187. *Wood Starches. Part II.* The Structure of the Sapwood Starch of the Maple* (Acer *Spp.*).

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Maple sapwood starch has been shown to contain *ca.* 19% of amylose. The methylated derivative, of high molecular weight, gave tetramethyl glucopyranose (3·3—3·4%), 2 : 3 : 6-trimethyl glucose (92—93%), and dimethyl glucoses (4—5%) on hydrolysis, corresponding to an average chain length of 26 glucose units for the amylopectin fraction. Periodate oxidation gave a somewhat lower figure (22 units) for this fraction.

In continuation of the investigations on wood starches,* a study has been made of a specimen of maple sapwood starch kindly supplied by Mr. W. G. Campbell, Forest Products Research Laboratory of the Department of Scientific and Industrial Research, Princes Risborough.

The light brown specimen (moisture content 15%; $[\alpha]_D$ +185° in perchloric acid) was purified by extraction with aqueous methanol (85%) which removed *ca.* 2·5% of the dry weight of the sample as a brown oil. Then, after hydrolysis with sulphuric acid (2%), an insoluble residue (10·5%) remained. The starch content, calculated from the reducing value of the hydrolysate, amounted to 86% of the dry weight. Chromatographic examination revealed the presence of glucose (96·4%) and xylose (3·6%), presumably derived from an accompanying xylan, so that the true starch content was *ca.* 84%.

The amylose content of the maple starch was found to be 16·5% (from the blue value), but the more accurate potentiometric titration method (Part I *) gave a value of 19%. Because of the limited supply of starch available, fractionation into amylose and amylopectin was not attempted. It may be recorded, however, that a typical crystalline amylose–butanol complex was isolated from a specimen of oak sapwood starch.

Determination of the proportion of non-reducing terminal groups by periodate oxidation (Brown, Halsall, Hirst, and Jones, *J.*, 1948, 27) gave an average chain length of 22 glucose units, compared with a value of 20 units determined by the same method for elm sapwood starch.

Methylated maple starch was found to have an apparent molecular weight of *ca.* 440 000 by the viscosity method. Hydrolysis of this product gave the following sugars as identified and estimated on the paper chromatogram (Part I *): tetramethyl glucopyranose (3·4%), 2 : 3 : 6-trimethyl glucose (91·6%), 2 : 3-dimethyl xylose from the contaminating xylan, 2 : 3-dimethyl glucose (5%), and a trace of another dimethyl glucose. The average chain length for the amylopectin component of the maple sapwood starch is therefore *ca.* 26 units.

Confirmation of this result and more precise identification of the products of hydrolysis were obtained by separating the products of hydrolysis on the cellulose column (Part I *) whence crystalline tetramethyl glucopyranose (3·3%), crystalline 2 : 3 : 6-trimethyl glucose (92·9%), and syrupy dimethyl glucoses (3·8%) were obtained.

The results of this investigation reveal that maple sapwood starch displays no unusual features, if the presence of xylose in the hydrolysed material is attributed to an associated xylan. The amylose content is similar to that of elm starch, and the fact that the amylopectin component has a larger average chain length is by no means surprising when it is recalled that the values recorded for other plant amylopectins vary from 20 units in maize and banana to 26 units in potato, sweet potato, and arrowroot amylopectins (Brown, Halsall, Hirst, and Jones, *J.*, 1948, 27).

Experimental

General Properties of Maple Sapwood Starch.—The starch was light brown and granular, and had $[\alpha]_D$ +185° (*c*, 1·0 in perchloric acid). The moisture content was 15%. Exhaustive extraction of the dry starch with boiling aqueous methanol (85%; 250 c.c.) in four operations

* Part I, *J.*, 1951, 3489.

removed *ca.* 2·5% of a dark brown oil. All subsequent work was carried out on this extracted material.

Hydrolysis.—The starch (91·6 mg.) was heated for 7 hours at 100° in a sealed tube with sulphuric acid (5 c.c.; 2%) containing acetic acid (2 drops). The undissolved material (9·78 mg.) was removed and the filtrate was neutralised with barium carbonate and diluted to 100 c.c. 5 C.c. consumed 2·47 c.c. of 0·0197N-iodine in the determination of aldose sugars by hypoiodite. From this result and with correction for the insoluble material (10·5%) the starch content of the sample was calculated to be 86·1%. The hydrolysate was deionised and examined on the paper chromatogram with the butanol–ethanol–water–ammonia solvent (Partridge, *Biochem. J.*, 1948, **42**, 238). Quantitative determinations gave 96·4% of glucose and 3·6% of xylose.

Chromatography with the acetic acid–ethyl acetate–water solvent (Jermyn and Isherwood, *Biochem. J.*, 1949, **44**, 402) gave glucose, xylose, and a faint trace of arabinose.

Periodate Oxidation.—Starch (0·2430 g.) in water (52·5 c.c.) containing potassium chloride (2·5 g.) was shaken in the dark with sodium periodate (7·5 c.c.; 0·293M) (Halsall, Hirst, and Jones, *J.*, 1947, 1427). The formic acid released was titrated after 168 hours (10 c.c. required 0·90 c.c. of 0·00943N-sodium hydroxide) and 192 hours (0·93 c.c.). The release of formic acid after 168 hours corresponds to 21·9 glucose units per end group, after correction for the amylose and the non-carbohydrate material. On the assumption that 5% of lignin was present, and with a correction for the acidic material produced on periodate oxidation, the chain length would be *ca.* 27 glucose units on the basis of the experiments described previously (Part I, *loc. cit.*).

Determination of the Amylose Content.—(a) *Colorimetrically.* Higginbotham and Morrison's method (*J. Text. Inst.*, 1949, **40**, T208) gave a blue value of 0·210. Based on a blue value of 1·40 for amylose this corresponds to an amylose content of 16·5% after correction for the non-starch components of the mixture.

(b) *Potentiometrically.* The method described previously (Part I, *loc. cit.*) was used on a sample (40·97 mg.): 100 g. of starch combined with 3·70 g. of iodine, corresponding to an amylose content of 19·0%.

Methylation of Maple Starch.—The specimen (4 g.) was dispersed in water (30 c.c.) and to the rapidly stirred suspension, in an atmosphere of nitrogen at room temperature, sodium hydroxide (14 c.c.; 30%) was added during 2 hours. With continued stirring, further alkali (50 c.c.) was added dropwise simultaneously with methyl sulphate (28 c.c.) in portions of 10, 10, and 8 c.c., at intervals of 20 minutes between each addition. Acetone (20 c.c.) was then added and the methylation procedure repeated with sodium hydroxide (70 c.c.) and methyl sulphate (28 c.c.). Acetone (100 c.c.) was added and the whole was mixed. Acetone was then removed by distillation but a satisfactory separation of the methylated starch was not obtained. The mixture was therefore cautiously neutralised with sulphuric acid and dialysed. One-quarter of this material was concentrated to a small volume and subjected to twelve more methylations. The product, isolated in the usual way (Part I, *loc. cit.*), was a greenish material (1·01 g.), soluble in acetone and chloroform and having $\eta_{sp.}^{20}$ 1·4 (*c*, 0·40 in *m*-cresol) corresponding to a molecular weight of 436 000 for the unmethylated starch ($Km = 1·3 \times 10^{-4}$) (Hirst and Young, *J.*, 1939, 1471) (Found: OMe, 43·5%).

The methylated product was fractionated by successive extractions under reflux with mixtures (50 c.c.) of light petroleum (b. p. 60—80°) and chloroform in the following proportions: 95 : 5, 90 : 10, 85 : 15, and 80 : 20. The product dissolved almost completely in the 85 : 15 solvent mixture and the fraction obtained (0·96 g.) was unchanged in methoxyl content and viscosity.

At a later stage, when the presence of a methylated pentose was detected in the hydrolysate of the methylated starch, another fractionation was attempted with the object of removing the methylated pentosan. The methylated starch (0·70 g.) was dissolved in chloroform (15 c.c.), and light petroleum added until the solution became turbid. 0·45 g. of material was precipitated which was boiled with methanol (100 c.c.) and centrifuged. The undissolved material amounted to 0·15 g. and the methanol solution gave 0·30 g. of solid on evaporation. The light petroleum–chloroform solution gave 0·17 g. of product. A portion of each fraction was hydrolysed and examined on the paper chromatogram, but the spot due to the dimethyl pentose was present in all the fractions in approximately constant amount.

Hydrolysis of Methylated Maple Starch and Separation of the Methylated Glucoses.—The methylated starch (0·61 g.) was boiled with methanolic hydrogen chloride for 7 hours, and after removal of the methanol the mixture of glucosides was hydrolysed for 7 hours at 100° with

hydrochloric acid (1%). The acid was neutralised with silver carbonate, and the solution was filtered and treated with hydrogen sulphide. After filtration through " Filter Cel," the filtrate was deionized with " Amberlite " resins and after removal of solvent a colourless syrup (0·62 g.) was obtained. When the syrup was examined on the paper chromatogram (Hirst, Hough, and Jones, *J.*, 1949, 928) and sprayed with aniline oxalate, spots were obtained corresponding to tetramethyl glucopyranose (brown; R_G = 1·00), 2 : 3 : 6-trimethyl glucose (brown; R_G 0·85), 2 : 3-dimethyl xylose (pink; R_G 0·78), 2 : 3-dimethyl glucose (brown; R_G 0·65), and a trace of another dimethyl glucose (brown; R_G 0·59) and 2-methyl xylose (pink; R_G 0·50). The di-, tri-, and tetra-methyl glucose fractions were estimated quantitatively by hypoiodite oxidation in the usual way (Part I, *loc. cit.*) as follows :

Titration (*c.c. of* 0·0101N-$Na_2S_2O_3$)

	(1)	(2)	Difference		Mean
Blank	9·86	9·78	—	—	—
Dimethyl glucose	9·48	9·38	0·38	0·40	0·39
Trimethyl glucose	3·07	3·20	6·79	6·58	6·66
Tetramethyl glucose	9·63	9·54	0·23	0·24	0·235

This gives 3·2% of tetramethyl glucose, 91·4% of trimethyl glucose, and 5·4% of dimethyl glucose (expressed as glucose). After correction for the trimethyl glucose arising from amylose (19%) the proportion of end-group is 3·82% (as glucose), giving an average chain length of 26·2 glucose units. Expressed as wt.% of each methylated glucose (not corrected for amylose) the figures are tetramethyl glucopyranose 3·5%, trimethyl glucose 91·5%, and dimethyl glucose 5·0%.

Separation on a Cellulose Column.—The syrupy hydrolysate (0·61 g.) was added to a column of powdered cellulose (Hough, Jones, and Wadman, *J.*, 1949, 2511), and the column eluted with light petroleum (b. p. 100—120°)–butanol (60 : 40) saturated with water in the usual way. The eluate was collected in 7-c.c. fractions (rate, *ca.* 9 c.c./hour) after the first 100 c.c. of eluate which contained no sugars had been discarded.

Tubes 40—80 which contained the tetramethyl glucose fraction were combined and concentrated. After dissolution in water and treatment with charcoal a partly crystalline material was obtained which on extraction with boiling light petroleum (b. p. 40—60°) gave crystalline tetramethyl glucopyranose (17 mg., 3·3%), m. p. 88—92°, $[\alpha]_D$ +107° (*c*, 1·0 in water; 30 minutes after dissolution), +83·5° (17 hours, constant). The trimethyl glucose fraction (0·475 g., 92·9%) appeared in tubes 260—350. Three recrystallisations from dry ether gave m. p. 95—100°; the m. p. after recrystallisation from butyl acetate was 100—115°, and $[\alpha]_D^{15}$ +70° (*c*, 1·1 in water).

After 500 tubes, the solvent was changed to a 50 : 50 mixture of light petroleum–butanol saturated with water. Dimethyl glucose (19 mg., 3·8%) was collected in tubes 670—770 and had $[\alpha]_D$ +58·5° (*c*, 1·0 in water).

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