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# REGIOSELECTIVE ENZYME-CATALYZED DEACETYLATION OF BENZYL PHENYL KETONE PERACETATES IN ORGANIC SOLVENTS+

**Virinder S. Parmar,* Ashok K. Prasad, Nawal K. Sharma, Kirpal S. Bisht, Hari N. Pati and Poonam Taneja**

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

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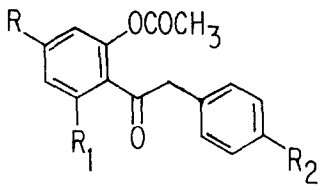
**Abstract-** Lipases from porcine pancreas and *Candida cylindracea*, suspended in different organic solvents have been used to deacetylate peracetates of benzyl phenyl ketones(desoxybenzoins). High regioselectivity in the reactions has been observed, also the nature of the solvent affects the rate of the reaction.

The realisation that enzymes can act as catalysts in neat organic solvents[1] has led to the introduction of a new fundamental variable into studies of enzyme-substrate interactions. It has been found that the nature of the solvent has a profound effect on substrate specificity,[2] regioselectivity[3] and enantioselectivity[4] of enzyme-catalyzed reactions. In the present study, we have investigated the regioselectivity of interesterification reactions with lipases from porcine pancreas (PPL) and *Candida cylindracea* (CCL) in anhydrous organic solvents for the following reasons : (i) these lipases are catalytically active in a number of anhydrous solvents and their behaviour in such media has earlier been investigated,[5] (ii) selectivity of these enzymes towards substrates varies with the reaction media, (iii) their cost is low and (iv) these enzymes require no added cofactors[6] and hence their use in organic reactions is easy.[7] The capabilities of PPL and CCL have been recognised for solving problems of different alcoholic group recognition within the same molecule mainly in case of carbohydrates[8] and aliphatic diols.[9] We have selected the peracetylated benzyl phenyl ketones **1-4** as target molecules for deacylation studies in four different anhydrous solvents [tetrahydrofuran (THF), diisopropyl ether (DIPE), acetone and acetonitrile] because these are the important starting materials for the synthesis of different classes of bioactive polyphenolics and little work has been done on the use of enzymes in protection-deprotection of hydroxyl groups in polyphenols.[10] Otherwise, selective protection of polyphenolics by chemical methods is cumbersome and the final yields are low.

+ A part of this work was presented at the IUPAC-NOST International Symposium on Enzymes in Organic Synthesis held in New Delhi (India) on 6-9 January 1992.

**The general reaction procedure** : A weighed amount of the polyphenolic peracetate ( 2-3 mmol) was dissolved in the dry organic solvent (20-30 ml) containing *n*-butanol (5 molar equivalent) and lipase (300-400 mg) was added. The reaction mixture was stirred at 42-45°C, the reaction was monitored by TLC and upon completion, it was quenched by filtering off the enzyme. The solvent was removed to dryness *in vacuo* and the product isolated by column chromatography or preparative thin layer chromatography. The CCL (Type VII) and PPL (Type II) were purchased from Sigma Chemical Co (USA) and used after keeping *in vacuo* over $P_2O_5$ for 10 hr. The organic solvents used were redistilled and dried over molecular sieves (4Å). The identification of the products was carried out on the basis of a detailed study of their colour reactions and spectral data.[11]




| | |
|---|---|
| **1** | $R=OCOCH_3$; $R_1=R_2=H$ |
| **2** | $R=OCOCH_3$;$R_1=H$;$R_2=OCH_3$ |
| **3** | $R=R_1=OCOCH_3$; $R_2=H$ |
| **4** | $R=R_1=R_2=OCOCH_3$ |
| **5** | $R=OH$; $R_1=R_2=H$ |
| **6** | $R=OH$; $R_1=H$; $R_2=OCH_3$ |
| **7** | $R=OH$; $R_1=OCOCH_3$; $R_2=H$ |
| **8** | $R=OH$; $R_1=R_2=OCOCH_3$ |
| **9** | $R=R_2=OH$; $R_1=OCOCH_3$ |

The results obtained with peracetates **1-4** are summarised below:

1. The study of enzymatic deacetylation in different anhydrous solvents revealed that the regioselectivity of the enzymes PPL and CCL remains almost the same in all the four solvents, *i.e.* diisopropyl ether, tetrahydrofuran, acetone and acetonitrile and the yields of the reactions are dependent on the nature of the solvent, maximum yields were obtained with PPL in THF.
2. Deacetylation of benzyl 2,4-diacetoxyphenyl ketone (**1**) and 4-methoxybenzyl 2,4-diacetoxyphenyl ketone (**2**) was highly regioselective giving benzyl 2-acetoxy-4-hydroxyphenyl ketone (**5**) and 4-methoxybenzyl 2-acetoxy-4-hydroxyphenyl ketone (**6**) in 65% and 73% yield, respectively. Deacetylation of benzyl 2,4,6-triacetoxyphenyl ketone (**3**) which has three acetoxyl groups is also regioselective giving exclusively benzyl 2,6-diacetoxy-4-hydroxyphenyl ketone (**7**) in 70% yield.
3. 4-Acetoxybenzyl 2,4,6-triacetoxyphenyl ketone (**4**) underwent deacetylation at position 4, and to a slower rate, at position 4' to yield 4-acetoxybenzyl 2,6-diacetoxy-4-hydroxyphenyl ketone (**8**) and 4-hydroxybenzyl 2,6-diacetoxy-4-hydroxyphenyl ketone (**9**) in 55% and 15% yield, respectively.

**Table 1. Effect of solvent on transesterification reactions between polyphenolic peracetates 1-4 and *n*-butanol catalysed by PPL and CCL**

| | | *Solvent [Time of reaction (hrs), Chemical yield (%)/Lipase]* | | | |
|---|---|---|---|---|---|
| *Substrate* | *Product(s) isolated* | Acetone | Acetonitrile | THF | DIPE |
| Benzyl 2,4 di-acetoxyphenyl ketone (**1**) | benzyl 2-acetoxy-4-hydroxyphenyl ketone(**5**) | 40,35 PPL | 50,35 PPL | 42,65 PPL | 45,60 CCL |
| 4-Methoxybenzyl 2,4-diacetoxy-phenyl ketone (**2**) | 4-methoxybenzyl 2-acetoxy-4-hydroxy-phenyl ketone (**6**) | 48,42 PPL | 50,35 PPL | 40,73 PPL | 45,65 CCL |
| Benzyl 2,4,6-tri-acetoxyphenyl ketone (**3**) | benzyl 2,6-diacetoxy-4-hydroxyphenyl ketone (**7**) | 40,40 PPL | 42,38 PPL | 40,70 PPL | 40,65 CCL |
| 4-Acetoxybenzyl 2,4,6-triacetoxy-phenyl ketone (**4**) | 4-acetoxybenzyl 2,6-diacetoxy-4-hydroxy-phenyl ketone (**8**) and | 48,18 PPL | 48,18 PPL | 45,55 PPL | 46,52 CCL |
| | 4-hydroxybenzyl 2,6-diacetoxy-4-hydroxy-phenyl ketone (**9**) | ---- | ---- | 45,15 PPL | 46,15 CCL |

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

These results indicate that the acetoxyl group at the *para* position to the carbonyl group is deacetylated predominantly over the one at the *ortho* position and catalytic activity of enzyme increases when non-polar solvent, like DIPE or THF is used. These observations and our similar results on polyacetoxy aryl-methyl ketones[12] suggest that the enzyme binds to the carbonyl function of the substrate in such a way that it inhibits the deacetylation of the acetoxyl group *ortho* to the carbonyl function and places the other acetoxyl groups near the serine residue of the active site of the lipase, thereby facilitating deacetylation at these positions.

To test the generality of the observed phenomena, we studied the regioselectivity of PPL and CCL on resorcinol diacetate and found that the enzyme failed to recognise the acetoxyl groups

in the molecule and deacetylation exclusively yielded only the dihydroxy compound, resorcinol. This may be because of the absence of directly attached carbonyl group to the aromatic nucleus through which it has been possible to recognise the different acetoxyl groups in the substrates **1-4** by the enzyme. In the course of these enzymatic deacetylation studies, we have prepared the peracetylated polyhydroxylated benzyl phenyl ketones **3** and **4** and the partially acetylated ketones **5** to **9** for the first time, these seven new compounds have been characterised from their spectral data.

**References and Notes**


1. Klibanov, A.M. *Trends Biochem. Sci.* **1989**, 14,141. Dordick, J.S. *Enzyme Microb. Technol.* **1989**, 11, 194.
2. Zaks, A.; Klibanov, A.M. *J. Am. Chem. Soc.* **1986**, 108, 2767. Gaestner, H.; Pingserver, A. *Eur. J. Biochem.*, **1989**, 181, 207. Ferjancic, A.; Pingserver, A.; Gaetner, A. *Appl. Microbiol. Biotechnol.* **1990**, 32, 651.
3. Rubio, E.; Fernandez-Mayorales, A.; Klibanov, A.M. *J. Am. Chem. Soc.* **1991**, 113, 695.
4. Sakumai, T.; Margolin, A.L.; Russell, A.J.; Klibanov, A.M. *J. Am. Chem. Soc.* **1988**, 110, 7236. Huber, J.E.; Klibanov, A.M. *J. Am. Chem.* Soc. **1989**, 111, 3094. Kisse, H.; Hayakawa, A.; Noritonio, H. *J. Biotechnol.* **1990**, 14, 239.
5. Klibanov, A.M. *Acc. Chem. Res.* **1990**, 23, 114. Dordick, J.S. *Enzyme Microb. Technol.* **1989**, 11, 194. Fourneron, J.D.; Chiche, M.; Pieroni, G. *Tetrahedron Lett.* **1990**, 31, 4875.
6. *Lipases;* Borgstorm, B.; Brockman, H.; Eds.; Elsevier: Amsterdam, 1984.
7. Chen, C.S.; Sih, C.J. *Angew. Chem. Int. Ed. Engl.* **1989**, 28, 695.
8. Ballestros, A.; Bernabe, M.; Cruzado, C.; Martin-Lomas, M.; Otero, C. *Tetrahedron* **1989**, 45, 7077. Sinay, P. *Pure Appl. Chem.* **1987**, 59, 445.
9. Xie, Z.F.; Suemune, H.; Sakai, K. *J. Chem. Soc., Chem. Commun.* **1988**, 1639. Fuganti, C.; Pedrocchi-Fantoni, G.; Servi, S. *Chemistry Lett.* **1990**, 1137.
10. Natoli, M.; Nicolosi, G.; Piattelli, M. *Tetrahedron Lett.* **1990**, 31, 7371.
11. Jain, A.C.; Arya, P.; Nayyar N.K. *Indian J. Chem.* **1984**, 23B, 1030, and references cited therein.
12. Parmar, V.S.; Prasad, A.K.; Sharma, N.K.; Singh, S.K.; Pati, H.N.; Gupta, S. *Tetrahedron* **1992**, 48, 6495. Parmar, V.S.; Prasad, A.K.; Sharma, N.K.; Bisht, K.S.; Sinha, R.; Taneja, P. *Pure Appl. Chem.* **1992**, 64, 1135.