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RESOLUTION OF α-METHYL AMINO ESTERS BY CHYMOTRYPSIN

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Abstract: The resolution of DL-α-MeTrp-OMe HCl, DL-α-MePhe-OMe HCl and DL-α-Me-pF-Phe-OMe HCl have been achieved using α-chymotrypsin at pH 5.0.

α-Alkyl amino acids are gaining importance in structure-activity relationship studies of peptide hormones[1]. Introduction of α-methyl residues into the naturally occurring sequence gives rise to peptide analogs having constrained conformation. In addition, analogs with α-methyl residues are often resistant to hydrolysis by proteolytic enzymes and thus are active for a longer time.

Resolution of several α-methyl-DL-amino acids has been carried out by fractional crystallization of diastereoisomeric salts[2,3] and by the action of carboxypeptidase A on the N-trifluoroacetyl derivatives[1]. The latter method works well but N-TFA derivatives are not always easy to prepare in good yield. Acylase, often used to resolve acyl amino acids, acts very slowly on most α-methyl substrates[2].

We have found that chymotrypsin hydrolyzes the L-isomers of some α-methyl amino acid esters having an unacylated amino group. The rate is slow compared to the corresponding simple α-amino acid esters, but sufficiently rapid to be a practical method for resolving a DL mixture. The hydrolysis of unacylated amino acid esters by chymotrypsin has been studied[4-5], but its action on unacylated α-methyl amino acid esters apparently has not been noted. Introduction of an α-methyl group into a good substrate for chymotrypsin, e.g. acetyl-L-tyrosine amide lowers the rate of enzymatic hydrolysis by a factor of 10^5, which is attributed not so much to decreased binding affinity but to an incorrect orientation of the bond to be cleaved[2]. We find that the introduction of an α-methyl group into an unacylated amino acid ester lowers the rate of hydrolysis also, but much less dramatically.

The stereospecificity of enzymatic action on α-alkyl amino acid derivatives cannot be assumed to be the same as for the natural amino acids[6]. In the present case, we show that chymotrypsin hydrolyzes only the L (S) isomer of α-methylphenylalanine methyl ester; the absolute configuration of L-α-methylphenylalanine has been determined[3,7], and we assume that this specificity is extended to α-methyltryptophan methyl ester.

DL-α-methyltryptophan methyl ester was prepared by the method of Brana et al.[8]. Although they claim a stereoselective synthesis, their product has a very low rotation and in our hands their procedure gave only the DL-mixture. DL-α-methylphenylalanine and the p-fluoro derivative were prepared by following the procedure of Stein et al.[9].

The resolution is described in detail for DL-α-methyltryptophan methyl ester. A solution of DL-α-methyltryptophan methyl ester (10.2 g, 40 mmoles) in 500 ml water at 37°C was adjusted to pH 5.0 by the addition of 0.5 N LiOH. α-Chymotrypsin[10] (500 mg) was added and the solution was stirred gently while the pH was maintained at 5.0 by the addition of 0.5 N LiOH with pH-stat. The uptake of base stopped after 40 hours; the solution was stirred with 1 g of charcoal and filtered to remove the enzyme, then adjusted to pH 10.0 with LiOH. Extraction with ethyl acetate yielded D-α-methyltryptophan methyl ester, 88% yield, crystallized as its hydrochloride. The aqueous solution was lyophilized and the solid residue, containing L-α-methyltryptophan together with about 4% of D-isomer from non-enzymatic hydrolysis, was reconverted to the methyl ester with thionyl chloride and methanol. Treatment with α-chymotrypsin as above for 30 hours gave L-α-methyltryptophan essentially free of the D-isomer. The results are summarized in the Table.

Table

Product	Reaction Time (h)	Yield, %	m.p.	$[\alpha]_D^{25}$
D-α-MeTrp-OMe HCl	40	88	160° dec.	-28.3° (C9.9, MeOH)
L-α-MeTrp-OMe HCl	30	72	159° dec.	+27.8° (C9.9, MeOH)
D-α-MePhe-OMe HCl	35	79	112-115°	-23.6° (C.8, NHCl)
L-α-MePhe-OMe HCl		66		
D-α-Me-p-F-Phe-Me HCl	35	78	142-144°	-11.2° (C1.0, MeOH)
D-α-Me-Phe				+19.3° (C1.0, H_2O)[11]

References and Notes

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11. D-α-MePhe was obtained by refluxing the methyl ester with 6 N HCl and neutralizing.

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