given for the benzyl ester-ether. The residual red oil was dissolved in acetone and methanol added till a slight cloudiness persisted. On cooling, 130 mg. of crystals melting 107–108° and giving no depression of melting point with benzyl 3-benzyloxy-5-cholenate was obtained. A small amount of impure 3-hydroxy-5-cholenic acid was recovered from the sodium bicarbonate solution after the steam distillation. In three trials using 0.5 g., 1.0 g., and 2.0 g. of 3-hydroxy-5-cholenic acid and 3 drops of concentrated sulfuric acid, about 120–150 mg. of the benzyl ester-ether was obtained. In one trial in which 10 drops of sulfuric acid were used, tar formation occurred.

The mother liquor from the ester-ether slowly thickened and solidified. The solid after several crystallizations from petroleum ether melted at 81.5–82.5° and was benzyl 3-hydroxy-5-cholenate.

Anal. Calcd. for $C_{31}H_{44}O_3$: C, 80.13; H, 9.55. Found: C, 79.99; H, 9.32.

In an attempt to prepare the benzyl ether by the action of benzyl chloride on the potassium alcoholate of methyl 3-hydroxy-5-cholenate in the dimethyl ether of ethylene glycol, only 3-hydroxy-5-cholenic acid could be isolated. A similar attempt in liquid ammonia, using sodium, likewise resulted in recovery of the free acid. When methyl 3-hydroxy-5-cholenate was treated with benzyl chloride in pyridine, under conditions similar to those used for tritylation (see below), no benzyl ether was obtained.

Methyl 3-Triphenylmethoxy-5-cholenate.—To 3.0 g. of methyl 3-hydroxy-5-cholenate was added 3.0 g. of trityl chloride and 5 ml. of anhydrous pyridine in a flask previously dried by a stream of dry hot air. This reaction was quite sensitive to traces of moisture and it was found most essential to have perfectly dry reagents and reaction vessels. The mixture was heated on a steam-bath for seven to eight hours under a condenser protected by a drying tube. On cooling, crystals formed in the flask. The reaction mixture was diluted with ice and extracted with ether. The ether solution was successively washed with water, 2% hydrochloric acid, half-saturated solution of sodium bicarbonate and water, ice being used throughout the washings. The ether was dried and removed. The pale yellow sirupy residue was taken up in acetone, methanol added to cloudiness and set aside. The blunt white rods which separated weighed 3.95 g. and melted 146–147°. A second crop of crystals was obtained, giving a total yield of 4.15 g. (86%). The recrystallized product melted 147.5–149°.

Anal. Calcd. for $C_{44}H_{54}O_3$: C, 83.76; H, 8.63. Found: C, 83.36; H, 8.65.

When 0.3 g. of the above trityl ether was refluxed for two and one-half hours with 2 ml. of glacial acetic acid, 65 mg. of crystals, m. p. 151–153° and giving no depression of m. p. when mixed with methyl 3-acetoxy-5-cholenate, was obtained. Some prisms, m. p. 158–161°, and giving no depression when mixed with trityl alcohol, were also obtained from the sirupy residue.

Summary

Several new esters and ethers of 3-hydroxy-5-cholenic acid are described, among which are the labile benzyl and trityl ethers.

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[CONTRIBUTION FROM THE DIVISION OF PLANT NUTRITION, COLLEGE OF AGRICULTURE, UNIVERSITY OF CALIFORNIA]

The Molecular Constitution of Glycogen and Starch from the Seed of Sweet Corn (*Zea Mays*)

BY W. Z. HASSID AND R. M. McCREADY

Morris and Morris[1] prepared a polysaccharide from the seed of *Zea mays*, Golden Bantam variety, which had the properties commonly associated with glycogen. The polysaccharide gave a red-brown coloration with iodine, its aqueous solution was opalescent, it was resistant to the action of alkali and had a high positive rotation. Since, with the exception of giving a clear solution, the properties of dextrins are similar to those described, these authors also compared the rate of enzymic hydrolysis and the cupric chloride crystallization patterns of this polysaccharide with dextrin from glycogens from animal sources. Using those two characteristics as definite criteria for distinction of dextrin from glycogen, Morris and Morris concluded that the polysaccharide isolated from the corn was apparently identical with glycogen. Although a substance bearing at least a superficial resemblance to animal glycogen is found in some lower plants—fungi, yeasts, bacteria—it has never been reported before in any of the higher plants. This finding is unique, since glycogen is *par excellence* the reserve polysaccharide of the animal world. It was therefore of considerable interest to study further this corn polysaccharide particularly in regard to its molecular constitution.

Haworth, Hirst and their collaborators[2,3,4,5] and also other workers[6] showed that the mole-

(1) D. L. Morris and C. T. Morris, *J. Biol. Chem.*, **130**, 535 (1939).

(2) W. N. Haworth, E. L. Hirst and M. D. Woolgar, *J. Chem. Soc.*, 177 (1935).

(3) W. N. Haworth and E. G. V. Percival, *ibid.*, 2277 (1932).

(4) E. L. Hirst and G. T. Young, *ibid.*, 951 (1939), also 1471 (1939).

(5) W. N. Haworth, E. L. Hirst and F. Smith, *ibid.*, 1914 (1939).

(6) W. Z. Hassid and I. L. Chaikoff, *J. Biol. Chem.*, **123**, 755 (1938); D. J. Bell, *Biochem. J.*, **31**, 1683 (1937).

cules of both starch and glycogen consist of repeating chains of glycopyranose units joined by α-glucosidic linkages between the first and fourth carbon atoms. The repeating chain in starch consists of 24 to 30 glucopyranose units, while in glycogen, it consists of 12 or, in some cases, 18 glucopyranose units. This was found to be true in the case of eight specimens of starches from different plant sources and about the same number of glycogens from various animal sources. Except for the difference in their chain lengths, a remarkably close structural relationship exists between glycogen and starch. Glycogen is attacked by the same plant amylases and, conversely, the animal enzymes, the glycogenolitic systems of the liver and muscle, will convert starch into glucose and lactic acid, respectively. The breakdown and synthesis of these two polysaccharides is accomplished through formation of the same intermediate glucose-1-phosphoric acid ester (Cori ester) by the action of phosphorylase.[7,8]

In the present investigation the polysaccharide described by Morris and Morris as glycogen was isolated together with starch from the Golden Bantam variety of corn, and for purposes of direct comparison the two polysaccharides were simultaneously studied. The starch, when treated with iodine solution gave a blue color similar to potato starch. In general, its properties were similar to those of other starches, except that its solubility in water was greater. It is of interest to note that the starch isolated by Haworth, Hirst and Woolgar[2] from waxy maize grains also was more soluble in water than potato starch. Using Haworth's gravimetric assay of the "end group," which was isolated by hydrolysis of the methylated corn glycogen and methylated corn starch, revealed the presence of 8.96 and 4.57% of tetramethylglucose, respectively. These values correspond to a repeating chain length of 12 glucopyranose units for glycogen and 25 glucopyranose units for starch, which agree well with glycogens and starches isolated from other sources. In view of the similarity of properties of the corn glycogen and its derivatives with glycogens and derivatives from other sources, and because of the fact that it possesses a repeating chain of the same length, the constitution of corn glycogen may be considered similar to that of animal glycogen.

(7) C. F. Cori, *Endocrinology*, **26**, 285 (1940).

(8) C. S. Hanes, *Proc. Royal Soc.*, **B129**, 174 (1940).

Experimental

Extraction of Glycogen and Starch from Sweet Corn.—Seeds of Golden Bantam sweet corn were ground in a flour mill to pass a 50-mesh sieve. Two hundred grams of the ground corn was soaked overnight in a liter of water under toluene, the water decanted and the material extracted five times with 1500-cc. portions of cold water with the aid of a mechanical stirrer each time for two hours. After each operation the extract was decanted, except for the last one, which was filtered through a cloth. The combined extract was concentrated to about 1200 cc. by boiling, and filtered through a cloth. using diatomaceous silica, "Hyflo-Super-Cel." One and a half volumes of 95% alcohol was added to the filtrate and the precipitate allowed to settle overnight. The main portion of alcohol was decanted and the precipitate filtered and dried *in vacuo* at 80°. The dry precipitate, consisting of a mixture of starch and glycogen, was dissolved in 10 parts of water and then 2 volumes of glacial acetic acid was added. The mixture was stirred and allowed to remain overnight in the ice box. The starch, which precipitated in 66% acetic acid, was collected on a Büchner funnel and sucked free of acetic acid. The 66% acetic acid filtrate was set aside for further isolation of glycogen. The starch was dissolved in water, adjusted with sodium hydroxide to *p*H 6.5 and reprecipitated by the addition of 1.5 volumes of alcohol. It was then filtered, washed with alcohol and dried *in vacuo* at 80°. The yield of starch was from 10 to 15% of the dry seeds.

The glycogen, soluble in the 66% acetic acid filtrate, was precipitated by the addition of a half volume of alcohol, and the mixture allowed to remain overnight at 0°. The glycogen was filtered, sucked free from the acetic acid–alcohol mixture, dissolved in water and adjusted with sodium hydroxide to *p*H 6.5. It was then reprecipitated with 2 volumes of alcohol, filtered, washed with alcohol and dried *in vacuo* at 80°. The yield of glycogen was from 3 to 5% of the dry corn.

Properties of Corn Glycogen.—The glycogen was soluble in cold water and formed an opalescent solution. When treated with iodine it gave a reddish-brown color. It did not reduce Fehling solution but, when oxidized with ferricyanide, gave a reducing value of 1.4% calculated as maltose.[9] Its specific rotation (*c*, 0.2) in water was $[\alpha]_D$ +190°; its N content, 0.17%.

Properties of Corn Starch.—The starch was soluble in cold water and was more opalescent than the glycogen. When treated with iodine solution it gave a blue coloration similar to potato starch. It had no action on Fehling solution and its reducing value with ferricyanide was 1.5% calculated as maltose. Owing to the opalescence of the solution, the specific rotation of the starch could not be carried out in water and was therefore made in sodium hydroxide. Its specific rotation (*c*, 0.5) in 0.5 *N* sodium hydroxide was $[\alpha]_D$ +152°; its N content, 0.07%.

Acetylation of Corn Glycogen.—Twenty grams of the dry corn glycogen was dissolved in one liter of hot water and precipitated by the addition of 2.5 liters of 95% alcohol. The precipitate was collected on a Büchner funnel, washed with alcohol and ether, and the slightly moist glycogen transferred to a flask and stirred mechanically

(9) W. Z. Hassid, R. M. McCready and R. S. Rosenfels, *Ind. Eng. Chem., Anal. Ed.*, **12**, 142 (1940).

TABLE I

HYDROLYSIS PRODUCTS OF METHYLATED CORN GLYCOGEN

Fraction	Wt., g.	$[\alpha]_D$ in water	$\eta^{16}D$	Constant[a]	"Tetra," %	"Tetra," g.	"Tri," g.	"Di," %	"Di," g.
I	1.470	+48.3°	1.4525	(a) 1.4432 (b) 1.4580	37.2	0.547	0.923	...	...
II	2.460	+56.9°	1.4570	(a) 1.4435 (b) 1.4584	9.4	0.232	2.228	...	...
III	2.560	+63.8°	1.4590	(a) 1.4437 (b) 1.5587	..	...	2.560	...	...
IV	0.418	+84.5°	1.4590	....	..	...	0.418	...	...
V	1.869	+82.3°	1.4630	....	..	...	0.971	48	0.898
	8.777					0.779	7.100		0.898

HYDROLYSIS PRODUCTS OF METHYLATED CORN STARCH

I	0.200	+72.0°	1.4459	(a) 1.4436 (b) 1.4590	85.2	0.170	0.030	..	...
II	1.753	+16.0°	1.4554	(a) 1.4422 (b) 1.4565	7.7	0.135	1.618	..	...
III	2.330	+57.2°	1.4584	(a) 1.4435 (b) 1.4585	..	...	2.330	..	...
IV	0.863	+63.3°	1.4584	....	..	...	0.863	..	...
V	1.603	+89.2°	1.4604	....	..	...	1.283	21	0.320
	6.749					0.305	6.124		0.320

[a] (a) and (b) are the $\eta^{16}D$ values of the "tetra" and the "tri" portions, respectively, present in these fractions, as estimated from rotational data.

with 250 cc. of pyridine for twenty-four hours. Two hundred cc. of acetic anhydride was then added gradually with stirring. The mixture was kept at room temperature for twelve hours, and then stirred at 60° for six more hours. The viscous solution was diluted with glacial acetic acid and poured into an excess of cold water. The precipitate was washed with cold water until free of acid and dried *in vacuo* at 80°. The acetylated polysaccharide was soluble in chloroform and acetone. Its specific rotation (*c*,1) in chloroform was $[\alpha]_D$ +173°. The acetyl content, $COCH_3$, was 44.5% (calculated $COCH_3$ content for the triacetate, $(C_6H_7O_5(CH_3CO)_3)_n$, 44.8%).

Methylation of Corn Glycogen.—The triacetate was simultaneously deacetylated and methylated as follows: 16 g. of the acetylated product was dissolved in 200 cc. of acetone, warmed to 55° and treated with 100 cc. of methyl sulfate and 300 cc. of 30% sodium hydroxide, these reagents being added in 10 equal portions at ten-minute intervals with vigorous mechanical stirring. At the end of this operation 200 cc. of boiling water was added and heated on a steam-bath. After evaporation of the acetone the partially methylated material separated out. It was dissolved in acetone and remethylated as before. After eight methylations the product was boiled twice with water, dried, and dissolved in chloroform. The solution was filtered through a Büchner funnel containing a thin layer of talc and the chloroform evaporated to a small volume. The methylated product was then precipitated by addition of low-boiling petroleum ether and dried *in vacuo* at 95°. Its specific rotation (*c*,1) in chloroform was $[\alpha]_D$ +218°. The methoxyl content, OCH_3 was 44.5% (calculated for $(C_6H_7O_2(OCH_3)_3)_n$, 45.6%).

The specific viscosity, $\eta_{sp.}$, at 25° of a 0.4% solution of the methylated corn glycogen in *m*-cresol was 0.09. This corresponds to an apparent molecular weight of 46,000 determined by Staudinger's formula with K_m 10^{-4}.[5]

Hydrolysis of Methylated Glycogen.—Nine grams of the methylated corn glycogen was boiled with 300 cc. of methanol, containing 1.6% of dry hydrogen chloride by weight, for eight hours under a reflux condenser. The hot solution was neutralized with lead carbonate, filtered when cold, and the filtrate evaporated to dryness. The residue was extracted with chloroform and after removal of the chloroform by evaporation, 9.5 g. of material was obtained (91.4% yield). The mixed methylglucosides were fractionally distilled by the method of Hirst and Young[10] from a flask fitted with a vacuum-jacketed fractionating column at a temperature between 100 and 230° and 10^{-4} mm. pressure. The fractions shown in Table I were obtained. In all cases methoxyl determinations were carried out as checks, but these results were not used in calculating the amount of "tetramethyl" derivative.

The relative proportions of the hydrolysis products, represented as percentage of the total recovery, were therefore: 2,3,4,6-tetramethylmethylglucoside, 8.9%; 2,3,6-trimethylmethylglucoside, 81%; dimethylmethylglucoside, 10%. The amount of 0.779 g. of tetramethylmethylglucoside (end group), obtained from a 91.4% yield of the theoretical total methylglucosides, was originally derived from 9.0 g. of the methylated polysaccharide. On this basis, applying a correction, the amount of tetramethylmethylglucoside becomes 0.853, which is equivalent to 0.806 g., or 8.96% tetramethylglucose. Using Haworth and Percival's[3] expression, $y = 236 \times 100/(204\ x + 46)$, in which x is the number of anhydroglucose residues and y the percentage of tetramethylglucose obtained from a fully methylated polysaccharide, for estimating the chain

(10) E. L. Hirst and G. T. Young, *J. Chem. Soc.*, 1247 (1938).

length, this proportion of "end group" corresponds to a repeating chain length of 12 glucose residues.

Identification of 2,3,4,6-Tetramethylglucose.—Fractions I and II (fission products of methylated corn glycogen, Table I) were combined and again fractionally distilled. The first fraction (0.4 g.) had an index of refraction, $n^{16}D$ 1.4445, showing pure tetramethylmethylglucoside. It was dissolved in 30 cc. of 5% hydrochloric acid and heated on a steam-bath for five hours. The acid solution was extracted with five 25-cc. portions of chloroform and the combined chloroform extract evaporated to a sirup. After adding a few cc. of petroleum ether and stirring, it set to a mass of crystals. Upon recrystallization from petroleum ether containing a trace of ether, fine, white crystalline needles were obtained. This product had a specific rotation (*c*, 0.5) in water $[\alpha]D$ +83°. Its methoxyl content, OCH_3, was 51.8% (calculated for $C_6H_8O_2(OCH_3)_4$, 52.6%).

Identification of 2,3,6-Trimethylglucose.—About 0.5 g. of fractions III and IV (fission products of methylated corn starch) was dissolved in 25 cc. of 5 *N* sulfuric acid, heated on the steam-bath for six hours and neutralized with barium carbonate. The solution was filtered and evaporated to dryness under reduced pressure. The residue was extracted with a mixture of equal parts of ether and benzene, filtered, and evaporated to dryness. After remaining at 0° for twenty-four hours the sirup set to a crystalline mass. Its methoxyl content, OCH_3, was 41.3% (calculated for $C_6H_9O_3(OCH_3)_3$, 41.9%). The specific rotation (*c*, 0.5) in water was $[\alpha]D$ +71°. The initial specific rotation in methanol containing 1% by weight of dry hydrogen chloride (*c*, 0.5) was $[\alpha]D$ +68°, and gradually changed over a period of twenty-four hours to a negative value of −28°. This polarimetric behavior is characteristic of 2,3,6-trimethylglucose.

Acetylation of Corn Starch.—The acetylation was similar to that described for the corn glycogen. The triacetate was soluble in chloroform and acetone. Its specific rotation (*c*, 1) in chloroform was $[\alpha]D$ +175°. The acetyl content $COCH_3$ was 44.8% (calculated content for the triacetate, $(C_6H_7O_5(CH_3CO)_3)_n$, 44.8%.

Methylation of Corn Starch.—The procedure was carried out as described in the case of corn glycogen. After eight methylations the product was precipitated from chloroform by the addition of petroleum ether. The methylated starch had a specific rotation (*c*, 1) in chloroform of $[\alpha]D$ +204°. Its methoxyl content OCH_3 was 44.8% (calculated for $(C_6H_7O_2(OCH_3)_3)_n$, 45.6%). The specific viscosity, $\eta_{sp.}$, at 25° of a 0.4% solution of the methylated starch in *m*-cresol was 0.1. This corresponds to an apparent molecular weight of 51,000, using $K_m = 10^{-4}$.

Hydrolysis of Methylated Starch.—Seven grams of the methylated starch was hydrolyzed with 225 cc. of 1.6% methyl alcoholic hydrogen chloride and the mixed methylglucosides isolated in the manner previously described. The methylglucosides (yield 90.2%) were fractionally distilled at 10^{-4} mm. pressure and 100 to 220° into fractions shown in Table I.

The analyses of hydrolysis products, expressed as percentage of total recovery, were as follows: 2,3,4,6-tetramethylmethylglucoside, 4.5%; 2,3,6 - trimethylmethylglucoside, 90.7%; dimethylmethylglucoside, 4.7%. The amount of tetramethylmethylglucoside, 0.305 g., due to the "end group" becomes 0.338 g., which is equivalent to 0.320 g. or 4.57% tetramethylglucose, taking into account that only 90.2% of the theoretical value of methylglucosides was obtained. This proportion of "end group" corresponds to a repeating unit of chain length of 25 glucose residues and is in agreement with starches isolated from other sources.

The identity of 2,3,4,6-tetramethylmethylglucoside and 2,3,6-trimethylmethylglucoside from the hydrolysis of the methylated starch was established as before by isolation from the respective fractions of crystalline 2,3,4,6-tetramethylglucose and 2,3,6-trimethylglucose.

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Summary

The molecular structure of a polysaccharide, apparently similar to glycogen, and of a starch, both simultaneously isolated from *Zea mays*, Golden Bantam variety, was studied.

The physical and chemical properties of the corn glycogen closely resembled those of animal glycogens. The proportion of tetramethylglucose (end group) isolated from the hydrolysis products of the methylated corn glycogen corresponded to a repeating unit of 12 glucose residues. This agrees with the chain lengths in glycogens isolated from animal sources. The corn glycogen may therefore be considered similar in molecular constitution with animal glycogen.

The repeating chain length in corn starch was found to be 25 glucose residues, thus agreeing with starches previously examined.

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