15. *Studies on Seed Mucilages. Part II. The Seed Mucilage of* Plantago arenaria.

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P. arenaria seeds yielded an acid polysaccharide of equivalent weight *ca.* 2000 which gave on hydrolysis *d*-xylose (70%), *l*-arabinose (9·5%), *d*-galactose (3·3%), and an aldobionic acid (13%) composed of *d*-galacturonic acid and *d*-xylose.

Hydrolysis of the methylated mucilage gave trimethyl xylopyranose (*ca.* 30%), 2-methyl xylose (*ca.* 23%), tetramethyl galactopyranose (*ca.* 4%), and a mixture (*ca.* 40%) composed chiefly of 3 : 4-dimethyl xylose but probably containing methylated arabinoses in addition.

It is concluded that the polysaccharide contains xylopyranose and galactopyranose " end groups " in the ratio of 9 : 1, the remaining xylose residues being linked by 1 : 2-β-linkages or triply linked with a free hydroxyl group on C_2.

Continuing our studies on seed mucilages of the *Plantago* family (J., 1940, 1501), we have now completed a preliminary study of the composition of the mucilage of the so-called dark Psyllium seed used in pharmacy. All the commercial samples we obtained were identified as *Plantago arenaria.* Anderson and Fireman (*J. Biol. Chem.*, 1935, **109**, 437) investigated light Psyllium—presumably the seeds known as ispaghula (*P. ovata*)—but did not report the results of any methylation experiments.

The seed mucilage, which was superficially similar to that from *P. lanceolata* already described, contained about 5·4% of ash (as sulphate), which fell on prolonged dialysis to 3·3%. Direct titration gave an acid equivalent of *ca.* 2000; the pentosan content of the mucilage was 90%, and the uronic anhydride content 7·5%. Hydrolysis with oxalic acid produced a mixture of sugars (87%) containing *l*-arabinose (9·5%) and *d*-galactose (3%), the remainder being *d*-xylose, together with an aldobionic acid (12%) composed of *d*-xylose and *d*-galacturonic acid. There is thus a marked difference in the composition of this mucilage and that of *P. lanceolata* (Part I, *loc. cit.*), for in that case analysis showed pentosan 72%, methylpentosan 11%, and uronic anhydride 15%, and although a small proportion of *d*-galactose was present, no arabinose could be detected in the products of hydrolysis.

The arenaria mucilage gave an acetate and a methyl derivative having strong negative rotations, a

fact, coupled with a knowledge of the products of hydrolysis, indicating a predominance of β-links. The methylated derivative was hydrolysed and as complete a separation of the methylated glycosides as could be achieved by fractional distillation and anilide formation gave the following results: 29·5% of the methylated polysaccharide appeared as trimethyl xylopyranose, so this proportion must constitute xylopyranose " end groups " in the polysaccharide. From the weight of tetramethyl galactopyranose anilide isolated, 4·5% of tetramethyl galactopyranose is estimated to be present in the hydrolysis products, so it would seem that all the galactose in the mucilage is accounted for by galactopyranose end groups. 40·5% of the hydrolysate (from which the galactose had been removed) was chiefly a dimethyl xylose. Methylation yielded trimethyl xylopyranose in good yield, so substitution on C_5 in the xylose molecule was excluded, and repeated failures to isolate a crystalline dimethyl xylose anilide negatived 2 : 3-dimethyl xylose as a possibility. Oxidation yielded a δ-lactone which has not so far been obtained crystalline (cf. 3 : 4-dimethyl δ-xylonolactone; Part I, *loc. cit.*), although the corresponding amide gave a positive Weerman reaction and oxidation yielded an unsymmetrical hydroxydimethoxyglutaric acid. These facts make it necessary to conclude that this fraction is mainly 3 : 4-dimethyl xylose (cf. Part I), but the failure to obtain crystalline derivatives does not exclude the possibility that the arabinose fragment is also present. A small quantity of a crystalline anilide, m. p. 170°, could be isolated from this fraction after removal of the tetramethyl galactose anilide. Analysis revealed that this was a dimethyl pentose anilide. The same substance was isolated from the hydrolysis products of the methylated mucilage of *Plantago ovata* and since this polysaccharide contains a higher proportion of arabinose than the polysaccharide under review, it is thought probable that this anilide is a dimethyl *l*-arabinose anilide. If so, it cannot be 2 : 3- or 2 : 4-dimethyl arabinose anilide (Smith, J., 1939, 744, 753) and 2 : 5-dimethyl arabinose does not appear to form an anilide (Smith, J., 1940, 1030), leaving 3 : 4- and 3 : 5-dimethyl arabinose as possibilities. This point will be investigated further when a sufficient quantity of the anilide is available.

The remainder of the hydrolysis product (23%) appeared as crystalline 2-methyl xylose (Bywater, Haworth, Hirst, and Peat, J., 1937, 1983), of which the crystalline *anilide* is described.

By taking into account the equivalent of the mucilage and the proportion of galactose, an approximation may be made to the composition of the chemical unit. Such a unit may contain nine xylopyranose and one galactopyranose end groups, ten xylopyranose linking units (joined by 1 : 2-β-linkages) and three arabinose linking units, eight xylose residues with free hydroxyl groups on C_2 at branching points of the structure, and two galacturonic acid residues, which appear to form a more stable union with xylose than with galactose or arabinose. This unit containing thirty-three components would have a molecular weight of 4476, an equivalent weight of 2238 and would give 9% of galacturonic acid and 10% of arabinose on hydrolysis. The methyl derivative would yield trimethyl xylose 31·8%, tetramethyl galactose 4·3%, dimethyl pentoses 42·6%, and monomethyl xylose 24·2%. These values are similar to those found experimentally, but further work will be necessary before the ramifications of the molecule can be worked out.

Experimental.

Preparation of the Mucilage.—*P. arenaria* seeds (1 kg.) were steeped in water (12 l.) for 24 hours with occasional stirring. After filtration through muslin and washing with water (4 l.) the product was precipitated by alcohol (2 vols.) with stirring, dehydrated with successive small quantities of alcohol, washed with ether, and dried in a vacuum to give a fibrous product (50 g.) (Found: ash, 5·4% as sulphate, 3·3% after dialysis in running water for 7 days). When the mucilaginous solution prepared as above was poured into acidified alcohol (50 c.c. of concentrated hydrochloric acid/l.), the acid polysaccharide used in the subsequent operations was obtained (Found: equiv., by titration, 2000; uronic anhydride, 7·5%; pentosan, 89·8%; methyl pentosan, nil; ash, 0·6%).

Hydrolysis of the Mucilage with Oxalic Acid.—The mucilage (16·1 g.) in 3% oxalic acid (250 c.c.) was heated at 100° for 20 hours. The insoluble residue (0·13 g.) was removed; the resulting solution showed $[\alpha]_D^{16°}$ + 34°. Neutralisation with calcium carbonate, filtration, and concentration at 45°/15 mm. yielded a syrup (15·5 g.), which was dissolved in water (40 c.c.) and precipitated in alcohol to yield a calcium salt X (2·2 g.) and a syrup Y (13·2 g.).

X appeared to be the calcium salt of an aldobionic acid. It showed $[\alpha]_D^{18°}$ + 64° in water (*c*, 1·2) [Found: Ca, 5·7. Calc. for $(C_{11}H_{18}O_{11})_2Ca$: Ca, 5·8%].

Hydrolysis for 24 hours in an autoclave at 120° with sulphuric acid (4%), followed by neutralisation with barium carbonate, yielded a glass, $[\alpha]_D^{17°}$ + 24° in water (*c*, 0·5), from which extraction with alcohol yielded *d*-xylose, $[\alpha]_D^{17°}$ + 24° in water (*c*, 1·1), and a residue of barium salt, $[\alpha]_D^{17°}$ + 14° in water (*c*, 0·8), which yielded mucic acid, m. p. 220°, on oxidation with bromine water.

The syrup Y (1 g.) gave *l*-arabinosediphenylhydrazone (0·15 g.), m. p. 196°, unchanged on admixture with an authentic specimen. *l*-Arabinose gave a 71% yield of this product, so the arabinose content of the

syrup is calculated to be 9·5% or 8% of anhydroarabinose in the mucilage. Crystalline *d*-xylose (7·1 g.), m. p. 142° alone or when mixed with *d*-xylose, $[\alpha]_D^{17°}$ + 80° (initial), + 17·4° (after 4 hours; constant) in water (*c*, 0·7), was removed by treatment with acetic acid. A portion (2 g.) of the resulting syrup, from which further quantities of *d*-xylose subsequently crystallised, gave *d*-galactosephenylmethylhydrazone (0·28 g.), m. p. 187°, unchanged on admixture with an authentic specimen. This corresponds to 8·8% of *d*-galactose in Y or 3·3% in the mucilage, since galactose (1 g.) yields galactosephenylmethylhydrazone (1·55 g.).

Acetylation of the Mucilage.—The mucilage (10 g.) was moistened with alcohol and dispersed in pyridine (150 c.c.). Acetic anhydride (50 c.c.) was added, followed by a further 50 c.c. in small portions; the mixture was heated at 95° for 20 hours and pyridine (60 c.c.) and acetic anhydride (40 c.c.) were then added. After standing at room temperature for 2 days, the acetate was precipitated by pouring into water; the product (15 g.) was washed with water, alcohol, and ether. Repeated extraction with acetone–chloroform (1 : 1) gave a jelly-like mass (6·5 g.) (Found: CH_3·CO, 33·5%) and a soluble fraction, $[\alpha]_D^{17°}$ − 61° in chloroform (*c*, 0·7) (Found: CH_3·CO, 38·1%).

Methylation.—Both acetates on methylation yielded similar products, so the acetate was methylated directly in four operations, as described in Part I (*loc. cit.*), without extraction with solvents (OMe, 33·9%), $[\alpha]_D^{17°}$ − 100° in chloroform (*c*, 0·3), after the first and (OMe, 35·3%), $[\alpha]_D^{17°}$ − 104° in chloroform (*c*, 0·3) after three further methylations. Fractional precipitation from chloroform solution by light petroleum gave fractions almost identical, in methoxyl content and specific rotation, with the starting material.

Typical Hydrolysis of the Methylated Polysaccharide.—The methylated compound (10 g.) (OMe, 34·9%; $[\alpha]_D^{17°}$ − 104°) was boiled with methyl-alcoholic hydrogen chloride (200 c.c.; 3%) until the rotation became constant ($[\alpha]_D^{18°}$ + 54°). After neutralisation with silver carbonate and filtration the liquid was concentrated to yield a non-reducing syrup (10·9 g.), which was separated into four fractions by distillation. The first two were refractionated from a Claisen flask with a vacuum-jacketed column, giving finally:

	Yield, g.	B. p.	n_D^{18}.
Fraction I	3·19	80—95°/0·02 mm. (bath temp.)	1·4400
,, II	3·80	95—115/0·02 mm. ,,	1·4563
,, III	1·84	115—130/0·02 mm. ,,	1·4660
,, IV	1·62	130—150/0·02 mm. ,,	1·4752

Fraction 1.—This had $[\alpha]_D^{16°}$ + 27° in chloroform (*c*, 0·6) (Found: OMe, 58·1. Calc. for $C_9H_{18}O_5$: OMe, 60·1%). A portion (2·1 g.) was hydrolysed at 100° for 2 hours with 2% nitric acid (25 c.c.), and the solution neutralised with barium carbonate and evaporated to dryness; the residue, repeatedly extracted with boiling acetone, gave a syrup (1·7 g.) which rapidly crystallised. The crystalline material (0·8 g.) had m. p. 89—90°, not depressed by authentic trimethyl xylopyranose, $[\alpha]_D^{19°}$ + 53° in water (*c*, 0·6), falling to + 20° (1 hour) (Found: C, 49·9; H, 8·5; OMe, 47·3. Calc. for $C_8H_{16}O_5$: C, 50·0; H, 8·3; OMe, 48·4%).

The porous tile used for removing the crystals yielded, on extraction with acetone, a syrup (0·85 g.) which subsequently crystallised and had $n_D^{20°}$ 1·4561, $[\alpha]_D^{19°}$ + 4° in water (*c*, 0·6), rising to + 18°, and therefore contained trimethyl β-xylopyranose. No crystalline anilide could be obtained from this syrup.

Methylation of Fraction II.—Fraction II (0·8 g.), $[\alpha]_D^{15°}$ + 46° in chloroform (*c*, 0·7) (OMe, 47·4%), was thrice methylated with silver oxide and methyl iodide to give an oil (0·7 g.), $n_D^{19°}$ 1·4408, $[\alpha]_D^{16°}$ + 45° in water (*c*, 0·6) (Found: OMe, 58·3%); this (0·36 g.) was hydrolysed with 2% nitric acid to give a syrup (0·28 g.), which yielded crystals (0·2 g.), m. p. 89°, unchanged by authentic trimethyl xylopyranose.

Examination of the Hydrolysis Products of Fraction II.—Hydrolysis of fraction II (1·2 g.) as for fraction I yielded a reducing syrup (1·0 g.), $n_D^{18°}$ 1·4768, $[\alpha]_D^{17°}$ + 33° in water (*c*, 0·6) (Found: OMe, 34·8. Calc. for $C_7H_{14}O_5$: OMe, 34·8%).

Isolation and Removal of Tetramethyl Galactopyranose Anilide.—Hydrolysed fraction II (3·7 g.) was heated with aniline (1·9 g.) in alcohol (10 c.c.) for 1·5 hours and six crops of tetramethyl galactose anilide were separated (0·585 g.), m. p. 195°, not depressed by authentic material, $[\alpha]_D^{17°}$ − 73° in acetone (*c*, 0·6) (Found: C, 61·2; H, 8·0; OMe, 39·6. Calc. for $C_{16}H_{25}O_5N$: C, 61·7; H, 8·1; OMe, 39·9%). A seventh crop of crystals (0·05 g.) had m. p. 170°, $[\alpha]_D^{11°}$ − 74° in acetone (*c*, 0·4), (Found: C, 61·3; H, 7·7; N, 6·25; OMe, 23·2. $C_{13}H_{19}O_4N$ requires C, 61·6; H, 7·5; N, 5·5; OMe, 24·5%).

The quantity of this material was too small for further investigation, but it is thought likely to be a dimethyl arabinose anilide, a similar product having been obtained from *Plantago ovata* mucilage, which contains a higher proportion of *l*-arabinose than *P. arenaria*.

Tetramethyl Galactopyranose Anilide from P. lanceolata *Mucilage.*—Although not reported in Part I, hydrolysed fraction II of the hydrolysis products of *P. lanceolata* mucilage (0·3 g.) gave tetramethyl galactopyranose anilide (0·07 g.), m. p. 195°, unchanged on admixture with an authentic specimen.

Lactone Formation.—The remaining syrupy anilides were hydrolysed with sulphuric acid as described by Smith (J., 1939, 744) and the syrup obtained (1·5 g.) (Found: OMe, 33·2. Calc. for $C_7H_{14}O_5$: OMe, 34·8%) was oxidised with bromine in the usual way. The product obtained after neutralisation with silver carbonate, treatment with hydrogen sulphide, and extraction with ether, on distillation at 140°/0·05 mm. (0·8 g.) had $n_D^{16°}$ 1·4618, $[\alpha]_D^{16°}$ + 46° (5 mins. in water, *c*, 0·6); + 37° (1·6 hrs.); + 31° (3 hrs.); + 29° (4 hrs.; constant

value) (Found: OMe, 35·5. Calc. for $C_7H_{12}O_5$: OMe, 36·1%. 0·0484 G. required 5·1 c.c. of N/20-sodium hydroxide. Calc. for $C_7H_{12}O_5$: 5·5 c.c.).

The lactone yielded an amide, $[\alpha]_D^{17°}$ + 34° in water (*c*, 1·6) (Found: OMe, 30·2. Calc. for $C_7H_{15}O_5N$: OMe, 32·1%), 0·12 g. of which gave 0·03 g. of hydrazodicarbonamide, m. p. 257°, when subjected to the Weerman test.

Attempts to isolate crystalline 3 : 4-dimethyl β-methylxyloside and its *p*-toluenesulphonate (Robertson and Speedie, J., 1934, 824) were unsuccessful.

Oxidation with Nitric Acid.—The above lactone (0·5 g.) was oxidised, and the products esterified as described in Part I (*loc. cit.*). Distillation gave a syrup (0·3 g.), b. p. 130°/0·05 mm. (bath temp.), $n_D^{16°}$ 1·4460, $[\alpha]_D^{15°}$ + 45° in methyl alcohol (*c*, 0·7) (Found: OMe, 53·0. Calc. for $C_9H_{16}O_7$: OMe, 52·5%), which had identical properties with the hydroxydimethoxyglutaric ester described in Part I. Treatment with methyl-alcoholic ammonia yielded an amide, $[\alpha]_D$ + 25° in water (*c*, 0·5), 0·05 g. of which gave hydrazodicarbonamide (0·012 g.) when subjected to the Weerman test.

Fraction III.—Methylation and hydrolysis. Fraction III (0·5 g.), $[\alpha]_D^{17°}$ + 56° in chloroform (*c*, 0·6) (Found: OMe, 41·8%), was methylated four times with the Purdie reagents to yield a mobile oil (0·45 g.), $n_D^{18°}$ 1·4404, which on hydrolysis yielded trimethyl xylopyranose (0·13 g.), m. p. 90°, not depressed on admixture with an authentic specimen, together with a syrup (0·17 g.), $[\alpha]_D^{18°}$ + 22·6° in water (*c*, 0·7), which later crystallised almost completely to yield trimethyl xylopyranose, m. p. 89°. Anilide formation with the above syrup gave tetramethyl galactopyranose anilide, m. p. 196° (1%). Fraction III is therefore a 1 : 1 mixture of dimethyl and monomethyl methylxylosides containing a small amount of methylated galactosides. Hydrolysis (3·15 g.) in the usual way gave a syrup (2·94 g.) (OMe, 27·0%), which was treated with aniline (1·61 g.) in alcohol (7 c.c.). Three crops of crystals were obtained. Crop 1 (0·87 g.) on recrystallisation from ethyl acetate and light petroleum had m. p. 140°, $[\alpha]_D^{17°}$ + 135° after 5 minutes in ethyl acetate [(10 c.c.) and acetic acid (0·4 c.c.) (*c*, 0·7)]; +113° (10 mins.); + 97° (20 mins.); + 93° (30 mins.); + 82·6° (24 hrs., constant value); $[\alpha]_D^{18°}$ + 240° in ethyl acetate (*c*, 0·7) (cf. 2 : 3-dimethyl xylose anilide; Hampton, Haworth, and Hirst, J., 1929, 1739) (Found: C, 60·6; H, 7·2; N, 6·1; OMe, 13·3. $C_{12}H_{17}O_4N$ requires C, 60·3; H, 7·1; N, 5·9; OMe, 13·0%). Hydrolysis of the anilide as previously described gave quantitatively 2-methyl xylose, m. p. 132—134°, not depressed on admixture with an authentic specimen prepared by the method of Robertson and Speedie (*loc. cit.*) (Found: C, 44·4; H, 7·4. Calc. for $C_6H_{12}O_5$: C, 43·9; H, 7·4%).

Crop 2 (0·04 g.) was tetramethyl galactopyranose anilide and crop 3 (0·07 g.) was a mixture of crops 1 and 2.

Hydrolysed fraction III was converted into the lactone, $[\alpha]_D^{17°}$ + 48° in water (*c*, 1·3) (initial); + 44° (3 hrs.; constant value), indicating the presence of some δ-lactone. The corresponding amide (0·1 g.) gave hydrazodicarbonamide (0·014 g.), m. p. 256°, on treatment according to Weerman.

Thus fraction III is composed of 2-methyl methylxylosides *ca.* 50% and tetramethyl methylgalactosides *ca.* 1%, the remainder being similar to fraction II.

Fraction IV. Isolation of 2-Methyl Xylose.—Fraction IV had $[\alpha]_D^{17°}$ + 75° in chloroform (*c*, 0·6) (Found: OMe, 33·2. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). Hydrolysis (2·54 g.) with 2% nitric acid gave a syrup, from which 2-methyl xylose (0·75 g.) crystallised, m. p. 134°, unchanged on admixture with authentic material, $[\alpha]_D^{18°}$ − 21° in water (*c*, 1·1) (initial); − 6° (5 mins.); + 19° (30 mins.); + 26·5° (90 mins.); + 36° (3 hrs. constant value) (Found: OMe, 18·2. Calc. for $C_6H_{12}O_5$: OMe, 18·9%).

0·1 G. yielded 0·03 g. of an osazone devoid of methoxyl, m. p. 160—161°, unchanged on admixture with *d*-xylosazone. The syrup (0·2 g.) from which the crystals had been removed also yielded *d*-xylosazone (0·06 g.). The syrupy residue crystallised almost completely to 2-methyl xylose on standing.

A search for the methylated uronic acid by ester determinations on the various fractions was unsuccessful. Fractions I, II, and III gave CO_2Me nil, fraction IV 1·2%, and the still residue 1%. It is concluded that decomposition had occurred during hydrolysis.

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