SYNTHESIS OF TRITIUM LABELLED CATECHOL

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SUMMARY

The preparation of tritium labelled catechol by catalytic reduction of tetrabromocatechol is described. Recrystallization of catechol (U-^{3}H) after silica gel column purification gave 68% yield of the desired product with 98% purity as assayed by high performance liquid chromatography.

Key Words: Catechol (U-^{3}H), Tetrabromocatechol, Tritium gas, Hydrogeneration apparatus, Instant thin-layer chromatography-silica gel, High performance liquid chromatography.

INTRODUCTION

Catechol (1,2-dihydroxybenzene) is present in tobacco leaf (1), and is the most abundant phenol in cigarette smoke condensate. About 0.2-0.5 mg catechol can be generated per cigarette from 85 mm non-filter and filter cigarettes (2,3).

Catechol has been shown to have biological activity, including cocarcinogenic activity with benzo(a)pyrene on mouse skin (4) and induction of sister chromatid exchange in human lymphocytes after treatment in culture (5). Studies of the metabolic fate of catechol in animals after exposure to whole cigarette smoke (6) necessitated the synthesis of tritium labelled catechol.

The multistep, noncatalytic reduction procedure for the synthesis of ^{14}C-labelled catechol and its derivatives have been reported (7, 8). We report here the one step synthesis of catechol (U-^{3}H) by catalytic reduction of tetrabromocatechol.

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EXPERIMENTAL

Materials

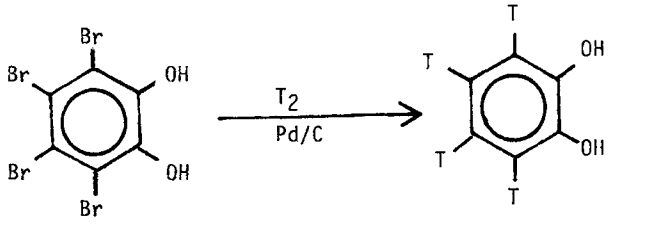
The IR spectra were obtained on a Beckman AccuLab 8 spectrometer. Ultraviolet spectra were taken in ethanol solutions using a Beckman model 25 spectrophotometer.

Radioactive disintegrations were measured on a Beckman LS-330 liquid scintillation counter. High performance liquid chromatography (HPLC) was done using a Waters Associates apparatus equipped with a 440 absorption detector (λ = 254) and a 3.9 mm x 30 cm μ porasil column.

Catechol and tetrabromocatechol were obtained from Aldrich Chemical Co., Milwaukee, WI. Palladium on carbon (10%) was purchased from Eastman Orgainc Chemicals, Rochester, NY. Tritium gas was obtained from Amersham Serale Corp., Arlington Heights, IL.

Instant thin-layer chromatographic assays were carried out on glass fiber sheets coated with silica gel (ITLC-SG), and these were purchased from Gelman Instruments Co., Ann Arbor, MI. Silica gel (60/200 mesh) was purchased from Davison Chemical, Baltimore, MD.

Synthesis of Tritium Labelled Catechol



Tetrabromocatechol Catechol (U-^{3}H)

Figure 1. Scheme of the synthesis.

A mixture of tetrabromocatechol (425 mg, 1 m mole), 10% palladium on charcoal (100 mg), triethylamine (1 ml, 10 m mole), and ethylacetate (10 ml) was placed in a round bottom flask, equipped with a three way valve. The valve was connected to 1) the low pressure hydrogenation apparatus, 2) the tritium gas reservoir, and 3) a vacuum-line. Air in the tubing between the hydrogenation apparatus and the flask was removed by vacuum, followed by purging with hydrogen gas. This was done four to five times to assure complete removal of the air from the flask and the tubing. Low pressure was created in the flask by applying vacuum and then closing the valve. Tritium gas (1 curie) was allowed to enter the flask. After 30 minutes, additional hydrogen gas from the hydrogenation apparatus was allowed to enter the flask. About 100 ml of hydrogen gas was consumed in six hours at room temperature and atmospheric pressure. The reaction was allowed to proceed for two additional hours to ensure the complete reduction of tetrabromocatechol.

The reaction mixture was filtered through a buchner funnel with celite, and the celite washed with 10 ml of ethyl acetate. The filtrate was transferred to a round bottom flask and the solvent evaporated to dryness using a rotary evaporator. The residue of the crude tritium labelled catechol was dried in a dessicator at room temperature for several hours before purification.

Purification of Catechol (U-^{3}H)

Purification of the catechol (U-^{3}H) was achieved by column chromatography (1.7 x 25 cm) on 25 g of silica gel eluted first with 200 ml benzene, followed by 200 ml of 20% ethyl acetate in benzene (v/v). Most impurities were eluted by benzene, whereas the majority of catechol (U-^{3}H) was found in the 20% ethyl acetate in benzene fraction. The catechol (U-^{3}H) was monitored by ITLC-SG sheets developed in a benzene solvent system. Visualization of spots was achieved by spraying with 50% sulfuric acid, followed by charring the ITLC-SG sheets. Recrystallization from benzene: n-heptane(1:1, v/v) gave 75 mg (68%) of purified tritium labelled catechol.

Identification and Analysis of Catechol (U-^{3}H)

The identity of synthesized tritium labelled catechol was confirmed by IR and UV spectra, melting points and chromatographic properities in comparison with commercially available non-radioactive catechol. Data were as follows: IR(KBr) 3440, 3310, 765, 750, 735 cm^{-1}; UV λ_{Max}^{EtoH} (ε) 214 (6476), 274 (2577); M.P. 103-107° (lit (9) 104-107°); Rf value of 0.63 on ITLC-SG sheets developed in benzene solvent system; retention time of 2.6 min on HPLC using ethyl acetate:hexane (60:40, v/v) as mobile phase.

The purity of catechol (U-^{3}H) was greater than 98%, and the specific activity determined to be 2.5 mCi/m mole.

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