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THE CONSTITUTION OF A SYNTHETIC XYLAN

I. GENERAL STRUCTURAL FEATURES

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ABSTRACT

The polysaccharide was obtained by polymerization of D-xylose at 140° C in the presence of phosphorous acid. Complete hydrolysis of the xylan followed by bromine oxidation showed that the synthetic polymer contained only xylose. Periodate oxidation resulted in formation of a considerable quantity of formaldehyde, the origin of which is not known. Periodate oxidation of borohydride-reduced xylan gave additional formaldehyde and, from this, the average D.P. of the polymer was estimated to be about 55. Complete hydrolysis of the polyalcohol gave ethylene glycol, glycerol, and xylose in a ratio of 2:9:1. Partial hydrolysis gave ethylene glycol, glycerol, and at least seven non-reducing components. Hydrolysis of the fully methylated xylan gave tri-, di-, mono-methyl xyloses and xylose in a 32:32:18:5 ratio, and a carbonyl band was evident in the infrared spectrum of the hydrolyzate. No such band was evident in the hydrolyzate of the unsubstituted xylan. The xylan is highly branched and contains some D-xylofuranose units.

In contrast to the relatively ordered structures of naturally occurring enzymatically synthesized polysaccharides, it has usually been found that synthetic polysaccharides have highly random and branched structures (1–5). Recent investigations (6) on a synthetic glucan (7) have shown that almost all possible linkages were present and that glucofuranose residues occurred in the polymer. In contrast to the apparently simpler structure of a synthetic xylan investigated by Bishop (8), the results reported in this paper from experiments on a synthetic xylan prepared by Mora and associates (9) indicate that its structure is complex.

Complete hydrolysis of the xylan gave only D-xylose, which was obtained in crystalline form. Bromine oxidation of the hydrolyzate followed by adsorption of the xylonic acid on anion exchange resin left no residue that reacted with *p*-anisidine trichloroacetate or ammoniacal silver nitrate spray reagents, thus indicating the absence of any polyols. The facile partial hydrolysis of the xylan suggested the presence of some xylofuranose units (Table I). The oligosaccharides surviving the mild acid hydrolysis were presumed to consist primarily of xylopyranose units. The specific rotation of the xylan, $[\alpha]_D^{22}$ 45°, suggested that both alpha and beta glycosidic linkages were probably present, but if some of the D-xylose residues were present as open-chain acetals this point is less certain.

The rapid production of formaldehyde (1.52 mg per xylose unit after 20 minutes and 2.35 mg after 210 minutes, with no significant change thereafter) upon periodate oxidation of the xylan under controlled conditions (pH 4.0–4.5, 5° C) indicated the oxidation of some primary hydroxyl groups. A structural feature to accommodate this observation cannot

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TABLE I

Partial hydrolysis of xylan: percent composition of hydrolyzate

Time (hr)	$[\alpha]_D$	Xylose	Disacch.	Tri- + tetra-sacch.	Oligosacch.
0	45°	—	—	—	100
0.5	46.5	9.0	2.6	4.4	84
1	50.3	9.8	2.8	4.4	83
1.5	50.8	10.0	3.0	4.6	83
2	50.8	10.1	3.0	4.7	83
4	50.8	10.5	3.1	4.6	82
6	51.4	11.1	3.1	4.9	81
27	52.4	11.7	3.2	5.2	80
48	53.0	12.5	3.3	5.9	78

be suggested with certainty at this point. Under the conditions of the oxidation, it does not seem probable that any appreciable overoxidation would have occurred. A possible explanation may be that there are present some open-chain xylose residues in which the C_4 and C_5 hydroxyls are not substituted. Periodate oxidation would result in the formation of formaldehyde from C_5 and the acetal system would be readily hydrolyzed by dilute acid. Periodate oxidation of the reduced ($NaBH_4$) xylan resulted in the liberation of additional formaldehyde, and by application of a reported procedure (10), the average degree of polymerization was estimated to be a minimum of 55. The assumption is made that the reducing end-group is not substituted at C_2 and hence gives 1 mole of formaldehyde on reduction and treatment with periodate.

The periodate uptake (0.91 mole per sugar residue, constant after 18 hours) indicated the presence of periodate-resistant xylose units in the xylan. Reduction ($NaBH_4$) of the xylan polyaldehyde followed by complete hydrolysis of the polyalcohol gave ethylene glycol (from non-reducing xylopyranose end-units and $1 \rightarrow 2$ linked pyranose units), glycerol (from internal xylopyranose and xylofuranose units, also from external xylofuranose units), and xylose in an approximate ratio of 2:9:1. About 7–8% xylose was not degraded. Since non-reducing xylofuranose end-units are present, the quantity of ethylene glycol formed in the periodate degradation compared to the estimated D.P. of the xylan cannot be used to calculate the approximate number of branch points in the molecule.

The controlled hydrolysis (11) of the polyalcohol gave ethylene glycol, glycerol, xylose, and at least seven oligosaccharide fragments with varying R_{xylose} values in several solvent systems. Free xylose is believed to have originated from relatively labile furanoside linkages. The oligosaccharides were composed of xylose and glycerol in varying ratios and further details concerning these will be reported later.

Methylation of the xylan was accomplished by employing successively the Haworth, Kuhn, and Purdie procedures. Only after extended and vigorous treatment with Purdie's reagents was the xylan fully methylated; methoxyl content 39.2%, no hydroxyl band in the infrared spectrum. The methylated xylan, $[\alpha]_D^{22}$ 42° (*c*, 8.2 in $CHCl_3$), was soluble in petroleum ether containing 4.5–5% chloroform. Hydrolysis of the methylated xylan gave tri-, di-, and mono-methyl xyloses and free xylose in a molar ratio of 32:32:18:5, respectively. The identity of the methylated sugars will be reported in a separate communication but it may be noted that the rotation, $[\alpha]_D^{22}$ 22.3° in water, of the trimethyl fraction indicated the presence of 37.5% 2,3,5-tri-*O*-methyl-D-xylofuranose; hence non-reducing xylofuranose end-units were present in the xylan. Since the methylated xylan was hydrolyzed with sulphuric acid under conditions known to minimize demethylation (12), the presence of xylose in the hydrolyzate indicates the occurrence of fully substituted residues in the xylan.

The infrared spectrum of the hydrolyzate of the methylated xylan showed absorption bands at 3400, 2930, 2825, 1725, 1650, 1460 cm^{-1}. The definite absorption at 1725 cm^{-1} suggested that aldehydic carbonyl groups might be present. It seemed unlikely that this absorption was due to furfural degradation products, since when a sample of the original xylan was hydrolyzed under similar conditions the band at 1725 cm^{-1} was absent. Xylose residues methylated at C_4 and C_5 would exist in the aldehyde form and could conceivably be responsible for absorption in the carbonyl region. As suggested previously, xylose residues occurring in the xylan in the open-chain form and not substituted at C_4 and C_5 would explain the rapid formation of formaldehyde upon periodate oxidation. The above spectroscopic observation lends support to this proposal.

EXPERIMENTAL

Chromatographic separations were carried out using the descending technique and Whatman No. 1 and 3MM paper. Solvent systems employed were A, ethyl acetate:acetic acid:water (8:2:2); B, ethyl acetate:pyridine:water (9:2:2); and C, butanone:water azeotrope. Spray reagents were D, *p*-anisidine trichloroacetate and E, ammoniacal silver nitrate solutions. Unless otherwise stated, evaporations were carried out under reduced pressure at a bath temperature of 40° C. Melting points are uncorrected and specific rotations were taken at 22±2° C.

Hydrolysis of Xylan

A quantity (750 mg) of xylan, $[\alpha]_D$ 45° (*c*, 2.0 in water), was dissolved in 0.2 *N* sulphuric acid (30 ml), and the solution boiled for 10 hours. The solution was neutralized ($BaCO_3$), deionized, and evaporated to a thick sirup which crystallized upon standing. After recrystallization from ethanol, D-xylose had m.p. and mixed m.p. 143–145°, $[\alpha]_D$ 18° (*c*, 1.8 in water).

Bromine Oxidation of Xylan Hydrolyzate

A portion (about 500 mg) of the hydrolyzate was oxidized with bromine in the usual manner until an aliquot of the solution no longer reduced Fehlings solution. After removal of excess bromine from the solution by aeration, the solution was passed through anion exchange resin. Evaporation of the neutral effluent left no residue which would react with either spray reagent D or E.

Partial Hydrolysis of Xylan

An aliquot (80 mg) of the polymer in 0.1 *N* hydrochloric acid (3 ml) was left at room temperature. The appearance of hydrolysis products was followed by paper chromatographic analysis in solvent A using the phenol – sulphuric acid method (13) to determine xylose and xylose oligosaccharides. The course of the hydrolysis was also followed polarimetrically. The change in the optical rotation and the extent of hydrolysis are summarized in Table I.

Periodate Oxidation, Reduction ($NaBH_4$), Complete Hydrolysis

Xylan (176 mg, 1.325 mmoles) was dissolved in water (20 ml) and the solution acidified with 6 *N* acetic acid (2 ml). After the solution was cooled, 0.5 *M* sodium periodate (5 ml) was added and the oxidation allowed to proceed at 5° C. Formaldehyde was determined (10) after $\frac{1}{3}$, $3\frac{1}{2}$, 12, 48, and 72 hours. After $\frac{1}{3}$ hour, 1.52 mg formaldehyde was produced per xylose unit and 2.43 mg after $3\frac{1}{2}$ hours, with no significant increase thereafter. After 72 hours, periodate consumption was determined and the xylan had consumed 0.905 mole of periodate per xylose unit. To the solution was added barium chloride and barium carbonate, and after filtration, sodium borohydride (200 mg) was added. The reduction was allowed to proceed overnight at room temperature, after which time the solution was acidified with hydrochloric acid and evaporated to dryness. Borate was removed by several evaporations with methanol and the residue hydrolyzed with sulphuric acid. The solution was deionized, concentrated, and the sirupy product chromatographed. Compounds corresponding to glycerol, ethylene glycol, and xylose were detected. The ratio of these was determined chromatographically using the periodate – chromotropic acid procedure (10) for the alcohols and the phenol – sulphuric acid method (13) for the determination of xylose. The ratio of ethylene glycol:glycerol:xylose thus found was about 2:9:1. A further quantity (650 mg) of xylan was treated as described above and the combined hydrolyzates resolved by paper chromatography (Whatman 3MM paper) using solvent A. The three compounds were located by spraying guide strips, and subsequently eluted. *Ethylene glycol*, 47 mg, was reacted with *p*-nitrobenzoyl chloride in pyridine solution to give the corresponding di-*p*-nitrobenzoate, m.p. and mixed m.p. 139–141° C. *Glycerol*, 435 mg, was similarly converted to the corresponding tri-*p*-nitrobenzoate, m.p. and mixed m.p. 188–190° C. D-*Xylose*, 70 mg, crystallized spontaneously and after recrystallization from ethanol had m.p. and mixed m.p. 143–144° C, $[\alpha]_D$ 17.7° (*c*, 1.4 in water).

Periodate Oxidation of Reduced ($NaBH_4$) Xylan and Determination of D.P. (10)

Xylan (230 mg) was dissolved in water (20 ml) and to the solution was added sodium borohydride (100 mg). The reduction was allowed to proceed at room temperature for 24 hours. The solution was acidified with acetic acid (2 ml), cooled to 5° C, and 0.5 *M* sodium periodate (5 ml) was added. Formaldehyde was determined (10) after $\frac{1}{3}$, $3\frac{1}{2}$, 12, 48, and 72 hours. After $3\frac{1}{2}$ hours, 4.20 mg formaldehyde had been formed per xylose unit, with no significant change thereafter. On the assumption that the extra formaldehyde formed in the periodate oxidation of the reduced xylan arose from the xylitol end-groups and that each such group produced 1 molecular proportion of formaldehyde, the approximate D.P. of the xylan was 55. The periodate consumption was determined after 72 hours and 0.92 mole of periodate had been consumed per pentose unit. The oxidation mixture was subsequently treated in the same manner as described in the previous section. Chromatography of the final product showed the presence of ethylene glycol, glycerol, and xylose. The ratio of these was determined and found to be about 2:10:1.

Partial Hydrolysis of Polyalcohol (11)

A quantity (100 mg) of xylan was dissolved in water (20 ml), cooled to 5° C, and 0.5 *M* periodic acid (5 ml) was added. After 72 hours, when 0.91 mole of periodate had been consumed per xylose unit, the solution was neutralized ($BaCO_3$), filtered, and sodium borohydride (75 mg) was added. The solution stood at room temperature overnight and was evaporated to dryness. Borate was removed by treatment with methanol containing 1% hydrogen chloride (14) and the residue was finally dissolved in 0.1 *N* sulphuric acid (30 ml) and left for 8 hours. The solution was neutralized ($PbCO_3$) and evaporated. Chromatographic examination of the sirupy residue indicated the presence of ethylene glycol, glycerol, xylose, and at least seven non-reducing components with a considerable range of R_{xylose} values. Further details of these periodate degradations will be reported separately.

Methylation of the Xylan

A quantity (4.0 g) of xylan was treated in the usual way with the Haworth methylating reagents, followed by two successive methylations using methyl iodide, silver oxide, and dimethylformamide (15). The product (4.59 g), a brittle, yellow glass, showed an OH band in the infrared spectrum and was therefore methylated three times with Purdie's reagents over extended periods (3–4 days). The final product (4.35 g) was dissolved in petroleum ether (30–60°) containing 5% chloroform, and a small quantity of insoluble material was removed by filtration. Evaporation of the solvent left a yellow, resinous solid which showed no OH character in its infrared spectrum and contained 39.2% methoxyl. Hydrolysis of a portion (80 mg) was effected using sulphuric acid (12). After neutralization ($BaCO_3$) and deionization, the solution was evaporated. Chromatographic examination using solvent C indicated the presence of tri-, di-, and mono-methyl xyloses and a small amount of free xylose. The ratio of the above-mentioned xylose methyl ethers was estimated chromatographically using the phenol – sulphuric acid reagent (13). The approximate molar ratio of tri-, di-, mono-methyl xyloses and xylose thus found was 32:32:18:5. The components of the hydrolyzate were subsequently separated by passage through a cellulose–hydrocellulose column using solvent C as the irrigant. The trimethyl fraction had a specific rotation of 22.3° in water, indicating that it was composed of 2,3,4- and 2,3,5-tri-*O*-methyl-D-xyloses in the ratio of 62.5:37.5. The identification of the other components will be reported later.

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REFERENCES

1. P. T. Mora, J. W. Wood, P. Maury, and B. G. Young. J. Am. Chem. Soc. **80**, 693 (1958).
2. P. W. Kent. Biochem. J. **55**, 361 (1954).
3. C. R. Ricketts and C. E. Rowe. J. Chem. Soc. 3809 (1955).
4. H. W. Durand, M. F. Dull, and R. S. Tipson. J. Am. Chem. Soc. **80**, 3691 (1958).
5. J. da S. Carvalho, W. Prins, and C. Schuerch. J. Am. Chem. Soc. **81**, 4054 (1954).
6. G. G. S. Dutton and A. M. Unrau. Can. J. Chem. **40**, 1196 (1962).
7. P. T. Mora and J. W. Wood. J. Am. Chem. Soc. **80**, 685 (1958).
8. C. T. Bishop. Can. J. Chem. **34**, 1255 (1956).
9. P. T. Mora, J. W. Wood, and V. W. McFarland. J. Am. Chem. Soc. **82**, 3418 (1960).
10. A. M. Unrau and F. Smith. Chem. & Ind. (London), 330 (1957).
11. I. J. Goldstein, G. W. Hay, B. A. Lewis, and F. Smith. Abstracts of Papers, 135th Meeting of the American Chemical Society, Boston, Mass. April, 1959. p. 3D.
12. I. Croon, G. Herrström, G. Küll, and B. Lindberg. Acta Chem. Scand. **14**, 1338 (1960).
13. N. Dubois, J. K. Hamilton, K. A. Gilles, P. A. Rebers, and F. Smith. Anal. Chem. **28**, 350 (1956).
14. D. R. Briggs, E. F. Garner, R. Montgomery, and F. Smith. Anal. Chem. **28**, 1333 (1956).
15. R. Kuhn, I. Löw, and N. Trischmann. Ber. **90**, 203 (1957).