230. *Pectic Substances. Part VII. The Constitution of the Galactan from* Lupinus albus.

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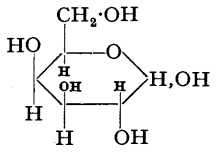
A galactan has been isolated from the pectin component of *Lupinus albus*. This polysaccharide is a linear polymer built up of *d*-galactose residues which, after methylation followed by hydrolysis, yields 2 : 3 : 6-trimethyl *d*-galactose together with a small amount of 2 : 3 : 4 : 6-tetramethyl *d*-galactose from the end group. The amount of this latter sugar corresponds to an average repeating unit of approximately 100 galactose residues. The isolation of this polysaccharide is further evidence that galactan is not converted into araban by a simple process of oxidation followed by decarboxylation of the resultant uronic acid residue.

Most samples of pectin on hydrolysis furnish a mixture of the three sugars *d*-galactose (I), *d*-galacturonic acid (II), and *l*-arabinose (III), and at one time it was considered possible that pectin was a single substance which possessed a cyclic structure built up from these residues. Ehrlich, however, demonstrated that pectin contained an araban as a separate entity, and subsequent work has indicated that at any rate most of the arabinose encountered in pectic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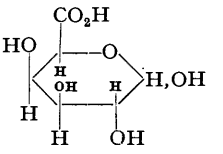
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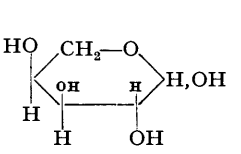
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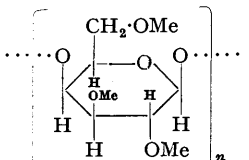
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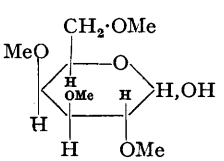
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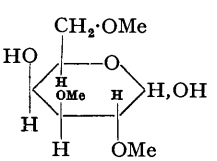
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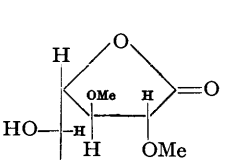

(VII.)

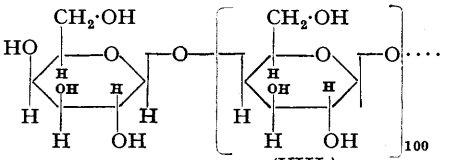
materials is present in the form of this araban component. It seemed probable that the galactose residues also might be present as a separate polysaccharide, and it is shown in the

present communication that the pectic material of the seeds of the white lupin does in fact contain a galactan, which can be separated from the pectic acid and the araban components. Since the conversion of (I) into (III) may proceed in Nature through (II), which then undergoes decarboxylation, it was of considerable interest to examine in detail the constitution of this galactan and to determine whether it was unbranched, as in pectic acid, or branched, as in araban, and thus to decide whether it could be structurally related to either the pectic acid or the araban component of pectin. In the former case the sugar residues should be of the pyranose form and linked through α-linkages, whilst in the latter case the *d*-galactose residues should be of the furanose form.

In common with many other plant materials the seed of the white lupin contains material which on hydrolysis is converted into *d*-galactose and *l*-arabinose (" The Principles of Plant Biochemistry ", M. W. Onslow, Cambridge University Press, 1931 Edtn., p. 72). Professor M. Skene of the University of Bristol had informed us that these seeds were a particularly rich source of pectin, and we investigated their carbohydrate content with the object of isolating the galactan component. We found that the carbohydrate fraction of the seed contained a pectin-like material in which the percentage of uronic acid component and of araban was much lower than any other sample of pectin we had hitherto encountered, and thus enabled us to obtain, in a relatively easy manner, a sample of the galactan. Associated with this galactan there was an araban which could be partly removed by extraction with 70% alcohol and a pectic acid which could be precipitated as calcium pectate. The araban showed the same ease of hydrolysis and high negative rotation as the araban associated with apple, citrus, and peanut pectins. The pectic acid component could be converted into a degraded product (see Morrel, Baur, and Link, *J. Biol. Chem.*, 1934, **105**, 1) which had properties close to those of the corresponding polysaccharides from apple and citrus pectins.

It was inferred, therefore, that we were dealing with a true pectin which was, however, particularly rich in galactan. This galactan, which could not be obtained completely free from adsorbed araban and pectic acid, had $[\alpha]_D$ + 38° in water. This value is low since the material still contained some 19% of pentosan and the corrected figure is therefore more likely to be about + 70°. After methylation with thallium hydroxide and methyl iodide (Menzies, *J.*, 1926, 937; Hirst and Jones, *J.*, 1938, 496) a methylated product was isolated which contained methylated araban and methylated galactan. The small quantity of pectic acid present was very difficult to methylate and was lost during the process (Hirst and Jones, *loc. cit.*). Methylated araban was separated from methylated galactan by extraction of the former from the mixture with ether. The ether-insoluble material had $[\alpha]_D^{20°}$ — 12° in methyl alcohol, this negative value being an indication that in methylated galactan the sugar residues are joined together by β-linkages and that the galactan cannot be directly related to pectic acid in which the sugar residues are joined together by α-linkages. Methylated galactan (IV) was resistant to hydrolysis and the rate of reaction corresponded to that of a typical galactopyranoside. Fractional distillation of the sugars formed on methanolysis of the methylated galactan led to the identification of tetramethyl *d*-galactopyranose (V), isolated as its crystalline anilide, and 2 : 3 : 6-trimethyl *d*-galactose (VI), identified after oxidation as its crystalline furanolactone (VII). The inferences are, therefore, that the galactan is built up of *d*-galactopyranose residues linked through carbon atoms 1 and 4 and that the molecule is linear. The optical rotatory power of the galactan points to the occurrence of β-galactosido-linkages, and the amount of tetramethyl galactose isolated suggests that there is approximately one end group per 100 galactose residues.

In the absence of evidence concerning molecular weight it is not possible at present to say whether this figure represents the molecular size or the size of the repeating unit in a larger molecule. The type of structure present is shown in (VIII). The methylated araban could



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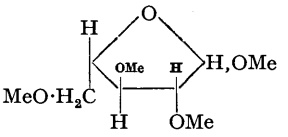

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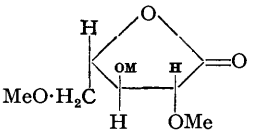

(X.)

not be obtained in a pure state, and a complete examination of it could not be attempted. Nevertheless, methanolysis of the crude material, which still contained much galactan, gave some 2 : 3 : 5-trimethyl methyl-*l*-arabinoside (IX) identified as crystalline 2 : 3 : 5-trimethyl

l-arabonolactone (X), the identity of which was confirmed by conversion into the corresponding crystalline amide. These arabofuranose residues were therefore terminal groups of the type previously found to be present in arabans associated with other pectic materials.

It is clear from these results that the araban cannot be formed directly from this galactan by processes of oxidation and decarboxylation at position 6. If phytochemical changes of this type do occur it would be necessary to postulate the participation of three types of galactan and two types of pectic acid in the reactions concerned (cf. Hirst, *J.*, 1942, 70). No evidence for the presence of isomeric pectic acids and galactans in pectic materials has yet been obtained, and it would appear much more likely that the various polysaccharides are formed by separate synthetic reactions. In addition, there remains the problem, still quite unsolved, as to whether the plant can utilise the *d*-galactose residues present in the galactan and convert them after hydrolysis into pectic acid and araban, or whether the synthesis proceeds from material other than *d*-galactose. On the basis of present knowledge the possibility cannot be ruled out that the simultaneous occurrence of the three stereochemically closely related substances *d*-galactose, *d*-galacturonic acid, and *l*-arabinose may have no immediate phytochemical significance.

Experimental.

Extraction of the Polysaccharides from the Seeds of Lupinus albus.—After being soaked overnight in water, the seeds were stripped of their skins and milled twice. The protein matter was then extracted by stirring the finely ground material with aqueous sodium chloride solution (10%) for 3 hours, followed by filtration and repetition of the process thrice. The solid residue was then stirred with aqueous sodium hydroxide (0·2%) thrice for 12 hours at a time. Addition of hydrochloric acid to the filtrate then gave no precipitate, indicating the absence of any appreciable quantities of protein. The solid residue was then boiled with aqueous sodium hydroxide (0·2%) for 3 hours, after which it was filtered and the filtrate poured into methylated spirit (4 vols.). The precipitated polysaccharide was filtered off, washed with alcohol, and dried in a vacuum at 90°. The resulting product was extracted with water, the solution was centrifuged to separate insoluble material, and the polysaccharide was precipitated by addition of methylated spirit acidified with hydrochloric acid. The precipitate was then filtered off, washed until free from acid, and dried in a vacuum at 90°. The product (*A*) was a non-reducing cream-coloured powder. Yield, 4%. $[\alpha]_D^{20°}$ + 52° (as sodium salt in water) [Found: furfuraldehyde, 26·5; uronic anhydride, 8% (corresponding to an equiv. of 2200 and 2% of the total furfuraldehyde); N, nil; OMe, nil; equiv., 1430 (by titration with 0·1N-sodium hydroxide)].

Hydrolysis of the Polysaccharide Material (A).—This material (15 g.) was boiled with N-sulphuric acid (250 c.c.) for 6 hours. Hydrolysis was then complete since the optical rotation and reducing power had become constant. A small quantity of flocculent material (0·8 g.) was removed on the centrifuge and the solution was neutralised with barium carbonate and filtered. The filtrate was concentrated under reduced pressure at 40° and poured into methyl alcohol (50 c.c.), and the precipitated barium salts were removed by filtration. The filtrate from the barium salts was concentrated under reduced pressure to a syrup which crystallised. The crystalline solid, m. p. 157° (3·6 g.), separated by filtration after trituration with methyl alcohol, was *d*-galactose since it gave mucic acid, m. p. 210°, after oxidation with nitric acid, and with methylphenylhydrazine it gave the phenylmethylhydrazone, m. p. and mixed m. p. with an authentic sample 186°.

After removal of crystalline galactose the filtrate was concentrated to a syrup and made up to 250 c.c. with water. An iodometric titration at this stage showed that the solution contained 10·3 g. of sugar calculated as hexose, or 8·6 g. calculated as pentose. Furfuraldehyde determinations on the solution indicated that pentose (3·9 g.) was present. Confirmation of this figure was obtained by determination of arabinose as its diphenylhydrazone by the method of Wise and Peterson (*Ind. Eng. Chem.*, 1930, **22**, 362). This determination indicated the presence of arabinose (3·9 g.). Other pentoses, except in traces, were therefore absent. Determination of galactose by the mucic acid method as modified by Wise and Peterson (*loc. cit.*) indicated the presence of galactose (6·1 g.). These figures for arabinose (3·9 g.) and for galactose (6·1 g.) are in agreement with the total sugar content obtained by iodometric titration and show that (*A*) contains approximately 6% of galactose residues and 26% of arabinose residues. Some pectic acid is also present.

(*A*) (100 g.) was shaken with 70% methyl alcohol (2½ l.) for 3 months. The slurry was then centrifuged and the supernatant liquid evaporated to dryness. The residual solid (0·1 g.) had $[\alpha]_D^{20°}$ − 119° (*c*, 1·0 in water) and yielded 32% of furfuraldehyde on distillation with 12% hydrochloric acid, corresponding to the presence of 64% of pentose.

Since (*A*) was possibly a mixture of an araban and galactan, a portion of it (3 g.) was boiled with 0·01N-oxalic acid at 100° until the optical rotation was constant. A small amount of insoluble material which separated appeared to be impure pectic acid; it had $[\alpha]_D^{20°}$ + 157° (in water) (Found: equiv., 370). The solution was filtered before and after neutralisation with calcium carbonate, and concentrated to dryness. The residual solid was extracted with methyl alcohol and the solution filtered and concentrated to a syrup (0·285 g.) which had $[\alpha]_D^{20°}$ + 91° in water and contained 36% of pentosan (calculated from the yield of furfuraldehyde obtained on distillation with 12% hydrochloric acid). The methyl alcohol-insoluble solid (2·0 g.) gave a negative test for pentoses and had $[\alpha]_D^{20°}$ + 77° in water. On hydrolysis with 1% sulphuric acid it gave *d*-galactose, m. p. and mixed m. p. 158°. The phenylmethylhydrazone had m. p. 184°. No other sugar could be detected.

Purification of (A) *by removal of calcium pectate.* Crude (*A*) contained some uronic acid (see above) which was largely removed in the following manner. The polysaccharide (80 g.) was dissolved in cold water and neutralised with 0·1N-sodium hydroxide, and calcium chloride solution was added to

precipitate the polyuronide as its calcium salt. The insoluble material was then removed on the centrifuge and the water soluble polysaccharide mixture (*B*) was precipitated by addition of alcohol (5 vols.); $[\alpha]_D^{20°}$ + 38° in water (Found: uronic anhydride, 2%; furfuraldehyde, 9·6%; equiv., 3060).

All attempts to separate completely the pentosan, galactan, and uronic acid fractions of (*A*) either by acid treatment or by fractional precipitation were unsuccessful. Accordingly, (*B*) (15 g.) was methylated by the standard thallous hydroxide–methyl iodide procedure (Menzies, *loc. cit.*; Hirst and Jones, *loc. cit.*). The methylation was completed by boiling with methyl iodide and silver oxide. The product (12 g.) (Found: OMe, 42%) was isolated in the usual manner.

Fractionation. The methylated product (12 g.) was dissolved in chloroform (50 c.c.) and fractionated by the portionwise addition of light petroleum (b. p. 40—60°). By this means the following fractions were obtained: Fraction I (0·9 g.), $[\alpha]_D^{20°}$ − 16° in methyl alcohol (Found: OMe, 40·3%). Fraction II (8·1 g.), $[\alpha]_D^{20°}$ − 12° in methyl alcohol [Found: furfuraldehyde (on boiling with 12% hydrochloric acid, see Bott and Hirst, *J.*, 1932, 2621), 3·1; OMe, 39·1%]. Fraction III (2 g.), $[\alpha]_D^{20°}$ − 12° in methyl alcohol (Found: OMe, 39·9%). Fraction IV (0·5 g.), $[\alpha]_D^{20°}$ − 13° in methyl alcohol (Found: OMe, 39·2%). Fraction V (0·4 g.), $[\alpha]_D^{20°}$ − 15° in methyl alcohol (Found: furfuraldehyde, 9·5; OMe, 37·5%).

Fractions I, II, III, and IV were combined and re-methylated with silver oxide and methyl iodide since their methoxyl content was still low. The product, isolated in the usual manner, was separated into two fractions by extraction with boiling methyl iodide. This separation of the methylated polysaccharide into methyl iodide-soluble and -insoluble fractions indicated that there was a substantial difference in the physical properties of the two components. The analytical figures and optical rotations, however, showed little difference between the two fractions. This was confirmed on examination of the products of methanolysis of the two fractions (see below). The solubility difference observed is due probably to difference in molecular weight and not to a substantial difference in chemical constitution. No pentose could be detected in the products of hydrolysis of either fraction.

The methyl iodide-soluble material (4·0 g.) had $[\alpha]_D^{20°}$ − 15·7° in methyl alcohol (Found: C, 51·0; H, 7·7; OMe, 39·5%). No acidic groups (uronic acids) were present. The methyl iodide-insoluble material (7·4 g.) had $[\alpha]_D^{20°}$ − 17° in methyl alcohol (Found: C, 51·6; H, 7·9; OMe, 41·6%). No acid groups (uronic acids) were present.

Hydrolysis of the Fraction Soluble in Methyl Iodide.—This polysaccharide derivative was very resistant to methanolysis even on prolonged boiling with fairly high concentrations of hydrogen chloride. Accordingly the methylated polysaccharide (3·9 g.) was dissolved in a solution (50 c.c.) containing glacial acetic acid (2 vols.), concentrated hydrochloric acid (1 vol.), and water (1 vol.) and heated on a water-bath until the rotation was constant (8 hours). The solution was partially neutralised with barium carbonate (to remove all the hydrochloric acid) and filtered, and the filtrate and washings were evaporated to dryness under reduced pressure to remove acetic acid and water. The solid residue was extracted exhaustively with chloroform and the extracts were concentrated under reduced pressure and converted into the methyl glycosides by boiling with methyl alcoholic hydrogen chloride (3%, 7 hours). The resulting methyl glycosides (3·9 g.) were isolated and fractionally distilled giving: Fraction 1 (0·30 g.), b. p. 129°/0·001 mm., $n_D^{20°}$ 1·4495 (Found: OMe, 54·1%). Fraction 2 (2·45 g.), b. p. 140—160°/0·001 mm., $n_D^{20°}$ 1·4568 (Found: OMe, 50·7%). Fraction 3 (0·38 g.), b. p. 160—175°/0·001 mm., $n_D^{20°}$ 1·4560 (Found: OMe, 50·6. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%). Fraction 4 (0·33 g.), b. p. 175—240°/0·001 mm., $n_D^{20°}$ 1·4686 (Found: OMe, 41·4%). Still residue (0·44 g.), not further examined.

Examination of the fractions. Fractions 1 and 2 (2·7 g.) were combined and hydrolysed with N-hydrochloric acid (20 c.c.) at 90—95°. $[\alpha]_D^{20°}$ + 20° (initial value) ⟶ + 81° (3 hours, constant value). The solution was cooled, neutralised with barium carbonate, filtered, and concentrated under reduced pressure to a solid which was exhaustively extracted with chloroform. On concentration the extracts gave a syrup (2·4 g.); $n_D^{21°}$ 1·4772; $[\alpha]_D^{20°}$ + 37° (in methyl alcohol) (Found: OMe, 40·9. Calc. for $C_9H_{18}O_6$: OMe, 41·8%). A sample of the syrup (0·039 g.) on dissolving in 3% methyl alcoholic hydrogen chloride (5 c.c.) showed a change of optical rotation identical with that given by 2 : 3 : 6-trimethyl *d*-galactose. $[\alpha]_D^{20°}$ + 36° (initial value); + 8° (8½ hours); + 0° (14 hours); − 16° (20 hours); − 26° (36 hours, constant value). The sugar (0·89 g.) was dissolved in water (5 c.c.) and bromine (1·5 c.c.) added. The solution was heated to 55° for 4 hours; a test sample after aeration to remove bromine then showed no reduction when heated with Fehling's solution. Bromine and hydrobromic acid were removed and the syrupy 2 : 3 : 6-trimethyl *d*-galactonolactone was isolated after removal of solvent at 40°. On standing, the lactone crystallised; it was purified by sublimation in a vacuum; m. p. 92°. Yield, 0·8 g. This product, further purified by recrystallisation from ether–light petroleum, had m. p. and mixed m. p. with an authentic sample, 98°. $[\alpha]_D^{20°}$ − 41° in water, initial value (Found: OMe, 41·0; equiv., 220. Calc. for $C_9H_{16}O_6$: OMe, 42·4%; equiv., 220). The lactone was converted quantitatively by solution in liquid ammonia into the amide, m. p. and mixed m. p. with an authentic specimen, 133°.

Fractions 3 and 4 were combined (0·7 g.) and hydrolysed with N-hydrochloric acid for 6 hours. The isolated sugars (0·55 g.) gave on oxidation crystalline 2 : 3 : 6-trimethyl *d*-galactonolactone, m. p. and mixed m. p. 98°, in 50% yield.

Hydrolysis of the Fraction Insoluble in Methyl Iodide.—A portion of the methylated polysaccharide (4·0 g.) was hydrolysed as described above and the methyl glycosides (4·5 g.) distilled giving: Fraction 5 (0·28 g.), b. p. 115—135°/0·001 mm., $n_D^{21°}$ 1·4532 (Found: OMe, 50·2%). Fraction 6 (1·59 g.), b. p. 135—145°/0·001 mm., $n_D^{21°}$ 1·4535 (Found: OMe, 51·4%). Fraction 7 (1·44 g.), b. p. 145—175°/0·001 mm., $n_D^{21°}$ 1·4548 (Found: OMe, 51·1%). Fraction 8 (0·47 g.), b. p. 175—190°/0·001 mm., $n_D^{21°}$ 1·4588 (Found: OMe, 48·8%). Fraction 9 (0·33 g.), b. p. > 190°/0·001 mm., $n_D^{21°}$ 1·4672 (Found: OMe, 43·5%). Still residue, 0·3 g. (not further examined).

Fractions 5, 6, 7, and 8 had substantially the same constants; they were combined and hydrolysed with N-hydrochloric acid for 4 hours ($[\alpha]_D^{20°}$ + 88° constant value). The solution was neutralised with

barium carbonate and the sugar isolated (3·5 g.); $n_D^{20°}$ 1·4770; $[\alpha]_D^{20°}$ + 36° (in methyl alcohol) (Found : OMe, 40·5. Calc. for $C_9H_{18}O_6$: OMe, 41·8%).

A sample of the syrup showed a change of rotation in cold 2% methyl alcoholic hydrogen chloride, similar to that shown by 2 : 3 : 6-trimethyl *d*-galactose. $[\alpha]_D^{20°}$ + 36° (initial value); + 5° (24 hours); 0° (36 hours); − 11° (56 hours); − 26° (70 hours, constant value). On oxidation with bromine (1 c.c.) in water (3 c.c.) the syrup (1·04 g.) gave 2 : 3 : 6-trimethyl *d*-galactonolactone, m. p. 97°, isolated in 85% yield. $[\alpha]_D^{20°}$ − 41° (initial value, in water) (Found : OMe, 41·2; equiv., 220. Calc. for $C_9H_{16}O_6$: OMe, 42·4%; equiv., 222).

A portion of the syrup (0·58 g.) was boiled with aniline (0·3 c.c.) and alcohol (3 c.c.) for 3 hours. On concentration a syrup was formed which crystallised only after 3 months. The crystals were tiled and purified by recrystallisation from absolute alcohol. Yield, 30 mg.; m. p. 189°, not depressed on admixture with an authentic specimen of 2 : 3 : 4 : 6-tetramethyl *d*-galactose anilide. Some 2 : 3 : 4 : 6-tetramethyl *d*-galactose (end group) is therefore present in the sugar syrup. No arabinose derivative could be detected in the products of hydrolysis.

Examination of Polysaccharide Fraction V.—Fraction V, the last fraction from the fractionation of the methylated polysaccharides with light petroleum (b. p. 40—60°), was purified by extraction with boiling light petroleum. The residual white solid (1·8 g.) [Found : dimethyl pentosan (by furfuraldehyde estimation), 19·4%; OMe, 40%] was subjected to methanolysis with boiling methyl alcoholic hydrogen chloride (3%) for 7 hours. The resulting methylglycosides (1·8 g.) were fractionally distilled giving : Fraction 10 (0·21 g.), b. p. 100°/0·01 mm., $n_D^{20°}$ 1·4355 (Found : OMe, 61%). Fraction 11 (0·24 g.), b. p. 100—120°/0·001 mm., $n_D^{20°}$ 1·4440. Fraction 12 (0·57 g.), b. p. 130—150°/0·01 mm., $n_D^{20°}$ 1·4568.

Fraction 10 (0·21 g.) was hydrolysed with N-hydrochloric acid (10 c.c.). $[\alpha]_D^{21°}$ − 47° (initial) fell to − 15° (constant value) in 2 hours. The syrupy sugar (0·19 g.) [Found : OMe, 43. Calc. for trimethyl *l*-arabinose ($C_8H_{16}O_5$) : OMe, 45·4%], after isolation, was oxidised with bromine (0·3 c.c.) in water (5 c.c.) for 2½ hours at 55°. Bromine was removed by aeration, the solution neutralised with silver carbonate, and the lactone isolated as a sticky solid which was purified on the tile and then by recrystallisation from ether–light petroleum; m. p. 29°, not depressed on admixture with an authentic specimen of 2 : 3 : 5-trimethyl *l*-arabonolactone. The lactone on treatment with methyl alcoholic ammonia gave 2 : 3 : 5-trimethyl *l*-arabonamide in quantitative yield, m. p. 134°, m. p. 135° on admixture with an authentic specimen of 2 : 3 : 5-trimethyl *l*- arabonamide.

The estimated yield of 2 : 3 : 5-trimethyl *l*-arabinose was about 8% of Fraction V, approximately one-third of the total pentosan content of the crude methylated polysaccharide. Fraction 11 was not further investigated. Fraction 12 (0·5 g.) on hydrolysis and oxidation (for details see under Fractions 1 and 2) gave 2 : 3 : 6-trimethyl *d*-galactonolactone, m. p. 98°, in 80% yield.

Identification of the Acidic Portion of the Polysaccharide as Pectic Acid by Preparation of the Degraded Poly-ester.—The polysaccharide (75 g.) was treated with boiling N-sulphuric acid at 90° for 6 hours. This process destroyed the araban and most of the galactan leaving a partly degraded pectic acid which separated as a flocculent precipitate. The insoluble portion (5 g.; equiv., 700) was filtered off and washed free from sulphuric acid, first with water and then with methanol. The solid was then boiled with 2% methyl alcoholic hydrogen chloride for 7 hours, and the insoluble residue was filtered off and washed free from hydrochloric acid with methanol. The solid was purified by dissolving in water and precipitating with ethanol. Yield 3 g. $[\alpha]_D^{20°}$ + 170° (in water) (Found : OMe, 17·9; equiv., 210. Calc. for methyl pectate : OMe, 16·3%; equiv., 190). On oxidation a sample gave mucic acid in 46% yield. Pectic acid was therefore a component of the mixture of polysaccharides (*A*).

The authors wish to thank Imperial Chemical Industries Ltd. for a grant.

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[*Received, December 2nd,* 1946.]