Zheng, J. K. et al. Cyclic tripeptides from the halotolerant fungus Aspergillus sclerotiorum PT06-1. J. Nat. Prod. 73, 1133-1137

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · JUNE 2010

Impact Factor: 3.8 · DOI: 10.1021/np100198h · Source: PubMed

Cyclic Tripeptides from the Halotolerant Fungus *Aspergillus sclerotiorum* PT06-1

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Received March 23, 2010

Eleven new aspochracin-type cyclic tripeptides, sclerotiotides A−K (**1**−**11**), together with three known compounds, JBIR-15 (**12**), aspochracin (**13**), and penicillic acid, were isolated from the ethyl acetate extract of the fermentation broth of the halotolerant *Aspergillus sclerotiorum* PT06-1 in a hypersaline nutrient-rich medium. Their structures were elucidated by spectroscopic analysis and chemical methods. Chemical transformations of **12** and **13** proved that sclerotiotides D−K (**4**−**11**) were artifacts probably formed during the fermentation or subsequent isolation steps. All 13 cyclic tripeptides have been evaluated for their antimicrobial and cytotoxic effects. Only sclerotiotides A (**1**), B (**2**), F (**6**), and I (**9**) and JBIR-15 (**12**) showed selective antifungal activity against *Candida albicans* with MIC values of 7.5, 3.8, 30, 6.7, and 30 μM, respectively.

Hypersaline environments might induce biosynthetic pathways of halotolerant microbes to produce structurally unique compounds.[1,2] In our previous studies, novel alkaloids were identified as secondary metabolites of halotolerant microorganisms.[3−5] Recently, two novel cyclic hexapeptides, sclerotides A and B, with antibiotic activity were obtained from the marine-derived halotolerant fungus *Aspergillus sclerotiorum* PT06-1 in a hypersaline nutrient-limited medium.[6] In order to further explore the effects of high-salt stress on the production of secondary metabolites of this strain, *A. sclerotiorum* PT06-1 was cultured in a nutrient-rich medium with 10% salt concentration. The fermentation broth exhibited distinct TLC and HPLC profiles from those in the nutrient-limited medium (Figure 1). Chemical investigation resulted in the identification of 11 new aspochracin-type cyclic tripeptides, sclerotiotides A−K (**1**−**11**), and three known compounds, JBIR-15 (**12**),[7] aspochracin (**13**),[8] and penicillic acid.[9] Chemical transformations of **12** and **13** proved that sclerotiotides D−K (**4**−**11**) were artifacts probably formed during the fermentation or subsequent isolation steps. The chemical diversity of these cyclic tripeptides was represented in the unsaturated fatty acid side chain, the constitution of amino acids, and *N*-methyl substitution in the amino acid moieties. These compounds did not show cytotoxicity against HL-60 and A549 cell lines, but selectively inhibited the growth of *Candida albicans* with MIC values of 7.5, 3.8, 30, 6.7, and 30 μM for sclerotiotides A (**1**), B (**2**), F (**6**), and I (**9**) and JBIR-15 (**12**), respectively. In addition, the ^{13}C NMR data of aspochracin (**13**) are reported here for the first time.

Sclerotiotide A (**1**), a pale yellow powder, had a molecular formula of $C_{22}H_{34}N_4O_4$ from the $[M + H]^+$ ion peak at *m*/*z* 419.2664 in the HRESIMS spectrum. A UV absorption at λ_{max} 296 nm revealed the presence of a conjugated substructure. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) of **1** showed four amide carbonyls and one *N*-methyl, indicating its peptide nature. Three characteristic α-methine signals at $\delta_{H/C}$ 4.77/55.9, 4.59/54.2, and 4.48/53.0 further supported a tripeptide structure. Except for the lack of an *N*-methyl group, the 1D NMR spectrum of **1** was similar to that of aspochracin (**13**),[8] indicating **1** as an analogue of **13**. The HMBC correlations (Figure 2) between *N*-Me (δ 2.96) and C-2 and C-4 indicated that the *N*-methyl was still present at the alanine residue, while the *N*-methyl group in the valine residue was absent. The absolute configurations of the amino acid residues of **1** were determined by Marfey's method.[10] HPLC analyses of derivatives of the hydrolysates with authentic samples (co-injection) revealed that the amino acids were L-*N*Me-Ala, L-Val, and L-Orn (Figure S31). Thus, sclerotiotide A (**1**) was thus elucidated as (2*E*,4*E*,6*E*)-*cyclo*-[(*N*Me-L-Ala)-L-Val-(N_α-octa-2,4,6-trienoyl-L-Orn)].

The molecular formula of sclerotiotide B (**2**) was determined as $C_{21}H_{34}N_4O_4$ on the basis of the molecular ion peak $[M + H]^+$ at *m*/*z* 407.2646 in the HRESIMS spectrum. Except for the fatty acid side chain, the ^{1}H NMR spectrum of **2** was similar to that of the known aspochracin (**13**).[8] There are only two double bonds in the side chain of **2** and both are *E* on the basis of the large coupling constants (J = 15.3, 15.0 Hz). Amino acid analysis disclosed L-*N*Me-Ala, L-*N*Me-Val, and L-Orn (Figures S29 and S30).[10] Sclerotiotide B (**2**) was thus elucidated as (2*E*,4*E*)-*cyclo*-[(*N*Me-L-Ala)-(*N*Me-L-Val)-(N_α-hexa-2,4-dienoyl-L-Orn)].

The molecular formula of sclerotiotide C (**3**) was deduced as $C_{24}H_{38}N_4O_4$ on the basis of the HRESIMS data, corresponding to an extra CH_2 compared to **13**. However, the chemical shifts of the ornithine unit in **13** were not duplicated in the spectra of **3**. These data implied that the ornithine unit in **13** was replaced by a lysine unit in **3**. Furthermore, the acidic hydrolysis experiment established the presence of L-Lys, L-*N*Me-Ala, and L-*N*Me-Val (Figure S32).[10] Therefore, sclerotiotide C (**3**) was identified as (2*E*,4*E*,6*E*)-*cyclo*-[(*N*Me-L-Ala)-(*N*Me-L-Val)-(N_α-octa-2,4,6-trienoyl-L-Lys)].

Sclerotiotides D (**4**) and E (**5**) were assigned the molecular formulas $C_{22}H_{34}N_4O_4$ and $C_{23}H_{36}N_4O_4$, respectively, on the basis of the HRESIMS data, indicating **4** and **5** as isomers of **12** and **13**. The NMR differences between **4** and **12** and between **5** and **13** were in the terminal double-bond region of the side chain, indicating that they were pairs of geometric isomers. The smaller $^3J_{(H6'-H7')}$ values of 11.4 Hz in **4** and 10.8 Hz in **5** indicated that they were the 6′-*Z* isomers of **12** and **13**, respectively. The deductions were confirmed by photoisomerization of **12** and **13** to **4** and **5**, respectively (Figures 3 and S34). Accordingly, sclerotiotides D (**4**) and E (**5**) were determined as (2*E*,4*E*,6*Z*)-*cyclo*-[L-Ala-(*N*Me-L-Val)-(N_α-octa-2,4,6-trienoyl-L-Orn)] and (2*E*,4*E*,6*Z*)-*cyclo*-[(*N*Me-L-Ala)-(*N*Me-L-Val)-(N_α-octa-2,4,6-trienoyl-L-Orn)], respectively.

The HRESIMS peak of sclerotiotide F (**6**) at *m*/*z* 421.2455 $[M + H]^+$ corresponded to the molecular formula $C_{21}H_{32}N_4O_5$. The differences in the NMR data from **13** occurred in the fatty acid side chain. An aldehyde signal at $\delta_{H/C}$ 9.62 (d, 8.2)/194.6 was present instead of a propenyl group in **13**, suggesting that the side chain of **6** was (2*E*,4*E*)-6-oxohexa-2,4-dienoic acid. The molecular formula of sclerotiotide G (**7**) was deduced as $C_{23}H_{36}N_4O_6$ from

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10.1021/np100198h
Published on Web 05/26/2010

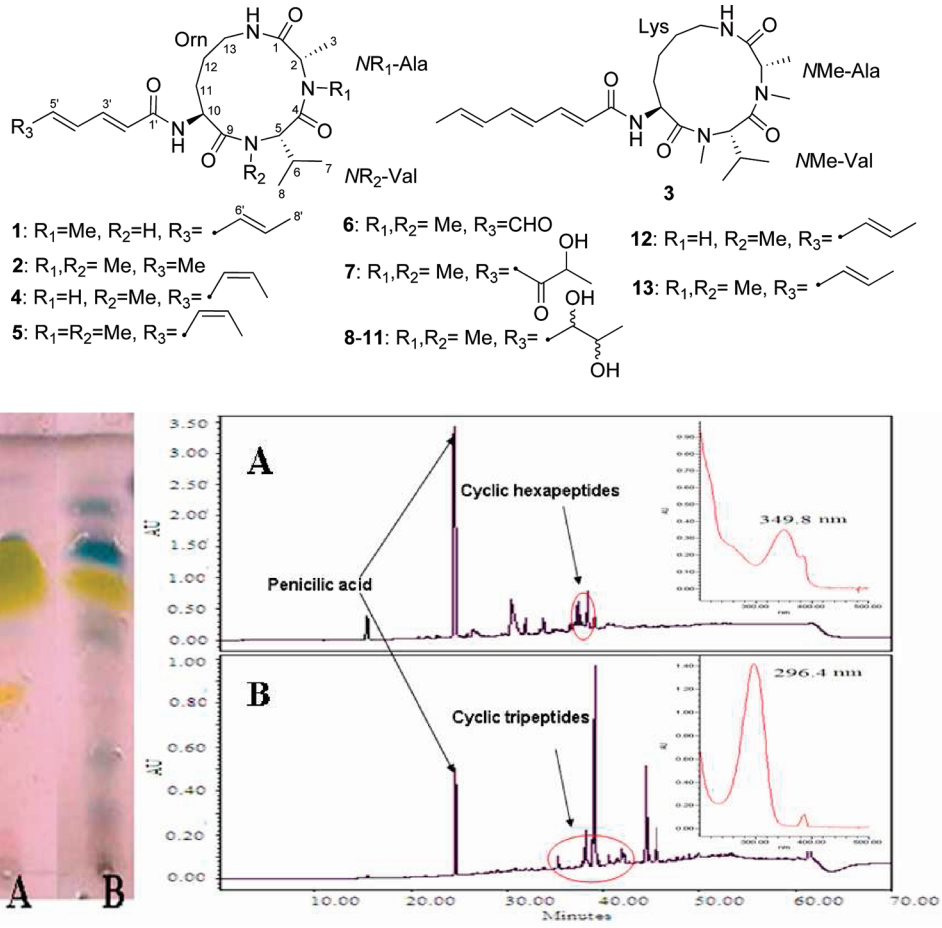

Figure 1. TLC and HPLC-UV profiles of secondary metabolites of *A. sclerotiorum* PT06-1 in hypersaline nutrient-limited medium (A) and hypersaline nutrient-rich medium (B).

the HRESIMS at *m*/*z* 465.2713 $[M + H]^+$. The UV absorptions at λ_{max} 216 and 275 nm were very similar to those for **6**, and the NMR spectra of **7** were almost the same as those of **6** except for the absence of the aldehyde signal in the side chain. Instead, an α,β-unsaturated carbonyl signal at δ_C 201.9 and a $-CH(OH)-CH_3$ spin system were displayed. When exposed to the air for 10 days, **13** produced **6** and **7** (Figures 3 and S34). The structures of sclerotiotides F (**6**) and G (**7**) were thus determined as (2*E*,4*E*)-*cyclo*-[(*N*Me-L-Ala)-(*N*Me-L-Val)-(N_α-6-oxohexa-2,4-dienoyl-L-Orn)] and (2*E*,4*E*)-*cyclo*-[(*N*Me-L-Ala)-(*N*Me-L-Val)-(N_α-7-hydroxy-6-oxoocta-2,4-dienoyl-L-Orn)], respectively.

Sclerotiotides H−K (**8**−**11**) were four isomers with the same molecular formula, $C_{23}H_{38}N_4O_6$, and the NMR data closely related to those of **7**. The only difference of the NMR data from those of **7** was a hydroxymethine instead of the carbonyl in the side chain. It is interesting that the NMR data of **8** and **9** were identical to those of **10** and **11**, respectively, suggesting that **8** and **10**, and **9** and **11**, were enantiotopic in the fatty acid moiety. The small $^3J_{(H6',H7')}$ of **8**−**11** (Table 1) indicated that all four compounds displayed *gauche*-conformations in the side chain.[11] The downfield shift of CH_3-8′ ($\delta_{H/C}$ 1.02/19.2) in **8** and **10** indicated the *threo*-configuration, whereas the upfield shift of CH_3-8′ ($\delta_{H/C}$ 0.95/18.2) in **9** and **11** indicated the *erythro*-configuration due to the steric hindrance between the methyl and the 5-amino-5-oxo-pentadienyl group.[12,13] In addition, **8**−**11** were produced by air oxidation of **13** (Figures 3 and S34). The structures of sclerotiotides H (**8**) and J (**10**), and I (**9**) and K (**11**), were therefore determined as *threo*- and *erythro*-(2*E*,4*E*)-*cyclo*-[(*N*Me-L-Ala)-(*N*Me-L-Val)-(N_α-6,7-dihydroxyocta-2,4-dienoyl-L-Orn)], respectively.

Compounds **1**−**13** were tested for cytotoxic effects on the HL-60 cell line using the MTT method[15] and on the A549 cell line using the SRB method.[16] The antimicrobial activities against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* were also evaluated by an agar dilution method.[17] Sclerotiotides A (**1**), B (**2**), F (**6**), and I (**9**) and JBIR-15 (**12**) showed selective antifungal activities against *C. albicans* with MIC values of 7.5, 3.8, 30, 6.7, and 30 μM, respectively, while no cytotoxicity nor antibacterial activities were observed.

Sclerotiotides A−K (**1**−**11**) belong to aspochracin-type cyclic tripeptides. So far, only three of these compounds have been reported in the literature.[7,8] It was proved in this paper that **4** and **5** could be formed from **12** and **13**, respectively, via a radical reaction[14] initiated by direct photoisomerization, while **6**−**11** could result from the air oxidation of **13** (Figure 3). Therefore, sclerotiotides D−K (**4**−**11**) were artifacts probably formed during the fermentation or subsequent isolation steps. It was also proved that the nutrients, especially nitrogen and carbon sources in a culture medium, affect the microbial secondary metabolites. Cyclic tripeptides were synthesized in the nutrient-rich medium, while cyclic hexapeptides were synthesized in the nutrient-limited medium by *A. sclerotiorum* PT06-1.

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Beckman DU 640 spectrophotometer. ^1H and ^{13}C NMR, DEPT, and 2D-NMR spectra were recorded on a JEOL JNM-ECP 600 spectrometer using TMS as internal standard, and chemical shifts were recorded as δ-values. ESIMS were measured on a Q-TOF Ultima Global GAA076 LC mass spectrometer. TLC and column chromatography (CC) were performed on plates precoated with silica gel GF_{254} (10−40 μm) and over silica gel (200−300 mesh, Qingdao Marine Chemical Factory)

Table 1. ^{1}H NMR Data for Compounds **1**−**11** (600 MHz, DMSO-d_6, TMS, δ ppm, J in Hz)

no.	1[a]	2	3[b]	4	5	6	7	8	9	10	11
Ala 2	4.77, q (6.9)	4.50, q (6.9)	4.42, q (6.9)	4.05, m	4.49, q (6.9)	4.52, q (7.3)	4.51, q (6.9)	4.50, q (7.1)	4.50, q (7.1)	4.50, q (7.1)	4.50, q (7.1)
3	1.52, d (6.9)	1.38, d (6.9)	1.43, d (7.3)	1.23, d (7.3)	1.37, d (6.9)	1.38, d (7.3)	1.39, d (6.8)	1.39, d (7.1)	1.39, d (7.1)	1.39, d (7.1)	1.39, d (7.1)
N-R_1	2.96, s	2.84, s	2.79, s	7.66, d (8.8)	2.83, s	2.84, s	2.85, s	2.84, s	2.84, s	2.84, s	2.84, s
Val 5	4.59, d (9.5)	4.97, d (10.2)	4.87, d (10.6)	4.81, d (10.6)	4.97, d (10.3)	4.99, d (10.1)	4.98, d (10.5)	4.98, d (9.9)	4.98, d (10.4)	4.98, d (9.9)	4.98, d (10.4)
6	2.21, m	2.21, m	2.24, m	2.16, m	2.21, m	2.22, m	2.22, m	2.22, m	2.22, m	2.22, m	2.22, m
7	0.89, d (6.4)	0.80, d (6.2)	0.81, d (6.2)	0.81, d (6.4)	0.79, d (5.9)	0.80, d (6.4)	0.80, d (6.4)	0.80, d (6.1)	0.80, d (6.1)	0.80, d (6.1)	0.80, d (6.6)
8	0.88, d (6.9)	0.63, d (6.5)	0.66, d (7.0)	0.63, d (6.9)	0.62, d (6.2)	0.64, d (6.4)	0.64, d (6.4)	0.63, d (7.1)	0.63, d (6.6)	0.63, d (7.1)	0.63, d (7.1)
N-R_2		2.84, s	2.73, s	2.88, s	2.83, s	2.84, s	2.85, s	2.84, s	2.84, s	2.84, s	2.84, s
Orn 10	4.48, d (5.5)	4.70, t (8.0)	4.88, overlap	4.73, t (7.8)	4.70, t (7.7)	4.73, dt (2.3, 8.2)	4.73, dt (1.8, 7.7)	4.72, t (8.5)	4.72, t (8.3)	4.72, t (8.3)	4.72, t (8.3)
10-NH		8.12, d (7.7)	8.11, d (8.0)	8.14, d (7.7)	8.14, d (7.7)	8.56, d (7.7)	8.47, d (7.3)	8.14, d (7.7)	8.15, d (7.7)	8.15, d (8.2)	8.14, d (7.7)
11	2.23, m; 1.64, m	1.95, m; 1.69, m	1.82, m; 1.67, m	1.95, m;1.67, m	1.94, m; 1.67, m	1.97, m;1.70, m	1.97, m; 1.70, m	1.96, m; 1.69, m	1.96, m; 1.68, m	1.96, m; 1.69, m	1.96, m; 1.68, m
12	1.67, m; 1.58, m	1.63, m; 1.47, m	1.25−1.65, m	1.69, m; 1.44, m	1.62, m; 1.45, m	1.63, m; 1.47, m	1.63, m; 1.47, m	1.63, m; 1.47, m	1.63, m; 1.47, m	1.63, m; 1.47, m	1.63, m; 1.47, m
13	3.36, m; 3.00, m	3.04, m; 2.84, m	1.25−1.65, m	3.05, m; 2.83, m	3.02, m; 2.83, m	3.01, m; 2.89, m	3.02, m; 2.89, m	3.04, m; 2.87, m	3.04, m; 2.86, m	3.04, m; 2.87, m	3.04, m; 2.86, m
13-NH		7.50, t (6.0)	7.44, q like (3.7)[c]	7.59, t (6.6)	7.49, t (6.0)	7.51, t (6.2)	7.52, t (6.0)	7.50, t (6.1)	7.50, t (6.1)	7.51, t (6.1)	7.50, t (6.1)
fatty acid 2′	5.92, d (14.6)	6.07, d (15.0)	6.15, d (14.6)	6.20, d (15.3)	6.18, d (15.0)	6.70, d (15.1)	6.88, d (14.6)	6.15, d (14.9)	6.15, d (14.9)	6.16, d (15.4)	6.15, d (14.8)
3′	7.21, dd (11.4,14.6)	6.97, dd (11.0, 15.0)	7.03, dd (11.0, 14.6)	7.12, dd (11.6, 15.3)	7.09, dd (11.4, 15.0)	7.26, dd (11.5, 15.1)	7.22, dd (11.5, 14.6)	7.00, dd (11.0, 14.9)	7.01, dd (11.0, 14.9)	7.01, dd (11.0, 15.4)	7.01, dd (11.0, 14.8)
4′	6.18, dd (11.4, 14.6)	6.17, dd (11.0, 15.0)	6.23, dd (11.0, 15.0)	6.32, dd (11.6, 14.9)	6.31, dd (11.4, 14.6)	7.42, dd (11.5, 15.1)	7.15, dd (11.5, 14.7)	6.28, dd (11.0, 14.9)	6.31, dd (11.0, 14.9)	6.29, dd (11.0, 15.6)	6.31, dd (11.0, 14.9)
5′	6.51, dd (10.6, 14.6)	6.09, m	6.56, dd (10.8, 15.0)	6.93, dd (11.4, 14.9)	6.90, dd (10.8, 14.6)	6.48, dd (8.2, 15.1)	6.64, d (14.7)	6.16, dd (5.5, 14.9)	6.12, dd (5.5,14.9)	6.16, dd (5.1, 15.6)	6.12, dd (5.0, 14.9)
6′	6.14, dd (11.2, 14.9)	1.79, d (6.6)	6.19, dd (10.8, 15.0)	6.13, t (11.4)	6.12, t (10.8)	9.62, d (8.2)		3.84, ‘q’ like (4.9,5.1)	3.93, ‘q’ like (4.4,4.7)	3.84, ‘q’ like (4.9,5.1)	3.93, ‘q’ like (4.4,4.7)
7′	5.90, dq (6.6, 14.9)		5.90, dq (6.6, 15.0)	5.67, dq (6.4, 11.4)	5.66, dq (7.3, 10.8)		4.25, dq (5.0, 6.9)	3.47, m	3.54, m	3.47, m	3.54, m
8′	1.82, d (6.6)		1.78, d (6.6)	1.78, d (6.4)	1.77, d (7.3)		1.19, d (6.9)	1.02, d (6.6)	0.95, d (6.0)	1.02, d (6.1)	0.95, d (6.6)
6′-OH								4.92, d (4.9)	4.95, d (4.4)	4.94, d (3.3)	4.95, d (3.8)
7′-OH							5.43, d (5.0)	4.55, d (4.9)	4.61, d (3.8)	4.58, d (4.9)	4.61, d (3.3)

[a] Recorded in a 1:1 mixture of $CDCl_3$ and CD_3OD. [b] CH_2-14 as δ 3.46, m; δ 2.82, m. [c] 14-NH.

Table 2. ^{13}C NMR Data for Compounds **1**−**11** and **13** (150 MHz, DMSO-d_6, δ ppm)

no.	1	2	3[b]	4	5	6	7	8	9	10	11	13
Ala 1	171.2, C	170.6, C	170.5, C	172.0, C	170.7, C	170.6, C	170.6, C	170.6, C	170.7, C	170.7, C	170.7, C	170.6, C
2	55.9, CH	54.4, CH	55.0, CH	50.8, CH	54.4, CH	54.5, CH	54.5, CH	54.4, CH	54.4, CH	54.4, CH	54.4, CH	54.4, CH
3	15.7, CH_3	16.2, CH_3	16.1, CH_3	18.7, CH_3	16.3, CH_3	16.3, CH_3	16.2, CH_3	16.3, CH_3	16.3, CH_3	16.3, CH_3	16.3, CH_3	16.3, CH_3
N-R_1	29.3, CH_3	29.7, CH_3	30.2, CH_3		29.7, CH_3	29.6, CH_3	29.6, CH_3	29.7, CH_3	29.7, CH_3	29.7, CH_3	29.7, CH_3	29.7, CH_3
Val 4	171.3, C	169.1, C	169.5, C	170.0, C	169.1, C	169.1, C	169.0, C	169.1, C	169.1, C	169.1, C	169.1, C	169.1, C
5	54.2, CH	57.5, CH	58.2, CH	57.0, CH	57.6, CH	57.6, CH	57.5, CH	57.6, CH	57.6, CH	57.6, CH	57.6, CH	57.6, CH
6	30.1, CH	26.3, CH	27.2, CH	25.9, CH	26.4, CH	26.4, CH	26.3, CH	26.4, CH	26.4, CH	26.4, CH	26.4, CH	26.4, CH
7	19.6, CH_3	19.8, CH_3	20.4, CH_3	19.6, CH_3	19.8, CH_3	19.8, CH_3	19.7, CH_3	19.8, CH_3	19.8, CH_3	19.9, CH_3	19.8, CH_3	19.8, CH_3
8	18.3, CH_3	17.6 CH_3	18.3, CH_3	17.7, CH_3	17.7 CH_3	17.7, CH_3	17.6, CH_3	17.7, CH_3	17.7, CH_3	17.7, CH_3	17.7, CH_3	17.7 CH_3
N-R_2		29.6, CH_3	30.0, CH_3	29.7, CH_3	29.6, CH_3	29.7, CH_3	29.7, CH_3	29.6, CH_3	29.6, CH_3	29.6, CH_3	29.6, CH_3	29.6, CH_3
Orn 9	171.7, C	171.8, C	171.1, C	171.9, C	171.8, C	171.5, C	171.4, C	171.8, C	171.8, C	171.8, C	171.8, C	171.8, C
10	53.0, CH	49.4, CH	47.7, CH	49.3, CH	49.5, CH	49.7, CH	49.7, CH	49.4, CH	49.4, CH	49.4, CH	49.4, CH	49.4, CH
11	29.6, CH_2	28.1, CH_2	28.3, CH_2	28.1, CH_2	28.1, CH_2	27.9, CH_2	27.8, CH_2	28.1, CH_2	28.1, CH_2	28.1, CH_2	28.1, CH_2	28.1, CH_2
12	22.1, CH_2	22.8, CH_2	18.6, CH_2	22.6, CH_2	22.8, CH_2	22.7, CH_2	22.6, CH_2	22.8, CH_2	22.8, CH_2	22.8, CH_2	22.8, CH_2	22.8, CH_2
13	39.5, CH_2	39.3, CH_2	25.0, CH_2	39.3, CH_2	39.2, CH_2	39.2, CH_2	39.1, CH_2	39.3, CH_2	39.3, CH_2	39.3, CH_2	39.3, CH_2	39.2, CH_2
fatty acid 1′	165.9, C	164.4, C	165.0, C	164.3, C	164.3, C	163.3, C	163.0, C	164.3, C	164.3, C	164.3, C	164.3, C	164.3, C
2′	121.8, CH	122.6, CH	124.2, CH	124.5, CH	124.4, CH	133.5, CH	130.2, CH	123.8, CH	123.9, CH	123.9, CH	123.9, CH	123.9, CH
3′	141.7, CH	139.5, CH	140.1, CH	139.5, CH	139.6, CH	136.3, CH	136.8, CH	139.3, CH	139.2, CH	139.3, CH	139.4, CH	139.5, CH
4′	127.4, CH	129.9, CH	128.5, CH	130.2, CH	130.2, CH	149.4, CH	139.6, CH	127.4, CH	127.8, CH	127.5, CH	127.9, CH	128.1, CH
5′	140.4, CH	136.7, CH	139.6, CH	134.0, CH	134.0, CH	135.9, CH	133.0, CH	142.5, CH	141.6, CH	142.5, CH	141.6, CH	139.0, CH
6′	131.1, CH	18.2, CH_3	131.9, CH	129.2, CH	129.2, CH	194.6, CH	201.9, C	75.1, CH	74.7 CH	75.1, CH	74.6 CH	131.5, CH
7′	134.4, CH		133.8, CH	130.1, CH	130.1, CH		71.5, CH	69.7, CH	69.4, CH	69.7, CH	69.4, CH	133.3, CH
8′	18.0, CH_3		18.7, CH_3	13.6, CH_3	13.6, CH_3		19.5, CH_3	19.2, CH_3	18.3, CH_3	19.1, CH_3	18.2, CH_3	18.3, CH_3

[a] Recorded in a 1:1 mixture of $CDCl_3$ and CD_3OD. [b] Lys 9 instead of Orn 9, and CH_2-14 as 36.7 ppm.

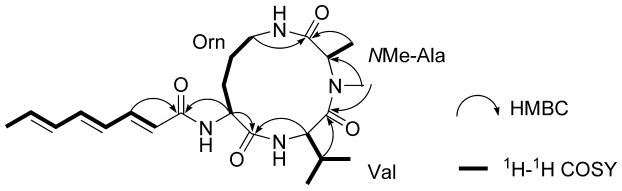

Figure 2. Selected two-dimensional NMR correlations for **1**.

and Sephadex LH-20 (Amersham Biosciences), respectively. Vacuum-liquid chromatography (VLC) was carried out over silica gel H (Qingdao Marine Chemical Factory). Semiprepartive HPLC was performed using an ODS column [Shin-pak ODS (H), 20 × 250 mm, 5 μm, 4 mL/min].

Fungal Material. *A. sclerotiorum* PT06-1 was isolated from salt sediments from the Putian Sea Salt Field, Fujian, China. It was identified according to its morphological characteristics and 18S rRNA sequences.[6] The voucher specimen is deposited in Dr. Zhu's laboratory at −80 °C. The producing strain was prepared on potato dextrose agar slants at 10% salt concentration and stored at 4 °C.

Fermentation and Extraction. *A. sclerotiorum* PT06-1 was incubated on a rotary shaker (160 rpm) at 28 °C for 16 days in 200 × 500 mL conical flasks containing liquid medium (150 mL/flask) composed of maltose (20 g/L), mannitol (20 g/L), monosodium glutamate (10 g/L), glucose (10 g/L), yeast extract (3 g/L), corn steep liquor (1 g/L), NaCl (80 g/L), $MgSO_4$ (5 g/L), KH_2PO_4 (5 g/L), NH_4Cl (5 g/L), KCl (5 g/L), and tap water after adjusting its pH to 7.0. The fermented whole broth (30 L) was filtered through cheesecloth to separate the supernatant from the mycelia. The former was concentrated *in vacuo* to about a quarter of the original volume and then extracted three times with EtOAc, while the latter was extracted three times with acetone. The acetone solution was evaporated under reduced pressure to afford an aqueous solution, which was then extracted three times with EtOAc. Both EtOAc solutions were combined and concentrated *in vacuo* to give an extract (50.2 g).

Purification. The extract (50.2 g) was subjected to vacuum-liquid chromatography on a silica gel column using step gradient elution with MeOH−$CHCl_3$ (0−100%). The collected materials were combined into six fractions based on TLC properties. Fraction 3 from the 50:1 $CHCl_3$−MeOH eluents was further separated on a Sephadex LH-20 column to give penicillic acid (402 mg). Fractions 4 and 5 were separated by ODS column chromatography (H_2O−MeOH gradient mixtures) into five subfractions, respectively. Subfraction 4-2 (206 mg), eluted with H_2O−MeOH (3:2), was separated by HPLC (35% MeOH) to yield compounds **6** (10 mg, t_R 15 min) and **7** (3 mg, t_R 16 min) and by HPLC (30% MeOH) to give **8** (8 mg, t_R 14 min), **9** (6 mg, t_R 16 min), **10** (8 mg, t_R 17 min), and **11** (13 mg, t_R 20 min). Further separation of Fr. 4.3 (3.2 g) and Fr. 5.3 (1.8 g) by CC (SiO_2; petroleum ether−AcOEt, 6:4) both afforded four subfractions. Fr. 4.3.3 (63 mg) was finally separated by HPLC (54% MeOH) to yield **4** (8 mg, t_R 14 min) and **12** (9 mg, t_R 15 min). Fr. 5.3.2 (447 mg) was separated by the same method to yield **5** (12 mg, t_R 18 min) and **13** (40 mg, t_R 19 min). Subfractions 4.4 and 5.4, eluted with H_2O−MeOH (1:4), were combined and purified by Sephadex LH-20 with $CHCl_3$−MeOH (1:1) and then HPLC with 60% aqueous MeOH to give **2** (4 mg, t_R 8 min), **1** (12 mg, t_R 13 min), and **3** (4 mg, t_R 18 min). All compounds were stored under nitrogen gas to avoid oxidation, and the vials were wrapped with foil to prevent exposure to light.

Sclerotiotide A (1): pale yellow, amorphous powder; $[\alpha]^{25}_D$ −44 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.9), 296 (4.3) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 419.2664 [M + H]$^+$ (calcd for $C_{22}H_{35}N_4O_4$, 419.2658).

Sclerotiotide B (2): pale yellow, amorphous powder; $[\alpha]^{25}_D$ −67 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.9), 259 (4.2) nm; ^{1}H NMR data, see Table 1; HRESIMS *m/z* 407.2646 [M + H]$^+$ (calcd for $C_{21}H_{35}N_4O_4$, 407.2658).

Sclerotiotide C (3): pale yellow, amorphous powder; $[\alpha]^{25}_D$ −57 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.0), 296 (4.4) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 447.2957 [M + H]$^+$ (calcd for $C_{24}H_{39}N_4O_4$, 447.2971).

Sclerotiotide D (4): pale yellow, amorphous powder; $[\alpha]^{25}_D$ −62 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.9), 296 (4.3) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 419.2650 [M + H]$^+$ (calcd for $C_{22}H_{35}N_4O_4$ 419.2658).

Sclerotiotide E (5): pale yellow, amorphous powder; $[\alpha]^{25}_D$ −84 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.9), 296 (4.4) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 433.2802 [M + H]$^+$ (calcd for $C_{23}H_{37}N_4O_4$, 433.2815).

Sclerotiotide F (6): pale yellow, amorphous powder; $[\alpha]^{25}_D$ −67 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.9), 270 (4.3) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 421.2455 [M + H]$^+$ (calcd for $C_{21}H_{33}N_4O_5$, 421.2451).

Sclerotiotide G (7): pale yellow, amorphous powder; $[\alpha]^{25}_D$ −59 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.1), 275 (4.3) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 465.2713 [M + H]$^+$ (calcd for $C_{23}H_{37}N_4O_6$, 465.2713).

Sclerotiotide H (8): pale yellow, amorphous powder; $[\alpha]^{25}_D$ −34 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 209 (4.0), 258 (4.2) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 467.2866 [M + H]$^+$ (calcd for $C_{23}H_{39}N_4O_6$, 467.2870).

Sclerotiotides I (9): pale yellow, amorphous powder; $[\alpha]^{25}_D$ −48 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 209 (4.0), 258 (4.2) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 467.2850 [M + H]$^+$ (calcd for $C_{23}H_{39}N_4O_6$, 467.2870).

Sclerotiotide J (10): pale yellow, amorphous powder; $[\alpha]^{25}_D$ −61 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 209 (4.0), 258 (4.2) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 467.2871 [M + H]$^+$ (calcd for $C_{23}H_{39}N_4O_6$, 467.2870).

Sclerotiotide K (11): pale yellow, amorphous powder; $[\alpha]^{25}_D$ −42 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 209 (4.0), 258 (4.2) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 467.2879 [M + H]$^+$ (calcd for $C_{23}H_{39}N_4O_6$, 467.2870).

Determination of the Absolute Configurations of Amino Acids by Marfey's Method.[10] Compounds **1**−**3**, **12**, and **13** (each 1 mg) were hydrolyzed in HCl (6 M; 1 mL) for 20 h at 110 °C. The solutions were then evaporated to dryness and redissolved in H_2O (250 μL). A 1% (w/v) solution (100 μL) of L-FDAA (1-fluoro-2,4-dinitrophenyl-5-L-alanine-amide) in acetone was added to an aliquot (50 μL) of the acid hydrolysate solution. After addition of $NaHCO_3$ solution (1 M; 20 μL) the mixture was incubated at 45 °C for 1 h. The reaction was quenched by the addition of HCl (2 M, 10 μL). Analyses of the FDAA-derivatized hydrolysates of compounds **2**, **12**, and **13** and standard FDAA-derivatized amino acids were carried out by HPLC (Waters 600E; solvents: A, water + 0.2% TFA; B, MeCN; linear gradient: 0 min 25% B, 40 min 60% B, 45 min 100% B; 30 °C; 1 mL/min; UV detection at λ 340 nm). Retention times of the amino acid derivatives were as follows: L/D-Orn, t_R 26.2/24.0 min; L/D-Ala, t_R 16.4/19.2 min; L/D-*N*Me-Ala, t_R 18.1/17.3 min; L/D-*N*Me-Val, t_R 26.3/28.8 min. Due to the poor discrimination between L-Orn and L-*N*Me-Val, they were eluted with the isocratic eluent (40% B), and retention times (min) were 12.4 and 13.1, respectively. The derivatized hydrolysates of **2** and **13** showed peaks designated as L-Orn, L-*N*Me-Ala, and L-*N*Me-Val. The hydrolysates of **12** consisted of L-Orn, L-Ala, and L-*N*Me-Val. Analyses of the FDAA-derivatized hydrolysates of compounds **1** and **3** were also carried out by HPLC (Shimadzu SCL-10A$_{VP}$) using the above-mentioned gradient elution and detection method. Retention times of the amino acids derivatives were as follows: L/D-Orn, t_R 28.9/27.1 min; L/D-Lys, t_R 30.4/32.1 min; L/D-Val, t_R 25.3/29.6 min; L/D-

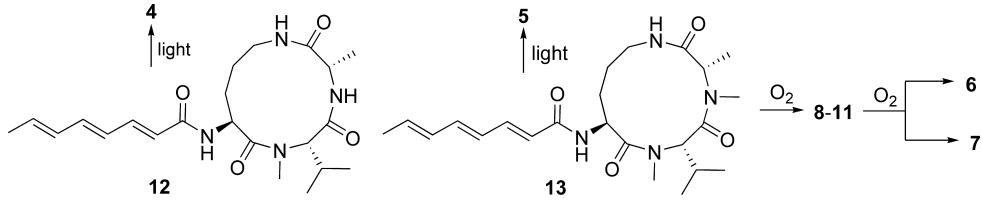

Figure 3. Photoisomerizations of compounds **12** and **13** and the oxidation of **13**.

*N*Me-Val, t_R 28.4/30.8 min; L/D-*N*Me-Ala, t_R 21.2/20.3 min. The hydrolysates of **1** consisted of L-Orn, L-Val, and L-*N*Me-Ala, while **3** contained L-Lys, L-*N*Me-Val, and L-*N*Me-Ala. All amino acids of these cyclopeptides were established as the L-configuration.

Chemical Transformation. Compounds **12** and **13** (1 mg each) were dissolved in 1 mL of MeOH−H_2O (1:1) and then exposed to daylight for 1 day. Compounds **4** (t_R 14.4 min) and **5** (t_R 17.9 min) were identified from the reaction mixtures of **12** and **13**, respectively, by HPLC (54% CH_3OH). In addition, **13** (1 mg) in MeOH−H_2O (1:1, 1 mL) was exposed to air for 10 days, and its reaction products were identified by HPLC (35% CH_3OH) as **6**, t_R 15.4 min, and **7**, t_R 16.4 min. Then using 30% CH_3OH, **8**, t_R 14.5 min; **9**, t_R 16.5 min; **10**, t_R 17.4 min; and **11**, t_R 20.2 min, were identified.

Acknowledgment. This work was supported by grants from National Basic Research Program of China (No. 2010CB833800), from the National Natural Science Foundation of China (Nos. 30670219 and 30770235), and from the Project of Chinese National Programs for High Technology Research and Development (2007AA09Z447). The cytotoxicity assay was performed at the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Supporting Information Available: Bioassay protocols used, NMR spectra of compounds **1**−**11**, HPLC profiles of acidic hydrolysates of **1**−**3**, **12**, and **13**, HPLC analysis of the products of photoisomerization of **12** and **13** and oxidation of **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP100198H