CXXXV. THE STARCH AND RELATED POLY-SACCHARIDES OF CERTAIN HARDWOODS.

I. THE PREPARATION AND PROPERTIES OF OAK AND WALNUT STARCH.

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So far as it can be ascertained the presence of starch in the parenchymatous cells of certain hardwoods has never been doubted. At the same time it is frequently admitted that the methods which have been used for its quantitative estimation are in the main unsatisfactory. Further it would appear that the polysaccharide itself has never actually been isolated from woody tissues and subjected to detailed chemical examination. In connection with the biological work [Fisher 1929], which has been in progress in this Laboratory for some years on the feeding habits of *Lyctus* powder-post beetles, it was shown [Campbell, 1929] that the larvae of this insect which bore in the sapwood of oak derive their nourishment from cell contents. Mer [1903] had already indicated that wood which is depleted of its starch is not attacked by *Lyctus canaliculatus* and *Anobium* sp. and this has recently been confirmed by Wilson [1933].

The importance of this biological work has rendered it desirable to enquire into the composition and properties of wood starch, and, if possible, to find reliable methods both of removing it from wood in commercial sizes and of estimating it quantitatively in experimental material. The substance is readily detected in woody tissues by means of iodine-potassium iodide solution, but in view of the absence of knowledge concerning its composition and properties a method has had to be developed for isolating it quantitatively from sawdust. The present communication is confined to a description of the general properties of wood starch and the method adopted for its isolation together with a record of preliminary evidence regarding its composition.

EXPERIMENTAL.

Microscopical examination has confirmed that the optical properties of the grains of starch in the parenchymatous cells of oak sapwood are similar to those of other starches. The grains are generally spherical or ovoid in shape and have a maximum diameter of 6-10 μ . Groups of grains suitable for microscopical examination were obtained free from the parent wood substance as follows. Small samples of dry sapwood sawdust were shaken with ether and, immediately the wood had settled, portions of the supernatant ether were placed on dry slides. When the ether evaporated large numbers of starch grains were found on the slides. In the dry condition the grains were opaque, but under reflected light their surfaces were brightly illuminated. On moistening with cold water the grains appeared suddenly to swell to a slight extent, whereupon they became

Fig. 1. Ray cell of oak sapwood ($\times 1000$) containing starch grains lightly stained with iodine-potassium iodide solution.

Fig. 2. Same cell after immersion in boiling water for 10 minutes.

Fig. 3. Same cell after final staining with iodine-potassium iodide solution.

translucent. Under crossed nicols a distinct black interference cross was discernible at approximately 600 diameters. The grains appeared to be structurally homogeneous and the presence of a hilum could not be detected.

Examination of wood starch in situ.

A small chip of oak sapwood was cut so as to expose a portion of a medullary ray. After moistening in cold water and staining lightly with a dilute iodine-potassium iodide solution a single cell containing upwards of 20 starch grains was brought into focus under the microscope. The individual grains were clearly discernible. After photographing, the chip was immersed in boiling water for 10 minutes and the same cell was brought into focus again. It appeared to be entirely devoid of starch, but on adding a drop of iodine solution the entire cavity of the cell and part of the walls stained a deep uniform blue. These results are illustrated in the accompanying series of photographs (Plate II, figs. 1-3) for assistance in the preparation of which the author is indebted to Messrs S. H. Clarke and W. R. Hutchins.

It would thus appear that the starch of oak wood occurs naturally in parenchymatous cells in the form of minute particles of a gel which is readily dispersed to a sol by hot water. It will be indicated later that under certain conditions it can be dispersed to some extent even by cold water. In the form of a sol the material diffuses out of the wood.

The isolation of wood starch.

The physical properties of the substance suggested that it might readily be removed from a wood meal by extraction with hot water, but early attempts to isolate it in a reasonably pure form from green oak sapwood were unsuccessful owing to contamination by soluble colouring matter, tannins, sugars *etc.* After experimenting with a number of different procedures the following proved to be satisfactory.

The green winter-felled sapwood of oak, previously found to contain starch by the iodine test, was converted to sawdust and dried as rapidly as possible in a current of air. When the moisture content reached approximately 12 % the material was screened and the portion which passed a 50-mesh and was retained by a 60-mesh screen was selected for extraction. Material of finer mesh was found to be unsatisfactory for reasons which will be apparent later. It was found by experiment that air-dry 50-60-mesh sapwood could be extracted with water at temperatures up to 60° in such a manner as to extract practically all the tannin and colouring matter and leave the starch grains apparently intact. Thereafter by extracting with water at 100° the starch could be removed with the minimum of contamination. The method used was as follows.

A series of 250 g. (air-dry) batches of the 50-60-mesh meal were extracted for 3 hours with constant stirring with 3 litres of water in 5-litre flasks maintained at 60° in a water-bath. After the wood had settled, the extract was siphoned off. The process was repeated usually four times until the final extract failed to give a tannin reaction with ferrous ammonium sulphate solution. Each extract was tested for starch with iodine-potassium iodide solution and it was found that while oak sapwood from one source gave no reaction in any of its 60° extracts a sample from a different source gave a definite starch reaction after the first extraction, but no reaction subsequently. So far, it has not been possible to explain this result for even after drying at 105° the second sample still yielded traces of starch at 60°. Examination of samples of the extracted wood meal under the microscope at this stage revealed that the ray cells still retained their starch in the form of grains.

The batches of wood meal were now subjected to a series of 3-hour extractions at 100° using 3 litres of water in each case. The resulting pale yellow colloidal solution, when cold, gave a pronounced starch reaction with iodine. Extraction was continued until the last extract gave no definite starch reaction. On the average four such extractions were sufficient. Microscopic examination of the wood at this stage revealed that all the starch grains had disappeared, but, on staining with iodine, cell walls and cavities here and there were coloured a pale blue. This was especially apparent in cells towards the interior of individual particles of wood. It was found that, if the 100° extractions were continued beyond this stage, another product, the colloidal solutions of which gave a royal purple colour with iodine was obtained. This will be referred to later.

The combined 100° extracts from groups of two batches of the original meal were concentrated *in vacuo* at 25–30° to a volume of 1.5 litres. The resulting dark brown colloidal solution was heated to approximately 70° in the water-bath, and acidified with 30 ml. of glacial acetic acid. An excess of hot 97 % ethyl alcohol was then added. The pale brown flocculent precipitate was allowed to settle and the supernatant alcoholic liquor containing tannin, colouring matter, soluble sugars *etc.*, was siphoned off. The product was then taken up in approximately 750 ml. of hot water and after the addition of a further 30 ml. of glacial acetic acid a further excess of hot alcohol was added. This operation was repeated 4–6 times until all colouring matter was removed, and the product was washed several times by decantation with alcohol until acid-free. It was then transferred to an extraction thimble and subjected to a series of 6-hour extractions with (1) absolute alcohol-dry benzene (1:2), (2) absolute alcohol and (3) dry ether. After removal of excess ether the product was transferred to a tared weighing bottle and the rest of the ether allowed to evaporate at room temperature, care being taken to stir frequently during this last operation. Final drying to constant weight was carried out in an air-oven at 105°. The final product was a pale pinkish buff impalpable powder which gave an intense blue coloration with iodine.

A sample of winter-felled English walnut sapwood was subjected to the same procedure as the oak and a final product of identical appearance was isolated. In the sample of walnut the starch was found to be confined to the outer layers of the sapwood, hence these only were converted. The actual yields obtained from typical extractions are recorded in Table I.

Table I. *Yields of starch from 50–60-mesh sapwood of winter-felled English oak and walnut.*

Results expressed as percentages by weight of original oven-dry wood meal.

	Oak sapwood					Walnut outer sapwood		
	1	2	3	4	5	1	2	3
Moisture content of original wood prior to extraction	11.39	11.25	11.13	11.15	11.14	9.37	8.87	8.87
Total loss in weight	10.62	9.93	10.65	Not determined	Not determined	8.94	10.60	9.17
Yield of starch	1.16	1.06	1.54	1.87	1.26	0.43	0.42	0.43
Average yield of starch	1.38					0.43		

The starch content of Lyctus frass.

It has already been shown [Campbell, 1929] that the frass of *Lyctus* powder-post beetles is so fine in texture that it readily passes a 120-mesh screen. A sample of frass was examined and found to contain traces of starch as revealed by the iodine test. A 200 g. (oven-dry) sample of each of 120-mesh oak sapwood and *Lyctus* frass obtained from a number of specimens of infested oak sapwood were extracted by the procedure outlined above (see Table II).

Table II. *Yields of starch from Lyctus frass and oak sapwood of the same particle size compared.*

	Sound oak sapwood 120-mesh	<i>Lyctus</i> frass from oak sapwood
Dry weight of original material	200 g.	200 g.
Total loss in weight	12.17 %	10.70 %
Yield of starch	0.90 %	0.28 %

The product in both cases was dark in colour and therefore probably contaminated to some extent by other substances. The preparation from material of such fine texture was much more difficult than from the 50–60-mesh material, the yield from the sound wood being lower than the average from 50–60-mesh material (Table I) although both were obtained from the same source. As would be expected the total loss in weight is greater in the fine material, owing to its state of division. It is noteworthy that total loss by exhaustive extraction with water is less in the frass than in the sound wood.

The physical and chemical properties of isolated wood starch.

On exposure to air the dried product from the sapwood of both oak and walnut absorbed moisture. After attaining an equilibrium moisture content of approximately 12 % there was no change in physical appearance. The starch was very slightly soluble in cold water, but more soluble in hot water to give a pale yellow colloidal solution. The solutions gave the characteristic blue colour with iodine which was discharged on heating and regenerated on cooling. There was no reduction of Fehling's solution until after hydrolysis with dilute HCl. When mixed with a little cold water on a glass plate the powder reverted to a brown-coloured gel which set hard on drying. In thin films the dried gel was almost colourless and transparent.

A sample of the powdered starch was dried to constant weight at 105°. After moistening with cold water it was again dried at 105° and yielded exactly the original weight.

It would thus appear that even in the solid form the material retains no more water at 105° than the powder.

Ultimate analyses of the product from the two different species of wood gave the following results.

Oak sapwood starch	Walnut sapwood starch
1. C, 43.95 %; H, 6.50 %	1. C, 44.29 %; H, 6.41 %
2. C, 44.11 %; H, 6.41 %	2. C, 43.96 %; H, 6.44 %
N, 0.12 %	N, 0.51 %
Mineral ash, trace	Mineral ash, 1.13 %

In view of the low yields of walnut material the following exploratory experiments were confined to the material from oak sapwood.

Specific rotation. A clear colloidal solution of the substance could only be obtained with difficulty. 0.4968 g. (oven-dry, 105°) was incorporated with 50 ml. of cold water and allowed to stand for several hours. The mixture was raised to 100°, but even after 4 hours at this temperature a considerable proportion of the starch remained undispersed. The mixture was transferred with a minimum of extra water to an alundum crucible of porosity R.A. 360 and filtered at the pump. The optical rotation of the pale yellow colloidal filtrate

was measured after noting its volume at 20°. The undissolved residue was dried at 105° and weighed; c was found by difference: found $[\alpha]_D^{20} + 164.3^\circ$ ($c = 0.286$). A portion of the solution gave the characteristic blue colour with iodine and did not reduce Fehling's solution.

0.4968 g. (oven-dry 105°) of the substance dissolved in 75–80 ml. of 2 % NaOH in about 2 hours to give a dark brown colloidal solution. This was made up to 100 ml.: found $[\alpha]_D^{20} + 128.8^\circ$ in a 25 mm. tube.

Action of takadiastase on oak sapwood starch. In the following experiments the actions of takadiastase on the soluble form of potato starch (B.D.H. preparation) and on oak sapwood starch were compared. The method used was similar to that used by Widdowson [1931]. Two samples of approximately 0.2 g. of each of the two substances were mixed with 100 ml. of water, heated in a water-bath for 30 minutes and cooled to room temperature. While the potato starch gave a clear solution the oak starch was not all dispersed. Part of it remained suspended as a gelatinous precipitate. 10 ml. of a 1 % solution of takadiastase were added to each sample and after acidifying with acetic acid and adding a little toluene the series was maintained at 38–40° in an air-oven. A blank consisting of 100 ml. of water and 10 ml. of 1 % takadiastase solution was subjected to the same treatment. At regular intervals a portion of one of each pair of samples was tested for starch with an iodine-potassium iodide solution. After 22 hours the potato starch solution gave a pale orange colour with iodine, but the wood starch solution gave a greenish colour. The suspended matter was stained a faint blue. A pale orange colour was only obtained with the wood starch solution after 64 hours at 38–40°. The remaining one of each pair of solutions and the blank were now removed from the oven, raised to boiling to remove toluene and destroy the enzyme and transferred to tared alundum filter crucibles (R.A. 360) with small quantities of water. There was a small residue in each case. This was washed with water and filtered at the pump. The residues were dried at 105° and weighed and the filtrates *plus* washings were examined optically after their volumes had been measured at 20° (Table III). In view of the slow rate of action of takadiastase in small concentrations on wood starch, the experiment was repeated using the modified technique advocated by Denny [1934]. Each sample weighed approximately 0.5 g. To each were added 25 ml. of water in a small conical flask and after heating at 100° for 30 minutes and cooling, 25 ml. of acetate buffer solution at p_H 4.5, 25 ml. of a 4 % takadiastase solution and a little toluene were added. The flasks were plugged with cotton-wool and incubated at 38–40°. A blank without starch was subjected to the same treatment. After 19 hours control solutions gave a pale orange-colour with iodine. The suspended matter in the wood starch solution stained faintly blue. A small amount of suspended matter in the potato starch solution, consisting apparently of the ruptured envelopes of starch grains, also stained blue. The solutions were boiled for 1 minute and filtered, the residues being washed and dried and the filtrates examined as before (Table III). While in the first experiment the takadiastase blank had no detectable rotation, in the second experiment a marked dextrorotation was observed, so that in this latter case the angular readings for the hydrolysed starch solutions were corrected to exclude the amount of deflection due to takadiastase. The observation of Denny [1934] that ordinary pharmaceutical preparations of takadiastase contain reducing substances was confirmed. Even after boiling in the presence of the buffer solution as described above reducing substances were still present: found $[\alpha]_D^{20} + 53.1^\circ$ ($c = 0.89$ by difference). A separate sample of the buffer solution in water was found to be optically inactive.

Table III. *The effect of takadiastase on the soluble form of potato starch and oak sapwood starch respectively at 38–40°.*

	Potato starch			Oak sapwood starch		
	1	2		1	2	
Dry wt. of original sample, g.	0.2396	0.50	0.50	0.1812	0.4937	0.4937
Wt. of takadiastase taken, g.	0.10	1.00 <i>plus</i> acetate buffer at p_H 4.5		0.10	1.00 <i>plus</i> acetate buffer at p_H 4.5	
Duration of hydrolysis, hours	22	19	19	64	19	19
Dry wt. of total residue, g.	0.0045	0.0138	0.0130	0.0351	0.0591	0.0595
Dry wt. of residue from takadiastase control, g.	0.0012	0.0075	0.0075	0.0012	0.0075	0.0075
Dry wt. of starch converted in g.	0.2363 =98.62 %	0.4937 =98.74 %	0.4945 =98.82 %	0.1473 =81.28 %	0.4421 =89.54 %	0.4417 =89.44 %
Final volume of solution in ml. at 20°	144.00	112.0	114.0	128.00	110.5	127.5
Rotation of solution at 20°	+0.10°	+0.74°	+0.73°	+0.08°	+0.71°	+0.63°
Correction for rotation of takadiastase	0.00°	+0.47°	+0.47°	0.00°	+0.48°	+0.41°
Corrected rotation (α) of sugar solution	+0.10	+0.27	+0.26	+0.08	+0.23°	+0.22°
c g. per 100 ml. of sugar solution	0.16	0.45	0.43	0.12	0.40	0.35
$[\alpha]_D^{20}$ of sugar solution	+62.5°	+60.0°	+60.5°	+66.6°	+62.5°	+62.8°

Table IV. *Determination of the starch contents of oak sapwood and of *Lyctus frass* from the same species by means of takadiastase.*

	Sound oak sapwood				<i>Lyctus frass</i> from oak sapwood	
	50–60-mesh (1)	50–60-mesh (2)	60-mesh (1)	60-mesh (2)	120-mesh (1)	120-mesh (2)
Dry weight of original sample, g.	1.8002	1.8002	1.7658	1.7658	1.8203	1.8203
Loss in weight, g.	0.1568	0.1611	0.1903	0.1930	0.0858	0.0871
Correction for residue from takadiastase, g.	0.0019	0.0019	0.0019	0.0019	0.0019	0.0019
Total loss of wood substance, g.	0.1587	0.1630	0.1922	0.1949	0.0877	0.0890
Average loss of wood controls, g.	0.1189	0.1189	0.1400	0.1400	0.0731	0.0731
Loss due to action of takadiastase, g.	0.0398	0.0451	0.0522	0.0549	0.0146	0.0159
Starch as % of oven-dry original sample	2.21	2.55	2.95	3.11	0.80	0.87

Determination of the starch content of wood by means of takadiastase.

A number of experiments were carried out in order to determine the effect of takadiastase on the starch in the parenchymatous cells of oak wood. 10 ml. of a 4 % solution of the enzyme at p_H 4.5 were found to remove all traces of the starch from parenchymatous cells of 2 g. (air-dry) of 60-mesh oak sapwood in 17 hours. The following method was used to determine the starch content of the wood and also of the *Lyctus* frass (Table II) from the same species.

Two 2 g. samples of each material were incorporated with 25 ml. of water in 250 ml. flasks and heated at 100° for 30 minutes. After cooling, 25 ml. of acetate buffer at p_H 4.5 and 10 ml. of 4 % takadiastase were added. A little toluene was now added, the flasks were plugged with cotton wool and incubated at 38–40° for 17 hours. Two further samples were subjected to the same treatment except that 10 ml. of water were substituted for takadiastase solution and a blank was introduced without the "wood flour." After incubation each flask was boiled for 1 minute and the contents filtered with 100 ml. of water. The residues were dried at 105° and weighed (Table IV).

Acid hydrolysis of oak starch.

11.7145 g. (oven-dry) of the starch were hydrolysed with 500 ml. of 2 % sulphuric acid at 100° for 6 hours. A dark brown precipitate formed at an early stage and at the end of the hydrolysis this was removed by filtration, washed with water till free from acid, dried at 105° and weighed. The hydrolysate was neutralised with hot barium hydroxide solution and barium carbonate [Wise *et al.*, 1933]. The inorganic barium salts were removed by filtration and the neutral sugar solution was concentrated *in vacuo* to a stage when a gummy precipitate formed. A small quantity of ethyl alcohol was added and the solution was filtered and concentrated *in vacuo* to a volume of 10–15 ml. Using the method of crystallisation from alcohol recommended by Anderson [1926] four crops of crystals were obtained. The combined yield was washed with alcohol and ether and dried to constant weight *in vacuo*. The yield was 7.2 g. which together with the unhydrolysed residue (6.14 %) and mineral ash (0.24 %) accounted for approximately 66 % of the starting material. The crystals had $[\alpha]_D^{20} + 57^\circ$ (in water), but no definite melting-point. A portion appeared to melt at 134–135° and the remainder at 175° with decomposition. The crystals obviously consisted of a mixture of sugars, but the rotation suggested that glucose was predominant. This was to some extent confirmed by the fact that under the conditions prescribed by Browne [1912, p. 349] a copious precipitate of glucosazone was obtained in 7 minutes.

Attempts to separate the components of the sugar mixture were unsuccessful, and it was therefore decided to examine the original substance for components other than anhydroglucose residues and to investigate the course of its hydrolysis with sulphuric acid polarimetrically.

On distillation with 12 % HCl the substance yielded small quantities of furfuraldehyde. When determined in a series of 1 g. samples by the Tollens method a yield of furfuraldehyde phloroglucide of 4.26 % (average of three determinations) was obtained. The substance also contained a trace of methoxyl. (Found OMe, 0.69 %, average of two determinations.) The Tollens naphthoresorcinol test for hexuronic acids [Browne, 1912, p. 384] gave a faint but positive result.

The amount of CO₂ given off by the substance on distillation with 12 % HCl was determined by the method of Dickson *et al.* [1930]. This when calculated as

uronic acid anhydride gave a result of 4.65 % (average of six determinations). On the usual assumption [Lefèvre and Tollens, 1907] that uronic acid anhydride residues yield in this way one-third of their weight of furfuraldehyde phloroglucide it can be calculated that the original substance may have contained 2.22 % of pentosan (calculated as xylan). It will be seen later that this is unlikely.

Graded hydrolysis of oak starch with sulphuric acid.

A series of 0.5 g. (oven-dry) samples of the original substance was hydrolysed with 100 ml. of 2 % H_2SO_4 at 100° for periods ranging from $\frac{1}{2}$ to 16 hours. At the end of each hydrolysis the unhydrolysed residue was filtered off and washed free from acid with water. The combined filtrate and washings was in each case cooled to 20° and examined for optical rotation before and after neutralisation with powdered barium carbonate (Table V).

Table V. *The influence of time of hydrolysis of oak sapwood starch with 2% H_2SO_4 at 100° on the specific rotation of the hydrolysis products in acid and neutral solution respectively.*

Duration of hydrolysis in hours	Residue %	$[\alpha]_D^{20}$ in acid solution	$[\alpha]_D^{20}$ in neutral solution
0	0	—	+164.3°
$\frac{1}{2}$	6.92	+155.6°	+159.5°
1	6.22	+124.4°	+124.4°
2	5.07	+88.4°	+116.3°
4	4.12	+62.6°	+46.1°
6	4.69	+50.6°	+59.0°
8	4.33	+50.8°	+58.8° unchanged after 12 hours
12	4.23	+63.1°	+63.1°
16	5.47	+64.3°	+64.3° $\rightarrow$ +55.7° after standing 24 hours

A specific rotation-time curve based on the results in Table V is given in Fig. 1.

Fig. 1.

DISCUSSION.

From the experimental evidence cited above it would appear that a reliable method has been found for isolating the starch present in the parenchymatous cells of certain hardwoods in a reasonably high degree of purity. The essential feature of this method is that success or failure depends on the colloidal condition of the wood substance prior to extraction with water. The wood starch grain is readily dispersed in water. It can be removed from the green wood in the form of a sol by warm water. If, however, the moisture content of the wood substance as a whole be rapidly reduced to a value of 12 % or less the partially dehydrated starch grains become much more stable towards water. Advantage can be taken of this fact to remove the greater part of certain other water-soluble wood components in the first instance, so that the starch is ultimately obtained in a pure condition. It has already been shown that, during drying, wood substance as a whole undergoes certain changes which are reflected in the results of comparative analyses of green and dried material [Campbell and Booth, 1930; 1931; O'Dwyer, 1931], but this would appear to be the first direct evidence of a change in the colloidal condition of a minor wood component due to partial dehydration.

Although the experimental evidence suggests that this important wood component is intermediate in composition between starch and the known wood hemicelluloses, to designate it as other than "wood starch" at this stage would merely lead to confusion. There are some grounds for the belief that both oak and walnut sapwood contain the same substance, although in widely differing amounts, and, from experimental work at present in progress, it would appear that good yields can be obtained from the endosperm of the acorn.

The amounts of starch that can be isolated from the cell cavities of woods (Table I) are surprisingly small, and in the case of oak sapwood it can be seen by comparison of the results in Tables I and IV that estimation by means of takadiastase gives a much higher figure. In this latter method it can be seen that the average particle size of the wood sample has a definite influence on the result. Although it is freely admitted that the yields of starch obtained gravimetrically can only be approximately quantitative the results in Table IV might be taken to indicate a high degree of inefficiency in the method of extraction and precipitation which were adopted were it not for the fact that the cell walls of oak sapwood appear to contain a related polysaccharide. Reference has already been made to the observation that, if the extraction of oak sapwood with water at 100° be continued after all but the last traces of starch have been removed from the cavities of parenchymatous cells, another product, the colloidal solutions of which give a royal purple colour with iodine, can be isolated. For the present, little is known concerning this latter product apart from the fact that it is much more difficult to disperse with water than the starch proper. So far, an amount corresponding to 1.2 % of the dry weight of the original wood has been obtained from oak sapwood but more recently it has been found that the extractions of the wood with water at 100° must be prolonged for a total period of not less than 90 hours after the removal of the starch before the whole of this second product is obtained and it is expected that the yield will be greater. It has been found that part at least of this substance is converted into reducing sugars by takadiastase, but no quantitative data are yet available. The frequent assumption that takadiastase acts on substances other than the starch of the cell contents of wood is therefore apparently justified.

A significant chemical difference between potato starch and wood starch is brought out by the results in Table III. The action of takadiastase is much slower on the latter, but the specific rotations in the second experiment suggest that under optimum conditions the conversion products are the same in both cases. It must, however, be concluded that only approximately 90 % of wood starch can be composed of anhydroglucose units. The specific rotations suggest that the conversion products contain a proportion of maltose in the case of both the potato starch and wood starch. A further point which emerges from the results is that takadiastase itself even after heating to 100° for a short period contains reducing substances. The omission of any correction for the presence of these substances is as Denny [1934] has pointed out certain to lead to high results in the determination of starch when the amounts of the enzyme used are considerable. When the enzyme is used in the proportions suggested by Widdowson [1931], however, no appreciable error appears to be introduced (Table III). It has been seen above that the general accuracy of the determination of the starch content of wood, even by the modified takadiastase method used above (Table IV), cannot be assessed until more is known concerning the related polysaccharides of the cell walls.

Graded hydrolysis of the starch of oak sapwood.

The results in Table V which are illustrated graphically in Fig. 1 afford further important evidence concerning the composition of wood starch. The efficacy of the experimental procedure employed has recently been pointed out by O'Dwyer [1934], who has shown that a definite indication can thereby be obtained of the nature of the acid residues in oak hemicellulose A. It can therefore be deduced from the results (Table V) that, in the course of the hydrolysis of oak starch by dilute sulphuric acid at 100°, there is no appreciable splitting off of acid residues until the 2-hour stage is reached. Since the specific rotation of the hydrolysis products at this stage is higher in neutral than in acid solution, it is reasonable to conclude that the acid residues which are split off are combined with sugar residues in all probability to form an aldobionic acid. As the hydrolysis proceeds the main part of the starch molecule continues to break down to sugars and the aldobionic acid simultaneously breaks down to uronic acid and sugar until a stage is reached (4 hours) at which the predominance of uronic acid is sufficient to lower the specific rotation of the whole after neutralisation. Because of its relative instability towards mineral acids, however, the uronic acid is rapidly broken down to CO₂ and a pentose which ultimately yields furfuraldehyde, until at the 6- and 8-hour stages the aldobionic acid again predominates. No observations were made between 8 and 12 hours, but it is not unlikely that between these times the cycle of events already recorded was repeated. The specific rotations in acid and neutral solution finally become constant after 12 hours' hydrolysis and after 16 hours the specific rotation is reduced to 55.7° after mutarotation. There can be little doubt that the end-product is *d*-glucose.

An interesting dip in the curve (Fig. 1) for the acid solution is observed between 6 and 8 hours. This is no doubt explained by the formation of xylose from the uronic acid. Incidentally it can be deduced from this curve that after 6 hours' hydrolysis with 2 % sulphuric acid the hydrolysis products are likely to consist of a mixture of glucose, maltose, xylose, aldobionic acid and uronic acid, so that the failure, recorded above, to separate the components of such a mixture is largely explained. The specific rotation of +57° obtained for the

products of hydrolysis of the large sample compares favourably with the value of $+59^{\circ}$ (Table V) for the small sample.

The data in Table V suggest strongly that the acid-building units in the original polysaccharide consist of an aldobionic rather than a uronic acid anhydride and that the anhydride in question is probably glucose-glucuronic anhydride. If the figures obtained for the amount of CO_2 given off on distillation with 12 % HCl be recalculated as aldobionic anhydride by means of the factor 7.7 an average result of 8.96 % of the dry weight of the original polysaccharide is obtained. This result, taken in conjunction with the results of the takadiastase experiments (Table III), is sufficient to account for 98.45 % of the original polysaccharide. Since, however, the yield of furfuraldehyde phloroglucide obtained from aldobionic acid anhydrides in general is not known, the actual percentage yield of the phloroglucide obtained above cannot at present be used to verify the position. No evidence has as yet been obtained to show whether the methoxyl groups determined are part of the molecule. For the present it can merely be stated that in all probability the starch of oak sapwood is composed of approximately 90 % of anhydroglucose residues, the remainder consisting of an aldobionic acid anhydride (probably glucose-glucuronic) which may or may not be partly methylated. The course of future work is obvious, and it will be pursued as soon as sufficient supplies of the polysaccharide are available.

*Wood starch as a source of nourishment for the larvae
of powder-post beetles.*

There would appear to be little doubt that the polysaccharide which has been isolated is the substance to which Wilson [1933] has referred as the food of *Lyctus* larvae. The experimental evidence suggests that it contains approximately 90 % of available carbohydrate which can be converted into sugars by enzymic hydrolysis. The author is indebted to his colleague Mr E. A. Parkin for showing that the polysaccharide can also be converted into sugars by the gut enzymes of *Lyctus* larvae. Full details concerning this result will be published elsewhere in due course. If the nitrogen associated with the product is available in the form of protein the food value of the starch will thereby be enhanced although as yet there would appear to be no evidence of the presence of proteolytic enzymes in wood-eating insects [Mansour and Mansour-Bek, 1934]. It is noteworthy that *Lyctus* frass contains apparent starch which amounts to slightly less than one-third of the amount which can be isolated from sound wood of the same particle size (Tables II and IV). This result in itself might be taken to indicate the disappearance of the polysaccharide due to larval activity were it not for the fact that the starch content of woods is normally subject to seasonal variations. It may be argued that the presence of starch in the frass of this insect indicates that part of the wood actually comminuted by the larvae cannot be ingested. If this view be accepted it can readily be calculated from the data in Table II that the proportion of the total borings actually discarded might amount to one-third in some cases. The fact that *Lyctus* frass contains starch at all, however, indicates that the accurate determination of the starch content of a sufficient number of samples from a given species of wood, is certain to yield valuable information concerning the so-called immunity starch level of certain woods [Wilson, 1933].

Having regard to the small amounts of starch which have now been shown to occur in certain woods and also to the variations in composition known to exist between different samples of the same species of wood it can readily be

appreciated why no significant differences in chemical composition could originally be detected between sound oak sapwood and *Lyctus* frass from the same species [Campbell, 1929]. Thus, despite recent criticisms [Wilson, 1933; Mansour and Mansour-Bek, 1934], the contention [Campbell, 1929], based on purely chemical evidence, that the source of larval nourishment is within the cells of the sapwood of oak and not in the cell wall substance, loses none of its force unless and until it be shown that the related cell wall polysaccharide referred to above is also a source of food. It may be repeated therefore that a complete solution of this question from the chemical point of view will only be attained when it is possible to feed the larvae of wood-boring insects on weighed samples of wood of known composition so that the losses in weight due to attack can be determined.

Another aspect of the feeding habits of *Lyctus* larvae merits consideration. It is asserted in some quarters [Fisher, 1928] that the insects only infest recently seasoned timber. It would be of interest to determine therefore whether, during the natural ageing of converted timber, the starch of the cell contents undergoes any chemical changes which might be likely to render it unsuitable to the insect. An early investigation of this question is contemplated.

The relationship of the starch of wood to certain other components.

The chief point of fundamental chemical importance which emerges from this exploratory examination of wood starch is that, until it can be proved to be other than a true chemical compound, this substance must be classified as an acid polysaccharide or hemicellulose. Hemicelluloses of this type have hitherto been considered to be of comparatively rare occurrence. The question naturally arises as to whether the starch of all "starch trees" is of the same composition. Much has been written on the chemistry of wood hemicelluloses and their relation to lignin, but the steps in the formation of these two wood components are still obscure. Buston [1934] has recently indicated after an extensive review of existing evidence that the hemicelluloses of lignified cell walls are probably not derived from pectin. The same author further postulates that because of their chemical composition such hemicelluloses must be derived from hexose polysaccharides. The composition of the wood starch which has now been isolated would appear not only to support this view but to provide a hitherto missing link between the hemicelluloses of the woody cell wall of certain species and the products of the chloroplasts of the leaf. The study of the chemistry of wood starch and the related polysaccharides in the cell walls is therefore being continued.

SUMMARY.

1. The starch present in the parenchymatous cells of certain woods is shown to resemble the known starches in some of its physical properties and to differ from them in others.
2. A method is described for the quantitative isolation of the substance in a high degree of purity from the sapwood of English oak and walnut.
3. Errors introduced into the determination of wood starch by means of takadiastase are discussed.
4. Wood starch is shown to be an acid polysaccharide approximately 90 % of which appears to be composed of anhydroglucose residues. It is suggested that the remainder consists of an aldobionic acid anhydride (probably glucuronic anhydride) which may or may not be partly methylated.

5. It is indicated that the cell walls of oak sapwood contain a polysaccharide which is chemically related to the starch of the cell contents.

6. Wood starch is considered to be a suitable source of nourishment for the larvae of *Lyctus* powder-post beetles.

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