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INVESTIGATION OF THERMOCHEMICAL FEATURES OF L-ALANYL-GLYCINE AND β-ALANYL-L-HISTIDINE (L-CARNOSINE)

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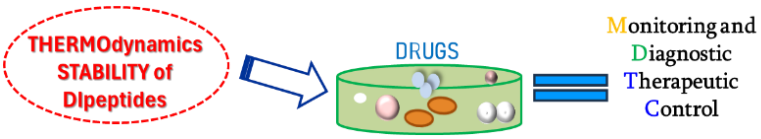
Thermal analysis of two dipeptides having alanine (Ala) as first term, using combustion calorimetry method and simultaneous TG-DSC measurements, have been carried out. The enthalpies of combustion and formation, the TG-DSC quantities related to the decomposition processes of these compounds, were compared with those of the free –α-amino acids contained in the dipeptides. Information about the stability and the influence of the components on the decomposition processes of the dipeptides was obtained.



INTRODUCTION

Dipeptides, compounds made up of two α-amino acids residues, are the smallest units of protein chains and play an essential role in the medicine sector, e.g. antihypertensive or vasodilatatory drugs, sport medicine and tumor therapy.[1] The peptide bond formation is the core for protein synthesis and plays a crucial reaction for life processes. Essentially, during the process of formation, dehydration reaction among aminoacids occurs, where each amino acid residue is linked via the acid amide (peptide) bond of its carboxyl group to the amino group of the other amino acid, releasing a molecule of water.[2]

Carnosine (β-alanyl-L-histidine) is first ever discovered biologically active dipeptide[3] and represents hydrophilic endogenous protein synthesized from 2 amino acids residues: β-alanine and L-histidine by the enzyme carnosine synthase.[4] Endogenous proteins have a key role in maintaining the structure and function of cells, tissues, and organs and refer to proteins that are naturally produced within an organism's cells. Carnosine is highly concentrated in muscle and brain tissues. It is naturally produced by the body in the liver from beta-alanine and histidine residues and plays an important role in muscular function, homeostasis, antioxidant defense, protective ability against diabetes, Alzheimer's and Parkinson's diseases. Carnosine is also known as neuropeptide due to its neurodegenerative disease protective role. Several studies showed that carnosine is a drug with anti-stress, antioxidative and pH-buffering properties, with the ability to protect against radiation damage. The main therapeutic

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effects of carnosine include antihypertensive potency, wound healing, anti-inflammatory and immunomodulating actions.[3] Also the ability of carnosine to suppress the growth of tumor cells has recently been reviewed.[5] In carnosine molecule the two amino acids, β-alanine (β-Ala) and L-histidine (L-His) residues are bound together by means of a peptide linkage.[6] With their potential biological activities, there is a growing interest to explore their fundamental properties. Carnosine is absent in plant, but rich in meat, beef being the high source of carnosine.[7]

L-Alanyl-glycine belongs to the class of organic compounds known as dipeptides. These are organic compounds containing a sequence of exactly two alpha-amino acids residues joined by a peptide bond.

L-alanyl-glycine is found in human urine. It is a breakdown product from endogenous and exogenous proteins.[8] L-Ala-Gly and β-Ala-His, likewise amino acids, having an acidic and a basic functional group in their molecule, appear in zwitterionic form.

Thermochemical study of the substances of biological interest (amino acids, peptides, proteins) by combustion calorimetry, in particular for the first two categories, it has been approached since the early 1930s and has been continued to this day. However, for most natural amino acids there are no consistent data for formation enthalpies and other thermodynamic parameters, or are very old.

On the other hand, differential scanning calorimetry is recognized as one of the most effective techniques in studying the thermal stability of proteins[9-13] being one of the most sensitive methods for characterizing protein depletion,[14] thus DSC determinations have become a key element in assessing the stability of peptides and proteins.

The present study focuses on obtaining the enthalpies of combustion and formation by combustion calorimetry method and the phase transition enthalpies using differential scanning calorimetry in order to bring more information about their thermal stability.

A single literature value of the enthalpy of formation of L-alanyl-glycine is reported in literature by Huffman *et al*.[15] in 1942 and for L-carnosine, despite its importance, reliable experimental data for the enthalpy of formation are not available.

The enthalpies of formation are a feature of the molecules stability, allowing the establishment of correlations of structure – molecular energy, with the structural clarifications resulting from these correlations. For this purpose, the experimental values of the formation enthalpies were compared with those obtained by estimation methods based on contributions of the atomic groups.

The parameters experimentally determined for the two peptides can serve as a model for a better understanding of proteins properties, in whose composition enters.

RESULTS AND DISCUSSION

Combustion energy

The results of combustion determination for the investigated dipeptides are presented in Tables 1 and 2. The corrections for the cotton thread and filament used in the combustion experiments were calculated considering $\Delta_c u°_{(cotton)}$ = 16709±5 J g^{-1} and $\Delta_c u°_{(Ni–Cr)}$ = 5.86 kJ g^{-1} (certified by the calorimeter's producer), the mass of cotton and the filament respectively. The values obtained for the combustion energy were reported to the standard state (T = 298.15 K and $P°$= 0.1 MPa). Corrections were made applying Washburn equation, previously described.[16-17]

Considering the combustion reactions, we have calculated the standard enthalpy of formation taking into account the following values: $\Delta_f H°_{CO2(g)}$= –393.51 ± 0.13 kJ mol^{-1}, $\Delta_f H°_{H2O(l)}$= –285.83 ± 0.042 kJ mol^{-1}.[18]

L-alanyl-glycine: $C_5H_{10}N_2O_3(s) + 6\ O_2(g) \rightarrow 5CO_2(g) + 5H_2O(l)+N_2(g)$ (1)

L-carnosine: $C_9H_{14}N_4O_3(s) + 11\ O_2(g) \rightarrow 9CO_2(g) + 7H_2O(l)+2N_2(g)$ (2)

by using the equation: $\Delta_c H° = \Delta_c U° + \Delta nRT$ (3)

where Δn is the change in the mole number of gaseous compounds during the combustion reaction:

$$\Delta n = \Sigma n_{products,g} - \Sigma n_{reactants,g} \quad (4)$$

$$\Delta_f H° = a\Delta_f H°_{CO2(g)} + b/2\Delta_f H°_{H2O(l)} - \Delta_c H° \quad (5)$$

Table 1

Results for typical combustion experiments for L-alanyl-glycine samples

Sample	1	2	3	4	5
m_{sample} [g]	0.049377	0.047896	0.056576	0.036476	0.032122
$m_{(Ni–Cr)}$ [g]	0.002525	0.002186	0.002397	0.002368	0.003000
m_{cotton} [g]	0.001224	0.001559	0.001233	0.001278	0.001427
ΔT [K]	0.3974	0.3872	0.4519	0.297	0.266
$\varepsilon_{calor}(-\Delta T_c)$ [J]	–926.88	–903.09	–1054.00	–692.71	–620.41
$-m_{wire}\Delta_c u°_{wire}$ [J]	14.79	12.80	14.04	13.87	17.57
$-m_{cotton}\Delta_c u°_{cotton}$ [J]	20.46	26.05	20.60	21.35	23.84
$-\Delta U_{decomp\mathrm{HNO3}}$ [J]	7.288	5.773	6.163	3.938	3.392
$-\Delta_c u$ [Jg^{-1}]	–17910.10	–17923.67	–17908.57	–17917.43	–17919.45
$-\Delta_c u°$ [Jg^{-1}]	–17911.26	–17924.83	–17909.73	–17918.59	–17920.61
$<\Delta_c u°>$[Jg^{-1}]	–(17917.01±6.4)				

*m_{sample} – sample mass, m_{wire} – mass of the ignition wire, m_{cotton}– mass of the cotton fuse. $V_{(\mathrm{bomb})}$ = 22 mL – internal volume of the calorimetric bomb, $m^{i}_{(\mathrm{H2O})}$ = 0.5 g – mass of water added to the bomb for dissolution of combustion gases; the pressure of oxygen in the bomb was 3.55±0.01 MPa, ΔT_c – corrected temperature rise due to combustion, ε_{calor} – energy equivalent of the calorimeter, Δu_{cotton} – energy of combustion of the cotton fuse, $\Delta U_{decomp\mathrm{HNO3}}$ – energy required for decomposition of the HNO_3 solution formed, $\Delta_c u°$– massic standard energy of combustion, $<\Delta_c u°>$ – mean value of standard molar energy of combustion, uncertainty represent the standard deviation of the mean.

Table 2

Results for typical combustion experiments for β-Alanyl-L-histidine samples

Sample	1	2	3	4	5
m_{sample} [g]	0.044851	0.050725	0.060701	0.055392	0.057233
$m_{(\mathrm{Ni–Cr})}$ [g]	0.001985	0.001078	0.004355	0.002137	0.002843
m_{cotton} [g]	0.001263	0.001180	0.001256	0.001164	0.001350
ΔT [K]	0.418	0.4682	0.5674	0.5134	0.5331
$\varepsilon_{calor}(-\Delta T_c)$ [J]	–974.93	–1092.02	–1323.39	–1197.44	–1243.39
$-m_{wire}\Delta_c u°_{wire}$ [J]	11.63	6.31	25.51	12.52	16.65
$-m_{cotton}\Delta_c u°_{cotton}$ [J]	21.10	19.72	20.99	19.45	22.56
$-\Delta U_{decomp\mathrm{HNO3}}$ [J]	8.10	9.84	12.56	11.76	12.14
$-\Delta_c u$ [Jg^{-1}]	–20826.68	–20820.99	–20828.83	–20828.14	–20827.81
$-\Delta_c u°$[Jg^{-1}]	–20827.24	–20821.55	–20829.39	–20828.70	–20828.37
$<\Delta_c u°>$[Jg^{-1}]			–(20827.05±3.7)		

In Table 3 are presented our data for the solid-state enthalpies of formation, together with literature values and those obtained by means of the group additivity method, with parameters recommended by Domalski and Hearing[19] and Salmon and Dalmazzone[20]. The enthalpies of formation for the studied dipeptides were compared with the values of their single components, alanine, glycine [21,23–24] and histidine.[22,24]

Table 3

Thermochemical data at T = 298.15 K and P^o = 0.1 MPa for the studied compounds

Compounds	$-\Delta_c U^o$ [kJ·mol^{-1}]	$-\Delta_c H^o$[kJ·mol^{-1}]	$-\Delta_f H^o$[kJ·mol^{-1}]		$-\Delta_f H^o$[kJ·mol^{-1}]
	(this work)*	(this work)*	(this work)*	(literature)	(calculated)
L-Ala-Gly	2618.57±1.8	2618.57±1.8	778.13±1.9	777.8[15]	777.9 [19] 778.4 [20]
β-Ala-His	4711.91±2.85	4711.91±2.85	830.49±2.9	–	740.4[19] 876.6[20]
L-Ala	1621.32±0.8	1621.94±0.8	559±0.95	560.17±1.7 [23]	–
L-Gly	974.86±1.5	977.34±1.5	524.26±1.4	527.5±0.5 [24]	–
L-His	3196.2 ± 2.4	3195.6 ± 2.4	451.7 ± 3.4	441.8 ± 2.6 [24]	–

*Uncertainty is standard deviations and includes the uncertainties of the enthalpies of formation of the reaction products H_2O and CO_2

The large negative values of the enthalpies of formation of the two dipeptides show their high stability, if the number of chemical bonds from their molecules is taken into account. The enthalpy of formation of carnosine is over 45 kJ/mol less negative than the calculated value. This proves that this molecule is rich enough in energy as to be involved in reactions with free radicals. Carnosine protects in this way biomolecules (such as nucleic acids and proteins) against oxidative stress.

Thermal analysis

Information regarding the thermal stability and mass loss of L-alanyl-glycine and L-carnosine were obtained by coupled TG-DSC (thermogravimetry – differential scanning calorimetry) technique using a Setaram Setsys Evolution 17 equipment.

Differential scanning calorimetry (DSC)

The DSC curves obtained in open alumina crucibles are presented in Fig. 1. The thermodynamic parameters of the investigated dipeptides and their single components obtained using the calorimeter TG-DSC SETSYS Evolution 17 are shown in Table 4.

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.[25]

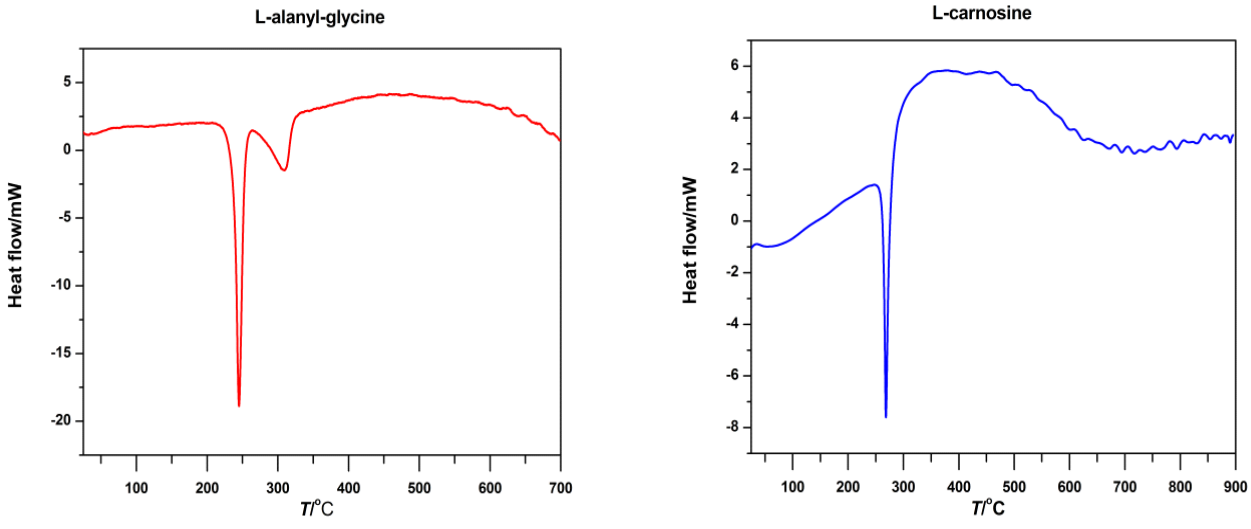


Fig. 1 – DSC scans of dipeptides obtained from calorimeter TG–DSC SETSYS Evolution 17, heating rate 10ºC /min, under Argon flow.

Table 4

Comparative DSC data of the studied dipeptides with their single components

Properties	Peak	Compounds				
		L-Ala-Gly	β-Ala-His	L-alanine[21]	L-glycine[21]	L-histidine[22]
T_{onset} [°C]	1	238.3	263.3	275.2	250.4	277.1
T_{max} [°C]		245.0	268.4	299.7	259.7	282.1
T_{end} [°C]		253.1	276.2	301.9	265.2	287.6
ΔH [Jg^{-1}]		621.28	615.76	857.11	906.35	320.81
T_{onset} [°C]	2	288.9	–			
T_{max} [°C]		309.3	–			
T_{end} [°C]		320.6	–			
ΔH [Jg^{-1}]		278.26	–			

The recorded peaks on the thermogram are assigned to L-alanyl-glycine decomposition, first peak with the onset temperature at 238ºC being attributed to the loss of methyl-amine from α-alanine,[26] while the second endothermic peak is corresponding to the decarboxylation and deamination processes respectively. However, the peaks are not well separated, which means that the first process extend along further decomposition processes. Rodante[27] states that α-alanine decomposes in a single process with the peak temperature of 301 ºC which overlaps with a minor difference the second peak of the analyzed alanyl-glycine (309 °C). The sum of the first and second enthalpy values (overall enthalpy) of the decomposition processes for L-alanyl-glycine is 131.45 kJ/mol, while α-alanine shows a value of 124.91 kJ/mol[25] for the single decomposition process, thus L-alanyl-glycine requires less energy for complete decomposition. α-alanine, as previously observed loses the carboxyl and amine groups simultaneously.[25]

Carnosine decomposes in a single step with the peak temperature at 268 °C requiring an energy of 161.35 kJ/mol more than that for α-alanine and L-alanyl-glycine, which means it is more stable than both compounds. In the case of carnosine, a small molecule (possibly CO) is evolved during the decomposition peak and other gaseous molecules (H_2O and NH_3) at higher temperatures.[22]

Thermogravimetric analysis

In Fig. 2 are plotted the temperatures and mass losses in the TG and DTG curves for the studied dipeptides.

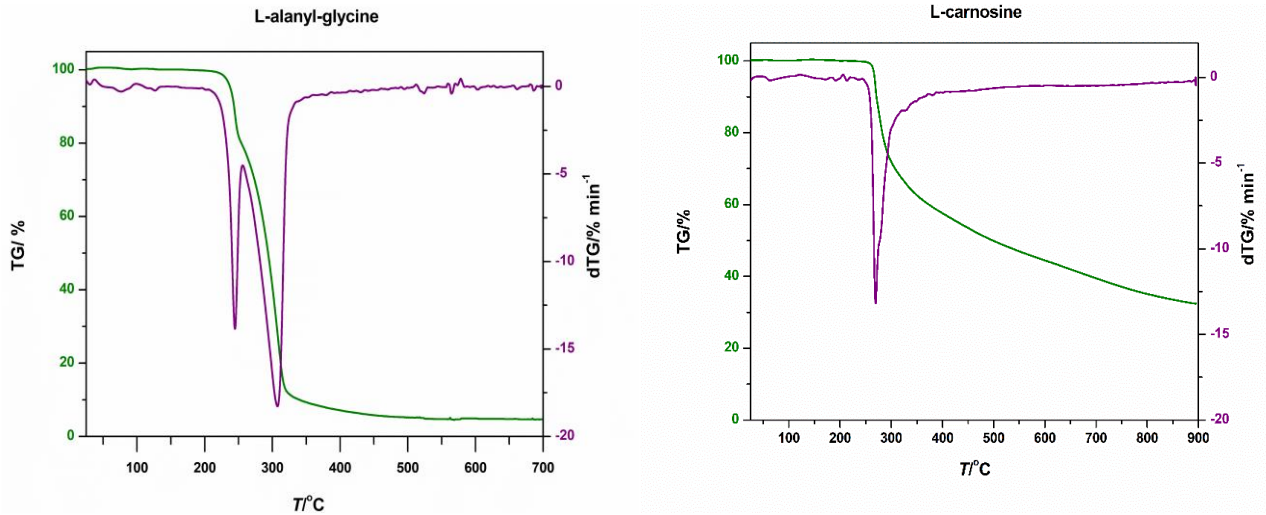


Fig. 2 – TG-DTG curves for dipeptides obtained from calorimeter TG–DSC SETSYS Evolution 17.

Table 5

Thermogravimetric data of L-alanyl-glycine (L-Ala-Gly) and β-Alanyl-L-histidine (L-carnosine)

Compounds	Mass [*m*g]	Δm_1 [%]	ΔT [°C]	Δm_2 [%]	ΔT [°C]	Δm_{total} [%]	ΔT [°C]
L-Ala-Gly	2.103	–20.1	210–255	–69.80	255–334	–95.4	60–700
β-Ala-His	0.998	–38.1	238–356	–29.4	356–900	–70.6	25–900

The TG curves (Fig. 2) of the dipeptide corroborate with the DSC result indicate two visible steps of weight loss of 95.4% up to 700ºC. Up to the temperature when the decomposition starts (~ 210 °C) the weight loss is insignificant (0.5%) while after the decomposition steps are finished (334 °C) the mass loss continues slowly up to 700 °C in proportion to only 5% of the total mass loss. Put in mathematical proportion with the methyl-amine molecular weight (31g·mol^{-1}), the weight loss assigned to the first decomposition process (20.1%) corresponds proportional of mass loss of 29.37 g·mol^{-1} which supports the explanation of the DSC first peak of decomposition. Also, the difference between the obtained 29.37 g·mol^{-1} value and the molecular weight of methyl-amine indicates that the first process is not completely finished when the further decomposition processes have been initiated. Comparing the temperature range within the decomposition occur, it was noticed that the dipeptide requires a narrower temperature range than α-alanine and the temperatures are shifted to lower values.

As regards the carnosine, there is also an insignificant mass loss (0.35%) up to the temperature at which decomposition starts (244 °C) but after the only visible step of mass loss on the DTG curve (between 244–305 °C) the mass loss continues in a large proportion of 29.4% from total mass loss up to 900 °C (Table 5). The TG results support the statement that a small molecule (possibly CO) is evolved during the decomposition peak and other gaseous molecules (H_2O and NH_3) at higher temperatures.[22]

There is a mutual influence of the two α-amino acids which makes the dipeptides quite different than the single component.[27] 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.

The processes recorded for L-carnosine, revealed by DSC and TG are essentially, if not exclusively due to decomposition. Bryan and Olaffson[28] state that the main decomposition reaction is decarboxylation, possibly preceded by deamination. Weiss *et al*.[29] consider that a single main reaction occurs, taking into account that a sharp peak is obtained during the DSC run. The weight loss in the case of decarboxylation reaction was the only one that would be about 28.4%, close to our value of 29.40% for carnosine.

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 glycine and α-alanine structures, decreasing the temperature range of the decomposition and the overall enthalpy of the dipeptides.

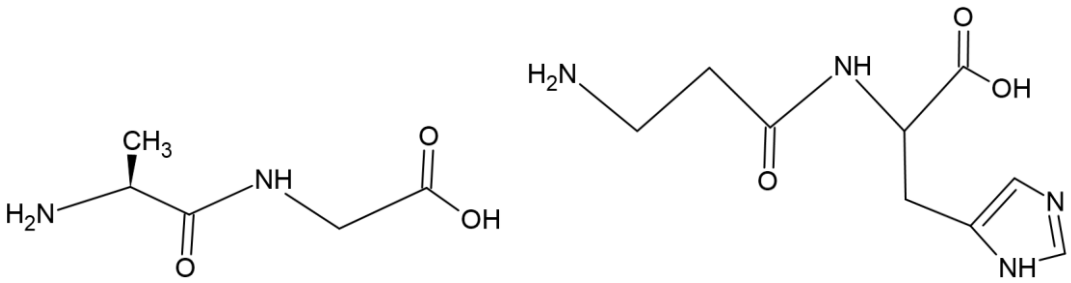
EXPERIMENTAL

Materials

Commercially available polycrystalline powders of L-carnosine (β-alanyl-L-histidine) (Fluka, purity ≥99.0%) and L-alanyl-glycine (Fluka, purity>99%) were used.

General information

L-alanyl-glycine and L-carnosine (β-alanyl-L-histidine) were purchased from Fluka and used without further purification. In Fig. 3 are shown the structural formulas of the studied dipeptides.



L-alanyl-glycine L-carnosine (β-alanyl-L-histidine)

Fig. 3 – Structural formulas of the studied dipeptides.

Method and equipment

Combustion calorimetry

An isoperibolic oxygen bomb calorimeter of Parr Instruments model 6200 type was used in order to perform the combustion experiments. A high precision electronic thermometer measures the temperature with a resolution of 0.0001 K. The jacket temperature is maintained constant for isoperibolic operation. The temperatures of the bucket and of the jacket are monitored by a microprocessor-based controller which make the required heat leak corrections resulting from differences between these two temperatures. These corrections are applied continuously throughout a test rather than as a final correction based on pre and post test measurements.

The calibration procedure of the combustion calorimeter was performed by means of ten combustion experiments with benzoic acid (CAS 65-85-0) supplied by Parr Company, having the energy of combustion under certificate conditions ($\Delta_c u^o$ = –26434.5 J g^{-1}). The energy equivalent of the calorimeter was determined as ε_{calor}= (10054.4 ± 1.4) J K^{-1}. The uncertainty associated with the average value of the energy equivalent is the standard deviation of mean.

The combustion bomb has an internal volume of 0.35 L, being a cylinder made of stainless steel. For samples ignition, a cotton thread was used. The completely equipped bomb (filament, fuse, sample, deionized water) was connected to the oxygen cylinder and flushed with oxygen. All combustion experiments were carried out in high purity oxygen 99.998% at 3.546 MPa and at T = 298.15 K and in the presence of 1 cm^3 of deionized water for saturation of the atmosphere.

The samples mass ranged between 0.3–0.6 g and were weighed with a Mettler-Toledo microbalance with an accuracy of ±2·10^{-6} g.

Simultaneous thermogravimetry-differential scanning calorimetry (TG–DSC) measurements

Thermal properties (mass change, temperature and enthalpy of transformations) of compounds were measured by a simultaneous TG–DSC SETSYS Evolution 17 analyzer from Setaram, in the temperature range from 20 to 900 °C with 10°C min^{-1}, in alumina crucibles, in argon atmosphere. The sample mass for TG-DSC measurements was about 1–4 mg. The error of TG measurement is ± 0.154%. All thermal analysis (TG-DSC) data were processed using Calisto software.

CONCLUSIONS

The studies performed in the present paper revealed original results regarding the investigated compounds, namely: through calorimetric combustion determinations, the values of combustion and formation enthalpies were obtained. In the literature, similar values were recorded only for L-alanyl-glycine, but date from 1942; for the other compound no similar measurements were made.

The mutual influence of the single amino acids makes the thermal behaviour of dipeptides quite different from those of free components. Combustion data indicate that the two studied dipeptides present high stability, according to the large negative values of the enthalpies of formation. Comparison of experimental values with those calculated by group contribution methods provided additional information on the contributions of different functional groups present in those molecules.

The thermal behavior of the compounds and the transformations occurring by heating revealed new information about the thermochemical stability, completing the data base in the field. These data could be used to understand the studied dipeptides behavior when they are involved in numerous biological processes in the living bodies, thus being a feedback for drug monitoring and diagnostic/therapeutic control.

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REFERENCES

1. A. Sallam, M. Krehenbrink and A. Steinbüchel, *BIOspektrum.*, **2012**, *18*, 102–104.
2. M. Vraneš, J. Panić, A. Tot, S. Papović, S. Gadžurić, Č. Podlipnik and M Bešter-Rogač, *J. Molec. Liquids*, **2021**, *328*, 115–250.
3. F. Iuliano, L. Csaderova, M. Labudova, M. Zatovicova, M. Barathova and J. Pastorek and D. Wells (Eds.), "Carnosine: Physiological Effects and Research Insights", Nova Science Pub Inc, New York, 2016, p. 41–82.
4. E. Baye, B. Ukropcova, J. Ukropec, A. Hipkiss, G. Aldini and B. de Courten, *Amino Acids*, **2016**, *48*, 1131–1149.
5. A. R. Hipkiss, S. P. Cartwright, C. Browley, S. R. Gross and R. M. Bill, *Chem. Central J.*, **2013**, *7*, 38, 1–9.
6. A. A. Boldyrev, "Bioinformservice*"*, Moscow, 2001.
7. A. A.Boldyrev, G. Aldini and W. Derave, *Physiol. Rew*., **2013**, *93*, 1803–1845.
8. Metabocard for Alanylglycine (HMDB0006899), https://hmdb.ca/metabolites/HMDB0006899
9. K. Gekko and H. Ito, *Biochem. J*., **1990**, *107*, 572–577.
10. F. Conejero-Lara and J. M. Sánchez-Ru´ız, *Eur. J. Biochem*., **1991**, *200*, 663–670.
11. S. A. Charman, K. L. Mason and W. N. Charman, *Pharm. Res*., **1993**, *10*, 954–962.
12. M. Madaisy Cueto, J. Dorta, O. Mungu´ıa and M. Llabrés, *Int. J. Pharm*., **2003**, *252*, 159–166.
13. M. Wasylewski, *Biochim. Biophys. Acta*, **2004**, *1702*, 137–143.
14. M. Gruebele, "Protein Folding, Misfolding and Aggregation", V. Muñoz (Ed.), in „Biomolecular Sciences series", Royal Society of Chemistry Publishingm, Cambridge, UK, 2008.
15. H. M. Huffman, *The J. Phys. Chem.*, **1942**, *46*, 885–891.
16. E. W. Washburn, *J. Res. Natl. Bur. Stand*., **1933**, *10*, 525–558.
17. D. Gheorghe, A. Neacsu, A. M. Sofronia and St. Perisanu, *Rev. Roum. Chim*., **2022**, *67*, 549–558.
18. CODATA Bulletin, Recommended Key Values for Thermodynamics, **1977**, Paris, France, *78*.
19. E. S. Domalski and E. D.Hearing, *J. Phys. Chem. Ref. Data*., **1993**, *22*, 805–1159.
20. A. Salmon and D. Dalmazzone, *J. Phys. Chem. Ref. Data*., **2007**, *36*, 19–58.
21. D. Gheorghe, Ph.D. Thesis, "Termodynamics characterization of some amino acids and their derivatives", Roumanian Academy Library, **2015.**
22. A. Neacsu, D. Gheorghe, I. Contineanu, A. M. Sofronia, F. Teodorescu and S. Perisanu, *J. Chem*., **2018**, 1–6.
23. I. Contineanu and D. I. Marchidan, *Rev. Roum. Chim*., **1984**, *29*, 43–48.
24. V. P. Vasilèv, V. A. Borodin and S. B. Kopnyshev, *Russ. J. Phys. Chem*., **1991**, *65*, 29–35.
25. F. Rodante, F. Raimondi and S. Vecchio, *Thermochim. Acta*., **1996**, *284*, 351–365.
26. F. Rodante, F. Raimondi and S. Vecchio, *Thermochim. Acta*., **1997**, *297*, 199–206.
27. F. Rodante, G. Marosu and G.Catalani, *Thermochim. Acta*., **1992**, *194*, 197–213.
28. A. M. Bryan and P. G. Olaffson, *Anal. Lett*., **1969**, *2*, 505–513.
29. I. M. Weiss, C. Muth, R. Drumm and H. O. K. Kirchner, *BMC Biophys*., **2018**, *11*, 1–15.