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# Aqueous Solutions Containing Amino Acids and Peptides. Part 22 - Free Energetic and Enthalpic Virial Coefficients at 25 °C for Some Interactions of Isofunctional Amides[1]

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*The excess free energies of aqueous solutions containing N-acetyl-L-prolinamide, N-acetyl-N-methyl-L-alaninamide and binary mixtures of these have been determined at 25 °C from isopiestic measurements. The corresponding excess enthalpies for the last two systems have been obtained from enthalpy of dilution measurements. The results derived have been considered using the Savage-Wood group additivity principle, and it is shown that this works remarkably well although deviations are observed which are probably significant.*

**KEY WORDS: Amino acids; peptides; osmotic coefficients; enthalpy of dilution; excess thermodynamic properties; homotactic interactions; heterotactic interactions; group additivity; N-acetyl-L-prolinamide; N-acetyl-N-methyl-L-alaninamide.**

## 1. INTRODUCTION

In 1976, Savage and Wood in a seminal paper[(1)] addressed the problem of the interactions occurring between solutes in a novel way. They suggested that when considering such interactions, as a first order approximation, an additivity of groups approach could be used. The es-

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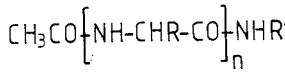
sential idea behind the approach is that if the net interactions between solutes is weak, then it can be considered to be the resultant of component interactions occurring between all groups on the sociating[2,3] molecules. If one considers only pairwise interactions between solute molecules, then the virial coefficient $x_{ij}$ representing the interactions of solutes $i$ and $j$ is given by an expression of the form

$$x_{ij} = \Sigma\, n_i^A n_j^B x_{ij}$$

where $n_i^A$ and $n_j^B$ are the numbers of defined groups of type $i$ on solute A and type $j$ on solute B, respectively, and $x_{ij}$ is a parameter representing the interaction of one group of type $i$ with one group of type $j$.

In the paper[1] in which this idea was first formulated a range of organic molecules in water was studied and the concept addressed to the interactions of these. Since that time, a considerable number of investigations on a wide range of solutes[4-19] have been presented in which the approach has been used and the idea has been applied not only to aqueous systems but also to some nonaqueous systems. It is now becoming clear, as anticipated by Savage and Wood,[1] that there are instances[7,8b,8c,9b,19] where the deviations from the additivity principle are significant and in some cases such departures from the anticipated norm have been interpreted in terms of specific chemical, including stereochemical, effects of the solutes.

We have used the additivity principle to rationalize results obtained in terminally substituted amino acids and peptides.



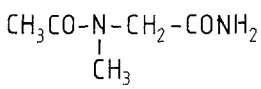
R = -H, $-CH_3$, $-CH(CH_3)_2$, $-CH_2CH(CH_3)_2$, $-CH_2C_6H_5$

R' = -H, $-CH_3$

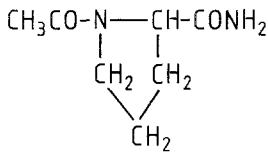
n = 1-3

For these, with one notable exception, the approach works rather well. We have also shown recently[16,18] that the principle is applicable to the heterotactic interactions of the terminally substituted $\alpha$-imino acids proline and sarcosine with $\alpha$-amino acid derivatives. (It should be mentioned that we have introduced the words homotactic to denote

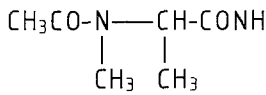
like solute-like solute interactions and heterotactic to denote like solute-unlike solute interactions.) In view of the importance of fairly small oligopeptide derivatives as pharmaceutically active compounds, there is considerable benefit to be gained in delineating the 'rules' which describe their interactions and partly with this in mind we have investigated the interactive behavior of two compounds, N-acetyl-L-prolinamide (PRO) and N-acetyl-N-methyl-l-alaninamide (MAL).



N-acetylsarcosinamide



N-acetyl-L-prolinamide



N-acetyl-N-methyl-L-alaninamide

These were chosen because using the usual[1] deconvolution of compounds into groups, both would have the same description, *i.e.*, they are what might be termed *isofunctional*. Consequently they give a useful test of the additivity principle, particularly as the prolyl residue is severely constrained by the presence of the pyrrolidine ring.

## 2. EXPERIMENTAL

### 2.1. Apparatus and Methods

The microcalorimetric method used has been described previously[9,20] as has the procedure for isopiestic investigations.[21]

### 2.2. Preparation and Purification of Materials

L-Alanine was a gift from Roche Products Ltd.

N-Phenylmethoxycarbonyl-N-methyl-L-alanine was prepared using a modification of an earlier procedure.[22] N-Phenylmethoxy-L-

alanine (22.3 g, 0.1 mol) was dissolved in dry tetrahydrofuran (THF) and the mixture cooled to 0 °C in an ice-salt bath with stirring. Methyl iodide (50 ml, 0.8 mol) was added with the subsequent, cautious addition of sodium hydride (50% dispersion, 14.4 g, 0.3 mol). The suspension was allowed to warm to ambient temperature and stirred for 24 h.

Ethyl acetate (200 ml) was added to the reaction mixture. Excess sodium hydride was quenched by the cautious, dropwise addition of water. The mixture was then evaporated *in vacuo* and the decoction partitioned between ether (200 ml) and water (500 ml). The ether extract was washed with aqueous sodium hydrogen carbonate (10% (*w*/*v*), 250 ml) and the combined aqueous extracts were acidified with cooling to pH 1-2. The product, which separated as an oil, was rapidly extracted thrice into ethyl acetate (250 ml). The organic extract was washed with distilled water, to remove excess acid, 5% aqueous sodium thiosulphate, to remove iodine, and saturated aqueous sodium chloride. The organic phase was dried over anhydrous magnesium sulphate, filtered, decocted and crystallized for a protracted period from ether-petrol; yield 21.6 g, 91%; *m.p.* 65-66 °C, $[\alpha]_D^{21}$ - 27.9 °C (*c*0.6, EtOH). [lit.[22] *m.p.* 65-66 °C; $[\alpha]_D^{25}$ - 29.2 (*c* 1, DMF)]. $R_f$ 0.74, *n*-butanol:acetic acid:water (4:1:1). δ ($CDCl_3$)[1] 10.53 (1H, *s*, COO*H*), 7.36 - 7.30 (5H, *m*, $C_6L_5$), 5.18 - 5.10 (2H, *m*, Ar$CH_2$), 4.44 - 4.31 (1H, *m*, $C^{\alpha}H$), 2.96 (3H, *s*, N$CH_3$), 1.41 (3H, *d*, *J*8, $C^{\beta}H_3$).

N-Phenylmethoxycarbonyl-N-methyl-L-alaninamide. N-Phenylmethoxy-carbonyl-N-methyl-L-alanine (21.4 g, 90 mmol) was dissolved into dry tetrahydrofuran (100 ml) and cooled to -15 °C with stirring. The addition of N-ethylmorpholine (NEM) (11.4 ml, 90 mmol) was followed by the addition of isobutylchloroformate (11.8 ml, 90 mmol) and the mixture stirred for a 5 min period prior to the addition of aqueous ammonia (0.88 S.G., 11.3 ml, 180 mmol). After stirring (20 min) at -15 °C the reaction mixture was allowed to warm to ambient temperature.

The *product* was isolated by a procedure analogous to that described[18] for N-phenylmethoxycarbonyl-L-prolinamide and recrystallized from ethyl acetate-petrol; yield 18.2 g, 85%; *m.p.* 69-70 °C, $[\alpha]_D^{20}$ -27.7 (*c*4, EtOH) $R_f$ 0.65, methanol:chloroform (1:9). Found: C, 60.75; H, 6.85; N, 12.00. $C_{12}H_{16}O_3N_2$ requires C, 61.00; H, 6.85; N, 11.85%. δ ($CDCl_3$)[1] 7.38 (5H, *s*, $C_6H_5$), 6.16 br (1H, *s*, $NH^{anti}$), 5.96 br (1H, *s*, $NH^{syn}$), 5.19 (2H, *s*, Ar$CH_2$), 4.85 (1H, *m*, $C^{\alpha}H$), 2.91 (3H, *s*, N$CH_3$), 1.39 (3H, *d*, *J*8, $C^{\beta}H_3$).

N-acetyl-N-methyl-L-alaninamide was prepared from the N-

phenylmethoxycarbonyl derivative in a manner analogous to that described[18] for N-acetyl-L-prolinamide.

N-Phenylmethoxycarbonyl-N-methyl-L-alaninamide (11.8 g, 50 mmol) was dissolved into aqueous acetic acid (80%(v/v), 100 ml) and deprotected by hydrogenolysis. Acetylation was executed using a solution of acetic anhydride (5.7 ml, 60 mmol) in pyridine (150 ml) at -15 °C.

The pure *product* was obtained by successive recrystallizations from ethanol-ether; yield 4.1 g, 57%; *m.p.* 133-134 °C, $[\alpha]_D^{21}$ - 73.5 (*c*2, MeOH). $R_f$ 0.39, (120:18:1:5) chloroform : methanol : acetic acid : water. Found: C, 50.25; H, 8.40; N, 19.75. Calculated for $C_6H_{12}O_2N_2$ C, 50.0; H, 8.4; N, 19.45%. δ ($D_2O$)[5] 4.81 (0.7H, *q*, *J*7, $C^{\alpha}H_{(trans)}$), 4.58 (0.3H, *q*, *J*7, $C^{\alpha}H_{(cis)}$), 2.89 (1.95H, *s*, $NCH_{3(trans)}$), 2.68 (1.05H, *s*, $NCH_{3(cis)}$), 2.02 (3H, *s*, $CH_3CO$), 1.33(0.9H, *d*, *J*7, $C^{\beta}H_{3(cis)}$), 1.25 (2.1H, *d*, *J*7, $C^{\beta}H_{3(trans)}$).

## 3. RESULTS

As in earlier investigations[9] the experimental data have been treated using the excess thermodynamic function concept. Using this it can be shown that the osmotic coefficient $\phi$ may be expressed as

$$\phi = 1 + (g_2 m + 2g_3 m^2 + \ldots)/RT \qquad (1)$$

where $g_2$, $g_3$, *etc.*, are coefficients representing, at least notionally, pairwise, triplet and higher order interactions and $m$ is the osmolality of the solution. For systems containing only one solute (A)

$$g_2 = g_{AA} \; ; \quad g_3 = g_{AAA} \qquad (2)$$

and for systems containing two solutes (A and B)

$$g_2 = g_{AA} y_A^2 + 2g_{AB} y_A y_B + g_{BB} y_B^2$$

$$g_3 = g_{AAA} Y_A^3 + 3g_{AAB} y_A^2 y_B + 3g_{ABB} y_A Y_B^2 + g_{BBB} y_B^3 \qquad (3)$$

In these $g_{ijk}$ is the virial coefficient of the *i*, *j*, *k* interaction and $y_A$ (= 1 - $y_B$) is the solute mole fraction of A. In the present experiments on binary solute mixtures the mixing fraction was fixed throughout the series. As in our earlier studies[9] rather than using Eq. (1) directly we

[5]The terminology used to describe nmr spectra has been given in Ref. 16.

**Table I.** Isopiestic Molalities of the Systems Investigated

| $m^a_{urea}$ | $\phi_{urea}$ | $m^a$ | $10^4\Delta^{a,b}$ |
|---|---|---|---|
| | PRO | | |
| 0.7175 | 0.9717 | 0.7228 | -31 |
| 0.7175 | 0.9717 | 0.7206 | -3 |
| 0.7175 | 0.9717 | 0.7216 | -16 |
| 0.9016 | 0.9653 | 0.9074 | -3 |
| 0.9016 | 0.9653 | 0.9065 | 6 |
| 0.9016 | 0.9653 | 0.9049 | 22 |
| 0.3393 | 0.9859 | 0.3407 | -30 |
| 0.4559 | 0.9813 | 0.4558 | 19 |
| 0.4559 | 0.9813 | 0.4554 | 28 |
| 0.4559 | 0.9813 | 0.4578 | -10 |
| 0.2881 | 0.9879 | 0.2887 | -13 |
| | MAL | | |
| 0.3879 | 0.9840 | 0.3908 | 19 |
| 0.4216 | 0.9827 | 0.4270 | -26 |
| 0.4638 | 0.9810 | 0.4696 | -17 |
| 0.5153 | 0.9791 | 0.5210 | 1 |
| 0.5688 | 0.9771 | 0.5743 | 19 |
| 0.6418 | 0.9744 | 0.6493 | 0 |
| 0.7402 | 0.9709 | 0.7484 | -3 |
| | MAL + PRO ($y_A$ = 0.5000) | | |
| 0.3879 | 0.9840 | 0.3908 | -37 |
| 0.4216 | 0.9827 | 0.4253 | -45 |
| 0.4638 | 0.9810 | 0.4680 | -43 |
| 0.5153 | 0.9791 | 0.5178 | 3 |
| 0.5688 | 0.9771 | 0.5715 | 12 |
| 0.6418 | 0.9744 | 0.6460 | 5 |
| 0.7402 | 0.9709 | 0.7454 | 14 |

[a] Units are mol-kg$^{-1}$. [b] $\Delta$ is the difference between the observed and fitted value [see Eq. (4)].

have recast it into a statistically more appropriate form for isopiestic measurements *viz.*[21]

$$[(m\phi)_{ref} - m] = [g_2 m^2 + 2g_3 m^3 + \ldots]/RT \qquad (4)$$

where $(m\phi)_{ref}$ is the product of the molality and osmotic coefficient of the reference solution used. In all of the experiments urea was used as the reference solute and osmotic coefficients of solutions of it were obtained from Ellerton and Dunlop's[23] expression.

**Table II.** Experimental Enthalpies of Dilution of the Systems Studied

| $m^a$ | $m'^a$ | $10^3 n^b$ | $-\Delta H^c$ | $\Delta^{c,d}$ |
|---|---|---|---|---|
| | | MAL | | |
| 0.3216 | 0.1587 | 0.6457 | 59.2 | -0.1 |
| 0.3216 | 0.1044 | 0.4823 | 58.5 | 0.7 |
| 0.3216 | 0.0646 | 0.3259 | 49.4 | -1.9 |
| 0.6986 | 0.2217 | 0.3231 | 339.1 | 1.1 |
| 0.6986 | 0.2708 | 1.3297 | 308.0 | -2.7 |
| 0.6986 | 0.1997 | 0.5512 | 145.3 | -3.9 |
| 0.3216 | 0.1718 | 0.5267 | 44.5 | -0.2 |
| 0.5022 | 0.0961 | 0.4898 | 111.2 | -0.6 |
| | | PRO + MAL ($y_A$ = 0.5039) | | |
| 0.4871 | 0.2344 | 0.9246 | 145.2 | -1.3 |
| 0.4871 | 0.1606 | 0.9783 | 196.5 | 0.2 |
| 0.4871 | 0.3275 | 1.3886 | 135.0 | 1.5 |
| 0.4871 | 0.0927 | 0.4593 | 109.7 | 1.9 |
| 0.4871 | 0.0628 | 0.2951 | 80.5 | -3.5 |

[a] Units: mol-kg$^{-1}$. [b] Units: mol. [c] Units: mJ. [d] $\Delta$ is the difference between the observed and fitted enthalpy change.

Table I lists the isopiestic data obtained for the three systems studied and in the final column we give the difference between the experimental dependent variable of Eq. (4) and that fitted. The coefficients obtained from the least squares fitting routine are given in Table II.

The enthalpy of dilution $\Delta H$ of a solution containing $n$ moles of solute(s) from an initial osmolality $m$ to a final osmolality $m'$ is given by [9]

$$\Delta H = n(m' - m)[h_2 + h_3(m' + m) + \dots] \qquad (5)$$

where $h_2$ and $h_3$ are the enthalpic analogs of $g_2$ and $g_3$. The links between these enthalpic terms and the enthalpic virial coefficients ($h_{AA}$, $h_{AB}$, *etc.*) are analogous to those given above for the free energetic coefficients.

In Table III we present the enthalpy of dilution data obtained and Table II includes the coefficients of Eq. (5) obtained from least-squares analysis.

**Table III.** The Coefficients of Eqs. (4) and (5)

| Solute(s) | $-g_2$ [a] | $g_3$ [b] | $h_2$ [a] | $h_3$ [b] |
|---|---|---|---|---|
| PRO | 110.8(3.9) [c] | - | - | - |
| PRO + MAL [d] | 124.4(9.4) | - | 615.9(12.1) | - |
| MAL | 170.6(20.5) | 35.5(23.2) | 586.8(42.5) | -51.8(46.7) |

[a] Units: J-kg-mol$^{-2}$. [b] Units: J-kg$^2$-mol$^{-3}$. [c] The parenthetical terms are 95% confidence limits. [d] The solute mol fraction of the first named compound was 0.5000 for the isopiestic measurements and 0.5039 during the enthalpy measurements.

**Table IV.** Homotactic and Heterotactic Free Energetic and Enthalpic Second Virial Coefficients

| | PRO | MAL | |
|---|---|---|---|
| PRO | -111(4) [b] | -108(31) | $g^a$ |
| | 660(28) [c] | 608(60) | $h^a$ |
| MAL | | -171(21) | $g^a$ |
| | | -587(43) | $h^a$ |

[a] Units: J-kg-mol$^{-2}$. [b] The parenthetical terms are 95% confidence limits. For the heterotactic interactions additivity in these limits was assumed. [c] Taken from Ref. 18.

## 4. DISCUSSION

Table IV gives the values of the homotactic and heterotactic second virial coefficients. The latter were obtained from the former, using Eq. (3) and its enthalpic analog. As mentioned in the Introduction, both of the solutes investigated are isofunctional and using the usual approximation[1] both contain 5 equivalent $CH_2$ groups, one primary amide group and one tertiary amide group. If the Savage and Wood additivity principle applied strictly the pairwise virial coefficients relating to a given thermodynamic property would be constant. It is apparent from the information given in Table IV that, although there are differences between the coefficients which are experimentally significant in some instances, generally the agreement is remarkably good and certainly within the normal range of deviations observed from group additivity correlations. The most obvious difference is that between the free energy coefficient for the homotactic interaction of

MAL and those for the other coefficients. The difference is almost certainly significant, particularly in view of the relative insensitivity of free energy coefficients. The most obvious molecular reason for the difference is the fact that the alaninamide is not conformationally restricted about the N-CH bond (the $\phi$ bond[24]) whereas the prolinamide is. Qualitatively, one imagines that the restricted rotation about the $\phi$ bond reduces the number of group interactions which are possible with a concomitant decrease in the attractive components to the free energetic coefficients for interactions involving the prolyl residue. Given the complexity of the net interactions, it is not possible *a priori* to state which of the various contributions will be perturbed, but the results obtained suggest that the group interactions which are most effected are those between amide groups. It is known[1,9,13,16,18] that both the group free energy and the enthalpy parameters for amide group-amide group interactions are large and negative and consequently, if these were 'switched off' to some extent because of conformational restriction, the free energy virial coefficient would be less negative than expected and the enthalpic coefficient would be more positive. The results obtained are consistent with this suggestion.

It should be mentioned that both of the solutes studied exhibit *cis-trans* isomerism and in principle these isomers could have different interactive properties. These would be very difficult to quantify but, using the usual nmr procedure,[25] we found that both the prolinamide and the alaninamide in water exist with about 25% of the *cis* isomer. A possible contribution to the observed interaction coefficients could come from shifts in the *cis-trans* ratio when two solutes interact but, since the energy of activation for rotation of an imino peptide bond is about 80 kJ-mol$^{-1}$, it seems very unlikely that the weak intermolecular interactions observed contain contributions from this source.

## ACKNOWLEDGMENTS

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