Carbohydrate Research, 185 (1989) 113–118
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

DETERMINATION OF THE SUBSTITUTION PATTERN OF MODIFIED POLYSACCHARIDES: PART I, BENZYL STARCHES

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(Received March 21st, 1988; accepted for publication, July 13th, 1988)

ABSTRACT

The distribution of substituents in benzylated starches has been investigated by g.l.c.–m.s. of the products obtained after reductive depolymerization of the methylated polysaccharides. Reductive degradation using triethylsilane causes *O*-debenzylation, the degree of cleavage depending on the type of Lewis acid used. The results are discussed in comparison to those of standard methylation analysis.

INTRODUCTION

Benzylation of starches confers the hydrophobic properties necessary for the solubility in organic solvents and compatibility with synthetic polymers. The distribution of the benzyl groups depends on the conditions of reaction and affects the physical properties of the products.

The application of methods which determine only the degree of substitution (d.s.), but not the positions substituted, cannot provide an explanation of the different properties of benzylated polysaccharides. The substitution patterns of, for example, hydroxyethyl, hydroxypropyl, and carboxymethyl derivatives of polysaccharides have been determined by standard methylation analysis[1–5]. Partial cleavage of benzyl residues occurs during hydrolysis. In contrast to standard methylation analysis, reductive depolymerization of polysaccharides[6,7] proceeds under mild conditions and gives, in one step, the partially methylated anhydroalditol acetates (**1** → **2**).

We have performed comparative studies of starch ethers and esters using both methods[8,9] and now report their application to benzylated polysaccharides.

RESULTS AND DISCUSSION

Benzylated starches having various d.s. values were methylated[10] with sodium hydroxide/methyl iodide in methyl sulfoxide. A portion of the methylated product

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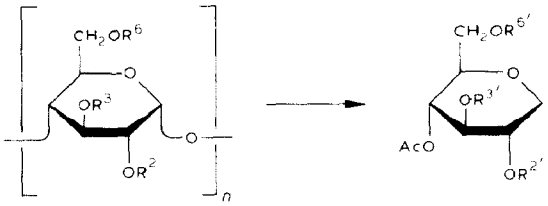

1 2

was hydrolysed, and the resulting products were reduced with $NaBD_4$ and acetylated. A second portion was reductively depolymerized, using various Lewis acids in combination with triethylsilane (Et_3SiH) as a hydride donor, and the resulting partially methylated anhydroglucitols were acetylated, fractionated by g.l.c., and identified by g.l.c.–m.s. When trimethylsilyl trifluoromethanesulfonate was the catalyst, the benzyl groups were partially cleaved. The extent of cleavage depended on the position substituted (2, 3, 6), the time of reaction, and amount of reagents, but was incomplete. The 2-*O*-benzyl group was the most stable, presumably reflecting the influence of the anomeric centre.

When the catalyst was a mixture of trimethylsilyl methanesulfonate and boron trifluoride etherate ($BF_3 \cdot OEt_2$)[11], all of the benzyl groups were cleaved and Fig. 1 shows the gas chromatogram of the resulting partially methylated 1,5-anhydroglucitol acetates.

Complete debenzylation results in higher volatility and uniformity of the reaction products. In addition, the calculation of the different molar responses in the flame-ionization detector [effective carbon response (e.c.r.)-concept[12,13]] is more reliable and the identification of the products by g.l.c.–m.s. is facilitated. However, information about undermethylation and the connection between the "anhydroglucose" units is lost. This last argument, however, is less relevant, since

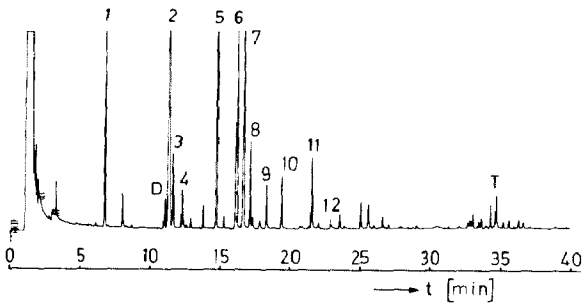

Fig. 1. Gas chromatogram of the products of reductive depolymerization of benzylated starch with complete loss of benzyl groups. G.l.c. conditions: 20-m capillary column with OV1; carrier gas, H_2 0.8 bar; temp. program: 3°/min from 100° to 260°. Key to derivatives of 1,5-anhydro-D-glucitol: *1* 2,3,4,6-tetra-*O*-methyl, *2* 4-*O*-acetyl-2,3,6-tri-*O*-methyl, *3* 6-*O*-acetyl-2,3,4-tri-*O*-methyl, *4* 3-*O*-acetyl-2,4,6-tri-*O*-methyl, *5* 3,4-di-*O*-acetyl-2,6-di-*O*-methyl, *6* 4,6-di-*O*-acetyl-2,3-di-*O*-methyl, *7* 2,4-di-*O*-acetyl-3,6-di-*O*-methyl, *8* 2,6-di-*O*-acetyl-3,4-di-*O*-methyl, *9* 2,3,4-tri-*O*-acetyl-6-*O*-methyl, *10* 3,4,6-tri-*O*-acetyl-2-*O*-methyl, *11* 2,4,6-tri-*O*-acetyl-3-*O*-methyl, *12* 2,3,4,6-tetra-*O*-acetyl. D,T.: dimers and trimers formed from cleared benzyl groups.

the gross structure of the starches is known. Furthermore, the cleaved benzyl groups form di- and tri-mers which may interfere in g.l.c.

The stability of the 6-*O*-benzyl group was investigated by reducing methyl 6-*O*-benzyl-2,3,4-tri-*O*-methyl-α-D-glucopyranoside. With $BF_3 \cdot OEt_2$ as the catalyst, no cleavage occurred but reduction was incomplete. When a larger excess of $BF_3 \cdot OEt_2$ and longer reaction times were used, partial loss of benzyl groups occurred. With trimethylsilyl triflate and trimethylsilyl mesylate/$BF_3 \cdot OEt_2$, cleavage of the benzyl groups was complete.

The mixtures of products were analyzed by capillary g.l.c., using an on-column injection system, in order to eliminate discrimination of less-volatile substances[14]. For quantification, the peak areas were corrected by using the e.c.r.-concept[12,13]. The constituents of the mixtures were identified by g.l.c.–m.s. [e.i. and c.i. (NH_3)]. The type and number of substituents were derived from the molecular weights of the products as obtained from the c.i.-mass spectra (Fig. 2). The mass spectra of some components have been described[15,16]. Table I contains the mass spectra of the other main anhydroglucitol derivatives formed by reductive depolymerization of benzyl starches. The data for 3,4-di-*O*-acetyl-1,5-anhydro-2,6-di-*O*-methyl-D-glucitol (*5*) differ from those reported[16].

The fragmentation of the partially methylated and acetylated anhydro-

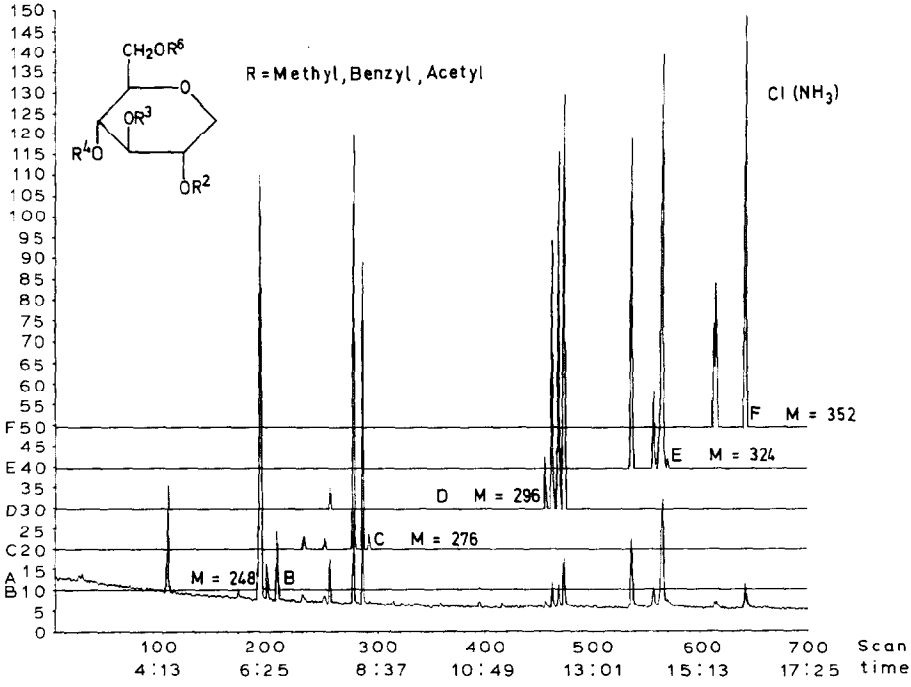

Fig. 2. Selected ion chromatogram [g.l.c.–m.s. (c.i.)] of the products obtained after reductive depolymerization of benzyl starch. Traces B–F, $[M + 1]^+$ ions. Number and type of substituents: mol. wt. 248, Me_3Ac; 276, Me_2Ac_2; 296, Me_3Bn; 324, Me_2AcBn; 352, $MeAc_2Bn$.

TABLE I

FRAGMENTATIONS OF METHYLATED AND ACETYLATED ANHYDROGLUCITOL DERIVATIVES[a] OBTAINED BY G.L.C.–M.S. (E.I. 70 eV)

Compound	m/z *(% of base peak)*
7	43(100), 71(17), 74(7), 87(15), 97(27), 103(2), 111(19), 129(10), 139(20), 147(4), 157(14), 171(7), 199(8), 231(6)
5	43(100), 58(17), 71(5), 74(6), 87(12), 97(59), 111(11), 125(5), 129(28), 139(3), 142(10), 143(2), 158(1), 171(30), 174(3), 184(6), 231(3)
9	43(100), 75(2), 87(5), 88(6), 97(22), 115(2), 139(15), 142(3), 157(11), 184(2), 199(10), 259(3)
11	43(100), 71(16), 74(9), 87(17), 97(8), 99(5), 103(5), 111(23), 115(3), 117(7), 124(4), 129(11), 139(4), 145(2), 157(3), 159(4), 171(3), 184(8), 199(1), 202(0.4), 231(1)
10	43(100), 58(25), 74(6), 87(6), 97(12), 103(4), 111(5), 115(2), 126(3), 128(3), 129(8), 142(15), 159(1), 170(13), 171(4), 184(1), 202(0.5), 212(4), 231(0.2)
12	43(100), 85(12), 87(2), 97(22), 103(5), 110(15), 111(5), 115(11), 127(7), 128(9), 129(2), 139(8), 145(2), 157(7), 170(21), 171(4), 187(1), 199(1), 212(7), 259(0.2)

[a]See Fig. 1.

glucitols during e.i.-m.s. is similar to that of the methyl glycosides[17–20]. The interpretation was corroborated by the mass shifts observed with the corresponding ethylated derivatives. Usually, the exocyclic residue is lost from the molecular ion, leading to fragments *m/z* 231 (*7*, *5*, *9*, and *10*) and 259 (*11* and *12*), on the basis of which the 6-substituent can be identified. Loss of AcOH (−60) or MeOH (−32) then occurs, preferentially from position 3, and with a greater probability for the former. The mass spectrum of 2,4-di-*O*-acetyl-1,5-anhydro-3,6-di-*O*-methyl-D-glucitol (*7*) contains secondary fragment ions at *m/z* 199, 171, and 111 due to the loss of MeOH and one or two molecules of AcOH, respectively. Acetylated fragments may also lose ketene (−42). In this way, *m/z* 129 is formed from *m/z* 171 (*5*). As a consequence of the increasing tendency to eliminate AcOH or ketene, the higher acetylated derivatives show more fragments with even mass numbers: M^+ *m/z* 332 → 212 (−2 × 60) → 170 (−42) → 110 (−60) in the mass spectrum of the 2,3,4,6-tetra-*O*-acetyl-1,5-anhydro-D-glucitol (*12*).

In addition to the energetically favourable cyclic oxonium ion fragments, two C_3 fragments are formed, as from hexopyranosides, involving C-2,3,4 (A, R^4O–CH=CH–CH=$\overset{+}{O}R^2$) or C-4,5,6 (B, $R^4\overset{+}{O}$=CH–$\dot{C}$H–CH_2OR^6).

A list of masses and intensities of 13 fragment ions has been reported[16] for 3,4-di-*O*-acetyl-1,5-anhydro-2,6-di-*O*-methyl-D-glucitol (*5*). Instead of *m/z* 86, 128,

and 141, we observed fragments one mass unit higher, and *m/z* 99 was absent. The ion *m/z* 142 arises from the loss of MeOH, AcOH, and ketene, *m/z* 129 is due to the elimination of AcOH and ketene after cleavage of the exocyclic residue, and *m/z* 87 originates from fragment A after loss of ketene.

Table II shows the quantitative results for the distribution of benzyl groups for two benzylated starches. 2-Substitution preponderates as expected for kinetically controlled alkylation reactions where the reaction rate corresponds to the acidity of the hydroxyl group. The formation of the anion is the rate-determining step.

Benzylated starches were also investigated by standard methylation analysis. However, the reproducibility of the results was unsatisfactory, due to partial loss of benzyl groups during hydrolysis. The d.s. values obtained were too low.

EXPERIMENTAL

Benzylated starches were obtained from Maizena GmbH Kleve. Methylation was carried out according to Ciucanu and Kerek[10] with 3 equiv. of NaOH and 3 equiv. of MeI per replaceable H.

Reductive depolymerization was carried out according to Gray *et al.*[6,7,11]. To a solution of the methylated benzyl starches (~1 mg) in dichloromethane (200 μL) in a screw-cap vial were added 5–10 equiv. of triethylsilane and Lewis acid [tri-

TABLE II

DISTRIBUTION OF SUBSTITUENTS IN THE BENZYL STARCHES BS-1 AND BS-2, DETERMINED BY THE REDUCTIVE-DEPOLYMERIZATION METHOD[a]

Position substituted	*(Mole%)*	
	BS-1[b]	*BS-2*[b]
--	66.1	51.2
2	14.8	19.0
3	8.8	8.5
6	6.0	11.9
2,3	0.9	1.1
2,6	1.9	4.6
3,6	0.8	1.2
Not defined	0.4	1.7
2,3,6	0.3	0.8
Unsubstituted AGU[c]	66.1	51.2
Monosubstituted AGU	29.6	39.4
Disubstituted AGU	4.0	8.6
Trisubstituted AGU	0.3	0.8
D.s.	0.39	0.59

[a]Values are corrected for branching of the natural starch. [b]Average value of 7 determinations. [c]"Anhydroglucose" unit.

methylsilyl triflate, $BF_3 \cdot OEt_2$, or a mixture of trimethylsilyl mesylate and $BF_3 \cdot OEt_2$ (5:1)], and the mixture was stirred for 2–24 h at room temperature. In reactions catalyzed by trimethylsilyl triflate, acetylation was performed by the addition of acetic anhydride (20 μL). After stirring for 2 h, the solution was washed with saturated aqueous sodium hydrogencarbonate and then with water, dried ($CaCl_2$), and subjected to g.l.c. Reaction mixtures containing $BF_3 \cdot OEt_2$ were quenched with methanol and deionized with Bio-Rad AG 501-X8 D resin, and the products were acetylated with *N*-methylimidazole and acetic anhydride. The solutions were washed as described above.

Standard methylation analysis. — Methylated benzyl starches were hydrolyzed with 2M trifluoroacetic acid at 120° for 2–4 h. After removal of the acid, the samples were reduced in 2M ammonia with $NaBD_4$ for 1 h at 60°. The solutions were acidified with acetic acid, and boric acid was removed as its methyl ester. Acetylation was carried out with acetic anhydride and pyridine in the usual way.

G.l.c. was performed on a Carlo Erba Fractovap 4160 instrument with an on-column injection system for quantitative analysis and a Carlo Erba Fractovap 2101 Ac with split injection and flame-ionization detector. G.l.c.–m.s. was performed with a Hewlett–Packard 5840 A/5985 A, Finnigan MAT 311 A, or Vacuum-Generators/70-250S instrument. For c.i., ammonia was used as reactant gas.

ACKNOWLEDGMENTS

We thank Maizena GmbH and the Bundesministerium für Forschung und Technologie (Projekt-Nr. 0319134 A) for financial support.

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