NEW GLUTARIMIDE ANTIBIOTICS, S-632-B_1 AND B_2

II. ISOLATION, PHYSICO-CHEMICAL PROPERTIES AND CHEMICAL STRUCTURE

TOSHIO OTANI, YOSHINORI MINAMI, HIROSHI MATSUMOTO and TERUYOSHI MARUNAKA

Biological Research Laboratory, Taiho Pharmaceutical Co., Ltd., Kawauchi-cho, Tokushima 771-01, Japan

ZHI-XIAN LOU and QI-WEI YU

Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences, Beijing, People's Republic of China

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Antifungal antibiotics S-632-A_1, A_2, B_1 and B_2 were extracted with ethyl acetate from the filtered broth of *Streptomyces hygroscopicus* S-632 and isolated through a combination of conventional and reversed-phase silica gel column chromatography. On the basis of the spectral data, S-632-B_1 and B_2 were found to be new members of the glutarimide family of antibiotics. The chemical structures of these components were elucidated as two stereoisomers of 3-(5,7-dimethyl-8,9-epoxy-2-hydroxy-4-oxo-6-decenyl)glutarimide.

A new soil isolate, *Streptomyces hygroscopicus* S-632, was found to produce four antifungal antibiotics, named S-632-A_1, A_2, B_1 and B_2, which were extracted from the filtered culture. Of these isolated antibiotics, S-632-B_1 (**1**) and B_2 (**2**) were shown to be new glutarimide antibiotics, which demonstrated cytotoxic activity *in vitro* against KB tissue culture cells as well as antifungal activity against *Saccharomyces* sp. S-632-A_1 (**3**) and A_2 (**4**) were identical to the known antifungal antibiotics, streptimidone[1] and 9-methylstreptimidone[2,3], respectively. The taxonomy of the producing organism, fermentation and biological activity of the antibiotics were described in a preceding paper[4]. The present paper describes the isolation, physico-chemical properties and structural elucidation of two new antibiotics.

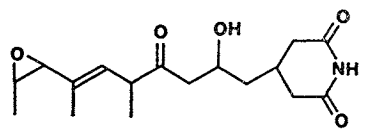
Microorganism and Fermentation

Producing organism and fermentation conditions used were essentially the same as those described in the preceding paper[4].

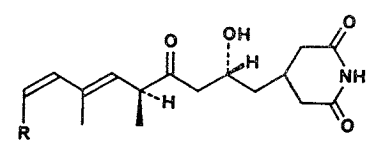
Isolation and Physico-chemical Properties

The fermentation broth was centrifuged and filtered to remove the mycelium. The filtered broth

Scheme 1. Structures of S-632-B_1 (**1**), B_2 (**2**), A_1 (**3**) and A_2 (**4**).



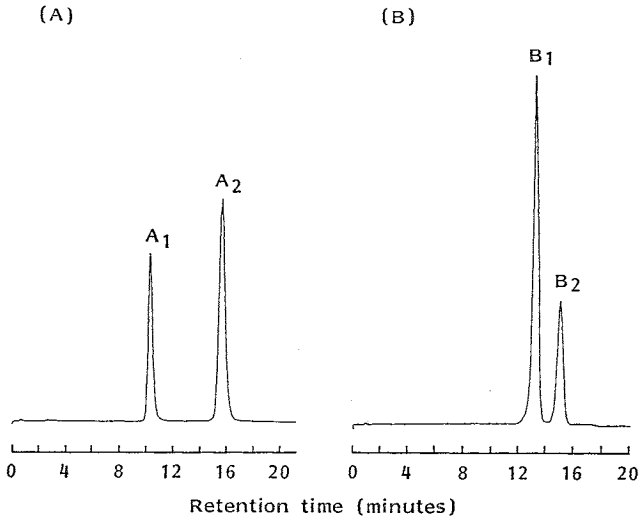
1 (and **2**)



3 (streptimidone) R=H
4 (9-methylstreptimidone) R=CH_3

(12.6 liters) was adjusted to pH 7.6 with 0.1 N sodium hydroxide and was extracted twice with 2 liters of ethyl acetate. The extract was dried over anhydrous sodium sulfate and evaporated to dryness *in vacuo* giving 6.0 g of oily residue. The residue was chromatographed on a silica gel column (45 × 6.0 cm, i.d.) developed with chloroform - acetone (4 : 1). The active eluate was separated into two major fractions. The appropriate active fractions were collected and evaporated *in vacuo* giving Fraction A (3.40 g) and Fraction B (0.42 g). The respective Rf values of Fractions A and B on TLC (Silica

Fig. 1. HPLC chromatograms of S-632-A_1 (3), A_2 (4), B_1 (1) and B_2 (2).



Column: Unisil Q C_{18} (250×4.6 mm, i.d.), mobile phase: (A) acetonitrile - water (20 : 80), (B) acetonitrile - water (35 : 65), flow rate: 1.0 ml/minute, detector: UV 210 nm, apparatus: Shimadzu LC-6A.

Table 1. Physico-chemical properties of S-632-B_1, B_2, streptimidone and 9-methylstreptimidone.

	S-632-B_1	S-632-B_2	Streptimidone[a]	9-Methyl-streptimidone[a]
Appearance	Pale yellowish oil	Pale yellowish oil	Colorless needle	Pale yellowish oil
$[\alpha]_D^{25}$	+68° (*c* 1.0, $CHCl_3$)	+73° (*c* 1.0, $CHCl_3$)	+238° (*c* 0.5, $CHCl_3$)	+105° (*c* 0.1, $CHCl_3$)
Molecular formula	$C_{17}H_{25}NO_5$	$C_{17}H_{25}NO_5$	$C_{16}H_{23}NO_4$	$C_{17}H_{25}NO_4$
Elemental analysis	C 62.76 (63.14)	C 62.59 (63.14)	C 65.66 (65.50)	C 65.88 (66.42)
Found (Calcd)	H 7.71 (7.79)	H 7.91 (7.79)	H 8.10 (7.90)	H 8.60 (8.20)
	N 4.12 (4.33)	N 4.23 (4.33)	N 4.78 (4.78)	N 4.38 (4.56)
HREI-MS				
Found	323.173	323.173	—	—
(Calcd)	(323.173)	(323.173)		
EI-MS (*m/z*)	323 (M^+), 305, 260 209, 198, 180, 125, 109, 97	323 (M^+), 305, 260, 209, 198, 180, 125, 109, 97	293 (M^+), 198, 180, 152, 138, 95, 67	307 (M^+), 225, 198, 180, 152, 109, 96
UV λ_{max} nm (ε)	206 (14,500), 282 (450)	207 (14,800), 280 (610)	232 (23,100), 291 (790)	231.5 (15,350), 283.0 (1,260), 291.0 (1,230)
IR ν_{max} cm^{-1}	3490, 2915, 1710, 1697, 1370, 1245	3350, 2970, 2920, 1725, 1705, 1250	3575, 3425, 1710, 1700, 1680	3475, 3225, 1725, 1710, 1650, 720

[a] These data were obtained from refs 1 and 2.

Table 2. Comparison of NMR spectral data of S-632-B_1 (**1**), B_2 (**2**) and A_2 (**4**).

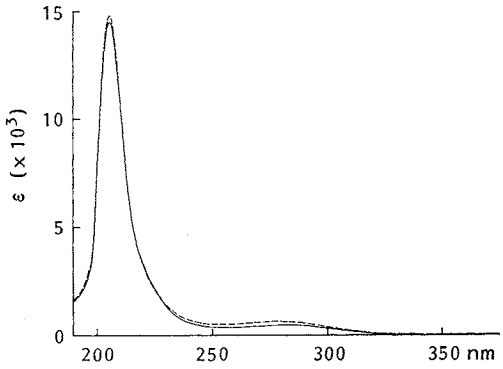
	1		**2**		**4** (9-Methylstreptimidone)[a]	
	^{13}C (25 MHz) ppm	^{1}H (400 MHz) ppm (J, Hz)	^{13}C (25 MHz) ppm	^{1}H (400 MHz) ppm (J, Hz)	^{13}C (25 MHz) ppm	^{1}H (400 MHz) ppm (J, Hz)
1	40.8 (t)	1.30 (ddd, J=13.9, 8.7, 2.6), 1.58 (ddd, J=13.9, 10.5, 4.8)	40.9 (t)	1.34 (ddd, J=14.1, 8.8, 2.1), 1.61 (ddd, J=14.1, 10.4, 4.8)	40.9 (t)	1.34 (ddd, J=14.0, 8.4, 2.0), 1.61 (ddd, J=14.0, 10.4, 4.8)
2	64.6 (d)	4.09 (m)	64.8 (d)	4.11 (m)	64.8 (d)	4.12 (m)
3	47.2 (t)	2.54 (d, J=5.3, 2H)	47.1 (d)	2.57 (dd, J=18.0, 3.4), 2.62 (dd, J=18.0, 8.2)	47.3 (t)	2.57 (dd, J=18.0, 3.2), 2.64 (dd, J=18.0, 8.2)
4	212.1 (s)		212.1 (s)		212.5 (s)	
5	46.4 (d)	3.42 (dq, J=9.8, 6.8)	46.4 (d)	3.43 (dq, J=9.9, 6.8)	46.9 (d)	3.44 (dq, J=9.6, 6.8)
5-CH_3	14.5 (q)	1.18 (d, J=6.8)	14.6 (q)	1.15 (d, J=6.8)	14.7 (q)	1.18 (d, J=6.8)
6	125.0 (d)	5.29 (dm, J=9.8, 1.3)	125.0 (d)	5.29 (dm, J=9.9, 1.3)	127.9 (d)	5.17 (dm, J=9.6)
7	132.3 (s)		132.2 (s)		135.7 (s)	
7-CH_3	16.3 (q)	1.74 (d, J=1.3)	16.4 (q)	1.75 (d, J=1.3)	16.2 (q)	1.85 (dd, J=1.5, 0.7)
8	59.0 (d)	3.35 (dd, J=4.2, 1.3)	58.8 (d)	3.37 (dd, J=4.3, 1.3)	132.7 (d)	5.81 (dm, J=11.7)
9	54.5 (d)	3.19 (dq, J=4.2, 5.3)	54.3 (d)	3.19 (dq, J=4.3, 5.3)	125.3 (d)	5.50 (dq, J=11.7, 7.2)
9-CH_3	12.3 (q)	1.12 (d, J=5.3)	12.0 (q)	1.13 (d, J=5.3)	17.3 (q)	1.78 (dd, J=7.2, 1.5)
2′	172.6 (s)		172.5 (s)		172.7 (s)	
3′	38.4 (t)	2.31 (m), 2.76 (m)	38.4 (t)	2.32 (m), 2.77 (m)	38.4 (t)	2.32 (m), 2.76 (m)
4′	27.1 (d)	2.48 (m)	27.1 (d)	2.48 (m)	27.1 (d)	2.48 (m)
5′	37.1 (t)	2.31 (m), 2.76 (m)	37.2 (t)	2.32 (m), 2.77 (m)	37.1 (t)	2.32 (m), 2.76 (m)
6′	172.6 (s)		172.4 (s)		172.6 (s)	

[a] These data were measured in this study.

gel 60 F_{254}, Merck No. 5715) were 0.57 and 0.36 with ethyl acetate as the developing solvent, and 0.31 and 0.21 with chloroform - acetone (4 : 1). In contrast to a single inhibition zone on bioautography with silica gel TLC, Fractions A and B could each be further separated into two components by reversed-phase chromatography. Thus, Fractions A (200 mg) and B (420 mg) were each dissolved in acetonitrile - water, and insoluble materials were removed by centrifugation. Each supernatant was then applied to an ODS column (50×1.5 cm, i.d., MM 1515, Yamazen Co.) and eluted with acetonitrile - water (30 : 70 for Fraction A and 20 : 80 for Fraction B) as an eluant. The active fractions corresponding to a single peak by HPLC were combined, evaporated to remove the organic solvent and lyophilized to give **3** (6 mg) and **4** (25 mg) from Fraction A, and **1** (19 mg) and **2** (6 mg) from Fraction B as pale yellowish oils.

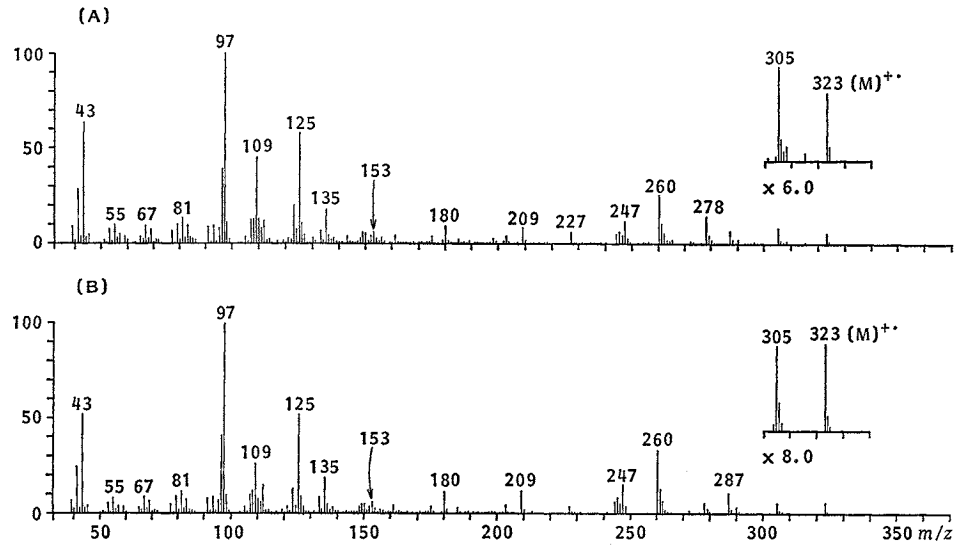
Typical HPLC chromatograms showing the separations of **1** and **2** as well as **3** and **4** are given in Fig. 1. All of these antibiotics gave positive reactions to iodine vapor, sulfuric acid, molybdophosphoric acid and Ehrlich reagent, and had negative reactions to ninhydrin and Tollens reagent. They were soluble in chloroform, ethyl acetate, acetone, methanol and ethanol but insoluble in water.

Fig. 2. UV spectra of S-632-B_1 (**1**) and B_2 (**2**).
— S-632-B_1, --- S-632-B_2.



Physico-chemical and spectral data of these antibiotics are summarized in Table 1. The molecular formulae of **1**, **2**, **3** and **4** were $C_{17}H_{25}NO_5$, $C_{17}H_{25}NO_5$, $C_{16}H_{23}NO_4$ and $C_{17}H_{25}NO_4$, respectively. They were determined by high-resolution electron impact mass spectrometry (HREI-MS) and elemental analysis. In the IR spectra of these antibiotics, a number of absorptions were

Fig. 3. EI-MS of S-632-B_1 (**1**) (A) and B_2 (**2**) (B).



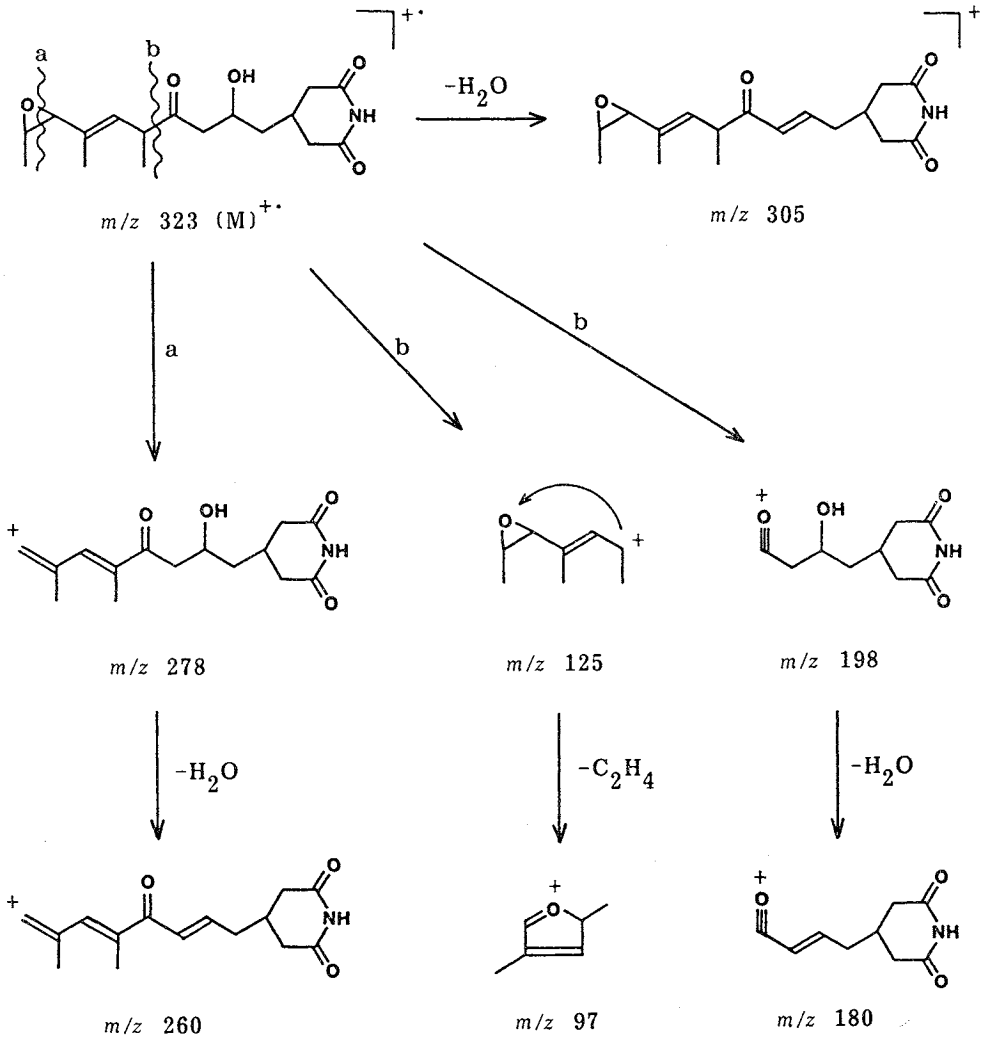
observed around 1700 cm^{-1}. The ^{1}H and ^{13}C NMR spectral data, as shown in Table 2, exhibited that all compounds possess the same glutarimide ring in their molecule. On comparison of the physico-chemical properties with those of known glutarimide antibiotics, compounds **3** and **4** were found to be identical with streptimidone[1] and 9-methylstreptimidone[2,3], respectively. Compounds **1** and **2** were clearly differentiated from the latter by their molecular formula, UV and NMR spectra.

Structural Elucidation of **1** and **2**

As shown in Fig. 2, the UV spectra of **1** and **2** had very similar absorption maxima at 206~207 and 280~282 nm, but these UV spectra were significantly different from that of **4**. Compounds **1** and **2** exhibited no absorption maxima at 232 nm, which corresponds to a conjugated diene system as present in **4** (9-methylstreptimidone).

The EI-MS of **1** and **2** gave the same molecular ion peaks (M^+) at *m/z* 323 together with appearance of the characteristic fragment ions at *m/z* 278, 260, 198, 180, 125 and 97, as shown in Fig. 3.

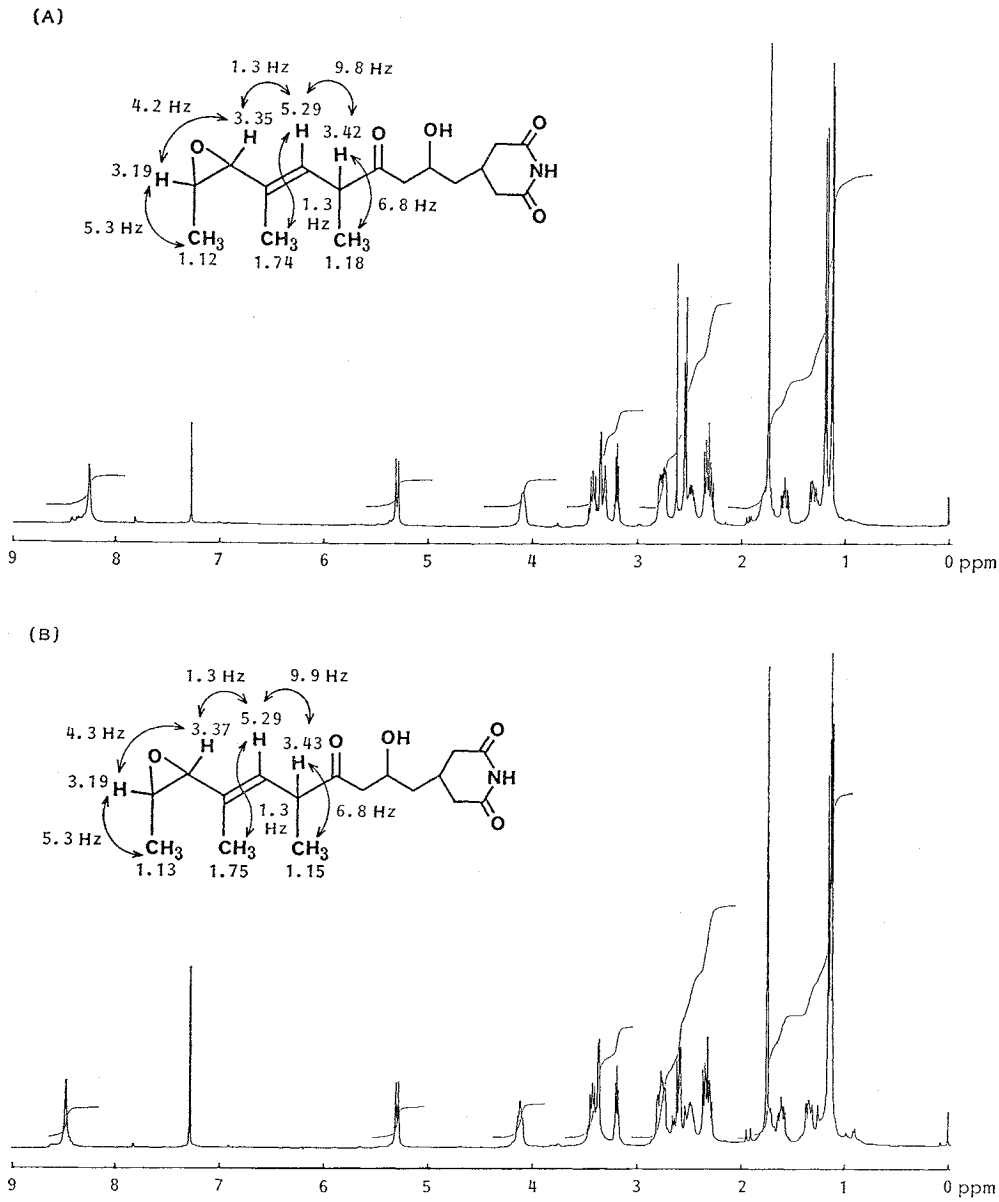
Fig. 4. Fragmentation of EI-MS of S-632-B_1 (**1**) and B_2 (**2**).



Ions at *m/z* 198 and 180 were also observed in the spectrum of **4** and provided evidence for the presence of the glutarimide moieties. The results obtained for **1** and **2** showed clearly that these compounds have one additional oxygen atom in their side chain, as compared with **4**. The structurally significant fragment ions are represented in Fig. 4.

A comparison of the ^{13}C chemical shift data given in Table 2 revealed the appearance of two new methine carbons at δ 59.0 and 54.5 in **1** (or δ 58.8 and 54.3 in **2**) instead of two olefinic carbons in **4**. The chemical shifts of these methine carbons and their large C-H J values (172~180 Hz) suggested the presence of epoxide rings. However, the ^{13}C NMR spectral data demonstrated no major differences between compounds **1** and **2**.

Fig. 5. ^1H NMR spectra of S-632-B_1 (**1**) (A) and B_2 (**2**) (B) in $CDCl_3$ at 400 MHz.



Further evidence for these structures was obtained by the detailed spin-decoupling experiments carried out in connection with ^{1}H NMR spectrometry. As shown in Table 2, the ^{1}H NMR spectra of **1** and **2** indicated two new methine protons at δ 3.35 and 3.19 for **1** (or δ 3.37 and 3.19 for **2**) instead of the two olefinic protons in **4**, and, furthermore, the secondary methyl signal observed at δ 1.78 in **4** was significantly shifted upfield to δ 1.12 in **1** (or δ 1.13 in **2**). On the basis of their spin-spin coupling networks, the connectivities of these protons were determined as illustrated in Fig. 5. Consequently, both **1** and **2** were considered to be 8,9-epoxide analogues of 9-methylstreptimidone. Thus, the structures of compounds **1** and **2** were both elucidated as 3-(5,7-dimethyl-8,9-epoxy-2-hydroxy-4-oxo-6-decenyl)glutarimide (**1**), and, accordingly, these compounds were proposed to be diastereoisomers.

In order to clarify the structural relationship between the two antibiotics, the stereochemistry was further confirmed. The double bonds at C-6 were determined to have the *E*-configuration, based on the allylic coupling constant of 1.3 Hz between the 6-H and 7-CH_3 in both compounds. The *J* values between 8-H and 9-H of the epoxide ring were 4.2 Hz in **1** and 4.3 Hz in **2**, indicating, for both, a *cis* substitution on the epoxide ring. The CD spectra of **1** and **2** were closely related to that of 9-methylstreptimidone, and exhibited a positive Cotton effect at the extremum of 290 nm in both compounds. The chirality at C-5 has been reported to provide a dominant contribution to the sign of this Cotton effect[5]. Therefore, compounds **1** and **2** can be assigned *S*-configuration at C-5 as also present in 9-methylstreptimidone.

Accordingly, at least for the chirality at C-5, the configurations of double bond and the relative configuration of epoxide, no structural distinction could be made between **1** and **2**. Thus, the chirality at C-2 or the absolute configuration of the epoxide could be different in the two compounds. However, these alternatives remain to be elucidated.

Experimental

Measurements

The optical rotations were measured in chloroform at 25°C on a Horiba SEPA-200 polarimeter. The UV spectra were taken in ethanol on a Shimadzu 265 spectrophotometer, and the IR spectra were run on KBr tablets on a Hitachi 260-50 spectrometer. The ^{1}H NMR spectra were recorded on a Bruker WH-400 spectrometer and ^{13}C NMR spectra were recorded on a Jeol FX-100 spectrometer in deuterochloroform, and the chemical shifts were shown in values downfield from TMS as an internal standard. The CD spectra were taken in MeOH on a Jasco J-500A spectrometer. The EI-MS was carried out with a Jeol DX-303 mass spectrometer equipped with DA-5000 data system.

Assay Methods for Antibiotics

The isolation of antibiotics was monitored both by the conventional paper-disk diffusion method using *Saccharomyces cerevisiae* IFO 0304 as the test organism and by HPLC with a Shimadzu LC-6A system. HPLC was performed with the following conditions: Column, Unisil Q C_{18} (250×4.6 mm, i.d., Gasukuro Kogyo Co.); mobile phase, acetonitrile - water (35 : 65 for compounds **1** and **2** or 20 : 80 for compounds **3** and **4**); flow rate, 1 ml/minute; detector, UV at 210 nm.

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