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Polyellisin, a novel polyketide from cultures of the basidiomycete *Polyporus ellisii*†

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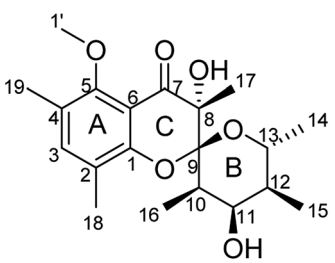
Polyellisin (**1**), an unprecedented polyketide possessing a tricyclic system sharing a spiroketal carbon, was isolated from cultures of the basidiomycete *Polyporus ellisii*. The structure with absolute configuration was elucidated by means of spectroscopic methods and the single crystal X-ray diffraction. Polyellisin showed NO production inhibition with an IC_{50} value of 17.2 μM.

Introduction

The basidiomycete *Polyporus ellisii*, belonging to the family Polyporaceae, is widely distributed in the Yunnan and Sichuan Provinces of China.[1] Its young fruiting body is used as a popular and delicious food in southwestern China, Japan and Korea. So far, the reports of chemical investigations on the species are not too many and have mainly been carried out by our research group. At first, a number of biologically active cerebrosides were isolated from its fruiting bodies.[2–4] After that, a number of ergosterols[5] and sesquiterpenoids[6] were obtained from cultures of this fungus in 2013. In our continuing search for structurally interesting and biologically active natural products from higher fungi,[5–12] an unprecedented polyketide, named polyellisin (**1**, Fig. 1), was isolated from cultures of the fungus *P. ellisii*. The structure was identified by means of spectroscopic methods, while its absolute configuration was determined by the single crystal X-ray diffraction. Polyellisin possesses a 6/6/6 tricyclic system sharing a spiroketal carbon. Its cytotoxicity against five human cancer cell lines and its ability to inhibit NO production were evaluated. Herein, the isolation, structural elucidation, and the biological activities of polyellisin are discussed.

Results and discussion

Polyellisin (**1**) had a molecular formula $C_{20}H_{28}O_6$ as determined on the basis of the positive HRESIMS, which showed a molecular ion peak at *m*/*z* 387.1779 (calcd for $C_{20}H_{28}O_6Na$, 387.1783), corresponding to seven degrees of unsaturation. The IR spectrum indicated the presence of hydroxy group (3438 cm^{-1}), carbonyl group (1708 cm^{-1}) and double-bonds (1623, 1593 cm^{-1}). The 1D NMR spectra, as well as the HSQC spectrum, revealed seven methyls (one methoxy), five methines, and eight quaternary carbons (Table 1). Of them, one olefinic proton at δ_H 7.18 (s, H-3), together with six olefinic carbon resonances indicated the presence of a five substituted benzene ring A (Fig. 1). The locations of substituent groups in the benzene ring were established by HMBC and ROESY spectra. In the ROESY spectrum, the olefinic proton at δ_H 7.18 (s, H-3) showed ROESY correlations to 2.21 (3H, s, Me-18) and δ_H 2.20 (3H, s, Me-19), indicating that the two methyls should be located at C-2 and C-4. While in the HMBC spectrum, the key HMBC correlation from H-3 to the oxygenated carbons at δ_C 153.0 (s) and 157.0 (s) revealed that these two olefinic carbons were assigned to C-1 and C-5. The carbonyl carbon at δ_C 191.8 (s) was identified to be connected to C-6 of the benzene ring. In the ^1H–^1H COSY spectrum, a partial moiety was established as shown in Fig. 2. In addition, a key HMBC correlation from δ_H 3.70 (1H, dq, J = 12.3, 6.2 Hz, H-13) to δ_C 107.8 (s, C-9) was detected. These data constructed a six-membered ether ring B (Fig. 2). Except the carbon resonances included in rings A and B, the rest including a carbonyl carbon at δ_C 191.8 (s, C-7), an sp^3 quaternary carbon at δ_C 76.2 (s, C-8), and a methyl carbon at δ_C 18.0 (q, C-17) are likely to build a six-membered ether ring C (Fig. 1), as deduced from the HMBC correlations from δ_H 1.44 (3H, s, Me-17) to C-7,



Polyellisin (**1**)

Fig. 1 Polyellisin (**1**) from cultures of the fungus *Polyporus ellisii*.

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Table 1 ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of **1**[a] in $CDCl_3$ (δ in ppm)

	δ_H (J in Hz)	δ_C, type
1		153.0, qC
2		120.5, qC
3	7.18 s	139.0, CH
4		125.2, qC
5		157.0, qC
6		112.0, qC
7		191.8, qC
8		76.2, qC
9		107.8, qC
10	2.30 qd (7.2, 3.1)	38.3, CH
11	3.58 br d (9.4)	74.9, CH
12	1.53 qd (7.0, 3.0)	42.0, CH
13	3.70 dq (12.3, 6.2)	68.0, CH
14	1.07 d (6.2)	18.9, CH_3
15	0.98 d (7.0)	13.8, CH_3
16	1.25 d (7.2)	13.2, CH_3
17	1.44 s	18.0, CH_3
18	2.21 s	15.8, CH_3
19	2.20 s	14.9, CH_3
OMe	3.76 s	61.0, CH_3

[a] Data were assigned by HSQC, HMBC, ^{1}H–^{1}H COSY and ROESY spectra.

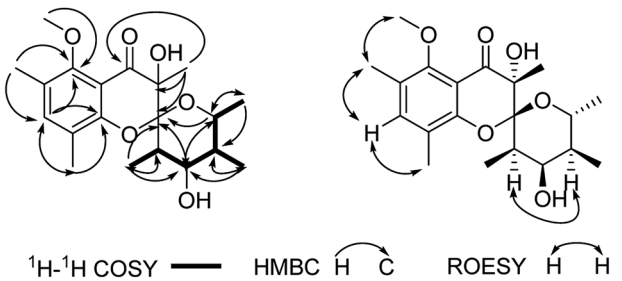

Fig. 2 Key 2D NMR correlations of **1**.

C-8, and C-9, as well as from the mass spectroscopic data. Therefore, the gross structure of **1** was established as depicted.

Since multiple chiral centers and adjacent quaternary carbons, the relative configuration of **1** could not be identified by the ROESY experiment. Only the ROESY correlation between H-10 and H-12 could indicated that the Me-15 and Me-16 were equatorial (Fig. 2). Fortunately, a single crystal X-ray diffraction not only confirmed the structure elucidation above but also established the absolute configuration as shown in Fig. 3.

Compound **1** was evaluated for its cytotoxicity against five human cancer cell lines, SK-BR-3 breast, SMMC-7721 hepatocellular carcinoma, HL-60 myeloid leukemia, PANC-1 pancreatic cancer and A-549 lung cancer, using the MTT method reported previously[13] with minor revision. Unfortunately, the results showed that **1** exhibited no significant inhibitory activities with IC_{50} values more than 40 μM. In addition, compound **1** was evaluated for its anti-inflammatory activity using the method of the NO production inhibition, as that we reported recently.[14] The result suggested that compound **1** possessed certain NO production inhibition with an IC_{50} value of 17.2 μM.

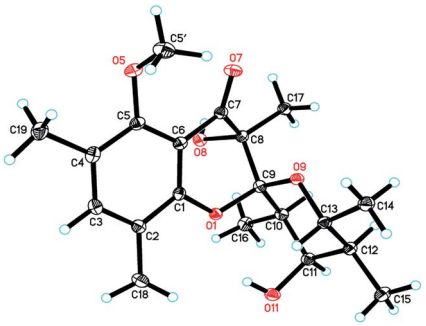

Fig. 3 ORTEP drawings of polyellisin (**1**).

Conclusions

In summary, the chemical investigation on the edible mushroom of *P. ellisii* was carried out, which resulted in the isolation of a novel polyketide, namely polyellisin (**1**). It possessed a tricyclic system sharing a spiroketal carbon, and the absolute configuration was determined by the single crystal X-ray diffraction. Polyellisin showed certain NO production inhibitory activity, suggesting a potential anti-inflammatory candidate.

Experimental section

General experimental procedures

The melting points were tested by a Putiantongchuang WRX-5A apparatus. Optical rotations were measured on a Jasco-P-1020 polarimeter. IR spectra were obtained by using a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were acquired with instrument of a Bruker DRX-500. HRESIMS was measured on an API QSTAR Pulsar spectrometer. Silica gel (200–300 mesh and 80–100 mesh, Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography (CC). Fractions were monitored by TLC (Qingdao Marine Chemical Inc., China) and spots were visualized by heating silica gel plates immersed in vanillin–H_2SO_4 in EtOH, in combination with Agilent 1200 series HPLC system (Eclipse XDB-C18 column, 5 μm, 4.6 × 150 mm). Preparative HPLC was performed on an Agilent 1100 series with a Zorbax SB-C18 (5 μm, 9.4 × 150 mm) column. MPLC was performed on Buchi apparatus equipped with Buchi fraction collector C-660, Buchi pump module C-605 and manager C-615.

Fungal material and cultivation conditions

Fruiting bodies of *P. ellisii* were collected at Jingdong, Yunnan Province, China in 2003 and identified by Prof. Zhu-Liang Yang (Kunming Institute of Botany). The voucher specimen (no. CGBWSHF00118) was deposited at herbarium of Kunming Institute of Botany. Culture medium was composed of glucose (5%), pork pepton (0.15%), yeast (0.5%), KH_2PO_4 (0.05%) and $MgSO_4$ (0.05%). Initial pH was adjusted to 6.0, the fermentation was first carried out on an Erlenmeyer flask for 6 days till the mycelium biomass reached to the maximum. Later

it was transferred to a fermentation tank (100 L) at 24 °C and 250 rpm for twenty days, ventilation was setted to 1.0 vvm (vvm: air volume/culture volume/min).

Extraction and isolation

The culture broth (80 L) was extracted four times with EtOAc. The organic layer was evaporated to give a crude extract (71 g). Then it was subjected to silica gel CC (200–300 mesh) eluted with petroleum ether (PE)–Me_2CO gradient system to afford fractions A–G. Fraction C, eluted with PE–Me_2CO (8/1), was separated by Sephadex LH-20 CC ($CHCl_3$–MeOH, 1/1), then applied to preparative MPLC with a reversed-phased C18 column (MeOH–H_2O, 50–100%) and preparative HPLC (MeCN–H_2O, 0–20%, 10 mL min^{-1}) to give **1** (4.8 mg).

Polyellisin (1). Colorless crystal ($CHCl_3$); mp 178 °C; $[\alpha]_D^{20}$ +105.3 (*c* 0.05 MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.61), 218 (3.70), 262 (3.16) nm; IR (KBr) ν_{max} 3483, 2972, 2932, 1708, 1623, 1593, 1474, 1381, 1298, 1090, 1025, 960 cm^{-1}; ^1H and ^{13}C NMR data (see Table 1); HRESIMS (pos.) *m*/*z* 387.1779 (calcd for $C_{20}H_{28}O_6Na$, 387.1783).

Crystallographic data of polyellisin (1). $C_{20}H_{28}O_6$, M = 364.42, orthorhombic, a = 7.6565 (2) Å, b = 9.8198 (3) Å, c = 24.8755 (7) Å, $\alpha = \beta = \gamma = 90.00°$, V = 1870.27 (9) $Å^3$, T = 100 (2) K, space group $P2_12_12_1$, Z = 4, μ(CuKα) = 0.779 mm^{-1}, 8819 reflections measured, 3262 independent reflections (R_{int} = 0.0387). The final R_1 values were 0.0311 ($I > 2\sigma(I)$). The final wR (F^2) values were 0.0790 ($I > 2\sigma(I)$). The final R_1 values were 0.0313 (all data). The final wR (F^2) values were 0.0792 (all data). The goodness of fit on F^2 was 1.059. Flack parameter = 0.07 (14). The Hooft parameter is 0.08 (6) for 1286 Bijvoet pairs. Crystallographic data for the structure of polyellisin (**1**) have been deposited with the Cambridge Crystallographic Data Centre (deposition no. CCDC 936912).

Cytotoxicity assay

All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates. Briefly, 100 μL adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with initial density of 1×10^5 cells per mL. Each tumor cell line was exposed to the test compound at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μM in triplicates for 48 h, with cisplatin (sigma, USA) as a positive control. After compound treatment, cell viability was detected and cell growth curve was graphed.

Anti-NO production assay

Murine monocytic RAW264.7 macrophages were dispensed into 96-well plates (2×10^5 cells per well) containing RPMI 1640 medium (Hyclone) with 10% FBS under a humidified atmosphere with 5% CO_2 at 37 °C. After 24 h of preincubation, cells were treated with serial dilutions of the test compounds, up to a maximum concentration of 25 μM (n = 2), in the presence of 1 μg mL^{-1} LPS for 18 h. The compounds were dissolved in DMSO and further diluted in medium to produce different concentrations. NO production in each well was assessed by adding 100 μL of Griess reagent (reagent A and reagent B, Sigma) to 100 μL of each supernatant from the LPS-treated or LPS- and compound-treated cells in triplicate. After 5 min incubation, the absorbance of samples was measured at 570 nm with a 2104 Envision Multilabel Plate Reader (Perkin-Elmer Life Sciences, Inc., Boston, MA, USA). MG-132 (Sigma-Aldrich, purity 98%) was used as a positive control (IC_{50} = 2.8 μM). Compound **1** (purity > 90%) were tested for inhibitory activity on NO production.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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