Summary

1. Desylacetophenone reacts with bromine to form a monosubstitution product, which can be isolated only if the reaction is carried out in the presence of potassium acetate. In the absence of the latter reagent, ring closure takes place with formation of triphenylbromofuran.

2. The structure of the bromo ketone has been determined.

McGILL UNIVERSITY
MONTREAL, CANADA

[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY OF THE UNIVERSITY OF UPSALA]

THE MOLECULAR WEIGHT OF BENCE-JONES PROTEIN

BY THE SVEDBERG AND BERTIL SJÖGREN

RECEIVED JULY 15, 1929 PUBLISHED DECEMBER 11, 1929

Certain diseases, especially myelomas, are characterized by the appearance in the urine of a peculiar protein called after its discoverer Bence-Jones protein.[1] The question of the origin of this abnormal product of cell life has caused much discussion. The comparative rareness of cases which are accompanied by the appearance of this protein has hampered the study of its properties. Recently such a case was reported from the Academic Hospital in Upsala and by courtesy of Professor G. Bergmark we received a sufficient quantity of urine for an ultracentrifugal investigation. In view of the fact that one of the theories about the origin of Bence-Jones protein considers it to be a product of protein cleavage, the determination of its molecular weight seemed to us to be of great interest. From analytical data Cohn has calculated a minimal molecular weight of 24,500,[2] but judging from the results of similar computations compared with the results of ultracentrifugal determinations no certain conclusion as to *the actual molecular weight in aqueous solution* can be drawn from his calculation.

Preparation of Material.—To 2400 cc. of urine were added 4800 cc. of saturated ammonium sulfate solution and some toluene as a preservative. After standing for twenty-four hours at 0° the precipitate was centrifuged off and washed repeatedly with a mixture of two parts of saturated ammonium sulfate and one part of water. The precipitate was then dissolved in the smallest possible volume of water and reprecipitated with ammonium sulfate solution to a saturation degree of 60%. After standing for twenty-four hours at 0°, the precipitate was filtered off and washed with ammonium sulfate solution of 60% saturation. This material was divided in two parts. One portion was dissolved in phosphate buffer of P_H 5.5 (0.095 *M* in KH_2PO_4 and 0.005 *M* in Na_2HPO_4) and dialyzed at 0° against the same buffer for six days, after which time the SO_4-reaction was negative; volume of solution, 50 cc., concentration 1.19% (Ma-

[1] See Wells, "Chemical Pathology," Saunders Co., Philadelphia and London, 5th ed., 1925, p. 596.

[2] Cohn, Hendry and Prentiss, *J. Biol. Chem.*, **63**, 764 (1925).

terial I). A second portion was dialyzed against pure water and then electrodialyzed. No precipitate was formed during the electrodialysis. The concentration of this solution was 1.28% and the volume 200 cc. (Material II).

The isoelectric point of Material II was measured by A. Tiselius in this Laboratory and found to be 5.18. In electrochemical respects, therefore, Bence-Jones protein is distinctly different from egg albumin and serum albumin, which have isoelectric points at P_H 4.6 and 4.9, respectively (according to recent determinations by Tiselius).

Specific Volume.—The partial specific volume was determined pycnometrically at 19.8°, as described in previous communications.[3] The data are shown in Table I.

TABLE I

PARTIAL SPECIFIC VOLUME OF BENCE-JONES PROTEIN AT 19.8°

Protein concn., %	Solvent	P_H of soln.	Partial spec. vol.
0.80	0.006 *M* in HAc, 0.014 *M* in NaAc	5.0	0.747
1.19	0.006 *M* in HAc, 0.014 *M* in NaAc	5.0	.749
1.32	Pure water	5.1	.751

These specific volumes are identical within the limits of error. The mean value 0.749 is also identical with the specific volumes of egg albumin, hemoglobin, serum albumin, serum globulin, phycocyan, phycoerythrin and edestin. As previously pointed out, this indicates a close relationship between all these proteins.[4]

Light Absorption.—The light absorption of solutions of Bence-Jones protein at various hydrogen-ion concentrations was measured with the Judd–Lewis spectrophotometer. Material II (electrodialyzed) was used and the measurements were carried out directly after making up the solutions. At the isoelectric point determinations were made in concentrations 0.06 and 0.12%, using a tube length of 2.0 cm. and in the concentration of 0.95% with 0.1-cm. tube length. All other determinations were performed on solutions of 0.05 and 0.10% using a tube length of 2.0 cm.

TABLE II

LIGHT ABSORPTION OF SOLUTIONS OF BENCE-JONES PROTEIN

Solvent	*M*	Solvent	*M*	P_H of soln.	Wave length of maximum, $\mu\mu$	Wave length of minimum, $\mu\mu$	ϵ/c at maximum
KCl	0.093	HCl	0.007	2.18	278	255	15.0
HAc	.017	NaAc	.003	3.94	278	253	13.0
HAc	.006	NaAc	.014	5.00	280	253	13.0
KH_2PO_4	.002	Na_2HPO_4	.031	7.74	280	252	13.0
Na_2HPO_4	.032	NaOH	.002	9.40	276	254	14.4
Na_2HPO_4	.050	NaOH	.008	10.85	278	255	17.0
Na_2HPO_4	.013	NaOH	.030	12.15	276	254	18.7
Na_2HPO_4	.010	NaOH	.036	12.45	278	255	18.7
NaOH	.05	NaCl	1%	12.66	276	256	18.6

[3] Svedberg and Chirnoaga, THIS JOURNAL, **50**, 1401 (1928).

[4] Svedberg and Sjögren, *ibid.*, **50**, 3321 (1928).

In Table II the positions of the maximum and the minimum of absorption and the extinction coefficients for the maximum are recorded. Figure 1 shows the absorption curves for three different hydrogen-ion concentrations.

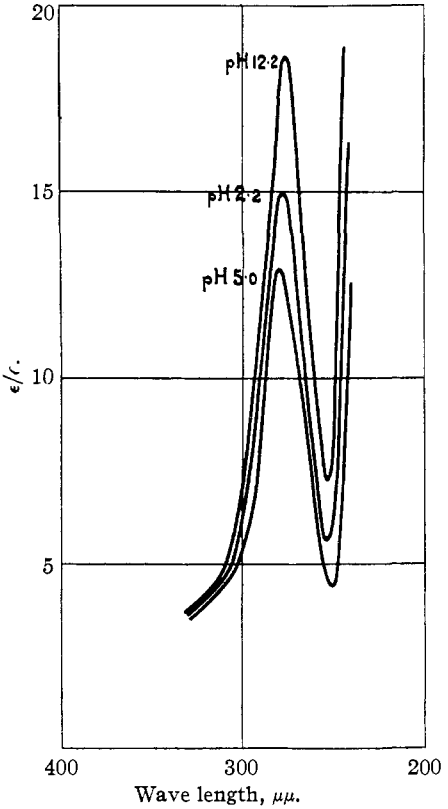


Fig. 1.

The position of the maximum and the minimum of the absorption curve is independent of P_H. The value of the extinction is independent of P_H within the region 3.94 to 7.74. In more acid and more alkaline solution, the extinction increases, indicating changes in the protein molecule. As will be seen from the molecular weight determinations, the molecule begins to break up into smaller units in the same P_H regions where the light absorption begins to rise. There is a distinct difference however between the acid and the alkaline hydrolysis. The rise in light absorption on the acid side is caused by the formation of strongly absorbing noncentrifugible decomposition products, the mass of the centrifugible substance remaining unchanged, while the rise in light absorption on the alkaline side is due to the formation of centrifugible decomposition products.

Determination of Molecular Weight

Sedimentation Equilibrium Method.—A number of runs were made at different hydrogen-ion concentrations in order to determine not merely the molecular weight but also the stability region of the protein. As shown in previous communications[5,6] the molecular weight is given by the relation

$$M = \frac{2RT \ln (c_2/c_1)}{(1 - V\rho)\omega^2 (x_2^2 - x_1^2)}$$

where R is the gas constant, T the absolute temperature, V the partial specific volume of the solute, ρ the density of the solvent, ω the angular

[5] Svedberg and Fåhraeus, THIS JOURNAL, **48**, 430 (1926).

[6] Svedberg and Nichols, *ibid.*, **48**, 3081 (1926).

velocity and c_2 and c_1 the concentrations at the distances x_2 and x_1 from the center of rotation.

An improved type of ultracentrifuge for medium speed was used for these determinations. The cells employed were made as follows: an ebonite plate 25 mm. in diameter and with a sectorial aperture of 5° cut from the periphery down to 7.5 mm. from the center of the plate was coated with a solution of rubber in benzene and after drying for a few minutes covered with two quartz plates of the same diameter and 5-mm. thick and slid into an ebonite collar. The latter was in its turn introduced into a steel collar threaded at the ends. By means of two steel guard rings and thin intermediate ebonite rings the quartz plates were firmly pressed together. Both the ebonite and the steel collar had openings coincident with the opening of the sectorial cell. The opening could be closed by an ebonite lid. Four different thicknesses were used for the ebonite middle plates, *viz.*, 2, 4, 8 and 16 mm., giving the corresponding four thicknesses of solution and thus permitting a wide range of different concentrations to be studied. This new type of cell has proved to be more reliable than the cemented one previously described.[7] The ebonite sector plates do not crack as the glass or quartz sector plates often do when exposed to the centrifugal force, the cell is easier to clean and the rubber is more resistant to salt solutions than the de Khotinsky cement. The ebonite collar around the quartz plates protects them from uneven strain.

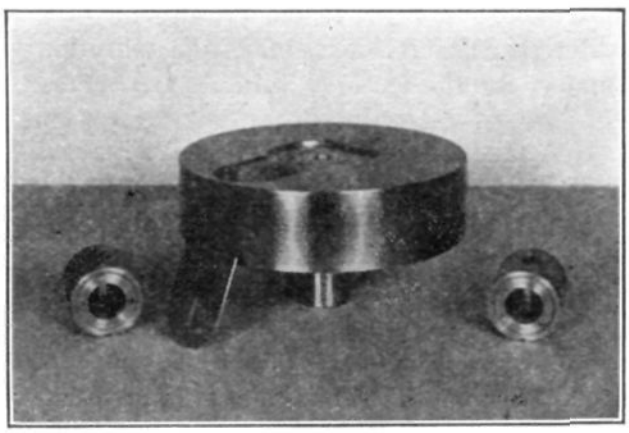


Fig. 2.

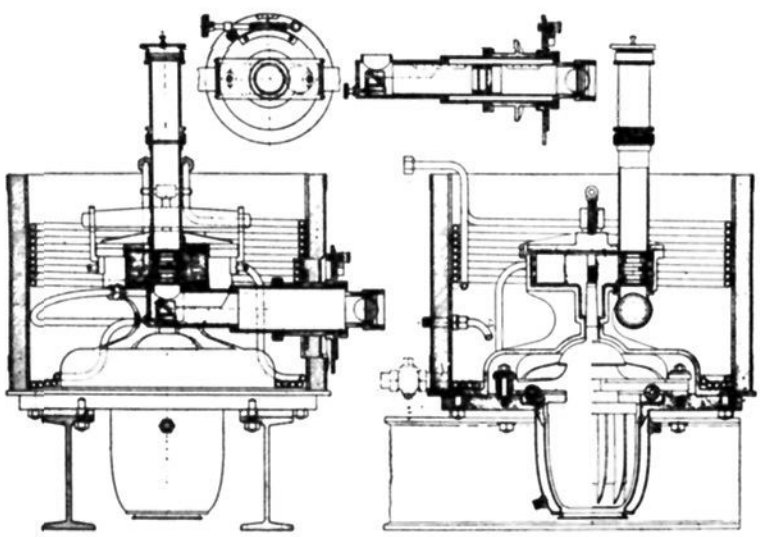


Fig. 3.

By choosing the inner diameter of the cell smaller, a thicker steel collar could be used which was less easily deformed by the centrifugal force. Optically the cell was defined by two steel diaphragms with sectorial apertures of 3°, one on each side of the cell. The rotor was a chromium–nickel steel cylinder 15 cm. in diameter and 4 cm. thick and was arranged to receive two cells. One of them was used for the solution to be studied; the other was

[7] Svedberg and Heyroth, THIS JOURNAL, **51**, 552 (1929).

filled with a non-centrifugible solution for standardizing the intensity of the illuminating lamp. Figure 2 shows the rotor with the two cells and one of the sector diaphragms removed. The rotor was supported upon a vertical steel shaft directly connected to the rotor of a special electric three-phase motor. The speed could be varied from 12,000 r.p.m. down to about 2000 r.p.m. by varying the frequency of the current feeding the motor. The centrifuge rotor as well as the rotor of the electric motor was surrounded by hydrogen of atmospheric pressure confined within a casing about the moving parts of the centrifuge. To further ensure constant temperature, the stator of the motor was water-cooled and the casing within which the centrifuge rotor moved was immersed in a water thermostat. Freedom from vibration was ensured by a special circular rubber support for the stator. Suitable arrangements for passing of the light beam through

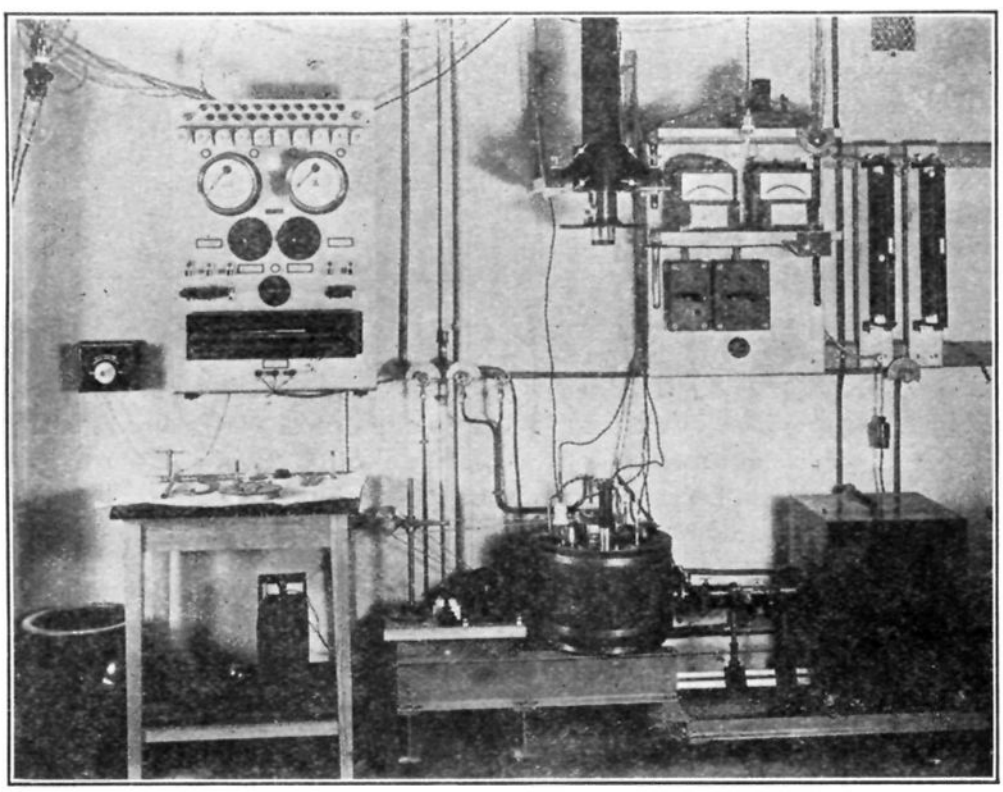


Fig. 4.

the centrifuge and for taking exposures were made. Figure 3 gives a diagram of the centrifuge and Fig. 4 a photographic view of the whole apparatus except the camera. The latter (Fig. 5) was placed in a room above the one where the centrifuge was mounted. The optical system used was about the same as previously employed.[8] In Fig. 4 the water-cooled house for the mercury lamp can be seen to the right, then the light filter and the centrifuge follow from right to left. The horizontal illuminating beam is reflected vertically through the centrifuge by a quartz prism. Above the centrifuge is the photographic lens and part of the camera. On the wall are mounted the controlling instruments.[9]

---

[8] Svedberg and Heyroth, THIS JOURNAL, **51**, 553 (1929).

[9] The motor and the generator for the variable three-phase current were delivered by Siemens, Berlin; most of the other parts of the equipment were manufactured in the workshops of the laboratory.

For the runs described below a wave length range 290–240$\mu\mu$ was isolated from the mercury arc by means of bromine and chlorine light filters.

In Table III the results of a typical run are given and in Table IV are collected the main data of all the runs.

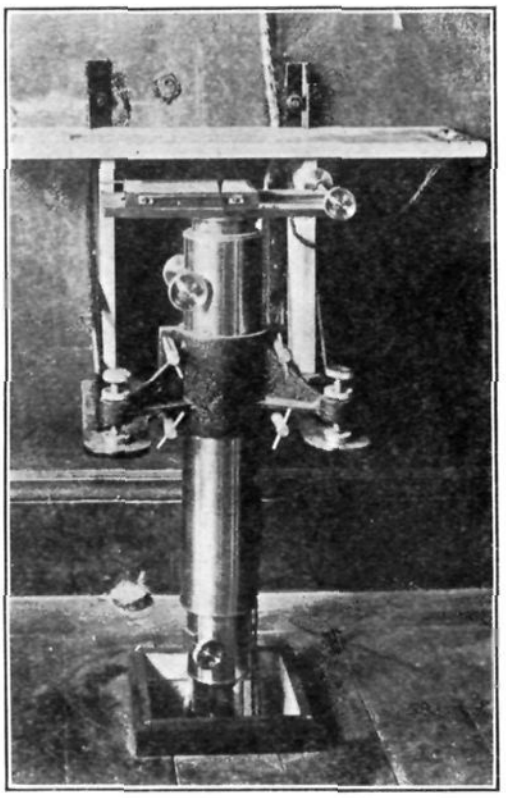


Fig. 5.

Within the P_H region 3.94 to 7.74, the molecular weight is independent of the hydrogen-ion concentration, with a mean value of 35,000 ± 1000. The constancy of the molecular weight as a function of distance from center of rotation shows that the Bence-Jones protein is homogeneous with regard to molecular weight within the same P_H region. At a P_H of 10.85 considerable decomposition has taken place, the values of the molecular weight ranging from 36,100 (the normal weight) to 16,400. An attempt was made to determine the molecular weight at P_H 12.66 (0.05 M in NaOH, 1% in NaCl) but during the long time required for reaching equilibrium, the light absorption increased enormously, indicating very strong hydrolysis. A sedimentation velocity run performed at the same P_H, which required only six hours of centrifuging, indicated a molecular weight of about one-half that of the normal protein.

TABLE III

BENCE-JONES PROTEIN, SEDIMENTATION EQUILIBRIUM RUN

Concn., 0.05%; phosphate buffer, P_H 5.5 (0.095 M in KH_2PO_4 and 0.005 M in Na_2HPO_4); V, 0.749; ρ, 1.006; T, 293.3; length of col. of soln., 0.525 cm.; thickness of col., 0.80 cm.; dist. of outer end of soln. from axis of rotation, 5.95 cm.; speed, 10,300 r.p.m. ($\omega = 343.3\pi$); standard, K_2CrO_4 $M/500$; source of light, mercury lamp; light filters, chlorine and bromine; aperture of objective, F:36; plates, Imperial Process; time of exposure, 45, 90 and 120 sec.; exposures made after 40, 45 and 51 hours of centrifuging.

Distances, cm.		Mean concn., %		Number of	
x_2	x_1	c_2	c_1	exposures	Mol. wt.
5.83	5.78	0.069	0.061	5	36,100
5.78	5.73	.061	.054	9	36,000
5.73	5.68	.054	.048	10	35,100
5.68	5.63	.048	.043	10	33,100
5.63	5.58	.043	.0382	10	35,500
5.58	5.53	.0382	.0342	10	34,600
5.53	5.48	.0342	.0305	10	34,700
				Mean	35,000

TABLE IV

BENCE-JONES PROTEIN, SUMMARY OF SEDIMENTATION EQUILIBRIUM MEASUREMENTS

Speed 10,000–11,000 r.p.m.

Solvent				P_H of soln.	Concn. of protein at start, %	Mol. wt.
	M		M			
HAc	0.017	NaAc	0.003	3.94	0.20	33,500
HAc	.006	NaAc	.014	5.08	.20	35,700
KH_2PO_4	.095	Na_2HPO_4	.005	5.50	.05	35,000
KH_2PO_4	.003	Na_2HPO_4	.031	7.74	.05	35,900
Na_2HPO_4	.050	NaOH	.008	10.85	.10	from 36,100 to 16,400

Sedimentation Velocity Method.—For the sedimentation velocity runs the high-speed oil turbine ultracentrifuge previously described[10] was used. New cells of a somewhat different type resembling those described above for the new medium-speed ultracentrifuge were employed.

For most of the runs light of the wave length region 290–240$\mu\mu$ was isolated from the mercury arc by means of bromine and chlorine filters. Using a 12-mm. cell the lowest concentration of protein that could still be studied with this illumination was 0.10%. In order to render possible the study of still lower concentrations, a cadmium spark lamp was constructed. It was fed by a 1-kilowatt transformer giving 25,000 volts with a condenser parallel to the spark. An image of the spark in natural size was thrown on a small diaphragm by means of a quartz condenser and a slightly converging beam of light was then sent through the centrifuge by means of a second quartz lens possessing a focal length double that of the quartz condenser. By using a chlorine filter of 100 cm. a wave length region 231–214$\mu\mu$ could be isolated from the light given off by the spark. As demonstrated by the curves in Fig. 1, the light absorption of the protein is much higher in this wave length region. As a matter of fact, concentrations down to 0.025% could be studied by means of the cadmium spark lamp in combination with the 100-cm. chlorine filter.[11]

In the steady state of sedimentation the centrifugal force per mole of protein, $M(1 - V\rho)\omega^2 x$, and the frictional force per mole, $f\mathrm{d}x/\mathrm{d}t$, where f is the molar frictional constant and the other symbols have the same significance as previously, are equal. If we define $s = \mathrm{d}x/\mathrm{d}t\ 1/\omega^2 x$ as the specific sedimentation velocity or sedimentation constant, we have

$$M = \frac{fs}{1 - V\rho}$$

[10] Svedberg and Nichols, THIS JOURNAL, 49, 2920 (1927); Svedberg, "Colloid Chemistry," 2d ed., Chemical Catalog Co., New York, 1928, p. 153.

[11] No bromine filter was used in this case because the light absorption of bromine is too high in the very short waved ultraviolet region. One of the functions of the long chlorine filter is that of absorbing the violet light.

If we assume that the molar frictional constant effective in sedimentation is the same as the one effective in free diffusion we are justified in using the relation $f = RT/D$, where D is the diffusion constant, and thus get for the molecular weight[10]

$$M = \frac{RTs}{D\,(1 - V\rho)}$$

The fact that in most cases hitherto studied the values of the molecular weights obtained by the sedimentation equilibrium and the sedimentation velocity method check within the limits of experimental error (hemoglobin, serum albumin, serum globulin, phycocyan, phycoerythrin, edestin) demonstrates that the above assumption is as a rule justified. Certain exceptions have been observed however. For hemocyanin the diffusion as measured from the sedimentation pictures was abnormally low at higher concentrations but became normal at high dilutions from about $^1/_3\%$ downward.[12] The sedimentation constant was only slightly affected by the forces which at higher concentrations depressed the diffusion. A similar effect has been observed for cellulose.[13] Recently several instances of abnormal diffusion persisting down to very low concentrations have been observed. In such cases the sedimentation velocity has always been perfectly in line with the molecular weight obtained from sedimentation equilibrium measurements. From these facts we must draw the conclusion that the value of the molar frictional constant derived from the diffusion formula where a perfectly free movement of the molecules is assumed is not necessarily the same as the molar frictional constant which is effective in the sedimentation of molecules in a field of force. In the latter case the molecules need not move independently of each other.

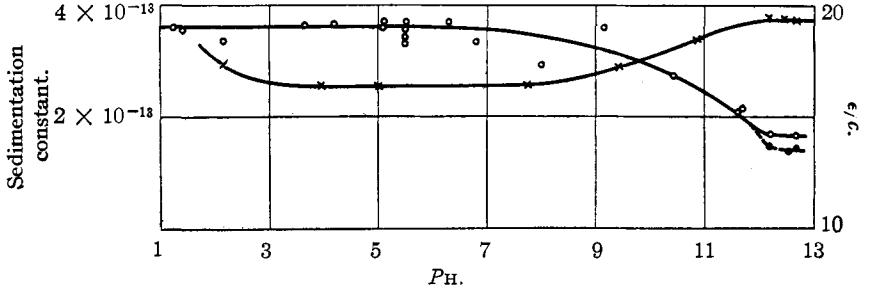
The determinations of sedimentation constant and diffusion constant for Bence-Jones protein have shown that the values of the diffusion constant are irregular and abnormally low even down to a few hundredths of a per cent. On the other hand, the values of the sedimentation constant were quite regular. In the following, therefore, only these latter determinations will be mentioned.

In order to explore the P_H stability region more in detail than was done by the equilibrium measurements, a series of determinations of the sedimentation constant was performed at different hydrogen-ion concentrations. The solutions were brought to the desired concentration and P_H immediately before starting the runs. In Table V the results are summarized. Figure 6 shows the relation between sedimentation constant and P_H graphically. In the same diagram are plotted the values of the extinction coefficient at the maximum of absorption as function of P_H. In the P_H region 3.5–7.5, both sedimentation and light absorption are practi-

[12] Svedberg and Chirnoaga, THIS JOURNAL, **50**, 1399 (1928).

[13] Stamm, unpublished investigations.

cally constant and no non-centrifugible substance is formed. The protein is very stable in this P_H region. At lower P_H values the light absorption increases, while the sedimentation constant remains unchanged. The



○, Sedimentation constant; ×, ϵ/c.

Fig. 6.

photograms of the solution during centrifuging indicate that at P_H values of 1.2–1.4, 40–35% of the protein is broken up into a non-centrifugible substance. This accounts for the rise in light absorption. At the acid

TABLE V

BENCE-JONES PROTEIN, SUMMARY OF SEDIMENTATION VELOCITY MEASUREMENTS

Time of centrifuging, 4 to 6 hours. Speed, 42,000–47,000 r.p.m.

Solvent	M		M	P_H of soln.	Concn. of protein, %	$s_{20} \times 10^{13}$
HCl	0.1	KCl	0.1	1.2	0.05	3.60
HCl	.04	KCl	.16	1.4	.06	3.56
HCl	.007	KCl	.093	2.15	.08	3.34
HAc	.018	NaAc	.002	3.63	.12	3.67
HAc	.015	NaAc	.005	4.20	.12	3.69
HAc	.006	NaAc	.014	5.08	.10	3.65
Pure water				5.10	.12	3.70
KH_2PO_4	.095	Na_2HPO_4	.005	5.5	.025[a]	3.43
KH_2PO_4	.095	Na_2HPO_4	.005	5.5	.10	3.71
KH_2PO_4	.095	Na_2HPO_4	.005	5.5	.78	3.63
KH_2PO_4	.095	Na_2HPO_4	.005	5.5	1.00	3.39
KH_2PO_4	.080	Na_2HPO_4	.020	6.30	0.12	3.42
KH_2PO_4	.050	Na_2HPO_4	.050	6.82	.12	3.35
KH_2PO_4	.005	Na_2HPO_4	.095	8.00	.11	2.94
Na_2HPO_4	.032	NaOH	.001	9.14	.12	3.64
Na_2HPO_4	.026	NaOH	.012	10.47	.060	2.79
Na_2HPO_4[b]	.050	NaOH[b]	.025	11.61	.055	2.13
Na_2HPO_4	.017	NaOH	.025	11.69	.055	2.17
Na_2HPO_4	.013	NaOH	.030	12.15	.045	1.49
Na_2HPO_4[b]	.013	NaOH[b]	.030	12.23	.04	1.69
		NaOH	.05	12.60	.04	1.40
Na_2HPO_4	.003	NaOH	.044	12.61	.04	1.44
NaOH[b]	.05			12.66	.04	1.69

[a] Cadmium light was used. [b] 1% in NaCl.

side, therefore, the molecule is directly hydrolyzed into small molecules and what still remains of bigger molecules shows the normal sedimentation of the protein. At P_H values higher than 7.5 the light absorption increases while the sedimentation constant decreases. Both curves have a small horizontal part in the P_H region 12.2–12.7 indicating that the centrifugible part of the protein has reached a second stability range. An increasing amount of non-centrifugible substance is being formed, amounting to about 18% at P_H 12.7. As an average value of 13 determinations of the sedimentation constant within the stability region including the isoelectric point we get 3.55×10^{-13}. Some of the determinations at high P_H values show a marked Donnan effect. While the two runs at P_H 11.61 and 11.69 gave practically the same sedimentation constant, 2.13 and 2.17, with and without 1% NaCl present, the runs from P_H 12.15 show a marked salt effect. Without salt the values of the sedimentation constant are 1.49, 1.40 and 1.44, at the P_H of 12.15, 12.60 and 12.61, while the values found in the presence of 1% NaCl are 1.69 and 1.69 at the P_H of 12.23 and 12.66. The value 1.69 is probably free from any influence of the Donnan effect.

Discussion of Results

The ultracentrifugal study of Bence-Jones protein has shown this substance to be uncommonly stable. Within the P_H range 3.5–7.5 there is no decomposition of the molecule. The sedimentation equilibrium method has given a mean value of 35,000 for the molecular weight, determinations being made in the P_H range 3.94–10.85. From P_H 3.94 to 7.74 the protein was found to contain only molecules of weight 35,000. A run at P_H 10.85 showed a drift in the values of the molecular weight from 36,100 at the bottom of the cell to 16,400 at the top of the cell. At this P_H, therefore, the protein is a mixture. The sedimentation velocity runs have shown that in the P_H region 1.2–3.5 there is hydrolysis of the molecule into a non-centrifugible substance. The remaining protein has the same sedimentation constant as in the P_H region, where it is perfectly stable. In the P_H region 7.5–12.2 the sedimentation decreases steadily. In the P_H range 12.2–12.7 the sedimentation is constant. Now it is of considerable interest to notice that the molecular weight of 35,000 found for the Bence-Jones protein by means of the sedimentation equilibrium method is practically identical with the molecular weight found by the same method for egg albumin,[6] *viz.*, 34,500, and that the sedimentation constant found for the Bence-Jones protein within the stability region which includes the isoelectric point is 3.55×10^{-13}, while the same constant found by Nichols for egg albumin is 3.32×10^{-13}.

The molar frictional constant $f = [M(1 - V\rho)]/s$ for Bence-Jones protein at 20° is 2.48×10^{16} and for egg albumin 2.63×10^{16}. The molar

frictional constant f_s for a substance of molecular weight 35,000 and possessing spherical molecules of the same specific volume as Bence-Jones protein can easily be calculated from the relation $f_s = 6\pi\eta N(3MV/-4\pi N)^{1/3}$, where η is the viscosity of the solvent, N the Avogadro constant and the other symbols have their usual significance. The value is 2.49×10^{16}. The corresponding value for egg albumin is 2.47×10^{16}. The ratio f/f_s may be taken as measure of the dissymmetry of the molecule. For a spherical molecule this ratio should be unity. From our experimental data we find for Bence-Jones protein $f/f_s = 1.00$ and for egg albumin 1.06. The data at our disposal also permit of the calculation of the radius of these spherical molecules. By means of the formula $r = (3MV/4\pi N)^{1/3}$ we get for Bence-Jones protein $r = 2.18\mu\mu$ and for egg albumin $2.17\mu\mu$.

The normal Bence-Jones protein, therefore, seems to have molecules of the same weight as egg albumin. Both proteins have spherical molecules of a radius of about $2.2\mu\mu$. The molecular weight value for the lightest component in the equilibrium run at P_H 10.85 was 16.400, which is not far from one-half the weight of the undecomposed protein. The sedimentation constant value found at high P_H values in the presence of salt was 1.69, while the value to be expected for a molecule of one-half the weight of the normal Bence-Jones protein and having about the same degree of dissymmetry as the other dissymmetrical protein molecules so far studied in this Laboratory is 1.70. It therefore seems probable that at high P_H values the molecule of the Bence-Jones protein is split up into halves. At the same time an ordinary hydrolysis producing non-centrifugible substance begins.

From the physiological point of view it is of interest to observe that native egg albumin also to some extent passes normal kidneys, just as the Bence-Jones protein does, while the appearance of serum albumin in the urine is accompanied by some change in the function of the kidneys. Now serum albumin has the double molecular weight of egg albumin and Bence-Jones protein. It may be, therefore, that the appearance of one or the other of these proteins in urine is more or less a question of permeability.

The expenses connected with these experiments have been defrayed by grants from the foundation "Therese and Johan Anderssons Minne" and from the Nobel Fund of Chemistry.

Summary

1. The ultracentrifugal methods have been applied to the study of the molecular weight and P_H stability range of Bence-Jones protein.

2. In the P_H range 3.5–7.5 the protein is stable and has a molecular weight of 35,000 ± 1000, a sedimentation constant of 3.55×10^{-13} and

a molar frictional constant of 2.48×10^{16} (both at 20°); a comparison with the molar frictional constant calculated for a spherical molecule of the same molecular mass and volume shows that the molecule of Bence-Jones protein is spherical and has a radius of 2.18$\mu\mu$.

3. At P_H values lower than 3.5 the molecule breaks up into some non-centrifugible substance, the remaining protein having the normal molecular weight. The acid hydrolysis of the protein is accompanied by a rise in light absorption.

4. At P_H values higher than 7.5 a slight alkaline hydrolysis sets in, producing a non-centrifugible substance, but the main change brought about by the high P_H is a splitting up of the molecule into a centrifugible substance which probably has a molecular weight equal to half that of the normal molecule. In the P_H range 12.2–12.7 the sedimentation constant has a value of 1.69×10^{-13}. The light absorption increases in the P_H range 7.5–12.2 and then becomes constant again.

5. A comparison between Bence-Jones protein and egg albumin with regard to molecular weight, sedimentation constant and molar frictional constant shows that the values are identical within the limits of experimental error. Although these two proteins are entirely different as to chemical composition and isoelectric point, their molecular mass and size are almost identical.

UPSALA, SWEDEN

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF BROWN UNIVERSITY]

STUDIES RELATING TO ALKYL TIN COMPOUNDS. III. SOME PROPERTIES OF TRIMETHYL TIN HYDROXIDE

BY CHARLES A. KRAUS AND RALPH H. BULLARD

RECEIVED JULY 17, 1929 PUBLISHED DECEMBER 11, 1929

Recently Kraus and Harada[1] have described compounds formed between trimethyl tin hydroxide and trimethyl tin halides corresponding to the formula $[(CH_3)_3SnOH]_2(CH_3)_3SnX$. In attempting to establish the constitution of these compounds, it was found necessary to study the properties of trimethyl tin hydroxide, and, particularly, its thermal decomposition. The present paper records the results of the latter investigation.

Preparation of $(CH_3)_3SnOH$.—According to Cahours,[2] trimethyl tin hydroxide is formed on treating trimethyl tin halides with a strong solution of an alkali. The hydroxide is readily soluble in water and sublimes at temperatures above 80°. Considerable difficulty was experienced in the preparation of the pure hydroxide, which was invariably accompanied by a large loss of material. As will be shown later, this loss was probably due

[1] Kraus and Harada, THIS JOURNAL, **47**, 2416 (1925).

[2] Cahours, *Ann.*, **114**, 377 (1860).