Structural Elucidation of Aculeximycin

IV. Absolute Structure of Aculeximycin, Belonging to a New Class of Macrolide Antibiotics

HIDEAKI MURATA^a, ISAKO OHAMA^a, KEN-ICHI HARADA^{*,a}, MAKOTO SUZUKI^a,
TAKAYA IKEMOTO^b, TOSHIRO SHIBUYA^b, TATSUO HANEISHI^c, AKIO TORIKATA^c,
YOSHIKO ITEZONO^d and NOBORU NAKAYAMA^d

^aFaculty of Pharmacy, Meijo University,
Tempaku, Nagoya 468, Japan

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

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

^dNippon Roche Research Center,
200 Kajiwara, Kamakura 247, Japan

(Received for publication January 19, 1995)

Aculeximycin (**1**) produced by *Streptosporangium albidum* possesses a 30-membered polyhydroxy macrocyclic lactone and five sugars including aculexitriose. We have described the determination of the planar structure of *N*-diacetylated aculeximycin (**2**) using degradation products, which were obtained by DBU-methanol treatment, ozonolysis and periodate oxidation. In order to determine the relative and absolute configurations of aculeximycin, first, the relative and absolute configurations of the degradation products **10**, **11**, **12** and **13** were determined. Rychnovsky's method was very useful to determine the relative configurations of these degradation products, and CD exciton chirality and the modified Mosher's methods were applied to determine their absolute configurations. From these results, fourteen out of the twenty asymmetric centers in aculeximycin were determined to be 5*S*, 17*R*, 20*S*, 21*R*, 23*R*, 24*R*, 29*S*, 30*R*, 31*S*, 34*R*, 35*S*, 36*S* and 37*R*. The absolute configurations at C-14 and C-15 on the hemiketal ring were confirmed using **12** obtained by the partial glycol bond cleavage of **9**. Absolute configurations of the remaining asymmetric centers were determined by spectral analysis of **15** and NOE experiment on **1**. From these results, the absolute configuration of **1** was determined to be 5*S*, 7*R*, 10*S*, 11*R*, 14*R*, 15*S*, 17*R*, 19*R*, 20*S*, 21*R*, 23*R*, 24*R*, 25*S*, 29*S*, 30*R*, 31*S*, 34*R*, 35*S*, 36*S* and 37*R*.

In the preceding paper, we described the planar structure of aculeximycin produced by *Streptosporangium albidum*. Aculeximycin (**1**), whose molecular weight is 1672, is a 30-membered macrolide antibiotic with five sugar moieties and twenty chiral centers that exist on the macrolide backbone. The absolute configurations of the sugar moieties have been determined to be *O*-6-deoxy- β -D-glucopyranosyl-(1 $\rightarrow$ 2)-*O*-[3-acetamido-2,3,6-trideoxy- β -D-arabino-hexopyranosyl-(1 $\rightarrow$ 3)]-6-deoxy- β -D-glucose pyranose (aculexitriose), D-mannose and L-vancosamine¹. The planar structure of aculeximycin is closely related to those of sporaviridins². However, the absolute configurations of these compounds have not been determined. In this paper, we report the determination of the absolute configurations at all chiral centers of aculeximycin as shown in Fig. 1. The studies were mainly performed using its degradation products obtained by the reaction sequence presented in Fig. 2^{3,4}) because these degradation products would be

partially available for the determination of the stereochemistry of sporaviridins.

In this study, the following sequence was planned for the establishment of the absolute stereochemistry of **1**: 1) Determination of the relative configurations of the degradation products, 2) Determination of the absolute configurations of the degradation products, 3) Determination of the absolute configurations of the remaining part that is not included in the previously mentioned degradation products, and 4) Establishment of the total absolute stereochemistry of **1** by evaluation of the above information. The degradation products of **1** possess three to six hydroxy and a few methyl groups on their carbon chains. It is difficult to determine the relative configurations by coupling constants in the ¹H NMR spectra of these degradation products because the conformation of the compounds cannot be confirmed. Although the formation of an acetamide from a 1,3-diol and/or a 6-membered hemiketal ring from a 1,3,5-triol

Fig. 1. Absolute configurations of aculeximycin (1) and *N*-diacetylated aculeximycin (2), and its degradation products (3~5). Fig. 2. Degradation procedure for *N*-acetylated pseudoaglycone I (4).

unit had been tried to fix the conformation⁵), it is not always easy to determine the conformation, except for a chair conformation, using ¹H NMR spectroscopy.

SCHREIBER *et al.* found that various per-acetonide derivatives are unsuitable for the ¹H NMR spectral analysis due to the overlap of the key proton resonances⁶).

Recently, RYCHNOVSKY *et al.* reported that the conformation of the 1,3-dioxane ring corresponded to the relative configuration of the 1,3-diol, and the chemical shifts of two acetonide methyl carbons can differentiate between chair (1,3-*syn*-diol) from twisted boat (1,3-*anti*-diol) conformations^{7~10}. Therefore, the relative configurations of the 1,3-diol units in the degradation products were determined using Rychnovsky's method. Additionally, because this method identified the conformation of the 1,3-dioxane rings, it was possible to determine the relative configuration of the methyl groups at C-2 in the 1,3-diols. Namely, in the case of *syn*-1,3-diol with acetonide, the configuration of the methyl group at C-2 can be determined by the coupling constants between H-1 and H-2, and H-2 and H-3, whereas the relative configuration of the methyl group at C-2 can be determined using the nuclear Overhauser effect (NOE) between methyl protons at C-2 and H-1 (or H-3) in the case of the *anti*-1,3-diol acetonide.

For the determination of the absolute configurations of asymmetric carbons bearing a hydroxy group in aculeximycin, modified Mosher's methods^{11,12} and CD exciton chirality were mainly used. The modified Mosher method is based on the evaluation of the different chemical shifts of diastereomeric compounds with (*S*)- and (*R*)- α -methoxy- α -(trifluoromethyl)phenylacetic acid (MTPA) esters. Although this method is very useful to determine the absolute configuration of a carbon with a functional group such as hydroxy and amino groups, all hydroxy groups except the target hydroxy group have to be masked with appropriate protection groups in the case of 1,3-polyols.

To use the CD exciton chirality method, a target compound must be converted into its dibenzoate derivative. It has been reported that the CD exciton chirality method was applied to an acyclic system¹³ and 1,3-diol systems including a primary alcohol, which exhibit the Cotton effect corresponding to the configurations (Table 1)^{14~17}, indicating that the CD exciton chirality method can be used without consideration of conformations. Although NAKANISHI *et al.* applied the CD exciton method to compounds with many chromophores, it was difficult to interpret the CD spectra^{18~20} that we obtained. However, if the relationship of two benzoate chromophores of a 1,3-polyol systems can be clarified, it is not difficult to interpret the CD spectrum. Indeed, the difference CD (DIF CD) method of allyl-polybenzoate systems was successfully applied²¹. Considering these facts, we applied the CD exciton chirality method to the degradation products with

Table 1. The sign of exciton CD cotton effects and absolute configuration of 1,3-ally, 1,3- and 1,2-dibenzoates.

(+) Cotton	(-) Cotton	A-value (average)
		Bz = p-bromobenzoyl : 17 ^{a,b} Bz = p-chlorobenzoyl : 10 ^{a,b}
		Bz = p-bromobenzoyl : 35 ^{a,b} Bz = p-chlorobenzoyl : 20 ^{a,b}
		Bz = p-bromobenzoyl : 3 ^c
		Bz = p-bromobenzoyl : 26 ^b Bz = p-chlorobenzoyl : 22 ^b Bz = benzoyl : 32 ^d
		Bz = p-bromobenzoyl : 20 ^b Bz = p-methoxybenzoyl : 16 ^e

^a N. HARADA *et al.*: J. Am. Chem. Soc. 113: 3842 (1991),

^b N. HARADA *et al.*: 33rd symposium on The chemistry of natural products symposium papers, p. 66 (1991), ^c N. C. GONNELLA *et al.*: J. Am. Chem. Soc. 104: 3776 (1982), ^d F. NISHIDA *et al.*: Chem. Pharm. Bull. 38: 2381 (1991). ^e H. UZAWA *et al.*: J. Org. Chem. 55: 119 (1990).

even-numbers of hydroxy groups and the modified Mosher method to the degradation products with odd-numbers of hydroxy groups.

Results

Absolute Stereochemistry of the Degradation Products **10**, **11**, **12** and **13**

The degradation product **10** possesses four hydroxy groups including two primary alcohols and three asymmetric centers at C-29, C-30 and C-31. The relative configuration at C-29/C-31 was established to be 29,31-*anti* using Rychnovsky's method because the two acetonide methyl carbons and one ketal carbon appeared at δ 23.6, 24.6 and 101.1, respectively, in the ¹³C NMR spectrum of **10a**. The *anti*-acetonide exists in the twisted boat conformation due to the 1,3-diaxial interaction that would be present in either chair conformation. The relative configuration at C-30/C-31 was determined to be 30,31-*syn* because irradiation of methyl protons at C-30 resulted in enhancement of the signals corresponding to H-29 (7.86%) and H-30 (6.63%) in the ¹H NMR spectrum of **10a**. Consequently, the relative configurations of **10** were determined to be 29,30-*anti* and 30,31-*syn*. The absolute configurations of **10** were determined using the CD exciton chirality method with **10b**. The CD spectrum of **10b** showed a large positive first Cotton effect at 247 nm ($\Delta\epsilon$ +27.5) and second negative Cotton effect at 230.8 nm ($\Delta\epsilon$ -12.7), indicating that the absolute

configurations of **10** were 29*S*, 30*S** and 31*R** (the asterisked absolute configuration of the degradation product is reversed in aculeximycin).

Compound **11** possesses four asymmetric centers at C-34, C-35, C-36 and C-37 of the aglycone. The relative configuration at C-35/C-37 was determined to be 35,37-*syn*, and the 1,3-dioxane ring existed in the chair conformation because two acetonide methyl carbons and one ketal carbon appeared at δ 19.4, 30.4 and 98.0, respectively, in the ^{13}C NMR spectrum of **11a**. The vicinal coupling constants between H-35 and H-36 ($J=10.4\text{ Hz}$), and between H-36 and H-37 ($J=7.4\text{ Hz}$) in the ^1H NMR spectrum of **11a** indicated that the three substituents at C-35, C-36 and C-37 are equatorially disposed. Eventually, the relative configurations at C-35/C-36 and C-36/C-37 were determined to be 35,36-*anti* and 36,37-*anti*, respectively. Although the reaction of **11** with 2,2-dimethoxypropane was used to determine the relative configuration at C-34/C-35, the resulting product was not **11b** but **11c**. Therefore, the relative configuration at C-34/C-35 was determined using **7a**, which possesses an acetonide at C-33/C-35. The appearance of two acetonide methyl carbon and one ketal carbon signals at δ 19.0, 30.3 and 98.4, respectively, indicated that the 1,3-dioxane ring of **7a** existed in the chair conformation. The vicinal coupling constants between H-33a and H-34 ($J=1.7\text{ Hz}$), and between H-34 and H-35 ($J=2.1\text{ Hz}$) showed that the substituent at C-34 was axially disposed (34,35-*syn*). From these results, the relative configurations of **7** were determined to be 34,35-*syn*, 35,36-*anti* and 36,37-*anti*. Although the determination of the configuration of **11** was initially attempted using the modified Mosher method, the preparation of **11b** failed as previously described. Consequently, the CD exciton chirality method was applied to **11d**, which was confirmed to be the 1,5-dibenzoate of **11** based on the following spectral data: H-33a (δ 4.50), H-33b (δ 4.38), H-35 (δ 3.63) and H-37 (δ 5.47). The CD spectrum of **11d** showed a negative first Cotton effect at 246.2 nm ($\Delta\epsilon -6.84$) and second positive Cotton effect at 229.4 nm ($\Delta\epsilon +3.89$), indicating that the absolute configurations of **11** were 34*R*, 35*S*, 36*R** and 37*R*⁽²²⁾.

In order to determine the relative configurations of **12**, **12a** having two primary alcohols protected with pivaloyl groups and four secondary alcohols protected with two acetonides, and **12b** having six alcohols protected with three acetonides, were prepared. In the ^{13}C NMR spectrum of **12a**, four acetonide methyl carbons appeared at δ 25.2, 24.8, 24.8 and 24.6, and two ketal carbons appeared at δ 100.9 and 100.6. Therefore, the relative

configurations at C-17/C-19 and C-21/C-23 were determined to be 17,19-*anti* and 21,23-*anti*, respectively. On the other hand, in the ^{13}C NMR spectrum of **12b**, six acetonide methyl carbons were observed at δ 30.5, 30.2, 25.3, 24.2, 19.5 and 19.4, and three ketal carbons were observed at δ 100.8, 98.7 and 98.4. Since the two 1,3-dioxane rings including the primary alcohol exist in chair conformations, the relative configuration at C-19/C-21 was determined to be 19,21-*anti*. Consequently, the relative configurations of the carbons bearing hydroxy groups in **12** were 17,19-*anti*, 19,21-*anti*, and 21,23-*anti*. The relative configurations at C-20 and C-24 were determined using **12b**. As the 1,3-dioxane ring including the primary hydroxy group at C-25 existed in the chair conformation, the relative configuration at C-23/C-24 was determined to be 23,24-*syn* by vicinal coupling constants between H-25a and H-24 ($J=2.3\text{ Hz}$), and between H-25b and H-24 ($J=1.1\text{ Hz}$). Irradiation of methyl protons at C-20 resulted in enhancement of the signals corresponding to H-21 (7.55%) in the difference NOE (DIF NOE) experiment. As the 1,3-dioxane ring including C-19 and C-21 existed in a twisted boat conformation, the relative configuration between C-20 and C-21 was determined to be 20,21-*anti*. Namely, the relative configurations of **12** were determined to be 17,19-*anti*, 19,20-*syn*, 20,21-*anti*, 21,23-*anti* and 23,24-*syn*.

The tetrabenzoate with two pivaloyl esters (**12c**) and the hexabenzoate (**12d**) derivatives were prepared to determine the absolute configuration of **12**. It was considered that **12c** and **12d** exist in the same conformation, because the coupling patterns of the oxymethine proton signals in the ^1H NMR spectrum of **12c** were similar to those in **12d**. The CD spectrum of **12c** showed a positive first Cotton effect at 247.6 nm ($\Delta\epsilon +42.2$) and a negative second Cotton effect at 229.6 nm ($\Delta\epsilon -19.6$) as shown in Fig. 3. Also, the CD spectrum of **12d** showed a positive first Cotton effect at 249.0 nm ($\Delta\epsilon +30.7$) and a negative second Cotton effect at 229.6 nm ($\Delta\epsilon -13.1$). It was expected that the difference of the amplitude of **12c** and **12d** at both Cotton effects is due to the presence or absence of a 1,3-dibenzoate at C-15/C-17 and C-23/C-25. The dibenzoates between C-15 and C-17, and between C-23 and C-25 were expected to exhibit the same sign of Cotton effects, because the relative configurations of all hydroxy groups in **12** were fortunately all *anti*. Actually, the DIF CD spectrum (**12d** - **12c**) showed a negative first cotton effect at 244.6 nm ($\Delta\epsilon -13.9$) and a positive second Cotton effect at 226.6 nm ($\Delta\epsilon +6.9$) as shown in Fig. 3, indicating that

Fig. 3. CD spectra of **12c** and **12d** and the difference CD (**12d**~**12c**) spectrum.

the absolute configurations at C-17 and C-23 of **12** were $17S^*$ and $23R$, respectively. Consequently, the absolute configurations of **12** were $17S^*$, $19R$, $20S$, $21R$, $23R$ and $24R$ (the asterisked absolute configurations of the degradation product is reversed in aculeximycin).

Compound **13** is a triol compound possessing a tetrahydropyran ring. The coupling constant (9.5 Hz) between H-10 and H-11, and NOEs between H-11 and H-7 (6.91%) and between H-11 and the methyl group at C-10 (0.72%) indicated that the tetrahydropyran ring exists in the chair conformation and that the three substituents on the ring are equatorially disposed. Thus the relative configurations at C-7/C-10 and C-10/C-11 of **13** are 7,10-*anti* and 10,11-*anti*, respectively. Although the relative configuration at C-10/C-11 of **13** was determined to be 10,11-*anti*, the relative configuration did not always coincide with the original one in aculeximycin because the chirality at C-11 was an artifact formed by DBU-MeOH treatment. The relative configuration between C-5 and C-7 has remained in question. The absolute configuration at C-5 of **13** was determined to be $5S$ using the modified Mosher method. Compounds **13a** and **13b** were prepared by protection of the two primary alcohols with pivaloyl groups followed by esterification at C-5 with (*R*)- and (*S*)-MTPA chloride, respectively. The absolute configuration at C-5 of **13** could be deduced by comparison of the chemical shifts in the ^1H NMR spectra of **13a** and **13b**. In comparison to **13a** with the (*R*)-MTPA ester, **13b** with the (*S*)-MTPA ester showed significant downfield shifts of H-7 ($\Delta\delta + 0.17$), H-11 ($\Delta\delta + 0.09$), H-13 ($\Delta\delta + 0.03$) and

Fig. 4. Application of the modified Mosher method to (a) **13a** and **13b**.

C-10~CH₃ ($\Delta\delta + 0.02$), and upfield shifts of H-3 ($\Delta\delta - 0.19$) as shown in Fig. 4, indicating that the configuration was S for C-5 of **13**. The assignment was also supported by the CD exciton chirality method for **13c**. The CD spectrum of **13c** showed a negative first Cotton effect at 247.4 nm ($\Delta\epsilon - 9.9$) and second positive Cotton effect at 229.0 nm ($\Delta\epsilon + 4.5$), indicating that the absolute configuration at C-5 was $5S$, because the tetrahydropyran ring exists between C-5 and C-13 and two 4-chlorobenzoates at C-3 and C-5 are not interacting with that at C-13. The relationship between the absolute configuration at C-5 and other chiral centers (C-7 and C-10) will be described later.

From the previously mentioned experiments, fourteen of the twenty asymmetric centers in aculeximycin have been determined as follows: $5S$, $17R$, $19R$, $20S$, $21R$, $23R$, $24R$, $29S$, $30R$, $31S$, $34R$, $35S$, $36S$ and $37R$. However, the absolute configurations at C-7, C-10, C-11, C-14, C-15 and C-25 remained unsolved. In our preceding paper, we described that aculeximycin possesses a six-membered hemiketal ring and four substituents are equatorially disposed on the ring. Namely, the relative

Fig. 5. Degradation procedure for *N*-diacetylated aculeximycin (**2**). Fig. 6. Observed nuclear Overhauser effects of **1** and partial models of **1** with 2*S* and 2*R* configuration.

configurations at C-14/C-15 and C-15/C-17 were *syn* and *anti*, respectively. Since the absolute configuration at C-17 had been determined to be 17*R*, the absolute configurations at C-14 and C-15 were determined to be 14*R* and 15*S*, respectively.

Absolute Configurations at C-7, C-10 and C-11

In order to determine the absolute configurations at C-7, C-10 and C-11, **2** was degraded to yield **15** by ozonolysis and cleavage of the glycols with sodium periodate followed by methanolysis (Fig. 5). The relative configuration at C-5/C-7 of **15** was established to be 5,7-*syn* by the $^{13}\text{C NMR}$ analysis of **15a**, because two acetonide methyl carbons and a ketal carbon appeared at δ 19.7, 30.5 and 98.6, respectively. Because 5*S* and 7,10-*anti* configurations had been determined, the absolute configurations at C-7 and C-10 were determined to be 7*R* and 10*S*, respectively. Moreover, the absolute configuration at C-11 could be determined to be 11*S* using the modified Mosher method for **15b** and **15c** (Fig. 5b).

Absolute Configuration at C-25

The absolute configuration at C-25 was determined

using rotating frame nuclear Overhauser and exchange spectroscopy (ROESY) of **1**. The presence of an NOE between the methyl group at C-26 (δ 1.36) and H-24 (δ 1.55) indicated that the two groups were spatially close (eclipsed) in a preferred conformation of **1** in the $\text{DMSO-}d_6$. Further, a proton signal of H-27 (δ 5.32) correlated with the methyl proton at C-24 (δ 0.61) and the methine proton of H-25 (δ 3.58) in the ROESY spectrum. Although the NOE correlation between H-25 and H-27 was explained in both the 25*R* and 25*S* configurations at C-25, another NOE correlation between H-27 and the methyl proton at C-24 was discrepant in the case of 25*R* configuration (Fig. 6). Consequently, the absolute configuration was deduced to be 25*S*.

Absolute Configuration of **1**

From the results of these experiments, the total absolute configurations of aculeximycin were determined to be 5*S*, 7*R*, 10*S*, 11*R*, 14*R*, 15*S*, 17*R*, 19*R*, 20*S*, 21*R*, 23*R*, 24*R*, 25*S*, 29*S*, 30*R*, 31*S*, 34*R*, 35*S*, 36*S* and 37*R*.

Discussion

Bioactive compounds having 1,3-polyol system are

widely distributed in nature and polyene and polyol macrolides are typical examples. Although the stereochemistries of the polyene macrolide amphotericin B²³⁾ and roxaticin²⁴⁾ have been determined by X-ray analysis, it has been very difficult to determine their structures with correct stereochemistry using conventional methods except for X-ray analysis. This may be due to the lack of available appropriate methodology. As will be described later, many trials for the establishment of the absolute stereochemistry have been carried out for polyene macrolides, whereas no systematic study has been done for polyol macrolides. Although the ozonolysis products of lienomycin were converted into their 6-membered 1,3-dioxane ring analogs to determine the relative configuration of the *sec*-hydroxyl groups, and converted into their 6-membered hemiacetal dibenzoates to establish their absolute configurations by the CD exciton chirality method²⁵⁾, the total absolute configurations of lienomycin have not been determined. The stereochemistry of pimaricin²⁶⁾ and nystatin A₁²⁷⁾ was determined by NOESY and ROESY analyses. This method was based on the proton-proton space contacts with the sugar as an internal chiral probe, but it was not easy to assign overlapped methylene signals in the ¹H NMR spectra. The relative stereochemistry for each 1,3-diol unit in micotycin²⁸⁾ was determined by NOE experiments for the tetrafolmylal derivative of micotycin, and the absolute configurations were established by the skillful synthesis of several 1,3-polyol fragments with the relative configurations.

NAKATA *et al.* reported a determination method for the relative configuration at C-3/C-5/C-7 of 3-hydroxy- δ -lactone and δ -enelactone using the signal pattern in their ¹H NMR spectra²⁹⁾, and MORI *et al.* reported a determination method for the absolute configuration at C-3 of the perbenzoate of 1-hydroxy-3,5,...polyol compounds using the difference CD between the perbenzoate and the corresponding allylic perbenzoate³⁰⁾. The combination of the methods with reiterative degradation enables one to determine the stereochemistry of the 1,3-polyol. For the application of these methods, however, one of the two primary hydroxy groups of a 1,3-polyol fragment must be changed to its carboxylic acid and the other remain as the free hydroxy group. It is probably difficult to apply these methods to the degradation products from aculeximycin.

The structural differences of the 1,3-polyol chain between polyol macrolides including sporaviridins and polyene macrolides is attributed to the presence or absence of a methyl group at C-2 of the 1,3-polyol moiety. Naturally, ozonolysis followed by periodative oxidation of a polyol macrolide gives 1,3-polyol fragments with a methyl group at C-2. Thus, the absolute configuration for the methyl group was required to be determined together with those for the 1,3-polyol for aculeximycin. Considering this point, the method used for lienomycin was suggested for our purpose, but was accompanied by the following difficulties: one must determine the coupling

constants of the methylene protons at C-2 and two oxymethine protons for determination of the relative configuration of the formed acetonide ring with a twisted boat conformation. Furthermore, the isolation of a desirable anomer of the resulting hemiacetal dibenzoates, which shows cotton effects in the CD spectrum, is required and the confirmation of its conformation is also needed for determination of the absolute configuration.

We adopted Rychnovsky's method for the determination of the relative configuration of the 1,3-polyol moieties of aculeximycin. This method is very useful because not only the relative configuration of the 1,3-diol but also the conformation of the acetonide ring (chair or twisted boat conformation) can be determined. Consequently, the relative configurations of the methyl groups at C-2 could also be determined by the coupling constants of the methine signals at C-1, C-2 and C-3 in the case of the chair conformation and by NOE between the methyl and one of the two oxymethine signals in the case of the twisted boat conformation. For the absolute configuration, we applied the CD exciton chirality method and/or the modified Mosher method according to the number of hydroxy groups of the degradation products. The CD exciton chirality method is used for even-numbered hydroxy groups. If the degradation product is applicable to the simple dibenzoate patterns illustrated in Table 1, the absolute configuration can be easily determined. The difference CD method is useful for more complicated cases. On the other hand, the degradation product with odd-numbered hydroxy groups is protected with pivaloyl and/or acetonide groups and a remaining free-secondary hydroxy group is changed to its (*R*)- and (*S*)-MTPA esters. Finally, both derivatives are examined by the modified Mosher method. The methods including NMR spectral and degradative studies for planar structure, Rychnovsky's method for relative structure, and the CD exciton method and modified Mosher's method for absolute structure could be used for other macrolide antibiotics.

Thus, we could determine the total structure of aculeximycin with the absolute stereochemistry as shown in Fig. 1 by a procedure composed of the previously mentioned methods. Aculeximycin is the first and only macrolide antibiotic whose absolute configuration has been determined using these methods. As shown in a preceding paper, the structure of aculeximycin is similar to those of the sporaviridins. For example, the partial structure from C-3 to C-17 of aculeximycin corresponds to that from C-5 to C-19 of the sporaviridins. Indeed, **13** from aculeximycin was identified with that from the sporaviridins based on optical rotation and ¹H NMR spectral data. The partial structure from C-20 to C-27 of sporaviridin is also similar to that from C-18 to C-25 of aculeximycin. The 6-membered hemiketal ring, which may play an important role in determining the conformation of the macrocyclic ring, is common not only to aculeximycin and sporaviridins but also to polyol macrolides, suggesting that this moiety of other com-

pounds may also have the same stereochemistry as that of aculeximycin. The absolute configuration of the sporaviridins and structural relationship between both compounds is being investigated.

Experimental

General

NMR spectra were recorded using a JEOL GX-400, GX-270, GSX-400 or Bruker ARX-500 NMR spectrometer. HRFAB and FAB mass spectra using xenon were obtained using a JEOL HX-110 spectrometer with glycerol or *m*-nitrobenzyl alcohol (NBA) as the 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 column for analysis. UV measurements were performed on a Shimadzu UV-2100 spectrophotometer. UV measurements were performed on 5~15 μ M methanol or ethanol solution and the concentrations were determined on the basis of the following approximate extinction coefficients: dibenzoate, ϵ 42800; tribenzoate, ϵ 64200; tetrabenzoate, ϵ 85600; hexabenzoate, ϵ 128400. CD spectra were recorded from 360~200 nm on a JASCO J-600 spectropolarimeter and DIF CD spectra were calculated using a JASCO J-600 data processing system.

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

The degradation procedure for *N*-acetylated pseudoaglycone I (4) was previously described²⁾. Degradation products 10~13 had been identified.

Compound 10a

Compound 10 (1.5 mg) was dissolved in 0.5 ml of pyridine, and 12 μ l of pivaloyl chloride was added. After 13 minutes, the reaction mixture was evaporated to dryness. The residue was applied on silica gel flash chromatography (benzene-ethyl acetate 1:1) to yield 3.5 mg of 10 with dipivaloyl esters. The dipivaloyl ester was dissolved in 1.0 ml of 2,2-dimethoxypropane, and a catalytic amount of *p*-toluene sulfonate was added. After 2 hours, the reaction mixture was diluted with 4.0 ml of diethyl ether and passed through a short column packed with basic alumina. The eluent was evaporated to yield 2.7 mg of 10a.

EI-MS m/z 357 ($M^+ - CH_3$, 2.4%), 57 (base peak). CI-MS (*iso* C₄H₁₀) m/z 373 ($M + H^+$, <0.5%), 315 ($M + H^+ - (CH_3)_2CO$, base peak). CI-MS (NH₃) m/z 390 ($M + NH_4^+$, 2.0%), 373 ($M + H^+$, 2.1%), 332 ($M + NH_4^+ - (CH_3)_2CO$, 10.0%), 315 ($M + H^+ - (CH_3)_2CO$, base peak). Identification of 10a was carried out by GC-MS analyses. The peak of 10a appeared at 5.1 minutes retention time. Compound 10a was given a ($M^+ - CH_3$) at m/z 357, which is a diagnostic ion for acetonide compounds, using EI. GC-MS conditions: Column; J & W Scientific Megabore DB-5 (1.5 mm i.d. $\times$

30 m), Injection temp.; 250°C, Column oven temp.; from 180°C to 250°C at 5°C/minute, Carrier gas; He (50 ml/minute), Makeup gas; He (20 ml/minute), Interval; 2.0 sec/scan. Ionization mode; EI. ¹³C NMR (100 MHz, CDCl₃) δ 101.1 (s, ketal carbon), 71.3 (d, C-31), 67.3 (d, C-29), 63.6 (t, C-32), 61.1 (t, C-27), 38.5 (d, C-30), 33.4 (t, C-28), 27.3 $\times$ 3 (q, pivaloyl methyl carbons), 27.2 $\times$ 3 (q, pivaloyl methyl carbons), 24.6 (q, acetonide methyl carbon), 23.6 (q, acetonide methyl carbon), 11.3 (q, C-30/CH₃). ¹H NMR (400 MHz, CDCl₃) δ 4.24 (1H, m), 4.20 (1H, m), 4.17 (1H, dd, $J=8.8, 1.3$ Hz), 4.12 (1H, m), 4.08 (1H, dt, $J=2.5, 6.9$ Hz), 3.44 (1H, ddd, $J=8.6, 8.4, 4.1$ Hz, H-29), 1.95 (1H, m, H-28a), 1.82 (2H, m, H-28a, H-30), 1.32 (6H, s), 1.19 (18H, s), 0.85 (3H, d, $J=6.8$ Hz, C-30/CH₃). DIF NOE (400 MHz, CDCl₃) C-30/Me (δ 0.85) irradi; H-29 (7.9%) at δ 3.44 and H-30 (6.6%) at δ 1.82. H-29 (δ 3.44) irradi; C-30/Me (4.3%) at δ 0.85, H-28a (2.4%) at δ 1.95 and H-30 (2.4%) at δ 1.82.

Compound 10b

Compound 10 (2.0 mg) was dissolved in 0.5 ml of pyridine, and 12 μ l of pivaloyl chloride was added. After 13 minutes, the reaction mixture was evaporated to dryness. The residue was applied on silica gel flash chromatography (benzene-ethyl acetate 1:1) to yield 3.0 mg of 10 with dipivaloyl esters. The dipivaloyl ester (3.0 mg) was dissolved in 1.0 ml of pyridine, and a catalytic amount of 4-dimethylamino pyridine and 10 μ l of 4-chlorobenzoyl chloride were added. After 2 hours, the reaction mixture was diluted with 2.0 ml of water and the solution was applied on Bond Elut C18 cartridge. The cartridge was washed with water, 50% methanol-water and 70% methanol-water. The compound 10b was then eluted with methanol and evaporated to dryness. The residue was dissolved in diethyl ether and passed through a short column packed with neutral alumina. The eluent was evaporated to yield 4.4 mg of 10b. CI-MS (NH₃) m/z 626 ($M + NH_4^+$, base peak), 470 ($M + NH_4 - C_7H_5O_2Cl^+$, 13.8%), 453 ($M + H - C_7H_5O_2Cl^+$, 82.8%). UV λ_{max}^{EtOH} 240.5 nm (ϵ 21400 $\times$ 2) c 2.266 $\times$ 10⁻⁶ mol/liter. CD λ_{ext} 247.6 nm (+27.5), 230.8 nm (-12.7), $A = +40.2$. ¹³C NMR (100 MHz, CDCl₃) δ 178.4, 178.1, 164.9, 164.7, 139.7, 139.5, 130.9, 130.9, 128.7, 128.6, 76.7 (C-29), 72.4 (C-31), 70.9 (C-32), 63.8 (C-27), 38.7 (pivaloyl C), 37.8 (C-30), 30.5 (C-28), 27.0 $\times$ 3 (q, pivaloyl Me), 27.0 $\times$ 3 (q, pivaloyl Me), 11.1 (q, C-30/Me). ¹H NMR (400 MHz, CDCl₃) δ 7.88 (2H, d, $J=8.5$ Hz), 7.83 (2H, d, $J=8.5$ Hz), 7.37 (2H, d, $J=8.5$ Hz), 7.29 (2H, d, $J=8.5$ Hz), 5.29 (1H, m, H-29), 5.62 (1H, dt, $J=3.4, 6.1$ Hz, H-31), 4.31 (2H, d, $J=6.1$ Hz, H-32ab), 4.15 (1H, m, H-27a), 4.06 (1H, m, H-27b), 2.33 (1H, m, H-30), 2.12 (2H, m, H-28ab), 1.16 (9H, s, pivaloyl Me), 1.12 (9H, s, pivaloyl Me), 1.19 (3H, d, $J=7.3$ Hz, C-30/Me).

Compound 11a

Compound 11 (2.0 mg) was dissolved in 1.0 ml of pyridine, and 10 μ l of pivaloyl chloride was added. After

15 minutes, the reaction mixture was evaporated to dryness. The residue was chromatographed on silica gel column eluted with benzene-ethyl acetate (7:3) to yield 1.4 mg of **11** with monopivaloyl ester. CI-MS (*iso* C₄H₁₀); *m/z* 289 (M+H⁺, base peak), 271 (M+H⁺+H₂O, 34.5%), 253 (M+H⁺-2H₂O, 20.7%), 169 (M+H⁺-*tert* C₄H₉COOH-H₂O, 34.5%), 151 (M+H⁺-*tert* C₄H₉COOH-2H₂O, 20.7%). The reaction products were dissolved in 1.0 ml of 2,2-dimethoxypropane and catalytic amount of *p*-toluene sulfonate was added. After 2 hours, the reaction mixture was diluted with 5 ml of diethylether and passed through a short column packed with basic almina. The eluent was evaporated to dryness. The residue was chromatographed on a silica gel column eluted with hexane-benzene (8:2) to yield 1.4 mg of **11a**. ¹³C NMR (100 MHz, C₆D₆); δ 98.0 (s, acetonide hemiketal), 74.3 (d, C-33), 73.9 (d, C-35), 64.8 (t, C-33), 40.5 (d, C-34), 38.8 (s, pivaloyl), 35.8 (t, C-38), 35.2 (d, C-36), 30.4 (q, acetonide methyl), 27.5 × 3 (q, pivaloyl methyl), 19.4 (q, acetonide methyl), 18.7 (t, C-39), 18.3 (t, C-49), 14.3 (q, C-40), 12.8 (q, C-49), 11.9 (q, C-50). ¹H NMR (400 MHz, C₆D₆) δ 4.31 (1H, dd, *J*=10.6, 8.9 Hz, H-33a), 4.24 (1H, dd, *J*=10.6, 5.3 Hz, H-33b), 3.65 (1H, dd, *J*=10.4, 1.8 Hz, H-35), 3.34 (1H, ddd, *J*=10.0, 7.4, 2.0 Hz, H-37), 1.60 (1H, m, H-39a), 1.55 (1H, m, H-38a), 1.48 (1H, m, H-38a), 1.47 (1H, m, H-36), 1.46 (3H, s, acetonide methyl), 1.43 (1H, m, H-39b), 1.37 (3H, s, acetonide methyl), 1.35 (1H, m, H-38b), 1.31 (1H, m, H-48b), 1.22 (9H, s, pivaloyl methyl), 0.92 (3H, t, *J*=7.1 Hz, H-40), 0.89 (3H, t, *J*=7.6 Hz, H-49), 0.51 (3H, d, *J*=6.6 Hz, H-50).

Compound 11c

The compound **11** (2.2 mg) was dissolved in 0.3 ml of 2,2-dimethoxypropane, and a catalytic amount of *p*-toluene sulfonate was added. After 3 hours, the reaction mixture was diluted with 5 ml of diethyl ether and passed through a short column packed with basic almina. The eluent was evaporated to dryness. The residue was chromatographed on a silica gel with benzene-hexane (7:3) to yield 1.6 mg of **11c**. EI-MS *m/z* 229 (M⁺-CH₃, 84.8%), 151 (66.7%), 131 (25.8%), 113 (62.0%), 109 (53%), 103 (base peak). CI-MS (*iso* C₄H₁₀) *m/z* 245 (M+H⁺, 43.8%), 229 (M+H⁺-CH₄⁺, 18.2%), 227 (M+H⁺-H₂O, 11.5%), 187 (M+H⁺-C₃H₆O, 81.8%), 169 (M+H⁺-C₃H₆O-H₂O, base peak), 151 (M+H⁺-C₃H₆O-2×H₂O, 23.6%). ¹³C NMR (100 MHz, C₆D₆) δ 98.0 (s), 77.9 (d), 74.3 (d), 64.3 (t), 42.3 (d), 35.6 (t), 35.3 (d), 30.4 (q), 19.6 (q), 18.6 (t), 17.0 (t), 14.3 (t), 30.4 (q), 19.6 (q), 18.6 (t), 17.0 (t), 14.3 (q), 12.7 (q), 11.8 (q). ¹H NMR (400 MHz, C₆D₆) δ 3.80 (1H, dd, *J*=10.7, 6.1 Hz), 3.66 (2H, dd, *J*=10.7, 2.7 Hz), 3.31 (1H, ddd, *J*=10.1, 8.2, 2.3 Hz), 2.26 (1H, OH), 1.8~1.5 (2H, m), 1.5~1.4 (6H, m), 1.37 (3H, s), 1.27 (3H, s), 0.97 (3H, t, *J*=7.3 Hz), 0.90 (3H, d, *J*=7.1 Hz), 0.47 (3H, d, *J*=6.59 Hz).

Compound 7a

Compound **7** (5.0 mg) was dissolved in 0.5 ml of 2,2-

dimethoxypropane, and a catalytic amount of *p*-toluene sulfonate was added. After 3 hours, the reaction mixture was diluted with 5 ml of diethyl ether and passed through a short column packed with basic almina. The eluent was evaporated to dryness. The residue was chromatographed on a silica gel with ethyl acetate-hexane (2:8) and (4:6) to yield 0.9 mg of **7a**. FAB-MS (NBA) *m/z* 412 (M+H⁺-H₂O, base peak). ¹³C NMR (100 MHz, C₆D₆) δ 98.4 (s), 94.4 (d, van C-1), 85.6 (d, C-35), 74.5 (d, C-37), 73.5 (d, van C-4), 64.3 (t, C-33), 62.8 (d, van C-5), 37.0 × 2 (d), 36.8 (d), 35.9 (t), 30.3 (q, acetonide methyl carbon), 29.8 (q, acetyl methyl carbon), 29.0 (q, methyl carbon at van C-3), 19.0 (q, acetonide methyl carbon), 18.9 (t), 16.0 (q, van C-6), 15.6 (t), 14.0 (q), 12.0 (q), 8.3 (q). ¹H NMR (400 MHz, C₆D₆) δ 4.85 (1H, dd, *J*=8.3, 6.1 Hz, van H-1), 4.1~3.9 (4H, H-33a, H-35, H-37, van H-5), 4.00 (1H, s, van H-4), 3.90 (1H, dd, *J*=12.0, 1.7 Hz, H-33b), 2.24 (1H, dd, *J*=14.9, 6.1 Hz, van H-2a), 2.05 (3H, s, acetyl methyl), 1.99 (1H, m, H-36), 1.66 (1H, dd, *J*=14.9, 8.3 Hz, van H-2b), 1.29 (3H, d, *J*=6.4 Hz, van C-6), 1.75 (1H, m, H-48a), 1.55~1.30 (4H, m, H-38, H-39), 1.40 (1H, m, H-48b), 0.99 (3H, t, H-49), 0.96 (3H, t, H-40), 0.75 (3H, d, *J*=7.1 Hz, H-50).

Compound 11d

Compound **11** (2.9 mg) was dissolved in 1.0 ml of pyridine, and 20 μl of 4-chlorobenzoyl chloride and a catalytic amount of 4-dimethylamino pyridine were added. After 18 hours, 2.0 ml of methanol and 1.0 ml of heptane were added, and the reaction mixture was evaporated to dryness. The residue was chromatographed on a silica gel with benzene-ethyl acetate (9:1). The favorable fraction was dissolved in 50% methanol-water and adsorbed on an ODS cartridge. The cartridge was washed with 50%, 70% and 80% methanol-water and eluted with 90% methanol-water to yield 0.8 mg of **11d**. CI-MS (NH₃) *m/z* 500 (M+2+NH₄⁺, 7.2%), 498 (M+NH₄⁺, 10.3%), 283 (M+2+H⁺, 16.4%), 281 (M+H⁺, 24.7%), 465 (M+2+H⁺-H₂O, 20.7%), 463 (M+H⁺-H₂O, 30.2%), 327 (M+2+H⁺-ClC₆H₄COOH, 32.8%), 325 (M+H⁺-ClC₆H₄COOH, base peak). ¹H NMR (400 MHz, CDCl₃) δ 7.95 (4H, d), 7.40 (4H, d), 5.47 (1H, ddd, *J*=10.3, 3.7, 2.9 Hz, H-37), 4.50 (1H, dd, *J*=11.1, 8.2 Hz, H-33a), 4.38 (1H, dd, *J*=11.1, 4.5 Hz, H-33b), 3.63 (1H, dd, *J*=9.8, 2.2 Hz, H-35), 1.80~1.25 (8H), 1.05 (3H, t), 0.95 (3H, d), 0.90 (3H, t). UV λ_{max}^{EtoH} 239.6 nm (ε 42800) *c* 3.8868 10⁻⁵ mol/liter. CD λ_{ext} 246.2 nm (Δε -6.84), 229.4 nm (Δε +3.89) A = -10.73.

Compound 12a

Compound **12** (5.4 mg) was dissolved in 1.0 ml of pyridine, and 50 μl of pivaloyl chloride was added. After 10 minutes, the reaction mixture was evaporated to dryness. CI-MS (*iso* C₄H₁₀) *m/z* 449 (M+H⁺, base peak), 431 (M+H⁺-H₂O, 25.9%), 413 (M+H⁺-2H₂O, 40.7%), 395 (M+H⁺-3H₂O, 10.3%). The residue was dissolved in 1.0 ml of 2,2-dimethoxypropane and a catalytic amount of *p*-toluene sulfonate was added. The reaction mixture was diluted with 5.0 ml of diethyl

ether and passed through a short column packed with basic alumina. The eluent was evaporated to dryness and the residue was chromatographed on a silica gel column eluted with benzene-ethyl acetate (9:1). The favorable fraction was chromatographed on silica gel eluted with benzene-ethyl acetate (95:5) to yield 2.5 mg of **12a**. CI-MS (*iso* C₄H₁₀) *m/z* 513 (M+H⁺−CH₄, 3.0%), 471 (M+H⁺−(CH₃)₂CO, 43.4%), 413 (M+H⁺−2×(CH₃)₂CO, base peak). FAB-MS (NBA) *m/z* 529 (M+H⁺, 10.0%), 513 (M+H⁺−CH₄ 8.3%), 471 (M+H⁺−(CH₃)₂CO, 15.0%), 413 (M+H⁺−2×(CH₃)₂CO, 28.3%), 57 (base peak). ¹³C NMR (100 MHz, CDCl₃) δ 100.9 (s, acetonide ketal), 100.6 (s, acetonide ketal), 67.7 (d), 67.5 (d), 66.4 (t), 64.9 (d), 64.4 (d), 61.5 (t), 4.36 (d), 38.0 (d), 36.5 (t), 35.7 (t), 35.6 (t), 27.6×6 (q, pivaloyl methyl), 25.2 (q, acetonide methyl), 24.8 (q, acetonide methyl), 24.8 (q, acetonide methyl), 24.6 (q, acetonide methyl), 12.4 (q), 8.6 (q). ¹H NMR (400 MHz, CDCl₃) δ 4.31 (1H, ddd, *J*=9.8, 7.0, 2.5 Hz), 4.23 (1H, ddd, *J*=11.8, 8.0, 5.9 Hz), 4.20 (1H, dt, *J*=11.8, 6.0 Hz), 4.12 (1H, dd, *J*=11.5, 6.2 Hz), 4.02 (1H, dd, *J*=11.5, 6.0 Hz), 3.75~3.88 (3H, m), 1.80 (1H, m), 1.75~1.55 (4H, m), 1.50~1.25 (3H, m), 1.50~1.46 (12H, s, acetonide methyl), 1.20 (18H, s, pivaloyl methyl), 0.86 (3H, d, *J*=7.1 Hz), 0.98 (3H, d, *J*=6.8 Hz).

Compound **12b**

Compound **12** (5 mg) was dissolved in 1.0 ml of 2,2-dimethoxypropane, and catalytic amount of *p*-toluene sulfonate was added. After 1 hour, the reaction mixture was diluted with 5.0 ml of diethyl ether and passed through a short column packed with basic alumina. The eluent was evaporated to dryness and the residue was chromatographed on a silica gel with benzene-ethyl acetate (9:1) to yield 4.7 mg of **12b**. EI-MS *m/z* 385 (M⁺−CH₃, 9.5%), 327 (M⁺−CH₃−(CH₃)₂CO, 1.1%), 129 (C₇H₁₃O₂⁺, 40.3%), 115 (C₆H₁₁O₂⁺, 55.3%), 59 ((CH₃)₂CO+H⁺, base peak). CI-MS (*iso* C₄H₁₀) *m/z* 401 (M+H⁺, 3.2%), 385 (M+H⁺−CH₄, 9.5%), 383 (M+H⁺−H₂O, 5.7%), 343 (M+H⁺−(CH₃)₂CO, 32.1%), 325 (M+H⁺−(CH₃)₂CO−H₂O, 37.9%), 285 (M+H⁺−2×(CH₃)₂CO, base peak), 267 (M+H⁺−2×(CH₃)₂CO−H₂O, 89.7%), 227 (M+H⁺−3×(CH₃)₂CO, 97.6%), 209 (M+H⁺−3×(CH₃)₂CO−H₂O, 91.4%). ¹³C NMR (100 MHz, CDCl₃) δ 100.7 (s), 98.7 (s), 98.3 (s), 71.0 (d, C-21), 67.6 (d, C-23), 66.9 (t, C-25), 65.2 (d, C-17), 64.6 (d, C-19), 60.0 (t, C-15), 40.8 (d), 38.9 (t), 38.1 (t), 33.0 (d), 32.3 (t), 30.5 (q), 30.2 (q), 25.3 (q), 24.2 (q), 19.4 (q), 19.4 (q), 11.6 (q, C-20/Me), 11.1 (q, C-24/Me). ¹H NMR (400 MHz, CDCl₃) δ 4.39 (1H, ddd, *J*=11.0, 5.4, 2.2 Hz, H-19), 4.24 (1H, dt, *J*=10.3, 2.0 Hz, H-23), 4.02 (1H, ddt, *J*=11.7, 9.5, 2.0 Hz, H-17), 3.85 (1H, dd, *J*=11.3, 2.3 Hz, H-25a), 3.69 (1H, dt, *J*=11.7, 2.2 Hz, H-15a), 3.66 (1H, m, H-15b), 3.62 (1H, ddd, *J*=10.3, 8.1, 1.5 Hz, H-21), 3.43 (1H, dd, *J*=11.4, 1.1 Hz, H-25b), 1.36 (1H, m), 1.69 (1H, ddd, *J*=14.0, 10.3, 1.8 Hz), 1.29 (1H, m), 1.30 (1H, m), 1.40 (1H, m), 1.05 (1H, m), 0.93 (1H, m), 1.50 (1H, m), 1.53

(3H, s), 1.50 (3H, s), 1.50 (3H, s), 1.47 (3H, s), 1.39 (3H, s), 1.38 (3H, s), 0.72 (3H, d, *J*=6.8 Hz, C-20/Me), 1.09 (3H, brs, C-24/Me), DIF NOE (400 MHz, CDCl₃); H-17 (δ 4.02) irra; H-15 (2.9%) at δ 3.67 and H-19 (2.2%) at δ 4.39. H-19 (δ 4.39) irra; H-17 (2.3%) at δ 4.02. C-20-Me (δ 0.72) irra; H-21 (7.6%) at δ 3.62. H-21 (δ 3.62) irra; C-20-Me (3.1%) at δ 0.72 and H-23 (1.5%) at δ 4.24. H-23 (δ 4.24) irra; H-21 (4.6%) at δ 3.62 and H-25 (2.2%) at δ 3.85.

Compound **12c**

Compound **12** (5.4 mg) was dissolved in 1.0 ml of pyridine, and 50 μl of pivaloyl chloride was added. After 10 minutes, the reaction mixture was evaporated to dryness. The residue was chromatographed on a silica gel column eluted with benzene-ethyl acetate (3:7). The favorable fraction was dissolved in 1.0 ml of pyridine, and 20 μl of 4-chlorobenzoyl chloride and a catalytic amount of 4-dimethylamino pyridine were added. After 18 hours, 2.0 ml of methanol and 1.0 ml of heptane were added, and the reaction mixture was evaporated to dryness. The residue was chromatographed on silica gel with benzene-ethyl acetate (9:1) to yield 2.0 mg of **12c**. FAB-MS (negative, NBA) *m/z* 1151 (M+NBA−H[−]), isotope pattern 1151:1152:1153:1154:1155:1156:1157=75:57:100:66:65:37 (calcd 64:42:100:60:63:33:20). UV λ_{max}^{MeOH} 240.2 nm (ε 85600) *c* 1.3189×10^{−5} mol/liter. CD λ_{ext}^{MeOH} 248.8 nm (Δε 28.9), 230.4 nm (Δε −16.2), A = +35.1. ¹H NMR (400 MHz, CDCl₃) δ 7.72 (2H, d), 7.69 (2H, d), 7.64 (2H, d), 7.55 (2H, d), 7.19 (2H, d), 7.18 (2H, d), 7.17 (2H, d), 7.01 (2H, d), 5.43 (1H, dt), 5.27 (1H, dt), 5.15 (1H, m), 5.02 (1H, ddd), 4.02~4.01 (2H, m, H-2~H-15), 3.86 (1H, dd, H-25a), 3.81 (1H, dd, H-25b), 2.4~1.8 (8H), 1.2 (18H, pivaloyl), 1.18 (3H, d, C-20-Me), 0.94 (3H, d, C-23-Me).

Compound **12d**

Compound **12** (4.0 mg) was dissolved in 1.0 ml of pyridine, and 20 μl of 4-chlorobenzoyl chloride and a catalytic amount of 4-dimethylamino pyridine were added. After 18 hours, 2.0 ml of methanol and 1.0 ml of heptane were added, and the reaction mixture was evaporated to dryness. The residue was chromatographed on a silica gel with benzene-ethyl acetate (9:1) to yield 2.0 mg of **12d**. FAB-MS (negative, NBA) *m/z* 1259 (M+NBA−H[−]), isotope pattern 1259:1260:1261:1263:1265:1266=55:45:100:70:92:60:53:30 (calcd 44:32:100:66:97:58:52:28). UV λ_{max}^{MeOH} (ε) 240.2 nm (128400) *c* 1.3022×10^{−5} mol/liter. CD λ_{ext}^{MeOH} 248.8 nm (Δε +28.9), 230.4 nm (Δε −16.2), A = +45.1. ¹H NMR (400 MHz, CDCl₃) δ 7.82 (2H, d), 7.80 (2H, d), 7.76 (2H, d), 7.75 (2H, d), 7.67, (2H, d), 7.67 (2H, d), 7.59 (2H, d), 7.28 (2H, d), 7.24 (2H, d), 7.23 (2H, d), 7.19 (2H, d), 7.06 (2H, d), 5.42 (1H, m, H-19), 5.34 (1H, m, H-23), 5.26 (1H, m, H-17), 5.10 (1H, m, H-21), 4.32 (1H, m, H-15a), 4.28 (1H, m, H-15b), 4.17 (1H, dd, H-25a), 4.08 (1H, dd, H-25b), 2.40~2.00 (8H), 1.11 (3H, d, C-20-Me), 1.06 (3H, d, C-24-Me)

DIF CD Spectrum of **12d** and **12c**

CD (**12c**): λ_{ext} 247.6 nm (+42.21), 230.6 nm (−19.62), $A = +61.83$.

CD (**12d**): λ_{ext} 249.0 nm (+30.68), 229.6 nm (−13.11), $A = +43.79$.

DIF CD (**12d** − **12c**): λ_{ext} 244.6 nm (−13.86), 226.6 nm (+6.88), $A = -20.74$.

Compounds **13a** and **13b**

The compound **13** (8.1 mg) was dissolved in 1.0 ml of pyridine and 30 μ l of pivaloyl chloride was added. After 7 minutes, methanol was added to the reaction mixture and evaporated to dryness. The residue was chromatographed on a silica gel with benzene-ethyl acetate (9:1) to yield 6.8 mg of **13** with dipivaloyl esters. CI-MS (*iso* C₄H₁₀) m/z 401 ($M + H^+$, 40.0%), 383 ($M + H^+ - H_2O$, base peak), 299 ($M + H^+ - \text{tert C}_4\text{H}_9\text{COOH}$, 12.1%), 281 ($M + H^+ - H_2O - \text{tert C}_4\text{H}_9\text{COOH}$, 7.6%). The compound (3.4 mg) was dissolved in 0.5 ml of pyridine and 0.5 ml carbon tetrachloride, and 40 μ l of (*R*)-(+)-MTPA-Cl and a catalytic amount of dimethylamino pyridine were added. After 3 hours, the reaction mixture was extracted with ethyl acetate and chromatographed on a silica gel with benzene-ethyl acetate (9:1) to yield 3.0 mg of **13a**. CI-MS (NH₃) m/z 634 ($M + NH_4^+$, base peak), 514 ($M + H^+ - \text{tert C}_4\text{H}_9\text{COOH}$, 2.6%), 383 ($M + H^+ - \text{MTPA} - OH$, 33.4%). ¹H NMR (400 MHz, CDCl₃) δ 7.52 (2H, dd, $J = 7.6, 3.7$ Hz, phenyl of MTPA), 7.40 (3H, m, phenyl of MTPA), 5.35 (1H, m, H-5), 4.18 (1H, ddd, $J = 10.7, 7.3, 4.4$ Hz, H-13a), 4.13 (1H, m, H-13b), 4.11 (1H, m, H-3a), 3.04 (1H, ddd, $J = 11.5, 7.8, 6.4$ Hz, H-3b), 3.54 (3H, s, OCH₃ of MTPA), 3.14 (1H, m, H-7), 2.85 (1H, dt, $J = 2.7, 9.8$ Hz, H-11), 2.10 ~ 1.24 (11H), 1.20 (9H, s, pivaloyl methyl), 1.17 (9H, s, pivaloyl methyl), 0.79 (3H, d, $J = 6.6$ Hz, C-10-CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 129.6, 128.5, 127.3, 80.0, 73.4, 71.5, 61.1, 60.2, 55.5, 39.3, 36.0, 32.7, 32.2, 31.9, 27.19 ($\times 3$), 27.16 ($\times 3$), 17.63.

Preparation of **13b** (4.0 mg) was achieved in the same manner as **13a**. ¹H NMR (400 MHz, CDCl₃) δ 7.52 (2H, dd, $J = 7.6, 3.7$ Hz, MTPA-ph), 7.40 (3H, m, MTPA-ph), 5.35 (1H, m, H-5), 4.21 (1H, ddd, $J = 10.5, 7.3, 4.6$ Hz, H-13a), 4.13 (1H, ddd, $J = 10.5, 8.4, 6.5$ Hz, H-13b), 4.04 (1H, dt, $J = 11.0, 5.6$ Hz, H-3a), 3.85 (1H, ddd, $J = 11.0, 8.3, 5.6$ Hz, H-3b), 3.54 (3H, s, MTPA-OCH₃), 3.31 (1H, m, H-7), 2.94 (1H, dt, $J = 2.4, 7.0$ Hz, H-11), 2.10 ~ 1.85 (4H), 1.80 ~ 1.68 (3HO), 1.40 ~ 1.24 (4H), 1.20 (9H, s, pivaloyl methyl), 1.18 (9H, s, pivaloyl methyl), 0.81 (3H, d, $J = 6.6$ Hz, C-10-CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 204.7, 129.6, 128.4, 127.3, 80.2, 73.8, 71.4, 61.2, 60.0, 55.4, 39.9, 34.9, 32.7, 32.3, 32.0, 27.2 ($\times 6$), 17.6.

Modified Mosher Method

$\Delta\delta = [\delta_{13b} - \delta_{13a}]$ $\Delta\delta$ (H-3a): −0.07, $\Delta\delta$ (H-3b): −0.19, $\Delta\delta$ (H-5): 0.00, $\Delta\delta$ (H-7): +0.17, $\Delta\delta$ (H-11): +0.09, $\Delta\delta$ (H-13a): +0.03, $\Delta\delta$ (H-13b): 0.00. From the results of these data, absolute configuration at C-5 was determined to be 5S configuration.

Compound **13c**

Compound **13** (2.2 mg) was dissolved in 1.0 ml of dry pyridine and 0.1 ml of 4-chlorobenzoyl chloride was added. After 30 minutes, the reaction mixture was evaporated to dryness. The residue was chromatographed on a silica gel to yield 2.0 mg of **13c**. CI-MS (NH₃) m/z 664 ($M + NH_4^+$, 37.9%), 491 ($M + H - C_7H_5O_2Cl^+$, base peak), 335 ($M + H - 2 \times C_7H_5O_2Cl^+$, 34.5%), isotope patterns m/z 664:666 = 1:1, m/z 491:493 = 9:6, m/z 335:337 = 3:1. UV $\lambda_{\text{max}}^{\text{EtOH}}$ 239.3 nm (ϵ 64200), c 130 μ g/100 ml. CD λ_{ext} 247.4 nm (+9.9), 229.0 nm (+4.5), $A = -14.4$.

Ozonolysis of **2**

Compound **2** (200 mg) dissolved in 10.0 ml of methanol was cooled in a dry ice-acetone bath (−78°C). Ozone was bubbled through the solution for 5~10 minutes. Excess ozone was then removed with a stream of nitrogen for 2 minutes. Sodium cyanoborohydride (323.4 mg) was added at −78°C and the mixture was stirred for 1 hour, then acetic acid (325 μ l) was added. After stirring had continued for an additional 30 minutes at room temperature, the solution was evaporated to dryness. The residue was dissolved in water and applied to an ODS silica gel column. The column was washed with distilled water to remove the reducing agent and then crude ozonolysis products were eluted with methanol to yield 211.9 mg. The crude ozonolysis products (211.9 mg) were chromatographed on a TOYOPEARL HW-40 column (22 mm i.d. $\times$ 855 mm) with methanol (flow rate 0.1 ml/minute) to yield 112.2 mg of the favorable fraction. FAB-MS (glycerol) m/z 1228 ($M + H^+$), (glycerol + NaCl) m/z 1250 ($M + Na^+$).

The fraction (72.4 mg) was treated with 271.5 mg of sodium periodate in 1.5 ml of 50% methanol-water and allowed to react for 3 hours at room temperature. Excess sodium periodate was decomposed with ethylene glycol (40 μ l). Sodium borohydride (77.9 mg) was added and the solution was stirred. After 30 minutes, the solution was neutralized with 120 μ l of 10% acetic acid-water and evaporated to dryness. The residue dissolved in water and adsorbed on an ODS silica gel cartridge. The column was washed with water and the reaction mixtures were eluted with methanol to yield 67.2 mg. The reaction mixture (67.2 mg) was chromatographed on a TOYOPEARL HW-40F column (22 mm i.d. $\times$ 890 mm) with 0.4 ml/minute of methanol to obtain 38.9 mg of **14** and 22.7 mg of **12** with the D-mannose part. FAB-MS (glycerol + NaCl) of **14**; m/z 708 ($M + Na^+$). FAB-MS (glycerol + NaCl) of **12** with D-mannose part; m/z 437 ($M + Na^+$).

Compound **14** (8.8 mg) was dissolved in 8 ml of 5% hydrogen chloride and heated under reflux for 4 hours. The reaction mixture was made neutral with silver carbonate then filtered. The solution was evaporated to dryness and the residue was adsorbed on a silica gel column eluted with 80% chloroform-methanol to yield 4.5 mg of **15**. FAB-MS (glycerol) m/z 251 ($M + H^+$), 154,

136. $[\alpha]_D^{MeOH}$ 5.48 (*c* 0.40). ^{13}C NMR (400 MHz, CD_3OD) δ 74.7 (d), 72.8 (d), 70.1 (d), 61.7 (t), 60.9 (t), 45.9, 41.7, 41.3, 37.4, 37.2, 29.9 (q), 6.4 (q).

Compound **15** (4.5 mg) was dissolved in 1.0 ml of pyridine, and 75 μ l of pivaloyl chloride was added. After 2 minutes, 0.3 ml of methanol was added and the reaction mixture was evaporated to dryness. The residue was chromatographed on a silica gel column with benzene-ethyl acetate (3:7) to yield 4.5 mg of **15** with dipivaloyl ester. HRFAB-MS (NBA+PEG#600) m/z 419.3067 ($M+H$)⁺ (Calcd for $C_{22}H_{43}O_7$, m/z 419.3009). Compound **15** with the dipivaloyl ester was dissolved in 1.0 ml of 2,2-dimethoxypropane, and a catalytic amount of *p*-toluene sulfonate was added. After 2 hours, the reaction mixture was diluted with 5.0 ml of diethyl ether and passed through a short column packed with basic alumina. The eluent was evaporated to dryness to yield 2.5 mg of **15a**. FAB-MS (glycerol) m/z 459 ($M+H$ ⁺, 30.4%), 443 ($M+H$ ⁺ - CH_4 , 8.7%), 401 ($M+H$ ⁺ - $(CH_3)_2CO$, base peak), 383 ($M+H$ ⁺ - $(CH_3)_2CO-H_2O$, 39.1%). ^{13}C NMR (100 MHz, $CDCl_3$) δ 98.6 (acetone ketal), 72.4, 69.6, 66.1, 62.3, 60.9, 39.3, 37.4, 36.0, 34.4, 33.2, 30.5 (acetone methyl), 27.8, 27.3 $\times$ 3 (pivaloyl methyl), 27.2 $\times$ 3 (pivaloyl methyl), 19.7 (acetone methyl), 15.5. 1H NMR (400 MHz, $CDCl_3$) δ 4.37 (1H, ddd, $J=11.0$, 8.6, 5.4 Hz), 4.30 (1H, ddd, $J=10.9$, 7.8, 6.1 Hz), 4.23 (1H, ddd, $J=11.0$, 5.7, 5.4 Hz), 4.14 (1H, ddd, $J=10.9$, 5.86, 5.13 Hz), 3.79 (1H, m), 3.61 (1H, m), 3.38 (1H, m), 1.80~1.60 (5H, m), 1.53 (3H, s, acetone methyl), 1.50~1.40 (4H, m), 1.36 (3H, s, acetone methyl), 1.25 (9H, s, pivaloyl methyl), 1.21 (9H, s, pivaloyl methyl), 1.20~1.10 (2H, m), 0.87 (3H, d, $J=6.8$ Hz).

Compounds **15b** and **15c**

Compound **16a** (2.5 mg) was dissolved in 1.0 ml of pyridine, and 1.0 mg of 4-dimethyl amino pyridine was added. After a few minutes, 70 μ l of (*R*)-MTPA-Cl was added and allowed to stand for 2 hours at room temperature. The reaction mixture was evaporated to dryness. The residue was chromatographed on a silica gel column with benzene-ethyl acetate (95:5) to yield 1.9 mg of **15b**. ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 130.1, 129.6, 128.7 $\times$ 2, 128.4, 127.3, 98.5, 69.0, 65.8, 60.7, 60.1, 55.4, 37.1, 36.1, 35.4, 33.8, 30.1, 28.3, 28.0, 27.2, 19.6. 1H NMR (400 MHz, $CDCl_3$) δ 7.51 (2H, m, phenyl of MTPA), 7.40 (3H, m, phenyl of MTPA), 5.15 (1H, m, H-11), 4.14 (3H, m, H-3ab, H-13a), 3.92 (2H, m, H-5, H-13b), 3.73 (1H, m, H-7), 3.52 (3H, m, OCH_3 of MTPA), 1.88 (1H, m, H-12), 1.85 (1H, m, H-10), 1.74 (2H, m), 1.44 (3H, m), 1.38 (3H, s, acetone methyl), 1.34 (3H, s, acetone methyl), 1.19 (18H, s, pivaloyl methyl), 1.14 (3H, m), 0.79 (3H, d, $J=6.8$ Hz, C-10-Me).

In the same manner as (*R*)-MTPA, compound **15c** (4.8 mg), which was the (*S*)-MTPA derivative, was prepared from **15a** (2.5 mg). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.5, 132.3, 129.6, 128.4, 127.4, 98.5, 69.0, 65.8, 60.7, 60.4, 55.4, 37.1, 35.7, 35.4, 33.8, 30.1, 28.2, 27.7, 27.2 $\times$ 6 (pivaloyl methyl), 19.7, 14.3. 1H NMR

(400 MHz, $CDCl_3$) δ 7.51 (2H, m, phenyl of MTPA), 7.40 (3H, m, phenyl of MTPA), 5.15 (1H, m, H-11), 4.14 (3H, m, H-3ab, H-13a), 3.92 (2H, m, H-5, H-13b), 3.73 (1H, m, H-7), 3.52 (3H, m, OCH_3 of MTPA), 1.88 (1H, m, H-12), 1.85 (1H, m, H-10), 1.74 (2H, m), 1.44 (3H, m), 1.38 (3H, s, acetone methyl), 1.34 (3H, s, acetone methyl), 1.19 (18H, s, pivaloyl methyl), 1.14 (3H, m), 0.79 (3H, d, $J=6.8$ Hz, C-10-Me).

References

- 1) MURATA, H.; K.-I. HARADA, M. SUZUKI, T. IKEMOTO, T. SHIBUYA, T. HANEISHI & A. TORIKATA: Structural elucidation of aculeximycin II. Structures of carbohydrate moieties. *J. Antibiotics* 42: 701~710, 1989
- 2) KIMURA, I.; Y. OTA, R. KIMURA, Y. YAMADA, Y. KIMURA, Y. SATO, H. WATANABE, Y. MORI, K.-I. HARADA, M. SUZUKI & T. IWASHITA: Structural investigation of antibiotic sporaviridin XIII. The total structures of *N*-acetylsporaviridins. *Tetrahedron Lett.* 28: 1921~1924, 1987
- 3) MURATA, H.; K. SUZUKI, T. TABAYASHI, C. HATTORI, Y. TAKADA, K.-I. HARADA, M. SUZUKI, T. IKEMOTO, T. SHIBUYA, T. HANEISHI, A. TORIKATA, Y. ITEZONO & N. NAKAYAMA: Structure elucidation of aculeximycin. III. Planar structure of aculeximycin, belonging to a new class of macrolide antibiotics. *J. Antibiotics* 48: 838~849, 1995
- 4) MURATA, H.; N. KOJIMA, K.-I. HARADA, M. SUZUKI, T. IKEMOTO, T. SHIBUYA, T. HANEISHI & A. TORIKATA: Structural elucidation of aculeximycin I. Further purification and glycosidic bond cleavage of aculeximycin. *J. Antibiotics* 42: 691~700, 1989
- 5) PAWALAK, J.; K. NAKANISHI, T. IWASHITA & E. BOROWSKI: Stereochemical studies of polyols from the polyene macrolide lienomycon. *J. Org. Chem.* 52: 296~2901, 1987
- 6) SCHREIBER, S. L.; M. T. GOULET & T. SAMMAKIA: Stereochemical studies of the skipped-polyol polyene macrolide class: NMR studies of a tetraformylal of micotycin A and B. *Tetrahedron Lett.* 28: 6005~6008, 1987
- 7) RYCHNOVSKY, S. D. & D. J. SKALITZKY: Stereochemistry of alternating polyol chains: ^{13}C NMR analysis of 1,3-diol acetoneides. *Tetrahedron Lett.* 31: 945~948, 1990
- 8) RYCHNOVSKY, S. C.; D. J. SKALITZKY, C. PATHIRANA, P. R. JENSEN & W. FENICAL: Stereochemistry of the macrolactins. *J. Am. Chem. Soc.* 114: 671~677, 1992
- 9) RYCHNOVSKY, S. C.; D. G. YANG & P. POWERS: Chair and twist-boat conformations of 1,3-dioxanes: Limitations of molecular mechanics force fields. *J. Org. Chem.* 58: 5251~5255, 1993
- 10) RYCHNOVSKY, S. C.; B. BOGERS & G. YANG: Analysis of two ^{13}C NMR correlations for determining the stereochemistry of 1,3-diol acetoneides. *J. Org. Chem.* 58: 3511~3515, 1993
- 11) OHTANI, I.; T. KUSUMI, M. O. ISHITSUKA & H. KAKISAWA: Absolute configurations of marine diterpenes possessing a xenicane skeleton. An application of advanced Mosher's method. *Tetrahedron Lett.* 30: 3147~3150, 1989
- 12) OHTANI, I.; T. KUSUMI, Y. KASHMAN & H. KAKISAWA: A new aspect of the high-field NMR application of Mosher's method. The absolute configuration of marine triterpene

- sipholenol-A. *J. Org. Chem.* 56: 1296~1298, 1991
- 13) HARADA, N.; A. SAITO, H. ONO, J. GAWRONSKI, K. GAWRONSKA, T. SUGIOKA, H. UDA & T. KURIKI: A CD method for determination of the absolute stereochemistry of acyclic glycols. 1. Application of the CD exciton chirality method to acyclic 1,3-dibenzoate systems. *J. Am. Chem. Soc.* 113: 3842~3850, 1991
- 14) HARADA, N.; A. SAITO, H. ONO, H. UDA, J. GAWRONSKI & K. GAWRONSKA: Determination of the absolute stereochemistry of acyclic glycols by the CD exciton chirality method. 33rd Symposium on the chemistry of natural products symposium papers pp. 464~471, 1991
- 15) GONNELLA, N. C.; K. NAKANISHI, V. S. MARTIN & B. SHARPLESS: General method for determining absolute configurations of acyclic allylic alcohols. *J. Am. Chem. Soc.* 104: 3775~3776, 1982
- 16) NISHIDA, F.; Y. MORI, N. ROKKAKU, S. ISOBE, T. FURUSE, M. SUZUKI, V. MEEVOOTISOM, T. W. FLEGEL, Y. YODHATHAI & S. INTARARUANGSORN: Structure elucidation of glycosidic antibiotics, glykenins, from *Basidiomycetes* sp. II. Absolute structures of unusual polyhydroxylated C26-fatty acids, aglycones of glykenins. *Chem. Pharm. Bull.* 38: 2381~2389, 1990
- 17) UZAWA, H.; Y. NISHIDA, H. OHKUI & H. MEGURO: Application of the dibenzoate chirality method to determine the absolute configuration of glycerols and related acyclic alcohols. *J. Org. Chem.* 55: 116~122, 1990
- 18) WIESLER, W. T. & K. NAKANISHI: Relative and absolute configurational assignments of acyclic polyols by circular dichroism. 1. Rationale for a simple procedure based on the exciton chirality method. *J. Am. Chem. Soc.* 111: 9205~9213, 1989
- 19) WIESLER, W. T. & K. NAKANISHI: Relative and absolute configurational assignments of acyclic polyols by circular dichroism. 2. Determination of nondegenerate exciton coupling interaction by assignment of prochiral aryloxy-methylene protons for ^1H NMR conformational analysis. *J. Am. Chem. Soc.* 112: 5574~5583, 1990
- 20) ZHOU, P.; N. ZHAO, D. N. RELE, N. BEROVA & K. NAKANISHI: A chiroptical/chemical strategy for conformational assignments of acyclic 1,3-skipped polyols: model 1,2,4,6-tetrols. *J. Am. Chem. Soc.* 115: 9313~9314, 1993
- 21) MORI, Y.; Y. KOHCHI, M. SUZUKI & H. FURUKAWA: Determination of absolute stereochemistry of acyclic 1,3-polyols by a difference CD method. *J. Am. Chem. Soc.* 114: 3557~3559, 1992
- 22) KAWAI, M.; U. NAGAI & M. KATSUMI: CD-spectra of *N,N'*-dibenzoylamino acids and related compounds: evidence for the conformation of acyclic diamino compounds. *Tetrahedron Lett.* 1975: 3165~3166, 1975
- 23) MECHINISKI, W.; C. P. SCHAFFNER, P. GANIS & G. AVITABILE: Structure and absolute configuration of the polyene macrolide antibiotic amphotericin B. *Tetrahedron Lett.* 1970: 3875~3876, 1970
- 24) MAEHR, H.; R. YANG L.-N. HONG, C.-M. LIU, M. H. HATADA & L. J. TODARO: Microbial products. 9. Roxaticin, a new oxo pentaene antibiotic. *J. Org. Chem.* 54: 3816~3819, 1989
- 25) JPAWLAK, J.; K. NAKANISHI, T. IWASHITA & E. BOROWSKI: Stereochemical studies of polyols from the polyene macrolide lienomycin. *J. Org. Chem.* 52: 2896~2901, 1987
- 26) LANCELIN, J.-M. & J.-M. BEAU: Stereostructure of pimaricin. *J. Am. Chem. Soc.* 112: 4060~4061, 1990
- 27) LANCELIN, J.-M. & J.-M. BEAU: Complete stereostructure of nystatin A_1 : A proton NMR study. *Tetrahedron Lett.* 30: 4521~4524, 1989
- 28) SCHREIBER, S. L. & M. T. GOULET: Application of the two-directional chain synthesis strategy to the first stereochemical assignment of structure to members of the skipped-polyol polyene macrolide class: mycoticin A and B. *J. Am. Chem. Soc.* 109: 8120~8122, 1987
- 29) NAKATA, T.; N. HARA, K. NAKASHIMA & T. OISHI: Determination of the stereostructure of the δ -lactones of 5,7-dihydroxy-2,3-unsaturated acid by ^1H NMR spectroscopy. *Chem. Pharm. Bull.* 35: 4355~4358, 1987
- 30) MORI, Y.; Y. KOHCHI, M. SUZUKI & H. FURUKAWA: Determination of absolute stereochemistry of acyclic 1,3-polyols by a difference CD method. *J. Am. Chem. Soc.* 114: 3557~3559, 1992