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

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ABSTRACT

Comb-like molecules having oligomeric side-chains were prepared by condensation of peracetylated 1,2-(ethyl orthoacetate) derivatives of maltose, maltotetraose, and maltohexaose with 2,3-di-*O*-phenylcarbamoyl derivatives of amylose and cellulose. The distance between the maltose, maltotetraose, and maltohexaose branch-points introduced into amylose was 3–4, 10, and ~17 D-glucose residues, respectively, and only slightly greater for cellulose. Thus, the branch frequency is considerably higher than that obtained previously by using the Bredereck and Helferich reactions. After saponification of the modified polysaccharides, the side chains of d.p. 4 and 6 attached to position 6 in the D-glucose residues of amylose and cellulose were easily extended by enzymic synthesis with potato phosphorylase.

INTRODUCTION

The orthoester method of glycosylation is very effective[1] for attaching D-glucose side-chains to position 6 of the D-glucose residues in amylose and cellulose. By using the 1,2-(ethyl orthoacetate) of 3,4,6-tri-*O*-acetyl-α-D-glucopyranose, a degree-of-branching was achieved at least as high as that previously[1] effected using tetra-*O*-acetyl-α-D-glucopyranosyl bromide under the conditions of the Bredereck[2,3] and the Helferich reactions[4,5]. The efficiencies of the last two methods were similar when the D-glucosyl bromide was used, but they differed widely for the acetylated glycosyl bromide derivatives of the higher malto-oligosaccharides[6]. The Bredereck reaction (silver perchlorate–nitromethane–*p*-dioxane) produced one branch per 25–55 residues, whereas the Helferich reaction (mercuric bromide–mercuric cyanide–acetonitrile–*p*-dioxane) gave only one branch per 100–150 residues of the backbone chain. Clearly, the conditions of the Bredereck reaction are more suitable for the less-reactive acetylated glycosyl bromides. We now report on the use of the relatively stable, peracetylated 1,2-(ethyl orthoacetate) derivatives of the maltosaccharides and reaction conditions (boiling chlorobenzene and catalytic amounts of 2,6-lutidinium perchlorate) previously used[1] for the corresponding glucose derivative.

*Chemical Synthesis of Branched Polysaccharides: Part IV[1].

RESULTS AND DISCUSSION

The extent of degradation during the preparation of peracetylated maltose 1,2-(ethyl orthoacetate) was first investigated. No degradation occurred (t.l.c.) if octa-*O*-acetyl-α-maltosyl bromide, prepared by treatment[6] of the octa-acetate with hydrogen bromide–acetic acid–red phosphorus, was immediately converted into the 1,2-(ethyl orthoacetate) by reaction[1] with ethanol and 2,6-lutidine (2,6-dimethylpyridine). The formation of the orthoester was verified by n.m.r. spectroscopy before and after acid hydrolysis[7,8].

Peracetylated maltose 1,2-(ethyl orthoacetate) was condensed with 2,3-di-*O*-phenylcarbamoyl derivatives of amylose or cellulose in boiling chlorobenzene with 2,6-lutidinium perchlorate as catalyst using a 1:1 molar ratio of the reactants. The extent of condensation could be assessed from the nitrogen content of the product. Thus, the products from 2,3-di-*O*-phenylcarbamoylamylose contained 4.91–5.36% of nitrogen (*cf.* 7% for starting material), indicating an average distance between the branches of 3.44–5.04 D-glucose residues. A corresponding separation distance of 2–3 D-glucose residues was obtained when tri-*O*-acetyl-α-D-glucopyranose 1,2-(ethyl orthoacetate) was condensed[1] with 2,3-di-*O*-phenylcarbamoylamylose.

The condensation with 2,3-di-*O*-phenylcarbamoylcellulose was less extensive, and the nitrogen contents (5.65–6.10%) of the products corresponded to average separation distances of 6.47–10.47 D-glucose residues. When maltotetraose (obtained[9] by degradation of amylose with an exo-amylase from *B. stutzeri*) was peracetylated and brominated by the procedure described above, the resulting glycosyl bromide contained (t.l.c.) only traces of the maltotriose derivative. However, the glycosyl bromide derived from maltohexaose (obtained[10] by degradation of amylose with an alpha-amylase from *B. subtilis*), or prepared from cyclodextrin[6], contained small amounts of shorter oligomers. No further degradation could be detected by t.l.c. during the conversion into the corresponding orthoesters, and n.m.r. spectroscopy revealed the typical peaks of the 1,2-(ethyl orthoester) which disappeared on acid hydrolysis.

A series of products (*OAO* and *OCO*, respectively) obtained by condensation of the foregoing orthoesters with the 2,3-di-*O*-phenylcarbamoyl derivatives of amylose and cellulose is shown in Table I. The products *HAO-3* and *HAO-4* were prepared, for purposes of comparison, by using the acetylated glycosyl bromide derivatives of maltotetraose and maltohexaose and the Helferich reaction.

The data in Table I indicate that a significantly higher degree-of-branching results when the orthoester method is used for glycosylation. Also, the maltotetraose derivative effected more branching than did the maltohexaose analogue (*cf. OAO-3/4* and *OAO-1/2*); the difference in branching for the products (*HAO-3/4*) from the Helferich reaction was not so marked.

The condensation products in Table I were saponified with sodium methoxide, and the resulting, branched amyloses were characterized by iodine binding and beta-amylolytic degradation. The data are shown in Table II. The reduced capacity for

TABLE I

PRODUCTS OF GLYCOSYLATION OF 2,3-DI-*O*-PHENYLCARBAMOYL DERIVATIVES OF AMYLOSE AND CELLULOSE WITH PERACETYLATED 1,2-(ETHYL ORTHOACETATE) AND GLYCOSYL BROMIDE DERIVATIVES OF MALTOTETRAOSE AND MALTOHEXAOSE

Compound	*D.p. of glycosylating agent*	*Mol. ratio of reactants*	*N* (%)	*Separation distance of branches* (D-*glucose residues*)
OAO-1	6	1:0.5	6.56	60.0
OAO-2	6	1:1	5.57	17.3
OAO-3	4	1:1	5.40	10.0
OAO-4	4	1:1	5.44	10.4
HAO-3	6	1:1	6.54	67.0
HAO-4	4	1:1	6.64	56.0
OCO-1	4	1:1	5.86	15.3
OCO-2	4	1:1	5.75	13.7

TABLE II

IODINE-BINDING PROPERTIES, AND DEGRADATION WITH beta-AMYLASE

Compound	*Iodine binding* (%)	λ_{max} (*nm*)	*beta-Amylolytic degradation* (%)
Amylose (d.p. 2000)	100	630	100
OAO-1'	17	585	31.2
OAO-2'	9	580	16.4
OAO-3'	7	574	9.7
OAO-4'	6	568	7.6
HAO-3'	17	603	13.8
HAO-4'	11	582	13.3

iodine binding, the shift of absorption maximum of the iodine complex to lower wavelengths, and the incomplete beta-amylolytic degradation clearly reflect the branched structure and fully accord with the results derived from nitrogen content. The relatively low degree-of-degradation of *HAO-3'* and *HAO-4'* by beta-amylase may arise from the fact that these products were synthesized in a homogeneous reaction mixture, whereas *OAO-1'–OAO-4'* were prepared under heterogeneous conditions. Thus, the branches may be distributed more uniformly along the main chain.

The branched amylose and cellulose derivatives in Table II were assessed as primers for phosphorylase by incubation with α-D-glucopyranosyl phosphate and potato phosphorylase. As controls, maltotetraose and an amylose carrying D-glucose side-chains (*OAG-II-2*)[1] were used. The rate of reaction was determined from the release of inorganic phosphate, and the results are shown in Fig. 1. No synthesis was observed with *OAG-II-2*. Thus, amylose that is densely branched with D-glucose residues does not act as a primer for phosphorylase. The priming activity was in the same order as the branch frequency shown in Table I, except for the cellulose products. The poor solubility of the latter substances in water may be the reason.

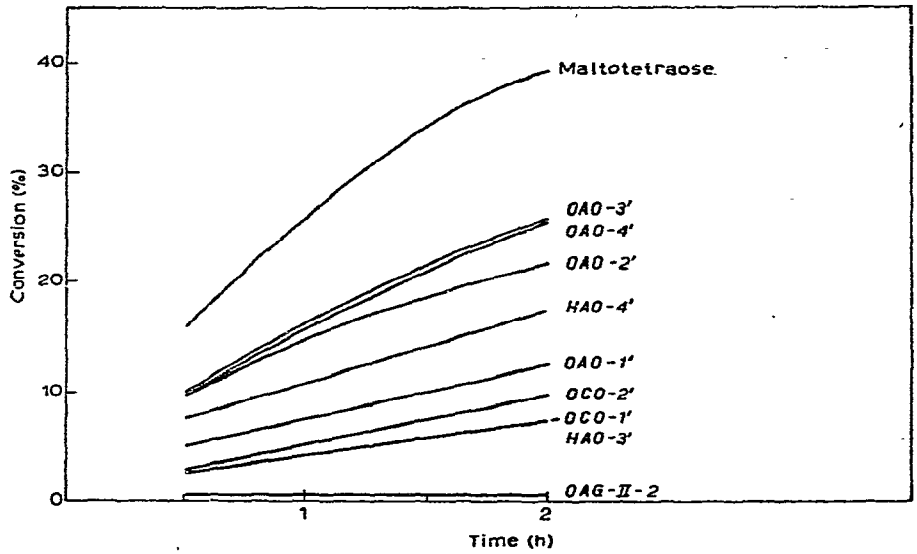

Fig. 1. Priming activity in phosphorolytic synthesis of amylose and cellulose comb-like derivatives with malto-oligomeric side-chains.

The Helferich, Bredereck, and orthoester methods employed for glycosylation are equally effective for introducing D-glucose side-chains into the 2,3-di-*O*-phenylcarbamoyl derivatives of amylose and cellulose. However, the methods differed considerably in their efficiency for introducing maltotetraose and maltohexaose side-chains. The Bredereck and Helferich reactions gave products containing one branch per 25 and 60–100 residues, respectively, of the main chain. The orthoester method gave products containing one branch per 10–15 residues. Thus, the orthoester method, especially when carried out with the relatively stable 1,2-(ethyl orthoacetate) derivatives, is the most suitable for introducing longer side-chains.

EXPERIMENTAL

2,3-Di-*O*-phenylcarbamoyl derivatives of amylose and cellulose were prepared as previously described[11]; see preceding paper[1], for the analytical data.

For calculation of the degree-of-branching from the N content, saponification procedure, and determination of iodine binding, see Part I[11]. For determination of priming activity in phosphorolytic synthesis, see Part II[6].

Preparation of orthoesters. — Acetobromination of maltose, maltotetraose, and maltohexaose was carried out as previously described[6], with a reaction time of 20 min.

A solution[12] of hepta-*O*-acetyl-α-D-maltopyranosyl bromide (7 g, 10 mmol) in nitromethane (10 ml), 2,6-lutidine (2.3 ml, 20 mmol), and ethanol (2.9 ml, 50 mmol) was stored at 37° for 45 h. 2M Silver nitrate (7 ml, 15 mmol), water (13 ml), and acetone (25 ml) were then added, and the solution was filtered, and diluted with chloroform and hexane. The organic layer was separated, washed twice with water,

and concentrated, and the residue was treated with ether–hexane to give 3,6,2′,3′,4′,6′-hexa-*O*-acetyl-α-D-maltopyranose 1,2-(ethyl orthoacetate) (3.9 g, 58.7%), m.p. 92–95°, $[\alpha]_D$ +30° (*c* 1.5, chloroform). N.m.r. data ($CDCl_3$, 220 MHz): δ 1.16 (m, 2 H, CH_3CH_2), 1.71 (s, 3 H, Me), and 3.50 (m, 3 H, CH_3CH_2).

Anal. Calc. for $C_{28}H_{40}O_{18}$: C, 50.60; H, 6.02. Found: C, 50.10; H, 6.25.

In a similar manner, the analogous peracetylated 1,2-(ethyl orthoacetate) derivatives of maltotetraose and maltohexaose were prepared. The products were purified by precipitation from chloroform solution with light petroleum.

Maltotetraose 1,2-(ethyl orthoacetate) dodeca-acetate (54.1%), m.p. 95–98°. N.m.r. data: δ 1.18 (m, 2 H, CH_3CH_2), 1.75 (s, 3 H, Me), and 3.55 (m, 3 H, CH_3CH_2).

Anal. Calc. for $C_{52}H_{72}O_{34}$: C, 50.32; H, 5.80. Found: C, 50.50; H, 5.92.

Maltohexaose 1,2-(ethyl orthoacetate) octadeca-acetate (51.7%). N.m.r. data: δ 1.18 (m, 2 H, CH_3CH_2), 1.75 (s, 3 H, Me), and 3.60 (m, 3 H, CH_3CH_2).

Anal. Calc. for $C_{76}H_{104}O_{50}$: C, 50.22; H, 5.72. Found: C, 49.8; H, 5.82.

T.l.c. was performed on silica gel (DC-Fertigfolien F 1500, LS 254, Fa., Schleicher and Schuell) with benzene–ethanol (95:5) and detection by charring with phenol–sulphuric acid at 110° for 30 min.

Condensation reactions. — (*a*) The condensation of the orthoacetate derivatives (1.25 mmol) of maltose, maltotetraose, and maltohexaose with the 2,3-di-*O*-phenylcarbamoyl derivatives (0.5 g, 1.25 mmol) of amylose and cellulose in boiling chlorobenzene (8 ml) containing 2,6-lutidinium perchlorate (0.005 mmol) was carried out as described in the preceding paper[1] (product yields, ~0.48 g).

(*b*) Following the procedure of Helferich and Zirner[4,6], a solution of 2,3-di-*O*-phenylcarbamoylamylose (0.5 g) in *p*-dioxane (7.5 ml) was treated with peracetylated α-D-maltotetraosyl bromide (3.1 g) or the maltohexaosyl analogue (4.6 g) in acetonitrile (1.5 ml) using a solution of $Hg(CN)_2$ (157 mg) and $HgBr_2$ (225 mg) in acetonitrile (2 ml); product yield, ~0.4 g.

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REFERENCES

1 Part III: B. Pfannemüller, G. C. Richter, and E. Husemann, *Carbohydr. Res.*, 56 (1977) 139–146.
2 H. Bredereck, A. Wagner, and G. Faber, *Angew. Chem.*, 69 (1957) 438.
3 H. Bredereck, A. Wagner, G. Faber, H. Ott, and J. Rauther, *Chem. Ber.*, 92 (1952) 1135–1139.
4 B. Helferich and J. Zirner, *Chem. Ber.*, 95 (1962) 2604–2611.
5 B. Helferich and W. Ost, *Chem. Ber.*, 95 (1962) 2612–2615.
6 B. Pfannemüller, G. C. Richter, and E. Husemann, *Carbohydr. Res.*, 47 (1976) 63–68.
7 R. D. Guthrie, A. D. Jenkins, and J. Stehlicek, *J. Chem. Soc., C*, (1971) 2690–2696.
8 D. Y. Gagnaire and P. J. A. Vottero, *Carbohydr. Res.*, 28 (1973) 165–170.
9 J. F. Robyt and R. J. Ackerman, *Arch. Biochem. Biophys.*, 145 (1971) 105–114.
10 B. Pfannemüller and W. Burchard, *Makromol. Chem.*, 121 (1969) 1–17.
11 B. Pfannemüller, G. C. Richter, and E. Husemann, *Carbohydr. Res.*, 43 (1975) 151–161.
12 N. K. Kochetkov, A. Y. Khorlin, and A. F. Bochkov, *Tetrahedron*, 23 (1967) 693–707.