# CARBON-14-LABELED 2,3,7,8- AND 1,2,7,8-TETRACHLORODIBENZOFURAN

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## SUMMARY

Both 2,3,7,8- and 1,2,7,8- tetrachlorodibenzofuran -U$^{14}$C, specific activity 57 mCi/mmol, have been obtained in low yield but at > 98% purity *via* Pschorr cyclization of *o*-phenoxyaniline -U$^{14}$C, chlorination of the resultant dibenzofuran and separation of the tetrachloro isomers by hplc. The lower yields obtained in the Pschorr cyclization of "hot" *o*-phenoxyaniline in comparison with the "cold" material are postulated to result from enhanced homolytic relative to heterolytic cleavage of the "hot" diazonium ion leading to a "hot" free radical which polymerizes. The completely anomalous results observed in the attempted palladium acetate-mediated cyclization of diphenyl ether- U$^{14}$C are likewise interpreted in terms of the intervention of a "hot" free radical.

Key words: Tetrachlorodibenzofuran -U$^{14}$C; 2,3,7,8- and 1,2,7,8-isomers; carbon-14; toxic environmental contaminants; free radical processes.

## INTRODUCTION

Interest in carrying out distribution and metabolism studies on the extremely toxic environmental contaminant 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDBF) (1,2) engendered a need for synthesis of the compound $^{14}$C-labeled. Because of its high toxicity a high specific activity (greater than 50 mCi/mmol) was required.

At the outset it was clear it would be most convenient to prepare the compound from uniformly labeled, appropriately substituted benzene starting materials. Further, since we had already shown that chlorination

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of dibenzofuran to the tetrachloro level in glacial acetic acid produced 2,3,7,8- and 1,2,7,8- TCDBF as the principal products and had demonstrated the feasibility of separating the isomers by high performance liquid chromatography (hplc) (2), consideration of the limitations in availability of suitably substituted, labeled starting materials indicated the most expeditious approach would be via chlorination of dibenzofuran -U-$^{14}$C.

A particularly attractive approach to preparation of labeled dibenzofuran appeared to be via the palladium acetate-mediated cyclization of diphenyl ether (3,4) which had been reported to go in excellent yield with non-radiolabeled material and which required the simplest starting materials, phenol and bromobenzene. In our hands, palladium acetate cyclization of "cold" diphenyl ether under conditions as described in the literature (3) went smoothly as reported to give uniformly good yields (70-75%) of dibenzofuran. With "hot" diphenyl ether (specific activity 55 mCi/mmol), however, strikingly different results obtained.

Diphenyl ether -U-$^{14}$C was prepared in 70-80% yields, comparable to those realized in the "cold" process, by Ullmann condensation of bromobenzene -U-$^{14}$C (specific activity 43-69 mCi/mmol) with "cold" phenol. In contrast, the corresponding reaction of "cold" bromobenzene with phenol-U-$^{14}$C (specific activity 60-70 mCi/mmol) afforded little or no product, a result which must be ascribed to instability of the "hot" phenolate anion under the reaction conditions. Two attempts to cyclize the diphenyl ether -U-$^{14}$C with one equivalent of palladium acetate in trifluoroacetic acid under the conditions used for the "cold" material afforded only traces (less than 0.1%) of dibenzofuran and less than 1% of recovered diphenyl ether, the crude product turning out to be a complex mixture of oligomeric substances. Qualitative, low resolution mass spectrometric analysis of the crude reaction product (electron impact; direct probe since the material had been undetectable in a gas chromatographic effluent) indicated the present of what appeared to be

discrete dibenzofuran dimers, trimers, tetramers and higher homologs with molecular weights up to and above 1000 as well as lesser amounts of apparently phenyl-substituted analogs of these oligomers.

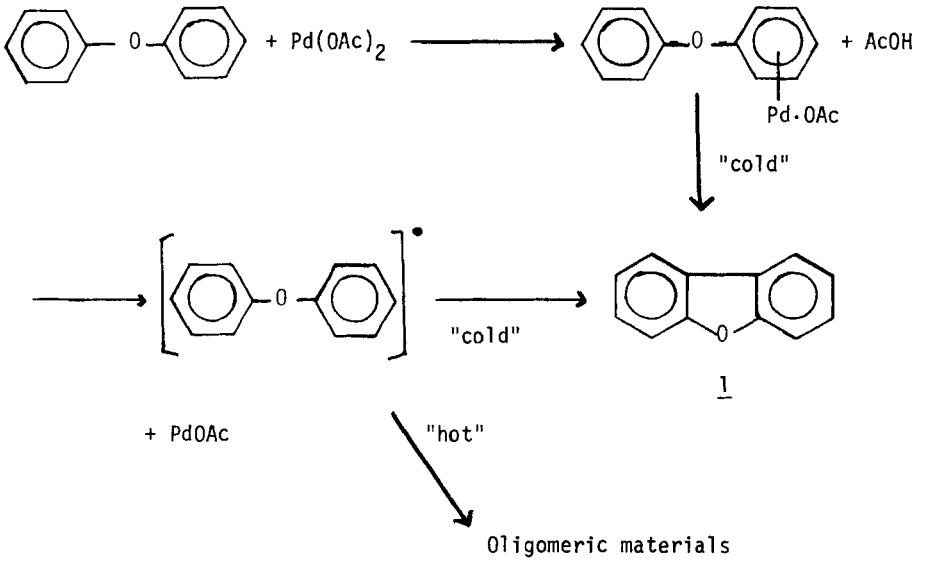
Varying amounts of diphenyl ether dimers have been reported to form when the "cold" reaction is carried out under different conditions (catalytic amount of palladium acetate, an inert solvent or, optimally, no solvent, at the much higher temperature of 150°C and under oxygen pressure) (5). The authors suggest their process involves reaction of oxygen to form an intermediate hydroperoxypalladium.diphenyl ether complex (5).

It appeared to us our results could be most plausibly rationalized in terms of a radical process. As shown in Scheme I, palladium acetate presumably reacts with diphenyl ether to effect loss of a hydrogen atom (or proton) and formation of an acetoxypalladium diphenyl ether complex. The complex may then undergo cyclization or other reactions either via direct homolytic displacement of palladium or via the free radical formed by homolytic cleavage of the palladium-carbon bond. A heterolytic process appears less likely. It seems reasonable to suppose that, owing to the excess energy contained in the "hot" diphenyl ether nucleus, the radiolabeled compound reacts with palladium acetate to form a "hot" free radical either directly or by rapid cleavage of a particularly unstable palladium complex. The energy deriving from the high level of radioactivity added to the energy inherently contained in a free radical could well alter the course of reaction and cause the finite-lived radical to enter into non-selective reaction processes.

It was thus clear that, first, we had to avoid use of a "hot" phenolate anion. Second, it appeared our chances of success would be greater if we avoided cyclization processes which might involve a free radical intermediate and focused instead on heterolytic processes. This

latter point ruled out consideration of the recently reported photochemical cyclization of o-chlorodiphenyl ethers(6) and directed our attention back to the conventional Pschorr cyclization which we had previously used for synthesis of "cold" polyCDBF's (2).

Scheme I




The limitations we had imposed meant we would need $^{14}C$-labeled o-chloronitrobenzene at a specific activity above 50 mCi/mmol as starting material. However, New England Nuclear, who contracted to supply this compound, found it, at a specific activity of 60 mCi/mM, to be too unstable to handle. It appeared the nitro substituent caused too much weakening of the carbon-chlorine bond. New England Nuclear resolved the problem by preparing both o-chloronitrobenzene -U-$^{14}C$ and phenol -U-$^{14}C$ at lower

(30 mCi/mmol) specific activities, immediately subjecting them to reaction and supplying us with the resultant o-phenoxynitrobenzene -U-$^{14}C$, 60 mCi/mmol. As expected, both reactants showed greater stability at the lower specific activity. Degradation was further avoided by their being promptly converted to the diphenyl ether derivative which proved to be eminently stable, possibly in part because the radioactivity was distributed over both rings.

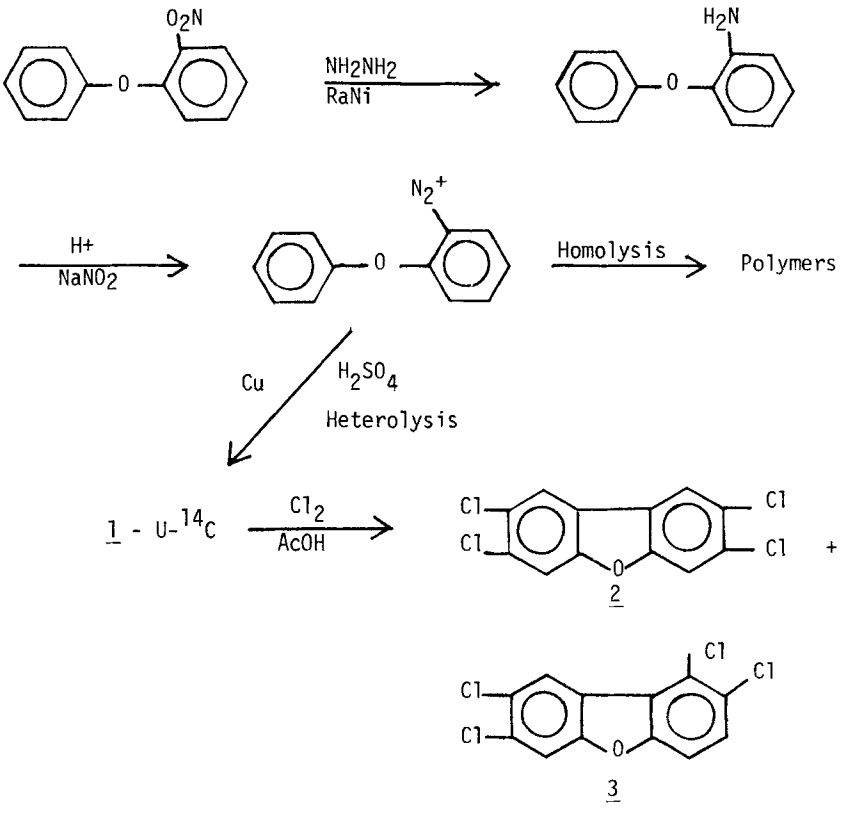
Reduction of the nitro groups by hydrazine and Raney nickel (2) proceeded uneventfully to give a good yield of o-phenoxyaniline -U-$^{14}C$ (Scheme II). The Pschorr cyclization was carried out in the normal way in aqueous sulfuric acid with diazotization by sodium nitrite since solubility did not present a problem as it did with polychloro-substituted analogs (2). Cyclization of the "hot" aniline did indeed afford dibenzofuran -U-$^{14}C$ (1-U-$^{14}C$) although the yield was much lower than was obtained with "cold" material. Yield was optimal when the cyclization was carried out at as low a temperature as possible and the solution of the diazonium salt was poured without delay into the boiling aqueous sulfuric acid containing the copper catalyst. As the solution of the "hot" diazonium salt stood, it rapidly deposited a precipitate of polymeric material with a consequent marked reduction in yield of 1-U-$^{14}C$.

We interpret these results as deriving from a competition between heterolytic and homolytic breakdown of the diazonium salt. Evidence that diazonium ions do indeed breakdown by the two competing processes has been discussed in detail (7,8). We postulate that with the "hot" diazonium ion homolytic breakdown to a free radical occurs at an enhanced rate.

Careful chlorination of 1-U-$^{14}C$ in hot glacial acetic acid gave, as with the "cold" material (2), a crude product containing (glc) 55% of a mixture of 2,3,7,8-TCDBF-U-$^{14}C$ (2) and 1,2,7,8-TCDBF-U-$^{14}C$ (3), 43% of tri-CDBF and small amounts of di- and penta-chloro materials. Hplc

separation of this mixture afforded both 2 and 3 at levels of purity > 98% by glc response.

Scheme II



## EXPERIMENTAL

Caution: In addition to normal precautions taken in dealing with radioactive materials, owing to its extreme toxicity special care must be exercised in working with 2,3,7,8- TCDBF. All work involving chlorinated DBF materials was carried out in glove boxes in an isolated toxic facility. Exhaust air was filtered. Contact with these compounds can cause chloracne and irreversible liver damage.

Mass spectral data were obtained at 70eV with a Hitachi Perkin-Elmer RMU-6D spectrometer and a direct probe. Glc data were obtained with a Varian Model 1700 gas chromatograph, hydrogen flame ionization detector at 325°C, injector temperature 275°C, helium flow rate 38 mL/min, 2m x 0.32 cm stainless steel column packed with Dexsil 300, oven program at 100-295°C at 12°/min, unless stated otherwise. Hplc was performed with a Dupont Model 830 instrument, UV detector at 280 nm, 0.62 x 25 cm Zorbax ODS column, methanol: 0.01 M aqueous phosphoric acid mobile phase 90:10 at a flow rate of 1.5 mL/min, column at 55°C and 1500 psi. Radioactivity was determined with an Amersham Searle Mark III liquid scintillation counter.

Uniformly $^{14}C$-labeled phenol was obtained from Amersham-Searle. Uniformly $^{14}C$-labeled bromobenzene and o-phenoxynitrobenzene were obtained from New England Nuclear.

o-Phenoxyaniline-U-$^{14}C$. Essentially as described for "cold" chloro-substituted analogs (2) a solution of 51.7 mCi (60.7 mCi/mmol, 0.85 mmol) of o-phenoxynitrobenzene -U-$^{14}C$ in 5 mL of 95% ethanol containing ca. 50 mg of activated Raney nickel was heated at reflux and treated, dropwise with stirring over a period of 15 min, with a solution of 162 mg of hydrazine hydrate (85% in water, 2.7 mmol) in 1.3 mL of 95% ethanol. Stirring and heating were continued for 1 hr, following which the reaction mixture was allowed to cool to room temperature, filtered and the solvent was removed from the filtrate by distillation. The process was repeated twice more to

yield a total of 412 mg (87%) of a brown solid residue, 95% o-phenoxyaniline and 5% starting material by glc response.

Dibenzofuran-U-$^{14}$C. - Cyclizing conditions adopted had in our hands afforded 60-70% yields of unlabeled dibenzofuran. To a solution of 143 mg (0.77 mmol) of the crude o-phenoxyaniline-U-$^{14}$C prepared in the preceding step in 1.5 mL of 1 N sulfuric acid was added, dropwise with stirring and cooling at 0°C, a solution of 79 mg (1.2 mmol) of sodium nitrite in 0.8 mL of water. Stirring was continued at 0°C for 3-5 min. (The longer the material was allowed to stir, and particularly if the temperature was allowed to rise slightly, the more polymeric precipitate formed. The precipitate could not be detected in a glc effluent. With increased precipitate formation yield of product suffered markedly.) The reaction mixture was then poured, rapidly with stirring, into 3.9 mL of boiling 1 N sulfuric acid containing ca.15 mg of copper catalyst (9). The resultant reaction mixture was heated under reflux with stirring for 3.5 hr., allowed to cool and extracted with 1 x 10 mL followed by 3 x 5 mL portions of chloroform. The combined organic layers were washed with 2 x 20 mL portions of 10% aqueous sodium hydroxide, once with water dried over anhydrous magnesium sulfate,and the solvent was removed by distillation. The crude product was chromatographed on a 1 x 10 cm silica gel column with elution by petroleum ether (b.p. 35-60°C): chloroform 9:1. The process was carried out 3 times with a total of 412 mg (2.23 mmol) of o-phenoxyaniline. The yield of product improved as we learned to curtail the time allowed for diazotization; however, because of the extremely low yield produced in the first run, an overall yield of only 48 mg (13%, 17.3 mCi) of dibenzofuran-U-$^{14}$C was obtained as a colorless crystalline solid, 98% pure by glc response.

2,3,7,8-Tetrachlorodibenzofuran-U$^{14}$C (2). - Essentially as previously described for the chlorination of cold dibenzofuran (2), a solution of the 48 mg (0.285 mmol) of dibenzofuran-U-$^{14}$C obtained in the preceding step in

0.5 mL of glacial acetic acid was treated, dropwise with stirring at 70°C over a period of 0.5 hr, with 1.6 mL of a 6% solution of chlorine (1.4 mmol) in glacial acetic acid. Stirring and heating at 70°C were continued and progress of the reaction was monitored by glc. Addition of chlorine solution was repeated in 1.5-1.6-mL portions per day until a total of 18 mL (15 mmol of chlorine) had been introduced and the reaction mixture was indicated by glc to contain 55% combined of 2,3,7,8- and 1,2,7,8-TCDBF, 43% of tri CDBF"S, 1% of diCDBF and 1% of pentaCDBF's. Chlorination was halted at this point based on earlier experience, in order to avoid formation of additional, difficultly separable, pentachloro material. Addition of 8.5 mL of water to the cooled reaction mixture gave a precipitate which was collected by centrifugation and dissolved in 25 mL of chloroform. The chloroform solution was washed successively with 25-mL portions of water, 10% aqueous sodium hydroxide and water, and dried over anhydrous sodium sulfate. The solvent was removed by distillation to provide 43 mg of light brown solid indicated by glc to consist of 80% combined of 2,3,7,8- and 1,2,7,8-TCDBF in approximately a 3:2 ratio, 15% of triCDBF's and 5% of pentaCDBF's. This material was dissolved in 1.5 mL of warm dioxan and the solution was injected in small (70 uL) portions into the hplc and chromatographed under the conditions described earlier. The 2,3,7,8-TCDBF peaks were collected, combined, and the solvent was removed by distillation to leave a white solid, 92% pure by glc. Two recrystallizations of this from chloroform afforded 9.0 mg of 2,3,7,8-TCDBF-U-$^{14}C$ as colorless crystals > 98% pure by glc response, glc retention time 17.5 min (peak height increased by spiking with authentic (2) "cold" 2,3,7,8-TCDBF), hplc retention time 23 min, specific activity 57 mCi/mmol.

1,2,7,8-Tetrachlorodibenzofuran-U-$^{14}C$ (3). - On standing in the methanol-0.01N phosphoric acid eluting solution, the collected and combined 1,2,7,8-TCDBF peaks deposited 1.5 mg of colorless crystals of 1,2,7,8-TCDBF-U-$^{14}C$, > 98% pure by glc response, glc retention time 17.2 min (checked with

authentic "cold" material (2) ), hplc retention time 30 min.

Reaction of Phenol -U-$^{14}$C with Bromobenzene. Our procedure, based on a classic preparation (10), consistently afforded 70-80% yields of unlabeled diphenyl ether. To a methylene chloride solution of 25.6 mg of phenol-U-$^{14}$C (amount determined by glc, Tenax 1m x 0.32 cm stainless steel column, helium flow rate 12 mL/min, 110° C isothermal, retention time 8.6 min; trimethylsilyl derivative on a 2 m x 0.32 cm Dexsil 300 column, helium flow 40 mL/min, 60° C isothermal, showed a retention time of 2 min), specific activity 110 mCi/mmol, was added 24.2 mg of "cold" phenol and the solution was evaporated to dryness under reduced pressure under nitrogen at room temperature. To the assumed 49.8-mg combined total phenol-U-$^{14}$C residue (0.53 mmol), calculated specific activity 57 mCi/mmol, was added 83 mg (0.53 mmol) of bromobenzene, 73 mg (0.53 mmol) of potassium carbonate and a small amount of copper catalyst (9). The reactants were intimately mixed and heated in a flask equipped with a reflux condenser under nitrogen for 3 hr at 210°C (bath temperature). The cooled reaction mixture was triturated with 4 mL of 10% aqueous sodium hydroxide and the mixture was extracted with 2 x 2 mL portions of methylene chloride. The organic extract was washed with water, dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The process was repeated several times with some variations in conditions to provide large amounts of recovered bromobenzene and yields ranging from 0-15% of diphenyl ether (amount determined by glc with the Tenax column, oven program 120-200°C at 6°/min, retention time 11.3 min). The specific activity of the diphenyl ether was 2.5 mCi/mmol suggesting selective, intramolecular degradation of the "hot" phenol.

Reaction of Phenol with Bromobenzene - U-$^{14}$C. - In similar fashion, a mixture of 170 mg (1.08 mmol) of bromobenzene-U-$^{14}$C (specific activity 69 mCi/mmol), 152 mg (1.62 mmol) of phenol, 224 mg (1.62 mmol) of potassium carbonate and a small amount of copper catalyst was heated for 2.5 hr at 205°C

(bath temperature). Workup as described for the reaction with phenol-U-$^{14}C$ afforded 167 mg (92%) of crude product as an oil indicated by glc response (Dexsil 300 column) to consist of 91% diphenyl ether -U-$^{14}C$ and 9% of bromobenzene - U-$^{14}C$. A repeat of the process gave comparable results.

Cyclization of Diphenyl Ether.- Essentially as described (3), a solution of 34 mg (0.2 mmol) of diphenyl ether (Aldrich) and 45 mg (0.2 mmol) of palladium acetate in 0.5 mL of trifluoroacetic acid was heated at reflux for 4 hr. The reaction mixture was filtered, the filtrate was evaporated in vacuo and the residue was taken up in 2 mL of methylene chloride. The solution was washed with 2 x 1 mL of 10% sodium hydroxide, water, dried over anhydrous magnesium sulfate and evaporated under reduced pressure to yield 25 mg (74%) of product as a solid indicated by glc to consist of 95% dibenzofuran and 5% diphenyl ether.

In order to simulate the radiolabeled starting material, an experiment was carried out in which 500 mg of diphenyl ether product from a reaction of "cold" bromobenzene and phenol, containing 90% diphenyl ether and 10% bromobenzene (glc) (2.65 mmol diphenyl ether), and 595 mg (2.65 mmol) of palladium acetate in 7 mL of trifluoroacetic acid were heated at reflux for 4 hr and the reaction mixture was worked up as before to give 330 mg (74% yield) of solid, 98% dibenzofuran by glc.

Two additional runs with "cold" material gave similar results.

Cyclization of diphenyl ether -U-$^{14}C$.- A solution of 182 mg of product from a reaction of bromobenzene - U-$^{14}C$ with phenol, containing (glc) 87% diphenyl ether -U-$^{14}C$ and 13% bromobenzene -U-$^{14}C$ (0.93 mmol diphenyl ether), and 210 mg (0.93 mmol) of palladium acetate in 2 mL of trifluoroacetic acid was heated at reflux for 4 hr. The reaction mixture was filtered and the filtrate was evaporated in vacuo to leave a black solid residue. The solid was dissolved in 4 mL of methylene chloride. Washing of this solution with 2 mL of 10% aqueous sodium hydroxide caused the precipitation of a large

amount of black solid. The black solid was collected by filtration, and it and the palladium salts precipitated from the reaction mixture were exhaustively extracted with chloroform. Glc analysis (Dexsil 300) of both the methylene chloride filtrate and the chloroform extract (reduced to a small volume) indicated the presence of no diphenyl ether, less than 0.1% dibenzofuran and no other chromatographable substances. The combined methylene chloride and chloroform solution was evaporated to dryness to give 107 mg of a black solid which was subjected to mass spectral analysis (direct probe) with the following results.

The product mixture was indicated to contain polymeric material, molecular weights above 1000, which showed fragmentation patterns involving stepwise loss of fragments with masses of 76 and 89-90. In addition, there appeared to be discreet dimers, trimers and tetramers present having molecular ions with masses centering around the following:

334 (major peak) - dibenzofuran dimer (?)

410 (major peak) - phenyl-substituted dimer (?)

486 (minor peak) - dimer substituted by two phenyls (?)

500 (major peak) - dibenzofuran trimer (?)

576 (major peak) - phenyl-substituted trimer (?)

652 (minor peak) - trimer substituted by two phenyls (?)

666 (major peak) - dibenzofuran tetramer (?)

A second attempt to carry out the process with diphenyl ether -U-$^{14}$C gave essentially the same results.

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