STUDIES ON TUBER HEMICELLULOSES

Part I. Hemicelluloses from the Tubers of *Asparagus adscendens*

BY P. S. RAO, F.A.SC. AND K. L. GAKHAR

(*Forest Research Institute, Dehra Dun*)

Received February 12, 1952

HEMICELLULOSES, as has already been reported,[1] form 13·1 per cent. of the carbohydrate portion of the tubers of *Asparagus adscendens* Roxb. Their isolation and examination 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] Following the procedure of Norris and Preece,[3] the hemicelluloses have been fractionated, and they have yielded only two fractions, namely A_2 and B_2.

Fraction A_2 is an amorphous greyish-white powder. It is soluble in hot water. Its solutions, though viscous, are not mucilaginous, and undergo easy hydrolysis with dilute mineral acids, indicating its hemicellulosic nature.[4] It is composed of xylose, glucose and glucuronic acid in the molecular ratio of 2:1:1.

Fraction B_2 is also amorphous but white in colour. It is also composed of the same sugars and uronic acid as A_2 but in the proportion of 1:1:2.

EXPERIMENTAL

Isolation and fractionation of the hemicelluloses.—From the debarked root powder of *Asparagus adscendens*, saponins, free sugars, mucilages and pectins were removed by extraction respectively with ethyl acetate, 70 per cent. alcohol, warm water and 0·5 per cent. ammonium oxalate solution, and the residual powder was taken for the extraction of the hemicelluloses.

The powder (100 g.) was heated with 4 per cent. sodium hydroxide (500 c.c.) at 45°–50° C. for 2 hours with frequent stirring. After filtering the mixture through a fine muslin, the residue was twice again extracted with the alkali, taking the same volume each time. The total alkaline extract was repeatedly filtered through glass wool, till a clear solution was

obtained. The filtrate was brownish-red in colour. In order to destroy the lignins, if any, the filtrate was treated with 10 g. of sodium hypochlorite and later just acidified with hydrochloric acid to liberate chlorine (method of Norman[2]). After 10 minutes, when no more chlorine was evolved, the hemicelluloses were precipitated by the addition of more acid and excess of alcohol (3 litres). The contents were allowed to stand for 3 hours, 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. The product was dried first in air and then in a desiccator. The yield was 6·1 per cent. on the weight of the debarked root powder taken.

The product was fractionated, following the method of Norris and Preece.[3] It was dissolved in 4 per cent. sodium hydroxide (500 c.c.) and treated with excess of glacial acetic acid, when the solution became turbid. When the solid settled down (6 hours), it was isolated in a centrifuge and washed successively with water and hot alcohol. It (Fraction A) was obtained in a yield of 2·5 per cent. on the weight of the debarked root powder. To the mother liquor (about 600 c.c. in volume), ethyl alcohol (300 c.c.) was added, when Fraction B separated out as a white substance. This fraction too was isolated, washed and dried as Fraction A, and was obtained in 3·6 per cent. yield. The mother liquor, left after the separation of B, did not give any hemicellulose (Fraction C) on treatment with excess of alcohol.

The above fractions were subjected to further fractionation. They were separately dissolved in hot 4 per cent. sodium hydroxide so as to form roughly 1 per cent. solution and filtered through glass wool to get a clear solution. The filtrate was then treated with Fehling's solution at the rate of 30 c.c. per every 100 c.c. However, 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. From the alkaline solutions they were recovered by acidification followed by the addition of excess of alcohol, and were purified by repeated dissolution in dilute alkali and reprecipitation by means of acid and alcohol. Ashless products were obtained after three such treatments. Fraction A_2 was obtained in a yield of 2·1 per cent. and B_2 in 3·2 per cent. on the weight of the original debarked root powder.

Hemicellulose A_2.—After purification A_2 was obtained as a greyish-white, amorphous powder, containing 5·2 per cent. of moisture. It was insoluble in alcohol, acetone, ether, etc., but was soluble in hot water, and the solution obtained was somewhat viscous but not mucilaginous. In

aqueous solution (C = 0·4 per cent.) it exhibited a specific rotation of + 15·7° at 18° C.

The hemicellulose responded to naphtho-resorcin test indicating the presence of uronic acid,[5] which when estimated according to the method of Dickson, Otterson and Link,[6] was found to be 24·5 per cent. on the basis of dry material. It did not contain any methoxyl group. Its pentosan content was estimated according to the method of Norris and Resch[7] (applying correction for the furfural which would be liberated from the uronic acid present), and was found to be 49·8 per cent. (zero-moisture basis).

For the identification of the constituent sugars and uronic acids, the purified material (2 g.) was boiled under reflux with 2 per cent. sulphuric acid (150 c.c.) for 2 hours, and the hydrolyzate examined. The latter was neutralized with barium carbonate, filtered and the filtrate analysed, adopting filter-paper chromatography (horizontal migration method of Rao and Beri[8]). Only glucose, xylose and glucuronic acid were detected. The identity of the sugars was confirmed by the preparation of cadmium bromide-cadmium xylonate (characteristic boat-shaped crystals) for xylose and glucosazone for glucose (m.p. 204°–06°). The identity of the uronic acid was also confirmed by the determination of the specific rotation of its barium salt,[9] $[\alpha]_D^{25°} = + 15·64°$, and by the preparation of saccharic acid (isolated as potassium hydrogen saccharate) by oxidation with nitric acid ($d = 1·15$).

Composition of Hemicellulose A_2.—Since the hemicellulose was constituted from only xylose, glucose and glucuronic acid, and since the uronic acid and xylose were found to be nearly 25 and 50 per cent. respectively, the composition of the hemicellulose might be taken to be xylose, glucose and glucuronic acid present in the ratio of 2 : 1 : 1.

Hemicellulose B_2 and Its Composition.—This hemicellulose (Fraction B_2) was white on purification, but was amorphous under the microscope. In aqueous solution (C = 0·67 per cent.), it had a specific rotation of + 21·02° at 18°. It also underwent easy hydrolysis when boiled with dilute sulphuric acid, indicating its hemicellulosic nature. It was examined just as in the case of hemicellulose A_2. The products of hydrolysis were just the same as those obtained from A_2, but the relative proportion was different. In this case xylose, glucose and glucuronic acid were present in the ratio of 1 : 1 : 2.

SUMMARY

The hemicelluloses of the tubers of *Asparagus adscendens* Roxb. have been isolated and chemically examined. They are composed of two fractions, A_2 and B_2 of Norris and Preece designation.

Both the fractions are constituted from the same sugars and uronic acid, *viz.*, xylose, glucose and glucuronic acid but in different proportions. In hemicellulose A_2 the ratio of these components is 2 : 1 : 1, while in B_2 it is 1 : 1 : 2.

REFERENCES

1. Rao, P. S., Beri, R. M. and Budhiraja, R. P. — *J. Sci. & Ind. Res.*, 1952, **11 B**, 127.
2. Norman, A. G. .. *Biochem. J.*, 1937, **31**, 1579.
3. Norris, F. W. and Preece, I. A. .. *Ibid.*, 1930, **24**, 59.
4. Rao, P. S. .. *Science & Culture*, 1951, **17**, 90.
5. Tollen, B. .. *Ber.*, 1908, **41**, 1788.
6. Dickson, A. D., Otterson, H. and Link, K. P. — *J. Am. Chem. Soc.*, 1930, **52**, 775.
7. Norris, F. W. and Resch, C. E. .. *Biochem. J.*, 1935, **29**, 1590.
8. (*a*) Rao, P. S. and Beri, R. M. .. *Proc. Ind. Acad. Sci.*, 1951, **33**, 368.
   (*b*) Rao, P. S., *et al.* .. *Ibid.*, 1951, **34**, 236.
9. Jones, J. K. N. and Hirst, E. L. .. *J. Chem. Soc.*, 1938, 1179.