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Structural Investigation of the Antibiotic Sporaviridin. VIII.[1] Carbon-13 Nuclear Magnetic Resonance Studies of Viridopentaoses and Related Compounds

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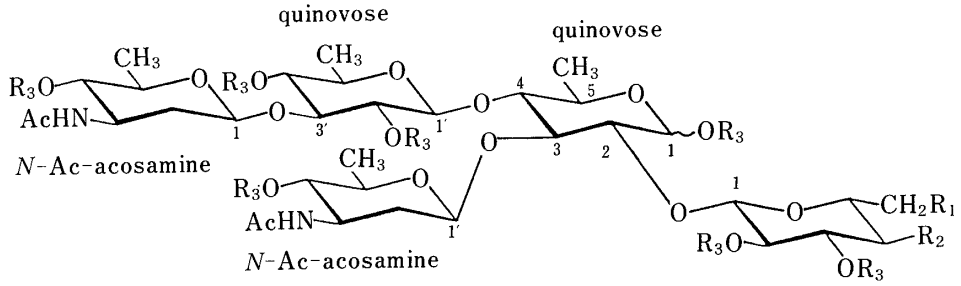
The significant features of the carbon-13 nuclear magnetic resonance spectra of viridopentaoses (**1A**, **1B** and **1C**), constituent pentasaccharides of *N*-acetylsporaviridin, and their degradation products (**2**—**9**) are discussed. The glycosidation shift was successfully applied to characterize the glycosidic linkages up to the tetrasaccharide level, whereas anomalous behavior was found in the pentasaccharides.

Keywords——sporaviridin; viridopentaose; ^{13}C-NMR; glycosidation shift

Sporaviridin (SVD) is a basic and water-soluble antibiotic containing oligosaccharide moieties.[2,3] In the previous paper we reported structural studies on the three constituent pentasaccharides, viridopentaoses A (**1A**), B (**1B**) and C (**1C**).[1] Carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectroscopy played a significant role in their structural characterization, and we now discuss in detail the ^{13}C-NMR spectra of **1A**, **1B** and **1C**, and their degradation products whose origins were described in Part VII.[1]



1

A: R_1=H, R_2=OH, R_3=H
B: R_1=H, R_2=NHAc, R_3=H
C: R_1=OH, R_2=OH, R_3=H

2

A: R_1=H, R_2=OAc, R_3=Ac
B: R_1=H, R_2=NHAc, R_3=Ac
C: R_1=OAc, R_2=OAc, R_3=Ac

Chart 1

Experimental

^{13}C-NMR spectra were recorded on a JEOL FX-100 spectrometer at 25 MHz (sweep width 6024 Hz, 8 K data points in the domain, pulse angle 45°). Tetramethylsilane (TMS) was used as an internal standard. Unless otherwise stated, samples were measured in CD_3OD. For other experimental conditions, see ref. 1.

Dihydroviridopentaose A (19A)——A solution of **1A** (50 mg) in MeOH (3 ml) was stirred with $NaBH_4$ (100 mg) at room temperature for 24 h. The reaction mixture was subjected to column chromatography on Sephadex LH-20 with MeOH to yield 53 mg of **19A**, which was precipitated from MeOH–Et_2O to give a white powder, mp 180—182 °C (dec.). $[\alpha]_D^{23}$ −20.6° (c=0.3, MeOH). Infrared spectrum (IR) ν_{max}^{KBr} cm^{-1}: 3350 (OH/NH), 1620 (CO). ^{1}H-NMR

(CD_3OD) δ: 1.95 (6H, s, $NCOCH_3$). Secondary ion mass spectrum (SIMS): *m/z* 839 $(M+K)^+$, 823 $(M+Na)^+$, 801 (MH^+). *Anal.* Calcd for $C_{34}H_{60}N_2O_{19}\cdot 3H_2O$: C, 47.77; H, 7.73; N, 3.28. Found: 47.81; H, 8.14, N, 3.11.

Dihydroviridopentaose B (19B)——A solution of **1B** (50 mg) in MeOH (3 ml) was stirred with $NaBH_4$ (100 mg) at room temperature for 24 h. The reaction mixture was subjected to column chromatography on Sephadex LH-20 with MeOH to yield 48 mg of **19B**, which was precipitated from MeOH–Et_2O to give a white powder, mp 183—186 °C (dec.). $[\alpha]_D^{23}$ −40.2° (*c*=0.3, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH/NH), 1620 (CO). ^{1}H-NMR (CD_3OD) δ: 1.97 (9H, s, $NCOCH_3$). SIMS: *m/z* 880 $(M+K)^+$, 864 $(M+Na)^+$, 842 (MH^+). *Anal.* Calcd for $C_{36}H_{63}N_3O_{19}\cdot 4H_2O$: C, 47.32; H, 7.78; N, 4.60. Found: C, 47.39; H, 8.14; N, 4.42.

Dihydroviridopentaose C (19C)——A solution of **1C** (50 mg) in MeOH (3 ml) was stirred with $NaBH_4$ (100 mg) at room temperature for 24 h. The reaction mixture was subjected to column chromatography on Sephadex LH-20 with MeOH to yield 53 mg of **19C**, which was precipitated from MeOH–Et_2O to give a white powder, mp 179—183 °C (dec.). $[\alpha]_D^{23}$ −43.0° (*c*=0.3, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH/NH), 1630 (CO). ^{1}H-NMR (CD_3OD) δ: 1.95 (6H, s, $NCOCH_3$). SIMS: *m/z* 855 $(M+K)^+$, 839 $(M+Na)^+$, 817 (MH^+). *Anal.* Calcd for $C_{34}H_{60}N_2O_{20}\cdot 3H_2O$: C, 46.90; H, 7.59; N, 3.22. Found: C, 46.89; H, 8.07; N, 3.09.

Abbreviations: Qui, D-quinovose residue; Glu, D-glucose residue; Vio, *N*-acetyl-D-viosamine residue; Aco, *N*-acetyl-D-acosamine residue.

Results and Discussion

The isolation, purification and principal physicochemical properties of viridopentaoses A, B and C were reported in the previous paper.[1] Their ^{13}C-NMR spectra are very similar to one another as shown in Fig. 1. Each component can immediately be identified as a pentasaccharide because of the presence of five anomeric carbon signals (A, 105.4, 101.7, 101.3, 100.9, 93.2; B, 105.4, 101.9, 101.5, 101.0, 93.4; C, 105.6, 101.7, 101.5, 101.0, 93.2 ppm) in the spectra. Among them, the highest field signals (93.2 and 93.4 ppm) are assignable to the anomeric carbons of the reducing monosaccharide residues. However, only the assignments given in Fig. 1 could be made in our initial studies. Therefore we attempted to elucidate the structures of the pentasaccharides on the basis of the ^{13}C-NMR chemical shifts of the constituent monosaccharides (**3**—**7**) shown in Chart 2 and the degradation products (**14**—**19**) shown in Charts 4 and 5.

Each component contains the following constituent monosaccharides: A, Qui : Aco (3 : 2); B, Qui : Aco : Vio (2 : 2 : 1); C, Qui : Aco : Glu (2 : 2 : 1).[4] First, the chemical shifts of these constituent monosaccharides were investigated. In view of the solubility properties of

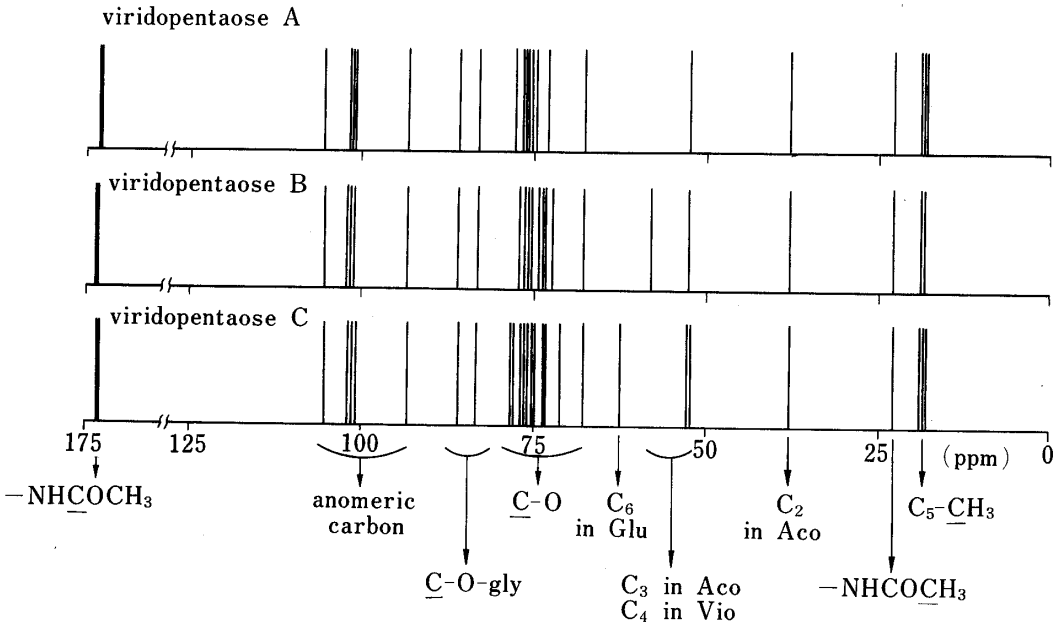
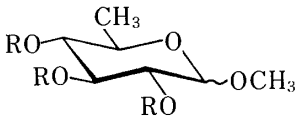
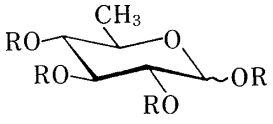


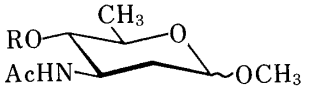
Fig. 1. ^{13}C-NMR Spectra of Viridopentaoses A, B, and C



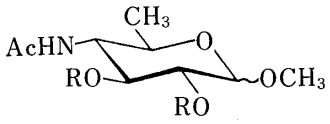
3 : R=H
9 : R=Ac



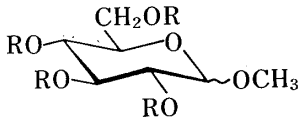
4 : R=H
10 : R=Ac



5 : R=H
11 : R=Ac



6 : R=H
12 : R=Ac



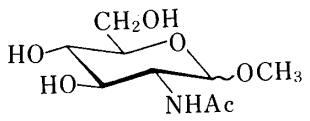
7 : R=H
13 : R=Ac

Chart 2

TABLE I. ^{13}C-NMR Spectral Data for 3—7 (ppm Values)

		C-1	C-2	C-3	C-4	C-5	C-6
3	α	100.9	73.5	74.7	77.1	68.4	18.0
	β	105.0	75.0	77.6	76.8	73.1	18.0
4	α	93.6	73.9	74.5	77.3	68.1	18.1
	β	97.7	76.3	77.6	76.9	73.1	18.1
5	α	98.7	36.9	49.7	76.5	69.5	18.3
	β	102.0	38.1	52.4	75.9	74.6	18.3
6	α	100.8	73.9	72.2	58.1	67.5	18.1
	β	104.9	75.2	75.5	58.1	72.1	18.2
7	α	100.6	72.9	74.6	71.1	72.9	62.1
	β	105.1	74.8	77.8	71.4	77.6	62.5

viridopentaoses and related compounds, measurements must be made in CD_3OD. The chemical shifts of anomeric pairs of methyl D-quinovoside (**3**), D-quinovose (**4**) and methyl D-glucoside (**7**) were assigned as shown in Table I by reference to the reported values in D_2O or pyridine-d_5.[5-7] In the case of methyl *N*-acetylviosaminide (**6**), the relationship of the difference of the chemical shifts between **6** and **3** was compared with that in the case of **7** and methyl *N*-acetylglucosaminide (**8**, Table II).[5,8] These data show that the carbon directly attached to the hydroxy group (α-carbon) and the carbon adjacent to the α-carbon (β-carbon)



8

Chart 3

TABLE II. ^{13}C-NMR Spectral Data for **3**, **6**, **7** and **8** (ppm Value)

	7[a]		**8**[b]		Δ=**7**—**8**	
	α	β	α	β	α	β
C-2	73.1	74.6	55.0	56.8	+18.1	+17.8
C-3	74.8	77.3	72.4	75.2	+2.4	+2.1
C-4	71.4	71.2	71.5	71.2	−0.1	0

	3		**6**		Δ=**3**—**6**	
	α	β	α	β	α	β
C-2	73.5	75.0	73.9	75.2	−0.4	−0.2
C-3	74.7	77.6	72.2	75.5	+2.5	+2.1
C-4	77.1	76.8	58.1	58.1	+19.0	+18.7

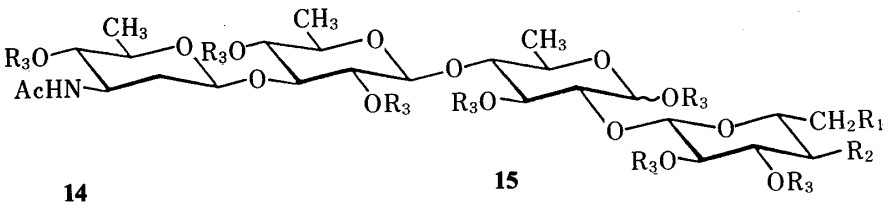
a) Measured in D_2O.
b) Measured in D_2O.

are shielded by 18—19 and 2—3 ppm, respectively, by the replacement of the hydroxy group with an acetoamide group. Consequently, the chemical shifts of **6** are as given in Table I. The assignments of methyl *N*-acetylacosaminide (**5**) were readily made by using off-resonance decoupling spectra.

Degradative reactions of **1A**, **1B** and **1C** gave several products as shown in Chart 4.[1] Acidic methanolysis under appropriate conditions afforded viridotetraoses A (**14A**), B (**14B**) and C (**14C**) or viridotrioses A (**16A**), B (**16B**) and C (**16C**). In the B series, methanolysis of **16B** yielded a disaccharide, methyl α-viridobioside B (**18B**). Treatment of **1A**, **1B** and **1C** with 5% NaOH (aq) in MeOH readily eliminated *N*-acetylacosamine to give unsaturated tetrasaccharides, **17A**, **17B** and **17C**, respectively.

In preference to the direct analysis of **1A**, **1B** and **1C**, we first investigated the assignments of their degradation products in the order of the di-, tri- and tetrasaccharides. Because these are all present in the B series, we first discuss the ^{13}C-NMR spectroscopic characteristic of the B series. Methyl α-viridobioside B (**18B**) is composed of a quinovose residue and an *N*-acetylviosamine residue, and is a methyl glycoside (55.5 ppm (q)). The ^{13}C-NMR spectrum of **18B** shows 15 signals, of which two anomeric carbons (105.8 and 100.9 ppm) correspond to those of **6β** and **3α**, respectively. The assignments of **18B** can be estimated by comparison of the chemical shifts of **18B** with those of **3α** and **6β** (Table III). In the spectrum of **18B**, C-2 of Qui is shifted 9.5 ppm downfield, while C-3 of Qui is shifted 1.2 ppm upfield. These shifts can be regarded as the glycosidation shift.[5,9-12] Therefore, the location of the glycosidic linkage is immediately assigned to C-2 of Qui.

Viridotriose B (**16B**) is a heterotrisaccharide in which one more quinovose residue is glycosidically linked to **18B**. The ^{13}C-NMR chemical shifts of **16B** are summarized in Table III. In the anomeric carbon region, two signals, 92.9 ppm (major) and 96.1 ppm (minor) due to the anomeric carbon of the reducing Qui are observed together with two other signals (105.2 and 104.4 ppm). The appearance of the signals (92.9 and 96.1 ppm) suggests that the reducing Qui is mainly present in the α-form. The anomeric configurations of Vio and the non-reducing Qui are obviously β, base on a comparison of the chemical shifts of **3β** and **6β**. Two signals of the carbon atoms attached to the glycosidic linkage appear at 87.1 and

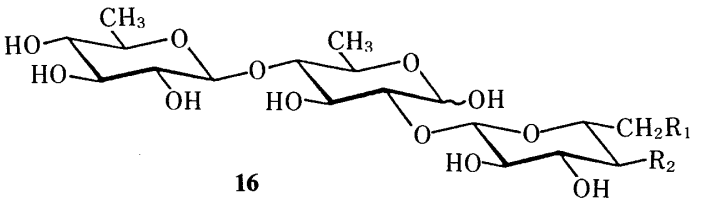


14

A: R_1=H, R_2=OH, R_3=H
B: R_1=H, R_2=NHAc, R_3=H
C: R_1=OH, R_2=OH, R_3=H

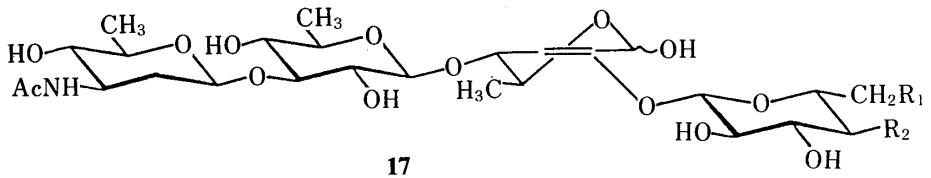
15

A: R_1=H, R_2=OAc, R_3=Ac
B: R_1=H, R_2=NHAc, R_3=Ac
C: R_1=OAc, R_2=OAc, R_3=Ac



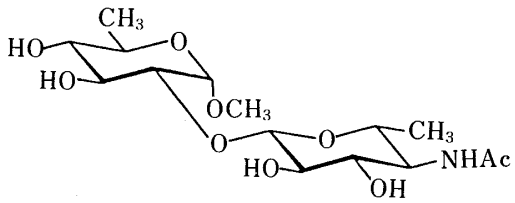
16

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



17

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



18B

Chart 4

TABLE III. ^{13}C-NMR Spectral Data for **1B**, **14B**, **16B**, **17B**, **18B** and **19B** (ppm Value)

	1B	**19B**	**14B**[a]	**17B**	**16B**[a]	**18B**
Vio						
C-1	105.4	105.1	105.2	105.5	105.2	105.8
C-2	74.9	75.2	74.8	75.0	74.9	75.1
C-3	75.7	76.1	75.9	75.7	75.9	75.9
C-4	58.1	58.2	58.0	58.0	58.1	58.1
C-5	72.3	72.3	72.2	72.6	72.2	72.3
C-6	18.4	18.3	18.3	18.4	18.3	18.4
Qui (Reducing)						
C-1	93.4	63.8	92.9	89.9	92.9	100.9
C-2	83.0	82.1	81.8	153.4	81.8	82.9
C-3	76.2[b]	76.3	71.9	101.1	71.8	73.5
C-4	76.9[b]	83.8	86.9[f]	80.7	87.1	77.0
C-5	67.8	68.1	66.4	67.7	66.4	68.4
C-6	18.4	19.7	18.0	18.2	18.0	18.1
Qui (Non-reducing)						
C-1′	101.0	104.5	104.4	104.7	104.4	
C-2′	73.2	75.0	74.1	75.0[g]	74.9	
C-3′	86.0	87.1	87.2[f]	87.2	77.3	
C-4′	75.7	76.0	74.8	75.2	76.4	
C-5′	73.2	72.9	73.0	72.7	73.3	
C-6′	18.4	18.3	18.0	18.4	18.0	
Aco						
C-1	101.9[c]	102.7[d]	102.1	102.4		
C-2	38.1	38.0[e]	37.9	38.0		
C-3	52.6	52.4	52.3	52.4		
C-4	75.3	75.7	75.5	75.2[g]		
C-5	74.9	74.8	74.8	74.6		
C-6	18.9	18.3	18.3	18.4		
Aco						
C-1′	101.5[c]	102.3[d]				
C-2′	38.1	37.8[e]				
C-3′	52.6	52.4				
C-4′	75.8	75.7				
C-5′	74.9	74.7				
C-6′	18.9	18.3				

a) Values in the α-anomer.
b)—*g*) Assignments may be reversed in each column.

81.8 ppm. The resonance at 81.8 ppm corresponds to C-2 in the reducing Qui and the resonance at 87.1 ppm is assigned to C-4 of the reducing Qui on the basis of the glycosidation shift (77.0→87.1 ppm). Consequently, the structure of viridotriose B is obtained as **16B**.

Viridotetraose B (**14B**) is a heterotetrasaccharide in which an *N*-acetylacosamine residue is glycosidically linked to **16B**, and is an anomeric mixture of the α form (C-1: 92.9 ppm, major) and the β form (C-1: 96.2 ppm, minor). The two characteristic signals at 102.1 and 87.2 ppm appear on attachment of an Aco to **16B** (Table III). The anomeric carbon (102.1 ppm) for Aco is readily assigned as β by comparison with the chemical shifts of **5β**. The glycosidation shift (77.3→87.2 ppm) is apparent at C-3 of the non-reducing Qui on comparing the chemical shifts of **16B** with those of **14B**. Therefore, the *N*-acetylacosamine residue is

linked through the glycosidic oxygen atom to C-3 of the non-reducing Qui. The assigned ^{13}C-NMR chemical shifts are given in Table III.

An alternative tetrasaccharide, **17B**, was also investigated (Table III). Although the reducing end of **17B** is free, it exists in a single form (89.9 ppm, probably α form).[13,14] The ^{13}C-NMR spectrum of **17B** except for the signals of the reducing Qui is consistent with that of **14B** and the only marked differences of chemical shifts are in the signals of the reducing Qui. The signals of C-1 and C-4 show a pronounced shielding, whereas the signals at C-2 and C-3 are strongly deshielded. The result of off-resonance decoupling indicated that the peak at 153.4 ppm is not split. These data indicated that **17B** is a 3-deoxy-hex-2-enopyranose derivative and the assignments of the individual carbons are presented in Table III.

TABLE IV. ^{13}C-NMR Spectral Data for **1A**, **14A**, **16A**, **17A** and **19A** (ppm Value)

	1A	**19A**	**14A**[a]	**17A**	**16A**[a]
Qui					
C-1″	105.4	105.1	105.3	105.4	105.2
C-2″	75.0	75.2	75.4	75.7	75.3
C-3″	77.0	78.0	77.3	77.4	77.2
C-4″	76.5	76.9	76.7	76.6	76.6
C-5″	73.0	73.2	73.1	73.4	73.0
C-6″	18.1	18.2	18.0	18.1	18.0
Qui (Reducing)					
C-1	93.2	63.7	92.9	89.8	92.9
C-2	82.9	82.0	81.9	153.3	81.8
C-3	75.7[b]	76.4	71.8	101.1	71.7
C-4	76.0[b]	83.8	86.9[f]	80.6	87.0
C-5	67.6	68.1	66.4	67.6	66.4
C-6	18.3	19.7	18.1	18.1	18.1
Qui (Non-reducing)					
C-1′	100.9	104.5	104.4	104.6	104.4
C-2′	73.0	75.0	74.0	74.5	74.8
C-3′	85.8	87.1	87.1[f]	87.2	77.2
C-4′	74.7	76.2	74.8	74.9	76.4
C-5′	73.0	72.7	73.0	72.7	73.3
C-6′	18.1	18.2	18.0	18.3	18.0
Aco					
C-1	101.7[c]	102.7[d]	102.1	102.3	
C-2	37.9	38.0[e]	37.9	38.0	
C-3	52.4	52.4	52.2	52.4	
C-4	75.0	75.7	75.4	75.1	
C-5	74.7	74.8	74.8	74.7	
C-6	18.3	18.2	18.2	18.3	
Aco					
C-1′	101.3[c]	102.3[d]			
C-2′	37.9	37.7[c]			
C-3′	52.4	52.4			
C-4′	75.0	75.6			
C-5′	74.7	74.7			
C-6′	18.9	18.2			

a) Values in the α-anomer.
b)—*g*) Assignments may be reversed in each column.

In the A series, the ^{13}C-NMR spectra of three degradation products, **14A**, **16A** and **17A** were investigated. Viridotriose A (**16A**) is a homotrisaccharide composed of D-quinovose. The chemical shifts of **16A** are shown in Table IV. In the anomeric carbon region, the signals due to the reducing Qui appear at 92.9 ppm for the α-anomer and at 96.1 ppm for the β-anomer together with two signals at 105.2 and 104.4 ppm due to two non-reducing Qui. The α-anomer is predominant over the β-anomer in CD_3OD solution. The configurations of the anomeric carbons for the non-reducing Qui are β, based on a comparison with the chemical shifts of the constituent monosaccharides. The glycosidation shifts (73.9→81.8 ppm and 77.3→87.0 ppm) are observed at C-2 and C-4 of the reducing Qui, respectively, whereas the signal at C-3 is shielded by 2.8 ppm.

TABLE V. ^{13}C-NMR Spectral Data for **1C**, **14C**, **16C**, **17C** and **19C** (ppm Value)

	1C	**19C**	**14C**[a]	**17C**	**16C**[a]
Glu					
C-1	105.6	105.3	105.4	105.0	105.4
C-2	74.6	75.7	74.8	74.5	74.8
C-3	78.1	77.6	77.6	78.1	77.5
C-4	71.1	71.2	71.2	71.0	71.2
C-5	77.7	78.3	77.6	77.7	77.5
C-6	62.5	62.5	62.4	62.3	62.4
Qui (Reducing)					
C-1	93.2	63.5	92.7	89.8	92.7
C-2	83.3	82.5[d]	82.1	153.3	82.1
C-3	76.2[b]	76.5	71.7	101.2	71.7
C-4	76.7[b]	83.1[d]	87.0[f]	80.4	87.0
C-5	67.8	68.1	66.4	67.6	66.4
C-6	18.2	19.7	18.0	18.2	17.9
Qui (Non-reducing)					
C-1′	101.0	104.1	104.4	104.6	104.4
C-2′	73.4	75.2	74.0	74.5	75.0
C-3′	85.9	87.1	86.8[f]	87.2	77.2
C-4′	74.9	76.3	75.0	74.9	76.4
C-5′	73.2	72.8	72.9	72.7	73.3
C-6′	18.3	18.1	18.0	18.3	17.9
Aco					
C-1	101.7[c]	102.7[e]	102.1	102.3	
C-2	38.1	38.0	37.8	38.0	
C-3	52.6	52.4	52.2	52.4	
C-4	75.9	75.2	75.4	75.7	
C-5	74.9	74.9	74.8	75.0	
C-6	18.3	18.3	18.2	18.3	
Aco					
C-1′	101.5[c]	102.3[e]			
C-2′	38.1	38.0			
C-3′	52.3	52.4			
C-4′	75.3	75.2			
C-5′	74.9	74.8			
C-6′	19.0	18.3			

a) Values in the α-anomer.
b)—*f*) Assignments may be reversed in each column.

Viridotetraose A (**14A**) is a heterotetrasaccharide in which an *N*-acetylacosamine residue is glycosidically linked to **16A** and is also an equilibrium mixture of the α-form (C-1: 92.9 ppm, major) and β-form (C-1: 96.2 ppm, minor) in CD_3OD solution (Table IV). Since the ^{13}C-NMR spectrum of **14A** is very similar to that of **14B**, the location of the new glycosidic linkage is C-3 of the non-reducing Qui linked to the reducing Qui at C-4. Moreover, because the spectroscopic features of an unsaturated tetrasaccharide, **16A** are also in good accord with those of **16B** except for Vio, the assignments are all straightforward. These data indicate that the reducing end is present in the α-form.

In the cases of **14C**, **16C** and **17C**, the ^{13}C-NMR spectroscopic features are in agreement with those of the A and B series except for the glucose residue. Therefore, the chemical shifts of the individual carbons are assigned as shown in Table V.

Viridopentaose B (**1B**) is a heteropentasaccharide in which one more *N*-acetylacosamine residue is glycosidically linked to C-3 of the reducing Qui in **14B** and is largely present as the α-anomer in CD_3OD solution. The ^{13}C-NMR spectrum of **1B** is shown in Fig. 1. The signals are arbitrarily assigned on the basis of the results discussed above (Table III). However, markedly anomalous behavior is apparent in comparing the chemical shifts of **1B** with those of **14B**. There are five signals in the anomeric carbon region. Among them the three signals at 105.4, 101.9 and 93.4 ppm correspond, as expected, to those of Vio, Aco and the reducing Qui, respectively. Although the signal at 101.5 ppm appears slightly upfield, it is assignable to C-1 of the newly introduced Aco with β-configuration. Therefore the remaining signal must be assigned to C-1 of the non-reducing Qui. Since this signal appears at 104.4 ppm in **14B**, it is shifted 3.4 ppm upfield in **1B**. Similar upfield shifts are also observed at C-1 of the non-reducing Qui in **1A** and **1C** (Tables VI and V).

In the ^{13}C-NMR spectra of peracetylated viridopentaoses (**2A**, **2B** and **2C**), these anomalous upfield shifts are also apparent. The chemical shifts of the anomeric carbons of **2A**, **2B** and **2C** are presented in Table VI together with those of peracetylated viridotetraoses (**15A**, **15B** and **15C**), and the chemical shifts of the anomeric carbons of the peracetylated constituent monosaccharides (**9**—**13**) are summarized in Table VII. For example, it is obvious that the three non-reducing residues in **15B** all have β-configuration by comparison of the chemical shifts of the anomeric carbons in **15B** with those of **9**, **11** and **12**. In the spectrum of **2B**, five signals appear in the anomeric region, of which the two signals of 101.7 ppm are assignable to C-1 of Vio and Aco, and the highest field signals (93.7 and 91.9 ppm) correspond to the α- and β-anomer of the reducing Qui, respectively. Consequently, the remaining two

Table VI. ^{13}C-NMR Spectral Data for **2** and **15** (ppm Value)

2A	101.7	101.5	99.4	98.9	91.8 (93.9)
2B	101.7	101.7	99.4	98.8	91.9 (93.7)
2C	101.7	101.7	99.4	98.8	91.8 (93.6)
15A	102.4	102.1	102.1		92.0 (93.4)
15B	102.4	102.4	102.2		92.0 (92.9)
15C	102.1	102.1	102.1		92.0 (93.2)

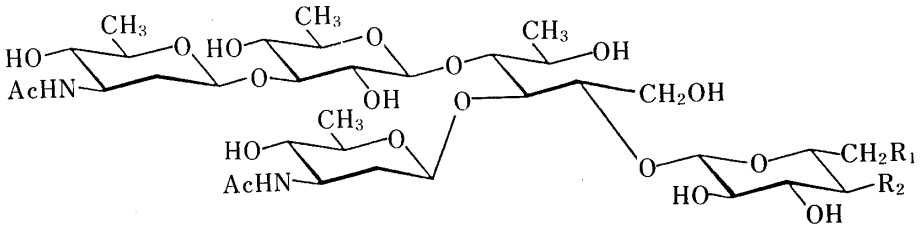
β-Anomer in parenthesis.

Table VII. ^{13}C-NMR Spectral Data for **9**—**13** (ppm Value)

	9	**10**	**11**	**12**	**13**
α	97.9	90.3	98.0	98.7	97.8
β	102.4	92.9	102.3	102.2	102.3

signals have to be assigned to C-1 of the non-reducing Qui and the newly introduced Aco. Although unequivocal differentiation of the signals cannot be performed at present, the signal of the anomeric carbon due to the non-reducing Qui is in any case shifted *ca.* 3 ppm upfield from that of **15B**. Similar anomalous upfield shifts are also apparent in the spectra of **2A** and **2C**.

This upfield shift in **1B** may be caused by a steric compression effect, when the second Aco is introduced at C-3 of the reducing Qui in **14B**. This effect is not observed at all below the tetrasaccharides. However, the effect disappears on reduction of **1B** in MeOH with $NaBH_4$. The ^{13}C-NMR chemical shifts of dihydroviridopentaose B (**19B**) are given in Table III. The signal of C-1 of the Qui in **19B** is readily assigned from the off-resonance spectrum and its upfield shift of 29.6 ppm on reduction of **1B**. In the anomeric carbon region four signals appear, of which the three signals at 105.1, 102.7 and 102.3 ppm can immediately be assigned to C-1 of Vio and two Aco by comparison of the chemical shifts of **19B** with those of **14B** and **5β**. Therefore, the remaining signal (104.5 ppm) must be assigned to C-1 of the non-reducing Qui. These results indicate that the constraint around C-1 of the non-reducing Qui in **1B** disappears upon reductive cleavage of the cyclic hemiacetal. Similarly, the anomalous upfield



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

Chart 5

shifts of C-1 of the non-reducing Qui in **1A** and **1C** disappear in dihydroviridopentaoses A (**19A**) and C (**19C**) (Tables IV and V).

An additional unusual feature is found in the ^{13}C-NMR spectrum of **1B** as compared with that of **14B**. In general, the resonance of carbon attached to a glycosidic linkage is observed in the range of 79—88 ppm, downfield of the usual range for pyranose carbon, except for anomeric carbon (glycosidation shift).[5] While there are, as expected, three signals (87.2, 86.9 and 81.8 ppm) with glycosidation shifts in **14B**, only two corresponding signals occur at 86.0 and 83.0 ppm in **1B** in spite of its being a pentasaccharide. These signals are tentatively assigned to C-3 of the non-reducing Qui and C-2 of the reducing Qui by reference to the results mentioned above, so that glycosidation shifts do not occur at C-3 and C-4 of the reducing Qui in **1B**. Further, similar behavior is recognized in **19B**. Although the signal at C-4 is considerably shifted downfield, glycosidation shift does not occur at all at C-3 of the reducing Qui. These phenomena in **1B** and **19B** are also caused by the introduction of the second Aco at C-3 of the reducing Qui in **14B**, in which the three glycosidic linkages are consecutively aligned at C-2, -3 and -4 of the reducing Qui in **1B** and **19B**. Usui *et al.* reported sterically hindered adjacent diglycosidation at C-4 and C-5 of the 2-deoxystreptamine residue in neomycin B.[8] Since the resonance of C-2 of the reducing Qui is not shifted upfield in **1B** and **19B**, the effect mainly involves C-3 and C-4 of the reducing Qui. In the cases of the A and C series, similar behavior is seen.

In conclusion, the glycosidation shift in ^{13}C-NMR spectroscopy is a useful empirical approach for the structural investigation of carbohydrates.[16,17] We have successfully applied

this method to characterize the glycosidic linkages up to the tetrasaccharide level, but anomalous behavior appears in the pentasaccharides. Previously we incorrectly assigned the ^{13}C-NMR signals of viridopentaoses because of inadequate consideration of this anomaly.[18,19] Although a reasonable explanation for this anomalous behavior can not be suggested at present, it may be caused by a steric constraint around the three consecutive glycosidic linkages, restricting their spatial arrangements.

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