Correction to Azaphilones from the Endophyte Chaetomium globosum.

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · AUGUST 2011

Impact Factor: 3.8 · DOI: 10.1021/np200556p · Source: PubMed

JOURNAL OF NATURAL PRODUCTS

ARTICLE

pubs.acs.org/jnp

Azaphilones from the Endophyte *Chaetomium globosum*

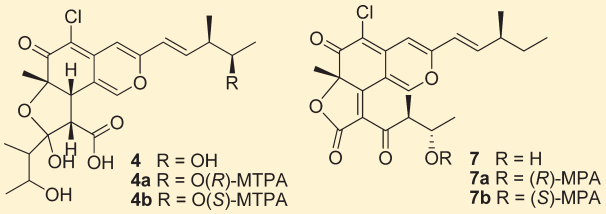
Warley S. Borges,[†,‡] Gabriela Mancilla,[§] Denise O. Guimarães,[†] Rosa Durán-Patrón,[§] Isidro G. Collado,[*,§] and Mônica T. Pupo[*,†]

[†]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, 14040-903, Ribeirão Preto, SP, Brazil

[§]Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, 11510, Puerto Real, Cádiz, Spain

Supporting Information

ABSTRACT: Six new azaphilones, 5′-epichaetoviridin A (**7**), 4′-epichaetoviridin F (**9**), 12β-hydroxychaetoviridin C (**10**), and chaetoviridins G−I (**11**−**13**), and six known azaphilones, chaetoviridins A−E (**1**−**5**) and 4′-epichaetoviridin A (**8**), were isolated from the endophytic fungus *Chaetomium globosum* cultivated in PDB medium for 21 days. The structure elucidation and the assignment of the relative configurations of the new natural products were based on detailed NMR and MS spectroscopic analyses. The structure of compound **1** was confirmed by single-crystal X-ray diffraction analysis. The absolute configurations of compounds **4**, **7**, **8**, and **12** were determined using Mosher's method. The antibiotic activity of the compounds was evaluated using an in vivo *Caenorhabditis elegans* infection model.



Endophytic fungi are microorganisms that live in the inter- and intracellular spaces of the tissues of apparently healthy host plants and do so in a variety of relationships, ranging from symbiotic to pathogenic.[1] Endophytes can inhabit the tissue of living plants for all or part of their life cycle and are considered relatively underexploited sources of novel bioactive compounds.[2,3]

As part of our bioprospecting studies on endophytes, we isolated several endophytic fungi that produce bioactive compounds.[4,5] The endophytic strain *Chaetomium globosum*, isolated from the leaves of *Viguiera robusta* (Asteraceae), was found to produce cytotoxic chaetoglobosins in Czapek medium.[6] Other recent studies on biologically active metabolites from the endophytic *C. globosum* from *Ginkgo biloba* led to the isolation of bioactive azaphilones.[7]

The azaphilones represent a structurally diverse family of natural products containing a pyrano-quinone structure. These compounds have been isolated from various fungi. Chaetoviridins were first characterized from the soil strain *C. globosum* var. *flavo-viriade*.[8] Seven chaetoviridins have been reported to date: chaetoviridins A−F (**1**−**6**) and 4′-epichaetoviridin A (**8**).[8,9]

Chaetoviridin A (**1**) inhibits tumor promotion by 12-*O*-tetradecanoylphorbol-13-acetate in a two-stage carcinogenesis model in mice.[10] Tomoda et al.[11] found that chaetoviridins A (**1**) and B (**2**) inhibited cholesteryl ester transfer protein. Chaetoviridin A (**1**) showed antifungal activity, inhibiting the growth of *Pyricularia oryzae* mycelia at 2.5 μg mL^{-1}.[8] Recently, Park et al.[12] reported that chaetoviridins A (**1**) and B (**2**) also display inhibitory activity against the in vitro growth of *Magnaporthe grisea* (rice blast) and *Pythium ultimum* mycelia with MIC values of 1.23 and 33.3 μg mL^{-1}, respectively. In addition, chaetoviridins A (**1**) and B (**2**) exhibited strong in vivo antifungal activity against *M. grisea* and *Puccnicia recondite* (wheat leaf rust).

In this paper, we describe the isolation and structural elucidation of six new azaphilones (**7**, **9**−**13**) along with six previously known azaphilones (**1**−**5**, **8**).[8,9] Structures were established on the basis of spectroscopic data, mainly 1D and 2D NMR. The structure previously reported for *epi*-chaetoviridin A has been revised and corrected to **8**. In addition, the in vivo anti-infective activities of the isolated azaphilones (**1**−**5**, **7**−**13**) were evaluated using the well-studied nematode *Caenorhabditis elegans* infected with the human opportunistic pathogen *Enterococcus faecalis*.

RESULTS AND DISCUSSION

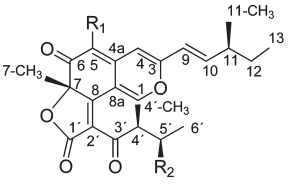
Cultured *C. globosum* produced the known chaetoviridins **1**−**5** and **8**[8,9] and six new chaetoviridins: 5′-epichaetoviridin A (**7**), 4′-epichaetoviridin F (**9**), 12β-hydroxychaetoviridin C (**10**), and chaetoviridins G−I (**11**−**13**).

The known chaetoviridins A−E (**1**−**5**) were identified by comparing their physical and spectroscopic data with literature values.[8,9] The structure of chaetoviridin A (**1**) was confirmed by single-crystal X-ray diffraction analysis (Figure 1).

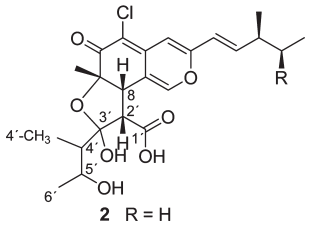
The spectroscopic data of compound **8** (Supporting Information) were coincident with those published in the literature for *epi*-chaetoviridin A, reported as the $C_{5'}$ epimer of **1**.[9] The epimerization at $C_{5'}$ was proposed on the basis of no correlation between H-4′ and H-5′ in the NOESY spectrum, which had been observed in **1**.[9] We applied the Mosher method to unambiguously establish the orientation of the hydroxyl group at $C_{5'}$. The

Received: February 7, 2011
Published: May 06, 2011

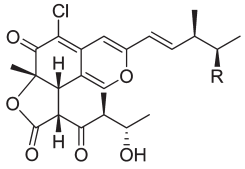
(*R*)- and (*S*)-MPA esters **8a** and **8b** were obtained by treatment of **8** with (*R*)- and (*S*)-MPA acids, respectively. Negative $\Delta\delta^{RS}$



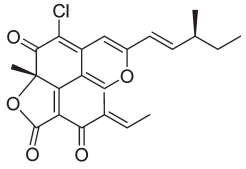
1 R_1 = Cl, R_2 = OH
6 R_1 = Cl, R_2 = H
12 R_1 = H, R_2 = OH
12a R_1 = H, R_2 = O(*R*)-MPA
12b R_1 = H, R_2 = O(*S*)-MPA



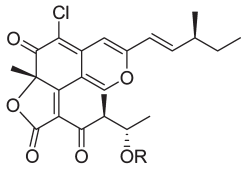
2 R = H
4 R = OH
4a R = O(*R*)-MPA
4b R = O(*S*)-MPA



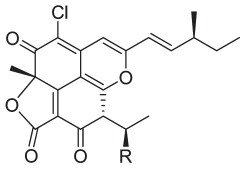
3 R = H
10 R = OH



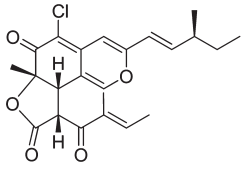
5



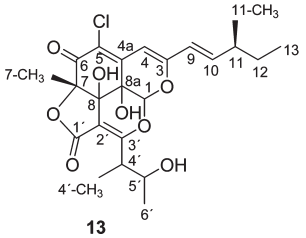
7 R = H
7a R = (*R*)-MPA
7b R = (*S*)-MPA



8 R = OH
8a R = O(*R*)-MPA
8b R = O(*S*)-MPA
9 R = H



11



13

($\delta_R - \delta_S$) values were found for H-4′ and H-4′CH_3 (−0.102 and −0.314, respectively), while a positive $\Delta\delta^{RS}$ value was found for H-6′ (+0.125). Following the MPA rules, these data indicated the *R*-configuration for $C_{5'}$ in **8**, which is identical to those described for **1**. In addition, $C_{4'}$, $C_{5'}$, and $C_{6'}$ chemical shifts of **8** are shielded compared to the same carbons in **1**, while in compound **7** $C_{5'}$ is deshielded compared to **1**. These data suggested that compound **8** must be the 4′-epimer, and not the 5′-epimer, of chaetoviridin A (**1**), as proposed by Phonkerd et al.[9] Therefore, the NMR data previously attributed to *epi*-chaetoviridin A[9] are in fact in agreement with the structure of 4′-epichaetoviridin A (**8**).

The HRMS and ^{13}C NMR spectra of compound **7** established its molecular composition as $C_{23}H_{25}ClO_6$, a chaetoviridin A (**1**) isomer. Its ^1H NMR spectrum showed signals very similar to those of **1** (Supporting Information). However, the upfield shift of the signal corresponding to H-4′CH_3 and the deshielded chemical shift of the signal corresponding to H-6′ suggested that this compound was 5′-epichaetoviridin A (**7**). This hypothesis was confirmed by the application of Mosher's method. In accordance with this method, the corresponding Mosher diastereoisomeric esters **7a** and **7b** were obtained by treatment of **7** with (*R*)- and (*S*)-MPA acids, respectively. Positive $\Delta\delta^{RS}$ ($\delta_R - \delta_S$)

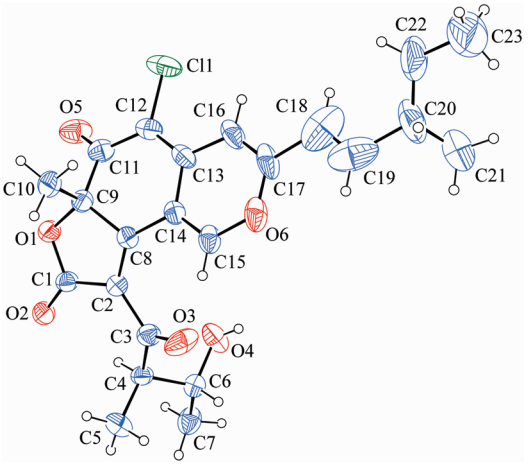


Figure 1. ORTEP drawing of the asymmetric unit of chaetoviridin A (**1**) with arbitrary atom numbering. The hydrogen atoms are represented as arbitrary radius spheres, and the ellipsoids of the non-H atoms are at the 50% probability level.

values were found for H-4′ and H-4′CH_3 (+0.101 and +0.105, respectively), while a negative $\Delta\delta^{RS}$ value was found for H-6′ (−0.175). Following the MPA rules, these data indicated the *S*-configuration for $C_{5'}$ in **7**.

The absolute configurations of C_7 and C_{11} in chaetoviridin A (**1**) have been reported as *S*.[8] Comparison of the NMR data of chaetoviridins B (**2**) and D (**4**) with those of **1** (Supporting Information) suggested the *S*-configuration for both positions in both compounds. On the other hand, the relative configuration of the C_8 and $C_{2'}$ positions of chaetoviridins B (**2**) and D (**4**) was suggested to be *cis* by Takahashi et al.[8] on the basis of the magnitude of the coupling constants (10.2 Hz in **2** and 9.7 Hz in **4**). A qualitative analysis of the NOE experiments confirmed this disposition. In particular, NOE interactions were detected between H-7CH_3, H-8, and H-2′, supporting the *S*-configuration for C_8 and $C_{2'}$. Finally, the orientation of the secondary hydroxyl group at C_{12} in chaetoviridin D (**4**) was established by the application of the Mosher's method. In accordance with this method, the corresponding Mosher diastereoisomeric esters **4a** and **4b** were obtained by treatment of **4** with (*R*)- and (*S*)-MTPA acids, respectively. The analysis by ^1H NMR of the resulting esters **4a** and **4b** revealed negative $\Delta\delta^{SR}$ ($\delta_S - \delta_R$) values for H-10, H-11, and H-11CH_3 (−0.14, −0.062, and −0.086, respectively) and a positive $\Delta\delta^{SR}$ value for H-13 (+0.043). Following the MTPA rules, these data indicated the *R*-configuration for C_{12} in chaetoviridin D (**4**). The absolute configuration of $C_{5'}$ could not be determined by this method, as it was not possible to derivatize the secondary alcohol linked to this carbon. On the basis of above data, the relative configurations of the chaetoviridins B and D were established as indicated in the structures of **2** and **4**.

Comparison of the NMR data of chaetoviridin C (**3**) with those of chaetoviridin A (**1**) (Supporting Information) suggested the *S*-configuration for C_7 and C_{11}. The relative configurations of the C_8−$C_{2'}$ positions of **3** were shown to be *cis* as in **2** and **4** by NOE difference spectra. In particular, NOE interactions were detected between H-7CH_3, H-8, and H-2′, supporting the *S*-configuration for C_8 and $C_{2'}$. The magnitude of the coupling constant between both protons H-4′ and H-5′ (9.1 Hz) was closer to that of **7** (8.1 Hz) than to that of **8** (3.0 Hz), suggesting

dx.doi.org/10.1021/np200110f |*J. Nat. Prod.* 2011, 74, 1182–1187

Table 1. ^{1}H NMR Data of Compounds 7 and 9−13 (400 MHz, $CDCl_3$)

	$\delta_{H,m}$ (J in Hz)					
position	7	9	10	11	12	13
1	8.87, s	8.79, s	7.38, s	7.33, s	8.77, s	6.00, s
4	6.57, s	6.57, s	6.50, s	6.49, s	6.01, s	6.15, s
5					5.32, s	
8			3.90, d (12.3)	4.11, d (12.2)		
9	6.10, d (15.9)	6.08, d (15.9)	6.12, d (15.9)	6.03, d (15.8)	5.95, d (15.9)	6.02, d (15.4)
10	6.63, dd (8.2, 15.9)	6.61, dd (7.7, 15.9)	6.62, dd (8.2, 15.9)	6.50, dd (8.1, 15.7)	6.50, dd (8.2, 15.9)	6.65, dd (7.7, 15.4)
11	2.30, m	2.29, m	2.45, m	2.25, m	2.24, m	2.27, m
12	1.46, m	1.45, m	3.82, m	1.41, m	1.42, m	1.45, m
13	0.92, t (7.2)	0.91, t (7.2)	1.19, d (6.1)	0.88, t (7.3)	0.89, t (7.2)	0.91, t (7.2)
7-CH_3	1.73, s	1.71, s	1.60, s	1.63, s	1.67, s	1.76, s
11-CH_3	1.10, d (6.6)	1.10, d (6.6)	1.11, d (6.7)	1.07, d (6.7)	1.06, d (6.2)	1.09, d (6.2)
2′			4.21, d (12.3)	4.37, d (12.2)		
4′	3.63, m	3.50, sext (6.7)	3.19, dq (6.7, 9.2)		3.65, m	3.31, m
5′	3.90, m	1.84, m 1.37, m	3.82, m	6.80, br q (6.9)	3.87, dq (6.1, 6.7)	3.64, dq (6.2, 6.7)
6′	1.33, d (6.6)	0.96, t (7.2)	1.24, d (6.1)	1.94, dd (1.1, 6.9)	1.16, d (6.7)	1.09, d (6.7)
4′-CH_3	1.02, d (7.2)	1.00, d (6.7)	1.05, d (6.7)	1.83, s	1.15, d (6.2)	1.11, d (6.2)

an *anti*-disposition between H-4′ and H-5′ and the *S*-configuration for $C_{4'}$ and $C_{5'}$. From these results, the relative configuration of chaetoviridin C was established as indicated in the structure of **3**.

Compound **9** was assigned the molecular formula $C_{23}H_{25}ClO_5$, as deduced from the HREIMS. The ^{1}H and ^{13}C NMR data of **9** (Tables 1 and 2) indicated a structure similar to that of chaetoviridin F (**6**).[9] However, there were some differences in chemical shifts between both compounds. In particular, the deshielded H-5′, H-6′, and 4′-CH_3 carbon signals suggested an opposite configuration at $C_{4'}$ between both compounds. On the basis of these data, the structure of **9** was assigned as 4′-epichaetoviridin F.

The molecular formula of compound **10** ($C_{23}H_{27}ClO_7$) was obtained by HREIMS and corroborated by ^{13}C NMR data. The spectroscopic data of this compound were similar to those of **3** (Supporting Information). However, the ^{1}H NMR spectrum (Table 1) showed a signal at δ 3.82 (m), which correlated in the COSY experiment with the signals corresponding to H-11 (δ_H 2.45) and H-13 (δ_H 1.19). Furthermore, the ^{13}C NMR spectrum (Table 2) showed the presence of an additional signal corresponding to a carbon atom attached to a hydroxyl group (δ_C 70.8), which was connected to the signal at δ 3.82 (H-12) in the HSQC experiment. These data suggested that compound **10** is a hydroxyl derivative of **3** at C_{12}. Due to the small amount obtained of this compound, it was not possible to determine the absolute configuration of the hydroxyl group by Mosher's method. However, the comparison of the NMR data of **10** (Tables 1 and 2) with those of chaetoviridin D (**4**) (Supporting Information) suggested the *R*-configuration for C_{12}. The configurations of C_7 and C_{11} were assigned to be *S*, similar to those reported for azaphilones.[8] On the other hand, the relative configurations of the C_8−$C_{2'}$ positions were shown to be *cis* as in **3** by NOE difference spectra. In particular, NOE interactions were detected between H-7CH_3 and H-8 and between H-2′ and H-8, supporting the *S*-configuration for C_8 and $C_{2'}$. Finally, the protons of the side chain at $C_{2'}$ showed chemical shifts very similar to those of **3** (Supporting Information). These data together with the magnitude of the coupling constant $J_{4'-5'}$ (9.3 Hz) suggested

Table 2. ^{13}C NMR Data of Compounds 7 and 9−13 (100 MHz, $CDCl_3$)

	δ_C, type					
position	7	9	10	11	12	13
1	152.2, C	151.7, C	146.2, C	147.0, C	152.7, C	96.1, C
3	157.1, CH	157.0, CH	157.2, CH	157.7, CH	155.4, CH	156.0, CH
4	105.3, CH	105.3, CH	105.2, CH	104.6, CH	107.9, CH	100.3, CH
4a	139.7, C	139.7, C	140.5, C	140.6, C	146.6, C	140.3, C
5	108.9, C	109.1, C	109.7, C	109.6, C	105.7, CH	125.2, C
6	183.1, C	183.3, C	184.4, C	184.7, C	190.1, C	185.8, C
7	87.9, C	87.5, C	83.4, C	83.5, C	87.7, C	86.2, C
8	164.8, C	164.1, C	42.5, CH	43.4, CH	165.2, C	72.9, C
8a	110.3, C	110.4, C	113.6, C	113.7, C	111.0, C	64.7, C
9	119.7, CH	119.7, CH	122.1, CH	120.1, CH	119.5, CH	121.7, CH
10	148.3, CH	148.1, CH	142.6, CH	145.8, CH	144.3, CH	147.9, CH
11	39.0, CH	39.0, CH	44.1, CH	38.8, CH	38.8, CH	38.8, CH
12	29.1, CH	29.1, CH	70.8, CH	29.1, CH	29.1, CH	29.2, CH
13	11.7, CH_3	11.7, CH_3	20.3, CH_3	11.7, CH_3	11.6, CH_3	11.8, CH_3
7-CH_3	26.2, CH_3	26.2, CH_3	23.5, CH_3	23.2, CH_3	26.4, CH_3	18.3, CH_3
11-CH_3	19.3, CH_3	19.3, CH_3	14.6, CH_3	19.3, CH_3	19.3, CH_3	19.0, CH_3
1′	167.8, C	167.4, C	168.8, C	168.4, C	168.4, C	167.9, C
2′	123.7, C	123.8, C	58.8, CH	51.6, CH	124.1, C	102.8, C
3′	200.8, C	201.0, C	207.0, C	191.4, C	201.1, C	167.7, C
4′	50.4, CH	45.1, CH	52.7, CH	137.5, C	50.8, CH	41.2, CH
5′	71.7, CH	25.0, CH_2	72.6, CH	144.8, CH	70.7, CH	69.8, CH
6′	21.7, CH_3	11.6, CH_3	22.4, CH_3	15.4, CH_3	21.3, CH_3	13.9, CH_3
4′-CH_3	14.2, CH_3	16.2, CH_3	13.0, CH_3	11.3, CH_3	13.5, CH_3	11.7, CH_3

an *anti*-disposition between H-4′ and H-5′ and the *S*-configuration for $C_{4'}$ and $C_{5'}$. On the basis of these data, the structure of **10** was assigned to 12β-hydroxychaetoviridin C.

Chaetoviridin G (**11**) has the molecular formula $C_{23}H_{25}ClO_5$ by HREIMS. Its ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) showed signals very similar to those of chaetoviridin E (**5**).[9] However, the appearance of two doublets at δ_H 4.11 and 4.37, showing a vicinal coupling (J = 12.2 Hz), suggested that **11** was the 8,2′-dihydro derivative of **5**. The configurations at C_7 and C_{11} were assigned to be *S*, similar to those reported for azaphilones.[8] The configurations at $C_{2'}$−C_8 were confirmed to be *cis* by NOE

dx.doi.org/10.1021/np200110f |J. Nat. Prod. 2011, 74, 1182–1187

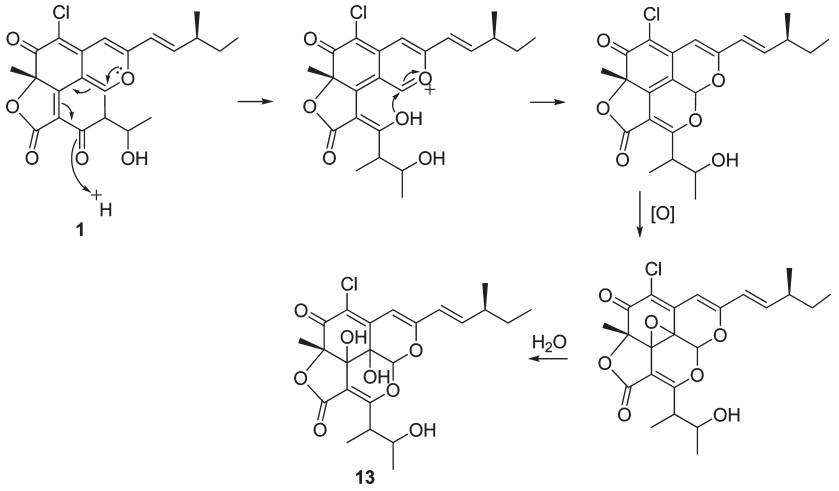


Figure 2. Proposed cyclization of **1** to yield compound **13**.

experiments. In particular, NOE interactions were detected between H-7CH_3 and H-8 and between H-8 and H-2′, which is only possible in the case of a β-disposition of the protons H-2′ and H-8. Finally, the geometry of the double bond $C_{4'}-C_{5'}$ was also inferred on the basis of NOE experiments. Irradiation of the signal at δ_H 1.94 (H-6′) led to enhancement of the signals at δ_H 1.83 (H-4′CH_3) and 6.80 (H-5′). These results indicated that the configuration of the $C_{4'}-C_{5'}$ double bond is *E*.

The molecular formula $C_{23}H_{26}O_6$ was assigned for chaetoviridin H (**12**) by HREIMS. This compound corresponds to a dechlorinated derivative of chaetoviridin A (**1**). The ^1H and ^{13}C NMR data of this compound were similar to those of **1** (Supporting Information), except for the presence of a signal at δ_H 5.31 (s). This singlet was connected to a sp^2 carbon at δ_C 105.7 (C_5) in the HSQC experiment and to three carbons at δ_C 111.0 (C_{8a}), 107.9 (C_4), and 87.7 (C_7) in the HMBC experiment. The remaining signals in the ^{13}C NMR spectrum along with the chemical shifts and coupling constants appearing in the ^1H NMR spectrum were consistent with the structure shown for compound **12**. The configurations of C_7 and C_{11} were assigned as *S* by comparison of the NMR data of **12** with those of **1** (Supporting Information). On the other hand, the orientation of the secondary hydroxyl group was established by the application of the Mosher's method. The (*R*)- and (*S*)-MPA esters **12a** and **12b** were obtained by treatment of **12** with (*R*)- and (*S*)-MPA acids, respectively. The negative value of $\Delta\delta^{RS}$ ($\delta_R - \delta_S$) for H-4′CH_3 (−0.063) and the positive value of $\Delta\delta^{RS}$ for H-6′ (+0.205) indicated the *R*-configuration for $C_{5'}$ in chaetoviridin H (**12**). Finally, the configuration at $C_{4'}$ was assigned as *S* on the basis of the magnitude of the coupling constant $J_{4'-5'}$ (6.1 Hz) and carbon chemical shifts similar to **1**.

The molecular formula of compound **13** was established as $C_{23}H_{27}ClO_8$ by HRESIMS, as well as ^1H and ^{13}C NMR data. NMR data for chaetoviridin I (**13**) were similar for the other isolated azaphilones except for the hydrogen at δ 6.00 (s, H-1), attached to the carbon at δ 96.1 in the HSQC experiment, suggesting a ketal group, and the quaternary carbons at δ 72.9 and 64.7. The hydrogen at δ 6.00 (s) showed a correlation in the HMBC experiment with a carbon at δ 156.0 (C-3). The methyl hydrogens at δ 1.76 (s, H-7CH_3) showed correlations with the carbon at δ 72.9, and the hydrogen at δ 6.15 (s, H-4) showed a correlation with the carbon at δ 64.7. On the basis of these HMBC correlations C-8 and C-8a positions were assigned for the carbons at δ 72.9 and 64.7, respectively. These data indicate modifications at the 1, 8, and 8a positions for the tricyclic moiety compared with the other azaphilones. Another difference for **13** was the absence of a ketone carbon at δ 191.4−207.0, which is characteristic of the related azaphilones bearing the side chain at C-2′. The correlation between the methyl hydrogens at δ 1.11 (d, 4′-CH_3) and the carbon at δ 167.7, not previously observed for the other compounds, suggested this carbon at δ 167.7 as C-3′. Compound **13** could be produced in acidic conditions through the cyclization of the hydroxyl group attached to C_3' at the C_1, producing the dihydropyran ring of chaetoviridin **13** (Figure 2).

The isolated compounds were tested in an in vivo pathogenicity assay that involves the infection of *C. elegans* with *Enterococcus faecalis*.[13] Using this assay the possibilities of discovering new antibacterial agents, or even prodrugs that need in vivo activation, is higher than traditional in vitro antibacterial assays. In this assay, the nematodes are transferred from their normal laboratory food, *Escherichia coli* strain OP50, to a lawn of *E. faecalis* strain MMH594 to establish an infection and subsequently to modified liquid brain-heart infusion medium in 96-well microtiter plates. *E. faecalis* causes a lethal infection in the *C. elegans* intestine, and the end point of the assay is dead worms. None of the compounds showed significant ability to promote nematode survival.

Thus, six new azaphilones together with six known azaphilones have been identified from the endophyte *C. globosum*. *C. globosum* from different biomes has already been reported as prolific producers of bioactive compounds. Other related, new cytotoxic azaphilone derivatives have been isolated from marine fish-derived *C. globosum*.[14−19] A different endophytic *C. globosum* strain produced the chemokine receptor CCR-5 inhibitor, with potential as an anti-HIV agent.[20]

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were determined with a Perkin-Elmer 341 polarimeter in $CHCl_3$. UV spectra were obtained in $CHCl_3$ solution on a GBC Cintra 101 diode array spectrophotometer, and IR spectra were recorded on a Perkin-Elmer Spectrum BX spectrophotometer, series FTIR (Fourier transform infrared). ^1H and ^{13}C NMR measurements were obtained on Varian Inova 400 and Varian Unity 600 spectrometers with $CDCl_3$ as solvent. Mass spectra of

1−**5** and **7**−**11** were recorded on gas chromatography/mass spectrometry Agilent-LR and Finningan Mat95 S instruments, and mass spectra of **12** and **13** were obtained on an UltrO-TOF (Bruker-Daltonics, Billarica, MA, USA). HPLC was performed with a Hitachi/Merck L-7100 apparatus equipped with a differential refractometer detector (RI-L7490) and an Intel Pentium computer for analytical system control, data collection, and processing (software Borwin). Purification by means of HPLC was carried out with a silica gel column (LIChroCART 250/10 LIChrospher SI60, 10 μm, 1 cm wide, 25 cm long, or LIChroCART 250/4 LIChrospher SI60, 5 μm, 0.4 cm wide, 25 cm long). TLC was performed on Merck Kiesegel 60 F_{254}, 0.25 mm thick. Si gel (Merck) type 9385 was used for column chromatography.

Fungal Material. The fungus was isolated from the leaves of *Viguiera robusta* and identified on the basis of its rDNA sequence as previously reported.[6] A voucher specimen has been deposited at "Laboratório de Química de Micro-Organismos", FCFRP, USP. The strain is maintained in both sterile water and silica gel (6−12 mesh, grade 40, desiccant activated) at 10 °C.

Culture Conditions. The general procedure adopted for cultivation of the microorganism followed the methodology described by Park et al.[12] *C. globosum* was grown in 40 Erlenmeyer flasks (500 mL) containing 200 mL of potato dextrose broth (PDB). Each flask was inoculated with 6 mycelium plugs (1 cm diameter each) from a 7-day-old culture grown on PDA agar and then incubated for 21 days at 25 °C on an orbital shaker at 150 rpm.

Extraction and Isolation. The culture broth (8 L) was separated from the mycelium by vacuum filtration and partitioned with ethyl acetate (EtOAc) three times. The mycelium was extracted by maceration with EtOAc. The organic solvent was evaporated under vacuum, and the EtOAc extracts were combined after TLC analysis. The crude combined EtOAc extract, a yellow oil (3.50 g), was separated by means of column chromatography using silica gel 60 eluted with *n*-hexane/EtOAc (7:3), EtOAc/MeOH (1:1), and MeOH 100%, yielding eight fractions. Subfraction 4 yielded compound **1** (1.87 g). Fraction 3 (89 mg) was subjected to HPLC using a LiChroCART 250-10−LiChrospher Si 60 (10 μm) column eluted with *n*-hexane/EtOAc (7:3) at a flow rate of 3 mL min^{-1}, yielding two subfractions. Subfraction 3.1 (62.4 mg) was submitted to HPLC purification in a normal-phase column (LiChroCART 250-4−LiChrospher Si 60, 5 μm) using *n*-hexane/EtOAc (4:1) as mobile phase at a flow rate of 3 mL min^{-1} to yield **5** (44 mg) and **11** (16 mg). Fraction 5 (80 mg) was also submitted to both HPLC purification procedures described for fraction 3. In the first HPLC step fraction 5 yielded two subfractions. In the second step subfraction 5.1 (28 mg) yielded **9** (22 mg). Fraction 6 (270 mg) was also submitted to the same first HPLC purification procedure described for fraction 3 to give five subfractions. Subfractions 2−5 correspond to compounds **3** (32 mg), **7** (12 mg), **8** (25 mg), and **2** (141 mg), respectively. Fraction 7 (501 mg) was subjected to silica gel 60 column chromatography eluted with *n*-hexane/EtOAc (2:3) and EtOAc 100%, yielding six subfractions. Subfraction 7.1 (65 mg) was subjected to normal-phase HPLC (LiChroCART 250-10−LiChrospher Si 60, 10 μm) with *n*-hexane/EtOAc (3:2) as mobile phase at a flow rate of 3 mL min^{-1}, yielding **12** (32 mg) and **13** (11 mg). Subfraction 7.5 (88 mg) was also subjected to normal-phase HPLC using the same conditions used for purification of fraction 1, except for *n*-hexane/EtOAc (1:4) used as mobile phase, yielding four subfractions. Subfraction 7.5.1 (6 mg) yielded **10** (4 mg) after purification in normal-phase HPLC (LiChroCART 250-4−LiChrospher Si 60, 5 μm, eluted with *n*-hexane/EtOAc (2:3) at flow rate of 1 mL min^{-1}), subfraction 7.5.2 (26 mg) yielded **4** after crystallization, and subfraction 7.5.3 (12 mg) also yielded **4** (total of 24 mg) after normal-phase HPLC purification (LiChroCART 250-4−LiChrospher Si 60, 5 μm, eluted with *n*-hexane/EtOAc (4:1) at flow rate of 1 mL min^{-1}).

*5′-Epichaetoviridin A (**7**):* orange, amorphous solid; $[\alpha]_D^{20}$ +40 (*c* 0.002, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε) 307 (0.66), 367 (0.53), 431 (0.15); IR (KBr) ν_{max} 3462, 2966, 2918, 1767, 1685, 1645, 1514, 756 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; EIMS *m*/*z* (%) 432 $[M]^+$ (4), 390 (16), 345 (25), 332 (100); HREIMS calcd for $C_{23}H_{25}ClO_6$ $[M]^+$ 432.1340, found 432.1344.

*4′-Epichaetoviridin A (**8**):* orange, amorphous solid; $[\alpha]_D^{20}$ +16 (*c* 0.002, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε) 306 (1.72), 367 (1.16), 430 (0.33); IR (KBr) ν_{max} 3477, 2965, 1765, 1621, 1513 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; EIMS *m*/*z* (%) 432 $[M]^+$ (14), 390 (52), 345 (42), 332 (100); HREIMS calcd for $C_{23}H_{25}ClO_6$ $[M]^+$ 432.1340, found 432.1343.

*4′-Epichaetoviridin F (**9**):* orange, amorphous solid; $[\alpha]_D^{20}$ +106 (*c* 0.002, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε) 306 (0.97), 365 (0.68), 430 (0.18); IR (KBr) ν_{max} 2967, 1768, 1621, 1512, 1219, 1167, 756, 458 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; EIMS *m*/*z* (%) 416 $[M]^+$ (26), 373 (34), 332 (100); HREIMS calcd for $C_{23}H_{25}ClO_5$ $[M]^+$ 416.1391, found 416.1378.

*12β-Hydroxichaetoviridin C (**10**):* yellow, amorphous solid; $[\alpha]_D^{20}$ −125 (*c* 0.002, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε) 293 (0.32), 389 (0.57), 409 (0.49), 434 (0.22); IR (KBr) ν_{max} 3422, 2975, 1774, 1718, 1617, 1560, 1516, 1247, 1094 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; EIMS *m*/*z* (%) 450 $[M]^+$ (69), 432 (10), 406 (86), 388 (16), 362 (100); HREIMS calcd for $C_{23}H_{27}ClO_7$ $[M]^+$ 450.1445, found 450.1450.

*Chaetoviridin G (**11**):* orange, amorphous solid; $[\alpha]_D^{20}$ +5.5 (*c* 0.002, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε) 295 (0.29), 388 (0.49), 404 (0.43), 431 (0.20); IR (KBr) ν_{max} 3400, 2929, 1713, 1634, 1520, 757 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; EIMS *m*/*z* (%) 416 $[M]^+$ (100), 381 (29), 357 (21), 345 (12), 337 (19), 263 (28), 83 (56), 55 (48); HREIMS calcd for $C_{23}H_{25}ClO_5$ $[M]^+$ 416.1391, found 416.1395.

*Chaetoviridin H (**12**):* yellow, amorphous solid; $[\alpha]_D^{20}$ +35 (*c* 0.003, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε) 260 (3.77), 338 (3.44); IR (KBr) ν_{max} 3458, 2964, 2927, 1763, 1682, 1621, 1532 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; HRESIMS calcd for $C_{23}H_{27}O_6$ $[M + 1]^+$ 399.1808, found 399.1808.

*Chaetoviridin I (**13**):* yellow, amorphous solid; $[\alpha]_D^{20}$ −51.7 (*c* 0.005, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε) 242 (4.31), 341 (4.11); IR (KBr) ν_{max} 3417, 2965, 1745, 1663, 1577 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 1 and 2; HRESIMS $[M + H]^+$ found 467.1467 (calcd for $C_{23}H_{28}ClO_8$, 467.1473); $[M + Na]^+$ found 489.1286 (calcd for $C_{23}H_{27}ClNaO_8$, 489.1292); $[M + K]^+$ found 505.1026 (calcd for $C_{23}H_{27}ClKO_8$, 505.1032).

*X-ray Crystallographic Analysis of **1**.* Orange crystals of **1** were obtained from *n*-hexane/EtOAc by slow evaporation. X-ray diffraction data were measured on a Bruker Smart CCD diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) at 297(1) K. Data collection was based on three ω-scan runs (starting = −34°) at values of ϕ = 0°, 120°, and 240° with the detector at 2θ = −32°. For each of these runs, 606 frames were collected at 0.3° intervals and 10 s per frame. An additional run at ϕ = 0° of 100 frames was collected to improve redundancy. The diffraction frames were integrated using the program SAINT, and the integrated intensities were corrected for Lorentz−polarization effects with SADABS. The structure was solved by direct methods and refined to all 3778 unique F_o^2 by full matrix least-squares calculations using SHELXL-97.[21] All the hydrogen atoms were placed at idealized positions and refined as rigid atoms. Final R_1 indices [2275 with $I > 2\sigma(I)$] 0.0670, wR_2 = 0.1676. *R* indices (all data) R_1 = 0.1168, wR_2 = 0.1983. $S[F_2]$ 1.067 for 278 refined parameters. Crystallographic data of chaetoviridin A have been deposited at the Cambridge Crystallographic Data Centre under the reference number CCDC 758082. Crystal data: $C_{23}H_{25}ClO_6$, *M* = 432.88, monoclinic, *a* = 12.3132 (15) Å, *b* = 5.5504(7) Å, *c* = 16.662 (2) Å, $\alpha = \gamma = 90°$, β = 108.050 (3)°, *V* = 1082.7 (2) $Å^3$, dimensions 0.55 × 0.12 × 0.05 mm, space group $P2_1$, *Z* = 2, *F*(000) = 456, μ = 0.213 mm^{-1}.

*Preparation of (R)- and (S)-MTPA Esters of Chaetoviridin D (**4a** and **4b**).* A solution of chaetoviridin D (**4**) (10 mg, 0.021 mmol) in dichloromethane was treated with DMAP (catalytic amount), DCC (9.26 mg,

0.045 mmol), and (*R*)-MTPA or (*S*)-MTPA (11.25 mg, 0.048 mmol), and the mixture was stirred at room temperature for 24 h. Evaporation of the solvent under reduced pressure yielded a residue that was purified by silica gel column chromatography eluted with *n*-hexane/EtOAc (7:3) to obtain 4.2 mg (0.006 mmol) of (*R*)-MTPA ester **4a** and 3.6 mg (0.005 mmol) of (*S*)-MTPA ester **4b**.

*Preparation of (R)- and (S)-MPA Esters of 5′-Epichaetoviridin A (**7a** and **7b**).* Treatment of a solution of **7** (10 mg, 0.023 mmol) in dichloromethane with DMAP (catalytic amounts), DCC (10.03 mg, 0.048 mmol), and (*R*)-MPA or (*S*)-MPA (8.65 mg, 0.036 mmol) as described above yielded 3.45 mg (0.006 mmol) of (*R*)-MPA ester **7a** and 2.90 mg (0.005 mmol) of (*S*)-MTPA ester **7b**.

*Preparation of (R)- and (S)-MPA Esters of 4′-Epichaetoviridin A (**8a** and **8b**).* Treatment of a solution of **8** (10 mg, 0.023 mmol) in dichloromethane with DMAP (catalytic amounts), DCC (10.03 mg, 0.048 mmol), and (*R*)-MPA or (*S*)-MPA (8.65 mg, 0.036 mmol) as described above yielded 4.04 mg (0.007 mmol) of (*R*)-MPA ester **8a** and 2.68 mg (0.005 mmol) of (*S*)-MTPA ester **8b**.

*Preparation of (R)- and (S)-MPA Esters of Chaetoviridin H (**12a** and **12b**).* Treatment of a solution of **12** (10 mg, 0.025 mmol) in dichloromethane with DMAP (catalytic amounts), DCC (10.88 mg, 0.052 mmol), and (*R*)-MPA or (*S*)-MPA (9.39 mg, 0.040 mmol) as described above yielded 6.50 mg (0.012 mmol) of (*R*)-MPA ester **12a** and 6.80 mg (0.012 mmol) of (*S*)-MTPA ester **12b**.

Antibacterial Assays. *Escherichia coli* OP50 and *Enterococcus faecalis* MMH594 were grown on brain-heart infusion (BHI, Difco) media at 37 °C. *C. elegans glp-4(bn2ts);sek-1(km4)* mutant animals were maintained using standard practices by feeding them with *E. coli* strain OP50.[22] Synchronized L4 stage to young adult worms were infected with *E. faecalis* according the protocol previously described.[13] All compounds were tested according to the protocol described by Moy et al.[13] at a final concentration of 100 and 50 μg mL^{-1} in 19% BHI, 80% M9 buffer, 80 μg mL^{-1} kanamycin, and 1% DMSO. Each compound was tested in triplicate using 96-well plates sealed with gas-permeable membranes (Breath-Easy, Diversified Biotech) at 25 °C, 80−85% relative humidity, without agitation for a total period of 168 h. Worm survival was scored at 3, 48, 120, and 168 h as described.[13] A positive control was carried out using ampicillin at a range of 25−0.39 μg mL^{-1}.

■ ASSOCIATED CONTENT

Supporting Information. Spectroscopic data of the compounds **1**, **2**, **3**, **4**, **4a**, **4b**, **5**, **7**, **7a**, **7b**, **8a**, **8b**, **9**−**11**, **12**, **12a**, **12b**, and **13**. Chromatograms of the isolation of compounds **2**−**5** and **7**−**13**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(I.G.C.) Tel: 34-956016365. Fax: 34-956016193. E-mail: isidro.gonzalez@uca.es. (M.T.P.) Tel: 55-16-36024710. Fax: 55-16-36024178. E-mail: mtpupo@fcfrp.usp.br.

Present Addresses

‡WSB Departamento de Química, Universidade Federal do Espírito Santo, 29075-910, Vitória, ES, Brazil.

■ ACKNOWLEDGMENT

The authors are grateful to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support and for granting research fellowships. This work was financed, in part, by grants from the Spanish Science Innovation and Technology Ministry through projects AGL-2009 13359-CO2-01 and PHB 2008-0067-PC. The authors acknowledge Dr. T. Soler of the Alicante University for the X-ray crystallographic analysis of chaetoviridin A and Prof. N. P. Lopes (FCFRP-USP) for the obtainment of HRESIMS spectra. We also thank Prof. F. M. Ausubel and T. I. Moy, Ph.D., from Department of Molecular Biology, Massachusetts General Hospital, and Department of Genetics, Harvard Medical School, Boston, MA, for the *C. elegans* assays.

■ REFERENCES

(1) Strobel, G.; Daisy, B.; Castillo, U.; Harper, J. *J. Nat. Prod.* **2004**, *67*, 257–268.

(2) Borges, K. B.; Borges, W. S.; Durán-Patrón, R.; Pupo, M. T.; Bonato, P. S.; González, I. G. *Tetrahedron: Asymmetry* **2009**, *20*, 385–397.

(3) Borges, W. S.; Borges, K. B.; Bonato, P. S.; Said, S.; Pupo, M. T. *Curr. Org. Chem.* **2009**, *13*, 1137–1163.

(4) Guimarães, D. O.; Borges, W. S.; Kawano, C. Y.; Ribeiro, P. H.; Goldman, G. H.; Nomizo, A.; Thiemann, O. H.; Oliva, G.; Lopes, N. P.; Pupo, M. T. *FEMS Immunol. Med. Microbiol.* **2008**, *52*, 134–144.

(5) Gallo, M. B. C.; Chagas, F. O.; Almeida, M. O.; Macedo, C. C.; Cavalcanti, B. C.; Barros, F. W. A.; Moraes, M. O.; Costa-Lotuffo, L. V.; Pessoa, C. O.; Bastos, J. K.; Pupo, M. T. *J. Basic Microbiol.* **2009**, *49*, 142–151.

(6) Momesso, L. S.; Kawano, C. Y.; Ribeiro, P. H.; Nomizo, A.; Goldman, G. H.; Pupo, M. T. *Quim. Nova* **2008**, *31*, 1680–1685.

(7) Qin, J. C.; Zhang, Y. M.; Gao, J. M.; Bai, M. S.; Yang, S. X.; Laatsch, H.; Zhang, A. L. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1572–1574.

(8) Takahashi, M.; Koyama, K.; Natori, S. *Chem. Pharm. Bull.* **1990**, *38*, 625–628.

(9) Phonkerd, N.; Kanokmedhakul, S.; Kanokmedhakul, K.; Soytong, K.; Prabpai, S.; Kongsearee, P. *Tetrahedron* **2008**, *64*, 9636–9645.

(10) Yasukawa, K.; Takahashi, M.; Natori, S.; Kawai, K.; Yamazaki, M.; Takeushi, M.; Takido, M. *Oncology* **1994**, *51*, 108–112.

(11) Tomoda, H.; Matsushisma, C.; Tabata, N.; Namatame, I.; Tanaka, H.; Bamberger, M. J.; Arai, H.; Fukazawa, M.; Inoue, K.; Omura, S. *J. Antibiot.* **1999**, *52*, 160–170.

(12) Park, J. H.; Choi, G. J.; Jang, K. S.; Lim, H. K.; Kim, H, T.; Cho, K. Y.; Kim, J. C. *FEMS Microbiol. Lett.* **2005**, *252*, 309–313.

(13) Moy, T. I.; Ball, A. R.; Anklesaria, Z.; Casadei, G.; Lewis, K.; Ausubel, F. M. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 10414–10419.

(14) Yamada, T.; Doi, M.; Shigeta, H.; Muroga, Y.; Hosoe, S.; Numata, A.; Tanaka, R. *Tetrahedron Lett.* **2008**, *49*, 4192–4195.

(15) Yasuhide, M; Yamada, T; Numata, A; Tanaka, R. *J. Antibiot.* **2008**, *61*, 615–622.

(16) Yamada, T.; Yasuhide, M.; Shigeta, H.; Numata, A.; Tanaka, R. *J. Antibiot.* **2009**, *62*, 353–357.

(17) Yamada, T.; Muroga, Y.; Tanaka, R. *Mar. Drugs* **2009**, *7*, 249–257.

(18) Muroga, Y.; Yamada., T.; Numata, A.; Tanaka, R. *Tetrahedron* **2009**, *65*, 7580–7586.

(19) Muroga, Y.; Yamada., T.; Numata, A.; Tanaka, R. *Helv. Chim. Acta* **2010**, *93*, 542–549.

(20) Yang, S. W.; Mierzwa, R.; Terracciano, J.; Patel, M.; Gullo, V.; Wagner, N.; Baroudy, B.; Puar, M.; Chan, T. M.; McPhail, A. T.; Chu, M. *J. Nat. Prod.* **2006**, *69*, 1025–1028.

(21) Sheldrick, G. M. *Acta Crystallogr. A* **2008**, *64*, 112–122.

(22) Lewis, J. A.; Fleming, J. T. In *Methods in Cell Biology*; Epstein, H. F.; Shakes, D. C., Eds.; Academic Press: San Diego, 1995; Vol. 48, Chapter 1, pp 3−29.