Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

POLYMERIZATION OF A CELLOBIOSE DERIVATIVE TO COMB-SHAPED OLIGOSACCHARIDES

VLADO MASURA AND CONRAD SCHUERCH

Department of Chemistry, State University College of Forestry at Syracuse University, Syracuse, New York 13210 (U. S. A.)

(Received March 3rd, 1970)

ABSTRACT

The Lewis acid-catalyzed polymerization of 1,6-anhydro-2,3-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-β-D-glucopyranose (hexabenzyl-1,6-anhydrocellobiose) leads to products of number average molecular weights of 6–7 × 10^3 and specific rotations as high as +80° (*c* 1, chloroform). Variations in reaction conditions affect the molecular weight and optical rotation of products significantly. Conditions giving maximum stereoregularity and molecular weight are similar to those observed with the corresponding maltose derivative. Debenzylation of products yielded nondialyzable hydrated oligosaccharides, presumably comb-shaped, of $[\alpha]_D^{25}$ +77.8° (*c* 1, water; observed) or $[\alpha]_D^{25}$ +84.3° (*c* 1, water; calculated for anhydrous weight). Analysis of the oligosaccharide, like the products from the corresponding maltose derivative, indicates the presence of 1.5 molecule of water for each repeating disaccharide unit. Presumptive evidence suggests that the products of highest optical rotation are highly stereoregular.

INTRODUCTION

There are three major polymer systems found widely distributed in nature, the proteins, polysaccharides, and nucleic acids. Of these, only the polysaccharides require for their synthesis a chain-forming reaction under complete steric control. The carbohydrates from which they are derived can be classed as multifunctional monomers of the AB_n type and can generate linear, cyclic, or branched polymers. There are 256 stereoregular, linear homopolysaccharides theoretically derivable from the aldohexoses alone, and a nearly infinite variety of copolymers and other less ordered structures.

Three stereoregular α-D-(1→6)-linked polysaccharides have been synthesized in this laboratory by the Lewis acid-catalyzed polymerization and debenzylation of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-gluco-, -manno-, and -galacto-pyranose[1–6]. We are now investigating the preparation of comb-shaped polysaccharides by polymerization of similar perbenzylated 1,6-anhydro disaccharides, such as 1,6-anhydro-maltose[7], -lactose, and -cellobiose. The present paper reports results on the last-mentioned polysaccharide. Comb-shaped polysaccharides are not common in nature, but the structure is approximated in certain arabinoxylans of wheat[8]. The ultimate objective is to be able to synthesize polysaccharides of known structure at will, so that one can investigate systematically the relationship between structure of polysaccharides and their physical and physiological properties.

In previous polymerizations of this series, high stereoregularities have been associated with the use of a fluorine-containing Lewis acid at the lowest temperature range at which the particular catalyst causes polymerization at reasonable rates. Phosphorus pentafluoride has generally been preferable to boron trifluoride, since it operates at lower temperatures at which termination and transfer processes are less prevalent. The solvent used for polymerization can affect stereoregularity but often its role is less important than the temperature and Lewis acid.

Although rigorous data on the rates of initiation, propagation, transfer, and termination are not yet available, qualitative observations suggest that the more slowly polymerizing monomers (anhydro-D-galactose and especially maltose derivatives) have a slower rate of propagation. The lower molecular weights of their polymers are presumably caused by the greater significance of transfer or termination processes. Molecular-weight distribution studies suggest that termination and transfer processes are not important, however, under optimum conditions with rapidly polymerizing monomers.

The present disaccharide derivative, 1,6-anhydro-2,3-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-β-D-glucopyranose (hexabenzyl-1,6-anhydrocellobiose, **1**), is the least suitable 1,6-anhydro sugar for the preparation of high molecular weight stereoregular polymers found to date. We assume that its rate of propagation is low under conditions which favor stereoregularity. There are at least four variables that can be altered in a given monomer–catalyst system to enhance polymerization rates. These are the use of higher temperatures or forcing conditions of high catalyst concentration at lower temperatures, and the use of high monomer and cocatalyst concentration. The latter presumably simplifies initiation and increases its rate but may influence propagation as well. By manipulating these variables, oligomers or polymers containing about 14 to 16 D-glucose units have been synthesized.

RESULTS AND DISCUSSION

The monomer **1** was prepared from cellobiose octaacetate by a conventional sequence of reactions[9–11] and was subjected to a series of polymerizations in methylene chloride at −78° with various concentrations of phosphorus pentafluoride from 2–60 mole %[2,7]. In all cases the conversions to petroleum ether-insoluble material were less than 25%. The lowest molecular weight products, obtained at catalyst-to-monomer ratios of 10% and less, had low optical rotations as well. The higher molecular weight products obtained at higher catalyst ratios lay only in the trimer and tetramer range (6 to 8 glucose residues) and had specific rotations from +63–70° (Fig. 1).

The yield of polymer and its molecular weight were slightly increased by the

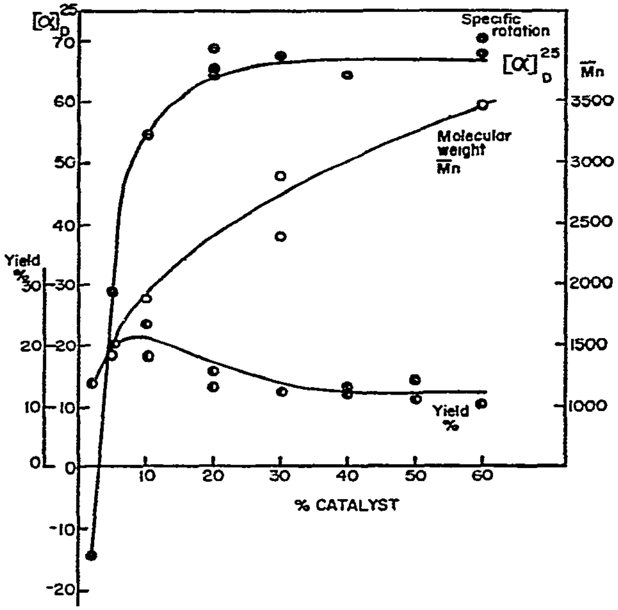

Fig. 1. The effect of catalyst concentration on the polymerization of 1,6-anhydro-2,3-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-β-D-glucopyranose.

use of benzoyl fluoride as cocatalyst (Fig. 2), and a series of polymerizations was performed at varying temperatures using 20 moles % of catalyst and cocatalyst, based

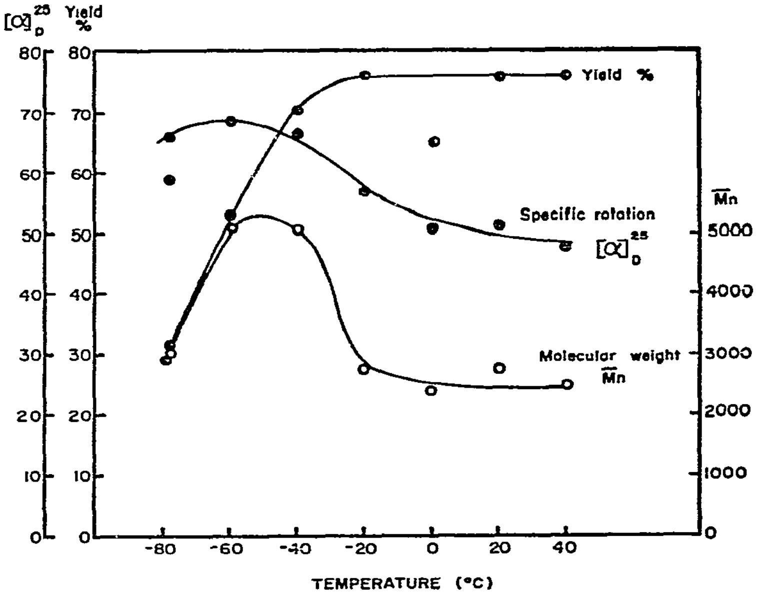

Fig. 2. The effect of temperature on the polymerization of 1,6-anhydro-2,3-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-β-D-glucopyranose. Conditions: 20 moles % catalyst and cocatalyst; solvent-to-monomer ratio 2:1.

on monomer (Fig. 2). Maximum conversions were obtained at temperatures of −20° and above, but at these temperatures the molecular weight and specific rotation of the polymers were substantially below the maximum values. The molecular weights were slightly above 5000 in the temperature range −40 to −60° and specific rotations ranged between +65 and +70° (*c* 1, chloroform) at temperatures from −78 to −40°. Essentially similar results were obtained at solvent-to-monomer volume-to-weight ratios 2:1 and 1:1, but, the conversions and optical rotations were somewhat higher at the higher concentration (Figs. 2 and 3).

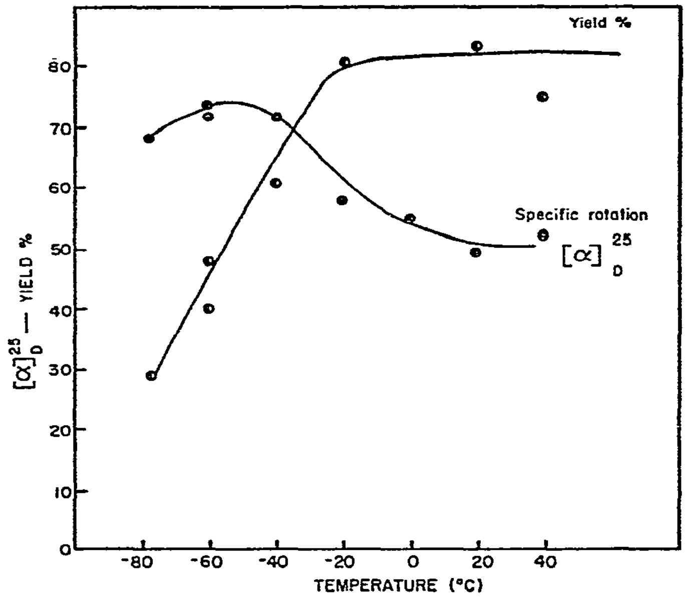

Fig. 3. The effect of temperature on the polymerization of 1,6-anhydro-2,3-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-β-D-glucopyranose. Conditions: 20 moles % catalyst and cocatalyst; solvent-to-monomer ratio 1:1.

The use of more cocatalyst than catalyst failed to produce any interesting results at −78° (Table I, Nos. 31–34), and the use of sulfur dioxide as solvent resulted in products of quite low specific rotation (Table I, Nos. 35–39).

A series of polymerizations was performed at −78° using equivalent amounts of catalyst and cocatalyst, but altering the catalyst-to-monomer ratios. It was found that at this temperature 40 to 60 moles % catalyst-to-monomer ratios gave higher molecular weights and specific rotations, and that lower and higher ratios gave poorer results (Fig. 4).

Similar experiments at −60° using both acetyl fluoride and benzoyl fluoride (Table I, Nos. 49–51, 53–56) as cocatalyst in 20 and 40 moles % catalyst, cocatalyst-to-monomer ratios, respectively, gave products of highest specific rotation, +77 to 79° (*c* 1, chloroform), and molecular weights above 5,800 (Table I, Nos. 51, 53, and 54). Most of product 54 remained, after three precipitations, with a molecular weight,

TABLE I

POLYMERIZATION OF 1,6-ANHYDRO-2,3-DI-*O*-BENZYL-4-*O*-(2-3,4,6-TETRA-*O*-BENZYL-β-D-GLUCOPYRANOSYL)-β-D-GLUCOPYRANOSE[a]

Expt. No.	*Catalyst*[b], *moles %*	*Cocatalyst*[b], *moles %*	*Temp., degrees*	*Conversion*	$[\alpha]_D^{25}$[c]	*Molecular weight*, $\overline{Mn}$
18	20.0	20.0	−60	52.7	68.0	5090
19	20.0	20.0	−40	70.5	66.8	5080
27[d]	20.0	20.0	−20	80.7	57.6	——
31	10.0	20.0	−78	13.5	45.9	2330
32	20.0	40.0	−78	32.3	49.5	2770
33	20.0	60.0	−78	35.7	45.8	3400
34	10.0	40.0	−78	16.7	32.7	1700
35	20.0	0.0	−60	33.4	36.2	——
36	40.0	0.0	−20	82.0	50.4	——
37	20.0	20.0	−60	30.9	29.4	——
38	40.0	40.0	−60	28.1	24.3	——
39	60.0	60.0	−60	48.5	42.1	——
47	80.0	80.0	−78	75.2	72.3	4700
49[e]	5.0	5.0	−60	21.1	65.2	2880
50[e]	10.0	10.0	−60	42.6	73.4	4350
51[e]	20.0	20.0	−60	70.3	77.2	5800
52	40.0	40.0	−78	69.3	74.3	4300
53	40.0	40.0	−60	70.7	79.0	——
54[d]	40.0	40.0	−60	72.1	78.0	7330[f]
55	20.0	20.0	−60	48.2	74.3	——
56[g]	20.0	20.0	−60	60.2	70.4	——

[a]The table refers to polymerizations of 0.43 g of monomer (0.5 mmoles). [b]The catalyst for all experiments was phosphorus pentafluoride. The cocatalyst was benzoyl fluoride, except for Expts. 49–52 in which acetyl fluoride was used. The solvent in all cases was dichloromethane, except for experiments 35–39 in which sulfur dioxide was used. [c]*c* 1, chloroform. [d]Dichloromethane:monomer = 1:1. [e]Solvent dried over sodium mirror. [f]After 3 fractional precipitations. [g]Dichloromethane:monomer = 1:1; phosphorus pentafluoride separated from 1-chloro-4-fluorobenzene.

$\overline{Mn}$, of 7,330. Fractionation of other products (Table I, Nos. 33 and 27) gave less soluble fractions of $[\alpha]_D^{25}$ +80–81° (*c* 1, chloroform). Some variation in optical rotation in products produced at temperatures of −78° to −60° is due to variable amounts of low molecular-weight material present. Repetition of a standard preparation without cocatalyst at −60° by T. Uryu followed by further precipitations led to the isolation of products having $[\alpha]_D^{25}$ +78–79°. Highly stereoregular high molecular-weight products in this series will probably have an $[\alpha]_D^{25}$ only slightly above +80–81° (*c* 1, chloroform). This value is surprisingly close to that of the product derived from the hexabenzyl ether of 1,6-anhydromaltose, $[\alpha]_D^{25}$ +96–97°, and might suggest the presence of a number of β-linkages in the maltose polymer. However, the specific rotation of the corresponding debenzylated maltose polymer is $[\alpha]_D^{25}$ +187–189° (*c* 1, water), indicating a high degree of stereoregularity.

A number of products resulting from these polymerizations were combined (Table I, Nos. 18, 19, and 47), $[\alpha]_D^{25} \cong +70.0°$ (*c* 1, chloroform), and debenzylated with sodium in liquid ammonia. The reaction proceeded normally, and the product

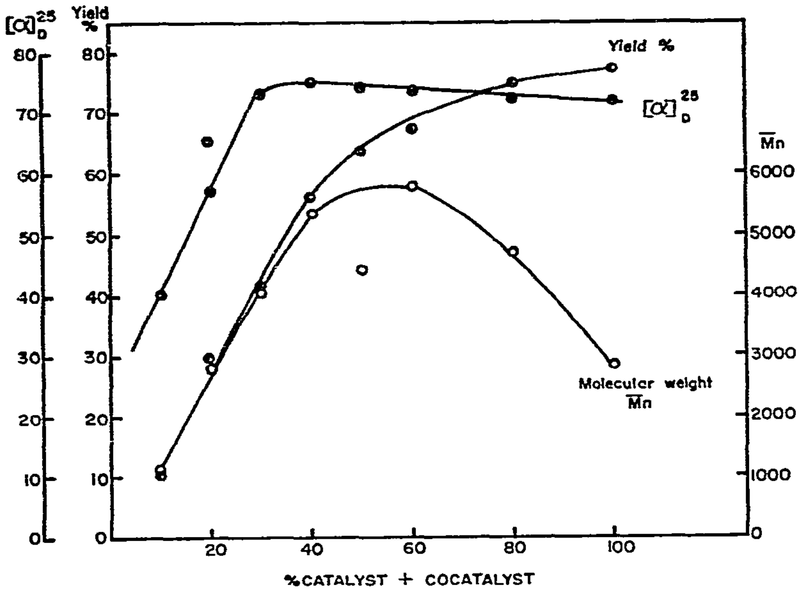

Fig. 4. The effect of quantity of catalyst and cocatalyst on the polymerization of 1,6-anhydro-2,3-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-β-D-glucopyranose.

was isolated by dialysis and freeze-drying. The yield of 66.5% probably suggests the loss of some low molecular-weight material of lower specific rotation and, therefore, it seems probable (from our experience with related polymers) that the specific rotation of the resulting polysaccharide, $[\alpha]^{25}_{D}$ +77.8° (*c* 1.0, water), is near the maximum to be expected. The carbon and hydrogen analysis was almost identical to that of the maltose polymer, and both correspond closely to that expected of a hydrated polysaccharide with 1.5 mole of water to each repeating disaccharide unit. Since the corresponding unbranched backbone of the α-D-(1→6)-linked glucan, mannan, and galactan have analyses corresponding to 0.5 mole of water per monosaccharide unit, this conclusion is not unreasonable. It appears clear that water constitutes a regular component in the solid state structure of these polysaccharides.

At the present time, the absolute stereoregularity of these comb-shaped polysaccharides cannot be considered established. However, the similarity of behavior of the monomers in polymerization, the identity of analysis of the hydrated polysaccharides, and the high specific rotation of the maltose polymer suggest that these comb-shaped polysaccharides may be as stereoregular as the corresponding products made from anhydro monosaccharides.

EXPERIMENTAL

Synthesis of monomer. — The monomer [1,6-anhydro-2,3-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl-β)-D-glucopyranose] was prepared from cellobiose octaacetate by treatment with phosphorus and bromine[10], phenyl glycoside formation, cyclization, reacetylation, and careful recrystallization[9] to give m.p. 145–146° and $[\alpha]_D^{25}$ $-54.2°$ (*c* 2.5, chloroform), agreeing closely with literature values[9]. Benzylation *via* Zemplén's procedure[11] was followed by steam distillation. Thin-layer chromatography on silica gel with 7:3 benzene–chloroform as eluant was used to show the presence of non-carbohydrate products from benzyl chloride. Chloroform containing 0.5% methanol was used as eluant to show the presence of incompletely benzylated products. At least two benzylations were required to obtain complete substitution which was confirmed by acetylation of the syrupy product followed by i.r. spectroscopy. When the product showed no carbonyl absorption at 1735–50 cm^{-1} and a single spot on thin-layer chromatography, it was crystallized from petroleum ether–ether, m.p. 86–87°, $[\alpha]_D^{25}$ $-19.7°$ (*c* 2.5, chloroform).

Anal. Calc. for $C_{54}H_{56}O_{10}$: C, 74.97; H, 6.52. Found: C, 74.36, 74.31; H, 6.54, 6.67.

Polymerizations. — All reagents and solvents were purified, and all polymerizations were performed as described earlier[1,2,5–7,12], usually on a scale of 5×10^{-4} moles of monomer (0.43 g) and at a solvent-to-monomer ratio 2:1 (vol./wt.). Polymerizations were stopped with methanol, and the product was extracted with chloroform. The extract was washed, dried, and concentrated. The concentrate (2 ml) was poured into petroleum ether (50 ml) and the precipitated polymer separated by centrifugation. The polymers formed cloudy solutions in benzene and were freeze-dried for isolation.

Anal. of products 51 and 54. Calc.: C, 74.97; H, 6.52. Found: C, 75.06, 74.72, H, 6.59, 6.60.

Number-average molecular weights were determined with a Mechrolab vapor-phase osmometer (Model M 301) in chloroform at concentrations of 15–60 g/l. The monomer was used as a reference.

Debenzylation. — Combined samples 18, 19, and 47 (Table I), $[\alpha]_D^{25} \cong +70°$ (*c* 2.5, chloroform), were dissolved in dry 1,2-dimethoxyethane (2 ml) and toluene (10 ml). The solution was dropped into stirred anhydrous ammonia (100 ml) containing dissolved sodium (1.5 g). The temperature was maintained at $-78°$ with a dry ice–isopropyl alcohol bath. After 3 h of stirring, ammonium chloride was added in portions to decolorize the blue solution, the solvent was evaporated under a stream of dry nitrogen, and the solids were triturated with methylene chloride and then dissolved in water. Dialysis against running distilled water for 4 days, followed by concentration of solution, gave an aqueous solution (20 ml) which was freeze-dried. The white powdery product weighed 0.148 g (66.5% yield); $[\alpha]_D^{25}$ $+77.8°$ (*c* 1.0, water, observed), $+84.3°$ (*c* 1.0, water, calculated on anhydrous weight).

Anal.: Calc. for $(C_{12}H_{20}O_{10})_n$: C, 44.43; H, 6.21; for $(C_{12}H_{20}O_{10}\cdot 1H_2O)_n$: C, 42.1; H, 6.48; for $(C_{12}H_{20}O_{10}\cdot 1.5H_2O)_n$: C, 41.05; H, 6.58; for $(C_{12}H_{20}O_{10}\cdot 2.0\,H_2O)_n$: C, 40.0; H, 6.67; Found: C, 41.14, 40.81; H, 6.33, 6.34.

ACKNOWLEDGMENT

The present work has been supported by Research Grant GM-06168 of the

Division of General Medical Sciences, National Institutes of Health, U. S. Public Health Service.

REFERENCES

1 J. Frechet and C. Schuerch, *J. Amer. Chem. Soc.*, 91 (1969) 1191.
2 E. R. Ruckel and C. Schuerch, *J. Org. Chem.*, 31 (1966) 2233.
3 E. R. Ruckel and C. Schuerch, *J. Amer. Chem. Soc.*, 88 (1966) 2605.
4 E. R. Ruckel and C. Schuerch, *Biopolymers*, 5 (1967) 515.
5 T. Uryu, H. Libert, J. Zachoval, and C. Schuerch, *Macromolecules*, 3 (1970), 345.
6 J. Zachoval and C. Schuerch, *J. Amer. Chem. Soc.*, 91 (1969) 1165.
7 B. Veruovic and C. Schuerch, *Carbohyd. Res.*, 14 (1970) 199.
8 A. S. Perlin, *Cereal Chem.*, 28 (1951) 370, 383.
9 E. M. Montgomery, N. K. Richtmyer, and C. S. Hudson, *J. Amer. Chem. Soc.*, 65 (1943) 1848.
10 P. G. Schuerer and F. Smith, *J. Amer. Chem. Soc.*, 76 (1954) 3224.
11 G. Zemplén, Z. Csüros, and S. Angyal, *Ber.*, 70 (1937) 1848.
12 J. Kops and C. Schuerch, *J. Polym. Sci., Part C*, (1965) 119.