APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1984, p. 119–124
0099-2240/84/010119-06$02.00/0

Vol. 47, No. 1

Stereoselective Metabolism of Anthracene and Phenanthrene by the Fungus *Cunninghamella elegans*

CARL E. CERNIGLIA[1]* AND SHEN K. YANG[2]

National Center for Toxicological Research, Food and Drug Administration, Jefferson, Arkansas 72079[1] and Department of Pharmacology, School of Medicine, Uniformed Services University of Health Sciences, Bethesda, Maryland 20814[2]

Received 29 August 1983/Accepted 21 October 1983

The fungus *Cunninghamella elegans* oxidized anthracene and phenanthrene to form predominately *trans*-dihydrodiols. The metabolites were isolated by reversed-phase high-pressure liquid chromatography for structural and conformational analyses. Comparison of the circular dichroism spectrum of the fungal *trans*-1,2-dihydroxy-1,2-dihydroanthracene to that formed by rat liver microsomes indicated that the major enantiomer of the *trans*-1,2-dihydroxy-1,2-dihydroanthracene formed by *C. elegans* had an *S,S* absolute stereochemistry, which is opposite to the predominately 1*R*,2*R* dihydrodiol formed by rat liver microsomes. *C. elegans* oxidized phenanthrene primarily in the 1,2-positions to form *trans*-1,2-dihydroxy-1,2-dihydrophenanthrene. In addition, a minor amount of *trans*-3,4-dihydroxy-3,4-dihydrophenanthrene was detected. Metabolism at the K-region (9,10-positions) of phenanthrene was not detected. Comparison of the circular dichroism spectra of the phenanthrene *trans*-1,2- and *trans*-3,4-dihydrodiols formed by *C. elegans* to those formed by mammalian enzymes indicated that each of the dihydrodiols formed by *C. elegans* had an *S,S* absolute configuration. The results indicate that there are differences in both the regio- and stereoselective metabolism of anthracene and phenanthrene between the fungus *C. elegans* and rat liver microsomes.

A considerable amount of information exists on the microbial metabolism of anthracene and phenanthrene. These compounds are widely distributed throughout the environment as a result of pyrolytic processes and as minor contaminants in wastewater effluents from coal gasification and liquification processes (2). Anthracene and phenanthrene and their metabolites are not acutely toxic, carcinogenic, or mutagenic; however, these compounds have been used as model substrates in studies on the environmental degradation of polycyclic aromatic hydrocarbons, since both structures are found in carcinogenic polycyclic aromatic hydrocarbons, such as benzo[*a*]pyrene, benz[*a*]anthracene and 3-methylcholanthrene.

Pure cultures of bacteria and microbial populations isolated from freshwater and marine environments have the ability to metabolize anthracene and phenanthrene (13, 15–17, 21, 22, 24). *Beijerinckia* sp. strain B-836 and *Pseudomonas putida* 119 mutant strains, deficient in dihydrodiol dehydrogenase activity, oxidized anthracene in the 1,2-positions to form (+)-*cis*-1*R*,2*S*-dihydroxy-1,2-dihydroanthracene (1, 16). Similar studies with phenanthrene as the substrate indicated that *Beijerinckia* sp. strain B-836 and *P. putida* 119 formed predominately optically pure (+)-*cis*-3*S*,4*R*-dihydroxy-3,4-dihydrophenanthrene and a trace amount of (+)-*cis*-1*R*,2*S*-dihydroxy-1,2-dihydrophenanthrene (3, 16, 18). The anthracene and phenanthrene *cis*-dihydrodiols are metabolized by *cis*-dihydrodiol dehydrogenases to dihydroxylated derivatives (20), which can be further oxidized to ring cleavage products (14).

Fungi metabolize aromatic hydrocarbons and other xenobiotics in a manner similar to mammalian enzyme systems (5, 25). It has been suggested that the transformation of xenobiotics by eucaryotic microorganisms has potential value in augmenting and facilitating mammalian drug metabolism studies (23). Although there have been numerous studies on the bacterial oxidation of anthracene and phenanthrene, very little is known about the metabolism of these compounds in fungi. A recent study indicated that *Cunninghamella elegans* oxidized anthracene predominately to *trans*-1,2-dihydroxy-1,2-dihydroanthracene and 1-anthryl sulfate (6). To our knowledge, there has not been a published report on the fungal oxidation of phenanthrene. In this study, we report on the initial oxidation of phenanthrene by *C. elegans* and the optical properties and the absolute configurations of the dihydrodiols formed from the fungal oxidation of anthracene and phenanthrene. In addition, we compare the differences in both the regio- and stereoselective metabolism of anthracene and phenanthrene between rat liver microsomes and the fungus *C. elegans*.

* Corresponding author.

MATERIALS AND METHODS

Chemicals. Anthracene and phenanthrene were obtained from Aldrich Chemical Co., Milwaukee, Wis. Solvents for high-pressure liquid chromatography (HPLC) were purchased from Burdick and Jackson Laboratories, Muskegon, Mich. All other chemicals were of reagent grade or of the highest purity available.

Microorganisms and culture conditions. Stock cultures of *C. elegans* ATCC 36112 were maintained on Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) slants and stored at 4°C. The spores or mycelia (or both) from several slants were used to inoculate 10 125-ml Erlenmeyer flasks containing 30 ml of Sabouraud dextrose broth. The flasks were incubated for 48 h at 25°C on a rotary shaker at 150 rpm. After 48 h of incubation, the mycelia were removed by aseptic filtration and transferred to 10 sterile 125-ml Erlenmeyer flasks containing 30 ml of Sabouraud dextrose broth. Three milligrams of either anthracene or phenanthrene was dissolved in 0.3 ml of dimethylformamide and added to each flask. Sterile control experiments were prepared by autoclaving the culture at 121°C for 40 min before adding either substrate. The flasks were incubated in the dark at 25°C for 24 h as described above.

Isolation and detection of anthracene and phenanthrene metabolites formed by *C. elegans*. After 24 h, the flask

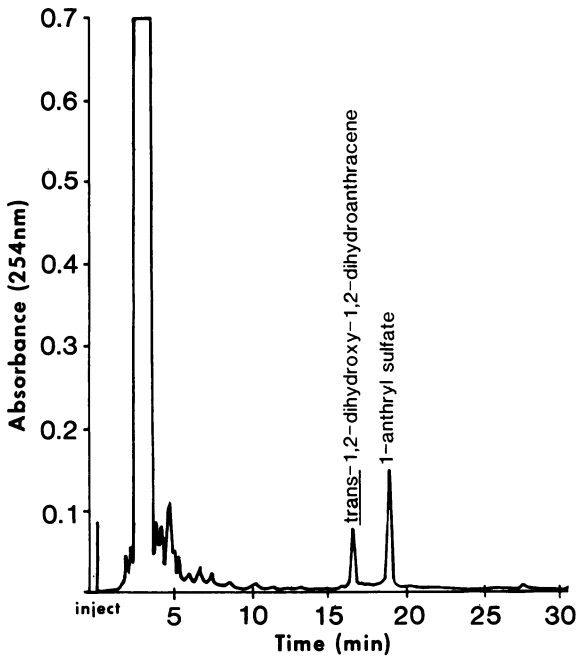

FIG. 1. HPLC elution profile of metabolites formed from anthracene by *C. elegans*.

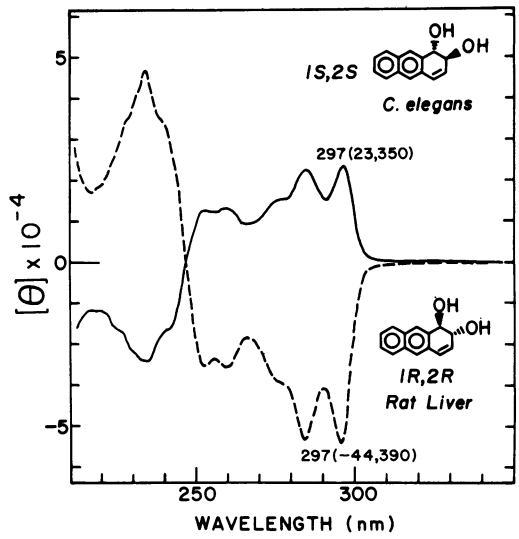

FIG. 2. CD spectra of *trans*-1,2-dihydroxy-1,2-dihydroanthracene formed from the metabolism of anthracene by *C. elegans* (——) and by liver microsomes from 3-methylcholanthrene-treated rats (------). The molecular ellipticity [θ] was calculated on the basis of an ϵ_{max} of 38,600 (16).

contents from either anthracene or phenanthrene incubations were pooled and filtered to separate the broth from the mycelia. The broth and the mycelia were each extracted with 6 volumes of ethyl acetate. The extracts were combined and dried over anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure at 40°C in the dark. Each residue was dissolved in methanol and analyzed by HPLC. Reversed-phase HPLC was performed with a Beckman system consisting of two model 100A pumps and a model 155-10 variable-wavelength absorbance detector adjusted at 254 nm. A 5-μm C_{18} ultrasphere ODS column (4.6 by 25 mm; Altex Scientific, Berkeley, Calif.) was used to separate anthracene and the anthracene metabolites. The separation was achieved with a programmed 60-min linear gradient of methanol-water (1:1 to 19:1, vol/vol) at a solvent flow rate of 1.0 ml/min. Phenanthrene metabolites were separated with a 5-μm Ultrasphere Cyano column (4.6 mm by 25 cm; Altex) with methanol-water (1:1, vol/vol) as the solvent at a flow rate of 1.0 ml/min.

Incubation of anthracene or phenanthrene with rat liver microsomes. Liver microsomes from 3-methylcholanthrene-pretreated immature male Sprague-Dawley rats (80 to 100 g of body weight) were prepared as previously described (12). Metabolites of anthracene or phenanthrene were obtained by incubation of anthracene or phenanthrene (8 μmol in 4 ml of methanol) at 37°C for 60 min in a 100-ml reaction mixture containing 5 mmol of Tris-hydrochloride (pH 7.5), 0.3 mmol of $MgCl_2$, 10 U of glucose 6-phosphate, and 100 mg of protein equivalent of liver microsomes from 3-methylcholanthrene-pretreated rats. Each reaction mixture was quenched with acetone (100 ml) and extracted with ethyl acetate (200 ml), and the organic solvent phase was dried with anhydrous $MgSO_4$ and evaporated under reduced pressure. Each residue was dissolved in methanol for HPLC separation of metabolites.

Physical and chemical analyses. The UV-visible absorption spectra of the metabolites were determined in methanol on a Beckman model 25 recording spectrometer. Mass spectra were obtained with a Finnigan model 4023 mass spectrometer operated at 70 eV of ionizing voltage with a solid probe. Direct probe mass spectrometry was performed on samples which were dissolved in 5 μl of methanol and dried in glass sample cups. Spectra were recorded as the probe temperature was increased ballistically from 30 to 300°C, monitoring the ion source temperature at 270°C. Circular dichroism

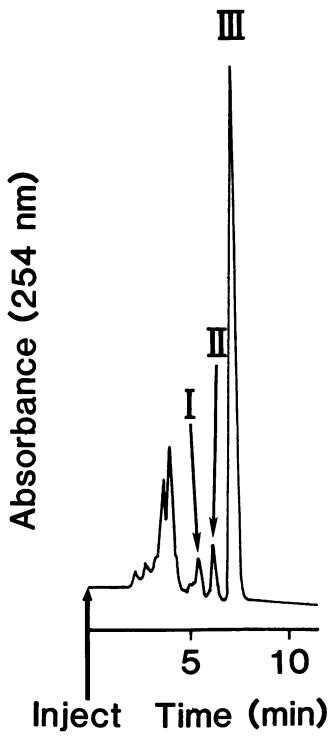

FIG. 3. HPLC elution profile of metabolites formed from phenanthrene by *C. elegans*.

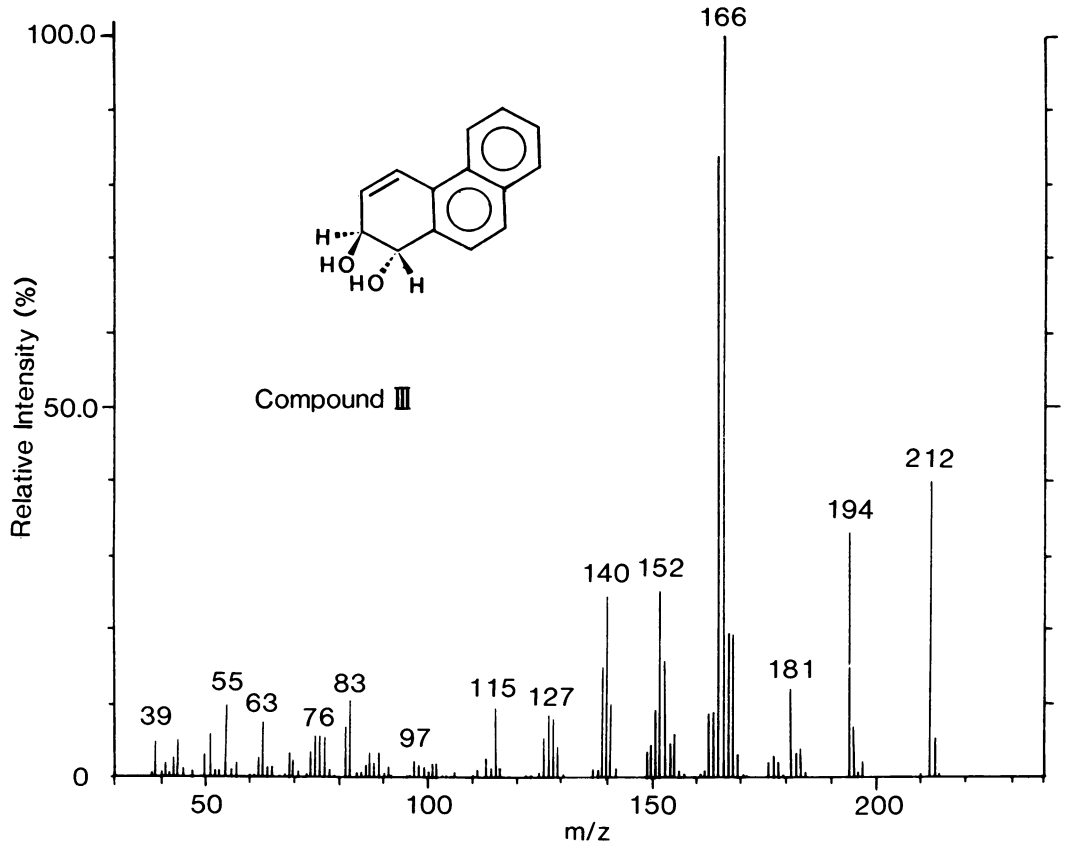

FIG. 4. Mass spectrum of compound III (*trans*-1,2-dihydroxy-1,2-dihydrophenanthrene) formed from phenanthrene by *C. elegans*.

(CD) spectra were determined with a quartz cell of 1-cm path length at room temperature on a Jasco 500A spectropolarimeter equipped with a Jasco DP-500 data processor.

The ^1H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker WM 500 spectrometer. The data were acquired under the following conditions: data size, 32,000; sweep width, 7,042 Hz; filter width, 17,800 Hz; temperature, 305°K; flip angle, 68°. The spectra were recorded in acetone-d_6. The chemical shifts are reported in parts per million (δ) downfield from the internal standard tetramethylsilane. Assignments were made via homonuclear decoupling experiments and shielding effects.

RESULTS AND DISCUSSION

C. elegans was incubated with anthracene for 24 h. The HPLC elution profile of the ethyl acetate-soluble anthracene metabolites formed by *C. elegans* is shown in Fig. 1. *C. elegans* oxidized anthracene predominantly to *trans*-1,2-dihydroxy-1,2-dihydroanthracene (16.5 min) and 1-anthryl sulfate (19.0 min). These results are similar to those described previously (6). The anthracene dihydrodiol was assigned as a *trans* isomer based on its identical rate of dehydration to the *trans* isomer formed from the mammalian metabolism of anthracene as compared to the *cis* isomer (16). To further confirm that the 1,2-dihydroxy-1,2-dihydroanthracene formed from anthracene by *C. elegans* had a *trans* configuration, the 500 ^1H MHz NMR spectrum of the fungal metabolite was recorded in this study. The following are the NMR spectral parameters obtained from first-order measurements for the fungal anthracene 1,2-dihydrodiol: (90% acetone-d_6)-δ 4.44 (*d*, $1H_2$), 4.82 (*d*, 1, H_1), 6.02 (*d*, 1, H_3), 6.60 (*d*, 1, H_4), 7.43 to 7.48 (*m*, 2, H_6, H_7), 7.58 (*s*, 1, H_{10}), 7.81 to 7.87 (*m*, 2, H_5, H_8), 8.03 (*s*, 1, H_9); $J_{1,2}$, 10.3 Hz; $J_{2,3}$, 2.2 Hz; $J_{2,4}$, 2.6 Hz; $J_{3,4}$, 9.9 Hz. The large coupling constant of 10.3 Hz between the carbinol protons is similar to that observed for the *trans*-1,2-dihydroxy-1,2-dihydroanthracene ($J_{1,2}$, 10.5 Hz) formed from anthracene by mammalian enzymes (16) and indicates that the fungal anthracene dihydrodiol is a *trans* isomer with both hydroxyl groups preferentially at the equatorial positions (26). The *cis* isomer formed by bacterial enzymes (16) has a relatively small coupling constant value ($J_{1,2}$, 4.6 Hz). The CD spectrum of *trans*-1,2-dihydroxy-1,2-dihydroanthracene formed from the metabolism of anthracene by *C. elegans* is shown in Fig. 2. Comparison of the CD data from the *trans*-1,2-dihydroxy-1,2-dihydroanthracene formed by *C. elegans* to that formed by liver microsomes from 3-methylcholanthrene-treated rats (Fig. 2) indicated that the fungal anthracene dihydrodiol is close to a mirror image to the corresponding anthracene 1*R*,2*R*-dihydrodiol formed by rat liver microsomes. The observed positive CD Cotton effect indicated that the fungal dihydrodiol was enriched in the 1*S*,2*S* enantiomer. However, it should be noted that a *trans*-1,2-dihydroxy-1,2-dihydroanthracene enriched in 1*S*,2*S* enantiomer ($[\theta]_{297}$, +40,700) has been isolated from the urine of rabbits (1).

C. elegans was grown in the presence of phenanthrene for 24 h. The ethyl acetate extractable metabolites were analyzed by HPLC (Fig. 3). Compound III was the major metabolite formed and had an HPLC retention time of 7.2 min. The UV spectral properties of compound III (λ_{max} at

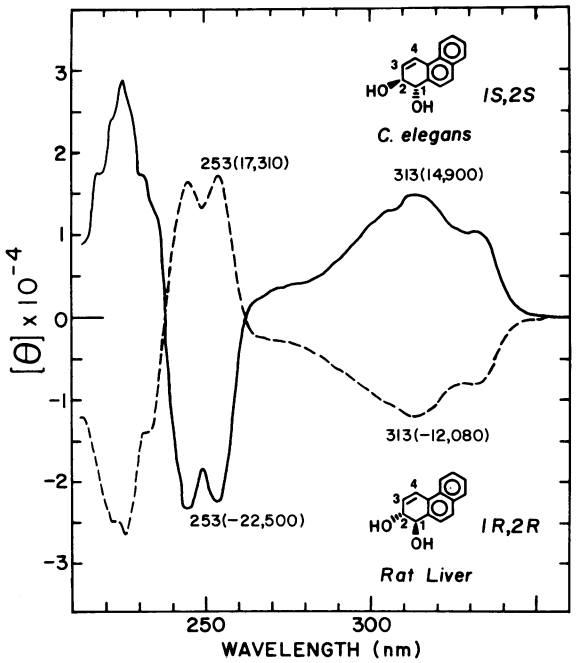

FIG. 5. CD spectra of *trans*-1,2-dihydroxy-1,2-dihydrophenanthrene formed from the metabolism of phenanthrene by *C. elegans* (——) and by liver microsomes from 3-methylcholanthrene-treated rats (------). The molecular ellipticity [θ] was calculated on the basis of an ϵ_{max237} of 40,264 (16).

236, 315, and 332 nm) are similar to those reported for *trans*-1,2-dihydroxy-1,2-dihydrophenanthrene (16). Mass spectral analysis of compound III (Fig. 4) gave a molecular ion (M^+) at *m/z* 212 and fragment ions at *m/z* 194 (M^+-H_2O) and *m/z* 166 (M^+-H_2O-CHO). The UV and mass spectral data indicated that compound III is 1,2-dihydroxy-1,2-dihydrophenanthrene. To determine the configuration and conformation of the dihydrodiol, 500 MHz ^1H NMR spectroscopic analysis was conducted for compound III. The assigned chemical shifts and coupling constants are as follows: (acetone-d_6) δ 4.50 (*d*, 1, H_2), 4.88 (*d*, 1, H_1), 6.21 (*d*, 1, H_3), 7.25 (*d*, 1, H_4), 7.48 (*dd*, 1, H_7), 7.54 (*dd*, 1, H_6), 7.82 to 7.86 (AB, 2, H_9, H_{10}), 7.88 (*d*, 1, H_8), 8.20 (*d*, 1, H_5) ppm; $J_{1,2}$, 11.6 Hz; $J_{2,3}$, 2.2 Hz; $J_{2,4}$, 2.6 Hz; $J_{3,4}$, 10.3 Hz; $J_{5,6}$, 8.6 Hz; $J_{6,7}$, 6.9 Hz; $J_{10,11}$, 10.3 Hz. The large coupling constant ($J_{1,2}$, 11.6 Hz) for the 1,2-dihydroxy-1,2-dihydrophenanthrene formed by *C. elegans* is similar to that reported for *trans*-1,2-dihydroxy-1,2-dihydrophenanthrene ($J_{1,2}$, 11.75 Hz) formed from phenanthrene by mammalian enzymes (16) and indicates that the fungal phenanthrene 1,2-dihydrodiol is a *trans* isomer with a diequatorial conformation.

The CD spectrum of *trans*-1,2-dihydroxy-1,2-dihydrophenanthrene formed by *C. elegans* was compared with that of the phenanthrene 1,2-dihydrodiol formed by liver microsomes from 3-methylcholanthrene-treated rats. Figure 5 indicates that the CD spectrum of the fungal dihydrodiol is enriched in the 1*S*,2*S* enantiomer and is a mirror image to the phenanthrene 1*R*,2*R*-dihydrodiol formed by rat liver microsomes. A phenanthrene *trans*-1,2-dihydrodiol formed from phenanthrene metabolism by liver microsomes from 3-methylcholanthrene-treated rats was reported to have an optical purity of 93% with an *R*,*R*/*S*,*S* enantiomer ratio of 96.5:3.5 (16).

Compound II (Fig. 3; retention time, 6.4 min) gave a UV absorption (λ_{max} 242, 249, and 258 nm) and mass spectrum (M^+ at *m/z* 212, *m/z* 194, and *m/z* 166) that were similar to those of *trans*-3,4-dihydroxy-3,4-dihydrophenanthrene (16). Since insufficient material was available for NMR analyses, the *trans* stereochemistry of compound II was based on the rate of acid-catalyzed dehydration compared with rates previously reported for *cis*- and *trans*-3,4-dihydroxy-3,4-dihydrophenanthrene (16). The rate of dehydration of compound II was similar to that reported for *trans*-3,4-dihydroxy-3,4-dihydrophenanthrene. In addition, all other known dihydrodiols produced by *C. elegans* have been shown to have a *trans* configuration (5). The CD spectrum of *trans*-3,4-dihydroxy-3,4-dihydrophenanthrene is shown in Fig. 6. The absolute configuration of the fungal phenanthrene 3,4-dihydrodiol is proposed to be 3*S*,4*S*. Since *trans*-3,4-dihydroxy-3,4-dihydrophenanthrene is a very minor metabolite in the mammalian metabolism of phenanthrene (4, 11, 19), a direct comparison of the CD spectra could not be made. However, a phenanthrene *trans*-3,4-dihydrodiol formed from the metabolism of phenanthrene by liver microsomes from 3-methylcholanthrene-treated rats was reported to have an optical purity of 97% enriched in the *R*,*R* enantiomer (19). Compound I (Fig. 3; retention time, 5.7 min) is a natural product formed by *C. elegans* and was found in control samples. Interestingly, there was no metabolism at the 9,10 positions (K-region) of phenanthrene as evidenced by the failure to detect *trans*-9,10-dihydroxy-9,10-dihydrophenanthrene (retention time, 6.7 min). *trans*-9,10-Dihydroxy-9,10-dihydrophenanthrene is a major metabolite in the mammalian metabolism of phenanthrene (4, 11, 19).

The results in this study indicate that *C. elegans* initially metabolizes anthracene and phenanthrene to form predominantly dihydrodiols with a *trans* configuration. Comparison of the CD spectra of *trans*-1,2-dihydroxy-1,2-dihydroanthracene and phenanthrene *trans*-1,2- and *trans*-3,4-dihydrodiols formed by *C. elegans* with those of the corresponding dihydrodiols of known absolute stereochemistry formed by

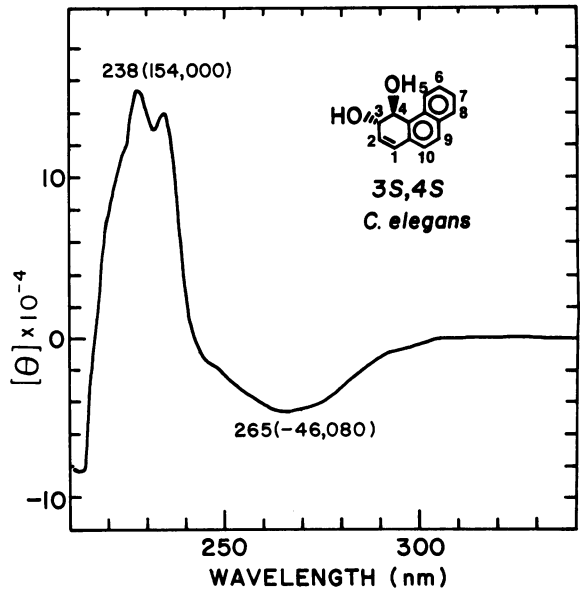

FIG. 6. CD spectrum of compound II (*trans*-3,4-dihydroxy-3,4-dihydrophenanthrene) formed from phenanthrene by *C. elegans*. The molecular ellipticity [θ] was calculated on the basis of an ϵ_{max} of 48,000 (16).

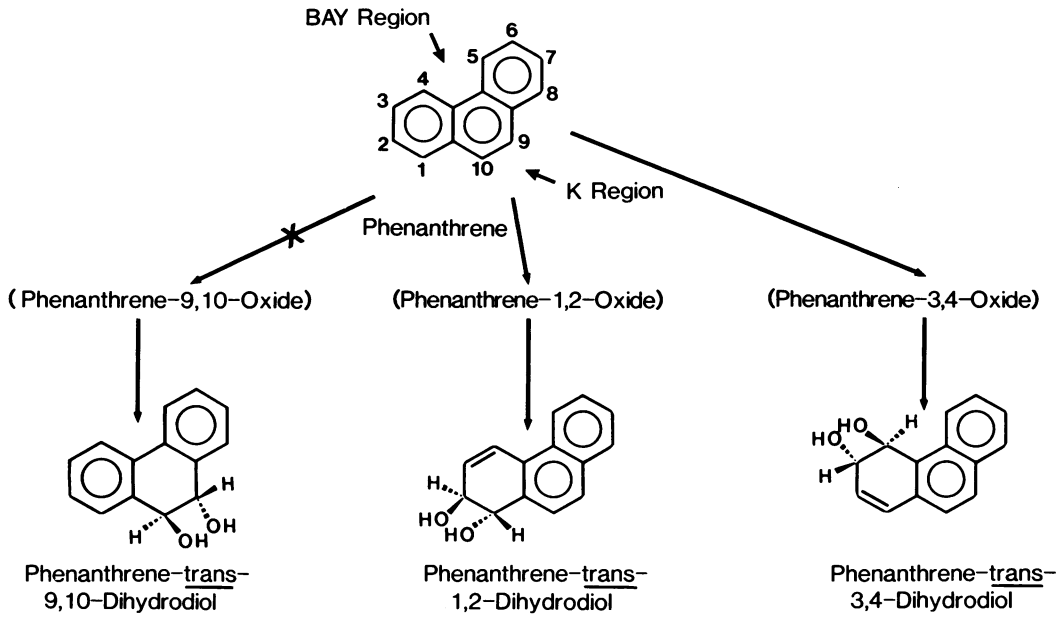

FIG. 7. Proposed pathways for the metabolism of phenanthrene by *C. elegans*.

rat liver microsomes indicated that the major enantiomeric dihydrodiol metabolites formed by *C. elegans* have *S,S* absolute stereochemistries. Since the anthracene *trans*-1,2-dihydrodiol and phenanthrene *trans*-1,2 and *trans*-3,4-dihydrodiols formed from anthracene and phenanthrene by rat liver microsomes have the *R,R* absolute configurations (3, 19), the results indicate that the stereoselective preference of cytochrome P-450 and epoxide hydrolase in *C. elegans* in the formation of *trans*-dihydrodiols is different from that in the rat liver microsomal system. Similar results were recently reported for the fungal oxidation of naphthalene (7). *C. elegans* oxidized naphthalene predominately to *trans*-1*S*,2*S*-dihydroxy-1,2-dihydronaphthalene, which had Cotton effects that were a mirror image to the naphthalene 1*R*,2*R*-dihydrodiol formed by rat liver microsomes.

C. elegans oxidized phenanthrene at the 1,2 and 3,4 positions to form phenanthrene *trans*-1,2- and *trans*-3,4-dihydrodiols (Fig. 7). There was no enzymatic attack at the 9,10 positions (K-region) of phenanthrene, which is a major site of metabolism in the mammalian oxidation of phenanthrene (4, 11, 19). This result suggests differences in the regioselectivity in the metabolism of phenanthrene by the cytochrome P-450 monooxygenase and epoxide hydrolase of *C. elegans* and rat liver microsomes. The lack of enzymatic attack at the K-region of phenanthrene has been found to be a general feature in polycyclic aromatic hydrocarbon metabolism by *C. elegans* (8–10).

ACKNOWLEDGMENTS

We greatly appreciate the technical assistance of L. David Pack and J. P. Freeman and Dwight W. Miller for the mass and NMR spectral measurements.

LITERATURE CITED

1. **Akhtar, M. N., D. R. Boyd, N. J. Thompson, M. Koreeda, D. T. Gibson, V. Mahadevan, and D. M. Jerina.** 1975. Absolute stereochemistry of the dihydroanthracene-*cis* and *trans*-1,2-diols produced from anthracene by mammals and bacteria. J. Chem. Soc., p. 2506–2511.
2. **Blumer, M.** 1976. Polycyclic aromatic compounds in nature. Sci. Am. **234**:34–44.
3. **Boyd, D. R., R. M. E. Greene, J. D. Neill, M. E. Stubbs, H. Yagi, and D. M. Jerina.** 1981. Racemization of phenanthrene 3,4-oxide. Absolute stereochemistry of *cis* and *trans*-phenanthrene-3,4-dihydrodiols. J. Chem. Soc. Perkin Trans. **1**:1477–1482.
4. **Boyland, E., and P. Sims.** 1962. The metabolism of phenanthrene in rabbits and rats: dihydroxy compounds and related glucosiduronic acids. Biochem. J. **84**:571–582.
5. **Cerniglia, C. E.** 1981. Aromatic hydrocarbons: metabolism by bacteria, fungi, and algae, p. 321–361. *In* E. Hodgson, J. R. Bend, and R. M. Philpot (ed.), Reviews in biochemical toxicology, vol. 3. Elsevier/North-Holland Publishing Co., New York.
6. **Cerniglia, C. E.** 1982. Initial reactions in the oxidation of anthracene by *Cunninghamella elegans*. J. Gen. Microbiol. **128**:2055–2061.
7. **Cerniglia, C. E., J. A. Althaus, F. E. Evans, J. P. Freeman, R. K. Mitchum, and S. K. Yang.** 1983. Stereochemistry and evidence for an arene oxide-NIH shift pathway in the fungal metabolism of naphthalene. Chem. Biol. Interact. **44**:119–132.
8. **Cerniglia, C. E., R. H. Dodge, and D. T. Gibson.** 1982. Fungal oxidation of 3-methylcholanthrene: formation of proximate carcinogenic metabolites of 3-methylcholanthrene. Chem. Biol. Interact. **38**:161–173.
9. **Cerniglia, C. E., P. P. Fu, and S. K. Yang.** 1982. Metabolism of 7-methylbenz(a)anthracene and 7-hydroxymethylbenz(a)anthracene by *Cunninghamella elegans*. Appl. Environ. Microbiol. **44**:682–689.
10. **Cerniglia, C. E., and D. T. Gibson.** 1979. Oxidation of benzo(a)-pyrene by the filamentous fungus *Cunninghamella elegans*. J. Biol. Chem. **254**:12174–12180.
11. **Chaturapit, S., and G. M. Holder.** 1978. Studies on the hepatic microsomal metabolism of ^{14}C-phenanthrene. Biochem. Phamacol. **27**:1865–1871.
12. **Chou, M. W., and S. K. Yang.** 1979. Combined reversed-phase and normal-phase high performance liquid chromatography and the purification and identification of 7,12-dimethylbenz[a]anthracene metabolites. J. Chromatogr. **185**:635–654.
13. **Colla, C., A. Fiecchi, and V. Treccani.** 1959. Ricerche sul metabolismo ossidativo microbico del' antracene e del fenantrene. Ann. Microbiol. **9**:87–91.
14. **Dagley, S.** 1971. Catabolism of aromatic compounds by microorganisms. Adv. Microbiol. Physiol. **6**:1–46.

15. **Evans, W. C., H. N. Fernley, and E. Griffiths.** 1965. Oxidative metabolism of phenanthrene and anthracene by soil pseudomonads: the ring fission mechanism. Biochem. J. **95**:819–821.
16. **Jerina, D. M., H. Selander, H. Yagi, M. C. Wells, J. F. Davey, V. Mahadevan, and D. T. Gibson.** 1976. Dihydrodiols from anthracene and phenanthrene. J. Am. Chem. Soc. **98**:5988–5996.
17. **Kiyohara, H., and K. Nagao.** 1978. The catabolism of phenanthrene and naphthalene by bacteria. J. Gen. Microbiol. **105**:69–75.
18. **Koreeda, M., M. N. Akhtar, D. R. Boyd, J. D. Neill, D. T. Gibson, and D. M. Jerina.** 1978. Absolute stereochemistry of *cis*-1,2-, *trans*-1,2- and *cis*-3,4-dihydrodiol metabolites of phenanthrene. J. Org. Chem. **43**:1023–1027.
19. **Nordquist, M., D. R. Thakker, K. P. Vyas, H. Yagi, W. Levin, D. E. Ryan, P. E. Thomas, A. H. Conney, and D. M. Jerina.** 1981. Metabolism of chrysenes and phenanthrene to bay-region diol epoxides by rat liver enzymes. Mol. Pharmacol. **19**:168–178.
20. **Patel, T. R., and D. T. Gibson.** 1974. Purification and properties of (+)-*cis*-naphthalene dihydrodiol dehydrogenase of *Pseudomonas putida*. J. Bacteriol. **119**:879–888.
21. **Rogoff, M. H., and I. Wender.** 1957. Microbiology of coal. I. Bacterial oxidation of phenanthrene. J. Bacteriol. **73**:264–268.
22. **Rogoff, M. N., and J. Wender.** 1957. 3-Hydroxy-2-naphthoic acid as an intermediate in the bacterial dissimilation of anthracene. J. Bacteriol. **74**:108–109.
23. **Rosazza, J. P., and R. V. Smith.** 1979. Microbial models for drug metabolism. Adv. Appl. Microbiol. **25**:169–208.
24. **Sherrill, T. W., and G. S. Sayler.** 1980. Phenanthrene biodegradation in fresh water environments. Appl. Environ. Microbiol. **39**:172–178.
25. **Smith, R., and J. Rosazza.** 1974. Microbial models of mammalian metabolism. Aromatic hydroxylation. Arch. Biochem. Biophys. **161**:551–558.
26. **Zacharias, D. E., J. P. Glusker, P. P. Fu, and R. G. Harvey.** 1979. Molecular structures of the dihydrodiols and diol epoxides of carcinogenic polycyclic aromatic hydrocarbons. X-ray crystallographic and NMR analysis. J. Am. Chem. Soc. **101**:4043–4051.