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Enzymatic oxidation of thioanisoles: isolation and absolute configuration of metabolites

Kevin J. Finn, Petr Cankař, Timothy R. B. Jones[†] and Tomas Hudlicky[*]

Department of Chemistry, Brock University, St. Catharines, Ontario, Canada L2S 3A1

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This paper is dedicated to Professor Peter Stanetty on the occasion of his 60th birthday

Abstract—Oxidation of *p*-bromothioanisole with toluene dioxygenase provides the corresponding diene diol **2** in good yield. Electrochemical reduction of **2** gives access to diene diol **3**, which is not accessible by direct bio-oxidation of thioanisole. Absolute configuration and enantiomeric purity are reported for the new metabolites.

1. Introduction

The oxidation of sulfur-containing compounds by various enzymes is well documented;[1] both monooxygenases and dioxygenases convert divalent sulfur to sulfoxides with high enantiomeric excess.[2] The prokaryotic dioxygenases, which process aromatic compounds to *cis*-dihydrodiols,[3] have shown remarkable selectivity in the oxidations of thiophene[4] and benzothiophene[4b,5] to the corresponding *cis*-diols, in most cases without attendant oxidation of the sulfur atom.

In connection with our study of selective electrochemical reduction of halogen versus sulfur functionalities, we became interested in halothioanisoles such as **1**, whose metabolites would be amenable to a variety of further reactions, as indicated in Figure 1. For example, oxidation of the thioether in diene diol **2** would provide sulfone **4** in which each carbon could be further functionalized by selective operations on the vinylsulfone moiety[6] as well as by tethered radical cyclizations[7] of the vinyl bromide. We were also interested in the directing effects of the thiomethyl group on the stereochemistry of the oxidation. In a review published in 1998 Boyd proposed that the larger of the two groups in *p*-substituted arenes directs the oxidation with a 2,3-

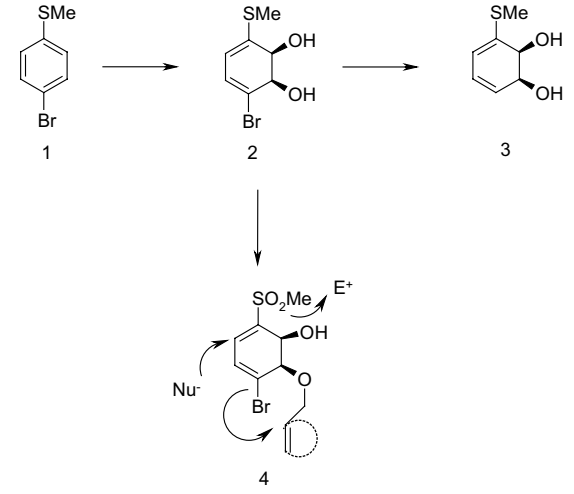

Figure 1. Oxidation of *p*-bromothioanisole and reactive options in metabolites.

regiochemistry and β-stereochemistry.[8] In this manuscript we report the synthesis, absolute configuration, and enantiomeric purity of diol **2** as well as the synthesis of diol **3**, which cannot be obtained by direct bio-oxidation of thioanisole with toluene dioxygenase.

2. Results and discussion

Boyd prepared the unstable diol **3** chemically from the homochiral diol **5** in a very low yield (~10–14%)[9]

* Corresponding author. Tel.: +1 905 688 5550x4956; fax: +1 905 984 4841; e-mail addresses: jonesy@chemiris.labs.brocku.ca; thudlicky@brocku.ca
† To whom correspondence regarding ^{19}F NMR should be addressed.

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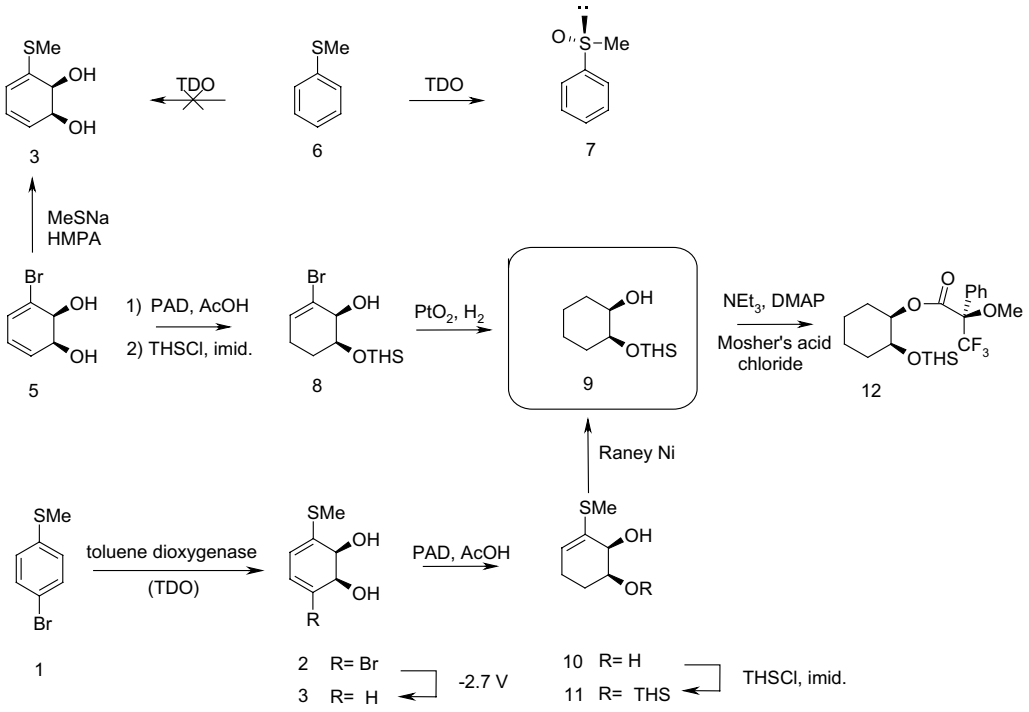
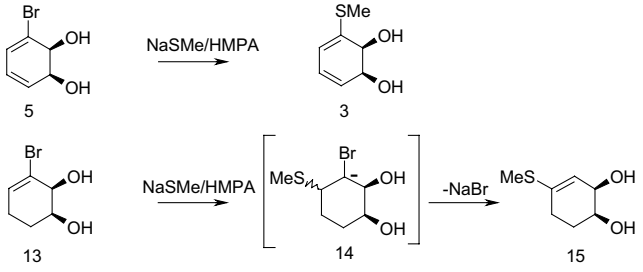


Scheme 1.

(Scheme 1). An improvement in yield (55%) was realized when the iodo analogue of **5** was used.[10] (*R*)-Sulfoxide **7** was also reported by Boyd et al.[11] from the fermentations of thioanisole **6** with *Pseudomonas* UV4.

When we subjected thioanisole **6** to whole-cell fermentation with the recombinant *E. coli* JM 109 (pDTG601), an organism developed by Gibson to over-express toluene dioxygenase (TDO),[12] we identified **7** as the major product (2.5 g/L, 92% enantiomeric excess)[13] together with trace amounts of **3**.[14] In a similar whole-cell oxidation of *p*-bromothioanisole with Gibson's organism, we obtained a relatively high yield (2.3 g/L) of diol **2**,[15,16] isolated as a crystalline compound by ethyl acetate extraction of the fermentation broth.[17] Diol **2**, unlike **3**, is quite robust, probably because of the electron flow from the two functionalities is mutually cancelled and does not contribute to the lability of either hydroxyl. Electrochemical reduction[18] of **2** at −2.7 V (Hg cathode) gave **3**, which was immediately subjected to diimide reduction with potassium azodicarboxylate (PAD)[19] and subsequently protected as the silyl ether **11**. Initial attempts to determine the absolute configuration of diol **2** were performed according to the precedent for vinyl halides to undergo substitution by sulfur nucleophiles by a putative addition–elimination mechanism mediated by HMPA.[20] Indeed, Boyd has reported the synthesis of cyclohexadiene-diol **3** by treatment of homochiral diol **5** with sodium thiomethoxide in HMPA, albeit in low yield, 1–5%[10] and 10–14%.[9] Under identical conditions, diol **13** was instead converted to the regioisomer **15**, the structure and connectivity of which was tentatively assigned on the basis of COSY ^{1}H NMR spectroscopy (Scheme 2). A plausible explanation for the formation of **15** may be the preference for a conjugate rather than the α-addition of sodium mercaptide to the olefin, as shown in Scheme 2.



Scheme 2.

In a second attempt to establish its absolute configuration, diol **2** was converted to silyl ether **11**, which was reduced in the presence of Raney nickel to ether **9**. Finally, homochiral diene diol **5** was partially reduced, protected as its THS ether, and hydrogenated in the presence of Adams' catalyst to provide mono-protected diol **9**, whose physical and spectral properties were compared with those of the material obtained from diol **2** as shown in Scheme 1. The sample of **9** obtained from **5** had a specific rotation of +10.6; the sample isolated from Raney nickel reduction of **11** had a specific rotation of +3. The mass spectrum of the latter showed high molecular weight impurities apparently transparent in ^{1}H and ^{13}C NMR experiments as the spectra of both compounds showed identical purity.

To clarify the observed discrepancy in specific rotation, we performed an additional proof of enantiomeric excess. Racemic *cis*-dihydroxy cyclohexane was converted to a monothexyl derivative and further transformed to Mosher's ester **12**, whose ^{19}F NMR spectrum displayed signals of equal intensity at −71.8 and −72.6 ppm. Both samples of **9** were converted to their Mosher esters and their ^{19}F NMR spectra displayed a single signal at −72.8 ppm. On the basis of this evidence we concluded that **2** possesses the β-absolute configuration as indicated in Scheme 1. As near as can be judged from the absence of the diastereomeric signal in **12**, the enantiomeric excess of diol **2** is therefore greater than 95%. We conclude that the whole-cell fermentation of *p*-bromothioanisole with *E. coli* JM 109 (pDTG601) produced the single stereoisomer represented in Scheme 1 and that the thiomethyl group rather than the bromine was the directing element in the biooxidation. This observation further supports Boyd's proposal that the larger of the two groups on the aromatic nucleus directs the regio- and stereochemistry of the oxidation. Further investigation of applications of these new metabolites in asymmetric synthesis is ongoing and will be reported in due course.

Acknowledgements

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References

1. (a) Holland, H. L. *Chem. Rev.* **1988**, 473–485; (b) Colonna, S.; Gaggero, N.; Carrea, G.; Pasta, P. *Chem. Commun.* **1998**, 415; (c) Holland, H. L. *Nat. Prod. Rep.* **2001**, *18*, 171–181; (d) Colonna, S.; Gaggero, N.; Carrea, G.; Pasta, P.; Alphand, V.; Furstoss, R. *Chirality* **2001**, *13*, 40–42; (e) Boyd, D. R.; Sharma, N. D.; Haughey, S. A.; Kennedy, M. A.; Malone, J. F.; Shepherd, S. D.; Allen, C. R.; Dalton, H. *Tetrahedron* **2004**, *60*, 549–559; (f) Boyd, D. R.; Sharma, N. D.; Gunaratne, N.; Haughey, S. A.; Kennedy, M. A.; Malone, J. F.; Shepherd, S. D.; Allen, C. R.; Dalton, H. *Org. Biomol. Chem.* **2003**, *1*, 984–994.
2. (a) Boyd, D. R. *J. Chem. Soc., Chem. Commun.* **1995**, 119–120; (b) Colonna, S.; Gaggero, N.; Carrea, G.; Pasta, P. *Chem. Commun.* **1997**, 439; (c) Holland, H. L. *J. Ind. Microbiol. Biotechnol.* **2003**, *30*, 292–301.
3. (a) Hudlicky, T.; Gonzalez, D.; Gibson, D. T. *Aldrichim. Acta* **1999**, *35*; (b) Boyd, D. R. *Nat. Prod. Rep.* **1998**, *15*, 309.
4. (a) Boyd, D. R.; Sharma, N. D.; Haughey, S. A.; Malone, J. F.; McMurray, B. T.; Sheldrake, G. N.; Allen, C. C. R.; Dalton, H. *J. Chem. Soc., Chem. Commun.* **1996**, 2363; (b) Boyd, D. R.; Sharma, N. D.; Gunaratne, N.; Haughey, S. A.; Kennedy, M. A.; Malone, J. F.; Allen, C. C. R.; Dalton, H. *Org. Biomol. Chem.* **2003**, *1*, 984–994.
5. (a) Boyd, D. R.; Sharma, N. D.; Boyle, R.; McMurray, B. T.; Evans, T. A.; Malone, J. F.; Dalton, H.; Chima, J.; Sheldrake, G. N. *J. Chem. Soc., Chem. Commun.* **1993**, 49; (b) Boyd, D. R.; Sharma, N. D.; Hempenstall, F.; Kennedy, M. A.; Malone, J. F.; Allen, C. C. R. *J. Org. Chem.* **1999**, *64*, 4005; (c) Boyd, D. R.; Sharma, N. D.; Harrison, J. S.; Kennedy, M. A.; Allen, C. C. R.; Gibson, D. T. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1264–1269; (d) Ref. 4b.
6. For recent examples, see: (a) Back, T. G.; Parvez, M.; Zhai, H. *J. Org. Chem.* **2003**, *68*, 9389–9393; (b) Chen, Y.; Evarts, J. B., Jr.; Torres, E.; Fuchs, P. L. *Org. Lett.* **2002**, *4*(21), 3571–3574; (c) Craig, D. C.; Edwards, G. L.; Muldoon, C. A. *Synlett* **1997**, 1318–1320; For comprehensive reviews, see: (d) Fuchs, P. L.; Braish, T. F. *Chem. Rev.* **1986**, *86*, 903; (e) Bäckvall, J. E.; Chinchilla, R.; Nájera, C.; Yus, M. *Chem. Rev.* **1998**, *98*, 2291.
7. For an excellent review of radical cyclizations in the synthesis of heterocycles, see: Bowman, W. R.; Fletcher, A. J.; Potts, G. B. S. *J. Chem. Soc., Perkin Trans. 1* **2002**, 2747–2762; For a review of free-radical annulation and cascade reactions, see: McCarroll, A. J.; Walton, J. C. *J. Chem. Soc., Perkin Trans. 1* **2001**, 3215–3229.
8. Boyd, D. R.; Sheldrake, G. N. *Nat. Prod. Rep.*, **1998**, 309.
9. Boyd, D. R.; Hand, M. V.; Sharma, N. D.; Chima, J.; Dalton, H.; Sheldrake, G. N. *J. Chem. Soc., Chem. Commun.* **1991**, 1630–1632.
10. Boyd, D. R.; Sharma, N. D.; Byrne, B.; Hand, M. V.; Malone, J. F.; Sheldrake, G. N.; Blacker, J.; Dalton, H. *J. Chem. Soc., Perkin Trans. 1* **1998**, 1935–1943.
11. Allen, C. R. R.; Boyd, D. R.; Dalton, H.; Sharma, N. D.; Haughey, S. A.; McMordie, R. A. S.; McMurray, B. T.; Sheldrake, G. N.; Sproule, K. *J. Chem. Soc., Chem. Commun.* **1995**, 119–120.
12. Zylstra, G. J.; Gibson, D. T. *J. Biol. Chem.* **1989**, *264*, 14940.
13. Holland, H. L.; Brown, F. H.; Larsen, B. G. *Biorg. Med. Chem. Lett.* **1994**, 647.
14. The phenolic derivative arising from the decomposition of thiomethyl-diene-diol **3** made by whole-cell fermentation of thioanisole was isolated in low yield (0.15 g/L) after flash chromatography. Physical (TLC) and spectral (^1H NMR) data are consistent with that of the compound arising from the decomposition of **3** synthesized by electroreduction of **2**.
15. For a detailed description of medium-scale whole-cell fermentation (>10 L) see: Endoma, M. E.; Bui, V. P.; Hansen, J.; Hudlicky, T. *Org. Proc. Res. Dev.* **2002**, *6*, 525–532.
16. Diol **2** and other metabolites derived from *p*-substituted thioanisoles have been prepared by enzymatic oxidations with *Pseudomonas putida* UV4. See: Boyd, D. R.; Sharma, N. D.; Byrne, B. E.; Haughey, S. A.; Kennedy, M. A.; Allen, C. C. R. *Org. Biomol. Chem.*, in press.
17. The spectral and physical data for **2**, **3**, and **10** are as follows: 1-Bromo-4-thiomethyl-(2*R*,3*S*)-dihydroxycyclohexa-4,6-diene **2**: mp 59–63 °C; $[\alpha]_D^{19} = -10.0$ (*c* 1.08, $CHCl_3$); $R_f = 0.26$ (hexanes–ethyl acetate, 1:1); IR (film) 3197, 2921, 1626, 1548, 1416, 1340, 1306, 1036 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 6.37 (d, $J = 6.1$ Hz, 1H), 5.35 (d, $J = 6.3$ Hz, 1H), 4.43 (dd, $J = 9.0$, 3.2 Hz, 1H), 4.32 (dd, $J = 7.5$, 1.4 Hz, 1H), 2.63 (d, $J = 9$ Hz, 1H), 2.26 (s, 3H), 2.24 (d, $J = 7.6$, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 142.8, 127.8, 120.3, 112.7, 73.6, 72.5, 14.4; EI-MS *m/z* (%): 238 (8), 236 (8), 220 (100), 218 (98), 205 (41), 203 (39), 177 (24), 175 (24), 157 (20), 142 (25), 109 (33), 96 (24), 45 (41). EI-HRMS calcd for $C_7H_9O_2SBr$ (M^+): 235.9501; found: 235.9506; Anal. Calcd for $C_7H_9BrO_2S$: C, 35.46; H, 3.83.

Found: C, 36.10; H, 3.88. After 72 h in transit, diol **2** was stable enough to provide the following elemental composition: Anal. Calcd for $C_7H_9BrO_2S$: C, 35.46; H, 3.83. Found: C, 36.10; H, 3.88.

1-Thiomethyl-(2*S*,3*S*)-dihydroxycyclohexa-4,6-diene **3**: mp 61–62 °C; $[\alpha]_D^{24} = +81.3$ (*c* 0.27, MeOH); $R_f = 0.4$ (hexanes–ethyl acetate, 30:70); IR (film) 3247, 2915, 2858, 1551, 1545, 1431, 1420, 1321, 1292, 1106 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ: 5.99 (dq, $J = 5.5$, 2 Hz, 1H), 5.72 (dd, $J = 9.5$, 3.6 Hz, 1H), 5.58 (d, $J = 5.6$ Hz, 1H), 4.25 (m, 1H), 4.02 (d, $J = 5.5$ Hz, 1H), 2.31 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ: 141.7, 125.4, 124.5, 114.0, 71.3, 69.1, 13.0. The crystalline diol **3** was found to decompose to its corresponding phenol at room temperature within 30 min, however, in dilute dichloromethane solution at −18 °C decomposition is significantly slowed and the diol **3** survives for several weeks.

1-Thiomethyl-(2*S*,3*S*)-dihydroxycyclohex-6-ene **10**: mp 91–93 °C; $[\alpha]_D^{24} = -104$ (*c* 0.75, $CHCl_3$); $R_f = 0.4$ (hexanes–ethyl acetate, 30:70); IR (film) 3249, 2944, 2913, 2890, 2829, 1623, 1434, 1356, 1327, 1100 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 5.57 (t, $J = 4$ Hz, 1H), 4.05 (s, 1H), 3.82 (d, $J = 4.2$ Hz, 1H), 2.55 (d, $J = 4.1$ Hz,1H), 2.40 (s, 1H), 2.19 (s, 3H), 2.15–2.10 (m, 1H), 1.85–1.60 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 134.6, 124.8, 69.4, 69.1, 25.7, 24.8, 15.1; EI-MS *m/z* (%): 160 (50), 142 (22), 127 (31), 116 (100), 95 (47), 87 (58), 68 (40), 55 (30), 45 (54). EI-HRMS calcd for $C_7H_{12}O_2S$ (M^+): 160.0554; found: 160.0558; Anal. Calcd for $C_7H_{12}O_2S$: C, 52.47; H, 7.55. Found: C, 52.74; H, 7.39.

18. (a) Hudlicky, T.; Claeboe, C. D.; Brammer, L. E.; Koroniak, L.; Butora, G.; Ghiviriga, I. *J. Org. Chem.* **1999**, *64*, 4909; (b) Hudlicky, T.; Frey, D. A.; Koroniak, L.; Claeboe, C. D.; Brammer, L. E., Jr. *Green Chem.* **1999**, *1*, 57–59.
19. Bui, V. P.; Nguyen, M.; Hansen, J.; Baker, J.; Hudlicky, T. *Can. J. Chem.* **2002**, *80*, 708–713.
20. Tiecco, M. *J. Org. Chem.* **1983**, *48*, 4795–4800.