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METHYLATION ANALYSIS OF MESOPHYLL, EPIDERMIS, AND FIBRE CELL-WALLS ISOLATED FROM THE LEAVES OF PERENNIAL AND ITALIAN RYEGRASS

ANDREW CHESSON, ALEX H. GORDON, AND JAMES A. LOMAX
Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB (Great Britain)
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ABSTRACT

Intact, finely milled mesophyll, epidermis, and fibre cell-walls prepared from the leaves of perennial and Italian ryegrass have been subjected to methylation analysis. Methylation of the cell-walls led to a consistently higher recovery of glucose residues than that obtained by analysis of monosaccharide residues as their alditol acetates. Values for other sugars were in close agreement. The partially methylated sugars formed were consistent with the presence, in order of decreasing concentration, of cellulose, (glucurono)arabinoxylan, xyloglucan, rhamnogalacturonan, (1→3),(1→4)-linked glucan, (1→4)-linked galactan, and (1→3),(1→6)-linked galactan. The relative proportions of these polysaccharides differed between the various types of cell. Arabinoxylan comprised 21.6%, 26.7%, and 36.5% of the total sugars recovered from mesophyll, epidermis, and fibre cell-walls, respectively. Mixed-linked glucan and rhamnogalacturonan were found in epidermis walls in amounts 2- to 3-fold higher than in other cell-walls. The xylan backbone of arabinoxylan was more heavily substituted in primary than in secondary-thickened (fibre) cell-walls. Arabinose, found largely as terminal residues in the cell-walls, carried various amounts of alkali-labile substituents, particularly at position 5. The extent of 5-substitution reflected the phenolic content and was substantially higher in fibre cell-walls. The methylation data, coupled with the analytical data for uronic acids and non-carbohydrate components, accounted for ~98% of the cell-wall dry matter.

INTRODUCTION

Plant cell-walls form the major part of the forage dry matter fed to ruminants and represent the roughage component of the diet. The composition of the cell-walls is related to their resistance to digestion by rumen micro-organisms. Although the phenolic content is important in determining digestibility, the structure of the cell-wall polysaccharides also can affect the rate of microbial degradation[1]. Whereas the analysis of forage plants or plant parts has been undertaken frequently, less attention has been paid to the composition of cell-walls from specific

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cell types. Gordon *et al.*[2] described the extraction of mesophyll cells from the leaves of ryegrass spp. and the gross composition of their cell-walls. Residual non-mesophyll cells have been further fractionated by centrifugation in aqueous medium of known density and cell-walls have been prepared from fractions arising from the epidermis and schlerenchyma[3]. Analysis of these cell-walls showed clear differences in polysaccharide composition which differed also from those in mesophyll cell-walls[2]. However, <90% of dry matter present was accounted for. It was suggested that much of this shortfall was due to incomplete hydrolysis of the structural polysaccharides. Methylation of cell-wall polysaccharides *in situ* overcomes the problems associated with the differential susceptibility of glycosidic linkages to acid hydrolysis and provides fuller information about polysaccharide structure[4].

We have applied methods, developed[4] for the quantitative methylation and acetalation–methylation of intact cell-walls, to the cell-wall polysaccharides from mesophyll, epidermis, and fibre cells isolated from the leaves of perennial and Italian ryegrass harvested at different stages of maturity.

EXPERIMENTAL

Plant material. — Samples of perennial ryegrass (*Lolium perenne* cv. Perma) and Italian ryegrass) *Lolium multiflorum* cv. RvP) were obtained from trial plots of the pure sward grown at the North of Scotland College of Agriculture. First cuts were made in early June and in October (early- and late-cut samples, respectively) and second cuts were made of both grasses in September from grass first cut in July. Leaves were removed from the fresh material and stored at −20°.

Cell-wall preparation. — Mesophyll cell-walls were prepared by the methods of Gordon *et al.*[2]. The non-mesophyll cells remaining after extraction of the mesophyll were freeze-dried, dry-milled, suspended in aqueous 56% (w/v) metrizamide (100 mL), and centrifuged overnight at 800*g* and 5°. The epidermal cells formed a band at the top of the tube and the fibre cells sedimented. Further centrifugations were required to obtain homogeneous suspensions before the cells were broken[3] to release cell contents.

Methylation analysis. — Whole cell-walls were dry-milled in liquid nitrogen, and samples (~10 mg) were suspended in methyl sulphoxide (1 mL) containing methyl β-D-allopyranoside (0.15 mg, internal standard) and methylated[4] with freshly prepared sodium methylsulphinylmethanide in methyl sulphoxide (1 mL) and methyl iodide. Quebrachitol, added after the methylation but before hydrolysis, was used as the second internal standard. Methylations were performed in triplicate.

The positions of alkali-labile linkages in the matrix polysaccharides of the cell-wall were located by the acetalation–methylation procedure[4].

G.l.c. of the partially methylated sugars was performed on Carlo-Erba (Fractovap 4160 and HRGC 5160 Mega) gas chromatographs with on-column injection

and silica columns (50 m × 0.3 mm) wall-coated with SP1000, CP-Sil88 (-Chrompack), or BP1 (chemically bonded phase of OV1, S.G.E.). Chromatogram peaks were integrated by using Spectra-Physics SP4270 and Trivector integrators, and data from the primary integrations were fed into a Prime 550 computer. Peaks were identified on the basis of their retention coefficients relative to those of the products derived from the added standards[5]. Information on the identity and amount of each partially methylated derivative obtained from each of the three columns was reconciled by using a computer-based procedure[6]. Where identifications were in doubt, mass spectrometry was used. The concentration of minor components in samples intended for m.s. was increased by the selective collection of effluent from a chromatograph equipped with an effluent splitter and fitted with a stainless-steel column (2 mm × 4 m) packed with 3% of SP2340 on Gas-Chrom Q (100–120 mesh).

RESULTS AND DISCUSSION

The results of methylation analysis of the polysaccharides in finely milled samples of the intact cell-wall are given in Tables I and II. The use of methyl β-D-allopyranoside as internal standard allowed recoveries to be compared with those obtained by analysis by the standard alditol acetate method[3]. When the contents of non-carbohydrate and uronic acid were included, 97–98% of the cell-wall could be accounted for. Much of the shortfall found previously[3], when sugar residues were determined after Seaman hydrolysis, was due to incomplete hydrolysis of cellulose. Recoveries of the pentoses generally were in good agreement and conditions of acid hydrolysis appeared to be optimal for their recovery. The difficulty of accounting for the total dry matter of grass-based feedstuffs when using methods other than those based on gravimetric analysis has been commented on[7]. Our results suggest that this is probably due to incomplete hydrolysis of wall polysaccharides.

The pattern of glycosidic linkages found for all three cell types, and for both grasses, was consistent with the presence of the polysaccharides previously identified in other members of the Gramineae. These include rhamnogalacturonan, (1→4)-galactan, 4-*O*-methylglucuronoarabinoxylan, xyloglucan, (1→3),(1→4)-β-D-glucan, and cellulose[8,9].

Arabinoxylan. — The xylose residues in the arabinoxylan were (1→4)-linked and the derived methylated sugars were the second most abundant, representing ~14% of mesophyll, ~19% of epidermis, and ~33% of fibre walls. The ratio of unsubstituted xylose residues to those carrying acid-labile (glycosidic) substituents at positions 2 or 3 varied greatly between cell types but remained constant within cell types, at ~1:1.4 for mesophyll, ~1:0.9 for epidermis, and ~1:0.3 for the fibre samples. The amounts of xylose residues with acid-labile substituents in primary and secondary-thickened cell types (6–7% dry matter) were similar despite a two-fold difference in the xylose content of mesophyll and fibre cells. This finding suggested that much of the xylose laid down during secondary-thickening was

TABLE I

METHYLATION ANALYSIS DATA FOR INTACT, PRIMARY, MESOPHYLL AND EPIDERMIS CELL-WALLS PREPARED FROM THE LEAVES OF ITALIAN (RvP) AND PERENNIAL (Perma) RYEGRASS

Sugar	*Composition[a] (%)*					
	Mesophyll cell-walls		*Epidermis cell-walls*			
	Perma (early cut)	*RvP (early cut)*	*Perma (early cut)*	*RvP (early cut)*	*RvP (regrowth)*	*RvP (late cut)*
2,3,4-Ara[a]	0.02	0.03	0.06	0.06	0.05	0.06
2,3,5-Ara	4.38	4.05	4.04	3.39	3.46	2 83
2,3-Ara	0.44	0.36	0.57	0.51	0.76	0.52
2,5-Ara	0.75	0.89	0.61	0.63	0.69	0.77
3,5-Ara	0.48	0.49	0.45	0.51	0.43	0.50
2-Ara	0.26	0.25	0.04	0.03	0.10	0.03
5-Ara	0.04	0.03	0.03	0	0.07	0.08
Total[c]	6.37 (7.47)	6.10 (6.79)	5.80 (6.34)	5.13 (6.32)	5.56 (6.43)	4.79 (6.03)
2,3,4-Xyl	0.79	0.88	0.76	0.59	0.74	0.24
2,3-Xyl	3.97	3.78	8.78	8.59	6.45	6.26
2,4-Xyl	0.04	0.05	0.08	0.08	0.06	0.04
2-Xyl	4.61	4.65	4.48	4.62	4.19	4.42
3-Xyl	1.32	1.81	2.28	2.87	2.10	2.27
Xyl	0.30	0.24	0.31	0.31	0.32	0.05
Total	11.04 (11.11)	11.41 (10.61)	16.69 (16.08)	17.06 (17.27)	13.86 (14.67)	13.73 (13.60)
2,3,4-Rha	0.25	0.26	0.08	0.14	0.12	0.10
3,4-Rha	0.21	0.17	0.14	0.12	0.17	0.15
2,4-Rha	0.16	0.15	0	0.06	0	0.08
3-Rha	0.37	0.36	0.30	0.38	0.46	0.54
Total	0.83 (0.56)	0.79 (0.90)	0.52 (0.43)	0.70 (0.45)	0.75 (0.45)	0.87 (0.40)
2,3,4-Fuc	0.02	0.06	0.09	0.10	0.07	0.09
2,3-Fuc	0.02	0.01	0.04	0.07	0.04	0
2,4-Fuc	0	0	0.06	0	0.06	0.06
Total	0.04 (0.09)	0.07 (0)	0.19 (0.12)	0.17 (0.18)	0.17 (0.47)	0.15 (1.10)
2,3,4,6-Man	trace	trace	trace	trace	trace	trace
2,3,6-Man	0.33	0.35	0.16	0.10	0.13	0.16
Total	0.33 (0.36)	0.35 (0.35)	0.16 (0.18)	0.10 (0.14)	0.13 (0.17)	0.16 (0.22)
2,3,4,6-Gal	0.99	1.40	1.35	1.34	1.39	1.76
2,3,4-Gal	0.62	0.76	0.24	0.32	0.32	0 47
2,3,6-Gal	0.63	0.68	0.22	0.26	0.38	0.34
2,4,6-Gal	0.18	0.15	0.05	0.26	0.04	0.02
2,3-Gal	0 03	0	0.09	0	0	0
2,4-Gal	0.04	0.09	0	0	0.14	0.17
Total	2.49 (2.38)	3.08 (2.78)	1.95 (2.08)	2.18 (2 16)	2.27 (2.41)	2.76 (2.74)
2,3,4,6-Glc	0.67	0.80	0.66	0.71	0.59	0.59
2,3,6-Glc	55.44	49.14	51.17	51.73	47.51	49.79
2,4,6-Glc	0.89	0.41	2.11	1.89	2.16	2.11
2,3-Glc	1.33	1.35	1.47	1.23	1.28	1.22
2,6-Glc	0.71	0.63	0.78	0.64	0.65	0.86
3,6-Glc	0.37	0.38	0.41	0.34	0.38	0.42
Total	59.39 (40.45)	52.71 (37.66)	56.60 (44.29)	56.54 (43.55)	52.57 (36.45)	54.99 (40.51)
Total neutral sugars	80.49	74.48	81.85	81.88	75.31	77.45
Uronic acid and non-carbohydrate[d]	18.51	24.98	15.66	14.86	22.78	19.69
Overall total	99 00	99.46	97.41	96.74	98.09	97.14

[a]Values are given as % dry matter and are based on "anhydro sugar" residues. [b]2,3,4-Ara denotes 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylarabinitol, *etc.* [c]Values in brackets are based on alditol acetate analysis. [d]Data taken from Gordon *et al.*[3]

TABLE II

METHYLATION ANALYSIS DATA FOR INTACT, SECONDARY-THICKENED FIBRE CELL-WALLS PREPARED FROM THE LEAVES OF ITALIAN (RvP) AND PERENNIAL (Perma) RYEGRASS

Sugar	*Composition*[a]			
	Perma (early cut)	*RvP (early cut)*	*RvP (regrowth)*	*RvP (late cut)*
2,3,4-Ara[b]	0.01	0.02	0.02	0.02
2,3,5-Ara	2.62	2.72	2.73	2.43
2,3-Ara	0.31	0.35	0.48	0.28
2,5-Ara	0.21	0.27	0.24	0.21
3,5-Ara	0.56	0.40	0.36	0.38
2-Ara	0	0	0.08	0.02
5-Ara	0.04	0.04	0.08	0.06
Total[c]	3.75 (3.67)	3.80 (3.58)	3.99 (3.53)	3.40 (3.14)
2,3,4-Xyl	1.09	0.90	0.44	0.24
2,3-Xyl	20.59	20.27	19.80	20.16
2,4-Xyl	0.08	0.08	0.01	0.06
2-Xyl	3.67	4.35	4.10	4.14
3-Xyl	1.61	1.74	1.76	1.23
Xyl	0.36	0.87	0.30	0.11
Total	27.40 (25.01)	28.21 (25.83)	26.40 (24.38)	25.94 (21.25)
2,3,4-Rha	0.05	0.07	0.04	0.04
3,4-Rha	0.04	0.04	0.08	0.04
2,4-Rha	0	0	0	0
3-Rha	0.11	0.15	0.19	0.14
Total	0.20 (0.17)	0.26 (0.15)	0.31 (0.22)	0.22 (0.22)
2,3,4-Fuc	0.04	0.05	0.04	0.04
2,3-Fuc	0.04	0.03	0.03	0.02
2,4-Fuc	0	0.04	0.03	0.04
Total	0.08 (0.09)	0.13 (0.09)	0.10 (0.95)	0.10 (0.93)
2,3,4,6-Man	trace	trace	trace	trace
2,3,6-Man	0.07	0.12	0.16	0.06
Total	0.07 (0.10)	0.12 (0.13)	0.16 (0.32)	0.06 (0.19)
2,3,4,6-Gal	0.51	0.52	0.54	0.49
2,3,4-Gal	0.07	0.17	0.13	0.10
2,3,6-Gal	0.11	0.26	0.25	0.14
2,4,6-Gal	0	0.02	0	0.02
2,3-Gal	0	0.13	0	0
2,4-Gal	0.10	0.14	0.18	0.12
Total	0.79 (0.83)	1.24 (1.04)	1.10 (0.82)	0.87 (0.89)
2,3,4,6-Glc	0.71	0.58	0.38	0.65
2,3,6-Glc	46.43	46.54	44.66	43.13
2,4,6-Glc	1.11	0.79	0.98	0.70
2,3-Glc	1.32	1.09	0.82	0.86
2,6-Glc	0.82	0.57	0.70	0.61
3,6-Glc	0.48	0.33	0.38	0.33
Total	50.87 (46.57)	49.90 (41.88)	47.92 (34.53)	46.28 (34.78)
Total neutral sugar	83.16	83.66	79.98	76.87
Uronic acid and non-carbohydrate[d]	14.60	14.71	17.93	21.16
Overall total	97.76	98.37	97.91	98.03

[a]Values are given as % dry matter and are based on "anhydro sugar" residues. [b]2,3,4-Ara denotes 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylarabinitol, *etc.* [c]Values in brackets are from alditol acetate analysis. [d]Data taken from Gordon *et al.*[3].

present in a linear polymer essentially free of glycosidic side-chains. Acid-labile (including glycosidic) substituents preponderated at position 3, whereas alkali-labile substituents preponderated at position 2 (Table III). Xylans from each of the three cell types were heavily substituted, with between 32–50% of all available sites carrying either acid- or alkali-labile side-chains. Fractionation of hemicellulosic polymers from *Zea mais*[10] suggested that acid-labile (glycosidic) substituents were not evenly distributed, and that xylan fractions with degrees of substitution higher and lower than the mean values obtained here can be isolated.

Methylation of intact cell-walls provides little direct information on the nature of substituent groups. However, the formation of 2,3,5-tri-*O*-methyl and 2,3-, 2,5- and 3,5-di-*O*-methyl derivatives of arabinofuranose is consistent with the presence of terminal residues and residues substituted at positions 5, 3, and 2, as in the arabinoxylans isolated from coleoptiles of *Zea mais*[10,11] and immature barley[12]. The amount of arabinoxylan present in the cell-walls of *Zea mais* was estimated to be ~34.5% of the total polysaccharides[9]. Calculated on the same basis, the values for arabinoxylan were ~21.6%, ~26.3%, and ~36.5% of the total neutral sugars found in mesophyll, epidermis, and fibre preparations, respectively. Two other partially methylated sugars formed from the glucuronoarabinoxylan from *Zea*[11], namely, 2,4-di-*O*-methylxylitol and 2,3,4-tri-*O*-methylgalactitol, were also formed from all of the cell types examined. 2,3,4-Tri-*O*-methylarabinitol, consistently detected at a low level but not confirmed by m.s., may have been derived from terminal arabinopyranosyl groups, or from a furanosyl residue with a free reducing group. The latter type of unit has been identified in lignin–carbohydrate complexes isolated from the sheep rumen[13].

Although most of the arabinose residues were terminal, they carried various amounts of alkali-labile substituents at position 5 (Table IV). As found previously[14,15], the extent of substitution at position 5 correlated with the phenolic

TABLE III

SUBSTITUTION OF XYLOSE RESIDUES FROM MESOPHYLL, EPIDERMIS, AND FIBRE CELL-WALLS PREPARED FROM THE LEAVES OF EARLY-CUT ITALIAN (RvP) AND PERENNIAL (Perma) RYEGRASS

Cell-wall		*Fraction of residues substituted at:*				*Total available sites substituted (%)*[a]
		O-2		*O-3*		
		Acid-labile	*Alkali-labile*	*Acid-labile*	*Alkali-labile*	
Mesophyll	Perma	0.15	0.24	0.44	0.14	48.5
	RvP	0.18	0.24	0.41	0.14	48.5
Epidermis	Perma	0.16	0.23	0.29	0.16	42.0
	RvP	0.19	0.19	0.29	0.15	41.0
Fibre	Perma	0.07	0.25	0.15	0.17	32.0
	RvP	0.09	0.29	0.19	0.21	39.0

[a]Assumed to be O-2 + O-3.

content of the sample and was substantially higher in the secondary-thickened fibre walls.

Glucan and xyloglucan. — An estimate of the cellulose content of the cell-walls was obtained by using the method of Updegraff[16] (Table V). From the difference between these values and those obtained for 4-linked glucose by methylation, it is evident that >20% of the glucose is present in non-cellulosic glucans. Mixed-linked glucans have been widely reported to occur in Gramineae[8] and the structures of some of these polysaccharides have been determined[17–21]. Examination[17] of β-D-glucans from coleoptiles of five species of grass indicated that 30.4–30.9% of the linkages were (1→3). Similar consistent values have been obtained for glucans extracted from the endosperm of ryegrass[19], immature barley[20], and *Avena*[21]. Applying this ratio (3:7) for 3- and 4-linked residues, and using values for the 2,4,6-tri-*O*-methyl derivative (Tables I and II), the (1→3),(1→4)-β-D-glucan content of the walls was estimated (Table V). Epidermis cell-walls proved to have a 2- to 3-fold higher content of mixed-linked glucan then either mesophyll or fibre cells-walls.

Xyloglucans also appear to be ubiquitous components of the cell-walls of vascular plants, including monocotyledons[22]. Characterisation of isolated xyloglucans from Gramineae walls[23–25] suggests that these polysaccharides share a common structure, differing only in fine detail. The formation of 2,3-di-*O*-methylglucitol, coupled with the high proportion of terminal xylose found in all of the samples, is strong evidence for the presence of polymers of similar structure in the ryegrass cell-walls. Assuming that 4-linked glucose, not otherwise ascribed to cellulose or mixed-linked glucan, is associated with xyloglucan, and that equimolar amounts of xylose and 4,6-linked glucose are present, then the xyloglucans represent ~10% of the cell-wall dry matter, with, perhaps, slightly higher concentrations in primary cell-walls (Table V). Terminal xylosyl groups were found in amounts insufficient to account for all of the branch points indicated by the recovery of 4,6-linked glucose. Some 4-linked xylose must be included in this polymer, probably further linked to terminal arabinosyl and galactosyl groups as found elsewhere[25].

Two other partially methylated glucose derivatives, namely 2,6-di-*O*-methyl- and 3,4-di-*O*-methyl-glucitol, routinely identified in cell-wall samples, cannot be readily ascribed. Both derivatives could arise as a result of undermethylation of cellulose. However, as the 2,6-derivative has been reported to be formed on methylation of both soluble and insoluble structural polysaccharides alike, this origin seems improbable[8,9].

Pectic polysaccharides. — The method of methylation analysis used here is applicable only to neutral sugar residues and provides no information about the nature of the linkages to the uronic acids. Work is in progress to develop these methods for the quantitative determination of acid (glycosidic)- and alkali-labile linkages to uronic acid residues in intact cell-walls. However, poly(galacturonic acid) has been found in other Gramineae[8,26], and 2-linked rhamnose was found in

TABLE IV

SUBSTITUTION OF ARABINOSE RESIDUES FROM MESOPHYLL, EPIDERMIS, AND FIBRE CELL-WALLS PREPARED FROM THE LEAVES OF EARLY-CUT ITALIAN (RvP) AND PERENNIAL (Perma) RYEGRASS

Cell-wall		*Fraction of residues substituted at:*						*Total available sites substituted (%)*[a]
		O-2		*O-3*		*O-5*		
		Acid-labile	*Alkali-labile*	*Acid-labile*	*Alkali-labile*	*Acid-labile*	*Alkali-labile*	
Mesophyll	Perma	0.08	0.09	0.16	0.15	0.11	0.25	28.0
	RvP	0.08	0.07	0.19	0.11	0.10	0.24	26.3
Epidermis	Perma	0.09	0.08	0.12	0.10	0.11	0.24	24.7
	RvP	0.10	0.07	0.13	0.08	0.11	0.24	24.3
Fibre	Perma	0.16	0.10	0.07	0.12	0.08	0.55	36.0
	RvP	0.12	0.14	0.08	0.15	0.09	0.55	37.7

[a]Assumed to be O-2 + O-3 + O-5.

TABLE V

CELLULOSE, MIXED-LINKED GLUCAN, AND XYLOGLUCAN IN MESOPHYLL, EPIDERMIS, AND FIBRE CELL-WALLS PREPARED FROM THE LEAVES OF ITALIAN (RvP) AND PERENNIAL (Perma) RYEGRASS

Cell-wall			*Cell-wall dry matter (%)*			
			Cellulose	*Non-cellulosic 4-linked glucose*[a]	*Mixed-linked glucan*[b]	*Xyloglucan*[c]
Mesophyll	Perma	(early)	44.3	11.1	3.0	11.0
	RvP	(early)	37.9	11.2	1.3	13.1
Epidermis	Perma	(early)	38.3	12.9	7.0	11.0
	RvP	(early)	38.8	12.9	6.3	10.9
	RvP	(regrowth)	34.8	12.7	7.3	10.2
	RvP	(late)	39.8	10.0	7.0	7.5
Fibre	Perma	(early)	40.0	6.4	3.7	6.4
	RvP	(early)	38.6	7.9	2.7	8.2
	RvP	(regrowth)	34.3	10.4	3.3	9.7
	RvP	(late)	34.8	8.3	2.3	8.5

[a]Difference between 4-linked glucose and cellulose values. [b]Calculated from 3-linked glucose, using the ratio (3:7) for 3- and 4-linked glucose. [c]4-Linked glucose not otherwise ascribed plus equimolar amounts of 4,6-linked glucose and xylose.

all samples examined consistent with the presence of rhamnose as a non-terminal component of rhamnogalacturonan. The formation of a high proportion of 3-*O*-methylrhamnose also suggests that a considerable proportion of the rhamnosyl residues carried side chains. A peak identified on CP-Sil88 as the 2,4-di-*O*-methyl-rhamnose derivative was found in mesophyll walls but rarely from other cell types. The identity of this sugar could not be confirmed by m.s. and it may be an artefact. Primary cell-walls had a greater content of rhamnose than fibre walls, but levels in mesophyll and epidermis were similar, despite the considerably greater amounts of uronic acid found in the latter. The poor agreement between alditol acetate and methylation results for rhamnose and fucose reflected the inadequate resolution of small amounts of these sugars achieved on the packed column used for the alditol acetate assay.

Galactose was present largely as terminal groups and, to a lesser extent, linked at positions 4 and 6. Traces of 3-linked galactose were also present, but the very small amounts of the derivatives were swamped by other peaks with similar retention coefficients (acetylated derivatives of 2,3,6-tri-*O*-methylglucitol on SP1000, xylitol on OV1-BP, and 2,3,6-tri-*O*-methylmannitol on CP-Sil88), which made quantification difficult. Some indication of possible branch-points was provided by the irregular detection of 3,6- and 4,6-linked residues. It was not possible to identify or quantify the galactose-rich polysaccharides in ryegrass cell-walls without the isolation of structurally relevant fragments. The partially methylated galactose residues suggested the presence of both a (1→4)-linked galactan and a (1→3),(1→6)-linked polymer as found in other Gramineae walls[9,27]. The extents to which either of these polymers is substituted with arabinose residues and to

which terminal galactosyl groups form minor components of other polysaccharides are unknown.

Plant maturity and polysaccharide structure. — Although the proportions of different cell types found in ryegrass leaves showed some change with maturity at time of harvest[3], the structure of their polysaccharides remains remarkably constant throughout the growing season (Tables I and II). Some small changes in sugar composition were discernible for the epidermis, but not for the fibre cell-walls from Italian ryegrass. The content of xylose, as a percentage of total recovered neutral sugars, decreased slightly with increasing maturity (20.8% in early-cut and 17.7% in late-cut grass), whereas that of galactose increased (2.7% in early-cut and 3.6% in late-cut grass). However, this variation did not alter the ratio of partially methylated derivatives. Similar results were obtained for perennial ryegrass.

The aerial parts of forage plants consumed by animals are composed of a heterogeneous mixture of cell types. Examination by microscopy of forage tissue undergoing digestion in the rumen revealed marked differences in the rates of digestion of cell-walls from these different cell types[28]. The isolation and chemical characterisation of cell-walls from different cells can aid considerably an understanding of the mechanism of microbial attack, often difficult to establish when whole plant tissue is examined. In our experience, a more satifactory description of the polysaccharide content of plant cell-walls can be obtained by quantitative methylation analysis of the intact material. This approach, at least for the Gramineae, enables virtually all of the dry-matter content to be accounted for and provides a sound basis for further structural and physiological investigations.

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