CXCII. THE NATURE AND COMPOSITION OF THE MUCILAGE OF THE SEED OF WHITE MUSTARD (*BRASSICA ALBA*).

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IN comparison with the immense bibliographies of starch and cellulose for example, the literature of the gums and mucilages is scanty. This, doubtless, is due in part to the great commercial importance of the former, but a contributory cause may be sought in the practical difficulties involved in dealing with the latter. The gums and mucilages are complex colloids which are not infrequently found to be mixtures of almost equally complex substances of closely similar physical and chemical properties. Separation of the constituents is consequently difficult, nor, when a separation has been achieved, is the characterisation of the components an easy matter. Nevertheless, increased knowledge of the chemical nature of such substances should be of interest to the plant biochemist and physiologist alike, and it is conceivable that the industrialist may also benefit ultimately as a result of such studies.

Comparatively modern researches on the plant mucilages may be said to date from the end of the nineteenth century, when Gans and Tollens [1888] investigated the mucilage of quince. Similar products were examined by Yoshimura [1895] and von Bitto [1895], and this work was supplemented later by investigations of the mucilage of the prickly pear [Harlay, 1902], and of linseed [Hilger, 1903; Neville, 1913].

The early researches were concerned chiefly with the nature of the sugars produced on hydrolysis, and in the case of linseed mucilage, for example, Hilger reported xylose, glucose, arabinose and galactose as constituent sugars. Neville also isolated the "barium salt of an organic acid," but was not convinced that the acid was a constituent of the mucilage, regarding it as an impurity. This observation is of peculiar interest in view of the fact that the existence of uronic acids in such plant materials was at that time unsuspected.

Re-awakened interest in the study of such plant products as pectin, hemicelluloses, gums and mucilages has followed the recognition of the wide occurrence of these acids, and recent investigations tend further to emphasise their importance. Galacturonic acid had been shown to be a constituent of

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pectin by Ehrlich [1917], and the occurrence of this acid and of glycuronic acid in various hemicelluloses was also demonstrated [O'Dwyer, 1926; Norris and Preece, 1930, and others]. Another member of the class, mannuronic acid, whose existence in plant products was foreshadowed by Cretcher and Nelson [1929] was shown to be a constituent of the cell-wall substances of *Laminaria* spp. by Bird and Haas [1931].

There is now reason to believe that in many plant products the uronic acid forms an aldobionic acid nucleus which is relatively resistant to hydrolysis. Thus, Butler and Cretcher [1929] isolated from gum arabic a galactose-glycuronic acid, and Anderson and Crowder [1930] prepared rhamnose-galacturonic acid from linseed mucilage by similar methods. Again, the specific carbohydrate of pneumococcus, isolated by Heidelberger and Goebel [1927] appears to be a polymeride of glucose-glycuronic acid.

The object of the present investigation of the mucilage of mustard seed was not solely the identification of the products of hydrolysis, but involved also the separation of the product into definite fractions. The mode of fractionation, described later, was not based on physical properties—precipitability by alcohol, acetone, *etc.*—as in the case of the hemicelluloses [Norris and Preece, 1930; Preece, 1930], but was effected by more truly chemical means, namely the action of alkali. We have been able to show that the mucilage is composed of cellulose together with acid polysaccharides which yield arabinose, galactose, rhamnose, galacturonic acid and glycuronic acid on hydrolysis, and contain methoxyl groups. The term "acid polysaccharides" has been very generally used by American workers in this field, to indicate polysaccharides which yield uronic acids in addition to simple sugars on hydrolysis. The term "polyuronide" advocated by Schryver has much to recommend it, but does not appear to have found wide acceptance. However, in the present confusion of nomenclature, any attempt at uniformity is desirable, and in this paper the term acid polysaccharide will be used.

By analogy with pectin, it was at first assumed that the methoxyl groups were bound to the carboxyl groups of the uronic acids by ester linkages. This view was, however, abandoned when it was found that the former resisted saponification by alkali, and it must therefore be assumed that these methoxyl groups are bound by ether linkages. A similar phenomenon was observed by O'Dwyer [1928] in the hemicelluloses of beech and oak [see also von Fellenberg, 1918; Anderson and Otis, 1930].

Evidence has been adduced that the rhamnose and at least some of the galactose combines with the uronic acid to form stable aldobionic acid nuclei, similar to those cited by Butler and Cretcher [1929] and Anderson and Crowder [1930]. Such aldobionic acids survive, at least in part, the hydrolytic action of 4 % sulphuric acid at 100° for 16–20 hours.

On soaking the mustard seed in cold distilled water, an extract is obtained which contains the mucilage, precipitable by alcohol, and also free sugars, amongst which galactose was identified. If, then, to this extract an equal

volume of saturated barium hydroxide solution is added, a gel is immediately precipitated, and after standing overnight, may be filtered off. The filtrate contains the free sugars above-mentioned, and also a water-soluble product, Fraction I. This fraction is precipitable by alcohol, yields on hydrolysis rhamnose, arabinose, galactose and galacturonic acid, and contains methoxyl groups.

The gel obtained by the action of barium hydroxide may be further fractionated by the action of sodium hydroxide solution, which effects its separation into cellulose, Fraction II, and a sodium hydroxide-soluble product, Fraction III, precipitable by alcohol, which yields on hydrolysis arabinose, galactose, galacturonic acid and glycuronic acid but no rhamnose. This fraction also contains methoxyl groups. It was found impossible to free the cellulose from substances which gave a positive naphthoresorcinol reaction; such substances, containing uronic acid and furfuraldehyde-yielding groups, may constitute an integral part of the cellulose itself, which may be of the nature of an oxy-cellulose; or the positive reaction may merely indicate that it is impossible to separate Fraction III completely.

All samples of the mucilage extract gave a strong positive reaction with benzidine and hydrogen peroxide, indicating the presence of peroxidase. In a histological examination of the testa, the sclerenchymatous palisade cells were found to give a similar strong peroxidase reaction, and these cells are only separated from the mucilage-containing cells of the cuticle by two rows of thin-walled collenchymatous cells. The possible significance of these observations is discussed later.

EXPERIMENTAL.

Preparation of the mucilage.

The dry seed is extracted with cold distilled water in the proportions of one part of seed to ten parts of water. The extraction is continued for 24 hours with efficient stirring or shaking, and the viscous extract obtained is filtered through muslin, while the seed is returned to the vessel for two additional extractions. The united extracts are then filtered through a bed of glass wool and muslin to remove foreign particles and the filtrate poured into alcohol, which precipitates a fibrous gelatinous product. Filtration, re-solution and reprecipitation of the mucilage may be carried out several times, the product then being dried in successive increasing concentrations of alcohol, and finally *in vacuo* over phosphorus pentoxide. Prepared thus, a snow-white fibrous product is obtained, corresponding in yield to about 2 % of the dry seed.

The mucilage swells in water and finally dissolves, giving an opalescent colloidal solution which gives no coloration with iodine, neither does it reduce Fehling's solution.

On heating the mucilage for a short time with dilute sulphuric acid a gel separated out, and was shown later to consist of cellulose. After more prolonged

hydrolysis and neutralisation with barium carbonate, barium salts of aldobionic acids, one of which contained rhamnose, were obtained. The residual solution after alcoholic precipitation of the barium salts, was shown to contain free sugars, namely, arabinose and galactose. The characterisation of these products is discussed in detail at a later stage.

Analysis of the mucilage.

Preliminary experiments had thus shown that the mucilage was composed of cellulose, together with compounds which contained carbohydrate and uronic acid residues. Analysis of the mucilage was then undertaken, the following determinations being made:

- (i) Ash.
- (ii) Cellulose.
- (iii) Methoxyl.
- (iv) Furfuraldehyde and methylfurfuraldehyde.
- (v) Uronic anhydride.
- (vi) Nitrogen (Kjeldahl).

Ash was determined by direct ignition at a dull red heat. Cellulose was determined by the method of Cross and Bevan [1889], with slight modifications [Norman, 1929, 2] chiefly designed to minimise manipulative losses. Methoxyl was determined by the Perkin [1903] modification of Zeisel's method. Furfuraldehyde and methylfurfuraldehyde were estimated by the phloroglucinol methods of Tollens and Kröber, and of Ellett and Meyer [Browne, 1912]. The probable inaccuracies involved in these estimations need no emphasis at this stage; the chief source of error in the estimation of methylfurfuraldehyde in the present instance is the presence of ω -hydroxymethylfurfuraldehyde from hexosic material, the results being somewhat high on this account. Uronic anhydride was estimated by the decarboxylation method of Nanji, Paton and Ling [1925], using a modified apparatus which gave accurate results with less than 0.1 g. of material.

Ash. Specimens of mucilage, prepared from different samples of seeds under slightly varying conditions, yielded varying quantities of ash. After dialysis of the mucilage in thin collodion bags of high permeability for 20–40 days, the ash in all cases fell to about 3.5 %.

Ash content of samples of mucilage.

Sample	Original ash %	After 20–40 days' dialysis: ash %
A	8.4	3.75
B	6.82	3.38
C	6.16	3.60
D	5.73	3.57
E	16.2	—

The ash of the dialysed mucilage was submitted to spectrographic analysis: calcium, magnesium and iron were present in abundance; strontium and

aluminium were also found. Sodium and potassium were conspicuous by their absence. The presence of silicon and phosphorus was demonstrated by chemical means.

It is not improbable that the undialysable ash forms an integral part of the mucilage, which may exist naturally as a calcium, magnesium or iron salt; silicic and phosphoric esters also, are of common occurrence in complex polysaccharides, as exemplified in the case of starch and some hemicelluloses.

The different samples of mucilage showed variations in the analytical results obtained, not only in the case of the ash, but also more particularly with regard to the cellulose and methoxyl contents; the hexosan content (by difference) likewise reflected these variations. The most strikingly contrasted samples were C and E, which gave the following results, calculated on an ash-free basis.

Analysis of samples of mucilage.

	(%) C	(%) E
Cellulose	45.70	55.60
Methoxyl	2.00	1.43
Uronic anhydride	20.43	17.24
Pentosan	6.73	6.13
Methylpentosan	7.14	6.44
Hexosan (by difference)	18.00	13.16
Nitrogen	—	0.66

Separation of the mucilage into three fractions.

To a large volume of an aqueous extract of the mucilage, an equal volume of a saturated solution of barium hydroxide was added, when a gel very rapidly formed. After standing overnight in absence of air, the gel was filtered off through muslin and subsequently freed from liquid as far as possible in a mechanical press. If insufficient barium hydroxide is added the gel cannot be obtained in a fibrous condition and is then difficult to treat. After re-extracting twice with saturated barium hydroxide, the united filtrates and the residue were treated separately.

(a) *The filtrate.* The excess barium hydroxide in the filtrate was precipitated as barium carbonate by passing carbon dioxide into the solution. After filtration, the solution was evaporated under diminished pressure and from time to time carbon dioxide was again passed into the solution, the evaporation being continued after further filtration until the volume of liquid was considerably reduced. Alcohol to a concentration of 75 % was then added with rapid stirring, and after standing overnight a white flocculum was filtered off, washed with absolute alcohol and air-dried. The product was then redissolved in water, the solution saturated with carbon dioxide and allowed to stand overnight in the ice-chest. On subsequent heating for 30 minutes on the water-bath, a fine suspension of barium carbonate settled out and was removed at the centrifuge. The object of this procedure was to prevent "saccharate" formation. The clear solution was again poured into alcohol and the precipitate, Fraction I, was dried in the usual manner.

It may be mentioned in passing that the alcoholic filtrate from the above precipitation was concentrated, primarily with the object of recovering the alcohol. It was found that the liquid concentrated to a syrup which reduced Fehling's solution and contained free sugars. Similarly, a strongly reducing syrup may be obtained by concentrating the alcoholic filtrate from freshly precipitated mucilage extract. The free sugars appear to be extracted from the seed at the same time as the mucilage and are always present in the watery extract of the seed. As this observation was not germane to the main object of the research, the sugars were not thoroughly investigated, although one of them was identified as galactose. It should be noted, however, that if the fractionation of the mucilage is carried out with fresh mucilage extract, these sugars will be found in the alcoholic filtrate from Fraction I, but will not be present if a solution of precipitated mucilage is used as the starting-point.

(b) *The gel.* This, which was a complex of cellulose with acid polysaccharides, was separated into two components by extraction for four hours with 4 % sodium hydroxide solution at 40–50°, this treatment being repeated until the residual cellulose gave only the faintest naphthoresorcinol reaction. Usually three extractions sufficed, although it was found almost impossible to free the cellulose entirely from uronide constituents. The fibrous cellulose was treated with acid to remove free alkali and washed with water until free from acid and soluble inorganic salts. A white fibrous product was obtained on drying in the usual manner and was designated Fraction II.

After filtration of this fraction, the united sodium hydroxide extracts were neutralised with glacial acetic acid and poured into an equal volume of acetone. The white gum obtained was redissolved in 4 % sodium hydroxide solution, filtered through paper pulp and reprecipitated as a copper gel by addition of Fehling's solution. The copper gel was decomposed with hydrochloric acid and again precipitated with acetone. Re-solution and reprecipitation followed, until a white product, free from copper, was obtained. This was dried in the usual manner and constituted Fraction III.

Analysis of Fraction I.

Fraction I is a white powder which swells in water and finally dissolves; the solution gives no iodine coloration, nor does it reduce Fehling's solution. The specific rotation $[\alpha]_D^{15} = +10^\circ$.

On analysis by the usual methods, the following results were obtained:

	%
Barium	9.45
Pentosan	6.80
Methylpentosan	10.00
Uronic anhydride	24.20
Methoxyl	4.90
Hexosan (by difference)	44.65
Nitrogen	nil

Assuming all the barium to be combined with the available carboxyl groups of the uronic acid, this percentage—9.45—would correspond to 24.3 % of

uronic anhydride, a very close approximation to the figure obtained above by decarboxylation. That the carboxyl groups of the uronic acid are free to combine with barium in this manner appears also to be indicated by the fact that they are not in ester combination with methoxyl. Thus, on treatment for 2 hours with 2% sodium hydroxide at 80°, the product still contained 3.5% of methoxyl, showing that these groups are resistant to hydrolysis, and are therefore probably in ether-linkage [von Fellenberg, 1918]. Moreover, on prolonged dialysis of the product through cellophane membranes, only traces of barium passed out, and this is not likely to be the case unless the substance is to be regarded as a barium compound. The balance of evidence is thus in favour of this last conclusion.

Hydrolysis of Fraction I.

70 cc. of 2.5% sulphuric acid were added to 3 g. of the product and the mixture was heated under reflux on a boiling water-bath for 4 hours. Neutralisation was effected by addition of saturated barium hydroxide solution, excess being removed by means of carbon dioxide. After filtration and evaporation under diminished pressure, the barium salt was precipitated by alcohol, and after drying weighed 0.9 g. The alcoholic filtrate was evaporated to dryness and the sugars extracted with successive portions of hot absolute, 80% and 60% alcohol. The fraction soluble in absolute alcohol contained a small amount of a sugar giving a positive orcinol reaction; the bulk of the sugar, however, dissolved in 60% alcohol.

Fraction soluble in absolute alcohol. Arabinose was present, and was isolated as the characteristic diphenylhydrazone melting at 200–201°. Xylose was absent; in repeated attempts to prepare cadmium xylonobromide, fine needle-shaped crystals were obtained entirely different from the characteristic boat-shaped crystals obtained from authentic xylose.

Fraction soluble in 80% alcohol. Galactose was present. The methylphenylhydrazone was prepared [van der Haar, 1920] and after several recrystallisations from 30% alcohol, melted at 189°. This was confirmed by admixture with an authentic specimen of galactose methylphenylhydrazone; the mixture again melted at 189°.

Fraction soluble in 60% alcohol. This consisted chiefly of galactose. On oxidation with nitric acid, sp. gr. 1.15, mucic acid was obtained, melting, after reprecipitation, at 219°.

The original syrup before extraction with alcohol gave negative results when tested for keto-hexoses by the Pinoff-Selivanoff method.

Treatment of the barium salt. The best means of identifying the methylpentose present in the fraction was afforded by an examination of the hydrolysis products of this barium salt. It has been stated that the methylpentose exists in relatively stable combination as an aldobionic acid and is therefore to be expected in the barium salt prepared as indicated. The barium salt was hydrolysed under more drastic conditions than in the original preparation as follows:

0.9 g. of the barium salt was boiled under reflux for 10 hours with 25 cc. of 5 % sulphuric acid, and after neutralisation with barium hydroxide, the solution was filtered, evaporated to a thin syrup and divided into two portions. After this treatment there was no precipitable barium in the syrup, indicating that the prolonged hydrolysis had broken up the aldobionic complex and destroyed the uronic acid as such. The only sugar likely to be present in the syrup was therefore the methylpentose, with, possibly a small amount of galactose. (The nature of the aldobionic acids is discussed on p. 1619.) The *p*-bromophenylosazone was prepared from the first portion of the syrup, according to the method indicated by van der Haar [1920]. The crude osazone was washed with ether followed by 70 % and then hot 95 % alcohol. There remained a yellow precipitate melting at 218°, and there was no depression of this melting-point on testing a mixture of the sample and an authentic specimen of rhamnose *p*-bromophenylosazone. The second portion of the syrup was freed from a trace of galactose by means of the methylphenylhydrazine, which was filtered off. Excess of methylphenylhydrazine was removed from the solution by shaking with ether, and the *p*-bromophenylosazone again prepared. This sample also melted at 218° and there is thus little doubt that the methylpentose in the barium salt is rhamnose.

Identification of the uronic acid. The method employed was that used by Heidelberger and Goebel [1927] in their study of the uronic acid from the specific polysaccharide of pneumococcus.

1.2 g. of Fraction I were dissolved in 12 cc. of 7.5 % hydrobromic acid containing 0.6 cc. of bromine, and heated on a boiling water-bath under reflux for 10 hours. The uronic acid produced on hydrolysis is immediately oxidised to a dicarboxylic acid, while free sugars are converted into the corresponding monocarboxylic acids. If, therefore, the dicarboxylic acid can be identified, the nature of the original uronic acid is known.

After standing for 24 hours a white precipitate was formed, which on purification weighed 0.06 g. and was found to be mucic acid, melting at 216°. The experiment was repeated with similar results and a control experiment with pure galactose yielded no trace of mucic acid. The original uronic acid from which the mucic acid was derived was galacturonic acid.

No other sugars or sugar acids could be detected by all the usual methods in the hydrolysis products of Fraction I, which therefore appears to consist of a barium salt or salts of polysaccharides which yield on hydrolysis rhamnose, arabinose, galactose and galacturonic acid, and also contain methoxyl groups. The plural is used advisedly, since there is no evidence that Fraction I is a definite chemical entity.

Analysis of Fraction II (cellulose).

This fraction was prepared from the mucilage not only by the alkali treatment outlined previously, but also more directly by acid hydrolysis as follows. An aqueous solution of mucilage was heated at the temperature of the boiling water-bath with 1-4 % sulphuric acid for 2 hours. At the end of this

period, a fibrous gel had separated and was filtered off, washed thoroughly until free from acid, dried in graded strengths of alcohol and finally to constant weight at 100°. The gel obtained by both the acid and alkali treatments was identified as cellulose by the following experiments.

(a) It was extraordinarily resistant to acid hydrolysis.

(b) It was soluble in concentrated zinc chloride solution.

(c) It gave a blue colour with chlor-zinc-iodine.

(d) 2 g. of the product were hydrolysed with 72 % sulphuric acid according to the method of Monier Williams [1921]. The only sugar present after hydrolysis was glucose as indicated by the characteristic phenylosazone, which melted sharply at 205°. The amount of glucose produced, as estimated by Fehling's method, corresponded to 80.5 % of the weight of gel taken, or 72.5 % of theory. The specific rotation of the purified sugar was $[\alpha]_D^{20} = +52.0^\circ$ in a second hydrolysis using 10 g. of the product.

(e) A further 10 g. of the product were acetylated by the method of Irvine and Hirst [1922]. A pure white granular product was obtained, and the acetyl groups therein were estimated by the saponification method indicated in the above-quoted paper.

	%
Original cellulose prepared by acid treatment: Found, CH_3CO	43.7
Original cellulose prepared by alkali treatment: Found, CH_3CO	45.3
Theoretical for cellulose triacetate: CH_3CO	44.8

The difference between the found and the theoretical figures is within the usual experimental error involved in such saponification methods, and hence it may be concluded that the acetate obtained is very closely similar to normal cellulose triacetate.

The cellulose obtained by alkali treatment was also subjected to analysis, with the following results:

	%
Ash	13.30
Furfuraldehyde	1.48 (ash-free basis)
Uronic anhydride	3.40 „

The presence of these substances would be responsible for the positive naphthoresorcinol reaction given by the cellulose, and the possible significance of this observation has been discussed previously.

Analysis of Fraction III.

Fraction III is a white powder, soluble in sodium hydroxide solution; it gives no coloration with iodine, nor does it reduce Fehling's solution.

The following analytical results, calculated on an ash-free basis, were obtained:

	%
Uronic anhydride	14.8
Pentosan	9.4
Methoxyl	3.4
Methylpentosan	nil
Nitrogen	nil
Hexosan (by difference)	72.4

It was impossible to determine the optical rotation owing to the highly opaque colloidal solution obtained even at considerable dilution.

Hydrolysis of Fraction III.

By similar methods (hydrolysis, preparation of substituted phenylhydrazine derivatives, *etc.*) to those already described for Fraction I, the following constituents were identified:

- (a) arabinose (diphenylhydrazone, m.p. 201°).
- (b) galactose (methylphenylhydrazone, m.p. 198°).

No other sugars were present, all the recognised tests giving negative results.

The characterisation of the uronic acids present in Fraction III calls for more detailed description. The method of procedure adopted was that of Heidelberger and Goebel [1927] previously described. In preliminary experiments using 1 g. and 3 g. respectively of the product, no mucic acid could be obtained, and it was at first thought that galacturonic acid was therefore not a constituent of the fraction. Moreover, attempts to isolate from the oxidation products (i) saccharic acid as potassium acid saccharate; and (ii) mannosaccharic acid as the diamide, were alike unsuccessful, and appeared to indicate the absence of both glycuronic and mannuronic acids from the original substance.

The precipitation of mucic acid is hindered, as is well known, by the presence of relatively large amounts of other organic matter, and as the fraction contains only 14 % of uronic anhydride it was thought possible that the small quantity of mucic acid had failed to come down. In further experiments, therefore, some of the sugars were removed by hydrolysis, and on neutralisation with barium hydroxide, the filtrate contained a barium salt which was precipitated by alcohol. 3 g. of this salt were submitted to bromine oxidation and yielded 0.32 g. of mucic acid, melting at 220°. The original presence of galacturonic acid was therefore inferred, and the above result confirmed by repetition.

The filtrate after separation of the mucic acid was examined for additional oxidation products and was evaporated to a syrup *in vacuo* at room temperature. Barium bromide was removed as it crystallised out and, after addition of 50 % potassium hydroxide, large amounts of potassium bromide were precipitated and filtered off. To the final clear filtrate a slight excess of glacial acetic acid was added, and after standing for three days, a fine crystalline precipitate was filtered off. On recrystallisation the product appeared as thick needle-shaped crystals with trapezoidal planes, identical with authentic crystals of acid potassium saccharate. Analysis of the crystals gave the following results:

0.0173 g. crystals yielded 0.0060 g. potassium sulphate, corresponding to 15.5 % potassium. Calculated: potassium 15.75 %.

The presence of glycuronic acid, in addition to galacturonic acid in Fraction III is therefore to be inferred.

No further sugars or sugar-acids were detected as constituents of this fraction, which as prepared is probably a sodium salt. The parent substance is composed of polysaccharides which yield on hydrolysis arabinose, galactose, galacturonic acid and glycuronic acid, and contain methoxyl groups. As in the case of Fraction I, there is no evidence that the product is a chemical entity; indeed it is most probable that both fractions are complex mixtures.

Isolation of barium salts of aldobionic acids.

It has been shown that hydrolysis of the mucilage for 2 hours with 4 % sulphuric acid suffices to precipitate the cellulose. In the experiment to be described, the hydrolysis, after separation of the cellulose, was continued for a further 16 hours, at the end of which period the solution was neutralised, while still warm, with barium carbonate. The precipitated sulphate was filtered off, the filtrate evaporated to small bulk under diminished pressure and poured into 95 % alcohol. The flocculum was separated by decantation and filtration, and after twice redissolving and reprecipitating was finally obtained as a white powder on drying in the usual manner.

The yield of salt from 50 g. of mucilage was only 0.85 g. so that an extensive examination was precluded. The following analytical results were obtained:

	%
Barium	21.70
On an ash-free basis:	
Uronic anhydride	51.00
Methylpentose	17.4
Hexose (by difference)	31.6
Furfuraldehyde	9.59
Furfuraldehyde from uronic anhydride (calc.)	8.50
Residual furfuraldehyde	1.09

The residual furfuraldehyde would correspond to less than 2 % of pentosan and may be neglected. For an aldobionic acid mixture of hexose-uronic acid and methylpentose-uronic acid in the proportions of 2:1, the calculated analytical results are as follows:

	%
Uronic anhydride	50.3
Methylpentose	16.1
Hexose	33.6

Having regard to the errors of the analytical methods involved, this is a sufficiently close approximation to the experimental figures above.

The product was obtained after prolonged hydrolysis under conditions which would destroy free uronic acids, and the results indicate that relatively stable aldobionic acid nuclei exist in the mucilage.

The operations described in the experimental section and the nature of the products obtained are indicated in the following scheme.

Scheme showing separation of mucilage into three fractions.

DISCUSSION.

From the foregoing it appears that the mucilage of mustard seed may be regarded as a type of compound cellulose in which the non-cellulosic moiety is composed of acid polysaccharides. The appearance of cellulose in the mucilage to the extent of roughly 50 % was unexpected, since there seems to be no reference in the literature to such a possibility. That there are other cases of its occurrence is indicated by experiments now proceeding on the mucilage of linseed, in which there appears to be a small proportion of cellulose, which has possibly been overlooked by other workers.

In the light of recent results by American workers, the existence in the mucilage of aldobionic acids was to be expected and it seems probable that a rhamnose-galacturonic acid and possibly a galactose-galacturonic acid are present. Challinor, Haworth and Hirst [1931], in a study of the galactose-glycuronic acid of gum arabic, have shown that the galactose residue is joined in the 6-position with the reducing group of the uronic acid residue. Such questions of linkage are outside the scope of the present communication, but it may be remarked in passing that in the case of rhamnose-galacturonic acid

neither the 5- nor the 6-position appears to be available for the purpose of linkage with the uronic acid residue.

The solubility relations of the complex polysaccharides constitute a physico-chemical problem of considerable interest. In the present instance cellulose, symbol of stability, completely insoluble in water, is rendered readily soluble by the presence of the acid polysaccharide moiety. The stability of the solution has been seen to be speedily affected by the addition of alkali or by warm dilute acid; the change, however, is not solely a physical one, but appears to involve some chemical rupture. On examination of the various products described in this paper, a possible correlation between solubility and the presence of uronic acids, or more particularly, carboxyl groups, suggests itself. Thus (i) cellulose, uronic anhydride content 3.4 %, is insoluble in water; (ii) Fraction III, uronic anhydride content 14.8 %, is soluble only with difficulty and gives a dense opaque solution; (iii) Fraction I, uronic anhydride content 26.7 % on a barium-free basis, is easily soluble in cold water.

The temptation to ascribe increasing solubility to increasing proportions of acid groups is considerable, but it is also probable that purely physical factors are concerned. In this connection it may be recalled that the solubilities of members of the pectin group are still difficult to explain; thus, pectic acid, with a uronic anhydride content of some 72 % is insoluble, whereas pectin in which the carboxyl groups are esterified, is comparatively readily soluble.

The probable mode of origin of uronic acids and pentoses from hexose material of the plant needs no prolonged discussion at this stage. The uronic acids may be produced by oxidation of the hexoses and in their turn suffer loss of carbon dioxide to yield pentoses [Haworth, 1929; Norman, 1929, 1; Norman and Norris, 1930]. The occurrence of the various hydrolysis products of the acid polysaccharides of the mucilage may be explained on these lines. Indeed, the mucilage itself seems to be an excellent example of an intermediate between cellulose, with all its hexosan units intact, and a typical pentosan, wherein the hexosan units of the original substance have suffered complete chemical change. In this light, the mucilage might be regarded as representing a half-way stage in the degradation.

Mild oxidation of pectin *in vitro* by means of hydrogen peroxide in the presence of a catalyst was shown by Norman and Norris [1930] to involve the production of substances of the nature of acid polysaccharides. The observation that a peroxidase system exists in cells closely associated with the mucilage-secreting cells of the mustard seed may indicate that oxidative processes do actually take place *in vivo* and may well account for the production of substances containing uronic acids.

The presence of a rhamnose-uronic acid in the mucilage constitutes a difficulty as its production from original hexose residues involves both oxidation and reduction; oxidation of a terminal CH_2OH group to produce the uronic acid, and reduction similarly to produce rhamnose. There is no experimental evidence which supports such a possibility, but it is conceivable that a

complete oxidation-reduction system may exist in the living organism which would be capable of effecting the complete change.

Whilst positive results have been recorded with reference to the composition and nature of the mucilage, the object of the present discussion has been largely to indicate the problems which still await solution.

SUMMARY.

1. The mucilage of mustard seed is shown to consist of a complex of cellulose and acid polysaccharides.

2. The cellulose may be readily separated by warming with dilute sulphuric acid.

3. By addition of barium hydroxide solution to the mucilage solution a gel and also a soluble fraction composed of rhamnose, arabinose, galactose and galacturonic acid are obtained.

4. The gel obtained above may be further separated by the action of 4 % sodium hydroxide into cellulose and a fraction composed of arabinose, galactose, galacturonic acid and glycuronic acid.

5. The acid polysaccharides contain methoxyl groups in ether-linkage.

6. Aldobionic acids, probably rhamnose- and galactose-galacturonic acids are present in the mucilage.

7. A peroxidase system is present in cells situated close to the mucilage-secreting cells and may be responsible for the production of uronic acid residues from pre-existing hexosan material.

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ADDENDUM.

While the above communication was in the Press, the existence of cellulose as a constituent of quince seed mucilage was reported by Cretcher and Renfrew [1932, *J. Biol. Chem.* **97**, 503].

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