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ENANTIOSELECTIVE RECOGNITION OF THE PHENACETYL MOIETY IN THE PEN G ACYLASE CATALYSED HYDROLYSIS OF PHENYLACETATE ESTERS

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(Dedicated to Prof. Cesare Cardani for its 70th birthday)

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Abstract:. Penicillin G acylase from E. coli (E.C. 3.5.1.11) immobilized on Eupergit C enantioselectively recognises phenyl acetates derivatives allowing the preparation of compounds of high ee to be used as side chains in the preparation of β-lactam antibiotics.

The enzyme Penicillin G Amidohydrolase (E.C. 3.5.1.11) (Pen G acylase) from *E. coli* ATCC 9637, catalyzes the hydrolysis/formation of amide and ester bonds in compounds

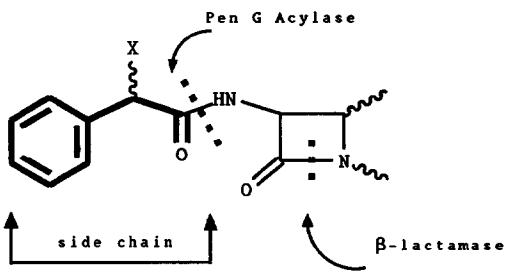
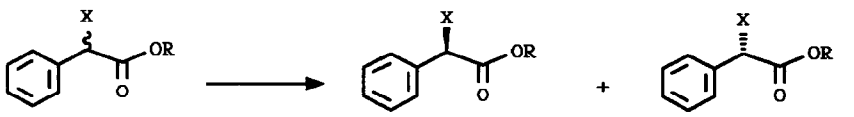


Figure 1

derived from phenyl acetic acid[1] and together with β-lactamase catalyzes the hydrolytic inactivation of penicillins and analogues (Figure 1). The application of preparations of this enzyme in a stable immobilized form for the production of 6-aminopenicillanic acid (6-APA) from penicillin G, constitute one of the largest utilizations of enzymatic

catalysis in the production of pharmaceuticals[2]. Application of this enzyme in the synthesis of penicillins[3] and cephalosporins[4] from 6-APA and 7-ADCA respectively with various phenylacetyl "side chains" is considered to become competitive with the chemical synthesis in the near future. The enzymatic activity of Pen G Acylase has been associated with the phenacetyl moiety and it has been shown that hydrolysis takes place in a variety of phenacetyl derivatives of primary amines[5] and alcohols[6], and that phenoxy acetyl derivatives are also accepted as substrates[7]. Among the side chains which characterize therapeutically important β-lactam antibiotics, some bear a chiral α-carbon, due to the presence of an amino group e.g. (ampicillin, amoxycillin, and cephachlor) or a hydroxyl group (cephamandol). The presence of a chiral carbon atom on the "side chain" of those β-lactams poses the question as to whether the amidase is able to selectively recognize the chain according to its chirality. In fact Pen-G acylases of different origin have been employed for the resolution of a number of amines and amino acids[5], aminoalkylphosphonic acids[8], primary and secondary alcohols[6] bonded to the phenacetyl chain, but there is surprisingly no information concerning the stereochemical demands of the phenyl acetyl moiety as depicted in Figure 1. We therefore studied the hydrolysis of a series of α-substituted phenyl acetic acid derivatives (Scheme 1), and we report here



1a X = OH, R = Me
2a X = OH, R = *i*Pr
3a X = OMe, R = Me
4a X = OCHO, R = Me
5a X = Me, R = Me
6a X = Me, R = *i*Pr
7a X = Br, R = Me
8a X = Cl, R = Me
9a X = OSi*t*ButMe$_2$, R = Me
10a X = OAc, R = Me
11a X = Et, R = Me

1b X = OH, R = H
3b X = OMe, R = Me
4b X = OCHO, R = Me
5b X = Me, R = H
7b X = Br, R = H
8b X = Cl, R = H

1c X = OH, R = Me
2c X = OH, R = *i*Pr
3c X = OMe, R = H
4c X = OCHO, R = H
5c X = Me, R = Me
6b X = Me, R = *i*Pr
7c X = Br, R = Me
8c X = Cl, R = Me

Scheme 1

on the results obtained (Table 1). In a typical procedure, 5 mmol of substrate in 50 mL of phosphate buffer at pH 8, stirred at 200 rpm was treated with 125 units of enzyme immobilized on Eupergit C[9] while the pH was manitained at the initial value by means of an automatic titrator. At 50% conversion the reaction was stopped by extraction with ethyl acetate. The acids obtained were analyzed by HPLC or GLC on chiral columns and ^{1}H NMR experiments with chiral shift reagents as such, or after conversion to the corresponding esters with CH_2N_2. The absolute configuration of the esters thus obtained was assigned by comparing the sign of the optical rotation with esters of known configuration[11]. Entry 1 shows that methyl mandelate is the substrate which was transformed at the highest rate, but that the products obtained were of very low enantiomeric excesses. The isopropylester (entry 2) was recovered in about 20% ee. Ongoing to the O-methyl derivative (entry 3), the reaction rate was slower, reaching 50% in 2.5 h, and both the acid and the recovered ester have an ee >98%. A similar result is

obtained with the O-formyl derivative in which the *S* enantiomer is hydrolysed first as in the preceeding examples. Surprisingly the acetate **10a** differing from **4a** of only one methyl group is not a substrate, like compounds with larger substituents **9a**. The structural requirement in the case of an alkyl substituent in the α-position is also very strict in that while the α-methyl derivative **5a** is hydrolyzed with a preference opposite to the one previously observed, compound **11a** is not transformed at all. Compound **5c** is obtained in an enantiomeric excess significant (76%) at 25% conversion, but lower (64%) at 50%. However the isopropyl ester **6a** is hydrolysed giving both the acid and the survived ester in >98% ee. The two halogen atoms as substituents are not accepted efficiently, but also in this case there is a difference in reactivity which can be correlated with the relative size of the substituents. Noteworthy also is the striking difference due to polarity as evident in comparing entry 1 and 3. Furthermore **3c** and **4c** have absolute configuration opposite than in **5c**. These results show that within the requirement for the general phenyl acetate structure, variation due to substituents in

Table 1: Mode of hydrolysis of esters 1a-8a with Pen-G acylase

Ester	Acid obtained	X	V_H	% ee of acids (*R*,*S*)#
1a	1b	OH	114	15 (*R*)[a]
2a	1b	OH	79	20 (*R*)[a]
3a	3c	OCH_3	33	>98 (*S*)[a,c]
4a	4c	OCHO[d]	1.4	>98 (*S*)[b,c]
5a	5b	CH_3	16	64 (*R*)[b,c]
6a	5b	CH_3	2.1	>98 (*R*)[b,c]
7a	7b	Br	11	3 (*R*)[b]
8a	8b	Cl	73	20 (*R*)[b]

Absolute configuration was determined by rotation sign, elution order or comparison with authentic samples. Ee of recovered acids is measured on the esters obtained after treatment with CH_2N_2.
At 50% conversion (automatic titrator)

V_H is the velocity of hydrolysis in μmol of substrate/minute x g of immobilized enzyme

a: HPLC on Chiracel OD; b: GLC on Megadex (β-cyclodextrine) capillary column

c: ^1H NMR with chiral shift reagent *tris*-[3(heptafluoropropylhydroxy-methylene)-*D*-camphorate],Eu(III).

d: reaction run at 1°C to avoid chemical hydrolysis of the formate ester which is prevalent at 25 °C as shown by control experiments.

Table 1

α-position strongly influence rate of hydrolysis, ee of products and hence enantiomer recognition. It remains open the question whether such a capacity will still be active in the ester-amide bond formation step. The fact that the formyl derivative (entry 4) has

the substituents required in an intermediate in the chemical synthesis of cephamandol, suggests a possible introduction of this side chain by the enzymic reaction with concomitant resolution of the formyl derivative.

REFERENCES

1. M. Cole, *Nature* (London), **1964**, *203*, 50; W. Kaufman and K. Bauer, *Naturwiss.*,**1960**, *47*, 474.
2. D.L. Regan, P. Dunnhil and M.D. Lilly, *Biotechnol. Bioeng.*, **1974**, *16*, 333; W. Dürckeimer, J. Blumbach, R. Lattrell and K.H. Scheunemann, *Angew. Chem. Int. Ed. Engl.*, **1985**, *24*, 180. 6,000 tons/year of 6-APA are estimated to be produced by enzymic hydrolysis using either Pen-G or Pen-V acylases.
3. S.K. Kaasgaard and P.B. Poulsen in " *Bioorganic Chemistry in Health Care and Technology*", U.K. Pandit and F.C. Alderweireldt Editors, NATO ASI Series vol. 207, pp 149, 1991.
4. E.M. Baldaro in " *Bioorganic Chemistry in Health Care and Technology*", U.K. Pandit and F.C. Alderweireldt Editors, NATO ASI Series vol. 207, pp 237, 1991.
5. D. Rossi, A. Romeo, G. Lucente, *J. Org. Chem.* **1978**, *43*, 2576; D. Rossi, A. Calcagni and A. Romeo, *J. Org. Chem.*, **1979**, *44*, 2222; C. Fuganti, D. Grasselli and P. Casati, *Tetrahedron Lett.*, **1986**, *27*, 3191.
6. C. Fuganti, P. Grasselli, P.F. Seneci, S. Servi and P. Casati, *Tetrahedron Lett.* **1986**, *27*, 2061; C. Fuganti, P. Grasselli, S. Servi, A. Lazzarini and P. Casati, *J. Chem. Soc. Chem. Commun.* **1987**, 538; C. Fuganti, P. Grasselli, S. Servi, A. Lazzarini and P. Casati, *Tetrahedron*, **1988**, *44*, 2575; H. Waldmann, *Tetrahedron Lett.* **1989**, *30*, 3057.
7. M.J. Zmijewski Jr., B.S. Briggs, A.R. Thompson and I.G. Wright, *Tetrahedron Lett.* **1991**, *32*, 1621.
8. V.A. Solodenko, T.N. Kasheva, V.P. Kukhar, E.V. Kozlova, D.A. Mironenko and V.K. Svedas, *Tetrahedron*, **1991**, *47*, 3989; W. Lothar, G. Fuelling, R. Keller, Eur. pat. appl. 382113, 1990 to Hoechst A.-G.
9. Pen-G Acylase immobilized on Eupergit C was from De.Bi. (Recordati). The same hydrolytic experiments were also run with Pen-G Acylase from *Kluyvera citrophila* (Antibioticos S.A. Madrid) immobilized on Agarose (ref. 10). The results obtained were similar.
10. J.M. Guisan, *Enzyme Microb. Technol.*, **1988**, *10*, 375
11. R. Chenevert and M. Létourneau, *Can. J. Chem.* **1990**, *68*, 314 and references therein.

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