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ILLUSTRATIONS OF THE POTENTIAL OF α-CHYMOTRYPSIN FOR THE RESOLUTION OF ALICYCLIC ACIDS AND ESTERS

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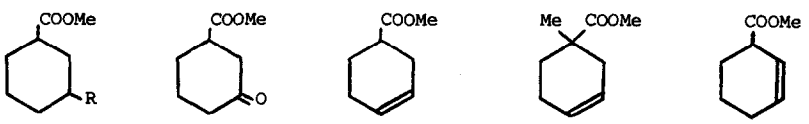
The aromatic-group specificity[1] of α-chymotrypsin (α-CT) has been exploited for the resolution of many racemic aromatic acids *via* hydrolysis of their esters.[2] In contrast, virtually no attention has been paid to the possibility of resolving aliphatic acids by this procedure. However, the optical purities achieved in the current study on representative cyclohexyl carboxylic acid esters demonstrate that the method holds considerable promise for the resolution of racemic alicyclic acid derivatives.

A survey of the rates of hydrolysis of homologous C_4-C_8 alicyclic esters showed that they were hydrolysed at adequate rates ($C_4 > C_6 > C_5 > C_7 > C_8$) in preparative-scale (1-2 g) reactions. Of the various alcohol moieties evaluated (Me, Et, n-Pr, i-Pr, n-Bu, cyclohexyl, p-$NO_2\phi$), methyl esters gave the best overall results. The racemic esters 1a-c, 2-5 were selected for the initial α-CT resolution study since they provided a representative structural variation and literature data on the optical purities and/or absolute configurations of the enantiomers were available.[3]

All reactions were carried out on up to 2 g of substrate using an aqueous solution containing 1 mg/ml of α-CT. The heterogeneous reaction mixture was stirred at room temperature



1 2 3 4 5

a,R = t-Bu; b,R = OH; c,R = THP; d,R = COOMe

under nitrogen and the pH maintained at 7.8 with a pH-stat or by periodic addition of 0.05N NaOH from a burette.[4] For reference purposes, each reaction was terminated at the 50% hydrolysis of substrate point since if the stereospecificity of hydrolysis was complete, total resolution would have occurred at this point. The residual ester was isolated by ether or

chloroform extraction of the pH 7.8 solution and the desired acid by a similar procedure from the subsequently acidified (pH 2) mixture. The results obtained are summarized in Table I.

Although different rates of hydrolysis of the enantiomers of 1a were anticipated,[5] the procedure was not preparatively viable in this case, owing to the almost complete insolubility of 1a in the aqueous reaction mixture.[6] In contrast, when the hydrophilic character of the substrate was increased by replacement of the t-butyl group by hydroxyl, as in 1b, significant resolution was achieved. The THP derivative 1c was twice as rapidly hydrolysed as 1b but increasing the bulk of the 3-substituent in this way had a marginal effect only on the degree of resolution. Appreciable resolution also occurs when sp^2-hybridized functions (compounds 2,3,5) are present. However, as expected from the literature,[1] when the carbon atom adjacent to the carbomethoxy group is fully substituted, as in 4, enzymic hydrolysis does not occur. In view of the ability of α-CT to distinguish between prochiral ester functions[12] its failure to hydrolyse the meso diester 1d to a chiral half ester was disappointing.

The degrees of resolution achieved following 50% hydrolysis by α-CT (Table I) improved dramatically following each successive α-CT treatment of the acid product (following reconversion to its ester) and of the unhydrolyzed ester recovered. Alternatively, by terminating the reaction after < or > 50% hydrolysis, up to 85% optically pure acid or ester can be obtained from one α-CT-catalyzed hydrolysis. The degrees of resolution attainable by these modifications compare favourably with, and in some cases exceed, the levels of optical purities reached during most early stages of a diastereomeric salt resolution. Furthermore, both R and S forms are isolable from the same reaction under very mild conditions.

Since the -COOMe groups of the substrates studied can be presumed to be oriented in the equatorial position at the active site,[5] the substituent or double bond is always on the same "side" (depicted as the rear in Table I) of the ring in the preferred conformation of the enantiomer hydrolysed faster.[13] This generalization may prove of value in assigning absolute configurations of substituted cyclohexanes and this aspect is being pursued further together with an extension of the study to racemic cyclobutyl, cyclopentyl and cycloheptyl carboxylic acid derivatives.

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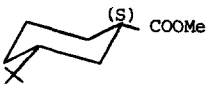
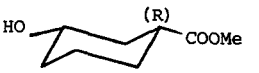
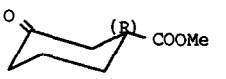
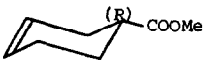
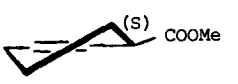
Table I. Optical Purities of Product Acid and Residual Ester Following 50% Hydrolysis of the (±) Methyl Ester by α-Chymotrypsin

(±) Substrates	$[\alpha]_D^{25}$ Acid (% opt.pur)	$[\alpha]_D^{25}$ Ester (% opt.pur)	Enantiomer hydrol. faster
1a	+[a]	-[a]	
1b	-4.1°(42)	+1.0°(n.d.)	
1c	-5.0°(50)	+4.0°(n.d.)	
2	-1.0°(9)	+2.2°(n.d.)	
3	+34°(44)[b]	-37°(47)	
4	-----[a]	-----[a]	
5	-80°(>70)[c]	+61°(n.d.)	
1d	0°	0°	R=S (COOMe)

For experimental details see text. Reaction periods of 18-30 hr. were required and 50-80% yields of products were isolated. Rotations were determined in $CHCl_3$ and optical purities and absolute configurations are based on literature data.[5,7-10] [a] Hydrolysis impracticably slow. [b] Resolved via α-phenethylamine salt. [c] Estimated following conversion to S(-)-2-methylcyclohexene during absolute configuration determination.[11]

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(13) The exception of 1a to this correlation is presumably due to the steric effect of the bulky t-butyl group.