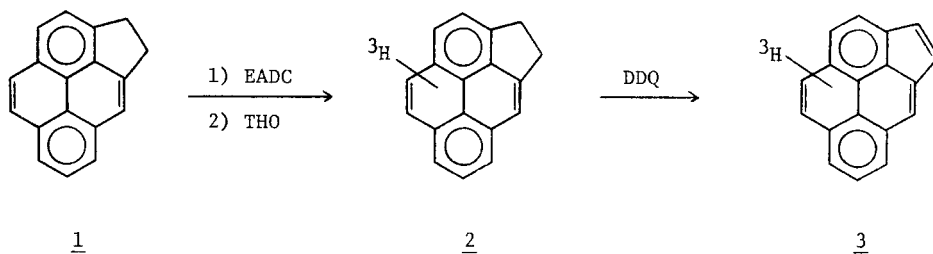


Synthesis of ^3H -Labeled Environmental Carcinogens:
Cyclopenta[*c,d*]pyrene-G- ^3H

Cyclopenta[*c,d*]pyrene (CPP) is a highly mutagenic¹ polycyclic aromatic hydrocarbon found in automobile exhaust,² carbon black,^{3,4} and a variety of soots.³ The widespread environmental distribution of this compound necessitates exhaustive *in vivo* and *in vitro* studies of CPP and its metabolites. Therefore, the synthesis of radiolabeled CPP was undertaken.

An initial attempt for the direct tritiation of CPP via a previously developed procedure⁵ using ethylaluminum dichloride (EADC) catalyst and tritiated water (THO) gave mostly dimeric products. This is not surprising, in view of the reactivity of the 3,4-exocyclic double bond⁴ and the reported sensitivity of CPP to acidic conditions.⁶ Therefore, a new synthetic route employing 3,4-dihydrocyclopenta[*c,d*]pyrene 1 was chosen.



Treatment of 1 with EADC, followed by quenching the resulting complex with 10 Ci of tritiated water (specific activity of 50 Ci/ml) gave 2 with a specific activity of 547 mCi/mole. Further, after dilution with pure unlabeled material, the tritiated 3,4-dihydrocyclopenta[*c,d*]pyrene (2) was converted to the final product by treatment with excess 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). The chemical and radiochemical purity of the final product was $\geq 98\%$ after

purification via column chromatography, affording a 57% yield of 3 (specific activity 293 mCi/mmole). It should be noted that a product with significantly higher specific activity could be obtained by using tritiated water with a specific activity of 500 Ci/ml.

Experimental

Nonradioactive CPP was prepared in this laboratory according to a previously published procedure.⁷ Hydrogenation⁴ of an ethanolic solution of CPP on 10% Pd/C catalyst, followed by purification via the picrate and subsequent column chromatography, gave 3,4-dihydrocyclopenta[c,d]pyrene 1 in 61% yield. Ethylaluminum dichloride was purchased from Ethyl Corporation, Baton Rouge, Louisiana. The tritiated water was purchased from Amersham Corporation, Arlington Heights, Illinois. The UV spectra were recorded using a Varian Superscan 3 spectrophotometer. Radioactivity was determined with a Packard Tri-Carb-2425 liquid scintillation spectrometer using solutions of Liquifluor[®] (New England Nuclear) and PCS[®] (Amersham Chemical Corporation). Radiochemical purity was determined by autoradiography and scanning of the TLC plates using a Packard Model 7201 radiochromatogram scanner. The purity and identity of the ³H-labeled compounds were confirmed by comparison with known standards. Silica gel plates (Brinkman 60F-254) were used for TLC and Ultrafilm ³H (LKB) film was used for the autoradiography.

3,4-Dihydrocyclopenta[c,d]pyrene-G-³H (2)

The tritiation of 1 using EADC and THO was carried out in a specially designed high vacuum reaction vessel⁸ suitable for handling 10 to 100 Ci amounts of tritiated water of relatively high specific activity (50 to 500 Ci/ml) without exposure to the atmosphere. Thus, to 13 mg (0.057 mmole) of 1, EADC (100 μ l, 0.10 mmole) was added through a septum and under a nitrogen atmosphere. After stirring for 5 min, THO (10 Ci, 0.2 ml) was added to quench the red-colored complex. The removal of labile tritium was achieved by azeotropic distillation

in *vacuo* using 10 x 2 ml methanol. The labile-free crude product was dissolved in benzene (25 ml) and dried over Na₂SO₄. After removal of the solvent, the residue was purified by chromatography on a silica gel column (15 mm x 150 mm) using cyclohexane-benzene (1:1) as the eluant. A yield of 8.65 mg (20.8 mCi, 547 mCi/mmole) of pure 2 was obtained. The chemical and radiochemical purity of 2 was ≥ 98% as determined by TLC (silica gel/cyclohexane, R_f = 0.18) and by UV analysis.

Cyclopenta[*c,d*]pyrene-G-³H (3)

The sample of 2 was first diluted with an equal weight of 1. Next, 17.3 mg (0.076 mmole) of 3,4-dihydrocyclopenta[*c,d*]pyrene in 40 ml benzene was heated to reflux (under argon), and then 40 mg of DDQ (0.176 mmole) in 10 ml of benzene was added. The mixture was allowed to reflux for 1 hr, cooled to room temperature, and then diluted with 50 ml of benzene and 100 ml of ether. The organic phase was washed with 3 x 20 ml of saturated sodium sulfite solution, 20 ml of sodium chloride solution, and dried over Na₂SO₄. After removal of the solvent, the residue was purified by chromatography on a column (10 mm x 100 mm) of Activity I Basic Alumina using benzene-cyclohexane (1:1) as the eluant. The desired compound appeared as an orange band (nonfluorescent with UV light). Removal of the solvent gave 9.8 mg (57% yield) of 3 (12.7 mCi, 293 mCi/mmole). The chemical and radiochemical purity was determined by TLC (silica gel/benzene, R_f = 0.59) and by comparison of the ultraviolet spectrum of 3 to that of a pure sample of CPP.

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