[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

Studies in the Physical Chemistry of Amino Acids, Peptides and Related Substances. VII. A Comparison of the Solubility of Amino Acids, Peptides and their Derivatives

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I. Introduction

The solubilities of α-amino acids[1] and certain of their derivatives[2] in water and ethyl alcohol have been reported previously. Although the solubility measurements themselves suggest certain generalizations, such as that solubility in water increases with the dipole moment, and diminishes with the length of the paraffin chain, the exceptional behavior of certain molecules has been noted and associated with differences in crystal lattice energy. If the ratio of solubility in two solvents be compared, this disturbing factor is eliminated and more precise rules may be deduced. One of these states that the ratio of the solubility in alcohol to that in water is increased threefold for each CH_2 group in hydrocarbon chains terminating in methyl groups. This rule holds not only for amino acids but for other sufficiently insoluble compounds such as the formyl amino acids, hydantoins and hydantoic acids.[2] In terms of mole fraction of solute in water, N_0, and ethanol, N_A, it may be written

$$\log N_0/N_A = K_2 + 0.49\ (CH_2)_n \qquad (1)$$

where n is the number of CH_2 groups and K_2 a constant depending upon the nature of the polar groups.

A further rule is implicit in the above equation, for it suggests that changes in the logarithm of the solubility ratio are additive not only for non-polar but for polar groups. Thus the change in the ratio of solubility from water to alcohol, tentatively ascribed to dipolar ionization, has been estimated by a comparison of α-amino acids and their hydantoic acids. Subtracting the logarithm of the solubility ratio of the uncharged hydantoic acids ($N\mu_A/N\mu_0$) from that of α-amino acids (N_{zA}/N_{z0}), we obtained

$$\log f_e = \log N_{zA}/N_{z0} - \log N\mu_A/N\mu_0 = 3.85 - 1.12 = 2.73 \qquad (2)$$

This calculation neglects the influence of the amide linkage and also the movement of the proton which results in the dipolar ion structure of amino acids and peptides. The amide group and the peptide linkage, which generally decrease solubility, are further considered in the present investigation, in which the above rules are extended to both solvents and solutes of different dipole moments. On the one hand, we have extended equation (1) to a variety of solvents, including higher alcohols, acetone and a solvent more polar than water, namely, formamide. On the other, we have extended the applicability of equation (2) to amino acids and peptides of long dipole moments.

II. Methods and Materials

The same methods of determining solubility were employed as have been described previously.[1,2] The concentrations of the hydantoic acids were usually determined by direct titration, advantage being taken of their acidic properties. The concentrations of the amino acids and peptides were determined by dry weight, or by Kjeldahl nitrogen analysis. In cases where the solubilities were very small, the concentrations were determined by digestion followed by direct Nesslerization. The solubility of amino acids in formamide was determined by precipitating the amino acids with one volume of alcohol and four volumes of ether. After standing for twenty-four hours, and washing, the insoluble amino acids were weighed. Nitrogen analyses and melting point determinations on the precipitates from formamide demonstrated that they were pure amino acids.

Solvents.—Density or boiling point determinations were always used to verify the purity of the solvents employed. In all cases the appropriate blank determination was made on the solvent. The butanol was redistilled and the fraction used boiled at 117–118°. The formamide prepared by crystallization at $-5°$ was neutral in reaction, free from ammonium salts, melted from -3 to $-1°$ and had a specific conductivity of 4×10^{-5}.

Substances.—The purification of the α-amino acids and the preparation of hydantoic acid, methylhydantoic acid, formylglycine and formylaminobutyric acid have been described previously.[1,2] The same method sufficed for all the hydantoic acids, save those of peptides. In the case of peptide hydantoic acids the reaction was carried out at room temperature for two hours before acidification. For convenience in comparing amino acids with their corresponding uramido acid derivatives, the uramido acid is designated as a hydantoic acid of the amino acid. The more accurate chemical names are given in parentheses in the description of preparations.

β-Alanine Hydantoic Acid (β-Uramidopropionic Acid).—There was a slight variation in the solubility of this compound, indicating decomposition during the solubility determination, or an impure product. The preparation

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[1] Cohn, McMeekin, Edsall and Weare, THIS JOURNAL, **56**, 2270 (1934).

[2] McMeekin, Cohn and Weare, *ibid.*, **57**, 626 (1935).

TABLE I

SOLUBILITY OF AMINO ACIDS AND PEPTIDES IN DIFFERENT SOLVENTS

Solvent	Density of satd. soln., ρ	Soly. moles per liter, C	Log soly. mole fraction, N	Density of satd. soln., ρ	Soly. moles per liter, C	Log soly. mole fraction, N
	Glycine			*dl*-α-Aminocaproic acid		
Water	1.0831	2.886	−1.247	0.9991	0.0866	−2.801
Formamide	1.13306	0.0838	−2.476	1.13089	.0173	−3.161
Methanol	0.78696	.00426	−3.762	0.78730	.00854	−3.458
Ethanol	.7851	.00039	−4.638	.7851	.00104	−4.215
Butanol	.80674	.0000959	−5.055	.80674	.000336	−4.512
Acetone	.78566	.0000305	−5.648	.78566	.0000793	−5.233
	β-Alanine			ϵ-Aminocaproic acid		
Water	1.1581	6.123	−0.816	1.0895	3.848	−0.975
5% Ethanol	1.1509	5.827	− .836	1.0834	3.733	− .983
10% Ethanol	1.1416	5.620	− .848	1.0794	3.625	− .991
20% Ethanol	1.1361	5.180	− .876	1.0662	3.439	− .998
40% Ethanol	1.0520	3.531	−1.011	1.0326	2.852	−1.050
60% Ethanol	0.9596	1.687	−1.283	0.9707	1.909	−1.185
70% Ethanol	.9086	0.814	−1.561			
80% Ethanol	.8638	.242	−2.033	.8742	0.485	−1.719
90% Ethanol	.8271	.0382	−2.759	.8304	.0713	−2.487
95% Ethanol				.80848	.0139	−3.134
Ethanol	.7856	.00189	−3.955	.7851	.00194	−3.947
	Triglycine					
Water	1.0229	0.309	−2.241			
80% Ethanol	0.8554	.000608	−4.636			
90% Ethanol	.8249	.0000381	−5.672			
Ethanol	.7851	.00000106	−7.206			

was repeated a number of times, always giving a product of the same properties, and melting between 168–169°.

Anal. Calcd. for $C_4H_8O_3N_2$: N, 21.2; eq. wt., 132.07. Found: N, 21.1; eq. wt., 132.1.

α-Aminocaproic Hydantoic Acid (α-Uramidocaproic Acid).—The pure substance melted at 169–170°.

Anal. Calcd. for $C_7H_{14}O_3N_2$: N. 16.08; eq. wt., 174.1. Found: N, 16.0; eq. wt., 174.2.

ϵ-Aminocaproic Hydantoic Acid (ϵ-Uramidocaproic Acid).—The pure substance melted at 179–180°.

Anal. Calcd. for $C_7H_{14}O_3N_2$: N, 16.08; eq. wt., 174.1. Found: N, 16.1; eq. wt., 174.6.

δ-Aminovaleric Hydantoic Acid (δ-Uramidovaleric Acid).—The pure substance melted at 179–180°.

Anal. Calcd. for $C_6H_{12}O_3N_2$: N, 17.49; eq. wt., 160.1. Found: N, 17.4; eq. wt., 160.8.

Diglycine Hydantoic Acid (Carbamidoglycylglycine).—The substance melted at 194–195°.

Anal. Calcd. for $C_5H_9O_4N_3$: N, 24.0; eq. wt.. 175.09. Found: N, 23.9; eq. wt., 175.0.

Triglycine Hydantoic Acid (Carbamidodiglycylglycine). The substance melted at 204–205°.

Anal. Calcd. for $C_7H_{12}O_5N_4$: N. 24.1; eq. wt., 232.08. Found: N. 24.0; eq. wt., 231.9.

β-Alanine was prepared by a modification of the method of Mulder.[3] β-Bromopropionic acid was allowed to stand for seven days with a large excess of ammonia. The ammonia was removed and the substance purified by crystallization. After three crystallizations from alcohol–water mixtures the pure substance melted at 197–198°. Nitrogen: found, 15.5; calcd., 15.7.

ϵ-Aminocaproic acid was prepared by the method of Wallach.[4] Later samples were obtained from the University of Illinois. After three crystallizations, the substance melted at 201–202°. Nitrogen: found, 10.56; calcd., 10.69.

Triglycine was prepared by the method of E. Fischer.[5] Nitrogen: found, 22.1; calcd., 22.2.

III. Experimental Results

The solubility measurements upon amino acids and peptides are recorded in Table I and those upon their derivatives in Table II. Apparent molal volumes, calculated from the densities of many of the solutions studied, are recorded in Table III. The values previously ascribed to the CH_2, CONH, COOH and NH_2 groups[6] yield satisfactory estimates of the apparent molal volumes, Φ, of the hydantoic acids.

The observed apparent molal volumes of the amino acids and peptides are smaller than those calculated, as a result of the electrostriction of solvent molecules due to the charged groups of dipolar ions.[6]

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[3] Mulder. *Ber.*, **9**, 1903 (1876).

[4] Wallach. *Ann.*, **312**, 171 (1900).

[5] Fischer. *Ber.*, **36**, 2982 (1903).

[6] Cohn, McMeekin, Edsall and Blanchard, THIS JOURNAL, **56**, 784 (1934).

TABLE II

SOLUBILITY OF HYDANTOIC ACIDS AND FORMYL COMPOUNDS IN DIFFERENT SOLVENTS

Solvent	Density of satd. soln., ρ	Soly. moles per liter, C	Log soly. mole fraction, N	Density of satd. soln., ρ	Soly. moles per liter, C	Log soly. mole fraction, N
	Formylglycine			Formyl-α-aminobutyric acid		
Water	1.0570	1.849	−1.432	1.0043	0.256	−2.325
Methanol	0.8280	0.710	−1.533	0.8222	.646	−1.539
Ethanol	.8023	.295	−1.762	.8018	.355	−1.674
Heptanol	.82412	.0347	−2.311	.82473	.0500	−2.152
	Hydantoic acid			α-Aminocaproic hydantoic acid		
Water	1.0112	0.329	−2.217	0.99727	0.00690	−3.903
Formamide	1.15405	.837	−1.462	1.13141	.165	−2.175
Methanol	0.79178	.0797	−2.488	0.79390	.1123	−2.335
80% Ethanol				.85947	.0867	−2.472
90% Ethanol	.82887	.0710	−2.485	.82920	.0803	−2.427
Ethanol	.7865	.0242	−2.851	.78885	.0477	−2.551
Butanol	.80674	.00643	−3.228	.80739	.01786	−2.785
Acetone	.78566	.00248	−3.737	.78588	.00463	−3.466
	α-Alanine hydantoic acid (Methylhydantoic acid)			β-Alanine hydantoic acid		
Water	1.00454	0.193	−2.451	1.00287	0.158	−2.539
Ethanol	0.7877	.0440	−2.590	0.78692	.0170	−3.002
	δ-Aminovaleric hydantoic acid			ε-Aminocaproic hydantoic acid		
Water	0.99763	0.0174	−3.502	0.99725	0.00690	−3.903
80% Ethanol				.85690	.0322	−2.910
90% Ethanol				.82619	.0228	−2.979
Ethanol	.78622	.00762	−3.350	.78639	.00756	−3.354
Butanol				.80643	.00278	−3.593
	Diglycine hydantoic acid			Triglycine hydantoic acid		
Water	1.0050	0.126	−2.638	1.0011	0.0446	−3.092
80% Ethanol	0.85695	.0220	−3.075			
90% Ethanol	.82564	.00851	−3.410			
Ethanol	.7857	.00115	−4.171	0.7851	.000077	−5.345

TABLE III

Solute	CH_2 groups, cc.	CONH groups, cc.	NH_2 + COOH groups, cc.	Apparent molal volume, cc. Calcd.	Obsd.
Hydantoic acid	16.3	20.0	40.7	77.0	77.6
α-Alanine hydantoic acid	32.6	20.0	40.7	93.3	94.2
β-Alanine hydantoic acid	32.6	20.0	40.7	93.3	95.8
α-Aminocaproic hydantoic acid	81.5	20.0	40.7	142.2	146.4
ε-Aminocaproic hydantoic acid	81.5	20.0	40.7	142.2	146.4
Diglycine hydantoic acid	32.6	40.0	40.7	113.3	112.4
Triglycine hydantoic acid	48.9	60.0	40.7	149.6	149.5

The aminocaproic hydantoic acids are so insoluble in water that no great confidence can be placed on the values of Φ estimated for this solvent. The apparent molal volumes of those molecules, sufficiently soluble to be studied in more than one solvent, vary to a small extent in the different solvents employed (Table IV).

Solubility in Alcohol–Water Mixtures.—Our previous communications have been concerned largely with α-amino acids and their derivatives. Two series of molecules, in which the ammonium groups are at a greater distance from the dissociated carboxyl groups, are here reported. In the one only non-polar CH_2 groups intervene between the charged groups, in the other (peptide) series, there is an alternation of CH_2 and CONH groups. Solubility in the first series appears to increase with increase in the dipole moment. Peptides of glycine on the other hand are less soluble the larger the number of glycine residues in the chain. This would appear to depend, at least in part, on close packing in the crystal lattice since peptides containing side chains of unequal lengths are often more soluble than those of glycine.[7] The hydantoic acids of both series

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(7) Fischer, "Untersuchungen über Aminosäuren, Polypeptide und Proteine," Julius Springer, Berlin, 1906, Vol. I, p. 333.

have been prepared and are far less soluble in water than the dipolar ions from which they were derived.

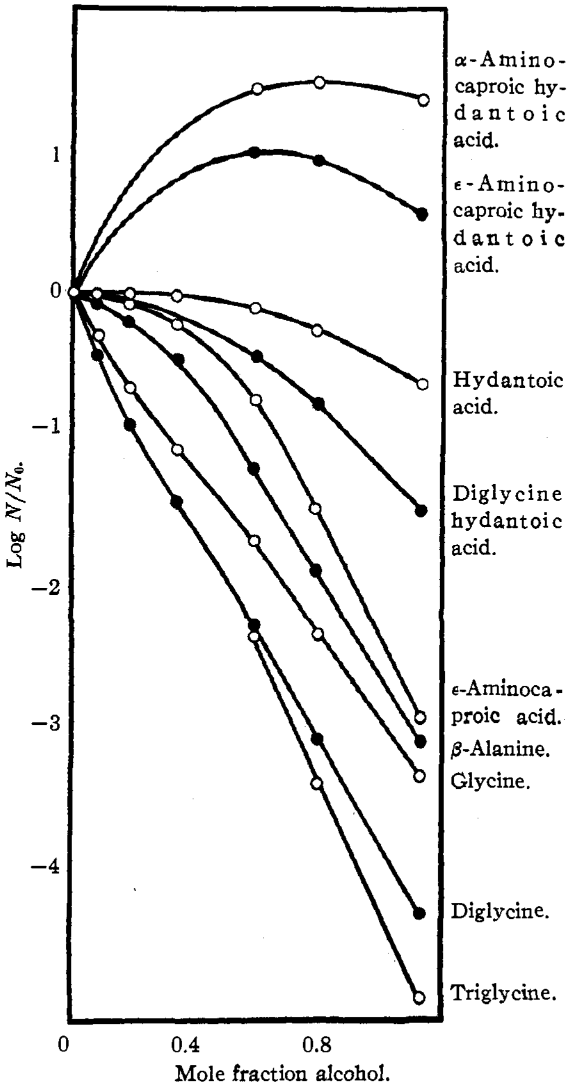

Fig. 1.

The ratio of the solubility, N, of these three classes of molecules in alcohol–water mixtures, to that in water, N_0, is plotted in Fig. 1 against mole fraction alcohol. The shapes of the glycine, diglycine and triglycine curves are very similar. All have steep segments at low mole fractions of alcohol, with comparable points of inflection in the range in which the solvent molecules are approximately equal in number. In systems containing larger amounts of ethanol the logarithm of the solubility may as a first approximation be considered to vary inversely as the mole fraction of alcohol. Straight lines have been drawn through these segments of the curves of glycine and its peptides.

TABLE IV

APPARENT MOLAL VOLUMES OF HYDANTOIC ACIDS AT 25°

Solvent	Concentration, moles per liter	Molal volume, cc.
	Hydantoic acid	
Formamide	0.837	79.8
Water	.250	77.6
Methanol	.0797	68.2
80% Ethanol	.1256	75.7
90% Ethanol	.071	76.1
Ethanol	.0242	(63.2)[a]
	α-Aminocaproic hydantoic acid	
Formamide	0.165	150.6
Water	.0069	(146.4)[a]
Methanol	.1123	139.2
80% Ethanol	.0867	144.8
90% Ethanol	.0803	146.7
Ethanol	.0477	138.5

[a] Concentration too low for accurate measurements of molal volume.

The curves describing the behavior of hydantoic acids in alcohol–water mixtures also form a family though their shape is quite different from that of the amino acids and peptides from which they were derived. The additional peptide linkage in the hydantoic acid of diglycine, as compared with hydantoic acid, is reflected in decreased solubility; and the additional CH_2 groups of α-aminocaproic hydantoic acid in increased solubility in systems rich in alcohol. The curve of the latter substance is very similar to that of formylleucine or benzamide. The isomer, ε-aminocaproic hydantoic acid, in which the CH_2 groups all lie between polar groups, behaves far more like formyl-α-aminobutyric acid, that is to say, like a molecule with two less CH_2 groups. Its solubility in 80% ethanol is approximately tenfold that in water and threefold that in ethanol, a type of behavior characteristic of a class of proteins, the prolamines.

The measurements on β-alanine and ε-aminocaproic acid cannot be considered as accurate as those upon the other substances studied, in part because their large dipole moments render difficult their purification, in part because the great solubility which results from these moments renders difficult accurate solubility measurements.

The curves for β-alanine and ε-aminocaproic acid clearly belong to a third family. In systems rich in alcohol these curves approach the straight lines characteristic of glycine and its peptides. Small amounts of ethanol, however, have but very slight precipitating action on these amino acids of long dipole moment, presumably because of their very great solubility and, therefore, the very high dielectric constants of their saturated solutions in water. Assuming that the dielectric constant increments of these two substances remain the same in concentrated as in dilute solution,[8] the dielectric constants of a saturated aqueous β-alanine solution would be 300 and of ϵ-aminocaproic acid 370, or more than four times that of water. A comparable calculation yields an estimate of 72 for the dielectric constant of 80% ethanol saturated with ϵ-aminocaproic acid. Only at alcohol concentrations higher than these do the compositions of the solutions containing these amino acids approach those of the pure solvents. The shapes of these curves in solutions containing small amounts of alcohol appear to depend in large part upon the high dielectric constants of the solutions.

Solubility in Pure Solvents.—The conditions that obtain in pure solvents are simpler than those in alcohol–water mixtures. Water is by far the best solvent for the amino acids. Their solubility in alcohols is smaller the longer the hydrocarbon chain of the latter, but is smaller in acetone than in butanol, although the former has a higher dielectric constant. The solubilities of the amino acids are also smaller in formamide than in water, despite its higher dielectric constant.

Certain of the less polar derivatives of the amino acids are more soluble in other solvents than in water. Thus hydantoic acid is more soluble in formamide than in water, as is the hydantoic acid of α-aminocaproic acid which is moreover more soluble in all of the alcohols than in water. The influence of the hydrocarbon chain of the solute in increasing solubility in a solvent with a hydrocarbon chain of somewhat the same length is also reflected by the measurements upon formyl α-aminobutyric acid.

Influence of the Hydrocarbon Chain.—The influence of the hydrocarbon chain is more readily examined, as we have previously shown[1,2] if we consider the solubility ratio. Values of log N/N_0 are plotted against the number of CH_2 groups, in the molecule $n CH_2$ in Fig. 2. The values for glycine, alanine, diglycine and hydantoic acid are from Studies II and III of this series.[1,2] Straight lines are drawn connecting the experimental values even when only two members of the series have been studied, since no exception has been found to the rule that provided CH_2 groups are in hydrocarbon chains terminating in methyl groups, the increase in log N/N_0 for each additional CH_2 group is, within the limits of error, identical, whether we are comparing amino acids or their derivatives (Table V). This influence of the CH_2 group is smaller for formamide than for methanol, and for methanol than for ethanol. There is no significant difference between the previously published coefficient for CH_2 groups between water and ethanol, 0.49,[1] and that for acetone. The coefficient for the higher alcohols is slightly greater. The value of 0.53 appears to be reached with butanol, and is not increased by further increase in the CH_2 groups of the solvent. It is probable that still greater influences of the CH_2 groups of the solvent might, however, be observed were we to study solutes of longer hydrocarbon chains.

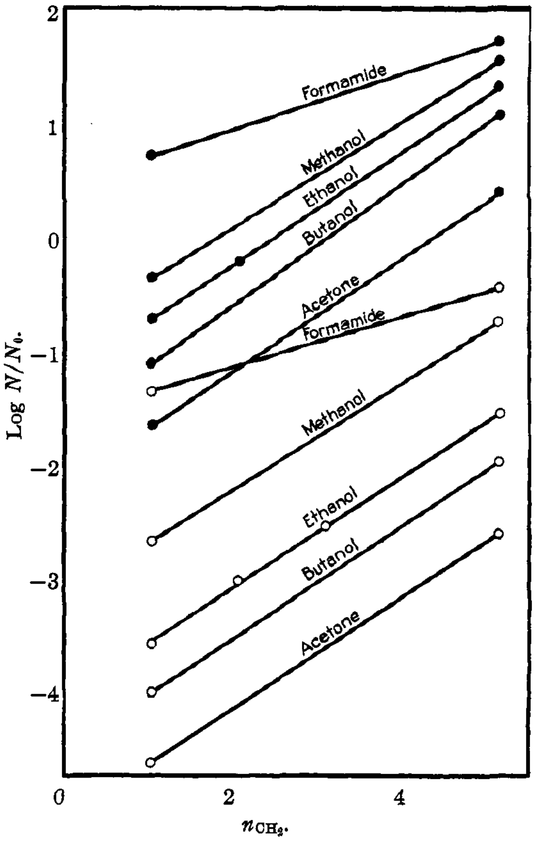

Fig. 2.—Solubility ratios of amino acids ○, and hydantoic acids prepared from them ●, in different solvents.

The influence of the CH_2 groups would appear to increase inversely as the dielectric constant of the solvent (Table IX), were we considering only the alcohols and acetone. Formamide, however,

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(8) Wyman and McMeekin, THIS JOURNAL, **55**, 908 (1933).

TABLE V

INFLUENCE OF THE CH_2 GROUPS OF THE SOLUTE UPON SOLUBILITY RATIO IN DIFFERENT SOLVENTS

Solute	Number of CH_2 groups, n_{CH_2}	Log. of soly. ratio, $\log N/N_0$	$\frac{\Delta \log N/N_0}{\Delta n_{CH_2}}$
	Formamide		
Glycine	1	−1.229	
α-Aminocaproic acid	5	−0.360	0.217
Hydantoic acid	1	+ .755	
α-Aminocaproic hydantoic acid	5	+1.728	.243
	Methanol		
Glycine	1	−2.515	
α-Aminocaproic acid	5	−0.657	0.464
Formylglycine	1	− .101	
Formyl α-aminobutyric acid	3	+ .786	.444
Hydantoic acid	1	− .271	
α-Aminocaproic hydantoic acid	5	+1.568	.460
	Ethanol		
Formylglycine	1	−0.330	
Formyl α-aminobutyric acid	3	+ .651	0.491
Hydantoic acid	1	− .630	.491
α-Alanine hydantoic acid	2	− .139	.496
α-Aminocaproic hydantoic acid	5	+1.352	.497
	Acetone		
Glycine	1	−4.401	
α-Aminocaproic acid	5	−2.432	0.492
Hydantoic acid	1	−1.520	
α-Aminocaproic hydantoic acid	5	+0.437	.489
	Butanol		
Glycine	1	−3.808	
α-Aminocaproic acid	5	−1.711	0.524
Hydantoic acid	1	−1.011	
α-Aminocaproic hydantoic acid	5	+1.118	.532
	Heptanol		
Formylglycine	1	−0.880	
Formyl α-aminobutyric acid	3	+0.173	0.527

has a higher dielectric constant than water, and we must therefore conclude that its behavior in these studies is determined, at least in part, by factors other than the dielectric constant.

Influence of Dipolar Ion Structure.—The same solubility ratios may be employed in estimating the influence of dipolar ion structure, by means of equation (2). This calculation is carried out in Table VI, which in conjunction with Table V demonstrates the additivity of the influence of the paraffin chain and of dipolar ionization. The value of 2.73, derived from the previous comparison[1] of all α-amino acids (corrected for the influence of the CH_2 group) with hydantoic and methyl hydantoic acid, holds also for the measurements in Table VI.

The comparison of the peptides of glycine with their hydantoic acids reveals no difference in the free energy change from water to ethanol with change in the dipole moment. This is contrary to what was expected on the basis of theoretical calculations, which suggested that this quantity would increase with increase in the dipole moment[1] (p. 2275).[9,10] The influence of dipolar ionization does not appear to be greater in the case of triglycine than of diglycine, or of diglycine than of glycine.

Influence of the Amide Group.—Hydantoic acids terminate in an amide group not present in the amino acids or peptides from which they are derived. In this, as in previous calculations, we have considered that the terminal $CONH_2$ group has but very small influence. This notion was deduced from a comparison of the solubility in water and ethanol of glycine with glutamine and asparagine. The measurements upon asparagine

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(9) Scatchard and Kirkwood, *Physik. Z.*, **33**, 297 (1932).

(10) Kirkwood, *J. Chem. Phys.*, **2**, 351 (1934).

TABLE VI

INFLUENCE OF DIPOLAR IONIZATION UPON THE SOLUBILITY RATIO IN DIFFERENT SOLVENTS

Substance	Log of soly. ratio log N/N_0	Influence of dipolar ionization
Formamide		
Glycine	-1.229	
Hydantoic acid	$+0.755$	-1.98
α-Aminocaproic acid	$-$ .360	
α-Aminocaproic hydantoic acid	$+1.728$	-2.09
Methanol		
Glycine	-2.515	
Hydantoic acid	-0.271	-2.24
α-Aminocaproic acid	$-$ .657	
α-Aminocaproic hydantoic acid	$+1.568$	-2.23
Ethanol		
Glycine	-3.391	
Hydantoic acid	-0.630	-2.76
α-Alanine	-2.856	
α-Alanine hydantoic acid	-0.139	-2.72
β-Alanine	-3.139	
β-Alanine hydantoic acid	-0.463	-2.68
α-Aminocaproic acid	-1.414	
α-Aminocaproic hydantoic acid	$+1.352$	-2.77
Diglycine	-4.367	
Diglycine hydantoic acid	-1.533	-2.83
Triglycine	-4.965	
Triglycine hydantoic acid	-2.253	-2.71
Butanol		
Glycine	-3.808	
Hydantoic acid	-1.011	-2.80
α-Aminocaproic acid	-1.711	
α-Aminocaproic hydantoic acid	$+1.118$	-2.83
Acetone		
Glycine	-4.401	
Hydantoic acid	-1.520	-2.88
α-Aminocaproic acid	-2.432	
α-Aminocaproic hydantoic acid	$+0.437$	-2.87

TABLE VII

SOLUBILITY RATIOS OF DIPOLAR IONS IN WATER AND ETHANOL

	Log N_A/N_0	Δ Log N_A/N_0
Glycine	-3.391	
Asparagine	-3.402	-0.011
Glutamine	-3.466	$-$ .064
Diglycine	-4.367	$-$ .976
Triglycine	-4.965	-1.574

are perhaps the more accurate and the difference between its solubility ratio and that of glycine must be considered within the errors of experiment. The influence of the amide group was, therefore, considered negligible.

If we assume the influence of dipolar ion structure on the solubility ratio to be that estimated in Table VI, we may subdivide the influence of the amide group into two parts. Thus, subtracting 2.73 from the value for glycine should yield -0.66 for its hypothetical uncharged isomer; a value not very different from that determined for glycol amide, which has the same composition though a different structure. The value for formylglycine is -0.33. The difference $+0.33$ might be considered to yield the effect of the carbonyl group. Comparable calculations could be made by comparing the other formyl compounds that have been studied with the corresponding amino acids.

Formylglycine and hydantoic acid differ by an NH group. The change in their solubility ratios is given below:

	Log N_A/N_0	Δ Log N_A/N_0
Formylglycine	-0.33	
Hydantoic acid	$-$ .63	-0.30

According to this calculation the influences of the CO and NH groups are opposite in sign, as might have been expected from the small apparent influence of terminal amide groups on the solubility ratios reported.

Influence of the Peptide Linkage.—Quite different is the influence of the peptide linkage. Glycylglycine and asparagine are isomers. The comparison of glycine and its peptides with their hydantoic acids (Table VI) indicates that variation in the dipole moment of the molecule has no measurable influence on the change in free energy in passing from water to ethanol. The great difference in Δ log N_A/N_0 between glycine and its peptides (Table VII) would therefore appear to be due to the CONH group between polar groups. The negative value of log N_A/N_0 for diglycine is larger than that for glycine by -0.976. The comparable value for triglycine, -1.574, is only -0.598 larger. The smaller influence of the second peptide linkage presumably is related to the greater distance of CH_2 and CONH groups in the larger molecule from the polar terminal groups.

Influence of CH_2 Groups between Polar Groups.—That CH_2 groups situated between polar groups have but slight influence on the solubility ratio has previously been demonstrated by comparison of aspartic with glutamic acid, and of asparagine with glutamine. Since there is no appreciable change in the solubility ratio, when instead of one CH_2 group two are present between polar groups, we assumed that the first also behaved as though it was not subject to the rule valid for CH_2 groups in the paraffin chain. With increase in the number of groups between polar groups intermediate behavior should obtain. This phenomenon has been investigated by studying the hydantoic acids of α, β, δ, and ϵ-amino acids. It will be observed that the larger the number of CH_2 groups between polar groups the greater the influence of each upon the solubility ratio (Table VIII). The coefficient per CH_2 group between the hydantoic acid of an α- and β-amino acid is but 0.167, whereas that between comparable δ- and ϵ-compounds is more than twice as great, namely 0.397, approaching that characteristic of the paraffin chain.

TABLE VIII

INFLUENCE OF THE POSITION OF GROUPS UPON THE SOLUBILITY RATIO IN ETHANOL AND WATER

	Number of CH_2 groups, n	Log of soly. ratio, $\log N/N_0$	$\frac{\Delta \log N/N_0}{\Delta n_{CH_2}}$
Hydantoic acid	1	-0.630	0.167
β-Alanine hydantoic acid	2	$-\ .463$	.308
δ-Aminovaleric hydantoic acid	4	$+\ .152$	.397
ϵ-Aminocaproic hydantoic acid	5	$+\ .549$	.495
α-Aminocaproic hydantoic acid	5	$+1.352$	

Comparison of the solubility ratio of ϵ-aminocaproic acid in water and ethanol with that of its hydantoic acid constitutes the one exception noted thus far to the rule deduced in Table VI, and suggests that CH_2 groups between the charged groups of a dipolar ion have a smaller influence than those between other polar groups at this distance from each other. This is not unexpected since the moment of the former is more than ten times that of the widely separated polar groups of this hydantoic acid.

	$\log N_A/N_0$	$\Delta \log N_A/N_0$
ϵ-Aminocaproic acid	-2.972	
ϵ-Aminocaproic hydantoic acid	$+0.549$	-3.521

Discussion.—The influence of the polar and non-polar groups of molecules upon solubility has been stressed previously.[11–13] "A comparison of various insoluble substances has proved that the spreading tendency depends upon the presence of certain active groups of radicles in the organic molecule, these being the groups which tend to increase the solubility of organic substances in water. For example, pentane, C_5H_{12}, is practically insoluble in water, but amyl alcohol, $C_5H_{11}OH$, is relatively soluble. Thus the hydroxyl groups in organic molecules exert strong attractive forces on the hydroxyl groups in the water molecules and these manifest themselves by an increase in solubility. Similarly the carboxyl group, COOH, tends to make the lower fatty acids much more soluble in water than the corresponding hydrocarbons"[13] (p. 161).

"Traube found that with molecules of aliphatic compounds having different lengths of hydrocarbon chains" the decrease in the surface tension of the pure liquid, divided by the osmotic pressure of the dissolved substance in the underlying solution, "for dilute solutions increases about threefold for each CH_2"[13] (p. 164). Our investigations confirm the applicability of Traube's rule[14] to the ratio of solubility in water and organic solvents.

The accuracy with which the above rule appears to hold even when solubility is not very low was, however, not foreseen. Thus in all the solvents studied there was no appreciable difference between the coefficient for the CH_2 group calculated from glycine and α-aminocaproic acid and from the far less soluble hydantoic acids (Table V), despite the high solubility of glycine and the high dielectric constant of its aqueous solution. This result may mean that the logarithm of the activity coefficient of glycine in saturated aqueous solution is not very large. Scatchard[15] estimates this quantity to be about -0.15 from freezing point determinations.

The CH_2 rule appears to hold for monoamino monocarboxylic α-amino acids as well as for their derivatives, but not for dicarboxylic acids, where the hydrocarbon chain terminates in a carboxyl group. The study of the hydantoic acids of β-, δ- and ϵ-amino acids suggests that interaction between the carboxyl, amide and CH_2 groups extends to the neighboring CH_2 groups, but not very much farther. The term interaction is used since the present studies yield no evidence as to whether polar groups are masking the effect of non-polar groups, or non-polar those of polar groups.

This interaction appears to extend to a greater distance, in the case of CH_2 groups between the charged groups of a dipolar ion. None the less, the fields of force set up must be restricted largely to the intervening atoms and cannot extend very far beyond them. Otherwise, the simple CH_2 rule would not apply to α-amino acids, for the co-

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(11) Freundlich, *Ergebnisse exakt. Naturwissenschaften*, **12**, 82 (1933).

(12) Hildebrand, "Solubility of Non-Electrolytes," Reinhold Publishing Corp., New York, N. Y., 2 ed., 1936.

(13) Langmuir, *Chem. Rev.*, **13**, 147 (1933).

(14) Traube, *Ann.*, **265**, 27 (1891).

(15) Scatchard and Prentiss, THIS JOURNAL, **56**, 1486 (1934).

efficient relating glycine and alanine would be different from that relating norvaline and norleucine.

Finally the results reported as solubility ratios may be transformed into calories and compared with the studies of Butler and his co-workers,[16] who have considered the free energy change, ΔF, from the gaseous state to aqueous solution. Our results recalculated (Table IX) suggest that the free energy increment for the CH_2 group in aqueous solutions referred to alcohols, ranges from 600 to 700 calories. Butler estimates the free energy in aqueous solution referred to the gaseous state as 200 calories per CH_2 group. Butler further estimates a change in free energy of about 6000 calories if NH_2 be substituted and 7400 calories if COOH be substituted in the paraffin chain.

The free energy in aqueous solution referred to alcohols due to dipolar ionization we estimate at −3000 to −4000 calories. This estimate is based on the comparison of the amino acids and the hydantoic acids and includes, as has previously been pointed out, effects due to the amide group and to the movement of the proton from the carboxyl to the ammonium group.

TABLE IX

Solvent	Dielectric constant[a]	Influence of CH_2 groups Δ log N/N_0	ΔF, cal.	Influence of dipolar ionization Δ log N/N_0	ΔF, cal.
Formamide	>84	+0.23	314	−2.03	−2768
Methanol	32.71	+ .44	600	−2.24	−3054
Ethanol	24.28	+ .49	668	−2.73	−3722
Acetone	20.83	+ .49	668	−2.87	−3913
Butanol	17.51	+ .53	723	−2.82	−3845
Heptanol	9.33	+ .53	723	....	....

[a] We are indebted to Dr. Wyman for measuring the dielectric constants of the solvents employed. The value for formamide is taken from "Int. Critical Tables," Vol. VI, p. 83.

These quantities define all the solubility measurements reported. They reveal no exceptional behavior such as was expected, due either to interaction between dipolar ions in concentrated aqueous solution, or to the changing ratio of dipolar ions and uncharged molecules in solvents of low dielectric constant. Moreover they do not reveal change in Δ log N/N_0 or of ΔF with change in the reciprocal of the dielectric constant, such as would be expected. The relations that obtain, however, appear to be far simpler than could have been predicted.

Summary

1. The solubilities of amino acids and peptides of different dipole moments have been studied in various solvents at 25°.

2. The logarithm of the ratio of solubility in any solvent, N, to that in water, N_0, is increased by a constant amount for each CH_2 group in hydrocarbon chains terminating in methyl groups. This amount is 0.53 for butanol and heptanol, 0.49 for acetone and ethanol, 0.44 for methanol and 0.23 for the most polar solvent studied, formamide.

3. When only one or two CH_2 groups are situated between polar groups the solubility ratio is but little affected. The larger the number of CH_2 groups situated between polar groups the more nearly the solubility is influenced, as in hydrocarbon chains ending in methyl groups.

4. CH_2 groups situated between the charges of a dipolar ion influence the solubility ratio far less than those between other polar groups. The electric moments of dipolar ions are generally more than fivefold as great as the moments of the carboxyl, hydroxyl, amine or amide groups.

5. Solutions of dipolar ions such as β-alanine and ϵ-aminocaproic acid are so concentrated and have such high dielectric constants that conditions existing in them must be considered different from those in the pure solvent.

6. The peptides of glycine are all less soluble than glycine and so are other α-amino acids and the hydantoic acids derived from them. Comparison of the solubility ratios of these dipolar ions with their hydantoic acids yields, as estimates of the influence of dipolar ionization upon the logarithm of the solubility ratio, −2.03 from water to formamide, −2.24 to methanol, −2.73 to ethanol, −2.82 to butanol and −2.87 to acetone.

7. The comparison of the peptides of glycine with their hydantoic acids has revealed no change in solubility ratio with change in the dipole moment of the peptide.

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[16] Butler, Ramchandani and Thomson, *J. Chem. Soc.*, 280 (1935).