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Four new compounds from *Neoboletus magnificus*

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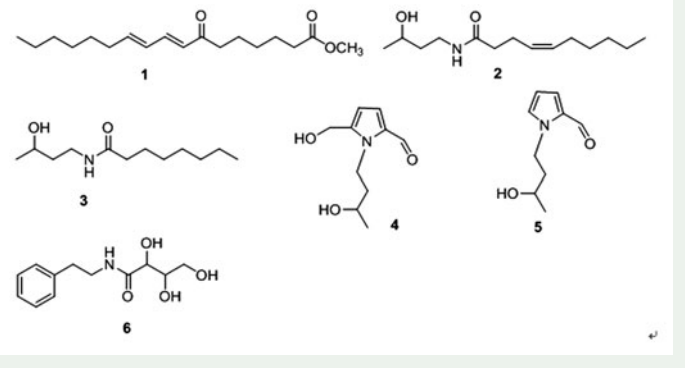
ABSTRACT

Four new compounds, compounds **1**, **2**, **4**, **6**, along with two known compounds **3**, **5**, were isolated from the methanol extract of the fruiting body of *Neoboletus magnificus*. The structures of compounds were elucidated by HRMS and NMR spectroscopic methods. The *in vitro* anti-inflammatory activity of the isolated compounds was evaluated.



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Boletaceae; neoboletus magnificus; pyrrole alkaloid; sphingolipid

1. Introduction

Boletales are of important source of medicinal mushroom resources, particularly in the *Boletaceae* family, which contains natural products with major representative such as butenolide (Duncan et al. 2003), sesquiterpene (Wada et al. 1995), steroid ingredients (Toi et al. 2007), nitrogen compounds (Kahner et al. 1998; Kawagishi et al. 2006), benzene derivatives (Song et al. 2009), sugar and fatty acids. Various natural compounds have wide ranges of bioactivities, such as anticancer, cytotoxicity, antibacterial activity and free radical scavenging ability (Takahashi et al. 1992; Yun et al. 2001; Bala et al. 2011; Kamo et al. 2004). Besides, (R)-3-hydroxybutanoyl-(R)-carnitine, Carnitine-esters from the mushroom *Suillus laricinus* promoted hyaluronan-degradation by human skin

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fibroblasts (Kawagishi et al. 2006). Two new peptides, tylopeptins A and B were isolated from the fruiting body of the mushroom *Tylopilus neofelleus*, which were shown to be active against some Gram-positive bacteria (Lee et al. 1999). In our continuing search for novel secondary metabolites with potential bioactivity from *Neoboletus magnificus,* four new compounds and two new natural compounds were obtained from this mushroom. In this manuscript, we describe the isolation, structural elucidation of these compounds.

2. Results and discussion

Compound **1** was isolated as a yellow oil. The molecular formula of compound **1** was determined to be $C_{18}H_{30}O_3$ by positive HR-EIMS (*m/z* 294.2203, calcd. 294.2195). The ^{13}C-NMR, DEPT, and HMQC spectra of compound **1** showed signals of two methyls, 10 methylenes, four methines, and two quaternary carbons including a ester bond (δ 176.0, C-1) and a ketone(δ 203.9, C-7), respectively. The presence of two conjugated *E, E*-form enone systems was revealed by the ^{1}H-NMR spectrum of **1** [δ 6.12 (1 H, d, $J = 15.6$ Hz, H-8), 7.23(1 H, dd, $J = 15.6$, 9.68 Hz, H-9), 6.27(2 H, ddd, $J = 21.44$, 15.2, 6.4, H-10, 11)]. Furthermore, ^{1}H–^{1}H COSY correlations from H-12 (δ_H 2.20) to H-11 (δ_H 6.25) and H-9 (δ_H 7.23) to H-10 (δ_H 6.27) revealed the presence of the C = C connected with one methylene (δ_C 34.1) and the C = C connected directly. This further proved the *trans*-geometry (*E*) of the double bond. The UV spectrum of **1** displayed a λ_{max} at 203 nm. The ^{1}H–NMR spectrum showed signals at 3.64 (3H), which can be attributed to the terminal methoxyl of aliphatic chains. HMBC correlations were observed at C-1/H-2, H-3, H-OCH_3; C-7/H-5, H-6, H-8, H-9. Further analysis of EI-MS data indicated that notable fragment ion peaks were observed at *m/z* 57, 71, 85 and 129. The locations of a carbonyl carbon (δ_C 203.9) at C-7 and a carbonyl carbon (δ_C 176.0) at C-1 were identified from the HMBC spectrum and EI-MS analysis. Based on the above data, compound **1** was determined to be (8*E*, 10*E*)-7-oxo-8, 10- heptadecadienoic acid.

Compound **2** was obtained as a yellow oil having a molecular formula of $C_{14}H_{27}NO_2$ deduced from the positive HR-ESIMS (*m/z* 264.1932 $[M + Na]^+$, calcd. 264.1934) and ^{13}C-NMR data, [α]25D-24.1(c 0.26, MeOH). IR absorption bands at 3314.79, 3096.87, 1646.39, 1555.88, 1383.65cm^{-1} indicated the occurrence of NH, OH and amide carbonyl groups. Its ^{1}H NMR data also displayed the diagnostic signals of the NH at δ_H 7.74 (br s) and the OH at δ_H 4.46 (d, $J = 4.8$ Hz), which was consistent with the carbon resonances at δ_C 171.62 (C-1), 63.81 (C-3′). The remaining protons were one olefinic signals at δ_H 5.29 (2 H, m, H-4, 5), two methyl signals at δ_H 1.02 (3 H, d, $J = 6.18$ Hz, H-4′), 0.84 (3 H, t, $J = 6.84$ Hz, H-10), eight methylenes at δ_H 3.06 (2 H, m, H-1′), 2.19 (2 H, dd, $J = 7.2$, 14.46 Hz, H-3), 2.05(2 H, t, $J = 7.74$ Hz, H-2), 1.97 (2 H, dd, $J = 6.9$, 13.92 Hz, H-6), 1.40 (2 H, m, H-2′), 1.23-1.26 (6 H, m, H-7, 8, 9), three methines at δ_H 5.29 (2 H, m, H-4, 5), 3.58(1 H, m, H-3′).

Its ^{13}C-NMR data revealed the presence of the C = C at δ_C 128.53(C-4), 130.28(C-5), supported by ^{1}H-NMR data δ_H 5.29 (2 H, m, H-4, 5). The ^{1}H–^{1}H COSY spectrum exhibited correlations from an amide proton (N-H, δ_H 7.74) to H-1′, from a hydroxy proton (O-H, δ_H 4.46) to H-3′,from H-4 to H-3, from H-5 to H-6. Additionally, HMBC correlations

between H-2 and H-3 to C-1, between H-2, H-3 and H-6 to C-4 confirmed a sphingolipid.

Moreover the *cis*-geometry (*Z*) of the double bond was determined from the δ value (23.27, 26.60) of the allylic carbon, since allylic carbon signals of *Z*- and *E*-isomers were observed at δ ca.27 and ca.32, respectively (Ishii et al. 2006). Hence, the structure of compound **2** was (*Z*)-N-(3-hydroxybutyl)-4- decene.

Compound **3**, a yellow oil was determined to have the molecular formula of $C_{12}H_{25}NO_2$ on the basis of positive HR-ESIMS (*m/z* 238.1775 $[M+Na]^+$, calcd. 238.1778) and ^{13}C NMR-data, [α]25D-22.1(c 0.24, MeOH). Analysis of its 1 D NMR data established the similar structure to compound **2**, especially when observing the ^{13}C-NMR data, compound **3** was less than a carbon carbon double bond compared to compound **2**. The HMBC correlations from C-1 to H-2 and H-3 and ^1H–^1H COSY correlations from an amide proton (N-H, δ_H 7.71) to H-1′, from H-2 to H-3 further indicated amide carbonyl bond between C-1′ and C-2. H-3′ gave ^1H–^1H COSY correlations to a hydroxy proton (O-H, δ_H 4.46, d, $J=4.62$ Hz), H-4′and H-2′, which C-2′ was connected to C-1′.Accordingly, the structure of compound **3** was concluded as N-(3-hydroxybutyl) octanamide.

Compound **4**, a yellow oil, was determined to have the molecular formula of $C_{10}H_{15}NO_3$ on the basis of positive HR-ESIMS (*m/z* 220.0945$[M+Na]^+$, calcd.220.0944) and ^{13}C-NMR data, [α]25D-28.8(c 0.15, MeOH). IR absorptions indicated the presence of OH (3414 cm^{-1}) and carbonyl groups (1656 cm^{-1}). The 1 D NMR and HMQC spectra of compound **4** showed three methines: C-3′(δ_C 63.65), C-3(δ_C 123.94), C-4 (δ_C 109.54). The carbon signals of C-2 (δ_C 131.45), C-3 (δ_C 123.94), C-4 (δ_C 109.54) and C-5 (δ_C 143.44) in the ^{13}C-NMR indicated a substituted pyrrole ring. This ^1H-NMR data showed two hydroxyls (O-H, δ_H 4.60, d, $J=4.7$ Hz; O-H, δ_H 5.30, brs) and an aldehyde hydrogen (CHO, δ_H 9.43, s). ^1H–^1H COSY correlations of H-1″/OH (δ_H 5.30), and the HMBC correlations from H-1′, H-3, H-4 and 2-CHO to the same carbon C-2, along with H-1″, H-1′, H-3 and H-4 to C-5 indicated that aldehyde group and C-1″ was respectively located C-2 and C-5. Meanwhile, which displayed C-1′ connected with the nitrogen atom of pyrrole ring. Additionally, OH (δ_H 4.60, d, $J=4.7$ Hz) was connected to C-3′ by ^1H–^1H COSY spectrum and HMBC correlations of H-4′, H-1′ and OH (δ_H 4.60) with C-2′. Thus, compound **4** was determined as 1-(3′-hydroxybutyl)-5-(hydroxymethyl)- 2-carbaldehyde-1H-pyrrole.

Compound **5** was isolated as a yellow oil having the molecular formula of $C_9H_{13}NO_2$ deduced from the positive HRESIMS ion at *m/z* 190.0830 $[M+Na]^+$ (calcd. 190.0838), [α]25D-26.1(c 0.28, MeOH). The NMR spectra of compound **5** obviously showed the presence of a substituted pyrrole ring. Careful comparison of the NMR data of compound **5** with those of compound **4** disclosed a less hydroxymethyl located in C-5 (δ_C 132.61). Therefore, the structure of compound **5** was established as 1-(3′-hydroxybutyl) -2-carbaldehyde-1H-pyrrole.

Compound **6** was obtained as a colorless oil, [α]25D2.6(c 0.13, MeOH). On the basis of positive HRESIMS ion at *m/z* 262.1050 $[M+Na]^+$ (calcd. for 262.1050), its molecular formula was $C_{12}H_{17}NO_4$. Its ^1H-NMR data displayed the phenyl group signals at δ_H 7.21 (3 H, m, H-3′, H-4′, H-5′), 7.29 (2 H, t, $J=7.68$ Hz, H-2′, H-6′), which was consistent with the carbon resonances at δ_C 126.16 (C-4′), 128.42 (C-3′, C-5′), 128.66 (C-2′, C-6′) and

139.50 (C-1′). Additionally, ^{1}H-NMR data indicated the diagnostic signals of NH at δ_H 7.80 (1 H, t, $J = 5.76$ Hz) and three hydroxyls at δ_H 4.45(t, $J = 5.52$ Hz), δ_H 4.80 (d, $J = 4.62$ Hz) and δ_H 5.58 (d, $J = 3.24$ Hz). ^{1}H–^{1}H COSY spectrum exhibited correlations from OH (δ_H 4.45) to H-7, from OH (δ_H 4.80) to H-6, from OH (δ_H 5.58) to H-5, from NH (δ_H 7.80) to H-2. Besides, HMBC correlations between H-2, H-5, H-6, NH (δ_H 7.80) and OH (δ_H 5.58) to C-4, between H-1 and H-2 to C-1′, between OH (δ_H 4.45), OH (δ_H 4.80), H-5 and H-8 to C-6 displayed the polyhydroxy-substituted amide long chain located in C-1′ of the benzene ring. Therefore, the structure of compound **6** was 2, 3, 4-trihydroxy-N-phenethylbutanamide.

3.1. General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter. IR spectra were obtained on a Tensor 27 spectrophotometer with KBr pellets. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. 1 D (^{1}H, ^{13}C, and DEPT) and 2 D (^{1}H-^{1}H COSY, HSQC, HMBC, and ROESY) NMR spectra were collected on a Bruker Avance III 500, 600 and 800 spectrometer. ESIMS and HRESIMS spectra were acquired on an Agilent G6230 spectrometer. EI and HREI spectra were acquired were obtained on Waters AutoSpec Premier P776.Semi-preparative HPLC separations were performed on an Agilent 1100 liquid chromatograph with a Waters X-Bridge Prep Shield RP18 (10×150 mm) column. Column chromatography (CC) was performed using silica gel (100–200 and 200–300 mesh, Qingdao Marine Chemical Co.Ltd., Qingdao, China) and Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden).Fractions were monitored by TLC (GF_{254}, Qingdao Marine Chemical Co. Ltd., Qingdao, China), and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in EtOH. All solvents were distilled prior to use.

3.2. Fungal materials

Fresh fruiting bodies of *Neoboletus magnificus* were collected from Yunnan Province, People's Republic of China, in July 2016. The fungal samples were identified by Research Fellow Zhu-Liang Yang from Kunming Institute of Botany, Chinese Academy of Science (CAS). A voucher specimen (KM20160702) was deposited in the school of Faculty of Life Science and Technology, Kunming University of Science and Technology, China.

3.3. Extraction and isolation

The fresh fruiting bodies (12 kg, wet weight) were cut into slices and extracted with 75% methanol (3×35 L) at room temperature for three times (30 d, 7 d, and then 7 d). Evaporation of the combined percolates under reduced pressure yielded a dark brown crude extract (300 g). The extract was then suspended in water and partitioned with EtOAc and n-Butanol, successively, yielding the fractions of EtOAc (19 g) and n-BuOH (119.1 g). The EtOAc extract was applied to an MCI gel column (eluted with

MeOH in water 10, 30, 40, 50, 60, 70, 80, 90 and 100%, successively) to afford 6 fractions (Fr.A-F) on the basis of TLC analysis. Fraction Fr C was subjected to a Sephadex LH-20 column (eluted with MeOH) to give subfractions Fr.C_1-Fr.C_3. Fr. C_2 (32 mg) was purified by semi-preparative RP-HPLC (ACN: H_2O, 42:58, v/v) to yield compounds **3** (1.1 mg, $t_R = 12.5$ min) and compound **2** (2 mg, $t_R = 22.8$ min). While Fr.F was chromatographed on a silica gel column (300–400 mesh) eluted with petroleum ether-acetone (100:1 to 20:1, v/v) to afford subfraction Fr.1- Fr.4. Compound **1** (1.3mg, $t_R = 40$ min) was further obtained from Fr.3 (17.3 mg) by using semi-preparative RP-HPLC (ACN: H_2O, 75:25, v/v).

The n-BuOH fraction was loaded on ODS CC with stepwise elution of MeOH-H_2O (10, 30, 50, 70 and 90%, successively) to get nine subfractions (Fr.G-O). Fr.K was separated repeatedly by CC over Sephadex LH-20, eluting with MeOH-H_2O (8:2, v/v) to give three subfractions (Fr.O_1-O_3). The subfraction Fr.O_2 (66.6 mg) was further purified by preparative HPLC with ACN: H_2O (14:86, v/v), to give compound **4** (7.0 mg, $t_R = 15.6$ min) and compound **6** (3.0mg, $t_R = 20$ min). Fr.M was chromatographed on a silica gel column (300–400 mesh) eluted with dichloromethane methanol (60:1, 30:1, 15:1, 10:1, v/v) to give four subfraction (Fr.M1- Fr.M4). Fr.M_1 (70 mg) was finely purified by semi-preparative HPLC with ACN: H_2O (11:89, v/v) to afford compound **5** (1.5mg, $t_R = 36$ min).

3.4. Anti-inflammatory activity assays

RAW 264.7 cells were used to evaluate the inhibitory activity toward NO production by LPS-activated macrophages (Kim et al. 2015; Baek et al. 2015). The RAW264.7 cells were seeded into 96-well plates, stimulated with 1 μg/ml LPS, and the test compound (final concentration 50 μM) was added. The drug-free group and L-NMMA-positive drug group were set as controls. After the cells were cultured overnight, the medium was assayed for NO production, and the absorbance was measured at 570 nm. MTS was added to the remaining medium for cell viability detection, eliminating the toxic effects of the compounds on the cells.

4. Conclusions

In this study, four new compounds, compounds **1**, **2**, **4**, **6**, along with two known compounds **3**, **5**, were isolated from the methanol extract of the fruiting body of *Neoboletus magnificus*. The structures of compounds were elucidated by extensive spectroscopic analyses including 1D- and 2D-NMR techniques.

Disclosure statement

No potential conflict of interest was by the authors.

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