Carbohydrate Research, 43 (1975) 151–161

SYNTHESIS OF COMB-LIKE DERIVATIVES OF AMYLOSE AND CELLULOSE HAVING (1→6)-LINKED D-GLUCOSE SIDE-CHAINS*

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(Received December 23rd, 1974; accepted for publication, February 17th, 1975)

ABSTRACT

D-Glucose side-chains were introduced into amylose and cellulose by condensation of tetra-*O*-acetyl-α-D-glucopyranosyl bromide with 6-trityl-2,3-dicarbanilate derivatives of the polysaccharides in nitromethane–*p*-dioxane in the presence of silver perchlorate (reaction *A*), and with the detritylated derivatives in acetonitrile–*p*-dioxane in the presence of mercuric cyanide and mercuric bromide (reaction *B*). The procedures were equally effective. The distance between the branch points in amylose was in the range 2–4 D-glucose residues, and 5–8 D-glucose residues in cellulose. Whereas considerable degradation of the backbone chain was found with reaction *A*, no degradation occurred in reaction *B* under suitable conditions. α-(1→6)-Links were preferably formed in reaction *B*, and β-(1→6)-links in reaction *A*.

INTRODUCTION

This paper is part of a series concerned with attempts to synthesize variously branched polysaccharides. Amongst the naturally occurring polysaccharides, there are two types of branched structures, namely, comb-like molecules which have a backbone carrying short side-chains, *e.g.*, dextrans, glucomannans, arabinogalactans, and xyloglucans, found as bacterial polysaccharides or as cell-wall constituents, and tree-like molecules having a more or less densely branched structure, *e.g.*, the reserve polysaccharides glycogen and amylopectin.

Apart from any possible biological function, very little is known about the effect of type of branching on the overall shape of the molecules in solution and in the solid state. Since the naturally occurring, branched polysaccharides either differ in their composition of sugars or in their type of branching, they cannot be compared directly. We have therefore prepared simpler substances which allow the different parameters to be varied independently, *i.e.*, lengths and frequency of branches, and monomer composition. Such polysaccharides could serve as model substances for comparative physico-chemical measurements.

*Chemical Synthesis of Branched Polysaccharides: Part I.

Husemann and Reinhardt[1,2] were the first to study the chemical synthesis of comb-like polysaccharides. They attached D-glucose, disaccharides, and maltose oligosaccharides as side chains to amylose by (1→6)-links, using the method of Bredereck[3,4] elaborated for disaccharide synthesis. Similar molecules having D-glucose side-chains were prepared by Schuerch *et al.*[5,6], using the cyclic acetal method. Thus, 4-*O*-α-D-glucopyranosyl-(1→6)-α-D-glucopyranan resulted from the cationic polymerization of fully benzylated 1,6-anhydromaltose, and 4-*O*-β-D-glucopyranosyl-(1→6)-α-D-glucopyranan from the corresponding 1,6-anhydrocellobiose derivative. In this way, highly stereoregulated products were obtained, but with low d.p. (14–32) after debenzylation. In another approach, Kochetkov *et al.*[7], using the ring-opening reaction[8] of cyclic orthoesters, glycosylated a partially acetylated cellulose with 3,4,6-tri-*O*-acetyl-α-D-glucopyranose 1,2-(*tert*-butyl orthoacetate) and attached β-D-glucopyranosyl units mainly at the secondary hydroxyl groups.

We now report on the synthesis of comb-like polysaccharides having (1→6)-linked D-glucose side-chains, which is based on previous work[1,2]. In order to investigate the influence of the backbone chain, amylose and cellulose, which differ markedly in their conformation, were used. Glycosylation was effected by the Bredereck method[3,4], which gives β-D links. The method used by Helferich and Zirner[9], which gives α-D links, was also studied.

RESULTS AND DISCUSSION

Bredereck's method[3,4] of glycoside synthesis involves the reaction of a tritylated sugar with an acetylated glycosyl halide in nitromethane in the presence of silver perchlorate. The reaction results in the exclusive formation[10–12] of β-glycosidic bonds, when equal molar proportions of the sugars and silver perchlorate are used, and is considered to involve an intermediate acyloxonium ion[13]. The participation of the 2-acyloxy group and the blocking of the positive charge by the perchlorate anion in a suitable solvent are mainly responsible for the stereospecificity of the reaction.

The modification of the Koenigs–Knorr reaction reported by Helferich *et al.*[9,14] involves the use of mercury salts as catalysts (cyanide, bromide) and acetonitrile as solvent. Acetonitrile was preferred to nitromethane as solvent, since better yields resulted and the formation of 1-cyano derivatives did not occur. The formation of α-glycosidic bonds by this procedure is noteworthy, since glycosides with *cis*-1,2-substituents are rarely obtained. The glycosyl cation is an intermediate in the reaction[13], and a mixture of α- and β-glycosides is therefore to be expected, as is usually found in Koenigs–Knorr reactions.

The glycosylation of the polysaccharides with tetra-*O*-acetyl-α-D-glucopyranosyl bromide (acetobromoglucose) was carried out using the Bredereck reaction with 2,3-di-*O*-phenylcarbamoyl-6-*O*-trityl-amylose and -cellulose, and the Helferich reaction with the detritylated products. The use of the phenylcarbamoyl groups instead of acetyl groups has several advantages: namely, (*a*) complete substitution of hydroxyl groups is possible in one step without degradation of the polysaccharides,

(*b*) the blocking groups are stable under the conditions of glycoside synthesis and migration does not occur, and (*c*) the degree of branching may be calculated from the decrease in nitrogen content.

As the polymer derivatives are insoluble in nitromethane or acetonitrile in the Bredereck reaction, *p*-dioxane was used as a solvent for the polysaccharide derivative and acetobromoglucose, and nitromethane for silver perchlorate. For the amylose compounds, a 3:1 *p*-dioxane–nitromethane mixture allowed initial homogeneity. For the cellulose compounds, a higher proportion of *p*-dioxane was necessary. Acetobromoglucose was always added last. The reaction products, silver bromide and trityl perchlorate, formed within a few minutes and were insoluble. The molar ratios of silver perchlorate–acetobromoglucose–trityl compound were 1:0.9:0.75.

The results obtained are shown in Table I.

TABLE I

GLYCOSYLATION OF AMYLOSE (BAG) AND CELLULOSE (BCG) BY THE METHOD OF BREDERECK

Compound	*N* (%)	z^a	$1/z^b$	*Yield* (%)
BAG 1	5.91	0.224	4.45	85.0
BAG 2	5.15	0.435	2.30	68.0
BAG 3	5.94	0.214	4.67	73.0
BCG 1	6.53	0.088	11.30	52.5
BCG 2	6.30	0.135	7.40	47.0
BCG 3	6.41	0.113	8.80	49.0

[a]Analogous to the degree of substitution (d.s.), see Experimental. [b]Indicates the number of D-glucose residues of the main chain carrying one D-glucose side-chain, *i.e.*, the degree of branching.

The values of $1/z$, derived from nitrogen determination, show that a higher degree of branching was obtained with amylose (BAG 1–3). The results are in good agreement with previous findings[1]. With cellulose (BCG 1–3), the branching reaction was less effective, and about half of the D-glucose units were incorporated*. The poorer solubility of the cellulose derivative, requiring a greater dilution of the reaction mixture, may be involved in limiting the extent of reaction.

Using the procedure of Helferich and Zirner[9], when solutions of 2,3-*O*-phenylcarbamoyl derivatives of amylose and cellulose in *p*-dioxane were treated with solutions of mercury salts and acetobromoglucose in acetonitrile at room temperature, reaction occurred. Data on the products are given in Table II. Good agreement was found between $1/z$ values determined from the acetyl and nitrogen contents, respectively. With the exception of HAG 2, the branch density obtained for amylose was as high as that in the product from the Bredereck reaction.

The data in Table III show the influence of the molar ratio of the components in the cellulose series.

*Cellulose is more difficult to tritylate than amylose, and a special procedure is necessary in order to obtain complete substitution[15].

TABLE II

GLYCOSYLATION OF AMYLOSE (HAG) BY THE METHOD OF HELFERICH

Compound	*Molar ratio amylose derivative/ acetobromoglucose*	*Acetyl (%)*	*1/z*	*N (%)*	*1/z*
HAG 1	1:1	—	—	5.57	3.20
HAG 2	1:1	3.06	13.20	6.63	14.50
HAG 3	1:2	14.11	2.22	5.17	2.32
HAG 4	1:2	15.50	2.32	5.35	2.68

TABLE III

GLYCOSYLATION OF CELLULOSE (HCG) BY THE METHOD OF HELFERICH[a]

Compound	*Molar ratio*		*Hg salts*	*N (%)*	*1/z*	*Yield (%)*
	Cellulose derivative	*Acetobromoglucose*				
HCG 1	1	1	0.5	6.42	8.90	77.5
HCG 2	1	2	0.5	6.18	6.35	87.5
HCG 3	1	2	0.75	6.37	8.22	86.5
HCG 4	1	3	0.5	6.00	4.90	68.5
HCG 5	1	3	0.75	6.30	7.40	80.5

[a]Reaction time, 48 h.

Increase in the proportion of the bromide results in an increase in branch density. With a three-fold molar excess of acetobromoglucose, cellulose can be glycosylated nearly as effectively as amylose. Increasing the ratio of mercury salts to 0.75 was unfavourable (*cf.* HCG 2 and 3, HCG 4 and 5). The yields obtained for cellulose products in the Helferich reaction are markedly better than those from the Bredereck reaction (BCG 1–3, Table I).

The influence of the reaction time on branch frequency, yield, and degradation of the main chain is shown in the data in Table IV, where the molar ratio of 2,3-di-*O*-phenylcarbamoylamylose to acetobromoglucose was 1:2.

TABLE IV

GLYCOSYLATION OF AMYLOSE BY THE METHOD OF HELFERICH

Compound	*Reaction time (h)*	*1/z*	*Yield (%)*	*[η] (ml/g) (Dioxane)*
Amylose 2,3-diphenylcarbamate				148.0
HAG 5	1	9.68	85.5	147.6
HAG 6	2	2.05	77.5	141.7
HAG 7	6	2.37	71.5	82.2
HAG 8	24	2.37	67.5	23.0

A high and final degree of branching was obtained after two hours of reaction. Almost no degradation occurred; a marked decrease of the viscosity was found only after prolonged reaction. Complex-salt formation from mercuric bromide and hydrogen bromide is mainly responsible for an increase in the acidity of the medium. The occurrence of side reactions, indicated by discolouration, was reflected by the decrease in yields for HAG 5–8. In respect to degradation, the results compare favourably with those for BAG 1–3 (Table I). Although reaction under Bredereck conditions is complete within a few minutes, a rather low viscosity, $[\eta] = 25–30$, was found for the product. Thus, even small amounts of the perchloric acid formed by hydrolysis are much more effective in promoting cleavage of α-(1→4)-bonds than are compounds formed from the mercury salts.

The products of the foregoing Bredereck and Helferich reactions were saponified with sodium methoxide. The resulting amyloses having D-glucose branches were readily soluble in water, whereas the cellulose derivatives swelled but were not completely soluble.

BAG 1–3 and HAG 1–4 were not attacked by beta-amylase, indicating that the enzyme is completely hindered by the presence of D-glucose side-chains. The product prepared by the reaction of 2,3-di-*O*-phenylcarbamoylamylose under Helferich conditions without the addition of acetobromoglucose (blank), followed by saponification, was completely degraded by beta-amylase.

The iodine-binding characteristics of the modified amylose were next examined. The results obtained by potentiometric titration and from the absorption spectra are summarized in Table V. The reference amyloses are synthetic samples of d.p. ~2000. The markedly reduced capacity for iodine binding in most BAG and HAG products is consistent with a highly branched structure. Longer, linear segments are found only in HAG 2, in accordance with $1/z$ evaluated from the nitrogen content.

TABLE V

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

Compound	*Iodine bound (mg/10 mg)*	*Iodine binding (%)*	λ_{max} *(nm)*
Amylose	1.99	100	650
BAG 1	0.02	1.05	—
BAG 2	0.03	1.50	—
BAG 3	0.045	2.35	535
Amylose	2.09	100	650
Blank	2.00	95.60	645
HAG 1	0.14	5.20	545
HAG 2	0.47	22.50	560
HAG 3	0.00	0.00	—
HAG 4	0.03	1.50	—

The $1/z$ values for BAG 2, BAG 3, and BCG 2 were determined by methylation analysis. The substances were methylated by the method of Hakomori[16,17], and the

products were hydrolyzed with 50% formic acid[18]. The reduced and acetylated monomers were separated by g.l.c. and the results are shown in Table VI. The degree of branching (1/z) obtained from the ratio of the peak areas tetra/di+tri and di/tri+tetra agree well for BAG 2 and confirms the value calculated from the nitrogen content. Small deviations are found for BCG 2, but for BAG 3, a somewhat lower branch-density was found as compared to that determined from nitrogen analysis. This is in accordance with the data in Table V, which showed that the 1/z values of BAG 1 and BAG 3 were not identical (as had been indicated by the nitrogen-determination results).

TABLE VI

DEGREE OF BRANCHING FROM METHYLATION ANALYSIS

Compound	*Product of hydrolysis (Methylglucose)*	T *value*[a]	*1/z*	
			from tetramethyl	*from dimethyl*
BAG 2	2,3,4,6	1.0(1.0)	2.5	2.1
	2,3,4	2.50(2.49)		
	2,3	5.40(5.39)		
BAG 3	2,3,4,6	1.0(1.0)	7.9	5.1
	2,3,4	2.65(2.49)		
	2,3	5.40(5.39)		
BCG 2	2,3,4,6	1.0(1.0)	6.2	5.2
	2,3,4	2.60(2.49)		
	2,3	5.40(5.39)		

[a]Figures in brackets taken from ref. 19.

TABLE VII

CHEMICAL SHIFTS (δ p.p.m.) OF H-1 OF SOME PERMETHYLATED POLYSACCHARIDES

Parent polysaccharide	*Type of linkage*	*H-1*
Pullulan[20]	α (1→4)	5.50
	α (1→6)	5.07
Dextran[20]	α (1→6)	5.08
Synthetic glucan[21]	α (1→6)	5.05
Pustulan[21–23]	β (1→6)	4.3(4.6)
BAG 2	α (1→4)	5.50
	α (1→6)	5.08
	β (1→6)	(4.3)
BAG 3	α (1→4)	5.59
	α (1→6)	5.13
HAG 6	α (1→4)	5.45
	α (1→6)	5.00
	β (1→6)	4.41

In seeking to determine the configuration of the (1→6)-linkages present in the amylose and cellulose derivatives, the n.m.r. spectra ($CDCl_3$, 220 MHz) of the methylated products of BAG 2, BAG 3, and HAG 6 were recorded. The resulting H-1 δ values are shown in Table VII, together with some reference data. Recent studies have shown that the type and configuration of glycosidic linkages in polysaccharides may be established by n.m.r. spectroscopy of methyl, acetyl, or benzoyl derivatives[20]. The peaks typical for α-(1→4)- and α-(1→6)-glycosidic bonds at δ 5.50 and 5.05–5.08, respectively, could be clearly identified. The signal for H-1*ax*, expected[21–23] to be in the range δ 4.3–4.6, was observed for BAG 2 and HAG 6, indicating that β-(1→6)-glycosidic bonds may also be present, but to a lesser extent, especially in HAG 6. The spectra, however, could not be interpreted unequivocally in this range, due to the neighbouring signals of the ring protons.

More information was obtained from i.r. spectroscopy. The bands characteristic[24,25] of α- or β-glycosidic bonds occur at 940–840 and ~750 cm^{-1}, respectively. The products from amylose (BAG and HAG) gave strong bands at 940 (945) cm^{-1}, characteristic for α-linkages, and those (BCG and HCG) from cellulose gave bands at 905 cm^{-1}, characteristic for β-linkages. Shoulders at 910 (BAG) and 905 cm^{-1} (HAG) presumably indicated the presence of β-linkages. The shoulder was more pronounced in the spectra of BAG than HAG products. Since a shoulder is also reported in this range for amylopectin (905 cm^{-1}) and glycogen (907 cm^{-1}), the interpretation is not unequivocal. On the other hand, the shoulders appearing in the spectra of all BCG and HCG products at 935 (940) and 860 (870) cm^{-1}, with the latter being more intense, are undoubtedly due to α-(1→6)-D-glucosidic linkages. This inference is supported when the band at 760 cm^{-1} is considered. This band, absent from the spectrum of cellulose, but fairly strong in that for amylose, is shown by BCG and HCG, but is considerably more pronounced in the spectrum of the latter.

The observation that α-(1→6)-linkages are formed in the polysaccharide derivatives obtained by the Helferich method agrees with the results reported for disaccharide syntheses. The formation of α-(1→6)-linkages under the conditions of the Bredereck reaction, modified by the addition of *p*-dioxane, is unexpected. As *p*-dioxane is as polar a solvent as nitromethane, the dissociation of silver perchlorate will scarcely be altered, but *p*-dioxane may (*a*) increase[4] the instability of the intermediate tetra-*O*-acetylglycosyl perchlorate; (*b*) affect the participation by AcO-2, so that the formation of a glycosyl cation is favoured; and (*c*) reduce the stabilising effect of nitromethane on certain conformations (*cf.* ref. 26).

The advantages of the Bredereck method are that the reaction is complete in a short time and that the tritylated 2,3-di-*O*-phenylcarbamoyl-polysaccharides are more soluble in *p*-dioxane, as compared to the detritylated substances. The disadvantages are that the procedures for the preparation, purification, and isolation of the substances and for the exclusion of moisture are time-consuming. Furthermore, the reaction with polysaccharides is not stereospecific, and degradation is considerable.

The advantages of the Helferich and Zirner method are that the reaction and the purification of the products are easy to perform. The extent of branching can be

controlled, degradation can be avoided by using short reaction times, and the yields are at least as high as those for the Bredereck method. A slight disadvantage is the lower solubility of the polysaccharide derivatives used.

Both methods are similar in their efficiency of glycosylation.

EXPERIMENTAL

6-O-Tritylamylose. — Amylose from potato starch was purified by repeated precipitation of the 1-butanol complex. A mixture of 20 g of amylose and 90 g of trityl chloride was dissolved in 350 ml of pyridine and stirred at 70–80° for 5 h.The dark-yellow solution was diluted with *p*-dioxane (120 ml) and precipitated in 8 l of methanol. The product was thoroughly washed with methanol and extracted with ether (Soxhlet). Final purification was achieved by precipitation from *p*-dioxane in methanol.

Anal. Calc. for $(C_{25}H_{24}O_5)_n$: C, 74.3; H, 5.94. Found: C, 74.36; H, 5.97.

2,3-Di-O-phenylcarbamoyl-6-O-tritylamylose. — To a solution of 20 g of tritylamylose in 160 ml of dry pyridine, 25 g of phenyl isocyanate were added. The solution was stirred for 60 h at 100°, then diluted with 100 ml of anhydrous *p*-dioxane, and precipitated in 8 l of methanol. The product was further purified by precipitation from *p*-dioxane in methanol.

Anal. Calc. for $(C_{39}H_{34}N_2O_7)_n$: C, 72.9; H, 5.30; N, 4.36. Found: C, 70.7; H, 5.49; N, 5.10.

2,3-Di-O-phenylcarbamoylamylose[27]. — A solution of 15 g of 2,3-di-*O*-phenylcarbamoyl-6-*O*-tritylamylose in 220 ml of anhydrous methanol was mixed with 2.15 ml of fuming hydrochloric acid and stirred at room temperature for 10–15 h. After precipitation of the product in methanol, the procedure was repeated. The air-dried product was further dried *in vacuo* over phosphorus pentaoxide.

Anal. Calc. for $(C_{20}H_{20}N_2O_7)_n$: C, 60.0; H, 5.00; N, 7.0. Found: C, 58.1; H, 5.38; N, 7.0.

6-O-Tritylcellulose[15]. — A mixture of 300 g of *N*-ethylpyridinium chloride and 150 ml of methyl sulphoxide was melted at 85°. After 18 g of cellulose (cotton linters) had been dissolved in this mixture, 111 ml of pyridine were added, with stirring, followed by 186 g of powdered trityl chloride. Stirring was continued at 80° for 4–5 h. The homogeneous solution was then poured into a tenfold amount of methanol, and the precipitate was collected, thoroughly washed with methanol, and extracted with ethanol for 3 days. The product was dried *in vacuo* at 60°.

Anal. Calc. for $(C_{25}H_{24}O_5)_n$: C, 74.3; H, 5.94. Found: C, 74.4; H, 6.13.

2,3-Di-O-phenylcarbamoyl-6-O-tritylcellulose. — This material was prepared by the procedure used for the corresponding amylose derivative.

Anal. Calc. for $(C_{39}H_{34}N_2O_7)_n$: C, 72.90; H, 5.3; N, 4.36. Found: C, 70.75; H, 5.5; N, 5.10.

2,3-Di-O-phenylcarbamoylcellulose. — Detritylation of the foregoing compound was carried out as described for the amylose series.

Anal. Calc. for $(C_{20}H_{20}N_2O_7)_n$: C, 60.0; H, 5.00; N, 7.0. Found: C, 60.2; H, 5.26; N, 7.0.

Condensation reactions. — Rigorously anhydrous conditions were essential for these reactions. The various cellulose and amylose derivatives, and acetobromoglucose were dried *in vacuo* over phosphorus pentaoxide.

Commercial anhydrous silver perchlorate (2 g) was dried by azeotropic distillation of a solution in benzene (12 ml). The product (1.7 g) obtained by precipitation with 8 ml of dry pentane was then dissolved in 14 ml of dry nitromethane and immediately used.

All solvents used were purified and dried by standard methods. *p*-Dioxane and *n*-pentane were stored over sodium wire, and nitromethane over a molecular sieve in a dark bottle. Acetonitrile was repeatedly refluxed over phosphorus pentaoxide, until colourless, and then distilled and redistilled from potassium carbonate with column fractionation. It was kept in a dark bottle.

(*a*) *2,3-Di-O-phenylcarbamoyl-6-O-tritylamylose*[3,4]. — A solution of 4 g of 2,3-di-*O*-phenylcarbamoyl-6-*O*-tritylamylose in 40 ml of *p*-dioxane was stirred with nitrogen. A solution of 1.7 g of $AgClO_4$ in 14 ml of dry nitromethane was added, and then a solution of 3.2 g of acetobromoglucose[28] in 5 ml of *p*-dioxane. A yellow precipitate of trityl perchlorate and silver bromide appeared immediately. The mixture was centrifuged in a closed vessel, and the brown supernatant solution was poured into ice–water containing sodium hydrogen carbonate and sodium thiosulphate. The greyish precipitate was collected, thoroughly washed with methanol, and extracted with ether for 5–10 h. Purification was effected by precipitation from solution in *p*-dioxane in methanol. The *p*-dioxane solution was centrifuged to remove traces of colloidal silver.

2,3-Di-*O*-phenylcarbamoyl-6-*O*-tritylcellulose and acetobromoglucose were subjected to a similar procedure, using 60 ml of *p*-dioxane, silver perchlorate in 20 ml of nitromethane, and acetobromoglucose in 5 ml of dioxane.

(*b*) *2,3-Di-O-phenylcarbamoylamylose*[9]. — To a stirred solution of 1 g of 2,3-di-*O*-phenylcarbamoylamylose in 10 ml of *p*-dioxane, a solution of 315 mg of $Hg(CN)_2$ and 450 mg of $HgBr_2$ in 5 ml of acetonitrile was slowly added, followed dropwise by a solution of 1.025 g of acetobromoglucose in 1 ml of acetonitrile. Stirring was continued for 2 h at room temperature. The product was then precipitated in 2 l of methanol to give a mobile oil, which was purified by several precipitations from solution in *p*-dioxane in methanol. The resulting syrupy product was air-dried and finely powdered in a mortar.

Likewise, 1 g of 2,3-di-*O*-phenylcarbamoylcellulose was dissolved in 20 ml of *p*-dioxane, and treated with 315 mg of $Hg(CN)_2$ and 450 mg of $HgBr_2$ in 8 ml of acetonitrile, and 1.025 g of acetobromoglucose in 7 ml of *p*-dioxane.

The amounts of the components given above correspond to molar ratios of polysaccharide/acetobromoglucose/$Hg(CN)_2$ or $HgBr_2$ of 1:1:0.5. Variations are indicated in Tables II and III. Where two salt additions were made, the first batch of

mercury salts was dissolved in 7 ml of acetonitrile, and the second in 3 ml of solvent. The acetobromoglucose was dissolved in 5 ml of acetonitrile.

Calculation of the degree of branching. — (a) *From nitrogen content.*

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

(*b*) *From acetyl content.*

$$z = \frac{\%Ac.400}{(3n+1).43.100 - \%Ac.(288n+42)}$$

For reactions with acetobromoglucose: $n = 1$.

Saponification of the acetyl and phenylcarbamoyl groups. — Methanol (15 ml) was slowly added to a boiling solution of 1 g of the polysaccharide derivative dissolved in 30 ml of *p*-dioxane with stirring under nitrogen. Then 6 ml of a solution of sodium methoxide in methanol (20%) were added dropwise and boiling was continued for 1 h. The product was collected, dried, purified by precipitation from solution in methyl sulphoxide with methanol, and finely powdered by treatment with acetone in a hot mortar.

Methylation analysis. — A commercial dispersion (100 mg) of sodium hydride in oil was repeatedly washed with light petroleum, and the solvent was removed under nitrogen. Anhydrous methyl sulphoxide (1 ml) was then added, and the mixture was stirred for 1 h. To the slightly green, homogeneous solution of the methylsulphinyl carbanion, a solution of 100 mg of the saponified polysaccharide in 7 ml of methyl sulphoxide was added dropwise. The mixture was stirred at room temperature under nitrogen for 10 min, and then 0.12 ml and 0.15 ml of methyl iodide were added at an interval of 2 h. After a further 6 h, the colourless solution was diluted with water, dialyzed for 3 days against running tap-water, and concentrated to dryness. The residue was repeatedly dissolved in a small volume of *p*-dioxane, and the solution was concentrated. The residue was finally isolated by lyophilization.

A solution of 100 mg of the methylated polysaccharide in 8 ml of formic acid[18] (50%) was heated for 18 h at 95°. Formic acid was removed by evaporation, and methanol (2 × 5 ml) was evaporated from the residue. The product was dried *in vacuo* over potassium hydroxide.

The usual procedures were applied for the conversion of the methylated sugars into their alditol acetates. Chloroform solutions of the alditol acetates were injected into a Varian Aerograph model 1520 B fitted with a glass column (0.32 × 152 cm) filled with 3% of ECNSS-M on gaschrom Q (100–120 mesh), a column temperature of 165°, and a nitrogen flow of 28 ml/min.

Potentiometric titrations[29]. — To a solution of a 10-mg portion of each branched amylose in 2.5 ml of methyl sulphoxide, 10 ml of 0.5M KCl and 10 ml of 0.5M KI were added, and the volume was made up to 100 ml with water. The titrating solution contained[30] 0.2 mg of iodine/ml, 0.05M KCl, and 0.05M KI.

Measurements were performed at 20° with a Metrohm Präzisions-Kompensator

E 388 (Metrohm AG, Herisau, Switzerland), which allows 0.1-mV readings. Platinum and calomel electrodes were used.

Absorption spectra of the amylose–iodine complex[29]. — To a solution of 5.0 mg of each branched amylose, 0.5 ml of methyl sulphoxide, 6 ml of water, and 3.5 ml of iodine solution (0.5225 g of iodine and 0.5225 g of KI in 1 litre of water) were added. The spectra were determined with a Zeiss DMR 21 spectrophotometer at 20°, using 1-mm cells.

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 of polysaccharide/200 mg of KBr).

ACKNOWLEDGMENTS

This work was supported by Deutsche Forschungsgemeinschaft. The authors thank Dr. I. Fromme, Max Planck Institute of Immunobiology, for gas-chromatographic analysis.

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