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Isoprenylated cyclohexanoids from the basidiomycete *Hexagonia speciosa*

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ABSTRACT

Nine oxygenated cyclohexanoids, speciosins L–T (**1**–**9**) as well as a 5*H*-furan-2-one metabolite, 5′-O-acetylaporpinone A (**10**), together with known analogs, speciosins A, B, D, E, F, I and K (**11**–**17**), and aporpinone A (**18**), were isolated from a scale-up cultures of the basidiomycete *Hexagonia speciosa*. Their structures were elucidated by analysis of spectroscopic data, including 1D and 2D NMR. Speciosin B (**12**) showed significant cytotoxicity against several tumor cell lines with IC_{50} values in the range 0.23–3.30 μM.

1. Introduction

The basidiomycete *Hexagonia speciosa* is a fungus of the family Polyporaceae widely distributed in the tropical and subtropical zones of China, such as the Hainan and Yunnan Provinces (Zhao, 1998). Our previous investigations of this fungus have reported a series of oxygenated cyclohexanoids (Jiang et al., 2009). In a continuing phytochemical study of this fungus, nine new oxygenated cyclohexanoids speciosins L–T (**1**–**9**) and a new 5*H*-furan-2-one metabolite 5′-O-acetylaporpinone A (**10**) were isolated from its scale-up cultures together with eight known analogs. Some of these isolates were evaluated for cytotoxic activities against five tumor cell lines, and speciosin B (**12**) showed significant cytotoxic activity. The isolation and structural elucidation of these new metabolites and their cytotoxicities are described herein.

2. Results and discussion

H. speciosa was isolated from its fruiting bodies collected in the Gaoligong Mountains of Yunnan Province, China, in July, 2007, and cultured in a modified PDA medium shaken at 150 rpm. After culturing for 25 days at 25 °C, the whole culture broth (63 L) was filtered and the filtrate was extracted thrice with EtOAc. The crude EtOAc extract (20.0 g) was subjected to repeated column chromatography to yield ten new compounds, speciosins L–T (**1**–**9**) and 5′-O-acetylaporpinone A (**10**), together with eight known metabolites, speciosins A, B, D, E, F, I and K (**11**–**17**), and aporpinone A (**18**) (Jiang et al., 2009; Levy et al., 2003).

Compound **1** was obtained as colorless oil, with the molecular formula $C_{10}H_{10}O_3$ as determined from HRESIMS (*m/z* 201.0525 $[M+Na]^+$, calcd for $C_{10}H_{10}O_3Na$, 201.0527), ^{13}C NMR and DEPT data. The IR spectrum showed absorptions for hydroxy groups (3405 and 3384 cm^{-1}), an acetylenic group (2225 cm^{-1}) and a double bond (1644 cm^{-1}) moiety. The ^1H NMR spectrum of 1 (Table 2) showed resonances for a terminal methylene [δ_H 5.87 (1H, dd, J = 17.4, 11.3 Hz, H-9), 5.69 (1H, dd, J = 17.4, 2.1 Hz, H-10a), 5.57 (1H, dd, J = 11.3, 2.1 Hz, H-10b)], a bis-substituted double bond [δ_H 5.58 (1H, m, H-2), 5.68 (1H, m, H-3)], and three oxymethine protons [δ_H 4.54 (1H, brs, H-1), 4.28 (1H, brd, H-4), 3.50 (1H, t, J = 2.1 Hz, H-5)]. The ^{13}C NMR spectrum (Table 1) indicates presence of 10 carbon resonances, including one terminal double bond at δ_C 129.1 and 117.3, a bis-substituted double bond at δ_C 129.4 and 126.8, as well as two acetylenic carbons (δ_C 88.3 and 83.0), three oxymethine carbons (δ_C 68.0, 64.1, and 63.4), and one quaternary carbon (δ_C 54.8). The ^1H-^1H COSY experiment delineated a spin system from H-1 to H-5. The above-mentioned data exhibited similarities with those of speciosin E (**14**) which suggested that both compounds possessed the same oxygenated cyclohexanoid skeleton (Jiang et al., 2009). The key difference between the two compounds was that the two oxygenated carbons in **14** were replaced by the terminal double bond moiety in **1**. The HMBC spectrum of **1** demonstrated the expected key correlations: from H-9 to C-7, C-8 and C-10 and from H-10 to C-8 and C-9. The relative configuration of **1** was the same as that of **14** based on their agreement with ^1H NMR coupling constants with references (Jiang et al., 2009; Nagata et al., 1992; Garbisch, 1964; Mühlenfeld and Achenbach, 1988; Pachler, 1971). Therefore, structure 1 was established shown in Fig. 1 and named as speciosin L.

Compound **2** was obtained as an oil and found to possess molecular formula $C_{13}H_{16}O_5$ based on the HRESIMS (found *m/z* 251.0912

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Table 1
^{13}C NMR spectroscopic data of speciosins L–T (**1**–**9**).

No.	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[a]	**7**[b]	**8**[a]	**9**[a]
1	68.0	68.2	68.1	68.1	152.4	74.1	67.1	69.8	202.5
2	129.4	129.5	129.3	129.4	117.2	34.2	31.4	28.6	33.7
3	126.8	126.8	126.9	126.9	118.4	34.6	29.1	28.6	32.1
4	63.4	63.4	63.4	63.4	151.0	70.7	70.3	66.9	64.0
5	64.1	63.9	63.8	63.8	119.4	39.9	35.4	34.4	159.4
6	54.8	54.5	54.4	54.3	111.3	44.8	40.5	37.7	127.0
7	88.3	80.4	82.2	82.7	82.3	31.4	30.7	30.4	126.1
8	83.0	85.8	87.3	86.2	92.9	123.4	127.4	129.4	148.1
9	117.3	27.1	69.1	67.3	64.8	133.7	131.5	132.3	71.6
10	129.1	17.5	26.0	26.4	67.2	26.1	70.2	71.4	29.7*
11		68.2	70.9	71.4		18.0	14.1	14.1	29.6*
CH_3CO		172.6		172.4			171.1	172.9	
CH_3CO		20.7		20.7			20.0	20.8	

* Interchangeable.
[a] Measured in CD_3OD.
[b] Measured in $CDCl_3$.

Table 2
^{1}H NMR spectroscopic data of speciosins L–P (**1**–**5**) in CD_3OD.

No.	**1**	**2**	**3**	**4**	**5**
1	4.54 (brs)	4.48 (brs)	4.52 (brs)	4.49 (brs)	
2	5.58 (m)	5.58 (m)	5.57 (dd, 10.4, 2.4)	5.57 (m)	6.66 (d, 8.7)
3	5.68 (m)	5.66 (m)	5.67 (ddd, 10.4, 4.3, 2.4)	5.67 (m)	6.63 (dd, 8.7, 2.3)
4	4.28 (brd, 4.8)	4.26 (brd, 4.6)	4.26 (brd, 4.3)	4.26 (brd, 4.0)	
5	3.50 (t, 2.1)	3.43 (t, 1.8)	3.48 (m)	3.46 (t, 1.5)	6.71 (d, 2.3)
9	5.87 (dd, 17.4, 11.3)	2.87 (m)			4.55 (dd, 6.9, 4.8)
10	5.69 (dd, 17.4, 2.1)	1.20 (d, 7.0)	1.41 (s)	1.46 (s)	3.69 (dd, 11.2, 4.8)
	5.57 (dd, 11.3, 2.1)				3.64 (dd, 11.2, 6.9)
11		4.09 (m)	3.49 (s)	4.09 (d, 15.9)	
		3.99 (m)		4.07 (d, 15.9)	
CH_3CO		2.06 (s)		2.07 (s)	

$[M-H]^-$, calcd for $C_{13}H_{15}O_5$, 251.0919) and NMR data. Its IR spectrum suggested the presence of carbonyl (1739 cm^{-1}), hydroxy (3406 and 3387 cm^{-1}), alkyne (2248 cm^{-1}) and methyl (1376 cm^{-1}) groups. A comparison of the ^{1}H, ^{13}C NMR spectroscopic data of compound **2** with those of **1** suggested that they shared the same partial structure: oxygenated cyclohexanoid. The NMR spectra of **2** exhibited one acetyl group (δ_C 172.6, 20.7; δ_H 2.06), one oxymethylene (δ_C 68.2; δ_H 4.09 and 3.99), one methine (δ_C 27.1; δ_H 2.87) and a secondary methyl (δ_C 17.5; δ_H 1.20). The HMBC spectrum displayed the correlations from H-9 to C-7, C-8, C-10 and C-11, from H-10 to C-8, C-9 and C-11, from H-11 to C-8, C-9, C-10 and the acetyl carbonyl carbon, which suggested 2 had a acetoxy group at C-11. Accordingly, compound **2** was determined and named as speciosin M.

Compound **3** was obtained as colorless oil. Its HRESIMS showed an $[M+Na]^+$ peak at *m/z* 249.0740, corresponding to the molecular formula $C_{11}H_{14}O_5Na$ (calcd 249.0738). The NMR spectra of **3** (Tables 1 and 2) showed features similar to those of **2**, suggesting they were analogs. The obvious differences were as follows: **3** had an oxygenated quaternary carbon (δ_C 69.1) rather than a methine in **2**, meanwhile, the acetyl signals were absent in **3**. The HMBC spectrum of **3** displayed correlations from H-10 to C-8, C-9 and C-11, from H-11 to C-8, C-9 and C-10. Thus, structure 3 was elucidated as shown in Fig. 1 and named speciosin N.

The HRESIMS of speciosin O (**4**) indicated a pseudomolecular ion $[M+Na]^+$ corresponding to molecular formula $C_{13}H_{16}O_6$. The NMR character was very similar to that of **3** except for an extra acetyl group. The HMBC spectrum showed a key correlation from H-11 (δ_H 4.09, 4.07) to an acetyl carbonyl carbon (δ_C 172.4), which indicated 4 was an acetyl ester of **3**. The relative configurations of **2**–**4** were the same as for of **1** on the basis of their coupling constants.

Speciosin P (**5**) was assigned the molecular formula $C_{10}H_{10}O_4$ by HRESIMS (found 193.0501 $[M-H]^-$, calcd for $C_{10}H_9O_4$, 193.0500), corresponding to six degrees of unsaturation. The ^{1}H NMR spectrum displayed signals for a 1,2,4-trisubstituted aromatic ring [δ_H 6.66 (d, J = 8.7 Hz, H-2), 6.63 (dd, J = 8.7, 2.3 Hz, H-3), 6.71 (d, J = 2.3, H-5)], one oxymethine [δ_H 4.55 (dd, J = 6.9, 4.8 Hz, H-9) and one oxymethylene [δ_H 3.69 (dd, J = 11.2, 4.8 Hz, H-10a), 3.64 (dd, J = 11.2, 6.9 Hz, H-10b)] functionality, respectively. Correspondingly, the ^{13}C NMR spectra displayed **10** resonances, including an aromatic ring (δ_C 152.4, 151.0, 119.4, 118.4, 117.2, 111.3), one oxymethine (δ_C 64.8), one oxymethylene (δ_C 67.2) as well as one alkyne bond (δ_C 92.9, 82.3). Finally, the linkage of the aromatic ring and oxymethine groups via a triple bond was established by the HMBC correlations of H-5 with C-7, H-9 with C-7 and C-8, and H-10 with C-8. Therefore, structure 5 was established as shown in Fig. 1.

Speciosin Q (**6**) was isolated as colorless oil. It possessed the molecular formula $C_{11}H_{20}O_2$ as established by positive HRESIMS (*m/z* 207.1361 $[M+Na]^+$, calcd for 207.1360). The NMR spectra (Tables 1 and 3) showed two methyl singlets, four aliphatic methylenes, a trisubstituted double bond, and three methines (two oxygenated). With the assistance of analysis of its 2D NMR spectra including ^{1}H–^{1}H COSY, heteronuclear single-quantum coherence (HSQC), and HMBC, **6** was shown to possess the same 6-isoprenylcyclohexane-1,4-diol framework as speciosin I (**16**). The relative configuration of **6** was deduced from the ^{1}H NMR characteristic coupling constants. Analysis of ^{1}H NMR spectrum indicated axial–axial coupling constants ($J_{a/a}$ 10.3, 10.3, 10.6, 10.6 Hz) of H-1/H-2*ax*, H-1/H-6, H-4/H-3*ax*, and H-4/H-5*ax*, which established that H-1 and H-4 were in axial position of a chair conformation for the cyclohexane ring. Thus, structure 6 was determined as shown in Fig. 1.

Speciosins R and S (**7**, **8**) were obtained as colorless oils. Their molecular formulas $C_{13}H_{22}O_4$ were determined by their HRESIMS. Their NMR spectra (Tables 1 and 3) showed two methyl singlets,

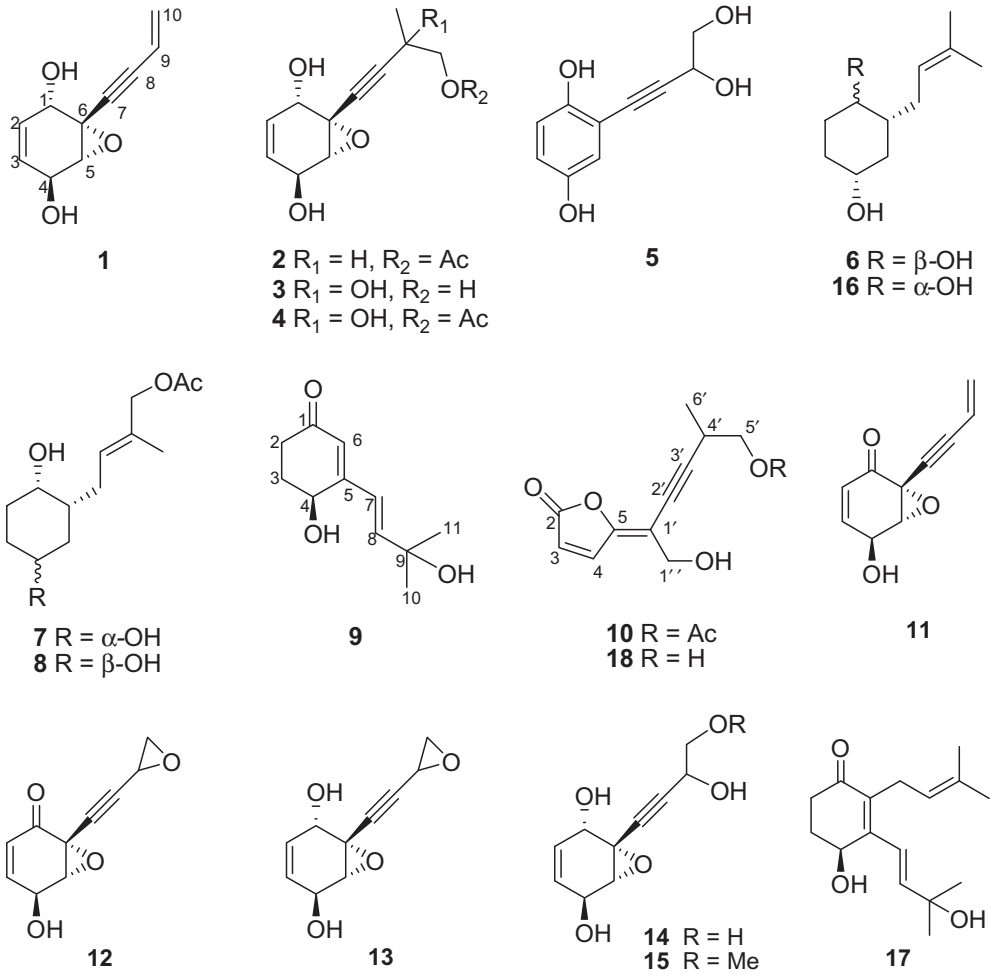

Fig. 1. Structures of compounds **1**–**18**.

Table 3
^{1}H NMR spectroscopic data of speciosins Q–T (**6**–**9**).

No.	**6**[a]	**7**[b]	**8**[a]	**9**[a]
1	3.14 (ddd, 10.3, 10.3, 4.4)	3.80 (brs)	3.80 (brs)	
2	1.90 (overlapped)	1.87 (ddd, 13.9, 6.8, 2.8)	1.84 (m)	2.70 (ddd, 17.2, 9.1, 7.8)
	1.30 (overlapped)	1.48 (overlapped)		2.33 (ddd, 17.2, 4.8, 4.8)
3	1.90 (overlapped)	1.77 (overlapped)	1.58 (m)	2.13 (m)
	1.30 (overlapped)	1.57 (m)	1.44 (m)	
4	3.50 (dddd, 10.6, 10.6, 4.4, 4.4)	3.59 (dddd, 11.9, 10.5, 4.1, 4.1)	3.94 (brs)	4.62 (dd, 4.1, 3.3)
5	1.95 (ddd, 12.5, 6.6, 3.7)	1.75 (overlapped)	1.64 (m)	
	0.94 (ddd, 12.5, 10.6, 10.6)	1.36 (ddd, 11.9, 11.9, 11.9)	1.47 (m)	
6	1.30 (overlapped)	1.48 (overlapped)	1.87 (m)	5.91 (s)
7	2.41 (m)	2.18 (ddd, 14.5, 14.5, 7.2)	2.16 (ddd, 14.0, 14.0, 7.0)	6.38 (d, 15.9)
	1.87 (overlapped)	2.07 (ddd, 14.5, 14.5, 7.9)	1.99 (ddd, 14.0, 14.0, 7.0)	
8	5.14 (t, 6.5)	5.47 (dd, 7.9, 7.2)	5.50 (t, 7.0)	6.60 (d, 15.9)
10	1.70 (s)	4.45 (s)	4.46 (s)	1.33 (s)
11	1.62 (s)	1.67 (s)	1.67 (s)	1.33 (s)
CH_3CO		2.07 (s)	2.03 (s)	

[a] Measured in CD_3OD.
[b] Measured in $CDCl_3$.

five aliphatic methylenes (one oxygenated), a trisubstituted double bond, and three methines (two oxygenated). The NMR spectroscopic data of **7** were similar to those of speciosin I (**16**), except for one oxymethylene (δ_C 70.2, C-10) instead of a methyl, and an additional acetyl group (δ_C 171.1, 20.0; δ_H 2.07, s). In the HMBC spectrum, the correlations from H-10 (δ_H 4.45, s) to C-11 and the acetyl carbonyl carbon, and from H-8 to C-10 and C-11 were observed. The relative configuration of 7 was deduced from the characteristic coupling constants. H-1 (δ_H 3.80, brs) showed small $J_{a/e,e/e}$ coupling with H-2 and H-6, indicated that H-1 was in an equatorial position. H-4 (δ_H 3.59) showed large $J_{a/a}$ coupling with H-3*ax* (J = 10.5 Hz) and H-5*ax* (J = 11.9 Hz), which indicated this H-4 was in an axial position. The ROESY correlation between H-8 and H-10 suggested that these protons were at the same side. From the data above, structure **7** was elucidated as shown in Fig. 1. According to analysis of the HSQC and HMBC spectra, the overall

Table 4
NMR spectroscopic data of 5′-o-acetylaporpinone A (**10**) in CD_3OD.

No.	δ_C	δ_H
2	170.8, s	
3	120.7, d	6.31 (d, 5.5)
4	141.9, d	8.08 (d, 5.5)
5	155.6, s	
1′	110.5, s	
2′	78.4, s	
3′	104.9, s	
4′	28.4, d	3.10 (m)
5′	68.2, t	4.15 (dd, 10.5, 6.8) 4.06 (dd, 10.5, 6.6)
6′	17.5, q	1.28 (d, 7.0)
1″	61.1, t	4.30 (s)
CH_3CO	172.7, s	
CH_3CO	20.7, q	2.08 (s)

structure of **8** was the same as **7**. However, H-4 (δ_H 3.94, brs) exhibited small coupling constants ($J_{a/e,e/e}$), which indicated that H-4 was in equatorial position. Thus, structure **8** was established as shown in Fig. 1.

The UV/Vis spectrum of speciosin T (**9**) exhibited absorption maxima at λ = 278 and 377 nm, indicative of the presence of a long conjugate system. The HRESIMS showed an $[M+Na]^+$ ion at m/z = 219.0992, corresponding to molecular formula $C_{11}H_{16}O_3$. Its IR spectrum showed unsaturated carbonyl and hydroxy absorptions at 1703 and 3405, 3397 cm^{-1}, respectively. The ^1H NMR spectrum showed a *trans*-double bond (δ_H 6.60, d, J = 15.9 Hz; 6.38, d, J = 15.9 Hz), a tri-substituted double bond (δ_H 5.91, s), one oxymethine (δ_H 4.62, dd, J = 4.1, 3.3 Hz), two methylenes and two methyls (δ_H 1.33, 6H, s). The following HMBC correlations were observed: from H-3 to C-1 and C-4, from H-4 to C-5, C-6 and C-7, from H-6 to C-2 and C-4, from H-7 to C-4, C-6 and C-9, from H-8 to C-5, C-9, C-10 and C-11. Therefore, structure **9** was assigned as shown in Fig. 1. Its absolute configuration was established by comparing its specific rotation with those of known analogs. Speciosin K (**17**) had an *S* configuration and gave a negative specific rotation value, −14.5 (*c* 0.13, $CHCl_3$) (Jiang et al., 2009). Another example, 4(*R*)-hydroxy-3-methyl-2-cyclohexen-1-one, has a specific rotation of +34.1 (*c* 1.0, $CHCl_3$), while the *S* enantiomer has a specific rotation of −35.2 (*c* 1.0, $CHCl_3$) (Galano et al., 2000). With a specific rotation value of −16.2 (*c* 0.41, MeOH), speciosin T was assigned to a 4*S* configuration.

The molecular formula of **10** was determined as $C_{13}H_{14}O_5$ by HRESIMS, indicating seven degrees of unsaturation. The NMR spectroscopic data of **10** (Table 4) were very similar to those of aporpinone A (**18**) (Jiang et al., 2009; Levy et al., 2003), except for an extra acetyl group linked at C-5′, which was determined by the HMBC spectrum and downfield esterification shift. Thus, the structure of **10** was established and named as 5′-O-acetylaporpinone A.

Compounds **2**, **3**, **6**, and **12**–**18** were tested for their toxicity effects in the human tumor cell lines HL-60, SMMC-7721, A-549, MCF-7, and SW480. Speciosin B (**12**) showed significant inhibitory activity against the five cell lines with IC_{50} values of 0.23 μM (HL-60), 0.70 μM (SMMC-7721), 3.30 μM (A-549), 2.85 μM (MCF-7), and 2.95 μM (SW480), and aporpinone A (**18**) showed weak inhibitory activities with IC_{50} values of 4.07 μM (SMMC-7721), 21.01 μM (A-549), and 10.45 μM (MCF-7). The other compounds were inactive (IC_{50} values >40 μM).

3. Concluding remarks

In conclusion, nine new oxygenated cyclohexanoids, speciosins L–T (**1**–**9**) as well as a new 5*H*-furan-2-one metabolite, 5′-O-acetylaporpinone A (**10**), were isolated from scale-up cultures of the basidiomycete *H. speciosa* together with known analogs, speciosins A, B, D, E, F, I and K (**11**–**17**), and aporpinone A (**18**). The discovery of compounds **1**–**9** is a further addition to that results for the diverse cyclohexanoids. Their presence as characteristic markers may be helpful in chemotaxonomical classifications. The cytotoxicity against several tumor cell lines of some compounds was also investigated.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured on JASCO P-1020 digital polarimeter. UV spectra were recorded on a Shimadzu UV-2401PC spectrophotometer. IR spectra were obtained on a Bruker Tensor 27 FT-IR spectrometer using KBr pellets. NMR spectra were acquired on Bruker DRX-500 and AV-400 instruments at room temperature. MS were recorded on a VG Autospec-3000 spectrometer and an API QSTAR Pulsar i spectrometer. Silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography (CC). Preparative HPLC was performed on an Agilent 1100 liquid chromatography system equipped with a Zorbax SB-C_{18} column (9.4 mm × 150 mm).

4.2. Fungal material and cultivation conditions

H. speciosa was isolated from its fruiting bodies collected in the Gaoligong Mountains, Yunnan Province, China, in July 2007, and identified by Prof. Zhu-Liang Yang, Kunming Institute of Botany. A voucher specimen (HFG 07061) was deposited in the Herbarium of the Kunming Institute of Botany. Culture medium consisted of potato (peel) (200 g), glucose (20 g), KH_2PO_4 (3 g), $MgSO_4$ (1.5 g), citric acid (0.1 g), and thiamine hydrochloride (10 mg) per L of deionized H_2O. The pH was adjusted to 6.5 before autoclaving, and the fermentation was carried out in a shaker at 25 °C and 150 rpm for 25 days.

4.3. Extraction and isolation

The entire culture broth of *H. speciosa* (63 L) was initially filtered, and the filtrate extracted (3× EtOAc, 60 L). The organic layer was concentrated under reduced pressure to give a crude extract (20.0 g), and this residue was subjected to silica gel CC (200–300 mesh) using a $CHCl_3$–MeOH gradient (100:0–0:100) to produce fractions A–D. Fraction B (13.2 g) was further purified by reversed phase chromatography on a C_{18} column (MeOH–H_2O, 20:80–100:0) to give six subfractions (B1–B6). Each subfraction was further purified by repeated silica gel CC. Compound **1** (28.0 mg), **4** (2.8 mg), 13 (221 mg), **15** (12.0 mg) were obtained from the subfraction B2 (2.1 g) by Sephadex LH-20 (MeOH), reversed-phased C_{18} CC (MeOH–H_2O 3:7) and preparative HPLC (CH_3CN–H_2O 2:8–4:6). Fraction B3 (6.5 g) was separated by silica gel CC eluting with petroleum ether–$EtOH_2$ (10:1–0:1, v/v) to give **12** (281 mg) and **18** (127 mg). Fraction B4 (500 mg) was purified over silica gel CC with petroleum ether–$EtOH_2$ (10:1–0:1), then by Sephadex LH-20 (MeOH) to provide **16** (192 mg). Compound **17** (16.7 mg) was obtained from B5 (1.5 g) by repeated silica gel CC and Sephadex LH-20 ($CHCl_3$–MeOH = 1:1). After repeated silica gel and Sephadex LH-20 CC, and preparative HPLC (CH_3CN–H_2O), **11** (10.0 mg), **6** (9.4 mg), **9** (4.2 mg), **2** (32.5 mg), **7** (7.0 mg), **10** (2.5 mg), **8** (6.8 mg) were obtained from fraction B6 (1.4 g). Fraction C (280 mg) was purified by reversed phase chromatography on a C_{18} column (MeOH–H_2O 3:7), Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) cc and preparative HPLC (CH_3CN–H_2O 2:8–4:6) to provide

compound **3** (19.0 mg) and **5** (14.0 mg). Compound **14** (122.8 mg) was isolated from Fraction D (600 mg) by successive reversed phase chromatography using a C_{18} column (MeOH–H_2O) and Sephadex LH-20 (MeOH).

4.4. Speciosin L (**1**)

Colorless oil; $[\alpha]_D^{23}$ +56.5 (*c* 1.36, MeOH); IR (KBr) ν_{max} 3405, 3384, 2924, 2225, 1644, 1385, 1225, 1033 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS (positive) *m/z* 201 $[M+Na]^+$, 379 $[2\,M+Na]^+$; HRESIMS *m/z* 201.0525 $[M+Na]^+$, calcd for $C_{10}H_{10}O_3Na$, 201.0527.

4.5. Speciosin M (**2**)

Colorless oil; $[\alpha]_D^{28}$ +136.6 (c 3.18, MeOH); IR (KBr) ν_{max} 3406, 3387, 2983, 2939, 2894, 2248, 1739, 1390, 1376, 1265, 1238, 1038 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; FABMS (negative) *m/z* 251 $[M-H]^-$; HRESIMS *m/z* 251.0912 $[M-H]^-$, calcd for $C_{13}H_{15}O_5$, 251.0919.

4.6. Speciosin N (**3**)

Colorless oil; $[\alpha]_D^{27}$ +89.9 (*c* 0.51, MeOH); IR (KBr) ν_{max} 3423, 2933, 2365, 2246, 1719, 1636, 1378, 1260, 1037 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS (positive) *m/z* 227 $[M+H]^+$; HRESIMS *m/z* 249.0740 $[M+Na]^+$, calcd for $C_{11}H_{14}O_5Na$, 249.0738.

4.7. Speciosin O (**4**)

Colorless oil; $[\alpha]_D^{23}$+161.0 (*c* 0.28, MeOH); IR (KBr) ν_{max} 3417, 2936, 1737, 1639, 1376, 1248, 1043 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS (positive) *m/z* 291 $[M+Na]^+$, 559 $[2\,M+Na]^+$; HRESIMS *m/z* 291.0840 $[M+Na]^+$, calcd for $C_{13}H_{16}O_6Na$, 291.0844.

4.8. Speciosin P (**5**)

Colorless oil; $[\alpha]_D^{26}$ −6.2 (*c* 0.41, MeOH); UV(MeOH) λ_{max} (log ε) 319 (3.69), 250 (3.97), 240 (3.94), 218 (4.18), 205 (4.26) nm; IR (KBr) ν_{max} 3374, 3362, 3347, 3149, 3111, 2920, 2231, 1612, 1208, 1083, 1004 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; FABMS (negative) *m/z* 193 $[M-H]^-$, 387 $[2\,M-H]^-$; HRESIMS *m/z* 193.0501 $[M-H]^-$, calcd for $C_{10}H_9O_4$, 193.0500.

4.9. Speciosin Q (**6**)

Colorless oil; $[\alpha]_D^{23}$ −23.1 (*c* 0.47, MeOH); IR (KBr) ν_{max} 3375, 2928, 1631, 1452, 1376, 1096, 1044 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 3; EIMS *m/z* 184 ($[M]^+$, 100), 166, 151, 133, 109; HRESIMS *m/z* 207.1361 $[M+Na]^+$, calcd for $C_{11}H_{20}O_2Na$, 207.1360.

4.10. Speciosin R (**7**)

Colorless oil; $[\alpha]_D^{27}$ +12.0 (c 0.40, MeOH); IR (KBr) ν_{max} 3406, 3387, 2936, 2867, 1734, 1443, 1377, 1260, 1023, 967 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 3; EIMS *m/z* 241 ($[M-H]^+$, 1), 240, 225, 182, 167, 149, 121, 79 (100); HRESIMS *m/z* 243.1617 $[M+H]^+$, calcd for $C_{13}H_{23}O_4$, 243.1596).

4.11. Speciosin S (**8**)

Colorless oil; $[\alpha]_D^{22}$ +18.0 (*c* 0.34, MeOH); IR (KBr) ν_{max} 3406, 3397, 3388, 2932, 1733, 1440, 1379, 1262 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 3; ESIMS *m/z* 265 $[M+Na]^+$, 507 $[2\,M+Na]^+$; HRESIMS *m/z* 265.1418 $[M+Na]^+$, calcd for $C_{13}H_{22}O_4Na$, 265.1415.

4.12. Speciosin T (**9**)

Colorless oil; $[\alpha]_D^{27}$ −16.2 (*c* 0.41, MeOH); UV($CHCl_3$) λ_{max} (log ε) 278 (4.09), 377 (2.83) nm; IR (KBr) ν_{max} 3405, 3397, 2972, 2928, 1703, 1659, 1632, 1593, 1383, 1358, 1258, 1197 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 3; EIMS *m/z* 178 ($[M-H_2O]^+$, 15), 163 (25), 136 (100), 121 (56); HRESIMS *m/z* 219.0992 $[M+Na]^+$, calcd for $C_{11}H_{16}O_3Na$, 219.0997.

4.13. 5′-O-acetylaporpinone A (**10**)

Colorless oil; $[\alpha]_D^{25}$ −8.0 (*c* 0.25, MeOH); IR (KBr) ν_{max} 3450, 2933, 2217, 1785. 1744, 1630, 1375, 1235 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 4; ESIMS (positive) *m/z* 273 $[M+Na]^+$, 523 $[2\,M+Na]^+$; HRESIMS *m/z* 273.0730 $[M+Na]^+$, calcd for $C_{13}H_{14}O_5Na$, 273.0738.

4.14. Bioassays

Five human cancer cell lines, human myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7, and colon cancer SW480 cells, were used in the cytotoxic assay. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method in 96-well microplates. The assay is based on the reduction of MTT by the mitochondrial dehydrogenase of viable cells to give a blue formazan product that can be measured spectrophotometrically (Mosmann, 1983). Briefly, 100 μL adherent cells were seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition, both with initial density of 1×10^5 cells/mL in 100 μL of medium. Each tumor cell line was exposed to the test compound at various concentrations in triplicate for 48 h, with 10-hydroxycamptothecin (Sigma) as positive control. After the incubation, MTT (100 μg) was added to each well, and the incubation continued for 4 h at 37 °C. The cells were lysed with 100 μL of 20% SDS-50% DMF after removal of 100 μL of medium. The optical density of the lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2011.03.011.

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