TABLE I

BASIC ESTER HYDROCHLORIDES $R-C(C_6H_5)(R')-CO_2R''\cdot HCl$

R	R′	R″	M. p., °C.[a]	Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Chlorine Calcd.	Chlorine Found
$-CH_3$	$-C_6H_5$	$-CH_2CH_2N(CH_3)_2$	178–180	$C_{19}H_{24}NO_2\cdot HCl$	68.36	68.48	7.24	7.15	10.63	10.42
$-C_2H_5$	$-C_6H_5$	$-CH_2CH_2N(CH_3)_2$[b]	164–165	$C_{20}H_{26}NO_2\cdot HCl$	69.05	69.16	7.53	7.45	10.19	10.21
$-C_2H_5$	$-C_6H_5$	$-CH_2CH_2N(C_2H_5)_2$	124–126	$C_{22}H_{29}NO_2\cdot HCl$	70.29	70.43	8.05	8.09	9.43	9.46
$-C_2H_5$	$-C_6H_5$	$-CH_2CH_2NC_5H_{10}$	146–147	$C_{23}H_{29}NO_2\cdot HCl$	71.23	71.25	7.77	7.89	9.14	8.92
$-C_2H_5$	$-C_6H_5$	$-CH_2CH_2CH_2N(C_2H_5)_2$	152–153	$C_{23}H_{31}NO_2\cdot HCl$	70.83	71.13	8.27	8.16	9.09	9.21
$-C_2H_5$	$-C_6H_5$	$-CH_2CH_2CH_2NC_5H_{10}$	143–144	$C_{24}H_{31}NO_2\cdot HCl$	71.70	71.98	8.02	8.12	8.82	9.00
$-C_2H_5$	$-C_6H_5$	$-CH(CH_3)CH_2N(CH_3)_2$	165–166	$C_{21}H_{27}NO_2\cdot HCl$	69.70	69.97	7.80	7.96	9.79	9.53
$-C_2H_5$	$-C_6H_5$	$-CH(CH_3)CH_2N(C_2H_5)_2$	117–118	$C_{23}H_{31}NO_2\cdot HCl$	70.83	70.81	8.27	8.16	9.09	8.96
$-C_2H_5$	$-C_6H_5$	$-CH(CH_3)CH_2NC_5H_{10}$[c]	218–219	$C_{24}H_{31}NO_2\cdot HCl$	71.70	71.53	8.02	8.04	8.82	8.88
$-C_2H_5$	$-C_6H_{11}$	$-CH_2CH_2N(CH_3)_2$	170–171	$C_{20}H_{31}NO_2\cdot HCl$	67.87	67.65	9.11	9.00	10.02	10.09
$-C_2H_5$	$-C_6H_{11}$	$-CH_2CH_2N(C_2H_5)_2$	185–186	$C_{22}H_{35}NO_2\cdot HCl$	69.17	69.27	9.50	9.67	9.28	9.23
$-C_2H_5$	$-C_6H_{11}$	$-CH_2CH_2NC_5H_{10}$	158–159	$C_{23}H_{35}NO_2\cdot HCl$	70.11	70.29	9.21	9.18	9.00	8.95
$-CH_2CH_2CH_3$	$-C_6H_5$	$-CH_2CH_2N(CH_3)_2$	176–177	$C_{21}H_{27}NO_2\cdot HCl$	69.70	69.85	7.80	7.64	9.79	9.73
$-CH_2CH=CH_2$	$-C_6H_5$	$-CH_2CH_2N(CH_3)_2$[d]	147–148	$C_{21}H_{25}NO_2\cdot HCl$	70.08	70.05	7.28	7.64	9.85	10.02

[a] All melting points are corrected. [b] The methiodide melted at 159–160°. *Anal.* Calcd. for $C_{21}H_{28}INO_2$: C, 55.63; H, 6.22; N, 3.09. Found: C, 55.61; H, 6.17; N, 2.90. [c] The methiodide melted at 211–213°. *Anal.* Calcd. for $C_{25}H_{34}INO_2$: C, 59.17; H, 6.75; N, 2.76. Found: C, 59.19; H, 6.75; N, 2.99. [d] *Anal.* Calcd. for $C_{21}H_{25}NO_2\cdot HCl$: bromine uptake, 1 mole. Found: bromine uptake, 0.98 mole.

A solution of 10 g. (0.04 mole) of 2,2-diphenylbutanoic acid in 25 ml. of thionyl chloride was refluxed for three hours. The solution was concentrated and then reconcentrated several times with benzene to remove the excess thionyl chloride. The residue was finally taken up in 40 ml. of dry benzene, decolorized with charcoal and refluxed for ten hours with 7.4 g. (0.08 mole) of dimethylaminoethanol. The reaction mixture was made alkaline with 10% sodium hydroxide, extracted with benzene and the benzene extracts washed well with water and finally concentrated at the water pump. The residue was taken up in dry ether and the hydrochloride of the basic ester precipitated with alcoholic hydrogen chloride. After washing with ether, the hydrochloride was recrystallized twice from ethyl acetate to give 8.3 g. (57%) of product, m. p. 164–165°.

Summary

A number of basically substituted esters of 2,2-diphenylalkanoic acids, 2,2-diphenyl-4-pentenoic acid and 2-cyclohexyl-2-phenylbutanoic acid have been prepared.

Procedures for the hydrolysis of certain tertiary nitriles to the corresponding acids have been investigated.

RENSSELAER, N. Y. RECEIVED AUGUST 20, 1948

[CONTRIBUTION FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

A Synthesis of Adenine. The Incorporation of Isotopes of Nitrogen and Carbon[1]

BY LIEBE F. CAVALIERI, JOHN F. TINKER AND AARON BENDICH

The earlier procedure[2] used for the synthesis of adenine labeled with isotopic nitrogen in the 1 and 3 positions[3] suffered from the disadvantage that the yields in certain steps were variable. This procedure as well as others[4,5] has been re-investigated and the synthesis now developed (Fig. 1) provides uniformly reproducible and satisfactory yields. This synthesis has been used for the incorporation of isotopes of carbon and of nitrogen into adenine.

Formamidine hydrochloride was prepared in nearly quantitative yield according to the reactions shown in Fig. 1. It will be noted that the atom per cent. excess of N^{15} in the formamidine hydrochloride is one half of that in the ammonia used, and that the isotope is distributed equally between the two nitrogen atoms. The condensation of formamidine hydrochloride and phenylazomalononitrile, to give I was carried out in *n*-butanol in the presence of sodium butoxide. In our hands, the yield of I was consistently lower when the condensation was carried out in ethanol containing sodium ethoxide.[2]

The previously described catalytic hydrogenation of 4,6-diamino-5-phenylazopyrimidine (I) to 4,5,6-triaminopyrimidine (II)[2] was found to be sensitive to impurities and variable yields were obtained. Upon investigation of other methods it was found that reduction with zinc and water proceeded smoothly with crude I.

A two-step cyclization of 4,5,6-triaminopyrimidine (II) to adenine (IV) was accomplished by

(1) The authors gratefully acknowledge the assistance of the James Foundation of New York, Inc., the National Cancer Institute of the U. S. Public Health Service, the United States Office of Naval Research and the Barker Welfare Foundation.

(2) Baddiley, Lythgoe and Todd, *J. Chem. Soc.*, 387 (1943).

(3) Brown, Roll, Plentl and Cavalieri, *J. Biol. Chem.*, **172**, 469 (1948).

(4) Traube, *Ann.*, **331**, 64 (1904).

(5) Hoffer, "Jubilee of Emil Barell." 428 (1946); *C. A.*, **41**, 4108 (1947).

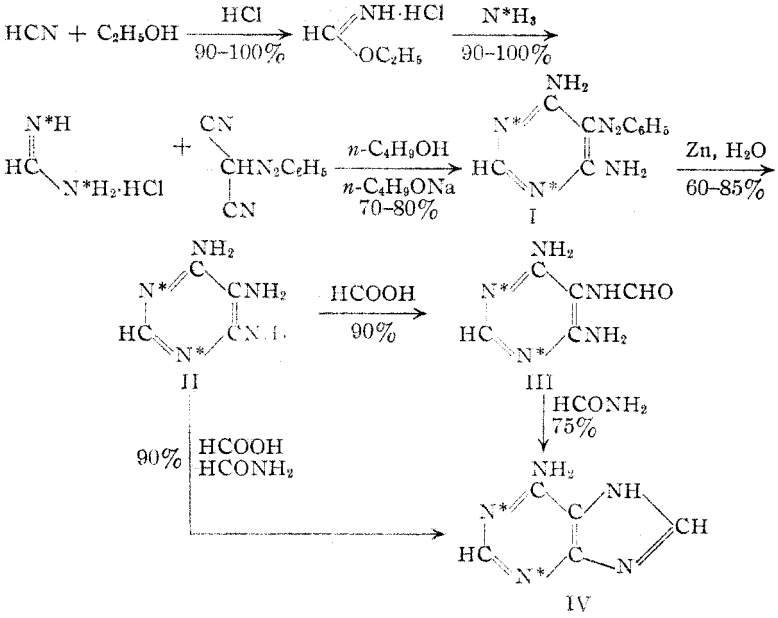

Fig. 1.—N* is N^{15}.

Baddiley, Lythgoe and Todd.[2] These investigators prepared the sulfur analog of III, 4,6-diamino-5-thioformylaminopyrimidine, by the reaction of II with potassium dithioformate and this derivative was cyclized to adenine by refluxing with either water or pyridine. In this Laboratory the product which resulted when the cyclization was carried out in water[3] revealed the presence of adenine and of 4,6-diamino-5-formylaminopyrimidine, when investigated by counter-current distribution. Cyclization with pyridine yielded, in addition to adenine, a small amount of 4,6-diamino-5-formylaminopyrimidine and unchanged starting material. The cyclization of 4,5,6-triaminopyrimidine (II) directly to adenine (IV) with formic acid[4] gave, in our hands, maximum yields of 35–40%. The conversion to adenine was most readily accomplished by heating II with a dilute solution of formic acid in anhydrous formamide.[6] Alternatively, the reaction could also be carried out in two steps. 4,6-Diamino-5-formylaminopyrimidine (III) was formed in quantitative yield when II was warmed gently with 98% formic acid. III was then cyclized to adenine in the presence of anhydrous formamide.

In hydrolysis experiments it was noted that when 4,6-diamino-5-formylaminopyrimidine (III) was heated with 1 *N* hydrochloric acid some adenine was produced together with a larger quantity of the hydrolysis product, 4,5,6-triaminopyrimidine. However, in the presence of concentrated hydrochloric acid, III was converted completely to 4,5,6-triaminopyrimidine.

Still another synthetic route which was investigated was based upon the condensation of malononitrile and thiourea to give 2-thio-4,6-diaminopyrimidine which was then nitrosated to 2-thio-4,6-diamino-5-nitrosopyrimidine.[4,6] The treatment of the latter compound with Raney nickel resulted in simultaneous desulfurization and the reduction of the nitroso group to yield 47% of 4,5,6-triaminopyrimidine (II). When 2-thio-4,5,6-triaminopyrimidine, prepared from the nitroso derivative by hydrosulfite reduction,[6] was desulfurized by Raney nickel treatment, a 78% yield of triaminopyrimidine (II) was obtained. The preparation of thiourea from isotopic ammonia is readily carried out but, although this synthesis could be useful for the incorporation of isotopic ammonia, the over-all yields based on malononitrile are less than for the method described in the experimental section.

The incorporation of isotopic carbon into malononitrile and thence into phenylazomalononitrile has also been accomplished (Fig. 2) and the incorporation of either C^{13} or C^{14} into the 4 and 6 carbons of the adenine molecule has been carried out by this synthesis.

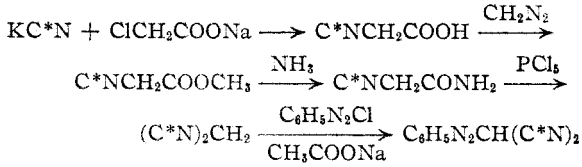

Fig. 2.—C* is C^{13}, C^{14}.

Experimental

Ethyl Formimino Ether Hydrochloride.—A four-necked flask was equipped with a mercury-sealed stirrer, a sintered glass gas-dispersion tube, a calcium chloride drying tube, and an inlet tube for the introduction of hydrogen cyanide. The flask was surrounded by a bath (alcohol-Dry Ice) kept at −10 to −20°. A mixture of one pound of commercial anhydrous ether and 225 cc. (3.86 moles) of absolute ethyl alcohol was placed in the flask. The stirrer was set in motion and the generation of hydrogen cyanide from 4 moles of sodium cyanide was begun.[7] When approximately 3.5 moles of the hydrogen cyanide was collected, a vigorous stream of anhydrous hydrogen chloride was passed in and continued for ten minutes after the first appearance of turbidity (total time, thirty to forty-five minutes). The mixture was then allowed to warm overnight, without stirring, during which time the bath temperature reached 10°. The bath was cooled to −20° and the solvent was removed through the gas dispersion tube by suction. The residual solid was washed with two one-half pound portions of ether, and the ether removed as before. The white crystalline product (285 g., 90% based on hydrogen cyanide, in an experiment where it was measured accurately) was dried briefly in air and stored in a tightly stoppered bottle. The compound slowly loses weight on exposure to air.

(6) Bendich, Tinker and Brown, THIS JOURNAL, **70**, 3109 (1948).

(7) "Organic Synthesis," Coll. Vol. I, John Wiley & Sons, Inc., New York, N. Y., 1932, p. 307.

Anal. Calcd. for $C_3H_7ON\cdot HCl$: Cl, 38.0. Found: Cl, 38.9.

Formamidine Hydrochloride.—Ethyl formimino ether hydrochloride (17.0 g., 0.18 mole) and 35 cc. of commercial absolute ethanol were placed in a dry Carius tube equipped with an inlet tube reaching an inch below the surface of the ethanol. The inlet tube was arranged so as to be easily detached. The tube was immersed in a Dry Ice-bath and connected to an ammonia generator. The generator consisted of a flask provided with a condenser and an inlet tube for the introduction of air. A drying tower filled with pellets of potassium hydroxide was placed on top of the condenser. Ammonium nitrate (8.8 g., 0.11 mole containing *ca.* 25 atom per cent. excess N^{15} in the ammonium group) which had been dried over calcium chloride was dissolved in 5–10 cc. of water and decomposed carefully by the addition of 11 cc. of 12 *N* potassium hydroxide. A brisk stream of air was drawn through the apparatus into an acid trap containing 5% aqueous boric acid and methyl violet indicator. Fifteen minutes after the addition of the potassium hydroxide, a water-bath was placed around the generating flask, the rate of air-sweep was decreased somewhat and the bath was warmed gradually to boiling in twenty minutes. After the bath had boiled for fifteen minutes, it was removed and the solution was boiled over a direct flame for fifteen minutes. Little or no ammonia remained in the generator.

The bomb tube, with its inlet tube detached and included, was sealed and heated for two hours at 100°, during which time the tube was shaken occasionally. The tube was cooled, opened and any excess ammonia was collected in the aqueous boric acid trap by drawing a stream of dry air through the inlet tube for thirty minutes while the tube was surrounded by a bath at 55–60°. Usually with this ratio of reactants no excess ammonia was collected. Ammonia which was not recoverable varied from 0 to 8%. The hot ethanolic solution was filtered (the residue is 99% pure ammonium chloride), the alcohol was evaporated by means of a stream of dry air, and the residual formamidine hydrochloride was dried *in vacuo* over phosphorus pentoxide and sulfuric acid.

Anal. Calcd. for $CH_4N_2\cdot HCl$: Cl, 44.1. Found: Cl, 43.8. (The analysis was carried out on material containing no excess N^{15}.)

4,6-Diamino-5-phenylazopyrimidine (I).—In a one-liter erlenmeyer flask was placed 29 cc. (46.5 g., 0.15 mole) of aniline and 85 cc. (1.02 moles) of concentrated hydrochloric acid. Ice was added to the mixture to maintain a temperature of 0 to 5°. Sodium nitrite (29.0 g., 0.50 mole, assay 97%) in 50 cc. of water was added in small portions, while stirring. After ten minutes, 51 g. (0.63 mole) of anhydrous sodium acetate in 100 cc. of water was added, followed by 33 g. (0.50 mole) of malononitrile[8] in 25 cc. of ethanol. After thirty minutes the phenylazomalononitrile[9] was collected by filtration, washed with cold water and dried in air. The material may be recrystallized from benzene (5 cc. per g.) although the crude material could be used for the following reaction. The yield of recrystallized material was 48–52 g., 55–60%. A three-necked flask was provided with a Hershberg stirrer, a reflux condenser (protected with a drying tube) and two separatory funnels (one on top of the other). Formamidine hydrochloride prepared from 0.11 mole of ammonia was placed in the flask. To this was added 20 g. (0.118 mole) of phenylazomalononitrile in 30 cc. of *n*-butanol (dried by distillation). In the bottom separatory funnel (connected to a drying tube) was placed 2.9 g. (0.13 mole) of sodium. Dry *n*-butanol (100–150 cc.) from the upper funnel was added to the sodium in small portions. The sodium butoxide solution was added to the reaction mixture in several portions, and the mixture refluxed gently for four hours. The mixture was cooled to 5° and the solid collected by filtration. The cake was washed alternately with water and alcohol and dried for a short time at 110°; yield, 15–18 g., 70–80%. This material was used in the next step without further purification, since the compound was difficult to recrystallize. When carefully dried ethanol was used as a solvent for the reaction[2] the yield was frequently as low as 30%.

4,5,6-Triaminopyrimidine Sulfate (II).—4,6-Diamino-5-phenylazopyrimidine (4.0 g., 0.0186 mole) was added to a boiling solution of 40 cc. of water and 6 cc. of ethyl cellosolve containing 4 g. of zinc dust. The mixture was boiled for 60–90 seconds while stirring. Sulfuric acid (6 cc. of 18 *N*) was added to the hot solution as rapidly as possible and the mixture decolorized with norite and filtered immediately. A lower yield resulted when the material was left in contact with acid. Upon cooling 2.4–3.4 g. (55–78%) of precipitate was collected. The 4,5,6-triaminopyrimidine sulfate thus obtained was used for the next step. The material could be recrystallized from 2 *N* sulfuric acid (20 cc. per g.) with an 85% recovery.

Anal. Calcd. for $C_4H_7N_5\cdot H_2SO_4\cdot H_2O$: N, 28.8. Found: N, 28.7.

4,6-Diamino-5-formylaminopyrimidine.—4,5,6-Triaminopyrimidine (1.0 g., 0.008 mole) was warmed gently with 2 cc. of 98% formic acid until all the solid had gone into solution. The excess formic acid was removed by evaporation at room temperature and the residue recrystallized from ethanol; yield, 1.15 g., 95%. The distribution constant in *n*-butanol–1 *N* phosphate, *p*H 6.5, for this material was 0.16.[3,10]

Anal. Calcd. for $C_5H_6ON_5$: C, 39.09; H, 4.51; N, 45.68. Found: C, 39.21; H, 4.61; N, 45.73.

4,6-Diamino-5-thioformylaminopyrimidine.—This compound was prepared according to the procedure of Baddiley, Lythgoe and Todd.[2] The distribution constant was 0.96.[10]

Anal. Calcd. for $C_5H_6N_5S$: C, 35.68; H, 3.99; N, 41.49; S, 18.79. Found: C, 35.49; H, 4.17; N, 41.39; S, 18.95.

Adenine Sulfate (IV).—(a) 4,5,6-Triaminopyrimidine sulfate (II) (0.78 g.) and 9 cc. of anhydrous formamide containing 0.3 cc. of 98% formic acid was heated in a bomb tube at 160–165° for two and one-half hours. The contents of the tube were chilled and the insoluble portion united with the residue on concentration of the filtrate *in vacuo* at *ca.* 150°. The residues were recrystallized from *ca.* 12 cc. of 2 *N* H_2SO_4; yield, 0.62 g. (95%).

(b) 4,5,6-Triaminopyrimidine sulfate (II) (5.0 g., 0.0207 mole) was dissolved in 25 cc. of anhydrous formic acid while warming the mixture gently. The solution was evaporated to dryness by warming gently in a stream of air. The 4,6-diamino-5-formylaminopyrimidine was transferred to a Carius tube and 20 cc. of formamide added. The tube was sealed and heated at 170° for two and one-half hours. The contents of the tube were cooled and the mixture filtered. The filtrate was evaporated to dryness and the two solid residues were recrystallized from 2 *N* sulfuric acid; yield, 3.1–3.5 g. (77–87%). The yield was lowered when the 4,5,6-triaminopyrimidine sulfate was boiled with formic acid or when the heating period in the formamide was greater than two and one-half hours.

Anal. Calcd. for $(C_5H_5N_5)_2{}^{11}\cdot H_2SO_4\cdot H_2O$: N, 36.3; S, 8.28. Found: N, 36.3; S, 8.50.

Atom per cent. excess N^{15}, 4.82. Counter-current distribution revealed that the adenine sulfate was 98–100% homogeneous with respect to ultraviolet absorbing impurities.[10]

Cyanoacetamide.[12]—In a 50-cc. flask was placed 590 mg. of sodium cyanide (90%) and approximately 2.7 millicuries of C^{14} in the form of sodium cyanide (147 mg.) containing about 80 mg. of sodium hydroxide. Five cc. of a 3.2 *N* solution of chloroacetic acid (neutralized with sodium carbonate) was added and the mixture was boiled

(8) "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 379.

(9) Topham, *J. Chem. Soc.*, 315 (1944).

(10) Tinker and Brown, *J. Biol. Chem.*, **173**, 585 (1948).

(11) Corrected for content of isotopic nitrogen.

(12) "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1932, p. 173.

gently for five minutes. The solution was allowed to cool somewhat and 2 cc. of concentrated hydrochloric acid was added. The mixture was evaporated to dryness *in vacuo* at 60–70°. The solid was extracted twenty times with 5-cc. portions of ether and the ether solution was added gradually to an ethereal solution of diazomethane (prepared from 4.5 g. of nitrosomethylurea). The solution was allowed to evaporate while standing overnight at room temperature. The residual oil was treated with 2 cc. of concentrated ammonia and warmed gently. An additional 2 cc. of ammonia was added and the solution was allowed to remain at room temperature overnight. The reaction mixture was evaporated to dryness *in vacuo* at room temperature and dried over phosphorus pentoxide for forty-eight hours; yield 400 mg., 42% over-all yield, based on the cyanide used.

Malononitrile.[8]—The cyanoacetamide was placed in a sublimation apparatus together with 400 mg. of phosphorus pentachloride. The system was evacuated by means of a water pump and the apparatus was immersed in a boiling water-bath. After the reaction had subsided, chloroform (cooled to −5° by means of a chloroform–Dry Ice-bath) was circulated through the cold finger. With the aid of an oil-bath the reaction mixture was heated to 160° and maintained there until the sublimation was complete (ten minutes).

Phenylazomalononitrile.—The malononitrile was allowed to melt from the cold finger into a 50-cc. glass-stoppered erlenmeyer and rinsed with a few cc. of methanol. Five cc. of 1 *M* phenyldiazonium acetate solution was added and the flask kept in an ice-bath overnight. The product was collected by filtration, washed with cold water and dried in the air; yield, 200 mg., 20% based on the cyanoacetamide used.

Adenine Sulfate.—The phenylazomalononitrile thus prepared was utilized in the preparation of adenine according to the directions described above. The final yield of adenine sulfate was 75[13] mg., which represents an over-all yield of 3.8% based upon the cyanide used. The product possessed an activity of approximately 7000 counts/min./milligram when a film of 1.93 micrograms/cm.^{2} was prepared by evaporation of an aqueous solution on an aluminum planchet and counted with a thin window (1.9 mg./cm.^{2}) Geiger–Müller counter.

Acknowledgment.—The authors wish to acknowledge the advice of Dr. George Bosworth Brown, the coöperation of Dr. Harold Beyer in the isotope determinations, and the assistance of Alice Angelos, Rosco Funk, Jr., and Thelma Kaplan.

Summary

Various syntheses of adenine have been investigated and a satisfactory procedure for the introduction of isotopes of nitrogen and carbon has been developed.

The preparation from isotopic carbon of cyanoacetamide and malononitrile is described.

(13) Of this amount, 32 mg. was obtained from the filtrate by washout dilution with 450 mg. of non-radioactive adenine.

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[CONTRIBUTION FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY, THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, PRINCETON, NEW JERSEY, AND THE DEPARTMENT OF PHYSICS,[1] UNIVERSITY OF PITTSBURGH, PITTSBURGH, PENNSYLVANIA]

The Hydration, Size and Shape of Tobacco Mosaic Virus[2a,2b]

BY H. K. SCHACHMAN[3] AND MAX A. LAUFFER

Introduction

On the basis of indirect evidence, several investigators have speculated about the degree of hydration of tobacco mosaic virus,[4a,b,c] but there has been no direct experimental attack on this problem. The ultracentrifuge furnishes a possible means for the direct determination of the density of the virus in solution by a study of the effect of varying solvent density on the change of sedimentation rate.[5a,b] The combination of this solution density with the apparent partial specific volume permits an evaluation of the hydration of the virus.

A certain amount of ambiguity arises in a precise evaluation of the size and shape of any molecule because of the difficulty in determining the relative contributions of hydration and anisometry to the physical chemical properties of the material in question. In the case of tobacco mosaic virus, the application of several physical technics, including ultracentrifugation, X-ray diffraction, viscosity, birefringence of flow and diffusion, has led to the evaluations of size and shape which were largely substantiated by direct observation in the electron microscope.[6] These computations, however, were predicated upon the assumption of little or no hydration of the virus particles, an assumption made because X-ray measurements could be interpreted to mean that the particles did not swell when placed in solution.[7]

In view of the apparent success of the centrifugation method of determining the hydration of viruses, it seemed worthwhile to determine the hydration of tobacco mosaic virus and then to re-examine the other physical chemical properties in the light of this result.

(1) Contribution No. 11p-47 of the Department of Physics, University of Pittsburgh.

(2) (a) Paper delivered before the division of Biological Chemistry at the 111th meeting of the American Chemical Society, April, 1947; (b) aided in part by a grant from the National Foundation for Infantile Paralysis.

(3) Junior Research Fellow of the National Institute of Health; present address: University of California, Berkeley California.

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(5) (a) J. E. Smadel, E. G. Pickels and T. Shedlovsky, *J. Exp. Med.*, **68**, 607 (1938); (b) D. G. Sharp, A. R. Taylor, I. W. McLean, Jr., D. Beard and J. W. Beard, *Science*, **100**, 151 (1944).

(6) M. A. Lauffer, THIS JOURNAL, **66**, 1188 (1944).

(7) J. D. Bernal and I. Fankuchen, *J. Gen. Physiol.*, **25**, 111 (1941).