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Polysaccharide Sequencing by Mass Spectrometry: Chemical Ionization Spectra of Permethyl Glycosylalditols[1]

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Chemical ionization (CI) mass spectra with isobutane and isobutane/ammonia as the reagent gases are reported for the six permethylated glucosylalditols and for two permethylated biosylalditols. Intense peaks corresponding to MH^+ or $(M + NH_4)^+$ were observed in all cases. In the isobutane CI spectra the ratio of abundances for the alditol ions that were formed by cleavage of the glycosidic bond on the alditol or glucosyl side of the glycosidic oxygen depended strongly on the position of the glycosidic linkage. When the linkage was 1→6, 1→2, 1→4, and 1→3 the ratio of intensities for the altitol$^+$ (A^+) and alditol hydrate$^+$ (AOH_2^+) ions were respectively 0.72, 0.37, 0.17, and 0.06. The fragmentation of a biosylalditol (**7**) was clearly consistent with the relative intensities for the A^+ and AOH_2^+ ions anticipated from the disaccharide results. The ratio of A^+ to AOH_2^+ was 0.16 for this 1→4 linked alditol.

Determination of the structure of oligosaccharides by mass spectrometry is directly analogous to the problem of sequencing polypeptides by the same technique. However, in the case of carbohydrates the details of the structure are considerably more subtle. Ideally one would like to obtain information about the molecular weight, subunit structure, and position sequence. Fortunately conventional techniques will give reliable information concerning subunit structure in unknown oligosaccharides. The information that must be obtained from the mass spectrum is thus reduced to molecular weight and structure and position sequence. Chemical ionization (CI) mass spectra of oligosaccharide peracetates using ammonia/isobutane[2] and isobutane[3] alone as reagent gases have been investigated in this regard.

The primary reagent ion in isobutane CI mass spectra is the *tert*-butyl cation ($C_4H_9^+$).[4] Proton transfer from this ion is considerably more exothermic than proton transfer from the ammonium ion or attachment of NH_4^+ to a molecule. The ammonium ion is the dominant reagent ion in ammonia/isobutane CI mass spectra.[2,5] The higher exothermicity of ionization in isobutane CI mass spectra results in extensive fragmentation of oligosaccharide peracetates so that the spectra resembled the electron ionization (EI) mass spectra of these molecules in many details.[3] In particular the intensity of ions with masses near the molecular weight of the molecule were quite low in both EI and isobutane CI mass spectra.

It has been possible to obtain sequence information from EI mass spectra of permethylated sugars.[6] The low intensity of the high mass ions is a distinct disadvantage in these cases and the high energies associated with the ionization process tend to cloud the structural information in the spectra.

Ammonia/isobutane chemical ionization mass spectra of oligosaccharide peracetates gave molecular weight and structure sequence information for di-, tri-, and tetrasaccharides.[2] The dominant fragment ions in these spectra corresponded to ammonium ion attachment to thermolysis fragments. The thermolysis fragments reliably produced information concerning the masses of the subunits and their sequence. However, it has not yet been possible to determine positions for the subunits in the chain from these spectra.

The permethyl derivatives of oligosaccharides are considerably less polar and more volatile than their acetate analogues. Thus it seemed reasonable to expect[2] that these derivatives might be used to give position sequence information in CI mass spectra. We have used the permethylated alditol derivatives because reduction of the carbonyl terminus of the oligosaccharide prior to methylation unequivocally tags the reducing end of the sugar for sequence analysis.

In this report we discuss the isobutane and ammonia/isobutane CI mass spectra of six permethylated glycosylalditols and two permethylated biosylalditols. The numerical designations, names, and molecular weights for these compounds are indicated in Table I.

Results and Discussion

Isobutane CI Spectra. The major ions in the isobutane CI mass spectra of the glucosylalditols, **1–6**, are presented in Table II. The most intense ion in every case corresponded to the protonated molecule. All six of the compounds in Table II showed very similar fragmentation patterns. Probable structures for the major ions and routes for their formation are illustrated for permethyl-2-*O*-β-D-glucopyanosyl-D-glu-

Table I. Names and Molecular Weights of Permethylated Glycosylalditols Examined in This Study

Compd	Name	Mol wt
1	2-*O*-β-D-Glucopyranosyl-D-glucitol	470
2	2-*O*-β-D-Glucopyranosyl-D-glucitol-*1-d*	471
3	3-*O*-β-D-Glucopyranosyl-D-glucitol-*1-d*	471
4	4-*O*-α-D-Glucopyranosyl-D-glucitol-*1-d*	471
5	6-*O*-β-D-Glucopyranosyl-D-glucitol	470
6	1-*O*-α-L-arabinopyranosyl-DL-xylitol	382
7	α-D-Glucopyranosyl-1→4-*O*-α-D-glucopyranosyl-1→4-*O*-D-glucitol	674
8	α-D-Glucopyranosyl-1→4-*O*-β-D-glucopyranosyl-1→1-*O*-DL-xylitol	630

citol (1) in Scheme I. In the spectrum of 1 and the rest of the glucosylalditols there are two major series of fragment ions, one derived from the glucosyl portion of the molecule—G^+, $(G - CH_3OH)^+$, $[G - (CH_3OH)_2]^+$—and the other derived from the alditol portion of the molecule—AOH_2^+, A^+, $(A - CH_3OH)^+$.

Since the glucopyranose end group was the same in all cases one would not expect the ions in the glucosyl series to carry significant information concerning the linkage position to the alditol. This is not true of the alditol ion series and indeed the relative intensities of alditol, A^+, and alditol hydrate ions, AOH_2^+, do vary strongly with the linkage position to the alditol. In the series **5, 2, 4,** and **3** in which the glycosidic linkage is 1→6, 1→2, 1→4, and 1→3 the ion intensity ratio for A^+/AOH_2^+ was respectively 0.72, 0.37, 0.17, and 0.06. Repeated measurements as well as the comparison between the spectra of 1 and **2** indicate that the reproducibility of these ratios is roughly ±15%. Since the ratios differ by roughly a factor of 2 in all cases it should be possible to reliably use the ratio of the A^+ (*m/e* 235) to AOH_2^+ (*m/e* 253) ions as an indication of the terminal linkage position in reduced and permethylated polysaccharides. The results for the 1→4 linked biosylalditol, **7,** are in complete agreement with this expectation (ratio of A^+ to AOH_2^+ 0.16).

The intensity ratio for the A^+ (*m/e* 191) and AOH_2^+ (*m/e* 209) for the 1→1 linked arabinosyl xylitol, **6,** was 0.65. This value is very to the ratio of A^+/AOH_2^+ for the terminally linked glycosyl alditol, **5** (0.72). The intensity ratio for the same ions for the xylitol containing trisaccharide, **8,** was 0.67, which further supports the utility of these ions in assignments of position sequence in premethylated alditols.

The formation of the A^+ ions in these spectra is easily understood by postulating protonation on the glycosidic oxygen followed by assisted cleavage to give the alditol cation. The ease of protonation as well as cleavage should be dependent on the linkage position in the alditol. Protonation on the glycosidic oxygen must also be responsible for the presence of the glucosyl ions G^+ and their fragments in the spectra.

The AOH_2^+ ions must arise by protonation at a site remote from the glycosidic oxygen[7,8] followed by elimination of the

Scheme I

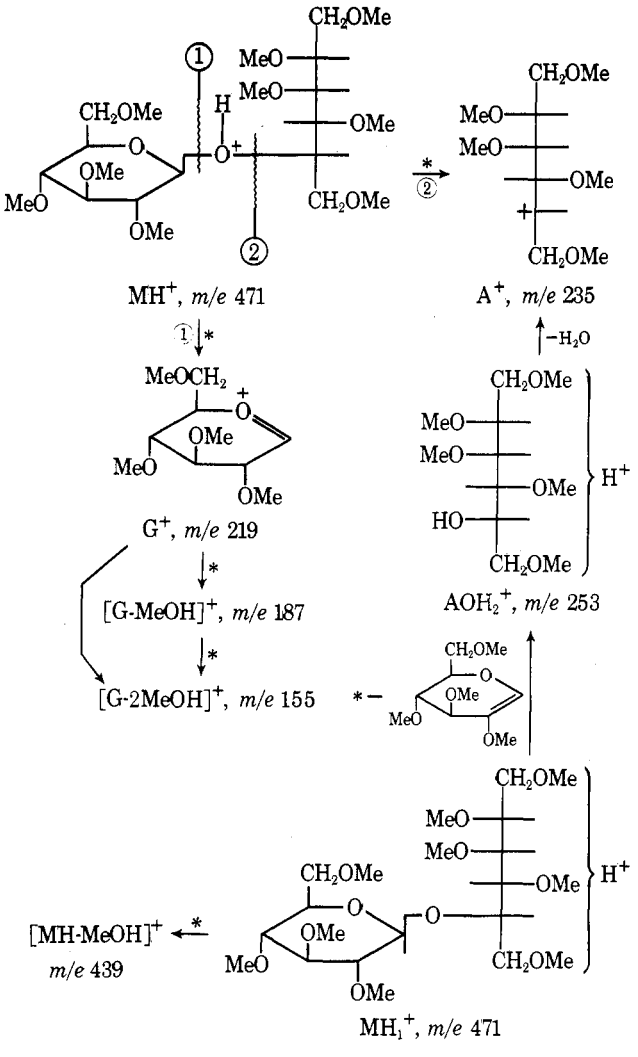


Table II. Monoisotopic Isobutane CI Mass Spectra of Permethylated Glycosylalditols[a]

Compd		MH^+	$MH - MeOH)^+$	AOH_2^+	A^+	G^+	$(G - MeOH)^+$	$(A - MeOH)^+$	$(G - 2MeOH)^+$	Other ions			
1	*m/e*	471	439	253	235	219	187		155	509	194	89	
	rel intensity	100	9	25	10	1	7		10	3	2	2	
2	*m/e*	472	440	254	236	219	187		155	502	263	89	80
	rel intensity	100	20	38	13	2	13		15	2.5	4	2	4
3	*m/e*	472	440	254	236	219	187		155	509	296	113	101
	rel intensity	100	10	40	2.5	2.5	16		12	1.5	3	1.5	1.5
4	*m/e*	472	440	254	236	219	187		155	509	296	93	
	rel intensity	100	6	24	4	1	8		2.5	1.5	2	1.5	
5	*m/e*	471	439	253	235	219	187		155	509	295		
	rel intensity	100	10	3.5	2.5	0.5	3.5		3	1.0	3		
6	*m/e*	383	351	209	191	175	143	159	111	277	245	211	201
	rel intensity	100	3	13	20	9	2	1	1.5	2.5	4	1.5	5
	m/e									193	177	145	
	rel intensity									2	1.5	5	

[a] Adduct ions, e.g., $(M + C_4H_9)^+$, whose intensity strongly depends on the precise conditions in the source have not been reported. The same is true for ions with less than 1% relative abundance. [b] A = alditol moiety, G = glycosyl moiety.

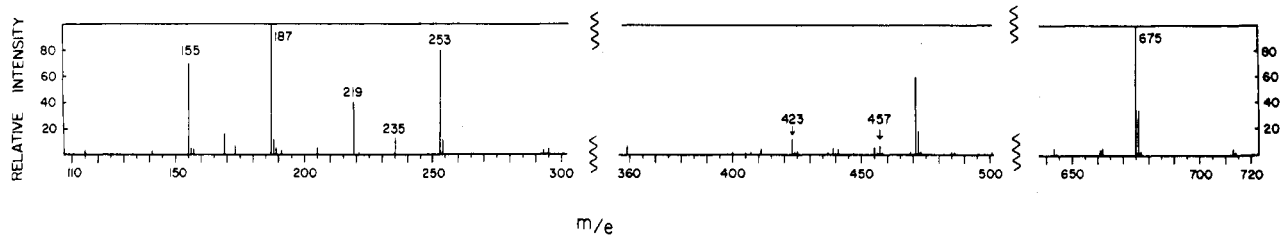


Figure 1. Isobutane CI mass spectrum of α-D-glucopyranosyl-1→4-*O*-α-D-glucopyranosyl-1→4-*O*-D-glucitol (7).

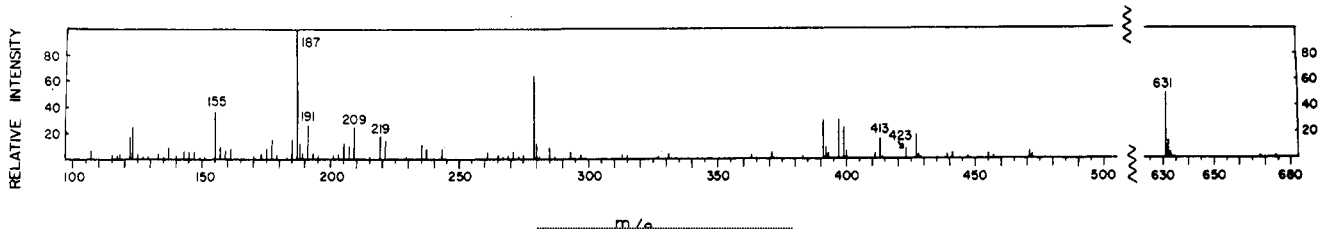


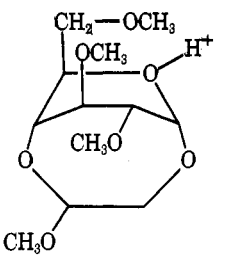
Figure 2. Isobutane CI mass spectrum of α-D-glucopyra[redacted]anosyl-1→1-*O*-DL-xylitol (8).

glucosyl residue with transfer of a hydrogen atom to the alditol fragment.

The metastable ion that appeared in all of the spectra of **1–5** at *m/e* 136.2 unequivocally connects the AOH_2^+ ion with the protonated molecule. The fact that we were unable to detect a metastable ion for the reaction $AOH_2^+ \rightarrow A^+ + H_2O$ supports the contention that the precursor to the AOH_2^+ ion is not protonated on the glycosidic oxygen and thus elimination of water from AOH_2^+ would require a proton rearrangement.

The isobutane CI mass spectra of the biosylalditols **7** and **8** are illustrated in Figures 1 and 2. In both cases the protonated molecule is a prominent ion in the spectrum. The two ion types seen in the glucosylalditol spectra, namely G^+ and A^+, are the prominent fragment ion series in both of these spectra. The fragments in the G series (*m/e* 155, 187, 219, and 423) and the A series (*m/e* 235, and 457) for **7** and *m/e* 191, 209, and 413 for **8** permit unequivocal determination of the mass sequence of the residues in the chain. The origins of the G and A series ions in these spectra are indicated in Scheme II.

Other than the sequence ions, the spectra of **7** and **8** both contain a prominent ion whose origin is not obvious. The prominent ion at *m/e* 471 in the spectrum of **7** corresponds to a protonated permethyl glucosylalditol. The ammonia/isobutane mass spectrum as well as GLC analysis gave no evidence of contamination of **7** with a permethylated disaccharide and thus this ion must have arisen by methoxy or methyl transfer in cleavage of the terminal glucosyl residue. The spectrum of **8** contains a prominent ion at *m/e* 279 which does not belong to the sequence ion series. The mass and isotope ratio suggested a formula for this ion of $C_{12}H_{23}O_7$ which would be compatible with a bicyclic structure involving the central glycosyl unit. Again the ammonia/isobutane spectra and GLC both indicated that the *m/e* 279 ion was not an artifact.



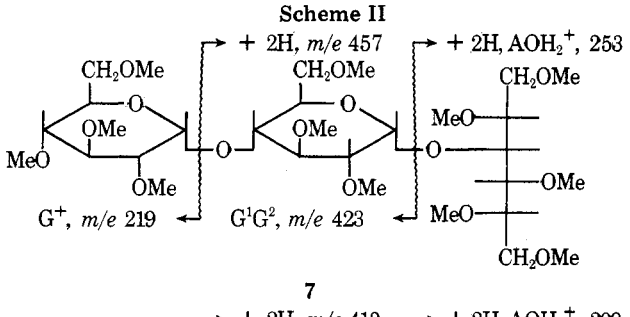
Ammonia/Isobutane CI Mass Spectra. The ammonia/isobutane mass spectra of this series of compounds have fewer prominent ions than their isobutane analogues in agreement with our expectations. The prominent ions in the ammonia/isobutane mass spectra of 1–8 are recorded in Table III.

These spectra are generally dominated by even mass ammonium ion complexes of molecules. Thus the fragments must have arisen by pyrolytic destruction of the original sugar derivatives.

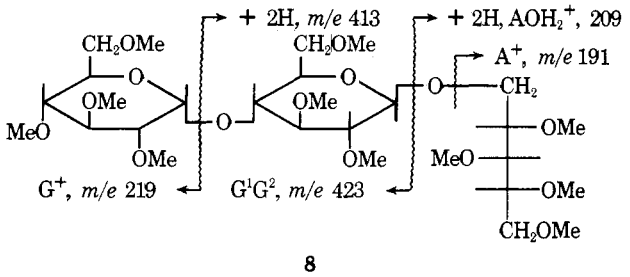
The fragments in the spectra of the biosylalditols exemplify the reactions that occur. The ion at *m/e* 472 in the mass spectrum of **7** would correspond to the ammonium ion complex of a permethyl glucosylglucopyranose. This molecule was probably formed by methyl or methoxyl transfer in the cleavage of the alditol terminus. The lower intensity of the same ion in the spectrum of **8** is consistent with structure dependence of the cleavage rate. The fact that the ion appears in both spectra indicates that it does not contain the alditol portion of the molecule. The ion at *m/e* 428 in the spectrum of **7** corresponds to $C_{18}H_{34}O_{10}$: NH_4^+ which could arise by a complex thermal fragmentation process removing Cl from the central glucosyl unit along with its attached alditol. The *m/e* 400 ion contains one less CO than that at 428, and could be a product of a similar fragmentation.

Proton transfer fragment ions also appear in the spectra as indicated in Table III; however, the intensity of these ions as

Scheme II



7



8

Table III. Monoisotopic Ammonia/Isobutane CI Mass Spectra of Permethylated Reduced Di- and Trisaccharides[a]

Compd		$(M + NH_4)^+$	MH^+	$(AOH + 2NH_4)^+$	AOH_2^+	A^+	Other ions				
1	*m/e*	488		270	253	235	450	439			
	rel intensity	100		4	2.5	8	5.5	3			
2	*m/e*	489	472	271		236	280	155			
	rel intensity	100	1	2		2.5	2.5	1			
3	*m/e*	489	472	271	254	236	296	210			
	rel intensity %	100	4.5	9.5			1.5	5.5	4	5	
4	*m/e*	489	472		254	236	110				
	rel intensity	100	3		1	2	2				
5	*m/e*	488	471	270	253	235	110	156			
	rel intensity	100	1.5	4.5	1	1	15	8			
6	*m/e*	400	383		191	236					
	rel intensity	100	2		1.5	5					
7	*m/e*	692					488	486	472	428	400
	rel intensity	100					12	14	25	20	8
8	*m/e*	648					414	408	302	296	157
	rel intensity	100					16	4.5	3.5	11	5

[a] Only the six most intense ions have been reported. The spectra were recorded with relatively low concentrations of the substrate in the source so that $(2M + NH_4)^+$ ions were insignificant.

well as that of the thermal fragment ions was low and generally of less structural utility than the corresponding ions in the isobutane spectra.

Summary. Isobutane CI mass spectra of permethylated saccharide alditols offer considerable promise for obtaining detailed oligosaccharide sequence information. Molecular weights and the mass sequence of monosaccharide units may be obtained directly from the spectra. The linkage position to a glucitol terminus may be inferred by the intensity ratios of alditol and alditol hydrate ions. On this basis the prospects for obtaining other position sequence information by examination of these spectra seems good.

Experimental Section

The permethylated oligosaccharide alditols used in this study were prepared by standard methods.[9] The deuterium labeled derivatives were prepared by $NaBD_4$ reduction of the parent oligosaccharide. Mass spectra were obtained by use of a solid probe inlet with an AEI MS-902 mass spectrometer equipped with an SRIC chemical ionization source. Spectra were recorded at a source pressure of 0.5 Torr (160 Pa). The source temperature was 150 °C. Ammonia/isobutane spectra were obtained with a 2:1 mixture of ammonia and isobutane at a total pressure of 0.5 Torr.

When isobutane was used as the reagent gas the intensity of the *tert*-butyl cation (*m/e* 57) exceeded the intensity of the most intense ion in the rest of the spectrum by at least a factor of 10. The recorded spectra were all obtained with the ^{13}C ion to *m/e* 58 off scale on the least sensitive range.

In the ammonia/isobutane spectra the ammonium ion (*m/e* 18) was always at least 10 times the intensity of any of the other ions in the spectrum.

Registry No.—**1**, 30608-25-4; **2**, 30608-28-7; **3**, 59907-27-6; **4**, 30608-29-8; **5**, 29923-20-4; **6**, 59907-28-7; **7**, 32581-17-2; **8**, 59907-29-8.

References and Notes

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