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KINETIC RESOLUTION OF p-HYDROXYPHENYL ACETAMIDES BY HYDROLYSIS WITH PEN-G ACYLASE

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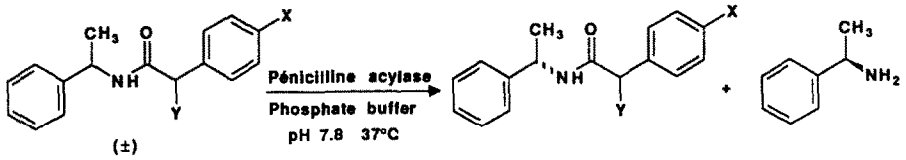
Abstract : The p-hydroxyphenylacetic amides of various amines were enantioselectively hydrolyzed with the aid of penicillin acylase.

Nowadays, there is an increasing demand for efficient processes for the synthesis of optically active compounds (1). For this purpose biocatalyst have widely been utilized (2) and in this field many examples of asymmetric esterification or transesterification catalyzed by hydrolytic enzymes are emerging as a method of choice for facile kinetic resolutions of racemic alcohols, acids and their derivatives. No such syntheses of chiral amines seem to have been so generally attempted despite the importance of chiral amines as chiral auxiliaries and catalyst and intermediates for pharmaceutical industry. With respect to the amidation reaction catalyzed by enzymes, only proteases such as subtilisin Carlsberg (3) and lipase from Candida cylindracea (4) have been applied with success in kinetic resolution of amino derivatives.

The enzyme penicillinacylase (E.C. 3.5.1.11) selectively transfers the phenylacetyl moiety of benzylpenicillin to water and is used in the production of 6-amino-penicillanic acid. Despite the wide industrial applications of this hydrolytic enzyme, little is know about its behaviour towards unnatural substrates (5). 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 alcohols (6), protected aminoacids utilized in peptides synthesis (7) and amines (8). In this communication, we reported that the enantioselective hydrolysis of amide bond is effectively sensitive to the nature of the phenyl acetyl moiety but accepts quite large substrates in the amino part.

Scheme 1



Firstly we have noted that the efficiency and the reproductivness of the resolution procedure with phenacetyl amide of α Me benzylamine is very dependent of the solubility of the amide substrate in the aqueous medium. In order to improve the solubility of the racemic amide in the phosphate buffer we have checked various substituted phenylacetamide possessing an hydrophilic character on the phenylacetyl residu. As one can see in Table I, Pen-G acylase shows different catalytic activity depending of the nature of the phenylacetyl group used. The best candidate for the kinetic resolution of amine being the p-hydroxy phenylacetyl group, both in activity and in enantioselectivity

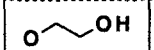
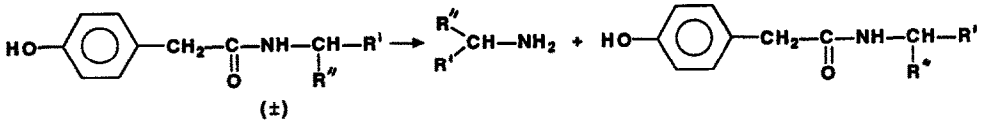
X	Y	enzyme quantity	Time	C %	ee % amide
H	H	3000 U	3h30	60	70
OH	H	3000 U	1h30	47	89
NH_2	H	10 000 U	4 days	67	17.3
	H	3000 U	1 week	<6	—
OH	SO_3H	10 000 U	3 days	0	—

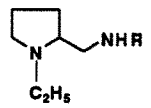
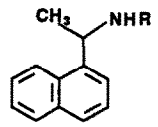
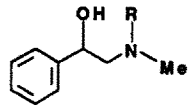
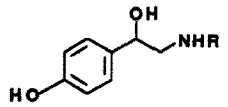
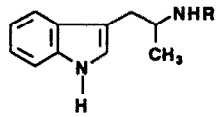
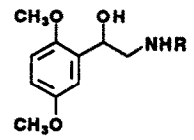
Table I : Influence of various N-acyl groups on the hydrolytic action of Pen-G acylase

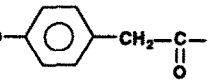
We have studied therefore the hydrolysis of a serie of racemic p-hydroxy phenylacetamides (scheme 2) and we report here on the results obtained (Table II). In a typical procedure, 2 mmol of substrate in 60 ml of phosphate buffer at pH 8, stirred magnetically was treated with 3000 units of enzyme (9) while the pH was maintained at the initial value by adjunction of NaOH O,1 N. At 50 % conversion the reaction was stopped by extraction with ethyl acetate and subsequent removal of the enzyme preparation by filtration on celite. The amine and the unreacted phenylacetamide are separated by classical working procedure.

Scheme 2



(±)

Substrate (a)	pH	Tp °C	t min	C % (b)	ee % amide (c)
	8.05	46	54	52	71(S)
	7.05	41	200	48	22(S)
	7.6	41	60	—	—
	7.6	32	63	53	98(S)
	7.6	39	80	58	76(S)
	7.45	41	80	50	93(S)

a) R = 

b) Determined by HPLC on Hypersil ODS C18 ; MeOH(55)/Phosphate buffer (45)

c) Determined by HPLC on a Chiralpak AD (Daicel) with hexane/alcool as eluent

Table II : Kinetic resolution of p-hydroxyphenylacetamides with Pen-G-acylase

The results obtained with the above sets of p-hydroxyphenylacetamides of structurally different amines, indicate the facile hydrolysis of the derivatives of primary amines, being, however, the enantioselectivity strongly depending upon some structural features. The above experiments, illustrate the synthetic utility of penicillinacylase in the preparation of chiral amine and aminoalcohols by means of the hydrolysis of the corresponding phenylacetamide.

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