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Thermal analysis of a series of dipeptides having α-alanine as the first term. Mutual influence of structures

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Abstract

Thermal analysis of a series of dipeptides having α-alanine as the first term was carried out by simultaneous TG–DSC measurements.

The thermal behaviour of these compounds was compared to that of the independent free α-amino acids contained in the dipeptides. The reciprocal influence of the two α-amino acids makes the dipeptides less stable than the single components.

The TG–DSC curves provide a rapid recognition of the presence of the compounds which retain the respective decomposition peaks of the independent free α-amino acids.

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

1. Introduction

The thermal analysis of organic compounds is usually combined with other analyses (UV, NMR, GC, GC-MS) to identify the products of thermal decomposition.

It is interesting to ascertain whether thermal analysis, used alone, could provide sufficient information on organic compounds.

It is well known that thermal analysis of solid phases provides physical measurements of the thermal decomposition process for organic compounds but gives no chemical information on the process being studied. The melting point, which is the simplest physical property most commonly used to characterize an organic compound, is unlikely to be applied to the α-amino acids.

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Indeed, during thermal reactions, these compounds form a range of products (CO_2, NH_3, linear and cyclic compounds); thus, quantitative measurements are difficult because of the wide temperature span over which the thermal processes take place. Moreover, the TG curves are subject to change with heating rate, so that different onset temperatures (T_0) for the same α-amino acids can be found in the literature [1–8].

In differential scanning calorimetry, the temperature of the lowest minimum inflection point of the decomposition curve (peak temperature) could provide valuable information in the analytical study of α-amino acids.

Ideally, the peak temperature is the temperature at which the decomposition reaction occurs most rapidly, but it is also the temperature at which the maximum rate of heat change between the sample and the environment takes place. Some α-amino acids can be identified on the basis of the peak temperature alone because these values are distinct and do not overlap with those of adjacent α-amino acids on the decomposition scale [1–8]. However, thermal analysis can supply useful information on the characterization of some organic compounds with similar structures.

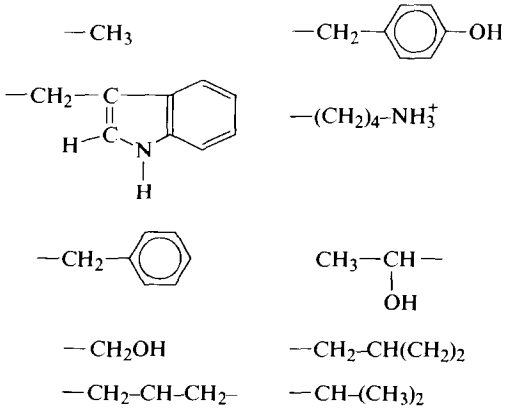
In this manner, it was possible in a recent work [9] to group some α-amino acids with similar structures by the shape and number of peaks of their thermograms. An attempt was also made to characterize the variation of different series of dipeptides, using the thermograms, in the solid phase, of the individual free α-amino acids [10].

The aim of this work is to determine whether thermal analysis can characterize a series of dipeptides having α-alanine as the common first term (structure 1).

The compounds studied in this work were: alanyl–alanine (AlaAla), alanyl–tyrosine (AlaTyr), alanyl–tryptophan (AlaTrp), alanyl–lysine (AlaLys), alanyl–phenylalanine (AlaPhe), alanyl–threonine (AlaThr), alanyl–serine (AlaSer), alanyl–leucine (AlaLeu), alanyl–proline (AlaPro) and alanyl–valine (AlaVal).

The variation of the compounds of this series was studied by comparing their thermograms with those of the independent α-amino acids which form the structures of dipeptides.

In structure 1, R is always $-CH_3$ while R′ represents the following groups:



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 were of purity > 99.99%, and the organic compounds > 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 decomposition of the samples, the TG–DSC system was flushed with nitrogen gas both below (flow rate, 30 ml min^{-1}) and above (flow rate, 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 thermodynamic parameters were calculated using the Stanton-Redcroft Data Aquisition System, Trace 2, Version 4.

The compounds (Polyscience) were used without purification and their purity (99%) is larger than that needed for application of the DSC technique [11–13]. The purity of the compounds was checked by HPLC measurements.

Simultaneous TG–DSC system is a very useful tool for investigating organic compounds since it combines, in a single run, weight loss and heat change processes. In this manner, transformations that occur even with small weight changes (chemical reactions, decomposition, vaporization, oxidation processes) can be distinguished from those occurring without change (melting, crystallization, polymorphic changes).

The quantities used to characterize the compounds were the onset temperature of decomposition (T_0) and the overall enthalpy of decomposition ΔH [9, 10].

This last value is obtained by adding the enthalpy values relating to different ranges of temperature and can be considered as the heat that the compound has exchanged with the external system, at constant pressure, up to its decomposition.

Finally, a kinetic TG-dynamic study of the decomposition processes for the studied compounds was carried out using the method of McCarty and Green [14]. Kinetic analysis includes the kinetic energy of activation E_a related to the decomposition processes, the frequency factor in A, and the reaction order.

In this study we considered, for the calculation of activation energy, mass losses consistently lower than 10%. Indeed, it has usually been considered [15] that the initial portion of the TG curves can be fitted by a first-order reaction equation.

3. Results and discussion

The trends in thermal behaviour for the compounds examined are given in Figs. 1–10. The values of the thermodynamic and kinetic quantities relating to the TG and

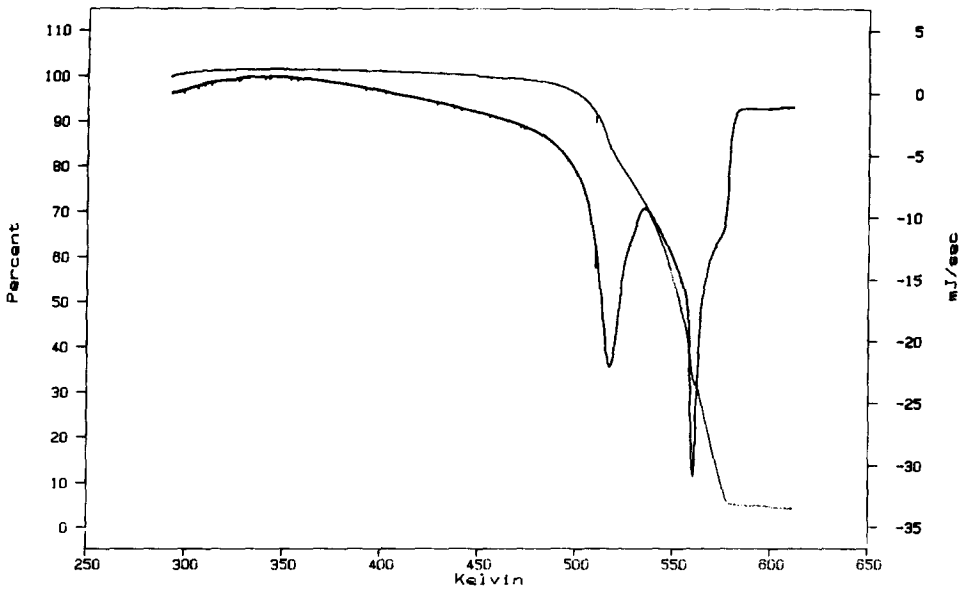


Fig. 1. DSC and TG curves of alanyl–alanine.

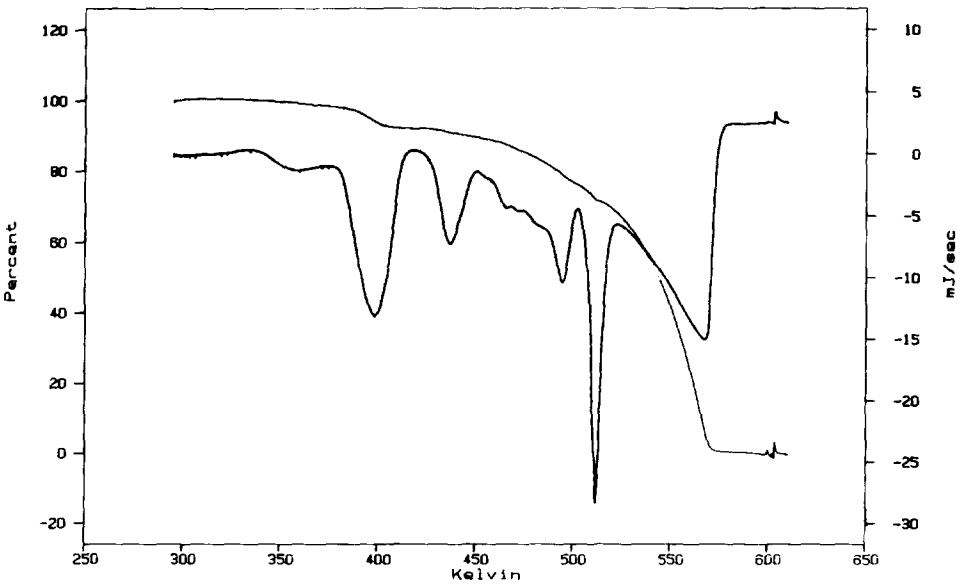


Fig. 2. DSC and TG curves of alanyl–valine.

DSC measurements are reported in Tables 1–3. The thermodynamic quantities, relating to the TG and DSC measurements for the decomposition and melting processes of the α-amino acids [9, 16], which are the components of the dipeptides, are reported in Table 4, below.

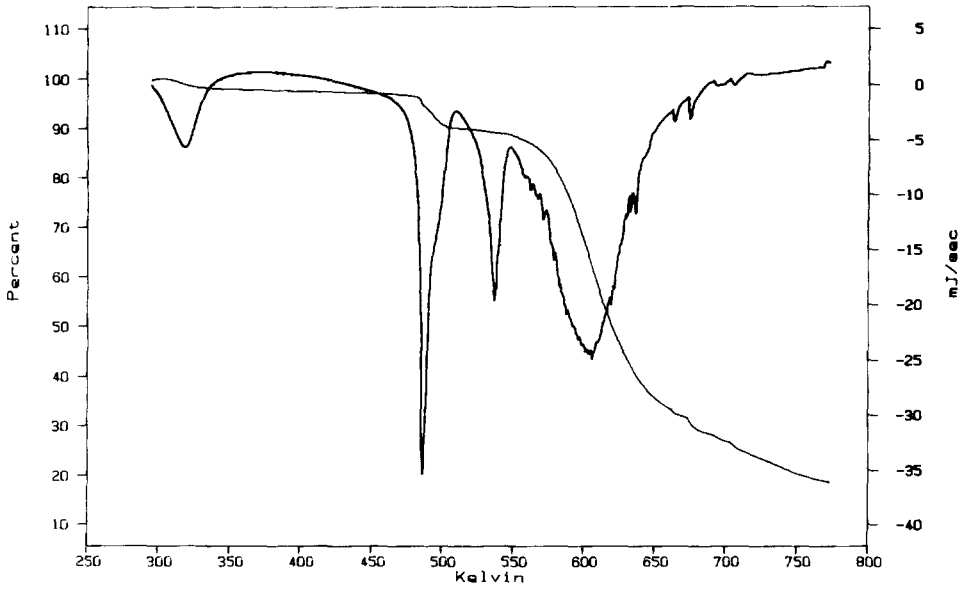


Fig. 3. DSC and TG curves of alanyl–lysine.

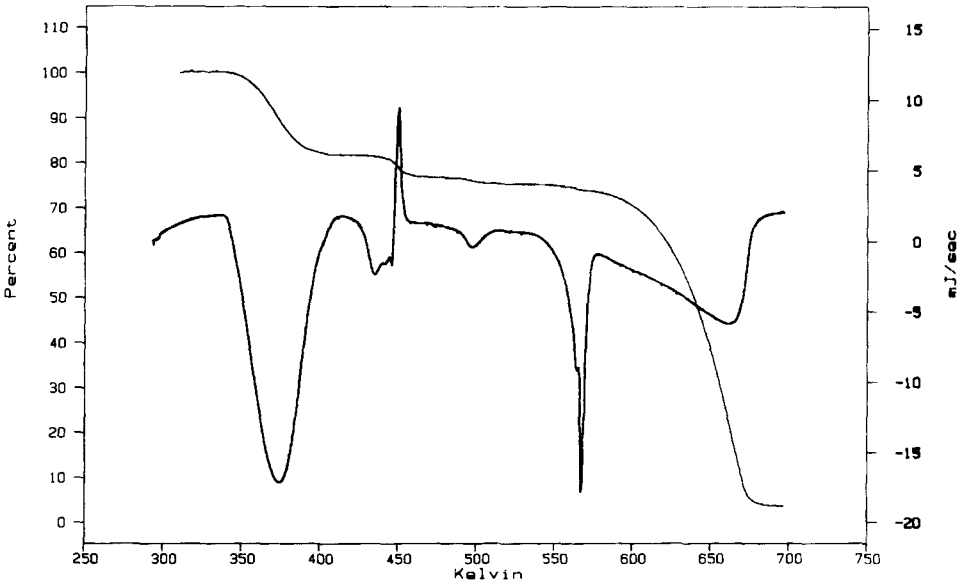


Fig. 4. DSC and TG curves of alanyl–tyrosine.

3.1. Thermal stability and enthalpy

Alanyl–alanine was used as a reference in the study of the series of dipeptides with α-alanine as the common first term; analysis of this compound can be carried out by

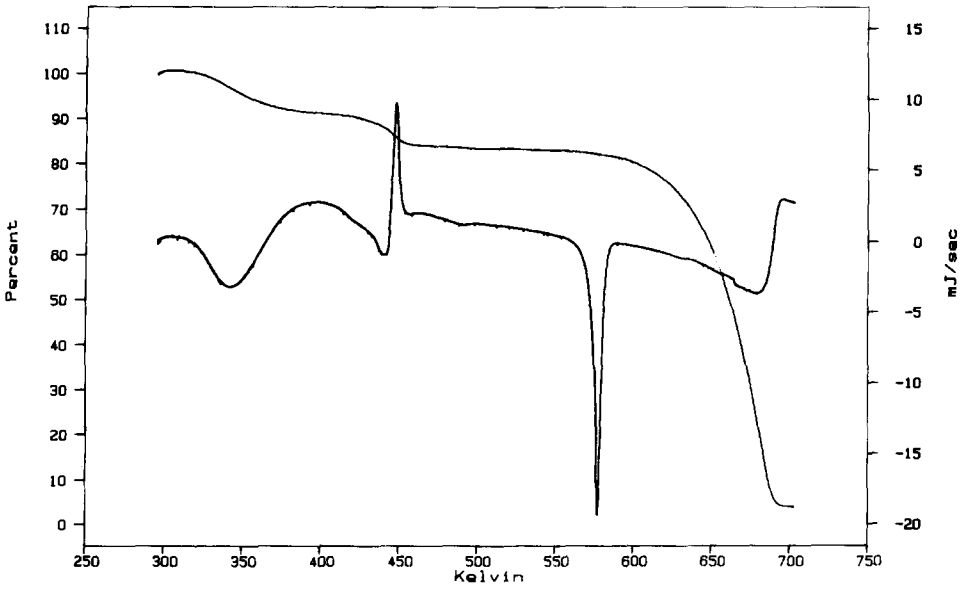


Fig. 5. DSC and TG curves of alanyl–tryptophan.

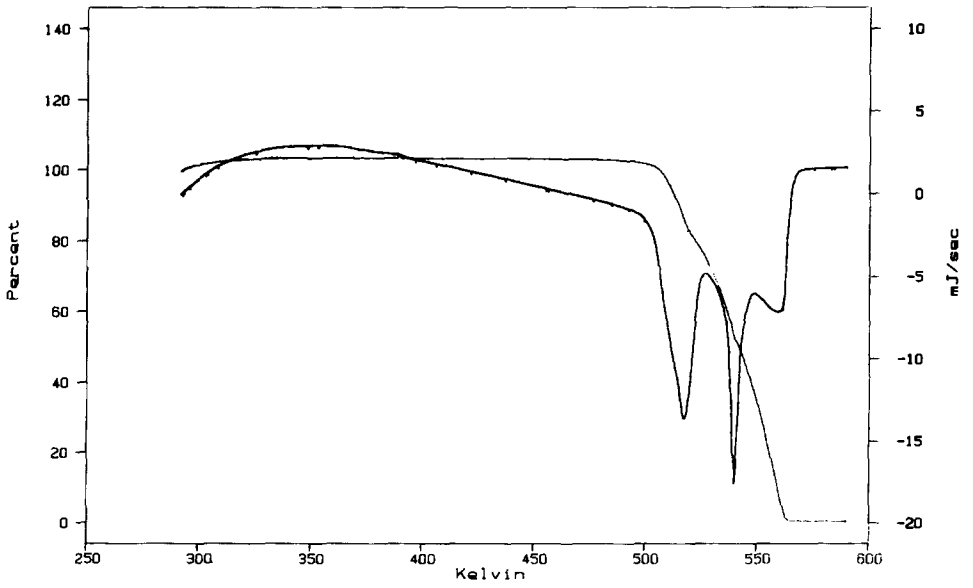


Fig. 6. DSC and TG curves of alanyl–leucine.

comparing its thermogram with that of α-alanine [9]. From this experimental evidence, it was observed that for alanyl–alanine (AlaAla) there are two weight loss processes and two endotherms. These processes occur within the same temperature range as the decomposition of α-alanine. The temperature ranges, which include the decomposition

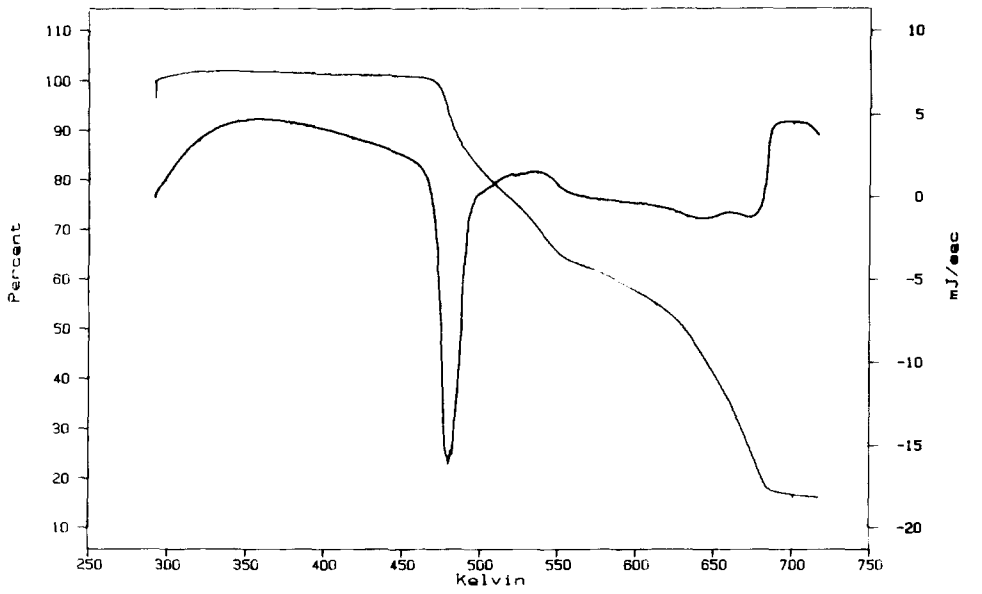


Fig. 7. DSC and TG curves of alanyl–serine.

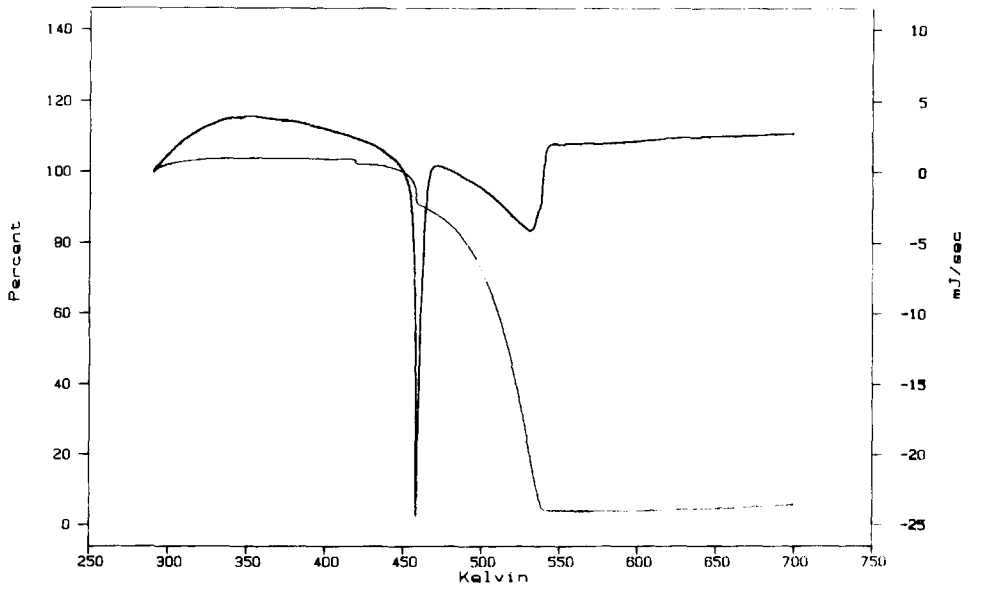


Fig. 8. DSC and TG curves of alanyl–proline.

and melting processes of the compound, are 501.00–525.00 K and 525.00–584.00 K with a weight loss (W%) of 28.01% and 65.84% respectively. α-alanine, however decomposes in a single process (490.00–631.00 K) with an overall weight loss of 100%.

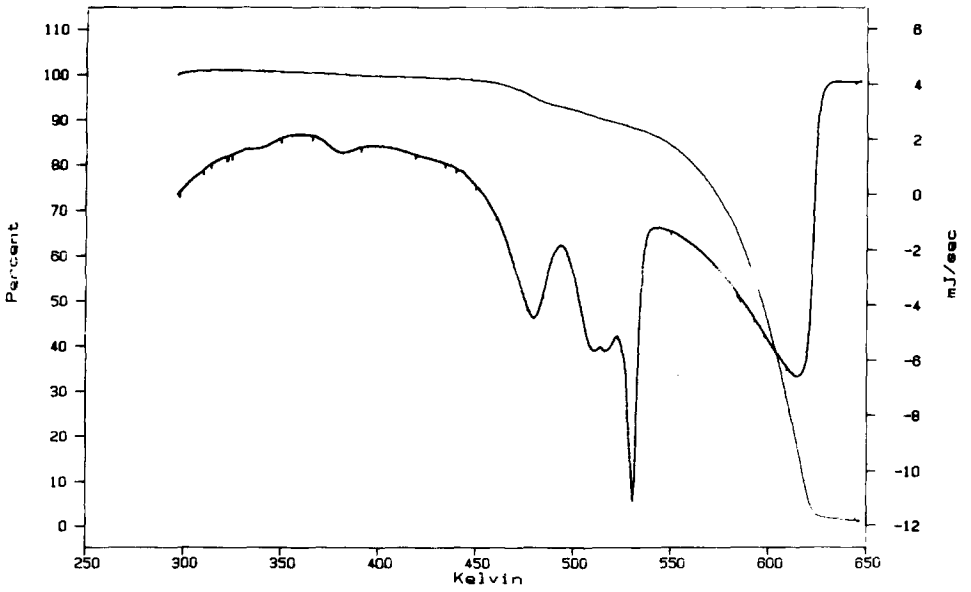


Fig. 9. DSC and TG curves of alanyl–phenylalanine.

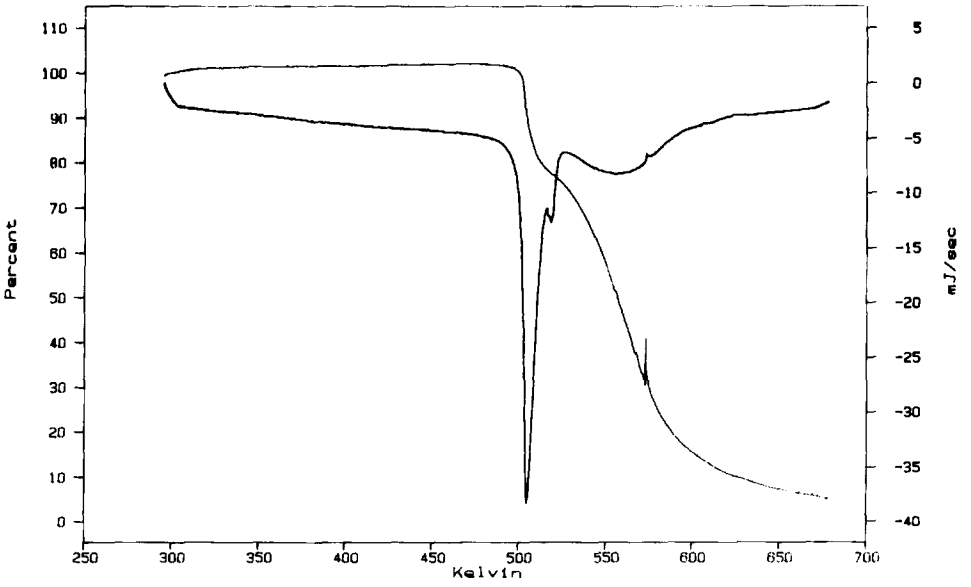


Fig. 10. DSC and TG curves of alanyl–threonine.

The sum of the first and second enthalpy values (overall enthalpy) of the decomposition processes for ananyl–alanine, is 114.76 kJ mol^{-1}, while α-alanine shows a value of 124.91 kJ mol^{-1} for the single process. The two peaks of this dipeptide at 517.00 and 560.00 K are below the peak of α-alanine (574.45 K). Thus alanyl–alanine has the same

Table 1
Extent of thermal decomposition of some dipeptides from TG measurements

Compound	Stage of decomposition							
	I			II			III	
	Onset/K	W/%	ΔT/K	W/%	ΔT/K		W/%	ΔT/K
AlaAla	501.00	28.01	501.00–525.00	65.84	525.00–584.00			
AlaVal	360.00	8.92	380.00–436.00	22.12	436.00–509.00		67.07	509.00–571.00
AlaLys	481.00	6.49	481.00–520.00	4.19	520.00–570.00		68.04	570.00–762.00
AlaTyr	354.95	18.13	354.00–387.00	3.63	387.00–470.00		71.65	470.00–750.00
AlaTrp	320.00	4.10	320.00–420.00	6.00	420.00–470.00		79.11	470.00–700.00
AlaLeu	506.21	21.85	506.00–530.00	65.84	530.00–563.00			
AlaSer	469.60	22.73	470.00–503.00	13.34	503.00–563.00		46.16	563.00–700.00
AlaPro	454.36	12.09	421.00–462.00	85.16	462.00–537.00			
AlaPhe	440.00	7.20	450.00–500.00	6.10	500.00–540.00		83.91	540.00–612.00
AlaThr	457.00	24.47	500.00–520.00	6.87	520.00–550.00		68.70	550.00–690.00

thermal stability as α-alanine, but requires less energy for complete decomposition. α-alanine, as previously observed [9], loses the carboxyl and amine groups simultaneously. The weight loss of the first decomposition process of alanyl–alanine, put in mathematical proportion with its molecular weight, acquires the value of 44 g mol^{-1}, related to the loss of the carboxyl group.

Alanyl–valine has three decomposition steps: the first has a weight loss of 8.92% (with a corresponding proportional weight loss of 17 g mol^{-1}) and can be related to the amino group. Valine, as previously observed [9], loses the carboxyl group and then the amino group. The overall enthalpy (Table 2) and the onset temperature of decomposition for this dipeptide are smaller than the corresponding values of the two independent α-amino acids (Table 4), showing the decreased thermal stability of the dipeptide. Moreover, its last peak (567.45 K) overlaps with the peak of valine (568.88 K), indicating that the structure of valine is not influenced by α-alanine.

The thermal behaviour of alanyl–lysine (Tables 1, 2, Fig. 3) compared with that of the lysine (Table 4, Fig. 11) shows that there is no influence of α-alanine on lysine. Indeed lysine shows (Fig. 11) an endotherm in the temperature range 333.15–353.15 K with a peak temperature of 339.00 K ($\Delta H = 7.12$ kJ mol^{-1}) and a weight loss of 2%. Subsequently (Table 4), an initial melting process occur in the temperature range 497.15–525.15 K ($\Delta H = 1.64$ kJ mol^{-1}) followed by two stages of decomposition, $\Delta T = 533.00$–569.00, $\Delta T = 569.00$–701.00, related to the decarboxylation and deamination processes.

Some noise peaks are seen in the enthalpy processes $\Delta T = 533.00$–569.00 K ($\Delta H = 38.16$, peak 532.00 K) and $\Delta T = 569.00$–673.00 ($\Delta H = 34.14$ kJ mol^{-1}, peak 603.00 K), probably due to the release of gas within the sample [17]. For the dipeptide (Fig. 3, Table 2), there is also an endotherm ($\Delta T = 296.00$–337.00 K, peak 318.00 K,

Table 2
Thermodynamic parameters of thermal decomposition of some dipeptides from DSC measurements

Compound	Stage of decomposition											
	I			II		III		IV		V		
	Onset/ K	ΔH/ kJ mol^{-1}	Peak/ K	ΔH/ kJ mol^{-1}	Peak/ K	ΔH/ kJ mol^{-1}	Peak/ K	ΔH/ kJ mol^{-1}	Peak/ K	ΔH/ kJ mol^{-1}	Peak/ K	$\Delta H_{overall}$/ kJ mol^{-1}
AlaAla	504.00	43.79	517.00	70.97	560.00							114.76
AlaVal	380.00	40.00	398.00	13.70	436.00	6.32	494.00	22.25	512.00	60.63	567.75	142.90
AlaLys	298.00	20.7	318.00	51.12	486.00	24.13	537.00	156.83	606.13			236.15
AlaTyr	342.00	208.38	374.00	− 12.54	450.00	42.61	567.00	114.62	662.36			353.07
AlaTrp	314.00	60.53	342.00	− 17.81	448.00	46.96	557.50	46.98	679.00			136.60
AlaLeu	504.21	49.79	518.00	28.21	540.00	21.72	559.00					99.72
AlaSer	472.00	83.43	481.00									83.43
AlaPro	431.64	67.90	458.22	99.30	530.00							167.20
AlaPhe	446.00	17.17	479.62	40.65	531.00	103.26	615.00					161.08
AlaThr	457.00	106.55	513.15									106.55

Table 3
Kinetic parameters of thermal decomposition of some dipeptides from TG measurements assuming first-order reaction

Compound	E_a/kJ mol^{-1}	ln (A/min^{-1})
AlaAla	151.17	34.76
AlaVal	61.01	17.51
AlaLys	142.30	34.19
AlaTyr	58.19	17.86
AlaTrp	34.37	10.63
AlaLeu	255.65	59.47
AlaSer	221.09	54.98
AlaPro	155.42	40.30
AlaPhe	59.88	12.76
AlaThr	312.16	74.36

$\Delta H = 20.77$ kJ mol^{-1}) with a weight loss of 2%. The third (537.00) and fourth (606.13 K) peaks of the DSC curve of the dipeptide are very close to those of pure lysine. Noise peaks also appear in the DSC curve of dipeptide. Moreover, the proportional weight loss of the second decomposition step (17 g mol^{-1}) indicates the loss of the amino group.

The first decomposition step of alanyl–tyrosine indicates a loss of one carboxyl (44 g mol^{-1}); the second step, the exothermic process, is characteristic of a thermal decomposition reaction involving loss of a diatomic gas molecule [18]. The dipeptide is 93.41% decomposed at 690.00 K while tyrosine is 74.36% decomposed at 875.00 K. The peak of tyrosine was not found in the thermogram of the dipeptide, thus indicating that α-alanine weakens tyrosine structure. Alanyl–tryptophan loses a CO group at 320.00 K. This compound, at 700.00 K, is 94% decomposed while tryptophan is 76.85% decomposed at 776.00 K.

The presence of five- and six-membered rings in alanyl–tryptophan and alanyl–tyrosine gives rise to similar thermograms (Figs. 4 and 5).

The first decomposition step of alanyl–leucine indicates loss of the carboxyl group. The overall enthalpy value is smaller than the sum of the enthalpy values of the two α-amino acids. The peak of leucine was not observed in the thermogram of the dipeptide, showing that the structure of leucine is modified by α-alanine.

Alanyl–serine has three decomposition steps in a temperature range that is smaller than those of the two components. The first step has a weight loss of 22.73% (with a corresponding proportional weight of 44 g mol^{-1}) and can be related to the carboxyl group. The dipeptides is 82% decomposed at 700.00 K while serine is 79% decomposed at 773.00 K; the structure of serine is influenced by α-alanine as can be inferred by the absence of its decomposition peak.

Proline is characterized by a single decomposition process, while alanyl–proline shows two decomposition steps, the proportional weight of the first (17 g mol^{-1}) being

Table 4
Thermodynamic parameters of thermal decomposition of some α-amino acids from TG–DSC measurements

Compound	Decomposition Stage	Onset/K	ΔT/K	W/%	ΔT/K	ΔH/kJ mol^{-1}	Peak/K
Valine	I	473.00	434.00–596.00	92.22	497.00–594.00	66.35	568.88
α-Alanine	I	542.67	490.00–631.00	100.00	483.00–601.00	124.91	574.45
Tyrosine	I	551.00	591.00–611.00	43.00	554.00–628.00	112.11	591.00
	II		621.00–637.00	0.60			
	III		637.00–875.00	30.76			
Phenylalanine	I	537.00	510.00–536.00	5.52	526.00–536.00	2.95	530.75
	II		536.00–569.00	41.42	536.00–569.00	39.61	544.00
	III		569.00–573.00	0.60	569.00–577.00	1.13	570.00
	IV		573.00–674.00	45.65	585.00–666.00	20.63	650.00
Tryptophan	I	565.00	543.00–590.00	17.45	550.00–590.00	76.85	570.00
	II		590.00–776.00	59.40	622.00–747.00	25.21	682.00
Threonine	I	520.00	501.65–600.00	93.32	499.00–583.06	85.43	540.00
Lysine	I	336.00	333.00–345.00	2.00	333.15–353.15	7.12	339.00
	II		533.00–569.00	18.38	497.15–525.00	1.64	515.00
	III		569.00–701.00	57.61	533.00–569.00	38.16	532.00
	IV				569.00–673.00	34.14	603.00
Leucine	I	548.00	505.00–615.00	99.30	505.00–601.00	121.08	578.95
Proline	I	512.00	491.00–603.00	94.50	500.00–591.00	103.33	506.85
Serine	I	496.00	486.00–542.00	57.80	486.00–542.00	68.36	508.36
	II		542.00–773.00	21.70			

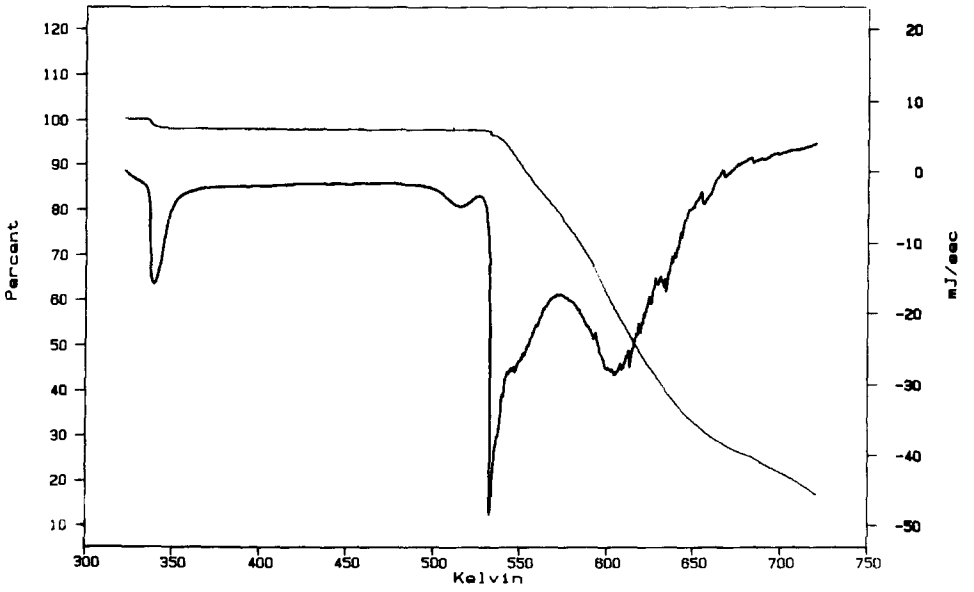


Fig. 11. DSC and TG curves of lysine.

related to the amino group. Taking as reference the temperature ranges and enthalpies relating to the decomposition and melting processes of the components (Table 4), it can be observed that the reciprocal influence destabilizes proline and α-alanine structures, decreasing the temperature range of the decomposition and the overall enthalpy of the dipeptides (Tables 1 and 2); this is confirmed by the fact that the peaks of the two independent α-amino acids are not recognized in the dipeptide thermogram.

The proportional weight loss of the first decomposition process of alanyl–threonine (45.05 g mol^{-1}) indicates loss of the CH_3–CHOH group. The absence of the peak of threonine in the thermogram of the dipeptide allows one to hypothesize that α-alanine influences the threonine structure.

Finally, for alanyl–phenylalanine, there are three stages of decomposition, the first of which (17 g mol^{-1}) can be related to the loss of the amino group. The values of the temperature ranges (Table 1) and of the overall enthalpy (Table 2) of this dipeptide are smaller than those of the corresponding quantities of the independent components (Table 3). This fact and the absence of the peaks of phenylalanine and α-alanine in the dipeptide thermogram, indicate that the structures of these α-amino acids are weakened by the reciprocal influence.

As only AlaVal and AlaLys retain the peaks of the independent α-amino acids, these two dipeptides cannot be compared with other compounds of the series. For the series, a thermal stability, referred to the onset temperature of decomposition for the studied dipeptides, can be written as: AlaLeu > AlaLa > AlaSer > AlaThr > AlaPro > Ala Phe > AlaTyr > AlaTrp.

Dipeptides with side chains containing aliphatic or polarized groups have larger thermal stability values than those containing aromatic groups. The overall enthalpy

scale shows the following order: AlaTyr > AlaPro > AlaPhe > AlaTrp > AlaAla > AlaThr > AlaLeu > AlaSer.

The two scales show an opposite order: the compounds having aliphatic chains show larger initial temperatures of decomposition, while for those having aromatic chains, a larger heat amount is required for their decomposition.

3.2. Kinetics

The activation energy values follow the order:
AlaThr > AlaLeu > AlaSer > AlaPro > AlaAla > AlaPhe > AlaTyr > AlaTrp.

It can be noted that, from the kinetic point of view, the dipeptides bearing five- and six-membered rings show activation energies smaller than those of the dipeptides bearing aliphatic chains.

This occurs because aromatic structures, in the first step of decomposition, stabilize the charge spread on the ions more than aliphatic chains do.

This hypothesis follows from the fact that, for AlaTyr and AlaTrp, the negative charge, by virtue of the loss of the carboxyl group, lies next to the aromatic rings, with a consequent activation energy requirement less than that of AlaPhe. Indeed this dipeptide loses the amino group in the first step of decomposition and the charge is farther away from the benzene ring.

4. Conclusion

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

The proportional weight loss indicates the first fragment of decomposition (carboxyl group, amino group and functional groups) and the beginning of the decomposition. The first fragment can also indicate which of the two structures has a prevailing influence.

Indeed, AlaAla, AlaLeu, AlaSer and AlaTyr lose the carboxyl group and AlaThr loses the CH_3–CH–OH functional group. For this reason, it can be hypothesized that the initial decomposition takes place in the second component.

AlaPro and AlaPhe lose the amino group, so a decomposition in α-alanine can be hypothesized with a consequent prevalence of the second component.

Finally, in the thermograms of AlaVal and AlaLys, peaks of the independent α-amino acids can be recognized. Thus it can be concluded that for these two compounds only, the TG–DSC curves represent a rapid tool, providing the first evidence for their presence.

Acknowledgements

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