THE SOLUBILITIES OF *l*-PROLINE AND *l*-HYDROXYPROLINE IN WATER, THE CALCULATED HEATS OF SOLUTION, AND THE PARTIAL MOLAL VOLUME OF *l*-HYDROXYPROLINE*

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The present work is a continuation of the previously reported studies on the solubilities of various amino acids in water and their thermodynamic properties (1). No precise solubility determinations on *l*-proline and *l*-hydroxyproline appear to have been carried out previously.

Due to the high solubility of *l*-proline and *l*-hydroxyproline, it was not feasible to carry out solubility determinations over as great a temperature range as in the previously reported studies. There is a tendency for these amino acids, especially hydroxyproline, to darken at higher temperatures.

It was necessary to modify somewhat the previously reported technique for the determination of solubility and partial molal volumes. A micro pipette of about 100 c.mm. capacity was used. It possessed a uniform capillary stem whose diameter was about 0.3 mm.; 1 mm. of the capillary corresponded to about 0.07 c.mm. The volume of the pipette was calibrated with the aid of mercury. The amount of amino acid solution which was contained in the pipette was estimated by measuring the distance on the stem which was not filled with the solution. For the estimation of density, the pipette was filled to a certain point on the stem and the tip of the pipette was wiped with wet cotton. The pipette was left in the balance room at 25° for 15 to 30 minutes, after which it was weighed on a Kuhlmann micro balance. The capillary stem and the tip of the pipette were so

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narrow that almost no loss of solution by evaporation was observed after standing in the balance room for a 2 hour period. The amino acid content of the solution, after thoroughly rinsing the pipette, was estimated on the basis of the nitrogen content. For this purpose the micro Kjeldahl method of Parnas and Wagner (2) was used.

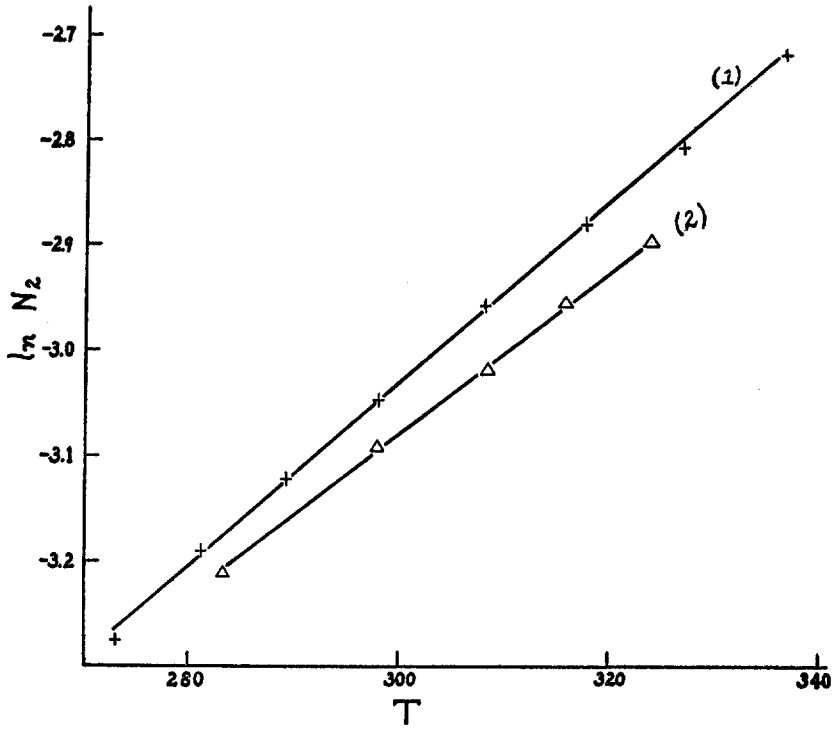

FIG. 1. Solubility of *l*-hydroxyproline (1) and *l*-proline (2). In the case of *l*-proline, the values of the ordinates are 1.5 less than the values indicated in the figure.

l-Proline was a commercial product. It was recrystallized 5 times from an alcohol-ether mixture. Both a commercial preparation of *l*-hydroxyproline and one which was prepared in this laboratory were used. They were recrystallized 3 times from 85 per cent alcohol. 14 solubility estimations of *l*-proline and 33 estimations of *l*-hydroxyproline were carried out.

The solubility-temperature relationships within the temperature

limits in which solubility estimations were carried out are represented graphically in Fig. 1. The coefficients of the equations which represent these relationships, together with the calculated values for ΔH, the heat of solution, are given in Table I. The solubility equations

TABLE I

Coefficients of Solubility Equations of l-Proline and l-Hydroxyproline*

Amino acid	a_1	$b_1 \times 10^2$	a_2	a_3	$b_3 \times 10^2$	a_4	$b_4 \times 10^2$	Maximum deviation†	Mean deviation‡	ΔH_{298} calculated§
								per cent	*per cent*	*cal.*
l-Proline....	3.1050	0.4206	1.0441	−0.2407	0.9686	−3.8586	0.7586	+2.53	±1.04	1340
l-Hydroxyproline...	2.4603	0.3891	0.3428	−1.6575	0.8959	−5.5906	0.8514	−1.53	±0.61	1506

* Solubility equations:

$$\log S = a_1 + b_1 t$$
$$\log m = a_2 + b_1 t$$
$$\ln m = a_3 + b_3 T$$
$$\ln N_2 = a_4 + b_4 T$$

† Maximum deviation of the observed from the calculated values.

‡ Calculated from the formula, mean deviation $= (\Sigma D^2/n)^{1/2}$.

§ See reference (1) for method of calculation.

TABLE II

Partial Molal Volumes of Solvent, $\bar{v}_1$, and of Solute, v_2, for Solutions of l-Hydroxyproline at 25°

m	$\bar{v}_1$	$\bar{v}_2$
2.202	18.29	80.40
2.370	18.23	81.75
2.546	18.17	83.16
2.755	18.09	84.78
3.022	18.05	85.85
3.289	17.99	86.56
3.579	17.94	87.68

are applicable only to the temperature ranges in which solubility estimations were carried out. The reported values for ΔH are perhaps more nearly correct than those which have been reported by Zittle and Schmidt (3).

Due to the tendency of *l*-proline to crystallize out of a saturated solution, it was not feasible to carry out density determinations. The partial molal volumes for *l*-hydroxyproline only are reported. These are given in Table II. On the basis of the apparent molal volumes calculated from the empirical atomic volumes given by Traube (4), the calculated molal volume of *l*-hydroxyproline is 85.2. The validity of the calculation is based on Traube's statement, "in den übrigen stickstoffhaltigen Ringen, wie Pyrrol, Pyrrolidin, Pyrazol, Trimethylenimid u.s.w. sind die Volumendekremente und Spannungen nicht gross."

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