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STRUCTURAL INVESTIGATION OF AN ANTIBIOTIC SPORAVIRIDIN II.[1] APPLICATION OF ^{13}C-NMR TO THE STRUCTURAL ELUCIDATION OF VIRIDOPENTAOSE B

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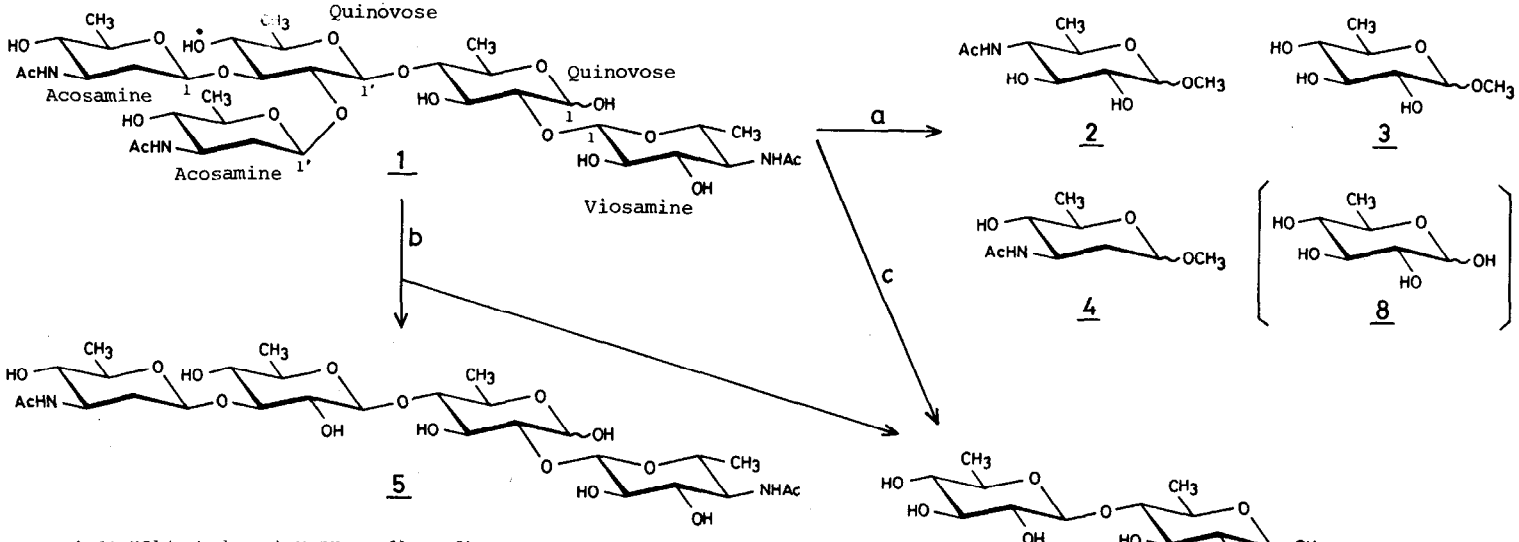
Summary: A new heteropentasaccharide named viridopentaose B was obtained by aqueous ammonia hydrolysis of N-acetylsporaviridin and the structure was established by the detailed analysis of ^{13}C-NMR spectra with those of further degradation products.

Sporaviridin (SVD) is a weakly basic antibiotic produced by *Streptosporangium viridogriseum*[2] and is considered to be a compound containing oligosaccharide moiety as the structural unit. In this paper we would like to report the isolation and structural elucidation of a new heteropentasaccharide, one of the degradation products of SVD, viridopentaose B, by the detailed analysis of ^{13}C-NMR spectra.

N-Acetylsporaviridin (N-Ac-SVD), a derivative obtained by treatment of SVD with acetic anhydride in MeOH, was subjected to mild hydrolysis with 7% NH_4OH. The resulting reaction products were separated by Sephadex LH-20 and silica gel column chromatography to give an aglycone moiety and three pentasaccharides. One of the three pentasaccharides was designated viridopentaose B (1, mp 207-209°(dec.), $C_{36}H_{61}N_3O_{19}\cdot 5H_2O$, $[\alpha]_D^{20}$ -31.7°(c 0.3, MeOH), IR(KBr): 3500-3200 cm^{-1} ($\nu_{OH/NH}$), 1650-1620 cm^{-1} (ν_{CO}), ^{1}H-NMR(CD_3OD): δ 1.95($NHCOCH_3$)), which gave each anomeric pair of methyl 4-acetamide-4,6-dideoxy-D-glucopyranoside(2, methyl N-acetyl-D-viosaminide)[3], methyl 6-deoxy-D-glucopyranoside(3, methyl D-quinovoside)[4], and methyl 3-acetamide-2,3,6-trideoxy-D-*arabino*-hexopyranoside(4, methyl N-acetyl-D-acosaminide)[1] on exhausive methanolysis.

Field desorption (FD) mass spectrum of 1 showed an important cluster ion peak $(M+Na)^+$ at m/z 862, which indicated the molecular weight of 1. Further, chemical ionization (CI) mass spectra of the permethylated viridopentaose B using isobutane and ammonia as reagent gases gave the valuable informations. Thus, a quasi-molecular ion ($M\cdot NH_4^+$) peak was observed at m/z 997 and the fragment ion peaks at m/z 781 and 582 were consistent with tetra- and tri-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
d: 8.0% HCl (aqueous)-MeOH 50°C 8hr

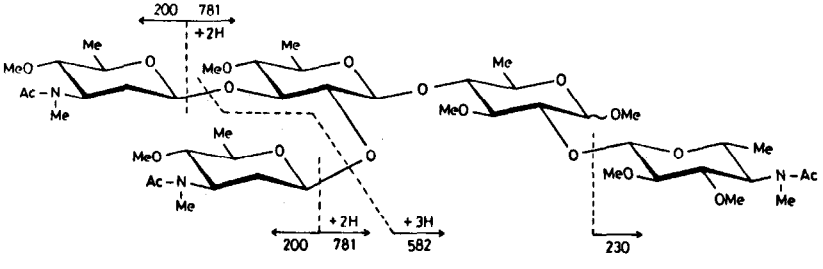


Figure Chemical ionization mass spectrum of permethylated viridopentaose B

Table ^{13}C-NMR chemical shifts of compounds 1 ~ 8 [a]

	1	5	6	7	Methyl N-acetylviosaminide (2) α	Methyl N-acetylviosaminide (2) β
Viosamine						
C-1	105.4	105.2	105.2	105.8	100.8	104.9
C-2	74.9	74.8	74.9	75.1	73.9	75.2
C-3	75.7	75.9	75.9	75.9	72.2	75.5
C-4	58.1	58.0	58.1	58.1	58.1	58.1
C-5	72.3	72.2	72.2	72.3	67.5	72.1
C-6	18.4	18.3	18.3	18.4[d]	18.1	18.2
					Quinovose (8) α	Quinovose (8) β
Quinovose						
C-1	93.4	92.9	92.9	100.9	93.6	97.7
C-2	83.0	81.8	81.8	82.9	73.9	76.3
C-3	73.2	71.9	71.8	73.5	74.5	77.6
C-4	86.0	87.2	87.1	77.0	77.3	76.9
C-5	67.8	66.4	66.4	68.4	68.1	73.1
C-6	18.4	18.0	18.0	18.1[d]	18.1	18.1
					Methyl quinovoside (3) α	Methyl quinovoside (3) β
Quinovose						
C-1′	101.0	104.4	104.4		100.9	105.0
C-2′	76.2[b]	74.1	74.9		73.5	75.0
C-3′	76.9[b]	86.9	77.3		74.7	77.6
C-4′	75.7	74.8	76.4		77.1	76.8
C-5′	73.5	73.0	73.3		68.4	73.1
C-6′	18.4	18.0	18.0		18.0	18.0
					Methyl N-acetylacosaminide (4) α	Methyl N-acetylacosaminide (4) β
Acosamine						
C-1	101.5[c]	102.1			98.7	102.0
C-2	38.1	37.9			36.9	38.1
C-3	52.6	52.3			49.7	52.4
C-4	75.3	75.5			76.5	75.9
C-5	74.9	74.8			69.5	74.6
C-6	18.9	18.3			18.3	18.3
Acosamine						
C-1′	101.9[c]					
C-2′	38.1					
C-3′	52.6					
C-4′	75.3					
C-5′	74.9					
C-6′	18.9					

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 Assignments may be reversed in each vertical column.

The degradative reactions of 1 by use of partial methanolysis conditions yielded tetrasaccharide 5, mp 235-237°(dec.) and trisaccharide 6, mp 216-219° (dec.). The latter was further led to disaccharide 7, mp 136-139°(dec.)(Scheme).

The ^{13}C-NMR chemical shifts of 1 could be assigned by comparison with those of 2, 3, 4, 5, 6, 7, and 8 (Table), as follows. The ^{13}C-NMR spectrum of 1 showed five signals due to anomeric carbons. The resonance at 93.4 ppm represented an anomeric carbon of the reducing D-quinovosyl residue(α-configuration). Three 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 101.0 ppm suggested the presence of the anomeric carbon in an α-configuration at first. However, the 3.4 ppm downfield shifts were observed at C-1 of the non-reducing D-quinovosyl residue in 5 and 6, when the acosamines were removed from 1 by selective methanolysis[5]. Consequently, the anomeric carbon of the non-reducing D-quinovosyl residue should be also in a β-configuration.

By considering glycosidation shift[6](83.0 ppm at C-2 and 86.0 ppm at C-4 in the reducing D-quinovose moiety) and the sterically hindered adjacent diglycosidation[5](76.2 and 76.9 ppm at C-2 and C-3 in the non-reducing D-quinovose moiety), 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.

Finally, it was proved that viridopentaose B was an O-(N-acetyl-β-D-acosaminopyranosyl)-(1→2)-O-[N-acetyl-β-D-acosaminopyranosyl-(1→3)]-O-β-D-quinovopyranosyl-(1→4)-O-[N-acetyl-β-D-viosaminopyranosyl-(1→2)]-α-D-quinovopyranose.

References

1. Part I. "Isolation and determination of D-acosamine from a basic antibiotic, Sporaviridin" K. I. Harada, S. Ito, and M. Suzuki, Carbohyd. Res., in press.
2. T. Okuda, Y. Ito, T. Yamaguchi, T. Furumai, M. Suzuki, and M. Tsuruoka, J. Antibiotics, Ser. A, 19, 85(1966).
3. C. L. Stevens, P. Blumbergs, F. A. Daniher, D. H. Otterbach, and K. G. Tatlor, J. Org. Chem., 31, 2822(1966).
4. M. E. Evans, L. Long, Jr., and F. W. Parrish, J. Org. Chem., 33, 1074(1968).
5. a) N. Yamaoka, T. Usui, H. Sugiyama, and S. Seto, Chem. Pharm. Bull.,(Tokyo), 22, 2196(1974). b) R. U. Lemieux, and H. Driguez, J. Am. Chem. Soc., 97, 4063(1975).
6. a) T. Usui, N. Yamaoka, K. Matsuda, K. Tuzimura, H. Sugiyama, and S. Seto, J. Chem. Soc. Perkin I, 2425(1973). b) P. A. J. Gorin, Carbohyd. Res., 39, 3(1975). c) K. Tori, S. Seo, Y. Yoshimura, M. Nakamura, Y. Tomita, and H. Ishii, Tetrahedron Lett., 4163(1976). d) R. Kasai, M. Suzuo, J. Asakawa, and O. Tanaka, Tetrahedron Lett., 175(1977).

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