[Chem. Pharm. Bull. 31(11)3829—3843(1983)]

Structural Investigation of the Antibiotic Sporaviridin. VII.[1] Structural Studies on the Constituent Pentasaccharides, Viridopentaoses

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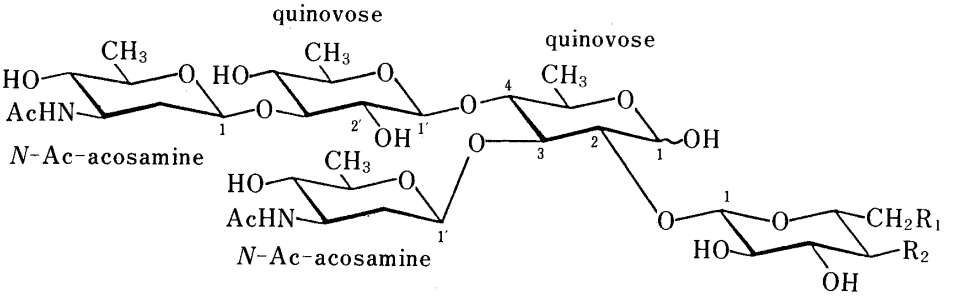
(Received February 9, 1983)

Sporaviridin is a basic and water-soluble antibiotic produced by *Streptosporangium viridogriseum*. During separation and purification of *N*-acetylsporaviridin, we found that this derivative was hydrolyzed by aqueous ammonia used as the eluent for column chromatography to yield glycosidic compounds containing an aglycone and three pentasaccharides, designated as viridopentaoses A, B and C. The purification of these compounds was performed as shown in Chart 2. They were concluded to be novel heteropentasaccharides (**1A**, **1B** and **1C**) containing two or three amino sugars, on the basis of chemical degradations and spectroscopic studies.

Keywords——sporaviridin; *Streptosporangium*; viridopentaose; secondary ion mass spectrometry; ^{13}C-NMR

Sporaviridin (SVD) is a basic and water-soluble antibiotic produced by *Streptosporangium viridogriseum* nov. sp.[2,3] and is a glycosidic compound containing an oligosaccharide moiety. In the previous paper we reported the individual sugar components derived from SVD.[1] We now describe the three constituent pentasaccharides of *N*-acetylsporaviridin (SVD-*N*-Ac); viridopentaoses A (**1A**), B (**1B**) and C (**1C**).



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

Chart 1

1. Isolation and Preliminary Characterization of Viridopentaoses

During separation and purification of SVD-*N*-Ac, a derivative obtained by treatment of SVD with acetic anhydride in MeOH, we found that SVD-*N*-Ac was hydrolyzed by aqueous ammonia used as an eluent for column chromatography to yield glycosidic compounds (probably containing an aglycone) and oligosaccharides. The ammonolysis products were separated by Sephadex LH-20 to give unchanged SVD-*N*-Ac, glycosidic compounds and an

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oligosaccharide fraction (Chart 2). The oligosacccharide fraction contained three components, which were separated by repeated column chromatographies on silica gel (Chart 2). Since they were later found to be pentasaccharides, they were designated viridopentaoses A, B and C. They were obtained as amorphous white powders, showing amide CO absorption (ν_{max} 1650—1660 cm^{-1}) in their infrared (IR) spectra. Their proton nuclear magnetic resonance (^{1}H-NMR) spectra indicate the presence of $NCOCH_3$ groups (δ 1.95 or 1.98) and anomeric protons (δ 5.00—5.50). The presence of five anomeric carbons (A, 105.4, 101.7, 101.3, 100.9,

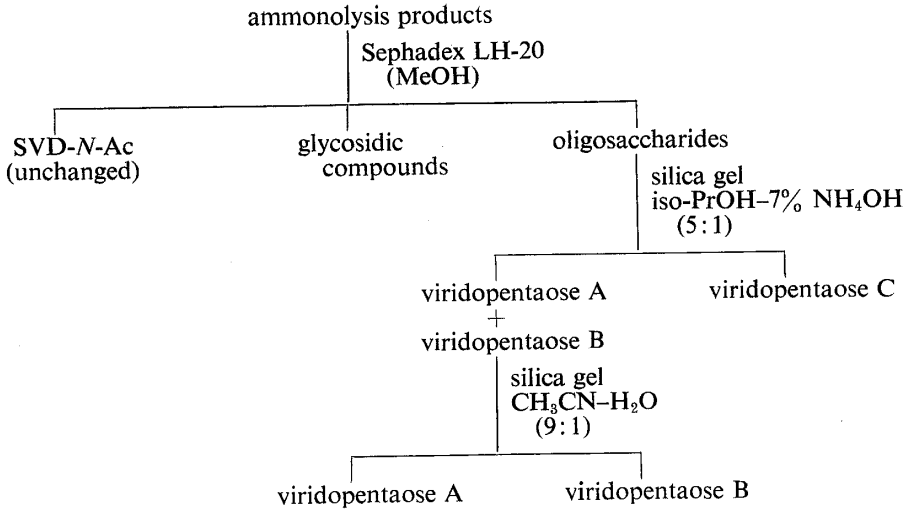

Chart 2. Isolation Procedure for Viridopentaoses A (**1A**), B (**1B**) and C (**1C**)

TABLE I. Physicochemical Properties of Viridopentaoses A, B and C

	Viridopentaose A	Viridopentaose B	Viridopentaose C
	White powder	White powder	White powder
mp	198—201 °C (dec.)	207—209 °C (dec.)	191—193 °C (dec.)
$[\alpha]_D$	−45.7° (c=0.3, MeOH)	−31.7° (c=0.3, MeOH)	−31.0° (c=0.3, MeOH)
IR	3200—3500 (OH/NH)	3200—3500 (OH/NH)	3200—3500 (OH/NH)
ν_{max}^{KBr} cm^{-1}	1650 (NHCO)	1620—1650 (NHCO)	1620—1660 (NHCO)
	1560 (NHCO)	1560 (NHCO)	1550 (NHCO)
^{1}H-NMR	1.25 (m, –CH–C$\underline{H}_3$)	1.15—1.35 (m, –CH–C$\underline{H}_3$)	1.25 (m, –CH–C$\underline{H}_3$)
(CD_3OD): δ	1.98 (s, NCOC$\underline{H}_3$)	1.95 (s, NCOC$\underline{H}_3$)	1.98 (s, NCOC$\underline{H}_3$)
	5.00—5.40 (m, anomeric proton)	5.05—5.40 (m, anomeric proton)	5.10—5.50 (m, anomeric proton)
Anal.	$C_{34}H_{58}N_2O_{19}\cdot 3H_2O$	$C_{36}H_{61}N_3O_{19}\cdot 5H_2O$	$C_{34}H_{58}N_2O_{20}\cdot 3H_2O$
Calcd	C, 47.89; H, 7.51; N, 3.29	C, 46.50; H, 7.64; N, 4.52	C, 47.00; H, 7.37; N, 3.23
Found	C, 48.12; H, 7.65; N, 3.25	C, 46.21; H, 7.60; N, 4.41	C, 46.70; H, 7.47; N, 3.12

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) was deduced from the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra. The principal physicochemical properties are summarized in Table I.

2. Molecular Weight Determination

Field desorption (FD) mass spectrometry has frequently been used to determine molecular weight for involatile natural products.[4] In most cases of involatile compounds, the protonated molecule (MH^+) and/or sodium addition ion $(M+Na)^+$ appear as molecular ion

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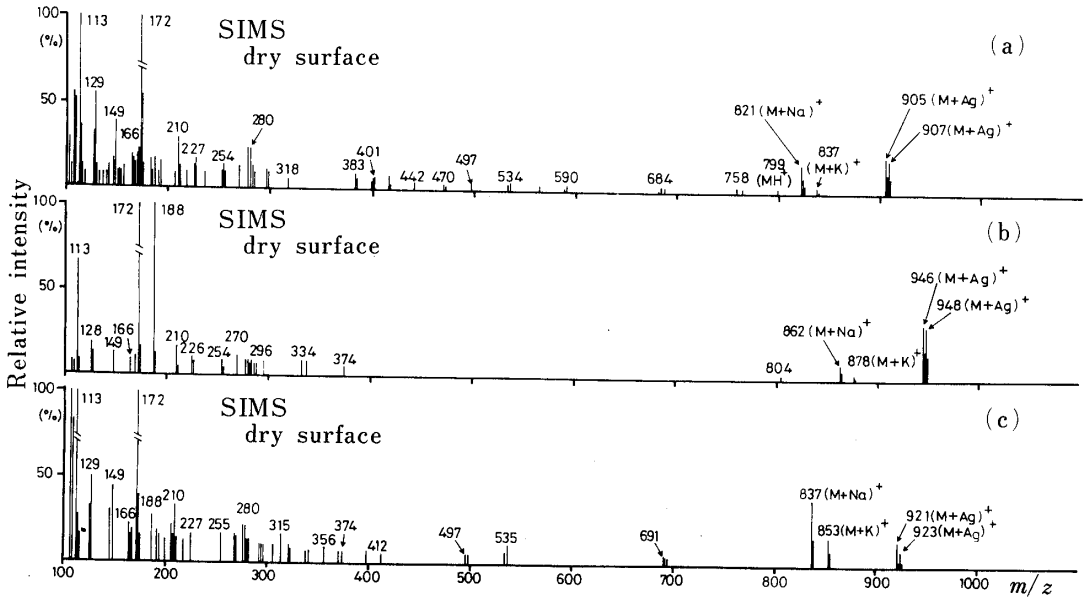

Fig. 1. Molecular Secondary Ion Mass Spectra of **1A** (a), **1B** (b) and **1C** (c) without Matrix

species in the FD spectra. The FD spectrum of **1A** shows MH^+ at *m/z* 799 and $(M+Na)^+$ at *m/z* 821 in the range of 17—21 mA emitter current. However, no reproducible molecular ion species was observed in the FD spectra of **1B** and **1C**.

Recently, sputtered type mass spectrometry such as plasma desorption (PD),[5] laser desorption (LD),[6] fast atom bombardment (FAB)[7,8] and secondary ion (SI)[9,10] mass spectrometry has been developed for the study of involatile and/or thermally labile compounds. These do not require heating of the sample for desorption or ionization. In particular SIMS and FABMS are useful because of their simple operation and good reproducibility.[11-13]

The molecular SI mass spectra of intact viridopentaoses A, B and C were investigated. The mass spectra of **1A** without matrix (dry surface) show four cationized molecular ion species, MH^+ at *m/z* 799, $(M+Na)^+$ at *m/z* 821, $(M+K)^+$ at *m/z* 837 and $(M+Ag)^+$ at *m/z* 905 and 907 and they correctly indicate the molecular weight (M.W. 798) (Fig. 1a). Because Ag-containing ions appear as doublet peaks due to the presence of Ag isotopes (107, 109), the silver addition ions, $(M+Ag)^+$ are valuable in the mass spectra of samples supported on silver. In the cases of **1B** and **1C**, three cationized molecular species, $(M+Na)^+$, $(M+K)^+$ and $(M+Ag)^+$ can be seen (Fig. 1b and 1c), so that the molecular weights of **1B** and **1C** were concluded to be 839 and 814, respectively. The matrix-assisted molecular SIMS of viridopentaoses has been preliminarily discussed,[14] and the details will be published elsewhere.

Two volatile derivatives, the peracetylated and permethylated derivatives were also examined by in-beam electron impact (EI), chemical ionization (CI) and FD mass spectrometry. Acetylation of **1A**, **1B** and **1C** with acetic anhydride-pyridine at room temperature gave the corresponding peracetylated derivatives. The FD mass spectra of **2A**, **2B** and **2C** show MH^+ at *m/z* 1135, 1134 and 1193 and $(M+Na)^+$ at *m/z* 1157, 1156 and 1215, indicating the presence of eight, seven and nine OH groups, respectively. Permethylation of oligosaccharides was performed by use of a modification of Hakomori's method.[15,16] The in-beam EI technique has been developed by the Dell and Ohashi groups.[17,18] The in-beam EI mass spectra generally exhibit MH^+ in place of the molecular ion (M^+). Protonated molecule peaks at *m/z* 939, 980 and 969 were obtained in the high-mass region for **3A**, **3B** and **3C**, respectively.

It is well known that CIMS is often more useful for molecular weight determination and

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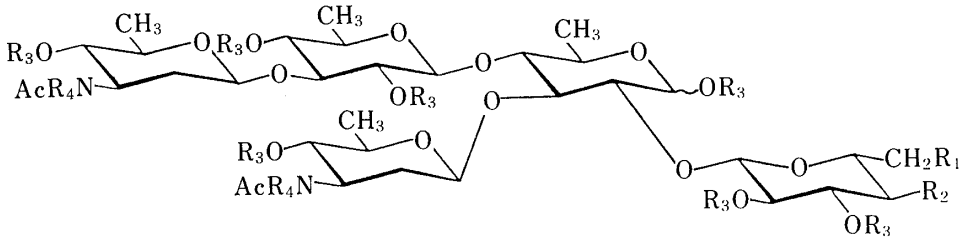


2A:	$R_1=H$,	$R_2=OAc$,	$R_3=Ac$,	$R_4=H$	**3A**:	$R_1=H$,	$R_2=OCH_3$,	$R_3=R_4=CH_3$
2B:	$R_1=H$,	$R_2=NHAc$,	$R_3=Ac$,	$R_4=H$	**3B**:	$R_1=H$,	$R_2=N(CH_3)Ac$,	$R_3=R_4=CH_3$
2C:	$R_1=OAc$,	$R_2=OAc$,	$R_3=Ac$,	$R_4=H$	**3C**:	$R_1=OCH_3$,	$R_2=OCH_3$,	$R_3=R_4=CH_3$

Chart 3

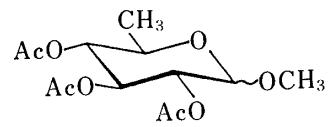
structural characterization of relatively involatile compounds than conventional EIMS. We have investigated the characterization of naturally occurring glycosides by this method.[19,20] The CI mass spectra of permethylated viridopentaoses A, B and C mediated with ammonia as a reagent gas were examined. In the cases of **3A** and **3B**, ammonium adduct ions $(M+NH_4)^+$ appear at *m/z* 956 and 997, respectively, together with MH^+ ions. Only the $(M+NH_4)^+$ ion is observed at *m/z* 986 in the CI mass spectrum of **3C**. When isobutane is used as a reagent gas, all the spectra show MH^+ ions in the molecular ion regions. Details of the mass spectrometric behavior under CI conditions will be published elsewhere.[21]

The molecular weights of **1A**, **1B** and **1C** were determined to be 798, 839 and 814, respectively, on the basis of the mass spectrometric studies mentioned above.

3. Identification of Exhaustive Methanolysis Products of Viridopentaoses A, B and C

In order to elucidate the constituent monosaccharides of viridopentaoses, exhaustive methanolysis was carried out. Viridopentaose A was heated under reflux with 1.6% hydrogen chloride in MeOH for 8 h. The solution was made neutral with silver carbonate, then filtered, and the filtrate was evaporated to dryness. The residue was acetylated with acetic anhydride in pyridine. The resulting acetylated derivatives were separated by repeated chromatographies on silica gel to yield two anomeric pairs of peracetylated methyl glycosides, **4** and **5′** in a molar ratio of 3 : 2. They were characterized as methyl 2,3,4-tri-*O*-acetyl-6-deoxy-D-glucopyranoside (methyl 2,3,4-tri-*O*-acetyl-D-quinovoside, **4**) and methyl 3-acetoamido-4-*O*-acetyl-2,3,6-trideoxy-*arabino*-D-hexopyranoside (methyl 3,4-diacetyl-D-acosaminide, **5′**),[22] which have been identified as sugar components of SVD.[1]

Analogously, methanolysis of viridopentaoses B and C followed by acetylation gave **4**, **5′** and methyl 4-acetamido-2,3-di-*O*-acetyl-4,6-dideoxy-D-glucopyranoside (methyl 2,3,4-triacetyl-D-viosaminide, **6**) in a molar ratio of 2 : 2 : 1, and **4**, **5′** and methyl 2,3,4-tetra-*O*-acetyl-D-glucopyranoside (**7**) in a molar ratio of 2 : 2 : 1, respectively.



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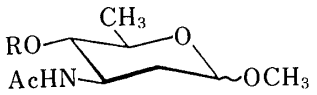
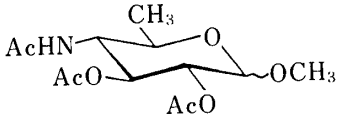


5 : R=H
5′ : R=Ac



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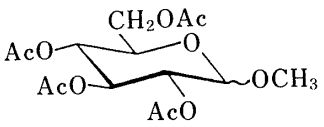

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Chart 4

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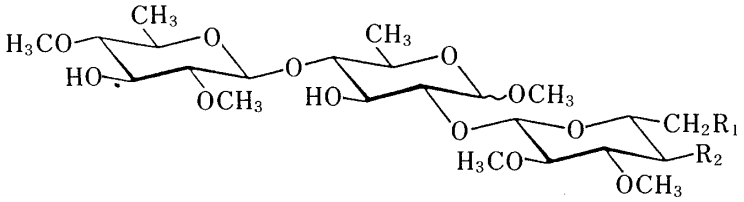
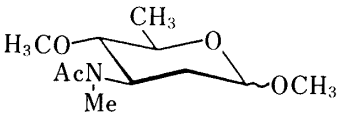
4. Identification of Intermonosaccharide Linkages (Branching Points) of Viridopentaoses

This was carried out using the classical method of permethylation followed by acidic methanolysis under two different temperature conditions. Treatment of permethylated viridopentaose A (**3A**) with 5% methanolic hydrogen chloride for 22 h at room temperature gave a mixture of partially methylated trisaccharide (**8A**), mp 120—130 °C, and methyl *N*-acetyl-*N,O*-dimethyl-D-acosaminide (**9**), which were separated by chromatography on Sephadex LH-20 (Chart 5). The elemental composition of **8A** were determined as $C_{24}H_{44}O_{13}$ from high resolution data under CI conditions. The CI mass spectrum of **8A** using isobutane as a reagent gas shows MH^+ at *m/z* 541 and has informative sequence ions at *m/z* 189 and 175. Compound **9** was identical with the permethylated derivative of methyl *N*-acetyl-α-acosaminide on the basis of ^{1}H-NMR and IR spectra and gas chromatography (GC).



8A: R_1=H, R_2=OCH_3
8B: R_1=H R_2=$N(CH_3)Ac$
8C: R_1=OCH_3, R_2=OCH_3



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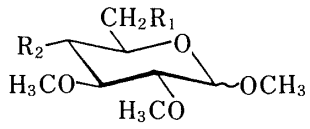
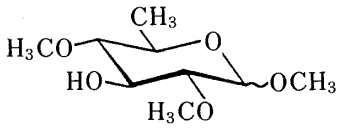


10A: R_1=H, R_2=OCH_3
10B: R_1=H, R_2=$N(CH_3)Ac$
10C: R_1=OCH_3, R_2=OCH_3



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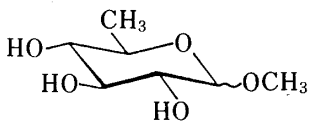

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Chart 5

In the same manner, acidic methanolysis of **3B** and **3C** at room temperature afforded **9** in common and **8B**, mp 140—142 °C, $C_{26}H_{47}NO_{13}$ and **8C**, mp 62—64 °C, $C_{25}H_{46}O_{14}$, respectively. These results indicate that the *N*-acetyl-D-acosamine residues are common to the three components, are terminal and are much more subject to hydrolysis than other constituent monosaccharides.

On the other hand, direct treatment of **3A** with boiling 5% methanolic hydrogen chloride for 7 h yielded a mixture of four methyl glycosides (**9**—**12**) which were separated by chromatography on silica gel. The isolation of **9** was expected from the previous experiment. Compound **10A** was confirmed to be permethylated quinovose by direct comparison with an authentic sample. The isolation of **10A** proves that one of the three quinovose residues is terminal and is linked through the glycosidic oxygen atom of another quinovose residue. The third methanolysis product, **11** was firmly established as methyl 2,4-di-*O*-methyl-α-D-quinovoside by its high resolution CI mass spectral data ($C_9H_{18}O_5$) and ^{1}H-NMR spectrum (H-1: δ 4.82, d, *J*=4 Hz, H-2: δ 3.19, dd, *J*=9.5 and 4 Hz, H-3: δ 3.90, t, *J*=9.5 Hz, H-4: δ 2.81, t, *J*=9.5 Hz, H-6: δ 1.31, d, *J*=6 Hz). These assignments were supported by spin-decoupling experiments. Base-catalyzed acetylation of **11** yielded a mono-acetate, whose ^{1}H-NMR spectrum shows a downfield shifts (δ 3.90→δ 5.32) of the H-3 proton. It was thus found that the second quinovose residue is linked to another monosaccharide at the C-3 position.

Since the remaining methyl glycoside **12** is more polar than other products, it was

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characterized as its triacetate. The acetate was found to be methyl 2,3,4-tri-*O*-acetyl-D-quinovoside by comparing its gas chromatogram and thin layer chromatogram with those of an authentic specimen. The isolation of methyl quinovoside demonstrates that the hydroxy groups at C-2, -3 and -4 in the third quinovose residue of viridopentaose A are glycosidically linked.

Acidic methanolysis of permethylated viridopentaoses B (**3B**) and C (**3C**) was similarly achieved. In the cases of **3B** and **3C**, methyl glycosides **9**, **11** and **12** were obtained in common and permethylated methyl *N*-acetylviosaminide (**10B**) and permethylated methyl glucoside (**10C**) were obtained, respectively. Compounds **10B** and **10C** were identical with the corresponding permethyl derivatives of methyl *N*-acetyl-D-viosaminide and methyl D-glucoside, respectively, on GC and TLC. These results suggest that a tetrasaccharide moiety composed of 2 mol of quinovose and 2 mol of *N*-acetylacosamine is common to the three pentasaccharides.[23]

5. Isolation and Structural Elucidation of Partial Methanolysis Products

In the previous section we demonstrated that the *N*-acetyl-D-acosamine residue is much more subject to hydrolysis than other constituent monosaccharides. Therefore, we attempted to isolate partial methanolysis products. Mild methanolysis of **1A** in MeOH with concentrated aqueous hydrochloric acid (4.5% w/v) at room temperature for 4 h gave, after neutralization and evaporation, a mixture which was fractionated chromatographically to yield a trisaccharide (**13A**) and methyl *N*-acetylacosaminide (**5**). Compound **13A**, mp 235—238 °C, composed of three quinovose residues, is designated as viridotriose A. Two cationized molecular ion species, *m/z* 479 $(M+Na)^+$ and *m/z* 563, 565 $(M+Ag)^+$ are observed in the SI mass spectrum of **13A**. The ^{13}C-NMR spectrum in CD_3OD shows two signals, 92.9 ppm (major) and 96.1 ppm (minor) due to the anomeric carbon of the reducing quinovose residue, indicating that the α-anomer is predominantly present.[24] The ^{1}H-NMR (100 MHz) chemical shifts of reducing quinovose residue in its octa-acetate (**13A′**) are summarized in Table II. These assignments were firmly supported by spin-decoupling experiments. These results indicate that the locations of intermonosaccharide linkages in the reducing quinovose residue are C-2 and C-4. Two anomeric protons, δ 4.54 (d, $J_{1,2}=8$ Hz) and δ 4.58 (d, $J_{1,2}=8$ Hz) due to the non-reducing quinovose residues appear in the ^{1}H-NMR spectrum of **13A′**. Additionally, the anomeric carbons of non-reducing quinovose residues are observed at 104.4 ppm and 105.2 ppm in the ^{13}C-NMR spectrum of **13A**. On the basis of NMR spectral studies, the intermonosaccharide linkages between the reducing quinovose and the non-reducing quinovoses in **13A** possess the β-configuration.

In the same way, **1B** was subjected to methanolysis to give viridotriose B (**13B**), mp 216—219 °C. Compound **13B** has been suggested to be composed of 2 mol of quinovose and 1 mol of *N*-acetylviosamine. The SI mass spectrum of **13B** shows unequivocally three molecular weight-related ions, *m/z* 520 $(M+Na)^+$, *m/z* 536 $(M+K)^+$ and *m/z* 604 and 606 $(M+Ag)^+$. Two signals, 92.9 ppm (major) and 96.2 ppm (minor) assignable to C-1 of the reducing quinovose residue are also apparent in the ^{13}C-NMR spectrum. The ^{1}H-NMR spectral behavior of the hepta-acetate (**13B′**) is very similar to that of **13A′**; significant signals are assigned in Table II, and signals of two protons at δ 4.58 (each 1H, d, $J_{1,2}=8$ Hz) were assigned to H-1 of the non-reducing quinovose and *N*-acetylviosamine residues. Consequently, two possible structures may be considered for viridotriose B in which the non-reducing quinovose and *N*-acetylviosamine residues are linked glycosidically with the β-configuration to either C-2 and C-4 of the reducing quinovose residue, respectively, or *vice versa*.

Because no spectroscopic method could resolve the problem, a further methanolysis of **13B** was performed. Methanolysis of **13B** in MeOH with concentrated hydrochloric acid (8%

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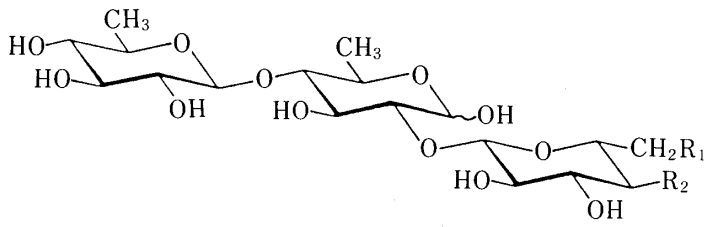
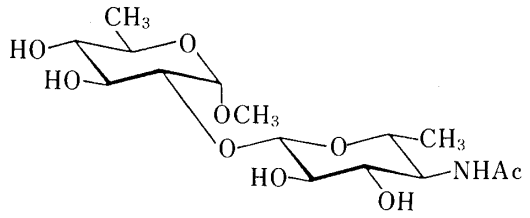
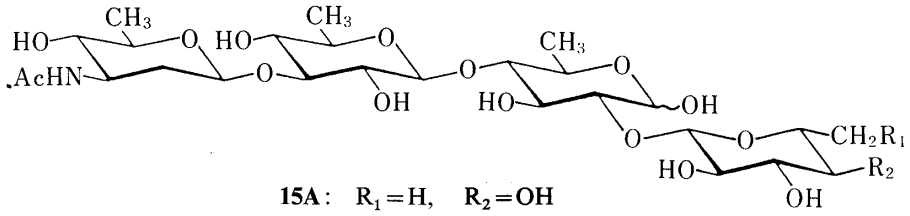


13A: R_1=H, R_2=OH
13B: R_1=H, R_2=NHAc
13C: R_1=OH, R_2=OH



14B



15A: R_1=H, R_2=OH
15B: R_1=H, R_2=NHAc
15C: R_1=OH, R_2=OH

Chart 6

TABLE II. ^{1}H-NMR Signals of the Reducing Quinovose Residues in **13A′**, **13B′** and **13C′**

	Chemical shifts (δ) (First-order coupling, Hz, in parentheses)						
	H-1 ($J_{1,2}$)		H-2[a] ($J_{2,3}$)	H-3[a] ($J_{3,4}$)	H-4[a] ($J_{4,5}$)	H-5[b]	H-6[a] ($J_{5,6}$)
	α	β					
13A′	6.22 d (4.0)	5.64 d (8.0)	3.80 dd (10.0)	5.36 t (10.0)	3.33 t (10.0)	—	1.26 d (6.0)
13B′	6.23 d (4.0)	5.65 d (8.0)	3.82 dd (10.0)	5.37 t (10.0)	3.35 t (10.0)	—	1.27 d (6.0)
13C′	6.22 d (4.0)	5.64 d (8.0)	3.82 dd (10.0)	5.36 t (10.0)	3.33 t (10.0)	—	1.26 d (6.0)

a) Chemical shifts in the α-anomer.
b) Unassignable.

w/v) at 50 °C for 8 h followed by neutralization, evaporation and chromatographic fractionation gave a disaccharide, **14B**, mp 264—266 °C. The CI mass and ^{1}H-NMR spectral data indicate that **14B** is composed of a quinovose and an *N*-acetylviosamine and is a methyl glycoside (δ 3.35, 3H, s, OCH_3). Therefore, **14B** is designated as methyl viridobioside B, whose constitution is established by the ^{1}H-NMR spectrum (100 MHz) of its tetra-acetate (**14B′**) (H-1, δ 4.85, 1H, d, J=4 Hz; H-2, δ 3.77, 1H, dd, J=10, 4 Hz; H-3, δ 5.45, 1H, t, J=10 Hz; H-4, δ 4.77, 1H, t, J=10 Hz; H-5, δ 3.92, 1H, dq, J=10, 6 Hz; H-6, δ 1.20, 3H, d, J=6 Hz; H-1′, δ 4.58, 1H, d, J=8 Hz). Several spin-decoupling experiments clearly supported these assignments. The configuration of the anomeric center in the quinovose residue can be assigned as α from the coupling constant ($J_{1,2}$=4 Hz) and the ^{13}C-NMR chemical shift in **13B** (100.9 ppm).

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Finally, comparison of the ^{1}H-NMR chemical shifts of the peracetate (**14B′**) with those of methyl 2,3,4-tri-*O*-acetyl-α-D-quinovoside indicates that the *N*-acetylviosamine residue must be glycosidically linked to C-2 of the quinovose residue in **14B**. Consequently, the structure of methyl α-viridobioside B is given as **14B**.

Methyl α-viridobioside B could also be obtained by the use of different conditions. Mild treatment of **1B** in MeOH with Amberlyst 15 at 50 °C for 8 h followed by chromatographic fractionation yielded **14B** in low yield (15%).

Viridotriose C (**13C**), mp 168—169 °C, obtained by methanolysis of **1C** is a heterotrisaccharide composed of 2 mol of quinovose and 1 mol of glucose residues. The ^{13}C-NMR spectrum of **13C** in CD_3OD suggests that the α-anomer (92.7 ppm) is predominant over the β-anomer (96.0 ppm). The ^{1}H-NMR spectroscopic behavior of the nona-acetate (**13C′**) is consistent with that of **13A′** and **13B′** (Table II). Although we could not obtain the corresponding disaccharide of **14B** in spite of some effort, the structure of viridotriose C was concluded to be **13C** on the basis of the spectroscopic evidence.

Further, milder methanolysis of **1B** in MeOH with concentrated aqueous hydrochloric acid (2.4% w/v) at 4 °C for 18 h provided mainly viridotetraose B, mp 235—237 °C together with **13B**. The silver addition ions $(M+Ag)^+$ appear at *m/z* 775 and 777 in the SI mass spectrum. The relation between viridotriose B (**13B**) and viridotetraose B is clear from the experiments presented above, namely viridotetraose B demands that the *N*-acetylacosamine residue is glycosidically linked to either C-3 of the reducing quinovose residue or C-3 of the non-reducing quinovose residue in **13B**. This was decided to be **15B** by consideration of the following CI mass spectral evidence. The diagnostic ion, *a* (*m/z* 374, Chart 7), derived from the disaccharide portion is definitely observed in both the isobutane and ammonia CI mass spectra of permethylated viridotetraose B. This conclusion was confirmed by the ^{13}C-NMR studies (Part XIII).

Similarly methanolysis of **1A** and **1C** gave viridotetraose A (**15A**), mp 207—209 °C and C (**15C**), mp 213—215 °C, respectively. Since their spectroscopic behavior is in accord with that of **15B**, their constitutions are established as **15A** and **15C**.[25]

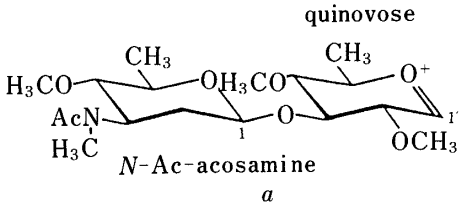

Chart 7

6. Alkaline Hydrolysis of Viridopentaoses

The degradative study discussed so far was bases on the use of acidic methanolysis. We next wanted to explore the possibility of obtaining additional evidence by examining the degradation of viridopentaoses by basic reagents. Alkaline hydrolysis of **1A** in MeOH with 5% NaOH (aq) at room temperature for 24 h, followed by neutralization with Amberlyst 15, gave a mixture which was separated on Sephadex LH-20 chromatography to yield an unsaturated tetrasaccharide (**16A**), mp 224—226 °C and *N*-acetylacosamine (**17**), mp 214—216 °C. The glycerol matrix-assisted SI mass spectrum of **16A** shows molecular ion species, $(M+K)^+$ at *m/z* 648, $(M+Na)^+$ at *m/z* 632 and MH^+ at *m/z* 610, indicating that the molecular weight of **16A** is 609. Comparison (Table III) of the ^{13}C-NMR spectra of **16A** and viridotetraose A (**15A**) provides significant structural information. Thus, marked differences of the chemical shifts are recognized only in the reducing quinovose. The signals at C-1 and C-4 exhibit a pronounced shielding, whereas the signals at C-2 and C-3 exhibit a remarkable

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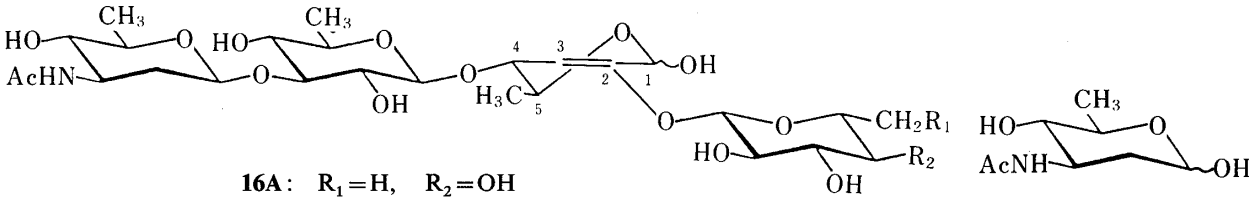
deshielding (the multiplicities in the off-resonance spectrum are singlet and doublet, respectively). These results can be interpreted as showing that **1A** liberates an *N*-acetylacosamine at C-3 on the reducing quinovose residue to form a 3-deoxy-hex-2-



16A: R_1=H, R_2=OH
16B: R_1=H, R_2=NHAc
16C: R_1=OH, R_2=OH

17

Chart 8

TABLE III. ^{13}C-NMR Chemical Shifts (ppm) of **15** and **16** Series in CD_3OD

		C-1	C-2	C-3	C-4	C-5	C-6
Unsaturated tetrasaccharide	A (**16A**)	89.8	153.3	101.1	80.6	67.6	18.1
	B (**16B**)	89.9	153.4	101.1	80.7	67.7	18.2
	C (**16C**)	89.8	153.3	101.2	80.4	67.6	18.2
Viridotetraose	A (**15A**)	92.9	81.9	71.8	87.1	66.4	18.1
	B (**15B**)	92.9	81.8	71.9	87.2	66.4	18.0
	C (**15C**)	92.7	82.1	71.7	87.0	66.4	18.0

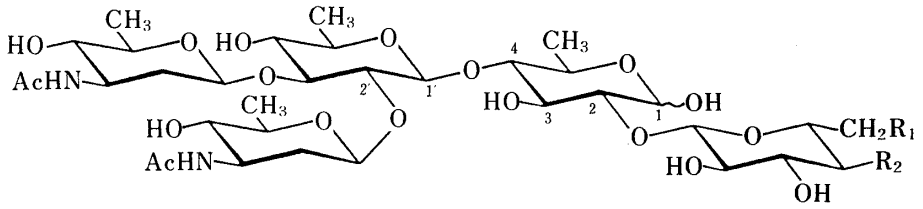
enopyranose derivative. The ^{1}H-NMR spectrum (100 MHz) in CD_3OD exhibits two signals δ 5.07 (s) and δ 5.33 (br s) due to H-1 and H-3 of the reducing quinovose residue, respectively. We therefore propose that the structure of the unsaturated tetrasaccharide is **16A**.

In the cases of **1B** and **1C**, the β-elimination also occurs under the same conditions to give unsaturated tetrasaccharides, **16B** and **16C**, respectively. The SIMS, ^{1}H-NMR and ^{13}C-NMR (Table III) spectral data support the proposed structures.[26]

From these results, it is evident that one of the two *N*-acetylacosamines is present at C-3 on the reducing quinovose residue in viridopentaoses.

7. The Constitutions of Viridopentaoses

Previously the structures of viridopentaoses were determined incorrectly as **18A**, **18B** and **18C** on the basis of analysis of the ^{13}C-NMR spectra.[27,28] Afterwards, we found that treatment of each pentasaccharide with 5% NaOH readily released *N*-acetylacosamine (**17**) to give unsaturated tetrasaccharides, **16A**, **16B** and **16C**, respectively, as described in the previous section. The formation of these degradation products suggests that the original position of one of the two *N*-acetylacosamines in viridopentaoses is not C-2 in the non-



18A: R_1=H, R_2=OH
18B: R_1=H, R_2=NHAc
18C: R_1=OH, R_2=OH

Chart 9

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TABLE IV. ^{1}H-NMR (360 MHz) Data for **2A**, **2B** and **2C**

	2A		**2B**		**2C**	
	Chemical shift (δ)	Coupling constant (Hz)	Chemical shift (δ)	Coupling constant (Hz)	Chemical shift (δ)	Coupling constant (Hz)
Quinovose						
H-1	6.14 (d)	$J_{1,2}=4.1$	6.13 (d)	$J_{1,2}=3.6$	6.15 (d)	$J_{1,2}=3.6$
H-2	3.70 (dd)	$J_{2,3}=9.5$	3.71 (dd)	$J_{2,3}=9.0$	3.72 (dd)	$J_{2,3}=9.9$
H-3	3.96 (t)	$J_{3,4}=9.5$	3.98 (t)	$J_{3,4}=9.0$	3.94 (t)	$J_{3,4}=9.9$
H-4	3.34 (t)	$J_{4,5}=9.5$	3.33 (t)	$J_{4,5}=9.0$	3.35 (t)	$J_{4,5}=9.9$
H-5	3.66 (dq)	$J_{5,6}=6.3$	3.69 (dq)	$J_{5,6}=6.3$	3.65 (dq)	$J_{5,6}=6.3$
H-6	1.24 (d)		1.24 (d)		1.25 (d)	
Quinovose						
H-1′	4.59 (d)	$J_{1,2}=8.1$	4.60 (d)	$J_{1,2}=8.1$	4.57 (d)	$J_{1,2}=8.0$
H-2′	4.93 (dd)	$J_{2,3}=9.9$	4.93 (dd)	$J_{2,3}=9.5$	4.93 (dd)	$J_{2,3}=9.5$
H-3′	3.75 (t)	$J_{3,4}=9.9$	3.75 (t)	$J_{3,4}=9.5$	3.76 (t)	$J_{3,4}=9.5$
H-4′	4.71 (t)	$J_{4,5}=9.9$	4.71 (t)	$J_{4,5}=9.5$	4.71 (t)	$J_{4,5}=9.5$
H-5′	3.54 (dq)	$J_{5,6}=6.3$	3.51 (dq)	$J_{5,6}=6.3$	3.50 (dq)	$J_{5,6}=6.3$
H-6′	1.20 (d)		1.19 (d)		1.20 (d)	
Acosamine						
H-1	4.51 (dd)	$J_{1,2ax}=9.5$	4.52 (dd)	$J_{1,2ax}=9.5$	4.52 (dd)	$J_{1,2ax}=9.9$
H-2_{ax}	1.46 (dt)	$J_{1,2eq}=1.8$	1.47 (dt)	$J_{1,2eq}=1.8$	1.46 (dt)	$J_{1,2eq}=1.8$
H-2_{eq}	2.12 (ddd)	$J_{2gem}=13.6$	2.10 (ddd)	$J_{2gem}=13.6$	2.12 (ddd)	$J_{2gem}=13.6$
H-3	4.09 (m)	$J_{2ax,3}=13.6$	4.08 (m)	$J_{2ax,3}=13.6$	4.10 (m)	$J_{2ax,3}=13.6$
		$J_{2eq,3}=4.5$		$J_{2eq,3}=4.5$		$J_{2eq,3}=5.0$
H-4	4.40 (t)	$J_{3,4}=9.9$	4.40 (t)	$J_{3,4}=9.5$	4.40 (t)	$J_{3,4}=9.9$
H-5	3.56 (dq)	$J_{4,5}=9.9$	3.56 (dt)	$J_{4,5}=9.5$	3.56 (dq)	$J_{4,5}=9.9$
H-6	1.22 (d)	$J_{5,6}=6.3$	1.22 (d)	$J_{5,6}=6.3$	1.23 (d)	$J_{5,6}=6.3$
NH	5.70 (d)	$J_{3,NH}=9.0$	5.73 (d)	$J_{3,NH}=9.0$	5.78 (d)	$J_{3,NH}=9.0$
Acosamine						
H-1′	4.95 (dd)	$J_{1,2ax}=9.4$	4.96 (dd)	$J_{1,2ax}=9.9$	4.96 (dd)	$J_{1,2ax}=9.9$
H-$2'_{ax}$	1.33 (dt)	$J_{1,2eq}=1.8$	1.34 (dt)	$J_{1,2eq}=2.0$	1.33 (dt)	$J_{1,2eq}=1.8$
H-$2'_{eq}$	2.26 (ddd)	$J_{2gem}=13.6$	2.28 (ddd)	$J_{2gem}=13.6$	2.27 (ddd)	$J_{2gem}=13.6$
H-3′	4.14 (m)	$J_{2ax,3}=13.6$	4.14 (m)	$J_{2ax,3}=13.6$	4.14 (m)	$J_{2ax,3}=13.6$
		$J_{2eq,3}=4.5$		$J_{2eq,3}=4.5$		$J_{2eq,3}=5.0$
H-4′	4.42 (t)	$J_{3,4}=9.9$	4.43 (t)	$J_{3,4}=9.5$	4.43 (t)	$J_{3,4}=9.9$
H-5′	3.52 (dq)	$J_{4,5}=9.9$	3.54 (dq)	$J_{4,5}=9.5$	3.50 (dq)	$J_{4,5}=9.9$
H-6′	1.26 (d)	$J_{5,6}=6.3$	1.26 (d)	$J_{5,6}=6.3$	1.27 (d)	$J_{5,6}=6.3$
NH	5.96 (d)	$J_{3,NH}=9.0$	6.01 (d)	$J_{3,NH}=9.0$	6.01 (d)	$J_{3,NH}=9.0$
	Quinovose (A)		Viosamine (B)		Glucose (C)	
H-1	4.60 (d)	$J_{1,2}=8.1$	4.57 (d)	$J_{1,2}=8.1$	4.67 (d)	$J_{1,2}=8.0$
H-2	4.97 (dd)	$J_{2,3}=9.5$	5.00 (dd)	$J_{2,3}=9.9$	5.02 (dd)	$J_{2,3}=9.5$
H-3	5.09 (t)	$J_{3,4}=9.5$	4.95 (t)	$J_{3,4}=9.9$	5.14 (dd)	$J_{3,4}=9.5$
H-4	4.78 (t)	$J_{4,5}=9.5$	3.91 (q)	$J_{4,5}=9.9$	5.04 (t)	$J_{4,5}=9.5$
H-5	3.50 (dq)	$J_{5,6}=6.3$	3.39 (dq)	$J_{5,6}=6.3$	3.65 (ddd)	$J_{5,6}=4.5$
H-6	1.16 (d)		1.19 (d)		4.03 (dd)	$J_{5,6'}=2.3$
					4.23 (dd)	$J_{6,6'}=13.6$
NH			5.62 (d)	$J_{4,NH}=9.9$		

reducing quinovose residue but C-3 in the reducing quinovose residue. Therefore, in order to confirm the structures of viridopentaoses, the ^{1}H-NMR (360 MHz) spectra of the peracetate **2A**, **2B** and **2C** were examined (Table IV). Full assignments, which were supported by conclusive spin-decoupling experiments, resolved the above problem. The chemical shifts of

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H-3 in the reducing quinovose residue and H-2 in the non-reducing quinovose residue indicate that the hydroxy group at C-3 is linked with an *N*-acetylacosamine residue, whereas the hydroxy group at C-2′ is acetylated. Consequently, the structures of viridopentaoses A, B and C should be **1A**, **1B** and **1C**, respectively.[29]

A detailed discussion of the ^{13}C-NMR spectra of viridopentaoses and related derivatives is given in Part VIII,[30] and an extensive CI mass spectrometric study of the permethylated derivatives is described in detail in Part IX.[31]

Experimental

All melting points were determined on a micro-melting point apparatus (hot-stage type, Yanagimoto MP-S3) and are uncorrected. Optical rotations were measured with a JASCO DIP-SL polarimeter. IR spectra were determined with a Hitachi IR-215 spectrometer. ^{1}H-NMR spectra were recorded on Hitachi R-24B (60 MHz), JEOL FX-100 (100 MHz) and Nicolet NT 360 spectrometers, and ^{13}C-NMR spectra were recorded on a JEOL FX-100 (25 MHz) spectrometer using tetramethylsilane as an internal standard. CI mass spectra were obtained using a Shimadzu LKB 9000B mass spectrometer. Operating conditions were as follows: ion source temperature 190 °C; electron energy 140 or 300 eV; reagent gas pressure 0.3 Torr, accelerating voltage 3.5 kV. High resolution CI mass spectra were recorded on a JEOL JMS-D-300 mass spectrometer coupled on-line to a JEC-980B computer at a resolution of approximately 5000 under the following conditions: ion source temperature 235—240 °C; electron energy 240 eV; accelerating voltage 3 kV; emission current 610 μA; reagent gas pressure about 0.3 Torr. SIMS, in-beam and FD mass spectra were obtained using a Hitachi M-80 mass spectrometer. GC analyses were performed on a Hitachi 164 machine with a flame ionization detector using a 2 m glass column packed with 3% JXR silicone on Gas-chrom Q. Helium was employed as a carrier gas at a flow rate of 40 ml/min. Thin layer chromatography (TLC) was performed on Merck pre-coated plates (Kieselgel 60 F_{254}). For column chromatography, Merck Kieselgel 60 (Art. 7729 or 7734) and Sephadex LH-20 (Pharmacia) were used.

Ammonolysis of SVD-*N*-Ac——A solution of crude SVD (30 g) in MeOH (300 ml) was treated with acetic anhydride (30 ml) and the reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated to dryness to yield crude SVD-*N*-Ac, as a pale yellow powder (30 g). When crude SVD-*N*-Ac (31.5 g) was subjected to column chromatography on silica gel using iso-PrOH–7% NH_4OH (5 : 1), ammonolysis occurred. The reaction mixture was concentrated to dryness and the residue was subjected to column chromatography on Sephadex LH-20. Elution with MeOH afforded unchanged SVD-*N*-Ac (10.3 g), a fraction containing glycosidic compounds (7.2 g) and a fraction containing oligosaccharides (6.0 g). The crude oligosaccharides (5.4 g) were separated as shown in Chart 2. Each component was subjected to column chromatography on Sephadex LH-20 to yield **1A**, 630 mg; **1B**, 695 mg; **1C**, 585 mg.

Acetylation of 1A——A solution of **1A** (50 mg) in pyridine (1 ml) was treated with acetic anhydride (0.5 ml) and the reaction mixture was allowed to stand for 2 d at room temperature, then concentrated to dryness. The residue was precipitated from AcOEt–Et_2O to give a white powder (48.7 mg), mp 160—163 °C (dec.). $[\alpha]_D^{20}$ +9.7° (c=0.3, MeOH). IR ν_{max}^{KBr} cm^{-1}: 1750, 1660 (CO). FDMS: m/z 1157 $(M+Na)^+$, 1135 (MH^+). *Anal.* Calcd for $C_{50}H_{74}N_2O_{27}\cdot 3H_2O$: C, 50.50; H, 6.73; N, 2.36. Found: C, 50.92; H, 6.76; N, 2.27.

Acetylation of 1B——A solution of **1B** (50 mg) in pyridine (1 ml) was treated with acetic anhydride (0.5 ml) and the reaction mixture was allowed to stand for 2 d at room temperature, then concentrated to dryness. The residue was precipitated from AcOEt–Et_2O to give a white powder (58.3 mg), mp 175—177 °C (dec.). $[\alpha]_D^{20}$ +18.3° (c= 0.3, MeOH). IR ν_{max}^{KBr} cm^{-1}: 1750, 1660 (CO). FDMS: m/z 1156 $(M+Na)^+$, 1134 (MH^+). *Anal.* Calcd for $C_{50}H_{75}N_3O_{26}\cdot 2H_2O$: C, 51.32; H, 6.76; N, 3.59. Found: C, 51.03; H, 6.99; N, 3.37.

Acetylation of 1C——A solution of **1C** (50 mg) in pyridine (1 ml) was treated with acetic anhydride (0.5 ml) and the reaction mixture was allowed to stand for 2 d at room temperature, then concentrated to dryness. The residue was precipitated from AcOEt–Et_2O to give a white powder (65.3 mg), mp 152—157 °C (dec.). $[\alpha]_D^{20}$ +11.6° (c=0.3, MeOH) IR ν_{max}^{KBr} cm^{-1}: 1740, 1660 (CO). FDMS: m/z 1215 $(M+Na)^+$, 1193 (MH^+). *Anal.* Calcd for $C_{52}H_{76}N_2O_{29}\cdot 3H_2O$: C, 50.08; H, 6.58; N, 2.25. Found: C, 50.18; H, 6.49; N, 2.31.

Methylation of 1A by Hakomori's Method——1) Dimsyl Carbanion: A mixture of NaH (0.4 g; a commercial sample of NaH was defatted with dry hexane beforehand) in dimethylsulfoxide (DMSO) (20 ml) was heated under an N_2 atmosphere at 60 °C for 2 h to yield greenish dimsyl carbanion. 2) A solution of **1A** (100 mg) in DMSO (5 ml) was then added to the dimsyl carbanion solution (6 ml) and the reaction mixture was stirred at room temperature for 6 h under an N_2 atmosphere. CH_3I (4 ml) was added to the solution below 20 °C and the whole was stirred overnight at room temperature. After dilution with ice-water, the mixture was extracted with $CHCl_3$. The organic layer was washed with 5% $Na_2S_2O_3$ (aq.) and water successively and dried over $MgSO_4$. The residue was chromatographed on a silica gel column with $CHCl_3$–MeOH (19 : 1) to yield a white powder (89.4 mg), mp 107—113 °C. $[\alpha]_D^{23}$ −16.5° (c= 0.4, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1635 (CO). MS: CI (iso-C_4H_{10}); m/z 939 (MH^+), CI (NH_3); m/z 956 $(M+NH_4)^+$, in-

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beam EI; *m/z* 939 (MH^+). *Anal.* Calcd for $C_{44}H_{78}N_2O_{19}\cdot 1/2H_2O$: C, 55.76; H, 8.34; N, 2.96. Found: C, 55.98; H, 8.75; N, 2.88.

Methylation of 1B by Hakomori's Method——A solution of **1B** (100 mg) in DMSO (5 ml) was added to dimsyl carbanion solution (6.4 ml) and the reaction mixture was stirred at room temperature for 6 h under an N_2 atmosphere. CH_3I (4 ml) was added to the solution below 20 °C and then the reaction mixture was stirred overnight at room tmperature. After dilution with ice-water, the mixture was extracted with $CHCl_3$. The organic layer was washed with 5% $Na_2S_2O_3$ (aq.) and water successively and dried over $MgSO_4$. The residue was chromatographed on a silica gel column with $CHCl_3$–MeOH (19 : 1) to give a white powder (81.7 mg), mp 174—185 °C. $[\alpha]_D^{23}$ −29.3° (*c*=0.4, $CHCl_3$), IR ν_{max}^{KBr} cm^{-1}: 1630 (CO). MS: CI (iso-C_4H_{10}); *m/z* 980 (MH^+), CI (NH_3); *m/z* 997 $(M+NH_4)^+$, in-beam EI; *m/z* 980 (MH^+). *Anal.* Calcd for $C_{46}H_{81}N_3O_{19}\cdot 2H_2O$: C, 54.43; H, 8.38; N, 4.14. Found: C, 54.30; H, 8.61; N, 4.07.

Methylation of 1C by Hakomori's Method——A solution of **1C** (100 mg) in DMSO (5 ml) was added to dimsyl carbanion solution (7.3 ml) and the reaction mixture was stirred at room temperature for 6 h under an N_2 atmosphere. CH_3I (4 ml) was added to the solution below 20 °C and then the reaction mixture was stirred overnight at room temperature. After dilution with ice-water, the mixture was extracted with $CHCl_3$. The organic layer was washed with 5% $Na_2S_2O_3$ (aq.) and water successively and dried over $MgSO_4$. The residue was chromatographed on a silica gel column with $CHCl_3$–MeOH (19 : 1) to afford a white powder (88 mg), mp 92—104 °C $[\alpha]_D^{23}$ −7.8° (*c*=0.8, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1625 (CO). MS: CI (iso-C_4H_{10}); *m/z* 969 (MH^+), CI (NH_3); *m/z* 986 $(M+NH_4)^+$, in-beam EI; *m/z* 969 (MH^+). *Anal.* Calcd for $C_{45}H_{80}N_2O_{20}\cdot 2H_2O$: C, 53.78; H, 8.36; N, 2.79. Found: C, 53.84; H, 8.51; N, 2.81.

Methanolysis of 1A——A solution of 100 mg of **1A** in 3 ml of 1.6% methanolic HCl was heated under reflux for 8 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was concentrated under reduced pressure to give a dark brown oil. A solution of the residue (106 mg) in pyridine (1 ml) was treated with acetic anhydride (2 ml) and the mixture was allowed to stand for 2 d at room temperature. The reaction mixture was then concentrated to dryness to yield 179 mg of a yellow oil. The residue was subjected to column chromatography on silica gel. Elution with C_6H_6–AcOEt (9 : 1) and C_6H_6–Me_2CO (7 : 3) successively afforded **4** (86 mg) and **5′** (40 mg).

Methanolysis of 1B——A solution of 100 mg of **1B** in 3 ml of 1.6% methanolic HCl was heated under reflux for 8 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was concentrated under reduced pressure to yield a dark brown oil. A solution of the residue (98 mg) in pyridine (1 ml) was treated with acetic anhydride (2 ml), and the mixture was allowed to stand for 2 d at room temperature. The reaction mixture was then concentrated to dryness to yield 163 mg of a yellow oil. The residue was chromatographed on a silica gel column with C_6H_6–AcOEt (9 : 1) and C_6H_6–Me_2CO (7 : 3) successively to yield **4** (42 mg), **5′** (48 mg) and **6** (18 mg).

Methanolysis of 1C——A solution of 100 mg of **1C** in 3 ml of 1.6% methanolic HCl was heated under reflux for 8 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was concentrated under reduced pressure to yield a dark brown oil. A solution of the residue (99 mg) in pyridine (1 ml) was treated with acetic anhydride (2 ml) and the mixture was allowed to stand for 2 d at room temperature. The reaction mixture was concentrated to dryness to yield 158 mg of a yellow oil. The residue was subjected to column chromatography on silica gel. Elution with C_6H_6–AcOEt (9 : 1) and C_6H_6–Me_2CO (7 : 3) successively afforded **4** (44 mg), **5′** (47 mg) and **7** (28 mg).

Preparation of Reference Compounds——All permethylated monosaccharides were prepared by Hakomori's method.

i) Methyl *N*-Acetyl-*N*-methyl-4-*O*-methyl-α-D-acosaminide (**9**): Colorless oil. High resolution mass spectrum: CI (iso-C_4H_{10}) Calcd for $C_{11}H_{22}NO_4$ (MH^+); *m/z* 232.1546. Found: *m/z* 232.1493. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1630 (CO). ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, d, *J*=6.5 Hz, H-6), 2.18 (3H, s, $NCOCH_3$), 2.82 (3H, s, N–CH_3), 3.33 (3H, s, OCH_3), 3.38 (3H, s, OCH_3), 4.73 (1H, t, *J*=3 Hz, H-1). GLC (150 °C): t_R 3.0 min (major), 3.5 min (minor).

ii) Methyl 2,3,4-Tri-*O*-methyl-α-D-quinovopyranoside (**10A**): Colorless oil. $[\alpha]_D^{25}$ +149.8° (*c*=0.5, MeOH, lit.[32] $[\alpha]_D^{12}$ +168.0° (*c*=1.05, MeOH). MS: CI (NH_3); *m/z* 238 $(M+NH_4)^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no OH. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, d, *J*=6.5 Hz, H-6), 3.38, 3.50, 3.55, 3.61 (each 3H, s, OCH_3), 4.74 (1H, d, *J*=4 Hz, H-1). GLC (120 °C): t_R 2.4 min.

iii) Methyl *N*-Acetyl-*N*-methyl-2,3-di-*O*-methyl-α-D-viosaminide (**10B**): Colorless oil. High resolution mass spectrum: CI (iso-C_4H_{10}) Calcd for $C_{12}H_{24}NO_5$ (MH^+); *m/z* 262.1653. Found: *m/z* 262.1623. ^{1}H-NMR ($CDCl_3$) δ: 1.16 (3H, d, *J*=6.5 Hz, H-6), 2.16 (3H, s, $NCOCH_3$), 2.81 (3H, s, N–CH_3), 3.43, 3.47, 3.52 (each 3H, s, OCH_3), 4.79 (1H, d, *J*=4 Hz, H-1). GLC (150 °C): t_R 3.9 min (minor), 4.7 min (major).

iv) Methyl 2,3,4,6-Tetra-*O*-methyl-α-D-glucopyranoside (**10C**): Colorless oil. $[\alpha]_D^{25}$ +139.5° (*c*=0.2, EtOH, lit.[32] $[\alpha]_D^{23}$ +139.1°). MS: CI (NH_3); *m/z* 268 $(M+NH_4)^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no OH. ^{1}H-NMR ($CDCl_3$) δ: 3.40 (6H, s, 2×OCH_3), 3.50, 3.52, 3.59 (each 3H, s, OCH_3), 4.82 (1H, d, *J*=4 Hz, H-1). GLC (120 °C): t_R 2.4 min.

Methanolysis of 3A——i) At Room Temperature: Compound **3A** (99 mg) was treated with 5% methanolic HCl (9 ml) at room temperature for 17 h and then the solution was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated to dryness. The residue was subjected to column chromatography on Sephadex LH-20. Elution with MeOH afforded **8A** (35 mg) and **9** (37 mg). **8A**: colorless crystals, mp 120—123 °C (from isopropyl ether–hexane). High resolution mass spectrum: CI (iso-C_4H_{10}) Calcd for $C_{24}H_{45}O_{13}$ (MH^+); *m/z* 541.2857. Found: *m/z* 541.2855. MS: CI (NH_3); *m/z* 558 $(M+NH_4)^+$, *m/z* 541 (MH^+). **9**: colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1630 (CO). ^{1}H-NMR ($CDCl_3$)

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δ: 1.29 (3H, d, J=6.5 Hz, H-6), 2.18 (3H, s, $NCOCH_3$), 2.82 (3H, s, N–CH_3), 3.33 (3H, s, OCH_3), 3.38 (3H, s, OCH_3), 4.73 (1H, t, J=3 Hz, H-1).

ii) Under Reflux: Compound **3A** (75 mg) was heated under reflux with 5% methanolic HCl (11 ml) for 3 h. After cooling, the solution was neutralized with Ag_2CO_3 and filtered. The filtrate was concentrated to dryness. The residue was subjected to column chromatography on silica gel. Elution with C_6H_6–Me_2CO (4:1→3:1→3:2) successively gave **9** (27 mg), **10A** (5 mg), **11** (7 mg) and **12** (12 mg).

Compounds **9**, **10A** and **12** (as the peracetates) were identified by comparison with the corresponding authentic samples (GLC and TLC). **11**, α-anomer: colorless oil. High resolution mass spectrum: CI (iso-C_4H_{10}) Calcd for $C_9H_{19}O_5$ (MH^+); m/z 207.1233. Found: m/z 207.1289. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 3450 (OH). ^{1}H-NMR ($CDCl_3$) δ: 1.31 (3H, d, J=6 Hz, H-6), 2.81 (1H, t, J=9.5 Hz, H-4), 3.19 (1H, dd, J_1=9.5 Hz, J_2=4.0 Hz, H-2), 3.41, 3.51, 3.59 (each 3H, s, OCH_3), 3.90 (1H, t, J=9.5 Hz, H-3), 4.82 (1H, d, J=4 Hz, H-1). 3-*O*-Acetate: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740 (CO). ^{1}H-NMR ($CDCl_3$) δ: 1.28 (1H, d, J=6 Hz, H-6), 2.10 (3H, s, –$OCOCH_3$), 2.88 (1H, t, J=10 Hz, H-4), 3.28 (1H, dd, J_1=10 Hz, J_2=3 Hz, H-2), 3.40, 3.42, 3.43 (each 3H, s, OCH_3), 3.70 (1H, dq, J_1=10 Hz, J_2=6 Hz, H-5), 4.78 (1H, d, J=3 Hz, H-1), 5.32 (1H, t, J=10 Hz, H-3).

Methanolysis of 3B——i) At Room Temperature: Compound **3B** (80 mg) was treated with 5% methanolic HCl (9 ml) at room temperature for 22 h and then the solution was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated to dryness. The residue was subjected to column chromatography on Sephadex LH-20. Elution with MeOH afforded **8B** (21 mg) and **9** (14 mg). **8B**: colorless crystals, mp 140—142 °C (from isopropyl ether–hexane). High resolution mass spectrum: CI (iso-C_4H_{10}) Calcd for $C_{26}H_{48}NO_{13}$ (NH^+); m/z 582.3126. Found: m/z 582.3131. MS: CI (NH_3); m/z 599 $(M+NH_4)^+$, 582 (MH^+).

ii) Under Reflux: Compound **3B** (98 mg) was heated under reflux with 5% methanolic HCl (15 ml) for 7 h. After cooling, the solution was neutralized with Ag_2CO_3 and filtered. The filtrate was concentrated to dryness. The residue was subjected to column chromatography on silica gel. Elution with C_6H_6–Me_2CO (9:1→4:1→3:2) successively gave **9** (31 mg), **10B** (9.5 mg), **11** (6.4 mg) and **12** (6 mg). They were identical with the corresponding authentic samples by GLC and TLC.

Methanolysis of 3C——i) At Room Temperature: Compound **3C** (80 mg) was treated with 5% methanolic HCl (10 ml) at room temperature for 22 h and then the solution was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated to dryness. The residue was subjected to column chromatography on Sephadex LH-20. Elution with MeOH afforded **8C** (20.1 mg) and **9** (12.7 mg). **8C**: white powder, mp 62—64 °C (from isopropyl ether–hexane). High resolution mass spectrum: CI (iso-C_4H_{10}) Calcd for $C_{25}H_{47}O_{14}$ (MH^+); m/z 571.2963. Found: m/z 571.2957. MS: CI (NH_3); m/z 588 $(M+NH_4)^+$, m/z 571 (MH^+).

ii) Under Reflux: Compound **3C** (93 mg) was boiled under reflux with 5% methanolic HCl (15 ml) for 7 h. After cooling, the solution was neutralized with Ag_2CO_3 and filtered. The filtrate was concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel. Elution with C_6H_6–Me_2CO (9:1→4:1→3:2) successively yielded **9** (32 mg), **10C** (6.4 mg), **11** (6 mg) and **12** (8 mg). They were identical with the corresponding authentic samples by GLC and TLC.

Viridotetraose A (15A)——A solution of **1A** (200 mg) in MeOH (3 ml) was treated with concentrated hydrochloric acid (0.15 ml) under ice-cooling and the reaction mixture was allowed to stand at 4 °C for 18 h. The solution was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated to dryness. The residue (191 mg) was subjected to column chromatography on silica gel. Elution with CH_3CN–7% NH_4OH (3:1) afforded **5** (61 mg), **15A** (104 mg) and **13A** (40 mg). Compound **15A** was purified by column chromatography on Sephadex LH-20 using MeOH as the eluent to give 65 mg of a white powder, mp 207—209 °C (dec.). $[\alpha]_D^{23}$ −18.7° (c=0.3, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3500—3200 (OH/NH) 1660 (CO) ^{1}H-NMR (CD_3OD) δ: 1.35 (12H, d, J=6 Hz, –CH–C<u>H</u>$_3$), 2.02 (3H, s, NCOC<u>H</u>$_3$), 5.29 (1H, d, J=3 Hz, anomeric H). SIMS: m/z 734, 736 $(M+Ag)^+$. *Anal.* Calcd for $C_{26}H_{45}NO_{16}\cdot 2H_2O$: C, 47.05; H, 7.44; N, 2.11. Found: C, 46.96; H, 7.55; N, 2.11.

Viridotetraose B (15B)——A solution of **1B** (200 mg) in MeOH (3 ml) was treated with concentrated hydrochloric acid (0.15 ml) under ice-cooling and the reaction mixture was allowed to stand at 4 °C for 18 h. The solution was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated to dryness. The residue (180 mg) was subjected to column chromatography on silica gel. Elution with CH_3CN–7% NH_4OH (3:1) afforded **5** (50 mg), **15B** (74 mg) and **13B** (31 mg). Compound **15B** was purified by column chromatography on Sephadex LH-20 using MeOH as the eluent to give 67 mg of a white powder, mp 235—237 °C (dec.). $[\alpha]_D^{23}$ −12.3° (c=0.3, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3500—3300 (OH/NH), 1650 (CO). ^{1}H-NMR (CD_3OD) δ: 1.34 (12H, d, J=6 Hz, –CH–C<u>H</u>$_3$), 2.01 (6H, s, NCOC<u>H</u>$_3$), 5.27 (1H, d, J=3.5 Hz, anomeric H). SIMS: m/z 775, 777 $(M+Ag)^+$. *Anal.* Calcd for $C_{28}H_{48}N_2O_{16}\cdot 3H_2O$: C, 46.53; H, 7.53; N, 3.88. Found: C, 46.73; H, 7.34; N, 3.88.

Viridotetraose C (15C)——A solution of **1C** (200 mg) in MeOH (3 ml) was treated with concentrated hydrochloric acid (0.15 ml) under ice-cooling and the reaction mixture was allowed to stand at 4 °C for 18 h. The solution was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated to dryness. The residue (240 mg) was subjected to column chromatography on silica gel. Elution with CH_3CN–7% NH_4OH (3:1) afforded **5** (67 mg), **15C** (90 mg) and **13C** (46 mg). Compound **15C** was purified by column chromatography on Sephadex LH-20 using MeOH as the eluent to give 62 mg of a white powder, mp 213—215 °C (dec.). $[\alpha]_D^{23}$ −19.0° (c=0.3, MeOH). IR ν_{max}^{KBr} cm^{-1}:

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3500—3300 (OH/NH), 1660 (CO). ^{1}H-NMR (CD_3OD) δ: 1.33 (9H, d, J=6 Hz, –CH–C$\underline{H}_3$), 2.00 (3H, s, NCOC$\underline{H}_3$). SIMS: m/z 750, 752 $(M+Ag)^+$. *Anal.* Calcd for $C_{26}H_{45}NO_{17}\cdot H_2O$: C, 47.20; H, 7.16; N, 2.21. Found: C, 47.23; H, 7.47; N, 2.39.

Viridotriose A (13A)——A solution of **1A** (100 mg) in MeOH (5 ml) was treated with concentrated hydrochloric acid (0.5 ml) and the reaction mixture was allowed to stand at room temperature for 4 h. The solution was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated to dryness, and the residue (103 mg) was subjected to column chromatography on silica gel. Elution with CH_3CN–7% NH_4OH (3:1) afforded **5** (49 mg) and **13A** (53 mg). Compound **13A** was purified by column chromatography on Sephadex LH-20 using MeOH as the eluent to give 38 mg of a white powder, mp 235—238 °C (dec.). $[\alpha]_D^{23}$ +11.3° (c=0.3, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3500—3200 (OH). ^{1}H-NMR (CD_3OD) δ: 1.30 (9H, d, J=6.0 Hz, –CH–C$\underline{H}_3$). SIMS: m/z 563, 565 $(M+Ag)^+$. *Anal.* Calcd for $C_{18}H_{32}O_{13}\cdot H_2O$: C, 43.90; H, 7.31. Found: C, 43.95; H, 7.02.

Viridotriose B (13B)——A solution of **1B** (100 mg) in MeOH (5 ml) was treated with concentrated hydrochloric acid (0.5 ml) and the reaction mixture was allowed to stand at room temperature for 4 h. The solution was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated to dryness, and the residue (102 mg) was subjected to column chromatography on silica gel. Elution with CH_3CN–7% NH_4OH (3:1) afforded **5** (50 mg) and **13B** (51 mg). Compound **13B** was purified by column chromatography on Sephadex LH-20 using MeOH as the eluent to give 41 mg of a white powder, mp 216—219 °C (dec.). $[\alpha]_D^{23}$ +4.7° (c=0.3, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH/NH), 1660 (CO). ^{1}H-NMR (CD_3OD) δ: 1.30 (9H, d, J=6 Hz, –CH–C$\underline{H}_3$), 1.99 (3H, s, NCOC$\underline{H}_3$), 5.23 (1H, d, J=4 Hz, anomeric H). SIMS: m/z 604, 606 $(M+Ag)^+$. *Anal.* Calcd for $C_{20}H_{35}NO_{13}\cdot H_2O$: C, 46.60; H, 7.18; N, 2.72. Found: C, 46.45; H, 7.33; N, 2.65.

Viridotriose C (13C)——A solution of **1C** (100 mg) in MeOH (5 ml) was treated with concentrated hydrochloric acid (0.5 ml) and the reaction mixture was allowed to stand at room temperature for 4 h. The solution was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated to dryness, and the residue (104 mg) was subjected to column chromatography on silica gel. Elution with CH_3CN–7% NH_4OH (3:1) afforded **5** (53 mg) and **13C** (50 mg). Compound **13C** was purified by column chromatography on Sephadex LH-20 using MeOH as the eluent to give 45 mg of a white powder, mp 168—169 °C (dec.). $[\alpha]_D^{23}$ +12.3° (c=0.3, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH). ^{1}H-NMR (CD_3OD) δ: 1.31 (6H, d, J=6 Hz, –CH–C$\underline{H}_3$). SIMS: m/z 579, 581 $(M+Ag)^+$. *Anal.* Calcd for $C_{18}H_{32}O_{14}\cdot H_2O$: C, 44.08; H, 6.99. Found: C, 44.05; H, 7.05.

Methyl α-Viridobioside B (14B)——i) A solution of viridotriose B (56 mg) in MeOH (5 ml) was treated with concentrated hydrochloric acid (1 ml) and the reaction mixture was allowed to stand at 50 °C for 8 h. The solution was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated to dryness. The residue (61 mg) was subjected to column chromatography on silica gel. Elution with CH_3CN–H_2O (9:1) afforded 10 mg of **14B**, which was purified by column chromatography on Sephadex LH-20 using MeOH as the eluent to give 8 mg of **14B**. The compound was recrystallized from AcOEt–MeOH to give colorless needles, mp 264—266 °C. $[\alpha]_D^{23.5}$ +46.7° (c=0.3, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3700—3100 (OH/NH), 1650 (CO). ^{1}H-NMR (CD_3OD) δ: 1.18 (3H, d, J=6 Hz), 1.24 (3H, d, J=6 Hz), 1.97 (3H, s, $NCOCH_3$), 3.35 (3H, s, OCH_3). MS: CI (iso-C_4H_{10}); m/z 366 (MH^+). CI (NH_3); m/z 383 $(M+NH_4)^+$. *Anal.* Calcd for $C_{15}H_{27}NO_9\cdot 1/2H_2O$: C, 48.12; H, 7.54; N, 3.74. Found: C, 47.98; H, 7.65; N, 3.66.

ii) A solution of viridopentaose B (200 mg) in MeOH (5 ml) was treated with Amberlyst 15 (20 ml) and the reaction mixture was stirred at 50 °C for 8 h. The mixture was filtered and the filtrate was concentrated to dryness. The residue (166 mg) was subjected to column chromatography on silica gel. Elution with CH_3CN–H_2O (9:1) afforded **14B** (14 mg), which was purified by column chromatography on Sephadex LH-20 using MeOH as the eluent to give 13 mg of **14B**.

Alkaline Hydrolysis of 1A——A solution of **1A** (100 mg) in MeOH (1 ml) was treated with 5% NaOH (aq.) (1 ml) and the mixture was allowed to stand for 24 h at room temperature. The solution was neutralized with Amberlyst 15 and filtered. The filtrate was concentrated *in vacuo*, and the residue was subjected to column chromatography on Sephadex LH-20. Elution with MeOH afforded **16A** (45 mg) and **17** (14 mg). Compound **16A** was recrystallized from AcOEt–MeOH to give colorless crystals, mp 224—226 °C (dec.). $[\alpha]_D^{23}$ −42° (c=0.3, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH/NH), 1620 (CO). ^{1}H-NMR (CD_3OD) δ: 1.28 (6H, d, J=6 Hz, –CH–C$\underline{H}_3$), 1.32 (6H, d, J=6 Hz, –CH–C$\underline{H}_3$), 1.97 (3H, s, NCOC$\underline{H}_3$), 4.41 (1H, d, J=8 Hz, anomeric H), 5.07 (1H, s, anomeric H), 5.33 (1H, s, H-3). SIMS: m/z 648 $(M+K)^+$, 632 $(M+Na)^+$, 610 (MH^+). *Anal.* Calcd for $C_{26}H_{43}NO_{15}\cdot 2H_2O$: C, 48.37; H, 7.28; N, 2.17. Found: C, 48.26; H, 7.59; N, 2.13. Compound **17** was recrystallized from AcOEt–MeOH to give colorless needles, mp 214—216 °C. $[\alpha]_D^{23}$ +26.9° (c=0.3, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 3275 (NH), 1635 (CO). ^{1}H-NMR (CD_3OD) δ: 1.21 (major), 1.27 (minor) (3H, d, J=6 Hz, H-6), 1.95 (3H, s, NCOC$\underline{H}_3$). MS: CI (NH_3); m/z 207 $(M+NH_4)^+$, 190 (MH^+). *Anal.* Calcd for $C_8H_{15}NO_4$: C, 50.79; H, 7.94; N, 7.41. Found: C, 50.40; H, 8.29; N, 7.18.

Alkaline Hydrolysis of 1B——A solution of **1B** (100 mg) in MeOH (1 ml) was treated with 5% NaOH (aq.) (1 ml) and the mixture was allowed to stand for 24 h at room temperature. The solution was neutralized with Amberlyst 15 and filtered. The filtrate was concentrated *in vacuo*, and the residue was subjected to column chromatography on Sephadex LH-20. Elution with MeOH afforded **16B** (44 mg) and **17** (12 mg). Compound **16B** was recrystallized from AcOEt–MeOH to give colorless crystals, mp 209—212 °C (dec.). $[\alpha]_D^{23}$ −46.7° (c=0.3, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH/NH), 1620 (CO). ^{1}H-NMR (CD_3OD) δ: 1.28 (6H, d, J=6 Hz, –CH–C$\underline{H}_3$), 1.32 (6H, d, J=6 Hz, –CH–C$\underline{H}_3$),

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1.93, 1.97 (each 3H, s, $NCOCH_3$), 4.42 (1H, d, $J=8$ Hz, anomeric H), 5.08 (1H, s, anomeric H), 5.31 (1H, s, H-3). SIMS: *m/z* 689 $(M+K)^+$, 673 $(M+Na)^+$, 651 (MH^+). *Anal.* Calcd for $C_{28}H_{46}N_2O_{15}\cdot 4H_2O$: C, 46.53; H, 7.47; N, 3.62. Found: C, 45.96; H, 7.42; N, 3.78.

Alkaline Hydrolysis of 1C——A solution of **1C** (100 mg) in MeOH (1 ml) was treated with 5% NaOH (aq.) (1 ml) and the mixture was allowed to stand for 24 h at room temperature. The solution was neutralized with Amberlyst 15 and filtered. The filtrate was concentrated *in vacuo*, and the residue was subjected to column chromatography on Sephadex LH-20. Elution with MeOH afforded **16C** (40 mg) and **17** (15 mg). Compound **16C** was precipitated from AcOEt–MeOH to give a white powder, mp 175—180 °C (dec.). $[\alpha]_D^{23}$ −35.7° ($c=0.3$, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH/NH), 1620 (CO). ^{1}H-NMR (CD_3OD) δ: 1.30 (12H, d, $J=6$ Hz, –CH–C$\underline{H}_3$), 1.97 (3H, s, $NCOCH_3$), 4.41 (1H, d, $J=8$ Hz, anomeric H), 5.07 (1H, s, anomeric H), 5.36 (1H, s, H-3). SIMS: *m/z* 664 $(M+K)^+$, 648 $(M+Na)^+$, 626 (MH^+). *Anal.* Calcd for $C_{26}H_{43}NO_{16}\cdot 3H_2O$: C, 45.94; H, 7.21; N, 2.06. Found: C, 46.27; H, 7.52; N, 2.06.

Acknowledgement The authors wish to thank Drs. M. Hayashi and K. Kinoshita, Research Laboratory, Toyo Jozo, Ltd., for their assistance in obtaining high-resolution CI mass spectrometric data, and Drs. H. Kambara and K. Shizukuishi, Hitachi Ltd., for SIMS, in-beam EI and FD mass spectral studies.

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