FULL PAPER

Stachartins A – E, Phenylspirodrimanes from the Tin Mine Tailings-Associated Fungus *Stachybotrys chartarum*

by **Wei-Xun Chunyu**[a])[†]), **Zhang-Gui Ding**[a])[†]), **Ming-Gang Li**[a]), **Jiang-Yuan Zhao**[a]), **Shao-Jie Gu**[b]), **Yuan Gao**[b]), **Fei Wang***[b]), **Jian-Hai Ding***[c]), and **Meng-Liang Wen***[a])

[a]) Key Laboratory for Microbial Resources, Ministry of Education, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, P. R. China (e-mail: mlwen@ynu.edu.cn)

[b]) BioBioPha Co., Ltd., Kunming 650201, P. R. China (e-mail: f.wang@mail.biobiopha.com)

[c]) College of Chemistry and Chemical Engineering, Ningxia Normal University, Guyuan 756000, P. R. China (phone/fax: +86-954-2079650, e-mail: dingjh_nx@126.com)

Eight phenylspirodrimane-type analogues, including five new compounds, named stachartins A – E (**1**, **3**, **6** – **8**), were isolated from cultures of the tin mine tailings-associated fungus *Stachybotrys chartarum*. Their structures were elucidated by spectroscopic methods including extensive 2D-NMR techniques.

Keywords: Stachartins A – E, Fungi, *Stachybotrys chartarum*, Phenylspirodrimane, Secondary metabolites.

Introduction

Microorganisms from extreme environments are a valuable source of secondary metabolites with structural novelty and biological activities [1]. Abandoned mine tailings with either high or low pH and high metal ion concentrations are man-made environments that have proven productive in the search for drug-like molecules (bioactive molecules, potential pharmaceutical agents, *etc.*) from natural products [2]. In the course of our investigation of the chemical diversity from extremophiles [3], the fungus *Stachybotrys chartarum* was isolated from a soil sample collected from the Datun tin mine tailings area, Yunnan, P. R. China. Chemical investigation of the AcOEt extract obtained from cultures of *S. chartarum* led to the isolation of five new phenylspirodrimanes, stachartins A – E (**1**, **3**, **6** – **8**), along with three known analogues (**2**, **4,** and **5**) [4 – 6]. Herein, we report the isolation and structure elucidation of these new phenylspirodrimanes.

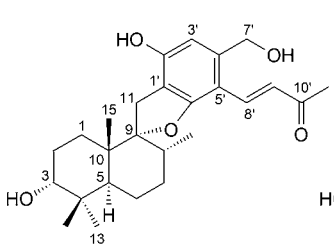
Results and Discussion

Compound **1** was obtained as colorless oil, and its molecular formula was determined as $C_{26}H_{36}O_5$ on the basis of the HR-ESI-MS data (*m*/*z* 451.2465 ($[M + Na]^+$, $C_{26}H_{36}NaO_5^+$; calc. 451.2460)), requiring nine degrees of unsaturation. The IR spectrum exhibited absorption bands at 3441, 3432, and 1622 cm^{-1} due to OH groups and α,β-unsaturated ketone group. Comparison of the ^1H- and ^{13}C-NMR spectroscopic data (*Table 1*) with those of F1839-I [7] showed the presence of the same phenylspirodrimane skeleton. The principal differences between these compounds were that the Me group of the known analogue was replaced by CH_2OH group at C(7′) in **1** and that the moiety of **1** at C(5′) was formed by aldol condensation with acetone; these differences were supported by the HMBCs H–C(7′) (δ(H) 4.47, 4.50 (*dd*, *J* = 12.9, 5.4 Hz))/C(3′) (δ(C) 108.4), H–C(8′) (δ(H) 7.61 (*d*, *J* = 15.9 Hz))/C(10′) (δ(C) 198.3), H–C(11′) (δ(H) 2.21 (*s*))/C(9′) (δ(C) 126.0), and H–C(9′) (δ(H) 7.15 (*d*, *J* = 15.9 Hz))/C(5′) (δ(C) 106.3). The substituent at C(4′) and C(5′) in **1** was determined by the ROESY correlations of H–C(9′) with H–C(6) and H–C(7), and from biogenetic considerations. The planar structure of **1** was further confirmed by 2D-NMR data (*Fig.*). On the basis of the ROESY correlations H–C(3)/Me(14), Me(14)/Me(15), Me(15)/H–C(8), and Me(13)/H–C(5), H–C(5)/H_α–C(7), H_α–C(7)/Me(12) determined that H–C(3), Me(14), and Me(15) as being in β-orientations, while Me(13), H–C(5), and Me(12) as being in α-orientations. Thus, compound **1** was elucidated as stachartin A.

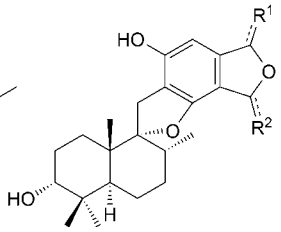
Compound **3** was obtained as colorless oil, and its molecular formula ($C_{23}H_{30}O_5$) was determined by HR-ESI-MS and NMR data (*Table 1*), which indicates nine degrees of unsaturation. The IR absorptions at 3433 and 1726 cm^{-1} were characteristic of OH and C=O functionalities. Comparison of the NMR spectroscopic data and

[†]) These authors contributed equally to this work and should be considered co-first authors.

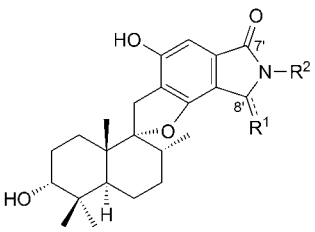
DOI: 10.1002/hlca.201600020



1



2 R^1 = O, R^2 = H
3 R^1 = H, R^2 = O



4 R^1 = =O, R^2 = H
5 R^1 = H, R^2 = H
6 R^1 = H, R^2 = CH(COOMe)$CHMe_2$
7 R^1 = H, R^2 = CH(COOMe)CH_2CHMe_2
8 R^1 = H, R^2 = CH(COOMe)CH_2–Ph

Table 1. ^{1}H- and ^{13}C-NMR data of **1** and **3** (DMSO, δ in ppm and J in Hz)

	1		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	1.65 – 1.71 (*m*) 0.86 – 0.93 (*m*)	23.9 (*t*)	1.62 – 1.70 (*m*) 0.86 – 0.93 (*m*)	23.9 (*t*)
CH_2(2)	1.77 – 1.83 (*m*) 1.34 – 1.39 (*m*)	25.0 (*t*)	1.74 – 1.83 (*m*) 1.32 – 1.39 (*m*)	25.0 (*t*)
H–C(3)	3.17 (br.)	73.4 (*d*)	3.16 (br.)	73.3 (*d*)
C(4)	–	37.3 (*s*)	–	37.4 (*s*)
H–C(5)	2.12 (*dd*, *J* = 12.3, 2.6)	40.3 (*d*)	2.03 (*dd*, *J* = 12.0, 2.0)	39.3 (*d*)
CH_2(6)	1.47 – 1.52 (*m*) 1.36 – 1.40 (*m*)	20.7 (*t*)	1.42 – 1.48 (*m*) 1.33 – 1.42 (*m*)	20.5 (*t*)
CH_2(7)	1.53 – 1.60 (*m*) 1.34 – 1.41 (*m*)	31.5 (*t*)	1.46 – 1.53 (*m*) 1.35 – 1.45 (*m*)	30.6 (*t*)
H–C(8)	1.73 – 1.83 (*m*)	36.3 (*d*)	1.72 – 1.82 (*m*)	36.6 (*d*)
C(9)	–	98.0 (*s*)	–	99.7 (*s*)
C(10)	–	41.9 (*s*)	–	41.8 (*s*)
CH_2(11)	3.00 (*d*, *J* = 16.4) 2.67 (*d*, *J* = 16.4)	30.5 (*t*)	3.03 (*d*, *J* = 16.4) 2.67 (*d*, *J* = 16.4)	30.7 (*t*)
Me(12)	0.58 (*d*, *J* = 6.5)	15.5 (*q*)	0.60 (*d*, *J* = 6.5)	15.4 (*q*)
Me(13)	0.90 (*s*)	29.0 (*q*)	0.87 (*s*)	28.6 (*q*)
Me(14)	0.79 (*s*)	22.2 (*q*)	0.78 (*s*)	22.5 (*q*)
Me(15)	0.94 (*s*)	15.6 (*q*)	0.93 (*s*)	15.9 (*q*)
C(1′)	–	112.1 (*s*)	–	113.3 (*s*)
C(2′)	–	155.0 (*s*)	–	159.7 (*s*)
H–C(3′)	6.37 (*s*)	108.4 (*d*)	6.35 (*s*)	100.5 (*d*)
C(4′)	–	143.1 (*s*)	–	149.8 (*s*)
C(5′)	–	106.3 (*s*)	–	98.6 (*s*)
C(6′)	–	162.6 (*s*)	–	159.4 (*s*)
CH_2(7′)	4.50 (*dd*, *J* = 12.9, 5.4) 4.47 (*dd*, *J* = 12.9, 5.4)	61.6 (*t*)	5.14 (*s*)	68.9 (*t*)
H–C(8′) or C(8′)	7.61 (*d*, *J* = 15.9)	136.0 (*d*)	–	168.0 (*s*)
H–C(9′)	7.15 (*d*, *J* = 15.9)	126.0 (*d*)		
C(10′)	–	198.3 (*s*)		
Me(11′)	2.12 (*s*)	28.6 (*q*)		
3-OH	4.10 (*d*, *J* = 3.1)	–	4.12 (*d*, *J* = 2.7)	–
2′-OH	9.96 (*s*)	–	10.58 (*s*)	–
7′-OH	5.19 (*t*, *J* = 5.4)	–		

analyses of the 2D-NMR spectra revealed the gross structure of **3** to be closely related to stachybotrylactone (**2**) [4]. The only difference was the position of the C=O group at C(8′) in **3** rather than at C(7′) in **2**. This assignment was confirmed by HMBCs of H–C(7′) (δ(H) 5.14 (*s*)/C(3′) (δ(C) 100.5), C(5′) (δ(C) 98.6), and C(8′) (δ(C) 168.0). Therefore, compound **3** was established to be stachartin B.

The molecular formula of compound **6** was determined as $C_{29}H_{41}NO_6$ on the basis of the HR-ESI-MS and NMR data, requiring ten degrees of unsaturation. The IR spectrum showed absorption bands for OH (3442 cm^{-1}), ester C=O (1742 cm^{-1}), and amide C=O (1670 cm^{-1}) groups. Comparison of the ^{1}H- and ^{13}C-NMR data of **6** (*Table 2*) with those of stachybotrin F [8] showed the presence of the same phenylspirodrimane skeleton. The distinction was attributed to C(9′), in which a propionic acid group of the known analogue was replaced by a ^{i}Pr group of **6**, as evident from H–C(9′) (δ(H) 4.61 (*d*,

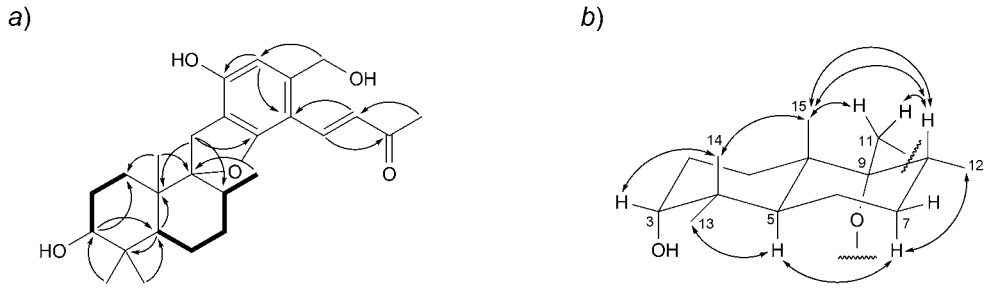


Figure. Selected *a*) HMBC (H → C) and COSY (▬), and *b*) ROESY (H ↔ H) correlations of **1**.

Table 2. ^{1}H- and ^{13}C-NMR data of **6** – **7** (CD_3OD, δ in ppm and J in Hz)

	6		**7**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	1.77 – 1.87 (*m*) 1.03 – 1.13 (*m*)	25.4 (*t*)	1.78 – 1.89 (*m*) 1.04 – 1.13 (*m*)	25.4 (*t*)
CH_2(2)	1.92 – 2.02 (*m*) 1.48 – 1.57 (*m*)	26.0 (*t*)	1.92 – 2.01 (*m*) 1.50 – 1.58 (*m*)	26.0 (*t*)
H–C(3)	3.34 (br.)	76.5 (*d*)	3.34 (br.)	76.4 (*d*)
C(4)	–	38.6 (*s*)	–	38.6 (*s*)
H–C(5)	2.12 (*dd*, *J* = 12.1, 2.5)	41.4 (*d*)	2.11 (*dd*, *J* = 12.0, 2.0)	41.4 (*d*)
CH_2(6)	1.46 – 1.63 (*m*)	22.1 (*t*)	1.44 – 1.61 (*m*)	22.1 (*t*)
CH_2(7)	1.49 – 1.64 (*m*)	32.2 (*t*)	1.48 – 1.64 (*m*)	32.2 (*t*)
H–C(8)	1.81 – 1.91 (*m*)	38.4 (*d*)	1.80 – 1.90 (*m*)	38.4 (*d*)
C(9)	–	99.9 (*s*)	–	99.9 (*s*)
C(10)	–	43.5 (*s*)	–	43.5 (*s*)
CH_2(11)	3.22 (*d*, *J* = 17.0) 2.85 (*d*, *J* = 17.0)	33.0 (*t*)	3.23 (*d*, *J* = 16.9) 2.84 (*d*, *J* = 16.9)	33.0 (*t*)
Me(12)	0.73 (*d*, *J* = 6.6)	16.0 (*q*)	0.72 d (*d*, *J* = 6.6)	16.0 (*q*)
Me(13)	0.98 (*s*)	29.0 (*q*)	0.97 (*s*)	29.0 (*q*)
Me(14)	0.88 (*s*)	23.0 (*q*)	0.87 (*s*)	23.0 (*q*)
Me(15)	1.04 (*s*)	16.6 (*q*)	1.04 (*s*)	16.6 (*q*)
C(1′)	–	119.4 (*s*)	–	119.3 (*s*)
C(2′)	–	155.4 (*s*)	–	155.3 (*s*)
H–C(3′)	6.67 (*s*)	102.3 (*d*)	6.67 (*s*)	102.2 (*d*)
C(4′)	–	133.9 (*s*)	–	134.2 (*s*)
C(5′)	–	114.5 (*s*)	–	114.6 (*s*)
C(6′)	–	157.6 (*s*)	–	157.6 (*s*)
C(7′)	–	171.5 (*s*)	–	171.7 (*s*)
CH_2(8′)	4.64 (*d*, *J* = 17.1) 4.33 (*d*, *J* = 17.1)	46.1 (*t*)	4.57 (*d*, *J* = 16.8) 4.27 (*d*, *J* = 16.8)	45.7 (*t*)
H–C(9′)	4.61 (*d*, *J* = 10.3)	61.8 (*d*)	5.02 (*dd*, *J* = 11.4, 4.7)	53.7 (*d*)
C(10′)	–	172.3 (*s*)	–	172.3 (*s*)
H–C(11′) or CH_2(11′)	2.34 – 2.45 (*m*)	30.1(*d*)	1.92 – 2.03 (*m*) 1.76 – 1.89 (*m*)	39.2 (*t*)
Me(12′) or H–C(12′)	1.04 (*d*, *J* = 6.5)	19.8 (*q*)	1.38 – 1.50 (*m*)	26.2 (*d*)
Me(13′)	0.89 (*d*, *J* = 6.6)	19.5 (*q*)	0.98 (*d*, *J* = 6.8)	23.4 (*q*)
MeO or Me(14′)	3.72 (*s*)	52.5 (*q*)	0.96 (*d*, *J* = 6.8)	21.4 (*q*)
MeO			3.71 (*s*)	52.9 (*q*)

J = 10.3 Hz)) exhibiting HMBCs to C(11′) (δ(C) 30.1), C (12′) (δ(C) 19.5), and C(13′) (δ(C) 19.8). The planar structure of **6** was further confirmed by 2D-NMR data. The absolute configuration of **6** was also postulated to be the same as stachybotrin F based on the similar specific rotation values ($[\alpha]_D^{20} = -45.1$ for **6** and $[\alpha]_D^{24} = -33.7$ for stachybotrin F), and biosynthetic considerations. Thus, compound **6** was established as stachartin C, as shown.

Compound **7** was established to have the molecular formula of $C_{30}H_{43}NO_6$ by the HR-ESI-MS at *m*/*z* 536.2988 ($[M + Na]^+$, $C_{30}H_{43}NNaO_6^+$; calc. 536.2988). This formula is 14 mass units larger than that of **6**, suggesting the presence of an extra CH_2 group. The ^{1}H- and ^{13}C-NMR spectra of **7** (*Table 2*) is very similar to those of compound **6**, with the exception of the presence of a extra CH_2 at δ(C) 39.2 which correlated with Me(13′) and Me(14′) in the

Table 3. ^{1}H- and ^{13}C-NMR data of **8** (CD_3OD, δ in ppm and J in Hz)

	δ(H)	δ(C)
CH_2(1)	1.76 – 1.86 (*m*), 1.02 – 1.11 (*m*)	25.4 (*t*)
CH_2(2)	1.91 – 2.01 (*m*), 1.48 – 1.57 (*m*)	26.0 (*t*)
H–C(3)	3.33 (br.)	76.4 (*d*)
C(4)	–	38.6 (*s*)
H–C(5)	2.09 (*dd*, *J* = 11.7, 2.5)	41.4 (*d*)
CH_2(6)	1.44 – 1.60 (*m*)	22.1 (*t*)
CH_2(7)	1.46 – 1.62 (*m*)	32.2 (*t*)
H–C(8)	1.77 – 1.88 (*m*)	38.4 (*d*)
C(9)	–	99.8 (*s*)
C(10)	–	43.4 (*s*)
CH_2(11)	3.20 (*d*, *J* = 17.0), 2.80 (*d*, *J* = 17.0)	33.0 (*t*)
Me(12)	0.66 (*d*, *J* = 6.5)	16.0 (*q*)
Me(13)	0.97 (*s*)	29.0 (*q*)
Me(14)	0.87 (*s*)	23.0 (*q*)
Me(15)	1.03 (*s*)	16.6 (*q*)
C(1′)	–	119.3 (*s*)
C(2′)	–	155.3 (*s*)
H–C(3′)	6.59 (*s*)	102.1 (*d*)
C(4′)	–	134.0 (*s*)
C(5′)	–	114.5 (*s*)
C(6′)	–	157.5 (*s*)
C(7′)	–	171.5 (*s*)
CH_2(8′)	4.48 (*d*, *J* = 16.7), 4.28 (*d*, *J* = 16.7)	46.3 (*t*)
H–C(9′)	5.25 (*dd*, *J* = 10.7, 5.6)	56.8 (*d*)
C(10′)	–	172.4 (*s*)
CH_2(11′)	3.47 (*dd*, *J* = 14.6, 5.6), 3.24 (*dd*, *J* = 14.6, 10.7)	36.4 (*t*)
C(12′)	–	138.2 (*s*)
H–C(13′)	7.21 – 7.27 (*m*)	129.7 (*d*)
H–C(14′)	7.21 – 7.27 (*m*)	129.6 (*d*)
H–C(15′)	7.12 – 7.19 (*m*)	127.9 (*d*)
H–C(16′)	7.21 – 7.27 (*m*)	129.6 (*d*)
H–C(17′)	7.21 – 7.27 (*m*)	129.7 (*d*)
MeO	3.72 (*s*)	52.9 (*q*)

HMBC spectrum indicating the presence of a ^{i}Bu group instead of a ^{i}Pr group as side chain in **6**. Thus, compound **7** was approved as stachartin D.

Compound **8** was isolated as pale yellow solid. The ^{13}C-NMR spectrum of **8** displayed a total of 33 C-atom signals, its HR-ESI-MS showed a pseudomolecular ion peak at m/z 570.2830 ($[M + Na]^+$) suggesting a molecular formula of $C_{33}H_{41}NO_6$. After we assigned the signals corresponding to the phenylspirodrimane skeleton and the lactam, in the ^{1}H-NMR spectrum, we confirmed the presence (*Table 3*) of signals at δ(H) 7.21 – 7.27 (*m*, H–C(13′) and H–C(17′)), 7.21 – 7.27 (*m*, H–C(14′), and H–C(16′)) and 7.12 – 7.19 (*m*, H–C(15′)) corresponding to an additional monosubstituted aromatic ring, and also the presence of a CH_2 group at 36.4 ppm (δ(H) 3.24, 3.47 (*dd*)) suggesting the presence of a Bn group as side chain. Therefore, compound **8** was elucidated as stachartin E.

The project was supported by the *National Natural Science Foundation of P. R. China* (Nos. 21102126, 21162039, 31260012, 21402169), the *Natural Science Foundation of Ningxia, P. R. China* (NZ15260), the Program for Leading Talents of Ningxia, P. R. China, and the *Scientific Research Foundation of the Higher Education Institutions of Ningxia, P. R. China* (NGY2015126).

Supporting Information

Additional Supporting Information (Spectroscopic data for compounds **1**, **3**, **6**, **7**, and **8** including ^{1}H-, ^{13}C-, and 2D-NMR spectra and ESI-MS/MS data) can be found in the online version of this article: http://dx.doi.org/10.1002/hlca.201600020.

Experimental Part

General

TLC: silica gel GF_{254} (*Qingdao Haiyang Chemical Co., Ltd*., Qingdao, P. R. China) and visualization by 10% H_2SO_4 in EtOH. Column chromatography (CC): SiO_2 (200 – 300 mesh, *Qingdao Haiyang Chemical Co., Ltd.*), *Sephadex LH-20* (*Pharmacia*, Piscataway, NJ, USA). MPLC: *BUCHI Sepacore* system (*BUCHI Labortechnik AG*, Switzerland), and columns packed with *RP-18* (40 – 75 μm, *Fuji Silysia Chemical Ltd.*, Kasugai, Aichi, Japan). HPLC: *Agilent 1100* series instrument equipped with *Agilent ZORBAX SB-C18* column (5 μm, 4.6 × 150 mm). Semiprep. HPLC: *Agilent ZORBAX SB-C18* column (5 μm, 9.4 × 150 mm; *Agilent Technologies*, CA, USA) for the sample preparation. M.p.: *Yuhua X-4* digital microdisplaying melting point apparatus. Optical rotations: *JASCO P-1020* digital polarimeter (*JASCO International Co*., Ltd, Tokyo, Japan). IR Spectra: *Tenor 27* spectrophotometer (*Bruker Optics GmbH*, Ettlingen, Germany) in KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Avance III 600*, *Bruker DRX-500*, and *Bruker AM-400* spectrometers (*Bruker Bio-Spin GmbH*, Rheinstetten, Germany); δ in ppm rel. to the solvent signals, J in Hz. HR-ESI- and HR-EI-MS: *API-Qstar-Pulsar-1* spectrometer (*MDS Sciex*, Concord, Ontario, Canada); in m/z.

Fungus Material and Cultivation Conditions

Stachybotrys chartarum was isolated from a soil sample collected from the Datun tin mine tailings area, Yunnan, P. R. China. A voucher specimen was deposited with Yunnan Institute of Microbiology, Yunnan University. The culture medium consisted of glucose (1.0%), peptone from porcine meat (0.5%), yeast powder (0.5%), KH_2PO4 (0.1%), and $MgSO_4 \cdot 7\ H_2O$ (0.02%). Fermentation was carried out on a shaker at 200 RPM for 15 days.

Extraction and Isolation

The culture broth (150 l) of *S. chartarum* was filtered, and the filtrate was extracted three times with AcOEt, while the mycelium was extracted three times with $CHCl_3$/MeOH (1:1). The AcOEt layer together with the mycelium

extraction was concentrated under reduced pressure to give a crude extract. The extract was subjected to CC over SiO_2 (200 – 300 mesh) eluted with a gradient of $CHCl_3$/MeOH (1:0 → 0:1) to obtain two fractions, *Frs. 1* and *2*. *Fr. 1* was separated by CC (*Sephadex LH-20*, $CHCl_3$/MeOH (1:1), MeOH) to give **2** (450 mg). *Fr. 2* was applied to MPLC (MeOH/H_2O, eluting from 1:9 to 1:1) to give *Subfractions A* and *B*. *Subfraction B* was separated by CC over SiO_2 ($CHCl_3$/MeOH 100:1 → 10:1) to give **3** (165 mg) and *Subfractions B.1 – B.4*. *Subfraction B.1* was isolated and purified repeatedly by CC (*Sephadex LH-20* (MeOH)), semiprep. RP-C_{18} HPLC with MeOH/H_2O (79 – 80%) to give **7** (23 mg), **8** (14 mg), and **6** (6 mg). *Subfraction B.2* was isolated and purified repeatedly by CC (*Sephadex LH-20* (MeOH)) to give **1** (13 mg), and **5** (224 mg). *Subfraction B.3* was separated by CC (*Sephadex LH-20* (MeOH)) to give **4** (79 mg).

Stachartin A (= **(3*E*)-4-[(2*R*,2′*R*,4a′*S*,6′*R*,8a′*S*)-3′,4′,4a′,5′,6′,7′,8′,8a′-Octahydro-4,6′-dihydroxy-6-(hydroxymethyl)-2′,5′,5′,8a′-tetramethyl-2′*H*,3*H*-spiro[1-benzofuran-2,1′-naphthalen]-7-yl]but-3-en-2-one**; **1**). Colorless oil. $[\alpha]_D^{20} = -169.4$ (c = 0.25, MeOH). IR (KBr): 3440, 2938, 2876, 1621, 1595, 1452, 1386, 1255, 988, 965. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 451.2465 ([M + Na]$^+$, $C_{26}H_{36}NaO_5^+$; calc. 451.2460).

Stachartin B (= **(2*R*,2′*R*,4a′*S*,6′*R*,8a′*S*)-3,3′,4′,4a′,5′,6,6′,7′,8′,8a′-Decahydro-4,6′-dihydroxy-2′,5′,5′,8a′-tetramethyl-2′*H*,8*H*-spiro[benzo[1,2-*b*:5,6-*c*′]difuran-2,1′-naphthalen]-8-one**; **3**). Colorless oil. $[\alpha]_D^{20} = -32.2$ (c = 0.25, MeOH). IR (KBr): 3433, 2955, 2941, 1726, 1623, 1460, 1330, 1247. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 409.1988 ([M + Na]$^+$, $C_{23}H_{30}NaO_5^+$; calc. 409.1991).

Stachartin C (= **Methyl 2-[(2*R*,2′*R*,4a′*S*,6′*R*,8a′*S*)-3′,4′,4a′,5′,6,6′,7′,8,8′,8a′-Decahydro-4,6′-dihydroxy-2′,5′,5′,8a′-tetramethyl-6-oxo-2′*H*-spiro[furo[2,3-*e*]isoindole-2,1′-naphthalen]-7(3*H*)-yl]-3-methylbutanoate**; **6**). Pale yellow solid. $[\alpha]_D^{20} = -45.1$ (c = 0.25, MeOH). IR (KBr): 3442, 2961, 2937, 2876, 1741, 1670, 1629, 1468, 1348, 1214, 1087. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS (pos.): 522.2827 ([M + Na]$^+$, $C_{29}H_{41}NNaO_6^+$; calc. 522.2832).

Stachartin D (= **Methyl 2-[(2*R*,2′*R*,4a′*S*,6′*R*,8a′*S*)-3′,4′,4a′,5′,6,6′,7′,8,8′,8a′-Decahydro-4,6′-dihydroxy-2′,5′,5′,8a′-tetramethyl-6-oxo-2′*H*-spiro[furo[2,3-*e*]isoindole-2,1′-naphthalen]-7(3*H*)-yl]-4-methylpentanoate**; **7**). Pale yellow solid. $[\alpha]_D^{20} = -43.5$ (c = 0.25, MeOH). IR (KBr): 3440, 2957, 2938, 2873, 1743, 1672, 1626, 1468, 1347, 1263, 1087. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS (pos.): 536.2988 ([M + Na]$^+$, $C_{30}H_{43}NNaO_6^+$; calc. 536.2988).

Stachartin E (= **Methyl 2-[(2*R*,2′*R*,4a′*S*,6′*R*,8a′*S*)-3′,4′,4a′,5′,6,6′,7′,8,8′,8a′-Decahydro-4,6′-dihydroxy-2′,5′,5′,8a′-tetramethyl-6-oxo-2′*H*-spiro[furo[2,3-*e*]isoindole-2,1′-naphthalen]-7(3*H*)-yl]-3-phenylpropanoate**; **8**). Pale yellow solid. $[\alpha]_D^{20} = -103.6$ (c = 0.25, MeOH). IR (KBr): 3440, 2955, 2937, 2875, 1743, 1672, 1626, 1468, 1348, 1083. ^{1}H- and ^{13}C-NMR: *Table 3*. HR-ESI-MS: 570.2830 ([M + Na]$^+$, $C_{33}H_{41}NNaO_6^+$; calc. 570.2832).

REFERENCES

[1] Z. E. Wilson, M. A. Brimble, *Nat. Prod. Rep.* **2009**, *26*, 44.

[2] A. A. Stierle, D. B. Stierle, B. Patacini, *J. Nat. Prod.* **2008**, *71*, 856; D. B. Stierle, A. A. Stierle, B. Patacini, *J. Nat. Prod.* **2007**, *70*, 1820; A. A. Stierle, D. B. Stierle, K. Kelly, *J. Org. Chem.* **2006**, *71*, 5357; A. A. Stierle, D. B. Stierle, K. Kemp, *J. Nat. Prod.* **2004**, *67*, 1392; D. B. Stierle, A. A. Stierle, J. D. Hobbs, J. Stokken, J. Clardy, *Org. Lett.* **2004**, *6*, 1049; A. A. Stierle, D. B. Stierle, E. Goldstein, K. Parker, T. Bugni, C. Baarson, J. Gress, D. Blake, *J. Nat. Prod.* **2003**, *66*, 1097.

[3] Z.-G. Ding, M.-G. Li, J.-Y. Zhao, J. Ren, R. Huang, M.-J. Xie, X.-L. Cui, H.-J. Zhu, M.-L. Wen, *Chem. Eur. J.* **2010**, *16*, 3902; Z.-G. Ding, M.-G. Li, J. Ren, J.-Y. Zhao, R. Huang, Q.-Z. Wang, X.-L. Cui, H.-J. Zhu, M.-L. Wen, *Org. Biomol. Chem.* **2011**, *9*, 2771; Z.-G. Ding, J.-Y. Zhao, M.-G. Li, J. Ren, R. Huang, Q.-M. Li, X.-L. Cui, H.-J. Zhu, M.-L. Wen, *J. Nat. Prod.* **2012**, *75*, 1994.

[4] W. A. Ayer, S. C. Miao, *Can. J. Chem.* **1993**, *71*, 487.

[5] Y. Li, C.-M. Wu, D. Liu, P. Proksch, P. Guo, W.-H. Lin, *J. Nat. Prod.* **2014**, *77*, 138.

[6] B. B. Jarvis, J. Salemme, A. Morais, *Nat. Toxins* **1995**, *3*, 10.

[7] K. Sakai, K. Watanabe, K. Masuda, M. Tsuji, K. Hasumi, A. Endo, *J. Antibiot.* **1995**, *48*, 447.

[8] X.-H. Ma, L.-T. Li, T.-J. Zhu, M.-Y. Ba, G.-Q. Li, Q.-Q. Gu, Y. Guo, D.-H. Li, *J. Nat. Prod.* **2013**, *76*, 2298.

Received January 21, 2016
Accepted June 13, 2016

