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Toluene Dioxygenase-Catalyzed Synthesis and Reactions of *cis*-Diol Metabolites Derived from 2- and 3-Methoxyphenols

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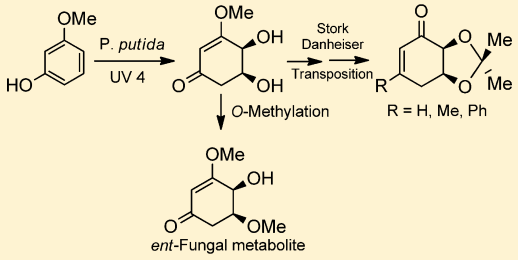
Supporting Information

ABSTRACT: Using toluene dioxygenase as biocatalyst, enantiopure *cis*-dihydrodiol and *cis*-tetrahydrodiol metabolites, isolated as their ketone tautomers, were obtained from *meta* and *ortho* methoxyphenols. Although these isomeric phenol substrates are structurally similar, the major bioproducts from each of these biotransformations were found at different oxidation levels. The relatively stable cyclohexenone *cis*-diol metabolite from *meta* methoxyphenol was isolated, while the corresponding metabolite from *ortho* methoxyphenol was rapidly bioreduced to a cyclohexanone *cis*-diol. The chemistry of the 3-methoxycyclohexenone *cis*-diol product was investigated and elimination, aromatization, hydrogenation, regioselective *O*-exchange, Stork–Danheiser transposition and *O*-methylation reactions were observed. An offshoot of this technology provided a two-step chemoenzymatic synthesis, from *meta* methoxyphenol, of a recently reported chiral fungal metabolite; this synthesis also established the previously unassigned absolute configuration.



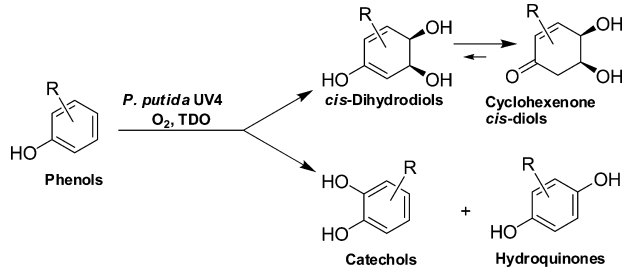
INTRODUCTION

The formation of enantiopure *cis*-dihydrodiol metabolites, from the biotransformation of monocyclic arene substrates, using mutant and recombinant bacterial strains as sources of toluene dioxygenase (TDO) and other arene dioxygenases, has been extensively reviewed in recent years.[1−17] A wide range of substituted benzenes has been used as substrates for TDO, resulting in *cis*-dihydrodiols with substituents including halogens, alkyl, aryl, heteroaryl, alkene, alkynyl, carboxylic acid, ester, ether, thioether, sulfoxide and nitrile groups. Continuing interest in the application of the resulting monocyclic *cis*-dihydrodiol bioproducts, as synthetic precursors of chiral natural products, chiral ligands and compounds of value in medicinal chemistry, is evident from the many recent publications on this topic by the groups of Banwell,[18] Hudlicky,[19] Lewis[20] and Stevenson.[21]

Until relatively recently, phenols were among the few types of substituted benzene substrates not found to yield the corresponding *cis*-dihydrodiol metabolites following TDO-catalyzed oxidation. Catechols and hydroquinones were, previously, identified as the major types of phenol derivatives isolated from bacterial arene dioxygenase-catalyzed oxidations (Scheme 1).[22−32] *cis*-Dihydrodiols were, however, postulated as possible intermediates during the formation of hydroquinones from phenols, but were not detected under the reaction conditions.[32] Following the first reports of *cis*-dihydrodiol metabolite formation from phenols, characterized as the more stable keto tautomers (cyclohexenone *cis*-diols),[33,34] a number of new family members of enantiopure cyclohexenone *cis*-diol metabolites (>20 examples) has recently been reported,[33−37] as a result of TDO-catalyzed *cis*-dihydroxylation or chemoenzymatic synthesis.

The difficulty in detecting cyclohexenone *cis*-diol metabolites from phenols may have been due to (i) competing formation of catechols, whose presence was known to inhibit TDO

Scheme 1. TDO-Catalyzed Oxidations of Monosubstituted Phenols to Yield Catechols, Hydroquinones and *cis*-Dihydrodiols/Cyclohexenone *cis*-Diols



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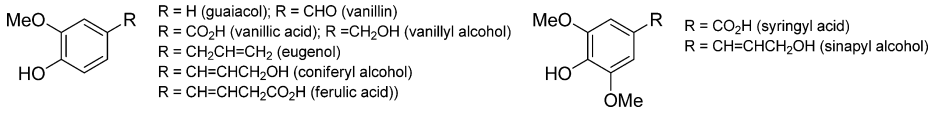


Figure 1. Structures of naturally occurring mono- and disubstituted guaiacols.

activity,[38] (ii) their instability, resulting in dehydration to yield hydroquinones and (iii) further metabolism including enzymatic alkene and/or ketone reductions to yield *cis*-triols and other bioproducts.[35,36] The majority of isolated cyclohexenone *cis*-diol metabolites have been derived from 3-substituted and 2,5-disubstituted phenols.[33−38] To date, only one example of a *cis*-diol metabolite has been reported from a 2-substituted phenol (*ortho* cresol)[34] and none from 4-substituted phenols.

Methoxyphenols are widely distributed in the environment, due to (i) their role as monomers in the biosynthesis of lignin in biomass, e.g., plants and trees, and (ii) their formation as a result of lignin biodegradation, pyrolysis and pretreatment in biorefinery operations.[39−41] Many of these naturally occurring phenolic products contain the *ortho* methoxyphenol moiety (guaiacol) with either one, e.g., vanillin, vanillic acid, eugenol, coniferyl alcohol, ferulic acid, or two, e.g., syringyl acid, sinapyl alcohol, additional substituents (Figure 1). Anthropogenic sources of substituted guaiacols, e.g., chloroguaiacols, are also prevalent in the environment as a result of the chlorine bleaching of wood pulp.[41,42]

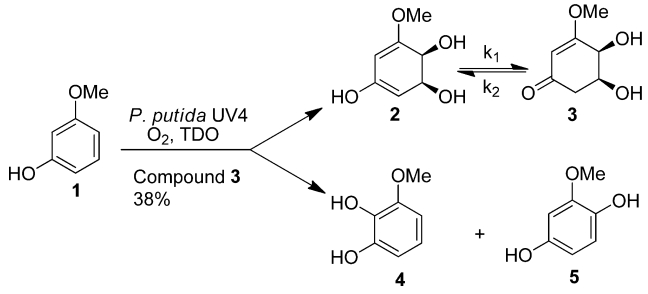
Guaiacol, is itself a naturally occurring product, also formed from the pyrolysis and biodegradation of wood or other plant material and is responsible for the flavor of many foods in our diet. It was also found to act both as a carbon and an energy source during bacterial metabolism of chloroguaiacols.[41,42] There has been considerable interest in a recent compound derived from a combination of guaiacol and vanillyl alcohol to yield bisguaiacol F (BGF) as a potential alternative to the endocrine disrupting compound bisphenol A (BPA).[43] Guaiacol is among the most abundant phenols in nature. Investigation of its metabolic breakdown pathways in bacteria, identification and reactions of the resulting metabolites, were major priorities of the current program.

A recent small-scale biotransformation of 3-methoxyphenol, using *Pseudomonas putida* UV4 whole cells, gave mainly a *cis*-dihydrodiol metabolite as its preferred keto tautomer (cyclohexenone *cis*-diol).[37] This observation prompted the current comprehensive biotransformation studies of 2- and 3-methoxyphenol substrates with emphasis on effective scale up, identification of the resulting metabolites, metabolic pathways and chemical reactions.

RESULTS AND DISCUSSION

Biotransformation of 3-Methoxyphenol 1 to Yield *cis*-Diols 2 and 3, Catechol 4 and Hydroquinone 5. Earlier small-scale biotransformations of 3-methoxyphenol **1**, using either the mutant *P. putida* UV4 or the recombinant *Escherichia coli* CL-4t strain, resulted in TDO-catalyzed formation of *cis*-dihydrodiol **2**, a major metabolite isolated as the preferred cyclohexenone *cis*-diol-tautomer **3** (Scheme 2).[36] A larger quantity of cyclohexenone *cis*-diol metabolite **3** was required, for studies of its reactivity and evaluation of its potential, as a new chiral pool compound. A large-scale biotransformation of 3-methoxyphenol **1** (96 g in 120 L) was therefore conducted using *P. putida* UV4. Concentration of the aqueous biotransformed material, followed by ethyl acetate extraction

Scheme 2. TDO-Catalyzed Oxidation of 3-Methoxyphenol 1 to Yield Cyclohexenone *cis*-Diol 3, via *cis*-Dihydrodiol 2, with Catechol 4 and Hydroquinone 5 as Minor Products



and column chromatography, yielded, mainly, 3-methoxycyclohexenone *cis*-diol **3** (45 g, 38% yield), via the undetected enol tautomer **2**. Catechol **4** and hydroquinone **5** were also detected, by GC−MS analysis of their silylated derivatives, among a mixture of unidentified minor metabolites.

Phenols are enol forms of cyclohexadienones. The kinetics and equilibrium constants for this tautomerism have been evaluated.[44] Because of the large gain in resonance energy, the enol form is by far the most stable tautomer, with an equilibrium constant $K > 10^{11}$. However, chemoselective removal of a non enolic C=C double bond removes the aromatic resonance energy and provides a novel entry to a new enol, **2**, with very different properties to the parent phenol. Although several methods for preparing enols have been reported, including hydration of alkynes,[45] hydrolysis of enol ethers,[46,47] decarboxylation of β-keto acids,[48] isomerization of allyl alcohols,[49] 1,4-addition of thioacetic acid to enals[50] and photochemical fragmentations,[51] the present enzymatic approach is novel, as it is the first method of asymmetric synthesis for preparing a chiral enol by a dearomatisation of a phenol. There is normally a large thermodynamic driving force for enols to tautomerise, to the corresponding carbonyl compounds, but since the activation energy for intramolecular migration of hydrogen is high, enols can be regarded as being metastable. Ethenol[47] and prop-1-en-2-ol[49] are sufficiently long-lived in a dilute solution of an aprotic solvent, at room temperature, to allow the NMR spectra of these species to be recorded. An *O*-deuterated enol with two fluorine atoms on the α-carbon has a half-life greater than 2 weeks in neutral methanol-d_4.[52] Cyclohexa-1,3-dienol has a half-life of 5 s,[53] further demonstrating the longevity of enols in aqueous media. However, the proton transfer is greatly facilitated in protic solvents, with most studies undertaken in water as solvent, catalyzed both by acid and base.[54]

DFT calculations (Table 1) indicated that the relative difference in free energy between enol **2** and ketone **3** in the gas phase was −76.5 kJ mol^{-1}. As such there was a huge thermodynamic driving force for forming the ketone tautomer and hence $k_1 \gg k_2$ (Scheme 2). ^{1}H NMR analysis of the vinylogous ester **3** failed to detect any of the enol tautomer **2**, when the signals for the ^{13}C satellites were clearly visible indicating that the equilibrium constant K for the tautomerism

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Table 1. DFT Calculated Gas Phase Differences in Free Energy of Ketones (3 and 11) with Respective Enols (2 and 10) and Comparison of Free Energy between Two Isomeric Ketones (3 and 26)

entry	ketone	enol	ΔG (kJ mol^{-1})
1	**3**	**2**	−76.5
2	**11**	**10**	−48.5
3	**3** → **26**		−11.7

reaction was less than 10^{-3}. For comparison purposes, keto−enol tautomerism in 2-cyclohexenone, with water as solvent, has an equilibrium constant K of 2.0×10^{-8},[53,55] which is well outside the range for ^{1}H NMR spectroscopy to detect the enol.

When the TDO-expressing recombinant strain *E. coli* (CL-4t) was used with the phenol substrate **1**, GC−MS analysis showed that *cis*-diol **3** and catechol **4** were again formed, consistent with TDO being the enzyme involved.

To provide further confirmation of the (4*S*,5*S*) absolute configuration of *cis*-diol-**3**, earlier assigned by ECD spectroscopy,[37] a stable derivative was synthesized. Hydrogenation of enone **3** using a palladium catalyst (Pd−C, MeOH) gave an oil (85% yield) which was identified as cyclohexanone *cis*-diol **6** (Scheme 3). This reaction was remarkably selective and only one diastereoisomer was detected. The observed relative configuration was consistent with delivery of hydrogen from the sterically less encumbered face of enone **3**.[56] The relative configuration of compound **6** was determined primarily from the magnitude of vicinal proton coupling constants associated with proton H-4 (*J* 5.6, 2.9, 2.9). The 5.6 Hz coupling constant was due to coupling of H-4 to the hydroxyl group, and the other two small coupling constants of 2.9 Hz were consistent with two axial−equatorial couplings, confirming a *cis*-relationship among protons H-3, H-4 and H-5, and hence establishing a *cis*-relationship between the OMe and OH groups. For unequivocal confirmation of the relative and absolute configuration of compound **6**, a suitable crystal of monocamphanate derivative **8**, for X-ray crystallographic analysis, was obtained by treatment with an excess of (1*S*)-camphanic chloride in pyridine solution. This procedure yielded the crystalline derivative **8** (Scheme 3); it was presumably derived from the dicamphanate **7**, which was clearly prone to facile elimination reactions under the basic reaction conditions. The X-ray crystal structure, confirmed that camphanate **8** had an equatorial OMe group and a pseudoaxial *O*-camphanate group in a *cis*-relationship in the solid phase and therefore a (1*S*,6*R*) absolute configuration. The (4*S*,5*S*) configuration for cyclohexenone *cis*-diol **3** and a (3*S*,4*S*,5*R*) configuration for cyclohexanone *cis*-diol **6**, were established from the stereochemical correlation sequence shown in Scheme 3. The same conformation of compound **8** was also dominant in solution, as determined by ^{1}H NMR spectroscopy. Proton H-1 showed a coupling constant of 4.3 Hz, when coupled to H-6, suggesting an axial−equatorial arrangement, and proton H-6 was coupled to H-5 with a coupling constant of 8.4 Hz, indicating that H-6 was axial and H-1 was equatorial. The tendency for electronegative allylic substituents to adopt pseudo axial positions, in six-membered rings, is well documented[57−59] and can be attributed to a combination of minimization of 1,2-allylic strain[60] and increased hyperconjugation $\pi-\sigma^*_{C-O}$,[61] even in this case with an electron-deficient alkene.

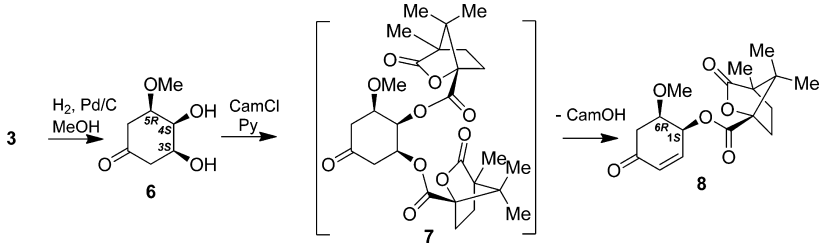
Biotransformation of Guaiacol 9 to Yield *cis*-Diols 10, 11, 13, 14, Methoxyhydroquinone 5 and Methoxycatechol 4. The bacterial biotransformation study of guaiacol substrate **9**, was conducted using *P. putida* UV4, a source of TDO and glucose as a carbon source (Scheme 4). The initial objective of this study was to compare the results with those observed earlier, using *ortho*-cresol as substrate, where the corresponding cyclohexenone *cis*-diol was obtained in very low yield (1%).[34] During an early small-scale biotransformation, surprisingly, the only keto *cis*-diol products isolated from guaiacol **9** were metabolites **13** and **14**. Intermediate **11**, the preferred tautomeric form of the initial *cis*-dihydrodiol metabolite **10**, was presumed to be reduced to *cis*-diol **14** and epimer **13**.

Results obtained from a later time course study of this *P. putida* UV4 biotransformation, using LC-TOFMS analysis of the crude culture medium, confirmed the presence of the major metabolites **11** and **14**, detected after 2 h, along with traces of partially separated metabolite **13** from diol **14**. After 20 h, metabolite **11** showed a 7-fold decrease, and the reduced bioproducts **13**/**14** a 7-fold increase in concentration; the epimeric ketone **12** was not detected. Only a very minor proportion of cyclohexenone *cis*-diol **11**, remained, when the biotransformation was terminated at 20 h, leaving a 1:10 mixture of *cis*-diol epimers **13**:**14** as major metabolites. The diastereoisomers **13** and **14**, separated by column chromatography, did not interconvert during extensive NMR experiments in CD_3OD solution.

The metabolic sequence, proposed in Scheme 4, involved an initial TDO-catalyzed *cis*-dihydroxylation, to yield guaiacol *cis*-dihydrodiol **10**. Enol-keto tautomerisation gave cyclohexenone *cis*-diol **11**, and a rapid ene reductase (ERED)-catalyzed reduction (hydrogenation) produced the cyclohexanone *cis*-diol **14**, followed by an epimerization to give isomer **13**. A similar type of enzymatic alkene reduction was observed, with other α,β-unsaturated ketones, e.g., 2-cyclohexen-1-one[62] and a 2-cyclohexen-1-one *cis*-diol,[34] using *P. putida* strains (M10 and UV4 respectively) both expressing ene reductase enzyme activity.

A large-scale biotransformation of guaiacol **9**, (96 g in 120 L), using glucose as carbon source and *P. putida* UV4, yielded

Scheme 3. Synthesis of (1*S*,6*R*) Monocamphanate 8 from (3*S*,4*S*,5*R*) Cyclohexanone *cis*-Diol 6



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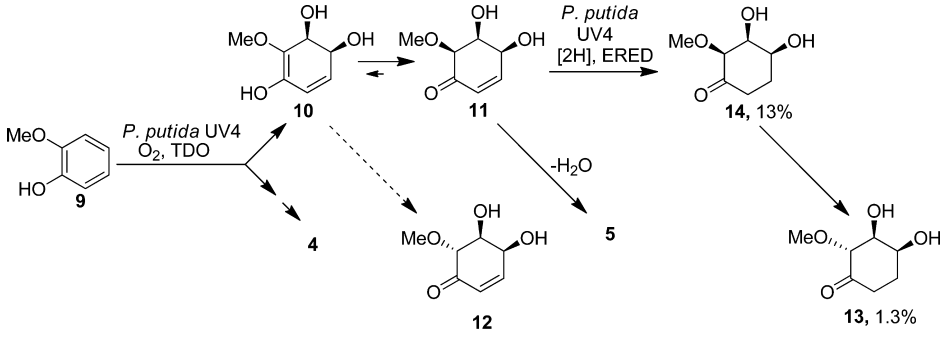
Scheme 4. Metabolism of Guaiacol 9 to Yield *cis*-Diols 10, 11, 13, 14, Catechol 4 and Hydroquinone 5



cyclohexanone *cis*-diols **14** (13% isolated yield) and **13** (1.3% isolated yield, Scheme 4), after separation by column chromatography. The relative stereochemistry of each of the diastereoisomers **13** and **14** was determined by the magnitude of the vicinal proton coupling constants. The major *cis*-diol **14** exhibited a small coupling constant ($J_{2,3}$) of 3.0 Hz, indicating a *cis*-axial−equatorial relationship between H-2 and H-3 protons. The minor isomer **13** showed a much larger value ($J_{2,3}$ 9.8 Hz), consistent with a *trans*-diaxial arrangement for H-2 and H-3 protons, and a corresponding *trans*-diequatorial relationship between the OMe and OH groups at C-2 and C-3 respectively. In both diastereoisomers, the conformer in which the methoxy group was equatorial predominated, and in each isomer two groups were equatorial and one was axial (Figure 2).

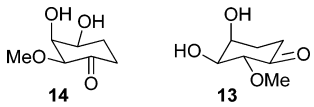
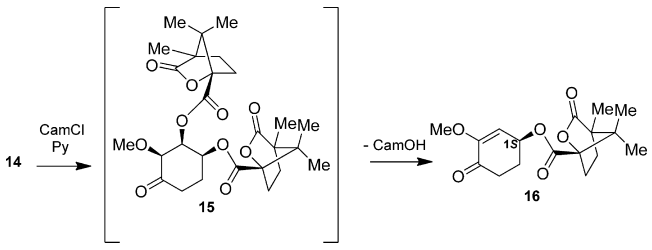


Figure 2. Preferred conformations of *cis*-diols **14** and **13**.

The absolute configuration of *cis*-diol **14**, was determined by single crystal X-ray crystal structure analysis of camphanate derivative **16**, followed by stereochemical correlation (Scheme 5). The undetected dicamphanate **15** eliminated camphanic

Scheme 5. Synthesis of (1*S*) Monocamphanate 16 from (2*S*,3*S*,4*S*) Cyclohexanone *cis*-Diol 14



acid (CamOH) under the reaction conditions, to give (1*S*) camphanate **16**. The absolute stereochemistry of diols **14** and **11** could be assigned as (2*S*,3*S*,4*S*) and (4*S*,5*S*,6*S*) respectively. In the solid state, the camphanate ester group in derivative **16** occupied a pseudoequatorial position. In solution, ^{1}H NMR spectroscopy showed that proton H-1 had vicinal coupling constants of 6.0 and 4.4 Hz to protons H-6 and H-6′, indicating that H-1 was axial in the dominant conformer, but a substantial amount of at least one other conformer was also present. This subtle difference in conformational preference, between camphanates **8** and **16**, indicates how finely balanced the stereoelectronic and steric factors are in these molecules.

Methoxycatechol **4** and methoxyhydroquinone **5** were later identified as very minor guaiacol metabolites, by GC−MS analysis of the freeze-dried residue after silylation with MSTFA. Catechol **4** could be formed via TDO-catalyzed dihydroxylation of guaiacol **9** at the C_1═C_6 bond followed by loss of water as proposed for the formation of catechols from other phenol substrates.[28] Hydroquinone **5** may be formed from the dehydration of intermediate cyclohexenone *cis*-diol **11** during the biotransformation.

In one of the experiments when a flask-scale biotransformation of guaiacol **9** was conducted, using *P. putida* UV4 and pyruvate as carbon source, a slightly higher concentration of cyclohexenone-*cis*-diol **11** than usual was observed, during the early phase of metabolism. Extraction and purification (PLC) resulted in partial decomposition and yielded mainly methoxyhydroquinone **5**. However, a very small sample of the residual cyclohexenone *cis*-diol **11** (<5 mg) survived the purification. The ^{1}H NMR spectrum of this impure sample showed chemical shift values (δ) and coupling constants (J) for the alkene protons, 6.76 (1 H, dt, J 10.3, 2.2, H-3), 6.04 (1 H, dd, J 10.3, 2.3, H-2) that were very similar to those found for the corresponding, but more stable, cyclohexenone-*cis*-diol metabolite from *ortho*-cresol.[34] The relative stereochemistry of diastereoisomer **11** was established by analysis of vicinal proton coupling constants. Coupling constants *J*-4,5 and *J*-5,6 were small, 3.9 and 2.3 Hz respectively indicating that H-4, H-5 and H-6 were *cis* to one another. The epimeric enone **12** remained undetected in these experiments. The NMR sample of the elusive metabolite **11** showed further evidence of instability in $CDCl_3$ solution and slowly decomposed forming methoxyhydroquinone **5** and other unidentified products, before full structural and stereochemical characterization could be carried out. Other researchers have noted the lability of structurally similar compounds, where facile dimerization occurred.[63] Further attempts to isolate the intermediate cyclohexenone-*cis*-diol **11**, by this method were unsuccessful and the absolute configuration of metabolite **11** was presumed to be identical to that of the major metabolite **14**.

Although compound **11**, was identified as the initial major cyclohexenone *cis*-diol metabolite, in the aqueous culture medium, it became evident that our efforts to extract and isolate a sample by PLC, were being thwarted by (a) the low conversions during the very early stages of the *P. putida* UV4 biotransformation, (b) it being an excellent substrate for ERED-catalyzed reduction and (c) its chemical/thermal instability. When an *E. coli* recombinant strain (CL4t), expressing TDO, but not the ERED enzyme, was used with

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guaiacol **9** as substrate, LC-TOFMS analysis showed that compound **11** was the only *cis*-diol metabolite formed. Since it was produced in an extremely low yield, and due to decomposition on attempted purification, it was not examined further.

Stability and Aromatization Studies of Methoxycyclohexenone *cis*-Diols. On the basis of earlier studies, it appeared that *cis*-dihydrodiols from nonphenolic monosubstituted arene substrates (e.g., **17**, R = OMe, Figure 3) were

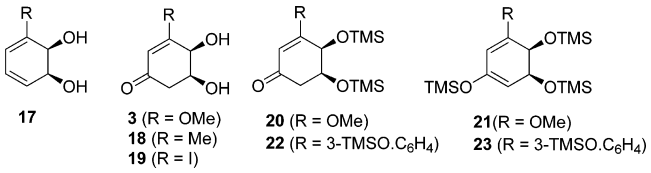

Figure 3. *cis*-Diol metabolites from monosubstituted benzenes **17**, from *meta*-phenols **3**, **18**, **19**, and their silylated derivatives **20**−**23** from the corresponding cyclohexenone *cis*-diols and *cis*-dihydrodiols.

generally much less stable,[64−67] at ambient temperature, compared with known members of the cyclohexenone *cis*-diol family of metabolites derived from *meta*-phenols or 2,5-disubstituted phenols.[33−37] During our attempts to purify cyclohexenone *cis*-diol **11**, it became evident that it was very labile and decomposed readily, when compared with some other members of this cyclohexenone *cis*-diol family; many having half-lives of several hours in 6 M perchloric acid,[34] thus indicating a much wider stability range for this type of phenol metabolite than anticipated.

Thermal dehydration of substituted benzene *cis*-dihydrodiols **17**, yielded both *ortho*- or *meta*-phenols, with preference being dependent upon the nature of the substituent group R. Furthermore, acid-catalyzed dehydration of *cis*-dihydrodiols **17** afforded mainly *ortho* rather than *meta* phenols,[33−37] and the trend reversed under base-catalyzed conditions.[67] Cyclohexenone *cis*-diols (e.g., **18** and **19**) were found to aromatize under both acidic and alkaline conditions, yielding the corresponding substituted 1,4-dihydroxybenzenes (hydroquinones) rather than 1,3-dihydroxybenzenes (resorcinols). In the present study, treatment of the methoxycyclohexenone *cis*-diols **3** and **11** under acid conditions (CF_3CO_2H) yielded methoxyhydroquinone **5** as the sole product.

As indicated in Table 1, the proportion of the less stable enol tautomer **2**, at equilibrium with cyclohexenone *cis*-diol metabolite **3**, would be extremely small. This could account our earlier unsuccessful attempts to observe or trap the elusive enol tautomers through formation of triazolinedione cycloadducts of the diene moiety, or methylation of the enol OH group with diazomethane.[34] Silylation and GC−MS analysis of a pure sample of cyclohexenone *cis*-diol **3** showed the expected major diTMS derivative **20** (ca. 95%) but also a minor proportion (ca. 5%) of a triTMS derivative **21** (Figure 3). Compound **21**, was a derivative of the less stable enol (*cis*-dihydrodiol) tautomer **2**, the initial metabolite of phenol **1** (Scheme 2). A very minor quantity of a diTMS derivative of hydroquinone **5** was also observed possibly due to dehydration of *cis*-diol **3** during the trimethylsilylation procedure.

Biphenyl dioxygenase-catalyzed *cis*-dihydroxylation of 3,3′-dihydroxybiphenyl earlier, yielded a cyclohexenone *cis*-diol metabolite of similar structure to compound **3**, but with the MeO group replaced by a 3-HO·C_6H_4 group.[33] A minor amount of the silylated (tetraTMS) product **23** was also observed, in the presence of the major silylated (triTMS) keto tautomer **22**, by GC−MS analysis.

The TDO-catalyzed *ipso* *cis*-dihydroxylation/dehydration route for the oxidation of phenols to catechols, proposed earlier by Gibson et al., involved an undetected triol intermediate, with the additional oxygen atom being derived from ^{18}O-labeled oxygen gas; this mechanism may also be involved in the formation of catechol **4**.[26,28] The formation of methoxyhydroquinone **5**, as a dehydration product from metabolites **3** and **11** (Schemes 2 and 4), provides strong support for a general pathway to hydroquinone metabolites from phenols, based on the instability of cyclohexenone *cis*-diol intermediates. An alternative biosynthetic pathway, for the naphthalene dioxygenase-catalyzed formation of hydroquinones and catechols, from the corresponding *ortho*- and *meta*-phenols, was, however, recently reported to involve monohydroxylation rather than *cis*-dihydroxylation and dehydration.[31] Thus, with *m*-cresol as substrate, monohydroxylation occurred via nucleophilic attack by ^{18}O-labeled water at the *para* position, to yield methylhydroquinone with incorporation of an ^{18}O-labeled oxygen atom and consumption of dioxygen. A similar pathway with water attacking at the *ortho* position was used, to explain the formation of catechols; the role of oxygen was to reoxidise the Fe(III) back to Fe(IV).

The isolated yields of *cis*-dihydrodiols **17**, obtained with monosubstituted benzene substrates and TDO as biocatalyst, were generally much higher than those obtained using disubstituted benzene substrates, due to (i) the increased steric requirements of the latter substrates at the active site, (ii) competition from alternative types of oxidation, particularly associated with *meta* disubstituted benzene substrates (*meta* effect).[68] It was expected that lower yields of cyclohexenone *cis*-diols, e.g., metabolite **3**, would be obtained and alternative pathways would operate, as 3-substituted phenols, e.g., **1**, are also *meta* disubstituted benzenes. The alkene and carbonyl groups associated with enones can both be reduced, under the biotransformation conditions,[36] leading to mixtures of bioproducts and hence decreased overall yields. Because metabolite **3** is a vinylogous ester, it has very different electronic properties to a typical enone and appeared to be resistant to biological reduction of both the alkene and carbonyl functional groups and to hydrolysis. This property, allied to its relative stability, may account for the isolated moderate yield of cyclohexenone *cis*-diol **3**. 3-Methoxyphenol **1** was thus one of the phenolic substrates selected for large scale (120 L) fermentation to yield cyclohexenone *cis*-diols. The sufficient quantity of metabolite **3** produced, made it possible to conduct a comprehensive study of its reactions and synthetic potential.

Synthetic Applications of *cis*-Diol Metabolite 3 Derived from 3-Methoxyphenol 1. Numerous synthetic applications of enantiopure *cis*-dihydrodiol metabolites, derived from substituted benzene substrates, have been reported, but, to date, relatively few reactions and applications of the new family of cyclohexenone *cis*-diol metabolites from phenols have appeared in the literature. These reactions include substitution of an iodine atom in cyclohexenone *cis*-diol **19** (Figure 3) by different atoms or groups (R = H, CN and CO_2Me) to provide a chemoenzymatic route to other family members.[34]

Hydrolysis of the vinylogous ester **3**, using aqueous 1 M sodium hydroxide, proceeded smoothly to give the enolate sodium salt **25** (Scheme 6). This process involved nucleophilic substitution of the methoxy group by a hydroxyl group, via an addition−elimination pathway, followed by the formation of

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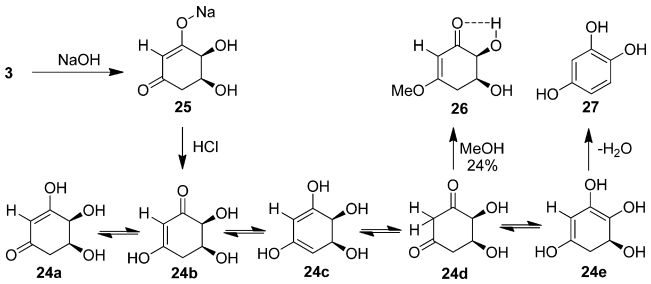
Scheme 6. Hydrolysis of Vinylogous Ester 3 and Esterification to Give Predominantly the Transposed Vinylogous Ester 26



enolate **25**, under the basic reaction conditions. Acidification (1 M HCl) and removal of water, under reduced pressure, yielded a very polar crude product, which was assumed to consist of diketone **24d** and its tautomers **24a−c** and **24e**. Attempts to obtain direct evidence of intermediates **24a−e** by NMR spectroscopy in D_2O as solvent, were unsuccessful. ^1H NMR analysis of the product showed very broad signals suggesting chemical exchange between these various species on the NMR time scale or aggregation. LC-TOFMS analysis did however show the presence of a single peak with the correct mass for any of the tautomeric intermediates **24a−e**. GC−MS analysis and trimethylsilylation of the tautomeric mixture, showed three peaks consistent with the presence of two triTMS keto tautomers (**24a**, **24b**) and one tetraTMS derivative (**24c** or **24e**) or both isomers coeluting. A triTMS derivative of 1,2,4-trihydroxybenzene **27** was also observed, which was assumed to be a dehydration product of the cyclohexenone *cis*-diol **24a** or its tautomers **24b−e**.

The crude product, **24a−e**, was extracted with methanol and the residue, obtained after concentration of the extract, was separated by PLC into a major less polar and a minor more polar compound (9:2). The major compound was identified as the transposed vinylogous ester **26**, by LC-TOFMS, NMR and IR spectroscopic analyses. It was less polar than its isomeric precursor **3**, presumably due to intramolecular hydrogen bonding between the transposed carbonyl group and the proximate OH group (Scheme 7). The minor compound was found to be the reformed vinylogous ester **3**. Gas phase DFT calculations, (Table 1), indicated that compound **26** was more stable than the isomeric compound **3**, by 11.7 kJ mol^{-1}, and in the minimized structure there was evidence of a hydrogen bond between the α-hydroxyl and ketone carbonyl groups. It was challenging to distinguish between compounds **3** and **26** by ^1H NMR spectroscopy. In both isomers, NOE was only observed between the methoxy group and the olefinic proton. Key proton carbon couplings, detected in HMBC experiments (Figure 4), confirmed the carbon connectivity of both isomers.

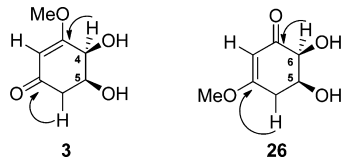
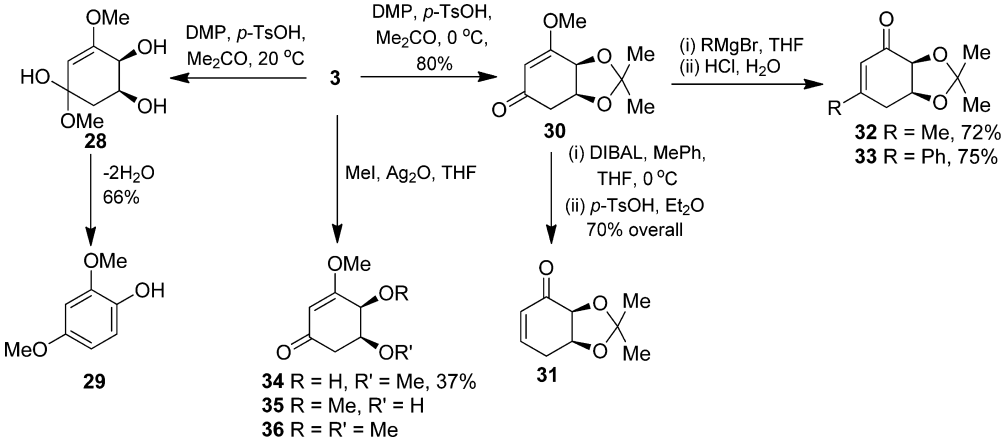


Figure 4. Key HMBC correlations for enone *cis*-diols **3** and **26** confirming the relative position of the carbonyl and methoxy groups in both isomers.

When the crude mixture of tautomers **24a−e** was dissolved in methanol-d_4, analysis of the ^1H NMR spectrum of the new vinylogous ester **26** indicated that the methyl group had been replaced, by CD_3 ($\mathbf{26}_{D3}$) and the vinyl proton signal had diminished in intensity. This result was consistent with CD_3 being incorporated into the product, after partial exchange of the vinyl proton for deuterium via diketone **24d** ($\mathbf{26}_{D4}$), by an addition−elimination reaction of methanol-d_4 with enol **24a**. Transposition of vinylogous esters, under acidic conditions, is well documented[69] and *O*-benzylation of unsymmetrical 1,3-dicarbonyl compounds, under basic conditions, gives transposition isomer mixtures.[70] The regenerated precursor **3** was also found to have incorporated a CD_3 group ($\mathbf{3}_{D3}$) and a vinyl deuterium atom ($\mathbf{3}_{D4}$). Notably, in both cases no deuterium was incorporated at carbons 4 and 6 suggesting limited contributions from enol tautomers **24c** and **24e** in methanol-d_4.

Under the strongly basic conditions that ketodiol **26** had been subjected to, there was a possibility of epimerization at C-6. The relative stereochemistry of diol **26** was established by analysis of vicinal coupling constants. The $J_{6,5}$ value of 3.0 Hz was consistent with an axial−equatorial vicinal coupling, confirming that the *cis* stereochemistry had been preserved. The signal for proton H-5 appeared as a ddd, *J* 3.1, 3.0, 2.8 Hz, indicating that H-5 was equatorial in the dominant conformer. Therefore, in diol **26** the proton on C-6 is axial and the hydroxyl group equatorial. This was in sharp contrast to

Scheme 7. Stork−Danheiser Transpositions of Compound 30 and Synthesis of the Fungal Metabolite 34



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compound **3**, where the hydroxyl group on C-4 was axial, presumably due to 1,2-allylic strain and hyperconjugation effects. Intramolecular hydrogen bonding, to the carbonyl group of diol **26**, was only possible, when the hydroxyl group was equatorial. The TDO-catalyzed synthesis of the vinylogous esters **3** and **11** and chemoenzymatic synthesis of diols **24a** and **26**, from metabolite **3**, add further members to the growing family of chiral cyclohexenone *cis*-diols derived from phenols and demonstrate their wide range of associated stabilities.

To further explore the chemistry of vinylogous ester **3**, it was necessary to protect the diol group as an acetonide. Initial efforts, using 2,2-dimethoxypropane, in the presence of *p*-TsOH at ambient temperature, proved to be unsuccessful. The only isolated product was 2,4-dimethoxyphenol **29** (Scheme 7). The mechanism for conversion of *cis*-diol **3** into phenol **29** is unclear; it may involve aromatization via formation and decomposition of the unstable hemiacetal intermediate **28**. When the protection step was repeated at a lower temperature (0 °C), cyclohexenone *cis*-diol **3** was successfully converted into the required acetonide **30** in good yield (82%), with only traces of dimethoxyphenol **29** present (Scheme 7).

Acetonide **30** underwent a Stork−Danheiser transposition, on reaction with nucleophiles followed by acid treatment.[71] Thus, reaction of acetonide **30** with DIBAL followed by treatment with *p*-TsOH in diethyl ether yielded the transposed cyclohexenone **31**, in an overall yield of 70%. The synthetic potential of this new route to enantiopure cyclohexenone **31** was evident from its earlier use as an intermediate in the total synthesis of natural products, e.g., (+)-clivonone,[72] (+)-trianthine[73] and the antibiotic (+)-palitantin.[74] Similarly, employing Grignard reagents (MeMgBr and PhMgBr) as nucleophiles, followed by acidic workup, resulted in hydrolysis of the enol ether and yielded the transposed cyclohexenones **32** (72% yield) and **33** (75% yield) respectively (Scheme 7). Recently, a new secondary metabolite, isolated from the phytopathogenic fungus *Peronophythora litchi*, was identified as the chiral *cis*-diol **34** but its absolute configuration was not determined.[75] On the basis of the structural similarities of the bacterial metabolite **3** and the fungal metabolite **34**, an attempted monomethylation (MeI, Ag_2O, CH_2Cl_2) under mildly basic conditions was carried out, which resulted in the formation of a mixture of monomethyl ethers **34**, **35** and dimethyl ether **36** (Scheme 7).

The monomethyl ether derivative **34**, was separated by PLC from the other products **35** and **36** and was obtained in modest yield (37%). A direct comparison of its NMR and IR data with that reported for the fungal metabolite, was in good agreement and confirmed its structure. Comparison of the optical rotation of the synthetic sample of (4*S*,5*S*) monomethyl ether **34** ($[a]_D$ −48.0, MeOH), derived from the enantiopure *cis*-diol **3**, with the reported value for the fungal metabolite ($[a]_D$ +28.4, MeOH),[75] suggested that the natural metabolite was of lower enantiopurity (ca. 60% *ee*) and had the opposite (4*R*,5*R*) absolute configuration. It is noteworthy, that despite the densely packed functionality of fungal metabolite **34**, it was synthesized in two steps from the commodity chemical 3-methoxyphenol **1**.

CONCLUSION

TDO-catalyzed *cis*-dihydroxylation of *meta* methoxyphenol **1** and *ortho* methoxyphenol **9**, using whole cell cultures of *P. putida* UV4, was found to yield the corresponding *cis*-diols **3** and **14** as the major metabolites respectively, along with methoxycatechol **4** and methoxyhydroquinone **5** as very minor products. The alkene bond in the initially formed conjugated enone *cis*-diol **11** was found to be rapidly reduced (hydrogenated), via ene reductase catalysis, yielding cyclohexanone *cis*-diol **14**, the major isolated bioproduct. A similar enzymatic reduction (hydrogenation) of the substituted alkene group in enone *cis*-diol **3** was not observed. The structures and absolute configurations of the chiral *cis*-diol bioproducts **3**, **11**, **14** and their derivatives were assigned by spectroscopic, X-ray crystallographic and stereochemical correlation methods. The isolated enone *cis*-diols **3** and **11** readily dehydrated to hydroquinone **5**, providing strong evidence that hydroquinones were formed from diols by an nonenzymatic pathway.

A large-scale biotransformation of phenol **1** provided sufficient quantities of cyclohexenone *cis*-diol **3** for further studies, viz. diol protection, aromatization, alkene hydrogenation, hydrolysis and re-esterification with transposition, *O*-methylation and Stork−Danheiser transposition with a range of nucleophiles. The versatility of enone *cis*-diol **3** was demonstrated in the synthesis and stereochemical assignment of the new type of cyclohexenone *cis*-diol **26**, and a fungal secondary metabolite **34**. The value of *cis*-diol **3** and its acetonide **30**, as useful additions to the chiral pool, was further demonstrated by the synthesis of (3a*S*,7a*S*) ketoacetonide **31**, which has been employed as a key intermediate for the synthesis of known alkaloids and an antibiotic.

EXPERIMENTAL SECTION

^1H and ^{13}C NMR spectra were recorded on 300, 400, and 500 MHz NMR spectrometers using the specified solvent. Chemical shifts (δ) are reported in ppm relative to $SiMe_4$ and coupling constants (J) are given in Hz. IR spectra were recorded either as KBr discs or as thin films on KBr plates. Mass spectra (EI) were run at 70 eV, on a heated inlet system. Accurate molecular weights were determined by the peak matching method, using heptacosafluorotributylamine as the standard reference and were accurate to $\pm 5 \times 10^{-6}$ ppm. Separations for TOFMS analyses were performed using a reverse phase column (C18, 5 mm, 150 × 2.1 mm) together with the corresponding guard column (5 mm, 12.5 × 2.1 mm). The mobile phase consisted of 95% methanol containing 0.1% formic acid in channel A, and 5% methanol containing 0.1% formic acid in channel B. The system was programmed to perform an analysis cycle consisting of 100% B for 1 min, followed by gradient elution from 100 to 5% B over a 14 min period, hold at 5% B for 10 min, return to initial conditions over 2 min and then hold these conditions for a further 8 min. The flow rate was 0.20 mL min^{-1} and the injection volume was 5 μL. MS experiments were carried out using ESI in positive ion mode with the capillary voltage set at 4.0 kV. The desolvation gas was nitrogen set at a flow rate of 8 L min^{-1} and maintained at a temperature of 350 °C. Optical rotation ($[\alpha]_D$) values were carried out on a polarimeter, using a specified solvent and concentration (g/100 mL) at the sodium D-line (589 nm) and ambient temperature. The measurements are in units of 10^{-1} deg cm^2 g^{-1}.

TLC was carried out on analytical plates. Visualization of bands/spots was at 254 nm with a UV lamp or by anisaldehyde- or permanganate-based stains. PLC was carried out on glass plates (20 cm × 20 cm) coated with silica gel (21 g silica gel in 62 mL water). Column chromatography was performed on type 60 (250−400 mesh). Phenol substrates **1**, **9**, hydroquinone **5**, catechol **4** and 1,2,4-trihydroxybenzene **27** were commercially available.

Geometry optimization and vibrational frequencies were evaluated in the gas phase by DFT calculations at the B3LYP/6-31G(d) level of theory. No imaginary frequencies were detected indicating that the structures were correctly optimized.

cis-Diols **3**, **11**, **13** and **14** were analyzed by NMR, LC-TOFMS and GC−MS (silylated). Catechol **4**, trihydroxybenzene **27**, and hydroquinone **5** were analyzed by GC−MS after silylation using *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) and a gas chromato-

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graph linked to a 5973 mass selective detector. The sample (1 μL) was injected in the split mode (20:1). The column used had dimensions (25 m × 0.2 mm × 0.33 μm). The GC oven used was maintained at 100 °C for 1 min and then ramped at 10 °C/min to 300 °C and held at this temperature for 5 min.

Small-scale biotransformations of 2-methoxyphenol **9** and 3-methoxyphenol **1** were carried out, using whole cell cultures of *P. putida* UV4 and *E. coli* (CL-4t), with glucose or pyruvate as a carbon source, under conditions reported earlier for other substituted phenol substrates.[34−37] For time course studies, the biotransformation was performed over 20 h and samples, collected at 2, 6 and 20 h intervals, were analyzed by LC-TOFMS.

Large-Scale Biotransformations of 2-Methoxyphenol 9 and 3-Methoxyphenol 1. 2-Methoxyphenol **9** and 3-methoxyphenol **1** (96 g, 0.77 mol) were each metabolized, using *P. putida* UV4, in a fermenter (pH 7.0, 30 °C, 400 rpm). D-Glucose was used as carbon source substrate (4.8 g/L) and air flow rate was maintained at 70% oxygen. Sodium hydroxide (2 M) was added, automatically, into the fermenter to maintain the pH. When the oxygen tension in the fermenter exceeded 50%, an additional quantity of D-glucose was added (1.64 g/min). Co-substrate addition was controlled, to maintain the oxygen tension in the fermenter in excess of 50%. The crude aqueous biomixture was centrifuged (30 000 rpm, 180 min); the aqueous supernatant solution was decanted off and concentrated at ∼40 °C under reduced pressure. The viscous concentrate was extracted with ethyl acetate (3 × 2.5 L) and the extract concentrated under reduced pressure to yield the crude mixture of bioproducts.

(4*S*,5*S*)-4,5-Dihydroxy-3-methoxycyclohex-2-enone 3. Isolated from the biotransformation of 3-methoxyphenol **1** by crystallization (EtOAc) of the crude mixture of bioproducts as colorless plates (45 g, 38%); the remaining mother liquor was retained for further study. Metabolite **3** was found to be indistinguishable from a sample reported earlier.[36] GC−MS analysis of a small sample of cyclohexenone *cis*-diol **3**, after treatment with MSTFA, showed the presence of disilylated keto derivative **20** as the major product (ca. 95%); *m*/*z* 302 (M^+, 1%), 287 (10), 187(14), 186(100), 147 (27), 73 (25) and the trimethylsilylated enol derivative **21** as the minor product (ca. 5%); *m*/*z* 375 (M^+, 11%), 374 (33), 359 (10), 286 (87), 285 (87), 284 (26), 271 (30), 269 (18), 254 (65), 239 (10), 191 (57), 147 (340, 133 (12), 75 (20), 73 (100).

(3*S*,4*S*,5*R*)-3,4-Dihydroxy-5-methoxycyclohexanone 6. To a solution of enone diol **3** (200 mg, 1.26 mmol) in MeOH (10 mL) was added 10% Pd/C (25 mg) and the mixture stirred overnight at room temperature under 1 atm of hydrogen. The catalyst was filtered off, the filtrate concentrated under reduced pressure, and the residue purified by column chromatography (90% EtOAc in hexane) to give the cyclohexanone **6** as a colorless oil (172 mg, 85%); R_f 0.21 (EtOAc); $[\alpha]_D$ −14.1 (*c* 0.7, $CHCl_3$); HRMS (LC-TOFMS) $[M + H]^+$ found 161.08091, $C_7H_{13}O_4^+$ calcd. 161.08084; $[M + Na]^+$ found 183.06273, $C_7H_{12}O_4Na^+$ calcd. 183.06273; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 2.53−2.63 (2 H, m, H-2, H-6), 2.73−2.80 (2 H, m, H-2′, H-6′), 3.0(2 H, bm, 2 × OH), 3.41 (3 H, s, OMe), 3.69 (1 H, m, H-5), 4.02 (1 H, m, H-3), 4.20 (1 H, ddd, *J* 5.6, 2.9, 2.9, H-4); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 41.8, 46.2, 57.4, 69.8, 70.3, 79.8, 206.0; IR (film) ν_{max}/cm^{-1} 3411, 2922, 1714, 1264, 1063.

(1a*S*,4a*R*,1*S*,6*R*)-(6-Methoxy-4-oxocyclohex-2-enyl)-4a,7a,7a-trimethyl-3a-oxo-2a oxabicyclo[2.2.1]heptane-1a-carboxylate 8. A solution of cyclohexanone diol **6** (62 mg, 0.39 mmol) in dry pyridine (0.5 mL) was treated with (1*S*)-camphanic chloride (208 mg, 0.96 mmol) and the mixture stirred at room temperature for 12 h. The pyridine was removed in vacuo, the residue dissolved in CH_2Cl_2 (10 mL) and the solution washed with brine (2 × 10 mL). It was dried (Na_2SO_4) and concentrated under reduced pressure to give a yellow oil, which was purified by PLC (40% EtOAc in hexane) to give camphanate **8** as a colorless crystalline solid (68 mg, 55%); R_f 0.36 (40% EtOAc in hexane); mp 129−131 °C (acetone/hexane); $[\alpha]_D$ +129.3 (*c* 0.5, $CHCl_3$); HRMS (LC-TOFMS) $[M + NH_4]^+$ found 340.17538, $C_{17}H_{26}NO_6^+$ calcd. 340.17546; $[M + Na]^+$ found 345.13050, $C_{17}H_{22}O_6Na^+$ calcd. 345.13086; $[M + K]^+$ found 361.10478, $C_{17}H_{22}O_6K^+$ calcd. 361.10480; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.98 (3 H, s, Me), 1.10 (3 H, s, Me), 1.13 (3 H, s, Me), 1.71 (1 H, ddd, *J* 13.2, 9.3, 4.3, H-5a), 1.94 (1 H, ddd, *J* 13.2, 10.7, 4.7, H-5a′), 2.07 (1 H, ddd, *J* 13.6, 9.5, 4.7, H-6a), 2.43 (1 H, ddd, *J* 13.6, 10.7, 4.2, H-6a′), 2.69 (1 H, dd, *J* 16.7, 3.9, H-5), 2.84 (1 H, dd, *J* 16.7, 8.4, H-5′), 3.41 (3 H, s, OMe), 3.95 (1 H, m, H-6), 5.88 (1 H, ddd, *J* 4.3, 4.3, 0.9, H-1), 6.20 (1 H, dd 10.1, 0.9, H-3), 6.82 (1 H, dd 10.1, 4.3, H-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 9.8, 16.7, 16.8, 29.1, 30.8, 31.1, 54.6, 55.0, 57.4, 67.7, 76.1, 91.1, 133.1, 141.8, 167.0, 177.8, 196.1; LR EI MS *m*/*z* 322 (M^+, 1%), 290 (9), 264 (49), 181 (19), 137 (20), 125 (100), 109 (24), 97 (47), 83 (88).

Crystal Data for 8. $C_{17}H_{22}O_6$, M = 322.4, monoclinic, *a* = 6.604(1), *b* = 19.939(3), *c* = 6.680(1) Å, *β* = 110.3(1), *U* = 1100.0(5) Å^3, *T* = 293(2) K, space group $P2_1$ (no. 4), Mo K*α* radiation, *λ* = 0.71073 Å, *Z* = 2, *F*(000) = 344, D_x = 1.298 g cm^{-3}, *μ* = 0.098 mm^{-1}, *ω* scans, 6.5° < 2*θ* < 50.0°, measured/independent reflections: 2005/1570, R_{int} = 0.014, direct methods solution, full-matrix least-squares refinement on F_o^2, 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_1 = 0.029 for 1455 data with $F_o > 4\sigma(F_o)$, 213 parameters, ωR_2 = 0.079 (all data), GoF = 1.06, CCDC 1025986. The absolute configuration was established as (1*S*,6*R*) relative to the known absolute configuration of the (1*S*)-camphanate group.

(4*S*,5*S*,6*S*)-4,5-Dihydroxy-6-methoxycyclohex-2-enone 11. The biotransformation of guaiacol **9**, with *P. putida* UV4 on a flask scale was carried out using pyruvate as carbon source for 2 h under previously reported conditions.[4b,5] LC-TOFMS analysis showed the presence of the transient cyclohexenone *cis*-diol **11** as a major product (>85%) and cyclohexanone *cis*-diol **14** as a very minor metabolite. Concentration of the aq. biomixture under reduced pressure and purification of the crude product by PLC (80% EtOAc in hexane) yielded hydroquinone **6** (12 mg) and metabolite **11** (ca. 5 mg); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 6.76 (1 H, dt, *J* 10.3, 2.2, H-3), 6.04 (1 H, dd, *J* 10.3, 2.3, H-2), 4.54 (1 H, dt, *J* 3.9, 2.4, H-4), 4.50 (1 H, dt, *J* 3.9, 2.3, H-5), 3.83 (1 H, d, *J* 2.3 Hz, H-6), 3.59 (3 H, s, OMe); HRMS (LC-TOFMS) $[M + H]^+$ found 159.06517, $C_7H_{10}O_4$ calcd. 159.06519$^+$; $[M + NH_4]^+$ found 176.01965, $C_7H_{14}O_4N^+$ calcd. 176.01973; $[M + K]^+$ found 197.02129, $C_7H_{10}O_4K^+$ calcd. 197.02107.

A 20 L aq. portion (from 120 L) of the biomixture, obtained using guaiacol **9** as substrate, was worked up as described earlier. The crude bioproduct mixture, on purification by column chromatography (20% hexane in EtOAc → 100% EtOAc) followed by multiple elution PLC (65% EtOAc in hexane), of the pooled major similar fractions, furnished *cis*-diol metabolites **13** and **14**. The minor fractions containing other bioproducts are currently under investigation.

(2*R*,3*S*,4*S*)-3,4-Dihydroxy-2-methoxycyclohexanone 13. Biotransformation of guaiacol **9** yielded compound **13**, a minor metabolite as a colorless oil (250 mg, 1.3%); R_f 0.45 (65% EtOAc in hexane, 2 elutions); $[\alpha]_D$ +92 (*c* 0.55, $CHCl_3$); HRMS (LC-TOFMS) $[M + H]^+$ found 161.08136, $C_7H_{13}O_4^+$ calcd. 161.08168; $[M + K]^+$ found 199.03869, $C_7H_{12}O_4K^+$ calcd. 199.03727; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.68 (1 H, tddd, *J* 14.4, 4.7, 2.4, 1.8, H-5), 2.18 (1 H, dddd, *J* 14.4, 6.2, 3.6, 2.5, H-5′), 2.27 (1 H, dddd, *J* 13.7, 4.7, 2.5, 0.7, H-6), 2.80 (1 H, tddd, *J* 13.7, 6.2, 1.3, 0.7, H-6′), 2.91 (1 H, t, *J* 1.9, OH), 3.09 (1 H, d, *J* 2.0, OH), 3.53 (3 H, s, OMe), 3.71 (1 H, ddd, *J* 9.8, 2.8, 1.8, H-3), 4.07 (1 H, dd, *J* 9.8, 1.0, H-2), 4.26 (1 H, m, H-4); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 27.5, 34.8, 59.7, 68.1, 76.4, 85.8, 207.2.

(2*S*,3*S*,4*S*)-3,4-Dihydroxy-2-methoxycyclohexanone 14. Major metabolite from guaiacol **9**, compound **14** was obtained as colorless needles (2.5 g, 13%); mp 124−125 °C (EtOAc/hexane); R_f 0.2 (EtOAc); $[\alpha]_D$ −54.2 (*c* 0.5, $CHCl_3$); HRMS (LC-TOFMS) $[M + H]^+$ found 161.08136, $C_7H_{13}O_4^+$ calcd. 161.08084; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 2.04 (1 H, m, H-5), 2.13 (1 H, dddd, *J* 12.5, 12.5, 10.2, 4.9, H-5′), 2.29 (1 H, dddd, *J* 14.1, 12.1, 6.5, 1.3, H-6), 2.45 (1 H, dddd, *J* 14.1, 5.0, 4.3, 0.5, H-6′), 2.49 (1 H, br s, OH), 2.78 (1 H, br s, OH), 3.51 (3 H, s, OMe), 3.80 (1 H, dd, *J* 3.0, 1.1, H-2), 4.10 (1 H, ddd, *J* 10.2, 5.0, 2.6, H-4), 4.34 (1 H, ddd, *J* 3.0, 3.0, 1.5, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 28.76, 35.31, 58.81, 69.70, 74.78, 84.51, 206.79; IR (film) ν_{max}/cm^{-1} 3418, 2926, 2856, 1731, 1494, 1452.

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(1a*S*,4a*R*)-(*S*)-3-Methoxy-4-oxocyclohex-2-enyl)4a,7a,7a-trimethyl-3a-oxo-2a-oxabicyclo[2.2.1] heptane-1a-carboxylate 16. A solution of cyclohexanone diol **14** (100 mg, 0.62 mmol) in dry pyridine (1 mL) was reacted with (1*S*)-camphanic chloride (342 mg, 1.58 mmol) as described for the synthesis of camphanate **8**. Camphanate **16** was obtained as a colorless crystalline solid (110 mg, 55%); mp 132−133 °C (acetone/hexane or EtOAc); $[\alpha]_D$ −114.5 (*c* 0.75, $CHCl_3$); HRMS (LC-TOFMS) $[M + H]^+$ found 323.14797, $C_{17}H_{23}O_6^+$calcd. 323.14891; $[M+ NH_3]^+$ found 340.17469, $C_{17}H_{25}O_6N^+$ calcd. 340.17546; $[M + Na]^+$ found 345.13014, $C_{17}H_{22}O_6Na^+$ calcd. 345.13086; $[M + K]^+$ found 361.1042, $C_{17}H_{22}O_6K^+$ calcd. 361.1048; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.97 (3 H, s, Me), 1.06 (3 H, s, Me), 1.13 (3 H, s, Me), 1.71 (1 H, ddd, *J* 13.5, 9.5, 4.2, H-5a), 1.94 (1 H, ddd, *J* 13.3, 10.9, 4.6, H-5a), 2.06 (1 H, ddd, *J* 13.8, 9.4, 4.6, H-6a), 2.17 (1 H, m, H-6), 2.37 (1 H, m, H-6), 2.44 (1 H, ddd, *J* 13.6, 10.8, 4.4, H-6a′), 2.55 (1 H, ddd, *J* 17.0, 7.8, 4.7, H-5), 2.79 (1 H, ddd, *J* 17.0, 9.0, 4.8, H-5′), 3.65 (3 H, s, OMe), 5.76 (1 H, m, H-1), 5.81 (1 H, d, *J* 4.5, H-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 9.8, 16.9, 17.0, 28.4, 29.0, 30.8, 34.5, 54.5, 54.9, 55.4, 69.5, 91.0, 111.3, 153.2, 167.4, 178.0, 192.6; LR EI MS *m*/*z* 322 (M^+, 1%), 170 (23), 139 (36), 124 (91), 109 (100), 93 (35), 109 (24), 89 (39), 81 (77).

Crystal Data for 16. $C_{17}H_{22}O_6$, M = 322.4, monoclinic, *a* = 6.071(3), *b* = 18.734(7), *c* = 7.024(3) Å, *β* = 98.10(1)°, *U* = 790.9(6) Å^3, *T* = 100(2) K, space group $P2_1$ (no. 4), Cu K*α* radiation, *λ* = 1,54178 Å, *Z* = 2, *F*(000) = 344, D_x = 1.354 g cm^{-3}, *μ* = 0.851 mm^{-1}, *ω* scans, 14.7° < 2*θ* < 133.1°, measured/independent reflections: 8710/2445, R_{int} = 0.032, direct methods solution, full-matrix least-squares refinement on F_o^2, anisotropic displacement parameters for non-hydrogen atoms; hydrogen atoms included at positions calculated from the geometry of the molecule using the riding model, with isotropic vibration parameters. R_1 = 0.036 for 2346 data with $F_o > 4\sigma(F_o)$, 213 parameters, ωR_2 = 0.096 (all data), GoF = 1.06, CCDC 1025987. The absolute configuration is established as (1*S*) relative to the known absolute configuration of the (1*S*)-camphanate group and independently from the anomalous scattering arising from the oxygen atoms; Flack parameter *x* = −0.05(14).

(2*S*,3*S*)-2,3-Dihydroxy-5-methoxycyclohex-6-enone 26. Enone *cis*-diol **3** (50 mg, 0.32 mmol) was treated with an aqueous solution of NaOH (1.5 mL, 1 M). The mixture was gently shaken, left at room temperature for 24 h, neutralized with 1 M HCl and an aliquot retained for LC-TOFMS analysis. The remaining reaction mixture was concentrated under reduced pressure, the crude product dried under a high vacuum. A small sample of the concentrate was silylated for GC−MS analysis and the remainder taken up in methanol (5 mL). The insoluble NaCl was filtered off, the filtrate concentrated, and the crude product, on purification by PLC (EtOAc) separated into two compounds. The major, less polar enone diol **26** was obtained as a light yellow oil (12 mg, 24%); R_f 0.35 (EtOAc); $[\alpha]_D$ +4.0 (*c* 1.0, $CHCl_3$); HRMS (LC-TOFMS) $[M + H]^+$ found 159.06544, $C_7H_{11}O_4^+$ calcd. 159.06519; $[M + Na]^+$ found 181.04713, $C_7H_{10}O_4Na^+$ calcd. 181.04729; ^{1}H NMR (400 MHz, CD_3OD) δ_H 2.59 (1 H, dd, *J* 18.1, 2.8, H-4), 2.93 (1 H, ddd, *J* 18.1, 3.3, 1.7, H-4′), 3.76 (3 H, s, OMe), 4.21 (1 H, d, *J* 3.0, H-2), 4.34 (1 H, ddd, *J* 3.0, 3.0, H-3), 5.43 (1 H, d, *J* 1.3, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 37.3, 57.1, 70.3, 76.0, 100.3, 177.3, 199.8; IR (film) ν_{max}/cm^{-1} 3397, 2659, 1658, 1603, 1386, 1227, 1199. The minor polar compound (3.1 mg, 6%); R_f 0.12 (EtOAc), was the starting enone *cis*-diol **3**.

LC-TOFMS analysis showed a strong peak consistent with the presence of tautomers **24a−e**; $[M + H]^+$ found 145.04977, $C_6H_9O_4^+$ calcd. 145.04954; $[M + Na]^+$ found 167.03145, $C_6H_8O_4Na^+$ calcd. 167.03148; $[M + K]^+$ found 183.00521, $C_6H_8O_4K^+$ calcd. 183.00542.

GC−MS analysis showed three peaks consistent with the presence of trimethylsilylated derivatives of tautomers **24a** or **24b** (triTMS) and **24c** or **24e** (tetraTMS) and 1,2,4-trihydroxybenzene **27** (triTMS).

Trimethylsilylated derivatives of trihydroxy tautomers **24a** and **24b**: Peak 1 (10.23 min); *m*/*z* 360 (M^+, 8%), 345 (18), 242 (12), 204 (27), 147 (23), 133 (11), 75 (13), 74 (8), 73 (100); Peak 2 (10.87 min); *m*/*z* 360 (M^+, 2%), 345 (13), 246 (9), 245 (20), 244 (99), 147 (38), 133 (15), 75 (11), 74 (9), 73 (100).

Trimethylsilylated derivative of tetrahydroxy tautomers **24c** or **24e**: (10.21 min); *m*/*z* 432 (M^+, 9%), 344 (11), 343 (28), 342 (11), 329 (10), 239 (17), 191 (19), 147 (24), 133 (7), 75 (13), 74 (9), 73 (100).

Trimethylsilylated derivative of 1,2,4-trihydroxybenzene **27**:(10.79 min); *m*/*z* 342 (M^+, 71%), 254 (11), 240 (11), 239 (17), 73 (100).

Following similar treatment of enone *cis*-diol **3** and employing CD_3OD instead of methanol yielded deuteriated compounds $\mathbf{3}_{D3}$, $\mathbf{3}_{D4}$ and isomers $\mathbf{26}_{D3}$, $\mathbf{26}_{D4}$. On separation by PLC, using methanol in the solvent mixture, the deuterium on the two hydroxyl groups was replaced with hydrogen.

cis-Diol **3**: HRMS (LC-TOFMS) $[M + H]^+$ found159.06534, $C_7H_{10}O_4^+$ calcd. 159.06519.

cis-Diol $\mathbf{3}_{D3}$: HRMS (LC-TOFMS) $[M + H]^+$ found 162.08391, $C_7H_8D_3O_4^+$ calcd. 162.08402.

cis-Diol $\mathbf{3}_{D4}$: HRMS (LC-TOFMS) $[M + H]^+$ found 163.08999, $C_7H_7D_4O_4^+$ calcd. 163.09029.

cis-Diol **26**: HRMS (LC-TOFMS) $[M + H]^+$ found 159.06544, $C_7H_{10}O_4^+$ calcd. 159.06519.

cis-Diol $\mathbf{26}_{D3}$: HRMS (LC-TOFMS) $[M + H]^+$ found 162.08372, $C_7H_8D_3O_4^+$ calcd. 162. 08402.

cis-Diol $\mathbf{26}_{D4}$: HRMS (LC-TOFMS) $[M + H]^+$ found 163.09037. $C_7H_7D_4O_4^+$ calcd. 163. 09029.

2,4-Dimethoxyphenol 29. To a stirred solution of enone *cis*-diol **3** (100 mg, 0.63 mmol) in a mixture of acetone (2 mL) and 2,2-dimethoxypropane (2 mL), was added *p*-TsOH monohydrate (6 mg, 0.03 mmol) at room temperature. After stirring the mixture for 2 h, the solvent was removed under reduced pressure and the residue dissolved in EtOAc (15 mL). The solution was successively washed with 5% aq. $NaHCO_3$ (10 mL), brine (10 mL), and dried (Na_2SO_4). Removal of solvent under reduced pressure gave a light brown colored oil, which was purified by column chromatography (66% EtOAc in hexane), to yield phenol **29** as a light yellow viscous oil (81 mg, 83%); R_f 0.21 (66% EtOAc in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 3.76 (3H, s, OMe), 3.85 (3H, s, OMe), 5.35 (1H, br s, OH), 6.39 (1H, dd, *J* 8.7, 2.7, H-5), 6.49 (1H, d, *J* 2.7, H-3),6.83 (1H, d, *J* 8.7, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 55.87, 55.94, 99.5, 104.3, 114.2, 139.9, 147.2, 153.6. The spectroscopic data of phenol **29** was in agreement with the literature.[76]

(3a*S*,7a*S*)-7-Methoxy-2,2-dimethyl-3a,4-dihydrobenzo[*d*]-[1,3]dioxol-5(7a*H*)-one 30. A solution of enone diol **3** (1.0 g, 6.33 mmol) in a mixture of acetone (4 mL) and 2,2-dimethoxypropane (8 mL), containing *p*-TsOH monohydrate (60 mg, 0.32 mmol), was stirred at 0 °C for 45 min. The solvent was removed under reduced pressure, the residue dissolved in EtOAc (35 mL) and the solution successively washed with 5% aq. $NaHCO_3$ (30 mL) and brine (30 mL). It was dried (Na_2SO_4) and concentrated under reduced pressure to give a yellow oil, which was purified by column chromatography (85% EtOAc in hexanes). Acetonide **30** was obtained as a white solid (1.0 g, 80%); mp 99−101 °C (EtOAc); R_f 0.55 (85% EtOAc in hexane); $[\alpha]_D$ −58.4 (*c* 0.5, $CHCl_3$); HRMS (ES): $[M + H]^+$ found 199.0975, $C_{10}H_{15}O_4^+$ calcd. 199.0970; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.37 (3 H, s, Me), 1.40 (3 H, s, Me), 2.64 (1 H, ddd, *J* 17.5, 2.7, 1.3, H-4), 2.86 (1 H, dd, *J* 17.5, 1.6, H-4′), 3.75 (3 H, s, OMe), 4.64−4.66 (2 H, m, H-3a, H-7a), 5.42 (1 H, s, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 25.4, 26.8, 37.6, 55.3, 71.3, 71.4, 101.8, 109.3, 170.9, 194.1; LRMS (ES): 199 (6), 221 (20), 259 (48), 413 (80), 484 (42), 691 (35), 803 (100), 1082 (10), 1194 (24).

(3a*S*,7a*S*)-2,2-Dimethyl-7,7a-dihydrobenzo[*d*][1,3]dioxol-4-(3a*H*)-one 31.[72−74] A solution of DIBAL in toluene (1 M, 421 *μ*L, 0.41 mmol) was added dropwise to a solution of acetonide **30** (59 mg, 0.30 mmol) in a mixture of THF and toluene (1:1, 5 mL) at 0 °C. After stirring for 45 min, a saturated aq. solution of NH_4Cl (0.3 mL) was added and the mixture stirred for another 1 h. Magnesium sulfate (200 mg) was added to the stirred reaction mixture and it was filtered through diatomaceous earth. The filtrate was concentrated in vacuo to yield a yellow oil, which was immediately dissolved in Et_2O (10 mL) and the solution treated with *p*-TsOH (5 mg). The reaction mixture was washed successively with saturated aq. $NaHCO_3$ (10 mL) and brine (10 mL), dried ($MgSO_4$) and concentrated in vacuo. The crude product obtained was purified by PLC (50% EtOAc in hexane) to

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yield enone **31** as a colorless oil (32 mg, 70%); R_f 0.55 (50% EtOAc in hexane); $[\alpha]_D$ +83.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.34 (3 H, s, Me), 1.40 (3 H, s, Me), 2.79 (1 H, dddd, *J* 20.4, 5.7, 2.9, 2.8, H-7), 2.88 (1 H, dddd, *J* 20.4, 4.8, 1.7, 1.5, H-7′), 4.29 (1 H, d, *J* 5.2, H-3a), 4.63 (1 H, dddd, *J* 5.7, 5.2, 1.7, 1.7, H-7a), 6.12 (1 H, ddd, *J* 10.3, 2.8, 1.5, H-5), 6.83 (1 H, dddd, *J* 10.3, 4.8, 2.9, 1.7, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 26.1, 27.6, 27.9, 73.0, 75.6, 109.4, 128.4, 146.4, 196.3.

(3a*S*,7a*S*)-2,2,6-Trimethyl-7,7a-dihydrobenzo[*d*][1,3]dioxol-4(3a*H*)-one 32. To a solution of acetonide **30** (290 mg, 1.46 mmol) in THF (6 mL) at 0 °C was added a solution of MeMgBr in Et_2O (3 M, 1.46 mL, 4.0 mmol). The reaction mixture was stirred at 0 °C for 2 h, diluted with Et_2O (20 mL), and then quenched with ice. It was washed with 0.5 M HCl (2 × 15 mL), the aq. layers extracted with Et_2O (2 × 15 mL) and the combined organic layers washed with brine (2 × 15 mL). The solution was dried ($MgSO_4$) and concentrated in vacuo to give a brown oil, which was purified by PLC (50% EtOAc in hexane) to yield enone **32** as a light yellow oil (192 mg, 72%); R_f 0.25 (50% EtOAc in hexane); $[\alpha]_D$ +42.8 (*c* 0.92, $CHCl_3$); HRMS (ES): $[M + H]^+$ found 183.1020, $C_{10}H_{15}O_3^+$ calcd. 183.1021; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.35 (3 H, s, Me), 1.40 (3 H, s, Me), 2.00−2.02 (3 H, m, HC═CMe), 2.76 (2 H, m, H-7, H-7′), 4.24 (1 H, d, *J* 5.1, H-3a), 4.61 (1 H, ddd, *J* 6.8, 3.5, 3.5, H-7a), 5.98 (1 H, dq, *J* 2.8, 1.4, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 24.6, 26.2, 27.6, 29.9, 32.8, 72.9, 74.8, 109.4, 125.3, 158.5, 196.1.

(3a*S*,7a*S*)-2,2-Dimethyl-6-phenyl-7,7a-dihydrobenzo[*d*][1,3]-dioxol-4(3a*H*)-one 33. Following the procedure given for the synthesis of compound **32**, acetonide **30** (65 mg, 0.33 mmol) was reacted with PhMgBr (3 M, 320 μL, 0.96 mmol) in Et_2O. The crude brown oil obtained was purified by PLC (50% EtOAc in hexane), to yield enone **33** as a white solid (60 mg, 75%); R_f 0.45 (50% EtOAc in hexane); mp 94 °C (EtOAc/hexane); $[\alpha]_D$ +18.8 (*c* 1.04, $CHCl_3$); HRMS (ES): $[M + H]^+$ found 245.1179, $C_{15}H_{17}O_3^+$ calcd. 245.1178; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.37 (3 H, s, Me), 1.43 (3 H, s, Me), 3.15 (1 H, ddd, *J* 19.4, 4.9, 2.4, H-7), 3.30 (1 H, dd, *J* 19.4, 1.9, H-7′), 4.36 (1 H, d, *J* 5.1, H-3a), 4.78 (1 H, ddd, *J* 5.1, 4.9, 1.9, H-7a), 6.50 (1 H, d, *J* 2.4, H-5), 7.40−7.45 (3 H, Ar), 7.54−7.58 (2 H, m, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 26.2, 27.6, 29.8, 73.0, 74.9, 109.6, 123.7, 126.5 (2C), 129.0 (2C), 130.7, 138.0, 155.4, 196.5; LRMS (ES): 217 (100), 245 (25), 451 (65).

(4*S*,5*S*)-4-Hydroxy-3,5-dimethoxycyclohex-2-enone 34. To a solution of enone *cis*-diol **3** (174 mg, 1.10 mmol) in CH_2Cl_2 (10 mL) was added Ag_2O (260 mg, 1.12 mmol) and methyl iodide (234 mg, 1.65 mmol). After stirring the reaction mixture for 12 h at room temperature, the insoluble salts were filtered off and the filtrate concentrated in vacuo, to yield a dark brown colored oil. The crude product on PLC (50% EtOAc in hexane, 3 elutions) separated into compounds **34**, **35** and **36**. Enone **34** was isolated as an oil (70 mg, 37%); R_f 0.26 (75% EtOAc in hexane); $[\alpha]_D$ −48.0 (*c* 0.67, MeOH); lit.[75] $[\alpha]_D$ +28.4 (*c* 1.62, MeOH); HRMS (ES): $[M + H]^+$ found 173.0811, $C_8H_{13}O_4^+$ calcd. 173.0814; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 2.52 (1 H, dd, *J* 16.6, 4.3, H-6), 2.70 (1 H, dd, *J* 16.6, 9.2, H-6′), 3.42 (3 H, s, OMe), 3.75−3.78 (1 H, m, H-5), 3.78 (3 H, s, OMe), 4.49 (1 H, d, *J* 3.5, H-4), 5.39 (1 H, s, H-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 39.2, 57.0, 57.1, 68.2, 78.3, 103.1, 178.3, 199.5; LRMS (ES): 173 (10), 217 (100), 304 (60); IR (film) ν_{max} /cm^{-1} 3434, 2944, 1637, 1607, 1458, 1378, 1231, 1099.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra of all compounds and HMBC spectra of compounds **3** and **26**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

This article is dedicated to the memory of the late Professor David T. Gibson (University of Iowa), a good friend and outstanding scientist.

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