Diketopiperazine Alkaloids Produced by the Endophytic Fungus *Aspergillus fumigatus* from the Stem of *Erythrophloeum fordii* OLIV.

by **Yu-Xi Liu**, **Shuang-Gang Ma**, **Xiao-Jing Wang**, **Nan Zhao**, **Jing Qu**, **Shi-Shan Yu***, **Jun-Gui Dai**, **Ying-Hong Wang**, and **Yi-Kang Si**

State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, No. 1 Xian Nong Tan Street, Beijing 100050, P. R. China (phone: +86-10-63165326; fax: +86-10-63017757; e-mail: yushishan@imm.ac.cn)

Four new diketopiperazine alkaloids, *rel*-(8*R*)-9-hydroxy-8-methoxy-18-*epi*-fumitremorgin C (**1**), *rel*-(8*S*)-19,20-dihydro-9,20-dihydroxy-8-methoxy-9,18-di-*epi*-fumitremorgin C (**2**), *rel*-(8*S*,19*S*)-19,20-dihydro-9,19,20-trihydroxy-8-methoxy-9-*epi*-fumitremorgin C (**3**), and (3*S*,8*S*,9*S*,18*S*)-8,9-dihydroxyspirotryprostatin A (**4**), together with the eight known compounds **5**–**12**, were isolated from the endophytic fungus *Aspergillus fumigatus*. The structures of the new compounds were determined by extensive spectroscopic methods including HR-ESI-MS, NMR, and CD experiments. Compound **12** showed weak inhibitory activity *in vitro* against the release of β-glucuronidase in rat polymorphonuclear leukocytes induced by the platelet-activating factor. None of the twelve compounds exhibited detectable cytotoxic activities toward five human tumor cell lines (HCT-8, Bel-7402, BGC-823, A549, and A2780) in the MTT assay.

Introduction. – *Erythrophloeum fordii* OLIV. (Leguminosae) is widely distributed in the south of China, and its bark and seed have been used in folk medicine to facilitate blood circulation and for dispersing blood stasis [1]. Basing on previous chemical and biological studies on this plant [2–6], we concentrated our efforts to investigate biogenetically related alkaloids among the secondary metabolites present in the roots of the endophytic fungus *Aspergillus fumigatus*. In our present study, four new diketopiperazine alkaloids, namely *rel*-(8*R*)-9-hydroxy-8-methoxy-18-*epi*-fumitremorgin C[1]) (**1**), *rel*-(8*S*)-19,20-dihydro-9,20-dihydroxy-8-methoxy-9,18-di-*epi*-fumitremorgin C[1]) (**2**), *rel*-(8*S*,19*S*)-19,20-dihydro-9,19*S*,20-trihydroxy-8-methoxy-9-*epi*-fumitremorgin C[1]) (**3**), and (3*S*,8*S*,9*S*,18*S*)-8,9-dihydroxyspirotryprostatin A[1]) (**4**), together with the eight known compounds brevianamide F (**5**) [7], fumitremorgin C (=(5a*S*,12*S*,14a*S*)-1,2,5a,6,11,12,14a-octahydro-9-methoxy-12-(2-methylprop-1-en-1-yl)-5*H*,14*H*-pyrrolo[1″,4″:4′,5′]pyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-5,14-dione; **6**) [8], 18-oxotryprostatin A (**7**) [9], cyclo(*N*′-prenyl-L-tryptophyl-L-prolyl) (**8**) [10], tryprostatin B (**9**) [11][12], 8,9-dihydroxyfumitremorgin C (**10**) [8], cyclo(L-isoleucyl-L-prolyl) (**11**) [7][13], and cyclo(L-leucyl-L-prolyl) (**12**) [7][13][14] were isolated from the AcOEt extract of a culture of *A. fumigatus* (*Fig. 1*). In this paper, we describe the

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.* For convenience, the stereodescriptors in the trivial names of **2** and **3** dot not follow the conventions for the denotation of relative configuration.

isolation, structural elucidation, and biological activities of these diketopiperazine alkaloids.

Results and Discussion. – 1. *Structure Elucidation*. The molecular formula of compound **1** was established as $C_{23}H_{27}N_3O_5$ according to the ion peak $[M+Na]^+$ at *m/z* 448.1850 in the HR-ESI-MS and ^{13}C-NMR data. Its IR absorption bands at 3300 cm^{-1} indicated the presence of an OH group. The ^{13}C-NMR data of **1** (*Table 1*) showed that its structure was similar to that of (8*S*)-8,9-dihydroxyfumitremorgin C (**10**) [15], except for the presence of the signal at δ(C) 56.5 (*Me*O–C(8)) ascribed to an additional oxygenated Me group in **1** (*Fig. 1*), which was also confirmed by the HMBC cross-peak from this MeO group at δ(H) 3.36 to C(8). Analysis of the ^{13}C- and ^{1}H-NMR data of **1** (*Tables 1* and *2*) and **10**, revealed that the most critical differences centered on the δ(C) values of C(3), C(4), C(8), C(9), C(12), C(18), and C(20), and the δ(H) values of

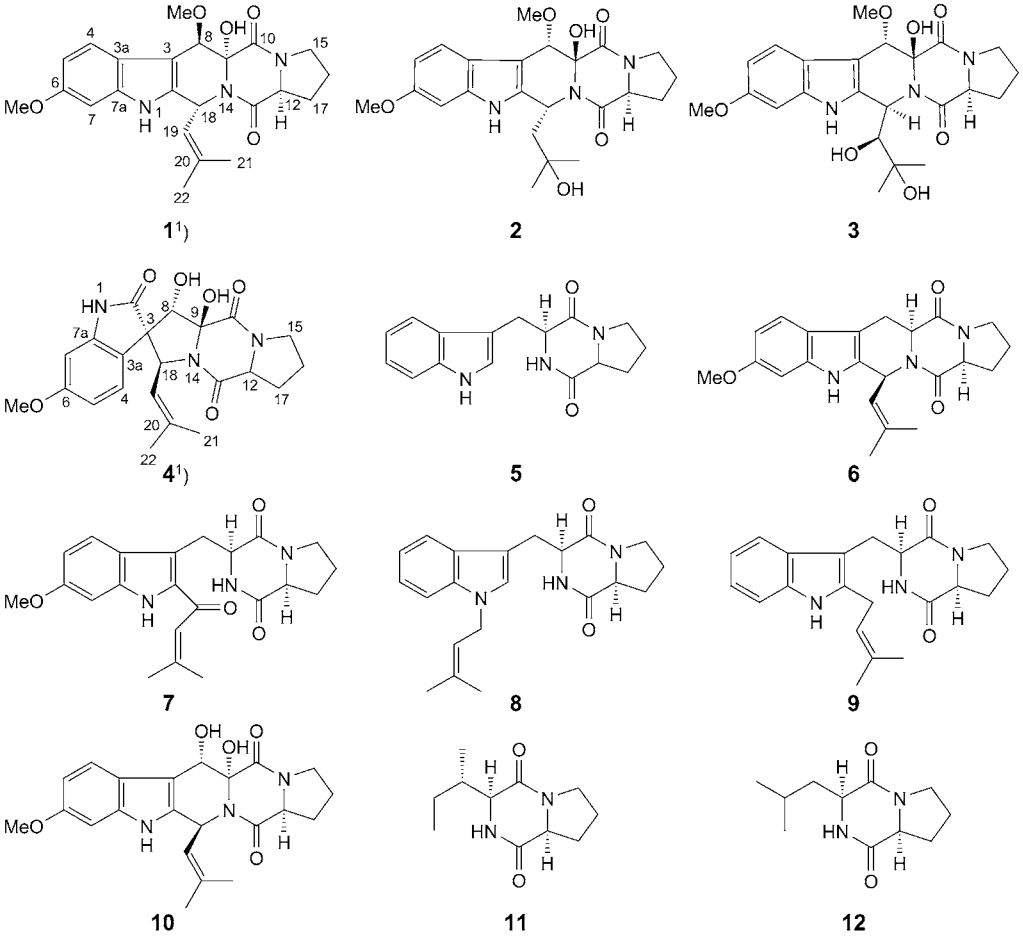

Fig. 1. *Compounds* **1**–**12** *produced by the endophytic fungus* Aspergillus funigatus

Table 1. *^{13}C-NMR Data* (125 MHz, $CDCl_3$) *of* **1**–**4**[1]). δ in ppm.

C-Atom	**1**	**2**	**3**	**4**
C(2)	133.6	135.1	129.3	181.4
C(3)	105.3	104.0	106.3	61.3
C(3a)	122.6	122.3	122.1	117.7
C(4)	118.6	118.4	118.3	127.2
C(5)	110.0	109.8	110.0	107.5
C(6)	156.4	156.2	156.4	160.6
C(7)	95.2	95.3	95.2	97.4
C(7a)	136.6	136.3	136.5	142.3
C(8)	77.0	76.8	76.2	75.4
C(9)	84.6	84.6	83.6	87.0
C(10)	165.8	165.9	165.1	165.1
C(12)	59.9	59.8	59.9	60.7
C(13)	167.0	166.8	167.2	168.9
C(15)	45.7	45.7	45.9	45.0
C(16)	22.0	22.0	22.0	23.2
C(17)	29.6	29.6	29.6	27.6
C(18)	49.0	47.5	53.2	57.3
C(19)	123.5	49.4	79.2	121.5
C(20)	137.8	71.3	73.1	139.0
C(21)	18.2	32.4	25.9	18.0
C(22)	26.0	28.7	29.0	25.2
*Me*O–C(6)	55.7	55.7	55.7	55.4
*Me*O–C(8)	56.5	56.6	56.7	

Table 2. *^{1}H-NMR Data* (500 MHz, $CDCl_3$) *of* **1**–**4**[1]). δ in ppm, J in Hz.

H-Atom	**1**	**2**	**3**	**4**
H–N(1)	7.84 (br. *s*)	9.60 (br. *s*)	9.99 (br. *s*)	8.43 (br. *s*)
H–C4	7.44 (*d*, *J* = 8.5)	7.42 (*d*, *J* = 9.0)	7.40 (*d*, *J* = 9.0)	6.96 (*d*, *J* = 8.5)
H–C5	6.82 (*dd*, *J* = 8.5, 2.0)	6.79 (*dd*, *J* = 8.5, 2.0)	6.80 (*dd*, *J* = 8.5, 2.0)	6.55 (*dd*, *J* = 8.5, 2.0)
H–C7	6.88 (*d*, *J* = 2.0)	6.87 (*d*, *J* = 2.0)	6.88 (*d*, *J* = 2.0)	6.43 (*d*, *J* = 2.0)
H–C8	4.73 (*s*)	4.70 (*s*)	4.71 (*s*)	4.82 (*s*)
H–C12	4.38 (*dd*, *J* = 15.5, 6.5)	4.38 (*dd*, *J* = 11.0, 6.0)	4.39 (*dd*, *J* = 10.5, 6.5)	4.62 (*t*, *J* = 8.5)
CH_2(15)	3.79, 3.71 (2*m*)	3.82, 3.70 (2*m*)	3.79, 3.67 (2*m*)	3.62, 3.62 (2*m*)
CH_2(16)	2.11, 1.98 (2*m*)	2.12, 1.97 (2*m*)	2.13, 2.02 (2*m*)	2.06, 2.04 (2*m*)
CH_2(17)	2.52, 1.99 (2*m*)	2.49, 1.99 (2*m*)	2.54, 2.05 (2*m*)	2.39, 2.14 (2*m*)
H–C(18)	6.65 (*d*, *J* = 9.5)	6.08 (*dd*, *J* = 8.0, 2.5)	6.28 (*d*, *J* = 12.5)	4.92 (*d*, *J* = 9.0)
H–C(19)	5.57 (*d*, *J* = 9.5)	2.03 (*dd*, *J* = 15.0, 3.0),	3.69 (*d*, *J* = 12.5)	4.89 (*d*, *J* = 9.0)
or CH_2(19)		2.33 (*dd*, *J* = 15.0, 8.0)		
Me(21)	2.05 (*s*)	1.33 (*s*)	1.61 (*s*)	1.10 (*s*)
Me(22)	1.79 (*s*)	1.55 (*s*)	1.42 (*s*)	1.60 (*s*)
MeO–C(6)	3.83 (*s*)	3.83 (*s*)	3.83 (*s*)	3.77 (*s*)
MeO–C(8)	3.36 (*s*)	3.35 (*s*)	3.32 (*s*)	
OH–C(9)	4.35 (br. *s*)	4.29 (br. *s*)		7.12 (br. *s*)

H–C(8), H–C(12), H–C(18), and H–C(19). These differences indicated that compound **1** had different relative configurations of the stereogenic centers at C(8) and C(18), compared to (8*S*)-8,9-dihydroxyfumitremorgin C (**10**). This deduction was

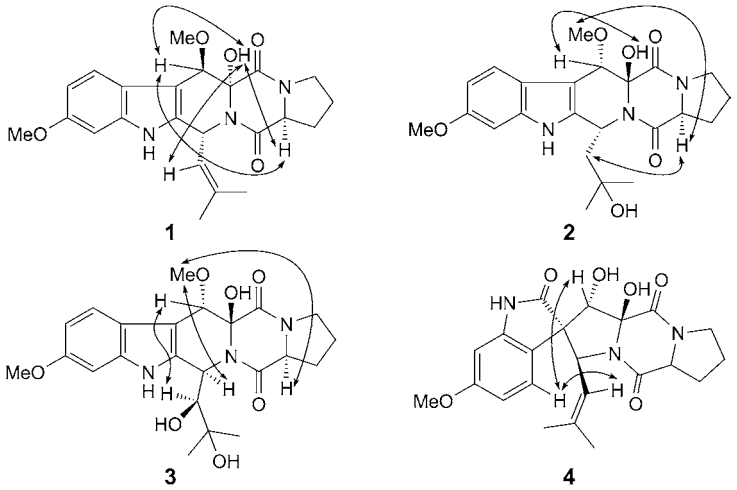

Fig. 2. *Key ROESY correlations for* **1**–**4**

confirmed by ROESY and NOE experiments. In the ROESY plot of **1**, correlations between OH–C(9) and H–C(12), between OH–C(9) and H–C(19), and between H–C(8) and H–C(12) were observed (*Fig. 2*). In addition, a NOE correlation H–C(8)/H–C(12) was observed in the NOE difference spectrum. Thus, the relative configuration of **1** was elucidated as shown in *Fig. 1*, and its structure was determined to be *rel*-(8*R*)-9-hydroxy-8-methoxy-18-*epi*-fumitremorgin C[1]).

The molecular formula of compound **2** was established as $C_{23}H_{29}N_3O_6$ according to the ion peak at *m*/*z* 466.1952 ($[M+Na]^+$) in the HR-ESI-MS and ^{13}C-NMR data. Its IR spectrum implied the presence of OH groups by the absorption band at 3339 cm^{-1}. The ^{13}C-NMR data of **2** (*Table 1*) showed that its structure was similar to that of **1**, except that the signals of the 2-methylprop-1-en-1-yl group of **1** were replaced by those ascribed to the 2-hydroxy-2-methylpropyl unit in **2** (*Fig. 1*). The difference indicated that compound **2** was a 19,20-hydrated derivative of **1**, which was confirmed by the HMBCs CH_2(19) (δ(H) 2.03 (*dd*))/C(2), C(18), C(20), C(21), and C(22), and Me(21) (δ(H) 1.33 (*s*))/C(20) and C(19). In addition, the relative configuration of **2** was elucidated by its ROESY experiment which revealed the correlations OH–C(9)/H–C(8), H–C(12)/MeO–C(8), and H–C(12)/CH_2(19) (*Fig. 2*). Accordingly, the structure of **2** was determined as *rel*-(8*S*)-19,20-dihydro-9,20-dihydroxy-8-methoxy-9,18-di-*epi*-fumitremorgin C[1]).

Compound **3** has the molecular formula $C_{23}H_{29}N_3O_7$ on the basis of the $[M+Na]^+$ ion peak at *m*/*z* 482.1905 in the HR-ESI-MS, and its molecular formula possessed only one O-atom more than that of **2**. Comparing the ^{13}C-NMR data of **3** with that of **2** (*Table 1*) revealed that the differences concerned mainly the δ(C) of C(18), C(19), C(20), and C(21). The ^{1}H-NMR data (*Table 2*) also showed that the signals at δ(H) 6.28 (*d*, H–C(18)) and δ(H) 3.69 (*d*, H–C(19)) of **3** were different from those at δ(H) 6.08 (*dd*, H–C(18)) and δ(H) 2.03, 2.33 (2*dd*, CH_2(19)) of **2**. These differences suggested that an additional OH group was located at C(19) in **3** (*Fig. 1*), which was

confirmed by the HMBCs H–C(19)/C(2), C(18), and C(22), and Me(22) (δ(H) 1.42)/C(19) and C(20). Additionally, the relative configuration of **3** was elucidated by a ROESY experiment which revealed the correlations H–C(19)/H–C(8), and MeO–C(8) (δ(H) 3.32)/H–C(12) and H–C(18) (*Fig. 2*). In the CD experiment of **3**, we used $[Mo_2(O_2CCF_3)_4]$ as an auxiliary to produce the induced CD spectrum [16], according to *Snatzke*'s method [17][18], which is applied well to determine the absolute configuration of the vicinal-diol moiety. On the basis of the positive *Cotton* effect observed at 300 nm, the absolute configuration at C(19) was established to be (*S*). Thus, the stucture of **3** was elucidated to be *rel*-(8*S*,19*S*)-19,20-dihydro-9,19,20-trihydroxy-8-methoxy-9-*epi*-fumitremorgin C[1]).

The molecular formula of compound **4** was established as $C_{22}H_{25}N_3O_6$ according to the ion peak at *m*/*z* 450.1641 ($[M+Na]^+$) in the HR-ESI-MS and ^{13}C-NMR data. Its IR spectrum implied the presence of OH groups on the basis of the absorption band at 3235 cm^{-1}. The ^{13}C-NMR data of **4** (*Table 1*) was similar to that of spirotryprostatin A (=(2*S*,3*S*,5a*S*,10a*S*)-1,5a,6,7,8,10a-hexahydro-6′-methoxy-3-(2-methylprop-1-en-1-yl)-spiro[5*H*,10*H*-dipyrrolo[1,2-*a*:1′,2′-*d*]pyrazine-2(3*H*),3′-[3*H*]indole]-2′,5,10(1′*H*)-trione) [19], except for the resonances of C(8) (δ(C) 75.4) and C(9) (δ(C) 87.0) which were shifted downfield in **4** by 41.1 and 28.5 ppm, respectively (*Fig. 1*). These results suggested that an additional OH group was located at C(8) of **4**, which was confirmed by the HMBC H–C(8)/C(18). The other additional OH group was located at C(9) of **4**, which was demonstrated by the presence of the signal at δ(H) 7.12 (br. *s*, OH–C(9)) and the disappearance of the signal at δ(H) 4.99 (*dd*, H–C(9)) of spirotryprostatin A. In addition, the relative configuration of **4** was elucidated by its ROESY experiment which revealed the correlations H–C(4)/H–C(8) and H–C(4)/H–C(19) (*Fig. 2*).

Based on the structural feature of **4** having vicinal diol moiety, we used $[Mo_2(O_2CCF_3)_4]$ as an auxiliary to produce the induced CD spectrum of **4**. According to the positive *Cotton* effect observed at 300 nm, the absolute configurations at C(8) and C(9) were determined to be (8*S*,9*S*). Combined with the established relative configuration (see above), the absolute configurations at C(3) and C(18) were determined as (3*S*,18*S*). Therefore, the structure of **4** was identified as (3*S*,8*S*,9*S*,18*S*)-dihydroxyspirotryprostatins A. As no correlations for H–C(12) were observed in the ROESY experiment, the configuration at this center could not be determined, but it is assumed to be (*S*) on the basis of biogenetic considerations.

2. *Anti-inflammatory Activity.* The anti-inflammatory and cytotoxic activities of compounds **1**–**12** were evaluated. The anti-inflammatory activities were assessed by measuring the inhibitory ratio of β-glucuronidase release in rat polymorphonuclear leukocytes (PMNs) induced by the platelet-activating factor (PAF) *in vitro* [20], and the inhibitory ratios of compounds **1**, **2**, **4**–**9**, and **12** were 16.7, 19.6, 6.1, 17.1, 15.9, 11.5, 12.5, 17.2, and 20.2%, respectively, at a concentration of 10 μM; ginkgolide B was used as a positive control, with an inhibitory ratio of 80.5% at 10 μM. The result suggested that compound **12** showed a weak inhibitory activity of β-glucuronidase release from rat PMNs induced by PAF. In addition, the experimental results of cytotoxic activities showed that compounds **1**–**12** exhibited no detectable cytotoxicity (IC_{50} 10 μM) toward five human tumor cell lines (HCT-8, Bel-7402, BGC-823, A549, and A2780) in the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay.

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Experimental Part

General. $[Mo_2(O_2CCF_3)_4]$ was purchased from *Acros Ltd*. DMSO (HPLC grade) was purchased from *Beijing Chemical Company* (Beijing, P. R. China), and dried with 4-Å molecular sieves. $CHCl_3$ (HPLC grade) was purchased from *Beijing Chemical Company* (Beijing). Prep. HPLC: *YMC-Pack ODS-A* column (20 × 250 mm, S-5 μm, 12 nm; MeOH/H_2O or MeCN/H_2O). Column chromatography (CC): silica gel (SiO_2, 200–300 μm; *Qingdao Marine Chemical Inc.*); macroporous resin (*HP20*, *Mitsubishi Chemical Industries Ltd.*); *C18* reversed-phase silica gel (SiO_2, 40–75 μm; *Fuji Silysia Ltd.*); *Sephadex LH-20* (*Amersham Pharmacia Biotech AB Ltd.*). Optical rotations: *Perkin-Elmer 343* polarimeter. UV Spectra: *Thermo Spectronic* spectrometer; in MeOH; λ_{max} ($\Delta\varepsilon$) in nm. CD Spectra: *Jasco-2000* spectropolarimeter; in $CHCl_3$; $\lambda([\theta])$ in nm IR Spectra: *Nicolet-Impact-400* spectrometer; ν in cm^{-1}. ^{1}H-, ^{13}C-, and 2D-NMR Spectra: *Bruker-AV500-III* spectrometer; in $CDCl_3$; δ in ppm rel. to Me_4Si as internal standard, J in Hz. ESI-MS: *Agilent-1100-LC-MSD-Trap-SL* instrument; in m/z. HR-ESI-MS: *Agilent-6520-Accurate-MS-Q-TOF* instrument; in m/z.

Fungal Material. The fungus *Aspergillus fumigatus* was separated from the stem of *Erythrophloeum fordii* OLIV. by *J.-G. D.* and identified by Dr. *Xian-Zhi Jiang* of the Institute of Microbiology, Chinese Academy of Sciences.

Extraction and Isolation. The liquid fermentation was applied to a *HP20* macroporous resin (eluted with H_2O and 95% EtOH), and then the 95% EtOH fraction (670 g) was extracted with AcOEt and BuOH. The AcOEt extracts (63.0 g) were applied to CC (SiO_2 AcOEt/acetone): *Fractions* A_1–A_8. *Fr.* A_4 (5.1 g) was subjected to CC (*Sephadex LH-20* MeOH) *Fr.* A_4B_1 (0.78 g), *Fr.* A_4B_2 (3.10 g), and *Fr.* A_4B_3 (1.15 g). *Fr.* A_4B_2 was separated by CC (*ODS* MeOH/H_2O 3:7) and then further purified by prep. HPLC (ODS) to yield with MeCN/H_2O 15:85 **1** (39 mg), **2** (23 mg), and **4** (10 mg), and with MeCN/H_2O 33:67 **3** (14 mg), **6** (32 mg), **10** (97 mg), **11** (31 mg), and **12** (6 mg). *Fr.* A_5 (4.6 g) was applied to CC (*Sephadex LH-20*; MeOH): *Fr.* A_5B_1 (0.65 g), *Fr.* A_5B_2 (2.50 g), and *Fr.* A_5B_3 (1.20 g). *Fr.* A_5B_2 was separated by CC (ODS, MeOH/H_2O 40:60), and then further purified by prep. HPLC (MeCN/H_2O 1:9): **5** (102 mg), **7** (8 mg), **8** (126 mg), and **9** (6 mg).

rel-*(8*R*)-9-Hydroxy-8-methoxy-18*-epi-*fumitremorgin C* (= *rel-(5a*R,*6*R,*12*R,*14a*R*)-1,2,3,5a,6,11,12,14a-Octahydro-5a-hydroxy-6,9-dimethoxy-12-(2-methylprop-1-en-1-yl)-5*H,*14*H-*pyrrolo[1'',2'':4',5']pyrazino[1',2':1,6]pyrido[3,4-*b*]indole-5,14-dione*; **1**). White amorphous power. $[\alpha]_D^{20}$ = +455.56 (*c* = 0.54, MeOH). UV (MeOH): 216 (0.85), 231 (1.30), 260 (0.61), 295 (0.80). CD (MeOH): 213 (+5.2), 232 (+13.6), 266 (+8.0), 295 (+4.8). IR: 3639, 3452, 3300, 2962, 2920, 2820, 1665, 1627, 1569, 950, 820. ^{1}H-NMR: *Table 2*. ^{13}C-NMR: *Table 1*. ESI-MS (pos.): 448 ($[M+Na]^+$), 464 ($[M+K]^+$), 873 ($[2M+Na]^+$). ESI-MS (neg.): 424 ($[M-H]^-$), 460 ($[M+Cl]^-$). HR-ESI-MS: 448.1850 ($[M+Na]^+$, $C_{23}H_{27}N_3NaO_5^+$; calc. 448.1843).

rel-*(8*S*)-19,20-Dihydro-9,20-dihydroxy-8-methoxy-9-*epi-*fumitremorgin C*[1]) (= *rel-(5a*R,*6*R,*12*S,*14a*R*)-1,2,3,5a,6,11,12,14a-Octahydro-5a-hydroxy-12-(2-hydroxy-2-methylpropyl)-6,9-dimethoxy-5*H,*14*H-*pyrrolo[1'',2'':4',5']pyrazino[1',2':1,6]pyrido[3,4-*b*]indole-5,14-dione*; **2**). White amorphous power. $[\alpha]_D^{20}$ = +317.69 (*c* = 0.51, MeOH). UV (MeOH): 216 (0.87), 227 (1.06), 259 (0.32), 294 (0.34). CD (MeOH): 229 (+4.1), 266 (+2.8), 297 (+0.7). IR: 3339, 2970, 2892, 2831, 1663, 1572, 954, 830, 807. ^{1}H-NMR: *Table 2*. ^{13}C-NMR: *Table 1*. HR-ESI-MS: 466.1952 ($[M+Na]^+$, $C_{23}H_{29}N_3NaO_6^+$; calc. 466.1949).

rel-*(8*S*,19*S*)-19,20-Dihydro-9,19,20-trihydroxy-8-methoxy-9-*epi-*fumitremorgin C* (= *rel-(5a*R,*6*R,*12*R,*14a*R*)-12-[(1*R*)-1,2-Dihydroxy-2-methylpropyl]-1,2,3,5a,6,11,12,14a-octahydro-5a-hydroxy-6,9-dimethoxy-5*H,*14*H-*pyrrolo[1'',2'':4',5']pyrazino[1',2':1,6]pyrido[3,4-*b*]indole-5,14-dione*; **3**). White amorphous power. $[\alpha]_D^{20}$ = +339.92 (*c* = 0.51, MeOH). UV (MeOH): 215 (0.94), 229 (1.26), 260 (0.53), 294

(0.66). CD (MeOH): 205 (−11.6), 229 (+2.5), 266 (+2.9), 300 (+0.3). IR: 3661, 2975, 2943, 2894, 2832, 1666, 1569, 953, 821. ^{1}H-NMR: *Table 2*. ^{13}C-NMR: *Table 1*. ESI-MS (pos.): 482 ($[M+Na]^+$), 498 ($[M+K]^+$). ESI-MS (neg.): 458 ($[M-H]^-$), 494 ($[M+Cl]^-$). HR-ESI-MS: 482.1905 ($[M+Na]^+$, $C_{23}H_{29}N_3NaO_7^+$; calc. 482.1898).

*(3*S*,8*S*,9*S*,18*S*)-Dihydroxyspirotryprostatin A* (=*(1*S*,2*S*,3*S*,5a*S*,10a*S*)-1,5a,6,7,8,10a-Hexahydro-1,10a-dihydroxy-6-methoxy-3-(2-methylprop-1-en-yl)spiro[5*H*,10*H*-dipyrrolo[1,2-a:1',2'-d]pyrazine-2(3*H*),3'[3*H*]indole]-2',5,10(1'*H*)-trione;* **4**): White amorphous power. $[\alpha]_D^{20}$ = +204.43 (c = 0.49, MeOH). UV (MeOH): 215 (1.33), 227 (1.56), 259 (0.40), 278 (0.48), 303 (0.07). CD (MeOH): 211 (+3.7), 225 (−3.8), 240 (+2.4), 261 (−0.7), 289 (−1.5). IR: 3235, 2970, 1686, 1636, 1601, 946, 802. ^{1}H-NMR: *Table 2*. ^{13}C-NMR: *Table 1*. ESI-MS (pos.): 450 ($[M+Na]^+$), 466 ($[M+K]^+$). ESI-MS (neg.): 426 ($[M-H]^-$). HR-ESI-MS: 450.1641 ($[M+Na]^+$, $C_{22}H_{25}N_3NaO_6^+$, calc. 450.1636).

Absolute Configurations of the Vicinal-Diol Moiety of **3** *and* **4** *by the* Snatzke *Method* [17][18]. The Diol/$[Mo_2(O_2CCF_3)_4]$ 1:1 mixture of compound **3** or **4** was subjected to CD measurement, at the concentration of 0.275 or 0.250 mg/ml, resp. The CD spectra of the Mo complexes were measured 30 min after mixing. The CD spectra of the compounds were subtracted from those of the Mo complexes, and the induced CD spectra of the compounds were obtained. The observed sign of the band at 300 nm was related to the absolute configuration of the vicinal-diol moiety of **3** or **4**.

Anti-inflammatory Activity. Based on reported procedures [20], the anti-inflammatory activities of compounds **1**–**12** were assessed by measuring the inhibitory ratio of β-glucuronidase release in rat polymorphonuclear leukocytes (PMNs) induced by the platelet-activating factor (PAF) *in vitro*. The tested compounds were dissoloved in DMSO at a concentration of 0.1 M and diluted with RPMI-1640 to 10^{-3} mol/l when used. The suspension of rat PMNs (245 µl) at a density of $2.5 \cdot 10^6$ cells ml^{-1} and test samples (2.5 µl) were incubated at 37° for 15 min and for another 5 min after the addition of 1 mM cytochalasin B (2.5 µl). Subsequently 2.5 µl of 0.2 µM PAF was added. The reaction was terminated in an ice bath after 10 min. The supernatant was obtained by centrifugation at 4000 rpm for 5 min. Then, 25 µl of supernatant and 2.5 mM of phenolphthalein glucuronic acid (=4-[1,3-dihydro-1-(4-hydroxyphenyl)-3-oxo-1-isobenzofuranyl]phenyl β-D-glucopyranosiduronic acid; 25 µl) were incubated with 0.1M AcOH buffer (pH 4.6; 100 µl) at 37° under 5% CO_2 for 18 h. The reaction was completed on addition of 0.3M NaOH (150 µl). The absorbance was read at 550 nm, and the inhibitory ratio (*IR*) was calculated as follows: $IR\,[\%] = (A_{PAF} - A_t)/(A_{PAF} - A_C) \times 100\%$, where A_{PAF}, A_t, A_{PAF}, and A_C refer to the cell level of PAF, test compounds, and control groups, resp.; ginkgolide B was used as the positive control.

REFERENCES

[1] China Flora Editing Group of China Science Academy, 'Flora Reipublicae Popularis Sinicae', Science Press, Beijing, 1997, Vol. 39, pp. 117–119.
[2] J. Qu, Y.-C. Hu, S.-S. Yu, X.-G. Chen, Y. Li, *Planta Med.* **2006**, *72*, 442.
[3] J. Qu, Y.-H. Wang, J.-B. Li, S.-S. Yu, Y. Li, Y.-B. Liu, *Rapid Commun. Mass Spectrom.* **2007**, *21*, 2109.
[4] N. Li, F. Yu, S. S. Yu, *Acta Bot. Sin.* **2004**, *46*, 371.
[5] F. Yu, N. Li, S.-S. Yu, *J. Asian Nat. Prod. Res.* **2005**, *7*, 19.
[6] D. Du, J. Qu, J.-M. Wang, S.-S. Yu, X.-G. Chen, S. Xu, S.-G. Ma, Y. Li, G.-Z. Ding, L. Fang, *Phytochemistry* **2010**, *71*, 1749.
[7] W. J. Han, X. L. Lu, Q. Z. Xu, X. Y. Liu, B. H. Jiao, *Acad. J. Second Military Med. Univ.* **2008**, *29*, 1234.
[8] D. H. Zhang, N. Dedi, N. Muhammad, X. D. Yang, S. Wha, *Nat. Prod. Sci.* **2007**, *13*, 251.
[9] M. Zhang, W.-L. Wang, Y.-C. Fang, T.-J. Zhu, Q.-Q. Gu, W.-M. Zhu, *J. Nat. Prod.* **2008**, *71*, 985.
[10] W. Y. Zhao, Y. P. Zhang, T. J. Zhu, Y. C. Fang, H. B. Liu, Q. Q. Gu, W. M. Zhu, *J. Chin. Antibiot.* **2006**, *31*, 749–752, 764.
[11] B. Wang, L. Chen, K. Kim, *Tetrahedron Lett.* **2001**, *42*, 1463.
[12] J. M. Schkeryantz, J. C. G. Woo, P. Siliphaivanh, K. M. Depew, S. J. Danishefsky, *J. Am. Chem. Soc.* **1999**, *121*, 11964.

[13] Y. Takaya, T. Furukawa, S. Miura, T. Akutagawa, Y. Hotta, N. Shikawa, M. Niwa, *J. Agric. Food Chem.* **2007**, *55*, 75.
[14] M. Adamczeski, A. R. Reed, P. Crews, *J. Nat. Prod.* **1995**, *58*, 201.
[15] W.-R. Abraham, H.-A. Arfmann, *Phytochemistry* **1990**, *29*, 1025.
[16] J. Frelek, A. Klimek, P. Ruskowska, *Curr. Org. Chem.* **2003**, *7*, 1081.
[17] L. D. Bari, G. Pescitelli, C. Pratelli, D. Pini, P. Salvadori, *J. Org. Chem.* **2001**, *66*, 4819.
[18] J. Frelek, G. Snatzke, *Fresenius' J. Anal. Chem.* **1983**, *316*, 261.
[19] C.-B. Cui, H. Kakeya, H. Osada, *Tetrahedron* **1996**, *52*, 12651.
[20] D.-M. Su, Y.-H. Wang, S.-S. Yu, D.-Q. Yu, Y.-C. Hu, W.-Z. Tang, G.-T. Liu, W.-J. Wang, *Chem. Biodiversity* **2007**, 4, 2852.

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