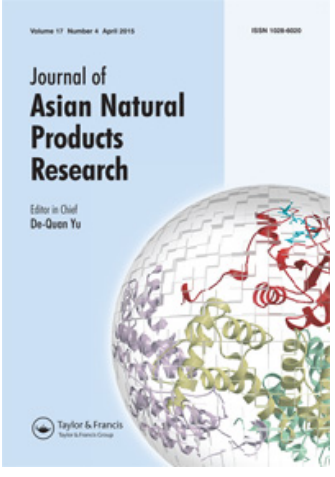




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# Two new phenol derivatives from Stereum hirsutum FP-91666

Yuan-Chang Duan[ab], Xin-Xin Meng[c], Yan-Long Yang[ab], Yin-He Yang[a] & Pei-Ji Zhao[a]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

[b] University of Chinese Academy of Sciences, Beijing 100049, China

[c] Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming 650500, China

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# Two new phenol derivatives from *Stereum hirsutum* FP-91666

Yuan-Chang Duan[ab], Xin-Xin Meng[c], Yan-Long Yang[ab], Yin-He Yang[a] and Pei-Ji Zhao[a]*

[a]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China;*
[b]*University of Chinese Academy of Sciences, Beijing 100049, China;*
[c]*Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming 650500, China*

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Two new phenol derivatives, 2-(3-methyl-2-buten-1-yl)-4-methoxyethyl-phenol (**1**) and 5-hydroxy-4-(hydroxymethyl)-2-(3-methylbut-2-en-1-yl)cyclohex-4-en-1-one (**2**), together with eight known compounds consisting of phenol derivatives (**3** and **4**), niacinamide (**5**), and five ergosta type compounds (**6**–**10**), were isolated from solid fermentation products of *Stereum hirsutum* FP-91666. Two new structures were elucidated by extensive spectroscopic methods, including 1D NMR and 2D NMR, and HR-EI-MS experiments.

**Keywords:** Stereaceae; *Stereum hirsutum* FP-91666; 2-(3-methyl-2-buten-1-yl)-4-methoxyethyl-phenol; 5-hydroxy-4-(hydroxymethyl)-2-(3-methylbut-2-en-1-yl)cyclohex-4-en-1-one



## 1. Introduction

The fungus of *Stereum* belongs to the Stereaceae family (basidiomycetes), and can produce diversiform secondary metabolites [1–3], especially produce structurally diverse sesquiterpenes [4]. In process of studying on the biosynthesis of vibralactone [5], which was produced by *Stereum vibrans* [6], we had searched analogous genes of biosynthetic vibralactone and found that *Stereum hirsutum* (*S. ostrea*) can produce vibaralactone type compounds [7,8]. According to genome data analysis, *S. hirsutum* would produce more secondary metabolites [9], so we investigated the chemical constituent of *S. hirsutum* FP-91666. And two new phenol derivatives, 2-(3-methyl-2-buten-1-yl)-4-methoxyethyl-phenol (**1**) and 5-hydroxy-4-(hydroxymethyl)-2-(3-methylbut-2-en-1-yl)cyclohex-4-en-1-one (**2**), together with eight known compounds were isolated from solid fermentation products of *S. hirsutum* FP-91666. This report describes the isolation and structural elucidation of these compounds.

## 2. Results and discussion

Compound **1** was obtained as colorless amorphism. The HR-EI-MS data indicated a molecular formula of $C_{14}H_{20}O_2$ based on the $[M]^+$ ion signal at *m/z* 220.1456. The $^{13}C$ NMR data (Table 1) revealed four quaternary carbons at $\delta_C$ 153.8, 135.6, 134.9, and 126.7, five methines at $\delta_C$ 127.9, 125.5, 121.8, 115.6, and 79.2, and one methylene ($\delta_C$ 30.1) and four methyls, and from $^1H$ NMR spectrum, three aromatic protons ($\delta_H$ 7.06 (brs), 7.07 (dd, 8.0, 2.1), and 6.81 (d, 8.0)) indicated the presence of three-substituted benzene ring; and two methyl singlets ($\delta_H$ 1.80 (s) and 1.81 (s)) and one $sp^2$ proton ($\delta_H$ 5.33–5.36 (m)) suggested compound **1** was prenylated phenol derivatives (Figure 1) [10].

*Corresponding author. Email: zhaopeiji@mail.kib.ac.cn

Table 1. $^{1}H$ and $^{13}C$ NMR spectral data of compounds **1** and **2**.

| | **1**[a] | | **2**[b] | |
|---|---|---|---|---|
| Position | $^{1}H$ | $^{13}C$ | $^{1}H$ | $^{13}C$ |
| 1 | – | 153.8, s | – | 212.7, s |
| 2 | – | 126.7, s | 2.38–2.44 (m) | 46.5, d |
| 3 | 7.06 (brs) | 127.9, d | 2.78–2.82 (m); 2.36 (d, 16) | 34.5, t |
| 4 | – | 135.6, s | – | 138.4, s |
| 5 | 7.07 (dd, 8.0, 2.1) | 125.5, d | – | 177.8, s |
| 6 | 6.81 (d, 8.0) | 115.6, d | 4.24 (s) | 54.0, t |
| 7 | 3.39 (d, 7.2) | 30.1, t | 2.38–2.44 (m); 2.10–2.16 (m) | 30.3, t |
| 8 | 5.33–5.36 (m) | 121.8, d | 5.06–5.09 (m) | 121.8, d |
| 9 | – | 134.9, s | – | 134.9, s |
| 10 | 1.80 (s) | 25.8, q | 1.68 (s) | 25.9, q |
| 11 | 1.81 (s) | 17.9, q | 1.63 (s) | 17.9, q |
| 12 | 4.24 (q, 6.4) | 79.2, d | 4.56–4.60 (m) | 61.3, t |
| 13 | 1.44 (d, 6.4) | 23.8, q | | |
| 12-$OCH_3$ | 3.22 (s) | 56.2, q | | |
| −OH | 5.12 (s) | – | | |

[a] The NMR data are obtained on Avance III 600 in $CDCl_3$.
[b] The NMR data are obtained on Bruker AV-800 in $CD_3OD$.

The HMBC experiment (Table 1) showed correlations between the proton at $\delta_H$ 4.24 (H-12) and carbons at $\delta_C$ 125.5 (C-5), 127.9 (C-3), 23.8 (C-13), 56.2 (12-$OCH_3$), and 135.6 (C-4), between the proton at $\delta_H$ 1.44 (H-13) and the carbons at $\delta_C$ 79.2 (C-12) and 135.6 (C-4), which indicated that one methoxyethyl group connected with prenylated phenol at position C-4 ($\delta_C$ 135.6) (Figure 2). C-12 was a chiral center, but its configuration cannot be determinated by current data. Together with other data (Table 1), compound **1** was elucidated to be 2-(3-methyl-2-buten-1-yl)-4-methoxyethyl-phenol (Figure 1).

Compound **2** was obtained as colorless amorphism. The HR-EI-MS data indicated a molecular formula of $C_{12}H_{18}O_3$ based on the $[M]^+$ ion signal at *m/z* 210.1250. The $^{13}C$ NMR data (Table 1) revealed four quaternary carbons at $\delta_C$ 212.7, 138.4, 177.8, and 134.9, two methines at $\delta_C$ 46.5 and 121.8, four methylenes ($\delta_C$ 54.0, 34.5, 30.3, and 61.3), and two methyls, which

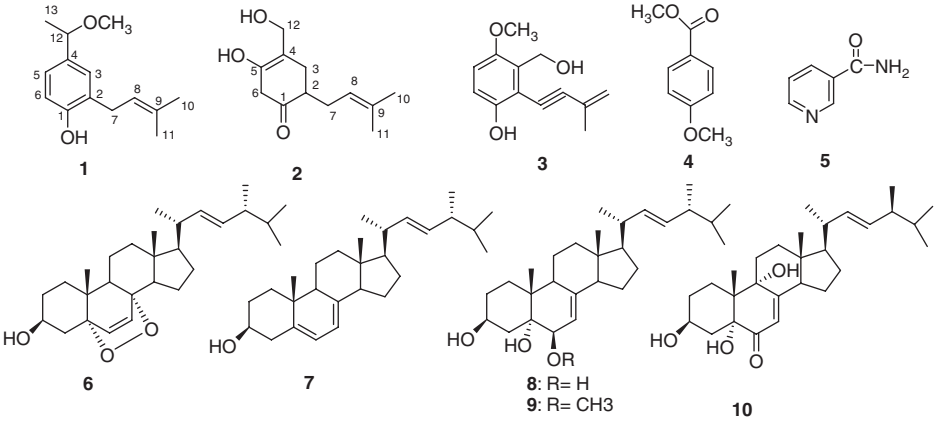


Figure 1. The compound structures from *S. hirsutum* FP-91666.

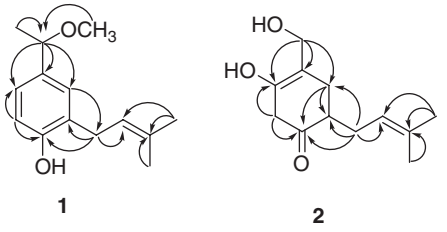


Figure 2. Selected HMBC correlations of new compounds **1** and **2**.

indicated that compound **2** was also prenylated phenol derivatives except the phenol was oxidized [10]. The detail structure was confirmed by 2D NMR. The HMBC experiment (Table 1) showed the proton at $\delta_H$ 5.06–5.09 (H-8) correlated with carbons at $\delta_C$ 17.9 (C-11), 25.9 (C-10), 30.3 (C-7), and the protons of methylene at $\delta_H$ 2.38–2.44 and 2.10–2.16 (H-7) with carbons at $\delta_C$ 121.8 (C-8) and 134.9 (C-9), and two methyl group protons at $\delta_H$ 1.68 (H-10) and 1.63 (H-11) with carbons at $\delta_C$ 121.8 (C-8) and 134.9 (C-9), which indicated the existence of an isopentenyl group. H-12 at $\delta_H$ 4.60 correlated with C-4, C-5, C-3, and H-2 correlated with C-1 and C-3, H-6 correlated with the C-1, C-4, and C-5, and H-3 correlated with the C-1, C-2, C-4 and C-5, which indicated compound **2** contained 5-hydroxy-4-(hydroxymethyl)cyclohex-4-en-1-one, which would come from oxidized phenol. These two units connected by C–C bond based on the correlations between protons of methylene at $\delta_H$ 2.38–2.44 and 2.10–2.16 (H-7) and C-2 (Figure 2). The configuration of C-2 is uncertain because H-2 was overlapped with H-7, and there is no NOE correlation between H-3 and H-7. Based on above data, compound **2** was determined to be 5-hydroxy-4-(hydroxymethyl)-2-(3-methylbut-2-en-1-yl)cyclohex-4-en-1-one (Figure 1).

In addition to the above-mentioned new phenol derivatives, eight known compounds including 3-(hydroxymethyl)-4-methoxy-2-(3-methylbut-3-en-1-yn-1-yl)phenol (**3**) [2], 4-methoxy-benzoic acid methyl ester (**4**) [11], niacinamide (**5**) [11], ergosterol peroxid (**6**) [11], (22*E*,24*R*)-ergosta-5,7,22-trien-3β-ol (**7**) [12], 3β,5α,6β-triol-ergosta-7,22-diene (**8**) [13], 3β,5α-diol-6β-methoxy-ergosta-7,22-diene (**9**) [14], and 3β,5α,9α-trihydroxy-ergosta-7,22-dien-6-one (**10**) [15] were also isolated and identified by comparing their NMR and MS data with those reported in the literatures.

## 3. Experimental

### 3.1 General experimental procedures

Optical rotations were measured on a Jasco DIP-370 digital polarimeter (JASCO, Tokyo, Japan). UV spectra were measured on a Shimadzu UV-2401PC spectrophotometer (Shimadzu, Tokyo, Japan), $\lambda_{max}$ (log $\epsilon$) in nm. IR spectra were scanned with Bruker Tensor-27 infrared spectrometer with a KBr disk (Bruker, Karlsruhe, Germany). NMR experiments were carried out on Bruker AM-400, Bruker DRX-500 NMR, Avance III 600, and Bruker AV-800 spectrometers (Bruker) with TMS as internal standard. ESI-MS and HR-EI-MS were recorded on a Finnigan LCQ Advantage mass spectrometer (Thermo, San Jose, CA, USA) and a VG Auto-Spec-3000 mass spectrometer (VG, Manchester, England). 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 Fine Chemicals, Uppsala, Sweden). Precoated silica gel GF254 plates (Qingdao Marine Chemical Factory) were used for thin-layer chromatography.

### 3.2 Fungal material

*S. hirsutum* FP-91666, stored in glycerol at −80°C in State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, was used to inoculate on YMG (yeast extract 4.0 g/l, malt extract 10.0 g/l,

glucose 4.0 g/l, agar 15.0 g/l, pH 7.3 before sterilization) media in plate at 26°C for 14 days to afford seed cultures. The *S. hirsutum* (15 l) was cultured on YMG dish at temperature of 26°C for 14 days.

### 3.3 Extraction and isolation

Solid fermentation products of *S. hirsutum* (15 l) was cut into small pieces and extracted exhaustively with mixture solution (EtOAc/MeOH/HAc, 80:15:5, v/v/v) by three times to obtain the rude extract. The extracts were dissolved in water, and extracted with EtOAc, and then with *n*-butanol three times, respectively. The extract of EtOAc section residue (6.85 g) was subjected to a column of silica gel G (200–300 mesh) using petroleum ether (PE)–EtOAc (10:1 → 6:4) and $CHCl_3$–MeOH (10:1 → 0:100) gradient solvent system to give 10 fractions (Fr. 1–Fr. 10). The fraction Fr. 1 (1.10 g) was placed in a column of silica gel (200–300 mesh) and eluted with PE–EtOAc (100:1 → 7:3) gradient solvent system to yield fractions of Fr. 1.1–Fr. 1.6. Fr. 1.2 (185 mg) was purified by silica gel (GF254) column using PE–acetone and Sephadex LH-20 (acetone) to produce compound **4** (56 mg). The fraction Fr. 3 (2.23 g) was subjected to a silica gel column (200–300 mesh) using PE–acetone (100:2 → 7:3) gradient solvent system to give fractions of Fr. 3.1–Fr. 3.6. Fr. 3.2 (399 mg) was purified with Sephadex LH-20 eluted with acetone to give compound **7** (33 mg). Fr. 3.6 (125 mg) was placed on a column of silica gel H using PE–acetone (20:1) isocratic solvent system to get compound **6** (2 mg). The fraction Fr. 4 (0.257 g) was subjected to a column of silica gel G (200–300 mesh) using PE–acetone (100:1 → 6:4) gradient solvent system to give fractions of Fr. 4.1–Fr. 4.7. Subsequently Fr. 4.3 (130 mg) was purified by silica gel column (200–300 mesh) with PE–EtOAc (25:1) isocratic solvent system to obtain compound **1** (3 mg). The Fr. 6 (0.32 g) was subjected to a column of silica gel (200–300 mesh) using PE–acetone (10:1 → 6:4) gradient solvent system to yield fractions of Fr. 6.1–Fr. 6.3. Fr. 6.3 (11 mg) was purified by Sephadex LH-20 ($CHCl_3$–MeOH; 1:1) and a column of silica gel (GF254) eluted by PE–acetone (20:1) gradient isocratic solvent system, to give compound **9** (2.8 mg). The fraction Fr. 7 (0.56 g) was placed to a column of silica gel (200–300 mesh) using PE–EtOAc (9:1 → 6:4) gradient solvent system to provide fractions of Fr. 7.1–Fr. 7.4. Fr. 7.2 (19 mg) was first subjected to Sephadex LH-20 ($CHCl_3$–MeOH; 1:1), and then purified by semi-preparative HPLC (LC3000 Semi-preparation Gradient HPLC System, Beijing, China) to yield compounds **2** (0.8 mg) and **3** (4 mg) (sample was performed in a RP-$C_{18}$ column (250 mm × 10 mm) at ambient temperature with a detection wavelength at 254 nm and a mobile phase of methanol/water (the water reduces from 50% to 0%) at a flow rate of 3 ml/min). Fr. 7.3 (22 mg) was purified by Sephadex LH-20 ($CHCl_3$–MeOH; 1:1), a column of silica gel (GF254) using PE–acetone (100:7) isocratic solvent system and Sephadex LH-20 (acetone) to provide compound **8** (2 mg). The fraction Fr. 8 (0.55 g) was placed on a column of silica gel (200–300 mesh) using PE–acetone (9:1 → 6:4) gradient solvent system to pick up fractions of Fr. 8.1–Fr. 8.6. Then Fr. 8.3 (21 mg) was purified by a column of silica gel H using ($CHCl_3$–MeOH; 30:1) isocratic solvent system to produce compound **10** (9 mg). Fr. 8.5 (10 mg) was subjected to Sephadex LH-20 $CHCl_3$–MeOH (1:1) and a column of silica gel (GF254) with $CHCl_3$–MeOH (30:1) isocratic solvent system to produce compound **5** (5 mg).

#### 3.3.1 *2-(3-Methyl-2-buten-1-yl)-4-methoxyethyl-phenol (**1**)*

Colorless amorphism, $[\alpha]_D^{22}$ −12.9 ($c = 0.70$, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$): 277 (3.17), 239 (3.04), 209

(2.85), 201 (2.85) nm; IR (KBr) $\nu_{max}$: 3440, 2923, 1621, 1111, 1036 $cm^{-1}$; $^{1}H$ and $^{13}C$ NMR spectral data see Table 1; EI-MS: *m/z* 220 $[M]^+$; HR-EI-MS: *m/z* 220.1456 $[M]^+$ (calcd for $C_{14}H_{20}O_2$, 220.1463).

### *3.3.2 3-Hydroxy-4-(hydroxymethyl)-6-(3-methylbut-2-en-1-yl)cyclohex-3-en-1-one (**2**)*

Colorless amorphism, $[\alpha]_D^{22}$ −6.6 ($c = 0.70$, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$): 202 (2.54) nm; $^{1}H$ and $^{13}C$ NMR spectral data see Table 1; EI-MS: *m/z* 210 $[M]^+$; HR-EI-MS: *m/z* 210.1250 $[M]^+$ (calcd for $C_{12}H_{18}O_3$, 210.1256).

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