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A STUDY OF THE PECTIN PRESENT IN THE BARK OF AMABILIS FIR (ABIES AMABILIS)

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ABSTRACT

A pectic material has been isolated from the bark of *Abies amabilis* (Dougl.) Forbes in a yield of 2%. On hydrolysis it yielded D-galacturonic acid, D-galactose, and L-arabinose in a ratio of 85:4:11, and also traces of rhamnose. The product, when submitted to several conventional fractionation methods, appeared homogeneous. Further resolution could be effected by acidification of an aqueous solution of the pectin, followed by ultracentrifugation. The insoluble portion (50%) was an electrophoretically homogeneous *galacturonan* with $[\alpha]_D$ +246°. The material remaining in solution (30%), here referred to as a *pectic acid*, had $[\alpha]_D$ +225° and on hydrolysis gave D-galacturonic acid, D-galactose, and L-arbabinose in a ratio of 74:7:19, as well as traces of rhamnose.

The structure of the galacturonan was established by partial hydrolysis and methylation. It consisted of α-D-galacturonic acid residues linked together by (1 → 4)-glycosidic bonds to a linear macromolecule. The same techniques were applied to the pectic acid. While a unique structural formula could not be assigned in this case, one probable alternative involved a framework of (1 → 4)-linked α-D-galacturonic acid residues together with a few residues of 1,2,4-linked L-rhamnose. Some of the galacturonic acid units carried at C-2 and C-3 side chains which were terminated by D-galactopyranose and L-arabinofuranose residues. A few of the latter also occurred as inner units, probably in the side chains. This appears to be the first time a pectic material has been resolved into a galacturonan and a pectic acid containing the four sugar residues usually found in pectins. It is probable that the pectin occurring to a limited extent in wood has a similar composition.

In previous investigations several polysaccharides were isolated from the bark of amabilis fir (*Abies amabilis*) (1), namely a cellulose (2), a galactoglucomannan (3), a glucomannan (4), and a xylan (5). The present study is concerned with the pectic substances also present in this bark. Pectin has previously been isolated from both wood and bark by Anderson and his co-workers (6–9). Painter and Purves (10) obtained pectin in a yield of 7% from white spruce inner bark. Similarly, inner bark of white birch has yielded a pectin in a yield of 3–4% (11). During the early stages of cell differentiation, the primary wall and the middle lamella consist largely of pectin. The pectic substances of cell walls in the phloem, cambial, sapwood, and heartwood regions have recently been studied by Thornber and Northcote (12).

The pectic group of polysaccharides is based on residues of D-galacturonic acid, D-galactose, L-arabinose, and L-rhamnose, with the galacturonic acid usually, albeit not always, predominating. A polymer containing only D-galacturonic acid residues (a galacturonan) seems to have been isolated only on one occasion, namely from sunflower heads (13). Lately, it has become evident that most of the so-called pectic acids probably also contain neutral sugar residues, such as D-galactose, L-arabinose, L-rhamnose, and L-fucose (14–18). The exact constitution of these heteropolymers is still unknown although the main features of one pectic acid have recently been established (17).

RESULTS AND DISCUSSION

When extractive-free fir bark was treated with chlorous acid for complete removal of polyphenolic material, a holocellulose was obtained in a yield of 50%. A mixture of

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polysaccharides, including a galactoglucomannan (3) and pectic material, was removed by extraction with hot water, the amount corresponding to a yield of 1.8% of the bark. The extract on hydrolysis gave galacturonic acid, galactose, glucose, mannose, arabinose, and xylose in a ratio of 10:8:20:44:10:8. Free boundary electrophoresis indicated that it consisted of at least three components. The solid residue was treated with hot 0.5% ammonium oxalate in water to give a crude ammonium pectate with $[\alpha]_D$ +120° in water and in a yield of 6.0% of the bark. On hydrolysis the product gave the same sugars as above, the ratio being 38:8:10:22:12:10. Boundary electrophoresis showed that it consisted of one major, fast-moving and three or four slower components.

Anderson and co-workers (19) have recently claimed that decarboxylation can easily occur on isolation of pectin by techniques such as those used here. Using different times of treatment with both water and ammonium oxalate, 16 samples were isolated. Yields, specific rotations, and sugar compositions remained practically constant throughout, showing that in the present case the pectic material underwent no chemical change during its isolation. When the crude ammonium pectate was subjected to chromatography on diethylaminoethylcellulose (20), three fractions were obtained. Water eluted a galactoglucomannan (52%), phosphate buffer an acidic arabinoxylan (7%), and sodium hydroxide a pectic acid (28%), containing galacturonic acid, galactose, and arabinose residues. Obviously, the crude ammonium pectate was a mixture of at least three different components.

The pectic acid was separated from the other polysaccharides by forming the insoluble calcium pectate which was washed with alkali and water. The purified product, obtained in a yield of 30%, had $[\alpha]_D$ +230° in water and on hydrolysis gave galacturonic acid, galactose, and arabinose in a ratio of 85:4:11, and also traces of rhamnose. Boundary electrophoresis indicated that the product was not homogeneous. Several attempts were made to effect further resolution by renewed treatment with calcium chloride, precipitation with cetyltrimethylammonium hydroxide, fractional precipitation with ethanol from an aqueous solution, and ion exchange chromatography on diethylaminoethylcellulose. In each case, the fractions obtained were unchanged in composition and optical rotation.

Yean and Goring (21) have recently shown that sodium lignosulfonate can be conveniently separated from neutral polysaccharides by an electrophoresis–convection technique. When the crude ammonium pectate was subjected to this procedure, the upper layers in the electrolyte vessel contained the galactoglucomannan, while the pectic acid was located at the bottom. Very little resolution could be achieved, however, with the purified product.The repeated failure to resolve the purified ammonium pectate any further made it tempting to conclude that the product must be chemically homogeneous in spite of the electrophoretic evidence. At this stage, however, it was discovered that the product could actually be resolved into two fractions. While several procedures were found to be applicable, they were all based on the different solubility in water of the two components. When an aqueous solution of the purified ammonium pectate was passed through a column with a cation exchange resin in the acid form, a slightly cloudy, colloidal solution was obtained. Ultracentrifugation at 40 000 g gave in 50% yield a precipitate, the sodium salt of which had $[\alpha]_D$ +246° in water and which on hydrolysis gave only galacturonic acid. The portion remaining in solution amounted to 30%, had $[\alpha]_D$ +225°, and contained, in addition to traces of rhamnose, residues of galacturonic acid, galactose, and arabinose in a ratio of 74:7:19. The first polysaccharide was obviously a *galacturonan*. The second will, somewhat arbitrarily, be referred to here as a *pectic acid*.

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The same fractionation was attained when a solution in water of the purified ammonium pectate was treated with dilute sulfuric acid or aqueous sodium chloride, respectively, followed by ultracentrifugation. On electrophoresis–convection of the ammonium pectate, a colloidal, viscous layer formed at the bottom of the vessel. When this layer was diluted with water and subjected to ultracentrifugation, the same two fractions were again obtained. Boundary electrophoresis showed that the galacturonan was homogeneous, while the pectic acid was probably still somewhat heterogeneous. Application of the four fractionation methods referred to above failed to effect any further resolution, showing that no separate galactan or araban was present. Gel filtration, using Sephadex G-200 was also of no avail, indicating the absence of low molecular weight material. The pectic acid was probably a mixture of closely related acidic polysaccharides, differing slightly in their content of galacturonic acid residues. Both polysaccharides were accordingly subjected to structural analysis.

When the galacturonan was hydrolyzed with a commercial pectinase enzyme, a series of four polymer-homologous oligomers of galacturonic acid were obtained, ranging from the di- to the penta-saccharide. The propylene glycol ester of the acidic polymer was reduced in water with sodium borohydride to the corresponding galactan (14). Reduction of the acetylated galacturonan with diborane in diglyme (22) proved less efficient. The reduced product, which still contained a few galacturonic acid residues, was methylated and hydrolyzed, giving, in a molar ratio of 1:350:1, 2,3-di-*O*-methyl-D-galacturonic acid, 2,3,6-tri-*O*-methyl-D-galactose, and 2,3,4,6-tetra-*O*-methyl-D-galactose, which were identified through crystalline derivatives. The number-average degree of polymerization of the *O*-methylgalactan was 450, as determined from osmotic pressure measurements. Only one nonreducing end group was thus present per average molecule.

These results show that the galacturonan was a linear polysaccharide containing at least 450 (1 → 4)-linked α-D-galacturonic acid residues. The unbranched nature of the galacturonan and its relatively high degree of polymerization are probably the reasons for its insolubility in water. When the pectic acid was subjected to a mild, partial acid hydrolysis, arabinose appeared immediately in the hydrolysate, soon followed by galactose. On prolonged hydrolysis, the four galacturonic acid oligomers, previously obtained from the galacturonan, were formed. In order to make available more of these compounds, the purified, but unfractionated, ammonium pectate was partially hydrolyzed. A combination of ion exchange and paper chromatography gave four pure oligomers. 4-*O*-(α-D-Galactopyranosyluronic acid)-D-galacturonic acid was tentatively identified by conversion to the known α-(1 → 4)-linked galactobiose (23). The corresponding galacturonotriose, on reduction, methylation, and hydrolysis, gave 1 mole of 2,3,4,6-tetra-*O*-methyl-D-galactose and 2 moles of 2,3,6-tri-*O*-methyl-D-galactose.

2-*O*-(α-D-Galactopyranosyluronic acid)-L-rhamnose was identified through its crystalline, fully methylated methyl glycoside dihydrate (17) and from the *O*-methyl sugars obtained on hydrolysis of the latter. The fourth oligosaccharide was a galacturonosyl rhamnosyl rhamnose. The nature of the linkage between the two rhamnose residues could not be determined with certainty. A similar trisaccharide has been isolated from alfalfa pectic acid (17). The nature of the first two oligosaccharides shows the presence of (1 → 4)-linked α-D-galacturonic acid residues. The isolation of the last two oligomers indicates that rhamnose was probably an integral part of the pectic acid.

A reduction of the pectic acid prior to its methylation would obviously make it impossible to distinguish between the galacturonic acid and the galactose residues. The polysaccharide was therefore methylated directly by the Haworth and the Purdie

methods, but under mild conditions in view of its lability towards alkali (24, 25). The mixture of *O*-methyl glycosides obtained on methanolysis was resolved into an acid and a neutral portion with the aid of exchange resin. The acid fraction was esterified, reduced with lithium aluminium hydride, and hydrolyzed. The neutral portion, finally, was also hydrolyzed. The neutral *O*-methyl sugars present in each fraction were separated by preparative paper chromatography. Seven of the compounds obtained were crystalline or were identified through crystalline derivatives. The remaining three were tentatively identified by other means. The methylation data are summarized in Table I.

TABLE I

Sugar constituents of the methylated pectic acid

Component	Mole %	Component	Mole %
Unknown sugars	1.8	D-Galacturonic acid	4.3
3-*O*-Methyl-L-rhamnose	2.6	2-*O*-Methyl-D-galacturonic acid	10.5
Di-*O*-methyl-L-arabinose	2.4	3-*O*-Methyl-D-galacturonic acid	9.7
2,3,5-Tri-*O*-methyl-L-arabinose	13.3	Di-*O*-methyl-D-galacturonic acid	0.3
2,3,4-Tri-*O*-methyl-D-galactose	1.2	2,3-Di-*O*-methyl-D-galacturonic acid	45.3
2,3,4,6-Tetra-*O*-methyl-D-galactose	7.2	2,3,4-Tri-*O*-methyl-D-galacturonic acid	1.4

The large amount of 2,3-di-*O*-methyl-D-galacturonic acid shows that the pectic acid contained a framework of (1 → 4)-linked D-galacturonic acid residues, probably, in view of the high specific rotation of the polysaccharide, in the α-D-modification. The presence of 2-*O*- and 3-*O*-methyl-D-galacturonic acid and of D-galacturonic acid indicates that the framework was partly branched through C-2 or C-3 or both, respectively. The 2,3,4-tri-*O*-methyl-D-galactose evidently originated from nonreducing end groups. The majority of the D-galactose and L-arabinose residues were present as nonreducing end groups, precluding the presence of a separate galactan or araban. Their number was only slightly less than that of galacturonic acid residues carrying branches. A small amount of inner arabinose and (1 → 6)-linked galactose residues were also present. The nature of the galacturonosyl rhamnose obtained on partial hydrolysis in conjunction with the presence of 3-*O*-methyl-L-rhamnose among the *O*-methyl sugars strongly indicate that a few rhamnose residues were an integral part of the galacturonan framework, with at least some of them serving as branching points through their 4-position. No evidence was obtained for the presence of (1 → 4)-linked galactose residues, such as those constituting the galactan occurring in seeds from *Lupinus albus* (26, 27).

It is not possible to assign a unique structural formula to the pectic acid on the basis of the present experimental evidence. One of several possibilities is a molecule consisting of a framework of (1 → 4)-linked α-D-galacturonic acid residues, carrying numerous branches containing D-galactopyranose and L-arabinofuranose residues, most of them present as end groups. Since partial acid hydrolysis produced no galacturonic acid oligomers containing (1 → 2)- or (1 → 3)-linkages, a direct attachment of side chain galacturonic acid residues to the backbone is not likely. The presence of (1 → 4)-linked galacturonic acid residues elsewhere in the side chains is, of course, not excluded.

The experimental evidence presented for the structure of the present pectic acid from fir bark is quite similar to that reported by Aspinall and Fanshawe (17) for their alfalfa pectic acid, and it is clear that these two polysaccharides are closely related. No other pectic acid has so far been studied in similar detail.

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CONCLUSIONS

To the best of the authors' knowledge, the present investigation represents the first successful resolution of a pectic material into a galacturonan and a pectic acid. There is little reason to doubt that in the future pectins from other sources will also prove amenable to this treatment. The earlier conclusion of Aspinall and Cañas-Rodriguez (14) that pectin might consist of "a mixture of acidic polysaccharides, one composed solely of D-galacturonic acid residues and the other or others containing both neutral sugar and D-galacturonic acid residues" appears eminently justified in view of the present results.

Bark obviously contains much more pectin than wood. No wood pectin has so far been subjected to a structural analysis, mostly, no doubt, because of the small quantities present. It is known, however, that the primary wall and middle lamella in wood cells are rich in galacturonic acid, galactose, and arabinose residues (12, 28–30). An α-(1 $\rightarrow$ 2)-linked-D-galacturonosyl rhamnose has been isolated from pine (31, 32) and beech (33) woods. The presence in wood of pectic material similar to that found in the fir bark is therefore quite likely.

EXPERIMENTAL

General experimental conditions were the same as in a previous paper (34). The following solvent systems were used for paper chromatography: (A) ethyl acetate, acetic acid, water (9:2:2 v/v), (B) ethyl acetate, acetic acid, water (18:7:8), (C) ethyl acetate, acetic acid, formic acid, water (18:3:1:4), (D) ethyl acetate, pyridine, water (8:2:1), and (E) 2-butanone, water, ammonia (90:8:2). For preparative purposes, Whatman No. 3MM filter paper with a wick of No. 50 paper was used. R_{GalA} values refer to the rate of movement of the sugars relative to galacturonic acid. M_G values refer to the rate of movement of a sugar relative to glucose on paper electrophoresis in 0.05 M borate solution. Specific rotations were determined in water (c, 1.0), unless otherwise stated.

Isolation of Crude Pectin

For isolation of crude pectin, the bark was first treated with chlorous acid as described elsewhere (1). The average yield of holocelluloses in several experiments was 50 $\pm$ 2%.

Batches of holocellulose (50 g) were stirred at 75° with water (1 l) containing 6% of potassium acetate for 3, 6, 9, and 12 h, respectively. Each of the four residues was extracted at the same temperature with 0.5% aqueous ammonium oxalate, aliquots being withdrawn after 3, 6, 9, and 12 h. Each of the 16 fractions thus obtained was purified by washing the insoluble calcium pectate with water. The purified ammonium pectates were all essentially similar, with average yield from the holocellulose 3.5%, $[\alpha]_D$ +195°, and the ratio of galacturonic acid, galactose, and arabinose 77:6:17.

For large-scale recovery of pectin, holocellulose (1 200 g) was stirred in water (18 l) containing 6% potassium acetate at 75° for 12 h. The residue was recovered by filtration and washed with hot water (20 l). After concentration to 3 l, the extract was added to ethanol (15 l). The precipitate formed was recovered by filtration, washed successively with 70% aqueous ethanol, ethanol, and petroleum ether, and finally dried *in vacuo* to give a white powder (42 g, 3.5% of holocellulose). The solid residue was extracted with 0.5% aqueous ammonium oxalate at 75° for 12 h. The extract was recovered in the usual way to give a white product (145 g, 12% yield) with $[\alpha]_D$ +120°.

Purification of Crude Ammonium Pectate

Crude ammonium pectate (113 g) was dissolved in water (2 l) and a 10% aqueous solution of calcium chloride (1.5 l) was added. The precipitate formed was washed with water and then suspended in water (4 l) to which 8% aqueous sodium hydroxide (2 l) was added. The suspension was stirred, and the solids were recovered by filtration, and washed with dilute acetic acid and water. The calcium pectate was treated with ammonium oxalate, insoluble calcium oxalate was removed by centrifugation, and the supernatant solution was concentrated and added to ethanol. The precipitate was recovered as described above to give 37 g of material (33% of crude product); $[\alpha]_D$ +230°; OCH_3, 0.9%; ash, 2.3%.

Chromatography on Diethylaminoethylcellulose (16, 20)

Purified ammonium pectate (400 mg) in aqueous solution was added to the top of a column containing diethylaminoethylcellulose in the phosphate form. The column was eluted in succession with 500 ml each of water, 0.025 M, 0.05 M, 0.10 M, and 0.25 M sodium dihydrogen phosphate, and, finally, using gradient elution, with water – 0.3 M sodium hydroxide (2 l). The fractions (15 ml each) were analyzed polarimetrically and by the phenol – sulfuric acid method (35). After deionization with exchange resins, the pectic material was recovered in the usual way.

Electrophoresis–Convection

The apparatus used has been described elsewhere (21). Ammonium pectate (500 mg) was dissolved in water (400 ml) and subjected to electrophoresis. After 5 h, a turbid, viscous layer was observed at the bottom of the vessel used. The experiment was terminated after 8 h, and fractions were carefully removed with a syringe, starting with the top layer.

Resolution of Purified Ammonium Pectate

Acidification.—Purified ammonium pectate (500 mg) was dissolved in water, and the solution was passed through a column containing Amberlite IR-120 exchange resin (acid form). The turbid eluate was concentrated and subjected to ultracentrifugation at 40 000 g. The supernatant, clear solution was treated with more exchange resin until no more turbidity was observed. The final, clear solution was concentrated and added to ethanol. The precipitate formed was recovered in the usual way to give a water-soluble *pectic acid* (160 mg); $[\alpha]_D$ +225°; OCH_3, 2.7%; ash, 0.35%. The solid residue was washed with water and subjected to the same ultracentrifugation, a process which was repeated six times, after which the material was washed with acetone and petroleum ether and dried *in vacuo*, yielding 250 mg of the water-insoluble *galacturonan*; $[\alpha]_D$ +246°; OCH_3, 0.6%; ash, 0.16%.

Alternatively, *N* sulfuric acid was added slowly to an aqueous solution of the ammonium pectate until a pH of 4.0 had been reached. The precipitate and solution were recovered as described.

Addition of aqueous sodium chloride.—A saturated solution in water of sodium chloride (10 ml) was added slowly to an aqueous, 1% solution of ammonium pectate (100 ml). The precipitate formed and the solution were treated as above.

Electrophoresis–convection.—The turbid, viscous solution obtained at the bottom after electrophoresis was diluted with water and subjected to ultracentrifugation. The precipitate and solution gave the same products as the other two methods.

Partial Acid Hydrolysis of the Purified Ammonium Pectate

Purified ammonium pectate (40 g) was dissolved in water (972 ml) to which 28 ml of concentrated sulfuric acid was added. The solution was heated on a steam bath for 3.5 h. After cooling, an equal volume of acetone was added. The precipitate formed was recovered by filtration and washed with aqueous acetone. The degraded product (18 g) gave only galacturonic acid on complete hydrolysis.

The filtrate and washings were neutralized with barium carbonate, treated with a cation exchange resin, and added to the top of a column containing Amberlite CG-45 (formate form) anion exchange resin. After washing with water until a Molisch test was negative, elution was continued with increasing concentrations of formic acid (0.001 *N* to 2 *N*). Eight fractions were collected and examined by paper chromatography (solvents A to D). Further resolution was effected with solvent B, using preparative paper chromatography.

Identification of Oligosaccharides

Galacturonotriose.—This trisaccharide (60 mg) had $[\alpha]_D$ +187°, R_{GalA} 0.04, 0.39, and 0.05 in solvents A, B, and C, respectively, and melted with decomposition at 135–142°. Partial hydrolysis gave galacturonic acid, galacturonobiose, and some galacturonotriose. The methyl ester methyl glycoside was reduced with sodium borohydride, methylated, and hydrolyzed, giving two sugars which were separated by paper chromatography (solvent E). One of the components (35 mg) was identified as 2,3,6-tri-*O*-methyl-D-galactose by conversion to the corresponding galactonolactone, which had melting point and mixed melting point 97–98°. The second compound (20 mg) was shown to be 2,3,4,6-tetra-*O*-methyl-D-galactose through its aniline derivative which had melting point and mixed melting point 192–194°.

Galacturonosyl rhamnosyl rhamnose.—This trisaccharide (90 mg) had $[\alpha]_D$ +118°, had R_{GalA} 0.10 in solvent C, and gave 1 mole of galacturonic acid and 2 moles of rhamnose on prolonged hydrolysis. Partial hydrolysis yielded rhamnose, galacturonic acid, and galacturonosyl rhamnose. After prior reduction with borohydride, only galacturonosyl rhamnose and a trace of galacturonic acid were obtained on hydrolysis.

Galacturonobiose.—This disaccharide (700 mg) had $[\alpha]_D$ +154° and R_{GalA} 0.23, 0.60, and 0.20 in solvents A, B, and C, respectively. It melted with decomposition at 125–135°. After treatment with trimethylorthoformate, esterification with diazomethane, and reduction with lithium aluminium hydride (23), a neutral disaccharide was obtained which gave only galactose on hydrolysis and had $[\alpha]_D$ +175°. The compound could not be induced to crystallize but was chromatographically and ionophoretically identical with an authentic sample of 4-*O*-(α-D-galactopyranosyl)-D-galactose (36).

2-O-(α-D-Galactopyranosyluronic acid)-L-rhamnose.—This oligosaccharide (300 mg) had $[\alpha]_D$ +108° and gave equimolar amounts of galacturonic acid and rhamnose on prolonged hydrolysis. The methyl ester methyl glycoside, after reduction with sodium borohydride, on hydrolysis gave galactose and rhamnose. When the disaccharide was directly reduced, galacturonic acid was the only reducing sugar obtained on hydrolysis. The fully methylated aldobiouronic acid, after recrystallization from chloroform – ethyl ether, gave methyl 2-*O*-(2,3,4-tri-*O*-methyl-α-D-galactopyranosyluronic acid)-3,4-di-*O*-methyl-L-rhamnopyranoside dihydrate which had m.p. 66–68° and $[\alpha]_D$ +98° (*c*, 0.5 in chloroform) (17). The compound (50 mg) was reduced with diborane in diglyme and hydrolyzed, giving equimolar amounts of two compounds which on demethylation gave galactose and rhamnose, respectively. They were tentatively identified from their rates of movement in solvent E as 2,3,4-tri-*O*-methyl-D-galactose and 3,4-di-*O*-methyl-L-rhamnose.

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Partial Enzymic Hydrolysis of the Galacturonan

An aqueous solution of the sodium salt of the galacturonan was treated with a commercial pectinase preparation (Nutritional Biochemical Corporation, Cleveland, Ohio) using the technique of Painter (37). The hydrolysate was resolved into five fractions using paper chromatography (solvent B). A plot of the degree of polymerization of each oligomer versus $R_{GalA}/(1 - R_{GalA})$ was linear.

Esterification and Reduction of the Galacturonan

The galacturonan (20 g) was esterified with propylene oxide and reduced with sodium borohydride as described earlier (11). After four such treatments, a product (13 g) was obtained which on hydrolysis gave galactose and galacturonic acid in a ratio of 94:6. When the fully acetylated galacturonan was reduced with diborane in diglyme (22) a product was obtained in 50% yield containing galacturonic acid and galactose residues in a ratio of 3:2. This method accordingly appeared to have no advantage in this case.

Methylation of the Galacturonan and Identification of Methylated Sugars

The reduced galacturonan (10 g) was methylated four times with dimethyl sulfate and alkali. The partly methylated product (8 g) was further methylated by the procedure of Kuhn and co-workers (38), yielding a product (3.5 g), which was further reduced with lithium aluminium hydride in tetrahydrofuran. After two further methylations with methyl iodide and silver oxide, the *O*-methylgalactan (2.0 g) had $[\alpha]_D$ +128° (*c*, 1.0 in chloroform) and OCH_3 42.7%. Methanolysis and hydrolysis gave a mixture containing one major and two minor constituents.

2,3,6-Tri-O-methyl-D-*galactose.*—The sirupy sugar (980 mg) had $[\alpha]_D$ +84°. The derived 2,3,6-tri-*O*-methyl-α-D-galactonolactone, on recrystallization from ethyl ether, had melting point and mixed melting point 97–98°.

2,3,4,6-Tetra-O-methyl-D-*galactose.*—This sirup (3 mg) had $[\alpha]_D$ +102°. It was converted to its aniline derivative which had melting point and mixed melting point 196–198°.

2,3-Di-O-methyl-D-*galacturonic acid.*—This compound (4 mg) was converted to its methyl ether methyl glycoside, reduced with lithium aluminium hydride, and hydrolyzed. The aniline derivative of the 2,3-di-*O*-methyl-D-galactose thus obtained had melting point and mixed melting point 130–132°.

Determination of the Molecular Weight of the Methylated Galactan

Osmotic pressure measurements were carried out with the *O*-methylgalactan dissolved in chloroform–ethanol (9:1 v/v) as described previously (39). Extrapolation to zero concentration gave a value of 0.28 for $(h/w)_{w\to 0}$, corresponding to a number-average molecular weight of 91 800 and a degree of polymerization of 450.

Methylation of the Pectic Acid

The pectic acid (8 g) was methylated for 20 h at 10° with dimethyl sulfate and alkali. Treatment with methyl iodide and silver oxide gave a product (1.5 g) which had $[\alpha]_D$ +118° (*c*, 1.0 in chloroform) and OCH_3 39.2%, not raised on further methylations. The methylated pectic acid (1.2 g) was directly hydrolyzed by refluxing with *N* sulfuric acid for 12 h. The neutralized (barium carbonate) and deionized solution was added to the top of a column containing Dowex 1-X4 (acetate form) anion exchange resin. Neutral sugars were removed with water. The eluate on concentration gave a sirup (270 mg) which was resolved by paper chromatography using solvent E. Acid sugars were eluted with 30% aqueous acetic acid, yielding a sirup (645 mg) which was treated with methanolic hydrogen chloride, reduced with lithium aluminuim hydride, and hydrolyzed. The mixture of neutral *O*-methyl sugars thus obtained was resolved as above.

Identification of Methylated Sugars

Neutral Series

3-O-Methyl-L-*rhamnose.*—This sugar (11 mg) had $[\alpha]_D$ +40° and gave rhamnose on demethylation. Crystals obtained from the sirup had m.p. 106–108°.

Di-O-methyl-L-*arabinose.*—This sugar (10 mg) had $[\alpha]_D$ +65° and gave arabinose on demethylation. The aniline derivative could not be induced to crystallize.

2,3,5-Tri-O-methyl-L-*arabinose.*—This sugar (60 mg) had $[\alpha]_D$ −36° and gave arabinose on demethylation. The 2,3,5-tri-*O*-methyl-L-arabonamide had melting point and mixed melting point 134–135°.

2,3,4,6-Tetra-O-methyl-D-*galactose.*—This sirupy compound (40 mg) had $[\alpha]_D$ +106°. The aniline derivative had melting point and mixed melting point 193–195°.

Acid Series

2-O-Methyl-D-*galactose.*—This compound (73 mg) gave no color reaction with triphenyltetrazolium hydroxide (40) and had $[\alpha]_D$ +80°. Crystals produced from the sirup had m.p. 145–146°. The aniline derivative had m.p. 159–161°.

Anal. Calcd. for $C_7H_{14}O_6$: OCH_3, 16.0. Found: OCH_3, 15.2.

3-O-Methyl-D-*galactose.*—This sugar (67 mg) had $[\alpha]_D$ +101° and gave a color reaction with triphenyltetrazolium hydroxide. Its M_G value was higher than that of 2-*O*-methylgalactose.

Anal. Calcd. for $C_7H_{14}O_6$: OCH_3, 16.0. Found: OCH_3, 15.2.

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*2,3-Di-O-methyl-*D*-galactose.*—The sirupy sugar (336 mg) had $[\alpha]_D$ +78°. The aniline derivative had m.p. 130–131°.

Anal. Calcd. for $C_8H_{16}O_6$: OCH_3, 29.8. Found: OCH_3, 28.9.

*2,3,4-Tri-O-methyl-*D*-galactose.*—This compound (11 mg) had $[\alpha]_D$ +107° and gave a crystalline aniline derivative with melting point and mixed melting point 164–166°.

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