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AQUEOUS SOLUTIONS CONTAINING AMINO ACIDS AND PEPTIDES. PART 21. THE ENTHALPIC COEFFICIENTS AT 298.15 K FOR THE INTERACTION OF *N*-ACETYL-L-PROLINAMIDE WITH SOME 2-(*N*-ACETYLAMINO)ACYL AMIDES

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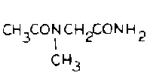
ABSTRACT

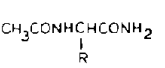
Enthalpies of dilution of *N*-acetyl-L-prolinamide and equimolal solutions of this with *N*-acetylglycinamide, *N*-acetyl-L-alaninamide, *N*-acetyl-L-valinamide and *N*-acetyl-L-leucinamide have been determined at 298.15 K using a microcalorimetric procedure. The results obtained were used to calculate the pairwise enthalpic virial coefficients for both like–like (homotactic) and like–unlike (heterotactic) solute interactions. It is shown that the data can be predicted rather well using a group additivity approach with parameters obtained from earlier studies on α-amino and α-imino acid derivatives.

INTRODUCTION

In some recent papers [1–8] from this laboratory we have presented the results of investigations into the interactions occurring in aqueous solutions between terminally substituted amino acids and peptides. The reasons for studying such systems have been outlined previously [1–3]. Most of the work presented has been concerned with solutions of the *N*-acetyl amides, *N*-acetyl-*N'*-methylamides and *N*-acetyl peptide amides of the α-amino acids glycine, L-alanine, L-valine, L-leucine and L-phenylalanine. However, in a recent study [8] we explored the consequences of incorporating a tertiary amide bond into a monomeric peptide molecule by examining the enthalpic behaviour of the terminally protected simplest α-imino acid sarcosine. Results were obtained for the homotactic (i.e., like solute–like solute) interactions of *N*-acetylsarcosinamide (SAR) (**I**) and for the heterotactic (i.e., like solute–unlike solute) interactions of SAR with *N*-acetylglycinamide (GLY) (**IIa**), *N*-acetyl-L-alaninamide (ALA) (**IIb**), *N*-acetyl-L-valinamide (VAL) (**IIc**)

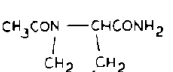


(I)



(II)

II a R = –H
II b R = –CH_3
II c R = –$CH(CH_3)_2$
II d R = –$CH_2CH(CH_3)_2$



(III)

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and *N*-acetyl-L-leucinamide (LEU) (**IId**). It was shown that the information obtained could be rationalised using a group additivity approach and values for the enthalpic parameters related to the various group interactions were estimated.

The reason we studied the sarcosyl derivative was because of its relationship with the biologically important α-imino acid residue proline. Proline is similar to sarcosine in that both contain a tertiary amide bond but they differ in that the geometry of proline is more limited because of the constraints induced by the presence of its five-membered pyrrolidine ring.

In this paper we present a study of the enthalpy of dilution of *N*-acetyl-L-prolinamide (PRO) (**III**) and of the enthalpies of dilution of equimolal solutions of PRO with GLY, ALA, VAL and LEU. The results obtained are compared with those given earlier [8] and the effects of the pyrrolidine ring on intermolecular interactions are briefly discussed.

EXPERIMENTAL

Apparatus and methods

The microcalorimeter used and its ancillary equipment have been described previously [9]. ^{1}H NMR spectra were recorded using a Jeol 220 MHz instrument at ambient temperature with TMS as internal reference.

Preparation and purification of materials

N-Phenylmethoxycarbonyl-L-proline

L-Proline (0.5 mol) was dissolved in aqueous sodium hydroxide (2 M, 250 ml) and the solution cooled below 5°C. Phenylmethylchloroformate (85 ml, 0.6 mol) and aqueous sodium hydroxide (2 M, 250 ml) were added synchronously from separate dropping funnels over a period of 30 min with vigorous mechanical stirring. The cold mixture was stirred for 2 h and then allowed to warm to room temperature. The resulting viscous liquid was washed twice with diethyl ether (250 ml). The aqueous phase was poured onto ice in a beaker and rapidly acidified to pH 1–2 with aqueous hydrochloric acid (6 M), with external cooling. The oily product was rapidly extracted with ethyl acetate (3 × 250 ml). The combined organic extract was washed with distilled water and with saturated aqueous sodium chloride, dried over anhydrous $MgSO_4$, filtered and evaporated in vacuo. The resulting oil crystallised after standing for a protracted period over P_2O_5 in vacuo: yield 86%; m.p. 81–2°C; (lit. [10] m.p. 78–80°C); R_f 0.76 (*n*-butanol/acetic acid/water, 4 : 1 : 1). $\delta(CDCl_3)$ 11.50 (1H, s, COOH), 7.3–7.16 (5H, m, C_6H_5), 5.18–5.04 (2H, m, $ArCH_2$), 4.49–4.32 (1H, m, α-CH), 2.32–1.25 (4H, m, β-, γ-CH_2), 3.69–3.48 (2H, m, δCH_2).

N-*Phenylmethoxycarbonyl-L-prolinamide*

N-Phenylmethoxycarbonyl-L-proline (0.18 mol) was dissolved in dry tetrahydrofuran (200 ml) and cooled to −15°C. *N*-Ethylmorpholine (22.8 ml, 0.18 mol) was added, followed by 2-methylpropyl chloroformate (23.6 ml, 0.18 mol) and stirring continued for 5 min at −15°C. Aqueous ammonia solution (0.88 S.G., 225 ml, 0.36 mol) was then added cautiously. The mixture was stirred for 20 min at −15°C and then allowed to warm to ambient temperature.

Solvent was evaporated under reduced pressure at an external temperature of less than 30°C. The oily residue was partitioned between ethyl acetate (2 × 300 ml) and water (150 ml) and the combined organic extracts washed till neutral with 10% (w/v) aqueous citric acid, then with 10% (w/v) aqueous sodium hydrogen carbonate, finally, with two portions of saturated aqueous sodium chloride and then dried over anhydrous $MgSO_4$. After filtration, the solvent was removed in vacuo and the residual oily solid crystallised from ethanol/petrol. The resulting colourless, crystalline solid was recrystallised from the same solvent mixture to give the product: yield 81%; m.p. 92–93°C; R_f 0.37 (methanol/chloroform, 1 : 9). Found: C, 62.9; H, 6.45; N, 11.45. Calc. for $C_{13}H_{16}N_2O_3$: C, 62.9; H, 6.5; N, 11.3%. $\delta(CDCl_3)$ 7.32 (5H, s, C_6H_5), 6.18–5.83 (2H, m, NH_2), 5.13 (2H, s, $ArCH_2$), 4.31 (1H, m, α-CH), 2.36–1.81 (4H, m, β-, γ-CH_2), 3.59–3.34 (2H, m, δ-CH_2).

N-*Acetyl-L-prolinamide*

N-Phenylmethoxycarbonyl-L-prolinamide (0.1 mol) was dissolved in aqueous acetic acid (80% v/v, 250 ml) and shaken overnight with hydrogen (1 atm) in the presence of 5% palladium–charcoal catalyst [11] (0.5 g). After hydrogen uptake was adjudged to be complete, the solution was filtered, cooled to −15°C, and added with stirring to a precooled mixture (−15°C) of acetic anhydride (10.4 ml, 0.11 mol) and pyridine (100 ml). After further stirring (15 min) at −15°C, solvent was evaporated under reduced pressure at minimal temperature. The resulting oil was lyophillised with toluene and then with ethyl acetate until crystallisation was induced. The colourless crystalline product was recrystallised from ethanol/ether to constant m.p.: yield 93%; m.p. 143–144°C, (lit. [12] m.p. 146–7°C); R_f 0.43 (chloroform/methanol/acetic acid/water, 60 : 18 : 2 : 3). Found: C, 54.05; H, 7.9; N, 18.1. Calc. for $C_7H_{12}N_2O_2$: C, 53.85; H, 7.75; N, 17.95%. $[\alpha]_D^{23}$81.0 (cl, EtOH). $\delta(CDCl_3)$ 7.13 (0.84H, s, $-NH_2$), 6.76 (0.16H, s, $-NH_2$), 6.49 (0.16 H, s, $-NH_2$), 5.97 (0.84H, s, $-NH_2$), 4.57 (0.84H, m, αCH), 4.29 (0.16H, m, α-CH), 3.66–3.56 (1H, m, δ-CH), 3.52–3.38 (1H, m, δ-CH), 2.54–1.81 (4H, m, β-CH_2, γ-CH_2), 2.09 (2.52H, s, CH_3CO), 2.05 (0.48H, s, CH_3CO).

N-Acetyl-L-valinamide has been previously synthesised [1]. The syntheses currently used for *N*-acetylglycinamide, *N*-acetyl-L-alaninamide and *N*-acetyl-L-leucinamide are described elsewhere [5]. Analytically pure, crystalline materials were thoroughly dried in vacuo over P_2O_5 prior to use. All

TABLE 1

Experimental enthalpies of dilution at 298.15 K

m (mol kg^{-1})	$10^3\,n$ (mol)	m' (mol kg^{-1})	$-q$ (J)	$10^4\,\Delta$ [a] (J)
N-Acetyl-L-prolinamide (PRO)				
0.9998	1.7812	0.3142	0.7383	+41
0.9998	1.0715	0.2109	0.5148	−82
0.6003	1.0746	0.2720	0.2202	+17
0.6008	1.0718	0.1863	0.2784	+17
0.6008	2.2399	0.3896	0.2990	+14
0.6008	0.6321	0.1228	0.1902	+8
0.3030	0.6536	0.1542	0.0623	−1
0.3030	0.9003	0.2041	0.0569	−10
0.3030	0.4645	0.1012	0.0603	−20
0.3030	0.3666	0.0704	0.0549	−6
PRO + *N*-acetylglycinamide				
y_A [b] = 0.4936				
0.7990	1.3889	0.3730	0.1131	−18
0.7990	1.5242	0.2601	0.1592	+24
0.7990	3.0309	0.5280	0.1539	−1
0.7990	0.8165	0.1666	0.1013	−12
y_A = 0.4980				
0.3515	0.4897	0.1101	0.0246	−9
0.3515	0.4937	0.1723	0.0183	+2
0.3515	0.2442	0.0675	0.0145	−7
0.3515	0.9924	0.2262	0.0255	0
PRO + *N*-acetyl-L-alaninamide				
y_A = 0.4961				
0.7995	1.0577	0.3724	0.1907	+67
0.7995	2.9947	0.5184	0.3656	+22
0.7995	1.5857	0.2650	0.3736	−32
0.7995	0.6536	0.1468	0.1887	−23
y_A = 0.4979				
0.4015	0.6801	0.1936	0.0628	−10
0.4015	0.5283	0.1280	0.0664	−33
0.4015	1.0837	0.2740	0.0623	−19
0.4015	0.4087	0.0830	0.0593	−24
PRO + *N*-acetyl-L-valinamide				
y_A = 0.5016				
0.2883	0.5779	0.0945	0.1028	+2
0.2883	0.5336	0.1833	0.0512	+4
0.2883	0.5702	0.1386	0.0792	−7
0.2883	0.2524	0.0509	0.0551	0
0.2883	0.5548	0.1123	0.0899	−1
0.2883	0.1861	0.0396	0.0423	+3

TABLE 1 (continued)

m (mol kg^{-1})	$10^3 n$ (mol)	m' (mol kg^{-1})	$-q$ (J)	$10^4 \Delta$ [a] (J)
PRO + *N*-acetyl-L-leucinamide				
$y_A = 0.5000$				
0.7900	1.4528	0.3890	0.6913	+5
0.7900	2.7933	0.5076	0.9311	+23
0.7900	1.4504	0.2432	0.9501	−44
0.7900	0.6624	0.1371	0.5171	+3
$y_A = 0.5018$				
0.3997	0.7750	0.1969	0.1849	+49
0.3997	0.5670	0.1365	0.1816	−10
0.3997	1.1034	0.2577	0.1856	+32
0.3997	0.3790	0.0765	0.1476	+9

[a] The difference between observed and calculated enthalpies.
[b] The solute mole fraction of PRO.

solutions were prepared using glass-distilled water which was subsequently deionised.

RESULTS

The general thermodynamic procedures used for the treatment of enthalpy of dilution data have been given elsewhere [1,3]. Briefly, the excess enthalpy (H^{ex}) of a solution containing 1 kg of solvent and two non-electrolytic solutes A and B may be represented as a virial expansion in molalities (m_A and m_B) of the solutes

$$H^{ex} = \left(h_{AA}m_A^2 + 2h_{AB}m_A m_B + h_{BB}m_B^2\right) + \left(h_{AAA}m_B^3 + 3h_{AAB}m_A^2 m_B + 3h_{ABB}m_A m_B^2 + h_{BBB}m_B^3\right) + \ldots \quad (1)$$

in which h_{ijk} is the enthalpic virial coefficient representing interactions of the solvated subscripted species. Equation (1) may be recast to a general form which is useful for solutions containing a single solute and for solutions containing two solutes at fixed solute mole fraction ($y_A = 1 - y_B = m_A/m$)

$$H^{ex} = h_2 m^2 + h_3 m^3 \ldots \quad (2)$$

where

m (the osmolality) $= m_A + m_B$

and

$$h_2 = h_{AA}y_A^2 + 2h_{AB}y_A y_B + h_{BB}y_B^2$$

$$h_3 = h_{AAA}y_A^3 + 3h_{AAB}y_A^2 y_B + 3h_{ABB}y_A y_B^2 + h_{BBB}y_B^3 \quad (3)$$

TABLE 2

The coefficients of eqn. (4) for the systems studied

Solutes		h_2 (J kg mol^{-2}) [a]	h_3 (J kg^2 mol^{-3}) [a]
A	B		
PRO	PRO	659.7 (28.4)	−41.9 (23.7)
PRO	GLY	219.1 (16.9)	−23.9 (14.6)
PRO	ALA	437.0 (5.9)	–
PRO	VAL	919.8 (5.0)	–
PRO	LEU	1229.1 (35.8)	−35.5 (31.7)

[a] The parenthetical terms are the 95% confidence limits of the coefficients.

The experimental enthalpy change (q) for a given dilution is

$$q = n(m' - m)[h_2 + h_3(m' + m) + \ldots] \quad (4)$$

with n being the total number of moles of solute and m' and m being the osmolalities after and before dilution.

Table 1 contains the primary experimental data for the systems studied and the coefficients of eqn. (4) are given in Table 2. These were obtained by a least-squares regression routine.

DISCUSSION

The homotactic virial coefficient for PRO is given in Table 3 along with the heterotactic coefficients for the interaction of PRO with GLY, ALA, VAL and LEU. In deriving these heterotactic coefficients the results in Table 2 were used along with the homotactic coefficients which were obtained earlier [1,3]. It is noteworthy that all of the virial coefficients are positive which is indicative of the interactions having major contributions from the hydrophobic regions of the solutes.

TABLE 3

Pairwise enthalpic virial coefficients for aqueous systems containing *N*-acetyl-L-prolinamide

Solute species		h_{AB} (J kg mol^{-2}) [a]	
A	B	Observed	Calculated
PRO	PRO	660 (28)	588
PRO	GLY	226 (52)	260
PRO	ALA	412 (34)	494
PRO	VAL	882 (46)	962
PRO	LEU	1273 (133)	1196

[a] The parenthetical term is the 95% confidence limit.

In our earlier papers on terminally blocked amino acids and small peptides we have established that a group additivity [13] approach can be used to some effect. The basic ideas behind and the assumptions of this approach have been discussed elsewhere [1,3,13–20] and need not be expanded here. As far as simple amino acid and peptide systems are concerned, we have shown [1–4] that for systems containing primary and secondary amide functions the enthalpic virial coefficients are represented quite well by the expression

$$h_{AB} = n^{A}_{CH_2} n^{B}_{CH_2} H_{CH_2-CH_2} + \left(n^{A}_{CH_2} n^{B}_{Pep} + n^{B}_{CH_2} n^{A}_{Pep}\right) H_{CH_2-Pep} + n^{A}_{Pep} n^{A}_{Pep} H_{Pep-Pep} \quad (5)$$

in which $n^{A}_{CH_2}$ and $n^{B}_{CH_2}$ are the numbers of equivalent CH_2 groups on the solutes A and B and n^{A}_{Pep}, n^{B}_{Pep} are the corresponding number of peptide (i.e., secondary and primary amide) groups on the solutes. In eqn. (5) H_{ij} is the enthalpic parameter representing the interaction of the subscripted i and j groups. When solutes containing the tertiary amide (iPep) function were considered [8] it was found necessary to introduce new parameters to represent its interactions. Three such parameters were needed, viz. $H_{CH-iPep}$, $H_{Pep-iPep}$ and $H_{iPep-iPep}$. The extended form of eqn. (5) including these is

$$h_{AB} = n^{A}_{CH_2} n^{B}_{CH_2} H_{CH_2-CH_2} + \left(n^{A}_{CH_2} n^{B}_{Pep} + n^{B}_{CH_2} n^{A}_{Pep}\right) H_{CH_2-Pep} + n^{A}_{Pep} n^{B}_{Pep} H_{Pep-Pep} + \left(n^{A}_{CH_2} n^{B}_{iPep} + n^{B}_{CH_2} n^{A}_{iPep}\right) H_{CH_2-iPep} + \left(n^{A}_{Pep} n^{B}_{iPep} + n^{B}_{Pep} n^{A}_{iPep}\right) H_{Pep-iPep} + n_{iPep} n_{iPep} H_{iPep-iPep} \quad (6)$$

The H_{ij} parameters in eqn. (6) were derived from information on the sarcosyl derivative using the earlier parameters on solutes containing only primary and/or secondary amidic functions. If we assume that these coefficients are completely transferable to prolyl-containing systems, i.e., we neglect any extra contributions from the presence of the restrained pyrrolidine ring such as *cis–trans* isomerism, then eqn. (6) becomes, for the homotactic PRO interaction

$$h_{PRO-PRO} = 25H_{CH_2-CH_2} + 10H_{CH_2-Pep} + H_{Pep-Pep} + 10H_{CH_2-iPep} + 2H_{Pep-iPep} + H_{iPep-iPep} \quad (7)$$

The corresponding expression for the heterotactic interaction of PRO with the other compounds studied is

$$h_{PRO-B} = \left(5H_{CH_2-CH_2} + H_{CH_2-Pep} + H_{CH_2-iPep}\right)n + \left(7.5\, H_{CH_2-CH_2} + 2H_{Pep-Pep} + 2H_{Pep-iPep} + 11.5\, H_{CH_2-Pep} + 1.5\, H_{CH_2-iPep}\right) \quad (8)$$

where now n is the number of equivalent CH_2 groups in the amino acid

residue of B. Equation (8) is linear in this number of methylene groups and the virial coefficients obtained are plotted in this way in Fig. 1. The solid line drawn in this figure was obtained using the earlier group parameters ($H_{CH_2-CH_2} = 25.0$, $H_{CH_2-Pep} = 80.5$, $H_{CH_2-iPep} = 28.5$, $H_{Pep-Pep} = -291.6$, $H_{Pep-iPep} = -273.2$ and $H_{iPep-iPep} = -289.0$. All coefficients have units of J kg mol^{-2}). We have given in the final column of Table 3 the calculated values for the virial coefficients shown in Fig. 1 and the calculated value for the homotactic virial coefficient. It is thus clear that the agreement between observed and predicted enthalpic virial coefficients is remarkably good, which seems to indicate that closure of the pyrrolidine ring has little effect on the parameters representing group interactions. This is perhaps a somewhat surprising conclusion but seems to indicate that the interactions occurring between the solutes when in water are dominated by solvation contributions rather than by direct intermolecular effects. Indeed, if the latter were the principal components it would be expected that the enthalpic virial coefficients would be negative, reflecting amide bond–amide bond interactions, and that the heterotactic coefficients would show little if any dependence on amino acid side-chain substitution.

It is our intention to investigate further α-imino acid derivatives and other thermodynamic functions both in aqueous and amidic solvent systems to see if confirmation of these preliminary conclusions can be obtained.

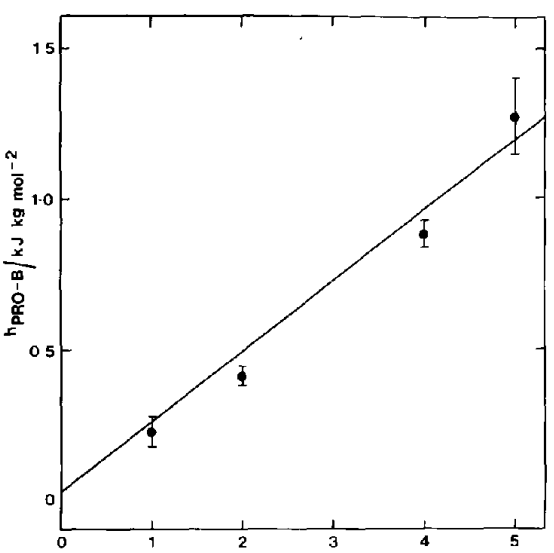

Fig. 1. The heterotactic enthalpic virial coefficients for the interaction of PRO with amino acid amides versus the number of equivalent methylene groups in the amino acid side chain (see eqn. 8). The bars on the points are the 95% confidence limits and the line is that predicted using the enthalpic group parameters (see text) obtained earlier.

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