STRUCTURAL INVESTIGATION OF THE ANTIBIOTIC SPORAVIRIDIN

X[1]. ISOLATION AND CHARACTERIZATION OF COMPONENTS OF *N*-ACETYLSPORAVIRIDINS

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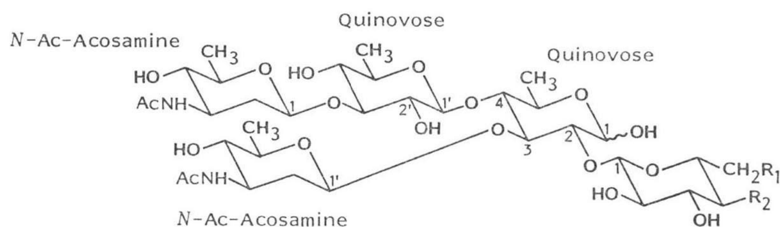
Isolation and characterization of the major components of *N*-acetylsporaviridins, a derivative obtained by treatment of sporaviridins with acetic anhydride in methanol were carried out. The isolation procedure as shown in Scheme 1 gave successfully six components whose molecular weights are all about 2,200. Each component was suggested to be a glycosidic compound consisting of a macrocyclic lactone, one of viridopentaoses, a D-glucose and an *N*-acetyl-L-vancosamine by various spectral data.

Sporaviridin, the first antibiotic produced by *Streptosporangium*, is basic and water-soluble, and exhibits strong inhibitory activity against Gram-positive bacteria, yeast and trichophyton.[2,3] Although various structural studies have been attempted since it was isolated in 1963, its structure remains unsolved. However, it has been considered to be a glycosidic compound in view of its biological properties and preliminary structural characterization. We have recently reported on the isolation, purification and structural determination of the three constituent oligosaccharides of *N*-acetylsporaviridin, designated as viridopentaoses A (**1A**), B (**1B**) and C (**1C**). They were established to be novel heteropentasaccharides containing two or three amino sugars on the basis of chemical degradations and spectroscopic studies.[1,4~6]

Very recently, sporaviridin has been found to be composed of at least six closely related components (hereafter referred to as sporaviridins) by chromatographic studies. In this paper we describe the isolation and characterization of the major components of *N*-acetylsporaviridins.

Isolation and Characterization

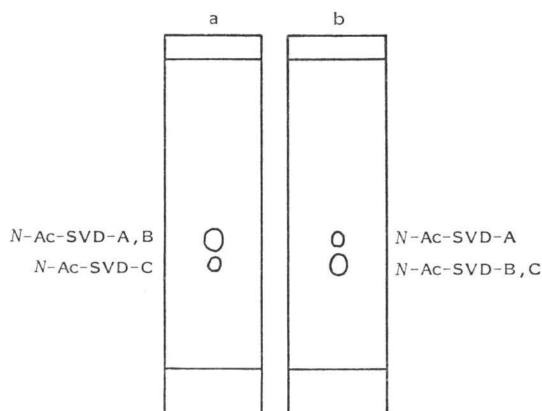
The fermentation and preliminary isolation of sporaviridins, and the taxonomic study of the producing strain have been already reported.[2,3] The sporaviridin complex was obtained as a amorphous pale yellowish powder. Because of its high polarity and instability the isolation of the individual



Viridopentaose A (**1A**) R_1 = H, R_2 = OH
Viridopentaose B (**1B**) R_1 = H, R_2 = NHAc
Viridopentaose C (**1C**) R_1 = OH, R_2 = OH

components as intact bases has not been succeeded. Therefore, we carried out the isolation of the components of *N*-acetylsporaviridins (*N*-Ac-SVD), a derivative obtained by treatment of sporaviridins with acetic anhydride in methanol. The presence of three components can be clearly recognized as indicated by the thin-layer chromatograms of *N*-Ac-SVD using the combination of the two solvent systems (Fig. 1). These components were named as *N*-Ac-SVD-A, B and C. They were separated by repeated column chromatographies on silica gel (Scheme 1) and were considered to be completely isolated. However, their analytical high performance liquid chromatograms using reversed-phase ODS silica gel column indicate that each consists of further two components (Fig. 2). These two components were isolated by repeated preparative HPLC as shown in Scheme 1. Finally, each component was further purified by column chromatography on Sephadex LH-20. Thus, the isolation procedure gave six components of *N*-Ac-SVD (*N*-Ac-SVD-A_1, A_2, B_1, B_2, C_1 and C_2) as single entities in sufficient quantities for characterization. But it is hard to separate directly these six components by only prepartive HPLC.

Fig. 1. Thin-layer chromatograms of *N*-acetylsporaviridins.
a: $CHCl_3$ - MeOH - H_2O (65: 35: 10) (lower layer).
b: EtOAc - 2-PrOH - H_2O (4: 3: 7) (upper layer.



The physico-chemical properties of the thus obtained *N*-Ac-SVD are summarized in Table 1. All components were obtained as amorphous white powder. They are closely similar one another

Scheme 1. Isolation procedure for *N*-acetylsporaviridins.
* HPLC condition: packing; Megapak SIL C_{18} (7.2×250 mm), solvent; CH_3CN - H_2O (7: 3), detection; UV 232 nm, flow rate; A 5.5 ml/minute, B 4.5 ml/minute, C 3.0 ml/minute.

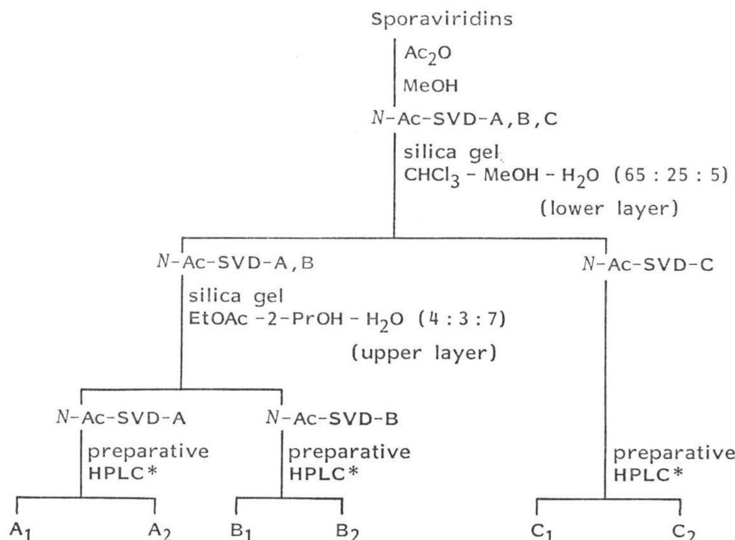


Fig. 2. High performance liquid chromatograms of *N*-Ac-SVD-A, B and C.
Packing; Finepak SIL C_{18} (4.6×250 mm), flow rate; 2.5 ml/minute, solvent; CH_3CN - H_2O (7: 3), detection; UV 232 nm.

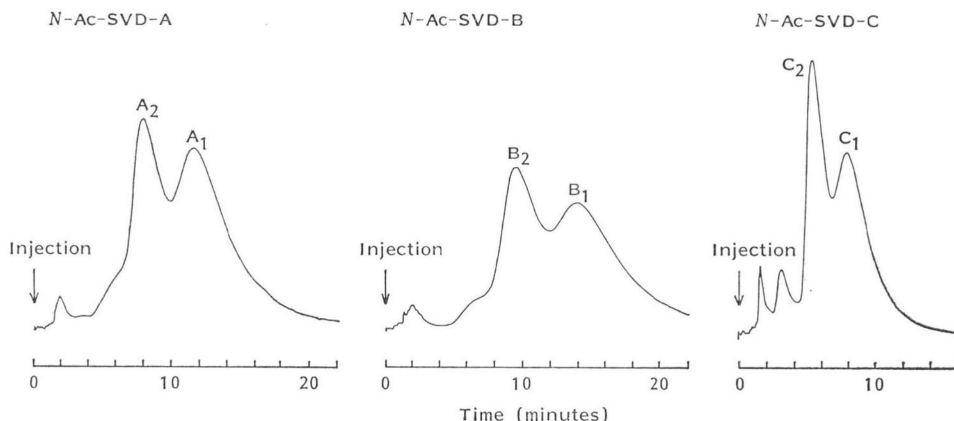
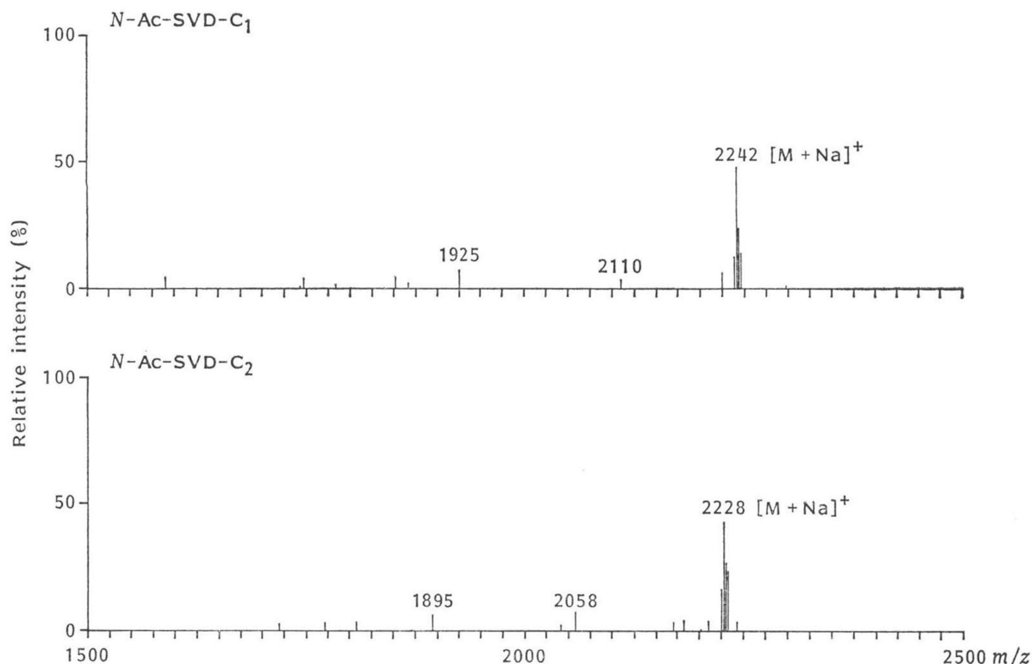


Fig. 3. Molecular secondary ion mass spectra of *N*-Ac-SVD-C_1 and C_2.

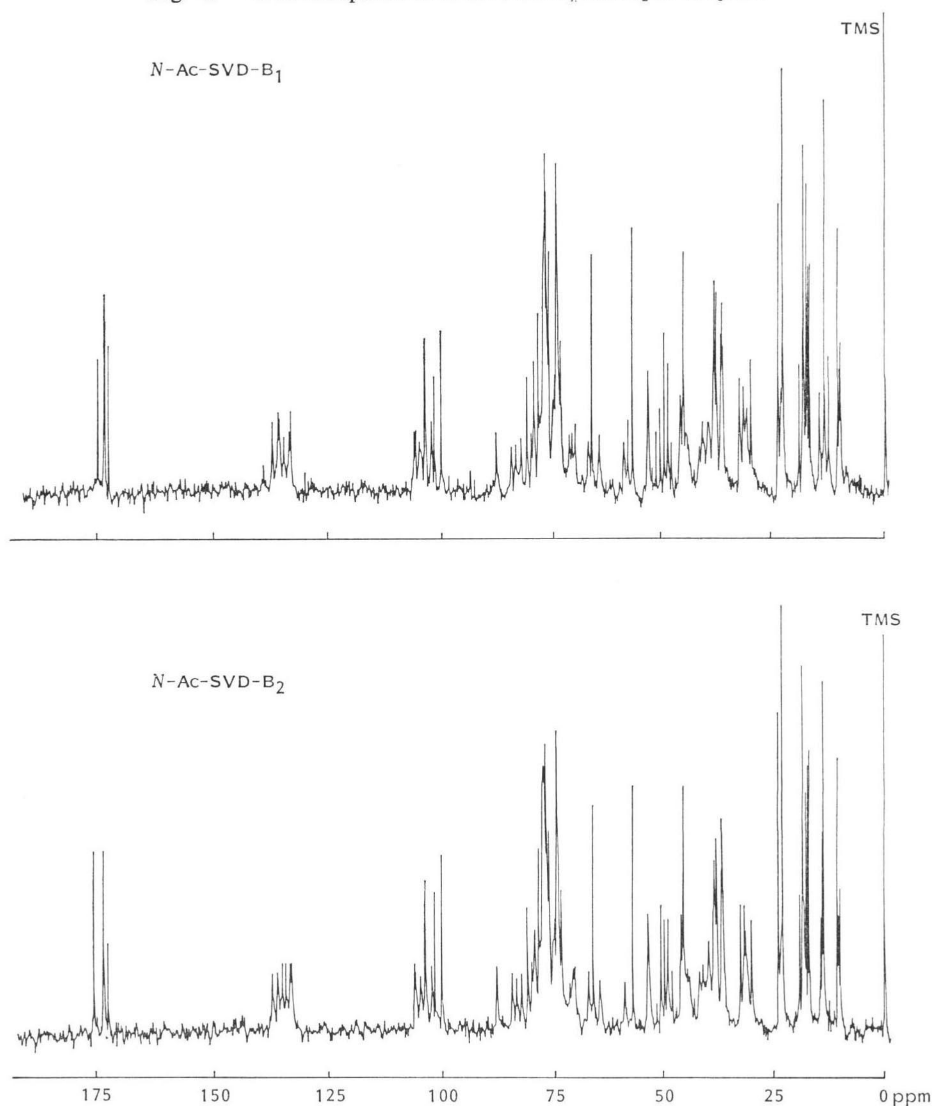


except for their molecular weights. Although no molecular ion species were observed using glycerol matrix alone in molecular secondary ion (SI) mass spectra of these components, addition of NaCl gave reproducible results. Fig. 3 shows the molecular ion regions in the molecular SI mass spectra of *N*-As-SVD-C_1 and C_2 as representative examples. The sodium addition ions, $[M+Na]^+$ appear at *m/z* 2,242 and 2,228, indicating that the molecular weights of *N*-Ac-SVD-C_1 and C_2 are 2,219 and 2,205, respectively. Analogously, the molecular weights of other components were determined by $[M+Na]^+$ ions in their molecular SI mass spectra (Table 1).

Table 1. Physico-chemical properties of *N*-acetylsporaviridins.

	A_1	A_2	B_1	B_2	C_1	C_2
Appearance	White powder	White powder	White powder	White powder	White powder	White powder
Mp (dec) (°C)	162~165	162~166	168~171	163~166	169~173	169~173
Anal (%) Found C	52.99	52.92	52.35	52.53	52.65	52.57
H	8.63	8.63	8.61	8.60	8.52	8.55
N	1.75	1.69	2.32	2.19	1.74	1.75
SIMS, m/z $[M+Na]^+$	2,226	2,212	2,267	2,253	2,242	2,228
$[\alpha]_D$ in MeOH	−18.5° (*c* 0.40)	−19.6° (*c* 0.28)	−25.0° (*c* 0.56)	−23.2° (*c* 0.81)	−24.9° (*c* 0.90)	−19.0° (*c* 0.90)
UV λ_{max}^{EtOH} nm (log ε)	232 (4.48)	232 (4.75)	232 (4.63)	232 (4.54)	232 (4.50)	232 (4.42)
IR ν_{max}^{KBr} cm^{-1}	3700~3000, 1705, 1630	3700~3000, 1700, 1630	3700~3050, 1705, 1630	3700~3050, 1705, 1630	3700~3050, 1700, 1630	3700~3050, 1700, 1630
^1H NMR (100 MHz, CD_3OD) (ppm)	1.98, 1.90	1.98, 1.90	1.98, 1.90	1.98, 1.90	1.98, 1.90	1.98, 1.90

Fig. 4. ^{13}C NMR spectra of *N*-Ac-SVD-B_1 and B_2 in CD_3OD.



The UV spectra of all components show maximums at 232 nm, which suggest the presence of a conjugated diene system in their molecules. The three characteristic bands, OH and NH groups at 3700 ~ 3000 cm^{-1} (very strong), ester carbonyl group at 1700 cm^{-1} and amide carbonyl group at 1630 cm^{-1} are observed in the IR spectra. The ^{1}H NMR spectra (100 MHz, CD_3OD) are very complicated and only three or four methyl signals due to $NHCOCH_3$ groups can be assigned.

Fig. 4 shows the ^{13}C NMR spectra of *N*-Ac-SVD-B_1 and B_2 as typical examples. Each ^{13}C NMR spectrum contains approximate 100 signals, including four or five carbonyl, six olefinic, seven anomeric, one hemiketal and three or four glycosidation-shifted carbons. The assignable signals are summarized in Table 2. Moreover, many signals (60 ~ 80 ppm; oxymethine and oxymethylene, 25 ~ 50 ppm: methine and methylene, 10 ~ 25 ppm: methyl carbons) are observed in the upper field regions but it is almost impossible to assign these signals at present. The similar spectral behavior is shown in the

Table 2. ^{13}C NMR spectral data of *N*-acetylsporaviridins.

	C=O	-C=C-	Anomeric	Hemiketal	*C*-O-gly
A_1	175.0, 173.3, 173.3, 172.6	136.0, 134.8, 134.3, 133.3, 132.2, 131.8	104.7, 104.4, 103.3, 102.0×2, 100.7, 98.5	99.8	86.3, 81.9, 80.7
A_2	175.9, 173.6, 173.5, 172.7	136.1, 134.8, 133.9, 133.0, 132.2, 131.7	104.7, 104.3, 103.3, 102.1×2, 100.7, 98.5	99.9	86.4, 82.7, 80.8
B_1	175.1, 173.6, 173.6, 173.4, 172.6	136.0, 134.8, 134.4, 133.5, 132.2, 131.9	104.6, 104.1, 103.4, 102.0×2, 100.7, 98.5	99.9	86.2, 82.8, 80.7
B_2	175.8, 173.6, 173.5, 173.4, 172.6	136.0, 134.8, 133.8, 133.0, 132.0, 131.6	104.7, 104.2, 103.2, 102.0×2, 100.6, 98.4	99.9	86.2, 82.7, 81.8, 80.6
C_1	175.1, 173.6, 173.3, 172.6	136.1, 134.7, 134.3, 133.3, 132.2, 131.7	104.6, 104.1, 103.1, 102.0×2, 100.6, 98.4	99.7	86.2, 83.4, 81.1, 80.6
C_2	175.7, 173.5, 173.3, 172.5	136.0, 134.7, 133.7, 132.9, 132.0, 131.7	104.6, 104.1, 103.1, 102.2×2, 100.4, 98.4	99.7	86.2, 83.3, 81.1, 80.0

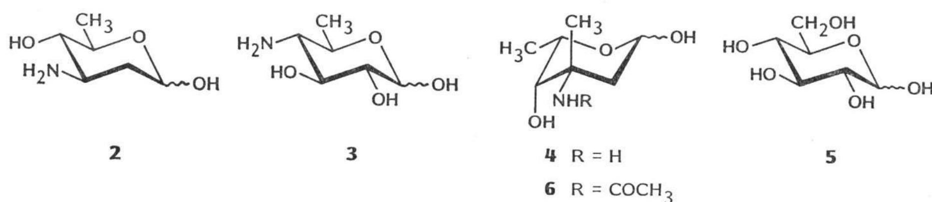
A and C series. The resonances in the lower field regions are also listed in Table 2.

Unfortunately, antimicrobial activity of all isolated components of *N*-Ac-SVD was greatly reduced in comparison with that of sporaviridins. This may be caused by the disappearance of the basicity on acetylation of amino groups in the carbohydrate moieties.

Structural Consideration

As mentioned above, each component is closely related one another and a glycosidic compounds containing seven monosaccharide units. There are three constituent amino sugars, D-acosamine (**2**), D-viosamine (**3**) and L-vancosamine (**4**) in their molecules.[4] Only primary amino groups in these amino sugars were acetylated under the conditions used in this study. Additionally, it was demonstrated viridopentaoses A, B and C correspond to *N*-Ac-SVD-A, B and C, respectively, and a D-glucose (**5**) and an *N*-acetyl-L-vancosamine (**6**) are common to all components by their degradative experiments.[7] Therefore, the carbohydrate moiety is composed of one of three viridopentaoses (**1**), a D-glucose (**5**) and an *N*-acetyl-L-vancosamine (**6**). On the other hand, the aglycone moiety has an ester or a lactone group and a hemiketal system. Two of the three double bonds form a conjugated diene in the aglycone moiety. These results suggest that *N*-Ac-SVD is a glycosidic compound consisting of a macrocyclic lactone as an aglycone.

Although several antibiotics possessing macrocyclic lactone above 30-membered ring such as copiamycin,[8] neocopiamycin,[9] scopafungin,[10] niphimycin,[11,12] azalomycin[13~16] and monazomycin[17] have hitherto been reported, no carbohydrate moieties are contained in their molecules except for monazomycin in which only one mannose is present. Recently, IKEMOTO *et al.* have reported the isolation of a sporaviridin-like antibiotic, aculeximycin from the fermentation broth of *Streptospo-*



rangium albidum.[18,19] However, these two antibiotics were shown to be clearly differentiated from each other by direct comparison.[19] Consequently, sporaviridins are not related to known antibiotic families.

At present, the further elucidation for the total structures of sporaviridins is in progress.

Experimental

General

Melting points were determined on a micro-melting point apparatus (hot-stage type, Yanagimoto MP-S3) and uncorrected. Optical rotations were measured with a Jasco DIP-181 polarimeter. IR and UV spectra were determined on a Hitachi IR-215 spectrometer and a Hitachi 200-10 double beam spectrophotometer, respectively. ^{1}H NMR and ^{13}C NMR spectra were measured on a Jeol JNM FX-100 (^{1}H: 100 MHz, ^{13}C: 25 MHz) spectrometer using tetramethylsilane (TMS) as an internal standard. SI mass spectra were obtained using a Hitachi M-80A mass spectrometer. Operating conditions were as follows: primary ions; Xe^{+}, accelerating voltage; 8 kV (primary) and 3 kV (secondary). HPLC was carried out on a Shimadzu LC-3A and Jasco Trirotar II systems with a Shimadzu SPD-2A and a Jasco Uvidec-100-II UV spectrophotometers as detectors, respectively. The separations were performed on a Jasco Finepak SIL C_{18} (analytical) and a Jasco Megapak SIL C_{18} (preparative). TLC was carried out on Merck pre-coated plates (Kieselgel 60 F_{254}). For column chromatography, Merck Kieselgel 60 (Art. 7734 and 7729) and Sephadex LH-20 (Pharmacia) were used.

N-Acetylation of SVD

A solution of sporaviridins (7 g) in dry MeOH (70 ml) was treated with acetic anhydride (35 ml). The reaction mixture was stirred overnight at room temperature and then was concentrated to dryness to yield crude *N*-Ac-SVD as a pale yellow powder (9.3 g). The crude *N*-Ac-SVD was separated as shown in Scheme 1.

Finally, each component was purified by column chromatography on Sephadex LH-20 to yield *N*-Ac-SVD-A_1, 376 mg; A_2, 546 mg; B_1, 230 mg; B_2, 222 mg; C_1, 418 mg; C_2, 625 mg.

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