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Novel biotransformations on peracylated polyphenolics by lipases immobilized in microemulsion-based gels and on carbohydrates by *Candida antartica* lipase

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ABSTRACT- The lipases from porcine pancreas (PPL) and *Candida cylindracea* (CCL), solubilized in AOT [sodium *bis*-(2-ethylhexyl) sulphosuccinate] water-in-oil (w/o) microemulsions and supported on gelatin have been used for selective deacylations of perpropanoates and peracetates of different classes of polyphenolics. In addition, lipase from *Candida antartica* (Novozym 435) has been used for regioselective propanoylation of 2-deoxy-D-ribose and D-ribose, the products on subsequent acetylation with acetic anhydride/pyridine afforded the corresponding tri- and tetraacyl derivatives, universal synthones for the synthesis of 2'-deoxyribonucleosides and ribonucleosides, respectively. An attempt has also been made to synthesise acylated erythrooxetanose using the same methodology.

INTRODUCTION

Water-in-oil microemulsions have merited attention as media for carrying out enzyme-catalysed reactions because of their interesting properties as hosts for biocatalysts. The unique properties of microemulsion systems allow hydrophilic enzymes to be incorporated into the nanometre-sized water core of droplets of microemulsions. By confining enzymes to an aqueous microdomain, they are protected from the potentially deleterious effects of organic solvents by a water and a surfactant shell and, therefore exhibit exceptionally high catalytic activity and substrate specificity even under conditions of water starvation.[1-4] One might expect, therefore that in view of the advantages offered by microemulsion systems, their use as media for enzymatic synthesis on a preparative scale might be more widespread. However, examples are relatively limited, partly because the scale-up of such processes can be problematic and also because of the difficulties associated with product recovery.

Immobilized biocatalysis in microemulsion-based gels (MBGs) is an area that is still in its infancy. However, recent innovations in this field[5-7] have made it possible to view biotransformations in MBG medium as a practical alternative, both in the Laboratory and on industrial scale. We have immobilized lipases from porcine pancreas (PPL) and *Candida cylindracea* (CCL) in MBGs and studied the hydrolysis of perpropanoates of different classes of polyphenolics with them; a high degree of selectivity leading to the formation of novel compounds, not accessible by purely synthetic reactions has been observed.

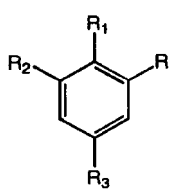
In addition, the lipase from *Candida antartica* has been used for selective propanoylation of the primary hydroxyl groups in 2-deoxy-D-ribose and D-ribose, the products on complete acetylation and coupling with silylated thymine, followed by deprotection lead to the corresponding nucleosides in

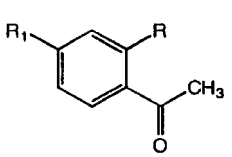
more than 90% overall yields.[8] We have also applied this methodology of regioselective enzymatic propanoylation, followed by chemical acetylation on D-erythrose thus aiming at acylated D-erythrooxetanose, which would be a universal synthon for oxetanonucleosides.[9]

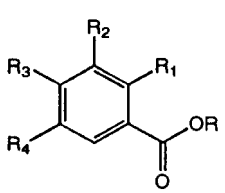
DEACYLATION OF PERACYLATED POLYPHENOLICS WITH IMMOBILIZED LIPASES

Di/trihydric phenols, alkyl benzoates and acetophenones are important starting materials for the synthesis of naturally occurring polyphenolics, i.e. chalcones, flavones, flavanones, chromones, isoflavones, etc. For the total synthesis of these natural products, selective protection/deprotection of the starting polyphenols or their precursors is always required which increases the number of steps, hence the final yields are quite low. We have earlier reported regioselectivity in deacetylation reactions with PPL and CCL in organic solvents on different classes of peracetylated polyphenolics having a nuclear carbonyl group.[10-14] Presently, we have studied hydrolysis of perpropanoates and peracetates of different classes of polyphenolics, e.g. catechol dipropanoate (**1**), resorcinol dipropanoate (**2**), hydroquinone dipropanoate (**3**), 2,6-dimethoxyhydroquinone dipropanoate (**4**), 2-*tert*-butylhydroquinone dipropanoate (**5**), phloroglucinol tripropanoate (**6**), 2-methylresorcinol dipropanoate (**7**), 2,4-diacetoxyacetophenone (**8**), ethyl 3,5-diacetoxybenzoate (**9**), ethyl 3,4,5-triacetoxybenzoate (**10**), methyl 2,4-diacetoxybenzoate (**11**) and ethyl 2,4-diacetoxybenzoate (**12**) in the presence of lipases immobilized in microemulsion-based gels (MBGs) in benzene. The results of deacylation reactions on compounds **1-12** are given in Table 1. The products **13-27** have been obtained in fairly good yields and are accompanied by high degree of selectivity, all the products have been fully characterized from their physical and spectral data. We have observed that lipases in MBGs catalyse the selective deacylations in polyphenolics much faster than in organic solvents.

We have earlier studied the selective deacylation of peracetates of different classes of polyphenolic compounds with PPL and CCL in dry organic solvents and observed that the lipase can recognise the different acetoxyl groups when there is a directly attached carbonyl group on the aromatic ring. But these lipases in organic solvents failed to differentiate between different acetoxyl groups in compounds lacking a ketonic function. Thus, resorcinol diacetate and 2-*tert*-butylhydroquinone diacetate underwent complete deacetylation.[14] Present results on di/trihydric phenolic esters **1-7** indicate that the same lipase, immobilised in microemulsion-based gels can differentiate one acyloxy group from others and that the deacylation reaction leads to partially protected phenols **13-19**, which are not easy to synthesise by purely chemical reactions.







1 $R=R_1=OCOCH_2CH_3$; $R_2=R_3=H$
2 $R=R_2=OCOCH_2CH_3$; $R_1=R_3=H$
3 $R=R_2=H$; $R_1=R_3=OCOCH_2CH_3$
4 $R=R_2=OCH_3$; $R_1=R_3=OCOCH_2CH_3$
5 $R=C(CH_3)_3$; $R_1=R_3=OCOCH_2CH_3$; $R_2=H$
6 $R=R_2=R_3=OCOCH_2CH_3$; $R_1=H$
7 $R=R_2=OCOCH_2CH_3$; $R_1=CH_3$; $R_3=H$
13 $R=OCOCH_2CH_3$; $R_1=OH$; $R_2=R_3=H$
14 $R=OCOCH_2CH_3$; $R_1=R_3=H$; $R_2=OH$
15 $R=R_2=H$; $R_1=OCOCH_2CH_3$; $R_3=OH$
16 $R=R_2=OCH_3$; $R_1=OCOCH_2CH_3$; $R_3=OH$
17 $R=C(CH_3)_3$; $R_1=OH$; $R_2=H$; $R_3=OCOCH_2CH_3$
18 $R=R_2=OCOCH_2CH_3$; $R_1=H$; $R_3=OH$
19 $R=OH$; $R_1=CH_3$; $R_2=OCOCH_2CH_3$; $R_3=H$

8 $R=R_1=OCOCH_3$
20 $R=OCOCH_3$; $R_1=OH$
21 $R=OH$; $R_1=OCOCH_3$

9 $R=CH_2CH_3$; $R_1=R_3=H$; $R_2=R_4=OCOCH_3$
10 $R=CH_2CH_3$; $R_1=H$; $R_2=R_3=R_4=OCOCH_3$
11 $R=CH_3$; $R_1=R_3=OCOCH_3$; $R_2=R_4=H$
12 $R=CH_2CH_3$; $R_1=R_3=OCOCH_3$; $R_2=R_4=H$
22 $R=CH_2CH_3$; $R_1=R_3=H$; $R_2=OH$; $R_4=OCOCH_3$
23 $R=CH_2CH_3$; $R_1=R_3=H$; $R_2=R_4=OH$
24 $R=CH_2CH_3$; $R_1=H$; $R_2=R_4=OCOCH_3$; $R_3=OH$
25 $R=CH_3$; $R_1=OCOCH_3$; $R_2=R_4=H$; $R_3=OH$
26 $R=CH_3$; $R_1=R_3=OH$; $R_2=R_4=H$
27 $R=CH_2CH_3$; $R_1=OCOCH_3$; $R_2=R_4=H$; $R_3=OH$

Table 1. Hydrolytic reactions catalysed by PPL immobilized in MBGs in dry benzene at 25-28°C on the peracylated polyphenolics 1-12.

Substrate	Reaction time (hrs)	Product(s) (%yield)
Catechol dipropanoate (**1**)	2	Catechol monopropanoate (**13**) (75)
Resorcinol dipropanoate (**2**)	2	Resorcinol monopropanoate (**14**) (70)
Hydroquinone dipropanoate (**3**)	2	Hydroquinone monopropanoate (**15**) (78)
2,6-Dimethoxyhydroquinone dipropanoate (**4**)	5	3,5-Dimethoxy-4-propanoyloxyphenol(**16**) (65)
2-*tert*-Butylhydroquinone dipropanoate (**5**)	5	2-*tert*-Butyl-4-propanoyloxyphenol (**17**) (55)
Phloroglucinol tripropanoate (**6**)	2	Phloroglucinol dipropanoate (**18**) (60)
2-Methylresorcinol dipropanoate (**7**)	4	2-Methylresorcinol monopropanoate(**19**) (50)
2,4-Diacetoxyacetophenone (**8**)	144	2-Acetoxy-4-hydroxyacetophenone (**20**) (60) *and* 4-Acetoxy-2-hydroxyacetophenone (**21**) (30)
Ethyl 3,5-diacetoxbenzoate (**9**)	24	Ethyl 5-acetoxy-3-hydroxybenzoate (**22**) (80) *and* Ethyl 3,5-dihydroxybenzoate (**23**) (10)
Ethyl 3,4,5-triacetoxybenzoate (**10**)	72	Ethyl 3,5-diacetoxy-4-hydroxybenzoate (**24**) (70)
Methyl 2,4-diacetoxybenzoate (**11**)	48	Methyl 2-acetoxy-4-hydroxybenzoate (**25**) (60) *and* Methyl 2,4-hydroxybenzoate (**26**) (20)
Ethyl 2,4-diacetoxybenzoate (**12**)	48	Ethyl 2-acetoxy-4-hydroxybenzoate (**27**) (40)

In our earlier studies involving lipase-mediated reactions on polyacetoxy aromatic ketones and benzopyrones in organic solvents, we have observed that all acetoxy groups other than the ones at *ortho* or *peri* position to the nuclear carbonyl group get deacetylated.[10,12-14] We have explained this high regioselectivity due to the formation of Schiff's base complex between the ε-amino group of the lysine residue in the active site of the enzyme and the keto group directly attached to the benzenoid ring.[15] In the present investigation, we have subjected 2,4-diacetoxyacetophenone (**8**) to PPL (immobilized in microemulsion-based gels)-catalyzed deacetylation and observed that there is no selectivity as both the products, *i.e.* 2-acetoxy-4-hydroxyacetophenone (**20**) and 4-acetoxy-2-hydroxyacetophenone (**21**) corresponding to deacetylation at *para* and *ortho* position, respectively in **8** are formed. This indicates that the lipase has a different orientation in the gel medium as compared to that in the organic solvents and the Schiff's base complex formation with the substrate is inhibited. To further confirm our enzyme-substrate Schiff's base complex formation hypothesis, we have recently studied deacetylation reactions of polyacetoxy ethyl and methyl benzoates.[15] As compared to the carbonyl group of aromatic ketones and benzopyrones, the ester carbonyl group in polyacetoxy aromatic acids esters cannot enter into Schiff's base complex formation that readily. As a result, the deacetylation in esters takes place at the *ortho*, *meta* and *para* acetoxy groups with equal ease in organic solvents.[15] Similar results have been observed in the present investigation involving immobilised lipase(in MBGs)-catalysed deacetylation reactions on the polyacetoxybenzoic acid esters **9-12**; the products **22-27** are obtained by deacetylation of acetoxy groups at *ortho, meta* and *para* positions. Of particular interest is the exclusive formation of the partially protected ethyl 3,5-dihydroxybenzoate (**22**) and that of ethyl gallate (**24**) in 80% and 70% yields, respectively from **9** and **10** by deacetylation of only one acetoxy group, i.e. only one of two symmetrical ones in **9** and only the middle one out of three acetoxy groups at adjacent positions in **10**, this is not easily feasible by non-enzymatic reactions.

IMMOBILISATION OF LIPASES IN MICROEMULSION-BASED GELS (MBGs)

Microemulsions containing lipase were prepared by stirring a mixture of PPL (0.25g) and reverse micellar solution of isooctane-water - AOT [sodium *bis*-(2-ethylhexyl) sulphosuccinate], which in turn was prepared by shaking a mixture of water (4ml), isooctane (25ml) and AOT (4.9 g). Gelatin (7g) was dissolved in water (10ml) preheated to 50°C and the above microemulsion containing enzyme was added to it with shaking while maintaining the temperature a little above 50°C, the gel transition temperature. The resulting microemulsion-based gels (MBGs) were subsequently cooled to room temperature and pelleted prior to use.

GENERAL REACTION PROCEDURE

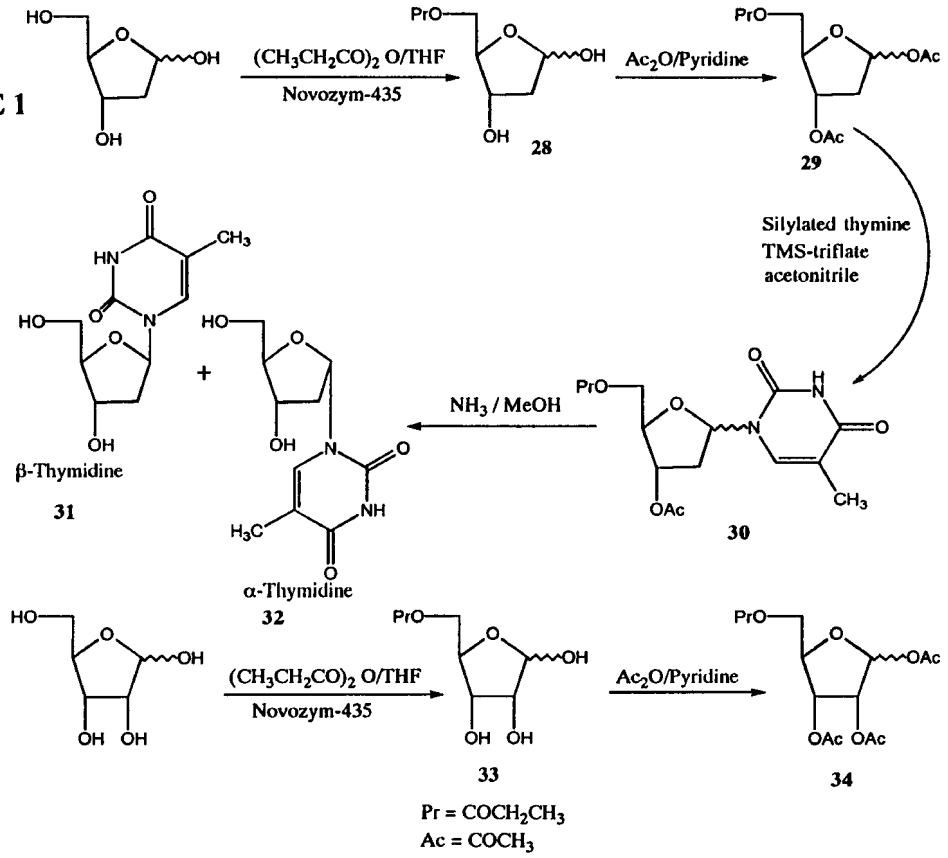
The perpropanoate of the polyphenolic compound (500 mg) was dissolved in dry benzene (20 ml) and pelleted MBGs (~15 g) added into it. The reaction mixture was left at 25-28°C and the progress of the reaction was monitored periodically by TLC. After completion of the reaction (2-4 hrs for 50-80% conversion), gel was removed by filteration, solvent removed under reduced pressure and the resulting residue purified by column chromatography or preparative TLC, followed by crystallization.

LIPASE-CATALYSED ACYLATION OF 2-DEOXY D-RIBOSE, D-RIBOSE AND D-ERYTHROSE

Tri-*O*-acyl 2-deoxy-D-ribofuranose and tetra-*O*-acyl D-ribofuranose are the ideal synthones for the synthesis of 2'-deoxyribonucleosides and ribonucleosides, respectively. However, only a few methods describing synthesis of 1,3,5-tri-*O*-acyl-2-deoxy-D-ribofuranose have been published so far, but none of them have found much capability because these strategies involve a number of steps and the overall yields of the desired products are very low. Thus 2-deoxy-1,3,5-tri-*O*-acetyl-D-ribofuranose was obtained by direct acetylation of 2-deoxy-D-ribose in only 2% yield after crystallization,[16] by acetolysis of methyl 2-deoxy-3, 5-di-*O*-acetyl-D-ribofuranoside[17] and by acetolysis of 6-*N*-acetyl-3',5'-di-*O*-acetyl-2'-deoxyadenosine[18] in a number of steps. We have developed a straight-forward and efficient one-pot chemo-enzymatic synthesis of 2-deoxy-1,3-di-*O*-acetyl-5-*O*-propanoyl-D-ribofuranose (**29**) from 2-deoxy-D-ribose. The versatility of this compound as a synthon in the synthesis of 2-deoxyribonucleosides has been proved by condensation with thymine to give, without any purification, the anomeric nucleosides **30** in high yield and high purity. This strategy of acylation has additionally been used for an analogous synthesis of 5-*O*-propanoyl-1, 2, 3-tri-*O*-acetyl-D-ribofuranose (**34**) from D-ribose.

The lipase from *Candida antartica* (Novozym-435) brings about the selective acylation of the primary -OH groups of 2-deoxy-D-ribose and D-ribose with propanoic anhydride in dry THF, subsequent addition of acetic anhydride and pyridine to the reaction mixture afforded 2-deoxy-1,3-di-*O*-acetyl-5-*O*-propanoyl-D-ribofuranose (**29**) and 5-*O*-propanoyl-1,2,3-tri-*O*-acetyl-D-ribofuranose (**34**) in 95% and 94% yields, respectively. The completion of the enzymatic conversions of unprotected pentoses into the monoacylated intermediates **28** and **33** are monitored by analytical TLC and their structures are confirmed from their NMR spectral data.[8] Therefore, this method can be useful for monoprotection of the primary hydroxyl group of a large variety of carbohydrate derivatives. We have obtained similar results using butanoic anhydride as acyl donor, thus showing that other acyl groups can be incorporated using the above described methodology. To evaluate the potential of tri-*O*-acyl-2-deoxy-D-ribofuranose derivative as a glycosyl donor in synthesis of anomeric mixtures of 2'-deoxyribonucleosides, a standard Vorbruggen coupling[19,20] was undertaken. Reaction with silylated thymine in acetonitrile using trimethylsilyl trifluoroethanesulfonate (TMS-triflate) as catalyst, followed by aqueous work-up afforded an anomeric mixture of 3',5'-di-*O*-acyl-2'-deoxythymine nucleosides in 90% overall yield

SCHEME 1



from 2-deoxy-D-ribose.[8] The anomeric mixture was deprotected with methanol saturated with ammonia to give ß-thymidine (**31**) and α-thymidine (**32**) (Scheme 1). The NMR data of these two nucleosides were identical in all respects with those earlier published, which unambiguously confirms the *erythro*-pentofuranose configuration of compounds **31** and **32.**

Some oxetanonucleosides (e.g. oxetanocin-A, *epi* nor-oxetanocin-A and their derivatives) having a four-membered ring sugar (oxetanose) moiety have been found to exhibit potent antiviral activities.[21-24] We have applied our above-developed chemo-enzymatic methodology involving acylation of the primary hydroxyl group to get triacyl D-erythrooxetanose, a synthon for oxetanonucleosides from D-erythrose. The propanoylation of D-erythrose catalysed by Novozym 435, followed by acetylation gave a complex mixture which was difficult to separate. Efforts are being continued to get partially protected D-erythrooxetanose by our chemo-enzymatic strategy reported in this paper.

CONCLUSIONS

Immobilization of lipases in microemulsion-based gels provides a novel means of performing biotransformations in apolar organic solvents. The enzyme contained in microemulsion-based gels not only possesses advantages of conventional water-in-oil microemulsion systems, but has the added advantages of straight-forward product isolation by filteration of the gel, followed by chromatographic purification of the product. We have observed that the rates of deacylation reactions catalysed by immobilized enzymes are much faster than the rates of corresponding lipase-catalysed reactions in organic solvents.

Using *Candida antartica* lipase in the key step, we have synthesised peracylated ribofuranose derivatives in high yields in a one-pot reaction from the parent pentose. The versatility of the 1,3,5-tri-*O*-acyl-2-deoxy-D-ribofuranose derivative as glycosyl donor in nucleoside synthesis has been confirmed.

It is a column-free methodology for synthesising protected nucleosides starting from pentose sugar, which significantly reduces the consumption of solvents in bulk in a routine chemical synthesis of nucleosides. Therefore, this strategy is attractive with regard to Enviromental-friendly (or Green) Chemistry/Technology.

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