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MICROBIAL OXIDATION OF NAPHTHALENE DERIVATIVES. ABSOLUTE CONFIGURATION OF METABOLITES[1]

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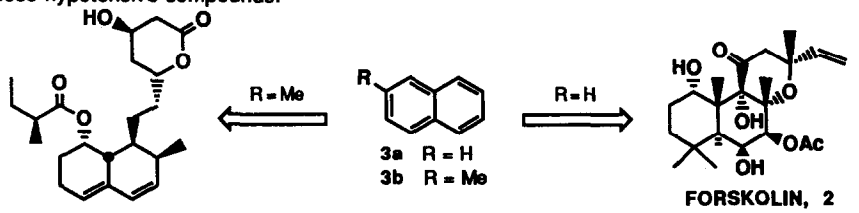
Abstract: 2-Methylnaphthalene was subjected to microbial oxidation by *Pseudomonas putida 39D* and *Pseudomonas putida NCIB 9816* organisms. The structure and absolute stereochemistry of the major metabolite is reported and its use in enantioselective synthesis is indicated.

The ability of certain microorganisms to oxidize aromatic hydrocarbons is well documented.[3,4] The major step in the bacterial metabolism of these hydrocarbons is the formation of the corresponding 1,2-dihydrodiol of specified regiochemistry.[3] The cis-relative stereochemistry of the diol is characteristic of the bacterial biodegradation of arenes in contrast to mammalian metabolism, which yields trans diols and arene oxides.[1,3] In most normal strains these dihydrodiols do not accumulate in the culture medium because of subsequent transformations in the degradative pathway, but in mutant strains the presence of an alternate carbon source and the inability of the organism to degrade the diols leads to their accumulation.The present investigation deals with the characterization of the dihydrodiols formed from methylnaphthalenes by *Pseudomonas putida 39D* and *Pseudomonas putida NCIB 9816* and the assignment of absolute stereochemistry.

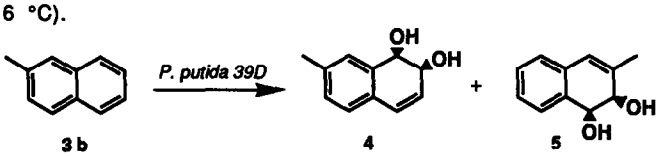
Recent studies from our laboratory have shown that certain arene cis-diols serve as outstanding chiral pool synthons in the preparation of complex oxygenated natural products such as prostaglandins,[1a] analogs of cyclohexene oxides,[1b] and carbohydrates.[1c] In connection with a project investigating a possible synthesis of compactin (**1**)[5,6] or forskolin (**2**)[5,6] from naphthalene or its derivatives, we sought to determine the regio- and stereochemistry of the microbial oxidation. The actual synthetic plan was postponed until crucial aspects of these degradations become known. The regiochemistry of the oxidation products would then determine the details of our synthetic strategy toward these hypotensive compounds.[5]



COMPACTIN, 1 **FORSKOLIN, 2**

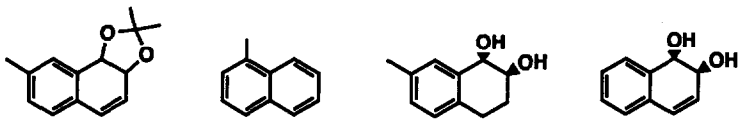
Pseudomonas putida strain *39D*, grown and induced under conditions previously reported,[1,3] was suspended in liquid medium, and a solution of 2-methylnaphthalene (**3b**) in dimethylsulfoxide was

added.[7] Two compounds were isolated from the culture medium, each of which crystallized as fine needles from warm hexane-ethyl acetate. They were identified as **4** and **5**[8] (m.p. of **4**: 104-105 °C; m.p. of **5**: 115-116 °C).



3b **4** **5**

Compound **4** was converted to the acetonide **6** by stirring with 2,2-dimethoxypropane and PTSA as catalyst.[9] This compound was also used as a substrate for *P. putida 39D*, but no further degradation was observed.



6 **7** **8** **9**

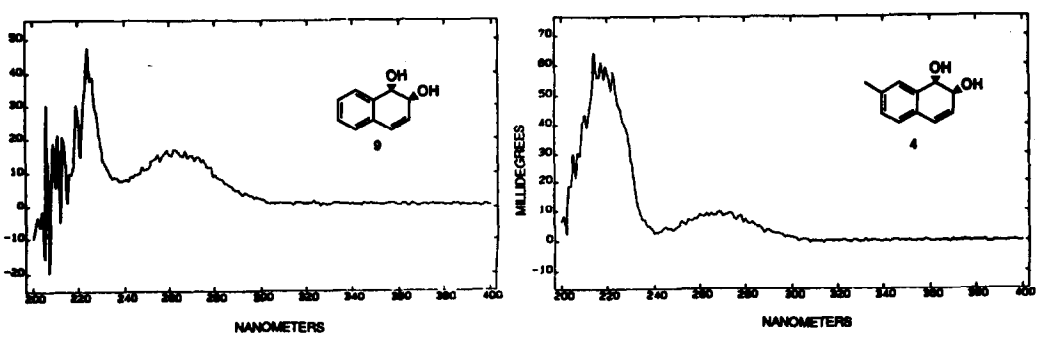
The same experimental conditions[7] were used for 1-methylnaphthalene (**7**), but only traces of the corresponding diol were observed. When *NCIB 9816* was grown in a liquid medium[10] in the presence of 2-methylnaphthalene, a single metabolite accumulated in an appreciably higher yield than observed for *P. putida 39D*.[11] The melting point, chromatographic mobility, and spectral details were identical to those given for compound **4**. This diol was hydrogenated (Pd/C (10%) in ethyl acetate) in order to increase its stability, and the dihydroxytetrahydronaphthalene **8**[12] was isolated.

The absolute stereochemistry of *cis*-dihydrodiol **4** was established by comparison of circular dichroism (CD) and optical rotatory dispersion (ORD) curves with those of its known analogue (+)-*cis*-1,2-dihydro-(1R,2S)-dihydroxynaphthalene (**9**).[13] (See Table 1 and Figure 1.)

Table 1: ORD measurements of naphthalene diols

λ (nm)	α(4)	α(9)
365	9.90	9.86
436	5.20	5.20
546	2.70	2.71
578	2.32	2.33
589	2.21	2.22

Figure 1: CD curves at room temperature in methanol solution.



Since the absolute stereochemistry of compound **9** is known, the sign and the essentially identical magnitudes of the curves can be used to determine the absolute stereochemistry of the chiral centers in compound **4**, which was therefore assigned as (+)-*cis*-1,2-dihydro-(1R,2S)-dihydroxy-7-methylnaphthalene.

The relative configuration of the asymmetric carbons in compound **4** agrees with previous observations of Ziffer and others,[14] and consequently compound **5** was tentatively assigned as (-)-*cis*-1,2-dihydro-(1R,2S)-dihydroxy-3-methylnaphthalene.

The inability to detect diol **5** when *Pseudomonas putida NCIB 9816* was used and the lower yield of **4** obtained with *P. putida 39D* suggest that the latter mutant manifests a decreased efficiency of its enzymatic system toward bicyclic aromatic substrates.[15] The isolation of diol **4** in preparatively useful yields bodes well for its utilization in enantiocontrolled synthesis of decalin-derived natural products such as compactin and forskolin through further transfer of chirality by chemical means. This endeavor as well as the confirmation of the absolute stereochemistry by X-ray analysis forms the current focus of our research effort.

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7. The suspention was aerated during 36 h at 28 °C. After centrifugation of cells at 5000 rpm, the aqueous solution was extracted several times with base-washed ethyl acetate, and the crude extract purified by chromatography.
8. The spectral properties of naphthalene diols are the following: Compound **4**: IR: 3200 (broad), 2930, 2850, 1440 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 2.02 (s, 1H), 2.34 (s, 4H), 4.34 (m, 1H), 4.62 (m, 1H), 5.97 (dd, J_1=4.2 Hz, J_2=9.7 Hz, 1H), 6.49 (d, J=9.7 Hz, 1H), 6.99 (d, J=7.6 Hz, 1Hz), 7.07 (d, J=7.6 Hz, 1H), 7.34 (s, 1H). ^{13}C-NMR ($CDCl_3$): 21.4 (CH_3), 68.0 (CH), 70.8 (CH), 126.9 (CH), 127.6 (CH), 127.7 (CH), 128.2 (CH), 129.0 (CH), 132.5 (C), 135.5 (C), 138.4 (C). Mass Spectrum (m/e (rel. int.)): 176 (17, M^+), 158 (100), 141 (13), 130 (49), 115 (50). Calcd. for $C_{11}H_{12}O_2$: 176.0837. Found: 176.0844.
   Compound **5**: IR: 3260 (broad), 2920, 2850, 1550, 1470 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 1.94 (s, 3H), 2.27 (broad, 2H), 4.01 (d, J=6.0 Hz, 1H), 4.62 (d, J=6.0 Hz, 1H), 6.20 (s, 1H), 6.94 (m, 1H), 7.17 (m, 2H), 7.45 (m, 1H). ^{13}C-NMR ($CDCl_3$): 20.4 (CH_3), 71.2 (CH), 71.6 (CH), 124.4 (CH and C), 126.0 (CH), 126.7 (CH), 127.4 (CH), 128.3 (CH), 134.6 (C), 137.2 (C). Mass Spectrum (m/e (rel. Int.)): 176 (23, M^+), 158 (90), 145 (13), 130 (100), 115 (78). Calcd. for $C_{11}H_{12}O_2$: 176.0837. Found: 176.0837.
9. ^{1}H-NMR ($CDCl_3$): 1.40 (s, 3H), 1.48 (s, 3H), 2.34 (s, 3H), 4.90 (dd, 1H), 4.98 (d, J= 6 Hz, 1H), 5.82 (dd, J_2= 9.2 Hz, 1H), 6.40 (d, J= 9.2 Hz, 1H), 7.02 (d, J= 7.6 Hz, 1H), 7.08 (d, J= 7.6 Hz, 1H), 7.24 (s, 1H).
10. Sodium succinate was used as the carbon source. Metabolism induction was done with naphthalene by placing a filter paper saturated with this compound on the lid of the petri dish. The induced cells were grown in liquid medium to which a solution of 2-methylnaphthalene in dimethylsulfoxide was added. A stream of air was then bubbled through the suspention for 48 hr at 28-30°C.
11. Yields of compound **4**: 20 mg/L with *P. putida 39D* and 350 mg/L with *NCIB 9816*.
12. IR: 3200 (broad), 2930, 1450 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 1.88 (m, 1H), 1.96 (m, 1H), 2.30 (s, 1H), 2.54 (m, 2H), 2.72 (m, 1H), 2.86 (m, 1H), 3.95 (br. s, 1H), 4.62 (br. s, 1H), 7.01 (d, 2H), 7.22 (s, 1H) ^{13}C-NMR ($CDCl_3$);136.1, 135.9, 133.0, 130.2, 129.0, 128.4, 70.0, 69.6, 26.5, 26.3, 20.8. Mass Spectrum (m/e (rel. int.)): 178 (10, M^+), 160 (50), 145 (28), 134 (100), 105 (40), 91 (35), 77 (20). HRMS for $C_{11}H_{14}O_2$: Calcd.: 178.0994, Found: 178.0997.
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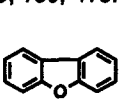

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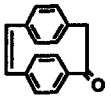

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