

STRUCTURAL INVESTIGATION OF AN ANTIBIOTIC SPORAVIRIDIN III¹.

STRUCTURES OF VIRIDOPENTAOSE A AND C

Ken-ichi Harada, Susumu Ito, Toshiaki Murase, and Makoto Suzuki*

Faculty of Pharmacy, Meijo University, Tempaku, Nagoya 468, JAPAN

Abstract — Viridopentaose A (1) and C (2) are new heteropentasaccharides, degradation products of sporaviridin. These structures were established by chemical degradative reactions, mass spectrometry, and ¹³C-NMR spectroscopy.

In the course of structural investigation of an antibiotic sporaviridin(SVD)², we obtained three heteropentasaccharides, viridopentaose A, B, and C on hydrolysis of N-acetylsporaviridin with aqueous ammonia. The structure of viridopentaose B has been determined with the detailed analysis of ¹³C-NMR spectra¹. The present communication deals with structural characterization of the remaining two pentasaccharides, viridopentaose A and C.

Viridopentaose A (1), mp 198-201°(dec.), C₃₄H₅₈N₂O₁₉·3H₂O, [α]_D²⁰ -45.7°(c 0.3, MeOH), IR(KBr): 3500-3200 cm⁻¹(OH/NH), 1650 cm⁻¹(CO), ¹H-NMR(CD₃OD): δ 1.98 (NHCOCH₃), was a faintly hygroscopic white powder. Acidic methanolysis of 1 with methanolic hydrogen chloride (1.6%, reflux, 8hr), followed by neutralization and evaporation gave a mixture which was fractionated chromatographically yielding each anomeric pair of methyl 3-acetamido-2,3,6-trideoxy-D-arabino-hexopyranoside (methyl N-acetyl-D-acosaminide, 3)³ and methyl 6-deoxy-D-glucopyranoside (methyl D-quinovoside, 4)⁴ in 2:3 molar proportions.

Field desorption (FD) mass spectrum of 1 showed a protonated ion peak (MH⁺) at m/z 799 and a cluster ion peak (M+Na)⁺ at m/z 821, which indicated the molecular weight of 1. Furthermore, chemical ionization (CI) mass spectra of the permethylated viridopentaose A using isobutane and ammonia as reagent gas gave the useful structural informations. Thus a protonated molecular ion peak (MH⁺) was observed at m/z 939 and the fragment ion peaks at m/z 740, 541, and 381 were consistent with tetra-, tri-, and di-saccharide ions, respectively, which were available for the determination of the sequence of the monosaccharide units mentioned above (Figure).

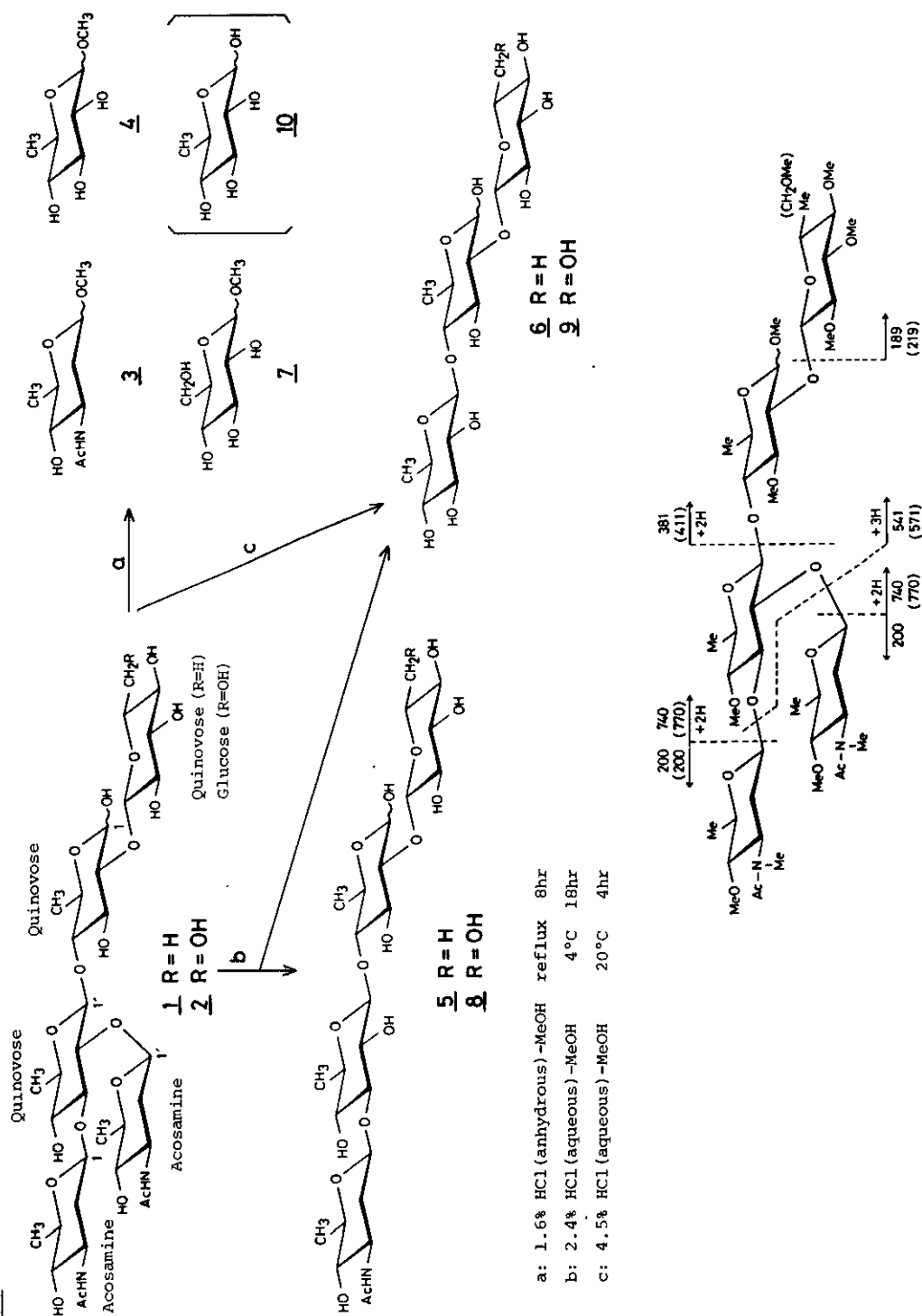


Figure Sequence ions in chemical ionization mass spectra of permethylated viridopentaose A and C

The degradative reactions of 1 by use of the condition(b) and (c) gave tetra-saccharide 5, mp 207-209°(dec.) and trisaccharide 6, mp 235-238°(dec.) (Scheme).

Viridopentaose C (2), mp 191-193°(dec.), $C_{34}H_{58}N_2O_{20} \cdot 3H_2O$, $[\alpha]_D^{20} -31.0$ (c 0.3, MeOH), IR(KBr): 3500-3200 cm^{-1} (OH/NH), 1660-1620 cm^{-1} (CO), 1H -NMR(CD_3OD): δ 1.98 (NHCOCH₃), was a faintly hygroscopic white powder. Acidic methanolysis of 2 using the condition(a) gave each anomeric pair of 3, 4, and methyl D-glucopyranoside 7 in 2:2:1 molar proportions.

FD mass spectrum of 2 provided a cationised cluster ion peak $(M+Na)^+$ at m/z 837 and in CI mass spectra of permethylated 2, a protonated molecular ion (m/z 969) was observed and the fragment ion peaks appeared at m/z 770 and 571 which corresponded to tetra- and tri-saccharide ions, respectively (Figure).

The partial methanolysis of 2 with the conditions described above yielded two products, 8, mp 213-215°(dec.) and 9, mp 168-169°(dec.).

The structures of viridopentaose A (1) and C (2) were established mainly on the basis of ^{13}C -NMR spectroscopic evidence as follows. The ^{13}C -NMR chemical shifts of 1 could be assigned by comparison with those of 3, 4, 5, 6, and 10 (Table). The ^{13}C -NMR spectrum of 1 showed five signals due to anomeric carbons. The resonance at 93.2 ppm represented an anomeric carbon of the reducing D-quinovosyl residue (α -configuration). Three (105.4, 101.7, 101.3 ppm) of the four remaining signals, except for that of the non-reducing D-quinovose moiety, were assignable to the anomeric carbon in a β -configuration as compared with the chemical shifts of the corresponding methyl glycosides. The last signal at 100.9 ppm suggested the presence of the anomeric carbon in an α -configuration. However, the 3.5 ppm downfield shifts were observed at C-1' of non-reducing D-quinovosyl residue in 5 and 6, when the acosamines were removed from 1 by selective methanolysis⁵. Accordingly, the anomeric carbon of the non-reducing D-quinovosyl residue should be also in a β -configuration.

By considering glycosidation shift⁶ (82.9 ppm at C-2 and 85.8 ppm at C-4 in the reducing D-quinovose moiety), the sterically hindered adjacent diglycosidation⁵ (75.7 and 76.0 ppm at C-2' and C-3' in the non-reducing D-quinovose moiety), and the structure of viridopentaose B¹, the four glycosidic linkages in 1 were determined at C-2 and C-4 positions of the reducing D-quinovose and at C-2' and C-3' positions of the non-reducing D-quinovose moiety. Consequently, it is proved that viridopentaose A (1) is an O-(N-acetyl- β -D-acosaminopyranosyl)-(1 \rightarrow 2)-O-[N-acetyl- β -acosaminopyranosyl-(1 \rightarrow 3)]-O- β -D-quinovopyranosyl-(1 \rightarrow 4)-O-[β -D-quinovopyranosyl-

Table ^{13}C -NMR chemical shifts of compounds 1 ~ 10^a

	<u>1</u>	<u>5</u>	<u>6</u>		<u>2</u>	<u>8</u>	<u>9</u>	
Quinovose				Glucose				
C-1"	105.4	105.3	105.2	C-1	105.6	105.4	105.4	
C-2"	75.0	75.4	75.3	C-2	74.9	74.8	74.8	
C-3"	77.7	77.3	77.2	C-3	78.1	77.6	77.5 ^g	
C-4"	76.5	76.7	76.6	C-4	71.1	71.2	71.2	
C-5"	73.0	73.1	73.0	C-5	77.7	77.6	77.5 ^g	
C-6"	18.1 ^b	18.0 ^e	18.0 ^g	C-6	62.5	62.4	62.4	
Quinovose				Quinovose				
C-1	93.2	92.9	92.9	C-1	93.2	92.7	92.7	
C-2	82.9	81.9	81.8	C-2	83.3	82.1	82.1	
C-3	73.0	71.8	71.7	C-3	73.2	71.7	71.7	
C-4	85.8	87.1 ^f	87.0	C-4	85.9	87.0	87.0	
C-5	67.6	66.4	66.4	C-5	67.8	66.4	66.4	
C-6	18.3 ^b	18.1 ^e	18.1 ^g	C-6	18.2 ^b	18.0	17.9	
Quinovose				Quinovose				
C-1'	100.9	104.4	104.4	C-1'	101.0	104.4	104.4	
C-2'	75.7 ^a	74.0	74.8	C-2'	76.2 ^a	74.0	75.0	
C-3'	76.0 ^a	86.9 ^f	77.2	C-3'	76.7 ^a	86.8	77.2 ^g	
C-4'	74.7	74.8	76.4	C-4'	74.9	75.0	76.4	
C-5'	73.0	73.0	73.3	C-5'	73.4	72.9	73.3	
C-6'	18.1 ^b	18.0 ^e	18.0 ^g	C-6'	18.3 ^b	18.0	17.9	
Acosamine				Acosamine				
C-1	101.7 ^d	102.1		C-1	101.5 ^d	102.1		
C-2	37.9	37.9		C-2	38.1	37.8		
C-3	52.4	52.2		C-3	52.3 ^e	52.2		
C-4	75.0	75.4		C-4	75.3 ^f	75.4		
C-5	74.7	74.8		C-5	74.9	74.8		
C-6	18.3 ^b	18.2 ^e		C-6	18.3 ^b	18.2		
Acosamine				Acosamine				
C-1'	101.3 ^d			C-1'	101.7 ^d			
C-2'	37.9			C-2'	38.1			
C-3'	52.4			C-3'	52.6 ^e			
C-4'	75.0			C-4'	75.9 ^f			
C-5'	74.7			C-5'	74.9			
C-6'	18.9 ^b			C-6'	19.0 ^b			
	Methyl N-acetyl- acosaminide (<u>3</u>)		Methyl quinovoside (<u>4</u>)		Methyl glucoside (<u>7</u>)		Quinovose (<u>10</u>)	
	α	β	α	β	α	β	α	β
C-1	98.7	102.0	100.9	105.0	100.6	105.1	93.6	97.7
C-2	36.9	38.1	73.5	75.0	72.9	74.8	73.9	76.3
C-3	49.7	52.4	74.7	77.6	74.6	77.8	74.5	77.6
C-4	76.5	75.9	77.1	76.8	71.1	71.4	77.3	76.9
C-5	69.5	74.6	68.4	73.1	72.9	77.6	68.1	73.1
C-6	18.3	18.3	18.0	18.0	62.1	62.5	18.1	18.1

^a ^{13}C -NMR spectra were recorded on a JEOL JNM-FX100 NMR spectrometer at 25.05MHz in CD_3OD with TMS as an internal reference.

b, c, d, e, f, g Assignments may be reversed in each vertical column.

(1+2)]- α -D-quinovopyranose.

In the same manner, we could also assign the ^{13}C -NMR chemical shifts of 2 (Table), which were compatible with those of 1, except for those of C-4, 5, and 6 of the D-glucose moiety. These results pointed out that viridopentaose C (2) had a D-glucose moiety in the place of a terminal D-quinovose moiety in viridopentaose A (1).

Therefore, it is concluded that viridopentaose C is an O-(N-acetyl- β -D-acosaminopyranosyl)-(1+2)-O-[N-acetyl- β -D-acosaminopyranosyl-(1+3)]-O- β -D-quinovopyranosyl-(1+4)-O-[β -D-glucopyranosyl-(1+2)]- α -D-quinovopyranose.

References

1. Part II. "Application of ^{13}C -NMR to the structural elucidation of viridopentaose B", K. I. Harada, S. Ito, and M. Suzuki, Tetrahedron Lett., in press.
2. T. Okuda, Y. Ito, T. Yamaguchi, T. Furumai, M. Suzuki, and M. Tsuruoka, J. Antibiotics, Ser. A, 1966, 19, 85.
3. K. I. Harada, S. Ito, and M. Suzuki, Carbohydr. Res., 1979, 75, C17.
4. M. E. Evans, L. Long, Jr., and F. W. Parrish, J. Org. Chem., 1968, 33, 1074.
5. a) N. Yamaoka, T. Usui, H. Sugiyama, and S. Seto, Chem. Pharm. Bull. (Tokyo), 1974, 22, 2196.
b) R. U. Lemieux and H. Driguez, J. Am. Chem. Soc., 1975, 97, 4063.
6. a) T. Usui, N. Yamaoka, K. Matsuda, K. Tuzimura, H. Sugiyama, and S. Seto, J. Chem. Soc. Perkin I, 1973, 2425.
b) P. A. J. Gorin, Carbohydr. Res., 1975, 39, 3.
c) K. Tori, S. Seo, Y. Yoshimura, M. Nakamura, Y. Tomita, and H. Ishii, Tetrahedron Lett., 1976, 4163.
d) R. Kasai, M. Suzuo, J. Asakawa, and O. Tanaka, Tetrahedron Lett., 1977, 175.

Received, 17th September, 1979