SPECIFICALLY TRITIATED ARENE OXIDES

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SUMMARY

Convenient methods have been developed for the preparation of specifically tritiated arene oxides. K-Region arene oxides of phenanthrene, benzo[a]anthracene and benzo[a]pyrene were prepared from tritiated cis dihydrodiols via the methoxy-dioxolane route and the non-K-region arene oxides, benzo[a]pyrene 7,8- and 9,10-oxide, were prepared via tritiated halohydrin esters.

INTRODUCTION

Arene oxides are now widely accepted as the initial oxidative metabolites of aromatic hydrocarbons in mammals [1]. The cytotoxic [2], mutagenic [3], and possibly carcinogenic [4] activity of these compounds has prompted widespread interest in the biochemistry of this class of compounds [cf. 1]. Radioactive arene oxides are invaluable in studying their binding to DNA, RNA, and protein as well as their detoxication by the enzymes epoxide hydrase and glutathione-S-epoxide transferase. Previously, tritiated arene oxides [5] were prepared from generally tritiated aromatic hydrocarbons by 1.) reaction with osmium tetroxide to form cis dihydrodiols,

2.) cleavage of the diols to dialdehydes, and 3.) cyclization to the desired arene

oxide [6]. This route is generally unsatisfactory in that the very cumbersome and

difficult reaction with osmium tetroxide must be conducted with radioactive mater-

ial. In addition, only K-region arene oxides can be prepared. The need for effici-

ent and general routes to labeled K-region and non-K-region arene oxides prompted

the present study.

DISCUSSION AND RESULTS

The initial synthetic route to K-region arene oxides via cyclization of dial-

dehydes [7] is not well suited to radiochemical synthesis in that there was no apparent

means to introduce radioactivity at a late point in the sequence, and intermediates

must be carefully purified at each stage. A more recent proceedure [8], consisting

of formation of methoxydioxolanes from cis dihydrodiols, conversion to halohydrin

acetates, and cyclization to the desired arene oxides, has the distinct advantage

that purification of intermediates is unnecessary. Furthermore, if radioactivity

could be introduced after the awkward step in which the cis dihydrodiol is formed

with osmium tetroxide, a highly convenient proceedure would be available. Formation

of cis dihydrodiols from polycyclic aromatic hydrocarbons with a bromine substitu-

ent at a non-K-region position was thus examined.

Oxidation of 3-bromophenanthrene, 7-bromobenzo[a]anthracene, and 6-bromobenzo[a]-

pyrene to cis dihydrodiols proceeded exceptionally smoothly. The bromodihydrodiols

are highly crystalline and are not succeptible to autoxidation as is the case for

the unsubstituted analogs [9,10]. Hydrogenolysis of the bromine substitutents with

tritium gas proceeded in high yield to provide the necessary tritiated dihydrodiols

for conversion to 3-^{3}H-phenanthrene 9,10-oxide, 7-^{3}H-benzo[a]anthracene 5,6-oxide,

and 6-^{3}H-benzo[a]pyrene 5,6-oxide. The sequence is illustrated for the benzo[a]-

anthracene 5,6-oxide in Scheme I.

The most efficient synthesis of non-K-region arene oxides is the halohydrin

ester route [11] as shown in Scheme II. Synthesis of the biochemically interesting

benzo[a]pyrene 7,8- and 9,10-oxides requires introduction of the label late in the

sequence. The aromatic ring of the halohydrin ester precursor was brominated

(route to 9,10-oxide shown) and conditions were developed for hydrogenolysis of the

aromatic bromine with tritium gas such that the sensitive halohydrin ester group was

SCHEME I
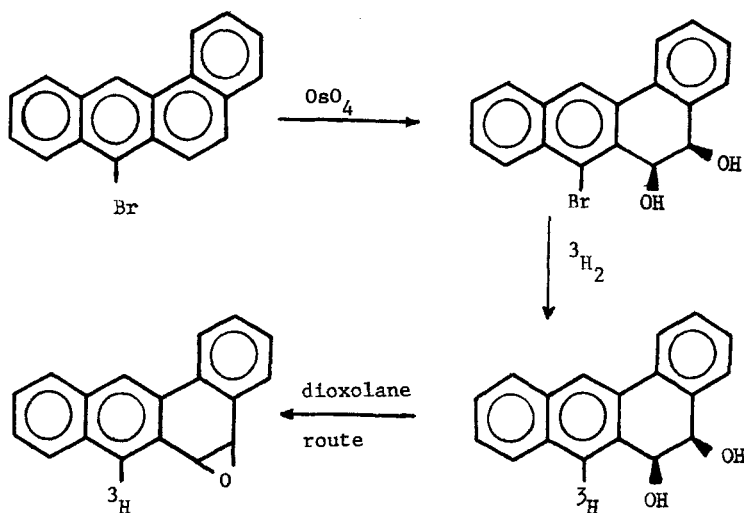
unaffected. Conversion to the desired arene oxides was as previously described [11]. Introduction of radioactivity at this late point in the synthesis has not added to the general difficulty in preparing highly reactive non-K-region arene oxides.

Introduction of radioactivity at specific ring positions in the aromatic portions of arene oxides precursors has been found a convenient proceedure for the preparation of specifically tritiated arene oxides. The labeled compounds are presently being employed to develop assays for the enzymes which metabolize arene oxides.

SCHEME II
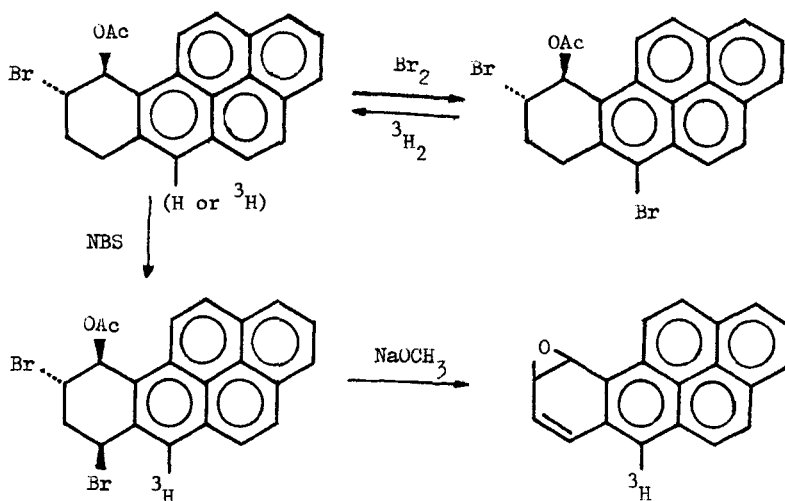
EXPERIMENTAL

Bromo cis Dihydrodiols 7-Bromobenzo[a]anthracene [12] and 6-bromobenzo[a]pyrene

[13] were prepared as previously described while 3-bromophenanthrene was the gift of

Dr. E. May at NIH [14]. Osmate esters of the dihydrodiols were formed by allowing the

hydrocarbons to react with equivalent amounts of osmium tetroxide in pyridine [9]

for 6 days at room temperature except for bromobenzanthracene which required a temp-

erature of 45°. The dihydrodiols were liberated from the osmate esters by reaction

with sodium bisulfite in 50% pyridine-water [15] and had the following properties:

cis-9,10-dihydroxy-9,10-dihydro-3-bromophenanthrene (90% yield, mp 159-152° from

benzene-ethyl acetate, nmr (acetone-d_6) of -C$\underline{H}$OH- 4.74 δ, Anal. ($C_{14}H_{11}BrO_2$) C,H);

cis-5,6-dihydroxy-5,6-dihydro-7-bromobenzo[a]anthracene (60% yield, mp 184-186°

from CCl$_4$, nmr (acetone-d_6) H$_5$=4.90 and H$_6$=5.53 δ with J$_{5,6}$=3.0 Hz, Anal. ($C_{18}H_{13}BrO_2$)

C,H); cis-4,5-dihydroxy-4,5-dihydro-6-bromobenzo[a]pyrene (91% yield, mp 225° (dec.)

from ethyl acetate, nmr (THF-d_8) H$_4$=5.10 and H$_5$=5.80 δ with J$_{4,5}$=4.0 Hz. A minor

amount ($\sim$10%) of an isomeric dihydrodiol was detected by tlc (silica gel, CHCl$_3$:

ethyl acetate, 3:1) from the reaction of 6-bromobenzo[a]pyrene, presumably the di-

hydrodiol at the 11,12 K-region.

Tritiated K-Region Arene Oxides. The brominated cis dihydrodiols were reduced

with tritium gas in the presence of 10% by weight of 10% Pd on carbon in tetrahydro-

furan solution containing 10% triethylamine. The reactions seemed to reach comple-

tion after 3 hrs, but were continued overnight. Conversion of the radioactive diols

to 3-^{3}H-phenanthrene 9,10-oxide, 7-^{3}H-benzo[a]anthracene 5,6-oxide, and 6-^{3}H-benzo[a]-

pyrene 4,5-oxide was as previously described [8]. Specific activities were $\sim$7mCi/mmole

which was about half the specific activity of the tritium gas. The arene oxides were

>98% radiochemically pure as judged by tlc on silica gel with benzene: chloroform:

ethyl acetate (1:1:1) containing 5% triethylamine as solvent.

6-^{3}H-Benzo[a]pyrene 9,10-Oxide. To a solution of 10-acetoxy-9-bromo-7,8,9,10-

tetrahydrobenzo[a]pyrene [11] (480 mg, 1.22 mmol), lithium acetate (1g), and ferric

chloride (20 mg) in 180 ml of acetic acid was added a solution of bromine (195 mg,

1.22 mmol) in 1.57 ml of acetic acid over a period of 15 min. with stirring at room

temperature. The reaction mixture was further stirred for 30 min and poured into

water (400 ml). The resulting solid was crystallized from benzene to provide 10-

acetoxy-6,9-dibromo-7,8,9,10-tetrahydrobenzo[a]pyrene: colorless needles (560 mg,

97%), mp 175-176°; pmr (100 MHz, CDCl$_3$) OCOCH$_3$ 2.10, 2H$_8$=2.20-2.90, 2H$_7$=3.20-3.80,

1H$_9$=4.82 (J$_{9,10}$=J$_{9,8}$=J$_{9,8'}$=3.0 Hz), 1H$_{10}$=7.05 (J$_{9,10}$=3.0 Hz), 6 aromatic protons=7.8-8.3,

and H$_5$=8.58 (J$_{4,5}$=9 Hz) δ; mass spectrum (electron impact, NO-N$_2$, m/e) M$^+$ (470, 472,

<u>Anal</u>. (C$_{22}$H$_{16}$Br$_2$O$_2$) C,H. Exact assignment of the position of substitution by bromine

in this dibromide, as well as that leading to the labeled 7,8-oxide in the next sec-

tion, is not possible from the pmr spectrum. Substitution by bromine at a K-region

position should result in a downfield singlet in the aromatic region. This is not

observed for either precursor. Instead, both brominated precursors show downfield

doublets indicative of bromine substitution adjacent to the K-region (i.e., positions

1, 3, or 6). The presence of meta coupling (J=3.4 Hz) for the signals tentatively

assigned for H$_1$ and H$_3$ strongly suggest the dibromides are substituted at C-6 of

the aromatic ring. A mixture of the dibromide (421 mg, 0.89 mmol), sodium

acetate (200 mg), and tetrahydrofuran (50 ml) was reduced with tritium gas in the

presence of 10% palladium on carbon for 4 days. Conventional work up provided

6-^{3}H-10-Acetoxy-9-bromo-7,8,9,10-tetrahydrobenzo[a]pyrene: recrystallized from

chloroform-petroleum ether to give colorless needles (346 mg, 98%), mp 162-164°;

specific activity, 85μCi/μmole. Conversion to 6-^{3}H-benzo[a]pyrene 9,10-oxide of

the same specific activity was as previously described [11].

6-^{3}H-Benzo[a]pyrene 7,8-Oxide. To a stirred mixture of 8-bromo-7-trifluoro-

acetoxy-7,8,9,10-tetrahydrobenzo[a]pyrene [11] (447 mg, 1 mmol) and acetic acid

(40 ml) was added a solution of bromine (160 mg, 1 mmol) in acetic acid (5 ml).

The reaction mixture was stirred at room temperature for 18 hr. and poured into

water (200 ml). The resulting solid was recrystallized from chloroform-petroleum

ether to provide 6, 8-Dibromo-7-trifluoroacetoxy-7,8,9,10-tetrahydrobenzo[a]pyrene:

colorless prisms (500 mg, 95%), mp 120-122°; pmr (100 MHz, CDCl$_3$) 2H$_9$=2.20-3.00,

H$_{10}$=3.52 (A$_2$B$_2$ triplet, J$_{9,10}$=J$_{9',10}$=6.5 Hz), H$_8$=4.70 (J$_{8,9}$=J$_{8,9'}$=3.0 and J$_{7,8}$=

4.0 Hz), H$_7$=6.68, 6 aromatic protons=7.6-8.3, and H$_5$=8.25 (J$_{4,5}$=10 Hz) δ: mass

spectrum (electron impact, NO-N$_2$, m/e) M$^+$ (524, 526, and 528); <u>Anal</u>. (C$_{22}$H$_{13}$Br$_2$F$_3$O$_2$)

C,H. A mixture of the dibromide (100 mg, 0.19 mmol), sodium acetate (50 mg), and

tetrahydrofuran (20 ml) was reduced with tritium gas in the presence of 10% palladium

on calcium carbonate (50 mg) for 25 hr. Conventional work up provided 6-^{3}H-8-bromo-

7-trifluoroacetoxy-7,8,9,10-tetrahydrobenzo[a]pyrene: colorless needles from chloro-

form-ethanol (75 mg, 88%), mp 169-170°; specific activity, 28.9 μCi/μmol. Some runs

required treatment of the sample with trifluoroacetic anhydride prior to recrystal-

lization. Conversion to 6-^{3}H-benzo[a]pyrene 7,8-oxide was as previously described

[11]. A 30% decrease in specific activity was associated with these steps. Inspec-

tion of the 100 MHz pmr spectrum of the aromatic ring brominated precursor for the

tritiation step did not allow a clear assignment to the position of the aromatic

bromine. Ring positions 1,3, or 6 appear possible, with C-6 as the most likely

position of substitution.

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