Tetrahedron Letters No. 30, pp 2711 - 2712. 0040-4039/78/0722-2711$02.00/0

THE DEPENDENCE OF RACEMIZATION IN PEPTIDE SYNTHESIS ON THE RELATIVE CONFIGURATION OF AMINO ACIDS

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(Received in UK 18 May 1978; accepted for publication 25 May 1978)

Racemization or epimerization in peptide synthesis was investigated by means of tests and found to depend on the mode of activation, the kind of protecting groups, the constitution of the carboxyl component, and the reaction conditions[1]. Furthermore, radiochromatographic investigation of epimerization[2] showed that the constitution of the amine component has the great influence on the degree of epimerization[3].

We have observed a hitherto unknown dependence of epimerization on the relative configuration of amino acids, in various peptides syntheses, e.g.:

$$\text{Ac.L.Leu + L.LeuOMe} \xrightarrow[k_1]{\text{DCC}} \text{Ac.Leu-LeuOMe; epim.degree } E_1 = \frac{\text{D-L}}{\text{L-L + D-L}} = 40\%$$

$$\text{Ac.L.Leu + D.LeuOMe} \xrightarrow[k_2]{\text{DCC}} \text{Ac.Leu-LeuOMe; epim.degree } E_2 = \frac{\text{D-D}}{\text{L-D + D-D}} = 13\%$$

The main cause of that phenomenon is thought to lie in the various rates of L- and D-amino ester acylation by the active acylamino acid derivative $/k_1 < k_2/$. The active derivative which has a labile chiral centre undergoes racemization in the proportion to its half-life time.

In the order to verify that hypothesis, we performed kinetic investigations of three reactions: Ac.L.Leu + L.LeuOMe*, Ac.L.LeuOMe + D.LeuOMe* and Ac.DL.Leu + DL.LeuOMe*. We used leucine esters, tritium-labelled in the methyl esters group[4], to determine the radioactive components of the reacting mixtures after separation by TLC. The degrees of conversion /yields/ and epimerization were calculated on the basis of the relative radioactivity of the spots which are a measure of mole fractions of each individual component. Results are listed in the Table.

EXTENT OF EPIMERIZATION DURING THE SYNTHESIS OF Ac.Leu-Leu.OMe DIASTEREOMERS

Synthesis : L + L									
Time hrs.	Yield %	E_1 %	Yield %	E_2 %	Yield %	L-L/D-D %	L-D/D-L %	$Q=\frac{L-D/D-L}{L-L/D-D}$	$\frac{E_1}{E_2}$
1.5	24.5	23.1	34.5	4.48	23.5	6.1	17.4	2.9	5.2
3	42.8	30.0	56.1	5.82	42.7	10.6	32.1	3.0	5.2
6	65.3	35.6	71.4	8.34	57.8	15.0	42.8	2.9	4.2
18	87.8	40.5	88.7	12.1	78.2	20.3	57.9	2.9	3.3
24	89.0	40.7	89.8	13.2	83.6	20.7	62.9	3.0	3.1

Conclusions :

1° The rate of peptide bond formation depends on the relative configuration of substrates - $k_1 < k_2$; peptides formed more rapidly undergo a lower degree of epimerization.

2° The epimerization degree increases with the conversion degree.

3° The epimerization degree in L-L peptide synthesis is higher than it is in L-D peptide synthesis, independently of the conversion degree;

4° The ratio of the epimerization degrees in syntheses of diastereomeric dipeptides $\frac{E_1}{E_2}$ is closely related to the stereoselectivity Q; final value $\frac{E1}{E_2} \cong Q$

Typical procedures: dioxane solutions TosOH.L.LeuOMe* /0.01 mmole in 0,1 ccm; sp.act. 6 mCi/mmole/, NEt_3 /0,01 mmole in 0,1 ccm/, Ac.Leu /0,01 mmole in 0,2 ccm/ and DCC /0,011 mmole in 0,1 ccm/ were mixed and left standing at 20°. Samples of the reacting mixture were separated on DC-Plastikfolien Kieselgel 60 Merck plates in the diisopropyl ether - isopropanol 10 : 1 system. Chromatograms were cut transversely into 2,5 mm wide fragments. Radioactivity was determined for each single fragment with an ISOCAP 300 scintillation counter[2,3].

The scintillating solution was composed of 950 ccm of toluene, 50 ccm of ethanol, 4 g of PPO and 0,2 g of POPOP.

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