

[CONTRIBUTION FROM THE CHEMISTRY LABORATORIES OF ARIZONA STATE UNIVERSITY, TEMPE, ARIZONA, AND IOWA STATE UNIVERSITY, AMES, IOWA]

## Hydration of the "V" Amylose Helix<sup>1</sup>

BY B. ZASLOW<sup>2</sup> AND RICHARD L. MILLER

RECEIVED MARCH 15, 1961

Structural changes resulting from the hydration of anhydrous "V" amylose and the amylose-iodine complex at 35° have been followed by means of X-ray diffraction powder patterns. Water vapor interacts initially with both of these substances at crystallographically equivalent sites on the exterior of the amylose helix. Subsequent reaction occurs by attack of water on the inside of the helix for the anhydrous "V" amylose, while cleavage into smaller groupings of helices results for the iodine complex. The "B" starch modification is a final product in both cases. The existence of a hydrated amylose-iodine complex having a pseudohexagonal lattice spacing of 13.6 Å. is reported.

### Introduction

The precipitate obtained by the reaction of amylose with 1-butanol has been characterized crystallographically,<sup>3</sup> and its dehydration has yielded two well-defined products: a hydrated "V" amylose modification with a 13.7 Å. pseudohexagonal lattice spacing<sup>3,4</sup> and an anhydrous "V" amylose with a 13.0 Å. lattice spacing.<sup>4</sup> Furthermore, the 1-butanol precipitate has been iodinated and dried to give an anhydrous iodine complex having unit cell dimensions that are equal to those of the anhydrous "V" modification.<sup>5</sup> In all of these compounds the amylose assumes a helical configuration having six glucose residues to a turn in the helix.<sup>3,5</sup> X-Ray diffraction intensities indicate that the iodine molecules are located within the helices in the iodine complex.<sup>4</sup>

Water is able to convert the "V" amyloses to the "B" form<sup>6,7</sup>; however, the details of this conversion have not been studied previously. A comparative hydration study on anhydrous "V" amylose and the anhydrous amylose-iodine complex could reasonably be expected to yield structural information about the "V" amylose helix; the helix exterior is the same in both cases, while the helix interior is exposed only in anhydrous "V" amylose because of the presence of iodine in the iodine complex. This investigation of the interaction between water vapor and the "V" amyloses and the amylose-iodine complex was undertaken to establish the nature of the hydration of these materials.

### Experimental

Conversion of anhydrous "V" amylose to the "B" starch modification proceeds too rapidly to be followed if liquid water is in direct contact with the amylose; therefore, hydration by water vapor was investigated. X-Ray diffraction diagrams were used to record changes in the samples. The general hydration procedure, except as indicated below, was to observe powder pattern variations with time starting with anhydrous "V" amylose and the anhydrous amylose-iodine complex. This was done first under a condition of 100% humidity and then under controlled vapor pressures fixed by crystal hydrate systems.

Experiments were conducted in a hydrating chamber which consisted of a large test tube and stopper. The sample and either pure water or the crystal hydrate system

TABLE I  
POWDER REFLECTIONS FROM THE HYDRATED AMYLOSE-  
IODINE COMPLEX

Indices	sin <sup>2</sup> θ (obsd.)	sin <sup>2</sup> θ (calcd.)	Intensities (obsd.)
200	0.00432	0.00427	28
101	.01063	.01033	1.0
111	.01348	.01246	5
220		.01281	
201		.01353	
310		.01388	
400	.01707	.01708	14
320	.02143	.02028	0.5
221		.02207	
401	.02641	.02634	1.5
420	.02985	.02989	60
510	.03339	.03309	0.8
331	.03852	.03808	28
600		.03843	
511	.04298	.04235	0.5
601	.04760	.04770	1.5
440	.05131	.05124	7
620	.05542	.05551	2
621	.06497	.06477	2
800	.06873	.06832	2
640	.08126	.08113	5
820	.08963	.08967	8
821	.09957	.09893	0.5
10.0.0	.1068	.1067	3
660	.1152	.1153	1.5
840	.1200	.1196	0.5
10.2.0	.1322	.1324	1.5
10.2.1	.1438	.1417	0.2
860	.1590	.1580	0.3
10.4.0	.1664	.1665	4
12.2.0	.1834	.1836	0.2
800	.1933	.2050	.2
14.0.0, 10.6.0	.2091	.2092	.5
12.4.0	.2238	.2220	.2
14.2.0	.2439	.2433	.2

were placed physically apart from each other into this container. The amount of sample was small compared to the quantity of material in the hydrate system in order to insure that the water vapor pressure was controlled by the latter. Temperature was maintained either by immersing the entire assembly in a thermostat for experiments involving minutes to a few days or by placing the assembly into an oven when longer hydration times were required. All experiments were run at 35°. Air was present inside the hydrating chamber.

After various time intervals portions of the samples were removed from the hydrating container and inserted into thin-walled glass capillaries having a 0.3 mm. diameter. The capillaries were sealed with Apiezon W wax, and powder diffraction diagrams were taken using Cu radiation with a camera having a 57.3 mm. radius. Temperature and pres-

(1) Supported in part by a grant from the Corn Industries Research Foundation, Inc.

(2) To whom inquiries concerning this publication should be sent: Arizona State University, Tempe, Arizona.

(3) R. E. Rundle and F. C. Edwards, *J. Am. Chem. Soc.*, **65**, 2200 (1943).

(4) R. E. Rundle, *ibid.*, **69**, 1769 (1947).

(5) R. E. Rundle and D. French, *ibid.*, **65**, 1707 (1943).

(6) R. S. Bear, *ibid.*, **64**, 1388 (1942).

(7) L. C. Spark, *Biochim. et Biophys. Acta*, **8**, 101 (1952).

TABLE II  
 FINAL AMYLOSE MODIFICATION LISTING

Hydrating system	Vapor pressure, mm.	Anhydrous "V" amylose initially in contact with water vapor	Anhydrous amylose-I <sub>2</sub> complex initially in contact with water vapor
CuSO <sub>4</sub> ·5H <sub>2</sub> O, CuSO <sub>4</sub> ·3H <sub>2</sub> O	16	Anhydrous "V" amylose	.....
NiCl <sub>2</sub> ·6H <sub>2</sub> O, NiCl <sub>2</sub> ·4H <sub>2</sub> O	21	Anhydrous "V" amylose	.....
KCr(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O, KCr(SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	24	Anhydrous "V" amylose	Anhydrous amylose-I <sub>2</sub>
Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O, Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O	26	Hydrated "V" amylose	Hydrated amylose-I <sub>2</sub>
MgSO <sub>4</sub> ·7H <sub>2</sub> O, MgSO <sub>4</sub> ·6H <sub>2</sub> O	28	Hydrated "V" amylose	.....
Na <sub>2</sub> C <sub>4</sub> H <sub>4</sub> O <sub>6</sub> ·2H <sub>2</sub> O, Na <sub>2</sub> C <sub>4</sub> H <sub>4</sub> O <sub>6</sub> ·2H <sub>2</sub> O dehydrate	37	Hydrated "V" amylose	.....
H <sub>2</sub> O (liquid)	42	"B" Modification	"B" Modification

sure could not be controlled while the capillaries were being filled or while the samples were in the X-ray beam, except that these substances were handled in atmospheres having humidities of less than 20%. In the course of this investigation it was necessary to prepare these substances: anhydrous "V" amylose, hydrated "V" amylose, the anhydrous amylose-iodine complex and a hydrated amylose-iodine complex. The hydrated amylose-iodine complex is characterized by the data given in Table I.

Amylose supplied by Stein Hall and Co. was used as the starting material for all of the compounds involved in the hydration and dehydration studies. Solution of this amylose was effected by a Soxhlet extraction with 1-butanol and water; the solid that appeared after a slow cooling with an excess of 1-butanol was washed with CCl<sub>4</sub> and digested in a 15% water in methanol mixture. The process of solution and reprecipitation using 1-butanol and water was repeated several times, and anhydrous "V" amylose was obtained by drying the wet butanol precipitate in a vacuum at 50°. Line intensities of the X-ray diffraction diagram of this material matched those previously reported.<sup>4</sup>

Hydrated "V" amylose was prepared by exposure of the anhydrous "V" amylose to water vapor for 30 minutes, and the best samples of the hydrated amylose-iodine complex resulted from the iodination of the hydrated "V" amylose, three days being required to complete the process at 35°. Material prepared by this method provided the Table I data; intensity values for the powder lines were determined from a series of X-ray films using timed exposures.

Anhydrous amylose-iodine complex was prepared by exposing the anhydrous "V" amylose to iodine vapor at 50° for one week in a closed system. One week of exposure at this temperature was found to be equivalent to three weeks of exposure at 35°. Since no change in lattice constant is involved, the process was followed by observing line intensities on X-ray powder diagrams. While the diffraction intensities approached those reported for the iodine complex<sup>4</sup> during the conversion process, the observed intensity values indicated that the iodination had not been completed.

The crystal hydrate systems are listed in Table II. These were made with reagent grade chemicals; weight analyses were used to check that roughly equal parts of crystal hydrate and dehydrate were present.

## Results

**The Diffraction Pattern of the Hydrated Amylose-Iodine Complex.**—The hydrated amylose-iodine complex is characterized by the observed  $\sin^2\theta$  values and intensity ratings in Table I; the powder lines have  $d$ -spacings that match the lines of the hydrated "V" amylose.<sup>3</sup> While most of the lines correspond to a small pseudo-hexagonal unit cell having a 13.6 Å. lattice spacing, the total data fit a larger hexagonal cell where  $a = b = 27.2$  Å. and  $c = 8.01$  Å. The Miller indices listed in Table I are those of the large hexagonal unit.

The ( $hkl$ ) intensities were converted to a structure factors and the electron density shown in Fig. 1 was obtained assuming that the iodine positions control the signs of the terms in the Fourier series and making the approximation that the projection has C<sub>6i</sub> symmetry. Beavers-Lipson strips were employed in performing the Fourier calculation using data from the planes of the small pseudo-unit only. This entailed the omission of the (320) and (510) planes from the series. Also omitted were the (220), (14.0.0) and (10.6.0) reflections, since there was no way of determining what fraction of the total observed intensities should be as-

signed to each of these terms. Detail shows up in this projection in the form of a ring around the iodine positions and a peak at the interstices between helices.

**Hydration Results.**—The hydration experiments are summarized in Table II, where vapor pressures of the hydrating systems are reported along with the final amylose modification present after exposure of anhydrous samples to these vapor pressures. Ten to twenty minutes were required to convert anhydrous "V" amylose to the hydrated amylose-iodine complex whenever these reactions took place.

Several steps were involved in the transformations that proceeded at 100% humidity. In the hydration of the anhydrous "V" amylose sample, the hydrated "V" amylose that formed first was transformed to an amorphous state during the next 10 hr. This was noted by the disappearance of the discrete diffraction pattern which occurred without change in  $d$ -spacings. Thereafter, the presence of some "B" modification was indicated, five days being required to effect this last change.

Similarly, the initial product that resulted upon the interaction of water with the anhydrous amylose-iodine complex under a 100% humidity condition was the hydrated amylose-iodine complex. "V" amylose impurity in the anhydrous amylose-iodine sample did not obscure the detection of the hydrated amylose-iodine, since several characteristic diffraction lines exist at large  $\sin \theta$  values for this compound. However, to avoid interference of "V" amylose impurity in the subsequent hydration, the study was continued on the hydrated amylose-iodine sample that had been prepared by iodinating the hydrated "V" amylose. It was found that many of the hydrated amylose-iodine lines faded on successive films, while other lines exhibited a pronounced broadening effect. The diffraction pattern on the film taken at the end of the third day was dominated by the (100) and (210) lines of the small hexagonal cell. Of the broadened lines, only (210) lent itself to a measurement of line width which, using the Scherrer formula, indicated an average particle size of  $3\pm$  Å.; maximum width was achieved after eight days and did not change after a two month exposure to water vapor. Lines corresponding to the "B" modification began to appear on the X-ray film taken after three days of exposure to water vapor and grew more intense on subsequent films.

It also was found that when the hydrated "V" amylose and hydrated amylose-iodine are exposed to the water vapor pressures above the KCr(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O, KCr(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O and the CuSO<sub>4</sub>·5H<sub>2</sub>O, CuSO<sub>4</sub>·3H<sub>2</sub>O crystal hydrate systems, no dehydration was observed during a two week period.

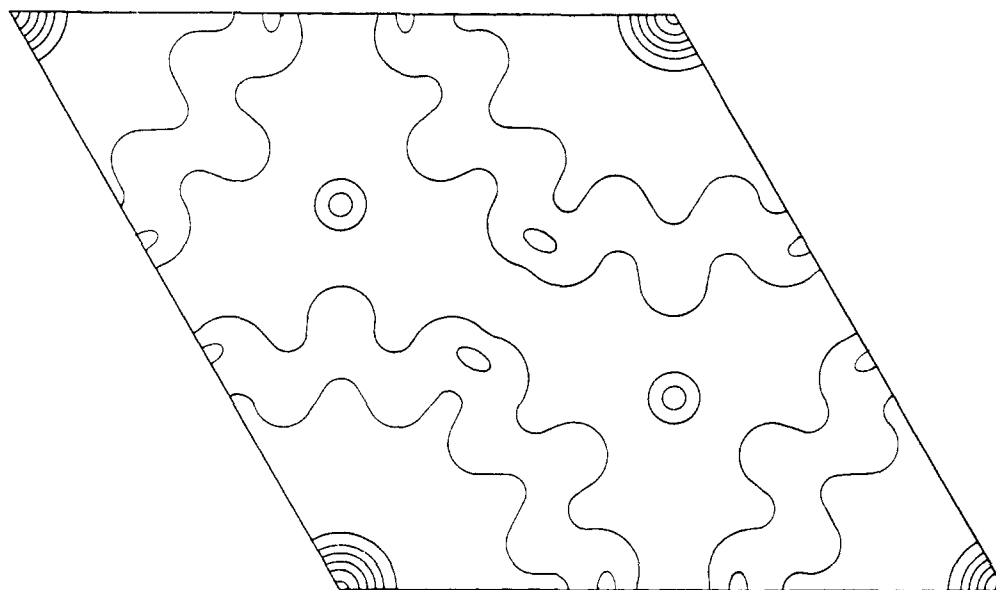


Fig. 1.—Fourier projection along the  $c$  axis. Contours are plotted at twice as large an electron density interval around the iodine positions than is used for the rest of the projection.

### Discussion

With respect to the conversion of the anhydrous "V" amylose to hydrated "V" amylose, three independent considerations establish that changes in composition of the amylose samples did not occur after they had been removed from the hydrating chamber to obtain X-ray photographs. This conversion process was consistently selective (see Table II), although all samples received similar handling; none of the powder patterns showed the simultaneous presence of  $d$ -spacings of both crystalline forms, and the stability of the hydrated "V" amylose in an atmosphere of low humidity was demonstrated by its inability to lose water to the copper sulfate system. Film sequences of slow and continuous change were obtained for the reactions between the hydrated helical amylose modifications and water at a vapor pressure of 42 mm., and the nature of the data indicates that the order of events was determined inside the hydrating chamber. For example, the line-broadening phenomenon was first observed on the film obtained at the end of one day of exposure of hydrated amylose-iodine to water vapor, and, in becoming more pronounced on films taken after longer exposures to water vapor, precludes the possibility that this effect was brought about by conditions the samples encountered during handling. Similar situations hold for the disappearance of the hydrated "V" amylose and the appearance of the "B" starch diffraction lines.

The first step recorded in the total hydration process is the same for both the anhydrous "V" amylose and the anhydrous amylose-iodine complex. Water vapor enters both structures at interstitial sites rapidly at the same rate, and, at 35°, requires a critical pressure between 24 and 26 mm. to effect an entry. The resultant hydrates are crystalline entities with similar lattice constants and undoubtedly are isomorphous. This process indicates that the interaction is unrelated to the presence of iodine; the pattern of hydration

confirms the crystallographic evidence which indicates that iodine lies in the center of the helices,<sup>4,8</sup> since no change in lattice constant would be expected if the water molecules enter within the helices. In this respect, the peak that shows up at the helix interstices in the electron density projection may be real. Density measurements give evidence that the hydrated "V" amylose contains one water molecule per glucose residue on the helix exterior.<sup>4</sup> The step-nature response to water vapor at only one pressure indicates that a specific interaction with each glucose residue takes place at an ordered site during hydration; this interaction is responsible for the discontinuity observed in the lattice expansion.

Since no further lattice expansion occurs upon continued hydration, the attack by water in the second stage of the process on the "V" amylose occurs within the helix, where a slow destruction of the helix is followed by the formation of the more extended "B" structure. That the former is completely destroyed before the latter begins to appear implies that the two structures are unrelated to each other. In the case of the iodine complex, the center of the helix is blocked to this attack by the presence of iodine molecules. Water acting over a longer period of time must penetrate to interstitial positions causing a cleavage of the amylose-iodine aggregate into smaller fragments. The 34 Å. particle size indicates that, on the average, seven helices exist in the new clusters. It is interesting that, as evidenced by the appearance of the "B" modification, the iodine complex is unstable to water vapor at high humidity; since the amylose-iodine complex is also not stable in an aqueous solution of  $I_2$  and amylose when the  $I^-$  concentration is repressed,<sup>9</sup> the neutral molecule is probably the principal species inside the helix when the complex

(8) W. J. James, D. French and R. E. Rundle, *Acta Cryst.*, **12**, 385 (1959).

(9) J. A. Thoma and D. French, *J. Am. Chem. Soc.*, **82**, 4144 (1960).

is formed from iodine vapor and solid "V" anylose.

**Acknowledgments.**—The authors wish to express their appreciation to Professor R. E. Rundle

for several valuable discussions and thank Mr. F. H. Schneider for assisting in the completion of some of the hydration experiments.

[CONTRIBUTION FROM MONSANTO CHEMICAL CO., RESEARCH DEPT., INORGANIC CHEMICALS DIV., ST. LOUIS, MO.]

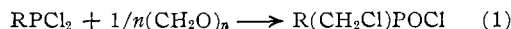
## Synthesis and Properties of Phosphinic and Phosphonic Acid Anhydrides\*

BY KURT MOEDRITZER

RECEIVED MAY 22, 1961

A new method for the preparation of phosphinic acid anhydrides,  $R_1R_2P(O)OP(O)R_2R_1$ , in good yields is described. It involves the action of paraformaldehyde on phosphinic chlorides. Several new symmetrical ( $R_1 = R_2$ ) and unsymmetrical ( $R_1 \neq R_2$ ) phosphinic acid anhydrides were prepared and their physical constants and characteristic infrared frequencies are reported. Regularities in their  $P^{31}$  (nuclear magnetic resonance) n.m.r. chemical shifts are discussed. The P-O-P bond in the phosphinic acid anhydrides is cleaved by  $PCl_3$  and by protolytic agents, such as HOH, HOR,  $HNR_2$ . It is shown that this new method can be used to prepare phosphonic acid anhydrides and anhydrides of other acids.

In connection with some other work in this Laboratory, Kabachnik's<sup>1</sup> reaction to prepare alkyl-chloromethylphosphinic chlorides from paraformaldehyde and alkyldichlorophosphine has been repeated.



The above authors suggested that paraformaldehydes or aldehydes in general react with alkyldichlorophosphines by the way of formation of an  $\alpha$ -chlorinated ester of triply connected phosphorus, which then undergoes intermolecular Arbuzov rearrangement to give the alkyl-chloromethylphosphinic chloride in yields varying from 36 to 50%.

In our Laboratory, Dr. L. C. D. Groenweghe independently observed that in the reaction of methyldichlorophosphine with paraformaldehyde a  $P^{31}$  n.m.r. peak was obtained which could not be accounted for by reaction 1. In repeating this reaction with several alkyl and aryldichlorophosphines the crude reaction products always exhibited three resonance peaks, indicating the presence of three different species of phosphorus compounds. One of the peaks could be assigned to unreacted alkyldichlorophosphine. The chemical shift of the second signal checked with that of a pure sample of alkyl-chloromethylphosphinic chloride, isolated from the reaction product by fractional distillation. The third peak represented a phosphorus compound, the chemical shift of which has not been reported in previous compilations of shift constants.<sup>2</sup> The latter resonance peak was observed again as the only signal given by a sample of residue of the alkyl-chloromethylphosphinic chloride distillation. This suggested that the residue consisted of a uniform phosphorus compound. High vacuum distillation of this dark, viscous residue yielded almost quantitatively a colorless liquid which was identified as alkyl-chloromethylphosphinic acid anhydride.

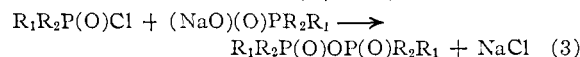
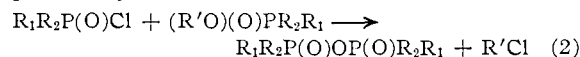
\* Presented at the 140th Meeting of the American Chemical Society, Chicago, Ill., Sept., 1961.

(1) M. I. Kabachnik and E. S. Shepeleva, *Izvest. Akad. Nauk S.S.S.R., Otdel. Khim. Nauk*, 862 (1953); *C. A.*, **49**, 843 (1955).

(2) H. S. Gutowsky, D. W. McCall and C. P. Slichter, *J. Chem. Phys.*, **21**, 279 (1953); H. S. Gutowsky and D. W. McCall, *ibid.*, **22**, 162 (1954); N. Muller, P. C. Lauterbur and J. Goldenson, *J. Am. Chem. Soc.*, **78**, 3557 (1956); J. R. Van Wazer, C. F. Callis, J. N. Shoolery and R. C. Jones, *ibid.*, **78**, 5715 (1956); H. Finegold, *Ann. New York Acad. Sci.*, **70**, 875 (1958).

This paper reports the utilization of the reaction to prepare phosphinic acid anhydrides in general. Such a method is of significance, since phosphinic acid anhydrides cannot be prepared by simple dehydration of the corresponding phosphinic acid.<sup>3</sup> It will be shown that this new method can be applied also to the preparation of anhydrides of other acids.

**Phosphinic Acid Anhydrides.**—Evidently, the phosphinic anhydride formation described above has occurred *via* the primarily formed phosphonic chloride (reaction 1), which in a second step reacted with paraformaldehyde to give the phosphinic anhydride. This assumption was confirmed when a slurry of paraformaldehyde in a phosphinic chloride in the mole ratio 1:1 was heated to 100–150°. A clear solution was obtained, which on distillation gave the corresponding phosphinic anhydride in good yield. The latter was found to be identical with an authentic sample prepared by one of the previously known methods.<sup>4,5</sup>



As compared to reactions 2 and 3, the new method described here offers the advantage of requiring phosphinic chloride only as starting material. Also, alkyl or aryl-chloromethylphosphinic anhydrides,  $R_1 = \text{alkyl or aryl}$ ,  $R_2 = \text{CH}_2\text{Cl}$ , can be prepared directly from an alkyl or aryldichlorophosphine by reaction with an excess of paraformaldehyde.

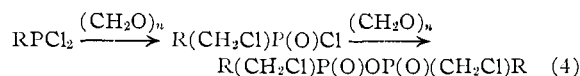


Table I lists the phosphinic acid anhydrides prepared by this new method and some of their physical data.

**Mechanism of the Reaction.**—Phosphorus halides are known to react with paraformaldehyde to give dihalomethyl ether, and if the reaction is pushed to its extreme limit, methylene halides are

(3) J. N. Collie, *J. Chem. Soc.*, **127**, 964 (1925).

(4) G. M. Kosolapoff, *J. Am. Chem. Soc.*, **73**, 4101, 5466 (1953).

(5) N. Kreutzkamp and H. Schindler, *Arch. Pharm.*, **293**, 296 (1960).