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Enantioselectivity and diastereoselectivity in the transesterification of secondary alcohols mediated by feruloyl esterase from *Humicola insolens*

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Abstract—Examination of the α and β selectivity of Ferrulic acid esterase (FAE) towards secondary alcohols which contain two stereocenters, at the α and β carbons, reveals that the factor governing the order is the R configuration of the stereogenic center which bears the alcoholic group.

Ferulic acid esterases[1] (FAE), whose physiological role is to hydrolyze ester linkage between hydroxycinnamic acids and sugars,[2] are a subclass of the carboxylic acid esterase (EC 3.1.1.1). Their active site and reaction mechanism are thought to be identical to those of carboxylic acid esterases, as deduced by crystal structure analysis.[3,4] These studies have shown that feruloyl esterases hydrolyze the esters via an acyl–enzyme intermediate, which is a well-known mechanism for serine hydrolases.[5] Despite their similarities with lipases, ferulic acid esterases do not show lipase activity[6] and exhibit high specificity for hydroxycinnamic acid esters.[7]

Recently,[8] we showed that feruloyl esterase from *Humicola insolens* catalyzes with good to excellent enantioselectivity the transesterification of secondary alcohols, which are substrates that bear no structural similarity to the natural substrates of this enzyme. The observed enantiopreference of FAE was *R*.

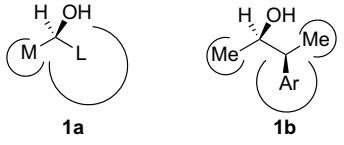

Figure 1.

Keywords: Feruloyl esterase; Enantioselectivity; Diastereoselectivity; Secondary alcohols.
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Numerous studies[9] have shown that the resolution of secondary alcohols catalyzed by lipases and esterases is based on the relative size of the substituents at the stereocenter (Fig. 1a), and their stereopreference can be predicted[10] by the 'Kazlauskas model', which has been validated experimentally. Although this model predicts accurately the stereoselectivity of a lipase towards alcohols containing one stereocenter (α-carbon), it does not include alcohols with two neighboring stereocenters (α and β). Elaboration of this rule to include the β-stereocenter has been done recently[11] (Figure 1b).

In our previous publication[12] we reported the effect of the β-stereocenter in the hydrolysis by *Candida rugosa lipase* (CRL) of acetates of secondary alcohols, both the enantio- and the diastereoselectivity. Our results were incorporated by Schmid in the proposal of his model.

In this work we have examined the α and β selectivity of Ferrulic acid esterase (FAE) towards secondary alcohols, which contain two stereocenters, at the α and β carbons. For this purpose we synthesized a 50:50 threo/erythro mixture of 3-phenyl-2-butanol (**1**) and a 65:35 threo/erythro mixture of 3-*m*-tolyl-2-butanol (**2**) and studied their enzymatic transesterifications with the activated acyl donor vinyl acetate (Fig. 2). Enantio- and diastereoselectivities of the transesterifications of the alcohols **1** and **2** were determined by gas chromatographic analysis of the reaction mixture by using a permethylated β-cyclodextrin 30×0.25 mm, chiral column. In the reaction of substrate **2** decaline was used as the internal standard for the calculation of the

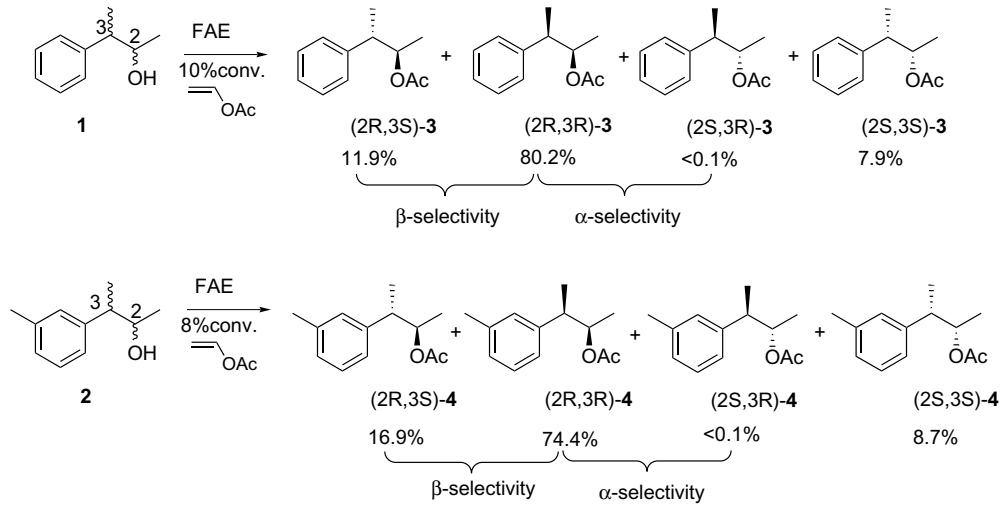

Figure 2.

Table 1. Asymmetric enzymatic transesterification of substrates **1** and **2** with FAE

Substrate	Time (days)	Conversion (%)	α-Selectivity (% ee)	β-selectivity (% ee)	Diastereoselectivity (% de)
1	3	10	>95	74	76
2	3	9	>95	66	66

enantio-selectivity and diastereoselectivity. The results of the enzymatic reactions are summarized in Table 1 and the chromatographic data in Figure 3.

The α-selectivity was determined from the relative amounts of the produced isomers (2*R*,3*R*)-**3**, (2*S*,3*R*)-**3** and (2*R*,3*R*)-**4**, (2*S*,3*R*)-**4**, coming from the reactions of substrates **1** and **2**, respectively. For example, the >95% value from substrate **1** was calculated as follows:

$$\left(\frac{[RR]-[SR]}{[RR]+[SR]}100\%\right).$$

Analogously was calculated and the >95% from substrate **2**. The β-selectivity was calculated from the relative amounts of the (2*R*,3*R*)-**3** (2*R*,3*S*)-**3** and (2*R*,3*R*)-**4** (2*R*,3*S*)-**4** isomers. For example, the 74% ee was calculated as follows:

$$\left(\frac{[RR]-[RS]}{[RR]+[RS]}100\%\right).$$

The diastereoselectivities of 76% de and 66% de—with erythro stereochemistry—for substrates **1** and **2**, respectively, were calculated as follows:

$$\left(\frac{([RR]+[SS])-([RS]+[SR])}{([RR]+[SS])+([RS]+[SR])}100\%\right).$$

The observed *R* stereoselectivity[8] of the α-stereocenter, for each individual diastereoisomer, is in accord with the proposed model by Kazlauskas[10] for the faster reacting enantiomer in the lipase catalyzed transesterification of secondary alcohols. As shown in Figure 1, for substrate **1**, the (*R*,*R*) stereoisomer reacts the fastest, followed by (*R*,*S*) and (*S*,*S*), which show small differences in reactivity, and finally by the (*S*,*R*) stereoisomer. Substrate **2** shows the same reactivity pattern with a small decrease

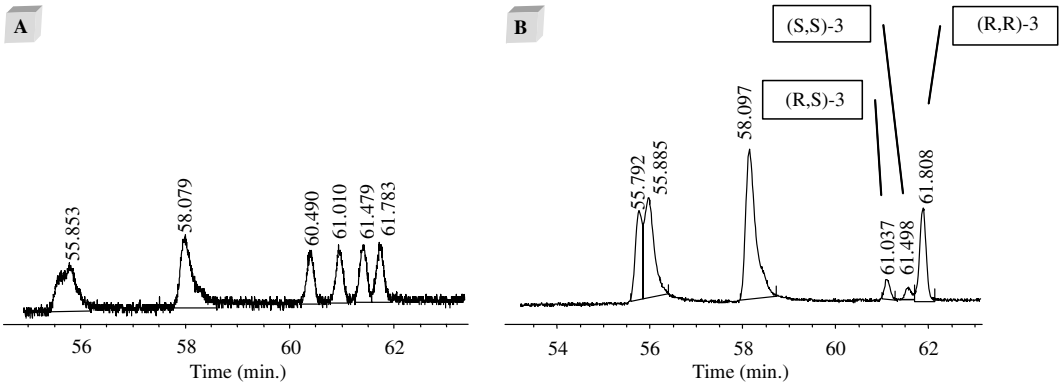

Figure 3. (A) GC analysis of the four diastereomeric acetates **3**. (B) Determination of the enantioselectivity and diastereoselectivity of the enzymatic transesterification using a permethylated β-cyclodextrin 30×0.25 mm, chiral column. The retention time of each individual stereoisomer was assigned by comparison with the corresponding esters **3** and **4** coming from the enzymatic reaction with CRL.[12]

in selectivity. Analogous studies performed with *Pseudomonas* fluorescence lipase (PFL)[13] for substrate **1** revealed that the reactivity pattern was $(R,S) > (R,R) > (S,S) > (S,R)$, that is the (R,S) isomer reacts faster than the (R,R) isomer rather than slower. Our recent studies[12] with CRL for substrates **1** and **2** proved the reactivity order to be $(R,R) > (S,S) > (R,S) > (S,R)$, a pattern that is similar to that of FAE, not PFL.

In conclusion, our results clearly demonstrate that the factor governing the order of reactivity in FAE catalyzed transesterifications of secondary alcohols is the *R* configuration of the stereogenic center, which bears the alcoholic group. The observed diastereoselectivity as well as the reactivity pattern can be accurately predicted by the model proposed by Pleiss and collaborators.[11]

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