

Enzymes for chiral synthesis

5330

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Judging from the burgeoning literature on enzyme catalysis, one would expect more chemists to take advantage of these exquisite tools. As Whitesides and Wong have remarked (1), "Enzyme assays seem (to an organic chemist) intrinsically sloppy and ill-defined, relative to methods based on GLC or HPLC. It is nonetheless possible, by an exercise of will, to overcome one's sense of distrust and unease in using these methods, and to obtain reproducible and reliable results." Many are available through commercial outlets; some are "home grown." Although enzymes are highly specific, they do accept other than their natural substrates; they can even function in nonaqueous media (2).

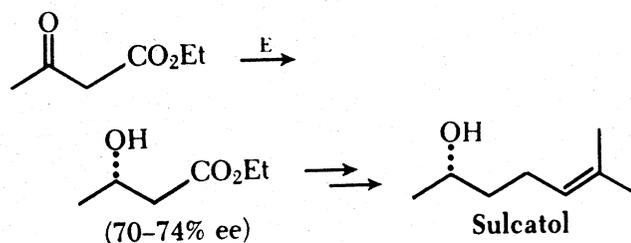
Here I review some recent work on asymmetric syntheses that were targeted for chiral insect pheromone structures. Pheromones are one of the modern tools for controlling insect pests. These chemicals are emitted by one sex of an insect species and are among the most biologically active substances known. For some insects, such as the gypsy moth, Japanese beetle, and several important rootworm species, the correct stereochemistry of the pheromone is critical. Thus the synthesis of chiral species is of special concern (4).

Enzyme-catalyzed asymmetric syntheses can be viewed as exemplifying two broad categories of reaction. In one, a prochiral center is reduced asymmetrically; in the other, a racemic material is resolved by selective reaction of one enantiomer. Excellent reviews of enzymes for chiral synthesis are available (5, 6).

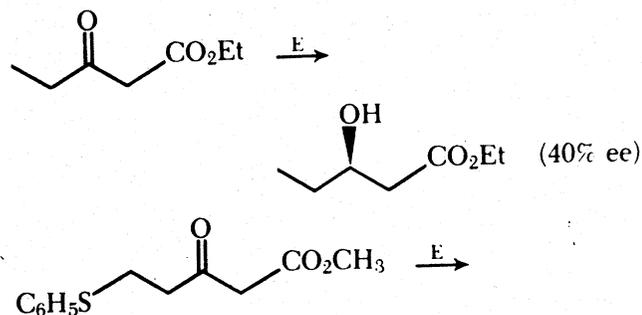
Asymmetric induction

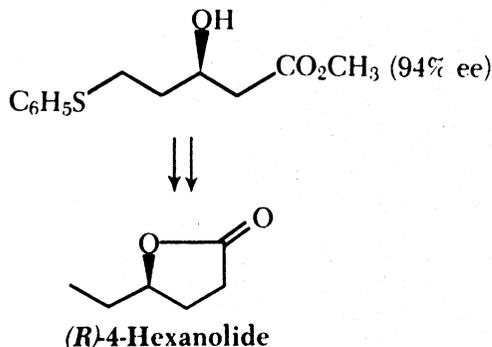
The reducing power of *Saccharomyces cerevisiae* (Baker's yeast) has been exploited frequently for the

preparation of chiral alcohols. Ethyl acetoacetate was reduced by Mori (7), achieving 70–74% enantiomeric excess (ee) of ethyl (S)-3-hydroxybutyrate that was then converted to sulcatol, the aggregation pheromone of the Ambrosia beetle, *Gnathotricus sulcatus*



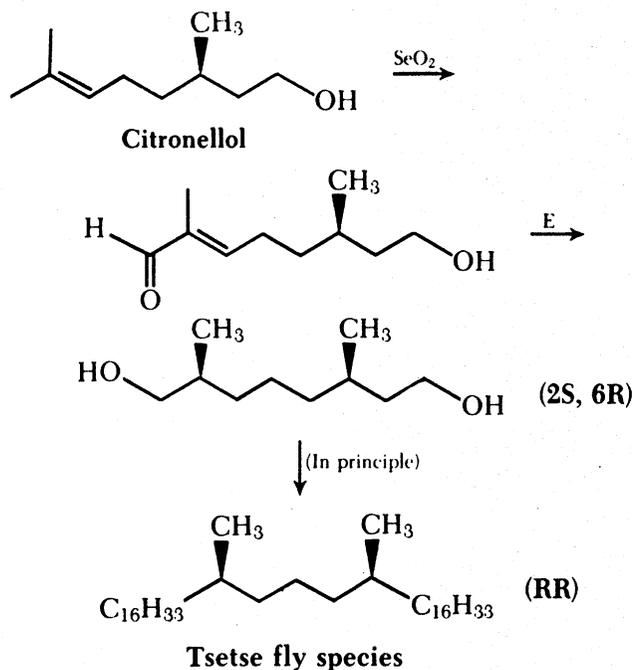
where E is the appropriate enzyme. Optical yields in this reduction reported from different laboratories vary, and this may reflect differences in yeast strain or conditions of reaction. In a subsequent study to produce (R)-4-hexanolide (a pheromone of the dermestid, *Trogoderma glabrum*), an enzymatic reduction of ethyl 3-oxopentanoate was sought.





For this substrate Baker's yeast produces the (*R*)-enantiomer, although the stereobias is much less. By enlarging the hydrophobic substituent (ethyl \rightarrow phenylthioethyl) and screening several reducing yeasts, a suitable reductant, *Pichia terricola*, was found that produced the desired configuration with 94% ee (8). Subsequent chemical elaboration of the carbomethoxy group to permit generation of the lactone ring was followed by reductive removal of the phenylthio unit.

An additional important use for reducing-enzymes is for the hydrogenation of double bonds that are conjugated to a carbonyl. For example, citronellol can be oxidized to an α, β -unsaturated aldehyde that undergoes Baker's yeast reduction at both carbonyl and carbon-carbon double bond, generating an (*S*)-configuration on the new saturated methyl branched carbon atom (9). Using this synthetic sequence, (*R*)- and (*S*)-citronellols have been converted to (2*S*,6*R*)- and (2*S*,6*S*)-2,6-dimethyl-1,8-octanediols.



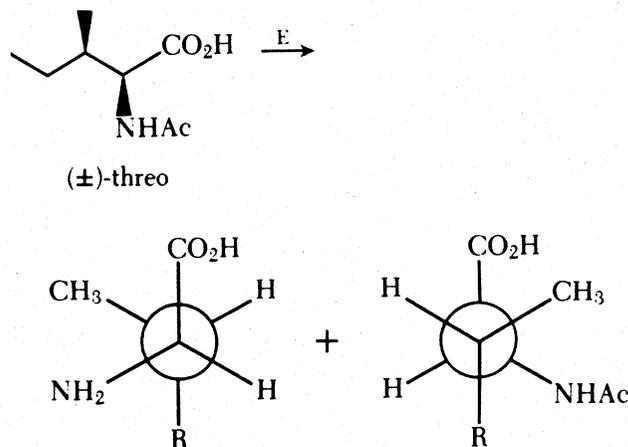
These diols can be viewed as useful chiral synthons for the many natural products that are 1,5-dimethylated acyclics such as pheromone structures of pine sawflies, red flour beetle, and tsetse fly species. The authors point out that one could use the (2*S*, 6*R*)-isomer to build the (*R*, *R*)-isomer of 17,21-dimethyl-heptatriacontane.

Note that these reductions require cofactors so that cells of cultures were used rather than an enzyme preparation. In contrast, hydrolyzing enzymes such as proteases, esterases, and lipases generally do not require cofactors, so powders derived from cultures can be used.

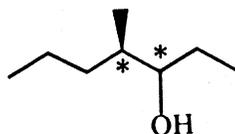
Kinetic resolution

Carbonyl reduction can in principle provide complete conversion to one enantiomer. On the other hand, resolutions are usually performed with the goal of obtaining pure quantities of both enantiomers. If one seeks a single enantiomer, the unwanted isomer must be transformed by stereospecific conventional processes to the desired one. Let me illustrate how kinetic resolution makes use of the preferential reaction of one of two enantiomers of a racemate.

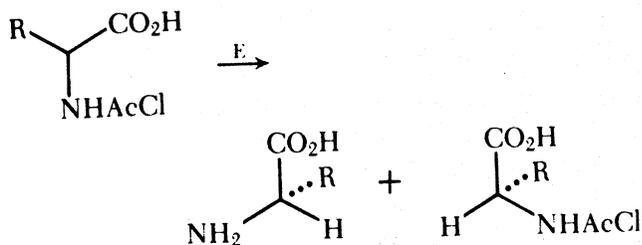
A strain of an *Aspergillus* species (Amano Co.) has been cultured for *N*-deacylase activity. Selectivity for (*S*)-acylated amino acids is nearly absolute. Racemic *threo*-2-amino-3-methylpentanoic acid was synthesized and converted to an acetamide (10). Treatment with deacylase produced the (2*S*, 3*R*)-amino acid. Further treatment of recovered undeacylated material provided a sample of the enantiomer as its amide that was then hydrolyzed to the (2*R*, 3*S*)-amino acid.



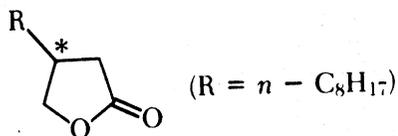
These amino acids were employed initially to obtain the remaining diastereomers by inverting the 2-position, then to prepare the enantiomers of *threo*-4-methyl-3-heptanol, a pheromone component of the smaller European elm bark beetle, *Scolytus multistriatus*.



In a similar fashion, racemic 2-aminodecanoic acid was resolved as its *N*-chloroacetyl derivative (11).

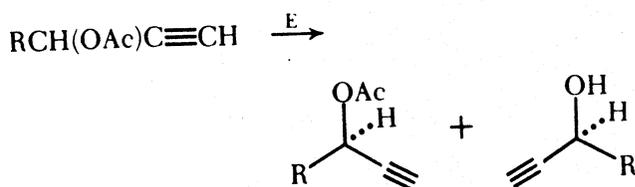


The amino-bearing carbon retained its configuration during deamination that replaces NH_2 with OH , and the carboxyl terminus was extended by conventional means that preserved the compound's stereochemistry. The ultimate products were the enantiomeric 4-dodecanolides that are produced in the pygidial glands of rove beetles.



This lactone was also synthesized by microbial reduction by another group (12).

The hydrolysis of acetates of alkynyl alcohols using *Bacillus subtilis* var *Niger* was studied (13).



The optical purities obtained depended on the alkyl group present and, for the 4-methyl-3-pentenyl unit, were only fair. Nevertheless, such a structure is convertible to the pheromone of the Japanese beetle (*Japonilure*)



and an improved enzymatic process would be a worthwhile objective, because the current industrial preparation begins with the unnatural isomer of glutamic acid.

Mathematical equations that describe enzymatically driven resolutions have been developed (14). These allow

calculation of the ratio of specificity constants for a pair of enantiomers and give a useful value for this ratio with stipulated provisions. A convenient form of this expression that may be more useful generally for synthetically oriented chemists has also appeared (15). Rate ratios (k_R/k_S) were calculated from the expression

$$\frac{k_R}{k_S} = \frac{\text{Ln}[(1 - C)(1 - ee)]}{\text{Ln}[(1 - C)(1 + ee)]}$$

where C is the fraction of racemic starting material converted and ee is the enantiomeric excess of residual starting material.

For hydrolysis, the ee was determined in the product and then related to the starting material:

$$ee_A = \frac{C(ee_B)}{1 - C}$$

where A refers to starting material and B refers to product.

The rate ratios are actually ratios of specificity constants with limitations to their validity (14), and k_R and k_S were employed for simplicity and the sake of comparison (15). Because the lipase-catalyzed resolutions described always resulted in faster reaction of (*R*)-enantiomers, the rate ratios are given as k_R/k_S . Operationally one need only determine the fraction of starting material converted (C) and the enantiomeric excess (ee) of the starting material. The product ee could, of course, be determined instead and transformed to starting material ee . A way to view this is as follows: If you had a set goal of 95% ee in your unreacted starting material, a rate ratio of 2.6 requires that the reaction proceed to 95% conversion. The product ee would only be 5. For an infinitely large rate ratio, one only needs to go to 50% conversion (in fact, one can go *only* that far). A "useful" rate ratio is subjective, and the ratio can be maximized by screening sources of enzymes, strain selection processes, optimizing reaction conditions, and even altering substrate structure as Mori's group had done to obtain (*R*)-4-hexanolide.

Lipases for kinetic resolutions

A number of pheromone structures are esters of methyl alkylcarbinols. In particular, 8-methyl-2-decanol is the parent alcohol for esters that have been identified for several closely related species of rootworm (genus *Diabrotica*) (16). These compounds contain two asymmetric centers, one involving the secondary alcohol (carbon 2) and the other designated as the hydrocarbon center (carbon 8). Table 1 shows how seven species respond to the stereoisomers with an (*R*)-hydrocarbon center. Those insects with trivial names are economic pests. The insects apparently do not respond to isomers with an (*S*)-hydrocarbon center—synthetics can be racemic at that site and, except for effects of dilution, elicit the same

response as the corresponding (*R*)-8 stereoisomer. However, responses to the carbinol site vary, and mixtures that contain blends of (*R*)-2 and (*S*)-2 materials show evidence of inhibition. Because the racemic alcohol can be readily synthesized (17), an efficient enzymatic resolution of the racemic carbinol center could be a valuable synthetic process. The ability to monitor these species selectively by using synthetics as baits in traps may depend critically on the stereochemical constitution of those chemicals.

We initially examined reactions involving 2-octanol and its esters as models using commercially available lipase preparations. Lipases have indeed been employed to perform resolutions (18), but the alcohols or esters involved were usually alicyclics. Enzymatic resolution of methyl *n*-alkylcarbinols is much more difficult and has only been occasionally reported (18). In addition, general screening of lipases for this purpose does not seem to have been done. We calibrated the enzymes for activity on olive oil (~85% triolein) using an initial rate assay. Considerable differences exist in these lipase preparations, and this can be attributed to the degree of purification as well as to intrinsic differences between the enzymatically active proteins. At this point, little is known of the structure or mechanism of action of triglyceride lipases. We opted therefore to treat these materials simply as undefined but potentially useful catalysts for organic synthesis.

An examination of the esterification of 2-octanol using octanoic acid in hexane at 30 °C with several lipases gave a strong indication that the *M. miehei* lipase sold by Novo Co. would be useful for our purposes (19).

Intriguingly, the esterification's stereochemical consequences depend on the length of the fatty acid chain (Figure 1). Acetic acid did not become esterified in the presence of the *M. miehei* lipase, and stereoselection for

(*R*)-2-octanol increased proceeding to hexanoic acid, diminished beyond nonanoic, and increased again to hexadecanoic. When we used 2-hexanol as the alcohol to be esterified, we obtained a similar profile albeit with overall lowered stereoselection. For example, the rate ratio calculated for 2-octanol and hexanoic acid was >50:1. The corresponding rate ratio for 2-hexanol was 9.5:1. The usual conceptualization of the mechanism of hydrolase activity invokes the intermediacy of an acyl-enzyme structure. Evidently information involving fatty acid chain length can be transmitted back to the vicinity of the acyl-bearing portion of the complex, resulting in altered selection for enantiomeric alcohols. This phenomenon may be general for ester hydrolases and does not appear to have been investigated.

We subsequently evaluated resolution via ester hydrolysis and transesterification using these lipases. Again, the octanoate ester appeared to be the best substrate for resolution. Transesterification with selected triglycerides gave interesting results, but we felt that work-up procedures would be more complicated. The reason for evaluating triglycerides as sources of acid residues for the resolution was that naturally occurring triglycerides such as vegetable oils or animal fats might prove to be cheaper reagents than organic fatty acids.

We used the information that we had obtained in evaluating esterification and hydrolysis of racemic 2-octanol to resolve 8-methyl-2-decanol, the pheromone precursor. Throughout these studies we monitored resolutions by derivatizing the recovered alcohols from reactions with (*S*)- α -methylbenzylisocyanate. The resulting diastereomeric carbamates are easily separated by capillary GLC. Typically, esterifications produced 93% (*S*)-alcohol and gave ester that was saponified to 95% (*R*)-alcohol in greater than 80% theoretical yield.

Table 1. Stereobias in biological activity

Insect	Response to carbinol center		
	2 (<i>R</i>)	2 (<i>S</i>)	Ester
<i>D. virgifera virgifera</i> (Western corn rootworm)	++	+	Propionate
<i>D. virgifera zea</i> (Mexican corn rootworm)	++	+	Propionate
<i>D. barberi</i> (Northern Corn rootworm)	++	I	Propionate
<i>D. porracea</i>	-	++	Propionate
<i>D. lemniscata</i>	+	++	Propionate
<i>D. longicornis</i>	I	++	Propionate
<i>D. cristata</i>	-	++	Acetate

D. Diabrotica; I, inhibitory.

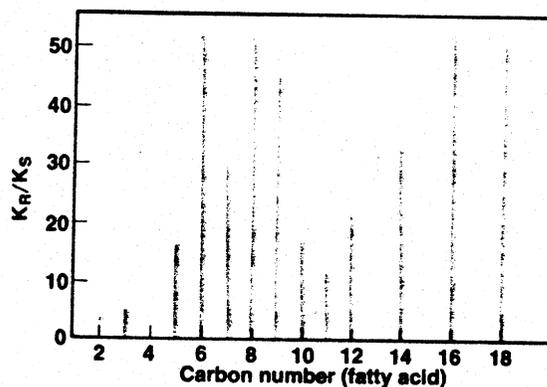


Figure 1. Enantiomeric ratio for esterification of racemic 2-octanol vs. fatty acid chain length using lipase for *M. miehei*. *, no reaction

Basically, if you are faced with the challenge of constructing an asymmetric center, you could screen available enzymatic activity monitoring your results with a suitable analysis and obtain an indication of the inherent bias of the catalysts. Beyond that, you can alter substrate structure, reaction medium, or even culture for strain variance to enhance the enzyme's stereoselection.

It's true that enzymes are expensive and relatively unstable. But these disadvantages must be weighed against the rate and degree of difficulty of a particular synthetic step. Moreover, there are techniques that can stabilize enzymes and promote their recovery.

Problems of longevity and reusability have been addressed by immobilization procedures (3), in which enzymes are deposited, adsorbed, included, and chemically bound. Deposition can be produced by salting with ammonium sulfate or adding acetone to a crude solution of relatively impure enzyme and a powdered solid such as diatomaceous earth. The recovered powder has enzymatic activity. When the powder is added to an aqueous reaction mixture, the enzyme tends to be released into the solution but cannot be recovered. For reactions in nonaqueous media, it is useful to adsorb the enzyme to ion-exchange resins. Inclusion into gels or capture by porous glass having controlled pore size have also been explored. Desorption (i.e., leaching) of enzyme activity seems to be slower than for simple deposition of the enzyme.

The most permanent technique involves bonding, often via the free amino groups from lysine residues present at the surface of globular proteins. Such methodology produces long-lasting activity in aqueous reaction mixtures, but the yield of activity is often low—one can imagine that the random binding to a solid support could occasionally produce a structure that can no longer undergo the many conformational changes necessary to catalyze the desired reaction.

Which immobilization technique to use depends on the particular reaction, the scale of the process, and the degree of concern about enzyme recovery.

Prospects

It seems likely that the current interest in enzyme technology will spur studies resulting in more complete information on the enzymes that in many cases are currently being generated by recipes designed empirically to satisfy an industrial customer. Better reference material that will tabulate reactions conducted with homogeneous, enzymatically active substances would be useful. New sources of enzymatic activity will lead to a greater range of choices of substrate structure. Enzymes that have been altered chemically may become available or will be prepared by the user. For example, we are currently working with *C. rugosa* lipase that has been derivatized to

add polyethylene glycol chains, thereby rendering the protein soluble in organic solvents. Such a procedure allows homogeneous reactions in benzene and methylene chloride and offers some interesting alternatives to conducting reactions with the native material (20). The potential for changing enzyme selectivity by such conditions has yet to be investigated. Finally, recombinant DNA methods would allow the evaluation of site-selective amino acid replacements that would lead to enzymes whose catalytic activity was better tailored for the desired substrate.

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