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Enzymatic Synthesis of Side Chain Benzyl Esters of L-α-Amino Dicarboxylic Acids

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Abstract: *Diesters of L-α-amino dicarboxylic acids were specifically hydrolysed by pronase at their α carboxylic group while the corresponding D-enantiomers remained unchanged. This enabled easy obtention of side chain benzyl esters of L-α-amino dicarboxylic acids starting from both optically active and racemic dibenzyl ester derivatives.*

The masking of side chain carboxyls of monoamino dicarboxylic acids as benzyl esters is routinely used for semipermanent protection of side chain functions during peptide synthesis. The specific introduction of selectively removable ester groups generally concerns aspartic and glutamic acids. Chemical approachs involve the kinetically controlled esterification of free amino acids taking advantage of the greatest reactivity of the side chain carboxyl [1,2]. The α carboxyl of aspartic and glutamic acids have been also protected by copper complexes [3] and as boroxazolinones [4,5] during esterification of the side chain carboxyls. Selective hydrolysis of dibenzyl esters in the α position by chemical [6] and enzymatic means [7-9] have been also proposed or attempted.

In the present study we report that dibenzyl esters of α-amino dicarboxylic acids are hydrolysed regiospecifically by pronase at the α-ester group and that the reaction is enantiospecific since only the L-enantiomers were hydrolysed while dibenzyl esters of the D-enantiomers remained unchanged. Consequently, pronase has been used to prepare side-chain monobenzyl esters of L-amino dicarboxylic acids from both L- and racemic dibenzyl esters and for optical resolution purposes.

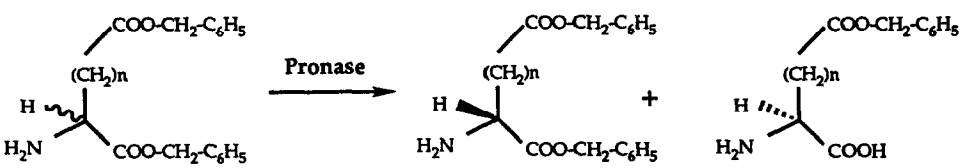
As shown in Table I, dibenzyl esters of L-aspartic acid and L-glutamic acid were substrates for pronase, which selectively catalysed hydrolysis of the α-ester group, while the side chain ester group remained unchanged. The reaction was enantiospecific since diesters of the corresponding D-enantiomers were inert toward the enzyme under the same experimental conditions.

Table I : Hydolysis by Pronase of Dibenzyl Esters to Monobenzyl Esters of α-Amino acids

Substrate	Product	Yield (%)[a]	$[\alpha]_D^{25}$ (reported)[8]
L-Asp-α,β-dibenzyl ester	L-Asp-β-benzyl ester	73	+30[b] (+29)
L-Glu-α,γ-dibenzyl ester	L-Glu-γ-benzyl ester	72	+22[c] (+22)
D, L-amino adipic acid-α,δ-dibenzyl ester	L-amino adipic acid-δ-benzyl ester	70	+10[d]
D, L-amino suberic acid-α,ω-dibenzyl ester	L-amino suberic acid-ω-benzyl ester	71	+18[d]

Conditions as indicated below
[a] calculated on the L enantiomer ; [b] (c = 1, CH_3COOH) ; [c] (c = 1, 1N HCl) ; [d] (c = 0.5, HCOOH).

The enzymatic reaction also took place with the higher homologues of α-amino dicarboxylic acids, which could be used as racemic mixtures due to the marked enantiospecificity of pronase (Scheme 1). This provides an attractive possibility for preparing optically pure side chain monoesters of amino dicarboxylic acids as intermediates for peptide synthesis.



Scheme 1: Representative Regiospecific and Stereospecific Enzymatic Hydrolysis of α-Amino Dicarboxylic Acid Dibenzyl Esters

While this work is of general applicability, more attention was focused on L-amino suberic acid-ω-benzyl ester, a key compound for synthesizing active analogues of deamino cysteinyl peptides with an ethylene linkage instead of a disulfide bond [10,11]. Enzymatic hydrolysis of D,L amino suberic acid dibenzyl ester (as well as the other lower homologues) was maximally activated by 10^{-2} M calcium ions. Cobalt ions at 10^{-3} M concentration caused a 2 to 3 fold enhancement of the reaction rate.

Pronase (E C 3.4.24.4.), is a commercial (Fluka, 6 U/mg at pH 7.5, 40°C and caseine as substrate) mixture of several *Streptomyces griseus* proteolytic enzymes, endopeptidases and exopeptidases commonly used for a complete hydrolysis of peptides and proteins.

The cooperative activating effect observed when using calcium and cobalt ions and the presence of a free amino group in the substrate molecule, strongly suggested that the main enzymatic activity in the regiospecific hydrolysis of dicarboxylic amino acid esters involved an aminopeptidase [12].

Typical procedure : D,L amino suberic acid dibenzyl ester (10^{-2} mol) in 300 ml aqueous ethanol (25%) at 25°C was treated with pronase (0.1 g). The apparent pH was adjusted to 7.2 and maintained at this level by the addition of aqueous alkali. The extent of the reaction was determined by analysing benzyl alcohol liberation by HPLC on suitable aliquots. When no further benzyl alcohol was released (at about 3 h) the pH was adjusted to 5.8 and ethyl acetate (100 ml) was added under vigorous stirring. After 3 h standing at 5°C, the L-amino suberic acid-ω-benzyl ester[13], poorly soluble in both aqueous and organic layers, was collected by filtration, washed with water, acetone, ethyl ether and dried at 40°C under vacuum (Table I).

The reaction mixture was also sampled to determine the enantiomeric excess of the unreacted dibenzyl ester using (S) camphosulfonyl chloride as chiral reagent (Fig. 1).

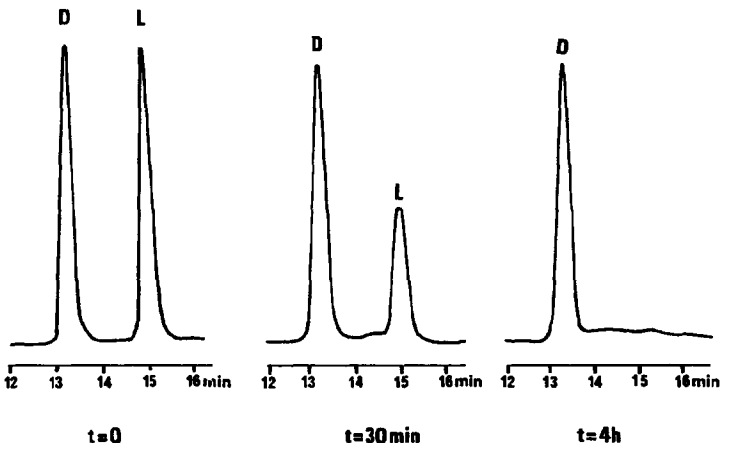


Figure 1: Progress of Pronase Catalysed Asymmetric Hydrolysis of Racemic Amino Suberic Acid Dibenzyl Ester.

HPLC analysis (Ultrasphere ODS 5mm column, 4.6 x 250 mm, eluted with acetonitrile : triethylamonium phosphate 2 10^{-3} M, pH 2.6; 7 : 3 by volume) of D and L amino suberic acid dibenzyl esters as (S) camphosulfonamides.

The other examples shown in Table I were treated under similar conditions. However, when esters of L-amino dicarboxylic acids were used as substrates the ethyl acetate step was deleted. Yields reported in Table I were calculated on the recovery of solid product while the conversion yields, calculated on the HPLC analysis of the reaction mixture, were nearly quantitative.

The monobenzyl esters listed in Table I were structurally characterized by comparison with authentic samples for L-aspartic acid-β-benzyl ester and L-glutamic acid-γ-benzyl ester. The side-chain monobenzyl esters of L-amino adipic acid and L-amino suberic acid, both obtained from racemic dibenzyl esters, yielded pure L-amino adipic acid ($[\alpha]_D^{25}$ +22, c= 0.1, 5N HCl)[14] and L-amino suberic acid ($[\alpha]_D^{25}$ +20, c= 0.1, 5N HCl)[14] after acid hydrolysis, thus confirming their L configuration. The position of the benzyl ester group was determined from the NMR spectra of the supposed side chain benzyl ester compared with the NMR spectra of L-glutamic acid γ-benzyl ester. In the racemic mixture of the dibenzyl ester the α-benzyl ester exhibited a diastereotopic CH_2 pair as an AB quartet at 5.2 ppm, whereas the ω-benzyl presented a singlet at 5.1 ppm. In the monoesters, only this last CH_2 singlet was present as in the γ-benzyl ester of L-glutamic acid.

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