SUPPORTING INFORMATION

New Families of Enantiopure Cyclohexenone cis-Diol, o-Quinol Dimer and Hydrate Metabolites from Dioxygenase-catalysed Dihydroxylation of Phenols

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General Procedure

1H and 13C NMR spectra were recorded on Bruker Avance DPX-300 and DPX-500 instruments. Chemical shifts (δ) are reported in ppm relative to SiMe4 and coupling constants (J) are given in Hz. Mass spectra were run at 70 eV, on a VG Autospec Mass Spectrometer, using a heated inlet system. Accurate molecular weights were determined by the peak matching method, with perfluorokerosene as the standard. CD spectra were recorded in spectroscopic grade acetonitrile using a JASCO J-720 instrument. A PerkinElmer 341 polarimeter was used for optical rotation ([α]D) measurements (ca. 20 ºC, 10–1 deg cm2 g–1). Flash column chromatography and preparative layer chromatography (PLC) were performed on Merck Kieselgel type 60 (250 - 400 mesh) and PF254/366 respectively. Merck Kieselgel type 60F254 analytical plates were employed for TLC. Phenol substrates 1b, 1c, 1d, and 1f, 3-methylcatechol 2c and (-)-((S))-camphanic chloride were purchased from Aldrich and used as received.

Small scale (0.2-4.00 g) shake flask biotransformations, with the whole cells of P. putida UV4 (TDO), were performed using methods described earlier1,2 for non-phenolic aromatic substrates. The biotransformation conditions were not optimised for the phenol substrates used in this study. The aq. culture medium, obtained after the biotransformation, was concentrated under reduced pressure at ca. 40 ºC, the concentrate repeatedly extracted (EtOAc), and the extract concentrated under reduced pressure to give the crude mixture of bioproducts. 1H NMR spectra of the bioproduct mixtures were routinely recorded, before further purification. The bioproducts were separated either by flash column chromatography and/or PLC. Catechols 2d and 2f isolated during the study showed identical physical and spectral characteristics to those reported.3–5 The enantiomeric excess (ee) values of metabolites 6b, 6c, 6d, and 6f were indirectly estimated from NMR spectroscopic analysis of their boronate derivatives. (-)-(S)- and (+)-(R)-2-(1-Methoxyethyl)benzene boronic acids were synthesised and used according to the literature method.6,7

Biotransformations of phenols and other substrates using P. putida UV4

(i) Substrate 2,5-dimethylphenol (p-xylene) 1d
The crude mixture of bioproducts (2.3 g) from substrate 1d (1.2 g), on separation / purification by PLC (EtOAc/hexane, 3:2, two elutions), gave three compounds. These were identified as cyclohexenone cis-diol 6dR, o-quinol dimer 11d and catechol 2d.

Enone cis-diol 6dR was obtained as a white crystalline solid (0.256 g, 17%), m.p. 134-36 ºC (EtOAc/hexane); Rf (0.29, EtOAc/hexane, 3:1); ee ≥ 98%; [α]D + 94 (c 1.05, in MeOH); HRMS (EI): Found 156.0785. requires C8H12O3156.0786; 1H-NMR (500 MHz, CDCl3) δ 5.94 (1H, dq, J = 3.1, 1.3 Hz, 2-H), 4.45 (1H, m, J = 3.1, 3.5, 9.3 Hz, 4-H), 4.25 (1H, m, J = 3.5, 2.4 Hz, 5-H), 2.71 (1H, d, J = 9.3 Hz, OH), 2.57 (1H, m, J = 2.4, 7.0 Hz, 6-H), 2.26 (1H, d, J = 4.8 Hz, OH), 2.05 (3H, d, J = 1.3 Hz, Me), 1.26 (3H, d, J = 7.0 Hz, Me); 13C NMR (125 MHz, CDCl3) δ 199.1, 159.0, 127.2, 76.3, 72.2, 47.0, 20.5, 11.7; IR(KBr) vmax/cm–1 1646 (α,β unsaturated ketone); CD λ 209 nm (Δε -13.23), λ 237 nm (Δε +10.00).
(1R,2S,3R,7S,8S,10R)-3,10-Dihydroxy-3,6,10,12-tetramethyltricyclo[6.2.2.0²,5]11,14-diene-4,9-dione 11d

Bis-ketol 11d was obtained as colourless plates (0.092 g, 7%), m.p. 202-04 °C (MeOH); (lit. 8 m.p. 190-91 °C); Rf (0.25, EtOAc/hexane, 1:1); ee ≥ 98%; [α]D +62.0 (c 0.58, in CHCl₃) (lit. 8 [α]D +45.7); HRMS (EI): Found 276.1363. requires C₁₆H₂₀O₄ 276.1361; ¹H-NMR (500 MHz, CDCl₃) δ 6.02 (1H, br s, 5-H), 5.80 (1H, dq, J = 3.4, 1.6 Hz, 11-H), 4.0 (1H, br s, OH), 3.32 (1H, m, J = 3.4, 6.6 Hz, 7-H), 3.15 (3H, m, 1-H, 2-H and 8-H), 2.24 (1H, br s, -OH), 2.02 (3H, d, J = 1.2 Hz, Me), 1.60 (3H, d, J = 1.6, Me), 1.30 (3H, s, Me), 1.25 (3H, s, Me); ¹³C NMR (125 MHz, CDCl₃) δ 212.8, 201.4, 156.4, 136.5, 128.3, 124.9, 73.2, 73.0, 56.9, 44.8, 44.2, 41.1, 32.0, 25.9, 22.4, 21.5. Compound 11d showed identical physical and spectroscopic characteristics to those reported. 8

3.6-Dimethyl-2,3-dihydroxybenzene 2d

Catechol 2d was isolated as a white crystalline solid (0.0125 g, 9%), m.p.101-102 °C; Rf (0.55, EtOAc/hexane, 2:3). It showed identical physical and spectroscopic characteristics to those reported. 3,4

(ii) Substrate 3-methylphenol (m-cresol) 1c

An ethyl acetate solution of the crude mixture of bioproducts, obtained from substrate 1c (0.75 g), was purified through a silica gel column. The ethyl acetate fraction collected was concentrated. The residue, on the purification by PLC (EtOAc), gave two compounds which were identified as cyclohexenone cis-diol 6c and 3-methylcyclohexene 2e (0.034 g, 4%).

(4R,5S)-4,5-Dihydroxy-3-methylcyclohex-2-enone 6c

Colourless crystalline solid (0.146 g, 15%), m.p. 80-81 °C (EtOAc/Et₂O); [α]D +62.0 (c 1.00, in MeOH); HRMS (EI): Found 192.4, 141.0, 130.0, 73.5, 67.8, 42.4; IR(KBr) νmax/cm⁻¹ 1644 (unsaturated ketone); CD λ 209 nm (Δε +5.82), λ 237 nm (Δε +8.73).

(iii) Substrate 2-methylphenol (o-cresol) 1b

The crude mixture of bioproducts obtained from substrate 1b (2 x 1.5 g) was subjected to flash column chromatography with increasing percentage of EtOAc in hexane. The fractions collected with EtOAc/hexane (2:1) were pooled together, evaporated, and the residue purified by PLC (EtOAc/hexane, 3:1) to give cyclohexenone cis-diol 6b.

(4S,5R,6S)-4,5-Dihydroxy-6-methylcyclohex-2-enone 6b

Colourless solid (0.041 g, 12%), m.p. 128-30 °C (EtOAc/hexane); Rf (0.32, EtOAc/hexane, 3:1); ee ≥ 98%; [α]D +223 (c 0.48, in MeOH); HRMS (EI): Found 142.0624. requires C₇H₁₀O₃ 142.0630; ¹H-NMR (500 MHz, CDCl₃) δ 6.66 (1H, m, 3-H), 6.50 (1H, d, J = 4.5, 2.5 Hz, 4-H), 4.27 (1H, m, J = 4.5, 2.5 Hz, 4-H); ¹³C NMR (75 MHz, CDCl₃) δ 199.4, 151.4, 132.9, 84.3, 76.9, 71.5, 48.0, 12.3; IR(KBr) νmax/cm⁻¹ 1668 (α,β unsaturated ketone); CD λ 200 nm (Δε +18.05), λ 232 nm (Δε +16.10).

(iv) Substrate 3-iodophenol 1f

The crude mixture of bioproducts from substrate 1f (0.8 g), on purification by PLC (EtOAc/hexane, 1:1, two elutions), gave three compounds. These were identified as cyclohexenone cis-diol 6f, ketol 13 and 3-iodocatechol 2f. 5 (0.067 g, 8%).

(4S,5S)-4,5-Dihydroxy-3-iodocyclohex-2-enone 6f

Colourless solid (0.390 g, 42%), m.p. 98-100 °C (MeCO); Rf (0.21, EtOAc/hexane, 1:1); ee ≥ 98%; [α]D +38 (c 0.91, in MeOH); HRMS (EI): Found 253.9468. requires C₇H₁₀O₃ 253.9440; ¹H-NMR (300 MHz, CDCl₃ + D₂O) δ 6.91 (1H, d, J = 1.3 Hz, 2-H), 4.52 (1H, dd, J = 1.3, 3.5 Hz, 4-H), 4.41 (1H, ddd, J = 3.5, 3.5, 5.9 Hz, 5-H), 2.82 (1H, dd, J = 5.9, 16.7 Hz, 6-H), 2.65 (1H, dd, J = 3.5, 16.7 Hz, 6-H); ¹³C NMR (125 MHz, CDCl₃) δ 192.4, 141.0, 130.0, 73.5, 67.8, 42.4; IR(KBr) νmax/cm⁻¹ 1644 (α,β unsaturated ketone).

Crystal data for 6f: C₇H₇IO₃, M = 254.0, monoclinic, a = 6.368(5), b = 7.456(6), c = 8.308(6) Å, β = 100.83(1)°, U = 387.4(5) Å³, T = 298(2) K, Mo-Kα radiation, λ = 0.71073 Å, space group P₂₁ (no. 4), Z = 2, F(000) = 240, Dₐ = 1.718 g cm⁻³, μ = 4.08 mm⁻¹, Bruker SMART CCD area detector diffractometer, ω/2θ scans, 5° < 2θ < 56°, measured/independent reflections: 3863/1572, R cryst = 0.021, direct methods solution, full-matrix least squares refinement on F², anisotropic displacement parameters for non-hydrogen atoms; all hydrogen atoms located in a difference Fourier synthesis but included at positions calculated from the geometry of the molecule using the riding model, with isotropic vibration parameters. R₁ = 0.032 for 1545 data with Fo > 4σ(Fo), 93 parameters, αFo = 0.091 (all data), GoF = 1.10, Flack x = 0.015 establishes the absolute configuration as (4S,5S), Δρ min,max = -0.63/0.71 e Å⁻³. CCDC reference number 721024.
(R)-4-Hydroxycyclohex-2-one 13

Light yellow oil (0.027 g, 7%); \( R_f (0.48, \text{EtOAc}) \); \([\alpha]_D +106.0 \) (c 1.25, in CHCl\(_3\)) (lit.\(^9,10\) \([\alpha]_D +110\)); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta 6.97 (1\text{H}, \text{dd}, J = 10.3, 1.8, 3.6 \text{ Hz}, 3\text{-H}), 5.97 (1\text{H}, \text{d}, J = 10.3 \text{ Hz, 2-H}), 4.59 (1\text{H}, \text{m, 4-H}), 2.51-2.64 (1\text{H}, \text{m, CCH}), 2.32-2.41 (2\text{H}, \text{m, HCH}), 1.81-2.08 (1\text{H}, \text{m, HCH}) \). The physical and spectroscopic data of hydroxycyclohexenone 13 were identical with those reported.\(^9,10\)

(v) Substrate (4R,5S)-4,5-dihydroxycyclohex-2-one 6a

The crude mixture of metabolites from substrate 6a was purified by flash column chromatography with an increasing percentage of EtOAc in hexane. The fractions collected with EtOAc/hexane (1:1), on evaporation, yielded (R)-4-hydroxycyclohexenone 13 (0.012 g, 23%); \([\alpha]_D +104.0 \) (c 0.5, in CHCl\(_3\)). The column was finally eluted with EtOAc/MeOH (9:1). The fraction was evaporated and the residual light yellow semi-solid crystallized to give cyclohexane-1,2,4-triol 14.

(1R,2S,4S)-Cyclohexane-1,2,4-triol 14

Light yellow transparent crystalline solid (0.026 g, 42%), m.p. 137-38 °C (EtOAc/EtOH); (lit.\(^12\) m.p. 137-38 °C);

\[ \text{C}_{18}H_{24}O_{6}, M = 336.4, \text{triclinic, } a = 6.581(8), b = 7.123(8), c = 11.041(13) \AA, \alpha = 102.33(2), \beta = 96.496(2), \gamma = 110.325(2) \text{°}, U = 462.69(9) \text{ Å}^3, T = 298(2) \text{ K, Mo-K\(_\alpha\) radiation, } \lambda = 0.71073 \text{ Å, space group } P1 (no. 1), Z = 1, F(000) = 180, D_s = 1.207 \text{ g cm}^{-3}, \mu = 0.09 \text{ mm}^{-1}, \text{Bruker SMART CCD area detector diffractometer, } \phi/\omega \text{ scans, } 3.8 < 2\theta < 56.5 \text{°, measured/independent reflections: 3590/3246, } R_{int} = 0.021, \text{direct methods solution, full-matrix least squares refinement on } F^2, \text{anisotropic displacement parameters for non-hydrogen atoms; all hydrogen atoms located in a difference Fourier synthesis but included at positions calculated from the geometry of the molecule using the riding model, with isotropic vibration parameters. } R_i = 0.049 \text{ for } 2834 \text{ data with } F_i > 4\sigma(F_i), 223 \text{ parameters, } \omega R_2 = 0.126 \text{ (all data), GoF = 1.05, } A_{\text{max}, \text{min}} = -0.16/0.18 \text{ e } \AA^{-3}. \text{CCDC reference number 721025. The absolute configuration was established as (4S,5R,6S) relative to the known configuration (1S) of the attached camphanate moiety.}
Substitution reactions of (4S,5S)-4,5-dihydroxy-3-iodocyclohex-2-enone 6fS

To a solution of enone cis-diol 6fS (0.100 g, 0.39 mmol) in anhydrous THF (10 mL) were added Bu3SnCN (0.186 g, 0.59 mmol), Pd(PPh3)4 (0.050 g) and Et3N (125 µL). The reaction mixture was refluxed until the starting material had been consumed (ca. 18 h). The cooled reaction mixture was loaded onto a column of silica gel and eluted with hexane containing an increasing percentage of EtOAc. The fractions collected with EtOAc/hexane (1:1) were evaporated and the residue purified by PLC (MeOH/CHCl3, 1:19) to give cyclohexenone cis-diol 6gS as a light yellow oil (0.034 g, 66%); [α]D217 = -217 (c 0.92, in MeOH); HRMS (EI) : Found 186.0528. requires C 8H10O5 186.0522; 1H-NMR (500 MHz, CD3OD)

\[ \delta = 6.68 (1H, s, 2-H), 4.73 (1H, d, J = 3.5 Hz, 4-H), 4.20 (1H, dd, J = 8.5, 3.6, 3.6 Hz, 5-H), 3.82 (3H, s, CO2Me), 2.79 (1H, dd, J = 8.5, 16.5 Hz, 6-H), 2.57 (1H, dd, J = 3.6, 16.5 Hz, 6´-H); 13C NMR (125 MHz, CDCl3) \delta 197.6, 139.6, 134.5, 117.9, 71.1, 69.3, 45.0. \]

Substitution reactions of (4S,5S)-4,5-dihydroxy-3-iodocyclohex-2-enone 6fS

A solution of cyclohexenone cis-diol 6fS (0.070 g, 0.28 mmol) in MeOH (6 mL) were added Pd(OAc)2 (0.013 g) and NaOAc.3H2O (0.076 g). The mixture was stirred, at room temperature under an atmosphere of carbon monoxide, until the reaction was complete (ca. 12 h). The reaction mixture was filtered, the filtrate concentrated, and the crude product purified by PLC (EtOAc/hexane, 3:1). Enone cis-diol 6hS was obtained as a colourless oil (0.034 g, 66%); [α]D217 = -48 (c 1.34, in MeOH); HRMS (EI) : Found 186.0528. requires C8H10O3 186.0522; 1H-NMR (500 MHz, CDCl3) \( \delta \) 6.68 (1H, s, 2-H), 4.73 (1H, d, J = 3.5 Hz, 4-H), 4.20 (1H, dd, J = 8.5, 3.6, 3.6 Hz, 5-H), 3.82 (3H, s, CO2Me), 2.79 (1H, dd, J = 8.5, 16.5 Hz, 6-H), 2.57 (1H, dd, J = 3.6, 16.5 Hz, 6´-H); 13C NMR (125 MHz, CDCl3) \( \delta \) 196.8, 166.2, 143.2, 133.6, 67.1, 64.9, 52.7, 41.3.

(4R,5S)-4,5-Dihydroxy-3-carbomethoxycyclohex-2-enone 6hS

A solution of cyclohexenone cis-diol 6fS (0.100 g, 0.39 mmol) in MeOH (8 mL), containing Et3N (65 µL) and 3% Pd/C (0.015 g), was stirred, overnight at room temperature, in an atmosphere of hydrogen. The catalyst was filtered off, the filtrate concentrated and the crude product purified by PLC (EtOAc/hexane, 3:1, two elutions). Enone cis-diol 6aS was obtained as a white crystalline solid (0.041g, 81%); m.p. 72-74 °C; [α]D217 = 0.28, (EtOAc); [α]D217 = -217 (c 0.92, in MeOH); HRMS (EI) : Found 128.0480. requires C6H8O3; 128.0473; 1H-NMR (500 MHz, CD3OD) \( \delta \) 6.73 (1H, m, J = 10.3, 2.9 Hz, 3-H), 5.88 (1H, dd, J = 10.3, 2.1 Hz, 2-H), 4.41 (1H, dd, J = 2.9, 5.3 Hz, 4-H), 4.13 (1H, dd, J = 5.3, 6.9, 3.7 Hz, 5-H), 2.55 (2H, m, J = 3.7, 5.3 Hz, 6-H and 6´-H); 13C NMR (125 MHz, CDCl3) \( \delta \) 200.4, 152, 130.7, 71.63, 69.0, 45.2; CD \( \lambda \) 205 nm (Ae = +11.80), \lambda 232 nm (Ae = -14.31).

The physical and spectroscopic data of enone cis-diol 6aS were identical with those reported for the opposite enantiomer.13

References
