2. Two lactones of *l*-mannonic acid were prepared: the common lactone having a butylene oxide ring and the less stable lactone having an amylene oxide ring.

3. Evidence for the structure of the two lactones was substantiated by the preparation of two distinct tetramethyl mannonic lactones.

4. *l*-Gluconic lactone was prepared for the first time in crystalline form and its constants were determined.

LINCOLN, NEBRASKA

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

THE MOLECULAR WEIGHTS OF PHYCOERYTHRIN AND OF PHYCOCYAN

BY THE SVEDBERG AND N. B. LEWIS[1]

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Phycoerythrin and phycocyan are closely related proteins of the globulin type which occur together in certain algae. They are both fluorescent and have characteristic colors; the color and fluorescence are quite strong even in 0.01% solution. On account of this strong light absorption both in the visible and the ultraviolet and of the relative ease of preparing them in a pure crystalline state they appeared to be particularly suitable for molecular weight determinations by means of the ultracentrifuge.

There is a series of references in the literature to these substances dating back to that of Kützing;[2] but they appear to have first been prepared in a pure state by Kylin.[3] In this paper he gives an account of their preparation from *Ceramium rubrum*, of their chemical properties, absorption spectra and a quantitative analysis of phycoerythrin. It also includes a complete list of the earlier references.

Kitasato[4] used another alga, *Porphyra tenera* (the sun-dried material being an article of commerce in Japan) as raw material, and the substances were prepared by a method analogous to that of Kylin. His observations in general, and his analysis of phycoerythrin, agree with those of Kylin. Further, he was able to make an analysis of phycocyan, which occurs in much smaller quantities (*Ceramium rubrum*, according to Kylin, about $^1/_{10}$ of that of phycoerythrin); his figures for this are very close to those for phycoerythrin.

Experimental

Preparation of Material.—*Ceramium rubrum* was extracted with water as described by Kylin. The extract, consisting of a solution of the mixed substances as well as

[1] Fellow of the International Education Board.

[2] Kützing, *Phycologia Generalis*, Leipzig, 1843.

[3] Kylin, *Z. physiol. Chem.*, **69**, 169 (1910).

[4] Kitasato, *Acta Phytochimica*, **2**, 75 (1925).

numerous impurities (slimy carbohydrates, etc.), was treated with about 20% of its weight of ammonium sulfate. The proteins thus precipitated were brought into solution by washing with distilled water. This solution was then treated with increasing amounts of ammonium sulfate up to 20% of its weight. After each addition it was allowed to stand for a day, filtered and the precipitate redissolved. In this way were formed four fractions with differing relative amounts of the two substances. The first fraction (the largest), consisting mainly of phycoerythrin, was recrystallized until the resulting crystals, as seen under the microscope, were seen to be entirely free from phycocyan; and the third and fourth, consisting almost entirely of phycocyan, recrystallized until they were free from phycoerythrin. The precipitate from Fraction 2, consisting of a mixture in which phycoerythrin predominated, was shaken with a little water and centrifuged. The deposit was found to be free from phycoerythrin crystals, which evidently dissolve more rapidly. The various mother liquors obtained, if dilute were discarded, if not, were separated into fractions in a similar manner. As these substances are decomposed on exposure to daylight, the liquids in all cases were kept in the dark, with toluene added as a preservative.

The pure crystalline substances were dissolved in as small a quantity of water as possible and dialyzed in collodium bags against water at 0° for periods of not less than a week, at the end of which time the specific conductivity at 20° had fallen to less than 10^{-4} ohm^{-1}, and the substances had commenced to precipitate in an amorphous form. The phycocyan was dissolved in a dilute sodium phosphate-potassium phosphate buffer solution (P_H = 7.0). The phycoerythrin dissolved again when the liquid was poured into a glass vessel (due probably to traces of alkali from the glass). This solution was filtered and part kept without buffer; the remainder in phosphate buffer (P_H = 6.8).

Specific Volume.—The partial specific volume at 19.4° of phycoerythrin was determined pycnometrically. The concentration of the sample was determined by drying to constant weight at 105°. The value for a dilute (0.84%) solution was found to be 0.746 which is very close to

TABLE I

EXTINCTION COEFFICIENTS ($\epsilon = 1/d \log I_0/I$) FOR PHYCOERYTHRIN (0.0097% SOLUTION)

Wave length, μμ	ε	Wave length, μμ	ε	Wave length, μμ	ε
635	0.0062	547	0.6565	496	0.6391
605	.0097	541	.6893	492	.5322
579	.2250	538	.6834	479	.3093
574	.5292	533	.6676	468	.1950
569	.7226	521	.5184	457	.1152
565	.7426	514	.4499	438	.0437
561	.6717	506	.5101	422	.0433
557	.6267	499	.6388	404	.0622

TABLE II

EXTINCTION COEFFICIENTS FOR PHYCOCYAN (0.0293% SOLUTION)

λ	ε	λ	ε	λ	ε
691	0.084	605	1.113	530	0.540
671	.125	593	0.856	521	.430
635	.758	579	.757	506	.312
632	.980	567	.787	485	.227
627	1.172	557	.894	457	.146
619	1.271	549	.895	415	.083
611	1.270	538	.792		

that for egg albumin and hemoglobin. For phycocyan, concentration was determined (a) by precipitating a weighed amount of solution with acid, filtering on a Gooch crucible and drying at 105°; (b) by drying

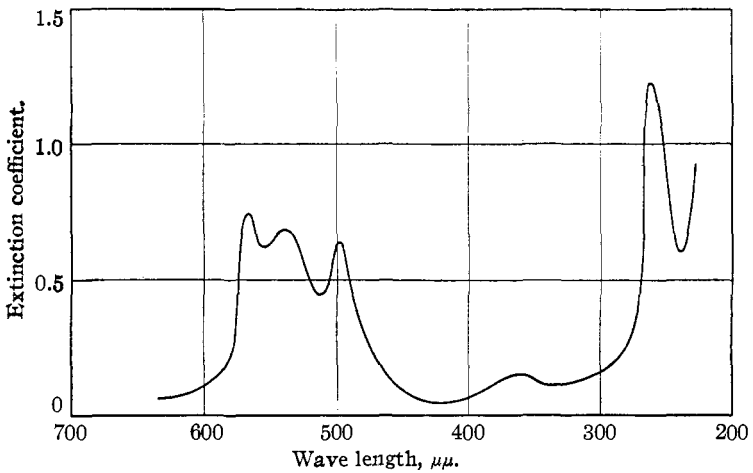

Fig. 1.—Light absorption for phycoerythrin (0.0097% solution).

at 105° and subtracting the calculated weight of phosphate in the volume of solution used. The two values agreed satisfactorily. The value found for the partial specific volume at 19.6° was 0.761 but this is rather inaccurate owing to the dilute solution used.

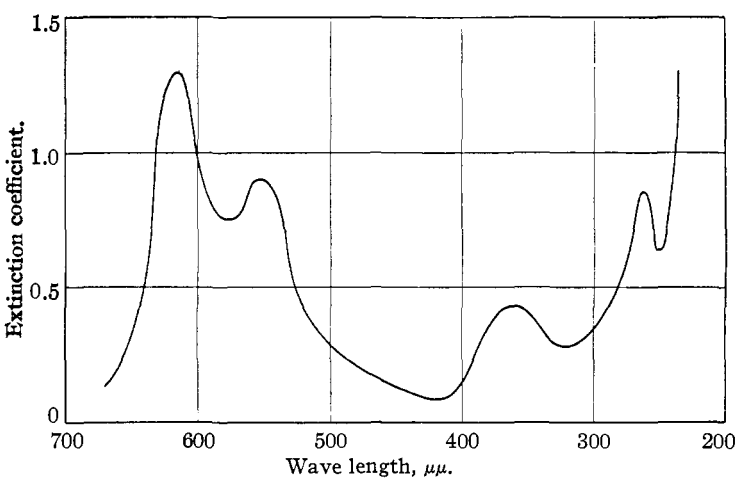

Fig. 2.—Light absorption for phycocyan (0.0293% solution).

Light Absorption.—(In dilute phosphate buffer solution, P_H = 6.8 and 7.0.) The absorption in the visible spectrum was measured with a König-Martens spectrophotometer and the results are given in Tables

I and II; for the ultraviolet a Judd-Lewis spectrophotometer was used; the values being of a lower order of accuracy they are not tabulated. The absorption curves obtained are shown in Figs. 1 and 2.

With phycoerythrin the absorption band in the visible was found to have three maxima, at 566, 540 and 497.5$\mu\mu$, agreeing with those obtained by Kylin[3] (569–565, 541–537 and 498–492$\mu\mu$) and Kitasato[4] (562, 526 and 495 $\mu\mu$, respectively). With phycocyan the maxima were found to be at 615 and 553$\mu\mu$, agreeing with those of Kylin (618–613 and 553–549$\mu\mu$, respectively) and Kitasato (614 and 546$\mu\mu$). In the ultraviolet both substances have bands with maxima at 262$\mu\mu$, and feebler, less well-defined ones at about 360$\mu\mu$ (Kitasato, approximately 270 and 330$\mu\mu$). With phycoerythrin the former band is much stronger than with phycocyan.

Determination of the Molecular Weight

A. By the Method of Sedimentation Equilibrium.—The apparatus and procedure have already been described.[5,6,7] With phycoerythrin photographs were taken in the visible spectrum (Wratten K3 filter), long-waved ultraviolet (Wratten or Corning nickel-glass filter), and short ultraviolet (quartz optical system, chlorine and bromine filters), the arrangements being as previously described.[5,6,7] In order to cut down the intensity of the mercury line 366$\mu\mu$ so as to get suitably long times of exposure, it was found convenient to insert a ground-glass screen between the mercury lamp and the water filter.

As standards, to correct for variation in intensity of the mercury lamp, solutions of potassium chromate of suitable strength were used, both for the long-waved and short-waved ultraviolet.

With phycocyan consistent results were not obtained with the short ultraviolet, possibly owing to a certain amount of decomposition during the run, so that the products formed would have absorption in the short ultraviolet, but not in the long ultraviolet or the visible, and hence the results would be affected only in experiments with the short ultraviolet. To increase the absorption in the long ultraviolet (since only a dilute solution of phycocyan was available), it was found necessary to use a cell of 8 mm. in thickness, as against 2 mm. in all other cases.

B. By the Method of Sedimentation Velocity.—The centrifuge and the method used have already been described.[8,9] Parallel light from the Pointolite lamp was used, with an Ilford three-color, green filter for phycoerythrin and a Lifa yellow filter for phycocyan. The plates used were Ilford

[5] Svedberg and Fåhraeus, THIS JOURNAL, **48**, 430 (1926).
[6] Svedberg and Nichols, *ibid.*, **48**, 3081 (1926).
[7] Svedberg, *Z. physik. Chem.*, **121**, 65 (1926).
[8] Svedberg, *ibid.*, **127**, 51 (1927).
[9] Svedberg and Nichols, THIS JOURNAL, **49**, 2920 (1927).

Screened Chromatic, developed for from two to three minutes with metol developer.

Results

A. Sedimentation Equilibrium.—The formula for the molecular weight, M, is given[5] by

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

where R is the gas constant, T the absolute temperature, V the partial specific volume of the protein, ρ the density of the solvent, c_2 and c_1 the

TABLE III

MOLECULAR WEIGHT OF PHYCOERYTHRIN

Concentration, 0.42 g. per 100 cc.; buffer solution P_H = 6.8 (0.01 M in KH_2PO_4 and Na_2HPO_4); standard, K_2CrO_4, $M/1200$; filter, Wratten ultraviolet; plates, Hauff Extra Rapid, developed for two minutes in metol developer for soft effects; source of light, mercury arc with ground-glass screen; aperture of objective, $f/22.7$; times of exposure, 15, 30 and 60 seconds; exposures made after 44, 48 and 51 hours of centrifuging. $\omega = 188.7\pi$; $V = 0.748$; $\rho = 1.001$; $T = 290.4$; length of column of solution = 0.53 cm.; thickness of column = 0.200 cm.; b, the distance of the outer end of solution from axis of rotation = 4.73 cm.

Distances, cm.		Mean concn. (orig. soln. = 1.000)		No. of expt. used	Mol. wt., thousands
x_2	x_1	c_2	c_1		
4.65	4.60	1.57	1.277	3	227
4.60	4.55	1.277	1.056	12	212
4.55	4.50	1.056	0.889	15	214
4.50	4.45	0.889	.747	18	208
4.45	4.40	.747	.632	15	206
4.40	4.35	.632	.542	15	192
4.35	4.30	.542	.467	15	196
4.30	4.25	.467	.394	15	212
4.25	4.20	.394	.336	15	203
				Mean,	206,000

TABLE IV

MOLECULAR WEIGHT OF PHYCOCYAN

Concentration, 0.12 g. per 100 cc.; buffer solution, phosphate mixture, P_H = 7.0; light source, Argenta lamp; filter, Wratten K3 (yellow); plates, Wellington Spectrum Panchromatic; development, 3 minutes in metol; aperture, $f/13.6$; times of exposure, 40, 60 and 90 secs.; exposures made after 40, 44 and 48 hours. $\omega = 239.7\pi$; $V\rho = 0.749$; length of column = 0.500 cm.; thickness of column = 0.200 cm.; $b = 4.73$ cm.; $T = 287°$.

Distances, cm.		Mean concn. (orig. soln. = 1.000)		No. of expt. used	Mol. wt., thousands
x_2	x_1	c_2	c_1		
4.63	4.58	1.258	1.088	11	105.5
4.58	4.53	1.088	0.953	11	97.6
4.53	4.48	0.953	.832	11	101.1
4.48	4.43	.832	.728	11	100.2
4.43	4.38	.728	.641	8	97.5
4.38	4.33	.641	.558	8	106.2
4.33	4.28	.558	.488	8	104.8
4.28	4.23	.488	.429	8	101.7
				Mean,	101,800

concentrations at distances x_2 and x_1, respectively, from the axis of rotation and ω the angular velocity.

The results of typical runs are given in Tables III and IV; the results for all the runs are summarized in Tables V and VI. It will be seen from Tables III and IV, and it was also the case in the other experiments, that there is no systematic variation in the values of the molecular weight with varying distance from the axis of rotation; in other words, the solutions consist of molecules of uniform size.

TABLE V

SUMMARY OF RESULTS FOR PHYCOERYTHRIN

Concn., g. per 100 cc.	P_H	Absorption band used	Plates used	Filter used	Mol. wt.
0.084	6.8	Visible	Ilford iso zenith	Wratten K3	209,000
.042	6.8	Visible	Ilford iso zenith	Wratten K3	209,400
.42	5.0	Long ultra-violet	Hauff ultra rapid	Corning nickel glass	217,400
.42	6.8	Long ultra-violet	Hauff extra rapid	Wratten nickel glass	206,000
.21	6.8	Short ultra-violet	Wellington ordinary	Chlorine and bromine	201,500
.21	6.8	Short ultra-violet	Hauff ultra rapid	Chlorine and bromine	202,900
				Mean	207,700

TABLE VI

SUMMARY OF RESULTS FOR PHYCOCYAN

Concn., g. per 100 cc.	P_H	Absorption band used	Plates used	Filter used	Mol. wt.
0.12	7.9	Visible	Wellington panchromatic	Wratten K3	112,600
.12	7.0	Visible	Wellington panchromatic	Wratten K3	101,800
.10	7.0	Visible	Ilford screened chrom.	Wratten K3	114,400
..	6.8	Long ultra-violet	Hauff extra rapid	Wratten nickel glass	100,000
..	6.8	Long ultra-violet	Hauff extra rapid	Wratten nickel glass	100,600
				Mean	105,900

B. Sedimentation Velocity.—The molecular weight is given by[9]

$$M = \frac{RT}{D(1 - V\rho)} \cdot \frac{1}{\omega^2 x} \cdot \frac{dx}{dt} = \frac{RT}{D(1 - V\rho)} \cdot s$$

where s is the specific sedimentation velocity, D the diffusion constant, x the distance from the axis of rotation and the other symbols have the same meaning as before. D can be calculated from the amount of blurring

of the boundary as previously described.[8,9] It must be pointed out that in these experiments, more particularly those with phycoerythrin, the values of D obtained are not very accurate, first, because the color-sensitized plates used do not appear to be as uniform, especially for green light, as the non-sensitized plates, thus making the determinations of concentration not so reliable; second, because with the relatively large sedimentation velocity and low diffusion constant, the time of centrifuging is shorter and thus the amount of diffusion much less; and third,

TABLE VII

PHYCOERYTHRIN

Concentration = 0.084%; phosphate buffer, P_H = 6.8; V = 0.755 at 30°; T = 303; length of column = 1.60 cm.; thickness of column = 0.20 cm.; exposure, 14 secs.; speed 39,050 r.p.m. (1302π).

Time interval, min.	Δx per 20 min., cm.	Mean x, cm.	$\omega^2 x \times 10^{-7}$	s, cm./sec. $\times 10^{13}$	D, cm.^{2}/day
20– 40	0.129	4.55	7.60	1.40	0.057
40– 60	.141	4.68	7.82	1.46	.046
60– 80	.134	4.82	8.05	1.34	.053
80–100	.147	4.96	8.29	1.42	.051
100–120	.152	5.11	8.54	1.42	.051
120–140	.159	5.26	8.79	1.44	.063
			Mean	1.413	0.0535

Calculated molecular weight, 235,000.

TABLE VIII

PHYCOCYAN

Concn. = 0.1%; phosphate buffer, P_H = 7.0; V = 0.755 at 30°; T = 303; length of column = 1.54 cm.; exposure = 15 sec.

Time interval, hours	Δx per half hour, cm.	Mean x, cm.	Speed, r.p.m.	$\omega^2 x \times 10^{-7}$	s, cm./sec. $\times 10^{13}$	D, cm.^{2}/day
$^1/_2$–1	0.090	4.56	39,100	7.64	6.42	(0.046)
1 –1$^1/_2$	.099	4.65	39,100	7.80	6.82	.0569
1$^1/_2$–2	.103	4.75	39,000	7.925	6.98	.0578
2 –2$^1/_2$	.106	4.85	39,000	8.105	6.98	.0626
2$^1/_2$–3	.109	4.97	39,000	8.29	7.01	.0617
3 –3$^1/_2$	.120	5.08	39,000	8.48	7.55	.0617
				Mean	6.96	0.0579

Calculated molecular weight, 107,000.

TABLE IX

Substance	P_H	Mean speed, r.p.m.	T	D, cm.^{2}/day	s, cm./sec.	M
Phycoerythrin	5.0	38,900	303	0.0528	1.435×10^{-12}	242,000
Phycoerythrin	6.8	39,100	303	.0614	1.435	208,000
Phycoerythrin	6.8	39,050	303	.0535	1.413	235,000
Phycoerythrin	6.8	28,100	300	.0538	1.370×10^{-12}	222,000
Phycocyan	7.0	38,950	303	.0606	7.01×10^{-13}	103,000
Phycocyan	7.0	39,000	303	.0579	6.96	107,000
				Mean for phycoerythrin		227,000
				Mean for phycocyan		105,000

because the value of D depends on the square of this quantity, so that any error in the determination of the latter has a correspondingly larger effect. The value of s, on the other hand, can be determined with a much higher degree of accuracy.

Details of typical runs are given in Tables VII and VIII and they are summarized in Table IX.

Fig. 3 gives a reproduction of the photographs taken during the centrifuging of the phycoerythrin at 39,000 r.p.m., that is with a mean centrifugal force of 90,000 times that of gravity, and shows the condition of the solution at 20, 40, ... and 140 minutes after the start of the run; the top row is the scale of concentrations.

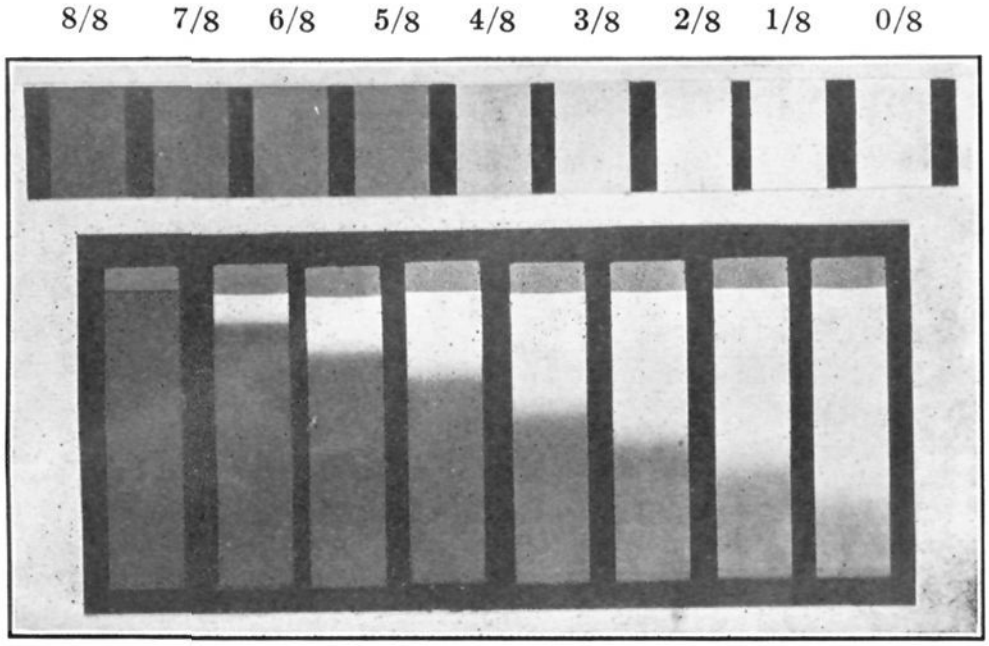

Fig. 3.

Fig. 4 shows the variation of concentration with distance after $^1/_2$, 1,. . . . . and $3^1/_2$ hours of centrifuging of phycocyan; the curves have been corrected for the decrease of concentration of the solution with time. The dotted curves for two and three hours represent the theoretical diffusion curves of a substance of only one molecular species. The deviation from the experimentally determined distribution is small and probably within the limits of experimental error. The same statement was also found to be true for the runs with phycoerythrin; that is, within the limits of experimental error, the molecules of both substances are uniform.

Examination of a Sample of Phycoerythrin Seventeen Years Old

A sample of Kylin's original phycoerythrin, which had also been prepared from *Ceramium rubrum*, was obtained through the courtesy of Pro-

fessor Mörner of the Biochemical Department. It had been kept for seventeen years in crystalline form under a saturated solution of ammonium sulfate covered with toluene.

Some of this substance was dialyzed for two weeks in a collodium bag against phosphate buffer solution ($P_H = 6.8$). The results of one run with this are shown in Fig. 5, which gives the variation of concentration with distance after 40, 65, 80 and 100 min. of centrifuging (speed 41,000–42,000 r. p. m.). The curves show marked divergences from those of a pure molecular species (see Fig. 4); Curve A shows the calculated curve for the pure substance after 80 minutes of centrifuging under the same conditions.

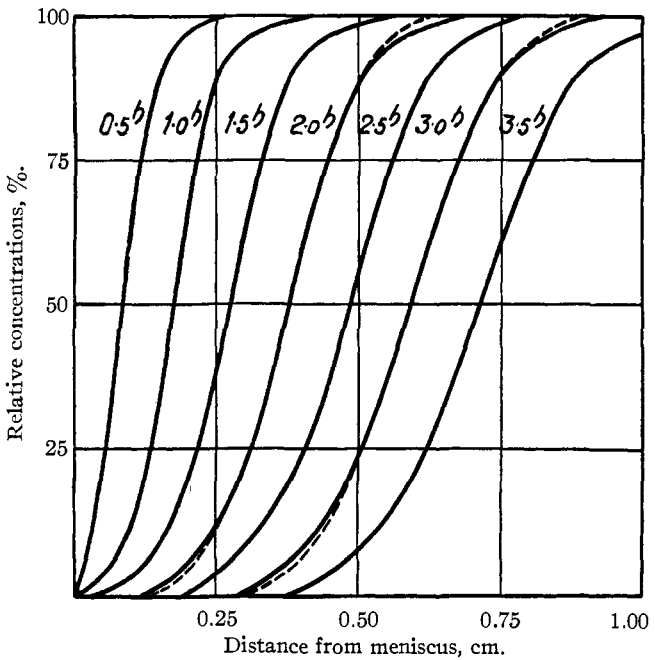

Fig. 4.

The curves show a point of inflection and evidently represent the distribution for a mixture of molecules of the normal size and of a smaller proportion of smaller ones so that the upper part corresponds to the distribution of the normal molecules and the lower to that of the smaller ones. From the amount of sedimentation corresponding to the lower part, it can be seen that these molecules were of $^1/_6$ or $^1/_8$ the weight of the normal ones. If we assume that the light absorption (in the green) per unit weight of these is the same as that for the normal molecules, an estimate can be made as to their proportion in the mixture. The values of s, the specific sedimentation velocity and of D, the diffusion constant for the normal molecules, were taken from the results of the

previous experiments and were corrected for any difference of temperature from 30°; those of s and D for molecules of $^1/_6$ and $^1/_8$ the size were calculated (assuming spherical molecules and the validity of Einstein's law) and the theoretical distribution curves for various mixtures then obtained. The experimental accuracy is, however, not great enough to decide whether the smaller molecules are those of $^1/_6$ or $^1/_8$ the normal size. In Fig. 5 the dotted curves show the calculated distributions for a mixture of 70% of molecules of weight 208,000 and 30% of 208,000/8. A mixture of 67% of 208,000 and 33% of 208,000/6 was found to agree equally well with the experimental results. A second run at 41,000 r. p. m. gave similar results, as did also one at 28,000 r. p. m.

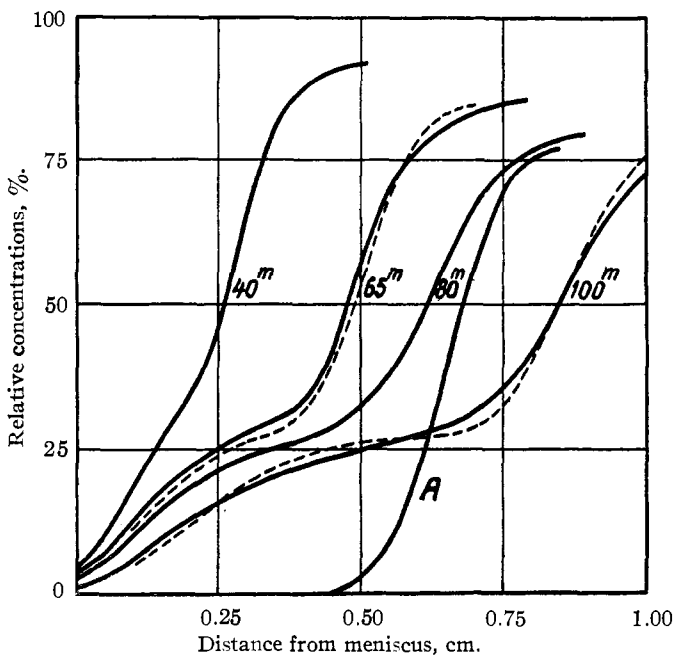

Fig. 5.

It was thought that the two kinds of molecules might be separable by recrystallization. Accordingly the material was recrystallized 6 times and the experiment repeated. The distribution curves still showed the presence of from 25 to 30% of the smaller molecules, so that very little, if any, separation had taken place.

Toward the end of the investigation the centrifuge was arranged so that photographs could be taken with short ultraviolet light. Under these conditions the recrystallized material gave qualitatively the same results as before, but the results were not suitable for quantitative information as to the composition of the mixture.

Discussion of Results

From the results it is considered that the most probable value of the molecular weight of phycoerythrin is 208,000 ± 8000 and of phycocyan 106,000 ± 5000, the former within the limits of experimental accuracy being double the latter. It is interesting to compare these values with those of the other two proteins already investigated in this Laboratory, namely, egg albumin and hemoglobin. We have for the molecular weight of egg albumin,[6] 34,500 ± 1000; for hemoglobin[10] it is 68,500 = 34,500 × 1.99; for phycocyan, = 34,500 × 3.07; and for phycoerythrin, = 34,500 × 6.03. The smaller molecules in the old sample of phycoerythrin may be of weight 208,000/6, that is, also approximating to 34,500.

The diffusion constant for phycoerythrin can be calculated from the Einstein formula

$$D = \frac{RT}{N} \cdot \frac{1}{6\pi r\eta}$$

where R is the gas constant, N the number of molecules in one gram molecule, η the viscosity of the solution at temperature, T (for the dilute solutions used this was taken as the same as that for pure water, that is, 0.0080 gm. cm.$^{-1}$ sec.$^{-1}$ at 30°), r the radius of the molecule (assumed spherical); r is calculated from the molecular weight M by the formula $M/N = {}^4/_3\ \pi\ r^3\rho$; ρ, the density, was assumed to be the reciprocal of the specific volume, the value being taken as 1.33. On this assumption $r = 3.95 \times 10^{-7}$ cm. The value of D calculated in this way is 0.061 cm.^{2}/day at 30°; experimental values were 0.061, 0.054 and 0.053, and the value obtained from the mean values of the molecular weight and specific sedimentation velocity was 0.061.

Thus the results agree very well with the assumption that the phycoerythrin molecule is spherical and has a density of 1.33. On the other hand, with phycocyan, which has practically the same diffusion constant and only half the molecular weight, the molecule must diverge widely from the spherical shape. Now Kitasato[4] found that these two substances had practically the same elementary composition, so that one could conclude that the phycoerythrin molecule is some sort of aggregation of two phycocyan molecules; or that it consists of an approximately spherical aggregation of smaller molecules and that phycocyan consists of an elongated-shaped aggregation of half the number of these smaller molecules.

Summary

1. The centrifugal sedimentation equilibrium and sedimentation velocity methods have been used to determine the molecular weights of phycoerythrin and phycocyan in dilute buffer solutions, using various

[10] Mean of determinations by Svedberg, Fåhraeus and Nichols.

hydrogen-ion concentrations and protein concentrations, and using visible, long-waved ultraviolet and short-waved ultraviolet light.

2. Within the limits of experimental error none of these factors influenced the values found for the molecular weight, the value of phycoerythrin being 208,000 ± 8000, and for phycocyan 106,000 ± 5000.

3. Within the limits of experimental error both of these substances consisted of a pure molecular species.

4. A sample of phycoerythrin 17 years old was found to consist of molecules of weight 208,000, together with about 30% of molecules of $^1/_6$ or $^1/_8$ of this weight; no marked separation of the two sizes of molecule was effected by recrystallization.

5. It was pointed out that the molecular weights of phycoerythrin and phycocyan, as well as that of hemoglobin, approximately were multiples of that of egg albumin.

UPSALA, SWEDEN

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE COLLEGE OF LIBERAL ARTS OF NORTHWESTERN UNIVERSITY]

PURE ARSENOBENZENE AND THE MOLECULAR COMPLEXITY OF ARSENOBENZENE AND ARSENOMETHANE

BY CHARLES SHATTUCK PALMER AND ALBERT B. SCOTT[1]

RECEIVED OCTOBER 28, 1927 PUBLISHED FEBRUARY 4, 1928

The structure of arseno compounds, which are probably the most important therapeutically and interesting chemically of all organic arsenicals, is not definitely known, although it has been quite generally accepted that they are analogous to the azo compounds, R—N=N—R. However, compounds of the two groups are quite different in solubility, ease of oxidation and reduction, action with chlorine and sulfur, etc. The published results on the molecular weights of symmetrical arseno compounds are conflicting and inadequate. The purpose of this investigation, therefore, was to determine the molecular weights of arseno compounds in associating and non-associating solvents in order to throw more light on the nature of their molecular structure.

Molecular weights for only three symmetrical compounds with the arseno linkage have been published. The first was for arsenomethane to which Auger,[2] in 1904, assigned the formula $(CH_3As)_4$ (I) from cryoscopic determinations on a solution of the yellow oil in benzene. But in 1926 Steinkopf, Schmidt and Smie[3] pointed out an error in his work and gave the

[1] Research Fellow under a grant from the Public Health Institute of Chicago. This paper is an abstract of a thesis presented by Albert B. Scott in partial fulfilment of the requirements for the degree of Master of Science at Northwestern University.

[2] Auger, *Compt. rend.*, **138**, 1705 (1904).

[3] Steinkopf, Schmidt and Smie, *Ber.*, **59**, 1463 (1926).