[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MCGILL UNIVERSITY, AND THE PULP AND PAPER RESEARCH INSTITUTE OF CANADA]

Distribution of Substituents in a Partially Methylated Xylan[1]

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The distribution of the substituents in two partly methylated xylans from esparto grass has been determined. The distributions corresponded to a ratio of the rate constants of the C_2- and C_3-hydroxyl groups of 2.5:1, provided the reactivity of the C_3-hydroxyl was doubled once the C_2-hydroxyl had been substituted. It is concluded that the low reactivity of the C_3-hydroxyl group in cellulose cannot be caused by steric hindrance from the hydroxymethyl group at C_5 which is missing in the xylan.

The relative reactivity of the hydroxyl groups present in polysaccharides is of considerable interest but has so far been studied in more detail only in the case of the etherification of cellulose, where in some cases[3-5] the C_2- and in others[6] the C_6-hydroxyl groups have been found to be most reactive. The C_3-hydroxyl invariably appears to possess the lowest reactivity. The present paper is concerned with the relative reactivity of the C_2- and C_3-hydroxyl groups in a xylan toward methylation.

The xylan, which was of the simplest possible type, was isolated from esparto grass (*Stipa tenacissima*), and had previously been found[7,8] to consist of (1 → 4)-linked β-D-xylopyranose residues, containing one branching point through C_2 per molecule. The present preparation yielded only D-xylose on hydrolysis and had a number-average degree of polymerization of 65.[9] By treating the xylan in the presence of aqueous alkali with dimethyl sulfate, two partly substituted *O*-methylxylans were obtained, one (A) containing 0.62 and the other (B) 1.55 methoxyl groups per anhydroglucose unit. Each polysaccharide, when hydrolyzed, yielded a mixture of xylose and methylated xyloses which was resolved on a column of charcoal and Celite[10] by gradient elution[11] with aqueous ethanol,[12] followed in the case of the mono-*O*-methylxyloses by further resolution by paper electrophoresis in a borate buffer.[13] The sugars thus obtained were fully characterized and their amount was estimated from the weight of each as obtained from the column, from the specific rotation of the appropriate fractions or by oxidation with hypoiodite.[14] The results are presented in Table I.

If the ratio between the first-order rate constants of the C_2- and C_3-hydroxyls, $k_2:k_3$, be assumed to average 2.0:1 and 2.5:1, respectively, and if it be further assumed that the C_3-hydroxyl becomes twice as reactive once the C_2-hydroxyl has been methylated,[6] the values presented in Table II are obtained.

TABLE I

AMOUNTS OF SUGARS (MG.) OBTAINED ON HYDROLYSIS OF THE METHYLATED XYLANS

Component	A	B
D-Xylose	539	93
2-*O*-Methyl-D-xylose	298	324
3-*O*-Methyl-D-xylose	104	132
2,3-Di-*O*-methyl-D-xylose	182	2377

TABLE II

FOUND AND CALCULATED PERCENTAGE MOLAR COMPOSITION OF METHYLATED XYLANS

	O-Methylxylan A			*O*-Methylxylan B		
Substitution	Found	Calcd.[a]	Calcd.[b]	Found	Calcd.[a]	Calcd.[b]
Unsubstituted	50.6	48.7	49.7	3.8	2.7	2.1
2-*O*-Methyl-	25.8	26.4	26.0	11.9	12.8	15.0
3-*O*-Methyl-	9.1	13.2	11.0	4.9	6.4	4.3
2,3-Di-*O*-methyl-	14.5	11.7	13.3	79.4	78.1	78.6

[a] $k_2:k_3 = 2:1$. [b] $k_2:k_3 = 2.5:1$.

Calculations showed that with the above assumptions the best agreement was obtained between found and calculated data. It is evident that the C_2-hydroxyl group throughout the methylation was 2 to 2.5 times as reactive as the C_3-hydroxyl, but that a better agreement between calculated and observed values was obtained when it was assumed that the reactivity of the C_3-hydroxyl was increased twofold as soon as the neighboring hydroxyl group had been substituted.[4] This situation has previously been encountered in the methylation of cellulose, where, however, the original ratio of k_2 to k_3 was closer to 3.5:1.[6]

Various explanations have been offered for the low reactivity of the C_3-hydroxyl group in cellulose. The electron density distribution in the glucose residue could presumably lower the dissociation of the C_3-hydroxyl[6] and the same effect could be caused by a strong hydrogen bond between this hydroxyl and the ring oxygen of a contiguous glucose residue, the presence of which has recently been suggested.[15]

Recently, the suggestion has been made[16] that the $-CH_2OH$ group at C_5 in cellulose should sterically hinder the C_3-hydroxyl group and thus account for its low reactivity. The glucose and xy-

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lose residues in cellulose and xylan are both presumed to exist in the preferred C1 conformation,[17] the only difference being the absence of the hydroxymethyl group at C_5 in the xylan. It is evident from the present results that the C_3-hydroxyl is two to three times less reactive than the C_2-hydroxyl group in both cellulose and xylan, and the above suggestion cannot therefore be adduced as an explanation for this phenomenon. The fact that the reactivity of the C_3-hydroxyl is so much enhanced once the possibility of the formation of an alkoxide ion from the relatively acidic hydroxyl group at C_2 is eliminated, instead strongly suggests that the main reason for the low reactivity of the C_3-hydroxyl is probably to be found in its low dissociation constant which is partially caused by the inductive effect of the C_2-alkoxide ion. This conclusion is further corroborated by results obtained in recent attempts to measure the dissociation constants of the hydroxyl groups of various glucose derivatives.[18] In this case, substitution at C_2 is claimed to enhance considerably the acidity of the C_3-hydroxyl group.

Experimental

All specific rotations are equilibrium values and were determined at 25° at a concentration of 2.0 in water, unless otherwise specified. Melting points are corrected. Evaporations were carried out *in vacuo* at 40–50°.

Paper Chromatography.—Solvents (v./v.) used for separating the sugars were (A) ethyl acetate–acetic acid–water (9:2:2), (B) butan-1-ol–pyridine–water (10:3:3) and (C) butanone–water (89:11). Separations were carried out by the descending technique on Whatman No. 1 filter paper. The spray reagent used was *o*-aminodiphenyl.[19] Electrophoretic separations were carried out on Whatman No. 3MM filter paper at 750 volts for 4 hr. in 0.05 *M* borate solution.

Preparation of the Xylan.—The xylan was prepared from esparto grass, essentially as described previously.[8] Old esparto grass (900 g.) was delignified with acid chlorite at 75° for 7 hr.[20] The white holocellulose was extracted with 24% aqueous potassium hydroxide (6 l.) to yield a crude product (200 g.). Hydrolysis followed by paper chromatographic examination (solvents A and B) indicated the presence of xylose in addition to minor amounts of glucose and arabinose residues. The hemicellulose was dissolved in 4% aqueous sodium hydroxide (5.1). On addition of Fehling's solution (10 l.) a blue precipitate was formed which was recovered by filtration on cheese-cloth and washed exhaustively with distilled water. The gelatinous precipitate was suspended in ice-cold *N* hydrochloric acid (2 l.) and the solution formed was poured with stirring into ice-cold ethanol (10 l.). The precipitate was recovered on the centrifuge and washed, first with 70% aqueous ethanol containing 10% concentrated hydrochloric acid (2 l.) and subsequently with water (3 × 2 l.) to remove the glucose-yielding component. After three precipitations as the copper complex, the hemicellulose contained no arabinose residues. Eight subsequent extractions on the centrifuge with water (2 l. each) finally removed also the glucose-containing polysaccharide, and left a material which was washed successively with ethanol and petroleum ether (b.p. 30–60°) and dried *in vacuo* to give a white powder (50 g.). The polysaccharide, $[\alpha]^{20}\text{D} - 90°$ (*c* 1.0 in 10% sodium hydroxide), yielded only xylose on hydrolysis and had a number-average degree of polymerization of 65 as determined by osmotic pressure measurements with the acetate derivative.[9,21]

Methylation of the Xylan. (**A**).—Xylan (3.0 g.) was dissolved in 19% sodium hydroxide (100 ml.) in an atmosphere of nitrogen. Dimethyl sulfate (20 ml.) was added dropwise over a period of 1 hr. with stirring and external cooling. After an additional period of 4 hr. at room temperature, the remaining alkali was neutralized with 8 *N* sulfuric acid (cooling) and the neutral solution was dialyzed against running tap water for 10 days. Freeze-drying yielded a white, fluffy material (2.7 g.), OMe, 13.6%.

(**B**).—Xylan (4.5 g.) was dissolved in 40% sodium hydroxide (45 ml.) and methylated in the same way with dimethyl sulfate (35 ml.) for 16 hr. at room temperature. After neutralization with sulfuric acid, the solution was heated to boiling and the precipitated, partly methylated xylan was recovered by filtration and washed with hot water. Drying *in vacuo* at 70° for 3 hr. yielded a brittle, white product (4.7 g.), OMe, 31.3%.

Hydrolysis of the Partly Methylated Xylans. (**A**).—The partly methylated xylan (1.5 g.) was dissolved in 72% sulfuric acid (15 ml.). After 1 hr. at room temperature, the solution was diluted to 8% acid concentration and boiled under reflux for 4 hr. The sirup obtained after neutralization (barium carbonate) was dissolved in 80% ethanol and the solution was filtered through Celite[22] and evaporated to dryness (1.3 g.).

(**B**).—The *O*-methylxylan (4.0 g.) was treated similarly to give a sirupy mixture of reducing sugars (3.9 g.).

Resolution of the Hydrolyzate.—The mixture of reducing sugars (A, 1.3 g.) was added to the top of a charcoal (Darco G-60)[23]–Celite[22] column (1:1, w./w., 3.5 × 55 cm.)[10] and the sugars were separated by gradient elution,[11,12] first with water (2 l.)–8% aqueous ethanol (2 l.) and subsequently with 8% ethanol (1 l.)–25% ethanol (1 l.). Fractions, 25 ml. each, were collected with an automatic fraction collector. Five main fractions were obtained, consisting of xylose, 3-*O*-methylxylose, a mixture of 3-*O*- and 2-*O*-methylxylose, 2-*O*-methylxylose and 2,3-di-*O*-methylxylose. The amount of each sugar was determined from the weight of the material obtained from the column and from the optical rotation of the solutions. A portion of the mixture of mono-*O*-methyl-xyloses was resolved by paper electrophoresis[13] in a 0.05 *M* borate buffer and the quantity of each sugar was determined by titration with hypoiodite.[14]

Identification of the Sugars.—All sugars were chromatographically (solvents A and C) or electrophoretically (borate buffer) identical with authentic specimens.

D-Xylose.—This fraction crystallized, m.p. and mixed m.p. 145–146°, $[\alpha]\text{D} +19°$.

2-*O*-Methyl-D-xylose.—The crystalline sugar had m.p. and mixed m.p. 133–134°, $[\alpha]\text{D} + 35°$.[24] *Anal.* Calcd. for $C_6H_{12}O_5$: OMe, 18.9. Found: OMe, 18.6.

3-*O*-Methyl-D-xylose.—This fraction, the second eluted from the column, crystallized, m.p. and mixed m.p. 102–103°, $[\alpha]\text{D} + 17.5°$.[25] *Anal.* Calcd. for $C_6H_{12}O_5$: OMe, 18.9. Found: OMe, 18.8.

2,3-Di-*O*-methyl-D-xylose.—The last fraction, $[\alpha]\text{D} + 22°$,[7] crystallized on seeding to give a product, a portion of which had m.p. 81–82°[26,27] while another portion melted over an interval of 89 to 103°.[28,29] The 2,3-di-*O*-methyl-*N*-phenyl-D-xylopyranosylamine had m.p. and mixed m.p. 123–125°, $[\alpha]\text{D} + 185°$ (*c* 2.0 in ethyl acetate).[7,30] Its infrared spectrum was identical with that of an authentic specimen.

Resolution of Hydrolyzate B.—The hydrolyzate from the second specimen (B) was resolved on charcoal-Celite as described above and the D-xylose, 2-*O*-, 3-*O* and 2,3-di-*O*-methyl-D-xyloses obtained were identified by chromatographic or electrophoretic means.

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Synthetic Polysaccharides. V. Polymerization of Various Aldoses

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Methods for polycondensation of aldohexoses (D-galactose, D-mannose), 2-deoxy-D-glucose (2-deoxy-D-arabino-hexose), aldopentoses (L-arabinose, D-xylose, D-ribose), L-rhamnose, and of a disaccharide (maltose: 4-O-α-D-glucopyranosyl-D-glucose) are reported, together with characterization data on the resulting polysaccharides.

The polymerization of glucose at elevated temperature in the presence of acid catalyst and in vacuum was reported previously.[1] The properties of the polyglucoses[2,3] and several derivatives[4,5] were also reported. Recently other polymerization methods resulting in similar polyglucoses have been reported.[6,7,8] We have extended our polycondensation method to several other simple carbohydrates, including the commonly occurring aldoses, the polymerization of which is reported in this paper.

The methods of polycondensation and the precautions which had to be employed in selecting the reaction conditions were essentially the same as those employed in the preparation of polyglucose.[1] The carbohydrates were carefully heated under vacuum and in the absence of oxygen but in the presence of an acid catalyst, until melted. When evolution of water indicated that polycondensation had occurred, the elevated temperature and high vacuum were maintained until the melt solidified upon advanced polymerization. The selection of the suitable temperature–time cycle at a certain acid catalyst concentration is critical, and different for each carbohydrate. The temperature should be sufficiently high to activate the polycondensation but should not cause side reactions such as decomposition, which is evidenced by browning of the melt. The preferred reaction conditions for each sugar are reported, together with characterization data on the resulting polysaccharides.

Experimental

The aldoses were commercial samples and were employed without further purification. The catalyst was phosphorous acid employed in a concentration of 0.82 weight % (based on the anhydrous sugar). This acid was selected for reasons discussed in ref. 1, and the above concentration was found convenient to find experimentally the preferred polymerization temperature–time cycle, without causing decomposition of the different sugars. In order to distribute the phosphorous acid uniformly in the sugar the former was added to the latter, either as a dilute aqueous solution (*e.g.*, 6 ml. of aqueous solution containing 0.205 g. of H_3PO_3 was added to 25 g. of L-rhamnose) or, as a more dilute solution in tetramethylene sulfone (TMS) (*e.g.*, 0.082 g. H_3PO_3 dissolved in 6 ml. of TMS was added to 10 g. of L-arabinose).

To eliminate traces of oxygen before heating, the vacuum system and polymerization flask containing the catalyzed sugar were evacuated and flushed with nitrogen successively five times followed by a final evacuation. Conditions employed and major changes observed during the polymerizations are summarized in Table I.

Polymerization was carried out on most of the sugars under conditions similar to those of the first stage of the "two-stage" method (expt. 5, Table I, ref. 1) except that stirring was eliminated. For D-galactose and 2-deoxy-D-glucose the conditions of the "solution melt" method were employed (*cf.* expt. 3, Table I of ref. 1); stirring was eliminated and a reflux condenser was not used. The TMS solvent lost on distillation was not replaced and nitrogen was not led into the system during the heating. The two methods can be used alternatively. The vacuum system was identical to that described for the polycondensation of glucose.[1]

During the heating, excess water and the TMS distilled off. As the temperature was increased, the mixture melted and began to boil, indicating that water of condensation was evolving. As the polymerization proceeded, the mass became more and more viscous and the bubbling slowly subsided. The resulting frothy mass slowly solidified, forming a brittle spongy resin on being cooled to room temperature at the completion of the reaction.

The polymer was powdered, and dissolved in sufficient water to give a 20% solution. The polymers of D-galactose, L-arabinose and D-xylose contained a small proportion (about 1%) of insoluble gel which was centrifuged off and discarded. The other polysaccharides were completely soluble. The aqueous solutions were neutralized with sodium bicarbonate, and 0.2% NaCl (wt./vol.) was added. One volume of the solution was then introduced in a thin stream through a capillary into 10 volumes of ice-cooled absolute ethanol. A white powdery precipitate resulted. To increase the yield of the precipitate, absolute ether was added to the supernatant. The combined precipitate was collected by centrifuging or by filtering, washed with ethanol, redissolved in water and finally freeze-dried. The only exception was the polymer of 2-deoxy-D-glucose (2-deoxy-D-arabino-hexose) which was first treated with Darco G-60 carbon, and then precipitated by introducing the aqueous solution (200 ml.) into a mixture of 2,800 ml. of cold ethanol, 4,000 ml. of ether and 160 ml. of chloroform. This modification was necessary since the polymer of 2-deoxy-D-glucose was fairly soluble in alcohol. The last column of Table I gives the yields of polysaccharide obtained by these methods. These polysaccharides were further dried for 24 hr. at 60° under vacuum before characterization.

Characterization.—Optical rotations were determined in water at 25.6°. The values are reported in Table II. No mutarotation was observed.

Dialysis of the polysaccharides was carried out in Visking cellophane tubing, against running distilled water for 48 hr. The residue was freeze-dried and the percentage loss upon dialysis is reported in Table II.

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