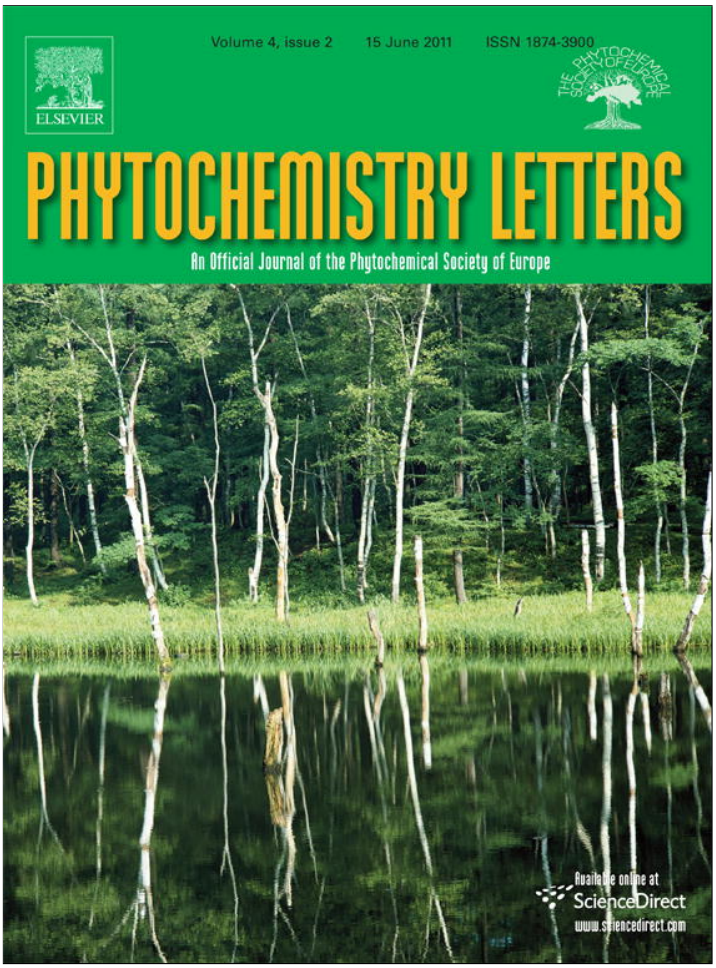

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Three new acorane sesquiterpenes from *Trichoderma* sp. YMF1.02647

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ABSTRACT

Three new acorane sesquiterpenes 1α-isopropyl-4α,8-dimethylspiro[4.5]dec-8-ene-2β,7α-diol (**1**), 1α-isopropyl-4α,8-dimethylspiro[4.5]dec-8-ene-3β,7α-diol (**2**) and 2β-hydroxy-1α-isopropyl-4α,8-dimethylspiro[4.5]dec-8-en-7-one (**3**) were isolated from the culture broth of *Trichoderma* sp. YMF1.02647. The structures were elucidated using spectroscopic data from 1D, 2D NMR and HRESIMS experiments, and the structure of **1** was further confirmed by a single-crystal X-ray diffraction analysis. Antitumor activity experiment showed that compound **2** had inhibitive activity against HL-60, A-549 and MCF-7 cell lines.

1. Introduction

The fungal genus *Trichoderma* is a widespread saprophyte that occurs almost ubiquitously. *Trichoderma* species have been studied extensively as potential sources of biocontrol agents (Katayama et al., 2001; Peltola et al., 2004; Harman et al., 2004). There is increasing circumstantial evidence implicating the existence of secondary metabolites as antibiotics that contribute to their biocontrol activities. The genus *Trichoderma* has afforded many types of bioactive substances including trichothecenes (Godtfredsen and Vangedal, 1964; Corley et al., 1994), gliotoxin (Wilhite and Straney, 1996), and other metabolite groups such as peptides, pyrones, lactones, isonitriles, and peptaibol (Andrade et al., 1996; Shirota et al., 1997; Nielsen, 2003; Garo et al., 2003; Ren et al., 2009). During the course of examining *Trichoderma* for natural products, we investigated the metabolites of strain *Trichoderma* sp. YMF1.02647. Herein, we report the isolation and structure elucidation of three new acorane sesquiterpenes (**1**–**3**) from *Trichoderma* sp. YMF1.02647.

2. Results and discussion

Strain YMF1.02647 was fermented in 18 L of PDB (potato dextrose broth) medium and the broth was concentrated and extracted with ethyl acetate to obtain brown crude extracts. The extracts were subjected to silica gel G and Sephadex LH-20 column chromatography to afford three new sesquiterpenoids (**1**–**3**).

Compound **1** (Fig. 1) showed in its mass spectrum a molecular ion $[M+Na]^+$ at *m*/*z* 261.1830, compatible with the molecular formula $C_{15}H_{26}O_2$. The ^{13}C NMR spectrum of compound **1** displayed 15 carbons, which were assigned by HSQC, HMBC, and DEPT experiments to the resonances of four methyls, three aliphatic methylenes, one olefinic, two oxygenated and three nonoxygenated methines, and two quaternary carbons. Detailed 2D NMR analysis showed that compound **1** is an acorane type sesquiterpenoid (Nawamaki and Kuroyanagi, 1996). In the HMBC spectrum (Table 1), isopropyl groups were assigned by the HMBC correlations from H-12 and H-13 to C-11 (δ 27.6) and C-1 (δ 68.5), and correlations from H-1 and H-11 to C-12 (δ 25.1) and C-13 (δ 21.7). The oxygenated methine H-2 correlated with carbons C-11 (δ 27.6) and C-4 (δ 43.3), and H-4 correlated with carbons C-3 (δ 41.1), C-5 (δ 46.7) and C-15 (δ 14.0), which together with the ^1H,^1H-COSY connections between H-11 and H-12, H-13, and between H-2 and H-1, H-3 provided the fragment 2-isopropyl-4-methylcyclopentanol. Similarly, ^1H,^1H-COSY also showed connections between H-7 and H-6, and between H-9 and H-10. In the HMBC spectrum (Table 1), 7-OH group was assigned by the HMBC correlations from H-6b (δ 1.34) to C-5 (δ 46.7), C-8 (δ 135.2), and methyl H-14 was correlated with C-7 (δ 68.0), C-8 (δ 135.2), C-9 (δ 124.5) and C-10 (δ 36.3). The relative configuration of **1** was unambiguously established by a single-crystal X-ray diffraction (Fig. 2), which was consistent with that of **1** in the solution as fixed by the ROESY spectrum: correlation between H-1 and H-4, H-10. So compound **1** was determined to be 1α-isopropyl-4α,8-dimethylspiro[4.5]dec-8-ene-2β,7α-diol.

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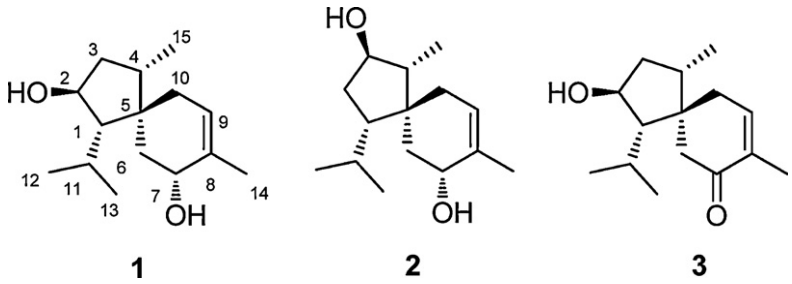

Fig. 1. Structure of the isolated acorane sesquiterpenes.

Compound **2** (Fig. 1) had the molecular formula $C_{15}H_{26}O_2$, as deduced by HRESIMS on a molecular ion $[M+Na]^+$ at *m/z* 261.1831. The ^{13}C NMR spectrum of compound **2** displayed 15 carbons, which were assigned by HSQC, HMBC, and DEPT experiments to the resonances of four methyls, three aliphatic methylenes, one olefinic, two oxygenated and three nonoxygenated methines, and two quaternary carbons. According to the NMR data (Table 1), compound **2** was similar to **1** except that the OH position in furan ring was different: 2-OH position in compound **1** instead of 3-OH position in compound **2**. In the HMBC spectrum (Table 1), the oxygenated methine H-3 (δ 3.77) was correlated with carbons C-1 (δ 57.3) and C-15 (δ 11.8), and methyl H-15 (δ 0.96) was correlated with carbons C-3 (δ 76.7), C-4 (δ 55.6) and C-5 (δ 46.0). In other parts, the correlations were the same as in compound **1** (Table 1). The relative configuration of **2** was established by the ROESY spectrum: correlations between H-1 and H-7, H-3 and H-15, between H-4 and H-10, and referred to compound **1** data from single-crystal X-ray diffraction (Fig. 2). Compound **2** was determined to be 1α-isopropyl-4α,8-dimethylspiro[4.5]dec-8-ene-3β,7α-diol.

Compound **3** showed in its mass spectrum a molecular ion $[M+Na]^+$ at *m/z* 259.1672, indicating a molecular weight corresponding to a molecular formula of $C_{15}H_{24}O_2$. Based on the NMR data (Table 1), compound **3** was similar to compound **1** except that the 7-OH in compound **1** was oxidized to be carbonyl group in compound **3**. In the HMBC spectrum (Table 1), the protons in methylene H-6 (δ 2.28 and 2.37) was correlated with carbons C-1 (δ 65.3), C-4 (δ 44.4), C-5 (δ 49.3), C-7 (δ 200), C-8 (δ 134.9), methyl H-14 was correlated with C-7 (δ 200.0), C-8 (δ 134.9) and C-9 (δ 144.1), and H-9 was correlated with C-5 (δ 49.3), C-10 (δ 38.1) and C-14 (δ 15.1). The relative configuration of **3** was established by the ROESY spectrum: correlations between H-1 and H-4, H-6, between H-2 and H-11, H-13, and referred to compound **1** data from single-crystal X-ray diffraction (Fig. 2). The skeleton of compound **3** was similar to AC-1, but the relative configuration of compound **3** was different from AC-1 (Nawamaki and Kuroyanagi, 1996). Based on the above data, compound **3** was elucidated to be 2β-hydroxy-1α-isopropyl-4α,8-dimethylspiro[4.5]dec-8-en-7-one.

Compounds **1**–**3** were evaluated for their cytotoxicity against HL-60, SMMC-7721, A-549, MCF-7 and SW480 cell lines using the MTT method (Mosmann, 1983). Compound **2** showed inhibiting activities against HL-60, A-549 and MCF-7 cell lines with IC_{50} value 22.8, 22.4 and 36.1 respectively. However, it did not show any inhibitory effect against SMMC-7721 and SW480 at 40 μM. Compounds **1** and **3** have no inhibitory activity against any of the tested cell lines at 40 μM.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with a Jasco DIP-370 digital polarimeter. NMR spectra were obtained (in $CDCl_3$) with Bruker AM-400 and Bruker DRX-500 NMR spectrometers with TMS as the internal standard at 293 K. ESIMS and HRESIMS were recorded on VG Auto-Spec-3000 mass spectrometer respectively. Column chromatography (CC) was performed on silica gel G (200–300 mesh, Qingdao Marine Chemical Factory, China) and Sephadex LH-20 (Amersham Pharmacia, Sweden).

Table 1
The NMR data of compounds **1**–**3** (in $CDCl_3$).

Position	**1**			**2**			**3**		
	^1H	^{13}C	HMBC	^1H	^{13}C	HMBC	^1H	^{13}C	HMBC
1	1.31 (m)	68.5	2, 6, 11, 12, 13	1.57 (m)	57.3	5, 6, 12, 13	1.38 (t, 6.0)	65.3	2, 5, 10, 11, 12, 13
2	4.18 (m)	73.8	4, 11	1.94 (m)	37.3	1, 3, 4, 11	4.14 (m)	73.4	5, 11
				1.68 (m)		1, 3, 5			
3	1.72 (m)	41.1	2, 5	3.77 (dt, 3.6, 9.2)	76.7	1, 15	1.73 (m)	41.8	\
	1.67 (m)		4, 15				1.67 (m)		1, 2, 4, 5, 15
4	1.89 (m)	43.3	3, 5, 6, 15	1.50 (m)	55.6	3, 5, 10	2.05 (m)	44.4	3, 5, 10,15
5	\	46.7	\	\	46.0	\	\	49.3	\
6a	1.76 (m)	33.8	\	1.72 (m)	33.2	1, 5, 7	2.37 (d, 17.0)	39.6	1, 4, 5, 7, 8
6b	1.34 (m)		5, 8	1.32 (m)		1, 4, 5, 7	2.28 (d, 17.0)		1, 4, 5, 7, 8
7	4.25 (m)	68.0	8, 9	4.25 (brs)	68.4	\	\	200.0	\
8	\	135.2	\	\	135.7	\	\	134.9	\
9	5.48 (brs)	124.5	5, 7, 14	5.43 (s)	124.7	10	6.65 (brs)	144.1	5, 10, 14
10a	2.17 (dm, 3.9)	36.3	4, 5, 8, 9	2.16 (m)	35.4	\	2.72 (d, 19.7)	38.1	
10b	1.86 (m)		5, 6	1.88 (m)		\	2.23 (d, 19.7)		4, 5, 8, 9
11	1.84 (m)	27.6	1, 2, 12, 13	1.63 (m)	29.9	1, 5, 12, 13	1.73 (m)	27.2	\
12	0.98 (d, 6.7)	25.1	1, 11, 13	0.92 (d, 6.5)	23.2	1, 11, 13	1.05 (d, 7.0)	24.8	1, 11, 13
13	1.03 (d, 6.7)	21.7	1, 11,12,	0.85 (d, 6.0)	22.9	1, 11, 12	1.00 (d, 7.0)	20.8	1, 11, 12
14	1.75 (s)	19.1	7, 8, 9, 10	1.75 (s)	18.5	7, 8, 9	1.75 (s)	15.1	7, 8, 9
15	0.86 (d, 6.7)	14.0	3, 4, 5	0.96 (d, 7.0)	11.8	3, 4, 5	0.83 (d, 7.0)	15.9	3, 4, 5

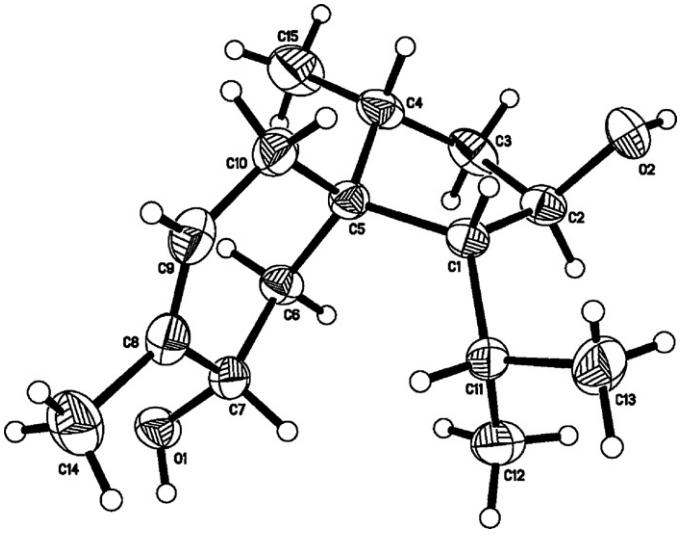

Fig. 2. X-ray structure of compound **1**.

3.2. Fungal material

Strain *Trichoderma* sp. YMF1.02647 was deposited in the culture collection of Key Laboratory for Conservation and Utilization of Bio-resource, Yunnan University, China. The strain was conserved on PDA (potato dextrose agar) medium.

3.3. Extraction and isolation

Strain YMF1.02647 was grown in shake culture (200 ml per 500 ml triangular flask) on PDB (potato dextrose broth) medium. After fermentation for 7 days at 26 °C at 180 rev/min, 18 L fermentation broth of YMF1.02647 was filtered. The fermentation broth was condensed and extracted by ethyl acetate. The ethyl acetate extract was concentrated to produce a crude extract (3.28 g). The extract was applied to silica gel G chromatography column (CC) (300 g) eluted with petroleum ether/acetone (20:1–1:1, v/v) to yield 16 fractions Fr.A1–Fr.A16. Fr.A1 was further separated on a Sephadex LH-20 column eluting with acetone to obtain five subfractions $Fr.A1_1$–$Fr.A1_5$, $Fr.A1_4$ was then purified on a silica gel G CC (20 g) eluted with chloroform to yield compound **3** (8 mg). Fr.A4 was applied to silica gel G CC (20 g) eluted with chloroform/acetone (20:1–1:1, v/v), to yield five fractions $Fr.A4_1$–$Fr.A4_5$. $Fr.A4_2$ was further separated on a Sephadex LH-20 column eluting with acetone to obtain four fractions $Fr.A4_{21}$–$Fr.A4_{24}$. $Fr.A4_{22}$ was purified on silica gel G CC (2 g, chloroform/acetone 200:1, v/v) to afford compound **1** (6 mg). Fr.A6 was separated on silica gel G CC (20 g) eluted with chloroform/acetone (20:1–1:1, v/v) to yield four fractions $Fr.A6_1$–$Fr.A6_4$ and $Fr.A6_2$ was further separated on a Sephadex LH-20 column eluting with acetone to obtain five fractions $Fr.A6_{21}$–$Fr.A6_{25}$. $Fr.A6_{24}$ was further purified on silica gel G CC (2 g, chloroform/acetone 100:1, v/v) to yield compound **2** (4 mg).

3.3.1. 1α-Isopropyl-4α,8-dimethylspiro[4.5]dec-8-ene-2β,7α-diol (1)

Colorless needles ($CHCl_3$); $[\alpha]_D^{26} + 4.6$ ($CHCl_3$, *c* 0.2); NMR data ($CDCl_3$) see Table 1; ESIMS: 261 $[M+Na]^+$; HRESIMS: $[M+Na]^+$ 261.1830, calcd for $C_{15}H_{26}O_2$ 261.1830.

3.3.2. 1α-Isopropyl-4α,8-dimethylspiro[4.5]dec-8-ene-3β,7α-diol (2)

Colorless powder; $[\alpha]_D^{26} + 18.0$ ($CHCl_3$, *c* 0.18); NMR data ($CDCl_3$) see Table 1; ESIMS: 261 $[M+Na]^+$; HRESIMS: $[M+Na]^+$ 261.1831, calcd for $C_{15}H_{26}O_2$ 261.1830.

3.3.3. 2β-Hydroxy-1α-isopropyl-4α,8-dimethylspiro[4.5]dec-8-en-7-one (3)

Colorless powder; $[\alpha]_D^{26} + 32.1$ ($CHCl_3$, *c* 0.18); NMR data ($CDCl_3$) see Table 1; ESIMS: 237 $[M+H]^+$, 259 $[M+Na]^+$; HRESIMS: $[M+Na]^+$ 259.1672, calcd for $C_{15}H_{24}O_2$ 259.1673.

3.4. Assay of antitumor activity

The antitumor activity of compounds **1**–**3** against cell lines (HL-60, SMMC-7721, A-549, MCF-7 and SW480) was assayed by the MTT method (Mosmann, 1983) in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Chinese Academy of Sciences.

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