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ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 48 (2007) 3255–3257

Substrate specificity and reaction mechanism of recombinant styrene oxide isomerase from *Pseudomonas putida* S12

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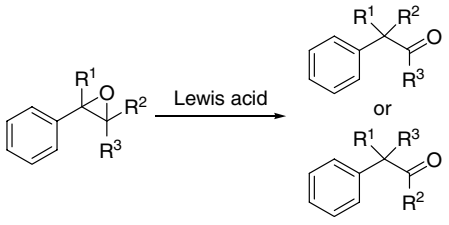
Received 23 February 2007; accepted 2 March 2007
Available online 12 March 2007

Abstract—To clarify the substrate specificity of the recombinant styrene oxide isomerase, various epoxides were subjected to the reaction. From the substituent effect on the rate of isomerization, the mechanism of the isomerase catalyzed reaction was estimated.

Epoxides are important and useful compounds in organic synthesis since they can be transformed into compounds with a variety of functional groups. One of the useful synthetic routes to aldehydes and ketones from epoxides is the Meinwald rearrangement[1] (Scheme 1). The reaction can be carried out in the presence of various Lewis acids (e.g., $BF_3{\cdot}Et_2O$,[2] $MgBr_2$,[2] aluminum-catalyst,[2] palladium species,[3] bismuth-catalyst[4] and $IrCl_3$[5]).

Styrene oxide isomerase (SOI) [EC 5.3.99.7] is the key enzyme in the metabolic pathway of styrene and catalyzes the Meinwald rearrangement type isomerization of styrene oxide to phenylacetaldehyde[6] (Scheme 2). It has been believed that SOI exhibits strict substrate specificity, and does not act on the epoxides other than styrene oxide[7] and indene oxide.[7] Thus, the application of SOI to the production of various aldehydes and ketones has not been reported. In this Letter, we would like to report the investigation to clarify the substrate specificity and the possible reaction mechanism of SOI.



Scheme 1. Meinwald rearrangement.

The styrene oxide isomerase gene (*styC*) was amplified by PCR using genomic DNA from *Pseudomonas putida* S12 (ATCC 700801) as the template, which was isolated by Hartmans et al.[8] as a styrene-degrading microorganism. As the *styC* gene of the strain was not sequenced, PCR primers were designed based on the reported sequences of *styC* from *Pseudomonas* sp. VBL120.[9] The forward and reverse primers used for the amplification were 5′-CCG*GAATTC*ATGCTTCATGCCTTCGAACGCAAAATGGCC-3′ and 5′-CCG*GAATTC*TCATTCCGCAGCAGCGTGCGGAACAGCTTT-3′, respectively. The primers were designed to introduce *Eco*R I sites (italics) at the 5′-end and 3′-end of the amplified *styC* genes. The amplified genes were then ligated into the *Eco*RI restriction sites in the multiple cloning site of pUC19, and the resulting recombinant plasmids (pStyC-S12) were introduced into *E. coli* JM109. The DNA sequence of *styC* from *P. putida* S12 was completely the same with that of *Pseudomonas* sp. VBL120. We then used cell-free extract of *E. coli* JM109 (pStyC-S12) to perform the biotransformation experiments of a series of epoxides. The reaction system consisted of 50 μl of cell-free extract (about 1 unit), 6 μmol of various epoxides (200 mM in dioxane), and 200 μM potassium phosphate buffer (pH 7.0) in a total volume of 1.0 ml. After incubation at 25 °C, 6 M HCl (50 μl) and 20 μl of 300 mM *m*-dimethoxybenzene in dioxane were added to the reaction mixture. The

Keywords: Styrene oxide; Isomerase; *Pseudomonas*; Substrate specificity.
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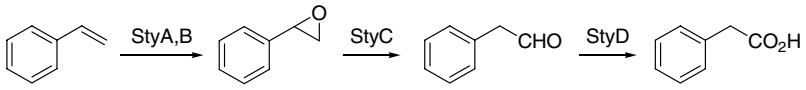
doi:10.1016/j.tetlet.2007.03.016



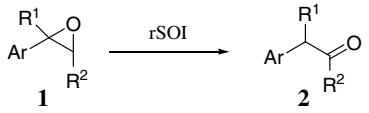
Scheme 2. Styrene metabolic pathway. StyA: Styrene monooxygenase large component, StyB: Styrene monooxygenase small component, StyC: Styrene oxide isomerase, StyD: Phenylacetaldehyde dehydrogenase.

remaining epoxides were hydrolyzed to glycols under an acidic condition, and products (aldehydes or ketones) were measured by gas chromatography (GC) using *m*-dimethoxybenzene as the internal standard.

To clarify the substrate specificity of the recombinant styrene oxide isomerase (rSOI) for various epoxides, the isomerase activities were determined and expressed relative to that of styrene oxide taken as 100%. As shown in Table 1, when R^1 group was changed to methyl (entry 2) and ethyl (entry 3), rSOI gave 2-phenylpropanal (**2b**, relative reactivity; 15%) and 2-phenylbutanal (**2c**, 7.6%), respectively. Although the reactivity of the epoxides (**1b** and **1c**) decreased compared to that of styrene oxide (**1a**), these are the first observation of the reactions of substituted styrene oxides. The lower reactivity must be due to the difference of the steric bulkiness of R^1 groups. Next, the effect of variation of aromatic part (Ar) was examined. The isomerase acted on 4-methylstyrene oxide (**1d**, 113%) and 4-chlorostyrene oxide (**1e**, 3.7%). The electron-donating substituent (entry 4) accelerated the rate of the reaction. In contrast, electron-withdrawing group (entry 5) reduced the rate. Recombinant SOI also acted on cyclic compounds, such as indene oxide (**1f**, 45%) and 3,4-dihydronaphthalene oxide (**1g**, 0.5%). On the other hand, the enzyme did not act on *cis* and *trans*-β-methylstyrene oxide ($R^1 = H$, $R^2 = CH_3$). It can be deduced that steric hindrance of R^2 group gave a critical effect on rSOI. To confirm the stereoselectivity of rSOI, the enzymatic reaction was performed using optically active styrene oxide. The enzyme converted (*S*)-styrene oxide two times faster compared to (*R*)-one (49% activity, relative to that of (*S*)-styrene oxide taken as 100%). This result is in agreement with the Nöthe's report.[10]

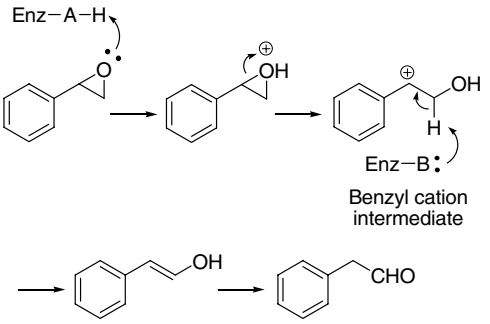
From the substituent effect on the rate of isomerization, it is supposed that the C–O bond cleavage of benzylic position forms the carbonium ion intermediate, which was stabilized by the resonance effect of the benzene ring (Scheme 3). The fact that the rates of isomerization of the compounds with an electron-donating substituent

Table 1. Substrate specificity of recombinant styrene oxide isomerase



Entry	Epoxide	Product	Relative activity[a] (%)
1	(**1a**)	(**2a**)	100
2	(**1b**)	(**2b**)	15
3	(**1c**)	(**2c**)	7.6
4	(**1d**)	(**2d**)	113
5	(**1e**)	(**2e**)	3.7
6	(**1f**)	(**2f**)	45
7	(**1g**)	(**2g**)	0.5

[a] The isomerase activities were determined and expressed relative to that of styrene oxide taken as 100.



Scheme 3. Estimated reaction mechanism of styrene oxide isomerase.

on the benzene ring was faster than that of the non-substituted compound supports the estimated reaction mechanism. The cationic intermediate is converted to the enol-type compound via the abstraction of a proton followed by keto–enol tautomerization to give the observed product, phenylacetaldehyde.

In conclusion, we have succeeded to demonstrate for the first time that styrene oxide isomerase is a valuable catalyst for the isomerization of various epoxides to the corresponding carbonyl compounds, although the enantioselectivity is not sufficiently high. The selectivity is expected to be improved by the introduction of mutations.[11] To this end, purification of the enzyme and cloning of the corresponding gene are required as well as to clarify the detail reaction mechanism.

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