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Stereoselective formation of a K-region dihydrodiol from phenanthrene by *Streptomyces flavovirens*

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Abstract. The metabolism of phenanthrene, a polycyclic aromatic hydrocarbon (PAH), by *Streptomyces flavovirens* was investigated. When grown for 72 h in tryptone yeast extract broth saturated with phenanthrene, the actinomycete oxidized 21.3% of the hydrocarbon at the K-region to form *trans*-9,10-dihydroxy-9,10-dihydrophenanthrene (phenanthrene *trans*-9,10-dihydrodiol). A trace of 9-phenanthrol was also detected. Metabolites isolated by thin-layer and high performance liquid chromatography were identified by comparing chromatographic, mass spectral, and nuclear magnetic resonance properties with those of authentic compounds. Experiments using [9-^{14}C]phenanthrene showed that the *trans*-9,10-dihydrodiol had 62.8% of the radioactivity found in the metabolites. Circular dichroism spectra of the phenanthrene *trans*-9,10-dihydrodiol indicated that the absolute configuration of the predominant enantiomer was (−)-9*S*,10*S*, the same as that of the principal enantiomer produced by mammalian enzymes. Incubation of *S. flavovirens* with phenanthrene is an atmosphere of $^{18}O_2$, followed by gas chromatographic/mass spectral analysis of the metabolites, indicated that one atom from molecular oxygen was incorporated into each molecule of the phenanthrene *trans*-9,10-dihydrodiol. Cytochrome P-450 was detected in 105,000 × *g* supernatants prepared from cell extracts of *S. flavovirens*. The results show that the oxidation of phenanthrene by *S. flavovirens* was both regio- and stereospecific.

Key words: Actinomycetes – Cytochrome P-450 – Dihydrodiol – K-region – Metabolism – Phenanthrene – Polycyclic aromatic hydrocarbons – *Streptomyces flavovirens*

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Abbreviations: CD, circular dichroism; DMF, *N,N*-dimethylformamide; GC/MS, gas chromatography/mass spectrometry; HPLC, high performance liquid chromatography; NMR, nuclear magnetic resonance; ODS, octadecylsilane; PAH, polycyclic aromatic hydrocarbon; TLC, thin-layer chromatography; TMS, tetramethylsilane; UV, ultraviolet

Phenanthrene, a polycyclic aromatic hydrocarbon (PAH) with three condensed rings, is distributed widely in the environment by anthropogenic processes such as the burning of wood and fossil fuels (Heitkamp and Cerniglia 1987; Guerin 1989; Shiaris 1989). Although phenanthrene is considered relatively nontoxic to mammals (Yoshikawa et al. 1985), it significantly reduces photosynthesis in green algae (Kusk 1981) and is extremely toxic to young rainbow trout (Black et al. 1983). Phenanthrene damages lysosomal membranes in the digestive cells of marine snails and causes digestive cell deletion in marine mussels (Pipe and Moore 1986a, b). It also decreases reproduction and increases mortality in crustaceans (Savino and Tanabe 1989). Phenanthrene itself is not known to be mutagenic or carcinogenic; however, Bücker et al. (1979) reported the K-region metabolites phenanthrene 9,10-oxide, 9-phenanthrol, and racemic phenanthrene *trans*-9,10-dihydrodiol to be weakly mutagenic.

Several bacteria are known to catabolize phenanthrene by metabolic pathways that proceed via either catechol or protocatechuic acid (Cerniglia 1984; Gibson and Subramanian 1984; Cerniglia and Heitkamp 1989). Tausson (1928) first reported the isolation from oil-polluted soils of Gram-negative bacteria that assimilate phenanthrene. *Pseudomonas* and *Flavobacterium* strains oxidize phenanthrene to phenanthrene 3,4-dihydrodiol, 3,4-dihydroxyphenanthrene, 1-hydroxy-2-naphthaldehyde, 1-hydroxy-2-naphthoic acid, and 1,2-dihydroxynaphthalene (Rogoff and Wender 1957; Colla et al. 1959; Evans et al. 1965). Further catabolism of 1,2-dihydroxynaphthalene by the *Pseudomonas* sp. appears to be by the naphthalene pathway (Evans et al. 1965). The 3,4-dihydrodiol produced from phenanthrene by *Beijerinckia* sp. and *Pseudomonas putida* has been shown conclusively to be the *cis* stereoisomer (Jerina et al. 1976). *Beijerinckia* sp. also produces a small amount of the *cis* stereoisomer of the 1,2-dihydrodiol (Jerina et al. 1976). *Aeromonas* sp. and several other bacteria also catabolize phenanthrene through 1-hydroxy-2-naphthoic acid but metabolize it further via 2-carboxybenzaldehyde, *o*-phthalic acid, and protocatechuic acid (Kiyohara et al. 1976; Cerniglia 1984; Cerniglia and Heitkamp 1989).

Among the PAHs, only naphthalene and biphenyl (Smith and Rosazza 1974; Trower et al. 1988) have been shown to be metabolized by streptomycetes. *Streptomyces flavovirens*, which we selected after screening several isolates with phenanthrene, has been shown to break down lignocellulose-containing cell walls in tobacco roots (Grossbard 1971) and Douglas-fir phloem (Crawford and Sutherland 1979; Sutherland et al. 1979). This species has also been reported to be an important nitrate reducer in some aquatic ecosystems (Mansour and Shady 1984). Although the strain we used does not have catabolic plasmids, it can accept plasmids from other streptomycetes during growth in soil (Rafii and Crawford 1988).

In this paper, we present data on the metabolism of phenanthrene by *S. flavovirens*. The principal metabolites are identified and the regio- and stereospecificity of the phenanthrene-metabolizing enzymes are demonstrated.

Materials and methods

Microorganism and growth conditions

Streptomyces flavovirens strain no. 28 (Sutherland et al. 1979) was maintained on slants of tryptone yeast extract agar consisting of 3.0 g of Bacto-Tryptone (Difco Laboratories, Detroit, MI, USA), 3.0 g of Bacto-Yeast Extract (Difco), 1.3 g of $K_2HPO_4 \cdot 3H_2O$, and 15 g of Bacto-Agar (Difco) per liter of distilled water. The pH was adjusted to 7.4 before sterilization.

Broth cultures were grown in tryptone yeast extract broth containing 50 mg $\cdot l^{-1}$ of Tween 80 (polyoxyethylene sorbitan monooleate, Difco). For each liter of the medium, 10 mg of phenanthrene was dissolved in 6.7 ml of *N,N*-dimethylformamide (DMF), autoclaved separately, and added aseptically before inoculation. The final concentration of phenanthrene was calculated to be 10 mg $\cdot l^{-1}$, in excess of its water solubility. For experiments requiring [9-^{14}C]phenanthrene, 0.23 μCi $\cdot l^{-1}$ of the radioactive compound was added with the nonradioactive phenanthrene. To isolate phenanthrene metabolites, multiple 2-l Erlenmeyer flasks containing 500 ml of culture medium and 5 mg of phenanthrene were incubated for 72 h at 28°C on a rotary shaker at 150 rpm.

Extraction of metabolites

After growth of the streptomycete with phenanthrene, the spent broth was extracted with an equal volume of ethyl acetate. The ethyl acetate extract was dried, concentrated under reduced pressure at 40°C with a rotary evaporator (Büchi Laboratoriums-Technik, Flawil, Switzerland), and redissolved in 500 μl of acetone for analysis by thin-layer chromatography or 500 μl of methanol for analysis by high performance liquid chromatography.

Thin-layer chromatography

TLC was performed on 20 × 20 cm glass plates coated with silica gel GF 1000 (Analtech, Inc., Newark, DE, USA). The plates were developed in a benzene:hexane (1:1, vol/vol) solvent system to separate the phenanthrene metabolites from nonmetabolized phenanthrene. The metabolites were scraped off, dissolved in methanol, and filtered to remove the silica gel. After the methanol had evaporated, the metabolites were redissolved in acetone and further resolved by TLC, using a benzene:ethanol (9:1, vol/vol) solvent system.

High performance liquid chromatography

Reversed-phase high performance liquid chromatography (HPLC) analyses typically were performed with a Perkin-Elmer Series 10 chromatograph (Perkin-Elmer, Inc., Norwalk, CT, USA) with a Zorbax octadecylsilane (ODS) column (25 cm × 4.6 mm; Du Pont Co., Wilmington, DE, USA). The mobile phase consisted of a 40-min linear gradient of methanol:water (from 50:50, vol/vol, to 95:5) and the flow rate was maintained at 1.0 ml $\cdot$ min^{-1} throughout. Phenanthrene metabolites eluting from the column were monitored with a Perkin-Elmer model LC-95 UV detector set at 254 nm.

To obtain UV absorption spectra of metabolites separated by HPLC, a Hewlett-Packard model 1040A diode array detector (Hewlett-Packard, Inc., Palo Alto, CA, USA) was employed. Data were collected and analyzed by a Hewlett-Packard model 300 computer.

During experiments using labeled phenanthrene, fractions from the HPLC analysis of a 72-h culture grown with [9-^{14}C]-phenanthrene were collected in scintillation vials at 30-s intervals. After the addition of 7 ml of Scintisol (Isolab, Inc., Akron, OH, USA) scintillation fluid, each fraction was counted for 1 min using a Packard Tri-Carb 2000CA liquid scintillation counter (Packard Instrument Co., Downers Grove, IL, USA).

Mass spectrometry

For identification of metabolites, mass spectrometry was performed by electron impact with a Finnigan MAT model 4023 quadrupole mass spectrometer (Finnigan MAT Corp., San Jose, CA, USA). Probe samples were analyzed with a platinum wire direct exposure probe employing a Vacumetrics DCI current programmer (Vacumetrics Corp., Ventura, CA, USA) to generate a 0 to 3 amp current ramp over 120 s.

Capillary column gas chromatography/mass spectrometry (GC/MS) was performed for analysis of acetylated derivatives of the metabolites. A DB5-30N bonded phase column (J & W Scientific, Folsom, CA, USA) was employed with a head pressure of 138 kPa of He. The column temperature was programmed to hold at 50°C for 1 min and then increase to 280°C at 20°C deg per min. Samples were injected via an OCI-3 on-column injector (Scientific Glass Engineering, Austin, TX, USA).

Nuclear magnetic resonance

^{1}H-NMR spectra were obtained with a Bruker model WM 500 NMR spectrometer (Bruker Instruments, Billerica, MA, USA). The phenanthrene dihydrodiol was dissolved in acetone-d_6 with a trace of D_2O for NMR spectral measurements. Chemical shifts were recorded in parts per million (ppm) relative to tetramethylsilane (TMS).

Circular dichroism

CD spectra (Cerniglia and Yang 1984) were obtained in methanol with a Jasco model 500A spectropolarimeter (Japan Spectroscopic, Tokyo, Japan). CD spectra were expressed as ellipticity for methanol solutions that read 1.0 absorbance at 264 nm in a UV-visible spectrophotometer with a quartz cell of 1-cm path length. They were compared with published spectra (Miura et al. 1968; Balani et al. 1986) to determine the absolute configuration of the enantiomers.

$^{18}O_2$ incorporation experiments

Cultures for experiments with $^{18}O_2$ were grown in 125-ml flasks containing 40 ml of tryptone yeast extract broth with phenanthrene.

The flasks were inoculated and sealed with rubber septa that had been sterilized in 70% ethanol. The atmosphere was replaced with $^{18}O_2$ as previously described (Heitkamp et al. 1988) and the cultures were incubated on a rotary shaker at 28°C for 72 h.

Before and after growth of the cultures, the relative concentrations of $^{18}O_2$ and $^{16}O_2$ in the headspaces of the flasks were determined by mass spectrometry (Heitkamp et al. 1988). Mass spectrometry for quantitation of the headspace gases was performed on a Finnigan MAT model 1015D quadrupole mass spectrometer with a Granville-Phillips model 203 variable leak valve (Granville-Phillips, Boulder, CO, USA). After the leak valve had been closed by a septum and evacuated, a 0.1-ml gas sample was injected into the inlet of the leak valve. The valve then was opened until the detection circuit of the mass spectrometer was nearly saturated.

After growth of the cultures, the metabolites were extracted with ethyl acetate and dried over anhydrous Na_2SO_4. The ethyl acetate was removed under reduced pressure and the residues were acetylated with acetic anhydride and pyridine (Cerniglia et al. 1989). The isotopic abundance of ^{16}O and ^{18}O species among the acetylated phenanthrene metabolites was determined by GC/MS (Heitkamp et al. 1988).

Cytochrome P-450 determination

Cytochrome P-450 activity was demonstrated in cell extracts prepared by sonic oscillation as previously described (Sutherland et al. 1981). After the mycelial debris had been pelleted by centrifugation at 25,000 × *g*, the supernatant was centrifuged at 105,000 × *g* for 2 h (Stevenson et al. 1983) and the new pellet was discarded. Protein in the extracts was determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard. The reduced carbon monoxide difference spectrum was determined by the method of Omura and Sato (1964) with an Aminco DW-2 double-beam spectrophotometer (American Instrument Co., Silver Spring, MD, USA).

Chemicals

Phenanthrene and [9-^{14}C]phenanthrene (10.9 mCi · mmol^{-1}) were obtained from Sigma Chemical Co., St. Louis, MO, USA. *N,N*-Dimethylformamide (DMF) was purchased from Burdick and Jackson Laboratories, Muskegon, MI, USA. Ethyl acetate and methanol were obtained from J. T. Baker Chemical Co., Phillipsburg, NJ, USA. Synthetic *trans*-9,10-dihydroxy-9,10-dihydrophenanthrene was prepared from phenanthrene-9,10-quinone (Aldrich Chemical Co., Milwaukee, WI, USA) by the procedure of Harvey et al. (1975).

Results

After the growth of *Streptomyces flavovirens* with [9-^{14}C]-phenanthrene, the metabolites were extracted with ethyl acetate and separated by HPLC (Fig. 1 A). The detectable ^{14}C-containing compounds eluted at 16, 23, 26, and 39 min (Fig. 1 B). The principal phenanthrene metabolite, which eluted at 16 min (Fig. 1 A and B), had 21.3% of the total radioactivity (62.8% of the metabolites). The retention time and the UV absorption spectrum, which had a maximum at 270 nm, were identical to those of synthetic phenanthrene *trans*-9,10-dihydrodiol. The 23-min and 26-min peaks had 6.6% and 6.0% of the radioactivity, respectively, but were not identified. Occasionally a trace of a compound eluting at 29 min with a retention time, UV absorption spectrum, and mass

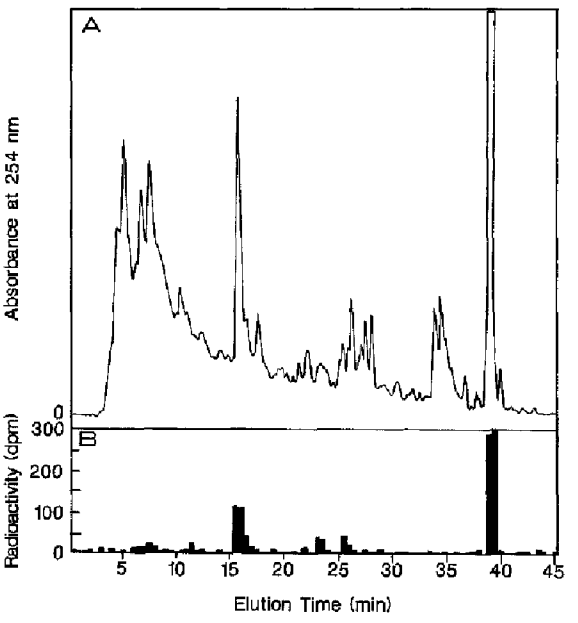

Fig. 1 A and B. HPLC elution profile of the metabolites produced during the growth of *Streptomyces flavovirens* with [9-^{14}C]phenanthrene. (**A**) UV absorbance at 254 nm and (**B**) radioactivity of the fractions eluting from the HPLC column. The 16-min peak represents the principal phenanthrene metabolite and the 39-min truncated peak (total height = 538 dpm) represents phenanthrene

spectrum similar to those of synthetic 9-phenanthrol was detected. The compound eluting at 39 min in Fig. 1 A and B had 66.1% of the radioactivity. Its retention time and UV absorption spectrum were identical to those of phenanthrene.

The mass spectrum of the principal metabolite eluting at 16 min (Fig. 2 A) shows a molecular ion (M^+) at m/z 212 and fragment ions at m/z 194 ($M^+ - H_2O$), 181 ($M^+ - CH_2OH$), 165 ($M^+ - H_2O$, $-CHO$), and 152 ($M^+ - CH_2OH$, $-CHO$). The mass spectrum was identical to that of synthetic phenanthrene *trans*-9,10-dihydrodiol.

The compound obtained by acidification of the phenanthrene 9,10-dihydrodiol metabolite had a mass spectrum (Fig. 2 B) with a molecular ion (M^+) at m/z 194 and a fragment ion at m/z 165 ($M^+ - CHO$). This mass spectrum was similar to that obtained with synthetic 9-phenanthrol.

To confirm the stereochemistry of the phenanthrene 9,10-dihydrodiol metabolite produced by *S. flavovirens*, ^{1}H-NMR analysis was conducted. The 500 MHz ^{1}H-NMR spectrum of the 9,10-dihydrodiol metabolite (acetone-d_6) was 4.60 (*s*, 2H, H_9, H_{10}), 7.31 – 7.38 (*m*, 4H, H_2, H_3, H_6, H_7), 7.70 (*d*, 2H, $J_{1,2}$ = 6.9 Hz, H_1, H_8) and 7.79 (*d*, 2H, $J_{2,3}$ = 6.9 Hz, H_4, H_5) ppm. The NMR spectrum of the metabolite was compared directly with that of synthetic phenanthrene *trans*-9,10-dihydrodiol and found to be virtually identical. Further confirmation of structure was accomplished by homonuclear decoupling experiments and nuclear Overhauser effect (NOE) difference experiments. The doublets at 7.70 and 7.79 ppm (H_1 and H_4, respectively) were irradiated independently; both experiments demonstrated that they were

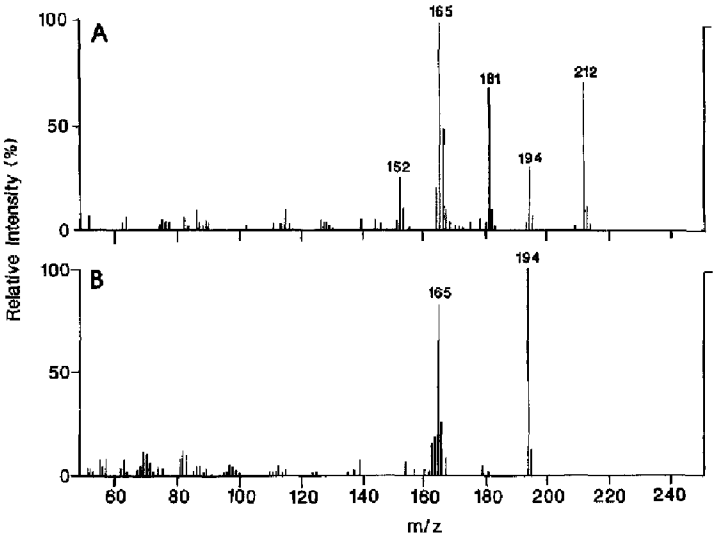

Fig. 2A and B. Mass spectra obtained by electron impact of **(A)** phenanthrene *trans*-9,10-dihydrodiol produced from phenanthrene by *S. flavovirens* and **(B)** 9-phenanthrol produced by acidification of the dihydrodiol

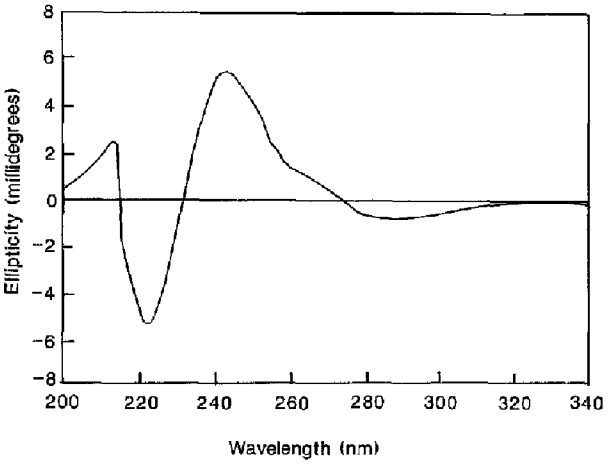

Fig. 3. Circular dichroism spectrum of the phenanthrene *trans*-9,10-dihydrodiol produced by *S. flavovirens*

coupled to the multiplet centered about 7.35 ppm. The resonance at 4.60 ppm (H_9, H_{10}) exhibited an NOE upon irradiation at 7.70 ppm (H_1, H_8). These experimental results were consistent with the phenanthrene *trans*-9,10-dihydrodiol structure.

The CD spectrum of the dihydrodiol (Fig. 3) shows a negative Cotton effect at 222 nm and positive Cotton effects at 212 and 243 nm. The CD spectral pattern of this metabolite was consistent with its identification as a *trans* rather than a *cis* dihydrodiol and indicated that it was the 9*S*,10*S* enantiomer (Miura et al. 1968; Balani et al. 1986).

For the experiment with $^{18}O_2$, phenanthrene *trans*-9,10-dihydrodiols from cultures grown under atmospheres containing $^{16}O_2$ and $^{18}O_2$ were extracted, derivatized by acetylation, and subjected to GC/MS. The mass spectrum of the *trans*-9,10-diacetoxy-9,10-dihydrophenanthrene derived from the culture grown under air containing $^{16}O_2$ (Fig. 4A) showed a molecular ion (M^+) at m/z 296 and fragment ions at m/z 236 ($M^+ - CH_3COOH$), 194 ($M^+ - CH_3COOH$, $-CH_2CO$), 178 ($M^+ - CH_3COOH$, $-CH_2COO$), and 165 ($M^+ - CH_3COOH$, $-CH_2CO$, $-CHO$). In the flasks containing $^{18}O_2$, the headspace contained 95.2 atom% of $^{18}O_2$ at the beginning before the growth of *S. flavovirens* and, due to slight leakage, 81.3 atom% of $^{18}O_2$ at the end. The mass spectrum of the *trans*-9,10-diacetoxy-9,10-dihydrophenanthrene derived from the culture grown under $^{18}O_2$ (Fig. 4B) shows a molecular ion (M^+) at m/z 298. Fragment ions were obtained that had m/z of 238 ($M^+ - CH_3CO^{16}OH$) and 236 ($M^+ - CH_3CO^{18}OH$); 196 ($M^+ - CH_3CO^{16}OH$, $-CH_2CO$) and 194 ($M^+ - CH_3CO^{18}OH$, $-CH_2CO$); 178 ($M^+ - CH_3COOH$, $-CH_2COO$); and 165 ($M^+ - CH_3COOH$, $-CH_2CO$, $-CHO$). The mass spectra in Fig. 4A and B indicate that only one oxygen atom from $^{18}O_2$ was added to the phenanthrene molecule during formation of the dihydrodiol.

A carbon monoxide difference spectrum was obtained for the reduced 105,000 × *g* supernatant from a cell extract of *S. flavovirens* grown with phenanthrene. The absorbance peak at 450 nm showed the presence of a low level (approximately 55 n*M*) of soluble cytochrome P-450 in the extract. The carbon monoxide difference spectrum of the reduced extract from a similar culture grown without phenanthrene also had an absorbance peak at 450 nm, indicating that phenanthrene was not required as an inducer.

Discussion

Phenanthrene can be converted to a *trans*-dihydrodiol by two enzymatic steps (Chaturapit and Holder 1978). The first step, catalyzed by a cytochrome P-450 monooxygenase, forms an arene oxide. The second step, catalyzed by an epoxide hydrolase, forms a *trans*-dihydrodiol. We have shown that *Streptomyces flavovirens* metabolized phenanthrene at the K-region, presumably via an arene oxide, to form phenanthrene *trans*-9,10-dihydrodiol (Fig. 5).

The detection of a soluble cytochrome P-450 in *S. flavovirens* was consistent with the involvement of a cytochrome P-450 monooxygenase in the initial oxidation

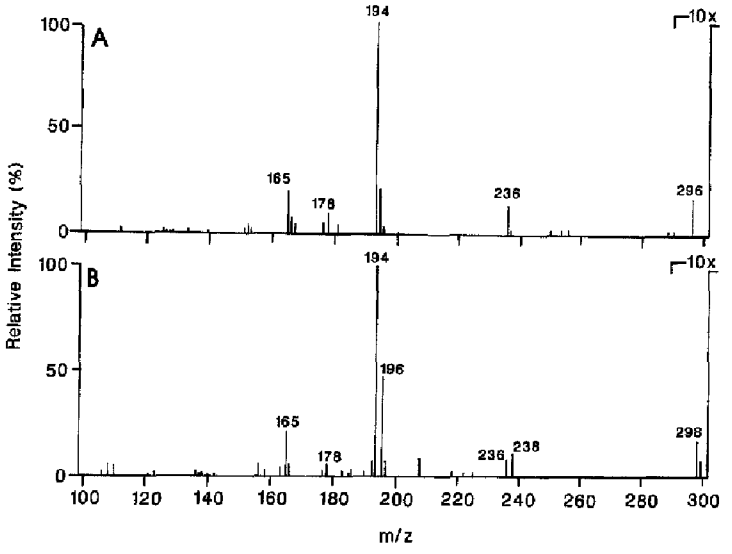

Fig. 4 A and B. Mass spectra, obtained by GC/MS, of *trans*-9,10-diacetoxy-9,10-dihydrophenanthrene produced by acetylation of the metabolites of *S. flavovirens* grown with phenanthrene (**A**) under $^{16}O_2$, (**B**) under $^{18}O_2$

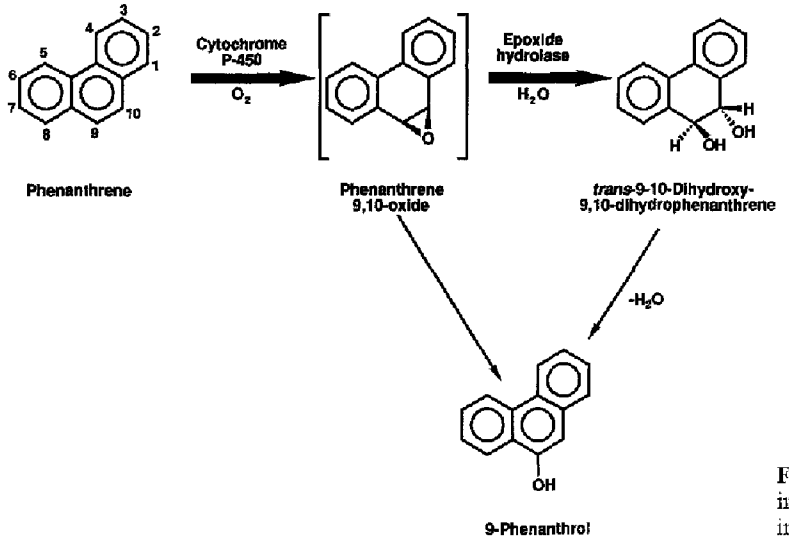

Fig. 5. Proposed pathway for phénanthrene metabolism in cultures inoculated with *S. flavovirens*. The *bold arrows* indicate the predominant pathway

step. Furthermore, the mass spectrum, obtained by GC/MS, of the acetylated derivative of the dihydrodiol produced under $^{18}O_2$ (Fig. 4) indicated that only one oxygen atom in the dihydrodiol came from O_2 and the other from water.

To demonstrate that the arene oxide was an intermediate, synthetic phenanthrene 9,10-oxide was added to cultures of *S. flavovirens* that had been grown without phenanthrene. A small amount of phenanthrene *trans*-9,10-dihydrodiol, which suggested epoxide hydrolase activity, was detected in the culture medium after 24 h (data not shown). Nevertheless, the nonenzymatic solvolysis of phenanthrene 9,10-oxide at pH 7 also may produce a *trans*-9,10-dihydrodiol (Bruice et al. 1976).

9-Phenanthrol (Fig. 5) could be produced either by the rearrangement of the arene oxide (Bruice et al. 1976; Chaturapit and Holder 1978) or by the dehydration of the dihydrodiol (Jerina et al. 1976). Direct hydroxylation of phenanthrene would also be theoretically possible (Tomaszewski et al. 1975).

The CD spectrum of the dihydrodiol produced by *S. flavovirens* was compared with that of synthetic phenanthrene (−)-*trans*-9*S*,10*S*-dihydrodiol (Miura et al. 1968; Balani et al. 1986). The comparison showed that the microbial metabolite had the (−)-9*S*,10*S* absolute configuration. In contrast, bacteria that utilize phenanthrene as a carbon source produce *cis*-3,4-dihydrodiols and occasionally also *cis*-1,2-dihydrodiols (Jerina et al. 1976; Cerniglia and Heitkamp 1989). Phenanthrene *trans*-9,10-dihydrodiol, with the 9*S*,10*S* enantiomer in predominance, has been reported to be a metabolite of the marine cyanobacterium *Agmenellum quadruplicatum* (M. L. Narro, C. E. Cerniglia, D. T. Gibson, and C. Van Baalen, Abstr. Annu. Meet. Amer. Soc. Microbiol. 1984, p. 156).

A constitutive isozyme of cytochrome P-450 has been detected in *S. griseolus* (O'Keefe et al. 1988). Although PAHs have not been shown to induce cytochrome P-450 activity in *Streptomyces* spp., several other compounds induce it. Veratrole and guaiacol induce cytochrome P-450 activity in *S. setonii* (Sutherland 1986), sulfo-

meturon methyl and chlorimuron ethyl induce it in *S. griseolus* (Romesser and O'Keefe 1986; O'Keefe et al. 1988), and genistein induces it in *S. griseus* (Sariaslani and Kunz 1986). The hydroxylation of naphthalene to 1-naphthol and of biphenyl to 2- and 4-hydroxybiphenyl has been demonstrated in extracts from genistein-induced cultures of *S. griseus* (Trower et al. 1988).

In rats and rabbits, the principal products of phenanthrene metabolism are 9*S*,10*S*-dihydrodiols (Boyland and Wolf 1950; Miura et al. 1968), although these animals also produce smaller amounts of *trans*-1,2- and *trans*-3,4-dihydrodiols (Boyland and Sims 1962). Liver microsomal preparations from rats, mice, and guinea pigs generally also produce *trans*-9,10-dihydrodiols (Chaturapit and Holder 1978). Microsomes from guinea pigs treated with 3-methylcholanthrene, however, produce mainly *trans*-1,2-dihydrodiols (Chaturapit and Holder 1978). The main products of phenanthrene metabolism in lobsters and sharks also are *trans*-9,10-dihydrodiols, although *trans*-1,2-dihydrodiols predominate in bony fishes (Solbakken and Palmork 1981).

The dihydrodiol produced from phenanthrene by *S. flavovirens* was similar to the principal dihydrodiol produced from phenanthrene by mammals (Miura et al. 1968; Balani et al. 1986). Since the CD spectrum indicates that the microbial metabolite had a 9*S*,10*S* absolute configuration, our results suggest that *S. flavovirens* metabolized phenanthrene in a regio- and stereospecific manner to produce a compound that is also typical of mammalian phenanthrene metabolism.

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