A STUDY OF CARBOHYDRATE DERIVATIVES

THESIS FOR DEGREE OF M. S.

ORSON D. BIRD

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Experimental part

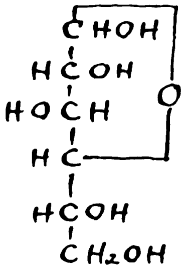
REVIEW OF LITERATURE.

The first recorded attempt to prepare alkyl ethers of the sugars was performed by Berthelot, in 1860, (Ann. Chim. Phys., 60, 103) who heated sugar with caustic potash and ethyl bromide, obtaining a substance which he called diethylglucosan ether. Fischer, in 1893, (Ber., 26, 2400) devised a practical method for alkylating sugars by the direct action of alcohol on the sugar in the presence of hydrochloric acid. But by this method only the terminal carbinol group of the sugar was etherified and the products, which were of a glucosidic nature, easily lost this alkyl group by hydrolysis. Purdie and Irvine (J. Chem. Soc., 83, 1021) were the first to prepare fully alkylated methyl ethers of the sugars which retained the aldehydic and ketonic properties of the parent sugars and resisted the action of hydrolyzing agents. This was accomplished in 1903, and really began the study of the methylated derivatives of sugars about which there has since been much interest shown.

Purdie and Irvine adopted a mixture of methyl iodide and silver oxide for their methylating reagent. Applying this to glucose directly they got no results since the aldehydic group of the glucose acted as a reducing agent and reduced the silver oxide to metallic silver. To avoid oxidation of the aldehydic group of the sugar they then began with a-methylglucoside, prepared after the method of Fischer, and applied their

methylation process to this. They obtained a trimethyl methylglucoside, boiling at 167-170° (17 m.m.) On hydrolysis three of the four methyl groups remained, the product being a trimethyl glucose. A further methylation of this substance in the same manner gave tetramethyl methylglucoside, boiling at 144-145° (17 m.m.). On hydrolysis with hydrochloric acid tetramethylglucose was obtained which distilled at 182-185° (17 m.m.). Recrystallization from ligroin gave crystals which melted at 81-83°.

Sucrose was also methylated by these workers and gave a thick, viscous syrup which on hydrolysis gave a substance identical with the tetra methyl glucose obtained from glucose, as shown by its melting point and analysis. This showed, they pointed out, that the constitution and linkage of the glucose group in sucrose is the same as that of the simple glucoside. They also oxidized tetramethylglucose with bromine water and obtained tetramethyl gluconic acid which had a lactone structure as evidenced by its characteristic reaction toward alkalie. Now lactone formation in the sugar molecule had been assumed by Fischer to take place on the gamma carbon atom so Purdie and Irvine reasoned that their tetramethylglucose formed a lactone, the unmethylated hydroxyl group must be in the gamma position, and therefore that the butylene oxide structure for the glucose residue in sucrose was correct. Hudson, in 1910, (J. A. C. S., 32, 345) working with lactones derived from sugars, proved beyond a doubt by a theoretical consideration of polarimetric data that the butylene oxide structure was correct, thus further substantiating Purdie and Irvine's views. This mass of evidence caused the following formula to be generally accepted for normal glucose:



Before the study of methylated sugars could go very far some reference compounds had to be prepared by which they could be isolated and recognized. Therefore Irvine and Moodie, in 1908, (J. Chem. Soc., 93, 95) prepared the oxime, anilide and chloro- derivatives of tetramethylglucose and also reached the conclusion that tetramethylglucose entered the same reactions as the parent sugar and as the simple glucoside.

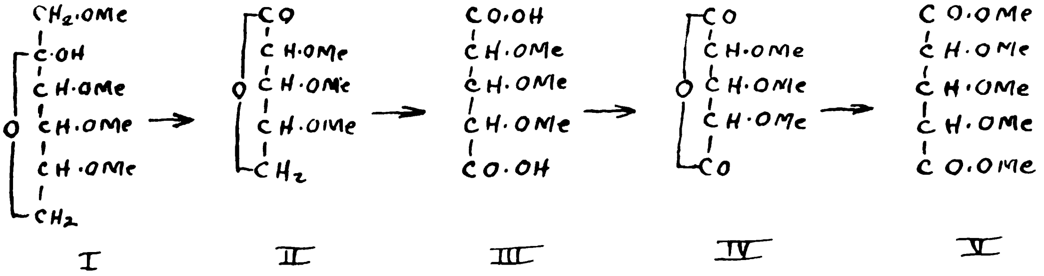
In all the first work done on methylation of carbohydrates the methyl iodide, silver oxide method of alkylation was used but in 1915 Haworth (J. Chem. Soc., 107, 11) developed a much simpler and less expensive process, using dimethylsulphate and sodium hydroxide as methylating agents. This method was applicable to both the reducing and non-reducing sugars directly. In the case of reducing sugars the reaction was carried out at a very low temperature at the start, during which time the glucoside was formed, and later the temperature could

be raised and the remaining hydroxyl groups alkylated. Haworth and Leitch, in 1918, (J. Chem. Soc., 113, 188) used this method of alkylation for an investigation of lactose and melibiose.

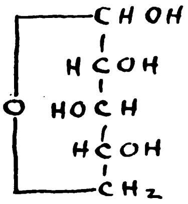
In 1914 Fischer prepared (Ber., 47, 1980) a new methyl glucoside very much greater in activity than the a and b forms previously known. He called this gamma methyl glucoside and concluded that it must have a different internal linking than either a or b forms. Almost simultaneously, that is in 1915, (J. Chem. Soc., 107, 524) Irvine, Fyfe and Hogg reported a similar reactive methyl glucoside which they had discovered while treating glucose with methyl alcohol and hydrogen chloride to form the ordinary glucoside. The compound reacted with acetone while neither a or b forms did so. Also it was very easily hydrolized. They methylated this gamma methyl glucoside with methyl iodide and silver oxide and obtained a tetramethyl gamma methyl glucoside which was purified by distillation. This compound reduced alkaline permanganate in the cold and was hydrolized by N/100 HCl at 40°, conditions which did not affect the normal alkylated glucosides. The product of hydrolysis, called tetramethyl gamma glucose, was not crystalline as ordinary tetramethylglucose, but a liquid, and was laevorotatory.

Continuing the work on active or gamma sugars, Hudson, in 1916 (J. A. C. S., 38, 1223) reported the isolation of an active form of galactose. Haworth, in

1923, (J. Chem. Soc., 123, 294) isolated tetramethyl gamma fructose thru the hydrolysis of methyl sucrose and fractional distillation thereof, and attempted to prove an amylene oxide structure for it. He argued that it should have the structure I (below) since on oxidation it gave trimethoxyvalerolactone II, and then trimethoxyglutaric acid III, which latter was identified as the the anhydride IV, and the dimethyl ester V:

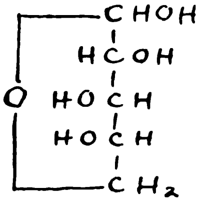


Work on the methyl derivatives of the pentoses was begun by Hirst and Purves in 1923(J. Chem. Soc., 123, 1352). They assigned the amylene oxide structure to the normal form of xylose.

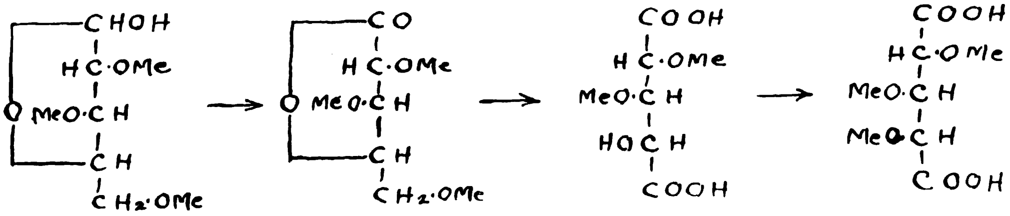


They proved this rather conclusively since the oxidation of normal trimethyl xylose with nitric acid gave a trimethoxyglutaric acid which would be possible only in the presence of an amylene oxide ring.

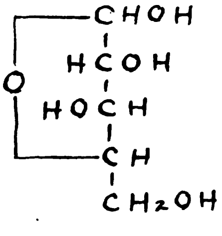
The normal form of arabinose was methylated by Hirst and Robertson in 1925(J. Chem. Soc., 127, 359). They ascribed the amylene oxide structure to it:



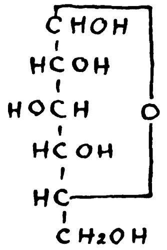
This argument was based on the fact that oxidation of normal trimethylarabinose with nitric acid gave almost quantitative yields of trimethoxy glutaric acid. The trimethyl derivative of gamma arabinose was prepared by Baker and Haworth in 1925, (J. Chem. Soc., 127, 365) and gave on oxidation with nitric acid a laevorotary lactone, which on further oxidation gave a dibasic acid with only two methoxyl groups. This, on complete methylation gave a product identical with Hirst's dibasic methoxy acid:



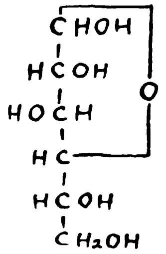
These reactions could be accounted for only by accepting for the active or gamma arabinose the following butylene oxide structure:



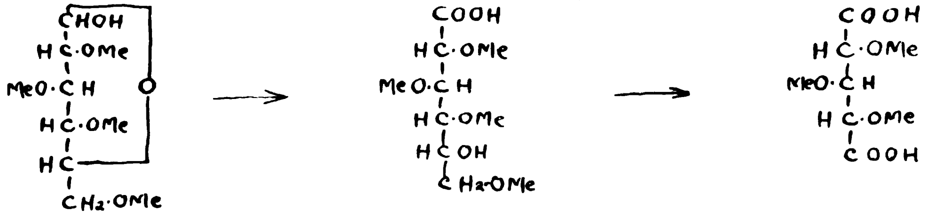
Charlton, Haworth and Peat in 1926, (J. Chem. Soc., 128, 96) summed up all the recent work done on methyl derivatives of the normal and gamma sugars and arrived at a new structural formula for normal glucose, such as is a constituent of sucrose. They observed a parellelism between the change in rotation on hydrolysis of the lactone from normal tetramethylglucose and those from the normal or amylene oxidic forms of galactose, xylose and arabinose. Therefore they ascribed to normal glucose the following amylene oxidic structure:



On the other hand they found that the lactone of tetramethyl gamma glucose corresponded in change of rotation to the lactones of gamma galactose, gamma xylose and gamma arabinose -- all of which have the butylene oxide structure. Therefore they assigned the butylene oxide structure to active or gamma glucose:



The presence of the amylene oxide ring in normal glucose was demonstrated beyond a doubt by Hirst (J. Chem. Soc., 128, 350) who oxidized tetramethylglucose with nitric acid to give xyloltrimethoxyglutaric acid which was identified as the crystalline diamide:



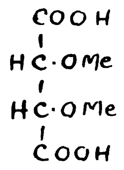
2:3:4:6 tetramethyl glucose.

tetramethylgluconic acid.

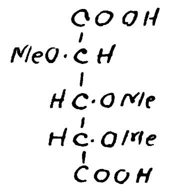
xylol trimethoxyglutaric acid.

The formation of this trimethoxy acid could only be possible assuming an amylene oxide structure for normal glucose.

Normal crystalline tetramethylfructose was oxidized by Haworth and Hirst in 1926 (J. Chem. Soc., 128, 1858) using nitric acid. The product they obtained was a mixture of the following:

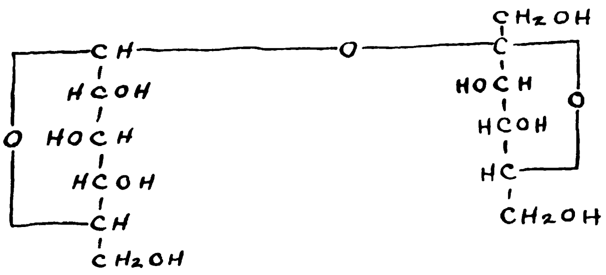


i-dimethoxy-succinic acid.



d-arabotrimethoxy-glutaric acid.

which was possible only upon assuming an amylene oxide structure for normal fructose. This being the case, the authors considered it imperative that the gamma fructose, which is found in the sucrose molecule, must contain the butylene oxide linking, thereby disproving Hawroth's earlier work on gamma fructose when he assigned the amylene oxide ring to gamma fructose. On the basis of this work the correct formula for sucrose would be:



A study of the methyl derivatives of the polysaccharides was begun in 1913 by Denham and Woodhouse (J. Chem. Soc., 103, 1735) who methylated cellulose by adding methyl sulphate to cellulose suspended in a 15% solution of sodium hydroxide. After three successive treatments in this manner a product was obtained having a methoxal content of 22.11%, which gave trimethylglucose on hydrolysis. In a later article (J. Chem. Soc., 111, 244) they report almost quantitative yields of trimethylglucose on hydrolysis of fully methylated cellulose, thus showing the cellulose molecule to be made up entirely of glucose residues.

Glycogen was partially methylated by Macbeth and Mackay in 1924. (J. Chem. Soc., 125, 513) This was accomplished by repeated treatments with sodium hydroxide and methyl sulphate and yielded a product of 37% methoxal content. Further treatments by either methyl sulphate or methyl iodide did not increase the methoxal content. On hydrolysis this partially methylated glycogen yielded trimethylglucose identical with that obtained from methylated cellulose.

Starch was fully methylated by Irvine and Macdonald in 1926, (J. Chem. Soc., 128, 1502) after twenty-four treatments with methyl sulphate and alkali. The final product had a methoxal content of 43.7% which agreed closely with that required for a trimethyl starch. This product on hydrolysis with methyl alcohol and hydrogen chloride gave 2:3:6 trimethyl methylglucoside melting at 57.5°.

EXPERIMENTAL.

Methylation of glucose.

The method of Haworth, (J. Chem. Soc., 107, 11) employing methyl sulphate and a solution of sodium hydroxide as methylating agents, was used exclusively. However, the temperature and time of the reaction and the relative proportions of methyl sulphate and sodium hydroxide were varied. Haworth's general method consists in adding three times the theoretical quantity of methyl sulphate, and sodium hydroxide in excess thru dropping funnels to a solution of the sugar in the minimum of water, the solution being stirred constantly by a mechanical stirrer during the operation. The vessel containing the sugar solution is surrounded by a water bath kept at 70° thruout the reaction, which lasts one hour, then the temperature is raised to 100° for thirty minutes to destroy the excess of methyl sulphate. The product, on cooling, is extracted twice with chloroform, the chloroform distilled off and the residue dried. In methylating the reducing sugars the temperature is kept at 50° during the initial stages of the reaction.

First methylation.

200 cc. of 30% sodium hydroxide and 100 cc. methyl sulphate were added slowly thru dropping funnels to 50 grams of glucose dissolved in the minimum of water, the reaction being kept at 35° until all the reagents had been

added, then it was raised to 100° for 30 minutes. The reaction mixture was distinctly alkaline at the completion of the reaction and some caromelization had taken place as evidenced by the burnt odor. The product was extracted with chloroform, the chloroform distilled off and the resulting dark brown syrup distilled "en vacuo". Approximately 2 cc. of a straw colored liquid was obtained which boiled at 144-146° (17 m.m.), which proved it to be tetramethyl methylglucoside.

Second methylation.

75 grams of sodium hydroxide in 250 cc. of water and 175 cc. methyl sulphate were added in the same manner as before to 50 grams of glucose in water solution, the temperature being maintained at 35° until all had been added then raised to 100° for half an hour. The reaction mixture remained neutral to litmus until after the final heating when it became just slightly acid. There was no caromelization and the resulting syrup after extracting with chloroform was a light golden color. After distilling off the chloroform 34.5 grams of the syrup remained. This was distilled at 24 m.m. and came over at 150-180°, there being no definite boiling point.

Hydrolysis of methylated glucose and determination of methoxal content.

The methylated methylglucoside obtained in the second methylation was hydrolized after the method used by Purdie and Irvine (J. Chem. Soc., 83, 1021). 3.5 grams of the material was dissolved in 50 cc. of 4% aqueous HCl, to which was added a little charcoal, and this was boiled under the reflux for one hour. The mixture was neutralized with barium carbonate and evaporated to dryness on the water bath. The residue was extracted with ether and the syrup resulting, after evaporating the ether, distilled at 160-190° (17 m.m.), no definite boiling point being evident. Three grams of a straw colored, viscous syrup was obtained. Determination of the methoxal content of the above syrup was made according to Perkin's modification of the original Zeisel method (J. Chem. Soc., 83, 1367). The hydriodic acid used was obtained from Merck & Co., of a sp. gr. 1.72. The alcoholic solution of silver nitrate was prepared according to the method of Pregl, (Die Quantitative Organische Microanalyse, p. 150) by boiling a solution of 20 g. silver nitrate in 500 cc. 95% alcohol under a reflux condenser for three hours and filtering off the separated silver oxide. Results of two determinations were as follows:

1. .0726 gave .2307 AgI = 41.9% OCH_3
2. .1029 gave .3325 AgI = 42.6% OCH_3

The calculated OCH_3 content of trimethylglucose, $C_6H_9O_3(OCH_3)_3$ is 41.89%, which corresponds closely with the above and identifies it as such.

Third methylation.

30 grams of the material from the second methylation above was remethylated using 37 grams sodium hydroxide in 225 cc. water and 87 cc. methyl sulphate. The temperature was maintained at 50-55° thruout the methylation and then raised to 100° for half an hour as before. The resulting product, after having been extracted with chloroform, dried and distilled, gave 27 g. of a water clear syrup boiling at 144° (17 m.m.). Methoxal determination gave the following results:

.1024 gave .4529 AgI = 58.53% OCH_3

The calculated OCH_3 content for tetramethyl methylglucoside is 62%. 27.5 g. of this material was hydrolized by heating with 200 cc. 4% HCl for one hour under a reflux, neutralized with barium carbonate, and evaporated to dryness on the water bath. After extracting with ether and removing the solvent by evaporation, a syrup was obtained which distilled at 180-185° (18 m.m.) to give 15.7 grams of a light straw colored, viscous material, which crystallized out almost completely on standing over night. Recrystallization from ligroin gave 7.83 grams of needle like crystals melting at 79-80°. This identifies the material as tetramethylglucose.

Preparation of the anilide of tetramethylglucose.

The anilide of tetramethylglucose was prepared according to the method of Wolfrom and Lewis (J. A. C. S., 50, 837). 15 cc. of absolute alcohol and 15 cc. redistilled aniline were added to 4.5 g. of the tetramethylglucose crystals and boiled gently under a reflux for two hours. On standing over night most of the material had crystallized out. The crystals were filtered out, washed with a little aniline and the filtrate evaporated "en vacuo" when more crystals separated. The combined crystals were recrystallized from absolute alcohol. 2.8 grams of crystals were obtained which melted at 124-125°.

Methylation of sucrose.

First methylation.

The method of McOwan (J. Chem. Soc., 128, 1737) was used for the methylation of sucrose. This is a modification of Haworth's general method employing more dilute solutions. The method is described as follows:

"165 grams sodium hydroxide dissolved in 550 cc. water and 183 cc. methyl sulphate are added simultaneously to 61 grams sucrose dissolved in the minimum of water. The reaction is kept at 35° during the addition of the reagents then raised to 60° for half an hour, 75° for half an hour, and finally boiled for half an hour. After cooling, the mixture is extracted with chloroform and the chloroform distilled off".

Thirty grams of a very viscous, straw colored syrup was obtained after one methylation by the above method.

Distillation of methylated sucrose.

As the methylated sucrose could not be distilled without decomposition in the ordinary vaccum distilling apparatus used for most organic compounds, a special, all-glass apparatus was constructed from Pyrex glass. All the joints were sealed except one where the material was admitted and a thermometer was introduced. This was closed by an inverted test tube, ground to fit the neck of the flask in which it was set, and containing a suspended thermometer which extended downward into the distilling flask so as to record the temperature at which the material distilled. The ground glass joint was made more air tight by a mercury seal. A McLeod gauge was incorporated in the apparatus, which made it possible to measure pressures below 3 m.m. accurately to 0.1 m.m.

The methylated sucrose described above was distilled in this apparatus and gave 15 grams of syrup distilling at 240-250° (1.6 m.m.). Methoxal determination gave the following results:

1. .1087 gave .3387 AgI = 41.13% OCH_3
2. .1063 gave .3317 AgI = 41.18% OCH_3

This indicated that the methylated sucrose was methylated beyond the penta-methyl stage, which required 37.6% OCH_3, but not quite to the hexa-methyl stage which called for 43.6% OCH_3.

Hydrolysis of partially methylated sucrose.

Twelve grams of the partially methylated sucrose was hydrolized according to the method of Haworth, (J. Chem. Soc., 117, 199) by dissolving in 110 cc. .4% HCl and heating in a water bath at 60° for six hours. Then it was neutralized with $BaCO_3$, evaporated to dryness, extracted with chloroformand dried. Eight and five tenths grams of syrup resulted which was distilled in three fractions as follows:

1.	160-170° (22 M.M.)	.97 g.	41.31% OCH_3.
2.	170-180° (19 M.M.)	3.65 g.	42.39% "
3.	180-190° (16 M.M.)	1.08 g.	42.37% "

These figures indicate the correct OHC_3 content for a trimethyl hexose, which proves that the original once methylated sucrose was hexamethyl sucrose. The large fraction of 3.65 g. was evidently largely trimethylglucose as its boiling point corresponds closely to that recorded for trimethylglucose, namely 180-185° (20 m.m.)

Second methylation of sucrose.

Fully methylated, octamethyl sucrose was prepared by three successive methylations of the sugar, using the same method as outlined above except that in the second methylation the sodium hydroxide was dissolved in 750 cc. of water and in the third methylation in 1000 cc.

cc. of water. After the three methylations 41 grams of syrup was obtained, 61 grams of sucrose having been used. On distillation, 18.3 grams of a straw colored, viscous syrup boiling at 177° (1.5 m.m.) was obtained. Methoxal determinations were as follows:

1. .1258 gave .5036 AgI = 52.84% OCH_3
2. .1236 gave .4911 AgI = 52.53% OCH_3

The theoretical OCH_3 content for octamethyl sucrose is 54.6%.

The success of this complete methylation of sucrose, using only sodium hydroxide and methyl sulphate as methylating agents, evidently depended upon increasing the dilution of the solution for each succeeding methylation. This was accounted for by the fact that highly methylated sucrose was only slightly soluble in a solution of sodium hydroxide but if a large enough volume of the alkalie solution were present, all would be dissolved and consequently acted upon by the methyl sulphate. This was borne out by Haworth, in 1916, (J. Chem. Soc., 117, 199) who reported that octamethyl sucrose could not be prepared by using sodium hydroxide and methyl sulphate alone, due to the insolubility of methylated sucrose in sodium hydroxide solution. Later(1920, J. Chem. Soc., 123, 309) he did prepare octamethyl sucrose with these reagents used in larger excess and in greater dilutions.

Methylation of starch.

Starch was partially methylated by a method reported by Irvine and Macdonald, in 1926 (J. Chem. Soc., 128, 1502). The method was recorded by them as follows:

"Thirty two grams starch were mixed with 150 cc. water and stirred slowly while 160 cc. of 12.5% sodium hydroxide was added. Then 140 cc. of 50% sodium hydroxide and 80 cc. methyl sulphate were run in simultaneously, taking place over a period of three hours and adjusted so as to maintain the alkaline reaction. The liquid was kept at 35° and vigorously stirred during the reaction. After the addition of the above it was heated at 100° for 40 minutes. After cooling, an equal volume of rectified spirit was added, and carbon dioxide gas passed thru the solution for a prolonged period. The precipitated sodium salts were removed by filtration thru linen, and the filtrate caustiously neutralized with dilute sulphuric acid. The liquid was evaporated to a thick syrup and the methylation repeated on this".

These workers obtained fully methylated starch (OCH_3 content: 43.7%) after twenty four methylations. They encountered three distinct stages during the methylations, which they thought almost waranted the existance of three compounds.

	%OCH_3	ratio of OCH_3 to initial OH
I. Dimethyl starch	32.7	6:9
II. Methylated starch	36.3	7:9
III. Trimethyl starch	43.7	9:9

The methylation of starch was carried out according to the above procedure, using the same amounts of material and reagents, but a simpler method of separation of the product was devised. Instead of precipitating and filtering off the sodium salts, then evaporating the filtrate which contained the partially methylated starch, it was found that by neutralizing the solution to litmus directly after the methylation process the methylated starch became insoluble and separated out as a curdy mass, leaving the salts in the solution which could be decanted off. This curdy mass was then emulsified in 12.5% sodium hydroxide and methylation repeated on this, using the same amounts of reagents as before. After eight methylations in this manner a product was obtained which when dried was very brittle but on standing soon took up some water and became plastic. This was purified by dissolving in chloroform and filtering out the insoluble material. After evaporating off the chloroform the residue was dissolved in ether, which upon evaporation left a flaky white powder. Methoxal determinations gave the following results:

1. .1064 gave .2974 AgI = 36.89% OCH_3
2. .1164 gave .3193 AgI = 36.21% OCH_3

These results indicate that the methylated starch obtained corresponds to fraction II. obtained by Irvine and Macdonald, which had an average methoxal content of 36.3%.

Methylation of glycogen.

Glycogen was partially methylated by the method of Irvine and Macdonald (J. Chem. Soc., 128, 1502) as applied to starch. The same changes were made in the method of separation of the product as in the case of metholated starch.

Starting with 32 grams of the material, there resulted, after six methylations and purification of the material with chloroform and ether, 15 grams of a grayish powder which very much resenbled the original substance. Methoxal determinations gave these results:

1. .1406 gave .3616 AgI = 33.94% OCH_3
2. .1240 gave .3236 AgI = 34.44% OCH_3

During the last two methylations the material became so insoluble in sodium hydroxide that is doubtful if any further methylation was taking place.

SUMMARY.

1. Glucose was methylated to the tetramethyl stage in one methylation, using a distinctly alkaline solution and keeping the temperature down to 35°, but considerable caromelization took place and the yield was very small. A good yield of tetramethyl glucose was obtained by two methylations, keeping the reaction just slightly alkaline and the temperature at 35° during the first methylation, and raising the temperature to 50-60° and increasing the alkalinity during the second methylation.

2. Hexamethyl sucrose was prepared by one methylation with sodium hydroxide and methyl sulphate. The product was successfully distilled at a pressure of 1.6 m.m. and came over at 240-250° with no appreciable decomposition. This product was hydrolized to a trimethyl hexose.

3. Sucrose was fully methylated after three methylations with sodium hydroxide and methyl sulphate, by increasing the dilution of the sodium hydroxide solution for each successive methylation. This distilled smoothly at 177° under 1.5 m.m. pressure.

4. Starch and glycogen were partially methylated, using the same method for both. The starch had a methoxal content of 36.5% after eitht methylations. By the same treatment glycogen had a 34.2% OCH_3 content after six methylations.