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Fungal Transformation of Fluoranthene

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The fungus *Cunninghamella elegans* ATCC 36112 metabolized approximately 80% of the 3-^{14}C-labeled fluoranthene (FA) added within 72 h of incubation. *C. elegans* metabolized FA to *trans*-2,3-dihydroxy-2,3-dihydrofluoranthene (*trans*-2,3-dihydrodiol), 8- and 9-hydroxyfluoranthene *trans*-2,3-dihydrodiol, 3-fluoranthene-β-glucopyranoside, and 3-(8-hydroxyfluoranthene)-β-glucopyranoside. These metabolites were separated by thin-layer and reversed-phase high-performance liquid chromatography and identified by ^{1}H nuclear magnetic resonance, UV, and mass spectral techniques. The major pathway involved hydroxylation to form a glucoside conjugate of 3-hydroxyfluoranthene and a glucoside conjugate of 3,8-dihydroxyfluoranthene which together accounted for 52% of the total ethyl acetate-soluble metabolites. *C. elegans* initially metabolized FA in the 2,3 position to form fluoranthene *trans*-2,3-dihydrodiol, which has previously been shown to be a biologically active compound in mammalian and bacterial genotoxicity tests. However, *C. elegans* formed predominantly glucoside conjugates of the phenolic derivatives of FA, which suggests that this fungus has the potential to detoxify FA.

Fluoranthene (FA), a polycyclic aromatic hydrocarbon (PAH) containing four fused rings, is formed during the combustion of fossil fuels and also occurs as a natural constituent of unaltered fossil fuels (18). PAHs are included in the priority pollutants listing of the U.S. Environmental Protection Agency since many are carcinogenic in laboratory animals and are potential health risks to humans (19). Since FA is consistently the most abundant of the PAHs measured in environmental samples, it has been used as an indicator PAH in the chemical analysis of surface soils, air particulates, estuarine and marine sediments, and aquatic samples for PAH contamination (18). The concentration of PAHs in sediments depends upon the distance of the site from industrialized regions, anthropogenic activities at the site, and the various aerial transport mechanisms at the site (8). Jones et al. (18) found that a typical range of FA in surface soils was 17 to 1,550 μg/kg; the highest concentrations were from industrialized and coal-mining districts.

Biodegradation of PAHs and detoxification of PAH-polluted sites by specific microorganisms may be both a cost-effective and a promising means of bioremediation of PAHs in the environment. However, there is a paucity of information on the bacterial degradation of PAHs containing four or more fused aromatic rings (3, 8). A bacterial community isolated from creosote waste was able to utilize FA as the sole carbon source (23). Recently Mueller et al. (22) and Weissenfels et al. (28) demonstrated that *Pseudomonas paucimobilis* and *Alcaligenes denitrificans*, respectively, can utilize FA as the sole source of carbon and energy. A *Mycobacterium* sp. has also been reported to be capable of extensive FA mineralization under cometabolic growth conditions (15–17; I. Kelley and C. E. Cerniglia, J. Ind. Microbiol., in press).

Fungi oxidize a wide variety of PAHs (4, 8). *Cunninghamella elegans* metabolizes PAHs to compounds which are less mutagenic than the parent compounds (7, 11). Although *C. elegans* initially metabolizes PAHs to *trans*-dihydrodiols, phenols, quinones, and dihydrodiol epoxides, the sulfate, glucuronide, and glucoside conjugates of the phenols have been identified as detoxification products of these primary metabolites (7, 9–11, 20, 21). Earlier studies in this laboratory have indicated similarities as well as differences between fungal and mammalian oxidation of PAHs (8). Fungal metabolic pathways have shown a greater tendency towards detoxification than towards bioactivation, which is more common in mammals (7, 9–11, 20, 21).

In this study, we report on the metabolism of FA by *C. elegans* and provide the first detailed investigation of the isolation and identification of the microbial metabolites of FA.

MATERIALS AND METHODS

Chemicals. FA was purchased from Fluka AG, Buchs, Switzerland. [3-^{14}C]FA (specific activity, 55.0 mCi/mmol; radiochemical purity, >98%) was obtained from Chemsyn Science Laboratories, Lenexa, Kans. High-performance liquid chromatography (HPLC)-grade solvents were purchased from Fisher Scientific Co., Pittsburgh, Pa. All other chemicals were of reagent grade and of the highest purity available.

Microorganism and culture conditions for the biotransformation of FA. Stock cultures of the fungus *C. elegans* ATCC 36112 were maintained on Sabouraud dextrose agar plates and stored at 4°C. Spores and mycelia from several plates were used to aseptically inoculate 125-ml Erlenmeyer flasks containing 30 ml of Sabouraud dextrose broth (Difco Laboratories, Detroit, Mich.). The flasks were incubated for 48 h at 24°C on a rotary shaker at 140 rpm. After 48 h of incubation, 20 mg of FA dissolved in 0.5 ml of dimethyl sulfoxide was added to a culture containing *C. elegans* (3 mg [dry weight]) in 30 ml of Sabouraud dextrose broth. Sterile control flasks were prepared by autoclaving similar cultures at 121°C for 40 min before adding FA. All flasks were incubated for an additional 72 h. After 72 h, the contents of the flasks were pooled and filtered aseptically to separate the broth from the mycelia. The mycelia were then extracted with three equal volumes of ethyl acetate. The extract was

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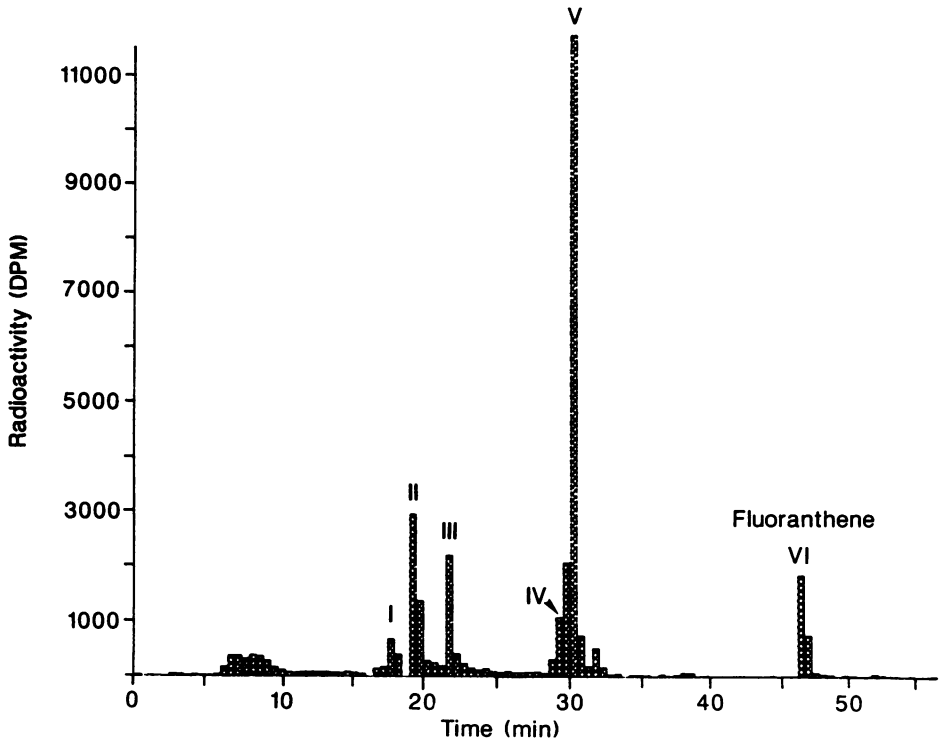

FIG. 1. HPLC elution profile of the ethyl acetate-soluble metabolites formed from [3-^{14}C]FA by *C. elegans*. Fractions eluting from the chromatograph were collected at 0.5-min intervals, and their radioactivities were measured by liquid scintillation.

dried over anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure at 38°C. The residue was dissolved in methanol and analyzed by HPLC and thin-layer chromatography.

Kinetic experiments were conducted as described above, with [3-^{14}C]FA (1.25 μCi) added to duplicate flasks. Additionally, 20 mg of unlabeled FA dissolved in 0.5 ml of dimethyl sulfoxide was added to each flask. After incubation for various periods, the entire contents were extracted and analyzed for metabolites.

Physical and chemical analysis. A Perkin-Elmer series 10 high-pressure liquid chromatograph equipped with an LC-95 UV-visible absorbance detector (The Perkin-Elmer Corp., Norwalk, Conn.), operated at 254 nm and fitted with a Zorbax ODS column (25 cm by 4.6 mm [inside diameter]; Du Pont Co., Wilmington, Del.), was used to separate the FA metabolites. A 40-min linear gradient of methanol-water (from 30:70 to 95:5 [vol/vol]) at a flow rate of 1.0 ml/min eluted the metabolites. Compounds were collected from repeated injections of the culture extracts. Fractions with similar compositions and HPLC retention times were pooled and concentrated in a Speed Vac concentrator (Savant Instruments, Inc., Hicksville, N.Y.).

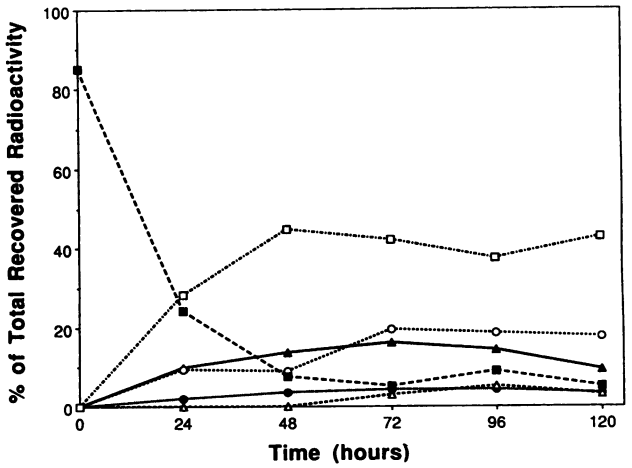

FIG. 2. Metabolism of [3-^{14}C]FA (■) and formation over time by *C. elegans* of FA metabolites I (●), II (▲), III (○), IV (△), and V (□).

In experiments with [3-^{14}C]FA, 0.5-ml fractions were collected every 0.5 min and added to scintillation vials containing 7.0 ml of scintillation fluid (Scintisol; Isolab Inc., Akron, Ohio). Radioactivity was determined on a Packard 2000CA Tri-Carb liquid scintillation counter (Packard Instrument Co., Downers Grove, Ill.).

UV-visible absorption spectra of the metabolites were determined in methanol on a model DU-7 spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.). Two FA metabolites (compounds II and V) were acetylated by the addition of 1.0 ml of pyridine and 1.5 ml of acetic anhydride and incubation of the mixture at 50°C for 24 h. Silica Gel F_{500} glass plates (E. Merck AG, Darmstadt, Federal Republic of Germany) were used for thin-layer chromatography analyses of the extracts. The solvent used for thin-layer chromatography was benzene-ethanol (9:1 [vol/vol]).

Mass spectral analyses were performed on a model MAT 4023 mass spectrometer (Finnigan Corp., San Jose, Calif.) with a platinum wire direct exposure probe and a desorption current programmer (Vacumetrics Corp., Ventura, Calif.). The mass spectrometer was operated in the electron impact

TABLE 1. Mass spectral properties of FA metabolites formed by *C. elegans*

Compound	Mass spectral properties {*m/z* (% relative intensity) [molecular ion]}
FA	203(25), 202(100)[M^+], 201(16), 200(19), 101(15), 100(11)
Metabolite I	252(17)[M^+], 235(23), 234(100), 218(44), 206(40), 205(20), 195(12), 189(24), 181(14), 177(17), 176(19), 165(23), 154(22), 152(16), 151(10), 70(18)
Metabolite II	397(0.14), 396(0.70)[M^+], 235(17), 234(100), 205(16), 176(10)
Metabolite II, acetylated	606(0.79)[M^+], 331(35), 276(5), 234(30), 233(18), 169(100), 127(15), 109(47)
Metabolite III	252(38)[M^+], 235(20), 234(100), 221(11), 206(40), 205(26), 195(17), 194(10), 181(13), 178(10), 177(22), 176(27), 165(28), 152(14), 151(12), 88(16)
Metabolite IV	236(21)[M^+], 219(19), 218(100), 205(15), 191(15), 190(64), 189(53), 187(12), 181(13), 179(38), 178(48), 177(14), 176(21), 165(18), 163(10), 152(11), 95(12), 94(13)
Metabolite V	381(0.14), 380(0.52)[M^+], 219(19), 218(100), 190(12), 189(34)
Metabolite V, acetylated	606(0.17), 548(0.46)[M^+], 534(0.24), 331(44), 234(14), 233(11), 218(12), 217(10), 189(18), 169(100), 127(20), 109(51)

(EI) ionization mode with a source temperature of 270°C and an electron energy of 70 V. The linear scan program was from *m/z* 50 to 650 in 1.5 s. The direct exposure probe current was programmed linearly from 0 to 3 A in 120 s.

The ^1H nuclear magnetic resonance (NMR) measurements were carried out at 500 MHz and 29°C on a model AM500 spectrometer (Bruker Instruments, Billerica, Mass.). Metabolites were dissolved in 0.6 ml of deuterated acetone. In some cases, 10 μl of D_2O was added to remove exchangeable protons before NMR spectral measurements. Chemical shifts are reported in parts per million by assigning the acetone resonance to 2.05 ppm. Typical spectral acquisition conditions were as follows: data size, 32K; flip angle, 80°; sweep width, 7 kHz; and relaxation delay, 0 s, except for spectra recorded under quantitative conditions, for which a 10-s relaxation delay was used. The number of scans varied from 32 to 300 for the glucosides and from 200 to 1,000 for the phenols. Typical conditions for nuclear Overhauser effect (NOE) measurements were as follows: relaxation delay, 6 s; presaturation time, 1.5 s; and number of scans, 1,000. Data were processed with an exponential function that resulted in line broadening of 0.4 Hz. For coupling constant measurements and examination of fine structure, data were zero filled to 64K and processed with a Lorentzian-to-Gaussian resolution enhancement by using Bruker parameters of −0.3 and 0.17. In cases with extensive overlap and tight coupling, computer simulation, with standard Bruker software, was used to measure spectral parameters.

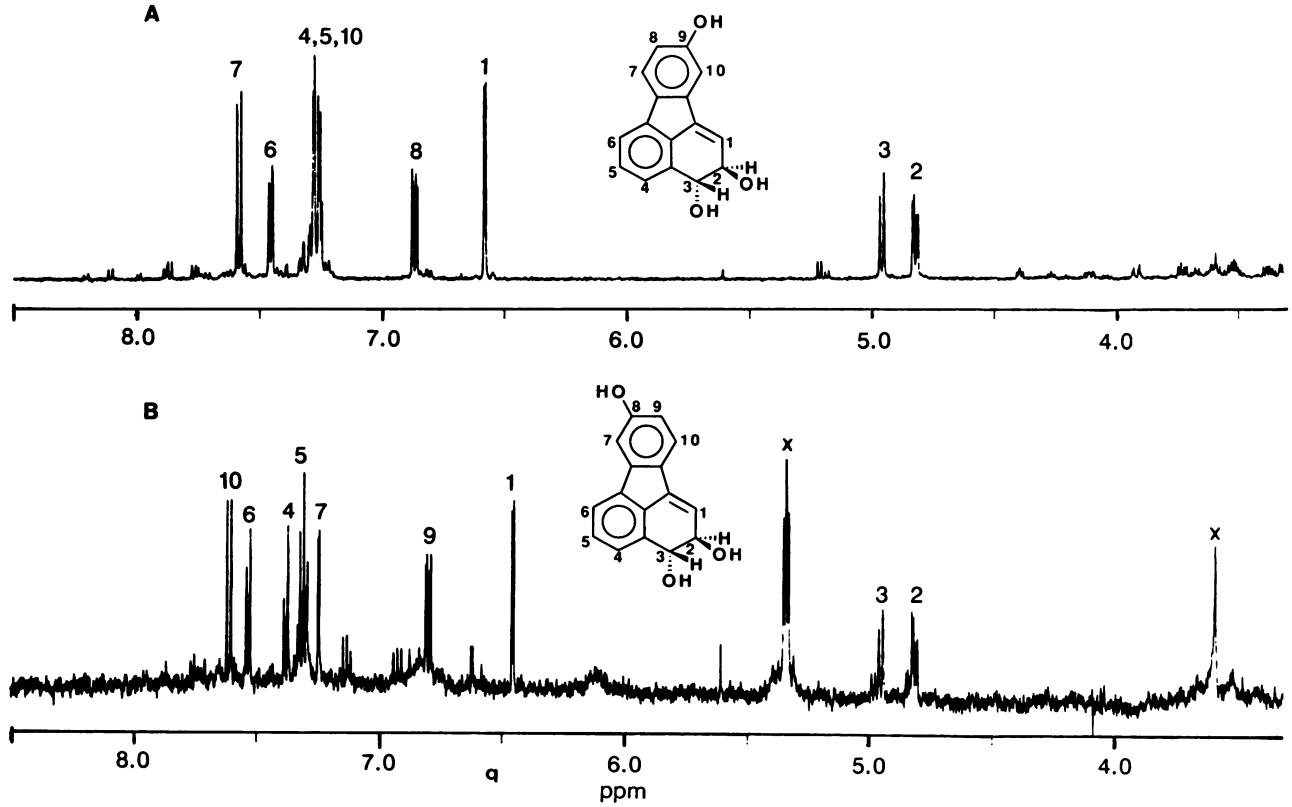

FIG. 3. The 500-MHz ^1H NMR spectra of 9-hydroxy-*trans*-2,3-dihydroxy-2,3-dihydrofluoranthene (metabolite I) (A) and 8-hydroxy-*trans*-2,3-dihydroxy-2,3-dihydrofluoranthene (metabolite III) (B).

TABLE 2. ^{1}H NMR chemical shifts (δ) of FA metabolites

Chemical shift	δ (ppm)					
	FA (VI)	Fluoranthene *trans*-2,3-dihydrodiol (IV)	3-(8-Hydroxyfluoranthene)-β-glucopyranoside[a] (II)	3-Fluoranthene-β-glucopyranoside[b] (V)	9-Hydroxyfluoranthene *trans*-2,3-dihydrodiol (I)	8-Hydroxyfluoranthene *trans*-2,3-dihydrodiol (III)
1	8.09	6.70	7.77	7.97	6.61	6.49
2	7.70	4.87	7.29	7.34	4.83	4.81
3	7.93	5.02			4.97	4.96
4	7.93	7.42	8.21	8.24	7.30	7.39
5	7.70	7.36	7.62	7.67	7.28	7.32
6	8.09	7.64	8.01	8.10	7.48	7.55
7	8.03	7.82[c]	7.47	8.00[c]	7.61	7.26
8	7.42	7.33[d]		7.35[d]	6.89	
9	7.42	7.40[d]	6.87	7.39[d]		6.82
10	8.03	7.81[c]	7.73	7.94[c]	7.27	7.63

[a] The chemical shifts (ppm) for the glucose moiety are as follows: 1', 5.18; 2', 3.67; 3', 3.59; 4', 3.51; 5', 3.60; 6a', 3.91; 6b', 3.73.
[b] The chemical shifts (ppm) for the glucose moiety are as follows: 1', 5.23; 2', 3.69; 3', 3.60; 4', 3.51; 5', 3.62; 6a', 3.92; 6b', 3.73.
[c,d] Assignments may be reversed.

TABLE 3. Three-bond coupling constants (*J*) of FA metabolites

Coupling constant	*J* (Hz)					
	FA (VI)	Fluoranthene *trans*-2,3-dihydrodiol (IV)	3-(8-Hydroxyfluoranthene)-β-glucopyranoside (II)[a,b]	3-Fluoranthene-β-glucopyranoside (V)[a,b]	9-Hydroxyfluoranthene *trans*-2,3-dihydrodiol (I)	8-Hydroxyfluoranthene *trans*-2,3-dihydrodiol (III)
1–2	7.0	3.2	7.5	7.7	3.2	3.2
2–3	8.2	8.0			8.0	8.0
4–5	8.2	7.5	8.4	8.4	7.5	7.5
5–6	7.0	7.5	6.9	6.9	7.5	7.5
7–8	7.6	7.5		7.1	8.2	
8–9	7.5	7.5		7.4		
9–10	7.6	7.5	8.2	7.3		8.2

[a] Coupling constants for the glucose moiety are in agreement with those characterized previously (5).
[b] The benzylic coupling constants (Hz) are as follows: J_{3-4}, −0.8; J_{3-6}, −0.8.

Otherwise, spectral parameters were first-order measurements. Resonance assignments are based on chemical shift and coupling constant measurements, integrations, selective decoupling experiments, long-range coupling constants, NOE measurements, and two-dimensional NMR measurements (COSY). The ability of hydroxy protons of the dihydrodiols to exchange with D_2O was a further basis for assigning structures. Assignments for the parent compound FA follow those previously reported for FA (2, 14).

RESULTS

Accumulation of FA metabolites. The reversed-phase HPLC elution profile of the ethyl acetate-extractable metabolites formed by incubation of [3-^{14}C]FA with *C. elegans* is shown in Fig. 1. FA was metabolized to five principal compounds, which eluted at 18.0, 19.5, 21.8, 29.0, and 30.3 min, and they are referred to as compounds I, II, III, IV, and V, respectively. FA eluted at 46.0 min.

Figure 2 illustrates the disappearance of FA and the production of ethyl acetate-extractable metabolites by *C. elegans* at 24-h intervals during 120 h of incubation. Of the total radioactivity added to the culture, 40% was recovered in the organic phase. At time zero extraction, 85% of the recovered radioactivity accounted for FA. However, FA decreased rapidly to 7.5% within 48 h. Metabolites I, II, III, and V accumulated to maximum levels at 48 and 72 h of incubation, ranging from 3.5 to 44.9% and 4.2 to 32.1% of the total radioactivity recovered by ethyl acetate-extractable metabolites, respectively. Figure 2 also illustrates that there was no apparent sequential pattern of metabolite production.

Identification of FA metabolites. The UV-visible absorption spectra for metabolites I and III showed absorption maxima at 227, 254, 263, 289, and 317 nm and at 234, 241, 253, 262, and 316 nm, respectively. EI mass spectra for FA and five metabolites are given in Table 1. EI mass spectra for metabolite I revealed that the molecular ion (*m/z* 252) [M^+] had characteristic fragment ions at *m/z* 234 [$M^+ - H_2O$] and at *m/z* 206 [$M^+ - H_2O - CO$]. Metabolite III appeared similar to metabolite I, except that the *m/z* 218 ion was not intense. The presence of the more intense *m/z* 221 ion suggested that this metabolite could be an isomer of metabolite I (Table 1). The HPLC retention times, UV-visible absorption, and mass spectra suggested that metabolites I and III were phenolic derivatives of fluoranthene *trans*-2,3-dihydrodiols.

The structural identification of metabolites I and III was done by NMR analyses. The NMR spectra for I and III are shown in Fig. 3A and B. The chemical shifts and coupling constants of the five principal metabolites and the parent compound FA are given in Tables 2 and 3, respectively. The principal methods used to elucidate the structures are illustrated for metabolite I, which was the most difficult one to analyze because of resonance overlap and multiple substitutions. Metabolite I has two proton resonances in the region

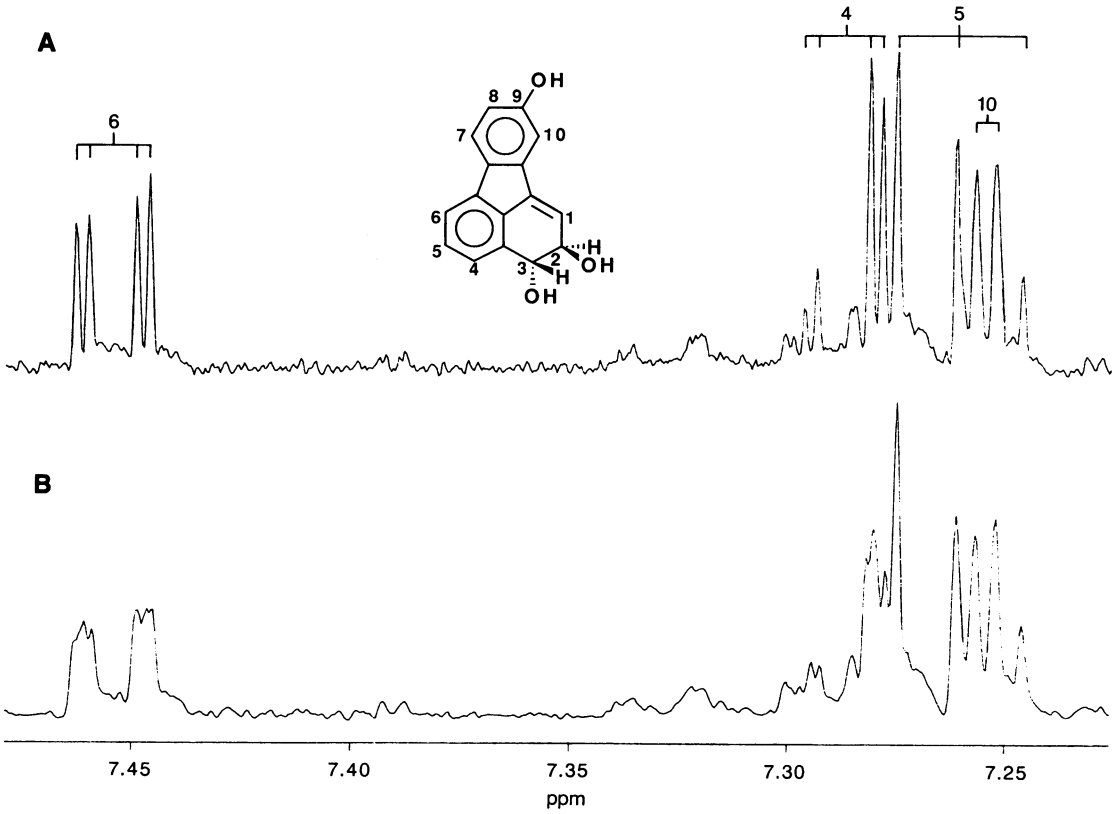

FIG. 4. (A) Expansion of resolution-enhanced segment of 500-MHz ^1H NMR spectrum of 9-hydroxy-*trans*-2,3-dihydroxy-2,3-dihydrofluoranthene obtained with selective decoupling of H_3 showing removal of benzylic couplings to H_4 and H_6. (B) Same as panel A, except obtained without decoupling.

characteristic of dihydrodiols (Fig. 3A), which sharpen upon addition of D_2O because of the exchange of hydroxy protons (not shown). Selective decoupling of one of the dihydrodiol protons (H_3) reveals two small long-range benzylic couplings with H_4 and H_6 in resolution-enhanced spectra (Fig. 4A and B). This can only occur with a 2,3 substitution. This method for resonance assignment has previously been demonstrated for dihydrodiols (13). The benzylic couplings also confirm that the adjacent ring system containing H_4, H_5, and H_6 is unsubstituted. The assignments in the tightly coupled part of the NMR spectrum (Fig. 4A and B) were confirmed by computer simulation. The presence of an additional substitution in the remaining ring system is demonstrated by the coupling pattern and the lack of one aromatic resonance. The key to determining the site of this substitution is an NOE measurement. Selective saturation of H_1 results in an NOE at the adjacent protons H_2 and H_{10} (Fig. 5A). Since H_{10} lacks an *ortho* coupling (Fig. 5A) and retains a *meta* coupling (Fig. 4B), substitution at C-9 is clearly indicated. The large upfield shifts of H_8 and H_{10}, compared with FA or fluoranthene 2,3-dihydrodiol, are characteristic of *ortho* effects of a phenol (Tables 2 and 3). Impurities in the sample, readily seen in the NMR spectra, did not interfere with the analysis. The J_{1-2} and J_{2-3} couplings of the dihydrodiol ring are 3.2 and 8.0 Hz, respectively, which are identical to those for synthetic fluoranthene *trans*-2,3-dihydrodiol (1). We therefore concluded that metabolite I was 9-hydroxyfluoranthene *trans*-2,3-dihydrodiol. Since coupling constants depend on conformation, we also concluded that the 9-hydroxy substitution did not alter the conformation of the dihydrodiol ring.

Procedures similar to those described above were used to elucidate structures and assign resonances of the other two dihydrodiol metabolites III and IV (Tables 2 and 3). Metabolite III (Fig. 3B) was found to be 8-hydroxyfluoranthene *trans*-2,3-dihydrodiol based on nearly identical values of the coupling constants J_{1-2} and J_{2-3}. The coupling constants differ from those of anthracene *trans*-dihydrodiols (6) because of a difference in the time-averaged conformation. Without a comparison with the synthetic standard (1), there would have been uncertainty in the distinction between possible *cis* and *trans* isomers. ^1H NMR spectral parameters of the metabolite IV, fluoranthene *trans*-2,3-dihydrodiol (Table 2), are consistent with those previously reported for the synthetic standard (1), although in the latter case many of the resonances were not resolved and no attempt was made to assign them.

Metabolite II appeared to be most concentrated (9.9 to 16.1%) at 72 h of incubation (Fig. 2). The UV-visible absorption spectrum of this metabolite showed absorption maxima at 225, 237, 273, 301, and 321 nm. Figure 6A shows the mass spectra obtained by direct-probe mass spectral analyses of metabolite II. A weak molecular ion was observed at *m/z* 396 [M^+] (Table 1). Strong fragment ions were observed at *m/z* 234 [M^+ —Glu], 205 [M^+ —Glu —HCO], and 176 [M^+ —Glu —HCO —HCO]. These strong lower-molecular-weight ions indicate a PAH diphenol.

Acetylation of metabolite II and subsequent EI mass spectral analyses confirmed that metabolite II was a glucoside conjugate of phenol. A weak molecular ion at *m/z* 606 [M^+] indicated the addition of five acetyl groups. Character-

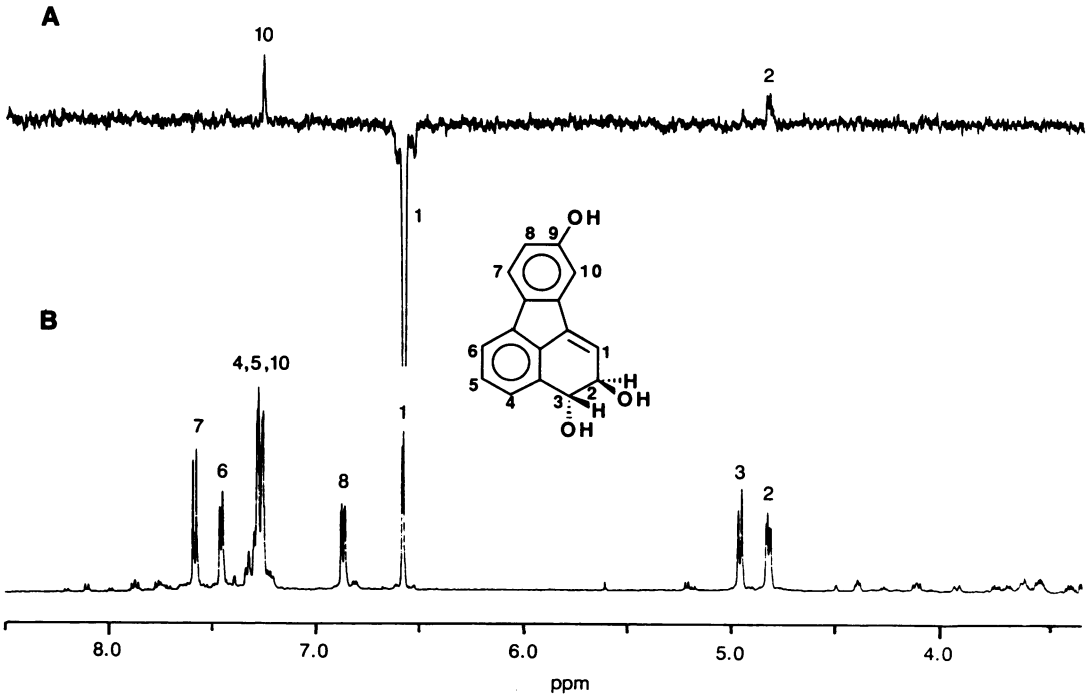

FIG. 5. (A) NOE difference spectrum of 9-hydroxy *trans*-2,3-dihydroxy-2,3-dihydrofluoranthene resulting from selective saturation of H_1 and showing NOEs for H_2 and H_{10}. (B) The 500-MHz ^1H NMR spectrum of the same compound is shown with the structure and resonance assignments.

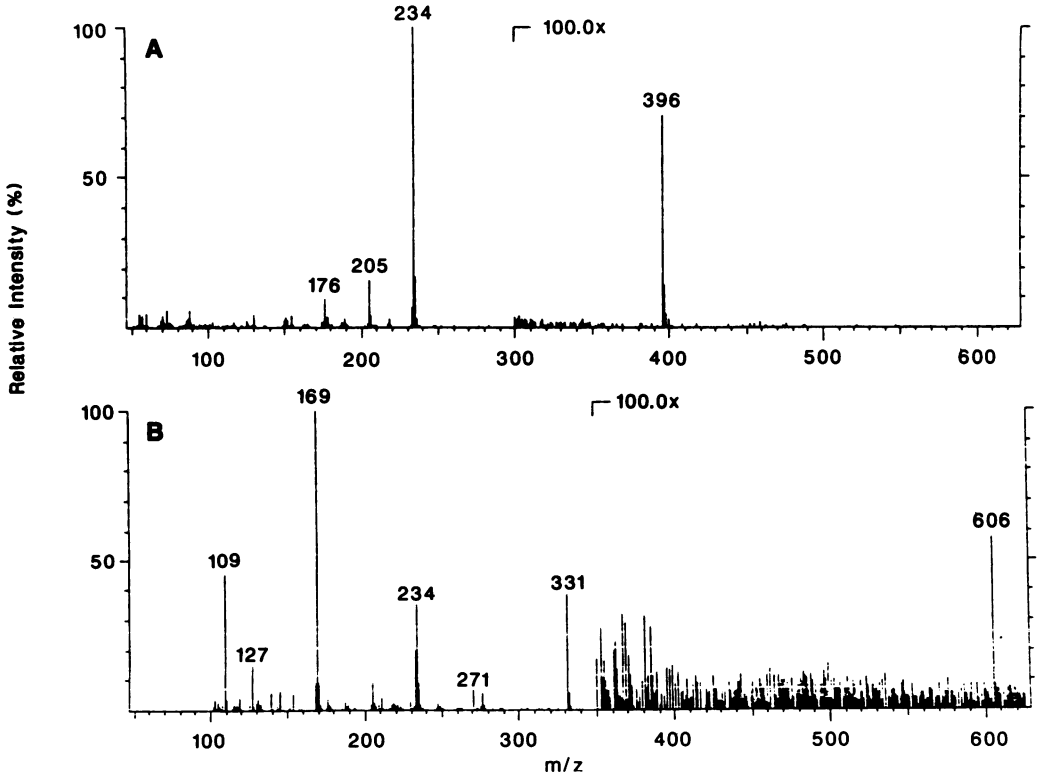

FIG. 6. Mass spectra of metabolite II produced from *C. elegans* cultures exposed to FA (A) and its acetylated derivative (B).

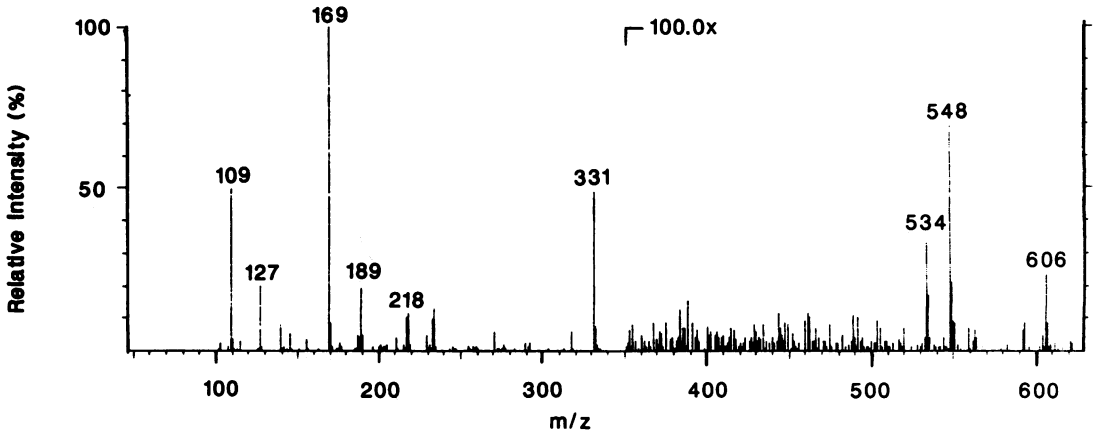

FIG. 7. Mass spectrum of acetylated derivative of metabolite V produced from *C. elegans* cultures exposed to FA.

istic ions for the acetylated glucose moiety were observed at *m/z* 331, 169, and 109 (27) (Table 1; Fig. 6B).

Metabolite V accounted for 44.8% of the total recovered radioactivity by ethyl acetate-extractable residue (Fig. 2) after 120 h of incubation. The UV-visible absorption spectrum of metabolite V showed absorption maxima at 225, 238, 282, 293, and 362 nm. This metabolite showed a very weak molecular ion at *m/z* 380 [M^+], with additional ions at *m/z* 218 [M^+ —Glu] and *m/z* 189 [M^+ —Glu —HCO] (Table 1). These strong, intense, lower-molecular-weight ions indicated a hydroxyfluoranthene. Also, in conjunction with the higher-molecular-weight weak ion of *m/z* 380, the spectrum indicated a glucoside of the hydroxyfluoranthene. Acetylation of metabolite V and subsequent EI confirmed that the compound was a glucoside conjugate. An apparent molecular ion was observed at *m/z* 548 [M^+], with typical acetylated glucose fragment ions at *m/z* 331, 169, and 109. Ions were also observed at *m/z* 218 [M^+ — Glu] and 189 [(M^+ —Glu, —[HCO] for the free phenol (27). Thus, a molecular weight of 548 was consistent with four acetyl groups attached to the glucose moiety (Fig. 7). Apparent impurity ions were also observed at *m/z* 606 and 534 and appeared to be very minor since these impurities showed up only at a magnification of ×100.

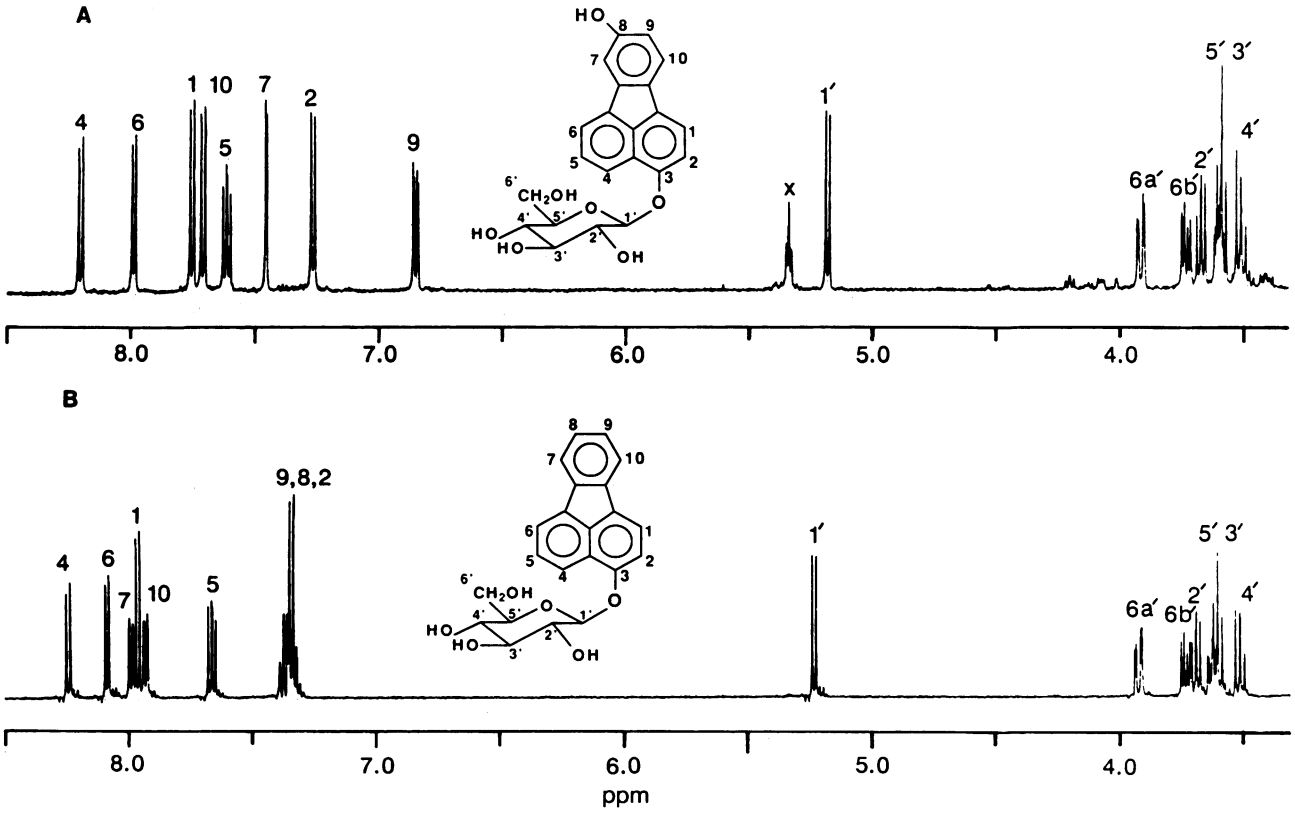

FIG. 8. The 500-MHz ^1H NMR spectra of metabolite II (A) and metabolite V (B) shown with the structure and resonance assignments.

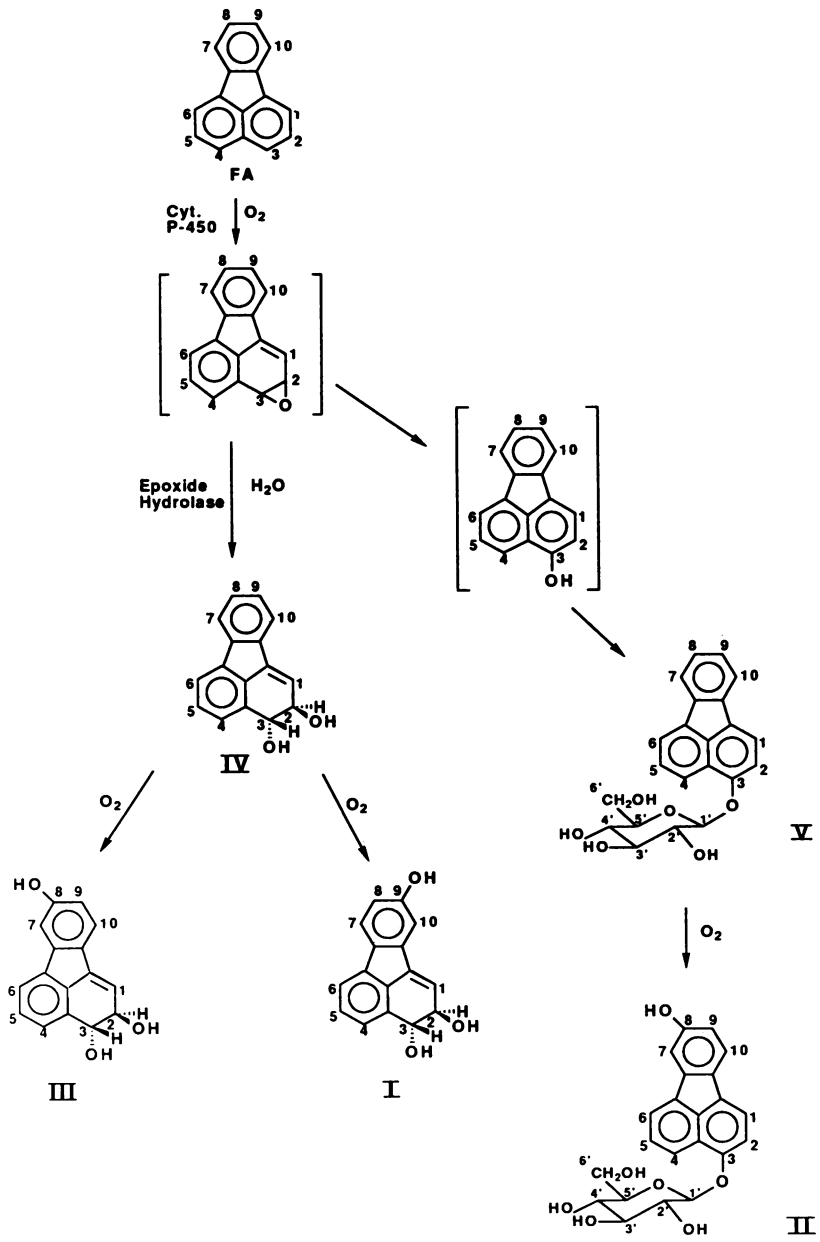

FIG. 9. Proposed pathways for the metabolism of FA by *C. elegans*. Structures shown in brackets are proposed intermediates and have not been characterized.

Further structural analyses were conducted by NMR to determine the identity of metabolites II and V. These metabolites gave aliphatic resonances in regions characteristic of aryl glucosides (Fig. 8A and B). The presence of a β-glucopyranose ring was confirmed by accurate measurement of the aliphatic coupling constants, by using procedures previously described for a phenanthrene glucopyranoside conjugate (5). The lack of an effect on the H_1 resonance in D_2O exchange experiments also confirmed the H_1 position of the glucose moiety as the site of substitution. It was apparent from the coupling pattern in the aromatic region (Fig. 8A and B) that the glucose was substituted at either the C-1 or C-3 position of the FA ring. This distinction was made by indirect methods. Our previous work on phenanthrene 1-β-glucoside had shown that glucose substitution caused a 0.6-ppm upfield shift of the aromatic proton *peri* to the substitution as well as an 0.9-Hz increase in the adjacent *ortho* coupling constant. Similar changes in the corresponding resonances of both of the FA glucosides are uniquely consistent with substitution at the C-3 position of FA for both glucosides (Table 2). Interestingly, saturation of the glucose H_1 resonance produced a large NOE at H_2 on the FA ring but no detectable NOE for H_4, which indicates that the conformation is such that H_1 is closer to H_2 than to H_4. One of the glucosides (metabolite II) had a hydroxy group at the C-8 position, based on an NOE to H_{10} when H_1 was saturated, a measurement similar to those shown in Fig. 5A and B. We observed that the NOEs between these protons

are much smaller than for bay-region protons in the phenanthrene glucopyranoside because of the closer position of protons in a bay region (5).

A minor metabolite, IV, accounted for 5.1% of the total recovered radioactivity during the 96-h exposure period. The UV-visible absorption spectrum of metabolite IV showed absorption maxima at 228, 248, 258, 276, and 307 nm. The UV-visible spectral characteristics were identical to those reported for synthetic fluoranthene *trans*-2,3-dihydrodiol (1). Direct-probe mass spectral analysis of metabolite IV produced molecular ions at *m/z* 236 [M^+], 218 [$M^+ - H_2O$], 190 [$M^+ - H_2O - CO$], and 189 [$M^+ - H_2O - HCO$], which indicated a PAH dihydrodiol (Table 1). The proton NMR spectra parameters (Table 2), including the measured coupling constants $J_{1,2}$ and $J_{2,3}$ (Table 3), were consistent with a PAH dihydrodiol. Thus, metabolite IV was found to be fluoranthene *trans*-2,3-dihydrodiol.

DISCUSSION

The proposed pathways for the metabolism of FA by *C. elegans* are given in Fig. 9. The initial oxidative attack on FA by *C. elegans* occurred predominantly at the 2,3 position, resulting in the formation of fluoranthene *trans*-2,3-dihydrodiol. This species and other fungi have been found to oxidize PAHs via cytochrome P-450 and catalyze monooxygenase and epoxide hydrolase reactions to form *trans*-dihydrodiols (6, 8). It is postulated that further oxidation of fluoranthene *trans*-2,3-dihydrodiol resulted in the formation of two stereoisomers, 9-hydroxyfluoranthene and 8-hydroxyfluoranthene *trans*-2,3-dihydrodiols. Phenolic derivatives of the dihydrodiols accounted for 24% of the total radioactive organic-soluble metabolites.

Rat liver microsomes also form fluoranthene *trans*-2,3-dihydrodiol, which is mutagenic toward *Salmonella typhimurium* (1, 24, 25). Babson et al. (1) found the major metabolite in rat liver microsomes to be fluoranthene *trans*-2,3-dihydrodiol. Several researchers have reported multistep processes occurring during the metabolism of FA, which contains baylike regions (24–26). These processes are similar to the multistep bioactivation process of other PAHs, which are catalyzed by cytochrome P-450 and epoxide hydrolase and form mutagenic and carcinogenic bay-region dihydrodiol epoxides (12, 26, 29). Babson et al. (1) therefore suggest that epoxide hydrolase plays a key role in converting fluoranthene 2,3-oxide to the fluoranthene *trans*-2,3-dihydrodiol, a major metabolite and a proximate mutagen in animal systems. Additionally, oxidation at the 2,3 position also yields the potentially mutagenic and toxic fluoranthene 2,3-quinone (1).

Although the 2,3 double bond of FA is the site of enzymatic attack by *C. elegans* to form *trans*-dihydrodiols, the formation of 3-hydroxyfluoranthene with subsequent glycosylation is the major pathway found in FA metabolism by *C. elegans*. The two glucoside conjugates, identified as 3-fluoranthene-β-glucopyranoside and 3-(8-hydroxyfluoranthene)-β-glucopyranoside, together accounted for 52% of the total ethyl acetate-extractable metabolites. Previous studies on fungal metabolism of PAHs have shown conjugation reactions in metabolic pathways leading to detoxification (11). Our study indicates that glycosylation of hydroxylated FA metabolites may be important in the detoxification of FA-contaminating wastes. This study indicates that *C. elegans*, unlike mammalian systems but similar to procaryotic systems, metabolizes FA rapidly and that the predominant metabolic pathway is beneficial for detoxification of this ubiquitous pollutant.

LITERATURE CITED

1. **Babson, J. R., S. E. Russo-Rodriguez, R. V. Wattley, P. L. Bergstein, W. H. Rastetter, H. L. Liber, B. M. Andon, W. G. Thilly, and G. N. Wogan.** 1986. Microsomal activation of fluoranthene to mutagenic metabolites. Toxicol. Appl. Pharmacol. **85**:355–366.
2. **Bartle, K. D., D. W. Jones, and J. E. Pearson.** 1967. High-resolution proton magnetic resonance spectra of fluoranthene, benzo[k]fluoranthene, and benzo[ghi]fluoranthene. J. Mol. Spectrosc. **24**:330–344.
3. **Barnsley, E. A.** 1975. The bacterial degradation of fluoranthene and benzo[a]pyrene. Can. J. Microbiol. **21**:1004–1008.
4. **Bumpus, J. A.** 1989. Biodegradation of polycyclic aromatic hydrocarbons by *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **55**:154–158.
5. **Cerniglia, C. E., W. L. Campbell, J. P. Freeman, and F. E. Evans.** 1989. Identification of a novel metabolite in phenanthrene metabolism by the fungus *Cunninghamella elegans*. Appl. Environ. Microbiol. **55**:2275–2279.
6. **Cerniglia, C. E., W. L. Campbell, P. P. Fu, J. P. Freeman, and F. E. Evans.** 1990. Stereoselective fungal metabolism of methylated anthracenes. Appl. Environ. Microbiol. **56**:661–668.
7. **Cerniglia, C. E., J. P. Freeman, G. L. White, R. F. Heflich, and D. W. Miller.** 1985. Fungal metabolism and detoxification of the nitropolycyclic aromatic hydrocarbon 1-nitropyrene. Appl. Environ. Microbiol. **50**:649–655.
8. **Cerniglia, C. E., and M. A. Heitkamp.** 1989. Microbial degradation of polycyclic aromatic hydrocarbons in the aquatic environment, p. 41–63. *In* U. Varanasi (ed.), Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment, vol. 2. CRC Press, Inc., Boca Raton, Fla.
9. **Cerniglia, C. E., D. W. Kelly, J. P. Freeman, and D. W. Miller.** 1986. Microbial metabolism of pyrene. Chem. Biol. Interact. **57**:203–216.
10. **Cerniglia, C. E., D. W. Miller, S. K. Yang, and J. P. Freeman.** 1984. Effects of a fluoro substituent on the fungal metabolism of 1-fluoronaphthalene. Appl. Environ. Microbiol. **48**:294–300.
11. **Cerniglia, C. E., G. L. White, and R. H. Heflich.** 1985. Fungal metabolism and detoxification of polycyclic aromatic hydrocarbons. Arch. Microbiol. **50**:649–655.
12. **Dipple, A., R. C. Moschel, and C. A. H. Bigger.** 1984. Polynuclear aromatic carcinogens, p. 41–163. *In* C. E. Searle (ed.), Chemical carcinogens, 2nd ed. American Chemical Society, Washington, D.C.
13. **Evans, F. E., P. P. Fu, T. Cairns, and S. K. Yang.** 1981. Long-range coupling constants for structural analysis of complex polycyclic aromatic hydrocarbons by high-field proton magnetic resonance spectrometry. Anal. Chem. **53**:558–560.
14. **Heffernan, M. L., A. J. Jones, and P. J. Black.** 1967. Proton magnetic resonance studies of non-alternate hydrocarbons. Aust. J. Chem. **20**:580–583.
15. **Heitkamp, M. A., and C. E. Cerniglia.** 1988. Mineralization of polycyclic aromatic hydrocarbons by a bacterium isolated from sediment below an oil field. Appl. Environ. Microbiol. **54**:1612–1614.
16. **Heitkamp, M. A., and C. E. Cerniglia.** 1988. Enhancement of polycyclic aromatic hydrocarbon degradation by a *Mycobacterium* sp. in microcosms containing sediment and water from pristine ecosystem. Appl. Environ. Microbiol. **55**:1968–1973.
17. **Heitkamp, M. A., W. Franklin, and C. E. Cerniglia.** 1988. Microbial metabolism of polycyclic aromatic hydrocarbons: isolation and characterization of a pyrene-degrading bacterium. Appl. Environ. Microbiol. **54**:2549–2555.
18. **Jones, K. C., J. A. Stratford, K. S. Waterhouse, and N. B. Vogt.** 1989. Organic contaminants in Welsh soils: polynuclear aromatic hydrocarbons. Environ. Sci. Technol. **23**:540–550.
19. **Keith, L. H., and W. A. Telliard.** 1979. Priority pollutants I—a perspective view. Environ. Sci. Technol. **13**:416–423.
20. **McMillan, D. C., P. P. Fu, J. P. Freeman, D. W. Miller, and C. E. Cerniglia.** 1988. Microbial metabolism and detoxification of 7,12-dimethylbenz[a]anthracene. J. Ind. Microbiol. **3**:211–225.

21. **Millner, G. C., P. P. Fu, and C. E. Cerniglia.** 1986. Microbial transformation of 6-nitrobenzo[a]pyrene. J. Toxicol. Environ. Health **19**:519–530.
22. **Mueller, J. G., P. J. Chapman, B. O. Blattmann, and P. H. Pritchard.** 1990. Isolation and characterization of a fluoranthene-utilizing strain of *Pseudomonas paucimobilis*. Appl. Environ. Microbiol. **56**:1079–1086.
23. **Mueller, J. G., P. J. Chapman, and P. H. Pritchard.** 1989. Action of a fluoranthene-utilizing bacterial community on polycyclic aromatic hydrocarbon components of creosote. Appl. Environ. Microbiol. **55**:3085–3090.
24. **Rastetter, W. H., R. B. Nachbar, S. Russo-Rodriguez, and R. V. Wattley.** 1982. Fluoranthene: synthesis and mutagenicity of four diol epoxides. J. Org. Chem. **47**:4873–4878.
25. **Rice, J. E., V. Bedenko, E. J. LaVoie, and D. Hoffman.** 1982. Studies on the metabolism of fluoranthene, 2-methylfluoranthene and 3-methylfluoranthene, p. 1009–1020. *In* M. Cook and A. J. Dennis (ed.), Polycyclic aromatic hydrocarbons: formation, metabolism and measurement. Battelle Press, Columbus, Ohio.
26. **Sims, P., and P. L. Grover.** 1981. Involvement of dihydrodiols and diolepoxides in the metabolic activation of polycyclic hydrocarbons other than benzo[a]pyrene, p. 117–181. *In* H. V. Gelboin and P. O. P. Tso (ed.), Polycyclic hydrocarbons and cancer, vol. 3. Academic Press, Inc., New York.
27. **Waller, G. R., and O. C. Dermer.** 1980. Biochemical applications of mass spectrometry, suppl. 1, p. 303. John Wiley & Sons, Inc., New York.
28. **Weissenfels, W. D., M. Beyer, and J. Klein.** 1990. Degradation of phenanthrene, fluorene and fluoranthene by pure bacterial cultures. Appl. Microbiol. Biotechnol. **32**:479–484.
29. **Wood, A. W., W. Levin, R. L. Chang, H. Yagi, D. R. Thakker, R. E. Lehr, D. M. Jerina, and A. H. Conney.** 1979. Polycyclic aromatic hydrocarbons, p. 531–551. *In* P. W. Jones and P. Lever (ed.), Third international symposium on chemistry and biology—carcinogenesis and mutagenesis. Ann Arbor Science Publishers, Ann Arbor, Mich.