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Note

Determination of the degree of polymerisation of xylans

R. J. STURGEON

Department of Brewing and Biological Sciences, Heriot-Watt University, Edinburgh EH1 1HX (Great Britain)

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Chemical methods for the determination of the number-average degree of polymerisation ($\overline{d.p.}_n$) of oligo-[1,2] and poly-saccharides[3] are based upon the measurement of the relative proportion of terminal reducing groups. This has been achieved by conversion of the reducing groups into the corresponding alditol with sodium borohydride and determining the ratio of alditol to reducing sugar after acid hydrolysis of the polymer, or by the release of formaldehyde from the terminal alditol residues on periodate oxidation[4]. When applied to xylans, the latter method requires knowledge of the linkage type since reduced (1→3)- and (1→4)-linked xylans liberate two and one mol. of formaldehyde, respectively.

An enzymic method[5] for the determination of the $\overline{d.p.}_n$ of glucans involved the sequence: reduction with sodium borohydride, acid hydrolysis, and determination of D-glucitol by using sorbitol (D-glucitol) dehydrogenase. We now report on an analogous method for xylans, since sorbitol dehydrogenase (E.C. 1.1.1.14) also oxidises xylitol[6,7] to an extent of ~70% (the oxidation of D-glucitol is quantitative).

Sorbitol dehydrogenase effects[7] the following reaction:

$$\text{Xylitol} + \text{NAD} \rightleftharpoons \text{D-Xylulose} + \text{NADH} + \text{H}^+.$$

Under the conditions used, the extinction for NADH became constant after 10–15 min, and the resulting calibration was linear up to 0.05 μmole of xylitol. The yield

TABLE I

THE EFFECT OF BORATE AND FORMATE IONS ON THE DETERMINATION OF XYLITOL[a]

Borate (*μmoles*)	*Xylitol assayed* (%)	*Formate* (*μmoles*)	*Xylitol assayed* (%)
0	100	0	100
0.04	99	1.5	80
2.0	60	14.7	78
5.0	46	147	37
10.0	33		

[a]Each cell contained 0.05 μmole of xylitol.

of NADH from xylitol did not vary with enzyme concentrations from 1.8–9 units. Using the extinction coefficient of NADH at 340 nm as 6.22×10^6 cm^2/mole, it was established that 0.78 μmole of NADH was produced per μmole of xylitol. Borate and formate ions interfered with the assay (Table I).

The determination of $\overline{\text{d.p.}}_n$ of xylo-oligosaccharides and xylans is given in Table II.

TABLE II

$\overline{\text{D.p.}}_n$ VALUES[a] OF SOME XYLO-OLIGOSACCHARIDES AND XYLANS

Substrate	$\overline{D.p.}_n$ *(Enzymic)*	$\overline{D.p.}_n$ *(Chemical or physiochemical)*
Milkweed floss xylan	170	172[10]
Yellow-birch xylan	218	207[12]
Aspen xylanitol	196	180[11]
Rhodymenia palmata xylan	94	86[b]
Caulerpa filiformis xylan	42	42[b]
Xylobiose	1.9	2
Xylotriose	2.9	3
Xylotetraose	4.2	4

[a]Average of three determinations. [b]Measured by the formaldehyde release method[4].

For the determination of the $\overline{\text{d.p.}}_n$ of glucans, the reduced end-group was measured, after total hydrolysis, by enzymic oxidation and the glucose liberated was determined by a reductometric procedure. For xylans, the xylose produced by acid hydrolysis of the reduced xylan can also be determined by enzymic oxidation after reduction with sodium borohydride. The method gives satisfactory results for small oligosaccharides and some xylans.

Wood hemicelluloses from milkweed floss[10], aspen[11], and yellow birch[12] contain D-glucuronic acid residues in addition to xylose and, on acid hydrolysis, the xylose is not completely liberated. Paper chromatography of the acid hydrolysates revealed xylose as the only neutral sugar and *O*-(D-glucopyranosyluronic acid)-xylose as the major acidic component together with small amounts of probably uronic acid and aldotriouronic acid. Since *O*-(D-glucopyranosyluronic acid)-xylitol (obtained by reduction of the aldobiouronic acid from aspen xylan) was not a substrate for sorbitol dehydrogenase, a correction, based on the reported uronic acid values[10–12], was necessary in the determination of the $\overline{\text{d.p.}}_n$ values for the hemicelluloses.

Other sources of error may be noted. If formic acid is used for hydrolysis, it must be completely removed from the hydrolysate since formate ion strongly inhibits the enzymic oxidation (Table I). Some reducing end-groups in hemicelluloses derived from softwoods after treatment with chlorous acid may have been oxidised to the aldonic acid. Moreover, the alkaline conditions used in the extraction of hemicelluloses and the *Caulerpa filiformis* xylan[13] could cause the formation of a xylosaccharinic acid end-group[14]. The occurrence of these reactions would decrease the

amount of xylitol formed on reduction of the polysaccharides with sodium borohydride and thereby lead to high $\overline{d.p.}_n$ values. The xylan from *Rhodymenia palmata*, with contains[15] both β-(1→4)- and β-(1→3)-linked xylose residues[15], was extracted under mildly acidic conditions, and any resulting degradation will produce terminal, reducing xylose residues and lead to a decrease in $\overline{d.p.}_n$ value. In the chemical estimation of $\overline{d.p.}_n$ of this type of molecule, but not for the enzymic procedure, it is necessary to know the ratio of (1→4) to (1→3) linkages present.

The enzymic method described has been applied to polysaccharides containing xylose and no glucose. Since this work was completed, a method has been reported for the estimation of xylitol in the presence of D-glucitol[16].

EXPERIMENTAL

Sorbitol dehydrogenase (from sheep liver) was purchased from C. F. Boehringer, and NAD from Sigma Chemical Company. Spectrophotometric measurements were made at 340 nm and 25°, using a Unicam SP800 spectrophotometer fitted with an external recorder. Formaldehyde was measured by the method of Vaskovsky and Isay[8], and xylose by the Nelson–Somogyi method[9]. Xylobiose, xylotriose, and xylotetraose were purified by preparative p.c. from a partial, acid hydrolysate of barley arabinoxylan.

Hydrolysis conditions. — For the $\overline{d.p.}_n$ determination of an oligosaccharide, ~2 mg of saccharide are required, whereas, for polysaccharides, 25 mg may be necessary to provide an adequate amount of xylitol. Oligosaccharides or polysaccharides containing only xylose were hydrolysed with 2M hydrochloric acid at 100° for 2–3 h. When uronic acids were also present, hydrolysis was effected with 90% formic acid at 100° for 2 h. The samples were diluted with water and evaporated to dryness under reduced pressure, and the residues were further hydrolysed with 2M hydrochloric acid at 100° for 3 h.

Reduction conditions. — The xylan (25 mg) was dissolved or suspended in water (1–2 ml) and treated with excess sodium borohydride (5 mg) at room temperature for 24 h. The excess borohydride was destroyed by the addition of hydrochloric acid to pH 4, and the solution was evaporated to dryness at 40°. The residue was hydrolysed with acid as described above. The hydrolysate was evaporated to dryness, and borate was removed from the residue by evaporation of methanol (5 × 2 ml) therefrom. The product was dissolved in water (5 ml), and aliquots (0.5 ml) of the solution (*A*) were reduced with sodium borohydride (3 mg) at room temperature for 24 h. The solution was freed from borate as described above and adjusted to a final volume of 5 ml (solution *B*). Xylitol was determined by using the enzymic procedure, and the $\overline{d.p.}_n$ was calculated from the formula $\overline{d.p.}_n$ = (μmoles of NADH in *B*/μmole of NADH in *A*)+1. Control experiments showed no destruction of either xylose or xylitol during the hydrolytic conditions.

Enzymic procedure. — The procedure is essentially similar to that previously described[5]. The following solutions were pipetted into a 1-cm silica cell: 0.1M sodium

pyrophosphate buffer (pH 9.5, 0.85 ml), 20mM NAD (0.1 ml), and a sample containing less than 20 μg of xylitol (0.05 ml). After measurement of the initial extinction, the reaction was started by the addition of sorbitol dehydrogenase (3.5 units, 0.02 ml), and readings were taken until the extinction became constant. Control experiments showed that there was a linear relation between the change in extinction and the xylitol concentration.

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