LABELED METABOLITES OF POLYCYCLIC AROMATIC HYDROCARBONS. VI. trans-7,8-DIHYDROBENZO[a]PYRENE-7,8,DIOL-G-^{3}H AND (±)-7α,8β-DIHYDROXY-9β,10β-EPOXY-7,8,9,10-TETRAHYDROBENZO[a]PYRENE-G-^{3}H

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Received on April 8, 1976

SUMMARY

The syntheses of trans-7,8-dihydrobenzo[a]pyrene-7,8-diol-G-^{3}H (V) and (±)-7α,8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene-G-^{3}H (VI) are described. The specific activities of V and VI were 158 mCi/mmole and 220 mCi/mmole, respectively. The key intermediate, trans-7,8,9,10-tetrahydrobenzo[a]pyrene-7,8-diol-G-^{3}H dibenzoate (III), was prepared by catalytic dehalogenation with tritium gas.

Key Words: trans-7,8-Dihydrobenzo[a]pyrene-7,8-diol-G-^{3}H, (±)-7α,8β-Dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene-G-^{3}H, Tritium, Polycyclic aromatic hydrocarbons, metabolites

INTRODUCTION

Among the major metabolites of benzo[a]pyrene (BP), trans-7,8-dihydrobenzo-[a]pyrene-7,8-diol (BP-7,8-diol) is unique in that, after incubation with hamster liver microsomes, it was observed to bind to DNA to an extent 10-fold greater than benzo[a]pyrene itself when subjected to similar metabolic activation [1]. The significance of this finding was revealed by the work of Sims and co-workers [2] which indicated that the BP-7,8-diol formed in a primary metabolic step is subsequently oxidized (microsomes) to a diol-epoxide which is the species responsible for the binding of the BP moiety to DNA. In particular, this was supported by the fact that BP-7,8-diol, after treatment with m-chloroperbenzoic acid, reacted in

vitro with isolated DNA to afford the same nucleoside-hydrocarbon conjugates as those obtained from BP-7,8-diol which was reacted after microsomal metabolic activation. Moreover these nucleoside-hydrocarbon products were chromatographically indistinguishable from those formed in vivo by whole cells treated with either BP or BP-7,8-diol.

The product of the BP-7,8-diol m-chloroperbenzoic acid reaction has been isolated and characterized as a 7,8-diol-9,10-epoxide [3]. Its stereochemistry was established when an isomeric diol-epoxide was prepared (by closure of a 9,10-bromohydrin) and on the basis of NMR spectrum, found to have the 7α,8β,9α,10α orientation [4]. The peracid product is therefore 7α,8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene.

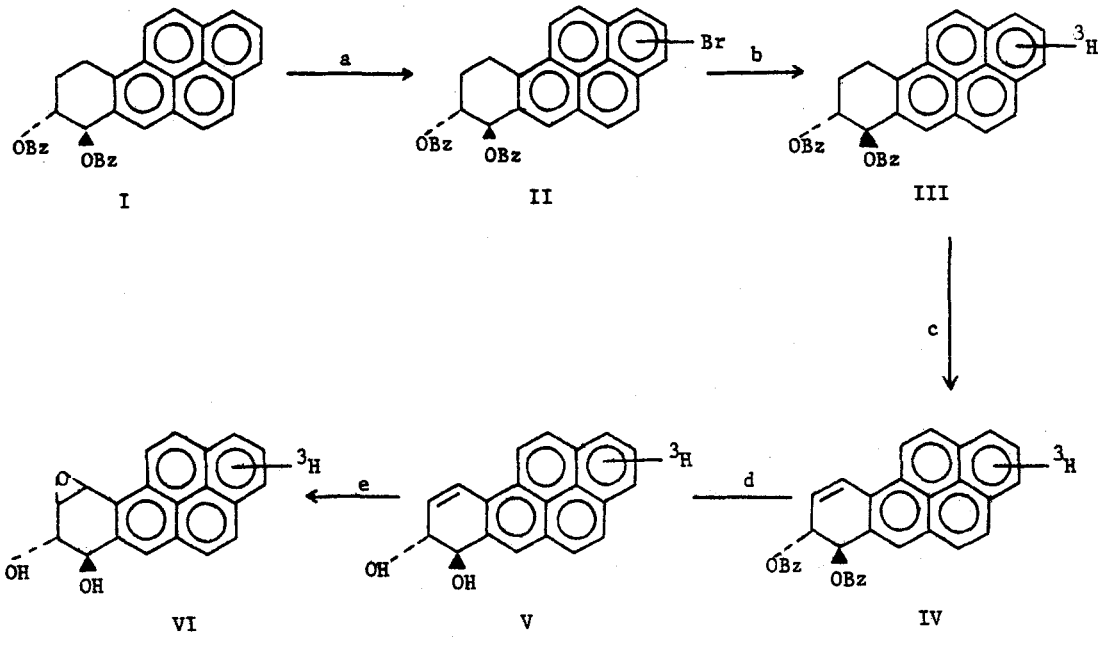
The 9α,10α-isomer was markedly more reactive toward nucleophiles in accordance with the expectation of Hulbert [5]. However, the chemically less reactive 9β,10β-isomer shows higher indices of biological activity in terms of mutagenic power [6]. Furthermore, incubation of the 7,8-diol with rat liver microsomes affords products characteristic of those resulting from hydration of synthetic 9β,10β-diol epoxide and different from those derived from the 9α,10α-isomer [6].

The above facts are consistent with the identification of 7α,8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene as the ultimate carcinogenic metabolite of BP. To aid in establishing further the role of BP-7,8-diol and the 9β,10β-diol epoxide in BP carcinogenesis, the title compounds were synthesized by methods reported herein.

The route, depicted in Scheme I used, in part, the reaction sequence originally reported for the preparation of unlabeled V and VI [3]. Tritium was introduced by catalytic hydrogenolysis of a nuclear monobrominated intermediate II. This product, which resulted from the treatment of I with the mild brominating agent--phenyltrimethylammonium tribromide (PTT), was separable (HPLC, crystallization) into two compounds. On the basis of bromine analyses, these were characterized as isomeric monobrominated derivatives [7]. However, for the present purpose, the isomer mix-

ture was used directly. Hydrogenolysis of II was conducted with Pd catalyst in dioxane solution using an equimolar amount of triethylamine as promoter.

SCHEME I



[a] PTT, HOAc/THF. [b] Dioxane/Et_3N, $^3H_2$, Pd black. [c] NBS, CCl_4, Bz_2O_2, reflux; boiling zylene. [d] $NaOCH_3$, MeOH/THF. [e] m-Chloroperbenzoic acid, THF.

EXPERIMENTAL

Tritium gas was obtained from Union Carbide Corporation, Nuclear Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Palladium black was purchased from Fisher Scientific Company, St. Louis, Missouri. IR spectra were determined with a Beckman Acculab I, using Nujol. UV spectra were recorded with a Cary 118 spectrophotometer. Radioactivity was determined in a Packard Model 3003 liquid scintillation counter using Econofluor™ (New England Nuclear) as the counting medium. Radiochemical purity was determined in a Packard Model 7201 radiochromatogram scanner. All experimental operations involving tritiated compounds were conducted under a

nitrogen atmosphere. High pressure liquid chromatography was performed with a Spectra-Physics 3500B using hexane--methylene chloride on a 3 mm x 250 mm Spherisorb silica column.

Monobromo-trans-7,8,9,10-Tetrahydrobenzo[a]pyrene-7,8-diol diol dibenzoate (II)

To a warm (40°) mixture of 1.0 g (2.0 mmole) of I [3] in 100 ml of acetic acid:tetrahydrofuran (3:2) was added 2.0 g (5.3 mmole) or phenyltrimethylammonium tribromide (PTT) in 1 ml of THF. After stirring at room temperature for 4 hr an additional 2.0 g of PTT was added. The resulting mixture was stirred for 44 hr afte which the excess PTT was decomposed by addition of saturated $NaHSO_3$ solution until a negative test for bromine was obtained with potassium iodide-starch paper. Gradua addition of water (500 ml) precipitated the product which was washed thoroughly wit water. After drying (in vacuo) at 50° for 4 hr, 970 mg of II was obtained as a ligh orange solid, m.p. 179-184°; TLC, R_f 0.44 silica gel/cyclohexane:acetone (3:2). Analysis by HPLC showed this product to contain 5% unreacted I with the remainder consisting of two brominated products present in the ratio of approximately 2:1. Crystals of each compound were deposited from an acetone solution of the product mixture. These were separated manually and used as seed for selective crystallization. Compound IIa was obtained as white prisms, m.p. 240-241°. Anal. Calcd for $C_{34}H_{23}BrO_4$: Br, 13.89. Found: Br, 13.92.

Compound IIb was collected as pale orange needles, m.p. 204-205°. Anal. Calcd. for $C_{34}H_{23}BrO_4$: Br, 13.89. Found: Br, 14.06.

trans-7,8,9,10-Tetrahydrobenzo[a]pyrene-7,8-diol-G-^{3}H dibenzoate (III)

The procedure described below exemplifies a typical tritiation run utilized for the preparation of III.

To a suspension of 5 mg of palladium black in 2 ml of dioxane [8] containing 7.2 µl of triethylamine was added 23.7 mg of II. The flask containing the above mixture was attached to a vacuum manifold, frozen with liquid nitrogen, and the entire system evacuated to 30 µ. The reaction mixture was then exposed to 50 Ci of tritium gas, after which it was thawed and the resulting suspension stirred for

2 hr while the uptake of tritium gas was monitored manometrically. After the reaction, the mixture was again frozen with liquid nitrogen and the unreacted tritium transferred to a uranium trap reservoir. The reaction vessel was then removed from the vacuum manifold and the contents transferred to a flask containing 340 mg of I in 40 ml of dioxane.

Water (60 ml) was added to the solution and the resulting solid removed by filtration. The solid was dissolved in 75 ml of boiling acetone, filtered, and the filtrate evaporated, at the boiling point to 30 ml. After chilling in an ice bath for 30 min, the product was collected by filtration, dissolved in 25 ml of boiling benzene, and this solution diluted with 90 ml of hexane. After cooling in ice for 30 min, the product was collected by filtration yielding 229 mg (63%) of III as white needles, specific activity 176.5 mCi/mmole.

Extension of the reaction time to 4-6 hr gave material with specific activities in the 400-500 mCi/mmole range.

trans-7,8-Dihydrobenzo[a]pyrene-7,8-diol-G-^{3}H (V)

To a suspension of 229 mg (0.46 mmole) of IV (176.5 mCi/mmole) in 30 ml of CCl_4 was added 82 mg (0.46 mmole) of N-bromosuccinimide. The mixture was heated under N_2 with stirring at 85° (bath temperature). Dibenzoyl peroxide (10 mg) was added to initiate the reaction which was allowed to proceed for 1 hr. On cooling, the precipitated succinimide was removed by filtration and the filtrate evaporated (*in vacuo*). Xylene (25 ml) was added to the residue and the resulting solution was heated at the boiling point under a gentle stream of N_2 for 20 min. The reaction mixture was evaporated (*in vacuo*) until solid began to appear. Dilution with acetone (10 ml), followed by cooling in an ice bath, precipitated the dihydrodiol dibenzate IV which was separated by filtration. Dark impurities were removed by washing the product with 5 ml of acetone, after which it was dissolved in 10 ml THF. An equal volume of methanol was added followed by 1 ml of 1 M sodium methoxide in methanol. The reaction mixture was heated at reflux under N_2 for 1 min. Water (1 ml) was added and the mixture concentrated (*in vacuo*) to one-half volume. Crude V, which was pre-

cipitated by addition of 30 ml water, was removed by filtration. The product was dissolved in 20 ml of hot THF and the solution percolated through a 15 mm x 15 mm column of charcoal and Celite (1:9). The column was eluted with 15 ml of ethanol and the combined eluates evaporated to dryness (in vacuo). The resulting solid residue was recrystallized from pyridine-ethanol (1:19) yielding 45 mg (34%) of V as pale yellow plates, specific activity 158 mCi/mmole; TLC, R_f 0.40, silica gel/benzene:1-propanol (4:1).

trans -7α,8β-Dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene-G-^{3}H (VI)

A solution of 143 mg (0.50 mmole) of V (230 mCi/mmole) in 50 ml of THF was treated with 860 mg (5.0 mmole) of purified m-chloroperbenzoic acid and the reaction allowed to proceed at room temperature for 1 hr. Ether (50 ml) was added to the reaction mixture and the whole extracted with cold 10% aqueous NaOH (3 x 50 ml) and water (1 x 50 ml). After drying (K_2CO_3), the solvents were removed (in vacuo) and the residue dissolved in 25 ml of THF-triethylamine (19:1). The solution was placed on a 15 mm x 120 mm column of SiO_2 and the column eluted with 40 ml of THF-triethylamine (19:1). Purified dioxane (10 ml) was added to the eluate and the solution evaporated at 50-60° under a N_2 stream to a volume of 3-5 ml. The product, which separated by crystallization, was removed by filtration. There was obtained 31 mg (21%) of VI as white crystals, specific activity 220 mCi/mmole; TLC, R_f 0.50, silica gel/THF:triethylamine (19:1).

ACKNOWLEDGEMENT

We gratefully acknowledge the support of this work by the National Cancer Institute, Contract No. NO1-CP-33387.

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7. Bromine analyses on the separated bromodiesters, IIa and IIb, the mixture of bromodiesters, II, and a mixture of bromodiols obtained by saponification showed that IIa and IIb were monobrominated and not brominated in the benzoate moiety. Furthermore, the NMR spectrum of a mixture of bromodiacetates obtained by acetylation of the bromodiols indicated that aliphatic H-C-Br was absent, thus establishing bromine substitution of the pyrene nucleus.

8. The dioxane was passed through a column of dry alumina and used immediately.