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Oxidation of Methyl-Substituted Naphthalenes: Pathways in a Versatile *Sphingomonas paucimobilis* Strain†

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Aromatic compounds with alkyl substituents are abundant in fossil fuels. These compounds become important environmental sources of soluble toxic products, developmental inhibitors, etc. principally through biological activities. To assess the effect of methyl substitution on the completeness of mineralization and accumulation of pathway products, an isolate from a phenanthrene enrichment culture, *Sphingomonas paucimobilis* 2322, was used. Washed cell suspensions containing cells grown on 2,6-dimethylnaphthalene in mineral medium were incubated with various mono-, di-, and trimethylnaphthalene isomers, and the products were identified and quantified by gas chromatography and mass spectrometry. The data revealed enzymes with relaxed substrate specificity that initiate metabolism either by methyl group monoxygenation or by ring dioxygenation. Congeners with a methyl group on each ring initially hydroxylate a methyl, and this is followed by conversion to a carboxyl; when there are two methyl groups on a single ring, the first reaction is aryl dioxygenation of the unsubstituted ring. Intermediates are channeled to primary ring fission via dihydrodiols to form methyl-substituted salicylates. Further evidence that there are multiple pathways comes from the fact that both phthalate and (methyl)salicylate are formed from 2-methylnaphthalene.

Alkylated aromatic hydrocarbons enter the ecosystem mostly from crude oil- and petroleum-derived materials. Many of these compounds are man-made products of large-scale industry and are used as fuels, solvents, detergent precursors, and commercial insect repellents (for example, methylnaphthalenes dispersed in aromatic solvents for mosquito control) (42, 47). Because of the abundance of methyl-substituted aromatic compounds and the formation of water-soluble products, the effects of these compounds on the biosphere may be greater than the toxic damage caused by the unsubstituted hydrocarbon counterparts (1, 10, 19, 29, 30, 37).

Removal of aromatic compounds from the biosphere is principally a microbial activity. Gibson et al. (20) first reported that unsubstituted structures undergo initial ring reductive dioxygenation to *cis*-dihydrodiols. With ring substituents, the positions exert a major effect on the first reaction site, the pathway, and the products. With a single methyl addition, ring dioxygenation may be retained, or methyl monoxygenation may first form the analogous benzylic alcohol. The most extensive studies have examined toluene, the simplest and most abundant methylated aromatic hydrocarbon. A recent summary of six known schemes for toluene metabolic patterns are reviewed in studies of fungal oxygenation (45). The products and genetics of both ring dioxygenation (the TOD pathway) (48) and side chain monooxygenation (the TOL pathway) (2) are well-documented. Data are also available for representative aromatized plant terpenes and homologs, including *p*-cymene (15), 3-ethyltoluene (28), and the xylene isomers (3, 16). The attention that has been devoted to methyl derivatives is limited (1, 5, 31, 39) compared with the attention that has been devoted to unsubstituted fused ring compounds (4, 9, 14, 27, 43). Moreover, although a very large number of strains have been isolated, few organisms have received the attention needed for even minimal biological and chemical characterization, and fewer still have been subjected to a genetic analysis to determine chromosome organization, regulation, and gene origin. The detailed data available suggest there is a limited variety of oxygenase catalytic centers in animal, plant, and microbial systems. Marked similarities among mono- and dioxygenase systems have led to the concept that there are enzyme superfamilies (36). Three oxygenase centers that contain metal (iron) prosthetic groups have been well-documented. The most abundant center, which was the first center documented, is a unique heme-thiolate monoxygenase center which now contains more that 500 related, but separate, protein sequences (36). Two other well-studied oxygenase reaction centers are the $Fe_2(\mu O)_2$ cluster, which was found first in methane monoxygenase and later in related nonheme di-iron enzymes (41), and disassociable ferrous, mononuclear nonheme iron coordination sites (25). The essential two-electron reducing power of both mono- and dioxygenase systems is provided primarily by flavoprotein reductases; here too, a limited number of structural patterns are found (17), and there is marked homology to the anaplerotic heterotrophic and photosynthetic flavoproteins (6, 17, 22, 23, 32).

A variety of bacterial strains and species have been used to identify products of methyl-substituted aromatic hydrocarbon metabolism. The profiles obtained are the principal sources of deduced oxygenation-oxidation patterns. Most previous studies on fused ring alkyl aromatic compounds have been concerned with the 1- and 2-methylnaphthalenes and the isomer effects on initial methyl monooxygenation (13, 31, 39, 46) compared with ring dioxygenation (7, 8, 14, 44, 46). *Pseudomonas putida* CSV86, for example, has been reported to convert 1- and 2-methylnaphthalenes to the respective methyl catechols by ring dioxygenation or to form naphthoic acids following methyl hydroxylation (31). In addition, the 2-methyl isomer has been reported to yield 4-hydroxymethyl catechol (31), implying

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that there is dual-pathway regulation. In vitro eucaryote oxygenation has also been found in hepatic microsomes (11, 26), fungi (13), and cyanobacteria (12), the role in plant cells is unclear.

Data on fused ring dimethyl structures are more limited; thus, the pathways are not understood as well (5, 21, 33). In early studies Barnsley (5) and other workers identified ring fission products obtained from fused ring aromatic compounds, and subsequently the process was well-documented by Eaton and Chapman (18). Barnsley (5) hypothesized that there is an initial 2-methyl hydroxylation, followed by oxidation to carboxylate and then a ring monohydroxylation to 1-hydroxy-6-methylnaphthalene-2-carboxylic acid. More recently, several workers have described dioxygenation to 1-hydroxy-2-naphthoic acid as an alternate product obtained from phenanthrene, 2-methylnaphthalene, or 2-naphthoic acid (4, 5, 34). Workers have also described methyl group oxidation of dimethylnaphthalene isomers by the naphthalene dioxygenase cloned from plasmid NAH7 (40) and by *Nocardia corallina* (*Rhodococcus* sp.) grown on aliphatic hydrocarbons (24, 38).

In this report we describe the effect of methyl-substituted aromatic isomers on the metabolic pathway(s) in washed cell suspensions of *Sphingomonas paucimobilis* 2322 grown on 2,6-dimethylnaphthalene (2,6-DMN) as a carbon and energy source. Our data documented that relaxed enzyme specificity and isomer substitution effects on mineralization pathways occur.

MATERIALS AND METHODS

Organism, cultures, chemicals, and products. *S. paucimobilis* 2322 from the collection of Peter Chapman (Gulf Ecology Division, U.S. Environmental Protection Agency) was isolated from a creosote-contaminated soil by enrichment on phenanthrene as the carbon source. The biological systematic properties of this organism were determined and fatty acid analyses and a 16S rRNA composition analysis were performed by Midi Laboratories, Newark, Del. Cells were grown in a liquid mineral salt medium (pH 7.0) containing (per liter) 3.24 g of K_2HPO_4, 0.87 g of NaH_2PO_4, 2.0 g of NH_4Cl, 123 mg of nitrilotriacetic acid, 200 mg of $MgSO_4 \cdot 7H_2O$, 12 mg of $FeSO_4 \cdot 7H_2O$, 3 mg of $MnSO_4 \cdot H_2O$, 3 mg of $ZnSO_4 \cdot 7H_2O$, and 1 mg of $CoCl_2 \cdot 6H_2O$. Solid media contained 2% agar (Difco). Cells were grown by incubating them at 30°C on a rotary shaker (180 rpm) in 2-liter Erlenmeyer flasks containing 400 ml of mineral salt medium supplemented with 1 g of a carbon source per liter. The cells were harvested from 48-h-old cultures by centrifugation (8,000 × *g*, 10 min), washed twice by suspending them in a volume equal to 1/10th the growth volume, collected by centrifugation, and resuspended (final volume, 100 ml) to an optical density at 600 nm of 2.0. For fermentation studies a hydrocarbon was added to a concentration of 1 g/liter to washed cell suspensions in individual Erlenmeyer flasks, and the flasks were incubated at 30°C for up to 72 h. After incubation, cells (and undissolved substrate, when present) were removed by centrifugation, and the supernatants were acidified to pH 2 to 3 with concentrated hydrochloric acid and extracted twice with equal volumes of ethyl acetate. The extracts were dried over anhydrous sodium sulfate and evaporated under reduced pressure at 30°C, and the materials recovered were methylated with an ethereal solution of diazomethane prior to gas chromatography-mass spectrometry (GC-MS) analysis. All chemicals were of the highest purity commercially available.

Analyses. GC-MS was performed with a Hewlett-Packard model 5890 series II gas chromatograph equipped with a model HP5971 mass-selective detector and a model HP 5965B infrared detector. Compounds were separated on a type HP-5 capillary column (25 m by 0.32 mm; film thickness, 0.25 μm) by using helium as the carrier gas at a flow rate of 23 cm/s. The column temperature was kept isothermally at 50°C for 1 min and then increased to 290°C at a rate of 5°C/min. The mass spectrometer was operated at an electron ionization energy of 70 eV, and the injector, transfer line, and analyzer temperatures were 150, 300, and 300°C, respectively. Instrumental library searches and comparison with available authentic compounds were used to identify possible metabolites. The relative amounts of metabolites were calculated from the peak areas of GC-MS total ion current chromatograms. The total ion current integrated responses (excluding the peak of residual starting material) were used as 100% internal standard values for each sample analyzed. Parallel processes without substrate were also included. Thin-layer chromatography was performed on 0.25-mm-thick silica gel (Merck Silica Gel 60 F254) plates by using methylene chloride-ethyl acetate (1:1) as the developing solvent, and the results were used for preliminary identification. Starting substrates and metabolites were detected under UV light and by exposure to iodine vapor. All of the chemicals used were of the highest purity available commercially, and identities were confirmed by GC-MS analysis.

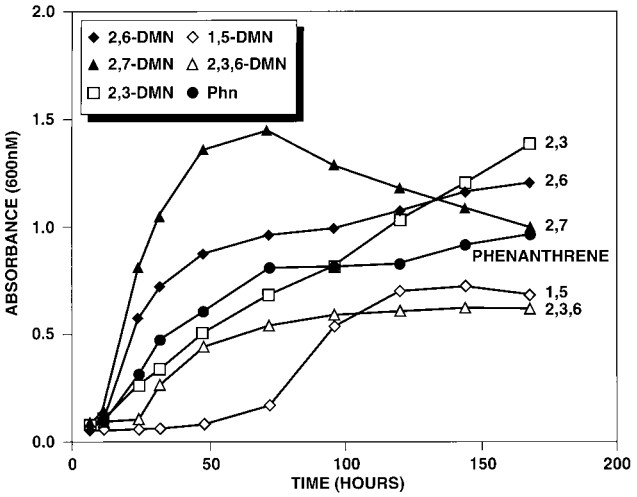

FIG. 1. Growth of *S. paucimobilis* 2322 in minimal salt medium containing aromatic compounds as carbon and energy sources. 2,3,6-DMN, 2,3,6-trimethylnaphthalene; Phn, phenanthrene.

RESULTS AND DISCUSSION

Culture growth. It was shown previously that *S. paucimobilis* 2322 grows well aerobically on several aromatic compounds, including 2,6-DMN, biphenyl, *o*-xylene, *p*-cymene, and acenaphthene, as well as phenanthrene (13a). To confirm that there are a number of growth substrates in the catabolic module, several compounds were tested, as shown in Fig. 1. As discussed below a number of related structures failed to support growth. With 2,6-DMN yellow metabolites were present in culture filtrates.

Metabolic pathway with 2,6-DMN. Harvested and washed cell suspensions oxidized a variety of methylnaphthalenes whose metabolites, as detected by thin layer chromatography, were later identified; the levels of these metabolites were determined by GC-MS, as shown in Table 1. The initial methyl group oxidation, a common event with the isomers tested, led to the proposed metabolic patterns shown in Fig. 2.

The fact that hydroxylation of 2,6-DMN occurred at the 2-methyl position was indicated by the presence of 2-hydroxymethyl-6-methylnaphthalene and its oxidation product, 6-methyl-2-naphthoic acid; the aldehyde intermediate was not detected in the reaction mixture (5, 21, 33). In contrast to the previous report of ring monooxygenation by Barnsley (5), a potential product of dioxygenation with properties of 1,2-dihydroxy-1-hydro-6-methyl-2-naphthoic acid was observed. The data support the pathway reported for naphthalene by Eaton and Chapman (18). The ring fission product appeared to be compound 35 or compound 36 (2-hydroxy-6-methylchromene-2-carboxylate or *trans*-2-hydroxy-5-methylbenzylidinepyruvate) as a *meta* fission product of 1,2-dihydroxy-6-methylnaphthalene (Fig. 2). A GC-MS analysis of the methylated derivative gave a relative amount of 4.2% (GC R_t = 16.60 min; MS *m/z* [%]: 234 [M^+] [3], 219 [1], 203 [6], 187 [10], 175 [100], 160 [2], 159 [5], 143 [6], 132 [10], 115 [13]). Compound 36 led to a salicylate (in this case, 5-methylsalicylate), a common intermediate. An alternate, dead end route appeared to be oxidation of compound 36 to compound 33 (2,6-naphthalene dicarboxylic acid) (Fig. 2). Further support came from a lack of growth

TABLE 1. GC-MS data for products formed from methylnaphthalenes by *S. paucimobilis* 2322 grown on 2,6-DMN[a]

Product (compound)	Relative amt (%)[b]	GC R_t (min)	*m/z* of major ion peaks (%)	Suggested structure
From 2,6-DMN				
1	27.5	11.34	166 (M^+) (34), 135 (21), 134 (100), 106 (23), 105 (11), 78 (17), 77 (18)	5-Methylsalicylic acid methyl ester[c,d]
2	3.2	16.24	172 (M^+) (100), 157 (23), 143 (11), 141 (8), 129 (32), 128 (29), 115 (10)	2-Hydroxymethyl-6-methylnaphthalene[e]
3	50.0	17.37	200 (M^+) (71), 185 (2), 169 (100), 155 (2), 141 (53), 139 (16), 115 (27)	6-Methyl-2-naphthoic acid methyl ester[d]
4	8.5	18.01	216 (M^+) (100), 201 (15), 187 (10), 186 (20), 173 (29), 158 (7), 145 (13), 129 (17), 119 (17), 115 (39)	6-Hydroxymethyl-2-naphthoic acid methyl ester[e]
5	0.5	18.58	234 (M^+) (12), 216 (29), 200 (68), 184 (27), 175 (29), 169 (100), 157 (87), 141 (60), 129 (47), 115 (52)	1,2-Dihydroxy-1-hydro-6-methyl-2-naphthoic acid methyl ester[e]
6	0.5	20.71	244 (M^+) (70), 213 (100), 185 (47), 170 (13), 169 (17), 154 (23), 126 (39), 115 (24)	Naphthalene-2,6-dicarboxylic acid dimethyl ester[d]
From 2,7-DMN				
7	36.1	11.34	166 (M^+) (34.5), 134 (100), 106 (26), 105 (20), 78 (19), 77 (18)	4-Methylsalicylic acid methyl ester[c,d]
8	1.0	16.18	172 (M^+) (100), 157 (33), 143 (22), 141 (38), 129 (64), 128 (50), 115 (25)	2-Hydroxymethyl-7-methylnaphthalene[e]
9	57.7	17.28	200 (M^+) (75), 185 (1), 169 (100), 155 (2), 141 (60), 115 (27)	7-Methyl-2-naphthoic acid methyl ester[e]
10	2.2	18.54	234 (M^+) (12), 216 (26), 200 (71), 184 (26), 175 (32), 169 (100), 157 (82), 141 (67), 129 (46), 115 (52)	1,2-Dihydroxy-1-hydro-7-methyl-2-naphthoic acid methyl ester[e]
From 2,3-DMN				
11	90.9	13.38	180 (M^+) (34), 165 (1), 149 (21), 148 (100), 141 (2), 120 (22), 105 (3), 91 (28)	4,5-Dimethylsalicylic acid methyl ester[c,d]
12	4.2	17.30	200 (M^+) (72), 185 (1), 169 (100), 155 (3), 141 (56), 138 (18), 115 (27)	3-Methyl-2-naphthoic acid methyl ester[c,d]
From 1,5-DMN				
13	26.0	11.47	166 (M^+) (35), 135 (24), 134 (100), 106 (31), 105 (22), 78 (18), 77 (17)	6-Methylsalicylic acid methyl ester[c,d]
14	44.0	17.32	200 (M^+) (74), 185 (3), 169 (100), 155 (2), 141 (58), 115 (32.7)	5-Methyl-1-naphthoic acid methyl ester[c]
From 1,6-DMN				
15	40.0	11.30	166 (M^+) (35), 134 (100), 106 (27), 105 (21), 94 (7), 78 (27), 77 (20)	5-Methylsalicylic acid methyl ester[c,d]
16	40.0	11.45	166 (M^+) (46), 134 (100), 106 (40), 105 (34), 94 (26), 78 (35), 77 (44)	6-Methylsalicylic acid methyl ester[c,d]
17	10.8	17.29	200 (M^+) (74), 185 (2), 169 (100), 155 (9), 141 (73), 128 (5), 115 (37)	5(6)-Methyl-2(1)-naphthoic acid methyl ester[e]
From 1,8-DMN				
18	2.0	11.05	166 (M^+) (41), 141 (15), 134 (100), 115 (14), 106 (65), 94 (35), 78 (40), 77 (31)	3-Methylsalicylic acid methyl ester[c,d]
19	67.0	15.67	170 (M^+) (75), 155 (3), 142 (52), 141 (100), 139 (21), 115 (35), 84 (10)	8-Methyl-1-naphthaldehyde[c]
20	3.0	16.79	172 (M^+) (41), 154 (73), 153 (100), 143 (13), 141 (21), 128 (30), 115 (22)	1-Hydroxymethyl-8-methylnaphthalene[e]
21	1.9	19.18	184 (M^+) (80), 155 (100), 139 (11), 127 (88), 101 (6), 77 (12)	1,8-Naphthalide[d]
From 1,2-DMN				
22	70.8	13.12	180 (M^+) (38), 149 (22), 148 (100), 120 (76), 105 (8), 91 (29)	3,4-Dimethylsalicylic acid methyl ester[c,d]
23	1.4	16.32	172 (M^+) (95.7), 157 (45), 154 (74), 143 (74), 141 (78), 129 (100), 128 (85), 115 (57)	2(1)-Hydroxymethyl-1(2)-methylnaphthalene[e]
From 1,4-DMN				
24	4.2	12.74	180 (M^+) (42), 165 (2), 155 (12), 149 (24), 148 (85), 120 (100), 105 (8), 91 (38)	3,6-Dimethylsalicylic acid methyl ester[c,d]
25	94.0	17.37	200 (M^+) (61), 185 (2), 169 (100), 141 (48), 115 (28)	4-Methyl-1-naphthoic acid methyl ester[c]
From 2,3,6-trimethylnaphthalene				
26	32.4	13.36	180 (M^+) (33), 165 (1), 149 (21), 148 (100), 120 (23), 105 (3), 91 (29)	4,5-Dimethylsalicylic acid methyl ester[c,d]
27	13.0	18.22	214 (M^+) (95), 199 (8), 183 (68), 182 (70), 155 (66), 154 (100), 139 (15), 128 (26), 115 (12)	3,6-Dimethyl-2-naphthoic acid methyl ester[e]
28	30.0	19.07	214 (M^+) (85), 199 (3), 183 (100), 169 (2.1), 155 (46), 139 (9), 128 (9), 115 (8)	6,7-Dimethyl-2-naphthoic acid methyl ester[e]

Continued on following page

TABLE 1—*Continued*

Product (compound)	Relative amt (%)[b]	GC R_t (min)	*m/z* of major ion peaks (%)	Suggested structure
From 2-methylnaphthalene				
29	1.6	9.47	152 (M^+) (46), 121 (37), 120 (100), 93 (14), 92 (49), 65 (24)	Salicylic acid methyl ester[c,d]
30	2.5	11.23	166 (M^+) (35), 134 (100), 106 (28), 105 (24), 78 (20), 77 (19)	4-Methylsalicylic acid methyl ester[c,d]
31	0.3	13.23	194 (M^+) (8), 163 (100), 149 (6), 135 (8), 115 (18), 109 (20), 77 (31)	Phthalic acid dimethyl ester[c,d]
32	5.8	15.77	186 (M^+) (57), 155 (100), 137 (11), 127 (85), 126 (14), 115 (8)	2-Naphthoic acid methyl ester[c,d]
33	7.5	16.98	202 (M^+) (28), 171 (18), 170 (100), 142 (7), 115 (19), 114 (55)	1-Hydroxy-2-naphthoic acid methyl ester[c,d]
34	58.0	17.31	220 (M^+) (9), 202 (27), 186 (2), 170 (7), 161 (37), 155 (3), 143 (100), 131 (27), 115 (95)	1,2-Dihydroxy-1-hydro-2-naphthoic acid methyl ester[e]

[a] Analyses were performed after methylation with diazomethane.
[b] Based on the total ion current response of the mass-selective detector.
[c] Identification was based on an instrument library match.
[d] Identification was based on a match of mass spectra (fragmentation and peak intensity) and capillary GC retention times with data for authentic samples.
[e] Tentative assignment of structure.

on or product formation from 2,6-naphthalene dicarboxylic acid by washed cells grown on 2,6-DMN.

2,7-, 1,5-, 1,6-, and 1,8-DMNs. The reactions of 2,7-DMN were similar to the reactions of the 2,6-DMN isomer, as indicated by the presence of 2-hydroxymethyl-7-methylnaphthalene, 7-methyl-2-naphthoic acid, 1,2-dihydroxy-1-hydro-7-methyl-2-naphthoate, and 4-methylsalicylate (Table 1 and Fig. 2); the data indicate that there was dioxygenation at the 1,2 positions, forming 6- and 7-methyl-2-naphthoates. These are characteristic fission products found in the benzoate-toluate pattern (35).

The 1,5-, 1,6-, and 1,8-DMN isomers gave analogous methylnaphthoate and methylsalicylate accumulations. When 1,6-DMN was used, GC-MS analysis revealed both 5- and 6-methylsalicylates (i.e., hydroxylation occurred at either the β-methyl group or the β-methyl group). The 1,8-DMN isomer products were "upper-pathway" products (i.e., 1-hydroxymethyl-8-methylnaphthalene, 8-methyl-1-naphthaldehyde, and 1,8-naphthalide, a spontaneously cyclized product of 8-hydroxymethyl-1-naphthoic acid obtained under acidic extraction conditions) (40).

2,3,6-Trimethylnaphthalene. With methyl groups on both rings and a third substituent at position 3−, methyl hydroxylation occurred as the first committed step, at either carbon 2− or carbon 6−, and oxidation to the carboxylate led to compounds 27 and 28 (the 3,6-dimethyl- and 6,7-dimethyl-2-naphthoates) (Table 1). The subsequent ring dioxygenation, however, appeared to be specific for the (renumbered) 2,3-dimethyl-6-naphthoate, for only 4,5-dimethylsalicylate was present. The *S. paucimobilis* products obtained from 2,3,6-trimethylnaphthalene are consistent with dioxygenation of the benzoate-toluate type (35).

1,2-, 1,4-, and 2,3-DMNs. As indicated above, placing both methyl groups on the same carbocyclic ring directed the initial reaction to dioxygenation of the unsubstituted aryl group and altered the early products. A subsequent *meta* ring fission should then have led to the respective dimethylsalicylates (that is, the 1,2-, 1,4-, and 2,3-DMNs and conversion to the respective 3,4-, 3,6-, and 4,5-dimethylsalicylic acids). The final products obtained from the salicylic acids converged regardless of whether one or both aryl ring substitutions occurred. An alternate oxidation, to the benzyl alcohols, yielded the apparent dead end compounds 23, 12, and 25, for neither salicylate nor phthalate derivatives were detected in the spent growth medium or after reactions with cell suspensions. The data are compatible with the presence of the phenanthrene dioxygenase, which apparently is coinduced in cells grown with 2,6-DMN.

2-Methylnaphthalene. 2-Methylnaphthalene, although not a growth substrate, was metabolized by washed cells grown on 2,6-DMN. The products indicate that initially either methyl hydroxylation or dioxygenation of an unsubstituted aryl ring occur. Thus, ring fission led to either 4-methylsalicylate or phthalate. Figure 2 shows the same dual pathways found in phenanthrene-grown cells.

Dioxygenation initiated on the unsubstituted aryl ring occurred by formation of a *cis*-dihydrodiol at the 1,2 carbons. As indicated in Fig. 2, the data suggest that dehydrogenation to the analogous vicinal 1,2-diphenol (a catechol) and ring fission to 4-methylsalicylate via compound 37 or 38 (2-hydroxy-7-methylchromene-2-carboxylate or *trans*-2-hydroxy-4-methylbenzylidinepyruvate) occur. A GC-MS analysis of the methylated product revealed a relative amount of 6% (GC R_t = 16.71 min; MS *m/z* [%]: 234 [M^+] [4], 219 [1], 203 [6], 187 [14], 175 [100], 160 [1], 159 [5], 143 [6], 132 [12], 115 [19]).

A β-methyl hydroxylation first led to phthalate or salicylate, depending on the reaction of compound 33. The presence of 2-naphthoate and 1-hydroxy-2-naphthoate recalls the Bradley monoxygenase hypothesis (5). When 2-naphthoate (compound 32) was used as the substrate, the products indicated that both mono- and dioxygenase pathways were present, based on identification of compounds 33 and 34, which led to phthalate (compound 31) or 4-methylsalicylate (compound 30). Phthalate generation from 1-hydroxy-2-naphthoate and 2-naphthoate is a well-established pathway in phenanthrene metabolism (4, 34).

To summarize, *S. paucimobilis* grown on phenanthrene generates phthalate via 1-hydroxy-2-naphthoate and salicylate in trace amounts in spent growth medium and washed cell suspensions. Thus, although the phenanthrene and 2,6-DMN pathways are considered separate, the two pathways seem to be coinduced by growth on either substrate, suggesting that there is a common regulation mechanism.

A comment on the growth substrates of strain 2322 is in order. In general, the 2-substituted dimethylnaphthalene isomers are suitable growth substrates, as are the 2,3,6-trimethyl

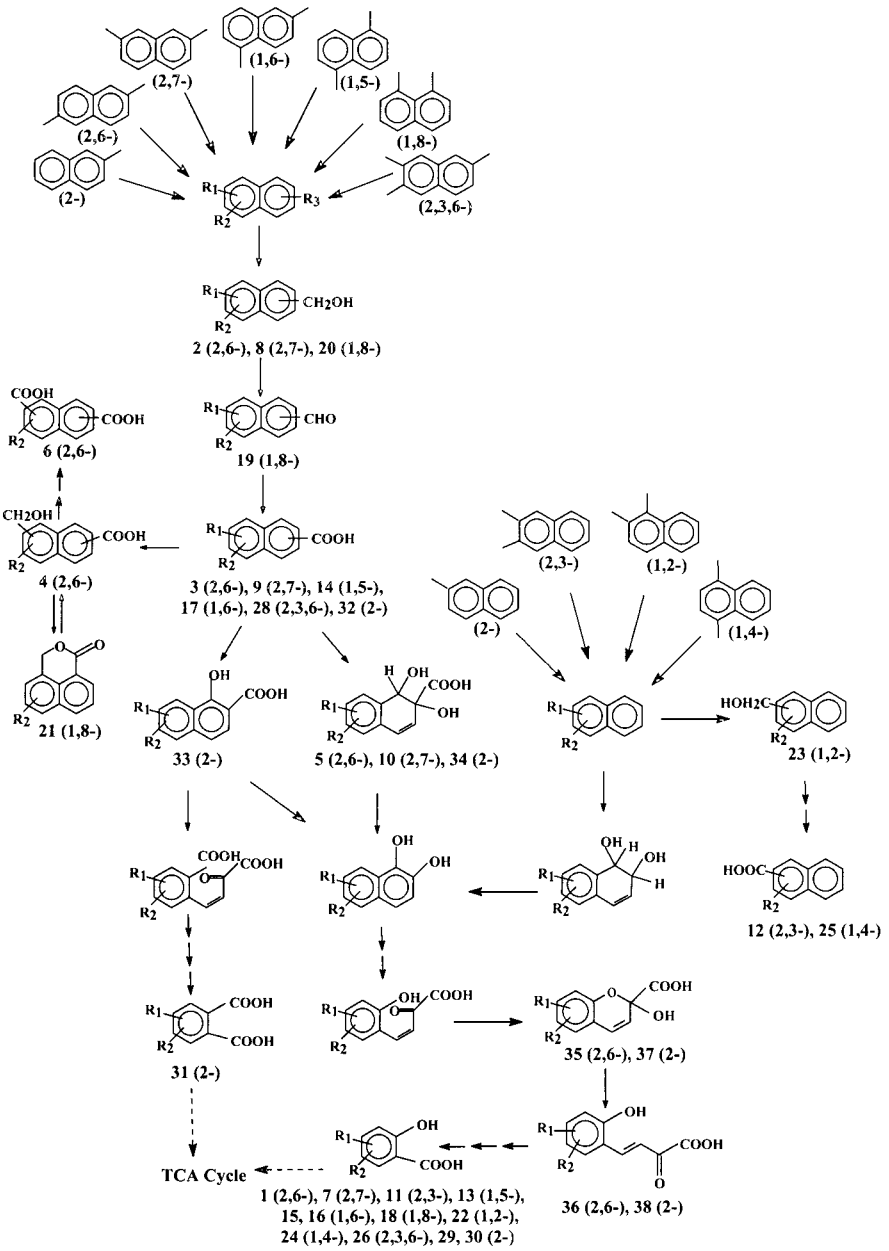

FIG. 2. Proposed pathway(s) for conversion of various methylnaphthalenes by *S. paucimobilis* 2322 grown on 2,6-DMN. The numbers of the metabolites detected are the numbers in Table 1, and the substitution patterns of the methylnaphthalene precursors are indicated in parentheses. TCA, tricarboxylic acid.

structure and phenanthrene. Naphthalene and mono-substituted 2-naphthalenes, however, do not support growth. Similarly, in strains of this type, neither 1-substituted dimethylnaphthalenes nor 1- or 2-naphthoate supports growth. The persistence of these compounds in nature requires more detailed consideration, along with the effects of other microbic strains (31), but this was beyond the scope of this work.

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