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ATTACHMENT OF (1→6)-LINKED D-GLUCOSE SIDE-CHAINS TO AMYLOSE AND CELLULOSE *via* 1,2-ORTHOESTERS*

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ABSTRACT

Comb-like derivatives of amylose and cellulose having (1→6)-linked D-glucose side-chains were prepared by condensation of 1,2-(*tert*-butyl orthoacetate) and 1,2-(ethyl orthoacetate) derivatives of 3,4,6-tri-*O*-acetyl-α-D-glucopyranose with 2,3-di-*O*-phenylcarbamoyl derivatives of amylose and cellulose in boiling chlorobenzene in the presence of catalytic amounts of 2,6-lutidinium perchlorate. The more-stable ethyl orthoacetate yielded a higher and more reproducible degree-of-branching than the *tert*-butyl orthoacetate. The distance between the branch points in the products was 2–3 D-glucose residues when 1 mol. of orthoester was used for each hydroxyl group in the polysaccharide. Little degradation of the backbone occurred, as shown by intrinsic viscosities and molecular weights determined by light-scattering measurements. Degradation increased with increasing molar ratio of orthoester. The branches were attached mainly (1→6)-β with a small proportion of (1→6)-α linkages as indicated by n.m.r. and i.r. spectroscopy.

INTRODUCTION

As part of our programme of synthesis of simple models of naturally occurring branched-polysaccharides, comb-like derivatives of amylose and cellulose have been prepared with D-glucose and malto-oligosaccharide side-chains[1,2]. The latter side-chains may be elongated in a controlled manner by phosphorolytic synthesis. Thus, the influence of short and long branches on the solution and solid-state properties of branched polysaccharides can be investigated.

In our preceding studies[1,2], the methods devised by Bredereck[3,4] and by Helferich[5,6] were employed for the coupling of acetobromo sugars to position 6 of D-glucose residues in appropriately substituted derivatives of amylose and cellulose. Although the Bredereck and Helferich reactions are equally efficient for introducing D-glucose side-chains, the attachment of malto-oligomers was not very successful,

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*Chemical Synthesis of Branched Polysaccharides: Part III[1,2].

especially with the Helferich reaction, and considerable degradation occurred when the Bredereck reaction was used.

We have therefore investigated the scope of the orthoester method, which was first demonstrated for the synthesis of branched polysaccharides by Kochetkov *et al.*[7]. Randomly substituted cellulose diacetate was glycosylated with 3,4,6-tri-*O*-acetyl-α-D-glucopyranose 1,2-(*tert*-butyl orthoacetate), when D-glucose branches were attached mainly to secondary positions as a result of acetyl migration during the reaction.

RESULTS AND DISCUSSION

The orthoester method of glycosylation has been extensively studied[8]. It generally leads to 1,2-*trans*-glycosides, and β-D-glycosidic bonds are formed preferentially[9-14]. The reaction mechanism is believed to involve an intermediate acyloxonium cation[11]. Boiling chlorobenzene, together with a catalytic amount of 2,6-dimethylpyridinium (2,6-lutidinium) perchlorate, proved to be the most effective reaction medium in disaccharide syntheses[9,14]. The orthoesters were prepared according to the convenient method described by Kochetkov *et al.*[14], involving tetra-*O*-acetyl-α-D-glucopyranosyl bromide, an alcohol, and 2,6-lutidine.

The 3,4,6-tri-*O*-acetyl-α-D-glucopyranose 1,2-orthoacetates were reacted with the 2,3-di-*O*-phenylcarbamoyl derivatives of amylose and cellulose. The urethane blocking-groups are stable during the condensation reaction and do not migrate. Since the polymer derivatives are insoluble in chlorobenzene, the reaction was heterogeneous. The use of a mixture of *p*-dioxane and chlorobenzene gave unsatisfactory results with respect to yield and branching efficiency. 2,6-Lutidinium perchlorate was always added last to the boiling suspension of the reactants.

Thus, 2,3-di-*O*-phenylcarbamoylamylose was condensed variously with 3,4,6-tri-*O*-acetyl-α-D-glucopyranose 1,2-(*tert*-butyl orthoacetate)[7,9]. The results are shown in Table I (*OAG-I, 1–4*), which also includes data for products obtained previously with acetobromoglucose and the methods of Bredereck (*BAG*) and Helferich (*HAG*).

TABLE I

PRODUCTS OF GLYCOSYLATION OF 2,3-DI-*O*-PHENYLCARBAMOYLAMYLOSE WITH 3,4,6-TRI-*O*-ACETYL-α-D-GLUCOPYRANOSE 1,2-(*tert*-BUTYL ORTHOACETATE)

Compound	*Molar ratio of reactants*	*N* (%)	*z*[a]	*1/z*[a]	*Yield* (%)
OAG-I-1	1:0.5	6.33	0.198	9.2	90.0
OAG-I-2	1:1	6.12	0.175	5.7	66.5
OAG-I-3	1:2	5.74	0.312	3.2	68.0
OAG-I-4	1:3	5.06	0.476	2.1	72.0
BAG-2	1:1.2	5.15	0.435	2.3	68.0
HAG-1	1:1	5.57	0.312	3.2	
HAG-3	1:2	5.17	0.435	2.3	

[a] The branch frequency *z* is the mean number of branches per main-chain residue; 1/*z* denotes the average number of main-chain residues separating the branches.

The branch frequency z, obtained from the nitrogen content, shows that the extent of branching increases with increase in the molar amount of the orthoester. Comparison of samples *OAG-I-2*, *BAG-2*, and *HAG-1* indicates that the orthoester reaction yields a lower branching frequency than do the previous methods, presumably due to the instability of the *tert*-butyl orthoester[9,15]. This supposition was supported by n.m.r. spectroscopy; the relative intensity of the peaks characteristic of orthoesters[16] varied from one preparation to the next.

Further experiments were therefore carried out with the more stable 1,2-(ethyl orthoacetate)[9] by using similar conditions, but extending the reaction time to 40 min to compensate for the lower reactivity.

Table II records data for the products (*OAG-II* and *OCG*, respectively) prepared by reaction of the 2,3-bis(phenylcarbamates) of amylose and cellulose with 3,4,6-tri-*O*-acetyl-α-D-glucopyranose 1,2-(ethyl orthoacetate).

TABLE II

PRODUCTS OF GLYCOSYLATION OF 2,3-DI-*O*-PHENYLCARBAMOYL DERIVATIVES OF AMYLOSE AND CELLULOSE WITH 3,4,6-TRI-*O*-ACETYL-α-D-GLUCOPYRANOSE 1,2-(ETHYL ORTHOACETATE)

Compound	*Molar ratio of reactants*	*N* (%)	*1/z*	*Yield* (%)
OAG-II-1	1:1	4.87	1.88	89.0
OAG-II-2	1:1	5.17	2.36	96.0
OAG-II-3	1:1	5.09	2.20	72.5
OAG-II-4	1:1	5.70	3.62	60.0
OAG-II-5	1:1	5.58	3.24	
OAG-II-6	1:1	5.60	3.30	95.0
OAG-II-7	1:0.5	6.51	10.80	78.0
OAG-II-8	1:1	5.02	2.07	91.0
OAG-II-9	1:2	4.39	1.39	
OAG-II-10	1:3	4.21	1.25	96.0
OCG-1	1:1	5.28	2.50	69.0
OCG-2	1:1	5.68	3.54	90.0
OCG-3	1:1	5.64	3.40	93.0

With a 1:1 molar ratio of reactants, the products (*OAG-II-1–OAG-II-6*) were as highly branched as those (*BAG-2* and *HAG-1*) previously obtained by the Bredereck and Helferich methods. The results demonstrate the reproducibility of the procedure and confirm the greater stability of the ethyl orthoester. The orthoester method is much more efficient than the formerly applied syntheses when applied to 2,3-di-*O*-phenylcarbamoylcellulose, for which the branch density obtained was only half of that for the corresponding amylose derivative.

That the branch period is dependent upon the molar ratio of the reactants is seen from the products *OAG-II-7–OAG-II-10*, which were prepared with the same sample of orthoester. As the molar ratio of ethyl orthoester was increased, the products (*OAG-II-9/10*) became increasingly soluble in chlorobenzene during the reaction, possibly due to degradation of the main chain. The influence of the excess of orthoester on the degree of polymerization (d.p.) was therefore studied by viscosity and light-scattering measurements on the saponified and recarbanilated products. The data in Table III show that an increase in the molar excess of orthoester parallels a decrease of the viscosity. This trend could be caused by the more numerous D-glucose side-chains and/or degradation of the amylose main-chain. From the weight-average molecular weight (M_w) measured by light scattering, the d.p. of the amylose backbone can be calculated by using the $1/z$ values obtained by nitrogen determination. The data given in the last column in Table III show that some degradation occurs, and this is more pronounced when a two- or three-fold excess of orthoester is present. Excess of acyloxonium cations formed from the orthoester are probably responsible for the cleavage of (1→4)-α-glycosidic bonds. The results of the experiments with a 1:1 molar ratio of reactants compare favourably with previous results[2] with the Bredereck reaction, for which the amylose was degraded to a d.p. of ~150. Essentially no degradation was found when the same substrate was branched under the mild conditions of the Helferich reaction (*HAG-9*).

Saponification of the products in Table II with sodium methoxide gave water-soluble amylose and cellulose derivatives having D-glucose side-chains. The amylose derivatives were not attacked by beta-amylase, indicating that the branch density is high enough to hinder the exo-enzyme. 2,3-Di-*O*-phenylcarbamoylamylose exposed to the condensation conditions but without orthoester, followed by saponification, gave a product that was fully degraded to maltose by beta-amylase.

The iodine-binding characteristics of some of the modified amyloses (*OAG-II-1*–*OAG-II-3*) were examined by potentiometric titration and absorption spectroscopy. The data are summarized in Table IV. Synthetic amylose of d.p. 2000 was used as reference.

The very low iodine-binding capacity and the position of the absorption maxima (λ_{max}) are in accordance with a highly branched structure for the products *OAG-II-1*–*OAG-II-3*.

The values of $1/z$ obtained from nitrogen determinations were substantiated by methylation analysis for *OAG-II-1*, *OAG-II-3*, *OAG-II-6*, and *OCG-3*. The results are shown in Table V and are in accordance with the data in Table II.

The configuration of the (1→6)-linkages present in the comb-like derivatives of amylose and cellulose were determined by n.m.r. spectroscopy ($CDCl_3$, 220 MHz) of the methylated products *OAG-II-1*, *OAG-II-3*, *OAG-II-6*, and *OCG-3*. The results are shown in Table VI, together with some reference data.

The signals at δ 5.51, 5.45, and 5.55 for the *OAG* products are typical for (1→4)-α-D-glycosidic linkages, whereas the peaks at δ ~5.05 show that (1→6)-α-D-glycosidic linkages (H-1*eq*) are also present. Clearly more pronounced, however, was a peak in the range δ 4.3–4.6, indicative of (1→6)-β-D-glycosidic linkages. In the spectrum of the branched derivative of cellulose, only one peak near δ 4.3 was observed. As expected from the reference data, no separate peaks for (1→4)-β and (1→6)-β linkages could be seen for *OCG-3*. A small proportion of (1→6)-α linkages was present, as shown by a small peak at δ 4.95.

TABLE III

VISCOSITY AND LIGHT-SCATTERING MEASUREMENTS ON SOLUTIONS OF THE TRICARBANILATED, BRANCHED PRODUCTS IN *p*-DIOXANE

Compound	*Molar ratio of reactants*	*N* (%)	*1/z*	[η] (*ml/g*)	M_w (10^{-3})	*d.p.*$_w$	*d.p. Main-chain*
				Branched molecules			
Substrate		7.00		136.0	410	790	790
OAG-II-7	1:0.5	6.51	10.80	115.8	385	742	679
OAG-II-6	1:1	5.60	3.30	101.0	395	761	584
OAG-II-8	1:1	5.02	2.07	102.3	454	875	590
OAG-II-9	1:2	4.39	1.39	64.8	393	653	380
OAG-II-10	1:3	4.21	1.25	60.6	300	578	321
HAG-9	1:1	5.46	2.92	135.0	545	1050	780

TABLE IV

IODINE-BINDING PROPERTIES OF THE COMB-LIKE MOLECULES DERIVED FROM AMYLOSE

Compound	*Iodine bound* (*mg/10 mg*)	*Iodine binding* (%)	λ_{max} (*nm*)
Amylose	2.01	100	630
OAG-II-1	0.18	8.5	555
OAG-II-2	0.17	7.6	551
OAG-II-3	0.15	7.0	557

TABLE V

DEGREE OF BRANCHING AS DETERMINED BY METHYLATION ANALYSIS

Compound	*Product of hydrolysis*[a]	T *value*[b]	*1/z from tetra-O-methylglucose*	*1/z from di-O-methylglucose*
OAG-II-1	2,3,4,6	1.0 (1.0)	2.5	2.3
	2,3,4	2.52 (2.49)		
	2,3	5.40 (5.39)		
OAG-II-3	2,3,4,6	1.0 (1.0)	3.0	2.4
	2,3,4	2.40 (2.39)		
	2,3	5.40 (5.39)		
OAG-II-6	2,3,4,6	1.0 (1.0)	3.2	2.9
	2,3,4	2.50 (2.49)		
	2,3	5.40 (5.39)		
OCG-3	2,3,4,6	1.0 (1.0)	4.4	5.5
	2,3,4	2.64 (2.49)		
	2,3	5.40 (5.39)		

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[a] Figures indicate location of methyl groups in the glucose derivatives. [b] Figures in brackets taken from Ref. 17.

TABLE VI

CHEMICAL SHIFTS (δ P.P.M.) OF H-1 OF SOME PERMETHYLATED POLYSACCHARIDES

Parent polysaccharide	*Type of linkage*	*H-1*
Pullulan[18]	(1→4)-α	5.50
	(1→6)-α	5.07
Dextran[18]	(1→6)-α	5.08
Synthetic glucan[19]	(1→6)-α	5.05
Cellulose[20]	(1→4)-β	4.31
Gentiobiose[20]	(1→6)-β	4.29
Pustulan[19,21,22]	(1→6)-β	4.30–4.60
OAG-II-1	(1→4)-α	5.51
	(1→6)-α	5.03
	(1→6)-β	4.35
OAG-II-3	(1→4)-α	5.45
	(1→6)-α	5.07
	(1→6)-β	4.25
OAG-II-6	(1→4)-α	5.55
	(1→6)-α	5.05
	(1→6)-β	4.27
OCG-3	(1→4)-β	
	(1→6)-β	4.25
	(1→6)-α	4.95

The results were supported by the i.r. spectra recorded on the unsubstituted samples. *OCG* products gave a signal at 760 cm^{-1}, typical of α-D-glycosidic bonds. For the *OAG* products, the presence of (1→6)-β-D linkages was indicated by a shoulder at 900 cm^{-1}.

Although the orthoester reaction is believed to proceed stereospecifically through an intermediate acyloxonium cation, the presence of (1→6)-α-D linkages, even in minor amounts, requires a glycosyl cation as intermediate.

In contrast to our previous methods for introducing branches, the techniques described here permit the attachment of D-glucose side-chains to cellulose as readily as to amylose. Branches can be incorporated on every second or third main-chain residue with equimolar amounts of orthoester per polysaccharide hydroxyl-group. No significant degradation occurs under these conditions. The finding that the ethyl orthoacetate, which is more stable than the *tert*-butyl analogue and acetobromoglucose, is still relatively reactive indicates that it should be possible to attach higher malto-oligosaccharides.

EXPERIMENTAL

2,3-Di-*O*-phenylcarbamoyl derivatives of amylose and cellulose were prepared as described in Part I[1].

Anal. Calc. for $(C_{20}H_{20}N_2O_7)_n$: C, 60.0; H, 5.0; N, 7.0. Found: C, 58.1; H, 5.38; N, 7.0 (amylose); C, 60.2; H, 5.26; N, 7.0 (cellulose).

The 1,2-(ethyl orthoacetate) of 3,4,6-tri-*O*-acetyl-α-D-glucopyranose[13], the *tert*-butyl analogue[14], and 2,6-lutidinium perchlorate[14] were prepared by literature procedures. The methods used for saponification and methylation analysis have been described in Part I[1].

Viscosity measurements were carried out in an Ostwald viscosimeter at 20°, and light-scattering measurements were performed on solutions of the tricarbanilated products (300 mg) in *p*-dioxane (50 ml).

The procedures for the potentiometric titration with iodine and for measurement of absorption spectra are described in Part I[1].

N.m.r. spectra were obtained with a Varian 220-MHz spectrometer for 10% solutions in $CDCl_3$ (internal Me_4Si) at 60°. I.r. spectra were obtained with a Perkin–Elmer 125 grating spectrophotometer on potassium bromide discs (2 mg/200 mg of KBr).

Acid hydrolysis of orthoesters[13]. — A solution of the orthoacetate (100 mg) in chloroform (10 ml) was shaken with 2M HCl (1 ml) for 3 min. Anhydrous $MgSO_4$ (500 mg) was added, the mixture was filtered, the solution was concentrated to dryness, and a solution of the residue in $CDCl_3$ (0.5 ml) was subjected to n.m.r. spectroscopy.

Condensation reactions. — Rigorously anhydrous conditions were essential. Reactants and solvents were carefully dried before use.

To a suspension of the thoroughly powdered 2,3-di-*O*-phenylcarbamoyl derivative (2.5 g, 6.25 mmol) of amylose or cellulose in chlorobenzene (14 ml), 3,4,6-tri-*O*-acetyl-α-D-glucopyranose 1,2-(ethyl orthoacetate)[13] (2.45 g, 6.25 mmol) was added, and the suspension was distilled at atmospheric pressure. When 1–2 ml of the solvent had been distilled, 2,6-lutidinium perchlorate (0.005 mmol) was added and the mixture was boiled under reflux for 40 min. The suspension was cooled to 70–80° and poured into methanol (500 ml), and the product was washed thoroughly. The air-dried product was purified by precipitation from solution in *p*-dioxane with water and dried at 60° (yield, 2.3 g).

The degree-of-branching was calculated from the nitrogen content by using the expression:

$$z = \frac{28.100 - 400.\%\mathrm{N}}{\%\mathrm{N}\,(288n + 42)},$$

where $n = 1$ for products obtained from the glucose orthoester.

Tricarbanilation. — To a suspension of the polysaccharide (100 mg) in pyridine (5 ml) at 100°, phenyl isocyanate (0.75 ml) was added after 30 min. After stirring for 8 h, the mixture was poured into methanol, and the product was purified by precipitation from solution in *p*-dioxane with water and dried at 60° (yield, 300 mg).

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