STRUCTURAL INVESTIGATION OF AN ANTIBIOTIC SPORAVIRIDIN III[1]. STRUCTURES OF VIRIDOPENTAOSE A AND C

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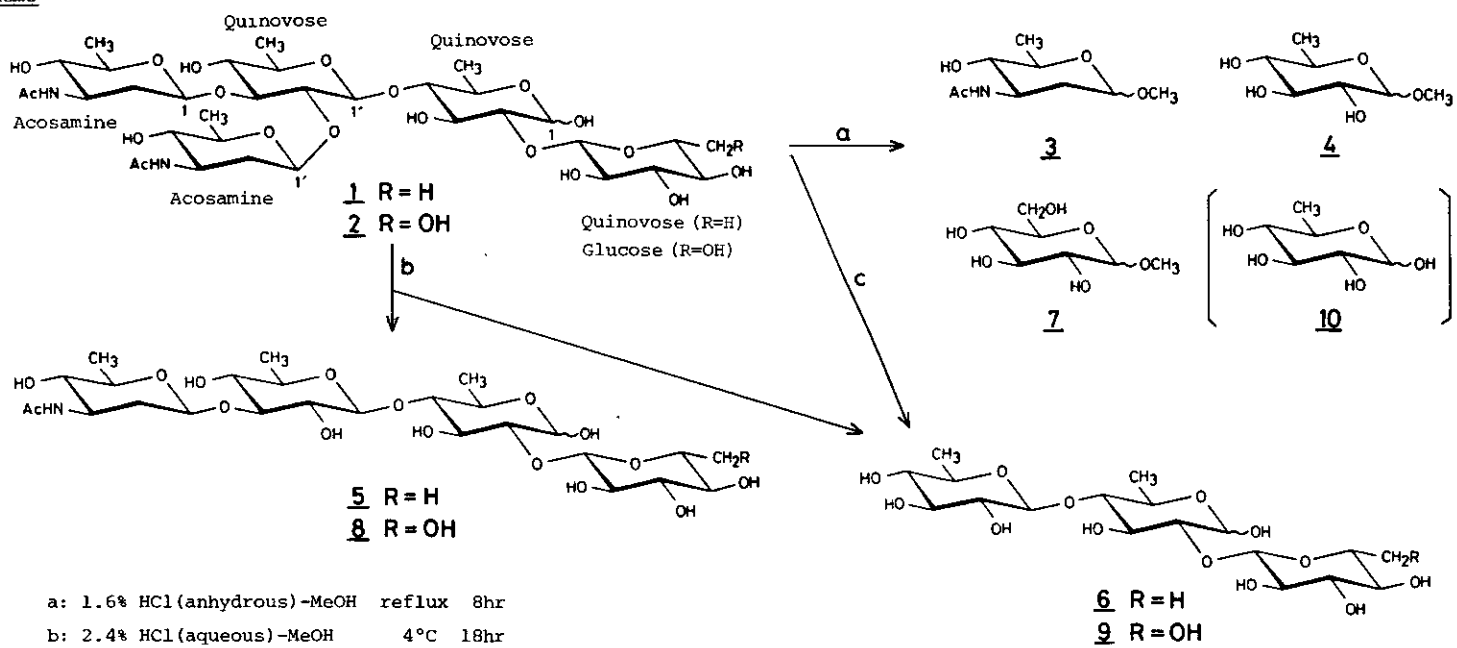
Abstract — Viridopentaose A (1) and C (2) are new heteropentasaccharides, degradation products of sporaviridin. These structures were established by chemical degradative reactions, mass spectrometry, and ^{13}C-NMR spectroscopy.

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 ^{13}C-NMR spectra[1]. The present communication deals with structural characterization of the remaining two pentasaccharides, viridopentaose A and C.

Viridopentaose A (1), mp 198-201°(dec.), $C_{34}H_{58}N_2O_{19}\cdot 3H_2O$, $[\alpha]_D^{20}$ -45.7°(c 0.3, MeOH), IR(KBr): 3500-3200 cm^{-1}(OH/NH), 1650 cm^{-1}(CO), ^{1}H-NMR(CD_3OD): δ 1.98 ($NHCOCH_3$), 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 moler 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).

Scheme



a: 1.6% HCl(anhydrous)-MeOH reflux 8hr
b: 2.4% HCl(aqueous)-MeOH 4°C 18hr
c: 4.5% HCl(aqueous)-MeOH 20°C 4hr

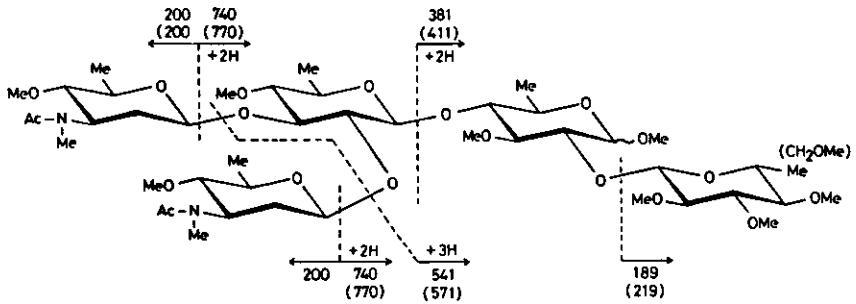


Figure Sequence ions in chemical ionization mass spectra of permethylated viridopentaose A and C

The degradative reactions of 1 by use of the condition(b) and (c) gave tetrasaccharide 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), ^{1}H-NMR(CD_3OD): δ 1.98 ($NHCOCH_3$), 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 moler 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 methanolysis[5]. Accordingly, the anomeric carbon of the non-reducing D-quinovosyl residue should be also in a β-configuration.

By considering glycosidation shift[6] (82.9 ppm at C-2 and 85.8 ppm at C-4 in the reducing D-quinovose moiety), the sterically hindered adjacent diglycosidation[5] (75.7 and 76.0 ppm at C-2' and C-3' in the non-reducing D-quinovose moiety), and the structure of viridopentaose B[1], 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→2)-O-[N-acetyl-β-acosaminopyranosyl-(1→3)]-O-β-D-quinovopyranosyl-(1→4)-O-[β-D-quinovopyranosyl-

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

	1	5	6		2	8	9
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[c]	74.0	74.8	C-2'	76.2[c]	74.0	75.0
C-3'	76.0[c]	86.9[f]	77.2	C-3'	76.7[c]	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 (3)		Methyl quinovoside (4)		Methyl glucoside (7)		Quinovose (10)	
	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.

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