广西植物 Guihaia May 2017, 37(5):617-620
http://journal.gxzw.gxib.cn
http://www.guihaia-journal.com

DOI: 10.11931/guihaia.gxzw201610012
引文格式: 周稚凡, 段媛昌, 李靖, 等. 毛韧革菌中的韧革菌素类似物 [J]. 广西植物, 2017, 37(5): 617-620
ZHOU ZF, DUAN YC, LI J, et al. Vibralactone derivatives from *Stereum hirsutum* FP-91666 [J]. Guihaia, 2017, 36(5): 617-620

Vibralactone derivatives from *Stereum hirsutum* FP-91666

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Abstract: In order to study the secondary metabolites of *Stereum hirsutum* FP-91666, four vibralactone derivatives were isolated from YMG fermentation broth products of this strain —— one new vibralactone derivative, vibralactone R(**1**), together with three known vibralactones (**2-4**) —— by the methods of silica gel chromatography, gel chromatography, and semi-preparative HPLC and so on. The new structure was elucidated by spectroscopic data including HR-ESI-MS experiments, 1D and 2D NMR.

Key words: phytochemistry, vibralactone, structure identification, chemical constituents, NMR

CLC number: Q946 **Document code**: A **Article ID**: 1000-3142(2017)05-0617-04

毛韧革菌中的韧革菌素类似物

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摘 要: 从毛韧革菌(*Stereum hirsutum* FP-91666)的 YMG 发酵液中分离得到 1 个新的韧革菌素类似物——韧革菌素 R(**1**)以及 3 个已知的类似物——韧革菌素(**2-4**)。利用硅胶色谱、凝胶色谱等方法,结合半制备型 HPLC 对该菌次生代谢产物进行研究得到这些化合物,并通过核磁共振(包括 1D-NMR、2D-NMR)、高分辨质谱实验(HR-ESI-MS)、紫外光谱等波谱学方法鉴定其结构。

关键词: 植物化学, 韧革菌素, 结构鉴定, 化学成分, 核磁共振

Stereum is basidiomycete fungus and belongs to Stereaceae family and can produce a variety of secondary metabolites (Nair et al, 1977; Dubin et al, 2000; Abraham 2001; Omolo et al, 2002). In the previous work (Duan et al, 2015), we used PDA medium to culture *S. hirsutum* FP-91666 and obtained some compounds from the strain. On the basis of the genome data, *S. hirsutum* could yield further more secondary metabolites (Lackner et al, 2012). In process of studying the biosynthesis of vibralactone (Zhao et al, 2013), which generated from *S. vibran* and inhibited the pancreatic lipase with an IC_{50} value of 0.4 μg · mL^{-1} (Liu et al, 2006), we had explored homologous genes of biosynthetic vibralactone, and learned that *S.*

Received date: 2016-10-15 **Accepted date**: 2016-12-09
Foundation item: Supported by National Basic Research Program of China "973" Program of China (2013CB127505); the Applied Basic Research Foundation of Yunnan Province (2013FA018).
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hirsutum can synthesize vibralactone-type compounds (Kim et al, 2009; Kim et al, 2010). In order to further explore its potential in production of new and active compounds, the OSMAC (one strain, many compounds) strategy was employed to mining the chemical diversity of this strain (Bode et al, 2002). And now one new vibralactone derivative, vibralactone R(**1**), together with three known vibralactones (**2**-**4**) were obtained from YMG fermentation broth products of *S. hirsutum* FP-91666. The present work describes the isolation and structure of four vibralactones (Fig. 1).

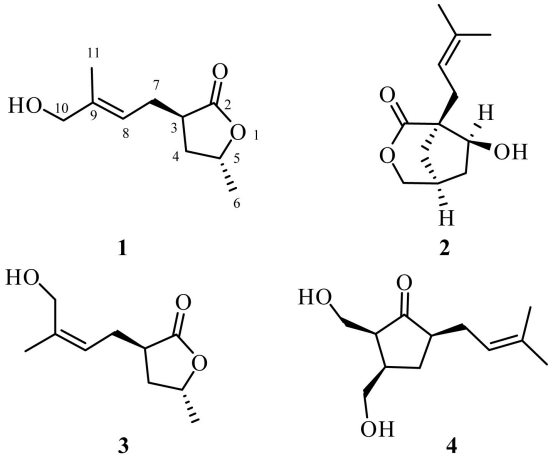

Fig. 1 Structures (**1**-**4**) isolated from *Stereum hirsutum* FP-91666

1 Materials and Methods

1.1 General

UV spectra were measured using a Shimadzu UV-2401 PC spectrophotometer (Shimadzu, Tokyo, Japan). NMR experiments were carried out on Bruker AM-400 and Avance 600 NMR spectrometers with tetramethylsilane (TMS) as an internal standard. ESI-MS and HR-ESI-MS were recorded on a VG Auto-Spec-3000 mass spectrometer (VG, Manchester, England) and a Finnigan LCQ-Advantage mass spectrometer (Thermo, San Jose, USA), respectively. Optical rotations were measured using a Jasco DIP-370 digital polarimeter (JASCO, Tokyo, Japan). Column chromatography was carried out on silica gel (G, 200 - 300 mesh and GF254) (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). Some fractions were purified by LC3000 Semi-preparation Gradient HPLC (Beijing Chuangxintongheng Science & Technology Co., Ltd, Beijing, China).

1.2 Fungal material

S. hirsutum FP-91666 was preserved in 20% glycerol at -80 ℃ in State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany. The strain was inoculated into 500 mL Erlenmeyer flasks, which contained 200 mL YMG broth (yeast extract 4.0 g · L^{-1}, malt extract 10.0 g · L^{-1}, glucose 4.0 g · L^{-1}, pH 7.3 before sterilization). After incubation at 26 ℃ for 7 d on a rotary shaker (180 r · min^{-1}), each primary culture was transferred into a 500 mL Erlenmeyer flask containing 250 mL the same broth and incubated at 26 ℃ for 21 d on a rotary shaker (180 r · min^{-1}).

1.3 Extraction and isolation

The extract of *n*-Butanol (10.51 g) of the culture broth (15 L) was separated on a column (silica gel G, 200-300 mesh, 150 g) and eluted with a petroleum ether (PE)-EtOAc (10 ∶ 1 to 6 ∶ 4) and $CHCl_3$-MeOH (10 ∶ 1 to 0 ∶ 100) gradient solvent system to yield nine fractions (Fr. 1-Fr. 9). Fr. 2 (0.86 g) was subjected on a column (silica gel G, 200-300 mesh, 60 g) using a PE-acetone (100 ∶ 4→0 ∶ 100) solvent system to produce six sub-fractions (Fr. 2.1 - Fr. 2.6). Fr. 2.2 was subjected on Sephadex LH-20 (MeOH) column and then purified by LC3000 Semi-preparation Gradient HPLC (RP-C_{18}, 250 mm × 10 mm, 5 μm, 210 nm, MeOH-H_2O from 50 ∶ 50 to 95 ∶ 5, a flow rate of 3.0 mL · min^{-1}) to yield **1** (5.6 mg) and **3** (4.2 mg). Fr. 2.3 was chromatographed on GF254 column using PE-acetone (100 ∶ 4→0 ∶ 100) and then purified by Sephadex LH-20 (MeOH) to produce **4** (2.1 mg). Fr. 3 (0.65 g) was subjected to a column (silica gel G, 200-300 mesh, 50 g) using a PE-EtOAc (100 ∶ 4→0 ∶ 100) solvent system to produce seven sub-fractions (Fr. 3.1 - Fr. 3.7). Fr. 3.1 was separated on a column (silica gel G, 200 - 300 mesh, 10 g) using $CHCl_3$-MeOH (100 ∶ 0→10 ∶ 1) and then purified by Sephadex LH-20 (MeOH)

column to yield **2** (5.2 mg).

2 Results

2.1 Vibralactone R (1)

Colorless oil; [α] = −6.8 (*c* = 1.3, MeOH); UV (MeOH) λ_{max}(log ε): 202 (3.44); ESI-MS: *m/z* 207 [M + Na]$^+$; HR-ESI-MS *m/z*: 207.099 4 [M + Na]$^+$(calc. 207.099 7).

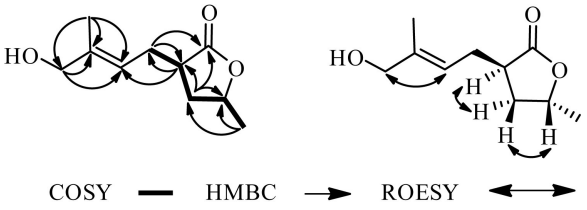

Fig. 2　Key ^{1}H−^{1}H COSY, HMBC and ROESY correlations for **1** supporting its structural assignments

Compound **1** was achieved as colorless oil. The HR-ESI-MS data revealed a molecular formula of $C_{10}H_{16}O_3$ based on the [M + Na]$^+$ ion signal at *m/z* 207.099 4 (calc. 207.099 7). The MS and NMR spectroscopic data of Compound **1** were substantially the same with those of vibralactone G (**3**) except that the chemical shifts of CH_2−10 (δ_H 4.02, δ_C 68.3) and CH_3−11 (δ_H 1.68, δ_C 13.9) were changed in Compound **1** (Wang et al, 2012). The 2D-NMR data revealed that the H-8 (δ_H 5.42) of methine correlated with the carbons at δ_C 68.3 (C-10), 39.5 (C-3), 28.4 (C-7) and 13.9 (C-11); H-10 (δ_H 4.02) of methylene correlated with the carbons at δ_C 138.0 (C-9), 120.9 (C-8) and 13.9 (C-11); H-11 (δ_H 1.68) of methylene correlated with the carbons at δ_C 138.0 (C-9), 120.9 (C-8) 68.3 (C-10) and 39.5 (C-2). The NOESY experiment (Fig. 2) showed NOE correlations between H-8 and H-10; H-5 and H-4b; H-4a and H-3a, supporting the relative configurations. Based on the above data, Compound **1** was determinated to be as shown in Fig. 1, and named as vibralactone R.

In addition, the three vibralactones D (**2**), G (**3**) and O (**4**) (Fig. 1) were identified by comparison of the MS and NMR data obtained with those reported in the literature (Wang et al, 2012; Chen et al, 2014).

Table 1　NMR Data of **1** and Vibralactone G (**3**) (in $CDCl_3$, 400 MHz)

Position	**1**		Vibralactone G (**3**)		
	^{1}H (*J* in Hz)	^{13}C	HMBC	^{1}H (*J* in Hz)	^{13}C
2	—	178.9, s	—	—	179.2, s
3	2.73 (1H, m)	39.5, d	2, 4, 5,7, 8	2.76 (1H, m)	39.4, d
4α	2.00 (1H, m)	34.5, t	2, 3, 5, 6, 7	2.02 (1H, m)	34.1, t
4β	2.11 (1H, m)	34.5, t	2, 3, 5, 6, 7	2.16 (1H, m)	34.1, t
5	4.66 (1H, m)	75.2, d	2 (weak)	4.67 (1H, m)	75.1, d
6	1.37 (3H, d, 6.4)	21.3, q	4, 5	1.36 (3H, d, 6.4)	21.2, q
7	2.53(1H, m)	28.4, t	2, 3, 4, 8, 9	2.46 (2H, m)	28.0, t
	2.31(1H, m)	28.4, t	2, 3, 4, 8, 9	2.46 (2H, m)	28.0, t
8	5.42 (1H, dt, 1.6, 7.5)	120.9, d	3, 7, 10, 11	5.29 (1H, t, 7.5)	123.0, d
9	—	138.0, s	—	—	138.2, s
10	4.02 (2H, s)	68.3, t	3 (weak), 8, 9, 11	1.82 (3H, s)	21.7, q
11	1.68 (3H, s)	13.9, q	3, 8, 9, 10	4.10 (2H, m)	61.3, t

2.2 Vibralactone D (2)

Colorless crystal; [α] = +17.4 (*c* = 0.8, MeOH); UV (MeOH) λ_{max}(log ε): 202.5 (3.64); ^{1}H-NMR (CD_3OD, 600 MHz) δ: 1.67 (1H, ddd, J_1 = 2.0, J_2 = 5.2, J_3 = 12.4 Hz, H-2β), 1.80 (1H, dd, J_1 = 2.5, J_2 = 12.4 Hz, H-2α), 2.26 (1H, m,

H-3), 1.47 (1H, ddd, J_1 = 2.7, J_2 = 4.6, J_3 = 14.0 Hz, H-4α), 2.40 (1H, m, H-4β), 3.95 (1H, dd, J_1 = 4.6, J_2 = 10.3 Hz, H-5), 2.08 (1H, dd, J_1 = 8.2, J_2 = 14.3 Hz, H-8β), 2.60 (1H, dd, J_1 = 6.7, J_2 = 14.3 Hz, H-8α), 5.06 (1H, m, H-9), 1.56 (1H, s, H-11), 1.61 (1H, s, H-12), 4.08 (1H, brd, J = 9.1 Hz, H-13a), 4.25 (1H, ddd, J_1 = 1.0, J_2 = 2.6, J_3 = 10.2 Hz, H-13b); ^{13}C-NMR (CD_3OD, 150 MHz) δ: 58.3 (C-1), 34.8 (C-2), 34.3 (C-3), 38.8 (C-4), 79.3 (C-5), 175.6 (C-7), 31.9 (C-8), 121.4 (C-9), 135.5 (C-10), 18.2 (C-11), 26.3 (C-12), 78.7 (C-13); ESI-MS: *m/z* 233 [M + Na]$^+$; HR-ESI-MS *m/z*: 233.1151 [M + Na]$^+$ (calc. 233.1154).

2.3 Vibralactone G (3)

Colorless oil; ESI-ME: *m/z* 207 [M + Na]$^+$; ^{1}H-NMRand ^{13}C-NMR see Table 1.

2.4 Vibralactone O (4)

Colorless oil; ESI-MS: m/z 213 [M + H]$^+$; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.05 (1H, overlap, H-2), 2.26 (1H, m, H-3), 1.23 (1H, m, H-4), 2.16 (1H, overlap, H-4), 2.15 (1H, overlap, H-5), 3.95(1H, m, H-6), 3.56 (1H, m,H-6), 3.80 (1H, m, H-7), 3.45 (1H, m, H-7), 2.04 (1H, overlap, H-8), 2.40 (1H, m), 5.00 (1H, t, J = 6.5 Hz, H-9), 1.57 (3H, s, H-11), 1.63 (3H, s, H-12); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 219.1 (s, C-1), 57.8 (d, C-2), 43.2 (d, C-3), 30.1 (t, C-4), 50.0 (d, C-5), 62.5 (t, C-6), 66.6 (t, C-7), 28.5 (t, C-8), 121.0 (d, C-9), 134.4 (s, C-10), 18.2 (q, C-11), 26.0 (q, C-12).

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(上接第 605 页 Continue from page 605)

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