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The Enzymatic Baeyer-Villiger Oxidation: A Study Of 4-Substituted Cyclohexanones

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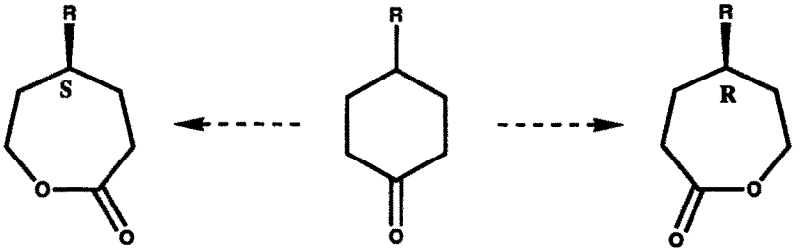
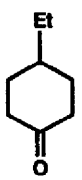
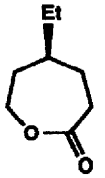
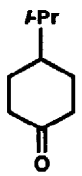
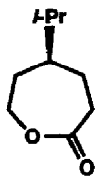
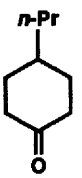
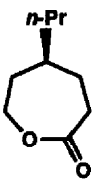
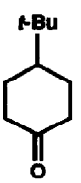
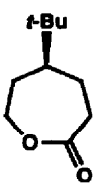
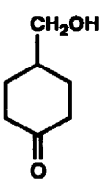
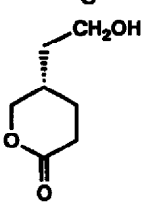
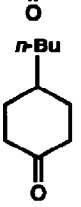
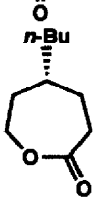
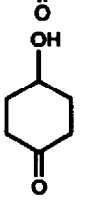
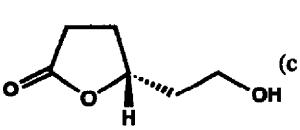
Abstract: A study of the enzymatic Baeyer-Villiger oxidation of a number of 4-substituted cyclohexanones utilizing the enzyme cyclohexanone oxygenase (E.C. 1.14.13.-), isolated from the bacteria *Acinetobacter* NCIB 9871, is described.

In a program aimed at the asymmetrization of symmetrical ketones[1] utilizing the enzymatic Baeyer-Villiger oxidation it was found that the reactions produced the Baeyer-Villiger products with high enantiomeric excesses in all the examples studied except for 4-methoxy cyclohexanone.[1a] The result of this experiment prompted an investigation of a series of 4-substituted cyclohexanones to examine the role substituents in the 4-position may play in the asymmetrization reaction depicted below. The overall objectives of this study were two-fold. From a synthetic point of view, it would extend the list of substrates that could be employed in the enzymatic Baeyer-Villiger reaction.[2,3] It was also of interest mechanistically because of the possibility of providing information about the dimensions of the active site or potentially important interactions within the active site of the enzyme. The following communication reports the findings of this investigation.



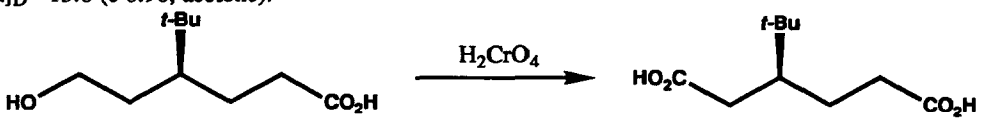
The enzyme cyclohexanone oxygenase (E.C. 1.14.13.-) was isolated from the bacteria *Acinetobacter* NCIB 9871 and purified according to the method described by Walsh.[4] The ketones (~1 mmol) were subjected to the enzymatic Baeyer-Villiger oxidation in a pH 8.0 glycine-NaOH solution utilizing approximately 10 mg (30 units) of cyclohexanone oxygenase.[1] A catalytic amount of $NADP^+$ and the glucose-6-phosphate/glucose-6-phosphate dehydrogenase $NADP^+$/NADPH recycling technique was employed to regenerate the required cofactor.[5] Shown in Table 1 are the results obtained with the series of 4-substituted cyclohexanones used for this investigation.

Table 1.

Substrate	Product	$[\alpha]_D$	% Yield	% E.e.
		-38° (c 5.55, $CHCl_3$)	84	>98
		-40° (c 0.44, $CHCl_3$)	60	>98
		-38° (c 6.41, $CHCl_3$)	80	>98
		-34.9° (c 0.78, $CHCl_3$)	17	>98
		-6.2° (c 5.66, $CHCl_3$)	80	>98
		+18.5° (c 2.74, $CHCl_3$)	70	52
		-6.80° (c 4.75, $CHCl_3$)	73	9.6

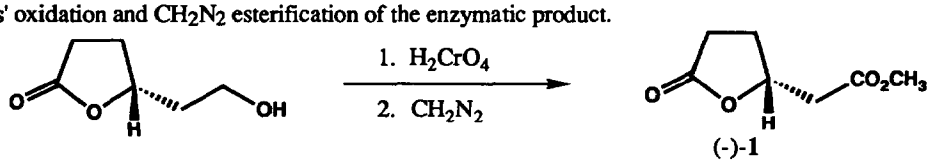
The enantiomeric excesses of the lactone products were determined by first transforming them to the hydroxy methyl esters with $NaOCH_3$ followed by conversion to either the (+)- or (-)-α-methoxy-α-(trifluoromethyl)phenylacetic acid esters. The enzyme derived products were then analyzed by ^1H, ^{13}C, and ^{19}F NMR spectroscopy and compared with their racemic counterparts obtained via the conventional peracid route. The five- and six-membered ring lactones shown in Table 1 are the isomeric lactones of the expected seven-membered ring lactones that had undergone hydrolysis under the reaction conditions and closed to the isomeric products on acidic work-up. The enantiomeric purities of these two lactones were analyzed by directly converting the alcohols to the (-)-α-methoxy-α-(trifluoromethyl)phenylacetic acid esters.[6]

The absolute configurations of the stereogenic centers in most of the lactones derived from the 4-substituted cyclohexanones are as shown. The configurational assignments are based on analogy with the 4-methyl and 4-*tert*-butyl products and on a predictive model that has evolved out of this and other work.[7] The 4-methyl product was shown to be *S* in the previous study.[1a] The 4-*tert*-butyl product also proved to have the *S* configuration. This was established by Jones oxidation of the hydroxy acid derived from the enzymatic Baeyer-Villiger oxidation which transformed it into the known (3*S*)-3-*tert*-butylhexanedioic acid ($[\alpha]_D$= 13.8 (c 0.90, acetone).[8]



There was a notable reduction in product E.e. when the substrate changed from 4-*n*-propyl to 4-*n*-butylcyclohexanone. The *n*-propyl derivative behaved in an analogous fashion to the other 4-alkyl derivatives, while the *n*-butyl compound only gave a product with a 52% E.e.. Additionally, based on of the rotation of the *n*-butyl lactone, it possibly has the *R* configuration. In going from *n*-propyl to *n*-butyl, the limit in terms of the length of the substrate that can be accommodated and still provide acceptable levels of enantioselectivity may have been reached or it may signal the point of change over in enantioselectivity. Studies to determine whether this is the limit or the point of change are actively being pursued.

Another conspicuous result was obtained from 4-hydroxycyclohexanone which produced a lactone with only a 9.6% enantiomeric excess. Not only was the enantiomeric excess low, but the enantiomer that was in excess was also opposite in configuration to that for most of the other lactones. The *R* configuration for the major enantiomer of the product from the 4-hydroxy ketone was assigned by comparison of the rotation of lactone **1** ($[\alpha]_D$ = - 3.65 (c 0.99, EtOH)).[8] Lactone **1** was obtained by Jones' oxidation and CH_2N_2 esterification of the enzymatic product.



(-)-**1**

The change for the 4-hydroxy compound is believed to be conformational in origin,[10,11] although hydrogen bonding-type phenomena may also be operative. In an effort to test if hydrogen bonding might be important, other candidates that could be capable of this phenomenon but would have a larger inherent preference for an equatorial arrangement of the substituent were assembled and screened as potential

substrates. The only acceptable substrate found was the 4-hydroxymethyl derivative. This compound provided a lactone with no erosion in E.e. (>98%) and presumably with the *S* configuration. Although not conclusive, this latter result does lend more support to the conformational rationale.

Efforts to rigorously establish the absolute configurations of the other products are currently underway. The syntheses of 4-substituted cyclohexanones with substituents that have *A*-values less than hydroxy are currently underway as well as the preparation of conformationally locked cyclohexanones with axial and equatorial 4-hydroxyls. The results of these and related studies will be reported in due course.

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