Phytochemistry, 1970, Vol. 9, pp. 871 to 874. Pergamon Press. Printed in England

CELL-WALL POLYSACCHARIDES OF SAGEBRUSH*

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(*Received* 12 *September* 1969)

Abstract—The woody tissues of *Artemisia tridentata vaseyana* were found to contain 34·5 per cent cellulose, 29·2 per cent *O*-acetyl-4-*O*-methylglucuronoxylan, 1·3 per cent glucomannan, 29·2 per cent Klason lignin and 7·6 per cent extractives.

INTRODUCTION

A PROGRAM has been developed in this laboratory for chemical analysis of the closely related varieties of sagebrush which grow abundantly in about 422,000 square miles of eleven western states.[1] The chemical investigation of these plants in the past has been mostly concerned with the nutritional characteristics[2–6] and the sesquiterpene lactone constituents.[7,8]

The first paper in this series deals with cell-wall polysaccharides of the woody tissues of *Artemesia tridentata vaseyana*, which is one of the common subspecies in Montana and the neighboring states.

RESULTS AND DISCUSSION

The woody stems of the sagebrush were separated from the bark leaving the interxylary cork layers that could not be readily removed. This material was analyzed for the general components shown in Table 1.

TABLE 1. COMPOSITION OF THE WOODY TISSUES OF *A. tridentata vaseyana*

Component	(%) Dry wood	(%) Extracted wood
Benzene–alcohol (2:1) soluble	5·0	
Alcohol soluble	1·4	
Water soluble	1·2	
Total extractives		7·6
Klason lignin		29·2
Cell-wall polysaccharides		66·4

* Part I in a projected series on the "Chemical Composition of Sagebrush".

† Established through a grant from the Hoerner Waldorf Corporation of Montana.

[1] A. A. BEETLE, *A Study of Sagebrush*, University of Wyoming Agricultural Experimental Bulletin 368 (1960).
[2] W. E. WATKINS and W. W. REPP, *Influence of Location and Season on Composition of New Mexico Range Grasses*, New Mexico Agricultural Experiment Station Bulletin 486 (1964).
[3] J. W. HAMILTON, *Chemical Composition of Certain Forage Plants*, Wyoming Agricultural Experiment Station Bulletin 356 (1958).
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[6] C. R. KINNEY, T. W. JACKSON, L. E. DEMYTT and A. W. HARRIS, *J. Org. Chem.* 6, 612 (1941).
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[8] C. STEELINK and J. C. SPITZER, *Phytochem.* 5, 357 (1966).

The cell-wall polysaccharides (holocellulose component) was hydrolyzed[9] to the sugars listed in Table 2 which indicated the presence of cellulose, "xylan" and a trace amount of "mannan". These polysaccharides were isolated by the alkaline extraction and fractionation scheme[10] shown in Fig. 1. This gave 51·24 per cent cellulose, 23·62 per cent 4-*O*-methylglucuronoxylan and 0·75 per cent glucomannan, based on the holocellulose.

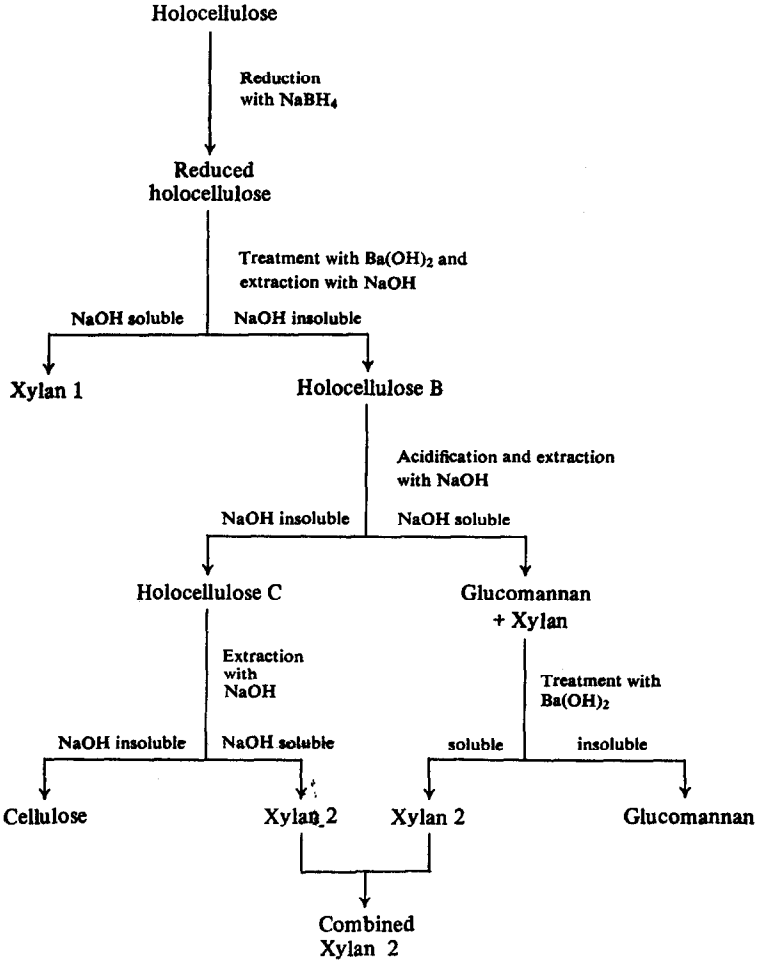

FIG. 1. ALKALINE FRACTIONATION OF THE HOLOCELLULOSE.

The cellulose content was also determined by direct extraction of the holocellulose fraction with sodium hydroxide.[11] This gave 53·5 per cent cellulose which is in agreement with the data obtained from systematic fractionation.

The holocellulose fraction was also extracted with methyl sulfoxide to give a small yield of *O*-acetyl-4-*O*-methylglucuronoxylan. This was used for the determination of acetyl

[9] M. L. LAVER, D. F. ROOT, F. SHAFIZADEH and J. C. LOWE, *Tappi* **50**, 618 (1967).
[10] A. BEELIK, R. J. CONCA, J. K. HAMILTON and E. V. PARTLOW, *Tappi* **50**, 78 (1967).
[11] ASTM, *Standards on Wood, Wood Preservatives and Related Materials*, alpha-cellulose in Wood, D 1103-50T, Philadelphia (1954).

groups, which are hydrolyzed under the alkaline condition of the systematic fractionation. Analysis of this product showed 12 per cent acetyl content.

Hydrolysis of the "xylan" and analysis of the hydrolyzate showed the presence of D-xylose and only a trace of D-glucose impurities. However, as usual, no 4-*O*-methyl-D-glucuronic acid was detected. The presence of this component was ascertained through decarboxylation and methoxyl determination of 4-*O*-methylglucuronoxylan. This gave a value of 17·8 per cent for the uronic acid residue in the deacetylated "xylan" which corresponds with 15·7 per cent in the acetylated product. On the basis of these data the *O*-acetyl-4-*O*-methylglucuronoxylan component contains an average of one uronic acid for each seven D-xylose units and about half of the D-xylose units contain an acetyl group.

TABLE 2. ANALYSIS OF THE CELL-WALL POLYSACCHARIDES

Sugar	(%) Holocellulose
Anhydro-D-glucose units*	52·5
Anhydro-D-xylose units*	32·8
Anhydro-D-mannose units*	0·8
4-*O*-Methyl-D-glucuronic acid†	5·9
Acetyl group†	5·3

* Determined by analysis of the hydrolysis products.
† Based on analysis of the functional groups.

The glucomannan on hydrolysis provided D-glucose and D-mannose in the ratio of 2:3. Assuming that on hydrolysis of the cell-wall polysaccharides all the D-xylose is derived from acetyl-4-*O*-methylglucuronoxylan and the D-mannose is derived from the glucomannan fractions, the percentage of each polysaccharide may be calculated as shown in Table 3.

TABLE 3. CELL-WALL POLYSACCHARIDES

Polysaccharide	Holocellulose (%)	Wood (%)
Cellulose	52	34·5
Acetyl-4-*O*-methylglucuronoxylan	44	29·2
Glucomannan	1·3	0·9

These data indicate that chemical composition of the woody tissues of sagebrush is similar to the general composition of the woods from arborescent angiosperms[12] except for a somewhat higher lignin and lower polysaccharides content which may be accounted for by the presence of the interxylary cork layers. Also the 4-*O*-methylglucuronoxylan is more on the acidic side although the ratio of the D-xylose to uronic acid residues of 7:1 falls within the range of 5 to 15:1 that has been reported for the hardwood "xylans".[12]

[12] T. E. TIMELL, *Advan. in Carbohydrate Chem.* **19**, 297 (1964).

EXPERIMENTAL

Plant Materials

Specimens of *Artemisia tridentata* Subsp. *vaseyana* were collected at Sec. 10, T.13.N., R.20.W., Montana meridian. Sound stems greater than 1 cm in diameter were used. The outerbark was thoroughly removed but the interxylary cork layer[13] was left in. The woody tissue was ground in a Wiley Mill to pass a 40-mesh screen.

Analysis of the Ground Wood

The ground wood was analyzed for the moisture content, extractives and Klason lignin[14] by standard methods. The extracted wood meal (105 g dry wt.) was delignified with acidic sodium chlorite[15, 16] to provide 78·6 g holocellulose with 3·1% residual lignin.

Analysis of the Holocellulose

The cell-wall polysaccharides were hydrolyzed and the hydrolyzate was analyzed by GLC following the method described by Laver and co-workers.[9] The value for each sugar was corrected for the loss due to decomposition and addition of a water molecule during hydrolysis. The cellulose content (53.5%) was determined by extraction with 18% NaOH according to the ASTM method.[11]

Isolation of the Polysaccharides

The terminal aldehyde groups of holocellulose (100 g) was reduced with sodium borohydride to prevent degradation under alkaline conditions. The reduced material was extracted with a modification of the $Ba(OH)_2$–NaOH procedure[10] in order to separate small amounts of glucomannan present (see Fig. 1). This provided 51·24 g cellulose which, on hydrolysis, gave 99·1% D-glucose, a trace amount of D-xylose as determined by GLC and no lignin. There were two "xylan" fractions (13·28 g and 10·34 g) which were combined for further study and a trace amount of glucomannan (0·75%).

Investigation of the "Xylan"

The "xylan" fraction (1·5 g) was purified twice by copper complexing[17,18] to give 1·28 g of a pure material which, when hydrolyzed, gave only D-xylose, a trace amount of D-glucose (0·6%), and no lignin. It had an ash content of 1·67% and $[\alpha]_D^{25} - 81·8°$ (c. 5% NaOH) compared with $[\alpha]_D^{25} - 83·8°$ for elm wood "xylan".[19] The i.r. spectrum was identical with that of a sample of hardwood "xylan". The percentage of 4-*O*-methyl-D-glucuronic acid residue which is decomposed on hydrolysis, was determined by decarboxylation[20] and methoxyl determination. This gave 4·14% CO_2 and 2·92% methoxyl content.

Direct extraction of the holocellulose (100 g) with methyl sulfoxide[12] gave a small amount (3·78 g) of *O*-acetyl-4-*O*-methylglucuronoxylan $[\alpha]_D^{25} - 82·0$ (c. 2% NaOH), 12% acetyl content.

Acknowledgements—The authors express their gratitude to Professor M. S. Morris for his interest in this work and to the Conservation and Forestry Experiment Station, School of Forestry, University of Montana, for financial support.

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