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The polysaccharides of agricultural lupin seeds*

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ABSTRACT

The polysaccharides of the seeds of four species of agricultural lupin have been shown to comprise galactans, arabinogalactans, arabinans, rhamnogalacturonans, and galactoxyloglucans. Low molecular weight compounds were present in the mixtures after methylation of the acidic polysaccharides. Three tri-*O*-acetyl-*O*-methylhexuronic acids, with one hydroxyl group unsubstituted, formed during methylation, hydrolysis, and acetylation of the acidic polysaccharides, were present in high and variable proportions.

INTRODUCTIONS

The seeds of lupins contain little or no starch. A century ago Schulze and Steiger[1] suggested that those of *Lupinus luteus* contained a "paragalaktan" and that it was mobilised during germination. The reserve rôle of galactans has since been confirmed[2]. Structural studies have been carried out on the polysaccharides of dewaxed flours from seeds of four agricultural lupins, namely, *L. angustifolius, L. albus, L. luteus,* and *L. mutabilis*, in order to assist companion studies on changes in the polysaccharides taking place during germination, to be reported elsewhere. It was hypothesised that the various structural features found in polysaccharides from seeds of any one of the four species of lupin might be present in those of the other three. This view has been largely confirmed: there were, of course, quantitative differences.

Structural features in complex, native, plant polysaccharides may be absent, or present in changed proportions, when procedures involve either deliberate or incidental fractionation or partial destruction[3]. Qualitative and quantitative comparisons between polysaccharides are more reliable when, as in the present work, unfractionated, and virtually total, rather than fractionated, polysaccharides are studied. As comparisons were to be made, routine methods had to be checked for suitability and to select conditions for their use.

RESULTS AND DISCUSSION

The polysaccharides were isolated by milling the seeds, after removing their coats, to give flours from which lipids, pigments, proteins, and oligosaccharides had been

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TABLE I

Neutral sugars (mol%) in hydrolysates of the water-soluble material extracted from the seeds of four agricultural lupins

Sugar	L. angustifolius	L. albus	L. luteus	L. mutabilis
Rha	3.3	0.9	0.9	1.2
Ara	4.1	2.2	2.6	0.8
Xyl	1.3	0.3	0.6	1.6
Man	1.6	1.1	3.5	2.3
Gal	76.7	64.8	87.6	62.0
Glc	3.0	30.7	4.7	31.8

removed. The ratios of raffinose and stachyose, relative to 100 for sucrose, were 19.7:31.6, 60.6:115.2, 110.9:33.3, and 94.2:28.9, respectively, for the oligosaccharides from the flours of *L. angustifolius, L. albus, L. luteus,* and *L. mutabilis.* The percentages of protein were 44.7, 47.6, 46.5 and 47.2, respectively. During the above extractions, ~2% of water-soluble carbohydrates, other than the oligosaccharides, passed into solution (see Table I).

Acid hydrolysis of complex heteroglycans and of hetero-linked glycans leads to the release of monosaccharides at rates that depend on the ways in which they are glycosidically linked, and on the individual sugars and there ring sizes. For quantitative comparisons, a balance has to be struck between the conditions leading to complete hydrolysis, and those giving acceptably low degradation of the monosaccharides after their liberation. Total avoidance of degradation is always impossible when acid hydrolysis is used, but its extent can be minimised[4]. The proportions of sugars released after different periods of acid hydrolysis of the polysaccharides from *L. angustifolius* are shown in Table II. The optimum period of hydrolysis with M H_2SO_4 at 100° was 6 h. Earlier, hydrolysis was incomplete: later, it was selective and there was considerable

TABLE II

Molar ratios of sugars obtained on hydrolysis of the total polysaccharides from the seeds of *Lupinus angustifolius* with M H_2SO_4 at 100° for different periods[a]

Parent sugar	*3 h*	*6 h*	*12 h*	*24 h*
Gal	700	748	720	662
Ara	124	120	111	72
Rha	19	27	29	21
Fuc	3	3	3	–
Xyl	22	21	17	13
Man[b]	4	8	9	9
Glc[b]	4	4	3	3

[a]Values corrected for detector response in g.l.c. and for extent of conversion into glycitol acetates. [b]Sugars detected as mannitol and glucitol may have been derived from D-fructose.

TABLE III

Neutral sugars (mol%) in hydrolysates obtained when the polysaccharides of lupin seeds were digested[5] successively with aq. 72% H_2SO_4 at 20° and M H_2SO_4 at 100°

Sugar	L. angustifolius	L. albus	L. luteus	L. mutabilis
Rha	3.0	3.2	5.4	4.2
Fuc	0.2	0.1	0.1	0.2
Ara	14.3	14.7	23.0	33.0
Xyl	2.8	4.0	4.6	7.2
Man	0.5	1.5	1.0	1.9
Gal	70.5	68.1	52.5	39.2
Glc	8.7	8.4	13.4	14.3

destruction of arabinose and xylose. Certain polysaccharides, such as cellulose, are highly resistant to the above conditions of hydrolysis. The flours were treated by the method of Saeman et al.[5] in order to hydrolyse celluloses (Table III).

The polysaccharides contain D-galacturonic and D-glucuronic acid residues which were reduced by successive treatments with 1-cyclohexyl-3-(2-morpholinoethyl) carbodi-imide metho-*p*-toluenesulphonate, then with sodium borohydride[6]. The meth-

TABLE IV

Neutral sugars (mol%)[a] in hydrolysates of lupin seed polysaccharides before and after their treatment with 1-cyclohexyl-3-(2-morpholinoethyl)carbodi-imide metho-*p*-toluenesulphonate and then with sodium borohydride

Sugar	L. angustifolius		L. albus		L. luteus		L. mutabilis	
	Before	*After*[b]	*Before*	*After*[b]	*Before*	*After*[b]	*Before*	*After*[b]
Rha + Fuc	5.1	4.4 4.3[c]	4.5	4.7	3.7	3.9	5.6	5.5
Ara	16.6	16.6 15.9[c]	18.4	18.4	24.2	24.2	27.9	27.9
Xyl	2.5	2.7 3.0[c]	4.5	4.6	5.8	6.0	6.1	6.3
Man	0.4	0.6 0.5[c]	0.3	tr	0.5	0.5	0.7	0.8
Gal	74.2	84.4 90.4[c]	71.7	82.4	64.4	73.5	58.2	69.5
Glc	1.2	1.2 2.1[c]	0.6	0.8	1.4	1.5	1.5	2.0

[a]Mol% values are for sugars prior to reduction of the polysaccharides. [b]Ratios for sugars after reduction are relative to a fixed value for arabinose before and after each reduction. [c]Values after a second, similar reduction.

TABLE V

Sugars in hydrolysates of methylated polysaccharides obtained after the polysaccharides of the seeds of *L. angustifolius* had been exposed to dimsyl-sodium for various periods prior to the addition of iodomethane[a]

Sugars and positions of glycosidic linkages	*1 h*	*3 h*	*6 h*	*12 h*	*24 h*	*1 week*
5-Ara*f*	4.9	5.1	9.0	11.9	12.2	12.6
3-Ara*f*	0.5	0.5	0.4	0.8	0.7	0.6
2-Ara*f*	0.8	0.8	0.8	1.0	1.3	0.6
2,5-Ara*f*	25.6	22.7	15.9	23.4	24.4	22.0
3,5-Ara*f*	2.8	2.5	2.0	3.6	5.3	6.6
2,3,5-Ara*f*	1.3	1.3	0.8	2.1	3.6	18.9
t-Ara*p*	0.5	tr.[h]	tr.	tr.	tr.	tr.
t-Ara*f*	12.3	11.4	14.7	18.7	36.0	43.1
6-Gal*p*	0.8	0.5	0.6	0.8	2.0	0.6
4-Gal*p*	100.0	100.0	100.0	100.0	100.0	100.0
3-Gal*p*	tr.	1.0	tr.	0.5	1.0	0.6
4,6-Gal*p*	4.1	?	3.0	3.9	3.6	7.6
3,6-Gal*p*	1.3	2.0	1.8	2.5	3.0	3.1
3,4-Gal*p*	1.0	1.5	1.4	2.3	2.6	3.1
2,4,6-Gal*p*	0.5	tr.	tr.	1.8	1.0	0.6
t-Gal*p*	2.6	2.8	5.8	5.7	8.6	7.9
2-Rha*p*	2.8	2.0	1.2	0.8	4.0	5.3
2,4-Rha*p*	1.5	1.8	2.6	3.4	2.6	3.1
2,3-Rha*p*	0.8	0.8	0.2	0.8	1.0	0.3
t-Rha*p*	1.8	0.5	0.4	0.3	1.3	1.6
t-Fuc*p*	1.8	1.3	0.2	tr.	2.6	tr.
4-Xyl*p*	0.8	0.8	0.6	0.8	0.7	0.6
2-Xyl*p*	0.5	0.5	0.6	0.5	0.7	0.3
2,4-Xyl*p*	0.5	0.3	0.4	0.3	0.3	0.3
t-Xyl*p*	2.3	2.5	2.2	4.9	6.6	5.3
4-Glc*p*	2.8	2.0	2.0	2.9	6.6	3.1
4,6-Glc*p*	tr.	tr.	1.6	4.7	3.3	2.2
3,4-Glc*p*	0.3	0.3	tr.	0.8	1.0	0.3
2,4-Glc*p*	1.0	1.5	2.0	3.6	2.0	1.9
t-Glc*p*	4.4	5.1	1.6	1.3	5.6	2.5
Unknown 1[b]	8.5	5.6	1.8	2.9	15.2	13.2
Unknown 2[b]	4.9	3.8	0.4	2.9	9.9	1.6
Compound *1*[c,d]	18.2	18.7	5.2	19.0	26.4	34.3
Compound *2*[c,e]	28.2	24.2	5.6	11.4	13.2	9.4
Compound *3*[f,g]	16.9	16.9	13.9	9.6	23.4	19.8

[a]Identity of the parent sugars, and positions of linkages, based on g.l.c. of glycitol acetates on OV-225 at 170°. Ratios are relative to a g.l.c. peak area of 100 for 2,3,6-tri-*O*-methyl-D-galactitol acetate. [b]Possibly a hexenuronic acid residue (linkage unknown). [c]Galacturonic acid residue; probably 4-linked. [d]Compound *1*: 2,3,6-tri-*O*-acetyl-4-*O*-methyl-D-galactonic acid derived by reduction of 1,4,5-tri-*O*-acetyl-3-*O*-methyl-D-galacturonic acid. [e]Compound *2*: 2,3,6-tri-*O*-acetyl-5-*O*-methyl-L-galactonic acid, derived by reduction of 1,4,5-tri-*O*-acetyl-2-*O*-methyl-D-galacturonic acid. [f]Glucuronic acid residue; linkage unknown. [g]Compound *3*: 2,3,6-tri-*O*-acetyl-5-*O*-methyl-L-gulonic acid, derived by reduction of 1,4,5-tri-*O*-acetyl-2-*O*-methyl-D-glucuronic acid. [h]Trace.

od is reported to lead to incomplete reduction[7]. In Table IV, the totals for non-acidic sugars in the hydrolysates of each polysaccharide before reduction are adjusted to 100. The values for L-arabinose released after reduction are each adjusted to retain the value prior to reduction on the assumption that the arabinose in the various polysaccharides was unaffected by the reductions. The only significant changes were in the proportions of galactose, which increased, indicating the presence in the reduced polysaccharides of *L. angustifolius, L. albus, L. luteus*, and *L. mutabilis* of 9.2, 10.0, 8.3 and 10.1%, respectively, of galacturonic acid residues that had been reduced. The polysaccharides from *L. angustifolius* were indicated by two successive reduction procedures to contain 12.4% of galactose derived from galacturonic acid (Table IV). Evidently, the galacturonic acid residues were not all reduced by one treatment, and, indeed, methylation analysis indicated a higher proportion of uronic acid (including glucuronic acid) residues than accounted for even after two reductions (see Table V). Estimated values for uronic acids in unmethylated polysaccharides should be regarded as minimal.

Checks were made on the Hakomori method of methylation to be used[8]. There was no change in the proportions of the various sugars found in hydrolysates of *L. angustifolius* polysaccharides which had been left in dimethyl sulphoxide (Me_2SO) for 12 h and then recovered. The polysaccharides were also treated with methylsulphinyl-methanide anion (dimsyl-sodium) in Me_2SO as in a standard methylation but without the subsequent addition of iodomethane. In hydrolysates of the dimsyl-treated polysaccharides, there was a slight fall in the xylose (from 1.7 to 0.9%), which may have been due to β-elimination of residues either directly, or indirectly, attached to O-4 of uronic acid residues. The same proportion of galacturonic residues was reduced by the carbodi-imide–borohydride method before and after the polysaccharides had been treated with dimsyl-sodium.

Other samples of the *L. angustifolius* polysaccharides were treated with dimsyl anion in Me_2SO for periods ranging from 1 h to 1 week before the addition of iodomethane. The methylated polysaccharides were then hydrolysed and the products determined by g.l.c. In Table V, ratios are shown relative to 100 for 4-linked galactose residues, as these were expected to be amongst the most resistant to the treatments with dimsyl-sodium. Treatment for 3 h was adopted as an acceptable period of exposure to the dimsyl anion before the addition of the iodomethane. The total value for each sugar, irrespective of the variety of substituted derivatives, was calculated for the polysaccharides treated for 3 h and for 1 week with dimsyl-sodium. The totals for the various partially methylated galactoses, arabinoses, xyloses, rhamnoses plus fucoses, and glucoses were 100, 41.1, 3.8, 5.9 (*i.e.*, 4.7 + 1.2), and 8.2, respectively, after treatment for 3 h, and 100, 84.5, 5.3, 8.3 (8.3 + tr.), and 8.1, respectively, after treatment for 1 week. The corresponding values for the unmethylated sugars in hydrolysates of the parent polysaccharide were 100, 22.3, 3.2, 6.7, and 1.5. The proportions of arabinose accounted for after methylation were 2–4 times greater than that found on hydrolysis of the unmethylated polysaccharides. These findings may be due to some of the arabinose residues being linked prior to methylation by acid-resistant glycosiduronic linkages. The higher proportion of methylated glucoses, derived from the methylated poly-

TABLE VI

Reproducibility of results when polysaccharides from the seeds of *L. angustifolius* were methylated under identical or similar conditions[a]

Sugar and position of glycosidic linkages	*Dimsyl-sodium 1st prep.*[b]					*Dimsyl-sodium 2nd prep.*[b]		*Dimsyl-lithium*[b]
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
5-Ara*f*	4.8	3.3	3.2	3.6	2.3	1.8	2.0	2.1
3-Ara*f*	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.2
2-Ara*f*	0.2	0.5	0.4	0.4	0.2	0.2	0.3	0.3
3,5-Ara*f*	1.6	0.9	1.4	1.6	1.7	1.4	1.0	2.0
2,3,5-Ara*f*	1.3	1.0	0.8	0.9	1.0	1.2	0.5	1.0
t-Ara*p*	0.2	0.4	0.3	0.2	0.2	0.2	tr.[e]	tr.
t-Ara*f*	4.1	3.6	3.8	4.8	4.6	3.8	4.5	3.7
6-Gal*p*	0.3	0.2	0.4	0.3	0.3	0.4	0.4	0.6
4-Gal*p*	40.2	41.3	38.9	41.4	40.6	43.5	39.6	48.8
3-Gal*p*	0.5	0.3	0.2	0.5	0.5	0.3	0.4	0.4
4,6-Gal*p*	2.0	2.7	2.8	2.6	2.4	2.8	3.5	2.9
3,6-Gal*p*	0.6	0.6	0.7	0.6	0.5	0.7	0.8	0.8
3,4-Gal*p*	0.5	0.4	0.5	0.3	0.6	0.5	0.9	0.6
2,4,6-Gal*p*	0.6	0.4	0.4	0.5	0.6	0.4	tr.	0.2
t-Gal*p* + 2-Xyl*p* and/or 4-Xyl*p*	1.7	1.5	1.6	1.6	1.7	1.6	1.5	1.7
2-Rha*p*	0.7	0.7	1.2	0.8	1.0	0.7	0.8	0.7
2,4-Rha*p*	0.6	0.8	0.9	1.2	0.9	0.6	0.7	0.9
2,3-Rha*p*	0.2	0.2	0.3	0.3	0.4	0.3	0.3	0.2
t-Rha*p*	0.4	0.3	0.2	0.1	0.2	0.4	0.2	0.3
t-Fuc*p*	0.6	0.2	0.3	0.5	0.5	0.6	0.5	0.4
t-Xyl*p*	1.1	1.2	0.9	1.1	1.1	1.0	1.0	1.1
4-Glc*p*	1.8	2.0	1.7	1.5	1.4	1.2	1.5	1.0
4,6-Glc*p*	0.8	0.9	0.7	0.7	0.6	0.8	0.5	0.7
3,4-Glc*p*	0.5	0.3	0.4	0.4	0.5	0.5	0.3	0.3
2,4-Glc*p*	0.5	0.4	0.6	0.5	0.3	1.3	0.7	1.1
t-Glc*p*	0.5	1.1	1.3	0.6	1.1	0.8	2.0	0.9
Unknown 1[c]	1.6	1.3	2.6	1.5	2.1	1.5	2.2	1.7
Unknown 2[c]	1.8	1.9	1.9	1.6	1.7	1.4	1.5	1.3
Compound *1*[c]	5.0	6.6	6.0	5.4	3.5	5.2	6.9	5.2
Compound *2*[c,d]	19.3	18.3	19.0	17.0	16.5	18.9	18.6	15.3
Compound *3*[c]	5.7	6.0	6.0	7.5	6.7	5.2	6.7	3.8

[a]Sugar residues and linkages identified, and methylated glycitol acetates determined, by g.l.c. on OV-225 at 170°. The total g.l.c. chart-areas are adjusted to 100. [b]See text. [c]See Footnotes *b–g* of Table V. [d]Possibly contaminated by 3-*O*-methyl-D-xylitol tetra-acetate. [e]Trace.

saccharides, compared to that of glucose, derived from the unmethylated polysaccharides, may be due to the swelling and dissolution of cellulose and other glucans in Me_2SO, thereby facilitating acid hydrolysis.

The dependability of values obtained by single methylation analysis was assessed by carrying out five separate, but simultaneous, methylations using dimsyl-sodium under identical conditions (Table VI, columns 1–5), and two others using separately prepared, but seemingly similar, preparations of dimsyl-sodium (columns 6 and 7). Dimsyl-lithium was also used (column 8). The values for methylations 1–5 were in good agreement and differed only slightly from those for methylations 6 and 7. When dimsyl-lithium was used, there was a slight increase in the proportion of the 4-linked galactose residues.

Commonly, after Hakomori methylations, the reaction mixtures are dialysed and the diffusible components are discarded. In the present work, the non-diffusible and diffusible components were recovered and determined quantitatively. Table VII compares the proportions and ratios of methylated sugars released on hydrolysis of the materials in each retentate with those present in the corresponding diffusate. Methylated sugar residues that were present mainly in the diffusate must have been components of partially depolymerised, methylated polysaccharide. The dialysis membrane did not retain oligosaccharides of d.p. <6.

The carbohydrates of low d.p. in the diffusates may have been derived from side chains attached to O-4 of uronic acid residues of the rhamnogalacturonans or of other acidic polysaccharides. Such side chains, or residues, may be released by β-elimination reactions during treatment of the polysaccharides with dimsyl-anion. Such elimination reactions would be promoted if the uronic acid residues were converted into inter-residue lactones (see below).

The structural features present in the various polysaccharides are shown in the "assemblages" **1**–**5** (*cf.* ref. 3). The term, assemblage, is preferred to "structure" since certainty of structure is rarely possible for plant polysaccharides. An assemblage incorporates one permutation of known structural features that is compatible with the available evidence and may be subject to modification as new evidence or ideas appear. The assemblages **1**–**5** should be viewed in conjunction with the data in Tables VIII (proportion of the structural features shown) and IX (proportions of the polysaccharides present in the seeds). Thus, in the seeds of each of the four lupins, there are two D-galactans, namely, 3,6-linked, with some 4-linkages[9] (**1**), present in low proportions, and 4-linked[10] having one in every 16–21 residues 6-substituted by L-arabinofuranose (**2**). The methylation evidence is compatible with the presence also of 5-linked L-arabinans with 2- and 3-linked side chains (**3**), rhamnogalacturonans[11] (**4**), and galactoxyloglucans[12] (**5**). The various polysaccharides mentioned are not necessarily independent in the natural state.

An indication of the breakdown of polysaccharides is given by the findings on dialysis of the various methylated polysaccharides. The 2,3-linked rhamnose and terminal fucose residues diffused through the membrane together with oligosaccharides having the residues shown in the second section of Table VII, including 3-, 6-, 3,4(or

TABLE VII

Methylated monosaccharide residues in hydrolysates of diffusible and non-diffusible products obtained after Hakomori methylation of seed polysaccharides from four agricultural lupins[a]

Sugar and position of glycosidic linkages	L. angustifolius		L. albus		L. luteus		L. mutabilis	
Residues present mainly or only in the non-diffusible components								
3-Ara	0.2/0	–	0.1/0	–	0.2/0	–	0.2/0	–
5-Ara	3.2/0.4	8	4.0/0.1	40	9.0/0.3	30	9.2/0.2	46
2,5-Ara	10.3/0	–	5.3/0	–	6.0/0	–	4.7/0	–
3,5-Ara	1.4/0.3	5	1.3/0.3	4	1.9/0.2	10	2.2/0.4	6
2,3,5-Ara	1.2/0.1	12	0.9/0.1	9	0.7/0	–	0.3/0	–
t-Ara*p*	0.2/0	–	0.7/0.4	2	1.0/0	–	0.2/0	–
t-Ara*f*	3.8/1.8	2	4.2/0.4	11	9.2/0.6	15	12.7/0.3	42
4-Gal	43/0.5	86	51/0.5	102	30/0.5	60	27/0.9	30
3,6-Gal	0.7/0	–	0.4/0	–	0.8/0	–	0.2/0	–
4,6-Gal	2.8/0	–	2.8/0	–	1.5/0	–	1.4/0	–
t-Gal	2.5/1.0	2.5	1.4/0.7	2	2.6/1.2	2	2.9/1.2	2
4-Glc	1.7/1.2	1.5	1.1/0.1	11	2.4/0.7	3	2.2/0	–
3,4-Glc	0.5/0.1	5	1.3/0	–	0.5/0	–	0.1/0	–
4,6-Glc	0.8/0	–	0.5/0	–	1.8/0	–	2.8/0	–
2,4-Glc	0.3/0	–	tr/0	–	tr/0	–	0.4/0	–
2,3,4-Glc or 2,4,6-Glc	0.4/0	–	0.7/0	–	0.6/0	–	0.3/0	–
t-Rha	0.4/0.1	4	0.3/0.1	3	0.2/0.1	2	0.7/0.1	7
t-Xyl	1.0/0.4	3	2.2/0.3	7	4.8/1.9	3	5.0/1.1	5
Residues present mainly or only in the non-diffusible components								
Compound *1*[b]	3.3/0	–	2.7/0	–	4.0/0.3	13	3.8/0	–
Compound *2*[b]	6.0/0	–	5.0/1.5	3	10.2/1.1	9	11.0/1	11
Compound *3*[b]	4.4/0.3	15	8.2/0.3	27	4.8/0.4	12	4.5/0.5	9
Residues generally present[d] *almost equally in the non-diffusible and diffusible components*								
2-Ara	0.2/0.3	1	0.4/0.3	1	0.6/0.2	3	0.8/0.3	3
3-Gal	0.3/0.2	1.5	(0.5/0)[c]	–	0.5/0.4	1	(0.2/0)	–
6-Gal	0.4/0.3	1	0.4/0.3	1	(0.3/0)[c]	–	0.4/0.3	1
3,4- or 3,5-Gal	0.5/0.2	3	(1.0/0)[c]	–	0.5/0.3	2	0.4/0.2	2
2,4,6- or 2,5,6-Gal	0.4/0.5	1	0.2/0.2	1	1.2/1.3	1	0.4/0.4	1
2-Rha	0.7/0.6	1	0.8/0.6	1	0.6/0.5	1	0.6/0.5	1
2,4-Rha	0.6/0.4	1.5	0.9/0.6	1.5	0.6/0.3	2	1.3/0.9	1.5
4-Xyl	0.3/0.1	3	0.1/0.1	1	0.1/0.1	1	0.3/0.1	3
2-Xyl	0.1/0.1	1	0.3/0.1	3	0.6/0.1	6	0.7/0.8	1

TABLE VII (continued)

Sugar and position of glycosidic linkages	L. angustifolius		L. albus		L. luteus		L. mutabilis	
Residues present mainly in diffusible components								
t-Glc	0.8/2.3	0.3	(0.5/0)[c]	–	(0.3/0)[c]	–	1.3/2.1	0.6
t-Fuc	0.6/0.7	1	0.3/1.1	0.3	0.6/0.4	1.5	0.3/0.6	0.5
2,3-Rha	0.3/1.3	0.2	0.2/0.9	0.2	0.1/1.4	0.1	0.2/2.1	0.1

[a]In each column, the relative amounts of (material in retentate)/(material in dialysate), where possible, are followed by the corresponding ratio. [b]See Footnotes *b–g* of Table V. [c]Exceptions are shown in brackets.

TABLE VIII

Structural assemblages of the polysaccharides of the seeds of agricultural lupins[a]

Residues	L. angustifolius	L. albus	L. luteus	L. mutabilis
Galactans **1**				
Gal*p a*	1	1	1	1
b	1	0	2	0
c	2	3	2	1
d	0	0	0	1
e	1	1	2	1
Arabinogalactans **2**				
Gal*p f*	15	18	20	19
g	1	1	1	1
Arabinans **3**				
Ara*f h*	2	3	0	0
j	5	1	0	0
k	1	0	0	0
l	0	1	1	1
Rhamnogalacturonans **4**				
Rha*p p*	1.3	1.5	2.3	3.7
r	1.7	4.0	5.4	2.2
Galactoxyloglucans **5**				
Glc*p s*	17	2	8	3
t	7	0	2	3
v	1	1	1	1
w	0	0	1	0

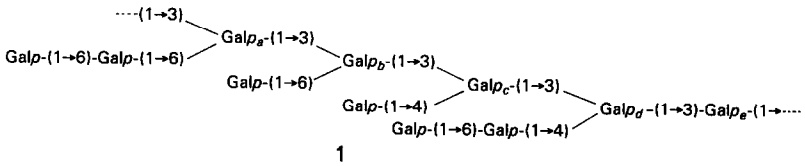
[a]See Table IX for ratios of the polysaccharides listed.

TABLE IX

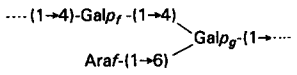
Relative proportions of the polysaccharides in lupin seeds[a]

Polysaccharide	L. angustifolius	L. albus	L. luteus	L. mutabilis
Galactan **1**	0.7	1.0	1.7	0.7
Arabinogalactan **2**	100.0	100.0	100.0	100.0
Arabinan **3**	7.4	7.9	29.8	33.9
Rhamnogalacturonan[b] **4**	1.6	1.6	2.0	2.2
Galactoxyloglucan **5**	1.9	1.0	6.0	10.3

[a]See Table VIII for proportions of the structural features. [b]Only rhamnose is included in the values shown.



1



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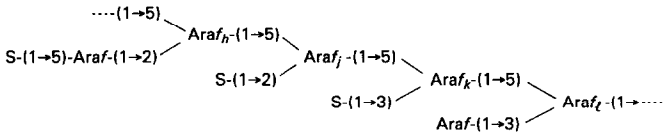

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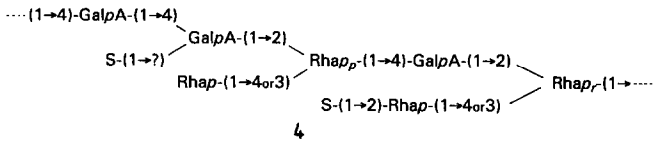

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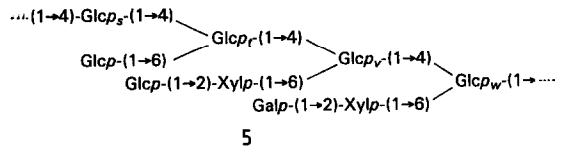

5

3,5)-, and 2,4,6(or 2,5,6)-linked galactose residues. About one-third of the terminal galactose residues were indicated to be in materials of low d.p. There is no proof of the presence or absence of galactofuranose residues[9], but their presence might have facilitated partial hydrolysis, yielding methylated oligosaccharides. However, most of these oligosaccharides were almost certainly derived by β-elimination of groups of main-chain or side-chain residues, or of single residues, attached to acidic residues of

rhamnogalacturonans. The 4-, 3,6-, and 4,6-linked galactose from the two types of galactan residues were mainly in the non-diffusible material, but about one-third of the terminal galactose residues were in material of low d.p.

In the hydrolysates of each of the various methylated polysaccharides, five of the components were present in substantial, but highly variable, proportions. These components had retention times relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol of 0.40, 0.94, 1.95, 2.42, and 2.52 in g.l.c. on column *a* (170°), and 0.61, 1.17, 1.78, 2.08, and 2.12 in g.l.c. on column *b* (185° for 5 min rising to 200° at 2°. min^{-1}). The last three compounds were found by g.l.c.–c.i.-m.s. to have masses of 336. The g.l.c.–e.i.-m.s. (Figs. 1 and 2) indicate the first compound to be 2,3,6-tri-*O*-acetyl-4-*O*-methyl-L-galactonic acid (derived by reduction of 1,4,5-tri-*O*-acetyl-3-*O*-methyl-D-galacturonic

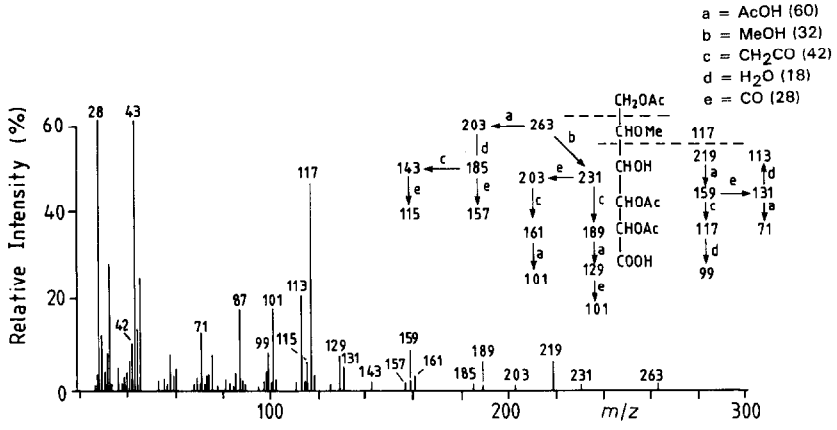

Fig. 1. E.i.-m.s. and fragmentation pathways of 2,3,6-tri-*O*-acetyl-5-*O*-methyl-L-galactonic and -L-gulonic acids.

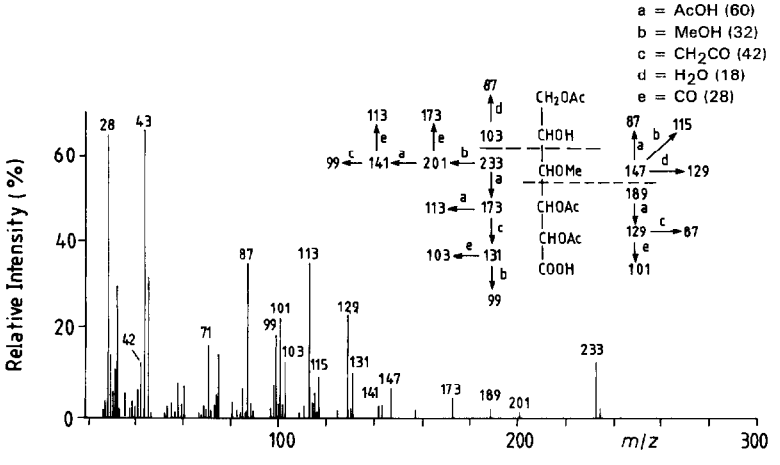

Fig. 2. E.i.-m.s. and fragmentation pathways of 2,3,6-tri-*O*-acetyl-4-*O*-methyl-L-galactonic acid.

acid residues) and the last two, identically substituted, compounds to be 2,3,6-tri-*O*-acetyl-5-*O*-methyl-L-galactonic acid (derived by reduction of 1,4,5-tri-*O*-acetyl-2-*O*-methyl-D-galacturonic acid residues) and 2,3,6-tri-*O*-acetyl-5-*O*-methyl-L-gulonic acid (derived by reduction of 1,4,5-tri-*O*-acetyl-2-*O*-methyl-D-glucuronic acid residues).

The unsubstituted hydroxyl group in each of the above three glyconic acids could have arisen from an intra- or inter-residue lactone formed when the polysaccharides were in the solution of dimsyl-sodium in Me_2SO. Hydrolysis of the methylated polysaccharides would also hydrolyse the lactones but, after borohydride reduction, the partially methylated glyconic acids derived from the uronic acids could reform lactones under the acetylation conditions. It is not surprising that the values for the glyconic acid derivatives varied since some degradation of uronic acids would be expected during acetylation. Apparently, there is much more of the uronic acid derivatives than is accounted for by the method used to estimate the parent unmethylated acid residues.

In spite of their abundance, the above three compounds have not been reported previously. In the present studies, the exposure of the polysaccharides to weak alkali when the protein was being removed would have saponified the lactones but would have been unlikely to cause β-elimination. There was a higher proportion of the three acids in the retentates than in the diffusates, and the presence in the latter indicated some degradation of the rhamnogalacturonans. Degradation of acidic polysaccharides is usually explained in terms of β-elimination following methyl esterification of acid residues, but the formation of the above acid derivatives indicates an additional, or alternative, route. Two components (unknowns 1 and 2, Tables V–VI) remain unidentified, but may be partially methylated and acetylated hexenuronates.

EXPERIMENTAL

General. — Solvents were removed either by rotary evaporation at $<40°$ or by freeze-drying using an Edwards High Vacuum Model 30P2 freeze-dryer. Ultrasonication during methylation was in a SC-121T Sonicor water-bath at 20°. G.l.c. was performed on a Perkin–Elmer Sigma 1 apparatus fitted with an injection-splitter, flame-ionisation and nitrogen–phosphorus detectors, and fused-silica columns (25 m × 0.31 mm i.d.) with (*a*) 0.19-μm OV-225 used at 170° for glycitol acetates and for aldononitrile acetates of methylated sugars, and at 190° for unmethylated glycitol acetates; (*b*) 18-μm WCOT SP-1000 used at 195° for unmethylated glycitol acetates; and (*c*) a fused-silica column (50 m × 0.32 mm i.d.) with 0.2-μm WCOT SP-2340 used at 185° for 5 min, then to 200° at 2°. min^{-1}, for glycitol acetates and aldononitrile acetates of methylated sugars. G.l.c.–e.i.-m.s. was performed on a Pye 104 chromatograph linked by a jet-separator to an A.E.I. MS 30 mass spectrometer. The column (150 × 4 mm) was packed with Chromosorb W AW DMCS 100–120 coated with 3% of OV-225, and used at 170° for 15 min then to 200° at 0.5°.min^{-1}. C.i. and additional e.i. were carried out using a fused-silica column (25 m × 0.31 mm i.d.) and 0.5-μm XE-60 with helium at 40 $cm.s^{-1}$, 150° for 2 min then 7°.min^{-1} to 150–240°. Methylation mixtures were dialysed in Visking dialysis-tubing (32/32) against frequent changes of distilled

water when the diffusates were retained, otherwise against running water for 2 days. The retentates were freeze-dried. The diffusates (3 × 700 mL) were combined, concentrated to 25 mL, and extracted[13] with CH_2Cl_2 (4 × 10 mL) and then $CHCl_3$ (4 × 10 mL). The extracts were combined and washed with water (4 × 10 mL), and the aqueous solutions were combined, concentrated to 25 mL, and then freeze-dried.

Isolation and examination of the polysaccharides. — Seeds of *L. angustifolius* Cv. Unicrop, *L. albus* Cv. Kievski mutant, *L. luteus* Cv. Weiko, and *L. mutabilis* Cv. LM139 were soaked in distilled water and their skins removed by hand. After milling, using an Apex Mill fitted with a 60-mesh, lipids and pigments were extracted (Soxhlet) by acetone and light petroleum (b.p. 60–80°). Oligosaccharides were extracted from the defatted flours with aq. 80% EtOH, and the extracts were concentrated and then subjected to h.p.l.c. using a Waters' "Carbohydrate analysis column" irrigated with 7:3 acetonitrile–water. The acetates were determined[14] using a Waters' R401 RI detector. The defatted flours were stirred with aq. 0.2% NaOH (3 × 12 h), recovered by centrifugation, washed with very dilute acetic acid and water, and freeze-dried to give the fluffy flours referred to earlier as "the polysaccharides". Protein was precipitated in the combined alkaline extract and in the wash liquors by adjusting the pH to 4.5. The materials were combined with further protein recovered by the Sevag method[15]. The values for protein, determined on replicate samples (200 mg) using a Kjeldahl procedure and a Technico autoanalyser, were obtained[16] by multiplying the values for nitrogen by 5.7. Water-soluble polysaccharides, recovered from the aqueous solutions by dialysis and freeze-drying, accounted for ~2% of the flours.

Studies of the effect of various procedures. — Monosaccharides were treated with M H_2SO_4 for periods ranging from 3 h to 1 week in order to establish the extent of degradation under potential conditions for the hydrolysis of the polysaccharides. Samples of the *L. angustifolius* polysaccharides were treated similarly for 24 h in order to determine the optimum conditions for their hydrolysis. Hydrolysates were neutralised[17] with 20% *N,N*-dioctylmethylamine in $CHCl_3$. The sugars were treated with $NaBH_4$ and then with Duolite C-225 (H^+) resin. Samples (10 mg) of the four flours were treated with aq. 72% H_2SO_4 for 1.5 h at 20°, then, after adjusting the acidity to 1M, the mixtures were heated at 100° for 2.5 h (Table III).

Sugars in hydrolysates were reduced conventionally with $NaBH_4$. The resulting alditols were heated at 100° with 1:1 (v/v) acetic anhydride–pyridine for periods ranging from 10 to 180 min. Fully acetylated *myo*-inositol was added as a reference standard after the acetylation. All of the sugars gave maximum values after 1 h: earlier, acetylation was incomplete; and later, there was degradation particularly of the pentoses and rhamnose. One hour was selected as the period for acetylation. Acetylated glycitols were recovered by dissolution in $CHCl_3$. Values determined by g.l.c. were corrected for degradation during preparation of the glycitol acetates, and also for differential molar responses by the flame-ionisation detector, by multiplying the peak areas on charts by 1.05, 1.14, 1.00, 1.00, 1.14, and 1.35, respectively, for deoxyhexoses (rhamnose and fucose), galactose, mannose, arabinose, xylose, and glucose. Acetylation using *N*-methylimidazole as catalyst[18] caused less degradation and was complete within a few

minutes. Aldononitrile acetates of methylated sugars were prepared from concentrated samples of hydrolysates of methylated polysaccharides by treatment with hydroxylamine hydrochloride (70 mg) and pyridine (2 mL) in Reacti-Vials in a Reacti-Therm Module for 1 h at 90°. Acetic anhydride (1.5 mL) was added and, after 1 h at 90°, the aldononitrile acetates were extracted into $CHCl_3$, and the extract was washed, dried, and examined by g.l.c.[19]. In this way, 2,3- could be distinguished from 3,4-di-*O*-methyl-D-xylose, and the quantification of certain other compounds inadequately separated as their glycitol acetates was assisted.

Reduction of the polysaccharides. — Samples (10 mg) of all four polysaccharides in water (10 mL) and MeOH (10 mL) were treated[6] for 3 h at pH 4.75 successively with 1-cyclohexyl-3-(2-morpholinoethyl)carbodi-imide metho-*p*-toluenesulphonate (210 mg), and then with 2M $NaBH_4$. The excess of hydride was destroyed with dilute aqueous acid and retentates were freeze-dried prior to hydrolysis. A sample of the *L. angustifolius* polysaccharides was given two such treatments.

Studies related to methylation analysis. — Dimsyl-sodium was prepared by the method of Hakomori[8], using an 80% suspension of NaH in white oil (Fluka). Dimsyl-lithium[20] was prepared using dry Me_2SO (20 mL) and adding a cold 1.6M solution (25 mL) of butyl-lithium in hexane (Fluka) then stirring for 2 h at 20° (ref. 7). The concentration of the dimsyl anion was determined by titrating aliquots with MeOH, using a 1% solution of triphenylmethane in Me_2SO as indicator[21]. The average molarities of the anion in dimsyl-sodium was 1.84, and in dimsyl-lithium was 1.58. Studies of the methylation conditions were made on the *L. angustifolius* polysaccharides. There was no indication of any oxidation when the polysaccharide was allowed to stand for 12 h in Me_2SO. Samples (15 mg) of the same polysaccharide were treated with dimsyl-sodium (2 mL) in Me_2SO (5 mL) with ultrasonication for periods ranging from 1 h to 1 week before adding CH_3I (2 mL) to frozen samples. Methylated polysaccharides were hydrolysed successively with aq. 90% HCO_2H (5 mL; 100° for 1 h) and then M H_2SO_4 (15 h at 100°). Hydrolysates of methylated polysaccharides were neutralised[22] using $Ba(OH)_2$ and then $BaCO_3$. Hydrolysates of unmethylated polysaccharides were neutralised[16] by shaking with 20% *N,N*-dioctylmethylamine in $CHCl_3$. Glycitol acetates were identified by g.l.c. and by g.l.c.–e.i.-m.s.[23].

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