[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY, UNIVERSITY OF WISCONSIN]

The Physical Chemistry of Secalin. Electrophoresis and Diffusion Constant Studies of the Prolamine of Rye[1]

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Those proteins in the cereal grains which are soluble in 60–80% ethyl alcohol, classified as prolamines, have been found to possess properties that are nearly identical in many respects. The prolamine in the cereal rye (*Secale Cereale*) has been described repeatedly as being identical with the gliadin in wheat. It was this close similarity of properties of these two prolamines that prompted studies of the electrophoretic mobilities and diffusion constant of the rye prolamine, since corresponding properties of the prolamine in wheat are fairly well worked out. The literature on this rye protein is not extensive, and a separate name was not suggested for it until Gortner[3] gave it the name of "secalin." Previous to this it shared with the prolamine from wheat the name of "gliadin."

Experimental

Preparation of Secalin.—Fresh rye grain was ground by a commercial mill into a meal of moderate degree of fineness. About 2000 g. of this rye meal was then placed in a four-liter bottle and covered with enough 70% alcohol to assure an excess present after the meal became thoroughly soaked. This mixture was allowed to stand for forty-eight hours with frequent stirring, filtered by suction, and washed thoroughly with 70% alcohol. The alcohol extract, which was dark red in color, was then poured slowly with constant stirring into a large quantity of iced water containing potassium chloride. Secalin, being insoluble in aqueous solutions, was immediately precipitated and settled out on the bottom of the containing vessel. The yellowish brown, sticky mass of secalin was reprecipitated in like manner several times. About 30–70 g. of impure wet secalin was obtained from the 2000 g. of rye meal.

Purification of Secalin.—The procedure used in purifying secalin was a modification of the method used by Dill and Alsberg[4] in their purification of gliadin from wheat. The alcoholic solution of secalin was placed in a cold room for three days at a temperature of −10°. Because of the large change in solubility with temperature, much of the secalin precipitated upon standing at this temperature. The solid secalin was filtered off and the supernatant liquid was three times subjected for three hours to an acetone–dry-ice bath (about −35°), until all the secalin had been precipitated. In so doing, the coloring matter and fat accumulated in the supernatant liquid. Finally this purified secalin was dissolved in 70% alcohol and twice dialyzed against fresh solvent through cellophane membranes. All dialyses were carried out in tightly stoppered bottles in a refrigerator. The outer liquid from the first dialysis was colored a slight green, but that from the second dialysis remained colorless and was therefore used as solvent for diluting the dialyzed protein when more dilute solutions were required.

An attempt was made to purify secalin by a method similar to that sometimes used in the purification of zein from corn. This method involves the extraction of coloring matter and fatty impurities by repeated shaking out of the impure alcohol extract with an immiscible organic solvent. Great difficulties were encountered in attempting to purify secalin by this method, especially in the selection of the best organic solvent. In the preparation of zein, low boiling point petroleum ether is used; with secalin, this solvent invariably resulted in the precipitation of the dissolved protein. The extent to which this precipitation took place was variable, sometimes great quantities of the protein being precipitated merely by the addition of ether to the extract, without shaking. Other times the protein was precipitated during shaking, resulting in a semi-permanent emulsion. As the emulsion broke and the ether rose, the precipitated protein rose with it, from which it could be separated only with great difficulty. Protein so precipitated was but slowly redissolved, if at all, in 70% alcohol. It had a pure white color while still wet, but rapidly turned gray upon drying and exposure to air. In attempting to find an organic solvent for the extraction of fat and coloring matter, many were tried, and finally carbon tetrachloride was chosen because it had the least precipitating effect upon secalin in 70% alcohol. Even it would at times precipitate secalin, but upon long standing in contact with the alcohol the protein would partially redissolve. Concentrated solutions of this protein in 70% alcohol were reddish-brown in color, becoming light yellow upon addition of more alcohol.

Other organic liquids were used in attempting to find a suitable solvent for the impurities, of which acetone, tetrachloroethane, oleic acid, and dioxane were found to have a precipitating effect. Ethyl oleate, decahydronaphthalene, glycerol, and ethylene glycol showed no precipitating effect. The precipitating reaction produced by acetone was very marked.

The Diffusion Apparatus.—The apparatus used in the determination of the diffusion constant of secalin was similar to that used by Tiselius and Gross,[5] but the concentration gradients were measured by the refractometric

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[1] This material comprises a portion of a thesis submitted to the Faculty of the University of Wisconsin in partial fulfilment of the requirements for the degree of Doctor of Philosophy, June, 1938.

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[3] R. A. Gortner, Fifth Colloid Symposium Monograph, A. C. S., 1924.

[4] D. B. Dill and C. L. Alsberg, *J. Biol. Chem.*, **65**, 279 (1925).

[5] A. Tiselius and D. Gross, *Kolloid-Z.*, **66**, 11 (1934).

method developed by Lamm,[6] in which a transparent uniform scale is photographed through the diffusion cell. The refractive index gradient at the diffusion boundary produces a distorted image of the scale, in which the displacement of the scale lines is proportional to the concentration gradient, provided the refractive index is a linear function of the concentration. The arrangement of the apparatus was similar to that described by Lamm and Polson.[7] The diffusion cell was nearly identical to the one used and described in detail by them, and by Svedberg.[8] The internal diameter of the diffusing solution arm was 1 cm. The linear scale was a photographic reduction of a large scale to a length of 53 mm., consisting of 200 divisions, every tenth being numbered. The source of illumination was a sodium vapor lamp. The final line displacement caused by the blurring of the boundary was read by means of a Gaertner micro-comparator. These line displacements were then plotted against the undeviated scale, and the curve so obtained was used in calculation of the diffusion constant.

The Electrophoresis Apparatus.—The apparatus used in obtaining the electrophoretic mobility was similar to that in general use, which employs the moving boundary method and is described in detail by Tiselius.[9] It consisted of a "U" tube having an inside diameter of 1 cm., accurately ground and polished cylindrical inside each arm. The electrode vessels that made connection to the top of each arm of the "U" tube had a combined capacity of 450 cc. The protein solution made connection at the bottom of the "U" through a three-way stopcock, in which the boundary was formed and from which it was moved into position in the two arms of the "U." The apparatus was immersed in the same water-bath at 25° and the same optical system was used as in the diffusion constant determinations. The movements of the boundaries were followed by the refractometric method developed by Lamm. Silver–silver chloride electrodes were used with an electrolyte solution of magnesium chloride in 70% alcohol. Currents between 2–3 milliamperes were used and the value taken was the mean of the current strength at the beginning and end of a time interval.

The protein solutions were prepared from purified secalin in 70% alcohol in concentrations from 0.4 to 1%. The solvent used for the solutions in electrophoresis was 62.9% by weight of ethyl alcohol solution, made 0.02 molar with respect to sodium acetate. The buffers were then made by adding varying amounts of acetic acid to this sodium acetate–alcohol–water solvent, in concentrations from 0.0022 to 0.18 *M*. The resulting *p*H values of all solutions and solvents were determined by means of the glass electrode after dialysis. Dialysis was carried out similarly to that described previously, the protein solution being dialyzed against buffers of the same concentration as that used as solvent. The outer liquid from the last dialysis served as the solvent into which the protein moved during electrophoresis. All conductivity measurements on solutions and solvents were made in a conductivity cell of the type designed by Washburn, using a Leeds and Northrup Jones conductivity bridge at 25°.

Calculations.—The distance between the scale divisions of the developed photographic plates was read to a thousandth of a millimeter. The normal undeviated scale, photographed through the solvent was computed and used as a reference scale. The differences between corresponding scale divisions of the deviated and "deviated" scale, called "*Z*" values, were plotted against the deviated scale. The curve so produced should approach the normal Gaussian or probability curve for a homogeneous dispersed substance.

In the calculation of the diffusion constant the equation used was

$$Dt = \frac{A^2}{4\pi H^2_{\max.}}\left(\frac{l-b}{l}\right)^2$$

Here D is the diffusion constant, t the time in seconds, A the area under the Gaussian curve, determined with a planimeter, $H_{\max.}$ the maximum ordinate of the curve, l the optical distance from the scale to the objective lens, and b the optical distance from the center of the diffusion cell to the scale. Zero time was chosen as that time at which the boundary first became visible in the cylindrical portion of the tube. The diffusion constants are expressed as $D \times 10^7$ cm.^{2} per sec.

In the calculation of the electrophoretic mobility, the Gaussian curves were not nearly as symmetric as the diffusion curves, being more irregular and jagged in outline. In an electrophoresis experiment there would be one such Gaussian curve from each scale picture taken corresponding to each interval of time, the difference between the pictures being mainly that of a displacement of the maximum ordinate along the scale in a direction depending upon the direction and time of flow of the current. Accumulated percentages of the areas under each curve for each 0.5 cm. abscissa interval were then calculated and plotted against the deviated scale, giving a series of "*S*" curves, one for each picture. The differences in the "*S*" curves would be a shift along the millimeter scale abscissa by an amount corresponding to the direction of current flow, to the velocity of migration and to charge on the protein particle. The value of u(cm.^{2} sec.$^{-1}$ volt^{-1}) was then calculated by $u = qkd/it = d/Ft$, where $F = i/qk$. In this equation, q is the cross sectional area of the "U" tube, k, the conductivity of the solution, t, the time in seconds, i, the mean current during the interval between photographic exposures, and d, the magnitude of the displacement in cm. of the 50% point in the "*S*" curves.

Results

Diffusion Constant.—The general law of linear diffusion was stated first by Fick in 1855 in two mathematical laws that bear his name. Fick's second law, $\partial c/\partial t = D\partial^2 c/\partial x^2$, defining the linear diffusion constant, allows of experimental verification. This partial differential equation and the corresponding theory are completely re-

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(6) O. Lamm, "Measurements of Concentration Gradients in Sedimentation and Diffusion by Refraction Methods," Dissertation, Upsala, Sweden, 1937.

(7) O. Lamm and A. G. Polson, *Biochem. J.*, **30**, 528–541 (1936).

(8) T. Svedberg, "Colloid Chemistry," A. C. S. Monograph Series 16, The Chemical Catalog Co., New York, N. Y., 1928.

(9) A. Tiselius, Inaugural Dissertation, University of Upsala, Upsala, Sweden, 1930, pp. 14–18.

viewed in many places [Williams and Cady[10]] and has for our problem a solution

$$C = C_0/2 + \frac{C_0}{2\sqrt{\pi D t}} \int_0^{\frac{x}{2\sqrt{Dt}}} e^{-(\alpha - x)^2/4Dt}\, d\alpha$$

Here the integral is of the form of the well-known probability integral and so can be evaluated from experimental results, provided it is assumed that the refractive index is exactly proportional to the concentration for all concentration ranges used. In Fig. 1 are shown four experimental typical curves obtained by plotting "Z" against the deviated scale. They are actually those obtained in diffusion experiment no. 4. The curves are symmetrical about the maximum ordinate, and the experimental points lie nicely on the smooth curve. In Table I are given the constants obtained from all such curves. The areas under the curves of Fig. 1 show a maximum deviation from the average area of 3%. In Table I the results tabulated are those obtained for secalin from the two described methods of preparation. The first three experiments were made on a solution containing 1.035 g. of secalin per 100 cc. of solution and prepared by the use of an organic solvent; the other experiments used secalin obtained by the method of low temperatures in concentration of 0.695 g. per 100 cc. of solution. Secalin obtained by either method gave nearly identical diffusion constants.

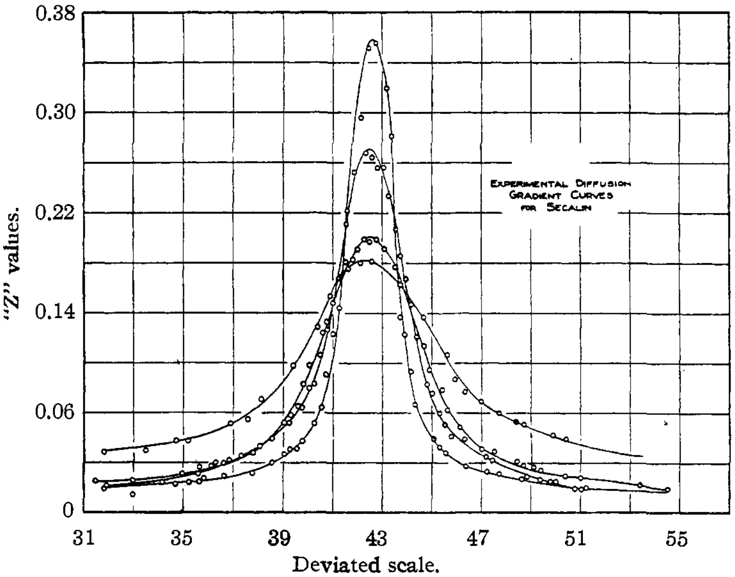

Fig. 1.—Experimental diffusion curves, showing the decrease in maximum ordinate with time as the boundary blurs.

The values of the diffusion constant decreased with the elapsed time of diffusion. Such a variation in diffusion constant with time seems to be inherent with the experimental method, probably explained in a large part by the mechanical inability to form a perfectly static boundary. Other investigators apparently have noticed this, because in nearly all cases the values are reported for those in which the elapsed time was sufficient for D to become fairly constant. In Table I the value of D for secalin decreases rapidly for the first 40,000 seconds and then in some cases becomes very nearly constant. Extrapolation of the diffusion constant–time curve to very large time intervals gives $D_{25} = 1.84 \times 10^{-7}$. An average of all such values for times 50,000 seconds or greater gives $D^{\text{av.}}_{25} = 1.91 \times 10^{-7}$. The curve obtained by the method of least squares for the combined values in experiments 1, 2, and 4 gives $D_{25} = (1.8 \pm 0.2) \times 10^{-7}$, when extrapolated to large time intervals. The value of the diffusion constant of secalin calculated to water solution at 25° by use of the equation $D^{\text{H}_2\text{O}}_{25} = D^{\text{alc.}}_{25}\, \eta^{\text{alc.}}_{25}/\eta^{\text{H}_2\text{O}}_{25}$, where $D^{\text{H}_2\text{O}}_{25}$ and $D^{\text{alc.}}_{25}$ are the diffusion constants in water and alcohol, and $\eta^{\text{H}_2\text{O}}_{25}$ and $\eta^{\text{alc.}}_{25}$ are their viscosities, gives $D^{\text{H}_2\text{O}}_{25} = 4.78 \times 10^{-7}$. For 20° the value is $D^{\text{H}_2\text{O}}_{20} = 4.3 \times 10^{-7}$.

This value of D for secalin compares favorably with the values of diffusion constants for gliadin, hordein, and zein. Williams and Watson[11] report the diffusion constants (20°) for three rather arbitrary fractions of zein as $D^{\text{H}_2\text{O}}_{20} = 3.0 \times 10^{-7}$, $D^{\text{H}_2\text{O}}_{20} = 4.0 \times 10^{-7}$, and $D^{\text{H}_2\text{O}}_{20} = 5.2 \times 10^{-7}$, in which the second fraction was present in the greatest amount. Lamm and Polson[7] report diffusion constants for three fractions of gliadin as $D^{\text{H}_2\text{O}}_{20} = 4.86 \times 10^{-7}$, $D^{\text{H}_2\text{O}}_{20} = 5.85 \times 10^{-7}$ and $D^{\text{H}_2\text{O}}_{20} = 6.72 \times 10^{-7}$. Quensel and Svedberg[12] report a diffusion constant for hordein from barley of 6.5×10^{-7}.

Sedimentation constants have been reported for zein by Williams and Watson of $s_{20} = 1.9 \times 10^{-13}$ for the predominant zein fraction, and

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(10) J. W. Williams and L. C. Cady, *Chem. Rev.*, **14**, 171 (1934).

(11) J. W. Williams and C. C. Watson, Cold Spring Harbor Symposia on Quantitative Biology, VI, 208 (1938).

(12) O. Quensel and T. Svedberg, *Compt. rend. trav. Lab. Carlsberg, Ser. chim.*, **22**, Vol. jubilaire S. P. L. Sørensen, 442–448 (1938).

TABLE I

EXPERIMENTAL DATA OBTAINED IN THE DETERMINATION OF DIFFUSION CONSTANT

Expt.	Time, sec.	$H_{\max.}$ × 10^2	Area, $A \times 10^{-2}$ cm.^{2}	Diff. constant, $D \times 10^7$
1	8,980	8.81	2.88	6.70
	15,950	7.36	2.66	4.61
	22,975	6.39	2.58	3.98
	30,280	5.92	2.33	2.88
	37,070	5.15	2.26	2.93
	43,940	4.90	2.16	2.49
	76,650	4.00	2.17	2.15
	82,960	3.82	2.16	2.18
	101,960	3.66	2.20	2.01
	129,500	3.29	2.11	1.79
	163,690	3.18	2.22	1.69
	170,610	3.06	2.20	1.72
2	18,020	7.52	2.18	2.62
	92,830	3.66	2.10	2.24
	97,292	3.54	2.11	2.06
	117,165	3.25	2.10	2.00
	131,995	3.07	2.10	2.00
	172,125	2.90	2.16	1.81
3	20,145	5.24	1.94	3.85
	67,005	3.25	1.58	1.98
	79,815	2.94	1.56	1.99
	98,410	2.72	1.60	1.96
	116,175	2.48	1.53	1.83
	149,570	2.30	1.45	1.50
4	11,272	3.76	1.25	5.62
	21,312	3.22	1.26	4.34
	32,260	2.83	1.29	3.70
	107,712	2.09	1.23	1.85
	120,272	1.95	1.23	1.92
	132,485	1.85	1.19	1.80
	150,192	1.77	1.18	1.71
	159,217	1.72	1.30	2.07
5	40,930	3.52	1.37	2.14
	48,430	3.05	1.23	1.94
	59,520	2.94	1.31	1.92
	67,310	2.60	1.26	2.01
	76,515	2.60	1.34	2.01
	122,507	2.10	1.27	1.72
	131,705	2.08	1.34	1.80
	143,400	1.99	1.32	1.75
	152,805	1.89	1.29	1.76

Krejci and Svedberg[13] give one for gliadin of 2.10×10^{-13}. Until secalin is actually centrifuged, its sedimentation constant can be assumed to lie between that of zein and gliadin, as does the diffusion constant. Then an approximate molecular weight can be calculated by the use of the formula $M = RTs/D(1 - Vd)$. Here M is the molecular weight of the sedimenting molecule, R, the gas constant, T, the absolute temperature, D, the diffusion constant, V, the partial specific volume of the protein, and d, the density of the solvent. The quantity V is taken as 0.722 (see Krejci and Svedberg[13]), and s is assumed to be 2.0×10^{-13}. Using these values for secalin gives an approximate molecular weight of 40,500. Lamm and Polson[7] calculated a value 27,500 for gliadin, while a value of 26,750 was reported by the use of the equilibrium centrifuge. Quensel and Svedberg[12] similarly calculated a molecular weight of 27,000 for hordein. Ultracentrifugal determinations with zein reported by Williams and Watson[11] give 40,000 for the molecular weight of this prolamine.

Electrophoresis.—The electrophoretic mobility of a protein particle varies with the *p*H of the solution, the ionic strength of the medium, etc. In general there is a certain *p*H for which the mobility becomes zero. This *p*H, which is called the isoelectric point, is definite for each protein as long as the ionic strength of the system is kept low and constant. The mobility of the particle increases as the *p*H is changed from the isoelectric point, but the direction of movement is opposite on the two sides of this point. Secalin shows its greatest mobility in the more acid solutions, and the mobility gradually decreases as the *p*H of the solutions increases, becoming zero at *p*H (in alcohol) of 6.67 ± 0.1. Further decrease in acidity produces a movement of protein particles again, but in a direction opposite to that of the original.

Two representative curves are plotted in Fig. 2, one for the change in mobility as current goes "up" on the right as one faces the "U" tube, and "down" as the current goes in the reverse direction. The mobilities are not exactly the same in both directions. The slope of the "up" curve is 1.30 and of the "down" curve is 1.47; thus the protein migrated about 11% faster in "up" direction than in "down." Zero mobility in the "up" direction is at a *p*H (alc.) of 6.74, and in the "down" direction of 6.60. The average of these two values gives an isoelectric point for the protein secalin at a *p*H (alc.) of 6.67. Practically all the experimental mobilities in Table II lie well within a range of ±0.1 of a *p*H unit from the curves of Fig. 2. Also practically any other straight line that reasonably could be drawn will have a zero mobility within ±0.1 *p*H unit of 6.67. The notation *p*H as used here for expressing hydrogen-ion concentration in alcohol–water solutions is defined in the same manner

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(13) L. Krejci and T. Svedberg, THIS JOURNAL, 57, 946 (1935).

TABLE II

ELECTROPHORETIC EXPERIMENTS WITH SECALIN

Expt.	*p*H (alc.)	Time, sec.	d/t (mm./sec.) $\times 10^3$	Current (milliamps.) (i)	Sp. cond. (K)	Mobility ($U = u \times 10^5$)
			"Up" Direction			
I-R	5.25	5875	0.638	2.05	3.615×10^{-4}	0.885
		5015	.737	2.05		1.008
		5315	.638	2.05		0.885
I-L		5845	.623	2.05		.865
		5275	.618	2.05		.856
II-R		6005	.760	2.15		1.005
		5700	.737	2.15		0.976
III-R	5.99	6165	.354	2.085	3.942×10^{-4}	.526
		5510	.333	2.075		.498
		5910	.324	2.070		.486
III-L		4320	.582	2.085		.866
		5480	.463	2.075		.693
IV-R	6.2	5170	.178	2.080	3.571×10^{-4}	.240
		4745	.200	2.070		.272
		5707	.219	2.070		.297
IV-L		5726	.279	2.070		.279
		4797	.371	2.070		.503
		5944	.267	2.105		.356
V-R	7.18	5287	− .187	2.050	3.605×10^{-4}	− .263
V-L		5257	− .196	2.050		− .278
		6615	− .112	2.050		− .158
		5690	− .165	2.050		− .232
VI-R		4920	− .228	2.380		− .271
		6591	− .220	2.400		− .260
		6309	− .185	2.400		− .219
VI-L		4910	− .126	2.385		− .150
		6630	− .330	2.400		− .390
		6240	− .228	2.400		− .269
VII-L		3262	− .270	2.105		− .362
		5223	− .436	2.090		− .589
		4870	− .448	2.100		− .601
			"Down" Direction			
I-R	5.25	5600	−0.735	2.055	3.615×10^{-4}	−1.016
		10245	− .437	2.070		−0.600
I-L		5560	− .570	2.055		− .788
II-R		5885	− .901	2.150		−1.192
		5390	− .683	2.150		−0.903
II-L		5930	− .773	2.160		−1.016
		5450	− .690	2.170		−0.905
III-R	5.99	4830	− .475	2.075	3.942×10^{-4}	− .710
		6100	− .493	2.095		− .732
		6005	− .233	2.115		− .342
III-L		6110	− .164	2.095		− .243
		5990	− .351	2.115		− .515
IV-R	6.2	5906	− .312	2.105	3.571×10^{-4}	− .416
IV-L		5095	− .259	2.075		− .350
		6063	− .231	2.090		− .311
V-L	7.18	6346	.260	2.050	3.605×10^{-4}	+ .366
		4417	.351	2.050		+ .495
		6080	.280	2.050		+ .394
VI-R		5460	.311	2.450		+ .360
		7515	.297	2.500		+ .339
		6975	.324	2.500		+ .367
VI-L		7514	.426	2.050		+ .482

as Haugaard and Johnson,[14] namely: "a water-alcohol mixture has the same hydrogen-ion activity as an aqueous solution when they both have the same potential difference against the hydrogen electrode."

In comparing this value of the isoelectric point with other investigators' results, it is necessary to convert all such data to an alcohol-water basis. Gortner[3] gives an isoelectric point for secalin at a *p*H of 5.43 (in water) obtained by methods of acid and base combining ratios. This value calculated to the alcohol basis by use of the tables of Haugaard and Johnson[14] gives an isoelectric point at a *p*H of 6.75, which is about the value obtained from these mobility experiments. Czonka, Murphy, and Jones[15] tabulated the results of isoelectric points obtained by a method similar to the critical precipitation method for a large number of proteins. The isoelectric point of secalin is given at a *p*H of 6.2, but no statement is made as to whether it was on the water or alcohol-water basis.

Gortner[3] gives isoelectric points for zein and gliadin at 6.29 and 7.11, respectively, calculated to the water-alcohol basis. Similarly, Williams and Watson give isoelectric points for their three fractions of zein, calculated to alcohol-water basis, at *p*H of 6.29, 7.16, and 6.95. Thus it would seem that the isoelectric points of the prolamines from the cereals so far studied lie within the same *p*H region.

Miscellaneous Experiments

Dielectric Constant and the Time of Relaxation.—A series of preliminary experiments was made in which the dielectric dispersion curve was determined for secalin over a frequency range of 24.2 kilocycles to 24.2 megacycles. The secalin used in these preliminary experiments was especially purified by electrodialysis at 10 milliamperes and 110 volts until the conductivity of the solution became equal to that of the solvent; the concentration of this solution was 0.204 g. of protein per 100 cc. of solution. The ratios of the dielectric constant change in the solution to that of solvent were plotted against the logarithms of their frequencies. The midpoints of the curves showing anomalous dispersion give as provisional values times of relaxation of $\tau_1 = 7.4 \times 10^{-7}$ sec., and $\tau_2 = 7.0 \times 10^{-8}$ sec., for the rotations about short and long axes. These figures, corrected to a process taking place in pure water at 25°, are 29×10^{-8} sec. and 2.7×10^{-8} sec., respectively. The ratio of major to minor axis from these times of relaxation is about ten to one for the secalin molecule. Details of similar experimental work and calculations for the protein zein are given in a recent article.[16]

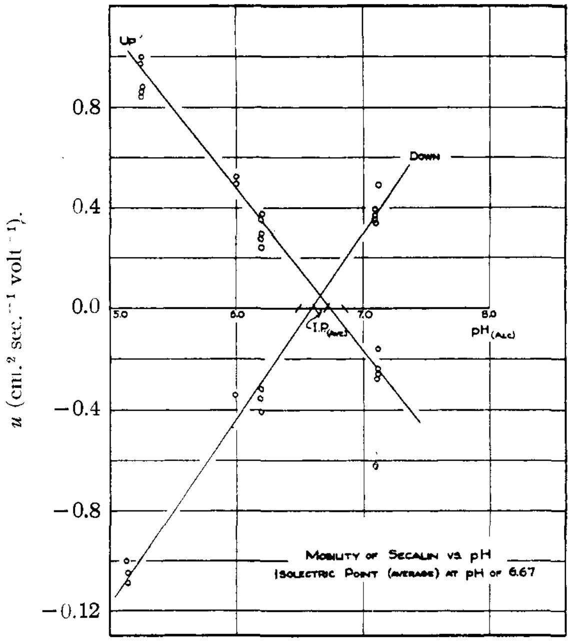

Fig. 2.—The effect of acidity upon the mobility of secalin in alcohol solution. The mobility becomes zero at *p*H of 6.67.

Optical Rotation and Refractive Index Increment.—The optical rotation for solutions containing 0.6950 and 0.5260 g. of secalin per 100 cc. of solution, respectively, gave specific rotations of −66.2 and −69.3° for an average of −67.7° at 25°. The refractive index increment ($\Delta\eta$ /g./100 cc.) was determined by the use of an immersion refractometer, giving an average refractive index increment of 1.51×10^{-3}.

The author wishes to express his thanks for the helpful advice of J. W. Williams throughout the course of this work. Grateful acknowledgment is also made to the Wisconsin Alumni Research Foundation for a scholarship which helped to make it possible to perform the work herein described.

Summary

The prolamine of rye grain was prepared and

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[14] G. Haugaard and A. H. Johnson, *Carlsberg Laboratory Reprints*, Vol. 18, No. 2.

[15] F. A. Czonka, J. C. Murphy and D. B. Jones, THIS JOURNAL, **48**, 766 (1926).

[16] M. A. Elliott and J. W. Williams, *ibid.*, **61**, 718 (1939).

purified by two different methods and certain of its physical constants were determined.

The diffusion constant calculated to the water basis at 25° was found to be 4.78×10^{-7} cm.^{2} per sec. (At 20°, $D = 4.3 \times 10^{-7}$ cm.^{2}/sec.)

A calculation by means of sedimentation methods, using this value of "D" and an estimated value of the sedimentation constant, gave a molecular weight for secalin of approximately 40,000. Thus the predominant constituent of secalin seems to belong in the egg albumin molecular weight class of molecules.

The ordinary isoelectric point for secalin as determined by electrophoretic mobilities was found to be at pH 6.67.

MADISON, WIS. RECEIVED JANUARY 6, 1940

[CONTRIBUTION FROM THE BAKER CHEMISTRY LABORATORY OF CORNELL UNIVERSITY]

Fluorochloroethanes as Solvents for the Cryoscopic Determination of Molecular Weights

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The purpose of this paper is to call attention to the suitability of fluoropentachloroethane, *sym*-difluorotetrachloroethane and *unsym*-difluorotetrachloroethane for use as solvents for the determination of molecular weights. These substances have large molecular freezing point depressions, are chemically inert and melt within convenient working ranges. Their solvent action is in general very good for non-associated substances. A representative list of qualitative solubilities of organic compounds in *sym*-difluorotetrachloroethane has been reported by Booth.[1]

Fluoropentachloroethane.—Fluoropentachloroethane was found to work particularly well as a molecular weight solvent using a semimicro procedure modeled after the Rast procedure as modified by Munster.[2] A sealed tube is necessary for the melting point determinations because of the high vapor pressure of this substance near its melting point. Small thin-walled "micro boiling point tubes" were used (4.5 × 60 mm.), and the fluoropentachloroethane was added by rolling it into small pellets which were dropped into the tube. In each case the ratio of the weight of the solute to the weight of the solvent was such as to give a depression of the freezing point between 9 and 15°.

From the observed values of the depression of the freezing point values of the freezing point constant k as calculated by the usual formula $k = \Delta TSM/s1000$ were obtained. The concentrations varied from 3–10%, and the values listed are the mean values of at least two determinations with an average deviation from the mean of about 0.1, except where otherwise indicated: benzyl bromide, 41.5; benzyl ether, 42.1; decachlorobutane, 42.6; p-dichlorobenzene, 42.1; 4,4′-difluorodiphenyl, 42.8; isoamyl acetate, 41.3; methyl benzoate, 41.1; naphthalene, 42.1; acetone, s/S = 0.0201, 0.0221, k = 31.5, 32.3; benzoic acid, s/S = 0.0230, 0.0483, 0.0682, k = 24.5, 22.0, 21.3; benzyl alcohol, s/S = 0.0485, 0.0691, k = 26.7, 23.6; n-butyl alcohol, s/S = 0.0304, 0.0512, k = 28.7, 23.5; butyraldehyde, s/S = 0.0149, 0.0220, 0.0226, 0.0379, k = 31.3, 33.5, 33.2, 27.7; α-naphthol, s/S = 0.0241, 0.0482, k = 38.3, 32.9; nitrobenzene, s/S = 0.0392, 0.0429, k = 35.8, 36.7, respectively.

Acetanilide, aniline and dimethylaniline were sparingly soluble in fluoropentachloroethane and hexachloroethane formed a crystalline solid phase of higher melting point. For the measurements in which there was complete solubility and no apparent association or compound formation, the average value of k was 42.0.

The fluoropentachloroethane used in this work was prepared by fluorinating pentachloroethane[3] and melted at 99.9° after purification by distillation. This substance also may be prepared readily from hexachloroethane and antimony trifluoride.[1,4]

sym- **and** *unsym*-**Difluorotetrachloroethane.**—These compounds do not work well with the semimicro procedure because of the difficulty of observing crystallization in the small tubes. However, their large freezing point depressions and convenient melting temperatures may be utilized in the usual macro procedure,[5] and their use for precise cryoscopic measurements is indicated. The constant k for *sym*-difluorotetrachloroethane was determined as 37.7 and for *unsym*-difluorotetrachloroethane as 38.6, using naphthalene and p-dichlorobenzene.

Naphthalene, s/S = 0.00140, 0.00140, 0.00127, k = 37.1, 36.7, 37.4; p-dichlorobenzene, s/S = 0.00129, 0.00121, 0.00134, k = 38.1, 38.0, 38.8; naphthalene, s/S = 0.000660, 0.00113, k = 38.2, 37.9; p-dichlorobenzene, s/S = 0.000794, 0.000863, 0.000919, k = 38.2, 39.4, 39.3, respectively.

The *sym*-difluorotetrachloroethane was prepared by adding fluorine to tetrachloroethylene[3] and melted at 24.7°. It also may be prepared from hexachloroethane and antimony trifluoride.[1,4] No direct methods for the preparation of *unsym*-difluorotetrachloroethane have appeared in the literature although it has been character-

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[1] Booth, Mong and Burchfield, *Ind. Eng. Chem.*, **24**, 328 (1932).

[2] Gattermann and Wieland, "Laboratory Methods of Organic Chemistry," The Macmillan Co., New York, N. Y., 1937, p. 86.

[3] Miller, THIS JOURNAL, **62**, 341 (1940).

[4] Locke, Brode and Henne, *ibid.*, **56**, 1726 (1934).

[5] Reilly and Rae, "Physico-Chemical Methods," Methuen and Co., Ltd., London, 1933, p. 439.