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Pen G Acylase Catalyzed Resolution of Phenylacetate Esters of Secondary Alcohols

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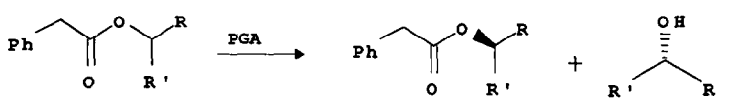
Abstract: Penicillin G acylase from *E. coli* (E.C. 3.5.1.11.) immobilized on Eupergit C is used for the kinetic resolution of phenyl acetate esters of secondary alcohols of pharmaceutical interest.

The enzyme Penicillin G Amidohydrolase (Pen G Acylase, PGA, E.C. 3.5.1.11) is well known for its industrial application in the hydrolysis of penicillin G, leading to the preparation of 6-amino penicillanic acid (6-APA)[1]. Due to the rather large production of the enzyme and hence its availability it is continuously finding new applications in biocatalysis[2]. The enzyme shows a strong affinity for the phenyl acetyl moiety and a high tolerance as far as the rest of the molecule is concerned. This is a desirable quality for an enzymatic system because a large number of substrates are accepted, thus widening the possible applications, but the low recognition of other parts of the substrates results in a low degree of enantioselectivity in the hydrolysis of phenyl acetate derivatives of racemic primary alcohols and amines giving after hydrolysis products of only low to medium enantiomeric excess [3,4]



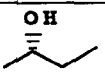
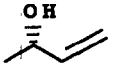
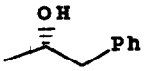
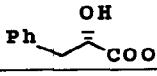
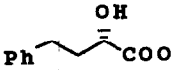
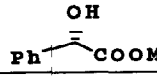
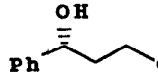
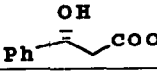
a **b** **c**

1	R = Et	R' = Me	**6**	R = COOEt	R' = Benzyl
2	R = Vinyl	R' = Me	**7**	R = COOEt	R' = Phenyl ethyl
3	R = Me	R' = Phenyl	**8**	R = COOMe	R' = Phenyl
4	R = Benzyl	R' = Me	**9**	R = CH_2CH_2Cl	R' = Phenyl
5	R = Et	R' = Phenyl	**10**	R = CH_2COOEt	R' = Phenyl

Scheme 1: Mode of hydrolysis of phenylacetyl esters of secondary alcohols

We have recently shown however that chirality present in the part of the molecule other than the phenyl acetyl moiety is well recognised allowing the resolution of racemic mandelic acid derivatives and products related to it[5]. We have now considered the resolution catalyzed by immobilised Pen G acylase of a group of phenylacetates of secondary alcohols with the aim of obtaining products enriched in one enantiomer and we report on the results obtained. As substrates the phenyl acetate esters of carbinols of current synthetic interest such as products **7c**, **9c** and **10c** key intermediates in the synthesis of therapeutically active enantiomeric forms of important drugs such as (R)-fluoxetine and various members of the ACE-inhibitors family were selected[6,7,8]. **Scheme 1** describes the mode of hydrolysis of the group of selected compounds giving as a result of partial kinetic resolution secondary alcohols of varying enantiomeric excess. Hydrolyses were run with 5 mmol of substrate in 50 mL of phosphate buffer at pH 8 which was added with 125 IU of enzyme immobilized on Eupergit C while the pH was maintained at the initial value with an automatic titrator. At 50% conversion the reaction was stopped by filtrating the enzyme off. The enantiomeric excess was evaluated in general on the alcohol obtained, with various methodologies as described in the experimental part. Experiments described in **Table 1**, entries 1-4 show the hydrolysis of esters occurring with the higher rate, giving alcohols of low enantiomeric excess.

Table 1: Products obtained from the PGA catalyzed hydrolysis of esters **1a-10a**

Entry	Product obtained	% Hydrolysis	e.e. (%)	E value	Method
1		50	26	2.15	a
2		50	10	1.3	a
3		50	40	4	a
4		50	56	6	a
5		12	94	36	a
6		50	92	79	a
7		25	94	44	b
8		50	90	58.5	b
9		18	>98	>100	a
10		32	36	2.5	b

Phenyl acetates of α-hydroxy esters (**6a**, **7a** and **8a**) are hydrolysed rather efficiently giving products of high e.e. The corresponding alcohols are obtained with the absolute configuration similar to the one shown by others for the analogous series of phenacetyl derivatives of α-amino esters[9]. Compound **7a** is not hydrolysed further then 25%. **7c** is the S-enantiomer of an intermediate used in the synthesis of homochiral drugs of the ACE-inhibitor class for which several enzymatic approaches are known[8]. Compounds **5c** and **9c** are obtained with good or excellent e.e., but the hydrolysis stops at modest conversions. This is particularly disappointing due to the special interest in compound **9c** which is an advanced intermediate in the synthesis of (R)-fluoxetine[6]. Compounds bearing an α-carboxylate seem particularly good substrates for the enzyme. Small structural modifications, as it is often the case, change dramatically the reaction rate and/or the enantiospecificity of the reaction (entries 8-10). The similarity of the way of hydrolysis of these compounds with the N-phenacetyl derivatives of α-aminoacids[9] have already been commented upon.The presence of the aromatic moiety seems beneficial for a good fit into the enzyme binding site, since compounds **1c** and **2c** have very low enantiomeric excesses. The same is true for the α-hydroxy ester group. The phenylacetate derivatives of less functionalized alcohols (entries 1 and 2, but to a minor extent also 3 and 4) apparently do not possess the steric and/or polarity requirements necessary for a good fit into the enzymic binding site.
Summarizing the behaviour of the immobilized PGA from these results, we can affirm that while the utility of the enzyme for the resolution of secondary alcohols seems to be ruled by more subtle requirements in terms of steric and polar character then with most of the widely used hydrolytic enzymes, the resolution process can occur with good efficiency when the conditions are met.

EXPERIMENTAL

Penicillin G amidase (PGA) immobilized on Eupergit-C was from Recordati-DE.BI. (Italy). HPLC analysis were run on a Merck-Hitachi L-6200 equipped with a UV detector L-4200 at 220 nm. Column Chiracel OD (DAICEL) with mixtures of hexane-*i*-propanol. GC analysis were run with a DANI 86.10 with a FID detector. Column Megadex 1 and Megadex 3 (MEGA, Italy), using He as carrier. The pH of the solution during hydrolysis was kept constant with an automatic titrator Metrohm E 526. Racemic alcohols were commercially available beside 7, prepared form racemic homophenylalanine through diazotation, and **10** prepared by H_2/10% Pd on charcoal reduction of the commercially available corresponding ketone **7**.

Preparation of phenylacetic acid esters.

The alcohol (10 mmol) was dissolved in methylene chloride or benzene (20 mL) containing 1 mL of pyridine. A slight excess of phenylacetyl chloride was then added dropwise and the mixture was refluxed for 2 h. The solution was then diluted with ethyl acetate and washed with sodium bicarbonate and water. If necessary the phenylacetate obtained was purified from the unreacted alcohol through column chromatography.

Hydrolysis of substrates

The ester (6 mmol) was dissolved in CH_3CN (6/10 mL) and diluted with 0.1 M phosphate buffer at pH 8.0 to a final concentration of about 150 mM (34/40 mL of buffer) at 27/28 °C. The enzyme (150 IU) was added and the mixture stirred at 200 rpm. The pH was maintained at 8.0 with the automatic addition of NaOH. At 50%

conversion, the reaction mixture was filtered through a sintered glass filter and extracted with ethyl acetate. The extract was passed through a short silica gel column and the two products isolated. The determination of the enantiomeric excess was performed with one of the following methods:

a): GC on Megadex 1. Comparison with retention times of the racemic mixture. The absolute configuration is determined by comparison with an authentic sample of the enantiomerically pure compound (entries 3,-6, 9).

b:) HPLC on Chiralcel OD. Comparison with retention times of the racemic mixture. The absolute configuration is determined by comparison with an authentic sample of the enantiomerically pure compound (entries 7, 8, 10).

The product analysed was the unhydrolysed phenacetyl-ester for entry 1 and 2, the alcohol from hydrolysis for all other entries. Product **2** was hydrogenated for comparison with an authentic sample of enantiomerically pure **1**. Product **7** was compared with a sample obtained from enantiomerically pure D-homophenylalanine Product **9** was compared with a sample obtained by resolution with lipase[6]

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