THE COMPOSITION OF FLAXSEED MUCILAGE

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C O N T E N T S

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INTRODUCTION

Mucilages are substances which swell up in water and give colloidal solutions which are slimy. They are distinguished from the pectic substances by the fact that they do not gelatinize. They are widely distributed in nature and they may occur in any organ of the plant. However, in some cases they are confined to certain cells such as mucilage canals or sacs. Some of the best-known examples of mucilage-bearing tissue are those in the root and flower of the hollyhock (Althea Rosea), in certain bulbs and tubers, in the berries of the mistletoe (Viscum Album), and in the seeds of the flax (Linium Usitatissimum).

The vegetable mucilages have as a whole been very little investigated. This is due to the fact that it is difficult to prepare them in a state of purity. Early investigators hydrolysed flaxseed mucilage and determined the presence of the various sugars in the hydrolytic products. In 1903 Hilger[1] hydrolysed it and found in the hydrolytic

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[1] Hilger, A., Zur Kentniss der Pflanzenschleime. Ber., 36: 3197-3203 (1903).

products d-galactose, l-xylose, and l-arabinose. In 1913 Neville[2] investigated the hydrolytic products and verified the presence of the sugars reported by Hilger and in addition d-glucose. In addition to sugars Neville found an acid residue to which were attached sugars. He was undecided as to whether this acid residue was part of the original mucilage molecule or was the result of some process going on during hydrolysis. He rather favored the view that the mucilage was a polysaccharide giving only sugars on hydrolysis. This view was also held by Onslow[3] who states that the difference between flaxseed mucilage and plant gums is that the gums on hydrolysis yield in addition to sugars other products of an acid nature. Abderhalden[4] states that flaxseed mucilage on hydrolysis yields in addition to sugars an acid complex which contains one pentose and one hexose.

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2 Neville, A., Linseed Mucilage. J. Agr. Sci., 5:113-128 (1913).

3 Onslow, M. W., Practical Plant Biochemistry, p. 62. Cambridge University Press (1920).

4 Abderhalden, E., Biochemisches Handlexikon, p. 65. II Band. Julius Springer, Berlin (1911).

PREPARATION AND PROPERTIES OF FLAXSEED MUCILAGE

Flaxseed mucilage is prepared by soaking the seed with four times their weight of water for 24 hours. After a few hours the seed swell up and a thick slimy solution of the mucilage results. This is separated from the seed by squeezing through cheese cloth and is precipitated by the addition of an equal volume of 95 per cent ethanol. On the addition of the alcohol a spongy mass results that occludes water and alcohol. The precipitated mucilage is pressed free of water and alcohol and may be further dehydrated by soaking in absolute ethanol, although this is not necessary. The final product is a stringy, fibrous mass.

By continued extraction with water as much as 6.4 per cent of the weight of the seed can be recovered as the dry mucilage. However, when large quantities of the mucilage are being separated it is impractical to continue extraction and the yield is about 5 per cent.[5]

The mucilage is inactive chemically. It does not reduce Fehling's solution and gives no reaction with phenylhydrazine, showing that there is no free aldehyde group.

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[5] Morrow, C. A., Biochemical Laboratory Methods. John Wiley and Sons, New York (1927), p. 234.

Its water solution is neutral to litmus, although it has the property of neutralizing small amounts of bases. Neville[6] found that .344-gram of the dried mucilage neutralized .0188-gram of sodium hydroxide. The mucilage gives a strong napthoresorcinol test[7] for hexose uronic acid. The water solution has a specific rotation of +10.5.

HYDROLYSIS OF THE MUCILAGE AND SEPARATION OF THE PRODUCTS

One kilogram of the crude mucilage was dissolved in 6 liters of 4 per cent sulphuric acid and heated in a boiling water bath for 20 hours. The solution was cooled and filtered from a small amount of insoluble material and the acids neutralized by the addition of an excess of calcium carbonate. The solution was heated in a boiling water bath and the precipitate of calcium sulphate filtered off. The resulting solution was clear but brown in color. It was concentrated in vacuo in a boiling water bath to a volume of 1,500 cc., 30 grams of Norit (activated wood charcoal) added, and the solution heated in a boiling water bath for 2 hours. After the Norit was filtered off, the resulting solution was a light amber color. The

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[6] Neville, A., Linseed Mucilage: J. Agr. Sci., 5:113-128 (1913).

[7] Browne, C. A., Handbook of Sugar Analysis, p. 383. John Wiley & Sons, New York (1912).

salts were precipitated as a gummy mass by the addition of three volumes of 95 per cent ethanol. After standing over night with fresh 95 per cent ethanol the salts were triturated with a pestle until granular, filtered, washed with alcohol and ether, and dried on a porous clay plate. The yield was 340 grams.

The purpose of this investigation was to establish the formula and structure of the acid nucleus. The above process separated the calcium salts of the acid nucleus from the sugars. The alcoholic sugar solution was not investigated, since previous workers had determined its composition.

PURIFICATION OF THE SALTS OF THE ACID NUCLEUS

The crude calcium salts prepared according to the preceding paragraph were purified according to the method of Heidelberger and Goebel.[8] Three hundred and forty grams of the salts were dissolved in 900 cc. of water, heated in a boiling water bath, and 1,800 cc. of 95 per cent ethanol added. A portion of the salts precipitated out as a gummy mass. The mixture was allowed to stand over night and the clear supernatant liquid poured off. This liquid was concentrated in vacuo in a boiling water bath to a volume of 200 cc.

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[8] Heidelberger, M., and Goebel, W. F., J. Biol. Chem., 74:3, 613-618.

and the salts precipitated by the addition of 4 volumes of 95 per cent ethanol. The yield was 24 grams. This was called fraction A. It was the part most soluble in a water--alcohol mixture. The solid residue containing the remainder of the salts after fraction A was separated was dissolved in 500 cc. of hot water and heated in a boiling water bath and 900 cc. of 95 per cent ethanol added. The precipitate was allowed to coagulate over night and the clear supernatant liquid poured off. This was concentrated in vacuo in a boiling water bath to a volume of 200 cc. and the salts precipitated by the addition of 4 volumes of 95 per cent ethanol. The yield was 65 grams. This was called fraction B. In a similar manner fraction C was separated. The yield of fraction C was 60 grams. The salts remaining insoluble after fraction C was separated were dissolved in water and precipitated by the addition of 4 volumes of 95 per cent ethanol. This last fraction, the part least soluble in a water--alcohol mixture, was called fraction D. The yield was 75 grams.

ANALYSIS OF THE SALTS OF THE ACID NUCLEUS

A portion of each fraction of salts was prepared for analysis as follows. Twenty grams were ground in an agate mortar and seived through an 80-mesh screen. Each fraction was then thoroughly mixed. The percentage moisture in each fraction was determined by drying samples of approximately .25-gram to constant weight in an Abderhalden vacuum drier over boiling toluene and the sample weights corrected for this.

The following standard analyses were run on the various fractions of the salts.

I. Ash (CaO)

In the case of calcium salts a .5-gram sample was weighed into a weighed porcelain crucible and ashed at low temperatures until all volatile matter had been driven off. At the end of this time the crucible was heated in a muffle for 10 minutes with a Meeker burner. The crucible was then allowed to cool and weighed, and the ash calculated as calcium oxide. The ash of the crude salts was generally brown and gave a qualitative test for iron. The purified salts gave a white ash.

In the case of barium salts a sample was ashed as above and the ash calculated as barium carbonate. Another sample was also run by precipitating the barium as the sulphate.

II. Uronic Acid.

The uronic acid was determined by the method of Lefèvre.[9] This method is based on the fact that on boiling with 12 per cent HCl the hexose uronic acid molecule breaks down, giving off CO_2 and furfural. The CO_2 is absorbed in potash bulbs and weighed. The yield of CO_2 is 100 per cent, so this method gives correct results for hexose uronic acid.

As is shown later, the hexose uronic acid present in the acid nucleus is galacturonic acid. The per cent of this was determined by oxidation with strong nitric acid according to Van der Haar.[10] Under these conditions galacturonic acid yields mucic acid which is insoluble. The yield of mucic acid is about 70 per cent of the galacturonic acid present. The accuracy of this method is questionable.[11]

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(a) Van der Haar, A. W., Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide und Aldehydesäuren, Gebrüder Borntraeger, Berlin, pp. 71-76 (1920).
(b) Lefèvre and Tollens, Ber., 40:4513 (1907).
(c) Lefèvre, Untersuchungen uber die Glucurensäure, Dissertation, Gottingen (1907).

10
Ibid 9 (a), p. 123.

11
Schorger, J. Ind. Eng. Chem., 8:498 (1916).

III. Pentoses. Methyl Pentoses.

Pentoses were determined according to "Methods of Analysis" A.O.A.C. (1925). It was necessary to correct the weight of the phloroglucide precipitate for furfural phloroglucide resulting from the decomposition of the galacturonic acid. This was done by using the factor determined by Lefèvre.[12] Methyl pentoses were determined by the method of Ellett and Tollens and Haywood,[13] and calculated as rhamnose hydrate from the table of Ellett.[14] The accuracy of the pentosan and methyl pentosan method is questionable.

IV. Reducing Sugar.

Total reducing sugar was determined by the method of Cajori.[15] Cajori's method is for free sugars such as glucose, maltose, etc. He states that the oxidation of glucose by iodine in sodium carbonate solution is complete in 25 minutes. It was found that the oxidation of the salts was not complete until 3 hours, so this modification was made in the procedure.

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12 Van der Haar, A. W., Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide und Aldehydesäuren, Gebrüder Borntraeger, Berlin, p. 75 (1920).

13 (a) Ellett and Tollens, Z. deut. Zuckerind., 42:19 (1905). (b) Haywood, U. S. Bureau of Chemistry, Bull. 105, p. 112, (1907).

14 Ibid 12, p. 82.

15 Cajori, J. Biol. Chem., 54:3, 617-627 (1922).

TABLE 1

RESULTS OF THE ANALYSIS OF CALCIUM SALTS OF THE ACID NUCLEUS

Fraction	Per cent					$\alpha]_D^{20^\circ}$
	Ash (CaO)	CO_2	Galactose	Methyl-Pentose	Reducing Sugar as CHO	
A	13.23	11.01	38.4	--	5.7	+92.0
B	12.07	11.27	36.6	35.3	5.57	+95.5
C	10.61	11.05	39.5	41.3	4.83	+94.4
D	10.16	11.27	45.2	37.2	4.70	+92.8

An inspection of the above table indicates that there is little difference in the composition of the various fractions. Deviations in the analyses of the various fractions particularly in the ash were probably due to the presence of calcium sulphate as an impurity. The data approximate the theory for an aldobionic acid consisting of one hexose uronic acid molecule and one sugar molecule, either a pentose or hexose, but the results are by no means conclusive.

It seemed best at this time to determine by qualitative tests which of the above substances were present in the salts.

DETERMINATION OF THE CONSTITUENTS OF THE ACID NUCLEUS

Since the napthoresorcinol test and the yield of CO_2 by the Lefèvre method indicated the presence of a hexose uronic acid, it seemed well at this time to identify this acid. This was done by the method of Heidelberger and Goebel.[16] Twenty grams of fraction D salts were dissolved in 200 cc. of 6 per cent hydrobromic acid containing 10 cc. of bromine. This mixture was heated under a reflux condenser in a boiling water bath for 10 hours. After 4 hours' heating a white crystalline precipitate began to settle out. After 10 hours the solution containing the precipitate was filtered. The yield of the insoluble white precipitate was 6.4 grams. This was identified as mucic acid (M.P. 210) by recrystallization in the regular way. This establishes the fact that the hexose uronic acid present in the acid nucleus is galacturonic acid.

Since the sugar present in the acid nucleus would be oxidized by the bromine to a pentonic or hexonic acid and remain in the filtrate from the mucic acid, this filtrate was boiled to remove excess bromine, an excess of lead carbonate was added until the solution was neutral to congo paper. Silver sulphate was then added in small quantities to remove

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16 Heidelberger, M., and Goebel, W. F., J. Biol. Chem., 74:3, 613-618.

the remaining bromide ions. The solution was then filtered from lead and silver bromides, hydrogen sulphide passed in to remove the lead and silver ions, and the solution concentrated in vacuo to a volume of 15 cc. To this solution containing the monobasic hexonic or pentonic acid was added 1 gram of phenylhydrazine hydrochloride. On standing over night a light-brown crystalline needle-like solid separated out. The crystals were filtered and recrystallized from hot 95 per cent ethanol. The product was a white crystalline solid whose melting point was 193°C. This was identified as rhamnonic phenyl hydrazide M. P. 186° - 191°C.

In order to further prove the presence of rhamnose in the acid nucleus 20 grams of D salts were dissolved in 100 cc. of 6 per cent sulphuric acid and hydrolysed in the autoclave at a gage pressure of one atmosphere for 8 hours. At the end of this time the flask was removed and the solution filtered. Three volumes of alcohol were then added to separate the salts from the sugars. The salts precipitated out, the sugars remaining in solution. The solution was dark brown in color. Attempts were made to ferment the water solution with yeast. There was no fermentation on standing over night in the incubator. A small amount of glucose solution was then added to see whether the yeast was active and fermentation resulted in 10 minutes. The solution was so dark in color that a rotation could not be made. The sugars were then oxidized with bromine and barium benzoate according to the method of Hudson and

Isbell.[17] The solution containing the oxidized sugar was concentrated in vacuo to a volume of 15 cc. and 1 gram of phenylhydrazine hydrochloride added. No precipitation occurred until a crystal of rhamnonic phenylhydrazide was added. When this was done a mass of needle-like crystals immediately separated out. These were filtered and recrystallized from hot alcohol. They melted at 193°C. The yield of rhamnonic phenyl hydrazide from the sugars was .6-gram. Some rhamnonic phenylhydrazide prepared from pure rhamnose by the method of Hudson and Isbell melted at 191°C. and had a similar crystalline structure to the one separated from the salts of the acid nucleus.

To further confirm the presence of rhamnose in the acid nucleus approximately 50 grams of mixed calcium and barium salts of the aldobionic acid were hydrolysed for 6 hours in six times their weight of 4 per cent sulphuric acid in the autoclave. The acids were neutralized by an excess of calcium carbonate and the salts separated from the sugars in the usual way. From the alcoholic sugar solution were isolated 7 grams of crystalline rhamnose hydrate M.P. 92° - 94°; $[\alpha]_D$ - 7.8°, constant after 10 minutes. The rhamnose was isolated by the method of Sands and Klaas.[18] It was found that for crystallization of the rhamnose hydrate to occur it was necessary to have

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17 Hudson and Isbell, J. Am. Chem. Soc., 51:7, 2225-2229 (1929).

18 Sands and Klaas, J. Am. Chem. Soc., 51:11, 3441-3446 (1929).

a small quantity of water present in the acetic acid solution of the sugar.

ISOLATION AND ANALYSIS OF THE PURE BARIUM SALT OF THE ALDOBIONIC ACID

Since the barium salt of the aldobionic acid can be more easily prepared pure than can the calcium salt, it seemed best to isolate the barium salt. Accordingly 200 grams of the crude mucilage were hydrolysed for 20 hours in a boiling water bath in 4 per cent sulphuric acid. At the end of this time the acids were neutralized by the addition of barium hydroxide and an excess barium carbonate and the salts precipitated in the usual way. The yield of barium salts was 40 grams.

TABLE 2

ANALYSIS OF THE BARIUM SALT OF THE ALDOBIONIC ACID

Constituent	Found	Theory*
	(per cent)	(per cent)
Barium (as $BaSO_4$).....	14.7	16.85
CO_2.....	10.90	10.80
Methyl Pentose as rhamnose hydrate.	34.9	44.6
Galactose.....	38.9	44.1
Reducing sugar as CHO.....	7.09	7.11
$\alpha]_D$.....	+79°	--

* -- Theory is for barium salt of an aldobionic acid consisting of one molecule of l-rhamnose and one molecule of d-galacturonic acid.

An examination of the results given in Table 2 shows that they agree very well with the theoretical values with the exception of the barium, which is low. The barium percentage given in the table was determined by precipitation as $BaSO_4$. It is probable that the error is due to the fact that a small amount of the aldobionic acid has been neutralized by calcium which does not give an insoluble sulphate precipitate. This is substantiated by the fact that when a sample of the salt was ashed and the ash assumed to be barium carbonate, the result for barium was 17.2 per cent, which is close to the theoretical. As has been stated before, the galactose and methyl pentose determinations are not quantitative.

OXIDATION OF THE ALDOBIONIC ACID TO A DIBASIC ACID

Thirty grams of fraction B calcium salts were dissolved in 220 cc. of .3 N iodine in barium iodide. To this mixture was added 330 cc. of barium hydroxide .4 N. The mixture was stirred and allowed to stand over night. The solution was then acidified with 6.2 cc. of concentrated sulphuric acid which was dissolved in 50 cc. of water. Excess lead carbonate was added to remove the hydriodic acid present. The precipitate was filtered off and the solution concentrated in vacuo to remove excess iodine. The last traces of iodine in solution were removed by the addition of a small amount of silver sulphate. Hydrogen sulphide was passed into the solution

to remove the silver and lead ions. The solution was filtered and the filtrate concentrated in vacuo to remove the hydrogen sulphide. The solution at this point contained some soluble sulphates. The sulphates were removed quantitatively by the careful addition of barium hydroxide. The solution was concentrated in vacuo in a boiling water bath to a volume of 50 cc. and the dibasic salt precipitated by the addition of 4 volumes of 95 per cent ethanol. The yield was 13 grams. This salt did not reduce Fehlings solution, showing the absence of a free aldehyde group.

TABLE 3

ANALYSIS OF THE DIBASIC CALCIUM SALT OF THE ALDOBIONIC ACID

Constituent	Found	Theory
	per cent	per cent
Ash (CaO).....	11.2	14.2
CO_2.....	10.55	11.1
Galactose.....	36.2	45.6

While the data in Table 3 approximate the theory for the calcium salt of an acid consisting of one galacturonic acid and one rhamnonic acid, the results are by no means conclusive. The deviations can be explained by the fact

that calcium sulphate was present as an impurity. Hence, it seemed best to oxidize the barium salt. This was done as follows. Fifteen grams of the barium salt were oxidized in the same manner as were the calcium salts. The yield of the dibasic barium salt of the aldobionic acid was 6.5 grams. The analysis of the dibasic barium salt is given in the following table.

TABLE 4

ANALYSIS OF THE DIBASIC BARIUM SALT OF THE ALDOBIONIC ACID

Constituent	Found	Theory*
	per cent	per cent
Barium (as $BaSO_4$).....	24.80	26.0
CO_2.....	8.85	8.94
Galactose.....	33.5	36.6
Methyl Pentose.....	--	0
$\alpha]_D$.....	$+72^0$	

* -- The theory is for the barium salt of a dibasic acid consisting of one rhamnonic and one galacturonic acid.

An examination of the above table shows that the experimental values agree closely with the theoretical values for the dibasic acid resulting from the oxidation of an aldobionic acid consisting of one galacturonic acid and one rhamnose.

STRUCTURE OF THE ALDOBIONIC ACID PRESENT IN FLAXSEED MUCILAGE

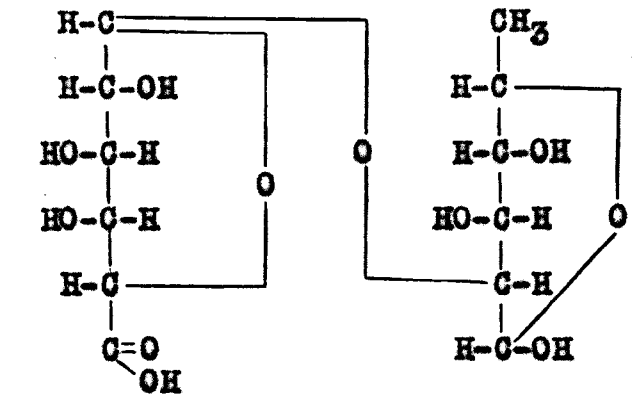
It is evident from the paragraph entitled "Determination of the Constituents of the Acid Nucleus" and the analyses given in tables 2 and 4 that the aldobionic acid contains l-rhamnose and d-galacturonic acid. The linkage between these two molecules involves the aldehyde group of the galacturonic acid and one of the secondary alcohol groups of the rhamnose. This is shown by the following facts:

1. The dibasic salts yield CO_2 on treatment with 12 per cent HCl. This is characteristic of hexose uronic acids, dibasic acids such as mucic acid and saccharic acid yielding no CO_2 by this treatment.

2. The dibasic salts give the napthoresorcinol test which is characteristic of hexose uronic acids.

3. The dibasic salts give a very low per cent of methyl furfural. If the union involved the aldehyde group of the rhamnose, on treatment with 12 per cent HCl the rhamnose would be converted into methyl furfural and give a large amount of methyl furfural phloroglucide.

On the basis of the above reasoning the following formula is given for the aldobionic acid present in flaxseed mucilage:



d-galacturonic acid　　l-rhamnose

CONCLUSION

1. It has been shown that the acid nucleus present in flaxseed mucilage is an aldobionic acid consisting of one molecule of l-rhamnose and one molecule of d-galacturonic acid, the sugar being linked to the acid by a glucosidic linking which involves the aldehyde group of the galacturonic acid.

2. The presence of l-rhamnose in flaxseed mucilage is established.

3. It is thus seen that flaxseed mucilage is similar in composition to plant gums in general since it gives on hydrolysis an aldobionic acid and various sugars.

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