STRUCTURAL ELUCIDATION OF ACULEXIMYCIN

I. FURTHER PURIFICATION AND GLYCOSIDIC BOND CLEAVAGE OF ACULEXIMYCIN

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A new insecticidal antibiotic, aculeximycin (ACM), was produced by an actinomycete identified as *Streptosporangium albidum*. ACM has been successfully isolated from culture filtrate by a combination of Diaion HP-20, Amberlite CG-50, reversed phase silica gel and Sephadex LH-20 chromatographies. It was found that ACM is a basic glycosidic antibiotic with a molecular weight of 1,672 including five monosaccharide units, three double bonds and a hemiketal ring by preliminary spectral analyses.

Treatment of ACM with 1,8-diazabicyclo[5,4,0]undecene-7 caused a glycosidic bond cleavage to give aculexitriose, pseudoaglycones I and II.

A screening for insecticidal antibiotics using mosquito larvae as a test organism led to the isolation of aculeximycin (ACM, **1**) which was found in the culture broth of an actinomycete identified as *Streptosporangium albidum*. ACM, which is a basic and water soluble compound, shows activity against insects, bacteria, yeast, and molds *in vitro* at low concentration. Because its biological properties and preliminary spectroscopic data are similar to those of a basic glycosidic antibiotic, sporaviridin (SVD), it is presumed that the structure of ACM is similar to that of SVD[1]. Although an isolation procedure for ACM had been reported by IKEMOTO *et al.*[2], it did not always give reproducible results and so it was necessary to improve the conventional procedure.

This paper deals with the isolation of ACM by the improved procedure, characterization of the purified ACM, and specific glycosidic bond cleavage of ACM using an organic base which had been successfully applied to the structure determination of SVD[3].

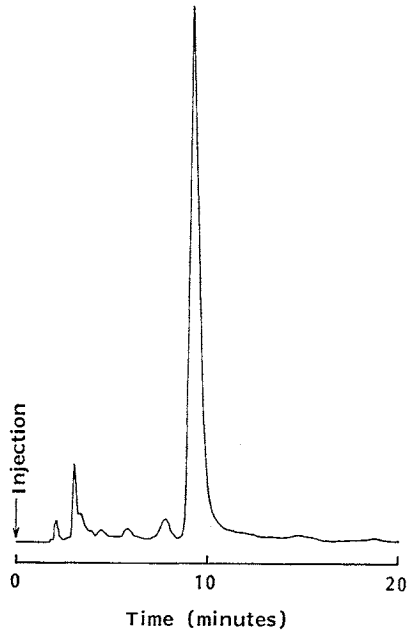
Isolation of ACM

In the conventional method, neutralized broth filtrate was adsorbed on a Diaion HP-20 column. The column was washed with deionized water followed by 50% aqueous acetone and the antibiotic was finally eluted with acidic 50% aqueous acetone (adjusted to pH 2 with hydrochloric acid). In this time purification of ACM was carried out by the conventional method, however, ACM was eluted with both neutral and acidic 50% aqueous acetone. These fractions contained brownish contaminants,

which could not be eliminated by the conventional procedure. So the active fraction was adjusted to pH 7.0 and lyophilized to give a brownish powder including approximately 11% pure ACM.

In order to remove efficiently the contaminants, Amberlite CG-50 (NH_4^+ form) was used. The powder was dissolved in distilled water and was adsorbed on Amberlite CG-50. The brownish contaminants were completely washed out with distilled water and then ACM was eluted with 0.05 N ammonia water. It should be here noted that the resultant eluate must be neutralized immediately because ACM is labile under basic conditions. Subsequently, the neutralized solution was adsorbed on reversed phase C_{18} silica gel (ODS). It is important to eliminate unnecessary contaminants as much as possible in this step. After washing inorganic salts and polar compounds with water and unfavorable degradation products of ACM with water - meth-

Fig. 1. HPLC of aculeximycin (**1**).



Column: Nucleosil $5C_{18}$ (4.6 mm i.d. × 150 mm), mobile phase: MeOH - 1 N aq NH_4Cl (70 : 30), flow rate: 1 ml/minute, detection: UV 220 nm.

Scheme 1. Improved isolation procedure for aculeximycin (ACM, **1**).

Broth

↓ adjusted to pH 6.8
Celite 12 kg, filtered

Broth filtrate

↓ adsorbed on Diaion HP-20
washed with water
eluted with 50% aqueous acetone
and acidic 50% aqueous acetone (pH 2, HCl)
neutralized
concentrated and lyophilized

Active fraction (brownish powder)

↓ adsorbed on Amberlite CG-50 (NH_4^+ form)
washed with water
eluted with 0.05 N NH_4OH
neutralized with 1 N HCl
adsorbed on silica gel ODS-W
washed with water and H_2O - MeOH (1 : 1 and 1 : 2)
eluted with MeOH
concentrated to dryness
chromatographed on Sephadex LH-20 eluted
with MeOH

ACM (**1**)

↓ treatment with Ac_2O - anhydrous MeOH
chromatographed on silica gel eluted
with $CHCl_3$ - MeOH - H_2O (65 : 35 : 5)
chromatographed on Sephadex LH-20 eluted
with MeOH

N-Acetylaculeximycin (**2**)

anol (1:2), ACM was finally eluted with methanol. The desired fraction was concentrated under reduced pressure and chromatographed on a Sephadex LH-20 column with methanol. The resulting ACM shows a single peak by HPLC (Fig. 1). The improved isolation procedure is summarized in Scheme 1. The new method is reproducible, facile and not time consuming when compared with the conventional one.

Physico-chemical Properties of ACM

ACM thus obtained is a basic, and white amorphous powder. It gives a protonated molecule $(M+H)^+$ at m/z 1,673 by secondary ion mass spectrometry (SI-MS). The molecular formula was estimated to be $C_{81}H_{144}N_2O_{33}$ based on mass and ^{13}C NMR spectral analyses of its degradation products. This was confirmed by high-resolution fast atom bombardment (HRFAB) mass measurement of the $(M+H)^+$ of ACM. The IR spectrum exhibits broad strong bands at 3600~3100 cm^{-1} due to multiple hydroxyl groups, and a characteristic band at 1680 cm^{-1} due to a carbonyl group. An absorption maximum at 218 nm (log ε 3.99) in its UV spectrum indicates the presence of a conjugated α,β-unsaturated carbonyl chromophore.

The ^{13}C NMR spectrum of ACM (Fig. 2) contains many peaks including a carbonyl carbon (168.7 ppm), six olefinic carbons (141.4, 140.5, 136.7, 134.0 and 123.2 ppm), five anomeric carbons (104.8, 103.6, 103.2, 102.2 and 95.2 ppm), and a hemiketal carbon (99.6 ppm). The ^1H NMR spectrum (Fig. 3) reveals the presence of four olefinic protons at δ 6.80 (t, J=6.9 Hz), 5.61 (dd, J=15.4 and 8.5 Hz), 5.51 (dd, J=15.4 and 4.9 Hz) and 5.45 (t, J=7.8 Hz), and two methyl protons at 1.52 and 1.58 ppm. The ^{13}C and ^1H NMR spectra showed that ACM possesses three double bonds, one of which is an isolated disubstituted double bond whose geometry was determined to be E by the coupling constant (J=15.4 Hz), and the remaining two are trisubstituted double bonds, one of which was in-

Fig. 2. ^{13}C NMR spectrum (25 MHz) of aculeximycin (1) in CD_3OD.

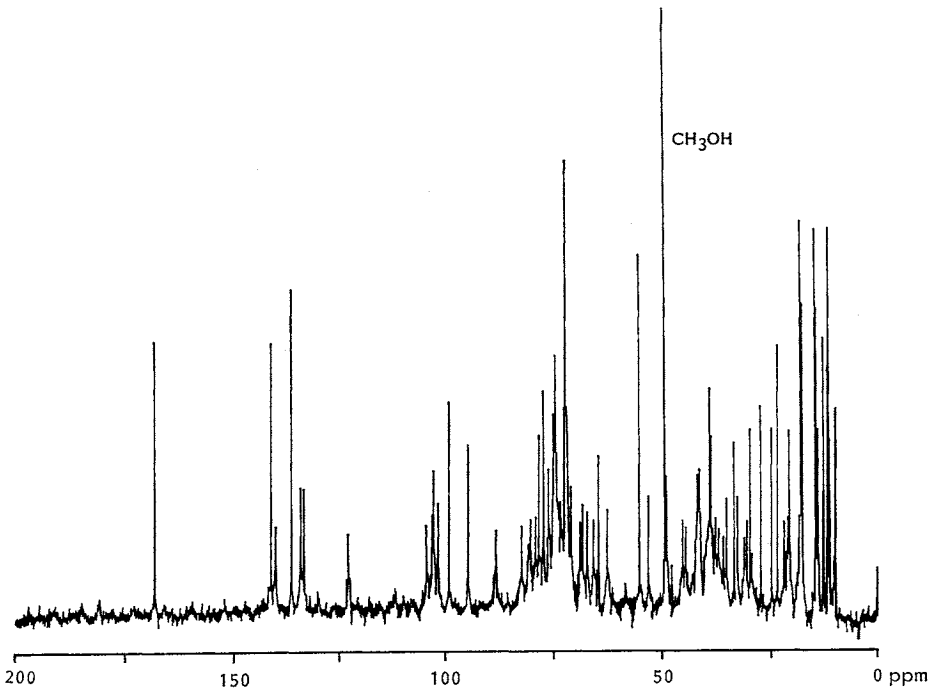


Fig. 3. ^{1}H NMR spectrum (400 MHz) of aculeximycin (**1**) in CD_3OD.

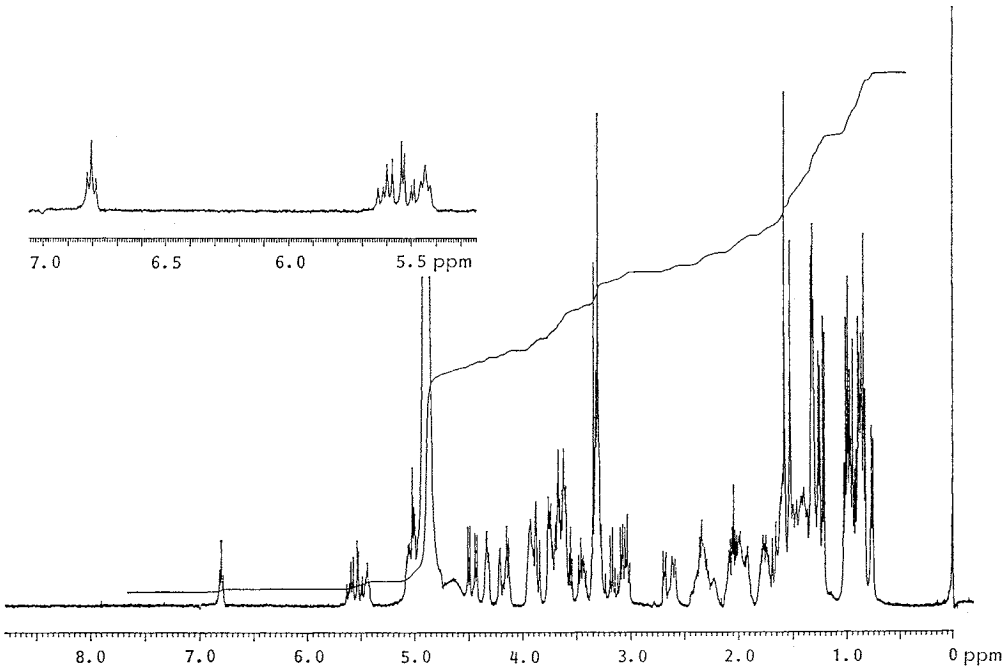


Table 1. Physico-chemical properties and spectral data of aculeximycin (**1**) and *N*-acetylaculeximycin (**2**).

	1	**2**
Nature	Basic, water soluble, amorphous white powder	Water soluble, amorphous white powder
$[\alpha]_D^{24}$	+1.75° (*c* 0.4, MeOH)	+26.32° (*c* 0.1, MeOH)
MP (°C, dec)	184~188	170~172
UV λ_{max}^{EtOH} nm (log ε)	218 (3.99)	218 (3.79)
IR (KBr) cm^{-1}	3600~3100, 1680, 1640	3600~3100, 1680, 1640
SI-MS (*m/z*)	1,673 $(M+H)^+$	1,779 $(M+Na)^+$
MW	1,672	1,756
HRFAB-MS	Calcd for $C_{81}H_{145}N_2O_{33}$: 1,673.9689	Calcd for $C_{85}H_{148}N_2O_{35}Na$: 1,779.9719
	Found: 1,673.9660	Found: 1,779.9750
^{13}C NMR (ppm)	168.7[a], 141.4[a], 140.5, 136.7[a], 134.6, 134.0, 123.3, 104.8, 103.6, 103.2, 102.2, 99.6[a], 95.2	173.6[a], 172.6[a], 168.7[a], 141.3[a], 140.3, 136.6[a], 134.4, 133.9, 123.3, 104.8, 103.0, 103.0, 102.6, 99.5[a], 96.1, 23.7, 23.6
^{1}H NMR (ppm)	6.80 (1H, t, J=7.0 Hz), 5.61 (1H, dd, J=12.8, 8.0 Hz), 5.51 (1H, dd, J=12.8, 5.0 Hz), 5.45 (1H, t, J=7.0 Hz)	6.80 (1H, t, J=7.0 Hz), 5.61 (1H, dd, J=12.8, 8.0 Hz), 5.51 (1H, dd, J=12.8, 5.0 Hz), 5.45 (1H, t, J=7.0 Hz), 1.90 (3H, s), 1.80 (3H, s)

[a] Quaternary carbon.

cluded in the α,β-unsaturated carbonyl moiety.

Acetylation of ACM in anhydrous methanol with acetic anhydride gave *N*-acetylaculeximycin (*N*-Ac-ACM, **2**) whose molecular weight was found to be 1,756 by SI-MS. This result demonstrates that ACM possesses two amino groups. Its molecular formula $C_{85}H_{148}N_2O_{35}$ was established by

HRFAB-MS. The ^1H NMR spectrum revealed also two acetyl methyl protons at 1.80 and 1.90 ppm and the ^{13}C NMR spectrum displayed two amide carbonyl carbon signals at 173.6 and 172.6 ppm, and acetyl methyl carbon signals at 23.6 and 23.7 ppm. The physico-chemical properties and spectral data of ACM and *N*-Ac-ACM are summarized in Table 1.

From these results, ACM is considered to be a basic glycosidic antibiotic with a molecular weight of 1,672 and possesses five sugars including two amino sugars, three double bonds, and a hemiketal ring. Therefore it seems that ACM has a structural resemblance to SVD obtained from the culture broth of *Streptosporangium viridogriseum*.

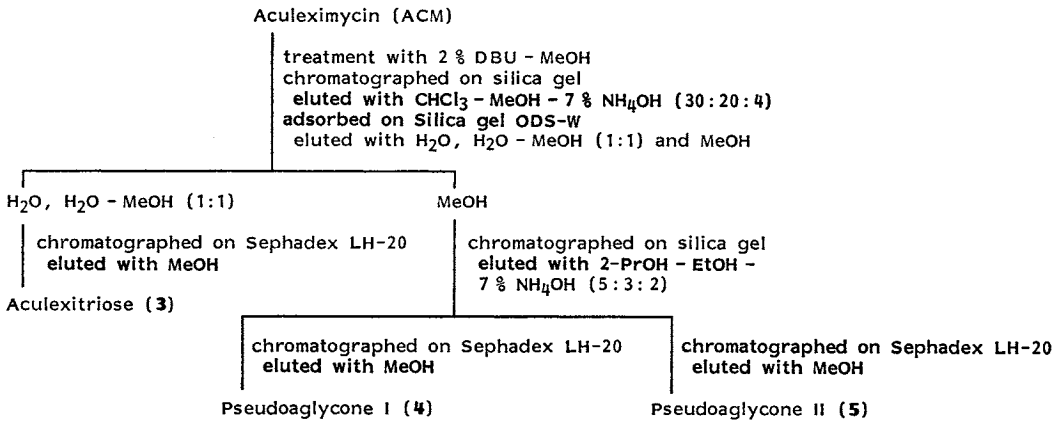
Glycosidic Bond Cleavage of ACM

During the structure elucidation of SVD, treatment of *N*-Ac-SVD with 1,8-diazabicyclo[5,4,0]undecene-7 (DBU) cleaved a glycosidic bond to give a constituent pentasaccharide, viridopentaose and an epimeric pair of pseudoaglycones. These degradation products were very useful for the elucidation of the total structure[3]. In this time a slightly modified condition was applied to ACM for the specific glycosidic bond cleavage.

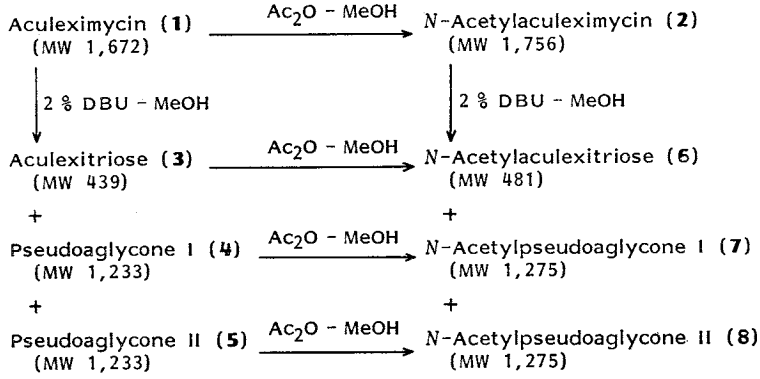
Treatment of ACM with 2% DBU - methanol yielded three products, one of which gave a characteristic carbohydrate color reaction (green) with 1% cerium(IV) sulfate 10% sulfuric acid on TLC (chloroform - methanol - 7% ammonia water (30 : 20 : 4)). As the remaining two products had still an UV absorption, they were considered to be aglycone moieties. The reaction mixture was chromatographed on a silica gel column to remove the reagent and then was adsorbed on reversed phase silica gel. The carbohydrate moiety was eluted with water and 50% aqueous methanol, and then the aglycone moieties were eluted with methanol. The aglycone moieties were further separated by silica gel chromatography (2-propanol - ethanol - 7% ammonia water (5 : 3 : 2)) to give each component. They were finally applied on a Sephadex LH-20 column with methanol (Scheme 2). Since the resulting carbohydrate moiety was found later to be a trisaccharide, it was designated aculexitriose (**3**). On the other hand, both aglycone moieties were tentatively named pseudoaglycones I (**4**) and II (**5**), because they were shown to be aglycones still possessing two monosaccharide units (Scheme 3).

Compound **3**, white amorphous powder, gave an $(M+H)^+$ ion at *m/z* 440 by SI-MS, indicating that its molecular weight is 439. The ^1H NMR spectrum was very complicated and the presence of

Scheme 2. Isolation procedure for aculexitriose (**3**), pseudoaglycones I (**4**) and II (**5**).



Scheme 3. Degradation scheme for aculeximycin (1).



methyl, methylene and oxymethine groups was recognized. Although the ^{13}C NMR spectrum taken in methanol-d_4 showed mainly 18 signals, several small signals were also found. In the anomeric carbon region, two signals at 93.5 ppm (major) and 96.3 ppm (minor) due to the reducing units were observed together with the other signals, 105.5 and 101.7 ppm (major), and 104.7 and 103.1 ppm (minor). The appearance of these signals suggested that the trisaccharide has a reducing end and the reducing unit is mainly in the α-form. Acetylation of aculexitriose (**3**) with acetic anhydride in anhydrous methanol gave *N*-acetylaculexitriose (**6**). Compound **6** showed $(M+H)^+$ and $(M+Na)^+$ ions at *m/z* 482 and 504, respectively by SI-MS. The molecular formula $C_{20}H_{35}NO_{12}$ was established by HRFAB-MS. The ^1H NMR spectrum revealed one acetyl methyl proton at 1.97 ppm and the ^{13}C NMR spectrum also displayed one amide carbonyl signal at 173.6 ppm and an acetyl methyl carbon signal at 22.9 ppm. The B/E linked scan spectra from the $(M+H)^+$ ions of **3** and **6** suggested the molecular weight of each constituent sugar (Fig. 4). The daughter ions of **3** were classified into two type ions due to glycosidic bond cleavage. The ions at *m/z* 422, 276 and 130 were oxonium type ions of trisaccharide, disaccharide and monosaccharide units, and the ions at *m/z* 294 and 148 were protonated ions of disaccharide and monosaccharide untis, respectively (Fig. 4B). The daughter ions of **6** were shifted upward by 42 amu in comparison with those of **3** (Fig. 4D). The results indicated that all daughter ions possess an amino sugar moiety. From these data, aculexitriose is a trisaccharide which is composed of two neutral sugars with a molecular weight of 164 and one amino sugar with a molecular weight of 147. Physico-chemical properties and spectral data of **3** and **6** are given in Table 2.

Physico-chemical properties and spectral data of pseudoaglycones I and II are summarized in Table 3. The molecular weights of the pseudoaglycones I and II were 1,233 because both gave $(M+H)^+$ ions at *m/z* 1,234 by SI-MS. These molecular formula were established to be $C_{63}H_{111}NO_{22}$ by HRFAB-MS. Their ^{13}C NMR spectra contain many peaks including a carbonyl carbon (I: 168.0 ppm, II: 168.5 ppm), six olefinic carbons (I: 141.5, 138.4, 137.5, 134.7, 133.7 and 122.9 ppm, II: 141.3, 138.5, 137.0, 134.6, 134.1 and 123.0 ppm) and two anomeric carbons (I: 103.7 and 95.3 ppm, II: 103.1 and 95.3 ppm). The ^1H NMR spectra reveal four olefinic proton signals (6.7~5.3 ppm). The UV spectra showed an absorption maximum at 218 nm (I: log 3.82, II: log 3.95) due to an α, β-unsaturated carbonyl chromophore. Treatment of the pseudoaglycones I and II with acetic anhydride in anhydrous methanol gave *N*-acetylpseudoaglycones I (**7**) and II (**8**), respectively. The molecular weights of **7** and **8** were found to be 1,275, because they gave $(M+Na)^+$ ions at *m/z* 1,298 by SI-MS. These

Fig. 4. SI-MS of **3** with glycerol (A), B/E linked scan spectrum of $(M+H)^+$ at *m/z* 440 from **3** (B), SI-MS of **6** with glycerol (C), B/E linked scan spectrum of $(M+H)^+$ at *m/z* 482 from **6** (D).

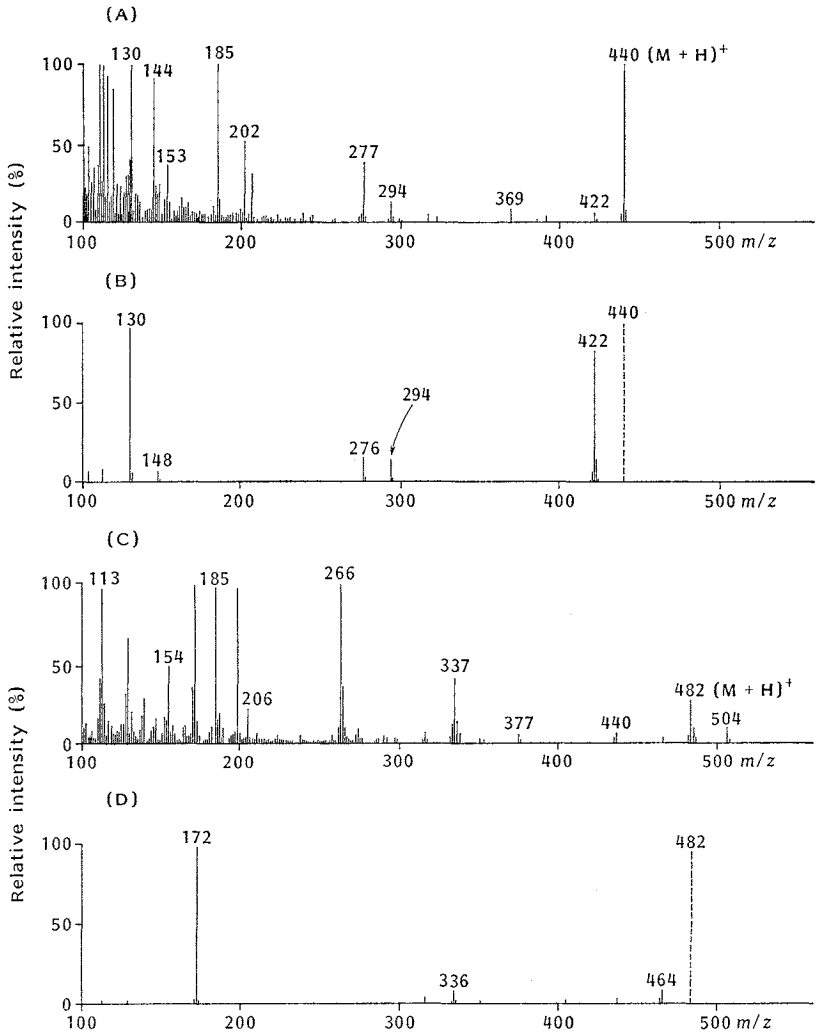


Table 2. Physico-chemical properties of aculexitriose (**3**) and *N*-acetylaculexitriose (**6**).

	3	**6**
Appearance	Amorphous white powder	Amorphous white powder
$[\alpha]_D^{24}$ (*c* 0.1, MeOH)	+12.9°	+16.5°
MP (°C)	198~200	162~166
IR (KBr) cm^{-1}	3600~3100	3600~3100, 1640
SI-MS (*m/z*)	440 $(M+H)^+$	482 $(M+H)^+$
MW	439	481
HRFAB-MS		Calcd for $C_{20}H_{36}O_{12}N$: 482.2227 Found: 482.2230
^{13}C NMR (ppm)	105.5, 101.7, 93.5, 83.4, 80.3, 77.4, 76.9, 75.7, 75.1, 74.5, 73.4, 73.1, 67.8, 52.5, 36.2, 18.3, 18.3, 18.2	173.6, 105.7, 102.4, 93.7, 83.5, 80.3, 77.6, 76.8, 76.2, 75.9, 75.1, 74.8, 73.1, 67.8, 83.3, 37.6, 22.9, 18.3, 18.2, 18.2

Table 3. Physico-chemical properties of pseudoaglycones I (**4**) and II (**5**).

	4	**5**
Appearance	Amorphous white powder	Amorphous white powder
$[\alpha]_D^{24}$ (*c* 0.1, MeOH)	+8.12°	+2.67°
MP (°C)	160~163	166~172
UV λ_{max}^{EtOH} nm (log ε)	218 (3.82)	218 (3.95)
IR (KBr) cm^{-1}	3600~3100, 1680, 1640	3600~3100, 1680, 1640
SI-MS (*m/z*, $(M+H)^+$)	1,234	1,234
MW	1,233	1,233
HRFAB-MS	Calcd for $C_{63}H_{112}NO_{22}$: 1,234.7645 Found: 1,234.7630	Calcd for $C_{63}H_{112}NO_{22}$: 1,234.7645 Found: 1,234.7720
^{13}C NMR (ppm)	168.0[a], 141.5[a], 138.4, 137.5[a], 134.7, 133.7, 122.9, 103.7, 101.0[a], 95.3	168.5[a], 141.3[a], 138.5, 137.0[a], 134.6, 134.1, 123.0, 103.1, 100.0[a], 95.3

[a] Quaternary carbon.

Table 4. Physico-chemical properties of *N*-acetylpseudoaglycones I (**7**) and II (**8**).

	7	**8**
Appearance	Amorphous white powder	Amorphous white powder
$[\alpha]_D^{24}$ (*c* 0.1, MeOH)	+18.13°	+15.43°
MP (°C)	148~152	152~155
UV λ_{max}^{EtOH} nm (log ε)	218 (3.98)	218 (4.07)
IR (KBr) cm^{-1}	3600~3100, 1680, 1640	3600~3100, 1680, 1640
SI-MS (*m/z*, $(M+Na)^+$)	1,298	1,298
MW	1,275	1,275
HRFAB-MS	Calcd for $C_{65}H_{114}NO_{23}$: 1,276.7750 Found: 1,276.7760	Calcd for $C_{65}H_{114}NO_{23}$: 1,276.7750 Found: 1,276.7720
^{13}C NMR (ppm)	172.6[a], 167.9[a], 141.3[a], 138.3, 137.3[a], 134.6, 133.6, 122.9, 103.5, 100.9[a], 96.3	172.7[a], 168.5[a], 141.3[a], 138.5, 137.0[a], 134.6, 134.1, 123.0, 103.1, 100.0[a], 96.3

[a] Quaternary carbon.

molecular formula were established to be $C_{65}H_{113}NO_{23}$ by HRFAB-MS. The ^1H NMR spectra of **7** and **8** revealed the presence of an acetyl methyl proton at 1.91 and 1.90 ppm, respectively and the ^{13}C NMR spectra exhibited an amide carbonyl carbon (**7**: 172.6 ppm, **8**: 172.7 ppm). By the same procedure used for ACM, treatment of *N*-Ac-ACM with 2% DBU - methanol gave **6**, **7** and **8** (Scheme 3).

The data mentioned above suggest that the pseudoaglycones are α,β-unsaturated lactones with a molecular weights of 1,233 and possess two other double bonds, a hemiketal ring, a neutral sugar and an amino sugar. Although these spectral data of **4** were closely similar to those of **5**, they can be differentiated by retention times in their HPLC. Their retention times of **4** and **5** are 5.8 and 9.0 minutes respectively on a Nucleosil $5C_{18}$ column using methanol - 1 N aq NH_4Cl (60:40) solvent system at flow rate of 1 ml/minute with UV detection at 220 nm. Therefore, compounds **4** and **5** are considered to be stereoisomers and to be an epimeric pair with respect to β-position of the hemiketal carbon in reference to a reaction mechanism in the treatment of *N*-Ac-SVD with DBU. Compounds **4** and **5** thus obtained will play a significant role in the structural elucidation of ACM. In the following paper, we will describe the structures of the carbohydrate moieties of ACM.

Experimental

General Procedures

All mp's 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-181 polarimeter. ^{1}H NMR spectra were recorded on Jeol JNM-GX400 (400 MHz), JNM-GX270 (270 Hz) and JNM-FX100 (100 MHz) spectrometers, and ^{13}C NMR spectra were recorded on Jeol JNM-GX400 (100 MHz) and JNM-FX100 (25 MHz) spectrometers using TMS as an internal standard. HRFAB-MS were obtained using a Jeol JMS-HX110 mass spectrometer. SI-MS were obtained using a Hitachi M-80 mass spectrometer. HPLC was carried out on a Hitachi 655 liquid chromatograph with a Hitachi 655A UV monitor. Separation was performed on a Chemco Pak Nucleosil $5C_{18}$ (4.6×150 mm). TLC was performed on Merck pre-coated plates (Kieselgel 60 F_{254}, DC-Fertigplatten RP-18 F_{254s}). For column chromatography, Amberlite CG-50 type I (Organo), Fuji Davison BW-200 (150~325 mesh), ODS-W (100~200 mesh) and Sephadex LH-20 (Pharmacia) were used.

Isolation of ACM (1)

Fermentation broth (340 liters) containing the antibiotic was adjusted to pH 6.8 with 6 N NaOH and filtered with the aid of infusorial earth (Celite, 12 kg), The filtrate was adsorbed on a Diaion HP-20 column. The column was washed with 60 liters deionized water followed by elution with 50% aqueous acetone (140 liters) and acidic 50% aqueous acetone (pH 2, 150 liters). The active fraction was neutralized to pH 7.0 and lyophilized to give 91.8 g of brownish powder.

The powder (30 g) was dissolved in 300 ml of distilled water. The solution was adsorbed on Amberlite CG-50 (NH_4^+ form, 12×8 cm grass filter) and washed with 3 liters of distilled water and the antibiotic was eluted with 0.05 N ammonia water (2 liters). The fraction was immediately neutralized with 1 N hydrochloric acid and adsorbed on an ODS silica gel column (4×32 cm). The column was washed with distilled water (2 liters) followed by water - methanol (1 : 1, 1 liter) and water - methanol (1 : 2, 1 liter), and then the antibiotic was eluted with methanol (3.5 liters). The methanol fraction was concentrated under reduced pressure to give 667.5 mg of white powder. The residue was chromatographed on a Sephadex LH-20 (1.5×80 cm) column with methanol. The active fraction was 467.6 mg of white amorphous powder.

N-Acetylation of ACM (1)

A solution of **1** (858.5 mg) in anhydrous methanol (5 ml) was treated with acetic anhydride (5 ml). The solution was allowed to stand for 2 hours at room temperature and was then concentrated to dryness to yield the crude *N*-acetyl derivative (1,260 mg) as a white powder. The derivative was chromatographed on a silica gel column with chloroform - methanol - water (65 : 35 : 5) and it was finally applied on a Sephadex LH-20 (methanol) column to yield 389.2 mg (*N*-Ac-ACM: **2**).

DBU Treatment of ACM (1)

Compound **1** (1,100 mg) was dissolved in 20 ml of 2% DBU - methanol, and the solution was allowed to stand for 12 hours. The reaction mixture was chromatographed on a silica gel column (2.8×80 cm) eluted with chloroform - methanol - 7% ammonia water (30 : 20 : 4) to remove the reagent and the desired eluate was concentrated to dryness to yield a mixture (1,090 mg) of aculexitriose (**3**), pseudoaglycones I (**4**) and II (**5**). The mixture was dissolved in water and adsorbed on an ODS column. The column was eluted with 200 ml of water, 200 ml of water - methanol (1 : 1), 200 ml of water - methanol (1 : 2), and 400 ml of methanol. The water and water - methanol (1 : 1) fractions were concentrated under reduced pressure to give 244.8 mg of **3**. The methanol fractions was concentrated to yield 850 mg of **4** and **5**. The mixture of **4** and **5** (850 mg) was separated by a silica gel column chromatography (2-propanol - ethanol - 7% ammonia water (5 : 3 : 2)). Finally, each component was purified by column chromatography on Sephadex LH-20 (methanol) to yield **3** (230.2 mg), **4** (281.0 mg) and **5** (172.5 mg).

N-Acetylation of Aculexitriose (3)

Compound **3** (29.1 mg) was treated with acetic anhydride - anhydrous methanol (4 ml) and allowed

to stand for 2 hours at room temperature. The solution was concentrated and chromatographed on a silica gel column (chloroform - methanol - water (65 : 25 : 5)) followed by Sephadex LH-20 (methanol) to yield **6**: 21.3 mg.

N-Acetylation of Pseudoaglycones I and II (**4** and **5**)

Compounds **4** and **5** (140.3 and 86.7 mg, respectively) were treated with acetic anhydride - anhydrous methanol (8 ml) and allowed to stand for 2 hours at room temperature. The solutions were concentrated and chromatographed on a silica gel column (chloroform - methanol - water (65 : 25 : 5)) followed by Sephadex LH-20 (methanol) to yield **7** (88.2 mg) and **8** (51.6 mg), respectively.

DBU Treatment of *N*-Ac-ACM (**2**)

Compound **2** (373.2 mg) was treated with 2% DBU - methanol and allowed to stand for 12 hours. The solution mixture were chromatographed on a silica gel column (chloroform - methanol - water (65 : 25 : 5)) to remove the reagent and the desired eluate was concentrated to dryness to yield a mixture (367.3 mg) of *N*-acetylaculexitriose (**6**), *N*-acetylpseudoaglycones I (**7**) and II (**8**). The reaction mixture (150.4 mg) was dissolved in 50 ml of water and adsorbed on an ODS column and eluted with 200 ml of water, 200 ml of water - methanol (1 : 1) and 400 ml of methanol. The water and water - methanol (1 : 1) fractions were concentrated under reduced pressure to give 43.0 mg of **6**. The methanol fraction was concentrated to yield 109.4 mg of **7** and **8**. The mixture of **7** and **8** was separated by an ODS silica gel column (methanol - water (8 : 2)). Finally, each component was purified by column chromatography on Sephadex LH-20 (methanol) to yield **6** (34.2 mg), **7** (20.2 mg) and **8** (26.1 mg).

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References

1) IKEMOTO, T.; H. MATSUNAGA, K. KONISHI, T. OKAZAKI, R. ENOKITA & A. TORIKATA: Aculeximycin, a new antibiotic from *Streptosporangium albidum*. I. Taxonomy of producing organism and fermentation. J. Antibiotics 36: 1093～1096, 1983
2) IKEMOTO, T.; T. KATAYAMA, A. SHIRAISHI & T. HANEISHI: Aculeximycin, a new antibiotic from *Streptosporangium albidum*. II. Isolation, physicochemical properties and biological properties. J. Antibiotics 36: 1097～1100, 1983
3) KIMURA, I.; K. YAMAMOTO, K. HARADA & M. SUZUKI: Structural investigation of the antibiotic sporaviridin XII. Isolation of pseudoaglycones from N-acetylsporaviridins under basic conditions. Tetrahedron Lett. 28: 1917～1920, 1987