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Microbial Oxygenation of Dialkylbenzenes (*1*)

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The use of the microorganism *Sporotrichum sulfurescens* (ATCC 7159) to oxygenate organic molecules has been extended to several dialkylbenzenes. Oxygenation of 1,4-di-*t*-butylbenzene (**1**) gave 4-*t*-butyl(1-hydroxy-2-methyl)isopropylbenzene (**2**) and 1,4-di-(1-hydroxy-2-methyl)isopropylbenzene (**3**); of 1,4-diisopropylbenzene (**4**) gave (*R,R*)-1,4-di-(1-hydroxy)isopropylbenzene (**5**); of 1,3-diisopropylbenzene (**6**) gave 1,3-di-(2-hydroxy)isopropylbenzene (**7**), 3-(1-hydroxy)isopropyl-(2-hydroxy)isopropylbenzene (**8**), and 1,3-di-(1-hydroxy)isopropylbenzene (**9**); and of *p*-isobutylisopropylbenzene (**20**) gave 1-(*p*-2-hydroxyisopropylphenyl)-2-methylpropan-2-ol (**15**) and 1-(*p*-1-hydroxyisopropylphenyl)-2-methylpropan-2-ol (**16**). Monohydroxydialkylbenzenes also served as useful substrates in this reaction as suggested by the fact that **2** is an intermediate in the formation of **3** from **1**. Oxygenation of 1-(*p*-isopropylphenyl)-2-methylpropan-2-ol (**14**), conveniently prepared from 2-(*p*-isopropylphenyl)propene (**12**) *via* oxygenative isomerization with thallium trinitrate to **13** followed by addition of methyl magnesium bromide, gave **15** and **16**. Oxygenation of 2-(*p*-isobutylphenyl)propan-2-ol (**18**) gave **15**, 2-(*p*-isobutylphenyl)-propan-1,2-diol (**21**), and 1-(*p*-2-hydroxyisopropylphenyl)-2-methylpropan-3-ol (**22**). Compound **16**, obtained from substrate **14**, was converted to (2*R*)-2-[4-(2-hydroxy-2-methylpropyl)phenyl]propionic acid (**11**), the enantiomer of a metabolite of the antiinflammatory agent, 2-(4-*i*-butyl)phenylpropionic acid (**10**).

INTRODUCTION

The microbial transformations of hydrocarbon molecules have been examined for their ability to sustain microbial growth (*2*), have been studied in relation to metabolic pathways (*2, 3*), and, occasionally, have been used as chemical reactions (*4*). Our own interests in this field have been in the preparation of unusual chemical intermediates *via* oxygenation reactions and in the nature of the oxygenation process, particularly as related to the introduction of stereochemistry into the products. The present report describes the side chain oxygenation of several dialkylbenzenes with the mold *Sporotrichum sulfurescens*, a microorganism that is known to oxygenate acyclic alkylbenzamides as well as numerous other substrates (*5*). In addition, the application of these results to the solution of a specific problem is presented as an illustration of the synthetic potential of this method

Previous studies of the microbial oxygenation of alkylbenzenes suggest that initial oxidative attack may occur at either the benzylic carbon or at the terminal carbon of the

alkyl side chain. Thus, oxygenation at the benzylic carbon may result in the formation of an alcohol (*6*), or, if further oxidation occurs, either a ketone or, in the case of a methyl group, a carboxylic acid (*7*). In those cases where oxidative attack occurs at the terminal carbon of the alkyl chain, either primary alcohols (*8*) or ω-phenylalkanoic acids may be obtained (*9*). More commonly, initial oxidation at this point is followed by the metabolic sequence of β-oxidation steps until the latter are inhibited by the presence of the aromatic ring as the carbon chain is successively shortened (*7a, c, 9a*). Only a few reports describe the use of microbial oxygenations of alkylbenzenes as a synthetic tool of organic chemistry. This is due, in part, to the fact that benzylic oxidations generally may be done in a more satisfactory manner with chemical reagents (*10*) and, in part, to the fact that the terminal oxygenations are difficult to control.

EXPERIMENTAL

General. Melting points were determined on a Fisher–Johns hot stage and are corrected. Magnesium sulfate was used as a drying agent. Infrared spectra were determined with either a Perkin–Elmer Infracord or Model 421 spectrophotometer. The NMR spectra, except where noted differently, were determined at 60 Mc with a Varian Model A-60A spectrometer, using tetramethylsilane as an internal standard. First-order analyses of the NMR spectra are reported. CD spectra were determined at 27°C on a Cary 60 ORD-CD instrument. Skellysolve B is the trade name for petroleum hydrocarbon fraction, bp 60–70°C, Skelly Oil Co. Florisil is the trade name for a synthetic magnesium silicate product of the Floridin Co. Nujol is the trade name for a high boiling fraction of paraffin oil.

Biotransformation procedure. The culture used in these experiments was *Sporotrichum sulfurescens* v. Beyma (ATCC 7159). The biotransformation procedure was the same for all experiments and has been described previously (*22*).

Oxygenation of 1,4-di-t-butylbenzene (**1**). The crystalline extract residues from oxygenation of **1** (20.0 g, 0.105 mole, purchased from Aldrich Chemical Co.) were dissolved in acetone, decolorized, and filtered through Celite. The still yellow filtrate was concentrated and crystallization was induced by addition of Skellysolve B. In this way a total of 5.219 g of product, mp 155–161°C, was obtained in three crops. Additional product, 0.693 g (total 5.912 g, 0.0266 mole, 25%), was obtained by chromatographing the remaining filtrate on a column of Florisil (1000 g, packed with Skellysolve B). An analytical sample of 1,4-di-(1-hydroxy-2-methyl)isopropylbenzene (**3**) was obtained after two additional recrystallizations from acetone–Skellysolve B and had mp 162–163°C (sealed capillary); ν_{OH} 3220, 3150 cm^{-1} in Nujol; $\delta_{d_6\text{-DMSO}}$ 7.27 (*s*, aromatic 4H), 4.53 (*t*, 2H, $J \simeq 5.5$ Hz, –OH), 3.42 (*d*, 4H, $J \simeq 5.5$ Hz, $-OCH_2-$, collapses to *s* upon addition of D_2O), 1.21 (*s*, 12H, $-CH_3$).

Anal. Calcd for $C_{14}H_{22}O_2$: C, 75.51; H, 10.31. Found: C, 75.63; H, 9.97.

Also obtained from the early fractions of the chromatography was a second crystalline product. This product was dissolved in Skellysolve B, decolorized, and crystallized by cooling in the freezer, 0.153 g, mp 105–111°C (sealed capillary). Three recrystallizations from cold Skellysolve B gave 4-*t*-butyl(1-hydroxy-2-methyl)isopropylbenzene (**2**) as colorless crystals, mp 116–119°C; ν_{OH} 3250, 3170 cm^{-1} in Nujol; δ_{CDCl_3} 7.28 (*s*, 4H, aromatic), 3.52 (*s*, 2H, $-CH_2O-$), 1.29 (*s*, 15H, $-CH_3$).

Anal. Calcd for $C_{14}H_{22}O$: C, 81.50; H, 10.75. Found: C, 81.18; H, 10.73.

Oxygenation of 1,4-diisopropylbenzene (**4**). The extract residues from oxygenation of **4** (25.0 g, 0.154 mole, purchased from Chemical Samples Co.) were chromatographed on Florisil (10.5 × 50 cm, 2-liter fractions) packed with Skellysolve B. Elution with 25% acetone–Skellysolve B gave the crystalline reaction product. Recrystallization from acetone–Skellysolve B gave, in three crops, 6.639 g (0.0342 mole, 22%) of product, mp 75–77°C. Two recrystallizations from acetone–Skellysolve B gave an analytical sample of 1,4-di-(1-hydroxy)isopropylbenzene (**5**), mp 76–77°C; $[\alpha]_D^{25°}$ + 19° (C, 0.9556, $CHCl_3$); CD maxima, nm ([θ]), 270.5 (88), 264 (84), 219 (5600) in cyclohexane; ν_{OH} 3280 cm^{-1} in Nujol; δ_{CDCl_3} 7.10 (*s*, aromatic 4H), 3.53 (*d*, $J \simeq 6.5$ Hz, 4H, $-CH_2O-$), 2.82 (six line pattern, $J \simeq 6.5$ Hz, 2H, –CH–), 1.18 (*d*, $J \simeq 6.5$ Hz, 6H, $-CH_3$).

Anal. Calcd for $C_{12}H_{18}O_2$: C, 74.19; H, 9.34. Found: C, 73.93; H, 9.19.

Resolution of 2-phenylpropionic acid and reduction to (2R)-2-phenyl-1-propanol. 2-Phenylpropionic acid was resolved by fractional crystallization of the salt formed with *l*-ephedrine according to the procedure of Roger and Neilson (*13*). The resolved acid had bp 119–120°C (0.55 mm); $[\alpha]_D$ −75° (C, 1.170, $CHCl_3$). Reduction of the acid (2.848 g) with lithium aluminum hydride gave the alcohol, 2.167 g after distillation, bp 102–104°C (8.0 mm), having $[\alpha]_D^{25°}$ + 16.96° (neat); lit. (*13*) bp 115°C (17 mm), $[\alpha]_D^{25°}$ + 16.7° (neat); CD maxima, nm ([θ]), 267 (73), 260 (96), 256 (84) 216 (1740) in cyclohexane.

Oxygenation of 1,3-diisopropylbenzene (**6**). The extract residues from oxygenation of **6** (25.0 g, 0.154 mole, purchased from Chemical Samples Co.) were chromatographed on Florisil (10.5 × 50 cm, 2-liter fractions) packed with Skellysolve B. Elution with 10–25% acetone–Skellysolve B gave, first, a crystalline product. This product was combined, decolorized, and recrystallized from acetone–Skellysolve B, 0.284 g, mp 122–130°C. Three further recrystallizations from acetone–Skellysolve B gave 1,3-di-(2-hydroxy)-isopropylbenzene (**7**) as colorless crystals, mp 138–139°C; ν_{OH} 3390 cm^{-1} in Nujol; $\delta_{d_6\text{-acetone}}$ 7.72 (*m*, 1H, aromatic), 7.30 (*m*, 3H, aromatic), 1.50 (*s*, 12H, $-CH_3$).

Anal. Calcd for $C_{12}H_{18}O_2$: C, 74.19; H, 9.34. Found: C, 74.49; H, 9.19.

Eluted second from the column were fractions of a viscous oil, which were combined and distilled under reduced pressure, 2.719 g, bp 132–134°C (0.10 mm). Tlc and vpc analyses of the distillate revealed that it was a mixture of two components. These were partially separated by chromatography on a silica gel column (3.8 × 40 cm, 200 g, 200-ml fractions) packed as a slurry in 10% ethyl acetate–Skellysolve B. Elution with 60% ethylacetate–Skellysolve B gave first a fraction containing only the faster-moving component (0.368 g), then three fractions containing a mixture (1.082 g), and finally five fractions of the slower-moving component (0.747 g). All fractions were viscous oils. The faster component was shown by its NMR spectrum to be 3-(1-hydroxy)isopropyl-(2-hydroxy)isopropylbenzene (**8**); $[\alpha]_D^{25°}$ + 4° (*c*, 0.8708, $CHCl_3$); ν_{OH} 3350^{-1} neat; δ_{CDCl_3} 7.6 (*m*, 4H, aromatic), 3.58 (*d*, $J \simeq 7$ Hz, 2H, $-CH_2O-$), 2.87 (six-line pattern, $J \simeq 6.5$ Hz, 1H, –CH–), 1.49 (*s*, 6H, $-CH_3$), 1.22 (*d*, $J \simeq 6.5$ Hz, 3H, $-CH_3$).

Anal. Calcd for $C_{12}H_{18}O_2$: C, 74.19; H, 9.34. Found: C, 73.92; H. 9.20.

The slower component was 1,3-di-(1-hydroxy)isopropylbenzene (**9**); $[\alpha]_D^{25°}$ +8° (*c*, 0.8270, CHC_{13}); ν_{OH} 3340 cm^{-1} neat; δ_{CDCl_3} 7.06 (*m*, 4H, aromatic), 3.58 (*d*, $J \simeq 7$ Hz, 4H, $-CH_2O-$), 2.85 (six-line signal, $J \simeq 7$ Hz, 2H, –CH–), 1.22 (*d*, $J \simeq 7$ Hz, 6H, $-CH_3$).

Anal. Calcd for $C_{12}H_{18}O_2$: C, 74.19; H, 9.34. Found: C, 74.01; H, 9.17.

p-Isopropylphenylpropanone (**13**). 2-(*p*-Isopropylphenyl)propene (**12**, 3.36 g, 0.021

mole, purchased from Aldrich Chemical Co.) was added slowly to a solution of thallium (III) nitrate (9.32 g, 0.021 mole) in 250 ml of methanol. A white solid precipitated immediately. The reaction mixture was stirred for 1 hr at room temperature. The white solid (4.62 g) was removed by filtration and the filtrate was poured into 0.5 *N* hydrochloric acid (200 ml). The mixture was stirred for 15 min, was filtered, and the methanol was removed. The aqueous residue was extracted with ether (3 × 100 ml). The ether extracts were dried with anhydrous sodium sulfate and the solvent was removed to leave an oil (3.65 g). The oil was distilled to give the desired product (**13**) as a colorless liquid (2.9 g, 78%): bp 85–88°C (0.75 mm); lit (*16b*) bp 113–115°C (10 mm); δ_{CDCl_3} 1.20 [*d*, 6, $J = 7$ Hz, $(C\underline{H}_3)_2CH$], 2.10 (*s*, 3, $OCCH_3$), 2.84 [*m*, 1, $J = 7$ Hz, $C\underline{H}(CH_3)_2$], 3.65 (*s*, 2, CH_2CO), 7.10 (*s*, 4); $\nu_{C=O}$ 1710 cm^{-1} neat.

1-(p-Isopropylphenyl)-2-methylpropan-2-ol (**14**). A solution of *p*-isopropylphenyl propanone (**13**, 2.5 g, 14.2 mmole) in 50 ml of ether was added dropwise to a solution of methylmagnesium bromide (60 mmole, 20 ml of 3 *M*) in ether (250 ml). The mixture was stirred at room temperature for 30 min, and then poured into an aqueous solution of ammonium chloride. The ether layer was removed and the aqueous layer was extracted with ether (2 × 75 ml). The combined ether extracts were dried with anhydrous sodium sulfate and the solvent was removed to leave an oil. The oil was distilled to give **14** as a colorless, viscous oil (2.5 g, 92%): bp 65–68°C (0.05 mm); δ_{CDCl_3} 1.23 [*d*, 6, $J = 7$ Hz, $(C\underline{H}_3)_2CH$], 1.22 [*s*, 6, $(C\underline{H}_3)_2COH$], 1.48 (*s*, 1, OH), 2.80 (*s*, 2, CH_2), 2.91 (*m*, 1, CH), 7.13 (*s*, 4, aromatic); ν_{OH} 3300 cm^{-1} neat.

Microbial oxygenation of 1-(p-Isopropylphenyl)-2-methylpropan-2-ol (**14**). The extract residues from oxygenation of **14** (total 14.0 g from three 10-liter fermentations, 0.073 mole) were chromatographed on Florisil (1500 g, 10.5 × 37.5 cm, 1500-ml fractions were collected) packed in Skellysolve B. Elution with 10% acetone–Skellysolve B gave first, crystalline 1-(*p*-2-hydroxyisopropylphenyl)-2-methylpropan-2-ol (**15**), 0.996 g after decolorization and crystallization from acetone–Skellysolve B, mp 95–98°C. Two recrystallizations from acetone–Skellysolve B gave an analytical sample of **15**, mp 100–101°C; ν_{OH} 3280 cm^{-1} in Nujol; δ_{CDCl_3} 7.25 (AB quartet, 4H, $J_{AB} = 8$ Hz, $\Delta\nu_{AB} = 17$ Hz, aromatic hydrogens), 2.72 (*s*, 2H, $-CH_2-$), 1.53 (*s*, 6H, $-CH_3$ of *i*-butyl group), 1.19 (*s*, 6H, $-CH_3$ of propyl group).

Anal. Calcd for $C_{13}H_{20}O_2$: C, 74.96; H, 9.68. Found: C, 75.09; H, 9.99.

The filtrates from crystallization of the above fractions were combined with the column fractions containing a mixture of products and rechromatographed on silica gel (250 g). In this way, an additional 0.616 g, mp 86–99°C (total 1.612 g, 0.00774 mole, 10.6%) of **15** was obtained, as well as an additional 1.649 g, mp 60–66°C, of product **16**, described below.

The pure fractions of a second, more polar product were combined in acetone, decolorized, and concentrated to an oil, which slowly crystallized. After allowing residual solvent to evaporate for 5 days, 5.301 g (6.950 g total, 0.00334 mole, 46%) of crystalline (2*R*)-1-(*p*-1-hydroxyisopropylphenyl)-2-methylpropan-2-ol (**16**) was obtained, mp 56–66°C; $[\alpha]_D^{25}$ +8° (C, $CHCl_3$, 1.031); ν_{OH} 3300 cm^{-1} in Nujol; δ_{CDCl} 7.03 (*s*, 4H, aromatic), 3.60 (*d*, $J = 7$ Hz, 2H, $-CH_2O-$), 2.86 (six-line pattern, $J \simeq 7$ Hz, 1H, $-\underset{|}{C}H-$), 2.67 (*s*, 2H, $-CH_2-$), 1.22 (*d*, $J = 7$ Hz, 3H, $-CH_3$), 1.16 (*s*, 6H, CH_3); CD maximum 220 nm ($[\theta] = 5100$) in methanol.

Two recrystallizations of a sample of **16** from acetone–Skellysolve B gave colorless crystals, mp 66–68°C; $[\alpha]_D^{25}$ +8° (*c*, 0.7994, $CHCl_3$); CD maxima, nm ([θ]), 262 (72), 257 (62), 220 (2750) in cyclohexane; NMR spectrum identical to the above.

Anal. Calcd for $C_{13}H_{20}O_2$: C, 74.96; H, 9.68. Found: C, 74.93; H, 9.68.

(2R)-1-(p-1-Hydroxyisopropylphenyl)-2-methylpropan-2-ol mono 2-methoxy-2-phenyl-3-trifluoromethylpropionate. A solution of (+)-2-methoxy-2-phenyl-3-trifluoromethylpropionyl chloride (0.125 g, 0.50 mmole) in pyridine (1 ml) was added to a solution of **16** (0.104 g, 0.50 mmole, of unrecrystallized material) in pyridine (3 ml). The resulting solution was left at room temperature for 2 hr. The reaction was quenched with water (20 ml) and the resulting mixture was extracted with methylene chloride. The organic phase was washed with 2 *N* HCl, with 5% sodium bicarbonate, and with saturated sodium chloride solution, then dried over $MgSO_4$. Tlc on silica gel microslides (50% ethyl acetate–Skellysolve B) showed only one product and a trace of starting alcohol. Removal of solvent gave the product as a viscous gum; δ_{CDCl_3} 7.34 (*s*, 5H, C_6H_5), 7.09 (*s*, 4H, C_6H_4), 4.45, 4.27 (AB of $ABMX_3$ system, 2H, $-OC\underline{H}_2CH$), 3.40 (*q*, $J \simeq 1$ Hz, 3H, $-OCH_3$), 3.13 (X of $ABMX_3$ system, 1H), 2.70 (*s*, 2H, benzylic $-CH_2-$), 1.25 (*d*, $J \simeq 6.5$ Hz, $-CH_3$), 1.18 (*s*, $-CH_3$). The 100-MHz spectrum (*18*) of this product mixture included two $ABMX_3$ systems, one for each diastereomer, which were slightly displaced from each other. The spectra were factored with the aid of spin decoupling and the results were checked by LAOCN3 computation. Peak height ratios of the AB portions of the spectra, corrected according to the theoretical intensities, indicated a 73:27 ratio of the two diastereomers (*18*).

(2R)-2-[4-(2-Hydroxy-2-methylpropyl)propionic acid (**11**). A standard chromic acid solution (2.5 ml, 3.65 *N*) was added dropwise to a solution of 2-[*p*-(2-methyl-2-hydroxypropyl)phenyl]propan-1-ol (**16**, 0.5 g, 0.00239 mole) in 50 ml of acetone. The reaction mixture was maintained at 10–15°C during the addition and then stirred at 20°C for 1 hr. The reaction mixture was poured into 300 ml of water and extracted with CH_2Cl_2 (5×75 ml). The combined extracts were washed with water (1×50 ml), were dried with anhydrous sodium sulfate, and the solvent was removed to leave a colorless oil which crystallized upon standing. The solid was recrystallized from chloroform–Skellysolve B to give a white solid (220 mg, 41%): mp 117–120.5°C, $[\alpha]_D^{25}$ −32° (*c*, 8.9 g/l EtOH), lit (*19*) mp 117–120.5°C, $[\alpha]_D^{25}$ +29.7° (absolute alcohol); δ_{CDCl_3} 1.23 [*s*, 6, $(CH_3)_2C$], 1.50 (*d*, 3, $J = 7$ Hz), 2.75 (*s*, 2, CH_3), 3.74 (*q*, 1, $J = 7$ Hz, CH), 7.25 (*s*, 4, aromatic); CD maxima, nm ([θ]), 227 (−10 650), 273 (−127.0) in ethanol.

Anal. Calcd for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16. Found: C, 69.97; H, 8.50.

p-Isobutylacetophenone (**17**). The procedure of Baddeley and Wrench (*20*) has been extensively modified (*23*). Acetyl chloride (184.8 g, 2.35 mole) was added slowly to a mixture of carbon tetrachloride (400 ml) and aluminum chloride (311.2 g, 2.41 mole) in a nitrogen atmosphere. The mixture was maintained at 3–4°C during this and subsequent additions. Isobutylbenzene (268 g, 2.0 mole), which had been previously dried with molecular sieves (Linde 3A), was added over a 1-hr period at such a rate that the temperature of the reaction mixture did not exceed 4°C. The reaction mixture was poured into 4 *N* HCl (1.2 liters). The mixture was extracted with CCl_4 (2×280 ml). The combined extracts were washed sequentially with 0.1 *N* HCl (240 ml), 1 *N* $NaHCO_3$ (300 ml), and water (400 ml). The organic phase was dried with anhydrous sodium sulfate and the solvent was removed to leave an oil (403 g). The oil was distilled to give

17 as a colorless liquid (333 g, 87%): bp 90–93°C (0.1 mm), lit (*20*) bp 120–123°C (12 mm); NMR ($DCCl_3$) δ 0.90 [*d*, 6, $J = 7.5$ Hz, $(CH_3)_2C$], 1.84 (*m*, 1, CH), 2.50 (*d*, 2, $J = 8$ Hz, CH_3), 2.53 (*s*, 3, CH_3CO), 7.14 (*d*, 2, $J = 8$ Hz, aromatic), 7.83 (*d*, 2, $J = 8$ Hz, aromatic); $\nu_{C=O}$ 1675 cm^{-1} neat.

Semicarbazone of **17**, colorless crystals from ethanol–water; mp 200–202°C.

Anal. Calcd for $C_{13}H_{19}N_3O$: C, 66.92; H, 8.21; N, 18.01. Found: C, 67.02; H, 8.18; N, 18.22.

2-p-Isobutylphenylpropan-2-ol (**18**). A solution of *p*-isobutylacetophenone (**18**, 25.0 g, 0.130 mole) in 50 ml of ether was added dropwise to a solution of methylmagnesium bromide (0.3 mole, 100 ml of 3 *M* in ether) in 200 ml of ether in a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 30 min, and then it was poured into a mixture of ice and concentrated HCl. The organic layer was separated and the aqueous phase was extracted with ether (2 × 100 ml). The combined organic phases were dried with anhydrous sodium sulfate. Removal of the solvent left a pale yellow oil (27.1 g, 99%). Distillation of this oil gave **18** as a colorless liquid (24.0 g, 87%): bp 84–85°C (0.1 mm); δ_{CDCl_3} 0.90 [*d*, 6, $J = 6$ Hz, $(C\underline{H}_3)_2CH$], 1.50 [*s*, 6, $(C\underline{H}_3)_2COH$], 1.76 (*m*, 1, CH), 2.36 (*s*, 1, OH), 2.43 (*d*, 2, $J = 6$ Hz, CH_2), 7.20 (AB quartet, 4, $J =$ 8 Hz, aromatic); ν_{OH} 3300 cm^{-1} neat.

p-Isobutylisopropenylbenzene (**19**). A mixture of 2-*p*-isobutylphenylpropan-2-ol (**18**, 10.0 g, 0.048 mole) and potassium hydrogen sulfate (10.0 g, 0.074 mole) was heated at 150°C for 1.5 hr. The product (**19**) was removed by distillation (7.5 g, 82%): bp 151–152°C (55 mm); δ_{CDCl_3} 0.90 [*d*, 6, $J = 6$ Hz, $(C\underline{H}_3)_2CH$], 1.86 (*m*, 1, CH), 2.13 (*s*, 3, CH_3), 2.46 (*d*, 2, $J = 7$ Hz, CH_2), 5.20 (*d*, 2, $J = 10$ Hz, $=CH_2$), 7.27 (AB quartet, 4, $J = 8$ Hz, aromatic); ν 890 cm^{-1} (CH_2 wag) neat.

p-Isobutylisopropylbenzene (**20**). *p*-Isobutylisopropenylbenzene (**19**, 7.25 g, 0.042 mole) in ethanol (100 ml) was hydrogenated in a Parr apparatus using 10% Pd/C (700 mg) at 45 psi of hydrogen. Removal of the catalyst and the solvent left an oil, which on distillation gave **20** as a colorless liquid (6.2 g, 85%): bp 71.5–73°C (2 mm); lit (*17*) bp 220°C; δ_{CDCl} 0.90 [*d*, 6, $J = 6$ Hz, isobutyl $(CH_3)_2C$], 1.20 [*d*, 6, $J = 7$ Hz, isopropyl $(CH_3)_2C$], 1.86 (*m*, 1, isobutyl CH), 2.42 (*d*, 2, $J = 7$ Hz, CH_2), 2.83 (*m*, 1, $J = 7$ Hz, isopropyl CH), 7.00 (*s*, 4, aromatic); mass spectrum, M^+ 176.

Oxygenation of p-isobutylisopropylbenzene (**20**). The extracts from oxygenation of **20** (2.5 g, 0.0142 mole) were chromatographed on Florisil (3.8 × 35 cm, 335-ml fractions) packed in Skellysolve B. Elution with 10–25% acetone–Skellysolve B gave a mixture of products **15** and **16** in the early fractions (Pool A, 0.029 g, 75% **15** and 25% **16**; Pool B, 0.061 g, 15% **15** and 85% **16**) and only product **16** (0.120 g) in the later fractions, as determined by NMR analysis. Total **15** was 0.031 g (1%) and total **16** was 0.179 g (6%).

Microbial oxygenation of 2-(p-isobutylphenyl)propan-2-ol (**18**). The extract residues from oxygenation of **18** (2.0 g, 0.0104 mole) were chromatographed on Florisil (3.8 × 35 cm, 335-ml fractions). Elution of the column was with increasing proportions of acetone in Skellysolve B. Fractions 8–10 (10% acetone–Skellysolve B), containing white crystalline material, were pooled, decolorized with activated charcoal, and crystallized from acetone–Skellysolve B, first crop, 0.407 g (0.00195 g, 19%) of crystals, mp 97–99°C. Recrystallization from acetone–Skellysolve B gave colorless crystals, mp 99–100°C, having spectral properties identical to those of **15** isolated from bioconversion of **14**. Fractions 11 and 12 were partially crystalline and by careful washing with

acetone–Skellysolve B, 0.019 g of crystals were obtained free of viscous, oily product. These crystals were recrystallized once from acetone–Skellysolve B, giving 2-(*p*-isobutylphenyl)propan-1,2-diol (**21**) as colorless crystals, mp 92–93°C; δ_{CDCl_3} 7.32, 7.07 (AB system, $-C_6H_4$), 3.65 (*d*, $J = 3$ Hz, 2H, $-C\underline{H}_2OH$), 2.47 (*d*, $J = 7$ Hz, 2H, Ar–$C\underline{H}_2$–CH), 1.50 (*s*, 3H, $-CH_3$), 0.90 (*d*, $J = 6$ Hz, 6H, $-CH_3$).

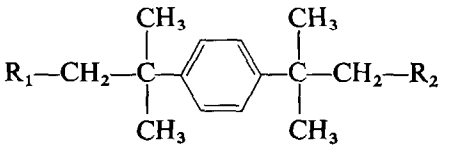
Anal. Calcd for $C_{13}H_{20}O_2$: C, 74.96; H, 9.68. Found: C, 74.73; H, 9.62.

The noncrystalline portion of fractions 11 and 12 consisted of two components, one of which was additional **21**. By subtraction of the NMR spectrum of **21** from the spectrum of the mixture, it was determined that the other component was 1-(*p*-2-hydroxyisopropylphenyl)-2-methylpropan-3-ol (**22**); signals were clearly seen at δ_{CDCl_3} 3.42 (*d*, $J = 6$ Hz, $-CHC\underline{H}_2OH$) and 1.52 (*s*, $-CH_3$) in the NMR, in addition to those present in the spectrum of **21**.

DISCUSSION

Several readily available dialkylbenzenes have been used as substrates with *S. sulfurescens* in the present study. The structures of the product obtained from fermentations of these substrates have been determined largely from the infrared and NMR spectra of the compounds. These spectra are very characteristic for the alkyl and hydroxyalkyl groups in question. Since the spectra are definitive but commonplace, the details are found mainly in the Experimental section and the discussion is presented in terms of the structures based on these data.

The oxygenation of 1,4-di-*t*-butylbenzene (**1**) was examined first. The substrate was completely consumed within 24 hr after addition to the growing culture. Although the addition of the wetting agent, Ultrawet DS-30, to fermentations of many amides often has increased the yield of products, with the present series of substrates a higher yield of product is obtained when the wetting agent is omitted from the fermentations. The only product isolated in quantity (25%) from this fermentation was 1,4-di-(1-hydroxy-2-methyl)isopropylbenzene (**3**). The intermediate monohydroxylated product **2**, detected



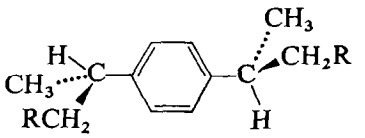
1: $R_1 = R_2 = H$

2: $R_2 = H$; $R_2 = OH$

3: $R_1 = R_2 = OH$

by the analysis of the fermentation in progress, was also isolated in a small amount from a large-scale conversion of **1**. The fate of the other 75% of the substrate is as yet undetermined.

The oxygenation of 1,4-di-*iso*-propylbenzene (**4**) was examined next. Again, a single main product (**5**) was isolated from the fermentation in yields of 20–22%. The presence of a compound that presumably is the monohydroxy intermediate also was detected by tlc analysis of this fermentation but was not isolated. It is clear from the nmr spectrum that product **5** must have the structure shown, resulting from oxygenation of one methyl group at each end of the molecule.



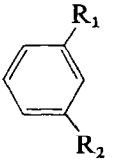
4: R = H

5: R = OH

A selectivity of the hydroxylating enzyme for one of the two methyl groups of each isopropyl group was shown by the fact that product **5** was optically active {$[\alpha]_D$ +19°}. Furthermore, hydroxylation must result in a chiral center of the same absolute configuration at both oxygenation sites or else a meso form of the product would result. Such a meso isomer has not been detected in the fermentation product.

The absolute configuration of **5** can be assigned as R by comparison of its circular dichroism (CD) spectrum to the CD spectrum of (2*R*)-2-phenylpropanol. Both compounds have positive $^1A_{1g} \rightarrow {}^1B_{2u}$ transitions near 265 nm and positive $^1A_{1g} \rightarrow {}^1B_{1u}$ transitions near 220 nm from which we conclude that they have the same absolute configuration. The absolute configuration of (2*R*)-2-phenylpropanol has been correlated in the present work with that of (2*R*)-hydratropic acid (*12*) by virtue of lithium aluminum hydride reduction of the acid to the alcohol, a reaction known to proceed with retention of configuration (*13*).

The oxygenation of 1,3-di-*iso*-propylbenzene (**6**) was then examined. Tlc examination of the product revealed it to be a mixture of at least three components. These were partially separated by column chromatography and samples containing the individual components were obtained. From the NMR spectra of these samples, it was clear that oxygenation had given a ditertiary diol (**7**), a primary–tertiary diol (**8**), and a diprimary diol (**9**). Complete stereochemical analysis of these products was made difficult by the



6: $R_1 = R_2 = -CH(CH_3)_2$

7: $R_1 = R_2 = -C(OH)(CH_3)_2$

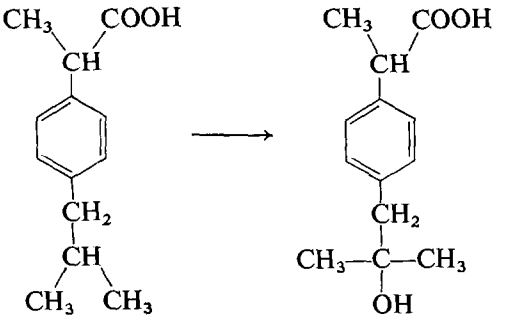
8: $R_1 = -C(OH)(CH_3)_2$; $R_2 = -CH(CH_3)CH_2OH$

9: $R_1 = R_2 = -CH(CH_3)CH_2OH$

incomplete separation achieved. Although the chiral molecules **8** and **9** were optically active {$[\alpha]_D$ +4° and +8°, respectively}, neither the optical purity of the compounds nor the presence or absence of a meso form of **9** was ascertained.

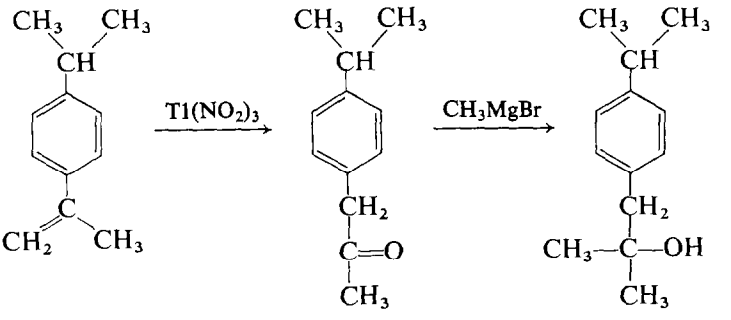
The results embodied in the above experiments have been applied to the solution of the following synthetic problem. The dextrorotatory compound, (2*R*)-2-[4-(2-hydroxy-2-methylpropyl)phenyl]propionic acid (**11**) has been isolated as a physiologically inactive metabolite of the analgesic and anti-inflammatory agent 2-(4-*i*-butyl)phenylpropionic acid (**10**) (*14*). A synthesis of the levorotatory form of the metabolite was desired in order to determine if this enantiomer was biologically active and also to confirm the structure of **11**.

The desired compound (**11**) has a single center of asymmetry in the propionic acid group and may be viewed as a dioxygenated dialkylbenzene. Our goal was to introduce this chiral center into the molecule *via* the microbial oxygenation reaction. Since the



10 **11**

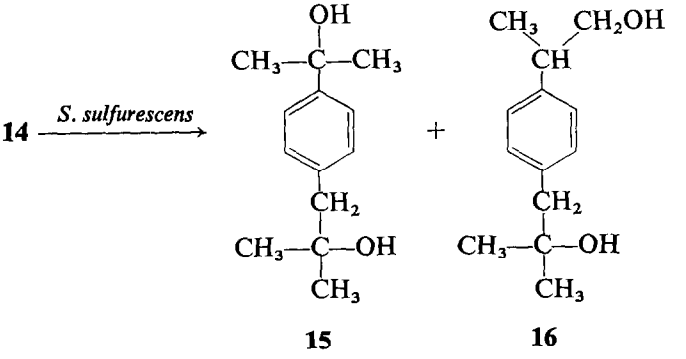
above microbial dihydroxylations proceeded stepwise, it was decided to introduce the tertiary hydroxyl group of the butyl group into the substrate first by chemical means and then allow the microorganism to introduce the second. Synthesis of the desired tertiary



12 **13** **14**

alcohol was accomplished in two steps, starting with *p*-isopropylisopropenylbenzene (**12**). Oxygenative isomerization of **12** with thallium trinitrate (*15*) gave ketone **13** in a manner greatly simplified in comparison to previous preparations of the compound (*16*). Reaction of **13** with methyl magnesium bromide gave alcohol **14**, the desired substrate.

Oxygenation of **14** with *S. sulfurescens* gave two products that could be separated by



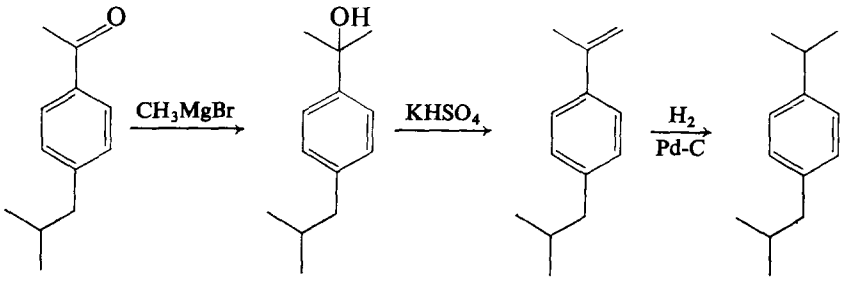
15 **16**

chromatography. The less polar product (**15**, 10.6%) was identified as the ditertiary diol by its NMR spectrum. The main product {**16**, 46%, $[\alpha]_D$ +8°}, also identified by its NMR spectrum, was the more polar tertiary–primary diol, desired for further chemical modification. In order to determine the optical purity of **16** the mono 2-methoxy-2-phenyl-3-trifluoromethylpropionate (*17*) was prepared. Selective ester formation at the primary hydroxyl function was essentially quantitative. The 100-MHz NMR spectrum of the diastereomeric mixture was difficult to analzye; however, from the AB portions of the two $ABMX_3$ systems ($-OCH_2\overset{|}{C}H-$) present in each it was possible to estimate a 73:27 ratio for the two diastereoisomers (*18*). Consequently, the optical purity of **16** is 46%.

Oxidation of **16** with a stoichiometric quantity of Jones reagent resulted in formation of the desired levorotatory (–) enantiomer of the metabolite acid (**11**, $[\alpha]_D$ –32°). The optical purity of this acid is likely of the order determined for the above alcohol. The optical purity of the dextrorotatory metabolite (*19*) {$[\alpha]_D$ +29.7°} must also be similar. It is not surprising, therefore, to find the sample of **11** enriched in (–) enantiomer also to be lacking in biological activity.

The absolute configuration of **16**, and therefore of the enantiomers of **11**, can be correlated with that of (*R*)-2-phenylpropanol on the basis of its CD spectrum just as was done with diol **5** above. The levorotatory and dextrorotatory enantiomers of metabolite **11** have the *R* and *S* configurations, respectively.

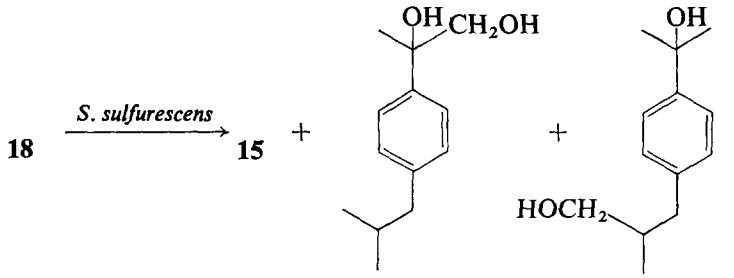
The hydrocarbon, *p*-isobutylisopropylbenzene (**20**), also was prepared for use as a substrate out of curiosity as to whether microbial hydroxylation would convert it to



17 **18** **19** **20**

the same products that were obtained from **14** above. Preparation of the hydrocarbon proceeded from *p*-isobutylacetophenone (*20*) (**17**) to the tertiary alcohol **18**. Dehydration of **18** gave **19**, which was catalytically hydrogenated to give the desired hydrocarbon (**20**). Although slightly longer than the literature preparation of **20** (*21*), this route yields intermediates that are useful as substrates also. Oxygenation of **20** with *S. sulfurescens* gave both **15** and **16** in a ratio of 1:6 and in a total yield of 7%. This may be compared to a ratio of 1:4.5 and a total yield of 56% from oxygenation of substrate **14**.

A final substrate examined was the tertiary alcohol, 2-(*p*-isobutylphenyl)propane-2-ol (**18**), conveniently available as an intermediate in the above preparation of **20**. Oxygenation of **18** gave mainly the ditertiary diol **15**, identical to the same compound isolated from oxygenation of **14**. Smaller amounts of two other diols were also obtained



21 22

as a mixture. From this mixture, one component partially crystallized, and from its NMR spectrum was assigned the glycolic structure **21**. Subtraction of the NMR spectrum of **21** from the spectrum of the diol mixture allowed assignment of structure **22** to the second component.

It is interesting to compare oxygenation of the isopropyl side chain, found in substrates **4**, **6**, **14**, and **20**, with oxygenation of the isobutyl group, found in **18** and **20**. The former is oxygenated exclusively (**4**) or predominantly (**6**, **14**, and **20**) on the primary carbon of the methyl group, whereas oxygenation of the latter occurs predominantly on the tertiary carbon of the isobutyl group. A possible explanation for these results is that there is a general preference of the oxygenating enzyme for oxygenation at the more highly substituted carbon but that this preference is counteracted by the benzene ring when it is too near the tertiary center. A similar preference for oxygenation at the more highly substituted carbon of several acyclic *N*-alkylbenzamides has also been observed (*5*). That preference was lost when the length of the alkyl chain was short (3–4 carbons from the amido group). Additional experiments are required in order to permit better understanding of these apparent preferences.

Isotopic labelling experiments have shown, in those cases studied, that oxygenation of alkyl side chains proceeds by direct substitution of oxygen for hydrogen, as opposed to dehydrogenation, forming an olefin that is then hydrated (*7b*). The oxygenation of 1,4-di-*t*-butylbenzene (**1**) is consistent with those results since formation of an olefin via dehydrogenation is impossible on the *t*-butyl groups. Dehydrogenation could conceivably occur between two methyl groups, giving a cyclopropyl intermediate, but that seems improbable.

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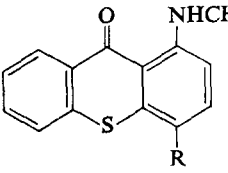
10. A notable exception is found in the oxidation of lucanthone (i) to the hydroxymethyl derivative, hycanthone (ii), in 60% yield by the organism *Aspergillus sclerotiorum* (*6a*). Here the microbial "reagent" clearly has an advantage in that oxidation of the sensitive amino and sulfide groups is



i: R = $-CH_3$
ii: R = $-CH_2OH$

avoided. The same organism carries out a similar oxidation of the methyl group in a series of 4-substituted 1-(3-chloro-4-methylphenyl)piperazines (*11*).
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23. We thank D. White for providing us with this modified procedure.