Optical Studies of the Soret Effect. Part III.[1] Entropies and Heat Capacities of Transfer of Norvaline, ε-Aminocaproic Acid, L-Proline, DL-Hydroxyproline, and Glycylglycine in Aqueous Solution

By M. Kennerley, H. J. V. Tyrrell, and M. Zaman

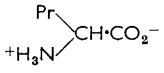
Soret coefficients for aqueous solutions of isoelectric norvaline, ε-aminocaproic acid, L-proline, DL-hydroxyproline, and glycylglycine have been measured over concentration ranges from about 0.1 molal upwards, at mean temperatures of 25 and 35°, using the optical beam-displacement method described in Part I. Entropies and heat capacities of transfer have been calculated from the results. On the basis of the qualitative model discussed in Part II, we conclude that (i) a terminal NH_3^+ group is more efficient in raising the " structural temperature " of the solvent round the solvated solute than a similar group placed elsewhere in an amino-acid molecule, and (ii) a significant increase in this effect probably occurs when a hydroxyl group is introduced into the molecule. The dipeptide glycylglycine has a higher heat capacity of transfer than has so far been observed for amino-acids themselves.

THE entropy of transfer S^* for a solute in a binary solution depends on several factors, one of which appears to be the relative size or mass of the solvent and solute molecules, and another the shape of the solute molecule.[2] The results reported in Part II [1] showed that, in aqueous solutions, structural changes induced in the surrounding solvent molecules by the diffusing entity

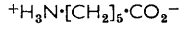
[1] Part II, H. J. V. Tyrrell and M. Zaman, *J. Chem. Soc.*, 1964, 6216.

[2] *E.g.*, H. Korsching, *Z. Naturforsch.*, 1965, **20**a, 968.

(either the solute molecule itself or the solvated solute) also have a considerable influence on S^*, and, even more clearly, on its variation with temperature. Such measurements on glycine and α- and β-alanine and their isomeric hydroxy-amides led to the conclusion that, when two isomeric solutes are compared, that with the higher S^* at 25° shows a smaller increase (and possibly a decrease) in S^* as the temperature increases. These observations are consistent with the belief that the diffusing molecules of this isomer induce a lower "structural temperature" in the surrounding solvent than do the molecules of the other isomer. Some clear indications of structural features in the molecule which might influence this "structural temperature" were obtained. For example, the presence of the α-methyl group in α-alanine increased S^*_{25} as compared with β-alanine over the whole concentration range for isoelectric solutions, while $(S^*_{35} - S^*_{25})$ was less for α- than for β-alanine except in the lower concentration range where it was almost the same. The apparently lower "structural temperature" round the diffusing α-alanine molecule may be due to "structure-building" around the α-methyl group, or to geometrical interference by this group with the interaction of the charged NH_3^+ group and the surrounding solvent molecules. The importance of this charged group in determining the magnitude of S^* for these zwitterionic solutes is shown by the observation (reported briefly in Part II) that glycine solutions to which one equivalent of base had been added, *i.e.*, sodium glycinate solutions, showed a much higher Soret coefficient at 25°, which decreased with increasing temperature, than did glycine solutions themselves. The effect of substitution of an alkyl group in the α-position has now been further studied by comparing data obtained in Part II with new measurements of S^*_{25} and S^*_{35} for two readily available substances, namely, norvaline (I) and ε-aminocaproic acid



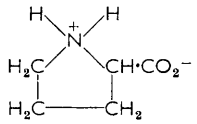
(I) $M = 117$



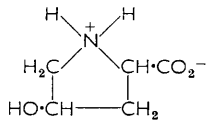
(II) $M = 131$

(II). Since, for ε-aminocaproic acid, the dielectric increment of the solutions is greater than for β-alanine solutions,[3] the charge centres are, on the average, further apart, and comparison of (II) with β-alanine and with glycine is therefore of interest. A compound which is, formally, closely related to norvaline is proline (III). In this compound the rotational freedom of the α-propyl group in norvaline has been largely removed by cyclisation, $-NH_3^+$ being at the same time changed to $>NH_2^+$. The commonly available isomer, L-proline, has therefore been studied to examine the effect of a structural change of this kind.

Agar and Price [4] showed that the conventional heat of transfer at 25° of the hydroxyethyltrimethylammonium ion, $Me_3(HO{\cdot}C_2H_4)N^+$, is less than that for the tetramethylammonium ion in spite of its higher molecular weight; this indicates that the hydroxyl group tends to raise the local structural temperature of the



(III)



(IV)

water in the vicinity of the diffusing ion. The possibility of such an effect existing in zwitterions has been examined by comparing values of S^* for L-proline solutions with those of DL-hydroxyproline (IV).

It was found in Part II that, at 25°, the entropy of transfer of glycine solutions displayed an unusual dependence on concentration. A possible analogy between this and the anomalous relationship found [5] between the frictional coefficient ξ for glycine and the macroscopic viscosity of its solutions was suggested. Since this anomaly was also observed for glycylglycine, but not for other amino-acids examined, it was desirable to see whether glycylglycine solutions displayed a similarly unusual dependence of S^*_{25} on concentration as did glycine solutions. The results of these experiments are also reported here.

EXPERIMENTAL

The techniques used for measurement of Soret coefficients, and of the refractive index parameters $(\partial n/\partial m)_T$, $(\partial n/\partial T)_m$, were described fully in Part II. The availability of a long path length (4 cm.) cell for the Rayleigh interferometer made it possible to measure $[(\partial n/\partial T)_{soln} - (\partial n/\partial T)_{solv}]$ over a narrow temperature range, and to avoid the necessity of using a Soret cell, with a 10-mm. plate separation, to measure $(\partial n/\partial T)_{soln}$ from the initial beam deflection in the median plane, which was the technique used earlier.

Norvaline (Light and Co.) was dissolved in hot water to form a saturated solution, and precipitated by adding 4 volumes of absolute alcohol and cooling in an ice bath. After three such precipitations it was dried under reduced pressure.

ε-Aminocaproic acid (B.D.H.) was dissolved in a minimum of water and decolourised with charcoal at 80°. The solution was filtered, 5 volumes of absolute alcohol were added, and the mixture was cooled to ice temperature. The crystals were filtered off and washed with cold alcohol. After 4 crystallisations from aqueous alcohol the crystals were dried at 80°.

L-Proline (Light and Co.) and DL-hydroxyproline were used directly (after drying) in the earlier experiments. Only comparatively small quantities of these solutes were available, and they were recovered after use for later experiments by evaporation to dryness under reduced pressure, dissolution in hot alcohol, and precipitation with dioxan.

Glycylglycine (Light and Co.) was purified by precipitation of the concentrated aqueous solution with alcohol three times, and drying at 80°.

[3] E. J. Cohn and J. C. Edsall, "Proteins, Amino Acids and Peptides," Reinhold, New York, 1943.

[4] C. D. Price, unpublished results, Cambridge, 1961, reported by J. N. Agar, *Adv. Electrochem. Electrochem. Eng.*, 1963, **3**, 96.

[5] H. D. Ellerton, G. Reinfelds, D. E. Mulcahy, and P. J. Dunlop, *J. Phys. Chem.*, 1964, **68**, 403.

RESULTS

Soret coefficients, σ, and entropies of transfer, S^*, for the solutions studied are summarised in Tables 1—5, together with the values of $(\partial n/\partial T)_m$ and $(\partial n/\partial m)_T$ (n = refractive index) which have been used in the calculation of these parameters. The activity coefficient terms $[1 + (\partial \ln \gamma)/(\partial \ln m)]$ at 25° were calculated as before from osmotic

TABLE 1

Soret coefficients (σ) and entropies of transfer for aqueous norvaline solutions (T_m = mean temperature of cell, τ = temperature interval across cell, a = cell height)

(a) $T_m = 25°$.

Molality (m)	$10^4(\partial n/\partial T)_m$	$(\partial n/\partial m)_T$	τ/a (deg. cm.$^{-1}$)	σ (deg.$^{-1}$)	S^* (cal. mole^{-1} deg.$^{-1}$)
0·1154	1·082	0·01986	14·13	7·03	4·21
0·2192	1·107	0·01949	14·08	6·87	4·16
0·2859	1·123	0·01925	14·13	6·80	4·14
0·3482	1·138	0·01902	14·22	6·77	4·16
0·4717	1·168	0·01860	14·04	6·61	4·09
0·5811	1·195	0·01821	14·08	6·54	4·12
		(b) $T_m = 35°$.			
0·0964	1·361	0·01970	12·89	7·02	4·34
0·1995	1·386	0·01933	12·82	6·72	4·20
0·3261	1·415	0·01889	12·31	6·55	4·15
0·4045	1·435	0·01859	12·87	6·53	4·16
0·5236	1·436	0·01817	12·45	6·53	4·22
0·6063	1·485	0·01797	12·87	6·30	4·11
0·6673	1·498	0·01770	12·48	6·34	4·17

TABLE 2

Soret coefficients (σ) and entropies of transfer for aqueous ε-aminocaproic acid solutions

(a) $T_m = 25°$.

Molality (m)	$10^4(\partial n/\partial T)_m$	$(\partial n/\partial m)_T$	τ/a (deg. cm.$^{-1}$)	σ (deg.$^{-1}$)	S^* (cal. mole^{-1} deg.$^{-1}$)
0·200	1·091	0·02293	8·26	6·84	4·00
0·500	1·143	0·02150	8·27	6·23	3·59
1·001	1·228	0·01940	8·32	5·40	3·19
1·446	1·301	0·01775	8·26	4·63	2·83
2·003	1·383	0·01590	8·33	3·72	2·37
2·495	1·453	0·01430	8·27	3·32	2·20
3·002	1·526	0·01290	8·44	2·69	1·85
		(b) $T_m = 35°$.			
0·261	1·370	0·02250	9·68	7·07	4·25
0·501	1·400	0·02139	9·54	6·78	4·04
1·014	1·462	0·01920	9·49	5·92	3·62
1·509	1·516	0·01745	9·21	5·15	3·26
1·978	1·568	0·01586	9·12	4·50	2·96
2·488	1·623	0·01418	9·09	3·88	2·65
2·875	1·664	0·01307	8·94	3·46	2·43

coefficient data derived from the following sources: (a) norvaline; isopiestic measurements of Smith and Smith;[6] (b) ε-aminocaproic acid; isopiestic measurements of Smith and Smith;[7] (c) L-proline and DL-hydroxyproline; isopiestic measurements of Smith and Smith;[7] (d) glycylglycine; isopiestic measurements of Ellerton, Reinfelds, Mulcahy, and Dunlop.[8] Figure 1 shows the thermodynamic factors for these solutes at 25°. No correction has been made for changes in the activity coefficient term at 35°; the possible effects of this neglect in the case of proline are discussed below.

[6] E. R. B. Smith and P. K. Smith, *J. Biol. Chem.*, 1937, **117**, 209.

[7] E. R. B. Smith and P. K. Smith, *J. Biol. Chem.*, 1940, **132**, 57, 133.

[8] H. D. Ellerton, G. Reinfelds, D. E. Mulcahy, and P. J. Dunlop, *J. Phys. Chem.*, 1964, **68**, 398.

TABLE 3

Soret coefficients (σ) and entropies of transfer for aqueous L-proline solutions

(a) $T_m = 25°$.

Molality (m)	$10^4(\partial n/\partial T)_m$	$(\partial n/\partial m)_T$	τ/a (deg. cm.$^{-1}$)	σ (deg.$^{-1}$)	S^* (cal. mole^{-1} deg.$^{-1}$)
0·0863	1·052	0·01928	13·55	6·42	3·84
0·1752	1·106	0·01892	13·34	6·42	3·87
0·2962	1·137	0·01842	13·49	6·38	3·89
0·4688	1·180	0·01770	13·49	6·22	3·85
0·6180	1·216	0·01710	13·55	6·16	3·87
0·8998	1·280	0·01596	13·44	6·04	3·89
1·2479	1·358	0·01474	13·58	5·87	3·91
1·6211	1·428	0·01338	13·62	5·69	3·91
2·0323	1·501	0·01200	13·58	5·68	4·02
		(b) $T_m = 35°$.			
0·0874	1·359	0·01900	12·48	6·54	4·04
0·2340	1·389	0·01844	12·41	6·35	3·97
0·428	1·430	0·01764	12·61	6·36	4·04
0·695	1·489	0·01654	12·55	6·48	4·22
0·967	1·542	0·01552	12·43	6·44	4·28
1·335	1·602	0·01442	12·39	6·40	4·40
1·652	1·651	0·01312	12·45	6·39	4·49
2·147	1·715	0·01164	12·47	6·39	4·74

TABLE 4

Soret coefficients (σ) and entropies of transfer for aqueous DL-hydroxyproline solutions

(a) $T_m = 25°$.

Molality (m)	$10^4(\partial n/\partial T)_m$	$(\partial n/\partial m)_T$	τ/a (deg. cm.$^{-1}$)	σ (deg.$^{-1}$)	S^* (cal. mole^{-1} deg.$^{-1}$)
0·0839	1·074	0·02174	13·53	5·45	3·23
0·1756	1·092	0·02140	13·44	4·08	2·41
0·3242	1·124	0·02084	13·58	3·87	2·29
0·4885	1·156	0·02020	13·58	3·82	2·27
0·6724	1·188	0·01950	13·53	3·87	2·30
0·9112	1·228	0·01862	13·53	3·89	2·33
1·2357	1·276	0·01736	13·53	3·89	2·35
1·6641	1·330	0·01600	13·49	3·91	2·39
2·0413	1·370	0·01480	13·53	3·92	2·43
		(b) $T_m = 35°$.			
0·098	1·357	0·02144	12·47	3·70	2·26
0·199	1·371	0·02107	12·52	4·50	2·75
0·308	1·387	0·02070	12·52	4·31	2·64
0·492	1·414	0·02000	12·52	4·16	2·55
0·641	1·434	0·01944	12·50	4·21	2·59
0·850	1·462	0·01866	12·47	4·27	2·61
1·467	1·539	0·01650	12·43	4·39	2·75
1·852	1·583	0·01528	12·52	4·33	2·75

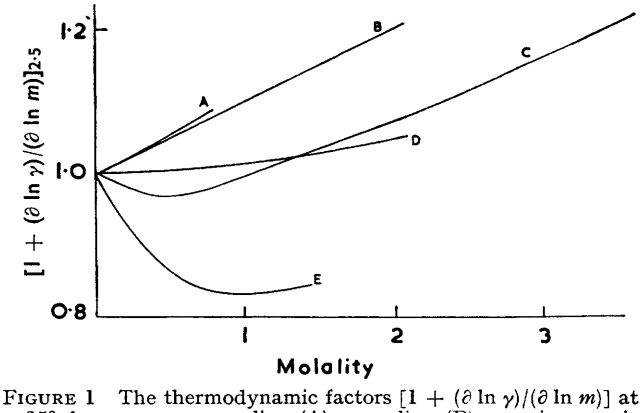


FIGURE 1 The thermodynamic factors $[1 + (\partial \ln \gamma)/(\partial \ln m)]$ at 25° for aqueous norvaline (A), L-proline (B), ε-aminocaproic acid (C), DL-hydroxyproline (D), and glycylglycine (E) solutions (sources shown in text)

TABLE 5

Soret coefficients (σ) and entropies of transfer for aqueous glycylglycine solutions

Molality (m)	$10^4\left(\frac{\partial n}{\partial T}\right)_m$	$\left(\frac{\partial n}{\partial m}\right)_T$	τ/a (deg. cm.$^{-1}$)	σ (deg.$^{-1}$)	S^* (cal. mole^{-1} deg.$^{-1}$)
		(*a*) $T_m = 25°$.			
0·102	1·090	0·02455	12·68	1·75	0·99
0·175	1·110	0·02417	13·28	1·98	1·09
0·396	1·163	0·02360	13·13	2·49	1·29
0·613	1·209	0·02210	13·00	2·72	1·36
0·772	1·245	0·02145	13·05	2·80	1·38
0·790	1·249	0·02138	13·06	2·78	1·37
0·947	1·282	0·02078	13·28	2·81	1·37
1·154	1·322	0·02005	12·67	2·96	1·46
1·175	1·325	0·01998	13·12	3·00	1·48
		(*b*) $T_m = 30°$.			
0·295	1·398	0·02336	11·74	3·60	1·97
0·507	1·432	0·02240	11·80	3·82	2·00
0·709	1·458	0·02152	12·26	3·86	1·97
0·896	1·480	0·02080	12·30	3·97	2·01
1·093	1·504	0·02011	12·26	4·00	2·02
1·308	1·534	0·01941	12·26	4·04	2·08

DISCUSSION

Norvaline is not very soluble in water, and the highest concentration which could be conveniently studied was 0·6 molal, the lowest being 0·12M. Over this range there was very little change in S^*_{25}, and the values found were higher than those obtained for ε-aminocaproic acid in spite of the higher molecular weight for this solute. S^*_{25} for the latter, however, falls quite rapidly with increasing m. There is a very clear difference in the variation of the entropies of transfer with temperature. The heat capacity of transfer C^*_{30}, defined for this purpose as $30{\cdot}3(S^*_{35} - S^*_{25})$, is very small for norvaline solutions but large for ε-aminocaproic acid solutions, even larger than that found in Part II for β-alanine solutions. Using the qualitative picture developed in Part II, it can be concluded that the diffusing entity in ε-aminocaproic acid solutions gives rise to a higher "structural temperature" in its vicinity than does the diffusing entity in β-alanine solutions. This might be expected in view of the higher polarity of the former.[3] Norvaline, on the other hand, induces a lower average "structural temperature" in the surrounding solute than does α-alanine, or any of the amino-acids with terminal polar groups which have yet been studied. This observation is consistent with the view that the alkyl group on the α carbon atom has a capacity for building up solvent molecules around it which increases with its size. This is a concept which has been developed from several lines of evidence,[9] but a possible alternative explanation for the present observations, which cannot be excluded on the evidence available, has already been mentioned. It is known that an increase in S^* with temperature is associated with the charged NH_3^+ grouping, both from the experiments [1] on sodium glycinate solutions (see above) and from the fact that S^* for trimethylglycine (betaine) solutions [10] decreases rapidly with increasing temperature. Steric hindrance, with the interaction of the NH_3^+ group and the water molecules surrounding it, should increase with the size of the α alkyl group, but the loss of rotational freedom of the hydrocarbon side-chain in going from norvaline to proline would be expected to reduce this effect. Table 3 shows the values of S^* for L-proline (M, 115). At 25°, they are less than those found for norvaline at a comparable concentration. C^*_{30} (Figure 2) is larger than for norvaline, though at the lowest concentration the difference is not great. The rapid increase in concentration with C^*_{30} for this solute is surprising and may be partly or wholly due to the fact that, of necessity,

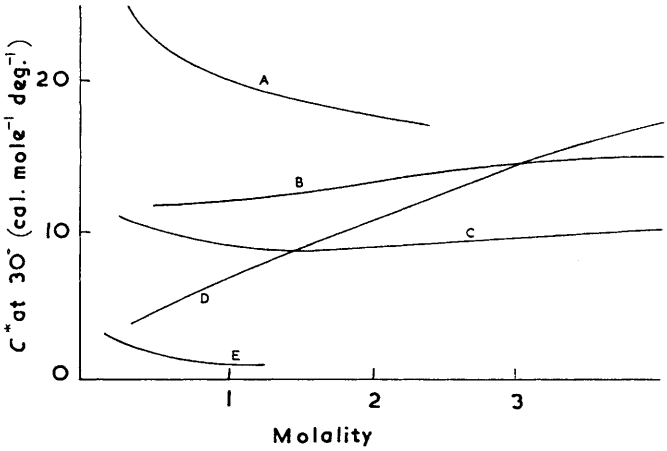


FIGURE 2 Mean heat capacities of transfer C^*_{30} (the curves have been smoothed; experimental values of C^*_{30} can be calculated if required from the tabulated data)

A, Glycylglycine; B, ε-aminocaproic acid; C, DL-hydroxyproline; D, L-proline; E, norvaline

S^*_{35} was calculated from experimental Soret coefficients using an activity coefficient term valid at 25°. Since, for proline solutions, γ increases rapidly with concentration at 25°, $[1 + (\partial \ln \gamma)/(\partial \ln m)]_{25}$ is considerably greater than unity at quite moderate concentrations. If this function decreases rapidly with temperature, that is, since,

$$\frac{\partial}{\partial T}\left(1 + \frac{\partial \ln \gamma}{\partial \ln m}\right) = -\frac{1}{RT^2}\frac{\partial \bar{L}_2}{\partial \ln m}$$

if the relative partial molar heat content $\bar{L}_2$ of the solute increases with m, then this apparent rise in C^*_{30} with concentration would be less. The values of C^*_{30} for L-proline shown in Figure 2 are most reliable at low concentrations, and, accepting these at their face value, it follows that the loss of rotational freedom of the hydrocarbon moiety in L-proline, compared with that in norvaline, leads to less interference with the interaction of the $>NH_2^+$ group and solvent molecules than was the case for the NH_3^+ group in norvaline. A possible alternative explanation of this difference between proline and norvaline is that structure-building round an alkyl side-chain is much more efficient in the presence of a terminal methyl group.

[9] *E.g.*, H. S. Frank and M. W. Evans, *J. Chem. Phys.*, 1945, **13**, 492, 507; G. Nemethy, I. Z. Steinberg, and H. Scheraga, *Biopolymers*, 1964, **1**, 43.

[10] H. J. V. Tyrrell and M. Kennerley, unpublished results.

Results in DL-hydroxyproline solutions (Table 4) show that S^*_{25} is low compared with the values found for proline solutions, a result which confirms Price's observation [4] on alkylammonium ions referred to earlier. At low concentrations, C^*_{30} for hydroxyproline is larger than for DL-proline, which appears to confirm that the hydroxyl group can itself act as a structure-breaker. As can be seen from Figure 2, at higher concentrations the heat capacity of transfer curves for the two solutes appear to cross; as explained above, this apparent discrepancy may be due to the use of a large activity coefficient term, valid at 25°, with Soret effect data at 35°.

Table 5 shows results for glycylglycine (M, 132) solutions. This dipeptide has a much lower value for S^*_{25} than ε-aminocaproic acid at all concentrations, although the molecular weights of the two solutes are similar. Also, C^*_{30} is high, comparable in fact to the values found for salt solutions. There is, however, no evidence that S^*_{25} shows a minimum at about 0·5 molal as does S^*_{25} for glycine solutions. Hence, the anomaly observed by Dunlop and his associates in the relationship between fractional coefficient and macroscopic viscosity for both glycine and glycylglycine solvents below 0·6M seems not to be related to the anomalous minimum in the S^*_{25}– concentration curve found earlier for glycine solutions.

In conclusion, it should be emphasised that the classification into structure-forming and structure-breaking solutes on the basis of S^* and C^* data will not necessarily give the same result as a similar classification on the basis of other physical properties. For example, measurements of the relative partial molar entropy of water gives the sum total of effects due to water closely bound to the solute, and to water in a possible structure-broken zone around the hydrated solute. The entropy of transfer, on the other hand, refers only to the water outside the diffusing entity, which is presumably a hydrated solute molecule. Careful comparison of different methods of classifying the effect of a solute upon solvent structure is essential and should be instructive.

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