

ENTHALPIES OF SUBLIMATION AND FUSION FOR N-ACETYL SUBSTITUTED GLYCINE, L-ALANINE, AND D-LEUCINE AMIDES*

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Enthalpies and entropies of sublimation for N-acetylglycine amide (NAGA), N-acetyl-L-alanine amide (L-NAAA), and N-acetyl-D-leucine amide (D-NALA) were determined from the dependence of their vapour pressures on temperature, as measured by the torsion-effusion method.

Enthalpies and temperatures of fusion were measured by differential scanning calorimetry (DSC) and entropies of fusion were derived. No solid-to-solid transitions were detected from r.t. to fusion. Enthalpies of sublimation and fusion were combined to evaluate enthalpies of vaporization of the melts.

The experimental results decreased inversely to the molecular mass. An interpretation of this trend in terms of the crystalline structure of these compounds is proposed.

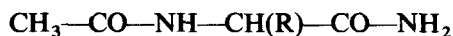
Reliable thermodynamic data on evaporation processes of low-molar-mass organic compounds are of great importance in several fields, ranging from organic chemistry to biochemistry and biophysics. In particular, enthalpies of sublimation and vaporization can be combined with enthalpies of solution at infinite dilution of solid and liquid compounds, respectively, to obtain enthalpies of solvation [1, 2].

Study of model compounds, such as amino acids [3], amides [4], and N-alkyl amides [5], can give insight into the factors affecting protein behaviour and stability in aqueous systems. Peptido-amides have recently been proposed by Lilley et al. as simple model molecules for polypeptide molecular interactions in solution [6]. By comparison with amino acids and their oligomers, in fact, they have the advantage

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of being uncharged, which means no long-range electrostatic interactions and the possibility of factorizing other intermolecular forces. Moreover, they are sufficiently soluble in water and polar solvents to allow their study in solution.

This paper reports some thermodynamic data associated with phase transitions of three peptido-amides represented by the general formula



in which R is: H for the N-acetylglycine amide, CH₃ for the N-acetyl-L-alanine amide, and C₄H₉ (branched) for the N-acetyl-D-leucine amide.

Experimental

The three peptido-amides were synthesized according to Blackburn et al. [6], apart from a slight modification for D-NALA [7]. Products were purified by several crystallizations from appropriate solvents [6], and dried under vacuum. Repeated polarimetric checks were performed to ascertain the absence of racemization in dry products.

High-purity indium (5N8, Koch-Light) and naphthalene (ref. mat. purity grade, Farmitalia Carlo Erba) were used as reference materials for calorimetric and vapour pressure measurements, respectively.

Vapour pressures were measured with a torsion-effusion assembly as previously described [8–10]. The pressure was determined for each temperature from the torsion angle α of a tungsten wire from which the effusion cell is suspended, through the relation:

$$P = 2K\alpha / (a_1 l_1 f_1 + a_2 l_2 f_2)$$

where a_1 and a_2 are the areas of the effusion holes, l_1 and l_2 the distances from the rotation axis, K is the torsion constant of the wire (length = 0.35 m, and diameter = 30 μm), and f_1 and f_2 are the geometrical constants introduced to correct the non-ideality of the effusion holes (with radius r and thickness R) and calculated through the relation [11]:

$$1/f = 0.9882 + 0.3490(R/r) + (R/r)^2$$

Temperatures were measured with a calibrated chromel-alumel thermocouple inserted directly below the cell of the torsion assembly, with a precision of $\pm 0.5\text{K}$. Pure naphthalene was used to test the reliability of the temperature-pressure data. An agreement of about 1.5% was observed with those of Colomina et al. [12]. Two runs on each solid compound were performed in the temperature range ($\Delta T = 30$ to

40 K) just below the melting points. At lower temperatures, the vapour pressures were too low for the sensitivity limits of our experimental assembly.

The enthalpies of fusion and the corresponding onset temperatures were measured with a Setaram DSC-111 G differential scanning calorimeter, with output recorded on a Sefram Servotrace recorder. Empirical equations giving the actual temperature of the sample as well as the sensitivity, $S/\mu\text{V mW}^{-1}$, of the heat-flux detector are reported elsewhere [13]. The heating rate was 1 deg/min^{-1} for all samples. Heats corresponding to the peaks were obtained by comparing their areas with others of approximately the same magnitude, whose heat as known from the sensitivity value and the chart speed of the recorder. All areas reported in this paper are referred to $250 \mu\text{V f.s.}$ and a chart speed of 25 mm min^{-1} . Areas were determined with a Salmoiraghi high-precision manual planimeter with an uncertainty of less than 0.2 per cent.

High-purity indium was used to check the sensitivity and sample temperature equations given by the supplier of the calorimeter. Agreement fell within the 0.5 per cent for the enthalpy and 0.05 per cent for the temperature.

Four to five runs were performed on each compound, taking a fresh sample for every fusion run and starting from r.t. to reveal possible solid-to-solid transitions.

Results and discussion

Dependence of vapour pressure on temperature follows the Clausius-Clapeyron equation, which can be written for our solid samples in the form:

$$\lg p = A - B/T = \Delta_{\text{sub}}S_T^\circ/2.303R - \Delta_{\text{sub}}H_T^\circ/2.303RT$$

in which standard quantities are referred to the mean temperature value of the explored range. Table 1 reports the experimental vapour pressure and temperature values. Two runs were performed per amide and processing of the data by the least squares method gave corresponding $\lg p$ vs. $1/T$ equations.

By weighting slopes and intercepts in proportion to the experimental points the following equations were obtained:

$$\text{NAGA} \quad \lg(p/\text{kPa}) = (15.37 \pm 0.30) - (7087 \pm 150)\text{K}/T \quad (378 \text{ to } 408 \text{ K})$$

$$\text{L-NAAA} \quad \lg(p/\text{kPa}) = (13.08 \pm 0.30) - (6012 \pm 150)\text{K}/T \quad (366 \text{ to } 411 \text{ K})$$

$$\text{D-NALA} \quad \lg(p/\text{kPa}) = (11.29 \pm 0.50) - (5295 \pm 200)\text{K}/T \quad (374 \text{ to } 401 \text{ K})$$

where the associated errors are estimated on the basis of the uncertainties of the pressure and temperature measurements. The sublimation enthalpy and entropy changes were derived by the second-law method. The results are presented in Table 3.

Table 1 Experimental vapour pressures measured by the torsion-effusion method

N-acetylglycine amide			N-acetyl-L-alanine amide			N-acetyl-D-leucine amide				
Cell	T, K	p, Pa	Cell	T, K	p, Pa	Cell	T, K	p, Pa		
A	378	0.457	A	366	0.467	A	374	1.622		
	380	0.588		378	1.380		378	2.089		
	382	0.630		380	1.820		380	2.291		
	383	0.575		383	2.630		383	2.754		
	385	0.790		392	5.500		385	3.163		
	387	0.933		393	6.918		387	3.890		
	389	1.148		402	13.80		389	4.365		
	390	1.380		407	19.50		391	5.495		
	392	1.820		410	21.88		393	6.607		
	393	2.290					395	8.912		
	396	2.818		B	370		0.575	398	10.47	
	398	3.630			375		0.933	401	14.12	
	399	4.365			379		1.479			
	401	4.677			380		1.820	B	375	1.622
	403	6.166			381		2.098		377	1.820
	406	9.332			382		2.400		379	2.042
					385		2.951		382	2.290
		389	4.266		383	2.519				
		391	4.680		385	2.951				
		394	8.318		386	3.467				
		399	10.96		388	3.890				
		400	12.30		390	4.365				
		410	21.88		392	5.248				
		411	23.44		395	8.912				
					396	9.550				
					397	11.22				
					399	13.18				
B	391	1.995								
	393	2.519								
	395	2.884								
	396	3.162								
	397	3.715								
	400	4.571								
	401	5.129								
	405	7.413								
407	10.00									
408	11.22									

The DSC temperatures and enthalpies of fusion measurements are given in Table 2, while mean values of fusion enthalpies and entropies are presented in Table 3, together with enthalpies of vaporization roughly evaluated from sublimation and fusion data by the relation

$$\Delta_{\text{sub}}H^{\circ} = \Delta_{\text{vap}}H^{\circ} + \Delta_{\text{fus}}H^{\circ}$$

The sublimation enthalpies and entropies markedly decrease as the length of the aliphatic side chain increases. Even if correlations between $\Delta_{\text{sub}}H$ and molecular structures of organic compounds cannot be extensively established [14], the observed trend seems in contrast with what one would expect from the molecular

Table 2 Experimental DSC data for temperatures and enthalpies of fusion

Δm , g	T_{fus} , K	A , mm ²	Q , J ^a	$\Delta_{\text{fus}}H^\circ$, kJ mol ⁻¹
N-acetyl-glycine amide (m.w. 116.13)				
0.00544	408.6	1520	1.218	26.00
0.00547	408.2	1516	1.214	25.77
0.00896	407.9	2420	1.939	25.13
0.00848	408.2	2248	1.801	24.66
N-acetyl-L-alanine amide (m.w. 130.15)				
0.00272	430.5	580	0.456	21.82
0.00839	430.9	1752	1.379	21.39
0.00579	431.6	1232	0.970	21.80
0.00746	430.9	1584	1.246	21.74
N-acetyl-D-leucine amide (m.w. 172.23)				
0.00545	403.9	810	0.651	20.57
0.00592	404.1	868	0.699	20.34
0.00688	404.0	988	0.796	19.92
0.00767	404.1	1112	0.896	20.12
0.00576	404.0	828	0.667	19.95

^a Sensitivity values were: 7.490, 7.623, and 7.448 $\mu\text{V mW}^{-1}$, respectively.

Table 3 Enthalpies and entropies of fusion and sublimation, and derived enthalpies of vaporization

Compound	T_{fus} , K	$\Delta_{\text{fus}}H^\circ$, kJ mol ⁻¹	$\Delta_{\text{fus}}S^\circ$, J deg ⁻¹ mol ⁻¹	$\Delta_{\text{sub}}H_T^\circ$, kJ mol ⁻¹	$\Delta_{\text{sub}}S_T^\circ$, J deg ⁻¹ mol ⁻¹	$\Delta_{\text{vap}}H$, kJ mol ⁻¹
NAGA	408.2 ± 0.3	25.6 ± 0.4	62.7 ± 1.0	135 ± 3	256 ± 6	110
L-NAAA	431.0 ± 0.4	21.7 ± 0.2	50.3 ± 0.5	115 ± 3	212 ± 5	93
D-NALA	404.0 ± 0.1	20.2 ± 0.3	50.0 ± 0.7	101 ± 3	178 ± 9	81

T : mean temperature value of vapour pressure measurements.

dimension effect, in particular when an aliphatic chain is concerned [15]. A rather constant contribution of the $-\text{CH}_2-$ group to the vaporization or sublimation enthalpy and entropy changes has, indeed, been observed for alcohols [16], mono- and dicarboxylic acids [17], esters [18] and primary amides [19] with long aliphatic straight chains.

However, what we found can be understood by taking the crystal structure of the three peptido-amides into consideration [20]. In the crystal of L-NALA (a mirror image of that of D-NALA), each molecule is involved in only five shared H-bonds,

and the molecular contacts in the crystal packing are assured by a series of alternate twistings of the molecules, whereas in that of L-NAAA each molecule shares six H-bonds with the neighbour ones and the interconnected molecules lie in compact layers. Finally, in the NAGA crystal, each molecule again shares six H-bonds with its nearest neighbours, but they are assembled in a tridimensional pattern. These structural factors probably overcome the molecular dimension effect in determining the sublimation enthalpies and entropies.

Furthermore, we can observe that enthalpies of fusion (Table 3) show a parallel decrease and that the average ratio between $\Delta_{\text{sub}}H$ and $\Delta_{\text{fus}}H$ is 5.2 ± 0.2 . Thus, the same structural factors can account for the decrease of both sublimation and fusion enthalpies in the series NAGA, L-NAAA and D-NALA. Entropies of fusion, ranging from 50 to 62 J deg⁻¹ mol⁻¹, are in agreement with Walden's rule, which suggests that the enthalpy of fusion divided by the melting point is equal to ca. 55 J deg⁻¹ mol⁻¹ [21].

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Zusammenfassung — Auf Grund der Abhängigkeit des Dampfdruckes von der Temperatur, gemessen mit Hilfe eines Torsions-Effusions-Verfahrens, konnten die Sublimierungsenthalpien und -entropien von N-Azetylglycinamid (NAGA), N-Azetyl-L-alaninamid (L-NAAA) und N-Azetyl-D-leucinamid (D-NALA) bestimmt werden. Schmelztemperatur und Schmelzwärme wurden mit einem Differential Scanning Kalorimeter gemessen und die Schmelzentropie errechnet. Es konnten keine fest- fest Phasenübergänge festgestellt werden. Zur Abschätzung der Verdampfungsenthalpie der Schmelze wurde eine Kombination von Schmelz- und Sublimierungsenthalpie verwendet. Die experimentellen Werte sinken umgekehrt zur molaren Masse. Zur Erklärung dieser Tendenz wird ein auf der Kristallstruktur beruhendes Modell empfohlen.

Резюме — Исходя из измеренной температурной зависимости давления паров амидов N-ацетилглицина, N-ацетил-L-аланина и N-ацетил-D-лейцина, найдены энтальпии и энтропии из сублимаций. С помощью дифференциальной сканирующей калориметрии были определены энтальпии и температуры плавления, а также установлены энтропии плавления. При этом не было обнаружено твердотельных переходов. Энтальпии сублимации и плавления были объединены с целью определения энтальпий испарения расплавов. Экспериментальные величины уменьшались обратно пропорционально молекулярному весу. Исходя из кристаллической структуры этих соединений, предложена интерпретация этого явления.