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REGIOSELECTIVE DEACYLATION OF POLYACETOXY ARYL-METHYL KETONES BY LIPASES IN ORGANIC SOLVENTS#

VS Parmar*, AK Prasad, NK Sharma, SK Singh, HN Pati and Suman Gupta

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

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Abstract- Lipases from porcine pancreas and *Candida cylindracea*, suspended in organic solvents have been used to study regioselective deacylation of polyacetoxy acetophenones. It has been observed that the hydrolysis of acetate groups at positions other than *ortho* predominates.

INTRODUCTION

Hydrolytic enzymes, particularly lipases have found widespread applications in organic synthesis because of low cost, versatile nature, easy use[1] and non-requirement of added cofactors.[2] They have been used in resolution of racemic alcohols,[3] esters[4] and production of chiral compounds from prochiral precursors *via* selective hydrolysis or transesterification in aqueous and non-aqueous media.[5] The regioselective capabilities of lipases have also been recognised for solving problems of different alcoholic group recognition within the same molecule mainly in case of carbohydrates[6,7] and aliphatic diols.[8,9] Only one example is reported on such studies on polyphenolics by Nicolosi *et al.*[10] who have carried out regioselective hydrolysis of flavone acetates in organic solvents.

Polyphenolics occur widely in nature and many of their analogues possess a variety of biological activities, *i.e.* antitumor, antiviral, antibiotic, antifungal, etc. Acetophenones are starting materials for the synthesis of different classes of natural polyphenolics, *viz* chalcones, flavones, flavanones, etc. and proper protection of polyhydroxy acetophenones is always required to achieve the total synthesis of compounds of these classes. In order to avert the problems in protection/deprotection in the synthesis of biophenolics, we report regioselective deacylation in polyacetoxy aryl-methyl ketones by lipases in organic solvents.

RESULTS AND DISCUSSION

We have studied enzymatic deacylation of six acetophenones, viz. 2,4-diacetoxyacetophenone (**1**), 2,5-diacetoxyacetophenone (**2**), 2,5-diacetoxy-4-methoxyacetophenone (**3**), 2,3,4-triacetoxyacetophenone (**4**), 2,4,6-triacetoxyacetophenone (**5**) and 2,6-diacetoxyacetophenone (**6**) by lipases from porcine pancreas (PPL) and *Candida cylindracea* (CCL) in different organic solvents.

From a preliminary screening of commercially available PPL and CCL in various organic solvents (di-isopropyl ether, tetrahydrofuran, acetone, acetonitrile and dimethylformamide) on 2,4- diacetoxyacetophenone

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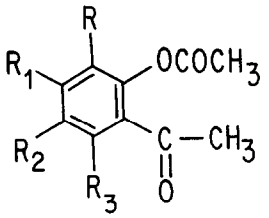

1. $R=R_2=R_3=H$; $R_1=OCOCH_3$
2. $R=R_1=R_3=H$; $R_2=OCOCH_3$
3. $R=R_3=H$; $R_1=OCH_3$; $R_2=OCOCH_3$
4. $R=R_1=OCOCH_3$; $R_2=R_3=H$
5. $R=R_2=H$; $R_1=R_3=OCOCH_3$
6. $R=OCOCH_3$; $R_1=R_2=R_3=H$
7. $R=R_2=R_3=H$; $R_1=OH$
8. $R=R_1=R_3=H$, $R_1=OH$
9. $R=R_3=H$; $R_1=OCH_3$, $R_2=OH$
10. $R=OCOCH_3$; $R_1=OH$; $R_2=R_3=H$
11. $R=R_2=H$; $R_1=OH$; $R_3=OCOCH_3$

(1), PPL in THF appeared best suited to our aim. In case of 2,5-diacetoxyacetophenone (2), the best results were obtained by CCL in di-iPE. The results obtained with six acetophenones are tabulated below:

Substrate	Reaction conditions	Product(s) (% yield)
2,4-Diacetoxyacetophenone (1)	THF, PPL, 42-45° 30 hrs	2-acetoxy-4-hydroxy-acetophenone (7) (80)
2,5-Diacetoxyacetophenone (2)	THF, PPL, 42-45° 45 hrs	2-acetoxy-5-hydroxy-acetophenone (8) (60)
2,5-Diacetoxy-4-methoxyaceto-phenone (3)	Di-iPE, CCL, 42-45° 50 hrs	2-acetoxy-4-methoxy-5-hydroxyacetophenone (9) (65)
2,3,4-Triacetoxyacetophenone (4)	THF, PPL, 42-45° 35 hrs	2,3-diacetoxy-4-hydroxy-acetophene(10) (55)
2,4,6-Triacetoxyacetophenone (5)	THF, PPL, 42-45° 30 hrs	2,6-diacetoxy-4- hydroxy - acetophenone (11) (78)
2,6-Diacetoxy-acetophenone (6)	THF, PPL, 42-45	No reaction

Salient features of the results obtained are:

a) Among the acetophenones studied, 2,6-diacetoxyacetophenone (6) does not react at all, possibly due to the inaccessibility of the functional group. The resistance of the acetoxy groups at the *ortho* positions towards hydrolysis is also observed in 2,4,6-triacetoxyacetophenone (5), where 2,6-diacetoxy-4-hydroxyacetophenone(11)[11] is obtained exclusively.

b) Transesterifications in 2,4-diacetoxyacetophenone (1), 2,5-diacetoxyacetophenone (2) and 2,5-diacetoxy-4-methoxyacetophenone (3) were found to behighly regioselective giving 2-acetoxy-4-hydroxyacetophenone(7)[12], 2-acetoxy-5-hydroxyacetophenone (8) and 2-acetoxy-5-hydroxy-4-methoxy-acetophenone (9), respectively in 65-80% yield.

c) 2,3,4-Triacetoxyacetophenone (4) underwent hydrolysis at much faster rate at position C-4 compared to hydrolysis at other positions. Thus triacetate 4 on hydrolysis gave 4-hydroxy-2,3-diacetoxyacetophenone (10) as the major product in 65% yield.

d) The acetophenones **8, 9 and 10** are new compounds as these have not been synthesised earlier. Thus our method of enzymatic hydrolysis may serve as a general method for the preparation of acetophenones with an acetoxyl group at the *ortho* position.

e) The reactions performed on all these compounds under the same conditions, but without adding the enzyme did not indicate any hydrolysis.

Our results indicate that 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. Thus, the three diacetates **1, 2** and **3** yield 4-hydroxy-2-acetoxyacetophenone (**7**), 5-hydroxy-2-acetoxyacetophenone (**8**)and 5-hydroxy-2-acetoxy-4-methoxyacetophenone (**9**) in 80%, 60% and 65% yield, respectively. These observations suggest that the enzyme binds to the carbonyl function[13,14] 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 regioselectivity of PPL and CCL on resorcinol diacetate in different organic solvents. Since this substrate has no carbonyl group, 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) exclusively.

Since acetophenone derivatives are important building blocks for the synthesis of various classes of natural products, regioselective deacylation of acetophenone peracetates should be of immense importance as it is complimentary to chemical hydrolysis which yields deacylation of the *ortho* acetoxy group.

EXPERIMENTAL

Porcine pancreas type II lipase and*Candida cylindracea* type VII lipase were purchased from Sigma Chemical Co (USA) and used after drying in vaccum over $CaCl_2$. All the solvents were completely dried before use. Preparative column chromatography and thin layer chromatography were performed on silica gel (60-80 mesh) and Merck Silica gel-G, respectively. Compounds were visualised either by leaving the developed TLC plate in iodine chamber or by spraying with alcoholic ferric chloride or conc. H_2SO_4. The UV spectra were recorded on Perkin Elmer model 554 and the IR spectra on a Shimadzu model 435 spectrophotometer using KBr disc or nujol film. The ^{1}H-NMR spectra were recorded either on Perkin-Elmer R-32 (90 MHz) spectrometer or on Jeol JNM FX-60 FT NMR spectrometer with reference to tetramethylsilane as internal standard. The chemical shifts are expressed in δ values and the J values are expressed in Hz.

Preparation of substrates. All the six acetates **1-6** were prepared by acetic anhydride-pyridine method either at room temperature or by heating below 100° in 80-85% yield. Known acetates were identified by comparison of their melting point and spectral data with those reported in the literature.

Procedure for Enzyme Hydrolysis. To a solution of 2,4-diacetoxyacetophenone (**1**, 3 mmol, 708 mg) in THF (30 ml) containing *n*-butanol (15 mmol, 1.38 ml), PPL (600 mg) was added and the suspension stirred at 42-45°, the progress of reaction was monitored by TLC. At the end, the reaction was quenched by filtering off the enzyme, the solvent was removed to dryness *in vacuo* and the product isolated by preparative column chromatography to give 2-acetoxy-4-hydroxyacetophenone (**7**). Deacylation reaction with other five peracetylated acetophenones (**2-6**) were also carried out in the similar way except in case of 2,5-diacetoxy-4-methoxyacetophenone, where CCL in di-iPE was used instead of PPL in THF.

2-Acetoxy-4-hydroxyacetophenone (7)[12] crystallized from ethyl acetate-petrol as white needles (465 mg, 80%), m.p. 87-88°; R_f 0.35 (benzene : ethyl acetate 19:1); ^{1}H-NMR ($CDCl_3$+DMF): 2.35 (3H, s, -$OCOCH_3$), 2.50 (3H, s, -$COCH_3$), 6.59(1H, d, J=3 H-3), 6.78(1H, dd, J=9 & 3, H-5) and 7.84(1H,d, J=9, H-6).

2-Acetoxy-5-hydroxyacetophenone (8), m.p. 93-95°; R_f 0.30 (benzene : ethyl acetate 19:1); ^{1}H-NMR ($CDCl_3$): 2.15 (3H, s, -$OCOCH_3$), 2.54 (3H, s, -$COCH_3$), 6.76(1H, d, J=10, H-3), 7.09 (1H, dd, J=10 & 3, H-4) and 7.28(1H,d, J=3, H-6).

2,5-Diacetoxy-4-methoxyacetophenone (3), m.p. 134-36°; R_f 0.60 (benzene : ethyl acetate 9:1); ^{1}H-NMR ($CDCl_3$) : 2.28 and 2.33 (2s, 3H each, 2X-$OCOCH_3$), 2.46 (3H, s,-$COCH_3$), 3.83 (3H,s,-OCH_3), 6.72 (1H, s, H-3) and 7.60 (1H,s, H-6).

2-Acetoxy-5-hydroxy-4-methoxyacetophenone (9), yellow viscous oil, R_f 0.35 (benzene : ethyl acetate 9:1); ^{1}H-NMR ($CDCl_3$) : 2.30 (3H, s, -$OCOCH_3$), 2.47 (3H, s, -$COCH_3$), 2.59(1H, bs, -OH), 3.88(3H,s,-OCH_3), 6.61(1H,s,H-3) and 7.47(1H,s, H-6).

2,3-Diacetoxy-4-hydroxyacetophenone (10), pale yellow viscous oil, R_f0.25 (benzene : ethyl acetate 49:1); ^{1}H-NMR ($CDCl_3$+DMF) : 2.20 (6H, s, 2X-$OCOCH_3$), 2.56 (3H, s, -$COCH_3$), 6.56(1H, d, J=10, H-5) and 7.48(1H, d, J=10, H-6).

2,4,6-Triacetoxyacetophenone (5), m.p. 70°, R_f 0.55 (benzene : ethyl acetate 49:1); ^{1}H-NMR ($CDCl_3$) : 2.20 (9H, s, 3X-$OCOCH_3$), 2.42 (3H, s, -$COCH_3$) and 6.36(2H, s, H-3 and H-5).

2,6-Diacetoxy-4-hydroxyacetophenone (11), [11]m.p. 112°; R_f 0.45 (benzene : ethyl acetate 19:1); ^{1}H-NMR ($CDCl_3$): 2.20 (6H, s, 2X-$OCOCH_3$), 2.40 (3H, s, -$COCH_3$) and 6.93 (2H, s, H-3 and H-5).

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