Two new pyridine derivatives and two new furan derivatives from *Irpex lacteus*

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**ABSTRACT**

Two undescribed disubstituted pyridine derivatives irpexidines A and B (1 and 2) and two undescribed alkylfuran derivatives irpexins K and L (3 and 4) were isolated from fermentation broth of *Irpex lacteus*. Their structures were established by extensive spectroscopic methods. The pyridine derivatives from this fungus were reported for the first time. The new compounds were evaluated for their cytotoxicity against Hela cancer cell and inhibitory activity on NO production.

**1. Introduction**

*Irpex lacteus*, belonging to the family Polyporaceae, is a pathogenic wood-decaying fungus (C. Novotny et al. 2009). Its crude polysaccharide fraction has long been used as a traditional Chinese medicine for the treatment of chronic glomerulonephritis in clinic (Dong et al. 2017). Previous chemical investigations on this fungus have reported a series of secondary metabolites including furan derivatives (Mayer et al. 1996; Duan et al. 2019; Wang Meng et al. 2020), tremulane type sesquiterpenoids (Mayer et al. 1996; Ding et al. 2013; Chen et al. 2018; Ding et al. 2018; Zhou et al. 2018; Ding et al. 2018; Ding et al.)

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2019; Chen et al. 2020; Wang et al. 2020), triterpenoids (Tang et al. 2019; 2019), alkaloids (Sadahiro et al. 2020), and polysaccharides (Zhang et al. 2012). Many of them possessed modified carbon skeletons. For instance, irlactins A–D are four tremulane type sesquiterpenoids featuring a ring-rearranged 6/6 backbone (Ding et al. 2013), while the triterpene irpexolidal possessed a 6/5/6/5/6/5-fused polycyclic skeletal system (Tang et al. 2019). Pharmacological studies on these metabolites have demonstrated rich biological activities such as NO production inhibition (Tang et al. 2019; 2019; Wang et al. 2020), antibacterial and antioxidant activity (Duan et al. 2019), cytotoxicity (Wang et al. 2020), and immunosuppressive activity (Wang et al. 2020). As our long-term research of fungal natural products (Chen and Liu 2017), the chemical constituents of the fungus I. lacteus was further studies. Two new disubstituted pyridine derivatives irpexidines A and B (1 and 2) and two new furan derivatives irpexins K and L (3 and 4), along with two known furan derivatives 5 and 6, were isolated from fermentation broth of I. lacteus (Figure 1). The structures were established by HRESIMS and 1D and 2D NMR spectroscopic data. Compounds 1 and 2 are two pyridine derivatives reported from this fungus for the first time. All compounds were evaluated for their cytotoxicity to human Hela cancer cell line. Herein, the isolation, structural elucidation and biological activity of these isolates were reported.

2. Results and discussion

Compound 1 was isolated as a colorless oil. Its molecular formula C_{11}H_{13}NO_{4} was determined on the basis of positive high-resolution electrospray ionization mass spectrometry (HRESIMS) with a molecular ion [M + H]^{+} at m/z 224.09171 (calcd 224.09173), corresponding to six degrees of unsaturation. The UV spectrum, with the absorptions at 220 and 265 nm, suggested the presence of an aromatic moiety. The IR spectrum showed the presence of an ester group (1732 cm\(^{-1}\)) and pyridine (1638 and 1607 cm\(^{-1}\)) absorptions (Horiuch et al. 2006). Its \(^{1}\)H NMR spectra displayed signals for two methoxy groups (\(\delta_{H} 3.62 \text{ and } 3.95\)) and three aromatic protons at \(\delta_{H} 8.03\) (1H, d,
\( J = 1.7 \text{ Hz}, \text{H-5})\), \( \delta_H 8.53 \) (1H, d, \( J = 5.0 \text{ Hz}, \text{H-6})\) and \( \delta_H 7.51 \) (1H, dd, \( J = 5.0, 1.7 \text{ Hz}, \text{H-3})\) were readily identified. The examination of the \( ^{13}\text{C} \) and DEPT NMR spectra recorded in methanol-\( d_4 \) showed the presence of four quaternary carbon atoms [\( \delta_C 148.7 \) (C-2), \( 153.9 \) (C-4), 166.6 (C-7) and 174.3 (C-10)]. The above data, especially signals at \( \delta_C 128.8 \) (C-3), \( \delta_C 126.5 \) (C-5), \( \delta_C 150.5 \) (C-6) suggested the presence of a pyridine moiety, while the coupling constants of H-3, H-5, and H-6 as described above indicated a disubstituted pyridine moiety. In the HMBC spectrum, one correlation from \( \delta_H 3.95 \) (3H, s, OMe-C7) to C-7 built a methyl formate moiety, the correlation of H-3) with C-7 suggested the methyl formate moiety to be located at C-2. Additionally, HMBC correlations of \( \delta_H 3.62 \) (3H, s, OMe-C10) with C-10, of \( \delta_H 2.73 \) (2H, t, \( J = 7.4 \text{ Hz}, \text{H-9})\) and \( \delta_H 3.02 \) (2H, t, \( J = 7.4 \text{ Hz}, \text{H-8})\) with C-10, as well as correlations between H-9 and H-8 in \(^1\text{H}-^1\text{H} \text{ COSY spectrum}, \text{confirmed the presence of a methyl propionate moiety. The HMBC correlations of H-8 with C-4, C-5, and of H-3, H-5 with } \delta_C 30.9 \) (C-8) indicated the methyl propionate moiety to be located at C-4. Finally, compound 1 was characterized as irpexidine A.

Compound 2 was isolated as a colorless oil. Its molecular formula was identified as \( \text{C}_{14}\text{H}_{19}\text{NO}_4 \) by the HRESIMS analysis, corresponding to six degrees of unsaturation. Both 1D and 2D NMR data showed similar patterns to those of 1, except three additional methylenes presented in 2. Analysis of the data suggested that a \( n \)-butyl ester moiety in 2 replaced the methyl ester in 1 as supported by the \(^1\text{H}-^1\text{H} \text{ COSY data and HMBC correlations. Detailed analysis of 2 D NMR data suggested that the other parts of 2 were the same to those of 1. Therefore, compound 2 was characterized as irpexidine B.

Compound 3 was isolated as a colorless oil. Its positive ion HRESIMS displayed a peak for a protonated molecular ion at \( m/z \) 241.10699, corresponding to a molecular formula \( \text{C}_{12}\text{H}_{16}\text{O}_5 \) for compound 3. The UV spectrum, with the absorption 255 nm suggested the presence of a conjugated moiety. The IR absorption bands at 1526, 1647 and 2959 cm\(^{-1} \) revealed the presence of furan moiety and methyl. In the 1H NMR spectrum, two olefinic protons at \( \delta_H 7.05 \) (1H, d, \( J = 3.3 \text{ Hz}, \text{H-3})\) and 6.28 (1H, d, \( J = 3.3 \text{ Hz}, \text{H-4})\) and one triplet for a methyl at \( \delta_H 0.86 \) (3H, t, \( J = 7.4 \text{ Hz}, \text{H-4})\) were readily identified. The 13C NMR and DEPT data, in association with HSQC data, revealed twelve carbon resonances ascribable for one CH\(_3\), five CH\(_2\), three CH and three non-protonated carbons. Of them, signals at \( \delta_C 144.5 \) (C-2), \( \delta_C 118.5 \) (C-3), \( \delta_C 108.2 \) (C-4), \( \delta_C 158.5 \) (C-5) were suggested to establish a furan moiety conjugated to a carboxylic acid group at \( \delta_C 160.0 \) (C-9). Furthermore, the HMBC correlations from \( \delta_H 4.01 \) (2H, t, \( J = 6.6 \text{ Hz}, \text{H-1})\) to \( \delta_C 172.1 \) (C-8), \( \delta_H 2.67 \) (2H, t, \( J = 7.4 \text{ Hz}, \text{H-7})\) to C-5 and C-8, from \( \delta_H 2.92 \) (2H, t, \( J = 7.3 \text{ Hz}, \text{H-6})\) to C-8, and from H-4 to \( \delta_C 23.4 \) (C-6), as well as \(^1\text{H}-^1\text{H} \text{ COSY data, suggested the presence of a long linear side-chain in the structure, and the side-chain was attached at C-5 of the furan nucleus. Therefore, compound 3 was established as irpexin K.

Compound 4 was isolated as a colorless oil. Its molecular formula \( \text{C}_{13}\text{H}_{20}\text{O}_4 \) was determined on the basis of the HRESIMS at \( m/z \) 263.12531 [M + Na]\(^+\) (calcd for \( \text{C}_{13}\text{H}_{20}\text{O}_4\text{Na}\)\(^+\), 263.12538), corresponding to four degrees of unsaturation. In the \(^1\text{H} \text{ NMR spectrum, two methyl group } \delta_H 1.17 \) (3H, d, \( J = 6.1 \text{ Hz}, \text{H-13})\) and \( \delta_H 2.08 \) (3H, s, H-10), one methoxy group \( \delta_H 3.20 \) (3H, s, OMe-C12), as well as two olefinic protons at
\( \delta_H 6.22 (1\text{H, d, } J = 3.1\text{ Hz, H-3}) \) and \( \delta_H 5.98 (1\text{H, d, } J = 3.1\text{ Hz, H-4}) \) were readily identified. The \(^{13}\)C NMR and DEPT data revealed thirteen carbon resonances ascribable for one methoxy group, two CH₃, three CH₂, four CH, and three non-protonated carbons. Of them, signals at \( \delta_C 151.9 \) (C-2), \( \delta_C 111.2 \) (C-3), \( \delta_C 106.9 \) (C-4), \( \delta_C 156.7 \) (C-5) were suggested to establish a furan moiety, while the coupling constants of H-3 and H-4 as described above indicated that the furan moiety was disubstituted. In the \(^1\)H-\(^1\)H COSY spectrum, four fragments were revealed. In the HMBC spectrum, the correlation from \( \delta_H 2.58 \) (2H, t, \( J = 7.3\text{ Hz, H-6} \)) to C-4 and C-5, as well as correlations from \( \delta_H 3.91\text{-}3.94 \) (1H, m, H-11) to C-2 and C-3 suggested the furan moiety was 2,5-disubstituted. Additionally, correlation from H-10 to \( \delta_C 43.2 \) (C-8) and 211.5 (C-9) suggested the presence of a ketone group. Due to limit amount available and weak absorptions in CD spectrum, the stereochemistry of 4 couldn’t be established currently. Compound 4 was, finally, identified as irpexin L, as shown.

In addition to the four new compounds reported, two known metabolites including 5-carboxy-2-furanpropanoic (5) (Votocek 1939) and 5-(methoxycarbonyl)-2-furanpropanoic acid (6) (Ancliff et al. 2003) were obtained. Their structures were determined by comparison of their spectroscopic data with the reported literature values.

The new compounds were evaluated for their cytotoxicity against Hela cancer cell and their inhibitory activity on NO production using the methods reported recently (Dai et al. 2020; Wang et al. 2020). However, none of these compounds exhibited any activity.

3. Experimental section

3.1. General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter. IR spectra were obtained with a Tenor 27 spectrophotometer using KBr pellets. 1D and 2D spectra were run on a Bruker Avance III 600 MHz spectrometer with TMS as an internal standard. Chemical shifts (\( \delta \)) were expressed in ppm with reference to the solvent signals. Mass spectra were recorded on an Agilent 6200 Q-TOF MS system. Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, People’s Republic of China), RP-18 gel (20–45 \( \mu \)m, Fuji Silysia Chemical Ltd., Japan), and Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd., Sweden). Medium Pressure Liquid Chromatography (MPLC) was performed on a Biotage SP1 equipment, and columns packed with RP-18 gel. Preparative High Performance Liquid Chromatography (prep-HPLC) was performed on an Agilent 1260 liquid chromatography system equipped with Zorbax SB-C18 columns (5 \( \mu \)m, 9.4 mm \( \times \) 150 mm or 21.2 mm \( \times \) 150 mm) and a DAD detector. Fractions were monitored by TLC (GF 254, Qingdao Haiyang Chemical Co., Ltd. Qingdao), and spots were visualized by heating silica gel plates sprayed with 10% \( \text{H}_2\text{SO}_4 \) in EtOH.

3.2. Fungal material

The fruiting bodies of I. lacteus were collected from the Wang-Tian-Shu Scenic Area, Xi-Shuang-Ban-Na, Yunnan Province in July 2014, and authenticated by Prof.
Yu-Cheng Dai of Beijing Forestry University. A voucher specimen of *I. lacteus* mycelium was deposited at the Higher Fungi Chemistry Group of School of Pharmaceutical Sciences, South-Central University for Nationalities (No. HFG 201407-SCUN201804.2).

### 3.3. Fermentation condition

This strain was cultured on PDA medium for 8 days, and then was cut into small pieces to incubate on solid rice medium. The rice medium was inoculated in 500-mL Erlenmeyer flasks, each containing 100 g of rice medium and 100 mL of water. Flask cultures were put into a high pressure sterilizing pot and sterilized at 121 °C for 15 minutes. Then, the strain of *I. lacteus* was cultured in the rice medium, and two hundred 500-mL Erlenmeyer flasks were incubated fixedly at 25 °C for 30 days in dark place.

### 3.4. Extraction and isolation

The culture of *I. lacteus* in rice medium (20 kg) was extracted five times with acetone to give a crude extract, which was partitioned into water and EtOAc layers. The EtOAc layer (72 g) was subjected to CC over silica gel (80–100 mesh) eluted with a solvent system of CHCl₃/MeOH (from 1:0 to 0:1) to obtain nine fractions (A–I). Fraction F (8 g) was separated by MPLC over RP-18 silica gel eluted with MeOH/H₂O (from 5:95 to 100:0, v/v) to give five sub-fractions (F₁–F₅). Fraction F₂ was subjected to CC over silica gel (200–300 mesh) and Sephadex LH-20 (MeOH), and then purified by prep-HPLC (CH₃CN/H₂O from 5:90 to 30:70 in 30 min, v/v) to give 1 (12 mg, retention time (t_R) = 23.6 min). Fraction F₃ was subjected to CC over silica gel (200–300 mesh) and Sephadex LH-20 (MeOH), and then purified by prep-HPLC (CH₃CN/H₂O from 10:90 to 25:75 in 18 min, v/v) to give 5 (17.6 mg, t_R = 6.6 min) and 6 (2.2 mg, t_R = 13.5 min). Fraction F₄ was subjected to CC over silica gel (600–800 mesh) and then purified by prep-HPLC (CH₃CN/H₂O from 25:75 to 45:55 in 40 min, v/v) to give 2 (4.8 mg, t_R = 25.2 min) and 3 (5.5 mg, t_R = 5.6 min). Fraction F₅ was subjected to CC over silica gel (200–300 mesh) and Sephadex LH-20 (MeOH), and then purified by prep-HPLC (CH₃CN/H₂O from 15:85 to 30:70 in 25 min, v/v) to give 4 (3.8 mg, t_R = 18.5 min).

### 4. Conclusion

In conclusion, four new compounds including two alkaloids and two alkylfuran derivatives were isolated from the fungus *Irpex lacteus*. The structure was determined by analysis of their NMR and HRESIMS data. The new modification of the isolated compound expand the chemical diversity of natural products family.

### Disclosure statement

No potential conflict of interest was reported by the authors.
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