The use of enzymes for the catalysis of key reactions in the synthesis of biologically active natural products and analogues

S.M. Roberts

Department of Chemistry, Exeter University, Exeter, Devon EX4 4QD.

Abstract
The incorporation of enzyme-catalysed reactions as key features in
multi-step organic syntheses is becoming more widespread. Various
hydrolase and oxidoreductase enzymes have been used in syntheses of
antibiotics and compounds of importance in inflammation and heart disease.

The employment of enzymes as catalysts in synthetic organic chemistry is
becoming widespread;   comprehensive recent reviews of the subject are available.[1]
Whole cell systems and isolated partially purified enzymes have been used for various
biotransformations and the 'pros and cons' of using one or other methodology have been
discussed.[2]   Both methods have been used to promote the hydrolysis of esters: thus (±)-
3-acetoxyoct-1-yne was hydrolysed to give oct-1-yn-3(S)-ol (e.e. 80-97%) and
recovered optically active ester using lyophilized bakers' yeast or the lipase from *Mucor
miehei*. The octyn-3(S)-ol has been used to make 13(S)-hydroxyoctadecadienoic acid
(coriolic acid) (1), a compound produced by rice as a defence against fungal disease.[3]

2-Azabicyclo[2.2.1]hept-5-en-3-one is available in both enantiomeric forms by
enantioselective hydrolysis of the racemic compound using selected micro-organisms.[4]
Both optically active lactams can be converted into the GABA-mimetic (2).[5]


(1)   (2)   (3)
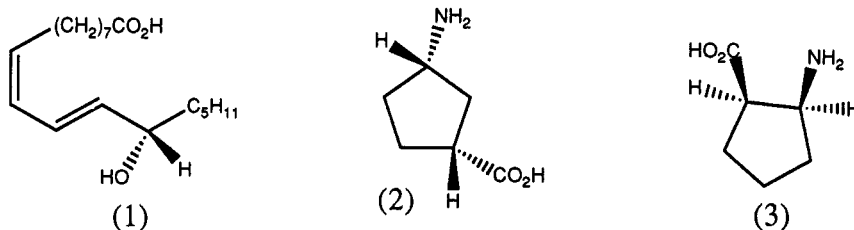
Similarly 6-azabicyclo[3.2.0]hept-3-en-7-one has been resolved using the
microorganism *Rhodococcus equi* and the one enantiomer has been converted into the
interesting anti-fungal antibiotic cispentacin (3).[6]

The hydrolysis of nitriles to carboxylic acids and carboxamides using nitrile
hydratases and nitrilases is an area of current interest.   The mild conditions employed
for the hydrolysis and the stability of other functional groups (*e.g.* esters) under the

reaction conditions means that further utilization of the technique in organic synthesis is assured. In one simple example methyl 3-cyanobenzoate is converted into the carboxylic acid (4) using nitrile hydratase SP361.[7] The hydrolysis of epoxides using microsomal fractions of liver extracts has been accomplished: an epoxide hydrolase readily available from a microorganism would be a benefit to the non-specialist.

The realization that certain hydrolases are relatively stable in some non-aqueous media has promoted a lot of work in the utilisation of these enzymes for the synthesis of esters and amides. For example cyclohexane carboxylic acid and (±)-bicyclo[3.2.0]-hept-2-en-6endo-ol have been reacted using immobilized *Mucor miehei* lipase in hexane containing a trace quantity of water to give, at *ca.* 50% conversion, the ester (5) and recovered (*S*)-alcohol. Both the ester and the alcohol have been used to make prostaglandin synthons.[8]

A similar strategy has been used to make peptides and selected derivatives such as the sweetener Aspartame.[9]

(4)   (5)   (6)   (7)
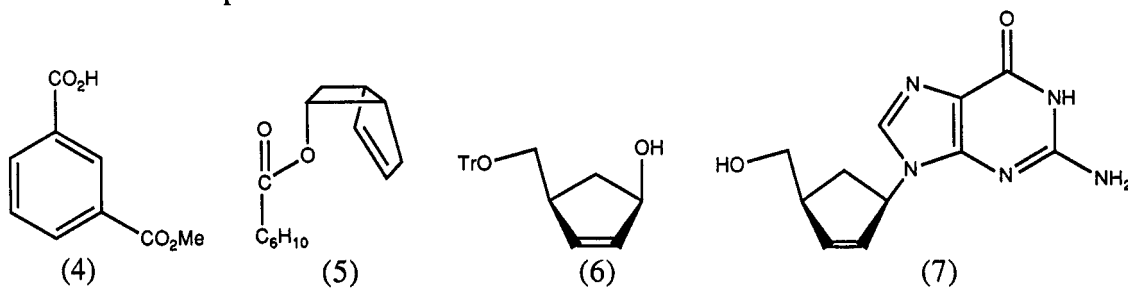
The kinetic resolution of *cis*-4-trityloxymethylcyclopent-2-en-1-ol using *Pseudomonas fluorescens* lipase and vinyl acetate is an example of a trans-esterification process of growing importance. The optically pure alcohol (6) has been converted very efficiently into the anti-HIV agent carbovir (7).[10]

A transamidation process can be used for the conversion of pig insulin into human insulin. This represented the first example of a biotransformation being employed to provide a compound for use in the clinic.[11]

Reduction of ketones to chiral secondary alcohols using microorganisms and dehydrogenase enzymes is well researched and often the stereochemistry of the product(s) from such a reductive biotransformation can be predicted. Simple acyclic dialkylketones, β-ketoesters and β-diketones are reduced to give secondary alcohols, β-hydroxyesters and β-hydroxyketones respectively. The reductive biotransformation of 7,7-dimethylbicyclo[3.2.0]hept-2-en-6-one using *Mortierella isabellina* gave 7,7-dimethylbicyclo[3.2.0]hept-2-en-6(*S*)endo-ol and 7,7-dimethylbicyclo[3.2.0]hept-2-en-6(*S*)exo-ol. Both alcohols are used to prepare complementary moieties of the naturally-occurring chemotactic and chemokinetic agent leukotriene-B4 (8).[12] In contrast reduction of 7,7-dimethylbicyclo[3.2.0]-hept-2-en-6-one with 3α,20β-hydroxysteroid alcohol dehydrogenase and NADH gave the corresponding 6(*S*)endo-alcohol and recovered optically active ketone. The former compound was transformed in seven steps into the pheromone (+)-eldanolide (9).[13]
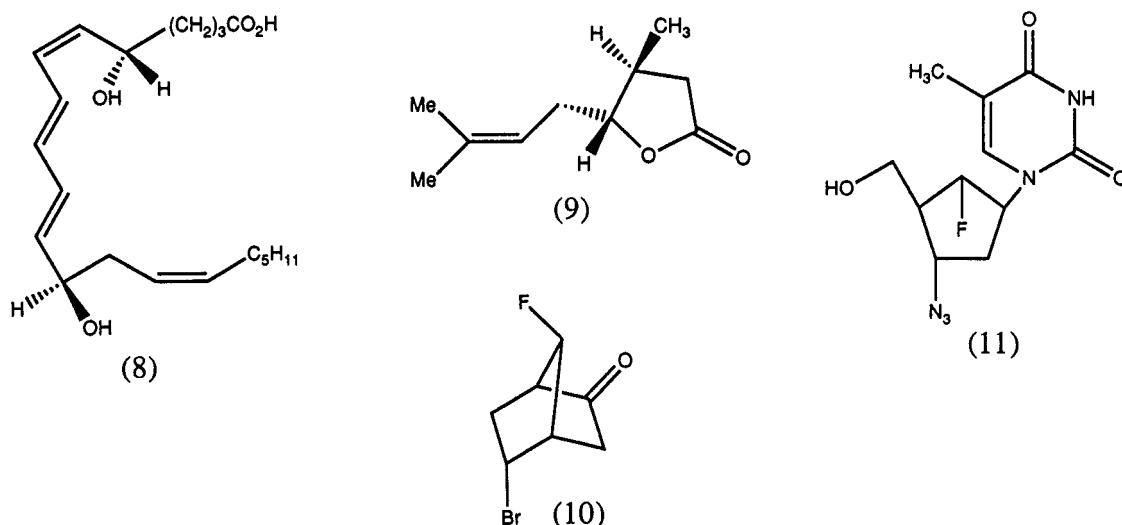
The oxidation of ketones to esters or lactones can be accomplished using either peracid or selected monooxygenase enzymes. The products obtained can be different depending on the technique selected. Thus peracid oxidation of 5*endo*-bromo-7*anti*-fluorobicyclo[2.2.1]heptan-2-one furnished 2-oxa-6*endo*-bromo-8*anti*-fluorobicyclo-[3.2.1]octan-3-one and 3-oxa-6*endo*-bromo-8*anti*-fluorobicyclo[3.2.1]octan-2-one, both in racemic form, in the ratio 1:3.5. In contrast oxidation of the same ketone with *Acinetobacter* NCIMB 9871 afforded optically active 2-oxabicyclooctan-3-one (40%) and optically pure ketone (10) (40%). The latter compound was converted into the AZT mimic (11).[14]

The potential profits that may be accrued by using a dehydrogenase and mono-oxygenase working in tandem (Figure 1) are being assessed.[15]

Figure 1
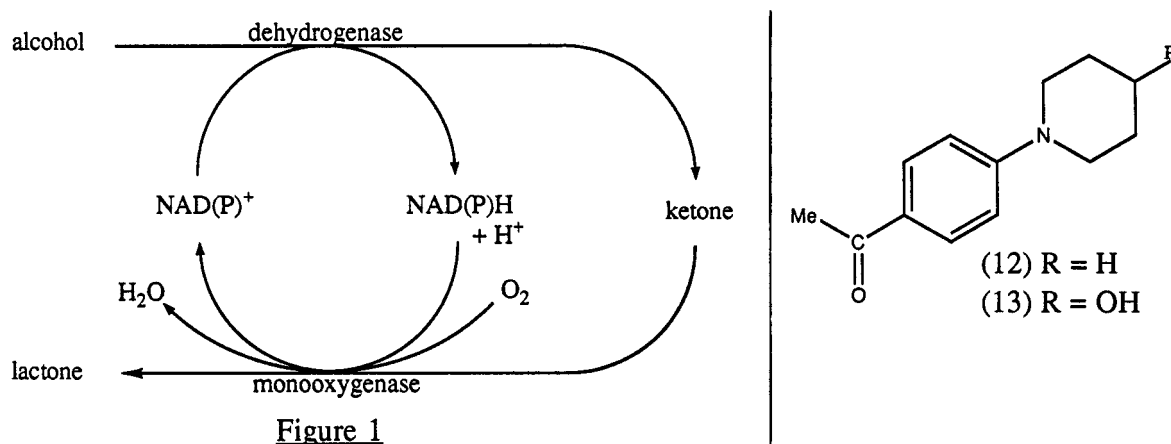
The oxidation of the aminoketone (12) to the alcohol (13) using *Beauveria sulfurescens* is an example of a highly regioselective oxidation of an ostensibly non-activated carbon atom using a microorganism.[16] The methodology is presently being adapted to provide syntheses of various biologically active alkaloids. There has been much interest in the fact that some pseudomonads convert benzene (and derivatives) into
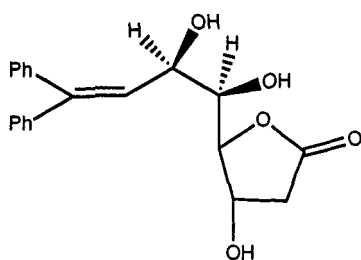
(14)
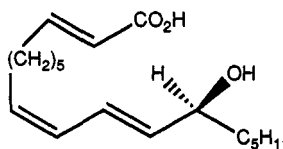
(15)

(substituted) cyclohexadiene-*cis* 1,2-diol.   The chiral dienediol derived from toluene has been very effectively used in the synthesis of various natural products.[17]   Cyclohexa-3,5-diene-*cis*1,2-diol itself has been converted into ribonolactone analogues (14).[18]

Finally soybean lipoxygenase has been shown to transform octadeca-2(E),9(Z)-12(Z)-trieneoic acid into the hydroxyacid (15)[19] and the ability of such compounds to undergo intramolecular Diels-Alder reactions is being investigated.

The above enzymes, as well as aldolases and nitrile ligases *etc.*, constitute a portfolio of chiral catalysts of growing importance in organic chemistry.

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