FUNGAL METABOLITES. PART 14†. NOVEL POTENT IMMUNOSUPPRESSANTS, MYCESTERICINS, PRODUCED BY *Mycelia sterilia*

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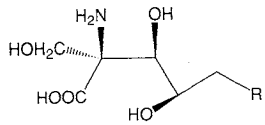
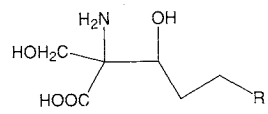
Mycestericins A, B, C, D and E were isolated from the culture broth of *Mycelia sterilia* ATCC 20349 along with thermozymocidin (=myriocin). Their structures were elucidated on the basis of spectroscopic studies and chemical evidence. The acetate of mycestericin C was identical with the acetate of 6,7-dihydromyriocin. Mycestericins suppressed the proliferation of lymphocytes in the mouse allogeneic mixed lymphocyte reaction, with a potency similar to that of myriocin.

We have very recently reported[2,3] that a metabolite of *Isaria sinclairii* (ATCC 24400) showed potent immunosuppressive activity. Its activities in the mouse allogeneic mixed lymphocyte and mouse allogeneic cytotoxic T lymphocyte assays were 10 times and 100 times those of cyclosporin A (CsA), respectively. The metabolite was identical with myriocin[4,5] (**1**, Fig. 1) and thermozymocidin[6], which are produced by *Myriococcum albomyces* (ATCC 16425) and *Mycelia sterilia* (ATCC 20349), respectively. Reduction of the ketone at C-14 of **1** to a methylene increased the activity 10-fold[7]. Therefore, it seemed possible that

Fig. 1. The structures of myriocin and mycestericins.



Myriocin (**1**)
R=$-CH\overset{E}{=}CH-(CH_2)_6-CO-(CH_2)_5CH_3$



Mycestericin D (**5**) and E (**6**)
R=$-CH\overset{E}{=}CH-(CH_2)_6-CO-(CH_2)_5CH_3$

Mycestericin A (**2**)
R=$-CH\overset{E}{=}CH-(CH_2)_4-CH\overset{E}{=}CH-\overset{R}{C}HOH-(CH_2)_5CH_3$

Mycestericin B (**3**)
R=$-CH\overset{E}{=}CH-(CH_2)_6-CHOH-(CH_2)_5CH_3$

Mycestericin C (**4**)
R=$-(CH_2)_8-CO-(CH_2)_5CH_3$

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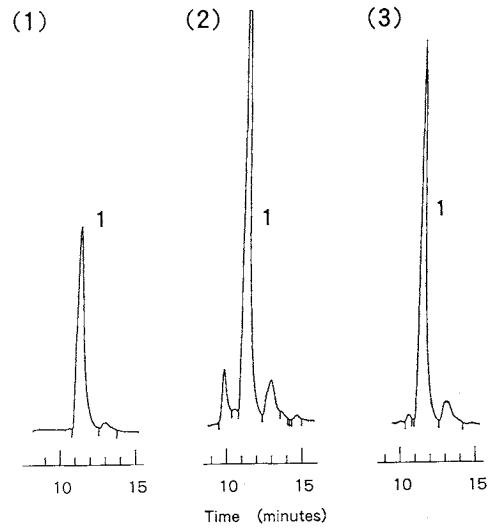
Fig. 2. Elution profiles of methanolic extracts from *I. sinclairii* (1), *M. sterilia* (2) and *M. albomyces* (3) on reverse-phase HPLC.



Each mycelium (wet weight 1 g) was extracted with MeOH (5 ml) and the extracts [the extract of (1) was concentrated to 0.3 ml] was subjected to HPLC analysis. Analytical conditions: eluent, MeOH-H_2O (75:25); flow rate, 1 ml/minute; detection, RI; injection, 10 μl; column, YMC-packed column AQ-312 (6 × 150 mm).

even more active immunosuppressants than **1** might be found in the culture broth of myriocin-producing microorganisms, such as the fungi, *I. sinclairii*, *M. sterilia* and *M. albomyces*. The MeOH extracts from mycelia of these three fungi showed immunosuppressive activity, and several minor compounds along with **1** as the major components were detected by HPLC analyses (Fig. 2). In order to investigate the minor components we selected *M. sterilia*, because this strain produced the greatest amount of **1** and the minor components, mycestericins, among the above three strains.

The present paper describes the isolation, the structural determination and the biological activities of mycestericins isolated from the culture broth of *M. sterilia*.

Results and Discussion

Isolation of Mycestericins

Mycestericins were isolated from the culture

Fig. 3. Isolation of mycestericins.

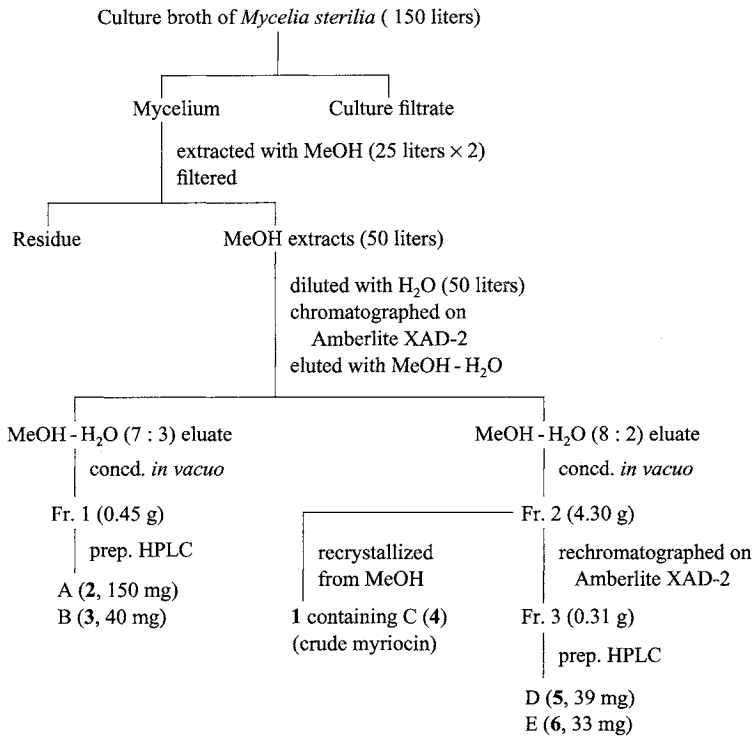


Table 1. Physico-chemical properties of myriocin (**1**) and mycestericins A, B, D and E (**2**, **3**, **5** and **6**).

Compound	1	2	3	5	6
MP (°C)	169~171	170~171	162~164	162~167	184~186
$[\alpha]_D$	+4.8°	−8.5°	−4.3°	−7.5°	−8.6°
	(*c* 0.29, MeOH)	(*c* 0.50, MeOH)	(*c* 0.28, MeOH)	(*c* 0.16, MeOH)	(*c* 0.06, MeOH)
IR (KBr) cm^{-1}	3400, 3250, 3125, 1710, 1670, 1660, 1605, 970	3400, 3270, 3180, 3130, 1630, 1605, 965	3390, 3260, 3260, 3150, 1625, 1605, 965	3375, 3275, 3100, 1710, 1625, 1580, 970	3075, 1710, 1660, 1580, 965
FAB-MS (*m/z*)					
$(M+H)^+$	402	402	404	386	386
$(M-H)^-$	—	400	402	384	—
HRFAB-MS (*m/z*)					
$(M+H)^+$					
Calcd:				386.2908	386.2908
Found:				386.2908	386.2910
HREI-MS (*m/z*)					
$(M-H_2O)^+$					
Calcd:	383.2672	383.2673	385.2830		
Found:	383.2666	383.2639	385.2824		
Molecular formula	$C_{21}H_{39}NO_6$	$C_{21}H_{39}NO_6$	$C_{21}H_{41}NO_6$	$C_{21}H_{39}NO_5$	$C_{21}H_{39}NO_5$

broth of *Mycelia sterilia* as shown in Fig. 3. The MeOH extract was subjected to Amberlite XAD-2 column chromatography to give Frs. 1 and 2. Preparative HPLC of Fr. 1 gave mycestericins A (**2**) and B (**3**), while Fr. 2 gave mycestericins D (**5**) and E (**6**) along with crude **1** containing mycestericin C (**4**).

Structural Determination

Mycestericin A (2)

Mycestericin A (**2**) was obtained as a white powder and its physico-chemical properties are summarized in Table 1. Based on the MS data, the molecular formula was determined as $C_{21}H_{39}NO_6$. The IR spectrum was similar to that of **1** except for the absence of the 1710 cm^{-1} absorption (C=O). The ^1H NMR spectra of **2** and its acetate were also similar to those of **1** and its acetate, respectively (Tables 2 and 4). These facts suggested the presence of partial structure I (Fig. 4) in **2**. The methylene proton signals at δ 2.43 (13-H_2 and 15-H_2) and 1.53 (12-H_2 and 16-H_2) of **1** were absent in the spectrum of **2**. On the other hand, the signals of trans olefinic protons [δ 5.59 (1H, dtd, J=15.3, 6.8 and 0.8 Hz) and 5.40 (1H, ddt-like, J=15.3 and 6.8 Hz)] and a methine proton on carbon bearing oxygen [(δ 3.95 (1H, br q, J=6.8 Hz)] appeared in the ^1H NMR spectrum of **2**. In the ^1H-^1H COSY spectrum of **2** cross peaks were observed for the signals at δ 5.59 (12-H)/δ 5.40 (13-H) and δ 2.03 (11-H_2); δ 5.40 (13-H)/δ 5.59 (12-H) and δ 3.95 (14-H); δ 3.95 (14-H)/δ 5.40 (13-H), δ 1.55~1.48 (15-H) and 1.45~1.38 (15-H); δ 2.03 (11-H_2)/δ 5.59 (12-H) and δ 1.39 (10-H_2) (Fig. 5). These results indicated the presence of partial structure II in **2**. Furthermore, signals due to $-CH_2CH_2CH_3$ [δ 0.90 (3H, deformed t)] and methylene [δ 1.38~1.25 (12H)] were observed.

The EI-MS of **2** showed dehydrated and fragment ion peaks at *m/z* 383 and 298, respectively, suggesting formation of γ-lactone followed by cleavage at C-14 to give an allylic alcohol ion (III, Fig. 4). Thus, the plane structure of mycestericin A was established as (*E*,*E*)-2-amino-3,4,14-trihydroxy-2-hydroxymethyleicosa-6,12-dienoic acid (**2**). The structure was supported by the ^{13}C NMR spectrum of **2** and its acetate (Tables 3 and 4).

In order to determine the absolute configuration at C-2 to C-4 and at C-14 of **2**, the benzoate CD

Table 2. ^{1}H NMR chemical shift assignments for myriocin (**1**), (*E*)-2-amino-3,4,14-trihydroxy-2-hydroxymethyl-eicos-6-enoic acid (**1a**) and mycestericins A, B, D and E (**2**, **3**, **5** and **6**) (600 MHz in CD_3OD).

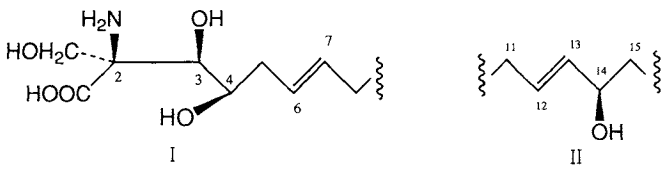
Proton	**1**	**1a**	**2**
3-H	3.77 d (1.0)	3.77 d (1.0)	3.79 d (0.8)
4-H	3.83 td (7.0, 1.0)	3.82 br t (7.2)	3.84 td (7.0, 0.8)
4-H_2	—	—	—
5-H_2	2.26 t (7.0)	2.26 t (6.8)	2.27 br t (7.0)
6-H	5.38 dt (15.2, 7.0)	5.38 dt (15.2, 7.0)	5.40 dt (15.3, 7.0)
7-H	5.52 dt (15.2, 6.5)	5.52 dt (15.2, 6.5)	5.53 dt (15.3, 6.8)
8-H_2	2.00 q (6.5)	2.00 q (6.5)	2.01 q (6.8)
9-H_2	1.27 br s	1.45～1.28 m	1.39 qui (6.8)
10-H_2	1.27 br s	1.45～1.28 m	1.39 qui (6.8)
11-H_2	1.27 br s	1.45～1.28 m	2.03 br q (6.8)
12-H	—	—	5.59 dtd (15.3, 6.8, 0.8)
12-H_2	1.53 qui (7.3)	1.45～1.28 m	—
13-H	—	—	5.40 dd (15.3, 6.8)
13-H_2	2.43 t (7.3)	1.45～1.28 m	—
14-H	—	3.48 m	3.95 br dt (6.8, 6.8)
15-H_2	2.43 t (7.3)	1.45～1.28 m	1.55～1.48 m, 1.45～1.38 m
16-H_2	1.53 qui (7.3)	1.45～1.28 m	1.38～1.25 m
17-～19-H_2	1.27 br s	1.45～1.28 m	1.38～1.25 m
20-H_3	0.90 t (6.5)	0.90 (6.8)	0.90 t (7.0)
21-H_2	3.99 d (11.0), 3.86 d (11.0)	3.99 d (11.0), 3.87 d (10.9)	4.00 d (11.1), 3.88 d (11.1)

Proton	**3**	**5**	**6**
3-H	3.79 d (1.0)	3.84 d (6.8)	3.85 d (6.8)
4-H	3.84 t (7.0)	—	—
4-H_2	—	1.53 m	1.41 dddd (13.8, 11.0, 9.0, 4.7), 1.70 dddd (13.8, 9.2, 7.1, 1.9)
5-H_2	2.27 (7.0)	2.24 dtd (14.2, 6.7, 5.9), 2.01 dtd (14.2, 7.1, 5.9)	2.27 dddd (13.8, 9.2, 6.2, 4.7), 2.05 dddd (13.8, 9.0, 7.1, 6.2)
6-H	5.39 dt (15.1, 7.0)	5.41 dt (15.4, 5.9)	5.42 dt (15.4, 6.2)
7-H	5.53 dt (15.1, 6.8)	5.46 dt (15.4, 5.9)	5.47 dt (15.4, 6.3)
8-H_2	2.00 br q (6.8)	1.97 q (6.2)	1.98 q (6.3)
9-H_2	1.47～1.25 m	1.38～1.24 m	1.38～1.24 m
10-H_2	1.47～1.25 m	1.38～1.24 m	1.38～1.24 m
11-H_2	1.47～1.25 m	1.38～1.24 m	1.38～1.24 m
12-H	—	—	—
12-H_2	1.47～1.25 m	1.54 q (7.2)	1.54 q (7.2)
13-H	—	—	—
13-H_2	1.47～1.25 m	2.44 t (7.2)	2.44 t (7.2)
14-H	3.50 q (4.0)	—	—
15-H_2	1.47～1.25 m	2.44 t (7.2)	2.44 t (7.2)
16-H_2	1.47～1.25 m	1.54 q (7.2)	1.54 q (7.2)
17-～19-H_2	1.47～1.25 m	1.38～1.24 m	1.38～1.24 m
20-H_3	0.90 t (7.0)	0.90 t (7.1)	0.90 t (7.0)
21-H_2	4.00 d (11.0), 3.87 d (11.0)	3.99 d (11.2), 3.82 d (11.2)	3.93 d (11.3), 3.81 d (11.3)

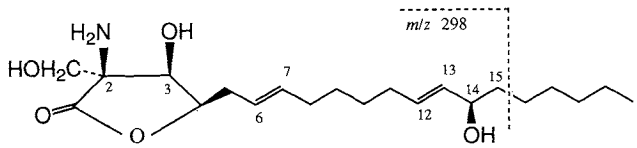
ppm and multiplicity (J=Hz).
qui: quintet.

chirality method was employed. Tribenzoyl-14-deoxytetrahydromycestericin A γ-lactone was synthesized by benzoylation of **2** followed by hydrogenolysis. The spectra (IR and ^{1}H NMR) and optical rotation were identical with those of an authentic sample (**12**, Fig. 6) derived from **1**. Furthermore, the CD spectra were virtually superimposable (Fig. 7). Consequently, the absolute configurations at C-2 to C-4 were determined to be 2*S*, 3*R* and 4*R*, as in **1**. On the other hand, *N*-acetyl-14-*O*-benzoylmycestericin A γ-lactone (**10**)

Fig. 4. Partial structures of **2**.

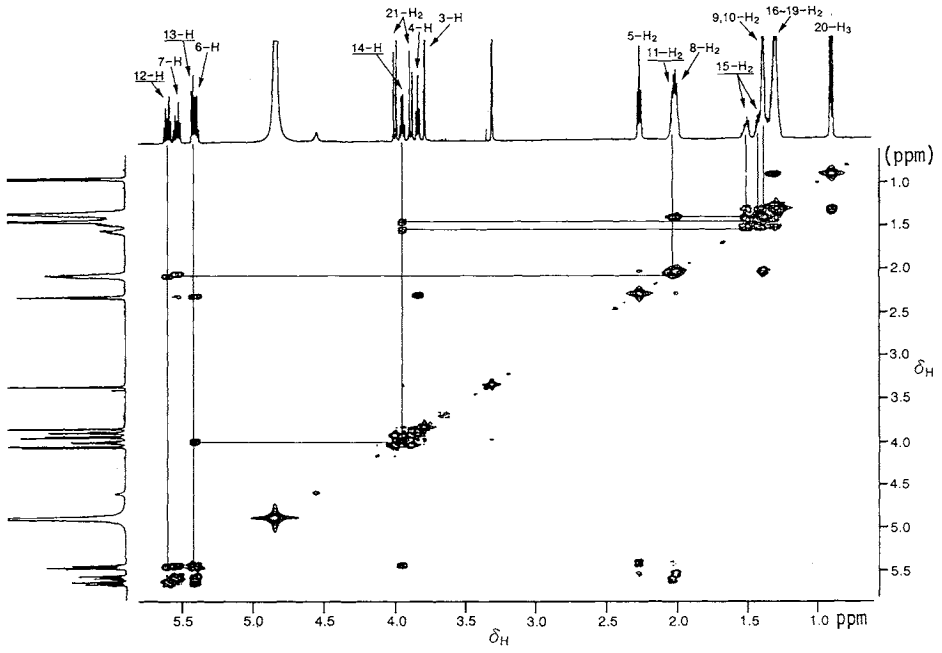


EI-MS *m/z* : 383 (M^+-H_2O)



III

Fig. 5. ^1H-^1H COSY spectrum of mycestericin A (**2**).



was synthesized by benzoylation of *N*-acetylmycestericin A (**8**) followed by partial hydrolysis. The CD spectrum showed a negative Cotton effect at 236 nm (Fig. 8), suggesting that the absolute configuration of the hydroxy group at C-14 is *R*[8,9]. Thus, the structure of mycestericin A was determined to be **2**, as shown in Fig. 1.

Mycestericin B (3)

Mycestericin B (**3**), $C_{21}H_{41}NO_6$, was obtained as an amorphous solid, and its physico-chemical

Table 3. ^{13}C NMR chemical shift assignments for mycestericins A, B, D and E (**2**, **3**, **5** and **6**) (75 Hz in CD_3OD).

Carbon	2	3	5	6	Carbon	2	3	5	6
1-C	173.49 (s)	—	—	—	12-C	[c]	[e]	[f]	[g]
2-C	71.32 (s)	71.38 (s)	64.71 (s)	62.54 (s)	13-C	132.39 (d)	[e]	43.53 (t)	43.55 (t)
3-C	[a]	[d]	71.01 (d)	71.79 (d)	14-C	[a]	[d]	214.44 (s)	214.47 (s)
4-C	[a]	[d]	[f]	[g]	15-C	[b]	[e]	43.53 (t)	43.55 (t)
5-C	[b]	[e]	[f]	[g]	16-C	[b]	[e]	[f]	[g]
6-C	126.95 (d)	126.78 (d)	130.63 (d)	130.57 (d)	17-C	[b]	[e]	[f]	[g]
7-C	[c]	134.85 (d)	132.07 (d)	132.26 (d)	18-C	[b]	[e]	[f]	[g]
8-C	[b]	[e]	33.63 (t)	33.64 (t)	19-C	23.70 (t)	23.72 (t)	23.60 (t)	23.62 (t)
9-C	[b]	[e]	[f]	[g]	20-C	14.45 (q)	14.45 (q)	14.39 (q)	14.40 (q)
10-C	[b]	[e]	[f]	[g]	21-C	65.16 (t)	65.12 (t)	64.71 (t)	62.54 (t)
11-C	[b]	[e]	[f]	[g]					

Chemical shifts are expressed as δ (ppm), with multiplicity in parentheses.
—: Not detected.
[a] >CHO: 70.49, 73.69 or 73.81 (each d).
[b] $>CH_2$: 26.65, 30.01, 30.04, 30.41, 33.07, 33.17, 33.62, 38.53 or 38.72 (each t).
[c] HC=CH: 134.61 or 134.68 (each d).
[d] >CHO: 70.44, 72.50 or 73.70 (each d).
[e] $>CH_2$: 26.81, 30.36, 30.58, 30.76, 33.08, 33.79, 38.48 or 38.71 (each t).
[f] $>CH_2$: 24.92, 30.03, 30.08, 30.22, 30.28, 30.60, 32.70 or 32.83 (each t).
[g] $>CH_2$: 24.93, 30.04, 30.09, 30.21, 30.60 or 32.84 (each t).

properties are summarized in Table 1. The ^1H NMR spectrum of **3** was similar to that of **1** (Table 2). However, the methylene proton signals at δ 2.43 (13-H_2 and 15-H_2) and δ 1.53 (12-H_2 and 16-H_2) in **1** were not observed in the spectrum of **3**. Instead, the signals of a methine proton on a carbon bearing oxygen (δ 3.50, 1H) was observed in the spectrum of **3**. These facts indicated the presence of a hydroxy group at C-14 in **3**. The spectral data of **3** were identical with those of (*E*)-2-amino-3,4,14-trihydroxy-2-hydroxymethyleicos-6-enoic acid[7] (**1a**).

The CD curve of tetrabenzoyldihydromycestericin B γ-lactone (**16**) showed good coincidence with those of tetrabenzoyltetrahydromycestericin A γ-lactone (**13**) and a tetrabenzoate (**14**) derived from tetrahydromyriocin (Fig. 9). Thus, the absolute configurations at C-2 to C-4 of **3** are 2*S*, 3*R* and 4*R*, respectively. Consequently, the structure of mycestericin B corresponds to **3**. However, the absolute configuration of the carbon bearing hydroxyl group at C-14 was not elucidated.

Mycestericin C (**4**)

HPLC analysis showed that crude **1** contained a minor component (**4**), whose Rf value on TLC was almost the same as that of **1**. The retention time of **4** identical with that of 6,7-dihydromyriocin[7]. However, it was difficult to isolate in a pure form by preparative HPLC. Therefore, the acetyl derivative of crude **1** was treated with OsO_4 and the non-reactive compound (mycestericin C triacetate) was purified by preparative TLC. The spectra (^1H NMR, IR and MS) and $[\alpha]_D$ value were identical with those of triacetyl-6,7-dihydromyriocin γ-lactone (Table 5). Consequently, mycestericin C (**4**) was determined to be (2*S*,3*R*,4*R*)-2-amino-3,4-dihydroxy-2-hydroxymethyl-14-oxoeicosanoic acid.

Mycestericin D (**5**)

Mycestericin D (**5**) was obtained as a white powder and its physico-chemical properties are summarized in Table 1. Based on the MS data, the molecular formula was determined as $C_{21}H_{39}NO_5$, suggesting that

Table 4. ^1H and ^{13}C NMR chemical shift assignments for triacetylmyriocin γ-lactone (**1b**) and tetraacetylmycestericin A γ-lactone (**7**).

	^1H NMR (600 MHz in $CDCl_3$)		^{13}C NMR (75 MHz in $CDCl_3$)		
Proton	**1b**	**7**	Carbon	**1b**	**7**
3-H	5.79 d (4.3)	5.79 d (4.4)	1-C	172.32 s	172.41 s
4-H	4.72 td (8.2, 4.3)	4.71 ddd (8.5, 4.8, 4.4)	2-C	62.73 s	62.63 s
5-H_2	2.5~2.3 m	2.44 ddd (14.8, 8.5, 6.9),	3-C	72.01 d	71.94 d
		2.35 ddd (14.8, 6.9, 4.8)	4-C	81.62 d	81.58 d
6-H	5.39 dt (15.2, 7.0)	5.40 dt (15.3, 6.9)	5-C	32.20 t	32.19 t
7-H	5.56 dt (15.2, 6.5)	5.56 dt (15.3, 6.7)	6-C	123.16 d	128.59 d
8-H_2	1.99 q (7.0)	2.05~1.98 m	7-C	135.04 d	134.94 d
9-H_2	1.27 br s	1.40~1.30 m	8-C	32.46 t	32.04 or 32.35 t
10-H_2	1.27 br s	1.40~1.30 m	9-C	[a]	28.48 or 28.68 t
11-H_2	1.27 br s	2.05~1.98 m	10-C	[a]	28.48 or 28.68 t
12-H	—	5.67 dtd (15.4, 6.7, 0.5)	11-C	[a]	32.04 or 32.35 t
12-H_2	1.55 qui (7.5)	—	12-C	[a]	134.03 d
13-H	—	5.37 ddt (15.4, 7.2, 1.4)	13-C	42.85 t	123.27 d
13-H_2	2.39 t (7.5)	—	14-C	211.38 s	75.04 d
14-H	—	5.17 dt (7.2, 6.9)	15-C	42.77 t	34.55 t
15-H_2	2.39 t (7.5)	1.66~1.58 m,	16-C	[a]	[b]
		1.56~1.49 m	17-C	[a]	[b]
16-H_2	1.55 qui (7.5)	1.32~1.22 m	18-C	[a]	[b]
17~19-H_2	1.27 br s	1.32~1.22 m	19-C	22.50 t	22.57 t
20-H_3	0.88 t (6.5)	0.88 t (7.0)	20-C	14.00 q	14.06 d
21-H_2	4.53 d (11.5),	4.52 d (11.5),	21-C	62.75 t	62.74 t
	4.50 d (11.5)	4.50 d (11.5)	-*C*OMe	170.13 s	170.44 s
-NH	6.03 s	6.10 s	-*C*OMe	169.95 s	170.18 s
-COMe	2.10	2.10 s	-*C*OMe	169.89 s	169.45 s
-COMe	2.05	2.05 s	-*C*OMe	—	168.89 s
-COMe	2.03	2.03 s	-CO*Me*	22.76 q	22.75 q
-COMe	—	2.02 s	-CO*Me*	20.37 q	21.41 q
			-CO*Me*	20.33 q	20.59 q
			-CO*Me*	—	20.34 q

ppm and multiplicity (J=Hz).
[a] 23.85, 23.91, 28.91, 28.97, 29.00, 29.11 or 31.63 (each t).
[b] 25.17, 29.03 or 31.73 (each t).

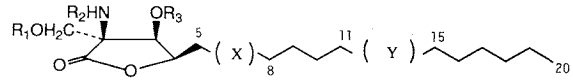
5 is the deoxy derivative of myriocin (**1**). The ^1H NMR spectrum of **5** was similar to that of **1**, except for the signals of a methine proton on carbon bearing oxygen (δ 3.83, 4-H) and allylic methylene protons (δ 2.26, 5-H_2) in **1** (Fig. 10).

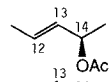
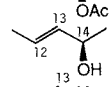
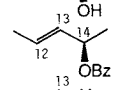
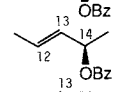
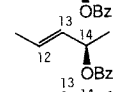
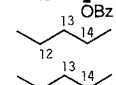
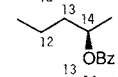
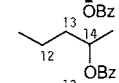
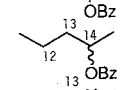
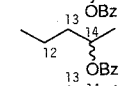
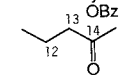
In the ^1H-^1H COSY spectrum of **5**, cross peaks were observed for the signals at δ 3.84 (3-H)/δ 1.53 (4-H_2), δ 2.24 (5-H) and δ 2.01 (5-H); δ 2.24 (5-H)/δ 5.41 (olefinic proton, 6-H) and δ 1.53 (4-H_2); δ 2.01 (5-H)/δ 5.41 (6-H) and δ 1.53 (4-H_2); δ 1.53 (4-H_2)/δ 2.24 (5-H), δ 2.01 (5-H) and δ 3.84 (3-H). These observations indicated the presence of a methylene group at C-4. Accordingly, the plane structure of mycestericin D was concluded to be **5**.

Mycestericin E (**6**)

Mycestericin E (**6**), $C_{21}H_{39}NO_5$, was obtained as a white powder and its physico-chemical properties are summarized in Table 1. Although the ^1H NMR spectrum of **6** was very similar to that of **5**, signals of methylene protons (δ 1.53, 2H) at C-4 of **5** appeared at δ 1.70 and δ 1.41 in the spectrum of **6**. The ^1H-^1H COSY spectrum of **6** showed cross peaks for the signals at δ 1.70 (4-H)/δ 2.27 (5-H) and δ 2.05

Fig. 6. The structures of myriocin and mycestericin derivatives.



Compound	R_1	R_2	R_3	X	Y
7	Ac	Ac	Ac		
8	H	Ac	H		
9	Bz	Ac	Bz		
10	H	Ac	H		
11	Bz	Bz	Bz		
12	Bz	Bz	Bz		
13	Bz	Bz	Bz		
14	Bz	Bz	Bz		
15	Bz	Bz	Bz		
16	Bz	Bz	Bz		
17	Ac	Ac	Ac		

[a] A mixture of 14-*R* and *S*.
[b] Absolute configuration at C-14 is not determined.

Fig. 7. CD spectra of **12**.

------ **12** derived from **1** (c 0.518×10^{-3} M), —— **12** derived from **2** (c 0.559×10^{-3} M).

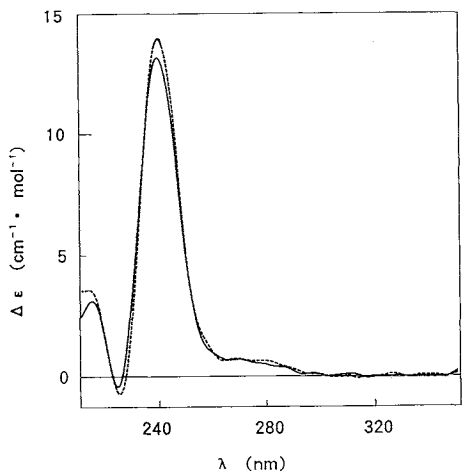
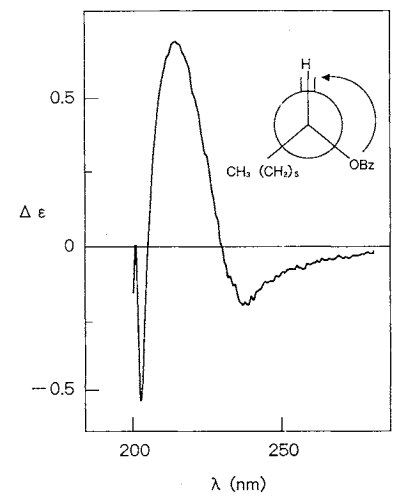


Fig. 8. CD spectrum of **10** (c 2.18×10^{-3} M).



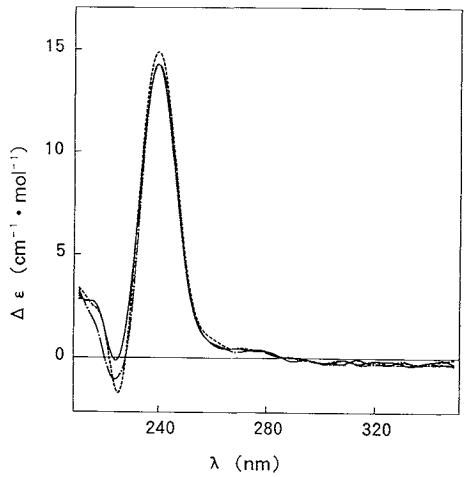
(5-H); δ 1.41 (4-H)/δ 2.27 (5-H), δ 2.05 (5-H) and δ 3.85 (3-H). Accordingly, the plane structure of mycestericin D (**6**) was concluded to be the same as that of **5** and the two compounds are in a diastereoisomeric relationship.

Effect of Mycestericins on Mouse Allogeneic Mixed Lymphocyte Reaction (MLR)

The effect of mycestericins A (**2**), B (**3**), D (**5**) and E (**6**) on mouse allogeneic MLR was examined in comparison with that of **1**. Table 6 shows the IC_{50} values of mycestericins and **1** on mouse allogeneic MLR. The potency of **2**, **3** and **5** was comparable to that of **1**, while **6** showed somewhat weaker activity. It appears that the hydroxy group at C-4 has not effect on the activity.

Fig. 9. CD spectra of **13**, **14** and **16**.

—— **13** (c 0.458×10^{-3} M), - - - - **14** (c 0.475×10^{-3} M), — — **16** (c 0.422×10^{-3} M).



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. UV and IR spectra were taken on a Shimadzu UV-2200 UV-VIS recording spectrophotometer and a Shimadzu IR-435 infrared spectrophotometer, respectively. CD spectra were measured on a Jasco J-720 spectrophotometer. The

Table 5. Physico-chemical properties of triacetyl-6,7-dihydromyriocin γ-lactone (a) and triacetylmycestericin C γ-lactone (b).

Compound	a	b
$[\alpha]_D$	+63.6° (c 0.27, $CHCl_3$)	+57.7° (c 0.24, $CHCl_3$)
IR ($CHCl_3$) cm^{-1}	3400 (–NH–)	3400
	1775 (γ-lactone C=O)	1775
	1755, 1700	1755, 1700
	1685 (C=O, –CONH–)	1685
HREI-MS (m/z)		
Found:	511.31387	511.31459
Calcd:	511.31465	511.31465
Formula (MW)	$C_{27}H_{45}NO_8$ (511)	$C_{27}H_{45}NO_8$ (511)
^1H NMR	δ ppm (300 MHz in $CDCl_3$)	δ ppm (300 MHz in $CDCl_3$)
	6.11 (1H, br s, –NH)	6.00 (1H, br s)
	5.79 (1H, d, J=4.2 Hz, 3-H)	5.79 (1H, d, J=4.2 Hz)
	4.72 (1H, ddd, J=9.0, 4.2, 4.2 Hz, 4-H)	4.71 (1H, ddd, J=9.0, 4.2, 4.2 Hz)
	4.52 (2H, s, 21-H_2)	4.53 (2H, s)
	2.38 (4H, t, J=7.4 Hz, 13-, 15-H_2)	2.38 (4H, t, J=7.4 Hz)
	2.10 (3H, s, –COMe)	2.10 (3H, s)
	2.05 (3H, s, –COMe)	2.05 (3H, s)
	2.03 (3H, s, –COMe)	2.02 (3H, s)
	1.76~1.65 (1H, m, 5-H)	1.75~1.64 (1H, m)
	1.65~1.47 (5H, m, 5-H, 12-, 16-H_2)	1.65~1.47 (5H, m)
	1.46~1.17 (18H, m, 6-~11-, 17-~19-H_2)	1.46~1.17 (18H, m)
	0.88 (3H, t, J=6.8 Hz, 20-H_3)	0.88 (3H, t, J=6.8 Hz)

Fig. 10. ^{1}H NMR spectra of **1**, **5** and **6**.

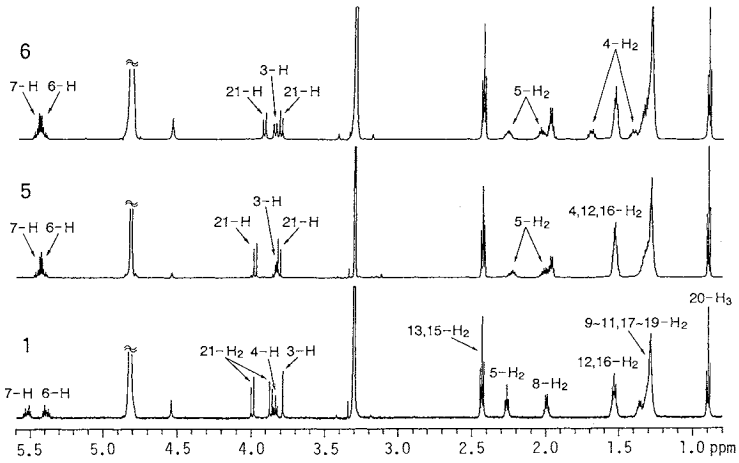


Table 6. Effect of myriocin and mycestericins on mouse allogeneic MLR.

Compound	IC_{50} (μg/ml)
Myriocin (**1**)	0.0032
Mycestericin A (**2**)	0.0036
B (**3**)	0.0025
D (**5**)	0.0022
E (**6**)	0.0062

concentration of benzoate derivatives was determined from the UV spectra by using a calibration curve for propyl benzoate. ^{1}H NMR and ^{13}C NMR spectra were taken on a JEOL FX-200, JEOL EX-270, Bruker AC-300 or Bruker AM-600 spectrometer with TMS as an internal standard. Mass spectra (EI-MS and FAB-MS) were taken on JEOL JMS-01SG, JEOL JMS-HX100 or JMS-HX110 spectrometer. HPLC was performed on a Shimadzu LC-8A system [eluent MeOH-H_2O (65:35 v/v); column temperature 40°C; UV detector (210 nm)]. Analytical HPLC was carried out with a YMC-ODS AM 313 column (6 mm i.d. × 250 mm) [flow rate 1.0 ml/minute]. Semi preparative HPLC was carried out with a YMC-ODS SH-343-5 column (20 mm i.d. × 250 mm) [flow rate 7.0 ml/minute]. Analytical TLC and preparative TLC were performed on Kieselgel 60 F_{254} (Merck) and Kieselgel 60 PF_{254} (Merck), respectively. Organic extracts of reaction mixtures were washed successively with 1 N HCl, 5% $NaHCO_3$ and saturated aqueous NaCl and dried over anhydrous magnesium sulfate unless otherwise specified.

Fermentation of *Mycelia sterilia* (ATCC 20349)

A seed medium (100 ml) containing glucose 3%, yeast extract (Difco) 0.5%, K_2HPO_4 0.03% and $MgSO_4 \cdot 7H_2O$ 0.05% at pH 5.5 was poured into a 500-ml Erlenmeyer flask and sterilized at 121°C for 20 minutes. Mycelium growing on the potato-dextrose agar (Difco) was inoculated into the medium and cultured at 40°C for 4 days on a reciprocal shaker (145 strokes/minute). The resultant culture was transferred to the same medium (5 liters) in a 10-liter jar fermentor which had been sterilized at 121°C for 30 minutes in advance. The seed fermenter was operated for 4 days at 40°C, with agitation at 150 rpm and with an air flow of 5 liters per minute.

For production, a 200-liter fermentor was used. The medium (150 liters) was composed of glucose 3%, L-glutamic acid monosodium salt 0.5%, K_2HPO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.2%, citric acid 0.05%, FS anti foam F-18 (Dow Corning Co., Ltd.) and 10 ml per liter of a trace element solution containing $FeSO_4 \cdot 7H_2O$ 0.1%, $MnCl_2 \cdot 4H_2O$ 0.1%, $ZnSO_4 \cdot 7H_2O$ 0.1%, thiamine hydrochloride 0.02%, pantothenic acid sodium salt 0.02% and riboflavin 0.02%. The production fermentor was inoculated with 4.5 liters of broth from the seed fermentor and operated for 10 days at 40°C, with an air flow rate of 150 liters/minute, a pressure of 0.8 kg/cm^2 and an agitator speed of 150 rpm.

Isolation and Purification

Mycestericins were isolated from the culture broth of *Mycelia sterilia* (ATCC 20349) as shown in Fig.

3. The culture broth (150 liters) was separated into filtrate and mycelium by filtration. The mycelium (6.2 kg) was extracted with MeOH (25 liters × 2). The MeOH extract was diluted with water (50 liters), and the mixture was passed through a column of Amberlite XAD-2 (18.4 liters). The column was washed with 50% (20 liters) and 60% aqueous MeOH (30 liters), then eluted with 70% (15 liters, Fr. 1, 0.45 g) and 80% aqueous MeOH (20 liters, Fr. 2, 4.3 g). A portion of Fr. 2 was concentrated to dryness and the residue was recrystallized from MeOH to give crude **1** containing **4**. On the other hand, most of Fr. 2 was rechromatographed on Amberlite XAD-2 (3 liters) with 80% aqueous MeOH to give Fr. 3 (0.31 g).

Fr. 1 was subjected to semi-preparative HPLC to give **2** (150 mg; retention time, 63 minutes) and **3** (40 mg; retention time, 91~92 minutes). Similar treatment of Fr. 3 gave **5** (39 mg; retention time, 92~93 minutes) and **6** (33 mg; retention time, 100~102 minutes).

Mouse Allogeneic Mixed Lymphocyte Reaction (MLR)

The effect of mycestericins on mouse allogeneic MLR was examined by the method described in our previous paper[2].

Tetraacetylmycestericin A γ-Lactone (**7**)

Acetic anhydride (1.0 ml) was added to a solution of **2** (20.0 mg) in pyridine (1.0 ml). The mixture was kept standing overnight, then poured into ice water and extracted with $CHCl_3$. The organic solution was washed, dried and concentrated to give an oily residue, which was purified by preparative TLC [solvent, EtOAc-*n*-hexane (8:2)] to yield **7** as an oil (24.6 mg, 89.4%). $[\alpha]_D^{25}$ +58.4° (*c* 0.50, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3400, 1780, 1755, 1725 (sh), 1685, 1600, 965. EI-MS *m/z* 551 (M^+), 491, 431, 261, 243, 129, 67, 43. HREI-MS calcd for $C_{29}H_{45}NO_9$: 551.3096, found: 551.3103. ^1H and ^{13}C NMR (see Table 4).

N-Acetylmycestericin A γ-Lactone (**8**)

Acetic anhydride (3.4 ml) was added to a suspension of **2** (80.0 mg) in absolute MeOH (1.3 ml) and the mixture was refluxed for 10 hours. The solvent was evaporated off and $CHCl_3$ was added to the residue. The organic solution was washed and concentrated to give an oily residue. The residue was purified by preparative TLC [solvent, $CHCl_3$-MeOH (85:15)] to give **8** as an oil (35.0 mg, 41.3%). ^1H NMR (200 MHz, $CDCl_3$) δ: 6.92 (1H, br s, -NH-), 5.62 (1H, dtt-like, *J*=15.3 and 6.3 Hz, 7-H), 5.62 (1H, dtd-like, *J*=15.3 and 6.3 Hz, 12-H), 5.43 (1H, dtt-like, *J*=15.3 and 6.3 Hz, 6-H), 5.41 (1H, ddt-like, *J*=15.3 and 6.3 Hz, 13-H), 4.59 (1H, br s, 3-H), 4.57 (1H, br t, *J*=6.3 Hz, 4-H), 4.01 (1H, qd-like, *J*=6.3 Hz, 14-H), 3.85 (2H, br s, 21-H_2), 2.55 (2H, br t, *J*=6.3 Hz, 5-H_2), 2.11 (3H, s, -Ac), 2.12~1.96 (4H, m, 8- and 11-H_2), 1.55~1.40 (2H, m, 15-H_2), 1.45~1.30 (4H, m, 9- and 10-H_2), 1.27 (8H, br s, 16-~19-H_2), 0.88 (3H, t, *J*=6.2 Hz, 20-H_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 174.53 and 173.80 (each s, 1-C and >C=O), 134.81 and 132.91 (each d, 7- and 12-C), 131.96 (d, 13-C), 123.64 (d, 6-C), 83.22 (d, 4-C), 73.29 and 72.34 (each d, 3- and 14-C), 67.53 (s, 2-C), 63.63 (t, 21-C), 37.31 (t, 15-C), 32.18 (t, 5-C), 31.87 and 31.79 (each t, 8- and 11-C), 29.19 and 25.44 (each t, 16-, 17- and 18-C), 28.34 and 28.24 (each t, 9- and 10-C), 22.99 (q, $-COCH_3$), 22.60 (t, 19-C), 14.08 (q, 20-C). EI-MS *m/z* 425 (M^+), 377, 340, 322, 292, 141, 67, 43. HREI-MS calcd for $C_{23}H_{39}NO_6$: 425.2779, found: 425.2786.

N-Acetyltribenzoylmycestericin A γ-Lactone (**9**)

Benzoic anhydride (105.8 mg) was added to a solution of **8** (20.0 mg) and *N*,*N*-dimethylaminopyridine (11.4 mg) in Et_3N (1.0 ml) and the mixture was kept standing overnight. Ice water was added and the mixture was extracted with $CHCl_3$. The organic solution was washed and concentrated to give an oily residue. The residue was purified by preparative TLC [solvent, $CHCl_3$-MeOH (95:5)] to give **9** as an oil (28.3 mg, 81.6%). IR ν_{max} ($CHCl_3$) cm^{-1}: 3400, 1780, 1725, 1685 (sh), 1600, 970, 710. ^1H NMR (200 MHz, $CDCl_3$) δ: 8.07~7.97 (6H, m, *o*-aromatic-H), 7.65~7.35 (9H, m, *m*- and *p*-aromatic-H), 6.33 (1H, br s, -NH-), 6.09 (1H, d, *J*=5.3 Hz, 3-H), 5.74 (1H, dtd-like, *J*=14.4 and 6.1 Hz, 12-H), 5.59~5.31 (4H, m, 6-, 7-, 13- and 14-H), 4.89 (1H, ddd, *J*=8.0, 5.6 and 5.3 Hz, 4-H), 4.80 (2H, s, 21-H_2), 2.60 (1H, br ddd, *J*=14.5, 8.0 and 5.6 Hz, 5-H), 2.48 (1H, br ddd, *J*=14.5, 5.6 and 5.6 Hz, 5-H), 1.99 and 1.93 (each 2H, br q, *J*=6.1 Hz, 8- and 11-H_2), 1.83~1.54 (2H, m, 15-H_2), 1.80 (3H, s, -Ac), 1.45~1.05 (12H, m, 9-, 10- and 16-~19-H_2), 0.86 (3H, t, *J*=6.3 Hz, 20-H_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 172.29 (s, 1-C), 169.67 (s, $-COCH_3$), 165.90, 165.80 and 164.73 (each s, $-COC_6H_5$), 134.93 (d, 7-C), 134.13 (d, 12-C), 133.79,

133.69, 132.67, 130.89, 129.82, 129.53, 129.09, 128.83 and 128.66 (aromatic-C), 128.48 (d, 13-C), 128.39, 128.27 and 126.25 (aromatic-C), 123.23 (d, 6-C), 81.44 (d, 4-C), 75.60 (d, 14-C), 72.22 (d, 3-C), 62.88 (t, 21-C), 62.64 (s, 2-C), 34.69 (t, 15-C), 32.35, 32.28, 32.0 and 31.72 (each t, 5-, 8-, 11- and 16-C), 29.07, 28.63, 28.39, 25.20 (each t, 9-, 10-, 17- and 18-C), 22.52 (t, 19-C), 22.52 (q, $-COCH_3$), 14.06 (q, 20-C). EI-MS *m/z* 737 (M^+), 615, 510, 493, 329, 122, 105, 77, 67, 51, 43. HREI-MS calcd for $C_{44}H_{51}NO_9$: 737.3565 (M^+), found: 737.3524.

N-Acetyl-14-*O*-benzoylmycestericin A γ-Lactone (**10**)

A solution of **9** (20.0 mg) in MeOH (1.5 ml) containing 1 N NaOH (0.17 ml) was stirred for 90 minutes, adjusted to pH 2 with 1 N HCl, and further stirring was continued for another 45 hours. The mixture was neutralized with 1 N NaOH and extracted with $CHCl_3$. The organic solution was washed and concentrated to give an oily residue. The residue was purified by preparative TLC [solvent, $CHCl_3$-MeOH (85:15)] to give **10** as an oil (5.7 mg, 39.7%). UV λ_{max} (MeOH) nm: 229 (ε 3,400). CD λ_{ext} (MeOH) nm: 236 ($\Delta\varepsilon$ −0.21). IR ν_{max} ($CHCl_3$) cm^{-1}: 3400, 3325 (sh), 1775, 1710, 1660, 970, 710. ^1H NMR (200 MHz, $CDCl_3$) δ: 8.04 (2H, ddd, *J*=7.1, 1.5 and 1.5 Hz, *o*-aromatic-H), 7.61~7.38 (3H, m, *m*-and *p*-aromatic-H), 6.48 (1H, br s, –NH–), 5.77 (1H, dtd-like, *J*=14.3 and 6.5 Hz, 12-H), 5.61 (1H, dtt-like, *J*=15.0 and 6.5 Hz, 7-H), 5.53 (1H, ddt-like, *J*=14.3 and 6.3 Hz, 13-H), 5.42 (1H, qd-like, *J*=6.3 Hz, 14-H), 5.41 (1H, dtt-like, *J*=15.0 and 6.5 Hz, 6-H), 4.67 (1H, d, *J*=2.1 Hz, 3-H), 4.60 (1H, td, *J*=6.5 and 2.1 Hz, 4-H), 3.90 (1H, d, *J*=11.6 Hz, 21-H), 3.85 (1H, d, *J*=11.6 Hz, 21-H), 2.55 (2H, br t, *J*=6.5 Hz, 5-H_2), 2.12 (3H, s, -Ac), 2.03 and 2.00 (each 2H, br q, *J*=6.5 Hz, 8- and 11-H_2), 1.85~1.50 (2H, m, 15-H_2), 1.55~1.10 (12H, m, 9-, 10- and 16-~19-H_2), 0.87 (3H, t, *J*=5.9 Hz, 20-H_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 174.17 and 173.97 (each s, 1-C and $-COCH_3$), 166.14 (s, $-COC_6H_5$), 135.00 (d, 7-C), 134.25 (d, 12-C), 132.79, 130.84 and 129.56 (aromatic-C), 128.51 (d, 13-C), 128.34 (aromatic-C), 123.43 (d, 6-C), 82.97 (d, 4-C), 75.77 (d, 14-C), 72.12 (d, 3-C), 67.60 (s, 2-C), 64.27 (t, 21-C), 34.71 (t, 15-C), 32.30 (t, 5-C), 32.01, 31.74 and 29.70 (each t, 8-, 11- and 16-C), 29.09, 28.58, 28.36 and 25.25 (each t, 9-, 10-, 17- and 18-C), 23.16 (q, $-COCH_3$), 22.60 (t, 19-C), 14.06 (q, 20-C). EI-MS *m/z* 511 $(M-H_2O)^+$, 481, 376, 359, 341, 219, 141, 105, 77, 67, 43. HREI-MS calcd for $C_{30}H_{41}NO_6$: 511.2935, found: 511.2953.

Tetrabenzoylmycestericin A γ-Lactone (**11**)

By a procedure similar to that used for the preparation of **9**, **11** (35.7 mg) was obtained as an oil in 68% yield from **2** (26.5 mg). $[\alpha]_D$ +28.6° (*c* 0.313, EtOH). CD λ_{ext} (EtOH) nm: 239 ($\Delta\varepsilon$ 10.6), 224 (−2.2). IR ν_{max} ($CHCl_3$) cm^{-1}: 2920, 2850, 1780, 1730, 1675 (sh), 1600, 970, 710. ^1H NMR (270 MHz, $CDCl_3$) δ: 8.06~7.23 (20H, m, aromatic-H), 6.82 (1H, s, NH), 6.23 (1H, d, *J*=4.6 Hz, 3-H), 5.75 (1H, dt, *J*=14.2 and 6.9 Hz, 14-H), 5.57~5.37 (4H, m, 6-, 7-, 12- and 13-H), 4.95 (overlap, 1H, dt, *J*=8~9 Hz, and 4.6 Hz, 4-H), 4.93 (overlap, 2H, s, 21-H_2), 2.66~2.44 (2H, m, 5-H_2), 2.10~1.89 (4H, m, 8- and 11-H_2), 1.84~1.50 (2H, m, 15-H_2), 1.45~1.18 (12H, m, 9-, 10- and 16-~19-H_2), 0.86 (3H, deformed, *J*=6.9 Hz, 20-H_3). EI-MS *m/z* 799 (M^+), 572, 556, 421, 341, 219, 105, 77, 67, 43. HREI-MS calcd for $C_{49}H_{53}NO_9$: 799.3723, found: 799.3716.

Tribenzoyl-14-deoxodihydromyriocin γ-Lactone (**12**)

By a procedure similar to that used for the preparation of **9**, **12** (28.2 mg) was obtained as an amorphous powder in 83% yield from 14-deoxo-6,7-dihydromyriocin[7]. $[\alpha]_D$ +54.2° (*c* 0.81, EtOH). CD λ_{ext} (EtOH) nm: 239 ($\Delta\varepsilon$ 14.0), 223 (−0.7). IR ν_{max} ($CHCl_3$) cm^{-1}: 2920, 2850, 1780, 1720, 1680 (sh), 1600, 710. ^1H NMR (270 MHz, $CDCl_3$) δ: 8.07~7.26 (15H, m, aromatic-H), 6.84 (1H, s, –NH), 6.25 (1H, d, *J*=4.6 Hz, 3-H), 5.00~4.9 (overlap, 1H, m, 4-H), 4.93 (overlap, 2H, s, 21-H_2), 1.93~1.69 (2H, m, 5-H_2), 1.62~1.25 (2H, m, 6-H_2), 1.25~1.22 (26H, m, 7-~19-H_2), 0.88 (3H, deformed t, *J*=6.3 and 6.9 Hz, 20-H_3). EI-MS *m/z* 683 (M^+), 621, 578, 440, 412, 122, 105, 77. HREI-MS calcd for $C_{42}H_{53}NO_7$: 683.3824, found: 683.3817.

Tribenzoyl-14-deoxytetrahydromycestericin A γ-Lactone (**12**) and Tetrabenzoyltetrahydromycestericin A γ-Lactone (**13**)

A solution of **11** (34.5 mg) in MeOH (30 ml) was hydrogenated over 5% Pd/C (3 mg) in the presence of $HClO_4$. The catalyst was filtered off and the filtrate was evaporated. The residue was purified by

preparative TLC (solvent, EtOAc-*n*-hexane, 1:2) to give **12** (4.7 mg) and **13** (24.4 mg).

12

$[\alpha]_D$ +54.2° (*c* 0.24, EtOH). CD λ_{ext} (EtOH) nm: 239 ($\Delta\varepsilon$ 13.2), 223 (−0.4). IR ν_{max} ($CHCl_3$) cm^{-1}: 2920, 2850, 1780, 1720, 1675 (sh), 1600, 710. ^1H NMR (270 MHz, $CDCl_3$) δ: 8.07~7.25 (15H, m, aromatic-H), 6.82 (1H, s, NH), 6.25 (1H, d, J=4.6 Hz, 3-H), 4.98 (1H, td, J=9.2 and 4.6 Hz, 4-H), 4.95 (2H, s, 21-H_2), 1.97~1.65 (2H, m, 5-H_2), 1.65~1.20 (2H, m, 6-H_2), 1.20~1.15 (26H, m, 7-~19-H_2), 0.86 (3H, deformed t, J=6.6 and 6.9 Hz, 20-H_3). EI-MS *m/z* 683 (M^+), 621, 578, 440, 412, 122, 105, 77. HREI-MS calcd for $C_{42}H_{53}NO_7$: 683.3824, found: 683.3828.

13

$[\alpha]_D$ +34.5° (*c* 1.80, EtOH). CD λ_{ext} (EtOH) nm: 239 ($\Delta\varepsilon$ 14.1). IR ν_{max} ($CHCl_3$) cm^{-1}: 2920, 2850, 1780, 1720, 1680 (sh), 1600, 710. ^1H NMR (270 MHz, $CDCl_3$) δ: 8.07~7.25 (20H, m, aromatic-H), 6.85 (1H, s, NH), 6.24 (1H, d, J=4.6 Hz, 3-H), 5.12 (1H, quintet, J=6.6 Hz, 14-H), 4.98 (overlap, 1H, td, J=8~9 Hz and 4.6 Hz, 4-H), 4.98 and 4.93 (2H, each d, J=11.5 Hz, 21-H_2), 1.97~1.55 (6H, m, 5-, 13- and 15-H_2), 1.45~1.15 (22H, m, 6-~12- and 16-~19-H_2), 0.86 (3H, deformed t, J=6.9 Hz, 20-H_3). EI-MS *m/z* 803 $(M)^+$, 718, 559, 410, 368, 122, 105, 77. HREI-MS calcd for $C_{49}H_{57}NO_9$: 803.4036, found: 803.4037.

Tetrabenzoyltetrahydromyriocin γ-Lactone (**14**)

By a procedure similar to that used for the preparation of **9**, **14** (24 mg) was obtained as an oil in 60% yield from tetrahydromyriocin (20 mg). $[\alpha]_D$ +34.8° (*c* 1.52, EtOH). CD λ_{ext} (EtOH) nm: 239 ($\Delta\varepsilon$ 14.0), 223 (−0.7). IR ν_{max} ($CHCl_3$) cm^{-1}: 3020, 2920, 2850, 1780, 1720, 1680 (sh), 1600, 710. ^1H NMR (270 MHz, $CDCl_3$) δ: 8.06~7.25 (20H, m, aromatic-H), 6.85 (1H, s, –NH), 6.24 (1H, d, J=4.6 Hz, 3-H), 5.11 (1H, quintet, J=6.9 Hz, 14-H), 5.00~4.9 (overlap, 1H, m, 4-H), 4.96 (overlap, 2H, s, 21-H_2), 1.92~1.64 (6H, m, 5-, 13- and 15-H_2), 1.64~1.21 (22H, m, 6-~12- and 16-~19-H_2), 0.86 (3H, deformed t, J=6.9 Hz, 20-H_3). EI-MS *m/z* 803 $(M)^+$, 741, 718, 681, 559, 532, 515, 410, 122, 105, 77. HREI-MS calcd for $C_{49}H_{57}NO_9$: 803.4036, found: 803.4039.

Tetrabenzoylmycestericin B γ-Lactone (**15**)

By a procedure similar to that used for the preparation of **9**, **15** (63 mg) was obtained as an oil in 63% yield from **3** (50 mg). $[\alpha]_D$ +28.7° (*c* 1.66, EtOH). CD λ_{ext} (EtOH) nm: 240 ($\Delta\varepsilon$ 13.0), 223 (−1.6). IR ν_{max} ($CHCl_3$): 2920, 2850, 1780, 1730~1670 (sh), 1600, 970, 710. ^1H NMR (270 MHz, $CDCl_3$) δ: 8.07~7.23 (20H, m, aromatic-H), 6.87 (1H, s, –NH), 6.23 (1H, d, J=4.6 Hz, 3-H), 5.60~5.40 (2H, m, 6- and 7-H), 5.11 (1H, quintet, J=6.6 Hz, 14-H), 4.97 (1H, td, J=8.3 and 4.6 Hz, 4-H), 4.94 (2H, s, 21-H_2), 2.70~2.45 (2H, m, 5-H_2), 1.92 (2H, q-like, J=6.6 Hz, 8-H_2), 1.75~1.50 (4H, m, 13- and 15-H_2), 1.45~1.10 (16H, m, 9-~12- and 16-~19-H_2), 0.86 (3H, deformed t, J=6.9 Hz, 20-H_3). EI-MS *m/z* 801 $(M)^+$, 716, 679, 558, 122, 105, 77. HREI-MS calcd for $C_{49}H_{55}NO_9$: 801.3879, found: 801.3862.

Tetrabenzoyldihydromycestericin B γ-Lactone (**16**)

By a procedure similar to that used for the preparation of **13**, **16** (23 mg) was obtained as an oil in 68% yield from **15** (33 mg). $[\alpha]_D$ +34.8° (*c* 1.56, EtOH). CD λ_{ext} (EtOH) nm: 239 ($\Delta\varepsilon$ 14.6), 224 (−1.4). IR ν_{max} ($CHCl_3$) cm^{-1}: 2920, 2870, 1780, 1730~1670 (sh), 1600, 710. ^1H NMR (270 MHz) δ: 8.06~7.25 (20H, m, aromatic-H), 6.85 (1H, s, NH), 6.24 (1H, d, J=4.6 Hz, 3-H), 5.11 (1H, quintet, J=6.6 Hz, 14-H), 5.00~4.9 (overlap, 1H, m, 4-H), 4.96 (overlap, 2H, s, 21-H_2), 1.92~1.64 (6H, m, 5-, 13- and 15-H_2), 1.64~1.21 (22H, m, 6-~12-, 16-~19-H_2), 0.86 (3H, deformed t, J=6.9 Hz, 20-H_3). EI-MS *m/z* 803 $(M)^+$, 718, 681, 559, 515, 122, 105, 77. HREI-MS calcd for $C_{49}H_{57}NO_9$: 803.4036, found: 803.4020.

Triacetylmycestericin C γ-Lactone (**17**)

Acetic anhydride (1 ml) was added to a solution of crude myriocin (100 mg) in pyridine (1 ml) and the reaction mixture was stirred at room temperature overnight, then poured into ice water and extracted with EtOAc. The organic solution was washed and concentrated to give an oily residue (115 mg). A solution of the residue (100 mg) in pyridine (5 ml) was treated with a solution of OsO_4 (57 mg) in pyridine (1 ml).

The mixture was stirred for 2 hours, then 1% aqueous $NaHSO_3$ (5 ml) was added. The whole was diluted with water, and extracted with EtOAc. The organic solution was washed and concentrated to afford a residue. The residue was purified by preparative TLC [solvent, EtOAc-*n*-hexane (1:2)] to give 6 mg of **17** as an oil (see Table 5).

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