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Preparative and mechanistic aspects of interesterification reactions on diols and peracetylated polyphenolic compounds catalysed by lipases+

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ABSTRACT - The lipases from porcine pancreas and *Candida cylindracea* have been successfully used in carrying out simple, efficient and potentially useful biotransformations on diols and peracetylated polyphenolic esters. It has been observed that the esterification reactions on 1,2-diols are highly regioselective and the primary hydroxyl group is exclusively esterified over the one at the secondary position. The results on lipase-mediated deacylation reactions on the mixed diesters of 1,2-diols suggest that during deacylation, the migration of acyl group takes place. Additionally, the lipase-catalysed deacetylations on peracetates of polyhydroxy aromatic esters substantiate the Schiff's base complex hypothesis proposed earlier by us for the regiospecific deacetylations on peracetates of polyhydroxyaryl-alkyl ketones and benzopyranones.

INTRODUCTION

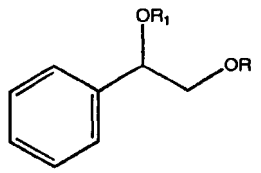
Enzymatic reactions are becoming increasingly popular among synthetic organic chemists and are being reported at an ever increasing rate (ref.1). The utility of hydrolases, especially lipases is well-known (ref. 2). In recent years, lipase-catalysed transformations have been studied for resolution of alcohols, esters and amides by several groups of workers (ref. 3). In the present study, lipases from porcine pancreas and *Candida cylindracea* have been successfully used in the regioselective acetylation/deacylation of 1,2-diols and their mixed esters, and deacetylation of peracetylated esters of polyhydroxy aromatic acids.

TRANSESTERIFICATIONS ON 1,2-DIOLS

Owing to the importance of diols in synthetic chemistry as important structural units for a large number of biologically active natural and synthetic compounds, a number of reports on biotransformations involving diols using different lipases have appeared. In the past, esterification reactions on different 1,2-diols have been reported in biphasic systems (ref. 4), acid anhydrides (ref. 5) and alkyl carboxylates (ref. 6,7). We have earlier demonstrated (ref. 8) that by using trifluoroethyl butyrate as the acylating agent in dry organic solvents, PPL and CCL catalyse regiospecifically the esterification of the primary alcoholic group in 1,2-diols. Also, it was observed that deacylation of the 1,2-diol diacetates by PPL or CCL in dry organic solvents containing *n*-BuOH results in the formation of 2-hydroxy -1-acetyloxyalkanes by remarkable regioselective deacetylation at the secondary acyloxy position (ref. 8). The formation of the products could either result from the direct alcoholysis at the secondary acyloxy position, or by migration of the acyl group from one position to the other

hydroxy group, set free by the lipase. In order to ascertain the mechanism of formation of the products during alcoholysis, we had carried out PPL-catalysed deacylation on 1-acetoxy-2-butanoyloxy-1-phenylethane (**1**) and the result supported the direct deacylation at the secondary acyloxy position (ref. 8) without any concurrent acyl migration. Similar investigations have also been made by other groups of workers in enzymatic hydrolysis of 1,2-diol diacetates in buffer medium and the products were mixtures of primary and secondary monoesters (ref. 9). In order to substantiate the mechanism of formation of the products during alcoholysis, we have now prepared 1-acetoxy-2-butanoyloxypentane (**2**), 1-acetoxy-2-butanoyloxyhexane (**3**) and 2-acetoxy-1-butanoyloxy-1-phenylethane (**4**) by PPL-catalysed highly regioselective acetylation of the primary hydroxy group of the corresponding 1,2-diols with vinyl acetate in dry acetone, followed by butyration of the secondary hydroxyl group chemically with butyric anhydride and 4-dimethylaminopyridine. The PPL-catalysed deacylation reactions on **2** and **3** resulted in the formation of mixtures of 2-butanoyloxypentan-1-ol (**5**) and 1-butanoyloxypentan-2-ol (**6**) in the ratio 2.2:1, and 2-butanoyloxyhexan-1-ol (**7**) and 1-butanoyloxyhexan-2-ol (**8**) in the ratio 2.4:1, respectively. The 2-acetoxy-1-butanoyloxy-1-phenylethane (**4**) did not show any indication of deacylation even after three days of incubation. The formation of the 1- and 2- butanoyloxyalcohols **5-8** clearly indicates that the lipase - catalysed deacylation in **2** and **3** takes place exclusively at the primary acyloxy position (as no compound carrying an acetoxy group was isolated) and also that the migration of the butanoyl group from the secondary to the primary hydroxyl group, set free by the lipase takes place to an appreciable extent.

The lipase-catalysed deacylations on 1-acetoxy-2-butanoyloxypentane (**2**) and 1-acetoxy-2-butanoyloxyhexane (**3**) are being reported for the first time by us.



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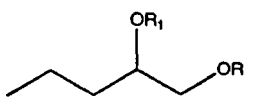
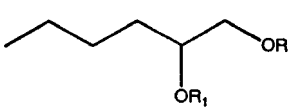
4 R=$COCH_3$, R_1=COC_3H_7



2 R=$COCH_3$, R_1=COC_3H_7
5 R=H, R_1=COC_3H_7
6 R=COC_3H_7, R_1=H



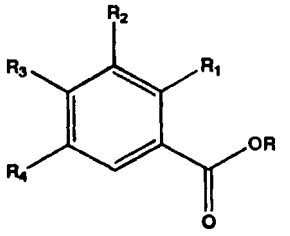
3 R=$COCH_3$, R_1=COC_3H_7
7 R=H, R_1=COC_3H_7
8 R=COC_3H_7, R_1=H

Table 1. Deacetylation reactions on peracetates of phenolic esters 9-16 with *Candida cylindracea* lipase in diisopropyl ether at 28 - 30°C.

Substrate	Product(s) (yield, %) (time of reaction, hrs)
Methyl 4-acetoxybenzoate (**9**)	Methyl 4-hydroxybenzoate (**17**) (95) (16)
Ethyl 4-acetoxybenzoate (**10**)	Ethyl 4-hydroxybenzoate (**18**) (97) (14)
Methyl 2-acetoxybenzoate(**11**)	Methyl 2-hydroxybenzoate (**19**) (90) (72)
Ethyl 2-acetoxybenzoate(**12**)	Ethyl 2-hydroxybenzoate(**20**) (94) (48)
Methyl 2,4-diacetoxybenzoate (**13**)	Methyl 2-acetoxy-4-hydroxybenzoate(**21**) (47) (16) *and* Methyl 2,4-dihydroxybenzoate (**22**) (49)
Ethyl 2,4-diacetoxybenzoate (**14**)	Ethyl 2-acetoxy-4-hydroxybenzoate (**23**) (42) (18) *and* Ethyl 2,4-dihydroxybenzoate (**24**) (52)
Methyl 3,5-diacetoxybenzoate (**15**)	Methyl 3-acetoxy-5-hydroxybenzoate (**25**) (25) (5) *and* Methyl 3,5-dihydroxybenzoate (**26**) (70)
Ethyl 3,5-diacetoxybenzoate (**16**)	Ethyl 3-acetoxy-5- hydroxybenzoate (**27**) (25) (3) *and* Ethyl 3,5-dihydroxybenzoate (**28**) (68)

DEACETYLATION OF PERACETATES OF POLYPHENOLIC ESTERS

Previously, regioselective deacetylations in peracetates of polyhydroxyaryl-alkyl ketones and benzopyranones, catalysed by lipases from porcine pancreas and *Candida cylindracea* have been studied by us (ref. 10-14). The regioselectivity demonstrated by lipases towards deacetylation of the acetoxy groups, other than those at the *ortho* positions was explained by suggesting that active site of the lipase contains a lysine residue (similar to the human pancreatic lipase) and the ε-amino group of the lysine residue forms a Schiff's base complex with the nuclear carbonyl of the substrate (Fig. 1). The formation of Schiff's base results in orienta-



9 $R=CH_3, R_1=R_2=R_4=H, R_3=OCOCH_3$
10 $R=C_2H_5, R_1=R_2=R_4=H, R_3=OCOCH_3$
11 $R=CH_3, R_1=OCOCH_3, R_2=R_3=R_4=H$
12 $R=C_2H_5, R_1=OCOCH_3, R_2=R_3=R_4=H$
13 $R=CH_3, R_1=R_3=OCOCH_3, R_2=R_4=H$
14 $R=C_2H_5, R_1=R_3=OCOCH_3, R_2=R_4=H$
15 $R=CH_3, R_1=R_3=H, R_2=R_4=OCOCH_3$
16 $R=C_2H_5, R_1=R_3=H, R_2=R_4=OCOCH_3$
17 $R=CH_3, R_1=R_2=R_4=H, R_3=OH$
18 $R=C_2H_5, R_1=R_2=R_4=H, R_3=OH$
19 $R=CH_3, R_1=OH, R_2=R_3=R_4=H$
20 $R=C_2H_5, R_1=OH, R_2=R_3=R_4=H$
21 $R=CH_3, R_1=OCOCH_3, R_2=R_4=H, R_3=OH$
22 $R=CH_3, R_1=R_3=OH, R_2=R_4=H$
23 $R=C_2H_5, R_1=OCOCH_3, R_2=R_4=H, R_3=OH$
24 $R=C_2H_5, R_1=R_3=OH, R_2=R_4=H$
25 $R=CH_3, R_1=R_3=H, R_2=OCOCH_3, R_4=OH$
26 $R=CH_3, R_1=R_3=H, R_2=R_4=OH$
27 $R=C_2H_5, R_1=R_3=H, R_2=OCOCH_3, R_4=OH$
28 $R_1=C_2H_5, R_1=R_3=H, R_2=R_4=OH$

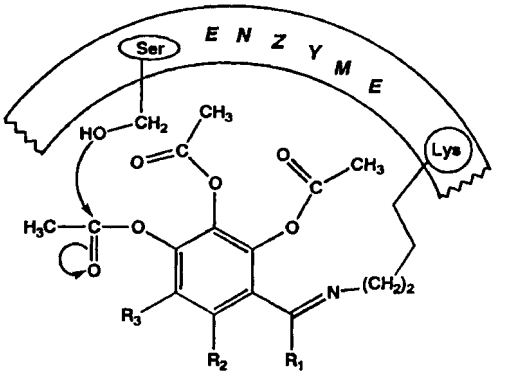


Fig.1. Proposed model of the action of PPL and CCL on polyacetoxyaryl/alkyl ketones.

tion of the different acetoxy groups in such a manner that the *ortho* acetoxy group is shielded by the bulk of the lipase and is not accessible to the hydroxyl group of the serine present in the active site. However, no direct evidence could be cited to support this hypothesis as the X-ray structure of PPL or CCL is not available and the Schiff's base complex could not be isolated as its formation is expected to be a dynamic process. Therefore to provide evidence to our suggested mechanism involving Schiff's base formation, we have carried out lipase-catalysed deacetylations of methyl and ethyl mono- and diacetoxybenzoates. Since the esters are not known to form the Schiff bases that easily, we would except much lower selectivity in the lipase-catalysed deacetylation of the peracetates of phenolic acid esters. The incapability of the aromatic acid esters to form Schiff's base complex with the lysine residue could result in random placement of different acetoxy groups near the hydroxyl group of serine present in the active site of the lipase. As a result, deacetylation at all the acetoxy positions may take place with equal ease. Results of CCL-catalysed deacetylations on methyl 4-acetoxybenzoate (**9**), ethyl 4-acetoxybenzoate (**10**), methyl 2-acetoxybenzoate (**11**), ethyl 2-acetoxybenzoate (**12**), methyl 2,4-diacetoxybenzoate (**13**), ethyl 2,4-diacetoxybenzoate (**14**), methyl 3,5-diacetoxybenzoate (**15**) and ethyl 3,5-diacetoxybenzoate (**16**) are summarized in Table 1.

Our results indicate that the ethyl or methyl monoacetoxybenzoates **9-12** are deacetylated to give the corresponding hydroxy esters in high yields, irrespective of the position of the nuclear carboalkoxy group from the

acetyloxy group, i.e. both the *ortho* acetoxy group (in **11** and **12**) and *para* acetoxy group (in **9** and **10**) are deacetylated with equal ease. In the case of methyl and ethyl esters of 2,4-diacetoxybenzoic acid (**13** and **14**), though there is preference towards deacetylation of the *para* acetoxy group to give the monoacetoxy esters **21** and **23**, respectively, the *ortho* acetoxy group is also deacetylated to give in addition the corresponding dihydroxybenzoic acid esters, **22** and **24**, respectively. It is interesting to note that the deacetylation of the ethyl and methyl esters of 3,5-diacetoxybenzoic acid (**15** and **16**) results in the formation of the corresponding 3-acetoxy-5-hydroxybenzoates **25** and **27**, respectively in 25% yield; however, the completely deacetylated esters **26** and **28**, respectively are obtained as major products. Partially protected esters, such as **25** and **27** are difficult to obtain by purely chemical means. The results obtained in CCL-catalysed deacetylation of the esters **9-16** in dry organic solvents provide support to our earlier proposed mechanism involving Schiff's base formation by the lysine residue of the enzyme with the nuclear carbonyl group in aryl-alkyl ketones and benzopyranones (Fig. 1).

In conclusion we have demonstrated that lipase-catalysed esterifications and deacetylations on 1,2-diols and their diacyl derivatives, respectively are carried out in a highly regioselective fashion. The deacylation studies on the peracetates of ethyl or methyl polyphenolic aromatic esters provide support to the mechanism of the enzymatic deacetylation of the peracetates of polyphenolic aromatic ketones . Interestingly, the phenolic acetate ester groups are hydrolysed exclusively over the carboalkoxy ester linkage in the same compound. The chemo- and regioselectivity and the high yields in the enzymatic reactions reported by us in this paper can be exploited for selective protection of such compounds in high yields in Synthetic Organic Chemistry.

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