# PREPARATION OF TRITIUM LABELED DIPHENYLHYDANTOIN OF HIGH SPECIFIC ACTIVITY

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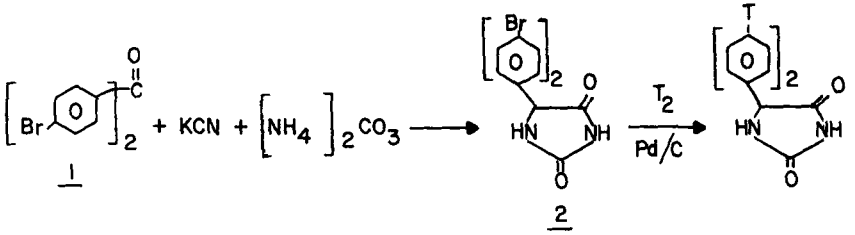
Since its introduction to medicine several years ago,[1] diphenylhydantoin (DPH) has been widely used in the treatment of epilespy, both in adults and children.[2] The effects of the drug is well correlated with the plasma levels. Low levels do not control seizures, whereas high levels have adverse effects.[3]

The sensitivity of radioimmunoassay (RIA) and its adaptability to routine assay of large numbers of samples, made RIA an attractive method for the analysis of plasma levels of DPH, especially in children.

The development of an RIA assay in these laboratories[4] required the preparation of tritium labeled DPH of high specific activity. This was accomplished by the scheme outlined in Chart 1. The modified Bucherer[5] reaction of 4,4'-dibromobenzophenone (1) with potassium cyanide and ammonium carbonate gave a 63% yield of 5,5-di(4-bromophenyl)hydantoin (2). Reductive titration of 2 afforded 5,5-di(phenyl-4-$^3$H)hydantoin

Chart 1

Preparation of Tritium Labeled Diphenylhydantoin




1 2

which was at least 98% radiochemically pure and had a specific activity of 56 Ci/mmol. The labeled DPH showed no decomposition after storage for two years in 10% ethanol-benzene solution at 4°C.

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## Experimental

### Preparation of 5,5-Di(4-bromophenyl)hydantoin

A 1.30 g (3.8 mmol) sample of 4,4'-dibromobenzophenone (Columbia Organic Chemicals) was dissolved in 16 g of acetamide with stirring and heating. Potassium cyanide (0.342 g, 5.26 mmol) was added and the stirring and heating continued until solution was complete. Ammonium carbonate (1.09 g, 11.3 mmol) was added and the solution immediately sealed in a Paar bomb (40 ml capacity) with glass liner. The sealed bomb was kept in an oven at 120° for 20 hours. It is essential that the entire bomb be heated uniformly, otherwise the ammonium carbonate sublimes from the solution and greatly reduced yields are obtained. After cooling, the contents of the bomb were suspended in water and the suspension acidified with hydrochloric acid. The mixture was filtered and the residue stirred with 50 ml of 5% sodium hydroxide and filtered. The filtrate was acidified, the precipitate collected and crystallized from ethyl acetate to give 973 mg (63%) of 5,5-di(4-bromophenyl)hydantoin. m.p. 308-309°, $C_{15}H_{10}Br_2N_2O_2$. Required m/e 409.909; Found m/e 409.911.

### Preparation of 5,5-Di(phenyl-4-$^3$H)hydantoin

To a solution of 10 mg (0.025 mmol) of 5,5-di(4-bromophenyl)hydantoin and 10 µl (0.07 mmol) of triethylamine in 1 ml of dioxane was added 10 mg of 10% Pd/C. The mixture was stirred in an atmosphere of tritium gas (25 Ci) until no more tritium was absorbed (ca. 3 hr). The catalyst was removed by filtration and the labile tritium removed in vacuo using ethanol as solvent. The residue was dissolved in 5 ml of ethyl acetate, washed with 3 x 1 ml of water and the ethyl acetate solution filtered through sodium sulfate. The ethyl acetate solution was chromatographed on a 20 x 20 ChromAR 1000 sheet using carbon tetrachloride-acetone (8:2) as solvent. The radioactive zone was cut from the chromatogram and eluted with ethanol. The residue obtained after removal of the ethanol was further purified by sublimation (180°/0.05 mm). The sublimate (1.5 mg) had a specific activity of 233 mCi/mg (56 Ci/mmol). Isotopic dilution analysis (crystallized from ethanol and water) indicated that the radio-

chemical purity was greater than 98%. The material was stored in 10% ethanol-benzene solution at about 4°.

After storage in this manner for two years no decomposition could be detected by radio tlc (silica gel HF, acetone-chloroform, 3:7).

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