Pergamon

PII: S0031-9422(97)00530-X

Phytochemistry, Vol. 47, No. 6, pp. 1131–1135, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

HYDROGENATED AZAPHILONES FROM *EMERICELLA FALCONENSIS* AND *E. FRUTICULOSA*

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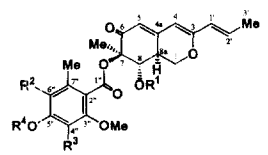
(*Received in revised form* 20 *May* 1997)

Key Word Index—*Emericella falconensis*; *E. fruticulosa*; ascomycetous fungus; hydrogenated azaphilones; falconensins.

Abstract—Six new hydrogenated azaphilones designated falconensins I–N, and a new azaphilone, monomethyldihydromitorubrin, were isolated as minor components from mycelia of *Emericella falconensis* and/or *E. fruticulosa* along with nine azaphilone derivatives, falconensins A–H and monomethylmitorubrin, and three hopane-type triterpenes. The structures of falconensins I–N and monomethyldihydromitorubrin were confirmed by spectroscopic investigation and chemical correlation.

INTRODUCTION

Recently, we isolated seven new hydrogenated azaphilones, falconensins A (**1**) to G (**7**) [1, 2], a new azaphilone, falconensin H (**8**) [3] and three hopane-type triterpenes [2] from the mycelial dichloromethane extract of the ascomycetous fungus, *Emericella falconensis* Horie, Miyaji, Nishimura and Ugadawa strain NHL 2999 (=ATCC 76117), isolated from Venezuelan soil in 1988 [4]. All of the above compounds were also isolated from the mycelium of *E. fruticulosa* (Raper and Fennell) Malloch and Cain, strain IFO 30841, the dichloromethane extract components of which are almost superimposable on those of *E. falconensis* on TLC. Falconensins A (**1**)–C (**3**) had inhibitory activity on 12-*O*-teradecanoylphorbol 13-acetate (TPA)-induced inflammation [5]. In the course of further investigations, six new hydrogenated azaphilones designated falconensins I (**9**)–N (**14**) and a new azaphilone, monomethyldihydromitorubrin (**15**), were isolated along with mitorubrin (**16**) and monomethylmitorubrin (**17**) from the mycelial extract of *E. falconensis* and/or *E. fruticulosa*. We now report on the structure determination of falconensins I (**9**)–N (**14**) and monomethyldihydromitorubrin (**15**).



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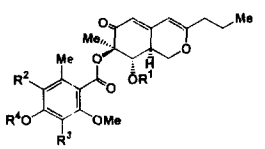
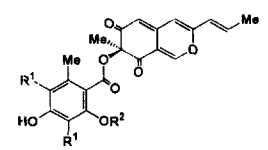
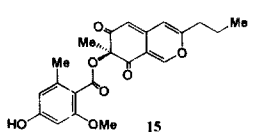
6 : R^1 = R^2 = R^3 = H, R^4 = Me
7 : R^1 = Ac, R^2 = R^3 = H, R^4 = Me
9 : R^1 = R^2 = R^3 = R^4 = H
11 : R^1 = R^3 = R^4 = H, R^2 = Cl
13 : R^1 = R^4 = H, R^2 = R^3 = Cl
18 : R^1 = R^4 = Ac, R^2 = R^3 = H



2 : R^1 = H, R^2 = R^3 = Cl, R^4 = Me
4 : R^1 = Ac, R^2 = R^3 = Cl, R^4 = Me
10 : R^1 = R^2 = R^3 = R^4 = H
12 : R^1 = R^3 = R^4 = H, R^2 = Cl
14 : R^1 = R^4 = H, R^2 = R^3 = Cl



8 : R^1 = Cl, R^2 = Me
16 : R^1 = H, R^2 = Me
17 : R^1 = R^2 = H



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RESULTS AND DISCUSSION

The molecular formula of falconensin I (**9**) was determined as $C_{22}H_{24}O_7$, on the basis of the high resolution mass spectrum ($[M]^+$ *m/z* 400). The fragment ion peak at *m/z* 235 ($C_{13}H_{15}O_4$) due to the azaphilone part was observed in the EI mass spectrum of **9**, as it was in falconensins A (**1**) and F (**6**) [1, 2]. The ^1H NMR spectrum of **9** (Table 1) was closely similar to that of **6**, except for the appearance of a phenolic proton at δ 6.18 in **9** and the disappearance of one of two methoxyl protons observed at δ 3.79 and 3.80 in **6**. Nuclear Overhauser enhancement (NOE) (17%) was observed at H-8a (δ 2.91) when the methyl signal at δ 1.47 was irradiated, whereas the NOEs of 7.4 and 2.4% were observed for the signal at δ 3.82 on C-1 and the methyl signal at C-7, respectively, when H-8a was irradiated. The relative configuration between H-

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Table 1. ^{1}H NMR chemical shifts of falconensins in $CDCl_3$

No.	1	2	5	6	9	10	11	12	13	14
1-H	3.84	3.82	3.84	3.81	3.82	3.80	3.88	3.81	3.85	3.81
	4.80	4.77	4.77	4.77	4.77	4.73	4.78	4.73	4.80	4.75
4-H	5.57	5.52	5.57	5.56	5.56	5.50	5.56	5.51	5.57	5.51
5-H	5.81	5.74	5.80	5.80	5.81	5.73	5.79	5.72	5.81	5.73
7-Me	1.49	1.49	1.58	1.47	1.47	1.47	1.46	1.46	1.50	1.49
8-H	4.72	4.71	4.75	4.73	4.73	4.72	4.73	4.73	4.73	4.69
8-OH	2.79	2.82	2.76	2.90	2.95	2.87	2.72	2.72	2.95	2.74
8a-H	2.88	2.86	2.88	2.87	2.91	2.84	2.88	2.83	2.90	2.85
1′-H	5.91	2.18	5.90	5.90	5.90	2.17	5.90	2.18	5.91	2.15
		2.25				2.20		2.24		2.22
2′-H	6.47	1.60	6.46	6.46	6.48	1.52	6.47	1.61	6.48	1.57
3′-H(Me)	1.87	0.95	1.87	1.87	1.88	0.95	1.88	0.95	1.88	0.95
3″-OMe	3.91[a]	3.91[a]	3.92[a]	3.80	3.80	3.82	3.88	3.83	3.92	3.92
4″-H			6.40	6.36	6.31	6.29	6.52	6.51		
5″-OMe	3.90[a]	3.90[a]	3.88[a]	3.79						
5″-OH					6.18	5.83	5.82	5.84	6.11	6.19
6″-H				6.32	6.29	6.29				
7″-Me	2.48	2.49	2.49	2.42	2.33	2.36	2.46	2.45	2.49	2.49

[a]Assignments may be reversed.

8 and H-8a was determined as *trans* from the coupling constant between these protons (J = 11 Hz). It was concluded that the azaphilone part of **9** was the same as that in **1** and **6**, including the relative stereochemistry, from the detailed analysis of the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2).

The fragment ion peaks of the benzoate part of falconensin I (**9**) appeared at m/z 165 ($C_9H_9O_3$) in the EI mass spectrum. These results indicated the lack of one *O*-methyl group in the benzoate part of **9** compared with **6**. It is confirmed that falconensin I (**9**) is the monodemethyl derivative of falconensin F (**6**), because of the identification with **6** by methylation of **9** with diazomethane. The absolute configuration of **9** was determined from the signs of the most bathochromic Cotton effect of the CD curves [$\Delta\varepsilon$: +7.8 (364 nm)] [2].

In order to determine the position of the methoxyl

Table 2. ^{13}C NMR chemical shifts of falconensins in $CDCl_3$

No.	1	2	5	6	9	10	11	12	13	14
1	68.5	68.7	68.6	68.6	68.5	68.7	68.5	68.7	68.5	68.7
3	160.7	168.5	160.3	160.3	160.8	168.5	160.4	168.3	160.6	168.4
4	102.7	100.8	102.8	102.8	102.8	100.9	102.8	100.8	102.7	100.8
4a	150.4	150.4	149.9	149.8	151.0	150.7	150.2	150.3	150.4	150.3
5	116.5	115.4	116.8	116.9	115.3	115.3	116.6	115.4	116.5	115.4
6	193.3	193.6	193.4	193.8	194.9	194.9	193.6	194.1	193.4	193.9
7	86.5	86.5	85.6	85.1	85.0	85.0	85.6	85.5	86.3	86.3
7-Me	16.9	16.9	16.8	16.9	16.9	16.8	16.8	16.8	16.9	16.9
8	70.1	70.1	69.9	70.0	69.9	69.9	69.8	69.9	70.0	70.1
8a	38.1	37.7	37.9	37.8	37.8	37.4	37.8	37.4	38.0	37.6
1′	125.4	36.5	125.5	125.6	125.4	36.6	125.4	36.5	125.4	36.5
2′	134.0	20.0	133.6	133.6	134.2	20.0	133.9	20.0	134.1	20.0
3′	18.4	13.6	18.4	18.6	18.4	13.6	18.4	13.6	18.4	13.6
1″	164.5	164.5	165.2	166.0	166.3	166.2	165.2	165.2	164.5	164.5
2″	126.4[a]	126.4[a]	116.1	116.2	114.8	115.1	113.2	113.3	117.9	117.9
3″	151.9	151.9	156.2[a]	157.6	158.0	157.9	155.5	155.4	152.1	152.
3″-OMe	62.5	62.5	56.3	56.3	56.2	56.2	56.5	56.4	62.5	62.5
4″	120.7	120.7	94.4	96.5	97.0	97.0	97.4	97.5	112.5	112.5
5″	154.3	154.3	156.7[a]	161.5	158.6	158.3	153.1	153.3	149.9	149.9
5″-OMe	60.6	60.6	56.3	55.4						
6″	126.6[a]	126.6[a]	117.4	107.3	110.0	109.9	117.2	117.1	122.6	122.6
7″	134.5	134.5	136.7	139.5	139.6	139.6	135.9	135.9	134.3	134.2
7″-Me	17.2	17.2	17.3	19.7	19.4	19.4	17.2	17.2	17.2	17.2

[a]Assignemnts may be reversed.

group, NOE experiments were performed on diacetate (**18**) derived from **9** by acetylation. NOE (23%) was observed on the aromatic proton at δ 6.52 when the methyl signal at δ 2.33 was irradiated, whereas 28% of NOE was observed on the aromatic proton at δ 6.47 when the methoxyl signal at δ 3.80 was irradiated. This result confirmed the structure of falconensin I as **9**.

The molecular formulae of falconensins K (**11**) and M (**13**) were determined as $C_{22}H_{23}O_7Cl$ and $C_{22}H_{22}O_7Cl_2$, respectively, on the basis of high resolution mass spectrum ($[M]^+$, *m/z* 434 and 346 for **11**; *m/z* 468, 470, and 472 for **13**). The fragment ion peak at *m/z* 235 ($C_{13}H_5O_4$) due to the azaphilone part was observed in the EI mass spectra of **11** and **13**, as in falconensin A (**1**) [1]. The ^1H NMR spectra of **11** and **13** (Table 1) were closely similar to those of falconensins E (**5**) and A (**1**), respectively, except for the appearance of a phenolic proton at δ 5.82 in **11** and δ 6.11 in **13**, and the disappearance of one of two methoxyl protons observed at δ 3.79 and 3.80 in **1** and δ 3.90 and 3.91 in **5**. The relative structures of the azaphilone part of **11** and **13** were shown to be the same as that of **1**, by detailed analysis of the ^1H and ^{13}C NMR spectra (Tables 1 and 2). The fragment ion peaks of the benzoate part of **11** and **13** appeared at *m/z* 199 and 201 ($C_9H_8O_3Cl$) and at *m/z* 233, 235, and 237 ($C_9H_8O_3Cl_2$), respectively, in the EI mass spectra. These results indicated the lack of one *O*-methyl group in the benzoate part of **11** and **13**, compared with **5** and **1**, respectively. The absolute structures of falconensins K and L were confirmed as shown in **11** and **13**, respectively, from detailed analysis of the 2-D NMR spectra and their CD curves [2].

The molecular formulae of falconensins J (**10**), L (**12**), and N (**14**) were determined as $C_{22}H_{26}O_7$, $C_{22}H_{25}O_7Cl$ and $C_{22}H_{24}O_7Cl_2$, respectively, on the basis of high resolution mass spectrum ($[M]^+$ *m/z* 402 for **10**; *m/z* 436 and 348 for **12**; *m/z* 470, 472 and 474 for **14**). The fragment ion peak at *m/z* 235 ($C_{13}H_{15}O_4$) due to the azaphilone part was observed in the EI mass spectra of **10**, **12**, and **14**, the same as that of falconensin A (**1**) [1]. The ^1H NMR spectra of **10**, **12**, and **14** (Table 1) were similar to those of **9**, **11**, and **13**, respectively, except for the appearance of a propyl group [**10**: δ 0.95 (3H), 1.52 (2H), 2.17 (1H) and 2.20 (1H). **12**: δ 0.95 (3H), 1.61 (2H), 2.18 (1H) and 2.24 (1H). **14**: δ 0.95 (3H), 1.57 (2H), 2.15 (1H) and 2.22 (1H)], and the disappearance of a 1-propenyl group [**9**: δ 1.88 (3H), 6.48 (1H) and 5.90 (1H). **11**: δ 1.88 (3H), 6.47 (1H) and 5.90 (1H). **13**: δ 1.88 (3H), 6.48 (1H) and 5.91 (1H)]. Falconensins J (**10**), L (**12**), and N (**14**) were assumed to be dihydro compounds, the side-chains of which were changed from a 1-propenyl residue into a propyl residue, of falconensins I (**9**), K (**11**), and M (**13**), respectively. The relative structures of **12**, **14**, and **16** were determined by detailed analysis of 2-D NMR spectra. The absolute structures of falconensins J (**10**), L (**12**), and N (**14**) were confirmed from the signs of the most bathochromic Cotton effect of the CD curves [$\Delta\varepsilon$: **10**: +6.9 (342 nm), **12**: +6.5 (343 nm), **14**: +10.7 (342 nm)] [2].

The molecular formula of monomethyldihydromitorubrin (**15**) was determined as $C_{22}H_{22}O_7$ on the basis of the high resolution mass spectrum ($[M]^+$ *m/z* 398). The ^1H NMR spectrum of **15** was similar to that of **16**, which had already been isolated from *E. falconensis*, except for the appearance of the signals of the propyl residue at δ 0.93 (3H, *t*) 1.61 (2H, *m*), and 2.32 (2H, *t*) in **15** instead of those assigned as 1-propenyl [1.94 (3H, *dd*), 6.00 (1H, *dq*) and 6.57 (1H, *dq*)] in **16**. Monomethyldihydromitorubrin (**15**) was assumed to be a dihydro compound, the side-chain of which was changed from 1-propenyl to propyl, of monomethylmitorubrin (**16**). The relative structure of **15** was determined by detailed analysis of the 2-D NMR spectra. The absolute configuration of monomethyldihydromitorubin (**15**) was confirmed to be the same as monomethylmitorubrin (**16**) from the comparison of the most bathochromic Cotton effect of the CD curves [$\Delta\varepsilon$: **15**: +6.9 (342 nm), **16**: +5.6 (366 nm)] [6].

EXPERIMENTAL

General. Mps: uncorr. ^1H (500 MHz) and ^{13}C (125 MHz) NMR: TMS as int. standard. LPLC: glass column (300 × 10 mm i.d.) with silica gel CQ-3 (Wako). HPLC: YMC-Pack SIL-06 (300 × 10 i.d. mm). TLC: pre-coated Kieselgel 60 F_{254} plates, detection by UV light at 254 and/or 365 nm.

Isolation of falconensin I (**9**)–*N* (**14**) *from* E. falconensis. Strain NHL 2999, was cultivated on Czapek medium supplemented with 0.2% yeast extract (30 l) using 120 Roux flasks at 26° for 28 days. Dried mycelia were extracted with CH_2Cl_2, the organic layer dried (Na_2SO_4) and then evapd *in vacuo*. The obtained extract (36.2 g) was dissolved in $CHCl_3$ and the filtrate concd by evapn. The residue (30.4 g) was chromatographed on silica gel with $CHCl_3$–Me_2CO (30:1) and (5:1) to give two frs. The latter was purified by HPLC with hexane–Me_2CO (17:7) to give falconensins I (**9**) (49 mg) and J (**10**) (13 mg), monomethyldihydromitorubrin (**15**) (8 mg) and monomethylmitorubrin (**16**) (47 mg). The former fr. was rechromatographed on silica gel with C_6H_6–Me_2CO (20:1), (15:1) and (10:1) to give frs 1–3, respectively. Fr. 1 was chromatographed on silica gel with hexane–Me_2CO (6:1), followed by purification by HPLC with hexane–Me_2CO (5:1) to afford falconensins M (**13**) (23 mg) and N (**14**) (12 mg). Fr. 2 was purified by LPLC with C_6H_6–Me_2CO (8:1) followed by purification by HPLC with hexane–Me_2CO (31:9) to afford falconensins L (**11**) (61 mg). Fr. 3 was purified by HPLC with hexane–Me_2CO (3:1) to afford falconensins K (**12**) (57 mg).

Falconensin I (**9**). Pale yellow crystalline powder, mp 121–123°. $[\alpha]_D^{20}$ 211° (*c* 0.33, MeOH). EI-MS *m/z* (rel. int.): 400.1529 $[M]^+$ (400.1522 for $C_{22}H_{24}O_7$, 7), 235 $[C_{13}H_{15}O_4]^+$ (7), 165 $[C_9H_9O_3]^+$ (51). UV λ_{max}^{MeOH}

nm (log ε): 244 sh (3.90), 280 sh (3.80), 344 (4.34). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1720 (COO), 1650 (CO). CD $\Delta\varepsilon^{20}$ (nm) (MeOH, $c = 9.0 \times 10^{-5}$): −1.7 (234), −2.0 (295), +1.2 (308 sh), +7.8 (364). ^{1}H NMR ($CDCl_3$) δ: 1.47 (3H, *s*, 7-Me), 1.88 (3H, *dd*, *J* = 7.0 and 1.5 Hz, 3′-H), 2.33 (3H, *s*, 7″-Me), 2.91 (1H, *dddd*, *J* = 13.0, 10.7, 5.2, and 1.8 Hz, 8a-H), 2.95 (1H, *d*, *J* = 2.1 Hz, 8-OH), 3.80 (3H, *s*, 3″-OMe), 3.82 [1H, *dd*, *J* = 13.0 and 9.6 Hz, 1-H (ax.)], 4.73 (1H, *dd*, *J* = 10.7 and 2.1 Hz, 8-H), 4.77 [1H, *dd*, *J* = 9.6 and 5.2 Hz, 1-H (eq.)], 5.56 (1H, *s*, 4-H), 5.81 (1H, *d*, *J* = 1.8 Hz, 5-H), 5.90 (1H, *dq*, *J* = 15.3 and 1.5 Hz, 1′-H), 6.18 (1H, *s*, 5″-OH), 6.29 (1H, *d*, *J* = 2.1 Hz, 4″-H), 6.31 (1H, *d*, *J* = 2.1 Hz, 6″-H), 6.48 (1H, *dq*, *J* = 15.3 and 7.0 Hz, 2′-H). ^{13}C NMR ($CDCl_3$): Table 2.

Falconensin J (**10**). Pale yellow crystalline powder, mp 96–98°. $[\alpha]_D^{20}$ +158° (*c* 0.33, MeOH). EI-MS *m/z* (rel. int.): 402.1672 $[M]^+$ (402.1677 for $C_{22}H_{26}O_7$, 7), 237.1121 $[C_{13}H_{17}O_4]^+$ (237.1126 for $C_{13}H_{17}O_4$, 9), 203 ($C_9H_{15}O_5$, 12), 165.0052 $[C_9H_9O_3]^+$ (165.0052 for $C_9H_9O_3$, 100). UV λ_{max}^{MeOH} nm (log ε): 226 sh (3.96), 251 (3.63), 321 (4.20). IR ν_{max}^{KBr} cm^{-1}: 3370 (OH), 1720 (COO), 1650 (CO). CD $\Delta\varepsilon^{20}$ (nm) (MeOH, $c = 1.4 \times 10^{-4}$): −1.8 (211), −1.4 (217), −1.1 (233), −1.8 (292), +3.7 (330 sh), +6.9 (342), +5.1 (356 sh). ^{1}H NMR ($CDCl_3$) δ: 0.95 (3H, *t*, *J* = 7.3 Hz, 3′-H), 1.47 (3H, *s*, 7-Me), 1.52 (2H, *tq*, *J* = 7.4 and 7.3 Hz, 2′-H), 2.10 (3H, *dt*, *J* = 14.7 and 7.4 Hz, 1′-H), 2.17 (3H, *dt*, *J* = 14.7 and 7.4 Hz, 1′-H), 2.36 (3H, *s*, 7″-Me), 2.84 (1H, *dddd*, *J* = 13.1, 10.5, 6.3, and 1.8 Hz, 8a-H), 2.87 (1H, *d*, *J* = 1.8 Hz, 8-OH), 3.80 [1H, *dd*, *J* = 13.1 and 10.7 Hz, 1-H (ax.)], 3.82 (3H, *s*, 3″-OMe), 4.72 (1H, *dd*, *J* = 10.5 and 1.8 Hz, 8-H), 4.73 [1H, *dd*, *J* = 10.7 and 6.3 Hz, 1-H (eq.)], 5.50 (1H, *s*, 4-H), 5.73 (1H, *d*, *J* = 1.8 Hz, 5-H), 5.83 (1H, *s*, 5″-OH), 6.29 (2H, *br s*, 4″-H, 6″-H). ^{13}C NMR ($CDCl_3$): Table 2.

Falconensin K (**11**). Pale yellow crystalline powder, mp 121–122°. $[\alpha]_D^{20}$ +110° (*c* 0.33, MeOH). Beilstein test: (+). EI-MS *m/z* (rel. int.): 434.1133 $[M]^+$ (434.1134 for $C_{22}H_{23}O_7{}^{35}Cl$, 11), 436.1102 $[M]^+$ (436.1102 for $C_{22}H_{23}O_7{}^{37}Cl$, 4), 235.0975 $[C_{13}H_{15}O_4]^+$ (235.0970 for $C_{13}H_{15}O_4$, 36), 199.0164 $[C_9H_8O_3{}^{35}Cl]^+$ (199.0163 for $C_9H_8O_3{}^{35}Cl$, 100), 201.0138 $[C_9H_8O_3{}^{37}Cl]^+$ (201.0132 for $C_9H_8O_3{}^{37}Cl$, 38). UV λ_{max}^{MeOH} nm (log ε): 250 sh (3.68), 288 (3.59), 349 (4.18). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1720 (COO), 1640 (CO). CD $\Delta\varepsilon^{20}$ (nm) (MeOH, $c = 1.3 \times 10^{-4}$): +1.9 (222), −0.7 (236), +0.8 (270), −1.0 (306), +3.2 (351 sh), +4.5 (364). ^{1}H NMR ($CDCl_3$) δ: 1.46 (2H, *s*, 7-Me), 1.88 (3H, *dd*, *J* = 7.0 and 1.5 Hz, 3′-H), 2.46 (3H, *s*, 7″-Me), 2.72 (1H, *d*, *J* = 2.4 Hz, 8-OH), 2.88 (1H, *dddd*, *J* = 12.9, 10.5, 5.4 and 1.8 Hz, 8a-H), 3.88 (3H, *s*, 3″-OMe), 3.88 [1H, *dd*, *J* = 12.9 and 10.6 Hz, 1-H (ax.)], 4.73 (1H, *dd*, *J* = 10.5 and 2.1 Hz, 8-H), 4.78 [1H, *dd*, *J* = 10.6 and 5.4 Hz, 1-H (eq.)], 5.56 (1H, *s*, 4-H), 5.79 (1H, *d*, *J* = 1.8 Hz, 5-H), 5.82 (1H, *s*, 5″-OH), 5.90 (1H, *dq*, *J* = 15.6 and 1.5 Hz, 1′-H), 6.47 (1H, *dq*, *J* = 15.6 and 7.0 Hz, 2′-H), 6.52 (1H, *s*, 4″-H). ^{13}C NMR ($CDCl_3$): Table 2.

Falconensin L (**12**). Pale yellow crystalline powder, mp 96–98°. $[\alpha]_D^{20}$ +110° (*c* = 0.42, MeOH). Beilstein test: (+). EI-MS *m/z* (rel. int.): 436.1286 $[M]^+$ (436.1287 for $C_{22}H_{25}O_7{}^{35}Cl$, 11), 438.1272 $[M]^+$ (438.1260 for $C_{22}H_{25}O_7{}^{37}Cl$, 4), 237 $[C_{13}H_{17}O_4]^+$ (32), 199.0166 $[C_9H_8O_3{}^{35}Cl]^+$ (199.0163 for $C_9H_8O_3{}^{35}Cl$, 100), 201.0124 $[C_9H_8O_3{}^{37}Cl]^+$ (201.0132 for $C_9H_8O_3{}^{37}Cl$, 34). UV λ_{max}^{MeOH} nm (log ε): 226 sh (4.01), 321 (4.23). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1720 (COO), 1660 (CO). CD $\Delta\varepsilon^{20}$ (nm) (MeOH, $c = 1.0 \times 10^{-4}$): −1.2 (234), +0.2 (262), −2.0 (306), +2.6 (329 sh), +6.5 (343), +5.1 (355 sh). ^{1}H NMR ($CDCl_3$) δ: 0.95 (3H, *t*, *J* = 7.6 Hz, 3′-H), 1.46 (3H, *s*, 7-Me), 1.61 (1H, *qt*, *J* = 7.6 and 7.5 Hz, 2′-H), 2.18 (1H, *dt*, *J* = 14.7 and 7.5 Hz, 1′-H), 2.24 (1H, *dt*, *J* = 14.7 and 7.5 Hz, 1′-H), 2.45 (3H, *s*, 7″-Me), 2.72 (1H, *d*, *J* = 2.5 Hz, 8-OH), 2.83 (1H, *dddd*, *J* = 13.1, 10.5, 5.0 and 1.8 Hz, 8a-H), 3.81 [1H, *dd*, *J* = 13.1 and 10.6 Hz, 1-H (ax.)], 3.83 (3H, *s*, 3″-OMe), 4.73 (1H, *dd*, *J* = 10.5 and 2.5 Hz, 8-H), 4.73 [1H, *dd*, *J* = 10.6 and 5.0 Hz, 1-H (eq.)], 5.51 (1H, *s*, 4-H), 5.72 (1H, *d*, *J* = 1.8 Hz, 5-H), 5.84 (1H, *s*, 5″-OH), 6.51 (1H, *s*, 4″-H). ^{13}C NMR ($CDCl_3$): Table 2.

Falconensin M (**13**). Pale yellow crystalline powder, mp 242–244°. $[\alpha]_D^{20}$ +195° (*c* = 0.25, MeOH). Beilstein test: (+). EI-MS *m/z* (rel. int.): 468.0742 $[M]^+$ (468.0742 for $C_{22}H_{22}O_7{}^{35}Cl_2$, 17), 470.0720 $[M]^+$ (470.0713 for $C_{22}H_{22}O_7{}^{35}Cl^{37}Cl$, 11), 472.0678 $[M]^+$ (472.0683 for $C_{22}H_{22}O_7{}^{37}Cl_2$, 2), 233 $[C_9H_8O_3{}^{35}Cl_2]^+$ (66), 235 $[C_9H_8O_3{}^{35}Cl^{37}Cl]^+$ $[C_{13}H_{15}O_4]^+$ (100), 237 $[C_9H_8O_3{}^{37}Cl_2]^+$ (20). UV λ_{max}^{MeOH} nm (log ε): 249 sh (3.86), 349 (4.41). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1720 (COO), 1650 (CO). CD $\Delta\varepsilon^{20}$ (nm) (MeOH, $c = 1.0 \times 10^{-4}$): +2.5 (220), −1.1 (241), +0.8 (270), −1.9 (308), +4.8 (347 sh), +8.3 (364). ^{1}H NMR ($CDCl_3$) δ: 1.50 (3H, *s*, 7-Me), 1.88 (3H, *dd*, *J* = 7.0 and 1.5 Hz, 3′-H), 2.49 (3H, *s*, 7″-Me), 2.74 (1H, *d*, *J* = 3.1 Hz, 8-OH), 2.90 (1H, *dddd*, *J* = 12.8, 11.0, 5.2 and 1.8 Hz, 8a-H), 3.85 [1H, *dd*, *J* = 12.8 and 10.4 Hz, 1-H (ax.)], 3.92 (3H, *s*, 3″-OMe), 4.73 (1H, *dd*, *J* = 11.0 and 3.1 Hz, 8-H), 4.80 [1H, *dd*, *J* = 10.4 and 5.2 Hz, 1-H (eq.)], 5.57 (1H, *s*, 4-H), 5.81 (1H, *d*, *J* = 1.8 Hz, 5-H), 5.91 (1H, *dq*, *J* = 15.6 and 1.5 Hz, 1′-H), 6.11 (1H, *s*, 5″-OH), 6.48 (1H, *dq*, *J* = 15.6 and 7.0 Hz, 2′-H). ^{13}C NMR ($CDCl_3$): Table 2.

Falconensin N (**14**). Pale yellow crystalline powder, mp 193–195°. $[\alpha]_D^{20}$ +147° (*c* = 0.30, MeOH). Beilstein test: (+). EI-MS *m/z* (rel. int.): 470.0905 $[M]^+$ (470.0899 for $C_{22}H_{24}O_7{}^{35}Cl_2$, 16), 472.0869 $[M]^+$ (472.0869 for $C_{22}H_{24}O_7{}^{35}Cl^{37}Cl$, 10), 474.0833 $[M]^+$ (474.0839 for $C_{22}H_{24}O_7{}^{37}Cl_2$, 2), 233 $[C_9H_8O_3{}^{35}Cl_2]^+$ (100), 235 $[C_9H_8O_3{}^{35}Cl^{37}Cl]^+$ $[C_{13}H_{15}O_4]^+$ (63), 237 $[C_9H_8O_3{}^{37}Cl_2]^+$ (64). UV λ_{max}^{MeOH} nm (log ε): 321 (4.41). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1740 (COO), 1650 (CO). CD $\Delta\varepsilon^{20}$ (nm) (MeOH, $c = 7.7 \times 10^{-5}$): −3.4 (217), +0.1 (237), −2.9 (293), +6.2 (331 sh), +10.7 (342), +8.3 (355 sh). ^{1}H NMR ($CDCl_3$) δ: 0.95 (3H, *t*, *J* = 7.6 Hz, 3′-H), 1.49 (3H, *s*, 7-Me), 1.57 (1H, *qt*, *J* = 7.6 and 7.4 Hz, 2′-H), 2.15 (1H, *dt*, *J* = 14.8 and 7.4 Hz, 1′-H), 2.22 (1H, *dt*, *J* = 14.8 and 7.4 Hz, 1′-

H), 2.48 (3H, *s*, 7″-Me), 2.79 (1H, *d*, $J = 2.6$ Hz, 8-OH), 2.85 (1H, *dddd*, $J = 13.1$, 10.7, 5.4 and 1.8 Hz, 8a-H), 3.81 [1H, *dd*, $J = 13.1$ and 10.7 Hz, 1-H (ax.)], 3.92 (3H, *s*, 3″-OMe), 4.69 (1H, *dd*, $J = 10.7$ and 2.6 Hz, 8-H), 4.75 [1H, *dd*, $J = 10.4$ and 5.4 Hz, 1-H (eq.)], 5.51 (1H, *s*, 4-H), 5.73 (1H, *d*, $J = 1.8$ Hz, 5-H), 6.19 (1H, *s*, 5″-OH). ^{13}C NMR ($CDCl_3$): Table 2.

Monomethyldihydromitorubrin (**15**). Pale yellow crystalline powder, mp 100–102°. $[\alpha]_D^{20}$ +105° ($c = 0.37$, MeOH). EI-MS *m/z* (rel. int.): 398.1364 $[M]^+$ (398.1366 for $C_{22}H_{22}O_7$), 218 ($C_{13}H_{14}O_3$, 13), 165 ($C_9H_9O_3$, 100). UV λ_{max}^{MeOH} nm (log ε): 215 (4.25), 258 (3.66 sh), 331 (4.04). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1710 (COO), 1660 (CO). CD $\Delta\varepsilon^{30}$ (nm) (MeOH, $c = 1.0 \times 10^{-4}$): +2.7 (226), −0.1 (240), −2.3 (274), +2.5 (331 sh), +3.0 (349). ^{1}H NMR ($CDCl_3$) δ: 0.93 (3H, *t*, $J = 7.6$ Hz, 3′-H), 1.52 (3H, *s*, 7-Me), 1.61 (2H, *m*, 2′-H), 2.32 (2H, *t*, $J = 7.3$ Hz, 1′-H), 2.33 (3H *s*, 7″-Me), 3.62 (3H, *s*, 3″-OMe), 5.51 (1H, *d*, $J = 1.2$ Hz, 4-H), 6.04 (1H, *s*, 5-H), 6.15 (1H, *br s*, 4″-H or 6″-H), 6.16 (1H, *br s*, 6″-H or 4″-H), 6.72 (1H, *br s*, 5″-OH), 7.85 (1H, *s*, 1-H). ^{13}C NMR ($CDCl_3$) δ: 13.4 (7″-Me), 19.9 (2′-C), 20.3 (3′-C), 22.3 (7-Me), 35.0 (1′-C), 56.0 (3″-OMe), 84.3 (7-C), 97.1 (4″-C), 106.9 (5-C), 108.9 (4-C), 109.8 (6″-C), 112.4 (8a-C), 115.3 (2″-C), 141.0 (7″-C), 143.3 (4a-C), 154.2 (1-C), 159.1 (3-C), 160.2 (3″- or 5″-C), 162.4 (5″- or 3″-C), 167.0 (1″-C), 193.4 (3- or 6-C), 193.4 (6- or 3-C).

Monomethylmitorubrin (**16**). Pale yellow crystalline powder. EI-MS *m/z* (rel. int.): 396 $[M]^+$ ($C_{22}H_{20}O_7$, 2), 165 $[C_{22}H_{20}O_7]^+$ (100), 165 $[C_9H_9O_3]^+$ (100). CD $\Delta\varepsilon^{20}$ (nm) (MeOH, $c = 4.7 \times 10^{-5}$): +4.0 (243), −3.0 (268), −2.7 (280), +5.6 (366). ^{1}H NMR ($CDCl_3$) δ: 160 (3H, *s* 7-Me), 1.94 (3H, *dd*, $J = 7.1$ and 1.5 Hz, 3′-H), 2.42 (3H, *s* 7″-Me), 3.72 (3H, *s*, 3″-OMe), 5.63 (1H, *d*, $J = 1.0$ Hz, 4-H), 6.00 (1H, *dq*, $J = 15.5$ and 1.5 Hz, 2′-H), 6.10 (1H, *s*, 5-H), 6.23 (2H, *br s*, 4″-H and 6″-H), 6.29 (1H, *br s*, 5″-OH), 6.57 (1H, *dq*, $J = 15.5$ and 7.1 Hz, 1′-H), 7.93 (1H, *br s*, 1-H).

Methylation of falconensin I (**9**). An Et_2O soln of CH_2N_2 was added to a soln of **9** (5 mg) in Et_2O (2 ml) and the reaction mixt. kept at room temp. overnight. Solvent was evapd *in vacuo*. The residue was purified by repeated LPLC with hexane–Me_2CO (8:1) to give falconensin I monomethyl ether (**6**) (2 mg). This compound was identified with naturally occurring falconensin F by the comparison of ^{1}H NMR, mass and CD spectra, and TLC behaviour.

Acetylation of falconensin I (**9**). Falconensin I (**9**) (15 mg) dissolved in pyridine (0.7 ml) and Ac_2O (0.7 ml) was kept at room temp. overnight. The reaction mixt. was poured into ice-H_2O and extracted with $CHCl_3$. The organic layer was washed with 0.5 M HCl and then H_2O, dried over Na_2SO_4 and then evapd *in vacuo*. The residue was purified by repeated LPLC with C_6H_6–Me_2CO (25:1) and/or hexane–Me_2CO (10:1) to give falconensin I diacetate (**17**) (14 mg).

Falconensin I diacetate (**17**). Pale yellow amorphous powder. EI-MS *m/z* (rel. int.): 484.1737 $[M]^+$ (484.1733 for $C_{26}H_{28}O_9$, 5), 277 $[C_{15}H_{17}O_5]^+$ (8), 235 $[C_{13}H_{15}O_4]^+$ (31), 207 $[C_{11}H_{11}O_4]^+$ (57), 165 $[C_9H_9O_3]^+$ (100). IR ν_{max}^{KBr} cm^{-1}: 1760 (COO), 1670 (CO). ^{1}H NMR ($CDCl_3$) δ: 1.57 (3H, *s*, 7-Me), 1.87 (3H, *dd*, $J = 8.1$ and 1.5 Hz, 3′-H), 2.15 (3H, *s*, 8-OAc), 2.27 (3H, *s*, 5″-OAc), 2.45 (3H, *s*, 7″-Me), 2.95 (1H, *dddd*, $J = 13.4$, 10.4, 4.9 and 1.8 Hz, 8a-H), 3.75 (3H, *s*, 3″-OMe), 3.94 [1H, *dd*, $J = 13.4$ and 11.0 Hz, 1-H (ax.)], 4.32 [1H, *dd*, $J = 11.0$ and 4.9 Hz, 1-H (eq.)], 5.57 (1H, *s*, 4-H), 5.86 (1H, *d*, $J = 1.8$ Hz, 5-H), 5.90 (1H, *dq*, $J = 16.5$ and 1.5 Hz, 1′-H), 6.08 (1H, *d*, $J = 10.4$ Hz, 8-H), 6.42 (1H, *dq*, $J = 16.5$ and 8.1 Hz, 2′-H), 6.47 (1H, *br s*, 4″-H), 6.52 (1H, *br s*, 6″-H).

Acknowledgements—We thank Prof. Makoto Miyaji of Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, and Dr Shun-ichi Udagawa of Tokyo University of Agriculture, for their kind advice. We are also grateful to Mrs T. Ogata, Mrs Y. Kawada and Mr H. Komiya of Hoshi University for elemental analysis and NMR and mass measurements. This work was supported in part by a Grant-in-Aid for Scientific Research (no. 07672296) from the Ministry of Education, Science, Sports and Culture, Japan.

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