ing lines from the normal to the enlarged partial diagram and vice versa.

Application to other Four-Component Systems.—The methods described above can be applied to systems composed of water and three salts with a common ion if the following method of plotting is followed. The figure appears in an equilateral triangular graph with vertices A, B and C and point of intersection of perpendiculars from these vertices to their opposite bases, O. Then a point is determined by plotting the mole fractions of salts A and B as coördinates along the axes OA and OB originating at the center O. The point is then moved parallel to the third axis OC, a distance equal to mole fraction of salt C.

Acknowledgment.—The author wishes to thank Professor J. J. Beaver for his constructive criticism of this paper.

Summary

A two-dimensional, quaternary phase diagram is presented in which compositions are described in all four constituents and in which graphical solutions to general phase separation problems may be made. The diagram is constructed by superimposing an orthogonal projection of a three dimensional Schreinemakers diagram upon a Jaenecke projection in such a way that the method of wet residues may be applied.

NEW YORK, N. Y. RECEIVED NOVEMBER 7, 1947

[CONTRIBUTION FROM THE METCALF RESEARCH LABORATORY, BROWN UNIVERSITY]

Properties of Electrolytic Solutions. XLIV. Conductance of Some Long Chain Salts in Ethylene Chloride, Pyridine and Nitrobenzene at 25°[1]

BY HAROLD L. PICKERING[2] AND CHARLES A. KRAUS

I. Introduction

Weaver[3] has measured the conductance of several long chain salts in ethylene chloride and nitrobenzene. In these solvents the salts behaved like normal electrolytes. It seemed worth while, therefore, to measure the conductance of a larger number of long chain salts. It was of particular interest to determine the limiting conductance of long chain ions and to find how the conductance of such ions depends on the number of carbon atoms that they contain, on the one hand, and their arrangement about the central nitrogen atom, on the other.

To this end, the conductance of a number of long chain salts was measured in ethylene chloride, pyridine and nitrobenzene. In addition, the conductance of several ordinary quaternary ammonium salts was determined in order to fill gaps in the list of ion conductances.

II. Experimental

1. **Salts.**—These were prepared according to conventional methods. It is important to use only well purified starting materials. Nitrates and picrates were prepared by metathesis of iodides with silver salts in methanol or ethanol. The following salts were prepared: (1) *n*-octadecylpyridonium nitrate (m. p., 79-80°), (2) di-*n*-octadecyldimethylammonium picrate (m. p., 75.5-76.2°), (3) di-*n*-octadecyldi-*n*-butylammonium picrate (m. p. 50.5-51.5°), (4) *n*-octadecyltri-*n*-butylammonium nitrate (m. p., 90.5-91.5°), (5) *n*-octadecyltri-*n*-butylammonium picrate (m. p., 42-43°), (6) *n*-octadecyltrimethylammonium iodide (m. p., 237-238.5°), (7) *n*-octadecyltrimethylammonium picrate (m. p., 134-135°), (8) *n*-propylpyridonium picrate (61-62°), (9) tetra-*n*-butylammonium picrate (m. p., 73.5-74.5°), (10) tetra-*n*-propylammonium picrate (m. p., 115-116°). Earlier preparations of tetra-*n*-butylammonium triphenylborofluoride (11) and formate (12) were measured after several recrystallizations.

The salts were recrystallized as follows: (1) hexane plus few drops of ethanol, (2) hot absolute ethanol, (3), (5) methanol plus few drops of ethanol, (4) dioxane-water followed by hexane-ethanol, (6), (7) absolute ethanol, (8) 99% ethanol, (9), (10), (12) 95% ethanol, (11) ethanol plus 10% hexane.

2. **Solvents.**—Solvents were prepared as described in earlier papers.[4] Solvent resistances were measured with a special parallel arm bridge permitting of precise measurements up to 10^7 ohms.

3. **Apparatus and Procedure.**—These were the same as those described in earlier papers of this series.[4]

III. Results

In Table I are given equivalent conductances at different concentrations (expressed in moles per liter of solution) for several salts in ethylene chloride. Similar data are given for solutions in pyridine in Table II and for solutions in nitrobenzene in Table III. All measurements were carried out at 25 ± 0.01°. In computations, the following values were employed for physical constants.

	Density	Viscosity	Diel. const.
Ethylene chloride	1.2455	0.00787	10.23
Pyridine	0.97792	.008824	12.01
Nitrobenzene	1.1986	.01811	34.5

IV. Discussion

1. Ethylene Chloride and Pyridine.—The data of Table I for ethylene chloride and those of Table III, for pyridine, have been analyzed

---

[1] This paper is based on a portion of a thesis presented by Harold L. Pickering in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in the Graduate School of Brown University, August, 1947.

[2] National Research Council Fellow, Brown University, 1946-1947. Present address: Research Laboratory, Stanolind Oil and Gas Company, Tulsa, Oklahoma.

[3] Weaver and Kraus, THIS JOURNAL, **70**, 1707 (1948).

[4] (a) Mead, Fuoss and Kraus, *Trans. Faraday Soc.*, **33**, 594 (1936), ethylene chloride; (b) Witschonke and Kraus, THIS JOURNAL, **65**, 2472 (1947), nitrobenzene; (c) Carignan and Kraus, *ibid.*, **71**, 2983 (1949), pyridine.

TABLE I

CONDUCTANCE OF SOME QUATERNARY AMMONIUM SALTS IN ETHYLENE CHLORIDE

$C \times 10^4$	Λ	$C \times 10^4$	Λ
A. N-Octadecylpyridonium nitrate		B. Di-*n*-octadecyldimethylammonium picrate	
29.25	6.728	12.79	12.92
3.359	15.53	3.059	20.75
1.804	19.70	2.034	23.50
0.7853	26.49	1.250	26.95
.4071	32.70	0.8192	30.01
.2027	39.78	.4463	34.25
.1070	45.88	.2192	38.60
		.1227	41.40
C. Di-*n*-octadecyldi-*n*-butylammonium picrate		D. N-Octadecyltri-*n*-butylammonium nitrate	
14.64	19.35	22.96	16.83
3.218	28.23	3.649	28.95
2.109	30.87	2.440	32.24
1.488	32.99	1.529	36.17
0.9060	35.78	0.7387	42.05
.5696	38.08	.4271	46.03
.2881	40.79	.2328	49.65
.1436	42.75	.09721	53.42
E. N-Octadecyltrimethylammonium formate		F. N-Octadecyltrimethylammonium iodide	
22.99	3.323	13.915	8.893
4.044	6.962	3.871	14.54
2.271	8.930	2.436	17.19
1.669	10.20	1.643	19.73
1.165	11.89	1.118	22.45
0.7884	14.00	0.8038	24.91
.4940	16.93	.4420	29.88
.2293	22.80	.1769	37.91
.1655	25.62		
G. N-Octadecyltrimethylammonium picrate		H. N-Propylpyridonium picrate	
17.34	10.96	37.63	11.13
3.840	18.70	4.471	23.37
2.193	22.59	2.805	27.42
1.597	25.00	1.806	31.67
1.200	27.29	1.017	37.74
0.8274	30.37	0.6386	42.88
.5738	34.15	.3482	49.51
		.2101	54.63
		.1350	58.49

TABLE II

CONDUCTANCE OF SOME QUATERNARY AMMONIUM SALTS IN NITROBENZENE

$C \times 10^4$	Λ	$C \times 10^4$	Λ
A. N-Octadecyltrimethylammonium picrate		B. N-Octadecyltri-*n*-butylammonium picrate	
23.59	22.80	18.43	21.83
10.42	24.05	9.397	22.59
4.459	24.92	4.472	23.23
2.402	25.36	2.264	23.63
1.669	25.57	1.156	23.91
0.8075	25.87	0.5718	24.11
.3536	26.09	.3041	24.30
.2060	26.16	.1411	24.43
C. Di-*n*-octadecyldimethylammonium picrate		D. Di-*n*-octadecyldi-*n*-butylammonium picrate	
20.78	20.65	30.54	19.85
9.645	21.66	10.24	21.20
4.909	22.29	5.767	21.70
2.355	22.78	3.710	22.00
1.194	23.07	2.309	22.26
0.6244	23.30	1.452	22.46
.3219	23.45	0.9777	22.60
.1530	23.56	.6178	22.74
		.3129	22.84
		.1601	22.94
E. Tetra-*n*-butylammonium triphenylborofluoride		F. N-Octadecylpyridonium nitrate	
21.91	20.79	53.21	23.04
10.59	21.70	26.10	26.07
5.795	22.23	12.79	28.40
2.848	22.71	6.618	29.97
1.291	23.07	3.255	31.16
0.7723	23.24	2.119	31.64
.3428	23.42	1.021	32.31
.1518	23.63	0.5094	32.73
		.2037	33.10

TABLE III

CONDUCTANCE OF SOME QUATERNARY AMMONIUM SALTS IN PYRIDINE

$C \times 10^4$	Λ	$C \times 10^4$	Λ
A. N-Octadecyltri-*n*-butylammonium picrate		B. Tetra-*n*-amylammonium picrate	
4.474	38.18	4.243	41.78
2.278	41.66	2.355	45.07
1.444	43.62	1.232	48.09
0.8643	45.46	0.6791	50.27
.4114	47.47	.3348	52.08
.1768	48.92	.2150	52.97
C. Tetra-*n*-propylammonium picrate		D. Tetraethylammonium picrate[a]	
2.913	49.76	5.6119	53.66
1.884	52.21	2.6677	59.17
1.104	54.72	1.6327	62.34
0.6816	56.55	0.77968	66.09
.5040	57.48	0.43674	68.27
.1912	59.56	0.21450	70.35

[a] Measurements by Dr. C. J. Carignan.

by the method of Fuoss[5] and values of the limiting conductance, Λ_0, and the dissociation constant, K, have been obtained. These values are collected in Table IV, where limiting conductances are given in column 2, cation conductances as determined by the method of Fowler[5] in column 3 and dissociation constants in column 4. In Figs. 1 and 2 are shown Fuoss plots for several salts in ethylene chloride and pyridine, respectively.

As may be seen from the figures, the salts measured in ethylene chloride and pyridine conform to the Fuoss relation within the limit of experimental error, and successive series are in good agreement.

---

[5] (a) Burgess and Kraus, THIS JOURNAL, **70**, 706 (1948); (b) Fowler and Kraus, *ibid.*, **62**, 2237 (1940).

TABLE IV

CONSTANTS FOR SALTS IN ETHYLENE CHLORIDE AND PYRIDINE

Salt	Λ_0	Λ_0^+	$K \times 10^4$
A. Ethylene Chloride			
$OctdMe_3NPi$	54.91	23.7	0.49
$Octd_2Me_2NPi$	47.73	16.5	0.78
$OctdBu_3NNO_3$	57.90	17.8	1.12
$Octd_2Bu_2NPi$	46.13	14.9	2.61
$OctdPydNO_3$	62.77	22.7	0.21
PrPydPi	71.84	40.6	.49
$OctdMe_3NI$	53.6	29.9[a]	.28
$OctdMe_3OCHO$	60.24	36.5[a]	.049

[a] Iodide and formate ions

Salt	Λ_0	Λ_0^+	$K \times 10^4$
B. Pyridine			
$OctdBu_3NPi$	50.79	17.1	12.0
$Octd_2Bu_2NPi$[a]	47.03	13.3	10.1
Am_4NPi	55.28	21.6	11.3
Pr_4NPi	62.11	28.4	11.2
Et_4NPi	73.31	39.6	10.4

[a] Privately communicated by Mr. E. J. Bair, of This Laboratory.

In ethylene chloride, the iodide and formate ions have rather low conductances; this is particularly true of the iodide. It is a striking fact that, in this solvent, the conductances of the chloride, bromide and iodide ions differ widely, being, respectively, 39.1, 33.8 and 29.9. The conductance of the iodide is 23% less than that of the chloride ion.

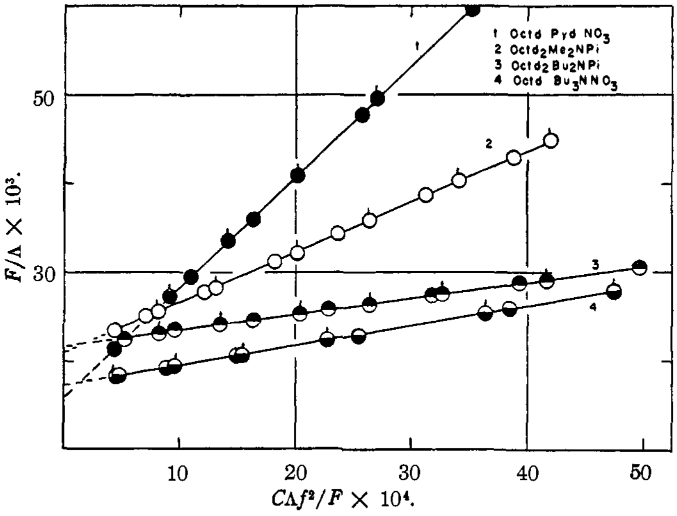

Fig. 1.—Fuoss plots for long chain salts in ethylene chloride.

The dissociation constant for octadecylpyridonium nitrate is about one half that of propylpyridonium picrate. The same is true of octadecyltributylammonium nitrate as compared with dioctadecyldi-*n*-butylammonium picrate. Octadecyltrimethylammonium formate is an especially weak salt, its constant being one-fifth that of the iodide.

The dissociation constants of the homologous series of quaternary ammonium picrates in ethylene chloride increase regularly with increasing numbers of carbon atoms in the substituent groups as Tucker has shown.[6] In this connection, it is of interest to note that the constant for dioctadecyldibutylammonium picrate is 2.61×10^{-4} as against 2.38×10^{-4} for tetra-*n*-amylammonium picrate. The constant for tetraethylammonium picrate is 1.59×10^{-4}.

In contrast to the regular change of the dissociation constant of the quaternary ammonium picrates in ethylene chloride, in pyridine the constant changes but little and, seemingly, irregularly. As may be seen from Table II, b, the constant for tetraethylammonium picrate is 10.4×10^{-4} while that for tetrabutylammonium picrate is 12.8×10^{-4}. But the constant for tetra-*n*-amylammonium picrate is only 11.3×10^{-4}, and that for dioctadecyldibutylammonium picrate is 12.0×10^{-4}. It is of interest to note that while in ethylene chloride the constant for tetra-amylammonium picrate is 7.4 times that of the corresponding tetramethylammonium salt, in pyridine the constants for the same two salts have a ratio of only 1.7. It is evident that the effect of chain length on the interaction of quaternary ions with the picrate ion in pyridine differs greatly from that in ethylene chloride. Whether the inversions found for the constants in pyridine are real or a result of experimental error remains uncertain as yet. Luder's conductance values for tetrabutylammonium picrate have subsequently been twice checked and confirmed independently. The symmetrical quaternary ammonium salts used in this investigation were the same as those used earlier by Tucker[1] except for their recrystallization.

2. Nitrobenzene.—Strong salts (*i. e.*, salts of large ions) are so highly dissociated in nitrobenzene that their dissociation constants cannot be evaluated. Values of Λ_0 may be determined by extrapolation of $\Lambda-\sqrt{C}$ plots. In Table V are given values of Λ_0 for the different salts measured; for the stronger salts, Λ_0 was obtained by extrapolation of the $\Lambda-\sqrt{C}$ plots. The weak salts were analyzed by the Fuoss method and values of the Λ_0 so obtained are given in column 2 and values of the dissociation constant, K, in the last column. Cation conductances are given in column 3. These are based on the conductance of the tetrabutylammonium triphenylborofluoride, it being assumed in accordance with Fowler[5b] that the two ions of this salt have the same conductance. Percentage deviations for the theoretical Onsager slope, Δ, are given in column 4.

Taylor and Kraus[7] measured the conductance of

---

[6] Tucker and Kraus, THIS JOURNAL, **69**, 454 (1947).

[7] Taylor and Kraus, *ibid.*, **69**, 1731 (1947).

TABLE V

CONSTANTS OF SOME QUATERNARY AMMONIUM SALTS IN NITROBENZENE

Salt	Λ_0	Λ_0^+	$\Delta\%$	$K \times 10^4$
Bu_4NPh_3BF	23.78	11.9	0.6	...
$Octd_2Bu_2NPi$	23.23	7.2	3.2	...
$OctdBu_3NPi$	24.64	8.6	4.1	...
$Octd_2Me_2NPi$	23.82	7.8	10.0	860
$OctdMe_3NPi$	26.50	10.5	19.4	430
$OctdPydNO_3$	33.39	10.8	146.	79

tetrabutylammonium triphenylborofluoride and found the slope of the Λ-$\sqrt{C}$ plot to be 4% less than the theoretical. It seemed worth while to measure this salt using another preparation.[8] The results of two series of measurements are shown graphically in Fig. 3. The slope of the plot is 0.6% below the theoretical. In other words, this salt conforms to the theoretical slope within the experimental error.

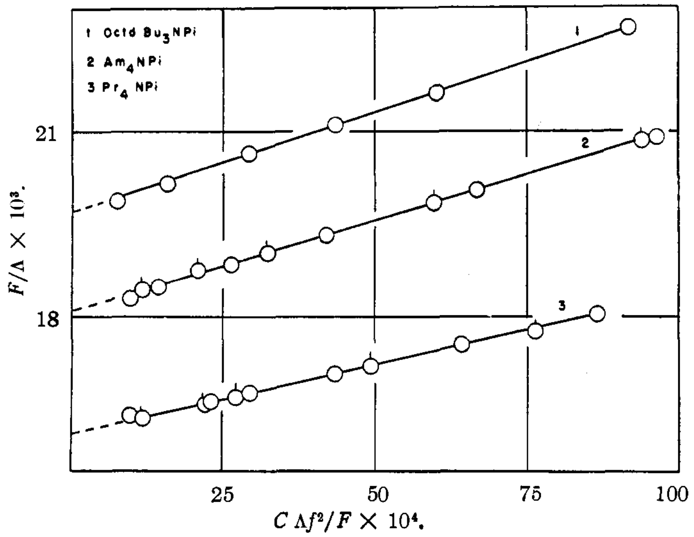

Fig. 2.—Fuoss plots for salts in pyridine.

The picrate ion in the ion pairs is smaller than the triphenylborofluoride ion. Dioctadecyldibutylammonium and octadecyltributylammonium picrate show slopes 3.2 and 4.1% greater than the theoretical. The picrates of the two methyl derivatives show markedly greater slopes. The slope of octadecylpyridonium nitrate is much greater than the theoretical. It may be pointed out that in ethylene chloride the dissociation constant of dioctadecyldimethylammonium picrate is approximately four times that of octadecylpyridonium nitrate; in nitrobenzene, the ratio of constants for the same two salts is approximately ten.

3. Conductance of Long Chain Cations.

—Thompson[9] has suggested that in comparing the conductance of the symmetrical quaternary ammonium ions as a function of the number of carbon atoms in the substituent alkyl groups, it is better to compare the reciprocal of ion conductances rather than the ion conductances themselves. In other words, it is best to compare equivalent ion resistances. He has shown that with the symmetrical quaternary ammonium ions in ethylene chloride, the reciprocal ion conductances vary approximately as a linear function of the number of carbon atoms from tetraethyl- to tetra-amylammonium, inclusive.

In comparing ion conductances as a function of carbon atom content in different solvents it is helpful to take the viscosities of the solvents into account. Accordingly, in Fig. 4, we have plotted the reciprocals of the product of ion conductance and viscosity for a number of quaternary ammonium ions as a function of the number of carbon atoms that they contain, for ethylene chloride, nitrobenzene and pyridine. To avoid confusion in the plots, the origin for each succeeding solvent has been displaced 8 carbon atoms to the right as indicated in the figure.

As may be seen from Fig. 4, the tetramethylammonium ion meets with much greater resistance in proportion to the number of carbon atoms that it contains than do the larger ions. The resistance of this ion, when adjusted for the viscosity of the solvent, is very nearly the same in ethylene chloride and nitrobenzene but it is much smaller in pyridine. The resistance with which an ion meets in its motion through a solvent medium is made up of two parts: first, a normal Stokes

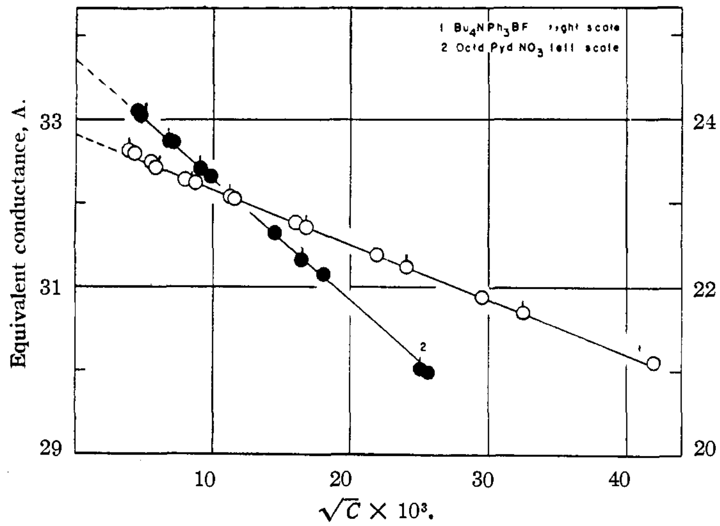

Fig. 3.—Λ-$\sqrt{C}$ plots for salts in nitrobenzene.

---

[8] Reynolds and Kraus, THIS JOURNAL, 70, 1704 (1948).

[9] W. E. Thompson, Thesis, Brown University, May, 1941.

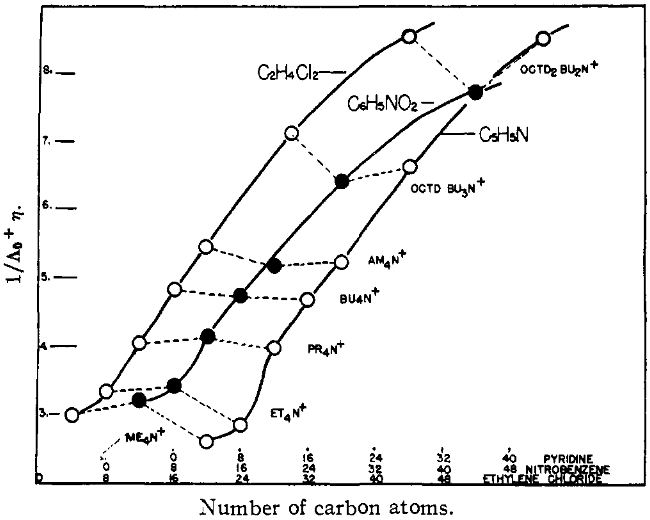

Fig. 4.—Plots of reciprocals of ion conductance–viscosity products for cations in ethylene chloride, nitrobenzene and pyridine as function of number of carbon atoms. (Note displacement of horizontal scales.)

frictional resistance and, second, a resistance due to the interaction of the charge on the ion with the dipole molecules of the solvent. The latter will depend on the size and shape of the ion and of the solvent molecules and the strength of the dipoles. It is to such factors that the tetramethylammonium ion owes its higher resistance in ethylene chloride and nitrobenzene as compared to that in pyridine.

In ethylene chloride, ion resistance varies as a linear function of the number of carbon atoms from the tetraethyl- to the tetra-amylammonium ion, inclusive. In other words, the introduction of a carbon atom causes the same increase in resistance, irrespective of the number of atoms already in the substituent groups. Even the octadecyltributylammonium ion deviates but little from the linear relation. With the dioctadecyldibutylammonium ion, the deviation is greater but not large.

For the smaller ions, the resistance curve in nitrobenzene parallels that in ethylene chloride. However, beginning with the tetrabutylammonium ion, the curve deviates from linearity and for the largest ion the deviation is very large. In contrast, the curve in pyridine deviates widely from that in ethylene chloride for smaller ions but parallels it for large ions. In going from the tetraethyl- to the tetrapropylammonium ion, the resistance change in pyridine is large in comparison with that in the other two solvents. The change in going from the tetramethyl- to the tetraethylammonium ion is exceptionally small. It would seem that the difference in ion resistance of smaller ions as between pyridine and the other two solvents must be ascribed to specific interaction of ions and solvent molecules. The difference in ion resistance for large ions as between nitrobenzene and the other two solvents may be ascribed to the compacting of the carbon atoms in the chains in the case of nitrobenzene. Indeed, the fact that the resistance of ions in ethylene chloride is proportional to the number of carbon atoms would seem to indicate that in this solvent the chains are extended. The same is true in pyridine but here we have differences in the case of smaller ions that must be attributed to other factors. In nitrobenzene, possibly due to weaker van der Waals interaction, the chains may no longer be extended and ion resistances would, accordingly, be smaller.

According to Walden's rule, the product of ion conductance and viscosity is a constant for large ions in solvents of different viscosities. From Fig. 4, it is evident that Walden's rule holds only as an approximation. Thus, for the seven quaternary ammonium ions, from tetramethyl- to dioctadecyldibutylammonium, inclusive, the maximum percentage differences for the $\Lambda_0\eta$ product in the three solvents is 19 (Me_4N^+), 16, 4, 3, 5, 10 and 9% ($Octd_2Bu_2N^+$). The product for the tetrabutyl- and the tetraamylammonium ions has the same value in pyridine and nitrobenzene while, for the dioctadecyldibutylammonium ion, it has the same value in ethylene chloride and pyridine. If Walden's rule is to be employed for the purpose of evaluating ion conductances in solvents where transport numbers cannot be determined experimentally, it would seem to be preferable to employ the tetrabutylammonium ion the $\Lambda_0^+\eta$ product of which is more nearly constant than is that of other ions. The tetraethylammonium ion yields unreliable results. The same can be said of the picrate ion as one of us has pointed out elsewhere.[10]

V. Summary

1. The conductances of seven long chain salts have been measured in ethylene chloride; five have been measured in nitrobenzene and one has been measured in pyridine. Cation conductances have been evaluated from the results of these measurements.

2. Several other salts have been measured in one or more of these solvents. Ion conductances have been derived from these measurements.

3. The conductance of tetrabutylammonium triphenylborofluoride in nitrobenzene has been remeasured. The slope of the $\Lambda_0-\sqrt{C}$ plot corresponds to the theoretical value within the limit of experimental error.

4. Plots are shown for the reciprocals of ion conductance viscosity products for the five symmetrical quaternary ammonium ions from tetramethyl- to tetraamyl, inclusive, and for octadecyltributyl- and dioctadecyldibutylammonium ions in ethylene chloride, nitrobenzene and pyridine.

---

[10] Kraus, *Ann. N. Y. Acad. of Sci.*, **51**, 789 (1949).

In ethylene chloride the equivalent resistance is a linear function of the number of carbon atoms from the tetraethyl- to the tetra-amylammonium ions, inclusive. Thereafter, resistance deviates slightly toward lower resistances as the number of carbon atoms increases. The resistance curve in nitrobenzene parallels that in ethylene chloride for smaller ions but deviates widely for large ions. The resistance curve for pyridine parallels that for ethylene chloride for large ions but deviates widely for small ions.

PROVIDENCE, R. I. RECEIVED APRIL 6, 1949

[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

Amino Acid Composition of α-Casein and β-Casein[2]

BY WILLIAM G. GORDON, WILLIAM F. SEMMETT, ROBERT S. CABLE AND MYRON MORRIS

The heterogeneity of cow's milk casein has been established by solubility studies and electrophoretic analysis. Warner[3] reviewed earlier work on this problem and described the chemical separation of casein into two mutually distinct components,[4] α-casein and β-casein, which occur in unfractionated casein in the approximate ratio of 4:1. The fractions isolated by Warner, although not electrophoretically homogeneous over the entire *p*H range, were purified, so that neither fraction contained any of the other. Comparison of the nitrogen and phosphorus contents of the isolated fractions with those of whole casein showed some significant differences; the values for β-casein, the minor component, differed from the values for whole casein more markedly than did those for α-casein.

The present paper deals with the amino acid analysis of α-casein and β-casein. Whole casein was also analyzed by the same methods for purposes of comparison. It has been possible to account for essentially all the nitrogen of each protein in terms of known amino acid residues and amide nitrogen.

Experimental

Proteins Used.—The samples of whole casein, α-casein and β-casein were prepared by Dr. Warner according to his published directions.[3] Electrophoretic analysis showed that each fraction was free of the other. In preliminary experiments, two preparations of each protein were analyzed for total nitrogen, phosphorus, lysine, tryptophan, tyrosine and amino nitrogen. The results indicated that there was no significant difference in composition between the two preparations. Therefore, in all subsequent experiments no distinction was made between different preparations of the same protein.

Methods of Analysis.—All analyses were carried out on air-dried protein samples; moisture determinations were made as suggested by Chibnall, *et al.*[5] True ash was determined according to Warner,[3] total nitrogen by the Kjeldahl method as used by Miller and Houghton[6] and phosphorus by the method of Fiske and SubbaRow[7] after digestion with sulfuric and nitric acids. Amino nitrogen values were obtained by the Van Slyke method as modified by Doherty and Ogg,[8] and amide nitrogen was estimated in Conway micro-diffusion cells according to the procedure suggested by Warner and Cannan.[9] In the latter procedure, a series of Conway vessels, each containing 10 mg. protein in 1 ml. 1.5 *N* sodium hydroxide in the outer compartment and 1.5 ml. 2% boric acid in the inner chamber, was set up at 35°. At intervals which ranged from thirty-five to sixty-five hours, vessels were removed from the oven, and the distilled ammonia was titrated with 0.01 *N* hydrochloric acid. The value at each reaction time was determined in triplicate. A progressive increase in ammonia liberated with time was observed, so that a linear extrapolation of the values to zero time was made to obtain the amide nitrogen figures.

Unless otherwise noted, hydrolysis of the proteins was carried out in 6 *N* hydrochloric acid in an oil-bath at 120° for twenty hours.

Lysine was determined by means of a specific decarboxylase both on total hydrolyzates and on catholytes obtained by ionophoresis of aliquots of the same hydrolyzates. Hanke's adaptation[10] of Gale's method[11] to the Van Slyke–Neill manometric apparatus was used. Ionophoresis was employed for the primary purpose of securing catholytes suitable for photometric determinations of arginine and histidine. A three-compartment cell patterned after that of Albanese[12] was constructed, and the general procedure of Gordon, Martin and Synge[13] was followed, with hydrolyzates prepared from 0.5 g. of protein. Arginine and histidine were then determined by Macpherson's modifications[14] of the Sakaguchi–Weber and Pauly reactions on the catholytes obtained after repeated (4 times) ionophoresis.[15] The values obtained for lysine in these catholytes were consistently lower (6 to 9%) than those found in the original hydrolyzates. On the

---

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) Preliminary reports of this work have been presented at meetings of the American Society of Biological Chemists, by title at the Chicago Meeting [*Federation Proc.*, **6**, 255 (1947)]; and orally at the Detroit Meeting [*ibid.*, **8**, 202 (1949)].

(3) Warner, THIS JOURNAL, **66**, 1725 (1944).

(4) A third component, γ-casein, may be present to the extent of a few per cent.; this point is discussed by McMeekin and Polis in "Advances in Protein Chemistry," Vol. V, Academic Press, Inc., New York, in press.

(5) Chibnall, Rees and Williams, *Biochem. J.*, **37**, 354 (1943).

(6) Miller and Houghton, *J. Biol. Chem.*, **159**, 373 (1945).

(7) Fiske and SubbaRow, *ibid.*, **66**, 375 (1925).

(8) Doherty and Ogg, *Ind. Eng. Chem., Anal. Ed.*, **15**, 751 (1943).

(9) Warner and Cannan, *J. Biol. Chem.*, **142**, 725 (1942).

(10) We are indebted to Prof. M. E. Hanke for details of his procedure [*Federation Proc.*, **5**, 137 (1946)], for a preparation of the enzyme and for a culture of *Bacterium cadaveris*.

(11) Gale, *Biochem. J.*, **39**, 46 (1945).

(12) Albanese, *J. Biol. Chem.*, **134**, 467 (1940).

(13) Gordon, Martin and Synge, *Biochem. J.*, **35**, 1369 (1941).

(14) Macpherson, *ibid.*, **36**, 59 (1942).

(15) After our analyses for the basic amino acids had been completed, the comprehensive paper on this scheme of analysis by Macpherson appeared.[51] Our experience confirms (a) the need for repeated ionophoresis of the catholyte to effect complete purification of the basic amino acid fraction, and, (b) the increased precision of the colorimetric methods when standards are used instead of calibration curves. In our hands, however, determination of lysine by difference on the basis of nitrogen analyses on the catholyte was not entirely trustworthy.