

Constitution of Polysaccharides, III. On Plant Mucilage, I.

By

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I. ISOLATION OF MUCILAGE.

Mucilages are widely distributed in the plant kingdom, they occur in some food materials utilized in Japan¹ and also in some barks and roots which are esteemed as important materials in making Japanese paper.¹

The study of their chemical composition is, therefore, interesting from the point of view of industrial and food chemistry. The material used in this investigation was obtained from the bark of the *Hydrangea paniculata*, Sieb.² It was extracted with cold water and then thrown down with alcohol from the solution.

The crude mucilage obtained in this way, still contained some crystals of calcium oxalate associated with it in the plant tissues.

(1) It was analysed after being washed with alcohol and ether successively, and dried in vacuo to constant weight:

$$\begin{array}{rcl} \text{C} = 41.1 & ; & \text{H} = 5.8 & ; & \text{Ash} = 19.7 . \\ & & 41.3 & ; & 6.2 & ; & 18.4 . \end{array}$$

(2) The precipitation of the mucilage from the aqueous solution with alcohol was repeated four times:

$$\begin{array}{rcl} \text{C} = 42.4 & ; & \text{H} = 6.3 & ; & \text{Ash} = 17.5 . \\ & & 42.6 & ; & 6.4 & ; & 18.0 . \end{array}$$

(3) The aqueous solution of mucilage was dialysed for two weeks with

¹ S. Yoshimura: Bull. Agric. College, Tokyo, 2, 207 (1895); G. Yoshii: On the Manufacture of Japanese Paper (in Japanese).

² S. Komatsu and H. Ueda: These Memoirs, 8, 59 (1925).

toluene to keep it from putrefaction by micro-organisms and then the substance precipitated with alcohol was analysed :

$$\begin{array}{rcl} \text{C} = 41.26 ; & \text{H} = 6.4 ; & \text{Ash} = 14.8 . \\ 41.3 ; & 6.3 ; & 14.5 . \end{array}$$

(4) 10 grm. of mucilage purified by repeated precipitations four times, dissolved in 170 c.c. of 1 % hydrochloric acid, filtered through silk cloth and precipitated with alcohol, washed with ether and then dried in vacuo :

$$\begin{array}{rcl} \text{C} = 42.2 ; & \text{H} = 5.6 ; & \text{Ash} = 14.2 . \\ 41.8 ; & 5.4 ; & 14.2 . \end{array}$$

(5) Mucilage (No. 4.) dissolved in water was left to stand one day in a cold place and then a mixture of acetone and absolute alcohol was added, whereby the mucilage was precipitated, and this separated by filtration, washed with alcohol and then with ether :

$$\begin{array}{rcl} \text{C} = 40.0 ; & \text{H} = 5.5 ; & \text{Ash} = 21.6 . \\ 39.3 ; & 5.0 ; & 21.5 . \end{array}$$

To the filtrate, ether was added to form the second precipitate which, after being separated from the mother liquor, washed with ether, and dried in vacuo, was analysed :

$$\begin{array}{rcl} \text{C} = 47.1 ; & \text{H} = 6.2 ; & \text{Ash} = 7.5 . \\ 46.9 ; & 6.3 ; & 9.2 . \end{array}$$

(6) The extract filtered through silk cloth many times, was centrifuged to separate it from needle crystals suspended in the solution, and the clear solution thus separated from the white mass deposited on the bottom of the vessel, was treated with alcohol to precipitate mucilage which, after being treated as usual, was analysed :

$$\begin{array}{rcl} \text{C} = 42.76 ; & \text{H} = 5.68 ; & \text{Ash} = 9.85 . \\ 42.09 ; & 5.48 ; & 11.56 . \end{array}$$

Thus, our mucilage prepared in the best conditions still contains 8 per cent of ash, whilst the usual one contains 14 per cent. Such a high content of ash should be naturally ascribed to the possible presence of the salts of other organic acids since it has been admitted that plant mucilage usually contains 5 per cent of ash. In the present case, it was due to the presence of calcium oxalate as indicated later. Consequently, when the sample was compared with others mentioned in the literature,¹

¹ W. Shirmer : Arch. Pharm., **250**, 230 (1912) ; R. Gans and B. Tollens : Lieb. Ann., **249**, 245 (1889) ; A. Hilger and Rothenfusser : Ber. D. Chem. Ges., **35**, 1841 (1902) ; A. Hilger : Ibid., **36**, 3198 (1903) ; A. Schmidt : Lieb. Ann., **51**, 29 (1844) ; Mulder : J. prak. Chem., **37**, 340 (1888) ; N. Kirchner and B. Tollens : Lieb. Ann., **175**, 215 (1875).

the result was poor in carbon and hydrogen content.

II. HYDROLYSIS WITH 1.5-2 % SULPHURIC ACID.

50 gm. of the crude mucilage were heated with 100 c. c. of sulphuric acid, the concentration of acid being adjusted to make 1.5 per cent, and set to boiling for 4-5 hours, the silky needle crystals which formed during the digestion were separated by filtration after the reaction was ended, and these weighed 11.2 gm. corresponding to 22 % of the sample.

It was grey in colour, treated with ether and the insoluble white residue was confirmed to be composed of pure calcium oxalate by analysis. On evaporating off the ether, there remained a resinous substance associated with calcium oxalate.

The acidic filtrate separated from calcium oxalate, neutralized with calcium carbonate, filtered and then concentrated under reduced pressure to a small volume.

The calcium salt (I) precipitated with alcohol from the concentrated solution, was separated, and washed with alcohol. It was then purified by repeated precipitation with alcohol from its aqueous solution. The yield was 27 gm. corresponding to 52 % of the crude mucilage.

The filtrate from the calcium salt (I) and the washings combined together, were concentrated under reduced pressure to a small volume.

It contained 7 gm. reducing sugar calculated as d-glucose, which corresponded to 14 % of the mucilage. l-Arabinose was isolated as osazone from the solution, which was confirmed to be l-arabinose osazone by determination of the melting point (160°).

The chemical nature of the other osazones, probably methyl pentose osazone, present in the residue which was with difficulty soluble in hot water, could not be examined owing to lack of material.

Thus, 50 gm. of the crude mucilage were decomposed by boiling with 1.5 % of acid into

- 52 % of the calcium salt of an organic acid I,
- 14 % of l-arabinose and other sugars,
- 22 % of calcium oxalate with a small amount of fatty substance,
- 12 % of other organic substances of unknown nature and treatment loss.

In other words, pentose molecule or pentosan combined in a particular way with the nucleus of the mucilage was split off by the digestion with dilute acid, resulting in a complex substance of acidic nature as one of the cleavage products. When, however, it was digested with 5 % or more of concentrated sulphuric acid for 4-5 hours in a flask with a

reflux condenser, the decomposition was proceeded further as noticed by many investigators in mucilages¹ so as to yield l-arabinose and d-galactose as cleavage products and the former by further action of the concentrated acid was decomposed into furfural. The authors have, thus, actually isolated d-galactose from the digested liquor after the usual treatments in the crystalline state from a glacial acetic acid solution, which was identified by determination of its melting point, of mutarotation in its aqueous solution and also by analysis :

$$C=39.94; H=6.72; \quad [\alpha]_D^{20} = \frac{1.93 \times 100}{2.41} = +80.13$$

III. CALCIUM SALT, I.

The substance purified by the repeated precipitations from the aqueous solution with alcohol, was a white amorphous powder and reduces Fehling's solution. The elementary and the proximate compositions of the substances were as follows :

$$C=43.64; 43.48; H=5.59; 5.55; \text{ash}=10.06; 9.61; Ca=6.12; \\ \text{Galactan}=15.7; \text{Pentosan}=16.09; \text{Methyl pentosan}=12.96; \\ \text{Methoxyl value}=3.78; CO_2=9.80^2.$$

The optical rotatory power of the aqueous solution shows :

$$[\alpha]_D^{20} = \frac{1.80 \times 100}{1 \times 2.504} = +71.9^\circ$$

In the calculation of the percentage of galactan and of the pentosans in the mucilage and also of the calcium salt I, by means of the mineral acids-digestion as usual, transforming them into mucic acid and furfural respectively, the occurrence of galactoronic acid should be taken in consideration since it yields by the treatments mucic acid on the one side, and on the other, furfural, and the evolution of carbon dioxide by digestion with conc. hydrochloric acid indicates the existence of galactoronic acid in the molecule.²

The calcium salt agrees in its essential properties with the mucilage ; they possess the property of swelling in contact with water, of being insoluble in alcohol and ether and are composed of polysaccharides. They, however, differ in sugar-content and also behaviour toward Fehling's solution ; the pentosan-galactan-ratio being 28 : 16 in the former and 21 : 8

¹ A. Hilger and Rothenfusser : *Loc. cit.*

² H. Meyer : *Analyse Org. Verb.*, 2 Auf. S. 564.

in the latter, and the phenomenon was due to the removal of the pentosan group from the mucilage molecule during the acid digestion.

Such relations between the mucilage and the calcium salt I, in these respects described above are comparable to those between crude pectin and pectic acid, and the latter substance according to F. Ehrlich¹ actually resulted by heating pectin with water under pressure, decomposing into araban and the calcium and the magnesium salts of pectic acid.

Moreover, referring to v. Fellenberg's investigation² in apple pectin, the authors came to have the conviction that the calcium salt I,³ approaches much more to pectic acid, in such a manner, indeed, as to allow of the conjecture that these substances, when examined in a state of purity, will prove identical with each other.

We, however, have no experimental evidence, at the present moment, to side with some chemists who believe that the so-called vegetable mucilages are nothing else than pectin substances, but are of the same opinion in attributing them to its nearest allies.

The behaviour of the salt I, toward sulphuric acid is an interesting phenomenon, being different with the concentration of the acid yielding reaction products which differ in composition as shown in the following experiments.

IV. HYDROLYSIS OF CALCIUM SALT I WITH SULPHURIC ACID.

I. 25 grm. of the salt I, were heated with 200 c. c. of 5 % sulphuric acid to boiling for 3.5 hours (the concentration of the acid becomes 3 %, since the acid was partly used for combination with 1.5 grm. of the calcium in the salt), and the calcium sulphate formed by the reaction was filtered off, and the filtrate was neutralized with calcium carbonate and filtered again. The filtrate containing 6 grm. of reducing sugar calculated as d-glucose from its reducing power of Fehling's solution, was concentrated under reduced pressure to a small volume.

The calcium salt of an organic acid (calcium salt II_a) was precipitated from the solution with 91% alcohol, washed with alcohol and then with ether. The yield was 18.2 grm. which corresponds to 72.8% of the salt I.

1 Chem. Ztg., **28**, 197 (1917).

2 Biochem. Z., **85**, 118 (1918).

3 The calcium salt I, which resulted from the mucilage by acid-digestion, splitting off pentosan (including methyl pentosan) only, should contain $21 - 14 = 7$ % of pentosan, and 7.8 % of galactan, while the actual observation was 29 and 16 % respectively (S. K.).

Calcium salt I yields $\left\{ \begin{array}{l} 73 \% \text{ of II}_a \text{ salt,} \\ 24 \% \text{ of reducing sugar,} \\ 3 \% \text{ of treatment loss and unknown} \\ \text{substances.} \end{array} \right.$

II. 20 gm. of the calcium salt I, were treated with 200 c.c. of 5 % sulphuric acid (using 130 c.c. of 10 % sulphuric acid and 70 c.c. of water) for 5 hours, the resulting substances were as follows:

6.1 gm. of reducing sugar calculated as d-glucose, 9.8 gm. of calcium salt II_b.

Calcium salt I yields $\left\{ \begin{array}{l} 49 \% \text{ of II}_b \text{ salt,} \\ 30 \% \text{ of reducing sugar,} \\ 21 \% \text{ of treatment loss and unknown} \\ \text{substances.} \end{array} \right.$

When strong acid was used in the digestion of the calcium salt, the hydrolysis proceeded with greater velocity, and, moreover, much treatment loss accompanied, owing to the partial decomposition of pentose into furfural, which split off from the complex groupings from the nucleus of the salt I.

V. THE CALCIUM SALT II_a AND II_b.

The calcium salts, II_a and II_b, purified by repeated precipitations with alcohol from their aqueous solutions, are a white amorphous powder, insoluble in alcohol and ether, and soluble in water, forming a viscous liquid. They reduce Fehling's solution.

Their elementary and proximate analysis was made with the following results:

	II _a		II _b	
C	41.31;	41.46 .	40.49;	40.02 .
H	5.59;	5.60 .	5.78;	5.84 .
Ash	11.55;	11.55 .	14.23;	14.55 .
CaO in ash	85		74	
Pentosan	23.5		23.6	
Methyl pentosan	17.5		13.9	
Galactan	17.7		20.8	
CO ₂	—		11.0	
$[\alpha]_D^{20}$	+67°		+58°	

As seen in the foregoing tables, when the calcium salt I was hydro-

lysed with more concentrated acid, the resulting calcium salt is rich in the content of hydrogen and ash, but poor in carbon.

The high percentage of ash content indicates that the salt contains much of a substance such as galactoronic acid which by treating with conc. hydrochloric acid, on the one hand, yields furfural, and on the other with conc. nitric acid, mucic acid. As a consequence, the calcium salt II_b , becomes a rich pentosan and galactan compared with the salt II_a .

VI. HYDROLYSIS OF THE CALCIUM SALTS II.

I. 3 gm. of the calcium salt II_a were heated with 3 % sulphuric acid (calculated as free acid) in a sealed tube at $135-145^\circ$ for 3 hours, the solution becomes brown and accompanied with the formation of a humic substance. The filtrate separated from the insoluble matter was concentrated under reduced pressure to a small volume after being neutralized with calcium carbonate, and then treated with alcohol to precipitate the calcium salt of an organic acid (Ca salt III_a). The filtrate separated the precipitate contains 1 gm. of reducing sugars calculated as d-glucose.

II. 5 gm. of the calcium salt II_b were treated with 2 % sulphuric acid in the manner described in I. After heating at $128-132^\circ$ for 2 hours, it was treated as usual, and the calcium salt III_b , 2.5 gm. of reducing sugar calculated as d-glucose and other organic acids of unknown nature were observed to be formed by the digestion.

The calcium salts III (a and b) are a white amorphous powder, reduce the Fehling solution and are soluble in water forming no viscous solution.

The research will be continued in the laboratory when new materials come to the hands of the present investigators.

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