FUNGAL METABOLITES. Part 12†. POTENT IMMUNOSUPPRESSANT, 14-DEOXOMYRIOCIN, (2*S*,3*R*,4*R*)-(*E*)-2-AMINO-3,4-DIHYDROXY-2-HYDROXYMETHYLEICOS-6-ENOIC ACID AND STRUCTURE-ACTIVITY RELATIONSHIPS OF MYRIOCIN DERIVATIVES

TETSURO FUJITA, KENICHIRO INOUE††, SATOSHI YAMAMOTO†††, TAKESHI IKUMOTO[a], SHIGEO SASAKI[a], RYOUSUKE TOYAMA[a], MASAHIKO YONETA[a], KENJI CHIBA[b], YUKIO HOSHINO[b] and TAKEKI OKUMOTO[b]

Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan
[a]Research Laboratory, Taito Company, Ltd., 1-26 Higashi Shriike-Shinmachi, Nagata-ku, Kobe 653, Japan
[b]Tokyo Research Laboratories, Yoshitomi Pharmaceutical Industries, Ltd., 3-7-25 Koyata, Iruma-shi, Saitama 358, Japan

(Received for publication September 6, 1993)

In order to investigate the structure-activity relationships, fourteen derivatives of myriocin ((2*S*,3*R*,4*R*)-(*E*)-2-amino-3,4-dihydroxy-2-hydroxymethyl-14-oxoeicos-6-enoic acid) were prepared and examined for immunosuppressive activity on mouse allogeneic mixed lymphocyte reaction *in vitro*. Among them, 14-deoxomyriocin ((2*S*,3*R*,4*R*)-(*E*)-2-amino-3,4-dihydroxy-2-hydroxymethyl-eicos-6-enoic acid) was the most potent. It also suppressed the generation of allo-reactive cytotoxic T lymphocytes in mice upon intraperitoneal administration, with a potency 10-fold greater than that of myriocin.

During our screening program of fungal extracts[1,2], the extract of *Isaria sinclairii* (ATCC 24400) was found to suppress lymphocyte proliferation in mouse allogeneic mixed lymphocyte reaction (MLR). We isolated the immunosuppressive principle, ISP-I, and showed that it is identical with myriocin[3,4] (thermozymocidin)[5], previously isolated from other fungi as an antifungal agent. Since myriocin is structurally very simple compared with other microbial products having immunosuppressive activity, such as cyclosporin A (CsA)[6] and FK-506[7,8], it is noteworthy that its immunosuppressive activity is 10 to 100 times more potent than that of CsA in terms of suppressing both lymphocyte proliferation in mouse allogeneic MLR *in vitro* and generation of allo-reactive cytotoxic T lymphocytes (CTL) in mice *in vivo*. We therefore prepared a number of derivatives with the aim of finding even more active compounds.

In the present paper, we describe the structure-activity relationships of fourteen myriocin derivatives, using mouse allogeneic MLR *in vitro* to evaluate potency. 14-Deoxomyriocin, (2*S*,3*R*,4*R*)-(*E*)-2-amino-3,4-dihydroxy-2-hydroxymethyleicos-6-enoic acid, the most active derivative on mouse allogeneic MLR, was also examined for ability to suppress the generation of allo-reactive CTL *in vivo*, in comparison with myriocin.

Chemistry

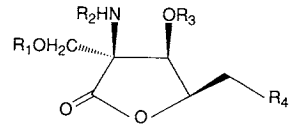
Various myriocin derivatives having the structures shown in Fig. 1 were synthesized as described below.

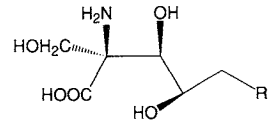
† See ref. 1.

†† Present address: Dept. of Pharmacognosy, Gifu Pharmaceutical University, 5-6-1 Mitahora-Higashi, Gifu 502, Japan

††† Present address: Pharmaceutical Research Laboratories I, Takeda Chemical Industries, Ltd., 2-17-85 Jusohonmachi, Yodogawa-ku, Osaka 532, Japan

Fig. 1. The structures of myriocin derivatives.





1 $R_1=R_2=R_3=Ac$,
$R_4=-CH=CH-(CH_2)_6-CO-(CH_2)_5CH_3$
2 $R_1=R_2=R_3=H$,
$R_4=-CH=CH-(CH_2)_6-CO-(CH_2)_5CH_3$
3 $R_1=R_2=R_3=H$, $R_4=-(CH_2)_8-CO-(CH_2)_5CH_3$
4 $R_1=R_2=R_3=H$,
$R_4=-CH=CH-(CH_2)_6-CHOH-(CH_2)_5CH_3$
5 $R_1=R_2=R_3=H$,
$R_4=-(CH_2)_8-CHOH-(CH_2)_5CH_3$
6 $R_1=R_3=H$, $R_2=Ac$,
$R_4=-CH=CH-(CH_2)_6-CO-(CH_2)_5CH_3$
15 $R_1=R_2=R_3=Ac$,
$R_4=-CH=CH-(CH_2)_6-C(S_2C_2H_4)-(CH_2)_5CH_3$
16 $R_1=R_2=R_3=Ac$, $R_4=-(CH_2)_{14}CH_3$
17 $R_1=R_2=R_3=Ac$, $R_4=-CH=CH-(CH_2)_{12}CH_3$
18 $R_1=R_2=R_3=Ac$, $R_4=-CH_2OH$

Myriocin $R=-CH=CH-(CH_2)_6-CO-(CH_2)_5CH_3$
7 $R=-CH=CH-(CH_2)_6-CHOH-(CH_2)_5CH_3$
8 $R=-(CH_2)_8-CO-(CH_2)_5CH_3$
9 $R=-(CH_2)_8-CHOH-(CH_2)_5CH_3$
10 $R=-(CH_2)_{14}CH_3$
11 $R=-CH=CH-(CH_2)_6-C(S_2C_2H_4)-(CH_2)_5CH_3$
12 $R=-CH=CH-(CH_2)_6-C(NOH)-(CH_2)_5CH_3$
13 $R=-CH=CH-(CH_2)_{12}CH_3$
14 $R=-CH_2OH$

Acetylation of myriocin with acetic anhydride in pyridine gave the triacetyl lactone (**1**), while treatment with acetic anhydride in MeOH gave the *N*-acetyl derivative (**6**). Treatment of myriocin with methanolic hydrogen chloride afforded the lactone (**2**), which was hydrogenated to give the dihydro lactone (**3**). Reduction of the lactone (**2**) with $NaBH_4$ yielded the 14-hydroxy lactone (**4**), which was converted into the dihydro-14-hydroxy lactone (**5**). Thus γ-lactone formation is favored under dehydrating or acidic conditions. Reduction of myriocin and the dihydro derivative (**8**) with $NaBH_4$ gave the 14-hydroxy acid (**7**) and the dihydro-14-hydroxy acid (**9**), respectively.

In order to evaluate the role of the 14-ketone group in immunosuppressive activity, 14-deoxo, 14-oxime and 14-ethylenedithioketal derivatives were prepared from myriocin. The triacetyl lactone (**1**) was converted into the 14-ethylenedithioketal[9] (**15**), which was treated with Raney nickel to afford the 6,7-dihydro-14-deoxo triacetyl lactone (**16**). Hydrolysis of compounds **16** and **15** gave the 6,7-dihydro-14-deoxo acid (**10**) and the 14-ethylenedithioketal acid (**11**), respectively. An oxime (**12**) was prepared in an usual manner from myriocin. Since desulfurization by Raney nickel of the 14-ethylenedithioketal (**15**) was accompanied with hydrogenation at the 6-double bond, a selective conversion of the keto group to a methylene group was attempted. The triacetyl lactone (**1**) was subjected to modified Clemmensen reduction[10] to give selectively the 14-deoxo triacetyl lactone (**17**), which in turn was hydrolyzed to afford the 14-deoxo acid (**13**).

Ozonolysis of compound **1** followed by reduction with $NaBH_4$ gave a cleaved triacetyl lactone alcohol (**18**), which was hydrolyzed to a 2-amino-2-hydroxymethyl-3,4,6-trihydroxy hexanoic acid (**14**).

Biological Evaluation

The effect of the synthesized compounds on mouse allogeneic MLR and that of compound **13** and myriocin on allo-reactive CTL generation in mice were examined by the methods described in our previous paper[1].

The following relationships between structures and suppressive activity on mouse allogeneic MLR

were noted.

1. From a comparison of the activities of unsaturated and saturated compounds, hydrogenation of the C-6 double bond decreases the immunosuppressive activity by over one order of magnitude.

2. Reduction of the C-14 ketone group to a hydroxy group does not affect the activity, except for **3**→**5**. However, the reduction to a methylene results in increased activity.

3. Lactonization of acids does not affect the activity, except for **8**→**3**.

4. Selective acetylation of the amino group or complete acetylation greatly decreases the activity.

5. Conversion of the ketone group to ethylenedithioketal or oxime, and ozonolysis at the double bond results in further decrease of the activity (Table 1).

Table 1. Effect of myriocin and its derivatives on mouse allogeneic mixed lymphocyte reaction.

	Compound			IC_{50} (μg/ml)
	Type	C(6)–C(7)	C(14)	
Myriocin	Acid	C=C	C=O	0.0032
1	tri Ac. Lactone	C=C	C=O	32
2	Lactone	C=C	C=O	0.005
3	Lactone	C–C	C=O	0.22
4	Lactone	C=C	CHOH	0.0020
5	Lactone	C–C	CHOH	0.035
6	*N*-Ac. Lactone	C=C	C=O	0.22
7	Acid	C=C	CHOH	0.0050
8	Acid	C–C	C=O	0.020
9	Acid	C–C	CHOH	0.022
10	Acid	C–C	CH_2	0.039
11	Acid	C=C	$C(S_2C_2H_4)$	0.75
12	Acid	C=C	C=N–OH	0.075
13	Acid	C=C	CH_2	0.00075
14	Acid	CH_2OH	—	0.135

Table 2. Effect of myriocin and **13** on generation of allo-reactive cytotoxic T lymphocytes (CTL) *in vivo*.

Compound	Dose (mg/kg)	Route	CTL activity	
			LU/Spleen	Inhibition (%)
Exp. 1				
Control			1,400±271	
Myriocin	0.01	ip	1,114±135	20.4
	0.03	ip	920±330*	34.3
	0.1	ip	427±229**	69.5
	0.3	ip	286±337**	79.6
Exp. 2				
Control			1,170±471	
13	0.001	ip	589±415	49.7
	0.003	ip	473±256*	59.6
	0.01	ip	443±141*	62.1
	0.03	ip	352±148**	69.9
	0.1	ip	158± 86**	86.5
	0.3	ip	50± 0**	95.7

Mice were immunized intraperitoneally with 10^7 EL-4 cells on day 0. Test compounds were administered on day 0 to 4. CTL activity was determined by ^{51}Cr release assay on day 10. One lytic unit (LU) was defined as the number of effector cells required by cause 25% lysis of 5×10^3 target cells. Results are shown as mean ± SD of 5 mice.

* $P<0.05$, ** $P<0.01$ (STUDENT's *t*-test.)

14-Deoxomyriocin, (2*S*,3*R*,4*R*)-(*E*)-2-amino-3,4-dihydroxy-2-hydroxymethyleicos-6-enoic acid (**13**) had the most potent immunosuppressive activity on mouse allogeneic MLR among the fourteen derivatives prepared at this time. It was also examined for suppressive activity on generation of allo-reactive CTL *in vivo*. As shown in Table 2, myriocin and **13** suppressed allo-reactive CTL generation in a dose-dependent manner. The minimum effective concentrations of myriocin and **13** were estimated to be 0.03 and 0.003 mg/kg/day, respectively. These results indicate that 14-deoxomyriocin, (2*S*,3*R*,4*R*)-(*E*)-2-amino-3,4-dihydroxy-2-hydroxymethyleicos-6-enoic acid (**13**) is 10-fold more potent than myriocin in its inhibition of immune responses *in vitro* and *in vivo*.

Experimental

General Methods

Melting points were determined on a Yanagimoto micro melting point apparatus without correction. Optical rotations were measured with a Jasco DIP-181 digital polarimeter. IR spectra were taken on a Shimadzu IR 435 infrared spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were taken on a JEOL FX-200 or Bruker AM-400 spectrometer with TMS as an internal standard. Mass spectra (EI-MS and FAB-MS) were measured on a JEOL JMS-01SG, Shimadzu GCMS-QP 2000 or JEOL JMS-HX110 spectrometer. For column chromatography on silica gel, Kieselgel 60 (70~230 mesh, Merck) was used. TLC was performed on Kieselgel 60 F_{254} (Merck). Organic solvent extracts were dried over anhydrous magnesium sulfate, and evaporation of solvents was performed under reduced pressure.

The Triacetyl Lactone (1)

Acetic anhydride (70 ml) was added to a stirred solution of myriocin (8.1 g) in pyridine (65 ml). After usual work-up, the reaction mixture gave an oily residue (10.08 g). The residue was purified by chromatography on a silica gel (300 g) column using EtOAc-*n*-hexane (7:3) as an eluent. The eluate was concentrated to give compound **1** (9.29 g, 91.4%) as an oil. IR ν_{max} ($CHCl_3$) cm^{-1}: 3450, 2930, 2860, 1780, 1760, 1710, 1690, 1380, 1220, 970; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.03 (1H, br s, –N*H*Ac), 5.79 (1H, d, J=4.3 Hz, 3-H), 5.56 (1H, dtt-like, J=15.2 and 7.0 Hz, 7-H), 5.39 (1H, dtt-like, J=15.2 and 7.0 Hz, 6-H), 4.72 (1H, td, J=8.2 and 4.3 Hz, 4-H), 4.53 (1H, d, J=11.5 Hz, 21-H), 4.50 (1H, d, J=11.5 Hz, 21-H), 2.5~2.2 (2H, m, 5-H_2), 2.39 (4H, t, J=7.5 Hz, 13- and 15-H_2), 2.10 (3H, s, Ac), 2.05 (3H, s, Ac), 2.03 (3H, s, Ac), 1.99 (2H, q, J=7.0 Hz, 8-H_2), 1.55 (4H, quintet, J=7.5 Hz, 12- and 16-H_2), 1.27 (12H, br s, 9-~11- and 17-~19-H_2), 0.88 (3H, t, J=6.5 Hz, 20-H_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 211.38 (s, 14-C), 172.32 (s, >C=O), 170.13 (s, >C=O), 169.95 (s, >C=O), 169.89 (s, >C=O), 135.04 (d, 7-C), 123.16 (d, 6-C), 81.62 (d, 4-C), 72.01 (d, 3-C), 62.75 (t, 21-C), 62.73 (s, 2-C), 42.85 (t, 13-C), 42.77 (t, 15-C), 32.46 (t, 8-C), 32.20 (t, 5-C), 31.63, 29.11, 29.00, 28.97, 28.91, 23.91 and 23.85 (each t, 9-~12- and 16-~18-C), 22.76 (q, –CO*C*H_3), 22.50 (t, 19-C), 20.37 (q, –CO*C*H_3), 20.33 (q, –CO*C*H_3), 14.00 (q, 20-C); EI-MS *m/z*: 509 (M^+, $C_{27}H_{43}NO_8$), 491, 382, 279, 129, 43.

HREI-MS Calcd for $C_{27}H_{43}NO_8$: 509.2990.
Found *m/z* 509.2998.

The Lactone (2)

A solution of myriocin (1.01 g) in MeOH (200 ml) was treated with 44% methanolic hydrogen chloride (4 ml). The mixture was kept standing overnight at room temperature, then the solvent was evaporated off. The residue was purified by chromatography on silica gel (100 g) using $CHCl_3$-MeOH (9:1), followed by recrystallization from $CHCl_3$-petroleum ether to give compound **2** (750 mg, 78.5%). MP 79~82°C; $[\alpha]_D^{25}$ +26.9° (*c* 1.00, MeOH); IR ν_{max} (KBr) cm^{-1}: 3300, 2920, 2850, 1765, 1710, 1605, 970; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.62 (1H, dtt-like, J=15.3 and 6.6 Hz, 7-H), 5.43 (1H, dtt-like, J=15.3 and 7.0 Hz, 6-H), 4.48 (1H, td, J=7.2 and 3.5 Hz, 4-H), 4.14 (1H, d, J=3.5 Hz, 3-H), 3.75 (1H, d, J=11.2 Hz, 21-H), 3.66 (1H, d, J=11.2 Hz, 21-H), 2.55 (2H, br t, J=7.0 Hz, 5-H_2), 2.39 (4H, t, J=7.4 Hz, 13- and 15-H_2), 2.01 (2H, br dt, J=7.0 and 6.8 Hz, 8-H_2), 1.54 (4H, quintet, J=7.1 Hz, 12- and 16-H_2), 1.38~1.26 (12H, m, 9-~11- and 17-~19-H_2), 0.88 (3H, t, J=7.0 Hz, 20-H_3); EI-MS *m/z*: 256 ($M-CH_2$–CO–*n*-C_6H_{13})$^+$,

102, 87, 55, 43.

Anal Calcd for $C_{21}H_{37}NO_5$: C 65.77, H 9.72, N 3.65.
Found: C 65.51, H 9.44, N 3.69.

The 6,7-Dihydro Lactone (**3**)

A solution of compound **2** (522 mg) in MeOH (150 ml) was subjected to hydrogenation over 5% palladium carbon (73 mg). The catalyst was filtered off and the solvent was evaporated. The residue was repeatedly recrystallized from EtOAc - hexane to give compound **3** (305.9 mg, 58.3%). MP 96~98°C; $[\alpha]_D^{25}$ +23.08° (*c* 0.838, MeOH); IR ν_{max} (KBr) cm^{-1}: 3400, 3320, 2925, 2855, 1770, 1720; ^1H NMR (400 MHz, $CDCl_3$) δ: 4.49 (1H, br t, J=7.0 Hz, 4-H), 4.13 (1H, br s, 3-H), 3.75 (1H, d, J=10.2 Hz, 21-H), 3.69 (1H, d, J=10.0 Hz, 21-H), 2.39 (4H, t, J=7.4 Hz, 13- and 15-H_2), 1.82 (2H, m, 5-H_2), 1.55 (4H, quintet, J=7.1 Hz, 12- and 16-H_2), 1.45~1.28 (18H, m, 6-~11- and 17-~19-H_2), 0.88 (3H, t, J=7.0 Hz, 20-H_3); EI-MS *m/z*: 258 $(M-CH_2-CO-n-C_6H_{13})^+$, 104, 85, 71, 58, 43

Anal Calcd for $C_{21}H_{39}NO_5$: C 65.42, H 10.20, N 3.63.
Found: C 65.20, H 10.00, N 3.59.

The 14-Hydroxy Lactone (**4**)

Sodium borohydride (100 mg) was added to a solution of compound **2** (200 mg) in MeOH (100 ml). After 30 minutes, the mixture was acidified to pH 2 with HCl - H_2O. The whole was extracted with $CHCl_3$. Evaporation of the solvent gave a residue, which was purified by chromatography on a silica gel (50 g) column using $CHCl_3$ - MeOH (15 : 1) to give compound **4** (117 mg, 58.2%). MP 55~56°C; $[\alpha]_D^{25}$ +22.09° (*c* 0.526, MeOH); IR ν_{max} (KBr) cm^{-1}: 3350, 2925, 2855, 1760, 1590, 975; ^1H NMR (400 MHz, $CDCl_3$) δ: 5.61 (1H, dtt-like, J=15.3 and 6.8 Hz, 7-H), 5.44 (1H, dtt-like, J=15.3 and 7.0 Hz, 6-H), 4.48 (1H, td, J=7.3 and 3.0 Hz, 4-H), 4.14 (1H, d, J=3.0 Hz, 3-H), 3.72 (1H, d, J=11.3 Hz, 21-H), 3.66 (1H, d, J=11.3 Hz, 21-H), 3.57 (1H, m, 14-H), 2.55 (2H, t, J=7.0 Hz, 5-H_2), 2.03 (2H, q, J=6.8 and 6.8 Hz, 8-H_2), 1.43~1.25 (20H, m, 9-~13- and 15-~19-H_2), 0.88 (3H, t, J=6.8 Hz, 20-H_3); EI-MS *m/z*: 300 $(M-n-C_6H_{13})^+$, 102,.87, 71, 55, 41.

Anal Calcd for $C_{21}H_{39}NO_5 \cdot \frac{1}{2}H_2O$: C 63.93, H 10.22, N 3.55.
Found: C 63.98, H 10.13, N 3.55.

The 6,7-Dihydro-14-hydroxy Lactone (**5**)

Compound **5** was obtained from **4** by the hydrogenation as well as from **3** by a similar procedure to that used for the preparation of **4** (54.3%). MP 83~84°C; $[\alpha]_D^{25}$ +22.75° (*c* 1.030, MeOH); IR ν_{max} (KBr) cm^{-1}: 3350, 2925, 2850, 1760, 1580; ^1H NMR (400 MHz, $CDCl_3$) δ: 4.49 (1H, td, J=7.8 and 3.5 Hz, 4-H), 4.11 (1H, d, J=3.4 Hz, 3-H), 3.74 (1H, d, J=11.2 Hz, 21-H), 3.69 (1H, d, J=11.2 Hz, 21-H), 3.58 (1H, m, 14-H), 1.83 (2H, m, 5-H_2), 1.49~1.29 (26H, m, 6-~13- and 15-~19-H_2), 0.89 (3H, J=6.8 Hz, 20-H_3); EI-MS *m/z*: 302 $(M-n-C_6H_{13})^+$, 224, 104, 87, 71, 55, 43.

Anal Calcd for $C_{21}H_{41}NO_5 \cdot \frac{1}{2}H_2O$: C 63.60, H 10.68, N 3.53.
Found: C 63.63, H 10.30, N 3.61.

The *N*-Acetyl Lactone (**6**)

Acetic anhydride (10 ml) was added to a solution of myriocin (100 mg) in MeOH (30 ml), and the mixture was kept standing overnight at room temperature. Water was added and the mixture was concentrated. The residue was purified by chromatography on silica gel (10 g) column using $CHCl_3$ - MeOH (9 : 1) to give compound **6** (60 mg, 56.6%). MP 105.5~107°C; $[\alpha]_D^{25}$ +34.6° (*c* 0.75, MeOH); IR ν_{max} (KBr) cm^{-1}: 3300, 2920, 2855, 1785, 1710, 1670, 1605, 975; ^1H NMR (400 MHz, $CDCl_3$) δ: 6.59 (1H, br s, –N*H*Ac), 5.62 (1H, dtt-like, J=15.2 and 6.8 Hz, 7-H), 5.42 (1H, dtt-like, J=15.3 and 7.0 Hz, 6-H), 4.67 (1H, dd, J=6.7 and 3.7 Hz, 21-OH), 4.60 (1H, br td, J=7.2 and 3.8 Hz, 4-H), 4.10 (1H, br dd, J=7.1 and 4.5 Hz, 3-H), 3.90 (1H, dd, J=11.7 and 3.7 Hz, 21-H), 3.85 (1H, dd, J=11.7 and 7.4 Hz, 21-H), 3.58 (1H, d, J=7.0 Hz, 3-OH), 2.58 (1H, dtd, J=15.8, 7.2 and 1.2 Hz, 5-H), 2.55 (1H, dtd, J=15.8, 7.1 and 1.5 Hz, 5-H), 2.39 (4H, t, J=7.5 Hz, 13- and 15-H_2), 2.12 (3H, s, –NH*Ac*), 2.00 (2H, q, J=6.7 Hz, 8-H_2), 1.55 (4H, quintet, J=7.2 Hz, 12- and 16-H_2), 1.38~1.25 (12H, m, 9-~11- and 17-~19-H_2), 0.88 (3H, t, J=7.0 Hz, 20-H_3).

Anal Calcd for $C_{23}H_{39}NO_6$: C 64.91, H 9.24, N 3.29.
Found: C 64.73, H 9.02, N 3.35.

The 14-Hydroxy Acid (**7**)

A solution of myriocin (150 mg) in MeOH (75 ml) was treated with $NaBH_4$ (75 mg) and the reaction mixture was worked up in the usual manner to give compound **7** (86.4 mg, 57.3%). MP 162~165 °C; $[\alpha]_D^{25}$ −10.1° (*c* 0.290, MeOH); IR ν_{max} (KBr) cm^{-1}: 3400~3170 (br), 2925, 2855, 1635, 970; ^1H NMR (400 MHz, $CDCl_3$) δ: 5.52 (1H, dtt-like, J=15.2 and 6.5 Hz, 7-H), 5.38 (1H, dtt-like, J=15.2 and 7.0 Hz, 6-H), 3.99 (1H, d, J=11.0 Hz, 21-H), 3.87 (1H, d, 10.9 Hz, 21-H), 3.82 (1H, br t, J=7.2 Hz, 4-H), 3.77 (1H, d, 1.0 Hz, 3-H), 3.48 (1H, m, 14-H), 2.26 (2H, br t, J=6.8 Hz, 5-H_2), 2.00 (2H, br q, J=6.5 Hz, 8-H_2), 1.45~1.28 (20H, m, 9-~13- and 15-~19-H_2), 0.90 (3H, t, J=6.8 Hz, 20-H_2); EI-MS *m/z*: 300 $(M-n\text{-}C_6H_{13})^+$, 102, 87, 71, 55, 41.

Anal Calcd for $C_{21}H_{41}NO_6$: C 62.50, H 10.48, N 3.47.
Found: C 62.32, H 10.48, N 3.38.

The 6,7-Dihydro Acid (**8**)

A solution of myriocin (500 mg) in MeOH (250 ml) was subjected to hydrogenation over 5% palladium carbon (400 mg). The reaction mixture was worked up in the usual manner to give compound **8** (375 mg, 75%). MP 154~155.5 °C; $[\alpha]_D^{25}$ −6.5° (*c* 0.108, MeOH); IR ν_{max} (KBr) cm^{-1}: 3400, 3250~3170, 2930, 2855, 1715, 1670; ^1H NMR (400 MHz, $CDCl_3$) δ: 3.99 (1H, d, J=11.0 Hz, 21-H), 3.87 (1H, d, J=10.9 Hz, 21-H), 3.81 (1H, br t, J=6.9 Hz, 4-H), 3.73 (1H, d, J=1.0 Hz, 3-H), 2.43 (4H, t, J=7.0 Hz, 13- and 15-H_2), 1.53 (4H, quintet, J=7.0 Hz, 12- and 16-H_2), 1.29 (20H, m, 5-~11- and 17-~19-H_2), 0.90 (3H, t, J=6.9 Hz, 20-H_3); EI-MS *m/z*: 258 $(M-CH_2\text{–}CO\text{–}n\text{-}C_6H_{13})^+$, 104, 85, 71, 55, 43.

Anal Calcd for $C_{21}H_{41}NO_6$: C 62.50, H 10.48, N 3.47.
Found: C 62.32, H 10.48, N 3.38.

The 6,7-Dihydro-14-hydroxy Acid (**9**)

A solution of compound **8** (300 mg) in MeOH (150 ml) was treated with $NaBH_4$ (150 mg) and the reaction mixture was worked up in the usual manner to give compound **9** (224 mg, 75%). MP 161~162°C, IR ν_{max} (KBr) cm^{-1}: 3300, 2925, 2855, 1630; ^1H NMR (400 MHz, $CDCl_3$) δ: 4.00 (1H, d, J=11.0 Hz, 21-H), 3.88 (1H, d, 11.0 Hz, 21-H), 3.81 (1H, br t, J=6.8 Hz, 4-H), 3.74 (1H, br s, 3-H), 3.49 (1H, m, 14-H), 1.55 (4H, m) and 1.43~1.25 (24H, m) (5-~13- and 15-~19-H_2), 0.90 (3H, t, J=6.9 Hz, 20-H_3); EI-MS *m/z*: 302 $(M-n\text{-}C_6H_{13})^+$, 224, 104, 87, 71, 55, 43.

Anal Calcd for $C_{21}H_{43}NO_6 \cdot \frac{1}{4}H_2O$: C 61.51, H 10.69, N 3.42.
Found: C 61.49, H 10.66, N 3.44.

The 14-Ethylenedithioketal Triacetyl Lactone (**15**)

1,2-Ethanedithiol (0.367 ml) and trifluoroborane etherate (0.012 ml) was added to a solution of compound **1** (1,509.5 mg) in dry dichloromethane (12.5 ml), and the mixture was stirred for 47 hours. The organic layer was washed with 5% $NaHCO_3$ and saturated aqueous NaCl, dried and concentrated to give an oily residue. The residue was purified by chromatography on a silica gel (50 g) column using EtOAc-*n*-hexane (6:4) to give compound **15** (1,538.9 mg, 88.7%) as an oil. IR ν_{max} ($CHCl_3$) cm^{-1}: 3400, 2950, 2850, 2700, 1780, 1750, 1680, 1500, 1370, 1230, 1050, 970. ^1H NMR (200 MHz, $CDCl_3$) δ: 6.07 (1H, br s, –N*H*Ac), 5.79 (1H, d, J=4.6 Hz, 3-H), 5.58 (1H, dtt-like, J=15.4 and 6.5 Hz, 7-H), 5.39 (1H, dtt-like, J=15.4 and 6.6 Hz, 6-H), 4.71 (1H, ddd, J=8.2, 5.4 and 4.6 Hz, 4-H), 4.52 (2H, s, 21-H_2), 3.26 (4H, s, 1'- and 2'-H_2), 2.44 (1H, ddd, J=14.9, 8.2 and 6.6 Hz, 5-H), 2.34 (1H, ddd, J=14.9, 6.60 and 5.4 Hz, 5-H), 2.10 (3H, s, Ac), 2.05 (3H, s, Ac), 2.03 (3H, s, Ac), 2.00 (2H, m, 8-H_2), 1.88 (4H, m, 13- and 15-H_2), 1.55 (m), 1.30 (m) and 1.40~1.20 (m) (16H, 9-~12- and 16-~19-H_2), 0.89 (3H, t, J=6.5 Hz, 20-H_3); ^{13}C NMR (100 MHz, $CDCl_3$), δ: 172.39 (s, >C=O), 170.14 (s, >C=O), 169.35 (s., >C=O), 168.83 (s, >C=O), 135.13 (d, 6-C), 123.05 (d, 7-C), 81.61 (d, 4-C), 71.92 (d, 3-C), 71.71 (s, 14-C), 62.69 (t, 21-C), 62.63 (s, 2-C), 43.45 and 43.45 (each t, 13- and 15-C), 39.39 and 39.39 (each t, 1'- and 2'-C), 32.53, 32.17 and 31.74 (each t, 5-, 8- and 18-C), 29.64, 29.43, 29.17 and 29.15 (each t, 9-~11- and 17-C), 26.89 and 26.89 (each t, 12- and 16-C), 22.75 (q, –CO*C*H_3), 22.63 (t, 19-C), 20.59 (q, –CO*C*H_3), 20.32 (q, –CO*C*H_3), 14.07 (q, 20-C); EI-MS *m/z*: 585 $(M)^+$, 500, 440, 398, 189, 160, 43.

HREI-MS Calcd for $C_{29}H_{47}NO_7S_2$: 585.27961.
Found: *m/z* 585.27914.

The 6,7-Dihydro-14-deoxo Triacetyl Lactone (**16**)

Raney nickel (22 g) was added to a solution of 14-ethylenedithioketal triacetyl lactone (1,422 mg) in dry EtOH (40 ml), and the mixture was refluxed for 90 minutes. After cooling, the Raney nickel was filtered off. The filtrate was concentrated to give a solid residue (1,062.7 mg). Acetic anhydride (5.0 ml) and pyridine (5.0 ml) were added to the residue. The mixture was stirred overnight at room temperature, diluted with water and extracted with EtOAc. The EtOAc layer was treated by a similar manner to the preparation of **15** to give a residue. The residue was purified by chromatography on a silica gel (33 g) column using EtOAc-*n*-hexane (7:3) to give compound **16** (862.2 mg, 71.4%) as an oil. IR ν_{max} ($CHCl_3$) cm^{-1}: 3400, 3010, 2910, 2850, 1775, 1750, 1680, 1500, 1460, 1370, 1270. ^1H NMR (200 MHz, $CDCl_3$) δ: 6.02 (1H, br s, –N*H*Ac), 5.80 (1H, d, J=4.4 Hz, 3-H), 4.72 (1H, dt, J=9.0 and 4.4 Hz, 4-H), 4.53 (2H, s, 21-H_2), 2.10 (3H, s, Ac), 2.05 (3H, s, Ac), 2.03 (3H, s, Ac), 1.65 (4H, m, 5- and 6-H_2), 1.26 (26H, br s, 7-~19-H_2), 0.88 (3H, t, J=6.5 Hz, 20-H_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 172.61 (s, >C=O), 170.15 (s, >C=O), 169.45 (s, >C=O), 168.96 (s, >C=O), 82.05 (d, 4-C), 72.22 (d, 3-C), 62.66 (t, 21-C), 62.66 (s, 2-C), 31.91 (t, 18-C), 29.68, 29.68, 29.68, 29.68, 29.68, 29.68, 29.60, 29.51, 29.36, 29.36 and 29.28 (each t, 7-~17-C), 28.75 (t, 5-C), 25.68 (t, 6-C), 22.79 (q, –CO*C*H_3), 22.70 (t, 19-C), 20.58 (q, –CO*C*H_3), 20.34 (q, –CO*C*H_3), 14.11 (q, 20-C); EI-MS m/z: 497 $(M)^+$, 454, 365, 322, 129, 112, 43.

HREI-MS Calcd for $C_{27}H_{47}NO_7$: 497.33541.
Found: m/z 497.33618.

The 6,7-Dihydro-14-deoxo Acid (**10**)

A solution of compound **16** (450.0 mg) in MeOH (21.7 ml) containing 1 N NaOH (5.43 ml) was refluxed under a nitrogen atmosphere overnight. The mixture was neutralized with 1 N HCl to give a precipitate. The precipitate was collected by filtration, which was washed with MeOH and water, and dried to give compound **10** (258.7 mg, 73.2%). MP 165.5~168.0 °C; IR ν_{max} (KBr) cm^{-1}: 3380, 3200, 2910, 2850, 1620, 1460, 1100, 1060; ^1H NMR (200 MHz, CD_3OD) δ: 4.00 (1H, d, J=11.1 Hz, 21-H), 3.87 (1H, d, J=11.1 Hz, 21-H), 3.81 (1H, br t, J=7.1 Hz, 4-H), 3.74 (1H, d, J=1.2 Hz, 3-H), 1.55 (m) and 1.28 (br s) (30H, 5-~19-H_2), 0.90 (3H, t, J=6.5 Hz, 20-H_3); FAB-MS m/z: 390 $(M+H)^+$, 372, 291, 277, 165, 104.

Anal Calcd for $C_{21}H_{43}NO_5$: C 64.74, H 11.13, N 3.60.
Found: C 64.27, H 11.41, N 3.62.

The 14-Ethylenedithioketal Acid (**11**)

A solution of compound **15** (72.6 mg) in MeOH (2.8 ml) containing 1 N NaOH (0.75 ml) was refluxed under a nitrogen atmosphere overnight. The mixture was neutralized with 1 N HCl and the solvent was removed. Water was added to give a precipitate. The precipitate was collected by filtration, washed with water and dried. MeOH was added and the mixture was heated. Insoluble material was filtered off and the filtrate was concentrated to give compound **11** (12.8 mg, 21.6%) as a white solid. MP 167.0~170.0°C; ^1H NMR (200 MHz, CD_3OD) δ: 5.54 (1H, dtt-like, J=15.1 and 6.3 Hz, 7-H), 5.39 (1H, dtt-like, J=14.9 and 6.6 Hz, 6-H), 4.0 (1H, d, J=11.0 Hz, 21-H), 3.86 (1H, d, J=11.0 Hz, 21-H), 3.84 (1H, br t, J=7.0, 4-H), 3.79 (1H, d, J=1.0, 3-H), 3.25 (4H, s, 1'- and 2'-H_2), 2.27 (2H, br t, J=6.5 Hz, 5-H_2), 2.00 (2H, q, J=6.4 Hz, 8-H_2), 1.88 (4H, m, 13- and 15-H_2), 1.45 (4H, m, 12- and 16-H_2), 1.30 (12H, m, 9-~11- and 17-~19-H_2), 0.91 (3H, t, J=6.6 Hz, 20-H_3); ^{13}C NMR (50 MHz, CD_3OD) δ: 173.41 (s, 1-C), 134.78 (d, 7-C), 126.83 (d, 6-C), 73.70 (d, 3-C), 72.54 (s, 14-C), 71.39 (s, 2-C), 70.42 (d, 4-C), 65.11 (t, 21-C), 44.71 and 44.71 (each t, 13- and 15-C), 40.33 and 40.33 (each t, 1'- and 2'-C), 38.73, 33.78, 32.94, 30.80, 30.60, 30.55, 30.24, 27.95, 27.95 and 23.69 (each t, 5-, 8-~12- and 16-~19-C), 14.45 (q, 20-C); FAB-MS m/z: 478 $(M+H)^+$, 418, 402, 185, 104, 93, 54.

The 14-Oxime (**12**)

Myriocin (200.9 mg) was added to a mixture of a solution of Na_2CO_3 (26.9 mg) in water (1.5 ml) and a solution of hydroxylamine hydrochloride (35.0 mg) in EtOH (3.5 ml), and the whole was heated at 70°C for 6 hours. After usual work-up, the mixture gave compound **12** (87.8 mg, 42.1%). MP 179.5~180.5°C; $[\alpha]_D^{28}$ −5.8° (c 0.509, MeOH); IR ν_{max} (KBr) cm^{-1}: 3200, 2910, 2850, 1620, 1460, 1320, 1100, 1050, 965; ^1H NMR (200 MHz, CD_3OD) δ: 5.54 (1H, dtt-like, J=15.6 and 6.2 Hz, 7-H), 5.38 (1H, dtt-like, J=15.4 and 6.4 Hz, 6-H), 4.00 (1H, d, J=11.2 Hz, 21-H), 3.86 (1H, d, J=11.2 Hz, 21-H), 3.84 (1H, br t, J=7.1 Hz,

4-H), 3.78 (1H, d, J=0.5 Hz, 3-H), 2.32 (2H, t, J=7.7 Hz, 13- or 15-H_2), 2.27 (2H, t, J=7.0 Hz, 13- or 15-H_2), 2.14 (2H, dd, J=7.8 and 6.8 Hz, 5-H_2), 2.00 (2H, q, J=6.6 Hz, 8-H_2), 1.49 (4H, m, 12- and 16-H_2), 1.30 (12H, m, 9-~11- and 17-~19-H_2), 0.90 (3H, t, J=6.6 Hz, 20-H_3); ^{13}C NMR (50 MHz, CD_3OD) δ: 173.44 (s, 1-C), 162.61 (s, 14-C), 134.64 (d, 7-C), 126.85 (d, 6-C), 73.56 (d, 3-C), 71.27 (s, 2-C), 70.49 (d, 4-C), 65.09 (t, 21-C), 38.68, 34.83, 33.71, 32.72, 30.77, 30.43, 30.09, 29.99, 28.07, 27.75, 26.71 and 23.59 (each t, 5-, 8-~13- and 15-~19-C), 14.42 (q, 20-C); EI-MS m/z: 398 $(M-H_2O)^+$, 328, 256, 128, 104, 18.

Anal Calcd for $C_{21}H_{40}N_2O_6$: C 60.55, H 9.68, N 6.72.
Found: C 60.26, H 9.47, N 6.57.

The 14-Deoxo Triacetyl Lactone (**17**)

Activated zinc powder (29.5 g) was added in small portions to a solution of compound **1** (7.084 g) in acetic anhydride (156 ml) saturated with hydrogen chloride during 4 hours with stirring and ice cooling. After the addition, stirring was continued for further 1.5 hours under ice cooling. The solution was sucked up into a pipette through cotton wool, poured into ice water and extracted with EtOAc. The organic layer was worked up in the usual manner to give an oily residue (7 g). The residue was purified by chromatography on a silica gel (140 g) using EtOAc-*n*-hexane (7:3) to give compound **17** (6.24 g, 81.7%) as an oil. IR ν_{max} ($CHCl_3$) cm^{-1}: 1780, 1755, 1685, 1500, 1030, 970; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.99 (1H, br s, –NH), 5.79 (1H, d, J=4.4 Hz, 3-H), 5.58 (1H, dtt-like, J=15.3 and 6.6 Hz, 7-H), 5.39 (1H, dtt-like, J=15.3 and 7.1 Hz, 6-H), 4.71 (1H, ddd, J=7.1, 5.6 and 4.4 Hz, 4-H), 4.52 (2H, br s, 21-H_2), 2.39 (2H, m, 5-H_2), 2.10 (3H, s, Ac), 2.05 (3H, s, Ac), 2.03 (3H, s, Ac), 2.01 (2H, m, 8-H_2), 1.26 (22H, br s, 9-~19-H_2), 0.88 (3H, t, J=6.4 Hz, 20-H_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 172.44 (s, >C=O), 170.17 (s, >C=O), 169.42 (s, >C=O), 168.89 (s, >C=O), 135.29 (d, 6-C), 122.96 (d, 7-C), 81.66 (d, 4-C), 71.93 (d, 3-C), 62.73 (t, 21-C), 62.64 (s, 2-C), 32.57, 32.18 and 31.91 (each t, 5-, 8- and 18-C), 29.68, 29.68, 29.68, 29.68, 29.68, 29.51, 29.36, 29.21 and 29.21 (each t, 9-~17-C), 22.77 (q, –$COCH_3$), 22.70 (t, 19-C), 20.58 (q, –$COCH_3$), 20.34 (q, –$COCH_3$), 14.11 (q, 20-C); EI-MS m/z: 495 $(M)^+$, 436, 376, 334, 265, 151, 129, 43.

HREI-MS Calcd for $C_{27}H_{45}NO_7$: 495.31975.
Found: m/z 495.31864.

The 14-Deoxo Acid, (2*S*,3*R*,4*R*)-(*E*)-2-Amino-3,4-dihydroxy-2-hydroxymethyleicos-6-enoic acid (**13**)

A solution of compound **17** (3.102 g) in MeOH (150 ml) containing 1 N NaOH (37.6 ml) was refluxed under a nitrogen atmosphere overnight. The mixture was neutralized with 1 N HCl to give a precipitate. The precipitate was washed with water and MeOH-water (1:1), and dried to give compound **13** (2.018 g, 83.2%). MP 186 °C; IR ν_{max} (KBr) cm^{-1}: 3400, 3150, 2925, 2850, 1660, 1565, 1525, 1465, 1405, 1360, 1260, 965; ^{1}H NMR (200 MHz, CD_3OD) δ: 5.54 (1H, dtt-like, J=15.4 and 5.9 Hz, 7-H), 5.38 (1H, dtt-like, J=15.4 and 6.1 Hz, 6-H), 4.00 (1H, d, J=11.0 Hz, 21-H), 3.86 (1H, d, J=11.0 Hz, 21-H), 3.83 (1H, br t, J=7.3 Hz, 4-H), 3.78 (1H, br s, 3-H), 2.26 (2H, br t, J=6.8 Hz, 5-H_2), 2.00 (2H, m, 8-H_2), 1.28 (22H, br s, 9-~19-H_2), 0.90 (3H, t, J=6.5 Hz, 20-H_3); FAB-MS m/z: 388 $(M+H)^+$, 104, 57, 45, 29.

Anal Calcd for $C_{21}H_{41}NO_5$: C 65.08, H 10.66, N 3.61.
Found: C 64.78, H 10.91, N 3.61.

Ozonolysis of Compound **1**

A solution of compound **1** (351.3 mg) in dichloromethane (25 ml) was ozonized at −60°C for 6 minutes, then concentrated to give an oily residue. To a solution of the residue in dioxane (21.2 ml) was added water (8 ml) and small pieces of dry ice, with stirring at room temperature. After disappearance of the dry ice, $NaBH_4$ (80.0 mg) was added to the mixture. After 30 minutes, the mixture was worked up in the usual manner to give compound **18** (106.0 mg, 48.5%). IR ν_{max} ($CHCl_3$) cm^{-1}: 3400, 3000, 2900, 1780, 1755, 1680, 1500, 1370, 1225, 1050; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.32 (1H, br s, –N*H*Ac), 5.81 (1H, d, J=4.6 Hz, 3-H), 5.06 (1H, ddd, J=9.0, 4.6 and 4.4 Hz, 4-H), 4.53 (2H, br s, 7-H_2), 2.11 (3H, s, Ac), 2.07 (3H, s, Ac), 2.03 (3H, s, Ac), 2.1~1.8 (4H, m, 5- and 6-H_2); EI-MS m/z: 317 $(M)^+$, 274, 185, 167, 43.

HREI-MS Calcd for $C_{13}H_{19}NO_8$: 317.11107.
Found: m/z 317.11177.

Hydrolysis of Compound **18**

A solution of compound **18** (70.0 mg) in MeOH (1.33 ml) and water (3.99 ml) was treated with 1 N

NaOH (1.33 ml). The mixture was refluxed under a nitrogen atmosphere overnight and applied to an IRC-50 (H^+ type 6 ml eluent: water - MeOH (8 : 2)) column. The eluate (30 ml) was concentrated to give a residue, which was taken up with water (2 ml) and filtered. The filtrate was concentrated until the solution became slightly turbid and the solution was kept standing for 3 hours to give compound **14** (14.0 mg, 30.3%) as colorless scales. MP 209.5~219.0 °C (dec.); ^{1}H NMR (200 MHz, D_2O, Ref. DSS) δ: 4.03 (1H, d, J=11.7, 7-H), 3.95 (1H, ddd, J=8.3, 5.1 and 1.2 Hz, 4-H), 3.92 (1H, d, J=11.7 Hz, 7-H), 3.78 (1H, d, J=1.2 Hz, 3-H), 3.67 (2H, br dd, J=7.1 and 6.2 Hz, 6-H_2), 1.87 (1H, ddt, J=14.2, 8.3 and 6.2 Hz, 5-H), 1.74 (1H, dtd, J=14.2, 7.1 and 5.1 Hz, 5-H); ^{13}C NMR (50 MHz, D_2O, Ref. DSS) δ: 175.45 (s, 1-C), 72.76 (s, 2-C), 72.51 (d, 3-C), 72.05 (d, 4-C), 66.43 (t, 7-C), 60.76 (t, 6-C), 38.58 (t, 5-C); FAB-MS m/z: 210 $(M+H)^+$, 185, 167, 115, 93, 75, 57.

Acknowledgment

We are grateful to Prof. TETSURO SHINGU, Kobe Gakuin University, Faculty of Pharmaceutical Sciences for NMR (400 MHz) measurement. This study was supported by a Grant-in-Aid for Scientific Research (No. 01870095 and No. 03557103) from the Ministry of Education, Science and Culture of Japan.

References

1) FUJITA, T.; K. INOUE, S. YAMAMOTO, T. IKUMOTO, S. SASAKI, R. TOYAMA, K. CHIBA, Y. HOSHINO & T. OKUMOTO: Fungal metabolites. Part 11. A potent immunosuppressive activity found in *Isaria sinclairii* metabolite. J. Antibiotics 47: 208~215, 1994
2) FUJITA, T.; K. INOUE, S. YAMAMOTO, T. IKUMOTO, S. SASAKI, R. TOYAMA, K. CHIBA, Y. HOSHINO & T. OKUMOTO: Isolation of ISP-I, a novel immunosuppressant produced by *Isaria sinclairii*. Abstracts of Papers of the 17th IUPAC International Symposium on the Chemistry of Natural Products, p. 68, New Delhi, Feb. 4~9, 1990
3) KLUEPFEL, D.; J. BAGLI, H. BAKER, M.-P. CHAREST, A. KUDELSKI, S. N. SEHGAL & C. VÉZINA: Myriocin, a new antifungal antibiotic from *Myriococcum albomyces*. J. Antibiotics 25: 109~115, 1972
4) BAGLI, J. F.; D. KLUEPFEL & M. ST-JACQUES: Elucidation of structure and stereochemistry of myriocin. A novel antifungal antibiotic. J. Org. Chem. 38: 1253~1260, 1973
5) ARAGOZZINI, F.; P. L. MANACHINI, R. CRAVERI, B. RINDONE & C. SCOLASTICO: Isolation and structure determination of a new antifungal α-hydroxymethyl-α-amino acid. Tetrahedron 28: 5493~5498, 1972
6) BOREL, J. F.: History of cyclosporin A and its significance in immunology. *In* Cyclosporin A. *Ed.* J. F. BOREL, pp. 5~17, Elsevier Biochemical Press, Amsterdam, 1982
7) TANAKA, H.; A. KURODA, H. MARUSAWA, H. HATANAKA, T. KINO, T. GOTO, M. HASHIMOTO & T. TAGA: Structure of FK-506: A novel immunosuppressant isolated from *streptomyces*. J. Am. Chem. Soc. 109: 5031~5033, 1987
8) KINO, T.; H. HATANAKA, M. HASHIMOTO, M. NISHIYAMA, T. GOTO, M. OKUHARA, M. KOHSAKA, H. AOKI & H. IMANAKA: FK-506, a novel immunosuppressant isolated from a *Streptomyces*. I. Fermentation, isolation, and physico-chemical and biological characteristics. J. Antibiotics 40: 1249~1255, 1987
9) HATCH, R. P.; J. SHRINNGARPURE & S. M. WEINREB: Studies on total synthesis of the olivomycins. J. Org. Chem. 45: 4172~4177, 1978
10) YAMAMURA, S. & Y. HIRATA: Zinc reduction of keto-groups to methylene groups. J. Chem. Soc. (C) 1968: 2887~2889, 1968