Carbohydrate Research, 47 (1976) 63–68

COMB-LIKE DERIVATIVES OF AMYLOSE HAVING (1→6)-LINKED MALTO-OLIGOSACCHARIDE SIDE-CHAINS*

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(Received March 24th, 1975; accepted for publication in revised form, September 15th, 1975)

ABSTRACT

The preparation of acetylated glycosyl bromide derivatives of the higher malto-oligosaccharides was studied by using β-cyclodextrin or linear malto-oligosaccharides of d.p. 6 and 7. The products were treated with 2,3-di-*O*-phenylcarbamoyl-6-*O*-tritylamylose in the presence of silver perchlorate (reaction A), and with 2,3-di-*O*-phenylcarbamoylamylose in the presence of mercuric cyanide and mercuric bromide (reaction B). After removal of the substituents, the branched molecules were characterized by their iodine-binding properties, beta-amylolytic degradation, and their priming activity in phosphorolytic synthesis. The distance between the branch points of the backbone chain was 25–55 and 100–150 units in the products from reactions A and B, respectively. Thus, the frequency of branching was considerably lower than in the comb-like molecules having D-glucose side-chains previously described.

INTRODUCTION

We have shown[1] that the glycoside syntheses devised by Bredereck[2,3] and Helferich and Zirner[4,5] can be employed for introducing D-glucose side-chains into amylose and cellulose at C-6. We now report on the similar introduction of malto-oligomers (d.p. ≥ 4). If these side chains could be further elongated by enzymatic synthesis with phosphorylase, then a great variety of branched polysaccharides would be available, differing in the backbone chain as well as in the distance between, and lengths of, the side chains. Amylose side-chains glycosidically linked at C-6 to an amylose main-chain would be especially interesting as intermediate structures between amylose and amylopectin.

In order to obtain well-defined, branched molecules suitable for phosphorolytic synthesis, it was necessary to carry out the condensation with acetylated glycosyl bromide derivatives of maltose saccharides of lengths as uniform as possible. This purpose required the availability of monodisperse malto-oligomers. Such compounds should serve to determine the extent of degradation on conversion into the acetobromo

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

derivatives and the applicability of the Bredereck and Helferich glycoside syntheses for the coupling of longer side-chains to an amylose main-chain.

RESULTS AND DISCUSSION

Preparation of acetylated glycosyl halide derivatives of malto-oligosaccharides. In investigating acetobrominolytic cleavage of acetylated β-cyclodextrin, the substrate was treated with freshly prepared hydrogen bromide–acetic acid, but the results were not reproducible. Under-bromination was frequently observed and this was due to traces of bromine, formed in the solutions within short periods of time. The interfering bromine could be scavenged by the addition of red phosphorus. Even commercial solutions of hydrogen bromide–acetic acid could then be used and the bromine content of the product was in the range calculated for acetylated maltoheptaosyl bromide (Br, 3.73%). The yields were raised to 95%, if, after brominolytic cleavage, the product was immediately dissolved in chloroform, thus minimising contact with water.

The chain lengths of acetylated glycosyl bromides obtained *via* cyclodextrins are restricted to 6 and 7. More-varied chain-lengths would be possible by the use of linear malto-oligomers. A mixture of maltohexaose and maltoheptaose, prepared by degradation of amylose with alpha-amylase of *B. subtilis* followed by fractionation on charcoal–Celite[6], was submitted to the usual procedure of acetobromination of D-glucose[7].

The results for this product (*M1*) are shown in Table I and compared to those obtained for a substance prepared from cyclodextrin (*C4*).

TABLE I

AVERAGE CHAIN-LENGTHS OF ACETYLATED GLYCOSYL BROMIDE DERIVATIVES OF MALTO-OLIGOMERS

Compound	*Br (%)*	*D.p. (Br)*	$\overline{M}_n$ *(d.p.) (osmometry)*	*D.p. (reduction)*	*D.p. of substrate*
M1	4.80	5.4	1795 (5.8)	6.0	6+7
C4	3.66	7.1	1538 (5.0)	5.9	7

The average degrees of polymerization (d.p.) were determined from the bromine content, by osmometry on solutions in *p*-dioxane, and, after saponification, from reducing end-group analysis. For *M1*, the various d.p. values agree quite well, indicating that degradation was not significant. The d.p. values found for *C4* are of the same order, but the somewhat lower bromine content may be explained by contamination with acetylated cyclodextrin.

It could be shown by t.l.c., using the appropriate compounds as references, that *C4* and *M1* consisted mainly of compounds of d.p. 6 and 7. Shorter oligomers (d.p. 3–5) were present only in minor amounts. The D-glucose and maltose derivatives were absent.

Thus, rather-uniform, brominated malto-oligomers can be obtained from cyclodextrin and linear malto-oligomers. Degradation can be minimised by using short reaction periods and storage times.

Introduction of malto-oligomeric side-chains into amylose. As in previous experiments with acetobromoglucose[1], 2,3-di-*O*-phenylcarbamoyl-6-*O*-tritylamylose was used for the condensation of the acetylated glycosyl bromide derivatives of the malto-oligomers in nitromethane–*p*-dioxane in the presence of silver perchlorate (Bredereck reaction). 2,3-Di-*O*-phenylcarbamoylamylose was used for the condensation in acetonitrile–*p*-dioxane in the presence of mercuric cyanide and mercuric bromide (Helferich reaction). It has been shown[1] that β-glycosidic bonds were preferentially formed in the former reaction and α-glycosidic bonds in the latter.

Examples of the products prepared by each method are given in Table II. The products *BAO-2* (Bredereck reaction) and *HAO-2* (Helferich reaction) were obtained from the same malto-oligomer derivative.

TABLE II

INTRODUCTION OF MALTO-OLIGOMERS INTO AMYLOSE

Compound	*Polysaccharide (g)*	*Glycosyl bromide (g)*	*Molar ratio*	*N (%)*	*1/z*	[η] (*ml/g*) p-*Dioxane*	*D.p.*
Substrate				7.00		137	790
BAO-2	2.0	8.55	1:1.2	5.95	25	29	150
HAO-2	0.5	5.30	1:2.0	6.50	58	130	750

An approximate value of the degree-of-branching (d.b.) was obtained from the decrease in nitrogen content. The calculations were based on an average branch-length of 6.5 D-glucose residues. The number of D-glucose residues of the main chain carrying one side-chain (1/*z*) was 25 for *BAO-2* and 58 for *HAO-2*. Thus, the branch density obtained with these longer acetobromo oligomers is considerably lower than when acetobromoglucose was used (1/*z*, 2–4). The d.b. was always higher when the Bredereck reaction was applied. The approximate d.p. of the backbone chain before and after branching was derived from viscosity measurements. No chain scission occurred under the conditions of the Helferich reaction, whereas the *BAO* samples were degraded to d.p. ~150.

Phenylcarbamoyl and acetyl groups were removed from the condensation products by saponification with sodium methoxide. The products prepared *via* the Bredereck reaction were readily soluble in water. This fact indicates that branches are present, since linear amyloses of d.p. ~150 are insoluble in water.

The products were further characterised by iodine binding and β-amylolytic degradation, phenomena that are sensitive to interruptions in the α-(1→4)-linked glycosidic chain (Table III).

The average chain-length between the branching points, *i.e.*, internal chain-length (i.c.l.), is given by the position of λ_{max} of the iodine complex. The average length of chains up to the first branching-point, *i.e.*, external chain-length (e.c.l.) is determined from the extent of degradation with beta-amylase. With low d.b., the i.c.l. and e.c.l. values are similar, as shown for *HAO-1* and *HAO-2*. When the d.b. increases, the values are different (see *BAO-1* and *BAO-2*). This result is due to the fact that only the longer chain-segments contribute to the iodine binding.

In order to examine whether the side chains could serve as primers for phosphorylase, D-glucopyranosyl phosphate and potato phosphorylase were incubated with samples of *BAO* and *HAO* products. As references, a mixture of linear malto-

TABLE III

IODINE-BINDING PROPERTIES AND DEGRADATION WITH BETA-AMYLASE

Compound	*Iodine binding* (%)	λ_{max} (*nm*)	*I.c.l.*	*beta-Amylolytic degradation* (%)	*E.c.l.*
Amylose (d.p. 2200)	100	645	—	100	—
BAO-1	51	575	55	15.5	23
BAO-2	18	517	28	5.7	8.5
HAO-1	81	615	160	19.8	150
HAO-2	70	605	115	15.7	120

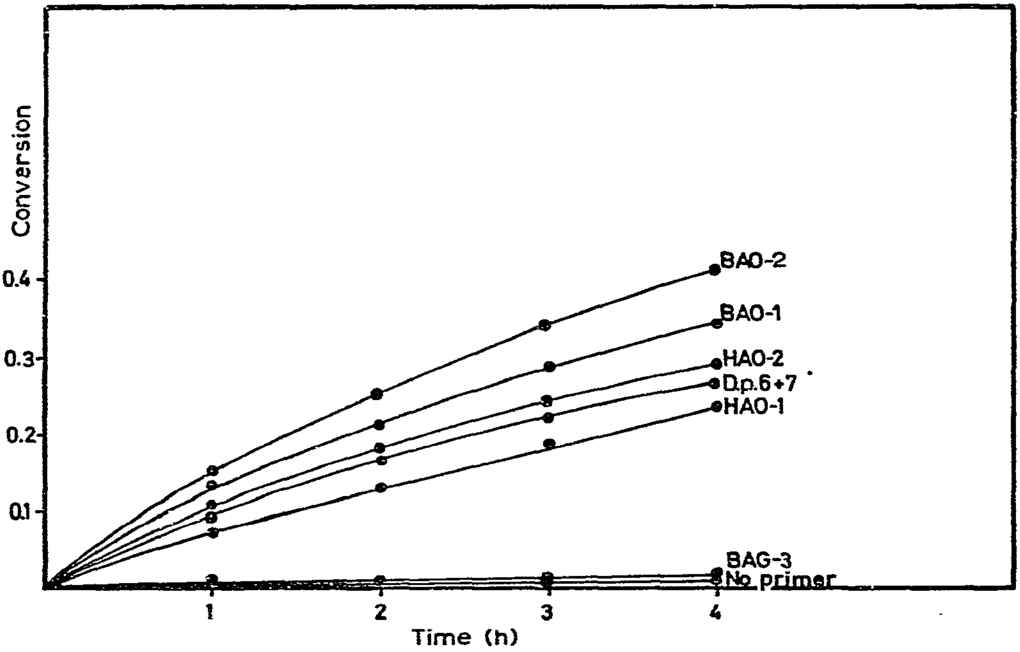

Fig. 1. Priming activity in phosphorolytic synthesis of amylose derivatives modified by the attachment of malto-oligomeric side-chains.

oligomers (d.p. 6 and 7) and an amylose carrying D-glucose side-chains[1] (*BAG-3*) were used. The rate of conversion was followed by determination of the inorganic phosphate released. From Fig. 1, it may be seen that no synthesis was observed with the blank or *BAG-3*. Thus, when the main chain is densely branched with D-glucose, the molecule will not act as a primer for phosphorylase. The priming activity was in the order *BAO-2*>*BAO-1*>*HAO-2*>*HAO-1*. This is in the same order as that for the d.b. values shown in Table III. A quantitative determination of $1/z$ from the kinetic measurements, using the linear primer as reference, is not possible, since the rate of synthesis is not linearly dependent on the concentration of end groups.

When D-glucose side-chains are attached to amylose[1], the Bredereck and Helferich reactions effect approximately the same d.b. However, as shown here, when oligosaccharides are attached, the Bredereck reaction effects a markedly higher d.b., but also causes more degradation.

EXPERIMENTAL

T.l.c. was performed on silica gel (DC-Fertigfolien F 1500, LS 254, Fa., Schleicher and Schuell), using benzene–ethanol (95:5) and detection by heating with phenol–sulfuric acid at 110° for 30 min[7].

Osmotic measurements were carried out in a vapor-phase osmometer (Fa. Knauer, Berlin), using solutions in chloroform or *p*-dioxane at 60°. Reducing values (maltose equivalent) were determined with the Nelson–Somogyi reagent[8,9].

The procedures for potentiometric titration with iodine and absorption spectra are described in Part I[1]. Acetobromination of linear malto-oligosaccharides was performed as described by Lemieux[10].

Acetobrominolytic cleavage of β-cyclodextrin acetate. — To a solution of β-cyclodextrin acetate[11] (1 g) in glacial acetic acid (6 ml), a saturated solution (30 ml) of hydrogen bromide in acetic acid containing red phosphorus (0.1 g) was added. After vigorous stirring for 15 min, the mixture was poured into chloroform (20 ml). The solution was repeatedly washed with aqueous sodium acetate and then water, dried (Na_2SO_4), and concentrated. The resulting syrup was poured into light petroleum (b.p. 60–80°), and purification of the product was effected by precipitation from solution in chloroform with light petroleum.

*Condensation of 2,3-di-*O*-phenylcarbamoyl-6-*O*-tritylamylose with acetobromo-malto-oligomers (Bredereck reaction[2,3]).* — To a solution of 2,3-di-*O*-phenylcarbamoyl-6-*O*-tritylamylose[1] (2 g) in dry *p*-dioxane (20 ml), solutions of silver perchlorate (0.8 g) in dry nitromethane (7 ml) and acetylated maltoheptaosyl bromide (8.55 g) in *p*-dioxane (5 ml) were added. The precipitation of trityl perchlorate was markedly slower as compared to previous experiments[1] with acetobromoglucose. After centrifugation and isolation, as previously described[1], the branched products were extracted with chloroform and precipitated from solution in *p*-dioxane with methanol.

*Condensation of 2,3-di-*O*-phenylcarbamoylamylose with acetobromo-malto-*

oligomers (*Helferich and Zirner reaction*[4]). — To a solution of 2,3-di-*O*-phenylcarbamoylamylose[1] (1 g) in dry *p*-dioxane (8 ml), a solution of $Hg(CN)_2$ (315 mg) and $HgBr_2$ (450 mg) in acetonitrile (5 ml) was slowly added, followed by a solution of acetylated maltoheptaosyl bromide (5.28 g) in *p*-dioxane (5 ml). After stirring for 24 h at room temperature, the mixture was poured into methanol. The product was collected, and purified by precipitation from solution in *p*-dioxane with methanol. The substances were dried *in vacuo* over phosphorus pentaoxide.

Degradations with beta-amylase. — Each digest (10 ml) contained a branched product (5 mg) dissolved in methyl sulphoxide (0.25 ml), 0.2M acetate buffer (pH 4.8, 2 ml), water (7.5 ml), and beta-amylase from barley, free of alpha-amylase (Serva, Heidelberg, 6 units/mg; 7.5 units, 0.25 ml). Aliquots (0.25–1.0 ml) were removed at intervals and reducing values were determined with the Nelson–Somogyi reagent[8,9].

Priming activity in phosphorolytic synthesis. — Each digest (10 ml) contained a branched product (2 mg) or the linear primer d.p. 6 and 7 (0.5 mg) dissolved in methyl sulphoxide (0.5 ml), D-glucopyranosyl phosphate (disodium salt, 500 mg) neutralized to pH 6.2 with acetic acid (4 ml), M sodium citrate buffer (pH 6.2, 1 ml), water (4 ml), and potato phosphorylase free of alpha-amylase[12] (0.24 unit/mg, 5 units, 0.5 ml). Syntheses were carried out at 37°. Aliquots (0.5 ml) were removed at intervals and analyzed for inorganic phosphate[13] (Fig. 1).

ACKNOWLEDGMENTS

This work was supported by Deutsche Forschungsgemeinschaft. The authors thank G. Pfister, W. Emmerling, and R. Müller for their assistance in the experimental work.

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