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Structure of the Primary Cell Walls of Suspension-Cultured *Rosa glauca* Cells

II. MULTIPLE FORMS OF XYLOGLUCANS

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ABSTRACT

Xyloglucans, characteristic hemicellulosic polysaccharides of plant primary walls, have been isolated from *Rosa glauca* suspension-cultured cells. The cell wall material was fractionated by two sequences of extraction based on solubilization of the hemicelluloses in alkaline and organic solvent systems, respectively. In both cases, only a part (about 50%) of the total xyloglucan could be extracted, the rest remaining tightly associated with cellulose and necessitating the use of acid to be solubilized. Purification of xyloglucans was effected by formation of a gel in appropriate mixtures of dimethyl sulfoxide and water. Further fractionation could be achieved on a cellulose column eluted with chaotropic solvents. This demonstrated the heterogeneity of xyloglucans in the primary cell walls. Analytical data show that all fractions are constituted with the same sugars: L-arabinose, L-fucose, D-galactose, D-xylose, and D-glucose, but their relative proportions differ, particularly the ratio of glucose to xylose which varies from 1.2 to 2 within the different xyloglucans. The structure of these hemicelluloses was established by methylation analysis and shown to consist of a (1 → 4)-linked glucan backbone which carries substituents on the O-6 of glucose. Here again, the multiple forms of xyloglucans was suggested by the various patterns of substitutions found on the different fractions. The configuration of the linkages were established by ^{13}C nuclear magnetic resonance spectroscopy and shown to be β for the glucan backbone, α for the xylosyl and fucosyl substituents, and β for the galactosyl substituents. These configurations agree with the specific rotation of the xyloglucan.

Xyloglucans are certainly the most characteristic polysaccharides found in primary cell walls. Quantitatively predominant (19) in the wall of dicots, they are also present in the wall of monocots but to a lesser extent (3). After the report of the presence of "amyloids" in several plant seeds (15), the description of the xyloglucan from *Tamarindus indica* was followed by the isolation of other xyloglucans from seeds at various origins (10, 24, 25). These hemicelluloses can be extracted with hot water or with alkaline solutions, generally 2.5 N NaOH or 4 N KOH. All these xyloglucans consist of D-glucose, D-xylose, and D-galactose in somewhat varied proportions. L-Arabinose and L-fucose may also be present although it has to be noted that none of the xyloglucans extracted with hot water contained L-fucose. The occurrence of xyloglucans is not restricted to seeds but has also been reported in several plant tissues as different as the bark of *Picea engelmanni* (21) or the cambium of *Populus tremuloides* and of *Tilia americana* (26). All these latter xyloglucans were isolated with alkaline solutions and showed D-glucose, D-xylose, and D-galactose as the predominant sugars with L-fucose and L-arabinose occasionally present. But in no instances in these xyloglucans were arabinose and fucose present at the same time as substituents. Seed xyloglucans and xyloglucans of various origins all show common structural characteristics. The only systématic differences are that in some seed xyloglucans, glucose has been reported as terminal nonreducing ends of side chains (25) a situation which has never been observed in other xyloglucans, and that they often lack terminal fucosyl residues which are present in most if not all cell wall xyloglucans.

The first demonstration of xyloglucan in suspension-cultured cells was reported by Aspinall *et al.* (1) in the culture medium of *Acer pseudoplatanus*. A similar hemicellulose was later characterized by McNeil *et al.* (19) in the primary walls of the same suspension cells. Other suspension-cultured cells revealed xyloglucans in their primary walls (9). In all cases, in addition to D-glucose, D-xylose, and D-galactose, terminal L-fucose was found to be present, and only occasionally L-arabinose.

Suspension-cultured cells have provided a source of xyloglucans for the study of their role in the structure of the plant cell wall (19). In fact, the main structural property of this hemicellulose is its particular capacity to establish strong intermolecular hydrogen bonds with cellulose or with other xyloglucan molecules. This hydrogen-bonding interaction with cellulose microfibrils is the first link in the cross-linking established through other hemicelluloses and pectic polysaccharides between cellulose microfibrils. Because of their presence in primary walls and because of their absence in secondary walls, xyloglucans have been suggested to play a key role in cell wall extension (19). However, according to McNeil *et al.* (19), the effect of auxin-induced pH variations during cell elongation (22) did not seem to affect the hydrogen-bond mode of attachment of xyloglucan to cellulose in a way which could justify the implication of this hemicellulose in cell elongation.

However, IAA was shown to induce the solubilization of a part of the xyloglucans from the cell walls of elongating pea stem (16). Xyloglucans were also solubilized in pea epicotyl tissues (8) where IAA seemed to be responsible for the release of several wall hemicellulose components. This is to be correlated with the solubilization of xyloglucans in low pH conditions (11). Although no connection was clearly established between the auxin-induced acidic pH and the action of a xyloglucanase, the existence of an insoluble xyloglucan and of a soluble one in etiolated mung bean hypocotyls (14) was ascribed to the hydrolysis of the hemicellulose (β 1 → 4)-linked cellulosic backbone by an enzyme. This is thus in favor of a role of xyloglucans in cell elongation. In this respect, the occurrence of different forms of xyloglucans in the cell walls of immature plants or suspension-cultured cells has been reported in *Glycine max* and *Vigna sesquipedalis* hypocot-

yls (12) and in soybean suspension-cultured cells (9).

In this paper, the occurrence of several xyloglucan fractions isolated from the suspension-cultured cells of *Rosa glauca* is reported. Their isolation and purification is discussed in relation to their main structural features determined by methylation analysis and ^{13}C NMR[1] spectroscopy.

MATERIALS AND METHODS

GLC was performed on a Packard-Becker instrument model 417 and a Hewlett-Packard 5710 A fitted with dual columns and flame ionization detector. Both apparatuses were connected to an integration-recorder Hewlett-Packard 3370 B or 3380 A. For GC-MS analysis, the GC System was a Girdel 3000 apparatus equipped with capillary columns. MS was performed on a MS 30 AEI apparatus connected to a Finnigan SS 100 MS computer. Specific rotations were measured with a Roussel and Jouan "Quick Polarimètre" at 589 nm. ^{13}C NMR spectra were recorded on a WP 100 Bruker Fourier transform spectrometer at 25.18 MHz.

Purification of Xyloglucans. *Gel formation.* To a solution of EA-S in DMSO (400 mg in 100 ml), water was added by portions of 10 ml. A gel formed after addition of 30 ml of water. When addition of water was continued beyond 70 ml, the gel redissolved. The gel was collected by centrifugation, dialyzed, and lyophilized to give 125 mg of XG-EA_S. The DMSO extracts from CA_2 and CA_3 were treated in the same way and lead to the formation of a gel.

Fractionation on Cellulose Column. The xyloglucan mixture XG-EA_S (120 mg) was solubilized in water (20 ml) and the insoluble residue (XG-EA_S-insoluble H_2O) was centrifuged. The solution was placed on top of a cellulose column (cellulose powder, Whatman CC 31, 5 g, 70 × 20 mm). The column was successively eluted with water (85 ml), 7 M urea (130 ml), and 0.5 N NaOH (45 ml) giving XG-$EA_{S\text{-}H2O}$ (50 mg), XG-EAS-urea (18 mg), and XG-EAS-NaOH (17 mg), respectively.

Methylation of Xyloglucans. Permethylation of the polysaccharides was according to Hakomori and Purdie. The sample (5–7 mg) was solubilized in freshly distilled DMSO (1 ml). To the solution, methylsulfinyl anion was added (sodium salt, about 2 M, 0.5 ml) and the mixture stirred under N_2 for 4 to 16 h at 20°C. Methyl iodide (0.6 ml) was then added over a period of 90 min. The excess of methyl iodide was evaporated, the mixture was dialyzed and then freeze dried, and the methylation analysis was carried out on the whole freeze-dried material.

Most of the time, a second methylation was performed to ensure completion of the permethylation using Purdie's procedure. The partially methylated polysaccharide was dissolved in methyl iodide (1.5 ml) and to the refluxing solution silver oxide was added by portions over a 3-h period. Reflux was maintained overnight and then the silver salts and unreacted oxide were centrifuged. For polysaccharides which were not soluble in DMSO, solubilization was performed in MMNO-DMSO (5) as previously described; after cooling to room temperature, methylation was carried out as before.

Analysis of Methylated Material. The permethylated polysaccharides were first hydrolyzed in 90% (v/v) formic acid at 100°C for 1 h and after evaporation of the acid a second hydrolysis step was performed with trifluoroacetic acid (2 N, 100°C, 4 h). Whenever the sample contained cellulose, 72% (w/w) H_2SO_4 was used at room temperature for 30 min followed by 2 N H_2SO_4 at 100°C for 6 h). The resulting partially methylated sugars were analyzed as their alditol partially acetylated derivatives.

GLC identification of the sugars was performed on a glass column (2 m × 3 mm) containing 3% SP 2340 on Chromosorb W-AW DMCS at 180°C for 2 min and then 2°C/min to hold at 220°C. GC-MS was performed using a capillary WCOT column coated with SP 2340 in the same conditions.

^{13}C NMR Spectroscopy of Xyloglucans. These were recorded at 25.18 MHz. The polysaccharide was dissolved in DMSO-d_6 at a concentration of 5 to 7% (w/v) depending on the solubility of the sample. The spectra were recorded at 85°C, and 79,000 scans and chemical shifts were measured relative to DMSO (39.6 ppm).

RESULTS AND DISCUSSION

Different Xyloglucans Are Present within the Cell Walls of *Rosa glauca*. Results of the general extraction sequences applied to the cell walls from *R. glauca* were presented in the accompanying paper (4) and give the overall composition of the numerous fractions obtained. From the analytical data provided by total hydrolysis of these fractions, one can anticipate the presence within mixtures of polysaccharides of a particular species belonging to any of the constitutive cell wall macromolecules. This is even more especially the case for some types of polysaccharides which have a characteristic qualitative and quantitative sugar composition. In this respect, xyloglucans can be relatively easily located in a fraction since in the primary wall of dicots, aside from a rather low proportion of glucuronoarabinoxylan (7), they are the only xylose-containing polymers.

Examination of the carbohydrate analysis of the different extracts showed that the concomitant presence of xylose and glucose in significant amounts (Glc/Xyl ≃ 2) began to be noticeable in the alkaline extracts but not in the previous steps of the extraction Sequence 1 (4). About 30% (w/w) of the total initial xylose of the walls was extracted between fractions E 1.25, E 2.5 and E 4.3 (6).

A large proportion (about 50%, w/w) of the xylose present in the starting wall material was still present in the alkaline residues indicating that another kind of xyloglucans was associating with cellulose (6). Moreover, a distinction between cellulosic glucose and hemicellulosic glucose can be made by the difference between the results given by total hydrolysis and selective hydrolysis with different H_2SO_4 concentrations (2). The relative amounts of arabinose and galactose which were found in these extracts and residues can also be regarded as resulting from the presence of arabinan-galactans and arabinogalactans accompanying the xyloglucans (4).

The same was true for extraction Sequence 2 (4) in which the alkaline extract EA comprised about 35% of the initial xylose of the wall. In all of the alkaline extracts, the ratios of glucose to xylose were between 1.9 and 2.5 which correspond to the expected proportions of these two sugars in xyloglucans (Table I). Moreover, analysis of the alkaline residues R 2.5, R 4.3, and RA by total hydrolysis and selective hydrolysis showed that a significant amount of xyloglucan was present in each of those residues (Table II, $0.7 \leq Glc/Xyl \leq 1.7$). In these residues because of the predominance of cellulose, the estimation of the hemicellulosic

Table I. *Presence of Xyloglucans in the Alkaline and Organic Solvent Extracts as Suggested by the Ratio of Glucose to Xylose*

Fractions[a]	Glc/Xyl[b]	Extraction[a]
E 1.25	2.5	Sequence 1
E 2.5	2.1	Sequence 1
E 4.3	2.3	Sequence 1
EA	1.9	Sequence 2
EM	2.9	Sequence 3

[a] The designation of fractions and Sequences 1 to 3 are according to Reference 4.

[b] Ratios established as the mean value of at least two experiments.

[1] Abbreviations: NMR, nuclear magnetic resonance; DMSO, dimethyl sulfoxide; EA, alkaline extract; RA, alkaline residue; RM, total MMNO/DMSO residue; EM, total MMNO/DMSO extract; XG, xyloglucan; MMNO, *N*-methylmorpholine *N*-oxide.

Table II. *Evidence of Xyloglucans Associated with Cellulose in the Extraction Residues*

Total hydrolysis was performed with 72% H_2SO_4 and then 2N H_2SO_4 in order to hydrolyze all polysaccharides including cellulose. Selective hydrolysis (2) with 2 N H_2O_4 allows complete hydrolysis of the hemicelluloses but not of the cellulose and therefore a distinction could be made between cellulosic and noncellulosic glucose.

Fractions[a]	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose	Glc/Xyl
Total hydrolysis								
R 2.5	2.5	1.5	15	6.5	Trace	15.5	59	9
R 4.3	2	1.5	14.5	6.5	Trace	14	61.5	9.5
RA	3	1	14	5.5	1	15	61	11
RM	1.5	1.5	8.5	9	3.5	8	68	7.5
Selective hydrolysis								
R 2.5	4	3	33	16		33	11	0.7
R 4.3	9	2	28	13		29	19	1.5
RA	9	2.5	28.5	12		28	20	1.7
RM	2.5	5	20	22.5	3	19.5	27.5	1.2

[a] As in Ref 4.

glucose was first obtained by selective hydrolysis (2 N H_2SO_4, 1 h, 120°C). However, by this method, the amount of glucose was always underestimated by comparison with that found when stronger conditions (2 N H_2SO_4, 6 h, 100°C) were applied on isolated and purified xyloglucans because of the particular resistance towards hydrolysis of the (β 1 → 4)-glucosyl backbone of these hemicelluloses.

That xyloglucans could be found in alkaline extracts of different concentrations could correspond to some kind of fractionation by degradation of the hemicellulose due to the alkaline nature of the extractant solution or by incomplete extraction. But the fact that xyloglucans remain in association with cellulose even after using 4.3 N NaOH is really the indication that another series of hemicelluloses is concerned. If this interpretation is correct, the extraction of the xyloglucans based on a completely different principle, that is with the solvent system MMNO (Sequence 3) (4), should also lead to two series of xyloglucans: the first soluble in the solvent and the second associated with cellulose. Indeed, the analytical results of the extract EM and of the residue RM (4) (Tables I and II) clearly showed the existence of soluble xyloglucans and cellulose-associated xyloglucans, representing about 1.5% (w/w) and 3% (w/w) of the cell wall, respectively. This means that about 35% (w/w) of the total initial xylose of the starting material was found in EM and about 65% (w/w) in RM.

These values are in very good agreement with those obtained in extraction Sequences 1 or 2 (4) and confirm the existence of at least two types of xyloglucans. It seems therefore that one is not directly associated to cellulose and that the other is more directly linked to cellulose and to polygalacturonic acid material (4). Are the two series of xyloglucans structurally different and are they made up of a spectrum of related polysaccharides?

Purification and Chemical Structure of the Xyloglucans. *Separation and Purification.* As a chaotropic reagent, DMSO is a good solvent of polysaccharides. The lyophilized EA (4) was suspended in DMSO and stirred for 24 h giving rise to a soluble mixture of polysaccharides EA-S and to an insoluble residue EA-NS (Fig. 1). No clear separation of the two main constituents of EA could be achieved with DMSO since xyloglucans and arabinogalactans still subsisted in both EA-S and EA-NS (Table III). However, the higher proportion of xylose in EA-S suggests that a xylan (7) must be present in addition to xyloglucans. Attempts to precipitate the xyloglucans from EA-S by acidification of the solution were unsuccessful. On the other hand, addition of water to the DMSO extract in order to selectively precipitate xyloglucans resulted in the formation of a gel which could easily be collected by centrifugation. The composition of the gel showed fucose, arabinose, xylose, mannose, galactose, and glucose, all

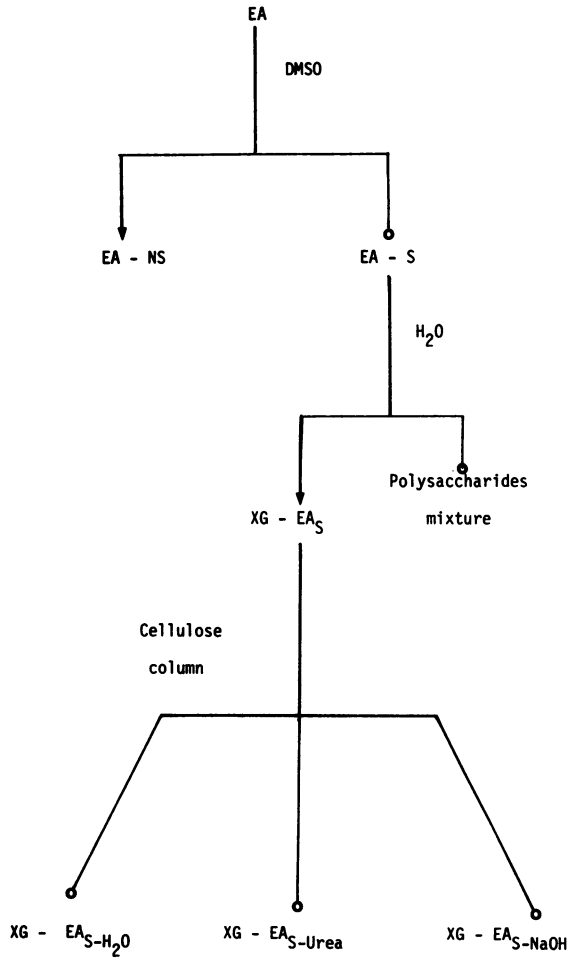

FIG. 1. Purification and fractionation of xyloglucans from the alkaline extract EA.

sugars which are found in xyloglucans, in the following molar proportions: 5.5:2:24.5:7:11:50, respectively. It is thus clear that the formation of a gel upon addition of water allowed an efficient separation of xyloglucans from arabinoxylans. This xyloglucan fraction is referred to as XG-EA_S (Tables III and IV). A second purification by solubilization in water and reprecipitation by addition of DMSO apported again a gel which was constituted of a purified xyloglucan. A fractionation of the purified XG-EA_S was performed on a cellulose column, taking advantage of the

Table III. *Effect of Gel Formation on Xyloglucan Purification*

The EA, which is essentially constituted of a mixture of xyloglucans and arabinogalactans, was fractionated first by DMSO which separated two kinds of xyloglucans (in EA-S and EA-NS) and then by formation of a gel upon addition of water (XG-EA_S).

Fractions	% of Fraction EA	Neutral Sugars							
		Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose	Glc/Xyl
		molar ratio							
EA	100	3	5	12	20	8	15	38	1.9
EA-NS	60	5.5	4	19	18	9.5	18	26	1.4
EA-S	40	0.5	6.5	10.5	40.5	6	10.5	25.5	0.5
XG-EA_S	12	<0.5	5.5	2	24.5	7	11	50	2

Table IV. *Sugar Composition of the Different Xyloglucans Isolated from EA*

Xyloglucan XG-EA_S obtained from EA was further separated into three subfractions (XG-$EA_{S\text{-}H20}$, XG-$EA_{S\text{-}urea}$, and XG-$EA_{S\text{-}NaOH}$) by elution on a cellulose column.

Fractions	% of Wall	Neutral Sugars								$[\alpha]_D^{20°C}$
		Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose	Glc/Xyl	
		molar ratio								
XG-EA_S	2.1	0.5	5.5	2	24.5	7	11	50	2	+10 (*C*, 10.8, DMSO)
XG-EA_S, insoluble H_2O	0.25	0.5	5	4.5	25	6	10	49	2	+10 (*C*, 10, DMSO)
XG-$EA_{S\text{-}H_2O}$	0.9		6.5	1	28.5	3	9.5	51.5	1.8	+15 (*C*, 12, H_2O)
XG-$EA_{S\text{-}urea}$	0.3		6.5	2	36	1.5	9	45	1.2	+20 (*C*, 5, H_2O)
XG-$EA_{S\text{-}NaOH}$	0.3		5	2	32.5	1.5	8	51.5	1.6	+9 (*C*, 4.4, H_2O)

ease with which xyloglucans hydrogen-bond to cellulose (1). Fraction XG-EA_S was solubilized in water, giving an insoluble residue (12%, w/w) and was placed on top of a cellulose column which was successively eluted with water, 7 M urea, and 0.5 N NaOH (Fig. 1). The elution was monitored by the Molish test for the presence of carbohydrates. The fractions eluted with urea and NaOH were dialyzed and freeze-dried. Three fractions, XG-$EA_{S\text{-}H20}$, XG-$EA_{S\text{-}urea}$, and XG $EA_{S\text{-}NaOH}$ were thus collected representing 42, 15, and 14% (w/w) of the starting material according to the elution with water, urea, and NaOH, respectively (Fig. 1). The rest of the polysaccharides placed on top of the column (17%, w/w) remained adsorbed and could not be eluted with the preceding eluents. Analytical data of the recovered fractions are given in Table IV. They all show a close sugar composition inasmuch as they all consist of xylose and glucose with lesser proportions of galactose, arabinose, fucose, and mannose. It must be noted that the ratio of arabinose to galactose does not correspond to the usual proportions of these two sugars in arabinogalactans where they are in a proportion close to unity, and therefore they must be integral parts of xyloglucans.

The ratios of glucose to xylose which are quite characteristic of a given xyloglucan are as low as 1.2 up to 2.0 (Table IV). The optical rotation of the different fractions varies from +9° up to +20°, but the increase in the optical rotation values is not in full agreement with the increase in the proportion of xylose, as expected from the positive contribution brought by the α-linked xylosyl residues. Thus, the fractionation by affinity on the cellulose column, the chemical analysis, and the physical constant of the optical rotation demonstrate that the purified alkaline extract XG-EA_S was in fact constituted of a mixture of several xyloglucans.

A similar behavior was observed for the xyloglucans associated with cellulose in the residues CA_2 and CA_3 obtained from RA (4) (Table V). Extraction of these residues with DMSO and addition of water to the soluble extract led to the formation of a gel which represented 5.3% (w/w) (XG-CA_2) and 3% (w/w) (XG-CA_3) of CA_2 and CA_3, respectively. According to the mode of obtaining of CA_2 and CA_3 by acidic treatments, xyloglucans could have undergone partial hydrolysis in CA_2, the isolation of which involved stronger acidic conditions than that of CA_3. Therefore, the higher yield of xyloglucans from CA_2 was unexpected unless one invokes the necessity of cleaving glycosidic bonds in order to release the xyloglucans from the hemicelluloses and pectic network in agreement with our model of polysaccharides interconnections in the cellulosic blocks (4). The sugar analysis of XG-CA_2 and XG-CA_3 shows the absence of the acid-labile constituents arabinose and fucose and a ratio of glucose to xylose of 1.6 and 1.9, respectively (Table V). The formation of a gel in DMSO-water mixtures is therefore a property of xyloglucans which does not involve the arabinosyl or fucosyl substituents. The conditions in which a gel can be formed with this family of polysaccharides were carefully investigated.

Conditions for Gel Formation by Xyloglucans in Water-DMSO Mixtures. To a 1% solution of xyloglucan in DMSO, water was progressively added by portions of 10 ml. A light flocculation began to take place upon addition of 30% (v/v) water for 70% (v/v) DMSO, and then a gel formation occurred. Addition of water up to 70% (v/v) water for 30% (v/v) DMSO did not increase the formation of the gel which seems to form in an all or none fashion. When more water was added beyond this proportion, the gel solubilized. Conversely, addition of DMSO resulted in the reformation of the gel. It was thus determined that the gel formation occurred in ratios of water to DMSO between 1:3 and 2:3. This provides a rapid and efficient method for the purification of xyloglucans. Also, neither the formation of the gel nor its stability was affected by pH variations since a gel could be obtained unchanged over a wide range of pH from 3 to 8.5. However, in the presence of 1.5 N NaOH, there was no gel formation. This agrees well with previous reports (19) showing that changing the pH from 2 to 7 did not alter the amount of xyloglucan fragments bound to cellulose.

The ability of xyloglucans to form gels has long been recognized although in different solvent conditions, in particular their property of forming gels over a wide pH range. This behavior

Table V. *Evidence of the Presence of Xyloglucans in the Cellulosic Residues and Their Composition*
XG-CA_2 and XG-CA_3 were extracted form the cellulosic residues CA_2 and Ca_3 by DMSO extraction.

Fractions	Glc/Xyl	
	Total hydrolysis[a]	Selective hydrolysis[b]
CA_2	19	2.1
CA_3	12	1.9

	% of wall	Neutral Sugar						
		Fucose	Arabinose	Xylose	Galactose	Glucose	Glc/Xyl	$[\alpha]_D^{20°C}$
		molar ratio						
XG-CA_2	1.3			37	4	59	1.6	+17 (*C*, 7, DMSO)
XG-CA_3	0.9		Trace	30	13	57	1.9	+26 (*C*, 2.6, DMSO)

[a] 72% (w/w) H_2SO_4, 12 h and then 2 N H_2SO_4, 6 h at 100°C.
[b] 2 N H_2SO_4, 1 h at 120°C.

corresponds to chain interaction by strong hydrogen bonds. That DMSO, which is a good solvent for neutral polysaccharides, and water, in which xyloglucans are highly soluble, constitute mixtures in which the solute reprecipitates certainly corresponds to a change in the solvent macromolecule hydrogen-bonding that results in self-association of xyloglucan chains. Conformational modifications have also to be invoked by analogy with what has been suggested in the melting behavior of extracellular schizophylan in water-DMSO mixtures studied by viscosimetry and ultracentrifugation (23). The stabilization of amylose and amylopectine helix structures in the gel state in mixtures of water and DMSO is another example of the role of hydrated DMSO in inducing greater polarity of the hydroxyl groups of amylose with the result of strengthening the hydrogen bonds between the adjacent glucose. This effect was even enhanced by chain branching as demosntrated by IR spectroscopy and electron spin resonance (18).

Do the Analytical Differences Observed in the Different Xyloglucan Fractions Correspond to Differences of the Molecular Structure? A first structural indication was provided by selective hydrolysis with oxalic acid (0.1 N, 2 h, 100°C) which cleaved all the fucose and arabinose residues and a little part of the xylose and galactose residues. This is in agreement with the results obtained by Simson and Timell (26) on xyloglucans from hardwood cambium and indicates that those residues certainly occupy nonreducing terminal positions in the side chains. Structural analysis by methylation was performed according to Hakomori on fractions purified from EA and RA. The completion of the permethylation was checked whenever possible by the disappearance of the band at 3450 cm^{-1} in the IR spectra of the products, but mostly by the reproducibility of the GLC analysis after one Hakomori methylation and after additional Purdie methylations. The proportions expressed in Table VI are calculated directly from the integration of the peak areas and were interpreted as molar ratios between the partially methylated, partially acetylated alditol derivatives without applying any molar response factor. This partly explains the lack of correspondance observed between the number of branched points on the (1 → 4)-linked glucopyranosyl backbone indicated by the 2,3-di-*O*-methyl glucose derivative and the number of end group indicated by the tri-*O*-methyl fucose, tri-*O*-methyl xylose, and tetra-*O*-methyl galactose derivatives. The proportion of terminal xylose residues predominates over the two others. According to the literature, terminal fucose is always linked to position C-2 of a galactose. This is also the case for *R. glauca* where the xyloglucans extracted from a material which had been previously treated with acid (CA_2 and CA_3) have lost their terminal fucose and accordingly show a loss of 3,4,6-tri-*O*-methyl galactose and a corresponding augmentation of 2,3,4,6-tetra-*O*-methyl galactose (Table VI). Because of the proportions of 3,4-di-*O*-methyl xylose, all of the xyloglucan fractions should have side chains of Fuc *p*-(1 → 2)-Gal *p*-(1 → 2)-Xyl *p*. The identity of 3,4-di-*O*-methyl xylose was ascertained by examination of the mass spectrum of the deuterated xylitol derivative obtained by reduction with sodium borodeuteride. From the methylation study, it can be concluded that no characteristic difference could be established between the hemicelluloses extracted by NaOH solution and those remaining in association with the cellulosic residues. The ratios (Table VI) of 2,3,6-tri-*O*-methyl glucose to 2,3-di-*O*-methyl glucose which express the degree of branching in xyloglucans are comprised between 0.45 to 0.60 as in most xyloglucans reported so far. The only significant difference occurs in the fraction eluted from the cellulose column with NaOH, XG-$EA_{S\text{-}NaOH}$, which showed twice the 3,4-di-*O*-methyl xylose than the other fractions and also a higher proportion of terminal xylose. The proportion of these two derivatives suggests the existence of side chains terminated by (1 → 2)-xylobiosyl end groups. The overall absence of distinctive structural features in the different xyloglucans analyzed was further confirmed by the examination of their ^{13}C NMR spectra where no conspicuous difference could be observed. All fractions showed characteristic chemical shifts in the anomeric region (Fig. 2) corresponding to β-linked glucopyranosyl residues (100.4, 102.5, and 102.8 ppm) and α-linked xylosyl residues (99.0–99.2 ppm) and a weak signal at 105.3 ppm assigned to the C-1 of galactopyranosyl residues and indicative of the β configuration of their linkage (5). That xylose and glucose units exhibit several signals of their anomeric carbon atoms corresponds to the different structural situations in which they can be involved in the polymers. This is again illustrated by the signals at 80 ppm assigned to the substituted C-2 of the internal xylosyl unit of the side chains and by the signals at about 62.1 and 69.8 ppm corresponding to the C-6 of internal unsubstituted glucose residues and C-6-substituted branched glucose residues, respectively. These data confirm that of Mori *et al.* (20), and the assignment of configuration of the principal sugar constituents is in good agreement with the observed and calculated values of the optical rotation of the xyloglucans.

CONCLUSION

Undoubtedly because of their ultrastructural position as junction polysaccharides between cellulose microfibrils and pectic polymers, xyloglucans must play an important role in the elongating primary cell wall of plants (4, 19). But this is certainly not the only biological role of these hemicelluloses since it appears that several forms of xyloglucans exist in the wall, and therefore one can anticipate different functions to be associated with the different macromolecular forms. The existence of xyloglucan fractions extracted in different conditions from the cotyledons

Table VI. *Methylation Analysis of the Different Xyloglucans Isolated from Rosa Glauca Cell Walls*

Partially Methylated Derivatives[a]	Recovered Carbohydrate					Glycosidic Linkages
	XG-EA$_{S-H20}$	XG-EA$_{S-urea}$	XG-EA$_{S-NaOH}$	XG-CA$_2$	XG-CA$_3$	
	mol %					
2,3,4-Me$_3$-Fuc	5.5	6.5	3.5			Fuc-*p*-(1 →
3,4,6-Me$_3$-Gal	7	7	3.5		Trace	→ 2)-Gal-*p*-(→
2,3,4,6-Me$_4$-Gal	2.5			13.5	7.5	Gal-*p*-(1 →
3,4-Me$_2$-Xyl	7.5	9	16	13.5	16.5	→ 2)-Xyl-*p*-(1 →
2,3,4-Me$_3$-Xyl	18	15.5	20	36	21.5	Xyl-*p*-(1 →
2,3-Me$_2$-Glc	39	40.5	35	24	36.5	→ 4,6)-Glc-*p*-(1 →
2,3,6-Me$_3$-Glc	17.5	19.5	21	13	18.5	→ 4)-Glc-*p*-(1 →
2,3,6-Glc/2,3-Glc	0.45	0.50	0.60	0.55	0.50	

[a] Identified as their partially acetylated alditol derivatives: 2,3,4-Me$_3$-Fuc = 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-L-Fucitol. Traces of 2,3,4,tri-*O*-methylmannitol were found in XG-EA$_S$ fractions.

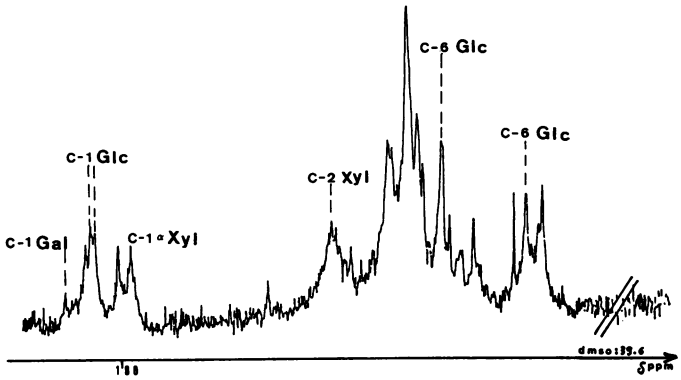

FIG. 2. ^{13}C NMR spectrum of XG-EA$_S$. The ^{13}C spectrum was recorded at 25.18 MHz. The concentration was 7% (w/v) in DMSO-d_6 and chemical shifts were measured relative to DMSO (39.6 ppm) at 85°C.

of several seeds (13, 25) and having different structures has been interpreted that these amyloids can have different biological functions. The lack of homogeneity of the alkaline extracts from cambial tissues (26) was revealed by examination on a DEAE-cellulose column and in addition a dispersion of mol wt values was observed by gel filtration of each fraction. This shows that the phenomenon of polymolecularity of hemicelluloses is a quite general characteristic of plant polysaccharides, not only between different tissues and at the level of a given tissue, but more, as we could see it, at the level of the wall of a single type of cell. The complexity of the architecture of a wall, even the primary wall, agrees with the idea of a multiplicity of polysaccharides within a given family. Reasons for that can be the variation of environment of each of the macromolecules within the wall which could justify that they can be extracted in different conditions.

Another reason is that, although they present a certain degree of homogeneity, suspension cells are not all of the same age in a culture. It is well known that in suspension cell cultures there is always a certain proportion of the cells which do not divide at the same rate as the others or which do not show the same synchronization as the bulk of the culture. Therefore, differences in their wall polysaccharides can reflect a difference in their development.

This should be particularly the case for xyloglucans since these polysaccharides are believed to play an important role in cell elongation. A turnover of hemicelluloses involving wall autolysis and breakage of the cross-linking polysaccharides has been suggested to take place during cell elongation (17). Other functions of xyloglucans in the wall could be envisaged in relation to their property of forming gels *in vitro*, which can result *in vivo* in a swelling ability which depends on the extent of cross-links with the other polymer components. This property represents another aspect of the exceptionally strong hydrogen-bonding interactions that xyloglucans can form by self-association or in association with cellulose.

The multiplicity of xyloglucans does not seem to be unique among the cell wall constituents since several fractions in other wall polysaccharides families have been obtained as in the pectic polymers or in the arabinan-galactan or arabinogalactan series. And it would be indeed surprising that the biosynthesis of these polysaccharides would be such that identical types of polysaccharides would be deposited throughout the length and thickness of the wall.

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