Carbohydrate Research, 206 (1990) 297–310
Elsevier Science Publishers B.V., Amsterdam

Linkage of phenolic acids to cell-wall polysaccharides of bamboo shoot

Tadashi Ishii* and Tadakazu Hiroi
Forestry and Forest Products Research Institute, P.O. Box 16, Tsukuba Norin Kenkyu Danchinai, Ibaraki, 305 (Japan)

(Received January 24th, 1990; accepted for publication in revised form, April 25th, 1990)

ABSTRACT

Hydrolysis of bamboo shoot cell walls with Driselase (a fungal enzyme preparation) gave xyloglucan and arabinoxylan oligosaccharides containing ferulic and *p*-coumaric acids, respectively. The structures of two oligosaccharides containing phenolic acids are here determined to be *O*-(4-*O*-*trans*-feruloyl-α-D-xylopyranosyl)-(1→6)-D-glucopyranose and *O*-[5-*O*-(*trans*-*p*-coumaroyl)-α-L-arabinofuranosyl]-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose, on the basis of n.m.r. spectroscopy, methylation analysis, and f.a.b.–m.s. The possible role of phenolic acid substituents in cell-wall architecture is discussed.

INTRODUCTION

Growing plant cell-walls contain polysaccharides which bear a small proportion of phenolic side-chains. Interest in these compounds arises from the fact that these side-chains appear to undergo *in vivo* oxidative coupling to yield cross-linked polysaccharides; such coupling may contribute not only to the control of cell-wall extensibility and cell growth[1–9], but also to decreased digestibility by ruminant-secreted enzymes[10–12].

The linkage between ferulic acid and cell-wall polysaccharides may be determined by analysis of low-molecular-weight carbohydrate esters released by treatment of cell walls with enzymes. Two feruloylated disaccharides have been isolated from cell walls of a suspension-cultured dicot (spinach)[13]. Later, feruloylated and *p*-coumaroylated arabinoxylan oligosaccharides were isolated from several members of the *Gramineae*[13–20].

We previously reported the presence of a feruloylated xyloglucan disaccharide and a *p*-coumaroyl arabinoxylan trisaccharide in Driselase hydrolyzates of growing bamboo shoot cell walls[21], but problems in their separation precluded a complete structural analysis. We have now purified a feruloylated xyloglucan disaccharide and a *p*-coumaroylated arabinoxylan trisaccharide and here report their full characterization and discuss the possible role of phenolic acids esterified to polysaccharides.

* To whom correspondence should be addressed.

0008-6215/90/$ 03.50

RESULTS

Characterization of compound **1**. — Compositional analysis by g.l.c. of alditol acetates showed that **1** consisted of xylose and glucose in the molar ratio of ~ 1:1. Both xylose and glucose had the D absolute configuration. Methylation analysis (Table I) of **1** gave two derivatives, 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylxylitol (derived from terminal xylopyranosyl residues), and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylglucitol (derived from 6-linked glucopyranosyl residues). Ferulic acid was the only phenolic acid released from **1** by alkali treatment and detected by h.p.l.c. The positive-ion fast-atom-bombardment mass spectrum (f.a.b.–m.s.) of the native material showed an intense ion at *m/z* 511 corresponding to the $(M + Na)^+$ quasimolecular ion of **1**. Weaker ions at *m/z* 489 and 527 corresponding to $(M + H)^+$ and $(M + K)^+$ quasimolecular ions, respectively, were also observed. The negative-ion spectrum of the native material showed an intense ion at *m/z* 487 $[(M - H)^-]$, indicating the molecular weight of **1** to be 488, and thus consistent with a molecule containing one ferulic acid, one pentose, and one hexose residue. During positive-ion f.a.b.–m.s., fragment ions at *m/z* 309 and 177, generated by loss of one hexose residue and one pentose residue, respectively, were observed, indicating that the ferulic acid was linked to the pentose residue. The positive-ion f.a.b. mass spectrum of the per-*O*-acetylated material showed ions at *m/z* 805 and 783 corresponding to $(M + Na)^+$ and $(M + H)^+$ quasimolecular ions, respectively, of a fully acetylated feruloylated disaccharide. In addition, fragment ions were observed at *m/z* 723, 435, and 219, generated by the cumulative loss of acetate, a hexose residue, and a pentose residue from the fully acetylated compound.

The n.m.r. spectra of **1** are shown in Figs. 1–4. The complete assignments of chemical shifts are given in Tables II–IV. The ^{1}H-n.m.r. spectrum (Fig. 1) was analyzed by using two-dimensional (2D-), ^{1}H homonuclear spectroscopy (COSY) (Fig. 2) and 2D *J*-resolved spectroscopy (data not shown). All chemical shifts and connectivities, except those of H-6 of α-D-glucose, are clearly observable. The signals of the ferulic acid protons in **1** were assigned as follows: δ 7.64 (d, $J_{7,8}$ 16.0 Hz, H-7), 7.34 (s, H-2), 7.12 (d,

TABLE I

Methylation analysis of compounds **1** and **2**, and oligosaccharide alditols **1** and **2**

Glycosyl residue	*Methylated derivative*	*Linkage positions*	*Compound*		*Glycosylalditol*	
			1 *mol%*	**2** *mol%*	**1** *mol%*	**2** *mol%*
Arabinofuranosyl	2, 3, 5	Terminal	[a]	34.2		40.9
Xylopyranosyl	2, 3, 4	Terminal	50.5		69.6	
	2, 3	4		15.6		
	2, 4	3		50.2		46.3
Glucopyranosyl	2, 3, 4	6	49.5			
Xylitol	1, 2, 3, 5	4				12.8[b]
Glucitol	1, 2, 3, 4	6			30.4[b]	

[a] Not detected. [b] In this case, the highly-volatile derivative was probably lost.

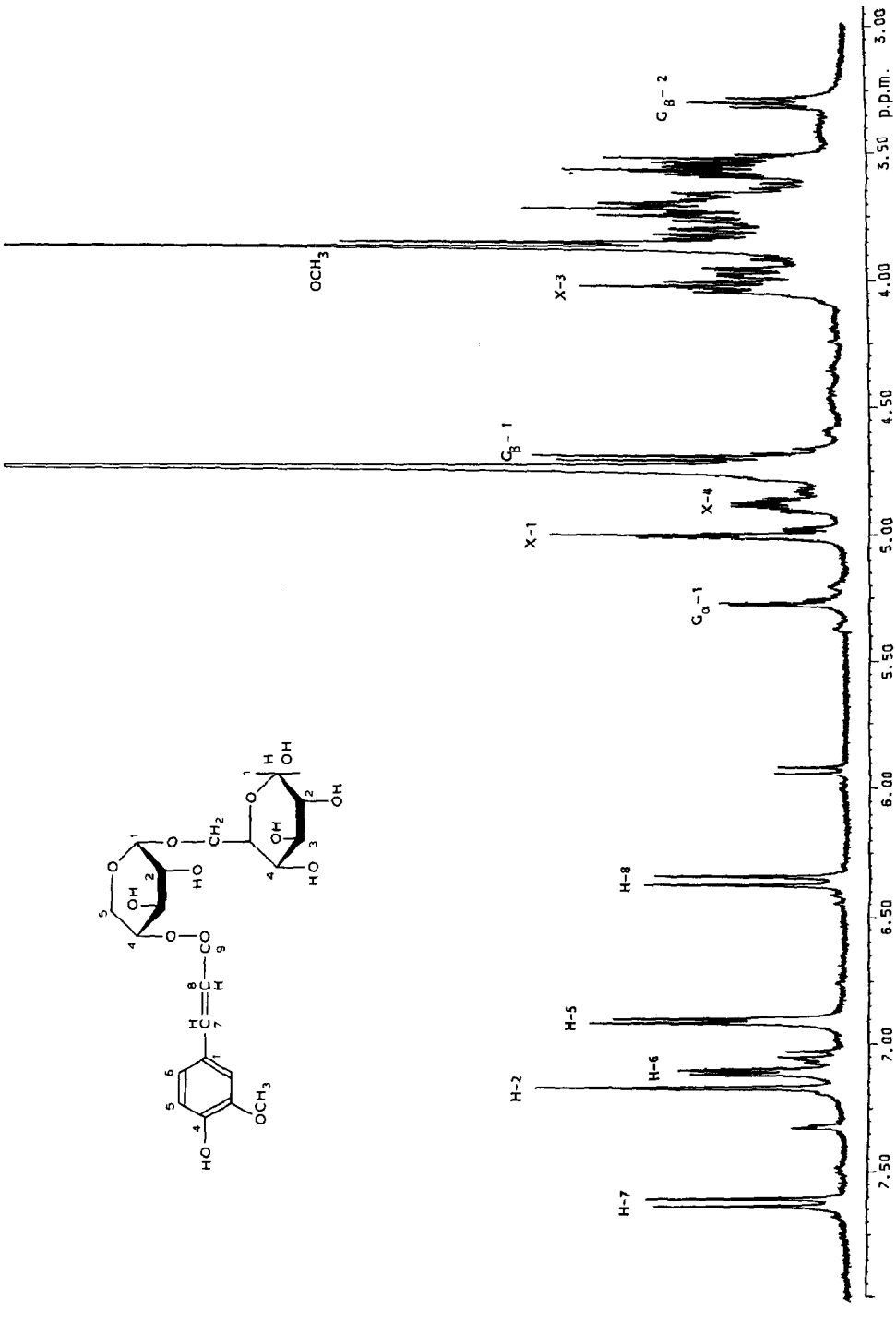


Fig. 1. Structure and 500-MHz ^{1}H-n.m.r. spectrum of **1**.

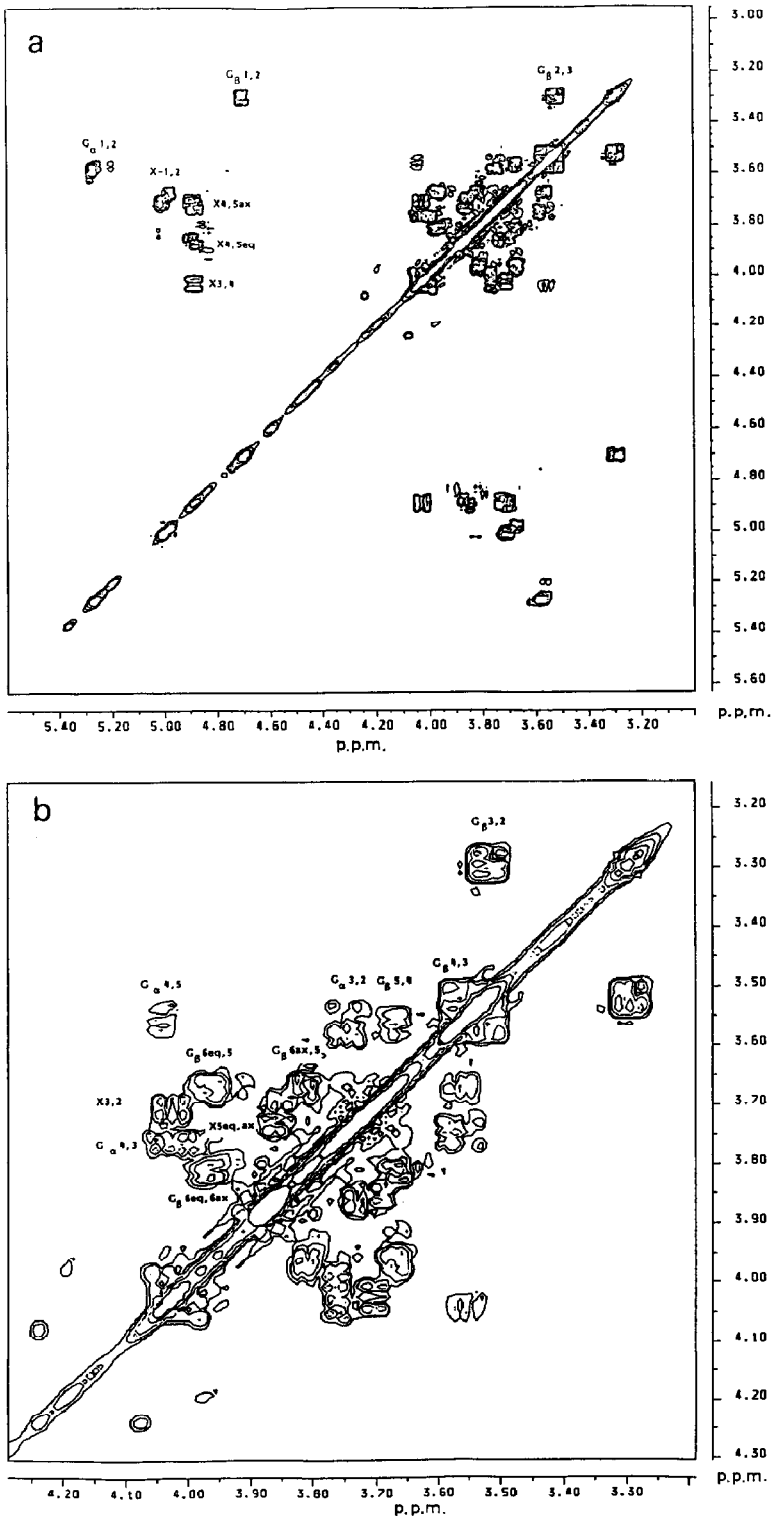
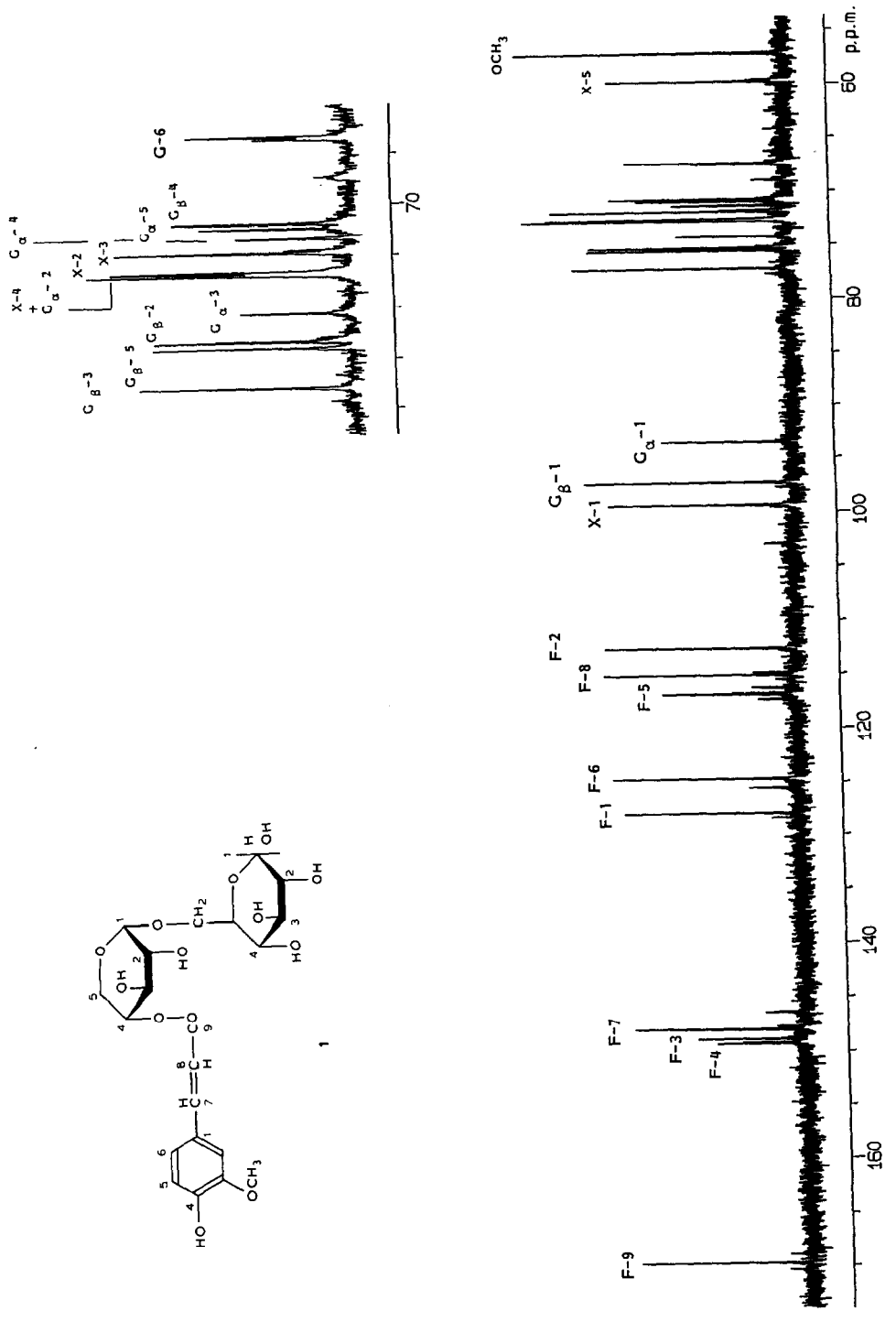


Fig. 2. 2D–COSY spectrum of **1**, (*a*) expanded region, (*b*) expanded upfield region.



1

Fig. 3. Structure and 100-MHz ^{13}C-n.m.r. spectrum of **1**.

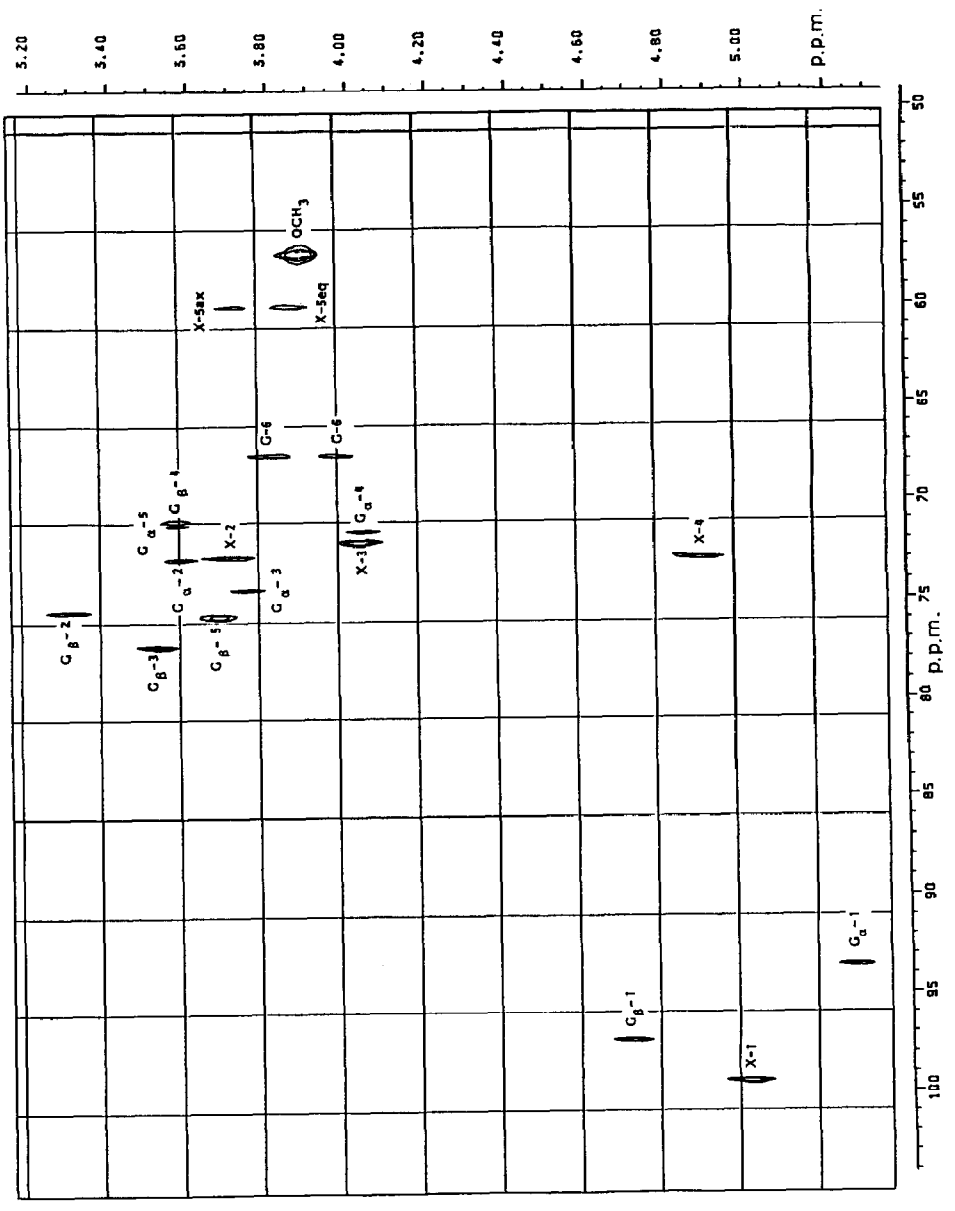


Fig. 4. 2D heteronuclear shift-correlated spectrum (expanded region) of **1**.

H-6), 6.91 (d, $J_{5,6}$ 8.0 Hz, H-5), and 6.37 (d, H-8). These assignments were based on a comparison with published values for ferulic acid[15]. The $J_{7,8}$ value of 16 Hz indicated that the ferulic acid occurred as the *trans* isomer. Integration of the signals for anomeric protons for α-D-xylose, α-D-glucose, and β-D-glucose gave the ratio 1:0.35:0.74. Doubling of peaks was observed for H-1 of xylose, as described by Vliegenthart *et al.*[22]. A comparison of the anomeric shifts and coupling constants for the xylose residue in **1** with those of methyl α- and β-D-xylopyranosides indicated that the D-xylose to be α-linked to glucose. The xylose was esterified at O-4, as indicated by the low-field resonances of H-4 (4.48 p.p.m.); H-4 in xylose would be expected to resonate at ~3.4–3.5 p.p.m.[23]. The reducing glucose residue was substituted at O-6; H-6 of the β anomer was deshielded relative to the corresponding proton in free glucose (H-6β *ax*, 0.17 p.p.m.; H-6β*eq*, 0.23 p.p.m.). The position of this linkage was unambiguously assigned by methylation analysis as described later.

^{13}C-N.m.r. spectroscopy confirmed the foregoing assignments (Fig. 3), see also Table IV). All of the resonances of **1** were assigned by 2D- heteronuclear, shift-correlated spectroscopy (Fig. 4). Proton assignments were transferred to the carbon spectrum by inspection of this plot. Some proton resonances not observable or not assignable in the 1D spectrum were then well separated and readily assigned. In the xylosyl residue, C-4 was deshielded by 2–4 p.p.m. in accordance with published data[23,24] (shifts due to ester linkage at CH_2OH and CHOH groups are −1.0 to +3.4 p.p.m.). For the α- and β-glucosyl residues, the signals for C-6 were shifted by 5.9 p.p.m. downfield, as compared with the corresponding signal in glucose. This shift agrees with the data of Usui *et al.*[25], who found that the glycosidation shift of α-linked glucobioses was similar to the methylation shift of methylated glucoses and was 3.2–7.4 p.p.m. downfield. Hence, the xylosyl residue was α-linked to D-glucose. Results obtained by 2D- heteronuclear, shift-correlated spectroscopy indicated that published values for the chemical shifts of C-5 and C-8 of the ferulic acid had been incorrectly assigned: the positions of cross peaks indicated that in fact C-8 resonates downfield from C-5, not upfield as previously suggested[15,16,18–20].

Compound **1** was reduced with $NaBD_4$ to give ferulic acid and the glycosylalditol **1**. Methylation analysis of the latter (**1**, Table I) revealed terminal xylopyranosyl and 6-linked glucitol residues, indicating that 6-linked glucose was present at the reducing terminus of **1**. By comparison of the retention time and mass spectrum of the per-*O*-methylated glycosylalditol **1** with those of the authentic compound, glycosylalditol **1** was determined to be α-D-xylopyranosyl-(1→6)-D-glucitol.

The proposed structure of compound **1** is thus *O*-(4-*O*-*trans*-feruloyl-α-D-xylopyranosyl)-(1→6)-D-glucopyranose (Fig. 5).

Characterization of compound **2**. — Compositional analysis by g.l.c. of the alditol acetates revealed arabinose and xylose in the molar ratio of ~1:2. The absolute configurations of arabinose and xylose were L and D, respectively. Methylation analysis (Table I) gave three derivatives, namely, 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol (derived from terminal arabinofuranosyl residues), 1,3,5-tri-*O*-acetyl-2,4-di-*O*-methylxylitol (derived from 3-linked xylopyranosyl residues), and 1,4,5-tri-*O*-acetyl-2,3-di-*O*-

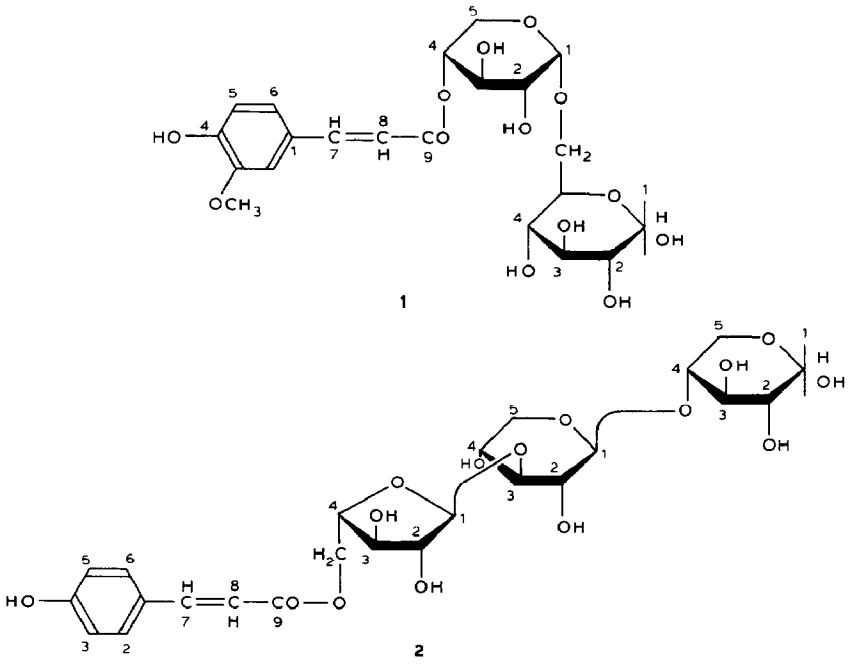


Fig. 5. Structures of compounds **1** and **2**.

methyl-xylitol (presumably derived from 4-linked xylopyranosyl residues). *p*-Coumaric acid was the only phenolic acid released from **2** by alkali treatment and detected by h.p.l.c.

The results of positive-ion and negative-ion f.a.b. mass spectra of the native compound indicated that the molecular weight of **2** to be 560, composed of one *p*-coumaric acid and three pentose residues. The positive-ion f.a.b. mass spectrum of the per-*O*-acetylated material showed ions at *m/z* 919 and 877 corresponding to $(M + Na)^+$ quasimolecular ions of a fully acetylated and underacetylated *p*-coumaroylated trisaccharide, respectively. In addition, fragment ions were observed at *m/z* 837, 621, 405, and 189, generated by loss of acetate and up to three pentose residues from the fully acetylated compound. A less-intense series of fragment ions derived from the underacetylated compound was also observed at *m/z* 579, 363, and 147; the expected fragment ion at *m/z* 795 was not observed, probably because of its low abundance.

Analysis of **2** by ^{1}H- and ^{13}C-n.m.r. spectroscopy was used to elucidate the primary structure. Proton and carbon chemical shifts of **2** are summarized in Tables II–IV. The proton spectrum was analyzed by using COSY and 2D *J*-resolved spectroscopy (data not shown). The ^{13}C-n.m.r. spectrum was completely assigned by 2D-heteronuclear, shift-correlated spectroscopy (data not shown).

The *p*-coumaric acid protons were assigned as follows: δ 7.71 (d, $J_{7,8}$ 16.1 Hz, H-7), 7.56 (d, $J_{2,3}$ 8.7 Hz, H-2), 6.94 (d, H-3), and 6.41 (d, H-8). The $J_{7,8}$ value of 16 Hz indicated that *p*-coumaric acid occurred as the *trans* isomer. In the ^{13}C-n.m.r. spectrum,

TABLE II

Assignments of signals in ^{1}H-n.m.r. spectra[a] of compounds **1** and **2**

Compound	*H-1*	*H-2*	*H-3*	*H-4*	*H-5$_a$*	*H-5$_e$*	*H-5*	*H-6$_a$*	*H-6$_e$*
1					*ppm*[b]				
α-D-Xylose	5.00	3.69	4.02	4.48	3.53	3.84			
α-D-Glucose	5.27	3.55	3.73	4.03			3.54		
β-D-Glucose	4.70	3.29	3.50	3.55			3.65	3.80	3.95
2									
α-L-Arabinose	5.36	4.23	4.07	4.42	4.34	4.51			
β-D-Xylose[b]	4.48	3.42	3.59	3.64	3.32	4.01			
α-D-Xylose	5.19	3.55	3.68	3.76	3.26				
β-D-Xylose	4.59	3.26	3.54	3.76	3.36	4.06			

[a] In D_2O at 27°, [b] Relative to internal acetone, and converted to the Me_4Si scale by applying the relationship δ Me_4Si(acetone) = 2.234.

TABLE III

^{1}H Coupling constants (J, Hz) of compounds **1** and **2**

Compound	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5a}$	$J_{4,5e}$	$J_{5,5}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6e}$	$J_{6,6}$
1										
α-D-Xylose	3.7	9.3	9.0	7.1	7.1	12				
α-D-Glucose	3.1	9.3	8.7				9.0			
β-D-Glucose	7.3	8.5	8.7				9.0	5.0	2.6	11.9
2										
α-L-Arabinose	1.4	3.3	5.6	5.4	2.6	11.1				
β-D-Xylose'	7.8	9.4	9.4	11.7	5.6	10.0				
α-D-Xylose	3.7	8.7								
β-D-Xylose	7.8	9.7	9.4	11.7	5.6	11.7				

the arabinofuranosyl C-5 was deshielded by 2.8 p.p.m., indicating the linkage of *p*-coumaric acid at that position.

Compound **2** was reduced with $NaBD_4$ to give *p*-coumaric acid and diglycosylalditol **2**. Methylation analysis (Table I) confirmed that 4-linked xylose was present at the reducing terminus of **2**. By comparison of the retention time and mass spectrum of per-*O*-methylated diglycosylalditol **2** with those of the authentic compound, diglycosylalditol **2** was determined to be α-L-arabinofuranosyl-(1→3)-D-xylopyranosyl-(1→4)-D-xylitol. The presence in the e.i. mass spectrum of an aJ_0 fragment ion at m/z 238 and absence of an aJ fragment-ion at m/z 252, indicated that penultimate glycosyl residue of diglycosylalditol **2** was 3-linked[26].

From these results, the proposed structure of **2** is *O*-[5-*O*-(*trans*-*p*-coumaroyl)-α-L-arabinofuranosyl]-(1→3)-*O*-β-D-xylopyranosyl)-(1→4)-D-xylopyranose (**2**) (Fig. 5). Mueller–Harvey *et al.*[18] recently have isolated the same *p*-coumaroylated arabinoxylan trisaccharide (compound **2**) from matured barley straw.

TABLE IV

Assignments of signals in ^{13}C-n.m.r.[a] spectra of compounds **1** and **2**

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-7*	*C-8*	*C-9*	*OCH3*
1						*p.p.m.*[b]				
α-D-Xylose	99.50	72.72	72.01	72.86	59.86					
α-D-Glucose	93.56	72.79	74.35	71.45	71.06	67.61[c]				
β-D-Glucose	97.45	75.44	77.31	70.86	75.70	67.51[c]				
Ferulic acid	128.16	112.72	148.96	149.24	116.93	124.77	148.11	115.26	169.92	57.23
2										
α-L-Arabinose	109.22	82.15[d]	77.85	82.51[d]	64.83[d]					
β-D-Xylose'	102.78	73.85	82.76[d]	68.75	66.05[d]					
α-D-Xylose	93.05	72.41[d]	72.00[d]	77.69[d]	59.86[d]					
β-D-Xylose	97.54	75.02	74.95	77.52[d]	64.03[d]					
p-Coumaric acid	127.44	132.74	116.97	159.32	116.97	132.74	147.42	114.95	170.26	

[a] In D_2O at 27°. [b] Values are chemical shifts relative to CD_3OD (49.30 p.p.m.). [c] These assignments may be interchanged. [d] These assignments were ambiguous in previously published work[18].

Contaminant in compounds **1** *and* **2**. — Compounds **1** and **2** were found to be accompanied by small amounts of isomers. The ^{1}H- and ^{13}C-n.m.r. spectra of these isomeric compounds bore close resemblance to those of **1** and **2**, respectively, except for the signals due to a phenolic acid moiety (Figs. 1 and 3). The ^{1}H-n.m.r. spectrum of **1** (Fig. 1) showed that the alkene proton signals at δ 5.93 and 7.05 p.p.m. (each 1 H, d) shifted upfield and that their coupling constant ($J_{7,8}$ 12.9 Hz) was smaller than that of **1** (δ 6.37 and 7.64 p.p.m., each 1 H, d, $J_{7,8}$ 16.0 Hz). This value indicates that the configuration of the alkene in the feruloyl moiety is *cis*. Analysis of ferulic acid liberated from **1** by alkali treatment by g.l.c.–m.s. showed *trans* and *cis* isomers in a molar ratio of ~3.5:1. The ^{1}H-n.m.r. spectrum of **2** (data not shown) also indicated the occurrence of the *cis-p*-coumaroyl moiety in **2**. Analysis of *p*-coumaric acid liberated from compound **2** by alkali treatment by g.l.c.–m.s. showed *trans* and *cis* isomers in a molar ratio of ~10:1. These *cis*-isomers might be artefacts formed from the *trans*-isomers during isolation. Sasaki *et al.*[27] recently isolated *trans*- and *cis*-isomers of hydroxycinnamic acid esters of phenethyl alcohol glycosides from *Rehmannia glutinosa* var. *purpurea* and proposed that the *cis*-isomers were artefacts.

DISCUSSION

The results presented here show that cell walls isolated from young growing *Gramineae* yield *O*-(4-*O*-*trans*-feruloyl-α-D-xylopyranosyl)-(1→6)-D-glucopyranose[1] and *O*-[5-*O*-(*trans-p*-coumaroyl)-α-L-arabinofuranosyl]-(1→3)-*O*-β-D-xylopyranosyl)-(1→4)-xylopyranose[2] after treatment with Driselase. This is the first report of the complete structural assignment of a feruloylated xyloglucan disaccharide. We previously reported the presence of a feruloylated xyloglucan disaccharide and a *p*-coumaroyl arabinoxylan trisaccharide in Driselase hydrolyzates of bamboo shoot cell-walls[21]. However, their structures were determined by using a mixture of the two compounds and only methylation analysis and ^{13}C-n.m.r. spectroscopy. The ^{1}H-n.m.r. spectrum was not assigned and assignment of the ^{13}C-n.m.r. spectrum was insufficient. We have now purified these two compounds and conducted the complete structural analysis, confirming the linkage positions between cinnamic acid derivatives and oligosaccharides.

The calculated yields of **1** and **2** from cell-walls of bamboo shoot were 0.039 and 0.016 mg/g of cell walls, respectively. The quantities of **1** and **2** released from cell-walls of bamboo shoot by treatment with Driselase accounted for 3.5% of ferulic acid and 4.3% of *p*-coumaric acids released by treatment with sodium hydroxide, respectively.

Our results demonstrate that ferulic acid is linked to xyloglucan in cell walls of a young, growing monocotyledonous plant. Even though it has been proposed earlier that xyloglucan bonds strongly to cellulose fibrils through hydrogen bonds[28], it seems probable that xyloglucan molecules bind not only to cellulose but also to other polysaccharides through diferuloyl bridges, which occur naturally in cell walls[4,5]. We have obtained a diferulic acid-rich fraction from Driselase hydrolyzates, which contained 52% diferulic acid residues in the total phenolic acids. Methylation analysis of

this fraction showed that it consisted mainly of arabinoxylan and a small amount of xyloglucan (unpublished data). Further studies are in progress to characterize this diferulic acid-rich fraction released by enzymic hydrolysis from bamboo shoot cell-walls.

EXPERIMENTAL

Plant material. — Shoots (~20 cm long) of Mouso-chiku bamboo (*Phyllostachys edulis*) were collected in Tsukuba, Ibaraki Prefecture, in May 1989. Bamboo shoots cell-walls (~80 g) were prepared from the youngest growing parts (5 kg) as described previously[19]. The cell walls contained 1.5 mg of phenolic acids per g of dry weight. The ratio of *p*-coumaric acid, ferulic acid, and diferulic acid determined by g.l.c. was 1:2.8:0.06 (mol/mol/mol). The phloroglucinol test on the cell walls indicated lignin to be absent.

Isolation of compounds **1** *and* **2**. — The cell walls (20 g) were suspended in 1.0 L of distilled water and incubated for 16 h at 30° after addition of 3 mL of a 60 $mg \cdot mL^{-1}$ solution of Driselase (purchased from Kyowa Hakko, Tokyo, and purified as described[13]), and the pH of the suspension was adjusted to pH 5.0 with AcOH. The suspension was heated for 15 min in a boiling-water bath to stop the reaction, and then centrifuged. The supernatant solution was concentrated. Polysaccharides were removed from the digestion products by addition of 5 volumes of EtOH, the precipitate was removed by centrifugation, and the supernatant was evaporated to dryness. The EtOH-soluble, Driselase digestion-products were chromatographed on a column (4.4 × 85 cm) of Sephadex LH-20 eluted with water. Fractions of 15 mL were collected and assayed for total carbohydrate and phenolic acid. A fraction, having K_{av} value 3.14, contained predominantly feruloylated oligosaccharides. These oligosaccharides were fractionated using preparative, reversed-phase h.p.l.c. with a 2.0 (i.d.) × 25 cm column [Sim-Pak Prep ODS(H) -kit from Shimadzu] at 40° eluted with 14% (v/v) aq. MeCN at $4 mL.min^{-1}$. The eluates were monitored at 260 nm, and a peak of u.v.-positive material eluting at 57–69 min was collected and lyophilized. This fraction was further purified by analytical normal-phase h.p.l.c. with a 0.6 (i.d.) × 15 cm column (Shim-Pack CLC-SIL from Shimadzu) at 40° eluted with 150:150:1 (v/v/v) 2-propanol–$CHCl_3$–AcOH at 0.5 $mL.min^{-1}$. The eluate was monitored at 320 nm, and peaks of u.v.-positive material eluting at 7.8, and 10.0 min were collected, and lyophilized to give **2** and **1**, respectively.

General methods. — Evaporations were conducted under diminished pressure at <40°. Total carbohydrate was determined by the phenol–H_2SO_4 method[29]. Alditol acetates were prepared[30] and analyzed[31] as described, except that hydrolysis in 2M CF_3CO_2H was performed for 20 min; g.l.c. was performed using a Shimadzu GC 14A instrument operated isothermally at 230° with a 30 m × 0.25 mm SP-2330 fused-silica column (Supelco). Absolute configurations were determined as described[32]. Per-*O*-methylation was performed by a modification[33] of the method of Hakomori[34], and per-*O*-methylated oligosaccharides and glycosylalditols were purified as described[35]. Glycosyl-linkage compositions were determined by g.l.c.–m.s. of per-*O*-methylated

alditol acetates[31]. Samples were per-*O*-acetylated for f.a.b.–m.s. by the method of Bourne *et al.*[36].

Alkaline hydrolysis and reduction of compounds **1** *and* **2**. — Compounds **1** and **2** (~300 μg of each) were separately dissolved in 250 μL of a 10 mg·mL^{-1} solution of $NaBD_4$ in 1.5M NH_4OH. After 3 h under N_2 in the dark at room temperature, the solutions were acidified with 6M HCl, and extracted with ether. The ether phases were washed with water and evaporated. The glycosylalditols **1** and **2** in the aq. phases were treated, per-*O*-methylated, and analyzed as described[19]. The ether extracts were fractionated by reversed-phase h.p.l.c. with an ODS column [Shim-Pack Prep-ODS(H)-kit), 0.46(i.d.) × 25 cm, from Shimadzu]. Samples were chromatographed in 30% aq. MeCN containing 0.05% $HClO_4$ at a flow rate of 1.0 mL·min^{-1}. Phenolic acids were monitored at 320 mm. Phenolic acids in u.v.-positive h.p.l.c. fractions were identified by using direct-insertion, electron-impact m.s. with an electron energy of 70 eV. A portion of the ether extracts was converted into O-trimethylsilyl derivatives by treatment with N,O-bis(trimethylsilyl)acetamide and analyzed by g.l.c.–m.s.

Mass spectrometry. — G.l.c.–m.s. was performed with a Hewlett–Packard 5890J g.l.c. coupled to a Jeol JMS-DX303 HF mass spectrometer, and a Jeol JMA-DA5000 data system; the ionization energy was 70 eV, emission current 300 μA, and source temperature 180°. For analysis of per-*O*-methylated alditol acetates, a 30-m × 0.25-mm SP-2330 column was used with a temperature program starting at 170° for 2 min, then increasing to 235° at 4°·min^{-1}. Per-*O*-methylated glycosylalditols were separated by using splitless injection and a 30-m × 0.25-mm fused-silica DB-1 column (J and W Scientific), with a temperature program starting at 50° for 3 min, then increasing to 150° at 30° min^{-1}, and finally to 340° at 6° min^{-1}. Silylated phenolic acids were separated by split injection and a 30-m × 0.25-mm fused-silica DB-1 column, with a temperature program starting at 180° for 2 min, then increased to 250° at 5° min^{-1}.

F.a.b.–m.s. was performed in the positive-ion and negative-ion mode by using a Jeol JMS-DX 303HF mass spectrometer. Native samples were dissolved in water and loaded into glycerol on a stainless-steel target. Per-*O*-acetylated samples were dissolved in MeOH and loaded into 3-nitrobenzyl alcohol. Xenon was used as the bombarding gas, and the gun was operated at 3 kV. Spectra were obtained at a scan rate that covered the mass range from 1 to 1800 units in 15 sec, using the Jeol JMA-DA 5000 data-system.

N.m.r. spectroscopy. — ^{13}C-N.m.r. spectra were recorded at 27° in D_2O with a Jeol GSX 400 spectrometer operated at 100 MHz, using the noise-decoupled mode and a deuterium lock. Chemical shifts were referenced to an internal standard of CD_3OD (49.30 p.p.m.). ^{1}H-N.m.r. spectra were recorded using a Bruker AM-500 spectrometer operated at 500 MHz and temperature at 30°. Samples were dissolved in D_2O (99.996 atom %), and chemical shifts are reported relative to internal acetone (δ 2.234 p.p.m.). 2D-COSY, 2D *J*-resolved spectroscopy, and 2D heteronuclear shift-correlated spectroscopy, were performed with a Bruker AM-500 spectrometer using standard Bruker 2D software.

ACKNOWLEDGMENTS

The authors thank Mr. M. Yanagisawa (Shimadzu, Inc.,) for giving invaluable suggestions about the separation of phenolic acid ester compounds, Dr. T. Tobita (Japan Tobacco, Inc.,) for the 500-MHz Bruker AM-500 spectroscopy, Dr. H. Imai (Tropical Agriculture Research Center) for using the negative-ion mode f.a.b–m.s., and Mrs. K. Ishizuka for providing fresh bamboo shoots. The authors also thank Dr. J. Thomas (Univeristy of Dundee, Scotland) for his critical reading of this manuscript and Mrs. M. Nishida for typing the manuscript. This work was supported by research grant (Biomedia Projects BMP-90-I-1-1) from the Ministry of Agriculture, Forestry, and Fisheries.

REFERENCES

1 T. J. Painter and H. Neukom, *Biochem. Biophys. Acta*, 158 (1968) 363–381.
2 T. Geissman and H. Neukom, *Helv. Chem. Acta*, 54 (1971) 1108–1112.
3 H. A. Stafford and M. A. Brown, *Phytochemistry*, 15 (1976) 465–469.
4 H. V. Markwalder and J. Neukom, *Phytochemistry*, 15 (1976) 836–837.
5 R. D. Hartley and E. C. Jones, *Phytochemistry*, 15 (1976) 1157–1160.
6 M. M. Smith and T. P. O'Brien, *Aust. J. Plant Physiol.*, 6 (1979) 201–219.
7 S. C. Fry, *Planta*, 146 (1979) 343–351.
8 S. C. Fry, *Phytochemistry*, 19 (1980) 735–740.
9 S. C. Fry, *Annual Rev. Plant Physiol.*, 37 (1986) 165–186.
10 X. Alibes, F. Munoz, and R. Faci, *Anim. Feed Sci. Technol.*, 10 (1984) 239–246.
11 H. Graham and P. Aman, *Anim. Feed Sci. Technol.*, 10 (1984) 199–211.
12 P. J. van Soest, A. Muscarenhes Ferneua, and R. D. Hartley, *Anim. Feed Sci. Technol.*, 10 (1984) 155–164.
13 S. C. Fry, *Biochem. J.*, 203 (1982) 493–504.
14 M. M. Smith and R. D. Hartley, *Carbohydr. Res.*, 118 (1983) 65–80.
15 A. Kato, J. Azuma, and T. Koshijima, *Chem. Lett.*, (1983) 137–140.
16 Y. Kato and D. J. Nevins, *Carbohydr. Res.*, 137 (1985) 139–150.
17 F. Gubler, A. E. Ashford, A. Bacic, A. B. Blakeney, and B. A. Stone, *Aust. J. Plant Physiol.*, 12 (1985) 307–317.
18 I. M. Harvey, R. D. Hartley, P. J. Harris, and E. H. Curzon, *Carbohydr. Res.*, 148 (1986) 71–85.
19 T. Ishii and T. Hiroi, *Carbohydr. Res.*, 196 (1990) 175–183.
20 A. Kato, J. Azuma, and T. Kosijima, *Agric. Biol. Chem.*, 51 (1987) 1691–1693.
21 T. Ishii, J. R. Thomas and, T. Hiroi, *Phytochemistry*, 29 (1990) 1999–2003.
22 J. F. G. Vliegenthart, L. Dorland, and H. van Halbeek, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 209–374.
23 T. McEwan, A. G. McInnes, and D. G. Smith, *Carbohydr. Res.*, 104 (1982) 161–168.
24 E. Petrakova and P. Kovac, *Carbohydr. Res.*, 101 (1982) 141–147.
25 T. Usui, N. Yamaoka, K. Matsuda, K. Tuzimura, H. Sugiyama, and S. Seto, *J. Chem. Soc., Perkin Trans. 1*, (1973) 2425–2432.
26 J. K. Sharp and P. Albersheim, *Carbohydr. Res.*, 128 (1984) 193–202.
27 H. Sasaki, H. Nishimura, M. Chin, and H. Mitsuhashi, *Phytochemistry*, 28 (1989) 875–879.
28 W. D. Bauer, K. W. Talmadge, K. Keestra, and P. Albersheim, *Plant Physiol.*, 51 (1973) 174–187.
29 M. Dubois, K. A. Gildes, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
30 P. Albersheim, D. J. Nevins, P. D. English, and A. Karr, *Carbohydr. Res.*, 5 (1967) 340–345.
31 W. S. York, A. G. Darvill, M. McNeil, T. T. Stevenson, and P. Albersheim, *Methods Enzymol.*, 118 (1985) 3–40.
32 G. J. Gerwig, J. P. Kamerling, and J. F. G. Vliegenthart, *Carbohydr. Res.*, 77 (1979) 1–7.
33 P. A. Sandford and H. E. Conrad, *Biochemistry*, 5 (1966) 1508–1516.
34 S. Hakomori, *Biochem. J.*, 55 (1964) 205–208.
35 T. J. Waeghe, A. G. Darvill, M. McNeil, and P. Albersheim, *Carbohydr. Res.*, 123 (1983) 281–304.
36 E. J. Bourne, M. Stacey, J. C. Tatlow, and J. M. Tedder, *J. Chem. Soc.*, (1949) 2976–2979.