STUDIES ON TUBER HEMICELLULOSES

Part II. Hemicelluloses from the Tubers of *Asparagus racemosus* and Their Complete and Partial Hydrolysis

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It has already been reported that the root powder (moisture content: 13·42 per cent.) of *Asparagus racemosus* Willd. contains 37·44 per cent. of free sugars and 2·10 per cent. of mucilages, while the total non-fibre carbohydrates amount to 52·89 per cent.[1] The other constituents of the non-fibre carbohydrates have now been examined and are found to be hemicelluloses (5·40 per cent.) and some insoluble polysaccharides (7·95 per cent.). The isolation and examination of the hemicelluloses are reported in this paper.

After the removal of saponins, free sugars, mucilages and pectins by successive treatments with ethyl acetate, 70 per cent. alcohol, hot water and ammonium oxalate solution, the root powder has been extracted for the isolation of the hemicelluloses with 4 per cent. sodium hydroxide according to the method of Norman.[2] On further fractionation as per the procedure of Norris and Preece,[3] the hemicelluloses have given rise to two fractions, namely, A_2 and B_2.

Both A_2 and B_2 are amorphous substances, which are almost white in colour with a greyish tinge. They are soluble in hot water, the solutions being slightly viscous but not mucilaginous. Further, they are readily hydrolysed by boiling dilute mineral acids, indicating their hemicellulosic nature.[4] They are composed of the same sugar and uronic acid, namely, glucose and galacturonic acid, but in different molecular proportions. In A_2 the molecular ratio of glucose to galacturonic acid is 10:1, while in B_2 it is 5:2.

The two hemicelluloses have been subjected to partial hydrolysis also. This degradation is best brought about by heating the material with 1·5 per cent. sulphuric acid for 3 hours on a boiling water-bath. It may be noted that direct boiling of the mixture under reflux even for a short time usually results in almost complete hydrolysis. Under the controlled conditions mentioned both A_2 and B_2 are hydrolysed to glucose and an aldobionic

acid. The latter has been isolated as its barium salt. On further hydrolysis it gives rise to *d*-glucose and *d*-galacturonic acid in equimolecular proportions.

It may be noted that the aldobionic acid present in the mucilage portion of these tubers is also a gluco-galacturonic acid.[5] It is interesting to note that in both the mucilage and the hemicellulose portions the aldobionic acids are composed of the same sugar and uronic acid; however, they differ in their resistance to hydrolysis by acids. The constitutional factors responsible for this difference are yet to be studied.

It is also interesting to note that amongst the three species of *Asparagus*, the tubers of which have been examined in some detail, *A. filicinus* does not seem to contain any hemicelluloses,[6] while the other two contain two fractions each.[7] Further, *A. adscendens* and *A. racemosus* differ as regards the nature of the sugars and also the uronic acids constituting the hemicelluloses. The hemicelluloses from *A. racemosus* are composed of glucose and galacturonic acid, while those of *A. adscendens* are constituted from xylose, glucose and glucuronic acid.

Experimental

Isolation and fractionation of the hemicelluloses.—The debarked root powder of *Asparagus racemosus* was extracted successively with ethyl acetate, 70 per cent. alcohol, warm water and 0·5 per cent. ammonium oxalate solution in order to remove saponins, free sugars, mucilages and pectins. The residual powder was worked up for the isolation of the hemicelluloses. It (100 g.) was heated with 4 per cent. sodium hydroxide (500 c.c.) at 45–50° C. for two hours with frequent shaking. The mixture was filtered through a fine muslin and the residue was twice again extracted with the alkali, taking the same volume each time. After clarification by repeated filtration through glass wool, the extract was treated with 10 g. of sodium hypochlorite and just acidified with hydrochloric acid. This treatment was to destroy the lignins, if present to any extent (method of Norman[2]). After 10 minutes when no more chlorine was evolved and when the hemicelluloses settled down, the supernatant liquid was decanted off and the rest centrifuged. The separated solid was washed first with small quantities of water, then with 60 per cent. alcohol and finally with hot absolute alcohol. It was then dried first in air and then in a desiccator. The yield was 5·40 per cent. on the weight of the debarked root powder taken.

The product was fractionated according to the method of Norris and Preece.[3] A 1 per cent. solution of the substance in 4 per cent. sodium

hydroxide (500 c.c. in volume) was treated with excess of glacial acetic acid and allowed to stand for 6 hours. The solid that settled down (Fraction A) was separated in a centrifuge, and washed successively with water and hot alcohol. Its yield on the basis of the root powder was 2·00 per cent. The mother-liquor (600 c.c.) was next treated with half its volume of ethyl alcohol, when Fraction B separated out as a greyish white substance. It was also isolated, washed and dried as Fraction A, and was obtained in 3·40 per cent. yield. On further treatment with excess of alcohol, the mother-liquor, left after the separation of B, did not give any more solid (Fraction C absent).

The above fractions were subjected to further fractionation. They were separately dissolved in hot 4 per cent. sodium hydroxide so as to form 1 per cent. solution and filtered through glass wool to remove the slight turbidity present. On treatment with Fehling's solution at the rate of 30 c.c. for every 100 c.c. of the alkaline solution, no precipitate separated out in either case, indicating the absence of Fractions A_1 and B_1. Hence the two hemicelluloses corresponded to Fractions A_2 and B_2 of Norris and Preece. On acidification, followed by the addition of excess of alcohol, the alkaline solutions precipitated the hemicellulose. Both A_2 and B_2 were purified by repeated dissolution in dilute alkali and reprecipitation by means of acid and alcohol. Three such treatments were required to get ashless products. The final yields of purified A_2 and B_2 were respectively 1·65 per cent. and 2·90 per cent. on the weight of the debarked root powder.

Hemicellulose A_2.—In the purified condition A_2 was greyish white in colour, and contained 9·26 per cent. of moisture. It was insoluble in alcohol acetone, ether, etc., but was soluble in hot water yielding viscous but non-mucilaginous solutions. In 0·5 per cent. aqueous solution its specific rotation at 18° C. was + 19·7°.

The hemicellulose contained uronic acid, as it responded to the naphthoresorcin test.[8] The uronic acid was quantitatively estimated according to the method of Dickson, Otterson and Link[9] and was found to be 8·8 per cent. on zero-moisture basis of the hemicellulose. It did not contain any methoxyl group or pentose.

The purified material (2 g.) was hydrolysed by boiling under reflux with 2 per cent. sulphuric acid (150 c.c.) for 2 hours. The hydrolysate was neutralized with barium carbonate, filtered and the filtrate examined for the sugars and the uronic acids adopting filter-paper chromatography (horizontal migration method of Rao and Beri.[10]) Only glucose and galacturonic acid could be detected. The identity of glucose was confirmed by the preparation of

its osazone with its characteristic crystal structure and melting point (204–206° C.). The identity of the uronic acid was also confirmed by the determination of the specific rotation of its barium salt $\{[\alpha]_D^{25°} = +24{\cdot}8°\}$[11] and its oxidation with nitric acid of sp. gr. 1·15 to produce mucic acid.

Composition of Hemicellulose A_2.—Since the hemicellulose contained only glucose and galacturonic acid, and the latter was found to be 8·8 per cent., the composition of the hemicellulose might be taken to be glucose and galacturonic acid present in the molecular ratio of 10:1.

Hemicellulose B_2 and Its Composition.—This fraction too was similar to Fraction A_2 in its colour, amorphous structure and solubility. It contained 8·2 per cent. of moisture. Its specific rotation in 0·3 per cent aqueous solution was + 27·1° at 18° C. It also underwent easy hydrolysis with dilute boiling sulphuric acid. The hydrolysate was examined as in the case of hemicellulose A_2, and was found to contain the same sugar and uronic acid, namely, glucose and galacturonic acid. In this case, the sugar and the uronic acid were present in the ratio of 5:2.

Partial Hydrolysis of A_2 and B_2.—The purified hemicellulose A_2 (5 g.) was heated in a round-bottom flask with 1·5 per cent. sulphuric acid (150 c.c.) on a boiling water-bath (98° C. at Dehra Dun altitude), when it rapidly went into solution. The course of the hydrolysis was followed by noting the optical rotation at frequent intervals. After 3 hours, when there was no more change in the rotation, the solution was neutralised with barium carbonate in the hot, concentrated to a small volume (40 c.c.), filtered and then treated with 95 per cent. alcohol (100 c.c.). The precipitated solid and the filtrate were examined separately.

From the filtrate alcohol was distilled off under reduced pressure. The resulting aqueous solution did not show the presence of any oilgosaccharides, since on further hydrolysis it did not undergo any change in reducing power or rotation. It contained only glucose.

The precipitate (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. It contained 16·40 per cent. of barium (estimated as barium sulphate) and liberated 9·89 per cent. of carbon dioxide on heating with 12 per cent. hydrochloric acid.[9] These data indicated that the substance was the barium salt of an aldobionic acid. [Barium aldobionate, $(C_{11}H_{19}O_{10}COO)_2Ba$, contains 16·21 per cent. of barium and liberates 10·38 per cent. of carbon dioxide]. The salt (2 g.) was further hydrolysed by

boiling with 3 per cent. sulphuric acid (200 c.c.) under reflux for 2 hours. As the separated barium sulphate did not interfere with the course of boiling, no attempt was made to remove it. On analysis the hydrolysate was found to contain only glucose and galacturonic acid in equimolecular proportion.

B_2 was also hydrolysed in the same way, and the same products were obtained.

SUMMARY

The hemicelluloses of the tubers of *Asparagus racemosus* Willd. have been isolated and chemically examined. Two fractions, *viz.*, A_2 and B_2 of Norris and Preece designation, have been obtained.

Both the fractions are constituted from the same sugar and uronic acid, namely, glucose and galacturonic acid but in different proportions. In hemicellulose A_2 the molecular ratio of these compounds is 10:1, while in B_2 it is 5:2. Under conditions of partial hydrolysis, both the fractions give rise to glucose and a gluco-galacturonic acid.

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