Determination of Relative Reactivities of Free Hydroxyl Groups in β-Cyclodextrin, Amylose, and Cellulose by Reductive-Cleavage Method

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β-Cyclodextrin, amylose, and cellulose were partially methylated and acetylated in order to examine the relative reactivities of 2-, 3-, and 6-OH groups to alkylation and acylation. The partially methylated samples of the polysaccharides were treated with excess of ethyl iodide and sodium hydroxide in dimethyl sulfoxide to convert all of the free hydroxyl groups to ethyl ether groups. The partially *O*-ethylated and *O*-methylated polysaccharides were reductively cleaved with triethylsilane in the presence of trimethylsilyl methanesulfonate and boron-trifluoride etherate (5 : 1 by mole) and the resulting 4-OH group was acetylated and benzoylated to form mixtures of eight 4-*O*-acyl-1,5-anhydroalditols. The relative ratio of the alditol esters were analyzed by gas chromatography to determine the degree of substitution at each position. A similar sequence of reactions was carried out with partially acetylated polysaccharides. The results indicated that the order of relative reactivities for methylation are 2-OH > 6-OH > 3-OH and for acylation are 6-OH > 2-OH > 3-OH regardless of the anomeric configuration.

Introduction

β-Cyclodextrin, amylose, and cellulose have 1,4-glycosidic linkage in common. However, β-cyclodextrin and amylose have α anomeric configuration whereas cellulose has β. Furthermore, β-cyclodextrin has a structure of highly ordered ring while amylose has a random folded arrangement. Cellulose, on the other hand, has a linearly sheeted structure with significant rigidity. Therefore, the reactivities of the free hydroxyl groups at 2-, 3-, and 6- positions may be expected to be different due to their overall availability toward an alkylating or acylating conditions. It is well known that the S_N reactivity of a primary hydroxyl group is much stronger than that of a secondary OH group. The 2- and 3-OH are secondary alcohols whereas the 6-OH is a primary alcohol.[1]

There are a few reports on the relative reactivities of the hydroxyl groups in 1,4-linked polysaccharides such as cellulose, amylose, and cyclodextrins. For example, acetylation of cellulose takes place largely at the 6-OH but etherification prefers the 2-OH with various etherifying agents. On the other hand, the base-catalyzed addition of acrylamide or methyl vinyl sulfone to cellulose produces a cellulose ether with substitution largely at the C-6 hydroxyl group.[2]

In order to compare the reactivities of 2-, 3-, and 6-OH in a polysaccharide having 1,4-linkage it is essential to develop a suitable method of determining the accurate molar ratio of each monosaccharide component derived from depolymerization. In addition, it is equally important to carry out each step without causing rearrangement of the *O*-substituent originally present. This is especially important for the analysis of *O*-acyl polysaccharide in which acyl migration is known to occur.[3] *O*-Alkyl (eg. methyl or ethyl) polysaccharides may be inert to such migration.

The typical procedure for the analysis involves the conversion of the free OH group to ether derivative.[3] For example, exhaustive ethylation of the free OH groups in a partially methylated β-cyclodextrin, cellulose, or amylose is carried out prior to depolymerization. Exhaustive methylation of a partially acetylated polysaccharide seems, in particular, to require conditions in which the acyl group does not migrate to other free hydroxyl group. Such conditions have been reported in literature.[4]

There are several methods for analyzing partially alkylated or acetylated polysaccharides. NMR spectroscopy seems to be the most useful one because the substrate will not undergo any structural change in the course of analysis.[5-9] However, this method has limited application due to the problems of solubility and viscosity. Furthermore, the value obtained by integration of individual peaks may have substantial error arising from the inherent problem of overlapping peaks.

On the other hand, chemical method of analysis, although very laborious, appears to provide a reliable means of determining both the degree of substitution (ds) and the distribution of substituents in a partially acetylated or methylated polysaccharide. Such analyses are consisted of initial methylation and ethylation of the free hydroxyl groups of an acetylated and methylated polysaccharide, respectively. Then the partially methylated and acetylated polysaccharide may be subjected to either hydrolysis[10] or reductive cleavage.[11] This results in monosaccharide derivatives which, after further transformation of the free OH group to a suitable functionality, may be analyzed by GLC-mass spectrometry.

In the analysis of the positions of substitution of *O*-acetyl groups in partially *O*-acetylated cellulose by the reductive-cleavage method we found that the acetyl group did not migrate throughout the process.[12] Therefore, the degree of substitution at each free hydroxyl group may be used as a measure of the relative reactivities of 2-, 3-, and 6-OH

groups. Furthermore, a previously established method for the analysis of partially methylated cellulose[11] may be applied to its anomeric counterparts, namely amylose and cyclodextrins.

In this paper we describe the relative reactivities of the hydroxyl groups in cellulose, amylose, and β-cyclodextrin, by the reductive-cleavage methodology.

Experimental Section

Instruments. ^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker DPX-400 FT NMR spectrometer in the Central Lab of Kangwon National University. Tetramethylsilane was used as an internal reference. Infrared (IR) spectra were recorded on a Perkin Elmer Model 1410R spectrometer. GC-CI (with NH_3) and GC-EI mass spectra were obtained using a JEOL JMS-AX 505 WA mass spectrometer at the probe temperature of 200 °C and at 70 eV. Gas-liquid chromatography (GLC) was carried out on a Hewlett-Packard 6890 gas chromatograph equipped with a split-splitless injector connected to a T-shaped splitter which, in turn, was connected to a HP-5 capillary column (30 m, film thickness 1.0 μm, ID 0.53 mm) and a HP 50+ capillary column (30 m, film thickness 1.0 μm, ID 0.53 mm), a flame-ionization detector for each column, and a Hewlett-Packard workstation. For retention indices experiments a Hewlett-Packard 5890A gas chromatograph was used. It was equipped with a split-splitless injector connected to a T-shaped splitter which, in turn, was connected to a HP-1 capillary column (30 m, film thickness 0.25 μm, ID 0.25 mm) and a Rtx-200 capillary column (30 m, film thickness 0.25 μm, ID 0.25 mm), a flame-ionization detector for each column, and a Hewlett-Packard workstation. Nitrogen was used as a carrier gas. The column temperature conditions are as follows: initial temperature, 110 °C; initial hold, 2 min; temperature increase, 2 °C/min; final temperature 240 °C; final hold, 20 min; injector temperature, 220 °C; detector temperature 250 °C.

Starting materials. β-Cyclodextrin, cellulose, cellulose triacetate, and amylose were all commercially purchased. Triethylsilane, trimethylsilyl methanesulfonate (Me_3SiO-SO_2CH_3), borontrifluoride etherate ($BF_3 \cdot OEt_2$), acetic anhydride, and benzoic anhydride were used as commercially available. Dichloromethane was distilled over CaH_2 prior to use.

Partial methylation of polysaccharides. An illustrative procedure. Cellulose and amylose were lyophilyzed and dried under vacuum (0.01 mm) at 60 °C for 48 h prior to use. A mixture of the polysaccharide (50 mg) and NaOH (20 mg) in DMSO (3 mL) was dissolved by sonication. It was then cooled in an ice-water bath. Methyl iodide (0.1 mL) was added. The mixture was gradually brought to room temperature. The solution was dialyzed with running water overnight. The resulting mixture was lyophilyzed to give a partially methylated polysaccharide (60 mg).

Ethylation of the free OH groups. Partially methylated polysaccharides (40-50 mg, 0.2-0.3 mmoles) was dissolved in DMSO (2 mL) by mild heating and sonication. Sodium hydroxide (0.12 g, 30 mmole) was added to the solution and stirred until the solid was dissolved (*ca.* 1-2 h). Ethyl iodide (3 mmoles) was added in one portion. The flask containing the solution was stoppered. The solution was stirred for 12 h. The solvent was distilled off under vacuum. The residue was dissolved in water (10 mL). The solution was extracted with chloroform (3 × 10 mL). The organic extract was dried over Na_2SO_4. The solvent was evaporated off. The ethylation was repeated with 1 mL of DMSO, 50 mg of NaOH, and 0.1 mL of EtI to ensure complete conversion of the remaining OH group. The yields of the partially ethylated and methylated polysaccharides were about 80%. The IR spectrum of the product showed complete disappearance of the OH group.

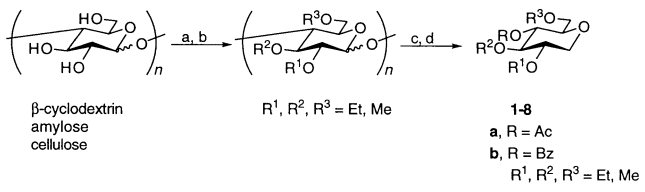
Partial acetylation of polysaccharides. The lyophilized polysaccharide (0.1 g) was suspended in pyridine (5 mL) and acetic anhydride (0.1 mL) was added. The mixture was stirred for 2 h and then poured into ice-water (50 mL). The milky mixture was stirred for 1 h and then extracted with chloroform (3 × 20 mL). The extract was washed with 3 N-HCl (3 × 10 mL) to remove the residual pyridine. The chloroform layer was dried over Na_2SO_4. The solvent was removed under vacuum to give a gel-like product (60%).

Partial hydrolysis of triacetates of cellulose and amylose. The triacetate (0.5 g) was dissolved in DMSO (5 mL) and 6 M-NaOH (1 mL) was added. The resulting solution was stirred at room temperature for 1 h and then poured into ice-water (20 mL). The mixture was neutralized with 1 M-HCl to pH 7 and then the mixture was dialyzed. After lyophilyzation a foamy solid was obtained (0.3 g).

Methylation of partially *O*-acetylated polysaccha-ride. The procedure is essentially similar to one in the literature.[4] A lyophilized sample (80 mg) was dissolved in trimethyl phosphate (4 mL) by sonication. Methyl triflate (0.35 mL) and 2,6-di-*tert*-butylpyridine (0.52 mL) were added. The solution was heated at 70 °C for 14 h and then dialyzed with running water. After lyophilization 90 mg (90%) of a partially *O*-acetylated and -methylated polysaccharide was obtained.

Acetyl-ethyl exchange reaction. The procedure described previously[12] has been modified as follow: a mixture of a partially *O*-acetylated and -methylated polysaccharide (30 mg) and NaOH (10 mg) in DMSO (2 mL) was stirred at 50 °C for 3 h. After cooling to 0 °C C_2H_5I (0.5 mL) was added. The solution was capped. The solution was stirred at 50 °C for 12 h and then dialyzed with running water. Lyophilyzation of the solution gave a partially *O*-ethylated and -methylated polysaccharide (20 mg).

Reductive cleavage of the polysaccharide derivatives. The procedure is essentially similar to one in the literature.[13] A sample of partially *O*-ethylated and -methylated polysaccharide (15 mg) was dissolved in dichloromethane (0.5 mL). Triethylsilane (70 μL), TMSOMs (60 μL), and $BF_3 \cdot OEt_2$ (10 μL) were added. The resulting solution was stirred for 24 h. After the typical workup and subsequent acetylation the mixture was analyzed by GLC. A protion of the reaction mixture was benzoylated with benzoic anhydride in pyridine at 50 °C for 24 h. The final product mixture was analyzed by



a, MeI, NaOH, DMSO. b, EtI, MeI, DMSO. c, Et_3SiH, Me_3SiOSO_2Me, $BF_3{\cdot}OEt_2$, CH_2Cl_2.

Scheme 1. Relative reactivity of methylation.

GLC.

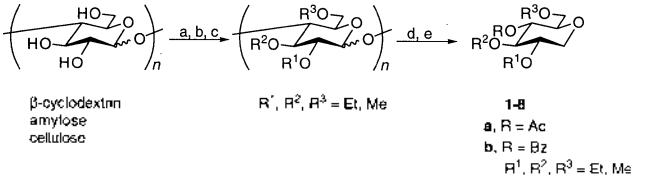
Results and Discussion

The partially methylated samples of β-cyclodextrin, amylose, and cellulose were prepared by treatment of the polysaccharides with MeI and NaOH in DMSO and then quenching by addition of water. The samples of ds 1-2 were obtained after dialysis and lyophilization (Scheme 1). The free OH groups in the partially methylated polysaccharides were converted to ethyl ether by reacting repeatedly (two or three times) with excess amount of EtI and NaOH in DMSO.

The partially acetylated samples of the polysaccharides were prepared similarly by treating the polysaccharides with acetic anhydride in pyridine (Scheme 2). The free OH groups in the partially acetylated samples were methylated with methyl triflate and 2,6-di-*tert*-butylpyridine in trimethyl phosphate. The acetyl group was converted to ethyl group by treatment with EtI and NaOH in DMSO.

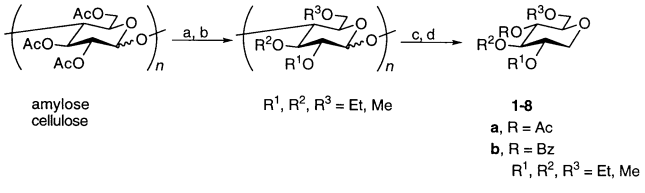
Partial hydrolysis of the triacetates of amylose and cellulose was also carried out in order to investigate the relative reactivities of the 2-, 3-, and 6-OAc groups (Scheme 3). Preparation of the partially acetylated samples of β-cyclodextrin by the partial hydrolysis of its triacetate was not successful due to acetyl group migration under the basic conditions, which should interfere the accurate quantification of the positional isomers.

Partially ethylated and methylated polysaccharides were



a, Ac_2O, pyridine. b. $MeOSO_2CF_3$, 2,6-di-tert-butylpyridine, $(MeO)_3PO$. c, EtI, NaOH, DMSO.

Scheme 2. Relative reactivity of acetylation.



a, MeI, NaOH, DMSO. b, EtI, NaOH, DMSO. c, Et_3SiH, Me_3SiOSO_2Me, $BF_3{\cdot}OEt_2$, CH_2Cl_2.

Scheme 3. Relative reactivity of hydrolysis of triacetate.

reductively cleaved with Et_3SiH and a mixture of $Me_3SiOSO_2CH_3$ and $BF_3 \cdot OEt_2$ (5 : 1 by mole) in dichloromethane. The resulting 2,3,6-trialkyl-1,5-anhydroglucitols, **1-8**, were divided into two portions. Each portion was either acetylated to give **a** series of compounds or benzoylated to give **b** series of compounds. The acetylation was usually carried out at room temperature with acetic anhydride in dichloromethane for 2-3 h, but the benzoylation was carried out at 45-50 °C with benzoic anhydride in pyridine for 24 h.

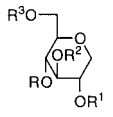
The percentage compositions of the eight anhydroalditols **1-8** after the reductive cleavage and subsequent acetylation and benzoylation are listed in Table 1. There is a slight variation between the acetates and benzoates. However, the overall trend is consistent with the values reported in the literature.

It is known that the reactivities of the free OH groups in cellulose differ depending on the reaction conditions. In gen-

Table 1. Mole percents of products derived by reductive cleavage of partially *O*-methylated and *O*-acetylated cellulose, amylose, and β-cyclodextrin and sebsequent acetylation and benzoylation

		Cellulose			Amylose			β-cyclodextrin	
		Me	Ac	Ac^{hyd}	Me	Ac	Ac^{hyd}	Me	Ac
1a		26.64	29.77	7.01	40.40	18.07	7.14	9.62	15.73
1b		31.54	31.64		49.69	28.47		8.11	19.52
2a		25.08	5.32	5.96	26.61	2.28	10.26	19.48	7.02
2b		19.83	6.34		23.58	2.42		19.77	10.15
3a		10.55	26.96	3.93	6 .21	25.17	3.36	.22	29.92
3b		13.58	19.81		6.31	24.62		5.86	28.08
4a		5.30	7.95	8.99	1.90	5.52	7.67	13.23	6.14
4b		7.17	11.74		2.00	8.91		13.03	7.72
5a		13.68	8.71	5.75	15.12	6.86	13.43	12.62	8.83
5b		10.89	9.29		11.14	5.95		13.46	8.05
6a		10.15	2.24	26.45	3.53	3.78	22.40	20.59	5.87
6b		8.88	2.28		2.64	3.78		22.11	7.66
7a		2.06	14.44	9.19	1.33	22.78	10.04	8.02	18.95
7b		2.75	12.41		1.33	17.11		7.90	12.26
8a		6.53	4.61	32.72	4.90	15.55	25.69	10.22	7.64
8b		5.36	6.49		3.35	10.07		9.76	6.57
ds	**a**	1.88	1.05	2.00	2.11	1.45	1.90	1.48	1.33
	b	1.98	1.05		2.28	1.20		1.45	1.22
x_2	**a**	0.759	0.292	0.774	0.883	0.476	0.658	0.479	0.386
	b	0.758	0.329		0.907	0.399		0.472	0.342
x_3	**a**	0.445	0.209	0.709	0.498	0.285	0.718	0.371	0.294
	b	0.550	0.244		0.593	0.222		0.349	0.324
x_6	**a**	0.672	0.547	0.516	0.724	0.694	0.525	0.629	0.653
	b	0.674	0.480		0.779	0.578		0.630	0.550
r_2	**a**	1.13	0.53	0.67	1.22	0.69	0.80	0.76	0.59
r	**a**	1.35[a]	0.29[c]	0.22[a]	4.28[a]	0.22[c]	0.60a	0.61[a]	0.21[c]
r_3	**a**	0.66	0.38	0.73	0.69	0.41	0.73	0.59	0.45
r	**a**	0.20[b]	0.20[d]	0.35[b]	0.38[b]	0.09[d]	0.45[b]	0.39[b]	0.23[d]
r_6	**a**	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Me: Partially methylated substrate. Ac: Partially acetylated substrate. Ac^{hyd}: Substrate prepared by partial hydrolysis of the triacetate of the polysaccharide. x_i: Degree of substitution at ith hydroxyl group. r_i: Ratio of degree of substitution. x_i/x_6. r: Ratio of mole percent: [a]**5a/6a**; [b]**7a/6a**; [c]**4a/3a**; [d]**2a/3a**.



a, R = $CH_3C{=}O$; b, $C_6H_5C{=}O$

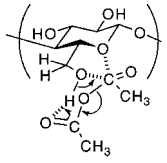
	R^1	R^2	R^3
1	Me	Me	Me
2	Me	Et	Me
3	Me	Me	Et
4	Et	Me	Me
5	Me	Et	Et
6	Et	Et	Me
7	Et	Me	Et
8	Et	Et	Et

eral, the 6-OH group reacts the most rapidly with acetic anhydride to give cellulose acetate. However, the 2-OH group frequently reacts first in etherification. Lenz analyzed a methylated cellulose (ds 0.99) and found that the relative reactivities were 2-OH > 6-OH > 3-OH.[14] The order of the relative reactivities was based on the composition of the monomethylated glucose unit being 50%, 41%, and 9% for 2-, 6-, and 3-OH, respectively, when the methylation was carried out with methyl iodide to monosodiocellulose.[14]

The values in Table 1 are corrected by the effective carbon response (ecr) factor.[15] Both acetylation and benzoylation were carried out with the same reaction mixture obtained after the reductive cleavage of the *O*-alkylated samples. Therefore, the values of **a** and **b** in Table 1 should give similar values for each component. The observed values are, however, slightly different although the ds values do not deviate much from each other. It is conceivable that either the acetylation or the benzoylation does not go to completion so that the relative percentages are different. It is also possible that the ecr corrections used for calculation for the mole percentage of the acetates **1a-8a** may not be suitable for the benzoates **1b-8b**. Whatever the cause of the discrepancy between the acetate and the benzoate, the relative mole percent of the benzoate should not be used as a base for the quantitative analysis by GLC using a flame ionization detector. There is no report in literature of the ecr values for benzoates of monosaccharides.

Based upon the x_i value in Table 1, which reflects the degree of substitution at the *i*th hydroxyl group, one can conclude that the trends of the reactivities are 2-OH > 6-OH > 3-OH for methylation and 6-OH > 2-OH > 3-OH for acetylation for cellulose and amylose. These trends are similar to the literature (eg. for methylation with methyl iodide the relative ratio was 2-OH, 5.0; 3-OH, 1.0; 6-OH, 2.5).[1] Therefore, it seems clear that the reactivities of the OH groups are not affected by the anomeric configuration of the glycosidic linkage in the polysaccharides. However, the order of the reactivities is 6-OH > 2-OH > 3-OH for both acetylation and methylation of β-cyclodextrin.

If we examine the values of the relative mole percent of the acetates **1a-8a**, a few observations should be pointed out. First, the relative ratios of **5a/6a** for the methylation are 1.35, 4.28, and 0.61 for cellulose, amylose, and β-cyclodextrin, respectively. The relative reactivity of the 2-OH is only slightly greater than that of the 6-OH in the case of cellulose, but it is about four times greater for amylose. On the other hand, the 2-OH is less reactive than the 6-OH for β-cyclodextrin. For acetylation the relative ratios of **4a/3a** for cellulose, amylose, and β-cyclodextrin are 0.29, 0.22, and 0.21, respectively. The ratio suggests that the relative reactivity of the 6-OH is 3-4 times greater than that of the 2-OH for the three polysaccharides. The ring oxygen atom may be involved in the transition state for acetylation of the 6-OH as shown:



Methylation, however, seems to be affected by both electronic and steric factors. For cellulose and amylose the electron density at the 2-C is greatly enhanced by both the oxygen atoms at 1-C and in the ring. This, in trun, will make the 2-OH group more nucleophilic. The oxygen atoms at 1-C and 2-C are cis to each other in amylose. The reactivity of the 2-OH should be enhanced due to the interaction of the lone pair orbitals through space. They are trans to each other in cellulose and the enhancement should be minimal. In the case of β-cyclodextrin all the 6-OH groups are one side of the circle composed of the seven glucopyranoside units. They are sterically open for attack on the methyl carbon of methyl iodide. Therefore, methylation at the 6-OH should be the most favorable reaction.

The relative reactivities of 2-, 3-, and 6-OAc groups toward the hydrolysis by NaOH in DMSO were also examined. The results are listed in Table 1. The ratios of the degree of hydrolysis of 2-OAc, 3-OAc, and 6-OAc are 0.67, 0.73, and 1.00, respectively. In contrast, they are 0.80, 0.73, and 1.00, respectively, for amylose. The results reflect that the order of the reactivity is 6-OAc > 3-OAc > 2-OAc for cellulose triacetate and 6-OAc > 2-OAc > 3-OAc for amylose triacetate. The order for the 2-OAc and 3-OAc are reversed for the cellulose and amylose esters. It seems difficult to explain the change in terms of the anomeric configuration.

Gray, *et al.*, applied the reductive-cleavage method for analysis of the positions of substitution of *O*-methyl or *O*-ethyl groups in partially methylated or ethylated cellulose.[16] They prepared acetate esters of the eight 1,5-anhydroalditols and identified each of them by GLC-mass spectra analysis with DB-5 capillary column. The method has the disadvantage because of a long retention time (40-63 min) and improper separation of **2a** and **4a** as well as **6a** and **7a** (Table 2). Although all of the eight components were independently synthesized and their retention times as well as mass spectra were established, a better analytical method based on the complete separation in the GLC is desirable. Therefore, we

Table 2. Linear temperature programmed gas-liquid chromatography retention indices of compounds **1-8** (**a**. acetate; **b**. benzoate)

Com	HP-1		HP-5		HP-50+		Rtx-200	
	a	**b**	**a**	**b**	**a**	**b**	**a**	**b**
1	1509.74	2034.39	1528.58	2073.71	1789.39	2483.88	1847.83	2412.87[a]
2	1555.59	2060.25	1570.27	2100.00	1818.96	2490.05	1879.78	2412.87[a]
3	1571.51[a]	2080.78	1586.92[a]	2120.76	1848.91	2525.76[a]	1884.09	2426.88[a]
4	1571.51[a]	2096.31	1586.92[a]	2135.14	1835.69	2525.76[a]	1902.99	2465.93[a]
5	1614.89[a]	2105.26	1629.80[a]	2143.99	1877.00	2527.76[a]	1914.26	2426.88[a]
6	1614.89[a]	2119.87	1629.80[a]	2156.40	1862.01	2527.76[a]	1934.24	2465.93[a]
7	1631.63	2143.20	1648.21	2180.69	1893.17	2566.93[a]	1939.23	2476.12[a]
8	1674.13	2164.99	1690.13	2200.00	1919.23	2566.93[a]	1968.27	2476.12[a]

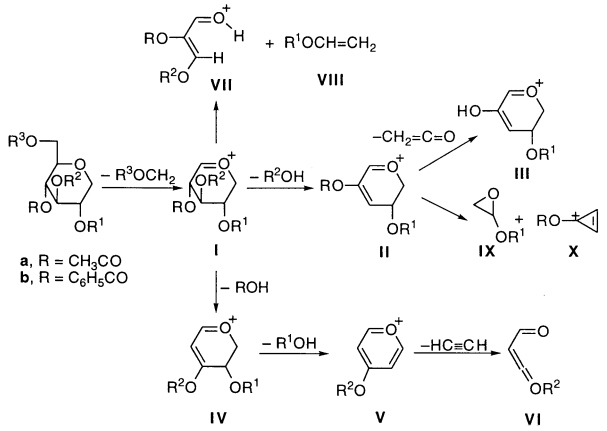
[a]Overlapped.

Table 3. Mass spectral fragmentation of 4-*O*-acyl-1,5-anhydro-glucitols. **1-8** (**a**. acetyl; **b**. benzoyl; relative intensity, %)

	I	II	III	IV	V	VI	VII	VIII	IX	X	R-CO	C_6H_5
1a	203	171	129	143	111	85	145	58	74	97	43	-
	(56)	(88)	(83)	(79)	(23)	(36)	(27)	(65)	(28)	(74)	(100)	
1b	265	233	129	143	111	85	207	-	74	-	105	77
	(4)	(6)	(1)	(15)	(11)	(7)	(10)		(2)		(100)	(21)
2a	217	171	129	157	125	99	159	58	74	97	43	-
	(36)	(58)	(50)	(86)	(8)	(32)	(30)	(55)	(32)	(56)	(100)	
2b	279	233	129	157	125	99	221	-	74	-	105	77
	(3)	(5)	(1)	(17)	(1)	(7)	(10)		(0.4)		(100)	(20)
3a	203	171	129	143	111	85	145	58	74	97	43	-
	(54)	(78)	(62)	(66)	(25)	(36)	(15)	(57)	(22)	(78)	(100)	
3b	265	233	129	143	111	85	207	-	74	-	105	77
	(5)	(8)	(1)	(13)	(6)	(5)	(7)		(2)		(100)	(19)
4a	217	185	143	157	111	85	145	72	88	97	31	-
	(33)	(40)	(41)	(61)	(23)	(26)	(14)	(38)	(12)	(52)	(100)	
4b	279	243	179	157	111	85	207	-	88	-	105	77
	(4)	(4)	(0.4)	(16)	(2)	(6)	(11)		(0.4)		(100)	(20)
5a	217	171	129	157	125	99	159	58	74	97	43	-
	(47)	(72)	(54)	(71)	(12)	(34)	(20)	(100)	(12)	(69)	(100)	
5b	279	233	129	157	125	99	221	-	74	-	105	77
	(4)	(6)	(1)	(14)	(2)	(6)	(7)		(2)		(100)	(17)
6a	231	185	143	171	125	99	159	72	88	97	43	-
	(21)	(33)	(46)	(69)	(12)	(28)	(20)	(78)	(28)	(41)	(100)	
6b	293	247	143	171	125	99	221	-	88	-	105	77
	(3)	(3)	(3)	(17)	(3)	(6)	(9)		(1)		(100)	(18)
7a	217	185	143	157	111	85	145	72	88	97	43	-
	(37)	(48)	(74)	(43)	(11)	(27)	(13)	(68)	(6)	(65)	(100)	
7b	279	247	143	157	111	85	207	-	88	-	105	77
	(4)	(5)	(1)	(13)	(3)	(6)	(8)		(0.4)		(100)	(18)
8a	231	185	143	171	125	99	159	72	88	-	43	-
	(29)	(45)	(65)	(58)	(16)	(28)	(14)	(66)	(23)		(100)	
8b	293	247	143	171	125	99	221	-	88	-	105	77
	(3)	(4)	(2)	(14)	(3)	(6)	(7)		(1)		(100)	(16)

examined the conditions for the most efficient GLC separation of 4-*O*-acyl-2,3,6-tri-*O*-alkyl-1,5-anhydro-alditols.

In order to explore the suitable conditions for separation and identification of **1-8** we prepared the benzoates (**b**) as well as the acetates (**a**) and examined a series of GLC conditions such as column, running temperature program, flow rate, etc. The linear temperature programmed gas-liquid chromatography retention indices of compounds **1-8** are listed in Table 2. The 4-*O*-acetyl derivatives of the 1,5-anhydroglucitol (**1a-8a**) were most efficiently separated with HP 50+ [(50%)-diphenyl-(50%)-dimethylsiloxane copolymer, intermediate polarity] and Rtx-200 (trifluoropropyl methylpolysiloxane copolymer, intermediate polarity) columns in 30 min. On the other hand, the baseline separation for the 4-*O*-benzoyl derivatives (**1b-8b**) could be achieved by HP-1 (dimethylsiloxane copolymer, nonpolar) and HP-5 [(5%)-diphenyl-(95%)-dimethylsiloxane copolymer, nonpolar] columns in 50 min.



Scheme 4

The EI-mass spectral fragmentation patterns of the benzoates and the acetates clearly established for structures of the eight components. The fragments from the benzoates and their relative intensities are listed in Table 3. There are 11 fragments which can be rationalized systematically as shown in Scheme 4. These fragments are definite evidence for the structural determination. The benzoates showed m/z 105 as the base peak for all eight components. Since the stability of the $C_6H_5CO^+$ is far greater than any other fragments, it is not surprising that only a few fragments with noticeable intensities are present in the mass spectra of the benzoates **1b-8b**. Although the relative intensities are rather small (5-20%) the pattern of the fragments clearly reflected the positions of methyl and ethyl groups. The intensities of the fragments from the acetates (Table 3) are much stronger than

those from the benzoates. If we are to depend on the only fragmentation pattern for determination of the structures of **1-8**, however, the benzoates seem to give better correlation than the corresponding acetates. The usefulness of the benzoates is their complete separation on the HP-1 and HP-5 column in 50 min.

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