THE CHEMISTRY OF GARLIC (ALLIUM SATIVUM L.)

Part III. The Reserve Polysaccharides

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Carbohydrates are generally stored as polymerised polysaccharides in various parts of the plant to be subsequently mobilised to meet any metabolic process that may arise. The storage of carbohydrate in this comparatively insoluble state, *e.g.*, as starch secures its safe retention. The colloidal carbohydrates may pass into the soluble, but colloidal and non-diffusible state, like inulin, which is the polymer of fructose.

Fructose seems to be a rather common unit in soluble polysaccharides. Johansson (1899) found several inulin-like carbohydrates in the Gramina. Parkin (1899) found, what he called inulin, fructose-yielding polysaccharides in the bulbs and tubers of several mono-cotyledons, sometimes together with starch. Mono-saccharides other than fructose also build up soluble polysaccharides. Glucose, mannose and galactose are all found as constituents of polymeric soluble carbohydrates.

It is quite possible that the reserve polysaccharides may act as emollients provided they are not attacked in the alimentary canal.

Experimental

1. Preliminary analysis

The various forms of carbohydrate present in Garlic are determined by the standard methods.

In this investigation, sugar determinations were carried out by following the conditions of Munson-Walker (1906) for reducing, using Bertrand’s method (1906) of determining the copper, by dissolving the cuprous oxide in ferric alum and titrating the reduced iron by a standard potassium permanganate solution. Reducing sugars, sucrose and starch (*Taka-diastase*)
were determined by the A.O.A.C. methods. Dextrins were determined by the method of Totingham et al. (1935). Table I presents the results of these experiments.

**Table 1**

Expressed as percentage of the fresh material

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<tbody>
<tr>
<td>Reducing Sugars</td>
<td>0.14</td>
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<tr>
<td>Sucrose</td>
<td>3.79</td>
</tr>
<tr>
<td>Starch</td>
<td>8.22</td>
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<tr>
<td>Dextrin</td>
<td>7.69</td>
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2. Examination of the soluble polysaccharide: Preparation of the soluble polysaccharide

250 g. of garlic was crushed to a fine paste in an end-runner. The paste was suspended in water (1.5 litre), stirred vigorously, and kept at 80° for 24 hours. The hot mixture was filtered through a cloth, and the filtrate was poured into three volumes of alcohol, and the precipitate allowed to settle by standing overnight. The supernatant layer was syphoned off and the carbohydrate was collected on the centrifuge, washed with absolute alcohol, dry ether and was finally dried in vacuum. The yield was 8 g. Nitrogen, phosphorus and ash were determined on the specimen. The results are N, 1.02, P, 1.10 and Ash, 0.6 per cent.

*Purification.*—The carbohydrate was brought into solution in water and made finally alkaline with sodium hydroxide and reprecipitated by pouring into alcohol. After two such purifications, the polysaccharide still analysed for nitrogen, phosphorus and ash as 1.00, 0.98 and 0.48 per cent. respectively. No further purification was attempted.

It is worthwhile to point out here that polysaccharide containing nitrogen as high as 4.2 per cent. (Black, 1931) from the pollen of Giant weed has been prepared. Similarly, Gough (1932) isolated a polysaccharide containing about a per cent. of nitrogen from Timothy grass pollen.

*Properties.*—

(1) The carbohydrate swells with water.

(2) In 2 per cent. sodium hydroxide it had \( \alpha_{5461} = -41^\circ \).

(3) It is not easily soluble in water, but dissolves readily on the addition of alkali.
Reactions.—

(1) The polysaccharide gave no colouration with a solution of iodine.
(2) It gave Molish’s test with sulphuric acid and alcoholic β-naphthol.
(3) Fehling’s solution was not reduced in the cold but was reduced readily if the carbohydrate was warmed previously with hydrochloric acid.
(4) Warming with concentrated hydrochloric acid and either orcinol or phloroglucinol did not produce any characteristic colour for pentoses.
(5) Methyl pentose are absent as the Rosenthaler’s reaction (1909) is negative.
(6) The polysaccharide failed to produce any characteristic colour for glycuronic acid with naphtho-resorcin (Neuberg and Kobel, 1931).
(7) It gave positive reaction for keto hexoses according to Selivanhoff (1887) using resorcinol and hydrochloric acid.

Hydrolysis of the Polysaccharide.—0.5 g. of the carbohydrate was hydrolysed with 50 ml. of one per cent. sulphuric acid for 3 hours on a boiling water-bath. After hydrolysis it gave a rotation of $+9^\circ$ (1 = 1 dm.). After neutralising the hydrolysate, reducing sugars were determined. This corresponds to 0.47 g. of glucose.

The reactions of the hydrolysate.—It gave no reactions for pentoses, methyl pentoses, glycuronic acid. But the test for keto hexoses was very strong.

Preparation of a barium salt from the hydrolysate.—Two grams of the polysaccharide were hydrolysed with 200 ml. of one per cent. sulphuric acid for 3 hours. The hydrolysate was neutralised with barium carbonate and filtered. The precipitate was washed with warm water and the filtrate concentrate to a thick syrup. The syrup was dissolved in 20 ml. of water and diluted with 200 ml. of methyl alcohol. The precipitate after standing for an hour was collected on the centrifuge and washed with methyl alcohol and dried in vacuo. It weighed 330 mg. The barium content was determined by the titrimetric method of King (1932) using 45 mg. of the barium salt. The barium content was found to be 26.8 per cent. It did not reduce Fehling's solution even on warming.

Identification of the products of hydrolysis.—The methyl alcoholic centrifugate after precipitation of the barium salt was concentrated in vacuo to
a syrup. The residue was again dissolved in water and evaporated to a syrup. This process was repeated twice over. Finally the syrup was dissolved in 20 ml. of water. More of the carbohydrate was hydrolysed and worked up in this way for examination.

The sugar solution gave rise to an ozazone m.p. 205°, identical with that of the glucose ozazone. Since glucose ozazone could arise from glucose, mannose, galactose and fructose, these units were tested in the hydrolysate. Glucose and galactose are absent because the hydrolysate failed to reduce the Nylander's reagent; oxidation of the hydrolysate or the solid polysaccharide with nitric acid resulted only in the formation of oxalic acid and no mucic acid could be detected. The hydrolysate in cold gave a phenyl hydrozone, m.p. 203–4° (mixed m.p. with an authentic specimen was not depressed). The filtrate after the collection of mannose at the pump, was shaken with benzaldehyde and refiltered. The final filtrate gave the characteristic red colour for keto hexoses with resorcinol and 12 per cent. hydrochloric acid, and the orange red precipitate with thio-barbutyric acid in 12 per cent. hydrochloric acid (Plaisance, 1916). The presence of mannose was confirmed by the preparation of the anilide, m.p. 180° (decomp.) from the hydrolysate (Irvine, 1910). The fructose was identified in the hydrolysate as the as-methyl phenyl ozazone, m.p. 156°, confirmed by a mixed melting point with an authentic specimen.

The above series of experiments establish that in addition to starch the reserve polysaccharide of garlic is composed of fructose, mannose and a non-reducing acid. Phosphorus and nitrogen were also found in the preparation. The ash from the polysaccharide gave positive tests for calcium.

Summary

1. The various forms of carbohydrate in the bulb of Garlic have been determined.

2. In addition to starch, the reserve polysaccharide is made up of mannose, fructose and a non-reducing acid.
REFERENCES

Bertrand

Black

Gough

Irvine

Johansson

King

Munson-Walker

Neuberg and Kobel

Parkin

Plaisance

Rosenthaler

Selivanhoff

Tottingham, Kertesz, Loomis and Phillips

. J. Allergy, 1931, 2, 161.