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CARBOHYDRATE CONSTITUENTS OF HEALTHY AND WOUND TISSUE IN THE SAGUARO CACTUS

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Abstract—In response to wounding or infection, the saguaro cactus forms a hard, ligniferous callus tissue. The major polysaccharides in the callus are cellulose and a xylan. In the healthy cortical tissue (pulp) are found carbohydrates derived mainly from glucose, galactose, xylose and arabinose; 70 per cent of the pulp polysaccharides are soluble in water. Galactose, which accounts for 31 per cent of all saccharide constituents in the pulp, is not present in the wound tissue.

INTRODUCTION

IN A PREVIOUS study,[1] we reported that the sequence of events which followed injury of the cortical tissue of the saguaro (*Carnegiea gigantea*, Br. and Rose) was as follows: (*a*) rapid increase in dopamine concentration at the site of injury; (*b*) enzymatic oxidation of dopamine to melanin; (*c*) rapid cell differentiation; (*d*) lignin and cellulose formation, concomitant with the formation of a variety of phenols and extractives. These results indicated that a major stimulation of the shikimic acid pathway (sugars → phenols) had probably occurred. If assumptions were correct, a major shift in normal carbohydrate metabolism also would have taken place. As a first step in tracing the abnormal carbohydrate metabolism, we decided to characterize the hydrolytic products of the polysaccharides in the healthy and wound tissue.

RESULTS

The analysis of the carbohydrate constituents of healthy and wound tissue was based on three fractions: the alcohol-soluble, the water-soluble and the water-insoluble fractions. In general, these fractions correspond to simple monomers and oligomers, low-molecular weight polysaccharides and high-molecular weight polysaccharides. Quantitative analysis of the monosaccharide moieties in each fraction was carried out by vapor phase chromatography, (VPC) based on the method of Sloneker.[2] Tissue extracts were hydrolyzed and inorganic ions removed by ion-exchange resins. The free sugars were reduced to alditols and converted to the alditol acetates. The latter were injected into the VPC analyzer. Areas under the peaks in the chromatogram were determined and assumed to be proportional to the concentration of monosaccharide. This assumption was validated by submitting standard solutions to analysis.

The results of the fractionation experiments are shown in Table 1.

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† Taken in part from the Ph.D. Thesis of M. J. Onore, University of Arizona, 1967.

[1] C. STEELINK, M. YEUNG and R. L. CALDWELL, *Phytochem.* **6**, 1435 (1967).

[2] J. H. SLONEKER, *Anal. Chem.* **37**, 1602 (1965).

TABLE 1. PERCENT OF CARBOHYDRATE IN TISSUE EXTRACTS*

Tissue	Alcohol-soluble fraction, %	Water-soluble fraction, %	Water-insoluble fraction, %
Healthy (cortical)	18	20	16
Wound (callus)	5·6†	0·5	64·7

* Based on dry tissue.
† The major portion of this fraction was probably non-carbohydrate extractive.

Since the cortical tissue contains normally 90 per cent water, the figures in Table 1 may be somewhat misleading on a dry weight basis. On a wet basis, the percentage of each sugar fraction in the living plant would be one-tenth of those indicated. In contrast, the callus tissue is practically devoid of water or water-soluble components; a property which admirably equips it to prevent water loss from the wounded area. The non-polysaccharide portion of the callus tissue is mainly lignin.[1]

In Tables 2 and 3 are summarized the results of the VPC analyses of the various fractions. These are based on the percentage of the total carbohydrates in the hydrolysate of each fraction.

TABLE 2. PERCENT OF MONOSACCHARIDES IN POLYSACCHARIDE FRACTION OF PULP

Monosaccharide	Alcohol soluble, %	Water soluble, %	Water insoluble, %
Xylose	5·4	—	13·3
Mannose	25·7	—	—
Galactose	1·3	55·0	37·0
Unknown	3·0	—	—
Rhamnose	—	5·4	4·8
Arabinose	—	20·3	10·3
Glucose	63·6	19·2	34·8

TABLE 3. PERCENT OF MONOSACCHARIDES IN POLYSACCHARIDE FRACTION OF CALLUS

Monosaccharide	Alcohol soluble, %	Water soluble, %	Water insoluble, %
Unknown*	34·0	—	—
Glucose	66·5	16·3	74·4
Rhamnose	—	4·2	—
Arabinose	—	11·5	—
Xylose	—	14·4	26·6
Mannose	—	18·8	—
Galactose	—	—	—

* Probably a desoxy hexose.

Subsequent to the VPC analysis, hydrolysates of the tissue fractions were subjected to paper chromatographic analysis. The primary purpose of these experiments was to detect sugars not susceptible to the VPC technique, such as uronic acids. The results of the paper chromatography confirmed the presence of the major saccharides found in the VPC study. However, significant amounts of sucrose were detected in the water-soluble and alcohol-soluble fractions of the cortical (healthy) tissue indicating incomplete hydrolysis. Sucrose would not appear as such in the VPC study, but rather show up as glucose and mannose (by hydrolysis and subsequent reduction of fructose to mannose and glucose). Small amounts of hexose-phosphate, desoxy sugars, uronic acids and di- and tri-saccharides (probably from incomplete acid hydrolysis of polysaccharides) were also detected.

Paper chromatographic analysis of the callus (wound) tissue fractions confirmed the presence of sugars reported in Table 3. Again, sucrose appeared in the alcohol-soluble portion. In the water-soluble portion, which constitutes a minute percentage of the total sugar content, galacturonic acid was a major constituent. In the water-insoluble fraction, which accounts for more than 90 per cent of the callus polysaccharides, the principal building blocks were glucose and xylose, as well as two uronic acids: galacturonic and 4-O-methyl-glucuronic.

To further elucidate the nature of the water insoluble callus polysaccharide, we treated the tissue to a procedure designed by Jermyn[3] to analyze holocellulose. The tissue was extracted with light petroleum to remove lipids, then alcohol/benzene to remove extractives, then sodium chlorite to remove lignin. The residue from these treatments, assumed to be holocellulose, was treated with NaOH to remove hemicellulose. As a result of these operations, it was shown that the holocellulose contained 66 per cent cellulose and 34 per cent hemicellulose. The latter, after hydrolysis and paper chromatography, was shown to contain xylose as the major constituent and lesser amounts of galacturonic and 4-O-methylglucuronic acids.

In addition to the above polysaccharides (and the lignin and polyphenols reported previously[1]), the callus tissue contained small quantities of fatty acid esters and minor amounts of C_{27} and C_{28} sterols. A lipid and neutral fraction were also observed in the pulp.

DISCUSSION

The results show that a major change in carbohydrate metabolism occurs when the healthy tissue is injured. From a mixture of relatively water-soluble polysaccharides (70 per cent), there is formed a polysaccharide matrix in the wound tissue that is more than 90 per cent water-insoluble. This matrix is a holocellulose, whose principal constituents are cellulose and a xylan containing some galacturonic and 4-O-methylglucuronic acids. In combination with the lignin and the lipid fraction, the holocellulose constitutes an admirable barrier to water loss, as well as to invasion by bacteria.

The work of Simionescu[4,5] on plant tumor formation closely parallels our results. His studies revealed that tomato plant tumor tissue has more lignin and less cellulose than healthy tissue. Radio carbon tracer studies showed that lignin is formed at the expense of glucose of the healthy tissue. In addition, there appeared to be a loss of pentosans during

[3] M. A. JERMYN, in *Modern Methods of Plant Analysis* (edited by K. PAECH and M. V. TRACEY), Vol. II, p. 201. Springer-Verlag, Berlin (1955).

[4] (*a*) C. SIMIONESCU, *Acad. Rep. Populare Romine, Feliala Iasi, Studii Cercetari Stiint. Chem.* **13**, 192 (1962); (*b*) C. SIMIONESCU, *Rev. Chim. (Bucharest)* **6**, 235 (1961).

[5] C. SIMIONESCU, M. GRIGORAS, A. CERNATESCU and S. GRIGORAS, *Bull. Inst. Politch Iasi* **2**, 115 (1956).

tumor formation, possibly indicating accelerated pentose–phosphate shunt activity as a result of increased respiration.

Judging from the composition of the Saguaro callus tissue, one can conclude that the polysaccharides are formed at the expense of the water-soluble glucans and xylans in the healthy cortical tissue. The fate of large amounts of galactose and arabinose remains to be assessed. It is quite possible that galactose may be epimerized to glucose[6] and subsequently utilized as a precursor to cellulose and lignin via the shikimic acid pathway. Or, it may be oxidized to galacturonic acid and incorporated into the callus xylan. A more detailed examination of these transformations would require the use of radio-carbon tracers. Such a study is now in progress in this Laboratory.

EXPERIMENTAL

Extraction of Plant Material

Mature saguaro cactus, which contained large amounts of callus tissue, were collected in the desert region near Tucson, Arizona. The pulp (healthy cortical tissue) was separated from the callus tissue. Each was ground in a Waring blendor for 2 min with enough hot ethanol to yield a mixture approximating 60–80 per cent ethanol in water. The filtrates from these mixtures contained the "ethanol-soluble" carbohydrate fraction. The residues from this treatment were then subjected to water extraction after being washed several times with 80 per cent ethanol. Dried tissue was suspended in water (50 ml per g of plant marc). The water suspension was refluxed for 4 hr with constant stirring. After being cooled, the suspension was filtered and the filtrate concentrated under vacuum. Polysaccharides were precipitated by addition of 3 volumes of 95 per cent ethanol and air-dried. The precipitate constituted the "water-soluble" fraction.

The residue from the alcohol and water treatments contained the "water-insoluble" polysaccharides. Both the ethanol-soluble and water-soluble fractions were extracted with light petroleum and ethyl ether to remove lipids.

Hydrolysis, Reduction and Acetylation

To 1·0 ml of dried polysaccharide was added 10 ml of 72 per cent H_2SO_4. The mixture was allowed to stand until it was homogeneous. Water was then added to dilute the acid to 1 per cent; the solution was refluxed 6 hr; Excess acid was neutralized with $BaCO_3$. The mixture was filtered and the filtrate shaken with Amberlite IR-120 (H) resin. After removal of the resin, the solution was neutralized with $NaHCO_3$.

Excess $NaBH_4$ was added to the neutral sugar solution with stirring over 10 min. After a further 10 min, acid Amberlite resin was slowly added until no more hydrogen was evolved. An additional amount of resin was added, and the solution stirred for 15 min. After removal of the resin, the solution was reduced to a thick syrup. Methanol was added and the solution flash-evaporated several times to remove boric acid.

The resulting polyhydric alcohols were mixed with a 1:1 v/v mixture of acetic anhydride-pyridine solution (1·0 ml of solution to 100 mg of alcohol) and refluxed for 4 hr. The solution was evaporated to dryness and the residue taken up in tetrahydrofuran. This solution after evaporation to a small volume, was ready for VPC analysis.

VPC Analysis

All analyses were carried out on an F and M Model 609 Flame Ionization Chromatograph equipped with disc integrator. Column temperature was 190°. The column, $\frac{1}{4}$ in. × 10 ft, was packed with 3 per cent ECNSS-M, an organo-silicone polyester especially prepared for this analysis[2] by Applied Science Laboratories, State College, Pennsylvania. Alditol acetates were identified by retention times, previously established with authentic monosaccharide samples. Areas under the peaks were assumed to be proportional to the concentration of monosaccharide; the results of analyses of standard solutions of sugars supported this assumption. The analytical procedure was also tested by subjecting a sample of commercial arabinogalactan (STRACTAN AF, No. 2 Stein, Hall and Company, Inc., 605 Third Ave., N.Y.) to degradation and analysis. The ratio of arabinose to galactose, as determined by the procedure, was 1/6·5, in close agreement with the manufacturer's analysis (1/6). However, considerable loss of total polysaccharide was noted as a result of the extensive sequence of steps in the analysis.

Delignification and Alkaline Extraction of the Callus Tissue

In order to determine the nature of the holocellulose in callus, the following procedure was followed:[3] The ground callus tissue (39 g) was extracted with light petroleum (60–100°) for 3 days, followed by a 3-day

[6] P. KARLSON, *Introduction to Modern Biochemistry*, 2nd edition, p. 301. Academic Press, N.Y. (1965).

extraction with benzene/ethanol (2/1). The residue (38 g) was suspended in 800 ml of water, to which 3 ml of glacial acetic acid and 7·5 g of $NaClO_2$ had been added. The suspension was heated on a steam bath for 1 hr; three fresh portions of acetic acid and $NaClO_2$ were added at intervals of 1 hr. After the delignified callus had been washed with 2 l. of cold water, it was air-dried. Its weight (27·5 g) indicated that 70 per cent of the callus was holocellulose.

To 1·0 l. of 12 per cent NaOH was added 18 g of holocellulose and the mixture stirred under N_2 for 48 hr. After filtration, the residue was re-treated. The combined filtrates were neutralized with HCl and treated with 95 per cent ethanol to precipitate the xylan, which constituted 5·3 g (34 per cent) of the holocellulose. The xylan was subsequently acid-hydrolyzed; paper chromatography of the hydrolysate revealed xylose as the major constituent, and galacturonic and *O*-methylglucuronic acids as minor constituents.

Paper Chromatography

Acid hydrolyzed samples of all tissue fractions were subjected to extensive paper chromatography in a variety of solvent systems. Spray reagents used to detect various classes of sugars were: uronic acids (aniline phthalate);[7] desoxy sugars and glycals (periodate);[8] ketoses (resorcinol-butanol);[9] sugar phosphates (ammonium molybdate);[10] uronic acids, sucrose and ketoses (naphthoresorcinol).[11]

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[7] J. K. BARTLETT, L. HOUGH and J. K. N. JONES, *Chem. & Ind.* 76 (1951).
[8] J. T. EDWARD and D. M. WALDRON, *J. Chem. Soc.* 3631 (1952).
[9] L. HOUGH and J. K. N. JONES, *J. Chem. Soc.* 4052 (1952), and previous papers.
[10] R. S. BANDURSKI and B. AXELROD, *J. Biol. Chem.* **193**, 405 (1951).
[11] R. M. HANN, E. B. TILDEN and C. S. HUDSON, *J. Am. Chem. Soc.* **60**, 1201 (1938).