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Identification of Metabolites from the Degradation of Fluoranthene by *Mycobacterium* sp. Strain PYR-1

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***Mycobacterium* sp. strain PYR-1, previously shown to extensively mineralize high-molecular-weight polycyclic aromatic hydrocarbons in pure culture and in sediments, degrades fluoranthene to 9-fluorenone-1-carboxylic acid. In this study, 10 other fluoranthene metabolites were isolated from ethyl acetate extracts of the culture medium by thin-layer and high-performance liquid chromatographic methods. On the basis of comparisons with authentic compounds by UV spectrophotometry and thin-layer chromatography as well as gas chromatography-mass spectral and proton nuclear magnetic resonance spectral analyses, the metabolites were identified as 8-hydroxy-7-methoxyfluoranthene, 9-hydroxyfluorene, 9-fluorenone, 1-acenaphthenone, 9-hydroxy-1-fluorenecarboxylic acid, phthalic acid, 2-carboxybenzaldehyde, benzoic acid, phenylacetic acid, and adipic acid. Authentic 9-hydroxyfluorene and 9-fluorenone were metabolized by *Mycobacterium* sp. strain PYR-1. A pathway for the catabolism of fluoranthene by *Mycobacterium* sp. strain PYR-1 is proposed.**

Fluoranthene is one of the principal polycyclic aromatic hydrocarbons (PAHs) in contaminated sediments (12). Since PAHs such as fluoranthene are increasingly widespread environmental pollutants and have been shown to be cytotoxic, mutagenic, and, in many cases, carcinogenic (4–6, 20–23, 27, 34–37), there is considerable interest in the fate of these compounds in the environment. Although microbial metabolic pathways for the degradation of PAHs containing up to three rings have been proposed (7, 11), until recently, little information has been available concerning the ability of microorganisms to metabolize the larger, more recalcitrant, high-molecular-weight PAHs (28). Biotransformation of the tetracyclic PAH fluoranthene by eukaryotes has been reported previously (1, 2, 31, 32), and bacteria that are able to oxidize or cooxidize fluoranthene have been isolated (3, 10, 13, 24, 26, 29, 30, 38, 39).

Research in this laboratory has demonstrated the ability of *Mycobacterium* sp. strain PYR-1 (15–18, 25, 33) to substantially mineralize fluoranthene in pure culture (24, 26) and significantly enhance mineralization of fluoranthene in sediments containing indigenous microorganisms (24). Recently, we reported the isolation and characterization of 9-fluorenone-1-carboxylic acid resulting from the oxidation and subsequent ring fission of fluoranthene by *Mycobacterium* sp. strain PYR-1 (26). In this report, additional metabolites are identified and the possible modes of degradation of fluoranthene by *Mycobacterium* sp. strain PYR-1 are discussed.

MATERIALS AND METHODS

Chemicals. Fluoranthene was purchased from Fluka AG, Buchs, Switzerland. 9-Hydroxyfluorene, 9-fluorenone, 9-hydroxy-1-fluorenecarboxylic acid, and 9-fluorenone-1-carboxylic acid were obtained from Aldrich Chemical Co., Milwaukee, Wis. Bacteriological media and reagents were purchased from Difco Laboratories, Detroit, Mich.; all solvents and other chemicals used were of the highest purity available.

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Culture conditions. The degradation of fluoranthene by *Mycobacterium* sp. strain PYR-1 has been described in detail previously (24). To monitor the disappearance of 9-fluorenone-1-carboxylic acid, 9-fluorenone, and 9-hydroxyfluorene, fluoranthene-adapted *Mycobacterium* cultures were incubated at 24°C in 125-ml flasks containing 30 ml of mineral salts medium with nutrients (18) and 250 μg of each of the test chemicals. Autoclaved controls were used in all experiments. The flasks were incubated in the dark for 1 week with shaking at 150 rpm. For each chemical tested, three replicate flasks and one control flask were sacrificed at various time intervals and extracted with ethyl acetate (24).

Physical and chemical analyses. Reversed-phase high-pressure liquid chromatographic (HPLC) analysis was performed with two HPLC pumps (model 100A; Beckman Instrument Co., Berkeley, Calif.), a 5-μm C_{18} Ultrasphere column (4.6 mm by 25 cm), and a spectrophotometer (model 100-40; Hitachi Scientific Instruments, Mountain View, Calif.). The mobile phase was a linear gradient of methanol-water (35 to 95% methanol [vol/vol]) over 40 min at 1.0 ml/min. For separation of acidic metabolites, 1% acetic acid was used in the mobile phase. UV absorbance was measured at 254 nm, and peak areas were integrated with an integrator (model C-R1A; Shimadzu Scientific Instruments, Columbia, Md.).

UV-visible absorption spectra were determined in methanol with a Beckman model DU-7 spectrophotometer. HPLC on-line absorbance spectra were obtained by using a 1040A diode array spectrophotometer (Hewlett-Packard, Inc., Palo Alto, Calif.) attached to the above-mentioned HPLC system.

Fluoranthene metabolites for chemical and physical analyses and identification were isolated as reported previously (26).

For gas chromatography-mass spectrometry (GC-MS) analysis, the acid-extractable residues were derivatized either by acetylation with acetic anhydride and pyridine or by methylation with diazomethane (19).

The metabolites and authentic compounds were analyzed by electron impact mass spectrometry via direct exposure probe (DEP-EI) and GCMS with a Finnigan MAT (San Jose, Calif.) model 4023 quadrupole mass spectrometer. The mass spectrometer was operated at 70 V of electron energy and a 270°C ion source temperature for all analyses. The DEP-EI

TABLE 1. Metabolites identified from the degradation of fluoranthene by *Mycobacterium* sp. strain PYR-1

Compound (metabolite)	Mol wt	UV λ_{max} (nm)	R_f[a]	R_t (min)[b]	
				HPLC	GC-MS
Fluoranthene	202	357, 341, 321, 285, 275, 233	0.93	42.4	
9-Fluorenone-1-carboxylic acid (I)	224	258, 254, 207	0.71	23.8	
9-Hydroxyfluorene (II)	182	269, 233, 225	0.63	25.1	
9-Fluorenone (III)	180	313, 295, 255	0.88	29.7	
7-Methoxy-8-hydroxy-fluoranthene (IV)	248	366, 349, 323, 290, 281, 238	0.68	33.7	
2-Carboxybenzaldehyde (V)	150	282, 272, 231		5.8	
Phthalic acid (VI)	166	278, 225		6.9	9:08
Adipic acid (VII)	146				6:18
9-Hydroxy-1-fluorenecarboxylic acid (VIII)	226	317, 271, 227		24.6	
Phenylacetic acid (IX)	136	257, 229			5:44
Benzoic acid (X)	122	273, 238			8:26
1-Acenaphthenone (XI)	168	250, 343		28.3	

[a] Values were obtained by thin-layer chromatography with the solvent benzene-ethanol (9:1), except for metabolite I for which benzene-acetone-acetic acid (85:15:5) was used.
[b] R_t, retention times were obtained with acidified solvents by HPLC and for methyl esters by GC-MS.

analyses employed a platinum wire probe and a Vacumetrics (Ventura, Calif.) DCI current programmer. The GC-MS analyses used a DB-1 (J & W Scientific, Rancho Cordova, Calif.) capillary column (0.25-mm diameter by 15 m) with 10 lb/in^2 of He head pressure. The column temperature was held at 50°C for 1 min and then programmed to 250°C at 10°C/min. All analyses were performed on samples dissolved in methanol.

^{1}H nuclear magnetic resonance (NMR) spectra were obtained at 500 MHz with a Bruker AM500 spectrometer at 29°C, with a sweep width of 7 or 3 kHz, a datum size of 32,000, a flip angle of 80°, and a relaxation delay of 0 s. For spectra recorded under quantitative conditions, a 10-s relaxation delay was used. The number of scans for the metabolites was typically 600, except when a nuclear Overhauser effect (NOE) experiment was performed on one of the metabolites and 16,000 scans were acquired for each spectrum. A 2-s saturation time was used for the NOE measurement. The metabolites were dissolved in deuterated acetone. The data were processed with a Lorenzian-to-Gaussian resolution enhancement, with Bruker parameters of −0.5 and 0.17, except for the NOE measurement, where exponential filtering that produced line broadening of 2 Hz was used. ^{1}H resonance assignments are based on chemical shift and coupling constant measurements, integrations, selective decoupling experiments, and NOE measurements. ^{1}H chemical shifts are reported in parts per million by assigning the residual acetone resonance to 2.05 ppm.

RESULTS

Isolation and characterization of metabolites. The extensive degradation of fluoranthene to CO_2 has been discussed previously (24). Approximately 78% of the added ^{14}C-fluoranthene was mineralized to $^{14}CO_2$ after 5 days of incubation. Minor amounts of acidic ethyl acetate-soluble metabolites and water-soluble metabolites increased steadily, while metabolites in neutral extracts decreased rapidly in the first 48 h, because of the rapid degradation of fluoranthene. Fluoranthene metabolites were detected in both the acidic and neutral fractions collected from the HPLC eluate. The respective HPLC and GC-MS retention times and UV absorption maxima of these metabolites are presented in Table 1. The acidic ethyl acetate fraction (4%) yielded the predominant metabolite, 9-fluorenone-1-carboxylic acid (0.65%) (Table 1, metabolite I) (26). An HPLC profile of the neutral ethyl acetate extract (20%), containing metabolites II, III, and IV, as well as the remaining fluoranthene, is presented in Fig. 1. These compounds were further purified by thin-layer chromatography, with benzene-ethanol (9:1) as the solvent.

The mass spectrum of metabolite II has a molecular ion at an *m/z* of 182 and fragment ions at *m/z*'s of 181 [$M^+ - 1$], 165 [$M^+ - 17$], 152 [$M^+ - 30$], and 76. The base peak is at an *m/z* of 181. The losses correspond to [$M^+ - H$], [$M^+ - OH$], and [$M^+ - H_2CO$], respectively (Fig. 2a). DEP-EI mass spectral analysis of authentic 9-hydroxyfluorene produced a spectrum similar to that of metabolite II.

The ^{1}H NMR spectrum of metabolite II is in full agreement with the structure of 9-hydroxyfluorene (Fig. 3a). The ^{1}H NMR chemical shifts for metabolite II are δ1 and 8, 7.62 ppm; δ2 and 7, 7.31 ppm; δ3 and 6, 7.37 ppm; δ4 and 5, 7.74 ppm; and δ9, 5.56 ppm. All *ortho* coupling constant *J* values are equal to 7.4 Hz.

Metabolite III was identified as 9-fluorenone by direct comparison of its HPLC retention time and UV (Table 1) and mass spectra with those of an authentic standard. The compound had a molecular ion at an *m/z* of 180 and fragment ions at *m/z*'s of 154 and 74.

Metabolite IV had a UV absorption spectrum similar to that of fluoranthene (Table 1). The R_f value of metabolite IV was 0.68 in benzene-ethanol (9:1). The structure of metabolite IV was determined by MS (Fig. 2b) and ^{1}H NMR spectroscopy (Fig. 3b). DEP-EI analysis of metabolite IV showed an apparent molecular ion [M^+] at an *m/z* of 248, fragment ions at *m/z*'s of 233 [$M^+ - 15$] and 205 [$M^+ - 43$], corresponding to probable losses of CH_3 and CO + CH_3, respectively, and a base peak at an *m/z* of 248. Fragment ions at *m/z*'s of 176, 103, and 88 were also observed for this metabolite. The molecular weight and fragmentation pattern suggested that metabolite IV might be a methoxy derivative of fluoranthene.

The 500-MHz ^{1}H NMR spectrum of metabolite IV consisted of eight aromatic protons and a three-proton singlet in the aliphatic region (Fig. 3b). Decoupling experiments indicated the presence of the three aromatic ring systems of fluoranthene, with substituents at C-7 and C-8. The chemical shift of the aliphatic singlet and the lack of any small long-range couplings to the aromatic protons were consistent with the presence of a methoxyl group and in agreement with

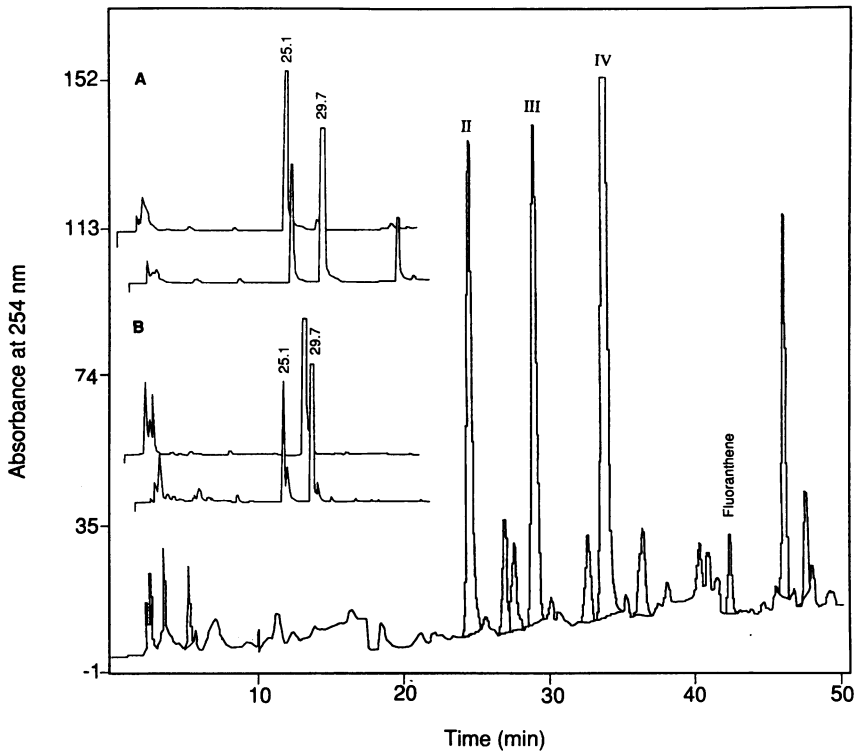

FIG. 1. HPLC profile of the neutral ethyl acetate extract from fluoranthene degradation by *Mycobacterium* sp. strain PYR-1 (Insets) *Mycobacterium* sp. incubated with 9-hydroxyfluorene (A) or 9-fluorenone (B). Lower band, live cells; upper band, autoclaved controls.

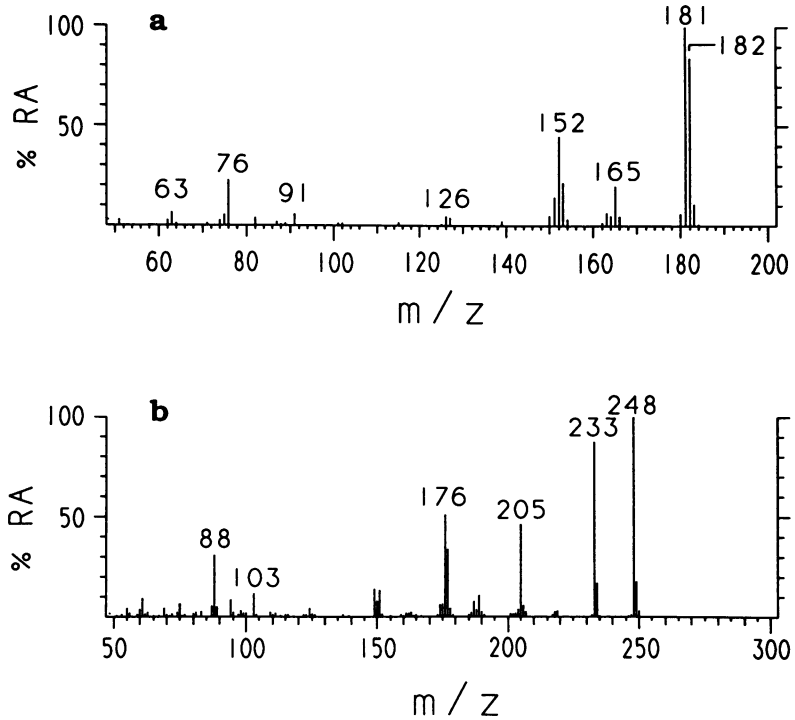

FIG. 2. Mass spectra of 9-hydroxyfluorene (a) and 8-hydroxy-7-methoxyfluoranthene (b) formed from fluoranthene degradation. RA, relative intensity.

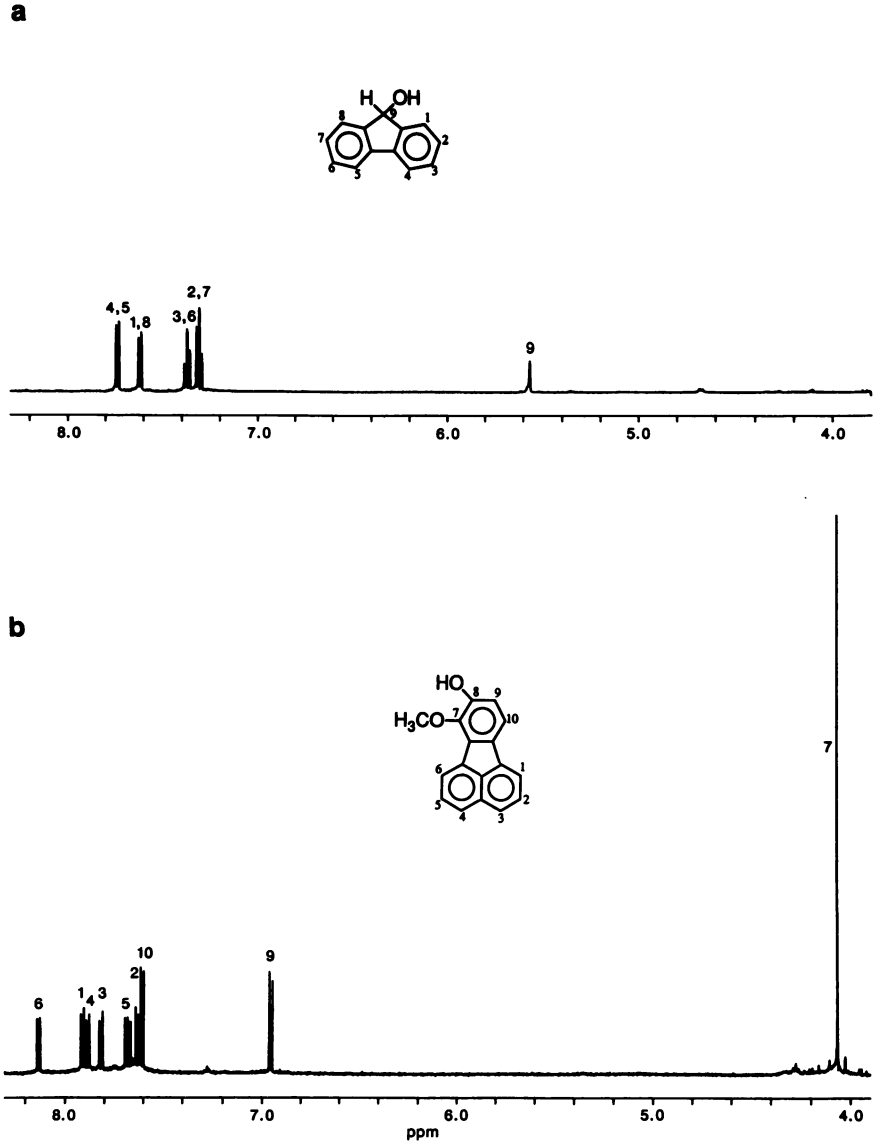

FIG. 3. Resolution-enhanced 500-MHz ^{1}H NMR spectra of 9-hydroxyfluorene (a) and 8-hydroxy-7-methoxyfluoranthene (b) formed from the metabolism of fluoranthene. Each sample was dissolved in deuterated acetone and is shown with the structure and resonance assignments.

the mass spectral data. The key experiment in elucidating the site of the methoxyl group was an NOE on H-6 as a result of selective saturation of the methoxy protons. This established that the methoxyl group was attached at C-7 of the fluoranthene ring system. The large upfield shifts of 0.5 and 0.4 ppm for H-9 and H-10, respectively, compared with the data reported for fluoranthene (31), are in good accord with *ortho*- and *para*-substituent effects from hydroxyl and methoxyl groups. ^{1}H NMR chemical shifts (δ in parts per million) and *ortho*-coupling constants (J in hertz) for metabolite IV are δ1, 7.91; δ2, 7.62; δ3, 7.81; δ4, 7.88; δ5, 7.68; δ6, 8.13; δ9, 6.96; δ10, 7.61; and $J_{1\text{-}2}$, 7.0; $J_{2\text{-}3}$, 8.4; $J_{4\text{-}5}$, 8.4; $J_{5\text{-}6}$, 7.0; $J_{9\text{-}10}$, 8.0, respectively.

Additionally, GC-MS analysis of acetylated and methylated residues of ethyl acetate extracts of the acidified aqueous phase revealed the presence of low-molecular-weight acids, including phthalic acid (metabolite VI). The phthalic acid methyl ester had a molecular ion at an m/z of 194, with fragment ions at m/z's of 77 and 133, and a base peak at an m/z of 163 [$M^+ - OCH_3$]. Additional minor acidic compounds found in the extract were the methyl esters of phenylacetic acid (metabolite IX), benzoic acid (metabolite X), and adipic acid (metabolite VII) (Table 1). The mass spectra of all of these compounds were identified by comparison to the National Institute of Standards and Technology mass spectral library.

Metabolites XI, V, and VIII were identified as 1-acenaphthenone, 2-carboxybenzaldehyde, and 9-hydroxy-1-fluorenecarboxylic acid by comparison of their GC and HPLC retention times and UV (Table 1) and mass spectra to those of authentic standards.

Biotransformation studies. Incubation of *Mycobacterium* sp. strain PYR-1 with 9-fluorenone-1-carboxylic acid resulted in the disappearance of this compound from the medium. The compound did not disappear from autoclaved controls. Although decarboxylation of the carboxylic acid

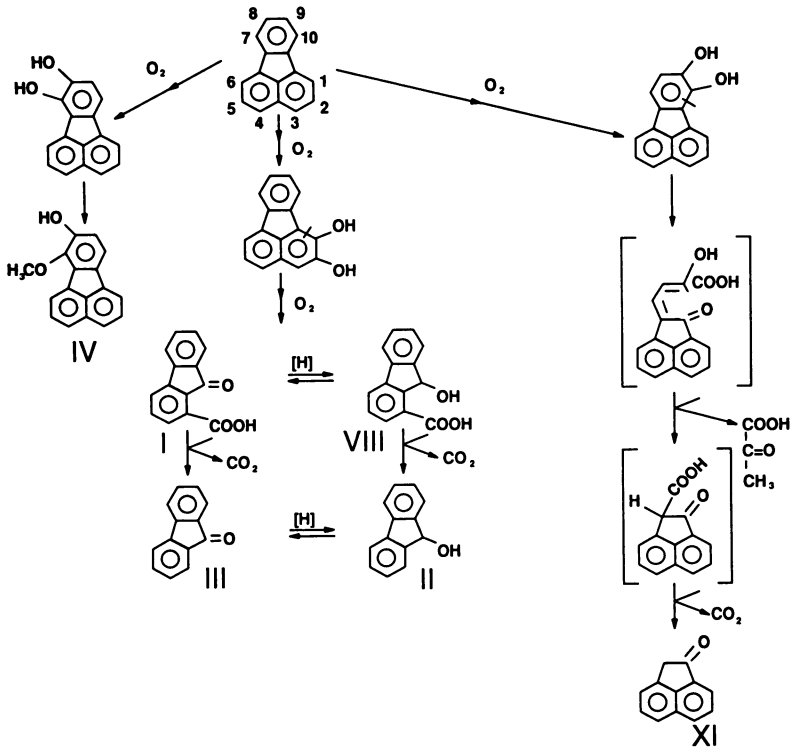

FIG. 4. Pathways proposed for the metabolism of fluoranthene by *Mycobacterium* sp. strain PYR-1. Compounds in brackets were not identified.

moiety should yield 9-fluorenone, neither 9-fluorenone nor 9-fluorenol was found in those extracts because of the rapid metabolism. However, both of these compounds were present in the neutral ethyl acetate extracts from fluoranthene metabolism. When the *Mycobacterium* sp. was incubated with commercially available 9-fluorenone, 9-fluorenol was formed. Conversely, 9-fluorenol incubated with *Mycobacterium* sp. strain PYR-1 resulted in the formation of 9-fluorenone (Fig. 1).

DISCUSSION

The observations that 9-fluorenone-1-carboxylic acid was apparently easily metabolized and did not accumulate to significant amounts, coupled with the metabolites found in the HPLC extracts, suggest that this compound is a metabolite of one of several pathways of fluoranthene mineralization by *Mycobacterium* sp. strain PYR-1.

The formation of 9-fluorenone-1-carboxylic acid (26) indicates an initial attack on the fused aromatic ring portion of the fluoranthene molecule, probably mediated by a dioxygenase in positions 1 and 2 via a dihydroxylated fluoranthene intermediate with subsequent *meta* cleavage of this intermediate (Fig. 4).

Although the abiotic conversion of a 9-fluorenone to 9-hydroxyfluorene is possible in the presence of a strong nucleophile, only minimal abiotic oxidation/reduction was observed in autoclaved controls. Therefore, the reaction was catalyzed enzymatically. The possible action of a reductase is suggested because it has been reported that NADPH–cytochrome P-450 reductase in the presence of NADPH was responsible for the reduction/oxidation of 9-fluorenone ⇆ 9-hydroxyfluorene by rat liver microsomes (8).

The detection of 8-hydroxy-7-methoxyfluoranthene and 1-acenaphthenone also suggests that dihydroxylation of the single benzene ring on the fluoranthene molecule occurred at positions 7,8 and 9,10. Dioxygenation at the 9,10 positions is speculated to lead to mineralization of fluoranthene by *Alcaligenes denitrificans* (39). Weissenfels et al. (39) identified 3-hydroxymethyl-4,5-benzocoumarin and acenaphthenone as intermediate compounds in the mineralization pathway of that bacterium. A similar pathway seems to occur in *Mycobacterium* sp. strain PYR-1. The dehydrogenation of a transient fluoranthene *cis*-7,8-dihydrodiol to form 7,8-dihydroxyfluoranthene with O-methylation at carbon 7 would form 7-methoxy-8-hydroxylfluoranthene. O-methylation of chlorinated phenols by a *Mycobacterium* sp. has been reported previously (14). Alternatively, cytochrome P-450 monooxygenation, followed by enzymatic hydration resulting in *trans*-7,8-dihydro-7,8-dihydroxyfluoranthene, and subsequent dehydrogenation and methylation of the hydroxyl moiety at C-7, could result in the formation of small amounts of 7-methoxy-8-hydroxyfluoranthene (Fig. 4). Monooxygenation is consistent with previous studies of pyrene and naphthalene degradation by *Mycobacterium* sp. strain PYR-1 (18, 25), which suggest the formation of small amounts of *trans*-dihydrodiol metabolites from the metabolism of PAHs. Methylation of a dihydroxylated intermediate could be brought about by a catechol *O*-methyltransferase (9).

The identification and isolation of metabolites that retain the intact fluorene configuration, as well as the identification of an initial oxygenated metabolite in which the single benzene ring has been attacked, indicate that several pathways operate simultaneously on the degradation of fluoranthene by *Mycobacterium* sp. strain PYR-1 (Fig. 4). The

ability of this microorganism to mineralize fluoranthene rapidly has been thoroughly demonstrated previously (24). The relatively low concentrations of metabolites, the rapid evolution of carbon dioxide, and the rapid disappearance of fluoranthene from the medium provide concrete evidence of the usefulness of this *Mycobacterium* sp. strain PYR-1 for bioremediation of some PAHs.

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REFERENCES

1. **Babson, J. R., S. E. Russo-Rodriguez, W. H. Rastetter, and G. N. Wogan.** 1986. *In vitro* DNA-binding of microsomally-activated fluoranthene: evidence that the major product is a fluoranthene N^2-deoxyguanosine adduct. Carcinogenesis **7**:859–865.
2. **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.
3. **Barnsley, E. A.** 1975. The bacterial degradation of fluoranthene and benzo[a]pyrene. Can. J. Microbiol. **21**:1004–1008.
4. **Bos, R. P.** 1987. Fluoranthene, a carcinogen? Mutat. Res. **189**:187.
5. **Bos, R. P., W. J. C. Prinsen, J. G. M. van Rooy, F. J. Jongeneelen, J. L. G. Theuws, and P. T. Henderson.** 1987. Fluoranthene, a volatile mutagenic compound, present in creosote and coal tar. Mutat. Res. **187**:119–125.
6. **Busby, W. F., Jr., M. E. Goldman, P. M. Newberne, and G. N. Wogan.** 1984. Tumorigenicity of fluoranthene in a newborn mouse lung adenoma bioassay. Carcinogenesis **5**:1311–1316.
7. **Cerniglia, C. E., and M. A. Heitkamp.** 1989. Microbial degradation of polycyclic aromatic hydrocarbons (PAH) in the aquatic environment, p. 41–68. *In* U. Varanasi (ed.), Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. CRC Press, Boca Raton, Fla.
8. **Chen, C., R. C. Lefers, E. L. Brough, and D. P. Gurka.** 1981. Interconversion of alcohol and ketone by cytochrome P-450 oxygenase; Fluoren-9-ol ⇄ fluorene-9-one. Fed. Proc. **40**:1540.
9. **Creveling, C. R., and B. K. Hartman.** 1982. Relationships between the cellular localization and the physiological function of catechol-*O*-methyltransferase, p. 479–486. *In* E. Usdin, R. T. Borchardt, and C. R. Creveling (ed.), Biochemistry of *S*-adenosylmethionine and related compounds. Macmillan Press, London.
10. **Foght, J. M., and D. W. S. Westlake.** 1988. Degradation of polycyclic aromatic hydrocarbons and aromatic heterocycles by a *Pseudomonas* species. Can. J. Microbiol. **34**:1135–1141.
11. **Gibson, D. T., and V. Subramanian.** 1984. Microbial degradation of aromatic hydrocarbons, p. 181–252. *In* D. T. Gibson (ed.), Microbial degradation of organic compounds. Marcel Dekker, New York.
12. **Grimmer, G., and F. Pott.** 1983. Occurrence of PAH, p. 61–128. *In* G. Grimmer (ed.), Environmental carcinogens: polycyclic aromatic hydrocarbons. CRC Press, Boca Raton, Fla.
13. **Groenewegen, D., and H. Stolp.** 1976. Mikrobieller Abbau von polyzyklischen aromatischen Kohlenwasserstoffen. Zentralbl. Bakteriol. **162**:225–232.
14. **Häggblom, M. M., L. J. Nohynek, and M. S. Salkinoja-Salonen.** 1988. Degradation and O-methylation of chlorinated phenolic compounds by *Rhodococcus* and *Mycobacterium* strains. Appl. Environ. Microbiol. **54**:3043–3052.
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.** 1989. Polycyclic aromatic hydrocarbon degradation by a *Mycobacterium* sp. in microcosms containing sediment and water from a 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. **Heitkamp, M. A., J. P. Freeman, D. W. Miller, and C. E. Cerniglia.** 1988. Pyrene degradation by a *Mycobacterium* sp.: identification of ring oxidation and ring fission products. Appl. Environ. Microbiol. **54**:2556–2565.
19. **Holder, C. L., W. A. Korfmacher, W. Slikker, H. C. Thompson, and A. B. Gosnell.** 1985. Mass spectral characterization of doxylamine and its rhesus monkey urinary metabolites. Biomed. Mass Spectrom. **12**:151–158.
20. **International Agency for Research on Cancer.** 1983. Polynuclear aromatic compounds, part 1. Int. Agency Res. Cancer Monogr. Eval. Carcinog. Risks Hum. **32**:355–364.
21. **Irvin, T. R., and J. E. Martin.** 1987. *In vitro* and *in vivo* embryotoxicity of fluoranthene, a major prenatal toxic component of diesel soot. Teratology **35**:65A.
22. **Kaden, D. A., R. A. Hites, and W. G. Thilly.** 1979. Mutagenicity of soot and associated polycyclic aromatic hydrocarbons to *Salmonella typhimurium*. Cancer Res. **39**:4152–4159.
23. **Kagan, J., E. D. Kagan, I. A. Kagan, P. A. Kagan, and S. Quigley.** 1985. The phototoxicity of non-carcinogenic polycyclic aromatic hydrocarbons in aquatic organisms. Chemosphere **14**:1829–1834.
24. **Kelley, I., and C. E. Cerniglia.** 1991. The metabolism of fluoranthene by a species of *Mycobacterium*. J. Ind. Microbiol. **7**:19–26.
25. **Kelley, I., J. P. Freeman, and C. E. Cerniglia.** 1991. Identification of metabolites from the degradation of naphthalene by a *Mycobacterium* sp. Biodegradation **1**:283–290.
26. **Kelley, I., J. P. Freeman, F. E. Evans, and C. E. Cerniglia.** 1991. Identification of a carboxylic acid metabolite from the catabolism of fluoranthene by a *Mycobacterium* sp. Appl. Environ. Microbiol. **57**:636–641.
27. **LaVoie, E. J., S. S. Hecht, V. Bedenko, and D. Hoffman.** 1982. Identification of the mutagenic metabolites of fluoranthene, 2-methylfluoranthene and 3-methylfluoranthene. Carcinogenesis **3**:841–846.
28. **Mahaffey, W. R., D. T. Gibson, and C. E. Cerniglia.** 1988. Bacterial oxidation of chemical carcinogens: formation of polycyclic aromatic acids from benz[*a*]anthracene. Appl. Environ. Microbiol. **54**:2415–2423.
29. **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.
30. **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.
31. **Pothuluri, J. V., F. E. Evans, J. P. Freeman, and C. E. Cerniglia.** 1990. Fungal transformation of fluoranthene. Appl. Environ. Microbiol. **56**:2974–2983.
32. **Pothuluri, J. V., R. H. Heflich, P. P. Fu, and C. E. Cerniglia.** 1992. Fungal metabolism and detoxification of fluoranthene. Appl. Environ. Microbiol. **58**:937–941.
33. **Rafii, F., W. R. Butler, and C. E. Cerniglia.** 1992. Differentiation of a rapidly growing, scotochromogenic, polycyclic-aromatic-hydrocarbon-metabolizing strain of *Mycobacterium* sp. from other known *Mycobacterium* species. Arch. Microbiol. **157**:512–520.
34. **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.
35. **Thilly, W. G., J. G. DeLuca, E. E. Furth, H. Hoppe, D. A. Kaden, J. J. Krolewski, H. L. Liber, T. R. Slopek, S. A. Slepikoff, R. J. Tizard, and B. W. Penman.** 1980. Gene-locus mutation assays in diploid human lymphoblast lines. Chem.

Mutagens **6:**331–364. Plenum
36. **Tuveson, R. W., J. Kagan, M. A. Shaw, G. M. Moresco, E. M. v. Behne, H. Pu, M. Bazin, and R. Santus.** 1987. Phototoxic effects of fluoranthene, a polycyclic aromatic hydrocarbon, on bacterial species. Environ. Mol. Mutagen. **10:**245–261.
37. **Van Duuren, B. L., and B. M. Goldschmidt.** 1976. Carcinogenic and tumor-promoting agents in tobacco carcinogenesis. J. Natl. Cancer Inst. **56:**1237–1242.
38. **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.
39. **Weissenfels, W. D., M. Beyer, J. Klein, and H. J. Rehm.** 1991. Microbial metabolism of fluoranthene: isolation and identification of ring fission products. Appl. Microbiol. Biotechnol. **34:**528–535.