THE CARBOHYDRATE COMPONENTS OF QUINCE SEED.
(CYDONIA VULGARIS)

-by-

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INTRODUCTION

Plant mucilages are naturally occurring polysaccharides of high molecular weight which form colloidal solutions in water. They are widespread in nature and may occur in any part of the plant, either as secondary membrane thickening material, or as an intercellular substance. The mucilage is sometimes present as a food reserve, and in plants living in arid conditions it may act as a water reservoir. The extremely hydrophilic nature of the mucilages extracted from the coatings of many seeds seems to indicate that here the function of the mucilage is to provide a moist environment, suitable for the germination of the seed.

Chemically, the plant mucilages may be divided into three classes:

1. Neutral polysaccharides, consisting of one or more sugar residues joined together through their reducing groups, giving substances of high molecular weight.

2. Polysaccharides containing uronic acid residues as well as other sugar residues.

3. Mucilages which occur in seaweed and consist of the salts of sulphate esters of sugar derivatives of high molecular weight.

The seed mucilages fall into the second category, and mucilages of this type are very similar to the water-soluble plant gums. One important difference is that, as a rule, the uronic acid residue present in mucilages is that of galacturonic acid, whereas glucuronic acid or methyl glucuronic acid is usually found in the plant gums. Exceptions/
Exceptions are known, however, for example the uronic acid present in gum tragacanth has been identified as galacturonic acid, not glucuronic acid.

Biologically, the plant gums and mucilages are quite different. The latter are present in the plant under normal conditions, whereas the plant gums are thought to be synthesised from starch granules after a plant has been injured either mechanically or by the invasion of fungi or bacteria. Whether the production of the gum is brought about by the invading fungi or bacteria, or whether the injury directly causes the plant to synthesise the gum is as yet unknown, but apparently the function of the gum is to seal off the injured part and protect the plant from further damage.

The constitution of a plant gum is usually invariable and is thought to be characteristic of the botanical species or variety of the plant from which the gum has been obtained. Some gums have been shown to be mixtures of polysaccharides, for example gum tragacanth \(^{(1,2)}\) is a mixture of a complex acid polysaccharide, tragacanthic acid, and a neutral polysaccharide composed mainly of L-arabinose residues. Usually, a plant gum consists solely of a neutral salt of a polyuronide which may readily be obtained in its acidic form by dehydration with acidified alcohol, giving a material which, after further drying, is suitable for analysis.

Many mucilages, however, are mixtures of polysaccharides. For example, the mucilage from white mustard seed \(^{(3)}\) has been shown/
shown to contain cellulose and two other materials, one of which was hydrolysed, giving, rhamnose, arabinose, galactose, glucose and glucuronic acid, while the other gave, after hydrolysis, arabinose, galactose, galacturonic acid and glucuronic acid. Linseed mucilage (4) has been fractionated by means of copper acetate solution to give a water-insoluble salt (45%) and a water-soluble fraction (55%).

The presence of cellulose has been observed in several other mucilages, including cress seed (5) and quince seed mucilages (6, 7, 8). In several of the plantago seed mucilages a material resistant to normal conditions of hydrolysis has been observed; under more severe conditions of hydrolysis this material gave D-glucose only (9) and presumably these again are examples of a polyuronide conferring solubility on the otherwise insoluble cellulose molecule.

The study of a polysaccharide which may be composed of several sugar residues and an uronic acid residue presents a problem of great difficulty to the investigator. Hydrolysis with dilute acid gives the sugars whose residues compose the molecule, as well as an aldobiuronic acid which consists of a monosaccharide linked with an uronic acid, the linkage here being more resistant to hydrolysis. The sugars most commonly found in such polysaccharides are D-galactose, D-mannose, L-rhamnose, L-fucose, L-arabinose and D-xylose. Obviously the analysis of a mixture of two or more sugars is no easy task, but in recent years the technique of paper chromatography (10, 11, 12, 13) has made what was once a very difficult problem much more easy.
of this technique the separation of the sugars in a mixture may be effected without the preparation of derivatives, and the sugars determined both qualitatively and quantitatively.

While the constituent sugars of a polysaccharide may thus be readily identified, the way in which they are combined in the polysaccharide is not indicated. The most fruitful line of investigation of this aspect of the problem is to methylate the polysaccharide, and then hydrolyse the methylated derivative. There will thus be obtained methylated sugars which will possess free hydroxyl groups only on those carbon atoms which were involved in linkages in the polysaccharide. If the methylated sugars so obtained can be identified and determined quantitatively, it will be possible to propose a structure for the gum or mucilage.

The successful methylation of a polyuronide often proves to be difficult. The method of Purdie and Irvine, using methyl iodide and silver oxide, is of great value, but it is usually preceded by several methylations according to the method of W.N. Haworth, using dimethyl sulphate and sodium hydroxide, in order that the polyuronide molecule becomes partially methylated and thus more soluble in organic solvents. Even by using these two methods successively, the introduction of methyl groups into the molecule is still difficult. In recent years, use has been made of the discovery of Menzies, that the thallium derivatives of alcohols react with methyl iodide to give thallous/
thallous iodide and the methyl ether of the alcohol. Hirst and co-workers have used this method with success in the study of several carbohydrates, e.g. cherry gum (17) and the mucilage present in the bark of the slippery elm (ulmus fulva) (18). With these methods available, complete methylation, though difficult, can usually be achieved.

After hydrolysis of the methylated polysaccharide, the sugar derivatives obtained must be separated from one another and identified. A reasonably good separation can usually be obtained by converting them into their methyl glycosides, followed by fractional distillation of the mixture in high vacuum. Unfortunately, comparatively large quantities are required for this method, and the danger of decomposing the less volatile fractions is considerable. Recently, the development of chromatography in the carbohydrate field has enabled the investigator to separate methylated sugars on a column of powdered cellulose (12). This technique has been and is being developed very rapidly and enables the research worker to work with much smaller quantities than previously, and causes no decomposition of the fractions being separated. Fully methylated, partially methylated and free sugars may also be separated by the method of solvent extraction of Brown and Jones (19).

By these methods it is possible to isolate pure methylated derivatives of the sugars which must then be identified. This identification is dependent upon the methods which have been developed for the elucidation of the structure of sugar derivatives and upon comparison with/
with derivatives of known structure. Thus the investigator of today is indebted to those earlier workers whose researches have provided him with such a large store of knowledge of the structure and properties of the monosaccharides and their derivatives.

In the elucidation of the structure of polysaccharides another important line of approach is afforded by the study of the products obtained after oxidation with periodic acid. This reagent attacks the linkage between adjacent carbon atoms possessing free hydroxyl groups, and gives valuable evidence concerning the mode of linkage of the sugar residues making up the molecule and also concerning the branching of chains in the molecule. This method has been used with success in the study of egg plum gum.

Mild hydrolysis of the polysaccharide under investigation often removes disaccharides whose structure may be determined by the usual methods of methylation and hydrolysis. This proves the position of the linkage between two of the sugars and consequently gives evidence concerning the order of arrangement of the sugar residues in the polysaccharide. F. Smith, in his study of gum arabic, isolated, after mild hydrolysis, the disaccharides 3-D-galactosido-L-arabinose(I) and 3-D-galactosido-D-galactose(II)
Smith also obtained, after mild hydrolysis, a degraded polysaccharide and reducing sugars, the latter consisting mainly of L-arabinose. The comparison of results obtained from the investigation of this degraded gum with those he obtained from the study of gum arabic itself gave valuable information about the structure of the polysaccharide.

Gum arabic has been studied more fully than any other plant gum or mucilage, and a review of the investigations of it gives an indication of the complexity of the problems involved in the elucidation of such polysaccharides.

Butler and Cretcher\(^{(24)}\) found that gum arabic, on acid hydrolysis, yielded D-galactose (2 moles), L-arabinose (3 moles), L-rhamnose (1 mole) with an aldobiuronic acid (1 mole). This aldobiuronic acid was found to consist of D-galactose and D-glucuronic acid. It was also found that the methyl ether of the aldobiuronic acid gave, on methanolysis, 2:3:4-trimethyl methyl D-galactoside and the methyl ester of 2:3:4-trimethyl methyl D-glucuronoside; the aldobiuronic acid was therefore concluded to be 6-\text{D-glucuronosido-} D\text{-galactose(III)}\(^{(25,23)}\)

\[\text{III}\]

F. Smith\(^{(23)}\) found that autohydrolysis of the gum gave L-arabinose, L-rhamnose and a disaccharide which was identified/
identified as 3-D-galactosido-L-arabinose(I). Removal of these labile residues left a degraded arabic acid which on further autohydrolysis yielded a small quantity of a disaccharide which was shown to be 3-D-galactosido-D-galactose(II).

The degraded gum was methylated and the methylated polysaccharide hydrolysed to give 2:3:4-trimethyl-D-galactose (5 moles), 2:4-dimethyl D-galactose (3 moles), 2:3:4-trimethyl D-glucuronic acid (3 moles) and 2:3:4:6-tetramethyl D-galactose (1 mole).

This evidence shows that the one molecule of tetramethyl galactose and the three molecules of trimethyl glucuronic acid are present as end groups in the methylated degraded gum. Since there are, out of the twelve residues present, four end groups, the molecule must be very branched. The 2:3:4-trimethyl galactose observed must have been derived from galactose linked in the degraded gum through carbon atoms 1 and 6. Similarly the 2:4-dimethyl galactose is a derivative of a galactose residue linked through positions 1, 3 and 6.

Smith later obtained, by controlled hydrolysis of the methylated degraded gum, the aldobiuronic acid derivative 6-(2:3:4-trimethyl D-glucuronosido) 2:3:4-trimethyl D-galactose(IV) which is evidence that the side chains consist of aldobiuronic acid units.
As a result of this evidence, Smith put forward the following structure (V) for the repeating unit of twelve sugar residues in gum arabic:

\[ \begin{array}{cccccccc}
\text{G} & \text{G} & \text{G} & \text{G} & \text{G} \\
\text{G} & \text{G} & \text{G} & \text{G} & \text{G} \\
\text{G} & \text{G} & \text{G} & \text{G} & \text{G} \\
\text{G} & \text{G} & \text{G} & \text{G} & \text{G} \\
\text{G} & \text{G} & \text{G} & \text{G} & \text{G} \\
\text{G} & \text{G} & \text{G} & \text{G} & \text{G} \\
\text{G} & \text{G} & \text{G} & \text{G} & \text{G} \\
\text{G} & \text{G} & \text{G} & \text{G} & \text{G} \\
\text{G} & \text{G} & \text{G} & \text{G} & \text{G} \\
\text{G} & \text{G} & \text{G} & \text{G} & \text{G} \\
\text{G} & \text{G} & \text{G} & \text{G} & \text{G} \\
\end{array} \]

where G represents D-galactopyranose
GLU represents D-glucuronic acid
a = 6, b = 3 or vice versa.

Hydrolysis of the methylated product from gum arabic itself gave more evidence as to the positions of the arabinose, rhamnose and galactose units in the gum. These sugars gave the following methylated derivatives:

- 2:3:4:6-tetramethyl D-galactose
- 2:3:4-trimethyl L-rhamnose
- 2:3:5-trimethyl L-arabinose
- 2:5-dimethyl L-arabinose
- 2:4-dimethyl D-galactose
- 2:3-dimethyl D-glucuronic acid
- 2:3:4-trimethyl D-glucuronic acid

From these results it is obvious that the labile rhamnopyranose, arabinofuranose and the 3-galactosido-L-arabinofuranose residues are attached to the skeleton structure of the degraded gum by glycosidic linkages. Since no 2:3:4-trimethyl D-galactose was obtained - only 2:4-dimethyl galactose - the three position of the galactose/
galactose must be engaged in the linkage of side chains consisting of labile residues.

From this evidence Smith proposed a structure (VI) for the repeating unit in gum arabic which would account for the methylated derivatives produced. Other structures are possible, but they are few and, if they are to remain in accordance with the evidence obtained, they must closely resemble Smith's.

\[
\begin{align*}
\text{G} & \quad 3 \text{G}_1 \quad 3 \text{G}_6 \\
\text{A} & \quad 3 \text{A}_1 \\
\text{A} & \quad 4 \text{A}_4 \\
\text{A} & \quad 4 \text{A}_4
\end{align*}
\]

\[
\text{VI}
\]

\[
\begin{align*}
\text{G} & = \text{D-galactopyranose} \\
\text{GL} & = \text{D-glucuronic acid} \\
\text{R} & = \text{L-rhamnopyranose} \\
\text{A} & = \text{L-arabofuranose}
\end{align*}
\]

Several of the other plant gums which have been investigated show a similarity in structure to gum arabic, for example egg plum gum \(^{(22)}\) yields an aldobiuronic acid identical with that from gum arabic and also gives a degraded gum which contains the same sugars in the same proportions as those present in degraded gum arabic, though it is apparently differently constituted.

The plant mucilages have not, as yet, received the same thorough investigation which the plant gums have, partly because it is often difficult to obtain a pure product/
product. Moreover, slightly different methods of extraction often give quite different products. Indeed, Bailey and Norris in their study of the mucilage from white mustard seed\(^{(26)}\) found that two extractions of the seeds, under identical conditions, did not yield identical products.

Few mucilages have been subjected to methylation studies, and, of them, probably the mucilage present in the bark of the slippery elm (ulmus fulva)\(^{(18,27)}\) has been studied in most detail. This material was found to contain some 10% of associated protein, but the carbohydrate material yielded, on hydrolysis with dilute acid, L-rhamnose, D-galactose and an aldobiuronic acid which consisted of L-rhamnose and D-galactose. Gill, Hirst and Jones proved the aldobiuronic acid to be 2-D-galacturonosido-L-rhamnose by means of the methylation procedure. This aldobiuronic acid is also obtained on hydrolysis of linseed mucilage\(^{(26)}\).

Methylation of the mucilage was achieved by employing the Menzies methylation technique\(^{(16)}\) which was mentioned previously. Hydrolysis of the methylated material gave a mixture of sugar derivatives whose glycosides were separated by distillation in high vacuum. These gave on hydrolysis, the following methylated sugars:-

\[
\begin{align*}
3:4- & \text{ dimethyl L-rhamnose} \\
4- & \text{ monomethyl L-rhamnose} \\
2:3:4:6- & \text{ tetramethyl D-galactose} \\
2:4:6- & \text{ trimethyl D-galactose} \\
2:3:6- & \text{ trimethyl D-galactose} \\
2:3:4- & \text{ trimethyl D-galacturonic acid} \\
2:3- & \text{ dimethyl D-galacturonic acid}
\end{align*}
\]
Despite this evidence, it is not yet possible to propose an unique structure for the mucilage or indeed to be certain that it contains a standard repeating unit. Much more evidence must be obtained before this can be done, though the following formula (VII) has been proposed and is in accordance with the experimental results:

\[ \text{VII} \]

where \( R = \text{L-rhamnopyranose} \)  
\( GA = \text{D-galacturonic acid (pyranose ring)} \)
\( G = \text{D-galactopyranose} \)

Of the seed mucilages, only those obtained from plantago seeds have been investigated by the methylation technique.\(^{29,9,30,31}\) Anderson found that hydrolysis of the mucilages from Indian wheat (\textit{plantago fastigiata})\(^{32}\) and light psyllium seeds (\textit{plantago ovata forsk})\(^{33}\) both gave D-xylose, L-arabinose and D-galacturonic acid.

Further work\(^{9}\) on the mucilage from the seeds of \textit{plantago ovata} showed that it contained an aldobiuronic acid consisting of D-galacturonic acid and L-rhamnose. After methylation studies this aldobiuronic acid was proved to be 2-D-galacturonosido-L-rhamnose.

The seed mucilage from the seeds of \textit{plantago arenaria} gave an aldobiuronic acid which was thought to be composed of xylose and galacturonic acid.\(^{29}\) It has, however, recently/
recently been shown to be 2-D-galacturronosido-L-rhamnose. Thus the mucilages from the bark of the slippery elm, linseed, plantago ovata seed and plantago arenaria seed all contain the same aldobiuronic acid. Along with this aldobiuronic acid hydrolysis of the mucilage from plantago arenaria gave the sugars L-arabinose, D-xylose and D-galactose.

Acetylation, followed by methylation by the Haworth technique, of the mucilage obtained by cold water extraction of the seeds from plantago ovata gave a material which yielded, on methanolysis, the following sugar derivatives:

- 2:3:4-trimethyl methyl D-xylopyranoside (6 moles)
- 2:3:5-trimethyl methyl L-arabofuranoside (1 mole)
- 2:4-dimethyl methyl D-xylopyranoside (1 mole)
- 3-monomethyl methyl D-xylopyranoside (4 moles)
- 2-monomethyl methyl D-xylopyranoside (1 mole)
- D-xylopyranoside (1 mole)

These results indicate that the sugar residues are linked as follows in the polysaccharide:

\[
\begin{align*}
X & \quad \Delta \quad \Delta \quad \Delta \\
1 & \quad 1 & \quad 1 & \quad 1 \\
2 & \quad 3 & \quad 4 & \quad 4
\end{align*}
\]

\(X = D-XYLOPYRANOSIDE\)

\(A = L-ARABOFURANOSIDE\)

It will be observed that no methylated uronic acids or rhamnose derivatives were found in the hydrolysis products of the methylated mucilage. Direct methylation of the polysaccharide showed that it could be separated into/
into a water-soluble partially methylated product containing uronic acid residues and an insoluble fraction containing no uronic acid. This gives support to the view that the mucilage may not be composed of a single acidic polysaccharide but consists of a mixture of a polyuronide molecule associated with a neutral polysaccharide.

Methylation of the mucilage from plantago arenaria seed\(^{(34)}\) gave a water insoluble product which on hydrolysis gave methylated sugars but again no methylated uronic acid, or methylated rhamnose. Assuming that the mucilage consists of a polyuronide molecule and a neutral polysaccharide, it has been suggested that possibly the two are associated by ester linkages which are disrupted by the strong sodium hydroxide solution used in methylation. Evidence in support of this was obtained when it was found that, by standing the mucilage in 2\(N\) sodium hydroxide solution for several days, followed by electrodialysis after the alkali had been neutralised, the uronic acid content of the undialysed material\((2.5\%)\) was considerably less than the value found before this treatment\((7.5\%)\). Electrodialysis of an aqueous solution of the mucilage gave an undialysed material whose uronic anhydride content was still 7.5%.

This short review of some of the mucilages which have been investigated will suffice to show that the information we possess about them is by no means complete, especially in the case of the seed mucilages.

The mucilage which is the subject of this investigation was obtained from the seeds of the quince, a small shallow rooted/
rooted tree of the order Rosaceae. It grows abundantly in semi-arid regions, the main source of quince seeds being Iran and Iraq. The quince is also grown in the United States, Switzerland and Japan, though the Japanese quince is a slightly different variety.

The leaf, fruits and flower of the quince closely resemble those of the apple and pear trees. Like the apple, the ripe fruit contains five ripened carpels which constitute a core, and each carpel contains six to fifteen seeds, arranged in two rows. The seeds are egg-shaped and reddish-brown in colour being 6-7mm. long. They are white within and are surrounded with a leather-like membrane, abounding in mucilage.

Quince seed mucilage is used commercially as a stabiliser and thickener, and also for its demulcent properties. It is employed in the manufacture of preserves and in the cosmetic industry as a component of hand, cleansing and hair-setting lotions.

For these purposes the mucilage solution is usually freshly prepared from the seeds by macerating them with 50 parts of boiling water for 30 minutes, then filtering the solution through muslin.

Quince seed mucilage is first mentioned in scientific literature in 1844, being part of a review of seed mucilages by Schmidt[6] Kirchner and Tollens[7] in 1876 published an account of their work on the purification of the mucilage. They also mentioned the isolation of cellulose from the mucilage by hydrolysis with dilute sulphuric/
sulphuric acid; the cellulose representing 30% of the mucilage hydrolysed. Bauer in 1893 reported that hydrolysis of the mucilage gave a solution of a sugar which could not be crystallised, but whose optical rotation was approximately equal to that of glucose and gave a phenylosazone, m.p. 204°C. (presumably glucosazone)

In more recent years quince seed mucilage has been investigated by Renfrew and Cretcher. They extracted quince seeds with alcohol and ether, and then soaked the seeds in water for 24 hours. After filtration the mucilage was precipitated by the addition of alcohol, it was then dried by washing with alcohol and acetone and pressed in a hydraulic press. To obtain a product which gave less ash on incineration, the precipitation was effected in alcohol containing 1% hydrochloric acid. The material obtained in this way gave the following figures on analysis:

<table>
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<th>Component</th>
<th>Content</th>
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<tr>
<td>Methoxyl content</td>
<td>3.3%</td>
</tr>
<tr>
<td>Uronic Acid content</td>
<td>27.8%</td>
</tr>
<tr>
<td>Pentose (corrected for uronic acid)</td>
<td>33.0%</td>
</tr>
<tr>
<td>Cellulose</td>
<td>33.0%</td>
</tr>
</tbody>
</table>

Hydrolysis of the mucilage with 0.5N sulphuric acid gave an insoluble material equal to one third of the weight of the mucilage treated. This material gave a 78% yield of glucose on hydrolysis by the method of Monier-Williams.

The solution obtained on hydrolysis, after neutralisation with barium carbonate, gave an alcohol insoluble salt whose analysis corresponded closely with that calculated for a barium/
barium salt of an aldobiuronic acid composed of 1 molecule of a hexuronic acid and 1 molecule of a pentose. This salt was found to have a methoxyl content of 5.5%, which indicates that it is a mixture of the salts of a monomethyl aldobiuronic acid (72%) and an unmethylated aldobiuronic acid (28%).

Hydrolysis of this aldobiuronic acid gave xylose and a mixture of a monomethyl uronic acid (60%) and an unmethylated uronic acid (40%). The investigators were unable to obtain either mucic acid or potassium acid saccharate by oxidation of the mixture of uronic acids.

The alcohol used in the precipitation of the barium aldobiuronate was found to contain arabinose, isolated as the diphenylhydrazone. No galactose was identified in the alcoholic solution.

This present investigation was undertaken in the hope that, with the aid of the many new techniques developed for the study of carbohydrates, some further knowledge might be gained concerning this interesting mucilage, and possibly some insight into its molecular structure obtained.
NOTE

All distillations described in the experimental section were carried out at 40°C/18mm. pressure unless otherwise stated.

All melting points quoted were uncorrected and were performed by the method described in Campbell's "Qualitative Organic Chemistry", page 7. By this method the powdered solid is heated in a capillary tube next to the bulb of a thermometer immersed in a pear-shaped flask containing dibutyl phthallate. This flask is heated by a micro-burner and the dibutyl phthallate stirred by convection currents. The thermometer reading at the moment of melting is quoted as the melting point.

Methoxyl determinations were carried out by the micro-Zeisel method as described in Pregl. Roth's "Quantitative Organic Microanalysis", page 171 (3rd Edition).

Free sugars were identified by paper chromatographic analysis by direct comparison with controls, using two solvents:

(1) The upper layer of a mixture of butanol/pyridine/water/benzene (5/3/3/1, V/V) as mobile phase, in the atmosphere of the lower layer. This solvent was used to identify galactose and glucose in the presence of each other and other sugars.

(2) The upper layer of a mixture of ethyl acetate/water/ acetic acid (3/3/1, V/V) as mobile phase in the atmosphere of the lower layer. This solvent was used to identify mannose/
mannose, arabinose and xylose in the presence of each other and other sugars.

Where other solvents are used, these are indicated.
EXPERIMENTAL

PREPARATION OF THE NEUTRAL MUCILAGE.

Quince seeds (400gm.) were soaked in water (8litres) at room temperature, with occasional stirring for 24 hours. The extremely viscous mucilage solution was filtered through muslin and concentrated to 2 l. This solution was poured into four volumes of methylated spirit, giving a gelatinous precipitate which was washed with acetone till crisp, then dried in a vacuum dessicator in the presence of calcium chloride. A 6.5% yield of a pale brown fibrous material was obtained.

Moisture Content. A weighed sample of the mucilage was dried in the presence of phosphoric oxide at 50°C (bath temperature) at a pressure of 0.01mm. mercury for 16 hours, then reweighed. The loss in weight of the sample during this treatment was taken as the moisture content.

*Found:* Moisture Content = 14.0%.

Residual Ash. A weighed sample of the mucilage was incinerated to constant weight in a platinum crucible and the ash weighed.

*Found:* Residual Ash = 16.4%.

Corrected for moisture = 19.1%.

Uronic acid Content. A weighed sample (ca.0.2gm.) of the mucilage was heated at 145°C (bath temperature) with 19% hydrochloric acid (30ml.) and the amount of carbon dioxide evolved estimated. The determination was carried out according to the method of Swenson, and McCready, and McIlvay.

*Found/*
Examination of the Alcohol after Precipitation of the Mucilage.

The methylated spirit used in the precipitation of the mucilage was concentrated to a syrup which reduced Fehling's solution. Study of this syrup by paper chromatography showed that it contained sugars which corresponded identically on paper chromatograms with authentic specimens of glucose and mannose.

PREPARATION OF THE ACIDIC MUCILAGE.

(a) The acidic mucilage was prepared as described above, except that the precipitation was effected in methylated spirit containing 1N hydrochloric acid (20ml/l). The precipitate was washed with methylated spirit till free from chloride ions, then with acetone till crisp, and finally dried in a vacuum desiccator in the presence of calcium chloride. The product was a brown fibrous material obtained in 6 per cent. yield.

(b) Quince seeds (200gm.) were soaked in water (4 l.) for 20 hours. The mucilage solution was filtered through muslin and concentrated to 2 l. This solution was poured into 4 volumes of glacial acetic acid and the mixture vigorously/
vigorously stirred. The precipitate obtained was filtered off, washed with methylated spirit till the washings were no longer acid, then with acetone till crisp and finally dried in a vacuum dessicator. The product was a white fibrous material, obtained in 6.3% yield.

**Found:**
- Moisture Content = 14.1%
- Residual Ash = 4.0%
- corrected for moisture = 4.4%
- Uronic Anhydride Content = 24.1%
- corrected for moisture = 28.1%
- Methoxyl Content (CH$_3$O) = 5.4%
- corrected for moisture = 6.3%

**HYDROLYSIS OF THE MUCILAGE (I):**

The neutral mucilage (3gm.) was heated at 100°C with sulphuric acid (160ml. 0.5N). At frequent intervals samples (ca. 15ml.) were withdrawn, filtered and cooled. 5ml. of each sample were added to sodium hydroxide solution (50ml. 0.1N) and iodine solution (10ml. 0.1N) added from a pipette. After standing for 45 minutes in the dark at room temperature, the solution was acidified with sulphuric acid (10ml. 1N) and the residual iodine determined by titration with standard sodium thiosulphate solution (0.05N). In this way the progress of the hydrolysis was followed.

The remainder of each sample was neutralised with barium carbonate, filtered and concentrated to small volume (ca. 2ml.). These concentrated solutions were poured into methylated/
methyleated spirit giving, in each case, a white precipitate which was removed at the centrifuge. The methylated spirit solutions were concentrated to syrups and the sugars present in each syrup determined by paper chromatography using standard sugar solutions for comparison.

**Results:**

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<thead>
<tr>
<th>Sample</th>
<th>Time of Withdrawal (hours)</th>
<th>0.1N Iodine Uptake</th>
<th>Sugars present in the sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.5 hours</td>
<td>1.16ml.</td>
<td>Arabinose, Xylose(trace) Galactose(trace).</td>
</tr>
<tr>
<td>II</td>
<td>1 &quot;</td>
<td>1.64ml.</td>
<td>Arabinose, Xylose Galactose(trace)</td>
</tr>
<tr>
<td>III</td>
<td>2 &quot;</td>
<td>2.50ml.</td>
<td>Arabinose, Xylose, Galactose, Glucose(trace).</td>
</tr>
<tr>
<td>IV</td>
<td>3 &quot;</td>
<td>2.98ml.</td>
<td>Arabinose, Xylose, Galactose, Glucose(trace).</td>
</tr>
<tr>
<td>V</td>
<td>4 &quot;</td>
<td>3.66ml.</td>
<td>Arabinose, Xylose, Galactose, Glucose.</td>
</tr>
<tr>
<td>VI</td>
<td>5 &quot;</td>
<td>4.08ml.</td>
<td>Arabinose, Xylose, Galactose, Glucose.</td>
</tr>
<tr>
<td>VII</td>
<td>6 &quot;</td>
<td>4.24ml.</td>
<td>Arabinose, Xylose, Galactose, Glucose.</td>
</tr>
<tr>
<td>VIII</td>
<td>7 &quot;</td>
<td>4.20ml.</td>
<td>Arabinose, Xylose, Galactose, Glucose.</td>
</tr>
</tbody>
</table>
In all samples the paper chromatograms showed the presence of uronic acids or uronic acid derivatives, but it was not found possible to identify these.

The alcohol-insoluble barium salt obtained from the first sample was washed with methylated spirit at the centrifuge, then dried in a vacuum desiccator giving a white powder A1.

The alcohol insoluble barium salt obtained from sample VIII was treated in a similar manner giving a white powder A2.

At the end of the hydrolysis the solid material remaining in the reaction flask was filtered off, washed thoroughly with water and dried in a vacuum desiccator, giving a brown horny material, R1.

Hydrolysis of A1

This solid was heated at 100°C with sulphuric acid (10ml, 2N) for 4 hours. The solution was filtered neutralised with barium carbonate and filtered again. The filtrate was concentrated to 1ml. and poured into excess methylated spirit giving a white precipitate which was removed at the centrifuge. The alcoholic solution was concentrated to a syrup which was found to contain arabinose, xylose, galactose and glucose (trace).

Hydrolysis of A2

This hydrolysis was carried out in the same manner as the hydrolysis of A1. The products were similarly treated giving an alcohol-insoluble barium salt and a syrup which contained xylose and galactose only.

Hydrolysis/
Hydrolysis of R₁

This material was heated at 100°C with sulphuric acid (2N) for 5 hours. The remaining solid was filtered off, washed thoroughly with water, then with methylated spirit and finally dried in a vacuum dessicator, giving a brown horny material, R₂.

The filtrate and aqueous washings were neutralised with barium carbonate, filtered and concentrated to 5ml. This concentrated solution was poured into excess methylated spirit, giving no precipitate. The alcoholic solution was concentrated to a syrup which was found to contain galactose, glucose, mannose and xylose with a trace of a uronic acid derivative.

Hydrolysis of R₂

A portion of this material was hydrolysed by the Monier-Williams' method for cellulose:-

R₂ (ca.0.4gm.) was immersed in sulphuric acid (4ml., 72%) for 7 days at room temperature. The solution was then diluted to 400ml. and heated at 100°C for 16 hours, then filtered. The filtrate was neutralised with barium carbonate, filtered again and concentrated to a syrup. Study of this syrup with paper chromatography showed the presence of glucose (large quantity), mannose (trace) and xylose (trace).

Hydrolysis of the Mucilage (II).

The acidic mucilage (3gm.) was heated at 100°C with sulphuric acid (160ml. 1N). At frequent intervals samples (ca. 7ml.)/
(ca. 7ml.) were withdrawn from the reaction flask, cooled and filtered. 5ml. of each sample were added to aqueous sodium hydroxide solution (50ml., 0.1N) and iodine solution (10ml. 0.1N) added. After standing in the dark for 45 minutes at room temperature the solution was acidified with sulphuric acid (10ml. 1N) and the residual iodine determined by titration with standard sodium thiosulphate solution (0.05N). In this way the progress of the hydrolysis was followed.

**Results:**

<table>
<thead>
<tr>
<th>Time of Hydrolysis</th>
<th>Iodine Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 hours</td>
<td>2.34 ml.</td>
</tr>
<tr>
<td>1.5 &quot;</td>
<td>4.04 &quot;</td>
</tr>
<tr>
<td>2.5 &quot;</td>
<td>4.60 &quot;</td>
</tr>
<tr>
<td>3.5 &quot;</td>
<td>5.10 &quot;</td>
</tr>
<tr>
<td>4.5 &quot;</td>
<td>5.38 &quot;</td>
</tr>
<tr>
<td>5.5 &quot;</td>
<td>5.74 &quot;</td>
</tr>
<tr>
<td>6.0 &quot;</td>
<td>5.76 &quot;</td>
</tr>
<tr>
<td>6.5 &quot;</td>
<td>5.78 &quot;</td>
</tr>
</tbody>
</table>

It was concluded that the hydrolysis was complete after 5.5 hours.

**HYDROLYSIS OF THE MUCILAGE (III).**

The acidic mucilage (9gm.) was heated at 100°C with sulphuric acid (600ml. 1N) for 7 hours. The solution was then filtered and the insoluble residue washed with water, then with methylated spirit, then with acetone and finally dried in a vacuum dessicator giving a brown horny material, P1 (2.2gm.).
The filtrate and aqueous washings were neutralised with barium carbonate, filtered and concentrated to 60ml. This concentrated solution was filtered through "filter-cel" and poured into excess methylated spirit giving a white precipitate which was removed at the centrifuge, washed thoroughly with methylated spirit and dried in a vacuum dessicator, giving a white powder, B (2.2gm.).

The alcohol used in the precipitation, together with the washings, was concentrated to dryness and the residue dissolved in water (500ml.). This solution was concentrated to 40ml. and poured into excess methylated spirit giving a faint white precipitate. After standing for 16 hours the coagulated precipitate was removed at the centrifuge. The alcoholic solution was concentrated to dryness, giving a white mass, (4.2gm.).

This solid was dissolved in water (100ml.) and the solution passed through a column of cation-removing ion-exchange resins ("Amberlite" 100-H), then through a column of anion-removing resins ("Amberlite" 1R-4B) and then concentrated. The final product was a syrup, S, (4.0gm.) which could not be evaporated to dryness.

**Hydrolysis of the Insoluble Residue (Pr)**

The water insoluble material (2.2gm.) obtained after the hydrolysis described above was stirred vigorously in water (150ml.), giving a gelatinous suspension. Sulphuric acid (150ml., 4N) was added and the whole heated at 100°C for 4 hours. The undissolved material was filtered off, washed/
washed with water, methylated spirit and finally with acetone. After drying in a vacuum desiccator the product was a brown fibrous material, F₂, (1.8 gm.).

The filtrate and aqueous washings were neutralised with barium carbonate, filtered, and the solution evaporated to dryness. The residue was exhaustively extracted with methanol and the methanolic extracts combined and evaporated to dryness, giving a white powder (0.25 gm.).

This was dissolved in water (2 ml.) and the solution was found to contain galactose, glucose (large quantity) and mannose.

Hydrolysis of F₂.

This material (0.5 gm.) was hydrolysed according to the Monier Williams' test for cellulose, described previously. The sugars found after hydrolysis were glucose (large quantity) and galactose (trace).

Separation and Identification of the Sugars present after Hydrolysis (III).

The syrup, S, (2 gm.), was fractionated on a column of powdered cellulose (65 x 3.5 mm.) according to the method of Hough, Jones and Wadman. The eluent used was the upper phase of a mixture of butanol, pyridine, water and benzene (5/3/3/1, V/V). Every fifth tube of eluate collected was evaporated to a syrup and the sugars present identified by paper chromatography. All tubes containing the same sugar, or mixtures of sugars, were combined/
combined and the solvent evaporated off. The syrups thus obtained were extracted with cold water and the aqueous solutions filtered slowly through filter paper to remove any oily residue. The filtrates were evaporated to dryness and the residues weighed.

The following fractions were obtained and identified, as stated, by direct comparison with controls on paper chromatograms:-

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Weight</th>
<th>Sugars Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.08gm.</td>
<td>Unidentified sugar $R_f$, 0.28.</td>
</tr>
<tr>
<td>2.</td>
<td>0.11gm.</td>
<td>Xylose and the above sugar.</td>
</tr>
<tr>
<td>3.</td>
<td>0.41gm.</td>
<td>Xylose</td>
</tr>
<tr>
<td>4.</td>
<td>0.19gm.</td>
<td>Xylose, arabinose and mannose.</td>
</tr>
<tr>
<td>5.</td>
<td>0.26gm.</td>
<td>Arabinose and mannose.</td>
</tr>
<tr>
<td>6.</td>
<td>0.25gm.</td>
<td>Arabinose, mannose and glucose.</td>
</tr>
<tr>
<td>7.</td>
<td>0.10gm.</td>
<td>Glucose</td>
</tr>
<tr>
<td>8.</td>
<td>0.28gm.</td>
<td>Glucose and galactose.</td>
</tr>
<tr>
<td>9.</td>
<td>0.10gm.</td>
<td>Galactose</td>
</tr>
</tbody>
</table>

Total Weight = 1.78gm.

Study of Fraction 1.

This syrup (0.08gm.) could not be crystallised. Its $R_f$ value, 0.28, was greater than any of the aldopentoses. It gave on development with aniline oxalate a red spot on the paper chromatogram; with urea oxalate it gave a grey-black spot, a colour specific for ketoses. A portion of the syrup gave a negative Seliwanoff test for ketoses.

$[\alpha]_D^{28°} = -24^° (c, 0.5, \text{ in water}).$

O-sazone
Osazone Formation

The syrup (0.05gm.), phenylhydrazine hydrochloride (0.1gm.) and sodium acetate (0.15gm.) were dissolved in water (1ml.). After heating in a boiling water bath for 5 minutes, an oil separated. This oil was filtered off and dissolved in alcohol. The alcoholic solution was concentrated to small volume and water added, dropwise, till cloudy. After standing at 0°C for 16 hours yellow crystals separated which were filtered off and washed with a little benzene, m.p. 153°C (d.).

Study of Fraction 3.

This partially crystalline material was identified on the chromatogram as xylose. It was dissolved in water, then concentrated to a syrup. Glacial acetic acid (2ml.) was added and dry ether (2 drops). After standing at room temperature for 3 hours, white crystals separated which were washed with cold dry ether, m.p. 144°C, not depressed on admixture with an authentic specimen of D-xylose.

\[ \alpha \] _D^\circ; 57° (15 minutes), 28° (50 minutes), 19° (120 minutes, constant). (c, 0.6 in water).

Osazone Formation.

The sugar (0.06gm.), phenylhydrazine hydrochloride (0.1gm.) and sodium acetate (0.15gm.) were dissolved in water (1 ml.), then heated in a boiling water bath. After 6.5 minutes yellow crystals separated which were filtered off, and washed with a little benzene, m.p. 163°, not depressed/
depressed on admixture with an authentic specimen of the phenyllosazone of D-xylose.

Study of Fraction 5.

Study by paper chromatography showed this fraction to consist of mannose and arabinose,

\[ [\alpha]_D^c 109^\circ (5 \text{ minutes}), 87^\circ (15 \text{ minutes}), 70^\circ (40 \text{ minutes}, \text{constant value}), (c, 0.5 \text{ in water}). \]

The specific rotation (constant value) corresponds to a mixture of 14 parts L-arabinose and 9 parts D-mannose (W/W), i.e. the ratio of arabinose to mannose is 1.56:1 (W/W).

**Phenylhydrazone Formation.**

The phenylhydrazone of mannose was prepared according to the method of Hirst, Jones and Wood.\(^{(38)}\)

Phenylhydrazone (re-distilled) (25ml.) was mixed with absolute alcohol (100ml.) containing glacial acetic acid (3ml.). Fraction 5, (0.215gm.), was dissolved in water (10ml.), the above reagent(10ml.) added and the mixture kept in a well-stoppered flask at 32°C for 16 hours with occasional shaking. It was then cooled to 0°C for 12 hours and the phenylhydrazone filtered off, washed with ice-cold alcohol (10ml.) dried at 100°C for 30 minutes and weighed. Yield, 0.0701gm., m.p. 198°C, not depressed on admixture with an authentic specimen of D-mannose phenylhydrazone.

The weight of mannose phenylhydrazone obtained corresponds to the presence of 0.077gm. D-mannose in the sample taken, according to the equation given by Hirst, Jones/
Jones and Wood:-

\[ y = 0.652x + 0.031 \]

where \( y \) = weight of mannose

\( x \) = weight of phenylhydrazone obtained.

Thus the weight of arabinose in the sample taken is, by difference, 0.136gm., and the ratio of arabinose to mannose in Fraction 5 is 0.136:0.077, which equals 1.76:1 (W/W). This ratio corresponds reasonably well with the ratio calculated from the specific rotation of the fraction (1.56:1).

Osazone Formation.

Attempts to prepare the osazone of arabinose from the filtrate after removal of the mannose phenylhydrazone were unsuccessful, the product being a brown oil which could not be crystallised.

Study of Fraction 7.

This syrup (0.10gm.) which could not be crystallised, was identified as glucose by paper chromatography.

\[ [\alpha]_D^{18} = 58^\circ \text{(equilibrium value), (c, 0.5 in water).} \]

Formation of the \( p \)-nitrophenylhydrazone. \(^{39}\)

The syrup (90mgm.) was dissolved in 96% ethanol (1.5ml.) and heated on a boiling water bath with \( p \)-nitrophenylhydrazine (0.1gm.) for 10 minutes. The alcohol was then distilled off and the residue recrystallised from ethanol. The product was yellow needles, m.p. 185°C, not depressed on admixture with the derivative similarly prepared from an authentic specimen of D-glucose.

Study/
Study of Fraction 9.

This syrup (0.1gm.) was identified on paper chromatograms as galactose. The syrup was dissolved in ethanol and the solution concentrated to a syrup. Ether (1 drop) was added, and, after standing at room temperature for 4 hours, white crystals separated. These were filtered off and washed with a little cold ether, m.p. 165°C, not depressed on admixture with an authentic specimen of D-galactose.

\[ [\alpha]_D^{16} = 82°(c, 0.3 \text{ in water}). \]

Oxidation of Fraction 9.

The remainder of the crystals (ca. 0.08gm.) was dissolved in nitric acid (5ml, 50%). The solution was evaporated to 2ml., over a period of 3 hours. This solution after standing overnight at 0°C deposited white crystals, m.p. 214°C (d), not depressed on admixture with an authentic specimen of mucic acid.

The Composition of Syrup 9.

If the assumption is made that Fraction 2 contains equal quantities of Fractions 1 and 3, and similarly Fraction 4, equal quantities of 3 and 5, Fraction 6 equal quantities of 5 and 7, Fraction 8 equal quantities of 7 and 9, then the following table indicates the weight of each sugar recovered from the cellulose column. The composition of Fraction 5 was taken to be that indicated by its specific rotation.
<table>
<thead>
<tr>
<th>Sugar</th>
<th>Weight</th>
<th>Percentage of the Total Weight Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unidentified sugar</td>
<td>0.135gm</td>
<td>7.6%</td>
</tr>
<tr>
<td>Xylose</td>
<td>0.560gm</td>
<td>31.5%</td>
</tr>
<tr>
<td>Arabinose</td>
<td>0.292gm</td>
<td>16.4%</td>
</tr>
<tr>
<td>Mannose</td>
<td>0.188gm</td>
<td>10.6%</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.365gm</td>
<td>20.5%</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.240gm</td>
<td>13.5%</td>
</tr>
<tr>
<td><strong>Total ...</strong></td>
<td><strong>1.780gm</strong></td>
<td><strong>100.1%</strong></td>
</tr>
</tbody>
</table>
INVESTIGATION OF THE ALDOBIURONIC ACID.

Analysis of the Barium Salt, B.

The alcohol - insoluble salt, B, prepared from the hydrolysis of the mucilage(III), had \([\alpha]_D^b = 55^\circ(c, 0.3\) in water), and gave the following figures on analysis:

- Barium content, 18.8%.
- Carbon dioxide, 11.3%.
- Methoxyl content, 8.6%.

For the barium salt of an aldobiuronic acid composed of a monomethyl hexuronic acid and a pentose, \(C_{12}H_{19}O_{11}Ba/2\), the calculated figures are:

- Barium content, 16.9%.
- Carbon dioxide, 10.3%.
- Methoxyl content, 7.6%.

Hydrolysis of the Barium Salt.

The barium salt(2.8gm.) was dissolved in water(30ml.) and sulphuric acid (30ml, 8N) added. The precipitate was filtered off and the filtrate heated in a stoppered flask at 100°C for 2 hours. The solution was neutralised with barium carbonate and filtered. The filtrate was evaporated to dryness giving a residue, U, which was exhaustively extracted with hot methanol. The methanolic solutions were combined and evaporated to a syrup, G, which was found to contain xylose and galactose.

Ratio of Xylose to Galactose in the Syrup, G.

This was determined by the technique of quantitative paper chromatography. The sugars were separated on a paper/
paper chromatogram and the paper strips containing the sugars determined by the method of Flood, Hirst and Jones (12). These strips were cut out and freed from traces of the solvent (butanol/pyridine/water/benzene, 5/3/3/1) by hanging them, in the presence of water, in a vacuum dessicator connected to the filter pump for 12 hours.

The sugars present in the strips were extracted by the flow of water from capillary tubes, according to the method of Laidlaw and Reid (13), and the sugar solutions collected in boiling tubes. The sugars present in the solutions were then estimated by periodate oxidation according to the method of Hirst and Jones (12). To each tube was added sodium metaperiodate solution (2ml. 0.25 molar) and they were then securely stoppered, and heated at 100°C for 20 minutes. The tubes were cooled, ethylene glycol (0.3ml.) added to destroy excess periodate and the formic acid produced by the oxidation titrated against standard sodium hydroxide solution, using methyl red-methylene blue as indicator.

Results:

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylose</td>
<td>0.900ml. sodium hydroxide(0.01N)</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.150ml.</td>
</tr>
<tr>
<td>Paper Blank</td>
<td>Nil.</td>
</tr>
</tbody>
</table>

Galactose gives 5 molecules of formic acid on oxidation and xylose 4, thus the molecular ratio of xylose to galactose in the syrup, G, is:

\[
\frac{0.900}{4} : \frac{0.150}{5} = 7.5 : 1.
\]
Investigation of the Residue U.

The residue after extraction with methanol was dissolved in water and shaken with cation-removing resin ("Amberlite IR 100-H"), filtered and concentrated to a syrup. This syrup was added to a short column (9") of the same resin and washed through with water. The issuing liquid was evaporated to a glass (1.1gm.) which was free from barium. This glass showed on paper chromatograms xylose and galactose together with three spots whose R.G. values were 0.29(yellow spot), 0.21(yellow spot), and 0.10(red spot). The solvent used was ethyl acetate/water/acetic acid (3/3/1) and the developing reagent aniline oxalate.

The uronic acid components of the remainder of the glass were purified by the method of Hough, Jones and Wadman(40)

The glass (1.06gm.) was dissolved in water (30ml.) and anion-removing resin ("Amberlite IR-4B") added portion-wise till the suspension was no longer acid. This suspension was then transferred to a glass column (18" x 1") containing a 6" depth of fresh resin, and the whole was washed with water till the washings no longer reduced Fehling's solution. The contents of the column were then transferred to a beaker and sulphuric acid (2N) added till the suspension was permanently acid. The resin was then filtered off and washed with water till the washings were free from sulphate ions. The filtrate and washings were/
were freed from sulphuric acid by the addition of barium acetate solution in slight excess and the barium sulphate formed filtered off. The filtrate was shaken with cation-removing resin ("Amberlite IR 100-H") to remove barium, filtered and the solution concentrated to dryness giving a brown glass, C, (0.48gm.). This glass gave paper chromatograms showing the presence of xylose (trace), a spot identical in both colour and position with that obtained from an authentic specimen of 4 methyl glucuronic acid, one identical with that obtained from an authentic specimen of glucurone, and one, yellow in colour (aniline oxalate), which had travelled further than glucurone. For these chromatograms the solvent used was ethyl acetate/water/acetic acid/formic acid (18/4/3/1, V/V).

Separation of the Uronic Acids.

The glass, C, (480mgm.) was dissolved in the minimum quantity necessary of the above solvent and added to a column of powdered cellulose (30cm. x 3cm.) moist with this solvent. The column was then eluated with this solvent and the eluate collected and examined in the usual fashion.

The following fractions were obtained and their probable identities deduced by direct comparison with controls on paper chromatograms, using ethyl acetate/water/acetic acid as solvent:-
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Weight</th>
<th>$R_F$ value</th>
<th>Probable identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>93mgm.</td>
<td>0.29</td>
<td>?</td>
</tr>
<tr>
<td>2.</td>
<td>86mgm.</td>
<td>0.21</td>
<td>glucurone</td>
</tr>
<tr>
<td>3.</td>
<td>53mgm.</td>
<td>0.15</td>
<td>xylose and 4 methyl glucuronic acid</td>
</tr>
<tr>
<td>4.</td>
<td>145mgm.</td>
<td>0.10</td>
<td>4 methyl glucuronic acid.</td>
</tr>
</tbody>
</table>

Total Weight = 377mgm.

**Study of Fraction 1.**

This brown syrup gave a positive naphthoresorcinol test for uronic acids and reduced Fehling's solution. $[\alpha]_D^2 = 72^\circ (25$ minutes), $21^\circ (235$ minutes, constant value), ($c, 0.3$ in water). Methoxyl Content ($\text{CH}_3\text{O}$)- nil.

**Reduction of Fraction 1.**

The syrup (90mgm.) was heated under reflux with gently boiling methanolic hydrochloric acid (5ml. 1%) for 7 hours. The solution was neutralised by the addition of an ethereal solution of diazomethane and the solvents distilled off, leaving a brown residue. This residue was treated with gently boiling ethereal lithium aluminium hydride (0.3gm. in 7ml. ether) for 4 hours. After cooling excess reagent was destroyed by the addition of water. The whole was acidified with sulphuric acid (2N), then neutralised with barium carbonate and filtered. Aluminium was removed as the hydroxide by the addition of barium hydroxide solution and barium and lithium as the carbonates ($\text{CO}_3$). The solution, after filtration, was concentrated to dryness and the residue heated at $100^\circ \text{C}/$
100°C with sulphuric acid (7ml., 2M) for 6 hours. The solution was neutralised with barium carbonate, filtered and concentrated to a syrup. Study of this syrup by paper chromatography showed the presence of xylose only.

The syrup had $[\alpha]_D^{16} = 20^\circ$ (c, 0.5 in water).

**Study of Fraction 2.**

This fraction was in the form of a brown glass (86mgm.), $[\alpha]_D^{16} = 20^\circ$, (c, 0.5 in water). This material gave on paper chromatograms a spot identical in colour (aniline oxalate) and position with that given by an authentic specimen of glucurone, when an acid solvent was used. In the alkaline solvent of butanol/ethanol/water/ammonia (40/10/49/1, V/V) it gave a spot corresponding to glucuronic acid, but no spot corresponding to the lactone. An authentic specimen of glucurone showed both spots with this solvent.

**Formation of the Isopropyldene Derivative**

Fraction 2. (80mgm.) was dissolved by shaking in dry acetone (5ml.) containing sulphuric acid (1 drop, 36N). The solution was kept for 24 hours, neutralised with barium carbonate, filtered and evaporated, leaving a solid residue. This solid was recrystallised from ether-light petroleum in colourless needles, m.p. 120°C, not depressed in admixture with a sample of the derivative obtained by similar treatment of an authentic specimen of glucurone.

**Study of Fraction 4.**

This fraction was a brown syrup (145mgm.), $[\alpha]_D^{16} = 38^\circ$ (c, 0.5 in water). Its Rf value was identical with that/
that of an authentic specimen of 4 methyl glucuronic acid,
and it gave the same colour of spot (aniline oxalate)
both in daylight and ultra-violet light.

Methoxyl content - 14.3% (calculated for a monomethyl
hexuronic acid, 14.9%).

Reduction of Fraction 4.
The syrup (0.1gm.) was reduced in exactly the same
fashion as Fraction 1. The product was a syrup which
gave paper chromatograms showing spots corresponding to
xylose (faint), 4 methyl glucuronic acid, and 4 methyl
glucose. This last spot corresponded to neither of the
spots given by 3 methyl and 2 methyl glucose.
REACTIONS OF THE MUCILAGE.

Reaction of the Mucilage with Barium Hydroxide.

The acidic mucilage (1gm.) was dissolved in water (100ml.). To this solution was added saturated aqueous barium hydroxide solution. No precipitation occurred no matter how much barium hydroxide solution was added.

Reaction of the Mucilage with Fehling's Solution.

The neutral mucilage (5gm.) was dissolved in water (500ml.). To this solution was added Fehling's solution (100ml.) with vigorous mechanical stirring, which was continued for a further 3 hours. The gel produced was filtered off and added to an equal volume of methylated spirit and the precipitate formed filtered off and washed with aqueous alcohol (1/1, V/V). The solid was then suspended in water and the suspension dialysed against running water till free from copper, by which time the bulk of the material had gone into solution. Methylated spirit (2 volumes) was added and the whole allowed to stand at room temperature for 16 hours. The precipitate was filtered off, washed with methylated spirit, then dried in the presence of phosphoric oxide at 50°C at a pressure of 12mm. mercury. The product was a white fibrous solid, $F_1$, (0.9gm.).

The filtrate obtained by removal of the copper complex was neutralised with acetic acid and dialysed against running water till free from copper. The solution was then concentrated to 10ml. and poured into excess/
excess methylated spirit giving a white precipitate which was removed at the centrifuge, washed with methylated spirit, then with acetone and finally dried in the presence of phosphoric oxide at a pressure of 12.mm. mercury at 50°C. The product was a grey powder F11 (1.4gm.).

Hydrolysis of F1.

F1 (0.1gm.) was hydrolysed according to the Monier-Williams' test for cellulose described previously (page 25). The solution, after hydrolysis, was neutralised with barium carbonate, filtered and concentrated to 10ml. All ions were removed by treatment with ion-exchange resins ("Amberlite IR 100-M" and "IR4B") leaving a solution which was found to contain glucose, xylose, arabinose and galactose (trace). Glucose was present in the greatest quantity.

Hydrolysis of F11.

F11 (40mg.) was heated at 100°C with sulphuric acid (10.6ml. 2N). The progress of the hydrolysis was followed by observing the optical activity of the solution at frequent intervals. The specific rotation, \([\alpha]_D^6\), rose from 0° to 68°, (constant value) in 34 minutes.

The solution was neutralised with barium carbonate, filtered and concentrated to 4ml. This solution was poured into excess ethanol and the white precipitate which formed was removed at the centrifuge. The alcoholic solution was concentrated to a syrup which was found to contain xylose, glucose, mannose and arabinose. The alcohol-insoluble barium salt gave a positive naphthoresorcinol test for uronic acids.
Second Fractionation with Fehling's Solution.

The procedure described above was repeated, giving $F_I$ (0.6gm.) and $F_{II}$ (1.7gm.).

$F_I$ was hydrolysed as described previously and gave the following sugars: - glucose, galactose, mannose, arabinose and xylose.

$F_{II}$ gave, on hydrolysis, xylose, arabinose, galactose and glucose (trace).

In view of the unsatisfactory nature of the gel formed on addition of Fehling's solution to a solution of the mucilage and the results described above, it was concluded that to attempt a fractionation of the mucilage by means of Fehling's solution would not be profitable.

Reaction of the Mucilage with Copper Acetate (I).

The neutral mucilage (1gm.) was dissolved in water (500ml.) and to this solution was added a saturated aqueous solution of copper acetate (250ml.). The bulky precipitate formed was filtered off and stirred in dilute hydrochloric acid (500ml., 2%). After the solid had been thoroughly dispersed, acetone (2l.) was added and the gelatinous precipitate filtered off and washed with acetone. It was then redisolved in water (250ml.) and reprecipitated by pouring into methylated spirit (1.5l.). This precipitate was filtered, washed with methylated spirit, then with acetone and dried in a vacuum desiccator in the presence of calcium chloride. The product was a brown fibrous material, QI, (0.8gm.).

The filtrate from the original precipitation was poured/
poured into acetone (2 l.), giving a fine white precipitate which was removed at the centrifuge, washed with acetone and dried in a vacuum dessicator in the presence of calcium chloride, giving a pale green powder, QII, (0.15 g.).

Hydrolysis of Qi.

Qi (0.15 g.) was heated at 100°C with sulphuric acid (20 ml., 2N) for 10 hours. The insoluble residue was filtered off and the filtrate neutralised with barium carbonate, filtered, and the solution concentrated to 4 ml. This concentrated solution was poured into excess methylated spirit, giving a white precipitate which was removed at the centrifuge. The alcoholic solution was concentrated to a syrup which was found to contain xylose, arabinose, galactose (trace) and glucose (trace).

The undissolved residue after hydrolysis was washed with acetone and dried in a vacuum dessicator in the presence of calcium chloride. It was then hydrolysed according to the Monier-Williams test for cellulose (page 25). After hydrolysis the solution was neutralised with barium carbonate, filtered and the filtrate concentrated to a syrup. This syrup was found to contain glucose only.

Hydrolysis of QII.

A portion of this material was heated at 100°C for 5 hours with sulphuric acid (50 ml., 2N). Hydrogen sulphide was passed through the solution and the copper sulphide produced filtered off. The filtrate was neutralised with barium carbonate and filtered again, then concentrated to 10 ml. This solution was added to excess methylated spirit.
spirit and the white precipitate formed removed at the centrifuge. The alcoholic solution was concentrated to a syrup which was found to contain galactose, glucose, mannose, arabinose and xylose. The ratio of the hexoses to the pentoses, judging by the intensity of the spots produced by development of paper chromatograms with aniline oxalate, was greater than in the hydrolysed solution of QI.

Repeated Treatments with Copper Acetate.

The polysaccharide from the copper complex could be readily dissolved in water and reprecipitated by copper acetate solution. After 5 precipitations, carried out in the manner described above, a fibrous material was obtained which was hydrolysed by heating at 100°C for 5 hours with sulphuric acid (2N). The insoluble residue was filtered off and the filtrate neutralised with barium carbonate, filtered again and concentrated to small volume. This concentrated solution was poured into excess methylated spirit, giving a white precipitate which was removed at the centrifuge. The alcoholic solution was concentrated to a syrup which was found to contain xylose and arabinose only.

The insoluble residue was hydrolysed according to the Monier Williams test for cellulose, described previously (page 25). The solution, after hydrolysis, was neutralised with barium carbonate, filtered and concentrated to a syrup, which was found to contain glucose only.
Reaction of the Mucilage with Copper Acetate(II).

Quince seeds (750gm.) were soaked in water (15 l.) for 20 hours and the solution filtered through muslin. To the filtrate was added saturated aqueous copper acetate solution (150ml./l mucilage solution). The resulting precipitate was filtered off and stirred in water (7.5 l.). After the solid had dispersed hydrochloric acid (300ml.10N) was added and the mixture stirred for 4 hours. The precipitate was filtered off and stirred in water (8 l.) for 4 hours, filtered off again and stirred in methylated spirit (2 portions, 4 l. each.). It was filtered once more, then redissolved in water.

This treatment was repeated 5 times and the final product, after washing with acetone, dried in a vacuum dessicator in the presence of calcium chloride, giving a brown fibrous material (35gm.).

**Found:**
- Moisture Content = 14.7%
- Residual Ash = 5.0%
  corrected for moisture = 5.9%
- Uronic Anhydride Content - \((C_6H_2O_6) = 25.4%\)
  corrected for moisture = 29.8%
- Methoxyl Content (dried sample) = 2.5%

Isolation of QII.

After removal of the insoluble copper salt by filtration, the filtrate was made slightly acid with acetic acid and dialysed against running water until the bulk of the copper had been removed. The solution was then/
then concentrated to 2 l. and poured into acetone (10 l.),
giving a flocculent green precipitate which was removed at
the centrifuge. This solid was redissolved in water (2 l.)
made slightly acid with acetic acid and dialysed against
running water till free from copper. It was then
concentrated to 250ml., and the solution poured into
methylated spirit (1 l.), giving a white precipitate
which was removed at the centrifuge, washed with
methylated spirit and then with acetone. It was dried
in a vacuum desiccator in the presence of calcium chloride,
giving a white powder (6.8gm.).

**Found:**
- Moisture Content = 17%
- Residual Ash = 13.4%
  corrected for moisture = 16.1%
- Uronic Anhydride Content \((C_6\text{H}_2\text{O}_6)\) = 23.6%
  corrected for moisture = 28.7%
- Methoxyl Content (dried sample) = 5.7%

**Hydrolysis of QI.**

QI (3gm.), from the second precipitation, was
heated at 100°C with sulphuric acid (160ml.0.5N). At
frequent intervals samples were withdrawn, filtered and
cooled. The progress of the hydrolysis was followed in
exactly the same manner as in the hydrolysis (I) of the
neutral mucilage (page 22). The sugars present in each
sample were determined also in the fashion described in
the hydrolysis (I) of the neutral mucilage.

**Results**
<table>
<thead>
<tr>
<th>Sample</th>
<th>Time of Withdrawal</th>
<th>Vol. of 0.1N iodine solution taken up by 5ml sample</th>
<th>Sugars present in the sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>30 mins.</td>
<td>2.60ml.</td>
<td>Arabinose and xylose (trace).</td>
</tr>
<tr>
<td>II</td>
<td>90 mins.</td>
<td>3.64ml.</td>
<td>Arabinose and xylose.</td>
</tr>
<tr>
<td>III</td>
<td>50 mins.</td>
<td>4.08ml.</td>
<td>Arabinose and xylose.</td>
</tr>
<tr>
<td>IV</td>
<td>210 mins.</td>
<td>4.22ml.</td>
<td>Xylose, Arabinose, galactose (trace) and glucose (trace)</td>
</tr>
<tr>
<td>V</td>
<td>270 mins.</td>
<td>4.30ml.</td>
<td>Xylose, arabinose, galactose and glucose (trace).</td>
</tr>
<tr>
<td>VI</td>
<td>330 mins.</td>
<td>4.44ml.</td>
<td>Xylose, arabinose, galactose and glucose.</td>
</tr>
</tbody>
</table>

The insoluble residue remaining after hydrolysis was filtered off and the filtrate neutralised with barium carbonate, filtered and concentrated to 5ml. This solution was poured into excess methylated spirit, giving a white precipitate which was removed at the centrifuge, washed with methylated spirit and dried in a vacuum desiccator giving a white powder, B.

The insoluble residue was washed with water, methylated spirit and acetone and finally dried in a vacuum desiccator giving a brownish horny material R.

Hydrolysis of the Barium salt (B).

The barium salt (0.1gm.) was heated at 100°C for 4 hours with sulphuric acid (20ml. 2N). The precipitate was filtered off and the solution neutralised with barium carbonate/
carbonate, filtered and concentrated to 4ml. This solution was poured into excess methylated spirit, giving a white precipitate which could not be removed at the centrifuge. The suspension was evaporated to dryness and the white residue extracted with methylated spirit. The alcoholic solution was concentrated to a syrup which was found to contain xylose, galactose (trace) and arabinose (trace).

Hydrolysis of the Insoluble Residue (R).

The insoluble residue (0.1gm.) was heated at 100°C with sulphuric acid (20ml. 2N) for 5 hours. The residue was removed by filtration.

The filtrate was neutralised with barium carbonate, filtered and concentrated to 5ml. This solution was poured into excess methylated spirit but gave no precipitate. The alcoholic solution was concentrated to a syrup which was found to contain galactose, glucose, mannose and xylose.

Hydrolysis of QII.

QII, (0.2gm.), from the second preparation was heated at 100°C with sulphuric acid (30ml. 2N) for 4 hours. The solution was neutralised with barium carbonate, filtered and concentrated to 5ml. This solution was poured into excess methylated spirit giving a white precipitate which was removed at the centrifuge.

The alcoholic solution was concentrated to a syrup which was found to contain mannose, xylose, arabinose, galactose (trace) and glucose (trace).
PREPARATION OF QI.

Quince seeds (1000gm.) were soaked in water (20 l.) with occasional stirring for 20 hours. The solution was filtered through muslin and saturated aqueous copper acetate solution (4 l.) added. The precipitate which formed was filtered off, washed by stirring in water (12 l.) containing hydrochloric acid (600ml., 10N). The solid was removed by filtration, stirred in water (6 l.), then in methylated spirit (3 l.) and finally removed by filtration. The product was a moist fibrous solid, QI, (327gm.).

A portion (40gm.) of this solid was dried in a vacuum dessicator giving a pale brown fibrous material (6gm.). Thus, the total dry weight of QI prepared was taken to be 50gm.

 METHYLATION OF QI.

QI (40gm., dry weight) was stirred in aqueous sodium hydroxide solution (320ml., 30%) and dimethyl sulphate (560ml.) and sodium hydroxide solution (800ml., 30%) added dropwise at room temperature over a period of 8 hours. After a further 4 hours stirring, the mixture was made slightly acid with acetic acid and dialysed against running water for 2 days.

The mixture was then concentrated to a slurry and treated again with the methylating reagents, as described above. After four treatments the mixture was heated to 90°C and filtered. The precipitate was washed quickly with/
with hot water, then dried in a vacuum desiccator, giving a dark fibrous material, $M_1$ (7.5 gm.).

The filtrate was made slightly acid with acetic acid and dialysed against running water till free from sodium sulphate. The solution was then concentrated to a syrup, $S_1$ (60 ml.).

**Further Methylation of $M_1$.**

This partially methylated material (5 gm.) was dispersed in aqueous sodium hydroxide solution (35 ml., 30%). Dimethyl sulphate (70 ml.) and sodium hydroxide solution (140 ml., 30%) were added dropwise at room temperature with vigorous stirring, over a period of 4 hours. The contents of the reaction flask were then heated to 90°C and filtered. The precipitate was washed quickly with hot water, then remethylated as described above. After 11 such methylations the product was dried in a vacuum desiccator giving a brown solid.

This solid was ground to a powder and heated in a boiling mixture of chloroform (750 ml.) and acetone (50 ml.), under reflux, for 6 hours. The undissolved material, $M_2$, was removed by filtration and the solution concentrated to 40 ml. This concentrated solution was poured into excess light petroleum, giving a white flocculent precipitate which was removed at the centrifuge, washed with petroleum and finally dried in the presence of phosphoric oxide and paraffin wax at 50°C (bath temperature) at a pressure of 0.01 mm. mercury. The product was a dark solid, $M_3$ (0.7 gm.).

*Found/*
The remainder of M₁ (2.5gm.) was dispersed in dioxan (50ml.), which caused it to swell and partially dissolve. Sodium hydroxide solution (140ml., 30%) and dimethyl sulphate (70ml.) were added dropwise, with vigorous stirring, at room temperature over a period of 4 hours. After the addition of the reagents the stirring was continued for a further 3 hours. Glacial acetic acid was added to the mixture until it was just acid to phenolphthalein and the bulk of the solvents was distilled off, leaving a slurry (50ml.). To this slurry was added hot water (250ml.) and the whole was heated to 90°C and filtered; the precipitate was washed quickly with boiling water.

This precipitate, the chloroform-soluble methylated material, M₂, and the chloroform-insoluble material, M₃, previously obtained, were combined and the whole methylated in dioxan, as described above, five times.

The product obtained was heated in a boiling mixture of chloroform (800ml.) and acetone (200ml.) for 6 hours. The undissolved material was removed by filtration and the solution concentrated to 50ml.; then added to excess light petroleum, giving a white flocculent precipitate which was removed at the centrifuge, washed with petroleum, then dried by heating at 50°C (bath temperature) in the presence of phosphoric oxide and paraffin wax at a pressure of 0.01mm. mercury for 16 hours. The product was a dark powder, M₄, (0.8gm.).

Found:
Found: Methoxyl Content = 36.5%.

Methanolysis of \( M_4 \), and subsequent hydrolysis of the methyl glycosides.

\( M_4 \) (0.1gm.) was heated at 100°C in a sealed tube with methanolic hydrochloric acid (5ml. 1.5N) for 12 hours. The undissolved material was removed by filtration and the solution concentrated to a syrup. This syrup was heated at 100°C with hydrochloric acid (12ml.1N) for 6 hours. The solution was then neutralised with silver carbonate and filtered. Silver present in the solution was removed as sulphide (\( H_2S \)) and the solution treated with ion-removing resins ("Amberlite" IR100-H and IR-4B) then concentrated to a syrup (83mgm.).

This syrup showed three yellow spots (aniline oxalate) on paper chromatograms. The \( R_{G'0} \) values were 0.24(faint), 0.58(faint), 0.83; Butanol/ethanol/water/ammonia was used as solvent. The spot of \( R_{G'0} \) value 0.83 was identical with that obtained from an authentic specimen of 2:3:6 trimethyl D-glucose. It was concluded that the three spots were caused by a trimethyl, a dimethyl and a monomethyl aldohexose.

Quantitative Estimation of the Sugars. The sugars present in the syrup were separated on a paper chromatogram and the paper strips containing the sugars cut out, according to the method of Flood, Hirst and Jones(12). The sugars were extracted from the strips with boiling water and estimated according to the method of Hirst, Hough/
Hough and Jones\(^{(12)}\)

The solutions were cooled and iodine solution \((1ml. 0.1N)\) and phosphate buffer \((2ml. pH 11.4)\)^{43} added. The solutions were contained in test tubes with ground glass stoppers which were moistened with potassium iodide solution \((1\%)\), to avoid loss of iodine. The solutions were left in the dark for 3 hours and then diluted to 25ml., acidified with sulphuric acid \((2ml.2N)\) and titrated with standard sodium thiosulphate solution \((0.01N)\). A blank was also run.

**Results.**

<table>
<thead>
<tr>
<th></th>
<th>Titre((0.01N) sodium thiosulphate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>9.122ml.</td>
</tr>
<tr>
<td>Monomethyl sugar</td>
<td>9.090ml.</td>
</tr>
<tr>
<td>Dimethyl sugar</td>
<td>9.060ml.</td>
</tr>
<tr>
<td>Trimethyl sugar</td>
<td>8.322ml.</td>
</tr>
</tbody>
</table>

These figures indicate that the molecular ratio of trimethyl to dimethyl to monomethyl aldohexose are 25:1.9:1. The methoxyl content of such a mixture would be 42.5%, whereas the value found for M\(_4\) was 36.5%. This discrepancy may be explained by presence of material resistant to the action of methanolic hydrochloric acid, which was possibly lignin.

**Further Methylation of M\(_4\).**

M\(_4\)(0.6gm.) was dissolved in methyl iodide \((15ml.)\) by refluxing gently on a water bath. Silver oxide \((8gm.)\) was added portionwise over a period of 6 hours and the heating continued for a further 3 hours. The methyl iodide/
Iodide was then removed by distillation and the residue heated with boiling chloroform, under reflux, on a water-bath, for 4 hours. The undissolved material was removed at the centrifuge and the solution concentrated to 20ml, and dried over sodium sulphate for 16 hours. The solution was then filtered and poured into excess light petroleum giving a white flocculent precipitate which was removed at the centrifuge, washed with petroleum and dried in a vacuum dessicator.

The product was a yellow powder which was re-methylated according to the above procedure. The methylated product was also extracted in the same fashion and precipitated in light petroleum. The precipitate was removed at the centrifuge, washed with petroleum and dried by heating at 50°C (bath temperature) in the presence of phosphoric oxide and paraffin wax at a pressure of 0.01mm. mercury.

The product was a pale yellow powder, M₅, (0.3gm.).

*Found:* Methoxyl Content = 44.4%.

\[
\left[\alpha\right]_D^o = -2.0.
\]

Methanolysis of M₅ and subsequent hydrolysis of the methyl glycosides.

M₅ (0.3gm.) was heated at 100°C in a sealed tube with methanolic hydrochloric acid (10ml. 2N) for 16 hours. The solvent was then removed by distillation and the residue heated at 100°C for 8 hours with hydrochloric acid (20ml. 1N). The solution was neutralised with silver carbonate, filtered, and silver present in the solution removed as silver sulphide (H₂S). The solution was then freed from all/
all ions by treatment with ion-removing resins ("Amberlite" IR4B and IR100-H) and finally concentrated to a syrup (0.29gm.). This syrup showed two spots on the paper chromatogram, one of R₂F⁺ value 0.54 (very faint) and one of R₂F⁻ 0.83. This last spot was identical with that obtained from an authentic specimen of 2:3:6 trimethyl D-glucose.

Separation and Identification of the Trimethyl Aldohexose.

The syrup (0.25gm.) was dissolved in the minimum necessary quantity of water and added to a column (40cm. x 4 cm.) of powdered cellulose moist with the upper layer of a mixture of butanol/water/pyridine/benzene (5/3/3/1) and the column eluated with this solvent. The eluate was collected in the usual manner and the contents of all tubes which contained the trimethyl aldohexose were combined. This solution was evaporated to dryness, giving a partially crystalline solid (0.21gm.).

This material was recrystallised from butyl acetate, giving white crystals, m.p. 120°C alone or admixed with an authentic specimen of 2:3:6 trimethyl D-glucose.

Found: \( [\alpha]_D^{14} = 72^\circ \) (equilibrium) \( (c = 0.4 \text{ in water}) \)

Specific Rotation in Methanolic Hydrochloric Acid.

The specific rotation, \( [\alpha]_D^{14} \), of the material in methanolic hydrochloric acid fell from 69° (initial value) to -34° (7 hours, constant value) \( (c, 0.6) \).

Further Methylation of \( S_1 \).

This concentrated solution of the water-soluble partially/
partially methylated material (40ml.) was remethylated by treatment with sodium hydroxide solution (840ml, 30%) and dimethyl sulphate (420ml.) in the usual manner. After treatment with these reagents, the solution was made slightly acid with acetic acid and dialysed against running water till free from sodium sulphate. The solution was then concentrated to dryness, giving a pale brown glassy solid. This material was ground to a powder and dried at 50°C (bath temperature) in the presence of phosphoric oxide at a pressure of 1 cm. mercury, for 10 hours. The product was a pale brown powder, S₂, (5.2gm.).

Found: Methoxyl Content = 31.3%.

Methanolysis of S₂ and hydrolysis of the methyl glycosides obtained.

S₂ (0.1gm.) was treated with methanolic hydrochloric acid in the same manner as in the methanolysis of M₄ (page 54). The products were hydrolysed with hydrochloric acid, and the solution neutralised and freed from all ions also in the same manner. The final product was a syrup (61mgm.) which gave paper chromatograms showing a series of spots from the starting line almost to the position of the solvent front. The solvent used was butanol/ethanol/water/ammonia. 9 spots could be clearly distinguished and these had the following colours (aniline oxalate) and R₄ values:—
<table>
<thead>
<tr>
<th>No.</th>
<th>R.L. value</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.20</td>
<td>Pink</td>
</tr>
<tr>
<td>2.</td>
<td>0.29</td>
<td>Brown</td>
</tr>
<tr>
<td>3.</td>
<td>0.38</td>
<td>Brown</td>
</tr>
<tr>
<td>4.</td>
<td>0.46</td>
<td>Red</td>
</tr>
<tr>
<td>5.</td>
<td>0.54</td>
<td>Brown</td>
</tr>
<tr>
<td>6.</td>
<td>0.63</td>
<td>Brown</td>
</tr>
<tr>
<td>7.</td>
<td>0.74</td>
<td>Red</td>
</tr>
<tr>
<td>8.</td>
<td>0.83</td>
<td>Red</td>
</tr>
<tr>
<td>9.</td>
<td>0.94</td>
<td>Red</td>
</tr>
</tbody>
</table>

**Extraction of S₃ with Chloroform.**

S₃ proved to be insoluble in boiling chloroform. A chloroform solution was, however, obtained by the following procedure:-

S₃ (0.65gm.) was dissolved in water (100ml.) and the solution concentrated to 40ml. This concentrated solution was extracted with hot chloroform (200ml.) using two "Quickfit and Quartz" continuous liquid extracts in series, the extraction being continued for 14 hours. The chloroform solution was then separated and concentrated to 2ml. This solution was poured into excess light petroleum giving a white flocculent precipitate which was removed at the centrifuge, washed with petroleum and dried at 50°C in the presence of phosphoric oxide and paraffin wax at a pressure of 1cm. mercury for 12 hours. The product was a white powder, S₃, (0.016gm.).

**Methanolysis of S₃ and hydrolysis of the products.**

S₃ (15mgm.) was treated with methanolic hydrochloric acid/
acid and then with hydrochloric acid in the usual way. The product, after all ions had been removed, was a yellow syrup. This syrup gave spots on a paper chromatogram identical with those previously obtained by similar treatment of S2.

Further Methylation of S2 (Thallium Method).

S2 (1gm.) was dissolved in water (100ml.) and thallous hydroxide solution (24ml., 1.5M) added. No precipitation occurred. The solution was evaporated to dryness and ground to a fine powder which was dried in the presence of calcium chloride in a vacuum desiccator for 16 hours. This powder was heated at 45°C with methyl iodide (20ml.) for 32 hours. The methyl iodide was removed by distillation and the residue ground to a powder and shaken with methanol (300ml.) for 4 hours. The solid was removed at the centrifuge and the methanol evaporated to dryness, leaving no residue.

The solid was then shaken with water (300ml.) giving a bright yellow colloidal solution of thallous iodide. The undissolved solid was removed at the centrifuge and hydrochloric acid (2N) added to the colloidal solution till it was just acid. This had the effect of dissolving the thallous iodide. This solution was dialysed against running water till free from thallium. The solution was then concentrated to dryness, giving a white glass (0.7gm.).

**Found:**

Methoxyl Content = 26.3%.
ISOLATION OF CELLULOSE FROM THE MUCILAGE SOLUTION.

The method employed was based on the method of "Residual Fibre", recommended by the Association of Agricultural Chemists, Washington, D.C., U.S.A.

Quince seeds (100 gm.) were soaked in water (2 l.) for 20 hours. The mucilage solution was filtered through muslin and concentrated sulphuric acid (36N) added, giving a 1.25% acid solution. This solution was heated at 100°C under reflux with vigorous mechanical stirring for 30 minutes. The solution was then cooled and the precipitate removed at the centrifuge and washed with water till free from acid.

This precipitate was heated at 100°C with sodium hydroxide solution (1 l. 1.25%), under reflux, with vigorous stirring for 30 minutes. Part of the finely divided precipitate was removed at the centrifuge and washed once with water. The slurry so obtained was suspended in water (200 ml.) and the suspension made slightly acid with acetic acid and dialysed against running water for 6 days. The suspension was then concentrated to a slurry which was freeze-dried. The product was a light grey mass, C1, (0.15 gm.).

Hydrolysis of C1.

(a) C1 (25 mgm.) was heated at 100°C with sulphuric acid (10 ml. 1N) for 4 hours. The insoluble residue was removed by filtration and the solution neutralised with barium carbonate, filtered and concentrated to a syrup. This syrup was found to contain xylose and galactose (trace).

(b)/
(b) $C_1$ (25mgm.) was hydrolysed according to the Monier-Williams method for cellulose (page 25). The sugars obtained were identified as glucose (large quantity), xylose and galactose (trace).

**Purification of $C_1$ (44)**

$C_1$ (0.1gm.) was stirred in syrupy phosphoric acid (40ml.) for 4 hours, the flask being cooled in running water. The solution obtained was poured into 6 volumes of water and the precipitate removed at the centrifuge and washed with water till the washings were no longer acid to methyl red. The moist solid obtained was freeze-dried giving a white cellular material, $C_2$, (80mgm.).

**Hydrolysis of $C_1$**

$C_2$ (20mgm.) was hydrolysed according to the Monier-Williams method for cellulose (page 25). The final product was a syrup which was found to contain glucose only.

**Quantitative Hydrolysis of $C_2$**

$C_2$ (9.9mgm.) was hydrolysed according to the Monier-Williams method for cellulose. Before neutralisation of the solution after hydrolysis, xylose (16.7mgm.) was added. The solution was then neutralised with barium carbonate, filtered, and concentrated to a syrup.

The ratio of glucose to xylose in this syrup was estimated by quantitative chromatography according to the method previously described (page 35); ethyl acetate/water/acetic acid was used as solvent.

**Results:**

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Titre (0.01N sodium hydroxide)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylose</td>
<td>1.196ml.</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.868ml.</td>
</tr>
<tr>
<td>Paper Blank</td>
<td>0.080ml.</td>
</tr>
</tbody>
</table>
The theoretical yield of formic acid from xylose is 4/150 molecules/gm. xylose, and from glucose 5/180 molecules/gm. glucose. Assuming total recovery of the xylose, then the weight of glucose in the syrup is:

\[
\frac{0.785}{1.116} \times \frac{180}{5} \times \frac{4}{150} = 11.32 \text{mgm (103% theory).}
\]

**Study of the Sulphuric Acid Extract.**

The sulphuric acid solution, which remained after removal of the precipitate in the isolation of the cellulose (page 61) was neutralised with barium carbonate, filtered and concentrated to 20ml. This solution was poured into excess methylated spirit, giving a white precipitate which was removed at the centrifuge, washed with methylated spirit and finally dried in a vacuum dessicator giving a pale grey powder (P1), (3gm.).

The alcoholic solution was concentrated to a syrup which was found to contain galactose, mannose, arabinose, xylose and glucose (trace).

**Hydrolysis of P1.**

P1 (1gm.) was heated at 100°C with sulphuric acid (25ml. 2N) for 2 hours. The solution was then filtered, neutralised with barium carbonate, filtered and concentrated to 6ml. This solution was poured into excess methylated spirit, giving a white precipitate which was removed at the centrifuge. The alcoholic solution was concentrated to a syrup, which was found to contain galactose, glucose, mannose, arabinose and xylose.

**Study of the Sodium Hydroxide Extract.**

The/
The sodium hydroxide solution used in the isolation of cellulose from the mucilage solution was made slightly acid with acetic acid and dialysed against running water for 10 days. The solution was then concentrated to dryness, giving a white powder, P₂.

**Hydrolysis of P₂**

P₂ was heated at 100°C with sulphuric acid (20ml, 1N) for 4 hours. The solution was neutralised with barium carbonate, filtered, and concentrated to 2ml. This solution was poured into excess methylated spirit giving a faint white precipitate which was removed at the centrifuge. The alcoholic solution was concentrated to a syrup which was found to contain galactose, glucose, mannose, xylose and arabinose (trace).
THE DEGRADED MUCILAGE.

Preparation.

Quince seeds (500gm.) were allowed to stand in water (10 l.) for 20 hours with occasional stirring. The solution was filtered through muslin and sufficient sulphuric acid (36N) added to give a 0.5N acid solution. This acidic solution was heated at 100°C for 30 minutes with vigorous mechanical stirring. The undissolved material was removed by filtration and the solution neutralised with barium carbonate, filtered and concentrated to 500ml. This solution was added to methylated spirit (3 l.) and the white precipitate which formed was removed at the centrifuge, washed with methylated spirit and dried in a vacuum desiccator.

This powder was dissolved in water, (600ml.), and sulphuric acid (0.1N) added till no further precipitation took place. The barium sulphate was removed by filtration and the solution concentrated to 300ml. and poured into methylated spirit (2 l.). The precipitate formed was removed at the centrifuge, washed with methylated spirit and finally dried in a vacuum desiccator in the presence of calcium chloride. The product was ground to a fine white powder, D, (13gm.).

Found:

\[ \alpha \]D \text{ (c) } = 33^\circ \\
Moisture Content = 7.6\% \\
Residual Ash = 7.2\% \\
corrected for Moisture = 7.8\%

Uronic/
Uronic Anhydride Content = 31.4%
corrected for moisture = 34.0%

\( \text{Methoxyl Content (dried sample)} = 9.0\% \).  

**Study of the Methylated Spirit after precipitation.**

The methylated spirit in which the barium salt of the degraded mucilage was precipitated was concentrated to a syrup which was found to contain glucose, mannose, arabinose and xylose.

**Hydrolysis of the Degraded Mucilage.**

The degraded mucilage (0.5042gm.) was dissolved in sulphuric acid (80ml. 1N). This solution was heated at 100°C, at frequent intervals the optical rotation was observed and thus the progress of the hydrolysis followed.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>([\alpha]_D^\circ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>32°</td>
</tr>
<tr>
<td>36</td>
<td>68°</td>
</tr>
<tr>
<td>64</td>
<td>75°</td>
</tr>
<tr>
<td>90</td>
<td>76°</td>
</tr>
<tr>
<td>125</td>
<td>76°</td>
</tr>
<tr>
<td>157</td>
<td>76°</td>
</tr>
<tr>
<td>182</td>
<td>76°</td>
</tr>
</tbody>
</table>

After 182 minutes the solution was cooled and rhamnose monohydrate (27.4mgm.) added. The solution was then neutralised with barium carbonate, filtered and concentrated to dryness, giving a white residue which was exhaustively extracted with methanol, and dried in a vacuum desiccator, giving a white powder (0.434gm.). The/
The methanolic solutions were combined and evaporated to dryness. The white residue obtained was dissolved in water (10ml.), and the aqueous solution treated with ion-removing resins ("Amberlite" IRA400-H and IR 4B2). The solution was finally concentrated to a syrup, D1, which was found to contain rhamnose, xylose, arabinose, glucose and galactose.

Quantitative Estimation of the Sugars given on Hydrolysis.

The sugars present in the syrup, D1, were estimated by quantitative chromatography in the usual way (page 35). The solvent used was butanol/pyridine/water/benzene.

Results:--

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Titre(0.01N NaOH)</th>
<th>Moles.of Formic Acid per gm. weight of sugar.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhamnose</td>
<td>0.582ml.</td>
<td>4/182</td>
</tr>
<tr>
<td>Xylose</td>
<td>1.406ml.</td>
<td>4/150</td>
</tr>
<tr>
<td>Arabinose</td>
<td>0.435ml.</td>
<td>4/150</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.328ml.</td>
<td>5/160</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.177ml.</td>
<td>5/160</td>
</tr>
</tbody>
</table>

Assuming total recovery of rhamnose, then the weight of xylose in the syrup is:

\[
\frac{1.406}{0.582 \times 150 \times 27.4 \text{ mgm.}} = 54.6 \text{ mgm.}
\]

Similarly, the weights of the other sugars in the syrup were found to be:

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Weight (mgm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabinose</td>
<td>16.9</td>
</tr>
<tr>
<td>Glucose</td>
<td>12.2</td>
</tr>
<tr>
<td>Galactose</td>
<td>6.6</td>
</tr>
<tr>
<td>Total</td>
<td>90.3</td>
</tr>
</tbody>
</table>
Methylation of the Degraded Mucilage.

The degraded mucilage (10gm.) was dissolved in water (200ml.) and thallous hydroxide (27gm. in saturated aqueous solution) added. The fine white precipitate which formed was removed at the centrifuge, washed with methanol and dried in a vacuum dessicator in the dark, giving a grey powder (1gm.).

The solution was concentrated to dryness, ground to a powder and dried in a vacuum dessicator in the dark, giving a cream coloured powder (28gm.).

These two solids were combined and stirred in gently boiling methyl iodide under reflux until the solution was no longer alkaline to litmus (24 hours). The methyl iodide was removed by distillation and the residue ground to a fine powder. This solid was extracted with boiling methanol (1 l.) for 4 hours and the undissolved material, T₁, removed at the centrifuge. The methanolic solution was evaporated to dryness and left no residue.

Extraction of T₁ and Methylation of the Product.

The solid, T₁, was stirred in water (2 portions, 2 l. each) at room temperature for 3 hours and the undissolved material, T₂, removed at the centrifuge. The aqueous solutions were combined and thallous hydroxide (14gm.) added and the solution evaporated to dryness. The residue was ground to a powder which was dried in a vacuum dessicator in the dark. This powder was then stirred in gently boiling methyl iodide till the/
the suspension was no longer alkaline to litmus. The methyl iodide was removed by distillation and the residue ground to a powder and then treated with boiling chloroform, under reflux, for 6 hours. The undissolved residue, T₃, was removed at the centrifuge and the chloroform solution was concentrated to 50ml. This solution was poured into excess light petroleum giving a flocculent white precipitate which was removed at the centrifuge, washed with petroleum and then dried in a vacuum desiccator. The product was a white powder, M₁, (1.7gm.).

Found: Methoxyl Content = 31.4%.

Extraction of T₂ and T₃.

The thallous iodide residues, T₂ and T₃, were combined and found to be completely insoluble in both boiling methanol and water. The solid was then stirred in hydrochloric acid (2 l., 0.1N) for 6 hours. The undissolved material was removed at the centrifuge and the solution neutralised with potassium hydroxide solution, then concentrated to 500ml. This solution was poured into methylated spirit (3 l.) giving a white precipitate which was removed at the centrifuge, washed with methylated spirit and dried in a vacuum desiccator, giving a white powder, T₄, (11.3gm.).

Found: Residual Ash = 62.3%.

Methylation of T₄.

T₄ was dissolved in water (300ml.) and thallous hydroxide (14gm. in saturated aqueous solution) added. The/
The solution was evaporated to dryness and the residue ground to a powder which was dried in a vacuum dessicator in the dark. This solid was stirred in gently boiling methyl iodide till the suspension was no longer alkaline to litmus, (36 hours). The methyl iodide was removed by distillation and the residue powdered and then heated in boiling chloroform (600ml.) under reflux for 6 hours. The undissolved residue, T5, was removed at the centrifuge and the chloroform solution concentrated to 20ml. This solution was poured into excess light petroleum, giving a white flocculent precipitate which was removed at the centrifuge, washed with petroleum and dried in a vacuum dessicator. The product was a white powder, M2, (0.45gm.).

Found: Methoxyl Content = 32.5%.

Extraction of T5.

T5 was extracted in exactly the same manner as T2 and T3. The hydrochloric acid solution was neutralised, concentrated and poured into methylated spirit as before. The white solid obtained was dried in the same manner giving a white powder, T6, (11.4gm.).

Methylation of T6.

T6 was dissolved in water (500ml.) and thallous hydroxide (14gm. in saturated aqueous solution) added. The solution was evaporated to dryness and the residue ground, dried and treated with methyl iodide as in the treatment of T4. After removal of the methyl iodide by distillation, the residue gave a chloroform-insoluble material/
material, T₇, and a chloroform-soluble white powder, M₃, (0.20 gm.) which was obtained by precipitation in light petroleum, in the usual manner.

**Found:** Methoxyl Content = 31.7%.

**Extraction of T₇.**

T₇ was extracted with hydrochloric acid and the hydrochloric acid solution treated in the same fashion as described previously. The product was a white powder, T₈, (15.0 gm.).

**Methylation of T₈.**

T₈ was treated directly with methyl iodide in the usual fashion, without any further addition of thallous hydroxide. There was obtained a chloroform-soluble white powder, M₃, (0.047 gm.), which was isolated by precipitation in light petroleum in the usual manner.

**Found:** Methoxyl Content = 31.6%.

**Further Methylation of M₃.**

M₃ (1.7 gm.) was dissolved in methyl iodide (30 ml.) and to the gently boiling solution was added, portionwise, silver oxide (3 gm.) over a period of 6 hours. The heating was continued for a further 3 hours. The methyl iodide was removed by distillation and the residue extracted by heating with boiling chloroform (2 portions, 0.5 l. each) for 3 hours. The undissolved residue was removed at the centrifuge and the chloroform solution concentrated to 15 ml. This solution was poured into excess light petroleum giving a flocculent white/
white precipitate which was removed at the centrifuge, washed with petroleum and dried in a vacuum desiccator, giving a white powder.

This powder was re-treated with methyl iodide and silver oxide as described above. After removal of the methyl iodide by distillation, the residue was extracted with chloroform (600mH.) in the same manner. The chloroform solution was concentrated to 30mL, and poured into excess light petroleum and the precipitate washed and dried in the usual fashion. The product was a pale yellow powder, M₄, (0.55gm.).

Found: Methoxyl Content = 31.1%.

Combination of the Methylated Fractions.

M₂, M₃ and M₄ were combined and intimately mixed by grinding in a mortar. The product, M₅, had [α]D⁺₉°, 69°, (c, 0.6 in chloroform), and gave no ash on incineration.

Hydrolysis of M₅(1)

M₅ (50mgm.) was hydrolysed by a two stage treatment:

1. M₅ (50mgm.) was dissolved in methanolic hydrochloric acid (3mL, 1.8%) and the solution heated in a sealed tube for 7 hours. The solvent was then distilled off, leaving a residue.

2. This residue was heated at 100°C with hydrochloric acid (5mL, 0.5N) for 8 hours. The solution was then cooled, neutralised with silver carbonate, filtered and the silver remaining in solution removed as the sulphide (H₂S). The solution was then treated with cation-removing resin ("Amberlite" IR 100-H) and concentrated to/
to a syrup.

This syrup was spotted on a paper chromatogram, using butanol/water/ethanol/ammonia as solvent. After elution and development (aniline oxalate) two red spots appeared which corresponded in position to the spots given by authentic specimens of 2,4-dimethyl xylose and 2-methyl xylose respectively. There was also trailing from the starting line for a length of 1.5 inches, which appeared bright red on the developed chromatogram.

Using this solvent and the technique of quantitative chromatography,\(^{(12)}\) the material causing this trailing was separated from the sugars and eluted from the paper strip. The solution obtained was concentrated to a syrup, which showed two bright red spots, \(R_f\) values 0.39 and 0.15, on a developed paper chromatogram which had been eluted with a solvent consisting of the upper phase of butanol/ethanol/water/acetic acid (40/10/50/5, V/V).

**Hydrolysis of \(M_g\): (II).**

\(M_g\) (1.14gm.) was hydrolysed in two stages:

1. \(M_g\) (1.14gm.) was heated at 100°C with methanolic hydrochloric acid (120ml. 1%) under reflux. After 4 hours the bulk of the polysaccharide had dissolved and the progress of the hydrolysis was followed by the polarimeter:

<table>
<thead>
<tr>
<th>Time</th>
<th>(\alpha^l / l_1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 hours 50 mins.</td>
<td>81°</td>
</tr>
<tr>
<td>4 hours 45 mins.</td>
<td>93°</td>
</tr>
<tr>
<td>5 hours 55 mins.</td>
<td>90°</td>
</tr>
<tr>
<td>6 hours 40 mins.</td>
<td>93°</td>
</tr>
<tr>
<td>7 hours 20 mins.</td>
<td>93°</td>
</tr>
</tbody>
</table>
After heating for 7 hours 20 minutes the solution was cooled and evaporated to dryness.

(2) The residue obtained was heated at 100°C with hydrochloric acid (60ml, 0.5N) until the optical rotation was constant, \([\alpha]_{D}^{2} 110^\circ\) (6 hours). The solution was filtered, neutralised with silver carbonate and filtered again. The silver remaining in solution was removed as sulphide (HgS) and the solution treated with cation-removing resins ("Amberlite" 1R100-H). The solution was then concentrated to dryness, giving a brown glassy solid, G₁, (0.81gm.).

Separation of the Products of Hydrolysis.

G₁ (0.7gm.) was dissolved in water, using the minimum quantity necessary. This solution was added to a column of powdered cellulose (7.5 x 3.5 cm.) moist with the upper phase of a mixture of butanol/ethanol/water/ammonia (20/5/25/2, V/V), and the column eluted with this solvent. The eluate was collected in the usual fashion.

The following fractions were obtained and their probable identities deduced as stated, by direct comparison with controls on a paper chromatogram. The papers were sprayed with aniline oxalate.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Weight</th>
<th>Colour of spot</th>
<th>Probable identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>19.2mgm.</td>
<td>brown</td>
<td>2:3:6-trimethyl glucose</td>
</tr>
<tr>
<td>II</td>
<td>17.3mgm.</td>
<td>red</td>
<td>2:4-dimethyl xylose</td>
</tr>
<tr>
<td>III</td>
<td>20.8mgm.</td>
<td>red</td>
<td>? R₉, 0.64.</td>
</tr>
<tr>
<td>IV</td>
<td>25.1mgm.</td>
<td>red</td>
<td>2-methyl xylose.</td>
</tr>
<tr>
<td>Total</td>
<td>82.4mgm.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
After removal of fraction IV, the column was eluted with water (500ml.) and the washings concentrated to dryness, giving a brown glassy residue, G2, (0.61gm.).

**Examination of Fraction I.**

This syrup gave on a paper chromatogram a spot identical with that given by an authentic specimen of 2:3:6-trimethyl glucose and different from the spots given by 2:3:4- and 2:4:6-trimethyl glucose.

\[ [\alpha]_D^0 = 63^\circ \text{(equilibrium value)} \]

This fraction was demethylated by the method of Hough, Jones and Wadman. The syrup (10mgm.) was heated at 100°C with hydrobromic acid (1ml., 48%) in a sealed tube for 7 minutes. The solution was cooled and diluted to 10ml. immediately, neutralised with silver carbonate and filtered. The silver remaining in solution was removed as the sulphide and the solution evaporated to a syrup. This syrup, by study by paper chromatography, was found to contain glucose and no other unmethylated sugar.

**Examination of Fraction II.**

This syrup gave, on the paper chromatogram, a spot identical with that given by an authentic specimen of 2:4-dimethyl xylose and different from that given by an authentic specimen of 2:3-dimethyl xylose.

\[ [\alpha]_D^0 = -10^\circ \text{(11mins.)}, 0^\circ \text{(23mins.)}, 9^\circ \text{(44mins.)}, 27^\circ \text{(77mins., constant)} \]

The syrup (10mgm.) was demethylated in the same fashion as Fraction I. The syrup obtained was found to/
to contain xylose and no other unmethylated sugar.

**Examination of Fraction III.**

This syrup gave two spots on a paper chromatogram when butanol/pyridine/water/benzene was used as mobile phase:

1. Red spot which corresponded with 2,3-dimethyl arabinose,
2. Yellow spot, which corresponded with the spot given by 2,3,4-trimethyl galactose.

The syrup (10mg.) was dimethylated by treatment with hydrobromic acid in the usual way. The resulting syrup was found to contain galactose and arabinose and no other unmethylated sugar.

**Examination of Fraction IV.**

This syrup gave a spot on the developed paper chromatogram identical with that obtained from an authentic specimen of 2-methyl xylose and different from that given by an authentic specimen of 3-methyl xylose.

$$\left[\alpha\right]_D^{15} 43^\circ.$$  

The syrup (10mg.) was demethylated in the usual way. Xylose was the only unmethylated sugar obtained.

**Examination of G2a (water-wash from column).**

A solution of this solid showed on the chromatogram an elongated heart-shaped cherry-red spot, Rf value 0.72, with very heavy brown trailing.

The remainder of G1 was dissolved in water (20ml.) and/
and shaken with cation-removing resin ("Amberlite" 1R100-H). The solution was then concentrated to a syrup which gave the same effect on paper chromatograms as the solution did before treatment with the resin.

Separation of the Methylated Uronic Acids.

The syrup obtained from G2 as described above was dissolved in water (5ml.) and the solution added to a column of powdered cellulose, moist with the upper phase of a mixture of butanol/ethanol/water/acetic acid (40/50/10/5 V/V). The column was then eluted with this solvent and the eluate was collected in the usual way.

Five fractions were obtained, whose Rf values were as stated:

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Weight</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.195gm.</td>
<td>0.72</td>
</tr>
<tr>
<td>B</td>
<td>0.088gm.</td>
<td>0.72 and 0.57</td>
</tr>
<tr>
<td>C</td>
<td>0.085gm.</td>
<td>0.57</td>
</tr>
<tr>
<td>D</td>
<td>0.016gm.</td>
<td>0.30</td>
</tr>
<tr>
<td>E (water wash)</td>
<td>0.096gm.</td>
<td>0.30</td>
</tr>
<tr>
<td>Total weight =</td>
<td>0.478gm.</td>
<td></td>
</tr>
</tbody>
</table>

The Rf values were obtained as the ratio of the distance on the chromatogram of the centre of the cherry-red heart-shaped spot from the starting to the distance travelled by tetramethyl glucose on the same paper. The extensive trailing behind the heart-shaped "head" was ignored.

Reduction of Fraction A.

This glass (0.19gm.) was heated under reflux with gently/
gently boiling methanolic hydrochloric acid \((12\text{ml}., 1\%\) for 12 hours. The solution was neutralised by the addition of an ethereal solution of diazomethane and the solvents removed by distillation. The residue was treated with gently boiling lithium aluminium hydride \((0.6\text{gm. in } 15\text{ ml. ether})\) for 4 hours. After cooling, excess reagent was destroyed by the addition of water. The whole was acidified with sulphuric acid \((2N)\), then neutralised with barium carbonate and filtered. Aluminium was removed as the hydroxide by the addition of barium hydroxide solution, and barium and lithium as the carbonates \((\text{CO}_2)\). The solution, after filtration, was concentrated to dryness and the residue heated at 100°C with sulphuric acid \((10\text{ml. } 2\text{N})\) for 6 hours. The solution was neutralised with barium carbonate, filtered and concentrated to 4ml. This solution was passed through a column of cation-removing resin \("\text{Amberlite} \text{ IR}100-\text{H}\)\), then through a column of anion-removing resin \("\text{Permutite Deacidite} \text{ F}\)\). The solution was then concentrated to a syrup, \(R_1\).

This syrup gave four spots on the paper chromatogram:

1. Brown spot (aniline oxalate) which corresponded with the spot given by an authentic specimen of 2:3:4-trimethyl glucose and to neither of the spots given by 2:3:6- and 2:4:6-trimethyl glucose.

2. Red spot which corresponded with the spot given by 2:3-dimethyl xylose and different from that given/
given by 2:4-dimethyl xylose.

(3) Brown spot (faint) which did not correspond with the spot given by 2:4-dimethyl glucose, being slightly behind it.

(4) Red spot (faint) which corresponded with the spot given by 3-methyl xylose, but different from that given by 2-methyl xylose.

Demethylation of R1.

The remainder of R1 was demethylated with hydrobromic acid in the usual fashion. The syrup obtained was found to contain glucose and xylose (trace) and no other unmethylated sugar.

Reduction of Fraction C.

This glass (0.1gm.) was reduced in the same fashion as Fraction A. After the removal of all ions the solution was concentrated to a syrup, R2, which gave 3 spots on the paper chromatogram:

(1) Red spot, corresponding with that given by 2:3-dimethyl xylose but different from that given by 2:4-dimethyl xylose.

(2) Red spot, corresponding with that given by 3-methyl xylose and different from that given by 2-methyl xylose.

(3) Red spot (very faint) corresponding with the spot given by xylose.

Demethylation of R2.

The remainder of R2 was demethylated by treatment with/
with hydrobromic acid in the usual manner, and gave a syrup which contained xylose and no other unmethylated sugar.

**Examination of Fraction E.**

A solution of this glass showed on the paper chromatogram, run in an alkaline solvent, a faint spot, corresponding with that given by xylose, and also trailing (red) near the starting line.
DISCUSSION.

This investigation of the mucilage from quince seeds was undertaken in the hope that some evidence would be obtained regarding the structure of the mucilage and in particular of the polyuronide portion. Little structural evidence was obtained, partly because the mucilage proved to be a mixture of polysaccharides not readily separable, and partly because these were themselves highly complex, being composed of a relatively large number of sugar residues. Moreover, no method of methylation of the polyuronide portion was found which would give a good yield of methylated material.

The mucilage was obtained by aqueous extraction of the seeds in approximately 6% yield, and found to have a high uronic anhydride content (23.1%).

The first significant results were obtained by hydrolysis of the mucilage. By treatment of the mucilage with sulphuric acid (1N) at 100°C there were obtained an undissolved material (24.4%), an alcohol-insoluble barium salt (24.4%) and an alcohol-soluble syrup (44.4%).

This syrup was found, by study by paper chromatography, to contain galactose, glucose, mannose, arabinose, xylose and an unidentified sugar. A partial separation of these sugars was obtained on a column of powdered cellulose, and pure specimens were obtained of galactose, glucose, xylose and the unidentified sugar. Galactose, glucose and xylose were identified as the D-forms by their optical/
optical rotation and the preparation of derivatives. D-galactose and D-xylose were obtained in crystalline form and their melting-points corresponded with those quoted for D-galactose and D-xylose.

The unidentified sugar corresponded to none of the aldopentoses on paper chromatograms, and had an Rf value of 0.28. It gave a red spot on the paper chromatogram when aniline oxalate was used as the developing reagent, and gave a grey-black spot with urea oxalate, a reagent specific for ketoses.\(^{(46)}\) It had a specific rotation, \([\alpha]_D^{16}, \) of -24° and was at first thought to be D-xylulose \([\alpha]_D^{16}, -33°\). However, the unknown sugar gave a negative Seliwanoff test for ketoses and its phenylosazone depressed the melting-point of an authentic specimen of the phenylosazone of D-xylose.

Arabinose and mannose were obtained as a mixture from the column, and the specific rotation of this mixture indicated that it consisted of 14 parts of L-arabinose and 9 parts of D-mannose. The mixture gave an insoluble phenylhydrazone, which was identified as mannose phenylhydrazone and was obtained in a yield which indicated the presence of 18 parts of arabinose and 10 parts of mannose in the mixture. No derivative of arabinose was isolated; attempts to prepare the phenylosazone of arabinose after the removal of mannose as the phenylhydrazone proved fruitless.

From the weights of the various fractions obtained from/
from the column, the composition of the parent syrup was estimated to be:

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unidentified sugar</td>
<td>7.6%</td>
</tr>
<tr>
<td>D-xylose</td>
<td>31.5%</td>
</tr>
<tr>
<td>L-arabinose</td>
<td>16.4%</td>
</tr>
<tr>
<td>D-mannose</td>
<td>10.6%</td>
</tr>
<tr>
<td>D-glucose</td>
<td>20.5%</td>
</tr>
<tr>
<td>D-galactose</td>
<td>13.5%</td>
</tr>
</tbody>
</table>

100.1%

These figures are only approximate; the percentage quoted for the unidentified sugar is almost certainly too high.

The mucilage was also hydrolysed with 0.5N sulphuric acid and samples were withdrawn at frequent intervals and the sugars present identified by paper chromatography. After 30 minutes heating arabinose was the only free sugar present in solution. Also in solution was a degraded polyuronide which was isolated as the alcohol-insoluble barium salt. This, on hydrolysis (2N sulphuric acid) gave arabinose, xylose, galactose, glucose and an aldobiuronic or uronic acid, removed as the barium salt.

As the hydrolysis with 0.5N acid proceeded, xylose was obtained in solution in addition to arabinose, then galactose and finally glucose. No mannose was obtained from this hydrolysis, which was allowed to continue until the sugar content was constant.

The insoluble residue remaining after hydrolysis gave/
gave, on further treatment with sulphuric acid (2N, 5 hours at 100°C), the sugars mannose, galactose, glucose and xylose. The bulk of the material remained unaffected by this treatment, and after hydrolysis by the Monier-Williams method for cellulose it gave glucose with traces of xylose and mannose. This indicated the presence of a glucan, resistant to fairly powerful hydrolysis and was probably cellulose, which Kirchner and Tollens; later Renfrew and Cretcher; reported to be present in quince seed mucilage.

By treatment of the mucilage solution according to a standard method for the isolation of cellulose, a material was obtained which hydrolysed to glucose with a trace of xylose. By dissolving this material in syrupy phosphoric acid in the cold and re-precipitating it in water, there was obtained a product which, on hydrolysis, gave glucose only (100%).

As mentioned in the introduction, the presence of cellulose in the aqueous extract from seeds has been observed on several occasions. However, its presence cannot be readily explained, since cellulose is completely insoluble in water and does not normally appear in solution with hemicelluloses when they are extracted from plants. This would suggest that in quince seed mucilage the polyuronide portion is linked chemically in some way with the cellulose, giving a water-soluble molecule. On treatment of the aqueous extract from quince seeds with hot/
hot dilute acid, material containing cellulose is precipitated almost immediately, and this appears to indicate that the linkage between the cellulose and the polyuronide is probably ruptured at once, and continued heating with acid proceeds to hydrolyse the polyuronide and leaves the cellulose relatively unaffected.

As regards the nature of this bond, one possibility is that there are ester linkages between the carboxylic acid groups of the uronic acids in the polyuronide portion and alcoholic groups in the cellulose. These ester linkages would be unstable in the presence of acid, more so than the normal ethereal linkages between sugar residues in a polyuronide.

The role of the mannose residues in the mucilage is difficult to understand. Mannose does not appear on hydrolysis of the mucilage with 0.5N acid and the mannose residues are left associated with the cellulosic material. Thus there would appear to be no mannose present in the polyuronide portion, nor does it appear to have any structural role in the cellulosic material. It may be, however, that extraction with water removes a mannan or galacto-mannan which is fairly resistant to hydrolysis. Galactomannans are quite often found in the endosperms of seeds, especially in those from leguminous plants, (e.g. carob bean\(^\text{47}\), guar seed\(^\text{48}\) and lucerne seed\(^\text{49}\)).

The aldobiuronic acid, which was obtained as the alcohol-insoluble barium salt after hydrolysis of the mucilage (1N sulphuric acid, 7 hours), proved to be very interesting/
interesting. This salt gave analytical figures (barium content, carbon dioxide evolved, methoxyl content) slightly higher than those calculated for the barium salt of an aldobiuronic acid consisting of a pentose sugar and a monomethyl hexuronic acid. This was taken to indicate that there was present a little of the barium salt of a simple uronic acid.

Hydrolysis of this aldobiuronic acid (4N sulphuric acid, 2 hours) gave the sugars xylose and galactose (molecular ratio 15:2), and an alcohol-insoluble barium salt. Barium was removed from this salt by treatment with cation-exchange resin, and the acidic material studied by paper chromatography. The paper chromatograms obtained, using an acidic solvent, indicated the presence of xylose and showed three other spots, one corresponding to that given by an authentic specimen of 4 methyl glucuronic acid, a second corresponding to that given by glucurone and a third appearing below the glucurone spot. The uronic acids were removed by anion-exchange resin which was washed free from non-acidic material, then treated with sulphuric acid, thus regenerating the uronic acids. The material obtained from this treatment showed only a trace of xylose on paper chromatograms, and all three of the spots described above.

The uronic acids were separated on a column of powdered cellulose and investigated.

The substance, A, giving a spot below glucurone on the chromatogram gave a positive naphthoresorcinol test for/
for uronic acids. It had $[\alpha]_D^{25} \, ^{72^0}(25 \text{ minutes})$ falling to $21^0(235 \text{ minutes, constant value}).$ It had no methoxyl content. Reduction of A by lithium aluminium hydride gave xylose, identified by paper chromatography. After separation from any non-reduced material on a paper chromatogram, this sugar had $[\alpha]_D^{25}, 30^0(c, 0.3 \text{ in water})$ and is presumably D-xylose.

This appears to indicate that the substance A is xyluronic acid (I), or rather the lactone (II), since it has such a high R value. This is analogous with glucuronic acid (III) and glucurone (IV).

![Chemical Structures](image)

The objection to this hypothesis is that a lactone such as (II) imposes quite a severe configurational strain on the molecule, whereas glucurone is strainless, and is indeed much more stable than glucuronic acid. Admittedly, no other lactone with less strain can be derived from xyluronic acid. The opening of the lactone ring may explain the rapid fall observed in the specific rotation. D-xyluronic acid has never been found in nature, though it has been obtained by synthesis$^{50}$ and the lactone (II) is unknown.

The substance, A, was at first thought to be the lactone/
lactone of a monomethyl glucuronic acid, but no methoxyl content was detected. Moreover, its colour on the developed chromatogram (aniline oxalate) is pale yellow, completely different from that given by an authentic specimen of 4-methyl glucuronic acid (brick red), whereas glucurone gives a colour identical with that given by glucuronic acid and galacturonic acid.

The material, B, which corresponded with glucurone on the paper chromatogram, was identified as such by its specific rotation and the isopropylidene derivative obtained from it, which was identical with that prepared from an authentic specimen of D-glucurone.

The third uronic acid, C, behaved on the paper chromatogram similarly to an authentic specimen of 4-methyl glucuronic acid. It had $\left[\alpha\right]_D^0 = 38^\circ\text{ (c, 0.5 in water)}$; the specific rotation found for 4-methyl glucuronic acid isolated from gum myrrh is $\left[\alpha\right]_D^0 = 45^\circ\text{ (c, 5.8 in water)}$.

Reduction of C gave a mixture which gave three spots on the paper chromatogram, the first corresponded with that given by 4-methyl glucuronic acid, the second (faint) corresponded with xylose, and the third corresponded with 4-methyl glucose and was different from either of the spots given by 2- and 3-methyl glucose.

The weights of the uronic acid fractions isolated from the column were:

A ... 93mgm.
B ... 86mgm.
C ... 145mgm.
There was also obtained, between B and C, a fourth fraction (53mg.) which appeared to consist of C with a little xylose. Assuming that this fourth fraction is mainly composed of C, then the weights of the three uronic acids are A - 93mg., B - 86mg., C - 198mg.

None of the ratios of two of these fractions to the third bears any relationship to the ratio of xylose to galactose (15:2) which was obtained by examination of the sugars produced by hydrolysis of the aldobriuronic acid. Thus, there is no circumstantial evidence as to which uronic acid residue is combined with which sugar residue. It was hoped that study of the degraded mucilage by the methylation procedure might provide some evidence as to the structure of the aldobriuronic acids.

The uronic acid usually found in mucilages and almost invariably in seed mucilages is galacturonic acid. 4-methyl glucuronic acid has been found in mesquite gum, aspen wood hemicellulose and in gum myrrh. It is most unusual for more than one uronic acid to be found in a gum or mucilage, though 4-methyl glucuronic acid and galacturonic acid have recently been discovered together in aspen wood hemicellulose.

As mentioned in the introduction, Renfrew and Cretcher in their study of quince seed mucilage, isolated an aldobriuronic acid whose methoxyl content (5.5%) indicated that it was composed of a mixture of a monomethyl aldobriuronic acid (72%) and an unmethylated aldobriuronic acid (28%). They were unable to isolate by oxidation of/
of the aldobiuronic acids, either mucic acid or potassium acid saccharate.

The methoxyl content of the aldobiuronic acid, found in this investigation is higher than the calculated value for a monomethyl aldobiuronic acid. Little significance can be attached to this result since the barium salt was isolated in alcohol, which is notoriously difficult to remove completely from solids, even by the most thorough drying.

Early in the investigation it became obvious that if a fractionation of the mucilage could be achieved, without degradation of any of the polysaccharides present, the research would become much more easy. With this object in mind the mucilage was treated with barium hydroxide, Fehling's solution and copper acetate. Bailey and Norris had been able to fractionate the mucilage from white mustard seed\(^{(26)}\) by the addition of saturated barium hydroxide solution to the mucilage solution. No precipitation, or gel-formation, was observed when barium hydroxide solution was added to the aqueous extract from quince seeds.

On the addition of Fehling's solution to a solution of the mucilage a gel was produced which could only be removed by filtration with great difficulty and could not be removed at the centrifuge. Two fractionations of the mucilage by this method gave different results, as well as a very poor recovery of material in each case, presumably owing to losses during dialysis to remove copper. In view/
view of this and the difficulty of manipulation of the gel, it was decided that Fehling's solution would not give a convenient means of fractionation.

The addition of saturated aqueous copper acetate solution to the mucilage gave a highly insoluble precipitate which could be washed with water, and could only be redissolved in water after almost complete removal of the copper. A sample of the neutral mucilage was dissolved in water and copper acetate solution added, giving an insoluble copper salt, QI, and a water-soluble fraction, QII. After removal of copper from the salt by washing in dilute acid (1%), QI could be redissolved in water and reprecipitated with copper acetate. After 5 such treatments the product, after removal of the copper, gave a material which, after hydrolysis, gave an insoluble residue, an alcohol-insoluble barium salt and a syrup which contained xylose and arabinose only. QII, obtained by precipitation in acetone, gave after hydrolysis an alcohol-insoluble barium salt and a syrup which contained galactose, glucose, mannose, arabinose and xylose.

In contrast to the results described above, it was found that, when copper acetate solution was added to the fresh aqueous extract from quince seeds, the product, QI, obtained gave after hydrolysis an insoluble residue, an alcohol insoluble barium salt and a syrup containing galactose, glucose, arabinose and xylose. Indeed, QI after 5 treatments with copper acetate solution appeared to/
to be little different from the acidic mucilage itself.

It was thought that the neutral mucilage used in the first fractionation may have suffered from enzymatic decomposition during its extraction and precipitation. However, treatment of the aqueous extract of the seeds with copper acetate provided a convenient method of obtaining polysaccharide suitable for methylation studies.

Methylation studies were carried out on Q1 prepared from fresh mucilage solution. The product was washed with dilute hydrochloric acid (1%), then with water and finally with methylated spirit. The final product was a moist fibrous solid.

This material was methylated by treatment with sodium hydroxide and dimethyl sulphate, and given 5 such treatments. After each treatment the mixture was dialysed to remove sodium sulphate, and the suspension, after dialysis, concentrated to a slurry. After the fifth treatment the water-insoluble material was removed by filtration and the solution again dialysed free from sodium sulphate, and concentrated to a syrup, which was subjected to remethylation, dialysed and concentrated to dryness, giving a brown glass.

The water-insoluble material was obtained in 19% yield and the water-soluble material also in 19% yield. This low recovery is presumably due to losses during dialysis, which indicates the presence of material of low molecular weight which is either present originally or is produced by the action of the strong alkali used in/
in methylation.

The insoluble methylated material was subjected to several more methylations by the Haworth technique and the chloroform-soluble portion of the material so obtained was methylated twice with Purdie's reagents. The final product had $[\alpha]_{D}^{20} = -2^0 (c, 0.1 \text{ in chloroform})$ and a methoxyl content of 44.4%. This product gave on hydrolysis a trimethyl aldohexose, with a very little dimethyl aldohexose. The trimethyl aldohexose was isolated by chromatography, using a column of powdered cellulose, and was identified as 2:3:6-trimethyl D-glucose by its specific rotation, melting point and its characteristic mutarotation in methanolic hydrochloric acid. This result and the negative specific rotation of the methylated material indicate that it is methylated cellulose. The specific rotation of fully methylated cellulose is usually higher (-10°).[53]

The water-soluble material, which had a methoxyl content of 31.3%, proved to be almost completely insoluble in chloroform. Hydrolysis of a portion of this material gave a syrup which, study by paper chromatography showed to contain at least nine different methylated sugars.

Also the subject of methylation studies was the degraded polyuronide which was isolated as the alcohol-insoluble barium salt, after hydrolysis of the mucilage solution with sulphuric acid (0.5N, 30 minutes). This degraded material, in its acidic form, had a uronic anhydride content of 34.0% and gave, on hydrolysis, xylose (9/
(9 moles), arabinose (3 moles), glucose (2 moles), galactose (1 mole), along with an aldobiuronic acid.

Methylation of the degraded mucilage was carried out by the thallium method. To an aqueous solution (10 gm. in 100 ml. water) was added thallous hydroxide (27 gm.) in saturated aqueous solution. A precipitate was formed which after separation and drying represented 2.7% of the combined weights of thallous hydroxide and degraded mucilage. This low yield was rather surprising since the thallium complexes are usually completely insoluble in water. The filtrate was concentrated to dryness and at no stage did it show any further sign of precipitation. The product was a cream coloured glass which was ground to a powder. The two solids were combined and treated with methyl iodide in the usual way. When the suspension was no longer alkaline to litmus, the methyl iodide was removed by distillation and the residue examined. It proved to be completely insoluble in methanol and other organic solvents. Extraction with water gave a solution of polysaccharide, and also some colloidal thallous iodide. To this solution was added a further quantity of thallous hydroxide (14 gm.) and the treatment described above repeated. After this treatment the thallous iodide residues were extracted with chloroform, giving a solution of a petroleum-insoluble material M1 (1.7 gm.) which had a methoxyl content of 31%.

The residues after extraction with chloroform were almost/
almost completely insoluble in water and completely insoluble in hot methanol. The total thallous iodide residues were combined and extracted with hydrochloric acid (0.1N). The solution obtained was neutralised with potassium hydroxide, concentrated and poured into excess methylated spirit, giving a white precipitate (11.3gm.), which gave a high ash on incineration (62%). This solid was dissolved in water, thallous hydroxide added and the methylation carried out as before, giving 0.43gm. chloroform-soluble material, of methoxyl content 32.5%.

A further extraction of the thallous iodide residues with hydrochloric acid, followed by treatment as usual, gave 0.20gm. methylated polysaccharide. A third extraction and methylation, without the addition of any more thallous hydroxide gave 47mgm. methylated polysaccharide.

The recovery of polysaccharide from these methylations is very poor and cannot be adequately explained. While the concentration to dryness of the degraded mucilage - thallous hydroxide solution is an exceedingly harsh treatment, and is liable to destroy carbohydrate material, it seems doubtful that it would destroy quite so much as the low recovery indicates. What is more probable is that the thallous iodide residues still contain polysaccharide which cannot be extracted, though why this should be so is not apparent.

Methylation of M1 (1.7gm.) by the Purdie method gave no increase in methoxyl content and a very poor yield (0.55gm.).
While concentrating the chloroform solution of methylated polysaccharide, obtained by extraction of the silver iodide residue after the first methylation, some charring appeared to take place, and this may account, at least in part, for the large loss of material. All the methylated derivatives of the degraded mucilage were combined. This mixture had \([\alpha]_D^{20} = 69^\circ\) (c, 0.6 in chloroform). Methanolyis of this mixture (1.1gm.) was carried out in the usual way with methanolic hydrochloric acid, followed by hydrolysis with hydrochloric acid of the glycosides and glycoside esters produced. This mixture (0.7gm.) of methylated sugars and methylated uronic acids was added to a column of powdered cellulose which was eluted with an alkaline solvent in order that the uronic acids would be held back and the methylated sugars separated. Four fractions of methylated sugars were obtained. These were in very small quantity and could only be determined by study of their specific rotations, comparison with controls on the paper chromatogram, and their product or products on demethylation. These fractions were deduced to be:

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Mass (mg)</th>
<th>Methylated Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>19</td>
<td>2:3:6-trimethyl-glucose</td>
</tr>
<tr>
<td>II</td>
<td>17</td>
<td>2:4-dimethyl-xylose</td>
</tr>
<tr>
<td>III</td>
<td>21</td>
<td>(2:3-dimethyl-arabinose)</td>
</tr>
<tr>
<td>IV</td>
<td>25</td>
<td>(2:3:4-trimethyl-galactose)</td>
</tr>
<tr>
<td></td>
<td>82</td>
<td>2-methyl-xylose</td>
</tr>
</tbody>
</table>

The column was washed through with water and the solution/
solution concentrated to dryness, giving a glassy solid 
(0.61gm.). This solid was dissolved in water and added 
to another column of powdered cellulose, which was eluted 
with an acidic solvent, in the hope that some separation 
of the methylated uronic acids might be achieved. Three 
fractions were obtained but study of them on paper 
chromatograms indicated that only poor separation had been 
effected. These fractions were:

A (193mgm.) - Rg value 0.72
B (88mgm.) - Rg values 0.72 and 0.57
C (85mgm.) - Rg value 0.57
D (16mgm.) - Rg value 0.30
E (96mgm.) - water wash, containing xylose (trace).

Fraction A was reduced with lithium aluminium hydride 
and the reduced material studied on paper chromatograms. 
These showed four spots:

(1) corresponding to 2:3:4-trimethyl glucose,
(2) corresponding to 2:3-dimethyl xylose,
(3) faint Rg 0.57 not corresponding to 2:4-dimethyl 
glucose, perhaps due to 3:4-dimethyl glucose,
(4) faint, corresponding to 3-methyl xylose.

Demethylation of the reduced material gave glucose 
and xylose (trace). These results indicate the presence 
in Fraction A of 2:3:4-trimethyl glucuronic acid, a 
dimethyl glucuronic acid, 2:3-dimethyl xyluronic acid and 
3-methyl xyluronic acid. These would also have been 
obtained by the reduction of a mixture of a fully 
methylated/
methylated and a partially methylated aldobiuronic acid consisting of a glucuronic acid residue, linked through carbon atom 1 to carbon atom 4 of a xylose residue. Such an aldobiuronic acid has recently been discovered in the hemicellulose from aspen wood.

Reduction of Fraction C gave a material which showed three spots on the paper chromatogram:

1. corresponding to 2:3-dimethyl xylose,
2. corresponding to 3-methyl xylose,
3. very faint corresponding to xylose.

Demethylation of the reduction products of C gave xylose and no other free sugar.

Evidence in support of the hypothesis that xyluronic acid is present in the mucilage is obtained from the reduction products of Fraction A and C. The reduction products of C cannot readily be explained without assuming the presence of xyluronic acid in the degraded mucilage.

Evidence in support of the hypothesis that xyluronic acid is present in the mucilage is obtained from the reduction products of Fraction A and C. The reduction products of C cannot readily be explained without assuming the presence of xyluronic acid in the degraded mucilage.

If we assume that Fraction B contains equal quantities of Fraction A and C, and ignoring Fractions D and E, we have the following figures for the products of hydrolysis of the methylated degraded mucilage:

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Weight</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>19mgm.</td>
<td>2:3:6-trimethyl-D-glucose.</td>
</tr>
<tr>
<td>II</td>
<td>17mgm.</td>
<td>2:4-dimethyl-D-xylose.</td>
</tr>
<tr>
<td>III</td>
<td>21mgm.</td>
<td>(2:3-dimethyl-arabinose (2:3:4-trimethyl)-galactose.</td>
</tr>
<tr>
<td>IV</td>
<td>25mgm.</td>
<td>2-methyl-D-xylose.</td>
</tr>
<tr>
<td>A</td>
<td>237mgm.</td>
<td>(2:3:4-trimethyl glucuronic acid (2:3-dimethyl xyluronic acid.</td>
</tr>
<tr>
<td>C</td>
<td>129mgm.</td>
<td>(2:3-dimethyl xyluronic acid (3-methyl xyluronic acid.</td>
</tr>
</tbody>
</table>
Traces of other materials in Fractions A and C have been ignored.

It will be seen that the total quantity of the methylated uronic acids is much greater than that of the methylated sugars, although the uronic anhydride content \((\text{C}_6\text{H}_8\text{O}_6)\) of the degraded mucilage before methylation was 34%. Undoubtedly the methylation procedure has caused further degradation of the material and it would be unwise to make any conjecture concerning the structure of the degraded mucilage from these results. Moreover, the methoxyl content of the methylated material was too low to assume that it was completely methylated.

From the identification of the methylated sugars which make up Fractions I, II, III, and IV, it follows that the sugar residues are linked as follows in the degraded mucilage.

\[
\begin{align*}
\text{GL} & \quad ; \quad \text{X} \quad ; \quad \text{A} \\
\text{GL} & \quad ; \quad \text{X}
\end{align*}
\]

where GL = D-glucose  \quad GA = \text{\textbeta}-galactose  \\
X = \text{\textbeta}-xyllose  \quad A = \text{\textalpha}-arabinose

The presence of 2:3:4-trimethyl glucuronic acid indicates that the glucuronic and 4-methyl glucuronic acid residues are linked through carbon atom 1 in the degraded mucilage. Similarly, xyluronic acid is linked through carbon atom 1, and these must be present as end groups.

Whether/
Whether the presence of 3-xyluronic acid indicates that some xyluronic acid residues are linked through carbon atoms 1 and 2 or that the residue was not fully methylated is uncertain. The second possibility is much more probable, however, in view of the low methoxyl content of the material which was hydrolysed.

This series of investigations indicates the complexity of quince seed mucilage and also the need for its further investigation.

Until the reason for the astonishing loss of material during the methylation of seed mucilages is known, it would be unwise to make any suggestions for further work which would indicate the structure of quince seed mucilage.

However, investigation, including study by methylation, of the aldobiuronic acids present could be made. It is also probable that valuable evidence concerning the modes of linkages between sugar residues in the mucilage would be obtained by isolation of oligosaccharides after mild hydrolysis (say by autohydrolysis) of the mucilage.
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1. Quince seed mucilage (in ca. 6% yield) was prepared by aqueous extraction of the seeds, followed by precipitation of the mucilage with alcohol.

2. The mucilage gave, on hydrolysis (1N sulphuric acid), an insoluble residue (24.4%), an alcohol-soluble syrup (44.4%) and an aldobiuronic acid, isolated as the barium salt (24.4%).

   The alcohol-soluble syrup contained xylose (ca. 31.3%), glucose (ca. 20.5%), arabinose (ca. 16.4%), galactose (ca. 13.5%), mannose (ca. 10.6%) and an unidentified sugar (ca. 7.6%).

   The insoluble residue on further hydrolysis (2N sulphuric acid) gave glucose, galactose, mannose and an insoluble residue. This remaining residue gave, on hydrolysis (Monier-Williams method), glucose and galactose (trace).

   The aldobiuronic acid gave, on hydrolysis (4N sulphuric acid), xylose, galactose, 4-methyl glucuronic acid, glucuronic acid and an unidentified uronic acid, apparently xyluronic acid.

3. Hydrolysis (0.5N sulphuric acid, 30 minutes) of the aqueous extract from quince seeds gave a degraded polyuronide, isolated as the alcohol-insoluble barium salt.

   Hydrolysis (1N sulphuric acid) of the degraded mucilage gave xylose (10.8%), arabinose (10.8%), glucose (3.4%), galactose (2.4%) and an aldobiuronic acid, removed as/
as the barium salt (86.2%).

4. A glucan, which hydrolysed completely to glucose, was isolated from the aqueous extract of quince seeds.

5. Methylation of the polysaccharide, precipitated from the mucilage solution by copper acetate, gave a water-soluble fraction and a water-insoluble fraction. Further methylation of this latter fraction gave a material which was shown to be methylated cellulose.

6. Methylation of the degraded mucilage gave a product which on hydrolysis gave 2:3:6-trimethyl glucose, 2:4-dimethyl xylose, 2:3-dimethyl arabinose, 2:3:4-trimethyl galactose, 2-methyl xylose, xylose (trace) and methylated uronic acids. The methylated uronic acids, after reduction, gave 2:3:4-trimethyl glucose, 2:3-dimethyl xylose, an unidentified dimethyl glucose, 3-methyl xylose and xylose (trace).
ACKNOWLEDGMENT.

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