

LABELED METABOLITES OF POLYCYCLIC AROMATIC HYDRO-CARBONS III. 3-, 7-, AND 9-HYDROXYBENZO [a] PYRENES-G-³H

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Definitive studies concerning the oxidative metabolism of benzo[a]pyrene (BP) necessitates the availability of highly pure radio-labeled oxygenated derivatives [1]. Since 3-, 7-, and 9-hydroxybenzo[a]-pyrenes (3-, 7-, and 9-HOBP) have been implicated in such studies [2], the need for these radiolabeled compounds is evident.

We now report a simple, rapid, and efficient method for the tritiation of BP phenols using ethylaluminum dichloride (EADG) catalyst with tritiated water as the isotope source. This procedure is a modification of the method reported by Garnett for the tritiation of aromatic hydrocarbons and substituted benzenes [3]. The labeled BP-phenols prepared are remarkably free of radioactive or nonradioactive impurities and require no purification. The chemical and radiochemical purity of all of the compounds produced was $\geq 98\%$; see the Figure below for a radiochromatogram

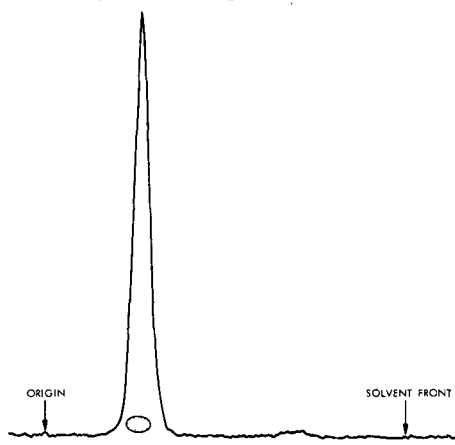


Figure - Radiochromatogram of 3-HOBP-G-³H

scan of 3-HOBP-G-³H. A summary of the results obtained for a number of experiments is given in the Table. As expected, higher specific activity products are obtained when high specific activity water is used, however no significant changes in chemical or radiochemical purity are observed. While studies concerning the distribution of tritium in these compounds have not yet been initiated, it should be noted that the data in the literature indicates that a random distribution is obtained when substituted benzenes are tritiated by this technique [3].

TABLE^{a/}

<u>Compound</u>	<u>Yield (%)</u>	<u>Specific Activity (mCi/mmol)</u>
3-HOBP	51	16.5
7-HOBP	73	11.6
9-HOBP	54	16.8
3-HOBP	68	144.5
7-HOBP	68	135.6
9-HOBP	47	213.2

a/ Reaction conditions: room temperature, 2 min, 13.4 mg (0.05 mmol) of HOBP, 30 μ l EADC, 370 μ l CS₂, and 83 μ l of ³H₂O at specific activity 5 Ci/ml or 26.4 Ci/ml.

EXPERIMENTAL

Previously published procedures were used to prepare 3-HOBP [4] and 9-HOBP [2b, 5]. The 7-HOBP was synthesized by a modification of the reported method [6]. These materials were analytically pure and free of chromatographically or spectroscopically detectable impurities. The high specific activity tritiated water was prepared by reducing platinum oxide in the presence of water according to the procedure outlined by Swain and Kresge [7], whereas, the tritiated water at specific activity 5 Ci/ml was purchased from New England Nuclear, Boston, Massachusetts. EADC was purchased from the Ethyl Corporation, Baton Rouge, Louisiana. UV spectra were recorded with a Cary 118 spectrophotometer. Radioactivity was determined

in a Packard Model 3003 liquid scintillation counter using Liquifluor™ (New England Nuclear) as the counting medium. Radiochemical purity was determined in a Packard Model 7201 radiochromatogram scanner. The tritiations and ensuing operations were performed under a nitrogen atmosphere. The general reaction procedure is exemplified by the preparation of 3-HOBBP-G-³H outlined below.

3-Hydroxybenzo[a]pyrene-G-³H

A 6 ml serum vial equipped with a stirring bar and protected from light (aluminum foil) was charged with 13.4 mg (0.05 mmol) of 3-HOBBP and sealed with a Teflon lined septum. The vial was then purged for 10 min with dry nitrogen, and 370 μ l of carbon disulfide and 30 μ l (0.3 mmol) of EADC was added. Upon addition of the EADC, a dark red-purple paste developed. After 2 min, 83 μ l (2.2 Ci) of tritiated water was added, specific activity 26.4 Ci/mmol, and the vial was shaken for an additional 2 to 3 min to insure good mixing. One ml of "cold" water was then added and the precipitated light yellow solid was extracted with benzene:ethyl acetate (1:1). The extract was washed with 3 by 10 ml portions of water to remove the labile tritium and dried (Na_2SO_4). TLC of the dried extract on silica gel using benzene:ethanol (19:1), R_f 0.27, showed no chemical or radiochemical impurities. Analysis of the solution by UV spectroscopy indicated 9.1 mg (68%) of 3-HOBBP-G-³H was obtained at a specific activity of 144.5 mCi/mmol.

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