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DETERMINATION OF THE PATTERNS OF SUBSTITUTION OF HYDROXYETHYL- AND HYDROXYPROPYL-CYCLOMALTO-HEPTAOSES

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ABSTRACT

2-Hydroxyethyl- and 2-hydroxypropyl-cyclomaltoheptaoses (HE-βCD and HP-βCD) with various molar degrees of substitution have been investigated by the reductive-cleavage method and methylation analysis. As expected for alkali-catalysed etherifications, mainly HO-2 was substituted.

INTRODUCTION

Cyclomalto-oligosaccharides (cyclodextrins, CDs), especialy βCD (cyclomaltoheptaose), are not very soluble in water due[1] to the strong hydrogen bond between HO-2 and O-3. Solubility in water, as required for biomedical applications, can be achieved by hydroxyalkylation. The products of such derivatization processes must be thoroughly characterised.

Hydroxyalkylated CDs are also good model compounds for the analysis of the corresponding derivatives of starch and cellulose. The mixtures of degradation products are less complex, because there are no terminal or branched glucose residues.

The molar degree of substitution (m.d.s.) can be calculated on the basis of n.m.r. data[2,3] or by cleavage of the substituent with hydriodic[4] or hydrobromic acid[5] and identification of the products. Pitha *et al.*[6] determined m.d.s. values for HP-CD by plasma desorption-m.s. However, they could not differentiate substituents at positions 2, 3, and 6, and assumed that HO-6 would be the most reactive as found for HE-celluloses[5,7] and HP-guar[8].

The patterns of substitution of hydroxyalkyl-starches and -celluloses have been investigated by acid hydrolysis and separation of the resulting glucose derivatives after trimethylsilylation or by methylation analysis[5,7,12]. Usually, HO-2 was found to possess the highest reactivity. However, at higher concentrations of alkali during the reaction with the alkylene oxide, HO-6 was the most reactive.

We have applied the reductive-cleavage method[13–15] to *O*-(2-hydroxy-3-phenoxypropyl)starch[16] and also to characterise CD derivatives[17], and now report

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its application to HE-βCD and HP-βCD in comparison with methylation analysis.

RESULTS AND DISCUSSION

Each HE-βCD and HP-βCD was methylated according to the method of Cincanu and Kerek[18]. The products were (*a*) subjected in sequence to hydrolysis, reduction with $NaBD_4$, and acetylation (standard methylation analysis[19]); and (*b*)

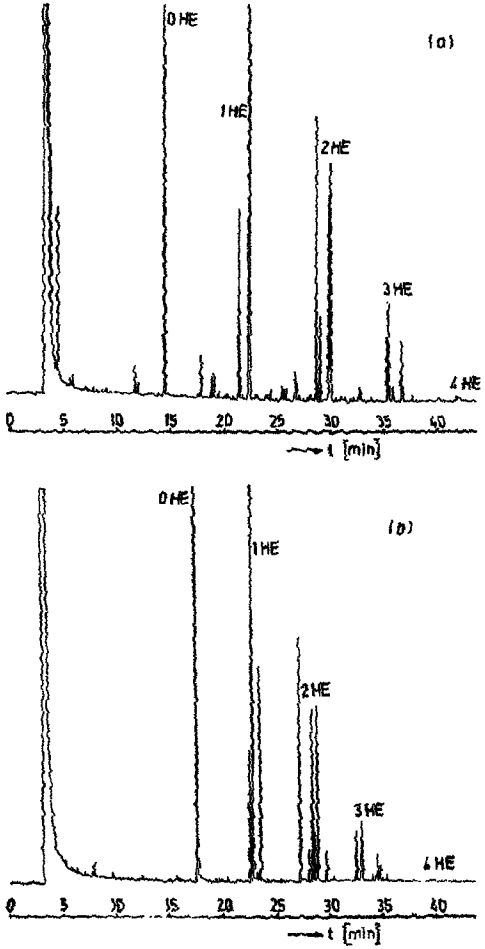

Fig. 1. Gas chromatograms of the mixtures of degradation products of HE-βCD (*6*) obtained by (*a*) reductive-cleavage and (*b*) methylation analysis; 40-m capillary column DB5; H_2 at 0.7 bar; temperature programs: (*a*) 130°, 4°/min → 270°; (*b*) 160°, 4°/min → 270°. 0–4 HE are the numbers of hydroxyethyl residues per glucosyl residue.

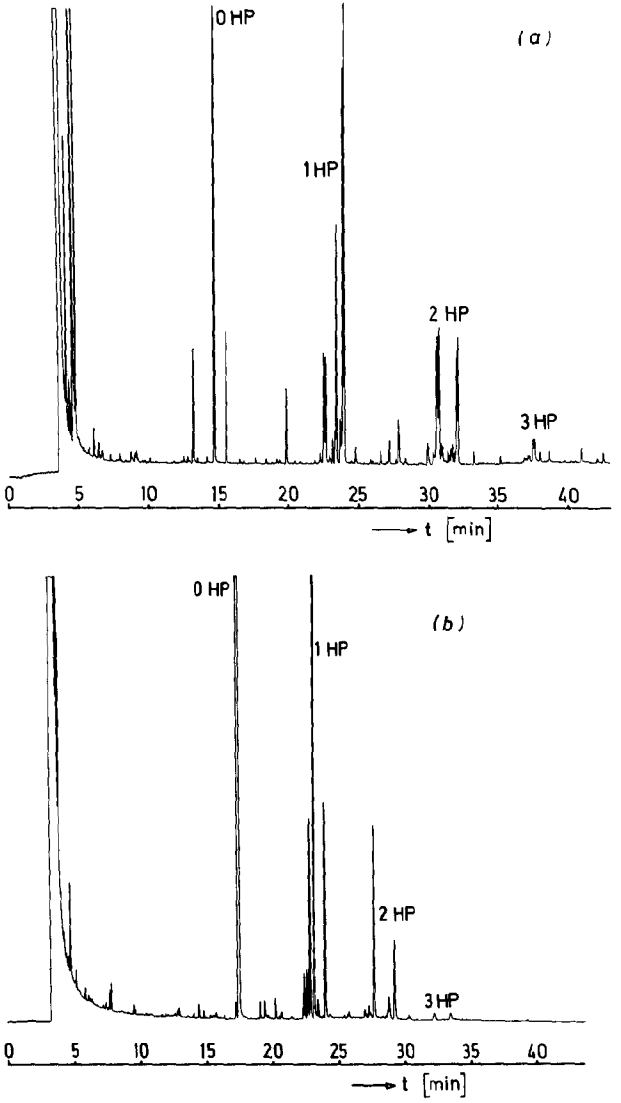

Fig. 2. Gas chromatograms of the mixtures of degradation products of HPCD 0.9 obtained by (*a*) reductive-cleavage and (*b*) methylation analysis. G.l.c. conditions as in Fig. 1. 0–3 HP are the numbers of hydroxypropyl residues per glucosyl residue.

cleaved to anhydroglucitols, using trimethylsilyl methanesulfonate–boron trifluoride etherate and triethylsilane, and then acetylated (reductive-cleavage[15]). Whereas HE groups are stable under reductive-cleavage conditions, HP groups may be cleaved due to the greater stability of the $[Me_2COMe]^+$ ion, which can be formed after cleavage. When 5 equiv. of triethylsilane and the catalyst mixture were applied for 24 h at room temperature, loss of substituents did not occur.

The mixtures of products from (*a*) and (*b*) were analysed by g.l.c. (Figs. 1 and 2) and g.l.c.–m.s. Two stereoisomers are formed when a hydroxyl group in a glucose residue reacts with propylene oxide. Only peak broadening was observed for the diastereoisomeric HP-glucitol ethers, and most of the diastereoisomeric anhydroglucitol derivatives were separated only partially.

Mass spectra. — The number of substituents in the products of degradation can be deduced from the c.i.-mass spectra on the basis of the increase of 44 mass units for every HE group and 58 mass units for an HP group, respectively. The position of substitution can be deduced from the c.i.-mass spectra. The fragmentation of the glucitol ethers follows the pathways outlined for partially methylated alditol acetates[20] and has been discussed for their 2-hydroxyethyl derivatives[5]. A small proportion of a fourth monosubstituted HP derivative was found, which had the same mass spectrum as 2-*O*-HP-glucitol. Presumably, this product was the *O*-(2-hydroxy-1-methylethyl) isomer formed by attack of the sugar anion on C-2 of the propylene oxide.

Ions of low *m/z* values characteristic for the substitution pattern of *O*-acetylated 1,5-anhydro-tri-*O*-methyl-D-alditols have been discussed[21] but, for deuterium-labelled methylated anhydroalditols, the origin of fragments becomes more ambiguous with decreasing *m/z* values[22]. The fragments A1 and A2 (Table I), resulting from consecutive losses of the exocyclic residue and elimination of R^3OH, are diagnostic for positional isomers[23]. In investigations of the products of reductive-cleavage of modified starches[16,24,25] and CDs[17], characteristic increased *m/z* values were found for some of the higher fragments when a methyl group was replaced by another alkyl substituent in a certain position. Selected fragments of 4-*O*-acetyl-1,5-anhydro-2,3,6-tri-*O*-methyl-D-glucitol and the corresponding fragments of the HE- and HP-derivatives are listed in Table I. The primary fragmentation step for most of these fragments is the cleavage of the exocyclic residue (→ A1). However, the 6-*O*-substituted derivatives can be recognised easily from their molecular mass (from c.i.-m.s.), the S fragment ions, and the ions B1′ and B2′.

In competition with the favoured elimination of R^3OH (→ A2) is the loss of HOAc (= R^4OH) to give A3, because acids are more easily eliminated than alcohols. The 2-*O*-HE derivatives give almost all of the selected fragment ions increased by the mass of substituent due to the relative stability of the 2-substituent. A4 consists of two isomers that contain only R^2 or only R^3, respectively. Because elimination is preferred from C-3, only a few fragment ions of the 3-substituted anhydroglucitols are increased. Thus, in addition to A4′, the primary fragment A1 [unsubstituted *m/z* 203) has *m/z* 247 (HE) or 261 (HP), and, after consecutive loss of HOAc, A3 (unsubstituted *m/z* 143) has *m/z* 187 (HE) or 201 (HP)]. For disubstituted derivatives, the *m/z* values are observed as listed for 2,3- and 2,6-disubstituted (HE,HP) derivatives. For the 2,2′-disubstituted products, originating from the reaction of a HE substituent with ethylene oxide, increases in *m/z* of 2×44 mass units are found. Additionally, there are some fragment ions originating from

TABLE I

SELECTED FRAGMENT IONS OF 4-*O*-ACETYL-1,5-ANHYDROGLUCITOLS WITH *O*-Me AND *O*-HE OR *O*-HP SUBSTITUENTS OBTAINED BY G.L.C.-M.S. (E.I., 70 eV)

Position of Me	*2,3,6*	*3,6*	*2,6*	*2,3*	*6*	*3*	*3,6*	*3,6*	*2,6*	*2,3*	*6*	*3*
HE	–	*2*	*3*	*6*	*2,3*	*2,6*	*2,2'*	–	–	–	–	–
HP	–	–	–	–	–	–	–	*2*	*3*	*6*	*2,3*	*2,6*
Fragment ion	m/z *(% of base peak)*											
M[a]	248	292	292	336	336	336	336	306	306	306	364	364
A1 (M − CH_2OR^6)	203	247	247	203	291	247	291	261	261	203	319	261
	7.8	6.5	5.9	19	3.5	9.6	17	6.8	2.6	10	0.6	8.2
A2 (A1 − R^3OH)	171	215	171	171	215	215	259	229	171	171	229	229
	16	6.2	36	36	18	7.4	6.1	2.9	29	22	5.7	2.5
A3 (A1 − HOAc)	143	187	187	143	231	187	231	201	201	143	259	201
	15	20	33	17	27	14	46	19	36	11	12	9.3
A4 (A2 − HOAc)	111	155	111	111	155	155	199	169	111	111	169	169
	7.6	6.9	9.0	19	16	7.4	13	4.7	7.1	12	8.7	4.5
A4' (A3 − R^2OH)	111	111	155	111	155	111	111	111	169	111	169	111
	7.6	11	6.2	19	16	16	35	14	6.5	12	8.7	17
A5 (A2 − CH_2=C=O)	129	173	129	129	173	173	217	187	129	129	187	187
	21	16	36	26	22	23	44	11	18	12	6.9	15
B1 (AcOCH= CH	129	173	129	129	173	173	217	187	129	129	187	187
CH=$\overset{+}{O}R^2$)	21	16	36	26	22	23	44	11	18	12	6.9	15
B1' CH=$\overset{+}{O}R^6$)	129	129	129	173	129	173	129	129	129	187	129	187
(AcOCH=CH	21	8.3	36	14	10	23	15	6.3	18	15	2.0	15
B2 (B1 − CH_2=C=O)	87	131	87	87	131	131	175	145	87	87	145	145
	21	3.8	24	15	6.3	9.3	10	4.7	17	8.2	2.2	6.2
B2' (B1' − CH_2=C=O)	87	87	87	131	87	131	87	87	87	145	87	87
	21	19	24	16	11	9.3	42	8.9	17	8.7	3.5	5.4
S1 ($CH_3CR=\overset{+}{O}CH_3$)[b]	–	59	59	59	59	59	59	73	73	73	73	73
		100	100	61	100	100	100	50	62	34	100	70
S2 ($CH_3CH=\overset{+}{O}CH_2$	–	–	–	–	–	–	103	–	–	–	–	–
CH_2OCH_3)							59					
S3 ($CHR=\overset{+}{O}CH_3$)[b]	–	–	–	–	–	–	–	59	59	59	59	59
								100	100	100	76	100

[a]Deduced from c.i.-m.s. [b]R = H (HE), R = Me (HP).

TABLE II

DISTRIBUTION OF SUBSTITUENTS IN HYDROXYETHYLCYCLOMALTOHEPTAOSES (HE-βCD) OBTAINED BY REDUCTIVE CLEAVAGE (*A*) AND STANDARD METHYLATION ANALYSIS (*B*)

Position substituted	*HE-βCD 0.6*		*HE-βCD 1.0*		*HE-βCD 1.6*	
	A	B	A	B	A	B
	(mol%)					
–	58.7	59.3	41.9	43.8	26.3	26.2
2	22.7	21.4	27.9	25.0	23.9	22.6
3	6.1	6.3	5.9	6.2	4.3	4.5
6	3.6	3.8	5.0	5.2	6.4	6.6
2,3	4.2	4.4	7.4	7.5	8.0	8.5
3,3′	Σ0.9	0.6	Σ1.6	1.2	Σ2.8	1.7
3,6		0.4		0.8		1.2
2,6	1.5	1.5	3.3	3.3	7.0	6.2
2,2′	1.3	1.3	3.6	3.1	7.3	6.6
6,6′		0.2		0.4		1.1
2,3,6	Σ0.9	0.3	1.3	0.9	1.5	2.2
2,2′,3		Σ0.5	1.0	1.6	Σ9.1	4.6
3,3′,6			Σ0.8	Σ0.2		0.4
3,3′,3″						0.1
2,2′,6				0.4		1.9
2,6,6′				0.2		1.1
3,6,6′						0.2
2,2′,2″						0.6
2,2′,3,3′ + 2,3,3′,6			Σ0.3	Σ0.2	1.0	1.2
2,3,6,6′					Σ1.6	0.3
2,2′,3,6(6,6′)						0.5
2,2′,2″,3 + 2,6,6′,6″						Σ0.5
2,2′,2″,6						0.4
2,2′,2″,2‴						0.3
Unsubstituted	58.7	59.3	41.9	43.8	26.3	26.2
ΣMonosubstituted	32.4	31.5	38.8	36.4	34.6	33.7
ΣDisubstituted	7.9	8.4	15.9	16.3	25.3	25.3
ΣTrisubstituted	0.9	0.8	3.1	3.3	10.6	11.1
ΣTetrasubstituted			0.3	0.2	2.6	3.2
ΣPentasubstituted					0.5	0.5
M.d.s.	0.51	0.51	0.81	0.80	1.30	1.33

TABLE III

DISTRIBUTION OF SUBSTITUENTS IN HYDROXYPROPYLCYCLOMALTOHEPTAOSES (HP-βCD) OBTAINED BY REDUCTIVE CLEAVAGE (*A*) AND STANDARD METHYLATION ANALYSIS (*B*)

Position substituted	*HP-βCD 0.6*		*HP-βCD 0.9*	
	A	B	A	B
	(mol%)			
–	54.4	57.9	35.7	41.2
2	24.4	23.3	29.7	27.7
3	4.8	4.9	6.3	5.0
6	5.6	4.9	7.1	6.5
2,3	6.4	5.1	11.3	9.9
2,6	3.6	2.6	5.8	6.0
3,6	0.6	0.7	1.8	1.5
2,3,6 (+2,2',x)	0.2	0.6	2.3	2.2
Unsubstituted	54.4	57.9	35.7	41.2
ΣMonosubstituted	34.8	33.1	43.1	39.2
ΣDisubstituted	10.6	8.4	18.9	17.4
ΣTrisubstituted	0.2	0.6	2.3	2.2
M.d.s.	0.57	0.52	0.88	0.81

the substituent: *m/z* 59 (S1) from the HE substituent is the base peak of all derivatives, with the exception of those that are 6-substituted where *m/z* 43 (Ac^+) is the base peak. For x,x'-substitution, the intensity of *m/z* 103 (S2, 59 + 44) increases. For all HP-derivatives, the base peak is *m/z* 59 (S3) formed by α-cleavage of the substituent with the exception of the 2,3-HP- disubstituted anhydroglucitol, where *m/z* 73 (S1) is the base peak. The relative intensity of the ion S3 is not listed for HE-derivatives, because the ion *m/z* 45 also has other origins.

Quantitative results. — The molar ratios of the components of the mixture of degradation products were obtained from the peak areas in g.l.c. after correction with molar response factors calculated by the e.c.r.-concept[26]. On-column injection was used to avoid diminution of the higher substituted less-volatile products. The compositions for three samples of HE-CD and two samples of HP-CD (m.d.s. 0.6–1.6) are summarized in Tables II and III. The results of methylation analysis and reductive cleavage agree for the HE-CDs, but the m.d.s. values calculated from reductive-cleavage data are higher for the samples of HP-CD than those found by methylation analysis. The distribution of the substituents is similar for HE-CDs and HP-CDs. As expected for such base-catalysed, kinetically controlled etherifications, HO-2 was substituted preponderantly. Further reaction of the hydroxyalkyl residues with alkylene oxide up to 2,2',2'',2'''-substitution was observed for HE-CD,

but not for the HP-CDs (highest m.d.s., 0.9). Here,the primary HO of the 2-HE is more reactive than the secondary HO of the 2-HP substituent. Therefore, the m.d.s. values of the HP-CDs are identical with their d.s. values, whereas they are higher for the HE-CDs. The m.d.s. values calculated from the observed composition are slightly lower than the average values, which also may deviate by ~10%. With increasing m.d.s., highly substituted hydroxyalkyl ethers, which have a relatively high contribution to the m.d.s., can escape detection due to their decreasing amount distributed over an increasing number of possible isomers.

Thus, reductive-cleavage is more simple and rapid than methylation analysis, but the 2-HP group is less stable under the former conditions.

EXPERIMENTAL

2-Hydroxyethyl- and 2-hydroxypropyl-cyclomaltoheptaoses were provided by Consortium für Elektrochemische Industrie GmbH, München (F.R.G.).

Methylation[18]. — The βCD derivative (~10 mg) was dried at 80° overnight, and a solution in dry dimethyl sulfoxide was treated with 6 equiv. of powdered NaOH and MeI in a screw-cap vial for 5 h. The products were extracted with $CHCl_3$ and washed 5 times with water.

Standard methylation analysis. — Methylated HE-βCDs and HP-βCDs (~2 mg) were hydrolysed with 2M trifluoroacetic acid at 120° for 3 h in a screw-cap V-vial. 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.

Reductive cleavage was carried out as described[14,15,24]. In order to avoid cleavage of HP-substituents, the mixture of trimethylsilyl mesylate and $BF_3 \cdot OEt_2$ (5:1) must be used as the Lewis acid and >5 equiv. must be avoided.

G.l.c. was performed on a Carlo Erba Fractovap 4160 instrument with an on-column injection system for quantitative analysis, a flame-ionization detector, and a Hewlett–Packard 3390 A integrator, or a Siemens Sichromat 2 instrument. G.l.c.–m.s. was performed with a Hewlett–Packard 5840 A-5985 A instrument (c.i., ammonia) and a Vacuum-Generators/70-250S instrument (e.i.).

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REFERENCES

1 W. SAENGER, *Angew. Chem.*, 92 (1980) 343–361.
2 F. F.-L. HO, R. R. KOHLER, AND G. A. WARD, *Anal. Chem.*, 44 (1972) 178–181.

3 K. KIMURA, T. SHIGEMURA, M. KUBO, AND Y. MARU, *Macromol. Chem.*, 186 (1985) 61–70.
4 P. W. MORGAN, *Anal. Chem.*, 18 (1946) 500–504.
5 B. LINDBERG, U. LINDQUIST, AND O. STENBERG, *Carbohydr. Res.*, 170 (1987) 207–214.
6 J. PITHA, L. SZABO, AND H. M. FALES, *Carbohydr. Res.*, 168 (1987) 191–198.
7 B. LINDBERG, U. LINDQUIST, AND O. STENBERG, *Carbohydr. Res.*, 176 (1988) 137–144.
8 M. MCNEIL AND P. ALBERSHEIM, *Carbohydr. Res.*, 131 (1984) 131–137.
9 J. W. MOURITS, H. G. MERKUS, AND L. DE GALAN, *Anal. Chem.*, 48 (1976) 1557–1562.
10 H. G. MERKUS, J. W. MOURITS, L. DE GALAN, AND W. A. DE JONGH, *Staerke*, 29 (1977) 406–410.
11 K. SOMMERMEYER, F. CECH, M. SCHMIDT, AND B. WEIDLER, *Krankenhauspharmazie*, 8 (1987) 271–278.
12 O. LARM, K. LARSSON, AND O. THEANDER, *Staerke*, 33 (1981) 240–244.
13 D. ROLF AND G. R. GRAY, *J. Am. Chem. Soc.*, 104 (1982) 3539–3541.
14 J. U. BOWIE, P. V. TRESCONY, AND G. R. GRAY, *Carbohydr. Res.*, 125 (1984) 301–307.
15 J.-G. JUN AND G. R. GRAY, *Carbohydr. Res.*, 163 (1987) 247–261.
16 P. MISCHNICK-LÜBBECKE, W. A. KÖNIG, AND M. RADELOFF, *Staerke*, 39 (1987) 425–431.
17 P. MISCHNICK-LÜBBECKE AND R. KREBBER, *Carbohydr. Res.*, 187 (1989) 197–202.
18 F. CIUCANU AND F. KEREK, *Carbohydr. Res.*, 131 (1984) 209–217.
19 H. BJÖRNDAL, C. G. HELLERQUIST, B. LINDBERG, AND S. SVENSSON, *Angew. Chem. Int. Ed. Engl.*, 9 (1970) 610–619.
20 H. BJÖRNDAL, B. LINDBERG, AND S. SVENSSON, *Carbohydr. Res.*, 5 (1967) 433–440.
21 E. BRÅTHEN, M. H. ENGEBRETSEN, AND O. KJOLBERG, *Int. Carbohydr. Symp., XIVth, Stockholm, 1988*, Abstr. A84.
22 M. S. KEMPER, Thesis, University of Minnesota, Minneapolis, 1988.
23 S. G. ZELLER, A. J. D'AMBRA, M. J. RICE, AND G. R. GRAY, *Carbohydr. Res.*, 182 (1988) 53–62.
24 P. MISCHNICK-LÜBBECKE AND W. A. KÖNIG, *Carbohydr. Res.*, 185 (1989) 113–118.
25 P. MISCHNICK-LÜBBECKE, Dissertation, University of Hamburg, 1987.
26 D. P. SWEET, R. H. SHAPIRO, AND P. ALBERSHEIM, *Carbohydr. Res.*, 40 (1975) 217–225.