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Thermal analysis of dipeptides. Part III. Decomposition processes of a series of dipeptides having α-alanine as first common term. Influence of the components

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Abstract

Thermal analysis of a series of dipeptides having α-alanine (Ala) as first term, using simultaneous TG–DSC measurements and kinetic calculations by the dynamic TG technique, have been carried out.

The TG–DSC and kinetic quantities, related to the decomposition processes of these compounds, were compared with those of the free α-amino acids contained in the dipeptides.

Some information on the influence of the components on the decomposition processes of the dipeptides was obtained.

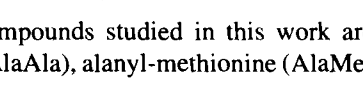
The first fragmentation indicates where the decomposition processes begin. © 1997 Elsevier Science B.V.

Keywords: Enthalpy; Kinetics; Simultaneous TG–DSC; Thermal stability

1. Introduction

Dipeptides, compounds made up of two α-amino acids, are the smallest units of protein chains. For this reason, dipeptides allow the reciprocal influences of different α-amino acids to be easily studied. In this laboratory, thermal behaviour of different series of dipeptides [1] and of a series having α-alanine as first common term [2] were studied.

The aim of this work is to determine whether, in decomposition processes, thermal analysis can highlight the influence of the components in a series of dipeptides still having α-alanine as first common term (structure 1). For this purpose, the quantities, obtained from TG–DSC curves, are compared with those obtained from TG–DSC curves of the free α-amino acids, which form the structures of dipeptides.



$$R-\underset{NH_3^+}{CH}-CONH-\underset{COO^-}{CH}-R'$$

The compounds studied in this work are: alanyl-alanine (AlaAla), alanyl-methionine (AlaMet), alanyl-aspargine (AlaAsn), alanyl-glycine (AlaGly), alanyl-isoleucine (AlaIso), alanyl-aspartic acid (AlaAsp), alanyl-glutamic acid (AlaGlu). In structure 1, R always represents $-CH_3$ while R' represents any of the following groups:

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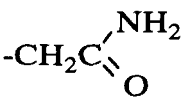
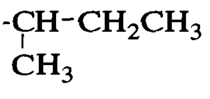
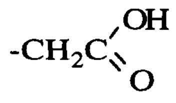
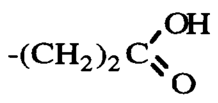
$-CH_3$	$-CH_2CH_2SCH_3$
 $-CH_2C\begin{smallmatrix}NH_2\\O\end{smallmatrix}$	$-H$
 $-\underset{CH_3}{CH}-CH_2CH_3$	 $-CH_2C\begin{smallmatrix}OH\\O\end{smallmatrix}$
 $-(CH_2)_2C\begin{smallmatrix}OH\\O\end{smallmatrix}$	

2. Experimental and procedure

The measurements were carried out on a Stanton–Redcroft 625 simultaneous TG–DSC connected to an Olivetti 250 computer. Instrument calibration was performed with standard indium, gallium, lead, tin, zinc, naphthalene and benzoic acid samples of known temperatures and enthalpies of melting. The metals possessed purity higher than 99.99% while that of organic compounds was higher than 99.95%.

For decomposition studies under dynamic conditions, the TG–DSC apparatus was set up as follows:

Samples (6–8 mg) were weighed into aluminium pans placed in a nitrogen-filled dry box. In order to avoid oxidative processes of the samples, the TG–DSC system was flushed with nitrogen gas both below (at flow rate of 30 ml min^{-1}) and above (at flow rate of 50 ml min^{-1}) the open pan.

In this way, the gases evolved during the thermal decomposition experiment were continuously removed. The heating rate was always 10 K min^{-1} and at least two runs were made for each compound.

All the quantities of the thermal decomposition processes were calculated using the Stanton–Redcroft Data Acquisition System, Trace 2, Version 4.

The compounds (Polyscience) were used without purification and their purity (99%) was higher than that needed for the application of DSC technique [3–5].

The purity of the compounds was checked by HPLC measurements.

The quantities used to study the thermal behaviour of dipeptides were: the extrapolated onset temperature of decomposition processes (T_e) and the peaks of DSC curves related to the same processes.

A kinetic TG-dynamic study of the decomposition processes for the studied compounds has been carried out using the method of McCarty and Green [6], the implementation of which is restricted to first-order reactions.

Kinetic analysis includes the evaluation of kinetic energy of activation (E_a) related to the decomposition process, the frequency factor ln A and the reaction order.

In this study, for the calculation of activation energy, a mass loss lower than 10% was considered. Indeed, it has been considered that the initial portion of the TG curves can be fitted by a first-order reaction equation [7].

3. Results and discussion

The trends in thermal behaviour for the dipeptides examined in comparison with those of the free α-amino acids [8] contained in them are given in Figs. 1–7. The values of the thermodynamic and kinetic quantities relating to TG and DSC measurements for the decomposition processes of this series of dipeptides are compared in Tables 1–3 with those of the α-amino acids [8,9].

3.1. Thermal stability and enthalpy

For alanyl-alanine (Fig. 1 and Table 1) there are two weight loss processes (mass loss in %) the first of which (28.01%), when put in mathematical proportion with its molecular weight, acquires the value of 44 g mol^{-1} (related to the loss of the carboxyl group).

On the contrary α-alanine decomposes in a single process with a weight loss of 94.15%. losing the carboxyl and amino groups simultaneously [8].

For AlaAla, the sum of the first and second enthalpy values of decomposition process is 113.48 kJ mol^{-1}, while α-alanine shows a value of 123.14 kJ mol^{-1} for the single process. From this experimental evidence, it can be concluded that dipeptide has less thermal

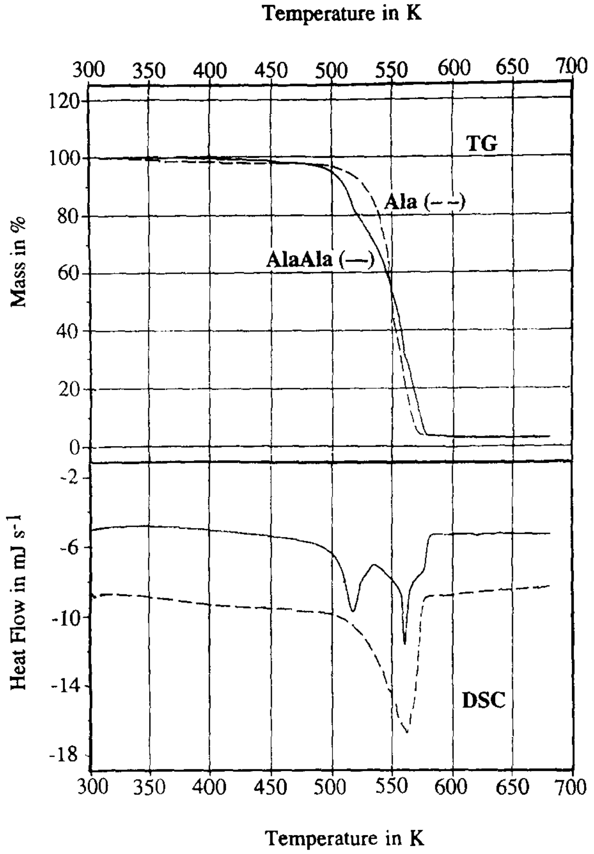


Fig. 1. Simultaneous TG–DSC curves of (———) – alanyl-alanine, and (- - -) – alanine.

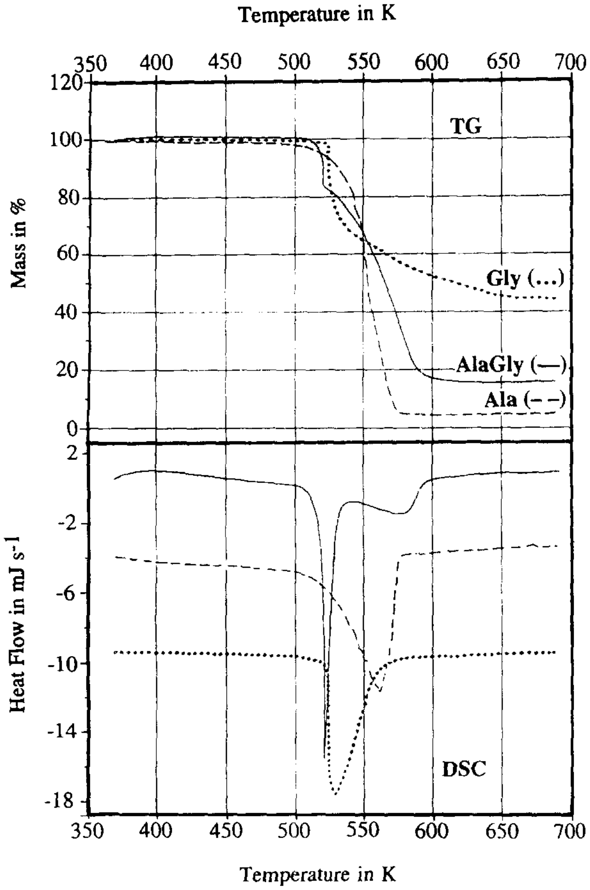


Fig. 2. Simultaneous TG–DSC curves of (———) – alanyl-glycine, (- - -) – α-alanine, and (· · ·) – glycine.

stability and requires less energy for its decomposition. Moreover, the second peak of the dipeptide (560.51 K) overlaps the peak of α-alanine (561.93 K) so that the α-alanine structure is recognized in alanyl-alanine.

From the TG–DSC curves of alanyl-glycine (Fig. 2), it can be seen that two decomposition steps occur: the first shows a weight loss of 21.18% (with a corresponding proportional weight loss of 31 g mol^{-1} which can be related to the CH_3NH_2 group of α-alanine). The first peak (520.06 K) and the extrapolated onset temperature of decomposition (523.20 K) of alanyl-glycine are close, respectively, to the peak (528.55 K) and to the extrapolated onset temperature of decomposition process (514.00 K) of glycine.

The thermal behaviour of alanyl-isoleucine (Fig. 3) shows a proportional weight loss of 44 g mol^{-1} in the first decomposition step that indicates the loss of the carboxyl group and a complex DSC curve with three peaks (505.97, 522.65 and 552.66 K) which are far away from those of α-alanine and isoleucine.

It can be observed that the mutual influence of the two α-amino acids destabilizes the dipeptide, lowering its extrapolated onset temperature of decomposition with respect to those of free α-amino acids (Table 1). This is confirmed by the fact that the peaks of the two α-amino acids are not recognized in the DSC curves of dipeptide (Fig. 3).

In the TG curve of alanyl-methionine (Fig. 4), the first decomposition indicates a loss of the amino group. The peaks of methionine and α-alanine were not found in the DSC curve of dipeptide.

The first step of AlaAsp (Fig. 5, Table 1) has a weight loss of 8.71% (with a corresponding propor-

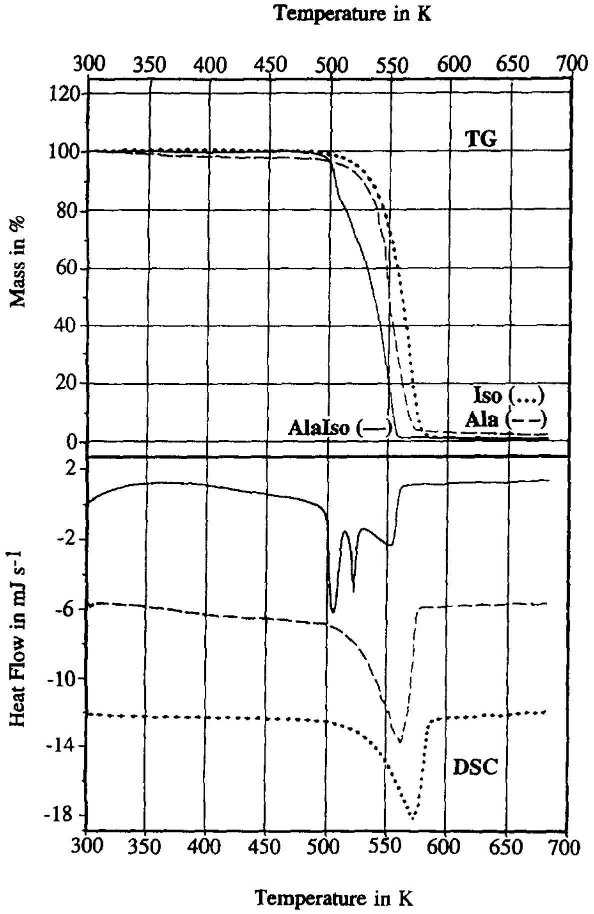


Fig. 3. Simultaneous TG–DSC curves of (———) alanyl-isoleucine, (- - -) – α-alanine, and (· · ·) isoleucine.

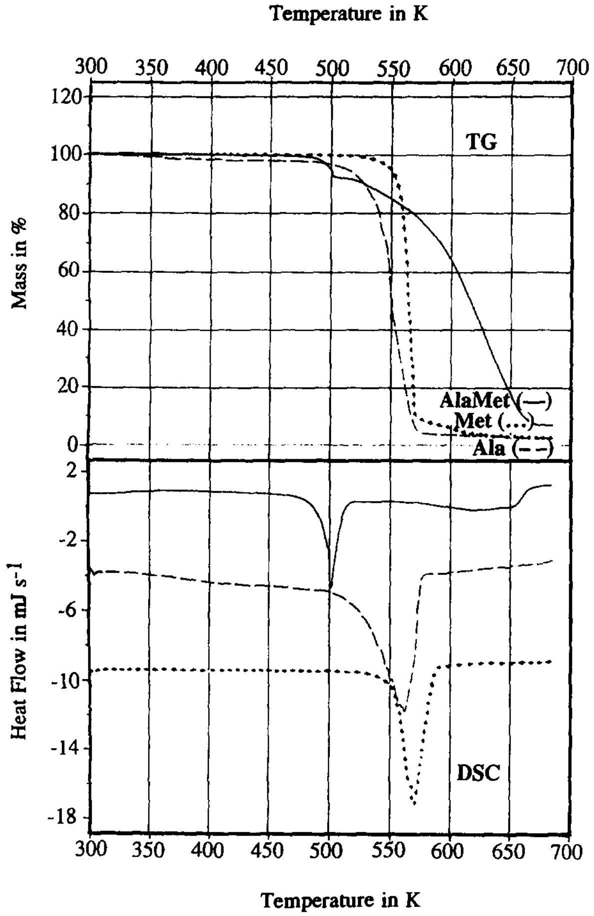


Fig. 4. Simultaneous TG–DSC curves of (———) – alanyl-methionine, (- - -) – α- alanine, and – (· · ·) – methionine.

tional weight of 17 g mol^{-1}) which can be related to the amino group. The absence of the three characteristic peaks of aspartic acid and that of α-alanine in the DSC curve of AlaAsp indicates that the thermal behaviour of the α-amino acids are modified by mutual influence.

Aspargine (Fig. 6, Table 1) shows three decomposition processes: the weight loss 11.64% in the first decomposition process can be ascribed to the loss of the second amino group [8]. Moreover, the presence of this group decreases the thermal stability of the aspargine with respect to that of aspartic acid (Fig. 5, Table 1). This behaviour is also recognized in thermal stability of AlaAsn with respect to that of AlaAsp.

The AlaAsn (Fig. 6, Table 1) also shows three decomposition processes, the first of which can be related to the loss of the CO group. The peaks of the two α-amino acids are not recognized in the DSC curve of the dipeptide: the structure of these α-amino acids are modified by the mutual influence.

Aspargine (Fig. 6, Table 1) shows three decomposition processes: the weight loss 11.64% in the first decomposition process can be ascribed to the loss of the second amino group [8]. Moreover, the presence of this group lowers the thermal stability of the aspargine with respect to that of aspartic acid (Fig. 5, Table 1). This behaviour is also recognized in thermal stability of AlaAsn with respect to that of AlaAsp.

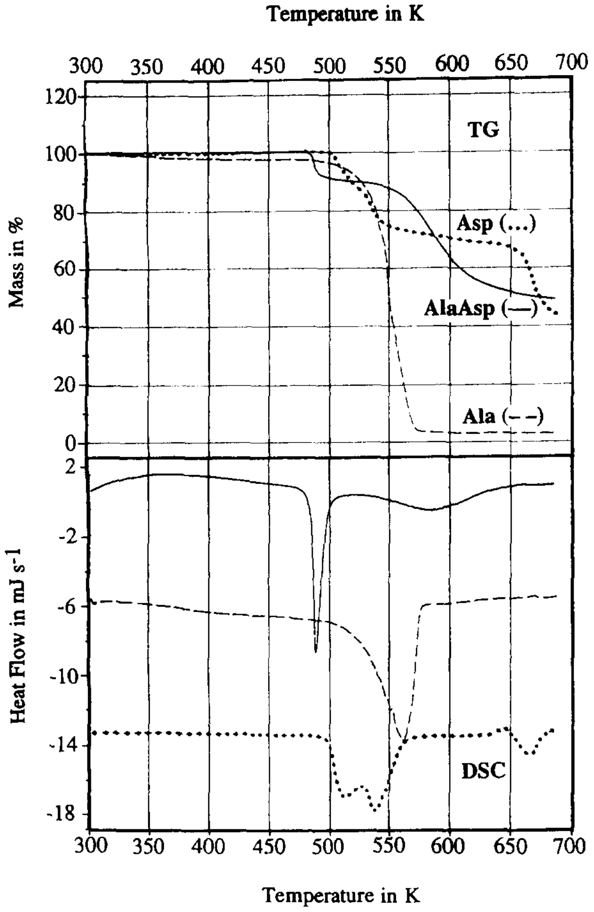


Fig. 5. Simultaneous TG–DSC curves of (————) – alanyl-aspartic acid, (- - -) – α-alanine, and (· · ·) – aspartic acid.

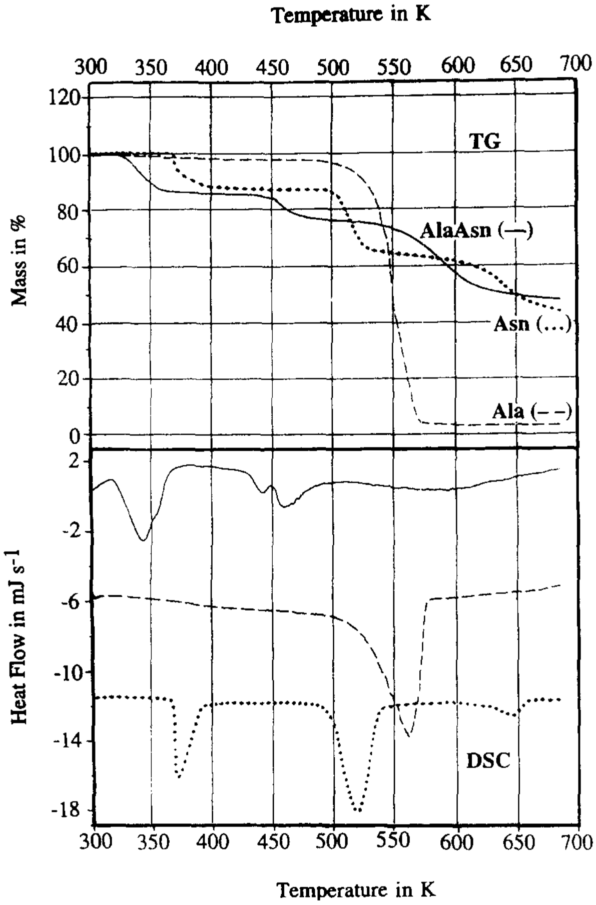


Fig. 6. Simultaneous TG–DSC curves of (————) – alanyl-aspargine, (- - -) – α- alanine, and (· · ·) – aspargine.

The AlaAsn (Fig. 6, Table 1) also shows three decomposition processes, the first of which can be related to the loss of the CO group. The peaks of the two free α-amino acids are modified by the mutual influence.

The TG curve of AlaGlu (Fig. 7) shows three decomposition steps, the first of which indicates the loss of the amino group; in the second one, the exothermic process (DSC curve) is characteristic of thermal decomposition reaction involving a loss of diatomic gas molecule [10]. The mutual influence of the two α-amino acids makes the thermal behaviour of AlaGlu quite different from those of free Ala and Glu.

For its symmetrical structure, alanyl-alanine can be assumed as reference compound in the study of the mutual influence of the components in the dipeptides. Variation in thermal stability of various dipeptides is represented by the difference $\Delta T_e = T_e^{AlaR} - T_e^{AlaAla}$, where T_e^{AlaR} is the extrapolated onset temperature of decomposition of each dipeptide and T_e^{AlaAla} that of alanyl-alanine.

This relative scale (Table 4) shows that dipeptides with side chains containing aliphatic groups have higher thermal stabilities than dipeptides bearing carboxyl or amino groups in side chains. Moreover, thermal stabilities of dipeptides containing aliphatic groups decrease as their length increases while for AlaGlu the introduction of only one methyl group in the side chain makes the thermal stability lower than those of AlaAsp and AlaAsn.

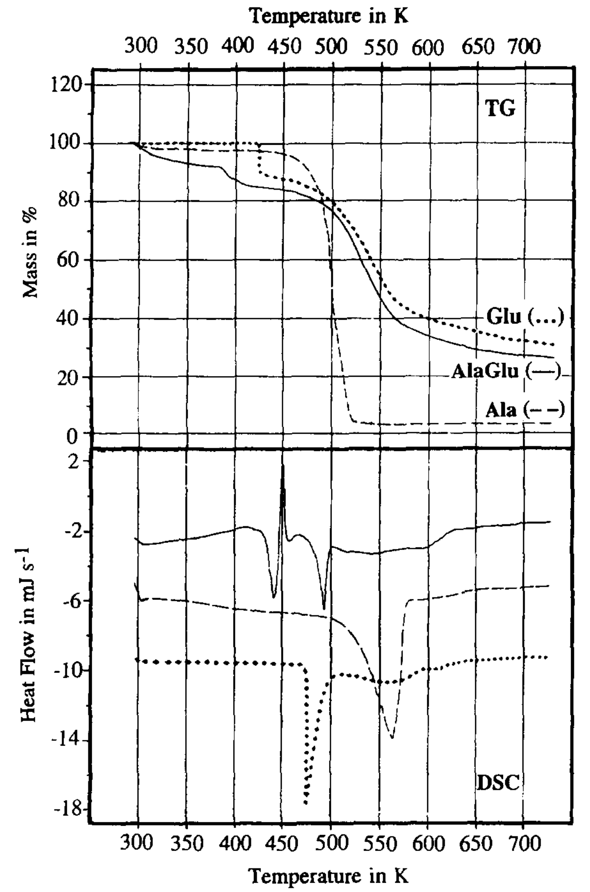


Fig. 7. Simultaneous TG–DSC curves of (———) – alanyl-glutamic acid, (- - -) – α-alanine, and (· · ·) – glutamic acid.

3.2. Kinetics

The relative scale of kinetic activation energies related to decomposition processes is also expressed as $\Delta E_a = E_a^{\mathrm{AlaR}} - E_a^{\mathrm{AlaAla}}$ (Table 4), where E_a^{AlaR} is the activation energy of each dipeptide and E_a^{AlaAla} that of AlaAla. From those values it can be noted that the mutual influence makes dipeptides with aliphatic chains more stable than those bearing, in the side chains, a second amino or carboxyl group.

A further contribution to the comprehension of the influence of the components in the dipeptides can be supplied from relations (1–1a) and (2–2a) which directly compare thermodynamic and kinetic data of dipeptides and free α-amino acids.

$$(T_e^{\mathrm{AlaR}} - T_e^{\mathrm{R}})/T_e^{\mathrm{R}} \tag{1}$$

$$(T_e^{\mathrm{AlaR}} - T_e^{\mathrm{Ala}})/T_e^{\mathrm{Ala}} \tag{1a}$$

$$(E_a^{\mathrm{AlaR}} - E_a^{\mathrm{R}})/E_a^{\mathrm{R}} \tag{2}$$

$$(E_a^{\mathrm{AlaR}} - E_a^{\mathrm{Ala}})/E_a^{\mathrm{Ala}} \tag{2a}$$

where T_e^{AlaR} and E_a^{AlaR} are the extrapolated onset temperatures and activation energies of decomposition processes of each dipeptide of the series while $T_e^{\mathrm{R}}, E_a^{\mathrm{Ala}}, T_e^{\mathrm{Ala}}$ and E_a^{Ala} are the extrapolated onset temperatures and activation energies of decomposition processes of each α-amino acid and of α-alanine.

The influence of α-alanine on the other α-amino acids and that of the different α-amino acids on the fixed first component were studied by means of the relationships (1, 1a) and (2, 2a) which supply percentage values of thermal stability variation (from thermodynamic and kinetic point of view) to the dipeptides (Tables 5 and 6) with respect to the single α-amino acids.

It can be seen (Table 5) that α-alanine lowers, in the dipeptides, the extrapolated onset temperatures of all α-amino acids (with the exception of glycine). The main influence is shown with regards to Glu and Asn. Thermal stability of α-alanine in dipeptide series is lowered (mostly by Glu and Asn) by other α-amino acids.

From a kinetic point of view (Table 6), α-alanine in dipeptides decreases (with respect to those of free α-amino acid) the activation energies of Asp, Glu and Asn, while it increases those of Gly, Iso and Met.

The activation energy of α-alanine is decreased by Iso, Asp, Glu and Asn, while Gly and Met increase it.

4. Conclusions

In general, the mutual influence of two α-amino acids make the dipeptides less stable than the single components.

The proportional weight loss could indicate the first fragment of decomposition (carboxyl group, amino group, functional group) and where the beginning of decomposition occurs.

AlaAla and AlaIso lose the carboxyl group and AlaAsn loses the CO group: the initial decomposition takes place in the second component.

Table 1
Extent of thermal decomposition of some dipeptides and their corresponding α-amino acids from TG measurements

Compounds	T_e^a /K	Stage of decomposition		
		I	II	III
		mass loss %	mass loss %	mass loss %
AlaGly	523.20	21.18	61.50	—
Gly [b]	514.00	65.02	—	—
AlaIso	499.41	21.65	73.38	—
Iso [b]	535.07	99.70	—	—
AlaMet	496.98	8.47	83.91	—
Met [b]	549.32	98.40	—	—
AlaAsp	484.95	8.71	43.06	—
Asp [b]	499.25	27.27	—	—
AlaGlu	307.88	7.70	7.02	56.28
Glu [b]	446.00	14.45	—	—
AlaAsn	321.61	13.85	9.80	28.72
Asn [b]	358.92	11.64	—	—
AlaAla	501.00	28.01	65.92	—
Ala [b]	541.68	94.15	—	—

[a] Extrapolated onset temperature.
[b] Values taken from [8].

Table 2
Thermodynamic parameters of thermal decomposition of some dipeptides and their corresponding α-amino acids from DSC measurements

Compounds	T_e [b]/ K	Stage of Decomposition					
		I		II		III	
		ΔH/ kJ mol^{-1}	Peak/ K	ΔH/ kJ mol^{-1}	Peak/ K	ΔH/ kJ mol^{-1}	Peak/ K
AlaGly	528.80	89.75	520.06	36.50	576.24	—	—
Gly [c]	524.00	77.30	528.55	—	—	—	—
AlaIso	500.00	54.69	505.97	16.81	552.65	49.52	552.66
Iso [c]	541.28	159.39	573.88	—	—	—	—
AlaMet	445.70	76.58	501.50	83.72	618.36	—	—
Met [c]	589.06	129.70	568.00	—	—	—	—
AlaAsp	477.00	94.05	487.58	62.24	585.00	—	—
Asp [c]	509.15	128.21	513.29 [a]	19.94	670.71	—	—
AlaGlu	431.59	37.36	440.30	−12.46	450.64	22.46	491.56
Glu [c]	482.14	77.09	470.07	29.77	556.84	—	—
AlaAsn	320.90	118.76	343.16	7.49	442.56	32.33	460.46
Asn [c]	362.53	43.43	365.31	107.82	520.30	—	—
AlaAla	504.00	42.30	517.00	71.18	560.51	—	—
Ala [c]	535.18	123.14	561.93	—	—	—	—

[a] First peak temperature; the second peak temperature is 537.61 K.
[b] Extrapolated onset temperature.
[c] Values taken from [8].

AlaMet, AlaAsp, AlaGlu, lose the amino group and AlaGly loses the CH_3NH_2 functional group, so an initial decomposition in α-alanine can be hypothesized.

A comparison between extrapolated onset temperature and kinetic activation energy values of decomposition processes, for both the series of dipeptides and of the free components contained in them, could

Table 3
Kinetic parameters of thermal decomposition of some dipeptides and their related α-amino acids from TG measurements assuming first-order reaction

Compounds	E/ kJ mol^{-1} K^{-1}	ln A/min^{-1}
AlaGly	301.31	69.22
Gly a	164.17	36.57
AlaIso	141.39	32.68
Iso [a]	118.85	26.43
AlaMet	228.98	54.58
Met [a]	199.13	43.09
AlaAsp	37.44	7.22
Asp [a]	275.42	64.65
AlaGlu	14.40	2.44
Glu [a]	27.58	5.10
AlaAsn	63.49	21.61
Asn [a]	186.90	60.43
AlaAla	109.93	25.37
Ala [a]	145.53	32.79

[a] Values taken from [8].

Table 4
Differences in extrapolated onset temperatures and activation energies of decomposition processes for a series of dipeptides with respect to the same processes of alanyl-alanine

Compounds	$T_e^{AlaR} - T_e^{AlaAla}$/ K	$E_a^{AlaR} - E_a^{AlaAla}$/ kJ mol^{-1}
AlaAla	0.00	0.00
AlaIso	−1.59	31.46
AlaMet	−4.02	119.05
AlaAsp	−16.05	−72.49
AlaGlu	−193.12	−95.53
AlaAsn	−179.39	−46.44
AlaGly	22.20	191.38

Table 5
Values taken from relations (1) and (1a)

Compounds	$(T_e^{AlaR} - T_e^{R})/T_e^{R}$ [a]	$(T_e^{AlaR} - T_e^{Ala})/T_e^{Ala}$ [a]
AlaGly	+0.02	−0.03
AlaIso	−0.07	−0.07
AlaMet	−0.09	−0.08
AlaAsp	−0.03	−0.10
AlaGlu	−0.31	−0.43
AlaAsn	−0.10	−0.41

[a] Here, R is each of α-amino acids linked to α-alanine in dipeptide structure.

Table 6
Values taken from relations (2) and (2a)

Compounds	$(E_a^{AlaR} - E_a^{R})/E_a^{R}$ [a]	$(E_a^{AlaR} - E_a^{Ala})/E_a^{Ala}$ [a]
AlaGly	+0.83	+1.07
AlaIso	+0.19	−0.03
AlaMet	+0.15	+0.57
AlaAsp	−0.86	−0.74
AlaGlu	−0.48	−0.90
AlaAsn	−0.66	−0.56

[a] Here, R is each of α-amino acids linked to α-alanine in dipeptide structure.

supply further information on the influence of the components on the decomposition processes.

Acknowledgements

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