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Three new diterpenes and two new sesquiterpenoids from the endophytic fungus *Trichoderma koningiopsis* A729

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ABSTRACT

Three new diterpenes koninginols A-C (**1–3**) and two new sesquiterpenoids 11-hydroxy-15-drimeneoic acid (**4**), koninginol D (**5**), together with twelve known compounds (**6–17**) were isolated from the endophytic fungus *Trichoderma koningiopsis* A729 derived from *Morinda officinalis*. Their structures were fully assigned with the aid of extensive spectroscopic analysis and reported data from the previous literatures. Koninginols A and B were disclosed as intriguing diterpene alkaloids, whereas the koninginol C was disclosed as one of the two examples of harziandione diterpene. All the isolates were evaluated for their antitumor activity, and compounds **1–2**, **4–6**, **11–13**, **16–17** were evaluated for their antibacterial activity, wherein the new compounds **1** and **2** exhibited significant antibacterial activity against *Bacillus subtilis* with MIC values of 10 and 2 μg/mL, respectively.

1. Introduction

Endophytic fungi have been emerged as fruitful resources for producing structurally fascinating and biologically active secondary metabolites [1], which were extensively used in medicinal industry to discover pharmaceutical drugs and as enlightening targets for synthetic chemists. The previously chemical investigation of the genus *Trichoderma* has resulted in the isolation of numerous bioactive metabolites including diterpenes [2,3], lipovelutibols [4], polyketides [5–9], and thiochromanone derivatives [10], which exhibited novel structural diversity and intriguing bioactivities. In our continuous search for biologically meaningful and structurally unique natural products from the genus *Trichoderma*, a detail chemical investigation of the fungus *T. koningiopsis* A729 led to the isolation of three new diterpenes koninginols A-C (**1–3**) and two new sesquiterpenoids 11-hydroxy-15-drimeneoic acid (**4**), koninginol D (**5**), together with twelve known compounds (**6–17**) (Fig. 1). The structures of all the compounds were adequately elucidated by careful analysis of NMR spectroscopic data or comparison with reported data. Among them, koninginols A and B were disclosed as intriguing diterpene alkaloids, whereas the koninginol C was disclosed as one of the two examples of harziandione diterpene. Moreover, compounds **1** and **2** exhibited significant antibacterial activity against *Bacillus subtilis* with MIC values of 10 and 2 μg/mL, respectively. In this work, the isolation, structural elucidation, and bioactivity of these compounds are described.

2. Experimental section

2.1. Experimental procedures

Optical rotations were recorded using an Anton Paar MCP-500 spectropolarimeter (Anton Paar, Graz, Austria). UV spectra were obtained by a Shimadzu UV-2600 spectrophotometer (Shimadzu, Kyoto, Japan). IR data were measured on a Shimadzu IR Affinity-1 spectrometer (Shimadzu, Kyoto, Japan). 1D and 2D NMR spectra were collected on a Bruker Avance-600 spectrometer with TMS as an internal standard (Bruker, Fällanden, Switzerland). HRESIMS spectra were acquired with a Thermo MAT95XP high resolution mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China) was used for column chromatography. TLC analysis was carried out on silica gel plate (Merck KGaA, Darmstadt, Germany). A Shimadzu LC-20 AT (Shimadzu, Kyoto, Japan) equipped with a SPD-M20A PDA detector (Shimadzu, Kyoto, Japan) was used for HPLC, a YMC-Pack ODS-A/AQ column (250 × 20 mm, 5 μm, 12 nm, YMC CO., Ltd, Kyoto, Japan) was used for semipreparative HPLC separation, and a YMC-Pack ODS-A

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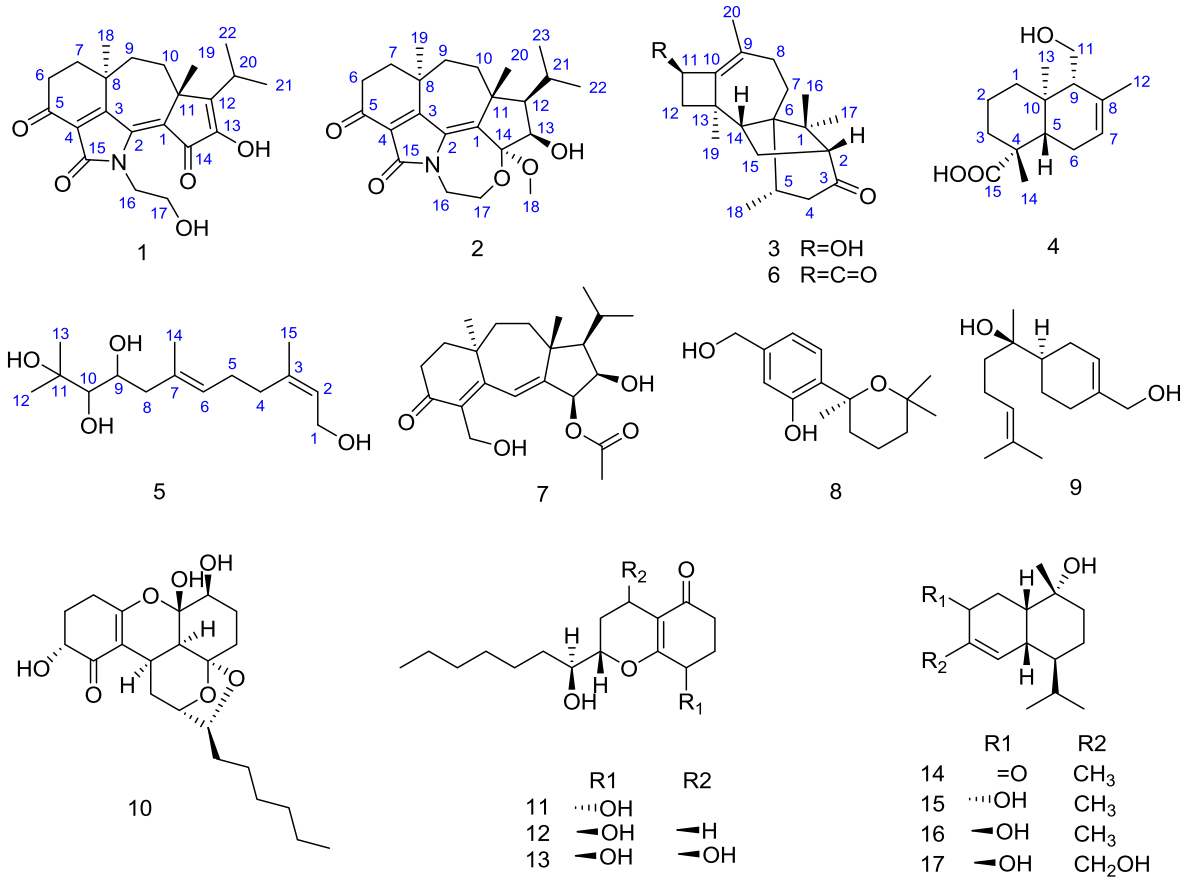

Fig. 1. The structures of compounds **1–17**.

column (250 × 20 mm, 5 μm, 12 nm, YMC CO., Ltd, Kyoto, Japan) was used for preparative HPLC separation. All solvents were analytical grade (Guangzhou Chemical Regents Company, Ltd., Guangzhou, China).

2.2. Fungal material

The fungal strain A729 was isolated from the fresh branches of the medicinal plant *Morinda officinalis,* which was collected in January 2015. The sequence data for this fungal strain has been submitted to Genbank (Accession No. KU529843). Using BLAST (nucleotide sequence comparison program) to search the Genbank database, A729 has 99% similarity to *Trichoderma koningiopsis* CQSQ1002 (Accession No. JQ040365). The strain was preserved at the Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Institute of Microbiology.

2.3. Fermentation, extraction, and isolation

The endophytic fungus *Trichoderma koningiopsis* A729 was cultivated in potato dextrose broth (potato 20%, glucose 2%, K_2HPO_4 0.3%, $MgSO_4{\cdot}7H_2O$ 0.15%, vitamin B 10 mg/L) at 28 °C for 7 days to prepare the seed culture. Then, the inoculated seed culture was transferred into each of a total of 100 flasks (1000 mL) containing 500 mL of potato dextrose broth. The liquid cultivation was then kept for 7 days at 28 °C and 120 r/m on a rotary shaker. The culture (50 L) was filtered to give the broth and mycelia. The fermented broth was subjected to macroporous resin column with ethanol as eluent. The EtOH fraction was evaporated under reduced pressure at a temperature not exceeding 40 °C to yield a dark brown gum (7.3 g), and it was then transferred to a silica gel column (100–200 mesh, 72 g) with two gradient systems of increasing polarity (petroleum ether/EtOAc, 5:1, 1:1; CH_2Cl_2/CH_3OH, 5:1, 2:1, 1:1, v/v) to yield six subfractions (A-F).

Fraction C (71.4 mg) was chromatographed over Sephadex LH-20 eluting with CH_2Cl_2/CH_3OH (1:1, v/v) and further isolated by semi-preparative HPLC (70% methanol in H_2O, 3 mL/min) to yield compound **8** (0.8 mg) and compound **6** (8.0 mg). Fraction D (189.4 mg) was subjected to Sephadex LH-20 and eluted with CH_2Cl_2/CH_3OH (1:1, v/v) to afford two major subfractions (D_1-D_2). Subfraction D_1 (48.3 mg) was further purified by semipreparative HPLC (70% methanol in H_2O, 3 mL/min) to obtain compound **3** (1.0 mg) and compound **14** (1.2 mg). Subfraction D_2 was followed by a silica gel column (100–200 mesh, 1.8 g) with a stepwise gradient elution of *n*-hexane/EtOAc (8:1, 5:1, 2:1, v/v) to yield compound **4** (38.5 mg). Fraction E (2.0 g) was chromatographed over Sephadex LH-20 eluting with CH_2Cl_2/CH_3OH (1:1, v/v) and followed by a silica gel column (100–200 mesh, 5 g) with two gradient systems of increasing polarity (*n*-hexane/EtOAc, 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, 0:1, v/v; CH_3OH, 100%, v/v) to afford eleven subfractions (E_1-E_{11}). Semipreparative HPLC (70% methanol in H_2O, 3 mL/min) analysis of subfractions E_3 (35.9 mg), E_8 (33.4 mg), E_{10} (78.1 mg), and E_{11} (120.2 mg) afforded compounds **2** (4.9 mg), **9** (0.9 mg), **12** (8.4 mg), **7** (0.9 mg), **17** (4.6 mg), and $E_{10\text{-}1}$ (10.6 mg), respectively. Subfraction E_4 was subjected to a silica gel column (100–200 mesh, 1.8 g) with a stepwise gradient elution of *n*-hexane/EtOAc (10:1, 5:1, 2:1, 1:1, 0:1, v/v) to obtain four major subfractions ($E_{4\text{-}1}$-$E_{4\text{-}4}$). Subfractions $E_{4\text{-}3}$ and $E_{4\text{-}4}$ were further purified by semipreparative HPLC (70% methanol in H_2O, 3 mL/min) to give compounds **11** (4.0 mg), **15** (2.2 mg), and **16** (8.2 mg), respectively. Subfractions E_5 and E_9 were further purified by semipreparative HPLC (70% methanol in H_2O, 3 mL/min) to obtain compound **5** (5.3 mg), $E_{9\text{-}3}$ (4.2 mg), and $E_{5\text{-}7}$ (2.5 mg), respectively. Semipreparative HPLC (65% MeCN in H_2O, 3 mL/min) analysis of subfractions $E_{10\text{-}1}$, $E_{9\text{-}3}$, and $E_{5\text{-}7}$ afforded

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of compounds **1** and **2** in $CDCl_3$.

No.	**1**		**2**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		129.3, C		148.6, C
2		143.7, C		135.6, C
3		168.7, C		168.0, C
4		125.6, C		124.5, C
5		192.5, C		193.3, C
6α	2.73 (1H, ddd, 19.5,	35.0, CH_2	2.71 (1H, ddd, 19.5,	35.0, CH_2
6β	14.0, 5.5)		13.7, 5.9)	
	2.65 (1H, ddd, 18.4,		2.58 (1H, m)	
	5.5, 2.0)			
7α	1.94 (1H, ddd, 13.8,	37.9, CH_2	1.85 (1H, ddd, 13.7,	37.7, CH_2
7β	5.5, 2.0)		5.9, 1.8)	
	2.27 (1H, dd, 13.8,		2.14 (1H, overlapped)	
	5.5)			
8		37.8, C		37.7, C
9α	1.73 (1H, ddd, 14.7,	35.2, CH_2	1.48 (1H, ddd, 14.6,	35.2, CH_2
9β	5.7, 2.2)		6.5, 1.7)	
	2.23 (1H, m)		2.14, (1H, overlapped)	
10α	2.01 (1H, m)	29.9, CH_2	2.14, (2H, overlapped)	34.5, CH_2
10β	2.14 (1H, ddd, 14.4,			
	5.5, 2.0)			
11		47.5, C		49.7, C
12		152.4, C	1.54 (1H, dd, 10.2, 4.5)	56.0, CH
13		149.8, C	4.17 (1H, d, 4.5)	73.2, CH
14		186.3, C		108.2, C
15		168.8, C		165.3, C
16a	4.01 (1H, ddd, 14.4,	47.4, CH_2	3.48 (1H, m)	44.1, CH_2
16b	8.0, 4.2)		4.31 (1H, dd, 14.9, 6.1)	
	4.06 (1H, dt, 14.4,			
	4.2)			
17a	3.87 (1H, ddd, 11.8,	61.1, CH_2	3.95 (1H, ddd, 12.7,	63.0, CH_2
17b	8.0, 4.1)		6.1, 1.4)	
	3.93 (1H, dt, 11.8,		4.04 (1H, 12.7, 8.4)	
	4.1)			
18	1.33 (3H, s)	26.0, CH_3	3.27 (3H, s)	49.6, CH_3
19	1.36 (3H, s)	27.2, CH_3	1.35 (3H, s)	27.6, CH_3
20	2.48 (1H, m)	26.2, CH_3	1.40 (3H, s)	27.2, CH
21	1.32 (3H, d, 7.0)	20.5, CH_3	2.14, (1H, overlapped)	25.0, CH
22	1.30 (3H, d, 7.0)	20.5, CH_3	1.12 (3H, d, 6.6)	23.4, CH_3
23			1.08 (3H, d, 6.6)	23.7, CH_3

compounds **1** (2.3 mg), **10** (1.0 mg), and **13** (5.1 mg), respectively.

Koninginol A (**1**): yellow powder; $[\alpha]_D^{25}$ −55.7 (*c* 0.1, MeOH). UV (MeCN) λ_{max} (log ε): 286.4 (3.26), 340.4 (3.31) 427.4 (3.40) nm. CD (0.12 mg/mL, MeCN): 218 (+12.9), 239 (+12.0), 270 (−4.85), 344 (+2.88), 448 (−8.66) nm. IR ν_{max}: 3361, 2926, 1699, 1668, 1645, 1456, 1396, 1142, 1121, 1011 cm^{-1}. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data, see Table 1. Positive ESIMS: *m/z* 386 $[M + H]^+$, HRESIMS: *m/z* 386.1963 $[M + H]^+$ (calcd for $C_{22}H_{28}NO_5$, 386.1962).

Koninginol B (**2**): yellow powder; $[\alpha]_D^{25}$ −53.0 (*c* 0.1, MeOH). UV (MeOH) λ_{max} (log ε): 207.4 (3.17), 238.8 (2.95), 360.2 (3.08) nm. CD (0.1 mg/mL, MeCN): 217 (+15.69), 251 (−8.59), 278 (+10.13), 310 (+14.82), 385 (−24.0) nm. IR ν_{max}: 3460, 2926, 1691, 1557, 1456, 1375, 1029 cm^{-1}. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data, see Table 1. Positive ESIMS: *m/z* 402 $[M + H]^+$, HRESIMS: *m/z* 402.2276 $[M + H]^+$ (calcd for $C_{23}H_{32}NO_5$, 402.2275).

Koninginol C (**3**): colorless oil; $[\alpha]_D^{25}$ + 25.8 (*c* 0.09, MeOH). UV (MeOH) λ_{max} (log ε): 204.8 (3.28) nm. CD (0.2 mg/mL, MeCN): 209 (−4.15), 292 (+4.93) nm. IR ν_{max}: 3347, 2928, 1695, 1015, 669 cm^{-1}. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data, see Table 2. Positive ESIMS: *m/z* 303 $[M + H]^+$, HRESIMS: *m/z* 303.2323 $[M + H]^+$ (calcd for $C_{20}H_{31}O_2$, 303.2319).

11-Hydroxy-15-drimeneoic acid (**4**): colorless oil; $[\alpha]_D^{25}$ + 131.9 (*c* 0.11, MeOH). UV (MeOH) λ_{max} (log ε): 206.2 (3.58), 243.0 (2.95) nm. CD (0.5 mg/mL, MeCN): 204 (−0.61), 239 (+0.24) nm. IR ν_{max}: 3356, 2936, 1695, 1238, 1028 cm^{-1}. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data, see Table 3. Negative ESIMS: *m/z* 251 $[M - H]^-$, HRESIMS: *m/z* 251.1656 $[M - H]^-$ (calcd for $C_{15}H_{23}O_3$, 251.1653).

Table 2
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compound **3** in $CDCl_3$.

No.	**3**		No.	**3**	
	δ_H (*J* in Hz)	δ_C		δ_H (*J* in Hz)	δ_C
1		51.8, C	11	4.92 (1H, m)	69.2, CH
2	2.21 (1H, d, 7.8)	59.9, CH	12*α*	2.16 (1H, dd, 10.6, 7.7)	48.2, CH_2
3		215.4, C	12*β*	1.41 (1H, m)	
4*α*	2.01 (1H, d, 16.6)	43.0, CH_2	13		40.8, C
4*β*	2.83 (1H, m)		14	2.42 (1H, dd, 11.0, 9.2)	55.4, CH
5	2.78 (1H, m)	30.2, CH	15*α*	1.37 (1H, d, 9.2)	25.6, CH_2
6		49.5, C	15*β*	1.94 (1H, dd, 11.0, 3.5)	
7*α*	1.43 (1H, d, 3.6)	31.0, CH_2	16	0.96 (3H, s)	25.2, CH_3
7*β*	1.10 (1H, s)		17	0.96 (3H, s)	23.4, CH_3
8*α*	2.37 (1H, m)	29.2, CH_2	18	1.08 (3H, d, 7.3)	20.9, CH_3
8*β*	1.81 (1H, m)		19	1.33 (3H, s)	21.3, CH_3
9		144.6, C	20	1.82 (3H, s)	21.2, CH_3
10		131.0, C			

Table 3
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compounds **4** and **5** in $CDCl_3$.

No.	**4**		**5**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1α	2.01 (1H, m)	40.2, CH_2	4.11 (2H, m)	59.4, CH_2
1β	1.10 (1H, m)			
2α	1.92 (1H, m)	19.6, CH_2	5.38 (1H, m)	124.7, CH
2β	1.50 (1H, m)			
3α	1.05 (1H, m)	38.0, CH_2		139.1, C
3β	2.16 (1H, m)			
4		43.8, C	2.10 (2H, m)	39.2, CH_2
5	1.44 (1H, m)	51.4, CH	2.19 (1H, m)	25.8, CH_2
			2.28 (1H, m)	
6α	2.47 (1H, m)	24.4, CH_2	5.20 (1H, m)	129.0, CH
6β	2.20 (1H, m)			
7	5.57 (1H, m)	124.6, CH		132.7, C
8		131.9, C	2.13 (1H, m)	44.8, CH_2
			2.60 (1H, m)	
9	1.88 (1H, m)	56.3, CH	3.71 (1H, ddd, 10.0,	69.4, CH
			6.9, 3.2)	
10		36.5, C	3.34 (1H, d, 6.9)	79.1, CH
11a	3.77 (1H, dd, 11.4,	60.9, CH_2		73.1, C
11b	4.3)			
	3.86 (1H, dd, 11.4,			
	3.7)			
12	1.77 (3H, s)	22.0, CH_3	1.30 (3H, s)	26.0, CH_3
13	0.80 (3H, s)	14.3, CH_3	1.25 (3H, s)	29.9, CH_3
14	1.24 (3H, s)	29.1, CH_3	1.67 (3H, s)	16.3, CH_3
15		183.6, C	1.66 (3H, s)	16.0, CH_3

Koninginol D (**5**): colorless oil; $[\alpha]_D^{25}$ −11.0 (*c* 0.1, MeOH). UV (MeOH) λ_{max} (log ε): 204.2 (3.39) nm. CD (0.2 mg/mL, MeCN): 206 (+2.61) nm. IR ν_{max}: 3383, 2924, 1667, 1383, 1236, 1173, 1047 cm^{-1}. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data, see Table 3. Positive ESIMS: *m/z* 295 $[M + Na]^+$, HRESIMS: *m/z* 295.1881 $[M + Na]^+$ (calcd for $C_{15}H_{28}NaO_4$, 295.1880).

2.4. Cytotoxicity assay

Compounds **1**–**17** were evaluated for their cytotoxic activity against HepG-2, MCF-7, SF-268, and A549 cell lines by the SRB (Sulforhodamine B) method [11]. The cells (180 μL) with a density of 3×10^4 cells/mL of media on 96-well plate were put under 37 °C at 5% CO_2 condition and incubated for 24 h. Then, 20 μL of various concentrations of compounds were added and further incubated for 72 h. After that, the cell monolayers were fixed by 50% (wt/v) trichloroacetic acid (50 μL) and stained for 30 min by 0.4% (wt/v) SRB, which was dissolved in 1% acetic acid. The unbound dye was removed by washing repeatedly with 1% acetic acid, and then dissolved into the protein-

bound dye in 10 mM Tris base solution (200 μL) for OD determination at 570 nm using a microplate reader. Adriamycin was used as a positive control possessing potent cytotoxic activity. All data were obtained in triplicate and presented as means ± S.D, and the IC_{50} values were calculated by the SigmaPlot 10.0 software with the use of a non-linear curve-fitting method.

2.5. Antibacterial assay

Antibacterial activity was evaluated against *Staphylococcus aureus* (CMCC 26003), *Bacillus subtilis* (CMCC 63501), and *Escherichia coli* (CMCC 44102) using the resazurin staining method [12]. The mid-logarithmic-phase tested bacteria were diluted to 2×10^6 cfu/mL with LB broth, meanwhile, compounds and ampicillin were diluted with DMSO to obtain the solutions with concentration ranging from 10 μg/mL to 1×10^3 μg/mL. Then, 20 μL of the tested compounds in the DMSO solution were added to 96-well flat-bottom microtiter plate and followed by adding 180 μL bacteria solution to each well. The 96-well plates were incubated at 37 °C for 24 h. After that, 20 μL resazurin in DMSO solution with the concentration of 0.2 mg/mL was added to each well. The lowest concentration of the tested compound that blue color did not turn to pink was determined as MIC. All assays were performed in triplicate, and the results presented as mean values of the three measurements. Ampicillin was used as a positive control.

3. Results and discussion

Compound **1** was obtained as a yellow powder. Its molecular formula was determined to be $C_{22}H_{27}NO_5$ based on the positive mode with an obvious HRESIMS ion peak found at *m/z* 386.1963 $[M + H]^+$, which corresponded to ten indices of hydrogen deficiency. The IR spectrum displayed strong absorptions at 3361 cm^{-1} and 1699 cm^{-1} that were characteristic for the hydroxyl and carbonyl functional groups. The ^1H NMR spectrum (Table 1) showed typical signals assignable to four methyl groups at δ_H (1.33, s, H_3-18), δ_H (1.36, s, H_3-19), δ_H (1.32, d, J = 7.0 Hz, H_3-21), and δ_H (1.30, d, J = 7.0 Hz, H_3-22). Moreover, the ^{13}C NMR (Table 1) and HSQC spectra of **1** displayed resonances for 22 carbons ascribed to four methyl, six methylene, one methine, and eleven quaternary carbons including six olefinic as well as three keto ones.

The HMBC correlations from H_2-6 to C-4 and C-8, H_2-7 to C-5, H_2-10 to C-1 and C-8, H_3-18 to C-7, as well as H_2-9 to C-3 and C-11, coupled with $^1H-^1H$ COSY cross-peak of **a** (C-6/C-7), were indicative of the existence of a cyclohexenone ring. In addition, the $^1H-^1H$ COSY spectrum could readily confirm the presence of other three independent spin systems **b** (C-9/C-10), **c** (C-16/C-17), and **d** (C-21/C-20/C-22). The aforementioned informative results in conjunction with close comparison of the NMR data for compound **1** with those for the known compound radianspene K [13,14] indicated that **1** should also share the similar guanacastane skeleton. The only significant difference between them was that a glycolyl group presented at the *N*-atom in **1**. The HMBC correlations (Fig. 2) observed from H-16 to C-2 and C-15, and the HRESIMS data, were also consistent with the assignment. The relative configuration of **1** was determined on the basis of the NOE correlations. The key correlation between H-9*α* and H_3-18 showed that these protons were cofacial and arbitrarily assigned as *α*-orientation. The correlation between H-9*β* and H_3-19 indicated that the methyl group of C-19 was *β*-orientated. The absolute configuration of **1** was determined by comparison of the experimental and simulated electronic circular dichroism (ECD) spectrum generated by time-dependent density functional theory (TDDFT) calculation. As expected, the calculated ECD spectrum was perfectly matched to the experimental one as shown in Fig. 3. Therefore, the absolute configuration of **1** was established as 8*R*, 11*R* and given a trial name as koninginol A.

The molecular formula of $C_{23}H_{31}NO_5$ was assigned to compound **2** as determined by an HRESIMS ion with the positive mode at *m/z* 402.2276 $[M + H]^+$, which corresponded to nine degrees of unsaturation. The IR spectrum showed the existence of hydroxyl and carbonyl moieties with characteristic absorption peaks observed at 3460 cm^{-1} and 1691 cm^{-1}, respectively. A comparison of its 1D (Table 1) and 2D NMR data with those of **1** suggested that **2** should also share a very similar structural core with that of **1**. One of the two differences between them was that the C-14 carbonyl carbon for **1** was replaced by a ketal carbon with the formation of a seven membered hetro-ring through the linkage of C-17-O-C-14 and attached with an additional methoxyl group for **2**. The other difference was the lack of a double bond at position C-12 for **2,** giving rise to two chiral centers at C-12 and C-13 position. The aforementioned conclusion was further confirmed by the key HMBC correlations (Fig. 2) from H_3-18 to C-14, H_2-16 to C-2, H_2-17 to C-14, and H_2-16 to C-15. Moreover, the key correlations between H-12 and H-13, H-13 and OMe-18, as well as H_2-7*α* and H_3-19 showed that these protons were cofacial and arbitrarily assigned as *α*-orientation, whereas the correlation between H_2-7*β* and H-20 indicated a *β*-orientation for Me-20. Satisfactorily, the calculated CD spectrum of the 8*S*, 11*R*, 12*R*, 13*R*, 14*S* isomer was closely matched with the experimental one observed as shown in Fig. 4. Therefore, the configuration of **2** was finally assigned to be 8*S*, 11*R*, 12*R*, 13*R*, 14*S* and given a trivial name of koninginol B.

Compound **3** was obtained as a colorless oil. It exhibited a molecular ion at *m/z* 303.2323 (calcd *m/z* 303.2319) in the positive HRESIMS spectrum, which was consistent with the molecular formula $C_{20}H_{30}O_2$ and indicated six indices of hydrogen deficiency. The IR spectrum showed broad absorption peaks at 3347 cm^{-1} and 1695 cm^{-1}, which accounted for the presence of a hydroxyl group and a carbonyl functionality, respectively. The ^{13}C NMR (Table 2) in conjunction with HSQC spectra adequately distinguished 20 carbon signals answering for five methyl carbons, five methylene carbons, four methine carbons, and six quaternary carbons. The NMR spectroscopic data (Table 2) and HMBC correlations (Fig. 2) together with COSY correlations observed for **3** exhibited almost the same results as those for harziandione [2] except for the replacement of the ketone unit by a free hydroxy moiety in the cyclobutane ring. This conclusion was further confirmed by the HMBC correlations from H-11 to C-12, C-13, and C-10 as well as the COSY consolation between H-11/H_2-12. The relative configuration of compound **3** was established by NOE experiment, wherein the key NOE correlation between H-11 and H_3-19 was observed and thus established the 11-OH was *β*-orientated. In light of the aforementioned evidence, the structure of **3** was concluded as shown in Fig. 2 and given the trivial name koninginol C.

Compound **4** was obtained as a colorless oil. Its molecular formula was established as $C_{15}H_{24}O_3$ based on the negative molecular ion $[M - H]^-$ at *m/z* 251.1656 ($C_{15}H_{23}O_3$, calcd for 251.1656) in the HRESIMS, indicating four degrees of unsaturation. The IR spectrum displayed characteristic resonances at 3356 cm^{-1} and 1695 cm^{-1}, which were responsive for the existence of hydroxyl and carbonyl functionalities, respectively. With the careful inspection of the ^{13}C NMR (Table 3) and HSQC spectra, fifteen carbon resonances including three methyl carbons [δ_C 22.0 (C-12), δ_C 14.3 (C-13), and δ_C 29.1 (C-14)], five methylene carbons [δ_C 40.2 (C-1), δ_C 19.6 (C-2), δ_C 38.0 (C-3), δ_C 24.4 (C-6), and δ_C 60.9 (C-12)], three methine carbons [δ_C 51.4 (C-5), δ_C 56.3 (C-9), and δ_C 124.6 (C-7)], four quaternary carbons [δ_C 43.8 (C-4), δ_C 36.5 (C-10), δ_C 131.9 (C-8), and δ_C 183.6 (C-15)] were definitely concluded. The proton/proton and proton/carbon connectivities were established with the aid of the ^1H–^1H COSY and HSQC spectra, which unambiguously revealed the presence of the following three spin systems: **a** (C-1/C-2/C-3), **b** (C-5/C-6/C-7), and **c** (C-9/C-11).

In the HMBC spectrum (Fig. 2), the correlations from H_2-2 to C-4 as well as H_2-3 to C-4 and C-15 suggested the connection of C-3-C-4-C-15. Moreover, the obvious HMBC correlations from H_3-14 to C-4 and H_2-3, H-5, H_3-14 to C-15 showed the linkage of C-14-C-4-C-5. The connections of C-11-C-9-C-8-C-12 could be readily rationalized by the critical correlations between H_2-11 to C-8 and C-9, as well as H_3-12 to C-7, C-8,

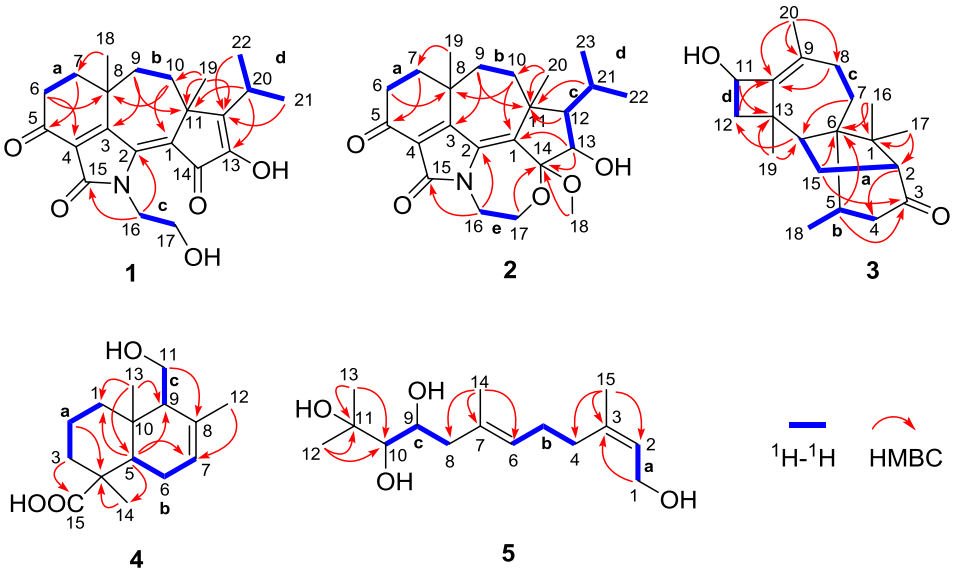

Fig. 2. ^{1}H–^{1}H COSYs and key HMBCs of **1**–**5**.

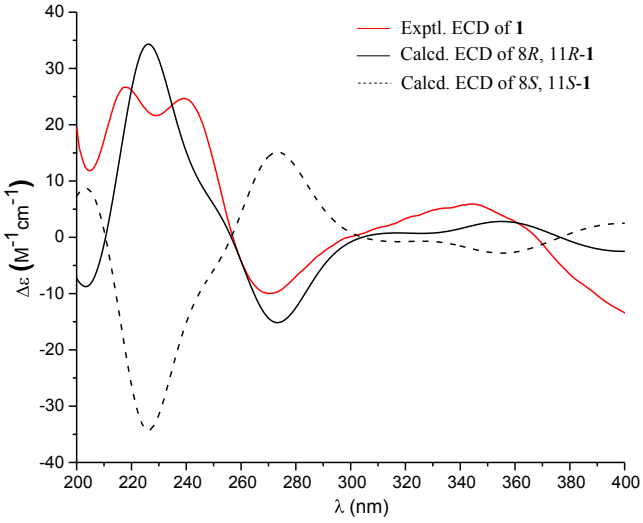

Fig. 3. Experimental and calculated ECD spectra of **1**.

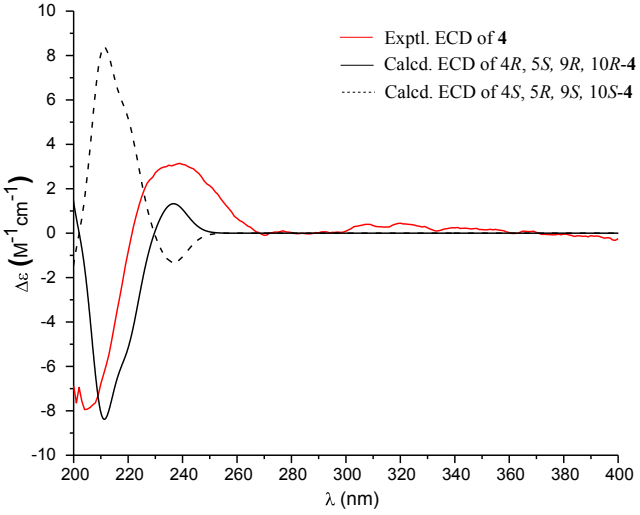

Fig. 5. Experimental and calculated ECD spectra of **4**.

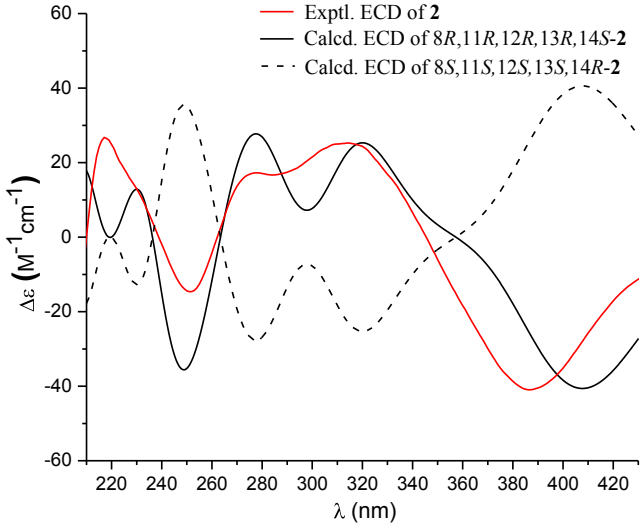

Fig. 4. Experimental and calculated ECD spectra of **2**.

and C-9. All of the aforementioned informative data were collectively pointed to that this compound should feature a drimane sesquiterpenoid skeleton and be closely related to 11,12-dihydroxy-15-drimeneoic acid [15], except that the methyl moiety of C-12 in the latter one was oxidized and transformed into a methoxyl functional group. The relative configuration of **4** was deduced by the analysis of NOESY correlations, which provided a series of essential interactions between H-5/H_3-14, H-5/H-9, strongly suggesting that these protons were cofacial and assigned as *α*-orientation. Additionally, the key NOESY correlation of H_3-13/H_2-11 evidenced that C-11, C-13, and C-15 also ought to be *β*-oriented. The absolute configuration of **4** was further evaluated by the calculated CD spectrum method, as expected, it provided a satisfactory agreement between the calculated CD spectrum of the 4*R*, 5*S*, 9*R*, 10*R* isomer and the experimental one (Fig. 5). Therefore, the configuration structure of **4** was conclusively assigned as 11-dihydroxy-15-drimeneoic acid as depicted in Fig. 1.

Compound **5** was also obtained as a colorless oil. Its molecular formula was determined to be $C_{15}H_{28}O_4$ based on the positive mode in the HRESIMS spectrum with a sodiated molecular ion peak at *m/z* 295.1881, indicating two degrees of unsaturation. The IR spectrum showed the existence of free hydroxyl moieties with a broad resonance

Table 4
Cytotoxic activities of compounds **1–17**.

Compounds	IC_{50} (μM)[a]			
	HepG-2	MCF-7	SF-268	A549
1	> 100	> 100	> 100	> 100
2	62.7 ± 0.7	69.8 ± 1.1	71.7 ± 0.4	46.6 ± 3.2
3–14	> 100	> 100	> 100	> 100
15	49.8 ± 1.0	65.5 ± 1.6	74.8 ± 2.4	31.3 ± 1.8
16	52.2 ± 0.4	53.9 ± 1.5	63.3 ± 2.1	22.2 ± 0.7
17	> 100	> 100	> 100	> 100
Adriamycin	1.18 ± 0.2	0.95 ± 0.1	0.57 ± 0.0	0.7 ± 0.0

[a] Values are expressed as the mean ± SD.

Table 5
Antibacterial activities of the tested compounds.

Compounds	MIC (μg/mL)	
	S. aureus (CMCC26003)	*B. subtilis* (CMCC63501)
1	> 100	10
2	40	2
4–6	> 100	> 100
11–13	> 100	> 100
16–17	> 100	> 100
Ampicillin	1	1

at 2926 cm^{-1}. Moreover, the ^1H NMR spectrum (Table 3) of **5** distributed a series of typic signals characteristic for four methyl groups at δ_H 1.30 (3H, s, H-12), 1.25 (3H, s, H-13), 1.67 (3H, s, H-14), 1.66 (3H, s, H-15), as well as two olefinic protons at δ_H 5.38 (1H, m, H-2) and 5.20 (1H, m, H-6). All proton resonances were then assigned to their corresponding carbons via the HSQC spectrum. As referring to the NMR and HRESIMS data in conjunction with a close comparison with previously reported agripilol D [16], it could be concluded that compound **5** should also be a sesquiterpenoid sharing a farnesane skeleton due to the similarity of their NMR profiles in most cases.

The ^1H–^1H COSY spectrum of **5** revealed the presence of three spin systems, namely, **a** (C-1/C-2), **b** (C-4/C-5/C-6), and **c** (C-8/C-9/C-10) as shown in Fig. 2. Based on the fragment **c**, the key HMBC correlations (Fig. 2) from H_3-13 to C-10, C-11 as well as H_3-12 to C-10, C-11 successfully identified the connection of C-11 to C-10, C-12, C-13 with a OH group attached at C-11 position. In addition, the HMBC cross peak between H_3-14 to C-6, C-7, and C-8 indicated the direct connection of C-7 to C-6, C-8, and C-14 with the consideration of fragment **b**. Moreover, the HMBC correlations from H_3-15 to C-2, C-3, C-4 and H_2-1 to C-3, coupled with $^1H-{}^1H$ COSY cross-peaks of **a**, further confirmed the linkage of C-15 to C-2, C-3, and C-4. Therefore, the planar structure of compound **5** was thus established, and it was given a trial name as koninginol D.

The twelve known compounds were identified as harziandione 2 (**6**) [2], radianspene B (**7**) [13], (*S*)-(-)-5-(hydroxymethyl)-2-(2′,6′,6′-trimethyltetrahydro-2*H*-pyran-2-yl)phenol (**8**) [17], hamanasol A (**9**) [18], trichodermatide A (**10**) [9], dihydropyran (**11**) [6], Keto diol (**12**) [7], 7-*O*-methylkoninginin D (**13**) [8], (1*S*,6*R*,7*S*,10*R*)-10-hydroxy-4(5)-muurolen-3-one (**14**) [19], 1*R*,3*S*,6*S*,7*R*,10*S*-7-isopropyl-4,10-dimethylbicyclo[4.4.0]dec-4-en-3,10-diol (**15**) [20], 1*R*,3*R*,6*S*,7*R*,10*S*-7-isopropyl-4,10-dimethylbicyclo[4.4.0]dec-4-en-3,10-diol (**16**) [21], coprinol (**17**) [22] by comparing their physicochemical and spectroscopic data with those of published values.

To further evaluate *in vitro* bioactivity, compounds were tested for cytotoxic and antibacterial activities, respectively. Compounds **1–17** were evaluated for their inhibitory activities against HepG-2, MCF-7, SF-268, and A549 cell lines, wherein compounds **2**, **15**, and **16** showed antiproliferative activities against A549 with IC_{50} values of 46.6, 31.3, and 22.2 μM, respectively (Table 4). In the antibacterial screening, compounds **1** and **2** exhibited significant antibacterial activity against *B. subtilis* with MIC values of 10 and 2 μg/mL, respectively (Table 5).

4. Conclusion

In summary, three new diterpenes koninginols A-C (**1–3**) and two new sesquiterpenoids 11-hydroxy-15-drimeneoic acid (**4**), koninginol D (**5**), together with twelve known compounds (**6–17**) were isolated from the endophytic fungus *Trichoderma koningiopsis* A729 derived from *Morinda officinalis*. Their structures were fully confirmed by NMR and HRESIMS analyses as well as the comparison with previously reported literatures. Among them, compounds **1** and **2** were intriguing diterpene alkaloids possessing guanacastane skeleton, whereas compound **3** contained a unique tetracyclic scaffold fusing four-, five-, six-, and seven-membered carbon rings to format a spiral skeleton. The cytotoxic assay clarified that compounds **2**, **15**, and **16** displayed inhibitory activities against A549 cell lines with IC_{50} of 46.6, 31.3, and 22.2 μM, respectively. It's worth to note that compounds **1** and **2** exhibited significant antibacterial activities against *B. subtilis* with MIC values of 10 and 2 μg/mL, respectively.

Conflicts of interest

There are no conflicts to declare.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.02.005.

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