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STRUCTURE OF HEMICELLULOSIC POLYSACCHARIDES OF *AVENA SATIVA* COLEOPTILE CELL WALLS

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Abstract—The glycosidic linkage compositions of intact and, in some cases, enzyme-degraded polysaccharides extracted from the cell walls of oat coleoptiles and subsequently purified have been examined. A major component is shown to be a glucuronoarabinoxylan similar in structure to those described for a variety of other monocots. The noncellulosic glucan component is a β-linked polymer containing both 1,4- and 1,3-linked glucosyl residues in a ratio of 2 to 1. Analysis of the oligosaccharide produced by 'lichenase' digestion of this β-glucan suggests that the the 1,3- and 1,4-glucosyl linkages repeat in regular fashion. A small amount of xyloglucan polysaccharides like those described for cell walls of dicots was also detected.

INTRODUCTION

A great deal of recent work has elucidated the structural characteristics of wall polysaccharides of dicot cells [1], and has led to a model for the molecular organization of dicot primary cell walls [2]. Primary walls of monocots, for which a detailed structural model is not yet available, were found in a survey of six cell cultures [3] and aleurone cells [4] to differ consistently from dicots in their major glycosidic linkages, which indicated that arabinoxylans are their principal matrix (noncellulosic) component. We have reported a fractionation of the cell walls of *Avena* coleoptiles [5] that shows glucuronoarabinoxylans (GAXs) and noncellulosic glucans to be major matrix polymers. The present paper analyzes the glycosidic linkages found in these products and shows their structure to be similar to polysaccharides that have been isolated from various older monocot tissues (see refs cited in [5]). We also offer an explanation of why the noncellulosic glucan component, which is a mixed-linked (β-1,3; β-1,4) glucan, was missed in the above-mentioned survey of monocot wall structure [3, 4].

RESULTS

The linkages found in different glucan and glucuronoarabinoxylan products obtained from *Avena* coleoptile walls by the previously described fractionation [5] are given in Tables 1 and 2.

Glucuronoarabinoxylan (GAX)

The linkages found (A2 and A3 in Table 1) conform with the structure that is familiar for this type of polysaccharide, consisting of a β-1,4 linked xylan backbone with substituents, primarily arabinose, in the 2- and/or 3-positions of about half of the backbone residues. The specific optical rotation of the GAX agrees with a β-linked backbone [5]. A minor fraction of the 2-substitutions must be the sites of attachment of glucuronic and 4-*O*-methylglucuronic acids, because these acids yield 2-linked aldobiouronic acids [6]. The occurance of 2-, 3- and 5-linked arabinose in amount much less than terminal arabinose suggests that some of the 2- or 3-positions of backbone xylose residues carry short side chains of 2 or more arabinose units while others carry a single arabinose unit. The extent of development of such side chains apparently differed between the 2 products that were analyzed. The minor amounts of terminal xylose and galactose may respresent arabinose side-chains that terminate with a xylose or galactose unit. Of the 2 methylation analyses, that of A2 is more satisfactory in that the number of terminal residues found (including allowance for uronic acids) corresponds relatively closely with the number of branch points found in the xylan backbone.

At neutral pH, under the conditions used to detect binding of arabinoxylan of barley aleurone walls to cellulose [4], none of the coleoptile GAX would bind to cellulose powder.

Glucans

These products contained both 1,3- and 1,4-linkages, in different proportions among the different fractions. The soluble glucans (G1 and G2, Table 2) were virtually unbranched. In view of the specific optical rotation of −7° found for G1 [5] it, and most likely the other glucans, are probably β-linked. These glucans are degraded by β-glucanases from *Streptomyces* [7] and *Rhizopus* [8] to yield small amounts of cellobiose and laminaribiose, respectively, plus in each case a larger amount of an oligosaccharide that chromatographed like a trisaccharide (Fig 1). The linkages that were found in the trisaccharide from *Rhizopus* glucanase digestion of G2 are given in Table 2, and show it to be a mixed-linked trisaccharide, probably (cf. [9]) cellobiosyl-(β-1,3)-glucose. The trisaccharide yielded by digestion with *Streptomyces* glucanase was not isolated and characterized but

Table 1. Linkage composition of arabinoxylans and of insoluble glucan fraction (G3)

Sugar	Linkage	Arabinoxylans A2	Arabinoxylans A3 (%)	'Glucan' G3
Xyl	*t*	2	5	12
	4	20	23	6
	2,4 + 3,4	34*	24*	9
	2,3,4	6	2	3
	total	**52**	**55**	**29**
Ara	*t*	30	28	3
	2	2	6	1
	3	3	5	1†
	5	4	6	<3‡
	total	**35**	**41**	**11**
Gal	*t*	n.d.	2	⩽3‡
	total	**7**	**3**	**6**
Glc	3	n.d.	n.d.	3
	4	n.d.	n.d.	37
	4,6	n.d.	n.d.	18
	total	**2**	**⩽0.5**	**54**
Rha	**total**	**2**	**⩽0.5§**	—
Uronic acid	**total**‖	**8**	**7**	**4**

Linkage positions are assigned from positions of methyl groups as in ref. [2] and the data represent mole % of the corresponding methyl derivatives that were recovered: *t* indicates non-reducing terminal groups; n.d., not detected. Values for **total** mole % of each monosaccharide are not sums of the methylated derivatives but come from independent determination of the overall monosaccharide composition; these provide a consistency check on recovery of methylated derivatives

* Mass spectroscopy indicated the presence of both 2,4- and 3,4-Xyl; these derivatives were not separated. † Includes some *t*-Glc.‡ These derivatives were not separated; from the definite presence of Gal in the overall monosaccharide composition the peak presumably represents t-Gal at least in part § A trace of fucose was also detected ‖ From ref. [5]. These figures represent % of total monosaccharides and uronic acids recovered by paper chromatography and are not included in estimating the % composition of monosaccharides by GLC ('total' figures given above). The uronic acid is almost entirely glucuronic and 4-*O*-methylglucuronic acid [5].

its chromatographic mobility was identical with that of authentic laminaribiosyl-(β-1,4)-glucose, which is known to be released from mixed-linked (β-1,3; β-1,4) glucans by this enzyme preparation [9]. The results show that both kinds of linkages occur interspersed within one glucan chain, as in the mixed-linked β-glucans of cereal seeds.

Glucans G1 and G2 are degraded by a commercial 'α-amylase' preparation from *Bacillus subtilis*, yielding a trisaccharide similar to that obtained with *Rhizopus* glucanase. This trisaccharide was isolated and methylated with results essentially identical to those shown for the G2 trisaccharide in Table 2. We found that the *B. subtilis* enzyme preparation also degraded lichenin to yield an apparently identical trisaccharide, plus a small amount of a second oligosaccharide which behaved as a pentasaccharide in passing a calibrated Biogel P-2 column, and upon methylation proved to contain three

Table 2. Linkage composition of coleoptile glucans and lichenin, and of oligosaccharides derived from them by enzymatic degradation

Sugar and linkage	Coleoptile glucans G1	Coleoptile glucans G2	Trisacc from G2 (%)	Lichenin ungraded	Lichenin trisacc	Lichenin pentasacc
t-Glc	1	⩽0.5	28	trace	30	18
3-Glc	21	34	34	28	35	22
4-Glc	76	66	38	72	35	60
4,6-Glc	2	—	-	- -	—	—
Xyl	1*	2*	-	—	-	—

Data are expressed as stated in Table 1. Data on oligosaccharides are for products obtained by treatment with *Rhizopus* glucanase; essentially identical results were obtained by degradation with *B. subtilis* 'α-amylase'

* From determination of total monosaccharide composition. These figures are not included in estimating the % linkage composition from recovery of methylated derivatives.

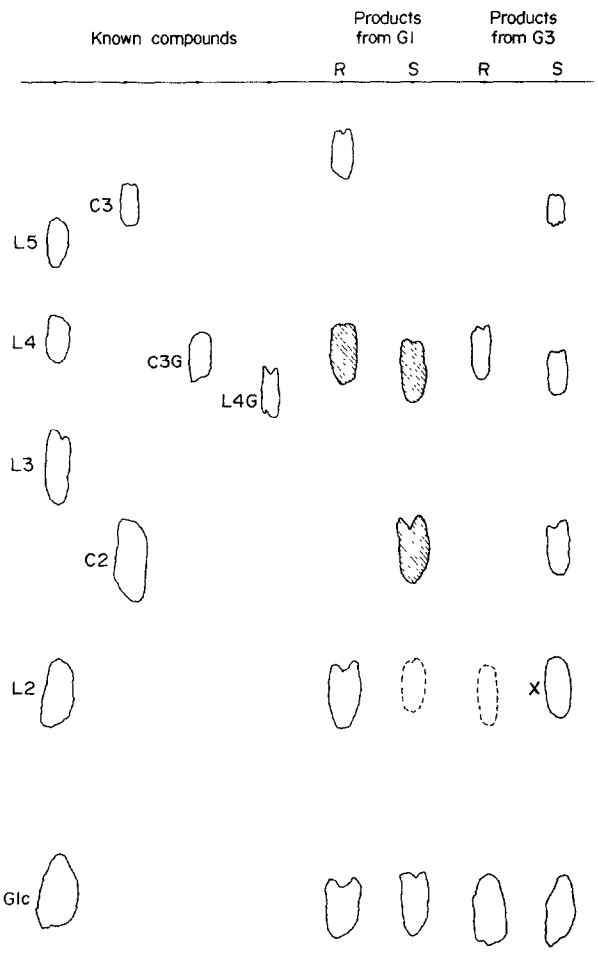

Fig. 1. Chromatographic separation of oligosaccharides released by treatment of glucans G1 and G3 with (S) *Streptomyces* β-1,4-glucanase and (R) *Rhizopus* β-1,3-glucanase. Comparison compounds: Glc, glucose; C2, cellobiose; C3, cellotriose; L2, laminaribiose; L3, laminaritriose; L4, laminaritetraose; L4G, laminaribiosyl-(β-1,4)-glucose; C3G, cellobiosyl-(β-1,3)-glucose. Line at top represents origin. X, pentose-containing oligosaccharide (red color with aniline phthalate reagent). All other spots gave a brown color reaction with aniline phthalate. Shaded spots indicate the major component(s); dashed lines indicate trace components.

Table 3. Linkage composition of oligosaccharides obtained by enzymatic degradation of insoluble glucan (G3)

Sugar and linkage	Trisaccharide		Pentasaccharide	
	found	predicted*	found	predicted*
	(%)			
t-Xyl	26	33	33	40
6-Glc	39	33	20	20
4-Glc	35	33	24	20
4,6-Glc	—	—	23	20

Degradation was by cellulysin as described in Experimental.
* Predicted for a trisaccharide and a pentasaccharide, respectively, derived from a xyloglucan chain, viz., Xyl-(1 → 6)-Glc-(1 → 4)-Glc and Xyl-(1 → 6)-Glc-(1 → 4)-[Xyl-(1 → 6)]-Glc-(1 → 4)-Glc.

(1,4)-linkages and one (1,3)-linkage for each non-reducing terminal glucose unit (Table 2). An apparently identical pentasaccharide was obtained from lichenin using the *Rhizopus* glucanase. These results indicate, in agreement with earlier findings [14–16, 25], that the *B. subtilis* amylase preparation contains a glucanase that can degrade mixed-linked glucans.

The water-insoluble glucan fraction (G3, Table 1) showed a lower ratio of 1,3- to 1,4-linked glucose than G1 or G2. G3 differed also in containing a substantial amount of xylose, much of which occurs as terminal residues, an appreciable proportion of 4,6-linked glucose residues (Table 1), and a minor amount of arabinose. The only terminal residues detected in sufficient number to account for these branch points are xylose. G3 evidently contains glucan chains substituted by xylose side groups, or by side chains that terminate in xylose. The linkages mentioned agree with the structure of xyloglucans of dicot primary walls [1], which possess a β-1,4-glucan backbone substituted in the 6-position with xylose units. This was confirmed by enzymatic degradation of G3, which yielded, upon fractionation on a Biogel P2 column, a pentasaccharide (29% of the solubilized carbohydrate) and a trisaccharide (6.6%) that contain xylose and glucose in the linkages typical of xyloglucans (Table 3).

The arabinose and the remainder of the xylose found in G3 exhibit the linkages characteristic of arabinoxylan, which presumably is admixed in this fraction.

DISCUSSION

The glucuronoarabinoxylans (GAXs) of *Avena* coleoptile walls are clearly similar to acidic polysaccharides that have been characterized from a number of monocot tissues (refs cited in [5]). Their linkages also resemble those in arabinoxylans of cultured monocot cells [3] and barley aleurone cells [4]. Some of these polysaccharides may also be GAXs, although that from barley aleurone was reported to contain no uronosyl residues [4]. The coleoptile GAXs contain a minor amount of terminal galactose, which from evidence obtained from fractionation [5] is attached to the GAX polymer, and must occur either as side groups or as the terminal groups of side chains.

The *Avena* coleoptile arabinoxylans differ from those of barley aleurone walls in several respects besides the presence of uronosyl groups. Aleurone arabinoxylan is composed of two subfractions, one rich in arabinosyl substitution and one whose xylan backbone is sparsely substituted. The latter material, which comprises about 70% of the total aleurone arabinoxylan, binds to cellulose powder at neutral pH [4], from which it was suggested that arabinoxylan in monocot walls associates with cellulose microfibrils like xyloglucan does in dicot walls [4]. However, the *Avena* coleoptile hemicellulose, when fractionated by any of several methods ([5], and unpublished data of S. Wada and P. M. Ray) yielded subfractions of similar arabinose: xylose ratio, and as noted above none of the isolated GAX would bind to cellulose. This is not surprising since the isolated GAX fractions possess a high degree of branching (arabinose substitution) of their xylan chains, like the aleurone arabinoxylan component that did not bind to cellulose [4].

In view of the large amount of GAX in the *Avena* coleoptile wall it seems likely that, as suggested for arabinoxylans of other monocot walls [3, 4], the GAXs comprise a significant structural element. The lack of strong tendency for coleoptile GAXs to bind to cellulose may indeed be important in the coleoptile cell wall's capability for extension growth, as compared with the aleurone cell [4]. In maize coleoptiles glucuronoxylan was reported to decrease during auxin-induced elongation, suggesting a possible role in cell wall extension [10].

Our results [5] indicate that the hemicellulosic glucan that is here shown to be mixed-linked (1,3- and 1,4-; probably β-), is also a prominent structural wall component. It is probably the glucan component that has been observed to decrease under auxin treatment [11], a breakdown that is thought to be involved in cell wall expansion during growth [12]. The enzymatic degradation of this glucan largely to the trisaccharide described in Table 4 indicates a fairly regular repeating structure composed of one β-1,3-linked glucose alternating with two β-1,4-linked glucose units. This would agree with the approximately 2:1 ratio of 1,4- to 1,3-linkages in the glucan indicated by the methylation results.

Although mixed-linked glucans have been widely encountered in monocot tissues (refs cited in [5]), this type of component was not detected in work on several cultured monocot cells [3] or barley aleurone [4], even

Table 4. Polysaccharide composition of oat coleoptile hemicellulose-B

	Hemicellulose-B subfractions				
	A1–A3	A4 + R	G1 + G2	G3	Totals
Mixed-linked glucan	—	5.5*	21	5.5	32
Xyloglucan	—	(—)	—	9.5	9.5
Glucurono-arabinoxylan	35	14.5		6.5	56
Mannan	—	1		—	1

Component polysaccharides are estimated in % of the total polysaccharide recovered in the fractionation of hemicellulose-B (ref. [5], Fig. 3) from considerations of linkage composition discussed in the text, and data on monosaccharide composition of the subfractions given in ref. [5], Table 1.
* Part of this could be contained in a glucomannan, in which case the amount of glucomannan would be greater than the figure for mannan given below.

though it is found in wall preparations from cultured aleurone cells of the grass *Lolium* [13]. In the work that indicated an absence of mixed-linked glucan [3, 4] the wall materials had been treated with an α-amylase preparation from *Bacillus subtilis* to remove starch. Our results and others [9, 14–16] indicate that this amylase preparation is contaminated with an enzyme ('lichenase') that breaks down mixed-linked glucans. This invalidates the conclusion that mixed-linked glucans do not occur in certain monocot walls [3, 4] and the inference that such glucans are not widespread monocot wall components [3].

The evidence presented regarding the water-insoluble glucan fraction G3 shows that it contains, in addition to 1,3- and 1,4-linked glucose, a component that has the same structure as the xyloglucans of dicot primary walls. The question of whether xyloglucans are present in monocot walls has heretofore been unsettled [3]. Their presence opens the possibility that extension growth of primary walls of monocots could involve a phenomenon similar to that observed in growing dicot walls, where solubilization of previously deposited xyloglucan occurs [17, 18].

The amount of xyloglucan in G3 can be estimated by considering that all of the 4,6-linked glucose in G3 (18 mole %, Table 1) is probably in xyloglucan, which should also contain at least half this amount (9%) of 4-linked glucose since as noted in Results the xyloglucan could be enzymatically degraded mainly to a pentasaccharide that contains one 4-linked glucose for every two 4,6-glucose units. The 4,6-linked glucose units must also be accompanied by an equal number of terminal residues, including the 12% of *t*-xylose that was found, plus 6% of other termini probably including galactose. These xyloglucan components add up to a total of 45 mole % of the G3 preparation, which agrees reasonably well with the yield of 35% of xyloglucan oligosaccharides obtained by enzymatic degradation of G3. Since G3 was obtained in 11% yield [5] from the hemicellulose-B fraction of the cell wall and the latter comprises 38% of the wall holocellulose [5], xyloglucan apparently constitutes at least 1.5% of the whole wall. This is undoubtedly an underestimate since xyloglucan probably occurs also in other wall fractions, including hemicellulose-A [5], and also was inevitably not fully recovered in the fractionation of hemicellulose-B by which G3 was isolated. Darville *et al.* [10] reported that whole maize coleoptile walls contain about 2.5 mole % of 4,6-linked glucose, which would suggest a xyloglucan content of as much as 5%. Xyloglucan cannot, however, be as important a component as in dicot primary walls [1], making it likely that biochemical mechanisms involving other polymers, such as mixed-linked glucan, are important in cell wall expansion [11, 12].

The G3 fraction also evidently contains some glucuronoarabinoxylan. If the composition of this component were similar to that of the isolated GAXs (A subfractions, ref. [5], Table 1) it would comprise about 30% of G3, with the remainder (25%) presumably comprising mainly mixed-linked glucan, which agrees approximately with the amount of 4- and 3-linked glucose (40%) found in G3 (Table 1) less the *ca* 9% of 4-linked glucose that should be contained in xyloglucan. A minimum estimate of the total amount of mixed-linked glucan in the hemicellulose-B fraction can be made by adding the estimated glucan in G3 to the amount of virtually pure glucan obtained in fractions G1 and G2 (ref. [5], Fig. 3). Similarly the amount of GAX can be estimated by adding the amount in G3, as estimated above, to the amounts represented by fractions A1–A3 and the fractional amounts of GAX apparently contained in mixed fractions A4 and R (ref. [5], Fig. 3 and Table 1). These evaluations are summarized in Table 4, which shows the polysaccharide composition of hemicellulose-B estimated as a percent of the total polysaccharides recovered during the fractionation of this material reported in [5]. Since hemicellulose-B is the principal matrix fraction of the coleoptile wall [5], Table 4 gives an idea of the composition of the wall matrix as a whole except for its pectic components, which from earlier data [5] appear to comprise not more than about 5% of the whole wall matrix.

EXPERIMENTAL

Polysaccharide analysis. Neutral sugar compositions of the two classes of purified polysaccharides were determined as described [19]. Glycosidic linkage compositions were determined by GLC of permethylated alditol acetates. Samples were methylated by the Hakomori method [20], and the permethylated polysaccharides were further derivatized and analyzed as described [21] except that the partially methylated aldoses were reduced with $NaBD_4$ rather than $NaBH_4$ to aid in MS analysis of otherwise symmetrical derivatives. Identification of partially methylated alditol acetates was based on co-chromatography with standards (synthesized by methylation and hydrolysis of known disaccharides and polysaccharides), and MS analysis. GLC was performed on a Varian series 1520 B instrument (Varian Associates, Los Altos, California). A 120 × 0.3 cm (outside diameter; *ca* 0.15 cm i.d.) copper column containing a mixture of 0.2% poly (ethylene glycol adipate), 0.2% poly (ethylene glycol succinate) and 0.4% silicone XF-1150 on Gas Chrom P (100–120 mesh) was used [21]. For alditol acetates temp. was programmed from 125 to 170° (1°/min), and for partially methylated alditol acetates, from 100 to 170° (1°/min); the nitrogen flow rate was 20 cm^3/min. The amounts of material in each peak were estimated by measuring the area under the peak with a Hewlett-Packard 9864A digitizer interfaced to a 9810 programmable calculator and applying appropriate response factors for each derivative [22].

Enzymic degradation. β-1,4-Glucanase from *Streptomyces* QM B814 [7], and β-1,3-glucanase from *Rhizopus arrhizus* S 176 G [8], were gifts of Dr. E. T. Reese, US Army Natick Laboratories, Natick, Mass.; 0.1 mg of enzyme was incubated with 0.5 mg of polysaccharide substrate at 45° in 1 ml of 100 mM NaOAc buffer, pH 4.5 for 4 hr. α-Amylase (0.1 mg) from *Bacillus subtilis* (Type II-A, Sigma) was incubated with 0.5 mg of substrate at 37° in 1 ml of 100 mM K phosphate, pH 7.0, for 5 hr. 'Cellulysin' (Grade B, Calbiochem) was desalted by passing through Sephadex G25; 20 mg of glucan G3 was incubated with shaking for 12 hr at 37° in 1.5 ml of acetate buffer (pH 4.5) containing 0.3 mg cellulysin, in the presence of thimerosal to prevent microbial growth. After incubation any insoluble material was removed by centrifugation and products were either deionized, freeze-dried, spotted on Whatman No. 1 filter paper sheets and chromatographed using *n*-PrOH–EtOAc–H_2O (7:1:2) (Fig. 1), or were passed through a 1.5 × 120 cm Biogel P-2 column with a flow rate of 8–10 ml/hr, at 50°, collecting 1.6 ml fractions. The column had been calibrated by passing known oligosaccharides through it. Carbohydrate in the effluent was determined by the anthrone method [23]. In each case the major peaks corresponded in elution volume to a trisaccharide and/or a pentasaccharide. The three top fractions of each of these peaks were pooled for methylation analysis which was performed as above. Laminaribiosyl-(β-1,4)-glucose and cellobiosyl-(β-1,3)-glucose for chromatographic comparisons were gifts of Dr. E. T. Reese (see [24]). Lichenin, used as a comparison substrate for the enzymatic degradations, was from Pfaltz and Bauer, Stamford, Conn.

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REFERENCES

1. Albersheim, P. (1976) in *Plant Biochemistry* (Bonner, J. and Varner, J. E. eds) 2nd edn, p. 225. Academic Press, New York.
2. Keegstra, K., Talmadge, K. W., Bauer, W. D. and Albersheim, P. (1973) *Plant Physiol.* **51**, 188.
3. Burke, D., Kaufman, P., McNeil, M. and Albersheim, P. (1974) *Plant Physiol.* **54**, 109.
4. McNeil, M., Albersheim, P., Taiz, L. and Jones, R. L. (1975) *Plant Physiol.* **55**, 64.
5. Wada, S. and Ray, P. M. (1978) *Phytochemistry* **17**, 923.
6. Ray, P. M. and Rottenberg, D. A. (1964) *Biochem. J.* **90**, 646.
7. Reese, E. T., Smakula, E. and Perlin, A. S. (1959) *Arch. Biochem. Biophys.* **85**, 28.
8. Reese, E. T. and Mandels, M. (1959) *Can. J. Microbiol.* **5**, 173.
9. Anderson, M. A. and Stone, B. A. (1975) *FEBS Letters* **52**, 202.
10. Darville, A. G., Smith, C. J. and Hall, M. A. (1977) in *Regulation of Cell Membrane Activities in Plants* (Marrè, E. and Ciferri, O. eds) p. 275. North-Holland, Amsterdam.
11. Loescher, W. H. and Nevins, D. J. (1973) *Plant Physiol.* **52**, 248.
12. Nevins, D. J. (1975) *Plant Cell Physiol.* **16**, 347.
13. Smith, M. M. and Stone, B. A. (1973) *Phytochemistry* **12**, 1361.
14. Huber, D. J. and Nevins, D. J. (1977) *Plant Physiol.* **60**, 300.
15. Moscatelli, E. A., Ham, E. A. and Rickes, E. L. (1961) *J. Biol. Chem.* **236**, 2858.
16. Reese, E. T. and Perlin, A. S. (1963) *Biochem. Biophys. Res. Commun.* **12**, 194.
17. Labavitch, J. and Ray, P. M. (1974) *Plant Physiol.* **54**, 499.
18. Jacobs, M. and Ray, P. M. (1975) *Plant Physiol.* **56**, 373.
19. Albersheim, P., Nevins, D. J., English, P. D. and Karr, A. (1967) *Carbohyd. Res.* **5**, 340.
20. Hakomori, S. (1964) *J. Biochem.* **55**, 205.
21. Talmadge, K. W., Keegstra, K., Bauer, W. D. and Albersheim, P. (1973) *Plant Physiol.* **51**, 158.
22. Sweet, D. P., Shapiro, R. H. and Albersheim, P. (1975) *Carbohyd. Res.* **40**, 217.
23. Dische, Z. (1962) in *Methods in Carbohydrate Chemistry* (Whistler, R. L. and Wolfrom, M. L. eds) Vol. 1, p. 477. Academic Press, New York.
24. Parrish, F. W., Perlin, A. S. and Reese, E. T. (1960) *Can. J. Chem.* **38**, 2094.
25. Kivilaan, A., Bandurski, R. S. and Schulze, A. (1971) *Plant Physiol.* **48**, 389.