Phytochemistry Letters 8 (2014) 137–140

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Three new koninginins from *Trichoderma neokongii* 8722

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ARTICLE INFO

Article history:
Received 9 January 2014
Received in revised form 25 February 2014
Accepted 4 March 2014
Available online 2 April 2014

Keywords:
Trichoderma neokongii
Chemical constituents
Structure identification
Koninginin

ABSTRACT

Three new fungal metabolites, named koninginins I (**1**), J (**2**) and K (**3**) together with four known koninginins A (**4**), B (**5**), D (**6**) and E (**7**), were isolated from solid fermentation products of *Trichoderma neokongii* 8722. Three new structures were elucidated by extensive spectroscopic methods, including 1D NMR and 2D NMR, and HR-ESI–MS experiments.

1. Introduction

Trichoderma can produce a lot of secondary metabolites using for biological control (Reino et al., 2008; El-Hasan et al., 2009; Stoppacher et al., 2010; Mukherjee et al., 2012). A new polyketide type compound, koninginin, was obtained from *Trichoderma* sp. (Reino et al., 2008). Koninginins A, B, C, E and G showed a growth inhibition of etiolating wheat coleoptiles (Cutler et al., 1989, 1991, 1999; Parker et al., 1995a, 1995b). Koninginins A, B, D and G also could affect the growth of some plant pathogen fungi (Dunlop et al., 1989; Ghisalberti and Rowland, 1993; Cutler et al., 1999). In order to search for new structure koninginin type compounds against plant diseases, the extract of *Trichoderma neokongii* 8722 was investigated and three new koninginin type compounds, together with four known koninginins were obtained. This report describes three new compounds structures.

2. Results and discussion

From the extracts of solid fermentation products of *T. neokongii* 8722, seven compounds including three new koninginins I-K (**1**–**3**) (Fig. 1) were identified. The structures of the known compounds were determined to be koninginins A (**4**) (Cutler et al., 1989), B (**5**) (Cutler et al., 1991), D (**6**) (Dunlop et al., 1989; Song et al., 2010), and E (**7**) (Parker et al., 1995a).

Compound **1** was obtained as colorless amorphism. The HR-ESI–MS data indicated a molecular formula of $C_{16}H_{24}O_5$ based on the $[M+H]^+$ ion signal at *m/z* 297.1699 (calc. 297.1697). The NMR data (Table 1) revealed one quaternary carbon at δ_C 199.5, 175.1 and 112.9, five methines at δ_C 67.1 (δ_H 4.39), 66.9 (δ_H 4.27), 78.1 (δ_H 4.11), 73.4 (δ_H 3.70) and 68.7 (δ_H 3.73), and seven methylenes and one methyl, which suggested compound **1** was koninginin type compound (Dunlop et al., 1989; Reino et al., 2008). According to the NMR and MS spectra, compound **1** had one more hydroxyl than koninginin D (Cutler et al., 1991; Song et al., 2010). A preliminary linear skeleton bearing two branches was deduced to be C-2–C-3–C-4 (–branch)–C-7–C-8–C-9–C-10-C-11 (–branch) from complete interpretation of key cross-peaks in the COSY spectrum (H-2/H-3/H-4; H-7/H-8/H-9/H-10/H-11) and key correlations in the HMBC spectrum: H-2 (δ_H 2.32 and 2.62) correlated with the carbons at δ_C 199.5 (C-1), 30.6 (C-3), 67.1 (C-4), H-3 (δ_H 1.97 and 2.18) with the carbons at δ_C 199.5 (C-1), 34.3 (C-2), 67.1 (C-4) and 175.1 (C-5), H-4 (δ_H 4.39) with the carbons at δ_C 175.1 (C-5), 112.9 (C-2), 34.3 (C-2) and 30.6 (C-3), and H-7 (δ_H 4.27) with the carbons at δ_C 199.5 (C-1), 175.1 (C-5), 112.9 (C-6), 78.1 (C-9), and 68.7 (C-15) (w), H-9 (δ_H 4.11) with the carbons at δ_C 66.9 (C-7) and 28.6 (C-8), H-16 (δ_H 1.16) with the carbons at δ_C 40.3 (C-14), 68.7 (C-15), and other correlations (Fig. 2). In the ^{1}H NMR spectrum, two intermediate coupling constants (J = 4.8, 6.6 Hz) were observed for H-4, which confirmed a pseudoequatorial position for H-4. Except for the signals of H-15 and C-15 for **1**, the NMR data were very similar to those of koninginin D (Dunlop et al., 1989; Song et al., 2010). According to a biogenetic perspective, and comparing the specific rotation, chemical shift and coupling constant of **1** with that of the

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http://dx.doi.org/10.1016/j.phytol.2014.03.004
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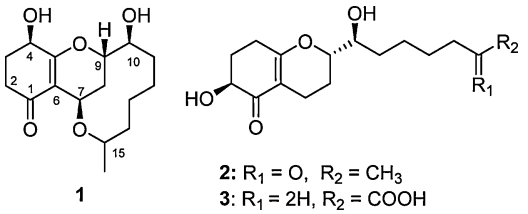

Fig. 1. The structures of compounds **1**–**3**.

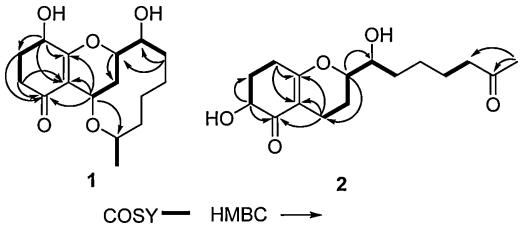

Fig. 2. HMBC and key correlations of compounds **1**–**2**.

literature data (Song et al., 2010), the relative configuration of **1** was same with koninginin D (Dunlop et al., 1989; Song et al., 2010). Based on above data, compound **1** was elucidated to be koninginin I.

Compound **2** was obtained as colorless powder. The HR-ESI–MS data indicated a molecular formula of $C_{16}H_{24}O_5$ based on the $[M+Na]^+$ ion signal at *m*/*z* 319.1519 (calc. 319.1516). The MS and NMR spectroscopic data of compound **2** were very similar to those of koninginin B except that one methylene (C-15) in koninginin B was oxidized to the ketone in compound **2** (Cutler et al., 1991; Liu and Wang, 2001). The 2D-NMR data (Table 2) showed the detail: H-2 (δ_H 2.14) correlated with the carbons at δ_C 209.0 (C-15) and 43.5 (C-14), H-14 (δ_H 2.46) with the carbons at δ_C 209.0 (C-15), 30.0 (C-16) and 23.4 (C-13), and other correlations (Fig. 2). In the ^{1}H NMR spectrum, two intermediate coupling constants (J = 6.8, 16.4 Hz) were observed for H-2, which confirmed a axial position for H-2. Except that one methylene (C-15) in koninginin B was oxidized to the ketone in compound **2**, the NMR data were very similar to those of koninginin B (Cutler et al., 1991; Liu and Wang, 2001). From a biogenetic perspective, the configuration of **2** should be identical to that of the co-occurring koninginin B. So, compound **2** was identified to be koninginin J.

Compound **3** was obtained as colorless oil. The HR-ESI–MS data indicated a molecular formula of $C_{16}H_{24}O_6$ based on the $[M+Na]^+$ ion signal at *m*/*z* 335.1466 (calc. 335.1465). The MS and NMR spectroscopic data of compound **3** were very similar to those of koninginin B except that terminal methyl (CH_3-16) in koninginin B was oxidized to the carboxyl group in compound **3** (Cutler et al., 1991; Liu and Wang, 2001). The 2D-NMR data (Table 2) showed the correlations between H-14 (δ_H 1.65) and carbons at δ_C 178.3 (C-16), 33.8 (C-15) and 29.1 (C-13), H-15 (δ_H 2.35) and carbons at δ_C 178.3 (C-16), 33.8 (C-15) and 24.7 (C-14). In the ^{1}H NMR spectrum, two intermediate coupling constants (J = 6.0, 18.0 Hz) were observed for H-2, which confirmed a axial position for H-2. Except that terminal methyl (CH_3-16) in koninginin B was oxidized to the carboxyl group in compound **3**, the NMR data were very similar to those of koninginin B (Cutler et al., 1991; Liu and Wang, 2001). From a biogenetic perspective, the configuration of **3** should be identical to that of the co-occurring koninginin B. So, compound **3** was identified to be koninginin K.

Compounds **1**–**7** were assayed for antifungal activity (*Gaeumannomyces graminis*, *Fusarinum moniliforme*, *Verticillium cinnabarium* and *Phyricularia oryzae*), but all compounds did not show the inhibition activity to the tested phytopathogenic fungi at 100 μg/disk. Nematicidal activity result indicated that only koninginin A (**4**) had weak activity against *Panagrellus redivivus* and *Caenorhabditis elegans*.

Table 1
NMR data of compounds **1** (in CD_3OD, *J* in Hz).

Position	^{1}H	^{13}C	HMBC
1	–	199.5	–
2	2.32 (1H, ddd, 4.8, 8.4, 16.8)	34.3	1, 3, 4
	2.62 (1H, ddd, 4.8, 7.8, 16.8)		1, 3, 4
3	1.97 (1H, m)	30.6	1, 2, 4
	2.18 (1H, m)		1, 2, 4
4	4.39 (1H, dd, 4.8, 6.6)	67.1	2, 3, 5, 6
5	–	175.1	–
6	–	112.9	–
7	4.27 (brs)	66.9	1, 5, 6, 9, 15 (w)
8	2.06 (1H, m)	28.6	6, 7
	1.57 (1H, m)		9
9	4.11 (1H, m)	78.1	7, 8, 5 (w)
10	3.70 (1H, m)	73.4	11, 12
11	1.66 (2H, m)	33.8	9, 10, 12
12	1.54 (2H, m)	26.9	9
13	1.44 (2H, m)	27.0	12, 14, 15
14	1.44 (2H, m)	40.3	12, 15
15	3.73 (1H, m)	68.7	13
16	1.16 (3H, d, 6.18)	23.7	14, 15

3. Experimental

3.1. General

UV spectra were measured on a Shimadzu UV-2401PC spectrophotometer, λ_{max} (log ε) in nm. NMR experiments were carried out on Bruker AM-400 and Bruker DRX-500 NMR spectrometers with TMS as internal standard. ESI–MS and HR-EI–MS were recorded on a Finnigan LCQ-Advantage mass spectrometer and a VG Auto-Spec-3000 mass spectrometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. Column chromatography was carried out on silica gel (G, 200–300 mesh and H, Qingdao Marine Chemical Factory, Qingdao, China), and Sephadex LH-20 (Pharmacia). Precoated silica gel GF254 plates (Qingdao Marine Chemical Factory, Qingdao, China) were used for thin layer chromatography (TLC).

3.2. Fungal material

The strain of *T. neokongii* 8722 was deposited in Southwest Forestry University. Four pathogenic fungi (*Gaeumannomyces graminis*, *Fusarinum moniliforme*, *Verticillium cinnabarium* and *Phyricularia oryzae*) were provided by Dr. Fan L. M. at Yunnan Agricultural University. The culture medium consisted of potato (peeled, 200 g), agar (15 g) and glucose (20 g), per L of deionized H_2O. *T. neokongii* 8872 (20 L) was cultured on potato-dextrose agar dish at temperature of 26 °C for 21 days.

3.3. Extraction and isolation

Solid fermentation products of *T. neokongii* 8722 (20 L) was cut into small pieces and extracted with mixture solution (EtOAc:-MeOH:HAc = 80:15:5, v/v/v) by three times to afford of rude extracts. The extracts were dissolved in water, and extracted with EtOAc and then *n*-butanol three times, respectively.

The EtOAc (31.0 g) residue was subjected to a column of silica gel G (200–300 mesh) using petroleum ether–EtOAc and $CHCl_3$–MeOH gradient solvent system to produce 11 fractions (Fr.1–Fr.11). The fraction Fr.5 (320 mg) was subjected to Sephadex LH-20 $CHCl_3$–MeOH (1:1) and subsequent purified by preparative TLC to give compound **5** (11 mg). The fraction Fr.8 (1.70 g) was subjected