

THE SOLUBILITY OF THE AMINO ACIDS IN WATER*

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Only a limited number of solubility data for the amino acids are given in the literature and many of the values are unreliable because precise experimental conditions were not observed or, at least, not reported. Because of the importance of solubility data, particularly for the isolation of amino acids, it is desirable to have more extensive information on this subject. The present paper deals with the solubility of twelve amino acids in water at five temperatures.

EXPERIMENTAL

Amino Acids—The *l*-aspartic acid used in these investigations was a Pfanstiehl Chemical Company product. The other amino acids were isolated from natural sources or synthesized in this laboratory. The purity of the samples was insured by repeated crystallization from water or from water and alcohol until the purified substances gave crystal-clear solutions, when dissolved in water, and theoretical amino nitrogen values, within the limits of experimental error, when analyzed by the Van Slyke procedure. The observed amino nitrogen value for glycine, 105.6 per cent, is in close agreement with the abnormality reported by other investigators. The specific rotations of *l*-aspartic acid and *l*-tyrosine agreed satisfactorily with the values in the literature (see Table I); but the figure found for *d*-glutamic acid was somewhat higher than that reported by Wood (1).

Method of Analysis—The quantity of amino acid dissolved in a solvent has been determined by carboxyl titration in the presence

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TABLE I

Specific Rotation of Amino Acids

The figures in parentheses indicate bibliographic citations.

Amino acid	Molal ratio of HCl to amino acid	[α] _D			
		Temperature	Experimental	Temperature	Literature
		°C.		°C.	
<i>l</i> -Aspartic acid.....	1.5	25.0	+24.1	25.0	+25.0 (2)
<i>d</i> -Glutamic ".....	1.55	27.0	+34.0	25.0	+30.9 (1)
<i>l</i> -Tyrosine.....	25.9	23.0	-8.25	20.0	-8.07 (3)

of formaldehyde (4-6), alcohol (7), and acetone (7); by Kjeldahl analysis for nitrogen (7-9); by specific rotation (10-12); and by gravimetric procedures (10, 13-15). In the present investigations a gravimetric method was employed because of its applicability to the active as well as the inactive amino acids. The belief that its inherent accuracy is at least as high as any of the methods listed above is confirmed by the experimental data summarized in Tables II and III.

Distilled water and an excess of the purified amino acid were placed in a stoppered, glass bottle which was protected with a rubber nipple or with DeKhotinsky cement. The latter cannot be used for temperatures much above 25°. Except at 0° the duplicate samples were heated or cooled 15° above and 15° below the temperature of the thermostat. In the determinations at 0° one bottle was cooled to about 15° and the other cooled in an ice-salt bath until ice appeared. This bottle was shaken thoroughly until the ice melted and then was placed immediately in the ice water thermostat.

Except with *dl*-alanine the bottles were rotated continuously or shaken at hourly intervals for periods of 12 to 77 hours, the time found to be necessary for the attainment of equilibrium. Because of its tendency to form a supersaturated solution 120 hours were required for reaching equilibrium with *dl*-alanine. The undissolved solid was allowed to settle by suspending the bottles for about an hour in the thermostat. Then aliquots were taken from the supernatant liquid by means of a 5, 10, or 25 cc. pipette fitted with a cotton filter and a grooved rubber stopper which was inserted in the bottle during the sampling. In order to avoid crystal-

TABLE II
Solubility of Amino Acids in Water

Amino acid	Average weight of aliquots from hot and cold sides	Average weight of solid in aliquot		Solid per 100 gm. water			
		Cold side	Hot side	Cold side	Hot side	Average	Deviation from mean
At 0.0°							
	gm.	gm.	gm.	gm.	gm.	gm.	per cent
Glycine	5.261	0.6547	0.6622	14.21	14.40	14.31	0.61
<i>dl</i> -Alanine	5.144	0.5583	0.5534	12.17	12.05	12.11	0.49
<i>dl</i> -Glutamic acid.	4.988	0.0414	0.0409	0.8369	0.8249	0.8309	0.72
<i>dl</i> -Valine	5.036	0.3281	0.3318	6.969	7.053	7.011	0.59
<i>dl</i> -Isoleucine.	4.969	0.0854	0.0855	1.749	1.751	1.750	0.06
<i>dl</i> -Phenylalanine.	4.976	0.0496	0.0498	1.007	1.011	1.009	0.19
<i>dl</i> -Norleucine.	4.976	0.0443	0.0453	0.8983	0.9187	0.9085	1.1
<i>d</i> -Glutamic acid.	9.959	0.0340	0.0345	0.3426	0.3476	0.3451	0.72
<i>dl</i> -Leucine.	4.974	0.0432	0.0437	0.8761	0.8862	0.8812	0.56
<i>dl</i> -Aspartic acid.	9.949	0.0313	0.0314	0.3156	0.3166	0.3161	0.15
<i>l</i> -Aspartic "	4.957	0.0108	0.0113	0.2184	0.2285	0.2235	2.3
<i>l</i> -Tyrosine	24.95	0.0057	0.0055	0.0229	0.0221	0.0225	1.8
At 25.0° ± 0.05°							
Glycine*				24.61	26.01	25.31	2.7
<i>dl</i> -Alanine	10.38	1.476	1.476	16.58	16.58	16.58	0.00
<i>dl</i> -Glutamic acid.	25.05	0.6458	0.6443	2.646	2.639	2.643	0.15
<i>dl</i> -Valine	25.18	1.746	1.742	7.452	7.432	7.441	0.12
<i>dl</i> -Isoleucine.	24.99	0.5340	0.5361	2.183	2.193	2.188	0.23
<i>dl</i> -Phenylalanine.	24.97	0.3487	0.3497	1.416	1.420	1.418	0.14
<i>dl</i> -Norleucine.	24.90	0.2913	0.2905	1.184	1.180	1.182	0.17
<i>d</i> -Glutamic acid.	9.972	0.0880	0.0875	0.8903	0.8852	0.8878	0.29
<i>dl</i> -Leucine.	24.90	0.2889	0.2925	1.174	1.188	1.181	0.58
<i>dl</i> -Aspartic acid.	9.964	0.0805	0.0808	0.8145	0.8175	0.8160	0.18
<i>l</i> -Aspartic "	9.947	0.0525	0.0542	0.5306	0.5479	0.5393	1.6
<i>l</i> -Tyrosine	24.95	0.0120	0.0119	0.0481	0.0477	0.0479	0.42

* The authors are indebted to Mr. T. W. Brophy who determined the solubility of glycine at 25.0°.

lization of the solute at 50° and 75° the pipette was maintained at these temperatures by suspending it in a glass tube immersed in the thermostat and the transfer of the solution from the bottle to the crystallizing dish was completed within 1.5 minutes. After

removal of a sample the pipette was rinsed, dried, and reheated to the temperature of the thermostat.

One aliquot was transferred to a glass-stoppered bottle and weighed. Three others from the same bottle were placed in glass, crystallizing dishes, each weighing about 30 gm. and of approximately 80 cc. capacity. Then these solutions were heated in an oven at 50–95° until the solvent had evaporated and the residual amino acid was constant in weight. For the most part the evaporations were made at the lower range of temperatures in order to avoid the slight decomposition observed in a few instances at the higher temperatures. Since Foreman (16) has shown that there is approximately a 3 per cent conversion of glutamic acid to pyrrolidonecarboxylic acid when a solution of this amino acid is evaporated to dryness at 81–83°, temperatures below 70° were used in our experiments to minimize this transformation.

A water thermostat was used for the temperatures at 25°, 50°, and 75° and an ice-water mixture at 0°. In working at 75° the water in the thermostat was covered with an inch layer of paraffin, which is liquid at this temperature, to diminish the rate of evaporation. The water in the 50° thermostat was maintained at a constant level by means of an automatic device. The temperatures maintained in the thermostats were constant within the following limits: 0.0° (no deviations were noted), 25.0° $\pm$ 0.05°, 50.0° $\pm$ 0.10°, and 74.96° $\pm$ 0.03°. A Beckmann thermometer and a Central Scientific Company 50° thermometer, graduated in 0.1°, were calibrated against a Bureau of Standards thermometer and used in measuring the thermostat temperatures.

DISCUSSION

The solubility data for the amino acids are given in Tables II to IV. The precision of the experimental values is indicated by the following data which were calculated from the determinations at 0°: deviation from the mean for the weight of the duplicate aliquots, maximum, 0.33 per cent; average, 0.17 per cent; deviation from the mean for the weight of solid in the triplicate aliquots (cold side) maximum, 2.4 per cent; average, 0.81 per cent; (hot side) maximum, 2.0 per cent; average, 0.64 per cent. Because of the larger quantity of amino acid in solution at the higher temperatures the mean deviations were smaller than at 0°.

TABLE III
Solubility of Amino Acids in Water

Amino acid*	Average weight of aliquots from hot and cold sides	Average weight of solid in aliquot		Solid per 100 gm. water			
		Cold side	Hot side	Cold side	Hot side	Average	Deviation from mean

At 50.0° ± 0.10°

	gm.	gm.	gm.	gm.	gm.	gm.	per cent
Glycine.....	5.535	1.583	1.588	40.06	40.23	40.15	0.20
dl-Alanine.....	5.251	0.9923	1.005	23.29	23.67	23.48	0.81
dl-Glutamic acid.....	5.075	0.3819	0.3843	8.137	8.192	8.165	0.33
dl-Valine.....	5.047	0.4321	0.4372	9.363	9.484	9.424	0.63
dl-Isoleucine.....	4.962	0.1432	0.1477	2.972	3.067	3.020	1.5
dl-Phenylalanine.....	4.966	0.1065	0.1074	2.191	2.210	2.201	0.45
dl-Norleucine.....	4.962	0.0877	0.0878	1.799	1.801	1.800	0.05
d-Glutamic acid.....	4.983	0.1083	0.1089	2.221	2.234	2.228	0.27
dl-Leucine.....	4.956	0.0857	0.0861	1.759	1.768	1.764	0.23
dl-Aspartic acid.....	4.988	0.1023	0.1027	2.094	2.102	2.098	0.19
l-Aspartic ".....	4.972	0.0614	0.0617	1.250	1.257	1.254	0.24
l-Tyrosine.....	9.918	0.0108	0.0110	0.1090	0.1110	0.1100	0.91

At 74.96° ± 0.03°

	gm.	gm.	gm.	gm.	gm.	gm.	per cent
Glycine.....	5.627	2.041	2.067	56.92	58.06	57.49	0.99
dl-Alanine.....	5.264	1.279	1.284	32.10	32.26	32.18	0.25
dl-Valine.....	10.00	1.155	1.194	13.06	13.56	13.31	1.9
dl-Isoleucine.....	4.941	0.2267	0.2283	4.809	4.844	4.827	0.35
dl-Phenylalanine.....	4.942	0.1756	0.1767	3.684	3.708	3.696	0.32
dl-Norleucine.....	4.914	0.1371	0.1381	2.870	2.891	2.881	0.34
dl-Leucine.....	4.918	0.1357	0.1359	2.838	2.842	2.840	0.14
dl-Aspartic acid.....	4.991	0.2280	0.2286	4.787	4.801	4.794	0.15
l-Aspartic ".....	4.970	0.1314	0.1313	2.715	2.713	2.714	0.04
l-Tyrosine.....	9.823	0.0234	0.0232	0.2388	0.2367	0.2378	0.46

* Since Foreman (16) has shown that there is approximately 3 per cent conversion of glutamic acid to pyrrolidonecarboxylic acid when an aqueous solution of this amino acid is heated for 2.5 hours at 81-83° it would appear that the determination of solubility of glutamic acid at 75° would not give accurate results.

The relation between solubility in gm. per 100 gm. of water and temperature in °C. is shown in Figs. 1 to 4. The experimental values at 0°, 25°, 50°, and 75°, and the calculated solubilities at

TABLE IV
Calculated Solubility of Amino Acids in Water at 100°

Amino acid	Temperature	Solubility			log N	ΔH (25° inter-vals)	ΔH (75-100°)	Solubility at 100°
	°C.	gm. per 100 gm. water	M per 100 gm. water	$\bar{n}$ (mole fraction)		calories	calories	gm.
Glycine	0	14.31	0.1908	0.03324	-1.4783			
	25	25.31	0.3375	0.05727	-1.2421	3600		
	50	40.15	0.5353	0.08790	-1.0560	3300		
	75	57.49	0.7665	0.12124	-0.9164	2900	2400	75.2
dl-Alanine	0	12.11	0.1361	0.02391	-1.6214			
	25	16.58	0.1863	0.03245	-1.4888	2000		
	50	23.48	0.2638	0.04533	-1.3436	2600		
	75	32.18	0.3616	0.06111	-1.2139	2700	2800	43.2
dl-Glutamic acid	0	0.8309	0.00565	0.00102	-2.9914			
	25	2.643	0.01798	0.00323	-2.4908	7550	(50-75°)	(At 75°)
	50	8.165	0.05135	0.00916	-2.0381	8100	8650	19.9
dl-Valine	0	7.011	0.05992	0.01067	-1.97224			
	25	7.441	0.06360	0.01132	-1.94692	381		
	50	9.424	0.08055	0.01429	-1.84466	1821		
	75	13.31	0.11376	0.02007	-1.69680	3075	4200	20.0
dl-Isoleucine	0	1.750	0.01346	0.00242	-2.6162			
	25	2.188	0.01683	0.00302	-2.5200	1450		
	50	3.020	0.02323	0.00416	-2.3809	2500		
	75	4.827	0.03713	0.00664	-2.1778	4200	6350	9.04
dl-Phenylalanine	0	1.009	0.00612	0.00110	-2.9586			
	25	1.418	0.00859	0.00154	-2.8125	2200		
	50	2.201	0.01334	0.00240	-2.6198	3450		
	75	3.696	0.02240	0.00402	-2.3958	4650	5840	6.53
dl-Norleucine	0	0.9085	0.00693	0.00125	-2.9031			
	25	1.182	0.00902	0.00162	-2.7905	1700		
	50	1.800	0.01374	0.00247	-2.6073	3250		
	75	2.881	0.02200	0.00394	-2.4045	4200	5050	4.70
d-Glutamic acid	0	0.3451	0.00235	0.00042	-3.3767			
	25	0.8878	0.00604	0.00109	-2.9626	6250	(50-75°)	(At 75°)
	50	2.228	0.01516	0.00272	-2.5654	7100	7900	5.34
dl-Leucine	0	0.8812	0.00673	0.00121	-2.9172			
	25	1.181	0.00902	0.00162	-2.7905	1900		
	50	1.764	0.01347	0.00242	-2.6162	3100		
	75	2.840	0.02168	0.00389	-2.4100	4300	5450	4.83
dl-Aspartic acid	0	0.3161	0.00238	0.000428	-3.3686			
	25	0.8160	0.00614	0.001104	-2.9570	6200		
	50	2.098	0.01577	0.002831	-2.5481	7300		
	75	4.794	0.03605	0.00645	-2.1904	7400	7460	9.94

TABLE IV—*Concluded*

Amino acid	Temperature	Solubility			log N	ΔH (25° inter- vals)	ΔH (75–100°)	Solubility at 100°
	°C.	gm. per 100 gm. water	M per 100 gm. water	x (mole fraction)		calo- ries	calories	
<i>l</i> -Aspartic acid	0	0.2235	0.00168	0.00030	–3.5229			
	25	0.5393	0.00406	0.00073	–3.1367	5800		
	50	1.254	0.00943	0.00169	–2.7721	6500		
	75	2.714	0.02041	0.00348	–2.4584	6530	6550	4.88
<i>l</i> -Tyrosine	0	0.0225	0.000124	0.000022	–4.6576			
	25	0.0479	0.000265	0.000048	–4.3188	5100		
	50	0.1100	0.000608	0.000109	–3.9626	6350		
	75	0.2378	0.001314	0.000236	–3.6271	7000	7530	0.492

100° were used in obtaining these curves. The most reliable solubility data from the literature were included in Figs. 1 to 4 for purposes of comparison. It is apparent that only a part of these data is in good agreement with our solubility values. It is to be expected from theoretical considerations that the solubilities obtained by a gravimetric process would be slightly higher than those resulting from the analytical procedures employed by the majority of the authors quoted.

Because of the experimental difficulties involved in measuring solubility at 100° the values at this temperature were calculated by means of the equation

$$\ln \frac{N_1}{N_2} = \frac{-\Delta H}{R} \left(\frac{1}{T_1} - \frac{1}{T_2} \right)$$

where N_1 and N_2 are the mole fractions of amino acid in solution at the absolute temperatures T_1 and T_2 respectively, R is 1.986, and ΔH is the differential heat of solution. ΔH , calculated over 25° intervals from 0–75°, and plotted against the corresponding temperature intervals gave curves from which the differential heat of solution at 100° was determined by extrapolation. ΔH could not be calculated over smaller temperature intervals from the available experimental data. Thus the values for ΔH are only of approximate accuracy but the error is not of serious significance since the resulting solubilities have an average difference of only

0.5 per cent for each per cent deviation in the values for ΔH . The calculated values for ΔH and for the solubility of the amino acids at 100° are given in Table IV.

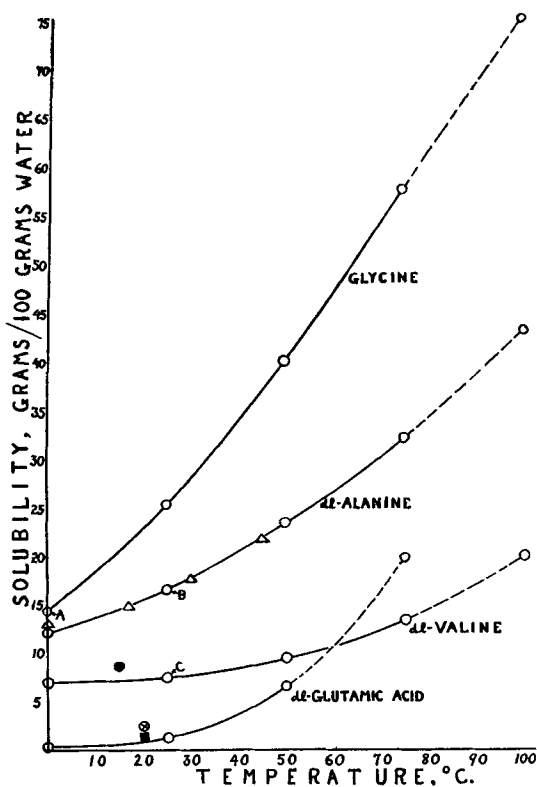


FIG. 1. Curves showing the solubility of glycine, *dl*-alanine, *dl*-valine, and *dl*-glutamic acid in water at temperatures from 0–100°. The data are plotted according to the following notation: ○, authors' experimental values at 0–75° and their calculated values at 100°; A, glycine, Shutt (6); B, *dl*-alanine, Holleman and Antusch (13); △, *dl*-alanine, Pellini and Coppola (10); ●, *dl*-valine, Slimmer (18); C, *dl*-valine, Hoppe-Seyler (19); ■, *dl*-glutamic acid, Wolff (20); and ⊕, *dl*-glutamic acid, Schulze (21).

It was of considerable interest to compare the experimental and the theoretical solubilities of the amino acids. If a nearly straight line is obtained when the logarithm of the mole fraction of the

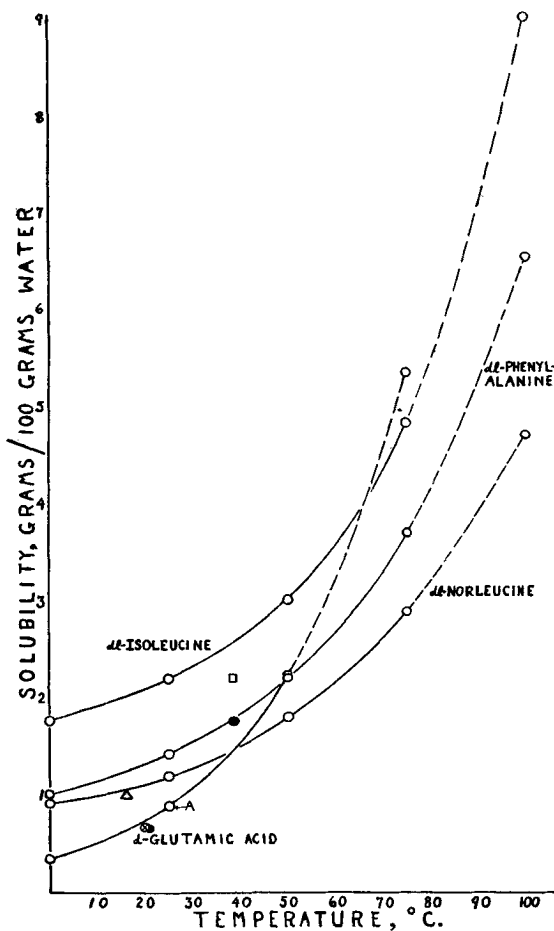


FIG. 2. Curves showing the solubility of *dl*-isoleucine, *dl*-phenylalanine, *dl*-norleucine, and *d*-glutamic acid in water at temperatures from 0–100°. The data are plotted according to the following notation: ○, authors' experimental values at 0–75° and their calculated values at 100°; □, *dl*-phenylalanine, Bayliss (22); △, *d*-glutamic acid, Fischer (23); A, *d*-glutamic acid, Pertzoff (7); ●, *d*-glutamic acid, Bayliss (22); ⊕, *d*-glutamic acid at 20°, Pfeiffer and Würzler (4) and at 21°, Pfeiffer and Angern (5). We were not able to find reliable values in the literature for *dl*-isoleucine and *dl*-norleucine.

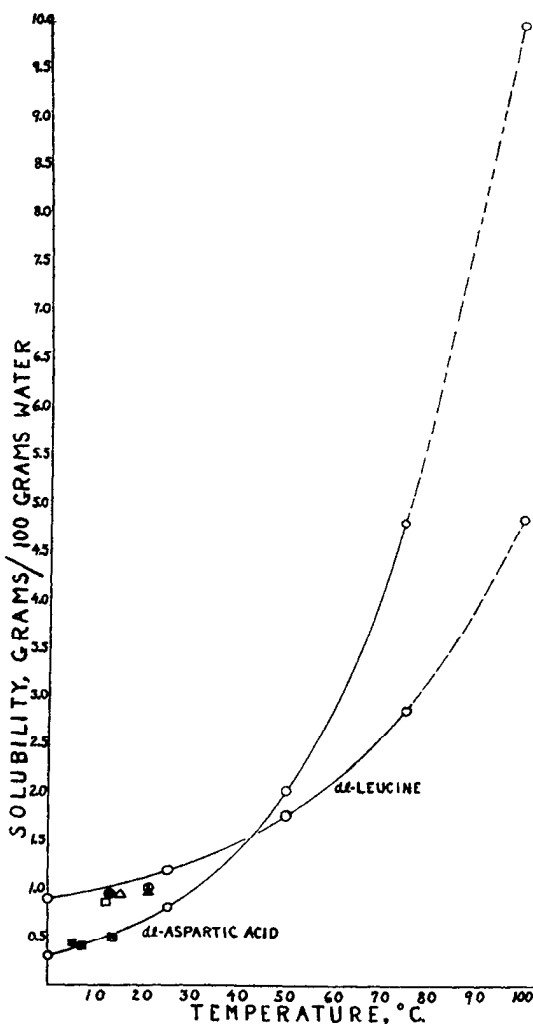


FIG. 3. Curves showing the solubility of *dl*-leucine and *dl*-aspartic acid in water at temperatures from 0–100°. The data are plotted according to the following notation: ○, authors' experimental values at 0–75°, and their calculated values at 100°; ⊕, *dl*-leucine, von Euler and Rudberg (8); ●, *dl*-leucine, Schulze and Likiernik (24); △, *dl*-leucine, Schulze and Likiernik (25); □, *dl*-leucine, Hüfner (26); ▲, *dl*-leucine, Schulze and Bosshard (27); ■, *dl*-aspartic acid, Michael and Wing (14); and ⊞, *dl*-aspartic acid, Pasteur (28).

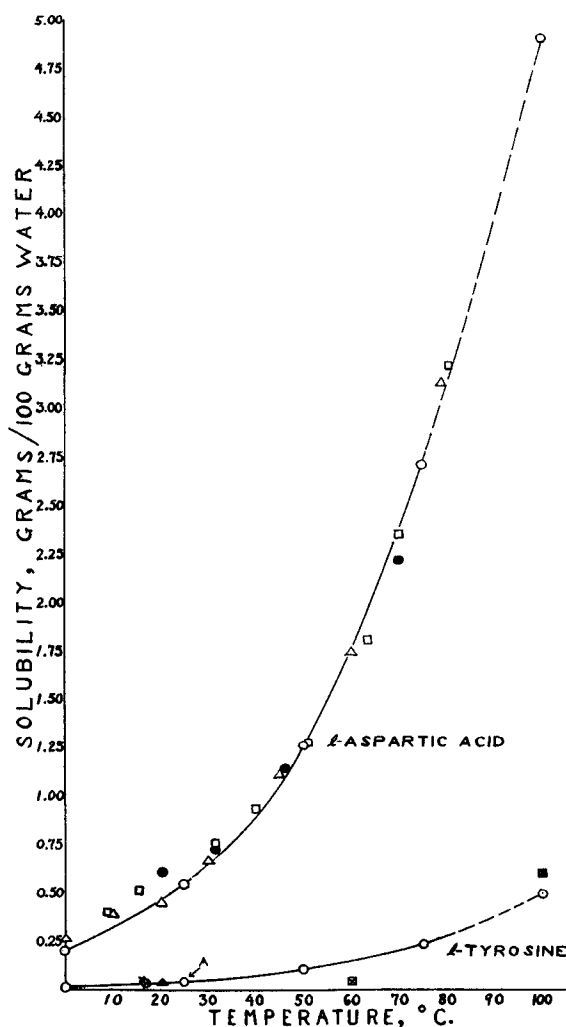


FIG. 4. Curves showing the solubility of *l*-aspartic acid and *l*-tyrosine in water at temperatures from 0–100°. The data are plotted according to the following notation: O, authors' experimental values at 0–75° and their calculated values at 100°; Δ , *l*-aspartic acid (5.380, the solubility at 100°, is not shown in the figure), Guareschi (29); $\square$, *l*-aspartic acid (0.2674, the solubility at 0.2°, and 5.3746, the solubility at 97.4°, are not shown in the figure), Bresler (11); $\bullet$, *l*-aspartic acid, Cook (12); $\boxtimes$, *l*-tyrosine, Brown and Millar (30); $\blacktriangle$, *l*-tyrosine, von Euler and Rudberg (8); Δ , *l*-tyrosine, Hitchcock (9); $\times$, *l*-tyrosine, Städelé (31); and $\otimes$, *l*-tyrosine, Erlenmeyer and Lipp (32).

amino acid in solution is plotted against the reciprocal of the absolute temperature it may be assumed that Raoult's law is obeyed. From an inspection of the curves shown in Figs. 5 to 8 it may be noted that nearly straight lines were obtained with glycine, *dl*-alanine, *d*- and *dl*-glutamic acids, and with *l*- and *dl*-aspartic acids. The curves with the other amino acids indicate deviations of considerable magnitude from the theoretical.

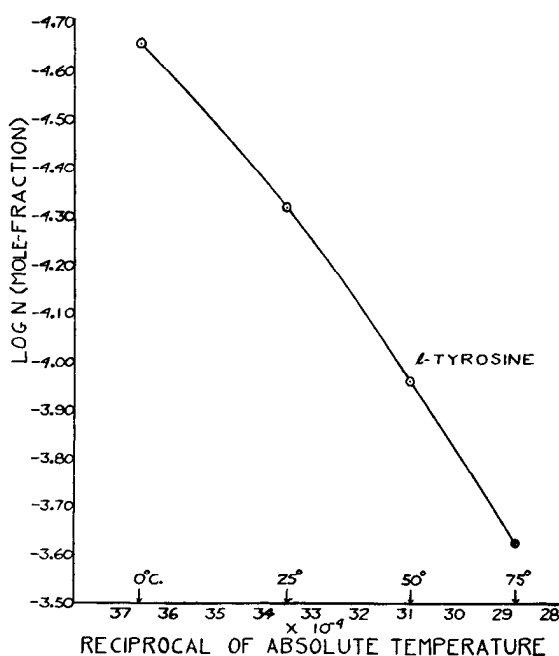


FIG. 5

In attempting to account for these deviations, normally it would be assumed for most solutes that solvation effects, heat of solution, and changes in the molecular species are the factors involved. The probability that amino acids exist in aqueous solution largely in the zwitter ion form has been strengthened by the recent studies of Wyman and McMeekin on the dielectric constants (17) of amino acid solutions and the dipole moments (33) of amino acid esters. Because of the electrical nature of the zwitter ion the

tendency to form associated molecules (micelles) is a probable inherent property of amino acids in solution. This assumption is borne out by the investigations of Hoskins, Randall, and Schmidt (34) and Cann (35). By means of conductivity and freezing point measurements of dilute aqueous solutions of aspartic and glutamic acids and the calculated activity coefficients of the undis-

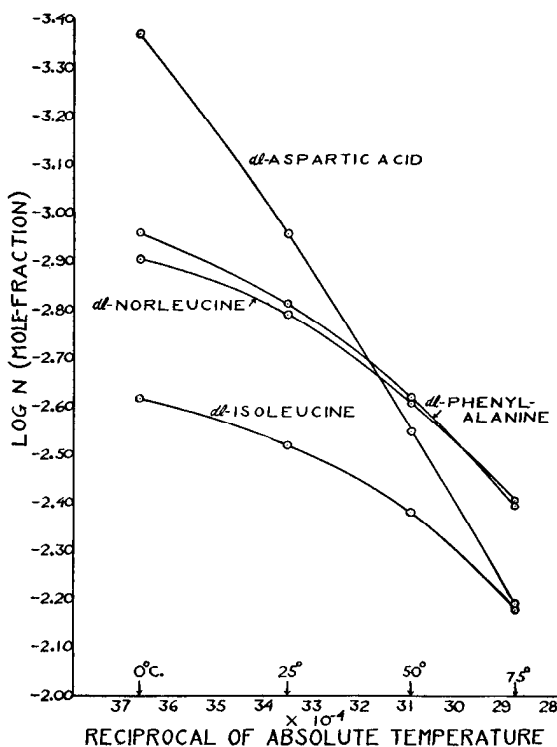


Fig. 6

sociated part of these molecules the former authors have shown that neutral aggregates (micelles) exist to a considerable extent. Furthermore, it was recognized that association increases with increasing stoichiometric concentration of amino acid and that the degree of association is markedly dependent upon temperature. Evidence of essentially the same character has been presented by Cann (35) to show the presence of micelles in glycine solutions.

If it is assumed that micellation is the principal cause of deviations from the theoretical solubilities predicted for amino acids it may be expected that those amino acids which show the greatest deviation from a straight line function will be the most highly micellated. However, direct evidence on this point cannot be presented since measurements of micellation are not subject to quantitative interpretation and the investigations of this phenomenon are at present limited to the three amino acids mentioned above.

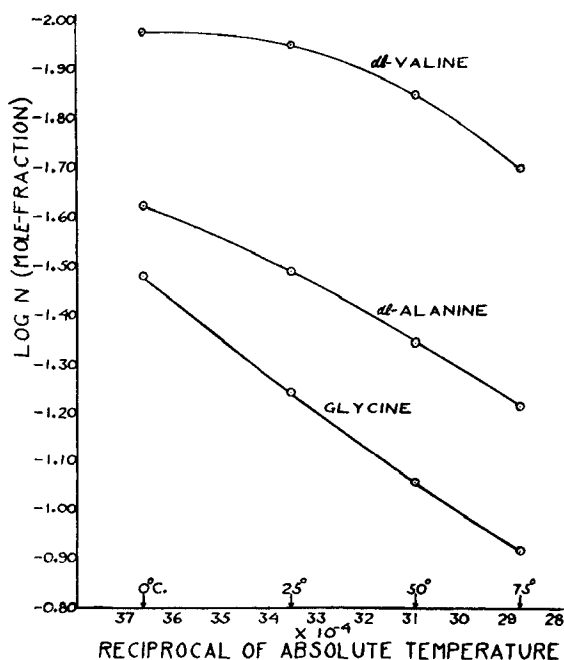


FIG. 7

In a recent paper Pertzoff (7) reported the solubility of *d*-glutamic acid at 25°. While our figure, 0.8878 gm. per 100 gm. of water, is in close agreement with his value, 0.8746 gm., our interpretation of the data differs somewhat from that of this author. Pertzoff obtained a straight line when his experimental value, the solubility at 20° found by Pfeiffer and Würzler (4), and that at 38.5° by Bayliss (22) were plotted as described above. The figure given by Pfeiffer and Angern (5) for the solubility of *d*-glutamic acid

at 21° does not fall on this line and was thus considered to be erroneous. Our results appear to verify the latter conclusion and the statement that approximately a straight line is obtained with the data for the temperatures from 20–38.5°. However, over the

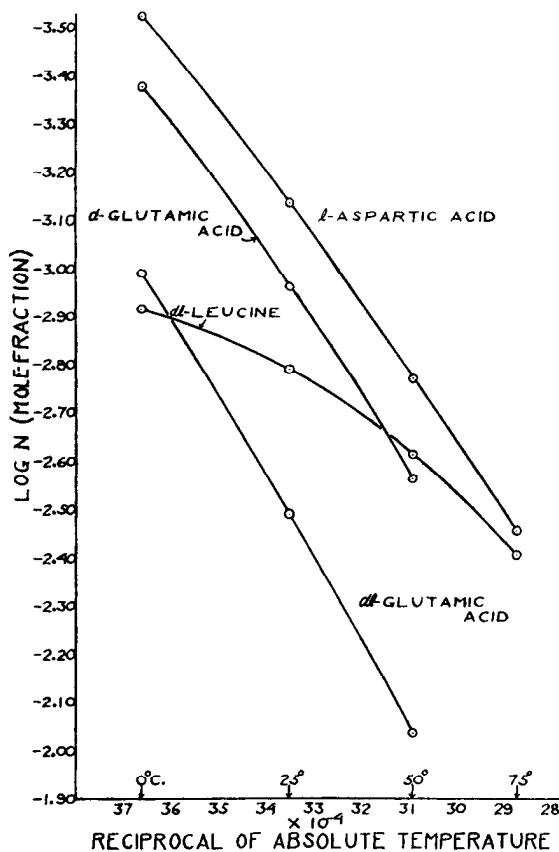


Fig. 8

temperature range from 0–100° it is apparent that there is a definite although small deviation from a straight line. The heat of solution, 9600 calories, which Pertzoff has calculated for *d*-glutamic acid appears to be somewhat high due to the use of the high solubility value reported by Bayliss at 38.5°.

SUMMARY

1. The solubility in water of three optically active amino acids and nine racemic forms has been determined at 0°, 25°, 50°, and 75°.

2. The differential heat of solution over 25° intervals from 0° to 75° and the solubility of these amino acids at 100° have been calculated.

3. Curves, showing the solubility of these amino acids in water from 0–100°, have been drawn with the authors' experimental and calculated values and the most reliable data reported in the literature by other workers.

4. The logarithms of the mole fractions were calculated from the solubility data and plotted against the reciprocals of the absolute temperatures. The six amino acids for which approximately straight lines were obtained were assumed to behave more nearly as perfect solutes than those amino acids which showed greater deviations from a straight line. Variations in the heat of solution and changes in the molecular species are known to cause solubility abnormalities. It was found that the calculated heat of solution varied widely over the range of temperatures investigated. It has been shown by other workers that certain amino acids are associated in aqueous solution. Since aspartic acid, glutamic acid, and glycine, amino acids known to be associated, exhibit less marked solubility abnormalities than other amino acids used in our experiments, it would appear (1) that amino acids of the latter class are probably more highly associated than those nearly normal in solubility and (2) that deviations from the theoretical solubility and associative phenomena are probably directly related.

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