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Antimicrobial polyketides from *Trichoderma koningiopsis* QA-3, an endophytic fungus obtained from the medicinal plant *Artemisia argyi*†

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Artemisia argyi is broadly cultivated as a medicinal plant in Qichun of Hubei province in central China. Five new fungal polyketides (**1–5**) and two known analogues (**6** and **7**) were isolated and identified from the culture extract of *Trichoderma koningiopsis* QA-3, an endophytic fungus obtained from the inner tissue of *Artemisia argyi* that was collected from Qichun. Their structures were elucidated by detailed interpretation of the spectroscopic data and the structures and absolute configurations of compounds **1–4** were confirmed by X-ray crystallographic analysis. Compounds **1–3** are tricyclic polyketides possessing octahydrochromene framework and having ketal unit in their structures, while compounds **4**/**7** and **5**/**6** are related bicyclic and tricyclic analogues, respectively. The antibacterial activities against human pathogen *E. coli* and seven marine-derived aquatic pathogens as well as against eight agro-pathogenic fungi for each of the isolated compounds were evaluated. Compounds **1–7** showed activity against human pathogen *Escherichia coli* (each with MIC 64 μg mL^{-1}), while **1** and **7** inhibited most of the tested aquatic bacteria and agro-pathogenic fungi (MICs ranging from 4 to 64 μg mL^{-1}), respectively.

Introduction

Artemisia argyi, a typical medicinal and industrial plant that is widely cultivated in Qichun in the Hubei Province in central China,[1] is frequently used for the treatment of diseases such as eczema,[2] hemorrhaging,[3] and inflammation.[4] Multiple active metabolites including sesquiterpenes,[5,6] triterpenes,[6–8] flavonoids,[9–11] and caffeoylquinic derivatives[12,13] as well as essential oils[14–16] were described from *A. argyi*. Endophytic fungi has been an essential source of novel and bioactivity natural products,[17–19] but the chemical constituents of endophytic fungi from *A. argyi* have not yet been reported. As part of our continuous research for new bioactive natural products from endophytic fungi,[20] a fungal strain *Trichoderma koningiopsis* QA-3, which was obtained from the inner tissue of *A. argyi*, attracted our attention. The organic extract of the fungal culture exhibited antimicrobial activity against several marine-derived pathogens in our primary screening. Chemical investigations on the fungal culture resulted in the isolation and identification of five new polyketides (**1–5**) and two known analogues (**6** and **7**) (Fig. 1). The structures of these compounds were determined by detailed analysis of the spectroscopic data and the structures and absolute configurations of compounds **1–4** were confirmed by single-crystal X-ray diffraction analysis. This paper describes the isolation, structure determination, stereochemical assignment, and antimicrobial activities of compounds **1–7**.

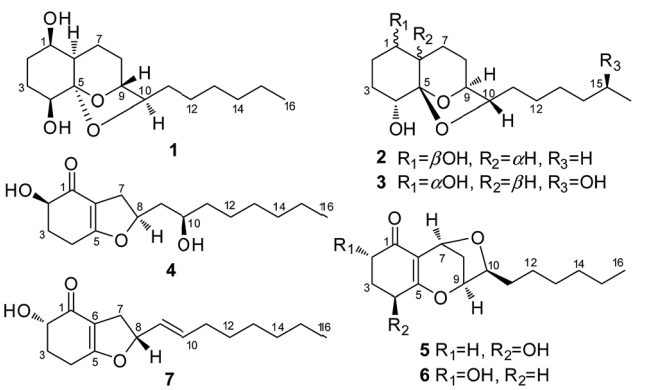

Fig. 1 Chemical structures of compounds **1–7**.

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† Electronic supplementary information (ESI) available: HRESIMS spectra of compounds **1–5**. Selected 1D and 2D NMR (**1–5**) and ECD (**3–5**) spectra (PDF). X-ray crystallographic files of **1–4** (CIF). CCDC 1566573–1566575, and 1566583 for compounds **1–4** respectively. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c7ra11122c

Results and discussion

The culture of *T. koningiopsis* QA-3 was exhaustively extracted with EtOAc to afford an organic extract, which was further purified by a combination of column chromatography on Sephadex LH-20, Si gel, and Lobar Li Chroprep RP-18 as well as by preparative TLC to yield compounds **1–7**.

Compound **1** was isolated as colorless crystals and its molecular formula was determined as $C_{16}H_{28}O_4$ on the basis of HRESIMS data, implying three degrees of unsaturation. The ^{1}H and ^{13}C NMR data of **1** indicated the presence of one methyl, nine methylenes, five methines (with four oxygenated), and one oxygenated non-protonated carbons as well as two exchangeable protons at δ_H 3.70 and 3.68 (Tables 1 and 2). Detailed analysis of the ^{1}H and ^{13}C NMR spectroscopic data as well as COSY and HMBC correlations (Fig. 2) indicated that the planar structure of **1** was same as that of koninginin A, a tricyclic fungal polyketide identified from the fungal strain *T. koningii* (ATCC no. 46314),[21] whose absolute configuration was determined to be 1*S*, 4*R*, 5*S*, 6*S*, 9*S*, and 10*S* by total synthesis[22] and by a single-crystal X-ray diffraction experiment.[23]

The relative configuration of compound **1** was determined by NOESY data. NOE correlations from H-8β to OH-1 and H-9 and from H-3β to OH-1 and OH-4 indicated the cofacial orientation of these groups (Fig. 3), while correlations from H-4 to H-6 and H-8α to H-10 suggested that these protons were on the other side of the molecule. However, the relative configuration at the chiral center C-5 could not be determined by NOESY spectrum since no diagnostic NOE could be observed. The relative and absolute configuration of **1** was unambiguously established by a single-crystal X-ray diffraction experiment using Cu Kα radiation (Fig. 4). The Flack parameter 0.0(5) allowed for the establishment of the absolute configuration of **1** as 1*R*, 4*S*, 5*R*, 6*R*, 9*R*, and 10*R*, which indicated the enantiomeric nature as that of koninginin A. On the basis of the above data, the structure of **1** was determined and it was named as *ent*-koninginin A.

Compound **2** was originally obtained as an amorphous powder. The molecular formula was assigned on the basis of

Table 2 ^{13}C NMR data for compounds **1–5** (125 MHz, measured in DMSO-d_6)

Position	1	2	3	4	5
1	71.0, CH	67.8, CH	71.0, CH	194.6, C	193.5, C
2	31.0, CH_2	24.6, CH_2	31.1, CH_2	70.6, CH	32.7, CH_2
3	26.6, CH_2	26.6, CH_2	26.7, CH_2	31.1, CH_2	29.8, CH_2
4	69.0, CH	69.3, CH	69.1, CH	22.0, CH_2	63.7, CH
5	108.6, C	108.4, C	108.6, C	176.4, C	172.3, C
6	41.5, CH	38.2, CH	41.5, CH	110.5, C	117.4, C
7	20.5, CH_2	18.2, CH_2	20.5, CH_2	37.2, CH_2	65.0, CH
8	26.6, CH_2	27.8, CH_2	26.6, CH_2	84.4, CH	31.7, CH_2
9	77.9, CH	77.3, CH	77.9, CH	43.2, CH_2	78.4, CH
10	77.6, CH	79.1, CH	77.7, CH	66.4, CH	84.9 CH
11	34.5, CH_2	35.0, CH_2	34.6, CH_2	30.1, CH_2	31.1, CH_2
12	25.0, CH_2	24.7, CH_2	25.4, CH_2	25.0, CH_2	25.8, CH_2
13	28.7, CH_2	28.6, CH_2	25.3, CH_2	28.8, CH_2	28.6, CH_2
14	31.3, CH_2	31.1, CH_2	39.0, CH_2	31.3, CH_2	31.2, CH_2
15	22.0, CH_2	21.9, CH_2	65.8, CH	21.9, CH_2	21.9, CH_2
16	13.9, CH_3	13.8, CH_3	23.6, CH_3	13.9, CH_3	13.9, CH_3

Table 1 ^{1}H NMR data for compounds **1–5** (500 MHz, *J* in Hz, measured in DMSO-d_6)

Position	1	2	3	4	5
1	3.70, br s	3.47, m	3.68, m (overlap)		
2α	1.39, m	1.73, m (overlap)	1.40, m	3.93, dd (11.1, 4.7)	2.21, m
2β	1.45, m (overlap)	1.40, m (overlap)	1.57, m		2.34, ddd (12.9, 7.7, 4.5)
3α	1.57, m	1.45, m	1.75, q (12.6)	2.10, m	2.01, m
3β	1.74, m	1.87, m	1.60, m (overlap)	1.76, dd (12.4, 5.3)	1.77, m
4α	3.34, dd (11.8, 4.4)	3.38, brs	3.36, br s	2.51, m	4.22, dd (12.6, 5.1)
4β				2.39, m	
6	1.60, m	1.90, m (overlap)	1.46, m (overlap)		
7α	1.95, m	1.39, m (overlap)	1.49, m (overlap)	2.78, m	4.78, d (4.6)
7β	1.49, m (overlap)	1.96, m	1.94, m	2.39, m	
8α	1.37, m (overlap)	1.76, m (overlap)	2.07, m	4.93, m	2.13, m
8β	2.07, m	1.56, dd (13.3, 5.7)	1.39, m		1.98, m
9α	4.15, br s	4.10, br s	4.16, br s	1.82, ddd (11.6, 9.7, 4.7)	4.98, br s
9β				1.63, m	
10	3.87, t (6.5)	4.03, t (6.3)	3.88, t (6.3)	3.50, m	3.96, t (7.0)
11a	1.46, m	1.36, m (overlap)	1.44, m	1.35, m	1.46, m
11b	1.36, m (overlap)	1.34, m (overlap)	1.36, m	1.23, m (overlap)	
12	1.24, m (overlap)	1.28, m (overlap)	1.24, m (overlap)	1.23, m (overlap)	1.29, m
13	1.24, m (overlap)	1.25, m (overlap)	1.26, m (overlap)	1.23, m (overlap)	1.26, m (overlap)
14	1.24, m (overlap)	1.25, m (overlap)	1.32, m (overlap)	1.23, m (overlap)	1.26, m (overlap)
15	1.24, m (overlap)	1.25, m (overlap)	3.55, br s	1.23, m (overlap)	1.26, m (overlap)
16	0.85, t (6.5)	0.86, t (6.8)	1.02, d (6.0)	0.84, t (6.7)	0.85, t (6.7)
1-OH	3.70, s	3.46, s	3.69, s		
2-OH				5.04, br s	
4-OH	3.68, s	4.88, s	4.04, d (5.4)		5.54, d (5.6)
10-OH				4.54, s	
15-OH			4.30, s		

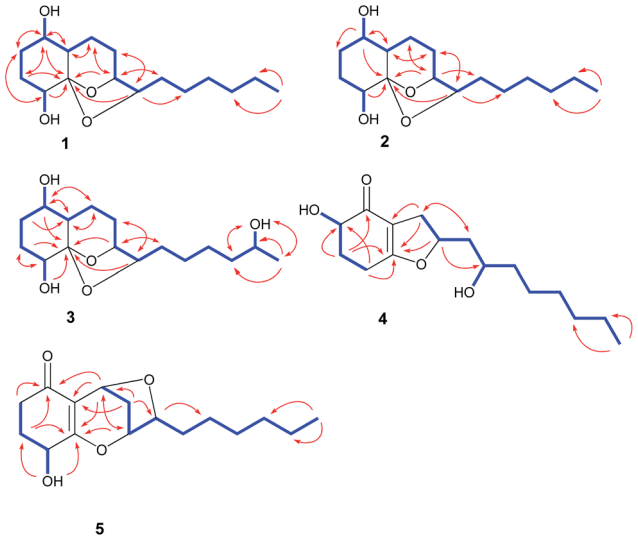

Fig. 2 Key HMBC (arrows) and COSY (bold lines) correlations for compounds **1–5**.

HRESIMS as $C_{16}H_{28}O_4$, indicating three degrees of unsaturation, same as that of **1**. The ^{1}H and ^{13}C NMR spectroscopic data of **2** (Tables 1 and 2) were very similar to those of **1**, except for minor differences in the chemical shifts for CH-1, CH_2-2, CH-6, and CH-10. Inspection of the COSY and HMBC correlations suggested that compound **2** contains same carbon and proton connectivity as that of **1** (Fig. 2). Upon slow evaporation of the solvent (MeOH : H_2O = 10 : 1) by storing in a refrigerator, quality single crystals of compound **2** were obtained, making feasible an X-ray diffraction analysis that unequivocally confirmed its relative and absolute configuration (Fig. 4). The final refinement of the Cu Kα data resulted in a 0.0(7) Flack parameter, allowing for the unambiguous assignment of the absolute configuration as 1*R*, 4*R*, 5*S*, 6*R*, 9*S*, and 10*S*. Thus, compound **2** was determined as 1,6-di-*epi*-koninginin A.

15-Hydroxykoninginin A (**3**) was initially obtained as an amorphous powder and its molecular formula was determined as $C_{16}H_{28}O_5$ (three degrees of unsaturation), with one oxygen atom more than **2**, on the basis of HRESIMS data. The ^{1}H and ^{13}C NMR spectroscopic data of **3** (Tables 1 and 2) was very similar to those of **2**, suggesting that **3** was an analogue of **2**. However, resonances for a methylene at δ_H 1.25 (2H, H-15) and δ_C 21.9 (C-15) in the NMR spectra of **2** disappeared in those of **3**. Instead, resonances for an oxygenated methine resonating at δ_H 3.55 (H-15) and δ_C 65.8 (C-15) were observed in the NMR spectra of **3**. This revealed that the methylene group (CH_2-15) in **2** was hydroxylated in **3**, which was confirmed by the COSY and HMBC correlations (Fig. 2).

In the NOESY experiments, NOEs from OH-1 to H-8α and H-9 and from H-3α to OH-1 and OH-4 indicated the cofacial orientation of these groups (Fig. 3), while correlations from H-1 to H-6 and from H-8β to H-10 suggested that these protons were on the other side of this molecule. However, the relative configuration of the chiral centers at C-5 and C-15 could not be determined by NOE spectrum. Using the same method, quality single

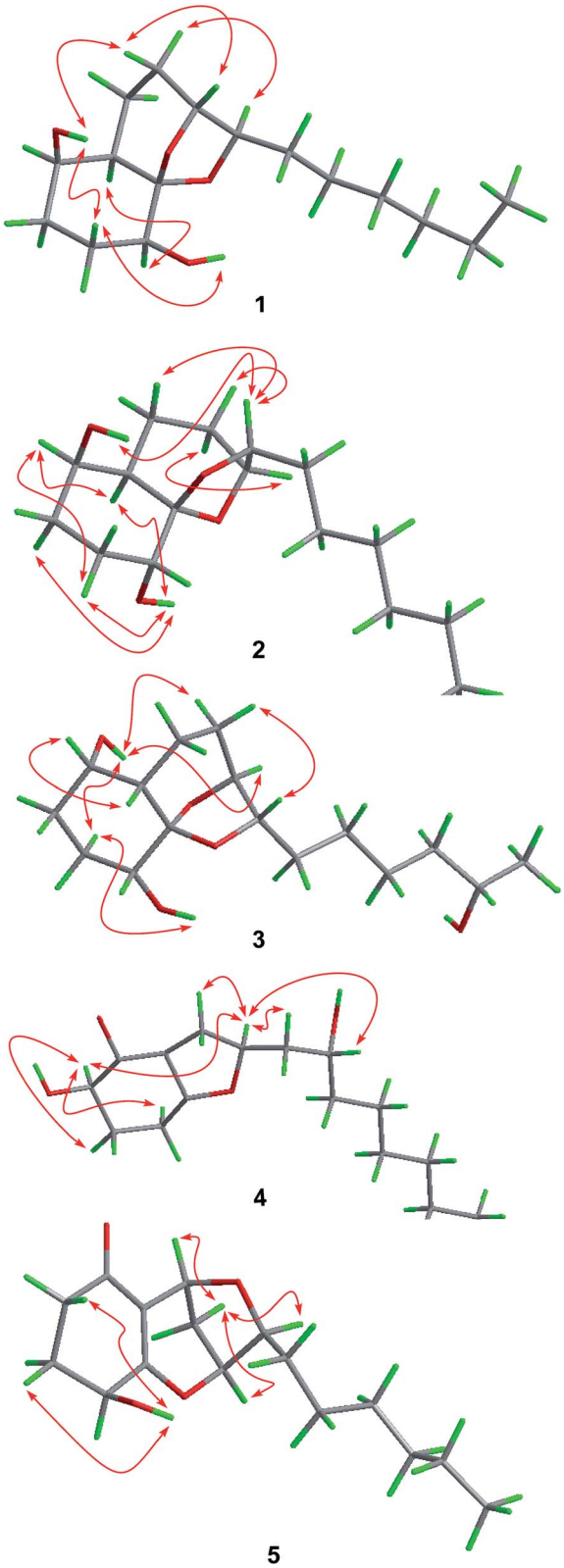

Fig. 3 Key NOESY correlations of **1–5**.

crystals of **3** were obtained, making feasible an X-ray diffraction analysis (Cu Kα radiation) that unambiguously confirmed its relative and absolute configurations (Fig. 4). The Flack

parameter 0.0(3) allowed for the establishment of the absolute configuration of **3** as 1*S*, 4*R*, 5*S*, 6*S*, 9*S*, 10*S*, and 15*S*.

HRESIMS analysis of **4** revealed two pseudo-molecular ion peaks at *m*/*z* 283.1907 $[M + H]^+$ and 305.1722 $[M + Na]^+$, consistent with the molecular formula $C_{16}H_{26}O_4$, and indicating the presence of four degrees of unsaturation. The skeleton of compound **4** was determined to be the same as that of koningiopisin D, a bicyclic polyketide isolated from the fungus *T. koningiopsis* YIM PH 30002,[24] by detailed analysis of the ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2). However, the OAc group connected to C-10 was replaced by OH group in compound **4**, as determined by the NMR data as well as supported by the HRESIMS data. The presence of OH group at C-10 was also determined by the COSY correlation from H-9 to H-10 and by the HMBC correlations from H-8 to C-10 as well as by the chemical shifts of CH-10 (δ_H 3.50 and δ_C 66.4).

The relative and absolute configuration of **4** were unambiguously established by a single-crystal X-ray diffraction experiment using Cu Kα radiation (Fig. 5). The Flack parameter 0.0(9) allowed for the establishment of the absolute configuration of **4** as 2*R*, 8*S*, and 10*R*. On the basis of the above data, the structure of **4** was determined, and was name as 10-deacetylkoningiopisin D.

Compound **5** was isolated as an amorphous powder and its molecular formula was assigned as $C_{16}H_{24}O_4$ on the basis of HRESIMS. Analysis of the NMR data (Tables 1 and 2) showed that **5** possessed a structural similarity with koninginin L (**6**), which was isolated from the fungus *T. koningii* 8662.[25] However, COSY correlations from H-4 to H-3 and the proton of 4-OH as well as HMBC correlations from the proton of 4-OH to C-3, C-4, and C-5 confirmed the presence of an OH group at C-4 in **5**. NOE correlations of H-8α with H-7, H-9, and H-10 revealed that these protons were on the same face of the molecule, same as those of koninginin L (**6**). Based on the octant rule for the cyclohexenones,[26–28] the positive Cotton effect (CE) at 306 nm in the ECD spectrum of **5** determined the absolute configuration of C-4 as *S*. The similar ECD curves of **5** and **6**, which showed negative CE around 265 nm and positive CE around 222 nm, assigned the absolute configuration as 7*S*, 9*S*, and 10*S*. Thus, the structure of **5** was determined and the trivial name koninginin T was assigned to this compound.

In addition to compounds **1–5**, two known compounds including koninginin L (**6**)[25] and trichoketide A (**7**)[29] were also isolated and identified from the fungal strain *T. koningiopsis* QA-3. Their structures were elucidated by comparing their NMR data with those reported in the literature.

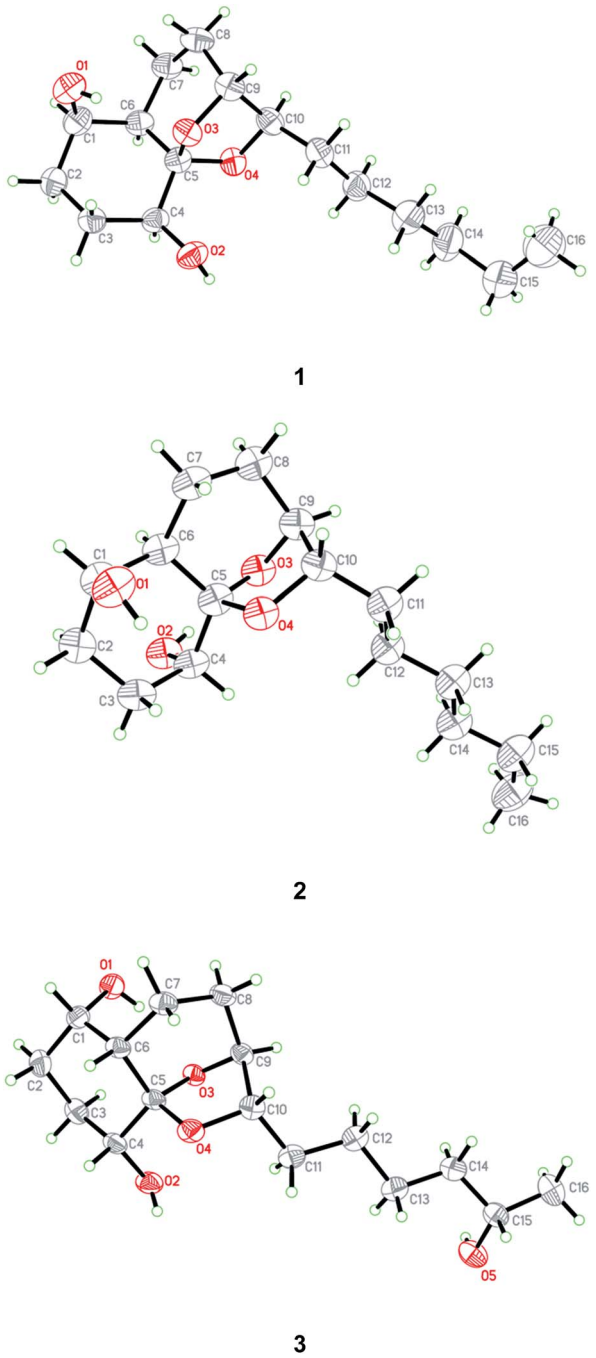

Fig. 4 X-ray crystallographic structures of compounds **1–3**.

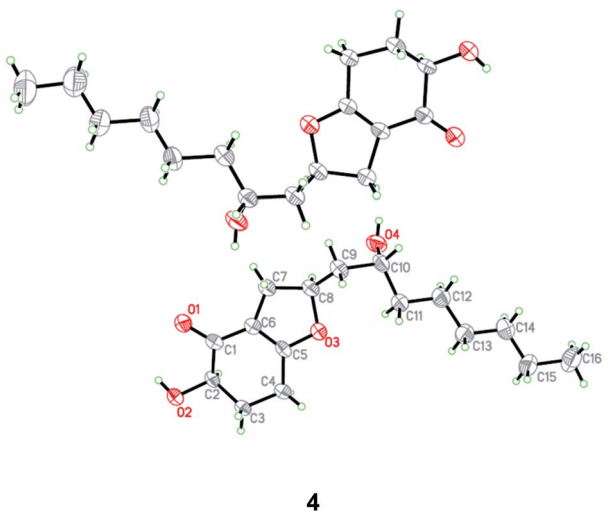

Fig. 5 X-ray crystallographic structures of compound **4**.

Table 3 Antibacterial activity of compounds **1–7** (MIC, μg mL^{-1})[a]

	1	2	3	4	5	6	7	Chloramphenicol
EC	64	64	64	64	64	64	64	2
ET	64	64	64	64	64	64	64	0.25
ML	64	64	—	—	—	—	64	2
PA	64	—	—	—	—	—	—	1
VAl	64	64	64	64	32	64	64	0.25
VAn	8	32	16	32	32	32	32	1
VP	64	—	—	—	—	—	64	2
VV	4	—	—	—	—	—	16	8

[a] EC: *E. coli*. ET: *E. tarda*. ML: *M. luteus*. PA: *P. aeruginosa*. VAl: *V. alginolyticus*. VAn: *V. anguillarum*. VP: *V. parahemolyticus*. VV: *V. vulnificus*. —: no activity.

Compounds **1–7** were assayed for their antimicrobial activities against human pathogen *Escherichia coli*, marine-derived aquatic bacteria (*Edwardsiella tarda*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Vibrio alginolyticus*, *V. anguillarum*, *V. parahemolyticus*, and *V. vulnificus*), and agro-pathogenic fungi (*Bipolaris sorokiniana*, *Ceratobasidium cornigerum*, *Colletottichum gloeosporioides* Penz, *Fusarium graminearum*, *F. oxysporum*, *Penicillium digitatum*, *Physalospora piricola* Nose, and *Valsa mali*). As presented in Table 3, compounds **1** and **7** showed activity against human pathogen *E. coli* and aquatic bacteria *E. tarda*, *V. anguillarum*, and *V. parahemolyticus*, with MICs ranging from 8 to 64 μg mL^{-1}. Compound **1** further showed activity against aquatic bacteria *M. luteus* and *P. aeruginosa* and agro-pathogen. In the antibacterial assays (Table 3), compounds **1–7** showed activity against human pathogen *E. coli* (each with MIC value 64 μg mL^{-1}) and activity against marine-derived aquatic bacteria *E. tarda*, *V. alginolyticus*, and *V. anguillarum* (with MICs ranging from 8 to 64 μg mL^{-1}) while compounds **1** and **7** further exhibited activity against *M luteus*, *V. parahemolyticus*, and *V. vulnificus* (with MIC values ranging from 4 to 64 μg mL^{-1}), and the activity of compound **1** against *V. vulnificus* was stronger than that of the positive control chloramphenicol (MIC, 4 μg mL^{-1} *vs.* 8 μg mL^{-1}). In the antifungal assays (Table 4), compound **7** showed a broad-spectrum activity against all of the eight tested agro-pathogenic fungi, and had potent activity against *C. cornigerum* and *P. digitatum*, both with MIC value 4.0 μg mL^{-1} (for the positive control amphotericin B, MIC = 1 and 0.5 μg mL^{-1}, respectively), while compounds **2–6** showed weak or moderate activity against *C. cornigerum*, *F. oxysporum*, and/or *P. digitatum*, with MICs ranging from 8 to 64 μg mL^{-1}.

Table 4 Antifungal activity of compounds **1–7** (MIC, μg mL^{-1})[a]

	1	2	3	4	5	6	7	Amphotericin B
BS	64	—	—	—	—	—	8	1
CC	8	64	64	32	—	16	4	1
CG	—	—	—	64	—	—	8	1
FG	—	—	—	—	—	—	64	0.25
FO	64	64	—	64	—	—	8	1
PD	32	64	64	8	16	—	4	0.5
PP	16	—	—	—	—	—	8	1
VM	—	—	—	—	—	—	64	1

[a] BS: *B. sorokiniana*. CC: *C. cornigerum*. CG: *C. gloeosporioides*. FG: *F. graminearum*. FO: *F. oxysporum*. PD: *P. digitatum*. PP: *P. piricola* Nose. VM: *V. mali*. —: no activity.

Conclusions

In summary, we isolated and characterized five new fungal polyketides including *ent*-koninginin A (**1**), 1,6-di-*epi*-koninginin A (**2**), 15-hydroxykoninginin A (**3**), 10-deacetylkoningiopisin D (**4**), and koninginin T (**5**), together with two known analogues, koninginin L (**6**) and trichoketide A (**7**) from the culture extract of endophytic fungus *T. koningiopsis* QA-3. Their structures were elucidated by detailed interpretation of the spectroscopic data and the structures and absolute configurations of compounds **1–4** were confirmed by X-ray crystallographic analysis. Compounds **1–3** are tricyclic polyketides possessing octahydrochromene framework and having ketal unit in their structures, while compounds **5**/**6** and **4**/**7** are related tricyclic and bicyclic analogues, respectively. The antimicrobial activities of compounds **1–7** were evaluated and compounds **1** and **7** inhibited human pathogen *E. coli* and all of the seven marine-derived aquatic bacteria with MIC values ranging from 4 to 64 μg mL^{-1}. In addition, compound **7** showed a broad-spectrum activity against all of the eight tested agro-pathogenic fungi.

Experimental section

General experimental procedures

Melting points were determined on an SGW X-4 micro-melting-point apparatus. Optical rotations were measured with an Optical Activity AA-55 polarimeter. The ECD spectra were recorded on a Chirascan spectropolarimeter, using CH_3OH as a solvent. UV spectra were recorded on a PuXi TU-1810 UV-visible spectrophotometer. 1D and 2D NMR spectra were recorded at 500 for ^1H and 125 MHz and ^{13}C in DMSO-d_6, respectively, on a Bruker Avance 500 spectrometer with TMS as internal standard. ESIMS and HRESIMS data were obtained on a Waters Micromass Q-TOF Premier and a Thermo Fisher Scientific LTQ Orbitrap XL spectrometers, respectively. Analytical HPLC were performed using a Dionex HPLC system equipped with P680 pump, ASI-100 automated sample injector, and UVD340U multiple wavelength detector controlled by Chromeleon software (version 6.80). Open column chromatography was performed with commercially available Si gel (200–300 mesh, Qingdao Haiyang Chemical Co.), Lobar LiChroprep RP-18 (40–63 μm, Merck), and Sephadex LH-20 (Pharmacia). TLC was carried out using silica gel GF254 (Qingdao Haiyang Chemical Factory) plates. All solvents used were distilled prior to use.

Fungal material

The endophytic fungus *T. koningiopsis* QA-3 was obtained from the inner tissue of the cultivated medicinal plant *Artemisia argyi* collected at Qichun of the Hubei Province in the central China, in July 2014, using a protocol as described in our previous

report.[30] Fungal identification was performed by analysis of its ITS region of the rDNA as described previously.[30] The resulting sequence was same (100%) to that of *T. koningiopsis* DMC 795b (compared with EU 718083.1), and has been deposited in GenBank (with accession no MF616361). The strain is preserved at the Key Laboratory of Experimental Marine Biology, Institute of Oceanology of the Chinese Academy of Sciences (IOCAS).

Cultivation

For chemical investigations, the fresh mycelia of *T. koningiopsis* QA-3 were grown on PDA medium at 28 °C for 7 days and were then inoculated for 30 days at room temperature in 90 × 1 L conical flasks with solid rice medium (each flask contained 70 g rice, 0.1 g corn flour, 0.3 g peptone, and 100 mL distilled water).

Extraction and isolation

The whole fermented cultures were extracted three times with EtOAc, which was evaporated under reduced pressure to afford an extract (76.7 g). The extract was fractionated by Si gel vacuum liquid chromatography (VLC) using different solvents of increasing polarity from petroleum ether (PE) to MeOH to yield ten fractions (Frs. 1–10) based on TLC and HPLC analysis. Purification of Fr. 5 (4.3 g) by reversed-phase column chromatography (CC) over Lobar LiChroprep RP-18 with a MeOH–H_2O gradient (from 10 : 90 to 100 : 0) yielded eight subfractions (Frs. 5.1–5.8). Fr. 5.6 (728.1 mg) was purified by CC on Sephadex LH-20 (MeOH) and then by recrystallization to obtain compound **1** (57.4 mg), and the residue was further purified by preparative TLC (plate: 20 × 20 cm, developing solvents: PE–EtOAc, 1 : 1) to obtain compound **2** (5.0 mg). Fr. 6(7.3 g) was fractioned by CC over Lobar LiChroprep RP-18 with a MeOH–H_2O gradient (from 10 : 90 to 100 : 0) and then purified by CC on Sephadex LH-20 (MeOH) and Si gel (PE–EtOAc, from 10 : 1 to 2 : 1) to yield compounds **6** (12.5 mg) and **7** (27.1 mg). Purification of Fr. 7 (10.2 g) by reversed-phase column chromatography (CC) over Lobar LiChroprep RP-18 with a MeOH–H_2O gradient (from 10 : 90 to 100 : 0) yielded fifteen subfractions (Frs. 7.1–7.15). Fr. 7.4 (1.9 g) was further purified on Si gel eluting with a CH_2Cl_2–MeOH gradient (from 100 : 1 to 10 : 1) and then by preparative TLC (plate: 20 × 20 cm, developing solvents: CH_2Cl_2–acetone, 5 : 1) to obtain compound **3** (61.2 mg). Fr. 7.5 (2.8 g) was purified by CC over Si gel eluting with a CH_2Cl_2–EtOAc gradient (from 20 : 1 to 2 : 1) and then by preparative TLC (plate: 20 × 20 cm, developing solvent: CH_2Cl_2/MeOH, 20 : 1) and Sephadex LH-20 (MeOH) to afford compounds **4** (12.1 mg) and **5** (14.0 mg).

***ent*-Koninginin A (1).** Colorless crystals; $[\alpha]_D^{25}$ −18.6 (*c* 1.18, MeOH); mp 82–84 °C; ^{1}H and ^{13}C NMR data: see Tables 1 and 2; ESIMS *m*/*z* 285.21 $[M + H]^+$, 302.23 $[M + H_2O]^+$; HRESIMS *m*/*z* 285.2062 $[M + H]^+$ (calcd for $C_{16}H_{29}O_4$, 285.2066).

1,6-Di-*epi*-koninginin A (2). Colorless crystals; $[\alpha]_D^{25}$ −60.0 (*c* 0.10, MeOH); mp 64–66 °C; ^{1}H and ^{13}C NMR data: see Tables 1 and 2; ESIMS *m*/*z* 285.21 $[M + H]^+$, 302.31 $[M + H_2O]^+$, 307.19 $[M + Na]^+$; HRESIMS *m*/*z* 285.2059 $[M + H]^+$ (calcd for $C_{16}H_{29}O_4$, 285.2066).

15-Hydroxykoninginin A (3). Colorless crystals; $[\alpha]_D^{25}$ −23.8 (*c* 0.84, MeOH); mp 99–101 °C; ECD (3.82 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 221 (+8.12), 258 (+11.16) nm; ^{1}H and ^{13}C NMR data: see Tables 1 and 2; ESIMS *m*/*z* 301.20 $[M + H]^+$, 318.23 $[M + H_2O]^+$, 323.18 $[M + Na]^+$; HRESIMS *m*/*z* 301.2005 $[M + H]^+$ (calcd for $C_{16}H_{29}O_5$, 301.2015).

10-Deacetylkoningiopisin D (4). Colorless crystals; $[\alpha]_D^{25}$ +40.1 (*c* 3.49, MeOH); mp 73–75 °C; UV (MeOH) λ_{max} (log ε) 271 (3.51) nm; ECD (3.39 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 264 (+34.25) nm; ^{1}H and ^{13}C NMR data: see Tables 1 and 2; ESIMS *m*/*z* 283.19 $[M + H]^+$, 305.17 $[M + Na]^+$; HRESIMS *m*/*z* 283.1907 $[M + H]^+$ (calcd for $C_{16}H_{27}O_4$, 283.1904) and *m*/*z* 305.1722 $[M + Na]^+$ (calcd for $C_{16}H_{26}O_4Na$, 305.1729).

Koninginin T (5). Colorless; amorphous powder; $[\alpha]_D^{25}$ −63.2 (*c* 0.48, MeOH); UV (MeOH) λ_{max} (log ε) 267 (3. 69) nm; ECD (3.26 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 222 (+49.21), 267 (−87.27), 306 (+35.99) nm; ^{1}H and ^{13}C NMR data: see Tables 1 and 2; ESIMS *m*/*z* 281.18 $[M + H]^+$; HRESIMS *m*/*z* 281.1750 $[M + H]^+$ (calcd for $C_{16}H_{25}O_4$, 281.1753).

X-ray crystallographic analysis of compounds 1–4 (ref. 31)

All crystallographic data were collected on an Agilent Xcalibur Eos Gemini CCD plate diffractometer equipped with graphite-monochromatic Cu-Kα radiation (λ = 1.54178 Å) at 293(2) K. The data were corrected for absorption by using the program SADABS.[32] The structures were solved by direct methods with the SHELXTL software package.[33] All non-hydrogen atoms were refined anisotropically. The H atoms were located by geometrical calculations, and their positions and thermal parameters were fixed during the structures refinement. The structures were refined by full-matrix least-squares techniques.[34]

Crystal data for compound 1. $C_{16}H_{28}O_4$, F.W. = 284.38, monoclinic space group *P*2(1)2(1)2(1), unit cell dimensions a = 5.4518(5) Å, b = 12.6676(11) Å, c = 23.036(2) Å, V = 1590.9(2) Å^3, $\alpha = \beta = \gamma = 90°$, Z = 4, d_{calcd} = 1.187 mg m^{-3}, crystal dimensions 0.30 × 0.08 × 0.05 mm, μ = 0.672 mm^{-1}, $F(000)$ = 624. The 2775 measurements yielded 1576 independent reflections after equivalent data were averaged, and Lorentz and polarization corrections were applied. The final refinement gave R_1 = 0.0626 and wR_2 = 0.1251 [$I > 2\sigma(I)$]. The Flack parameter was 0.0(5) in the final refinement for all 2775 reflections with 1576 Friedel pairs.

Crystal data for compound 2. $C_{16}H_{28}O_4$, F.W. = 284.38, monoclinic space group *P*2(1), unit cell dimensions a = 11.7535(11) Å, b = 5.4477(5) Å, c = 12.7020(12) Å, V = 784.55(13) Å^3, $\alpha = \gamma = 90°$, β = 105.281(4)°, Z = 2, d_{calcd} = 1.204 mg m^{-3}, crystal dimensions 0.21 × 0.08 × 0.05 mm, μ = 0.681 mm^{-1}, $F(000)$ = 312. The 2155 measurements yielded 935 independent reflections after equivalent data were averaged, and Lorentz and polarization corrections were applied. The final refinement gave R_1 = 0.0679 and wR_2 = 0.1055 [$I > 2\sigma(I)$]. The Flack parameter was 0.0(7) in the final refinement for all 2155 reflections with 935 Friedel pairs.

Crystal data for compound 3. $C_{16}H_{28}O_5$, F.W. = 300.38, monoclinic space group *P*2(1), unit cell dimensions a = 5.4895(4) Å, b = 13.2356(12) Å, c = 11.0897(10) Å, V = 805.34(12) Å^3,

$\alpha = \gamma = 90°$, $\beta = 91.805(2)°$, $Z = 2$, $d_{calcd} = 1.239$ mg m^{-3}, crystal dimensions 0.23 × 0.20 × 0.10 mm, $\mu = 0.739$ mm^{-1}, $F(000) = 328$. The 1989 measurements yielded 1701 independent reflections after equivalent data were averaged, and Lorentz and polarization corrections were applied. The final refinement gave $R_1 = 0.0443$ and w$R_2 = 0.1117$ [$I > 2\sigma(I)$]. The Flack parameter was 0.0(3) in the final refinement for all 1989 reflections with 1701 Friedel pairs.

Crystal data for compound 4. $C_{16}H_{26}O_4$, F.W. = 282.37, monoclinic space group $P2(1)$, unit cell dimensions a = 5.7605(5) Å, b = 9.1002(8) Å, c = 31.056(3) Å, V = 1627.6(2) Å^3, $\alpha = \gamma = 90°$, $\beta = 91.3470(10)°$, $Z = 4$, $d_{calcd} = 1.152$ mg m^{-3}, crystal dimensions 0.32 × 0.10 × 0.05 mm, $\mu = 0.657$ mm^{-1}, $F(000) = 616$. The 4825 measurements yielded 2388 independent reflections after equivalent data were averaged, and Lorentz and polarization corrections were applied. The final refinement gave $R_1 = 0.1255$ and w$R_2 = 0.2966$ [$I > 2\sigma(I)$]. The Flack parameter was 0.0(9) in the final refinement for all 4825 reflections with 2388 Friedel pairs.

Antimicrobial assays

Antimicrobial evaluation against human pathogen *Escherichia coli* IOCAS-1, marine-derived aquatic bacteria (*E. tarda* QDIO-2, *M. luteus* QDIO-3, *P. aeruginosa* QDIO-4, *V. alginolyticus* QDIO-5, *V. anguillarum* QDIO-6, *V. parahemolyticus* QDIO-8, and *V. vulnificus* QDIO-9) and plant-pathogenic fungi (*B. sorokiniana* QDAU-7, *C. cornigerum* QDAU-8, *C. gloeosporioides* Penz QDAU-9, *F. graminearum* QDAU-10, *F. oxysporum* QDAU-5, *P. digitatum* QDAU-11, *P. piricola* Nose QDAU-12, and *V. mali* QDAU-13) was carried out by the microplate assay.[35] The aquatic pathogens were obtained from the Institute of Oceanology, Chinese Academy of Sciences, while the plant pathogens were provided by the Qingdao Agricultural University. Chloramphenicol and amphotericin B were used as the positive control against bacteria and fungi, respectively.

Conflicts of interest

There are no conflicts to declare.

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