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Potentially useful lipase-catalysed transesterifications

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Abstract. Organic reactions catalysed by commercially available lipases, reported in recent years from our own laboratory and those published by others are described, confining the discussions to esterification and hydrolytic reactions only. The text gives an insight into the variety of substrates accepted by these lipases and their potential applications to catalyse reactions regio-, chemo-, enantio- and diastereoselectively to produce compounds of industrial, commercial, pharmaceutical and synthetic interest.

Keywords. Biocatalysis; enzymes; lipases; transesterifications.

1. Introduction

The immense potential of lipases to catalyse various kinds of organic reactions *in vitro* is now being recognised (Simon *et al* 1985; Whitesides and Wong 1985; Jones 1986; Yamada and Shimizu 1988; Wong 1989). Chemists now love to use enzymes if these can serve their purpose for certain obvious reasons, i.e.

(a) high selectivity can be achieved by putting in relatively less effort if enzymes are used,
(b) conditions (which are environmentally friendly) required for carrying out such reactions can easily be maintained,
(c) many of the enzymes are now commercially available and are fairly inexpensive.

However, an inherent shortcoming is that enzymes require aqueous conditions in which most of the organic substrates do not dissolve resulting in poor product yields. Recent work by different teams of workers has resulted in an important realisation that quite a few enzymes can work in organic solvents containing little or no added water (Klibanov 1986, 1989; Halling 1987; Deetz and Rozzell 1988; Zaks and Russell 1988; Dordick 1989).

Biocatalysis in organic solvents has revealed some beneficial phenomena, e.g. the stability of enzymes is more in organic media (Zaks and Klibanov 1984; Aldercreutz and Mattiasson 1987); substrate specificity of enzymes can be regulated by the solvent (Zaks and Klibanov 1986; Russell and Klibanov 1988); enantioselectivity can be controlled by the choice of solvent (Sakurai *et al* 1988); the enzymes, being insoluble in organic solvents can be filtered out after the reaction is complete and reused etc.

Many of the commercially available lipases are versatile with regard to their catalytic activity, i.e. they accept a variety of structurally different compounds as substrates; porcine pancreatic lipase (PPL) and *Candida cylindraceae* lipase (CCL)

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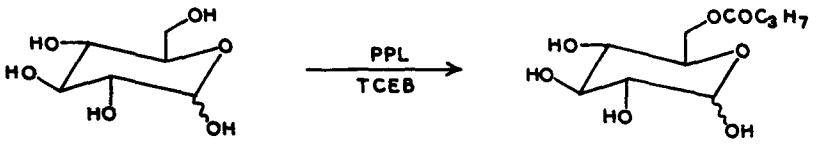
are among such broad spectrum lipases which have widely been used to catalyse an array of reactions, among which esterification and hydrolysis are more common.

Here, we intend to give some representative examples of lipase-catalysed reactions, including some of our own findings.

2. Lipase-catalysed reactions

2.1 *Esterification reactions catalysed by lipases*

Klibanov and others examined the acylation of a range of sugars catalysed by PPL in pyridine, e.g. galactose (1) was acylated with PPL and trichloroethyl butyrate (TCEB) with 95% regiospecificity to give 6-0-butyroyl galactose (2) in 60% isolated yield. Regioselective acylation of sugars is a fundamental and difficult task in organic chemistry (Sujihara 1983). The efficient preferential acylation of primary over secondary hydroxyl group can only rarely be carried out with free sugars. This usually requires protected sugars (Bollenback and Parrish 1971), thereby necessitating cumbersome protection and deprotection steps (Haines 1981). It was observed that lipases have the potential to catalyse such reactions in organic solvents (Zaks and Klibanov 1984).



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Fatty acid esters of sucrose are currently utilised as anticancer agents, biodegradable surfactants and emulsifiers in foods and cosmetics (Khan 1984). Such esterifications are now known to be catalysed by PPL and active esters in organic solvents (Cesti *et al* 1985).

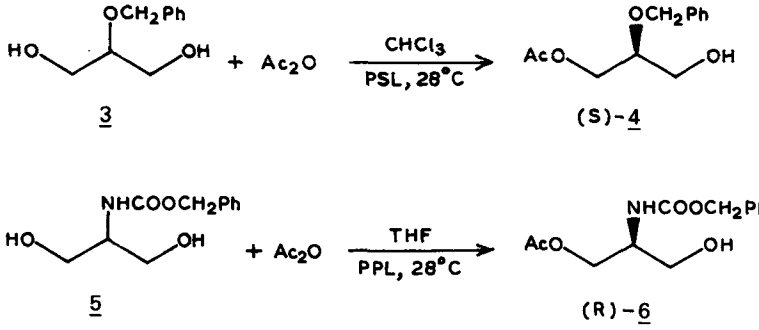
Ethyl D-glucopyranoside can be esterified with C_8–C_{18} fatty acids in yields of 85–95% of the 6-O-monoesters using immobilised CCL as catalyst. The surfactant properties of these esters have been found to be similar to those of common non-ionic surfactants (Ladner and Whitesides 1984). Regioselective acylation of the primary hydroxyl group in alkyl furanosides and pyranosides leading to the formation of fatty acid esters of carbohydrates having potentially important applications in detergents, food preparations, cosmetics and pharmaceuticals has been carried out using PPL (Hennen *et al* 1988; Bjorkling *et al* 1989; Carrea *et al* 1989; Adelhorst *et al* 1990; Chauvin and Plusquellec 1991).

Double acylation of sugars in the presence of PPL, CCL and *Pseudomonas fluorescens* lipase (PFL) leads to the formation of 6-O-, 2-O- and 3-O-di/triacylated sugars. The acylation does not take place at the 4-O-position (Colombo *et al* 1991). PFL is also known to catalyse the acylation of sugar moieties of nucleosides at the primary hydroxyl group (Uemura *et al* 1989). Regioselective esterification of methyl-4,6-O-benzylidene-α- and -β-D-glucopyranosides to give β-monoesters was carried out using PFL. The regioselectivity of the enzyme is established as being dependent on the anomeric configuration of the glucosidic acceptor (Chin *et al* 1992).

Esterification and transesterification reactions catalysed by lipases *in vitro* have been utilised to accomplish the synthesis of a variety of compounds having synthetic, pharmaceutical and commercial importance. In these reactions, the lipases show average to excellent regioselectivity, chemoselectivity, enantioselectivity and diastereoselectivity too, depending on the substrates used.

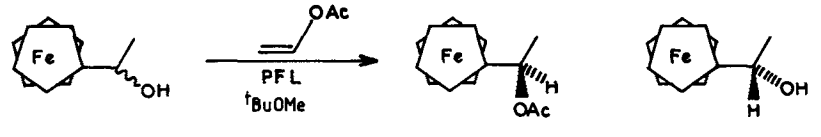
Lipases are used for the resolution of recemic acids by esterifying them with an alcohol; CCL catalyses the esterification of the *R*-isomer of the racemic α-halo substituted aliphatic acids in hexane with the overwhelming preference over its *S*-counterpart (Brandstorm 1983).

Chiral (*R*)- or (*S*)-3-O-acetyl-2-O-benzylglycerol (4) and (*R*)- or (*S*)-3-O-acetyl-2-N-(benzyloxycarbonyl) serinol (6) are considered to be useful building blocks for the preparation of enantiomerically pure biologically active molecules, such as phospholipids (Baer *et al* 1952), platelet-activating factor (Suemune *et al* 1986), phospholipase A_2 inhibitors (Dennis 1987) and sphingolipids (Koyke *et al* 1987). To prepare these chiral synthons, the prochiral diols 2-O-benzylglycerol (3) and 2-O-(benzyloxycarbonyl)serinol (5) were chosen as substrates and the acylation was done using acetic anhydride with a lipase from *Pseudomonas* species (PSL) and PPL at room temperature to yield (*S*)- (4) and (*R*)- (6) esters respectively in excellent yields (Wang *et al* 1988).



Stereospecific acylation of terpenic alcohols by aliphatic anhydrides has been carried out using PPL, CCL and PFL. The lipase is able to recognise the structural dissymmetry located three atoms away from the bond to be created and best results are obtained when two substituents are of very different size. With the allylic alcohols, even when the dissymmetry is located five atoms away, the enzyme displays a relatively high selectivity (Fourneron *et al* 1990).

PFL has been used for enantioselective acylation of an organometallic compound, i.e. ferrocene derivative (7), which is used as an optically active catalyst and ligand (Boaz 1989).

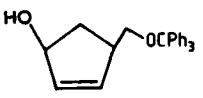


The chemoenzymatic enantioselective synthesis of epichlorohydrins which serve as versatile C_3-synthons in the synthesis of an array of important molecules has been achieved using PPL (Chen *et al* 1990b). Enantiomerically pure (*R*, *R*) and (*S*, *S*) 1,2-cycloalkane diols can be prepared by both, the enzymatic hydrolysis or esterification using PFL; these find uses in the synthesis of optically active crown ethers or as auxiliaries for the preparation of chiral bidentate ligands (Seemayer and Schnieder 1991).

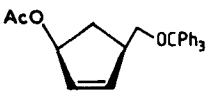
Enantioselective esterification of racemic 2-hydroxy acids using CCL in dry organic solvents with primary alcohols as nucleophiles, followed by chemical reduction leads to the formation of optically active 1,2 diols, which are useful intermediates in the synthesis of antibodies, optically active polymers and chiral solvents (Parida and Dordick 1991). PPL catalyses the enantioselective O-acylation of amino alcohols to yield chiral aminoalcohols having useful pharmaceutical properties (Gotor *et al* 1988; Skorey *et al* 1989).

Dialkyl amphiphile (lipid) coated *Pseudomonas fragi* lipase has been used to resolve the racemic alcohols in anhydrous organic solvents (Okahata *et al* 1988).

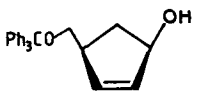
Enantioselective esterification of racemic 2-methyl alkanoic acids using CCL leads to the formation of (*S*)-esters and (*R*)-acids. Optically pure 2-alkyl alkanoic acids are useful building blocks for the synthesis of biologically active compounds with branched chain structures, e.g. pheromones (Engel 1991). Enantioselective acylation of 4-*cis*-hydroxycyclopent-2-enylmethyl triphenyl methyl ether (9) to give optically pure (+)-ester (10) and (−)-9 alcohol having optical purities > 95% has been reported. These esters are used to prepare (+)-carbovir (11), a chemotherapeutic agent for the treatment of AIDS infection. These esters are used as valuable precursors of various classes of carbocyclic nucleosides (Evans *et al* 1992).



(±)−9



(+)−10



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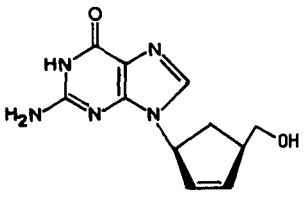
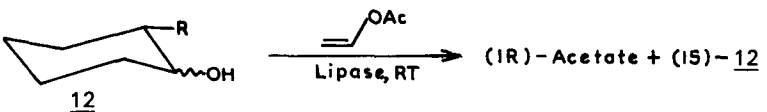


(+)−11

Resolution of cyclohexanols (12) (Laumen *et al* 1990), 4-hydroxy-2-alkylcyclopentenones (13) (Babiak *et al* 1990), β-hydroxytosylates (14) (Chen and Liu 1989), secondary alcohols (Hsu *et al* 1990) and *endo*-norbornene lactone (15) (Janssen *et al* 1990) has been achieved by transesterification reactions catalysed by PSL, PPL and CCL.



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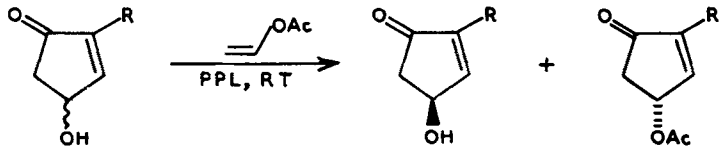

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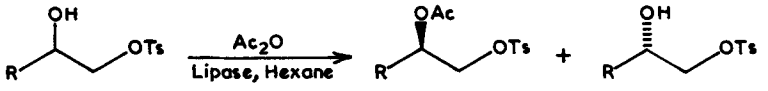
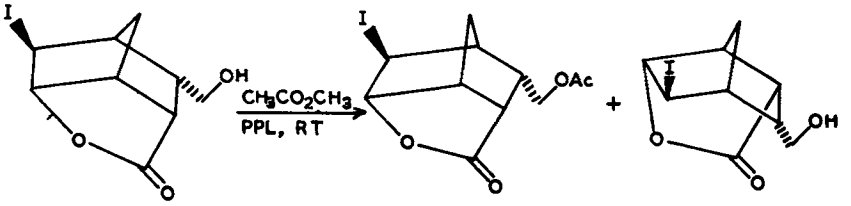
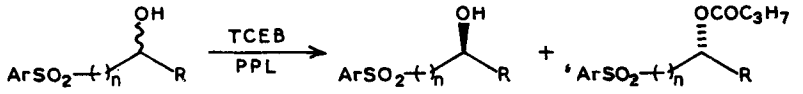


(±) 14



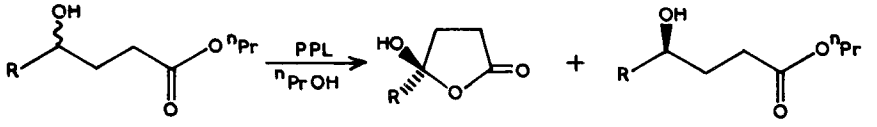
(±)-15 (+) (−)

Resolution of several cyanohydrins lead to the isolation of enantiomerically pure synthons which can be converted to compounds of pharmaceutical interest, viz. *S*-(−)-propanolol, an adrenergic blocking agent. Such transesterifications are catalysed by PPL (Wang *et al* 1989). *β*-, *γ*- and *δ*-hydroxysulphones (16), which are excellent building blocks for the synthesis of chiral lactones, desepoxiasperdiol, prostaglandins etc. are resolved by transesterification using PPL (Chinchilla *et al* 1990). PPL or *Chromobacterium viscosum* lipase (CVL) catalyses the transesterification reaction between ethyl esters of carboxylic acids and tributyl stannyl ethers of primary and secondary alcohols (Therisod 1989).



(R,S)-16 (S)-16 (R)-ester

Intramolecular transesterification is also known to be catalysed by PPL when *n*-propyl esters of (*R*,*S*)-4-hydroxyalkanoic acids (17) in diethyl ether at 20°C give (*S*)-4-alkylated lactones (18) of high optical purity (> 80%) and optically pure (*R*) 4-hydroxyalkanoic *n*-propyl esters, which are widely used as intermediates in the synthesis of natural products and are important widespread flavour compounds (Huffer and Schreier 1991).

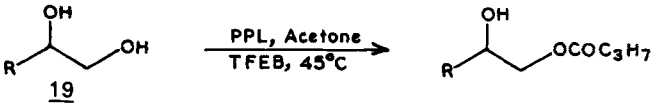


(R,S)-17 (S)-18 (R)-17

Cis- and *trans*-4-hydroxycyclopent-2-enylmethanol derivatives are resolved by esterifying them with vinyl acetate in the presence of PFL to yield (1*R*,4*S*) acetate and (1*S*,4*S*) alcohol with enantiomeric excess (*ee*) > 95%. The products obtained are extremely useful building blocks for the preparation of optically pure carbocyclic nucleosides (Roberts and Shoberu 1991).

CVL, absorbed on Hyflo Super Gel catalyses the irreversible transesterification of substituted monohydric phenol with vinyl acetate in a mixture of cyclohexane:THF (95:5) (Nicolosi *et al* 1992).

We have carried out a few interesting transesterification reactions catalysed by the lipases, PPL and CCL in different organic solvents. In the 1,2-alkanediols (19), the acylation takes place exclusively at the primary hydroxyl group (Parmar *et al* 1993c); also in the case of butane-1,3-diol (20), the acylation takes place regioselectively at the primary hydroxyl. In the case of D-panthenol (21), which has two primary hydroxyl groups and one secondary hydroxyl group, the one at the far end position of the asymmetric carbon is acylated regioselectively and N-(3'-butanoyloxy)propyl-3,3-dimethyl-2,4-dihydroxybutanamide (22) is obtained in 94% yield (Parmar *et al* 1993c).



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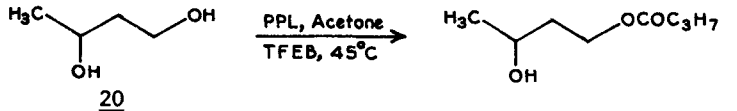
R = Ph, Me, Et and ^{n}Pr



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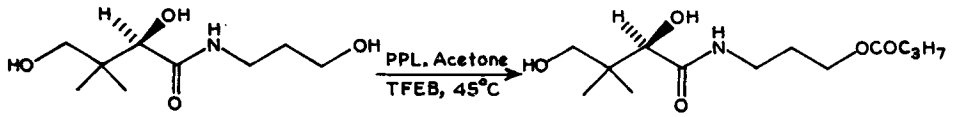

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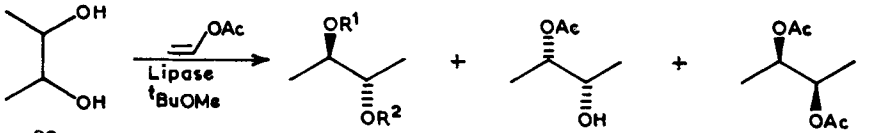
To reasonably explain the regioselective acylation of D-panthenol, we have proposed (Parmar *et al* 1993c) a schematic model for the active site of the enzyme PPL taking a clue from the two models put forward for CCL by two other groups of workers (Bhalerao *et al* 1991; Hult and Norin 1992). The lipase from *Pseudomonas fluorescens* (Lipase P) exhibits diastereo- and enantioselectivity towards the transesterification of *meso* and DL mixture of butane-2,3-diol (23). The reaction yields monoacetates



23 24 25 26

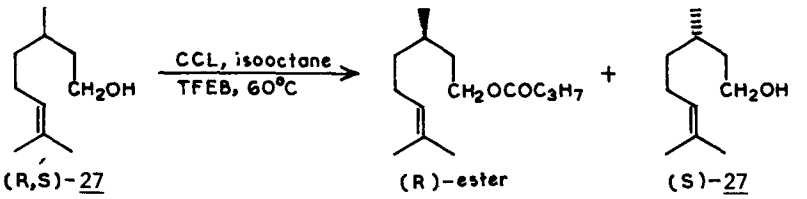
meso + dl

R1 = H, R2 = Ac

R1 = Ac, R2 = H

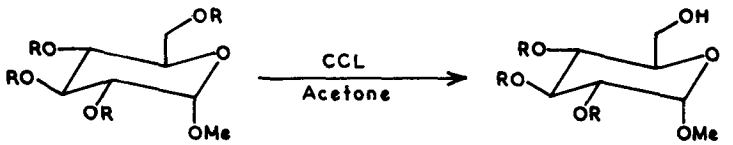
of *meso* diol (24), monoacetate of (2*S*, 3*S*)diol (25) and diacetate of (2*R*, 3*R*) diol (26). The (*R*, *R*) diacetate (26) thus obtained has the *ee* of > 98% (Bisht *et al* 1993).

We have also studied the temperature effect and solvent effect on the lipase-catalysed transesterification reaction to optimise the conditions for desired stereoselectivity. Using the racemic β-citronellol (27) as substrate, we have established that the best *R*/*S* ratio is obtained in isooctane among an array of solvents screened with CCL as the catalyst. The best enantioselectivity was displayed at 60°C in this reaction (Parmar *et al* 1992c).



2.2 *Hydrolytic/deacylation reactions catalysed by lipases*

Lipases have the potential to catalyse transesterifications as well as hydrolytic reactions. A number of studies aimed at selective protections and deprotections with lipases have been carried out successfully in carbohydrate synthesis. An example that spells success is the selective deprotection of the primary 6-O- ester group from the fully protected glucose derivative 1-O-methyl-2,3,4,6-tetra-O-pentanoyl-D-glucopyranoside (28) using CCL in acetone which affords the deprotected product (29) in 90% yield (Sweers and Wong 1986).



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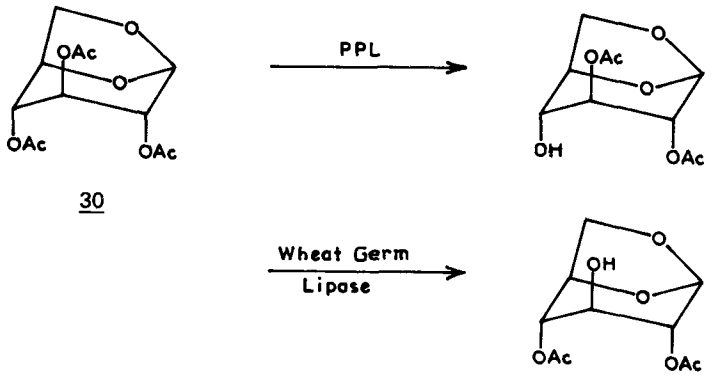
Enzyme-catalysed deacylation of 1,6-anhydro-2,3,4-tri-O-acetyl-D-glucopyranose (30) led to the preferential hydrolysis of either the C-3 acyl group when wheat germ lipase was used or the C-4 acyl group when PPL was used (Zemek *et al* 1987).

Selective cleavage of 1-O-acyl group of per-O-acylated derivatives of carbohydrates was achieved by using immobilised PPL (Chin *et al* 1992).

CCL has been used to hydrolyse the racemic acetate of *endo* norborneol. After 40% conversion, the (+)-*endo* norborneol was obtained with 90% *ee*. The racemic *exo* acetate of norborneol was hydrolysed very slowly without any stereoselectivity. The (+)-*endo* norborneol is a useful intermediate in the synthesis of nucleoside analogues (Eichberger *et al* 1986). Enantio- and regioselective hydrolysis of racemic 2,7-diacetoxy bicyclo [2·2·1] heptane to (−)-monoacetate is facilitated by PPL, CCL and PFL. The acetoxy group located on the methano bridge is preferentially hydrolysed (Chenevert *et al* 1990). Using CCL again, the resolution of racemic butyrates of *endo*



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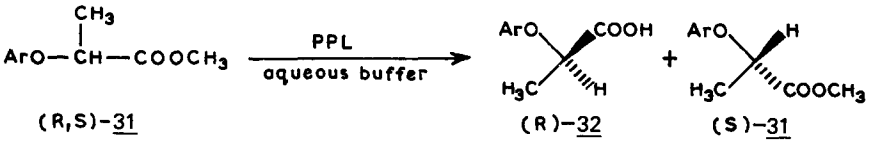
bicyclo [2·2·2] octanols and *endo*-bicyclo [2·2·1] heptan-2-ols is achieved. The enantioselection of resolution depends on the steric factors of substituents and increases with decrease in the size of substituents (Konigsberger *et al* 1989). Steroselective hydrolysis, catalysed by CCL of 2,6-diacetoxybicyclo [3·3·1] nonane gives (+)-(1*S*, 2*R*, 5*S*, 6*R*)-6-acetoxybicyclo [3·3·1] nonane-2-ol and (−)-(1*R*, 2*S*, 5*R*, 6*S*)-2,6-diacetoxybicyclo [3·3·1] nonane, which are used in the synthesis of optically active crown ethers and podands (Naemura *et al* 1989). Bicyclo [3·2·0] hept-2-en-6-ols, central building blocks for the synthesis of chiral cyclobutane and cyclopentane systems have been prepared using CCL and PFL with > 99% *ee* by the resolution of their acetates and/or butyrates (Klempier *et al* 1990). 6-Acetoxy-7,7-disubstituted bicyclo [3·2·0] hept-2-ene is enantioselectively hydrolysed by PPL, CCL and *Pseudomonas cepacia* lipase to yield (*R*)-alcohol regardless of the configuration of the main framework of the substrate. It was always the enantiomer possessing (*R*)-configuration at the acetate-bearing carbon atom which preferentially reacted in case of both the diastereomeric substrates. The products obtained are found to be the intermediates for the synthesis of numerous bioactive compounds, such as pheromones and leukotrienes (Cotterill *et al* 1991).

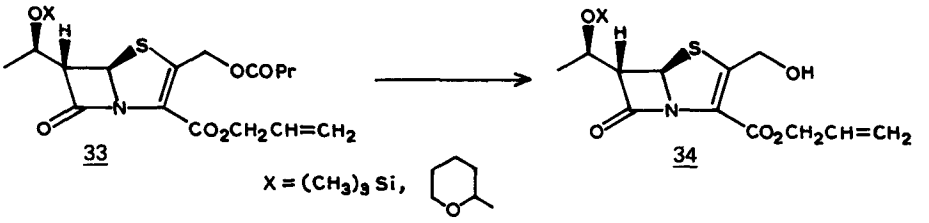
The racemic acetates or *n*-butyrates of substituted carboxylic esters are enantioselectively hydrolysed by PPL, CCL and PFL (Miyazawa *et al* 1989). Enantioselective hydrolysis of α-chlorophenoxy methyl propanoates (31) catalysed by PPL leads to the formation of corresponding optically active α-chlorophenoxypropanoic acids (32) which serve as plant growth regulators (Chenevert and D'Astous 1988).



(R,S)-31 (R)-32 (S)-31

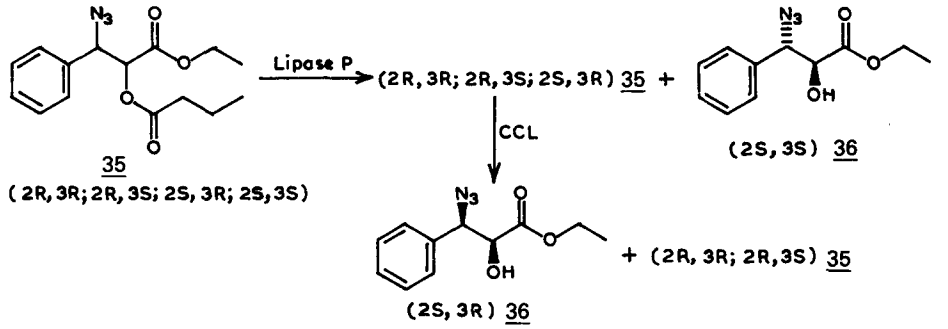
Ar = o-ClC6H4, m-ClC6H4 and p-ClC6H4

CVL and lipoprotein lipase from *Pseudomonas* species facilitate the selective hydrolysis of the mixed esters (33) to the corresponding monodeacylated derivatives (34), which in several steps can be converted into the valuable penem antibiotic FCE 22101 and FCE 22891 in high yield, without involving the expensive protecting groups (Altamura *et al* 1989).



PPL enantioselectively hydrolyses the esters of racemic epoxy secondary alcohols leading to the formation of optically active epoxy secondary alcohols (Marples and Rogers-Evans 1989). *Aspergillus niger* hydrolyses the acetate of racemic (α-phenyl)-1-(2-phenylethyl)-4-piperidinemethanol to give the (−) hydrolysed product (Nieduzak and Carr 1990). Acetic or butyric esters of α- and β-hydroxyaldehydes are stereoselectively hydrolysed by PPL producing the corresponding optically pure *R*-alcohols and *S*-esters (Bianchi *et al* 1989). *Mucor javanicus* lipase is known to hydrolyse stereoselectively the *meso* dibutyrates of substituted tetrahydrofuran diols leading to the formation of corresponding monoesters. Substituted tetrahydrofurans are important subunits in many naturally occurring polyether antibiotics (Estermann *et al* 1990).

Enantiomerically and diastereomerically pure 3-azido-2-hydroxy-3-phenylpropanoate (36) has been separated from a mixture of racemic, *threo*- and *erythro*-3-azido-2-butanoyloxy-3-phenyl propanoate (35) by hydrolysing it in the presence of lipase P from *Pseudomonas* species or CCL (Honig *et al* 1990).

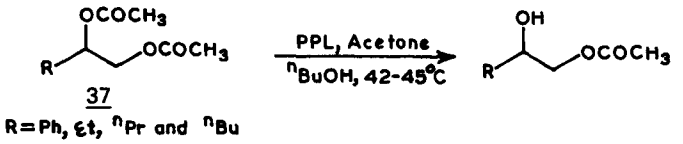


Cis-1,2-*bis*-(butyryloxymethyl)cyclopropane under optimised conditions in presence of PPL hydrolyses to optically pure *cis*-(1*R*,2*R*)-1-hydroxymethyl-2-butyryloxymethyl cyclopropane, an important synthon for the synthesis of seaweed pheromone, called dictyopterene (Grandjean *et al* 1991).

Regioselective deacylation of 5,5-disubstituted *bis*-acyloxymethyl barbiturates in water-saturated diisopropyl ether is carried out by PPL, CCL and PFL to give the corresponding N-acyloxymethyl barbiturates which are used for the synthesis of *R*-(−)-mephobarbital (sedative) and *S*-(+)-hexobarbital (anaesthetic) (Murata and Achiwa 1991). PPL catalyses the hydrolysis of 3,5-*cis*-diacetoxy-4-*trans*-benzyloxymethyl cyclopentane to (−)-monoester, a synthetic precursor of (−)-aristeromycin

(LeGrand and Roberts 1992). *Mucor miehei* lipase helps in enantioselectively hydrolysing (*R*, *S*)-3-acetoxyoct-1-yne to yield (*S*)-oct-1-yn-3-ol and the latter has been used to prepare an antifungal agent, namely coriolic acid (Chen *et al* 1990a).

We have studied regioselective deacetylation of aliphatic diacetates (37), where we have established that it is the secondary acetoxy which is exclusively deacetylated (Parmar *et al* 1993c).



37

R=Ph, Et, nPr and nBu

We have recently carried out the enzymatic deacetylation of peracylated polyphenols and related compounds. In the course of our studies, we have found that polyphenolic peracetates in suitable organic solvents are regioselectively deacylated by PPL and CCL at positions *para* and *meta* to the carbonyl group and not at the *ortho* or *peri* position (Parmar *et al* 1992a, b, 1993a, b; Bisht *et al* 1994). This phenomenon is complimentary to chemical hydrolysis. We have tried to rationalise this fact by proposing an enzyme–substrate complex model where the carbonyl function plays an important role. In addition to serine, the lipase may also contain lysine (having free ξ-amino group) in its active site. The substrate may bind covalently to the enzyme through the formation of a Schiff's base by reaction between the carbonyl group of the substrate and the ξ-amino group of the lysine residue. It is assumed that Schiff's base is formed in such a way that the hydroxyl group of serine residue can approach the *para* acetoxy group and to a lesser extent the *meta* acetoxy group, but the *ortho* acetoxy group is buried in active site near the lysine residue and is not accessible to the serine residue (Parmar *et al* 1992a). These reactions are of general applicability in the synthesis of different types of biopolyphenolics.

We have studied the deacylation of different amino phenol diacetates with CCL and PPL. These lipases have exhibited an overwhelming chemoselectivity by hydrolysing the phenolic acetate group (Parmar *et al* 1992d).

3. Conclusion

Enzymes offer a promising method to manipulate a synthetic organic reaction in a desired fashion. With cheaply available commercial enzymes, the reactions are economical and environment-friendly. Intelligently designed and accidently hit upon methods using lipases have paid off and have attracted researchers to explore newer and cheaper means for performing various kinds of reactions which were otherwise difficult, and in some cases, not even feasible.

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