Thiodiketopiperazines Produced by the Endophytic Fungus *Epicoccum nigrum*

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Thirteen new thiodiketopiperazines, epicoccin I (**1**), ent-epicoccin G (**2**), and epicoccins J−T (**3**−**13**), together with six known diketopiperazines (**14**−**19**), have been isolated from the endophytic fungus *Epicoccum nigrum*. The structures of **1**, **2**, and **10** were confirmed by X-ray crystallography, and the absolute configurations of **2**, **4**, **6**, and **8** were assigned using Moshers' method. Compounds **2**, **6**, **12**, and **17** showed potent activities in vitro against the release of β-glucuronidase in rat polymorphonuclear leukocytes induced by platelet-activating factor, with IC_{50} values of 3.07, 4.16, 4.95, and 1.98 μM, respectively. None of the 19 compounds exhibited detectable cytotoxic activities toward six tumor cell lines (A549, Be-l7402, BGC-823, HCT-8, HCT-116, and A2780) in the MTT assay.

In previous reports, we described the structures of several new phloroglucinol derivatives, phloroglucinol glycosides, flavanols, and stilbenes with vasodilator activities,[1] isolated from the roots of *Lysidice rhodostegia* Hance (Fabaceae), a shrubbery plant that has been used for the treatment of ache, fractures, and hemorrhage for a long time in China. The interest in new bioactive metabolites has prompted a thorough examination of *Lysidice rhodostegia* Hance's secondary metabolites, and this examination has grown to embrace some endophytic fungi associated with the plant. The endophytic fungus *Epicoccum nigrum* was obtained from the leaves of *L. rhodostegia*. Several compounds with diketoperazine moieties and a rare cross-ring sulfur bridge have previously been isolated from *E. nigrum*,[2] and some of them showed inhibitory effects on HIV-1 replication in C8166 cells. In our study, 13 new thiodiketopiperazines, named epicoccin I (**1**), ent-epicoccin G (**2**), and epicoccins J−T (**3**−**13**), were isolated from the EtOAc extract of a culture of *E. nigrum*. The thiodiketoperazine group is the nucleus of an important class of fungal metabolites that are of moderate pharmacological and chemical interest. The structural types of thiodiketopiperazines include gliotoxins,[3] BDA,[4] epicorazines,[5] rostratins,[6] exserohilones,[7] vertihemiptellides,[8] verticillins,[9] sporidesmins,[10] and chaetocin.[11] Some of them displayed antibacterial, antioxidant,[3b] antiangiogenic,[3c] and cytotoxic activities.[5c,6,8] Although thiodiketopiperazines are frequently encountered in nature, compounds containing both a cross-ring sulfur(disulfide) bridge and an *S*-methyl group like those in **1**, **6**, **7**, **8**, and **9** have not been previously reported from natural sources. Compounds **2**, **6**, **12**, and **17** showed potent activities in vitro against the release of β-glucuronidase in rat polymorphonuclear leukocytes induced by platelet-activating factor. Details of the isolation, structure elucidation, and biological activities of these compounds are reported herein.

Results and Discussion

Epicoccin I (**1**) was obtained as white powder; its molecular formula was established as $C_{19}H_{20}N_2O_5S_2$ by HRESIMS. The ^1H and ^{13}C NMR data of **1** (Tables 1 and 2) showed the presence of three exchangeable protons (δ_H 5.07, 5.48, and 6.09), one methyl group, two methylenes, 10 methines (four bonded to oxygen or nitrogen), and six quaternary carbons including two carbonyl carbons.

The ^1H−^1H COSY data showed the presence of two isolated proton spin systems. A series of ^1H−^1H COSY cross-peaks from

H-5 (δ_H 5.93) through H-6 (δ_H 5.88) to H-7 (δ_H 5.59) and then to H-8 (δ_H 4.55) and H-9 (δ_H 4.74) and HMBC correlations from both H-5 and H-9 to C-4 (δ_C 133.6) revealed the presence of a six-membered ring in **1** (C-4−C-9). Another series of ^1H−^1H COSY cross-peaks from H-5' (δ_H 4.19) through H-6' (δ_H 6.0) to H-7' (δ_H 5.78) and then to H-8' (δ_H 4.44) and H-9' (δ_H 3.99), together with HMBC correlations from both H-5' and H-9' to C-4' (δ_C 77.2), demonstrated the presence of another six-membered ring in **1** (C-4'−C-9'). In addition, HMBC correlations from H-3b to C-1 and C-5 and from H-3b' to C-1' and C-5', coupled with the molecular formula, the amide carbonyl signals (δ_C 160.9, C-1; δ_C 164.9, C-1'), and the structural features of known compounds **14**, **15**, **16**, **17**, and **18**, indicated the presence of a 6−5−6−5−6 diketopiperazine (DKP) skeleton.

The chemical shift of the methyl group (δ_H/δ_C 2.08/13.8) and the HMBC cross-peak from the methyl protons to C-2 indicated it was connected to the sulfur atom attached to C-2, while the chemical shifts of C-2' (δ_C 72.9) and C-5' (δ_C 43.2), together with HMBC correlations from H-5' (δ_H 4.19) to C-2', indicated the presence of an internal sulfur bridge from C-2' to C-5'. The chemical shifts for OH-8 (δ_H 5.48), OH-4' (δ_H 6.09), and OH-8' (δ_H 5.07), in conjunction with HMBC correlations from OH-8 to C-7, C-8, and C-9, from OH-4' to C-3', C-4', C-5', and C-9', and from OH-8' to C-7', C-8', and C-9', indicated that three hydroxy groups were located at C-8, C-4', and C-8'. Analysis of the NMR data (Tables 1 and 2) for the left portion of **1** revealed structural similarities to methylthiogliotoxin.[3c] The olefinic carbon signals (δ_C 127.3, C-6'; δ_C 128.2, C-7') indicated the formation of a double bond between C-6' and C-7'. This information, along with the aforementioned two hydroxy groups at C-4' and C-8' and an internal sulfur bridge from C-2' to C-5', indicated the right portion of **1** to be a highly oxidized phenylalanine residue with a double bond, two hydroxy groups, and an internal sulfur bridge. These data in conjunction with HMBC correlations (Figure 2) indicated the planar structure of **1** as shown in Figure 1.

The X-ray data allowed the determination of the relative configuration of epicoccin I (**1**). In addition, the presence of sulfur atoms in **1** and the value of the Flack parameter 0.04(6)[13] determined by X-ray analysis allowed assignment of the absolute configuration of all stereogenic carbons in **1** as 2*R*, 8*S*, 9*S*, 2'*R*, 4'*S*, 5'*S*, 8'*S*, and 9'*R*. The compound was named epicoccin I.

The molecular formula of compound **2** was established as $C_{20}H_{26}N_2O_6S_2$ by accurate mass measurement. Comparison of the NMR data of **2** (Tables 1 and 3) with those of epicoccin G indicated that the planar structure of **2** was identical to that of epicoccin G.[2b]

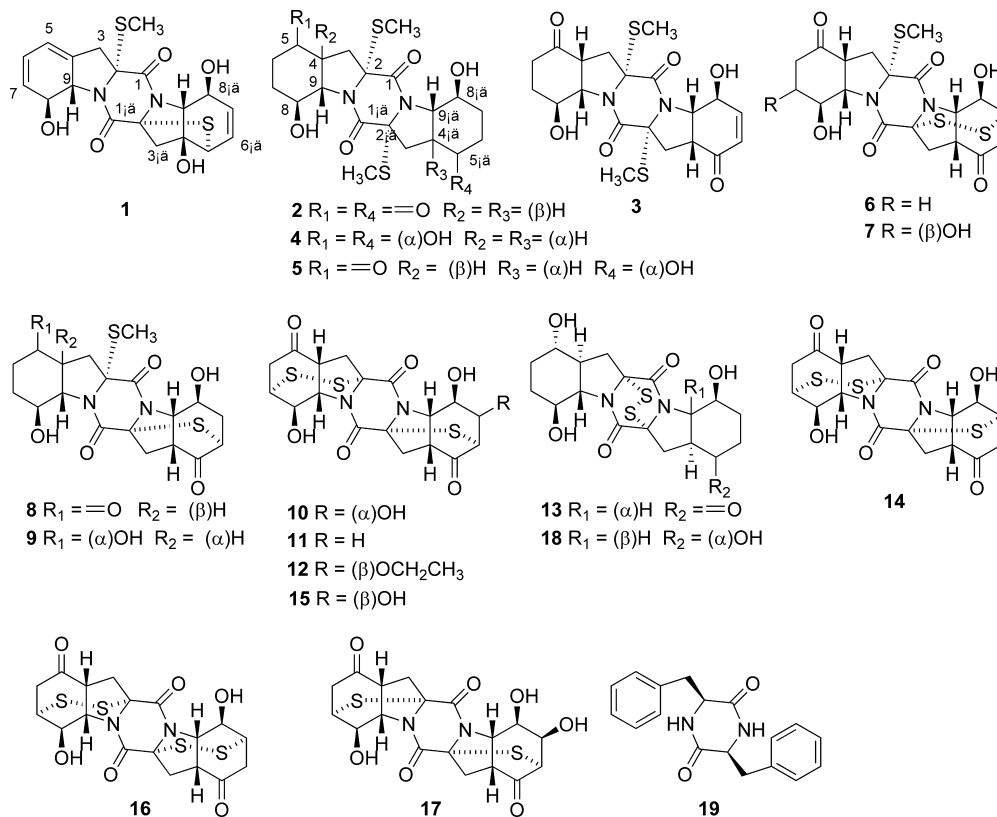* To whom correspondence should be addressed. E-mail: yushishan@imm.ac.cn. Tel: +86-10-63165324. Fax: +86-10-63017757.

Figure 1. Structures of compounds **1**−**19**.

Table 1. ^{13}C NMR Data for Compounds **1**−**13** in DMSO-d_6 (125 MHz for **1**−**13**)[a]

no.	1	2	3	4	5	6	7	8	9	10	11	12	13
1	160.9	161.5	165.7	169.4	169.3	162.0	162.0	161.7	161.8	161.1	161.8	161.8	162.7
2	74.0	71.5	71.6	72.6	71.4	71.5	71.6	72.1	73.5	73.5	73.7	73.6	75.6
3	38.6	34.1	34.3	35.1	34.4	34.3	34.5	33.7	34.4	45.9	45.7	45.5	33.4
4	133.6	44.0	44.1	45.3	43.8	44.2	44.0	44.2	45.5	43.3	43.2	43.1	48.3
5	118.9	207.7	207.8	69.5	207.4	207.6	207.3	207.5	69.5	207.9	207.9	207.8	69.0
6	123.4	33.8	33.8	33.5	33.7	33.9	43.4	33.8	33.5	38.0	38.0	37.9	33.0
7	130.4	25.8	25.9	32.8	25.8	25.6	65.7	25.7	32.7	45.5	45.5	45.4	30.9
8	73.6	63.4	63.3	72.3	63.3	64.5	68.0	63.3	72.0	64.8	64.9	64.7	69.9
9	68.9	64.7	64.7	69.6	64.6	65.6	64.0	65.6	70.5	62.5	62.4	59.9	70.7
1′	164.9	161.5	165.5	169.4	165.5	165.0	165.2	162.6	166.3	161.1	161.1	160.8	164.4
2′	72.9	71.5	71.1	72.6	72.9	73.2	73.3	72.4	72.5	71.1	71.1	73.3	76.5
3′	53.9	34.1	36.0	35.1	35.0	46.5	46.6	52.6	53.3	52.3	52.0	51.9	32.6
4′	77.2	44.0	41.3	45.3	45.4	42.9	43.0	45.9	45.8	45.4	46.1	45.4	46.7
5′	43.2	207.7	195.1	69.5	69.5	208.0	208.1	205.2	205.2	205.4	205.3	203.3	208.7
6′	127.3	33.8	129.7	33.5	33.4	38.0	38.2	45.2	45.2	52.7	45.0	50.3	33.8
7′	128.2	25.8	146.5	32.8	32.7	45.0	45.1	40.6	41.0	73.7	41.7	85.7	25.2
8′	62.3	63.4	61.7	72.3	72.2	66.3	66.3	61.5	61.8	68.0	61.2	61.7	60.6
9′	65.6	64.7	64.6	69.6	69.6	61.8	61.9	62.8	62.8	60.5	63.2	59.9	65.6
2-SCH$_3$	13.8	14.2	14.3	14.4	14.2	13.7	13.7	13.7	14.1				
2′-SCH$_3$		14.2	14.8	14.4	14.4								
7′-CH$_2$-												64.0	
7′-CH$_3$												14.9	

[a] Assignments based on the HSQC and HMBC experiments.

The relative configuration of **2** was assigned by comparison of its ^{1}H NMR data with those of epicoccin G.[2b] Due to the presence of two secondary alcohol units in **2**, its absolute configuration was determined by Mosher's method.[14] Compound **2** was separately esterified with (*S*)- and (*R*)-MTPA chloride to give the (*R*)- and (*S*)-MTPA esters, respectively. The striking difference in proton chemical shifts between these esters showed that C-8 (8′) possessed the *S* configuration ($\Delta\delta$ values $= \delta S - \delta R$ are given in Figure 5). This information, along with the aforementioned relative configuration, defined the absolute configurations of other stereogenic carbons in **2** (Figure 5). This was further confirmed by a single-crystal X-ray crystallographic analysis, and the perspective ORTEP plot of **2** is shown in Figure 4. In addition, compound **2** and

epicoccin G had identical ^{1}H and ^{13}C NMR spectra but opposite specific rotations, making them enantiomers.[2b] The solid data of spectroscopic analysis and X-ray diffraction allowed revision of the absolute configuration of epicoccin G.[2b] Thus, compound **2** was a new compound and named ent-epicoccin G.

HRESIMS analysis indicated that the molecular formula of epicoccin J (**3**) was $C_{20}H_{24}N_2O_6S_2$. The ^{13}C NMR data of **3** revealed marked similarities to **2** except that the carbon signals for C-6′ (δ_C 129.7) and C-7′ (δ_C 146.5) were shifted downfield. Additionally, the olefinic proton signals (δ_H 6.09, H-6′; δ_H 6.87, H-7′) indicated a double bond between C-6′ and C-7′. Further analysis of the ^{1}H−^{1}H COSY, HMQC, and HMBC data verified the structural similarities and differences between **3** and **2**, leading to the proposed structure

Table 2. ^{1}H NMR Data for Compounds **1−5** in DMSO-d_6 (500 MHz for **1−5**)[a]

no.	1	2	3	4	5
3a	3.11, d (15.5)	2.76, br d (13.5)	2.77, br d (13.5)	2.34, br d (13.0)	2.77, br d (13.5)
3b	2.87, d (15.5)	2.27, dd (13.5, 8.0)	2.28, dd (13.5, 8.5)	1.98, dd (13.0, 11.5)	2.28, dd (13.5, 8.0)
4		2.94, br dd (8.0, 8.0)	2.94, br dd (8.5, 7.5)	2.12, m, (overlap)	2.96, br dd (8.0, 8.0)
5	5.93, br d (2.5)			3.43, m	
6a	5.88, dd (9.5, 2.5)	2.59, ddd (17.0, 11.5, 5.5)	2.59, ddd (17.5, 12.5, 5.5)	1.83, m	2.60, ddd (17.5, 12.5, 5.5)
6b		2.19, m, (overlap)	2.20, m, (overlap)	1.25, m, (overlap)	2.21, m, (overlap)
7a	5.59, br d (9.5)	2.14, m, (overlap)	2.15, m, (overlap)	1.95, m	2.18, m, (overlap)
7b		1.88, m, (overlap)	1.88, m, (overlap)	1.25, m, (overlap)	1.92, m, (overlap)
8	4.55, br d (13.0)	4.31, m, (overlap)	4.30, m, (overlap)	3.50, m	4.33, m, (overlap)
9	4.74, d (13.0)	4.29, br d (8.0), (overlap)	4.28, br d (7.5), (overlap)	3.34, dd (11.5, 11.5)	4.30, br d (8.0), (overlap)
3′a	2.73, d (9.5)	2.76, br d (13.5)	2.76, dd (13.5, 7.5)	2.34, br d (13.0)	2.30, br d (12.5)
3′b	2.63, d (9.5)	2.27, dd (13.5, 8.0)	2.46, br d (13.5)	1.98, dd (13.0, 11.5)	1.98, dd (12.5, 11.5)
4′		2.94, br dd (8.0, 8.0)	3.08, br dd (7.5, 7.5)	2.12, m, (overlap)	2.09, m, (overlap)
5′	4.19, d (6.0)			3.43, m	3.41, m
6′a	6.0, dd (9.0, 6.0)	2.59, ddd (17.0, 11.5, 5.5)	6.09, d (10.0)	1.83, m	1.82, m
6′b		2.19, m, (overlap)		1.25, m, (overlap)	1.23, m, (overlap)
7′a	5.78, dd (9.0, 2.5)	2.14, m, (overlap)	6.87, dd (10.0, 4.5)	1.95, m	1.89, m
7′b		1.88, m, (overlap)		1.25, m, (overlap)	1.23, m, (overlap)
8′	4.44, m	4.31, m, (overlap)	4.54, m	3.50, m	3.47, m
9′	3.99, br s	4.29, br d (8.0), (overlap)	4.41, br d (7.5)	3.34, dd (11.5, 11.5)	3.32, dd (11.5, 11.5)
2-SCH$_3$	2.08, s	1.96, s, (overlap)	1.90, s	2.11, s	1.92, s
5-OH				4.95, s	
8-OH	5.48, s	5.32, s	5.31, d (2.0)	6.12, s	5.33, d (3.0)
2′-SCH$_3$		1.96, s, (overlap)	1.89, s	2.11, s	2.06, s
4′-OH	6.09, s				
5′-OH				4.95, s	4.93, d (5.0)
8′-OH	5.07, s	5.32, s	5.67, d (6.5)	6.12, s	6.19, s

[a] Chemical shifts δ_H mult. (*J* in Hz)
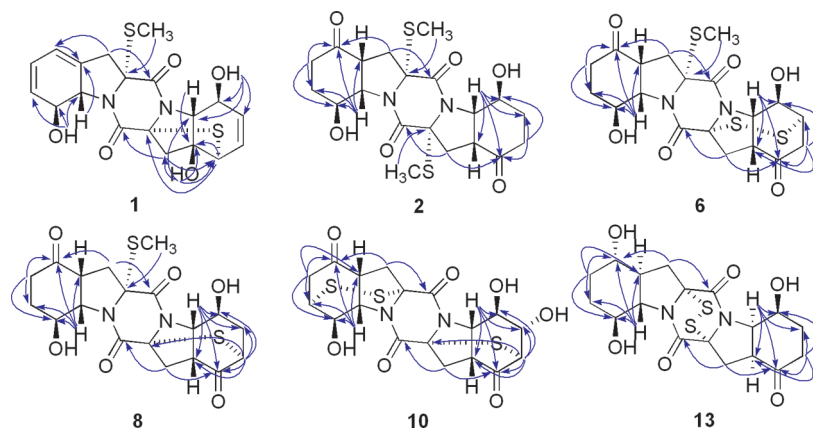
Figure 2. Key HMBC correlations of **1**, **2**, **6**, **8**, **10**, and **13**.

of **3**. The NOESY correlations and the coupling patterns for H-4 (δ_H 2.94, br dd, *J* = 8.5, 7.5 Hz) and H-9 (δ_H 4.28, br d, *J* = 7.5 Hz), and H-4′ (δ_H 3.08, br dd, *J* = 7.5, 7.5 Hz) and H-9′ (δ_H 4.41, br d, *J* = 7.5 Hz), indicated that the relative configuration of **3** was the same as **2**. The negative specific rotation of **3**, in conjunction with the configuration of **2**, indicated the gross structure of **3** shown in Figure 1. The compound was named epicoccin J.

The molecular formula of epicoccin K (**4**) was established as $C_{20}H_{30}N_2O_6S_2$ by HRESIMS. The ^{13}C NMR spectrum of **4** was similar to that of **2**, except for differences associated with the presence of two hydroxy groups at C-5/5′ (δ_C 69.5) and the absence of two carbonyl groups at C-5/5′ (δ_C 207.7). These changes were also confirmed by HMBC correlations from OH-5/5′ to C-5/5′.

The relative configuration of **4** was elucidated on the basis of [1]H−[1]H coupling constants and NOESY data. The 11.5 Hz vicinal coupling constant for H-4/4′ and H-9/9′ revealed their *trans* relationship, compared to the *trans* configuration deduced from the large coupling constant of 12.0 Hz for the corresponding protons in rostratin A.[6] On the basis of the ring juncture, the spatial orientation of the relevant protons was confirmed by the NOESY spectrum, which showed correlations between SCH$_3$/SCH$_3$′, H-3b/

3b′, H-4/4′, and H-8/8′, indicating that they were on the same face (α) of the molecule. The NOESY spectrum showed correlations between H-5/5′ and H-9/9′, thereby confirming that the two groups were located on the β-face.

To solve the absolute configuration of **4**, we considered ways to regioselectively acylate **4** to produce a mono (bis) MTPA ester.[6] To achieve selective monoacylation of one of the two (symmetrical) secondary hydroxy groups in the cyclohexane-1,4-diol moiety, compound **4** was dissolved in pyridine-d_5 in an NMR tube dried under a gentle argon stream, and a baseline ^{1}H NMR spectrum was acquired. The lower half of the NMR tube was maintained at approximately 0 °C, and after 10 min, (*R*)-MTPA chloride was added. The tube was shaken to afford even mixing and maintained at 0 °C. Spectra were recorded every 15 min to monitor the rate of acylation. This reaction temperature was maintained for 30 min until the reaction had gone to completion. Excess (*R*)-MTPA chloride was destroyed by adding a drop of D$_2$O, and [1]H−^{1}H COSY NMR data of the resulting (*S*)-MTPA ester (**4b**) were recorded, confirming that selective acylation had occurred at C-5(5′). Similarly, compound **4** was selectively acylated at C-5(5′) with (*S*)-MTPA-Cl to generate (*R*)-MTPA ester **4a**, the ^{1}H NMR spectrum

Table 3. ^{1}H NMR Data for Compounds **6−10** in DMSO-d_6 (500 MHz for **6−10**)^a

no.	6	7	8	9	10
3a	2.83, br d (13.5)	2.79, br d (13.5)	2.82, br d (13.5)	2.36, br d (12.5)	2.81, dd (13.0, 8.5)
3b	2.34, dd (13.5, 8.5)	2.39, dd (13.5, 8.5)	2.35, dd (13.5, 8.5)	2.03, dd (12.5, 11.5)	2.53, br d (13.0)
4	3.01, br dd (8.5, 8.5)	3.04, br dd (8.5, 8.5)	2.99, br dd (8.5, 8.0)	2.17, m, (overlap)	3.08, br dd (8.5, 8.5)
5				3.43, m	
6a	2.56, ddd (17.5, 10.5, 5.5)	2.55, dd, (17.0, 8.5), (overlap)	2.60, ddd (17.5, 11.5, 5.5)	1.84, m	3.07, dd (14.5, 6.0)
6b	2.23, br d (17.5)	2.53, m, (overlap)	2.22, br d (17.5)	1.23, m, (overlap)	2.44, br d (14.5)
7a	2.06, m	4.09, ddd, (8.5, 4.5, 4.5)	2.09, m	1.93, m	3.73, br dd (6.0, 4.0)
7b	1.86, m		1.88, m	1.23, m, (overlap)	
8	4.31, m, (overlap)	4.27, m	4.35, m, (overlap)	3.47, m	4.57, m
9	4.29, br d (8.5), (overlap)	4.34, dd (8.5, 4.5)	4.34, m, (overlap)	3.40, dd (11.5, 11.5)	4.46, m, (overlap)
3'a	2.79, dd (13.0, 9.5)	2.81, dd (13.0, 7.0)	3.01, dd (12.5, 5.5)	3.04, dd (12.5, 7.0)	2.99, dd (11.5, 6.5)
3'b	2.49, br d (13.0)	2.46, br d (13..0)	2.80, br d (12.5)	2.83, br d (12.5)	2.89, br d (11.5)
4'	3.08, br dd (9.5, 8.0)	3.09, br dd (7.0, 7.0)	3.11, br dd (5.5, 5.5)	3.14, br dd (7.0, 5.0)	3.04, br dd (6.5, 6.5)
6'a	3.06, dd (21.0, 5.0)	3.06, br d (14.5)	3.58, br d (9.5)	3.61, br d (9.5)	3.68, d (7.5)
6'b	2.44, br d (21.0)	2.42, br d (14.5)			
7'a	3.70, m	3.71, m	2.72, br dd (15.0, 5.5)	2.70, br dd (15.0, 5.0)	3.94, br dd (7.5, 5.0)
7'b			2.45, br dd (15.0, 9.5)	2.48, br dd (15.5, 9.5)	
8'	4.44, m, (overlap)	4.44, m, (overlap)	4.46, m	4.45, m	4.22, br dd (3.0, 3.0)
9'	4.46, br d (8.0), (overlap)	4.46, br d (7.0), (overlap)	4.56, dd (5.5, 5.5)	4.59, dd (5.0, 5.0)	4.49, m, (overlap)
2-SCH$_3$	1.96, s	1.95, s	1.98, s	2.13, s, (overlap)	
5-OH				4.97, d (5.0)	
7-OH		5.15, s			
8-OH	5.33, s	5.35, s	5.37, s	6.15, s	6.06, d (3.0)
7'-OH					4.71, d (5.0)
8'-OH	6.03, s	6.04, s	5.49, s	5.51, d (2.5)	5.63, d (3.0)

^{a} Chemical shifts δ_H mult. (J in Hz).
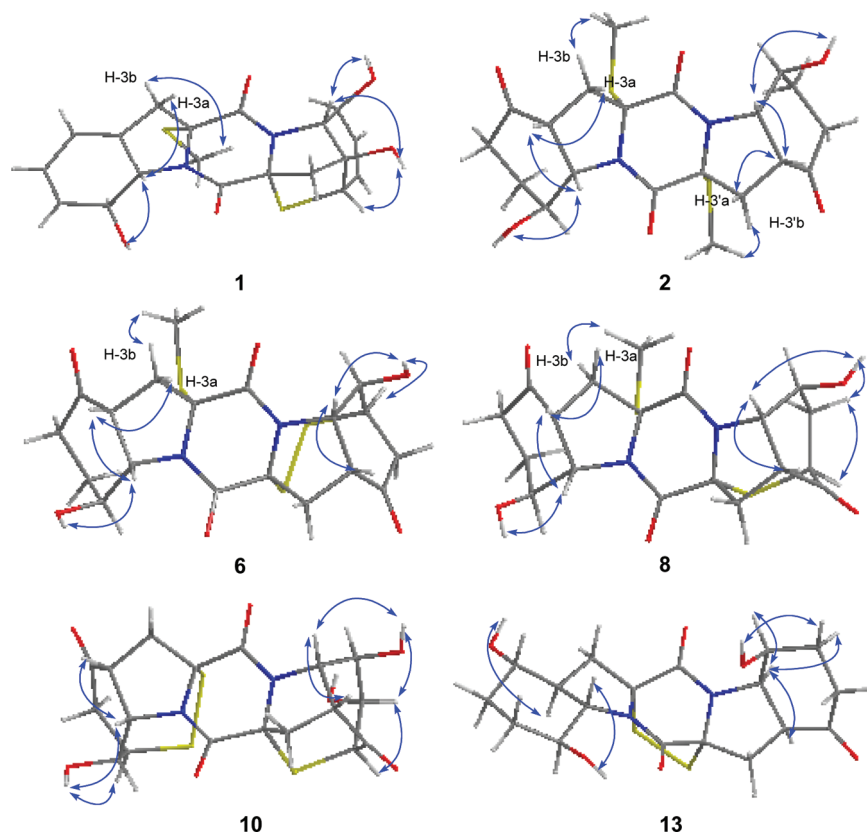
Figure 3. Key NOE (or NOESY) correlations of **1**, **2**, **6**, **8**, **10**, and **13**.

of which is shown in Figure S43 (Supporting Information). Analysis of the proton chemical shift $\Delta\delta$ values between the (S)- and (R)-MTPA esters demonstrated that C-5(5′) had the S configuration [$\Delta\delta$ values = $\delta S - \delta R$ are given in Figure 6]. This information, along with the aforementioned NOESY data, defined the absolute configurations of the other stereogenic carbons in **4** (Figure 6). The compound was named epicoccin K.

HRESIMS analysis indicated that the molecular formula of epicoccin L (**5**) was $C_{20}H_{28}N_2O_6S_2$. The ^{1}H and ^{13}C NMR data of

5 (Tables 1 and 2) revealed the presence of three exchangeable protons (δ_H 4.93, 5.33, and 6.19), two methyl groups, six methylenes, seven methines (three of which was oxygenated), and five quaternary carbons including three carbonyl carbons. Compound **5** displayed NMR data (Tables 1 and 2) almost identical to **2** from C-1 to C-9 and **4** from C-1′ to C-9′. The relative configuration of **5** was elucidated on the basis of ^{1}H−^{1}H coupling constants and NOE difference experiments compared to those of **2** and **4**. Considering the absolute configuration established for **2** by X-ray
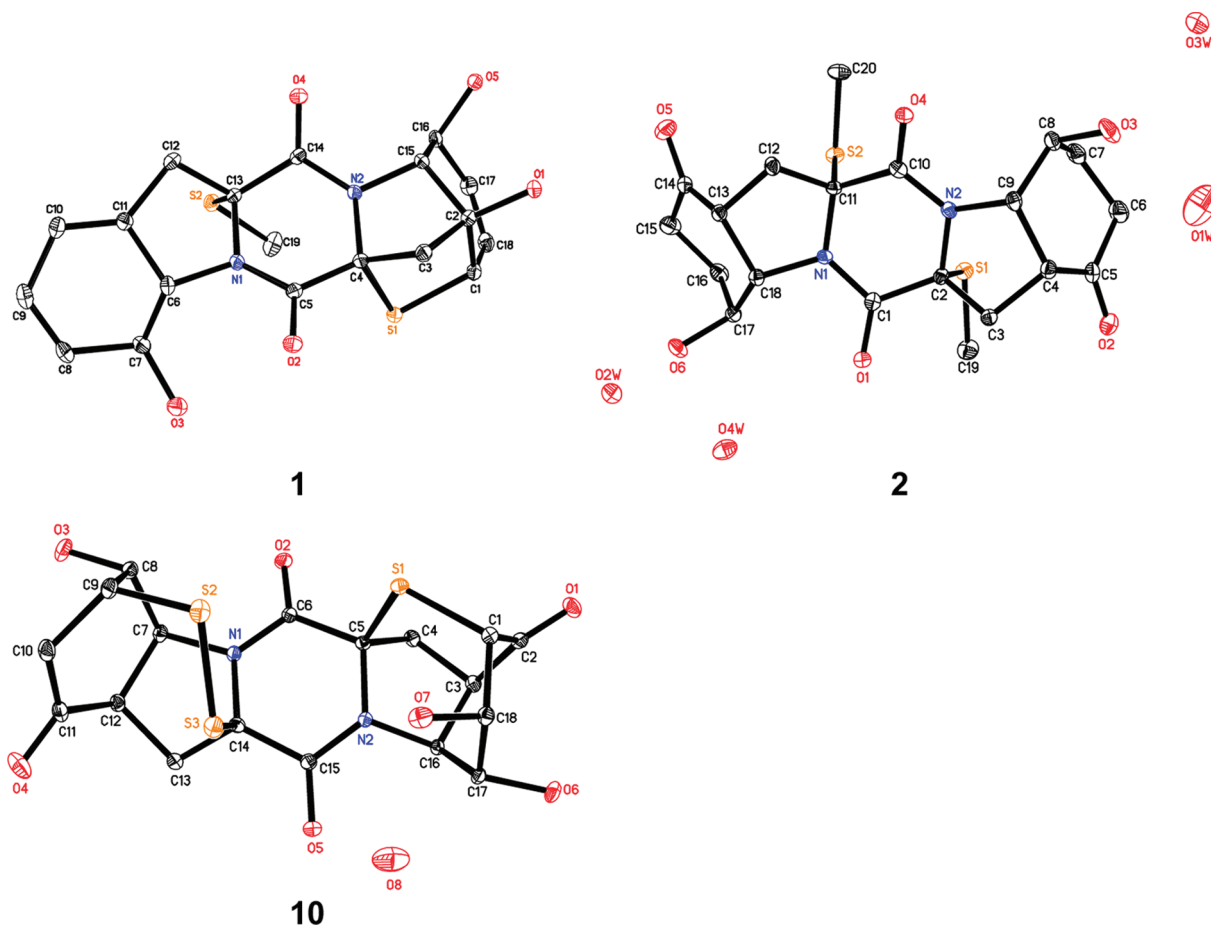
Figure 4. ORTEP drawing of the X-ray crystal structures of **1**, **2**, and **10**.
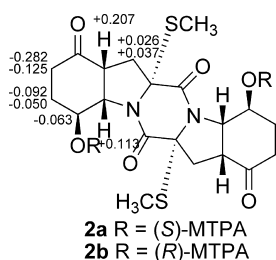

Figure 5. Relative and absolute configuration of ent-epicoccin J (**2**): $\Delta\delta$ values ($\delta S - \delta R$) for the two MTPA esters **2a** and **2b**.
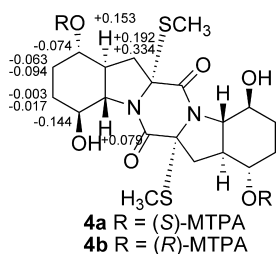

Figure 6. Relative and absolute configuration of epicoccin K (**4**): $\Delta\delta$ values ($\delta S - \delta R$) for the two MTPA esters **4a** and **4b**.

data and **4** by Mosher's method, the absolute configuration of epicoccin L was presumed to be $2R$, $4R$, $8S$, $9S$, $2'R$, $4'S$, $5'S$, $8'S$, and $9'S$.

HRESIMS analysis indicated that epicoccin M (**6**) had the molecular formula $C_{19}H_{22}N_2O_6S_3$. The ^1H and ^{13}C NMR data of **6** (Tables 1 and 3) were identical to those of **2** from C-1 to C-9 and **16**[2a] from C-1' to C-9', respectively. The HMBC cross-peak (Figure 2) from the methyl protons to C-2 indicated that the methyl group was connected to the sulfur atom attached to C-2, whereas the chemical shifts of C-2' (δ_C 73.2) and C-7' (δ_C 45.0) revealed the presence of an internal disulfide bridge from C-2' to C-7'. The chemical shifts and coupling patterns for H-4 (δ_H 3.01, br dd, $J = 8.5$, 8.5 Hz) and H-9 (δ_H 4.29, br d, $J = 8.5$ Hz), and H-4' (δ_H 3.08, br dd, $J = 9.5$, 8.0 Hz) and H-9' (δ_H 4.46, br d, $J = 8.0$ Hz), of **6** indicated that the relative configuration of **6** was the same as that of **2** from C-1 to C-9 and **16**[2a] from C-1' to C-9'. This conclusion was further supported by NOESY correlations (Figure 3).

The absolute configuration of **6** was also assigned by interpretation of chemical shift data derived from the corresponding C-8 (8') (R)- and (S)-MTPA esters. Using Mosher's acylation described above for ent-epicoccin G (**2**), the (R)- and (S)-MTPA esters at C-8 (8') were produced. Significant chemical shift differences between (R)- and (S)-MTPA esters **6a** and **6b** defined C-8 (8') as S (Figure 7). The aforementioned relative configuration allowed assignment of the absolute configurations of other stereogenic carbons in **6** (Figure 7). The compound was named epicoccin M.

The molecular formula of epicoccin N (**7**) was deduced to be $C_{19}H_{22}N_2O_7S_3$ by HRESIMS analysis. The NMR data of **7** (Tables 1 and 3) revealed structural similarities to **6** except that the signal for C-7 (δ_C 65.7) was shifted downfield, indicating the presence of a C-7 hydroxy group in **7**. This was confirmed by an HMBC cross-peak between OH-7 and C-7. H-6a and H-7 showed a large vicinal coupling constant (8.5 Hz) consistent with two *trans* diaxial protons. The coupling constant (4.5 Hz) for H-7 and H-8 in conjunction with NOESY correlations between OH-7 and OH-8 indicated that the two hydroxy groups were oriented on the same side of the cyclohexanone ring. The positive specific rotation data of **7** were
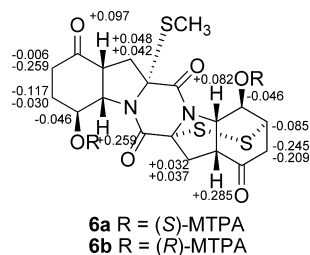
Figure 7. Relative and absolute configuration of epicoccin M (**6**): $\Delta\delta$ values ($\delta S - \delta R$) for the two MTPA esters **6a** and **6b**.
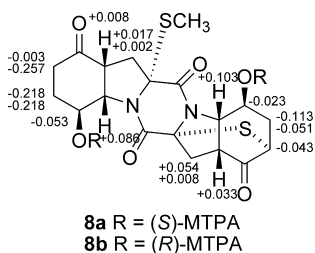

Figure 8. Relative and absolute configuration of epicoccin O (**8**): $\Delta\delta$ values ($\delta S - \delta R$) for the two MTPA esters **8a** and **8b**.

similar to those of **6**. Thus, the gross structure of epicoccin N was determined to be that shown in Figure 1.

For epicoccin O (**8**), $[\alpha]^{20}_D$ +106.3 (*c* 0.1, MeOH), HRESIMS analysis indicated the molecular formula as $C_{19}H_{22}N_2O_6S_2$. Compound **8** displayed spectroscopic data (Tables 1 and 3) similar to those of **2** from C-1 to C-9 and **17**[2b] from C-1′ to C-9′. However, NMR signals for C-6′ (δ_C 45.2), C-7′ (δ_C 40.6), and C-8′ (δ_C 61.5) of **8** were shifted upfield relative to those of **17**. These differences indicated that the β-hydroxy group at C-7′ in **17** was eliminated, which was supported by HMQC correlations between a diastereotopic methylene pair (δ_H 1.30 and 1.85) and C-7′. Analysis of the proton−proton coupling constants ($J_{H\text{-}4/H\text{-}9} = 8.0$ Hz and $J_{H\text{-}4'/H\text{-}9'} = 5.5$ Hz) indicated that both the C-4−C-9 and C-4′−C-9′ ring junctures in **8** were *cis*. The above data in conjunction with the NOESY correlations indicated that the relative configuration of **8** was as shown in Figure 3. Using the identical Mosher method, the absolute configuration of **8** was readily defined by analysis of the NMR shift data from the corresponding C-8(8′) (*R*)- and (*S*)-MTPA bis-esters. The significant proton chemical shift differences between the (*R*)- and (*S*)-MTPA esters **8a** and **8b** demonstrated that C-8(8′) possessed the *S* configuration (Figure 8). This information, along with the aforementioned NOESY data, defined the absolute configuration of the other stereogenic carbons in **8** (Figure 8). The compound was named epicoccin O.

The NMR data of epicoccin P (**9**) (Tables 1 and 3) revealed structural similarities to **8**, except that the signal for C-5 (δ_C 69.5) was shifted upfield, indicating elimination of a carbonyl group in **8** and the formation of a hydroxy group at the C-5 position in **9**. This was supported by HRESIMS analysis and HMBC correlations between OH-5 (δ_H 4.97) and C-5 (δ_C 69.5). Analysis of the proton−proton coupling constants indicated that the C-4−C-9 ring juncture was *trans* ($J_{H\text{-}4/H\text{-}9} = 11.5$ Hz), whereas the C-4′−C-9′ ring juncture had the *cis* configuration in **9** ($J_{H\text{-}4'/H\text{-}9'} = 5.0$ Hz). The above data in conjunction with chemical shifts and NOESY correlations, as compared with those of **4** from C-1 to C-9 and **8** from C-1′ to C-9′, indicated the structure of **9** to be that as shown in Figure 1. The compound was named epicoccin P.

Comparison of the NMR data of epicoccin Q (**10**) ($C_{18}H_{18}N_2O_7S_3$) (Tables 1 and 3) with those of epicoccin B (**15**)[2a] indicated that H-6′ and C-8′ of **10** were deshielded by $\Delta\delta_H$ +0.52 and $\Delta\delta_C$ +4.8 ppm, respectively, whereas H-7′ and H-8′, and C-6′

and C-7′, were shielded by $\Delta\delta_H$ −0.41 and −0.21 and $\Delta\delta_C$ −0.9 and −4.4 ppm, respectively. These differences revealed that an α-hydroxy group was located at C-8′ in **10**.

The X-ray data allowed determination of the relative configuration of epicoccin Q. In addition, the presence of sulfur atoms in **10** and the value of the Flack parameter 0.03(6) determined by X-ray analysis allowed assignment of the absolute configuration of all stereogenic carbons in **10** as 2*R*, 4*R*, 7*R*, 8*R*, 9*S*, 2′*R*, 4′*R*, 6′*S*, 7′*S*, 8′*R*, and 9′*S*. The compound was named epicoccin Q.

Epicoccin R (**11**) had the molecular formula $C_{18}H_{18}N_2O_6S_3$, one oxygen atom less than **15** (HRESIMS). The NMR data of **11** (Tables 1 and 4) revealed structural features similar to those of **15**,[2a] except that the C-7 hydroxy group in **15** was reduced in **11**. The spectroscopic data and specific rotation of **11** were similar to those of **15**. Thus, the relative and absolute configuration of **11** were presumed to be the same as those of **15**. The compound was named epicoccin R.

HRESIMS analysis indicated that epicoccin S (**12**) had the molecular formula $C_{20}H_{22}N_2O_7S_3$. The ^1H NMR data of **12** (Table 4) were similar to those of **15**.[2a] However, resonances at δ_H 1.08 (3H, t, *J* = 7.0 Hz, 7′-CH$_3$) and 3.46 (2H, q, *J* = 7.0 Hz, 7′-CH$_2$−) revealed the presence of an *O*-CH$_2$CH$_3$ unit. Comparison of the ^{13}C NMR data of **12** (Table 1) with those of **15**[2a] indicated new carbon signals (δ_C 64.0, 14.9) for the *O*-CH$_2$CH$_3$ unit. HBMC correlations from the methylene protons to C-7′ confirmed the connection between C-7′ and the *O*-CH$_2$CH$_3$ moiety. The relative configuration was confirmed by the NOESY correlations and chemical shifts. The specific rotation of **12** was similar to that of **15**. On the basis of the above results, the absolute configuration of epicoccin S was deduced to be the same as that of **15**, and the gross structure of epicoccin S was established as shown in Figure 1. However, epicoccin S might be an artifact of the extraction process.

HRESIMS analysis showed the molecular formula of epicoccin T (**13**) to be $C_{18}H_{22}N_2O_6S_2$. The ^1H and ^{13}C NMR data of **13** (Tables 1 and 4) revealed the presence of three exchangeable protons (δ_H 5.03, 5.48, and 6.02), six methylenes, seven methines, two heteroatom-bonded sp^3 quaternary carbons, and three carbonyl carbons including one ketone. These above data revealed structural similarities to rostratin A (**18**)[6] from C-1 to C-9 and rostratin B[6] from C-1′ to C-9′. Assignment of the absolute configuration of **13** was determined by comparison of its CD spectrum with that of **18**. The disulfide group of **18** has been shown to have a strong positive Cotton effect near 270 nm.[15] Compound **13** showed the same sign as **18** at this characteristic CD absorbance. Thus, the absolute configuration of the disulfide bridge carbons in **13** are likely *R*. These data in conjunction with chemical shifts and NOESY correlations, as compared with those of rostratin A[6] from C-1 to C-9 and rostratin B[6] from C-1′ to C-9′, indicated the structure of **13** to be that shown in Figure 1.

The known compounds were identified as epicoccins A (**14**), B (**15**), C (**16**), and E (**17**),[2] rostratin A (**18**),[6] and (3*S*,6*S*)-3,6-dibenzylpiperazine-2,5-dione (**19**)[16] by comparison of experimental and literature spectroscopic data. Metabolites **1**−**18** could be postulated to be L-phenylalanine-derived DKPs with a unique framework. This hypothesis was confirmed using Marfey's methodology[17] to determine the absolute configuration of **19**. The acid hydrolysate of **19** was treated with Marfey's reagent, 5-fluoro-2,4-dinitrophenyl-L-alanine amide (FDAA), and the resulting FDAA derivatives were analyzed by reversed-phase HPLC. Peaks in the chromatogram were identified by comparing the retention times with those of the FDAA derivatives of the authentic amino acids. The Marfey's reagent derivative of the amino acids liberated from **19** showed peaks matching L-Phe. Therefore, the Phe residue in **19** was assigned the L-configuration. Epicoccins mainly consist of fungal metabolites with the unique basic diketopiperazine ring. For epicoccin I and related co-occurring compounds, we have postulated

Table 4. ^{1}H NMR Data for Compounds **11**−**13** in DMSO-d_6 (500 MHz)[a]

no.	11	12	13
3a	2.84, dd (13.0, 8.5)	2.84, dd (13.0, 8.0)	2.56, dd (14.0, 13.0)
3b	2.54, br d (13.0)	2.54, br d (13.0)	2.34, br d (14.0)
4	3.11, br dd (8.5, 8.5)	3.13, br dd (8.0, 8.0)	2.02, dddd (13.0, 11.5, 11.5, 5.0)
5			3.36, m
6a	3.06, br d (21.5)	3.06, br d (21.5)	1.83, m
6b	2.47, br d (21.5)	2.47, br d (21.5)	1.24, m (overlap)
7a	3.76, m	3.76, m	1.89, m
7b			1.24, m (overlap)
8	4.53, m (overlap)	4.55, m (overlap)	3.59, m
9	4.48, br d (8.5)	4.48, br d (8.0)	3.36, dd (11.5, 9.5)
3′a	3.04, dd (11.5, 6.5)	3.01, dd (11.0, 7.0)	2.92, dd (14.5, 7.5)
3′b	2.86, br d (11.5)	2.85, br d (11.0)	2.77, br d (14.5)
4′	3.11, br dd (6.5, 6.5)	3.10, br dd (8.0, 7.0)	3.23, br dd (7.5, 7.5)
6′a	3.58, m	3.42, br s	2.69, ddd (17.0, 12.5, 4.5)
6′b			2.28, ddd (17.0, 4.0, 3.5)
7′a	2.49, m (overlap)	4.10, br d (3.0)	1.93, m
7′b	2.49, m (overlap)		1.66, br ddd (17.0, 12.5, 4.0)
8′	4.48, m (overlap)	4.57, m	4.84, m
9′	4.53, br d (6.5)	4.56, br d (8.0)	4.37, dd (7.5, 3.0)
5-OH			5.03, d (5.0)
8-OH	6.07, d (3.5)	6.07, d (3.5)	6.02, s
8′-OH	5.52, s	5.54, s	5.48, d (4.0)
7′-CH$_2$-		3.46, q (7.0)	
7′-CH$_3$		1.08, t (7.0)	

[a] Chemical shifts δ_H mult. (*J* in Hz)

Table 5. Inhibition Effect of Compounds **2**, **6**, **12**, and **17** on PAF-Induced Release of β-Glucuronidase from Rat PMNs (in vitro)[a]

compound	2	6	12	17	ginkgolide B[b]
IC$_{50}$ (μM)	3.07	4.16	4.95	1.98	2.35

[a] The rest of the compounds exhibited no anti-inflammation effect.
[b] Positive control.

a biogenetic pathway in which phenylalanine would be enzymatically oxidized to its benzene oxide first[18] and further reduced to cyclohexanol.

The anti-inflammatory activity of compounds **1**−**19** was assayed by measuring inhibition of the platelet-activating factor (PAF)-induced release of β-glucuronidase from rat polymorphonuclear leukocytes (PMNs) in vitro. Compounds **2**, **6**, **12**, and **17** displayed anti-inflammatory activities (Table 5), with IC$_{50}$ values of 3.07, 4.16, 4.95, and 1.98 μM, respectively [positive control ginkgolide B (BN52021), IC$_{50}$ 2.35 μM]. However, all test compounds were inactive in the HCT-8, HCT-116, Bel-7402, BGC-823, A549, and A2780 cell lines.

Experimental Section

General Experimental Procedures. Optical rotations and CD spectra were recorded on a JASCO P-2000 polarimeter and a JASCO-2000 spectropolarimeter. UV spectra were measured on a JASCO V650 spectrophotometer. IR spectra were recorded on a Nicolet 5700 FT-IR spectrometer by the microscope transmission method. NMR spectra were obtained on an INOVA-500 spectrometer. Chemical shifts are given in δ (ppm) with solvent (DMSO-d_6) peaks used as reference. ESIMS were measured on an Agilent 1100 Series LC/MSD ion trap mass spectrometer. HRESIMS data were recorded on an Autospec Ultima-TOF mass spectrometer. Preparative HPLC was performed on a Shimadzu LC-6AD instrument with an SPD-10A detector, using a YMC-Pack ODS-A column (2 × 25 cm, 5 μm). Sephadex LH-20 (Amersham Pharmacia Biotech AB Factory) and ODS (45−70 μm, Merck) were used for column chromatography.

Fungal Material. The fungal strain of *E. nigrum* was isolated from the leaves of *L. rhodostegia*, collected in June 2007 at Nanning, Guangxi Province, People's Republic of China. The isolate was identified by Dr. Xianzhi Jiang and assigned the accession number LJJ028 in Professor X. Liu's culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The GenBank accession number was HM055593. Plugs of agar supporting mycelial growth were cut and transferred aseptically to a 1000 mL Erlenmeyer flask containing 400 mL of PDA medium (200 g of potato and 25 g of commercial sugar in 1000 mL of water). After incubation for 3 days at 28 °C on a rotary shaker at 110 rpm, 20 mL of culture liquid was transferred as a seed into each 1000 mL flask containing culture liquid (400 mL). The cultivation was then kept for 7 days at 28 °C.

Extraction and Isolation. The mycelium (2.4 kg) from the culture (255 L) was extracted with EtOH, and the extract and filtrate of the culture were mixed together. The mixture was concentrated to 10 L in vacuo below 50 °C and successively extracted with EtOAc and *n*-BuOH. The EtOAc extract (33.5 g) was applied to a Sephadex LH-20 column eluted with methanol to give three fractions (fraction A, 1.47 g; fraction B, 25.14 g; fraction C, 6.35 g). Fraction B (25.14 g) was chromatographed over ODS, eluting with a gradient of increasing MeOH (15−30%) in H$_2$O to afford four subfractions (B1−4). Fraction B1 (5.0 g) was separated by RP-HPLC using 20% CH$_3$CN in H$_2$O to yield **18** (45 mg). Fraction B2 (5.2 g) was chromatographed over ODS with a gradient of increasing MeOH (15−30%) in H$_2$O to afford seven subfractions (B2-1−B2-7). Fractions B2-3 (1.1 g), B2-4 (750 mg), B2-5 (750 mg), and B2-6 (300 mg) were chromatographed separately over Sephadex LH-20 with MeOH and separated by RP-HPLC using 25% CH$_3$CN in H$_2$O to afford pure compounds **1** (30 mg), **8** (7 mg), and **13** (9 mg) from B2-3, **4** (40 mg), **3** (10 mg), and **12** (35 mg) from B2-4, **2** (91 mg), **5** (30 mg), and **6** (82 mg) from B2-5, and **10** (15 mg), **17** (12 mg), and **9** (7 mg) from B2-6. Fraction C (6.35 g), eluted with 10% MeOH in H$_2$O, was chromatographed over ODS, eluting with a gradient of increasing MeOH (10−50%) in H$_2$O to afford four subfractions (C1−4). Fractions C2 (200 mg) and C3 (1.12 g) were purified by RP-HPLC with 30% CH$_3$CN in H$_2$O to yield pure compounds **15** (35 mg) from C2 and **14** (62 mg) from C3. Fraction C4 (3.6 g) was purified by RP-HPLC using 30% CH$_3$CN in H$_2$O to afford **7** (15 mg), **11** (10 mg), **16** (182 mg), and **19** (40 mg).

Epicoccin I (1): colorless needles (MeOH−H$_2$O); [α]$^{20}_D$ +147.7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 205 nm; IR ν_{max} 3467, 3276, 1644, 1412, 1073 cm^{-1}; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 1; ESIMS *m/z* 443 [M + Na]$^+$; HRESIMS *m/z* 443.0717 [M + Na]$^+$ (calcd for C$_{19}$H$_{20}$N$_2$O$_5$S$_2$Na, 443.0711).

X-ray Crystallographic Analysis of 1[19]. Upon crystallization from MeOH−H$_2$O (1:1) using the vapor diffusion method, colorless crystals of **1** were obtained. A crystal (0.28 mm × 0.20 mm × 0.20 mm) was separated from the sample and mounted on a glass fiber, and data were collected using a MM007HF + CCD (Saturn724+) area detector with a graphite monochromator and Mo KR radiation, λ = 0.71073 Å at 173(2) K. Crystal data: C$_{19}$H$_{20}$N$_2$O$_5$S$_2$, *M* = 420.49, space group monoclinic, *P*2$_1$; unit cell dimensions were determined to be *a* = 10.823(2) Å, *b* = 8.2414(16) Å, and *c* = 11.223(2) Å; *V* = 902.1(3) Å^3, *Z* = 2, *D*$_{calcd}$ = 1.548 mg/m^3, *F*(000) = 440. The 10 911

measurements yielded 4101 independent reflections after equivalent data were averaged and Lorentz and polarization corrections were applied. The structure was solved by direct methods using the SHELXL-97 program, expanded using difference Fourier techniques, and refined by the program SHELXL-97 and full-matrix least-squares calculations. The final refinement gave $R_1 = 0.0358$ and $wR_2 = 0.0808$.

Ent-epicoccin G (2): colorless needles (MeOH−H$_2$O); $[\alpha]^{20}_D$ −141.5 (c 0.1, MeOH); UV (MeOH) λ_{max} 205 nm; IR ν_{max} 3465, 2919, 1705, 1640, 1419, 1071 cm^{-1}; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 1; ESIMS m/z 455 [M + H]$^+$, 477 [M + Na]$^+$, 453 [M − H]$^-$, 489 [M + Cl]$^-$; HRESIMS m/z 477.11381 [M + Na]$^+$ (calcd for C$_{20}$H$_{26}$N$_2$O$_6$S$_2$Na, 477.1130).

Preparation of (R)- and (S)-MTPA Esters from 2. After dissolving in pyridine (0.5 mL) under a gentle argon stream, compound 2 (1.5 mg, 3.5 μmol) was placed in a dried, clean NMR tube, and (S)-MTPA chloride (2.0 μL, 10.6 μmol) was added and immediately shaken until uniformly mixed. The NMR tube was kept overnight at room temperature. The residue was purified by RP-HPLC using 80% CH$_3$CN in H$_2$O to afford the corresponding (R)-MTPA ester 2b (1.1 mg): ^{1}H NMR (500 MHz, DMSO-d_6, assigned by its ^{1}H−^{1}H COSY spectrum) δ_H 2.763 (1H, br d, J = 13.0 Hz, H-3/3′a), 2.363 (1H, dd, J = 13.0, 8.0 Hz, H-3/3′b), 2.829 (1H, br dd, J = 8.0, 8.0 Hz, H-4/4′), 2.453 (1H, m, H-6/6′a), 2.344 (1H, m, H-6/6′b), 2.321 (1H, m, H-7/7′a), 2.260 (1H, m, H-7/7′b), 5.852 (1H, m, H-8/8′), 4.326 (1H, br d, J = 8.0 Hz, H-9/9′). In an identical fashion, compound 2, (R)-MTPA chloride, and pyridine were combined in an NMR tube and allowed to stand overnight. ^{1}H NMR measurements showed the quantitative conversion of compound 2 to the anticipated (S)-MTPA ester 2a (1.3 mg): ^{1}H NMR (500 MHz, DMSO-d_6, assigned by ^{1}H−^{1}H COSY spectrum) δ_H 2.789 (1H, br d, J = 13.5 Hz, H-3/3′a), 2.400 (1H, dd, J = 13.0, 8.0 Hz, H-3/3′b), 3.036 (1H, br dd, J = 8.0, 8.0 Hz, H-4/4′), 2.328 (1H, m, H-6/6′a), 2.062 (1H, m, H-6/6′b), 2.271 (1H, m, H-7/7′a), 2.168 (1H, m, H-7/7′b), 5.789 (1H, m, H-8/8′), 4.439 (1H, br d, J = 8.0 Hz, H-9/9′).

X-ray Crystallographic Analysis of 2[20]. Upon crystallization from MeOH−H$_2$O (1:1) using the vapor diffusion method, colorless crystals of 2 were obtained. A crystal (0.57 mm × 0.40 mm × 0.34 mm) was separated from the sample and mounted on a glass fiber, and data were collected using a MM007HF + CCD (Saturn724+) area detector with a graphite monochromator and Cu KR radiation, λ = 1.54178 Å at 173(2) K. Crystal data: C$_{20}$H$_{34}$N$_2$O$_{10}$S$_2$, M = 526.61, space group monoclinic, $P2_1$; unit cell dimensions were determined to be a = 10.949(2) Å, b = 8.7488(17) Å, and c = 13.036(3) Å; V = 1232.7(4) Å^3, Z = 2, D_{calcd} = 1.419 mg/m^3, F(000) = 560. The 12 282 measurements yielded 4399 independent reflections after equivalent data were averaged and Lorentz and polarization corrections were applied. The structure was solved by direct methods using the SHELXL-97 program, expanded using difference Fourier techniques, and refined by the program SHELXL-97 and full-matrix least-squares calculations. The final refinement gave $R_1 = 0.0372$ and $wR_2 = 0.0955$.

Epicoccin J (3): white powder; $[\alpha]^{20}_D$ −75.9 (c 0.1, MeOH); UV (MeOH) λ_{max} 205 nm; IR ν_{max} 3412, 2924, 1700, 1666, 1397, 1073 cm^{-1}; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 1; ESIMS m/z 453 [M + H]$^+$, 475 [M + Na]$^+$, 487 [M + Cl]$^-$; HRESIMS m/z 453.1149 [M + H]$^+$ (calcd for C$_{20}$H$_{25}$N$_2$O$_6$S$_2$, 453.1154).

Epicoccin K (4): white powder; $[\alpha]^{20}_D$ −87.9 (c 0.1, MeOH); UV (MeOH) λ_{max} 205 nm; IR ν_{max} 3353, 2929, 1637, 1411, 1102 cm^{-1}; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 1; ESIMS m/z 459 [M + H]$^+$, 481 [M + Na]$^+$, 493 [M + Cl]$^-$; HRESIMS m/z 459.1671 [M + H]$^+$ (calcd for C$_{20}$H$_{31}$N$_2$O$_6$S$_2$, 459.1623).

Procedure for the in-NMR-Tube Selective Mosher's Reaction to Produce Mono-MTPA Esters.[6] The sample is dissolved in pyridine-d_5 in an NMR tube dried under a gentle argon stream. A ^{1}H NMR spectrum is recorded as a reference. The NMR tube is precooled to 0 °C, and a calculated amount of MTPA chloride is added. The NMR tube is rigorously shaken until the liquids are evenly mixed. The ^{1}H NMR spectrum is recorded every 15 min. When evidence of acylation is observed, the NMR tube is maintained at that temperature until complete conversion has been observed. The acylation reaction is quenched by adding one drop of D$_2$O (to hydrolyze excess MTPA chloride). The ^{1}H NMR and ^{1}H−^{1}H COSY spectra are acquired to derive ^{1}H NMR data of the MTPA esters, resolving the overlapping MTPA product signals when necessary.

Preparation of (R)- and (S)-MTPA Esters from 4. Following the procedure summarized above, compound 4 (2.5 mg, 5.2 μmol) was acylated in an NMR tube with (S)-MTPA chloride (4.0 μL, 21.2 μmol) in pyridine-d_5. The selective acylation was achieved at 0 °C for 30 min, with compound 4 being transformed entirely into the (R)-MTPA ester 4b: HPLC/MS m/z 913 [M + Na]$^+$; ^{1}H NMR (500 MHz, pyridine-d_5, assigned by its ^{1}H−^{1}H COSY spectrum) δ_H 2.490 (1H, br d, J = 13.0 Hz, H-3/3′a), 2.311 (1H, br d, J = 13.0 Hz, H-3/3′b), 2.700 (1H, m, H-4/4′), 5.300 (1H, m, H-5/5′), 2.275 (1H, m, H-6/6′a), 1.544 (1H, m, H-6/6′b), 2.242 (1H, m, H-7/7′a), 1.544 (1H, m, H-7/7′b), 3.970 (1H, m, H-8/8′), 3.970 (1H, m, H-9/9′). In the same fashion, compound 4 was treated with (R)-MTPA chloride in pyridine-d_5 to give the expected (S)-MTPA ester 4a: 1H NMR (500 MHz, pyridine-d_5, assigned by its ^{1}H−^{1}H COSY spectrum) δ_H 2.682 (1H, br d, J = 13.0 Hz, H-3/3′a), 2.645 (1H, br d, J = 13.0 Hz, H-3/3′b), 2.853 (1H, m, H-4/4′), 5.226 (1H, m, H-5/5′), 2.212 (1H, m, H-6/6′a), 1.450 (1H, m, H-6/6′b), 2.212 (1H, m, H-7/7′a), 1.527 (1H, m, H-7/7′b), 3.826 (1H, m, H-8/8′), 4.049 (1H, m, H-9/9′); ^{1}H NMR (500 MHz, pyridine-d_5, assigned by spin decoupling) of 4, as a reference spectrum for the selective Mosher's reaction, δ_H 3.011 (1H, br d, J = 13.0 Hz, H-3/3′a), 2.287 (1H, br d, J = 13.0 Hz, H-3/3′b), 2.768 (1H, m, H-4/4′), 3.716 (1H, m, H-5/5′), 2.266 (1H, m, H-6/6′a), 1.645 (1H, m, H-6/6′b), 2.093 (1H, m, H-7/7′a), 1.564 (1H, m, H-7/7′b), 3.807 (1H, m, H-8/8′), 3.662 (1H, m, H-9/9′).

Epicoccin L (5): white powder; $[\alpha]^{20}_D$ −88.0 (c 0.1, MeOH); UV (MeOH) λ_{max} 205 nm; IR ν_{max} 3348, 2926, 1700, 1640, 1406, 1110 cm^{-1}; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 1; ESIMS m/z 457 [M + H]$^+$, 479 [M + Na]$^+$, 455 [M − H]$^-$, 491 [M + Cl]$^-$; HRESIMS m/z 479.1262 [M + Na]$^+$ (calcd for C$_{20}$H$_{28}$N$_2$O$_6$S$_2$Na, 479.1286).

Epicoccin M (6): white powder; $[\alpha]^{20}_D$ +339.4 (c 0.1, MeOH); UV (MeOH) λ_{max} 205 nm; IR ν_{max} 3373, 2923, 1665, 1403, 1000 cm^{-1}; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 1; ESIMS m/z 471 [M + H]$^+$, 493 [M + Na]$^+$, 469 [M − H]$^-$, 505 [M + Cl]$^-$; HRESIMS m/z 471.0717 [M + H]$^+$ (calcd for C$_{19}$H$_{23}$N$_2$O$_6$S$_3$, 471.0718).

Preparation of (R)- and (S)-MTPA Esters from 6. After dissolving in pyridine (0.5 mL) under a gentle argon stream, compound 6 (2.5 mg, 5.2 μmol) was placed in a dried, clean NMR tube and (S)-MTPA chloride (2.0 μL, 10.6 μmol) was added and immediately shaken until uniformly mixed. The NMR tube was kept overnight at room temperature. The residue was purified by RP-HPLC using 80% CH$_3$CN in H$_2$O to afford the corresponding (R)-MTPA ester 6b (0.5 mg): ^{1}H NMR (500 MHz, DMSO-d_6, assigned by its ^{1}H−^{1}H COSY spectrum) δ_H 2.815 (1H, br d, J = 13.0 Hz, H-3a), 2.448 (1H, dd, J = 13.0, 8.0 Hz, H-3b), 2.839 (1H, br dd, J = 8.0, 8.0 Hz, H-4), 2.355 (1H, m, H-6a), 2.322 (1H, m, H-6b), 2.297 (1H, m, H-7a), 2.252 (1H, m, H-7b), 5.901 (1H, s, H-8), 4.420 (1H, br d, J = 8.0 Hz, H-9), 2.810 (1H, br d, J = 13.0 Hz, H-3′a), 2.417 (1H, dd, J = 13.0, 8.5 Hz, H-3′b), 2.926 (1H, br dd, J = 8.5, 8.5 Hz, H-4′), 2.734 (1H, m, H-6′a), 2.625 (1H, m, H-6′b), 4.236 (1H, m, H-7′), 5.901 (1H, s, H-8′), 4.564 (1H, br d, J = 8.0 Hz, H-9′). Treatment of 6 in the same manner with (R)-MTPA chloride in pyridine showed a quantitative conversion to the (S)-MTPA ester 6a (0.7 mg): ^{1}H NMR (500 MHz, DMSO-d_6, assigned by its ^{1}H−^{1}H COSY spectrum) δ_H 2.863 (1H, br d, J = 13.0 Hz, H-3a), 2.490 (1H, dd, J = 13.0, 8.0 Hz, H-3b), 3.098 (1H, br dd, J = 8.0, 8.0 Hz, H-4), 2.349 (1H, m, H-6a), 2.063 (1H, m, H-6b), 2.267 (1H, m, H-7a), 2.135 (1H, m, H-7b), 5.855 (1H, s, H-8), 4.517 (1H, br d, J = 8.0 Hz, H-9), 2.847 (1H, br d, J = 13.0 Hz, H-3′a), 2.449 (1H, dd, J = 13.0, 8.5 Hz, H-3′b), 3.211 (1H, br dd, J = 8.5, 8.5 Hz, H-4′), 2.525 (1H, m, H-6′a), 2.380 (1H, m, H-6′b), 4.151 (1H, m, H-7′), 5.855 (1H, s, H-8′), 4.646 (1H, br d, J = 8.5 Hz, H-9′).

Epicoccin N (7): white powder; $[\alpha]^{20}_D$ +229.2 (c 0.1, MeOH); UV (MeOH) λ_{max} 205 nm; IR ν_{max} 3408, 2923, 1667, 1408, 1073 cm^{-1}; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 1; ESIMS m/z 487 [M + H]$^+$, 509 [M + Na]$^+$, 485 [M − H]$^-$, 521 [M + Cl]$^-$; HRESIMS m/z 487.0671 [M + H]$^+$ (calcd for C$_{19}$H$_{23}$N$_2$O$_7$S$_3$, 487.0667).

Epicoccin O (8): white powder; $[\alpha]^{20}_D$ +106.3 (c 0.1, MeOH); UV (MeOH) λ_{max} 205 nm; IR ν_{max} 3415, 2929, 1725, 1663, 1412, 1053 cm^{-1}; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 1; ESIMS m/z 461 [M + Na]$^+$, 437 [M − H]$^-$, 473 [M + Cl]$^-$; HRESIMS m/z 439.1005 [M + H]$^+$ (calcd for C$_{19}$H$_{23}$N$_2$O$_6$S$_3$, 439.0998).

Preparation of (R)- and (S)-MTPA Esters from 8. After dissolving in pyridine (0.5 mL) under a gentle argon stream, compound 8 (1.0 mg, 2.3 μmol) was placed in a dried, clean NMR tube and (S)-MTPA chloride (1.0 μL, 5.3 μmol) was added and immediately shaken until

uniformly mixed. The NMR tube was kept overnight at room temperature. The residue was purified by RP-HPLC using 80% CH$_3$CN in H$_2$O to afford the corresponding (*R*)-MTPA ester **8b** (0.2 mg): ^{1}H NMR (500 MHz, DMSO-d_6, assigned by its ^{1}H$-^1$H COSY spectrum) δ_H 2.842 (1H, br d, *J* = 13.0 Hz, H-3a), 2.490 (1H, br d, *J* = 13.0, H-3b), 2.921 (1H, br dd, *J* = 8.0, 8.0 Hz, H-4), 2.490 (1H, m, H-6a), 2.331 (1H, m, H-6b), 2.279 (1H, m, H-7a), 2.279 (1H, m, H-7b), 5.862 (1H, s, H-8), 4.481 (1H, br d, *J* = 8.0 Hz, H-9), 3.040 (1H, br d, *J* = 13.0 Hz, H-3′a), 2.842 (1H, br d, *J* = 13.0 Hz, H-3′b), 2.896 (1H, br dd, *J* = 8.5, 8.5 Hz, H-4′), 2.765 (1H, d, *J* = 9.0 Hz, H-6′), 2.946 (1H, m, H-7′a), 2.738 (1H, m, H-7′b), 5.883 (1H, s, H-8′), 4.807 (1H, br d, *J* = 8.5 Hz, H-9′). Treatment of **8** in the same manner with (*R*)-MTPA chloride in pyridine showed a quantitative conversion to the (*S*)-MTPA ester **8a** (0.2 mg): ^{1}H NMR (500 MHz, DMSO-d_6, assigned by its ^{1}H$-^1$H COSY spectrum) δ_H 2.859 (1H, br d, *J* = 13.0 Hz, H-3a), 2.492 (1H, br d, *J* = 13.0 Hz, H-3b), 2.929 (1H, br dd, *J* = 8.0, 8.0 Hz, H-4), 2.490 (1H, m, H-6a), 2.074 (1H, m, H-6b), 2.061 (1H, m, H-7a), 2.061 (1H, m, H-7b), 5.809 (1H, s, H-8), 4.567 (1H, br d, *J* = 8.0 Hz, H-9), 3.094 (1H, br d, *J* = 13.0 Hz, H-3′a), 2.850 (1H, br d, *J* = 13.0 Hz, H-3′b), 2.929 (1H, br dd, *J* = 8.5, 8.5 Hz, H-4′), 3.722 (1H, d, *J* = 9.0 Hz, H-6′), 2.895 (1H, m, H-7′a), 2.625 (1H, m, H-7′b), 5.860 (1H, s, H-8′), 4.904 (1H, br d, *J* = 8.5 Hz, H-9′).

Epicoccin P (9): white powder; [α]$^{20}_D$ −74.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 205 nm; IR ν_{max} 3395, 2931, 1725, 1648, 1415, 1109 cm^{-1}; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 1; ESIMS *m/z* 463 [M + Na]$^+$, 439 [M − H]$^-$, 475 [M + Cl]$^-$; HRESIMS *m/z* 441.1164 [M + H]$^+$ (calcd for C$_{19}$H$_{25}$N$_2$O$_6$S$_2$, 441.1154).

Epicoccin Q (10): colorless needles (MeOH−H$_2$O); [α]$^{20}_D$ +537.8 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 205 nm; IR ν_{max} 3446, 2951, 1701, 1650, 1418, 1050 cm^{-1}; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 1; ESIMS *m/z* 493 [M + Na]$^+$, 469 [M − H]$^-$, 505 [M + Cl]$^-$; HRESIMS *m/z* 471.0362 [M + H]$^+$ (calcd for C$_{18}$H$_{19}$N$_2$O$_7$S$_3$, 471.0354).

X-ray Crystallographic Analysis of 10[21]. Upon crystallization from MeOH−H$_2$O (1:1) using the vapor diffusion method, colorless crystals of **10** were obtained. A crystal (0.15 mm × 0.10 mm × 0.09 mm) was separated from the sample and mounted on a glass fiber, and data were collected using a MM007HF + CCD (Saturn724+) area detector with a graphite monochromator and Mo KR radiation, λ = 0.71073 Å at 173(2) K. Crystal data: C$_{18}$H$_{20}$N$_2$O$_8$S$_3$, *M* = 488.54, space group monoclinic, *P*2$_1$; unit cell dimensions were determined to be *a* = 6.5869(13) Å, *b* = 8.1866(16) Å, and *c* = 17.513(4) Å; *V* = 940.5(3) Å^3, *Z* = 2, D_{calcd} = 1.725 mg/m^3, *F*(000) = 508. The 12 690 measurements yielded 4211 independent reflections after equivalent data were averaged and Lorentz and polarization corrections were applied. The structure was solved by direct methods using the SHELXL-97 program, expanded using difference Fourier techniques, and refined by the program SHELXL-97 and full-matrix least-squares calculations. The final refinement gave R_1 = 0.0360 and wR_2 = 0.0799.

Epicoccin R (11): white powder; [α]$^{20}_D$ +477.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 205 nm; IR ν_{max} 3427, 2931, 1726, 1668, 1412, 1052 cm^{-1}; ^{1}H NMR data, see Table 4; ^{13}C NMR data, see Table 1; ESIMS *m/z* 477 [M + Na]$^+$, 453 [M − H]$^-$, 489 [M + Cl]$^-$; HRESIMS *m/z* 455.0411 [M + H]$^+$ (calcd for C$_{18}$H$_{19}$N$_2$O$_6$S$_3$, 455.0405).

Epicoccin S (12): white powder; [α]$^{20}_D$ +682.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 205 nm; IR ν_{max} 3443, 2973, 1730, 1673, 1408, 1054 cm^{-1}; ^{1}H NMR data, see Table 4; ^{13}C NMR data, see Table 1; ESIMS *m/z* 499 [M + H]$^+$, 521 [M + Na]$^+$, 497 [M − H]$^-$, 533 [M + Cl]$^-$; HRESIMS *m/z* 499.0675 [M + H]$^+$ (calcd for C$_{20}$H$_{23}$N$_2$O$_7$S$_3$, 499.0667).

Epicoccin T (13): white powder; [α]$^{20}_D$ −221.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 205 nm; CD (MeOH) 265 nm (Δε +0.06); 228 nm (Δε −0.24); IR ν_{max} 3371, 2938, 1664, 1377, 1112 cm^{-1}; ^{1}H NMR data, see Table 4; ^{13}C NMR data, see Table 1; ESIMS *m/z* 449 [M + Na]$^+$, 425 [M − H]$^-$, 461 [M + Cl]$^-$; HRESIMS *m/z* 427.1001 [M + H]$^+$ (calcd for C$_{18}$H$_{23}$N$_2$O$_6$S$_2$, 427.0998).

Absolute Configuration of 19. A standard amino acid (0.2 mg, Sigma) was dissolved in 30 μL of H$_2$O and treated with 1% 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA) in acetone (60 μL) and 6% Et$_3$N in 30 μL of H$_2$O at 40 °C for 1 h. After cooling to room temperature, the derivative was analyzed by reversed-phase HPLC with detection by UV absorption at 340 nm. The column [Agilent C18, 5 μm, 4.6 × 150 mm] was eluted with a linear gradient of (A) CH$_3$CN and (B) 0.05% aqueous TFA from 10% to 40% (A) over 30 min followed by isocratic elution with 40% A at a flow rate of

1 mL/min. The standards gave the following retention times in min: 21.7 for FDAA, 31.4 for L-Phe, and 34.3 for D-Phe. (3*S*,6*S*)-3,6-Dibenzylpiperazine-2,5-dione (**19**, 1 mg) was dissolved in 6 N HCl (0.5 mL) and heated at 110 °C in a sealed vial overnight to yield the corresponding amino acids. The cooled reaction mixture was evaporated to dryness under reduced pressure, and HCl was removed from the residual acid hydrolysate by repeated evaporation from frozen H$_2$O (1 mL). The amino acid mixture was then treated in the same manner as the standards above (1% FDAA and 6% Et$_3$N). The mixture of FDAA derivatives was filtered, and the filtrate was diluted with H$_2$O and analyzed by HPLC. The FDAA derivative of the amino acid liberated from **19** showed a peak at 31.4 min, matching the retention time of L-Phe.

Anti-inflammatory Activity Assay. See ref 22.

Supporting Information Available: 1D and 2D NMR spectra of compounds **1−19**, 1D NMR spectra of known compounds **14−19**, ^{1}H NMR spectra of (*R*)- and (*S*)-MTPA derivatives of **2**, **4**, **6**, and **8**, HPLC analysis chromatograms of FDAA derivatives, and IR and MS spectra of compounds **1−19**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Hu, Y. C.; Wu, X. F.; Gao, S.; Yu, S. S.; Liu, Y.; Qu, J.; Liu, J.; Liu, Y. B. *Org. Lett.* **2006**, *7*, 2269–2272. (b) Gao, S.; Feng, N.; Yu, S. S.; Yu, D. Q.; Wang, X. L. *Planta Med.* **2004**, *70*, 1128–1134. (c) Gao, S.; Liu, J.; Fu, G. M.; Hu, Y. C.; Yu, S. S.; Fan, L. H.; Yu, D. Q.; Qu, J. *Planta Med.* **2007**, *70*, 163–166. (d) Gao, S.; Fu, G. M.; Fan, L. H.; Yu, S. S.; Yu, D, Q. *J. Integr. Plant. Biol.* **2005**, *47*, 759–763. (e) Gao, S.; Yu, S. S.; Yu, D. Q. *Chin. Chem. Lett.* **2004**, *15*, 313–315. (f) Gao, S.; Fu, G. M.; Fan, L. H.; Yu, S. S.; Yu, D. Q. *Chin. J. Nat. Med.* **2005**, *3*, 144–147. (g) Wu, X. F.; Hu, Y. C.; Gao, S.; Yu, S. S.; Pei, Y. H.; Tang, W. Z.; Huang, X. Z. *J. Asian Nat. Prod. Res.* **2007**, *9*, 471–477. (h) Wu, X. F.; Hu, Y. C.; Yu, S. S.; Jiang, N.; Ma, J.; Tan, R. X.; Li, Y.; Lv, H. N.; Liu, J.; Ma, S. G. *Org. Lett.* **2010**, *12*, 2390–2393.

(2) (a) Zhang, Y. G.; Liu, S. C.; Che, Y. S.; Liu, X. Z. *J. Nat. Prod.* **2007**, *70*, 1522–1525. (b) Guo, H. J.; Sun, B. D.; Gao, H.; Chen, X. L.; Liu, S. C.; Yao, X. S.; Liu, X. Z.; Che, Y. S. *J. Nat. Prod.* **2009**, *72*, 2115–2119.

(3) (a) Beecham, A. F.; Fridrichsons, J.; Mathieson, A. M. *Tetrahedron Lett.* **1966**, *27*, 3131–3138. (b) Li, X. F.; Kim, S. K.; Nam, K. W.; Kang, K. M.; Choi, H. D.; Son, B. W. *J. Antibiot.* **2006**, *59*, 248–250. (c) Lee, H. J.; Lee, J. H.; Hwang, B. Y.; Kim, H. S.; Lee, J. J. *Arch. Pharm. Res.* **2001**, *24*, 397–401.

(4) Moncrief, J. W. *J. Am. Chem. Soc.* **1968**, *90*, 6517–6518.

(5) (a) Deffieux, G.; Baute, M. A.; Baute, R.; Filleau, M. J. *J. Antibiot.* **1978**, *31*, 1102–1105. (b) Deffieux, G.; Filleau, M. J.; Baute, R. *J. Antibiot.* **1978**, *31*, 1106–1109. (c) Kleinwachter, P.; Dahse, H. M.; Luhmann, U.; Schlegel, B.; Dornberger, K. *J. Antibiot.* **2001**, *54*, 521–525.

(6) Tan, R. X.; Jensen, P. R.; Williams, P. G.; Fenical, W. *J. Nat. Prod.* **2004**, *67*, 1374–1382.

(7) Sugawara, K.; Sugawara, F.; Strobel, G. A. *J. Org. Chem.* **1985**, *50*, 5631–5633.

(8) Isaka, M.; Palasarn, S.; Rachtawee, P.; Vimuttipong, S.; Kongsaeree, P. *Org. Lett.* **2005**, *7*, 2257–2260.

(9) Minato, B. H.; Matsumoto, M.; Katayama, T.; Verticillin, A. *J. Chem. Soc., Chem. Commun.* **1971**, *1*, 44–45.

(10) Jamieson, W. D.; Rahman, R.; Taylor, A. *J. Chem. Soc. C* **1969**, *11*, 1564–1567.

(11) Safe, S.; Taylor, A. *J. Chem. Soc., Perkin Trans. 1* **1972**, *4*, 472–479.

(12) (a) Strobel, G. A. *Microbes Infect.* **2003**, *5*, 535–544. (b) Zhang, H. W.; Song, Y. C.; Tan, R. X. *Nat. Prod. Rep.* **2006**, *23*, 753–771.

(13) Flack, H. D. *Acta Crystallogr. A* **1983**, *39*, 876–881.

(14) (a) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543–2549. (b) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.

(15) Nagarajan, R; Woody, R W. *J. Am. Chem. Soc.* **1973**, *95*, 7212–7222.

(16) Sun, Y.; lv, A. L.; Tian, L.; Wei, L.; Pei, Y. H. *J. Shenyang Pharm. Univ.* **2007**, *24*, 546–559.

(17) Summers, M. Y.; Kong, F. M.; Feng, X. D.; Siegel, M. M.; Janso, J. E.; Graziani, E. I.; Carter, G. T. *J. Nat. Prod.* **2007**, *70*, 391–396.

(18) Rastetter, W. H.; Chancellor, T.; Richard, T. J. *J. Org. Chem.* **1982**, *47*, 1509–1512.

(19) Crystallographic data for compound **1** have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC-758494). Copies of the data can be obtained, free of charge, on application to the director, CCDC, 12 Union Rd., Cambridge CB21EZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

(20) Crystallographic data for compound **2** have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC-762009).

(21) Crystallographic data for compound **10** have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC-762008).

(22) Liu, J.; Liu, Y. B.; Si, Y. K.; Yu, S. S.; Qu, J.; Xu, S.; Hu, Y. C.; Mang, S. G. *Steroids* **2009**, *74*, 51–61.

NP1000895