SYNTHESIS OF A BRANCHED GLUCAN FROM CELLULOSE

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ABSTRACT

D-Glucosylation of cellulose acetate (DS_{Ac} 2.2) with 3,4,6-tri-*O*-acetyl-α-D-glucopyranose 1,2-(*tert*-butyl orthoacetate), followed by saponification and reduction with sodium borohydride, gave a water-soluble glucan. Periodate oxidation and partial acetolysis data revealed that the polysaccharide contained a main chain of the cellulose type, branched (at approximately each second residue) with β-D-glucopyranosyl residues mainly at the secondary hydroxyl groups of chain D-glucose residues. The predominant D-glucosylation of secondary hydroxyl groups was explained by inter-monomeric acetyl migration in the cellulose derivative during the reaction process.

RESULTS AND DISCUSSION

Polymerization[1–4] or polycondensation[5,6] of sugar orthoesters under glycosylation conditions[7] have been successfully employed in our laboratory for the synthesis of polysaccharides of predetermined structure. On the basis of the experience thereby gained, the partial synthesis of polysaccharides by glycosylation of synthetic or natural polysaccharide derivatives seemed feasible. We now describe this approach in the synthesis of a branched glucan from cellulose.

Cellulose acetate having a degree of substitution (*DS*) of 2.2 was used as starting material. It was obtained by the known[8,9], two-step, partial hydrolysis of commercial cellulose triacetate with acid. According to the literature data[8,9], the resulting diacetate contains free primary and secondary hydroxyl groups in the ratio *ca.* 1:1. We have recently demonstrated[10,11] that glycosylation of both primary and secondary hydroxyl groups can be most successfully effected by treatment with sugar *tert*-butyl orthoacetates in boiling chlorobenzene in the presence of a catalytic amount of 2,6-dimethylpyridinium perchlorate. Such conditions were used for the glycosylation of cellulose diacetate by 3,4,6-tri-*O*-acetyl-α-D-glucopyranose 1,2-(*tert*-butyl orthoacetate) (**1**).

The starting cellulose acetate was insoluble in boiling chlorobenzene, but the resulting gel gradually dissolved as the glycosylation by orthoester **1** proceeded. The polysaccharide product was subjected to alkaline deacetylation with simultaneous borohydride reduction to give a water-soluble glucan.

The structure of the synthetic glucan (*S*-3) was deduced from the following data. Total hydrolysis with acid afforded glucose. Partial hydrolysis with acid was accompanied by the formation of a precipitate, which apparently consisted of cellulose and cellulose derivatives of low *DS*, while the water-soluble portion of the hydrolysate contained only glucose (paper chromatography). Acetolysis of *S*-3, with subsequent saponification of the products, gave cellobiose and laminaribiose (identified by paper chromatography) and, possibly, sophorose; gentiobiose was not detected.

Oxidation of glucan *S*-3 in unbuffered solution with 10mM sodium metaperiodate proceeded slowly, with concomitant over-oxidation. The highly branched character of the polysaccharide was indicated by the high yield of formic acid (see Table I). The considerable proportion of unoxidized glucose indicates that secondary hydroxyl groups were preponderantly glycosylated.

TABLE I

PERIODATE OXIDATION[a] OF GLUCAN *S*-3

Time (*h*)	*Periodate reduction*	*Formic acid liberation*	*Formaldehyde liberation*	*Unoxidized* D-*glucose* (%)[b]
	(*moles per "anhydro-glucose" unit*)			
0.5	0.16	0.07	0.020	—
1	0.50	0.14	0.030	—
3	0.63	0.19	0.035	—
6	0.72	0.24	0.035	—
19	0.74	0.31	0.035	—
22	0.76	0.31	0.040	—
28	0.90	0.33	0.040	—
44	1.02	0.35	0.042	—
128	1.22	0.37	0.042	35
142	1.20	0.39	—	32

[a]With unbuffered, 10mM sodium metaperiodate at 20°; [b]determination by the method given in Ref. 12.

The D-glucitol residue at the reduced end of the polysaccharide chain can undergo periodate oxidation with formation of one or two moles of formaldehyde, depending on the presence and position of substituents and on the course of oxidation. The liberation of formaldehyde in the initial stages of the oxidation (35 mmoles per "anhydro-glucose" unit within the first 6 hours, see Table I) was associated with the oxidation of D-glucitol residues due to rapid scission of primary–secondary glycol groups. Thus, the number-average degree of polymerization ($\overline{DP}_n$) of glucan *S*-3 is between 30 and 60 units. From the amount of periodate-resistant D-glucose, glucan *S*-3 contains 35 branch points (substituted at the secondary hydroxyl groups) and 30 unsubstituted (1→4)-linked D-glucose residues per 100 "anhydro-glucose" units. Periodate oxidation of a polysaccharide having this structure should produce 0.35 mole of formic acid upon reduction of 1.0 mole of oxidant per "anhydro-glucose" unit. This is in good agreement with the observed values (44 h, 1.02 mole of oxidant reduced,

0.35 mole of formic acid liberated, see Table I). More-prolonged treatment caused a slow increase in the consumption of periodate and liberation of formic acid, possibly by over-oxidation or because of the presence of a few branch points at position 6. If all of the excess of periodate reduced was due to the latter feature, four such branches (calculated from the yield of formic acid at the end of oxidation) per 100 "anhydro-glucose" units would be present. Apparently, the proportion of (1→6)-linkages is even less.

Thus, the synthetic glucan *S*-3 has a $\overline{DP}_n$ of not less than 30–35 and an average branching-number of 0.5 per "anhydro-glucose" unit of the main chain, 90–100% of the branch β-D-glucopyranose residues being attached to the secondary hydroxyl groups of the D-glucose residues in the cellulose chain.

The preferred glycosylation of secondary hydroxyl groups in the cellulose derivative is possibly due to acetyl migration during the reaction, resulting in the blocking of primary hydroxyl groups. Under similar reaction conditions, we have observed acetyl migration when 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose is subjected to glycosylation[11]. In the case of cellulose diacetate, intramonomeric migration seems unlikely from stereochemical considerations, but migration could occur between neighbouring D-glucose residues.

This new approach to polysaccharide synthesis can be used for more-selective substitution if non-migrating, protecting groups are employed.

EXPERIMENTAL

Cellulose diacetate (DS_{Ac} 2.2). — This material was obtained by a two-stage deacetylation[8,9] of commercial cellulose triacetate. The latter polymer contained 62% of bound acetic acid, and its specific viscosity was 0.46, corresponding to $DP \sim 100$. The partially saponified cellulose acetate contained[13] 37% of acetate, corresponding to DS_{Ac} 2.2.

Glycosylation of cellulose diacetate. — Cellulose diacetate (0.24 g) was dissolved in absolute acetone (8 ml), and the acetone was gradually replaced by addition of absolute chlorobenzene[10,11] and distillation. Chlorobenzene was distilled off to adjust the final volume to 7 ml (the mixture was a gel under liquid). The ortho-ester[10,11] **1** (1.00 mmole, 0.405 g) and 2,6-dimethylpyridinium perchlorate[10,11] (0.01 mmole) were then added. A solution of 3.00 mmoles of orthoester **1** in 150 ml of absolute chlorobenzene was added dropwise to the boiling reaction mixture during 1.5 h, with simultaneous distillation of the solvent at a rate that maintained constant volume of the mixture (the gel dissolved during this period). The polysaccharide derivative was precipitated by addition of methanol (200 ml), filtered off, washed with methanol, and treated with 0.25M sodium hydroxide (35 ml) containing sodium borohydride (50 mg) for 48 h at 20°. The homogeneous solution was acidified with acetic acid, treated with cation-exchange resin QU-2 (H^+), and evaporated to dryness *in vacuo* at 30–35°. Repeated distillation of methanol from the residue removed boric acid, and the residue was then dissolved in water (30 ml). Ethanol

(150 ml) was added, and the resulting precipitate was filtered off and washed with ethanol and ether to yield 150 mg of polysaccharide *S*-3, $[\alpha]_D$ 0.0° (*c* 2.0, water) −1.80° (*c* 2.5, 3M sodium hydroxide), which showed no i.r. carbonyl absorption.

Periodate oxidation. — The sample of polysaccharide was dissolved in water and the concentration was determined by the method described in Ref. 14. A solution containing 24.4 mg of polysaccharide and 1.00 mmole of sodium metaperiodate per 100 ml was stored at 20° in darkness. Oxidant consumption was determined by a modified procedure[15]. Titration with mM sodium hydroxide, using a mixture of 0.1% Bromocresol Purple and 0.1% Bromothymol Blue[16], was used for the determination of formic acid. Formaldehyde[17] and unoxidized D-glucose[12] were determined by literature methods. The results are presented at Table I.

Partial hydrolysis. — A solution of polysaccharide *S*-3 (5 mg) in 1.5 ml of 50mM sulphuric acid was heated in a sealed tube for 16 h at 100° (precipitate formation occurred after 5–6 h). The reaction mixture was neutralized with Amberlite IRA-410 (HCO_3^-) resin, and the resin and the precipitate were filtered off. Examination of the filtrate by paper chromatography, using butyl alcohol–pyridine–water (6:4:3), revealed glucose only.

Acetolysis. — The polysaccharide (5 mg) was subjected to acetolysis[18], and the products were saponified and analysed by paper chromatography in the solvent system mentioned above. Cellobiose, laminaribiose, and, possibly, sophorose were detected. Gentiobiose could not be detected.

REFERENCES

1 A. F. Bochkov, I. G. Yazlovetsky, and N. K. Kochetkov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1968) 1812.
2 N. K. Kochetkov, A. F. Bochkov, and I. G. Yazlovetsky, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1968) 1818.
3 N. K. Kochetkov, A. F. Bochkov, and I. G. Yazlovetsky, *Carbohyd. Res.*, 9 (1969) 49.
4 N. K. Kochetkov and A. F. Bochkov, *Carbohyd. Res.*, 9 (1969) 61.
5 N. K. Kochetkov, A. F. Bochkov, I. G. Yazlovetsky, and V. I. Snyatkova, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1968) 1802.
6 N. K. Kochetkov, A. F. Bochkov, and I. G. Yazlovetsky, *Carbohyd. Res.*, 5 (1967) 243.
7 N. K. Kochetkov, A. J. Khorlin, and A. F. Bochkov, *Tetrahedron*, 23 (1967) 693.
8 L. J. Tanghe, L. B. Genung, and J. W. Mench, *Methods Carbohyd. Chem.*, 3 (1963) 198.
9 C. J. Malm, L. J. Tanghe, B. C. Laird, and C. D. Smith, *J. Amer. Chem. Soc.*, 74 (1952) 4105.
10 N. K. Kochetkov, A. F. Bochkov, and T. A. Sokolovskaya, *Dokl. Akad. Nauk SSSR*, 187 (1969) 96.
11 N. K. Kochetkov, A. F. Bochkov, T. A. Sokolovskaya, and V. I. Snyatkova, *Carbohyd. Res.*, 16 (1971) 17.
12 G. W. Hay, B. A. Lewis, and F. Smith, *Methods Carbohyd. Chem.*, 5 (1965) 381.
13 L. J. Tanghe, L. B. Genung, and J. W. Mench, *Methods Carbohyd. Chem.*, 3 (1963) 201.
14 M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350.
15 L. M. Likhosherstov and L. E. Brossar, *Khim. Prir. Soedin.*, (1967) 7.
16 F. Brown, T. G. Halsall, E. L. Hirst, and J. K. N. Jones, *J. Chem. Soc.*, (1948) 27.
17 V. E. Vaskovsky and S. V. Isay, *Anal. Biochem.*, 30 (1969) 25.
18 M. L. Wolfrom and A. Thompson, *Methods Carbohyd. Chem.*, 3 (1963) 143.