Potential applications of enzyme-mediated transesterifications in the synthesis of bioactive compounds

VS Parmar*, AK Prasad, NK Sharma, KS Bisht, Rita Sinha and Poonam Taneja

Department of Chemistry, University of Delhi, Delhi-110 007 (India)

ABSTRACT- The yeast lipase *Candida cylindracea* (CCL) and porcine pancreatic lipase (PPL) have been used for regioselective deacylation of peracetylated benzopyrones, diphenylpropenones and acetophenones for the first time. The deacylation study on different classes of polyphenols has revealed that the presence of carbonyl group attached to the aromatic ring is needed by the lipases to exhibit regioselectivity towards hydrolysis of acetoxyl groups. The acetoxyl groups at positions other than the one at *ortho* position to the carbonyl group get selectively hydrolysed by PPL in organic solvents. The transesterification reactions using triflouroethylbutyrate (TFEB), catalysed by PPL and CCL on some polyols in dry organic solvents were also performed. It was found that the primary hydroxyl is acylated. In D-panthenol, the oxidised dextrorotatory form of which is a major constituent of vitamin B-complex, the primary hydroxyl group at the far end of the asymmetric carbon atom gets exclusively acylated. This work should be of importance in the synthesis of building blocks of biologically active natural products which may provide structural leads to anti AIDS and anticancer agents.

INTRODUCTION

Hydrolytic enzymes, particularly lipases have found widespread applications in organic synthesis because of low cost, wide-versatility, easy use(ref. 1) and non-requirement of added cofactors. They have been used in the resolution of racemic alcohols(ref.2), esters(ref.3) and in the production of chiral compounds from prochiral precursors *via* selective hydrolysis or transesterification in aqueous and non-aqueous media. The regioselective capabilities of lipases have also been recognized for solving problems of different alcoholic group recognition within the same molecule in the case of carbohydrates(ref.4,5,6), only one example is reported by Nicolosi *et al.* (ref.7) on the regioselective hydrolysis of flavone acetates in organic solvents.

We have successfully used lipases from porcine pancreas and *Candida cylindracea* for regioselective deacylation of the peracetates of a range of polyphenolics (acetophenones, chalcones, flavones, flavanones, coumarins and chromanones). Lipases have also been used by us for recognition of a primary hydroxyl group in presence of another primary and secondary hydroxyl group in the same molecule by transesterification with trifluoroethyl butyrate (TFEB) in organic solvents. Our studies on selective protection/deprotection of polyphenols and polyols should be of utility in the synthesis of different types of biologically active compounds.

DEACYLATION OF ACETOPHENONES

Aryl-alkyl ketones are important starting materials for the synthesis of naturally occurring polyphenolics, i.e. chalcones, flavones, flavanones, chromones, etc. For the total synthesis of these natural products, proper protection/deprotection of acetophenones is always required which increases the number of steps, hence the final yields are quite low. In order to avert this problem, we have studied regioselective deacylation in seven differently substituted acetophenones 1-7 by lipases from porcine pancreas and *Candida cylindracea* in different organic solvents (tetrahydrofuran, di-isopropyl ether, acetone and acetonitrile) and the results are shown in Table 1.
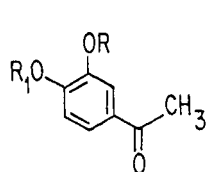
1 R = R₁ = COCH₃

8 R = R₁ = H
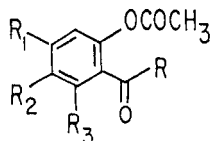
2   R = CH₃; R₁ = R₂ = H; R₃ = OCOCH₃

3   R = CH₃; R₁ = R₃ = OCOCH₃; R₂ = H

6   R = C₂H₅; R₁ = R₃ = OCOCH₃; R₂ = H

7   R = C₃H₇; R₁ = R₂ = OCOCH₃; R₃ = H

9   R = CH₃; R₃ = OCOCH₃; R₁ = OH; R₂ = H

12 R = C₂H₅; R₃ = OCOCH₃; R₁ = OH, R₂ = H

13 R = C₃H₇; R₂ = OCOCH₃; R₁ = OH; R₃ = H

14 R = C₃H₇; R₁ = R₂ = OH; R₃ = H
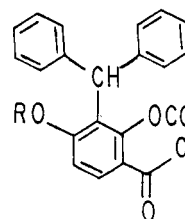
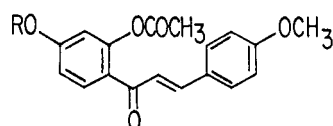4 R = COCH₃

10 R = H
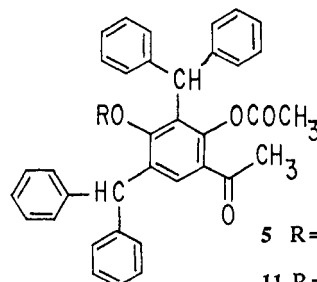
5 R = COCH₃

11 R = H

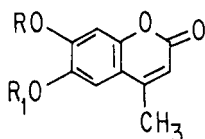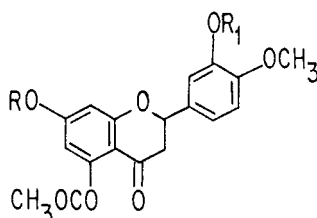TABLE 1. Hydrolysis studies on the peracetates of acetophenones *1-7* with lipases

Substrate	Reaction conditions	Product(s) (% yield)
3,4-Diacetoxy-acetophenone(1)	Di-iPE, PPL, 42-45° 32 hrs	3,4-dihydroxy-acetophenone(8)(80%)
2,6-Diacetoxy-acetophenone(2)	THF, PPL, 42-45° 60 hrs	No reaction
2,4,6-Triacetoxy-acetophenone(3)	THF, PPL, 42-45° 30 hrs	2,6-diacetoxy-4-hydroxyacetophenone (9) (78%)
2,4-Diacetoxy-3-benzhydrylacetophenone (4)	THF, PPL, 42-45° 38 hrs	2-acetoxy-4-hydroxy-3-benzhydrylaceto-phenone (10) (70%)
2,4-Diacetoxy-3,5-dibenzhydryl-acetophenone(5)	THF, PPL, 42-45° 42 hrs	2-acetoxy-4-hydroxy-3,5-dibenzhydryl-acetophenone (11) (63%)
2,4,6-Triacetoxy-propiophenone(6)	THF, PPL, 42-45° 40 hrs	2,6-diacetoxy-4-hydroxypropiophenone (12) (60%)
2,4,5-Triacetoxy-butyrophenone(7)	THF, CCL, 42-45° 48 hrs	2,5-diacetoxy-4-hydroxybutyrophenone (13) (65%) and 2-acetoxy-4,5-dihydroxybutyro-phenone (14)(20%)

## DEACYLATION OF CHALCONES, BENZOPYRANONES AND COUMARINS

Chalcones, benzopyranone derivatives, coumarins and chromanones occur widely in nature and many of their analogues possess a variety of biological activities, i.e. antitumor, antiviral, antibiotic, antifungal, etc. Aiming to simplify the total synthesis of these naturally occurring polyphenolics, we have studied selective protection of different model compounds representing four different groups of natural products, i.e. 2',4'-diacetoxy-4-methoxychalcone (15), 5,7,3'-triacetoxy-4'-methoxyflavanone (16), 5,7-diacetoxy-3-methoxyflavone (17), 6,7-diacetoxy-4-methylcoumarin (18) and 5,7-diacetoxy-2,2-dimethylchromanone (19). Since acylation of polyphenols according to the lipase-catalysed irreversible transesterification procedure using active ester is not possible due to inactivation of the enzyme by the free phenolic hydroxyl(s), we have studied the selective deacylation of the polyphenol peracetates 15-19 by PPL and CCL in tetrahydrofuran and di-isopropyl ether. The results of the hydrolysis reactions are described in Table 2.

15 R = COCH₃

20 R = H

18 R = R₁ = COCH₃

24 R = H; R₁ = COCH₃

25 R = R₁ = H

16 R = R₁ = COCH₃

21 R = H; R₁ = COCH₃

22 R = R₁ = H
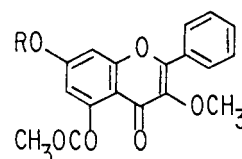
17 R = COCH₃
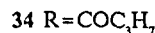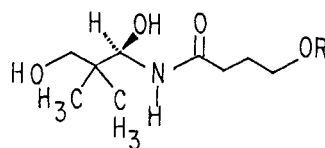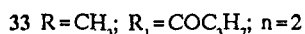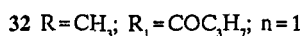
23 R = H
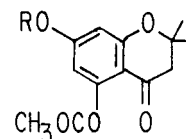
19 R = COCH₃

26 R = H

TABLE 2. Hydrolysis studies on the polyphenolic peracetates *15-19* with lipases

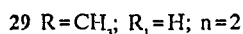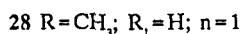| Substrate	Reaction conditions	Product(s) (% yield)
2',4'-Diacetoxy-4-methoxychalcone(15)	THF, PPL, 42-45° 32 hrs	2'-acetoxy-4'-hydroxy-4-methoxychalcone (20) (50%)
5,7,3'-Triacetoxy-4'-methoxyflavanone(16)	THF, PPL, 42-45° 48 hrs	5,3'-diacetoxy-7- hydroxy-4'-methoxyflavanone (21) (55%) and 5-acetoxy-7,3'-dihydroxy-4'-methoxy-flavanone (22) (23%)
5,7-Diacetoxy-3-methoxyflavone (17)	Di-iPE, CCL, 42-45° 32 hrs	5-acetoxy-7-hydroxy-3-methoxyflavone (23) (65%)
6,7-Diacetoxy-4-methylcoumarin (18)	THF, PPL, 42-45° 38 hrs	6-acetoxy-7-hydroxy-4-methylcoumarin (24) (65%) and 6,7-dihydroxy-4-methylcoumarin (25)(15%)
5,7-Diacetoxy-2,2-dimethylchroma-none (19)	THF, PPL, 42-45° 32 hrs	5-Acetoxy-7-hydroxy-2,2-dimethylchroma-none (26) (73%)

Our results indicate that in case of acetophenones **1-7**, the acetoxyl group *ortho* to the carbonyl function is not hydrolysed at all by the enzyme, whereas the group at the *para* position is hydrolysed preferentially over the one at the *meta* position. Similarly in case of benzopyranone derivatives, the acetoxyl group at the position 5 *(peri)* is not hydrolysed by the enzyme. These observations suggest that the enzyme binds to the carbonyl function of the substrate in such a way that it inhibits the hydrolysis of the acetoxyl group *ortho* to the carbonyl function and places the other acetoxyl groups, preferentially the one at *para* position near the serine residue at the active site of the lipase, thereby facilitating hydrolysis at this position. To support our hypothesis of enzyme selectivity, we investigated PPL/CCL-catalysed hydrolysis of resorcinol diacetate in different organic solvents. Since this substrate has no carbonyl function attached directly to the aromatic ring, one would expect that the enzyme should show no preference for one acetoxyl group over the other. Indeed we found that the enzymatic hydrolysis of resorcinol diacetate gives the dihydroxy compound, resorcinol in quantitative amounts.

LIPASE-CATALYSED TRANSESTERIFICATION OF POLYOLS

We have studied selective protection of aliphatic hydroxyl groups in compounds **27-30** of synthetic importance by two approaches, (I) by reacting these alcohols with active ester (2,2,2-triflouroethyl butyrate, TFEB), and (II) by converting the diols and triols to the corresponding peracetates, followed by selective hydrolysis in different organic solvents using PPL and CCL by the above experimental procedure used for deacylation of phenolic peracetates.

$$\underset{|}{\overset{\overset{\displaystyle OH}{|}}{R}}-CH-(CH_2)_n-OR_1$$

27 R = C_6H_5; R_1 = H; n = 1

28 R = CH_3; R_1 = H; n = 1

29 R = CH_3; R_1 = H; n = 2

31 R = C_6H_5; R_1 = COC_3H_7; n = 1

32 R = CH_3; R_1 = COC_3H_7; n = 1

33 R = CH_3; R_1 = COC_3H_7; n = 2

30 R = H

34 R = COC_3H_7
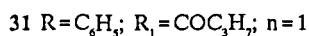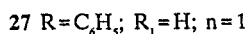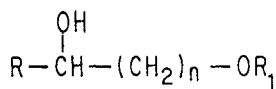
(I). Transesterification reactions using TFEB were carried out in presence of PPL and CCL in different organic solvents, i.e. pyridine, dimethylformamide, acetonitrile, acetone, tetrahydrofuran and isooctane, the results obtained with four polyols **27-30** are summarised below:

(a) The comparative studies in different organic solvents revealed that PPL suspended in acetone is best suited for maximum conversion, though the rigioselectivity remains the same in all the solvents used.

(b) Transesterification in the three diols **27-29** is highly regioselective and acylation takes place at the primary hydroxyl group. Thus diols **27-29** exclusively give 2-phenyl-2-hydroxyethyl butyrate (**31**), 2-hydroxypropyl butyrate(**32**) and 3-hydroxybutyl butyrate (**33**), respectively.

(c) The rate of reaction in 1,2-propanediol (**28**) is found to be more as compared to that of 1-phenyl-1,2-ethanediol (**27**) and 1,3-butanediol (**29**), may be because the primary hydroxyl group is nearer to the asymmetric carbon atom in **28**.

(d) D-Panthenol (**30**) has two primary hydroxyl groups and one secondary hydroxyl group. In this case only the primary hydroxyl group at the far end from the asymmetric centre gets acylated.

(e) No appreciable enantioselectivity is observed in any of the three diols either with PPL or CCL.

(II). No appreciable conversion was observed in the deacetylation of diacetates of 1,2-propanediol, 1,3-butanediol and D-panthenol triacetate. 1-Phenyl-1,2-diacetoxyethane on enzymatic hydrolysis yielded 1-phenyl-1-hydroxy-2-acetoxyethane in 55% yield with CCL in di-isopropyl ether.

REFERENCES

1. C.S.Chen and C.J.Sih, Angew.Chem.Int.Ed.Eng.,**28**, 695, (1989).

2. A.J.Pearson, H.S.Bansal and Y.S.Lal,J.Chem.Soc.,Chem.Commun., 519, (1987).

3. B. Cambon and A.M. Klibanov, Biotechnol.Bioeng, **26**, 1449, (1984).

4. A. Bellesteros, M. Bernabe, C.Cruzado, M.Martin-Lomas and C. Otero,Tetrahedron, **45**, 7077, (1989).

5. P. Sinay, Pure Appl. Chem. **59**, 445, (1987).

6. M. Therisod and A.M. Klibanov, J. Am. Chem. Soc, **108**, 5638, (1986).

7. M. Natoli, G. Nicolosi and M. Piattelli, Tet.Lett., **31**, 7371, (1990).