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

Studies in the Physical Chemistry of Amino Acids, Peptides and Related Substances. VI. The Densities and Viscosities of Aqueous Solutions of Amino Acids

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1. Introduction

The simpler crystalline amino acids, peptides and proteins are for the most part constituted of the same chemical groups. The number of CH_2 and CONH groups, of ammonium and carboxyl groups, their spatial arrangement and, therefore, the size and shape of the molecules differ widely. None the less, certain properties are independent of the size, others of the shape of molecules. Thus amino acids are relatively small molecules, whereas even the smallest of the proteins have colloidal dimensions. Despite this discrepancy in size, their densities and viscosities in aqueous solution are of the same order of magnitude. These properties of proteins have been abundantly investigated. Since the structure of proteins is as yet incompletely apprehended, we have now supplemented their study by measuring the densities and viscosities of amino acids of known structure.

The partial specific volumes of most proteins are close to 0.75.[1] With the exception of gelatin all of the proteins studied by Svedberg have partial specific volumes between 0.731[2] and 0.759.[3] That of gelatin is 0.682.[4] Gelatin contains over 25% glycine, and glycine is both the smallest and the densest of the amino acids. The apparent specific volume of glycine in dilute aqueous solution is 0.583 cc. All other amino acids are less dense, the volume occupied per gram increasing with the number of CH_2 groups in the molecule to 0.684 for *d*-alanine, 0.770 for *d*-valine, and 0.817 for *l*-leucine.[5,6] These values for the amino acids thus differ far more from each other than do those of the proteins whose specific volumes are calculable from those of the amino acids that they contain.[6]

The molal volumes of amino acids and proteins differ enormously. The molal volume of glycine has been estimated to be 57 cc.,[6] and that of egg albumin to be 25,500 cc.[1] Both molecules are considered to be spherical, the radius of the former being 2.82 Å.,[6] that of the latter 21.6 Å.[1] The viscosity of such a protein as egg albumin is, however, not very different from that of glycine when both solutes occupy the same volume fraction of solution. Relative viscosities are plotted in Fig. 1, against the product of the molal volume, V, and concentration, C, in one liter. The coefficient φ generally employed to denote volume fraction is, therefore, $VC/1000$.

The similarity between the curve of glycine (curve 5) and that of egg albumin (curve 6) is consistent with the notion that the viscosity of spherical molecules is independent of their volume. Einstein's theory demands that viscosity be independent of the molecular volume, provided molecules are large, spherical and uncharged. All proteins are large, some are spherical, but even at their isoelectric points, where the net charge is zero, these molecules bear electrically charged groups, which give rise to electrostriction of the solvent and electrostatic forces with ions or zwitterions.

That egg albumin approximately obeys Einstein's law, relating viscosity to concentration, was suggested by Loeb.[7] Hemoglobin and serum albumin, whose molecules—according to Svedberg[1]—are larger and not quite spherical, are, however, more viscous than demanded by the coefficient of Einstein's equation.[8] None the less, hemoglobin[9] like egg albumin[10] obeys Poiseuille's law, relating viscosity to pressure. A great many protein molecules are, however, highly asymmetrical in shape, and give rise both to anomalous viscosity and to double refraction of flow,[11] in this respect resembling the long chain polymers studied by Staudinger and his collaborators.[12] Staudinger estimates that anomalous viscosity occurs only in very long molecules, and this attribute of the behavior of certain proteins will not, therefore, be

(1) Svedberg, *Kolloid Z.*, **51**, 10 (1930).
(2) Svedberg, THIS JOURNAL, **50**, 1399 (1928).
(3) Svedberg, *ibid.*, **50**, 525 (1928); **51**, 3523 (1929).
(4) Krishnamurti and Svedberg, *ibid.*, **52**, 2897 (1930).
(5) Cohn, *Science*, **79**, 83 (1934).
(6) Cohn, McMeekin, Edsall and Blanchard, THIS JOURNAL, **56**, 784 (1934); *J. Biol. Chem.*, **100**, Proc. xxviii (1933).
(7) Loeb, *J. Gen. Physiol.*, **4**, 73 (1921-22).
(8) Cohn and Prentiss, *ibid.*, **8**, 619 (1927).
(9) Loughlin and Lewis, *Biochem. J.*, **26**, 476 (1932).
(10) Boehm and Signer, *Helv. Chim. Acta*, **14**, 1370 (1931).
(11) Von Muralt and Edsall, *J. Biol. Chem.*, **89**, 315, 351 (1930); Edsall, *ibid.*, **89**, 289 (1930).
(12) Staudinger, "Die hochmolekülaren organischen Verbindungen," Julius Springer, Berlin, 1932.

reflected by the behavior of the amino acids of which they are composed.

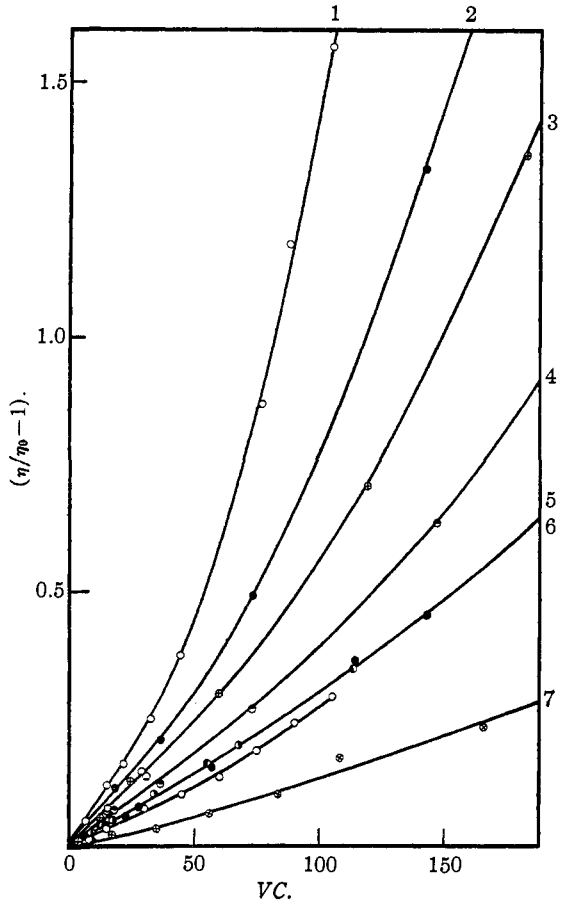

Fig. 1.—Relative viscosity of amides, acids, amino acids and proteins as a function of the volume fraction of the solute: (1) serum albumin O; (2) lysylglutamic acid ●; (3) ε-aminocaproic acid ⊕, hemoglobin O; (4) β-alanine ◓; (5) glycine ●, propionic acid ◑, propionamide ◐; (6) egg albumin O; (7) formic acid ⊕, urea ⊗. The results of Hedestrand [*Z. anorg. allgem. Chem.*, **124**, 153 (1922)] on glycine at 18° are plotted as well as ours. The measurements on formic and propionic acids are those of Reyher,[21] those of urea and propionamide are those of Dunstan and Mussell,[27] others are from this Laboratory.

II. Materials and Methods

The purification of the amino acids, and of the amino acid derivatives that have been studied, have elsewhere been reported.[13,14] The viscosities were measured at 25° in Ostwald type viscosimeters, and the densities in pycnometers of approximately 10 cc. capacity. These densities have been employed not only in the correction of the viscosity measurements, but also in the estimation of molal volumes.

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

(14) McMeekin, Cohn and Weare, *ibid.*, **57**, 626 (1935).

III. Molal Volumes of Aliphatic Acids, Amides and Amino Acids

In sufficiently dilute solution the apparent molal volumes of the aliphatic acids, amides, amines and amino acids, increase by almost exactly 16.3 cc. for each additional CH_2 group, and those of the peptides and amino acid derivatives by 20 cc. for each CONH group in the molecule, at 25°. These estimates, originally made by Traube,[15] we have confirmed recently for all the above classes of substances.[6] The volumes of the first members of these series have been estimated to be 35.0, 38.3 and 40.7, respectively,[6] for formic acid, formamide and carbamic acid. As a result of electrostriction of the solvent due to their zwitterionic structure, the observed apparent molal volumes, Φ, of amino acids (Table I) are far smaller than the volumes given by the relation

$$V_{\text{amino acid}} = V_{NH_3^+ + COO^-} + V_{CH_2} = 40.7 + 16.3n_{CH_2} \quad (1)$$

Values of V (Table II) are therefore tentatively employed in estimating molecular volumes, on the assumption that variations in Φ on the whole reflect compression of solvent rather than solute molecules. The orientation of water molecules in the neighborhood of the charged groups of the amino acids might be expected to lead to behavior characteristic of a larger molecule than that given by the above calculation, whereas the apparent molal volume is far smaller, as is the case with electrolytes.

The sodium salts of aliphatic and amino acids are strong electrolytes. The volume fraction that they occupy has been estimated by tentatively assuming Webb's value[16] for the radius of the sodium ion of 1.505 Å. This leads to the volume of 8.7 cc. per gram atom of sodium. Traube's estimate of the volume occupied by the hydrogen atom is 3.1 cc. The difference, 5.6 cc., has been employed as representing the difference in volume between the sodium salts and the corresponding free acids or amino acids. Though only rough approximations, they should suffice for the comparisons of members of the same series, and certainly more nearly yield the volumes occupied by the molecules than would apparent or partial

(15) Traube, *Samml. chem. u. chem.-techn. Vortr.*, **4**, 255 (1899).

(16) Webb, THIS JOURNAL, **48**, 2589 (1926).

molal volumes uncorrected for electrostriction of the solvent.

IV. Apparent Molal Volumes of Amino Acid Solutions

The apparent molal volume is a function of the concentration. It has recently been suggested that Φ is linear in the square root of the concentration in the case of strong electrolytes,[17] and in the concentration in the case of uncharged molecules. Without entering into the controversy as to whether certain uncharged molecules, such as urea and sucrose, obey the square root law,[18] the density determinations reported in this paper (Table I) indicate that this law does not hold for all electrolytes. Variation of the apparent molal volume of the sodium salt of glycine, and also of

TABLE I

THE DENSITIES, APPARENT MOLAL VOLUMES AND VISCOSITIES OF AMINO ACID SOLUTIONS

Concn., C	Density, ρ	Apparent molal volume Obsd. Φ	Calcd. Φ	Rel. viscosity, $\eta/\eta_0 - 1$
	Glycine $\Phi = 43.25 + 0.83\ C$			
0.250	1.00500	43.48	43.46	1.038
.500	1.01285	43.64	43.67	1.076
1.000	1.02822	44.04	44.08	1.153
2.000	1.05775	44.85	44.91	
2.500	1.07172	45.33	45.33	1.452
	Na Glycine $\Phi = 42.4 + 3.89\ \sqrt{C}$			
0.250	1.01026	44.40	44.35	1.093
.500	1.02309	45.14	45.15	1.186
1.000	1.04793	46.32	46.29	1.402
2.000	1.09594	47.75	47.90	2.016
4.000	1.18523	50.15	50.18	4.700
	Glycine hydrochloride $\Phi = 67.0 + 2.54\ \sqrt{C}$			
0.250	1.00793	68.28	68.27	
.500	1.01854	68.78	68.79	
1.000	1.03927	69.52	69.54	
2.000	1.07929	70.61	70.59	
4.000	1.15534	72.17	72.08	
	α-Alanine $\Phi = 60.6 + 0.60\ C$			
0.250	1.00423	60.64	60.75	
.375	(1.00773)		60.83	1.108
.500	1.01123	60.94	60.90	
.750	(1.01822)		61.05	1.213
.999	1.02499	61.29	61.20	
1.500	1.03874	61.47	61.50	1.49
	β-Alanine $\Phi = 58.6 + 0.72\ C$			
0.250	1.00465	58.92	58.78	1.067
.500	1.01221	58.96	58.96	1.123
1.000	1.02703	59.27	59.32	1.264
2.000	1.05534	60.10	60.04	1.632
4.000	1.10807	61.49	61.48	3.050
	α-Amino-n-butyric acid			
0.250	1.00378	76.52		
.500	1.01075	76.96		1.187
1.000	1.0242	76.19		
1.500	1.03767	76.26		1.746
1.800	1.0454	76.47		
	α-Aminoisobutyric acid			
0.250	1.00338	78.12		1.096
.500	1.0100	77.46		1.196
1.000	1.02370	75.69		1.421
	β-Amino-n-butyric acid			
1.000	1.02415	76.25		1.398
1.500	1.03733	76.49		1.705
	ϵ-Aminocaproic acid $\Phi = 104.35 + 0.72\ C$			
0.500	1.01041	104.72	104.71	1.297
1.000	1.02338	105.10	105.07	1.705
1.500	1.03620	105.32	105.43	2.360
2.000	1.04822	105.84	105.79	3.194
2.500	1.06016	106.18	106.15	4.615
	Na ϵ-Aminocaproic acid $\Phi = 106.00$			
0.250	1.00877	106.68		1.192
.500	1.02032	106.94		1.430
1.000	1.04252	107.98		2.064
2.000	1.08418	109.87		
3.000	1.12078	112.19		
	ϵ-Aminocaproic acid hydrochloride $\Phi = 135.4$			
0.250	1.00518	135.56		
.500	1.01320	135.74		
1.000	1.02897	136.08		
2.000	1.05903	136.99		
4.000	1.11254	139.11		
	Formylglycine			
0.350	1.0084	70.89		
.376	(1.00925)			1.060
.753	1.02150	70.82		1.133
1.506	1.04553	71.08		1.296
	Formyl α-aminobutyric acid			
0.108	1.00002	103.70		1.031
.162	1.00144	104.07		1.047
.215	1.00288	104.60		1.066
	Methylhydantoic acid			
0.150	1.0028	94.20		1.043
.193	1.00454	93.99		1.054
	Glycylglycine $\Phi = 76.8 + 1.30\ C$			
0.250	1.01086	77.16	77.13	1.092
.500	1.02453	77.38	77.45	1.189
1.000	1.05120	78.18	78.10	1.412
1.500	1.07749	78.70	78.75	
	Lysylglutamic acid $\Phi = 172.4$			
0.0860	1.00593	172.67		1.111
.1720	1.01476	172.85		1.206
.3440	1.03223	173.49		1.489
.6718	1.06269	178.03		2.330

Values in parentheses are interpolated.

(17) Redlich and Klinger, *Monatsh.*, **65**, 137 (1934).
(18) Gucker, *Chem. Rev.*, **13**, 111 (1933).

its hydrochloride, is approximately linear in the square root of the concentration. On the other hand, the values of Φ for the sodium salt of ϵ-aminocaproic acid (Fig. 2) and of its hydrochloride[6] are not even linear in the concentration, although there is no reason to believe that they are not strong electrolytes. It would appear that molecules containing a large number of non-polar groups do not obey the square root law, even if strong electrolytes.

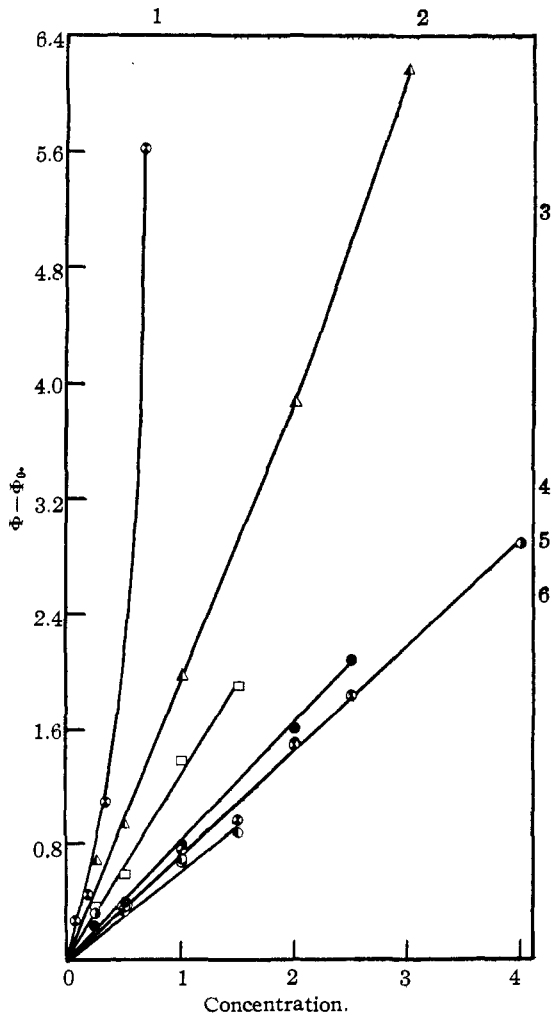

Fig. 2.—Increase in apparent molal volume as a function of the concentration: (1) lysylglutamic acid ⊕; (2) sodium ϵ-aminocaproic acid ▲; (3) glycylglycine □; (4) glycine ●; (5) ϵ-aminocaproic acid ⊖, and β-alanine ◑; (6) α-alanine ◐.

The apparent volumes of the amino acids increase more with concentration the shorter the hydrocarbon chain[19] and the greater the dipole moment (Fig. 2). The apparent molal volume of the α-aminobutyric acids does not increase with concentration, and that of α-alanine increases less than that of glycine, which contains one less CH_2 group, or than that of β-alanine with its higher dipole moment. ϵ-Aminocaproic acid with both a large number of CH_2 groups and a larger dipole moment has the same coefficient as β-alanine, and a smaller one than glycylglycine, which has roughly the same dipole moment, but whose chain is made up in part of the CONH group. The deviations from a linear relation between Φ and concentration may readily be examined by comparing the calculated and observed values in Table I. Were only the low concentrations of ϵ-aminocaproic acid and its salts considered, higher values of Φ_0 and lower slope constants would result.

The forces reflected by density determinations[20] are perhaps too intricate to be analyzed at this time. Instead of assuming that the volumes of the solute molecules change in the systems that we have investigated, it has seemed preferable to assume that the molecular volume, V, of the solute remains constant, and that increased densities reflect forces between solvent and solute, than to attribute changes in the density to changes in the volume of solute molecules, though either assumption is unquestionably too simple.

V. Viscosity of Solutions of Amino Acids, Aliphatic Acids and Amides

The viscosity of solutions of amino acids is related to the shape of their molecules. The viscosities of β-alanine, ϵ-aminocaproic and lysylglutamic acids are far greater than that of glycine at the same volume fraction (Fig. 1). This effect is not primarily related to the greater dipole moments of the longer molecules—as is demonstrated by comparison with less soluble isomeric α-amino acids—but to their longer hydrocarbon chains. This influence of the paraffin chain upon viscosity is comparable to that of aliphatic acids and amides. Indeed, measurements of the viscosity of glycine, of propionic acid, and of propionamide fall on the same curve (Curve 5 in Fig. 1), indicating that the zwitterionic condition of the amino acids, which so profoundly affects the density of their solutions,[6] has a smaller effect on their viscosity.

(19) This is confirmed not only by our measurements but by those of Dalton and Schmidt, *J. Biol. Chem.*, **103**, 549 (1933), and of Wyman, THIS JOURNAL, **56**, 536 (1934).

(20) Bridgman and Dow have studied the compressibility of certain of these amino acids, *J. Chem. Phys.*, **3**, 35 (1935).

The salts of the amino acids also behave much as do the salts of the fatty acids long since studied by Reyher[21] and Lauenstein.[22] The sodium salts of glycine and ϵ-aminocaproic acid differ by four CH_2 groups per mole. None the less, at the same volume fraction of solute the sodium salts of these two amino acids have the same viscosity as do the sodium salts of aliphatic acids (Fig. 3). A single curve describes the viscosity–concentration function of all of these sodium salts,[23] although sodium acetate, propionate and butyrate are slightly more viscous at the same volume fraction than sodium valerianate or capronate. Solutions of the sodium salts of the amino acids have viscosities most nearly like those of sodium valerianate. The shape of the anion would appear to exert a small influence in comparison with that due to a net charge.

The viscosities of these salts are far greater than those of the free aliphatic acids or amino acids, plotted in Fig. 1. In solutions, in which the solute occupies one-tenth the total volume (VC = 100), the sodium salt of ϵ-aminocaproic acid is more than one-third again as viscous as the free acid, and sodium glycinate two and one-half times as viscous as glycine. These observations are consistent with, and tend to explain, the well-known fact that amino acids and proteins[24,25,26] have minimal viscosities in the isoelectric condition.

Although the viscosities of glycine and amino acids of longer hydrocarbon chains are very different, no great difference is apparent in the form of the concentration function for the various free amino acids. The volume fractions at which various amino acids are isoviscous may, therefore, be compared by means of a coefficient of viscosity, K, independent of concentration for any given solute. For amino acids with long hydrocarbon chains, and salts, K is large since but low concentrations suffice to yield solutions isoviscous with more spherical uncharged molecules. Values of VC for the amino acids multiplied by these coefficients are plotted in Fig. 4 against relative viscosity. For present purposes these viscosity curves may be considered to coincide satisfactorily with each other, as well as with those of the aliphatic amides studied by Dunstan and Mussell,[27] and n-caproamide measured in this Laboratory. The measurements of Reyher[21] upon the aliphatic acids, though not quite as satisfactory, yield coefficients[28] also represented in Table II.

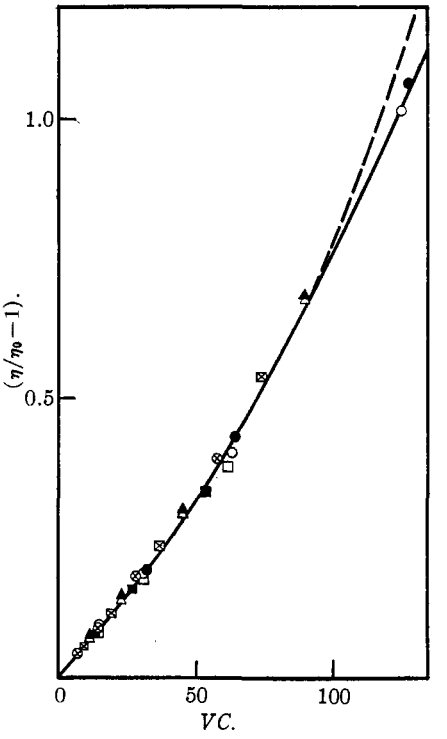

Fig. 3.—Relative viscosity of sodium salts of aliphatic acids and amino acids as a function of the volume fraction of the solute: Na glycine ○; Na ϵ-aminocaproic acid ●; Na acetate ⊗; Na propionate ⊠; Na n-butyrate △; Na isobutyrate ▲; Na isovalerianate ■; Na capronate □.

The viscosity of aqueous glycine solutions is increased by 0.152 per mole in all solutions of less than molar concentration (Table I). For all dilute glycine solutions we may, therefore, write

$$\frac{(\eta/\eta_0 - 1)}{C} = 0.152 = \frac{2.67\ V}{1000} = \frac{2.5 \times 1.068\ V}{1000} \quad (2)$$

The agreement of the coefficient 2.67 with the comparable coefficient of the Einstein equation,

(21) Reyher, *Z. physik. Chem.*, **2**, 744 (1888).

(22) Lauenstein, *ibid.*, **9**, 417 (1892).

(23) The sodium salts of a great many dicarboxylic acids were also studied by Reyher[21] and Lauenstein.[22] It is interesting to note that the viscosity of these molecules at the same volume fraction is always approximately one-third lower than that of the sodium salts of the monocarboxylic acids, although in the case of such a polyvalent acid as citric acid viscosity is greater at the same volume fraction for the disodium than for the monosodium salt, and for the trisodium salt even than for the sodium salts of the aliphatic monocarboxylic acids.

(24) Pauli and Handovsky, *Biochem. Z.*, **18**, 340 (1909); *ibid.*, **24**, 239 (1910).

(25) Loeb, "Proteins and the Theory of Colloidal Behavior," McGraw-Hill Book Co., New York, 1922, 1924.

(26) Hardy, *J. Physiol.*, **33**, 281 (1905).

(27) Dunstan and Mussell, *J. Chem. Soc.*, **97**, 1935 (1910).

(28) The shapes of the curves of formic acid,[21] formamide and urea[27] are somewhat different from those of the higher amides, the amino acids, or the proteins, and are, therefore, not plotted in Fig. 2.

2.5, must be considered fortuitous because of the small size and zwitterionic structure of the glycine molecule. None the less it seemed convenient to define the viscosity coefficient, K, so that

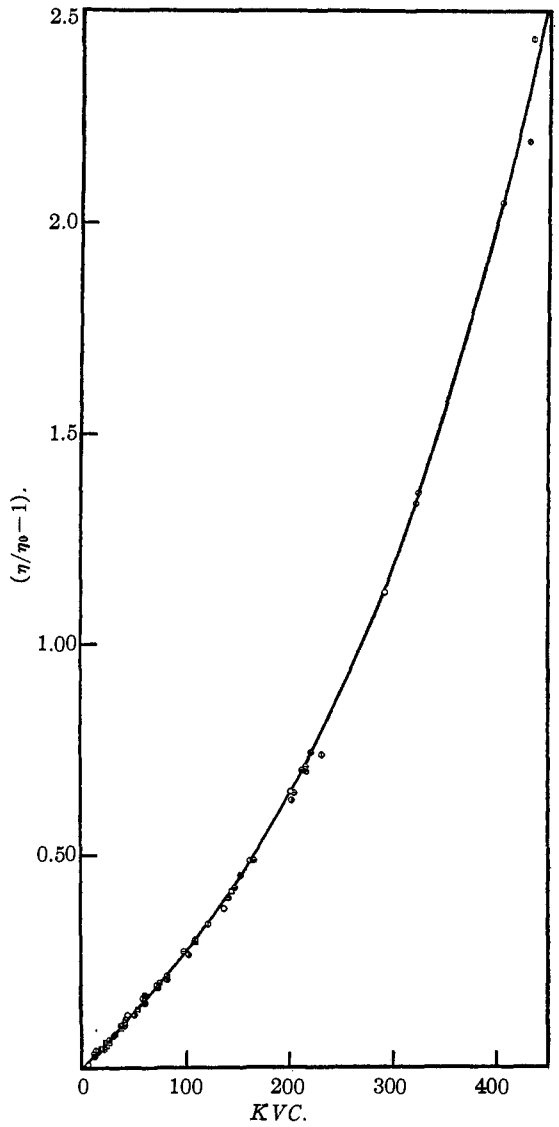

Fig. 4.—Relative viscosity as a function of the volume fraction and the constant, K, characteristic of the solute and independent of concentration: glycine ●; β-alanine ◑; α-alanine ◐; β-amino-n-butyric acid ◓; α-aminoisobutyric acid ◒; α-amino-n-butyric acid ◒; ε-aminocaproic acid ◕; lysylglutamic acid ◕; acetamide ○; propionamide ⊙; butyramide ⊖; caproamide ⊕; formylglycine ◻; methylhydantoic ◧; formyl α-amino-butyric acid ◨; glycylglycine □.

it describes deviation from the behavior of the large spherical ideal solute, contemplated by that equation. Since $VC/1000$ is the volume fraction occupied by the solute (often written φ), K for

TABLE II

	App. molal vol.[a] obsd. Φ_∞	Est. molal vol. calcd. V	Constant K	2.5 VK
Formamide[27]	38.0	39.3	0.34	33
Formic acid[21]	34.7	35.0	.39	34
Urea[27]	44.3	44.0	.54	59
Acetamide[27]	55.0	54.6	.83	113
Acetic acid[21]	50.7	51.3	.88	113
Glycine	43.25	57.0	1.07	152
Formalglycine	70.6	71.3	1.00	178
Propionic acid[21]	67.9	67.6	1.11	188
Propionamide[27]	71.0	70.9	1.07	190
β-Alanine	58.6	73.3	1.38	253
n-Butyric acid[21]	84.3	83.9	1.21	254
n-Butyramide[27]	87.0	87.2	1.20	262
α-Alanine	60.6	73.3	1.48	271
Methylhydantoic acid	94.2	93.5	1.20	281
Formyl-α-amino-n-butyric acid	103.5	103.9	1.16	302
Na Glycine	42.4	62.6	2.27	355
β-Amino-n-butyric acid	76.4	89.6	1.58	354
Glycylglycine	76.8	93.3	1.55	362
α-Aminoisobutyric acid	78.1	89.6	1.65	370
α-Amino-n-butyric acid	76.5	89.6	1.65	370
Caproamide	119.2	119.8	1.39	416
ε-Aminocaproic acid	104.35	122.2	1.76	538
Na ε-aminocaproic acid	106.00	127.8	2.32	741
Lysylglutamic acid	172.4	211.5	2.26	1195

[a] These values were obtained by extrapolation of apparent molal volumes to zero concentration from the values in Table I. The values previously reported were for the most part slightly higher and derived from measurements at finite, for the most part quarter molecular, concentrations.

glycine is 1.068. For an ideal larger spherical molecule K is unity.

The longer the hydrocarbon chain of the solute, and the more viscous the solution, the greater the value of K and the greater the deviation from a linear relation between viscosity and concentration. The viscosities of the larger amino acids are, as we have seen (Fig. 4), related to each other and to glycine in terms of the coefficient K, which describes behavior not only in dilute but in concentrated solutions. This coefficient may also be employed, however, in estimating what Staudinger calls the specific viscosity coefficient[12] in the extremely dilute range, in which the increase in viscosity per mole is a constant, by means of the relation

$$\frac{(\eta/\eta_0 - 1)}{C} = \frac{2.5\,KV}{1000} \qquad (3)$$

Values of $2.5\,KV/1000$ for amino acids and aliphatic acids and amides are plotted in Fig. 5 against the number of CH_2 groups in the molecule. Whereas the relative viscosities of long chain

amino acids divided by the concentration fall on curves, which are steeper the greater the concentration and the longer the hydrocarbon chain of solute molecules, values for this ratio estimated by means of the above equation, fall on a straight line. An equation comparable to that employed by Staudinger defines the viscosity relations of amino acids of long dipole moment

$$\frac{2.5\,KV}{1000} = \eta_{NH_3^+ + COO^-} + \eta \times n_{CH_2} = 0.052 + 0.10 \times n_{CH_2} \quad (4)$$

The first term yields the viscosity due to the ammonium and carboxyl groups, the second the increment for each additional CH_2 group in the molecule.

Staudinger assumes that the greater viscosity of long molecules is a direct function of their length. If the molecules be considered as cylinders of radius r, and length l, one may substitute for V in the above equation the expression $\pi r^2 Nl$, where N is Avogadro's number. For amino acids, having the radius 2.61 Å., of the paraffin chain[6] we have

$$\frac{2.5\,K}{1000} \times \pi r^2 Nl = 0.00474\,K \times 2.61^2\,(3.15 + 1.26 \times n_{CH_2}) \quad (5)$$

From this point of view K should deviate from unity the more, the greater the length of the molecule deviates from the diameter.

Were the length of the molecule alone responsible for viscosity, however, branched chain amino acids should be less viscous than their straight chain isomers. Although α-amino-*n*-butyric acid may possibly be slightly more viscous than α-aminoisobutyric acid, β-aminobutyric acid is considerably less viscous. β-Alanine is also slightly less viscous at the same volume fraction than α-alanine. The slope constant, $\eta \times n_{CH_2}$, appears to be the same for α- and β-amino acids, but the intercept constant is greater for the former, namely 0.07.

Comparable calculations for aliphatic acids and amides yield a similar equation

$$\frac{2.5\,KV}{1000} = 0.035 + 0.076 \times n_{CH_2} \quad (6)$$

These results are also plotted in Fig. 4. The first right hand term yields the specific viscosities of the first members of the series, formic acid and formamide. These molecules, as well as urea, have extraordinarily low viscosities.

The smaller increment per CH_2 group for aliphatic acids and amides than for amino acids might be attributed to greater rotation around free bonds leading to shorter and more nearly spherical molecules. This would at first thought appear to be contrary to the expectation as a result of the electrostatic attraction between the oppositely charged groups of the amino acids, but this expectation has not been confirmed by dielectric constant measurements.[29] More probably the larger increment in the case of the amino acids is related to the influence of polar groups on the paraffin chain.

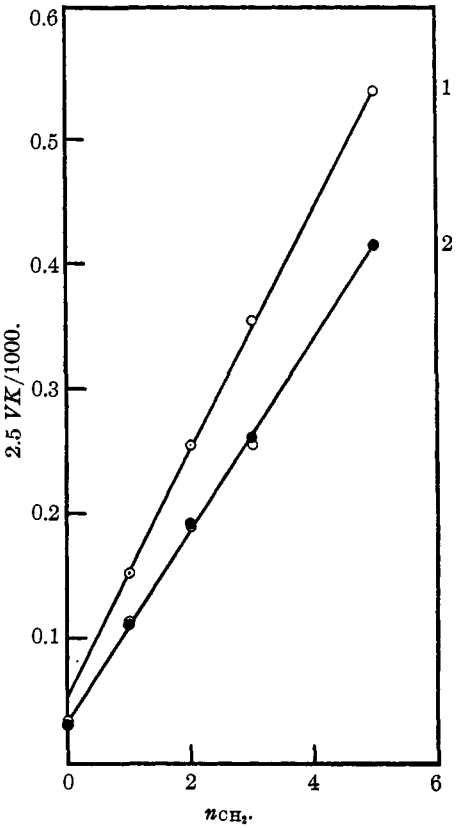

Fig. 5.—Specific viscosity in dilute solution as a function of the number of CH_2 groups in the molecule. (1) amino acids ⊙; (2) aliphatic acids O and amides ●.

Comparable observations may be made with respect to the CONH group estimated as increasing $\left(\frac{\eta/\eta_0 - 1}{C}\right)$ by 0.065 from the comparison of acetic acid with formylglycine and 0.048 from that of butyric acid with the formyl derivative of aminobutyric acid. The identical calculation in the case of zwitterions, glycylglycine and β-alanine is 0.108.[30]

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

(30) The same point is made by comparing the non-zwitterionic isomer of glycylglycine, methylhydantoic acid, with α-alanine.

Not only is the increment in viscosity per CH_2 group greater the more polar the solute, but it varies somewhat with the solvent. Staudinger[31] has studied the viscosity of aliphatic acids in benzene and carbon tetrachloride at 20°.

Although the equations relating structure and viscosity apply only in very dilute solution, they render possible the calculation of K for any member of a series in any solvent for which the specific viscosity due, respectively, to a repeating element, such as the CH_2 group, and to the remaining terminal groups, η_T, are known from the relation

$$K = 400 \frac{(\eta_T + \eta \times n_{CH_2})}{(V_T + V \times n_{CH_2})} \quad (7)$$

which for amino acids of long dipole moment is

$$K \times 400 \frac{(0.05 + 0.10 \times n_{CH_2})}{(40.7 + 16.3 \times n_{CH_2})} \quad (8)$$

Given K and V, viscosity can be estimated even in very concentrated solutions by use of such graphs as Figs. 3 or 4, or an expansion of equation (3) comparable to that previously employed for the Einstein equation

$$(\eta/\eta_0 - 1) \times 0.4 = K\varphi + (K\varphi)^{2.8} \quad (9)$$

where φ is equal to $VC/1000$.

The curve drawn in Fig. 3 is constructed on the basis of this equation. It holds not only for all viscosities for amino acids and amides up to three and a half times the viscosity of water (Fig. 4), but also for the few more concentrated measurements that have been made (Table II), which extend beyond the coördinates of the figure.

Combining equation (9) with equation (7) enables us to estimate the viscosity of any aliphatic amide, amino acid or peptide of the series, some members of which have been investigated. The dotted curve in Fig. 2 is also constructed on the basis of equation (9). It appears to hold also for the sodium salts of aliphatic and amino acids when they occupy up to 10% of the volume of the solution. At higher volume fractions their viscosities appear to increase less rapidly than the exponential term of this equation.[32]

Lysylglutamic acid is the most viscous of the zwitterionic molecules of known structure that have been investigated, and has the largest value of V and of K. Its specific viscosity is, however, somewhat greater than can be accounted for on the basis of its length or of the constants deduced for the groups that it contains.

From this point of view lysylglutamic acid might be considered constituted of glycylglycine and an amino acid with six CH_2 groups, and the specific viscosity estimated to be their sum, or (0.36 + 0.65) 1.01. The observed value, though of the right order of magnitude, is higher, namely, 1.20. This may depend on the stretched condition of this molecule in solution, previously ascribed[33] to electrostatic repulsion between the two positively charged ammonium groups on the one side of the peptide linkage, and the two negatively charged carboxyl groups on the other. The high viscosity of lysylglutamic acid would thus appear to be consistent with Werner Kuhn's[34] view that, as a result of free rotation at the valence angles of long chains, the paraffin chain is usually not stretched in solution.[35]

The polypeptide chain must be considered considerably twisted to yield the spherical molecules characteristic of certain proteins.[36] Egg albumin is, as we have seen (Fig. 1), less viscous than glycine at the same volume fraction, and hemoglobin than ϵ-aminocaproic acid. Without considering in detail the concentration function, or the possibilities of hydration in the case of the proteins,[37,38] it seems probable, as in the case of the amino acids and peptides, that it is the relation of the polar to non-polar groups,[27] and the shape of the molecules rather than their size, which determine viscosity.

Summary

1. The densities and viscosities of amino acids and peptides and of certain of their salts have been measured at 25°.

2. The law relating the apparent molal volume to the square root of the concentration of strong electrolytes appears to hold for the sodium salt and the hydrochloride of glycine, but not for salts of amino acids of long hydrocarbon chain.

3. The increase in apparent molal volume of the free amino acids with concentration is greater the greater the dipole moment and the smaller the hydrocarbon chain.

(31) Staudinger, *Z. Elektrochem.*, **40**, 434 (1934).

(32) Even the most viscous salts so far investigated are given by diminishing the constant of this term by 5%. The viscosity of the very small molecule formamide is also approximately given by this equation if the value of K of the exponential term is multiplied by 3.4.

(33) Greenstein, Wyman and Cohn, THIS JOURNAL, **57**, 637 (1935).

(34) Kuhn, *Kolloid Z.*, **68**, 2 (1934).

(35) See pp. 106–108, Cohn, "Annual Review of Biochemistry," Stanford Univ., Calif., Vol. IV, 1935.

(36) Except when spread on a surface layer where they assume the dimensions of the stretched chain.

(37) Arrhenius, *Medd. Vetenskapsakad. Nobelinst.*, **3**, No. 13 (1916); *Biochem. J.*, **11**, 112 (1917).

(38) Kunitz, *J. Gen. Physiol.*, **17**, 365 (1934).

4. The viscosities of dilute solutions of amino acids of long dipole moments increase with the length of the hydrocarbon chain, according to the equation $(\eta/\eta_0 - 1) = (0.052 + 0.10 \times n_{CH_2})\ C = 2.5\ K\varphi$, where φ is the volume fraction occupied by the solute, C its concentration per liter of solution, and V its molal volume. K is defined by this equation and represents deviation from the behavior of a large ideal spherical solute molecule.

5. The comparable equation for aqueous solutions of aliphatic acids and amides is $(\eta/\eta_0 - 1) = (0.035 + 0.076 \times n_{CH_2}) = 2.5\ K\varphi$.

6. Measurements upon aliphatic amides, amino acids and peptides even up to viscosities more than three times that of water are given by the equation $(\eta/\eta_0 - 1) = 2.5\ K\varphi + (2.5\ K\varphi)^{2.8}$.

7. The sodium salts of the amino acids are all more viscous than the free amino acids, behave much as do the sodium salts of aliphatic acids and have the same viscosity at the same volume fraction. The above equation holds for the salts of amino and aliphatic acids up to solutions as concentrated as 10% with K equal to 2.3.

8. Those proteins which obey Poiseuille's law and are approximately spherical have roughly the same viscosity at the same volume fraction as amino acids and peptides. The higher viscosities of protein salts than of isoelectric proteins may be compared with the higher viscosity of the sodium salts of the amino acids and with the higher viscosity of the sodium salts of citric acid.

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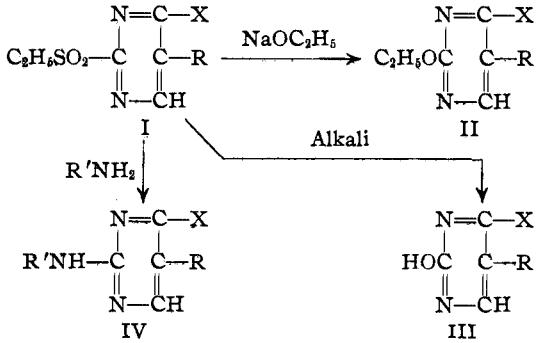
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

Researches on Pyrimidines. CXLIX. Reactions of Some 2-Ethylsulfonylpyrimidines

BY JAMES M. SPRAGUE[1] AND TREAT B. JOHNSON

A study of the action of chlorine on some ethylmercaptopyrimidines has resulted recently in the preparation of a series of new ethylsulfonylpyrimidine compounds.[2] These are the first representatives of this type to be described in the pyrimidine literature. In an attempt to establish the structure of these interesting compounds, it was observed that an ethoxyl group was easily substituted for an ethylsulfonyl group by the action of alcoholic alkali upon 2-ethylsulfonyl-4-ethoxy-5-methylpyrimidine.[2] This has led to a more detailed study of the behavior of the ethylsulfonylpyrimidines toward such reagents as sodium ethoxide, alkali, ammonia and aniline.

It has been found that the ethylsulfonyl group when substituted in a pyrimidine molecule behaves in a manner analogous to the corresponding chloropyrimidines. Thus this group may be replaced easily by an ethoxyl group when the ethylsulfonylpyrimidine (I) is treated with a cold alcoholic solution of sodium ethoxide. Consequently, it was not possible to prepare an ethoxysulfone derivative (I, X = OC_2H_5) from a chlorosulfone (I, X = Cl) by reaction with sodium ethoxide; instead the ethylsulfonyl group and chlorine were simultaneously replaced by ethoxyl. 2,4-Diethoxy-5-methylpyrimidine (II, X = OC_2H_5, R = CH_3) was formed from both 2-ethylsulfonyl-4-ethoxy-5-methyl (I, X = OC_2H_5, R = CH_3) and 2-ethylsulfonyl-4-chloro-5-methylpyrimidine (I, X = Cl, R = CH_3). Likewise, 2,4-diethoxy-5-bromopyrimidine was obtained from 2-ethylsulfonyl-4-ethoxy-5-bromo- (I, X = OC_2H_5, R = Br) and from 2-ethylsulfonyl-4-chloro-5-bromopyrimidine (I, X = Cl, R = Br).



The aminosulfone, 2-ethylsulfonyl-4-amino-5-carbethoxypyrimidine (I, X = NH_2, R = $CO_2C_2H_5$) reacts with sodium ethoxide to give 2-ethoxy-4-amino-5-carbethoxypyrimidine (II, X = NH_2, R = $CO_2C_2H_5$). Also, when 2-ethylsulfonyl-4-chloro-5-carbethoxypyrimidine (V, X

(1) Sterling Professorship of Chemistry Research Assistant (1935–36).

(2) Sprague and Johnson, THIS JOURNAL, 57, 2252 (1935).