Novel Mammalian Cell Cycle Inhibitors, Tryprostatins A, B and Other Diketopiperazines Produced by *Aspergillus fumigatus*

II. Physico-chemical Properties and Structures

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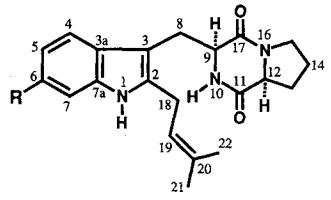
Two novel diketopiperazines named tryprostatins A and B and a new natural product belonging to the diketopiperazine series, designated as demethoxyfumitremorgin C, together with four known diketopiperazines, fumitremorgin C, 12,13-dihydroxyfumitremorgin C, fumitremorgin B and verruculogen, are new M phase inhibitors of the mammalian cell cycle, which were isolated from the secondary metabolites of *Aspergillus fumigatus*. The structures of tryprostatins A, B and demethoxyfumitremorgin C were determined mainly by the use of spectroscopic methods especially by detailed analyses of their ^{1}H and ^{13}C NMR spectra with the aid of 2D NMR techniques including pulse field gradient heteronuclear multiple-bond correlation (PFG-HMBC) spectroscopy. Their absolute configurations were determined on the basis of the optical rotational values and CD spectra.

In the course of our screening for new mammalian cell cycle inhibitors, we have previously reported several new compounds such as thiazoline derivatives[1] and acetophthalidin[2] as new inhibitors of the mammalian cell cycle. In the continuation, we have recently isolated two novel diketopiperazines named tryprostatins A (**1**) and B (**2**)[3] and a new natural product belonging to the diketopiperazine series, designated as demethoxyfumitremorgin C (**3**), as well as fumitremorgin C (**4**) from the secondary metabolites of *Aspergillus fumigatus* BM939 (FERM P-15067) together with 12,13-dihydroxyfumitremorgin C (**5**), fumitremorgin B (**6**) and verruculogen (**7**). These diketopiperazines provide new M phase inhibitors of the mammalian cell cycle. Taxonomy, fermentation, isolation and biological properties of **1**~**7** were reported in a previous paper[4]. In this paper, we report the structure elucidation of three new natural products, **1**~**3**, and the identification of the known diketopiperazines, **4**~**7**.

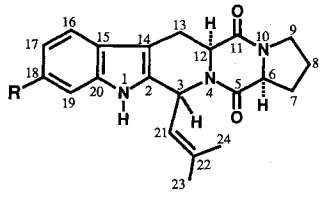
Physico-chemical Properties of Three New Natural Diketopiperazines

Tryprostatins A (**1**) and B (**2**) were obtained both as pale yellow crystalline solids having melting points

Fig. 1. Structures of **1**~**7**.



1 R=CH_3O
2 R=H



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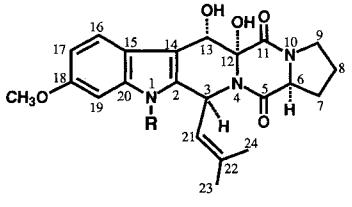
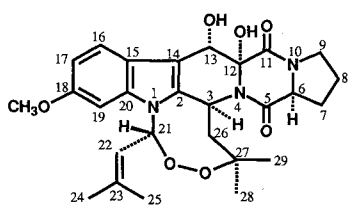
4 R=CH_3O



5 R=H
6 R=Isoprenyl



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120~123°C and 102~105°C, respectively, and showed optical rotational values, $[\alpha]_D$ −69.7° ($CHCl_3$) for **1** and $[\alpha]_D$ −71.1° ($CHCl_3$) for **2**, respectively. Demethoxyfumitremorgin C (**3**) was obtained as pale yellow needles, mp 210~212°C, and showed optical rotational value of $[\alpha]_D$ +8.0° ($CHCl_3$). The physico-chemical properties of **1**~**3** are summarized in Table 1.

Planar Structures of Three New Natural Diketopiperazines

Tryprostatin A (**1**) had the molecular formula, $C_{22}H_{27}N_3O_3$, which was determined by HR-EI-MS (Found *m/z* 381.2050 (M^+), Calcd for $C_{22}H_{27}N_3O_3$ 381.2050) and agreed well with its ^1H and ^{13}C NMR data (Table 2). The UV spectrum of **1** revealed the presence of a 6-*O*-methylindole chromophore in **1** with the absorption maxima at 227 (ε 24540), 270 (5450) and 297 nm (6590)[3,5]. In the IR spectrum, **1** showed multiple absorption at 1670 and 1655 cm^{-1} (Table 1), which are typical of amide groups[3,5~7]. These findings together with the absence of the amide II band near 1550 cm^{-1} in the IR spectrum suggested the presence of diketopiperazine system[6,7] in **1**.

In the ^1H NMR spectrum, **1** showed signals due to an 1,2,4-trisubstituted benzene ring (δ 7.34 d, J=8.8 Hz, 4-H; δ 6.76 dd, J=8.8, 2.4 Hz, 5-H; and δ 6.83 d, J=2.4 Hz, 7-H), a methoxy (δ 3.83 s) and two tertiary methyl (δ 1.75 s, 22-H_3 and δ 1.78 s, 21-H_3) groups and two exchangeable (δ 7.88 br s, 1-H and δ 5.65 br s, 10-H) and an olefinic (δ 5.29 br dd, J=7.0, 6.5 Hz, 19-H) protons along with signals due to several methylene and methine groups (Table 2). The ^{13}C NMR spectrum of **1**, analyzed by the DEPT method, indicated the presence of two amide carbonyls (δ 169.35 s, C-11 and 165.82 s, C-17), an oxygen-bearing sp^2 carbon (δ 156.37 s, C-6) and a methoxy (δ 55.77 q, OCH_3) and two methyl (δ 25.76 q, C-21 and δ 17.98 q, C-22) groups together with four sp^2 methines, five quaternary sp^2 carbons and two sp^3 methine and five methylene groups (Table 2).

Detailed analyses of the ^1H and ^{13}C NMR spectra (Table 2) with the aid of ^1H-^1H COSY, ^1H-^{13}C COSY and difference NOE experiments, coupled with the structural information from the UV and IR spectra, led us to postulate the existence of partial structures A, B, and C in **1** (Fig. 2).

We then measured the pulse field gradient heteronuclear multiple-bond correlation (PFG-HMBC) spectrum of **1** in order to determine its total structure. In the PFG-HMBC spectrum, the oxygen-bearing sp^2 carbon at δ 156.37 (C-6) showed long-range correlations with the benzene protons, 4-H (δ 7.34) and 7-H (δ 6.83), and the methoxy proton at δ 3.83 in the partial structure A, while the quaternary sp^2 carbon at δ 122.30 (C-3a) correlated with the 4-H, 5-H and 7-H protons and the amide 1-H proton at δ 7.88 (see the partial structure A in Fig. 3). Therefore, they were assigned respectively to

Table 1. Physico-chemical properties of tryprostatins A (**1**), B (**2**) and demethoxyfumitremorgin C (**3**).

Characteristics	1	2	3
Appearance	Pale yellow crystalline solid	Pale yellow crystalline solid	Pale yellow needles
MP	120-123°C	102-105°C	210-212°C
$[\alpha]_D$	$[\alpha]_D^{27}$ -69.7°(c 0.70, $CHCl_3$)	$[\alpha]_D^{27}$ -71.1°(c 0.63, $CHCl_3$)	$[\alpha]_D^{30}$ +8.0°(c 0.2, $CHCl_3$)
Molecular Formular	$C_{22}H_{27}N_3O_3$	$C_{21}H_{25}N_3O_2$	$C_{21}H_{23}N_3O_2$
EI-MS *m/z*	381 (M^+, 26%), 228 (M^+-153, base preak)	351 (M^+, 20%), 198 (M^+-153, base peak)	349 (M^+, base peak), 294 (31%), 251 (46%), 182 (42%)
HR-EI-MS	M^+	M^+	M^+
Found (*m/z*)	381.2050	351.1943	349.1801
Calcd (*m/z*)	381.2050	351.1944	349.1799
	M^+-$C_7H_9N_2O_2$ (153)	M^+-$C_7H_9N_2O_2$ (153)	
Found (*m/z*)	228.1372	198.1279	
Calcd (*m/z*)	228.1387	198.1282	
UV λ_{max}^{MeOH} nm (ε)	227 (24540) 270 (5450) 297 (6590)	226 (23780) 277 (8690) 298 (sh, 7180)	226 (24950) 282 (9000) 291 (sh, 7450)
IR ν_{max}^{KBr} cm^{-1}	3365, 3270, 3070, 2965, 2930, 2880, 2860, 1670, 1655, 1460, 1430, 1160	3370, 3270, 3070, 2970, 2930, 2875, 2860, 1670, 1655, 1460, 1435, 1160	3445, 3260, 3050, 2952, 2930, 2880, 2850, 1660, 1655, 1460, 1410, 1140

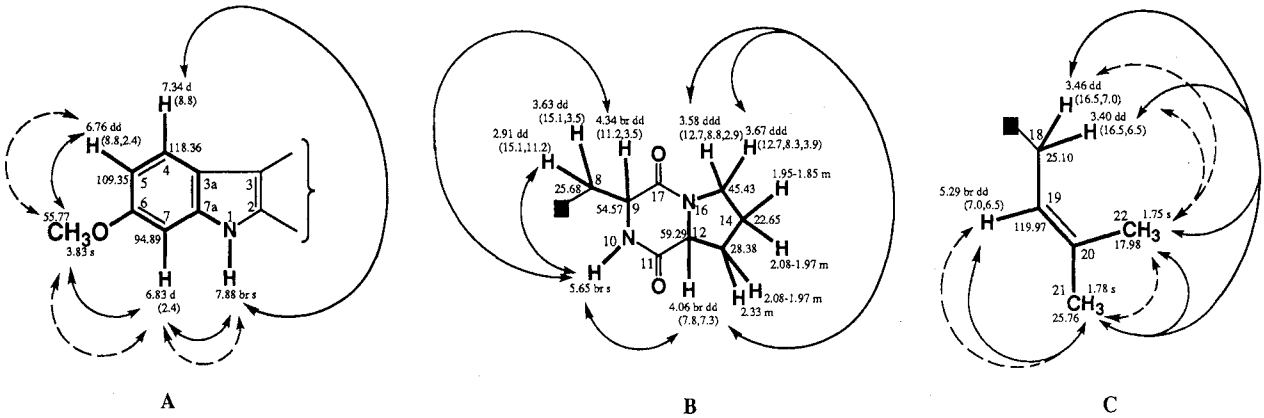
Table 2. 500 MHz ^{1}H and 125 MHz ^{13}C NMR data for **1** and **2** in chloroform-d[a].

Positions	**1**		**2**	
	δ_H (J in Hz)	δ_c	δ_H (J in Hz)	δ_c
1 (NH)	7.88[b),c)] br s	--------	8.03[b),c)] br s	--------
2	-----	135.11 s	-----	136.43 s
3	-----	104.46 s	-----	104.61 s
3a	-----	122.30 s	-----	127.97 s
4	7.34 d (8.8)	118.36 d	7.47 d (7.7)	117.74 d
5	6.76 dd (8.8, 2.4)	109.35 d	7.09 t (7.7)	119.91 d
6	-----	156.37 s	7.16 t (7.7)	121.87 d
7	6.83 d (2.4)	94.89 d	7.31 d (7.7)	110.79 d
7a	-----	136.28 s	-----	135.44 s
8	2.91 dd (15.1, 11.2) 3.63 dd (15.1, 3.5)	25.68 t	2.96 dd (15.0, 11.0) 3.68 dd (15.0, 3.5)	25.61 t
9	4.34 br dd (11.2, 3.5)	54.57 d	4.37 br dd (11.0, 3.5)	54.57 d
10 (NH)	5.65 br s	--------	5.64 br s	-------
11	-----	169.35 s	-----	169.35 s
12	4.06[d)] br dd (7.8, 7.3)	59.29 d	4.06[d)] br dd (8.0, 7.5)	59.27 d
13	2.33 m 2.08-1.97 m	28.38 t	2.33 m 2.08-1.97 m	28.34 t
14	2.08-1.97 m 1.95-1.85 m	22.65 t	2.08-1.97 m 1.95-1.85 m	22.64 t
15	3.67 ddd (12.7, 8.3, 3.9) 3.58 ddd (12.7, 8.8, 2.9)	45.43 t	3.68 overlapped 8-H 3.59 ddd (12.0, 8.5, 3.0)	45.41 t
17	-----	165.82 s	-----	165.80 s
18	3.46[e)] dd (16.5, 7.0) 3.40[e)] dd (16.5, 6.5)	25.10 t	3.49[e)] dd (17.0, 7.0) 3.44[e)] dd (17.0, 6.5)	25.12 t
19	5.29[f)] br dd (7.0, 6.5)	119.97 d	5.31[f)] br dd (7.0, 6.5)	119.70 d
20	-----	135.25 s	-----	135.48 s
21	1.78[g)] s	25.76 q	1.79[g)] s	25.74 q
22	1.75[g)] s	17.98 q	1.75[g)] s	17.98 q
OCH_3	3.83[h)] s	55.77 q	-----	--------

[a] Signal assignments were based on the results of ^{1}H-^{1}H and ^{1}H-^{13}C COSY, PFG-HMBC and difference NOE experiments. [b,e] and [f] NOE's were observed with 7-H, 18-H_2 and 19-H, with 8-H_2 and 22-H_3, and with 21-H_3, respectively, in the difference NOE experiments. [c,d] and [g] Long-range ^{1}H-^{1}H couplings were observed with 4-H and 7-H, with 9-H, 10-H, and 15-H_2, and with 18-H_2 and 19-H, respectively, in the ^{1}H-^{1}H COSY. [h] NOE's and ^{1}H-^{1}H couplings were observed with 5-H and 7-H in the difference NOE experiments and in the ^{1}H-^{1}H COSY, respectively.

Fig. 2. Partial structures, A, B and C, and NMR data for **1**.

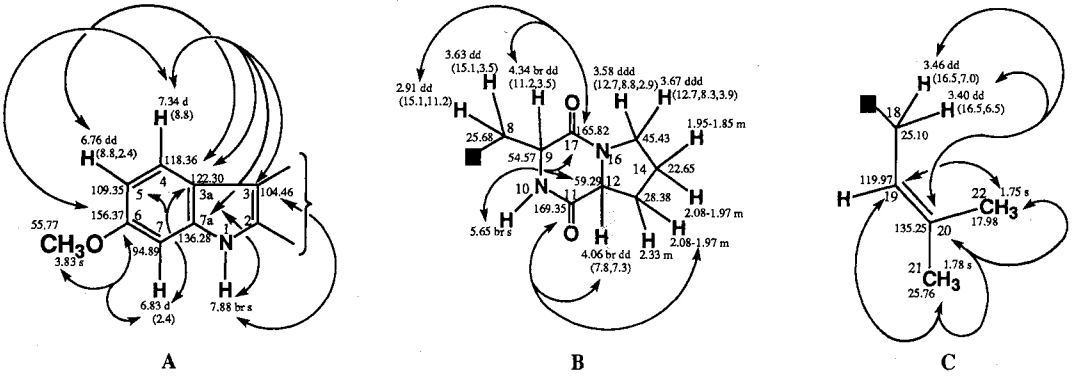
Bold lines indicate spin systems obtained by the analyses of ^{1}H-^{1}H and ^{1}H-^{13}C COSY's. Solid line arrows indicate long-range ^{1}H-^{1}H couplings observed in the ^{1}H-^{1}H COSY. Dashed line arrows indicate NOE's observed in the difference NOE experiments.



A B C

Fig. 3. Partial structures, A, B and C, and NMR data for **1**.

Solid line arrows indicate long-range ^{1}H-^{13}C correlations observed in the PFG-HMBC spectrum.

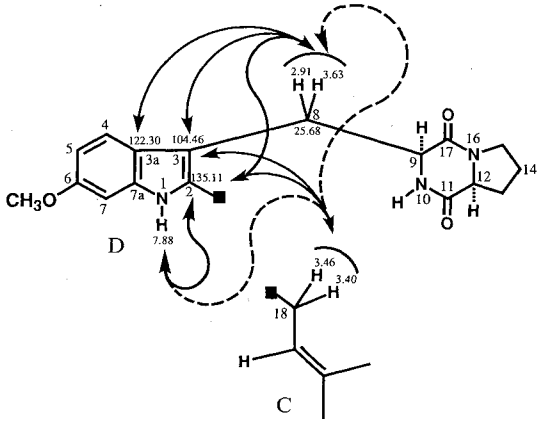


C-6 (δ 156.37) and C-3a (δ 122.30) in the partial structure A. Two quaternary sp^2 carbons at δ 104.46 and at δ 136.28 were considered to be either C-3 or C-7a in the partial structure A, respectively, according to their long-range correlations both with 4-H and the correlation between the former carbon (δ 104.46) and 1-H (Fig. 3). Eventually, they were assigned respectively to C-3 (δ 104.46) and C-7a (δ 136.28) in view of their chemical shift values. For the partial structure B, two amide carbonyls, C-11 (δ 169.35) and C-17 (δ 165.82), were assigned respectively on the basis of their long-range correlations observed in the PFG-HMBC spectrum, as shown by solid arrows on the partial structure B in Fig. 3. Similarly, the quaternary carbon at δ 135.25 could be assigned to C-20 in the partial structure C (Fig. 3), which showed long-range correlations with 18-H_2 (δ 3.40 and 3.46), 21-H_3 (δ 1.78) and 22-H_3 (δ 1.75).

Next, in the PFG-HMBC spectrum, 8-H_2 (δ 2.91 and 3.63) in the partial structure B showed long-range correlations with C-3 and C-3a in the partial structure A and with a quaternary sp^2 carbon at δ 135.11 (C-2) which, in turn, further correlated with 1-H in the partial structure A, as shown in Fig. 4. Thus the partial structure B could be linked with its C-8 to the C-3 carbon of the partial structure A to form the partial structure D and the quaternary carbon at δ 135.11 could be assigned to C-2 in the partial structure D (Fig. 4). The methylene protons 18-H_2 (δ 3.40 and 3.46) in the partial structure C showed long-range correlations with the C-2 (δ 135.11) and C-3 (δ 104.46) carbons in the partial structure D in the PFG-HMBC spectrum. In addition, in the difference NOE experiments, NOE's were observed between 1-H (in the partial structure D) and 18-H_2 (in the partial structure C) and between 8-H (δ 2.91, in the partial

Fig. 4. Connectivities between partial structures for **1**.

Solid line arrows indicate long-range ^{1}H-^{13}C couplings observed in the PFG-HMBC spectrum. Dashed line arrows indicate NOE's observed in the difference NOE experiments.



structure D) and 18-H_2, as shown in Fig. 4. Therefore, the partial structure C must be linked at C-2 of the partial structure D and thus the planar structure of **1** was deduced.

Tryprostatin B (**2**) is also a nitrogen-containing compound and its molecular formula was determined to be $C_{21}H_{25}N_3O_2$ by HR-EI-MS (Found m/z 351.1943 (M^+), Calcd for $C_{21}H_{25}N_3O_2$ 351.1944), which is consistent with the data of its ^{1}H and ^{13}C NMR spectra (Table 2). In the UV spectrum, **2** showed absorption maxima at 226 (23780), 277 (8690) and 298 (sh, 7180), which are typical of indole chromophore[3,6]. The IR spectrum of **2** closely resembled that of **1** in the functional group region, indicating the presence of diketopiperazine system in **2**.

The ^{1}H NMR spectrum of **2** was very similar to that of **1**, but it was characterized by the disappearance of the methoxy proton signal (δ 3.83) in **1** and the appearance of characteristic signals due to 1,2-disubstituted benzene protons in the δ 7~7.6 region, instead of the signals due to 1,2,4-trisubstituted benzene protons in **1** (Table 2). In parallel, the ^{13}C NMR spectrum of **2**, analyzed by the DEPT method, was also similar to that of **1** and showed the lack of the methoxy (δ 55.77) and the oxygen-bearing sp^2 quaternary carbon (δ 156.37, C-6) signals in **1**, accompanied with the appearance of a new sp^2 methine carbon signal (δ 121.87, C-6) and the significant changes in the chemical shifts of several sp^2 carbons (Table 2).

The above observations, coupled with the molecular formula of **2**, $C_{21}H_{25}N_3O_2$, which is CH_2O less than that of **1**, led us to consider that **2** may be a demethoxy derivative of **1**. Eventually, its final structure was determined by extensive analyses of the ^{1}H and ^{13}C NMR spectra (Table 2) with the aid of ^{1}H-^{1}H and ^{1}H-^{13}C COSY's, PFG-HMBC and difference NOE experiments.

Demethoxyfumitremorgin C (**3**) was obtained as pale yellow needles and its molecular formula was determined to be $C_{21}H_{23}N_3O_2$ by HR-EI-MS (Found m/z 349.1801 (M^+), Calcd for $C_{21}H_{23}N_3O_2$ 349.1799), coupled with its ^{1}H and ^{13}C NMR data (Table 3). The UV and IR absorption (Table 1) of **3** suggested, like in the case of **2**, that **3** is also a diketopiperazine derivative with an indole chromophore.

The ^{1}H NMR spectrum of **3** resembled that of **4** except for the absence of the methoxy proton signal (δ 3.83) in **4** and the presence of signals due to 1,2-disubstituted benzene protons, instead of the signals due to 1,2,4-trisubstituted benzene protons in **4** (Table 3). From those findings and in view of the molecular formula of **3**, $C_{21}H_{23}N_3O_2$, which is CH_2O less than that of **4**, com-

Table 3. 500 MHz ^{1}H and 125 MHz ^{13}C NMR data for **3** and **4** in chloroform-d[a].

Positions	3		4	
	δ_H (J in Hz)	δ_c	δ_H (J in Hz)	δ_c
1 (NH)	7.92[b),c)] br s	--------	7.89[b),c)] br s	--------
2	-----	133.48 s	-----	132.20 s
3	6.03[d)] br d (9.8)	51.00 d	5.98[d)] br d (9.5)	51.04 d
5	-----	169.53 s	-----	169.55 s
6	4.12[e)] br dd (8.0,7.5)	59.25 d	4.10[e)] br dd (9.5,7.5)	59.25 d
7	2.41 m	28.58 t	2.40 m	28.61 t
	2.24 m		2.23 m	
8	2.06 m	23.06 t	2.06 m	23.06 t
	1.94 m		1.94 m	
9	3.64 (2H) m	45.43 t	3.65 (2H) m	45.43 t
11	-----	165.70 s	-----	165.76 s
12	4.19[f)] br dd (11.7, 5.0)	56.84 d	4.18[f)] br dd (11.5, 5.0)	56.80 d
13	3.57 dd (15.7, 5.0)	21.88 t	3.51 dd (16.0, 5.0)	21.96 t
	3.13 br dd (15.7, 11.7)		3.10 ddd(16.0,11.5,1.0)	
14	-----	106.44 s	-----	106.26 s
15	-----	126.26 s	-----	120.77 s
16	7.58 br d (7.5)	118.34 d	7.43 d (9.0)	118.88 d
17	7.15 br t (7.5)	120.07 d	6.81 dd (9.0, 2.2)	109.51 d
18	7.20 br t (7.5)	122.20 d	-----	156.54 s
19	7.34 br d (7.5)	111.16 d	6.85 d (2.2)	95.32 d
20	-----	136.16 s	-----	137.05 s
21	4.92[g),h)] br d (9.8)	123.99 d	4.91[g),h)] dm (9.5)	124.20 d
22	-----	134.31 s	-----	134.00 s
23	1.65[i)] s	25.70 q	1.64[i)] s	25.72 q
24	2.01[j)] s	18.15 q	1.99[j)] s	18.11 q
OCH_3	-----	--------	3.83[k)] s	55.79 q

[a] Signal assignments were based on the results of ^{1}H-^{1}H COSY, PFG-HMQC, PFG-HMBC and difference NOE experiments. [b,f,g] and [j] NOE's were observed with 3-H and 19-H, with 3-H, 6-H and 13-H$_\alpha$ (δ 3.57), with 13-H$_\beta$ (δ 3.13) and 23-H_3, and with 3-H and 23-H_3, respectively, in the difference NOE experiments. [c,d,e,h] and [i] Long-range ^{1}H-^{1}H couplings were observed with 16-H and 19-H, with 13-H$_\beta$ and 23-H_3, with 12-H, with 23-H_3 and 24-H_3, and with 24-H_3, respectively, in the ^{1}H-^{1}H COSY. [k] NOE's and long-range ^{1}H-^{1}H couplings were observed with 17-H and 19-H in the difference NOE experiments and in the ^{1}H-^{1}H COSY, respectively.

pound **3** was considered to be a demethoxy derivative of **4**. This was further supported by the comparison of ^{13}C NMR spectrum of **3** with that of **4**. The ^{13}C NMR spectrum of **3**, analyzed by the DEPT method, showed the disappearance of the methoxy (δ 55.79) and the oxygen-bearing sp^2 quaternary carbon (δ 156.54, C-18) signals in **4**, the appearance of a new sp^2 methine carbon signal (δ 122.20, C-18) and the significant changes in the chemical shifts of several sp^2 carbons (Table 3). These were closely similar to the case of **2** and **1** as seen in Table 2, supporting that **3** is a demethoxy derivative of **4**.

Finally, the planar structure of **3** was confirmed by detailed analyses of its ^1H and ^{13}C NMR spectra (Table 3) with the aid of ^1H-^1H and ^1H-^{13}C COSY's, PFG-HMBC and difference NOE experiments.

Stereochemistry of Three New Natural Diketopiperazines

The relative stereochemistry of tryprostatins A (**1**) and B (**2**) were determined on the basis of the difference NOE experiments. In the difference NOE experiments, irradiation of 12-H (or 9-H) caused the NOE increase at 9-H (or 12-H) for both **1** and **2**. Therefore, the protons, 9-H and 12-H, in both **1** and **2** could be in the *cis*-relations.

Then, the absolute configurations of tryprostatins A (**1**) and B (**2**) were concluded to be 9*S*,12*S* by comparison of their optical rotational values ($[\alpha]_D$ $-69.7°$ for **1** and $-71.1°$ for **2**, respectively, both in chloroform) with those of deoxybrevianamide E (9*S*,12*S*, $[\alpha]_D$ $-59°$ in chloroform)[6] and its C-9 epimer (9*R*,12*S*, $[\alpha]_D$ $-30°$ in chloroform)[8]. Furthermore, **1** and **2** showed positive Cotton effects, λ_{238} +1.5, λ_{272} +1.0, λ_{296} +0.3 ($\Delta\varepsilon$) for **1** and λ_{232} +3.0, λ_{272} +0.3, λ_{292} +0.2, λ_{303} +0.3 ($\Delta\varepsilon$) for **2**, respectively, in the CD spectra in methanol solutions. These were very similar to the CD characteristics of deoxybrevianamide E recorded in the literature[6], further supporting the 9*S*,12*S* configurations of **1** and **2**.

The relative stereochemistry of demethoxyfumitremorgin C (**3**) was also determined by the difference NOE experiments. In the difference NOE experiments, irradiation of 12-H increased intensities of the 3-H and 6-H signals and irradiation of 3-H (or 6-H) caused the NOE increase at 12-H, indicating that 3-H, 6-H and 12-H in **3** are all in the *cis*-relations.

The absolute configuration of **3** could be determined by comparison of its CD spectrum with that of fumitremorgin C (**4**). In the CD spectra, **3** and **4** showed Cotton effects, λ_{222} -12.0, λ_{241} -1.0, λ_{271} +7.5 ($\Delta\varepsilon$) for **3** and λ_{226} -12.0, λ_{244} -2.0, λ_{272} +8.6, λ_{301} +6.0 ($\Delta\varepsilon$) for **4**, respectively. The similar CD characteristics corresponding to their respective UV absorption indicated the same configuration for **3** and **4**. Therefore, the absolute configuration of **3** was determined to be 9*S*,12*S*,18*S*, the same as that of **4**.

Identification of Four Known Diketopiperazines

Four known diketopiperazines, fumitremorgin C[9] (**4**), 12,13-dihydroxyfumitremorgin C[10] (**5**), fumitremorgin B[11~13] (**6**) and verruculogen[5] (**7**), were obtained from MeOH all as colorless needles respectively having melting points, 246~249°C (**4**), 163~165°C (**5**), 216~217°C (**6**) and 242~244°C (**7**), and were identified respectively according to their UV, IR, EI- or FAB-MS, ^1H and ^{13}C NMR data and by a comparison of their optical rotational values and/or CD data with those in the literature[5,9~13].

Discussion

The present work has provided three new natural diketopiperazines, tryprostatins A (**1**), B (**2**) and demethoxyfumitremorgin C (**3**), together with four known diketopiperazines, fumitremorgin C (**4**), 12,13-dihydroxyfumitremorgin C (**5**), fumitremorgin B (**6**) and verruculogen (**7**), as the new group of M phase inhibitors of mammalian cell cycle[4]. Among them, **1** and **2** are novel diketopiperazine derivatives. The molecules of **1** and **2** are composed from a 2-isoprenyl-L-tryptophan moiety and a L-proline residue, forming a diketopiperazine unit, which are distinguished from the molecules of fumitremorgin series[14] by the opening of the centeral heterocyclic ring at the C-N bond between the 18 and 10 positions. Only few kinds of natural products structurally related to **1** and **2** such as deoxybrevianamide E (**8**) have been reported[6], and the present result provides the first example of natural product belonging to this novel class as an inhibitor of the mammalian cell cycle. On the other hand, **3** was isolated for the first time from a natural source.

The isolation of tryprostatins A (**1**), B (**2**) and demethoxyfumitremorgin C (**3**) is also of considerable biogenetic interest. Tryprostatins A and B may be biogenetic intermediates of the verruculogen and fumitremorgin series[14] similar to the role of deoxybrevianamide E in the biogenesis of brevianamides[15,16]. L-Tryptophan and L-proline are well known as efficient precursors of the fumitremorgins[14], but the biogenetic pathways from these precursors to the fumitremorgins are still unknown. The co-occurrence of **1**, **2**, **3** and **4**~**7** in the secondary metabolites of *Aspergillus fumigatus* suggested also the possible intermediacy of **3** in the biogenesis of the verruculogen and fumitremorgins.

Experimental

General Instrumental Analyses

Melting points were measured using a Yanagimoto micro melting point apparatus and were uncorrected. Optical rotations and CD spectra were determined both in MeOH solutions respectively on a JASCO DIP-370 polarimeter and a JASCO J-270 CD/ORD spectropolarimeter. UV spectra were taken with a Hitachi 220A spectrophotometer in MeOH solutions and IR spectra were recorded on a Shimadzu FTIR-8100M Fourier transform infrared spectrophotometer in KBr discs. EI-MS (ionization voltage, 70 eV, accelerating voltage, 3 kV), HR-EI-MS and FAB-MS were measured respectively on Hitachi M-80A, Hitachi M-80 and JEOL JMS-HX110 mass spectrometers using a direct inlet system. Glycerol and triethanolamine were used as matrices respectively in positive and negative FAB-MS measurements. ^{1}H and ^{13}C NMR spectra were taken on a JEOL GSX-500 spectrometer with tetramethylsilane as an internal standard and chemical shifts are recorded in δ values. Multiplicities of ^{13}C NMR signals were determined by the DEPT method and are indicated as s (singlet), d (doublet), t (triplet) and q (quartet). 2D NMR spectra (^{1}H-^{1}H and ^{1}H-^{13}C COSY, PFG-HMQC and PFG-HMBC spectra) were measured on JEOL GSX-500 or α-400 spectrometers by the use of JEOL standard pulse sequences and collected data were processed by JEOL standard software. Difference NOE spectra were obtained by the use of a JEOL standard pulse sequence with irradiation for 5 seconds.

Diketopiperazines

The diketopiperazines, tryprostatins A (**1**) and B (**2**), demethoxyfumitremorgin C (**3**), fumitremorgin C (**4**), 12,13-dihydroxyfumitremorgin C (**5**), fumitremorgin B (**6**) and verruculogen (**7**), used in the present study were isolated from the fermentation broth of *Aspergillus fumigatus* BM939 by the combined use of solvent extraction, silica gel column chromatography, preparative TLC and repeated-preparative HPLC as described in the preceding paper[4].

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