for the preparation of 4-nitrocatechol were optimized employing nonradioactive 4-nitrophenol. Variation in the acetonitrile-water solvent compositions did not significantly affect product yield. 4-Nitrophenol was more soluble in acetonitrile; however, the purification of 4-nitrocatechol, after photolysis, was greatly facilitated when water was used as the solvent. The product, after crude extraction, was purified by high pressure liquid chromatography. The identity of the product was confirmed by infrared, nuclear magnetic resonance and mass spectrometry. The chemical purity of the product was determined by ultraviolet spectroscopy and liquid and thin-layer chromatography. Radiochemical purity was confirmed by autoradiography of a two dimensional thin-layer chromatogram.

EXPERIMENTAL.

Instrumentation. The photolyses were carried out in a 15 X 180 mm quartz vessel fitted with a straight bore water jacket condenser. A nitrogen sparging tube (2 mm o.d.) was inserted to the bottom of the vessel via the condenser. Irradiations were performed in a Rayonet model RPR-204 photochemical reactor providing approximately 60 watts of 2537 Å ultraviolet energy.

Liquid chromatography was conducted using a Waters Associates M-6000 chromatography pump and a 0.7 cm i.d. X 91.5 cm long stainless steel column packed with Waters Associates Durapack Carbowax 400 on Porasil C (36-57 micron mesh). The solvent was redistilled chloroform[1] at a flow rate of 8 ml/min. The detector was an ultraviolet flow through monitor utilizing a 2537 Å light source. The detector output was displayed on a strip chart recorder. Sample introductions were made via a 240 μl loop using a switch valve. Eluate fractions were collected manually with respect to the recorder response. Ultraviolet, infrared and mass spectrometry were obtained using standard instruments. The nuclear magnetic resonance data was obtained on a Varian A-60-A equipped with a Digilab NMR-3 Fourier transform system.

Radiochemical measurements were performed using standard liquid scintillation techniques. The fluor solution was 2,5-diphenyloxazole (5.0 g) and 1,4-phenylenebis (5-phenyloxazole) (400 mg) in toluene (680 ml) and absolute ethanol (285 ml). Counter efficiency was determined by the internal standard method.

Thin-layer chromatography was carried out using 500 μ silica gel HF, on glass plates. Ultraviolet light (2537 Å)

[1] Distillation effectively removed the 1% ethanol added as a stabilizer to commercial chloroform.

was used to visualize the developed chromatograms. Autoradiography confirmed that the two dimensional chromatogram[2] yielded a single radioactive spot. In solvent system A, benzene:methanol:acetic acid (100:10:1), the product had an Rf of 0.38. In solvent system B, toluene:ethyl acetate: acetic acid (5:4:1), the product had an Rf of 0.68.

Synthesis of 4-nitrocatechol. The reaction vessel was charged with 79.04 mg (0.57 mMoles, 1.0 mCi) of 4-nitrophenol-UL-^{14}C (Mallinkrodt, specific activity 1.76 mCi/mMoles), 5 ml water and 1 ml 30% hydrogen peroxide (8.8 mMoles). After flushing with nitrogen, the sample was irradiated for 1 hour. The temperature in the irradiation chamber reached 45-50°C during this period, necessitating the water condenser.

The irradiated solution was transferred to a 50 ml separatory funnel with about 10 ml of water. This aqueous solution was then partitioned 5-6 times with 5 ml portions of chloroform to remove unreacted nitrophenol. The combined chloroform layers (which gave a negative hydrogen peroxide test[3]) were taken to dryness, redissolved in 1 or 2 ml of water and transferred back to the reaction vessel with water to yield 5 ml total volume. A 1.0 ml portion of 30% hydrogen peroxide was added and the photolysis repeated (1 hour).

This procedure was repeated on the second photolysis solution and the chloroform extract was again photolyzed as described. The third photolysis solution was worked up as before. At the end of the third photolytic reaction the chloroform extract contained 2.8% of the initial ^{14}C label.

[2] Dithiothreitol (0.01%) was added to each chromatographic solvent system to inhibit oxidation of the nitrocatechol on the surface of the thin layer plate.

The combined aqueous extracts from the three photolysis reactions were extracted 10-12 times with 10 ml portions of ethyl ether. After the ether extraction, the aqueous layer contained 17% of the initial radioactive carbon. The combined ether extracts were treated cautiously with solid sodium bisulfite to destroy the hydrogen peroxide present[3]. The ether solution was then dried over sodium sulfate, filtered, reduced in volume to about 2 ml, and purified by liquid chromatography.

The unreacted starting material and an unidentified component were eluted from the Carbowax 400 column in the first 120 ml. This fraction accounted for 6.1% of the initial ^{14}C label. The desired product (center cut) was collected between 160 ml and 200 ml. A second uncharacterized reaction product was observed at an elution volume of 560 ml to 960 ml, accounting for about 3% of the ^{14}C label.

The overall yield for the preparation after final purification was 48.9%. The specific activity of the product was 1.25 mCi/mg or 1.76 mCi/mMoles. The product was estimated to be in excess of 99% purity by ultraviolet spectroscopy and liquid chromatographic elution studies in collaboration with the radiochemical data.

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[3] A drop of solution to be tested was placed on a piece of filter paper followed by a drop of aqueous potassium iodide. A brown color constituted a positive test.

with the liquid chromatographic studies.

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