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ENANTIOSELECTIVE MICROBIAL HYDROLYSIS OF DISUBSTITUTED CYCLIC CARBONATES

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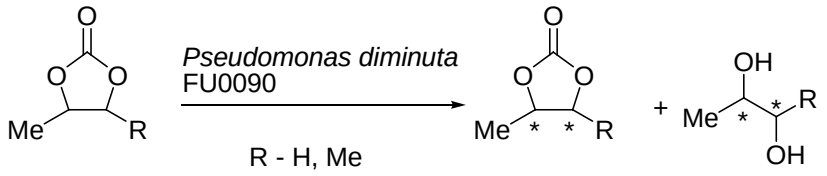
Abstract – *Pseudomonas diminuta* (FU0090), a bacterium, efficiently catalyzes the hydrolysis of five-membered cyclic carbonates bearing two substituents, which are methyl and another groups to give the corresponding optically active 1,2-diols.

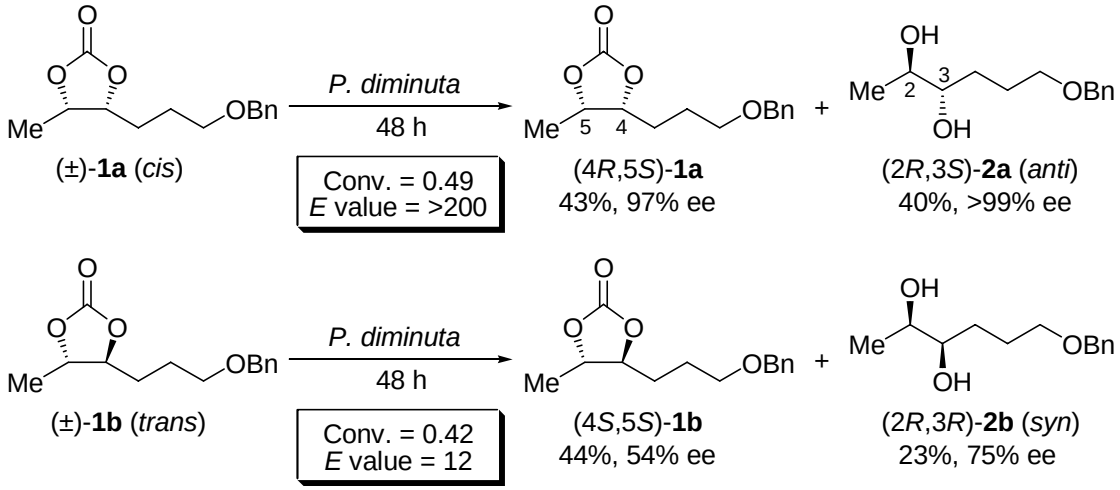
Optically active 1,2-diols are important intermediates for the synthesis of natural products, and many synthetic procedures for such compounds have been developed. Although the asymmetric dihydroxylation of olefins is one of the most popular ways to prepare chiral diols,[1] this method does not always satisfactorily work in terms of the enantioselectivity in some cases. For example, the oxidation of (*Z*)-disubstituted olefins is not a suitable tool for the preparation of optically active *anti*-1,2-diols.[2]
Enzymatic hydrolysis of diacetates and esterification of diols are the representative biochemical methods to prepare such compounds.[3] However, the reactions produce a mixture of more than two compounds (diol, diacetate, and two monoacetates) which causes difficulty with the purification. Recently, the kinetic resolution of cyclic carbonates with hydrolytic enzymes is one of the attractive methods for preparing of optically active diols.[4] We have already reported the enzyme-mediated hydrolysis of cyclic carbonates, and have accomplished the efficient preparation of various kinds of optically active diols.[5,6] Porcine pancreas lipase (PPL, Type II from Sigma) catalyzes the hydrolysis of monosubstituted cyclic carbonates to afford the corresponding optically active 1,2-diols.[5] On the other hand, *Pseudomonas diminuta* (FU0090), which is a bacterium isolated from a soil and classified by NCIMB Japan Co., Ltd., hydrolyzes



Scheme 1

the C_2-symmetrical five-membered cyclic carbonate with a dimethyl group, and then the resulting (*R*,*R*)-2,3-butanediol and the unreacted (*S*,*S*)-substrate were obtained.[6] The enzyme, however, has a high substrate specificity for the side chain of the substrate, and the reaction of the substrate bearing a diethyl group was not hydrolyzed at all. During our studies on this microbial reaction, we observed that even disubstituted substrates could be enantioselectively hydrolyzed when one of the substitutions was a methyl group. In this report, we tried to apply the microbial hydrolysis to various five-membered *cis*- and *trans*-cyclic carbonates bearing a methyl and another group, and then prepared the corresponding optically active 1,2-diols with two chiral centers (Scheme 1). In particular, the optically pure *anti*-1,2-diols were obtained from the *cis*-substrates.

We selected the racemic diastereomers of the *cis*- and *trans*-4-(3-benzyloxy)propyl-5-methyl-1,3-dioxolan-2-ones ((±)-**1a** and (±)-**1b**, respectively) as the representative substrates.[7] A typical experimental procedure of the microbial reactions is as follows. Fifty mL of sterilized nutrient medium (10% glucose, 7% peptone and 5% yeast extract in 0.1 M phosphate buffer (pH 6.5)) was inoculated with *P. diminuta* and incubated for 48 h at 30 °C. To the suspension of grown cells, 10 mM of the substrate (ca. 84 mg) was added and the incubation was continued for several days. Extraction of the broth with ethyl acetate followed by purification by column chromatography on silica gel gave the products. In these cases, *P. diminuta* catalyzed the hydrolysis of both substrates to give

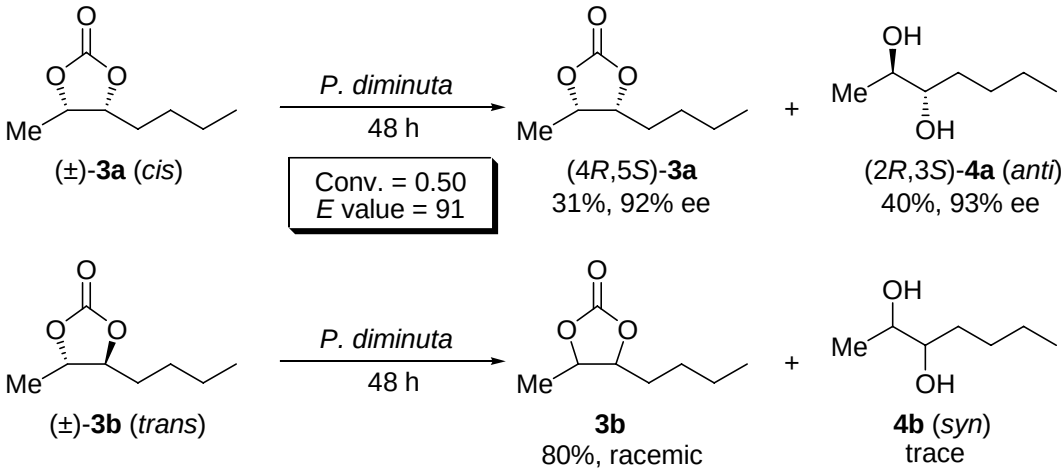


Scheme 2

the corresponding diols (**2a**) and (**2b**) (Scheme 2). Although, in the case of the *trans*-form substrate (±)-(**1b**), (*4R*,*5R*)-**1b** was preferentially hydrolyzed to give the (*2R*,*3R*)-6-benzyloxyhexan-2,3-diol (**2b**), the hydrolysis proceeded with moderate enantioselectivity, and the conversion and *E* value[8] of the reaction for 48 h were 0.42 and 12, respectively.[9] To determine the stereochemistry of the resulting (2*R*,3*R*)-**2b**, the sign of the optical rotation ($[\alpha]_D^{22}$ = +11.5 (c 0.72, MeOH), 75% ee) was compared with that of the authentic sample (2*S*,3*S*)-(**2b**)($[\alpha]_D^{27}$ = –13.8 (c 1.16, MeOH), 77% ee), which was transformed from (*E*)-6-benzyloxy-2-hexene with AD-mix-α and $CH_3SO_2NH_2$ in *t*-BuOH-H_2O.[1]

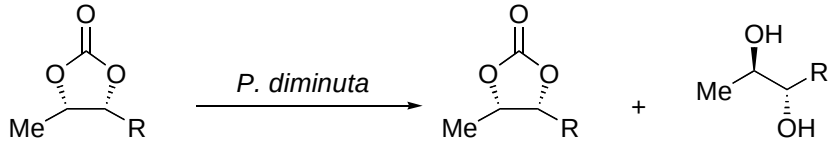
Interestingly, the enantioselectivity of the hydrolysis of the *cis*-substrate (±)-(**1a**) was almost perfect, and the reaction for 48 h gave the remaining cyclic carbonate (*4R*,*5S*)-(**1a**)(97% ee) in 43% yield, $[\alpha]_D^{26}$ = +10.9 (c 1.68, MeOH), and the resulting optically pure diol (*2R*,*3S*)-(**2a**) in 40% yield, $[\alpha]_D^{25}$ = –14.0 (c 2.54, MeOH) (conv. = 0.49, *E* value = >200).[10,11] Although we have already reported the hydrolysis of the *meso*-carbonate bearing a dimethyl group as the symmetrical substrate, this is the first example of the enantioselective hydrolysis of the *cis*-disubstituted carbonates. It is noteworthy that asymmetric dihydroxylation of (*Z*)-6-benzyloxy-2-hexene with AD-mix-α procceded with very low enantioselectivity to give (*2R*,*3S*)-**2a** in only 18% ee. These results show that the microbial hydrolysis apparently has some advantage for the preparation of optically active *anti*-1,2-diol with high ee.

The difference in the reactivity between the diastereomers is noticeably observed during the hydrolysis of the substrates bearing a butyl group as a substituent ((±)-**3** in Scheme 3). While the *trans*-isomer (±)-(**3b**) was scarcely hydrolyzed at all, the hydrolysis of the *cis*-substrate (±)-(**3a**) smoothly proceeded to give optically active compounds, (*4R*,*5S*)-(**3a)** (92% ee) in 31% and (2*R*,3*S*)-(**4a**) (93% ee) in 40 % yields (reaction for 48 h; conv. = 0.50, *E* value = 91).



Scheme 3

Table 1. Microbial hydrolysis of several *cis*-cyclic carbonates[a]



R	time/h	carbonate			diol			conv.[b]	*E* value[c]
			yield/%	ee/%		yield/%	ee/%		
3-butenyl	48	**5**	46	63	**8**	42	95	0.40	75
pentyl	96	**6**	77	18	**9**	14	>99	0.15	>200
heptyl	96	**7**	84	13	**10**	11	>99	0.12	>200

[a]The reaction was performed using 10 mM of the substrate.
[b]Calculated by ee(carbonate)/[ee(carbonate)+ee(diol)].
[c]Calculated by ln[(1-conv.)(1-ee(carbonate))]/ln[(1-conv.)(1+ee(carbonate))].

Then, we focused on the examination of the microbial reaction using several *cis*-form cyclic carbonates, and these results are summarized in Table 1. As expected, all substrates were hydrolyzed with high enantioselectivity. The reaction of the substrate bearing an unsaturated substituent (R = 3-butenyl, (±)-**5**, entry 1) gave a result similar of that of (±)-**3a**. The hydrolysis of (±)-**5** for 48 h smoothly proceeded to afford the optically active (*4R*,*5S*)-**5** (63% ee) and (2*R*,3*S*)-**8** (95% ee) in 46% and 42% yields, respectively (conv. = 0.40, *E* value = 75). The optically active *anti*-diol is an important precursor for the synthesis of a biologically active deoxysugar, D-amicetose.[12] The substrates (±)-(**6**)(R = pentyl) and (**7**)(R = heptyl) bearing a longer chain were hydrolyzed with further excellent enantioselectivities, and the *E* values were over 200. For the reactions going 96 h, the resulting (2*R*,3*S*)-**9** and **10** were obtained in their optically pure forms, although the reactions proceeded very slowly.

Based on all of our observations, we can formulate an empirical rule for predicting the enantioselectivity in this microbial reaction as shown in Figure 1. First, a methyl group at C-5 position of the substrates is necessary for the enantioselective reaction because we have found that monosubstituted cyclic carbonates (R^1 = H), such as 4-(2-benzyloxy)ethyl-1,3-dioxolan-2-one (**11**) and 4-methyl-1,3-dioxolan-2-one (**12**) in Figure 2, were smoothly hydrolyzed without enantioselectivity. Second, the enzyme prefers

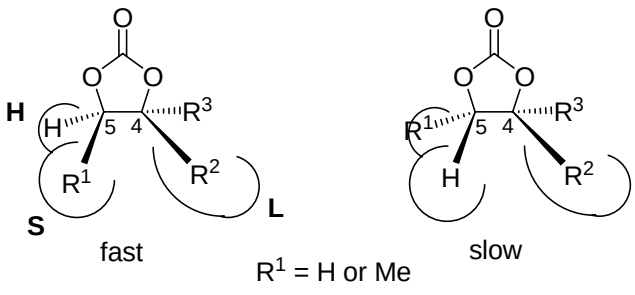


Figure 1

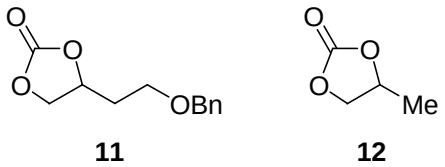


Figure 2

(5*R*)-substrates as well as in the reaction of the C_2-symmetrical substrate with a dimethyl group.[6] These results indicate that the enzyme apparently distinguishes the stereochemistry at the C-5 position substituted with a methyl group and the *cis*-(5*R*)-substrate is most suitable for the active site of the enzyme (R^1 = Me, R^2 = alkyl, R^3 =H). In the case of the fast reactive enantiomer, the methyl group would locate at the S (small)-pocket, with hydrogen in H-site, and with R^2 group in L (large)-pocket. In the reaction of *trans*-substrates, the elongation of the substituent (R^3) at the C-4 position decreases both the reactivity and the enantioselectivity. Because the introduction of a benzyloxy group on the side chain could improve the reactivity, the oxygen atom of the substrates could play an important role for the interaction between the substrates and the enzyme.

In conclusion, we have established the microbial enantioselective hydrolysis of cyclic carbonates bearing two substituents, which are a methyl and another group, as a new route to optically active diols. In particular, this is the first report for the enantioselective hydrolysis of *cis*-cyclic carbonates, which are favorably hydrolyzed with enantioselectivity to give the corresponding optically pure *anti*-diols. Further investigations for applying the method and the study on the mechanism are now in progress.

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9. The ee of (2*R*,3*R*)-**2b** was determined by HPLC analysis of the corresponding bis-(+)-MTPA ester. Conditions of the HPLC analysis: column, Zorbax-Sil (0.46 mm x 25 cm, DuPont Instruments); eluent, hexane/AcOEt = 90/10; flow rate, 0.5 mL/min; retention time, 36 (2*S*,3*S*) and 39 (2*R*,3*R*) min. To determine the ee of (4*S*,5*S*)-**1b**, the cyclic carbonate was hydrolyzed with K_2CO_3 to afford the corresponding (2*S*,3*S*)-**2b**.
10. To determine the stereochemistry of the resulting (2*R*,3*S*)-**2a**, the sign of the optical rotation was compared to that of the authentic (2*S*,3*R*)-**2a**, $[\alpha]_D^{25}$ = +12.9 (c 1.34, MeOH). The authentic sample was stereoselectively synthesized from ethyl L-lactate in 7 steps. The details will be reported elsewhere.
11. The ee of (2*R*,3*S*)-**2a** was determined by HPLC analysis of the corresponding bis-(+)-MTPA ester. Conditions of the HPLC analysis: column, Zorbax-Sil (0.46 mm x 25 cm, DuPont Instruments); eluent, hexane/AcOEt = 90/10; flow rate, 0.5 mL/min; retention time, 30 (2*R*,3*S*) and 32 (2*S*,3*R*) min. To determine the ee of (4*R*,5*S*)-**1a**, the cyclic carbonate was hydrolyzed with K_2CO_3 to afford the corresponding (2*S*,3*R*)-**2a**.
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