STUDIES ON PLANT MUCILAGES

Part III. Mucilage from the Tubers of *Curculigo orchioides*

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In continuation of the work in progress in these laboratories on plant mucilages, the examination of the tubers of *Curculigo orchioides* Gaertn. has been undertaken. *Curculigo orchioides* is a small herbaceous plant growing in the hotter regions of India and Ceylon. The tuberous root of this plant, which is called *Kalimusli* in Hindi, is largely used in the Ayurvedic and Unani systems of medicine as a tonic and for piles and diarrhœa.

The fresh roots are soft and pliable, but become hard and brittle on drying. When dried roots are powdered and passed through a 80-mesh sieve, most of the fibrous bark is retained by the sieve, while the core powder passes through. The following are the results of a proximate analysis of the core powder:—

Moisture	10·42	per cent.
Fat	1·92	,,
Colouring matter	3·28	,,
Proteins	9·81	,,
Fibre	15·70	,,
Nonfibre carbohydrates	53·20	,,
Inorganic matter (by difference)	5·67	,,
Ash content	5·51	,,

Carbohydrates are, therefore, the main constituent of the tubers. Their further examination has shown that they are composed of free sugars (7·56 per cent. on the weight of the air-dried core powder), mucilages (8·12 per cent.), hemicelluloses (20·51 per cent.), and other polysaccharides (17·01 per cent.). The free sugars, which are extractable with 70 per cent. alcohol, are found to be composed of only xylose and glucose. The residue, left after the extraction of the free sugars, when treated with warm water, yields a somewhat sticky viscous solution, which on treatment with alcohol, precipitates a greyish substance. The latter, unlike hemicelluloses, undergoes hydrolysis with difficulty to simple sugars and uronic acid and hence

belongs to the class of mucilages. The constituent sugars are mannose and glucose, while the uronic acid is found to be glucuronic acid. Quantitative estimations have shown that mannose, glucose and glucuronic acid are present in the ratio of 6 : 9 : 10 in the mucilage molecule.

Experimental

Composition of the Root Powder.—The tubers were obtained from Sambalpur circle (Orissa State). After drying they were ground in a pulverizer and passed through a 80-mesh sieve. The bark was retained by the sieve, while the inner core powder passed through it. The powder thus obtained was a little darkish brown in colour. Its proximate analysis was carried out according to standard methods,[1] and the results have already been presented under the theoretical introduction. The ash contained Na^+, K^+, Ca^{++}, Mg^{++}, and Fe^{+++} as the basic radicals and CO_3^{--}, SO_4^{--}, and PO_4^{---} as the acid radicals. Some silica was also present.

Estimation and Identification of Free Sugars.—The root powder (50 g.) was taken in a big soxhlet extractor and extracted with 500 c.c. of absolute alcohol till the extract removed no more of the colouring matter. After the removal of colouring matter, the extraction was continued with 70 per cent. alcohol for six hours. When the extraction was over, alcohol was distilled off from the extract, the aqueous solution was clarified with alumina cream, the volume was made up to a known volume and the sugars analysed both qualitatively and quantitatively. The solution contained only glucose and xylose. Glucose was detected by the formation of the characteristically crystalline phenyl osazone, melting at 204°–06° and the diphenyl hydrazone melting at 161°. The presence of xylose was established by the formation of the characteristic boat-shaped crystals of cadmium bromide-cadmium xylonate on oxidation with bromine and treatment with cadmium carbonate. Further, when a small portion of the sugar solution was evaporated to dryness and the dry product treated with benzaldehyde and methanolic hydrogen chloride, dimethylacetal of dibenzylidene *d*-xylose separated, confirming the presence of xylose.[2] The total sugars were estimated according to the method of Allihn and were found to be 7·56 per cent. Xylose was determined by estimating the amount of furfural liberated, when the sugar solution was boiled with 12 per cent. hydrochloric acid at 175°–80° and was found to be 25·68 per cent.

Isolation of the Mucilage.—The root powder (50 g.) was treated with warm water (1 l) for 3 hours. After removal of the suspended impurities by filtration through silk and subsequent centrifuging a moderately viscous solution was obtained. It was poured with stirring into alcohol (2 l)

containing a few c.c. of concentrated hydrochloric acid. The mucilage separated out as a greyish mass. It was again dissolved in water and reprecipitated from alcohol containing a little hydrochloric acid. The process was repeated thrice and the ash content was determined every time to indicate the purity. The final product, which was fairly free from ash, was dehydrated by tituration first with warm absolute alcohol and then with ether. The resultant powder was finally dried in a vacuum desiccator. The yield was 4·06 g. On powdering it looked greyish and was amorphous under the microscope. Its solution was faintly acidic and did not reduce Fehling's solution either in the cold or on heating. In 0·5 per cent. solution in water it had a specific rotation of 99·6° at 30°.

Estimation of Uronic Anhydride.—This was done according to the method of Dickson, Otterson and Link[3] by boiling 3 g. of the mucilage with 100 c.c. of 12 per cent. hydrochloric acid (sp. gr. 1·06). Calculated on the basis of dry material, the mucilage contained 39·56 per cent. of the anhydride.

Hydrolysis of the Mucilage.—The purified air-dried mucilage (3 g.) was boiled under reflux with 200 c.c. of 5 per cent. sulphuric acid for four hours. The temperature was gradually raised to the boiling point to avoid any local heating and consequent charring. The resultant hydrolyzate was dark brown in colour due to the formation of furfuraldehyde from the liberated uronic acid. However, partial decomposition of uronic acid was minimized, if not altogether eliminated, by conducting the hydrolysis 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. For the characterization of the uronic acid and the other products, the hydrolyzate, about 200 c.c., was neutralized in the hot with barium carbonate, the precipitated barium sulphate was filtered off and the filtrate concentrated to 100 c.c. under vacuum. On adding 300 c.c. of alcohol to the concentrate, the barium salt of the uronic acid separated out. After filtration, the residue and the filtrate were examined separately for the identification of the uronic acid and the sugars respectively.

Nature and Relative Proportion of the Sugars.—From the filtrate alcohol was distilled off and the residuary aqueous solution was made up to 200 c.c. in a standard flask. When a small part of the sugar solution was treated with well-cooled phenylhydrazine solution in acetic acid, mannose phenylhydrazone melting at 188° separated out. The mixture was left overnight in a frigidaire to completely precipitate the hydrazone and the latter filtered

off. The filtrate, after the addition of a little more phenylhydrazine acetat was heated on a water-bath to see if any osazone would separate out. about fifteen minutes, phenyl glucosazone crystallized out with its chara teristic structure and melting point (204°–06°). The sugar solution did n respond to Pinoff's and Seliwanoff's tests[4, 5] indicating the absence of fructos It did not produce any mucic acid on oxidation with nitric acid showing th galactose was absent. Tests for pentoses were also negative. Hence manno and glucose were the only sugars present in the hydrolyzate.

Quantitatively the total sugars in the solution were estimated accordi to Allihn's method. Mannose was determined as follows (method Bourquelot and Herissey).[6] 50 c.c. of the sugar solution was concentrate to about 20 c.c. and treated in the cold with a well-cooled mixture of 2 c. of phenylhydrazine, 1 c.c. of glacial acetic acid and 6 c.c. of water. Th mixture was allowed to stand with occasional shaking for 4 hours at temperature below 10°. The precipitated hydrazone was filtered in a glas sintered crucible, washed with 10 c.c. of absolute alcohol and 10 c.c. of ethe The hydrazone was dried at 100° for half an hour in a steam oven and weighe The amount of the hydrazone, when multiplied by 0·666, gave the amount mannose. The latter was found to be 40·13 per cent. of the sugars. Henc mannose and glucose were present in the ratio of 2:3.

Nature of the Uronic Acid.—The barium uronate obtained was purifie by repeated precipitations from aqueous solution by means of alcohol. Th purified salt contained 25·37 per cent. of barium, barium uronate requirin 26·40 per cent. of the metal. On oxidation with nitric acid (1·15 d.) it di not produce any mucic acid, thereby indicating that it was not galacturoni acid. In aqueous solution (c=0·4 per cent.), the salt showed a specifi rotation of + 17·76° at 25°, indicating that it might be barium glucuronat (the specific rotation of barium glucuronate is reported to be + 15·0° a 19°).[7] For confirmation, the free uronic acid was liberated from the bariu salt by treatment with just the required amount of sulphuric acid, and it specific rotation determined. It was found to be + 37·74° at 25° (glucuroni acid has a specific rotation of + 35·2° at 23°).[8] Hence the barium salt wa concluded to be barium glucuronate in a somewhat impure state.

Relative Proportion of the Sugars and the Uronic Acid in the Mucilag Molecule.—As already seen, the mucilage contained nearly 40 per cent. o uronic acid and 60 per cent. of sugars. The latter were composed o mannose and glucose in the molecular ratio of 2:3. Since the uronic aci and the sugars are nearly of the same molecular weight, the mucilag

molecule may be said to be constituted from mannose, glucose and glucuronic acid in the molecular ratio of 6 : 9 : 10.

Summary

The mucilage from the tubers of *Curculigo orchioides* Gaertn. is constituted from mannose, glucose and glucuronic acid in the molecular ratio of 6 : 9 : 10. It is present to an extent of 8·12 per cent.

The results of a proximate analysis of the root powder are also given.

References

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