

## STUDIES ON PLANT MUCILAGES

### Part II. Partial Hydrolysis of the Mucilage from the Tubers of *Asparagus filicinus*

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IN the constitutional studies of gums and mucilages, partial or graded hydrolysis plays an important part. In several cases the change proceeds progressively, the component sugars getting detached one by one, and the final product is an aldobionic acid.

The mucilage from the tubers of *Asparagus filicinus* Buch.-Ham. ex D. Don, as already reported,<sup>1</sup> is composed of glucose, fructose, mannose and a uronic acid. With a view to obtaining a further knowledge regarding the mucilage molecule, it has been subjected to partial hydrolysis, as in the case of the mesquite gum,<sup>2</sup> by heating with 4 per cent. sulphuric acid at 80° for 3 hours. Amongst the products of hydrolysis, a polysaccharide and an aldobionic acid have been isolated.

The polysaccharide is composed of mannose, glucose and fructose in the molecular ratio of 9:8:2. It is soluble in water and also in 70 per cent. alcohol, and undergoes ready acetylation. The aldobionic acid undergoes further hydrolysis yielding mannose and glucuronic acid in equal proportion. It is, therefore, a manno-glucuronic acid. In this connection it may be noted that a manno-glucuronic acid ( $\beta$ -*D*-glucuronosido-2-*D*-mannose) was isolated from damson and cherry gums through partial hydrolysis,<sup>3,4</sup> and it remains to be seen whether these two aldobionic acids are identical or different.

From the results recorded above, it looks as though the mucilage molecule splits itself up into a manno-glucuronic acid and a polysaccharide. The latter still contains mannose, and hence it appears that a portion of the total mannose constituting the mucilage molecule is united to the uronic acid to form a resistant nucleus and the rest attached probably through different linkages.

#### EXPERIMENTAL

*Partial Hydrolysis of the Mucilage.*—The mucilage (10 g.) was taken in a round bottom flask of 500 c.c. capacity and to it was added 4 per cent.

sulphuric acid (200 c.c.). The material almost immediately swelled into a thick dough, which was subsequently heated in a water-bath at 80° for 3 hours. As the heating progressed the contents became more and more fluid and after three hours they broke up into a thin colloidal solution and a coagulated precipitate. At this stage heating was stopped, the mixture cooled and filtered through a fine muslin. The filtrate was then passed through a fluted filter, when a clear solution was obtained. It was then neutralized with barium carbonate and concentrated to about 50 c.c. in an evaporating dish in the presence of barium carbonate. After filtration, the concentrate was cooled and treated with 95 per cent. alcohol (150 c.c.) when the barium salt of a complex uronic acid separated out. The barium salt and the sugar solution were treated separately.

The barium salt was purified by repeated dissolution in water and precipitation by means of alcohol. After the third precipitation, it was filtered, dehydrated using hot absolute alcohol and dried in a vacuum desiccator. On analysis it was found to contain 15·8 per cent. of barium (estimated as barium sulphate). On heating with 12 per cent. hydrochloric acid (method of Dickson, Otterson and Link for the estimation of uronic acid<sup>5</sup>) it liberated 4·64 per cent. of carbon dioxide. These data indicated that the substance was the barium salt of an aldobionic acid. (Barium aldobionate contains 16·21 per cent. of barium and liberates 5·19 per cent. of carbon dioxide).

The filtrate, left after the removal of barium aldobionate, was concentrated to about 30 c.c. by distillation under reduced pressure and then treated with 120 c.c. of alcohol, when a dark-coloured resinous mass separated out. The supernatant liquor, which contained some sugars, was decanted off, and the resinous residue, on trituration with hot methyl alcohol, became quite crisp. The solid was further purified by repeated precipitations from aqueous solutions by means of alcohol, till it attained a constant specific rotation. The product finally obtained was almost colourless with a pale yellowish tinge. It was amorphous under the microscope and did not melt below 250°. The substance responded to Dreywood reaction by developing a green colour, when treated with anthrone and sulphuric acid, thereby indicating its carbohydrate nature<sup>6</sup> (Found in a sample dried at 110° in vacuum: C, 43·91 and H, 5·82;  $(C_6H_{10}O_5)_n$  requires C, 44·44 and H, 6·17%). The polysaccharide was freely soluble in water and aqueous alcohols up to 70 per cent. strength. Above this strength the solubility decreased gradually so that in alcohols of 90 per cent. strength and above the material was almost insoluble. In other organic solvents too it was insoluble. In 0·5 per cent. solution, its specific rotation  $[\alpha]_D^{20}$  was  $-14\cdot45^\circ$ . It reduced Fehling's solution, 100 mg. liberating 221·2 mg. of cuprous oxide.

The substance underwent ready acetylation when it was heated with acetic anhydride and anhydrous sodium acetate on a boiling water-bath for 6 hours. The acetyl derivative was freely soluble in chloroform and other organic solvents. However, on heating with hydroxy solvents, it used to undergo decomposition to some extent, probably through partial deacetylation. From chloroform solution it came out as an almost colourless substance with a slight yellow tinge and was found to be microcrystalline under the microscope. On heating it sintered at  $165^{\circ}$  and decomposed at about  $180^{\circ}$ . It contained 46.85 per cent. of  $-\text{COCH}_3$ . In chloroform solution its specific rotation was  $-15.0^{\circ}$ .

*Hydrolysis of the Polysaccharide.*—The polysaccharide (2 g.) was dissolved in 4 per cent. sulphuric acid (100 c.c.) and mildly refluxed for 4 hours on a wire gauze. By this treatment the polysaccharide underwent complete hydrolysis. After neutralization with barium carbonate the hydrolysate was made up to 200 c.c. and analyzed for the constituent sugars. It was found to contain all the three sugars present in the original mucilage, namely, mannose, glucose and fructose. The presence of fructose was detected by its response to Pinoff's<sup>7</sup> and Seliwanoff's tests<sup>8</sup> and was confirmed by the formation of the characteristic osazone melting at  $151^{\circ}$ , when treated with methyl phenyl hydrazine according to the method of Bourquelot and Herissey<sup>9</sup>. The total sugars were estimated employing Fehling's solution and the total aldoses by the iodine-oxidation method of Willstätter and Schudel.<sup>10</sup> The difference between the total aldose content and that of mannose gave the amount of glucose, since these were the only two aldoses present. The balance of the sugars was obviously fructose. According to these estimations, mannose was found to be 47.4, glucose 42.1 and fructose 10.5 per cent., corresponding to 9, 8 and 2 molecules of mannose, glucose and fructose, respectively.

*Hydrolysis of the Aldobionic Acid.*—The barium salt of the aldobionic acid (1 g.) was dissolved in 20 c.c. of 2.5 N sulphuric acid and heated under reflux on a boiling water-bath for 20 hours. The small amount of the barium sulphate that separated as a result of the initial decomposition of the barium aldobionate did not interfere with the subsequent heating, and no attempt was, therefore, made to remove it. After the heating was over, the hydrolysate was neutralized with barium carbonate and filtered. The filtrate, when poured into alcohol, gave an insoluble barium salt (0.4 g.). It had  $[\alpha]_D^{20} + 13.8^{\circ}$  (in water  $c=1$ ); the specific rotation of barium glucuronate is reported to be  $+15.0^{\circ}$  at  $19^{\circ}$ <sup>11</sup>. (Found: Ba, 25.6 per cent.; Barium glucuronate,  $\text{Ba}(\text{C}_6\text{H}_9\text{O}_7)_2$ , requires Ba, 26.3 per cent.). The barium salt, on oxidation

with nitric acid (1·15 d.) did not give any mucic acid, showing thereby that it was not derived from galacturonic acid.

The alcoholic filtrate from the barium glucuronate was distilled to remove all the alcohol. The aqueous solution on treatment with phenyl hydrazine in the cold very readily formed mannose phenyl hydrazone. No other sugars could be detected. The specific rotation of the sugar solution was  $14\cdot1^\circ$ , indicating that it contained only mannose.

#### SUMMARY

The mucilage from the tubers of *Asparagus filicinus*, when subjected to partial hydrolysis by heating with 4 per cent. sulphuric acid at  $80^\circ$  for 3 hours, breaks into an aldobionic acid and a polysaccharide. The aldobionic acid is composed of mannose and glucuronic acid, while the polysaccharide is constituted from two molecules of fructose, eight molecules of glucose and nine of mannose.

The polysaccharide does not melt or decompose below  $250^\circ$  and has a specific rotation of  $-14\cdot45^\circ$ . It undergoes easy acetylation when treated with acetic anhydride and anhydrous sodium acetate. The acetyl derivative decomposes at about  $180^\circ$  and has a specific rotation of  $-15\cdot0^\circ$  in chloroform solution.

#### REFERENCES

1. Rao, P. S. and Rozdon, O. N. .. *Proc. Ind. Acad. Sci.*, 1950, 31, 441.
2. Anderson, E. and Sands, L. .. *J. Amer. Chem. Soc.*, 1926, 48, 3172.
3. Hirst, E. L. and Jones, J. K. N. .. *J. Chem. Soc.*, 1938, 1174.
4. Jones, J. K. N. .. *Ibid.*, 1939, 558.
5. Dickson, A. D., Otterson, H. and Link, K. P. .. *J. Am. Chem. Soc.*, 1930, 52, 775.
6. Dreywood, R. .. *Ind. Eng. Chem. (Anal.)*, 1946, 18, 499.
7. Pinoff, E. .. *Ber.*, 1905, 38, 3317.
8. Seliwanoff, T. .. *Ibid.*, 1887, 20, 181.
9. Bourquelot, E. and Herissey, H. .. *Compt. rend.*, 1899, 129, 339.
10. Willstätter, R. and Schudel, G. .. *Ber.*, 1918, 51, 780.
11. Jones, J. K. N. and Hirst, E. L. .. *J. Chem. Soc.*, 1938, 1179.