Anal. Calcd. for $C_{16}H_{15}O_2Br$: Br, 25.1. Found: Br, 24.6.

The acid was then prepared by an alkaline hydrolysis of the ester, and crystallization from benzene–ligroin; m. p. 107–108°.

3-(4'-Bromophenyl)-indanone VI.—To a solution of 7.9 g. of the above acid in 50 cc. of carbon disulfide was carefully added 5.2 g. of phosphorus pentachloride, and, after warming, all the solvent was removed by reducing the pressure. The residual oily chloride was taken up in 100 cc. of carbon disulfide, and, at 10–15°, 6.7 g. of anhydrous aluminum chloride was added. After stirring the mixture at room temperature for three hours and appropriate manipulation, 5 g. of the indanone, m. p. 59–60°, was secured, recrystallizing from benzene–petroleum ether.

Oxidation.—To a boiling solution of 4 g. of the indanone in 30 cc. of acetic acid was added 4 g. of chromium trioxide; the 4'-bromo-2-benzoylbenzoic acid, m. p. 172–173°, was isolated in the usual way. A mixed melting point with an authentic sample[6] showed no depression.

2-Bromo-3-(4'-bromophenyl)-indanone X was secured in both stereoisomeric forms as described.[1] The reduction to VI was carried out by adding the substance to an ethereal methylmagnesium iodide solution, refluxing for two hours, and appropriate manipulation. The indanone VI was readily isolated. Since one of the stereoisomers can be converted into the other, this fixes the structure of both forms of the dibromoindanones.

Summary

When unsymmetrical β,β-diarylpropionic acids are cyclized to indanones, by the use of anhydrous aluminum chloride on their acid chlorides, ring closure takes place with the unsubstituted phenyl group.

Certain discrepancies in the literature have been cleared up, and corrected structures assigned to two bromoindanones.

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

The Sedimentation and Diffusion Behavior of Certain Nucleic Acid Preparations[1]

BY HOWARD G. TENNENT AND CHARLES F. VILBRANDT

Introduction

Nucleic acids have a marked influence on biological processes because of their position in cell nuclei. As Hammarsten has pointed out, a large part of this influence is exerted through their physical properties. These have received much less attention than the organic chemistry of nucleic acids. Hammarsten[1a] measured the osmotic pressures, freezing points, viscosities, conductivities and Donnan equilibria of aqueous solutions of sodium thymonucleate, and considered the effect of added electrolytes on some of these properties. Later experiments showed that addition of large quantities of various salts caused a parallel decrease in the viscosity of sodium thymonucleate solutions and in the intensity of their streaming birefringence; this effect was reversible on removal of the salts.[2] The electrophoretic behavior of thymonucleic acid has been reported.[3] From diffusion measurements the following molecular weights have been calculated: 3000 for pancreas polynucleotide,[4] 37,000 for tobacco mosaic virus nucleic acid,[5] 17,000 for one preparation of yeast nucleic acid,[5] and 1300 for another.[4] The sedimentation behavior of sodium thymonucleate in the ultracentrifuge was found to depend upon the method by which the material was prepared.[6] Sodium thymonucleate has been shown to exist in solution as long thin rods of high molecular weight (200,000–500,000) by viscosity and streaming birefringence measurements[7] and by sedimentation and diffusion experiments.[8] Astbury and Bell[9] have made X-ray diffraction analyses of several nucleic acids.

In this report we present the results of sedimentation velocity, diffusion and partial specific volume measurements with solutions of sodium thymonucleate, thymonucleic acid, yeast nucleic acid, pancreas polynucleotide and barium thymate samples. From these data information as to their molecular sizes and shapes has been obtained.

(1) More complete details of this work are to be found in Theses of the authors submitted to the faculty of the University of Wisconsin in partial fulfillment of the requirements for the Ph.D. degree in June, 1942.

(1a) E. Hammarsten, *Biochem. Z.*, **124**, 353 (1924).

(2) J. P. Greenstein and W. V. Jenrette, *J. Nat. Cancer Inst.*, **1**, 77 (1940).

(3) E. Stenhagen and H. Teorell, *Trans. Faraday Soc.*, **35**, 743 (1939).

(4) K. Myrbach and E. Jorpes, *Z. physiol. Chem.*, **237**, 159 (1935).

(5) H. S. Loring, *J. Biol. Chem.*, **128**, lxi (1939).

(6) G. Schmidt, E. G. Pickels and P. A. Levene, *ibid.*, **127**, 251 (1939).

(7) R. Signer, T. Caspersson and E. Hammarsten, *Nature*, **141**, 122 (1938).

(8) K. O. Pedersen, in Svedberg and Pedersen, "The Ultracentrifuge," Oxford University Press, Oxford, 1940.

(9) W. T. Astbury and F. O. Bell, "Cold Spring Harbor Symposia on Quantitative Biology," Vol. VI, 109 (1938).

Materials[10]

The materials used in the sedimentation and diffusion studies may be briefly described.

Sodium Thymonucleates (STN): STN-1 was prepared by Bailey[11] by using a modification of the Hammarsten method. In the solid state it was a white, asbestos-like, fibrous material. It dissolved slowly but completely in water to give clear, highly viscous solutions. STN-2 was prepared by Caspersson by using the Hammarsten method.[1] Its appearance in the solid state was identical to that of STN-1. It dissolved slowly in water to give slightly cloudy solutions, which were not quite as viscous as those of STN-1 at comparable concentrations. STN-3 was prepared by Carter and Hall,[12] also using the method of Hammarsten. Its appearance in the solid state was identical to that of STN-1. Like STN-2 it dissolved slowly in water, giving cloudy solutions which had a lower viscosity than did solutions of STN-1 for comparable concentrations.

Thymonucleic Acids (TNA): TNA-1 was prepared by Bailey. TNA-2 was prepared by Gulland.

Yeast Nucleic Acid (YNA): YNA-1 was prepared by Caspersson.

Pancreas Polynucleotide (PPN): PPN-1 was prepared by Caspersson.

Barium Thymate (BT): BT-1 was prepared by Gulland.

The aqueous solutions of the sodium thymonucleates and barium thymate were neutral to litmus, whereas the others were acid (*p*H 2–3). With the exception of the sodium thymonucleates, all samples dissolved readily in water to give clear solutions whose viscosities were not noticeably different from that of water; a few of these solutions contained a trace of insoluble residue which was removed by filtration. With the exception of barium thymate, these materials were dissolved in 1% aqueous solution of sodium chloride; 1% barium chloride was used for the barium thymate solutions.

Experimental

Sedimentation Velocity Measurements.—Measurements of sedimentation velocity were made in a standard Svedberg oil-driven ultracentrifuge. The sodium thymonucleates were centrifuged at 42,000 r. p. m., corresponding to a centrifugal force of about 120,000 times gravity. Experiments on the other samples were made at 60,000 r. p. m., or 250,000 times gravity. The scale line displacement method of observation was used in most of the experiments; in a few the Philpot schlieren method was used. The sedimentation velocity constants were calculated by the equation

$$s = \frac{\Delta x}{\Delta t}\frac{1}{\omega^2 x}$$

and, for purposes of comparison, reduced to the basis of sedimentation in water at 25° by the expression

$$s^0_{25} = s\frac{\eta^s_t}{\eta^0_{25}}\frac{1 - V_{25}\rho^0_{25}}{1 - V_t\rho_t}$$

Definition of the symbols and the description of the apparatus and of methods used in the evaluation of the data are to be found in the recent monograph of Svedberg and Pedersen.[13]

Diffusion Measurements.—Diffusion constants were measured in cells similar to that described by Lamm and Polson.[14] In all experiments the scale line displacement method was used to observe the blurring of the boundary caused by diffusion. All measurements were made at 25°. Diffusion constants were calculated by using the first and second moments of Wiener's classical equation. The calculation has been described by Lamm.[15] These constants were converted to those corresponding to a process taking place in water at 25° by multiplying them by the relative viscosity of the solvent used.

Apparent Specific Volume Measurements.—Apparent specific volumes were measured pycnometrically and calculated by the equation

$$v_1{}^a = \frac{m_0 - (m - h)}{\rho_0 h}$$

in which m_0 and m are the weights of solvent and solution, respectively, contained in the pycnometer, h is the weight of solute present in m grams of solution, and ρ_0 is the density of the solvent. The pycnometer was made by blowing a bulb in the end of a piece of Pyrex capillary tubing of 0.5 mm. internal diameter. The tubing was cut off to leave a 5-cm stem on the bulb. Evaporation of water from solutions in the pycnometer was prevented by a wire plug inserted in the capillary. A fine scratch on the stem served as a reference mark. The pycnometer contained only 4.4 cc., permitting the use of a microbalance for weighings without overloading. A pipet ending in a fine capillary was used to fill and empty the pycnometer. Weighings were made with calibrated weights on a balance whose sensitivity was 55 divisions per milligram, and were accurate to within 0.01 mg. To calibrate the pycnometer, it was filled with recently boiled redistilled water so that the stem was full to a point slightly above the reference mark. After equilibration of the pycnometer and contents to 25.00 ± 0.005° in a water-bath, the distance from the meniscus in the capillary to the reference mark was measured within 0.02 mm. with a cathetometer. The filled pycnometer was then weighed. Subtraction of the weight of the empty pycnometer gave the weight of water contents corresonding to the measured height of the meniscus in the capillary. The process was repeated five times for different meniscus heights. The weight changed linearly with height within the limits of experimental error, proving that the capillary was uniform. For an apparent specific volume determination the weights of solvent and solution corresponding to measured meniscus heights were found in

(10) Of the eight samples of nucleic acid derivatives whose molecular kinetic properties were studied, seven were supplied by Professor W. T. Astbury of the University of Leeds, England. X-Ray investigations of these substances in the dried condition have been made in his laboratory, and the results of these analyses are to appear in another place. Descriptions of the methods of preparation are available only in the case of the thymonucleates, but the names of the persons who supplied the others can be given. We wish to acknowledge our indebtedness to him for placing these interesting materials at our disposal and to express our regret that the results of the X-ray investigations cannot be printed in a report to accompany this one.

(11) K. Bailey, private communication.

(12) R. O. Carter and J. L. Hall, THIS JOURNAL, **62**, 1194 (1940).

(13) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford University Press, Oxford, 1940.

(14) O. Lamm and A. Polson, *Biochem. J.*, **30**, 528 (1936); Polson, *ibid.*, **31**, 1903 (1937).

(15) O. Lamm, *Nova Acta Regiae Soc. Sci. Upsaliensis*, **4**, No. 6, 10 (1937).

a similar manner. The weight of solvent (m_0) corresponding to the solution meniscus height could then be calculated by using the weight *versus* height increment determined for water. Since the densities of the solvents were little different from that of water, the error introduced by using this increment was negligible.

The nucleic acid samples used in the determinations of apparent specific volumes were purified by dissolving them in water, centrifuging or filtering to remove insoluble material, freezing the clear solutions, and removing the ice by sublimation in a vacuum desiccator. The solid samples obtained in this way were washed several times with alcohol and dry ether and dried for several days over phosphorus pentoxide in a vacuum desiccator. The solutions on which apparent specific volume determinations were made were prepared by weighing the dried nucleic acids rapidly with a microbalance[16] and dissolving them in the proper amounts of solvent. The weights of the resulting solutions were measured on an analytical balance so that the nucleic acid concentrations in weight per cent., could be calculated. Dilutions of these solutions were made by weight with an analytical balance.

Results

The optical methods which were used to observe sedimentation and diffusion are applicable only when the refractive indices of the solutions vary linearly with the solute concentration. Refractive index measurements were made at 25° with a Bausch and Lomb dipping refractometer on solutions of several concentrations of STN-3. The results of these measurements given in Table I show that the optical methods were valid over the range of concentrations used for the sodium thymonucleates. There were not sufficient amounts of the more soluble samples available for corresponding measurements at higher concentrations.

TABLE I

REFRACTIVE INDEX INCREMENT DATA FOR SAMPLE STN-3 IN 1% SODIUM CHLORIDE AT 25°

Concn. of STN-3 (weight per cent.)	Refractive index	$(n - n_0)/c$
0.0000	1.33427	
.0497	1.33435	0.00161
.0993	1.33443	.00161
.1489	1.33452	.00168
.2014	1.33459	.00159
.2511	1.33466	.00155
.3008	1.33475	.00160
.3470	1.33482	.00159

Sedimentation and diffusion constants were determined for each sample at several concentrations. Measurements were continued to the lowest concentrations at which accurate values could be obtained. The results are given in Table II.

(16) The dried nucleic acids were quite hygroscopic and gained weight very rapidly in air. The inaccuracy in their weights because of this behavior was the chief source of error in the determination of their apparent specific volumes.

TABLE II

VARIATION OF SEDIMENTATION CONSTANT AND DIFFUSION CONSTANT WITH CONCENTRATION AT 25°

Sample	Concn. (wt. per cent.)	s^0_{25} (in S)	D^0_{25} (in 10^{-7} cm.^{2}/sec)
STN-1	0.29	6.4	0.61
	.19	7.3	...
	.10	9.4	...
	.06	12.0	...
	.05	13.0	...
	.04	14.1	...
	.025	17.1	...
STN-2	.30	7.0	...
	.26	..	1.0
	.11	8.0	1.0
	.08	8.3	...
	.055	8.7	...
STN-3	.26	7.8	0.96
	.17	8.0	.95
	.11	8.4	...
	.09	8.9	...
	.086	9.1	...
	.07	9.3	...
TNA-1	1.0	1.8	21.4
	0.75	2.2	21.0
	.5	..	21.9
TNA-2	.75	..	22.8
	.5	..	23.3
YNA-1	1.0	..	22.5
	0.75	..	24.0
	.5	..	26.3
PPN-1	1.0	2.7	17.2
	0.75	2.4	17.3
	.5	2.4	19.0
BT-1	1.0	..	29.3
	0.75	..	29.4
	.5	..	31.1

Samples TNA-2 and YNA-1, in repeated experiments, gave no diagrams from which sedimentation constants could be calculated. The shape of the curves obtained from diffusion experiments on TNA-2 and YNA-1 indicated that these samples were polydisperse. This probably explains their sedimentation behavior. The rapid diffusion of Sample BT-1 made measurement of its sedimentation constant impossible.

Three apparent specific volume determinations were made for each sample of nucleic acid. One was made with each of two concentrated solutions (to check the accuracy of the method) and the third on a system prepared by dilution of one of these solutions. The preparations of the two concentrated solutions, including the purification of

the nucleic acid, were entirely independent of each other. The values obtained are listed in Table III. Since v_1^a for all samples was found to be

TABLE III

VALUES OF APPARENT SPECIFIC VOLUME AT 25°

Sample	Concn. (wt. per cent.)	v_1^a (cc./gram)
STN-1	0.300	0.53
	.379	.55
	.103	.55
STN-2	.454	.56
	.356	.55
	.069	.56
STN-3	.346	.55
	.488	.54
	.094	.54
TNA-1	.830	.57
	.875	.57
	.092	.56
TNA-2	.729	.58
	.548	.57
	.099	.55
YNA-1	.787	.53
	.793	.54
	.096	.55
PPN-1	.896	.51
	.818	.52
	.092	.52
BT-1	.745	.42
	.629	.40
	.098	.38

independent of concentration within experimental error, it is equivalent to the true partial specific volume.

Molecular weights of the samples for which sedimentation constants could be obtained were calculated by the well-known equation

$$M = RTs/D(1 - v\rho)$$

This relationship is derived by assuming that the frictional resistance to sedimentation is equal to the frictional resistance to diffusion. When the solute molecules are highly asymmetric, this assumption is probably valid only in very dilute solutions. Consequently, sedimentation and diffusion constants should be determined at several concentrations and the values obtained by extrapolation to infinite dilution used in the calculation of molecular weight. It can be seen from Table II that the sedimentation constants of the sodium thymonucleates increase with decreasing concentration in such a way that extrapolation to infinite dilution is not possible. Therefore, in the calculation of molecular weights of the sodium thymonucleates, it was necessary to assume that at the same finite concentration the frictional resistances to sedimentation and diffusion were equal. The molecular weights were calculated by using the diffusion constants measured at the lowest concentrations at which accurate values could be obtained and the sedimentation constants measured at the same concentrations. For the other samples the sedimentation and diffusion constants were found to be independent of concentration within experimental error, and averaged values of each were used. Table IV lists the sedimentation and diffusion constants and partial specific volumes from which the quantities in Table V are calculated.

TABLE IV

VALUES OF SEDIMENTATION CONSTANTS, DIFFUSION CONSTANTS, AND PARTIAL SPECIFIC VOLUMES AT 25° USED TO CALCULATE QUANTITIES IN TABLE V

Sample	s^0_{25} (in S)	D^0_{25} (in 10^{-7} cm.²/sec.)	v_1^a (in cc./gram)
STN-1	6.4	0.61	0.55
STN-2	8.0	1.0	.55[a]
STN-3	7.8	0.95	.55[a]
TNA-1	1.8	21.5	.57
PPN	2.4	18.4	.52

[a] Average value for the three sodium thymonucleates was chosen.

TABLE V

MOLECULAR WEIGHTS, FRICTIONAL RATIOS, AXIAL RATIOS AND MOLECULAR DIMENSIONS OF NUCLEIC ACIDS

Sample	M	f/f_0	a/b	Cross sectional diameter in Å.	Length of Molecule, Å.
STN-1	580,000	8.0	400	13	5200
STN-2	430,000	5.3	170	16	2720
STN-3	450,000	5.6	200	15	3000
TNA-1	4,800[a]	1.1	3	14	42
PPN	6,700	1.2	4	14	56

[a] Sedimentation equilibrium measurements give M = 4100.

The frictional ratios, f/f_0, were calculated by the equation[12]

$$\frac{f}{f_0} = \frac{1}{6\eta^0_{25}}\left[\frac{4R^2T^2}{3\pi^2N^2}\right]^{1/3}\left[\frac{(1 - V\rho^0_{25})}{D^2sV}\right]^{1/3}$$

The shape factors, a/b, were computed from the frictional ratios by the Perrin equation[17]

$$\frac{f}{f_0} = \frac{[1 - (b/a)^2]^{1/2}}{(b/a)^{2/3} ln\ 1 + \dfrac{[1 - (b/a)^2]^{1/2}}{b/a}}$$

which is based on the assumption that the molecules in solution act as oblong ellipsoids of revolution whose major to minor axes have the ratio a/b. It is not certain that this equation is valid for molecules as asymmetric as the sodium thy-

(17) F. Perrin, *J. phys. rad.*, [7] 7, 1 (1936).

monucleates. The actual dimensions of the ellipsoid of revolution were calculated by using the equation

$$b = \left[\frac{4MV}{\pi Na/b}\right]^{1/3}$$

The molecular weights of samples TNA-2, YNA-1 and BT-1 could not be calculated because their sedimentation constants could not be measured. The diffusion constants of these samples, however, are approximately the same as that of TNA-1. If the shapes of the molecules of all these samples are assumed to be similar, it follows that the molecular weights are of the same order of magnitude.

Discussion

The striking differences in molecular weights and shape factors between the sodium thymonucleates and the other samples are probably due to differences in the methods by which the materials were prepared. This will be discussed more fully in another report.

The results we have obtained from sedimentation and diffusion experiments agree very well with the ideas of Astbury and Bell with respect to the physical structure of the nucleic acids. Their dimensions for the cross section of the polynucleotide column are 16 × 7 Å.; we have found that the cross-sectional diameters of all the samples for which the axial ratios, a/b, could be calculated are approximately 15 Å. The fact that molecules of all these samples in solution have practically the same cross sectional diameters regardless of their length suggests that the nucleic acids of low molecular weight are degradation products which result from cutting long columns into smaller fragments of the same cross section.

Note added December 21, 1942.—The Referee was kind enough to call to our attention the results of some viscosity and double refraction of flow studies with sodium thymonucleate preparations by A. Wissler (Dissertation, Bern, 1940) from which the length of the molecule was calculated to be 4700 Å., with a probable error of 10%. It will be seen that this value is in good agreement with the ones deduced in this report. Also, it indicates that the calculation of shapes of molecules from the Perrin equation gives a reasonable result, even in the case of extremely asymmetrical units.

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Summary

1. Measurements of sedimentation velocity, diffusion and apparent specific volume were made on solutions of eight nucleic acid preparations.

2. From these measurements molecular weights, frictional ratios, shape factors and molecular dimensions were calculated wherever possible (solutions of five of the eight preparations gave measurable sedimentation constants).

3. The three sodium thymonucleates, which had been prepared without the use of strong reagents or elevated temperatures, gave similar molecular constants. They were present in solution as long molecules whose molecular weights are in the neighborhood of 500,000.

4. The other materials (thymonucleic and yeast nucleic acids, pancreas polynucleotide, and barium thymate) have much lower molecular weights. They vary between 3000 and 7000.

5. The cross-sectional diameters of the molecules of the five substances for which shape factors can be calculated are identical within the limits of experimental error, regardless of the molecular length. These values agree very well with the dimensions calculated from X-ray data for the same samples in the solid state.

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