Tetrahedron: Asymmetry Vol. 4, No. 12, pp. 2469-2474, 1993
Printed in Great Britain

0957-4166/93 $6.00+.00
Pergamon Press Ltd

MICROBIAL OXIDATION OF 2-BROMOSTYRENE BY *PSEUDOMONAS PUTIDA* 39/D. ISOLATION AND IDENTIFICATION OF METABOLITES

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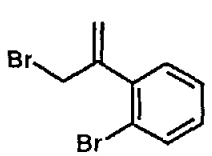
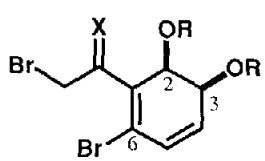
(*Received in UK* 17 *September* 1993; *accepted* 1 *November* 1993)

Abstract: 2-Bromostyrene was subjected to microbial oxidation by two microorganisms. Oxidation by *Pseudomonas putida* 39/D yielded a mixture of ring dihydroxylated (1*S*,2*R*)-4-bromo-3-ethenylcyclohexa-3,5-diene-1,2-diol (**4a**) (optical purity o.p.>92%) and side chain dihydroxylated (1*R*)-1-(2-bromophenyl)ethan-1,2-diol (**5a**) (o.p. 91%) (ratio **4a**:**5a** = 1:4) in a yield of 100 mg/L. *Pseudomonas sp.* NCIB 9816-11 afforded only **5a**.

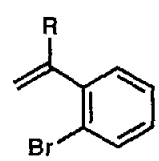
Introduction. As part of our studies directed toward the chemoenzymatic synthesis of taxol, we considered improvements in Wender's reported synthesis of the taxol skeleton.[2] We intended to prepare a nonaromatic ring C synthon similar to Wender's intermediate **1** that would be both optically pure and amenable to coupling with verbenone. Compounds such as **2a**, **2b**, and **2c** would be, with protection of the diol unit, ideally suited for such coupling. In all these diols, the configuration of the C-2 hydroxyl corresponds to that of the C-7 hydroxyl of taxol, and their functionalities should permit the elaboration to ring C of taxol. Either *o*-bromostyrene **3a** or its α-methyl derivative **3b** could yield diene diol **2** according to the published protocols of aromatic dihydroxylation.[3,4] Furthermore, Boyd[5] has recently determined that prediction of the outcome of microbial oxidation of disubstituted benzenes can be made with some degree of confidence.



1



2a (R=H, X=H_2)
2b (R=H, X=O)
2c (R=H, X=CH_2)

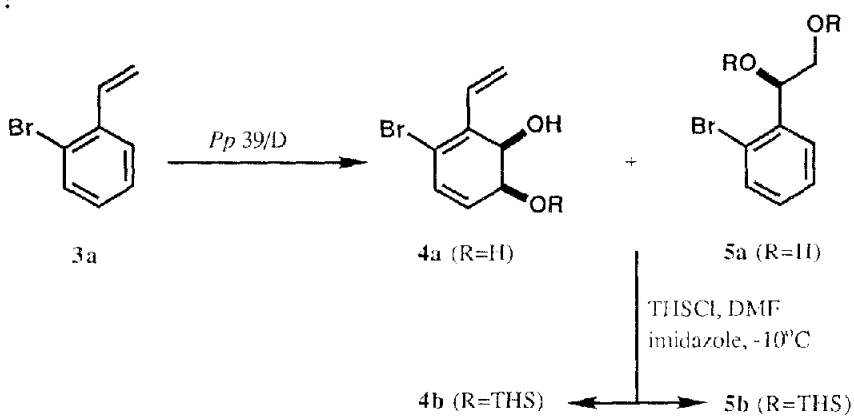


3a (R=H)
3b (R=CH_3)

Diol metabolites derived from aromatic compounds have risen to prominence in recent years as chiral synthons as evidenced by a number of reviews in the area of synthetic applications.[6] In this manuscript we report on the isolation and identification of those metabolites from *o*-bromostyrene and discuss the relevance of such compounds to taxol synthesis.

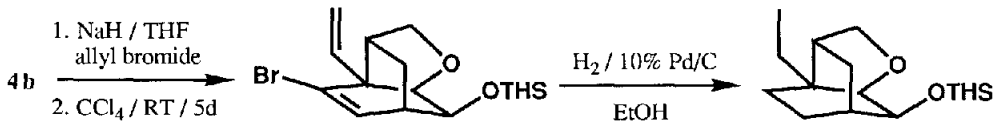
Results and Discussion. The microbial dihydroxylation of 2-bromostyrene (**3a**) by fermenting *Pseudomonas putida* 39/D,[3] induced by toluene vapor according to an established protocol,[7] afforded a 1:4 mixture of diols **4a** and **5a** in a yield of 100 mg/L of culture from 1.4 g/L of 2-bromostyrene, accompanied by approximately 100 mg/L of toluene-2,3-dihydrodiol from the oxidation of the toluene used as an inducer. Because of similar Rf-values and the tendency of **4a** toward aromatization, the chromatographic separation of the mixture proved difficult.

Scheme 1:



Reaction of a 1:2 mixture of **4a** and **5a** at −10 °C with dimethylthexylsilyl chloride (THSCl) and imidazole in DMF afforded the monoprotected alcohol **4b** and the disilylated compound **5b** (Scheme 1). To determine the absolute configuration and the enantiomeric excess of **4a**, compound **4b** was transformed at −25 °C to its allyl ether, which underwent a facile Diels–Alder cycloaddition at room temperature to give tricyclic ether **6** in 25% yield (Scheme 2). Hydrogenation with 10% palladium on carbon catalyst afforded the known ether (−)-**7**, whose optical purity was determined as >92% by comparison with literature data.[7] Diol **5a** was dehalogenated with sodium in refluxing ethanol and yielded (−)-(1*R*)-phenylethyleneglycol ($[\alpha]_{546}^{20}$ 41.4, optical purity 91%) of known absolute configuration.[8]

Scheme 2:



6

7: $[\alpha]_D^{20}$ -29.5
lit. $[\alpha]_D^{20}$ -30.2 (e.e.>98%) [7]

The low yield of diols **4a** and **5a** as well as the unfavorable product ratio in the dihydroxylation by *P. putida* 39/D prompted us to investigate the hydroxylation reaction by other dioxygenase-containing microorganisms. Incubation of **3a** with the dehydrogenase-deficient organism *Pseudomonas sp.* NCIB 9816-11, known for its ability to oxidize naphthalene,[9] afforded the product of side-chain dihydroxylation, **5a**, exclusively.

Conclusion. A comparison of the rate of dihydroxylation of a series of styrene derivatives (styrene,[7] 2-chlorostyrene,[7] and 2-bromostyrene) clearly reveals the low activity of *P. putida* 39/D dioxygenase towards substrates bearing bulky substituents in an *ortho*-position. These results may be explained by steric interactions that prevent the larger substrates from readily entering the active site of the dioxygenase. Electronic factors can be largely excluded because styrene itself as well as all of the halobenzenes are excellent substrates for toluene dioxygenase.[10] With ring oxidation impeded by steric hinderance, oxidation of the side chain predominates. Whereas only about 5% of phenylethyleneglycol was formed from styrene,[7] 65% was obtained from 2-chlorostyrene,[7] and 80 % from 2-bromostyrene.

To attain our goals in terms of production of **2a**, other organisms or substrates will be investigated. We will report on these accomplishments in due course.

Experimental Section

General: NMR spectra were determined in $CDCl_3$ (unless otherwise stated) on a *Bruker* WP-270 or a *Varian* Unity 400, ^1H at 270 MHz or 400 MHz and ^{13}C at 68 MHz or 100 MHz. Coupling constants are given in Hertz. IR spectra were obtained from neat oils unless otherwise noted. All solvents used in reactions were dried according to standard procedures. Flash column chromatography was performed on Merck silica gel (grade 60, 230–400 mesh). Elemental analyses were performed by Atlantic Microlabs, Norcross, GA.

Microbial oxidation of 2-bromostyrene by *Pseudomonas putida* 39/D. Fermentations were carried out as described earlier.[7] From 14.0 g (76.5 mmol) of 2-bromostyrene (**3a**), 2.1 g of crude extract was obtained. Flash chromatographic separation of 1.95 g of the extract with a gradient of 20–50% ethyl acetate in hexane afforded 330 mg of a mixture of **4a** and **5a**, and 660 mg (3.0 mmol) of **5a**. Recrystallization of **5a** from ethyl acetate/hexane afforded an analytically pure crystalline sample. Chromatography of 50 mg of the mixture of **4a** and **5a** with benzene/ethyl acetate/methanol (60:30:1) yielded 15.6 mg of pure **4a**.

(1*S*,2*R*)-4-Bromo-3-ethenylcyclohexa-3,5-diene-1,2-diol (4a). ^1H-NMR (270 MHz) δ 6.82 (1H, dd, *J*=17.5, 11.0), 6.12 (1H, dd, *J*=10.0, 2.8), 5.83 (1H, dt, *J*=10.0, 3.0), 5.64 (1H, d, *J*=17.5), 5.42 (1H, d, *J*=11.1), 4.55 (1H, m), 4.47 (1H, m), 2.35 (2H, s); ^{13}C-NMR (68 MHz) δ 134.54, 134.08, 133.33, 129.69, 121.77, 117.70, 70.35, 67.61.

(−)-(1*R*)-1-(2-Bromophenyl)ethan-1,2-diol (5a). mp 118–120 °C. $[\alpha]_D^{20}$ −7.5 (c 0.99, $CHCl_3$). ^1H-NMR (270 MHz, CD_3OD) δ 7.58 (1H, dd, *J*=7.7, 1.5), 7.52 (1H, dd, *J*=8.0, 1.1), 7.35 (1H, td, *J*=7.6, 1.0), 7.16 (1H, td, *J*= 7.7, 1.7), 5.18 (1H, dd, *J*=7.9, 2.9), 3.90 (1H, dd, *J*=11.3, 2.9), 3.55 (1H, dd, *J*=11.4, 7.9), 3.02 (1H, s). ^{13}C-NMR (68 MHz, CD_3OD) δ 142.22, 133.55, 130.04, 129.35, 128.61, 123.08, 74.71, 67.27. MS *m/z* (relative intensity) 218 (M^+, 5), 216 (M^+, 5), 187 (65), 185 (100), 77 (100). Anal. Calcd for $C_8H_9BrO_2$: C, 44.27; H, 4.18. Found: C, 44.16; H, 4.17.

Debromination of diol 5a was performed according to a literature procedure for the corresponding chlorinated compound.[7] Thus, from **5a** (216 mg, 0.986 mmol), 93 mg (0.67 mmol, 68%) of **(−)-(1*R*)-1-phenylethan-1,2-diol** was obtained as a white crystalline material. $[\alpha]_D^{20}$ −35.6 (c 0.55, EtOH), $[\alpha]_{5461}^{20}$ 41.4 (c 0.55, EtOH). Other physical data were in accordance with the literature.[7]

Protection of the mixture of 4a and 5a. The mixture of **4a** and **5a** (300 mg, 1.38 mmol) and imidazole (211 mg, 3.1 mmol) were dissolved in DMF (2 ml). Dimethylthexylchloride (483 mg, 2.7 mmol) was added dropwise at −10 °C by syringe, and the mixture was stirred at 10 °C. After 24 h the starting material was consumed as judged by TLC, and a white precipitate had formed. The mixture was diluted with 3 ml of brine, extracted with diethyl ether (2 mL, 4 times), the combined organic phases was washed with saturated copper sulfate solution (2 mL, 2 times) and brine (2mL), then dried over sodium sulfate. Flash chromatography on 20 g of silica gel eluting with a gradient of 0–5% ethyl acetate in hexane afforded **4b** (180 mg, 0.36 mmol, 26%) and **5b** (212 mg, 0.59 mmol, 43%) as colorless oils.

(+)-(1*R*,6*S*)-3-Bromo-6-{dimethyl-(2,3-dimethylbut-2-yl)siloxy}-2-ethenylcyclohexa-2,4-dienol (4b). $[\alpha]_D^{20}$ +109 (c 3.87, $CHCl_3$); $[\alpha]_{5461}^{20}$ +127 (c 1.32, $CHCl_3$). ^{1}H-NMR (400 MHz) δ 6.85 (1H, dd, *J*=17.6, 11.0), 6.09 (1H, dd, *J*=10.0, 2.8), 5.68 (1H, dt, *J*=10.0, 1.9), 5.67 (1H, d, *J*=17.5), 5.42 (1H, d, *J*=11.0), 4.65 (1H, m), 4.32 (1H, dt, *J*=5.5, 1.9), 2.71 (1H, s), 1.68 (1H, septet, *J*=6.9), 0.932 (3H, d, *J*=6.9), 0.928 (3H, d, *J*=6.9), 0.91 (6H, s), 0.202 (3H, s), 0.19 (3H, s). ^{13}C-NMR (100.57 MHz) δ 135.00, 133.55, 132.57, 129.62, 121.58, 117.51, 71.27, 67.88, 34.43, 25.29, 20.54, 20.44, 18.87, 18.82, –2.37, –2.64. MS *m/z* (relative intensity) 360 (M^+, 0.6), 358 (M^+, 0.4), 275 (22), 273 (22), 257 (100), 255 (100), 194 (100), 179 (100). HRMS (CI+) calcd for $C_{16}H_{26}BrOSi$ [M-17] 341.0936, found 341.0984.

(–)-(1*R*)-1,2-Bis-{dimethyl-(2,3-dimethylbut-2-yl)siloxy}-1-(2-bromophenyl)ethane (5b). $[\alpha]_D^{20}$ –29.4 (c 1.17, $CHCl_3$); $[\alpha]_{5461}^{20}$ –35.3 (c 1.17, $CHCl_3$). IR (ν) 2955, 2865, 1462, 1250, 1125, 1082, 820 cm^{-1}. ^{1}H-NMR (270 MHz) δ 7.57 (1H, dd, *J*=7.7, 1.7), 7.49 (1H, dd, *J*=1.2, 8.0), 7.30 (1H, dt, *J*=1.2, 7.5), 7.11 (1H, dt, *J*=1.8, 7.8), 5.13 (1H, dd, *J*=6.8, 3.5), 3.66 (1H, dd, *J*=10.3, 3.7), 3.56 (1H, dd, *J*=10.3, 6.8), 1.66 (2H, septet, *J*=6.9), 0.92 (3H, d, *J*=6.9), 0.90 (3H, d, *J*=6.9), 0.87 (3H, d, *J*=6.9), 0.86 (3H, d, *J*=6.9), 0.83 (12H, br. s), 0.17 (3H, s), 0.05 (3H, s), 0.04 (3H, s), –0.06 (3H, s). ^{13}C-NMR (68 MHz) δ 141.96, 132.39, 129.51, 128.83, 127.34, 122.38, 75.21, 68.53, 34.40, 25.59, 25.34, 20.75, 20.58, 19.02, 18.77, –2.48, –3.17 (several CH_3 in the thexyl groups have not been resolved). MS (CI+) 487 (M^+–15), 485 (M^+–15). Anal. Calcd for $C_{24}H_{45}BrO_2Si_2$: C, 57.46; H, 9.04. Found: C, 57.55; H, 9.03.

(–)-(1*R*,4*R*,6*S*,9*S*,10*S*)-8-Bromo-9-ethenyl-10-{dimethyl-(2,3-dimethylbut-2-yl)siloxy}-2-oxatricyclo[4.3.1.0^{4,9}]dec-7-ene (6). A solution of **4b** (160 mg, 0.445 mmol) in THF (3.0 ml) was added slowly to a stirred slurry of hexane-washed NaH (78 mg, 3.25 mmol) in THF (3 ml) cooled to –25 °C. A solution of allyl bromide (393 mg, 3.25 mmol) in THF (3 ml) was added slowly and the mixture stirred at –25 °C for 48 h, after which time TLC showed complete disappearance of **4b**. The reaction was quenched at –25 °C with brine (10 ml), then extracted with Et_2O, dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo and dissolved in 100 ml of CCl_4. The resulting solution was kept at room temperature for 5 days. After removal of the solvent in vacuo, flash chromatography on silica gel eluting with a 0–3% ethyl acetate/hexanes gradient provided the adduct **6** (45 mg, 0.113 mmol, 25%) as a colorless oil: $[\alpha]_D^{20}$ –50.9 (c 1.69, $CHCl_3$). IR (ν) 2960, 2890, 2870, 1465, 1250, 1118, 830 cm^{-1}. ^{1}H-NMR (270 MHz) δ 6.58 (1H, d, *J*=7.6), 5.98 (1H, dd, *J*=17.5, 10.9), 5.34 (1H, dd, *J*=9.0, 0.75), 5.27 (1H, dd, *J*=17.5, 0.9), 3.88 (1H, dd, *J*=7.4, 4.7), 3.83 (1H, d, *J*=7.1), 3.62 (1H, d, *J*=7.4), 3.57 (1H, dt, *J*=6.75, 2.0), 2.42 (1H, ddt, *J*=7.7, 5.5, 2.0), 2.22 (1H, dd, *J*=9.9, 4.5), 1.87 (1H, ddd, *J*=12.7, 3.5, 1.3), 1.55–1.73 (3H, m), 0.93 (3H, d, *J* = 6.9), 0.91 (3H, d, *J*=6.8), 0.87 (3H, s), 0.86 (3H, s), 0.13 (s, 3H), 0.09 (s, 3H). ^{13}C-NMR (78 MHz) δ 139.17, 135.52, 121.51, 116.09, 74.76, 74.47, 71.44, 56.73, 39.68, 39.56, 34.79, 32.65, 25.55, 20.87, 20.57, 19.01, 18.81, –2.24, –3.00. MS *m/z* (relative intensity) 385 (M^+–15, 0.8), 383 (M^+–15, 0.6), 315 (35), 313 (32), 297 (12), 295 (10), 285 (28), 283 (25), 73 (100). HRMS (CI^+) calcd for $C_{18}H_{28}O_2BrSi$ [M-15] 383.1042, found 383.1034.

(−)-(1*R*,4*R*,6*R*,9*S*,10*S*)-9-Ethyl-10-{dimethyl-(2,3-dimethylbut-2-yl)siloxy}-2-oxatricyclo [4.3.1.0^{4,9}]decane (7). Hydrogenolysis of **6** was performed according to a literature procedure for the corresponding chlorinated compound.[7] Thus, from **6** (26 mg, 0.065 mmol), **7** (11.1 mg, 0.034 mmol, 52%) was obtained as a colorless oil: $[\alpha]_D^{20}$ −29.5 (c 0.741, $CHCl_3$). All other physical data were in accordance with the literature.[7]

Acknowledgements. K.K. gratefully acknowledges an Erwin Schrödinger Fellowship of the Austrian Fonds zur Förderung der wissenschaftlichen Forschung. This work was supported by Genencor International, Inc., and TDC Research, Inc. We thank Gregg Whited of Genencor International for logistical support.

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