PREPARATION OF UNIFORMLY ^{14}C-LABELED AND ^{13}C-ENRICHED CATECHOL AND HYDROQUINONE VIA PHENOL

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SUMMARY

Catechol-U-^{14}C and hydroquinone-U-^{14}C were prepared from phenol-U-^{14}C by mononitration followed by chromatographic separation of *o*- and *p*-nitrophenol-U-^{14}C. The nitrophenols were separately hydrogenated, and the resulting aminophenols were converted by diazotization followed by hydrolysis to the title diols. Phenol-U-^{14}C was synthesized efficiently from benzene by nitration, reduction, diazotization and hydrolysis. A similar series of reactions beginning with benzene-U-^{13}C gave phenol, catechol and hydroquinone containing more than 88% total carbon-13.

Key Words: Benzene metabolites, Catechol-U-^{13}C, Catechol-U-^{14}C, Hydroquinone-U-^{13}C, Hydroquinone-U-^{14}C

INTRODUCTION

The great industrial importance of benzene and the widespread potential for human exposure to it, coupled with its serious hematotoxicity, have catalyzed extensive study of its metabolism and disposition in living systems [1]. By 1949, it was recognized that primary benzene metabolites include phenol, catechol and hydroquinone [2]. Other metabolites which have been identified include 1,2,4-trihydroxybenzene, *trans,trans*-muconic acid, *trans*-benzene dihydrodiol, phenylmercapturic acid, phenyl- and hydroxyphenylsulfuric acids and phenyl- and hydroxyphenylglucuronic acids [1], many of which undoubtedly arise via further transformation of the three phenolic metabolites mentioned above. In spite of much study, however, the pattern of benzene metabolism

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in specific target organs under varying exposure conditions remains obscure, and continues to be actively studied [3].

To facilitate such studies, we have prepared quantities of the previously unreported title compounds, with a facile synthetic scheme utilizing phenol as an intermediate.

RESULTS AND DISCUSSION

The Figure illustrates the procedures used for the preparation of the title compounds. Benzene-^{14}C (45.1 mCi/mmole) or benzene-^{13}C (90 atom %) was nitrated using the procedure of Spitzer and Stewart [4], providing nitrobenzene-U-^{14}C or -U-^{13}C (2) in yields of 99% and 95%, respectively. Nitrobenzene-U-^{14}C was converted to N-phenylacetamide-(phenyl-U-^{14}C) (3a) in 96% yield by catalytic hydrogenation in acetic anhydride. Compound 3a-phenyl-U-^{14}C could be stored

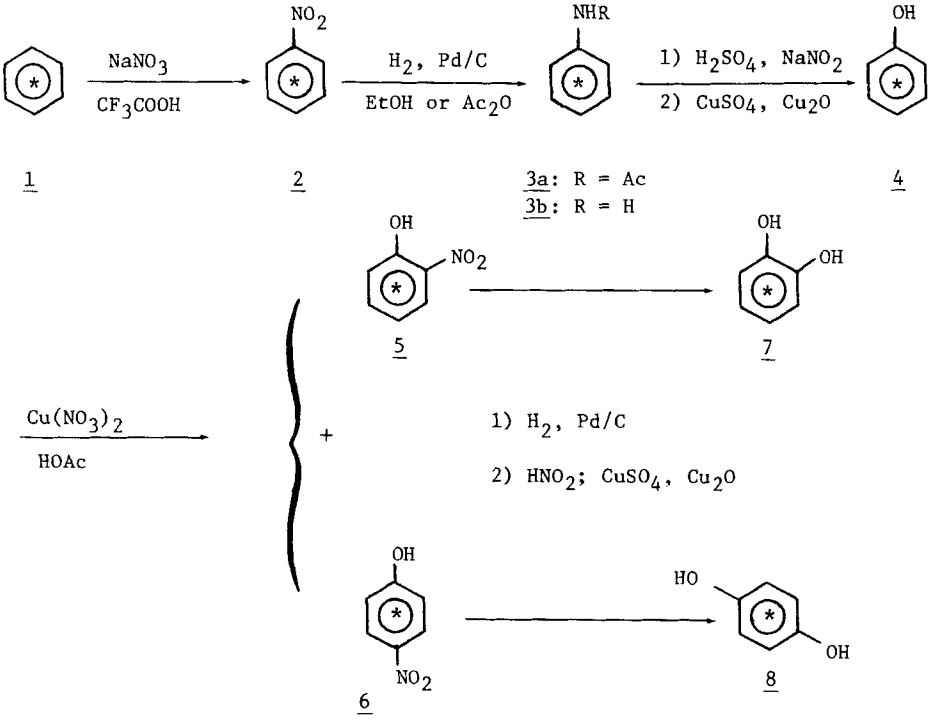

Figure. Synthesis of Labeled Phenol, Catechol and Hydroquinone.

and handled without the precautions necessitated by the oxygen sensitivity and volatility of aniline. Nitrobenzene-U-^{13}C, on the other hand, was quantitatively reduced to 3b-U-^{13}C in ethanol and used immediately and without purification in the succeeding step.

N-Phenylacetamide-(phenyl-U-^{14}C) was hydrolyzed by refluxing in dilute sulfuric acid, and the cooled mixture was diazotized by addition of sodium nitrite. Following the procedure of Cohen et al. [5], treatment of the phenyldiazonium salt with aqueous cupric sulfate and cuprous oxide provided, after purification by vaccuum distillation, 187 mCi phenol-U-^{14}C in 99% purity, for an overall yield of 68% from benzene-^{14}C. Phenol-U-^{13}C (749 mg) was produced from aniline-U-^{13}C by diazotization and $CuSO_4$/CuO treatment in an overall yield from benzene-^{13}C of 84%.

Although reports exist for the preparation by rather lengthy procedures of specifically labeled catechol and hydroquinone [6], no reference to syntheses of the uniformly labeled compounds appears to have been made. Our quick entry into these systems commenced with the mononitration of phenol-U-^{13}C or -U-^{14}C by the $Cu(NO_3)_2$/HOAc reagent of Menke [7]. Contrary to this report of exclusive ortho nitration, we consistently obtained a mixture of o- and p-nitrophenol (5 and 6) in a ratio of 1:2 to 2:3, which was not significantly affected by lowering reaction temperatures below 25°C. The product isomers were separated by preparative TLC on silica gel, which also served to allow isolation of a small amount of dinitrophenol by-product.

The mononitrophenols were independently reduced by catalytic hydrogenation in ethanol, and the resulting aminophenols immediately converted to the ^{13}C- or ^{14}C-labeled dihydroxybenzenes 7 and 8 using the procedure employed earlier for conversion of the labeled anilines to phenols. These reactions, and the product purifications using careful preparative TLC or column chromatography, were

conducted with degassed solvents and as much as possible under inert atmosphere. Despite these precautions, losses due to oxidative decomposition usually accompanied the process, especially in the case of catechol. Overall yields of ^{14}C-labeled catechol and hydroquinone from phenol were 3.1% and 25%, respectively; those for ^{13}C-labeled catechol and hydroquinone, 7% and 14%, respectively.

The specific activity of both catechol-U-^{14}C and hydroquinone-U-^{14}C was 37.9 mCi/mmol. Examination of the mass spectral molecular ion series (m/e = 116, 115, 114, 113) revealed total carbon-13 contents for catechol-U-^{13}C and hydroquinone-U-^{13}C to be 88.1% and 88.4%, respectively. The infrared spectra of the ^{13}C-enriched compounds revealed, as expected, the shift of most absorbances to lower wavenumbers as compared to nonenriched standards (see Experimental).

EXPERIMENTAL

Benzene-U-^{14}C (45.1 mCi/mmol) was purchased from Pathfinder Laboratories, St. Louis, Missouri. Benzene-U-^{13}C (ca. 90% enrichment) was purchased from KOR Isotopes, Cambridge, Massachusetts. IR spectra were recorded with a Beckman Acculab I, either neat or in Nujol mull. Radioactivity was determined in a Packard Model 3003 liquid scintillation counter using Econofluor™ (New England Nuclear); radiochemical purity was determined using a Packard Model 7201 radiochromatogram scanner and by autoradiographic methods. Preparative TLC was carried out using Whatman PK5F plates, 20 x 20 cm x 1 mm dimensions.

Carbon-13 enrichments of appropriate compounds were determined by mass spectrometry using the isotopic abundance patterns of the molecular ion clusters. Spectra were obtained with a Varian/MAT 311-A instrument using a direct insertion probe. Gas chromatographic analyses were performed on a Varian Series 2400 instrument equipped with flame ionization detector.

Nitrobenzene-U-^{14}C (2)

A 0.693 g (8.87 mmol, 400 mCi) sample of benzene-^{14}C was transferred (in vacuo) into a suspension of 0.808 g (9.5 mmol) of $NaNO_3$ in 25 ml of trifluoroacetic acid (TFA). The reaction mixture was stirred 18 hr at room temperature, after which time GLC analysis (20% SP-2100 on 100/120 Supelcoport) indicated complete reaction. Most of the TFA was removed by vacuum distillation at -20°C. The residue was neutralized with cold 2 N NaOH and the nitrobenzene-U-^{14}C was extracted with 3 x 20 ml of ether. After drying the combined extracts over $MgSO_4$ and distillation of the solvent at atmospheric pressure, the nitrobenzene was vacuum distilled into a trap maintained at -45°C, affording 1.080 g (8.78 mmol, 396 mCi, 99%) of product of ≥ 98% purity (GLC).

N-Phenylacetamide-(phenyl-U-^{14}C)(3a)

A 1.204 g (9.79 mmol, 37.6 mCi/mmol) sample of nitrobenzene-U-^{14}C was dissolved in 20 ml of acetic anhydride and 0.1 g of 10% Pd/C was added. The mixture was hydrogenated at 40 psi H_2 for 35 min. After filtration of the catalyst the solvent was removed by vacuum distillation at -30°C. The residue was triturated with 10 ml of 1:1 ether:hexane and after filtration and drying, the yield of N-phenylacetamide-(phenyl-U-^{14}C) was 1.256 g (9.29 mmol, 352 mCi, 96%). The radiochemical purity was 98% by TLC (silica gel, hexane:acetone 3:1, R_f 0.19).

Phenol-U-^{14}C (4)

A 0.960 g (7.1 mmol, 269 mCi) portion of N-phenylacetamide-(phenyl-U-^{14}C) was dissolved in 5 ml of ethanol and 6 ml of 6 N H_2SO_4. After refluxing for 6 hr, the reaction mixture was cooled to room temperature and evaporated in vacuo. The residue, dissolved in 15 ml of 17% H_2SO_4 and colled to 3°C, was diazotized by dropwise addition of a solution of 0.662 g (9.6 mmol) of $NaNO_2$ in 3 ml of H_2O. After stirring an additional 45 min with cooling, a few crystals of urea were

added to decompose excess nitrous acid.

A solution of 28 g $CuSO_4 \cdot 5H_2O$ in 200 ml of water was added, followed by 0.95 g of Cu_2O. After overnight stirring, the resulting mixture was extracted with 4 x 15 ml of ether. The combined extracts were dried ($MgSO_4$), and most of the solvent was removed by vacuum distillation at -50°C. Phenol-U-^{14}C was collected by vacuum distillation at 50°C into a -45°C trap, affording 0.464 g (4.93 mmol, 187 mCi, 70%); radiochemical purity 99% by TLC (silica gel, hexane:acetone 3:1, R_f 0.35).

o-and p-Nitrophenol-U-^{14}C (5 and 6)

A 0.447 g (4.75 mmol, 180 mCi) sample of phenol-U-^{14}C was dissolved in 2 ml of glacial acetic acid and added to a suspension of 0.648 g (2.75 mmol) $Cu(NO_3)_2 \cdot 3H_2O$ in 2 ml of glacial acetic acid at 25°C. After stirring 1 hr, ice chips were added and the products were extracted with 4 x 10 ml of ether:hexane (3:1). The solvents were removed from the combined extracts (in vacuo) and the product isomers separated by preparative TLC (hexane:acetone 5:2, R_f ortho 0.47; para 0.35), affording 0.110 g (30 mCi, 17%) o-nitrophenol-U-^{14}C and 0.257 g (70 mCi, 39%) p-nitrophenol-U-^{14}C; radiochemical purity of each ∿ 98% by TLC.

Catechol-U-^{14}C (7)

A 0.092 g (0.66 mmol, 25 mCi) sample of o-nitrophenol-U-^{14}C was hydrogenated (40 psi H_2) in 10 ml of ethanol with 25 mg of 10% Pd/C as catalyst. A TLC taken after 30 min indicated completion of reaction. The mixture was filtered into 3 ml of 4 N H_2SO_4 and most of the solvent was evaporated (in vacuo) to remove ethanol. After cooling to 0-5°C, 0.080 g of $NaNO_2$ in 2 ml of water was added and stirring was continued for 1 hr. Sufficient solid urea was added to destroy excess nitrous acid and 20 ml of a solution of $CuSO4 \cdot 5H_2O$ (2.8 g) was added, followed by 0.095 g of Cu_2O. After stirring 45 min at 5°C, the mixture was extracted with

4 x 10 ml of ether. Normal workup provided 16 mCi of product of ca. 80% radiochemical purity. Column chromatography on 8 g of silica gel eluted with degassed hexane:ether (9:1) provided 13 mCi of material ca. 95% pure. Final preparative TLC (methanol:benzene:acetic acid 30:60:1) afforded 4.5 mCi (18% yield, specific activity 37.9 mCi/mmol) of catechol-U-^{14}C, radiochemical purity ≥ 98%.

Hydroquinone-U-^{14}C (8)

A 0.126 g (0.91 mmol, 34.4 mCi) sample of p-nitrophenol-U-^{14}C was reduced at 40 psi of H_2 in 20 ml of Et_2O with 0.050 g of 10% Pd/C. After 1.2 hr the catalyst was filtered off and the filtrate was stipped of solvent at < 20°C (in vacuo). The residue was immediately dissolved in 2 ml of 6 N H_2SO_4 and treated at 5°C for 3 hr with 84 mg of sodium nitrite dissolved in 2 ml of water. Excess nitrous acid was decomposed with urea and the solution was treated with 3.67 g of $CuSO_4 \cdot 5H_2O$ in 33 ml of H_2O followed by 87 mg of Cu_2O. After stirring overnight at 25°C, the reaction mixture was worked up in the same manner as the o-isomer reaction.

Purification was accomplished by careful chromatography on a column of siliga gel (1.1 x 17 cm) eluted with 20-30% acetone:hexane, giving hydroquinone-U-^{14}C, 22.1 mCi (64% yield), which was 98% pure by TLC (silica gel, benzene:methanol:acetic acid 30:15:1).

Nitrobenzene-U-^{13}C (2)

Nitration of benzene-U-^{13}C (0.75 g, 8.9 mmol) was carried out in the same manner as that of the ^{14}C-labeled compound, giving 1.09 g (95% yield) of pure product.

Phenol-U-^{13}C (4)

Nitrobenzene-U-^{13}C (1.09 g, 8.45 mmol) was dissolved in 30 ml of 95% EtOH and reduced at 40 psi H_2 with 100 mg of 10% Pd/C. Hydrogen uptake ceased after

40 min, and the mixture was filtered, the solvent was removed (in vacuo) and the residue was dissolved in 20 ml of 6 N H_2SO_4. The aniline-U-^{13}C was diazotized at 0-5°C for 2 hr with 840 mg of sodium nitrite. After destruction of excess nitrite with solid urea, a solution of 23.6 g of $CuSO_4 \cdot 5H_2O$ dissolved in 330 ml of water was added, followed by 860 mg of Cu_2O. After agitating overnight at room temperature, the product was extracted three times with ether and the combined extracts were dried ($MgSO_4$) and evaporated at < 10°C, giving phenol-U-^{13}C (749 mg, 89% yield) as a slightly off-white solid.

o- and p-Nitrophenol-U-^{13}C (5 and 6)

A 745 mg (7.45 mmol) sample of phenol-U-^{13}C was nitrated by the same procedure used for the phenol-U-^{14}C. Preparative TLC purification as before gave p-nitrophenol-U-^{13}C (334 mg, 31% yield) and o-nitrophenol-U-^{13}C (248 mg, 24% yield).

Catechol-U-^{13}C (7)

A 213 mg (1.53 mmol) sample of o-nitrophenol-U-^{13}C was reduced, diazotized and converted to pyrocatechol-U-^{13}C by the procedure used for the ^{14}C-labeled sample outlined above. Initial column chromatographic purification gave 96 mg of red solid. Recrystallization from degassed benzene:hexane gave 46.5 mg (28% yield) of pinkish crystals, m.p. 101-104°C. TLC showed one spot, R_f 0.46 (silica gel, benzene:methanol:acetic acid 30:15:1). Mass spectrum: m/e 116 (100%), 115 (74.76%), 114 (26.74%), 113 (4.88%), 98 (13.87%), 87 (20.43%), 86 (26.57%), 69 (46.32%), 68 (64.32%), 67 (32.03%), 57 (18.45%), 55 (16.45%) and 44 (27.99%). IR (^{13}C-enriched/natural abundance) 3235/3235, 3175/3175, 1570/1625, 1480/1520, 1445/1475, 1360/1370, 1235/1260, 1075/1100, 1020/1040, 750/765, 740/750 cm^{-1}.

Hydroquinone-U-^{13}C (8)

Following the procedure used for the synthesis of the ^{14}C-labeled compound, 334 mg (2.3 mmol) of p-nitrophenol-U-^{13}C was reduced, diazotized and treated with $CuSO_4/Cu_2O/H_2O$ to give 123 mg (46% yield) of hydroquinone-U-^{13}C, m.p. 170-171°C. Mass spectrum: m/e 116 (100%), 115 (79.53%), 114 (24.14%), 113 (5.05%), 86 (18.7%), 58 (18.5%), 42 (10.11%) and 29 (22.43%). IR (^{13}C-enriched/natural abundance): 3120/3120, 1440/1510, 1320/1345, 1210/1250, 1170/1200, 1080/1085, 965/1000, 800/815, 730/745 cm^{-1}.

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