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

Studies in the Physical Chemistry of Amino Acids, Peptides and Related Substances. III. The Solubility of Derivatives of the Amino Acids in Alcohol–Water Mixtures

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That polar molecules are more soluble in polar solvents and non-polar molecules in non-polar solvents, has become axiomatic. Probably the most polar organic molecules are the amino acids. They are extremely soluble in water and increase its dielectric constant by as much as 23 per mole, even when the amino group is in the α-position. This effect is most readily accounted for by the hypothesis, repeatedly affirmed by diverse investigations, that a proton has migrated from the carboxyl to the amino group, leaving the former negatively, the latter positively, charged. The so-called zwitterionic structure of the amino acids accounts not only for their amphoteric properties, but for their high density in the solid state and even higher specific densities in aqueous solution, a phenomenon due to electrostriction of the solvent. The influence of amino acids both upon the dielectric constant and upon electrostriction is, moreover, greater if the amino group is situated at a greater distance from the carboxyl group. Under these circumstances solubility in water is also increased.

The solubility in water of α-amino acids tends to be smaller the longer the hydrocarbon chain. The solubility in alcohol, however, tends to be greater the longer the hydrocarbon chain. Consequently the ratio of solubility in alcohol to that in water is smallest for the smallest of the amino acids, glycine, and largest for the longest of the amino acids, α-aminocaproic acid. Molecules of the same dipole moment but longer hydrocarbon chains have greater ratios. Expressed in moles per liter this ratio is 0.000135 for glycine, and 0.012 for α-aminocaproic acid. Increase in the dipole moment, as when the amino group is in the ϵ-position, enormously increases the solubility both in water and alcohol, and diminishes the solubility ratio to 0.0005, though not to a value as small as that of glycine. Amino acids like salts are far more soluble in water than in alcohol. That this is due to the zwitterionic nature of the neutral amino acid molecule is best demonstrated, however, by comparing the behavior of the amino acids, considered in the last communication,[1] with that of derivatives of the amino acids in which their zwitterionic nature is destroyed. Four such series of compounds have been prepared and investigated: (1) the amides of the amino acids in which the carboxyl group is combined, and feebly basic molecules result; (2) the formyl compounds of the amino acids in which the amino group is combined, and feebly acidic molecules result; (3) the hydantoic acids which possess both the amino and carboxyl groups; and (4) the hydantoins, ring compounds which may be derived from them.

All of these molecules contain the CONH group characteristic of peptides and proteins, but not of the monoamino monocarboxylic acids previously reported. We have therefore studied the amides of the dicarboxylic acids, which are isomeric with the hydantoic acids. These studies have resulted in certain generalizations, and quantitative rules regarding the influence of the hydrocarbon chain upon solubility.

I. Methods and Materials

The method of determining solubility has been described previously.[1] The concentration of the solutions was determined either by the method of dry weight. by Kjeldahl nitrogen analyses. or by direct titration. advantage being taken of the pronounced acidic properties of the compounds; often two of these methods were employed.

The formyl derivatives of the amino acids were prepared by the general method described by Fischer and Warburg,[2] the hydantoins by the procedures described by Dakin,[3] and the amides by the method of Fischer as modified by Yang and Rising.[4] The product in all cases. unless otherwise noted. was recrystallized from hot water.

Formylglycine.—Glycine, protected with a drying tube, was refluxed on a water-bath for three hours with one and one-half times its weight of commercial water-free formic acid and then concentrated as far as possible under reduced pressure. This procedure was repeated several times with fresh acid. The final crystalline mass was sucked dry. washed with a small quantity of ice water and then recrystallized from hot water: yield. 60%: m. p. 152–153°.

Formyl-*dl*-leucine.—The reaction mixture was extracted with ethyl acetate and the extracts concentrated: yield 75%; m. p. 114–115°.

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

[2] Fischer and Warburg. *Ber.*, **38**. 3997 (1905).

[3] Dakin. *Am. Chem. J.*, **44**. 48 (1910); *J. Biol. Chem.*, **8**. 25 (1910); *Biochem. J.*, **12**, 290 (1918); *J. Biol. Chem.*, **44**. 499 (1920); Dakin and Dudley, *ibid.*, **17**, 29 (1914).

[4] Yang and Rising. THIS JOURNAL. **53**. 3183 (1931).

Formyl-*dl*-α-aminobutyric Acid.—The purified product melted at 154–155°.

Hydantoic acid was prepared by heating an aqueous solution of glycine and potassium cyanate on a steam-bath for thirty minutes. The cooled solution was carefully acidified with hydrochloric acid until it was faintly acid to congo red paper, whereby the substance crystallized on further cooling. The purified product melted at 169–170°.

***dl*-Methylhydantoic acid** was made from *dl*-alanine and potassium cyanate. After three crystallizations from water it melted at 169–170°.

Hydantoin was prepared by heating an aqueous solution of glycine and potassium cyanate on a steam-bath for thirty minutes. The solution was acidified with hydrochloric acid until distinctly acid to congo red paper and then boiled gently for thirty minutes. A little charcoal was added and the solution filtered. On cooling hydantoin crystallized. The purified product melted at 217–218°.

Anal. Calcd. for $C_3H_4O_2N_2$: N, 28.0. Found: N, 27.7.

Hydantoin of *dl*-α-aminobutyric acid was prepared in a manner similar to hydantoin. To obtain ethylhydantoin it was necessary to heat ethyl hydantoic acid to 100° for thirty minutes. The purified product melted at 118–120°.

Anal. Calcd. for $C_5H_8O_2N_2$: N, 21.8. Found: N, 21.3.

Hydantoin of *dl*-Leucine.—The purified product melted at 208–209°.

Anal. Calcd. for $C_7H_{12}O_2N_2$: N, 17.9. Found: N, 17.5.

Hydantoin of aspartic acid was prepared by the method of Lippich.[5] The purified substance melted at 215–217°.

Anal. Calcd. for $C_5H_6O_4N_2$: N. 17.7. Found: N. 17.5.

Glycine amide was prepared by treating the ethyl ester of glycine dissolved in 250 cc. of absolute methyl alcohol with dry ammonia gas at 0°. The reaction proceeded at room temperature for days and the alcohol and ammonia were removed under reduced pressure. The substance was recrystallized from chloroform. The product melted at 66–68°.

Anal. Calcd. for $C_2H_6ON_2$: N. 37.8. Found: N, 37.0.

***dl*-Leucine amide** was prepared in a similar manner as was glycine amide. The substance melted at 105–108°.

Anal. Calcd. for $C_6H_{14}ON_2$: N, 21.5. Found: N. 21.9.

***l*-Aspartic acid** was purified by recrystallizing from water and from alcohol–water mixtures. The optical rotation of the preparation was $(\alpha)^{25}\mathrm{D}$ +25.0° when dissolved in 1.5 moles of normal hydrochloric acid.

***d*-Glutamic acid** was purified by crystallizing twice from water and once from 50% alcohol. The optical rotation of the preparation was $(\alpha)^{25}\mathrm{D}$ +32.4° when dissolved in 4.53 moles of hydrochloric acid. The values for the solubility of *d*-glutamic acid in water as reported by Dalton and Schmidt,[6] Pertzoff[7] and Dunn, Ross and Read[8] are in excellent agreement with each other and with our results.

***l*-Asparagine** was purified by crystallization from water. The substance gave an optical rotation $(\alpha)^{25}\mathrm{D}$ +37.2° when it was dissolved in hydrochloric acid (10 volume per cent.).

Anal. Calcd. for $C_4H_8N_2O_3$: N, 21.2. Found: N. 20.9.

***d*-Glutamine.**—We are indebted to Dr. Hubert B. Vickery of the Connecticut Agricultural Experiment Station for the very excellent preparation of glutamine which he placed at our disposal.

Glycylglycine was purified by crystallizing from water and 50% alcohol.

Anal. Calcd. for $C_4H_8N_2O_3$: N, 21.2. Found: N, 21.1.

II. Melting Points of Amino Acids and their Derivatives

The melting points of amino acids are higher than those of most organic compounds, reflecting, as Pfeiffer[9] and Bjerrum[10] suggested, their zwitterionic structure. Amino acid melting points are not accurately known since they decompose when melting, but all are in the neighborhood of 300°, whereas their derivatives melt sharply, and at lower temperatures. With the exception of the hydantoins, ring compounds containing two CONH groups, the melting points of all that have been prepared are lower than 200°.

The contrast between the melting points of the aliphatic amines and acids and the amino acids is very great. Relatively few organic compounds have melting points as high as those even of derivatives of amino acids. Among these are sucrose and urea, both of which are more soluble in water than in alcohol, and whose behavior, it has been suggested, approximates the law deduced for ions, relating the apparent molal volume to the square root of the concentration.[11] The derivatives of urea which, like the hydantoins, contain several CONH groups have melting points ranging up to 235°. Oxamide, the diamide of oxalic acid, with the same composition as formyl urea, has a density of 1.667, and melts at 419°, or nearly as high as a salt of low melting point, such as lithium iodide (446°), which is relatively soluble in alcohol.

III. The Ratio of the Solubility in Alcohol and Water

Urea is one of the few substances sufficiently polar to increase the dielectric constant of water,

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(5) Lippich, *Ber.*, **41**, 2953 (1908).

(6) Dalton and Schmidt, *J. Biol. Chem.*, **103**, 549 (1933).

(7) Pertzoff, *ibid.*, **100**, 97 (1933).

(8) Dunn, Ross and Read, *J. Biol. Chem.*, **103**, 579 (1933).

(9) Pfeiffer, *Ber.*, **55**, 1762 (1922).

(10) Bjerrum, *Z. physik. Chem.*, **104**, 147 (1923).

(11) Gucker, *Chem. Rev.*, **13**, 111 (1933). See also Redlich and Klinger, *Monatsh.*, **65**, 137 (1934).

TABLE I

MELTING POINTS OF AMINO ACIDS AND RELATED SUBSTANCES

Amino acids	Melting points, °C.	Aliphatic amines and acids	Melting points, °C.
Glycine	290	Ethylamine	−80.6
dl-Alanine	297	Propylamine	−83.0
dl-Valine	292	Amylamine	−55.0
dl-Leucine	332	Hexylamine	−19.0
dl-α-Aminocaproic acid	327	Acetic acid	16.6
Glycylglycine	262–264	Propionic acid	−22.0
dl-Phenylalanine	318–320	Valeric acid	−34.5
l-Tyrosine	342–344	Caproic acid	− 9.5
Certain amino acid derivatives		Certain organic compounds	
Glycyl amide	66– 68	Acetamide	81
Alanyl amide	71– 72	Butyramide	116
Leucyl amide	105–108	Benzamide	130
Formylglycine	152–153	Acetanilide	114
Formylglycylglycine	168–170	Acetnaphthalide	159
		Phenylthiourea	154
Formyl-α-aminobutyric acid	154–155	Urethan	48
Formylleucine	114–115		
		Ethyl allophanate	191
Hydantoic acid of glycine	169–170	Glucose	146
		Sucrose	186
Hydantoic acid of alanine	169–170	Urea	133
		Formyl urea	169
Hydantoin of glycine	217–218	Biuret	193
		Acetyl urea	217
Hydantoin of α-aminobutyric acid	118–120	Acetyl biuret	194
Hydantoin of leucine	208–209	Carbonyl diurea	233
		Allantoin	235
Hydantoin of aspartic acid	215–217		

The melting points of the amino acids have recently been critically examined by Dunn and Brophy [*J. Biol. Chem.*, **99**, 221 (1932)]. The organic compounds, including the aliphatic amines and acids, are from Vol. I, "International Critical Tables." Yang and Rising[4] report the melting point of alanyl amide, Gorski the melting point of formyl urea [*Ber.*, **29**, 2046 (1896)]. The measurements of the remaining amino acid derivatives, as well as of acetyl biuret, carbonyl diurea and ethyl allophanate, have been made in this department.

though its effect per mole is only one-tenth that of glycine. In order to account for this effect it has been suggested that urea is a zwitterion. There are various strong arguments against this view, including the observation that urea does not produce electrostriction of the solvent comparable to that due to amino acids.[12] Presumably the high dipole moments[13] and heats of formation[14] of urea and the amides depend upon resonance of an electron but not the shifting of a proton. If the increment in dielectric constant per mole of solute, δ, is proportional to the square of the dipole moment, μ, a view consistent with that of Wyman,[15] Kirkwood[16] and Kuhn and Martin,[17] the following relation results

$$\mu_{\text{urea}} = \mu_{\text{glycine}} \sqrt{\frac{\delta_{\text{urea}}}{\delta_{\text{glycine}}}} = 15 \times 10^{-18} \sqrt{\frac{2.8}{23}} = 5.2 \times 10^{-18} \text{ e. s. u.} \quad (1)$$

If a higher value were adopted for the dipole moment of glycine, or if allowance were made for the fact that δ is negative when the ratio (dipole moment/molal volume) is not greater than for water, this estimate would be somewhat greater. The dipole moment of tetraethyl urea—which cannot conceivably be a zwitterion—is reported to be 3.3,[18] or close to that of the amide group,[14,19] whereas the moment of symmetrical dimethyl urea is reported to be 5.1×10^{-18} e. s. u.[18]

According to Seidell,[20] 1130 g. of urea is dissolved by 1 kilogram of water, or 10.17 moles in a liter at 25°. The solubility in alcohol is 0.7756 mole per liter. The ratio is, therefore, only approximately one-tenth as compared with one-ten thousandth for glycine, and one-hundredth for α-aminocaproic acid.

Influence of the CH_2 Group.—Urea may be regarded as the first member of the series of amides of the amino acids.[12] It contains no CH_2 group. Leucyl amide, the largest member of the series, contains 5 CH_2 groups. Instead of being ten times as soluble in water as in alcohol, as is urea, the increased number of CH_2 groups renders the molecule more soluble in alcohol than in water. Indeed it is appreciably soluble even in benzene. Instead of behaving like a highly polar molecule its behavior strongly reflects the hydrocarbon chain. The amides of the mono-amino monocarboxylic acids, like urea, are all extremely soluble. Their study is therefore not readily susceptible of theoretical interpretation, since their solubility ratios may deviate widely from their activity coefficients.

Three formyl compounds and three hydantoins of the amino acids have been studied: those of glycine, α-aminobutyric acid and leucine. In these series, as in the others investigated, solubility in water tends to decrease, and in alcohol to

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(12) Cohn, McMeekin, Edsall and Blanchard, THIS JOURNAL, **56**, 784 (1934).

(13) Pauling and Sherman, *J. Chem. Phys.*, **1**, 606 (1933).

(14) Kumler and Porter, THIS JOURNAL, **56**, 2549 (1934).

(15) Wyman, *ibid.*, **56**, 536 (1934).

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

(17) Kuhn and Martin, *Ber.*, **67**, 1526 (1934).

(18) Devoto, *Gazz. chim. ital.*, **63**, 491 (1933).

(19) Zahn, *Trans. Faraday Soc.*, **30**, 804 (1934).

(20) Seidell, "Solubilities of Inorganic and Organic Compounds," D. Van Nostrand Company, New York, 1928.

increase, with increase in the hydrocarbon chain. In comparing solubility in two solvents, influences due to the solid state are largely eliminated, and a quantitative relation obtains regarding the influence of terminal CH_2 groups upon the solubility ratio. This rule, roughly stated, is that *the ratio of the solubility in alcohol to that in water is increased three-fold for each* CH_2 *group in hydrocarbon chains terminating in methyl groups.*[21]

It follows that if the logarithm of the solubility ratio be plotted against the apparent molal volume, a straight line results, whose slope is just under one-half and is conveniently given as equal to 0.03 times the volume of the CH_2 group. This rule holds also for the α-amino acids, as has already been demonstrated.[1] The solubility ratios of all of these substances, calculated as mole fractions, are represented in Fig. 1. The parallel lines that have been drawn appear to describe adequately all of the measurements upon the α-amino acids and their compounds.

TABLE II

Vol. fr. alc. in solvent, v_2	Density of soln., ρ	Solubility		Log. of soly. ratio, $\log N_2/N_2^0$
		Moles per liter, C	Mole fr., $N_2 \times 10^{-3}$	
		l-Aspartic acid		
0.0	0.9994	0.0375	0.679	
.2	.9729	.0149	.307	−0.345
.4	.9457	.00675	.161	− .625
.5	.9272	.00441	.115	− .771
.6	.9064	.00264	.0769	− .946
.7	.8826	.00149	.0490	−1.142
.8	.8550	.00070	.0263	−1.412
.9	.8262	.00021	.0096	−1.851
1.0	.7851	.0000116	.00068	−2.999
		d-Glutamic acid		
0.0	1.0002	0.0585	1.06	
1.0	0.7851	.0000185	0.00108	−2.992
		l-Asparagine		
0.0	1.0072	0.186	3.40	
.2	0.9761	.0750	1.55	−0.342
.4	.9473	.0306	0.732	− .667
.6	.9068	.0105	.306	−1.046
1.0	.7851	.000023	.00135	−3.402
		d-Glutamine		
0.0	1.0124	0.291	5.38	
1.0	0.7851	.0000315	0.00184	−3.466
		Glycylglycine		
0.0	1.0779	1.512	30.04	
.2	1.0019	0.531	11.26	−0.426
.4	0.9541	.152	3.661	− .914
.6	.9082	.0362	1.055	−1.455
.8	.8554	.00374	.143	−2.323
.9	.8250	.000493	.0225	−3.126
1.0	.7851	.0000222	.00129	−4.367

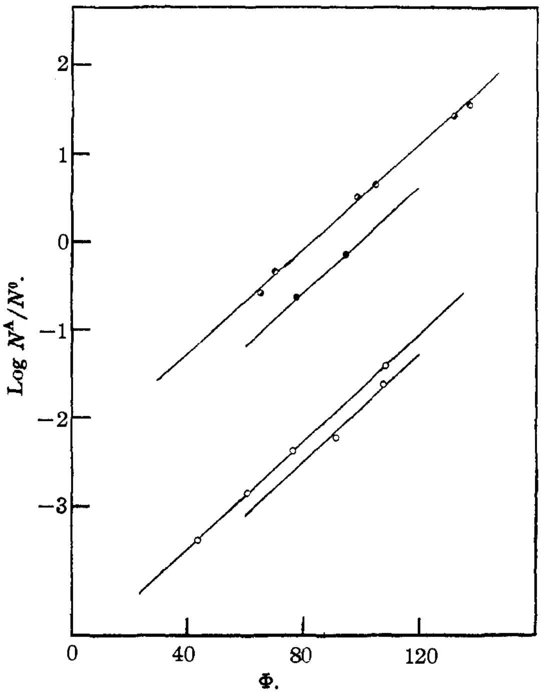

Fig. 1.—Relation between length of hydrocarbon chain and the ratio (solubility in alcohol/solubility in water): ○, amino acids; ●, hydantoic acids; ◒, hydantoins of amino acids; ◓, formyl amino acids.

Exceptions to this rule are not lacking. Thus α-amino acids with branched chains behave as though their hydrocarbon chains were shorter than their normal isomers. Aspartic and glutamic acid, and their amides, asparagine and glutamine, are also α-amino acids.[22] The CH_2 groups in these molecules are situated between highly polar groups. Although glutamic acid contains one CH_2 group more than aspartic acid and glutamine one more than asparagine, the ratio of solubility in alcohol to that in water is not measurably increased in either series (Table II). At a boundary between water and a higher alcohol, aliphatic acids, amides or amino acids would so orient that the hydrocarbon chain extended into the less polar solvent. It seems doubtful whether

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[21] Traube [*Ann.*, **265**, 27 (1891)] "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 partial osmotic pressures of the dissolved substance in the underlying solution, "for dilute solutions increases about three-fold for each CH_2" [Langmuir, *Chem. Rev.*, **13**, 164 (1933)], and Langmuir states that his studies "are completely in accord with the general relationships found by T. Traube."

[22] Dr. Jeffries Wyman has kindly measured the dielectric constants of solutions of both asparagine and glutamine, and obtained values of δ, respectively, of 20.2 and 19.6 as compared with 22.6 for glycine.

dicarboxylic acids or their amides would extend into the alcoholic solution to the same extent.

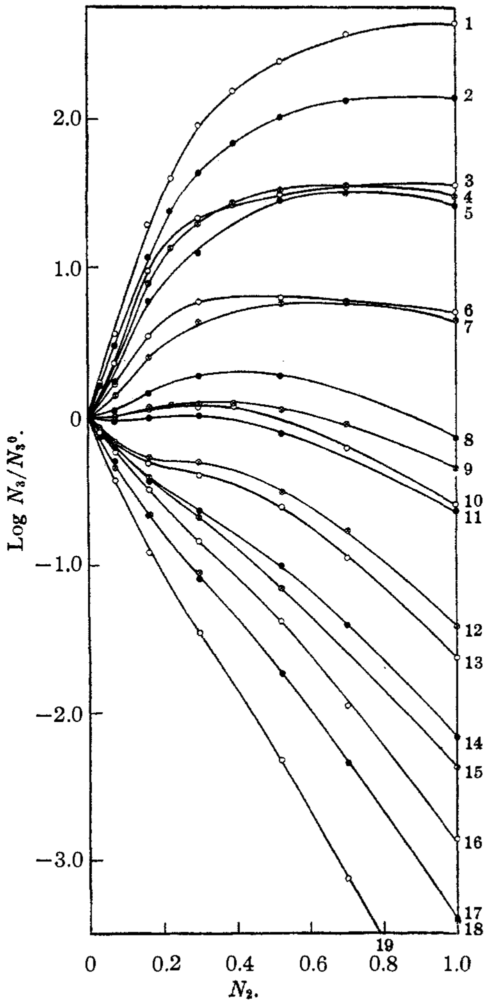

Fig. 2.—Compounds: 1, acetnaphthalide ○; 2, acetanilide ●; 3, formylleucine ○; 4, benzamide ⊗; 5, hydantoin of leucine ●; 6, hydantoin of α-amino-*n*-butyric acid ○; 7, formyl-α-aminobutyric acid ⊗; 8, methylhydantoic acid ●; 9, formylglycine ⊗; 10, hydantoin ○; 11, hydantoic acid ●; 12, amino-*n*-caproic acid ⊗; 13, *l*-leucine ○; 14, *dl*-valine ●; 15, amino-*n*-butyric acid ⊗; 16, alanine ○; 17, glycine ●; 18, *l*-asparagine ⊗; 19, glycylglycine ○.

In order to investigate the influence of a CH_2 group between polar groups in a molecule not a zwitterion, the hydantoin of aspartic acid was prepared. This molecule differs from α-aminobutyric hydantoin by substitution of a methyl by a carboxyl group. As a result the value of log N_3^A/N_3^0 changes from +0.521 to −0.190. The comparable value for the first member of the series, hydantoin, is −0.585. The differences from it are 1.106 for 2 CH_2 groups, but only 0.395 for one CH_2 and a terminal carboxyl group. The comparable differences for glycine, aspartic acid and α-aminobutyric acid, all α-amino acids, are 1.016 and 0.392. These differences between ratios are remarkably consistent, demonstrating no difference in this respect between zwitterions and uncharged molecules, and indicating that *a CH_2 group between two polar groups does not measurably increase the solubility ratio between alcohol and water.*

Another kind of exception, characteristic of α-aminobutyric acid and its derivative, occurs when the crystal lattice energy is abnormal, and is generally revealed by the density of the solid. Comparison is completely satisfactory only if all are relatively insoluble. Thus among the hydantoins, that derived from α-aminobutyric acid is much the most soluble. Compared with hydantoin the difference in log N_3^A/N_3^0, divided by the difference in the number of CH_2, groups, is 0.55; compared with leucylhydantoin it is 0.45. Comparison of the less soluble first and last members of this series yields 0.50, in excellent agreement with the value 0.49 derived from the hydantoic acids.

In subsequent calculations the influence of a terminal CH_2 group upon the solubility ratio will be calculated by the relation

$$\log N_3^A/N_3^0 = K_2 + 0.49\,(CH_2)_n = K_2' + 0.03\,\Phi \quad (2)$$

where Φ is the apparent molal volume, n the number of CH_2 groups in the molecule, and K_2 or K_2' a constant, depending only on the nature of the polar groups.

IV. Solubility in Alcohol–Water Mixtures

Solubility in three-component systems is more complicated than in the two-component systems thus far considered. Beside the forces between solute molecules and the two solvent species, there are the forces between the solvent molecules themselves. Moreover, if the affinity of the solute is far greater for the one than for the other solvent, a redistribution of solvent molecules may occur in the neighborhood of solute molecules so that the solvent as a whole can no longer be considered a uniform medium.

On the other hand, the forces between the solute and one of the solvents may be so much greater than the other that extremely simple relations obtain. Thus in the case of the α-amino acids previously considered,[1] the solubility ratios of all those of straight chains fall on the same curve provided there is subtracted from the solubility ratio of each the quantity $0.03\, v_2^2 V_{CH_2}$.

The solubility ratios of derivatives of amino acids in alcohol–water mixtures are given in Table III and represented graphically in Fig. 2. The points in absolute alcohol are the same as in Fig. 1. The shapes of the curves indicate, however, that the solubility of molecules containing the larger number of CH_2 groups is increased by alcohol by an amount far greater than the second power of its volume fraction. Indeed at low alcohol concentrations the logarithm of the solubility of the larger molecules studied, such as formylleucine, increases almost proportionately with the mole fraction of alcohol in the solvent.

TABLE III

Vol. fr. alc. in solvent, v_2	Density of soln., ρ	Soly. moles per liter, C_3	Log. of soly. ratio, $\log N_3/N_3^0$	Log $N_3/N_3^0 - 0.03v_2^2\Phi$	$v_2\left(\frac{K_1v_1}{1+v_1}\right)K_2$
Hydantoin					
0.0	1.0105	0.397	−0.0		
.2	.9846	.348	−0.002	−0.080	−0.054
.4	.9579	.340	0.052	−0.261	−0.249
.6	.9207	.294	0.072	−0.632	−0.645
.7	.8923	.249	0.054	−0.904	−0.951
.9	.8304	.0987	−0.210	−1.794	−1.870
1.0	.7867	.0324	−0.585	−2.541	−2.543
Hydantoin of α-amino-*n*-butyric acid					
0.0	1.0223	0.863	0.0		
.2	1.0118	1.233	0.222	0.103	0.110
.4	1.0092	2.053	0.542	0.066	0.035
.6	1.0008	2.725	0.772	−0.298	−0.306
.8	0.9713	2.408	0.800	−1.103	−1.052
.9	0.9222	1.971	0.774	−1.634	−1.649
1.0	0.8969	0.988	0.521	−2.452	−2.448
Hydantoin of leucine					
0.0	0.9972	0.0124	0.0		
.2	.9730	.0186	0.233	0.075	0.113
.4	.9470	.0444	0.679	0.048	0.034
.6	.9107	.0951	1.096	−0.324	−0.318
.8	.8610	.162	1.448	−1.077	−1.087
.9	.8314	.154	1.503	−1.692	−1.703
1.0	.7922	.100	1.421	−2.524	−2.525
Formylglycine					
0.0	1.0570	1.849	0.0		
.2	1.0402	1.680	0.004	−0.081	−0.080
.4	1.0078	1.633	0.056	−0.283	−0.287
.5	0.9824	1.560	0.076	−0.454	−0.454
.6	.9572	1.459	0.090	−0.672	−0.677
.7	.9310	1.312	0.092	−0.946	−0.969
.8	.8989	1.055	0.052	−1.304	−1.344
.9	.8556	0.712	−0.048	−1.764	−1.832
1.0	.8023	0.295	−0.330	**−2.448**	−2.457
Formyl-α-aminobutyric acid					
0.0	1.0043	0.256	0.0		
.2	0.9816	.313	0.145	0.019	0.021
.4	.9600	.475	0.397	−0.107	−0.120
.6	.9297	.666	0.635	−0.498	−0.490
.8	.8812	.691	0.768	−1.246	−1.213
.9	.8492	.586	0.769	−1.780	−1.767
1.0	.8018	.355	0.651	−2.496	−2.497
Formylleucine					
0.0	1.0006	0.185	0.0		
.2	0.9807	0.364	0.359	0.195	0.212
.4	.9714	1.163	0.975	0.318	0.200
.6	.9622	1.936	1.326	−0.151	−0.129
.7	.9554	2.113	1.421	−0.590	−0.466
.8	.9385	2.156	1.491	−1.136	−0.947
.9	.9183	2.070	1.534	−1.790	−1.625
1.0	.8726	1.792	1.556	−2.548	−2.544
Hydantoic acid					
0.0	1.0112	0.329	0.0		
.2	0.9835	.272	−0.029	−0.122	−0.116
.4	.9575	.246	−0.008	−0.381	−0.379
.6	.9166	.211	0.009	−0.829	−0.855
.8	.8619	.124	−0.106	−1.596	−1.652
1.0	.7865	.0242	−0.630	−2.958	−2.958
Methylhydantoic acid					
0.0	1.0045	0.193	0.0		
.2	0.9795	.187	0.042	−0.071	−0.060
.4	.9541	.212	0.162	−0.290	−0.285
.6	.9160	.223	0.270	−0.747	−0.746
.8	.8649	.173	0.274	−1.535	−1.566
1.0	.7877	.044	−0.139	−2.965	−2.963
Aspartic acid hydantoin					
0.0	1.0016	0.0705	0.0		
1.0	0.7878	0.0141	−0.190		

The smallest members of these three series, hydantoin, hydantoic acid and formylglycine, are less soluble per volume of solvent the larger the fraction of alcohol. The hydantoins and formyl compounds of aminobutyric acid and of leucine are all more soluble in alcohol than in water. However, if solubility be expressed as moles per liter, the solubility maximum occurs not in alcohol, but in alcohol–water mixtures. The maximum for the two derivatives of leucine and for formyl-α-aminobutyric acid occurs in 80% alcohol. The hydantoin of α-aminobutyric acid is most soluble in 60% alcohol. This substance is approximately 3 times as soluble in this alcohol–water mixture as in either pure solvent, and suggests a type of behavior which is characteristic of a class of proteins, the prolamines. The member of the series containing two less CH_2 groups is more than ten times as soluble in water as in alcohol, and that containing two more CH_2 groups is very nearly ten times as soluble in alcohol as in water.

Amino acids such as glycine may be compared with inorganic salts because of their far smaller solubility in alcohol than in water. Amino acid derivatives, such as formylleucine, may be compared with such organic molecules as benzamide. The measurements of Holleman and Antusch[23] upon benzamide, acetanilide and acetnaphthalide are included in Fig. 2, since they indicate the characteristic form of the solubility curve of uncharged organic molecules. The solubility curves of formylleucine and of benzamide are almost superimposed, precisely as would be those of glycine and thallous chloride, or cystine and barium iodate.

Slight differences in the shapes of the various curves are more readily examined if one plots log N_3/N_3^0 − 0.03 $v_2^2\Phi$ against v_2 or N_2. So plotted the results of all the α-amino acids superimpose, excepting in strongly alcoholic solutions, where amino acids of branched chains diverge somewhat from their straight chain isomers. In strongly alcoholic solution the amino acid derivatives of each series converge, whether derived from straight chain or branched amino acids. In systems poor in alcohol and rich in water, however, the quantity log N_3/N_3^0 − 0.03 $v_2^2\Phi$ is greater the longer the hydrocarbon chain. Apparently the forces between the hydrocarbon chain and alcohol as solvent are under these circumstances large in comparison with those between water and the polar terminal groups.

Although empirical expressions may be derived, which describe the observed solubility relations of amino acid derivatives, they cannot, at the present, be deduced from such theoretical considerations as those developed by van Laar,[24] Scatchard[25] and Hildebrand.[26] The expression which has been found adequate to describe the ratio of solubility in water and in alcohol may be written

$$\log N_3^A/N_3^0 - 0.03\Phi = K_2' \qquad (3)$$

where K_2' is the intercept in Fig. 1. The contours of the solubility curves are roughly given if the right-hand side of this expression has the form

$$\log N_3/N_3^0 - 0.03v_2^2\,\Phi = v_2\left(\frac{K_1v_1}{1+v_1} - K_2'\right) \qquad (4)$$

in which the term containing K_1 vanishes both in water and in alcohol and has the following values for the amino acid derivatives studied.[27]

TABLE IV

Substance	Φ	K_1	K_2'
Hydantoin	65.2	5.12	2.543
Hydantoin of aminobutyric acid	99.1	6.76	2.448
Hydantoin of leucine	131.5	6.96	2.525
Formylglycine	70.6	4.64	2.457
Formylaminobutyric acid	104.9	5.86	2.497
Formylleucine	136.8	8.12	2.544
Hydantoic acid of glycine	77.6	5.36	2.958
Hydantoic acid of alanine	94.2	6.00	2.963

Whereas K_2' is independent of the size of the molecule and depends upon the structure of terminal groups, the value of K_1 increases with increase in the hydrocarbon chain.[28] The value of K_1/Φ is 0.066 ± 0.013 for all the substances studied. Variation in the ratio K_1/Φ depends upon the fact that it also reflects changes in the volume fraction occupied by the solute.

V. Influence of Zwitterionic Structure

Our concern at the moment is less with the influence of the hydrocarbon chain on the behavior of uncharged molecules than with differences in the behavior between charged and uncharged molecules. This problem is perhaps best approached through the study of uncharged isomers of amino acids and peptides. The amides of dicarboxylic acids form such a series with the hydantoic acids, asparagine and glycylglycine being isomeric with methylhydantoic acid. Of the three isomers asparagine is an α-amino acid, and glycylglycine has a far greater dipole moment, whereas the neutral molecule of methylhydantoic acid, presumably, bears no electric charges. The apparent specific and molal volumes of most organic substances are greater in aqueous solution than in the solid state. The apparent molal volume of methylhydantoic acid is almost the same in dilute aqueous solution as in the solid state. In this it behaves much as does urea, and very differently from amino acids, peptides and proteins, whose specific density appears far greater

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[23] Holleman and Antusch, *Rec. trav. chim.*, **13**, 277 (1894).

[24] Van Laar, *Z. physik. Chem.*, **72**, 723 (1910); **83**, 599 (1913).

[25] Scatchard, *Chem. Rev.*, **8**, 321 (1931).

[26] Hildebrand, "Solubility," The Chemical Catalog Co., New York, 1924.

[27] Activity coefficients of water in alcohol–water mixtures, calculated from the vapor pressure measurements of Dobson [*J. Chem. Soc.*, **127**, 2866 (1925)], may also be described by an equation of this form with K_1 equal to 0.96 and K_2 equal to 1.86.

[28] The agreement between observed and calculated solubility ratios becomes still closer if for the right-hand side of equation (4) we substitute the expression: $v_2(K_1v_1/(1 + K_3v_1) - K_2)$. When the value of K_3' approaches unity that of K_1 approaches its value in equation (4). The more complicated equation describes differences in solvent action at certain alcohol–water mixtures of substances, the ratios of whose solubility in water and alcohol are entirely comparable. Theoretically, this effect should still be separated from that due to change in the volume occupied by solute molecules.

in solution as a result of electrostriction of the solvent.

DENSITY AND APPARENT VOLUMES OF CERTAIN SUBSTANCES

Substance	Density in solid state	App. molal vol. in solid state	App. molal vol. in aqueous soln.	Δ
Urea	1.335[a]	44.9[a]	44.3	0.6
Methylhydantoic acid	1.398	94.4	94.2	.2
l-Asparagine	1.543[a]	85.5[a]	78.0	7.5
Glycylglycine	1.552	85.1	77.2	7.9

[a] "International Critical Tables," Vol. I.

The solubility relations of the isomers also may be contrasted. Although the apparent molal volumes of α-aminobutyric acid and of *l*-asparagine are very similar, the solubility ratios are very different, whereas those of *l*-asparagine and glycine are very similar although the apparent molal volume of the latter is far smaller. The fact that asparagine and glutamine have the same solubility ratio in water and alcohol has suggested that CH_2 groups placed between polar groups do not increase the ratio of solubility in alcohol to that in water. Provided the CH_2 groups in asparagine are considered without effect, the very comparable curves for the solubility ratios of glycine and asparagine may be understood (Fig. 2). The CH_2 groups in hydantoic acid and in glycine are also situated between polar groups.

The logarithm of the solubility ratios, log N_3^{A}/N_3^0, of asparagine is -3.402 and of glutamine -3.466. Subtracting the value -0.630 of hydantoic acid yields -2.77 and -2.84 as the differences in solubility ratio due to zwitterionic structure. If we assume tentatively that this comparison eliminates specific chemical effects, and that it reveals the electrostatic forces due to the charged groups of the amino acids, these are given by dividing by the difference in the reciprocals of the dielectric constants.

$$K_2\left(\frac{1}{D}-\frac{1}{D^0}\right) = \left\{ -\begin{matrix} 2.77 \\ 2.84 \end{matrix}\right.$$

All of the substances compared have sufficiently low solubilities in both solvents, that the solubility ratios may be considered to yield activity coefficients. The activity coefficients due to electrostatic forces may, therefore, be taken as 97 or 100, in excellent agreement with that deduced from the change in solubility of α-monoamino monocarboxylic acids in systems containing small volume fractions of alcohol (see reference (I), Table III).

The comparable calculation for glycylglycine yields -3.74 for the difference due to the peptide zwitterion, and 131 for K_2.

These calculations are subject to criticism on the grounds that although asparagine, glycylglycine and hydantoic acid contain no terminal CH_2 groups, and may therefore be compared as the first members of series,[29] the shift of a proton in the zwitterions yields different terminal groups, and further that the CONH group is terminal in asparagine and glutamine, but not in hydantoic acid and in glycylglycine. We shall consider the influence of this group from the point of view of position and structure in a subsequent communication. For the moment it should suffice to point out that addition to the solubility ratio of hydantoic acid of a term of the form of the electrostatic force effect (log $N_3^{\mathrm{A}}/N_3^0 - 97\ (1/D - 1/D^0)$) does not yield as high results for change in solubility with change in dielectric constant as has been observed for α-amino acids in systems containing small volume fractions of alcohol, whereas the change observed for asparagine is still greater. These effects are in the direction expected from this study of the derivatives of the amino acids, which are more soluble in such systems the larger the proportion of non-polar groups in their molecules.

Summary

1. Derivatives of the amino acids which are not zwitterions have been prepared and studied.
2. The melting points of all the derivatives are lower, and the apparent molal volumes higher, than of the amino acids.
3. The ratio of the solubility in alcohol to that in water is increased approximately three-fold for each terminal CH_2 group in the molecule. This rule applies both to amino acids and to their derivatives studied.
4. A CH_2 group situated between strongly polar groups, as in aspartic acid and asparagine, does not affect measurably the solubility ratio.
5. A comparison of asparagine and hydantoic acid yields an estimate of the effect of zwitterionic structure upon the solubility ratio.
6. The solubility of amino acids in alcohol–water mixtures varies in the same manner as salts, whereas the solubility of amino acid derivatives varies in the same manner as other uncharged organic molecules.

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[29] Since they also have the same apparent molal volumes, the differences ascribed to electrostatic forces would be the same whether calculated in terms of K_2, or K_2' in equation (2).