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Remarkable Effect of D-Sorbitol on the Second Stage in the PLE-Catalyzed Hydrolyses of a σ-Symmetric Diester, *cis*-Cyclohex-4-ene-1,2-bis(methyl acetate)

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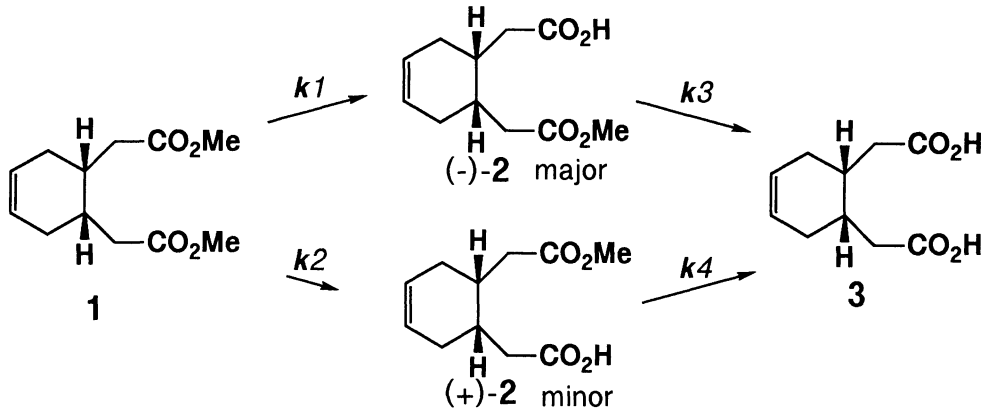
Enantioselective hydrolysis of *cis*-cyclohex-4-ene-1,2-bis(methyl acetate) with porcine liver esterase (PLE) in the presence of D-sorbitol afforded a chiral monoester in a highly enantioselective manner. During the enzymatic hydrolyses, D-sorbitol efficiently accelerate the second stage; hydrolysis of the resultant minor monoester toward the dicarboxylic acid leaving the desired major monoester.

As an improvement technique for the low enantioselectivities of enzymatic hydrolysis of the prochiral dicarboxylic diesters, exploitation of organic solvents and additives seems to be promising.[1] Previously, Sih *et al.* reported an optical purity enhancement of monoesters using esterases [porcine liver esterase (PLE) and porcine pancreatic lipase] of low to moderate stereoselectivity for enantioselective hydrolysis of *meso* diol diesters.[2] This attractive procedure should be constituted of the consecutive enantioselective hydrolyses; hydrolysis of diester toward its chiral monoester ("first stage") followed by further hydrolysis of the resultant minor monoester toward the achiral diol leaving the major monoester ("second stage"). Thus, we anticipated that efficient artificial rate control of the first and/or second stage(s) during the consecutive hydrolytic process of a dicarboxylic diester might be possible by exploiting suitable additives into an enzymatic reaction system.

Herein we report the first example of the additive effect on the second stage in the PLE-catalyzed hydrolyses of a *meso* dicarboxylic diester involving the optical purity enhancement of the resultant monoester.

Enzymatic hydrolyses[3] of *cis*-cyclohex-4-ene-1,2-bis(methyl acetate) **1** (1 mmol) with PLE (Sigma Type I, 800 units) at 24 °C in 0.1 M phosphate buffer solution (pH 7.5, 30 mL) were carried out in the presence of several additive compounds (1-40 mol equiv. to **1**) such as D-sorbitol, β-cyclodextrin, D-glucosamine hydrochloride, *n*-octyl-β-D-thioglucoside, diglyme, H_3BO_3, and acetone or without any additive. Among several compounds, D-sorbitol (40 mol equiv.) was proved to be most effective in order to obtain monoester (-)-**2** in a highly enantioselective

manner. The chemical yield of (-)-2 decreased in inverse proportion to the rise of its enantiomeric excess (e.e.) value and reaction time. This expected phenomenon might be rationalized in terms of promoting the second stage in the consecutive PLE-catalyzed hydrolysis process. It is intriguing to learn how D-sorbitol is involved in the kinetics of the PLE-catalyzed hydrolyses of diester **1**. Thus, the rate constants[4] of all enzymatic hydrolysis stages were defined to be *k1*, *k2*, *k3*, and *k4* as shown in Scheme 1 and then the hydrolyses were examined in detail.



Scheme 1.

First, we attempted to plot chemical yields (%) of diester **1**, monoesters (-)-2 and (+)-2, and dicarboxylic acid **3** as well as e.e. value of (-)-2 as a function of the reaction time under similar conditions described above in the absence and presence of D-sorbitol (40 mol equiv. to **1**). All results are depicted in Fig. 1. Because it was documented that esterase-catalyzed hydrolysis of

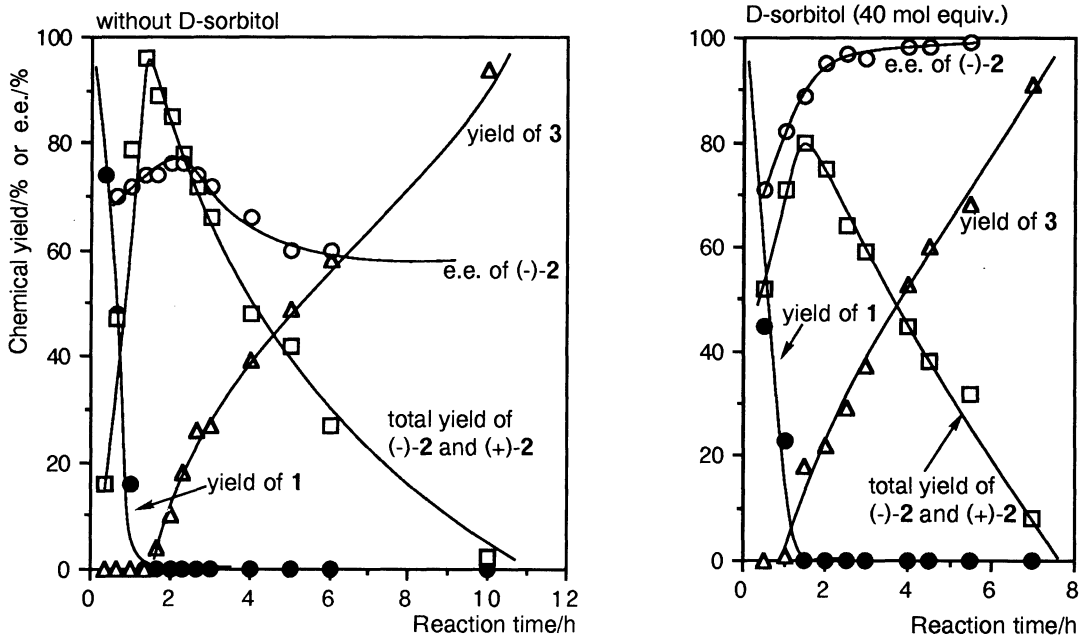


Fig. 1. Time course of PLE-catalyzed hydrolyses of dimethyl ester **1**.

dicarboxylic diesters, in general, terminates at the corresponding monoester stage,[5] hydrolysis of diester **1** with PLE seems to be interesting. The resultant monoesters (-)-**2** and (+)-**2** readily undergo further hydrolysis to give the dicarboxylic acid **3** without regard to the presence or absence of D-sorbitol. In the case without D-sorbitol, the e.e. value of (-)-**2** does not increase in proportion to the rise of chemical yield of dicarboxylic acid **3**. However, when D-sorbitol is present, the e.e. value of (-)-**2** increases obviously in proportion to the rise of production of **3**.

Subsequently, the chemical yields of monoesters (-)-**2** and (+)-**2** as a function of the reaction time were plotted in a semi-logarithm manner to understand more clearly the difference due to configuration in the kinetics of their enzymatic hydrolyses between the case with and without D-sorbitol. As shown in Fig. 2, the concentration of (-)-**2** and (+)-**2** decreases in a pseudo first order mode, respectively, after the first hydrolysis of **1** was terminated.[4] The slope of each line means the rate constant *k3* or *k4* of the further hydrolysis of each corresponding monoester (-)-**2** or (+)-**2**. The kinetic parameters involving the ratio *k1/k2* or *k4/k3* were also calculated (Table 1). The rate constant *k3* is almost same under both reaction conditions with and without D-sorbitol. On the other hand, the *k4* value with 40 mol equiv. of D-

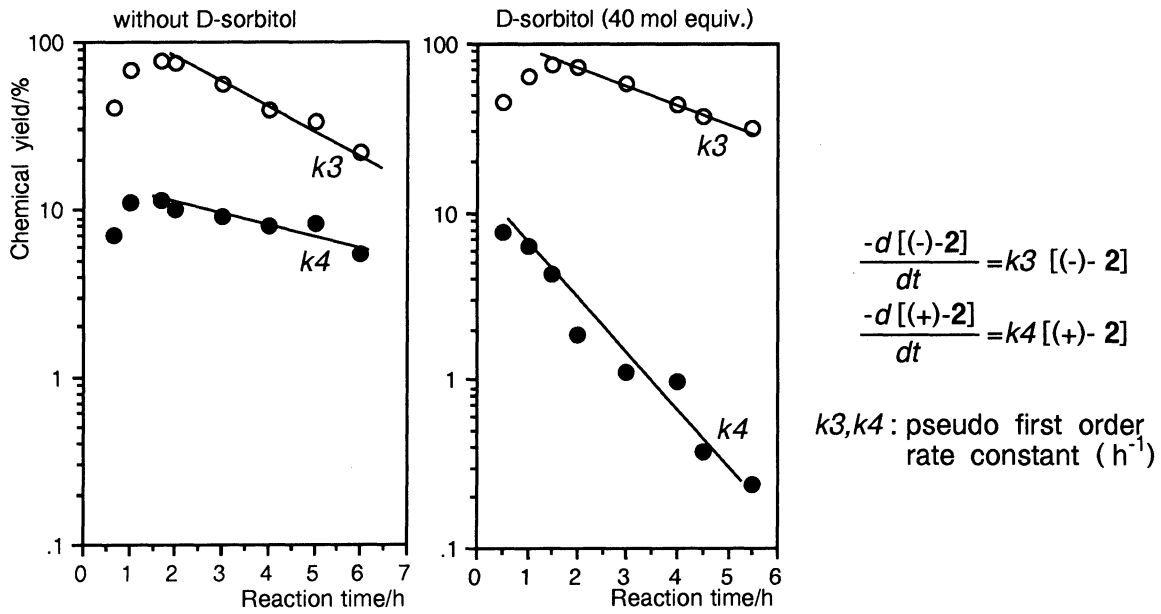


Fig. 2. Kinetic analysis of PLE-catalyzed hydrolyses.

Table 1. Kinetic parameters

Additive	*k1 / k2* [a]	*k4 / k3* [b]
none	5.71	0.75
D-sorbitol (20 mol equiv.)	5.21	3.48
D-sorbitol (40 mol equiv.)	5.85	3.82

a) $k1/k2 = \frac{100+\text{e.e.}}{100-\text{e.e.}}$ (at the initial stage of the reaction)

b) Caluculated from the slopes in Fig. 2.

sorbitol exhibited ca. four times of that without one. Although the ratio $k1/k2$ value does not change between both conditions, the ratio $k4/k3$ in the presence of D-sorbitol increased remarkably to ca. five times of that without D-sorbitol. Consequently, we can virtually obtain monoester (-)-**2** (94% e.e. and 70% yield) useful for asymmetric synthesis of (+)-carbacyclin, (+)-isocarbacyclin, monoterpenoids, and other natural products.[6]

Thus, we demonstrated that D-sorbitol accelerates enantioselectively the second stage ($k4$ stage) in the PLE-catalyzed hydrolysis of **1**. Generalization for this additive effect or the esterase-catalyzed hydrolysis of various prochiral σ-symmetric dicarboxylic diesters is now undertaken.

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4) The stage to form enzyme-substrate complex is included in the rate constant in this report.

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