NOTES

LABELED METABOLITES OF POLYCYCLIC AROMATIC HYDROCARBONS II. 4,5-DIHYDROBENZO[a]PYRENE-4,5-EPOXIDE-G-^{3}H VIA CIS 4,5-DIHYDROBENZO[a]PYRENE-4,5-DIOL-G-^{3}H

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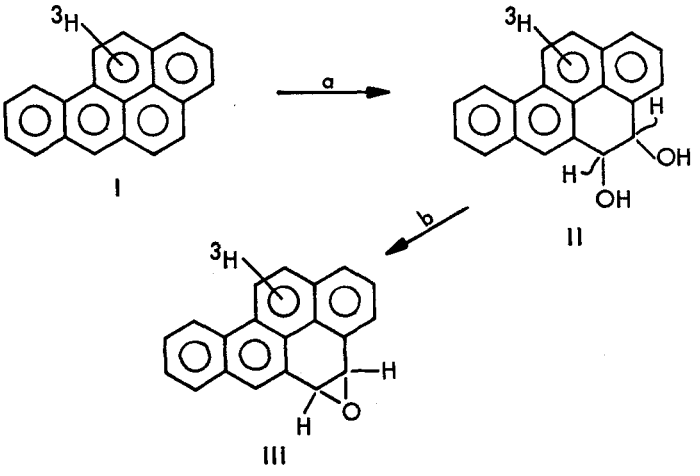
Recent investigations concerning the oxidative metabolism of benzo[a]-pyrene (BP) have implicated various epoxides as intermediates [1]. However, more conclusive results regarding the role of BP epoxides in carcinogenesis necessitates the availability of labeled compounds for further studies [2].

Therefore, we report the synthesis of 4,5-dihydrobenzo[a]pyrene-4,5-epoxide-G-^{3}H from BP-G-^{3}H as outlined in Scheme I. The pathway described is a

SCHEME I



I II III

[a] OsO_4, pyridine; $KHCO_3$, Na_2SO_3; Ac_2O, pyridine; CH_3OH, NH_3.
[b] $CH_3C(OCH_3)_3$; $(CH_3)_3SiCl$; $NaOCH_3$.

combination of the methods reported recently for the synthesis of unlabeled 4,5-dihydrobenzo[a]pyrene-4,5-epoxide [3,4] and requires purification of the intermediate diacetate and final product via column chromatography. Chemical and radiochemical purity were established using high pressure liquid chromatography (HPLC). An 8.5% overall yield of chemically and radiochemically pure epoxide was realized.

EXPERIMENTAL

Benzo[a]pyrene-G-^{3}H was obtained from Amersham/Searle Corporation, Arlington Heights, Illinois, at a specific activity of 500 mCi/mmol. IR spectra were determined with a Beckman Acculab I, using Nujol. UV spectra were recorded with a Cary 118 spectrophotometer. All experimental operations were conducted under a nitrogen atmosphere. Yields reported are based on starting BP-G-^{3}H.

High pressure liquid chromatography was performed with a Du Pont 830, fitted with a 1 meter ODS permaphase column. Elution was by a buffered methanol: water gradient ranging initially from 30:70 to a final concentration of 70:30; the solvent gradient was changed at 3% per minute with a column temperature at 50°C and a column pressure at 450 psi. Solvent mixtures were buffered to pH 9.0 by the addition of 0.1 M tris and triethylamine (0.4% v/v) to stabilize the column. The eluent was collected in 0.4 ml fractions and the radioactivity was determined in a Beckman LS-355 scintillation counter with Aquasol™ (New England Nuclear) as the counting medium. Other radioactivity measurements were determined in a Packard Model 3375 scintillation counter using ECONOFLUOR™ (New England Nuclear) as the counting medium.

cis-4,5-Dihydrobenzo[a]pyrene-4,5-diol-G-^{3}H (II)

To 650 mg (2.6 mmol) of I and 0.7 ml of pyridine was added 900 mg (3.5 mmol) of osmium tetroxide in 70 ml of benzene. The mechanically stirred reaction mixture was protected from light (aluminum foil) and held at room temperature for 72 hr. The benzene supernatant was decanted from the light brown residue. The solid

was then washed with 5 x 50 ml portions of benzene and dried under vacuum (1 mm) at room temperature yielding 1.3 g (98%) of osmate ester.

A solution containing 25 g of sodium sulfite and 25 g of potassium bicarbonate in 250 ml of water was added to the osmate ester with fast mechanical stirring. After 0.5 hr, 100 ml methanol was added and stirring was continued for 3 hr. The resulting reaction mixture was filtered and the solids washed with 150 ml of water, followed by 150 ml of methylene chloride and finally with 200 ml of hot tetrahydrofuran. The aqueous filtrate and wash were combined and extracted with 4 x 50 ml of methylene chloride. The methylene chloride extracts were combined, washed with 75 ml of water, and subsequently combined with the tetrahydrofuran extracts, dried (Na_2SO_4) and concentrated (in vacuo) to near dryness. The residue was dissolved in 40 ml of absolute ethanol and concentrated (in vacuo).

To this residue a mixture of 5.0 ml of pyridine and 30 ml of acetic anhydride was added. The mixture was stirred overnight at room temperature followed by precipitation of the crude diacetate by addition of 150 ml of water. After stirring 2 hr the solids were filtered and column chromatographed on silica gel (E. Merck), 2 cm x 20 cm (packed using hexane), eluting stepwise with 400 ml of hexane:benzene (1:1), 400 ml of hexane:benzene (1:3), 400 ml of benzene, 400 ml of benzene:ethyl acetate (99:1), and 200 ml of benzene:ethyl acetate (19:1). Fifteen fractions (120 ml each) were collected and those fractions containing pure diacetate were identified by thin-layer chromatography (R_f 0.23, silica gel/benzene). These fractions were combined and concentrated (in vacuo) yielding 315 mg (34%) of cis-4,5-dihydrobenzo[a]pyrene-4,5-diol-G-^{3}H diacetate, m.p. 215°, lit. 214-215° [5]. The IR and UV spectra were consistent with expected results.

The diol diacetate was stirred for 30 min with 125 ml of methanol at room temperature. The mixture was then cooled (-5°C) and ammonia gas was bubbled through the solution for 15 min. The resulting mixture was stirred overnight and the diol isolated by evaporation of the solvent (in vacuo). Recrystallization

from benzene yielded 180 mg (24%) of gray crystalline II, 215° (decomp.), lit. 200-215° (decomp.) [5]. The IR and UV spectra were consistent with expected results.

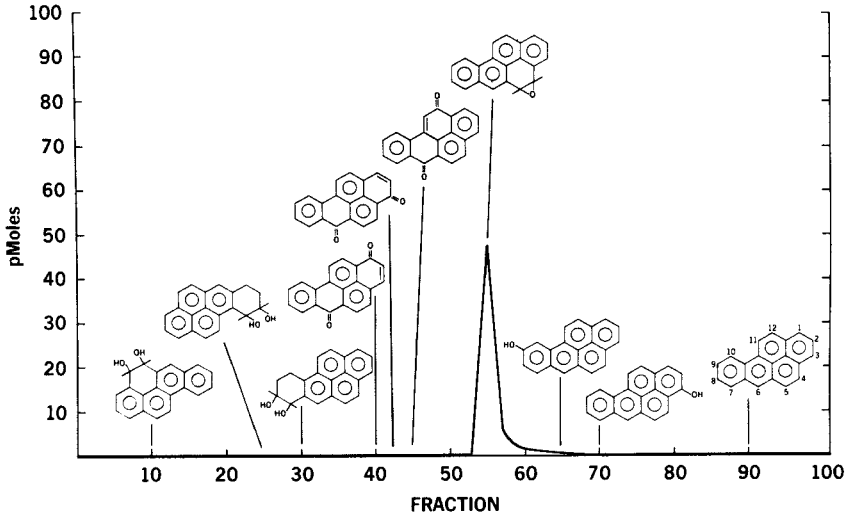
4,5-Dihydrobenzo[a]pyrene-4,5-epoxide-G-^{3}H (III)

To 180 mg (0.63 mmol) of II was added 0.3 ml (24.0 mmol) of trimethylorthoacetate, 5 mg (0.04 mmol) of benzoic acid and 8 ml of benzene. The mixture was distilled for 3 hr at a 1 ml per hour distillation rate, cooled to room temperature and 50 mg of sodium carbonate added. The resulting mixture was filtered and the filtrate concentrated (in vacuo) yielding the crude ortho ester as a light yellow-green viscous oil. The ester was dissolved in 6 ml of methylene chloride containing one drop of triethylamine, the solution cooled to 0°C and 0.25 ml (2.7 mmol) of trimethylchlorosilane added. The chloro-acetate precipitated as a yellow-green solid upon refrigeration overnight (5°C). Removal of the solvents (in vacuo) gave crude chloro-acetate which was used without further purification.

A solution of the chloro-acetate in 25 ml of tetrahydrofuran was slowly added to a cold (-78°C) magnetically stirred suspension of 500 mg (9.2 mmol) of sodium methoxide in 10 ml of tetrahydrofuran. The resulting orange solution was stirred at -78°C for an additional 2 hr and stored overnight (-5°C). Ether (125 ml) was added to this solution and the resulting solids filtered. The filtrate was washed with 2 x 50 ml of cold water, dried (K_2CO_3), filtered and concentrated (in vacuo) yielding the crude epoxide as an orange-brown solid. The solid was dissolved in 20 ml of benzene and adsorbed on a dry 2 cm x 20 cm column of neutral alumina, grade IV. The column was eluted with benzene (containing 1 ml of triethylamine per liter) under nitrogen pressure (4 psig). The first 200 ml of eluent, the zone preceding an orange band, was concentrated (in vacuo) yielding yellow-orange crystalline III. Recrystallization from hexane:benzene (1:1) afforded 60 mg (8.5%) of yellow-gold crystalline III m.p. 160-160.5° (decomp.), softening from 156°; lit. 150°, softening from 135° [3]; specific activity 408 mCi/mmol;

TLC, R_f 0.8, alumina/benzene:ethyl acetate (4:1). The IR and UV spectra were identical to those reported in the literature [3,4]. The HPLC scan indicating chemical and radiochemical purity is shown in the figure below. The retention volumes of possible impurities are indicated by the structures shown.

HIGH PRESSURE LIQUID CHROMATOGRAPHY OF
^{3}H-BENZO (a) PYRENE-4, 5-OXIDE



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