SYNTHESIS OF HIGH SPECIFIC ACTIVITY BENZO[*a*]PYRENE-6-*t* AND ITS K-REGION OXIDIZED DERIVATIVES

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SUMMARY

The potent carcinogen benzo[*a*]pyrene specifically tritiated in the 6-position was synthesized through hydrogenation of 6-bromobenzo[*a*]pyrene with tritium gas. The benzo[*a*]pyrene-6-*t* was then employed in the synthesis of benzo[*a*]pyrene-6-*t* 4,5-oxide of high specific activity *via* the corresponding *cis*-diol, quinone and *trans*-diol. Also, BaP-6-*d* and BaP-G-*t* were employed in the synthesis of the corresponding K-region oxidized derivatives (*cis*- and *trans*-diols, quinones, and oxides). Loss of the isotopic label was generally minimal except in the transformations involving the high specific activity BaP-6-*t* compounds.

INTRODUCTION

Recent evidence suggests that carcinogenic polycyclic hydrocarbons are activated metabolically (1,2). However, the nature of the active metabolite(s) and the positions in the hydrocarbon molecule involved in binding to cellular macromolecules are still unknown. The low level of *in vivo* binding requires the use of radioactivity, and hydrocarbons randomly labelled with tritium have been employed in studies of metabolism and binding (3-7).

This study is directed towards synthesis of high specific activity tritiated benzo[*a*]pyrene 4,5-oxide required for studies on the involvement of this intermediate in the binding of BaP to DNA *in vivo* (8). The preparation of the parent hydrocarbon, BaP[1], specifically labelled in the 6-position through reaction of the corresponding Grignard reagent with tritiated water was recently described (9). However, the level of radioactivity was too low to provide accurate data at the low levels of binding generally encountered in *in vivo* studies (3). Accordingly, an alternative method capable of providing much higher specific activities was sought.

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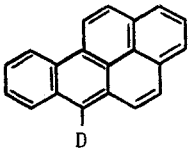
[1]Abbreviations: BaP, benzo[*a*]pyrene; THF, tetrahydrofuran; DMF, dimethylformamide; TNF, 2,4,7-trinitrofluorenone.

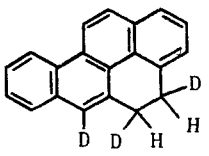
EXPERIMENTAL

Materials and methods: 6-Bromo-BaP was synthesized from BaP by the published procedure (10), chromatographed on neutral alumina, and recrystallized from benzene (mp 222-223°). BaP was purchased from Koch-Light. The BaP-G-*t* and the BaP-6-*t* (synthesized from 6-Br-BaP according to the procedure described in the paper) were purchased from Amersham-Searle Corp. All solvents were freshly distilled. Nmr spectra were obtained on Varian T-60 and Bruker HX 270 spectrometers. The radioactive samples were counted in a solution of 40 ml Packard Permafluor per liter toluene on a Packard Tricarb liquid scintillation spectrometer, Model 3330. All reactions were conducted under a nitrogen atmosphere, and precautions were taken to maintain anhydrous conditions.

Benzo[a]pyrene-6-d: 6-Br-BaP (1.3 g, 4.2 mmol) partially dissolved in DMF (150 ml) and triethylamine (30 ml) was stirred for 18 hr at ambient temperature in the presence of a 10% palladium/charcoal catalyst in an atmosphere of deuterium gas. The catalyst was removed by filtration through Filter-Cel, washed with 30 ml $CHCl_3$ and the filtrate and washings concentrated to dryness *in vacuo*. The residue was taken up in 50 ml dry benzene, filtered, and reconcentrated to give 800 mg (75%) of BaP-6-*d*. Tlc on silica gel in benzene-hexane (2:1) developed with TNF gave a greenish-black spot (R_f 0.55) for BaP-6-*d* (I) with only a trace of the corresponding 4,5-dihydro-BaP-6-*d* (II) (R_f 0.58, orange; nmr, benzylic δ 3.43). The 270 MHz nmr spectrum was



I



II

essentially that of BaP except for the absence of the characteristic singlet at δ 8.42 for the 6-proton. Chromatography on Florisil eluted with hexane and recrystallization from benzene-hexane afforded pure BaP-6-*d* as yellow needles, mp 179.5-180°.

Substitution of other solvents for DMF-trimethylamine afforded a higher proportion of *II*; in benzene or ethyl acetate the latter became the predominant product. Regioselective hydrogenation of BaP in the 4,5-bond is somewhat surprising in view of the report that hydrogenation over a Pt catalyst affords 7,8,9,10-tetrahydro-BaP as the principal product (II).

Benzo[a]pyrene-6-t: Reaction of 6-Br-BaP (80 mg, 0.24 mmol) according to the above procedure appropriately scaled down afforded BaP-6-*t* which was diluted with cold BaP (580 mg) and taken up in 60 ml of pure benzene. This material

(total activity = 5 Ci; specific activity = 1.97 Ci/mmol) was employed without further purification in subsequent experiments. However, if BaP-6-*t* is itself to be employed in biological experiments, further purification by the chromotographic method described for BaP-6-*d* is recommended. Chromatography on 45% acetylated cellulose using ethanol - methylene dichloride (2:1) has also been found to be effective (12).

Cis-4,5-Dihydrobenzo[a]pyren-4,5-diol-6-t: The procedure was adapted from that described for the preparation of the unlabelled *cis*-diol (method B) (13) with appropriate modification and precautions for handling the radioactive materials. Reaction of the solution of BaP-6-*t* (56.8 ml plus cold BaP to bring total BaP to 1g) with OsO_4 (1g) in 14 ml pyridine over 5 days furnished the osmate ester. Decomposition with sodium bisulfite and precipitation with water (125 ml) furnished the crude *cis*-diol. Purification of the latter was accomplished *via* the diacetate which was dissolved in 20 ml of benzene and chromatographed on Florisil (15 g) in hexane. Pure *cis*-diol diacetate (460 mg) was obtained as a white solid on elution with 1200 ml of benzene. Tlc on silica gel in ethyl acetate-benzene (1:9) gave a single fluorescent spot. Specific activity was 1.77 Ci/mmol.

Conversion to the *cis*-diol took place smoothly on bubbling ammonia into a solution of the diacetate in methanol (200 ml) for 2 hr at 0°. Addition of ice-water (300 ml) gave a white precipitate which was separated by filtration and dried to afford the pure *cis*-diol (289 mg). Tlc on silica gel in ethyl acetate-benzene (1:9) gave a single spot whose R_f matched that of the *cis*-diol of BaP.

Benzo[a]pyren-4,5-dione-6-t: To the pure dry *cis*-diol was added consecutively triethylamine (2 ml), DMSO (16 ml), and pyridine-SO_3 (1.6 g), and the resulting solution stirred at room temperature for 30 min. Addition of icewater (30 ml) precipitated the tritiated quinone (284 mg). Tlc on silica gel in ethyl acetate-benzene (1:9) gave a single red spot whose R_f matched that of BaP 4,5-dione.

Trans-4,5-Dihydrobenzo[a]pyren-4,5-diol-6-t: The BaP-6-*t* 4,5-dione diluted with twice the weight (568 mg) of cold quinone was extracted in a Soxhlet apparatus into a solution of $LiAlH_4$ (1 g) in refluxing anhydrous ether (400 ml) over 2 days. In some experiments longer times were required for complete extraction. Workup by the conventional procedure (13) gave the crude *trans*-diol which, like the *cis*-diol, was purified through formation of the diacetate and chromatography on Florisil. The pure *trans*-diol diacetate (816 mg) was obtained as white solid on elution with benzene (200 ml) and ether (800 ml). The purity was confirmed by its 60 MHz nmr spectrum and tlc on silica gel in comparison with the pure unlabelled diacetate. Specific activity was 524 mCi/mmole.

Conversion to the *trans*-diol was effected by ammonolysis in methanol essen-

tially according to the procedure for the *cis*-isomer. The *trans*-diol was obtained as a pale pink solid (566 mg). Tlc on silica gel in ethyl acetate-benzene (1:9) gave a single spot.

4,5-Epoxy-4,5-dihydrobenzo[a]pyrene-6-t: Reaction of the *trans*-diol (566 mg) with the dimethylacetal of DMF (450 mg) in DMF (4.2 ml) and THF (7 ml) at reflux for 7 hr, followed by workup according to the procedure described (13) gave the pure BaP-6-*t* 4,5-oxide (232 mg). Tlc on low activity neutral alumina in ethyl acetate-benzene (1:9) gave a single spot with R_f corresponding to that of the pure 4,5-oxide. Specific activity was 400 mCi/mmole.

4,5-Epoxy-4,5-dihydrobenzo[a]pyrene-6-d: The BaP-6-*d* was transformed through the *cis*-4,5-dihydrodiol, the 4,5-dione, and the *trans*-4,5-dihydrodiol to BaP-6-*d* 4,5-oxide following the methods employed for the synthesis of the analogous tritiated derivatives. The 270 MHz nmr spectra of each of these products corresponded closely with those of the unlabelled K-region oxidized derivatives of BaP, except for the virtual absence of the singlet peak corresponding to the proton in the 6-position. Within the limits of the analytical method the overall loss of the deuterium label was <6%.

4,5-Epoxy-4,5-dihydrobenzo[a]pyrene-G-t: Cold BaP (1g) was added to a solution of BaP-G-*t* (specific activity 25 Ci/mmol, 5 mCi in 1 ml benzene) labelled by a conventional exchange procedure. This solution was then employed in the synthesis of BaP-G-*t* 4,5-oxide via the same sequence of reactions utilized in the synthesis of the specifically tritiated and deuterated analogs. Dilution was made at the quinone stage by addition of an equal weight (420 mg) of cold BaP-4,5-dione. The specific activity of the pure BaP-G-*t* 4,5-oxide was 0.59 mCi/mmol, and the overall percentage loss of radiactivity was only 6.3%.

DISCUSSION

Synthesis of BaP-6-*t* of high specific activity and its transformation into BaP-6-*t* 4,5-oxide *via* the corresponding *cis*- and *trans*-diols and quinone was conveniently achieved by the procedures described.

The specificity of tritium incorporation in the 6-position of BaP-6-*t* is of some importance for subsequent biological experiments. Determination by chemical means, e.g. iodination (9), requires assumptions regarding regiospecificity and efficiency of reaction which may not be entirely justified. The 270 MHz nmr spectral analysis of the BaP-6-*d* obtained in the parallel experiment with deuterium in place of tritium gas provides a more accurate direct experimental measurement. On this basis, it is estimated that 94-96% of the isotopic label is present in the 6-position. Direct tritium nmr analysis has also been performed on a sample of BaP-6-*t* synthesized by the method described

herein; there were two signals in the tritium spectrum which indicated that 96% of the tritium was in the 6-position with the remaining 4% in the 1-position. The small discrepancy between the two analytical methods probably reflects experimental variation, e.g. differences in catalyst activity, pressure, temperature, time, selective enrichment during purification, etc.

In principle, BaP-6-*t* derivatives of considerably higher specific activity than described herein are attainable, since a 20 fold dilution was made at the BaP-6-*t* stage and an additional 3-fold dilution at the quinone stage. The stability of the labelled compounds under the conditions employed may, of course, be expected to provide a practical limitation. Since the BaP-6-*t* 4,5-oxide, 400 mCi/mmole, in benzene solution stored under nitrogen in a freezer exhibited little significant decomposition over several weeks it is likely that considerably higher levels of tritium are possible.

The overall loss of 38% of the radioactivity in the high spec. activity BaP-6-*t* series contrasts with the minimal loss of the isotopic label observed in the analogous BaP-6-*d* and BaP-G-*t* series. Thus, the decrease in activity associated with the BaP-6-*t* series appears more a function of their high specific activity than an inherent feature of the chemical transformation involved. While it was impractical to assess the change in activity at all stages, due to the low solubility of the free diols and the quinones in the counting solution, it appears that the principal losses occured during operations involving prolonged heating and/or strongly basic media. Tentatively, we assume that the stability of the BaP-6-*t* derivatives is unlikely to pose any serious problems in biochemical experiments conducted under mild conditions.

Studies in progress on the binding of BaP-6-*t* 4,5-oxide to nucleic acids and on the metabolism and binding of BaP-6-*t* in cells in culture (14) appear to confirm this assumption.

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REFERENCES

1. Heidelberger, C., Adv. Cancer Res. 18, 317-366 (1973).
2. Sims, P., Adv. Cancer Res. 20, 165-274 (1974).
3. Duncan, M.E., and Brookes, P., Int. J. Cancer, 6, 496-505 (1970).
4. Diamond, L., Int. J. Cancer, 8, 451-462 (1971).
5. Huberman, E., Selkirk, J., and Heidelberger, C., Cancer Res. 31, 2161-2167 (1971).
6. Wang, I., Rasmussen, R., and Crocker, T., Biochem. Biophys. Res. Commun. 49, 1142-1149 (1972).
7. Huberman, E., and Sachs, L., Int. J. Cancer, 11, 412-418 (1973).
8. Baird, W., Harvey, R.G., and Brookes, P., Cancer Res., 35, 54-57 (1975).

9. Warshawsky, D., and Calvin, M., Biochem. Biophys. Res. Commun. 63, 541-547 (1975)
10. Dewhurst, F., and Kitchen, D., J. Chem. Soc. Perkin I, 710-712 (1972).
11. Lijinsky, W., and Zechmeister, L., J. Amer. Chem. Soc. 75, 5495-5497 (1953).
12. Evans, A.E., personal communication.
13. Harvey. R.G., Goh, S.H., and Cortez, C., J. Amer. Chem. Soc. 97, 3468-3479 (1975).
14. King, H.W.S., Thompson, M.H., Osborne, M.R., Harvey, R.G., and Brookes, P., Biol. Interact., submitted.