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Structural Investigation of the Antibiotic Sporaviridin. IX.[1] Chemical Ionization Mass Spectral Studies of Permethylated Viridopentaoses and Their Degradation Products

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Sequence determination of permethylated viridopentaoses and their degradation products was performed by chemical ionization mass spectrometry (CIMS) using isobutane and ammonia as reagent gases. The CI mass spectra show MH^+ or $(M+NH_4)^+$ ions in the molecular ion region. Fragmentation involving carbon–carbon bond fission rarely occurs. As a major fragmentation, the glycosidic linkage is cleaved between the glycosidic oxygen atom and the anomeric carbon atom. Resulting fragment ions in the isobutane spectra can be classified into oxonium and protonated (with methyl or hydrogen transfer) ions, whereas fragment ions in the ammonia spectra can be divided into oxonium (occasionally with attachment of an ammonia molecule) and ammonium adduct (with methyl or hydrogen transfer) ions. These results are shown to be useful for sequencing unknown oligosaccharides.

Keywords——viridopentaose; chemical ionization mass spectrometry; sequence determination; reagent gas; fragmentation

Although chemical ionization mass spectrometry (CIMS) has frequently been used as a complementary method to electron impact mass spectrometry (EIMS), it is evident that CIMS is more effective for molecular weight determination and structural characterization of relatively involatile compounds than EIMS.[2] We have investigated the characterization of naturally occurring glycosides by this method.[3–6] One of the characteristic features of CIMS is that its fragmentations are similar to acidic reactions in solution, so that controlled fragmentations yielding useful structural information are expected to occur. Therefore, CIMS is a useful method for structural characterization of peptides and oligosaccharides, especially for sequence determination. We have described a fundamental study on the CI mass spectrometric behavior of permethylated readily available oligosaccharides.[7] However, because the samples examined were homooligosaccharides or heterooligosaccharides with constituent monosaccharides having the same molecular weight, it was hard to overcome the isomeric weight problem.

In the previous papers we reported three heteropentasaccharides, viridopentaoses A, B and C, which are carbohydrate moieties of the antibiotic sporaviridin.[1,8,9] Sequence information, apart from the positions of linkages, could be successfully obtained by CIMS. Moreover, since the constituent monosaccharides have different molecular weight, it is possible that these oligosaccharides may be useful as model compounds for CIMS studies.

In the present paper we wish to discuss the CI mass spectrometric behavior of permethylated viridopentaoses and their degradation products in connection with their structural elucidation.

Experimental

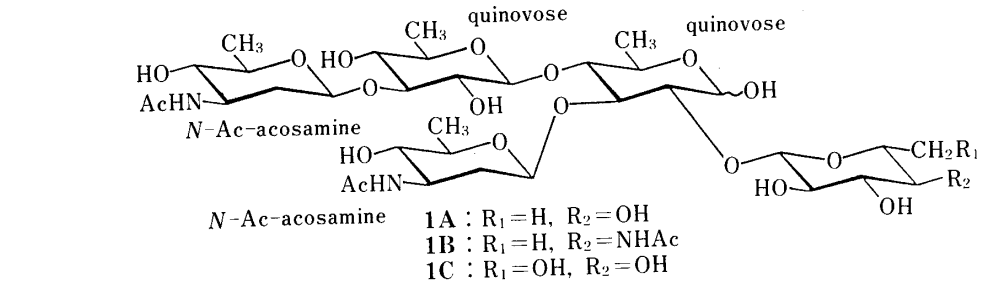
Chemical ionization mass spectra were recorded on a Shimadzu LKB 9000A mass spectrometer under the

following conditions: accelerating voltage 3.5 kV; electron energy 140 eV; emission current 250 or 500 mA. The ion source temperature was maintained at 120—150 °C. Reagent gas pressure was approximately 0.1 Torr. All the samples were introduced with a direct-insertion probe. The reagent gases used, iso-C_4H_{10} and NH_3, were obtained from Takachiho Trading Co., Ltd.

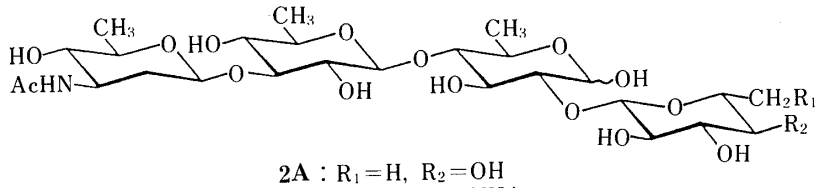
Permethylation of oligosaccharides was performed by a modification of Hakomori's method. The origins of all oligosaccharides studied were described in Part VII.[9]

Results and Discussion

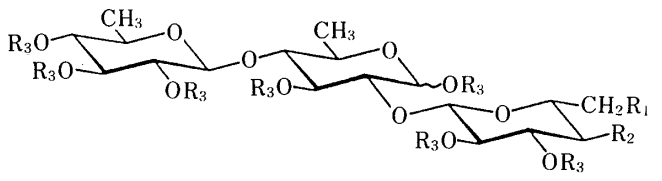
Viridopentaoses A (**1A**), B (**1B**) and C (**1C**) are ammonolysis products obtained from the antibiotic sporaviridin. These compounds have been determined to be novel heteropentasaccharides containing two or three amino sugars on the basis of some chemical degradative reactions and spectroscopic evidence. In particular, acidic methanolysis of viridopentaoses yielded structurally informative products, viridotetraoses A (**2A**), B (**2B**), C (**2C**), viridotrioses A (**3A**), B (**3B**), C (**3C**) and methyl α-viridobioside B (**4B**).[9]



1A : R_1=H, R_2=OH
1B : R_1=H, R_2=NHAc
1C : R_1=OH, R_2=OH

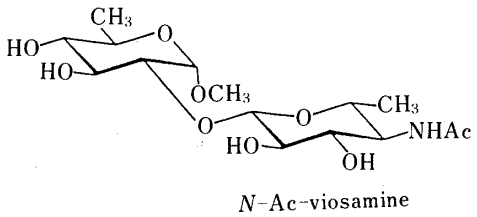


2A : R_1=H, R_2=OH
2B : R_1=H, R_2=NHAc
2C : R_1=OH, R_2=OH



3A : R_1=H, R_2=OH
3B : R_1=H, R_2=NHAc
3C : R_1=OH, R_2=OH

5A : R_1=H, R_2=OCH_3, R_3=CH_3
5B : R_1=H, R_2=$N(CH_3)Ac$, R_3=CH_3
5C : R_1=OCH_3, R_2=OCH_3, R_3=CH_3



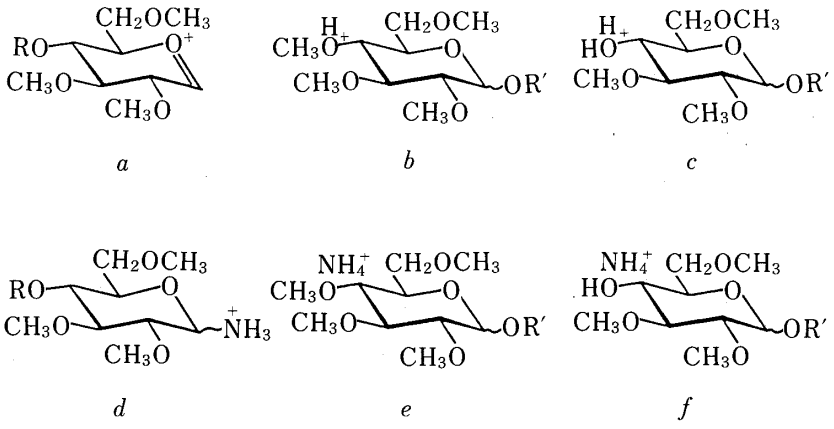
N-Ac-viosamine
4B

Chart 1

Although the CI mass spectra of the disaccharide, **4B** could be successfully measured without leading it to a volatile derivative, it was impossible to obtain the CI mass spectra of the intact oligosaccharides, **1**—**3** under CI conditions on account of their low volatility. In view of the molecular weights of the three pentasaccharides (**1A**: 798, **1B**: 839, **1C**: 814), we chose to prepare permethylated derivatives to increase the volatility of these three oligosaccharides for our present study.

We have already described the CI mass spectrometric behavior of permethylated neutral tri- and tetrasaccharides using isobutane and ammonia as reagent gases.[7] The results obtained can be summarized as follows (Chart 2): 1) Ammonia CI mass spectra exhibit unambiguously an ammonium adduct molecular ion $(M+NH_4)^+$, whereas isobutane CI

mass spectra give considerable information concerning fragment ions. 2) Fragmentations are mainly restricted to the glycosidic linkage between the glycosidic oxygen atom and the anomeric carbon atom.[10] 3) Resulting fragment ions in isobutane spectra can be divided into oxonium type ions, *a*, and protonated ions, which are formed by methyl or hydrogen transfer with cleavages of glycosidic bonds (*b* and *c*).[11,12] 4) Fragment ions in ammonia spectra appear as oxonium type ions with attachment of an ammonia molecule, *d* ($a+NH_3$) and ammonium adduct ions (*e* and *f*), which are also produced with methyl or hydrogen transfer. 5) Molecular ion and fragment ion species are usually accompanied with demethanolated ions.



R=CH_3 or glycosyl, R'=glycosyl

Chart 2

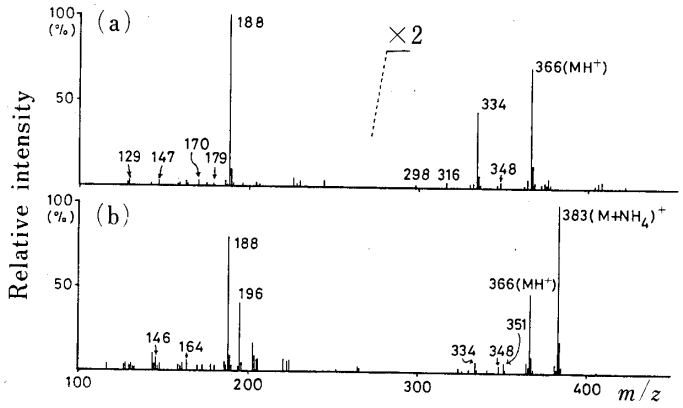


Fig. 1. CI Mass Spectra of Methyl α-Viridobioside (**4B**)
(a) isobutane; (b) ammonia.

We applied these results for the sequence analysis of viridopentaoses and their degradation products. Figure 1 shows the CI mass spectra of **4B** using isobutane and ammonia as reagent gases. In the molecular ion region the protonated molecule (MH^+) is observed at *m/z* 366 in the isobutane CI mass spectrum, whereas both MH^+ and $(M+NH_4)^+$ appear clearly in the ammonia CI mass spectrum. While the viosamine-derived oxonium type ion (*m/z* 188) occurs in common in both spectra, the quinovose-derived ion (*m/z* 196, $(178+NH_4)^+$) is only recognized in the ammonia spectrum. These results show that the quinovose residue is a reducing end. The CI mass spectra of permethylated viridotrioses A (**5A**), B (**5B**) and C (**5C**) have been discussed in detail; the CI mass spectrometric behavior of

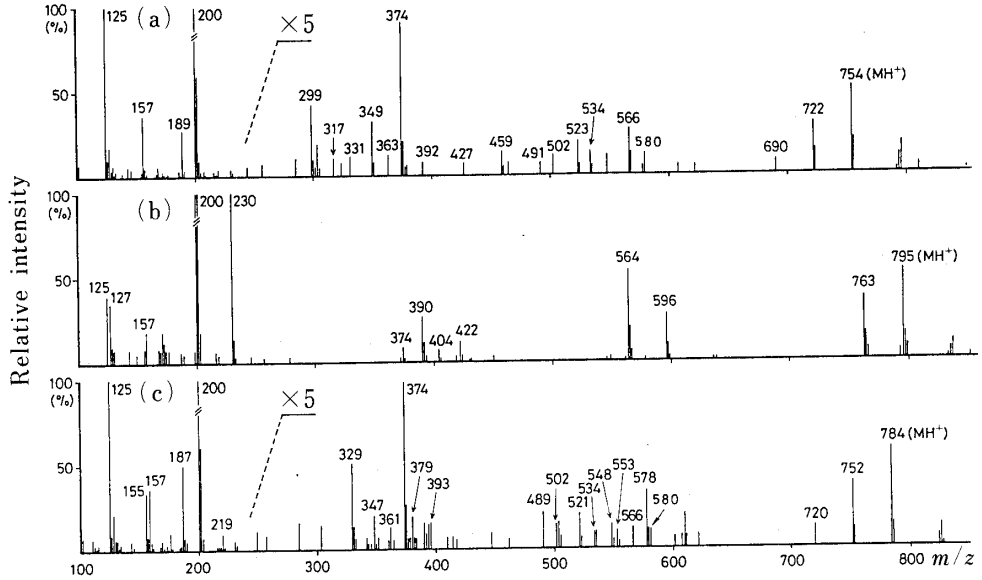
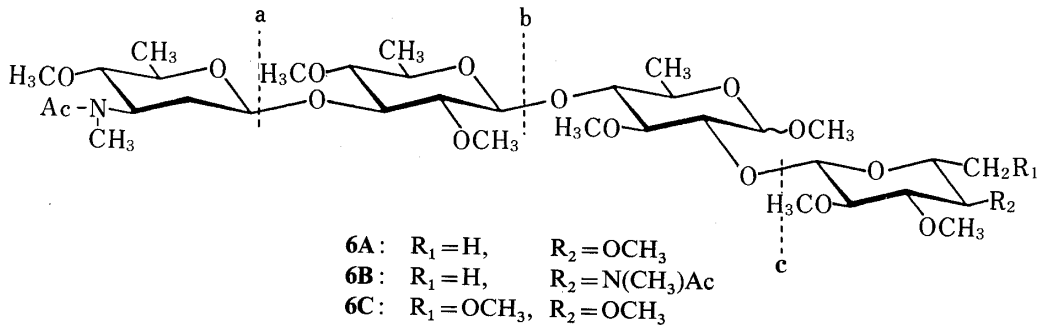


Fig. 2. CI Mass Spectra of Permethylated Viridotetraoses (a) A (**6A**); (b) B (**6B**); (c) C (**6C**) Using Isobutane as a Reagent Gas

the aminosugar-containing compounds, **5B**, is considerably different from those of the other two compounds.[7]

The isobutane CI mass spectra of permethylated viridotetraoses A (**6A**), B (**6B**) and C (**6C**) are shown in Fig. 2. The MH^+ ions appear at *m/z* 754, 795 and 784, respectively. The other ions at *m/z* 722, 690, 763, 752 and 720 in the higher mass region are generated by losses of methanol from these molecular ion species. The ions beyond MH^+ correspond to molecular ion cluster such as $(M+C_3H_3)^+$, $(M+C_3H_5)^+$, $(M+C_3H_7)^+$ and $(M+C_4H_9)^+$. The fragmentations are mainly restricted to the glycosidic linkages (a—c, Chart 3) between glycosidic oxygen and the anomeric carbon atoms. Consequently, the fragment ions can be classified into three groups which are assigned as tri-, di- and monosaccharide sequence ions. They are summarized in Table I. The trisaccharide ions are of protonated type, formed by methyl or hydrogen transfer with the cleavage of the glycosidic linkage a or c. The trisaccharide ions at *m/z* 566 and 580, formed by the cleavage of the glycosidic linkage c, are common to **6A** and **6C**, however these ions cannot be recognized in the spectrum of **6B**. The alternative trisaccharide ion appears at *m/z* 596 with cleavage of the glycosidic linkage a in the case of **6B**. Although the trisaccharide ions at *m/z* 555 and 585 themselves do not appear, corresponding demethanolated ions appear at *m/z* 523 and 553 in the spectra of **6A** and **6C**, respectively. The disaccharide ions are mainly produced by the cleavage of the linkage b, so that two kinds of sequence ions are formed. One kind is an oxonium type ion at *m/z* 374 in common for all three components and the other kind consists of counterpart ions with methyl or hydrogen transfer. Because the latter ions contain the reducing end, they frequently appear as demethanolated ions (**6A**, *m/z* 349, 363; **6B**, *m/z* 390, 404; **6C**, *m/z* 379, 393). The monosaccharide-derived ions all appear as oxonium-type ions at *m/z* 200, 189, 230 and 219 which are the counterparts of the trisaccharide sequence ions. Among them, the acosamine-derived ion is very intense at *m/z* 200 in common for all three components. This behavior is consistent with that seen in acidic methanolysis of permethylated viridopentaoses.[9]

The ammonia CI mass spectra of **6A**, **6B** and **6C** clearly show both molecular-related ions, $(M+NH_4)^+$ ions at *m/z* 771, 812 and 801 and MH^+ ions at *m/z* 754, 795 and 784 (Fig. 3). The resulting fragment ions are mainly ammonium-type ions, which can also be classified into three groups, tri-, di- and monosaccharide sequence ions (Table II). While the cleavages



6A: $R_1=H$, $R_2=OCH_3$
6B: $R_1=H$, $R_2=N(CH_3)Ac$
6C: $R_1=OCH_3$, $R_2=OCH_3$

Chart 3

TABLE I. Diagnostic Ions (m/z) from the Isobutane CI Mass Spectra of **6A**, **6B** and **6C**

	Molecular ion region	Trisaccharide ion				Disaccharide ion			Monosaccharide ion	
		a		c			b		a	c
		H	CH_3	H	CH_3		H	CH_3		
	754 (MH^+)	555*	—	566	580	374	381*	395*	200	189
	↓	↓		↓			↓	↓		↓
6A	722	523		534			349	363		157
	↓	↓		↓			↓	↓		↓
	690	491		502			317	331		125
	795 (MH^+)	596	—	—	—	374	422	436*	200	230
6B	↓	↓					↓	↓		
	763	564					390	404		
	784 (MH^+)	585*	—	566	580	374	411*	425*	200	219
	↓	↓		↓	↓		↓	↓		↓
6C	752	553		534	548		379	393		187
	↓	↓		↓			↓	↓		↓
	720	521		502			347	361		155
		↓						↓		
		489						329		

Asterisks indicate the presence of a demethanolated ion.

of linkages a or c produce trisaccharide ions (ammonium adduct ions) and monosaccharide ions (ammonia addition ions for the neutral sugars or oxonium ions for the amino sugars), the cleavage of linkage b forms two disaccharide ions, ammonium adduct and oxonium-type ions. Because the proton affinity of the acetoamide group is higher than that of ammonia, aminosugar-containing fragment ions are frequently observed without attachment of ammonia or ammonium ion.

The isobutane CI mass spectra of permethylated viridopentaoses A (**7A**), B (**7B**) and C (**7C**) are presented in Fig. 4. The MH^+ ions are definitely observed at m/z 939, 980 and 969, respectively. The loss of a methanol molecule from these ions produces the ions at m/z 907, 948 and 937, respectively. In the higher region than MH^+, molecular ion cluster occurs. Since these compounds possess four glycosidic linkages in the molecule, the fragmentations are more complex than is the case below the tetrasaccharide level. The resulting fragment ions are chiefly produced by cleavages of the glycosidic linkages, producing protonated or oxonium ions. They can be divided into four groups, tetra-, tri-, di- and monosaccharide sequence ions, as summarized in Table III and Chart 4. The tetrasaccharide ions are formed by cleavage of

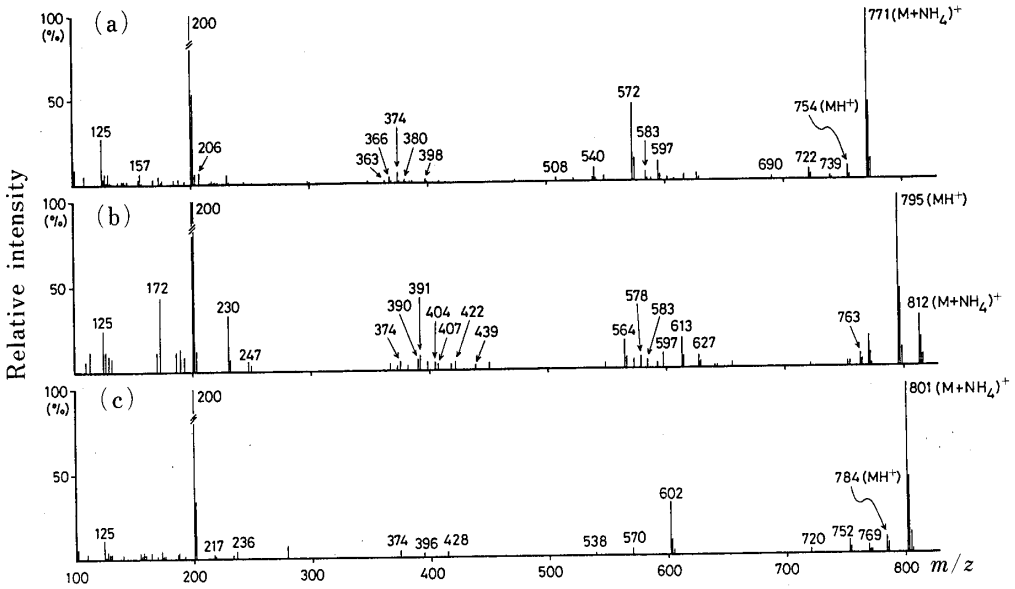


Fig. 3. CI Mass Spectra of Permethylated Viridotetraoses (a) A (**6A**); (b) B (**6B**); (c) C (**6C**) Using Ammonia as a Reagent Gas

TABLE II. Diagnostic Ions (m/z) from the Ammonia CI Mass Spectra of **6A**, **6B** and **6C**

	Molecular ion region		Trisaccharide ion				Disaccharide ion			Monosaccharide ion	
			a		c			b			
	$(M+NH_4)^+$	(MH^+)	H	CH_3	H	CH_3		H	CH_3	a	c
6A	771 ↓ 739	754 ↓ 722 ↓ 690	572 ↓ 540 ↓ 508	—	583	597	374	398 ↓ 366	412* ↓ 380	200	206
6B	812	795 ↓ 763	613	627	583	597	374	439 ↓ 407	(436) ↓ 404	200	247 (230)
6C	801 ↓ 769	784 ↓ 752 ↓ 720	602 ↓ 570 ↓ 538	—	—	—	374	428 ↓ 396	—	200	236

Asterisks indicate the presence of a demethanolated ion.
Ions in parentheses appear without attachment of ammonia or ammonium ion.

the glycosidic linkage a or g. The cleavage of the linkage a with hydrogen transfer produces three different ions at m/z 740, 781 and 770. In the case of **7A** only, the methyl-transfer ion is recognized at m/z 754. In contrast to the above behavior the cleavages of linkage g in **7A** and **7C** occur mainly with methyl transfer, so that common ions are observed at m/z 765. The trisaccharide ions result from the fission of the glycosidic linkages b, c, and f. The formation of the ions at m/z 552 and 566 for **7A** and **7C** involves two-bond fission. Similarly, the loss of two *N*-acetylacosamine moieties with hydrogen transfer gives the sequence ions at m/z 541, 582 and 571 in each spectrum, but no corresponding methyl-transfer ions are found in the

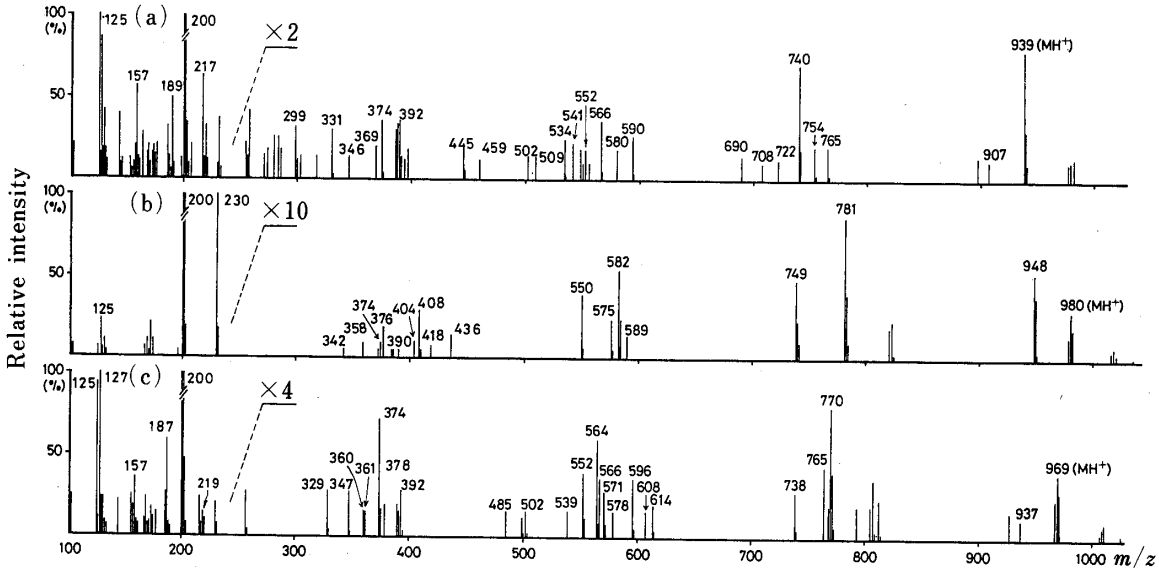
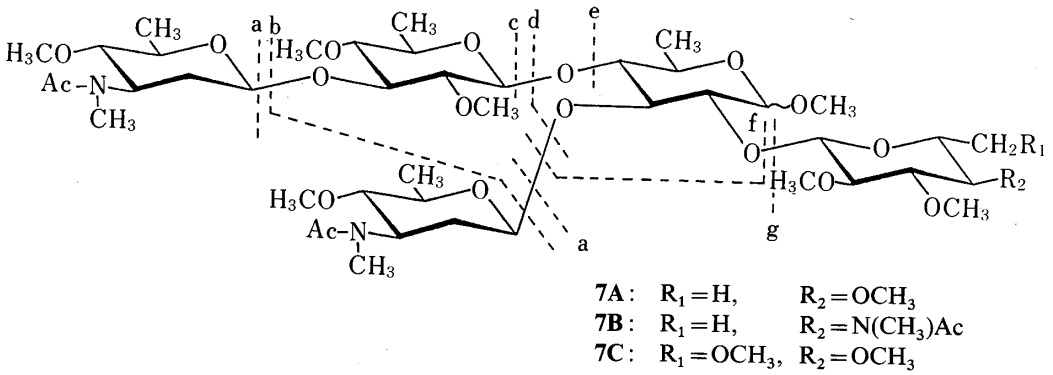
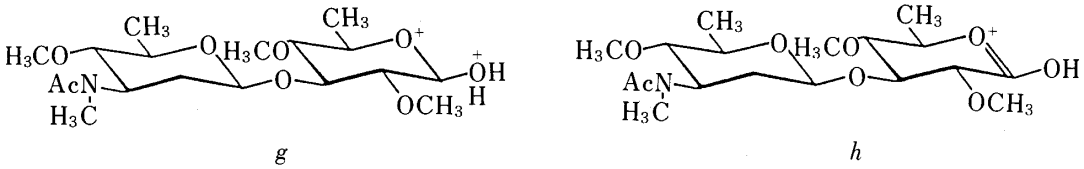


Fig. 4. CI Mass Spectra of Permethylated Viridopentaoses (a) A (**7A**); (b) B (**7B**); (c) C (**7C**) Using Isobutane as a Reagent Gas



7A: $R_1=H$, $R_2=OCH_3$
7B: $R_1=H$, $R_2=N(CH_3)Ac$
7C: $R_1=OCH_3$, $R_2=OCH_3$

Chart 4



g *h*

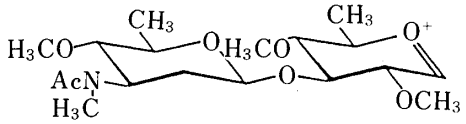


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Chart 5

spectra. The cleavage of the glycosidic linkage c generates two series of methyl- and hydrogen-transfer trisaccharide ions. When linkage d is cleaved, three disaccharide ions are observed at *m/z* 408, 422 and 436 in **7B** only, because of the presence of the amino sugar in its molecule.

TABLE III. Diagnostic Ions (m/z) from the Isobutane CI Mass Spectra of **7A**, **7B** and **7C**

	Molecular ion region	Tetrasaccharide ion				Trisaccharide ion						Disaccharide ion					Monosaccharide ion	
		a		g		b		c		f		c	e	d			a	g
		H	CH_3	H	CH_3	2H	CH_3+H	H	CH_3	2H	CH_3+H			2H	CH_3+H	$2CH_3$		
7A	939 (MH^+) ↓ 907	740 ↓ 708	754 ↓ 722 ↓ 690	—	765	541 ↓ 509	—	566 ↓ 534 ↓ 502	580 ↓ 548	552	566	374	392 390	—	—	—	200	189 ↓ 157 ↓ 125
7B	980 (MH^+) ↓ 948	781 ↓ 749	—	—	—	582 ↓ 550	—	607* ↓ 575	621* ↓ 589	—	—	374 ↓ 342	390	408 ↓ 376	422* ↓ 390 ↓ 358	436 ↓ 404	200	230
7C	969 (MH^+) ↓ 937	770 ↓ 738	—	—	765	571 ↓ 539	—	596 ↓ 564	610* ↓ 578	552	566	374	392 390	—	—	—	200	219 ↓ 187 ↓ 155

Asterisks indicate the presence of a demethanolated ion.

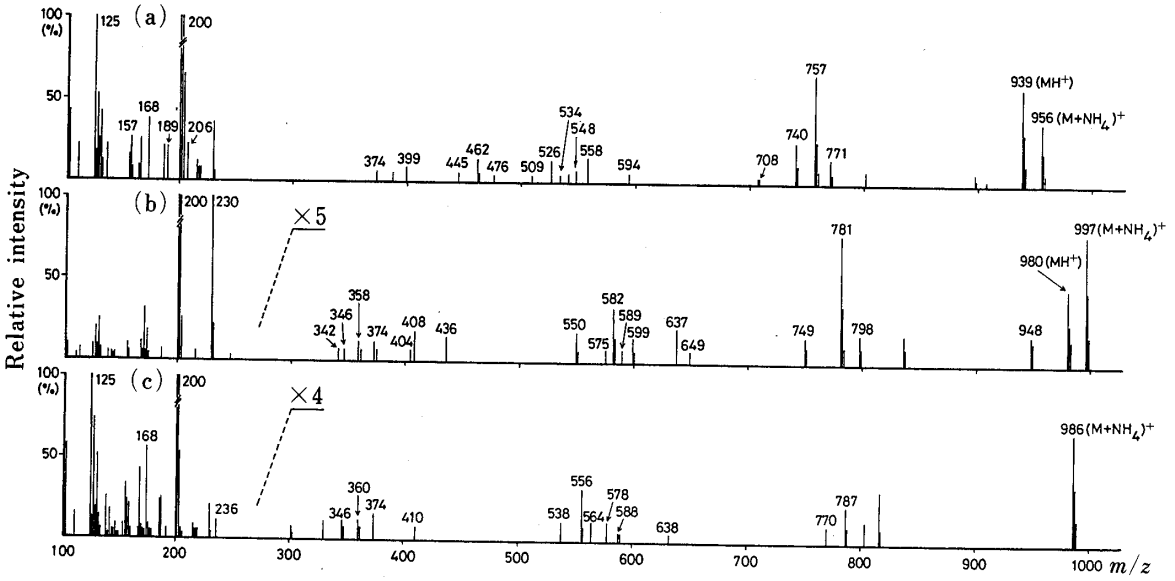

Fig. 5. CI Mass Spectra of Permethylated Viridopentaoses (a) A (**7A**); (b) B (**7B**); (c) C (**7C**) Using Ammonia as a Reagent Gas

TABLE IV. Diagnostic Ions (m/z) from the Ammonia CI Mass Spectra of **7A**, **7B** and **7C**

	Molecular ion region	Tetrasaccharide ion		Trisaccharide ion				Disaccharide ion				Monosaccharide ion	
		a		b		c		c	d			a	g
		H	CH_3	2H	CH_3+H	H	CH_3		2H	CH_3+H	$2CH_3$		
7A	956 $(M+NH_4)^+$	757 (740)	771	558	—	566*	580*	374	—	—	—	200	206
	↓	↓		↓		↓	↓						
	939 (MH^+)	708		526		534	548						
7B	997 $(M+NH_4)^+$	798 (781)		599 (582)	—	607*	621*	374 (408)	—		(436)	200	(230)
	↓	↓		↓		↓	↓				↓		
	980 (MH^+)	749		550		575	589				404		
	↓												
	948												
7C	986 $(M+NH_4)^+$	787 (770)		588	—	596*	610*	374	—	—	—	200	236
						↓	↓						
						564	578						

Asterisks indicate the presence of a demethanolated ion.
Ions in parentheses appear without attachment of ammonia or ammonium ion.

No ion of this type is observed in the case of **7A** or **7C**. The disaccharide ion at m/z 374 in common for the three components is an oxonium ion formed by the cleavage of linkage c. The ions at m/z 392 and 390 are formed by the fission of linkage e between the glycosidic oxygen atom and reducing ring. That is, the glycosidic linkage between the reducing and non-reducing quinovoses is cleaved at both sides (c and e) of the glycosidic oxygen atom to produce three disaccharide ions, *g* (m/z 392), *h* (m/z 390) and *i* (m/z 374) (Chart 5). Similar fragmentations have been recognized in the CI mass spectra of basic macrolide antibiotics.[5] The monosaccharide ions all appear as oxonium-type ions at m/z 200, 189, 230 and 219, the counterparts of the tetrasaccharide ions.

Figure 5 shows the ammonia CI mass spectra of **7A**, **7B** and **7C**. In the molecular ion region, the $(M+NH_4)^+$ and/or MH^+ ions appear abundantly. The fragmentations are very

limited as compared with those in the isobutane CI mass spectra. The sequence ions are summarized in Table IV. Although these ions are mainly found upwards by 17 u as compared with those in isobutane CI, the aminosugar-containing fragment ions occur without attachment of ammonia or ammonium ion.

In conclusion, sequence determination of permethylated viridopentaoses and their degradation products was performed by CIMS using isobutane and ammonia as reagent gases. The CI mass spectra show the MH^+ or $(M+NH_4)^+$ ions in the molecular ion region, and the latter ions clearly indicate the molecular weight. Fragmentation involving carbon–carbon bond fission rarely occurs. Because these oligosaccharides are composed of constituent monosaccharides with different molecular weights, it is obvious that the glycosidic linkage is mainly cleaved between the glycosidic oxygen atom and the anomeric carbon atom. Thus, this mass spectrometric behavior is similar to the chemical behavior in acidic methanolysis of permethylated oligosaccharides. As expected, the fragment ions in isobutane spectra consist of oxonium and protonated (with methyl or hydrogen transfer) ions, and fragment ions in the ammonia spectra are also composed of two groups, oxonium (occasionally with attachment of an ammonia molecule) and ammonium adduct (with methyl or hydrogen transfer) ions. These results should be valuable for sequencing studies on unknown oligosaccharides.

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