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CHARACTERIZATION OF CYCLOMALTO-HEXAOSE AND -HEPTAOSE DERIVATIVES BY THE REDUCTIVE-CLEAVAGE METHOD

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ABSTRACT

The substitution patterns of cyclomalto-hexaose and -heptaose derivatives carrying alkyl, acyl, and carbamoyl substituents have been investigated by the reductive-cleavage method. The modified cyclomalto-hexaoses or -heptaoses were treated with triethylsilane and trimethylsilyl trifluoromethanesulfonate to give the corresponding 1,5- and 1,4-anhydroglucitol derivatives that were acetylated or, in the case of acetyl derivatives, trifluoroacetylated and analysed by g.l.c.–m.s. For the alkylated compounds, minute amounts of products formed by under- or over-alkylation, or of isomeric components, could be detected. Partial reduction of the acyloxy groups to alkyloxy groups and cleavage of acyl substituents were observed. Carbamoyl substituents were stable under the conditions of reductive cleavage.

INTRODUCTION

The low reactivity of HO-3 of each glucose residue in cyclomalto-hexaose (α-CD) and -heptaose (β-CD) can be ascribed to hydrogen bonding and steric hindrance due to their direction in the cavity[1,2]. Efforts have been made to prepare symmetrical mono- and di-substituted derivatives[3], *e.g.*, 2,6-di-*O*-pentylated α- and β-CD, which, after acetylation of HO-3, can be used in capillary g.l.c. as chiral stationary phases that interact with high enantioselectivity with chiral substrates[4,5].

Regioselective derivatization depends on the reaction conditions and the substituent; thus, the pentyl group can be introduced more selectively than the smaller methyl group.

High-field n.m.r. spectroscopy has been used[6] to prove the uniformity of modified CDs. Spencer *et al.*[7] located all, and assigned most, of the resonances for the heterotopic protons and carbon atoms in the unsymmetrically substituted derivatives of β-CD after separation from the main symmetrical derivative. However, <5% of impurities are difficult to detect due to the complexity of the spectra and the large number of possible, unsymmetrically substituted CDs, especially for

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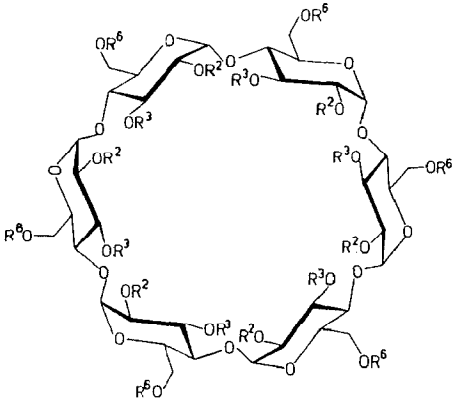
derivatives with C_4–C_6 alkyl substituents. Furthermore, f.a.b. mass spectra could not be obtained for the highly hydrophobic derivatives of CDs. Gray and co-workers[8–10] introduced reductive depolymerization of methylated polysaccharides for structural analysis, and we have applied this method to starch and cellulose ethers and esters[11,12]. We now report the application of reductive depolymerization for the characterization of substituted CDs.

RESULTS AND DISCUSSION

Treatment of substituted cyclomaltohexaose (**1**) with triethylsilane in the presence of a Lewis acid affords the corresponding 1,5-anhydro-D-glucitol



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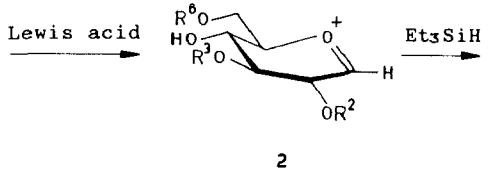

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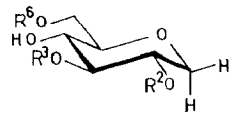

3

	R^2	R^3	R^6
Alkyl derivatives[a]			
4	Me	OH	Me
5	Me	Me	Me
6	Me	Pn	Me
7	Bu	OH	Bu
8	Bu	Bu	Bu
9	Pn	OH	Pn
10	Pn	Pn	Pn
11	Pn	Me	Pn
12	Hx	OH	Hx
13	Hx	Pn	Hx
14	Hx	Hx	Hx
15	^{i}Pn	OH	^{i}Pn
Carbamoyl derivatives			
16	Pn	CONHPh	Pn
17	Pn	$CONEt_2$	Pn
Acyl derivatives			
18	Pn	Ac	Pn
19	COBu	COBu	COBu

[a] Pn = pentyl ; Hx = hexyl ; ^{i}Pn = isopentyl

derivative **3** (*via* **2**) which, after acetylation or trifluoroacetylation, was amenable to g.l.c.–m.s. and could be quantified by capillary g.l.c. Peak areas were corrected with the molar response factors, using the e.c.r. concept[13,14]. Methylation, as usually performed in the structural analysis of polysaccharides, is unnecessary when the substituents are stable under reductive cleavage conditions and the derivatives are soluble in dichloromethane.

Trimethylsilyl trifluoromethanesulfonate was used as the Lewis acid. Trimethylsilyl methanesulfonate/boron trifluoride etherate could not be used, since "anhydroglucose" units with HO-3 unsubstituted were recovered incompletely from the mixture of degradation products.

Alkyl derivatives (**4–15**). — All alkyl substituents were stable under the conditions of reductive cleavage. Fig. 1 shows the gas chromatograms of the degradation products of 2,6-di-*O*-pentyl-α-CD (**9**) and 2,3,6-tri-*O*-pentyl-β-CD (**10**). The minor peaks are the 5-*O*-acetyl-1,4-anhydro-D-glucitol derivatives, which were formed when trimethylsilyl trifluoromethanesulfonate was used as the Lewis acid[9]. The quantitative results are listed in Table I. Whereas only traces of underalkylation could be detected in the preparation of **10**, the disubstituted derivative **9** contained ~6 mol% of tripentylated "anhydroglucose" units. In Table I, the composition of two samples with high and low uniformity are listed. Monopentylated "anhydroglucose" units were identified also. Even so, the selectivity of the pentylation reaction was higher than that of methylation, for which 10 mol% of overmethylated "anhydroglucose" units were found in commercially available 2,6-di-*O*-methyl-β-CD, corresponding to 30–40% of unsymmetrically substituted β-CD. Much higher selectivity was achieved with isopentylation (→**15**); there was only 1.8 mol% of overalkylated and no mono- or isomeric di-substituted "anhydroglucose" units.

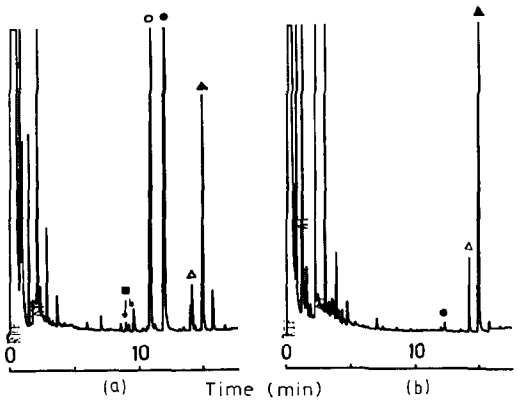

Fig. 1. Gas chromatograms of the degradation products of (*a*) heptakis(2,6-di-*O*-pentyl)cyclomaltoheptaose and (*b*) heptakis(2,3,6-tri-*O*-pentyl)cyclomaltoheptaose. G.l.c. conditions: 25-m capillary column of CP Sil8-CB; H_2 0.8 bar; temperature program: 50°, 30°/min → 160°, 5°/min → 280°. 1,5-Anhydro-D-glucitol derivatives: ■, tri-*O*-acetyl-*O*-pentyl-; ●, 3,4-di-*O*-acetyl-2,6-di-*O*-pentyl-; ▲, 4-*O*-acetyl-2,3,6-tri-*O*-pentyl-; ○,△, 5-*O*-acetyl-1,4-anhydro-D-glucitol derivatives.

TABLE I

DISTRIBUTION OF SUBSTITUENTS IN CYCLOMALTO-HEXAOSE AND -HEPTAOSE DERIVATIVES OBTAINED BY THE REDUCTIVE-CLEAVAGE METHOD

One type of substituent

Compound	*CD*	*Monosubstituted*			*Disubstituted*			*Trisubstituted*
		2	*3*	*6*	*2,6*	*2,3*	*3,6*	*2,3,6*
4	β	—	—	—	89.3	0.6	—	10.1
5	α	—	—	—	7.3	—	—	92.7
	β	—	—	—	5.4	0.6	0.8	93.2
7	α	—	—	—	84.7	—	—	15.3
8	α	—	—	—	2.2	—	—	97.8
9	α		Σ1.2		90.6	—	—	8.2
	β	—	—	—	94.4	—	—	5.6
	β	1.9	—	8.8	74.4	0.4	0.9	13.5
10	α	—	—	—		Σ0.3		99.7
	β	—	—	—		Σ0.4		99.6
12	α		(Σ<0.2)		85.0	Σ0.5		14.5
14	α	—	—	—	0.2	—	—	99.8
15	β	—	—	—	98.2	—	—	1.8

Second type of substituent at position 3

Sample (synthesised from)	*CD*	*Degree of 3-substitution (mol %)*
6 (4)	β	98.9
11 (9)	α	99.9
16 (9)	α	99.6
17 (9)	β	73.2
13 (12)	α	100.0

The 2,6-di-*O*-alkylated CDs **4** (Me), **9** (pentyl), and **12** (hexyl) were subsequently 3-substituted. The degrees of substitution are shown in the second part of Table I. Nearly quantitative substitution was achieved in most reactions.

Carbamoyl derivatives (**16** *and* **17**). — Carbamoyl substituents were also stable under conditions of reductive cleavage. The degrees of 3-substitution for 2 different types are given in Table I.

Acyl derivatives (**18** *and* **19**). — Triethylsilane does not usually attack acyl groups[15]. However, under the conditions of reductive cleavage, acyloxy groups can be reduced to alkyloxy groups, depending on the position of substitution[11,12]. Thus, most of the 6-*O*-acetyl groups were reduced to ethyl groups. Furthermore, for β-CD valerate, a minor proportion of the acyl residues were reduced but most were cleaved, resulting in a complex mixture of anhydroglucitol derivatives.

The reductive-cleavage method is a rapid and simple procedure for determining the degree of substitution in alkyl and carbamoyl derivatives of cyclomalto-oligosaccharides, and <0.1 mol% under- or over-substituted products can be detected by capillary g.l.c.–m.s.

EXPERIMENTAL

Cyclomalto-hexaose and -heptaose derivatives. — 2,6-Di-*O*-methyl-β-CD was purchased from Aldrich; α-CD and β-CD were alkylated according to Ciucanu and Kerek[16], and then using sodium hydride as the base[4]. The *N*-phenylcarbamate was prepared with an excess of the corresponding isocyanate in pyridine at 100° and purified by gel chromatography. All other derivatives investigated were synthesised by Wenz and von der Bey[17].

Reductive cleavage. — The procedure of Gray *et al.*[9] was used. For cyclomalto-oligosaccharides carrying acetyl groups, a modified procedure was applied. After reductive cleavage, the reaction mixture was quenched with methanol, deionised with a mixed-bed resin [Bio-Rad AG 501-X8(D)], and concentrated to dryness, and the residue was treated with trifluoroacetic anhydride (100 μL) in dichloromethane (100 μL) for 1 h at room temperature. Reagents were removed, and the products were dissolved in dichloromethane for g.l.c.

G.l.c. — Quantitative g.l.c. was carried out on a Carlo Erba Fractovap 4160 gas chromatograph, equipped with an on-column injection system, a flame-ionisation detector, and an integrator 3390A from Hewlett–Packard. See Fig. 1 for the conditions.

G.l.c.–m.s. — E.i. mass spectra were obtained with a Carlo Erba 2150 gas chromatograph coupled to a Finnegan MAT-311-A mass spectrometer. C.i. (ammonia) mass spectra were obtained with a Hewlett–Packard HP-5840-A/5985-A g.l.c.–m.s. system.

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