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Transformation of 1- and 2-Methylnaphthalene by *Cunninghamella elegans*

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Cunninghamella elegans metabolized 1- and 2-methylnaphthalene primarily at the methyl group to form 1- and 2-hydroxymethylnaphthalene, respectively. Other compounds isolated and identified were 1- and 2-naphthoic acids, 5-hydroxy-1-naphthoic acid, 5-hydroxy-2-naphthoic acid, 6-hydroxy-2-naphthoic acid, and phenolic derivatives of 1- and 2-methylnaphthalene. The metabolites were isolated by thin-layer and reverse-phase high-pressure liquid chromatography and characterized by the application of UV-visible absorption, ^{1}H nuclear magnetic resonance, and mass spectral techniques. Experiments with [8-^{14}C]2-methylnaphthalene indicated that over a 72-h period, 9.8% of 2-methylnaphthalene was oxidized to metabolic products. The ratio of organic-soluble to water-soluble metabolites at 2 h was 92:8, and at 72 h it was 41:59. Enzymatic treatment of the 48-h aqueous phase with either β-glucuronidase or arylsulfatase released 60% of the metabolites of 2-methylnaphthalene that were extractable with ethyl acetate. In both cases, the major conjugates released were 5-hydroxy-2-naphthoic acid and 6-hydroxy-2-naphthoic acid. The ratio of the water-soluble glucuronide conjugates to sulfate conjugates was 1:1. Incubation of *C. elegans* with 2-methylnaphthalene under an $^{18}O_2$ atmosphere and subsequent mass spectral analysis of 2-hydroxymethylnaphthalene indicated that hydroxylation of the methyl group is catalyzed by a monooxygenase.

Naphthalene and methylnaphthalenes are among the most toxic components in the water-soluble fraction of crude and fuel oils (1, 2, 12, 21). These compounds have also been identified in commercial mosquito repellants (22, 23) and in an aromatic hydrocarbon solvent, Aerotex 3470 (18). There has been concern about the occurrence, transport, and environmental fate of naphthalene and methyl-substituted naphthalenes because they have been shown to accumulate in vertebrate and invertebrate freshwater and marine organisms (1, 13). The toxic effects of these compounds could be due to the naphthalene and its methyl-substituted derivatives or one or more of their biotransformation products or both. The mechanism of the toxicity of naphthalene and methyl-substituted naphthalenes has not been determined.

The metabolism of 1- and 2-methylnaphthalene has been investigated in bacteria, cyanobacteria, rats, and rainbow trout (3, 8, 11, 14, 15, 19, 20; C. E. Cerniglia, J. P. Freeman, J. R. Althaus, C. Van Baalen, Arch. Microbiol., in press). Bacterial strains isolated from oil-polluted estuarine waters and various pseudomonads have the ability to grow on 1- and 2-methylnaphthalene as the sole source of carbon and energy. Resting cell suspensions oxidize 1-methylnaphthalene to 3-methylsalicylic acid (8). Treccani and Fiecchi identified 1,2-dihydroxy-1,2-dihydro-7-methylnaphthalene as an intermediate in the oxidation of 2-methylnaphthalene by *Pseudomonas desmolyticum* (19). Williams et al. (20) reported that 2-methylnaphthalene is metabolized in pseudomonads to 4-methylcatechol and further degraded via the *meta* pathway. We recently showed that three cyanobacterial strains grown photoautotrophically in the presence of either 1- or 2-methylnaphthalene oxidized both compounds at the methyl group to form 1- and 2-hydroxymethylnaphthalene, respectively (Cerniglia et al., in press).

Kaubisch et al. (11) reported the in vitro metabolism of 1- and 2-methylnaphthalene with guinea pig hepatic microsomes. Oxidation occurs primarily at the methyl substituent to form 1- and 2-naphthoic acids, respectively. Dihydrodiols and methylnaphthols are minor products. In contrast, Breger et al. (3) showed that hepatic microsomes of rats and rainbow trout metabolize 2-methylnaphthalene to three isomeric dihydrodiols. In addition, phenolic metabolites and their conjugates were also found.

Although the fungal metabolism of naphthalene has been extensively investigated (5–7, 9, 17), similar studies on methyl-substituted naphthalenes have not been reported. The present paper describes the transformation of 1- and 2-methylnaphthalene by the fungus *Cunninghamella elegans*.

(A preliminary report of this study has been published [C. E. Cerniglia and K. J. Lambert, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, O79, p. 252].)

MATERIALS AND METHODS

Microorganism 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 and mycelia from several slants were used to inoculate 30 125-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 a 48-h incubation, the mycelia were removed by aseptic filtration and transferred to 30 sterile 125-ml Erlenmeyer flasks containing 30 ml of Sabouraud dextrose broth. Three milligrams of 1- or 2-methylnaphthalene 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 1- or 2-methylnaphthalene. All of the flasks were incubated at 25°C.

Isolation and detection of 1- and 2-methylnaphthalene metabolites formed by *C. elegans*. At various times, the flask contents from each 1- or 2-methylnaphthalene incubation and control flask were pooled and filtered to separate the broth from the mycelia. The broth and the mycelia were then extracted with 6 volumes of ethyl acetate. The extracts were

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combined and dried over anhydrous sodium sulfate; the solvent was evaporated under reduced pressure at 40°C. The residue from each incubation was dissolved in methanol and analyzed by thin-layer chromatography (TLC) and high-pressure liquid chromatography (HPLC).

TLC was performed on Silica Gel 60 F_{254} glass plates developed with chloroform-acetone (4:1). Starting material and metabolites were detected under UV light (254 nm) and by spraying with Gibbs reagent (2,6-dichloroquinone-4-chloroimide in methanol [2%, wt/vol]). Reverse-phase HPLC was performed with a Beckman system consisting of two model 100A pumps and a model 155-10 variable-wavelength absorbance detector adjusted to 254 nm. A 5-μm C_{18} Ultrasphere ODS column (4.6 mm by 25 cm; Altex Scientific, Berkeley, Calif.) was used, and the separation was achieved with one of three solvent systems. System A consisted of a methanol-water linear gradient (45 to 95%, vol/vol; 30 min) at a flow rate of 1 ml/min. System B consisted of a methanol-water linear gradient (45 to 95%, vol/vol; 30 min) containing 1% acetic acid at a flow rate of 1 ml/min. System C consisted of a methanol-water linear gradient (55 to 45%, vol/vol) containing 1% acetic acid at a flow rate of 1.5 ml/min.

In experiments with [8-^{14}C]2-methylnaphthalene, 0.5-ml fractions were collected at 0.5-min intervals in scintillation vials, and 7.0 ml of Aquasol-2 (New England Nuclear Corp., Boston, Mass.) was added to each vial. The radioactivity present in each fraction was determined in a Beckman LS-250 liquid scintillation counter.

Physical and chemical analyses. UV-visible absorption spectra of the metabolites were determined in methanol on a Beckman model 25 recording spectrophotometer or a Gilford System 2600 UV-Vis microprocessor-controlled spectrophotometer. Mass spectra were obtained with a Finnigan model 4023 mass spectrometer operated at an ionizing voltage of 70 eV and a source temperature of 270°C. 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 while scanning the mass/charge region of *m/z* 35 to 750.

The ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker WM 500 spectrometer. The data were acquired under the following conditions: datum size, 32,000; sweep width, 7,042 Hz; filter width, 17,800 Hz; temperature, 305K; 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 through consideration of shielding effects and coupling patterns and homonuclear decoupling experiments.

Kinetics of 2-methylnaphthalene metabolism by *C. elegans*. [8-^{14}C]2-methylnaphthalene (0.5 μCi, 250 nmol) was added to nine 125-ml Erlenmeyer flasks containing 30 ml of Sabouraud dextrose broth with cells of *C. elegans* and incubated as described above. At the appropriate times, the mycelial pellets were removed, and the culture filtrate was extracted with ethyl acetate. The organic extract was dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure at 40°C. The residue was dissolved in methanol and analyzed by HPLC with solvent system B. To determine water-soluble metabolites, the aqueous layer was extracted several times until no radioactivity was detected in the ethyl acetate extracts. Residual ethyl acetate in the aqueous layer was removed by bubbling nitrogen through the medium. Aliquots (1.0 ml) of the aqueous phase were added to 7.0 ml of Aquasol-2, and the amount of radioactivity was determined with a Beckman LS-250 liquid scintillation counter.

Deconjugation experiments. After a 48-h incubation in the presence of [8-^{14}C]2-methylnaphthalene, the cells were filtered as described above, and the culture filtrate was extracted with 6 equal volumes of ethyl acetate. The aqueous layer was divided into three 3-ml portions, diluted 1:1 with 0.2 M sodium acetate buffer (pH 4.5), and incubated with 3×10^3 Fishman units of β-glucuronidase (type H-1; Sigma Chemical Co., St. Louis, Mo.). The second sample was incubated with 15 U of arylsulfatase (type V; Sigma) and 12.5 μmol of D-saccharic acid 1,4-lactone (an inhibitor of β-glucuronidase). The third sample was incubated without an enzyme and served as a control. Each reaction mixture was incubated for 24 h at 37°C on a rotary shaker operating at 150 rpm. Samples were then extracted with 3 equal volumes of ethyl acetate, dried in vacuo at 30°C, and analyzed by HPLC as described above for the separation of 2-methylnaphthalene metabolites.

Oxygen-18 incorporation. Cells of *C. elegans* were incubated with 2-methylnaphthalene for 4 h as described above except that a rubber stopper was used to seal the flask. The contents of the flask were degassed by a vacuum pump and then by flushing with nitrogen. Oxygen-18 (98.0 atom% ^{18}O; Mound Facility, Monsanto Corp., Miamisburg, Ohio) was introduced into the flask with a cannula. The isotopic composition of the atmosphere was determined at the beginning and end of the experiment and was ca. 98% $^{18}O_2$. The $^{16}O_2/^{18}O_2$ ratio was determined using a Varian-MAT CH5-DF mass spectrometer. 2-Hydroxymethylnaphthalene was isolated by HPLC, and its isotopic abundance was calculated from the relative intensities of the ^{16}O and ^{18}O species (*m/z* 158 and 160, respectively) with a Finnigan model 4023 mass spectrometer.

Metabolism of 1- and 2-naphthoic acids by *C. elegans*. Cells of *C. elegans* were prepared as described above and incubated with either 1- or 2-naphthoic acid (3 mg). After 24 h of incubation, each culture filtrate was extracted with ethyl acetate. The ethyl acetate-soluble material was concentrated and dried as previously described. The residue was dissolved in methanol and analyzed by HPLC with solvent system C.

Chemicals. 1-Methylnaphthalene (99.9%) and 2-methylnaphthalene (99.9%) were purchased from Chem Service, West Chester, Pa. [8-^{14}C]2-methylnaphthalene (98%) with a specific activity of 5.0 mCi/mmol was purchased from California Bionuclear Corp., Sun Valley, Calif., and was further purified by preparative silica gel TLC with benzene-hexane (1:1, vol/vol) as the eluants. 1-Naphthoic and 2-naphthoic acid were purchased from Fluka Chemical Corp., Hauppauge, N.Y. 1-Hydroxymethylnaphthalene and 2-hydroxymethylnaphthalene were prepared by reducing 1- and 2-naphthaldehyde (Aldrich Chemical Co., Milwaukee, Wis.) with a slight excess of sodium borohydride as described by Cerniglia et al. (in press). Solvents for HPLC were purchased from Burdick and Jackson Laboratories, Muskegon, Mich.

RESULTS AND DISCUSSION

Isolation and identification of 1- and 2-methylnaphthalene metabolites. The materials eluted from TLC region I from the 1- and 2-methylnaphthalene incubations (R_f, 0.98; Fig. 1B and D) each gave one chromatographic peak with an HPLC retention time of 31.0 min and were identified as 1- and 2-

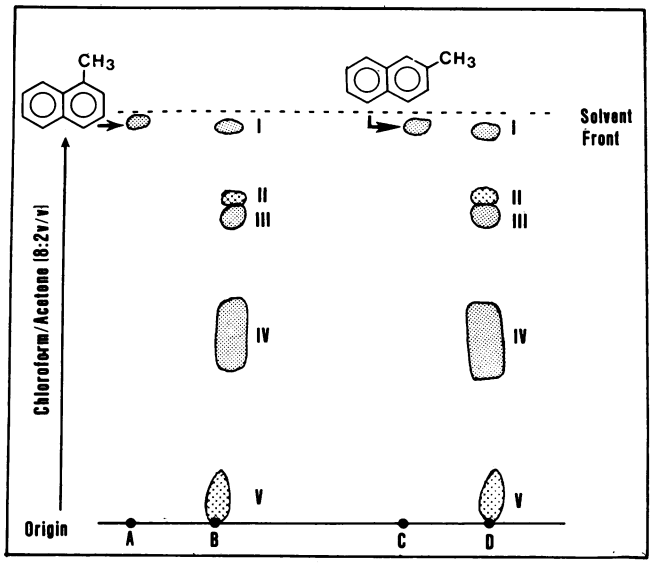

FIG. 1. Schematic representation of thin-layer chromatogram of metabolites formed by *C. elegans* when grown in the presence of 1- and 2-methylnaphthalene. Compounds were detected as described in the text. (A) 1-Methylnaphthalene standard; (B) 1-methylnaphthalene metabolites formed by *C. elegans*; (C) 2-methylnaphthalene standard; (D) 2-methylnaphthalene metabolites formed by *C. elegans*.

methylnaphthalene based on a mass spectral analysis (M^+ at *m/z* 142), the UV absorption spectrum, and the HPLC retention time which were identical to those of 1- and 2-methylnaphthalene, respectively (Tables 1 and 2).

The materials eluted from region II (R_f, 0.80; Fig. 1B and D) gave a blue color when sprayed with Gibbs reagent after exposure to ammonia vapor, indicating that each compound was hydroxylated on the aromatic ring. When each extract was analyzed by HPLC (retention time, 22.5 min), one product was detected. The UV absorption and the mass spectrum (M^+ at *m/z* 158 and 129 [loss of CHO]) indicated that each compound was a phenolic derivative of 1- and 2-methylnaphthalene (Tables 1 and 2). Since only trace amounts were formed, insufficient material was available for ^1H NMR analysis to determine the location of the hydroxyl group on the aromatic ring.

The materials eluted from TLC region III (R_f, 0.76; Fig. 1B and D) from the 1- and 2-methylnaphthalene incubations each gave a yellow color with Gibbs reagent and had an R_f value identical to those of authentic 1- and 2-hydroxymethylnaphthalene, respectively. To confirm that *C. elegans* oxidized 1- and 2-methylnaphthalene at the methyl group to form hydroxymethyl derivatives, each extract was examined by HPLC; and after repeated injections, sufficient material was obtained for structure elucidation. The HPLC elution profile of the materials eluted from region III (Fig. 1D) formed from 2-methylnaphthalene by *C. elegans* gave one chromatographic peak which had an HPLC retention time (17.1 min) and UV absorption spectrum (Table 2) identical to those of authentic 2-hydroxymethylnaphthalene. The structure of this metabolite was further confirmed by mass spectrometry (M^+ at *m/z* 158, *m/z* 141 [loss of OH], and *m/z* 129 [loss of CHO] [Fig. 2]) and 500-MHz ^1H NMR spectroscopy. The NMR spectrum (Table 2) for 2-hydroxymethylnaphthalene shows a doublet at 4.80 ppm and a D_2O-exchangeable triplet at 4.32 ppm, indicating a hydroxy-

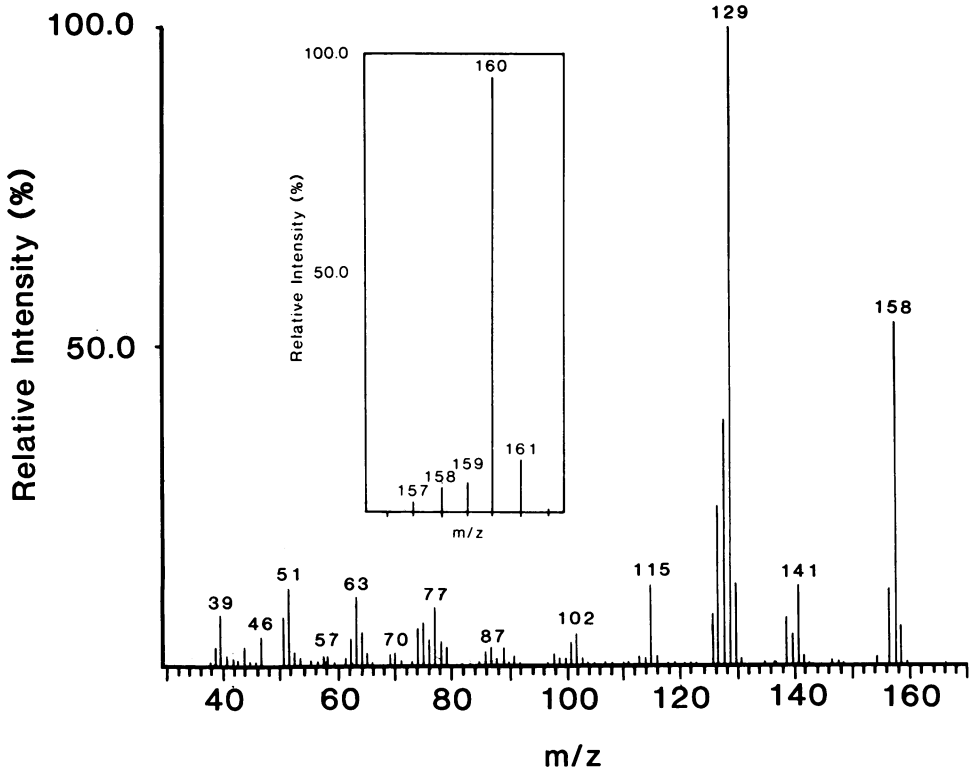

FIG. 2. Mass spectrum of compound III (Fig. 1D) formed from 2-methylnaphthalene by *C. elegans*. (Inset) Mass spectrum of 2-hydroxymethylnaphthalene formed by *C. elegans* grown in the presence of a 98% $^{18}O_2$ atmosphere.

TABLE 1. HPLC retention times and UV-visible absorption, mass spectral, and NMR data of 1-methylnaphthalene metabolites isolated from the extracts of *C. elegans*

Compound	Assignment	Hplc Retention Time (min)	UV-Visible Absorption Maxima (nm)	Mass Spectra M/E (%)	Nuclear Magnetic Resonance
I	1-Methylnaphthalene	31.0 [1,2]	**221**, 270, 276, 283, 305, 314, 319	142(100)141(69)115(28)	ND*
II	1-Methyl-(?)-Naphthol	22.5 [1,2]	272, **280**, 322	158(100)157(37)129(25) 128(25)82(12)64(11)63(23) 53(12)	ND
III	1-Hydroxymethylnaphthalene	16.8 [1,2]	**224**, 269, 280, 292, 304, 313	158(54)157(12)141(12)130(13) 129(100)128(39)127(25)115(13) 63(11)51(12)	(Acetone-d_6) s 8.14(d,1,H_8),7.90(d,1,H_5) 7.81(d,1,H_4),7.59(d,1,H_2),7.54-7.48 (m,2,$H_{6,7}$),*7.45(dd,1,H_3)*,5.11(d,2,CH_2)4.31 (t,1,OH)ppm; $J_{2,3}$=6.9, $J_{3,4}$=8.2, $J_{2,4}$=0.8, $J_{5,6}$=7.3, $J_{7,8}$=8.2, $J_{CH_2\,OH}$=5.6 Hz
IVa	(?)-Hydroxy-1-naphthoic acid	12.1[1],11.8[2],5.3[3]	**240**, 283	188(100)171(36)143(15)	ND
IVb	5-Hydroxy-1-naphthoic acid	12.1[1],13.3[2],6.1[3]	**246**, 278, 334	188(100)171(18)125(16)116(14) 115(45)91(12)	(Acetone-d_6) 8.53(d,1,H_4),8.49(d,1,H_4), 8.49(d,1,H_8), 8.26(d,1,H_2) 7.54(dd,1,H_3) 7.42 (dd,1,H_7), 7.00(d,1,H_6)ppm; $J_{2,3}$= 7.3, $J_{2,4}$= 1.5, $J_{3,4}$= 8.4, $J_{4,8}$= 1.1, $J_{6,7}$= 7.5, $J_{6,8}$= 0.9, $J_{7,8}$= 8.6, Hz

ND = Not determined
1 - System A (Methods)
2 - System B (Methods)
3 - System C (Methods)

TABLE 2. HPLC retention times and absorption, mass spectral, and NMR data of 2-methylnaphthalene metabolites isolated from the extracts of *C. elegans*

Compounds	Assignment	Hplc Retention Time (min)	UV-Visible Absorption Maxima (nm)	Mass Spectra M/E (%)	Nuclear Magnetic Resonance
I	2-Methylnaphthalene	31.0 [1,2]	**223**, 276, 306, 313, 319	142(100)141(69)115(28)	ND*
II	2-Methyl-(?)Naphthol	22.5[1,2]	**272**, 280, 322	158(58)157(16)141(14)130(13) 129(100)128(40)127(26)115(12) 84(13)50(12)	ND
III	2-Hydroxymethylnaphthalene	17.1[1,2]	**224**, 272, 304, 318	158(59)157(14)141(13)130(14) 129(100)128(40)127(27)115(13) 63(10)51(12)	(Acetone-d_6) s 7.88-7.83(m,4,$H_{1,4,5,8}$), 7.52-7.43(m,3,$H_{3,6,7}$) 4.80(d,2,CH_2), 4.32 (t,1,OH)ppm; J_{CH_2OH} = 5.6 Hz
IVa	6-Hydroxy-2-Naphthoic Acid	12.1[1],11.8[2],5.3[3]	**240**, 283	188(100)171(56)143(28)115(15)	(Acetone-d_6) s 8.74(s,1,H_1),7.97(d,1,H_3) 7.96(d,1,H_8),7.76(d,1,H_4),7.28(s,1,H_5), 7.24(d,1,H_7)ppm; $J_{3,4}$=8.8, $J_{5,7}$=2.5 $J_{7,8}$=8.8 Hz
IVb	5-Hydroxy-2-Naphthoic Acid	12.1[1],13.3[2],6.1[3]	**246**, 278, 334	188(100)171(21)158(20)143(11) 116(17)115(45)114(11)	(Acetone-d_6) s 8.58(s,1,H_1),8.32(d,1,H_4) 8.02(d,1,H_3),7.57(d,1,H_8),7.42(dd,1,H_7), 7.07(d,1,H_6)ppm; $J_{1,3}$=1.6, $J_{3,4}$=8.8 $J_{6,7}$=7.2, $J_{7,8}$=8.2 Hz

ND = Not determined
1 - System A (Methods)
2 - System B (Methods)
3 - System C (Methods)

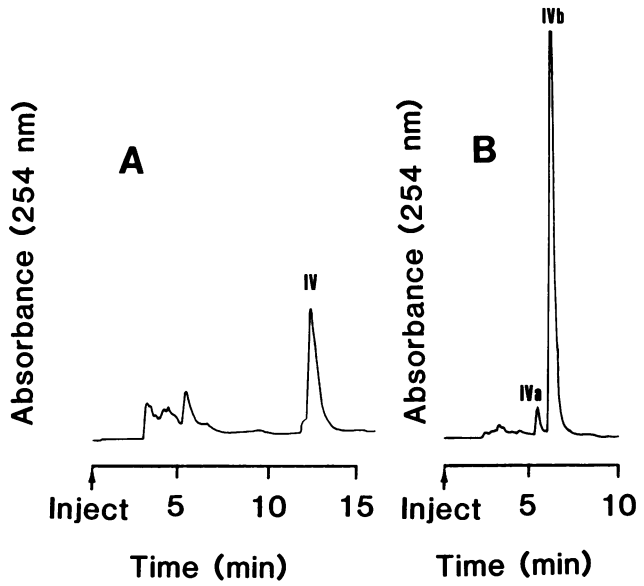

FIG. 3. HPLC elution profile of region IV (Fig. 1D) formed from 2-methylnaphthalene by *C. elegans*. (A) HPLC elution of region IV with solvent system A; (B) HPLC elution profile of region IV with solvent system C.

methyl substituent. The assignments for protons H_1 and H_3 were made by nuclear Overhauser difference spectrum experiments (10). The assigned chemical shifts and coupling constants are indicated in Table 2. All mass and NMR spectral patterns are consistent to those of authentic 2-hydroxymethylnaphthalene. Chromatographic, absorption, mass, and ^1H NMR spectral data (Table 1) similar to those described above were found for 1-hydroxymethylnaphthalene (Fig. 1B, region III).

The materials in region IV (R_f, 0.50 to 0.60; Fig. 1B and D) which were eluted from the TLC plate of 1- and 2-methylnaphthalene incubations each gave a blue color with Gibbs reagent. HPLC analysis of each extract gave a broad chromatographic peak with an HPLC retention time of 12.1 min (Fig. 3A; data shown for 2-methylnaphthalene incubations, Fig. 1D). Separation into two components (compounds IVa and IVb) was achieved by an acidic reverse-phase HPLC mobile phase (Fig. 3B). Mass spectral analysis of each compound was identical and showed a molecular ion at *m/z* 188 and fragment ions at *m/z* 171 (loss of OH), *m/z* 159 (loss of CHO), and *m/z* 143 (loss of COOH) (Tables 1 and 2). The mass fragmentation pattern indicated that this compound was a phenolic derivative of 2-naphthoic acid. The mass spectral data for compounds separated in region IV (Fig. 1B and D) are shown in Tables 1 and 2. Two isomeric hydroxynaphthoic acids were formed from the 1- and 2-methylnaphthalene incubations. To determine the position of the hydroxy group on the aromatic ring for each of these hydroxynaphthoic acids, ^1H NMR analysis was conducted. Compounds IVa and IVb formed from 2-methylnaphthalene by *C. elegans* (Fig. 3B) were identified as 6-hydroxy-2-naphthoic acid and 5-hydroxy-2-naphthoic acid, respectively. The position of the hydroxyl substituent on the aromatic ring for compound IVb was determined by decoupling and nuclear Overhauser enhancement techniques. The downfield *meta*-coupled singlet at 8.58 ppm ($J_{1,3}$ = 1.6 Hz) is indicative of a proton *ortho* to the carboxyl group (10). Proton decoupling of the singlet produced the loss of *meta* coupling in the doublet at 8.02 ppm (H_3) (Table 2). Proton decoupling of the resonance H_3 produced the loss of an *ortho* coupling ($J_{3,4}$ = 8.8 Hz) in the resonance at 8.32 ppm (H_4). The confirmation of the assignment of H_8 (7.57 ppm) was determined by a nuclear Overhauser enhancement difference spectrum. The position of the H_6 (7.07 ppm) being upfield is consistent with the *ortho* effect of a hydroxyl group. The substitution on the aromatic ring system for compound IVa (6-hydroxy-2-naphthoic acid) was determined by similar NMR techniques (Table 2). Similar chemical analyses as described above were used for the separation of compounds in region IV (Fig. 1B) formed from 1-methylnaphthalene by *C. elegans*. 5-Hydroxy-1-naphthoic acid was found to be the major isomer formed by *C. elegans* from 1-methylnaphthalene. Insufficient material was available to confirm the position of the hydroxyl group on the aromatic ring of the minor isomer. Assigned chemical shifts and coupling constants for each of the hydroxynaphthoic acids are given in Tables 1 and 2. The

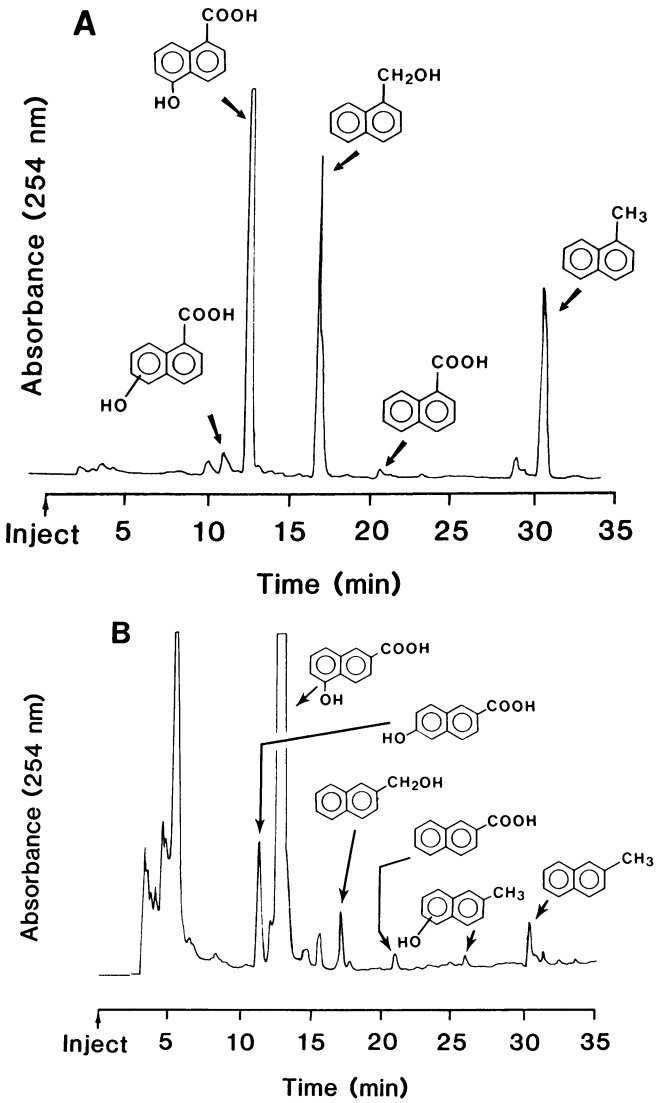

FIG. 4. HPLC elution profiles of the metabolites formed from 1-methylnaphthalene (A) and 2-methylnaphthalene (B) by *C. elegans*. The metabolites were separated with solvent system B. Compounds were collected and identified as described in the text.

materials that eluted from TLC region V (Fig. 1) were medium-extractable compounds and natural products produced by *C. elegans*. In a separate experiment, 1- and 2-naphthoic acids were identified as minor metabolites of 1- and 2-methylnaphthalene, respectively. This evidence was based on identical HPLC retention times (21.5 min) (Fig. 4A and B) and UV and mass spectral (M^+ at *m/z* 172, M^+ at *m/z* 155 [loss of OH], and M^+ at *m/z* 127 [loss of COOH]) properties that were identical to authentic 1- and 2-naphthoic acids, respectively.

When cells of *C. elegans* were incubated with either 1- or 2-naphthoic acid as described previously and analyzed by HPLC with system C, results identical to those described above were found.

The HPLC elution profiles showing the identified metabolites formed from the metabolism of 1- and 2-methylnaphthalene by *C. elegans* are shown in Fig. 4. These metabolites were not detected in autoclaved control samples.

Kinetics of 2-methylnaphthalene metabolism. The time course for the metabolism of [8-^{14}C]2-methylnaphthalene is shown in Fig. 5. Samples were taken over a period of 72 h, and the amounts of ethyl acetate- and water-soluble metabolites were determined. The percentage of organic-soluble and water-soluble metabolites is shown in Fig. 5A. The total amount of 2-methylnaphthalene metabolized increased from 2.3% at 8 h to 9.8% at 72 h. The ratios of organic-soluble to water-soluble metabolites were 92:8 at 2 h and 41:59 at 72 h, respectively. Treatment of the 48-h aqueous phase with either β-glucuronidase or arylsulfatase indicated that 60% of the water-soluble metabolites were glucuronide and sulfate conjugates. The ratio of glucuronide to sulfate conjugates was 1:1. HPLC analysis of the hydrolysis products from β-glucuronidase or arylsulfatase incubations indicated that 5- and 6-hydroxy-2-naphthoic acids were the predominant conjugates. Previous studies have also shown that *C. elegans* has the ability to form glucuronide and sulfate conjugates of phenolic aromatic hydrocarbons (4).

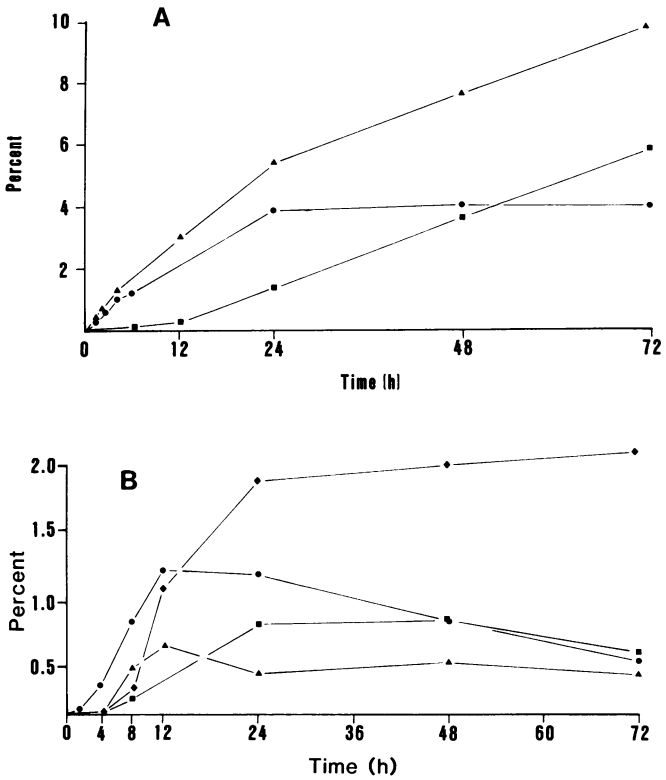

FIG. 5. (A) Kinetic analysis of 2-methylnaphthalene metabolism by *C. elegans*. Symbols: ▲, total 2-methylnaphthalene metabolites, ●, ethyl acetate-soluble metabolites, ■, water-soluble metabolites. (B) Kinetic analysis of ethyl acetate-soluble 2-methylnaphthalene metabolites formed by *C. elegans*. Symbols: ●, 2-hydroxymethylnaphthalene; ◆, 5-hydroxy-2-naphthoic acid, ■, 6-hydroxy-2-naphthoic acid, ▲, 2-naphthoic acid. Data shown as percentage of initial radioactivity.

The formation of the identified organic-soluble metabolites of 2-methylnaphthalene was determined (Fig. 5B). 2-Hydroxymethylnaphthalene accounted for 75.7% of the 2-methylnaphthalene metabolites after 4 h of incubation. The concentration of this metabolite increased for 8 h of incubation, followed by a subsequent decline in concentration between 24 and 72 h. 5-Hydroxy-2-naphthoic, 6-hydroxy-2-naphthoic, and 2-naphthoic acids accounted for 47.6, 18.0, and 10.0%, respectively, of the total organic-soluble metabolites after 48 h of incubation (Fig. 5B). 2-Methylnaphthols accounted for less than 2% of the total organic-soluble metabolites during the course of the incubation.

Oxygen-18 incorporation into 2-methylnaphthalene. *C. elegans* was grown in the presence of 2-methylnaphthalene for 4 h under an atmosphere of 98% oxygen-18. After 4 h of incubation, the 2-hydroxymethylnaphthalene formed was isolated as described above, and the percent incorporation of oxygen-18 into 2-hydroxymethylnaphthalene was determined by mass spectrometry. As described earlier, the 2-hydroxymethylnaphthalene formed in the presence of $^{16}O_2$ showed a parent ion at *m/z* 158 (Fig. 2). The 2-hydroxymethylnaphthalene formed in the presence of $^{18}O_2$ showed a 90% increase in the ion at *m/z* 160, which indicated ca. 95% incorporation of oxygen-18 into 2-hydroxymethylnaphthalene (Fig. 2, inset).

The present investigation indicates that *C. elegans* oxidized 1- and 2-methylnaphthalene, which are among the most water-soluble and toxic components of petroleum, at the methyl group to form 1- and 2-hydroxymethylnaphthalene, respectively. Oxygen-18 incorporation experiments indicated that molecular oxygen is necessary for enzymatic attack at the methyl group. Other metabolites identified are shown in Tables 1 and 2. Interestingly, *C. elegans* formed predominately hydroxymethyl derivatives of 1- and 2-methylnaphthalene, 1- and 2-naphthoic acids, and various hydroxy-1- and -2-naphthoic acids. 1-Methylnaphthol and 2-methylnaphthol were minor metabolites. In earlier work we showed that phenolic derivatives of 1- and 2-methylnaphthalene are toxic to the cyanobacterium *Agmenellum quadruplicatum* PR-6 by the algal lawn bioassay technique whereas the naphthoic acids and hydroxynaphthoic acids are not toxic to the cyanobacterium (Cerniglia et al., in press). This suggests that the transformation of 1- and 2-methylnaphthalene by *C. elegans* may function as a detoxification pathway. From an environmental standpoint, the transformation of methylnaphthalenes by *C. elegans* to detoxified products could be very important in their biodegradation in natural habitats.

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