A Simple Method for the Study of Racemization in Peptide Synthesis

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RACEMIZATION of isoleucine at the α-carbon atom yields its diastereomer, alloisoleucine. The formation of D-alloisoleucine from L-isoleucine during peptide synthesis was observed by Photaki and du Vigneaud[1] and also by Nesvadba, Honzl, and Rudinger.[2] Since the currently used Spackman–Stein–Moore method[3] of quantitative amino-acid analysis permits a convenient measurement of the amounts of alloisoleucine and isoleucine, an attempt was made to use the coupling of acetyl-L-isoleucine to glycine ethyl ester for a study of the influence on racemization of different tertiary amines.[4] Several excellent model peptides[5] were suggested and are applied for the same purpose,

TABLE

Method of coupling (coupling reagent)	Solvent	Tertiary amine[a]	Extent of racemization[b,c]
Dicyclohexylcarbodi-imide[7]	Chloroform	Triethylamine	9
	Chloroform	*NN*-Di-isopropylethylamine	10
	Chloroform	Tribenzylamine	10
	Chloroform	None[d]	4
	Dimethylformamide	Triethylamine	37
	Dimethylformamide	*NN*-Di-isopropylethylamine	25
	Dimethylformamide	Tribenzylamine	5
N-Ethyl-5-phenylisoxazolium-3′-sulphonate[8]	Acetonitrile	Triethylamine	20
	Acetonitrile	*NN*-Di-isopropylethylamine	12
	Acetonitrile	Tribenzylamine	4

[a] Glycine ethyl ester hydrochloride and tertiary amine were applied in equimolar amounts, both in 20% excess.
[b] $\frac{100 \times \text{alloisoleucine}}{\text{isoleucine} + \text{alloisoleucine}}$
[c] Less than 1% racemization occurred during hydrolysis of a sample of the acetyl-L-isoleucine preparation used in these experiments. The hydrolysis of pure acetyl-L-isoleucylglycine ethyl ester also proceeded with less than 1% racemization.
[d] Free glycine ethyl ester was used.

but it was felt that the present suggestion has the advantage of simplicity.†

The experiments are summarised in the Table. Samples taken from the neutral fraction of the reaction mixtures were hydrolyzed with constant-boiling hydrochloric acid in evacuated, sealed ampoules at 110° for 16 hr., and the hydrolysates applied to the long column of a Beckman Spinco amino-acid analyser. The extent of racemization is expressed as the percentage of alloisoleucine in the sum of isoleucine and alloisoleucine.

The acetyl-L-isoleucylglycine ethyl ester model allows no conclusion on factors which play a role in racemization if the latter proceeds through β-elimination or simply through the abstraction by base of a proton from the α-carbon. On the other hand, it can give valuable information in the probably most general case, in racemization through an azlactone intermediate. The results shown confirm the observations of Anderson[6] on the role of tertiary bases in the racemization of activated acylamino-acids and also support previous findings[4] from this laboratory on the effect on racemization of some hindered amines. The easy and straightforward manner in which the quantitative information on racemization was collected suggests that the same model and approach might also be useful in the study of several additional factors such as concentration, temperature, solvent, amino-component, *etc.*, which influence racemization in peptide synthesis.

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† Acetyl-L-alloisoleucine could serve equally well. Derivatives of threonine or hydroxyproline seem to be less advantageous. The acetyl group probably has a moderate influence in the formation of an oxazolinone intermediate, while in benzoyl amino-acids, azlactone formation is probably enhanced and more racemization may take place than with acylpeptides.

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