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A SUBSTRATE MODEL FOR THE ENZYMATIC RESOLUTION OF ESTERS OF BICYCLIC ALCOHOLS BY CANDIDA CYLINDRACEA LIPASE

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Abstract - By evaluation of results obtained from enzymatic resolution of twenty five structurally different esters of secondary alcohols possessing a bicyclo[2.2.1]heptane or bicyclo[2.2.2]octane framework a model was developed which is proposed as an aid for the design of substrates to obtain good acceptance and high enantioselection by *Candida cylindracea* lipase.

INTRODUCTION

In recent years chemoenzymatic methodologies employing hydrolytic enzymes have become of increasing importance for the synthesis of optically active building blocks[1-7]. From the large number of esterases, proteases and lipases hitherto explored, some emerged by possessing the desired properties: A high specificity for asymmetric features and low restrictions for the substrate structure as a whole. Within the group of enzymes which meet these criteria, α-chymotrypsin[8], pig liver esterase[9] and lipases from porcine pancreas[10] and from *Candida cylindracea*[11] probably caused the broadest impact on organic synthesis.

In some cases, the evaluation of results obtained from a larger or smaller number of substrates converted with a particular enzyme led to model conceptions by rationalizing general rules with the intention to predict sense and magnitude of the enantioselection of the enzyme and to facilitate a redesign of substrates, which initially were transformed with unsufficient selectivity or speed.

In principle two different concepts have been applied:

1) The design of an *active site model* of the enzyme, possessing "sites" and "pockets" with distinct properties, which requires knowledge on the interaction between the substrate and the active site of the enzyme itself. In most cases this comprises some uncertainties, especially if the accurate structure of the active site is not elucidated by X-ray analysis. Among hydrolytic enzymes, this approach has been carried out for α-chymotrypsin[12], pig liver esterase[13-16] and *Pseudomonas flourescens* lipase[17].

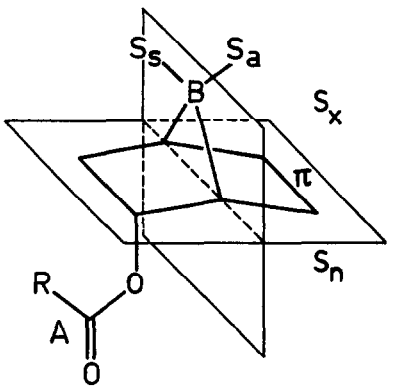
2) The design of a *substrate model* results in elaboration of a more or less specific structure which a substrate should come to as close as possible to ensure an optimal enantioselection and reaction rate by the enzyme. Such a model has been developed by Tamm *et al.*[18] for pig liver esterase. In contrast to the former method, the latter makes use of the relationship between substrate structure and optical purities of products, which can be established the more accurate the more the structure is rigid, thus avoiding conformational changes of the substrate caused by matching to the active site of the

enzyme. For this reason esters of bicyclic alcohols are almost ideally suited due to their fixed geometry.
We wish to present here a *substrate model* for *Candida cylindracea* lipase[19] obtained by evaluation of our results from enzymatic resolution of twenty five structurally different esters of bicyclic alcohols[11,20-22] possessing a bicyclo[2.2.1]heptane or bicyclo[2.2.2]octane framework. These compounds are a valuable starting material for the synthesis of a variety of monocyclic substances of biological interest[28].

RESULTS AND DISCUSSION

Analysing the relationship between substrate structure - individual regions of the main skeleton as depicted in figure 1 were consequently filled by substituents[23] with different steric requirements[11,20-22] - and the enantioselection of the enzyme - given as the enantiomeric ratio (E)[24] - the following set of rules was deduced:

Figure 1



Region	Requirements	Reference
A	Ester must be *endo*-configurated.	11
Site of reaction	R variable, may be *n*-alkyl, preferably *n*-C_3H_7.	11, 20
B	May contain hetero atoms (O).	21
Bridge	Must be small.	22
S_a S_s *anti-syn*	A methylene bridge may carry an ester, ether or acetal group.	22
Substituents	These must be small.	
S_x	This region may be covered.	11
exo-Substituents	Substituents may be large.	
S_n	This region must not be occupied.	11
endo-Substituents	Substituents (if any) must be very small.	
Π	π-Electrons in this region enhance the	11, 22
Π-Site	enantiomeric ratio.	

A, Site of Reaction
Only esters possessing an *endo*-configurated alcoholic center are resolved with high enantioselection (E varies from 10 to >100 depending on the remaining structural features of the substrate), with (*R*)-configurated centers being

cleaved preferentially[11]. In contrast, no clear preference for one enantiomer was found with *exo*-derivatives[25] where E remains below 5. Upon extension of the chain length of the acid moiety (R) a significant increase in the rate of hydrolysis from acetate to octanoate was found on *endo*-norborn-5-en-2-yl esters coming along with a slight decrease in enantioselection[11]. As a consequence, *n*-butyrates proved to be most advantageous substrates with respect to reaction rate, enantioselection and ease of handling.

B, Bridge

The bridge may consist of a hetero atom (O)[21], however, it must be small. A switch from bicyclo[2.2.1]heptanes[11] to bicyclo[2.2.2]octanes[22] cuts the enantiomeric ratio roughly into half.

S_a and S_s, *anti*- and *syn*-Substituents

Going in line with requirements for the bridge, any substituents in this region - esters, ethers or acetals - have a strong negative impact on the enantioselection[22]. If any substitution is neccessary, steric requirements should be kept on a minimum to retain a "flat" basic framework.

S_x, *exo*-Substituents

This region may be filled completely with even bulky substituents (e.g. a dimethyl-dioxolane moiety), which generally increase E slightly combined with a weak decline in the reaction rate[11].

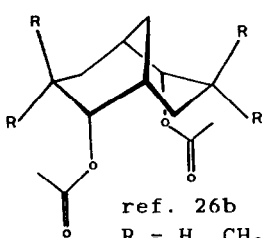
S_n, *endo*-Substituents

Any substitution in this area should be kept very small to avoid a non-acceptance of the substrate[11]. Therefore, it may regarded as a forbidden zone.

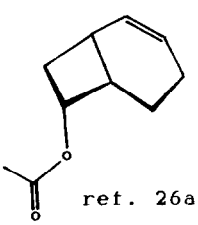
Π, Π-Site

A comparison of results obtained from substrates bearing double bonds in this region with those of their corresponding saturated counterparts[11,23] leads to the conclusion that π-electrons significantly enhance the enantiomeric ratio[28].

In conclusion, we believe that this model is not only applicable for esters possessing a bicyclo[2.2.1]heptane and bicyclo[2.2.2]octane framework but also gives good results for an expected direction of asymmetric hydrolysis when applied to substrates having similar more[26] or less[27] rigid structures which may roughly be superimposed upon the main framework of the model, if some uncertainties are accepted. For the following illustrating examples the preferred hydrolysed enantiomer is drawn.



ref. 26b
R = H, CH_3



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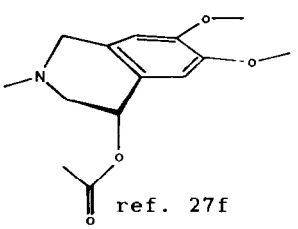

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