

The Synthesis of ^{14}C -uniformly Ring Labeled 3,3',5,5'-Tetra-*Tert*-Butyldiphenoquinone

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SUMMARY

*^{14}C -uniformly ring labeled 3,3', 5,5'-tetra-*tert*-butyldiphenoquinone was synthesized from 2,6-di-*tert*-butylphenol. Chemical purity was established by mixed melting point, thin-layer and gas-liquid chromatography. Radiochemical purity was determined using autoradiography in conjunction with thin-layer chromatography and liquid scintillation techniques. Structure was confirmed by elemental analysis, ultraviolet-visible and infrared spectroscopy and nuclear magnetic resonance.*

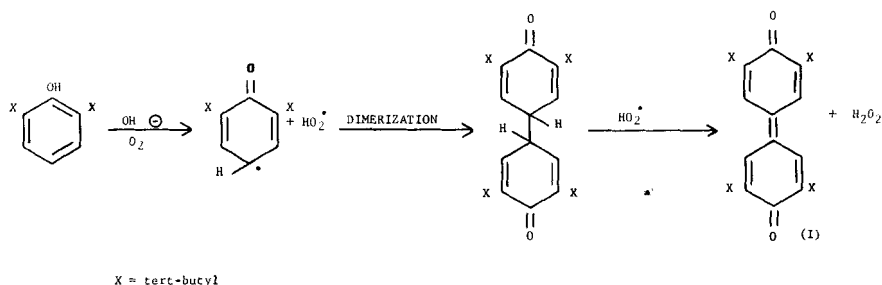
INTRODUCTION.

3,3', 5,5'-Tetra-*tert*-butyldiphenoquinone has been reported to be a major metabolite of probucol ***, an effective hypocholesterolemic agent in several laboratory animals ^(1, 2). Since probucol shows promise as a potential serum cholesterol lowering agent, it is important to study the distribution and excretion of the compound's metabolites. The ^{14}C -uniformly ring labeled diphenoquinone was synthesized for these metabolite studies which are currently in progress.

The synthesis of the diphenoquinone from 2,6-di-*tert*-butylphenol has been reported by several investigators ⁽³⁻⁵⁾. A modification of the work of Kharasch and Joshi ⁽⁵⁾ was utilized in this radiochemical synthesis. The sequence of reactions, as previously reported by the above authors, is shown

*** 4,4'-(isopropylidenedithio)bis(2,6-di-*tert*-butylphenol), The Dow Chemical Company, Zionsville, Indiana 46077.

in Figure 1. In this base catalyzed reaction, 2,6-di-*tert*-butylphenol was dissolved in *tert*-butyl alcohol, and the reaction mixture was agitated in the presence of oxygen to give the diphenoquinone. The diphenoquinone is reported to be a dark red crystalline material with a purple reflex, having a melting point of 245-247° C, a molecular weight of 408, and a molecular formula of $C_{28}H_{40}O_2$.



EXPERIMENTAL.

^{14}C -Uniformly Ring Labeled Diphenoquinone ⁽⁵⁾. — Thirty-seven milligrams of uniformly ^{14}C -ring labeled 2,6-di-*tert*-butylphenol *, 1.035 mCi (28.3 μ Ci/mg), was accurately weighed into a graduated 15-ml flask, followed by 163 mg unlabelled 2,6-di-*tert*-butylphenol ** for a total of 200 mg (9.7×10^{-4} mole) of 2,6-di-*tert*-butylphenol. *Tert*-butyl alcohol (2.5 ml) and 0.3 ml KOH (67% w/v solution) was added. The vessel was placed in a water bath at 37° C, and oxygen was passed into the solution to allow good agitation of the mixture. *Tert*-butyl alcohol (0.5 ml) was added to the reaction at 14 and 28 minutes to keep the volume of the reactants at 2.8 ml. After 42 minutes, 5 ml of deionized water was added, and the mixture was filtered through a small Buchner funnel. The precipitate was then washed with six 5-ml aliquots of water and allowed to dry. The yield of the dry precipitate was 148 mg (72%).

Purification of the Crude Product. — The precipitate was placed in a 25-ml Erlenmeyer flask, and 6 ml of redistilled chloroform and 10 ml of absolute ethanol was added. The chloroform was evaporated at 70° C. The solution was allowed to cool slowly with continued evaporation to a volume of 8 ml. After 55 hours the crystals were separated, washed with four 10-ml portions of deionized water and dried under nitrogen. After recrystallizing twice from the chloroform-ethanol mixture, the final yield of the purified crystals was 131 mg (64%). The activity of the product was 5.97 μ Ci/mg and the specific activity was 2.4 mCi/mmole as determined by liquid scintillation counting.

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Chemical and Radiochemical Purity. — The melting point of the synthesized compound was found to be 245.5° C as compared to previously reported values of 245-247° C. A mixed melting point of the synthesized compound with an authentic sample of the diphenquinone gave no melting point depression. Purity was also determined by thin-layer chromatography (TLC) according to the following procedure: Two samples of the ¹⁴C-diphenquinone were accurately weighed on a Kahn microbalance. Each sample was placed into a 1-ml, Class A, volumetric flask and dissolved in spectrophotometric grade benzene. Four 20- μ l samples (1 μ g/ μ l) were placed on separate 20 \times 20 cm silica gel plates * along with 10 μ g of unlabeled diphenquinone under nitrogen. Two solvent systems were utilized, hexane-benzene (80 : 20), and hexane-ether (97 : 3), in a two dimensional system, in that order, to develop the plates. The plates were dried between runs under nitrogen. One of the plates was examined under shortwave UV light after exposure to I₂ vapor, and only one spot could be detected at the R_f of the diphenquinone. Previous work on the TLC methods showed that impurities were visibly detectable by either method at a level of 0.2 μ g.

The synthesized diphenquinone and an authentic sample were each dissolved in acetone for purity studies using gas chromatography. These samples were injected separately and as a mixture at a level of 2 to 4 μ g using flame ionization and 2 to 4 ng using the electron capture detector.

A gas chromatograph ** with the hydrogen flame ionization detector was used for the first method of analysis. The glass column, 2 feet long by 3.5 mm I.D., contained 1% OV-1 on Chromosorb W ***, 100/120 mesh, as the stationary phase. The temperature of the column was 195° C, isothermal, injector 295° C, detector 295° C, and the argon carrier flow rate 80 cc/minute. The hydrogen flow rate was 35 cc/minute and the air flow 350 cc/minute. The retention times and responses, under these conditions, for the two samples and the mixture were identical. The second method utilized the same gas chromatograph with a ⁶³Ni. electron capture detector. The glass column, 4 feet long by 3.5 mm I.D., contained 1% OV-17 on Chromosorb W, 100/120 mesh, as the stationary phase. The temperature of the column was 210° C, isothermal, injector 290° C, and the nitrogen flow rate 150 cc/minute. The resulting chromatogram contained one peak.

Two of the plates from the thin-layer chromatography methods previously discussed were subjected to autoradiography. No-Screen Medical X-Ray Film **** was placed over the developed plates and exposed for a period of 90 minutes. A major spot representing the diphenquinone was detectable. The plates were exposed for an additional 12.5 days to allow detection of radio-

* Bakerflex 1B-F, J. T. Baker Chemical Co., Phillipsburg, N. J.

** Barber-Colman, Series 5000, Barber-Colman Co., Rockford, Ill.

*** John-Manville Products, Co. New York, N. Y.

**** Kodak (NS-54T), Eastman Kodak Co., Rochester, N. Y.

impurities at a level of 1/2 of 1 %. The resulting radiograms revealed impurities at an R_f of 0.28 and 0.39 in the hexane-ether system. The R_f of the diphenoquinone in the hexane-ether system is 0.55. Using the autoradiogram as a template, the radioactive spots, as well as the remaining silica gel, were scraped from the plates, and their activity was determined by liquid scintillation counting techniques. A ^{14}C -toluene internal standard was used to determine the absolute disintegration of the samples. The diphenoquinone was found, by the above technique, to be 99.8 % radiochemically pure.

Proof of Structure. — The structure of the synthesized diphenoquinone was confirmed by elemental analysis and a comparison of infrared spectrum and ultraviolet-visible spectrum to that of previously published information^(5, 6). Also, to further prove the structure of the diphenoquinone, the NMR spectrum was determined. The carbon-hydrogen analysis* of the ^{14}C -synthesized diphenoquinone revealed the following: Calculated for $\text{C}_{28}\text{H}_{40}\text{O}_2$: Carbon, 82.4 %; Hydrogen, 9.8 %. Found: Carbon, 82.14 %; Hydrogen, 9.87 %.

The ultraviolet and visible spectrum was analyzed from 200 to 600 nm. The resulting peaks ($\lambda_{\text{max.}}^{\text{iso-octane}}$ 260, 270 and 418 nm), are in agreement with previously reported information⁽⁵⁾. An $\epsilon(\text{max})$ determination was also conducted. The results 4,300, 4,300, and 84,000 for the 260, 270 and 418 nm peaks, are also in agreement with results previously published⁽⁵⁾.

The infrared spectrum analysis showed a carbonyl bond stretching frequency $\nu(\text{C}=\text{O})$ at $1,605\text{ cm}^{-1}$ (strong) in agreement with previously reported information on the infrared carbonyl stretching frequency of the diphenoquinone^(5, 6).

The nuclear magnetic resonance spectrum was also determined. Two peaks were found at 1.37 δ and 7.73 δ for the compound. These peaks corresponded to the Aliphatic protons on the *tert*-butyl groups of the compound which contain a total of thirty-six protons as compared to the four Aromatic protons found on the benzene rings of the labeled compound, respectively. An integration of the peaks showed a 9 to 1 ratio of the first peak to the second downfield peak. Since these were the only protons detected by the instrument, the structure of the synthesized compound was well substantiated.

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