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Use of Enzymes as Catalysts to Promote Key Transformations in Organic Synthesis

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Use of enzymes as catalysts to promote key transformations in organic synthesis

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The field of biotransformations has developed rapidly over the past eight years. The use of esterases and lipases is now widespread; these enzymes are of particular importance in the production of optically active building blocks for organic synthesis as well as in large-scale processes involving the transesterification of fats. The latter area (i.e. the catalysis of esterification processes) has stimulated research into the properties of immobilized enzymes and the use of enzymes in low-water systems. In related work, enzymes have been used for the preparation of peptides and small proteins.

Redox enzymes have been investigated extensively, particularly with regard to the stereocontrolled reduction of ketones to secondary alcohols. The methods for using commercially available enzymes of this type have become increasingly 'user-friendly'. The controlled oxidation of hydrocarbon units is another area that has deserved increased attention. For example, oxidation of benzene and simple derivatives by *Pseudomonas* sp. has been researched by a number of U.K. groups.

These recent advances in enzyme-catalysed reactions (using both whole-cell systems and partly purified protein) for the transformation of unnatural substrates is discussed and some areas of interest for the future are outlined.

1. Introduction

Over the past eight years there has been a massive increase in the amount of research aimed at studying the use of enzymes in organic synthesis. Much of this new work has been done in non-specialist laboratories with simple, whole-cells systems (e.g. yeasts) or commercially available hydrolase or oxidoreductase enzymes (Jones 1986).

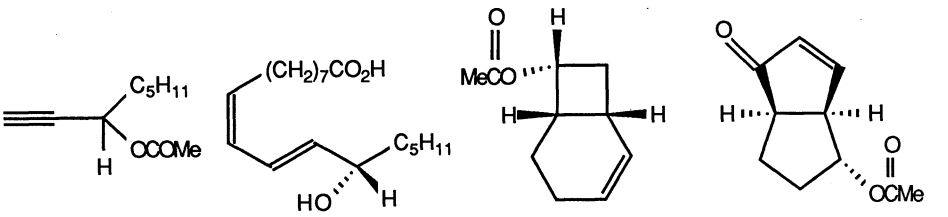
The first part of this review will illustrate some of these new processes; particular attention will be given to situations in which the enzyme catalysed reaction is one step, often the key step, in a multi-stage sequence of transformations. The discussion will be divided into the use of hydrolase enzymes (lipases, esterases, amidases, etc.), oxidoreductase enzymes (principally dehydrogenases) and other biocatalysts (aldolases, etc.). The second, shorter part of the article will deal with the use of multi-enzyme systems for the transformation of simple starting materials into relatively complex end-products.

2. Use of enzyme-catalysed reactions as key transformations in multi-stage sequences of reactions

2.1. *Hydrolases*

Lipases and esterases can be employed to catalyse the hydrolysis of a wide range of substrates. The enzyme catalysed hydrolysis is often preferred to a 'chemical' hydrolysis when optically active compounds are required from racemic or mesomeric substrates. For example,

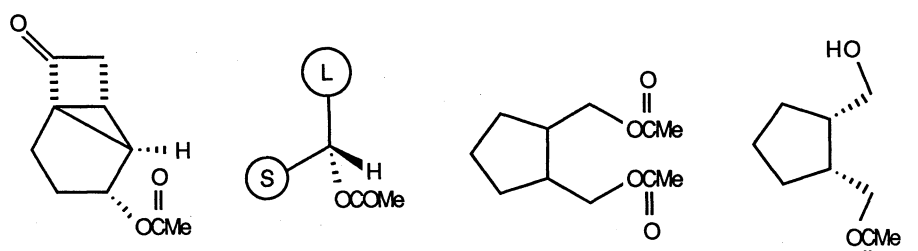
the simple ester 3-acetoxyoct-l-yne (**1**) is hydrolysed by *Mucor miehei* lipase (Chan *et al.*, 1988) or lyophilized yeast (Glänzer *et al.* 1987) to provide 3(*S*)-oct-1-yn-3-ol and recovered optically active ester. 3(*S*)-Oct-1-yn-3-ol has been converted into the natural product 13-hydroxy-



1 **2** **3** **4**

octadeca-9(Z),11(E)-dienoic acid **2** (13-HODE) in five simple chemical steps. 13-HODE may prove to be an important chemorepellant *in vivo* (Buchanan *et al.* 1986).

More complex esters can also be resolved by using lipases. The acetate **3** is readily available from cyclohexa-1,3-diene in four steps. Hydrolysis with porcine pancreatic lipase or *Mucor miehei* lipase proceeds until half of the racemic substrate has been consumed, whereupon the hydrolysis essentially stops. Separation of the recovered ester and the product alcohol gives optically pure materials. The 1(*R*), 6(*S*), 7(*R*)-bicyclo[4.2.0]oct-2-en-7-ol produced in this way has been converted into the potentially useful unsaturated ketone **4** in four steps via the strained tricyclic ketone **5** (Cotterill *et al.* 1988*a*). Sufficient research work has been done on



5 **6** S = small alkyl L = large alkyl **7** **8**

the resolution of racemic esters that the stereo-selectivity of the process can be predicted with some accuracy, at least in qualitative terms. For example, work with the *Mucor miehei* lipase on substrates **1, 3** and a number of other systems (Cotterill *et al.* 1988*c*) suggests that the enzyme preferentially hydrolyses the enantiomer with the structure described in **6**.

Meso-compounds, such as the cyclopentane derivative **7**, have also been hydrolysed in a selective fashion using hydrolase enzymes. The corresponding products, e.g. **8**, can be obtained in high optical purity and in almost quantitative yield (Kasel *et al.* 1985).

So far, esters of chiral alcohols have been described as the substrates for the enzymatic hydrolysis step. An equally large body of work has been documented revealing that esters with chirality present in the acid portion can also be usefully transformed by using enzymes. For example racemic α-amino esters have been hydrolysed with very high stereoselectivity by using hydrolase enzymes such as α-chymotrypsin (Clement & Potter 1971).

The work of Jones in Canada and Ohno in Japan provides some of the most elegant examples in this area of endeavour. Jones has studied the hydrolysis of a wide range of alicyclic

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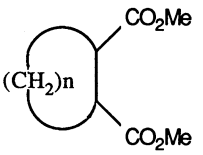
esters of type **9** and has shown that porcine pancreatic lipase can give rise to optically pure monoesters in many instances (Sabbioni & Jones 1987). Ohno has studied the hydrolysis of



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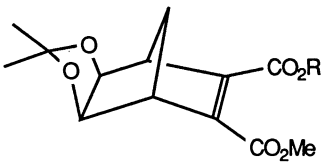
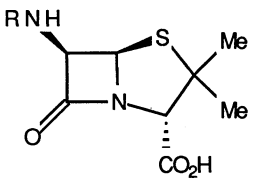


10 R = Me

11 R = H

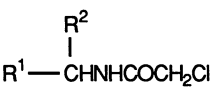
complex diesters such as the tricyclic compound **10**, with pig-liver esterase as the catalyst, and he has shown that the optically active products, e.g. **11**, can be converted, with conventional stereocontrolled chemical transformations, into important natural products such as the carbocyclic nucleoside neplanocin A and some β-lactam antibiotics (Ohno *et al.* 1986).

Amidases have been used for some time for the hydrolysis of amide bonds in sensitive molecules. The conversion of penicillin-G **12** into 6-amino-penicillanic acid **13** by using a bacterial amidase is one example (Lagerlöf *et al.* 1976). The employment of acylases for the



12 R = $PhCH_2CO$

13 R = H



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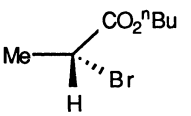

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stereoselective hydrolysis of chloroacetamides **14** has been explored (Baldwin *et al.* 1976) as has the use of an amidase from *Pseudomonas putida* for the enantiospecific hydrolysis of racemic α-aminoamides (Meijer *et al.* 1985).

One very important development in the field of enzyme-catalysed reactions that has emerged over the past few years is the much-increased use of lipases and amidases (proteases) in low-water systems for the *synthesis* of esters and amides. Publications by Klibanov and co-workers drew attention to the potential of this strategy. The American team showed, among other things, that reaction of racemic 2-bromopropanoic acid with *n*-butanol catalysed by porcine pancreatic lipase (or yeast lipase) in hexane containing a trace of water gave unreacted optically active acid and the ester **15** (Kirchner *et al.* 1985). Similarly the racemic secondary alcohol **16**, is esterified with high selectivity by using cyclohexane carboxylic acid and lipozyme (Godtfredsen 1988) to give the ester **18** and recovered optically active alcohol (E. L. A. Macfarlane & S. M. Roberts, unpublished results). Stereoselective transesterification is also possible and the one enantiomer of the acetate **17** reacts preferentially to furnish the ester **18**.

The possibilities that emerge from the use of proteases and lipases for the synthesis of small peptides (Barbas & Wong 1988) are just beginning to emerge. A trypsin-catalysed trans-amidation process has been used to convert porcine insulin into human insulin for use in the clinic (Larner & Pohl 1984).

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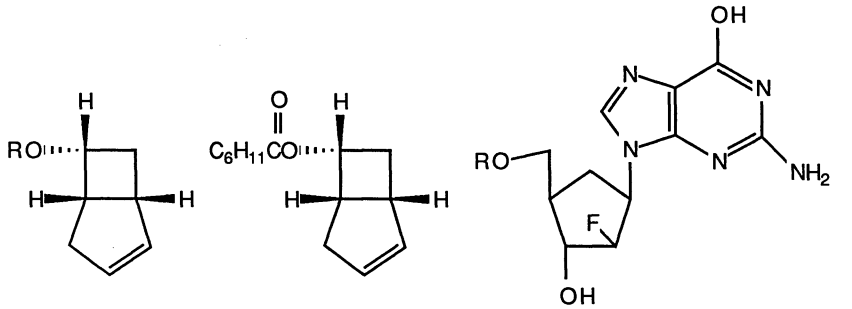


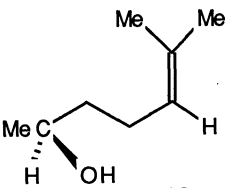
(±) - **16** R = H
(±) - **17** R = COMe

18

(±) - **19** R = PO_3^{2-}
(±) - **20** R = H

$MeCOCH_2CH_2CHCMe_2$

21



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A kinase derived from a virus was used to effect the synthesis of the racemic carbocyclic nucleotide **19** by regioselective phosphorylation of the 5′-hydroxyl group of the corresponding nucleoside. Enantioselective hydrolysis of the 5′-phosphate moiety was achieved by using a snake-venom nucleotidase to produce the optically active nucleoside **20**, which proved to be an extremely potent anti-herpes agent (Roberts *et al.*, 1988).

2.2. *Oxidoreductase enzymes*

Most of the work in this area has been concentrated on the reduction of ketones into chiral secondary alcohols with dehydrogenase enzymes. The enzymes can be used as part of a whole-cell system or as partly purified protein: in the latter case the appropriate co-factor (NADH or NADPH) must be added to the reaction mixture and co-factor recycling must be arranged. The pros and cons of using a whole-cell system as opposed to an isolated enzyme have been discussed elsewhere (Butt & Roberts 1987).

Simple ketones can be reduced efficiently by using this methodology; the achiral alkenone **21** is converted into the optically active natural product sulcatol **22** with a dehydrogenase enzyme from *Thermoanaerobium brockii* with NADPH as the co-factor and *iso*-propanol as the sacrificial alcohol in the recycling system (Keinan *et al.* 1986). The same reduction can be accomplished with whole-cell systems (Belan *et al.* 1987).

More complex ketones with preexisting chiral centre(s) can also be reduced effectively. The usefulness of the strategy will be illustrated with the bicycloheptenones **23** and **24**, both readily available from a [2+2] cycloaddition reaction of the appropriate ketene and cyclopentadiene.

The racemic ketone **23** is reduced with high enantioselectivity by the fungus *Mortierella ramanniana* to give 1(*S*), 5(*R*), 6(*S*)-bicyclo[3.2.0]hept-2-en-6 *endo*-ol and an optically active ketone. Both products have been converted into prostaglandins by enantiocomplementary syntheses (Butt *et al.*, 1985). Prostaglandins and analogues of these natural products are being

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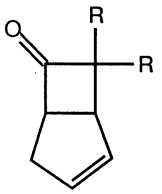
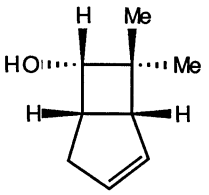


23 R = H
24 R = Me



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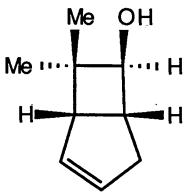

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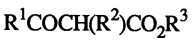
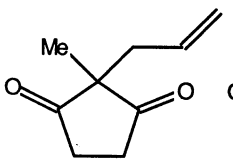


27

investigated as potential cytoprotective agents and as compounds as possible agents for the control of certain forms of heart disease. The ketone **23** is also reduced by the enzyme *Thermoanaerobium brockii* dehydrogenase (with NADPH as co-factor and glucose and glucose dehydrogenase as the co-factor recycling system) to give optically pure 1(*S*), 5(*R*), 6(*S*)-bicyclo[3.2.0]hept-2-en-6 *endo*-ol (Roberts 1988).

In contrast, the ketone **24** is not affected by the *Thermoanaerobium brockii* dehydrogenase enzyme. However, effective enantioselective reduction of this ketone is achieved by using commercially available 3α, 20β-hydroxysteroid alcohol dehydrogenase (employing NADH as the co-factor and ethanol/horse-liver alcohol dehydrogenase as the recycling system) to furnish the secondary alcohol **25** and recovered optically active ketone. The alcohol **25** has been converted into (+)-eldanolide, the pheromone of the sugar cane borer, a pest infesting the sugar cane and maize crops in West Africa (Butt *et al.* 1987). Alternatively the ketone **24** can be added to a fermentation of the microorganism *M. ramanniana* to give the alcohol **25** and the diastereoisomer **26**. Both alcohols have been converted into leukotriene-B_4, a naturally occurring substance that has been implicated as an important factor in the onset and maintenance of inflammatory disorders such as psoriasis (Davies *et al.* 1985).

The reduction of β-keto esters **27** has received a lot of attention. Bakers' yeast has often proved to be the catalyst of choice for work in this area. Thus reduction of ethyl 3-oxobutanoate ((27) $R^1 = CH_3$, $R^2 = H$, $R^3 = C_2H_5$) with yeast provides the 3(*S*)-hydroxybutanoate (Ehrler *et al.* 1986). Substituents can be tolerated at the 2- and 4-positions and the product(s) derived from the reduction can be predicted with some accuracy (Sih & Chen 1984). The conversion of achiral cyclic 1,3-diketones into optically active synthons has been the subject of some intensive research investigations by Brooks *et al.* (1987) and others, following earlier work of relevance to the synthesis of steroids (Kieslich 1976). Thus the dione



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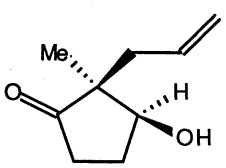

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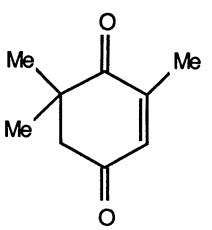

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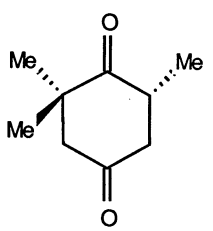

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28 was reduced by yeast to give the hydroxyketone **29** which was, in turn, converted into the naturally occurring compound coriolin (Brooks *et al.* 1985). More recently the researchers have concentrated on the reduction of 1,3-dione moieties in larger ring systems.

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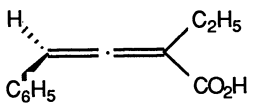
Reduction of carbon–carbon double bonds in αβ-unsaturated carbonyl compounds can be accomplished with reductase or hydrogenase enzymes in whole-cell systems. The dione **30** afforded the compound **31** on incubation with bakers' yeast (this reaction has been conducted on a substantial scale (Leuenberger *et al.* 1976), and *Clostridium* species contain a hydrogenase that will effect conversion of the allene **32** into the acid **33** (Simon *et al.* 1981). Reduction reactions of this type have not been fully exploited for the preparation of new, useful synthons.



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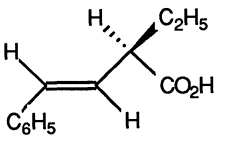

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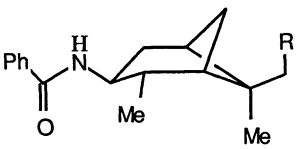
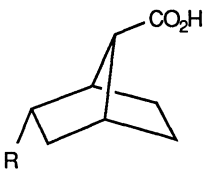


34 R = H
35 R = OH

The hydroxylation of an acyclic or an alicyclic compound in a regioselective and stereoselective fashion at a carbon centre remote from functional groups is not readily accomplished by chemical reagents. Mono-oxygenases in whole-cell systems are capable of introducing one or more hydroxy groups at ostensibly non-activated positions in a molecule but the prediction of the site of oxidation is not straightforward. One of the methyl groups attached to the bridging carbon atom in the pinane derivative **34** is hydroxylated with good selectivity by the microorganism *Beauvaria sulfurescens* to give the alcohol **35** (Archelas *et al.* 1986). Similarly the carboxylic acid **36** has been converted into the optically active hydroxy acid **37** (57% yield) (Yamazaki & Maeda 1985).

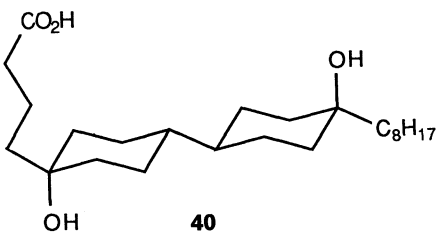


36 R = H
37 R = OH

38 R = H
39 R = OH

Cyclohexylcyclohexane **38** is converted into the diol **39** with good selectivity by using *Cunninghamella blakesleeana* and other microorganisms. The diol **39** was transformed into the more complex substance **40** by a series of standard chemical transformations in an attempt to prepare an antagonist of the leukotrienes-B (Davies *et al.* 1986).

Recent papers in the literature describing the conversion of benzene into the diol (**42**, R = H) with an oxygenase system in the microorganism *Pseudomonas putida* have created a lot of



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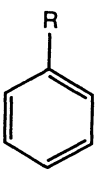

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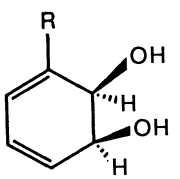

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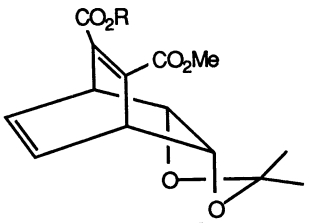
excitement. The diol has been converted into polyphenylene (Taylor 1985) and into the natural product (±)-pinitol (Ley *et al.* 1987). Cyclohexa-3,5-diene-1,2-*cis*-diol (**42**, R = H) has recently been transformed into a variety of bicyclic molecules (for example, the diester **43**) by way of Diels–Alder reactions on appropriately protected material (Cotterill *et al.* 1988*b*). Interestingly, treatment of the diester **43** with pig-liver esterase gave an optically active mono-ester which was tentatively assigned the structure **44**. Mono-substituted benzene derivatives,

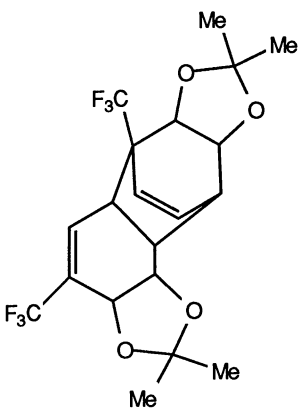


43 R = Me
44 R = H

e.g. **41**, R = CH_3 (Hudlicky *et al.* 1988), and di-substituted benzene derivatives (Taylor *et al.* 1987) are also biotransformed with selected microorganisms to give cyclohexa-1,3-diene derivatives. The bonus accrued is that from mono-substituted benzenes, and di-substituted benzenes possessing two different substituents, chiral products are formed often displaying high enantiomeric excess. Interesting chemical properties are observed for some of these substances: after protection as an acetal, 3-trifluoromethylcyclohexa-3,5-diene-1,2-diol (**42**, R = CF_3) readily dimerizes, even at low temperature; to give the polycyclic compound **45** (C. Pittol, R. J. Pryce, S. M. Roberts, G. Ryback & J. O. Williams, unpublished results). Conversion of chlorobenzene into 3-chloro-cyclohexa-3,5-diene-1(*S*)2(*S*)-diol provided an early stage intermediate for prostaglandin synthesis (T. Hudlicky, personal communication).

The formation of hydroxylated derivatives of heterocyclic aromatic compounds is also currently being investigated.

There are a number of other oxidoreductase enzymes that will attract some attention over the next few years. Cyclohexanone oxygenase from *Acinetobacter* sp. performs the equivalent of a chemical Baeyer–Villiger reaction on cyclic ketones. For example, cyclohexanone formed the corresponding caprolactone **46** (Walsh & Chen 1988). The full potential of the oxidation system should be explored.



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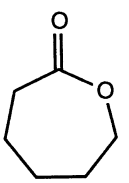

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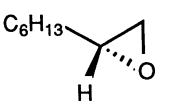

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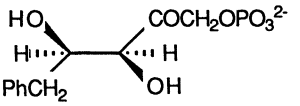
The stereoselective epoxidation of allylic alcohols and some homoallylic alcohols is best done with the Sharpless method (Gao *et al.* 1987). On the other hand, stereoselective epoxidation of alkene units remote from hydroxy groups (or any other functionality) can be effected by using microorganisms: the conversion of oct-1-ene into the epoxide **47** with *Pseudomonas oleovarans* provides one example (de Smet *et al.* 1981). More fundamental research is needed in this area.

2.3. *Other biotransformations*

There are a plethora of other bioconversions that are becoming used for the synthesis of new, potentially interesting substances. Only a small number of examples are cited in this review to whet the appetite of the reader; two recently published books (Davies *et al.* 1989; Wong & Whitesides 1989) highlight many of the other exciting possibilities for future research.

Mandelonitrile lyase, isolated from bitter almonds and immobilized on cellulose, catalyses the addition of HCN to a variety of aldehydes to furnish the corresponding (*R*)-cyanohydrins **48** (Effenberger *et al.* 1987).

48



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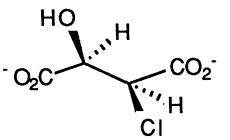

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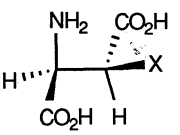
One of the most useful reactions in organic chemistry is the aldol reaction, and good progress has been made in finding conditions under which aldolase enzymes catalyse the formation of the requisite carbon–carbon bond. For example, dihydroxyacetone phosphate reacts with 2-phenylethanal, under catalysis by an aldolase enzyme, to give the adduct **49**; a wide variety of aldehydes undergo a similar transformation (Wong 1986; Effenberger & Straub 1987).

The addition of water and ammonia to αβ-unsaturated acids such as derivatives of fumaric acid has been studied in recent years. Thus chlorofumaric acid is hydrated to give L-threochloromalic acid **50** on catalysis with pig-heart fumarase (Findeis & Whitesides 1987). The use of 3-methylaspartate ammonia lyase for the conversion of halofumarates to amino acids such as **51** has been described recently (Akhtar *et al.* 1987) as has the employment



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of cloned *E. coli* aspartate transaminase for the conversion of various aromatic and aliphatic α-ketoacids into the corresponding L-α-amino acids (Baldwin *et al.* 1987).

3. Multi-step enzyme-catalysed reactions for the conversion of simple substrates into complex products

Some enzymes can perform more than one reaction on a suitable substrate. The work of Baldwin in Oxford provides an example. The conversion of the tripeptide **52** into iso-penicillin-*N* **53** is well documented (although the details of this rather complex transformation are not

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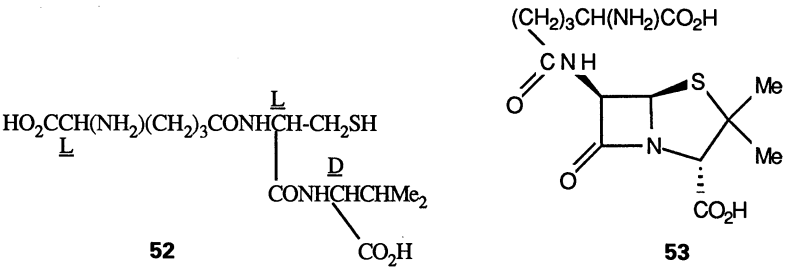

52 53

yet known). The Oxford group have shown more recently that unnatural substrates are also accepted by the enzyme and transformed into new potentially important antibiotics (Baldwin 1985).

There are a number of ways of obtaining optically active amino acids by means of enzymes-catalysed reactions (see above). One method that highlights the mutual compatibility of enzymes, thus allowing them to be used in the same reaction vessel, is shown in figure 1. The enzyme cofactor recycling system is particularly noteworthy (Schmidt-Kastner & Egerer 1984).

Last, but not least, the recent work of Whitesides at Harvard, and others, has focused

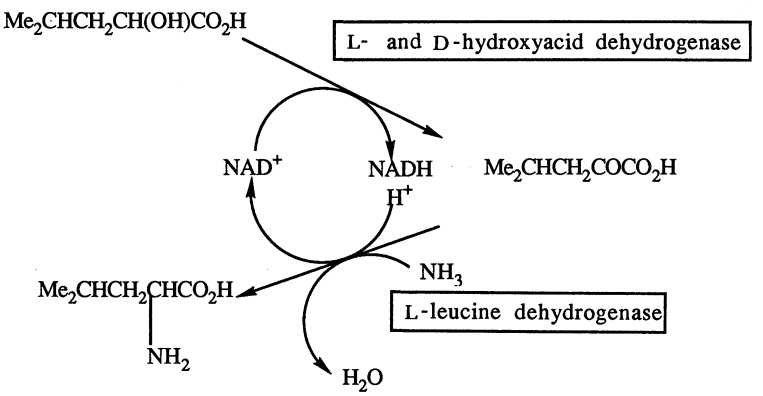

FIGURE 1. A multiple-enzyme system for the production of optically active amino acids.

attention on the possibilities involving the formation and further transformation of relatively unstable compounds by using a series of enzyme-catalysed transformations (figure 2) (Card *et al.* 1986).

The latter figure illustrates, far better than words, the tremendous potential of using coupled enzyme-catalysed reactions in organic synthesis.

4. SOME OF THE POSSIBILITIES FOR THE NEXT TEN YEARS

The employment of hydrolase and oxidoreductase enzymes for the formation of compounds, particularly optically active substances, useful for the synthesis of interesting and potentially important molecules will continue to become more popular. Other transformations (e.g. enzyme-catalysed aldol and other carbon–carbon bond-forming reactions) will become more routinely used in non-specialist laboratories. The use of various enzymes in low-water systems will be fully investigated.

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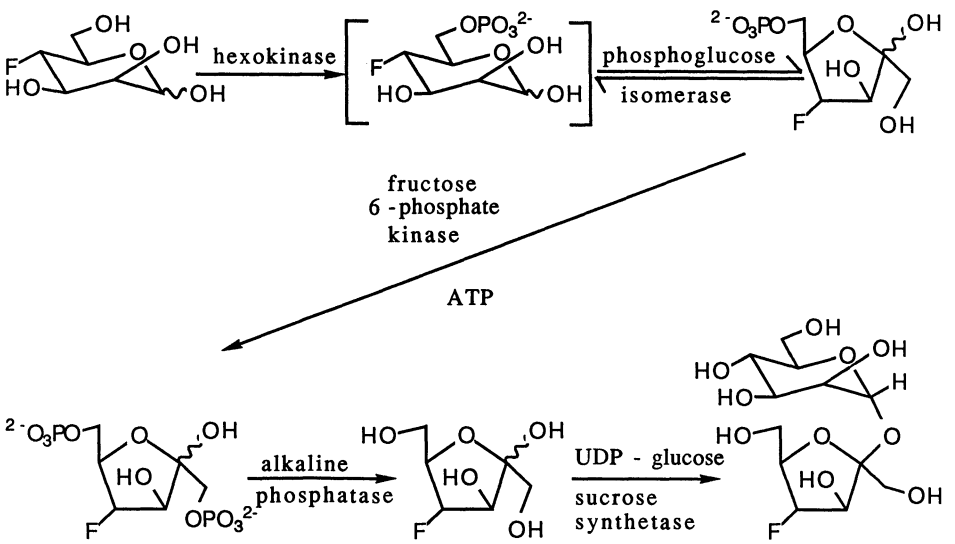

FIGURE 2. Enzyme-catalysed preparation of a sugar mimic.

Microorganisms will be modified by genetic engineering to allow processes to occur that are foreign to the wild-type organism. The amino acid sequence of naturally occurring enzymes will also be altered to provide modified catalytic activities (Clarke *et al.* 1987): the possibility of using antibodies as catalysts will be explored further (Napper *et al.* 1987).

Thus the prospects are that enzyme-catalysed reactions will become widely accepted as 'tools of the trade' in synthetic organic chemistry. Enzymes and whole-cell systems will be used by more and more research scientists working on a relatively small scale (less than 10 g of substrate). Obviously the number of biotransformations that need to be scaled up to be of use in the preparation of large quantities of material will increase; probably by the year 2000 we shall see double the number of large-scale biotransformation processes of industrial significance.

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