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Convenient synthesis of chiral lignin model compounds via optical resolution: four stereoisomers of guaiacylglycerol-β-guaiacyl ether and both enantiomers of 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxy-phenoxy)-propan-1-one (erone)

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ABSTRACT

Guaiacylglycerol-β-guaiacyl ether (GGE) is one of the most important phenolic compound for studying the chemistry and biochemistry of lignin. GGE contains two asymmetric carbons at the alpha and beta positions of its side chain; therefore, theoretically it can exist as four different stereoisomers. It has been proposed that a Gram-negative bacterium, *Sphingobium* sp. SYK-6 (formerly referred as *Sphingomonas paucimobilis* SYK-6), degrades GGE enantiospecifically via cleavage of the ether bond. The cleavage was thought to proceed in two steps, each catalyzed by a different enantiospecific enzyme. In the first step, the alcohol residue at the alpha position of the side chain in GGE was thought to be oxidized enantiospecifically by four distinct Cα-dehydrogenases (LigD, LigN, LigL and LigO), to produce two enantiomers of 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-propan-1-one (erone). To study this enantiospecific degradation step by the four dehydrogenases, we synthesized all four stereoisomers of GGE and both enantiomers of erone and separated them into enantiopure samples. The stereoisomers and our synthetic methods to prepare them are useful both for microbial and chemical investigations of lignin degradation.

Introduction

Lignin is a major component of vascular plants and one of the most abundant aromatic substances in nature. Lignin degradation by microbes is an essential process in the Earth's carbon cycle. It is known that the degradation of native lignin is initiated by the action of lignin peroxidase, manganese peroxidase, and laccase secreted by white rot fungi.[1] Bacteria definitely play an important role in the complete mineralization of the low-molecular-weight lignin-derived compounds in soil. Lignin consists of various intermolecular linkages between phenylpropane units and contains a number of asymmetric carbons.[2] The structural nature of lignin is thought to be racemic macromolecule, therefore, its side chain parts constructed by asymmetric carbons consist of both enantiomeric forms.[3] Microbes, especially bacteria, have a variety of stereospecific enzymes to degrade various stereoisomers of lignin substructures, but little is known about the relationship between the chirality of lignin molecules and microbial lignin catabolism.[4] Because the β-*O*-4 substructure is abundant (50–70%) in lignin, guaiacylglycerol-β-guaiacyl ether (GGE) has been mainly used for model studies on the microbial and chemical degradation of lignin. *Sphingobium* sp. SYK-6 (formerly referred as *Sphingomonas paucimobilis* SYK-6) can catabolize a wide variety of lignin-derived low molecular-weight phenolic compounds. In the catabolism of GGE, cleavage of the ether bond is the critical step for degradation, and our previous studies suggest that it proceeds enantiospecifically through two enzymatic reactions. In the first step, the alpha carbon of the GGE side chain is oxidized by four Cα-dehydrogenases (LigD, LigL, LigN, and LigO) to produce erone.[5] Then, the ether bond in erone is enantiospecifically cleaved by two different glutathione-S-transferases (LigE and LigF).[6] Conversion of GGE to erone was also thought to proceed in a stereospecific manner, with each Cα-dehydrogase acting on a single GGE stereoisomer. However, the definite stereospecificity of the enzymes remained unclear due to the unavailability of pure stereoisomers of GGE and erone. Here, we describe that preparation of the following enantiopure chiral compounds: four stereoisomers of GGE [(α*S*,β*R*)-*erythro*-GGE,

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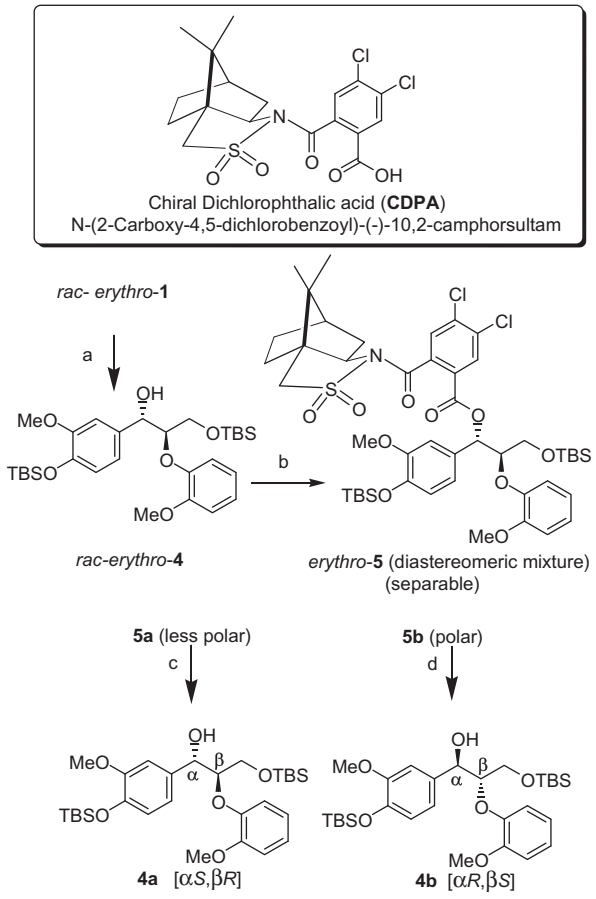
(αR,βR)-*threo*-GGE, (αR,βS)-*erythro*-GGE, and (αS,βS)-*threo*-GGE] and both enantiomers of erone [(βS)-erone and (βR)-erone]. In our recent study, using these compounds, we succeeded in proving the stereospecificity of four Cα-dehydrogases (LigD, Lig L, LigN, and LigO).[5]

Results and discussion

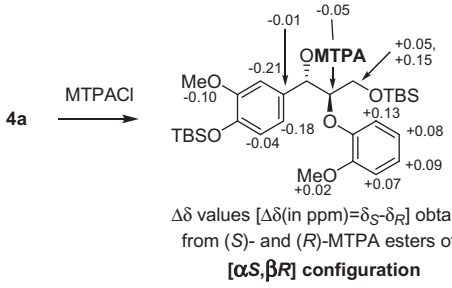
Synthetic methods to produce racemic lignin model compounds with β-*O*-4 linkage including GGE have been reported in previous studies.[7] In addition, several synthetic approaches to prepare chiral 8-*O*-4′ type neolignans, whose chemical structures are quite similar to GGE, have been reported.[8] However, stereoselective syntheses of GGE in enantiopure form and determinations of their stereochemistry have not been published to date. It was thought that the most obvious route to enantiopure GGE (accompanied with determination of its stereochemistry) would be optical resolution of racemic *erythro*-GGE. Furthermore, it was thought that the *erythro*-GGE **1** could be converted into *threo*-GGE and erone without inversion of stereochemistry at the β-carbon atom. To resolve racemic *erythro*-GGE, chiral diclorophthalicacid (CDPA) was selected as a chiral auxiliary (Scheme 1). CDPA is a powerful resolving agent for racemic alcohols.[9] We planned to convert the racemate into the diastereomeric mixture by condensing the chiral secondary alcohol at benzylic position of GGE **1** with CDPA.

The synthesis of the racemic *erythro*-GGE **1** commenced with acetovanilone in accordance with the previously described procedure.[7] After selective protection of phenolic hydroxyl group and primary hydroxyl group at γ-position of racemic *erythro*-GGE with *t*-butyldimethylsilyl (TBS) groups, the resulting benzylic alcohol **4** was esterified with CDPA in the presence of *N*,*N*′-dicyclohexylcarbodiimide and 4-dimethylaminopyridine. ^{1}H NMR measurements revealed that the obtained ester **5** was a ca. 1:1 mixture of diastereomers. High-performance thin-layer chromatography analysis of the mixture showed a clear difference between R_f values. The value of less polar moiety **5a** was 0.29 and that of the more polar moiety **5b** was 0.27, when hexane/ethyl acetate (5/1) was used as the eluent. Fortunately, the separation of the diastereomers was possible with a few repetitions of silica gel open column chromatography using above eluent.

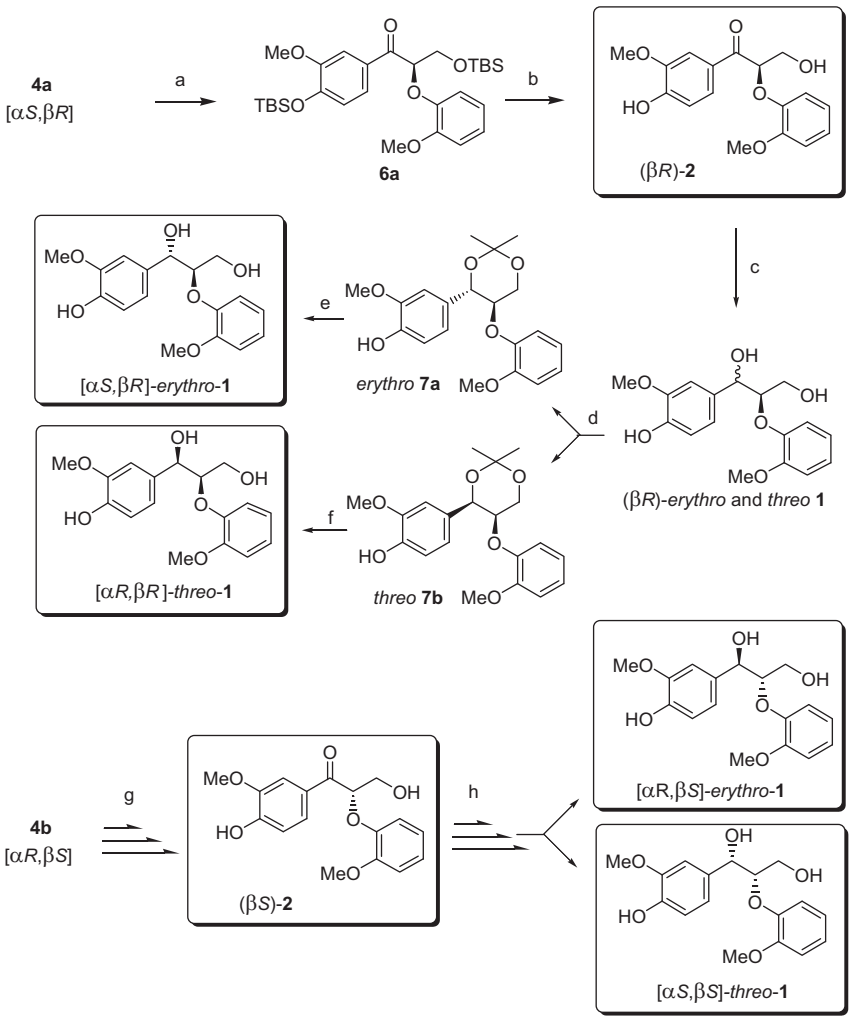
The absolute configuration of the less polar compound **5a** was determined by application of the modified Mosher's method.[10] Treatment of **4a**, which was obtained by removing the CDPA group of the less polar ester **5a**, with (*R*)-(−)- and (*S*)-(+)-2-methoxy-2-trifluoro-2-phenylacetyl chloride (MTPACl) gave the (*S*)- and (*R*)-MTPA esters of **4a**. The ^{1}H NMR data for each diastereomer were assigned by analysis of the ^{1}H–1H COSY spectra, and the chemical shift differences ($\Delta\delta = \delta_S - \delta_R$) were shown in Scheme 2. Δδ values for protons on the left side had negative signs, whereas positive signs were observed for protons on the right side, suggesting that C-α possessed an *S*-configuration. An irregular value was observed on C-β(−0.05); however, it was attributed to the anisotropic effect of aromatic ring, so it can be neglected in the determination of absolute configuration.[11] Given the already known relative configuration, the absolute configuration of **4a** was assigned as αS*β*R. Similarly, antipode **4b**, whose absolute configuration is αR*β*S, was obtained from **5b** by removing the CDPA group. Scheme 3 shows the synthetic steps to prepare enantiopure **2** and **1**.[12] For the synthesis of enantiopure **2**, (αS,βR)-*erythro*-**4a** was oxidized by tetra-*n*-propylammonium perruthenate in the presence of *N*-methylmorpholine-*N*-oxide and 4 Å molecular sieves, and the TBS groups of the obtained α-keto compound **6a** were removed to afford optically pure (βR)-**2**, whose optical rotation is (−), ($[\alpha]_D$ −28.5). Applying the same procedure to the enantiomer, (αR,βS)-*erythro*-**4b**, delivered the optically pure (βS)-**2**, whose optical rotation is (+), ($[\alpha]_D$ +27.9). Reduction of the benzylic ketone in (βR)-**2** with sodium borohydride gave a diastereomixture of (βR)-**1**[(αS,βR)-*erythro*-**1** and (αR,βR)-*threo*-**1**]. It was difficult to separate these isomers by silica gel column chromatography, so the 1,3-diol was converted by acetonization into six membered ring structures **7a** and **7b**, which enhanced the separation of these diastereomers by silica gel chromatography. After separation of the diastereomeric mixture, conformational analysis of both compounds were performed by NMR spectroscopy, revealing that the more polar acetonide, which had a small coupling constant (J = 2.0 Hz), corresponded to (αR,βR)-*threo*-**1**, whereas the less polar acetonide, which had a large coupling constant (J = 8.8 Hz), corresponded to (αS,βR)-*erythro*-**1**. Removal of the acetonide in **7a** and **7b** under acidic conditions[13] delivered the desired optically pure (αS,βR)-*erythro*-**1**, whose optical rotation is (−), ($[\alpha]_D$ −8.5), and optically pure (αR,βR)-*threo*-**1**, whose optical rotation is (−), ($[\alpha]_D$ −39.2).



Scheme 1. Resolution of (±)-*erythro*-GGE (**1**). Reagents and conditions: (a) TBSCl, imidazole 99%; (b) DCC, DMAP 75%; (c) K_2CO_3, MeOH, 100%; (d) K_2CO_3, MeOH 100%.



Scheme 2. Determination of stereochemistry of **4a** by advanced Mosher's method.



Scheme 3. Syntheses of both enantiomers of **2** and all four stereoisomers of **1**. Reagents and conditions: (a) TPAP, NMO, Ms 4 Å, 88%; (b) TBAF, THF 100%; (c) $NaBH_4$; (d) 2,2-DMP, PPTS 79% (2 steps) separable mixture (1:1); (e) 1 N-HCl, THF 89%; (f) 1 N-HCl, THF 93%; (g), (h) same procedure for **4a**.

Finally, applying the same procedure (β*S*)-**2** gave the enantiomers, optically pure (α*R*,β*S*)-*erythro*-**1**, whose optical rotation is (+), ($[\alpha]_D$ +8.7), and optically pure (α*S*,β*S*)-*threo*-**1**, whose optical rotation is (+), ($[\alpha]_D$ +43.8). The specific rotations of all six chiral compounds obtained in this study are summarized in Table 1.

Finally, the stereospecificities of the four recombinant Cα-dehydrogenases, LigD, LigL, LigN and LigO, were determined by chiral high-performance liquid chromatography with the enantiopure GGE stereoisomers that were synthesized in this study.[5] The results revealed that LigD and LigO converted (α*R*,β*S*)-GGE and (α*R*,β*R*)-GGE into (β*S*)-erone and (β*R*)-erone, respectively, whereas LigL and LigN transformed (α*S*,β*R*)-GGE and (α*S*,β*S*)-GGE to (β*R*)-erone and (β*S*)-erone, respectively. The biological reason for the enantiometric degradation of GGE in *Sphingobium* sp. SYK-6 is unclear, and we intend to investigate the same in our future work. The above chiral compounds and synthetic methods are useful for studies on stereospecific catabolism of lignin and neolignans and their reactivities during various chemical processes.

Table 1
Specific rotations of enantiopure GGE and erone

GGE		Erone	
Configuration	Specific rotation	Configuration	Specific rotation
Erythro [α*S*, β*R*]	$[\alpha]_D$ −8.5	[β*R*]	$[\alpha]_D$ −28.5
Erythro [α*R*, β*S*]	$[\alpha]_D$ +8.7		
Threo [α*R*, β*R*]	$[\alpha]_D$ −39.2	[β*S*]	$[\alpha]_D$ +27.9
Threo [α*S*, β*S*]	$[\alpha]_D$ +43.8		

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Supplementary data

Supplementary data (^1H and ^{13}C NMR spectra for the complete synthesis of the compounds) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.12.016.

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12. The both enantiomers of (α*S*,β*R*)-*erythro*-**1** and (α*R*,β*S*)-*erythro*-**1**, can be obtained directly, from **4a** and **4b** by standard TBS removal procedure (TBAF, THF rt) in quantitative yields.
13. Although the epimerization of α-hydroxyl group might be predicted during the acetonide removal of compounds **7a** and **7b** in acidic condition, the corresponding isomer (*threo*-**1** and *erythro*-**1**, respectively) was not detected by ^{1}H NMR analysis of crude reaction mixture.