STUDIES ON PLANT MUCILAGES

Examination of the Root Powder of *Asparagus filicinus*

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Asparagus filicinus Buch.-Ham. *ex* D. Don. is an under-shrub occurring widely in tropical and temperate regions of the Himalayas (7,000 to 10,000 ft. high) in the Punjab and Kashmir and extending upto Bhutan and Assam. The root of this plant, called *Chirya musli* or *Nari musli*, swells up like gum tragacanth, when its powder is treated with water.

The fresh root is pliable and its bark can be readily peeled off. The debarked roots, when dried and powdered, gives an almost colourless material. However, when once the roots become dry, the bark becomes so firmly attached to the flesh that it is not easy to separate it. In this case the bark can be separated by powdering the entire root and passing through a 80-mesh sieve, when most of the fibrous matter will be retained by the sieve while fine powder passes through. The powder thus obtained is of a light yellow colour and can be decolourised further by soaking in absolute alcohol for a day or so and pressing out the solvent.

A small amount of a yellow colouring matter is present in the bark, and it can be easily isolated by extraction with absolute alcohol. It gives a deep yellow colour in acid and a crimson red in alkali solutions.

The root powder readily swells in water forming highly viscous colloidal solutions, far more viscous than those of starch, tamarind seed jellose, gum tragacanth or gum arabic of the same strength. In 2 per cent. solution it is 9 times more viscous than gum tragacanth and 27 times more so than tamarind seed jellose, while the viscosity of starch or gum arabic is almost negligible.

In higher concentrations the viscosity of the solutions rises enormously; for instance, the product obtained by the addition of 5 g. of the powder to 100 c.c. of water is a thick dough. Unlike other gums which take considerable time and heat for swelling, the *Chirya musli* powder swells almost immediately in water and forms a homogeneous paste on slight warming.

Aqueous solutions of the powder, on drying, leave continuous, elastic and transparent films, a property which is very much valued in sizing.

Experiments in bulk-sizing indicate that the powder can be used in place of starch in warp-sizing, and in conjunction with tamarind seed kernel powder, it is reported to give satisfactory results. Both for stiff and glaze finishing it is found to be very good, possessing certain advantages even over starch.

Experiments have also been conducted to examine the suitability of the powder in printing pastes, and the results show that it can be used as a printing base just like other gums both for acid and basic dyes.

The main constituent of the root powder is an acid polysaccharide which swells up in water and forms slimy solutions on dilution. Hence it belongs to the class of plant mucilages. It is present in the roots to an extent of 70 per cent. Besides this the roots contain glucose and fructose as free sugars, which can be extracted with 70 per cent. alcohol. The mucilage is isolated and purified by treating the root powder with water to form a thin colloidal solution, filtering through silk, centrifuging the filtrate, and precipitating the mucilage with excess of acidified alcohol.

On boiling with 12 per cent. hydrochloric acid, the mucilage liberates carbon dioxide corresponding to 4·76 per cent. of uronic anhydride (Dickson, Otterson and Link's method[1]). When refluxed with 5 per cent. sulphuric acid for 6 hours, it yields mannose, fructose, glucose and uronic acid. Quantitative estimation of the sugars has shown the mannose, glucose and fructose to be present in the ratio 5 : 4 : 1. Mannose has been estimated according to the method of Bourquelot and Herissey by its conversion into the insoluble hydrazone,[2] while the total aldoses have been determined by the iodine-oxidation method of Willstätter and Schudel.[3] The difference between the total aldose content and that of mannose gives the amount of glucose, since these are the only two aldoses present. The balance of the sugars is obviously fructose. The specific rotation of the hydrolysate is in conformity with the relative proportion of the sugars. Since the uronic acid content is only 4·76 per cent. of the total sugars it appears that there is one uronic acid for every 20 sugar units.

On account of the small proportion of uronic acid, its isolation for characterisation is a matter of some difficulty. However, from the products of hydrolysis, a small amount of the acid has been isolated as the barium salt. Its oxidation with nitric acid has not produced any mucic acid, indicating that it is not galacturonic acid. The barium salt has a specific rotation of $+12{\cdot}16°$ indicating that it might be the barium salt of glucuronic acid.

EXPERIMENTAL

The Root Powder

(*a*) *From fresh roots.*—For this experiment fresh roots were secured in the month of July from plants raised in the experimental garden attached to the Chemistry and Minor Forest Products Branch of the Forest Research Institute. They were washed well to remove any adhering dirt, and the outer bark was peeled off mechanically with a knife. The debarked roots (1 kg.) were taken, cut into halves lengthwise and then into small bits and dried in the sun, when they were reduced to 120 g. losing 880 g. of moisture during the course of four days. The dry material was ground to a fine powder in a pulverizer and passed through a 80-mesh sieve. The powder thus obtained (sample I) was light yellow in colour and the yield was 115 g.

(*b*) *From dry roots.*—The roots were obtained from the Kagan Forest Division, Abbottabad. The dry roots (1 kg.) were ground to a fine powder in a pulverizer. The outer bark and the inner core had different degrees of hardness and hence underwent pulverization at different rates. When the core powder was sufficiently fine as to pass through a 80-mesh sieve, the bark was still in a crushed stage and most of it did not pass through the sieve. Thus an initial separation of the root bark and the core powder could be conveniently effected. The portion that passed through the 80-mesh sieve was about 900 g. and was pale yellow in colour. This was soaked in absolute alcohol (4 l.) for two days and filtered under suction. The residue was washed with further quantities of absolute alcohol till the fitrate was no longer yellow or yellowish in colour. It was then dried in a flat basin in the laboratory itself. The final product (sample II) was almost white with a pale yellowish tinge and the yield was a little less than 900 g. The colour was examined under the Lovibond tinctometer against absolute white and was found to be 1·3 yellow and 0·8 red. It was an amorphous powder and had no definite melting point. The viscosity of a 1 per cent. solution was found to be 3,780 Engler's seconds, and it increased so rapidly with concentration that a 5 per cent. solution was no longer fluid but a thick dough.

The proximate analysis of the two samples was carried out according to the methods adopted by the Association of the Official Agricultural Chemists and those given in Allen's *Commercial Organic Analysis*. For the estimation of free sugars extraction with water was not feasible, because the mucilage also would get into solution. So hot boiling 70 per cent. alcohol was used, since only sugars and not the mucilage would be soluble in alcohol of this concentration. The following results were obtained:—

	Sample I	Ssmple II
	%	%
Moisture ..	13·90	15·4
Colouring matter ..	0·30	..
Free sugars ..	2·18	..
Fat ..	1·17	1·2
Carbohydrates (*minus* free sugars) ..	58·82	70·0
Proteins ..	6·12	5·9
Fibre ..	12·85	3·5
Inorganic matter (by difference) ..	4·66	4·0
Ash ..	3·90	3·9

The ash contained Na^{+}, K^{+}, Ca^{++}, Mg^{++} and Fe^{+++} as the metallic radicals and CO_3^{--}, SO_4^{--} and O^{--} as the acid radicals. Some silica was also present.

Sample II was used for all further experiments except the isolation of the colouring matter.

Isolation of the Colouring Matter

The root powder containing the bark (1 kg.) was refluxed successively on two days with alcohol (4 l.) for 12 hours, when almost all the colouring matter went into solution, imparting a brownish yellow colour. From the combined extract, the solvent was distilled off, when a resinous, brownish yellow mass was left behind. When the latter was treated with ether, only the colouring matter went into solution, which was recovered from the etherial extract by distilling off the solvent. Though yellow in colour, the solid was still sticky and resinous, and as a step for further purification, it was dissolved in 4 per cent. sodium hydroxide when a thick red solid appeared (sodium salt). It was filtered under suction and washed with small quantities of absolute alcohol and ether. The yield of the product was 3·0 g. For the liberation of the colouring matter, the salt was dissolved in glacial acetic acid and kept in an ice-box for crystallisation. After two days, a brownish yellow solid separated out. The yield of the purified sample was 1·0 g. The solid was not, however, crystalline under microscope and resisted all attempts at crystallisation from all solvents. It was freely soluble in alcohol, acetone, glacial acetic acid and sulphuric ether. It gave a deep red coloration with sodium hydroxide in alcoholic solution and a buff-coloured precipitate with ferric chloride. It readily dissolved in aqueous sodium hydroxide imparting a crimson red colour which changed to yellow when acidified by both organic as well as inorganic acids. The acid-alkali colour changes were quite reversible.

Free Sugars

After the removal of the colouring matter with hot absolute alcohol the root powder (50 g.) was extracted in a soxlet with 70 per cent. alcohol for the isolation of the free sugars. After distilling off the alcohol, the residuary aqueous solution was clarified with alumina cream, made up to a known volume and analysed for the constituent sugars. It contained 2·18 per cent. of reducing sugars and had a specific rotation of −45·45°. It responded to Pinoff's[4] and Seliwanoff's[5] tests, indicating the presence of fructose. Further, when a portion of the solution was concentrated and treated with methyl phenyl hydrazine in alcoholic solution and kept for 24 hours, the methyl phenyl fructosazone was obtained. The latter, when crystallised from hot benzene, separated as needle-shaped, orange-coloured crystals melting at 151°. Furthermore, when another portion of the concentrate was treated with diphenyl hydrazine in alcoholic solution, glucose diphenyl hydrazone was produced. The hydrazone crystallised from hot water as small colourless prisms melting at 161°. Mixed melting point with an authentic sample was undepressed. No other sugars could be detected. Since only glucose and fructose were present, the specific rotation shows that the two sugars were approximately in the ratio of 1 : 2.

Isolation of the Mucilage

The fibre-free sample of the root powder (50 g.) was treated with warm water (5 l.), when a moderately viscous colloidal solution was obtained. After the removal of the suspended impurities by filtration through fine silk and subsequent centrifuging, the filtrate was poured with stirring into alcohol (10 l.) containing 100 c.c. of concentrated hydrochloric acid. The mucilage separated out as fine white flakes. A portion of it was dried and the ash content was found out. The rest of the mucilage was again dissolved in water and reprecipitated by alcohol containing hydrochloric acid. The process was repeated thrice and the ash content was determined every time. The final product was dehydrated by trituration first with warm absolute alcohol (500 c.c.) and then with 200 c.c. of ether. The resultant pale white cream was finally dried in a vacuum desiccator. The yield was 32·5 g. On powdering it looked greyish white and was amorphous under microscope. On heating, it decomposed at about 200°. Its solution was faintly acidic and did not reduce Fehling's solution either in the cold or on heating but gave a blue jelly-like precipitate. The moisture content of the mucilage was 15·01 per cent. The methoxyl content, as found out by Zeisel's method, was negligible. (Found in a sample dehydrated at 105° C, in vacuum: C, 43·25, 43·59; H, 6·00, 6·54%.)

The mucilage swelled in water, giving rise to a thick mucilaginous solution and was found to be insoluble in alcohol, acetone, glacial acetic acid, sulphuric ether, etc. The specific rotation was $-12{\cdot}4°$ at 20°.

Estimation of Uronic Anhydride

This was done according to the method of Dickson, Otterson and Link[1] by boiling 3 g. of the purified mucilage with 100 c.c. of 12 per cent. hydrochloric acid of specific gravity 1·06 and estimating the evolved carbon dioxide by absorption in barium hydroxide of known strength (0·2 N). The excess of barium hydroxide was determined by titration against standard hydrochloric acid using phenolphthalein as indicator. The amount of carbon dioxide evolved, when multiplied by 4, gave the amount of uronic anhydride. Calculated on the basis of dry material the mucilage contained 4·76 per cent.

Hydrolysis of the Mucilage

The purified air-dried mucilage (5 g.) was boiled under reflux with 200 c.c. of 5 per cent. sulphuric acid for 4 hours. The temperature was gradually raised to the boiling point to avoid any local heating and consequent charring. The resultant hydrolysate was brown in colour due to the presence of furfuraldehyde formed from the liberated uronic acid. At the end of the hydrolysis almost the whole of the substance was in solution except a small amount of fibrous matter. After cooling the contents were filtered through a tared filter-paper and the residue estimated by drying and weighing. The reducing sugars in the filtrate, including the uronic acid, were estimated as glucose according to the method of Allihn and were found to be 106·8 per cent. on the basis of pure anhydrous material. The percentage would have been higher but for the inevitable decomposition of some portion of the uronic acid during boiling with sulphuric acid. With a view to minimizing, if not eliminating, the partial decomposition of the uronic acid, the hydrolysis was brought about by conducting the heating at the temperature of boiling water-bath (98°) for a long time (18 hours) and subsequently on a wire-gauze for 2 hours. The latter direct heating was to ensure the completion of the hydrolysis. Under these conditions the uronic acid did not seem to undergo any appreciable decomposition, and the mucilage yielded 110·2 per cent. of reducing sugars expressed as glucose.

For the characterisation of the individual sugars and the uronic acid, the latter was removed from the hydrolysate as follows:—

The acid hydrolysate was neutralized in the hot with barium carbonate and allowed to stand. The precipitated barium sulphate was filtered and the filtrate concentrated to 100 c.c. under vacuum. On adding 300 c.c. of absolute

alcohol to the concentrate the barium salt of uronic acid was precipitated. This was filtered off and from the filtrate the alcohol was distilled off under reduced pressure. The residuary aqueous solution was directly filtered into a standard flask of 200 c.c. capacity, the volume made up and the solution was examined both qualitatively and quantitatively for the constituent sugars.

Nature and Relative Proportion of the Sugars

When a small part of the sugar solution obtained above was treated with phenylhydrazine and heated, glucosazone, melting at 205° and having the characteristic crystalline structure, was readily produced. However, when the treatment was done in well-cooled solutions, the colourless mannose phenyl hydrazone, m.p. 188°, was also obtained. The solution responded to Pinoff's and Seliwanoff's tests indicating the presence of fructose. This was confirmed by the formation of fructose methyl phenylosazone. It did not produce any mucic acid on oxidation with nitric acid, indicating the absence of galactose. Tests for pentoses were also negative.

The total sugars were estimated in the sugar solution according to Allihn's method. The total aldoses were determined according to the method of Willstätter and Schudel.[3] To an aliquot portion of the sugar solution containing approximately 100 mg. of sugar was added three times the volume of 0·1 N iodine required for oxidation. With vigorous stirring 1·5 as much 0·1 N sodium hydroxide as iodine was then dropped into the mixture which was subsequently left aside for about 15 minutes. After the addition of sulphuric acid to slight acidity, the excess of iodine was titrated with 0·1 N thiosulphate solution. From the amount of iodine consumed the amount of the aldoses was calculated (1 c.c. of 0·1 N iodine = 9·00 mg. of hexose), and was found to be 89·8 per cent. of the total sugars.

Mannose was estimated according to the method of Bourquelot and Herissey. The sugar solution was concentrated under reduced pressure so as to be about 6 per cent. After estimating the total sugars accurately, 15 c.c. of the solution were treated with a solution of 1·2 c.c. of phenyl hydrazine and 1·2 c.c. of glacial acetic acid made up to 6 c.c. with water and allowed to stand for 8 hours at a temperature not above 10°. The precipitated hydrazone was filtered in a glass-sintered crucible, washed with 15 c.c. of ice-cold water and finally with 10 c.c. of absolute alcohol and 10 c.c. of ether. The hydrazone was dried at 100° for half an hour in a steam-oven and weighed. The amount of the hydrazone, when multiplied by 0·666, gave the amount of mannose. The latter was found to be 49·5 per cent. of the sugars.

Relative Proportion of the Sugars and the Uronic Acid

As already observed the total aldoses amounted to nearly 90 per cent. of the total sugars in the products of hydrolysis of the mucilage so that the ketose, namely, fructose would come to 10 per cent. The mannose formed about 50 per cent. of the total sugars and so glucose would amount to 40 per cent. Therefore, fructose, glucose and mannose were in the ratio of 1 : 4 : 5, and this was confirmed by the specific rotation, $[\alpha]_D^{20}$, of the sugar solution, which was found to be $+19{\cdot}0°$. A mixture containing 5 molecules of mannose, 4 of glucose and 1 of fructose requires the specific rotation to be $+18{\cdot}96°$. Further, the mucilage contained 4·76 per cent. of uronic acid. Hence it appears as though there is one molecule of uronic acid present for every 20 molecules of the sugars. The composition of the mucilage may, therefore, be tentatively represented as 1 molecule of uronic acid, 2 of fructose, 8 of glucose and 10 of mannose.

Nature of the Uronic Acid

The barium salt of the uronic acid isolated from the products of hydrolysis of the mucilage was purified by dissolution in water and reprecipitation by means of alcohol. The salt readily dissolved, when freshly precipitated, but on drying it assumed a horny structure and became much less soluble. It contained 25·2 per cent. of barium, barium uronate requiring 26·4 per cent. of the metal. Its specific rotation at 20° was $+12{\cdot}16°$ while that of barium glucuronate is reported to be $+15{\cdot}0°$ at 19°.[6] The barium salt now isolated might, therefore, be barium glucuronate, of course in an impure state. On oxidation with nitric acid, it did not produce any mucic acid, indicating that it was not derived from galacturonic acid.

Experiments on Sizing, Finishing, etc.

These were conducted at our request at the David and Edward Textile Mills of E. D. Sassoon & Co., Ltd., Bombay, to whom our thanks are due. The fibre-free root powder was tried in bulk-sizing in combination with tamarind seed powder and was found to give good results.

Experiments on finishing indicated that a $1\frac{1}{2}$ per cent. solution gave good stiffness.

In calico-printing too a 4 per cent. solution of the substance was reported to be satisfactory for use as a base.

Summary

The roots of *Asparagus filicinus* contain a mucilage to an extent of 60 per cent. The latter is composed of a uronic acid, fructose, glucose and

mannose, which are in the ratio of 1 : 2 : 8 : 10. The uronic acid seems to be glucuronic acid.

The fibre-free root powder was found to give satisfactory results in bulk-sizing and finishing in the textile industry. It was also found to be satisfactory as a base in calico-printing.

References

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