interest in the present study. They are also thankful to the National Research Council of Canada for financial support. I. C. also wishes to express his gratitude to McGill University for the award of the Harold Hibbert Memorial Fellowship for 1959.
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Synthetic Polysaccharides. V. Polymerization of Various Aldoses

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RECEIVED DECEMBER 2, 1959

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.

(1) P. T. Mora and J. W. Wood, THIS JOURNAL, **80**, 685 (1958).
(2) P. T. Mora, J. W. Wood, P. Maury and B. G. Young, *ibid.*, **80**, 693 (1958).
(3) P. T. Mora, *J. Polymer Sci.*, **23**, 345 (1957).
(4) J. W. Wood and P. T. Mora, THIS JOURNAL, **80**, 3700 (1958).
(5) P. T. Mora, E. Merler and P. Maury, *ibid.*, **81**, 5449 (1959).
(6) F. Micheel and W. Gresser, *Chem. Ber.*, **91**, 1241 (1958).
(7) H. W. Durand, M. F. Dull and R. S. Tipson, THIS JOURNAL, **80**, 3691 (1958).
(8) J. da S. Carvalho, W. Prins and C. Schuerch, *ibid.*, **81**, 4054 (1959).

TABLE I

POLYMERIZATION OF SUGARS

Carbohydrate	G.	Temperature, °C. Bath	Temperature, °C. Internal	Time, hr.	Press., μ	Observation	Yield, % polysaccharide[c]
D-Galactose	20[a]	140–170	107–141	1	1	Melted, heating contd.	74.3
		140	133	18.6	1	Frothed, solidified	
D-Mannose	25	109[b]	58[b]	0.5	30	Melted	
		109	100	4.3	30	Frothed, solidified	84.4
2-Deoxy-D-glucose	10[a]	78[b]	61[b]	0.5	30	Melted, melt boiled	
(2-deoxy-D-arabino-		105	73	0.4	30	Boiled	
hexose)		105–108	75–92	1.2	2–5	Solidified	43.0
		80	79	2	1	No more vapor evolved	
L-Arabinose	25	123–140	56–118	1.4	8	Remained solid	67.2
		140	120–125	4.2	8	Remained solid	
D-Xylose	25	140[b]	114[b]	1	12	Melted, solidified	72.8
		140	114–120	1	12		
		120	120–113				
D-Ribose	10	105[b]	88[b]	0.7	5	Melted	
		105–133	88–125	1.6	5	Frothed, solidified	81.9
		133	125	4.5	5		
L-Rhamnose	25	107[b]	81[b]	1	6	Melted	64.7
		120	81–117	8.1			
Maltose	25	128[b]	97[b]	1	10	Melted	60
(4-O-α-D-glucopyrano-		128–155	97–139	2.5		Solidified	
syl-D-glucose)		155	140–145	2			

[a] "Solution melt" method (with tetramethylene sulfone) was used for the polymerization of D-galactose and 2-deoxy-D-glucose, while the remainder of the sugars were polymerized by the "stage I" step of the "two-stage" method. For a detailed description of the methods see ref. 1. [b] Gradually increased up to this temp. [c] Recovered by precipitation in ethanol, or for 2-deoxy-D-glucose in a mixture of EtOH, $(Et)_2O$, $CHCl_3$, followed by freeze drying.

TABLE II

CHARACTERIZATION OF POLYSACCHARIDES

Polymer	$[\alpha]^{25.6}D$ in H_2O	Loss on dialysis, %	$[\eta]$, dl./g.	$\overline{M}_n$[a]	Reducing power,[b] %	IO_4^- consumed moles per anhydro-sugar unit 48 hr.	IO_4^- consumed moles per anhydro-sugar unit 72 hr.	HCOOH produced 48 hr.	HCOOH produced 72 hr.	Infrared absorption frequencies,[c] $cm.^{-1}$		
D-Galactose	+42.4°	17	0.09	18,700	81	1.17	1.34	0.34	0.37			
D-Mannose	+42.1	64	.04	17,900	93	1.58	1.79	.75	.81	880s	800s	
2-Deoxy-D-glucose	+75.0		.03	7,100	99[d]	0.36	0.49	.04	.04	880vs	800s	720m
L-Arabinose	+27.1	11	.07	6,300	97	.67	.69	.13	.13	840m	780s	720s
D-Xylose	+53.5	76	.05	3,300	93	.87	.89	.39	.34			
D-Ribose	− 8.6	26	.06	5,700	93	.70	.79	.22	.22	780s	760m	
L-Rhamnose	−40.4	83	.04	1,500	103	1.08	1.11	.35	.38	830m	800m	
Maltose	+105.5	31	.06	8,300	104	1.11	1.12	.32	.40	840s	760s	

[a] Assuming one reducing end group. [b] Reducing power of sugar recovered after hydrolysis in N HCl for 1 hr. [c] Absorption bands in the "finger print" region: vs = very strong, s = strong, m = medium. [d] Hydrolysis 1 hr. 0.1 N HCl.

The intrinsic viscosity was measured in aqueous solutions at 26.7° in Ubbelohde viscometers. Kinetic energy corrections were not applied.

Number average molecular weight ($\overline{M}_n$) was determined by a reducing end group method,[1] based on the assumption that there was one reducing end group per polymer molecule, and that the reducing power of the end group is equal to that of the monosaccharide, as determined in a separate experiment.

Hydrolysis of Polysaccharides.—The polysaccharides were heated at 100° in N HCl for 1 hour. After neutralization, the reducing power was compared to that which would be expected upon complete conversion to the corresponding monosaccharide and the values are reported in Table II as % reducing power recovered. The polymer of 2-deoxy-D-glucose decomposed under these conditions and, therefore, milder hydrolysis conditions (1 hr., 0.1 N NCl) had to be employed. Concentrated hydrolysis products (10%) of the polysaccharides were investigated by paper chromatography so that at each starting point 0.65 mg. was applied to the paper. The corresponding monomers were indicated as the major products of hydrolysis and only very small proportions of other components were present. These had lower R_f-values and were obviously reversion products, since they were also formed by heating the monomers with acid. From the maltose polymer, D-glucose was the hydrolysis product. L-Rhamnose and its polymer did not show any evidence of reversion during treatment with acid. The unhydrolyzed polysaccharides did not have any movable component upon paper chromatography.

Periodate oxidation experiments were carried out under previously reported conditions[1] (0.0374 N $NaIO_4$, 4°, dark). The periodate consumed and the formic acid produced were determined after 48 and 72 hr., and the values were reported in Table II.

Infrared absorption spectra were determined in Nujol mull, and the distinguishable absorption band frequencies in the "finger print" region[9] (700–950 $cm.^{-1}$) are reported. However, these bands were relatively minor ones, and generally there was a high over-all absorption in this region.

Results and Discussion

Polysaccharides were produced in good yield upon polycondensation of several aldoses under the experimental conditions summarized in Table I.

The synthetic polysaccharides contained high molecular weight non-dialyzable fraction in different amounts: L-arabinose polymer contained

(9) S. A. Barker, E. J. Bourne, M. Stacey and D. H. Whiffen, *J. Chem. Soc.*, 171 (1954).

89% non-dialyzable material while the L-rhamnose polymer had only a 17% non-dialyzable fraction. The number average molecular weight, as determined by reducing end group titration, also showed great variation: from 1,500 for L-rhamnose polymer up to about 18,000 for the polysaccharides from D-galactose and D-mannose. This number average molecular weight, however, may be a misleadingly low figure, at least as far as experiments on polyglucose indicated.[10] Furthermore, number average molecular weights emphasize the low end of the molecular weight distribution of polycondensation products with broad molecular weight distribution. The weight average molecular weight of the poly-disperse polyglucoses prepared by similar method was found to be much higher than the number average molecular weight, and unfractionated polyglucoses with $\bar{M}_w$ of about 200,000 were obtained.[3]

The polysaccharides were reconverted to the original monomer on acid hydrolysis (in the case of the polymer of maltose to glucose), as the percentage recovery of reducing power and paper chromatography experiments indicated. There were no detectable degradation products present, except a small proportion of reversion products (dimers and other oligosaccharides) which were also formed upon similar aqueous acid treatment of the nonosaccharides. L-Rhamnose and its polysaccharide did not show any detectable reversion when heated in aqueous solution in acid, probably because of the absence of the primary hydroxyl group, which is known to be the most reactive under such conditions.[11]

The mechanism of polycondensation of the aldoses is assumed to be essentially similar to that which was postulated for polyglucose.[1] Because of the simultaneous availability of several functional groups (hydroxyls) on the monomer, such polycondensation should lead to highly branched structures.

The two aldohexose polymers, produced from D-galactose and D-mannose, behaved similarly to polyglucose on periodate oxidation (*cf.* Table II) except that with the D-galactose polymer the lower amount of periodate consumed and formic acid produced gave indication of a higher degree of branching, while the D-mannose polymer appeared to be less branched than polyglucose (for periodate oxidation of polyglucose see refs. 2 and 5). The lower temperature and the shorter heating cycle in the polymerization of D-mannose might be the cause of the lower degree of branching, if the arguments which were presented in connection with the periodate oxidation of polyglucose[2] are correct; or the difference might be due to stereochemical differences, such as the reduced simultaneous spatial availability of the second and third *cis*-hydroxyls on the D-mannose for polymerization. Apparently the D-mannose polysaccharide is of lower molecular weight, as indicated by the dialysis and the intrinsic viscosity figures.

The polysaccharide of 2-deoxy-D-glucose is quite different from polyglucose: it is fairly soluble in alcohol and it is very sensitive to acid hydrolysis. The 2-deoxy-D-glucose monomer itself was found to be more sensitive to heat than D-glucose, hence the reason for the relatively low temperature selected for polymerization. The periodate consumption of the polysaccharide was very low, and since there are no three neighboring hydroxyls present, there was no significant amount of formic acid produced. The moles of periodate consumed should correspond to the sum of the 1→6-linear units and of the non-reducing end groups. Since such units represent only about half of the monomers (*cf.* the IO_4^- value after 72 hr. oxidation) the other half has to be branched or linked through either the third or the fourth hydroxyl, or both. The 1→6-linkage is expected to be favored, as in polyglucose.[2,11]

The aldopentoses L-arabinose, D-ribose and D-xylose, behaved quite similarly upon polymerization, and the polysaccharides were also similar, except that the molecular weight of the D-xylose polymer was lowest. This might be because of the shorter heating cycle in the polymerization, although the temperature was higher than with the other aldopentoses. The polymers of the aldopentoses consumed less periodate and produced less formic acid than the aldohexose polymers, since there were fewer neighboring hydroxyls available. Structural investigation of polyxylose[12] prepared by similar polycondensation but at lower temperature[13] showed that the polymer is highly branched and that the frequency of the 1→4-linkage is highest, followed by the 1→2-linkage.

L-Rhamnose demonstrated resistance to polymerization, as the low molecular weight and the high amount of dialyzable product indicated, and it did not show signs of reversion upon heating with aqueous acid. This was expected, since there is no primary hydroxyl available for polymerization in this hexose and the primary hydroxyls are the most reactive in reversion and in polycondensation.[2,11] It was remarkable that 35–38 mole per cent. formic acid was produced from the polymer, and this could only come from non-reducing end groups. Since each branching has to terminate in a non-reducing end group according to our postulated mechanism for the polycondensation of aldoses, this means that somewhat more than one-third of the monomeric units are branched in this polymer. When one new branching is produced one attacking point is lost for the periodate ion (except when the linear link was 1→3, when upon further branching there will be no decrease in the periodate consumption), but at the same time two new sites are introduced which will be available for periodate attack, because an equivalent number of non-reducing end groups are formed. Therefore, the moles of periodate consumed per anhydro sugar unit must have a slightly

(10) We thank Dr. F. Smith for bringing this to our attention and for his number average molecular weight estimate on our polyglucose sample by using formaldehyde determination after periodate oxidation, to estimate reducing end groups. His value was approximately double our $\bar{M}_n$ figure obtained by reducing power titration. Part of this difference might be due to our assumption that the reducing power of the end group is similar to that of the low molecular weight sugar.

(11) H. Frahm, *Ann.*, **555**, 187 (1944).

(12) C. T. Bishop, *Can. J. Chem.*, **34**, 1255 (1956).

(13) C. K. Ricketts and C. E. Rowe, *J. Chem. Soc.*, 3809 (1955).

higher value than unity, and this was found experimentally.

The polymerization product from maltose is similar to polyglucose. The hydrolysis product was D-glucose, and the physical characteristics of the polymer were similar to that of a polyglucose produced by a low temperature short polymerization cycle. The lower amount of periodate consumption and formic acid production of the polymer from maltose, as compared to that of a polyglucose produced at higher temperature and upon longer heating,[2,5] indicate, however, that the polymer of maltose is more branched than polyglucose. The somewhat higher restriction of initial mobility of the dimer during the melt polymerization might account for this phenomenon. In any case, rearrangement of the polysaccharide linkage and randomization with respect to sequence of linkages, and establishment of α-β and pyranose-furanose equilibrium are expected to give a product essentially similar to that of polyglucose.[1-3,13]

The polymers of aldoses are apparently all highly branched, the aldohexose polymers being more branched than the aldopentose polymers because of the higher functionality of the monomer.[14]

The different linkages are expected to be distributed randomly in all the polysaccharides, since there is no reason for them to be otherwise in a chemically catalyzed polymerization. Also, since the reactivities of the functional groups are more alike at high temperature, their simultaneous availability for condensation should lead to more branching, and if the activating energy is high enough to cause condensation at each hydroxyl, eventually all possible linkages are to be expected. However, structural (methylation) studies should be carried out to substantiate this and other arguments presented above. It is further assumed that the amount of the α- and β-glycosidic links, and the furanose and pyranose rings, correspond to the equilibrium concentration established during the polymerization.[2,3]

The above experiments are described in order to illustrate the general utility of our polycondensation method for aldoses. By this method a large number of new synthetic polysaccharides became available for structural and biological investigations. Results of such investigations will be reported in due course, as well as further polymerization experiments with restricted functional groups on monomers leading to different polymers.

(14) *Cf.* P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953, Chapter IX, pp. 347-398.

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

Methylation Studies on Dialdehydes Obtained from Methyl Glycosides by Periodate Oxidation[1]

BY IRWIN J. GOLDSTEIN AND F. SMITH

RECEIVED OCTOBER 12, 1959

Evidence is presented to support the view that the so-called "dialdehydes" produced by the periodate oxidation of methyl glycosides exist in the cyclic form as derivatives of dioxane.

In previous communications[2-4] it was shown that the so-called dialdehydes formed by periodate oxidation of methyl glycosides exist in the cyclic acetal form as derivatives of dioxane. This conclusion was reached from infrared and polarimetric measurements and also because the "dialdehydes" reacted not as carbonyl but as hydroxy compounds. Thus the so-called dialdehyde formed by periodate oxidation of methyl α-L-rhamnopyranoside gave rise to a crystalline di-*p*-nitrobenzoate, and methylation with silver oxide and methyl iodide furnished a dimethyl ether.

This paper is concerned with methylation studies on three additional "dialdehydes" (I, IV and VII) which further support the view that these substances exist in the cyclic form as derivatives of dioxane.

The "dialdehyde" I, prepared from methyl β-L-arabinopyranoside by periodate oxidation, gave upon treatment with silver oxide and methyl iodide a sirupy product, 2(D″), 3,5-trimethoxy-1,4-dioxane (III),[5] the methoxyl content of which indicated that two methoxyl groups had been introduced during methylation. Furthermore, the rotation ($[\alpha]^{24}D$ +124° in ethanol) of III was comparable with that of the starting material which showed $[\alpha]^{20}D$ +125° in water. This similarity in rotation suggested[3] that the oxidation product I possessed the cyclic structure II.

The compound D′-methoxy-D-methoxymethyldiglycolic aldehyde (IV) obtained by periodate oxidation of methyl 6-*O*-methyl-α-D-galactopyranoside also reacted in the cyclic form (V) because upon methylation with silver oxide and methyl iodide 2(D″),3,5-trimethoxy-6(L″)-methoxymethyl-1,4-dioxane (VI)[5] was formed. The compound VI which showed $[\alpha]^{22}D$ +150° (in ethanol) as compared with $[\alpha]^{21}D$ +143° (in ethanol) for the starting

(1) Paper No. 4215 Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul 1, Minn. This work was sponsored by the Office of Ordnance Research, U. S. Army.

(2) J. E. Cadotte, G. G. S. Dutton, I. J. Goldstein, B. A. Lewis, F. Smith and J. W. Van Cleve, THIS JOURNAL, **79**, 691 (1957).

(3) I. J. Goldstein, B. A. Lewis and F. Smith, *ibid.*, **80**, 939 (1958).

(4) I. J. Goldstein, B. A. Lewis and F. Smith, *Chemistry & Industry*, 595 (1958).

(5) Groups designated by D″ and L″ in this and other formulations of dioxane compounds correspond to positions below and above the plane of the ring of the original formula written according to the Haworth convention (*cf.* ref. 3).