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HYDROLYSIS OF N-PHENYLACETYL DERIVATIVES OF AMINO COMPOUNDS BY BENZYLPENICILLIN--ACYLASE. STERIC COURSE OF THE REACTION

A. Romeo - G. Lucente - D. Rossi - G. Zanotti

Centro di Studio per la Chimica del Farmaco (C.N.R.) - Roma, Italy.

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Much work has been carried out on the hydrolytic properties of the benzyl penicillin-acylase enzyme of Escherichia Coli[1-9] A.T.C.C. 9637. We have previously shown the hydrolytic activity with regard to a number of acylamido acids.[4,7] In this paper we are concerned with the activity of acylase with regard to other substrates, using a high enzyme/substrate ratio. Working in such conditions it was found that acylase acts on a number of N-phenylacetyl derivatives of amines (2-aminobutane, p-hydroxyamphetamine, 1,1,1-trifluoro-2-aminopropane) of α-amino alcohols (alaninol, α-aminobutanol) and of α-aminonitriles (alaninonitrile, butyronitrile, valinonitrile); it was also possible to enzymatically split phenylacetyl-D-alanine and phenylacetyl-α-aminoisobutyric acid (see Table 1).

From the above it is clear that the acylase of Escherichia Coli is able to hydrolyze a large number of N-phenylacetyl derivatives. In the case of the racemic or optically active compounds examined, it was observed that acylase acts on both enantiomorphs.

Data obtained from enzymatic reactions carried out limiting the time, to avoid the complete hydrolysis of racemic substrates are reported in Table 1. The non hydrolyzed N-phenylacetyl derivatives were recovered; they consisted mainly of that isomer which was the least rapidly hydrolyzed.

The substrate stereospecificity of the enzyme may be evaluated from Table 1 comparing the rotation data found for N-phenylacetyl derivatives recovered with those of R isomers. To determine the steric course of the enzymatic hydrolysis in the case of the N-phenylacetyl derivatives of the α-amino nitriles, the recovered optically active N-phenylacetylamino nitriles (see Table 1) were transformed into the methylesters of the corresponding amino acids. The specific rotations of the nitriles and of the methylesters obtained may be seen from Table 2.

As regards stereospecificity in the case of the examined racemic compounds the S isomers are more rapidly split, and only in the case of N-phenylacetyl-valinonitrile is the D(R) form more rapidly hydrolyzed.

TABLE 1

Substrates	Substrate mg.	Enzyme preparation -mg.	0.1M phosphate buffer - ml.	Incubation hr.	% hydrolysis	$[\alpha]_D^{20}$ of substrates recovered	R isomers
N-phenylacetyl-2-aminobutane	390	6.00	90	24**	62	-10 (c,4)	-17 (c,2)
N-phenylacetyl-(R),(S)-p-Hydroxyamphetamine	200	3.00	360+40ml MeOH	24**	77	+23 (c,2)	+23 (c,2)
N-phenylacetyl-(R),(S)-1,1,1-trifluoro-2-aminopropane	200	0.50	180	10**	63	-18 (c,3)	
N-phenylacetyl-D,L-alaninonitrile	400	0.30	70	14*	49	+37 (c,2)	
N-phenylacetyl-D,L-α-aminobutyronitrile	300	0.25	120	14*	39	+11 (c,4)	
N-phenylacetyl-D,L-valinonitrile	300	0.70	180	14*	34	-11 (c,4)	
N-phenylacetyl-D,L-alaninol	150	0.70	20	24**	54	+12 (c,4)	+15 (c,3)
N-phenylacetyl-D,L-α-aminobutanol	300	1.30	60	24**	30	+6 (c,5)	+34 (c,4)
N-phenylacetyl-D-alanine	100	0.70	20	20**	37		
N-phenylacetyl-α-aminoisobutyricacid	50	0.30	9	20**	70		

The specific activity of the enzyme preparation, used by us, is 1,500 μ mole (N-phenylacetyl-L-alanine)/hour mg. enzyme protein at 37° and pH 7.0 (0.1M phosphate buffer). The specific activity was determined by using two methods: ninhydrin determination of released L-alanine; gas-chromatographic determination of phenylacetic acid by esterifying with diazomethane (internal standard methyl benzoate). The % hydrolysis was determined by using the gas chromatographic method. The enzyme preparation contains some foreign protein; however, in all probability they contained only one enzyme to hydrolyze N-phenylacetyl derivatives. The incubation temperature was: * 30°; ** 37°.

All $[\alpha]_D^{20}$ determinations were carried out in methanol. The substrates used were prepared from known amino compounds; all the products obtained by chemical methods or by enzymatic hydrolysis showed correct elemental analysis and spectroscopic data.

TABLE 2

	Derivatives corresponding to:		
	N-phenylacetyl alanine	N-phenylacetyl-α-aminobutyric acid	N-phenylacetyl valine
	$[\alpha]_D^{20}$	$[\alpha]_D^{20}$	$[\alpha]_D^{20}$
Recovered Nitriles	+84 (c,1)	+40 (c,3)	-46 (c,1)
Methylesters from recovered nitriles	+16 (c,1)	+4 (c,4)	-21 (c,1)
Methylesters D-isomers	+56 (c,1)	+49 (c,1)	+32 (c,1)

The nitriles used have specific rotation of the same sign but higher than that shown in Table 1, since they have been crystallized, thus increasing the optical purity of the predominant isomer. This procedure is necessary in view of the fact that in the transformation reaction into methylesters (CH_3OH, HCl) partial racemisation occurs.

O.R.D. curves were carried out for D isomers of the three methylesters; all these compounds give a negative Cotton effect.

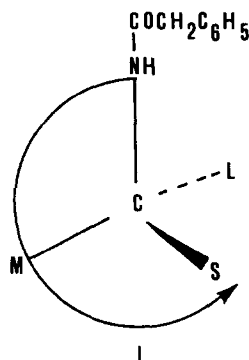
The O.R.D. curves of the methylesters obtained from the recovered nitriles show that,in terms of shape and sign of the Cotton effect curve, they are identical to those of the D isomers of the methylesters of the N-phenylacetylalanine and N-phenylacetyl-α-aminobutyric acid, but antipodal to that of the D isomer of N-phenylacetylvaline.

All the $[\alpha]_D^{20}$ determinations were carried out in methanol.

The compounds so far considered, to evaluate the steric course of the hydrolysis reactions, contain only one asymmetric carbon atom, corresponding to that to which the phenylacetamido group is bonded; for only two of the compounds examined by us are the absolute configurations unknown (N-phenylacetyl-1,1,1-trifluoro-2-aminopropane; α-benzyloxy-phenaceturic acid [4]). In attempting to determine which of the two isomers of the N-phenylacetyl-1,1,1-trifluoro-2-aminopropane is split more rapidly by acylase, we considered the results obtained in the case of the N-phenylacetyl-p-hydroxyamphetamine and N-phenylacetyl-2-aminobutane (compounds with known absolute configuration). Model I corresponds to the isomers which are most easily split by acylase in the previously mentioned two cases. In model I the molecule is viewed from the side remote from the large group (among the three groups bonded to the asymmetric carbon atom bearing the phenylacetamido group) and the sequence from phenylacetamido group to medium group to small group involves an anticlokwise rotation.

According to the hydrolysis data in the case of the N-phenylacetyl-1,1,1-trifluoro-2-aminopropane, the more rapidly hydrolyzed isomer has positive rotation; if model I be regarded as valid in this case too, S(+) configuration may be assigned to that isomer.

To use model I in the case of the α-benzyloxy-phenaceturic acid, account was taken of the results of the enzymatic hydrolysis of compounds with known configuration containing a clearly hydrophilic function close to the carbon atom to which the phenylacetamido group is bonded. It was seen that in the case of the N-phenylacetyl derivatives of the α-amino acids the most easily hydrolyzed isomer corresponds, with reference to model I, to that in which the hydrophilic functional group is in the "large" position; such group should probably also be bulkier in aqueous solution as a result of hydration.



According to the hydrolysis data in the case of α-benzyloxy-phenaceturic acid the more rapidly hydrolyzed isomer has positive rotation; if the above mentioned considerations be applied with reference to model I, S(+) configuration can be assigned.

Considering the hydrophilic character of the nitrilic group, the steric course of the enzymatic hydrolysis of the N-phenylacetyl derivatives of the α-aminonitriles tested, do not seem to agree with the scheme previously adopted (using model I) as far as valinonitrile is concerned. In fact the enzyme splits the L-forms of the N-phenylacetyl derivatives of the alaninonitrile and of the α-aminobutyronitrile and the D-form of the N- phenylacetyl-valinonitrile more rapidly.

By way of preliminary approximation it may, however, be observed that the steric course of the hydrolysis of the N-phenylacetyl-valinonitrile may be in accordance with model I if consideration be taken of the size of the isopropylic group and of the lower hydrophylicity of the nitrilic group.

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