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Synthesis of a Broad Array of Highly Functionalized, Enantiomerically Pure Cyclohexanecarboxylic Acid Derivatives by Microbial Dihydroxylation of Benzoic Acid and Subsequent Oxidative and Rearrangement Reactions

Andrew G. Myers,* Dionicio R. Siegel, Daniel J. Buzard, and Mark G. Charest

Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138

myers@chemistry.harvard.edu

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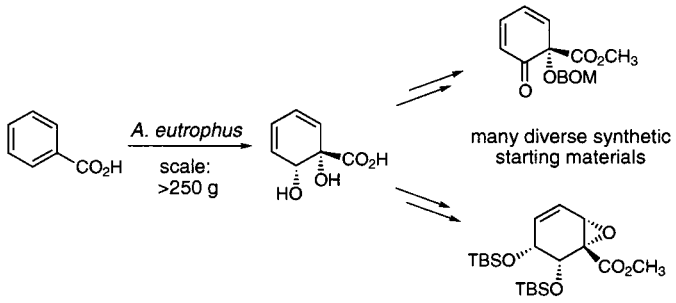
ABSTRACT



We have found that the 1,2-dihydroxylation of benzoic acid with *Alcaligenes eutrophus* strain B9, first reported in 1971 by Reiner and Hegeman, is readily adapted for the preparation of tens to hundreds of grams of (1*S*,2*R*)-1,2-dihydroxycyclohexa-3,5-diene-1-carboxylic acid of >95% ee. This unique substrate undergoes many specific oxidative and rearrangement processes. Among these are transformations of unanticipated chemical novelty and many products that have not been previously described.

Microbial cis 2,3-dihydroxylation of halo- and alkylbenzene substrates provides access to valuable chiral synthetic intermediates that would be difficult to obtain by other means. These intermediates, in turn, have been used as starting materials for the preparation of a wide variety of complex target structures.[1] In the context of studies directed toward the preparation of the tetracycline antibiotics and related biologically active compounds, we were led to explore a different microbial oxidation process as a potential source of synthetic starting materials. In 1971, Reiner and Hegeman reported that a mutant strain of *Alcaligenes eutrophus*, when grown in the presence of benzoic acid, accumulated the 1,2-*cis*-dihydroxylation product **1** (Scheme 1), presumably due to a defect in the metabolic pathway that normally produces

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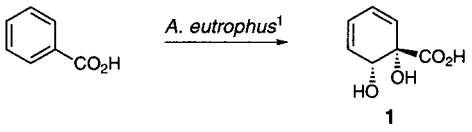
Scheme 1



1

catechol from benzoate. The product was shown to be optically active, but the extent and sense of asymmetric induction were not determined.[2] Subsequently, Widdowson et al. showed that a mutant strain of *Pseudomonas putida* also produced a 1,2-*cis*-dihydroxylation product when incubated with benzoic acid.[3] They determined that the stereochemistry of this product was 1*S*,2*R* and prepared the corresponding acetonide methyl ester as a substrate for Diels−Alder and hetero Diels−Alder reactions.[4] In the course of our own studies, we found that the fermentation of benzoic acid with the original mutant organism (*A. eutrophus* strain B9) is easily adapted for the preparation of tens to hundreds of grams of the (1*S*,2*R*)-dihydroxylation product, without the need for specialized apparatus, and that the product, a compound of >95% ee, undergoes many specific oxidative and rearrangement processes. Among these are transformations of unanticipated chemical novelty and many products that have not been previously described. The combined microbial and synthetic transformations make available a wide range of valuable enantiomerically pure synthetic starting materials.

Whole-cell, microbial dihydroxylation of benzoic acid was conducted by a modification of the original procedure of Reiner and Hegeman,[2] using the microorganism *A. eutrophus*, strain B9. The procedure developed gave rise to a higher yield of product and a more concentrated product solution and was simpler to conduct. Essentially, the modification entailed the use of a single-batch process, omitting a cell-harvesting step, with alternating feeds of benzoic and succinic acids throughout the incubation (∼18 h, 30 °C). We used routine and inexpensive apparatus (diagrams are provided for the production of 36- and 270-g batches of product, see Supporting Information) and isolated the product by simple extraction (ethyl acetate), followed by drying (sodium sulfate) and concentration. Trituration of the solid residue with dichloromethane afforded pure **1** as a white powder, indefinitely stable when stored in the solid state at −20 °C. The optical rotation of our fermentation product $[\alpha]_D$ −114.8 (*c* 0.5 in EtOH), lit.[4] $[\alpha]_D$ −106 (*c* 0.5 in EtOH) was nearly identical to that formed from *P. putida*, establishing its configuration as 1*S*,2*R*. Through a sequence of methylation (CH_2N_2), hydrogenation of both olefins of the diene (Pd−C, ethyl acetate), and (mono) Mosher esterification of the secondary alcohol,[5] we established that **1** had >95% ee (^{1}H NMR analysis, comparison to material prepared from a racemic precursor).

The optically pure diol **1** can be selectively protected in various ways (Figure 1). The corresponding methyl ester (**2**)

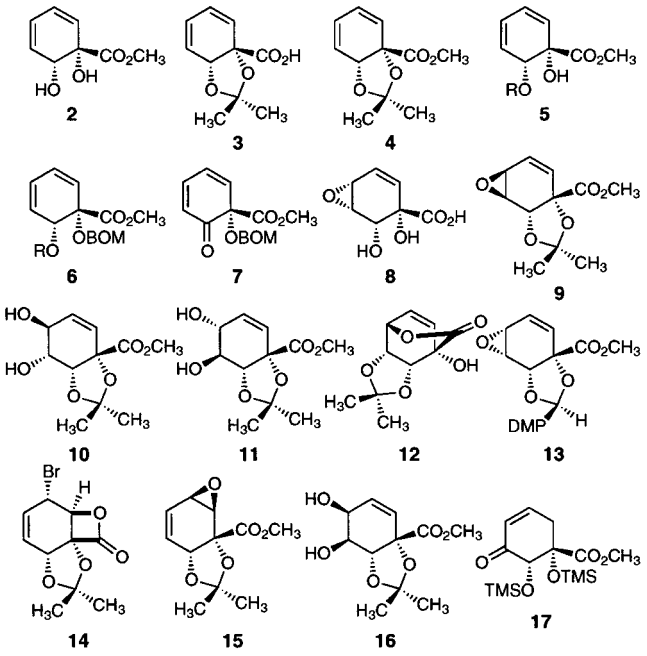


Figure 1.

can be formed using diazomethane, as previously described,[3,6] or more practically on large scale with dimethyl sulfate and cesium carbonate. Acetonide formation has been described[3,6] and is highly efficient using either the acid **1** (forming **3**, 98%) or the methyl ester (forming **4**, 96%) as starting material. The secondary hydroxyl group of the methyl ester **2** was found to react selectively with silylating and acylating agents (∼1 equiv, quantitative yield for **5**, R = TMS, and 91% yield for **5**, R = pivaloyl), but protection of the tertiary alcohol (with a group that did not subsequently migrate when the secondary hydroxyl group was revealed) proved challenging. Eventually, we discovered that alkylation of the sodium salt (NaH, THF, −65 °C) of the trimethylsilyl-protected intermediate **5** (prepared in situ, not chromatographically stable) with benzyloxymethyl iodide[7] produced the corresponding BOM ether **6** efficiently (R = TMS, 90% yield after chromatographic isolation) and that this intermediate could be cleanly deprotected with triethylamine trihydrofluoride to give the stable secondary alcohol **6** (R = H, quantitative). In a noteworthy transformation, oxidation of the latter product with the Dess−Martin periodinane[8] furnished the crystalline 2,4-cyclohexadienone **7** in quantitative yield, after flash column chromatography. Substituted

(2) Reiner, A. M.; Hegeman, G. D. *Biochemistry* **1971**, *10*, 2530.
(3) Rossiter, J. T.; Williams, S. R.; Cass, A. E.; Ribbons, D. W. *Tetrahedron Lett.* **1987**, *28*, 5173.
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(8) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155.

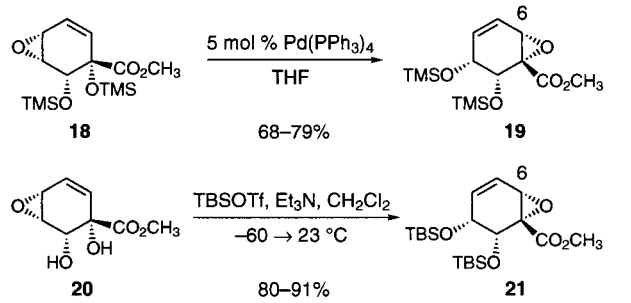
2,4-cyclohexadienones are often not stable to isolation, undergoing rapid Diels−Alder dimerization, and, to our knowledge, have not been prepared practically in optically active form before. Dimerization of **7** did occur slowly at 23 °C ($t_{1/2}$ = 4 h, initial concentration 0.6 M, $CDCl_3$), forming a single Diels−Alder adduct (X-ray analysis, see Supporting Information), consistent with the proposal that **7** was of high optical purity.

Virtually any position of the 1,3-cyclohexadiene nucleus of **1** and its protected forms can be oxidized stereoselectively in simple one- and two-step procedures. The products, allylic epoxides, bromides, lactones, etc. (Figure 1), are well disposed for further facile synthetic elaboration. Epoxidation can be conducted either before or after protection of the diol and carboxylic acid groups, with the ordering determining the stereoselectivity of the reaction. Thus, hydroxyl-directed epoxidation of **1** with *m*-CPBA (1.2 equiv) in ethyl acetate at 23 °C provided the α-oriented allylic epoxide **8** exclusively (91%, after washing with CH_2Cl_2), whereas epoxidation of the acetonide methyl ester **4** produced the stereoisomeric, *β*-epoxide **9** as a crystalline solid (*m*-CPBA, 1.5 equiv, CH_2Cl_2, 23 °C, 91% of a 3:1 mixture of **9** and **15**). Both products **8** and **9** are activated toward invertive 1,2-addition of nucleophiles to the allylic position of the epoxide. For example, perchloric acid-mediated hydrolysis of the methyl ester derived from **8** (CH_2N_2) in aqueous acetonitrile at 23 °C followed by acetonide formation provided the crystalline diol acetonide **10** in 79% yield, whereas **9** produced the stereoisomeric diol acetonide **11** under similar conditions (76%, structure confirmed by X-ray analysis). Epoxide opening followed an intramolecular pathway when **8** was treated with perchloric acid in anhydrous acetone at reflux; acetonide formation occurred concomitantly and with a different regiochemistry than before, providing the lactone acetonide **12** in 64% yield. Because the epoxy acid **8** was sensitive to acid-catalyzed aromatization (and the corresponding methyl ester **20** more so), to protect the diol it was necessary to use neutral conditions. This was achieved by ketalization using 2,4-dimethoxybenzyl methyl ether (1.5 equiv) and DDQ (2.0 equiv) in dichloromethane at 23 °C for 24 h, furnishing a 1.5:1 mixture of the diastereomeric acetals **13** (75%, major stereoisomer (*β*) depicted).[9] The 2,4-dimethoxyphenyl acetal was useful not only as a protective group but also for its ability to promote nucleophilic addition of organometallic reagents (e.g., vinyllithium intermediates) to the adjacent ester group, an observation of some interest, should it prove to be general.

Selective functionalization of the more hindered olefin of the diene system was accomplished by bromolactonization of **3** with NBS (2 equiv) in a mixture of toluene and dichloromethane (1:20) at 23 °C for 2 h. The crystalline bromo *β*-lactone **14** was obtained in 69% yield after purification by column chromatography. This regiochemical outcome in the halolactonization reaction, although not unprecedented,[10] was useful here, for it provided an entry into an alternative allylic epoxide series. Thus, exposure of **14** to anhydrous sodium methoxide in methanol at 23 °C furnished the epoxide **15** in 81% yield. By contrast, osmylation of the diene system of the acetonide methyl ester **4** (catalytic osmium tetroxide, stoichiometric NMO) proceeded with predominant attack upon the less hindered olefin and with complete selectivity for the *β*-face (regioselectivity 5:1, major isomer **16**, 90% isolated yield).

One of the more interesting and useful transformations we developed in this study was discovered serendipitously, arising from an effort to produce the cyclohexenone **17** by the rearrangement of the bis-trimethylsilyl ether **18** (BSTFA, 83%) in the presence of a palladium catalyst.[11] In the event, only trace quantities of **17** were formed (5 mol % of Pd(PPh_3)$_4$, THF, 23 °C, 16 h); the rearranged, isomeric allylic epoxide **19** was produced instead, in 68−79% yield after chromatographic isolation (Scheme 2). We believe that this

Scheme 2



rearrangement, which did not occur in the absence of the palladium catalyst or with triphenylphosphine alone, is novel. Speculation upon the mechanism of this transformation should take into account the fact that essentially the same rearrangement occurs, and more efficiently, within the methyl ester **20**, in the presence of *tert*-butyldimethylsilyl triflate (3 equiv) and triethylamine (10 equiv) in dichloromethane at −40 → 23 °C, forming the epoxide **21** (80−91%). We defer mechanistic conjecture at this point, other than to offer that the system is well disposed for intramolecular 1,3-transfer of a silyl group, be this promoted by a Lewis acid (silyl triflate) or in a nucleophilic process (alkoxy *π*-allylpalladium intermediate). Evidently, and not surprisingly, the more substituted epoxide (**21**) is favored thermodynamically. Resubjection of **21** to the reaction conditions did not lead to detectable levels of the regiosiomeric epoxide. The great advantage of the transformation is that it transfers the position of the allylic epoxide so as to allow for nucleophilic additions to C6, of potential utility in tetracycline synthetic studies.

In summary, practical, large-scale microbial hydroxylation of benzoic acid provides convenient access to the diol **1** in high optical purity. Apart from simplification of the original fermentation procedure of Reiner and Hegeman, we have shown that an extraordinarily wide array of highly function-

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alized starting materials can be synthesized from **1** by operationally simple protocols. Regio- and stereoselective epoxidation reactions, epoxide-opening reactions, dihydroxylation reactions, lactonizations, and a novel allylic epoxide rearrangement reaction provide several points of entry into many of the substitution patterns that can be imagined for the cyclohexanecarboxylic acid skeleton. In addition, a practical synthesis of optically active cyclohexadienones was developed. The utility of these findings in synthetic chemistry is apparent and potentially further enhanced with the recognition that additional amplification of the substrate pool is possible by the use of substituted benzoic acids as an alternative feedstock in the microbial oxidation step.[2,5]

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Supporting Information Available: Experimental protocols and apparatus diagrams for fermentive production of **1** and listings of selected spectral data (^{1}H NMR, ^{13}C NMR, IR, HRMS, X-ray). This material is available free of charge via the Internet at http://pubs.acs.org.

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