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

Studies in the Physical Chemistry of Amino Acids, Peptides and Related Substances. IV. The Distribution Coefficients of Amino Acids between Water and Butyl Alcohol

BY ALBERT ENGLAND, JR., AND EDWIN J. COHN

Butyl alcohol has been employed in the extraction of amino acids from aqueous solutions by Dakin[1] and several subsequent investigators. Dakin carried out a continuous extraction in a Kossel–Kutscher apparatus, either at atmospheric or reduced pressure, and noted that only monoamino monocarboxylic acids were carried over by the butyl alcohol. It has since been noted by H. B. Vickery and in this Laboratory that trivalent amino acids are also extracted in the neighborhood of their isoelectric points; dicarboxylic acids near P_H 3, and the basic amino acids at alkaline reactions.[2,3]

Although only free amino acids apparently are extracted by butyl alcohol, their complete extraction has not always been accomplished readily. Thus Dakin encountered difficulty in extracting all of the monoamino monocarboxylic acids from hydrolyzed gelatin and resorted to propyl alcohol to effect the separation. Although propyl alcohol is ordinarily miscible with water, the amino acids brought about the necessary separation into two layers. The gelatin hydrolysate contained large amounts of glycine, whereas the other proteins analyzed by Dakin were richer in amino acids of longer hydrocarbon chains. Accordingly it seemed desirable to study the influence of the concentration of the amino acid, and the length of its hydrocarbon chain, upon extraction.

Instead of employing continuous extraction the distribution of amino acids between water and butyl alcohol at given volume and temperature has been investigated quantitatively. The distribution coefficients calculated from the ratio of concentrations in the two liquid phases yield in dilute solution their relative fugacity, and have thermodynamic significance.

Materials and Methods

The amino acids were derived from the same sources, and purified by much the same procedures as those employed in the solubility studies of amino acids in mixtures of water and ethyl alcohol.[4] Results with α-aminovaleric acid are reported in this paper, although no preparation has been found which gives a satisfactory solubility in water. That employed in this investigation was a recrystallized Eastman Kodak product, as were the glycine, alanine, valine and α-aminobutyric and caproic acids. The sample of *l*-leucine was from Hoffmann–LaRoche.

The butyl alcohol was an Eastman Kodak product, further purified by repeated distillation. Only the fraction boiling from 116.5 to 117.5° was employed. The residue from butyl alcohol thus purified was very small, and contained no nitrogen.

Definite volumes of water and butyl alcohol were employed, and the two phases containing some definite amount of dissolved amino acid were equilibrated for approximately forty-eight hours in a constantly rotated separatory flask immersed in a water thermostat at 25.0°. The alcohol was generally saturated with water before being mixed with the aqueous phase in which the amino acid was dissolved, so as to diminish the immediate interchange of liquid between the phases. After equilibration the upper layer was withdrawn by pipet from the separatory flask, before the lower layer was run out. Aliquots were then taken for the various analyses.

The butyl alcohol was largely removed by vacuum distillation from the aliquots employed in estimating the distribution coefficients of the amino acids. The amino acid concentrations in the respective phases were then determined by Kjeldahl nitrogen analysis.

The amino acids cause a redistribution of the butyl alcohol and the water in the two phases and this was estimated by means of a Bausch and Lomb dipping refractometer. Hill and Malisoff[5] have studied the solubility of butyl alcohol in water, and Munch[6] the refractive index of such solutions. A curve constructed from their data was employed as a basis for estimating the concentration of butyl alcohol in the systems containing amino acids. Aliquot parts of each phase were, however, first freed from the amino acids by distillation. In analyzing for butyl alcohol a 5-cc. aliquot was usually distilled into a 100-cc. volumetric flask, distilled water being added to ensure complete washing over of the alcohol, and a sufficient amount of water to effect its solution. The volumetric flask was then filled to the mark, and the amount of butyl alcohol it contained estimated by determining its refractive index, also at 25°. The high solubility of water in butyl alcohol, approximately 9.5 moles per liter at 25°, makes it necessary to attain great accuracy if the changes in its solubility due to low concentrations of amino acids are to be followed. The high solubility of many amino acids, and low solubility of butyl alcohol, 0.973 mole per liter at 25°, in water renders greater the changes in concentration of the various components of this phase, which

(1) Dakin, *Biochem. J.*, **12**, 290 (1918); *J. Biol. Chem.*, **44**, 499 (1920); *Z. physiol. Chem.*, **130**, 159 (1923).

(2) Cohn, *Ergebn. Physiol.*, **33**, 781 (1931).

(3) Calvery, *J. Biol. Chem.*, **94**, 613 (1932).

(4) Cohn, McMeekin, Edsall and Weare, THIS JOURNAL, **56**, 2270 (1934).

(5) Hill and Malisoff, *ibid.*, **48**, 918 (1926).

(6) Munch, *ibid.*, **48**, 994 (1926).

was therefore more readily investigated by the rather simple procedures employed in this work.

Influence of the Hydrocarbon Chain in Three-Phase Systems.—The difference in concentration of an amino acid distributed between butyl alcohol and water is greater the smaller the hydrocarbon chain. This depends less on large differences in the solubility of the amino acids in the alcohol phase, than on the much greater solubility of amino acids with short hydrocarbon chains in the aqueous phase. By mutual interaction the butyl alcohol diminishes the solubility of amino acids in water, and the amino acids also diminish the solubility of butyl alcohol in the water.

The solubilities in water of glycine, alanine, α-aminobutyric and α-aminocaproic acids have been reported previously.[4] The solubility of these amino acids in water saturated both with respect to the amino acid and butyl alcohol has now been determined.

TABLE I

SOLUBILITY IN WATER SATURATED BOTH WITH BUTYL ALCOHOL AND AMINO ACIDS

	Glycine	Alanine	α-Amino-butyric acid	α-Amino-caproic acid
Amino acid soly. in water, C_3^0	2.886	1.660	1.800	0.087
Amino acid soly. in water satd. with butyl alcohol, C_3	2.443	1.316	1.614	0.067
Butyl alcohol soly. in water satd. with amino acid, C_2	0.44	0.55	0.77	0.95
$(\log C_2/C_2^0)/C_3$	− .14	− .19	− .063	− .16
$(\log C_3/C_3^0)/C_2$	− .16	− .18	− .061	− .12

Glycine, which is the most soluble in water of the α-amino acids, has the greatest influence in diminishing the solubility of the butyl alcohol. It is this effect which leads to the separation into two phases of lower alcohols in the presence of high concentrations of the amino acids.

The salting-out effect in these saturated solutions is smaller than in dilute solution (Fig. 1), excepting perhaps in the case of α-aminocaproic acid which is very insoluble. In solutions dilute with respect to amino acids the related influence of butyl alcohol upon amino acid solubility is more accurately determined.

Butyl alcohol saturated with water contains approximately 9.5 moles of the latter at 25°. In the presence of appreciable concentrations of the smaller amino acids the water in the butyl alcohol is appreciably reduced, though by no means to the same extent as is butyl alcohol in water. Ethyl alcohol containing approximately 15% of water by volume would contain approximately the same number of moles of water as the butyl alcohol phase in the systems we are considering. Moreover, the solubility of the amino acids would be of the same order. The solubilities of the above amino acids in 80% ethyl alcohol are compared in the accompanying table with butyl alcohol saturated with water and amino acid.

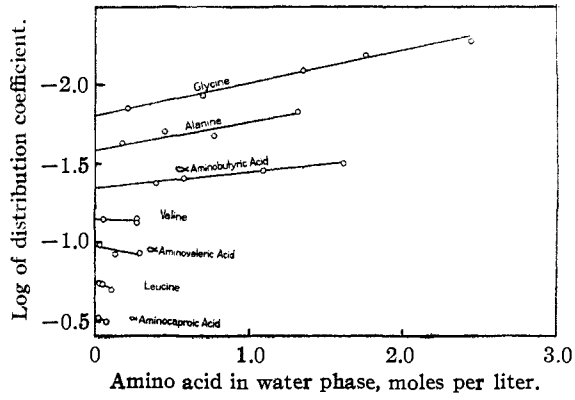

Fig. 1.—Distribution coefficients of amino acids between butyl alcohol and water.

TABLE II

	Glycine	Alanine	α-Amino-butyric acid	α-Amino-caproic acid
Soly. in 80% EtOH	0.0278	0.0359	0.0668	0.0130
Soly. in BuOH satd. with water	.0129	.0198	.0513	.0204

Although amino acids tend to be more soluble in water the shorter the hydrocarbon chain, their solubilities tend to be greater in alcoholic solutions the longer their hydrocarbon chains,[7] though α-aminobutyric acid because of its loose packing in the solid state is always more soluble than would otherwise be expected. These relations obtain also in the butyl alcohol systems, but appear not to be of sufficient magnitude to facilitate the separation of the aliphatic α-amino acids from each other.

The influence of the hydrocarbon chain upon the distribution coefficient in these three-phase systems is graphically represented by the hollow circles in Fig. 2. The two- to three-fold increase

(7) Cohn, *Naturwissenschaften*, **20**, 663 (1932).

in the distribution coefficient due to each CH_2 group is reminiscent of the threefold increase in the ratio of the solubilities in water and ethyl alcohol associated with each additional CH_2 group in amino acids and their derivatives.[4,8]

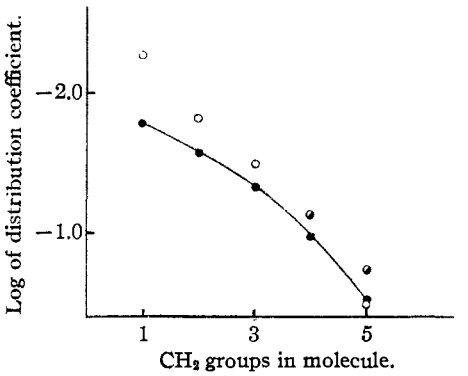

Fig. 2.—○, *n*-Amino acids in saturated solution; ●, *n*-amino acids at infinite dilution; ◐, branched amino acids at infinite dilution.

Influence of Amino Acid Concentrations in Two-Phase Systems.—The distribution of amino acids between butyl alcohol and water is a function of the amino acid concentration. Thus glycine has a higher distribution coefficient the lower its concentration (Table III). The logarithms of the distribution coefficients of the various amino acids studied are represented in Fig. 1. Within the limit of accuracy of the present measurements they vary proportionately as the concentration of amino acid in the water phase. Straight lines have therefore been drawn through the points at various concentrations of the amino acids. The range of concentrations for the smaller and more water soluble amino acids is sufficiently large so that the slope may be considered reasonably well known. It diminishes from just over 0.2 in the case of glycine to 0.1 for α-aminobutyric acid. The measurements upon valine show no appreciable drift in distribution coefficient with concentration, and those with amino acids of still longer hydrocarbon chain suggest slopes in the opposite direction, though the range of concentrations studied, and the accuracy of the measurements, do not permit a final decision on this point.

The logarithms of the distribution coefficients at infinitely small amino acid concentrations—that is, the intercepts of Fig. 1—are plotted in Fig. 2 against the number of CH_2 groups in the molecule. A very regular relation appears to obtain between the straight chain compounds. Isomers of branched chains such as valine and leucine behave like shorter molecules, an effect noted also in the solubility relations in mixtures of water and ethyl alcohol.[4]

(8) McMeekin, Cohn and Weare, THIS JOURNAL, 57, 626 (1935).

TABLE III

DISTRIBUTION COEFFICIENTS OF α-AMINO ACIDS BETWEEN BUTYL ALCOHOL AND WATER AT 25°

Amino acid concentration, moles per liter: Water phase	Alcohol phase	Distribution coefficient	Log of distribution coefficient
	Glycine		
0.208	0.0029	0.0142	−1.848
0.701	.0083	.0119	−1.925
1.353	.0108	.0081	−2.092
1.760	.0113	.0065	−2.187
2.443	.0129	.0053	−2.276
	Alanine		
0.174	0.0041	0.0237	−1.625
.454	.0090	.0198	−1.703
.769	.0163	.0212	−1.674
1.316	.0198	.0150	−1.824
	α-Aminobutyric acid		
0.396	0.0166	0.0420	−1.377
0.577	.0228	.0394	−1.405
1.094	.0386	.0352	−1.454
1.614	.0513	.0318	−1.498
	Valine		
0.054	0.0038	0.0722	−1.142
.054	.0039	.0725	−1.140
.268	.0193	.0722	−1.142
.271	.0206	.0762	−1.118
	α-Aminovaleric acid		
0.022	0.0023	0.105	−0.979
.128	.0153	.120	− .921
.270	.0302	.112	− .951
	Leucine		
0.023	0.0043	0.183	−0.737
.047	.0088	.187	− .728
.101	.0204	.202	− .695
	α-Aminocaproic acid		
0.020	0.0061	0.310	−0.509
.067	.0204	.328	− .484

The influence of variation in amino acid concentration upon the concentration of butyl alcohol in the water phase has been estimated by measurements of the refractive index. The results are reported in Fig. 3. Increasing concentration of amino acids has a diminished effect both upon the concentration of butyl alcohol in water, and its logarithm. The salting-out of the non-electrolyte by the amino acids appears to diminish as the electrostatic forces in the system increase. Glycine has a greater salting-out effect than α-amino-

butyric acid, the value of the ratio $(\log C_2/C_2^0)/C_3$ being -0.20 for the former and -0.12 for the latter in dilute solution, a value of the same order as that calculated in Table I for α-aminocaproic acid. In their saturated solutions this ratio is reduced only to -0.14 for glycine and -0.19 for alanine, but to -0.06 for α-aminobutyric acid.

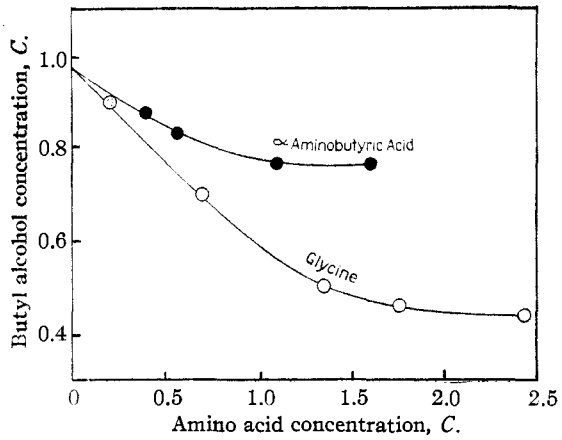

Fig. 3.—Influence of amino acids upon solubility of butyl alcohol in water.

The amino acids of still longer hydrocarbon chains, which in any case have far smaller solubilities in water, have far less influence on the solubility of butyl alcohol in water. The very profound influence of glycine and alanine in salting out butyl alcohol unquestionably accounts for the difficulties encountered by Dakin in his efforts to extract the monoamino monocarboxylic acids from gelatin, which contains 34% of these amino acids. Comparable difficulties might be expected with fibroin, which contains 65% glycine and alanine, whereas most other proteins thus far investigated contain not more than 11% amino acids of short hydrocarbon chain. It is apparently the length of the hydrocarbon chain that influences the solubility in alcohol–water mixtures of substances alike in other respects.

Summary

1. The distribution coefficients of α-amino acids between butyl alcohol and water have been measured at 25°.

2. The concentration in the water phase tends to be smaller and in the alcohol phase to be greater the longer the hydrocarbon chain.

3. Amino acids with branched chains tend to behave in butyl alcohol–water as in ethyl alcohol–water mixtures like smaller molecules than their straight chain isomers.

4. Butyl alcohol diminishes the solubility of amino acids, and amino acids diminish the solubility of butyl alcohol in water. The salting-out effect is greatest for the smallest amino acid, glycine.

5. Salting-out appears to diminish with increasing concentration presumably because of electrostatic forces.

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Studies of Multivalent Amino Acids and Peptides. III. The Dielectric Constants and Electrostriction of the Solvent in Solutions of Tetrapoles

BY JESSE P. GREENSTEIN, JEFFRIES WYMAN, JR., AND EDWIN J. COHN

I. *Introduction.*—Amino acids in the neutral state bear a positive $-NH_3^+$ and a negative $-COO^-$ group and the distance of separation of these charges determines predominantly the values of the dipole moment the dissociation constants and the amount of electrostriction of the solvent. The naturally occurring α-amino acids, with the exception of cystine, contain but one positive and one negative charge in the isoelectric state. This condition obtains moreover in the case of even the largest polypeptides so far prepared, containing respectively 18 and 19 simple amino acid residues.[1] These substances, although having a linear dimension comparable to the diameter of proteins, are nevertheless simple dipoles.[2] On the other hand, proteins must be considered to be multipoles. Egg albumin for example contains approximately 27 ammonium and 27 carboxyl groups.[3] The interpretation of protein behavior

(1) Fischer, *Ber.*, **40**, 1754 (1907); Abderhalden and Fodor, *ibid.*, **49**, 561 (1916).

(2) The length of peptides in the solid state is given roughly by multiplying the number of amino acid residues in the chain by 3.5 Å. [Astbury, *Trans. Faraday Soc.*, **29**, 193 (1933)]. The diameter of egg albumin according to Svedberg is 44 Å. [Svedberg, *Kolloid-Z.*, **51**, 10 (1930)].

(3) Cohn, *Physiol. Rev.*, **5**, 349 (1925).