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CHEMICAL SYNTHESIS OF A (1→2)-D-GLUCOPYRANAN

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ABSTRACT

1,2-Anhydro-3,4,6-tri-*O*-benzyl-α-D-glucopyranose was polymerized with a number of Lewis acids. Phosphorus pentafluoride at −60° caused polymerization to a product rich in β linkages. Other Lewis acids at higher temperatures gave perbenzylated polysaccharides of lower molecular weight with less stereoselectivity. Debenzylation of the most-regular derivative gave a polysaccharide whose specific rotation was +14.7° and whose ^{13}C-n.m.r. spectrum had six absorptions corresponding to those of natural (1→2)-β-D-glucopyranans and additional minor peaks presumably due to some α-anomeric configurations. It was estimated to have ~90% of β linkages.

INTRODUCTION

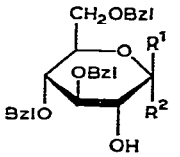
Agrobacterium tumefaciens is an organism that generates crown-gall tumors by transfer of its DNA to the plant cell. As it is possible to proliferate the tumor in tissue culture and regenerate plants from the tissue, this system constitutes a possible vector for the transfer of genetic information. The transfer of DNA is preceded by interaction of plant and bacterial cell-walls by a mechanism that is not understood[1]. It has, however, recently been emphasized[2] that the taxonomically related bacterial genera *Rhizobium* and *Agrobacterium* are able to synthesize and secrete (1→2)-β-D-glucopyranans of low molecular weight and to infect and stimulate tissue proliferation in dicotyledonous plants. The suggestion has been made[2] that perhaps these phenomena are related and that the (1→2)-β-D-glucopyranans may thus play a significant role in the interaction of these bacteria and their hosts.

Synthetic polysaccharides have been used to investigate immunological and allergic reactions in experimental animals and the interaction of plant lectins with carbohydrates[3]. It, therefore, appeared that the chemical synthesis of a (1→2)-β-D-glucopyranan might provide a useful model substance to investigate plant–pathogen and plant–symbiont relationships. We have recently synthesized 1,2-anhydro-3,4,6-tri-*O*-benzyl-α-D-glucopyranose[4] (3). A completely stereoselective polymerization of this monomer would be expected to form a perbenzylated (1→2)-β-D-glucopyranan from which the parent polysaccharide could be derived.

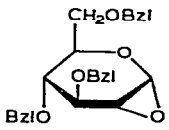
We now report a preliminary survey of polymerizations of 3 and the syntheses of a (1→2)-D-glucopyranan rich in β linkages.

RESULTS AND DISCUSSION

On repeating the synthesis of 3, it was established that conversion of 3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl chloride (**1**) into the corresponding glycosyl fluoride produces a mixture of anomers (**2**). The mixture of anomers was treated directly with base to effect ring closure. In this reaction, sodium hydride in oxolane (tetrahydrofuran) was found to give better yields of 3 than the published method using



1 R^1 = H; R^2 = Cl
2 R^1, R^2 = H, F



3

potassium *tert*-butoxide as base. As *trans* elimination is necessary for ring closure[3], presumably only the β-fluoride (β-**2**) is effective in monomer synthesis, unless some α⇄β equilibration occurs during ring closure. Purification of 3 was effected by liquid chromatography and subsequent crystallization. The m.p. of the product and the ^{1}H- and ^{13}C-n.m.r. spectra corresponded to those of the earlier preparation and was improved. Polymerizations under a variety of conditions and with a number of catalyst systems are summarized in Table I. The polymers produced were white powders of relatively low intrinsic viscosity, and molecular weights probably $<11{,}000$. Optical rotation was used as a criterion of stereoregularity for screening, with large negative values indicating high proportions of β linkages. Generally, the rate of polymerization was low. Cationic polymerization at low temperatures, using phosphorus pentafluoride (PF_5) as catalyst, gave the most useful results, yielding a predominantly β-linked polymer having the highest molecular weight and at the highest percent conversion. Cationic polymerizations at temperatures from 0 to −40° with boron trifluoride etherate, antimony pentachloride, and phosphorus pentafluoride gave much-less stereoregular polymers of lower molecular weight in lower yields. These results suggest the presence of carbonium-ion rather than trialkyloxonium-ion intermediates, and enhanced termination-rates, as have been proposed for other polymerizations of anhydro sugars performed under similar circumstances[3]. Catalysis with phenylmagnesium bromide and phenylmagnesium bromide complexed with L-(−)-sparteine gave unpromising yields and products.

The best conditions found for these cationic polymerizations are somewhat different from those most effective for 1,6-anhydro sugar derivatives[3], with higher phosphorus pentafluoride and lower monomer concentrations. Further experimenta-

TABLE I

POLYMERIZATION OF 1,2-ANHYDRO-3,4,6-TRI-*O*-BENZYL-α-D-GLUCOPYRANOSE[a]

Polymer expt. number	*Catalyst*	*Mole % to monomer*	*Monomer to solvent Ratio g/100 mL*	*Temp (degrees)*	*Time (h)*	*Yield (%)*	$[\eta]_{CHCl_3}^{25}$ *dL/g*	$[\alpha]_D$[b] *(deg.)*	M_w[c]
1	PF_5	2	42.3	−78	15	6		−14.7	10,700
2	PF_5	2	20.3	−60	4	9		−15.5	
3	PF_5	5	11.4	−60	4	34	0.08	−21.3	10,000
4	PF_5	5	13.0	−60	5	53	0.08	−21.1	10,200
5	PF_5	5	12.9	−60	6	60		−23.7	10,700
6	PF_5	5	14.9	−40	3.75	25	0.11	+15.2	5,900
7	$SbCl_5$	3	15.0	−40	5	<1%		+40±15	1,500
				0	24				
8	$BF_3 \cdot O(Et)_2$	10	10.5	0	47.5	10		+43.5	1,500
9	C_6H_5MgBr	2	10.7	RT	70.0	2		+24.8	1,500
10	C_6H_5MgBr[d]	2	10.5	RT	240	trace			

[a]Polymerizations 1–8 performed in dichloromethane, polymerizations 9 and 10 in benzene. [b]At room temperature in chloroform. [c]Approximated by gel-permeation chromatographic analysis. [d]Sparteine (1.2 mol) added to 1.0 mol of PhMgBr.

tion is required to establish that these conditions are indeed optimal. The rate of polymerization at −60° is rather low (Table I). After 4 h, conversion is at only 34% and 6 h are required for it to reach 60%. During this period, there is little change in molecular weight. Although the rate of propagation in this system appears low, initiation is thus probably rate-determining.

The molecular-weight distribution of the low-temperature polymers, as estimated by gel-permeation chromatography, were approximately bell-shaped and ranged from a high molecular weight of 100,000 (d.p. ~230) to a low molecular weight of 3,000 (d.p. ~6). The distributions were not perfectly symmetrical; the curves fell off in the lower-molecular-weight range faster than they rose in the higher-molecular-weight range. These results are similar to those obtained for the polymerization of 1,2-anhydro-3,4,6-tri-*O*-benzyl-β-D-mannopyranose by cationic catalysis. Other products (6–10, Table I) were more complex in molecular-weight distribution.

In order to understand more fully the structure of the polymeric (1→2)-D-glucopyranans prepared, a typical PF_5-catalyzed, low-temperature polymer (P-5, Table I) was debenzylated with sodium in liquid ammonia. The debenzylated polymer was purified by dialysis. Salts and material of low molecular weight passed through a UM2 filter (nominal rating of 1,000), but polymer was collected. Approximately 20% of the debenzylated polymer passed through the filter; product having d.p. >5 was thus collected. The polymer was isolated by freeze-drying as a light-yellow powder with a specific rotation of +14.7°.

The specific rotation to be expected of a pure β-linked (1→2)-D-glucopyranan of this size is somewhat difficult to estimate. The values for oligomers from dimer to hexamer have been found to decrease regularly from +15 to +5. The usual extrapolation (trimer to hexamer values) against 1/d.p. would give a predicted specific rotation of $[\alpha]_D = -2 \pm 1°$ for a high polymer. However, a (1→2)-β-D-glucopyranan[6] from *Agrobacterium tumefaciens IIBV7* of d.p. ~80 and with at least 8% of branches has been found to have a specific rotation of −12°. The branching sites or side chains

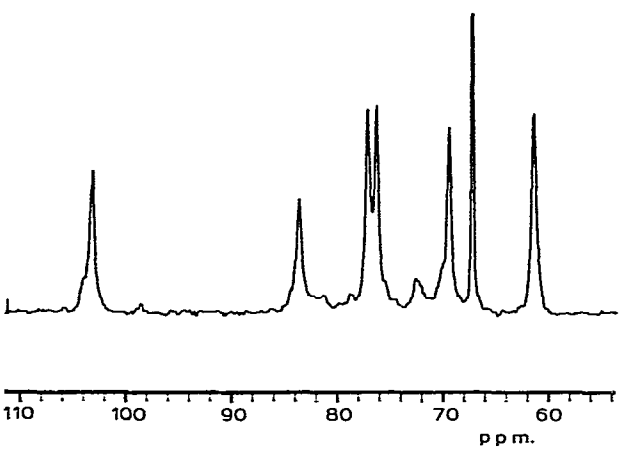


Fig. 1. The ^{13}C-n.m.r. spectrum of synthetic (1→2)-D-glucopyranan, $[\alpha]_D$ +14.7°.

may, however, contribute a greater negative rotation to this polymer. As the specific rotation of 2-*O*-(α-D-glucopyranosyl)-α-D-glucose (α-kojibiose)[7] is +162°, a small content of α linkages should produce a large effect on specific rotation. It thus appears probable that the synthetic glucan is ~90% β-linked.

The ^{13}C-n.m.r. spectrum (Fig. 1) is consistent with this estimate. It consists of six major peaks that correspond to those found by Gorin for (1→2)-β-D-glucopyranans isolated from natural sources. A small peak 4.5 p.p.m. upfield from the main anomeric peak (δ 103.1) lies in the region expected for an α-anomeric carbon atom in the chain, and corresponds in size to ~10% of the main anomeric peak. Minor peaks, presumably due to the presence of some α linkages, are also evident in the region of the spectrum covered by non-anomeric carbon atoms. No extraneous peaks arising from aromatic residues or fortuitous carbonyl groups were observed. No evidence of a free reducing or non-reducing end group was apparent. As the weight-average degree of polymerization of the perbenzylated polysaccharide was 23–26, the degradation during debenzylation apparently was not very severe.

The stereoselectivity of the cationic polymerization of 3 appears to be somewhat greater than that of 1,2-anhydro-3,4,6-tri-*O*-benzyl-β-D-mannopyranose[5], but further improvement in steric control, or enzymic purification of product, is necessary to obtain a pure (1→2)-β-D-glucan for physiological studies.

EXPERIMENTAL

General methods. — ^{1}H-N.m.r. spectra were determined with a Varian A-60-A spectrometer with solutions in chloroform-*d*, with tetramethylsilane (Me_4Si) as the internal standard, or in D_2O with acetone or 1,4-dioxane as the internal standard. ^{13}C-N.m.r. spectra were determined with a Varian XL-100-15 spectrometer in the pulsed Fourier-transform–proton-noise-decoupled mode on solutions in chloroform-*d* with Me_4Si as the internal standard, or in D_2O with 1,4-dioxane as the internal standard.

Melting points, viscosities, and optical rotations were determined and chromatography performed as described previously[5,8]. Molecular-weight distributions were analyzed by high-pressure gel-permeation chromatography. A Waters model 200 chromatograph equipped with a u.v.-detector (fixed at 245 nm), a pump model 6000, and a Valvco septumless injector (0.5 mL) was used. Six 30-cm-long stainless-steel columns packed with micro Styragel (2×10^6, 1×10^5, 1×10^4, 1×10^3, and $1 \times$ 500 Å) were used as packing. The flow rate was 2.0 mL/min. Calibration curves were made by using standard samples of polystyrene (Waters).

*Modified synthesis of 1,2-anhydro-3,4,6-tri-*O*-benzyl-α-D-glucopyranose* (3). — 3,4,6-Tri-*O*-benzyl-α-D-glucopyranosyl chloride[4] (5.2 g) and silver fluoride (Alfa Division, Ventron, 2.1 g, 1.5 eq) with a magnetic stirring rod were placed in two separate round-bottomed flasks (50 mL), and the flasks connected to a vacuum line through a Y-tube with stopcock. The solids were dried under high vacuum and acetonitrile (15 mL, dried over calcium hydride) was distilled into each flask. The

apparatus was sealed and removed from the vacuum line, and the solutions were combined and stirred in the dark for 24 h. The apparatus was reattached to the vacuum line and acetonitrile was distilled off at low temperature and replaced by oxolane (distilled and stored over calcium hydride) (25 mL). The mixture was filtered into a thoroughly dried, round-bottomed flask containing a stirring bar. Sodium hydride (Alfa Division, Ventron, 1.6 g, 6 eq.) as a dispersion in oil was rinsed with pentane and added to the filtrate. The flask was evacuated and the mixture stirred for 24 h. The solvent was removed under vacuum and benzene added to the solid residue. Undissolved material was separated by centrifugation and the supernatant solution was chromatographed in small batches by elution with benzene on poly(vinyl acetate)[9] (Fractogel PVA 6000, EM Laboratories, Inc.). Compound 3 was collected in the appropriate fractions, isolated by freeze-drying, and recrystallized from ether–petroleum ether without any special apparatus to exclude moisture[4] (yield 2.7 g); m.p. 82.0–82.5°, $[\alpha]_D$ +31.1° (*c* 0.5, chloroform, room temp.); lit.[4] m.p. 77–78°, $[\alpha]_D^{25}$ +31.1° (*c* 0.5, chloroform); ^{1}H-n.m.r.: δ 7.50–7.18 (m, 15 H, aromatic H), 4.98 (d of d, 1 H, $J_{1,2}$ 2.5 Hz, H-1), 4.88–3.45 (m, 11 H), 3.05 (d, 1 H, H-2). ^{13}C-n.m.r.: δ 138.5, 138.3, 137.8, 128.5, 127.9 (aromatic); 79.1; 77.5, 75.0, 74.4, 73.6, 72.3, 69.6, 68.4, and 52.5 (C-2).

Anal. Calc. for $C_{27}H_{28}O_5$: C, 74.98; H, 6.53. Found: C, 75.07; H, 6.61.

*Polymerization of 1,2-anhydro-3,4,6-tri-*O*-benzyl-*D*-glucopyranose* (3). — The transfer of the catalysts and solvents, and all the polymerizations, were performed under high-vacuum conditions as reported for related preparations[5,8]. Solvents were dried over calcium hydride before transfer. The cationic polymerizations were terminated at the polymerization temperature by adding cold methanol. Reprecipitation into petroleum ether was repeated twice. The anionic-polymerization solutions were washed with water several times, dried with anhydrous magnesium sulfate, concentrated, and poured into petroleum ether.

All of the polymers were freeze-dried from benzene, and the supernatant solutions from precipitations were evaporated and further dried under vacuum for spectral analysis.

*Preparation of (1→2)-*D*-glucopyranan.* — Polymer 5 (Table I) (0.5 g) was dissolved in toluene and the solution stirred with liquid ammonia as described for related debenzylations. Freshly cut sodium metal (1.7 eq) was added slowly. After the characteristic blue color had remained for 1 h, ammonium chloride was added to decolorize the solution. The ammonia was removed in a stream of nitrogen, and the residue dissolved in water. The water solution was extracted with toluene, concentrated *in vacuo* to 5 mL, and dialyzed until ion-free with a UM-2 membrane (Diaflo Ultrafilter, Amicon Co., nominal rating 1000). The glucan was isolated by freeze-drying from distilled water; yield 0.17 g (80%) $[\alpha]_D^{25}$ +14.7° (*c* 0.3, water); ^{13}C-n.m.r.: δ 103.9, 103.1 (β-C-1), 98.6 (α-C-1), 83.7 (C-2), 77.3 (C-5), 76.4 (C-3), 72.8 (α-C-8), 67.4 (1,4-dioxane), 69.6 (C-4), and 61.6 (C-6).

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