Phytochemistry, 1965, Vol. 4, pp. 631 to 634. Pergamon Press Ltd. Printed in England

SHORT COMMUNICATION

COMPARISON OF THE HEMICELLULOSES FROM PLANTS BELONGING TO TWO DIFFERENT PLANT FAMILIES

BLANCHE D. E. GAILLARD

Laboratory of Animal Physiology, Agricultural University, Wageningen, The Netherlands

(*Received* 9 *December* 1964)

Abstract—The composition of corresponding linear and branched polymers from the hemicelluloses of three Gramineae: *Lolium perenne* (grass), *Triticum vulgare* (wheat) and *Zea mais* (maize) and three Leguminosae: *Trifolium pratense* (red clover), *Medicago sativa* (lucerne) and *Glycine max* (soya bean) were compared.

The corresponding polymers from the three species of Gramineae were very similar as were those from the Leguminosae. However there was a distinct difference between the two plant families. The linear polymers of the Gramineae contained more arabinose and less glucuronic acid than those from the Leguminosae. The greatest difference between the two plant families was found in the branched polymers, those from the Gramineae containing a high percentage of xylose and rather small amounts of arabinose, galactose and uronic acid, whereas those from the Leguminosae contained relatively large amounts of uronic acid, galactose and arabinose and little xylose. In the branched polymers from the Gramineae the uronic acid was linked to xylose, whereas in those from the Leguminosae it was linked to arabinose.

INTRODUCTION

IN PREVIOUS WORK[1] the xylans from the hemicellulose A fractions from several annual plants were compared. Certain differences were found between the xylans from cereals (corn stalk and wheat-straw) and those from legumes (alfalfa and soya bean stalks). The cereal xylans had L-arabinose and D-glucuronic acid units attached to the xylan chain. Those from the legumes showed a slightly higher uronic acid content and contained no arabinose at all. The purpose of the present work was to see whether similar differences could be found between corresponding polymers from the hemicellulose B fractions from Gramineae and Leguminosae.

Hemicellulose A is normally considered to be that fraction of the total hemicellulose which is precipitated when an alkaline extract of holocellulose is neutralized. When purified by precipitations from alkali this fraction is generally believed to be homogeneous.

Hemicellulose B is obtained from the original filtrate of the hemicellulose A preparation by precipitation with ethanol. This fraction is a mixture of several different polymers, both linear and branched. The separation of these individual polymers in a pure state is a tedious procedure generally achieved by repeated fractional precipitation from aqueous solution with ethanol or acetone. Recently[2] it has been shown that it is possible to separate the linear heteroxylans and glucans from the branched polymers in hemicellulose B fractions by dissolving the mixture in concentrated calcium chloride solution and precipitating the linear polymers with an iodine-potassium iodide solution. The branched polymers are then recovered from the filtrate.

In all cases so far investigated the branched fraction appeared to be a single polymer as

[1] R. L. WHISTLER and B. D. E. GAILLARD, *Arch. Biochem. Biophys.* **93**, 332 (1961).
[2] B. D. E. GAILLARD, *Nature* **191**, 1295 (1961).

judged by ultracentrifugation and microelectrophoresis. The linear fraction from hemicellulose B was composed largely of two polymers. An attempt to separate these on a Sephadex column gave no clear cut results. The composition of the different fractions obtained from the column however, suggested the presence of a neutral glucan and of a heteroxylan containing varying amounts of arabinose and uronic acid side units. The hydrolysates of the first fraction contained glucose only, the following fractions yielding uronic acid, arabinose and xylose with decreasing amounts of glucose, and the last fractions contained only the first three sugars with no glucose at all.

The separation between the hemicellulose A and B fractions is not sharp. When purifying the hemicellulose B mixture by reprecipitation it can be dissolved in a smaller volume of alkali than was originally needed for the extraction of the polysaccharides from the holocellulose. Upon neutralization this alkaline solution yields again a precipitate which by definition is also hemicellulose A. Usually this precipitate is discarded as it represents only a small amount. For soya bean, this second precipitate was purified by several reprecipitations from alkali and its monosaccharide composition was compared with that of the first precipitated hemicellulose A. It was found that although neither contained arabinose, unlike the xylan from the purified hemicellulose B fraction, the first precipitate contained 6·6 per cent of uronic acid whereas the second only contained 1·2 per cent.

In the present investigation the monosaccharide composition of some linear polymers and the branched polymer from red clover, lucerne and soya bean stalks (Leguminosae) was compared with that of the corresponding fractions from grass, wheat and maize stalks (Gramineae).

RESULTS AND DISCUSSION

Table 1 shows the composition of the purified hemicellulose A, and both the linear and the branched polymer from the purified hemicellulose B for the three Leguminosae and the three Gramineae. The linear polymer from the hemicellulose B could not be isolated in sufficient amounts free from glucose, but as the glucose in these fractions probably is present as a separate neutral glucan the composition of the xylan is calculated on a glucose free basis. The percentages of glucose in the hydrolysates are given between brackets.

As was found previously [1] the hemicelluloses A from the species of Leguminosae, unlike those of the Gramineae, contained no arabinose at all. The uronic acid content of the polymers, on the other hand, is higher in the hemicelluloses A from the Leguminosae.

The heteroxylans from the hemicellulose B fractions show similar differences. For both groups of plants xylose is the main constituent. In this fraction, however, the amounts of arabinose attached to the main xylan chain are higher than in the corresponding hemicellulose A fractions and the amounts of uronic acid are lower. Again the linear xylans from the hemicellulose B fractions from the Leguminosae contain less arabinose and more uronic acid than do those from the Gramineae.

The branched polymers, apart from being highly branched, appear to be quite different from any of the linear ones. In addition to xylose, arabinose and uronic acid they contain appreciable amounts of galactose. In these branched polymers the differences between those from species of the Leguminosae and the Gramineae are very distinct. Whereas in the Gramineae xylose is still the major component, it is only a minor constituent in the Leguminosae. In its place we find larger amounts of uronic acid, arabinose and galactose. Although

TABLE 1. COMPOSITION OF THE LINEAR A AND B AND THE BRANCHED B POLYMERS FROM SOME GRAMINEAE AND LEGUMINOSAE

	T. pratense (red clover) (%)	*M. sativa* (lucerne) (%)	*G. max* (soya bean) (%)	*L. perenne* (grass) (%)	*T. vulgare* (wheat) (%)	*Z. mais* (maize) (%)
Linear A						
uronic acid	4·7	6·6	6·6	1·9	2·1	2·4
galactose	—	—	—	—	—	—
arabinose	—	—	—	12·9	5·7	5·3
xylose	95·3	93·4	93·4	85·2	92·2	92·3
glucose	—	—	—	—	—	—
Linear B						
uronic acid	1·0	1·5	4·3	0·4	0·3	0·1
galactose	—	—	—	—	—	—
arabinose	10·8	8·3	8·3	16·5	11·1	10·9
xylose	88·2	90·2	89·2	83·1	88·6	89·0
glucose	(11·6)	(11·4)	(4·0)	(11·0)	(12·0)	(21·5)
Branched B						
uronic acid	20·6	22·3	24·6	5·4	7·9	12·8
galactose	34·5	31·1	34·3	7·7	9·8	8·7
arabinose	27·6	34·2	24·0	23·8	26·5	24·4
xylose	17·3	3·1	3·4	63·1	55·8	54·1
glucose	—	9·3	6·9	—	—	—
rhamnose	—	—	6·8	—	—	—

the complete structure of the branched polymers has not been investigated, a difference was observed in the way the uronic acid is linked to the molecules from the two families. In the hydrolysates of the branched polymers from the Gramineae the usual aldobiouronic acid was shown on the chromatograms, whereas with the Leguminosae none could be found. This indicates that in the case of the Leguminosae the uronic acid is probably bound to an arabinofuranose. In digests by the enzymes of rumen protozoa on the branched polymers of the Leguminosae an aldobiouronic acid was demonstrated by paper chromatography. After elution and hydrolysis this aldobiouronic acid indeed gave a distinct spot of arabinose on the chromatograms.

Whether the differences in hemicellulose composition between the Gramineae and the Leguminosae are a reflection of differences between monocotyledons and dicotyledons has yet to be investigated.

EXPERIMENTAL

Isolation of the hemicelluloses. The hemicellulose A fractions were prepared and purified as described by Whistler and Gaillard[1] and the hemicellulose B mixtures as described by Whistler and Lauterbach.[3]

Separation of branched and linear polymers from the hemicellulose B fraction. One gram of the hemicellulose B mixture was dissolved in 100 ml of calcium chloride solution (s.g. 1·3) and clarified by a short centrifugation at 20,000 *g*. To this solution was then added 15 ml of an aqueous solution of I_2 (3%) and KI (4%). The dark-blue precipitate was left to settle for

[3] R. L. WHISTLER and G. E. LAUTERBACH, *Arch. Biochem. Biophys.* 77, 62 (1958).

two hours and collected by centrifugation at 20,000 *g* for 2 hr. The clear brown supernatant was neutralized with sodium thiosulphate and poured, with stirring, into five volumes of ethanol to precipitate the branched polymer. To remove the calcium this precipitate was dissolved in 5 ml of 0·1 N hydrochloric acid and reprecipitated with 25 ml of ethanol. The polysaccharide was filtered off, washed with ethanol and ether and finally dried over calcium chloride in a vacuum desiccator.

The dark blue precipitate containing the linear polymer was washed with the calcium chloride solution containing (15%) iodine–potassium iodide solution. The washed precipitate was dissolved in 100 ml of hot water, the iodine was neutralized with sodium thiosulphate and the polymer reprecipitated by pouring the solution into five volumes of ethanol. To remove calcium the precipitate was dissolved under nitrogen in as little N KOH as possible, neutralized with N HCl and again precipitated in five volumes of ethanol. The precipitate was collected and dried as described above.

Sephadex column. In trying to separate the linear polymers from the hemicellulose B fractions, columns of different grades of Sephadex were used; Sephadex 200 gave the best results. The columns were prepared by soaking the Sephadex in water and the polysaccharides were also eluted with water.

Enzyme digest. An aqueous extract of the enzymes of *Epidinium ecaudatum* was prepared as described by Bailey *et al.*[4] The extract was dialysed against distilled water and finally freeze dried. The digest contained 5 mg of extract and 10 mg of polysaccharide in 1 ml of water. The digest was kept at 39 for 24 hr.

Analytical methods. Ultracentrifugation was performed at 1–1·5% concentration in 0·1 M citrate buffer pH 3·5 or borate buffer pH 9·6 at 60,000 rev min in a Spinco model E centrifuge. Micro electrophoresis was carried out in a Kern LK 30 apparatus at 2·5% concentration in a 0·1 M borate buffer solution at pH 9·6.

To determine the constituent sugars the polysaccharides of the hemicellulose B group were hydrolysed in N H_2SO_4 at 100° for 3 hr. The hemicelluloses A were hydrolysed with the 72% sulphuric acid procedure described by Whistler and Gaillard.[1] The sugars were separated by paper chromatography using Sleicher and Schull paper No. 2040*a*. The solvent was butanol: ethanol: water (7:2:2) and the indicator aniline hydrogen phosphate. The eluted sugars were determined by the method of Hagedorn and Jenssen.

Uronic acids were estimated by CO_2 evolution as described by Gaillard[5] or by titration (Whistler and Feather[6]).

Acknowledgement—The author wishes to thank Mrs. D. H. I. Pierie-Estourgie for technical assistance in this work.

[4] R. W. BAILY, R. T. J. CLARKE and D. E. WRIGHT, *Biochem. J.* **83**, 517 (1962).
[5] B D. E. GAILLARD, *J. Sci. Food. Agr.* **9**, 170 (1958).
[6] R. L. WHISTLER and M. S. FEATHER, *Meth. Carbohydrate Chem.* **1**, 468 (1962).