LABELED METABOLITES OF POLYCYCLIC AROMATIC HYDROCARBONS. V. trans-7,8-DIHYDROBENZO[a]PYRENE-7,8-DIOL-7-^{14}C AND (±)-7α,8β-DIHYDROXY-9β,10β-EPOXY-7,8,9,10-TETRAHYDROBENZO[a]PYRENE-7-^{14}C

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SUMMARY

trans-7,8-Dihydrobenzo[a]pyrene-7,8-diol-7-^{14}C (VI) and (±)-7α,8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]-pyrene-7-^{14}C (VII) were prepared at a specific activity of 53.9 mCi/mmole by a multi-step synthetic sequence using K^{14}CN as the labeled precursor. The overall radiochemical yields for (VI) and (VII) were 18 and 7%, respectively.

Key Words: trans-7,8-Dihydrobenzo[a]pyrene-7,8-diol-7-^{14}C, (±)-7α,8β-Dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene-7-^{14}C, Carbon-14, 3-(1-Pyrenyl)-1-bromopropane, Polycyclic aromatic hydrocarbons, Metabolites

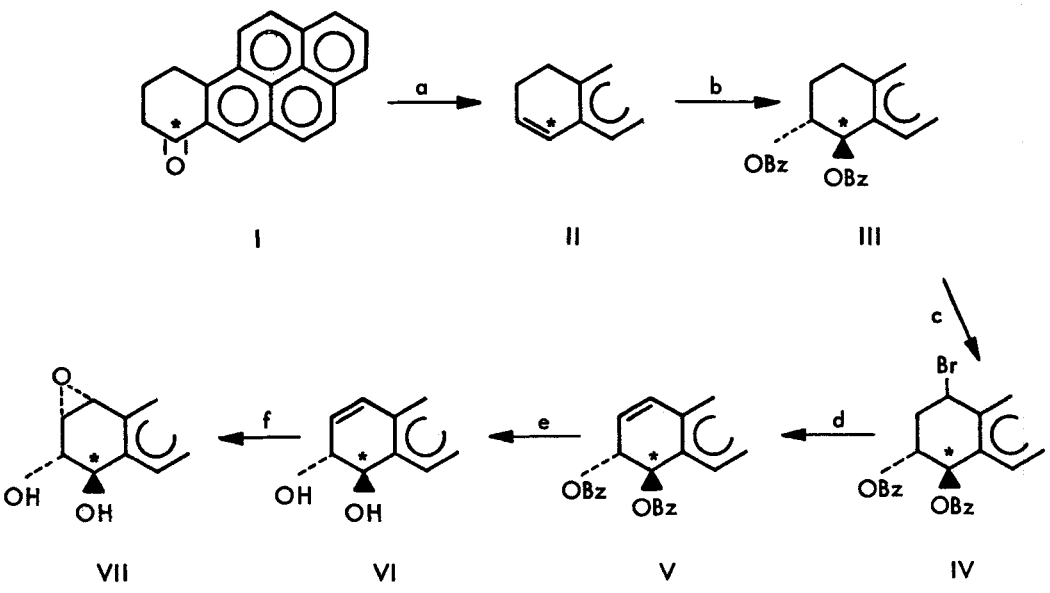
INTRODUCTION

Recent studies concerning the metabolic activation of the carcinogen benzo[a]pyrene (BP) have indicated that trans-7,8-dihydrobenzo[a]pyrene-7,8-diol (VI) is produced initially by formation of the requisite arene oxide followed by enzymatic ring opening [1-5]. Subsequent epoxidation of the diol at the isolated 9,10 double-bond by the microsomal mono-oxygenase gives a diol-epoxide [3] which has been suggested to be intimately involved in the mechanism of carcinogenesis by BP [3,5,6].

To facilitate further investigations regarding the importance of the role of these metabolites in the metabolic activation of BP, we now report the synthesis of the title compounds, VI and VII. The synthesis and physicochemical characterization of VI and VII [7] have been reported [3,6,8]. The multi-step

pathway, depicted in Scheme I, is derived from the procedures reported recently for the preparation of 7-hydroxybenzo[a]pyrene-7-^{13}C [9] and unlabeled VI and VII [8]. Thus, 3-(1-pyrenyl)-1-bromopropane was converted to 9,10-dihydrobenzo-[a]pyrene-7(8H)-one-7-^{14}C (I) in an overall radiochemical yield of 63.5% using K^{14}CN as the labeled starting material. Sodium borohydride reduction of I followed by dehydration of the intermediate alcohol gave a 91% yield of the corresponding olefin (II), which in turn was converted to the dibenzoate (III) in 74% yield. Bromination of III with N-bromosuccinimide and subsequent dehydrobromination of the resulting bromodibenzoate (IV) in boiling xylene afforded (V), which was saponified to yield trans-7,8-dihydrobenzo[a]pyrene-7,8-diol-7-^{14}C (VI) in an overall radiochemical yield of 18% (based on K^{14}CN). Treatment of VI with excess m-chloroperbenzoic acid gave (±)-7α,8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene-7-^{14}C in 40% yield.

SCHEME I



[a] $NaBH_4$, EtOH, reflux; H_2O; HOAc/HCl, reflux. [b] AgOBz, I_2, benzene, reflux. [c] NBS, CCl_4, Bz_2O_2, reflux. [d] Boiling xylene. [e] $NaOCH_3$, MeOH/THF. [f] m-Chloroperbenzoic acid, THF.

EXPERIMENTAL

Potassium cyanide-^{14}C was obtained from New England Nuclear, Boston, Massachusetts, at a specific activity of 54.2 mCi/mmole. 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 were conducted under a nitrogen atmosphere.

9,10-Dihydrobenzo[a]pyrene-7(8H)-one-7-^{14}C (I)

This compound was synthesized from 3-(1-pyrenyl)-1-bromopropane using the procedure previously described for the preparation of the carbon-13 labeled material [9]. Thus, from 300 mg (4.54 mmole) of $K^{14}CN$ was obtained 780 mg (63.5%) of I, specific activity 54.2 mCi/mmole, as yellow-brown needles; TLC, R_f 0.57 silica gel/cyclohexane:dioxane (3:2).

trans-7,8-Dihydrobenzo[a]pyrene-7,8-diol-7-^{14}C (VI)

To a suspension of 780 mg (2.89 mmole) of I in 20 ml of ethanol was added 1 g (27 mmole) of sodium borohydride and the mixture was heated at gentle reflux for 10 min. Water was added dropwise to the clear solution with continued heating and stirring until the gummy deposit of inorganic salts redissolved. An additional 40 ml of water was then added to completely precipitate the product which was collected by filtration and washed with a minimum amount of methanol and briefly air-dried on the filter. The crude alcohol was then dissolved in about 15 ml of hot glacial acetic acid and the solution heated to boiling. Concentrated hydrochloric acid was added to the hot solution and the mixture was held at reflux for 5 min. Water, 30 ml, was added to the cooled reaction mixture and the resulting crude olefin was collected by filtration and dissolved in 25 ml of benzene. After drying (Na_2SO_4 and K_2CO_3) the solution was percolated through a 2 cm x 8 cm column of neutral Al_2O_3 (Woelm, Activity I). The column was eluted

with an additional 50 ml of benzene and the combined eluates evaporated (in vacuo) to give 670 mg (91%) of II.

To 670 mg (2.64 mmole) of II dissolved in 50 ml of benzene was added 1.2 g (5.28 mmole) of freshly prepared silver benzoate. The resulting suspension was heated to reflux and a solution of 670 mg (2.64 mmole) of iodine in 40 ml of benzene was added all at once. The reaction mixture was refluxed for 8 hr, after which the suspended silver iodide was removed by filtration through celite. The filtrate was evaporated (in vacuo) and the residue was triturated with 5 ml of acetone. The resulting solid (dibenzoate) was collected by filtration, washed with 5 ml of acetone and subjected to column chromatography on silica gel (E. Merck), 2 cm x 12 cm, eluting with 70 ml benzene. The characteristic yellow-green fluorescent product band was collected and the solvent evaporated at the boiling point until a volume of 20 ml was attained. To this solution was added 60 ml of hexane and the whole was chilled (ice) for 15 min. The resulting crystalline solid was collected by filtration yielding 965 mg (74%) of III as pink needles.

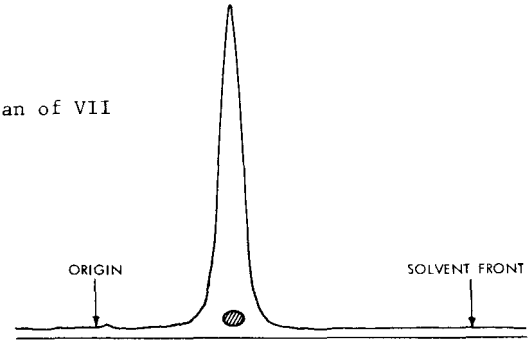
To a solution of 965 mg (1.90 mmole) of III in 100 ml of boiling carbon tetrachloride was added 350 mg (1.90 mmole) of N-bromosuccinimide and 10 mg of benzoyl peroxide. The stirred mixture was refluxed for 1 hr, cooled, and the succinimide removed by filtration. The filtrate was evaporated to dryness (in vacuo) and the residue was dissolved in 30 ml of hot xylene. The resulting solution was heated to boiling (nitrogen sweep) for 20 min, cooled, and concentrated (in vacuo) to a volume of 10 ml. Acetone, 15 ml, was added and the mixture was chilled (ice) for 1 hr yielding crude crystalline V which was collected by filtration, washed with cold acetone and dissolved in 20 ml of tetrahydrofuran (THF). To this solution was added 20 ml of methanol and 2 ml of 1 M sodium methoxide in methanol (2.00 mmoles). The mixture was heated for 5 min at 60°, 1 ml of water was added and the solution was concentrated to 20 ml. Additional water (40 ml) was added and the mixture was chilled (ice) to precipitate the product completely. The precipitate of crude VI was collected by filtration and dissolved in 30 ml

of hot THF. This solution was passed through a 2 cm x 2 cm column of charcoal and celite (1:9). The column was eluted with an additional 30 ml of ethanol and the combined eluates evaporated (in vacuo) to dryness. The residue was crystallized from 30 ml of 2% pyridine in ethanol yielding 233 mg (42%, based on III) of VI as light yellow plates, specific activity 53.9 mCi/mmole; TLC, R_f 0.40 silica gel/benzene:1-propanol (4:1). No detectable impurities were observed by TLC and a radiochromatogram scan indicated that the radiochemical purity was ≥ 98%.

(±)-7α,8β-Dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene-7-^{14}C (VII)

To a solution of 117 mg (0.41 mmole) of VI in 25 ml of THF was added 700 mg of m-chloroperbenzoic acid. After standing at room temperature for 1 hr, 75 ml of ether was added to the mixture and the resulting solution was extracted with 3 x 25 ml of cold 10% NaOH and 25 ml of water. The organic layer was dried (K_2CO_3), evaporated to dryness (in vacuo), and the residue dissolved in 25 ml of THF and triethylamine (19:1). This solution was subjected to column chromatography on silica gel (E. Merck), 1.5 cm x 10 cm (packed using THF:triethylamine, 19:1), eluting with additional THF:triethylamine (19:1). The first 10-ml fraction was discarded. To the following 30-ml fraction, which contained the product, was added 10 ml of dioxane and the mixture was evaporated at 40-50° in a nitrogen stream to a volume of 5 ml at which point crystallization occurred. Collection of the product by filtration gave 50 mg (40%, based on VI) of VII as white prisms, specific activity 53.9 mCi/mmole; TLC, R_f 0.50 silica gel/THF:triethylamine (19:1). The chemical and radiochemical purity was ≥ 98%, see the Figure below.

Figure -
Radiochromatogram Scan of VII



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