

329. *The Hemicelluloses of Esparto Grass (Stipa tenacissima, L.). The Arabinose-rich Fraction.*

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An arabinose-rich fraction has been isolated from esparto-grass hemicellulose. This gave on hydrolysis D-xylose (12 parts), L-arabinose (5 parts), D-glucose (1 part), and D-galactose (1 part). Hydrolysis of the methylated polysaccharide gave 2:3:5-trimethyl L-arabinose (1 part), 2:3-dimethyl D-xylose (3 parts), and 2-methyl D-xylose (1 part), together with smaller quantities of 2:3-dimethyl L-arabinose, 2:3:4:6-tetramethyl D-galactose, 2:4:6-trimethyl D-galactose, and 2:4-dimethyl D-galactose. It is concluded that the majority of the L-arabofuranose residues are present as side-chains attached to a main chain of 1:4-linked D-xylopyranose residues.

THE presence of combined arabinose (*ca.* 7%) associated with esparto xylan has been known for some time and early work appeared to indicate that arabinose was present in the furanose form as an end group in a molecule comprising 18—20 xylopyranose residues (Haworth, Hirst, and Oliver, *J.*, 1934, 1917). More recently it was found that, by repeated fractional precipitation as the copper complex under carefully controlled and very mild conditions, a xylan devoid of arabinose residues could be obtained from esparto (Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289). This polysaccharide was shown to consist of a singly branched molecule containing $75(\pm 5)$ D-xylopyranose units. These residues are linked through C₍₁₎ and C₍₄₎ except that the single branching point is formed by a 1:3-union. The mode of occurrence of the arabinose residues in the hemicellulose fraction remained obscure and the present investigation was undertaken to obtain fuller information on this point. Possibilities which could be envisaged were that the arabinose residues might be present in an associated araban of the type found in pectic substances (Hirst and Jones, *J.*, 1939, 452) or in a xyloaraban or araboxylan such as that recently isolated from wheat flour (Perlin, *Cereal Chem.*, 1951, 28, 352). In the latter instance the arabofuranose residues were attached as side chains to a main chain of 1:4-linked D-xylopyranose units.

Crude esparto xylan was extracted from delignified esparto grass by the method of Chanda *et al.* (*loc. cit.*), and the hemicellulose thus obtained was extracted with hot 70% aqueous alcohol. This gave an arabinose-rich fraction, which on hydrolysis yielded xylose (12 parts), arabinose (5 parts), glucose (1 part), and galactose (1 part). Although many methods of extraction and fractionation were investigated the separation of an araban in a pure state was not achieved and we regard it as doubtful whether such a polysaccharide composed solely of arabinose residues does in fact occur in esparto hemicellulose.

The material richest in arabinose residues (hereinafter called polysaccharide A) was methylated, first by Fear and Menzies's method (*J.*, 1926, 937) and then with methyl iodide and silver oxide. The methylated derivative (OMe, 36.6%) was hydrolysed successively with methanolic and with aqueous hydrochloric acid, and the products of hydrolysis were chromatographed on a cellulose column. Although complete separation of all the fractions was not achieved, the presence of the following sugars was established by the isolation of crystalline derivatives (quantitative analyses indicated that these were present in the proportions shown in parentheses): 2:3:5-trimethyl L-arabinose (29), 2:3:4:6-tetramethyl D-galactose (4), 2:3-dimethyl D-xylose (100), 2:3-dimethyl L-arabinose (6), 2:4:6-trimethyl D-galactose (8), 2-methyl D-xylose (34), and 2:4-dimethyl D-galactose (8). The trimethyl pentose fraction was shown chromatographically to yield on demethylation a small quantity of xylose in addition to arabinose. It is likely therefore that the fraction contained, as is to be expected, a little 2:3:4-trimethyl xylose but attempts to form derivatives were unsuccessful. No methylated derivatives of glucose were isolated.

The small proportion of 2:3-dimethyl arabinose present together with the complete absence of a monomethyl arabinose preclude the existence of a multi-branched araban of

Separation of Methylated Sugars.—The syrupy hydrolysate (5.5 g.) was fractionated on a cellulose column (90 × 3.4 cm.) (Hough, Jones, and Wadman, *J.*, 1949, 2511), elution being carried out with (a) light petroleum (b. p. 100–120°)–butanol (7 : 3), saturated with water, (b) light petroleum–butanol (1 : 1), saturated with water, and (c) butanol, partly saturated with water. The fractions obtained (see Table) were examined by paper chromatography with butanol–ethanol–water–ammonia (40 : 10 : 49 : 1; top layer) as solvent, and in a number of cases the sugars produced after demethylation were examined (Hough, Jones, and Wadman, *J.* 1950, 1702).

Analysis of hydrolysate of methylated polysaccharide A.

Fraction	Wt. of material eluted (g.)	[α] _D ²⁰	Paper chromatography		Sugar given after demethylation *
			R _F *	Sugar	
1	0.053	—	{ 1.00 (t) 0.95	Tetramethyl glucose Trimethyl pentose	—
2	0.265	–26.8°	0.95	Trimethyl pentose	{ Arabinose, xylose (trace), glucose (trace)
3	0.307	–18.3	0.95	Trimethyl pentose	Arabinose, xylose (trace)
4	0.023	+23	{ 0.95 0.88	Trimethyl pentose Tetramethyl galactose	—
5	0.056	+79.2	.88	Tetramethyl galactose	—
6	0.116	–18.2	{ 0.88 (t) 0.82 0.74 (t)	Tetramethyl galactose Unknown Dimethyl xylose	{ Galactose (trace) Arabinose Xylose (trace)
7	0.068	+30.8	{ 0.82 0.74	Unknown sugar Dimethyl xylose	—
8	1.907	+30.0	0.74	Dimethyl xylose	—
9	0.063	+46	{ 0.74 0.70	Dimethyl xylose Trimethyl galactose	Xylose, galactose
10	0.065	+54.7	{ 0.74 (t) 0.70 0.64 (t)	Dimethyl xylose Trimethyl galactose Dimethyl arabinose	—
11	0.209	+85.2	{ 0.70 0.64	Trimethyl galactose Dimethyl arabinose	—
12	0.103	—	{ 0.64 (t) 0.38	Dimethyl arabinose Monomethyl xylose	—
13	0.559	+34	0.38	Monomethyl xylose	—
14	0.043	—	{ 0.38 0.36	Monomethyl xylose Dimethyl galactose	—
15	0.119	+71.6	0.36	Dimethyl galactose	Galactose
16	0.067	—	{ 0.36 0.30 (t)	Dimethyl galactose Unknown	—
17	0.104	—	{ 0.15 0.13 (t)	Xylose Arabinose	—

* t = trace.

Identification of fractions. 2 : 3 : 5-Trimethyl L-arabinose was identified in fractions 2 and 3 by conversion into 2 : 3 : 5-trimethyl L-arabonamide (m. p. and mixed m. p. 135–136°). An attempt to prepare the aniline derivative of 2 : 3 : 4-trimethyl D-xylose from fraction 3 was unsuccessful. 2 : 3 : 4 : 6-Tetramethyl D-galactose was identified in fraction 5 by conversion into 2 : 3 : 4 : 6-tetramethyl D-galactosylaniline (m. p. and mixed m. p. 194–195°). 2 : 3-Dimethyl D-xylose was identified in fraction 8 by conversion into 2 : 3-dimethyl D-xylosylaniline (m. p. and mixed m. p. 120–122°) and 2 : 3-dimethyl D-xylonamide (m. p. and mixed m. p. 131–132°). 2 : 4 : 6-Trimethyl D-galactose and 2 : 3-dimethyl L-arabinose were identified in fraction 11 by conversion into 2 : 4 : 6-trimethyl D-galactosylaniline (m. p. and mixed m. p. 165–166°) and 2 : 3-dimethyl L-arabonamide (m. p. and mixed m. p. 159–160°) respectively. 2-Methyl D-xylose from fraction 13 crystallised completely from acetone (m. p. and mixed m. p. 134–136°) and was converted into 2-methyl D-xylosylaniline (m. p. and mixed m. p. 124–125°). 2 : 4-Dimethyl D-galactose from fraction 15 was recrystallised from acetone containing 1% of water, giving the monohydrate (m. p. and mixed m. p. 93–96°), and was characterised as 2 : 4-dimethyl D-galactosylaniline (m. p. and mixed m. p. 209–210°) and 2 : 4-dimethyl D-galactonamide (m. p. and mixed m. p. 163–165°).

Quantitative Estimation of Methylated Sugars.—After identification of the fractions obtained from the column the relative proportions of the different sugars were calculated, the results being tabulated below. In the case of fractions containing mixtures of sugars the relative proportions were estimated approximately from the intensities and colours of the spots given on paper chromatograms.

Sugar	Wt. (g.)	Parts per 100 parts of 2 : 3-di- methyl xylose	Sugar	Wt. (g.)	Parts per 100 parts of 2 : 3-di- methyl xylose
Trimethyl pentose	0.636	32	Trimethyl galactose ...	0.160	8
Tetramethyl galactose	0.080	4	Monomethyl xylose ...	0.680	34
Dimethyl xylose	2.000	100	Dimethyl galactose ...	0.160	8
Dimethyl arabinose ...	0.120	6			

On the assumption that trimethyl xylose was present in the same quantity as in the hydrolysate of methylated arabinose-free xylan, 32 parts of trimethyl pentose would contain 3 parts of trimethyl xylose and 29 parts of trimethyl arabinose.

The authors thank Messrs. Alex. Cowan and Sons Ltd. for financial assistance, including the award of a scholarship to one of them (R. W. M.), and the Distillers Company Ltd. for a grant.

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[Received, February 11th, 1953.]