THE REACTION BETWEEN AN OXIDIZED STARCH AND ACID METHYL ALCOHOL

by

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Signature of Author.....

Department of Chemistry, April 7, 1941

Signature of Professor in charge of Research..

Signature of Chairman of Department Committee on Graduate Students.....

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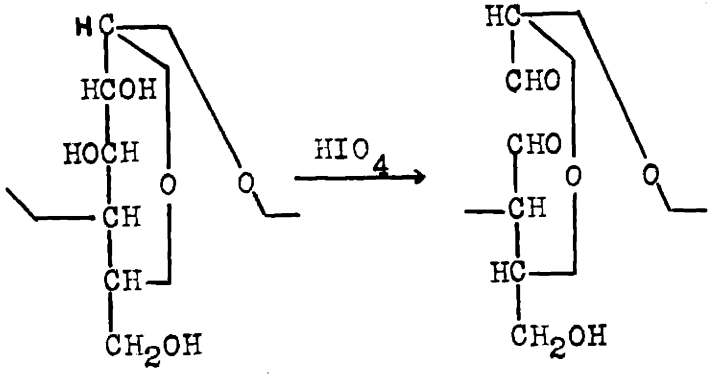
PART I.

THE PROBABLE STRUCTURE OF A CRYSTALLINE PRODUCT

I. INTRODUCTION

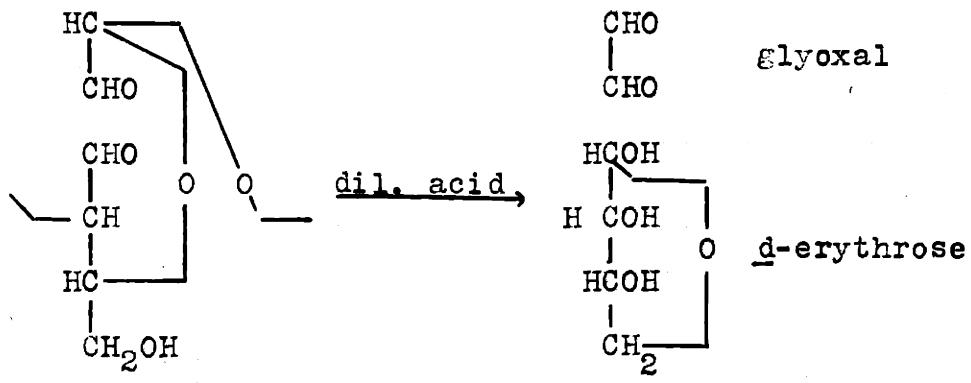
In 1937, Jackson and Hudson (1) submitted starch and cellulose to the action of one mole of periodic acid, and assumed that the reaction could be formulated as follows:



(I) Starch unit, or cellulose, with β glycosidic linkage

(II) Oxidized starch unit.

This assumption was in line with the structures for starch and cellulose worked out by Haworth and his collaborators (2) and with the behavior of periodates toward glycols, made familiar by Malaprade (3). In a later paper, Jackson and Hudson (4) hydrolyzed starch oxidized in this manner to glyoxal and d-erythrose, isolated in 33% and 22% yields respectively, and positively identified them in the form of various well known derivatives. As the hydrolysis took place under very mild conditions the above yields were unaccountably low. Glyoxal was also



found, in low yield, as a product of the hydrolysis of periodate oxidized starch, and identified, by Caldwell and Hixon (5). The same phenomenon of a low yield was observed by Grangaard and Purves, whose unpublished notes show a recovery of only 50% of the theoretical amount of glyoxal tetramethylacetal after boiling the oxidized starch with 10% methyl alcoholic hydrogen chloride for three hours. More prolonged boiling (six hours) increased the yield either very slightly or not at all. The latter workers also examined the residue from which the glyoxal tetramethylacetal had been recovered and found it to be soluble in ether or chloroform, and strongly laevorotatory. This solubility in organic solvents was surprising, in view of the fact that the expected product, methyl d-erythroside is insoluble in organic solvents. Suitable manipulation of a solution of these residues in ether caused the precipitation of a methoxyl-containing crystalline material, melting at 148°-148.5° C, in a yield which amounted to somewhat less than 1% of the weight of the starch (corn) used.

The primary objects of the present thesis were to repeat the isolation of this crystalline material and and to decide whether it was derived from an integral part of the starch molecule, or from some adventitious impurity peculiar to corn starch. When it was found that this substance could be obtained from several species of starch and also from carefully purified soluble potato starch, always in the same small yield, the detailed study of its structure was undertaken. Semi-micro and micro technique were used, since only 4.5g. of the material was accumulated during the research.

The succesful outcome of this study led to a new interpretation of the action of acid-methanol on periodate-oxidized starches, and to the support of this interpretation by further experimental work which is outlined in Part II of this thesis. The final results rendered the unexpected observations of Jackson and Hudson and of Grangaard and Purves readily understandable.

Some of the earlier work was published in the form of a note by Grangaard, Michell and Purves (6), a reprint of which is included in this thesis.

II. DISCUSSION OF RESULTS.

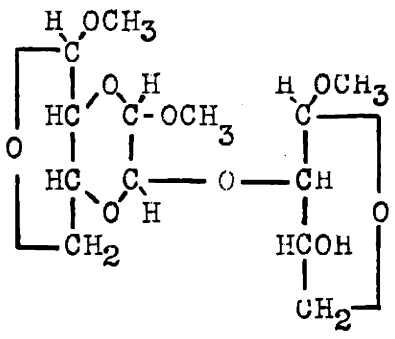
The isolation of a definite chemical entity in suspiciously low yields from a complex polysaccharide like starch always suggests that the substance originated in some associated impurity. This possibility was reduced by the observation that the crystalline material, m.p., 148°-148.5° C, isolated by Dr. Grangaard from cornstarch could be obtained from several other cereal and root starches, the impurities in which were not likely to be uniform. The recovery of the crystals from a soluble potato starch then shifted the balance of evidence in favor of the possibility that they were derived from an integral part of the starch molecule. This was because the soluble starch had been extensively purified by fractional precipitation (7) prior to oxidation, and the oxidized product had been further purified by dialysis, prior to treatment by acid methanol. The dialyzable crystals (mol.wt., 322), had therefore been produced in the latter treatment, were certainly not derived from a dialyzable material, and most probably had not originated from a high molecular weight, non-dialyzable impurity.

The very strong probability that the crystals originated in the macromolecule of all starches justified an intensive research on their chemical structure. Analysis gave the molecular formula $C_{10}H_{13}O_6(OCH_3)_3$,

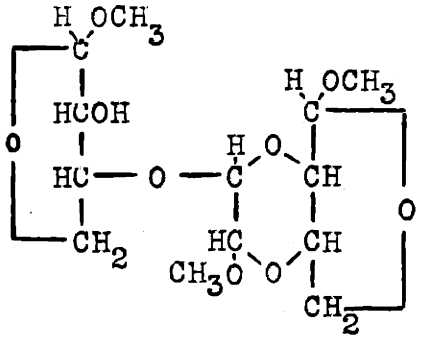
while a neutral reaction to litmus and stability to alkali indicated the absence of carboxyl and ester groups. The probable absence of aldehyde or α-hydroxyketone groups was suggested by stability toward Fehling's solution. Mild acid treatment, however, readily gave a strongly reducing syrup. The presence of acetal linkings was therefore suspected. Standard tests with bromine, and tetranitromethane(8), revealed no unsaturation. The complete absence of double bonds and carbonyl or carboxyl groups was finally established by the transparency of the substance to ultraviolet light. This led to the immediate suspicion that all the oxygen atoms were present as ether or glycosidic bonds, or as hydroxyl groups. The preparation of a monoacetate revealed the presence of one hydroxyl group, the failure of which to give a marked coloration with ferric chloride pointed to its non-aromatic character.

At this point the formula of the crystals could be written $C_{10}H_{12}O_5(OCH_3)(OH)$, where the five oxygen atoms were involved in ether or acetal linkages. Since double bonds were absent, application of the "Condensation Number" relationship due to Frèrejacque (9) showed that three cyclic structures must be present. Further suggestions as to the structure were derived from a consideration of the possibilities inherent in the structure of periodate oxy-starch. The latter consists of alternating erythrose (C_4 unit) and glyoxal (C_2 unit) residues, linked through the second or third positions of the

erythrose resisues. Furthermore, Hockett and Maynard(10) have pointed out the tendency of erythrose to assume a 1,4 or furanose configuration. Two erythrose units with two rings, plus one glyoxal unit forming part of another ring caused by treatment with acid methanol, might conceivably give a structure such as (III) or (IV) either of which would satisfy all the evidence so far gathered from the study of the crystals.



(III)



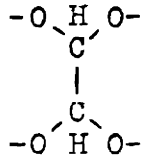
(IV)

Further experiments were guided by this tentative hypothesis.

The nature of the hydroxyl group was discovered by the method of Oldham and Rutherford (11), who found that the p-toluenesulfonic acid ester of a primary alcohol is unstable to sodium iodide in acetone solution at 100°C, resulting in the substitution of an iodine atom for the acid group. On the other hand, the p-toluenesulfonic acid ester of a secondary alcohol is stable to this treatment. The p-toluenesulfonic acid ester of the

crystalline substance was prepared and, on treatment with sodium iodide, 84.5% of the material was recovered unchanged. This was taken as ample evidence that the substance was not a primary alcohol. It will be noted that the formulae (III) and (IV), above, represent secondary alcohols.

Prolonged treatment of the substance with a 10% methanolic hydrogem chloride solution, followed by steam distillation, removed one mole of glyoxal tetramethylacetal, estimated as the water-insoluble glyoxal-bis-2,4-dinitrophenylhydrazone. This derivative was found to be identical with an authentic sample. A two-carbon fragment, therefore, must have been present in the molecule as



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Only eight carbon atoms remained unknown in the original substance.

Although the substance was stable to an aqueous solution of trisodium paraperiodate at a pH of 4.1 at room temperature, it was found, by the microtechnique of Rappoport and Reifer (12), that six moles of potassium metaperiodate were consumed by one mole of the subatance in acid solution at a pH of 1, at 100°C. From this it was assumed that hydrolysis had taken place, exposing hydroxyl groups to the action of the periodate. The consumption of six moles of periodate can be effected by four possible types in the sugar series, namely, a keto-

octose, an aldoheptose, a heptaalcohol (straight chain), or two moles of an aldotetrose. The chances that starch should give rise to the first three types are very remote whereas the presence of a group, which, on hydrolysis should give an aldotetrose, is very apparent in Hudson's proposed structure for periodate oxidized starch(4). Therefore, it seemed very reasonable that one mole of the substance should contain two separated chains of four carbon atoms each.

Four distinct attempts were made to prove the presence of these erythrose groups by direct methods but all were unsuccesful. It is believed, in this laboratory, that the great instability of d-erythrose to heat and dilute acid, observed originally by Ruff(13), and implied by Hudson (4) is the reason behind the difficulty of preparation of derivatives of erythrose, as well as the intense blackening of periodate oxidized starch on boiling in acid methanol. Final proof of the C_4-C_2-C_4 carbon skeleton present in the crystals was obtained by the degradation of an isomer to a C_4-C_2 unit, as described in Part II.

The name assigned to structure (III) is 2,7-dimethoxy-3-(2-methylerythrofuranosido-)hexahydrofuro(3,4)-p-dioxin. It is emphasized that closure of the dioxan ring to the right (IV), would have allowed the left-hand erythroside unit to attach itself through the second, and not the third hydroxyl group. One attempt to discriminate between these possibilities, by the graded hydrolysis of the p-toluenesulfonic ester, was unsuccesful and is described

in the amplification. This point, together with the exact configuration of the eight asymmetric carbon atoms in the structure has therefore not been proven. In Part II, some reasons for choosing certain configurations about the asymmetric carbon atoms, based on carbon models, will be given. It is, nevertheless, stressed that no definite conclusions can be drawn from the use of carbon models in the present case.

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III. EXPERIMENTAL PART

(a) The oxidation of starches with trisodium paraperiodate.

The procedure used by Grangaard, Michell and Purves(6) was closely followed with the various kinds of water-insoluble starches, and is described in detail in the amplification. A solution of trisodium paraperiodate, ($Na_3H_2IO_6$), buffered to a pH of 4.1 by acetic acid, was substituted for the potassium metaperiodate (KIO_4) in a phthalate buffer of the same pH, which was previously used. Potato starch, made water-soluble by digestion in acid ethanol according to the method of Baird, Haworth and Hirst(7) and carefully purified by Prof. Purves was also available. In this case, the oxidation with sodium periodate took place in a homogeneous system and dialysis was necessary to remove the sodium iodate produced by the reduction of the periodate. The yield, in this case, was 86% of theory. The commercial (Mallinckrodt) potato starch, used as a source of the crystalline material for further experiments, contained 11.7% moisture, 0.29% ash, and 0.56% nitrogen(Dumas). The latter figure was assumed to correspond to approximately 3.5% protein. When allowance was made for these constituents, the yield of oxidized starch, which contained 0.28% ash, was nearly 104% of theory, and the analysis corresponded to $C_6H_8O_5 \cdot \frac{1}{2}H_2O$, rather than $C_6H_8O_5$.

Anal. Calcd. for $C_6H_8O_5$: C,45.0;H,5.03.

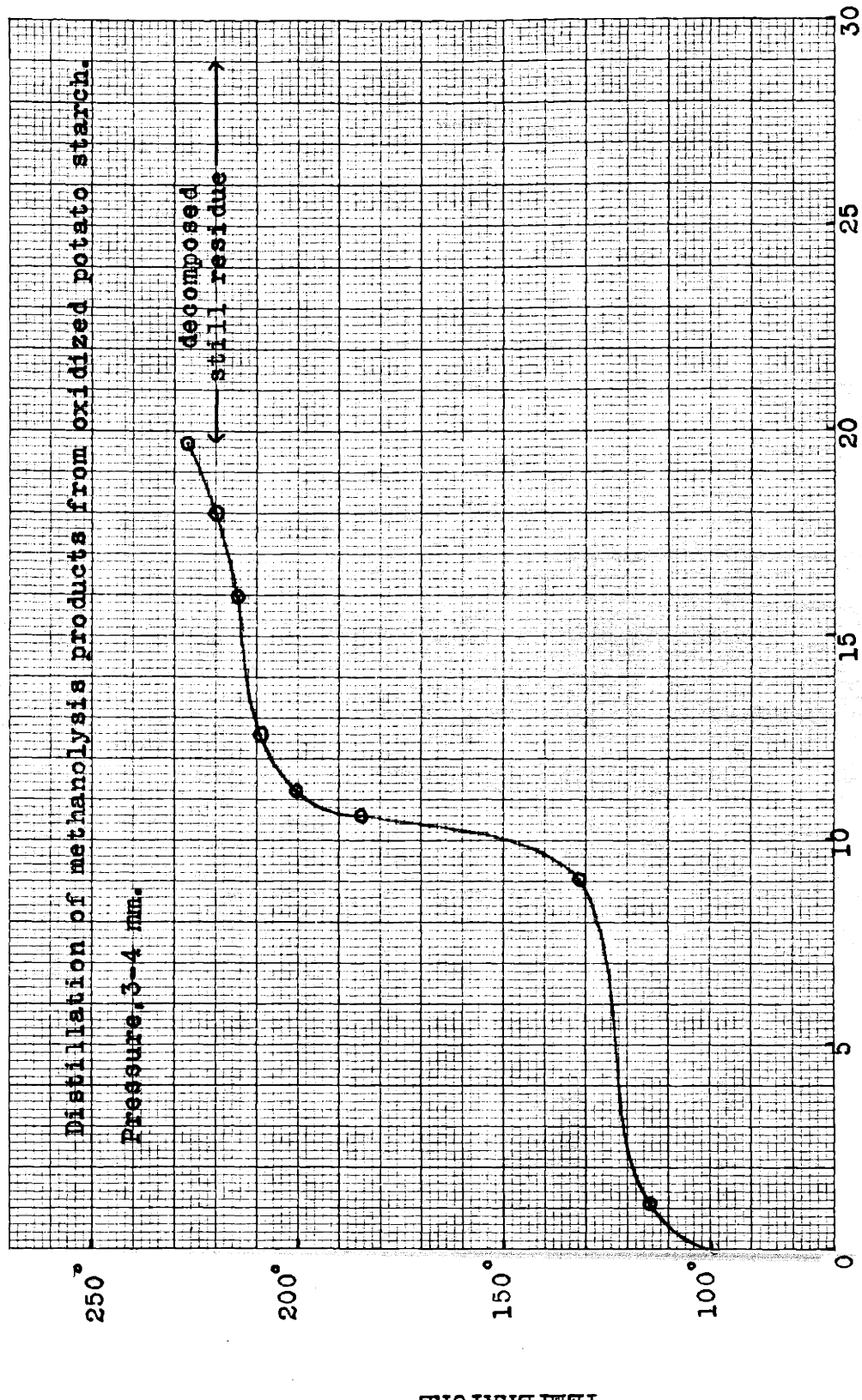
Calcd.for $C_6H_8O_5.\frac{1}{2}H_2O$:C,42.61;H,5.32

Found: C, 42.35,42.30; H,5.32,5.47

(Analysis by Mrs.C.K.Fitz.)

(b) Preparation of the crystalline material.

The procedure outlined by Grangaard,Michell and Purves (6) was used, although certain modifications were introduced and adhered to for the remainder of the investigation. After the dry oxidized starch (e.g.50g.) had been boiled under reflux for three hours with dry methanol containing 10% of hydrogen chloride, the acid was neutralized with lead carbonate and the solvent was replaced with water. Chloroform was substituted for ether in the extraction of the aqueous solution, and this extract after drying and the removal of the chloroform under reduced pressure (water-pump) was fractionally distilled at a pressure of 3-4mm. (Weight of extract,31.0g.) A small quantity of glyoxal tetramethylacetal usually came over at 30°-50° C although the greater bulk of this substance was known to have remained in the aqueous portion. Two main fractions were isolated, one boiling at 110°-120° C (which will be discussed in Part II) and the other between 185° and 220° C. A charred residue, which had decomposed above 220° C remained. (See distillation curve, p.12) The high-boiling fraction, on freezing in dry ice and trituration with ether, gave a white,


Distillation of methanolysis products from oxidized potato starch.
Pressure, 3-4 mm.
decomposed
still residue
TEMPERATURE
250°
200°
150°
100°
0
5
10
15
20
25
30
YIELD, GRAMS

flaky product, which, after two recrystallizations from ether, melted at 148°-148.5° C. These modifications resulted in raising the yield of this substance from somewhat less than 1% up to 1.6%, based on the weight of the starch used. Yields, 9.1g. of each main fraction, and 9.3g. of undistilled residue.

The earlier method (loc. cit.) was used in the preparation of this crystalline substance from corn, wheat, arrowroot, potato and soluble (potato) starch. The yields, based on the weight of the starch used, varied between 0.53% and 0.74%, except in the case of soluble starch, where the yield was 0.22%. In all cases the melting point and mixed melting points were the same, i.e. 148°-148.5° C.

This substance was neutral to litmus and did not reduce Fehling's solution, but did so vigorously after mild acid hydrolysis. The substance was found to be completely transparent to ultraviolet light, in methanol solutions of varying concentrations. It was stable to cold aqueous sodium periodate at a pH of 4.1, was soluble in the usual organic solvents, butwas only slightly soluble in ether, and was about 0.8% soluble in water at room temperature.

Anal. Calcd. for $C_{10}H_{13}O_6(OCH_3)_3$: C, 48.43; H, 6.86; CH_3O, 28.9; Mol.Wt., 322.2

Found: C, 48.2, 47.77, 47.75; H, 6.61, 6.59, 6.63; CH_3O, 28.9, 29.1, 29.2; Mol.Wt., 323, 321 (see note, p.14)

(Analyses by Mrs. C.K. Fitz)

Note: In the note by Grangaard, Michell and Purves, the molecular weight was determined, by Rast's method, to be 426 because the molecular depression constant for camphor was taken as 49.5 instead of 40.0. This error led to the assignment of the erroneous formula $C_{13}H_{16}O_8(OCH_3)_4$ for the substance. The new molecular weight figures were found ebullioscopically in carbon tetrachloride (see amplification).

(c) Preparation of the acetate.

A sample of the substance, 381.2 mg., was placed in a small flask, and 5ml. of a 1:1 mixture of especially purified, dry pyridine(14) and acetic anhydride was added. The flask was stoppered and warmed for three minutes by dipping in boiling water. The solution was set aside for five days in a desiccator, and then poured onto crushed ice. A precipitate appeared at once, was separated by filtration, washed with water and dissolved in hot water containing about 20% alcohol. After standing overnight in an ice-box, long white crystals appeared. M.P., 120°-120.5° C(Corr.). Mixed M.P. with original substance, 107°-113° C. Yield, 310 mg., or 72% of theory.

Anal, Calcd. for $C_{10}H_{12}O_5(OCH_3)_3(O.CO.CH_3)$:

C, 49.5; H6.59; CH_3O, 25.6; mol.wt., 364.

Found: C, 49.13, 49.13; H, 6.71, 6.85; CH_3O, 24.8, 25.0; mol.wt., 365, 355, 351.

(Analyses by Mr. Julius Kovitz)

In an acetyl determination by the method of Kunz and Hudson (15), 13.8mg. and 21.5mg. samples required 2.65 ml. and4.10 ml. of 0.01425 N potassium hydroxide solution respectively.

Acetyl, Calcd. for $C_{10}H_{12}O_5(OCH_3)_3(O.CO.CH_3)$, 11.82%

Found, 11.8%, 11.7%.

A sample of the acetate (70mg.) was dissolved in dry methanol and five drops of N/1 barium methylate added, which were sufficient to make the solution redden phenol-red indicator. After standing for twenty-four hours, the solution was evaporated, and the residue recrystallized from ether. The melting point was 148°-148.5° C(corr.) and was not depressed by admixture with some of the original substance which had not been acetylated.

(d) Preparation of the p-toluenesulfonic ester.

A sample of the substance, 300mg., was placed in a small tube and dissolved in 4.1g. of dry pyridine. This was added to a solution of 2.15g. of carefully purified p-toluenesulfonyl chloride in 3.1g. of pyridine, cooled to 0° C. The mixture was stirred, corked and set aside in a dark place. These proportions were 1 mole of substance to 12.3 moles of p-toluenesulfonyl chloride and 92 moles of pyridine, which were ratios found advisabl by Cramer and Purves (16) and Hudson, Hann and Maclay (17). After one week, the mixture

was poured into an aqueous 5% acetone solution at 0°C, and a heavy precipitate appeared at once. This was washed with water at 0°C., dried, and recrystallized twice from petroleum ether. M.P., 87°-88° C (corr.).

Anal., Calcd. for $C_{10}H_{12}O_5(OCH_3)_3.SO_2.C_7H_7$: CH_3O, 19.54; S, 6.72 .

Found: CH_3O, 19.5, 19.6; S, 6.8, 6.8 .

(e) Treatment of the p-toluenesulfonic ester with sodium iodide.

A piece of 10mm. Pyrex tubing, 10 cm. in length, was sealed at one end, cleaned and dried. Into this was placed exactly 105mg. (1 mole) of the ester, 51.2mg. (1.5 moles) of sodium iodide and 3.2 ml. of pure acetone. The tube was sealed and placed in boiling water, surrounded by a wire gauze cage, for two hours. On cooling, a very small amount of crystalline material appeared which was separated and found to weigh less than 3mg. The acetone was evaporated and the residue was refluxed several times with petroleum ether (after the addition of 2ml. water to extract the sodium iodide). A total of 88.7mg. or 84.5% of the ester was recovered in a pure condition and a mixed melting point with the original ester was not depressed.

(f) Estimation of the "glyoxal" content of the substance.

A sample of the substance, 168.4mg., was weighed into the flask of a clean, dry "acetyl" apparatus, built

according to the specifications of Clarke (18) and equipped for small-scale steam-distillation. To this 15 ml. of 10% methanolic hydrogen chloride was added and the mixture refluxed under a glass-jointed cold-finger for 24 hours. During this time, the solution turned a dark brown in color. After cooling, the solution was neutralized with a strong solution of sodium methylate in dry methyl alcohol, and a few extra drops added to make the mixture alkaline to bromthymol blue indicator. About 5 ml. of water was added and the solution boiled until 10 ml. of methyl alcohol had been collected in the receiver, which was cooled in ice-water. Steam was then run in, in a steady stream, and the level of the liquid in the flask maintained constant at 10 ml. At least 180 ml. of faintly cloudy distillate was collected, and made up to a volume of 200 ml. The glyoxal tetramethylacetal present in this distillate was hydrolyzed to glyoxal and estimated as glyoxal-bis-2,4-dinitrophenylhydrazone in one operation. Aliquots, 25 ml., of the distillate, contained in 50 ml. beakers were heated on a steam bath with 5 ml. of concentrated hydrochloric acid, while 0.19g. of thesolid hydrazine was stirred in with a glass rod until solution was complete. The estimation was finished according to the methods of Grangaard and Purves (19).

Found, 0.81 and 0.84 moles of glyoxal per mole of substance.

Analysis of hydrazone: Calcd.for $C_{14}H_{10}N_8O_8$; N,26.8%.
Found; 11.64 mg. gave 2.87 ml. nitrogen at 729.8mm.
and 26.5 C.: N,27.0%

(g) Simultaneous acid hydrolysis of the substance and titration with potassium metaperiodate.

A solution of the substance was prepared to contain 0.000368 g. per ml. of water. Mixtures, including 1 ml. volumes of this solution,5ml. of a 0.08% aqueous solution of potassium metaperiodate and 3.3 ml. of 5% sulfuric acid were made up in each of three 25ml. Erlenmeyer flasks. The flasks were allowed to stand on a steam bath for 40 minutes, then cooled, and 6.6 ml. of 12% dipotassium hydrogen phosphate was added to bring the pH up to 6.8 . A single crystal of pure potassium iodide was added to each flask and the iodine which was released was titrated with N/200 sodium thiosulfate solution,using a freshly prepared starch indicator. Two blank determinations were run simultaneously. The blanks each required 6.66 ml. of thiosulfate solution, and the other solutions required 3.98ml. and 3.94ml. and 3.93ml. respectively.

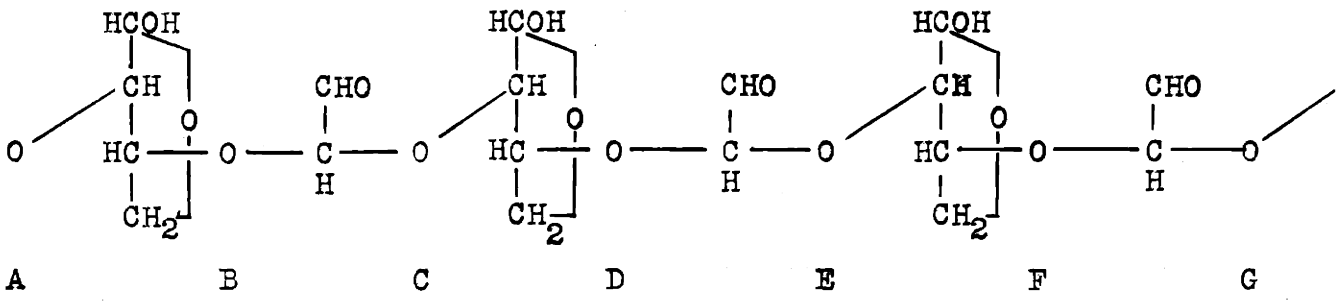
These data show that one mole of the hydrolyzed substance consumed 5.97, 6.05 and 6.08 moles of periodate in the oxidation.

PART II.

THE PROBABLE STRUCTURES OF OTHER PRODUCTS AND AN INTERPRETATION OF THE REACTION

I. INTRODUCTION

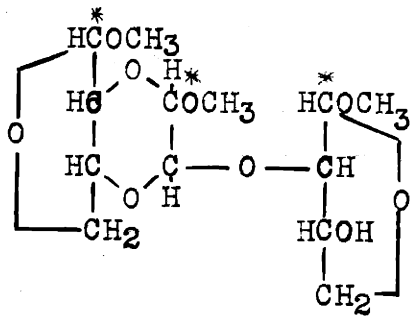
Inspection of the accepted structural unit for periodate oxidized starch,(Fig.(II),PartI.), will show that it may also be represented in the following manner (V) when the tendency to form a furanoid ring is recalled (10).



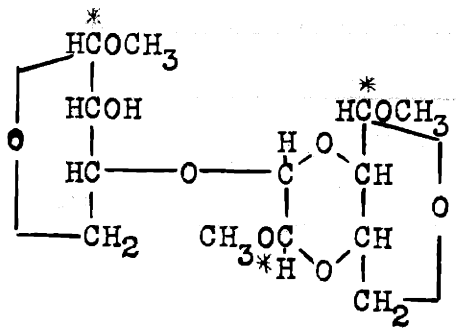
(V)

The acetal linkages A to G would be expected to undergo methanolysis when the material was boiled in 10% methanolic hydrogen chloride, and the same operation would methylate the exposed reducing groups in the erythrose and glyoxal units. If scission should first take place at A, a new hydroxyl group shoul appear in the second position of the adjacent erythrose residue. Cyclization of this hydroxyl group with the aldehyde group of the glyoxal residue between B and C,accompanied by scission of the bond D, with subsequent methylation of the three hemiacetal groups, would give one

of the alternative structures (III) allotted to the crystalline product of methanolysis studied in Part I.



(III)



(IV)

*, Glycosidic carbon atoms.

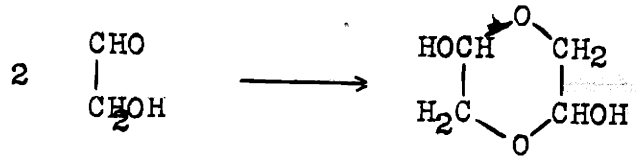
Similarly, scission first at D and later at A would give rise to the alternative structure (IV).

Equal amounts of (III) and (IV) would not be expected because the bonds A to G alternate between the second and third positions of the erythrose residues and might not cleave at the same rate. The yield of any single individual of the type (III) or (IV) would also be reduced by the fact that eight methyl glycosidic configurations are theoretically possible from the three asymmetric carbon atoms marked by red stars. Furthermore, a maximum yield of such isomers demands that links of the type A and D should undergo methanolysis selectively, while links of the type B and C are untouched. The probability of such an occurrence is not high. All these factors combine to make the small yield (1.6%) of

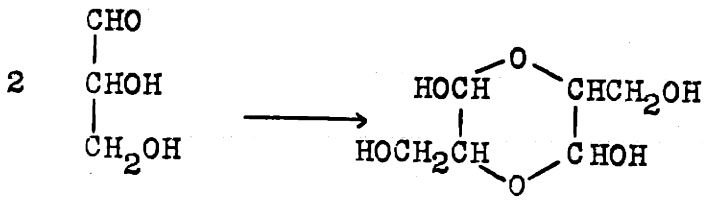
the crystalline fraction readily understandable.

If the above hypothesis is valid, isomers corresponding to the crystalline fraction would undoubtedly arise as a result of methanolysis. Homologues of greater and smaller molecular weight, corresponding to different priorities of scission of the bonds A to G, might also be expected. These and other possibilities were experimentally studied in the following work.

It should be mentioned that the formation of dioxan rings by hydroxyaldehydes has been reported several times in the chemical literature. For example, both glycolaldehyde and glyceric aldehyde will form the intermolecular compounds shown (20).



glycolaldehyde



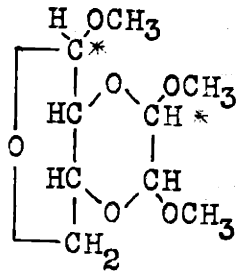
glyceric aldehyde

II. DISCUSSION OF RESULTS

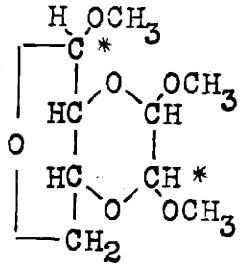
It will be remembered from Part I, that the methanolysis of periodate oxidized starch produced a high-boiling glassy distillate, B.P., 185°-220° C, from which the crystalline fraction was isolated. Although the residual liquid fraction was somewhat discolored by traces of volatile decomposition products from the residue in the still, elementary analysis, methoxyl content and molecular weight agreed with the formula $C_{10}H_{13}O_6(OCH_3)_3$ which was assigned to the crystalline substance. Structural identity with the crystals was strongly supported by the high yield of combined glyoxal recovered from the glassy fraction. The latter was, undoubtedly, isomeric with the crystals, as the foregoing theory required, and probably was a mixture of isomers. As such, it differed from the crystalline material slightly in boiling point and markedly in optical rotation, having $[\alpha]_D^{20°}$ -53.6° instead of $[\alpha]_D^{20°}$ -4.3°, in water.

In addition to the above high-boiling fraction, a mobile, colorless fraction, $n_D^{20°}$ 1.4488, came over in clean-cut fashion at 116°-119° C, 3-4mm. On working up, this yielded a pure crystalline fraction, M.P. 97°-98° C, $[\alpha]_D^{20°}$ -59.1° (in water), and a liquid fraction, $n_D^{20°}$ 1.4489, $[\alpha]_D^{20°}$ -91.7° (in water). These two fractions on analysis for carbon, hydrogen and methoxyl both agreed with the molecular formula $C_6H_7O_3(OCH_3)_3$ which was checked by molecular weight determinations. The isolation

isolation of approximately one molecular proportion of glyoxal tetramethylacetal left only a four-carbon fragment in the molecule unaccounted for, but by using the reasoning outlined in Part I, the following structural formula stands out as most probable.



(VI)



(VII)

This structure can very easily be reproduced with carbon models and seems to be quite strainless.

By reference to the structure given for oxidized starch (V), it is obvious that this structure can very readily be obtained, on methanolysis, by the preferential rupture of the bonds A,C,E,G,&c. The asymmetry of the carbon atoms carrying stars, in (VI), makes four isomers possible. Furthermore, had the bonds B,D,F,&c. been ruptured , another four isomers (VII) might be expected, making a total of eight. It was fortunate that one of these crystallized out of the liquid mixture. It is interesting to note that, by the use of carbon models, the dioxan ring can only exist in the "bath" form and

never in the "chair" form, when derived from the familiar cyclic structure for glucose. Reference to Pattersons Ring Index (21) shows that these substances may be regarded as isomeric forms of 2,3,7-trimethoxy-hexahydrofuro(3,4)-p-dioxin.

When the high-boiling liquid fraction, B.P. 195°-205° C, from the methanolysis of periodate oxidized starch (III and IV) was submitted to the action of acid methanol, the product included a fraction, B.P. 120°-135° C(3-4mm), $n_D^{20°}$ 1.4505, $[\alpha]_D^{20°}$, -73.2° (in water), that was apparently not quite homogeneous. This, perhaps, was because the very small quantity available reduced the efficiency of the fractional distillation. Nevertheless, complete analysis, including that for combined glyoxal, showed that it consisted of the 2,3,7-trimethoxy-hexahydrofuro(3,4)-p-dioxin mixture (VI and VII), isolated directly from oxidized starch, b.p., 116°-119° C, $n_D^{20°}$, 1.4488, $[\alpha]_D^{20°}$, -90.5°.

The conversion of (III) and (IV) into (VI) or (VII) in 27.5% yield , is entirely in keeping with the progressive breakdown of a long acetal chain, and definitely puts the choice of two four-carbon residues in structures (III) or (IV), instead of an eight-carbon residue, on a sound basis. (See Part I)

Another deduction from theory was that structures analogous to (III) and (IV), but of greater molecular complexity, should be formed in the initial stages of the methanolysis of starch by the preferential scission

of widely spaced bonds like A and G. An attempt to support this deduction is described in the Amplification, because the products of mild methanolysis (1% acid methanol, instead of 10%) were amorphous materials, which, although soluble in organic solvents, could not be purified to solids of narrow melting range, but rather, had the melting characteristics peculiar to high molecular weight polymers.

The isolation of two fractions from the products of methanolysis of periodate oxidized cellulose, the lower boiling member of which gave physical and analytical data which corresponded closely to the lower boiling fraction derived from oxidized starch, is an important lead in the development of the above theory to explain the mechanism of the formation of these products from starch. A crystalline portion could not be isolated from the high-boiling fraction from periodate oxidized cellulose. One possible explanation of this is that the configuration about the third, starred, carbon atom in the furodioxin ring (VII) is dependent upon the particular polysaccharide used, i.e., the crystalline material from starch retains the α glycosidic bond in starch, and the material from cellulose retains the characteristic β glycosidic bond.

The "long-chain" structure of cellulose is now generally accepted(22), and the supposition that the two distinct substances, derived from periodate oxidized starch, should be formed as a result of a long-chain

structure in starch is logical. The existence of a long chain of anhydroglucose units in starch has been demonstrated by Haworth and others (2) and found to contain from 24 to 30 glucose units. Regardless of new theories about the way in which these chains are combined in the starch macromolecule (see amplification), the proven existence of such chains affords a reasonable explanation of the mode of derivation of the substances here obtained from oxidized starch.

The low yields of glyoxal and of erythrose obtained by Jackson and Hudson(4), and the 50% yield of glyoxal tetramethylacetal observed by Purves and Grangaard, now may be explained on the basis that both these fragments of periodate oxidized starch are partially protected in the form of the low and high-boiling substances described above.

III. EXPERIMENTAL PART

(a) Examination of the liquid portion of the high-boiling fraction.

After isolation of the crystalline portion of the high- boiling fraction by trituration with cold ether, the solvent was removed by evaporation under reduced pressure (water-pump), and the residue distilled. This material boiled at 195°-205° C at 2-3 mm. and was a deep yellow glass at room temperature, extremely viscous at 80° C. Specific rotation, $[\alpha]_D^{20°}$, -53.6° (in water, C, 0.966)

Anal., Calcd. for $C_{10}H_{13}O_8(OCH_3)_3$: C, 48.43; H, 6.86; CH_3O, 28.9; mol.wt., 322.2

Found: C, 48.8, 48.7; H, 7.42, 7.23; CH_3O, 32.3, 32.5; mol.wt., 320

Glyoxal content: 233.1 mg. subst. gave 28.1mg. and 26.9mg. glyoxal-bis-2,4-dinitrophenylhydrazone; theory, 30.25 mg. Yield, 93% and89%.

(b) Examination of the low-boiling fraction.

(A) Crystalline fraction.

The low-boiling fraction was distilled through a Podbielniak column (23) and about 95% of the material came over between 116° and119.5° C at 5 mm. This was a water-white liquid; $n_D^{20°}$, 1.4488; Sp.g., 20/20, 1.204; specific rotation, $[\alpha]_D^{20°}$, -90.5° (in water, C, 0.613); mol. wt., 226; M.R.(calcd.), 49.22, M.R.(found), 49.05.

On standing overnight this liquid solidified into a white waxy material. A solution of the solid (3g.) in 20 ml. of a 3:10 ether-petroleum ether mixture, after two days at 0°C. yielded a bulky white solid, amounting to 18.8% of the original mixture. The precipitate, on recrystallization from petroleum ether, and sublimation, appeared as rosettes of extremely fine silky needles, m.p., 97°-98° C. (corr.). Specific rotation, $[\alpha]_D^{20°}$, -59.1°, (in water, C, 0.220).

Anal., calcd. for $C_6H_7O_3(OCH_3)_3$: C, 49.09; H, 7.32; CH_3O, 42.3; mol.wt., 220.2

Found: C, 49.10, 49.0; H, 7.52, 7.35; CH_3O, 41.5, 41.6; mol.wt., 216, 209.

Glyoxal content: 119.4 mg. subst. gave 27.9 mg. and 28.25 mg. of glyoxal-bis-2,4-dinitrophenylhydrazone; theory, 28.36 mg. Yield, 98% and 99%.

(c) Examination of low-boiling fraction.

(B) Liquid fraction.

The mother-liquors, from which the crystalline portion had been separated, were evaporated free of ether and the residue distilled under reduced pressure. The distillate was a clear colorless liquid, b.p., 117°-118° C., at 4 mm.; $n_D^{20°}$, 1.4489, Sp.g., 20/20, 1.209. Specific rotation, $[\alpha]_D^{20°}$, -91.7° (in water, C, 0.781).

Anal., Calcd. for $C_6H_7O_3(OCH_3)_3$: C, 49.09; H, 7.32; CH_3O, 42.3; mol.wt., 220.2; M.R., 49.22

Found: C, 49.14, 48.84; H, 759, 7.52; CH_3O, 41.6, 42.3;
mol.wt., 223; M.R., 48.85

Glyoxal content: 116.2 mg. gave 29.3 mg. and 30.7 mg. of glyoxal-bis-2,4-dinitrophenylhydrazone; theory, 27.44 mg. Yield, 107% and 112%. (Note: It was later discovered that the glyoxal tetramethylacetal estimation gave high results when a trace of grease from a ground glass joint passed over during the steam distillation.)

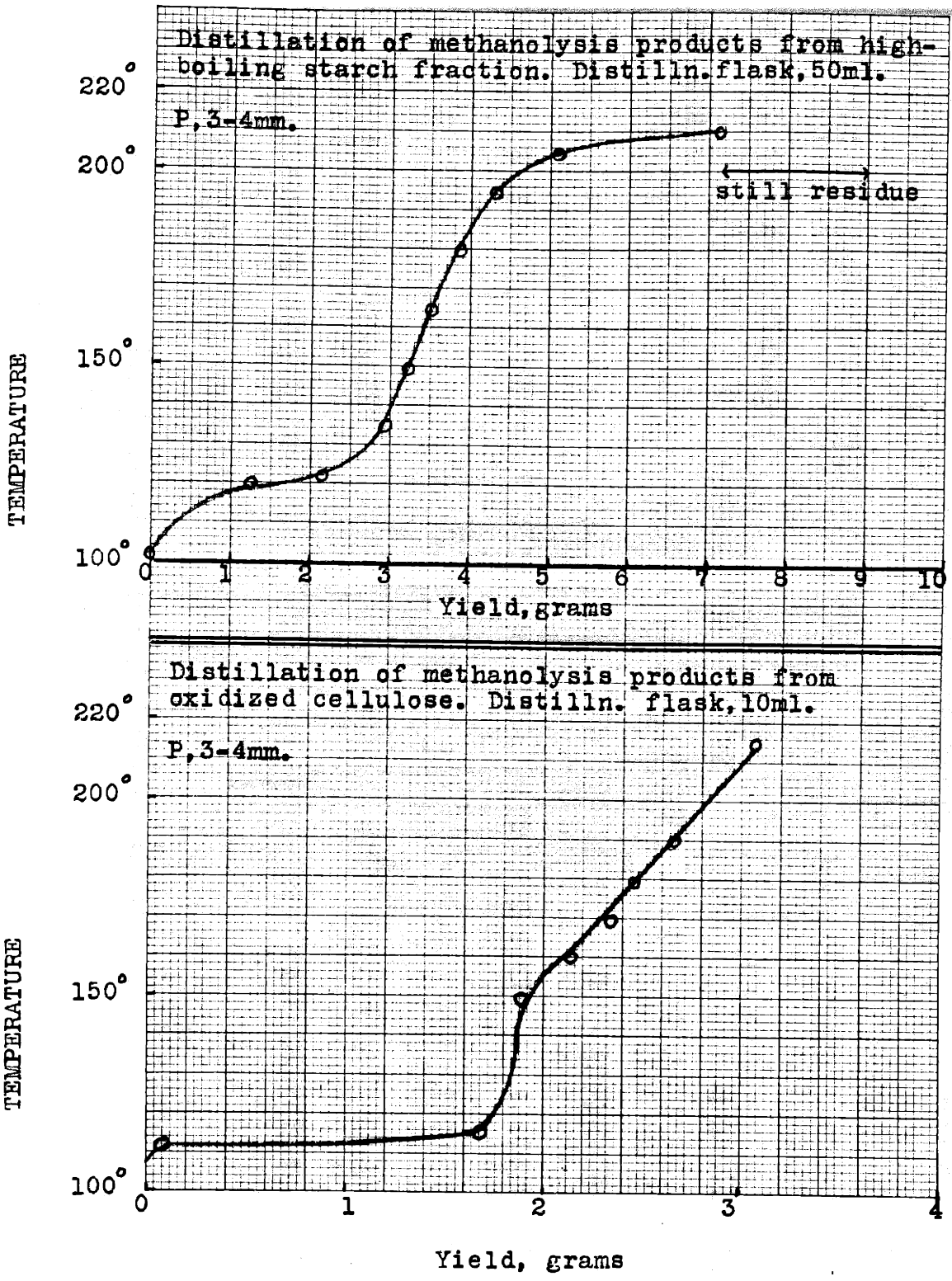
(d) Treatment of the high-boiling fraction (liquid portion) with 10% methanolic hydrogen chloride.

A sample of the high-boiling fraction (liquid portion), weighing 16g. was added to 133g. of a 10% solution of hydrogen chloride in dry methyl alcohol. While still cold, a brown color developed immediately. The solution was refluxed for three hours. After the first five minutes, the color had become a black-brown. On cooling, the acid was neutralized with lead carbonate and the alcohol replaced with water. The aqueous solution was extracted with chloroform and the extract evaporated under reduced pressure. A small quantity of glyoxal tetramethylacetal distilled off at 30°-50° C at 3-4mm. The residues then weighed 8.5g. Fractionation at a pressure of 3-4mm. gave 2.7g. of a liquid boiling at 102°-135° C, of which the greater proportion distilled between 120°-135° C; $n_D^{20°}$, 1.4505; $[\alpha]_D^{20°}$, -73.2° (in water, C, 0.902).

Anal., Calcd. for $C_6H_7O_3(OCH_3)_3$: C, 49.09, H, 7.32; CH_3O, 42.3
Found, for crude material: C, 48.7, 48.25; H, 7.59, 7.70;
CH_3O, 41.5, 42.6
A second fraction boiled between 135°-235° C., of which by far the greater proportion boiled between 195°-210° C. Yield, 4.7g. (See distillation curve, p.32)

(e) Treatment of periodate oxidized cellulose with 10% methanolic hydrogen chloride.

Oxidized cellulose, (28.0g.) was added to 500 g. of a 10% solution of hydrogen chloride in dry methanol, and refluxed for five hours. The products were worked up in the usual manner except that ether was used for the extraction of the aqueous solution of the residues instead of chloroform. The ether extract, after evaporation of the ether, was distilled at 3-4 mm. Equal yields of two fractions were obtained, the first, a very pale yellow liquid boiling between 112°-115° C.; $n_D^{20°}$, 1.4466; $[\alpha]_D^{20°}$, -88.1° (in water, C, 0.942). The very small yield, due to accidental loss, prevented further purification.
Anal., Calcd. for $C_6H_7O_3(OCH_3)_3$: C, 49.09; H, 7.23; CH_3O, 42.3
Found; C, 48.8, 48.2; H, 6.69, 7.56; CH_3O, 41.05, 40.85
Glyoxal content: 128.0 mg. gave 32.4 mg. and 32.7 mg. of glyoxal-bis-2,4-dinitrophenylhydrazone; theory, 24.3 mg. Yield, 133%, and 135%.
Between 150°-215° C., at 3-4 mm., a second fraction came



over, corresponding in boiling point to the higher boiling fraction from methanolyzed periodate oxidized starch. Trituration with cold ether did not result in the precipitation of a solid fraction.

SUMMARY

(1.) Corn, wheat, arrowroot, soluble and insoluble potato starches were oxidized by an aqueous solution of trisodium paraperiodate, and the oxidized starches were boiled under reflux for three hours with methanol containing 10% of hydrogen chloride. The products in each case gave a small yield, up to 1.6%, of a crystalline material, m.p., 148°-148.5° C., $[\alpha]_D^{20°}$, -4.3° in water.

(2.) When the products from the methanolysis of oxidized potato starch were fractionally distilled, the main fractions boiled at 116°-119° C. and 195°-205° C., at a pressure of 3-4 mm., and were obtained in yields of 14.2% and 16.0% respectively. Detailed chemical study showed that the high-boiling fraction consisted of a methylerythroside unit combined in a dioxan ring with a glyoxal residue, to which in turn a second methylerythroside unit, and a methoxyl group were attached. This fraction consisted of a mixture of isomeric 2,7-dimethoxy-3-(2-(3)-methylerythrofuranosido)-hexahydrofuro(3,4)-p-dioxins. The above crystalline material was one of these isomers.

(3.) The second fraction derived from the methanolysis of the oxidized starch was shown to be a methylerythroside residue condensed in a dioxan ring with a glyoxal

unit to which two methoxyl groups were attached. The fraction was a mixture of isomeric 2,3,7-trimethoxy-hexahydrofuro(3,4)-p-dioxins. One of these isomers was isolated in a pure crystalline condition, m.p., 97°-98° C., $[\alpha]_D^{20°}$, -59.1 (in water). The liquid mixture boiled at 116°-119° C, $n_D^{20°}$, 1.4488 .

(4.) When the first fraction was further degraded by methanolysis, a 27.5% yield of the second fraction b.p., 102°-135° C, $n_D^{20°}$, 1.4505, was obtained, but the small amount prevented the isolation of the crystalline isomer.

(5.) All the data summarized in (2), (3) and (4) agreed with the hypothesis that the fractions had been produced by a random scission of the acetal bonds during the methanolysis of the oxidized starch. The latter was assumed to consist of uniform linear molecules in which two different types of acetal linkage occurred in alternation.

(6) Further support for the hypothesis in (5) resulted from the isolation of similar fractions, prepared from periodate oxidized cellulose by similar treatment with acid methanol.

AMPLIFICATION

I. THE CONSTITUTION OF STARCH, AND RECENT THEORIES CONCERNING ITS CHEMICAL STRUCTURE.

Starch, as well as cellulose, has long been known to give a very high yield of glucose on hydrolysis. The occurrence of the two substances in plant material led to the earlier belief that they should be very similar in chemical structure. Cellulose, we now know, is composed of long chains of glucopyranose units joined by β-glycosidic bonds between the 1,4 positions of these units (22); the chains are unbranched and the resultant linear polymer exhibits the physical characteristics associated with such polymers, namely, fibre or film-forming properties and tensile strength. Starch, on the other hand, exhibits none of these properties. It has been found to be composed of glucopyranose units joined together by α-glycosidic bonds in the 1,4 positions (2,22) but will never form films, comparable with those of cellulose, nor fibres. Furthermore, starch, regardless of source, may be easily divided into two quite distinct fractions. Some excellent work, done very recently by Meyer (24), has shown that by steeping in water at 70°C., a portion of starch consisting of 10-20% of the whole will go into"solution", while the remainder is insoluble. This phenomenon has also been observed by many others (24a); the soluble portion is now generally

referred to as "amylose", and the insoluble portion as " amylopectin".

Amylose has been found by Meyer to be a straight chain of glucopyranose units joined by α-glycosidic bonds, and having a molecular weight varying between 10,000 and 60,000. This portion alone has cellulose-like properties. Methylated amylose may be spun into threads, or spread into films of remarkable tensile strength (25). Amylopectin, on the other hand, has been found by the same author to have none of these properties. Whereas amylose is completely hydrolyzed to maltose by β-amylase, amylopectin is only 54% hydrolyzed under the same conditions. The residual "dextrin" may be hydrolyzed by the enzyme α-glucosidase, but again, the material resists complete hydrolysis, and another dextrin is obtained. These dextrins are of high molecular weight as is amylopectin itself, which varies between 50,000 and 1,000,000. Although Meyer's theories agree in outline with a great mass of earlier work, the details have not yet been confirmed.

The methylation of starch gives, on hydrolysis, a high yield of 2,3,6-trimethylglocose, as well as a very small quantity of 2,3,4,6-tetramethylglucose, the latter, presumably, resulting from an "end-group" in which the hydroxyl group on the fourth carbon atom is exposed to methylation. By measurement of the amount the tetramethylglucose derived from starches, Haworth (2)

has for some time been estimating the length of the chain of glucose units, on the assumption that starch is a long straight chain composed exclusively of glucose units. The results vary between 23-30 glucose units for different starches, and a minimum molecular weight for these starches is calculated to be between 4000 and 5000. These numbers are completely at variance with Staudinger's viscosity and osmotic pressure measurements (26) of starch solutions, which give molecular weights between 300,000 and 700,000.

Besides deriving a small quantity of tetramethylglucose and a large proportion of trimethylglucose from methylated starches, Irvine (27) has obtained a small quantity of 2,3-dimethylglucose and 2,6-dimethylglucose. This clue led Staudinger to propose a structure for starch(26) which tentatively would account for the high molecular weight of starch as well as its diverse methylated derivatives. This structure contains a branched chain in which the branches which radiate from the central chain are composed of chains of 24-30 glucose units attached to the central chain (also composed of glucose units) by means of glycosidic oxygen bonds. These oxygen bonds may be of two types. One pendant chain is attached by an oxygen bridge between the first carbon atom of the nearest glucose unit, and the primary hydroxyl group on one of the glucose residues in the central chain. The next pendant chain is

attached in the same manner, but to the hydroxyl group on the third carbon atom of the next glucose unit in the central chain. This alternating form of linking to pendant chains is repeated until a very large branched molecule is obtained. Such an arrangement, if methylated, should give, on hydrolysis, a small yield of 2,3-dimethylglucose and 2,6-dimethylglucose in the proportion of one mole of each to every 48 to 60 moles of 2,3,6-trimethylglucose. Although the actual yields of these dimethylglucoses are in this order of magnitude, the quantitative data are still unreliable. Recent chemical evidence by Hirst(27a) supports the view that starch is a branched chain polymer in which the branches are attached by definite chemical (glycosidic) bonds. The earlier views of Haworth that the high molecular weight of starch was due to association of macromolecules containing 24-30 glucose units seem to have been disproven by these recent developments. Physical data, especially X-ray studies (28), support the theory that starch has a branched chain structure, this, mainly, from the impossibility of obtaining "fibre" diagrams from starch. Staudinger's viscosity data also shows that samples of starch which have a high degree of polymerization have molecular cross-sections which are far too large to correspond to a molecule consisting of a long straight chain. For example, starches which have a degree of polymerization corresponding to "meso"

and "eucolloid" cellulose (i.e.,chain lengths between 250A and 2500A or over) show, nevertheless, behaviour corresponding to "hemicolloid" cellulose (i.e.,a chain length less than 250 A).

Another important example of the heterogeneity of starch,and which does not fit in even with Staudinger's proposed structure, is provided by the observation of Schardinger (29) that two low-molecular weight, non-reducing dextrins may be obtained from starch by the action of Aerobacillus Macerans, without degrading the starch into simple reducing sugars. Hudson has confirmed this(30) and has shown definitely that these dextrins are not a result of synthetic activity by the bacilli, but are formed by the action of a hitherto unknown enzyme, which they secrete. Freudenberg(31,32), by chemical data, and Kratky(33) by X-ray data, have shown that these two dextrins are composed of 5,and6-membered rings, each member of which is an anhydroglucose unit. The ring formation effectively blocks potential aldehyde groups, thereby explaining the lack of reducing power shown by the dextrins. The manner in which these rings are incorporated in the starch molecule is a matter of conjecture,and thus far, no satisfactory suggestion has been made.

To summarize, we see that starch is heterogeneous, not only in a physical and macroscopic sense, but in the chemical sense as well. The smaller portion (20%)

may consist of linear molecules, intimately intertwined with much larger three-dimensional structures built up in unknown ways from macrocyclic rings and highly branched chains. Whatever the details may be, the methylation data proves that the average branch in the chain of many starches contains 24-30 uniformly linked glucose units. Such a vague and complex structure must of necessity complicate the interpretation of the data obtained by starch chemists and this is especially true of the oxidation results, briefly reviewed in the next section.

II. THE ACTION OF OXIDIZING AGENTS ON STARCH.

The oxidation of starch by almost every conceivable oxidizing agent has been studied for many years, but the great diversity of products, and the difficulty of characterizing the nature of the oxidations, have made it impossible to draw any definite conclusions. Not only the heterogeneity of starch but also the non-specificity of the usual oxidant for any particular region of the molecule have contributed to this situation. Most of the literature is due to technologists whose interest was usually extended only to the production of slightly modified starches with desirable physical properties. The present review is restricted to isolated researches which contain suggestions as to the nature

of the oxidations which took place.

A considerable amount of work with hydrogen peroxide has been reported (34-37), the general effect being to oxidize and hydrolyze starch down to dextrins, maltose, and glucose and to give certain acids and aldehydes. Oxidation with chromic acid has given a 10% yield of furfural (38). Hachihama (39) obtained a yield of 49% of saccharic acid and 10% oxalic acid by the oxidation of starch with 35% nitric acid. This oxidation was no mild affair, but involved a complete breakdown of the starch molecule. An excellent study of the oxidizing action of bromine on starch was made by Felton (40). The products were considered to contain carboxyl groups arising from the oxidation of primary alcohol groups (i.e., position six in the anhydroglucose unit), ketone groups from secondary alcohol groups, and oxidation of glycol groups (i.e.,the hydroxyl pair at positions two and three) which resulted in the degradation of the starch chain and the formation of non-uronic carboxyl groups. Such a series of oxidation products were certainly to be expected from a non-specific agent such as bromine. Szniewski, on the other hand, showed that under certain conditions, soluble starch was oxidized by bromine in the presence of barium carbonate, so that only the primary alcohol group was affected and converted to a carboxyl group (41).

Other authors (42,43) studying the action of hypohalites on starch, reported simply the random oxidation of starch with increasing appearance of carboxyl groups.

Only one type of oxidizing agent has been reported which apparently has a specific effect on one definite region of the starch molecule. This is periodic acid and its salts, the action of which was discussed in Part I of this thesis. Hudson's work has shown, and the research outlined in this thesis has confirmed to a great extent, that starch or cellulose which has been oxidized by periodic acid, contains aldehyde groups in the 2,3 positions of the glucose unit, and no other manifestation of oxidation has taken place, such as the appearance of carboxyl groups. It may be mentioned that close control of acidity and of temperature are necessary to avoid a random oxidation, even in the case of periodates. One other oxidizing agent has a specificity similar to that of periodic acid, namely, lead tetraacetate, which was first studied by Criegee (44). The reaction between polysaccharides and lead tetra-acetate, has not yet appeared in the chemical literature.

An approach to the specificity of periodic acid is possibly provided by Omori (35) who remarks that the splitting of starch by a combination of a heavy metal and hydrogen peroxide "....is governed by a mechanism different from that of diastase hydrolysis".

Such a statement might possibly be associated with an observation by Fischer (45) that a trace of lead tetraacetate in the presence of a large amount of hydrogen peroxide will split glycols into dialdehydes in the same manner as if lead tetraacetate were present in molar proportions. It may be that the observations of Omori and Fischer will lead to useful and cheap oxidizing systems, the action of which, on starch, is similar to that of the expensive periodic acid.

III. ADDITIONAL EXPERIMENTAL DETAILS.

(1) The preparation of trisodium paraperiodate (46)

A solution of 240 g. of sodium hydroxide (C.P.) pellets in 1000 ml. of distilled water was prepared, and 50 g. sodium iodide was added. The solution was made up to 2000 ml. and placed in a 3000 ml. beaker. This was warmed to 80°C., and 80 ml. of bromine added slowly from a dropping funnel with its tip beneath the surface of the liquid. The addition took about one hour, and the solution was stirred mechanically during the operation. When about two thirds of the bromine had been added, a cloudy precipitate appeared, which increased rapidly when all the bromine had been added. The mixture was allowed to cool slowly, with stirring, to 40°C. It was then separated by filtration through a sintered glass funnel, and the filtrate heated to 95°C for about one half hour. At this stage, more precipitate came down, and was separated when cold. The combined mass of periodate was washed in the funnel with four 50 ml. portions of water, suction being applied only when the material had been in contact with the water for ten minutes. Some stirring of the sludge, while washing, was found advantageous. The periodate was finally sucked dry and then dried completely in an oven at 50°C overnight. Yield, 85 g., or 84 % of theory.

(2) Estimation of purity of the periodate. (47)

The following is a typical example of the estimation of sodium periodate, and is the method followed in the determination of the amount of periodate comsumed during the oxidation of starch.

A sample of the prepared salt, 0.0771g.,was dissolved in water with a few drops of conc.hydrochloric acid. This was neutralized with dilute sodium hydroxide until just red to phenolphthalein. About 10 ml. of a freshly prepared (important!) saturated solution of sodium bicarbonate was added and the color vanished. Standard arsenite solution (10 ml.) was added , together with 1 g. of pure sodium iodide, and the excess arsenite titratd with N/10 iodine.

Titration, 4.60 ml. of 0.1040N iodine.
But 10 ml. arsenite is equivalent to 9,55 ml. 0.1040N iodine. Therefore,amount of iodine released by the action of periodate on sodium iodide was 9.55-4.60, or 4.95 ml. of 0.1040 N iodine.

Weight of periodate present; $\frac{1040 \times 294 \times 4.95}{1000 \times 20 \times 1000}$ or 0.0756 g.

Purity of preparation; $\frac{.0756 \times 100}{.0771}$, or 98.2%

(3) Determination of moisture, protein and ash in potato starch.

A sample of Mallinckrodt potato starch,2.3054 g., was placed in an oven at 110° C and left overnight. The

loss in weight was 0.2671 g., increasing, after two more days in the oven to 0.0.2691 g. Moisture, 11.7%. A sample of dried starch was burned for an estimation of nitrogen by the method of Dumas. The starch, 0.35586 g. gave 0.84 ml. of nitrogen at 25°C and 744.0 mm. This corresponded to 0.63% nitrogen or 0.56% in starch of 11.7% moisture. On the assumption that protein varies between 15% and 18% nitrogen, the above result was taken to represent the presence of approximately 3.5% protein in starch. Two samples of starch, on ignition, left ash amounting to 0.28% and 0.29% of the original weight of starch.

(4) <u>The oxidation of starch with trisodium paraperiodate.</u>

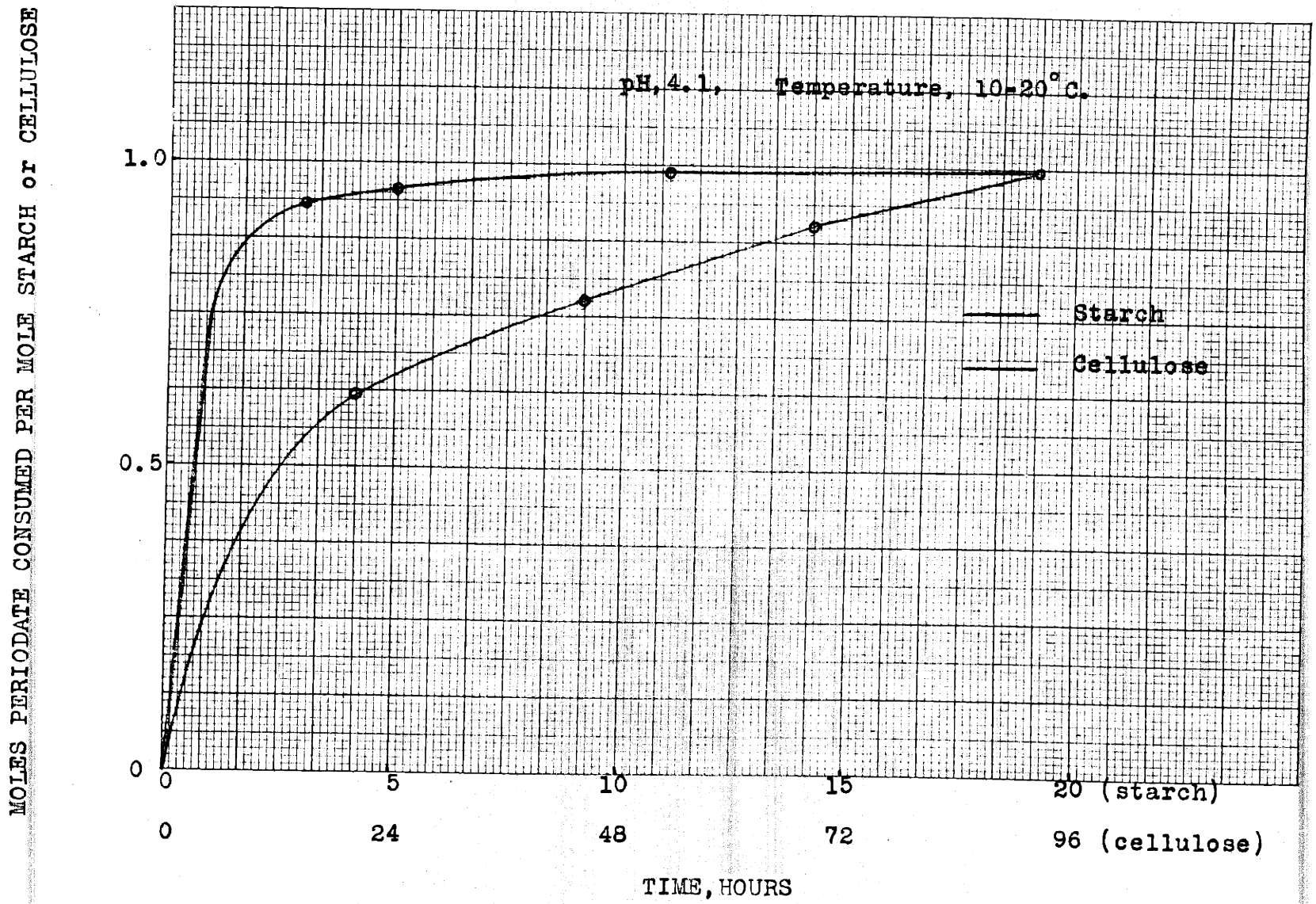
One third mole of sodium periodate (100g. of 98% pure substance) was added to a solution of 150 ml. glacial acetic acid in 1500 ml. distilled water. After stirring for thirty minutes, the pH of the solution was 4.1. The solution was cooled to 5°C. and one third mole of potato starch (71.0 g. starch, corrected for a total impurity of 15.5%) was added slowly. The mixture was mechanically stirred for 10 hours and the temperature maintained between 5-15 C. (Above 25°C, on one occasion, the starch went partially into solution, giving an unfilterable gel). At the end of this time, titration of a 10 ml. sample of the supernatant liquid (as described on the previous page) showed that all of the periodate

had been consumed. (See oxidation curve, p.49) The mixture was separated by filtration, and the oxidized starch washed eight times with 500 ml. portions of distilled water, after which, no test for iodate in the washings could be observed (i.e., acidification of washings and addition of potassium iodide and starch). The product was dried by stirring in two 400ml. portions of acetone and then in two 400 ml. portions of petroleum ether, and sucked dry. The white, fluffy material was dried to constant weight in a vacuum desiccator over phosphorus pentoxide. Yield, 64.5g. Ash, 0.28% Theory, 62.4%

(5) The methanolysis of oxidized starch.

(Based on Dr. Grangaard's unpublished work.)

A fresh stock of synthetic methanol was found to be sufficiently free of water that it could be used without further precautions, but in case of uncertainty, dry methanol could be obtained by a single distillation through an efficient column or by refluxing over magnesium ribbon for a few hours prior to distillation. Dry oxidized starch(50g.) was added to a solution of 41g. of hydrogen chloride in 410g. of dry methanol, and the mixture allowed to reflux under a ground-in glass-jointed condenser for three hours. After the first 20 minutes the material had gone into solution with the development of a very dark brown color. During this time the oxidized starch passed through a gummy, stringy


pH, 4.1, Temperature, 10-20° C.
1.0
0.5
0
MOLES PERIODATE CONSUMED PER MOLE STARCH or CELLULOSE
Starch
Cellulose
0
5
10
15
20 (starch)
0
24
48
72
96 (cellulose)
TIME, HOURS

stage, which, on dissolving, left a very small quantity of powdery material which eventually went into solution. In one case, oxidized starch which had been stored over calcium chloride for three months, passed directly into solution without going through the gummy stage; the time required for complete solution was one hour instead of twenty minutes.

After three hours refluxing, the solution was cooled , and neutralized with a slight excess of lead carbomate; neutrality was confirmed by a negative reaction to congo-red paper. The liquid was filtered off and the inorganic material washed thoroughly with methanol, the washings being added to the filtrate. About 100 ml. of water was added at this stage ,and the solution stirred with about 10 g. decolorizing charcoal. After filtration, the methanol was distilled off under reduced pressure, at 50°C. The aqueous residue was extracted with chloroform until the last two extracts were colorless, and the extract boiled down under vacuum to remove all the chloroform. Usually a few grams of glyoxal tetramethylacetal came over at this stage, when the residues were being heated to 100°C under vacuum by an oil-pump. This temperature was not high enough to remove any material boiling higher than the glyoxal tetramethylacetal; weight of remainder, 31.0 g.

Dr. Grangaard found that glyoxal tetramethylacetal can not be removed from aqueous solution by chloroform extraction. The slight yield of this acetal in the above mentioned chloroform extract may therefore be connected

with the high concentration of other acetals, also present.

(6) The determination of molecular weight.

(a) By the method of Rast (48a), as modified by Pregl (48b).

The micro method outlined by Pregl was followed exactly, except that slightly larger samples of camphor and solute were used. The camphor was a very pure sample supplied by the Eastman Kodak Co., m.p., 178.8° C. A sample of 1,3,5-tri-p-anisylbenzene was used as a standard with which to obtain the molecular depression constant of the camphor. This gave the very high constant of 49.5, due, as it was later discovered, to the standard compound being very impure. Using naphthalene as a reference substance, another student found the cryoscopic constant for the same camphor to be 40.0. Results, for crystalline $C_{10}H_{13}O_8(OCH_3)_3$:

Conc., %	dt	M.W.	(M.W., $\frac{40.0 \times 1000 \times C}{dt}$)
4.2	4.8°	350	
6.1	7.2°	339	
8.6	10.0°	344	
11.1	12.8°	347	

Note: these resulte were later discarded in favor of those obtained by the ebullioscopic method.

(b) By the ebullioscopic method of Swietoslawski (49).

This method involved the measurement of the change in the difference between the temperature of a boiling

liquid and its vapor on addition of the substance , the molecular weight of which, is desired. Carbon tetrachloride,(10 ml.) was the solventused, and its ebullioscopic constamt was first determined by using phenanthrene as a reference substance. These determinations gave an average constant of 64.3 . Another sample of carbon tetrachloride gave 65.0 .

Results;

Wt. sample	dt	K	M.W.
87.0 mg.	0.110°	64.3	321
76.7 mg.	0.097°	65.0	323
Calcd.for $C_{13}H_{22}O_9$,			322.2

(7) The determination of acetyl content.

(a) By the method of Kunz and Hudson (15).

Samples of the acetate of $C_{13}H_{22}O_9$, weighing 13.8mg. and 21.5 mg. respectively, were weighed into two 125 ml. flasks. To each was added 10 ml. of acetone and 25 ml. of 0.01425 N potassium hydroxide solution. The flasks were corked and set aside for two hours at room temperature. Two blanks were set up similarly. The solutions were titrated with standard acid.

Titration: Alkali consumed by first sample,2.65 ml.N/100 sodium hydroxide solution. Second sample,4.10 ml.

Analysis; 1. $\frac{2.65 \times 43 \times 0.01425 \times 100}{1000 \times 0.0138}$ or 11.8% acetyl.

2. $\frac{4.1 \times 43 \times 0.01425 \times 100}{1000 \times 0.0215}$ or 11.7% acetyl.

Theory, 11.82%

(b) By the method of Clarke (18).

An all-glass steam-distillation apparatus was built, according to the description by Clarke. A sample (10-20 mg.) of the acetate was weighed out on a small piece of cigarette paper and dropped carefully into the clean dry flask. Alcoholic potassium hydroxide, (N/1, 2ml.) was added and the mixture brought to a boil. This was acidified by the addition of 18 ml. of an aqueous solution of magnesium sulfate and sulfuric acid (100g. magnesium sulfate and 1.5g. sulfuric acid, made up to 180 ml.) Steam was run in gently while the flask was heated to maintein a constant volume of boiling liquid. The rate of flow of the distillate was carefully controlled to 20-25 drops per minute. The first 50 ml. of distillate was titrated with N/100 alkali. Standardization of the apparatus was performed with pure triacetyl- -glucosan. On the average it was found that 98.3% of the acetic acid came over with the first 50 ml. of the distillate.

A typical case: Sample, 13.1 mg. Titration, 1.80 ml. of 0.0210N potassium hydroxide.

Acetyl content; $\frac{43 \times 0.021 \times 1.80 \times 1000}{0.0131 \times 1000}$ or 12.6% acetyl

(8) The determination of combined glyoxal.

The special all-glass apparatus used for the determination of acetyl groups was found entirely suitable for this experiment. The apparatus consisted of a 50 ml. distilling flask with a standard ground-glass joint at the neck, into which either a "cold-finger" or an inlet tube for the introduction of steam, could be placed. The side arm, containing a bulb which served admirably for the elimination of froth, was sealed to a simple water-jacketed condenser. After very careful cleaning and drying of the apparatus, a weighed quantity of the substance to be estimated was introduced and 15 ml. of 10% methanolic hydrogen chloride was added. The mixture was refluxed for 24 hours under the cold finger, while the end of the condenser was protected by a tube filled with calcium chloride. On cooling, the solution was neutralized with e predetermined volume of a solution of sodium methylate in dry methyl alcohol, and a few extra drops added to bring the mixture definitely to the alkaline side, as tested by bromthymol blue indicator. Before steam was introduced, the mixture was boiled until about 10 ml. of methyl alcohol had come over, in order to prevent too violent distillation when heated by steam. The distillate was collected directly in a 250 ml. volumetric flask, cooled in ice-water. A gentle but steady flow of steam was passed in, while the flask was heated to maintain

the volume at not over 10 ml. Above 10 ml. volume, for some reason, extensive frothing took place. At least 180 ml. of distillate was collected and the volume made up to 250 ml. This distillate, when it contained glyoxal tetramethylacetal was always faintly cloudy; a solution of the pure synthetic product of the same concentration was also cloudy, but not to the same extent as in the former case. In some early experiments, the ground-glass joint was sealed with vaseline, and some of the vaseline came over with the steam. This tended to give rather high results, but reasonably accurate data were obtained by refraining from the use of vaseline. The quantity of the substance to be analyzed for glyoxal content was always chosen so that the glyoxal tetramethylacetal, when made up to 250 ml., was present in a concentration close to 0.39 mg. per ml. A standard solution, of the same strength, was made up from pure synthetic glyoxal tetramethyl-acetal, for purposes of comparison.

The analysis for glyoxal was carried out by the addition of 25 ml. aliquots of the steam distillate to 50 ml. beakers, along with 5ml. of concentrated hydrochloric acid, and then stirring in 0.19 g. of 2,4-dinitrophenylhydrazine in each mixture. The beakers were heated for one half hour on the steam-bath, during which time the precipitation of the flocculent orange product was clearly visible, and after which time

the termination of the reaction was obvious. After transferring to weighed crucibles, washing with 2N hydrochloric acid and water, and drying in an oven at 110°C the precipitates were weighed.(52,19)

(9) Attempts to isolate a derivative of erythrose from the high-boiling crystalline material.

(a) The preparation of brucine-d-erythronate.

After the methanolysis of 168.4 mg. of the crystalline substance,(see p.16),the aqueous solution from which the glyoxal tetramethylacetal had been steam distilled was presumed to contain methyl erythroside, and an attempt to isolate this material as brucine-d-erythronate was made, after the manner of Hudson (4) and others(53,54).

The solution was made slightly acid to bromthymol blue with hydrochloric acid and refluxed for three hours to hydrolyze the acetal to the free sugar. Water was added to the cold, filtered solution to bring the volume to 50 ml. and then 1.2 ml. of bromine. The mixture was set aside for four days in a dark cupboard. The bromine was removed by aeration and the absence of bromine proved by the addition of phenol to a small portion of the solution .No precipitate of tribromophenol was observed. Silver carbonate removed the halogen ions and the mixture was filtered. Brucine was added to the filtrate until alkaline to bromthymol blue, and the mixture warmed on a steam-bath for three hours. After

cooling, the excess brucine was removed by extraction with chloroform, and the aqueous portion evaporated to a syrup. Addition of alcohol at this stage, if brucine-d-erythronate had been present, would have given a precipitate. On the contrary, a small amount of material which proved to be sodium chloride was the only substance recovered.

This experiment was abandoned for several reasons:
1. The yield, at best, according to Hudson's example could not be greater than 22%.
2. After 24 hours refluxing in acid methanol, and further heat treatments in aqueous acid, the possibility that the heat sensitive erythrose (55) should survive was very remote.
3. The acute lack of material necessitated working on a nearly micro scale, which, in view of the low yields which were expected left very little hope for success.

(b) The preparation of erythrosazone.

The isolation of erythrosazone, by acid hydrolysis of the crystalline substance and treatment of the product with phenylhydrazine, seemed to be a reasonable plan of attack, especially since the solubility of erythrosazone in hot water should lead to an easy means of separation of the material from the accompaning glyoxalosazone. A sample of the substance, 143.5 mg., was warmed on a steam bath for one hour with 8.3 ml. of 2% sulfuric acid. On cooling, the solution was made

just alkaline to phenolphthalein and then just acid to the same indicator with glacial acetic acid. Excess phenylhydrazine was added and the mixture set aside at 40°C for 24 hours. A heavy amorphous precipitate was separated by filtration, and boiled for a few minutes in water. A portion of the material dissolved to give a pale yellow solution, which, on long cooling, yielded a yellow amorphous product, m.p., 137°-140°C (corr.). This form of recrystallization was repeated several times, but in no case was the melting point raised higher than 137°-140°C. The substance, if erythrosazone, should have meltd at 164°-165°C (55-60), and should also crystallize from hot benzene. The latter was attempted but only a gelatinous mass was the result. It was concluded that the acid hydrolysis of the substance was so injurious to the substance that any erythrosazone formed in this semi-micro scale experiment was too small in amount for isolation and identification.

(c) The effect of hydrolysis on the rotation of the crystalline substance.

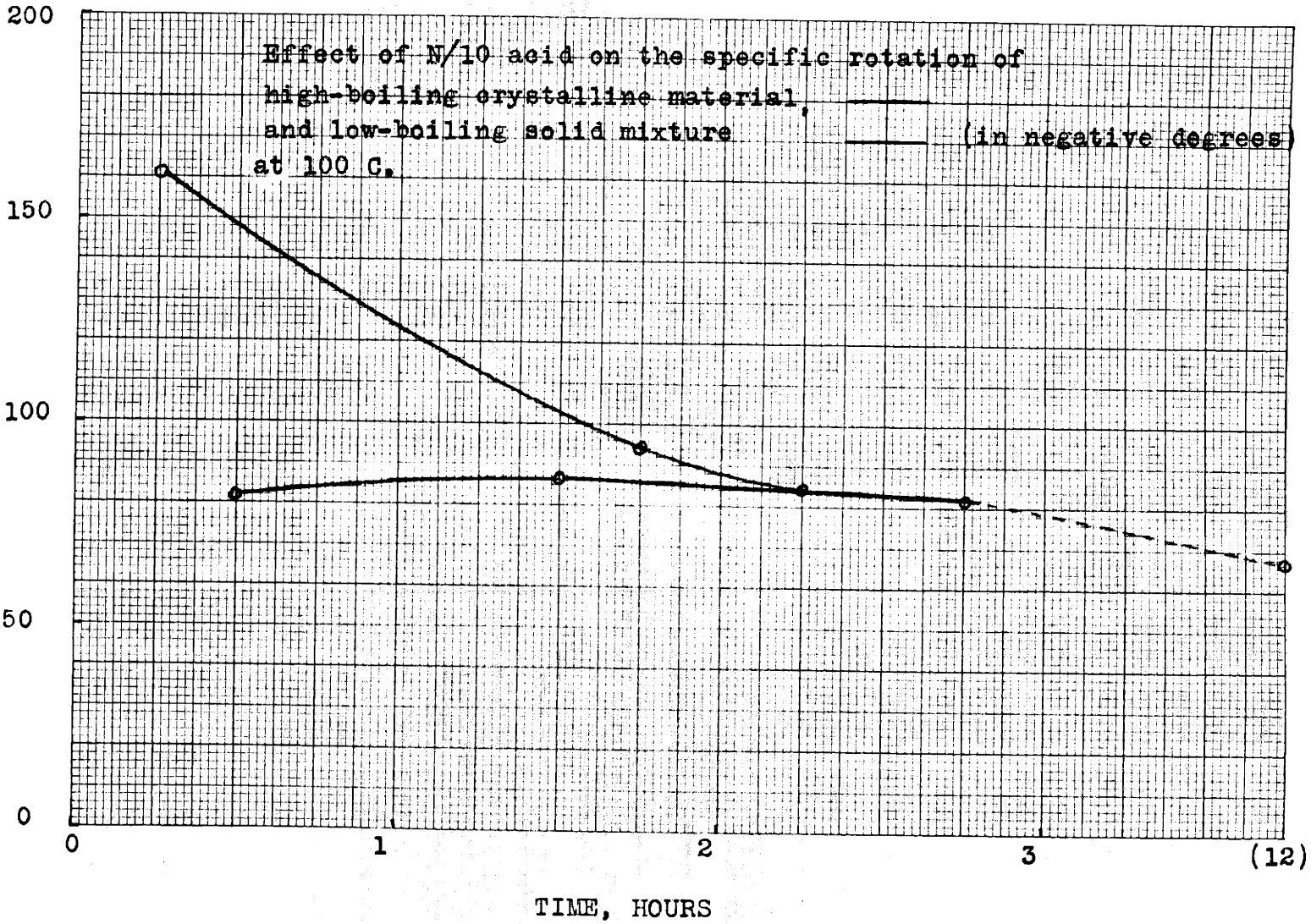
Pure d-erythrose has a specific rotation in water of -14.5°, after three days standing, or after a few hours in 1.5% sulfuric acid (55). After that time, Ruff observed gradual decomposition, accompanied by the development of a brown discoloration. A 3.685% solution of the crystals in N/10 hydrochloric gave

a high positive rotation $[\alpha]_D^{20°}$ +61.1° (based on $C_{13}H_{22}O_9$) after going completely into solution (one half hour); the rotation increased to +64.3° after one hour on the steam bath, then gradually decreased to +61.1° after 2¼ hours, and reached +50.5° after 12 hours, with extensive decomposition as evidenced by the brown discoloration. Based on a hypothetical content of erythrose from its calculated proportion in the substance, these figures became +81.97°, 86.3°, 81.97°, and 67.77° respectively.

The hydrolysis of a sample of the pure low-boiling isomeric mixture ($C_9H_{16}O_6$), gave equally strange results. A 3.633% solution in N/10 hydrochloric acid turned a deep yellow color immediately on going into solution (15 min.). Based on the above formula, the specific rotations at the beginning, and after 1¾ and 2¼ hours, respectively, on the steam bath, were: -87.9°, -51.3°, and 46.4° Based on the theoretical quantity of erythrose present, these figures became -161°, -94.2° and -85.1° respectively.

In both cases, attempts to follow the course of the change in rotation with time were given up because the solutions became too dark to read, and because brown precipitates in each solution signified decomposition, making further readings unreliable.

It is interesting to note that after 2¼ hours of hydrolysis the high and low-boiling substances gave rotations which were equal in magnitude, but opposite in


Effect of N/10 acid on the specific rotation of
high-boiling crystalline material, ——
and low-boiling solid mixture —— (in negative degrees)
at 100 C.
200
150
100
50
0
0
1
2
3
(12)
SPECIFIC ROTATION, DEGREES
TIME, HOURS

sign, and that subsequent change in rotation of the high-boiling substance was slow; the shape of the curve for the low-boiling mixture suggests that this too had reached a rotation which might remain steady for some time. (See graph, p.60)

No explanation of these phenomena is offered, nor for the fact that at no time did the rotations approximate to that of -14.5° expected for erythrose. Apparently the latter failed to survive the hydrolysis in substantial amount, while other optically active substances of an unknown nature did so.

(d) Oxidation of the substance with nitric acid.

The experiments of Hirst and coworkers (61-64), on the oxidation of methylated sugars, by means of nitric acid, to yield methylated dibasic acids, seemed to be a reasonable method for isolating a four-carbon dibasic acid by a similar oxidation of the crystalline substance. A sample of the material, 476.4 mg., was treated with 6.5 ml. of nitric acid (sp.g., 20/20, 1.14) for six hours at 80° C. The greater part of the nitric acid was then removed by steam distillation, and the aqueous residue treated in the hope of isolating a derivative of tartaric acid. Potassium acetate and ethyl alcohol were added after the manner of Reid(65) and Nelson(66), and the solution set aside for two days in an ice-box at 0° C. After this time, long needles appeared

which proved to be potassium nitrate. No other product was obtained. The procedure was repeated with a synthetic mixture of d-tartaric and oxalic acids in the same proportion as they had been expected in the first oxidation. Again, potassium nitrate was the only product precipitated out, due apparently to the incomplete removal of the nitric acid. No reference to the action of dilute aqueous nitric acid on tartaric acid could be found in the literature. Tartaric acid, untreated with nitric acid was precipitated out quantitatively as the acid potassium salt, by the above treatment.

(e) The graded hydrolysis of the p-toluenesulfonic ester.

This experiment was designed to differentiate between structure III (which should give 3-p-toluenesulfonyl erythrosazone) and structure IV (giving 2-p-toluenesulfonyl erythrose hydrazone) in Part I, on the assumption that the p-toluenesulfonyl-substituted erythrose group in either of these two structures could be hydrolyzed off and treated separately with p-nitrophenylhydrazine.

A sample of the p-toluenesulfonic ester of the high-boiling crystalline compound, (174 mg.) was dissolved in 6 ml. of methanol and 10 ml. of N/10 hydrochloric acid was added. The mixture was warmed on a steam bath for 20 minutes, then cooled, and the volume doubled by the addition of water. This solution was extracted five

times with equal volumes of chloroform, and the extract evaporated under reduced pressure. The residue was a gummy mass which did not crystallize on cooling and scratching with a glass rod. The residue was found to be soluble only in 95 ml. of 75% acetic acid. To this was added 200 mg. of p-nitrophenylhydrazine (3½ moles) and the mixture was stirred until all the reagent went into solution. The solution was placed in an oven at 50°C., but no precipitate appeared after two days. The color of the solution was a much darker (red) than a solution of the p-nitrophenylhydrazine alone. The volume of the solution was reduced under vacuum to 25 ml., and left to stand overnight at room temperature. After two days a very slight, red, precipitate appeared, but in far too small a quantity to permit of isolation and analysis.

(10) <u>The mild methanolysis of periodate oxidized starch, and attempted fractionation of the products.</u>

A 25g. sample of periodate oxidized starch which had been in a vacuum desiccator over calcium chloride for two months was refluxed in a 1% methanolic hydrogen chloride solution. The oxidized starch took one hour to dissolve. At the end of this time, the solution was cooled, neutralized, filtered and 50 ml. of N/10 sodium hydroxide added. The methanol was distilled off and when the temperature had risen to 80°C., steam was

run in to remove the glyoxal tetramethylacetal. After 100 ml. of aqueous distillate had come over, the distillation was stopped. It was noticed that a heavy tar had appeared in the flask. The liquid in the flask was extracted with 750 ml. of chloroform, in which the tar was insoluble. The extract was evaporated to 300 ml., and 300 ml. of ether added. A brownish precipitate appeared at once which, on removal by filtration, and drying, was found to fuse at 155°-160° C. (fraction A). The filtrate was evaporated to a volume of 50 ml. and cooled in an ice-bath. On addition of 200 ml. of ether, a heavy precipitate was formed, which on separation and warming to room temperature, resolved itself into a brown oil, (fraction B) and a white amorphous powder (fraction C), which were separable mechanically. Fraction B, on drying in an oven at 50° C., exuded much ether and finally appeared as a brittle, microcrystalline mass; m.p., 130°-140° C.
Fraction A was refluxed for three hours in 1% acid methanol, but the resultant solid gave the same m.p. as before. Fraction B was treated in a similar manner, resulting in a very viscous liquid, insoluble in water. Fraction C, m.p. 140°-160° C., was suspected of being the high-boiling crystalline material (structure III, Part I), but was found to be completely insoluble in hot ether.

It was inferred, from the melting point ranges of these fractions, and their high insolubility in water, that they were high molecular weight fragments from the partial methanolysis of oxidized starch.

(11) The oxidation of cellulose with trisodium paraperiodate.

Sodium periodate, (54.4g., or 0.2 moles) was added to a solution of 80 ml. of glacial acetic acid in 800 ml. of water, and brought to a temperature of 20°C. Purified cotton linters, (32.4g. or 0.2 moles) were added and the mixture was set, under constant agitation, in a room at 20 C. After four days, titration of aliquots of the liquid showed that all the periodate had been consumed. The liquid was filtered off and the oxidized cellulose washed free of iodate, and dried over phosphorus pentoxide. Yield, 28.0g. (See graph, p.49)

IV. Comments on the applicability of Kuhn's treatment of the degradation of long-chain linear polymers.

Kuhn has advanced, on a mathematical basis, a means of calculating the yield of an n-membered fragment, resulting from the random degradation of a long-chain molecule, in which the repeating units are joined together by bonds of uniform strength (67).

With the optimum degree of scission in each case, the maximum yield of a three membered fragment is 50%, and of a two membered fragment, 66%, when each fragment is assumed to be stable in the reaction medium. These

calculated yields are less when the fragments are assumed to be exposed to further degradation. Suppose the degradation is known to have reached an extent of 50% of the total possible number of bonds which might be ruptured. In such a case, by Kuhn's calculations, the yield of a three membered fragment, exposed to further degradation, is 18.75% and of a two membered fragment, 25%.

The yields of the three and the two membered fragments from oxidized starch were 16% and 14.2% respectively, but it is obvious that Kuhn's assumptions cannot be applied, without modification, to the case in hand. In the first place it is not known whether the 50% yield of glyoxal tetramethylacetal actually represents a 50% degradation of the molecule, and in the second place, it is not known whether the bonds in the high-boiling fraction are of the same resistance to degradation as the bonds in the low-boiling fraction. It is known that the specific rotation of the former changes very slowly on warming in dilute aqueous acid, whereas the latter changes rapidly under the same conditions. (See graph, p.60) It would be interesting to observe whether the same phenomenon occurs on warming in 10% methanolic hydrogen chloride, or, better still, to obtain some kind of quantitative estimation of the relative resistances of the bonds in the two substances to rupture by acid methanol. When this is done, Kuhn's assumptions could be modified, and new theoretical yields calculated.

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The Isolation of a Crystalline Substance from Starches Oxidized by Periodate

By D. H. Grangaard, J. H. Michell and C. B. Purves

By degrading various periodate oxy-starches with acid methyl alcohol,[1] we have isolated a white, crystalline, levorotatory compound with the formula $C_{13}H_{16}O_8(OCH_3)_4$ and m. p. 150–150.5° (corr.). Although stable to further oxidation with periodate or Fehling's solution, the substance readily reduced the latter after mild acid hydrolysis and appeared to be the methyl acetal of an aldehyde or ketone. This in turn was possibly a fragment of a still larger chemical unit which was oxidized with the starch. No conclusive evidence was obtained to indicate whether the crystals were derived from an integral part of the starch macromolecule or from an associated impurity.

The crystalline material was obtained in 0.7 to 0.9% yield from high grade commercial specimens of corn, wheat, potato and arrowroot starches. It was also isolated from soluble potato starch[2] which after being oxidized was dialyzed against water for four days. Preliminary experiments with xylan gave none of the substance while with cellulose the trace of apparently crystalline material observed was too minute to be identified with certainty.

This note is published because only two of us (J. H. M. and C. B. P.) intend to continue the investigation.

(1) Jackson and Hudson, This Journal, **60**, 989 (1938).
(2) Baird, Haworth and Hirst, *J. Chem. Soc.*, 1201 (1935).

Experimental

Sodium periodate, 34.0 g. or 0.116 mole of $Na_3H_2IO_6$ was shaken at room temperature with 500 cc. of water and 50 cc. of glacial acetic acid until as much as possible had been dissolved. Starch, 20 25 g. air dry or 0.114 mole dry, was then added and the mixture, *p*H about 4.2, gently agitated until the next day, when titration[3] showed that all of the periodate had reacted. The oxy-starch was filtered off, washed free of iodate, completely dried in a desiccator and suspended in dry methanol containing 10% of hydrogen chloride. Five hours of heating under a reflux condenser completed the degradation and after the removal of acid as the lead salt the solvent was evaporated. The viscid, levorotatory residue was dissolved in 70 cc. of 0.2 *N* aqueous caustic soda, the solution was almost saturated with sodium chloride and extracted with a total volume of 1500 cc. of diethyl ether. After drying, the extract yielded a pale yellow oil which partly crystallized when alternately cooled in dry-ice and allowed to thaw. The same crystals also separated from the high-boiling fraction of the oil (b. p. 170–180° at 3–4 mm.). Two recrystallizations from ether left the m. p constant at 150–150.5° (corr.) and not depressed by admixture with specimens derived from other starches. The yield of pure material was 0.15 to 0.17 g. for the different starches and either sodium or potassium periodate, buffered to *p*H 4.2 with acetate or phthalate, was used in the preparation.

Anal. Calcd. for $C_{13}H_{16}O_8(OCH_3)_4$: C, 48.11; H, 6.60; OCH_3, 29.24; mol. wt., 424. Found for two independent preparations: C, 48.21, 47.77, 47.75; H, 6.61, 6.59, 6.63; OCH_3, 28.87, 29.09, 29.20; mol. wt. (Rast), 419, 425, 428, 432 for 3–10% solutions.

The substance was sparingly soluble in water and petroleum ether but dissolved more readily in other solvents. The specific rotation at 26° in dioxane was −7.1° (sodium light, *C*, 0.911).

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AUTOBIOGRAPHY

The author was born on July 30th.,1915, in the village of Empingham, county of Rutland, England. He was brought to Canada in 1916, and lived in Kingston and Toronto, Ontario, for the greater part of his childhood. In 1930, he moved to Hamilton, Ontario, where his home is at present situated. In Toronto, he attended Kent Public School, and the University of Toronto Schools; in Hamilton, he attended the Westdale Secondary School for two years, returning to the University of Toronto Schools for his final high school year. He matriculated in 1933, and entered Trinity College in the University of Toronto during the same year. He graduated in chemistry in 1937, with the degree of Bachelor of Arts. During 1938, he studied at the Banting Institute, wrote a thesis entitled "The Preparation of Optically Active Fats", and received the degree of Master of Arts. During the summers of 1936 and 1937 he was a research assistant at the Ontario Research Foundation, Toronto, in the Textile Research department. In June, 1938,he went to the Massachusetts Institute of Technology, and for two years was a Teaching Fellow in the Department of Chemistry. He was given a Graduate Scholarship during his final year. He has accepted a position in the Research and Development Laboratories of Canadian Industries Limited, at Beloeil, Quebec.