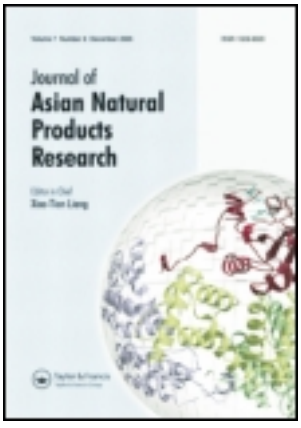

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

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Published online: 25 Jun 2013.

To cite this article: Jian-Hai Ding, Tao Feng, Zheng-Hui Li, Jing Si, Hai-You Yu, Hong-Bin Zhang & Ji-Kai Liu (2013) Four new sesquiterpenoids from cultures of the fungus Funalia trogii, Journal of Asian Natural Products Research, 15:8, 828-832, DOI: 10.1080/10286020.2013.807251

To link to this article: http://dx.doi.org/10.1080/10286020.2013.807251

Journal of Asian Natural Products Research, 2013
Vol. 15, No. 8, 828–832, http://dx.doi.org/10.1080/10286020.2013.807251

Four new sesquiterpenoids from cultures of the fungus *Funalia trogii*

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Four new drimane sesquiterpenoids, named as funatrols A–D (**1**–**4**), together with isodrimenediol (**5**), were isolated from cultures of the fungus *Funalia trogii*. The new structures were elucidated by means of spectroscopic methods. All compounds were tested for their cytotoxicities against five human cancer cell lines.

Keywords: *Funalia trogii*; fungus; drimane sesquiterpenoids

1. Introduction

Funalia trogii (Berk.) Bondartsev & Singer, a white-rot basidiomycete, is widely distributed in temperate China as both parasitic and saprotrophic [1–4]. The water extract of *F. trogii* was reported to exhibit anti-cancer properties [5,6] and a protective effect on deltamethrin-induced liver toxicity in rats [7]. However, the chemical constituents of the formation of *F. trogii* have not been investigated so far. As our ongoing investigation on structurally interesting and biologically active natural products from higher fungi [8–12], an EtOAc extract of cultures of the *F. trogii* was subjected to investigation, which resulted in the isolation of four new drimane sesquiterpenoids, named funatrols A–D (**1**–**4**), together with a known compound, isodrimenediol (**5**) (Figure 1) [13]. Their structures were established by extensive spectroscopic data analysis. Meanwhile, all compounds were evaluated for their cytotoxicities against five human cancer cell lines.

2. Results and discussion

Compound **1**, a colorless oil, gave a molecular formula of $C_{15}H_{26}O_3$ by HR-EI-MS at *m/z* 254.1883 $[M]^+$, with three degrees of unsaturation. The ^{1}H nuclear magnetic resonance (NMR) spectral data (Table 2) showed the presence of three tertiary methyls (δ_H 0.80, 0.85, and 1.03), two oxymethylenes (δ_H 2.73, 3.21, 3.41, and 3.61) and an oxymethine (δ_H 3.29). The ^{13}C NMR and DEPT experiments (Table 1) displayed 15 carbon resonances comprising one oxygenated quaternary carbon, two oxygenated methylenes, one oxygenated methine, as well as three methyls, four methylenes, two methines, and two quaternary carbons. The above-mentioned data exhibited similarities with those of isodrimenediol (**5**) [13], which suggested that compound **1** possessed a drimane skeleton same as that of **5**. Compound **1** was readily identified as an oxygenated derivative of **5** at the double bond between C-8 and C-12 by carbon resonances at δ_C 61.6 (s, C-8) and 51.7 (t, C-12), as supported by the HMBC correlations from H-12 at δ_H 3.21 (1H, br s) and 2.73 (1H, d, $J = 3.4$ Hz) to C-7 (δ_C 36.0, t), C-8 (δ_C 61.6, s), and C-9 (δ_C 54.0, d) (Figure 1). Detailed analysis of other 2D NMR correlations (HSQC, HMBC, ^{1}H–^{1}H

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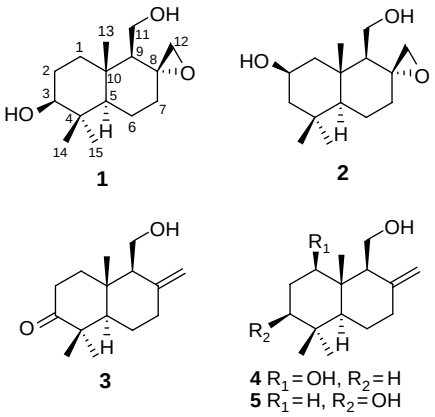

Figure 1. Structures of compounds **1**–**5**.

COSY) suggested that the other parts were the same as those of **5** (Figure 1). The relative configuration was elucidated by the rotating-frame nuclear Overhauser effect correlation spectroscopy (ROESY) experiment and by comparison with that of **5**. The ROESY correlation between H-12 and H-13 indicated the α orientation of 8,12-epoxy moiety (Figure 2). Therefore, compound **1** was established to be funatrol A, as shown in Figure 1.

HR-EI-MS (observed at *m/z* 254.1895, calcd at *m/z* 254.1882, $[M]^+$) analysis of compound **2** demonstrated that it had the same molecular formula of $C_{15}H_{26}O_3$ as that of **1**. The 1D NMR data (Tables 1 and 2) were very similar to those of **1**. Analysis of HSQC and HMBC spectra showed that obvious differences were resulted from the location of one hydroxyl group. The HMBC correlations of H-2 with C-1/C-3/C-4/C-10, and of H-1 and H-3 with C-2 revealed that this hydroxy group should be located at C-2 in **2** other than at C-3 in **1**. In ROESY spectrum, the correlation between H-2 and H-3α suggested that the OH group at C-2 was β orientation. Therefore, compound **2** was established to be funatrol B.

Compound **3** was obtained as a colorless oil with the molecular formula of $C_{15}H_{24}O_2$ based on the HR-EI-MS at *m/z* 236.1783 $[M]^+$. The infrared spectroscopy (IR) absorption bands at 3441 and 1705 cm^{-1} indicated the presence of OH and CO groups, respectively. The ^1H NMR data (Table 2) exhibited signals corresponding to three tertiary methyls (δ_H 1.13, 1.05, and 0.94), a terminal double bond (δ_H 4.73 and 5.02), and an oxymethylene (δ_H 3.83). The ^{13}C and DEPT NMR spectra (Table 1) displayed 15 carbons, including a ketone carbonyl group, two olefinic carbons, two sp^3 quaternary carbon resonances, two methines, four methylenes (oxygenated one), and three methyls. The data suggested that

Table 1. ^{13}C NMR spectral data for compounds **1**–**4**.

No.	**1**	**2**	**3**	**4**
1	36.7 (t)	44.9 (t)	37.6 (t)	77.1 (d)
2	27.2 (t)	67.6 (d)	34.8 (t)	28.6 (t)
3	78.3 (d)	46.6 (t)	216.6 (s)	40.0 (t)
4	39.0 (s)	32.7 (s)	38.7 (s)	33.1 (s)
5	53.8 (d)	53.1 (d)	55.2 (d)	53.7 (d)
6	21.3 (t)	21.7 (t)	25.0 (t)	24.2 (t)
7	36.0 (t)	36.1 (t)	37.5 (t)	38.0 (t)
8	61.6 (s)	61.6 (s)	146.7 (s)	149.1 (s)
9	54.0 (d)	54.9 (d)	58.2 (d)	57.8 (d)
10	38.8 (s)	38.9 (s)	48.1 (s)	44.9 (s)
11	58.8 (t)	59.0 (t)	59.0 (t)	61.4 (t)
12	51.7 (t)	51.9 (t)	107.8 (t)	106.8 (t)
13	15.7 (q)	18.2 (q)	15.0 (q)	9.3 (q)
14	28.3 (q)	24.2 (q)	22.0 (q)	21.4 (q)
15	15.5 (q)	33.7 (q)	26.1 (q)	33.0 (q)

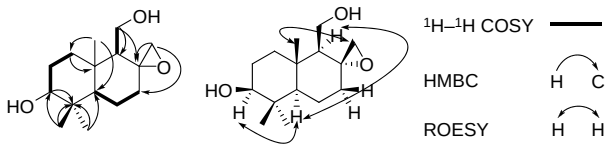

Figure 2. Selected 2D NMR correlations of **1**.

3 was a drimane-type sesquiterpenoid similar to **5** [13], except for the OH-3 was oxidated into a carbonyl carbon at C-3 in **3**, which was supported by the HMBC correlations from H-1, H-2, H-14, and H-15 to C-3 at δ_C 216.6 (s). The other 2D NMR data (HSQC, HMBC, ^{1}H–^{1}H COSY) suggested that the other parts of **3** were the same to those of **5**, while the relative configuration of **3** was also in accordance with that of **5** based on the ROESY correlations. Accordingly, compound **3** was determined to be funatrol C.

Based on the HR-EI-MS at *m/z* 238.1947 $[M]^+$, compound **4** was suggested to possess the same molecular formula of $C_{15}H_{26}O_2$ as that of **5** [13], while the NMR data (Tables 1 and 2) were also closely related to those of **5**. The only difference was the position of the OH group at C-1 in **4** rather than at C-3 in **5**. This was confirmed by HMBC correlations of H-2, H-3, H-5, and H-13 with C-1, as well as the ^{1}H–^{1}H COSY cross peaks from H-1 to H-3. The ROESY correlation between H-1 and H-5 indicated the β orientation of OH-1. Detailed analysis of other 2D NMR data (HSQC, HMBC, ^{1}H–^{1}H COSY, ROESY) suggested that the other parts were the same as those of **5**. Hence, compound **4** was established to be funatrol D.

All compounds were evaluated for their cytotoxicities against five human cancer cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method as reported previously

Table 2. ^{1}H NMR spectral data for compounds **1**–**4**.

No.	**1**	**2**	**3**	**4**
1a	1.72[a] (m)	1.83[a] (m)	2.04[a] (m)	3.61 (dd, 6.5, 9.3)
1b	1.36 (m)	1.63[a] (m)	1.77 (m)	
2a	1.70[a] (m)	4.21 (m)	2.66 (m)	1.67 (m)
2b	1.58[a] (m)		2.44 (m)	
3a	3.29 (m)	1.68 (m)		1.43[a] (m)
3b		1.49 (m)		1.36 (m)
5	1.08 (d, 12.6)	1.15 (d, 12.4)	1.65 (dd, 2.9, 3.1)	1.11 (dd, 2.7, 12.6)
6a	1.86[a] (m)	1.87[a] (m)	1.74 (m)	1.75 (m)
6b	1.56[a] (m)	1.60 (m)	1.55 (m)	1.44[a] (m)
7a	1.98 (m)	1.97 (m)	2.51 (m)	2.32 (m)
7b	1.44 (m)	1.42 (m)	2.10 (m)	2.04 (m)
9	1.85[a] (m)	1.88[a] (m)	2.05[a] (m)	2.00 (m)
11a	3.61 (m)	3.62 (m)	3.83 (m)	3.87 (m)
11b	3.41 (dd, 10.7, 11.0)	3.46 (dd, 10.3, 11.5)		3.81 (m)
12a	3.21 (br. s)	3.23 (br. s)	5.02 (s)	4.74 (s)
12b	2.73 (d, 3.4)	2.74 (d, 3.4)	4.73 (s)	4.32 (s)
13	0.85 (s)	1.10 (s)	0.94 (s)	0.79 (s)
14	1.03 (s)	1.05 (s)	1.05 (s)	0.81 (s)
15	0.80 (s)	0.95 (s)	1.13 (s)	0.87 (s)

[a] Signals were partially overlapped.

[14]. No compound showed significant activity (IC_{50} values $> 40\,\mu M$).

3. Experimental

3.1 General experimental procedures

Optical rotations were recorded on a Jasco P-1020 digital polarimeter (Jasco International Co., Ltd, Tokyo, Japan). UV data were obtained on a Shimadzu UV-2401A spectrophotometer (Shimadzu Corporation, Kyoto, Japan). IR spectra were obtained on a Bruker Tensor27 FT-IR spectrometer with KBr pellets (Bruker Optics GmbH, Ettlingen, Germany). NMR spectra were obtained on Bruker AV-400 and DRX-500 instruments, and a Bruker Avance III 600 MHz spectrometer with tetramethylsilane as an internal standard at room temperature (Bruker BioSpin GmbH, Rheinstetten, Germany). Mass spectra were recorded on a VG Autospec-3000 mass spectrometer and an API QSTAR Pulsar I spectrometer (MDS Sciex, Concord, ON, Canada). Silica gel (200–300 mesh, Qingdao Marine Chemical, Ltd, Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Upssala, Sweden) were used for open column chromatography (CC). Fractions were monitored by thin layer chromatography. Spots were visualized by heating silica gel plates immersed in Vanillin–H_2SO_4 in ethanol.

3.2 Fungal material and cultivation conditions

F. trogii was provided by Prof Yu-Cheng Dai, Institute of Microbiology, Beijing Forestry University, and fermented by Mr Zheng-Hui Li, Kunming Institute of Botany. A voucher specimen is deposited in the Institute of Microbiology, Beijing Forestry University. The culture medium consisted of glucose (5%), peptone from porcine meat (0.15%), yeast powder (0.5%), KH_2PO_4 (0.05%), and $MgSO_4$ (0.05%). Fermentation was carried out on a shaker at 160 revolutions per minute for 25 days.

3.3 Extraction and isolation

The culture broth (25 liters) was filtered, and the filtrate was extracted three times with EtOAc while the mycelium was extracted three times with CH_3Cl–MeOH (1:1). The EtOAc layer, together with the mycelium extraction, was concentrated under reduced pressure to give a crude extract (8.0 g), and this residue was subjected to CC over silica gel (200–300 mesh) eluted with a gradient of CH_3Cl–MeOH (1:0 → 0:1, v/v) to obtain seven fractions (1–7). Fractions 5 and 6, eluted with petroleum ether–acetone (15:1–8:1, v/v), were separated repeatedly by reversed-phase RP-18 (MeOH–H_2O, 3:7–9:1, v/v) CC, followed by Sephadex LH-20 (Me_2CO) to afford **5** (6.0 mg), **3** (0.7 mg), **4** (6.2 mg), **1** (3.5 mg), **2** (5.0 mg), respectively.

3.3.1 Funatrol A (*1*)

A colorless oil; $[\alpha]_D^{25}$ − 22.1 ($c = 0.35$, MeOH); IR (KBr) ν_{max} 3425, 2936, 1451, 1384, 1101, and 1036 cm^{-1}; for ^1H (400 MHz) and ^{13}C NMR (100 MHz) spectral data ($CDCl_3$), see Tables 1 and 2; HR-EI-MS: *m/z* 254.1883 $[M]^+$ (calcd for $C_{15}H_{26}O_3$, 254.1882).

3.3.2 Funatrol B (*2*)

A colorless oil; $[\alpha]_D^{25}$ − 4.0 ($c = 0.25$, MeOH); IR (KBr) ν_{max} 3425, 2934, 1452, 1118, 1079, and 1035 cm^{-1}; for ^1H (400 MHz) and ^{13}C NMR (100 MHz) spectral data ($CDCl_3$), see Tables 1 and 2; HR-EI-MS: *m/z* 254.1895 $[M]^+$ (calcd for $C_{15}H_{26}O_3$, 254.1882).

3.3.3 Funatrol C (*3*)

A colorless oil; $[\alpha]_D^{24}$ − 20.6 ($c = 0.07$, MeOH); IR (KBr) ν_{max} 3441, 2931, 1705, 1645, 1457, 1385, and 1110 cm^{-1}; for ^1H (500 MHz) and ^{13}C NMR (150 MHz) spectral data ($CDCl_3$), see Tables 1 and 2; HR-EI-MS: *m/z* 236.1783 $[M]^+$ (calcd for $C_{15}H_{24}O_2$, 236.1776).

3.3.4 *Funatrol D (4)*

A colorless oil; $[\alpha]_D^{25} - 11.0$ ($c = 0.31$, MeOH); IR (KBr) ν_{max} 3422, 3210, 2937, 1640, 1461, 1086, and 1049 cm^{-1}; for ^1H (400 MHz) and ^{13}C NMR (100 MHz) spectral data ($CDCl_3$), see Tables 1 and 2; HR-EI-MS: *m/z* 238.1947 $[M]^+$ (calcd for $C_{15}H_{26}O_2$, 238.1933).

3.4 *Cytotoxicity assay*

Five human cancer cell lines: breast cancer SK-BR-3, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, pancreatic cancer PANC-1, and lung cancer A-549 cells were used in the cytotoxic assay. Cells were cultured in Roswell Park Memorial Institute-1640 medium or in Dulbecco's-modified Eagle medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone) in 5% CO_2 at 37°C. The cytotoxicity assay was performed according to the MTT method in 96-well microplates [14]. Briefly, 100 μl of adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before addition of test compounds, while suspended cells were seeded just before drug addition with initial density of 1×10^5-cells/ml. Each tumor cell line was exposed to the test compound at the concentrations of 0.0625, 0.32, 1.6, and 8 μM in triplicates for 48 h, with cisplatin (Sigma-Aldrich, St Louis, MO, USA) as positive control. After compound treatment, cell viability was detected and a cell growth curve was graphed. IC_{50} values were calculated by Reed and Muench's method [15].

Acknowledgments

This work was financially supported by National Basic Research Program of China (973 Program, 2009CB522300), the National Natural Science Foundation of China (U1132607).

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