26. *The Hemicellulose of* Phormium tenax (*N.Z. Flax*). *Part II. The Constitution of the Aldotrionic Acid.*

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The " aldopolyonic " acid of Phormium hemicellulose has been re-examined and found to consist of an aldotrionic acid constituted of a terminal residue of D-glucuronic acid and two D-xylose residues, one of which is linked through positions 1, 2, and 4, and the other through positions 1 and 4. Tentative formulæ for the aldotrionic acid and the hemicellulose are proposed and discussed.

Nitric acid oxidation of the methylated aldotrionic ester gave *meso*-xylodihydroxymethoxyglutaric acid (1 part), 2 : 3 : 4-trimethyl saccharo-δ-lactone (*ca.* 1 part), dimethoxysuccinic acid (*ca.* 4%), and an α-hydroxy-acid believed to be xylohydroxydimethoxyglutaric acid (unsymm.) (1 part). Partial hydrolysis of the methylated aldotrionic ester (2% methanolic hydrogen chloride at 120°) gave 2 : 3-dimethyl methylxyloside and a monomethyl methylxyloside.

In Part I (*J.*, 1945, 796) a preliminary investigation of the structure of the hemicellulose extracted from *Phormium tenax* (N.Z. flax) by 4% sodium hydroxide was reported. Methanolysis of the methylated polysaccharide gave 2 : 3-dimethyl methylxyloside, 2 : 3 : 4-trimethyl methylxyloside (*ca.* 11%, end-group), and the methyl ester of a resistant acid nucleus (aldopolyonic acid). The yield of end-group indicated a chain length of 9—10 xylose units for the main xylose chain, while an upward mutarotation on methanolysis of the methylated polysaccharide indicated a predominance of β-linkages. Accordingly it was deduced that Phormium hemicellulose is constituted of a chain of 9—10 D-xylose residues united in 1 : 4-β-linkage and terminated at the reducing end by an " aldopolyonic " acid.

Oxidation of the barium salt of the methylated aldopolyonic acid with nitric acid, esterification, and distillation of the mixture of esters (1·27 g.) obtained, yielded dimethyl xylohydroxydimethoxyglutarate (unsymm.) (*ca.* 30%), together with some dimethyl dimethoxysuccinate and other oxidation products tentatively identified as 2 : 3 : 4-trimethyl saccharo-δ-lactone methyl ester and 2 : 3-dimethyl saccharo-γ-lactone methyl ester.

The methylated aldopolyonic ester has now been subjected to further examination by two methods : (*a*) methanolysis by 2% methanolic hydrogen chloride for 21 hours at 120° in a sealed tube, and (*b*) nitric acid oxidation under the conditions employed in Part I (*loc. cit.*) but on a larger scale.

Although the drastic conditions of methanolysis in method (*a*) led to considerable degradation and not more than 60% of distillable products was obtained, hydrolysis was incomplete, one-fourth of the methylated aldopolyonic ester being recovered from the distillate. Approximately one-third of the distillate consisted of 2 : 3-dimethyl methylxyloside, identified by conversion into 2 : 3-dimethylxylose anilide, m. p. 145°. In addition a small amount (*ca.* 5%) of an unidentified monomethyl methylxyloside was isolated. No free uronic ester was recovered.

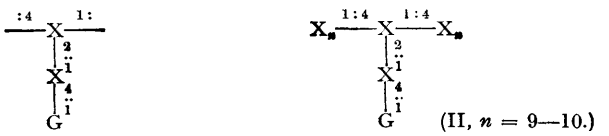
Nitric acid oxidation of the methylated aldopolyonic ester (4·35 g.) was more fruitful. The mixture of esters obtained on esterification of the oxidation products yielded on distillation

dimethyl *meso*-xylodihydroxymethoxyglutarate (1 part) (identified as the *amide*, m. p. 163—164°, optically inactive), the methyl ester of 2 : 3 : 4-trimethyl saccharo-δ-lactone (*ca.* 1 part), dimethyl dimethoxysuccinate (*ca.* 4%; identified as the amide, m. p. 270°), and a syrup believed to consist mainly of dimethyl xylohydroxydimethoxyglutarate (unsymm.) (1 part). Treatment of the syrup with methanolic ammonia yielded a syrupy *α-hydroxy-amide* (Weerman's test) which had rotation $[\alpha]_D$ +26° in water and methoxyl, 33% {cf. xylohydroxydimethoxyglutaramide (unsymm.), $[\alpha]_D$ + 27° in water, methoxyl, 31·0% (Mullan and Percival, *J.*, 1940, 1506)}. This amide was reported by the latter workers as a syrup, but was obtained by Mauger in Part I of this series (*loc. cit.*) in crystalline form, m. p. 140°.

In support of the view that the syrup has the above composition there is the following evidence: (*a*) the isolation of 2 : 3-dimethyl xylose from the products of partial hydrolysis of methylated aldopolyonic ester, (*b*) the isolation of approximately 30% of xylohydroxydimethoxyglutarate from the products of the oxidation described in Part I.

The dimethyl *meso*-xylodihydroxymethoxyglutarate could arise only from the oxidation of 3-methyl xylose. Dimethyl xylohydroxydimethoxyglutarate (unsymm.) could have its origin in 2 : 3- or 3 : 4-dimethylxylose, but the isolation of 2 : 3-dimethyl xylose from the hydrolysis products of the methylated aldotrionic ester indicates that 3 : 4-dimethyl xylose is not the source. The methyl ester of 2 : 3 : 4-trimethyl saccharo-δ-lactone arises from 2 : 3 : 4-trimethyl glucuronic acid which must be present as a terminal unit in the methylated aldotrionic acid.

It is concluded that the aldopolyonic acid of Phormium hemicellulose is in reality an aldotrionic acid consisting of a terminal residue of D-glucuronic acid and two D-xylose residues, one of which is linked through positions 1, 2, and 4 and the other through positions 1 and 4. Possible formulæ for the aldotrionic acid (I), and for the hemicellulose (II), are given below:



(I.) (II, n = 9—10.)

X = D-xylopyranose. G = D-glucuronic acid.

Since Phormium hemicellulose exhibits no reducing properties it is evident that the reducing group of the terminal xylose residue of the aldotrionic acid cannot be free but must be involved in linkage to a second aldotrionic acid unit or to a xylose chain as in formula (II). The calculated equivalent (2816—3080) for formula (II) is in good agreement with the observed equivalent (*ca.* 3000) of the hemicellulose. A final decision on the state of aggregation must await determination of the molecular weight of the polysaccharide.

Experimental.

(Micro-analyses are by Weiler and Strauss, Oxford.)

Methylation.—"Total" hemicellulose (50 g.) extracted from *Phormium tenax* var. *Ngaro* leaves (800 g., air dry) according to the procedure described in Part I (*loc. cit.*), dissolved in sodium hydroxide (750 c.c., 4%), was methylated with methyl sulphate (600 c.c.) and sodium hydroxide (1700 c.c., 30%) at 40—45°, 10 additions being made at 15-minute intervals. The solution was neutralised by sulphuric acid (20%) at 0° and made just alkaline with sodium hydroxide. On warming, partly methylated hemicellulose rose to the surface and was filtered off. The filtrate was concentrated at below 50°, and the combined precipitate and concentrate were re-methylated as before. After 5 methylations the methylated product was separated by filtration of the hot solution. Yield, 72 g. (corr. for ash) (Found: OMe, 34·8%).

The crude product was dissolved in water (600 c.c.) and precipitated by acidification with sulphuric acid (20%) at 0°, and the precipitate taken up in acetone and methylated with methyl iodide (288 c.c.) and silver oxide (88 g.) at 45° during 8 hours. The product, which was now soluble in methyl iodide, was re-methylated with methyl iodide (200 c.c.) and silver oxide (60 g.). Extraction with chloroform and precipitation with light petroleum gave a cream powder (39·4 g.) (Found: OMe, 36·7%; ash, nil).

Methanolysis.—Fully methylated hemicellulose (39·4 g.; OMe, 36·7%) was heated with 2% methanolic hydrogen chloride (400 c.c.) for 8½ hours at 100°. Final rotation: $[\alpha]_D^{20°}$ +55·1° (*c*, 0·96; constant). Neutralisation with silver carbonate, filtration, and evaporation of the solvent yielded a syrup (38·5 g.) which was heated with 3 times the theoretical quantity of saturated barium hydroxide solution for 2 hours at 60°. Excess of barium was removed by carbon dioxide, the filtrate evaporated to dryness under reduced pressure, and the resultant syrup taken up in dry methanol. Addition of ether and light petroleum precipitated the barium salt as a hygroscopic cream powder which was purified by repeated precipitation by ether–light petroleum from solution in methanol. Yield, 10·2 g. (Found: OMe, 32·3; Ba, 13·0%). Evaporation of the combined filtrates gave a syrup (26·9 g.) (Found: OMe, 45·4. Calc. for dimethyl methylxyloside, $C_8H_{16}O_5$: OMe, 48·0%).

Esterification of the Barium Salt.—The barium salt (10·2 g.) was refluxed with 1% methanolic

hydrogen chloride (250 c.c.) for 7 hours. Neutralisation by silver carbonate, filtration, and evaporation of the filtrate gave a yellow syrup which still contained barium. The syrup was taken up in acetone and filtered. Evaporation of the filtrate gave aldopolyonic methyl ester as a yellow syrup (9·54 g.), $n_D^{21°}$ 1·4660°, $[\alpha]_D^{20°}$ +87·5° (*c*, 0·8 in chloroform) (Found : OMe, 44·0%).

Nitric Acid Oxidation of Aldopolyonic Methyl Ester.—The methyl ester (4·35 g.), dissolved in " A.R." nitric acid (60 c.c., *d* 1·20), was oxidised according to the procedure described in Part I (*loc. cit.*). The resultant syrup was esterified by refluxing it with 3% methanolic hydrogen chloride (50 c.c.) for 6 hours. Hydrogen chloride was removed by silver carbonate. Ether extraction of the evaporated filtrate and concentration gave a neutral, yellow syrup (3·87 g.).

Fractionation of the Methyl Esters.—The mixture of esters (3·87 g.) was distilled from a vacuum-jacketed Vigreux column in the following fractions (b. p.s recorded are bath temperatures).

Fraction (1). 0·15 G., pale yellow liquid, b. p. 113—123°/0·1 mm., $n_D^{21°}$ 1·4449 (Found : OMe, 49·1%). Treatment with methanolic ammonia at 0° gave dimethoxysuccinamide (15 mg.) which darkened above 200° and had m. p. 270° (decomp.), not depressed by admixture with an authentic specimen. Concentration of the mother liquor gave a second crop (0·11 g.) of dimethoxysuccinamide, m. p. and mixed m. p. 270°.

Fraction (2). 1·73 G., pale yellow liquid, b. p. 140—150°/0·1 mm., $n_D^{22°}$ 1·4523 (Found : OMe, 46·1%). On trituration with alcohol–ether–light petroleum, crystals (0·67 g.) separated. These had m. p. 105—106° and $[\alpha]_D^{21°}$ +100° ⟶ +52° (*c*, 0·85 in methanol; constant after 20 hours); cf. 2 : 3 : 4-trimethyl saccharo-δ-lactone methyl ester, m. p. 105—106°, $[\alpha]_D$ +102° ⟶ +52° in methanol (Smith, *J.*, 1939, 1732) (Found : C, 48·4; H, 6·4; OMe, 50·1. Calc. for $C_{10}H_{16}O_7$: C, 48·4; H, 6·5; OMe, 50·0%). Treatment of the mother liquor with methanolic ammonia gave crystals (0·64 g.) of **meso-*xylodihydroxymethoxyglutaramide***, m. p. 163—164° (decomp.) after darkening at *ca.* 155°, $[\alpha]_D^{15°}$ ±0° (*c*, 0·47 in water) (Found : C, 37·2; H, 6·4; N, 14·5; OMe, 17·1. $C_6H_{12}O_5N_2$ requires C, 37·5; H, 6·3; N, 14·6; OMe, 16·1%). The amide gave a positive Weerman test.

Fraction (3). 1·23 G., yellow liquid, b. p. 150—160°/0·1 mm., $n_D^{22°}$ 1·4616 (Found : OMe, 42·9%). On standing, this fraction deposited crystals which were separated by trituration with alcohol–ether. Yield, 0·35 g., m. p. 105—106° alone or on admixture with authentic 2 : 3 : 4-trimethyl saccharo-δ-lactone methyl ester. $[\alpha]_D^{21°}$ +100° ⟶ +52° (*c*, 0·8 in methanol) (Found : OMe, 50·0. Calc. for $C_{10}H_{16}O_7$: OMe, 50·0%). The mother liquor, treated with methanolic ammonia at 0°, gave a crystalline amide (0·51 g.), m. p. 163—164° (decomp.) after darkening at *ca.* 155°, not depressed by admixture with *meso*-xylodihydroxymethoxyglutaramide from fraction (2); optically inactive in water.

Fraction (4). 0·10 G., extracted from the column and side-arm of the distilling flask with ether, yellow liquid, $n_D^{20°}$ 1·4646 (Found : OMe, 38·5%). Trituration with alcohol–ether–light petroleum gave 2 : 3 : 4-trimethyl saccharo-δ-lactone methyl ester (0·05 g.), m. p. and mixed m. p. 105—106°. Addition of methanolic ammonia to the mother liquor at 0° gave crystals (12 mg.) of *meso*-xylodihydroxymethoxyglutaramide, m. p. 163—164° (decomp.) not depressed by admixture with sample from fraction (2).

Fraction (5). 0·23 G., still residue, b. p. >180°/0·1 mm., a dark brown, resinous mass, partly charred. Extraction with methanol, filtration through charcoal, and evaporation, gave a brown syrup (0·13 g.) (Found : OMe, 31·4%). This fraction was treated with methanolic ammonia but it was not possible to isolate a crystalline amide.

Examination of the Combined Mother Liquors.—Concentration of the combined mother liquors yielded a yellow syrup (1·1 g.) which had $[\alpha]_D^{18°}$ +26° (*c*, 0·86 in water). Cf. xylohydroxydimethoxyglutaramide, a syrup, $[\alpha]_D$ +27° in water (Mullan and Percival, *loc. cit.*) (Found : C, 40·9; H, 6·9; OMe, 33·0. $C_7H_{14}O_5N_2$ requires C, 40·8; H, 6·8; OMe, 31·0%). The *amide* (0·083 g.) gave hydrazodicarbonamide (0·037 g.) when subjected to the Weerman test for α-hydroxy-amides.

Methanolysis of the Aldopolyonic Ester.—Methylated aldopolyonic methyl ester (4·5 g.) was heated with 2% methanolic hydrogen chloride (50 c.c.) for 21 hours in a sealed tube at 120°. Neutralisation with silver carbonate, filtration and evaporation of the filtrate yielded a brown syrup (4·35 g.) which was distilled in the following fractions : Fraction (i), 0·28 g., b. p. 95—105°/0·04 mm., $n_D^{20°}$ 1·4530 (Found : OMe, 46·7%); Fraction (ii), 1·12 g., b. p. 115—120°/0·04 mm., $n_D^{20°}$ 1·4530—1·4546 (Found : OMe, 47·2%); Fraction (iii), 0·30 g., b. p. 124—130°/0·04 mm., $n_D^{20°}$ 1·4546—1·4586 (Found : OMe, 44·6%); Fraction (iv), 0·76 g., b. p. 170—200°/0·04 mm., $n_D^{20°}$ 1·4586—1·4641 (Found : OMe, 43·0%). The still residue (1·4 g.) was refluxed with 4% methanolic hydrogen chloride (50 c.c.) for 13 hours at 100°. Neutralisation with silver carbonate, filtration, and evaporation of the filtrate gave a syrup (1·1 g.) which, on distillation, yielded fraction (v) (0·24 g.), b. p. 140—200°/0·04 mm., $n_D^{20°}$ 1·4575 (Found : OMe, 49·6%). The final still residue [Fraction (vi), 0·87 g.] was a brown resin.

Fractions (i) *and* (ii). These gave negative tests for uronic acid (naphtharesorcin). They were combined (1·4 g.) and hydrolysed by heating them with hydrochloric acid (100 c.c., 3%) at 100° for 8 hours; $[\alpha]_D$ (initial) +45° ⟶ +33° (*c*, 1·4; 6 hours). Neutralisation with barium carbonate, evaporation, and extraction of the residue with ether gave a pale yellow syrup which was freed from traces of barium by extraction with acetone and filtration. Removal of the solvent gave a syrup (0·8 g.) (Found : OMe, 34. Calc. for dimethylxylose, $C_7H_{14}O_5$: OMe, 34·8%). Refluxing with alcoholic aniline and distillation of the solvent converted the syrup to a crystalline anilide, m. p. 145°, not depressed by admixture with authentic 2 : 3-dimethyl xylose anilide, m. p. 145° (Hampton, Haworth, and Hirst, *J.*, 1929, 1748). 2 : 4-Dimethyl xylose forms an anilide, m. p. 170° (Hirst and Jones, *J.*, 1946, 783); 3 : 4-dimethyl xylose does not form a crystalline anilide.

Examination of fractions (iii), (iv), (v), *and* (vi). An attempt to prepare crystalline amides by treatment of these individual fractions with methanolic ammonia was unsuccessful. The four fractions were combined and heated with saturated barium hydroxide solution (25 c.c.) for 3 hours at 60°. Excess of barium was removed by carbon dioxide, and the filtered solution evaporated to dryness under reduced pressure. The residue was taken up in dry methanol, and the barium salt (*A*) precipitated by addition of ether. The barium salt was thrice redissolved in methanol and precipitated by ether to effect purification. Yield, 0·68 g. (Found : OMe, 28·8; Ba, 19·1%). The combined filtrates, on evaporation, yielded a pale yellow syrup (*B*) (1·01 g.); $n_D^{11°}$ 1·4640 (Found : OMe, 30·0%).

Esterification of Barium Salt (A).—Barium salt (*A*) (0·64 g.) was esterified by refluxing it with 1% methanolic hydrogen chloride (50 c.c.) for 7 hours. The barium chloride which separated on cooling was filtered off, and the filtrate neutralised with silver carbonate, filtered, and evaporated under reduced pressure. Traces of barium were removed by solution of the resultant syrup in acetone and addition of ether. Evaporation of the filtrate yielded a brown syrup (0·49 g.); $n_D^{14°}$ 1·4681 (Found: OMe, 45·0%) (cf. methylated aldopolyonic methyl ester, $n_D^{21°}$ 1·4660; OMe, 44·0%).

Hydrolysis and Oxidation.—The methyl ester (0·48 g.) from the barium salt (*A*) was heated with N-sulphuric acid (30 c.c.) for 20 hours at 100° in order to remove the ester methoxyl group and at the same time hydrolyse the oligosaccharide. The solution was neutralised by barium carbonate, filtered, and evaporated to dryness under reduced pressure. The residue was dissolved in water (5 c.c.) and oxidised with bromine (1 c.c.) until it was non-reducing to Fehling's solution. Bromine was removed by aeration, and the solution neutralised by silver oxide. Silver bromide was filtered off, and traces of silver were removed by hydrogen sulphide. Evaporation of the filtrate gave a syrup which was esterified by boiling for 8 hours with 1% methanolic hydrogen chloride (50 c.c.). Neutralisation with silver carbonate, filtration, and evaporation yielded a syrup (0·34 g.); $[\alpha]_D^{20°}$ +72° (*c*, 1·32 in methanol) (Found: OMe, 43·2%; equiv., 495). This fraction was evidently unhydrolysed oligosaccharide.

Examination of Syrup (B).—Syrup (*B*) (0·86 g., $n_D^{11°}$ 1·4640, OMe, 30·0%) was heated with N-sulphuric acid (50 c.c.) for 3 hours at 100°. The solution was neutralised with barium carbonate and filtered, and the filtrate evaporated to dryness under reduced pressure. Extraction of the residue with hot acetone and evaporation of the solvent yielded a crystalline mass (0·52 g.). Addition of ether to a solution of the latter in methanol precipitated barium salt (*C*) (0·39 g.) (Found: OMe, 25·3; Ba, 17·6%). Evaporation of the ether–methanol filtrate and washings gave a colourless, reducing syrup (0·12 g.); $n_D^{18°}$ 1·4786, $[\alpha]_D^{16°}$ +28° (*c*, 0·6 in water) (Found: OMe, 20. Calc. for monomethyl xylose: $C_6H_{12}O_5$: OMe, 18·9%). This fraction was lost owing to an unexpected interruption of the water services and could not be further examined.

Esterification of Barium Salt (C).—The barium salt (*C*) (0·36 g.) was esterified by heating it with 2% methanolic hydrogen chloride (30 c.c.) for 5 hours at 100°. Neutralisation with silver carbonate, filtration, and evaporation of the filtrate gave a syrup (0·28 g.) (Found: equiv., 350). This fraction was evidently unhydrolysed oligosaccharide.

CANTERBURY UNIVERSITY COLLEGE, CHRISTCHURCH, N.Z. [*Received, April 13th*, 1948.]