STRUCTURAL ELUCIDATION OF ACULEXIMYCIN

II. STRUCTURES OF CARBOHYDRATE MOIETIES

HIDEAKI MURATA, KEN-ICHI HARADA and MAKOTO SUZUKI*

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

TAKAYA IKEMOTO and TOSHIRO SHIBUYA

Teikyo University School of Medicine,
2-11-1 Kaga, Itabashi-ku, Tokyo 173, Japan

TATSUO HANEISHI and AKIO TORIKATA

Fermentation Research Laboratories, Sankyo Co., Ltd.,
1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140, Japan

(Received for publication June 13, 1988)

Treatment of aculeximycin with 2% 1,8-diazabicyclo[5,4,0]undecene-7 (DBU) - methanol yielded three products, aculexitriose, pseudoaglycones I and II. The structural elucidation of aculexitriose was carried out by spectral analyses (MS, NMR (^{1}H-^{1}H 2D NMR spectroscopy, nuclear Overhauser effect)) and chemical degradations of aculexitriose and its derivatives. The structure of aculexitriose was established to be a branched trisaccharide, *O*-6-deoxy-β-D-glucopyranosyl-(1→2)-*O*-[3-amino-2,3,6-trideoxy-β-D-*arabino*-hexopyranosyl-(1→3)]-6-deoxy-D-glucopyranose. On the other hand the pseudoaglycones I and II were stereoisomers with respect to a chiral center newly formed by the DBU reaction. The pseudoaglycones contain one neutral sugar and one amino sugar, which turned out to be D-mannose and L-vancosamine, respectively.

Aculeximycin (ACM), which was produced by *Streptosporangium albidum*, is active against insect, bacteria, yeast and molds *in vitro* at low concentration[1]. In the preceding paper we reported isolation, characterization and glycosidic bond cleavage of ACM[2]. So ACM is considered to be a basic glycosidic antibiotic with 1,672 of molecular weight and possesses five sugars including two amino sugars, three double bonds, and a hemiketal ring. ACM has a structural resemblance to sporaviridin[3] obtained from culture broth of *Streptosporangium viridogriseum*.

Treatment of ACM with 2% 1,8-diazabicyclo[5,4,0]undecene-7 (DBU) - methanol produced three products, aculexitriose, pseudoaglycones I and II. And the followings become clear after the investigation on preliminary spectroscopic data of the products, 1) aculexitriose is a trisaccharide containing an amino sugar, 2) the pseudoaglycones, counterparts of aculexitriose, are stereoisomers with respect to a chiral center newly formed by the DBU treatment, and 3) the pseudoaglycones contain still one neutral sugar and one amino sugar. The report describes the structural elucidation of aculexitriose and the constituent sugars in the pseudoaglycone moieties.

Structure of Aculexitriose

Aculexitriose was isolated as white amorphous powder from the 2% DBU - methanol reaction mixture by the procedure previously described[2]. The molecular weight of aculexitriose was determined to be 439 by the presence of a protonated molecule $(M+H)^+$ at *m/z* 440 and an $(M+Na)^+$

ion at *m/z* 462 in its secondary ion mass spectrum (SI-MS) with glycerol as a matrix. And the ^{13}C NMR spectral data (Table 1) indicates that aculexitriose has three 6-deoxyhexose units including one amino sugar. Upon acetylation in anhydrous methanol and in pyridine, aculexitriose afforded *N*-acetylaculexitriose (**1**) and hepta-*N*,*O*-acetylaculexitriose (**2**), respectively. Compound **1** showed $(M+H)^+$ and $(M+Na)^+$ ions at *m/z* 482 and 504 by SI-MS, respectively. The ^1H NMR spectrum revealed one acetyl methyl signal at 1.97 ppm and the ^{13}C NMR spectrum displayed one amide carbonyl signal at 173.6 ppm and acetyl methyl carbon signal at 22.9 ppm.

Table 1. ^{13}C NMR (25 MHz, CD_3OD) spectral data of aculexitriose.

Assignment	Chemical shift (ppm)[a]
Anomeric carbon	105.5, 101.7, 93.5
Oxymethine carbon	83.4, 80.3, 77.4, 76.9, 75.7, 75.1, 74.5, 73.4, 73.1, 67.8
Quaternary carbon	53.5
Methylene carbon	36.2
Methyl carbon	18.3×2, 18.2

[a] Values in the main anomer (α-anomer).

In order to elucidate the constituent monosaccharides of aculexitriose, exhaustive methanolysis of **1** was carried out. Compound **1** was heated under reflux with 5% methanolic hydrogen chloride for 4 hours. The solution was made neutral, then filtered, and the filtrate was evaporated to dryness. The residue was acetylated with acetic anhydride in pyridine. The resulting acetylated derivatives were separated by silica gel chromatography to yield two anomeric pairs of peracetylated methyl glycosides, **3** and **4** in a molar ratio of 2 : 1. Compound **3** was further separated to **3a** and **3b** by silica gel column chromatography (hexane - acetone (7 : 3)) and then they were recrystallized from benzene - cyclohexane. Compound **4** was also separated to **4a** and **4b** by silica gel chromatography (toluene - ethyl acetate - ethanol (30 : 10 : 3)) and, **4a** and **4b** were recrystallized from benzene - hexane. The physico-chemical properties, as well as ^1H NMR spectral data are summarized in Tables 2 and 3.

Compounds **3a** and **3b** gave $(M+NH_4)^+$ ions and $(M+H-MeOH)^+$ ions at *m/z* 322 and 273 by chemical ionization mass spectrometry (CI-MS) with ammonia as a reagent gas. The elemental analysis and mass spectral data for **3a** and **3b** indicate that their formulae are $C_{13}H_{20}O_8$. The ^1H NMR spectra reveal the presence of three acetyl methyl signals. The chemical shifts of oxymethines indicate that the three acetyl groups were attached at C-2, C-3 and C-4. The resonances at 1.20 ppm and 3.88 ppm also indicate that **3a** contained a 6-deoxypyranose ring. The presence of consecutive *trans* diaxial protons (2-H～5-H) displaying large coupling constants shows that the substituents at C-2～C-5 are equatorially disposed (*gluco* configuration). In the same manner as **3a**, the proton signals of **3b** are assigned as shown in Table 3. The differences of $J_{1,2}$ coupling constant between **3a** and **3b** were consistent with α- and β-anomers. The absolute stereochemistries of **3a** and **3b** were determined by comparing the specific optical rotation values with those of authentic samples[4~7]. Therefore, compound **3a** is methyl 2,3,4-tri-*O*-acetyl-6-deoxy-α-D-glucopyranoside and **3b** is its β-anomer. Thus one of the constituent sugars of ACM is 6-deoxy-D-glucose (D-quinovose, Scheme 1).

Compound **4** gave $(M+H)^+$ and $(M+H-MeOH)^+$ ions at *m/z* 246 and 214 by CI-MS with ammonia. Since the compounds **4a** and **4b** showed $(M+H)^+$ ions at *m/z* 246.1349 and 246.1348, respectively under CI (*iso*-C_4H_{10}) conditions, their formulae were established to be $C_{11}H_{19}NO_5$. In the ^1H NMR spectrum the anomeric proton signal (4.71 ppm) of **4a** was coupled (J=3.7 and 1.2 Hz) with a 2-H_{eq} at 2.23 ppm (1H, ddd) and a 2-H_{ax} at 1.59 ppm (1H, ddd). The methyl signal at 1.18 ppm was coupled (J=6.1 Hz) to an 5-H at 3.90 ppm (1H, dq), which in turn was coupled (J=8.8 Hz) to an 4-H at 4.47 ppm (1H, dd). As the resonance at 4.43 ppm (3-H) was coupled to an NH at 5.56

Table 2. Physico-chemical properties of **3**, **4**, **8** and **9**.

Compound	MP (°C) (literature)	Appearance	Formula	CI-MS (NH_3) (m/z)	$[\alpha]_D$ (°) (literature)	IR $\nu_{max}^{CHCl_3}$ cm^{-1}
3a	76~78 (81~82)[5]	Colorless needles	$C_{13}H_{20}O_8$	322 $(M+NH_4)^+$ 273 $(M+H-MeOH)^+$	+119.6 (*c* 0.4, $CHCl_3$) (+112.0 (*c* 1.2, $CHCl_3$))[5]	1750
3b	103~105 (102~103)[6]	Colorless needles	$C_{13}H_{20}O_8$	322 $(M+NH_4)^+$ 273 $(M+H-MeOH)^+$	−23.0 (*c* 0.3, EtOH) (−19.0 (*c* 1.6, EtOH))[7]	1750
4a	162~163 (162~163)[8,9]	Colorless needles	$C_{11}H_{19}NO_5$	246 $(M+H)^+$ 214 $(M+H-MeOH)^+$	+204.0 (*c* 0.1, MeOH) (+194.0 (*c* 0.6, MeOH))[10]	3420, 1720, 1670
4b	184~186 (180)[9]	Colorless needles	$C_{11}H_{19}NO_5$	246 $(M+H)^+$ 214 $(M+H-MeOH)^+$	+39.0 (*c* 0.3, $CHCl_3$) (+23.4 (*c* 1.0, $CHCl_3$))[9]	3420, 1720, 1670
8	67~71 (65~66)[11]	Colorless prisms	$C_{15}H_{22}O_{10}$	380 $(M+NH_4)^+$ 331 $(M+H-MeOH)^+$	+48.8 (*c* 0.6, $CHCl_3$) (+49.0 (*c* 1.1, $CHCl_3$))[11]	1745
9a	157~161 (166~168)[4,12]	Colorless needles	$C_{12}H_{21}NO_5$	260 $(M+H)^+$ 228 $(M+H-MeOH)^+$	−209.7 (*c* 0.1, MeOH) (−220.0 (*c* 0.3, MeOH))[4]	3450, 1730, 1670
9b	123~124 (123~124)[4]	Colorless needles	$C_{12}H_{21}NO_5$	260 $(M+H)^+$ 228 $(M+H-MeOH)^+$	−82.4 (*c* 0.3, MeOH) (−83.0 (*c* 0.3, MeOH))[4]	3450, 1730, 1670

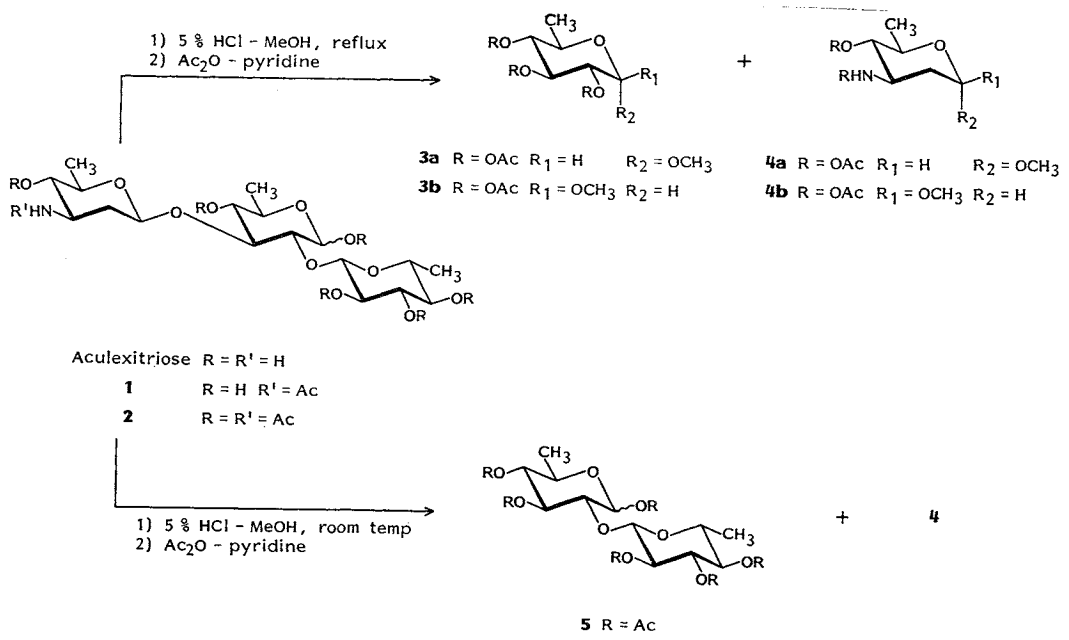
Table 3. ^1H NMR (400 MHz, $CDCl_3$) spectral data of **3**, **4**, **8** and **9**.

Compound	1-H ($J_{1,2}$)[a]	2-H ($J_{2,3}$)	3-H ($J_{3,4}$)	4-H ($J_{4,5}$)	5-H ($J_{5,6}$)	6-H ($J_{5,6'}$)	6'-H ($J_{6,6'}$)	OCH_3	$OCOCH_3$
3a	4.88 d (3.8)	4.96 dd (9.5)	5.43 dd (9.8)	4.80 t (9.8)	3.88 dq (6.4)	1.20 d —	—	3.40 s	2.07 s, 2.03 s, 2.00 s
3b	4.39 d (8.1)	4.96 dd (9.8)	5.16 dd (9.5)	4.82 t (9.5)	3.57 dq (6.1)	1.25 d —	—	3.50 s	2.05 s, 2.04 s, 2.00 s
8	4.72 d (1.7)	5.24 dd (3.2)	5.34 dd (10.0)	5.28 dd (9.3)	3.97 ddd (5.4)	4.29 dd (2.4)	4.13 dd (12.2)	3.41 s	2.16 s, 2.11 s, 2.04 s, 1.99 s

Compound	1-H ($J_{1,2ax}$)	2-H_{eq} ($J_{1,2eq}$)($J_{2eq,2ax}$)	2-H_{ax} ($J_{2eq,3}$)	3-H ($J_{2ax,3}$)	4-H ($J_{3,4}$)	5-H ($J_{4,5}$)	6-H ($J_{5,6}$)	NH ($J_{3,NH}$)	OCH_3 CCH_3	$OCOCH_3$ $NCOCH_3$
4a	4.71 br d (3.7)	2.23 ddd (1.2)(13.2)	1.59 ddd (4.4)	4.43 m (12.0)	4.47 dd (10.3)	3.90 dq (8.8)	1.18 d (6.1)	5.56 br d (7.3)	3.34 s	2.08 s 1.92 s
4b	4.47 br d (9.5)	2.30 ddd (2.0)(12.7)	1.48 dt (4.6)	4.15 m (12.7)	4.45 dd (11.5)	3.57 dq (12.5)	1.24 d (6.1)	5.67 br d (8.6)	3.49 s	2.08 s 1.93 s
9a	4.79 br d (4.4)	2.37 br d (<1)(13.6)	2.01 dd —	—	4.92 br s —	4.13 br q (<1)	1.36 d (6.6)	5.59 br s —	3.33 s 1.72 s	2.19 s 1.87 s
9b	4.56 dd (9.8)	2.25 ddd (2.4)(12.7)	1.73 br d —	—	5.02 br s —	3.90 dq (1.0)	1.21 d (6.4)	5.51 br s —	3.53 s 1.64 s	2.18 s 1.90 s

[a] J=Hz.

Scheme 1. Degradation scheme for aculexitriose.



ppm (1H, br d) besides the resonance at 2-H_{eq}, 2-H_{ax} and 4-H, an acetamide group was attached at C-3. Therefore, an acetoxy group was located at C-4. By the presence of the consecutive *trans* diaxial protons (3-H~5-H) displaying large coupling constants, we determined that **4a** has the *arabino* configuration. In the same manner as **4a**, the proton signals of **4b** are assigned as shown in Table 3. The difference of $J_{1,2}$ coupling constant between **4a** and **4b** was based on that of their anomeric forms. The absolute stereochemistries of **4a** and **4b** were determined by comparing the optical rotation value of authentic samples[4,8~10]. Therefore, compound **4a** is methyl 3-acetamido-4-*O*-acetyl-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside and **4b** is its β-anomer. Therefore, another constituent sugar of ACM is 3-amino-2,3,6-trideoxy-D-*arabino*-hexopyranose (D-acosamine, Scheme 1). As the results of these data, the constituent monosaccharides of aculexitriose were determined to be D-quinovose and D-acosamine in a molar ratio of 2 : 1.

The CI-MS of **2** shows a $(M+H)^+$ ion at *m/z* 734 (13.0%) together with some prominent fragment ions at *m/z* 674 (100%), 273 (17.4%) and 214 (67.4%). The presence of the fragment ions at *m/z* 273 and 214, which were assigned to oxonium type ions of 2,3,4-tri-*O*-acetylquinovose and 3,4-di-*N*,*O*-acetylacosamine, showed that one of D-quinovoses and D-acosamine were located at the terminal position of aculexitriose. Fig. 1 shows the ^{1}H NMR spectrum of **2** dissolved in chloroform-d_1. Judging from the spectrum, compound **2** is an anomeric mixture. The signals for the anomeric protons of the reducing D-quinovose residue appeared at δ 6.21 (d, $J_{1,2}$=3.9 Hz) and 5.61 (d, $J_{1,2}$=8.1 Hz) in a ratio of 74 : 26, respectively. The resonance at 4.63 ppm with the large coupling constant ($J_{1,2}$= 7.8 Hz) is assigned to the anomeric proton of the nonreducing β-D-quinovose residue. Also the resonance at 4.56 ppm (dd, $J_{1,2ax}$=9.3 Hz and $J_{1,2eq}$=2.0 Hz) is assigned to the anomeric proton of the β-D-acosamine residue. The correlated homonuclear ^{1}H-^{1}H 2D NMR spectroscopy (COSY) of **2** confirmed the resonance assignments presented in Table 3. Comparison of the chemical shifts in the ^{1}H NMR spectral data of the sugars I and II with those of **3a** and **3b**, respectively, indicated that C-2

Fig. 1. ^{1}H NMR spectrum of hepta-*N*,*O*-acetylaculexitriose (**2**).

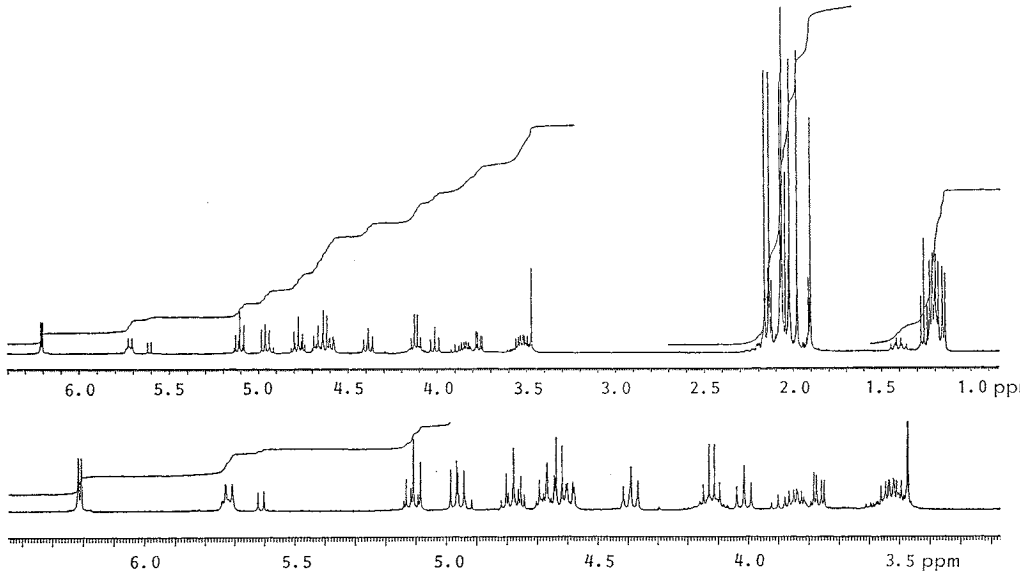
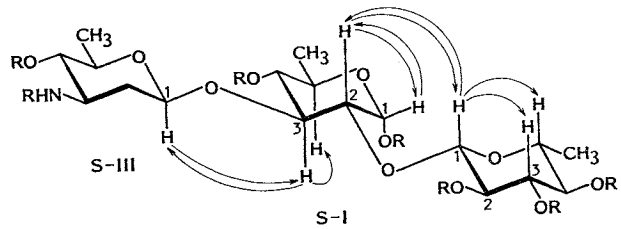


Fig. 2. NOE's of hepta-*N*,*O*-acetylaculexitriose (**2**).



Proton irradiated		NOE's observed
S-I	1-H	S-I, 2-H (5.6%)
	2-H	S-I, 1-H (6.9%), S-II, 1-H (4.3%)
	3-H	S-I, 5-H (weak), S-III, 1-H (weak)
S-II	1-H	S-I, 2-H (weak), S-II, 3-H (weak), S-II, 5-H (weak)
S-III	1-H	S-I, 3-H (weak)

S-I: Sugar I, S-II: sugar II, S-III: sugar III.

and C-3 of the sugar I and C-1 of the sugar II form glycosidic bonds. Analogously comparison of chemical shift of the sugar III with those of **4b**, indicated that C-1 of the sugar III is glycosidically linked. In the nuclear Overhauser effects (NOE's) experiments (Fig. 2), irradiation of the 2-H signal of the sugar I (3.77 ppm) gave a 4.3%-enhancement of the signal at 4.63 ppm (1-H in the sugar II), so the sugar II is attached to C-2 of the sugar I. Furthermore, the sugar III was attached to C-3 of the sugar I because weak enhancement of the signal at 4.56 ppm (1-H in the sugar III) appeared on irradiation of the 3-H signal of the sugar I (4.02 ppm).

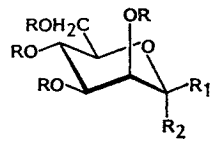
In order to confirm which sugar moiety is located at C-2 or C-3 of the sugar I, we tried to obtain a partial methanolysis product of aculexitriose. Treatment of **1** with 5% methanolic hydrogen chloride at room temperature followed by acetylation with acetic anhydride in pyridine gave methyl di-*N*,*O*-acetyl-D-acosaminide (**4**) and hexa-*O*-acetyldisaccharide (**5**). Compound **5**, composed of two quinovose residues, gave a $(M+NH_4)^+$ ion at *m/z* 580 by CI-MS with ammonia. The ^{13}C NMR spectrum revealed two anomeric carbon signals at 90.5 and 101.0 ppm. The ^1H NMR spectral data of **5** are summarized in Table 4, whose assignments were firmly supported by spin-decoupling experiments. Comparison of the chemical shifts in the ^1H NMR spectrum of **5** with those of **2** indicated that C-3 of the sugar I was acetylated and C-2 of the sugar I was glycosidically linked. The orientation of anomeric proton of the sugar II moiety was assigned on the basis of its *J* value of 7.8 Hz (β-linkage). The mass spectral and ^1H NMR spectral data finally established the structure of the disaccharide to be *O*-6-deoxy-β-D-glucopyranosyl-(1→2)-6-deoxy-D-glucopyranose (Scheme 1). As the result of these data, the structure of aculexitriose was established to be *O*-6-deoxy-β-D-glucopyranosyl-(1→2)-*O*-[3-amino-2,3,6-trideoxy-β-D-*arabino*-hexopyranosyl-(1→3)]-6-deoxy-D-glucopyranose (Scheme 1).

Constituent Monosaccharide in Pseudoaglycones

As described previously, the following became clear that pseudoaglycones I and II are stereoisomer containing two sugars. In order to elucidate the constituent monosaccharides, exhaustive methanolysis of pseudoaglycone I was carried out. It was heated under reflux with 5% methanolic hydrogen chloride for 6 hours. The solution was made neutral, then filtered, and the filtrate was evaporated to dryness. The residue was separated by Sephadex LH-20 chromatography to yield a decomposed aglycone moiety and two methyl glycosides **6** and **7**. Both methyl glycosides **6** and **7** were acetylated with acetic anhydride in pyridine. The resulting acetylated derivatives were separated by repeated silica gel chromatographies to yield **8**, **9a** and **9b**. The physico-chemical properties as well as ^1H NMR spectral data are summarized in Tables 2 and 3. Compound **8** gave a $(M+NH_4)^+$ ion and a $(M+H-MeOH)^+$ ion at *m/z* 380 and 331 by CI-MS with ammonia, respectively. The ^1H NMR spectral data exhibit a methoxyl signal (3.41 ppm), four acetyl methyl signals (2.16, 2.11, 2.04 and 1.99 ppm), and three consecutive *trans* diaxial protons (3-H~5-H) displaying a large coupling constant. The anomeric proton signal (4.72 ppm) was coupled (1.7 Hz) with a 2-H at 5.24 ppm (1H, dd), which in turn was coupled (3.2 Hz) with 3-H at 5.34 ppm (1H, dd). The absolute stereochemistry of **8** was determined by comparing with an authentic sample[11]. Therefore, compound **8** is methyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (Fig. 3).

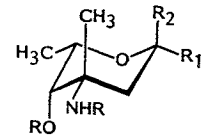
Compounds **9a** and **9b**, which were an anomeric pair of the amino sugar, gave a $(M+H)^+$ at *m/z* 260 by CI-MS with ammonia. Their formulae of **9a** and **9b** were established to be $C_{12}H_{21}NO_5$ by elemental analysis or high resolution (HR)CI-MS. The ^1H NMR spectra of **9a** and **9b** showed a methoxy signal, an acetoxy methyl signal, an acetamide methyl signal and a singlet methyl signal. 1-H and 2-H appear as an ABX system which allowed the structure assignment of the α-anomer (**9a**: $J_{1,2ax}=4.4$, Hz $J_{1,2eq}<1$ Hz) and the β-anomer (**9b**: $J_{1,2ax}=9.8$ Hz, $J_{1,2eq}=2.4$ Hz). As a doublet methyl signal (1.36 ppm) was coupled only with 5-H, which in turn was coupled with a 1-H broad singlet at 4.92 ppm (4-H), the substituent (CH_3COO) at C-4 is axially disposed. So the remaining acetamide and methyl groups should be disposed at C-3. In order to determine the relative configuration of the methyl and acetamide groups (*lyxo* or *xylo*) at C-3 and the absolute configuration

Fig. 3. Structure of **8**.



8 R=OAc R_1=H R_2=OCH_3

Fig. 4. Structures of **9a** and **9b**.



9a R=OAc R_1=H R_2=OCH_3
9b R=OAc R_1=OCH_3 R_2=H

of **9**, it was compared with methyl 3-acetamido-4-acetyl-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranoside (methyl di-*N*,*O*-acetyl-α-L-vancosaminide), which had been obtained during the course of the structure elucidation of sporaviridin and the structure had been already determined by X-ray analysis[4]. The optical rotation value, mp, IR and ^{1}H NMR spectra of **9a** are identical with those of an authentic sample[4,12]. Therefore, **9a** and **9b** were identified as methyl di-*N*,*O*-acetyl-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranoside and its β-anomer (Fig. 4), respectively. In the same manner as pseudoaglycone I, methanolysis of pseudoaglycone II also gave D-mannose and L-vancosamine, as constituent monosaccharides.

As the results of the extensive experiments mentioned above, it was found that ACM possesses aculexitriose (*O*-6-deoxy-β-D-glucopyranosyl-(1→2)-*O*-[3-amino-2,3,6-trideoxy-β-D-*arabino*-hexopyranosyl-(1→3)]-6-deoxy-D-glucopyranose), D-mannose and L-vancosamine as the carbohydrate moieties. It has been already reported that sporaviridin includes a pentasaccharide, viridopentaose, D-glucose and L-vancosamine[4]. These results remind us an interest in the structural correlation between ACM and sporaviridin. The structural investigations of the pseudoaglycones are in progress at present.

Experimental

General Procedures

All mp's 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. ^{1}H NMR spectra were recorded on a Jeol JNM-GX400 (400 MHz) spectrometer, and ^{13}C NMR spectra were recorded on a Jeol JNM-FX100 (25 MHz) spectrometer using TMS as an internal standard. SI-MS were obtained using a Hitachi M-80 mass spectrometer. CI-MS were obtained using a Shimadzu GC-MS QP-1000 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, Fuji Davison BW-200 (150~325 mesh), ODS-W (100~200 mesh) and Sephadex LH-20 (Pharmacia) were used.

Methanolysis of *N*-Acetylaculexitriose (1)

Compound **1** (41.9 mg) was dissolved in 3 ml of 5% methanolic hydrogen chloride and heated under reflux for 4 hours. The solution was made neutral with silver carbonate, then filtered, and the filtrate was evaporated to dryness. The residue was acetylated with acetic anhydride in pyridine. The reaction mixture was concentrated under reduced pressure to yield a mixture of peracetylated methyl glycosides (58.4 mg). A mixture of the peracetylated methyl glycosides (90.2 mg) was chromatographed on a silica gel column (toluene - ethyl acetate - ethanol (30 : 10 : 3)) to yield **3a** and **3b**: 43.4 mg and **4a** and **4b**: 16.7 mg.

A mixture of **3a** and **3b** was applied on a silica gel column (hexane - acetone (7 : 3)) to yield **3a**:

17.8 mg and **3b**: 6.8 mg. A mixture of **4a** and **4b** was chromatographed on a silica gel column (toluene - ethyl acetate - ethanol (30 : 10 : 3)) to yield **4a**: 11.5 mg and **4b**: 2.0 mg. Compounds **3a**, **3b**, **4a** and **4b** were recrystallized from benzene - cyclohexane or benzene - hexane.

Compound **3a**: Colorless needles; mp 76~78°C; $[\alpha]_D^{24}$ +119.6° (*c* 0.4, $CHCl_3$); *Anal* Calcd for $C_{13}H_{20}O_8$: C 51.31, H 6.63, Found: C 50.98, H 6.65; IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1750; CI-MS (NH_3) *m/z* 322 $(M+NH_4)^+$, 273 $(M+H-MeOH)^+$; ^1H NMR (400 MHz, $CDCl_3$) see Table 3.

Compound **3b**: Colorless needles; mp 103~105°C; $[\alpha]_D^{24}$ −23.0° (*c* 0.3, EtOH); *Anal* Calcd for $C_{18}H_{20}O_8$: C 51.31, H 6.63, Found: C 50.89, H 6.56; IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1750; CI-MS (NH_3) *m/z* 322 $(M+NH_4)^+$; ^1H NMR (400 MHz, $CDCl_3$) see Table 3.

Compound **4a**: Colorless needles; mp 162~163°C; $[\alpha]_D^{24}$ +204.0° (*c* 0.1, MeOH); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3420, 1720, 1670; CI-MS (NH_3) *m/z* 246 $(M+H)^+$, 214 $(M+H-MeOH)^+$; HRCI-MS (*iso*-C_4H_{10}) Calcd for $C_{11}H_{20}NO_5$: 246.1336, Found: 246.1349; ^1H NMR (400 MHz, $CDCl_3$) see Table 3.

Compound **4b**: Colorless needles; mp 184~186°C; $[\alpha]_D^{24}$ +39.0° (*c* 0.3, MeOH); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3420, 1720, 1670; CI-MS (NH_3) *m/z* 246 $(M+H)^+$, 214 $(M+H-MeOH)^+$; HRCI-MS (*iso*-C_4H_{10}) Calcd for $C_{11}H_{20}NO_5$: 246.1336, Found: 246.1348; ^1H NMR (400 MHz, $CDCl_3$) see Table 3.

Peracetylation of *N*-Acetylaculexitriose (**1**)

Compound **1** (82.6 mg) was treated with acetic anhydride - pyridine (1 : 1, 4 ml) and allowed to stand for 2 hours at room temperature. The solution was concentrated and chromatographed on Sephadex LH-20 (methanol) and silica gel (toluene - ethyl acetate - ethanol (15 : 5 : 3)) to yield 57.8 mg (**2**: hepta-*N*,*O*-acetylaculexitriose).

Hepta-*N*,*O*-acetylaculexitriose (**2**): White amorphous powder; mp 123~127°C; $[\alpha]_D^{24}$ +51.3° (*c* 0.1, MeOH); *Anal* Calcd for $C_{32}H_{47}NO_{18}$: C 52.38, H 6.41, N 1.91, Found: C 52.19, H 6.94, N 1.81; IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1760, 1680; CI-MS (NH_3) *m/z* 734 (13.0%), 674 (100%), 273 (17.4%), 214 (67.4%); ^{13}C NMR (25 MHz, $CDCl_3$) δ 171.5, 170.2, 169.8, 169.6×2, 169.55, 169.2, 100.9, 99.6, 90.7, 78.7, 76.0, 75.5, 73.6, 73.2, 73.1, 72.0, 70.6, 70.1, 67.3, 49.1, 37.8, 23.2, 21.2, 21.0, 20.9×2, 20.6, 20.5, 18.0, 17.5, 17.1; ^1H NMR (400 MHz, $CDCl_3$) see Fig. 1 and Table 4.

Table 4. ^1H NMR (400 MHz, $CDCl_3$) spectral data of hepta-*N*,*O*-acetylaculexitriose (**2**) and hexa-*O*-acetyldisaccharide (**5**).

		2 (α-anomer)	**5** (α-anomer)
S-I	1-H	6.21 d (3.9)[a]	6.22 d (3.9)[a]
	2-H	3.77 dd (9.5, 3.9)	3.85 dd (10.0, 3.9)
	3-H	4.02 t (9.5)	5.37 dd (10.0, 9.8)
	4-H	4.67 dd (10.1, 9.5)	4.76 t (9.8)
	5-H	3.84 dq (10.1, 6.1)	3.92 dq (9.8, 6.1)
	6-H	1.15 d (6.1)	1.21 d (6.1)
S-II	1-H	4.63 d (7.8)	4.55 d (7.8)
	2-H	4.96 dd (9.5, 7.8)	4.89 dd (9.8, 7.8)
	3-H	5.11 t (9.5)	5.09 t (9.8, 9.5)
	4-H	4.78 t (9.5)	4.80 t (9.5)
	5-H	3.57 dq (9.5, 6.1)	3.54 dq (9.5, 6.4)
	6-H	1.19 d (6.1)	1.17 d (6.4)
S-III	1-H	4.56 dd (9.3, 2.0)	
	2-H_{ax}	1.40 dt (12.8, 9.3)	
	2-H_{eq}	2.19 ddd (12.8, 4.9, 2.0)	
	3-H	4.12 m	
	4-H	4.39 t (9.6)	
	5-H	3.52 dq (9.6, 6.1)	
	6-H	1.22 d (6.1)	
	NH	5.72 d (8.5)	

S-I: Sugar I, S-II: sugar II, S-III: sugar III.

[a] *J*=Hz.

Partial Methanolysis of *N*-Acetylaculexitriose (**1**)

Compound **1** (8.6 mg) was dissolved in 5% methanolic hydrogen chloride and allowed to stand for 4 hours at room temperature. The solution was made neutral with silver carbonate, then filtered, and the filtrate was evaporated to dryness. The residue was acetylated with acetic anhydride - pyridine (1 : 1) overnight at room temperature. The reaction mixture (11.5 mg) was adsorbed on a silica gel column equilibrated with benzene - acetone (9 : 1). Compound **5** was eluted with benzene - acetone (9 : 1) to yield 5.7 mg and compound **4** was eluted with toluene - ethyl acetate - ethanol (30 : 10 : 3) to yield 1.8 mg.

Compound **5**: White amorphous powder; mp 184~195°C; $[\alpha]_D^{24}$ +60.8° (*c* 0.1, MeOH); *Anal* Calcd for $C_{24}H_{34}O_{15}$: C 51.25, H 6.05, Found: C 50.83, H 6.18; IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1780; ^{13}C NMR (25 MHz, CD_3OD) δ 170.6, 170.1×2, 169.8×2, 169.4, 101.0, 90.5, 75.8, 73.5, 73.2, 72.8, 71.6×2, 70.2, 67.3, 20.8, 20.4×4, 17.3, 17.2; ^1H NMR (400 MHz, CD_3OD) see Table 4.

Methanolysis of Pseudoaglycone I

Pseudoaglycone I (200.0 mg) was dissolved in 5% methanolic hydrogen chloride (3 ml) and heated under reflux for 4 hours. The solution was made neutral, then filtered, and the filtrate was concentrated under reduced pressure to yield 201.2 mg. The methanolysis products (201.2 mg) was applied on Sephadex LH-20 (methanol) to yield decomposed pseudoaglycone (140.5 mg), **6** (21.2 mg) and **7** (19.3 mg).

Peracetylation of **6**

Compound **6** (16.3 mg) was treated with acetic anhydride - pyridine (1 : 1, 1 ml) and allowed to stand for 2.5 hours at room temperature. The solution was concentrated and chromatographed on Sephadex LH-20 with chloroform - methanol (1 : 1) to yield **8**: 22.0 mg. Compound **8** was recrystallized from ether - hexane.

Compound **8**: Colorless prisms; mp 67~71°C; $[\alpha]_D^{24}$ +48.8° (*c* 0.6, $CHCl_3$); *Anal* Calcd for $C_{15}H_{22}O_{10}$: C 49.72, H 6.12, Found: C 49.75, H 6.17; IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1745, 1230; CI-MS (NH_3) *m/z* 380 $(M+NH_4)^+$, 331 $(M+H-MeOH)^+$; ^{13}C NMR (25 MHz, $CDCl_3$) δ 170.7, 170.0, 169.9, 169.7, 98.6, 69.5, 69.1, 68.4, 66.2, 62.6, 55.3, 20.9, 20.7×3; ^1H NMR (400 MHz, $CDCl_3$) see Table 3.

Peracetylation of **7**

Compound **7** (10.0 mg) was treated with acetic anhydride - pyridine (1 : 1, 1 ml) and allowed to stand for 6 hours at room temperature. The solution was concentrated and chromatographed on silica gel with toluene - ethyl acetate - ethanol (30 : 10 : 3), followed by application on silica gel with hexane - acetone (7 : 3) to yield **9a**: 7.3 mg, **9b**: 4.2 mg. Compounds **9a** and **9b** were recrystallized from benzene - cyclohexane and benzene - hexane, respectively.

Compound **9a**: Colorless needles; mp 157~161°C; $[\alpha]_D^{24}$ −209.7° (*c* 0.1, MeOH); *Anal* Calcd for $C_{12}H_{21}NO_5$: C 55.58, H 8.16, N 5.40, Found: C 55.25, H 8.21, N 5.36; IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1730, 1670, 1240; CI-MS (NH_3) *m/z* 260 $(M+H)^+$, 228 $(M+H-MeOH)^+$; ^{13}C NMR (25 MHz, $CDCl_3$) δ 171.8, 169.4, 98.4, 74.6, 62.7, 55.1, 54.3, 35.3, 24.4, 23.8, 20.8, 17.3; ^1H NMR (400 MHz, $CDCl_3$) see Table 3.

Compound **9b**: Colorless needles; mp 123~124°C; $[\alpha]_D^{24}$ −82.4° (*c* 0.3, MeOH); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1730, 1670, 1240; CI-MS (NH_3) *m/z* 260 $(M+H)^+$, 228 $(M+H-MeOH)^+$; HRCI-MS (*iso*-C_4H_{10}) Calcd for $C_{12}H_{22}NO_5$: 260.1492, Found: 260.1511; ^{13}C NMR (25 MHz, $CDCl_3$) δ 171.4, 169.4, 99.7, 73.2, 68.2, 56.6, 55.5, 37.9, 24.2, 22.0, 20.8, 17.4; ^1H NMR (400 MHz, $CDCl_3$) see Table 3.

Acknowledgment

We wish to express our thanks to Dr. MITSUO HAYASHI (Medical Research Laboratories, Toyo Jozo Co., Ltd.) for measurement of HRCI-MS and to Dr. YUJI MORI (Faculty of Pharmacy, Meijo University) for measurement of ^1H NMR spectra.

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