Structural Elucidation of Aculeximycin

III. Planar Structure of Aculeximycin, Belonging to a New Class of Macrolide Antibiotics

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(Received for publication January 19, 1995)

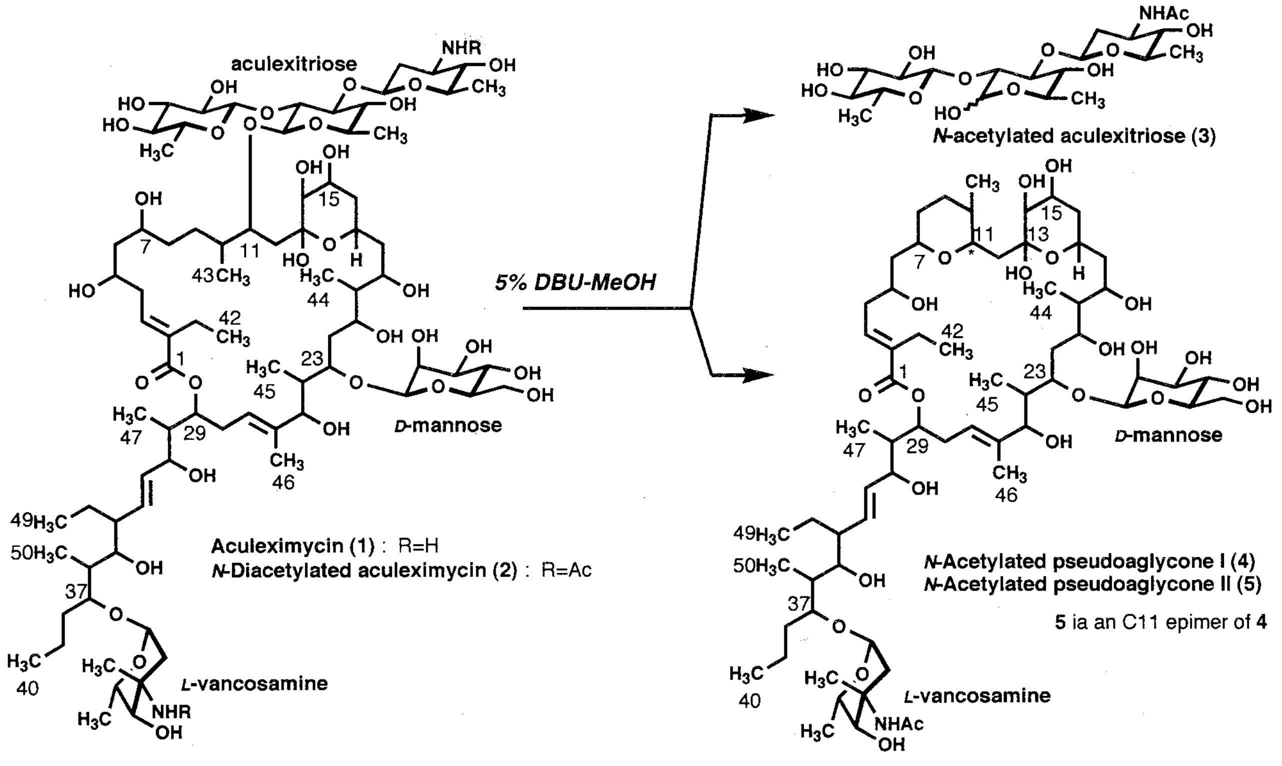
The planar structure of aculeximycin (**1**) produced by *Streptosporangium albidum* has been determined by spectral methods and chemical degradations such as 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU)-methanol reaction, ozonolysis, and periodative oxidation. The antibiotic consists of a 30-membered polyhydroxy lactone ring, an α, β-unsaturated ester group, an intramolecular hemiketal, an oligosaccharide (aculexitriose), a neutral sugar and an amino sugar. The structure of aculeximycin is closely related to those of sporaviridins produced by *Streptosporangium viridogriseum*. We consider that aculeximycin and sporaviridins belong to a new class of macrolide antibiotics, which is different from the polyol macrolides produced by *Streptomyces*.

In general, macrolide antibiotics are classified into 12, 14 or 16-membered ring macrolides according to size of the macrocyclic lactone ring of the aglycone. Because the term "macrolide" has been broadly interpreted at the present time, polyene and polyol macrolides are also placed in the same category[1]. Original macrolide antibiotics possess strong activity against Gram-positive bacteria but not against fungi. Polyene macrolides such as amphotericin B and nystatin have been employed in antifungal therapy, but they have undesirable effects. Polyol macrolides such as copiamycin[2,3], azalomycin[4~7], guanidylfungin[8,9], niphimycin[10~12] (scopafungin[13]), amycins[14], shurimycin[15] and malolactomycin[16] are characterized by the presence of a larger (32, 36 or 40-membered) lactone ring with a guanidyl group at the end of the side chain. Although the polyol macrolides do not contain three to eight conjugated carbon-carbon double bonds, they exhibit antimicrobial and antifungal activities. Azalomycin F has actually been employed in antifungal therapy for external use. Recently, opportunistic fungal infections are becoming more serious in the compromised patients and antifungal agents have been urgently required.

While the aforementioned macrolides are mainly produced by *Streptomyces*, one group of macrolide compounds named sporaviridins[19,20] are produced by *Streptosporangium*. The structures of the sporaviridins are different from those of polyol macrolides in that they possess a 34-membered macrocyclic lactone with seven sugars including a pentasaccharide viridopentaose instead of the malonyl half ester and the guanidyl group found in the polyol macrolides. Later, another new macrolide antibiotic, aculeximycin, was isolated from a strain of *Streptosporangium albidum* during insecticidal screening. It was considered that aculeximycin and sporaviridins belong to a new class of antifungal macrolide antibiotics. Although the planar structures of sporaviridins had been determined, their stereochemistries have remained unresolved. Our goal was to determine the absolute stereochemistry and conformation for aculeximycin and sporaviridins. We have based the structural elucidation of aculeximycin on the degradative reactions used for sporaviridins, because these degradation products would be available for determination of the stereochemistry of both antibiotics.

We have reported the physico-chemical properties of aculeximycin (**1**) and its *N*-diacetylated derivative (**2**)[21]. Compound **1** was considered to be a basic

Fig. 1. Structures of aculeximycin (1), *N*-diacetylated aculeximycin (2), and its degradation products (3~5) using 5% DBU-MeOH.



glycosidic antibiotic with a molecular weight of 1672 ($C_{81}H_{144}N_2O_{33}$) and possessed five sugars including two amino sugars, a hemiketal ring and three carbon-carbon double bonds. Treatment of **2** with 5% 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU)-methanol gave *N*-acetylated aculexitriose (**3**) and an epimeric pair of counterparts named *N*-acetylated pseudoaglycones I (**4**) and II (**5**) (Fig. 1). Compound **3** was determined to be *O*-6-deoxy-β-D-*gluco*-pyranosyl-(1→2)-*O*-[3-acetamido-2,3,6-trideoxy-β-D-*arabino*-hexopyranosyl-(1→3)]-6-deoxy-D-*gluco*-pyranose and compounds **4** and **5** still contained a D-mannose and an *N*-acetyl-L-vancosamine[22]. In the present study, **4** and **5** were further degraded and a combination of the resulting degradation products allowed us to construct the planar structure of aculeximycin.

Results

Preliminary Characterization of *N*-Acetylated Pseudoaglycone I (4) by NMR Techniques

Compound **4** has the molecular formula $C_{65}H_{113}NO_{23}$ (MW 1275), which was determined by high resolution fast atom bombardment mass spectrometry (HRFAB-MS). The ^1H and ^{13}C NMR spectral data of **4** in DMSO-d_6 are summarized in Table 1. Isotope shifts in the ^{13}C NMR signals, as observed by the chemical shift differences between DMSO-d_6 and DMSO-d_6 containing water solutions, led to identification of hydroxyl-bearing carbons. The proton and/or carbon connectivities were elucidated by ^1H-^1H correlation spectroscopy (COSY), ^1H detected heteronuclear multiple bond coherence spectroscopy (HMBC) and homonuclear Hartmann-Hahn spectroscopy (HOHAHA) experiments. Fig. 2 shows the partial structures deduced by the NMR experiments.

Firstly, the partial structures around three carbon-carbon double bonds including an α, β-unsaturated carbonyl moiety were determined. The geometry of an isolated disubstituted double bond at C-32~C-33 was determined to be *E* by the coupling constant (J=15.2 Hz) and the two trisubstituted olefinic geometries at C-2~C-3 and C-26~C-27 were determined to be *E*, because the allylic methylene (δ 19.6; C-41) at C-2 and the methyl (δ 9.59; C-46) at C-26 signals were shifted to a higher field due to γ-effects[23]. The coupling constants of olefinic protons at H-3 (t, 7.0 Hz), H-27 (t, 7.0 Hz), H-32 (dd, 12.8, 8.0 Hz) and H-33 (dd, 12.8, 5 Hz) showed that these olefinic carbons were linked to the methylene (C-4), methylene (C-28), methine (C-31) and methine

Table 1. ^{1}H and ^{13}C NMR spectral data (DMSO-d_6) of *N*-acetylated pseudoaglycones I (**4**) and II (**5**).

	Compound **4**					Compound **5**				Compound **4**				Compound **5**		
	^{13}C			^{1}H	position	^{13}C		^{1}H		^{13}C		^{1}H	position	^{13}C		^{1}H
1	168.8	s	-	-	V3-Ac	168.8	s	-	34	47.5	d	2.01	34	47.6	d	2.10
2	165.5	s	-	-	1	165.5	s	-	35	42.1	d	1.19	24	42.0	d	1.20
3	140.5	s	-	-	26	140.5	s	-	36	41.8	t	1.21		41.8	t	1.21, 1.31
4	136.6	d	-	6.45 (t)	3	136.5	d	6.48	37	41.2	d	1.24	20	41.1	d	1.23
5	135.1	s	-	-	2	135.1	s	-	38	41.1	t	1.41, 1.51		40.1	t	1.18, 1.46
6	133.8	d	-	5.38 (dd)	33	133.8	d	5.40	39	41.1	t	1.17		39.8	t	1.16, 1.67
7	131.5	d	-	5.45 (dd)	32	131.5	d	5.40	40	40.3	t	1.15, 1.66		39.1	t	1.68, 2.52
8	120.1	d	-	5.32 (t)	27	131.0	d	5.32	41	38.6	d	1.71	30	38.3	d	2.23
9	101.5	d	OR	4.91	M1	101.5	d	4.90	42	38.4	t	2.23		36.4	t	1.87
10	99.0	s	OH	-	13	99.0	s	-	43	36.7	d	1.87	36	34.7	d	1.73
11	94.0	d	OR	4.78	V1	94.0	d	4.84	44	35.0	t	1.73, 1.83	16	34.0	t	1.20
12	79.5	d	OH	2.92 (d)	14	78.9	d	2.93	45	34.1	d	1.22	10	32.7	d	1.23
13	78.6	d	OH	3.58	25	78.4	d	3.57	46	32.8	t	1.12, 1.28	9	32.6	t	1.08
14	78.1	d	OR	3.50	11	78.2	d	3.47	47	32.8	t			32.5	t	1.69
15	77.8	d	OR	3.32	17	77.8	d	3.31	48	31.4	t	1.29, 1.39	38	31.5	t	1.33
16	75.7	d	OR	3.78	37	76.0	d	3.71	49	30.8	t	1.56, 1.89		30.6	t	1.56, 1.89
17	75.5	d	OH	3.12	35	75.5	d	3.11	50	27.3	t	2.15, 2.60	28	27.1	t	2.12, 2.57
18	74.5	d	OR	4.46	23	74.5	d	4.41	51	23.8	q	1.77	V3-Ac	23.5	q	1.77
19	74.2	d	OH	3.47		74.2	d	4.88	52	23.1	q	1.53	V3-Me	23.0	q	1.50
20	74.1	d	OH	4.93	29	73.8	d	3.45	53	20.9	t	1.05, 1.09	48	21.0	t	1.06, 1.58
21	71.7	d	OH	3.93	21	71.6	d	3.88	54	19.6	t	2.20, 2.26	41	19.5	t	2.19, 2.25
22	70.9	d	OH	3.73	M2	70.6	d	3.73	55	18.6	t	1.25, 1.42	39	18.2	t	1.21, 1.37
23	70.9	d	OH	3.45		70.6	d		56	17.4	q	1.08 (d)	V6	17.3	q	1.05
24	70.7	d	OH	3.45		70.5	d	3.47	57	17.1	q	0.81(d)	43	17.0	q	0.79
25	70.4	d	OH	3.62	V4	70.4	d	3.61	58	14.0	q	0.85(t)	40	14.1	q	0.85
26	69.0	d	OH	3.43	15	68.9	d	3.43	59	13.4	q	0.93(t)	42	13.4	q	0.91
27	68.4	d	OH	4.04	31	68.5	d	4.01	60	12.0	q	0.79(t)	49	11.9	q	0.76
28	67.1	d	OH	3.37		66.6	d	3.40	61	11.3	q	0.72(d)	50	11.7	q	0.71
29	65.6	d	OH	4.34	19	65.7	d	4.31	62	11.0	q	0.96(d)	44	10.9	q	0.94
30	64.7	d	OR	3.40		64.8	d	3.48	63	9.6	q	1.27(s)	46	9.5	q	1.24
31	63.4	d	OR	3.95	V5	63.3	d	3.92	64	9.4	q	0.65(d)	45	9.3	q	0.62
32	61.6	t	OH	3.48, 3.70	M6	61.2	t	3.49, 3.68	65	9.1	q	0.83(d)	47	9.0	q	0.84
33	53.8	s	-	-	V3	53.8	s	-								

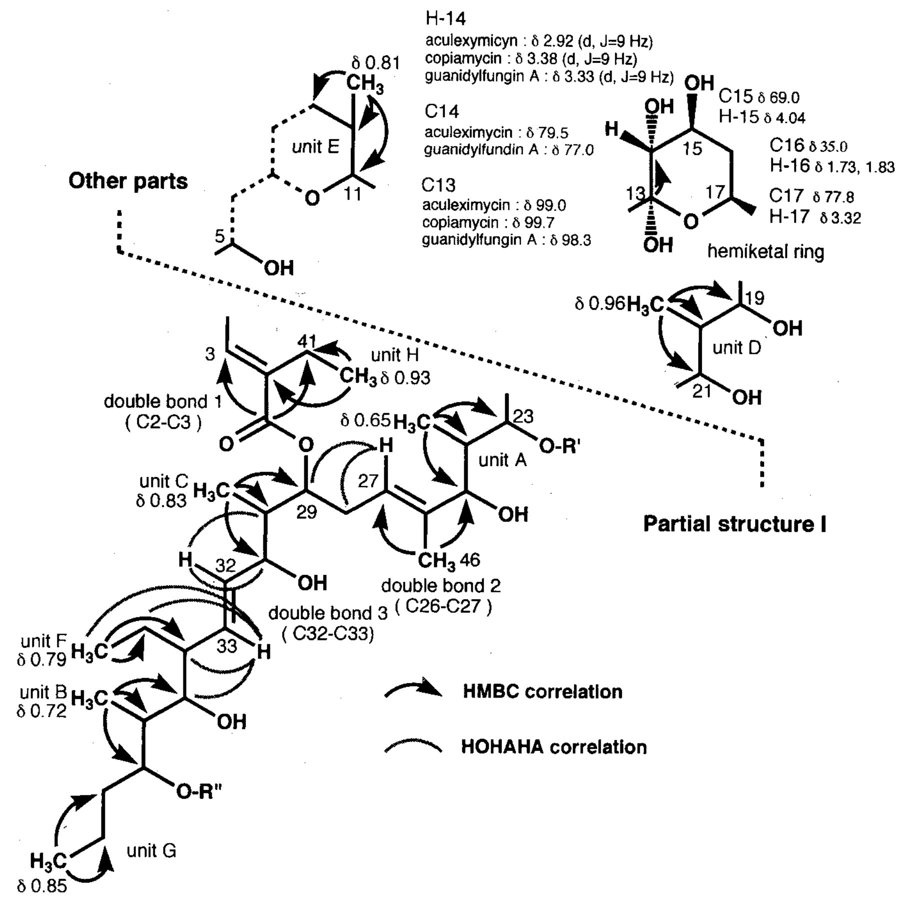
V: vancosamine moiety, M: mannose moiety.

(C-34) carbons, respectively. The oxymethine proton signal at δ 2.92 (d, 9.0 Hz) could be assigned to H-14 and the presence of a six-membered hemiketal ring (Fig. 2) was supported by comparison with the published NMR data for polyol macrolides[2~7,15,16]. In order to assign the 2-methyl-1,3-diol units (A~H), HMBC correlations from eight splitting methyl signals were used. The 1,3-diol units A and C were attached to the double bond units 2 and 3, respectively. Moreover, the appearance of an oxygenated methine proton at δ 4.93 (H-29) suggested that the methine carbon was connected with the double bond unit 1 by an ester linkage. Structural information about another aspect of these olefins was given by HOHAHA correlations from the olefinic protons. A proton signal at δ 5.32 (H-27) in the double bond unit 2 correlated with methylene (δ 2.15, 2.60; H-28ab) and oxymethine (δ 4.93; H-29) protons in the 1,3-diol unit C. Two proton signals around δ 5.40 (H-32, H-33) correlated to oxymethine (H-31) in the 1,3-diol unit C, a methine proton (H-34) in 1,3-diol unit F and oxymethine protons in the 1,3-diol unit B. Although the structure of the backbone (C-4~C-22) corresponding to half of a lactone ring has been unknown because of signal overlapping, we understood the positions of the unit containing three double bonds and the ester linkage. Subsequently, degradative experiments were carried out based on the information mentioned above.

Ozonolysis of *N*-Acetylated Pseudoaglycone I (4)

Compound **4**, on ozonization in methanol followed by decomposition of the ozonide with sodium cyanoborohydride, afforded a mixture of degradation products **6**, **7** and **8** (Fig. 3). Compound **6** gave a protonated molecule, $(M+H)^+$ at m/z 390 and the formula was estimated to be $C_{20}H_{29}NO_6$ by HRFAB-MS. The ^{1}H, ^{13}C NMR and mass spectra of **6** showed that **6** possesses an α-linked L-vancosamine moiety (δ 4.96, br d, 4.4 Hz). The ^{1}H NMR spectra of **6** and its deuteracetyl derivative (**9**) indicated that the structure of **6** is 2-ethyl-4-methyl-1,3,5-heptanetriol with the sugar moiety at C-5. Compound **6** corresponds to the backbone C-33~C-40 of

Fig. 2. Partial structures of *N*-acetylated pseudoaglycone I (**4**) by 2D NMR spectra.



partial structure I.

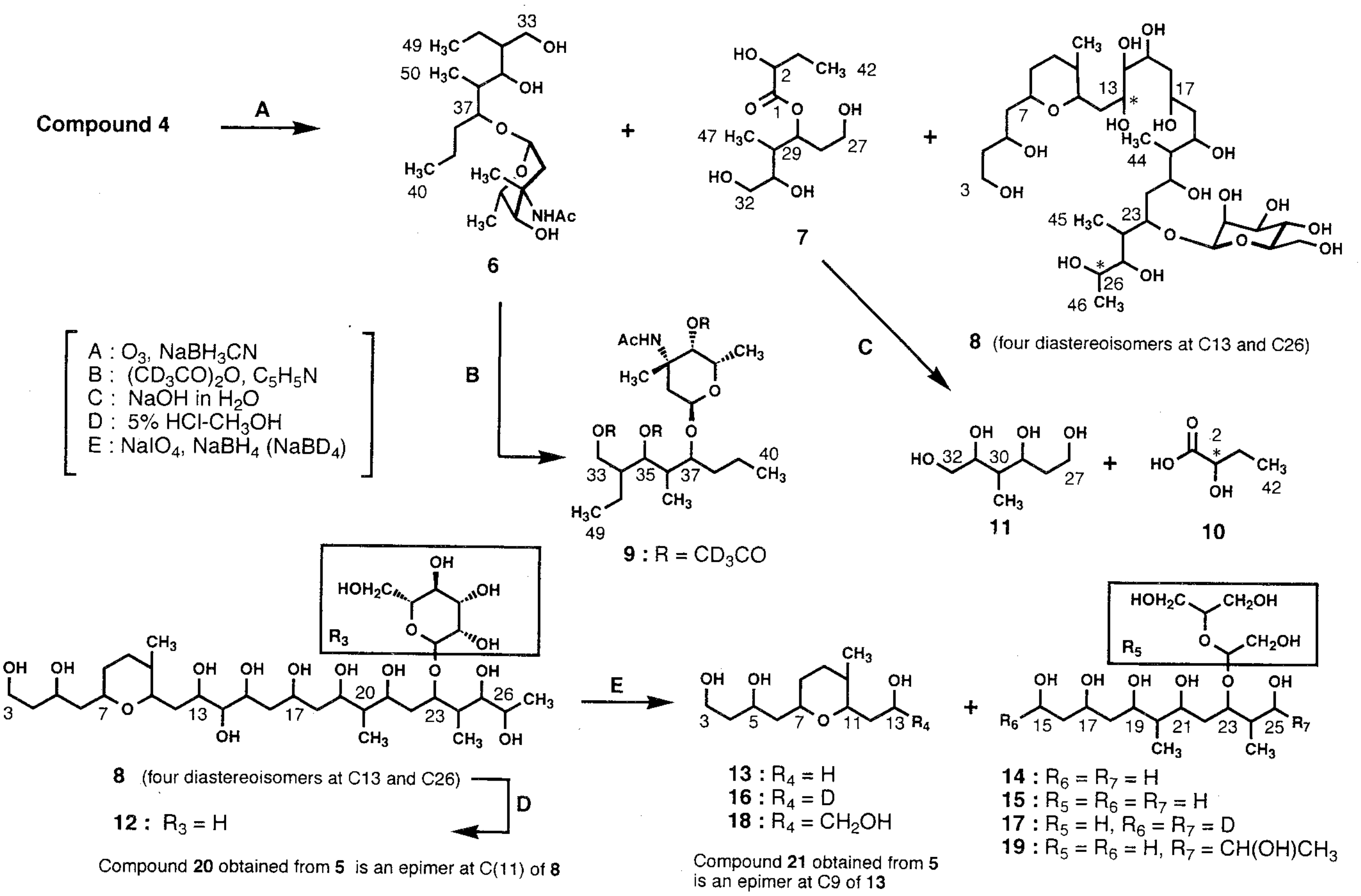
Compound **7** gave the $(M+H)^+$ and $(M+H-CH_3CH_2CH(OH)COOH)^+$ at *m/z* 251 and 147 by FAB-MS, respectively, and it was obtained as a mixture of a few stereoisomers, which were formed by ozonization of the trisubstituted olefin followed by reduction. Each stereoisomer of **7** could not be isolated due to easy hydrolysis of an ester bond and/or acyl migration during their separation. Since the linked position of the ester bond had been elucidated (partial structure I), **7** was subjected to alkaline hydrolysis to yield an acid (**10**) and an alcohol (**11**). Compound **10** was identified as 2-hydroxybutyric acid by GC-MS analysis of its methyl ester derivative. The ^1H and ^{13}C NMR spectra of **11** indicated that it is a single compound and 3-methyl-1,2,4,6-hexanetetraol. Based on partial structure I, the structure of **7** was determined to be an ester of 2-hydroxy butyric acid with 3-methyl 1,2,4,6-hexanetetraol at C-4. Compound **8** was obtained as a mixture of four stereoisomers (**8a**, **8b**, **8c** and **8d**) at C-13 and C-26, of which **8b**, a major product, was isolated by HPLC. The HMBC correlations of **8b** indicated that the linking position of D-mannose is at C-23. Namely, a partial structure between C-23 and C-26 which is a terminal carbon of **8b**, was found by HMBC correlations from two methyl proton signals at C-26 (δ 1.10) and C-24 (δ 0.78), and a cross peak between the anomeric proton of D-mannose at δ 4.83 and the oxymethine carbon at C-23 was observed in the HMBC spectrum. Because **8** had a 1,2-glycol unit at C-25~C-26 and a 1,2,3-triol unit in the six-membered hemiketal ring (C-13~C-17), periodative oxidation of **8** and its methanolysis product (**12**) was carried out.

Periodative Oxidation of 8 and 12

Compound **8** was exhaustively treated with sodium periodate in 50% methanol - water followed by reduction with sodium borohydride to give **13** and **14**. On the other hand, **12** was oxidized with sodium periodate and reduced with sodium borohydride to give **13** and **15** (Fig. 3). The molecular weights of **13**, **14** and **15** were determined to be 232, 414 and 280, respectively.

Compound **13** possessed two oxygenated methylene and three oxygenated methine carbons. Because three

Fig. 3. Degradation scheme for *N*-acetylated pseudoaglycone I (**4**).



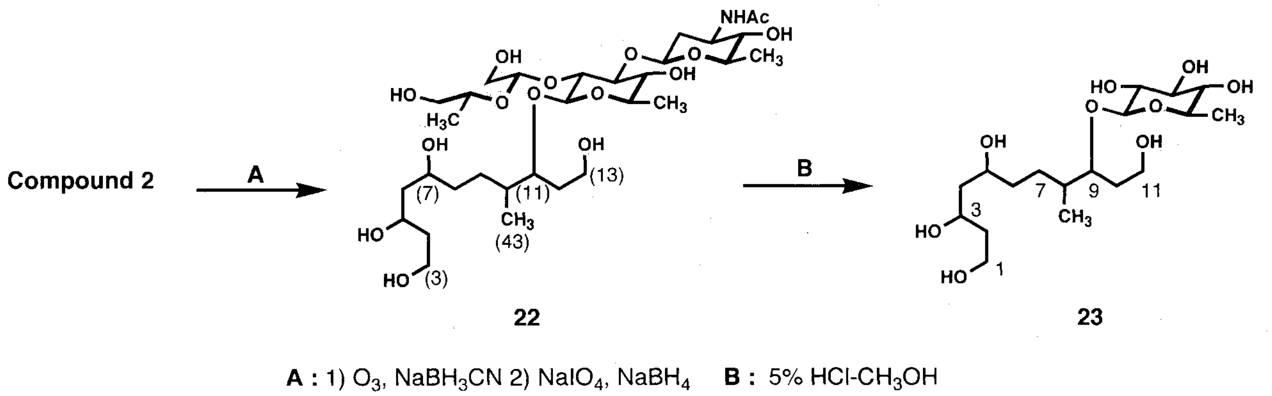
acetyl groups were introduced on acetylation of **13**, a cyclic ether structure such as a tetrahydropyran ring was included in **13**. Indeed, the structure of **13** was determined to be 3,7-anhydro-4-methyl-1,3,7,9,11-undecanpentanol by ^{1}H-^{1}H COSY analysis. The coupling constant (9.5 Hz) between H-10 and H-11 and NOE between H-7 and H-11 indicated that the tetrahydropyran ring exists in a chair conformation and three substituents on the ring are equatorially disposed. The difference in the molecular weights between **14** and **15** corresponded to a $C_5H_{10}O_4$ unit, which was formed by periodative oxidation of the mannose moiety. The linking position of the mannose moiety had been already elucidated to be C-23. Compound **15** was characterized as 1,3,5,7,9,11-undecanhexanol with two methyl groups by ^{1}H and ^{13}C NMR spectral analyses. The positions of the two methyl groups were determined to be C-20 and C-24 by EI-MS fragmentation of **15**.

Structure of *N*-Acetylated Pseudoaglycone I (**4**)

Because **13** ($C_{12}H_{24}O_4$) and **15** ($C_{13}H_{28}O_6$) were derived from **12** ($C_{28}H_{56}O_{12}$) with sodium periodate, a unit of $C_3H_4O_2$ was lost during the degradation step. The structure of **12**, therefore, was established by deuterium labeling and partial glycol bond cleavage experiments. Compound **12** was treated with sodium periodate in 50% methanol-water (1 : 1) followed by reduction with sodium borodeuteride ($NaBD_4$) to give two deuterium labeled products **16** and **17**. Compounds **16** and **17** gave the $(M+H)^+$ at *m/z* 234 and 283 by CI-MS, respectively.

The structure of **16** was determined to be 1-monodeuter-3,7-anhydro-4-methyl-1,3,7,9,11-undecanpentanol, because the appearance of a signal at H-13ab of **16** (δ 3.64, 2H $\times$ 0.5, m) was different from that of **13** (δ 3.67, 2H, t), but other signals resembled each other. In the same manner, **17** was characterized as 1,11-dideuter-2,6-dimethyl-1,3,5,7,9,11-undecanhexanol, because the appearance of a signal at H-15ab (δ 3.68, 2H $\times$ 0.5, br t), H-25a (δ 3.42, 1H $\times$ 0.5, br d) and H-25b (δ 3.59, 1H $\times$ 0.5, br d) of **17** was different from that of **15** (δ 3.70: 2H, t, δ 3.44: 1H, dd, and δ 3.61: 1H, dd, respectively), but other signals resembled each other. These deuterium labeling experiments showed that an oxymethylene at

Fig. 4. Degradation products of *N*-acetylated aculeximycin (**2**).



C-13 of **13** and two oxymethylenes at C-15 and C-25 of **15** were newly formed by periodate oxidation, and an oxymethylene at C-3 of **13** had been formed by ozonolysis.

In order to obtain degradation products without the loss of $C_3H_4O_2$, **12** was partially oxidized with 1.5 moles of sodium periodate to give **18** and **19** in addition to **13** and **15**. The molecular weight of **18** was found to be 262, which is 30 amu larger than that of **13**. Deuteracetylation of **18** indicated the presence of four hydroxy groups in the molecule. The ^1H NMR spectrum of **18** was similar to that of **13**, in which eight proton signals (H-3ab at δ 3.68, H-5 at δ 3.86, H-7 at δ 3.49, H-11 at δ 3.12, H-13 at δ 3.83, H-14a at δ 3.44 and H-14b at δ 3.51) were observed at δ 3.0~4.0. By a decoupling experiment, the structure of **18** was determined to be 4,8-anhydro-5-methyl-1,2,4,8,10,12-dodecanhexanol, which corresponds to **13** with a $-CH_2OH$ unit at C-13. The molecular weight of **19** was found to be 324, which is 44 amu larger than that of **15**. A doublet signal at δ 1.20 (C-26~CH_3) and multiplet signals at δ 4.25 (H-25) and 3.82 (H-26) were observed in the ^1H NMR spectrum of **19** instead of oxymethylene proton signals at δ 3.61 and 3.44, which correspond to the signals at H-25ab in **15**. The structure of **19**, therefore, was characterized to be 6,10-dimethyl-1,3,5,7,9,11,12-tridecanheptanol, which corresponds to **15** with a $-CH(OH)CH_3$ unit at C-25. As a result of these experiments, the structure of **12** was determined by connecting C-14 of **18** to C-15 of **19**. Consequently, we could assemble the partial structures I and **8** (or **12**) into the structure of **4**.

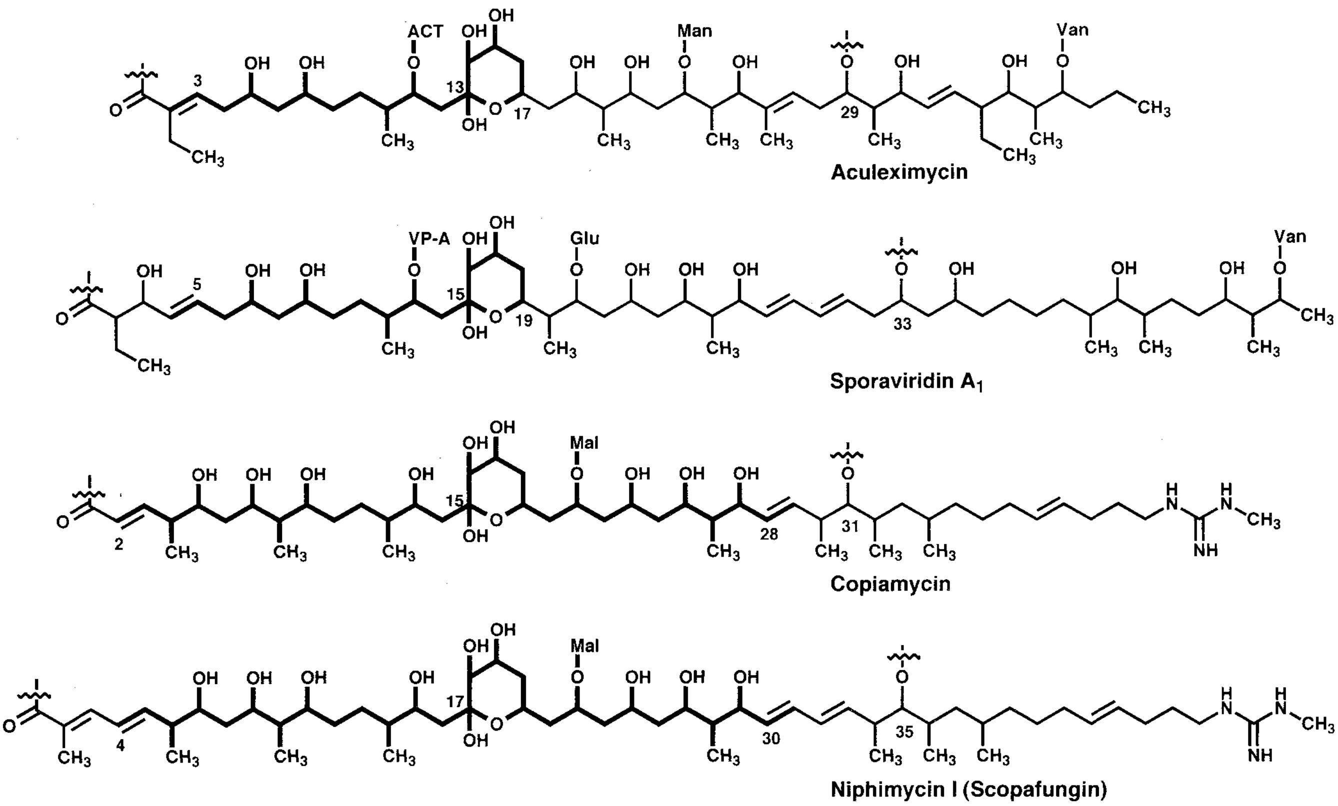
Structure of Aculeximycin (**1**)

Ozonolysis of **5** yielded **20** (MW 746) in addition to **6** and **7**, the same degradation products from **4**. Treatment of **20** with sodium periodate followed by sodium borohydride gave **21** (MW 232) and **14**. The structural difference between **4** and **5** was derived from that between **13** and **21**. The ^1H and ^{13}C NMR spectral analysis clarified that **21** is an epimer at C-11 of **13** by comparison with the coupling patterns at H-11 (**21**: dt, 12.1, 4.3 Hz, **13**: dt, 2.2, 9.5 Hz). The location of the remaining glycosidic linkage of **3** was assumed to be at C-11 in consideration of a mechanism of a specific glycosidic bond cleavage with DBU[19] and the resulting epimeric mixture of **4** and **5**. In order to prove the formation of the tetrahydropyran ring by 5% DBU-MeOH treatment, **2** was treated by the sequence of ozonolysis and periodative oxidation to yield **22** in addition to **6** and **7** (Fig. 4). Compound **22** gave the $(M+Na)^+$ ion at *m/z* 708, and the formula was estimated to be $C_{31}H_{59}NO_{15}$ by HRFAB-MS. Treatment of **22** with 5% methanolic hydrogen chloride at room temperature gave **23**, whose molecular formula was found to be $C_{18}H_{36}O_9$ by HRFAB-MS. The ^1H and ^{13}C NMR spectra indicated that **23** is 9-(6-deoxy-β-D-glucopyranosyl)-8-methyl-1,3,5,9,11-undecanpentanol. From this result, it was proved that *N*-acetylated aculexitriose (**3**) is connected at C-11 of **2** with a β-anomeric linkage. Thus, the total structure of aculeximycin was determined as shown in Fig. 1.

Discussion

The structure of aculeximycin (30-membered) is closely related to those of sporaviridins (34-membered). The segment of C-3~C-17 in aculeximycin has a good homology with that of C-5~C-19 in sporaviridins (Fig. 5). This new class of macrolides possesses an intramolecular hemiketal, an oligosaccharide, which is located at the β-position of a hemiketal carbon, a neutral sugar and an amino sugar (L-vancosamine) at the end of the side chain. On the other hand, polyol macrolides such as copiamycin (32-membered), azalomycins, guanidylfungins, niphimycin (scopafungin), amycins, shuri-

Fig. 5. Structures of aculeximycin, sporaviridin A_1, copiamycin and niphimycin I (scopafungin).



ACT : aculexitriose, **VP-A** : viridopentaose A, **Man** : mannose, **Glu** : glucose, Van : vancosamine, **Mal** : $COCH_2COOH$

mycin (36-membered) and malolactomycin (40-membered) all consist of a macrocyclic polyhydroxy lactone ring with groups of a malonyl monoester and an intramolecular hemiketal and a side chain with mono-, di- or tri-substituted guanidine as their terminal moiety. It seems that an amino sugar such as L-vancosamine and a neutral sugar in aculeximycin correspond to the guanidyl group and the malonyl monoester in polyol macrolides, respectively. However, in polyol macrolides there is no moiety equivalent for the oligosaccharide moiety of aculeximycin. Another difference in the structural features between aculeximycin and polyol macrolides is the ring size of the macrocyclic lactone, which is related to the position of the ketone group masked as a hemiketal. KOSHINO *et al.* reported that the ketone group is located at the (n/2-1) position in n-membered polyol macrolides[16]. The ketone positions of aculeximycin and sporaviridins could not be adapted for the equation. We consider that the difference in ring size is correlated to the conformation of the macrocyclic lactone. Elucidation of the stereochemistry of aculeximycin using its degradation products is in progress.

Experimental

General Methods

NMR spectra were recorded on a JEOL GX-400, 270, GSX-400 or Bruker ARX-500 NMR spectrometer. HRFAB and FAB mass spectra using xenon were obtained on a JEOL HX-110 spectrometer using a glycerol matrix. EI and CI mass spectra were recorded on a Shimadzu QP-1000 spectrometer. HPLC was carried out on a Shimadzu LC-9A with a Shimadzu SPD-2A spectrometer and/or Erma ERC-7512 as the detector. The separation was performed on a Cosmosil 5C18 or a Nucleosil 5C18 as a column for HPLC analysis.

Isolation and Purification of **1**, **2**, **3**, **4** and **5**

Aculeximycin (**1**), N-diacetylated aculeximycin (**2**), N-acetylated aculexitriose (**3**), N-acetylated pseudoaglycone I (**4**) and II (**5**) were isolated as previously described[21].

Aculeximycin (**1**): white amorphous powder. $[\alpha]_D^{25}$ $+1.75°$ (c 0.4, MeOH). mp $184\sim188°$. UV λ_{max} (EtOH) 218 nm (log ε 3.99). IR (KBr) $3600\sim3100$, 1680, 1640 cm^{-1}. HRFAB-MS: m/z 1673.9660 $(M+H)^+$ (Calcd for $C_{81}H_{145}N_2O_{33}$, 1673.9689). ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.1, 140.8, 139.1, 134.2, 133.8, 131.9, 119.6, 103.1, 101.3, 100.2, 100.1, 97.8, 93.5, 86.1, 79.1, 78.9, 78.6, 76.6, 76.4, 76.4, 75.5, 74.7, 74.1, 73.6, 73.3, 73.1, 72.7, 72.7, 71.7, 71.1, 70.7, 70.5, 70.4, 70.2, 69.9, 69.1, 68.5, 67.2, 66.9, 65.3, 64.0, 63.3, 61.1, 53.6, 51.8, 47.5, 43.9, 43.8, 41.6, 41.0, 38.0, 37.6, 36.9, 36.3, 35.7, 34.8, 34.0, 31.3, 28.3, 27.2, 22.6, 20.6, 19.8, 18.7, 17.8, 17.6, 17.6, 17.1, 14.5, 14.1, 13.5, 12.1, 11.4, 10.6, 10.0, 9.8, 9.1 (four peaks are included in the solvent peaks). ^{1}H NMR (500 MHz, DMSO-d_6) Signals at δ 6.48 (H-3), 2.16 (H-4a), 2.29 (H-4b), 3.74 (H-5), 1.41 (H-6), 3.45 (H-7), 1.35 (H-9), 1.95 (H-10), 3.65 (H-11), 4.33 (H-19), 1.77 (H-20), 3.45 (H-21), 4.57 (H-23), 1.55 (H-24), 3.56 (H-25), 5.24 (H-27), 2.10 (H-28a), 2.56 (H-28b), 4.88 (H-29), 1.76 (H-30), 4.06 (H-31), 5.47 (H-32), 5.36 (H-33), 1.99 (H-34), 3.11 (H-35), 1.87 (H-36), 3.79 (H-37) 1.78 (H-38a), 1.95 (H-38b), 1.20 (H-39a), 1.37 (H-39b), 0.86 (t, C-40-CH_3 (H-40)), 2.21 (H-41ab), 0.92 (t, C-2-CH_3 (H-42)), 0.76 (d, C-10-CH_3 (H-43)), 0.74 (d, C-20-CH_3 (H-44)), 0.61 (d, C-24-CH_3 (H-45)), 1.36 (s, C-26-CH_3 (H-46), 0.83 (d, C-30-CH_3 (H-47)), 1.14 (H-48a), 1.55 (H-48b), 0.79 (t, C-34-CH_3 (H-49)) and 0.71 (d, C-36-CH_3 (H-50)) were clearly assigned.

N-acetylated Pseudoaglycone I (**4**): white amorphous powder. $[\alpha]_D^{25}$ +18.13° (*c* 0.1, MeOH). MP 148~152°. UV λ_{max} (EtOH) 218 nm (log ε 3.98). IR (KBr) 3600~3100, 1680, 1640 cm^{-1}. HRFAB-MS: *m/z* 1276.7760 $(M+H)^+$ (Calcd for $C_{65}H_{114}NO_{23}$, 1276.7750). ^{13}C NMR and ^{1}H NMR see Table 1.

HMBC Correlations of 4 (400 MHz, DMSO-d_6)

The carbonyl carbon signal at δ 165.5 (C-1) was correlated with H-3 (δ 6.45, C-3: δ 136.6) and H-41ab (δ 2.26, 2.20, C-41: δ 19.6). The olefinic carbon signal at δ 140.5 (C-26) was correlated with H-46 (δ 1.27, C-46: δ 9.6), H-27 (δ 5.32, C-27: δ 120.1) and H-25 (δ 3.85, C-25: δ 78.6). The olefinic carbon signal at δ 131.5 (C-32) was correlated with H-31 (δ 4.04, C-31: δ 68.4) and H-33 (δ 5.38, C-33: δ 133.8). The hemiketal carbon signal at δ 99.0 (C-13) was correlated with δ 2.92 (H-14, C-14: δ 79.5).

The doublet methyl signal at δ 0.65 (H-45) was correlated with C-23 (δ 74.5, H-23: δ 4.46), C-24 (δ 42.1, H-24: δ 1.19) and C-25 (δ 78.6, H-25: δ 3.85). The doublet methyl signal at δ 0.72 (H-50) was correlated with C-35 (δ 75.5, H-35: δ 3.12), C-36 (δ 36.7, H-36: δ 1.87) and C-37 (δ 75.6, H-37: δ 3.78). The doublet methyl signal at δ 0.83 (H-47) was correlated with C-29 (δ 74.1, H-29: δ 4.93), C-30 (δ 38.6, H-30: δ 1.71) and C-31 (δ 68.4, H-31: δ 4.04). The doublet methyl signal at δ 0.96 (H-44) was correlated with C-19 (δ 65.6, H-19: δ 4.34), C-20 (δ 41.2, H-20: δ 1.24) and C-21 (δ 71.7, H-37: δ 3.93). The doublet methyl signal at δ 0.81 (H-43) was correlated with C-9 (δ 32.8, H-9: δ 1.12, 1.28), C-10 (δ 34.1, H-10: δ 1.22) and C-11 (δ 78.1, H-11: δ 3.50). The triplet methyl signal at δ 0.79 (H-49) was correlated with C-48 (δ 20.9, H-48: δ 1.09, 1.05) and C-34 (δ 47.5, H-34: δ 2.01). The triplet methyl signal at δ 0.79 (H-49) was correlated with C-48 (δ 20.9, H-48: δ 1.09, 1.05) and C-34 (δ 47.5, H-34: δ 2.01). The triplet methyl signal at δ 0.85 (H-40) was correlated with C-39 (δ 18.6, H-39: δ 1.42, 1.25) and C-38 (δ 31.4, H-38: δ 1.38, 1.29). The triplet methyl signal at δ 0.93 (H-42) was correlated with C-41 (δ 19.6, H-41: δ 2.20, 22.6) and C-2 (δ 135.1).

Ozonolysis of 4, Production of Ozonolysis Products 6, 7 and 8

N-acetylated pseudoaglycone I (**4**) (265 mg) dissolved in methanol (10 ml) was cooled in a dry ice-acetone bath (−78°C). Ozone, produced in a Nippon Ozone model 0-1-2 type ozone generator (80 V), was bubbled through the solution for 5~10 minutes. Excess ozone was then removed with a stream of nitrogen for 2 minutes. Sodium cyanoborohydride (350 mg) was added at −78°C, and the mixture was stirred for 1 hour, then acetic acid (350 μl) was added. After stirring was continued for an additional 30 minutes at room temperature, the solution was evaporated to dryness. The residue was dissolved in water (30 ml) and applied to a C18 silica gel column (22 mm i.d. × 110 mm). The column was washed with distilled water (150 ml) to remove the reducing agent, and then crude ozonolysis products were eluted with methanol (150 ml) to yield 235 mg (**6**, **7** and **8**).

Separation of Ozonolysis Products 6, 7 and 8

The mixture (235 mg) was chromatographed on a TOYOPEARL HW-40 column (11 mm i.d. × 870 mm) with methanol (flow rate 1.0 ml/minute) to yield 204 mg of a mixture of **6** and **8** and 16 mg of a mixture of **7** and **8**. The mixture of **6** and **8** (95.1 mg) was chromatographed on YMC gel AM 120-S50 ODS column (11 mm i.d. × 250 mm) with 75% methanol-water (flow rate 1.0 ml/minute) to yield 32 mg of **6** and 62.3 mg of **8**. The mixture of **7** and **8** was adsorbed on a silica gel column (5 mm i.d. × 60 mm). The column was eluted with chloroform-ethanol (8 : 2) to yield 2.9 mg of **7**, then with methanol to yield 2.6 mg of **8**.

Compound **6**: white amorphous powder. $[\alpha]_D^{25}$ −101.6° (*c* 0.24, MeOH). IR ($CHCl_3$) 3420, 1660, 1510 cm^{-1}. HRFAB-MS *m/z* 390.2854 $(M+H)^+$ (Calcd for $C_{20}H_{40}NO_6$, 390.2856). ^{13}C NMR ($CDCl_3$) δ 170.6 (s), 94.9 (d), 78.6 (d), 75.8 (d), 72.9 (d), 63.8 (t), 63.6 (d), 54.5 (s), 42.9 (d), 37.4 (d), 35.4 (d), 31.6 (t), 24.1 (q), 23.0 (q), 19.2 (t), 17.1 (t), 15.4 (t), 14.1 (q), 12.1 (q), 10.4 (q). ^{1}H NMR ($CDCl_3$) δ 6.15 (1H, s, van C-3-NH), 4.96 (1H, d, J=4.4 Hz, van H-1), 4.21 (1H, q, J=6.6 Hz, van H-5), 3.86~3.73 (3H, m, H-33ab, H-35), 3.70 (1H, d, J=9.8 Hz, H-37), 3.38 (1H, s, van H-4), 2.21 (1H, d, J=14.2 Hz, van H-2a), 1.94 (3H, s, van C-3-CH_3), 1.96~1.93 (1H, m, van H-2b), 1.60~1.30 (8H, m), 1.24 (3H, d, J=6.6 Hz, van H-6), 0.96 (3H, t, J=7.1 Hz, H-49), 0.94 (3H, t, J=7.1 Hz, H-40), 0.75 (3H, d, J=6.8 Hz, H-50), van = vancosamine moiety.

Peracetylation (Perdeuteracetylation) of 6

Compound **6** (10 mg) was treated with acetic anhydride (deuteracetic anhydride)-pyridine (1 : 1) and allowed to stand for 3 hours at room temperature. The solution was concentrated and chromatographed on a silica gel column (5 mm i.d. × 75 mm) with benzene-ethyl acetate (4 : 6) to yield 6.5 mg (9.3 mg) of peracetylated **6** or perdeuteracetylated **6** (**9**).

Peracetylated **6**: EI-MS *m/z* 271 (17%), 228 (51%), 169 (57%), 109 (base peak); CI-MS (NH_3) m/z 516 ($M+H^+$, 29%), 412 (40%), 228 (base peak).

Perdeuteracetylated **6** (**9**): EI-MS m/z 231 (51%), 172 (57%), 109 (base peak). CI-MS (NH_3) m/z 525 ($M+H^+$, 43%), 462 (49%), 231 (base peak). ^{13}C NMR (100 MHz, CD_3OD) δ 172.4 (s), 171.4 (s), 170.6 (s), 169.7 (s), 94.8 (d), 76.6 (d), 75.3 (d), 74.2 (d), 64.2 (t), 63.2 (d), 54.6 (s), 40.8 (d), 36.3 (d), 35.9 (t), 31.3 (t), 24.7 (q), 23.9 (q), 19.8 (t), 18.3 (t), 17.5 (q), 14.0 (q), 12.3 (q), 10.8 (q). ^1H NMR (C_6D_6) δ 5.48 (1H, s, van NH), 5.18 (1H, dd, J=10.1, 2.4 Hz, H-35), 5.03 (1H, d, J=4.4 Hz, van H-1), 4.87 (1H, s, van H-4), 4.17 (1H, q, J=6.4 Hz, van H-5), 4.04 (2H, d, J=6.7 Hz, H-33), 3.70 (1H, dt, J=8.7, × Hz, H-37), 2.65 (1H, d, J=13.8 Hz, van H-2a), 2.19 (1H, dd, J=13.8, 4.7 Hz, van H-2b), 2.10 (1H, m, H-36), 1.85 (3H, s, van C-3-Ac), 1.78 (1H, m, H-34), 1.55 (3H, s, van C-3-CH_3), 1.65~1.14 (6H, H-48ab, H-38ab, H-39ab), 1.06 (3H, d, J=6.4 Hz, van H-6), 0.99 (3H, t, J=6.4 Hz, H-49), 0.79 (3H, t, J=7.4 Hz, H-40), 0.73 (3H, d, J=7.1 Hz, H-50).

Methanolysis of **6**

Compound **6** (10 mg) was dissolved in 1.0 ml of methanolic hydrogen chloride and heated under reflux for 1 hour. The solution was made neutral with silver carbonate, then filtered. The solution was evaporated to dryness and the residue was adsorbed on a silica gel column (5 mm i.d. × 75 mm). The methanolysis product of **6** (4.2 mg) was eluted with benzene-ethyl acetate (3 : 7), followed by methyl vancosaminide (4.3 mg) which was eluted with methanol.

Methanolysis products of **6**: CI-MS (NH_3) m/z 205 ($M+H^+$, base peak), 187 (5%), 169 (10%); EI-MS m/z 143 (9%), 131 (6%), 113 (9%), 103 (26%), 95 (9%), 85 (14%), 73 (40%), 67 (6%), 55 (base peak). The fragment ion at m/z 131 corresponded to the α-cleavage between C-34 and C-35 by the hydroxyl group at C-35. The fragment ion at m/z 73 corresponded to the α-cleavage between C-6 and C-37 by the hydroxyl group at C-37. The fragment ion at m/z 103 corresponded to the α-cleavage between C-35 and C-36 by the hydroxyl group at C-35. Moreover, fragment ions at m/z 113 (131-H_2O), 95 (131-2×H_2O), 85 (103-H_2O) 67 (103-2×H_2O) and 55 (73-H_2O) were observed.

Hydrolysis of **7**

Compound **7** (5 mg) was treated with 5% sodium hydroxide-water for 1 hour under reflux condition. The solution was then acidified with dilute hydrochloric acid-water and extracted with ether. After evaporation of the ether layer residue, methanol and 10% trimethylsilyl diazomethane in hexane were added. After 3 minutes, 2 µl of the reaction solution was analyzed by GC-MS (conditions see below). On the other hand, the water layer was evaporated to dryness, and the residue was chromatographed on a TOYOPEARL HW-40F column with methanol and a silica gel column with chloroform-ethanol (8 : 2) to yield 1.8 mg of **11**.

GC-MS Conditions: Column, Waters Porapak Type Q 80~100 mesh (3 mm i.d. × 1.0 m); Carrier gas, He (flow rate 50 ml/minutes); Column oven temp., 150~200°C (5°C/minute); Injector temp., 300°C; Separator temp., 250°C; Ion source temp., 250°C; Ionization mode, EI, CI (*iso* C_4H_{10}), CI (NH_3). Methyl esters of lactic acid (t_R 10 minutes), 2-hydroxybutyric acid (14.5 minutes) and 3-hydroxybutyric acid (16.5 minutes) were used as standard samples.

Compound **11**: $[\alpha]_D^{25}$ −25.1° (c 0.45, MeOH). CI-MS (*iso* C_4H_{10}) m/z 165 $(M+H)^+$, 147, 129, 111; ^{13}C NMR (100 MHz, CD_3OD) δ 73.8 (d, C-31), 73.4 (d, C-29), 66.1 (d, C-32), 61.2 (t, C-27), 42.1 (t, C-30), 38.7 (t, C-28), 11.1 (t, C-47). ^1H NMR (400 MHz, CD_3OD) δ 3.95 (1H, ddd, J=6.7, 5.7, 2.7 Hz, H-31), 3.72 (1H, dd, J=7.1, 6.1 Hz, H-27), 3.72 (1H, ddd, J=9.8, 7.1, 3.0 Hz, H-29), 3.54 (1H, dd, J=14.8, 6.7 Hz, H-32a), 3.48 (1H, dd, J=14.8, 5.7 Hz, H-32b), 1.80 (1H, ddt, J=14.1, 4.0, 7.1 Hz, H-28a), 1.66 (1H, d-quin, J=2.7, 7.1 Hz, H-30), 1.61 (1H, m, H-28b), 0.9 (3H, d, J=7.1 Hz, H-47).

HPLC analysis and separation of **8**: HPLC analysis was performed on a Develosil ODS-5 column (5 µm, 4.6 mm i.d. × 250 mm) with 40% methanol-water (1.0 ml/minute) as a mobile phase at 35°C (RI detection). Peaks of compound **8** appeared at 12.2 (a), 13.1 (b), 17.6 (c) and 18.6 (d) minutes. Separation of **8** (16.5 mg) was chromatographed on YMC gel AM120-S50 (11 mm i.d. × 920 mm) with 40% methanol-water (0.2 ml/minutes) to yield 5.0 mg of **8b**.

Compound **8b**: HRFAB-MS m/z 747.4392 $(M+H)^+$ (Calcd for $C_{34}H_{67}O_{17}$, m/z 747.4381). ^{13}C NMR (100 MHz, CD_3OD) δ 102.8 (d), 85.3 (d), 79.2 (d), 77.9 (d), 77.7 (d), 77.5 (d), 75.2 (d), 73.1 (d), 72.6 (d), 72.1 (d), 72.1 (d), 69.3 (d), 68.9 (d), 68.6 (d), 68.4 (d), 67.9 (d), 66.8 (d), 63.2 (t), 60.2 (t), 45.1 (d), 45.0 (t), 43.9 (t), 42.9 (t), 41.1 (d), 41.0 (t), 39.8 (t), 38.2 (t), 37.0 (d), 33.9 (t), 33.3 (t), 20.6 (q), 18.2 (q), 11.0 (q), 10.9 (q). ^{13}C NMR (100 MHz, DMSO-d_6) δ 101.1 (d, OR; δ 4.83), 83.0 (d, OR; δ 3.13), 76.6 (d, OR; δ 4.27), 76.2 (d, OH; δ 2.46), 75.4 (d, OR; δ 3.43), 75.2 (d, OH; δ 2.97), 73.8 (d, OR; δ 3.48), 70.8 (d, OH; δ 3.42), 70.7 (d, OH; δ 3.61), 70.7 (d, OH; δ 3.69), 70.0 (d, OH; δ 3.63), 66.9 (d, OH; δ 3.42), 66.5 (d, OH; δ 4.12), 66.2 (d, OH; δ 3.93), 65.8 (d, OH; δ 3.71), 65.3 (d, OH; δ 3.68), 64.1 (d, OH; δ 3.84), 61.4 (t, OH; δ 3.67, 3.47), 58.0 (t, OH; δ 3.49), 4.38 (d), 4.36 (t), 43.2 (d), 42.3 (d), 40.4 (d), 40.1 (t), 39.0 (d), 37.0 (t; δ 2.08, δ 1.30), 35.3 (d; δ 1.30), 32.3 (t; δ 1.18, 1.72), 31.4 (t; δ 1.18, 1.63), 20.5 (q; δ 1.10), 17.7 (q; δ 0.80), 10.4 (q; δ 0.79), 9.9 (q; δ 0.78).

Methanolysis of **8**

Compound **8** (36.5 mg) was dissolved in 3.0 ml of methanolic hydrogen chloride and heated under reflux for 2 hours. The solution was made neutral with silver carbonate, then filtered. The solution was evaporated to dryness and the residue was adsorbed on a Bond Elut C18 cartridge (6 cc). Methyl mannoside (8.6 mg) was eluted with 20 ml of water, followed by compound **12**

(25.3 mg) which was eluted with 50% methanol - water.

Compound **12b**: FAB-MS *m/z* 607 $(M+Na)^+$, 585 $(M+H)^+$. ^{13}C NMR (100 MHz, CD_3OD) δ 85.2 (d), 79.1 (d), 77.9 (d), 77.7 (d), 77.6 (d), 73.1 (d), 72.4 (d), 70.1 (d), 69.9 (d), 68.6 (d), 68.5 (d), 66.7 (d), 60.2 (t), 45.1 (t), 45.0 (d), 13.7 (t), 43.0 (t), 41.2 (t), 41.0 (d), 40.1 (t), 39.8 (t), 38.2 (t), 37.0 (d), 33.8 (t), 33.3 (t), 20.3 (q), 18.2 (q), 11.5 (q), 11.2 (q).

Preiodative Oxidation of 12

Compound **12** (22.7 mg) was treated with 18.6 mg of sodium periodate in 7 ml of 66% methanol - water and allowed to stand for 1 hour at room temperature. Excess sodium periodate was decomposed with ethylene glycol (20 μl). The solution was diluted with 2 ml of methanol and 12.6 mg of sodium borohydride was added. The solution was stirred for 30 minutes before neutralization with 60 μl of 10% acetic acid and centrifuged for 3 minutes at 2,000 rpm. The supernatant was evaporated to dryness. The residue was adsorbed on a Bond Elut C-18 cartridge. The column was washed with water and the reaction mixtures of **13** and **15** were eluted with methanol. The reaction mixture was chromatographed on a silica gel column with chloroform - methanol - water (75 : 25 : 3) and (65 : 25 : 3) to yield 7.0 mg of **13** and 14.5 mg of **15**. Compound **15** (2.0 mg) was treated with acetic anhydride-d_6 in pyridine to yield 1.9 mg of perdeuteracetylated **15**. Compound **8** (4.6 mg) was degraded using sodium periodate (3.0 mg) in the same manner as **8** to yield 1.6 mg of **13** and 2.2 mg of **14**.

Compound **13**: $[\alpha]_D^{25}$ +23.9° (*c* 0.43, MeOH). HRFAB-MS *m/z* 233.1766 $(M+H)^+$ (Calcd for $C_{12}H_{25}O_4$, 233.1753). EI-MS *m/z* 187 (4.6%), 169 (7.3%), 151 (2.5%), 143 (15.8%), 125 (10.5%), 75 (base peak), 57 (50.9%). ^{13}C NMR (100 MHz, CD_3OD) δ 82.4 (d, C-11), 77.5 (d, C-7), 68.4 (d, C-5), 60.2 (t, C-3), 60.1 (t, C-13), 44.7 (t, C-6), 40.6 (t, C-4), 3.70 (t, C-12), 36.6 (d, C-10), 33.9 (t, C-9), 33.4 (t, C-8), 18.1 (q, C-43). ^1H NMR (400 MHz, CD_3OD) δ 3.93 (1H, m, H-5), 3.69 (2H, t, H-3ab), 3.67 (2H, t, H-13ab), 3.49 (1H, m, H-7), 3.12 (1H, dt, *J*=2.2, 9.5 Hz, H-11), 1.66 (1H, m, H-8a), 1.64 (1H, m, H-4a), 1.79 (1H, m, H-9b), 1.72 (1H, m, H-4b), 1.54 (1H, m, H-6a), 1.52 (1H, m, H-12a), 1.36 (1H, m, H-6b), 1.32 (1H, m, H-8a), 1.30 (1H, m, H-10), 1.27 (1H, m, H-9a), 1.19 (1H, m, H-12b), 0.85 (3H, d, *J*=6.4 Hz, H-43).

Compound **14**: FAB-MS (glycerol) *m/z* 437 $(M+Na^+$, 3.4%), 415 $(M+H^+$, 6.8%), 323 $(M+H-C_3H_8O_3{}^+$, base peak), 281 $(M+H-C_5H_{10}O_4{}^+$, 16.9%). FAB-MS (glycerol + NaCl) *m/z* 437 $(M+Na^+$, base peak), 323 $(M+H-C_3H_8O_3{}^+$, 62.7%), 281 $(M+H-C_5H_{10}O_4{}^+$, 12.9%). ^{13}C NMR (100 MHz, CD_3OD) δ 103.8 (d), 81.3 (d), 75.5 (d), 71.4 (d), 70.1 (d), 67.1 (d), 65.2 (t), 64.8 (t), 63.3 (t), 63.0 (t), 60.3 (t), 45.0 (d), 43.1 (t), 41.5 (t), 41.1 (d), 38.2 (t), 12.4 (q), 11.2 (q). ^1H NMR (400 MHz, CD_3OD) δ 4.80 (1H, dd, man H-1), 4.17 (1H, m, H-23), 4.15 (1H, m, H-19), 3.96 (1H, m, H-21), 3.94 (1H, m, H-17), 3.74 (1H, m, man H-5), 3.71 (2H, t, H-15), 3.64 (1H, dd, H-25a), 3.62 (4H, d, man H-4ab, H-6ab), 3.54 (2H, d, man H-2ab), 3.46 (1H, dd, H-25b), 1.89 (1H, m, H-24), 1.69 (2H, m, H-16), 1.67 (1H, m, H-22a), 1.64 (1H, m, H-18a), 1.52 (1H, m, H-20), 1.45 (1H, m, H-18b), 1.41 (1H, m, H-22b), 0.92 (3H, d, H-45), 0.91 (3H, d, H-44), man : mannose residue.

Compound **15**: EI-MS *m/z* 221 (0.1%), 217 (0.1%), 203 (0.1%), 199 (1.3%), 191 (0.1%), 185 (3.7%), 181 (1.9%), 177 (0.2%), 173 (1.9%), 167 (6.2 %), 163 (1.1%), 159 (1.8%), 155 (4.9%), 149 (1.9%), 141 (19.3%), 137 (2.9%), 133 (1.1%), 123 (7.9%), 119 (4.1%), 115 (80.1%), 101 (base peak), 97 (20.2%), 89 (52.3%), 83 (28.1%), 75 (62.1%), 71 (55.9%), 57 (74.1%). The fragment ion at *m/z* 221 corresponded to the α-cleavage between C-16 and C-17 by the hydroxyl group at C-17. The fragment ion at *m/z* 177 corresponded to the α-cleavage between C-18 and C-19 by the hydroxyl group at C-19. The fragment ion at *m/z* 119 corresponded to the α-cleavage between C-20 and C-21 by the hydroxyl group at C-21. The fragment ion at *m/z* 75 corresponded to the α-cleavage between C-22 and C-23 by the hydroxyl group at C-23. The fragment ion at *m/z* 89 corresponded to the α-cleavage between C-17 and C-18 by the hydroxyl group at C-17. The fragment ion at *m/z* 133 corresponded to the α-cleavage between C-19 and C-20 by the hydroxyl group at C-19. The fragment ion at *m/z* 191 corresponded to the α-cleavage between C-21 and C-22 by the hydroxyl group at C-21. The fragment ion at *m/z* 235, which is not observed, corresponded to the α-cleavage between C-23 and C-24 by the hydroxyl group at C-23. Moreover, fragment ions were observed at *m/z* 203 (221-H_2O), 167 (221-2 × H_2O), 147 (221-3 × H_2O), 159 (177-H_2O), 141 (177-2 × H_2O), 123 (177-3 × H_2O), 101 (119-H_2O), 83 (119-2 × H_2O), 115 (133-H_2O), 97 (133-2 × H_2O), 173 (191-H_2O), 155 (191-2 × H_2O), 137 (191-3 × H_2O), 217 (235-H_2O), 199 (235-2 × H_2O), 181 (235-3 × H_2O) and 163 (235-4 × H_2O). CI-MS (*iso* C_4H_{10}) *m/z* 281 $(M+H^+$, base peak). ^{13}C NMR (100 MHz, CD_3OD) δ 72.1 (d), 69.8 (d), 69.5 (d), 65.9 (t), 60.1 (t), 44.9 (d), 42.6 (d), 43.1 (t), 41.3 (t), 40.1 (t), 11.3 (q), 10.8 (q). ^1H NMR (400 MHz, CD_3OD) δ 4.19 (1H, dt), 3.98 ~ 3.94 (3H, m), 3.76 (2H, t, H-15ab), 3.62 (1H, dd, H-25a), 3.45 (1H, dd, H-25b), 1.75 ~ 1.40 (8H), 0.92 (6H, d, H-44, H-45).

Perdeuteracetylated **15**: ^1H NMR (400 MHz, CD_3OD) δ 5.06 (1H, dt, *J*=9.3, 4.2 Hz, H-19), 4.98 (1H, dt, *J*=10.5, 3.4 Hz, H-23), 4.93 (1H, m, H-17), 4.83 (1H, ddd, *J*=9.8, 6.4, 2.9 Hz, H-21), 4.09 (2H, t, H-15ab), 4.02 (1H, dd, H-25a), 3.88 (1H, dd, H-25b), 2.04 (1H, m, H-24), 1.91 (2H, m, H-16ab), 1.89 (1H, m, H-18a), 1.87 (1H, m, H-20), 1.84 (1H, m, H-22a), 1.81 (1H, m, H-18b), 1.76 (1H, m, H-22b), 0.96 (3H, d, H-45), 0.95 (3H, d, H-44).

Deuterium Labeling Experiment of 12

Compound **12** (13.1 mg) was treated with 28.8 mg of sodium periodate in 5 ml of 66% methanol - water and allowed to stand for 1 hour at room temperature. Excess sodium periodate was decomposed with ethylene glycol

(15 μl). The solution was diluted with 2 ml of methanol and 13.9 mg of sodium borohydride was added. The solution was stirred for 1 hour before neutralization with 300 μl of 10% acetic acid. The solution was evaporated to dryness. The residue was adsorbed on Bond Elut C18 cartridge. The column was washed with water and the reaction mixtures of **16** and **17** were eluted with methanol. The reaction mixture was chromatographed on a silica gel column with chloroform - methanol - water (100 : 25 : 3) and benzene - acetone (1 : 1) to yield 4.3 mg of **16** and 6.3 mg of **17**.

Compound **16**: CI-MS (*iso* C_4H_{10}) *m/z* 234 ($M+H^+$, base peak), ^{13}C NMR (100 MHz, CD_3OD) δ 82.4 (d), 77.5 (d), 68.4 (d), 60.2 (d), (60.0, weak), 44.8 (d), 40.6 (t), 36.9 (t), 36.6 (d), 33.9 (t), 33.4 (t), 18.0 (q). ^1H NMR (400 MHz, CD_3OD) δ 3.92 (1H, m, H-5), 3.68 (2H, t, H-3ab), 3.64 (1H × 0.5, m, H-13ab), 3.48 (1H, m, H-7), 3.11 (1H, dt, H-11), 1.92 (1H, m), 1.80 (1H, m), 1.75 ~ 1.60 (3H), 1.60 ~ 1.48 (2H), 1.38 ~ 1.20 (4H), 0.84 (3H, d, H-43).

Compound **17**: CI-MS (*iso* C_4H_{10}) *m/z* 283 ($M+H^+$, base peak), ^{13}C NMR (100 MHz, CD_3OD) δ 72.2 (d), 68.9 (d), 69.7 (d), 67.0 (d), (66.1, 65.9, 65.7, 65.5: weak), (60.2, 59.9, 59.8, 59.7: weak), 45.2 (d), 43.3 (t), 42.8 (d), 41.5 (t), 40.3 (t), 11.5 (q), 11.0 (q). ^1H NMR (400 MHz, CD_3OD) δ 4.18 (1H, dt, H-21), 3.96 (1H, m, H-23), 3.94 (1H, m, H-17), 3.83 (1H, dt, H-19), 3.68 (2H × 0.5, br t, H-15ab), 3.59 (1H × 0.5, br d, H-25a), 3.42 (1H × 0.5, br d, H-25b), 1.70 ~ 1.60 (5H), 1.60 ~ 1.40 (3H), 0.92 (6H, d, H-44, H-45).

Partial Periodative Oxidation of 12

Compound **12** (33.5 mg) was treated with a 1.5 equivalent amount of sodium periodate in 5 ml of 66% methanol - water and allowed to stand for 1 hour at room temperature. Excess sodium periodate was decomposed with ethylene glycol (20 μl). The solution was diluted with 2 ml of methanol and 16.5 mg of sodium borohydride was added. The solution was stirred for 30 minutes before neutralization with 200 μl of 10% acetic acid and centrifuged for 3 minutes at 2,000 rpm. Fractions A (**13** and **18**, 14.1 mg) and B (**15** and **19**, 12.2 mg) were obtained using a Bond Elut C18 cartridge and a silica gel column (90% chloroform - methanol). Fraction A was chromatographed on a silica gel column with chloroform - methanol (14 : 1) to yield 3.3 mg of **18**, 3.1 mg of **13** and 4.6 mg of the mixture. Fraction B was chromatographed on a silica gel column and eluted with a stepwise gradient of chloroform - methanol (85 : 25), (80 : 20) and (70 : 30) to yield 4.2 mg of **15** and **19**. The mixture was applied to TOYOPEARL HW-40F column chromatography to yield < 1.0 mg of **19**.

Compound **18**: CI-MS (NH_3) *m/z* 263 ($M+H^+$, base peak). ^{13}C NMR (100 MHz, CD_3OD) δ 83.9 (d), 70.6 (d), 72.0 (d), 68.3 (d), 66.8 (t), 60.1 (t), 44.9 (t), 40.7 (t), 37.9 (t), 36.5 (d), 33.8 (t), 33.3 (t), 18.0 (q). ^1H NMR (400 MHz, CD_3OD) δ 3.89 (1H, m, H-5), 3.83 (1H, m, H-13), 3.68 (2H, t, H-3ab), 3.51 (1H, dd, H-14a), 3.49 (1H, m, H-5), 3.44 (1H, dd, H-14b), 3.12 (1H, dt, H-11), 1.90 (1H, ddd, H-12a), 1.78 (1H, m), 1.70 ~ 1.60 (4H), 1.60 ~ 1.40 (2H), 1.40 ~ 1.20 (3H), 0.83 (3H, d, H-43). By irradiation at δ 3.83 (H-13) the splitting patterns at δ 3.51 (H-14a), 3.44 (H-14b), 1.90 (H-12a) were changed to the doublet, doublet and double-doublet, respectively. By irradiation at δ 1.90 (H-12a) the splitting patterns at δ 3.12 (H-11) and 3.83 (H-13) were changed to the triplet and simplified multiplet.

Compound **19**: CI-MS (*iso* C_4H_{10}) *m/z* 325 ($M+H^+$, base peak). EI-MS *m/z* 207 (10.5%), 185 (4.5%), 167 (7.5%), 159 (10.3%), 141 (50.0%), 133 (6.9%), 123 (20.7%), 115 (82.8%), 101 (41.4%), 97 (31.0%), 83 (24.1%), 75 (48.3%), 57 (base peak). The fragment ion at *m/z* 75 corresponded to the α-cleavage between C-24 and C-25 by the hydroxyl group at C-25 or between C-17 and C-18 by the hydroxyl group at C-17. The fragment ion at *m/z* 133 corresponded to the α-cleavage between C-22 and C-23 by the hydroxyl group at C-23. The fragment ion at *m/z* 177, which is not observed, corresponded to the α-cleavage between C-20 and C-21 by the hydroxyl group at C-21 or between C-21 and C-22 by the hydroxyl group at C-21. The fragment ion at *m/z* 119 corresponded to the α-cleavage between C-19 and C-20 by the hydroxyl group at C-19. Moreover, fragment ions were observed at *m/z* 159 (177-H_2O), 141 (177-2 × H_2O), 123 (177-3 × H_2O), 115 (133-H_2O), 97 (133-2 × H_2O), 57 (75-H_2O), 101 (119-H_2O) and 83 (119-2 × H_2O). The structure of 6,10-dimethyl-1,3,5,7,-9,11,12-tridecanheptanol gives fragment ions at *m/z* 159, 141 and 123 by α-cleavage but that of 2,6-dimethyl-1,3,-5,7,9,11,12-tridecanheptanol does not give these ions.

Ozonolysis and Periodative Oxidation of *N*-Acetylated Pseudoaglycone II (5)

Ozonolysis of **5** was achieved in the same manner as that of **4** to give **6**, **7** and **20**, which is an epimer of **8** at C-11. Periodative oxidation of **20** was achieved in the same manner as that of **8** to give **14** and **21**.

Compound **21**: $[\alpha]_D^{25}$ −66.0° (*c* 0.70, MeOH). ^1H NMR (400 MHz, CD_3OD) δ 3.92 (1H, dt, *J* = 12.1, 4.3 Hz, H-11), 3.87 (1H, m, H-5), 3.76 (1H, m, H-7), 3.67 (2H, t, H-3ab), 3.65 (1H, dt, H-13a), 0.74 (3H, d, H-43). ^{13}C NMR (100 MHz, CD_3OD) δ 75.7 (s, C-11), 69.4 (s, C-7), 68.9 (d, C-5), 60.6 (t, C-3), 60.5 (t, C-13), 17.7 (q, C-43).

Ozonolysis and Periodative Oxidation of *N*-Acetylated Aculeximycin (2)

Ozonolysis of **2** was achieved in the same manner as that of **4** to give **6**, **7** and **22**, which is an epimer of **8** at C-11. Periodative oxidation of **22** was achieved in the same manner as that of **8** to give **13** and **23**.

Compound **22**: HRFAB-MS (glycerol) *m/z* 708.3815 $(M+Na)^+$ (Calcd for $C_{31}H_{59}NO_{15}Na$, *m/z* 708.3782).

Compound **23**: $[\alpha]_D^{25}$ −4.44° (*c* 0.23, MeOH). HRFAB-MS (glycerol) *m/z* 397.2445 $(M+H)^+$ (Calcd for $C_{18}H_{37}O_9$, *m/z* 397.2438). FAB-MS (glycerol) *m/z*

397 $(M+H)^+$, 251 $(M+H-C_6H_{10}O_4)^+$. ^{13}C NMR (100 MHz, CD_3OD) δ 104.9 (d, quino C-1), 83.1 (d), 78.0 (d, quino C-3), 77.1 (d, quino C-4), 75.8 (d, quino C-2), 73.1 (d, quino C-5), 71.9 (d), 69.4 (d), 60.1 (t), 45.1 (t), 41.0 (t), 38.5 (d), 36.5 (t), 33.5 (t), 30.0 (t), 18.2 (q, quino C-6), 15.1 (q), quino = quinovose moiety. ^1H NMR (400 MHz, CD_3OD) δ 4.31 (1H, d, J=7.81 Hz, quino H-1), 3.92 (1H), 3.80~3.60 (5H), 3.32 (1H, dd, J=9.3, 9.0 Hz, quino H-3), 3.29 (1H, dd, J=9.0, 6.1 Hz, quino H-5), 3.16 (1H, dd, J=9.3, 7.8 Hz, quino H-2), 2.98 (1H, t, J=9.0 Hz, quino H-4), 1.88 (1H, m, H-8), 1.80~1.50 (7H), 1.69 (1H), 1.40 (1H), 1.25 (3H, d, J=6.1 Hz, quino H-6), 1.20 (1H), 0.91 (3H, d, H-43).

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