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CARBOHYDRATE COMPOSITION OF THE POLYSACCHARIDES OF THE TEA PLANT

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The carbohydrate composition of the various groups of polysaccharides isolated from the ethanol-insoluble material of the bark and wood of the stems and roots of the tea plant was studied. The hot water-soluble polysaccharide fraction of bark contained a higher proportion of pectic substances compared with that of the wood. Starch was the main component of the hot water-soluble fraction of root-wood. The composition of hemicelluloses and ∝-cellulose from the different tissues were qualitatively similar, although there were quantitative differences.

A technique has been developed in this laboratory for chemical analysis of the polymers of the tea plant. Most of the chemical investigations of this plant in the past have been concerned with composition of the flush.

This paper deals with detailed chemical composition of the polysaccharides of the various tissues of the plant and is an essential corollary to the earlier study on changes in the polysaccharides of the tea plant during post-prune growth (Selvendran & Selvendran 1972). Such information is useful for an understanding of the physiology and biochemistry of the plant during growth, recovery from pruning, fertilizer uptake, as well as during attack by insects and microorganisms.

MATERIALS AND METHODS

Plant Material

All material (bark and wood of stems and roots, 1-1.5 cm thick used in this investigation was collected from two-year-old plants (Clone KEN 16/3) grown in a field near the laboratory (1500 m elevation). Processing of the material was begun within 1 hr of harvesting.

Preparation and analysis of the ethanol-insoluble material (EIM)

The tissues were extracted with 80% (v/v) boiling aq. ethanol and the EIM was fractionated into the component polysaccharides by the method described before (Jermyn & Isherwood 1956; Selvendran & Perera 1971a).

Hydrolysis of polysaccharide fractions

The polysaccharides were hydrolysed by refluxing with 2N H_2SO_4. Direct addition of 2N H_2SO_4 to the polysaccharide fractions resulted in considerable precipitation, which could only be dissolved after protracted heating. This difficulty was overcome by first dissolving the polysaccharide (100 mg) in 72% sulphuric acid

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(0.7 ml) and allowing the solution to stand at room temperature for 1 hr. The solutions were then made to 10 ml and boiled under reflux. Hot-water-soluble polysaccharides were hydrolysed for 5 hr and the others for 16 hr. The hydrolysates were neutralized with solid $BaCO_3$ (care was taken to keep the pH of the solution below 6), filtered and the precipitates washed with distilled water. The filtrates and washings (approximately 50 ml) were concentrated to a small volume, passed through a small cation exchange colum (2 cm x 1 cm diameter) of Dowex AG50 (8 - 10% cross-linked; H^+ form; 200-400 mesh) and the effluents concentrated and taken up in 1 ml distilled water.

Paper chromatographic analysis of hydrolysates

The sugars present in the hydrolysates were separated by descending paper chromatography using ethyl acetate-pyridine-water (2:1:2, by vol. top layer) and ethyl acetate-acetic acid-water (3:1:3 by vol.) as developing solvents (Jermyn & Isherwood 1949). The position of sugar spots was revealed by spraying with aniline hydrogen phthalate reagent of Wilson (1959) and their concentrations were determined by the Park and Johnson method (1949), after extracting them from appropriate areas of similar, but unsprayed paper. As the aldopentoses, aldohexoses and uronic acids have different reducing values, separate standard graphs were prepared for each group of sugars using xylose, glucose and galacturonic acid as standards.

RESULTS AND DISCUSSION

The chemical composition of the polysaccharides isolated from the EIM of the various tissues are given in Tables 1-4. Root wood and stem wood contained a very small quantity of ammonium oxalate soluble pectic acid which was, therefore, not analysed. The hot water-soluble fraction of the bark of roots and stems contained a higher percentage of pectic substances compared with the wood. The presence of glucose in the hydrolysates of the hot water-soluble fraction suggests the presence of starch in them. Root-wood contains the highest starch content relative to that of the other organs. Mannans (and fructosans) which are sometimes regarded as reserve material in plants were not present in any of the tissues. The composition of the $(NH_4)_2\ C_2O_4$-soluble pectic acid from stem and root bark is similar to the corresponding fraction from tea flush (Selvendran & Perera 1971b). The composition of the hemicelluloses and α-cellulose from the various tissues compare well with the corresponding fractions isolated from other plant tissues (Thornber & Northcote 1061). It is interesting to note that the hydrolysates of the hemicellulose and α-cellulose fractions of wood contain a higher percentage of xylose and glucose respectively compared with the bark. It is probable that the uronic acid detected in the hemicellulose and α-cellulose preparations from the various tissues is methyl glucuronic acid (Thornber & Northcote 1961). The disaccharides detected in the hydrolysates of the α-cellulose and some of the hot water-soluble polysaccharide fractions are probably incompletely hydrolysed cellobiose or 'similar units'.

Detailed studies on the changes in the polysaccharides of the tea plant during post-prune growth indicated that the hemicelluloses from the various tissues do not appear to function as reserve food. They seem to function only as structural material (Selvendran & Selvendran 1972). This may be due to their structural constitution.

TABLE 1—*Percentage composition of the polysaccharide fractions extracted from the EIM of root wood*

Polysaccharides present	Polysaccharide fractions			
	Hot water soluble	Hemicellulose A	Hemicellulose A	∝Cellulose
Glucosan	95.0	8.8	23.4	92.8
Galactan	0.8	4.3	0.3	—
Xylan	0.6	67.5	54.1	0.8
Araban	1.1	2.9	21.8	2.9
Polygalacturonic acid	1.1	—	—	—
Uronic acid	—	14.8	0.3	0.1
Rhamnan	0.1	1.5	0.1	—
'Disaccharide'	1.3	—	—	3.4

TABLE 2—*Percentage composition of the polysaccharide fractions extracted from the EIM of root-bark*

Polysaccharides present	Polysaccharide fractions				
	Hot water soluble	Ammonium oxalate-soluble	Hemicellulose A	Hemicellulose B	∝ Cellulose
Glucosan	31.4	—	18.4	48.2	74.4
Galactan	18.1	19.8	12.6	16.1	3.9
Xylan	0.2	—	19.6	19.3	1.6
Araban	25.3	14.2	11.4	6.1	3.5
Polyagalacturonic acid	24.9	63.9	—	—	—
Uronic acid	—	—	37.9	8.9	11.7
Rhamnan	0.1	2.1	0.05	1.4	0.7
'Disaccharide'	—	—	—	—	4.2

TABLE 3—*Percentage composition of the polysaccharide fractions extracted from the EIM of stem wood*

Polysaccharides present	Polysaccharide fractions			
	Hot water-soluble	Hemicellulose A	Hemicellulose B	∝Cellulose
Glucosan	51.8	2.9	31.5	88.7
Galactan	7.7	8.2	8.5	1.9
Xylan	4.3	57.0	34.9	1.4
Araban	12.1	2.0	8.2	4.8
Polygalacturonic acid	8.9	—	—	—
Uronic acid	—	28.5	16.1	—
Rhamnan	0.03	1.4	.08	—
'Disaccharide'	14.9	—	—	3.2

TABLE 4—*Percentage composition of the polysaccharide fractions extracted from the EIM of stem bark*

Polysaccharides present	Polysaccharide fractions				
	Hot water-soluble	Ammonium oxalate-soluble	Hemicellulose A	Hemicellulose B	∝Cellulose
Glucosan	27.9	—	9.9	51.3	65.7
Galactan	17.3	43.2	10.2	1.1	3.5
Xylan	4.0	—	39.7	28.0	0.5
Araban	16.8	23.2	11.0	17.4	0.2
Polygalacturonic acid	33.0	33.1	—	—	—
Uronic acid	—	—	29.0	1.0	27.9
Rhamnan	1.0	0.5	0.2	1.2	—
'Disaccharide'	—	—	—	—	2.2

A knowledge of the composition of the polysaccharides from the various tissues would help us to elucidate the food relations of insects which feed on the tea plant. This subject is becoming increasingly important in view of the damage caused to tea plants by wood borers such as termites and Shot-hole Borer (Cranham 1966). Little is known as to what substances they feed on. It is known, however, that some wood-borers (termites) can break down cell wall components and use the resulting products as a source of nourishment (Wigglesworth 1965). Some insects (Shot-hole Borer?) are unable to hydrolyse cell-wall material and must rely for food upon cell contents or the breakdown products of wood caused by other agencies such as fungi (eg *Monacrosporium ambrosium*). It would be of interest to devise artificial feeding experiments to determine whether the wood-borers which plague the tea plant could discern differences in the various groups of polysaccharides. Such studies may throw light on the feeding habits of these insects.

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