is given in the equation which follows for corn sirup of 20 Brix with a dilution of the sirup, 50.0 ml. to 500.0 ml.

$$\frac{12.02/\text{ml. titration}}{50.0/500.0 \times 20.00 \times 1.08096 \times \text{factor}} \times 100$$

This calculation requires the use of a factor, which is specific for the D.E. before D.E. is known. This impasse is avoided by obtaining D.E. additions in advance and applying the required addition to a given calculation based on Brix solids. The additions to be applied were derived from the data which appear in Table VI and are shown in Table VII.

The additions of D.E. required to correct for the overestimation of solids in corn sirup and corn sugar by the Brix hydrometer are surprising and unexpectedly simple. The data are particularly useful in the analysis of such sirups as first and second greens obtained in the manufacture of dextrose. Hydrol, corn sugar molasses, or second greens will vary in D.E. within the range of 68 to 75, and the salt within the range of 6 to 10% dry basis. Thus, the D.E. of the sugar itself with range from 75 to 88 D.E. obtained by the vacuum procedure for solids and the arbitrary addition of 0.5 D.E. to the D.E. obtained by the above method of analyses will be within 0.1 D.E., well within the tolerance of the procedure for the reducing sugar determination.

Laboratories in the corn wet milling industry determine a very large number of D.E.'s daily, particularly if refined corn sugar or dextrose is manufactured. To save time and expedite results to the plant, most laboratories have large tables with coordinates of Brix and "milliliters of titration," which give the D.E. for the specific condition involved. In the tables, the Brix values, 20° C., have been adjusted for the effect of salt and the D.E. value obtained carries the correction for the overestimation of solids by the Brix hydrometer. Abbreviated forms of the two tables are shown (Table VIII for corn sirup and Table IX for corn sugar sirups).

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PLANT PECTIN ANALYSIS

Determination of Pectic Substances by Paper Chromatography

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Physical methods of purifying several fruit and vegetable pectinic acids resulted in polymers containing nonuronide sugar units. The sugars, α-D-galactose, β-L-arabinose, α-L-rhamnose, and α-D-xylose, were separated from the hydrolysis products of apricot pectin by thick-paper chromatography and identified by comparing their x-ray powder diagrams with those of authentic specimens. Two widely different molecular species of galacturonic acid–containing polymers were separated from apricot polysaccharides. Attempts to resolve copper-purified apricot pectinic acid by further copper precipitation, dialysis, paper electrophoresis, and fractionation of the acetate into different molecular species were unsuccessful. Nonuronide sugars appear to be incorporated into the galacturonan molecule of pectic substances from most plant materials.

THE PECTIC POLYSACCHARIDES exist in plants in close physical union with araban and galactan. This association is so firm that upon extraction of polysaccharides from plants, a triad containing galacturonan, araban, and galactan is obtained. Because of the facility of paper chromatography for detection of small amounts of sugars in complex polymers, researches on composition of pectic substances have been stimulated in recent years.

Reports of composition of most fruit and vegetable pectins indicate that they are mixtures of polysaccharides or molecules apparently containing sugar residues other than galacturonic acid (*1, 4, 5, 7, 13, 16, 20, 24*). Many attempts to isolate a pure galacturonan from a wide variety of plant materials were unsuccessful unless strong chemical procedures were applied, which were suspected of hydrolyzing glycosidic linkages and degrading one or more of the carbohydrate polymers.

The question of composition and structure of undegraded pectic substances appears to be open to further investigation. Mainly, the unanswered question is whether pectin is a pure galacturonan admixed with the carbohydrates araban and galactan, or whether the so-called galacturonan is a complex carbohydrate containing some nonuronide sugars as part of the molecule.

The experiments reported here indicate the difficulties involved in attempts to prepare pure galacturonan from some natural materials and suggest that families of soluble complex galacturonic acid–containing polysaccharides occur in some plants.

Experimental

Analyses. Pectic substances isolated by precipitation from aqueous solution by ethyl alcohol were dried in vacuo at 60° C., ground to pass 60-mesh, humidified to an equilibrium moisture content of about 10%, and analyzed. Methods of analyses used were mainly those described by Owens *et al.* (*21*). Carbomethoxy (ester methoxyl) analyses were made by saponification, moisture analyses by oven drying in vacuo, ash analyses by incineration at 600° C., anhydrouronic acid analyses by a colorimetric carbazole method (*14*), and acetyl analyses by a hydroxamic acid color reaction (*15*). Rotations are specific rotations at 25° C. using the *D*-line of sodium. Intrinsic viscosities are those extrapolated to infinite dilution. Quantitative sugar analyses were done from reflection densities of colored spots on paper chromatograms (*17*).

Qualitative Chromatography. Polyuronides (1.0 gram in 50 ml. of 1*N* sulfuric acid) were boiled under reflux

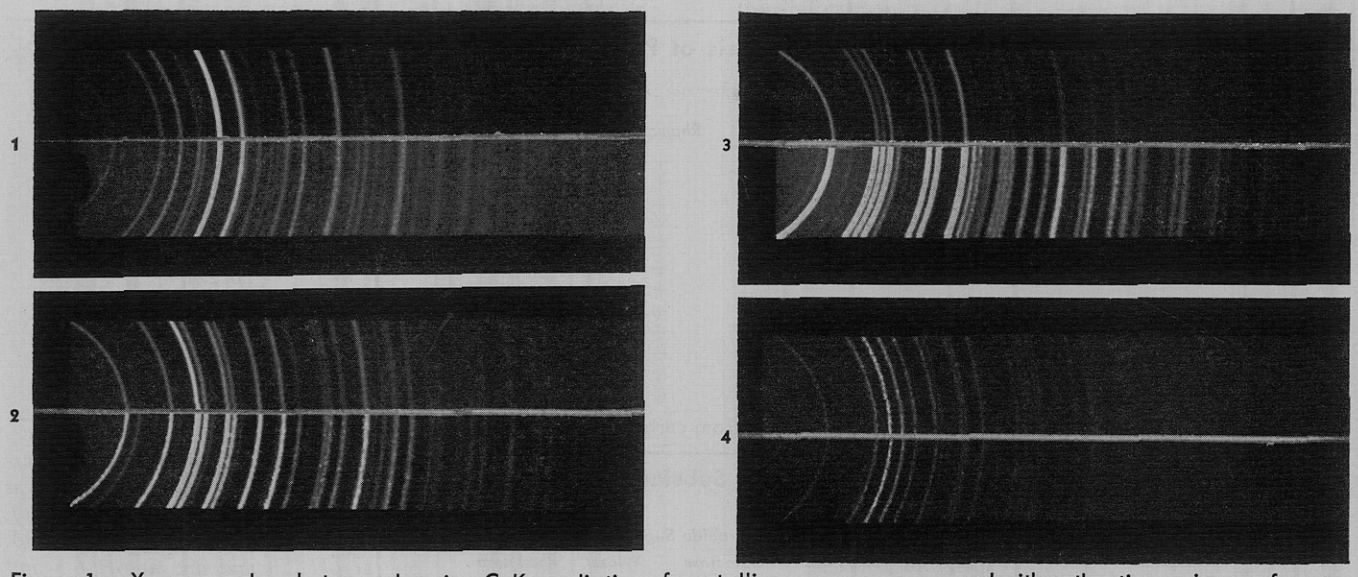

Figure 1. X-ray powder photographs using CuK_α radiation of crystalline sugars as compared with authentic specimens of sugars
Upper halves: unknowns. Lower halves: β-L-arabinose, α-D-galactose, α-L-rhamnose hydrate, and α-D-xylose, respectively

for 2.5 hours. The cooled solution containing the partial hydrolysis products of the polymer was neutralized with barium carbonate and the barium sulfate removed by filtration. The filtrate was concentrated to 10% solids for quantitative chromatography. When testing for sugars alone, 3 volumes of ethyl alcohol were added to precipitate the insoluble barium salts. The filtered solution was evaporated to a sirup for qualitative chromatography. Five-microliter spots of sirup were placed on the starting line of a sheet of Whatman No. 1 filter paper, and the chromatogram was developed in the ascending direction with a miscible solvent of ethyl acetate, pyridine, and water—5, 2.5, and 3 volumes, respectively. Sugars and galacturonic acid were revealed as brown or red spots on the dried chromatogram after dipping in ethyl acetate solution containing 2% each of aniline and trichloroacetic acid, followed by heating for 5 minutes at 85° C.

Extraction and Isolation of Pectin. Two hundred and fifty grams of fresh sliced pectin-containing material, 10 grams of paper pulp, 1500 ml. of water, and sulfuric acid to pH 2 were heated together at boiling for 20 minutes. The disintegrated mass was filtered with suction on canvas precoated with a diatomaceous silica filter-aid. The cooled filtrate was poured into 2 volumes of 95% ethyl alcohol and left standing for 15 minutes, after which the precipitated pectin was strained and pressed on a nylon cloth. The pressed cake was shredded by hand, stirred in 5 volumes of 60% ethyl alcohol, pressed, shredded, washed with 95% ethyl alcohol, and dried in vacuo. The dried product was ground to pass 60-mesh for analysis.

Purification by Copper Precipitation. Pectic substances are quantitatively precipitated from aqueous solution by cupric ion and thus freed from copper-soluble polysaccharides (*19*). Pectinic acid (5 liters of 0.5% solution adjusted to pH 5.2) was precipitated with 250 ml. of 15% cupric sulfate solution. The precipitate was centrifuged, washed twice by suspending it in water, and recentrifuged. The precipitate was dissolved in 1 liter of water, when the pH was adjusted to 1.5 with hydrochloric acid. The pectinic acid was precipitated from this solution by pouring it slowly into 2 volumes of 95% ethyl alcohol. The gelatinous precipitate was strained from the solution on nylon cloth, pressed by hand, shredded in 10 volumes of 70% ethyl alcohol–1% hydrochloric acid, and pressed. This was repeated twice to remove copper ions, followed by washing three times in 70% ethyl alcohol to remove mineral acid. A final washing in 95% ethyl alcohol further dehydrated the pectic materials so that they dried in a fluffy condition.

Identification of Sugars. The sirup containing the partial hydrolysis products of 10 grams of purified apricot pectinic acid was applied to thick papers (Whatman No. 103, 0.03 inch thick) for large-scale separations of sugars for identification purposes. The technique employed has been described (*18*). The areas occupied by the sugars were excised in strips and eluted with water by capillary flow. The sugar solutions were treated batchwise with charcoal and ion exchange resins. Upon slow evaporation the sugars crystallized. The crystals were identified by comparing their x-ray powder diagrams with those of authentic specimens (Figure 1). No attempt was made here to crystallize and identify galacturonic acid as the acidic constituent of these pectinic acids. Qualitative paper chromatography of acid hydrolysis products has demonstrated that the sole acidic constituent was galacturonic acid (*8*). All of the polymers examined here contained only galacturonic acid as the acidic constituent.

Analyses of several copper-purified pectinic acids from various sources are shown in Table I. Pectinic acid showing 90% anhydrouronic acid and containing 10% carbomethoxyl, when corrected for 4.5% methylene (the oxygen of the carbomethoxyl is measured in the anhydrouronic acid), would show a corrected anhydrouronic acid content of about 94.5%. Several reports in the literature expressed analyses as galacturonic acid and as methoxyl, apparently not taking into account the anhydrous character of polymerized sugars or that only the methylene of the methoxyl should be used to correct for anhydrouronic acid.

The citrus pectins are those showing the highest anhydrouronic acid content and the highest accounted-for material. The other fruit and vegetable pectins are lower in anhydrouronic acid. Sugar beet pectin contains 4.5% acetyl, apricot pectin 2.3%, and the citrus pectins only 0.2%. Corrections in the anhydrouronic acid of these pectinic acids shown in Table I for acetyl were not made, but they would not be changed by more than a few per cent.

Purified pectinic acids from several sources are similar in nonuronide sugar composition. Some of these pectinic acids showed traces of several other sugars not identified, but a large group showed mainly the four sugars positively identified. Coleman *et al.* (*7*) and Carrao (*4*, *5*) demonstrated the presence of arabinose, galactose, xylose, and rhamnose among the hydrolysis products of pectic substances from coffee, grapes, and persimmons, respectively. In sisal pectic acid Aspinall and Canas-Rodrigues (*1*) found the above sugars plus D-glucose, 2-*O*-methyl-L-fucose, and 2-*O*-methyl-D-xylose. Newbold and Josyln (*20*) and McCready, Jeung, and Maclay (*16*) demonstrated the presence of rhamnose, galactose, and arabinose among the hydrolysis products of highly purified

Table I. Sugars Liberated by Partial Acid Hydrolysis of Purified Pectinic Acids

Source	Anhydrouronic Acid[a]	Arabinose	Galactose	Rhamnose	Xylose
Orange	92.1	+	+	+	0
Grapefruit	91.7	+	+	+	0
Lemon	90.4	+	+	+	0
Fig	87.1	+	+	+	0
Carrot	76.7	+	+	+	0
Apple	88.0	+	+	+	+
Peach	86.8	+	+	+	+
Pea pod	84.8	+	+	+	+
Apricot	83.1	+	+	+	+
Pear	82.6	+	+	+	+
Sugar beet	82.3	+	+	+	+
Avocado	79.0	+	+	+	+

[a] Content corrected for water, ash, and methylene impurity from carbomethoxyl.

Table II. Composition of Apricot Pectic Substances

Treatment	Anhydro-uronic Acid,[a] %	Nonuronide Sugars, %			
		Arabinose	Galactose	Xylose	Rhamnose
Unpurified	47.2	10.0	1.7	1.0	1.0
Oxalate extracted	...	17.0	3.5	1.0	1.0
Acid–ethyl alcohol ppt.	62.0	14.4	1.9	1.1	1.0
Copper ppt.					
1X	75.5	9.2	2.3	1.1	1.1
2X	76.0	9.0	2.0	1.0	1.0
Copper ppt. 1X and dialyzed	78.0	6.0	2.0	1.0	1.0
Pectic acid	...	7.2	2.5	0.9	0.9
Polygalacturonase-treated, dialyzed	24.0	8.0	13.0	2.0	2.0
Copper-soluble fraction	18.6	8.0	32.2	6.9	2.3

[a] Water- and ash-free basis.

Table III. Fractionation of Acetylated Apricot Pectinic Acid

Fraction	Weight, Grams	Rotation
1	0.028	
2	0.031	
3	0.211	+134
4	0.278	
5	0.325	
6	0.271	+166
7	0.330	
8	2.38	
9	2.28	+132
10	0.88	
Residue	0.45	+124

citrus pectic acids. Researches on tomato fruits (*24*), passion fruits (*13*), and other fruit and vegetable materials showed the presence of nonuronide sugars. Some of the reports have included the four sugars positively identified here plus glucose, mannose, and unidentified sugars from some pectinic acids.

Solms, Büchi, and Deuel (*22*) and Büchi and Deuel (*3*) isolated soluble galacturonic acid–containing carbohydrates from grapes that yielded an aldobiuronic acid, 2-α-D-galacturonopyranosido-L-rhamnopyranose. Hostettler and Deuel (*9*) showed that plantago mucilage contains a polymer which yields upon hydrolysis an aldobiuronic acid, 4-α D - galacturonopyranosido - D - xylose. Several gums and mucilages have been reported to contain galacturonic acid, galactose, arabinose, xylose, rhamnose, and other sugars. Jones and Smith (*11*) have suggested that gums might be regarded as carbohydrate polymers which contain D-glucuronic acid as the acidic constituent, and mucilages as those which contain D-galacturonic acid as the acidic moiety. An inspection of these definitions and the ones for pectic substances (*12*, *23*) reveals that none is adequate for the known carbohydrates in plants. There are many known exceptions to suggested classifications and it is likely that families of plant carbohydrates containing less than 10% and up to 100% of galacturonic acid and many kinds of nonuronide sugars may occur in plants.

The composition of apricot pectinic acid was of particular interest because of its low anhydrouronic acid content and the presence of four nonuronide sugars. Pectinic acid extracted from commercial Blenheim apricot purée with boiling water was isolated as described and purified by dissolving in water and precipitating in acidified ethyl alcohol.

Blenheim apricot purée was also extracted with ammonium oxalate–oxalic acid at pH 3.6 described by Bishop for sunflower heads (*2*) and isolated as described.

The partially purified apricot pectinic acid was dissolved in 0.5% solution, adjusted to pH 5.2, and precipitated with copper sulfate as described. A part was prepared for analysis and a part was dissolved, filtered, and precipitated a second time with copper and purified for analysis (Table II).

The copper-containing aqueous solution from the once copper-precipitated apricot pectinate was adjusted to pH 7. A precipitate of insoluble copper salts was removed by filtration. The solution was passed through a Dowex 50(H) ion exchange column to remove cupric ions, concentrated to 5% solids, and precipitated with 4 volumes of 95% ethyl alcohol. The product was washed with 95% ethyl alcohol and worked up as described. The compositions of the pectinic acid precipitated by cupric ion and that soluble as a copper salt are shown in Table II.

The insoluble fraction of copper-precipitated apricot pectinic acid was subjected to further tests for homogeneity. Two solutions of 0.5% pectinic acid, one at pH 2.8 and one at pH 6.5 and both containing phenyl mercuric nitrate as a preservative, were dialyzed in Visking cellulose sausage casings against distilled water for 12 days. The contents of the cellulose sausage casings were precipitated with ethyl alcohol; the pectinic acid was hydrolyzed with acid and sugars were determined in the products by qualitative chromatography. Both preparations contained like amounts and kinds of the four sugars previously described. Upon hydrolysis the material outside of the bags showed traces of arabinose. Analyses of the insoluble fraction are shown in Table II.

Purified apricot pectinic acid was converted to pectic acid by de-esterification at 25° C. with sodium hydroxide at pH 12. The pectic acid was isolated by precipitation with sulfuric acid at pH 1.5, and the precipitate was pressed and washed with water and then with ethyl alcohol (Table II).

The purified apricot pectic acid was dissolved in water and hydrolyzed with 0.01% of purified fungal polygalacturonase (*10*) at pH 4.5 and 25° C. for 2 days. After hydrolysis the solution was dialyzed in a cellulose sausage casing for 5 days. The content of the casing was concentrated and a polymer was precipitated with 3 volumes of ethyl alcohol. The analyses of the polygalacturonase-treated, dialyzed polymer are shown in Table II.

The results of the fractionation and purification of apricot pectinic acid shown in Table II indicate that pectinic acid mixtures were easily purified from an anhydrouronic acid content of 47% to 76% with copper precipitation. Further attempts to increase the anhydrouronic acid value by a second copper precipitation did not change the pattern of the sugar components. Most of the arabinose can be hydrolyzed from apricot pectinic acid by boiling in 0.02*N* oxalic acid for 2.5 hours. Because the arabinose moiety appears to be a furanoside and very labile to acid hydrolysis,

quantitative changes in amounts readily occur whenever acid conditions are used to extract or purify pectic substances. For these reasons the composition of pectic substances as they exist in apricots may not be accurately represented as shown in Table II.

Analyses of the copper-insoluble and copper-soluble materials as shown in Table II show that at least two groups of galacturonic acid–containing carbohydrates occur in apricot pectic substances. The copper-soluble fraction showed 18.6% of anhydrouronic acid and a low intrinsic viscosity of 0.86. The low molecular weight and the insufficient number of acid groups permit it to remain soluble in the presence of cupric ion. This fraction may not be homogeneous but may be a mixture of several molecular species that have similar solubility properties.

Analysis of the copper-insoluble apricot pectinic acid showed 76% anhydrouronic acid and an intrinsic viscosity of 2.66. The copper-precipitated lemon pectinic acid yielded 85% anhydrouronic acid, and a molecular weight of 35,000 based on an intrinsic viscosity of 5.5. These materials both have high molecular weights and high anhydrouronic acid contents and precipitate quantitatively from aqueous solution with cupric ion.

Under the conditions of this experiment, citrus polygalacturonic acid hydrolyzed completely to dialyzable fragments, whereas the polygalacturonase-treated apricot pectic acid yielded an undialyzable residue containing 24% anhydrouronic acid. If the nonuronide sugars were not part of the apricot pectic acid molecule, it would have been hydrolyzed to dialyzable galacturonic and oligouronic acids, leaving only the nonuronide carbohydrate residues.

Copper-purified apricot acid was subjected to paper electrophoresis in an attempt to show the presence of polymers such as araban and galactan. A 1% solution was applied on a sheet of Whatman 3MM filter paper as a line in the center of a 40-cm. filter paper strip, which had been previously immersed in buffer solution and blotted. The moist paper strip was mounted between glass plates and subjected to a potential of 300 volts for 6 hours. The carbohydrate materials moved in a broad band 10 cm. in width and showed no separation. Attempts to make a separation in several different buffer solutions gave similar results.

The apricot pectinic acid was further tested for homogeneity by attempted fractionation of the acetate. Eight grams of apricot pectinic acid yielded 9 grams of acetate by the method of Carson and Maclay (*6*). Fractionation was done by using 7.5 grams of acetate dissolved in 500 ml. of chloroform. Skellysolve B was added in 50-ml. increments, and the precipitate when formed was centrifuged. The solvent was decanted and the residue dried in vacuo. Fractions were collected after addition of ten 50-ml. increments of solvent and a residue of 0.45 gram remained dissolved. Results are summarized in Table III. Hydrolysis and chromatography of these fractions failed to show that a fractionation of molecular species had been made. Galacturonic acid and the nonuronide sugars occurred in the same proportions as in the original acetate. The optical rotation of combined fractions 2, 3, and 4 was +134; fractions 5 and 6, +166; fractions 8 and 9, +132. The residue gave a rotation of +124. The very small changes in rotation and the fact that there was no trend in the rotation changes indicated that no major fractionation of molecular species was accomplished. It appears that the fractionation was made on the basis of molecular weight.

Discussion

There is little doubt that pectic substances examined here and elsewhere contain polymerized nonuronide sugars. Determination of their mode of linkage has not yet been established, because pectic substances are difficult to purify and labile to alkali, resist exhaustive methylation, and are associated with araban, galactan, and other polysaccharides. An exception was the pectin from sunflower heads, which was extracted with oxalate and proved to be a pure galacturonan (*2*). The oxalate procedure was applied to apricots, but the resulting pectic substances contained nonuronide sugars in proportions comparable to those extracted by other means. Studies of polymers such as the copper-soluble fraction high in nonuronide sugars may permit the characterization of these materials.

Many researches reported nonuronide sugars among the hydrolysis products of pectic substances other than the four identified in this work. The composition of pectic substances from various plants appears to be more complex than results of analysis made on the materials shown here. There were traces of unidentified reducing substances among the hydrolysis products of several of the pectinic acids shown in Table I and the nonuronide composition of these selected pectinic acids as they occur in plants is by no means settled.

Mucilages contain all of the constituents found in these pectic substances and traces of a number of other sugars. Some of the so-called pectic substances might be inseparable from mucilages, and the reverse may also be true. The definitions do not describe the carbohydrate polymers as they occur in plants, and polymers containing from high to low contents of galacturonic acid occur.

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