769. *Wood Starches. Part I.**

By W. G. Campbell, J. L. Frahn, E. L. Hirst, D. F. Packman, and [the late] E. G. V. Percival.

The occurrence of starch in wood is discussed, together with methods of isolating and purifying sapwood starches.

The granular starch from elm sapwood has been shown to contain 20·5% of amylose. The methylated starch was hydrolysed and the products separated chromatographically, both on paper strips and on a cellulose column. The yield of tetramethyl glucopyranose (4·3%) indicates that in the amylopectin component there are 20 glucose residues per terminal group. This conclusion is supported by the results of oxidation with periodate.

Because of its characteristic colour reaction with iodine, starch is the most readily recognized reserve foodstuff of trees. It occurs for the most part in the form of simple granules measuring up to 10 μ in diameter and enclosed within specialized cells in leaves, stems, roots, and seeds. In woody stems starch is only rarely found in heartwood. In sapwood, however, it is frequently found in abundance in the parenchymatous tissue, a continuous system of living cells comprising laterally-arranged elements in the rays and vertically-arranged elements surrounding the sap-conducting vessels of hardwoods (wood parenchyma) and the resin-canals of softwoods (epithelium). It is only occasionally found in the lumina of dead cells such as the fibres of hardwoods. According to Phillips (*Forestry*, 1938, **12**, 15) the parenchymatous tissue of hardwoods can account, in some cases, for as much as 30% or more of the total tissue volume. In softwoods the proportion of parenchymatous tissue is, in the average, very much smaller than this. No entirely satisfactory analytical method for accurate determination of the amount of starch present in a sample of wood has so far been evolved. Estimated starch

* This paper was presented in abstract at the International Congress of Pure and Applied Chemistry in New York, September, 1951.

contents recorded in the literature vary from approximately 0·5 to over 5% of the dry weight of total wood substance. This wide range of results is not surprising when it is realized that the starch content of wood is subject to natural fluctuation. In the deciduous trees of the north temperate zone, for example, this fluctuation is seasonal, as demonstrated first by Cockerham [*Proc. Leeds Phil. Soc.* (*Sci.*), 1930, **2**, 64] for the wood of a 20-year old sycamore (*Acer pseudoplatanus*) by microscopical observations of thin sections. In general, starch content is minimal in early summer, and maximal in later summer. During the winter months it declines somewhat, but rises again to a maximum in spring just before the ascent of sap and bud burst.

The isolation of starch from wood for chemical study was first accomplished at the Forest Products Research Laboratory, Princes Risborough, in 1933 (*Rep. Forest Products Research Board*, H.M.S.O., London, 1933, 30; 1934, 31) and the first wood starch preparations from the sapwood of oak and walnut were described by Campbell (*Biochem. J.*, 1935, **29**, 1068) in March 1935. In June of the same year Niemann, Roberts, and Link (*J. Biol. Chem.*, 1935, **110**, 727) presented their well-known paper on the isolation and characterization of a starch polysaccharide from the woody tissue of the apple tree. In all of these preparations and in others which followed, notably those of Sullivan (*J. Assoc. Off. Agric. Chem.*, 1935, **18**, 621) and of Spoehr and Milner (*J. Biol. Chem.*, 1936, **116**, 493), the native starch granules were first rendered soluble *in situ* and recovered in an amorphous form from wood extracts, various devices being used to remove soluble impurities such as tannins, colouring matters, and other polysaccharides extracted from the wood at the same time. It was early realized (Campbell, *Nature*, 1935, **136**, 299; Spoehr and Milner, *loc. cit.*) that prolonged extraction of wood with hot water gives rise to polysaccharide preparations resembling starch in their colour reaction with iodine but yielding uronic acids and xylose on acid hydrolysis in addition to glucose. In other words, such preparations are probably mixtures containing, in part, starch from the lumina of parenchyma cells and polyuronides from cell walls. That the prolonged action of hot water on wood substance brings about considerable chemical breakdown of the major cell-wall components of wood before all of the polysaccharides which stain blue with iodine are removed is shown by the data in the following Table.

Analysis of English oak sapwood (Quercus *sp.*) *before and after prolonged extraction with hot water renewed at 3-hourly intervals.*

(All results are expressed as percentages by weight of original, oven-dried wood.)

	Original wood	Residue after extraction with water: at 60° for 15 h.	at 100° for 27 h.*	at 100° for 117 h.†	at 100° for 141 h.‡
Loss in wt.	—	6·68	11·78	25·10	28·30
Sol. in cold water	4·68	0·29	0·31	0·76	0·12
Sol. in hot water	7·34	2·17	0·92	1·51	0·66
Sol. in 1% NaOH	22·54	15·92	14·87	17·53	16·18
C–B Cellulose [a]	51·46	51·05	50·99	44·88	40·78
Total pentosan	21·51	21·16	20·07	15·58	13·47
Pentosan in cellulose	12·51	11·73	11·68	7·14	5·34
Lignin	20·01	19·48	18·66	15·92	15·67
Total methoxyl	5·83	5·60	5·55	4·44	4·17
OMe in lignin	4·25	4·17	3·91	3·32	3·28
CO_2 as uronic anhydride	4·53	*b*	3·69	2·16	1·68

* Greater part of original starch and some polyuronides recovered from extracts (Campbell, *loc. cit.*).
† Starch and polyuronides recovered from extract (Spoehr and Milner, *loc. cit.*).
‡ Residue no longer gave starch reaction with iodine.
[a] Cross and Bevan cellulose. [b] Not determined.

O'Dwyer (*Biochem. J.*, 1934, **28**, 2116; 1936, **31**, 254) has shown that, even after extensive extraction of oak sapwood with hot water to the point at which neither extract nor residue gives a blue colour with iodine, it is possible to extract with 4% aqueous sodium hydroxide a hemicellulose A which gives such a colour and yields small amounts of glucose on hydrolysis in addition to xylose and a methylated uronic acid. The presence of glucose residues in a hemicellulose extracted from mesquite wood was later established by Sand and Nutter (*J. Biol. Chem.*, 1935, **110**, 17). There may be some grounds, therefore, for the belief that there are close constitutional relations between the cell content starch of wood and the polyuronides of the cell walls, but it is now realized that the possible transformation of starch into

polyuronides and xylan is, on configurational grounds, not explicable on any such simple basis as the oxidation followed by decarboxylation *in situ* of primary alcoholic groups at the sixth carbon position of glucose residues (Campbell, *loc. cit.*).

In view of the realization that the true constitution of wood starch would be difficult, if not impossible, to determine on pre-solubilized material extracted from the wood with solvents, means were sought for the isolation of starch in its granular form. A simple technique was eventually evolved and the first 100 g. of grains were isolated from oak sapwood at Princes Risborough in 1939. The grains were of a pale buff colour due to occluded tannin, and the preparation contained a proportion of finely-divided fibrous cell wall material. Bleaching was effected by suspending the grains in chlorine water for 5 minutes, followed by treatment with sulphurous acid, and the products of chlorination were removed by cold 2% aqueous sodium sulphite. This treatment was repeated four or five times until no pink colour appeared after addition of sodium sulphite. Thorough washing with water, acetone, and ether yielded a pure white starch of $[\alpha]_D^{20}$ +154·8° (*c*, 0·47 in 0·1N-sodium hydroxide), containing 8—10% of finely divided cellulosic material. This starch was acid to litmus and could not be freed from acid by repeated washing. It has been suggested by Samec (*Monatsh.*, 1929, **53/54**, 852) that, when sulphurous acid is used in the bleaching of starches, a sulphonic acid group is introduced into the molecule.

Owing to the war, work was interrupted from the Autumn of 1939 until 1946 (*Rep. Forest Products Res. Board*, 1939—1947, 24). It was found later that the acid condition of this early starch preparation was responsible for some hydrolytic degradation during the long period of storage. In 1948, the technique for isolating granular starch from wood was improved and the structural investigations described below were carried out on fresh material from the elm (*Ulmus campestris*).

Microscopic examination of the elm sapwood starch revealed great variations in the shape of the granules, which ranged from roughly cubic and tetrahedral to spherical and dumb-bell shaped. The larger granules measured up to 10—12 μ along their longer axes. The starch was pale brown, owing to the adsorption of coloured impurities, and, together with a small proportion of fibrous material, probably included lignin, gallotannin, and anthocyanins. The view that lignin and gallotannin at least were merely incidental impurities is supported by the fact that, while some granules appeared not to absorb ultra-violet light of λ about 2800 Å, others manifested varying degrees of absorption. It thus seems that the granules were unevenly contaminated with the impurities, but it is difficult to decide whether these completely impregnated or merely encrusted the granules. We are indebted to Professor H. Erdtman and Dr. P. Lange of the Royal Institute of Technology, Stockholm, who prepared and interpreted the ultra-violet absorption photomicrographs.

The starch content of the air-dried sample of starch (moisture, 12·2; mineral ash, 0·96%) $[\alpha]_D$ +149° (*c*, 0·21 based on actual starch content, in 0·5N-sodium hydroxide) was determined by hydrolysis and paper-strip chromatography. Glucose was the only reducing sugar which could be detected and when estimated quantitatively (Flood, Hirst, and Jones, *J.*, 1948, 1679) accounted for 76·6% of the starch sample. An insoluble, flocculent, brown residue, probably lignin, which remained after hydrolysis of the sample, accounted for a further 4·6% of the total, the remaining 5—6% being represented mainly by the gallotannin impurity. The absence of uronic anhydride residues in the sample was demonstrated (method of Swenson, McCready, and McClay, *Ind. Eng. Chem. Anal.*, 1946, **18**, 290), the yield of carbon dioxide obtained (0·45%) having no structural significance (Campbell, Hirst, and Young, *Nature*, 1938, **142**, 912).

The gallotannin which appeared to be strongly adsorbed on the wood starch lattice was found to interfere seriously with many of the prescribed analytical techniques normally applied to starches. For example, during the dispersion of elm sapwood starch samples in potassium hydroxide solution prior to potentiometric iodine titration (Bates, French, and Rundle, *J. Amer. Chem. Soc.*, 1943, **65**, 142) for the assay of the amylose content, the associated gallotannin was atmospherically oxidized to ellagic acid. After neutralization of the disperison with hydrochloric acid (Wilson, Schoch, and Hudson, *ibid.*, p. 1381) and the addition of potassium iodide a pale purple colour soon developed which slowly intensified during the following hour. This effect was shown to be caused by the gradual liberation of free iodine by the oxidation of potassium iodide by ellagic acid. The lignin impurity also caused the same effect but to a quantitatively minor degree. In preparing the elm sapwood starch dispersions for potentiometric iodine titrations it was essential to remove this liberated iodine otherwise grossly low values for the amylose content would have resulted. This was accomplished

by the careful addition of dilute sodium thiosulphate solution until the purple colour was just discharged. Potentiometric iodine titrations were then conducted in the normal manner. Elm sapwood starch was thus shown to contain 20·5% of amylose. The efficacy of this procedure in providing results of the desired degree of accuracy was demonstrated when results identical within the limits of experimental error were obtained for the amylose content (18·7%) of a sample of barley starch before and after the addition of an authentic sample of gallotannin.

The blue value (0·337) (Hassid and McCready, *J. Amer. Chem. Soc.*, 1943, **65**, 1154) estimated for elm sapwood starch roughly confirms the amylose content as determined by the above modification to the prescribed procedure for potentiometric iodine titration. In the absence of definite figures for the blue values of the pure amyloses and amylopectins of wood starches, calculation of the amylose content from the blue value of elm starch is based on the arbitrarily chosen blue values of 1·35 for amylose and of 0·05 for amylopectin (Haworth, *J.*, 1946, 543). The blue value of 0·337 thus corresponds to an amylose content of 22·2%. An attempted purification of the starch by dispersion in sodium hydroxide solution (2N.) followed by neutralization and precipitation into alcohol yielded a product the blue value (0·311) of which corresponded to an amylose content of 20·2%.

The presence of an amylose component in elm sapwood starch was confirmed by its isolation as the butanol complex in typically spherocrystalline form (Schoch, *J. Amer. Chem. Soc.*, 1942, **64**, 2957). The fractionation procedure recommended by Higginbotham and Morrison (*J. Text. Inst.*, 1949, **40**, 208 T) was employed and the sample of amylose thus obtained was found to absorb 19·6% of its own weight of iodine as determined by potentiometric iodine titration. It is not claimed that this value represents that of the pure amylose component of elm sapwood starch. Further reprecipitations from butanol-saturated water would, no doubt, have increased the capacity of the product to absorb iodine, but continued efforts to purify the amylose were rendered impracticable by the relatively small quantity of wood starch available. However, the value of 19·6% uptake of iodine does at least approach that of 21·4—21·5% determined by Higginbotham and Morrison (*Chem. and Ind.*, 1947, **66**, 45) for pure amyloses from various sources.

The granular starch was methylated directly in the absence of air by the repeated application of methyl sulphate and concentrated sodium hydroxide solution (Hirst and Young, *J.*, 1939, 1471). The value of the methoxyl content of the product could not be increased beyond 44·1% (Calc. for $C_9H_{16}O_5$: OMe, 45·6%) by continued treatment with these reagents followed by fractionation from chloroform solution by the addition of light petroleum. Gallotannin and other coloured impurities had obviously been removed during methylation, to yield a snow-white derivative, $[\alpha]_D$ +208° (in chloroform), $\eta^{20}_{sp.}/c$ 3·0 (in *m*-cresol). Hydrolysis of the methylated starch and separation of the products on a paper chromatogram (Hirst, Hough, and Jones, *J.*, 1949, 928) indicated the presence of (*a*) 2 : 3 : 4 : 6-tetramethyl glucose, (*b*) 2 : 3 : 6-trimethyl glucose, and (*c*) two dimethyl glucoses, as well as faint traces of (*d*) a monomethyl glucose and of (*e*) glucose itself. The measured R_G values of the sugars corresponded reasonably well with the reported values, but confirmation of their identity was obtained by running authentic samples against the hydrolysate. Quantitative estimation of each of the major products of hydrolysis separated on a paper chromatogram was effected by hypoiodite oxidation in sodium hydroxide–phosphate buffer (pH 11·4; Ingles and Israel, *J.*, 1948, 810). The results indicated that of the total weight of the mixed methylated sugars obtained by hydrolysis of the methylated amylopectin component, some 5·3% was represented by tetramethyl glucopyranose, on the assumption that the methylated starch contained 20·5% of methylated amylose. The amylopectin of elm sapwood starch thus appears to contain 20 glucose residues per non-reducing terminal residue.

Confirmation of this result was obtained by separation of the hydrolysis products of the methylated starch, using a cellulose column (Hough, Jones, and Wadman, *J.*, 1949, 2511), two operations being necessary because of the resistance of the dimethyl and trimethyl methylglucosides to hydrolysis. The weights thus estimated of the various methylated sugars recovered from the columns were (from 2·17 g.): 2 : 3 : 4 : 6-tetramethyl glucose, 0·0917 ($\pm$0·0032) g.; 2 : 3 : 6-trimethyl glucose, 1·7534 g.; 2 : 3-dimethyl glucose (?), 0·0886 g.; 3 : 6- and/or 2 : 6-dimethyl glucose (?), 0·1062 g.; monomethyl glucose and glucose obtained as a mixture, 0·0195 g. The total recovery was of the order of 95%. The limiting values (20 $\pm$ 1) for the number of glucose residues per non-reducing terminal residue in the amylopectin of elm sapwood starch were arrived at by calculations based on the upper and lower limiting values for the recovery of 2 : 3 : 4 : 6-tetramethyl glucose together, respectively, with the total actual and total possible recoveries of the methylated sugars from the columns.

The calculations were made on the assumptions that 20·5% of methylated amylose was present in the methylated starch and that the methylated amylose yielded 0·4% of tetramethyl glucopyranose on hydrolysis. Due allowance was also made for the effects of demethylation of tetramethylglucopyranose by the reagents used for the hydrolysis of the methylated starch to the mixture of free sugars. It was found that, under the conditions of hydrolysis, pure tetramethyl glucopyranose yielded 1·7% of trimethyl glucose.

An apparent molecular weight (approx. 5×10^5) for methylated elm sapwood starch was calculated from its viscosity measured in *m*-cresol ($\eta^{20}_{sp.}/c = 3$) (Hirst and Young, *loc. cit.*). An apparent value of about 2×10^5 was similarly calculated for the acetyl derivative (CH_3·CO, 44·9; calc. for $C_{12}H_{16}O_8$, 44·8%), $[\alpha]_D$ +167° (in chloroform), $\eta^{20}_{sp.}$ 0·32 (*c*, 0·47 in *m*-cresol) [by use of Staudinger's equation with the value ($0·93 \times 10^{-4}$) of the constant k_m, given by Staudinger and Husemann (*Annalen*, 1937, **527**, 95)]. Some degradation of the polysaccharide probably occurred in this case, however, owing to the conditions of acetylation employed. Estimation of the reducing end-group of the unsubstituted starch by colorimetric measurement of the products of reduction of 3 : 5-dinitrosalicylic acid (Meyer, Noelting, and Bernfeld, *Helv. Chim. Acta*, 1948, **31**, 103) suggested a molecular weight of about $1·2 \times 10^5$. This value was obtained for the sample of elm sapwood starch from which the impurities had been partly removed by dispersion in sodium hydroxide solution followed by neutralization and precipitation into alcohol. The apparent molecular weight obtained for the sample thus recovered is appreciably lower than the corresponding values of 2×10^5 and $2·5 \times 10^5$ obtained for similarly treated potato and waxy maize starches respectively, but this may have been due to the persistence of a reactive impurity. An attempt was also made to estimate the reducing end-group by hypoiodite oxidation in a buffer medium (pH 10·6), but the results were rendered invalid by the presence of traces of gallotannin which was found to absorb iodine rapidly under the conditions of the experiment. It therefore appeared that, of the methods employed, viscosity determinations of the methyl derivative provided the most reliable data for the estimation of the molecular weight of elm sapwood starch.

It follows, therefore, that elm sapwood starch contains some 20% of amylose, that the amylopectin component possesses a high molecular weight, and that the proportion of non-terminal to terminal glucose residues in the latter component is approx. 20 : 1. Elm sapwood starch is thus constituted similarly in these respects to the majority of starch varieties for which data have been recorded. As with the amylopectins of other starches, it is clear that the amylopectin of elm sapwood starch possesses a branched-chain structure such as the so-called " laminated " structure (Haworth, Hirst, and Isherwood, *J.*, 1937, 578). The relatively high yield (9·5%) of dimethyl glucoses obtained by chromatographic separation of the products of hydrolysis of methylated elm sapwood starch is, however, at first sight inconsistent with such a formula. Only a small proportion of the yield of dimethyl glucoses could have arisen by demethylation of 2 : 3 : 6-trimethyl glucose during hydrolysis of the methylated starch, since pure 2 : 3 : 6-trimethyl glucose yielded only 1·5% of dimethyl glucoses under conditions identical with those used for the hydrolysis. The yield of dimethyl glucoses in excess of that which can be accounted for directly on the basis of a simple branched-chain structure probably arose, therefore, by undermethylation of the starch in the first place.

The periodate oxidation method of determining the proportion of terminal to non-terminal glucose residues in unmethylated starches (Brown, Halsall, Hirst, and Jones, *J.*, 1948, 27) was applied to granular elm sapwood starch. It was found that reliable results consistent with those provided by the end-group assay of the methylated starch were obtained only after the complete removal from the granules of the adsorbed gallotannin since this material was found to react with periodate to produce excessive quantities of acidic material. Removal of gallotannin was effected most efficiently without simultaneous degradation of the starch by repeated batchwise extraction of the granules with boiling 85% aqueous methanol (Schoch, *J. Amer. Chem. Soc.*, 1942, **64**, 2954). An aqueous solution of the extracted material gave reactions to tests applied for the detection of gallotannin. The residual granules retained their buff colour owing, probably, to the persistence of lignin which was also found to react with periodate with the formation of acidic material. However, direct compensation could be made for the quantity of acidic material liberated by the lignin during the periodate oxidation, by isolating a sample of lignin which was obtained as a flocculent, brown residue after complete acid hydrolysis of the starch and treating it with periodate under the same conditions. It was thus demonstrated that the amylopectin component of elm sapwood starch, on oxidation with periodate, liberated acidic material equivalent to one mole of formic acid per 20 glucose units, in agreement with the results of the methylation experiments.

EXPERIMENTAL.

Isolation of Granular Starch from Various Hardwoods.—Sapwood from trees freshly-felled between January and April was first converted into coarse sawdust and air-dried as rapidly as possible at room temperature. Rapid drying is necessary in order to kill the starch-bearing cells, Wilson (*Forestry*, 1935, **9**, 96) having emphasized that, even after a tree is felled, its parenchyma cells continue to live at the expense of their starch foodstuffs. Delay in drying of the wood may thus result in enzymic degradation or actual disappearance of the starch. The sawdust was then screened to remove particles larger than 20 mesh and smaller than 60 mesh. 200-G. batches of 20/60-mesh wood, suspended in 2·25 l. of cold water, were agitated with a high-speed propeller-type stirrer for approx. 45 minutes in order to dislodge the starch grains from open parenchyma cells. The liquor containing the starch grains was filtered off, made up to 2·25 l. with water, and added to a fresh batch of wood. Agitation was carried out as before and the various steps repeated using successive batches of wood up to 4 or 5 in number until a relatively concentrated suspension of grains was obtained. Filtration of this suspension through a Jena G.1, and subsequently a Jena G.2, sintered-glass filter was effective in separating relatively large wood particles and in yielding a starch suspension contaminated by a proportion of fine wood particles. By agitation with an excess of water and then storage, wood particles larger than the starch grains settled out first. When the supernatant liquor appeared to be free from wood, *i.e.*, after examination of samples at frequent intervals under the microscope, it was siphoned off and set aside to allow the starch grains to settle. This process of agitation with water, settling, and siphoning was repeated until all particles which settled more rapidly than starch grains were removed. After final settlement from suspension in water, the starch grains were washed with acetone, collected on a silk filter, washed with ether, and air-dried. Typical yields, on an oven-dried original wood basis, were 1·2% from English oak sapwood (*Quercus* sp.), 0·075% from maple wood (*Acer* sp.), and 0·16% from elm sapwood (*Ulmus campestris*). These yields are, of course, not quantitative, as much of the original starch remained enclosed in unruptured cells. The granular starch from oak contained about 8—10% and the elm starch approx. 7% of fibrous material insoluble in perchloric acid.

The starch grains isolated from the woods referred to above were buff-coloured owing to the occlusion of tannin and of other colouring matter such as anthocyanins which could not be removed by cold organic sovents. The influence of these and other impurities in the constitutional investigation of wood starch will be dealt with later.

Hydrolysis of granular wood starch with 12% hydrochloric acid gave rise to carbon dioxide in yields which in no case exceeded 0·5%. In accordance with previous findings this is without structural significance. It may, therefore, be concluded that the granular wood starches, although contaminated with particles of cell-wall material in loose admixture, are of true cell content origin and, unlike the solubilized starches described previously (Campbell, *Biochem. J.*, 1935, **29**, 1068), they contain no significant proportion of intimately admixed polyuronides derived from wood cell walls.

Properties of Elm Sapwood Starch.—Examination under the microscope revealed that only traces of fibrous material were admixed with the granules. The presence of anthocyanins in the preparation was indicated by treating the granules (0·2 g.) for 30 seconds with boiling ethyl-alcoholic hydrochloric acid (10 ml.; 1%) and then allowing the mixture to cool for 30 minutes. The pink colour which developed was replaced by a green colour on the addition of a slight excess of sodium hydroxide solution (Robinson and Robinson, *Biochem. J.*, 1929, **23**, 35; 1931, **25**, 1687). The gelation point of the starch was 54—55°. Above this temperature it formed with water a pale brown paste (1%) which was rather more mobile than potato starch paste (1%). The granules dispersed with some difficulty in N-sodium hydroxide, to give a solution which was deep brown owing mainly to the presence of gallotannin. The specific rotation, $[\alpha]_D^{19}$ +149° (*c*, 0·21 based on actual starch content, in 0·5N-sodium hydroxide), was determined with difficulty owing to the brown colour of the dispersion. The optical rotation was therefore also measured in solution in perchloric acid: $[\alpha]_D^{17}$ +191° (*c*, 0·51 in 4·8N-perchloric acid), to be compared with potato starch $[\alpha]_D^{17}$ +197° (*c*, 0·49 in 4·8N-perchloric acid). The gallotannin impurity was also responsible for a positive reaction of the starch to Fehling's test. A weak paste of the starch with water gave a deep blue colour with iodine solution. The colour was discharged by boiling the mixture but reappeared on cooling.

Hydrolysis.—The air-dried starch (11·31 mg.) was hydrolysed by heating it at 100° with sulphuric acid (0·5 c.c.; 5%) in a sealed tube for 6—7 hours. After neutralization with barium carbonate, only glucose could be detected on a paper-strip chromatogram. The glucose thus separated was estimated by the method of Flood, Hirst, and Jones (*loc. cit.*), with D-ribose as the reference sugar. The yield of glucose (9·63 mg.) accounted for 76·6% of the air-dried starch sample.

After larger-scale hydrolysis of the air-dried starch (0·1154 g.) with boiling sulphuric acid (10 c.c.; 5%) for 7 hours, a copious, flocculent, red-brown residue was collected on a Gooch crucible and dried to constant weight (5·3 mg., thus accounting for a further 4·6% by weight of the sample). The residue gave a positive reaction to Mäule's test (*Beiträge wiss. Bot.*, 1900, **4**, 166) for lignin. The optical rotation of the filtrate expressed in terms of the specific rotation of the calculated yield of glucose was $[\alpha]_D^{19}$ +51°

The yield of carbon dioxide obtained by decomposing the starch with boiling 12% hydrochloric acid for 4—5 hours (Swenson, McCready, and McClay, *loc. cit.*) was 0·45% by weight of the starch sample used.

The blue value measured at 20° and calculated on the basis of the actual starch content of the sample was 0·337 (Hassid and McCready, *loc. cit.*).

Potentiometric Determination of Amylose.—The method used was essentially that described by Bates, French, and Rundle (*loc. cit.*). The starch (19·94 mg.) was dispersed in potassium hydroxide (10 c.c.; 0·5N.), and the solution neutralized with hydrochloric acid (0·5N.) (Wilson, Schoch, and Hudson, *loc. cit.*). Potassium iodide (10 c.c.; 0·5N.) was added and the solution set aside with occasional gentle stirring for

1 hour. The purple colour which developed was easily discharged by the addition of sodium thiosulphate (5·44 c.c.; 0·001N.) and the volume made up to 100 c.c. with water. Potentiometric iodine titration of the resulting dispersion indicated that the amount of iodine taken up by 1 g. of starch was 0·044 g. If 21·5% is the amount of iodine taken up by pure amylose (Higginbotham and Morrison, *loc. cit.*), the amylose content of the sample of elm sapwood starch was 20·5%.

Separation of Amylose.—Granular elm sapwood starch (1·7 g.) was made into paste with hot water (30 c.c.) and added, with vigorous mechanical stirring, to a mixture of pyridine (30 c.c.) and water (40 c.c.) at 92° (Higginbotham and Morrison, *loc. cit.*). Some difficulty was experienced in dispersing the sample and, after 8 hours' stirring at 92°, there remained a copious, dark-brown residue (0·38 g.) which, moistened with water, gave a blue colour with iodine. Water (100 c.c.) at 90° was added to the hot, clear dispersion after centrifuging and the mixture allowed to cool undisturbed in a Dewar vessel for 6 days. The precipitated brown amylose–pyridine complex was separated at the centrifuge, washed well with cold, butanol-saturated water, and treated as described below. The brown centrifugate was evaporated under reduced pressure at 40° to a volume of 75 c.c., heated with animal charcoal for 20 minutes at 50°, and recentrifuged. The clear dispersion of elm sapwood amylopectin was precipitated into 95% alcohol (1 l.) and the residue after centrifuging dried *in vacuo* over calcium chloride, to give a fluffy, pale brown powder (0·75 g.; blue value, 0·13).

The moist amylose complex was dispersed in butanol-saturated water (200 c.c.) at 90°. The dispersion was centrifuged while hot and allowed to cool during 15 hours from 90° to room temperature in a flask well-lagged with cotton-wool. The precipitated amylose–butanol complex was separated at the centrifuge and the residue washed three times with cold butanol-saturated water. Two further similar reprecipitations involving six washings yielded a pale brown micro-spherocrystalline product (Schoch, *loc. cit.*). The amylose–butanol complex was decomposed by addition of water (40 c.c.) and removal of the butanol at 35°/15 mm. The residual aqueous solution was made up to 50 c.c. with water. The amylose content (0·1912 g., 55%) of the resulting solution was determined by hydrolysis of an aliquot portion with sulphuric acid and estimation of the liberated glucose with Somogyi's copper reagent. The product was not actually isolated in the solid state because of the possibility of retrogradation. Appropriate volumes of the aqueous solution were therefore used for the determination of the following properties of the sample of elm sapwood amylose: $[\alpha]_D^{17}$ +194° (*c*, 0·52 in water); blue value, 1·22; iodine uptake (measured potentiometrically), 19·6%.

Acetylation of Elm Sapwood Starch.—The starch was prepared for acetylation in a manner similar to that described by Haworth, Hirst, and Webb (*J.*, 1928, 2681) for potato starch. The air-dried starch (0·75 g.) was made into a paste with hot water (40 c.c.). After cooling, the paste was poured with stirring into 95% alcohol (400 c.c.), and the precipitated starch collected at the centrifuge, washed well with alcohol, and dried *in vacuo* over calcium chloride, to give a fluffy, cream-coloured powder (0·65 g.). The product (0·62 g.) was mixed with glacial acetic acid (6·5 c.c.) through which chlorine gas had been bubbled for 30 seconds. The mixture was stirred for 30 minutes at 20°, and then acetic anhydride (22 c.c. containing an amount of sulphur dioxide roughly equivalent to the chlorine in the glacial acetic acid) was added. Stirring was continued at room temperature for 1 hour, after which the temperature was raised to 55°. After 2—3 hours at this temperature, the starch dissolved, leaving a very fine greenish-brown precipitate suspended in the medium. This was removed at the centrifuge and the clear, colourless supernatant liquid poured with stirring into ice-cold water (1 l.). The precipitated starch acetate was collected at the centrifuge, washed first with water until free from acid, then with alcohol and ether, and dried *in vacuo* over calcium chloride. The white product (0·91 g., 83%) had $[\alpha]_D^{17}$ +167° (*c*, 1·0 in chloroform), $\eta_{sp.}^{20}$ 0·32 (*c*, 0·47 in *m*-cresol) (Found: CH_3·CO, 44·9. Calc. for $C_{12}H_{16}O_8$: CH_3·CO, 44·8%). The acetate was completely and easily soluble in acetone and in chloroform and, on evaporation of the solutions, clear, fragile films were formed.

In a previous experiment, the acetate of oak sapwood starch was prepared after preliminary dispersion of the starch in pyridine as recommended by Pacsu and Mullen (*J. Amer. Chem. Soc.*, 1941, **63**, 1487). Acetylation was effected by treatment with acetic anhydride, and the product was isolated and dried as described above for elm sapwood starch acetate. The brown oak starch acetate thus prepared was insoluble in acetone, chloroform, and *m*-cresol, although in the presence of these solvents the solid swelled considerably to form gelatinous lumps. Because of the unsatisfactory nature of this product it was considered desirable for the acetylation of elm sapwood starch to use the more drastic method described above.

Direct Methylation of Granular Elm Sapwood Starch.—The wood starch was methylated under conditions similar to those used by Hirst and Young (*loc. cit.*) for rice starch. The granules (2·5 g. of actual starch) were made into a smooth cream with water (20 c.c.), and sodium hydroxide (14 c.c.; 30% by wt.) was added. The air in the flask was displaced by nitrogen, a steady stream of the gas being maintained throughout. Sodium hydroxide (56 c.c.; 30%) and methyl sulphate (28 c.c.) were then added gradually at room temperature with vigorous mechanical stirring. After 15 hours' stirring at room temperature the mixture was again treated with sodium hydroxide (70 c.c.; 30%) and methyl sulphate (28 c.c.). A tendency for the mixture to froth was prevented by the addition of decyl alcohol. After a further 15 hours' stirring at room temperature, acetone (50 c.c.) was added and the mixture was evaporated first on the steam-bath at atmospheric pressure and finally at 75°/400 mm. As the acetone evaporated the partly methylated starch separated on the surface of the alkaline solution as an insoluble, spongy mass. The brown liquid was siphoned from beneath the insoluble product and neutralized by the cautious addition of sulphuric acid (50%) with cooling in an ice-bath. The neutral solution was dialysed against running tap-water for 3 days and then evaporated under reduced pressure at 40° to 15—20 c.c. This somewhat viscous solution was added to the bulk of the partly methylated starch dissolved in acetone (30 c.c.), and the product remethylated by the gradual addition of sodium hydroxide (70 c.c.; 30%) and methyl sulphate (28 c.c.). After 15 hours' stirring, the product was isolated as

described above, except that the hot alkaline liquor separated from the insoluble partly methylated starch was discarded on this and subsequent occasions.

The methylation procedure was repeated a further 11 times, the last twice at 40°. The product finally isolated was washed 3 times with boiling water and then dissolved in acetone. The solution was separated from inorganic material at the centrifuge and evaporated to dryness under reduced pressure at 30—35°. The residue was taken up in chloroform (150 c.c.), and the solution filtered through a G.3 sintered-glass filter. The filtrate was evaporated to 80 c.c. and poured slowly with vigorous stirring into light petroleum (800 c.c.; b. p. 60—80°). A pale-yellow oil (2·86 g., 91%) separated which solidified overnight (Found: OMe, 42·9. Calc. for $C_9H_{16}O_5$: OMe, 45·6%).

Fractionation of the Methylated Starch.—The methylated starch (2·85 g.) was dissolved in chloroform (100 c.c.), and light petroleum (b. p. 40—60°) was slowly added with thorough mixing. After the addition of 600 c.c. of petroleum, a pale yellow oil (fraction I) separated which solidified after decantation of the supernatant liquid and the addition of fresh petroleum. The bulk of the methylated starch in solution was precipitated by the addition of a further 100 c.c. of light petroleum, to yield fraction II, a pale yellow oil which solidified similarly. Further slight precipitation occurred on the addition of more light petroleum to a total volume of 1 l. The oily precipitate was combined with the residue obtained on evaporation of the chloroform–light petroleum mixture to dryness. The product (fraction III; 0·08 g.) was discarded.

Fraction I (0·21 g.) (Found: OMe, 43·7%) had $[\alpha]_D^{17}$ +207° (*c*, 0·51 in chloroform), $\eta_{sp.}^{20}/c$ 3·26 (*c*, 0·41 in *m*-cresol), corresponding to an apparent *M* of 494,000 (see Hirst and Young, *J.*, 1939, 1475). Fraction II (2·48 g.) (Found: OMe, 44·1%) had $[\alpha]_D^{17}$ +208° (*c*, 0·88 in chloroform); $\eta_{sp.}^{20}/c$ 3·08 (*c*, 0·45 in *m*-cresol), corresponding to an apparent *M* of 465,000. Fraction I was not further investigated.

Hydrolysis of Methylated Elm Sapwood Starch and Separation of Methylated Glucoses.—(a) *By paper chromatography.* The methylated starch (fraction II; 52 mg.) was hydrolysed at 100° with methanolic hydrogen chloride (1 c.c.; 4%) for 6 hours. The resulting methylglycosides were hydrolysed at 100° with aqueous hydrochloric acid (5 c.c.; 4%) for 6 hours. The hydrolysate was neutralized with silver carbonate and filtered. Silver ions were removed from the filtrate with hydrogen sulphide. After further filtration, the solution was shaken alternately with Amberlite resins IR-100 and IR-4B, and then evaporated to dryness under reduced pressure at 40°. The residual syrup (43 mg.) was dissolved in water (0·1 c.c.), and the components of the syrup were separated on a paper chromatogram and quantitatively estimated (see following Table) by the method of Hirst, Hough, and Jones (*loc. cit.*). The method was modified slightly by conducting the alkaline hypoiodite oxidations of the methylated sugars in the presence of a sodium hydroxide–phosphate buffer (pH 11·4) (Ingles and Israel, *loc. cit.*) in place of the carbonate–bicarbonate buffer (pH 10·6). The qualitative paper chromatograms and the marginal strips of the quantitative chromatograms were developed with aniline oxalate. On the assumption that the methylated starch contained 20·5% of methylated amylose, the figures in the Table indicate that the ratio of non-terminal to terminal groups in the amylopectin component was 20 : 1.

Sugar indicated	Estimated R_G value	Sugar in mixture, % by wt.
2 : 3 : 4 : 6-Tetramethyl glucose	1·0	4·3
2 : 3 : 6-Trimethyl glucose	0·84	86·5
2 : 3-Dimethyl glucose	0·60 } estimated together	9·3
2 : 6-Dimethyl glucose and/or 3 : 6-dimethyl glucose	0·53 }	
Monomethyl glucose	0·29	trace
Glucose	0·11	trace

(b) *On a cellulose column.* The methylated starch (fraction II; 2·17 g.) was boiled with methanolic hydrogen chloride (100 c.c.; 1%) for 7 hours, after which the solution was cooled and cautiously neutralized with silver carbonate. The mixture was filtered and the insoluble residue of silver salts washed well with hot dry methanol. The filtrate and washings were concentrated to a syrup (2·41 g., 96%) which was dried in a vacuum over phosphoric oxide. The syrupy methylglycosides were hydrolysed at 95—100° with hydrochloric acid (100 c.c.; 2%) for 14 hours. After cooling, the solution was neutralized with silver carbonate and filtered, and silver ions were removed with hydrogen sulphide. The solution was filtered through a layer of "Filter Cel" and concentrated under reduced pressure at 40° to a syrup (2·18 g., 92·3%) which partly crystallized during 2—3 days.

A column of powdered cellulose (67 × 2·2 cm.) was prepared and washed as described by Hough, Jones, and Wadman (*loc. cit.*). The solvent was a 1 : 1 mixture of purified light petroleum (b. p. 100—120°) and *n*-butanol, saturated with water. The mixture of methylated glucoses (2·18 g.) from the above hydrolysis was dissolved in this solvent (5 c.c.), and the solution added dropwise to the centre of the top of the column, each drop being allowed to soak in before the next was added. The column was eluted with the solvent, and the eluate was collected in the receiving tubes which were changed automatically at 12-minute intervals. 4—5 C.c. of the eluate were collected in each of 300 tubes. The contents of every tenth tube were concentrated in a glass dish over a water-bath, any residue was dissolved in acetone, and small portions of each solution transferred in chronological order to the starting-lines of paper chromatograms which were then developed in the usual manner. The distribution of the sugars in the tubes was thus determined. The residues in the glass dishes were returned quantitatively to the respective tubes.

Tubes 20—30 contained 2 : 3 : 4 : 6-tetramethyl glucose, and after a gap of 20 tubes, 2 : 3 : 6-trimethyl glucose appeared (50—90). A further gap of 10 tubes was followed by the appearance of an apparently homogeneous dimethyl glucose (100—130), then a mixture of dimethyl glucoses (130—160), followed by an apparently homogeneous dimethyl glucose (160—200). Tubes 200—300 contained no reducing sugars. The column was finally washed with water (500 c.c.), to yield traces of monomethyl glucose and glucose. The eluates were grouped appropriately and the solvent was removed at 35°/20 mm. The residues in each case were dissolved in water and filtered through "Filter Cel." The filtrates were treated with charcoal at 35° for 15 minutes and refiltered through "Filter Cel." The solutions were evaporated at 40°/20 mm. and the residual syrups dissolved in acetone and filtered to remove traces of waxy impurities. After the removal of acetone, the syrups were dried *in vacuo* over phosphoric oxide for several days. The following fractions were thus obtained: (1) tetramethyl glucose (0·1911 g.); (2) trimethyl glucose (1·8032 g.); (3) dimethyl glucose (0·0581 g.); (4) mixed dimethyl glucoses (0·0658 g.); (5) dimethyl glucose (0·0816 g.); (6) monomethyl glucose; and glucose (0·0195 g.).

Fraction (1). The syrup showed no tendency to crystallize during 2 weeks although examination on a paper chromatogram indicated the presence of one sugar only (R_G 1·0) which corresponded to an authentic sample of 2 : 3 : 4 : 6-tetramethyl glucose. Quantitative determination by hypoiodite oxidation indicated the presence of 0·0949 g. of tetramethyl glucose in the syrup, the remainder of the syrup being represented mainly by trimethyl methylgucoside. The methylglucosides in fraction (1) (0·1857 g.) were hydrolysed at 95—100° with hydrochloric acid (17 c.c.; 2%) for 10 hours. The products (0·162 g., 90%) were isolated as described previously for the bulk hydrolysis of the methylated starch. The hydrolysis products were separated by use of the apparatus, method, and solvents previously described. The eluate was collected in a total of 250 tubes, each containing 4—5 c.c. Tubes 20—40 contained tetramethyl glucose. A gap of 20 tubes was followed by the appearance of trimethyl glucose (60—160). Although the remaining tubes (160—250) appeared to contain no reducing sugars, their contents were combined and evaporated to dryness. The residue examined on a paper chromatogram, sprayed with aniline oxalate, provided two spots of roughly equal intensities corresponding with 2 : 3- and 3 : 6-dimethyl glucose. The column was washed with water (500 c.c.) and the eluate evaporated to dryness. No reducing sugars were detected when the residue was examined on a paper chromatogram.

The following fractions were isolated and dried as described previously: (1*a*) tetramethyl glucose (0·0885 g.); (1*b*) trimethyl glucose (0·0682 g.); (1*c*) dimethyl glucose (0·0051 g.).

Fraction (1*a*) (a syrup) crystallized almost completely after the removal of traces of solvent. Examination by paper chromatography indicated the presence of a single substance (R_G 1·0) which corresponded to tetramethyl glucopyranose. Hypoiodite oxidation indicated that the fraction was 97% pure. The crystals (0·0847 g.) were recrystallized twice from light petroleum (b. p. 40—60°) and recovered in the form of long needles (0·041 g.), m. p. 85—87°, not depressed on admixture with an authentic sample of tetramethyl glucopyranose, $[\alpha]_D^{17}$ +96° (initial), +83° at equilibrium (*c*, 0·2 in water) (Found: OMe, 51·9. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%). The total recovery from the hydrolysate of the methylated starch was 91·7 (±3·2) mg., corresponding to one non-reducing end-group in 20 ± 1 residues in the amylopectin component.

Fraction (1*b*) (a syrup) partly crystallized on drying. Paper chromatography indicated the presence of one reducing sugar only, corresponding to 2 : 3 : 6-trimethyl glucose. Hypoiodite oxidation indicated that the fraction was 94·8% pure, $[\alpha]_D^{18}$ +85° (initial), +67° at equilibrium (*c*, 0·5 in water) (Found: OMe, 39·2. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). Fraction (1*b*) (0·031 g.) was dissolved in methanolic hydrogen chloride (10 c.c.; 1%) at room temperature. The rotation of the solution in a 2-dm. polarimeter tube was observed at intervals: $[\alpha]_D^{17}$ +64° (initial), $[\alpha]_D^{17}$ −36° (8 hours, constant).

Fraction (2). This partly crystallized on drying. Paper chromatography indicated the presence of a single sugar corresponding to 2 : 3 : 6-trimethyl glucose. Hypoiodite oxidation indicated that the fraction was 93·5% pure. The crude material (53·5 mg.) was treated with hydrochloric acid (3 c.c.; 2%) at 95—100° for 8 hours. The solution was neutralized and evaporated to a syrup as previously described. Examination of the residue on a paper chromatogram revealed the presence of very faint traces of dimethyl glucoses along with 2 : 3 : 6-trimethyl glucose. Fraction (2) (1·6 g.) was recrystallized twice from dry ether and the sugar recovered in the form of fine needles (0·71 g.), m. p. 113—115° with previous softening, alone or on admixture with an authentic specimen of 2 : 3 : 6-trimethyl D-glucopyranose (Found: OMe, 41·2. Calc. for $C_9H_{18}O_6$: OMe, 41·9%), $[\alpha]_D^{19}$ +98° (initial), +68° at equilibrium (*c*, 1·0 in water), $[\alpha]_D^{17}$ +69° (initial), −42° (final, 14 hours) (*c*, 1·1 in 1% methanolic hydrogen chloride).

Fraction (3). This did not crystallize. Hypoiodite oxidation indicated that the syrup contained 95·6% of dimethyl glucose. The position and colour of the spot on a paper chromatogram sprayed with aniline oxalate corresponded to 2 : 3-dimethyl glucose.

Fraction (4). This did not crystallize. Analyses as in the preceding paragraph indicated 94·8% of dimethyl glucose, of which two were present. These were separated on a paper chromatogram. Analysis by the method of Hirst, Hough, and Jones (*loc. cit.*) indicated that fraction (4) consisted of 53% of 2 : 3-dimethyl glucose. The remainder was probably represented by 3 : 6- and/or 2 : 6-dimethyl glucoses.

Fraction (5). This did not crystallize. Analysis as above indicated 94·3% of dimethyl glucose and the presence of one sugar only, but it is possible that the syrup consisted of 3 : 6- and/or 2 : 6-dimethyl glucoses.

Fraction (6). A paper chromatogram, when sprayed with aniline oxalate, revealed the presence of glucose and another sugar (R_G 0·20) which corresponded to a monomethyl glucose and gave a

red-brown spot. 3-Methyl glucose (R_G 0·19), which gave a brown spot, was used as the "control" sugar.

Partial Demethylation of 2 : 3 : 4 : 6-*Tetramethyl and* 2 : 3 : 6-*Trimethyl Glucoses.*—Samples of the appropriate sugars (50 mg.) were treated first with methanolic hydrogen chloride (1·5 c.c.; 1%) at 100° for 7 hours and then with aqueous hydrochloric acid (4 c.c.; 2%) at 100° for 14 hours. The samples were thus subjected to slightly more drastic treatment than the methylated starch during hydrolysis. The products were isolated in the usual way and the components of each syrup separated on paper chromatograms and determined with buffered hypoiodite. The results are collected in the Table.

	0·01N-$Na_2S_2O_3$ (c.c.).	Methylated sugar, %
2 : 3 : 4 : 6-*Tetramethyl glucose*		
Tetramethyl glucose	2·248	98·3
Trimethyl glucose	0·041	1·7
2 : 3 : 6-*Trimethyl glucose*		
Trimethyl glucose	2·611	98·5
Dimethyl glucose	0·042	1·5

Solvent Extraction of Gallotannin from Granular Elm Sapwood Starch.—It was found, after initial failures to remove the gallotannin impurity from the granules by a variety of methods including solvent extraction using a wide range of solvents, that treatment with boiling 85% aqueous methanol (Schoch, *loc. cit.*) produced the desired effect. Four successive extractions of the granules (0·8 g.) for 4 hours each with fresh batches (50 c.c.) of this solvent removed 6·4% of solid material based on the weight of the air-dried granules. The extracted material in aqueous solution gave the following positive reactions for gallotannin: (*a*) the addition of neutral ferric chloride solution gave a greenish colour, (*b*) a brown colour developed immediately on addition of sodium hydroxide solution, (*c*) a sparse reddish precipitate in the 2 : 2′-dipyridyl test (Feigl, *Ind. Eng. Chem., Anal.*, 1946, **18**, 62).

The residual starch granules retained their buff colour. On hydrolysis with sulphuric acid, a sample of the granules yielded a flocculent, brown residue of lignin-like material which, after being washed and dried, was treated with periodate as described below.

Periodate Oxidation.—(a) *Granular elm sapwood starch extracted with* 85% *aqueous methanol.* The granules (0·3120 g., containing 0·2942 g. of starch and 0·0178 g. of lignin) were suspended in water (52·5 c.c.) containing potassium chloride (2·5 g.), and sodium metaperiodate solution (7·5 c.c.; 0·293M.) was added. The oxidation and subsequent titrations were conducted as prescribed by Halsall, Hirst, and Jones (*loc. cit.*) for the determination of formic acid liberated by periodate oxidation of starches. "Corrected" titres: 1·482 c.c. of 0·01N-sodium hydroxide (172 hours); 1·571 c.c. (192 hours); 1·75 c.c. (268 hours); 1·922 c.c. (314 hours). These correspond with the formation of 3·91 mg. of formic acid after oxidation for 150 hours.

(b) *Lignin isolated after hydrolysis of elm sapwood starch.* The lignin sample (0·0215 g.) was treated with periodate under conditions identical with those described above for the oxidation of granular elm sapwood starch. "Corrected" titres: 0·289 c.c. of 0·01N-sodium hydroxide (167 hours); 0·29 c.c. (185 hours); 0·33 c.c. (262 hours); 0·49 c.c. (424 hours). From the results of this experiment it may be calculated that the lignin-like impurity (0·0178 g.) present in the starch sample oxidized with periodate was responsible for the generation of acidic material equivalent to 0·64 mg. of formic acid after oxidation for 150 hours.

The combined results of periodate oxidation experiments (a) and (b) thus indicate that the amylopectin component of elm sapwood starch liberated 3·27 mg. of formic acid after oxidation for 150 hours. This corresponds to the formation of 1 mole of formic acid per 10 glucose residues in that component.

Some of the work upon which this paper is based formed part of the programme of the Forest Products Research Board and the authors are indebted to the Department of Scientific and Industrial Research for permission to communicate the results.

Thanks are expressed to the Commonwealth Scientific and Industrial Research Organization for a grant (J. L. F.) and to the Distillers Company Limited and Imperial Chemical Industries Limited for grants.

Forest Products Research Laboratory,
Princes Risborough, Bucks.
The University of Edinburgh.

[*Received, August* 17*th*, 1951.]