Pergamon

Tetrahedron: Asymmetry, Vol. 8, No. 7, pp. 975–977, 1997

Printed in Great Britain
PII: S0957-4166(97)00059-1 0957-4166/97 $17.00 + 0.00

New metabolites from toluene dioxygenase dihydroxylation of oxygenated biphenyls

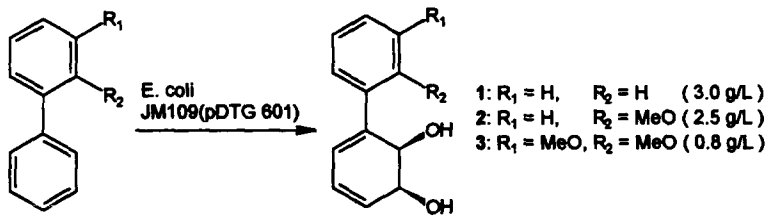
David Gonzalez,[a] Valeria Schapiro,[b] Gustavo Seoane[b] and Tomas Hudlicky[a,*]

[a] Department of Chemistry, University of Florida, Gainesville, FL 32611, USA
[b] Facultad de Química, Universidad de la República, Gral. Flores 2124, Montevideo, Uruguay

Abstract: Direct microbial oxidation of 2-methoxybiphenyl and 2,3-dimethoxybiphenyl by *E. coli* JM109 (pDTG601) furnished enantiomerically pure 3-(2-methoxyphenyl)-(1*S*,2*R*)-3,5-cyclohexadiene-1,2-diol (**2**) and 3-(2,3-dimethoxyphenyl)-(1*S*,2*R*)-3,5-cyclohexadiene-1,2-diol (**3**) respectively as the only reaction products. © 1997 Elsevier Science Ltd

Although strains of *Beijerinckia* and *Pseudomonas* are known to oxidize biphenyl to 3-phenyl-(1*S*,2*R*)-3,5-cyclohexadiene-1,2-diol (**1**),[1] few substituted biphenyl diol metabolites have been isolated and fully characterized. Most of the work in this class of compounds has been done on mono- and polychlorinated diols derived from the biooxidation of PCBs.[2a] In addition, Furukawa[2b] and collaborators have recently reported that strains of *Pseudomonas* are capable of oxidizing biphenyls bearing a wide range subtituents on one ring. Specifically, they found that hydroxylated biphenyls were oxidized to the corresponding bis-catechols and that the oxidation took place exclusively on the non-substituted aromatic ring.[3]

As a part of our ongoing program in practical chemoenzymatic synthesis of natural products we became interested in diols **2** and **3** as asymmetric synthons (Scheme 1).[4,5] We have found that these interesting metabolites can be cleanly prepared on multigram scale by direct microbial oxidation of the corresponding aromatic substrates with *E. coli* JM109 (pDTG601), a recombinant microorganism introduced by Gibson and collaborators that overexpresses the genetic information for the enzyme toluene dioxygenase from several *Pseudomonas* strains (Scheme 1).[6,7]

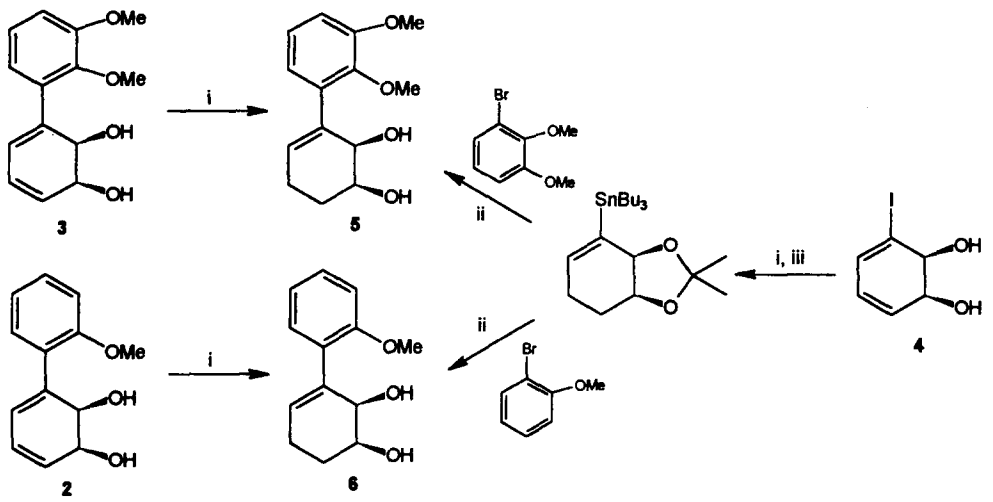


Scheme 1.

Although derivatives of diols such as **2** and **3** can be obtained by chemical modification of diol **4** by means of palladium mediated cross-coupling methodology,[8] the yields are frequently low and the procedures involve the use of environmentally unacceptable reagents and large amounts of expensive catalysts (Scheme 2). However, because the absolute stereochemistry of diol **4** is known,[9] the chemical process was used here to determine the absolute configuration of the new metabolites.

Diols **2** and **3** were isolated from the microbial broth by extraction with ethyl acetate and purified by flash chromatography.[7,10,11] To minimize the tendency toward aromatizacion the disubstituted olefin in both **2** and **3** was reduced with diimide generated from PAD (potassium azodicarboxilate) in the

* Corresponding author. Email: hudlicky@chem.ufl.edu



i. PAD, HOAc, 90%; ii. (a) $Pd(PPh_3)_4$, toluene, 30%, (b) Amberlyst 15 (wet), MeOH-10% H_2O, 45°C, 80%; iii. (a) dimethoxypropane, *p*-TsOH, (b) *s*-BuLi, then $ClSnBu_3$, 60%.

Scheme 2.

presence of acetic acid. Independently, diol **4**, derived from iodobenzene, was converted to its tributyltin derivative according to Ley's procedure[8] and coupled with either 2-bromodimethylcathechol to give **5** or with 2-bromoanisol to furnish **6**, for comparison with the reduced metabolite.

The absolute stereochemistry of diols **2**[10] and **3**[11] was thus unequivocally proven by comparison of the optical rotation of the reduced derivatives **5**[11] and **6**[10] respectively to the corresponding value obtained for the same compounds prepared from the known diol **4** (Scheme 2). Since the values obtained in either cases were identical within the experimental error we assigned the stereochemistry of **2** and **3** as shown.

In summary, two new metabolites from the microbial oxidation of oxygen-containing biphenyls are reported. The resulting diols are obtained as the sole product of the biotransformation and constitute, to our knowledge, the first example of this class of metabolites isolated from oxygenated biphenyls. Their use in new synthetic ventures will be reported in due course.

Acknowledgements

We thank Dr. David Gibson for providing us with a sample of *E. coli* JM109 (pDTG601) and Mary Ann Endoma for her assistance in biooxidation techniques. Financial support by TDC Research Inc., National Science Foundation (CHE-9315684 and CHE-9521489), The University of Florida, CSIC-Uruguay and CONICYT-Uruguay (Project 060/94) are gratefully acknowledged.

References

1. (a) Ziffer, H.; Kabuto, K.; Gibson, D. T.; Kobal, V. M.; Jerina, D. M. *Tetrahedron* **1977**, *33*, 2491; (b) Gibson, D. T.; Roberts, R. L.; Wells, M. C.; Kobal, V. M. *Biochem. Biophys. Res. Commun.* **1973**, *50*, 211; (c) Catelani, D.; Sorlini, C.; Treccani, V. *Experientia* **1971**, *27*, 1173.
2. (a) Safe, S.H. in *Microbial Degradations of Organic Compounds* Gibson, D. T., Ed., Marcel Dekker, New York, **1984**, 361; (b) Furukawa, K.; Tomizuka, N.; Kamibayashi, A. *Appl. Environ. Microbiol.* **1979**, *38*, 301.
3. Kimura, K.; Kato, H.; Nishi, A.; Furukawa, K. *Biosci. Biotech. Biochem.* **1996**, *60*, 220.
4. Gonzalez, D.; Schapiro, V.; Seoane, G.; Hudlicky, T., Abboud, K. *J. Org. Chem.* **1997**, *62*, 1194.

5. For comprehensive reviews of arene *cis*-diol chemistry see: (a) Brown, S. M.; Hudlicky, T. *Organic Synthesis: Theory and Applications* Hudlicky, T., Ed., JAI Press, Greenwich, CT, **1993**, *2*, 113; (b) Widdowson, D. A.; Ribbons, D. W.; Thomas, S. D. *Janssen Chimica Acta* **1990**, *8*, 3; (c) Carless, H. A. J. *Tetrahedron: Asymm.* **1992**, *3*, 795; (d) Hudlicky, T.; Reed, J. W. in *Advances in Asymmetric Synthesis* Hassner, A., Ed., JAI Press, Greenwich, CT, **1995**, 271; (e) Hudlicky, T. in *Green Chemistry: Designing Chemistry for the Enviroment, ACS Symposium Series* Anastas, P. T.; Williamson, T., Eds., Washington, DC, **1996**, *626*, 180; (f) Hudlicky, T.; Thorpe, A. J. *J. Chem. Soc., Chem Commun.* **1996**, 1993; (g) Hudlicky, T. *Chem. Rev.* **1996**, *96*, 3; (h) Hudlicky, T.; Entwistle, D. A.; Pitzer, K. K.; Thorpe, A. J. *Chem. Rev.* **1996**, *96*, 1195; (i) Grund, A. D. *SIM News* **1995**, *45*, 59.
6. Zylstra, G. J.; Gibson D. T. *J. Biol. Chem.* **1989**, *264*, 14940.
7. **Typical procedure for microbial transformations**: *E. coli* JM109 (pDTG601) was grown overnight at 35°C in an enriched medium containing ampicillin (100 mg/L). The preculture was then transferred to a 12 L fermentor containing 8 L of a similar medium, and the cells were grown to an OD=70 (λ=660 nm). The finely ground substrates were added in small portions, and their metabolic transformation was monitored by observing the oxygen consumption and CO_2 production by the culture. Diol production was checked by measuring the characteristic absorbance peak in the UV region (λ=270 nm). After all metabolic activity ceased (or no more diol formation was observed), the reaction was stopped and the pH was adjusted to 8.0 with conc. NH_4OH. The cells were separated from the broth by centrifugation (7000 rpm, 20 minutes), and the resulting clear solution (8 L) was extracted with ethyl acetate (5×1 L). The organic layer was dried with $MgSO_4$, and the solvent was evaporated at 20°C. The crude diol was purified *via* flash chromatography on deactivated silica gel (10% water) using hexane/ethyl acetate (7:3) as the eluent to render in each case a pure compound: diol **1** (3 g/L), diol **2** (2.5 g/L), and diol **3** (0.8 g/L).
8. Ley, S. V.; Redgrave, A. J.; Taylor, S. C.; Ahmed, S.; Ribbons, D. W. *Synlett* **1991**, 741.
9. Boyd, D. R.; Hand, M. V.; Sharma, N. D.; Chima, J.; Dalton, H.; Sheldrake, G. N. *J. Chem. Soc., Chem. Commun.* **1991**, 1631.
10. *Data for* **2**: white crystalline solid; mp 77–78°C; R_f=0.3 hexane/ethyl acetate, 50:50; $[\alpha]_D^{28}$+160 (c 1.74, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.25 (m, 2H), 6.95 (m, 2H), 6.07 (m, 2H), 5.92 (m, 1H), 4.51 (bs, 1H), 4.42 (bd, *J*=5 Hz, 1H), 3.86 (s, 3H), 3.31 (bs, 1H), 2.81 (bs, 1H), ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.3, 139.2, 130.8, 130.0, 129.1 (d.i.), 124.3, 123.9, 121.3, 110.8, 69.7, 69.4, 55.7. *Data for* **6**: white crystalline solid; mp 82.5–83.0°C; R_f=0.3 hexane/ethyl acetate, 50:50; $[\alpha]_D^{23}$ −80.7 (c 1.0, $CHCl_3$); IR ($CHCl_3$) 3564, 3020, 2937, 2840, 1598, 1489 1464, 1436, 1246, 1222, 1206; ^1H NMR (300 MHz, $CDCl_3$) δ 7.19 (m, 1H), 7.09 (dd, *J*=7.4, 1.4Hz, 1H), 6.85 (m, 2H), 5.79 (t, *J*=3.7 Hz, 1H), 4.42 (bd, *J*=2.8 Hz, 1H), 3.88–3.79 (m, 1H), 3.76 (s, 3H), 2.61 (bs, 2H), 2.24 (m, 2H), 1.78 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0, 156.2, 137.4, 131.0, 130.4, 128.7, 121.1, 110.6, 69.3, 68.7, 55.3, 25.1, 24.4; (LRMS (CI/CH_4) *m/z* (rel intensity) 220 (M^+, 12), 203 (100), 175 (8); HRMS calcd for $C_{13}H_{16}O_3$: 220.1099. Found: 220.1098.
11. *Data for* **3**: Yellow oil, R_f=0.3 hexane/ethyl acetate, 50:50; $[\alpha]_D^{24}$+62.5 (c 0.8, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.07 (t, *J*=8.0 Hz, 1H), 6.89 (td, *J*=7.0, 1.4 Hz, 2H), 6.12 (m, 2H), 6.02 (m, 1H), 4.49 (bs, 2H), 3.89 (s, 3H), 3.84 (s, 3H), 3.38 (bs, 1H), 2.58 (bs, 1H). *Data for* **5**: white crystalline solid; mp 66.0–67.0°C; R_f=0.3 hexane/ethyl acetate, 50:50; $[\alpha]_D^{23}$ −62.9 (c 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.03 (t, *J*=17.7 Hz, 1H), 6.87 (d, *J*=7.1 Hz, 1H), 6.77 (dd, *J*=7.4, 0.8 Hz, 1H), 5.89 (t, *J*=3.6 Hz, 1H), 4.42 (bs, 1H), 3.94–3.89 (m, 1H), 3.87 (s, 3H), 3.70 (s, 3H), 2.60 (bs, 2H), 2.30 (m, 2H), 1.88 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.5, 145.8, 136.6, 135.8, 130.5, 124.5, 122.5, 111.6, 69.3, 69.0, 61.0, 55.8, 25.2, 24.2; LRMS (CI/CH_4) *m/z* (rel intensity) 250 (M^+, 100), 232 (35), 206 (93); HRMS calcd for $C_{14}H_{18}O_4$: 250.1205. Found: 250.1205.

(Received in USA 16 December 1996; accepted 26 January 1997)