# **418.** *Polysaccharides. Part XXVI. Xylan.*

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Earlier work of two of the authors has shown that xylan consists of linked chains of xylopyranose units terminated at one end by units of arabofuranose. Removal of this terminal group exposes what was formerly a penultimate group of xylopyranose and this now becomes the new end-group in these linked chains. This new end-group has been evaluated by assay of the crystalline trimethyl xylopyranose and the former estimate of the basal chain length is confirmed.

It was established by Haworth, Hirst, and Oliver (J., 1934, 1917) that *l*-arabofuranose was present in xylose as a terminating unit, there being one such unit to 18—20 *d*-xylopyranose residues. The significance of this observation was discussed and an opportunity was taken to examine again the validity of the end-group method in the determination of polysaccharide structure. It was also emphasised that a straight terminated chain is only one of a number of possible structures for a methylated polysaccharide which has been shown to yield a recognisable " end-group ". The work now described represents an attempt to decide between these various structural arrangements or to limit their number. Xylan was chosen for this purpose, since the terminating group in this polysaccharide is distinguished sharply from the remaining residues of the chain in that it is a configurationally different sugar and exists in a different ring form (furanose). Inasmuch as a furanoside link is more susceptible to acid hydrolytic agents than is a pyranoside union it was conjectured that conditions might be found which would enable the arabofuranose unit to be removed by hydrolysis from the xylan molecule without disturbance of the xylopyranoside linkages, and these conditions have been realised. Treatment of xylan with 0·2% aqueous oxalic acid at 100° effects complete removal of the arabinose fragment without the liberation of any detectable xylose.

The arabinose-free xylan so prepared showed an enhanced reducing power. It was methylated by the method of Haworth, Hirst, and Oliver, and a careful fractionation of the product in a chloroform–ether–light petroleum mixture showed it to be essentially homogeneous. The main fraction of methylated arabinose-free xylan (which was subsequently hydrolysed) showed a methoxyl content of 38·3% (calculated for a dimethyl pentosan, 38·9%) and had $[\alpha]_D^{21^\circ} - 91{\cdot}2^\circ$ in chloroform (methylated xylan has $[\alpha]_D^{20^\circ} - 98{\cdot}3^\circ$).

Complete hydrolysis of methylated arabinose-free xylan was achieved by boiling with 1·2% methyl-alcoholic hydrogen chloride and the resulting mixture of methylated methylxylosides was separated by fractional distillation. The yield of trimethyl methylxylopyranoside was estimated as 7%, corresponding to a chain length of 18—19 xylose units in the polysaccharide. Indisputable proof is thus afforded that in the process of removing the arabinose residue from xylan no material degradation of the basal chain units occurs.

In a number of preliminary experiments, nitric acid solutions of various dilutions were also used as hydrolytic agents, but the results were not so conclusive as in the experiments with oxalic acid. No hydrolysis of the xylopyranoside linkages occurred when xylan was boiled with N/200-nitric acid, but, at this concentration of acid, the removal of the arabinose residue was only partly effected. The product, referred to in the experimental section as xylan A, yielded on methylation and subsequent hydrolysis a trimethyl pentose fraction which was shown to be a mixture of trimethyl arabofuranose and trimethyl xylopyranose. The partial removal of arabinose from xylan by nitric acid hydrolysis does not materially affect the viscosity of derivatives of the xylan A so produced.

It is clear from the experiments described that the hydrolytic removal of the arabofuranose end-group from xylan exposes a xylose unit which now functions as an end-group and gives rise to the trimethyl methylxyloside isolated after methylation and hydrolysis. It follows that xylan must contain the structure arabofuranose-xylopyranose-(main chain) and that a continuous loop of xylose units with side-chains of arabofuranose residues is excluded as a possibility.

Methylated arabinose-free xylan shows a viscosity which is only half of that of methylated xylan. This fall in viscosity is accompanied by a corresponding increase in the reducing power of the polysaccharide. Table I makes this clear and it is to be observed that chain length as determined by the end-group method is substantially the same for xylan, xylan A, and arabinose-free xylan.

The observations recorded point to a structure for xylan in which primary chains of the form arabofuranose-(xylose)$_{16\text{-}17}$-xylose are linked together by some type of union involving the free xylose reducing group and a hydroxyl group in a second chain. The fact that 2-monomethyl xylose always accompanied the trimethyl pentose and that the amounts of each are similar lends some support to this hypothesis and further suggests that the link occurs between the reducing group of one primary chain and the 3-position of a xylose residue in another chain. The nature of this linkage between the primary chains is still a matter for speculation. It would seem at least to be a link which is relatively stable to the action of the alkaline methylating agents, although this stability (as measured by the degree of aggregation) is adversely affected under the conditions described by the removal of the arabofuranose. Nevertheless the latter cannot itself participate in the linkage, inasmuch as it functions as an end-group.

Experience indicates that this assembly of primary terminated chains into larger complexes is a structural development common to most, if not all, polysaccharides (Haworth, Hirst, and Oliver, *loc. cit.*; Haworth, Hirst, and Isherwood, this vol., p. 784). The views expressed and their detailed formulation in these and in earlier papers from this laboratory are supported in the present case. In addition, the significance of the effect of the terminating non-reducing unit (the arabofuranose) of the primary xylan chains upon the tendency to mutual combination of those chains may assume importance and must be taken into account in any attempt to determine the nature of the linkages responsible for the union of several chains.

## EXPERIMENTAL.

*Removal of Arabofuranose Residue from Xylan.*—The xylan used in the following experiments was prepared from esparto half-stuff kindly supplied to us by Messrs. Alexander Pirie & Co. of Bucksburn. It conformed exactly in its properties with the samples of xylan previously described (Hampton, Haworth, and Hirst, J., 1929, 1739; Haworth, Hirst, and Oliver, *loc. cit.*). Dilute nitric acid was found to be a suitable hydrolytic agent. In order to ascertain the conditions most favourable for removal of the arabinose residue without degradation of the xylan, preliminary experiments were performed as follows: A solution of xylan (*c*, 1·0) in nitric acid of various strengths was boiled for 1 hour, and the solution was then made alkaline by addition of 12% aqueous sodium hydroxide so that in the final solution the concentration of sodium hydroxide was 6%. The rotation of the solution was then determined. With nitric acid concentrations between N/150 and N/200 the final rotation was $[\alpha]_D^{20^\circ}$ − 102°, but with acid stronger than N/100 lower rotations were observed and hydrolysis of the xylose residues took place. In 6% sodium hydroxide solution the rotation of ordinary xylan is − 108°. It appeared therefore that hydrolysis by N/200-nitric acid would be most likely to remove the arabofuranose without disturbance of the rest of the molecule.

| | | | | | | |
|---|---|---|---|---|---|---|
| Strength of $HNO_3$ ............... | N/1 | N/10 | N/50 | N/100 | N/150 | N/200 |
| Final $[\alpha]_D$ ........................ | +20° | −50° | −85° | −98° | −102° | −102° |

Xylan (20 g.) was boiled for 1 hour with N/200-nitric acid (2 l.). The colloidal solution was cooled and centrifuged. The solid was triturated successively with water, alcohol, and ether and dried in a vacuum (17·5 g.). In this way 175 g. of xylan A were collected, $[\alpha]_D^{21^\circ}$ − 111° in 6% sodium hydroxide solution (*c*, 1·3), iodine number 1·5 (the original xylan had $[\alpha]_D^{22^\circ}$ − 109·5° and an iodine number 1·1).

The clear liquid in the centrifuge tubes was neutralised with sodium carbonate and concentrated to a small volume. Addition of alcohol precipitated some xylan which had remained in solution and removal of the solvent then left a syrup, which was extracted with boiling 90% alcohol. The alcohol was distilled off, leaving a strongly reducing syrup. Estimation of the reducing power of this crude syrup by Fehling's solution showed that 5% of the original xylan had been converted into reducing sugar (calculated as arabinose). Proof that the reducing sugar was arabinose was obtained by dissolving the syrup (0·5 g.) in water (2 c.c.) and adding alcohol containing benzylphenylhydrazine (0·65 g.). The solution was warmed (15 mins.) and then kept at room temperature for 2 days. The yield of arabinose benzylphenylhydrazone (m. p. 171°, alone or when mixed with an authentic sample) was 0·5 g. But estimation of the reducing power (Fehling's method; Cole's ferricyanide method) of the syrup showed that 0·5 g. contained 0·3 g. of reducing sugar (calculated as arabinose). Now 0·3 g. of arabinose gave under similar conditions 0·54 g. of arabinose benzylphenylhydrazone.

It appears, therefore, that all the reducing sugar formed in the hydrolysis was arabinose, and the amount of it was such that the acid-treated xylan could retain only a portion of its original arabinose content. To distinguish this acid-treated xylan from ordinary xylan it is proposed to refer to it as xylan A.

*Methylation of Xylan A.*—Xylan A (10 g.) was ground to a paste with water (280 c.c.), water (600 c.c.) was then added, and potassium hydroxide (720 g.) was dissolved in the mixture. The heat liberated brought about solution of the xylan. Methylation was then carried out at 20° by gradual addition of methyl sulphate (800 c.c.), with stirring. The mixture frothed badly and this was controlled as far as posisble by addition of a few drops of amyl alcohol from time to time. After the completion of the reaction the mixture was heated at 100° for 1 hour, diluted with boiling water (1·5 l.), and filtered. The crude methylated xylan collected on the filter had OMe, 30%. After three successive methylations the product was thoroughly washed with boiling water and obtained as a white powder [ash content, 4·5%; OMe (corrected for ash content), 38·5%; yield, 95 g. from 150 g. of xylan].

For further purification the methylated material (94 g.) was dissolved in chloroform (1500 c.c.). The solution was centrifuged to remove insoluble inorganic impurities, and ether (1500 c.c.) was added to the clear solution. This precipitated fraction I (29 g.) as a brown jelly. A second fraction was then precipitated by addition of light petroleum to the solution (fraction II) (64 g.). These fractions were further separated by repetition of the process, giving finally the four fractions described in the following table.

| Fraction. | Weight, g. | Ash, %. | OMe, %.* | $[\alpha]_D^{20^\circ}$ in $CHCl_3$. | Apparent *M.W.*† |
|---|---|---|---|---|---|
| 1 | 10·5 | 15 | 38·5 | — | — |
| 2 | 15·1 | 2·3 | 37·0 | — | — |
| 3 | 31·5 | 0·8 | 38·1 | −92° | 86 units |
| 4 | 29·8 | 0·8 | 38·0 | −92 | 88 |

* Corrected for ash content.

† From viscosity in *m*-cresol, using Staudinger's formula with $k = 10^{-3}$.

A portion of fraction (3) was further purified until it was ash-free. It then had m. p. 202° (ash-free methylated xylan containing the arabinose residue has m. p. 197°).

*Hydrolysis of Methylated Xylan A.*—The methylated derivative (fraction 3 and part of fraction 4; weight, 40·34 g.) was boiled with 1·2% methyl-alcoholic hydrogen chloride (1300 c.c.). After 10 hours the whole of the methylated xylan A had dissolved and after 15 hours the rotation became constant at $[\alpha]_D^{18^\circ} + 57^\circ$. The product was isolated in the usual way (98% yield of methylated pentosides) and fractionated by distillation in a high vacuum, as follows:

| Fraction ............ | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Weight, g. ......... | 0·766 | 0·850 | 1·117 | 1·255 | 5·475 | 11·898 | 12·821 |
| $n_D^{21^\circ}$ .................. | 1·4388 | 1·4421 | 1·4475 | 1·4535 | 1·4555 | 1·4560 | 1·4560 |

The methoxyl content of fraction (1) (59·7%) showed it to consist entirely of trimethyl methylpentoside (Calc.: OMe, 60·2%), while the constancy of refractive index suggests that fractions (6) and (7) consist entirely of dimethyl methylpentoside. The yield of trimethyl methylpentoside, calculated on the basis of the following detailed examination of the fractions, corresponded to a chain length of 18—20 pentose units for xylan A.

*Examination of the Trimethyl Methylpentoside.*—Hydrolysis of fraction (1) (trimethyl methylpentoside) with N/15-hydrochloric acid at 100° was followed polarimetrically: $[\alpha]_D^{20^\circ} - 21^\circ$ (initial value); − 21° (30 mins.); − 14° (60 mins.); − 4° (90 mins.); + 1° (120 mins.); + 4° (150 mins.); + 5° (180 mins., constant for 60 mins.). The strength of the acid was then raised to N, and the hydrolysis continued. After a further 30 minutes the rotation had fallen to − 2°. From these results it was evident that the syrup was not pure trimethyl methylxyloside. That both trimethyl xylopyranose and trimethyl arabofuranose were present was proved in the following way, fraction (2), which was known to contain 20% by weight of dimethyl methylxyloside, being used. The syrup (1·3 g.) was heated with N/15-hydrobromic acid at 100° until the rotation was constant (4 hours). During this period any methylfuranoside present was hydrolysed. To the solution were added calcium carbonate (1 g.) and bromine (0·4 c.c.). After 3 days at 0° the solution was non-reducing. The excess of bromine was removed by aeration and the solution (X) was retained for further examination after extraction with chloroform. Evaporation of the chloroform extract left a syrup, which was distilled, giving: (a) 0·13 g., $n_D^{16^\circ}$ 1·4420, b. p. 100°/1 mm. (bath temp.); (b) 0·3 g., $n_D^{16^\circ}$ 1·4462, b. p. 100—110°/1 mm. (bath temp.); (c) 0·32 g., $n_D^{17^\circ}$ 1·4560, b. p. 110—140°/0·1 mm. (bath temp.).

Hydrolysis of (a) with N-sulphuric acid at 100° was complete in 30 minutes ($[\alpha]_D + 16°$, final value). The neutralised solution was evaporated to dryness, and the product extracted with ether. After recrystallisation from ether–light petroleum it had m. p. 90° alone or when mixed with 2 : 3 : 4-trimethyl xylopyranose (yield, 0·03 g.). This proved that 2 : 3 : 4-trimethyl methylxyloside was present in the products obtained by hydrolysis of methylated xylan A.

Fraction (c) (above) was hydrolysed by N-hydrobromic acid at 85° and the reducing sugar so obtained was oxidised by bromine. The resulting 2 : 3-dimethyl xylonolactone was isolated as the corresponding crystalline amide, m. p. 133°, alone or when mixed with an authentic sample of 2 : 3-dimethyl xylonamide (for details, see Haworth, Hirst, and Oliver, *loc. cit.*). The presence of 2 : 3-dimethyl methylxyloside in fraction (2) was thus confirmed.

The neutral aqueous solution (X) (see above) remaining after extraction by chloroform was evaporated to dryness, and the residue made slightly acid and extracted with chloroform. Removal of the chloroform left a syrup, which was distilled, giving crystalline 2 : 3 : 5-trimethyl arabonolactone (0·1 g.), m. p. after recrystallisation from ether–light petroleum, 29°, $[\alpha]_D^{19°} - 38°$ in water $\longrightarrow - 36°$ after 115 hours (Found : OMe, 47·4. Calc., 48·2%). Some 2 : 3 : 5-trimethyl arabofuranose was therefore present in the hydrolysis products. The relative proportion of trimethyl arabofuranose and trimethyl xylopyranose cannot be determined accurately, but in view of the rotations observed during the hydrolysis of fraction (1) the two sugars were present probably in the proportion of 2 to 1 respectively.

The identity of the 2 : 3-dimethyl methylxyloside, which formed the major part of the distillate (see above), was proved by its conversion into the lactone and amide of the corresponding 2 : 3-dimethyl xylonic acid. The amide, obtained in good overall yield, had m. p. 133°, $[\alpha]_D^{20°} + 46°$ in water (*c*, 1·2) (Found: OMe, 32%. Calc. for $C_7H_{15}O_5N$: OMe, 32·1%).

*Monomethyl Xylose.*—After distillation of the bulk of the dimethyl methylxyloside there remained a small amount of syrup, which distilled slowly at bath temp. 130°/0·02 mm., giving monomethyl methylxyloside (1·87 g. from 40·3 g. of mixed methylpentosides obtained after hydrolysis), $n_D^{18°}$ 1·4720 (Found : OMe, 34·5. $C_7H_{14}O_5$ requires OMe, 34·8%). The hydrolysis of this substance by N-hydrochloric acid at 95° was followed polarimetrically : $[\alpha]_D^{19°} + 84°$ (initial value, *c*, 1·6); 69° (15 mins.); 53° (30 mins.); 40° (45 mins.); 31° (75 mins.); 29° (115 mins., constant value). The product was isolated in the usual manner and was recrystallised from ether, giving 2-monomethyl xylose, m. p. 131°, $[\alpha]_D^{20°} + 34°$ in water (*c*, 0·9) (Found : OMe, 21·1. Calc. for $C_6H_{12}O_5$ : OMe, 18·9%). The identity of the sugar was confirmed by comparing it with a sample of 2-monomethyl xylose prepared by Robertson and Speedie's method (J., 1934, 825). This had m. p. 133°, $[\alpha]_D^{20°} + 34°$ in water (*c*, 2·2), and a mixed m. p. gave no depression.

This series of experiments showed that the hydrolysis products from methylated xylan A consisted of 2 : 3-dimethyl methylxyloside (85—90%), 2 : 3 : 5-trimethyl methylarabinoside (2% approx.), 2 : 3 : 4-trimethyl methylxyloside (4% approx.), and 2-monomethyl methylxyloside (6—10%). The whole of the arabinose had not been removed, but wherever it had been eliminated by the acid-treatment of the original xylan a terminal xylopyranose unit was found instead. Further experiments were then made in which the whole of the arabinose was removed without degradation of the xylan molecule. Oxalic acid was found to be a convenient reagent for this purpose.

*Preparation of Arabinose-free Xylan.*—Xylan (15 g.) was made into a paste with water (400 c.c.). Water (1600 c.c.) was added, and the mixture heated at 100° for 2 hours. Oxalic acid (4·5 g.) was then added and the heating was continued for a further 5 hours. At this stage the reducing power (Fehling's solution) indicated the formation of 8% by weight of arabinose and further heating in the presence of oxalic acid brought about no increase in the reducing power. The xylan was then isolated in the manner described above and the arabinose in the aqueous-alcoholic supernatant liquor was separated as the benzylphenylhydrazone (yield of arabinose-free xylan, 180 g. from 210 g. of xylan).

*Methylation of Arabinose-free Xylan.*—The methylations were conducted exactly as described above. The methylated derivative so prepared was submitted to fractional precipitation, giving :

| Fraction. | Weight, g. | Ash, %. | $[\alpha]_D$ in $CHCl_3$. | Apparent *M.W.* (by viscosity). | OMe, %.* |
|---|---|---|---|---|---|
| 1 | 0·65 | 7·5 | — | — | 38 |
| 2 | 13·4 | 5·4 | — | — | 38 |
| 3 | 39·8 | 1·9 | −91° | 44 units | 38·3 |
| 4 | 15·5 | 0·2 | −90 | 46 | 38·1 |

* Corrected for ash-content.

Hydrolysis of fraction (3) by 1·2% methyl-alcoholic hydrogen chloride at the boiling point produced a non-reducing syrup, which was fractionally distilled, giving ultimately the following fractions :

| Fraction. | Weight, g. | $n_D^{18\cdot5}$. | Estimated content of trimethyl methylpentoside, g. | Estimated content of monomethyl methylpentoside, g. |
|---|---|---|---|---|
| A * | 0·76 | 1·4430 | 0·71 | — |
| B | 1·08 | 1·4475 | 0·69 | — |
| C | 3·35 | 1·4515 | 1·26 | — |
| D | 30·77 | 1·4570 | — | — |
| E | 3·53 | 1·4585 | — | 0·35 |
| F | 0·79 | 1·4650 | — | 0·42 |
| G | 1·20 | 1·4720 | — | 1·20 |
| | | | Totals 2·66 | 1·97 |

* Found : OMe, 58·9%. Still residue 0·84 g.

From these figures the calculated chain length is 18 units. Similar hydrolyses carried out on fractions (2) and (4) gave almost identical results.

*Identification of Hydrolysis Products.*—(1) A portion of fractions (A) and (B) (mixed together) was hydrolysed with N/15-hydrochloric acid at 100°, the reaction being followed polarimetrically : $[\alpha]_D^{21°}$ + 14° (initial value) ; + 17° (30 mins.) ; + 19° (60 mins.) ; + 20° (150 mins., constant value). The final equilibrium value was not altered when the strength of the acid was increased to N and the heating continued for a further period (340 minutes). The solution was neutralised and the product isolated in the usual way, giving crystalline 2 : 3 : 4-trimethyl xylose, m. p. 89° alone or when mixed with an authentic sample, $[\alpha]_D^{22°}$ + 17°, equilibrium value in water (Found : OMe, 49·8. Calc. for $C_8H_{16}O_5$ : OMe, 48·4%) (yield, 30% of the theoretical). The low yield was caused by difficulties during crystallisation due to contamination with 2 : 3-dimethyl xylose.

(2) The 2 : 3-dimethyl methylxyloside was identified, as before, by its conversion into 2 : 3-dimethyl xylonamide, m. p. 133°. In addition a portion of it was methylated, giving 2 : 3 : 4-trimethyl methylxyloside (yield, quantitative), and the latter substance was hydrolysed to 2 : 3 : 4-trimethyl xylose, m. p. 90° (yield, 90% of the theoretical).

(3) From fraction (G) after hydrolysis with N-hydrochloric acid at 100° crystalline 2-monomethyl xylose, m. p. 131°, was isolated (yield, 80% of the theoretical) (Found : C, 44·3 ; H, 7·4 ; OMe, 21·0. Calc. for $C_6H_{12}O_5$ : C, 43·9 ; H, 7·3 ; OMe, 18·9%).

*Reducing Power of Xylan, Xylan A, and Arabinose-free Xylan.*—It is shown above that the apparent molecular weight (by viscosity methods) of methylated arabinose-free xylan is approximately half that of ordinary methylated xylan. The figures in Table I show that the decrease in viscosity is accompanied by increase in reducing power. In each case cited, however, the chemical chain-length determined by the end-group method is the same, namely, 18 units approximately. The iodine number is the no. of c.c. of N/10-iodine required by 1 g. of substance, and the copper number (determined by the method of Knecht and Thomson, *J. Soc. Dyers and Col.*, 1920, **36**, 255) is the number of g. of copper reduced by 100 g. of substance.

TABLE I.

| | | | Apparent chain length estimated by : | | |
|---|---|---|---|---|---|
| Substance. | Copper No. | Iodine No. | Iodine No. | Viscosity of Me derivative. | End-group assay of Me derivative. |
| Original xylan | 0·37 | 1·67 | 91 | 92 | 18—19 |
| Xylan after treatment with dilute $HNO_3$ (xylan A) | 0·50 | 1·98 | 77 | 88 | 18—20 |
| Arabinose-free xylan (oxalic acid treatment) | 0·71 | 2·75 | 55 | 44 | 18—19 |

These viscosity data are not employed in any other than a comparative sense inasmuch as the factor used in their calculation may not be applicable to xylan. It is by coincidence perhaps that the apparent chain length is similar to that given by the iodine number.

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