Phytochemistry, Vol. 28, No. 8, pp. 2105–2107, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00

XYLOGLUCAN ISOLATED FROM SUSPENSION-CULTURED SYCAMORE CELL WALLS IS *O*-ACETYLATED*

LAURA L. KIEFER, WILLIAM S. YORK, ALAN G. DARVILL and PETER ALBERSHEIM

Complex Carbohydrate Research Center, Department of Biochemistry, The University of Georgia, U.S.D.A.. Russell Research Building, P.O. Box 5677, Athens, GA 30613, U.S.A.

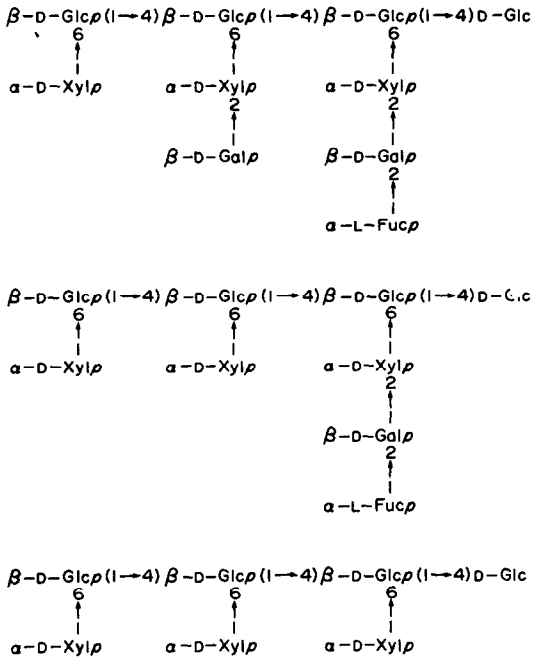
(*Received* 5 *September* 1988)

Key Word Index—Cell wall; ^{1}H NMR; xyloglucan; *O*-acetate.

Abstract—Xyloglucan oligosaccharides were enzymically released from purified cell walls isolated from suspension-cultured sycamore cells. The *O*-acetyl substitution pattern of these oligosaccharides was determined by ^{1}H NMR spectroscopy. This analysis revealed that most of the 2-linked galactosyl residues of the decasaccharide and nonasaccharide fragments of cell wall xyloglucan are *O*-acetyl substituted, while the heptasaccharide fragment does not contain *O*-acetyl substituents. This acetylation pattern does not differ significantly from that of the same xyloglucan oligosaccharides enzymically released from the xyloglucan secreted by suspension-cultured sycamore cells.

INTRODUCTION

Xyloglucans are hemicellulosic polysaccharides present in the cell walls of higher plants [1]. Xyloglucans are found in association with cellulose microfibrils in primary (growing) cell walls [2], and they probably contribute to the structural integrity of the cell wall. Endoglucanases (1,4-β-D-glucan 4-glucanohydrolases, EC 3.2.1.4) capable of cleaving xyloglucan into repeating unit oligosaccharides have been isolated from auxin-treated pea stems [3] and various fungi [4]. The major products [5] obtained when the xyloglucan from sycamore extracellular polysaccharides (SEPS) is treated with the endoglucanase from *Trichoderma viride* are decasaccharide **1**, nonasaccharide **2**, and heptasaccharide **3**. Evidence has been obtained that oligosaccharide fragments of xyloglucans are involved in the control of plant cell elongation [6]. Addition of as little as 10^{-9} M of a fraction rich in nonasaccharide **2** to the growth medium inhibits 2,4-D stimulated elongation of excised pea stem segments [6]. Furthermore, the nonasaccharide has been detected in the culture filtrate of suspension-cultured spinach cells at physiologically significant concentrations [7].



2

3

Recently, through the use of ^{1}H NMR and FABMS, it was determined that most of the decasaccharide and nonasaccharide repeating units of SEPS xyloglucan have one or two *O*-acetyl substituents on the 2-linked galactosyl residue [8]. The heptasaccharide does not contain galactosyl residues and is not *O*-acetylated. Solubilization of xyloglucans from plant cell walls with alkali would remove any *O*-acetyl substituents that might be present. Therefore, as alkali extraction is the only convenient method to obtain polymeric xyloglucan from cell walls [2], *O*-acetyl substituents have not been found in these polymers.

The physical properties of the xyloglucan of the walls of growing plant cells are likely to be affected by the presence of acetyl substituents. These substituents may affect the rheological properties of xyloglucans [9], and may also alter their susceptibility to enzymic cleavage [10]. The potential that *O*-acetyl substituents have for affecting the ability of xyloglucan to modulate or control cell elongation prompted us to look for their presence in cell wall bound xyloglucan.

RESULTS AND DISCUSSION

Purified walls of suspension-cultured sycamore cells were treated with a fungal endoglucanase to release oligosaccharide fragments from the cell wall xyloglucan [5]. The oligosaccharides were separated into size classes by gel-permeation chromatography on Bio-Gel P-2

*Part 27 in the series 'Structure of Plant Cell Walls'. For part 26, see ref. [15].

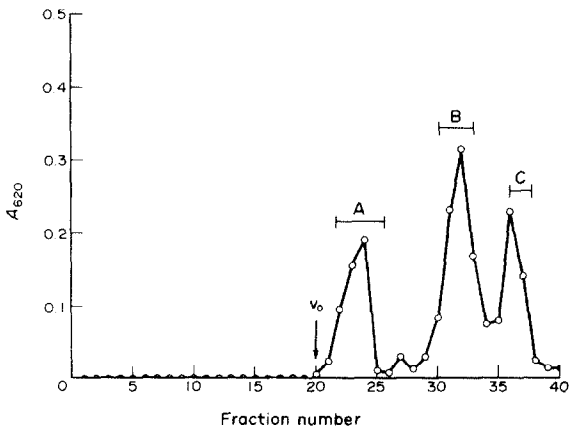


Fig. 1. Gel permeation chromatography on Bio Gel P-2 of xyloglucan oligosaccharides (16 anthrone glucose equivalents) released from purified sycamore cell walls by the *T. viride* 1,4-β-D-glucan 4-glucanohydrolase. The oligosaccharides were previously desalted by gel permeation chromatography on Sephadex G-15, and thus no smaller oligosaccharides eluting after fraction # 40 of the P-2 column were detected. Fractions of the P-2 column were pooled as indicated. Peak A consists mainly of xyloglucan oligosaccharides with more than 15 glycosyl residues. Peak B contains a 5:1 mixture of nonasaccharide **2** and decasaccharide **1**, and peak C heptasaccharide **3**.

(Fig. 1) [5]. Carbohydrate-rich material was eluted from the column and pooled as indicated in Fig. 1.

The 500 MHz ^{1}H NMR spectra of the pooled P-2 fractions were recorded and compared to the previously described spectra of the xyloglucan oligosaccharides released from SEPS by the same endoglucanase [8]. The spectrum of the material eluted as peak B (Fig. 1) indicated that it was composed of a mixture of the decasaccharide **1** and nonasaccharide **2** in the ratio 1:5 [8]. Peak C was composed of heptasaccharide **3**. Using published assignments of ^{1}H NMR signals that are diagnostic for specifically located *O*-acetyl substituents of the xyloglucan oligosaccharides [8], it was determined that the 2-linked galactosyl residues of the oligosaccharides in fraction B (from cell wall xyloglucan) are *O*-acetylated at C-3, C-4, and C-6 (Table 1, Fig. 2). Thus, the pattern of *O*-acetylation in the oligosaccharides of xyloglucan oligosaccharide fraction B is very similar to that of the corresponding SEPS xyloglucan oligosaccharides. The total degree of *O*-acetylation of xyloglucan oligosaccharides in fraction B is slightly less than that of the corresponding SEPS fraction. However, this difference is no more pronounced than differences typically observed between individual SEPS xyloglucan preparations. No *O*-acetylation of the heptasaccharide was observed, regardless of the source (cell wall xyloglucan peak C or SEPS).

The observation that SEPS and cell wall xyloglucan were found to have comparable *O*-acetyl substitution patterns suggests that they are synthesized by the same cellular machinery, and that the primary difference between these two types of xyloglucan is that cell wall xyloglucan is bound to the cellulose microfibrils of the cell wall, while SEPS xyloglucan is secreted into the surrounding medium [11]. Thus, SEPS is a convenient source of intact, soluble, *O*-acetylated xyloglucan and it appears to be an appropriate model for cell wall xyloglucan.

EXPERIMENTAL

Colorimetric assays. Hexose-containing carbohydrates were detected by the anthrone method [12] and amounts were quantitated and reported relative to the response of 1 mg of glucose (i.e. as anthrone glucose equivalents). Uronic acids were quantitated by the *m*-hydroxy biphenyl assay [13].

Isolation of xyloglucan oligosaccharides from sycamore cell walls. EPG-pretreated sycamore cell walls (1.8 g dry wt) were suspended in 200 ml 50 mM NaOAc and incubated overnight with *Trichoderma viride* endoglucanase (55 units). The suspension was centrifuged, and the resulting supernatant soln collected and passed through a QAE-Sephadex column as described [5]. The uronic acid-free eluant was desalted on Sephadex G-15,

Table 1. Comparison of the *O*-acetyl substitution pattern* of xyloglucan oligosaccharides

Structure	SEPS xyloglucan oligosaccharides†	Cell wall xyloglucan oligosaccharides†
4,6-Di-*O*-acetyl galactosyl‡	10	5
4-*O*-Mono-acetyl galactosyl	5	5
3,6-Di-*O*-acetyl galactosyl	16	9
3-*O*-Mono-acetyl galactosyl	6	8
6-*O*-Mono-acetyl galactosyl	34	35
Total percentage of 2-linked galactosyl residues with one or two *O*-acetyl substituents	71	62

*Expressed as normalized per cent of galactosyl residues having the indicated *O*-acetyl substituents.

†Mixtures of xyloglucan nona- and decasaccharides obtained either from endoglucanase treatment of xyloglucan from the culture filtrate (SEPS) or from the cell walls of suspension cultured sycamore cells.

‡The 2-linked galactosyl residues were the only residues where *O*-acetylation was detected.

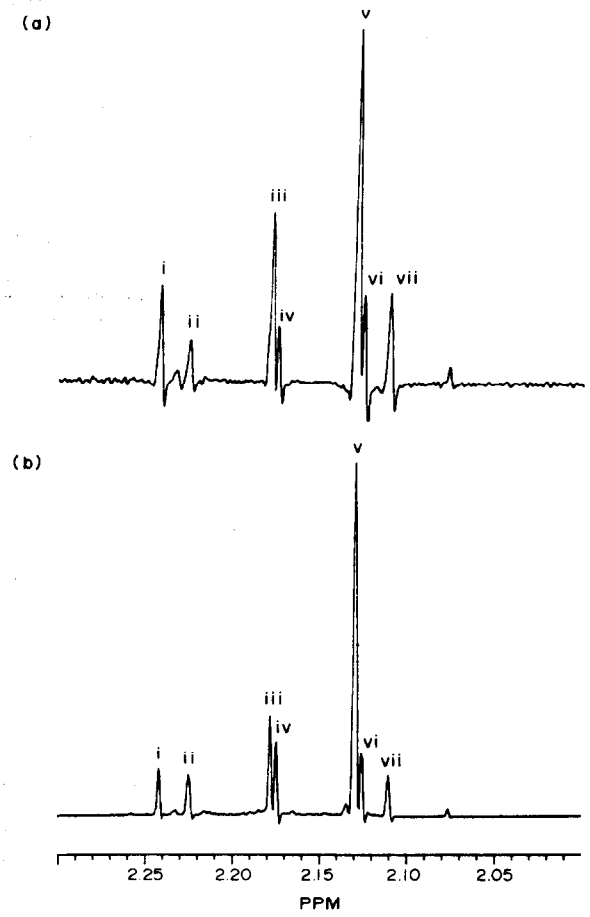


Fig. 2. *O*-Acetyl region of the resolution enhanced 500 MHz ^{1}H NMR spectra of the mixture of **1** and **2** isolated from (A) SEPS xyloglucan and (B) sycamore cell wall xyloglucan. Signals correspond to the methyl protons of the *O*-acetyl substituents located at: (i) C-4 of 4,6-di-*O*-acetyl β-D-galactosyl residues; (ii) C-4 of 4-*O*-acetyl β-D-galactosyl residues; (iii) C-3 of 3,6-di-*O*-acetyl β-D-galactosyl residues; (iv) C-3 of 3-*O*-acetyl β-D-galactosyl residues; (v) C-6 of 6-*O*-acetyl β-D-galactosyl residues; (vi) C-6 of 3,6-di-*O*-acetyl β-D-galactosyl residues; (vii) C-6 of 4,6-di-*O*-acetyl β-D-galactosyl residues. No acetone was added to samples when *O*-acetyl methyl protons were being quantitated, as the acetone signal coincides with those of the *O*-acetyl methyl protons at C-4 of 4-*O*-acetyl β-D-galactosyl residues.

concd, and chromatographed in H_2O on a column of Bio-Gel P-2 (85 × 1.6 cm). Fractions were obtained yielding a mixture in peak B of the nonasaccharide **2** and decasaccharide **1**, (Fig. 1, frs 30–33, ~3.8 anthrone glucose equivalents) and heptasaccharide **3** in peak C (Fig. 1, fractions 36–37, ~1.8 anthrone glucose equivalents). The structure of the oligosaccharide components contained in peak A are currently under investigation.

^{1}H NMR of the nona- and decasaccharide mixture and of the heptasaccharide. 500 MHz ^{1}H NMR spectra of oligosaccharides eluting in peak B and peak C (5–10 mM in D_2O) were recorded at 27°. A spectral width of 5000 Hz was used with 32 000 data points, and up to 464 scans were averaged. Chemical shifts are reported in ppm relative to 4,4-dimethyl-4-silapentane-1-sulphonate (DSS). Me_2CO was used as an int. standard (δ2.225). Fourier transformation was performed without resolution enhancement and integrals of signals diagnostic for *O*-acetyl substituents [8] were calculated. Alternatively, free induction decays were multiplied by a Gaussian resolution enhancement function [14] and Fourier transformed. Signal heights were measured from the resolution enhanced spectra and compared, in order to estimate the relative intensity of signals that were inadequately resolved for quantitation by integration. The results of these two approaches were combined to generate the data describing the relative proportions of the *O*-acetyl substituted forms in the cell wall nonasaccharide (Table 1).

Acknowledgements—This work was supported by U.S. Department of Energy grant # DE-FG09-85ER13426 and National Science Foundation grant # DMB-8545798. The authors thank D. Gollin for the supply of EPG-treated sycamore cell walls and J. Thomas for the purified 1,4-β-D-glucan 4-glucanohydrolase from *T. viride*.

REFERENCES

1. McNeil, M., Darvill, A. G., Fry, S. C. and Albersheim, P. (1984) *Annu. Rev. Biochem.* **53**, 625.
2. Hayashi, T., Polonenko, D. R., Camirand, A. and Maclachlan, G. (1986) *Plant Physiol.* **82**, 301.
3. Verma, D. P. S., Maclachlan, G. A., Byrne, H. and Ewings, P. (1975) *J. Biol. Chem.* **250**, 1019.
4. Coughlan, M. P. (1985) *Biotech. Genet. Eng. Rev.* **3**, 39.
5. Bauer, W. D., Talmadge, K. W., Keegstra, K. and Albersheim, P. (1973) *Plant Physiol.* **51**, 174.
6. York, W. S., Darvill, A. G. and Albersheim, P. (1984) *Plant Physiol.* **75**, 295.
7. Fry, S. C. (1986) *Planta.* **169**, 443.
8. York, W. S., Oates, J. E., Van Halbeek, H., Darvill, A. G., Albersheim, P., Tiller, P. R. and Dell, A. (1988) *Carbohydr. Res.* **173**, 113.
9. O'Neill, M. A., Morris, V. J., Selvendran, R. R., Sutherland, I. W. and Taylor, I. T. (1986) *Carbohydr. Res.* **148**, 63.
10. Biely, P., MacKenzie, C. R., Puls, J. and Schneider, H. (1986) *Bio/Technology* **4**, 731.
11. Becker, G. E., Hui, P. A. and Albersheim, P. (1964) *Plant Physiol.* **39**, 913.
12. Dische, Z. (1962) *Methods Carbohydr. Chem.* **1**, 477.
13. Blumenkrantz, N. and Asboe-Hansen, G. (1973) *Anal. Biochem.* **54**, 484.
14. Ernst, R. R. (1966) *Adv. Magn. Res.* **2**, 1.
15. Ishii, T., Thomas, J., Darvill, A. and Albershcim, P. (1989) *Plant Physiol.* **89**, 421.