Identification of Stereoisomers of Methyl Hexopyranosides by Mass Spectrometry of Their Trifluoroacetates

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The mass spectra of the trifluoroacetates of methyl hexopyranosides and their partially deuterated derivatives were measured. The trifluoroacetyl derivatives were found to be very useful for identification of some stereoisomeric hexoses. The mass spectra were characterized by abundant peaks in the high mass range, the presence of a recognizable molecular ion, and sensitive spectral differences due to different stereoisomers. Glucose, galactose and mannose were distinguished from each other by comparing the intensities of the fragment ions due to the loss of trifluoroacetyl groups or cleavage of the pyranose ring. Alfa and β-anomers were distinguished by differences in the abundance of fragment ions produced by the loss of glycosidic methoxyl groups.

Monosaccharides have been examined by measuring the mass spectra of their methyl,[1,2] acetyl,[3] trimethylsilyl (TMS)[4-6] and isopropylidene derivatives.[7,8] However, use of the mass spectra of these derivatives has the disadvantages of the absence of a molecular ion and small differences in the specral patterns of different stereoisomers (*e.g.* epimers or anomers). Chizhov *et al.*[9] reported that the trifluoroacetyl (TFA) derivatives of alditols give sufficiently intense molecular ions to allow direct determination of their molecular weights. TFA derivatives were previously used in gas chromatographic analysis of methyl glycosides because of their high volatility.[10,11] The present paper reports studies on the mass spectra of the TFA derivatives of methyl hexopyranosides. The major fragmentation processes are proposed on the basis o results of deuterium-labeling experiments.

Experimental

Methyl α-D-glucopyranoside (Ia) and methyl β-D-glucopyranoside (Ib) were obtained from Sigma Chemical Co. Methyl α-, and β-D-galactopyranosides (IIa and IIb) were prepared by refluxing D-galactose with 1% methanolic HCl and were separated by Dowex (1 × 4) column chromatography as described by Matsushima and Miyazaki.[12] Methyl α-, and β-D-mannopyranosides (IIIa and IIIb) were obtained in a similar way. Deuteriomethyl α-, and β-D-galactopyranosides (VIa and VIb) were also prepared with the use of methanol-d_4 instead of methanol.

Methyl α-, and β-D-Glucopyranoside-2-d and Deuteriomethyl α-, and β-D-Glucopyranoside-2-d(IVa, IVb, Va, and Vb). Deuteration of the C_2 position was carried out as described by Lemieux and Stevens.[13] 2-*O*-benzyl-D-glucose[14] (750 mg) was dissolved in 3 ml of deuterium oxide (d, 99.5%) and lyophilized. The treatment was repeated three times. The lyophilized material was then dissolved in 3 ml of 1.7 M NaOD, and left to stand for 10 days at room temperature. The solution was neutralized with acetic acid-d_1 and extracted five times with 10 ml volumes of ethyl acetate. The extracts were combined and evaporated to dryness. The residue was recrystallized from ethyl acetate to yield 670 mg of 2-*O*-benzyl-D-glucose-2-*d*; mp, 176—178 °C; NMR(D_2O) ppm, 5.26(1-H_{eq}, singlet), 4.70 (1-H_{ax}, singlet), 7.4 (phenyl protons). 2-*O*-benzyl-D-glucose-2-*d*(600 mg) was dissolved in 20 ml of 95% ethyl alcohol and subjected to hydrogenolysis for 24 h under atmospheric pressure with 5% Pd on charcoal. The reaction mixture was filtered and evaporated to yield 540 mg of D-glucose-2-*d*; syrupy; NMR (D_2O) ppm, 5.24 (1-H_{eq}, singlet), 4.94 (1-H_{ax}, singlet). No detectable phenyl proton. Methyl α-, and β-glucopyranoside-2-*d*(IVa and IVb) and deuteriomethyl α-, and β-glucopyranoside-2-*d*(Va and Vb) were derived from D-glucose-2-*d* using acidic methanol and deuteriomethanol, respectively. Each anomer was purified by Dowex 1 column chromatography as described above.

Methyl α-, and β-D-glucopyranoside-6-d_2*(VIIa and VIIb).* To a solution of methyl D-glucuronic acid methyl ester (100 mg) in 2 ml of water was added dropwise a solution of $NaBD_4$ (50 mg) in 3 ml of cold water. The mixture was left to stand for 6 h at room temperature, treated with Dowex 50 (H^+ form) and IR-45 (OH^-), and evaporated to dryness. VIIa and VIIb were separated on a column of Dowex 1.

Trifluoroacetylation of Sugars. The dried material (3 mg) was allowed to react with a mixture of 0.1 ml of ethyl acetate and 0.2 ml of trifluoroacetic anhydride at 100 °C for 8 min.[10] The reaction mixture was then evaporated under a stream of nitrogen at 40 °C and the residual trifluoroacetic acid was removed as an azeotropic mixture with carbon tetrachloride.

Mass spectra were measured on a Hitachi model RMS-4 mass spectrometer, temperature of the ion source being 200°C, ionizing potential 70 eV and accelerating voltage 3 kV. Samples were vaporized in a 1 l glass reservoir at 140 °C. Mass spectra were recorded on a photographic chart. Peak intensities were expressed in percentage of that of total ions above *m/e* 100 ($\% \sum_{100}$).

Results and Discussion

The physical properties of the methyl hexopyranosides used are listed in Table 1. The deuterium contents in the deuterated sugars were determined from the isotope peaks. The deuterium content at position C_2 of IVa was 95.8% and that at C_6 of VIIa 97.1%. The deuterium content in the methoxyl group of VIa was 98.2%.

The major fragment ions of the TFA derivatives of methyl glucosides, galactosides and mannosides are listed in Table 2. The TFA derivatives of hexopyranosides all showed a molecular ion (*m/e* 578), whereas the acetyl,[3] methyl[1,2] and TMS derivatives[4-6] of monosaccharides gave $(M-CH_3CO)^+$, $(M-CH_3O)^+$ and $(M-CH_3)^+$, respectively, as the highest ions.

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Table 1. Physical properties of methyl hexopyranosides

Compound	mp, °C	$[\alpha]_{\rm D}^{17}$ in H_2O
Ia	167—8	+158.2
Ib	107—8	− 28.6
IIa	112—3	+193.5
IIb	175—8	0
IIIa	191—2	+ 84.6
IIIb	66—9	− 64.0
IVa	167—9	+160.9
IVb	105—7	− 30.9
Va	165—7	+152.7
Vb	106—7	− 29.2
VIa	108—111	+193.9
VIb	174—7	+ 0.8
VIIa	166—8	+156.2
VIIb	103—6	—

Table 2. Relative intensities ($\%\sum_{100}$) of major fragment ions of methyl *O*-TFA hexopyranosides. *m/e* 177 was base peak in all the compounds tested.

Fragment ions *m/e*		Ia	Ib	IIa	IIb	IIIa	IIIb
M^+	578[a)]	0.05	0.11	0.06	0.14	0.04	0.08
g	547[b)]	0.04	0.02	0.07	0.04	0.08	0.02
a	465	1.54	1.49	1.42	1.48	1.78	1.00
d	405	4.30	4.17	3.20	4.37	4.94	3.66
	404	2.02	1.75	0.89	1.18	2.11	1.57
b	351	0.75	1.07	1.70	1.36	1.27	0.65
h	319[b)]	2.06	1.05	1.70	0.96	2.20	0.92
e	291[c)]	2.50	2.50	1.85	1.88	2.94	2.64
j	265[c)]	2.92	2.64	0.99	1.75	3.03	2.90
k	252[c)]	1.35	1.39	0.55	0.92	1.54	1.46
c	237[c)]	1.99	2.35	0.99	2.16	3.48	2.82
	221	1.30	1.26	0.58	0.82	1.58	1.31
i	205[b)]	0.41	0.21	0.28	0.16	0.41	0.21
	196	1.47	1.36	0.63	1.33	1.13	1.63
l	193	2.44	2.64	1.22	2.39	2.73	2.54
m	183	1.97	1.77	0.98	1.73	2.72	2.09
f	177	13.21	11.87	8.33	14.60	12.90	12.49
n	170	4.92	2.73	1.80	1.88	7.55	4.10
p	157	6.30	3.72	2.73	3.37	8.62	5.23
o	153	2.30	2.50	1.43	2.80	2.32	2.69
	149	5.93	5.68	4.42	8.93	6.00	6.64
	129	3.62	2.80	2.01	3.28	4.17	3.39
	109	2.25	1.75	1.60	2.62	2.19	2.32

a) Peak intensity of β-anomer is greater than that of the corresponding α-anomer. b) Peak intensity of α-anomer is greater than that of the corresponding β-anomer. c) Intensities decrease in the order of mannoside, glucoside and galactoside.

A typical mass spectral pattern of the TFA derivative of methyl α-D-glucopyranoside is shown in Fig. 1-b, with that of the corresponding TMS derivative (Fig. 1-a) for comparison. Relatively abundand peaks were observed in the high mass range (above *m/e* 177) of the spectra of the TFA derivatives, whereas the mass spectra of the methyl,[1,2] acetyl[3] or TMS derivatives were characterized by peaks of extremely low intensity in the high mass range. TFA groups may effectively stabilize the molecular and fragment ions in the high mass range.

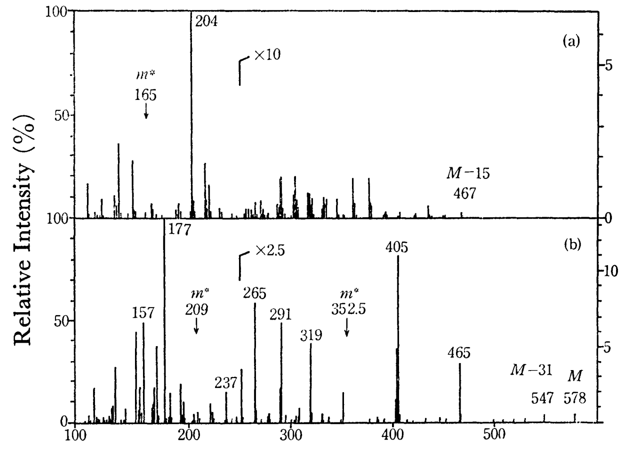

Fig. 1. Mass spectra of (a) TMS- and (b) TFA-derivatives of methyl α-D-glucopyranoside.

Fragmentation Process. Three modes of fragmentation are presented. The fragmentation mode in series **A** (Scheme 1) was produced by sequential elimination of TFA groups. A similar sequential elimination of TFA groups was reported in the mass spectra of TFA alditols.[9] In the course of the fragmentation, ring scissions occurred at C_1–C_2 and C_5–O bonds to produce the fragment ions *m/e* 405(**d**) or 404. The fragment ion **d** further released TFA groups to yield the ions **e** and **f**. These degradation processes were confirmed by the presence of the following metastable ions: *m/e* 352.5 (*m/e* 465→405), *m/e* 209.0 (*m/e* 405→291) and *m/e* 107.5 (*m/e* 291→177). All the deuterium atoms of IVa

Scheme 1. Fragmentation due to elimination of TFA groups and ring scission at C_1–C_2 and C_5–O (series **A**).
Relative intensities are shown in parentheses.

Molecular ion	$\xrightarrow{-\text{TFA}}$ **a**	$\xrightarrow{-\text{TFA}}$ **b**	$\xrightarrow{-\text{TFA}}$ **c**[c)]
Ia	465 (1.54)	351 (0.75)	237 (1.99)
IVa	466 (1.33)	351 (0.36) 352 (0.51)	237 (1.22) 238 (1.08)
VIa	468 (1.61)	354 (1.24)	240 (1.73)
VIIa	467 (1.14)	353 (0.78)	238 (0.40) 239 (2.62)

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	d	$\xrightarrow{-\text{TFA}}$ **e**	$\xrightarrow{-\text{TFA}}$ **f**
Ia	405 (4.30)	291 (2.50)	177 (13.21)
IVa	406 (4.58)	292 (2.53)	178 (12.61)
VIa	405 (4.39)	291 (3.11)	177 (13.89)
VIIa	406 (2.32) 407 (4.58)	292 (0.93) 293 (2.49)	178 (7.07) 179 (4.57)

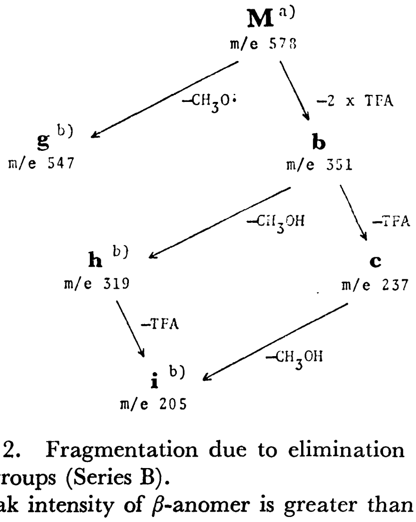
c) Intensities decrease in the order of mannoside, glucoside and galactoside.

TABLE 3. FRAGMENT IONS DERIVED FROM METHYL α-GLUCOPYRANOSIDE AND ITS DEUTERATED COMPOUNDS BY RING SCISSIONS (SERIES **C**)

Compound	Fragment ion, *m/e* ($\%\Sigma_{100}$)						
	j	**k**	**l**	**m**	**n**	**p**	**o**
Ia	265 (2.92)	252 (1.35)	193 (2.44)	183 (1.97)	170 (4.92)	157 (6.30)	153 (2.30)
IVa	265 (0.70)	252 (0.13)	193 (0.34)	183 (0.80)	170 (0.55)	157 (6.47)	153 (1.51)
	266 (2.07)	253 (1.23)	194 (2.15)	184 (1.47)	171 (5.32)		154 (1.14)
Va	265 (0.39)	252 (0.15)	193 (0.38)	183 (0.52)	170 (0.95)	160 (6.04)	153 (1.63)
	266 (1.90)	253 (1.18)	194 (2.14)	186 (0.72)	171 (0.50)		154 (1.14)
				187 (1.42)	173 (0.37)		
					174 (5.54)		
VIIa	265 (3.04)	252 (1.65)	193 (0.72)	183 (2.50)	170 (4.26)	157 (6.31)	153 (1.20)
			194 (0.55)		171 (1.78)		154 (1.20)
			195 (1.79)				155 (2.20)

and VIIa remained in fragment **a**, and 2-**d** (IVa) and 6-d_2 (VIIa) were still retained in **d** and **b**, respectively. The fragmentation, **c**→**f**, seems unlikely, because of the difference in the deuterium contents of the two fragments. The deuterium content at the C_2 position of fragment ion **c** was reduced to 47%, but not the deuterium content of fragment ion **f**. During the fragmentation from $\mathbf{M}^+$ to **c**, elimination of TFA groups caused only a little loss of the hydrogens at the C_6 position. After the ring scissions at C_1–C_2 and C_5–O bonds, sequential elimination of TFA groups was not accompanied by removal of the hydrogen at the C_2 position. Scarcely any fragment ions due to loss of F-, CF_3-, or CF_3CO-radicals were detected.

The second fragmentation mode in series **B** was characterized by elimination of the methoxyl group from the parent molecule or the fragments **b** or **c** without ring opening (Scheme 2).



Scheme 2. Fragmentation due to elimination of methoxyl groups (Series B).
a) Peak intensity of β-anomer is greater than the corresponding α-anomer. b) Intensity of α-anomer, greater than the corresponding β-anomer.

The third mode of fragmentation in series **C** included several kinds of ring scissions. Simultaneous cleavage of C_1–C_2 and C_5–O linkages produced fragment ion *m/e* 405 from the tris-TFA fragment (**a**, *m/e* 465) (Scheme 1). The path was confirmed by the loss of deuteriomethoxyl groups from the corresponding fragment ion **a** of VIa and VIb. Other ring scissions produced some characteristic ions, *m/e* 265, 252, 193, 183, 170, 157 and 153, from the fragments **a**, **b** and **c** (Table 3).

Differentiation of Anomers. The relative intensities of the molecular ions of β-anomer were more than twice those of α-anomers (Table 2). Similar phenomena have been observed with the methyl ethers of methyl hexosides.[1,2] However, the difference in abundance of the high mass fragments of the methyl derivatives was not so large. The TFA derivatives of gluco-, galacto- and mannopyranosides gave reproducible results when they were introduced into a mass spectrometer through a heated reservoir or a gas chromatograph. The two anomers of the TFA derivatives were also distinguished by the difference in abundance of the fragment ions produced by the loss of glycosidic methoxyl groups (series **B**, Scheme 2, Table 2). The relative intensities of the fragment ions, **g**, **h** and **i**, derived from the α-anomers were about twice as large as those of the corresponding fragments from β-anomers. The difference in abundance of these ions may be caused by steric effects of the configuration of methoxyl groups.

Differentiation of Epimers. Differences in the spectra of some epimers have been reported.[2,3,7] Kochetkov and Chizhov[2] studied methylated hexosides and reported that the abundance of the fragment *m/e* 149 from galactose and that of the fragment *m/e* 145 from mannose were relatively large. DeJongh and Biemann[7] found differences in the spectra of the isopropylidene derivatives of glucose, galactose and mannose. However, no significant difference was observed between the spectra of acetylated hexoses.[3]

The TFA derivatives of glucose, galactose and mannose could be distinguished from each other by the abundance of various fragments (Table 2). In particular, differences in the abundance of the fragment ions, **c**, **e**, **j** and **k**, could be used to differentiate these monosaccharides. The two former ions are produced in the fragmentation process of series **A**, and the two latter in the process of series **C**.

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