[CONTRIBUTION NO. 306 FROM THE DEPARTMENT OF CHEMISTRY, KANSAS AGRICULTURAL EXPERIMENT STATION, KANSAS STATE COLLEGE]

The Chlorination of Starch with Phosphorus Pentachloride[1]

BY H. N. BARHAM, E. S. STICKLEY AND M. J. CALDWELL

During recent years, various factors have combined to stimulate increased interest in the chemical utilization of starch. One promising approach to the problem would appear to be the preparation of reactive chlorine derivatives of starch. From exploratory experiments in which sulfur monochloride, sulfuryl chloride, phosphorus trichloride, phosphorus pentachloride, etc., were used as chlorinating agents, it was found that only phosphorus pentachloride did not usually result in the formation of black, intractable tars of low chlorine content. The present article describes the production and properties of yellow, solid, chlorinated, high molecular weight starch derivatives by the action of phosphorus pentachloride, or phosphorus pentachloride and phosphorus oxychloride. The chlorination was successful only when suitable pressures and reactant concentrations were employed, with the temperature being raised in well-defined stages. A tentative reaction mechanism is suggested.

Experimental

Preparation of Reaction Mixtures.—Cornstarch, dried for twelve hours *in vacuo* at 100°, was used in all experiments. The starch was intimately mixed with phosphorus pentachloride which first had been ground to a fine powder. The grinding and blending were done under anhydrous conditions in a cabinet (15″ × 18″ × 24″), coated inside with a chemically resistant enamel and constructed so that the worker could use his arms and hands, protected by rubber sleeves and gloves, to carry out necessary manipulations. Observations could be made through a full-vision window at the top. The desiccant used was phosphorus pentoxide, both on the open trays inside and at the vents through which dry air could be passed to sweep out phosphorus pentachloride vapors. The starch sample was weighed accurately before being placed in the cabinet, while the approximate weight of the phosphorus pentachloride was determined on a balance placed inside. The reaction mixture was weighed accurately after its removal from the cabinet.

Preparation of Pentachlorostarch.—A mixture of 40 g. of dried starch (0.247 equivalent mole of anhydro-glucose) and 540 g. of phosphorus pentachloride (2.59 moles) was placed in a 1-liter, round-bottom reaction flask attached to a condenser leading to a receiver cooled in an ice-bath. Ground glass connections were used throughout and the reaction mixture was protected against atmospheric moisture by the use of a drying train. Heating was carried out in three steps. It was essential that a first stage of the reaction be completed before the temperature could be increased if a destructive reaction was to be avoided. The length of time required by the first stage depended upon the temperature; about fifteen to seventeen hours was sufficient at 100–105°. During the second stage of the reaction the mixtures were heated at 125–135°; a minimum of about twenty-four hours heating was essential. The third stage, during which the chlorine content of the products increased only slightly and a major portion of the excess phosphorus pentachloride sublimed, was carried out at 160–170° over periods exceeding eight hours. The completion of any stage could be gaged roughly by the appearance of the reaction mass and the rate at which the phosphorus chloride products distilled. Upon completion of the reaction, the residue in the reaction flask was treated with ice water to remove the residual phosphorus pentachloride at low temperature. Later, the organic product was repeatedly scrubbed with cold water, filtered and dried.

Anal. Calcd. for $C_6H_5O_2Cl_5$: Cl, 61.90. Found: 62.2; 62.4. The chlorine content of products was determined by sodium peroxide fusion in a Parr bomb and subsequent weighing as silver chloride. Its variation among different pentachlorostarch products was between 60 and 63%.

Physical Properties of Pentachlorostarch.—The pentachlorostarch products are yellow to orange-yellow, amorphous solids. When heated, they become tarry through decomposition and finally char. At room temperature, they are insoluble in water and acid solutions, very slightly soluble in alcohol and ligroin, and very soluble in ethylene dibromide, ethylene dichloride, ethyl bromide, dioxane, ether, benzene and acetone. A solution of the pentachlorostarch in an equal weight of acetone is notably viscous.

Analysis of $POCl_3$–PCl_3 Mixtures.—The liquid phosphorus chloride products were analyzed by fractional distillation in a column of 8 mm. in internal diameter and 64 mm. in length. This column was packed with single-turn glass helices and was surrounded by a silvered, highly evacuated jacket. Calibration showed that the column operated efficiently as an 11-plate column under a 10:1 reflux ratio.

The Finkelstein[2] Reaction.—Five and two-tenths grams of pentachlorostarch was refluxed for two hours with 110 cc. of 1 *N* sodium iodide solution in acetone (a 10% excess of sodium iodide). The finely divided brown precipitate, consisting principally of sodium chloride formed during the reaction, was filtered from the solution, after which the acetone was removed from the filtrate by distillation and evaporation. The organic residue was transferred to a mortar in which it was ground under aqueous sodium thiosulfate. After repeated trituration with water, it was filtered and dried *in vacuo*. The products were hygroscopic, dark brown solids having a gritty texture.

Anal. Calcd. for $C_6H_5O_2Cl_3$: Cl, 49.37. Found: Cl, 50.3.

Refluxing for twelve instead of two hours caused a decrease in chlorine content to 44.4%; standing at room temperature for six days caused a decrease to 47.6%.

Rates of Formation of Phosphorus Oxychloride and Hydrogen Chloride at 60°.—The apparatus used for this purpose was so designed that samples of the absorbed volatile products could be removed for analysis at will. In the assembly, a 500-cc. reaction flask was connected by a 75° gooseneck to an adapter, the outlet of which led to a three-way stopcock. The stopcock could be turned so that two parallel branches could be connected alternately into the system. Each of the branches consisted of a trap, followed by an absorption bulb; the trap and the bulb were sealed together to form a single unit. The parallel branches were connected to the stopcock by means of spherical ground-glass joints.

Small volumes of concentrated sulfuric acid in the traps served to prevent the movement of moisture from the absorption bulbs into the reaction flasks; sodium hydroxide solution of approximately 2 *N* concentration absorbed all vapors which passed through the traps. Analyses for total phosphorus and chlorine were made upon

---

[1] Acknowledgment is made to Sharples Chemicals Inc. for their support of this work.

[2] Finkelstein, *Ber.*, **43**, 1528 (1910).

stock solutions prepared by mixing the contents of the trap and absorption bulb and diluting to volume. The phosphorus was weighed as magnesium pyrophosphate and the chlorine as silver chloride. Since no phosphorus trichloride was formed at 60°, the quantities of phosphorus oxychloride and hydrogen chloride formed could be calculated. The pressure maintained was 240 mm.

Solvent-Extraction of the Product.—At the end of each reaction at 60°, the solid mass in the reaction flask was extracted with carbon bisulfide in a Soxhlet extractor to remove the unused phosphorus pentachloride. The sacks which held the solid residue during extraction were made of "Fiberglas," stitched with glass fibers. A moisture flask and an absorption bulb, equipped with suitable safety vessels and containing, respectively, concentrated sulfuric acid and approximately 2 *N* sodium hydroxide solution, were connected in series to the vapor outlet of the condenser. The absorbents served to fix phosphorus oxychloride and hydrogen chloride vapors which escaped condensation in the extraction apparatus. Upon completion of the extraction, the phosphorus oxychloride and hydrogen chloride still retained by the solvent were also recovered in the above manner and the total phosphorus and chlorine determined.

Part I

Numerous experiments have shown that an intimate mixture of starch and finely powdered phosphorus pentachloride react, under anhydrous conditions and at atmospheric pressure, to form starch derivatives which contain 60–63% chlorine and to produce phosphorus oxychloride, phosphorus trichloride and hydrogen chloride. In order that the reaction proceed uniformly to completion, it was essential that the phosphorus pentachloride concentration be high, approximately 10 molecules for each glucose unit, and that increases in the reaction temperature be made stepwise. If the temperature exceeded the boiling point of phosphorus oxychloride by more than a few degrees during the first few hours of the reaction, the whole mass turned black and the formation of phosphorus oxychloride and phosphorus trichloride ceased. The reaction was explosive when the temperature was increased rapidly above about 107°.

The analysis of samples of the liquid distillate taken at intervals during the course of the reaction showed the presence of phosphorus trichloride from the beginning of the reaction. By the analysis of the total liquid distillate obtained during the reaction, it was determined that the phosphorus trichloride and phosphorus oxychloride were produced in the approximate molar ratio of 2:3.

The formation of phosphorus trichloride and phosphorus oxychloride in this ratio suggested that they were produced in accordance with the equation

$$C_6H_7O_2(OH)_3 + 5PCl_5 \longrightarrow C_6H_5O_2Cl_5 + 5HCl + 2PCl_3 + 3POCl_3$$

Upon this basis, a mixture composed of 40 g. of starch and 560 g. of phosphorus pentachloride should result, upon completion of the reaction, in the calculated quantities of the products and unused reactants listed in the second column of Table I. In the third column are given for comparison results of an experiment in which the stated quantities were used.

TABLE I

A COMPARISON OF REACTION DATA WITH CORRESPONDING VALUES CALCULATED ON THE BASIS OF THE CHEMICAL EQUATION

	Calcd. values	Reaction data
Weight of organic product, g.	71	70.5
Weight of reclaimed PCl_5, g.	303	176
Volume of liquid distillate, cc.	111	108
Molar ratio: $PCl_3/POCl_3$	2/3	2/3.1
Chlorine in product, %	61.9	62.3

The comparison provided by Table I emphasizes the probability that the over-all reaction is in essential agreement with the above equation. It will be noted, however, that only 176 g. of the calculated 303 g. excess phosphorus pentachloride was recovered. This may be explained by the difficulty encountered in the complete sublimation of unused phosphorus pentachloride out of the reaction mixture.

Polyhydric alcohols and miscellaneous sugars, including aldohexoses, an aldopentose, a ketohexose and disaccharides also were chlorinated under similar conditions to determine the extent to which carbon-bound hydrogen would be replaced. There was the possibility that the greater hydroxyl content of the sugars and alcohols might alter the sequence of replacement reactions exhibited by carbohydrate polymers, with the result that the carbon-bound hydrogen atoms of the former would show greater resistance to substitution. The results indicate that the chlorination of sugars and polyhydric alcohols approaches completion with the elimination of the hydroxyl groups. To the extent that this occurs, the number of chlorine atoms attached to a given carbon atom is limited to one. The data obtained for glucose, sucrose and mannitol illustrate the extent to which the chlorine content of the low molecular weight compounds exceeds that which corresponds to the hydroxyl content.

Anal. Glucose: calcd., 65.01; found, 66.0. Sucrose: calcd., 57.90; found, 60.1. Mannitol: calcd., 72.64; found, 72.5.

It will be noted that, of the three compounds, sucrose showed the greatest difference between the calculated and observed chlorine contents. This may be attributed to the oxygen bridge in sucrose; if one hydrogen atom of a bridge-carbon had been replaced, the calculated chlorine content would become 60.86 instead of 57.90%.

Two series of experiments are described to show the influence of the pressure and the bulk phosphorus pentachloride concentration upon the nature of the products and the course of the reaction. In one series, the 10:1 ratio of reagents was used and the previously described heating procedure applied, while the pressure was varied; the pressures used were 200, 400, 750 and 1020 mm. Pressures were controlled by means of a glass valve pressure regulator.[3] In the second series, lower proportions of phosphorus pentachloride were used in the reaction mixtures, the pressure was atmospheric, and again the heating was carried out in steps; the reactant ratios used were 10:1, 5:1, 4:1 and 3:1. It was found that these two variables have, qualitatively, several of the same effects upon the course of reaction and the properties of the final products. The results which were noted either from a lowering of the reactant ratio below 10:1 or from decreasing the pressure below atmospheric pressure are as follows: (1) The chlorine content and weight of the organic product

---

[3] Caldwell and Barham, *Ind. Eng. Chem., Anal. Ed.*, **14**, 485 (1942).

decreased. (2) The products became progressively less stable, tending to split off hydrogen chloride and to become very dark in color. (3) The products exhibited increasing heterogeneity with respect to color and solubility. (4) The ratio of phosphorus trichloride to phosphorus oxychloride formed during the reactions decreased sharply, dropping as low as 2:7.5 for a reaction of a 10:1 mixture at 200 mm. pressure, and to 2:11 in the case of a 3:1 reaction mixture reacting at atmospheric pressure. It was noted further that, when the temperature was increased above 107°, before sufficient time had been allowed for the completion of the first stage of the reaction, reaction mixtures containing the lower proportions of phosphorus pentachloride blackened much more rapidly than the 10:1 mixtures. The initial addition of quantities of phosphorus oxychloride sufficient to moisten the reaction mixtures also served to decrease their sensitivity in this respect. However, the moistening of the reaction mixtures did not serve to eliminate the lack of stability nor the heterogeneity which characterize products not formed in the proper sequence of reactions.

From the above results, the following seem probable. (1) The availability of phosphorus pentachloride to the organic reactant, which is determined by the bulk concentration of phosphorus pentachloride and the concentration of phosphorus oxychloride, determines to a considerable extent the course of the reaction and the nature of the products. (2) If the phosphorus oxychloride reaction predominates, products of low stability result. (3) If the phosphorus trichloride reaction predominates, the products consist principally of carbon. (4) For ultimate stability of the product, the phosphorus oxychloride and phosphorus trichloride reactions should occur at comparable rates. (5) Increased temperature favors the phosphorus trichloride reaction. (6) The formation of the relatively stable pentachlorostarch requires the substitution of at least one carbon-bound hydrogen atom. (7) Double bonds in the ring are not stable.

Part II

Another series of experiments was carried out at lower temperatures to learn whether the phosphorus oxychloride and phosphorus trichloride reactions could be resolved and, at the same time, to study further the stability of compounds containing fewer than five chlorine atoms per glucose unit. At temperatures of 60 to 80°, and at pressures of 200 to 400 mm., reaction mixtures of phosphorus pentachloride and starch in a 3:1 ratio were found to react continuously over periods as long as thirty days. Red-brown, solid, organic products, hydrogen chloride and phosphorus oxychloride were formed. No phosphorus trichloride was obtained under these conditions. Furthermore, raising the temperature as high as 97° or the pressure to 600 mm. or higher, at any time during the first eight days of the reaction, resulted in spontaneous blackening of the reaction mass and stoppage of the evolution of phosphorus oxychloride. After fourteen days at 60° and 240 mm. pressure, however, even if the pressure was raised to atmospheric and the temperature increased to 100°, the mass remained red-brown in color and phosphorus oxychloride continued to be formed. For purposes of comparison, a study was made also of the reaction of 10:1 PCl_5–starch mixtures at 60° and 240 mm. pressure and, as in the 3:1 reaction, no phosphorus trichloride was formed.

The following are some significant facts observed in the above experiments. The reaction of calculated equivalent quantities of phosphorus pentachloride upon starch at temperatures below 97° and at pressures less than 600 mm. affects the hydroxyl groups of the glucose units, but does not cause the substitution of carbon-bound hydrogen atoms. Furthermore, the solvent-extracted organic product always weighed less than the starch used, consistently possessed a low chlorine content, and contained an appreciable quantity of bound phosphorus. In no case was more than 50% of the calculated phosphorus pentachloride converted into phosphorus oxychloride in thirty days or less. Moreover, more than two moles of hydrogen chloride were evolved for each mole of phosphorus oxychloride produced during the reaction proper.

No satisfactory method was found for the separation of the solid reaction mass into organic and inorganic components without considerable alteration of the organic product. During the carbon bisulfide extraction, the reaction continued with the formation of phosphorus oxychloride; in some instances, depending on the length of the initial reaction time, relatively small quantities of hydrogen chloride also were produced.

A completely satisfactory material balance was difficult to obtain. This was probably due to the loss of highly reactive substances during the long reaction periods and the incomplete separation of the products from the unused phosphorus pentachloride. Changes occurring during solvent-extraction proved to be the most troublesome. The material balance recorded below was obtained only after an extensive search for sources of error.

Because of the apparent stripping of the glucose units of their hydroxyl groups and, to a lesser extent, their hydrogen atoms as a consequence of reaction with phosphorus pentachloride, a study was made of the rates of formation of phosphorus oxychloride and hydrogen chloride during the course of reaction. For this purpose, 3:1 and 10:1 PCl_5–starch mixtures were allowed to react for periods as long as thirty days at a temperature of 60° and a pressure of 240 mm. During the reactions, the volatile products were removed as they were formed by absorption in sodium hydroxide solution. The hydrolyzed products were removed for analysis generally at twenty-four hour intervals. At the end of the reaction period, the organic product, freed from excess phosphorus pentachloride and most of the $POCl_3$-forming structure by extraction with carbon bisulfide, was analyzed immediately for chlorine and phosphorus.

Material Balance. Reaction of Phosphorus Pentachloride and Starch in a 3:1 Ratio at 60°.—Using 40 g. of starch and 154 g. of phosphorus pentachloride, 126 g. of material remained in the reactor at the end of the reaction period. During the reaction, 41.1 and 25.1 g. of phosphorus oxychloride and hydrogen chloride, respectively, were formed. The material loss was therefore only 1.8 g.

The weights of solvent-extracted product, phosphorus oxychloride formed during solvent-extraction and reclaimed phosphorus pentachloride were 35.8, 14.5 and 75.7 g., respectively, giving a total of 126 g. and therefore showing no loss. The weights of phosphorus and chlorine in these substances are smaller than those taken by only 0.007 and 0.042 gram atom, respectively.

The solvent-extracted organic product contained 6.1% chlorine and 0.5% phosphorus from which its —O—PCl_4 and C—Cl contents may be calculated to be 0.006 and 0.037 equivalent, respectively.

The HCl *versus* $POCl_3$ ratio formed during reaction was 2.57 and during reaction and carbon disulfide extraction 1.90, while the ratio of HCl + C—Cl and total phosphorus oxychloride was 2.00.

It would be reasonable to expect that the rates of formation of phosphorus oxychloride and hydrogen chloride during reaction would be dependent upon the degree of subdivision of the solid reactants and the uniformity of mixing, as well as upon the bulk concentration, temperature and pressure. It was found that the volatile products were produced uniformly but the curves expressing their rates of formation shifted to some extent from experiment to experiment. The dotted-line and solid-line curves of Fig. 1 represent the rates of formation of hydrogen chloride and phosphorus oxychloride from 3:1 and 10:1 PCl_5–starch mixtures, respectively. The dotted-line and solid-line curves of Fig. 2 represent ratios of the rates of formation of hydrogen chloride and phosphorus oxychloride from 3:1 and 10:1 mixtures, respectively. For 3:1 reactant mixtures, the extremes in different experiments of the HCl:$POCl_3$ ratios, corresponding to the nearly linear portion of the curve, were about 2.5 to 3.2. The most nearly reproducible curves were obtained when the phosphorus pentachloride was ground to a maximum fineness by a given method and thoroughly mixed with the starch.

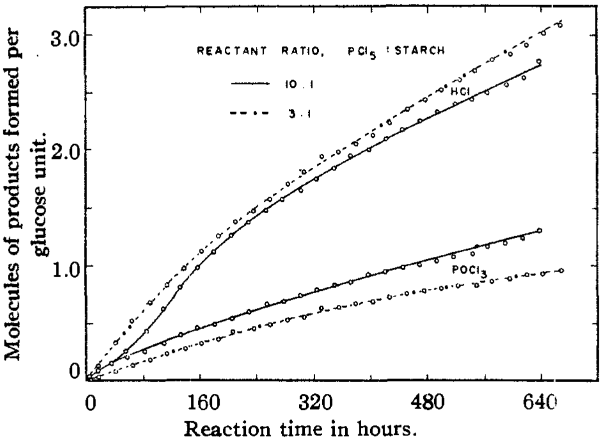


Fig. 1.—Effects of concentration of reactants upon the rates of formation of HCl and $POCl_3$.

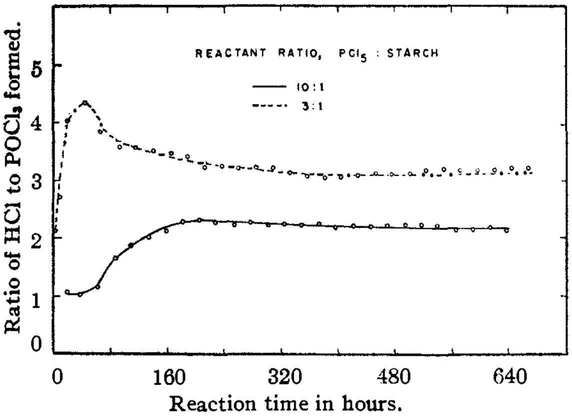


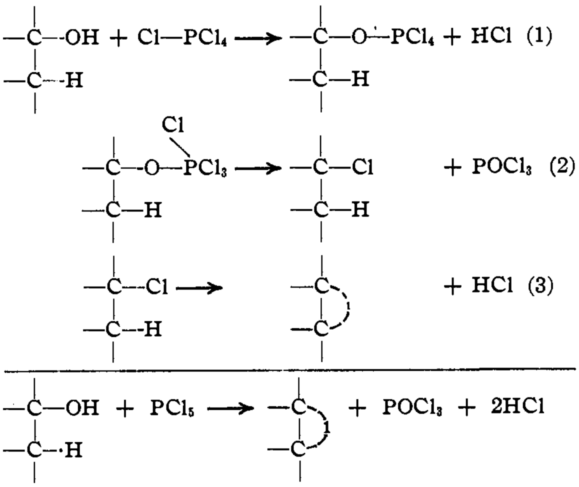
Fig. 2.—Effects of concentration of reactants upon the HCl:$POCl_3$ ratio during the course of reaction.

In the above experiments, further evidence has been obtained for the stripping of the carbon skeleton of the glucose unit. One important indication of this is the relative rates of formation of hydrogen chloride and phosphorus oxychloride. In the usual reaction between a carbinol group and phosphorus pentachloride, hydrogen chloride and phosphorus oxychloride are formed in a 1:1 ratio. However, in the PCl_5–starch reaction at low temperature and pressure, they were formed in a ratio greater than 2:1, except during the first few days of the reaction of the 10:1 PCl_5–starch mixture (Figs. 1 and 2). There are a number of reasons for not ascribing the relatively low rate of phosphorus oxychloride formation to occlusion in the solid mixture. After thirty days, the 3:1 and 10:1 PCl_5–starch mixtures were still yielding hydrogen chloride and phosphorus oxychloride in molar ratios of approximately 3:1 and 2:1, respectively. In the 3:1 reaction mixture, an excess of 0.626 mole of hydrogen chloride was formed during the reaction proper. The retention of 0.626 mole of phosphorus oxychloride was not possible since less than that amount of phosphorus pentachloride was available for its formation. Further, the low pressure employed, the uniform rates of hydrogen chloride and phosphorus oxychloride formation, and the failure of the reaction mixture to increase in fluidity preclude any appreciable hold-up of phosphorus oxychloride.

During the carbon bisulfide extraction of the reaction mass, phosphorus oxychloride was always evolved (Table II), accompanied at times by relatively small amounts of hydrogen chloride. This could not have resulted from the continued reaction of phosphorus pentachloride on the organic product nor from its reaction with moisture, because it was accompanied by insufficient amounts of hydrogen chloride.

The material balance covering the reaction proper and the carbon bisulfide extraction of the reaction mass also indicate a stripping of the carbon skeleton. In the experiment describing the reaction of phosphorus pentachloride and starch in a 3:1 ratio at 60°, about one-third of the total phosphorus oxychloride was evolved during solvent-extraction but the amount of hydrogen chloride formed was not appreciable. The ratio of the total hydrogen chloride to total phosphorus oxychloride was slightly less than 2.0. If, however, the carbon-bound chlorine had been evolved as hydrogen chloride, the ratio would have become almost exactly 2.0. Further, had this occurred, the stripping would have been complete stoichiometrically. The extracted organic products weighed less than the starch used and their chlorine and phosphorus contents were small. Moreover, a balance is struck only if four atoms of the residual chlorine in the extracted product are associated with each atom of phosphorus. During the reaction proper, the bound phosphorus in the 3:1 reaction mixture reached a maximum during the early stages (two to six days, Fig. 2), after which it decreased steadily to a fairly constant value after about eight days. On the other hand, in the 10:1 reaction mixture, the bound phosphorus was constant for the first few days, after which it increased gradually to about the eighth day where it became nearly constant. The chlorine content of the product could not be correlated with the reaction time; the highest value obtained was 6.79%, after a reaction period of 300 hours.

The preceding observations, which have been interpreted to be indicative of the formation and subsequent decomposition of an organic-phosphorus complex, are taken as sufficient evidence for the postulation of the following series of reactions:



The relatively rapid evolution of hydrogen chloride at low temperature shown by the curves of Figs. 1 and 2 may be accounted for by assuming that steps (1) and (3) take place more rapidly than does step (2). The latter, as shown in Fig. 1, does not differ greatly from a zero order reaction. In accordance with this mechanism, the extracted organic product is considered to consist of glucose units which, to the extent of the reaction, have lost their hydroxyl groups and somewhat less than an equal number of hydrogen atoms. Smaller amounts of the intermediates of steps (1) and (2) are also assumed to be present. In the event that the formation of stable double bonds in the ring is not possible, condensation might be expected to occur as the result of free radical formation. There is the further possibility that the organic structure of step (2) is more stable if it involves carbon atom 6; this could account for the appreciable amount of carbon-bound chlorine in the product. Each residue of the glucose units of starch which has undergone complete reaction with phosphorus pentachloride would contain, if all the chlorine had been eliminated as hydrogen chloride, only four hydrogen atoms. The hydrogen content of the product would be 3.73%.

As shown in Figs. 1 and 2, there are two effects of an increased bulk concentration of phosphorus pentachloride. At the higher phosphorus pentachloride concentration, hydrogen chloride was evolved at a definitely slower and phosphorus oxychloride at a more rapid rate. These differences are consistent with the idea that the greater concentration of phosphorus pentachloride accelerates step (1) with the formation of the organic phosphorus complex, the decomposition of which is responsible for the increased production of phosphorus oxychloride. At the same time, the high concentration of phosphorus pentachloride effects a retardation of step (3), thereby accounting for a decrease in the evolution of hydrogen chloride. During the first two days of the reaction of the 10:1 reaction mixture, step (3) could not have occurred to an appreciable extent.

Structure of Pentachlorostarch

Investigations of recent years have led many to the view that non-glutinous starches consist of branched and linear polymers of anhydro-glucose. Measurements on cornstarch have indicated that it contains about 70% of the branched and 30% of the linear polymers. There is general agreement that the linkage of the linear molecules or fragments is 1,4, while it is believed the linkage at points of branching is 1,6. The linkages of the molecules as well as the functional groups of the repeating unit might be subject to attack in the formation of pentachlorostarch.

The chlorine content of numerous products indicates that there is only a limited action of phosphorus pentachloride on the oxygen linkages. The data in Table II illustrate the tendency of the chlorine content to approach 61.92%, the calculated value for a product formed without degradation. The chlorine content would be 62.28%, if it is calculated upon the assumption that the average degrees of polymerization of the branches and linear molecules are 25 and 250, respectively, and that only the 1,6-linkages are ruptured. Complete degradation would yield a product containing 72.72% Cl. Cellulose showed no evidence of degradation when chlorinated with phosphorus pentachloride. The calculated chlorine content of pentachlorocellulose of a chain length of 150 is 61.99%; that found was 61.5%.

TABLE II

EFFECT OF REACTION TIME UPON THE CHLORINE CONTENT OF THE PRODUCT. REACTANTS: 40 G. STARCH AND 560 G. PCl_5

Reaction temp., °C.	Reaction time in hours 1	2	3
70			16
80			48
90			24
100	17	44	48
110		12	24
125–135	25	36	72
135–160	8	8	24
Cl, %	60.0	62.3	63.3

All experiments designed to degrade the pentachlorides subsequent to their formation were unsuccessful. They were inert even to 50% sulfuric acid solution.[4] Rudy[5] found that tri-trichloroacetyl starch and cellulose resisted all efforts to hydrolyze them and attributed their stability to steric hindrance.

Chromatographic adsorption experiments, in which were used alcohol, ether, benzene, ligroin–benzene mixtures, and ethylene dichloride as solvents and $MgO + SiO_2$, Al_2O_3, MgO, $CaCO_3$ and CaO as adsorbates, showed no evidence of banding. Although the experiments were not exhaustive, they do suggest the possibility that degradation, to whatever extent it might have occurred, was not random but is ordered in such a manner as to leave the final components more homogeneous in molecular configuration than the original starch. These results may be explained by either a chemical or physical depolymerization. The former is favored, however, since an increase of the chlorine content above 61.92% appears to be accompanied by a corresponding increase in the phosphorus oxychloride formed.

The approximate equivalence of the hydroxyl groups of starch and the phosphorus oxychloride formed indicates that three of the five chlorine atoms of the pentachloroglucose unit are located on carbon atoms 2, 3 and 6. Results obtained in the chlorination of sugars and polyalcohols support the view that the two remaining chlorine atoms are attached to carbon atoms 1 and 4, 1 and 5, or 4 and 5.

If only one chlorine atom is attached to a given carbon atom, there can be no more than two pairs of adjacent carbon atoms per pentachloroglucose unit capable of double bond formation through chlorine elimination. Both pairs of adjacent carbon atoms could be in the ring or one in the ring and one in the side chain. When subjected to the Finkelstein reaction, pentachlorostarch loses only two chlorine atoms by direct elimination to give an unsaturated trichloride. This behavior, together with evidence that double bonds in the ring are not stable, suggests that a fourth chlorine atom is attached to carbon 5.

Scattergood and Pacsu[6] have pointed out that, of eight conformations of a pyranose molecule, there is only one, a *trans* form, from which all the *d*-hexopyranose molecules and their derivatives may be derived. A section of a model molecule was constructed from the Fisher–Hirschfelder atom models according to this conformation to learn where it would place the fifth chlorine atom and to determine whether it was compatible with experimental results. The following observations were made from the model. (1) The 1,4-oxygen bridge is so well screened that it is inaccessible to water molecules. (2) The more exposed 1,6-linkages are more readily accessible to the relatively large phosphorus pentachloride molecules than are the 1,4-linkages. (3) Chlorine atoms on carbon atoms 1, 2, and 3 are so closely packed that they are not replaceable, while those on carbon atoms 5 and 6 are replaceable by iodine. (4) Stable double bond formation in the ring is not possible, but it can occur between carbon atoms 5 and 6. (5) The model is consistent with the formation of tars and black solids through condensation of unsaturated fragments formed under conditions which favor the phosphorus oxychloride reaction. (6) The fifth chlorine atom can exist on carbon atom 1 without interference but not on carbon atom 4.

Summary

1. Through reaction with phosphorus pentachloride, a stable chlorine derivative of starch has been prepared which contains five chlorine atoms per glucose unit. From chemical evidence, four of the chlorine atoms of pentachlorostarch may be tentatively assigned to carbon atoms 2, 3, 5 and 6; the space relationships of the atom model formula indicates a greater likelihood of the fifth chlorine atom being attached to carbon atom 1 than to carbon atom 4. Degradation of the polymer during the chlorination process was slight.

2. Chlorine was introduced into the glucose unit in two ways: through reaction of the phosphorus pentachloride with the carbinol groups with the formation of phosphorus oxychloride, and through the reaction of phosphorus pentachloride with carbon-bound hydrogen atoms with the formation of phosphorus trichloride. The

---

(4) Monier-Williams, *J. Chem. Soc.*, **119**, 803 (1921); Barkey, Doctorate Thesis, 1943, Massachusetts Institute of Technology.

(5) Rudy, *Cellulosechemie*, **13**, 49 (1932).

(6) Scattergood and Pacsu, THIS JOURNAL, **62**, 903 (1940).

formation of a stable product required that the phosphorus oxychloride and phosphorus trichloride reactions occur at comparable rates. When the phosphorus trichloride reaction predominated, carbon was formed spontaneously; when the phosphorus oxychloride reaction predominated, the product exhibited instability through the elimination of hydrogen chloride. At least one carbon-bound hydrogen atom had to be replaced by chlorine if the loss of hydrogen chloride was to be blocked.

MANHATTAN, KANSAS RECEIVED DECEMBER 31, 1945

[CONTRIBUTION FROM THE WESTERN REGIONAL RESEARCH LABORATORY[1]]

Action of Sulfating Agents on Proteins and Model Substances. I. Concentrated Sulfuric Acid

BY HENRY C. REITZ, ROBERT E. FERREL, HEINZ FRAENKEL-CONRAT AND HAROLD S. OLCOTT

The method of preparation, properties and possible practical applications of a derivative of wheat gluten obtained by the action of cold, concentrated sulfuric acid have recently been described.[2] The sodium, potassium and ammonium salts of this derivative possess the unique property of absorbing almost immediately 100–300 times their weight of cold water to give stiff gels. Because information that might lead to an understanding of this property would be of theoretical and practical value, a study has been made to determine the specific groups in proteins that react with sulfuric acid.

The effect of sulfuric acid on proteins has not previously been investigated in detail. Uchino[3] kept silk fibroin in contact with concentrated sulfuric acid at 7–8° for an extended period of time and followed the extent of hydrolysis by amino nitrogen determinations. There appeared to be no measurable hydrolytic changes until after the twelfth day. Harris, Mease and Rutherford[4] treated wool with various concentrations of sulfuric acid, observed that sulfate was bound non-ionically, and suggested that combination occurred with the amino groups. However, evidence obtained in the present study indicates that the amino groups are not involved. Instead, the bound sulfate can be accounted for almost quantitatively as acid sulfate esters of the hydroxyl groups of the β-hydroxyamino acids and of hydroxyproline. In addition, a small portion of the bound sulfate is accounted for by the reaction of sulfuric acid with sulfhydryl groups to form thiosulfates, and with part of the phenolic rings of tyrosine to form sulfuric acid esters or sulfonic acids in varying proportions, depending upon the conditions of the reaction.

The participation of the hydroxyl groups of the aliphatic hydroxyamino acids is in accord with the known reaction of sulfuric acid with alcohols to form alkyl acid sulfates.[5] Since the amino and many other polar groups of proteins do not react, sulfuric acid appears to be more selective in reacting with the aliphatic hydroxyl groups of proteins than other reagents so far investigated.[6] In contrast to this selectivity, another sulfating agent, the addition product of pyridine and chlorosulfonic acid, reacts with most types of polar groups in proteins, as will be demonstrated in a subsequent paper.[7]

In general the reaction was performed by mixing proteins or model substances with concentrated sulfuric acid at temperatures below 0°. The mixtures were allowed to warm to room temperature, poured over ice, brought to neutrality with sodium hydroxide, dialyzed to remove inorganic sulfates and dried from the frozen state. Yields varied from 100 to 130% of the weight of the original material. The products were analyzed for nitrogen, bound sulfate and, in some cases, for total sulfur, amino nitrogen and tyrosine. With materials that could not be isolated by dialysis, the extent of reaction was measured, in solutions from which excess sulfuric acid had been removed as barium sulfate, by determining, after acid hydrolysis, the ratio of sulfate sulfur to nitrogen.

Since serine, threonine, hydroxyproline and cysteine were found to react more slowly than proteins, they were mixed with sulfuric acid at room temperature and allowed to stand for varying lengths of time before continuing with the analyses.

Aliphatic Hydroxyl Groups—The amounts of sulfate bound by a series of proteins are compared in Table I with their contents of aliphatic hydroxyamino acids. The materials used varied

---

(1) Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Not copyrighted.

(2) Reitz, Ferrel, and Olcott, *Ind. Eng. Chem.*, **36**, 1149 (1944).

(3) Uchino, *J. Biochem.* (Japan), **20**, 65 (1934).

(4) Harris, Mease and Rutherford, *J. Res. Natl. Bur. Stand.*, **18**, 343 (1937).

(5) Suter, "Organic Chemistry of Sulfur," John Wiley and Sons, Inc., New York, N. Y., 1945, Chap. I.

(6) Gordon, Martin and Synge, *Biochem. J.*, **37**, 538 (1943), reported that treatment of wool with methyl sulfate in alkaline solution caused partial methylation of the aliphatic hydroxyl and the phenolic groups. Blackburn and Phillips, *ibid.*, **38**, 171 (1944), found that methylation with this reagent in neutral or acid solution also introduced some bound sulfate into wool and suggested that the reaction involved the hydroxyl groups of hydroxyamino acids and proximal "activated" peptide bonds, as well as carboxyl and other groups.

(7) Reitz, Ferrel, Olcott and Fraenkel-Conrat, THIS JOURNAL, **68**, 1031 (1946).