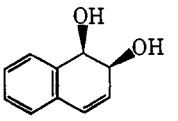
Communications to the Editor

Absolute Stereochemistry of the (+)-*cis*-1,2-Dihydroxy-3-methylcyclohexa-3,5-diene Produced from Toluene by *Pseudomonas putida*

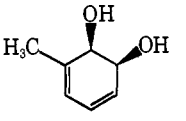
Sir:

Both animals and bacteria oxidize aromatic hydrocarbons to dihydrodiols.[1] This metabolic pathway in mammals occurs with the incorporation of one atom of molecular oxygen and leads, initially, to the formation of arene oxides. Subsequent addition of water, catalyzed by the enzyme epoxide hydrase, yields trans dihydrodiols.[2] The absolute stereochemistries of several trans dihydrodiols, formed from mono- and polycyclic aromatic substrates, have been determined.[3] In contrast, the dihydrodiols formed during the bacterial degradation of aromatic hydrocarbons have a cis stereochemistry,[4] and both atoms of oxygen in the diols originate from molecular oxygen.[5] To date, only the absolute stereochemistry of the cis dihydrodiol (**1**) from naphthalene has been determined.[6] To ascertain the steric and electronic factors that affect enzyme–substrate binding, we wished to determine the absolute stereochemistry of a number of cis dihydrodiols that are formed from aromatic substrates by *Pseudomonas putida*. A mutant strain of this organism (*P. putida* 39/D) lacks the enzyme that dehydrogenates cis dihydrodiols to catechols. Consequently, when *P. putida* 39/D is grown on glucose in the presence of an aromatic hydrocarbon, the corresponding cis dihydrodiol accumulates in the culture medium. In this way, cis dihydrodiols have now been obtained from benzene, toluene, *p*-chloro-, *p*-fluoro-, *p*-bromotoluene, ethylbenzene, chlorobenzene, and cyanobenzene. In addition a cis dihydrodiol from biphenyl has been obtained with a *Beijerinckia sp.* mutant. On the basis of their nmr spectra and their dehydration to known phenols, these 1,2-diols were demonstrated to have the remaining substituent(s) at the 3 or the 3 and 6 positions. Since the problem of establishing absolute stereochemistry for each of these metabolites is not trivial, our approach has been to determine unequivocally the absolute stereochemistry of one of the metabolites, (+)-*cis*-1,2-dihydroxy-3-methylcyclohexa-3,5-diene (**2**), in order that the stereochemistries for the other cis dihydrodiols could be assigned by spectroscopic measurements.

Hydrogenation of **2** in the presence of Pd and triethylamine yields a mixture of *cis,cis*- and *cis,trans*-3-methylcyclohexane-1,2-diols (**3a** and **4a**) which, as the C-1 monobenzoates (**3b** and **4b**), were readily separated *via* chromatography on silica gel. The major isomer ($[\alpha]^{25}D$ −25°, chloroform) was assigned the cis,cis structure **3a** based on proton nmr spectra. The



1



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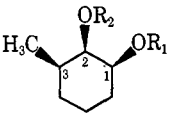
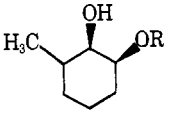


3a, $R_1 = R_2 = H$
b R_1 = PhC(=O); $R_2 = H$
c, $R_1 = R_2$ = PhC(=O)



4a, R = H
b, R = PhC(=O)

coupling constants $^3J_{2,3}$ and $^3J_{1,2}$ in the diol **3a** (2.6 and 2.6 Hz, respectively) and in the monobenzoate **3b** (2.5 and 2.5 Hz, respectively) require a cis,cis stereochemistry. To support this assignment the cis,trans diol **4a** was prepared by oxidation of 3-methylcyclohexene with OsO_4–H_2O_2, a reagent which is known to attack from the least hindered side.[7] The coupling constants $^3J_{2,3}$ and $^3J_{1,2}$ were 9.0 and 3.0 Hz, respectively. The large $^3J_{2,3}$ in **4a** requires trans stereochemistry and thus confirms the assignment of cis,cis relative stereochemistry for **3a**. Oxidation of **3a** with Jones reagent and purification of the resulting diacid *via* its bis(dicyclohexyl)ammonium salt yielded the known[8] (−)-2(*R*)-methyladipic acid (found $[\alpha]^{25}D$ −14.2°, ethanol; lit.[8] $[\alpha]^{25}D$ −13.4°). From knowledge of the absolute stereochemistry of the asymmetric carbon atom in (−)-2-methyladipic acid and the relative stereochemistry of **3a**, the absolute stereochemistries of **2** and of **3a** are assigned as 1*S*,2*R* and 1*S*,2*R*,3*R*, respectively. The assignment of **2** is novel in that it is based on an asymmetric center introduced during conversion of **2** to **3a**.

The optical property most strongly associated with the absolute stereochemistry of dihydrodiols is their circular dichroism (CD) spectrum; **2**, for example, shows a positive chiroptical effect with $\theta_{270} = +1985°$. The sign of the chiroptical effect of homoannular cisoid dienes has been shown theoretically and experimentally to be related to the skew sense of the diene.[9] Since these dihydrodiols, however, can exist in either of two conformations in which the skew sense of the diene differs, absolute stereochemistry cannot be assigned from a CD spectrum alone. Furthermore, several recent publications have claimed that hydroxyl groups[10] and/or axial substituents[11] allylic to the diene profoundly affect the CD spectrum. These considerations prompted us to seek an alternate procedure to assign stereochemistry by a spectroscopic method. The "dibenzoate chirality rule" of Nakanishi and Harada[12]

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was found to apply for **3c**. The CD spectrum of **3c** showed a negative chiroptical effect at 237 nm and a positive band at 222 nm. The "dibenzoate chirality" rule, in conjunction with the established relative stereochemistry, requires therefore a 1*S*,2*R*,3*R* stereochemistry which agrees with that obtained by chemical methods.[13]

The techniques described here for assigning absolute stereochemistry to diol **2** are presently being applied to the other dihydrodiols obtained with *P. putida* 39/D. Interestingly, the diol groups in (+)-*cis*-1(*R*),2(*S*)-dihydroxy-1,2-dihydronaphthalene (**1**) and dihydrodiol **2** have the same absolute stereochemistry when the methine CH in **1** and the methyl group in **2** are treated as the same position.[14] All of the monosubstituted benzenes which are substrates for the mutant yield dihydrodiols with a high degree of optical activity, while disubstituted benzenes such as *p*-chloro- and *p*-bromotoluene yield optically inactive diols. In contrast, *p*-fluorotoluene is converted to a diol with high optical activity. Seemingly, the enzyme has a bulk tolerance at only one of the ring positions adjacent to the double bond at which the diol group is introduced. Thus, in the toluene series of substrates, the bulky chloro and bromo groups may be confused with methyl, while the fluoro group is sufficiently small that one mode of binding predominates and a highly optically active product is produced. Determination of absolute stereochemistry for this series of dihydrodiols will provide a test of this hypothesis.

(13) The absolute stereochemistry of the Diels–Alder adduct between the diacetate of **2** and *p*-bromophenyltriazolinedione has been deduced by X-ray crystallography and agrees with the assignment made here. We are indebted to Dr. R. E. Davis, Department of Chemistry, University of Texas at Austin, for disclosure of this information to us prior to publication.

(14) The diol (**1**) from naphthalene was obtained from a different *Pseudomonas* mutant.[6]

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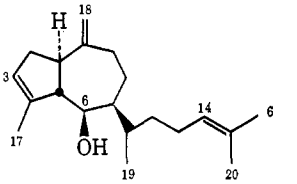
Marine Natural Products. VIII. Pachydictyol A, an Exceptional Diterpene Alcohol from the Brown Alga, *Pachydictyon coriaceum*[1]

Sir:

Marine algae of the family Dictyotaceae, particularly the genera *Dictyopteris*[2–4] and *Taonia*,[5] are known for the interesting secondary metabolites they produce. *Pachydictyon coriaceum*, a member of this family, is found on the Pacific coast of California, south from Santa Barbara to Baja California. We report here the structure of one of the constituents of *P. coriaceum*, which we have found to have mild antibiotic activity *vs. Staphyloccus aureus*.

Hexane extraction of the air dried seaweed followed by column chromatography on silica gel resulted in the isolation of a new diterpene alcohol, pachydictyol A (**1**) (0.7% dry weight of alga), $[\alpha]^{30}D$ +106° (cyclohexane). A composition of $C_{20}H_{32}O$ was indicated by the mass spectrum of **1** (M^+ = *m/e* 288) and confirmed by elemental analysis of its α-naphthylurethane derivative (mp 114–115°). The infrared spectrum of **1** showed bands at 2.87 (OH), 6.12 (C=C), and 11.23 μ (C=CH_2).



Nmr spectra of **1** and added increments of Eu(dpm)$_3$ were analyzed using a recently developed graphical method.[6] The following chemical shift assignments were made: δ 5.30 (1 H, m, H-3), 5.10 (1 H, t, H-14), 4.72 (2 H, s, H-18), 3.85 (1 H, broad s, H-6), 1.75 (3 H, d, H-17), 1.67 (3 H, s, H-20), 1.60 (3 H, s, H-16), 0.97 (3 H, d, H-19).

Pachydictyol A formed an acetate with difficulty and was oxidized with Jones reagent to a ketone (ir 5.88 μ, 7-ring C=O).

The structure, including absolute stereochemistry, of **1** was obtained by an X-ray crystallographic analysis of its *p*-bromophenylurethane derivative (mp 107–109°, needles from ethanol). Weissenberg and precession photographs showed monoclinic symmetry and space group $P2_1$. The unit cell constants, a = 13.392 (9), b = 19.28 (2), c = 5.195 (5) Å, and β = 103.45 (2)°, were determined from a least-squares fit of 12 carefully centered high angle reflections, using Mo $K\alpha_1$ radiation (λ 0.70926 Å). The density of the crystal measured by flotation was 1.22 g cm^{-3}, while that calculated for two molecules of $C_{27}H_{36}O_2NBr$ in a unit cell is 1.204 g cm^{-3}.

Intensity data were collected on a computer controlled Picker four-circle diffractometer, using Mo $K\alpha$ radiation made monochromatic by Bragg reflection from a graphite crystal. Reflections having 2θ values up to 38° (1005 unique reflections) were collected by the 2θ–θ scan technique at a scan rate of 1°/min and a scan range of 1.9°. During the period of data collection, three standard reflections were measured after every 75 reflections. At the end of data collection the crystal turned yellowish brown with the decomposition showing up equally in the three standard reflections as a monotonic 15% loss of intensity. The intensities were scaled to the initial standards and corrected for Lorentz polarization effects.

The structure was solved by the heavy-atom method and refined by full-matrix least-squares calculations, using 951 reflections which were greater than 2σ (the

(1) Paper VII in this series: *J. Org. Chem.*, submitted for publication.

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