208. *A Preliminary Study of the Polyuronide Hemicellulose of* Phormium tenax *(N.Z. Flax).*

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The polyuronide hemicellulose extracted from *Phormium tenax*, a lignified fibre, gave on hydrolysis *d*-xylose and *d*-glucuronic acid, the latter as component of a resistant acid nucleus.

Methanolysis of the methylated polysaccharide gave 2 : 3 : 4-trimethyl methylxyloside (*ca.* 11%, end group), 2 : 3-dimethyl methylxyloside, and the methyl ester of a methylated *aldo*-polyuronic acid.

Nitric acid oxidation of the latter gave *d-xylo*-hydroxydimethoxyglutaric acid, *d*-dimethoxysuccinic acid, 2 : 3 : 4-trimethyl δ-saccharolactone, and 2 : 3-dimethyl γ-saccharolactone.

It is concluded that the hemicellulose is constituted of a main chain containing 9 or 10 xylo-pyranose residues united by 1 : 4-β-linkages, terminated at the reducing end by a complex, highly branched acid nucleus.

The first recorded application of methylation methods to a study of the constitution of a polyuronide hemicellulose has recently been reported by Granichstadten and Percival (J., 1943, 54), who examined the hemicellulose from Iceland Moss (*Cetraria islandica*), a non-lignified tissue. The methylated product, on hydrolysis, yielded a mixture of trimethyl glucoses, which appeared to be united by β-linkages in the main chain, together with end groups of galactopyranose and glucopyranose. Although uronic acid (5%, probably *d*-glucuronic) was present in the unmethylated hemicellulose, Granichstadten and Percival were unable to isolate its methylated derivative from the hydrolysis products of the methylated hemicellulose.

The investigation of a polyuronide hemicellulose extracted from New Zealand flax, a typical lignified fibre, is now recorded. Hemicelluloses from lignified tissues consist of a uronic acid (generally *d*-glucuronic) united to a series of *d*-xylose units with which *d*-glucose may be associated. They resemble the gums and mucilages in that, on hydrolysis, they yield sugar units and a more resistant portion, an " aldobionic acid," which contains the uronic acid component.

" Total " hemicellulose extracted from *Phormium* leaves by 4% sodium hydroxide, after preliminary extractions with water and 0·5% ammonium oxalate to remove pectin and water-soluble substances, was precipitated by acetic acid and alcohol as a cream, amorphous powder. Hydrolysis with 3% sulphuric acid yielded *d*-xylose and glucuronic acid. Glucose was not detected.

Methylation of the hemicellulose with methyl sulphate and sodium hydroxide yielded a product containing OMe, 32·6% (constant after 5 methylations). Fractional precipitation from chloroform with light petroleum demonstrated the homogeneity of the methylated derivative. Subsequent methylation with Purdie's reagents increased the methoxyl content to 36·8%.

Methanolysis (2% hydrogen chloride) gave a mixture of methylxylosides and the methyl ester of a methylated acid nucleus, the latter being separated as the barium salt. The mixture of xylosides, fractionally distilled, yielded *ca.* 11% of 2 : 3 : 4-trimethyl methylxyloside (end group, identified by hydrolysis to crystalline 2 : 3 : 4-trimethyl xylose), together with 2 : 3-dimethyl methylxyloside (main fraction, identified by conversion into 2 : 3-dimethyl xylonamide). A small proportion of monomethyl methylxyloside present in the residues from distillation is attributed to incomplete methylation rather than to a branched-chain structure.

From the above evidence the authors conclude that the *Phormium* hemicellulose examined consists of a chain containing approximately 10 xylopyranose units united by 1 : 4-glycosidic linkages and terminated at the reducing end by a resistant acid nucleus henceforth referred to as " *aldo*-polyuronic acid." Change in the rotation of the methylated hemicellulose from negative to highly positive on methanolysis suggests a predominance of β-linkages.

From the same end-group assay a possible chain length of approximately 18 units may be deduced for the total hemicellulose, including the *aldo*-polyuronic acid unit.

The methylated barium salt prepared directly from the methanolysis products of fully methylated hemicellulose was oxidised with nitric acid according to Hirst and Purves (J., 1923, **123**, 1352). After removal of the nitric acid, the oxidation products were esterified, and the resultant methyl esters fractionally distilled. Methyl esters of the following were isolated : 2 : 3 : 4-trimethyl δ-saccharolactone, 2 : 3-dimethyl γ-saccharolactone, *d-xylo*-hydroxydimethoxyglutaric acid, *d*-dimethoxysuccinic acid, and an acid which may be *d-xylo*-(unsymm.) dihydroxymethoxyglutaric acid, the last in small yield only. The above esters were converted into crystalline amides for identification.

For purposes of comparison, an authentic sample of 2 : 3-dimethyl methylxyloside was oxidised with nitric acid under the same conditions and esterified. Methyl *d-xylo*-hydroxydimethoxyglutarate (unsymm.) was the major product.

It may be deduced from the foregoing that the methylated *aldo*-polyuronic acid contains dimethyl xylose

(2 : 3 or 3 : 4), 2 : 3-dimethyl glucuronic acid (or glucose), and 2 : 3 : 4-trimethyl glucuronic acid, the latter in a terminal position. Failure to detect glucose among the hydrolysis products of the unmethylated hemicellulose, however, makes a glucose origin for the saccharolactone appear improbable.

Allocation of a constitutional formula to the *aldo*-polyuronic acid would be premature. It is evidently of complex structure and highly branched.

It is not claimed that the length of the main xylose chain is invariably 9—10 units as found for the sample examined. In an earlier investigation by one of us (R. J. M., Ph.D. Thesis, 1938, University of Birmingham) of a different sample of *Phormium* hemicellulose a length of 5 units for the xylose main chain was estimated. It is possible that the size of the repeating unit varies with the age of the plant, or that the hemicellulose may have been degraded during extraction or subsequent treatment.

Experimental.

Extraction.—Phormium tenax, var. Ngaro, leaves (800 g. air-dry), finely pulverised, were extracted with cold water (7 l.) for 24 hours, pressed, and the residue extracted with ammonium oxalate (5 l.; 0·5%) for 7 hrs. on a boiling water-bath to remove pectic substances. The residue, washed and pressed, was extracted three times with 4% sodium hydroxide solution, twice for 48 hours at room temperature (6 l.), and finally for 2 hours at 100° (6 l.). The combined sodium hydroxide extracts, filtered, were concentrated to 3 l. at 25°, excessive alkalinity being prevented by periodical addition of acetic acid. Acidification with glacial acetic acid to pH 4 gave a colloidal solution from which "total hemicellulose" was precipitated by addition of an equal volume of alcohol.

The product was redissolved in sodium hydroxide (4%), acidified, and reprecipitated by excess alcohol; yield 51·7 g. (dry wt.), ash 15·9%. Twice redissolved and reprecipitated, the hemicellulose was obtained as a cream, amorphous powder (Found: OMe, 2; ash, 9·5%; equiv., 2230). The ash content could not be further reduced by this means.

Hydrolysis.—Hemicellulose (5·0 g.) was heated with 3% sulphuric acid (250 c.c.) for 4 hours at 100°. Insoluble material was removed by filtration, and the filtrate, freed from sulphuric acid by precipitation as barium sulphate, was examined for sugars. Xylose (as cadmium xylono-bromide; xylosephenylhydrazone, m. p. 116°; and xylose *p*-nitrophenylhydrazone, m. p. 160°) and glucuronic acid (separated as barium salt by the method of van der Haar, and identified by nitric acid oxidation to acid potassium saccharate) were the only sugars identified. The naphtha-resorcin test for uronic acid was positive.

Methylation.—Crude hemicellulose (50 g., 15·9% ash) was treated with methyl sulphate (180 c.c.) and sodium hydroxide (540 c.c.; 30%) in acetone at 40—45° with vigorous stirring, ten additions being made at 15-min. intervals; 30 mins. after the last addition the bath temperature was raised to 100° and maintained thereat for 30 mins. to remove acetone and excess of methyl sulphate. The solution was neutralised by sulphuric acid (50%) at 0° and made just alkaline with sodium hydroxide. On warming, partially methylated hemicellulose rose to the surface as a brown precipitate, was skimmed off, and the filtrate concentrated at below 40°. Combined precipitate and concentrate were remethylated as before.

After the third methylation the methylated product was freed from sodium sulphate by extraction with 50% aqueous acetone. A representative sample after 5 methylations contained OMe, 32·1% (corr. for ash); ash, 10·7%; and after seven methylations 32·3% and 4%, respectively.

Isolation of Methylated Hemicellulose.—After the seventh methylation the bulk of the methylated hemicellulose was separated as sodium salt by hot filtration. Acidification of the filtrate with sulphuric acid gave a further precipitate (C) (2·64 g. Found: OMe, 30·2; ash, 0·4%), soluble in chloroform, insoluble in light petroleum, which proved to be partially methylated hemicellulose.

The main fraction, as sodium salt, was dissolved in water, acidified at 0° with sulphuric acid (50%), and extracted with chloroform. Two fractions were obtained. (A) Chloroform-soluble: 25 g. [Found: OMe, 32·3 (corr. for ash); ash, 4%]; (B) chloroform-insoluble: 8·3 g. [Found: OMe, *ca.* 38 (corr. for ash); ash, 67%].

Methylation with methyl iodide. Fraction (A) (5·0 g.) was methylated with methyl iodide and silver oxide in the usual way; yield, 4·7 g. (Found: OMe, 36·8; ash, 4%). Similar treatment of fraction (C) increased OMe to 36·2%; ash 0·5%.

Typical hydrolysis of (*A*). Fully methylated hemicellulose (A) was heated with 2% methyl-alcoholic hydrogen chloride (1220 c.c.) for 7 hrs. at 100°. Initial rotation: $[\alpha]_D$ − 5·6° in $CHCl_3$ (*c*, 1·0); 3 hrs. + 51·5°; 6 hrs. + 71·5°; 7 hrs. + 72° in 2% MeOH–HCl (*c*, 1·0) (const. value). Neutralisation with silver carbonate and evaporation of the filtrate yielded a syrup, which was heated with excess of saturated baryta at 60° for 2 hours. Excess of barium was removed by carbon dioxide. The filtrate, evaporated to dryness under reduced pressure, was exhaustively extracted with boiling ether, the residue taken up with water and extracted with chloroform. Combined ethereal and chloroform extracts, on evaporation, yielded a mixture of methyl xylosides as a pale yellow syrup (5·25 g.). The aqueous solution from the chloroform extraction, evaporated to dryness, gave 4·54 g. of methylated barium salt as a hygroscopic yellow glass (Found: Ba, 14·8; OMe, 27·6%), non-reducing to Fehling's solution.

Fractionation of the methylxylosides. The xyloside mixture (5·25 g.), distilled from a vacuum-jacketed Widmer flask, yielded, after refractionation, the following final fractions:

Fraction.	Yield (g.).	B. p. (bath temp.).	$n_D^{17°}$.	Wt. of trimethyl methylxyloside (g.).
I	0·042	140—143°/16 mm.	1·4426	0·04
II	0·140	144—146/14 mm.	1·4468	0·10
III	0·453	146—150/14 mm.	1·4507	0·18
IV	0·227	150—152/14 mm.	1·4505	0·10
V	0·325	152—155/14 mm.	1·4522	0·10
VI	0·098	152—155/14 mm.	1·4539	0·02
VII	0·199	155—190/14 mm.	1·4550	0·02
			1·4562 *	
VIII	3·103	150—158/16 mm.	1·4562	— (Di)
IX	0·293	100—170/0·03 mm.	1·4600 *	—
Still residue	0·252			
	5·132			0·56

* Last drop.

Constants used in calculation: Trimethyl methylxyloside, $n_D^{17°}$ 1·4426; dimethyl methylxyloside, $n_D^{17°}$ 1·4562.

The yield of trimethyl methylxyloside (0·56 g.) is equal to 10·9% of the xyloside mixture and corresponds to a chain length of approx. 10 anhydro-xylose units. A value of approx. 18 units for the total hemicellulose (xylose chain + *aldo*polyuronic acid) may be deduced.

Methanolysis of a further portion of (A) and distillation of the products gave similar results.

Identification of dimethyl methylxyloside. The xyloside (1·00 g.; OMe, 47·8%; $n_D^{17°}$ 1·4562) from the above fractionation was hydrolysed by 3% hydrobromic acid (18 c.c.) at 85° for 1 hr. Bromine (1 c.c.) was added, and heating continued for 12 hours at 75°, the solution then being non-reducing. Bromine was removed by aeration, and the lactone extracted with chloroform. Evaporation of solvent and distillation of the syrup yielded dimethyl γ-xylonolactone, $n_D^{19°}$ 1·462 (0·50 g.). Treatment with methyl-alcoholic ammonia gave 2 : 3-dimethyl xylonamide (0·47 g.), m. p. (recrystallised from ethyl acetate), 133° (alone or mixed with authentic specimen); $[\alpha]_D^{12°}$ + 46·5° in water (*c*, 1·0) (Found : OMe, 32·0. Calc. : OMe, 32·1%).

Identification of trimethyl methylxyloside. A portion (0·30 g.) of the main trimethyl-containing fractions, once methylated with Purdie's reagents, yielded a syrup, $n_D^{20°}$ 1·4430, which was hydrolysed with 2% nitric acid (10 c.c.) during 1 hr. at 100°. The hydrolysate, neutralised with barium carbonate and evaporated, was extracted with boiling acetone and boiling ether. Concentration of the extracts gave a syrup {0·15 g.; $[\alpha]_D^{22°}$ + 21° in $CHCl_3$ (*c*, 0·61); Found : OMe, 47·9%}, from which crystals of 2 : 3 : 4-trimethyl xylose, m. p. and mixed m. p. 89—90°, rapidly separated.

Examination of still residue. The still residue (0·25 g.) combined with fraction IX (0·29 g.; $n_D^{17°}$ 1·4600; OMe, 41·5%), three times methylated with Purdie's reagents, gave a syrup (0·43 g.), $n_D^{17°}$ 1·4530, $[\alpha]_D^{18°}$ + 66° in chloroform (*c*, 1·6) (Found : OMe, 54·8%). It is assumed that the residue and final fraction consisted of dimethyl methylxyloside in admixture with a little monomethyl methylxyloside due to incomplete methylation.

Esterification of Partially Methylated Barium Salt with Methyl Iodide.—Crude methylated barium salt (4·54 g.) from the methanolysis, purified by solution in methyl alcohol and filtration from extraneous barium carbonate, was treated with Purdie's reagents. Solvent extraction and evaporation yielded the methyl ester of hemicellulose " aldobionic " acid as a pale yellow syrup (2·67 g.) (Found : OMe, 44·0%); $n_D^{14·7°}$ 1·4654; $[\alpha]_D^{12°}$ + 84° in $CHCl_3$ (*c*, 1·04).

Nitric Acid Oxidation of Methylated Barium Salt.—Methylated barium salt (2·00 g. Found : Ba, 12·4; OMe, 31·9%) from methanolysis of a second portion of methylated hemicellulose, fraction (A), was dissolved in A.R. nitric acid (33 c.c., *d* 1·20) in a 300-c.c. flask. The temperature, initially raised to 90°, was lowered to 80° when reaction commenced and maintained at 80° for 6 hrs. The clear, almost colourless solution was diluted with an equal volume of water, and the greater part of the nitric acid removed by distillation at 45—50° under reduced pressure during the continued addition of water (3 l.). On concentration, a pale yellow syrup, which still reacted acid to Congo-red paper, was obtained. This was taken up in dry methyl alcohol, and the solvent evaporated in order to remove the last trace of water.

Esterification of the oxidation products. The pale yellow syrup which finally resulted was esterified by refluxing with methyl-alcoholic hydrogen chloride (30 c.c., 3%) for 6 hrs. Hydrogen chloride was removed by silver carbonate. After evaporation of solvent the syrup still contained some silver nitrate. Ether extraction and concentration gave a neutral syrup (1·27 g.). A further portion of light brown syrup (0·16 g.) was extracted from the silver residues by hot methyl acetate.

Fractionation of the methyl esters. The mixture of esters (1·27 g.) was distilled from a vacuum-jacketed Widmer flask in the following fractions :

Fraction (1), 0·21 g., pale yellow, mobile liquid, b. p. 115—122° (bath temp.)/0·07—0·10 mm.; $n_D^{19°}$ 1·4453; $[\alpha]_D^{11°}$ + 87° in methyl acetate (*c*, 3·57) (Found : OMe, 54·6%). A mixture of approx. 38% dimethyl dimethoxysuccinate (OMe, 60%), 60% dimethyl *xylo*-hydroxydimethoxyglutarate (OMe, 52·5%), and 1—2% dimethyl *xylo*-dihydroxymethoxyglutarate (OMe, 41·9%) requires OMe, 55%.

Fraction (1) (0·16 g.) was dissolved in dry methyl alcohol (2·5 c.c.) and the solution saturated with dry ammonia. A pale green colour developed in 48 hours, and clusters of long, colourless needles were deposited. The crystals (0·02 g.) became dark brown at 200°, melted to a dark liquid at 270°, and decomposed at about 285° (Found : N, 18·0. Calc. for $C_6H_{12}O_4N_2$: N, 15·9%); these properties agree with those of dimethoxysuccinamide (Haworth, Hirst, and Miller, J., 1927, 2440).

A second crop of crystals (0·01 g.) was deposited after a further 2 days. These separated as small clusters of fine, colourless needles but different in appearance from the first crop. The crystals darkened slightly at 126° and melted at 127°; $[\alpha]_D^{19°}$ + 28° (30 mins.) ⟶ + 40° (const. value, 7 hrs.) in water (*c*, 0·63) (Found : OMe, 29·6%). This crop may be *xylo*-dihydroxymethoxymethylglutaramide (Calc. : OMe, 29·8%), m. p. not recorded.

The liquid remaining after removal of the second crop was concentrated to half volume and became moss-green in colour, indicating the presence of a *xylo*-derivative (Pryde and Williams, *Biochem. J.*, 1933, **27**, 1197). Fan-shaped clusters of colourless, narrow blades (0·15 g.) were slowly deposited; m. p. 140°, not depressed by *xylo*-hydroxydimethoxyglutardiamide prepared by oxidation of 2 : 3-dimethyl methylxyloside.

Fraction (2), 0·39 g., pale yellow syrup, b. p. 130—150° (bath temp.)/0·02—0·03 mm.; $n_D^{9·5°}$ 1·4657 (last drop); $[\alpha]_D^{11°}$ + 75° in methyl acetate (*c*, 1·54) (Found : OMe, 44·1%). This fraction also was apparently a mixture of esters. Treatment with methyl-alcoholic ammonia and concentration gave an amide (0·21 g.) after 3 days; narrow, blade-like crystals, m. p. 140° not depressed by *xylo*-hydroxydimethoxyglutardiamide prepared by oxidation of 2 : 3-dimethyl methylxyloside. The residual solution, on standing, yielded colourless needles, m. p. 155—156°.

Fraction (3), 0·08 g., yellow syrup, $n_D^{9·5°}$ 1·4657; soluble in ether; extracted from side arm and column of Widmer flask. Large, colourless plates (0·05 g.), m. p. 101°, separated on standing (cf. 2 : 3-dimethyl-γ-saccharolactone, m. p. 101°). Amide formation yielded colourless needles (0·07 g.), similar to those obtained from fraction (2), second crop, m. p. 155—155·5°, $[\alpha]_D^{18°}$ + 30·5° in water (*c*, 0·18) (Found : N, 12·1; OMe, 21·8. Calc. for $C_8H_{16}O_6N_2$: N, 11·9; OMe, 26·3%), believed to be 2 : 3-dimethyl saccharamide (cf. Smith, J., 1940, 1035).

Fraction (4), 0·03 g., flat, colourless, hexagonal plates, m. p. 105—106°, sparingly soluble in ether, readily soluble in methyl alcohol, extracted from column and side arm of Widmer flask by methyl alcohol; $[\alpha]_D^{?°}$ + 85° in methyl alcohol (*c*, 0·51). This was evidently 2 : 3 : 4-trimethyl-δ-saccharolactone methyl ester (Robertson and Waters, J., 1931, 1709; Smith, J., 1939, 1732; Hirst and Jones, *loc. cit.*).

Fraction (5), 0·33 g., brown still residue (Found : OMe, 35·2; ash, 4·8%). Methyl-alcoholic ammonia gave a reddish-brown syrup from which colourless needles, m. p. 155—156°, slowly separated.

Examination of the methyl acetate extract. This extract (0·16 g.) of the methyl esters prepared from the oxidation products on distillation yielded :

Fraction (I), 0·01 g., yellow syrup, b. p. 95—110° (bath temp.)/0·01 mm., $n_D^{13°}$ 1·4508; $[\alpha]_D^{8·4°}$ + 75° in methyl acetate (*c*, 0·28); compare fraction (2), $n_D^{12°}$ 1·4507, from oxidation of 2 : 3-dimethyl methylxyloside. This fraction probably consists of a mixture of the methyl esters of *xylo*-hydroxydimethoxyglutaric and *xylo*-dihydroxymethoxyglutaric acids, together with a small proportion of trimethyl δ-saccharolactone.

Fraction (II), 0·03 g., yellow syrup which did not distil at 160°/0·02 mm. and was extracted from the column. Treatment with methyl-alcoholic ammonia gave an amide (0·04 g.), colourless needles, m. p. 155°.

Fraction (III), still residue (0·11 g.), a brown syrup which contained silver. Methyl acetate extraction gave a syrup (0·04 g.) free from silver. The amide (0·05 g.) slowly deposited colourless needles, m. p. 156°.

Nitric Acid Oxidation of 2 : 3-*Dimethyl Methylxyloside.*—For purposes of comparison 2 : 3-dimethyl methylxyloside (1·00 g.) was oxidised by nitric acid according to the procedure already described. The resultant mixture of methyl esters (1·14 g.) was distilled from a Widmer flask in the following fractions :

Fraction (1), 0·68 g., colourless, mobile liquid, $n_D^{10°}$ 1·4479; $[\alpha]_D^{8°}$ + 123° in methyl acetate (*c*, 2·64) (Found : OMe, 45·6%). Treated with methyl-alcoholic ammonia, it deposited a crystalline amide (0·39 g.) after 9 days; colourless, elongated, hexagonal prisms, m. p. 140° to a dark brown melt; $[\alpha]_D^{14°}$ + 24° in water (*c*, 0·51) (Found : C, 41·0; H, 6·7; N, 13·5; OMe, 31·2. Calc. for $C_7H_{14}O_5N_2$: C, 40·8; H, 6·8; N, 13·6; OMe, 31·0%). This was evidently *xylo*-hydroxydimethoxyglutardiamide, m. p. not recorded (Nelson and Percival, J., 1942, 58).

The residual yellow solution, on concentration, gave a further crop of colourless crystals (0·10 g.), identical with the first crop; m. p. and mixed m. p. 140°.

Fraction (2), 0·10 g., pale yellow syrup, $n_D^{9°}$ 1·4503. On amide formation a few crystals identical with the amide from fraction (1) were deposited after 14 days.

Fraction (3), 0·17 g., a yellow syrup remained in the column and side arm of the Widmer flask at 200°/0·02 mm.; $n_D^{13°}$ 1·4507. On amide formation, clusters of colourless, rod-like crystals (0·02 g.) were deposited. These darkened above 200° and melted at 270°, identical with dimethoxysuccinamide. The residual orange solution did not crystallise.

The still residue (0·05 g.) could not be induced to form a crystalline amide.

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