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# Identification of Mutagenic Dihydrodiols as Metabolites of Benzo(*j*)fluoranthene and Benzo(*k*)fluoranthene[1]

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## ABSTRACT

The metabolism of the environmental agents benzo(*j*)-fluoranthene and benzo(*k*)fluoranthene was investigated using supernatants from the livers of Aroclor 1254-pretreated rats, which are effective in activating benzo(*j*)fluoranthene and benzo(*k*)fluoranthene to metabolites mutagenic toward *Salmonella typhimurium* TA 100. Six bands of metabolites of benzo(*j*)fluoranthene were separated by high-pressure liquid chromatography, and each band was tested for mutagenicity toward *S. typhimurium* TA 100 with activation. The major mutagenic band contained two dihydrodiols, one of which was identified as 9,10-dihydro-9,10-dihydroxybenzo(*j*)fluoranthene by comparison to a synthetic reference standard. 9,10-Dihydro-9,10-dihydroxybenzo(*j*)fluoranthene was mutagenic toward *S. typhimurium* TA 100 with activation, presumably as a result of conversion to the corresponding dihydrodiol-epoxide. The major dihydrodiol metabolite of benzo-(*k*)fluoranthene was identified, by comparison to a synthetic standard, as 8,9-dihydro-8,9-dihydroxybenzo(*k*)fluoranthene. This dihydrodiol, which could also be converted to a dihydrodiol-epoxide, was mutagenic toward *S. typhimurium* TA 100 with activation. The results of this study indicate that metabolism to dihydrodiols is one pathway in the activation of benzo(*j*)fluoranthene and benzo(*k*)fluoranthene to ultimate mutagens for *S. typhimurium* TA 100.

## INTRODUCTION

Benzofluoranthenes such as benzo(*j*)fluoranthene and benzo(*k*)fluoranthene (Chart 1) are among the most commonly detected environmental polynuclear aromatic hydrocarbons, occurring in gasoline engine exhaust, urban air, cigarette smoke, soil, water, and broiled and smoked foods (2, 5, 6, 18). The levels of these compounds in the environment are comparable to those of benzo(*a*)pyrene. Benzo(*j*)fluoranthene, benzo(*k*)fluoranthene, and the related isomers benzo-(*b*)fluoranthene and benzo(*g,h,i*)fluoranthene are mutagenic toward *Salmonella typhimurium* TA 100 with activation (9). Benzo(*j*)fluoranthene and benzo(*b*)fluoranthene have been shown to be complete carcinogens on mouse skin, whereas benzo(*k*)fluoranthene was inactive at the same dose (9, 17). Benzo(*k*)fluoranthene was carcinogenic when tested by s.c. injection in mice (8).

Despite the ubiquitous occurrence of benzofluoranthenes in the environment, little is known about their metabolic activation. Only one such study has appeared in which the binding of dibenzo(*a,e*)fluoranthene to nucleic acids was investigated (14). In the present study, we have evaluated the potential of B(j)F-9,10-dihydrodiol[4] and B(k)F-8,9-dihydrodiol (Chart 1) to contribute to the mutagenicity of benzo(*j*)fluoranthene and benzo(*k*)fluoranthene toward *S. typhimurium* TA 100.

## MATERIALS AND METHODS

**Apparatus.** High-pressure liquid chromatography was performed with a Waters Associates, Inc., Model ALC/GPC-204 high-speed liquid chromatograph equipped with a Model 6000A solvent delivery system, a Model 660 solvent programmer, a Model U6K septumless injector, a Model 440 UV/visible detector using 254 nm, and Columns 1 (3.9-mm × 30-cm $C_{18}$-μBondapak; Waters Associates, Inc., Milford, Mass.) and 2 (4.6-mm × 250-mm LiChrosorb RP-18, 10 μm; EM Reagents, Cincinnati, Ohio). Solvent programs were as follows: benzo(*j*)fluoranthene metabolites, Column 1, 50% methanol in $H_2O$ for 20 min, then linear to 80% methanol in $H_2O$ in 40 min at 2 ml/min; benzo(*k*)fluoranthene metabolites, Column 2, 50% methanol in $H_2O$ for 15 min, then linear to 100% methanol in 60 min at 2 ml/min. UV spectra were run on a Cary Model 118 spectrometer. Mass spectrometry was done with a Hewlett-Packard Model 5982A instrument.

**Chemicals.** Benzo(*j*)fluoranthene and benzo(*k*)fluoranthene were synthesized (3, 13) as were B(j)F-9,10-dihydrodiol and

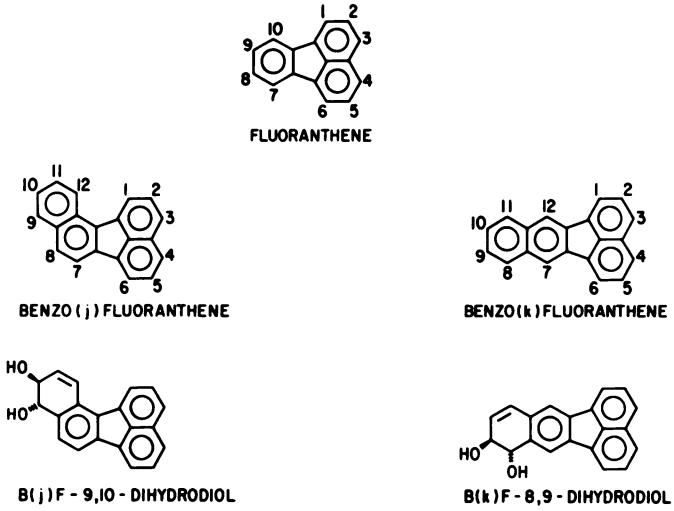


Chart 1. Structures of fluoranthene, benzo(*j*)fluoranthene, benzo(*k*)-fluoranthene, B(j)F-9,10-dihydrodiol, and B(k)F-8,9-dihydrodiol.

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[4] The abbreviations used are: B(j)F-9,10-dihydrodiol, 9,10-dihydro-9,10-dihydroxybenzo(*j*)fluoranthene; B(k)F-8,9-dihydrodiol, 8,9-dihydro-8,9-dihydroxybenzo(*k*)fluoranthene; TCPO, 1,1,1-trichloropropene oxide.



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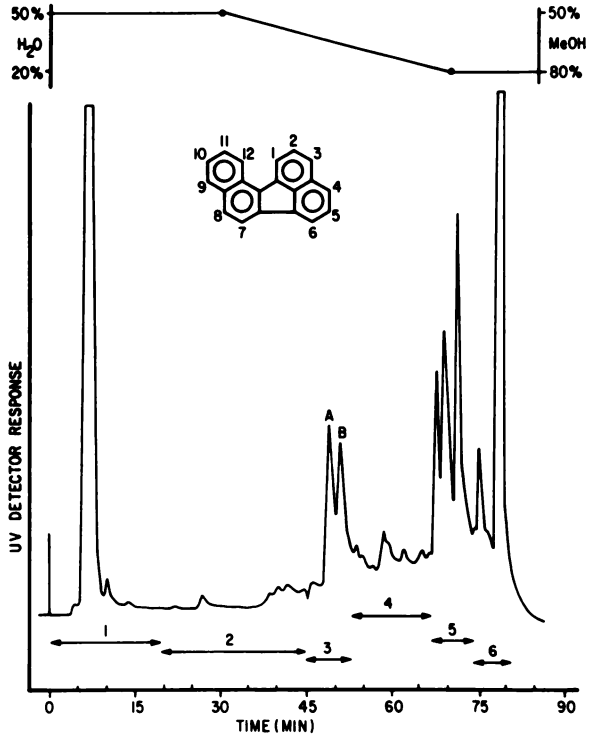


Chart 2. High-pressure liquid chromatogram of metabolites formed from benzo(*j*)fluoranthene upon incubation with rat liver 9000 × *g* supernatant. Metabolite Bands 1 to 6 were assayed for mutagenicity (see Table 1). *Peaks A* and *B* are mutagenic dihydrodiols; *Peak B* is B(j)F-9,10-dihydrodiol.

Table 1

*Mutagenicity of benzo(j)fluoranthene metabolites*

Each band was collected and after concentration was redissolved in 150 μl dimethyl sulfoxide for assay; 50 μl of each solution were applied to each of 2 replicate plates. Dimethyl sulfoxide control; 140 His revertants/plate.

| Metabolite band[a] | *S. typhimurium* TA 100[b] $His^+$ revertants/plate |
|---|---|
| 1 | 177 ± 5 |
| 2 | 275 ± 25 |
| 3 | 662 ± 16 |
| A | 465 ± 19 |
| B | 767 ± 25 |
| 4 | 265 ± 7 |
| 5 | 207 ± 25 |
| 6 | 2[illegible] ± 5 |

[a] See Chart 2 for metabolite bands.
[b] Metabolites formed from 2.5 mg benzo(*j*)fluoranthene.

B(k)F-8,9-dihydrodiol.[5] Benzofluoranthenes and their metabolites were purified by high-pressure liquid chromatography prior to mutagenicity assays. $NADP^+$ and glucose-6-phosphate were obtained from Sigma Chemical Co., St. Louis, Mo. TCPO was obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis. Aroclor-1254 was procured from Analabs, Inc., New Haven, Conn.

**Metabolism *in Vitro*.** The *in vitro* metabolism studies were performed using the identical S-9 mix used in the mutagenicity assays. The S-9 mix contained, per ml, 100 μmol of potassium phosphate buffer (pH 7.4), 8.0 μmol of $MgCl_2$, 1.65 μmol of KCl, 5.0 μmol of glucose-6-phosphate, 4.0 μmol of NADP, and 0.5 ml of S-9 fraction. Incubations were performed at 37° for 20 min using a 25-ml Erlenmeyer flask to which were added 250 μg of compound in 10 μl of dimethyl sulfoxide and 2 ml of S-9 mix. The effect of epoxide hydrase inhibition was examined

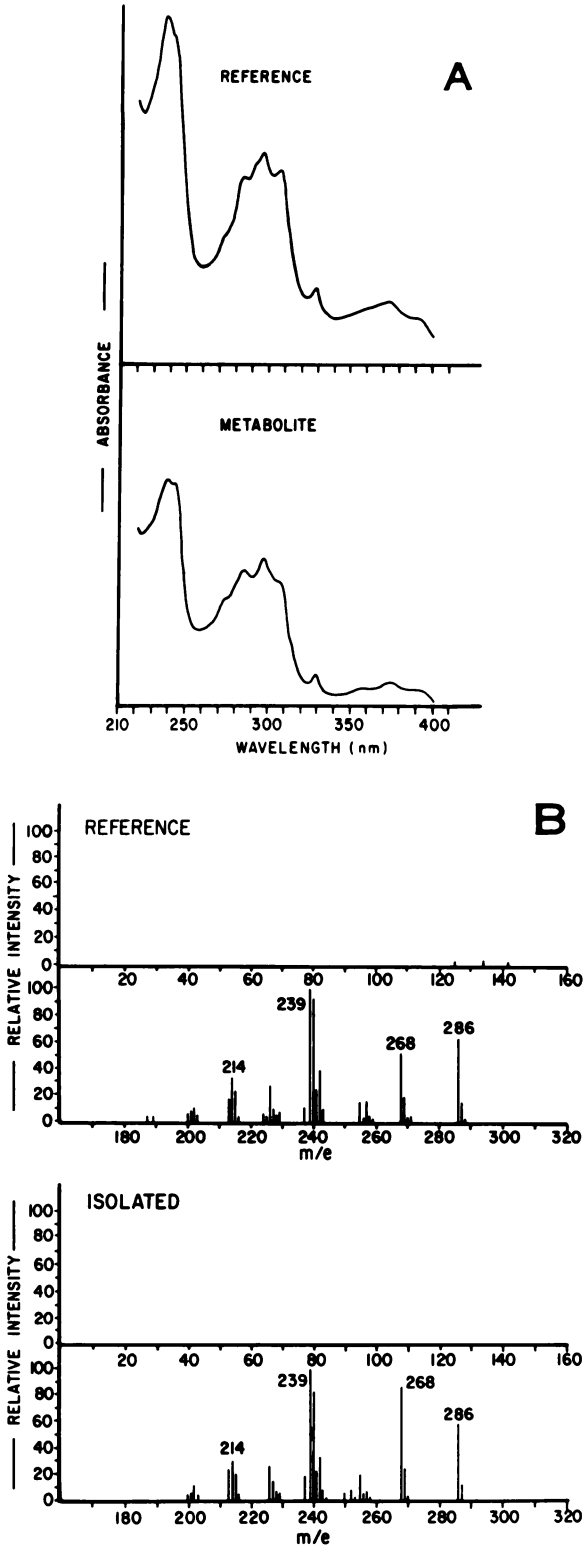


Chart 3. UV and mass spectra of synthetic B(j)F-9,10-dihydrodiol and isolated dihydrodiol metabolite of benzo(*j*)-fluoranthene.

[5] S. Amin, V. Bedenko, E. LaVoie, S. S. Hecht, D. Hoffmann. Syntheses of dihydrodiols as potential proximate carcinogens of benzofluoranthenes, submitted for publication to *Journal of Organic Chemistry*.



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by using $2.0 \times 10^{-3}$ M TCPO in these incubation mixtures. The incubations were terminated by addition of 2 ml of ice-cold acetone. The mixture was then extracted 5 times with 10-ml aliquots of ethyl acetate. The ethyl acetate solution was concentrated under reduced pressure, below 40° prior to analysis or purification of the metabolites by reverse-phase high-pressure liquid chromatography.

For isolation of the metabolite bands for mutagenicity assays, 2.5 mg of benzo(*j*)fluoranthene were incubated with 20 ml of S-9 mix. After extraction, bands were collected from the entire sample by repetitive injections on Column 1. Each band was concentrated by evaporation under reduced pressure at 30°, and the residue was dissolved in 150 μl of dimethyl sulfoxide. For mutagenicity assays, 50 μl were added to each of 2 plates for each band.

**Mutagenicity Assays.** Mutagenicity studies were performed as previously described (4) using *S. typhimurium* TA 100 (TA 1535/pK*m* 1101) provided by Dr. Bruce Ames of the University of California, Berkeley, Calif. The S-9 fraction used in both mutagenicity assays and in metabolic studies was obtained from the livers of male Fischer 344 rats weighing 300 to 500 g which had been treated 5 days prior to sacrifice with Aroclor-1254 (500 mg/kg).

## RESULTS AND DISCUSSION

A high-pressure liquid chromatogram of the metabolites of benzo(*j*)fluoranthene formed *in vitro* is shown in Chart 2. Six bands of metabolites were collected and tested for mutagenicity toward *S. typhimurium* TA 100 with activation. The results

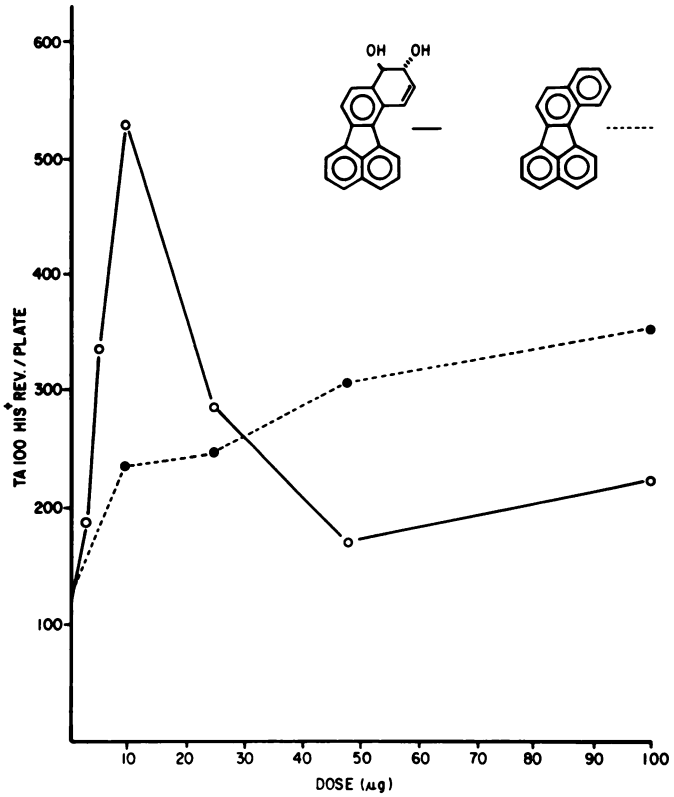


Chart 4. Mutagenicity toward *S. typhimurium* TA 100 of B(j)F-9 (○), 10-hydrodiol and benzo(*j*)fluoranthene (●) in the presence of rat liver 9000 × *g* supernatant.

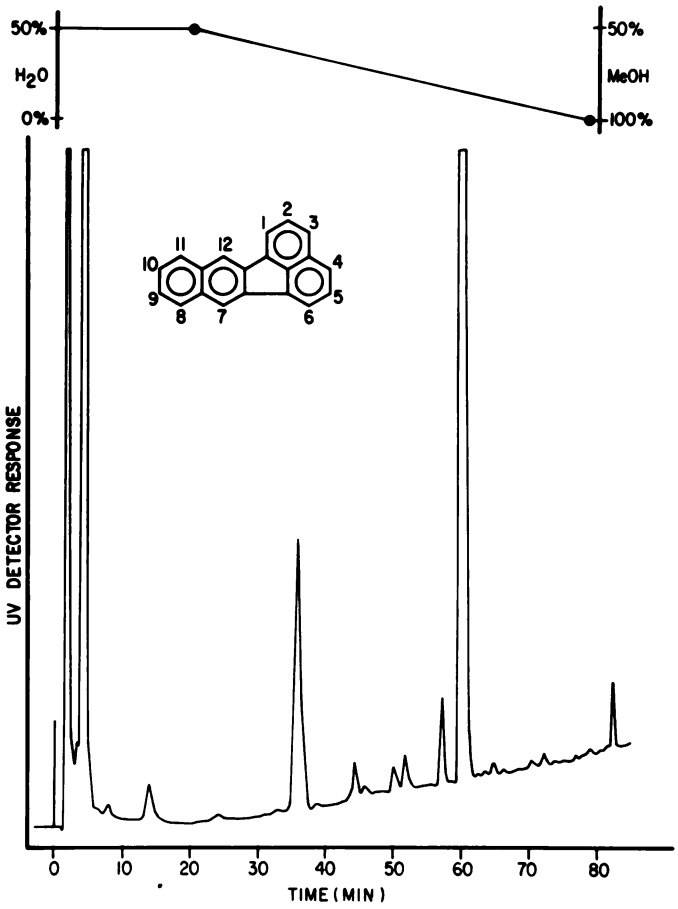


Chart 5. High-pressure liquid chromatogram of metabolites formed from benzo(*k*)fluoranthene upon incubation with rat liver 9000 × *g* supernatant. The *peak* eluting at 36 min is B(k)F-8,9-dihydrodiol.

are summarized in Table 1. Band 3 had the highest mutagenic activity, suggesting that this band contained proximate mutagenic metabolites of benzo(*j*)fluoranthene. When tested separately, each of Peaks A and B of Band 3 was mutagenic (Table 1).

For structural elucidation, incubations were carried out in the presence of the epoxide hydrase inhibitor TCPO. Peaks A and B of Band 3 disappeared, indicating that they were dihydrodiols. This was confirmed by mass spectrometry. Synthetic B(j)F-9,10-dihydrodiol had the identical retention volume on high-pressure liquid chromatography as Peak B. The UV and mass spectra of Peak B were also identical to synthetic B(j)F-9,10-dihydrodiol as shown in Chart 3A and 3B, confirming the identity of this mutagenic metabolite. The structure of the other mutagenic dihydrodiol (Peak A) is not known. The comparative mutagenic activities toward *S. typhimurium* TA 100 of B(j)F-9,10-dihydrodiol and benzo(*j*)fluoranthene are illustrated in Chart 4, which indicates that B(j)F-9,10-dihydrodiol is one proximate mutagen of benzo(*j*)fluoranthene.

A high-pressure liquid chromatogram of the metabolites formed from benzo(*k*)fluoranthene *in vitro* by rat liver 9000 × *g* supernatant is shown in Chart 5. The relative simplicity of the metabolic pattern may be a consequence of the symmetry of benzo(*k*)fluoranthene. The major metabolite, eluting at 36 min, disappeared when incubations were done in the presence of TCPO, while the minor peaks eluting at 45 to 55 min increased



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in intensity. Thus, the former was a dihydrodiol while the latter were probably phenolic metabolites. Synthetic B(k)F-8,9-dihydrodiol was shown to have the same retention volume on high-pressure liquid chromatography as this metabolic dihydrodiol. The metabolic dihydrodiol also had identical UV spectrum and mass spectrum as illustrated in Chart 6A and 6B to synthetic

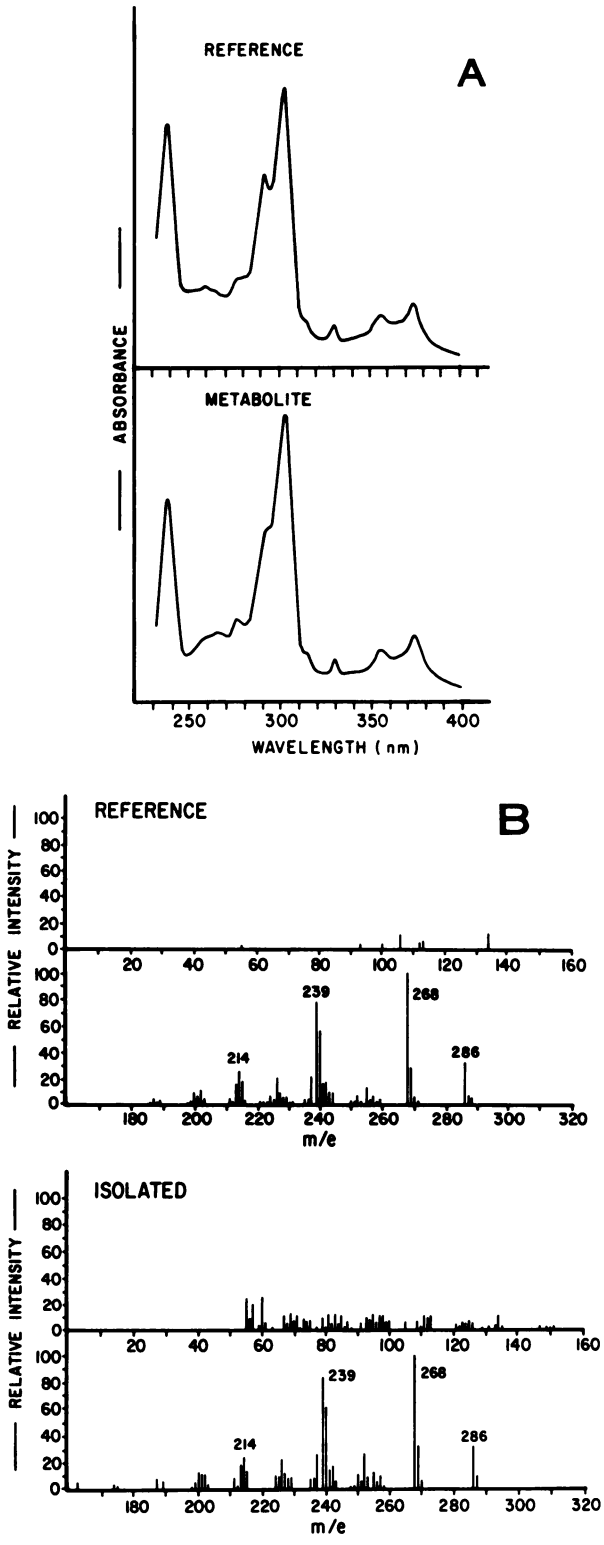


Chart 6. UV and mass spectra of synthetic B(k)F-8,9-dihydrodiol and isolated dihydrodiol metabolite of benzo(*k*)fluoranthene.

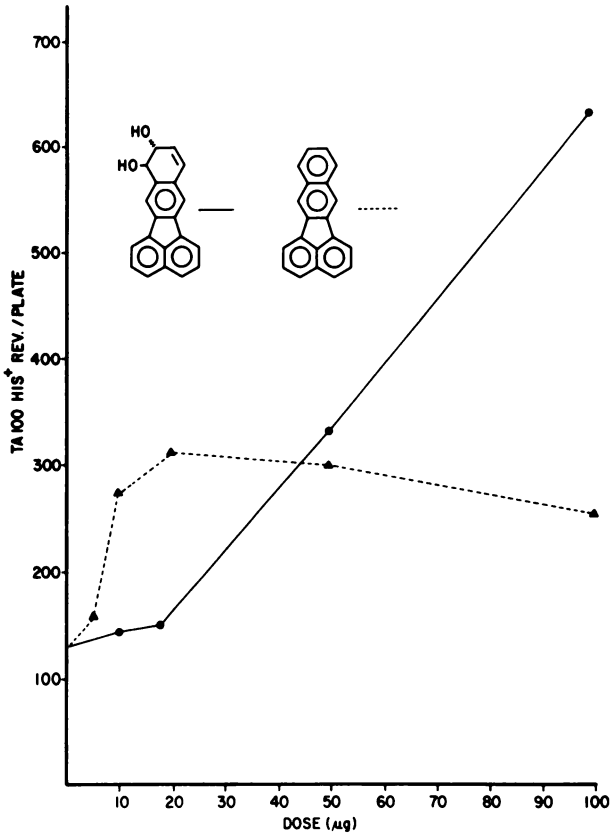


Chart 7. Mutagenicity toward *S. typhimurium* TA 100 of B(k)F-8,9-dihydrodiol (●) and benzo(*k*)fluoranthene (▲) in the presence of rat liver 9000 × *g* supernatant.

B(k)F-8,9-dihydrodiol, confirming its identity. The comparative mutagenicity of synthetic B(k)F-8,9-dihydrodiol and benzo(*k*)fluoranthene toward *S. typhimurium* TA 100 with activation is shown in Chart 7.

The results of this study indicate that dihydrodiols are proximate mutagenic metabolites of benzo(*j*)fluoranthene and benzo(*k*)fluoranthene. B(j)F-9,10-dihydrodiol and B(k)F-8,9-dihydrodiol were mutagenic only in the presence of rat liver homogenate, indicating that their mutagenic activities resulted from conversion to dihydrodiol-epoxides, as observed with other hydrocarbons (1, 7, 11, 12, 15, 16). Other metabolites may also contribute to the mutagenicity of benzo(*j*)fluoranthene and benzo(*k*)-fluoranthene. These include benzo(*j*)fluoranthene-9,10-epoxide and benzo(*k*)fluoranthene-8,9-epoxide which are the likely precursors to the observed dihydrodiols, but they were not isolated or synthesized in the present study.

The structure of B(j)F-9,10-dihydrodiol is similar to those of dihydrodiols which are proximate forms of other carcinogenic polynuclear aromatic hydrocarbons and can form dihydrodiol-epoxides with one carbon of the epoxide ring in a 3-sided "bay region" (1, 7, 16). The presumed ultimate mutagen derived from B(j)F9,10-dihydrodiol, 9,10-dihydro-9,10-dihydroxy-11,12-epoxybenzo(*j*)-fluoranthene would have the epoxide ring in a 4-sided, "pseudo-bay region," in which the additional side is derived from the 5-membered ring. In contrast, B(k)F-8,9-dihydrodiol would be converted to a "linear" dihydrodiol-epoxide structurally related to those derived from oxidation of the 8,9- and/or 10,11-positions of benz(*a*)anthracene. Such a dihydrodiol-epoxide would not be expected to be a potent



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carcinogen (7, 15, 16). B(j)F-9,10-dihydrodiol and B(k)F-8,9-dihydrodiol are currently being bioassayed on mouse skin for carcinogenicity.

## REFERENCES


1. Bartsch, H., Malaveille, C., Tierney, B., Grover, P. L., and Sims, P. The association of bacterial mutagenicity of hydrocarbon-derived "bay-region" dihydrodiols with the Iball indices for carcinogenicity and with the extents of DNA-binding on mouse skin of the parent hydrocarbons. Chem. Biol. Interact. *26:* 185–196, 1979.
2. Baum, E. J. Occurrence and surveillance of polycyclic aromatic hydrocarbons. *In*: H. V. Gelboin and P. O. P. T'so (eds.), Polycyclic Hydrocarbons and Cancer, Vol. 1, pp. 45–70. New York: Academic Press, Inc., 1978.
3. Buu-Höi, N. P., Lavit, D., and Lamy, J. A new synthesis of dibenzo(*a,c*)anthracene, benzo(*k*)fluoranthene, and benzo(*b*)fluoranthene. J. Chem. Soc. (Lond.), 1845–1849, 1959.
4. Hecht, S. S., El-Bayoumy, K., Tulley, L., and LaVoie, E. Structure-mutagenicity relationships of *N*-oxidized derivatives of aniline, *o*-toluidine, 2′-methyl-4-aminobiphenyl, and 3,2′-dimethyl-4-aminobiphenyl. J. Med. Chem., *22:* 981–987, 1979.
5. Hoffmann, D., and Wynder, E. L. Organic particulate pollutants-chemical analysis and bioassays for carcinogenicity. *In*: A. C. Stern (ed.), Air Pollution, Ed. 3, Vol. 2, pp. 361–455. New York: Academic Press, Inc., 1977.
6. International Agency for Research on Cancer. Certain polycyclic aromatic hydrocarbons and heterocyclic compounds. IARC (Int. Agency Res. Cancer) Monogr. *3:* 69–90, 1973.
7. Jerina, D. M., Yagi, H., Lehr, R. E., Thakker, D. R., Schaeffer-Ridder, M., Karle, J. M., Levin, W., Wood, A. W., Chang, R. L., and Conney, A. H. The bay region theory of carcinogenesis by polycyclic aromatic hydrocarbons. *In*: H. V. Gelboin and P. O. P. T'so (eds.), Polycyclic Hydrocarbons and Cancer, Vol. 1, pp. 173–188. New York: Academic Press, Inc., 1978.
8. Laccassagne, A., Buu-Höi, N. P., Zajdela, F., Lavit-Lamy, D., and Chalvet, O. Activité cancérogène d'hydrocarbures aromatiques polycycliques a noyau fluoranthène. Acta Unio. Int. Contra Cancrum, *19:* 480–496, 1963.
9. LaVoie, E. J., Bedenko, V., Hirota, N., Hecht, S. S., and Hoffmann, D. A comparison of the mutagenicity, tumor-initiating activity, and complete carcinogenicity of polynuclear aromatic hydrocarbons. *In*: P. W. Jones and P. Leber (eds.). Polynuclear Aromatic Hydrocarbons, pp. 705–721. Ann Arbor, Mich.: Ann Arbor Science Publishers, 1979.
10. LaVoie, E. J., Hecht, S. S., Bedenko, V., Amin, S., Mazzarese, R., and Hoffmann, D. On the metabolic activation of the environmental carcinogens benzo(*j*)fluoranthene and benzo(*b*)fluoranthene. Proc. Am. Assoc. Cancer Res., *20:* 81, 1979.
11. Malaveille, C., Bartsch, H., Tierney, B., Grover, P. L., and Sims. P. Microsome-mediated mutagenesis of the dihydrodiols of 7,12-dimethylbenz(*a*)anthracene: high mutagenic activity of the 3,4-dihydrodiol. Biochem. Biophys. Res. Commun., *83:* 1468–1473, 1978.
12. Malaveille, C., Tierney, B., Grover, P. L., Sims, P., and Bartsch, H. High microsome-mediated mutagenicity of the 3,4-dihydrodiol of 7-methylbenz(*a*)anthracene in *S. typhimurium* TA 98. Biochem. Biophys. Res. Commun., *75:* 427–433, 1977.
13. Nenitzescu, C. D., and Avram, M. The polymerization of 1,2-dihydronaphthalene and dehydrogenating condensation of 1,2,3,4-tetrahydronaphthalene. J. Am. Chem. Soc., *72:* 3486–3490, 1952.
14. Perin-Roussel, O., Ekert, B., Zajdela, F., and Jacquignon, P. Binding of dibenzo(*a,e*)fluoranthene, a carcinogenic, polycyclic hydrocarbon without K-region, to nucleic acids in a subcellular microsomal system. Cancer Res., *38:* 3499–3504, 1978.
15. Slaga, T., Huberman, E., Selkirk, J. K., Harvey, R. G., and Bracken, W. M. Carcinogenicity and mutagenicity of benz(*a*)anthracene diols and diol-epoxides. Cancer Res., *38:* 1699–1704, 1978.
16. Wood, A. W., Levin, W., Chang, R. L., Yagi, H., Thakker, D. R., Lehr, R. E., Jerina, D. M., and Conney, A. H. Bay region activation of carcinogenic polycyclic hydrocarbons. *In*: P. W. Jones and P. Leber (eds.), Polynuclear Aromatic Hydrocarbons, pp. 531–551. Ann Arbor, Mich.: Ann Arbor Science Publishers, 1979.
17. Wynder, E. L., and Hoffmann, D. Carcinogenicity of benzofluoranthenes. Cancer (Phila.), *12:* 1194–1199, 1959.
18. Wynder, E. L., and Hoffmann, D. Tobacco and Tobacco Smoke. New York: Academic Press, Inc., 1967.




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