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Two New Alkaloids from *Pleurotus ostreatus* (Jacq. :Pers.) Roll

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Abstract: *Pleurotus ostreatus* (Jacq. :Pers.) Roll is a grey edible mushroom (*Agaricochaete*), containing a variety of vitamins, minerals and different kind of secondary metabolites. Two new terpenoid indole alkaloids (Terpendole N and Terpendole O) were isolated from the fermentation of *P. ostreatus*. Their structures were elucidated by 1D, 2D NMR, HR-ESI-MS, and ECD.

Keywords: *Pleurotus ostreatus* (Jacq. :Pers.) Roll; alkaloids; spectroscopic analyses.

1. Introduction

Higher fungi are commonly seen in our daily life because many of them are widely cultivated as edible fungi. It has been reported that higher fungi can produce many types of bioactive components, such as polysaccharides, terpenes, sterols, unsaturated fatty acids, minerals, and vitamins [1-5], and have various bioactivities, including anti-inflammatory, anti-tumor, cardiovascular preventive, anti-parasitic, anti-diabetic, anti-microbial, and anti-oxidative effects. Extensive attentions have been paid on higher fungi as it can produce novel structures and possess the potential to be developed as therapeutic products. For example, lentinan-the polysaccharide extracted from *Lentinula edodes* and *Coriolus versicolor*-has been marketed as an anti-tumor agent in Japan [6].

Edible fungi are widely distributed all over the world and have been used for thousands of years in China, in which there are 936 species, 23 varieties, 3 subspecies and 4 variants regarding the edible fungi [7].

Pleurotus ostreatus (Jacq. :Pers.) Roll is a edible fungus in *Agaricochaete*. As a common grey edible mushroom, it contains a variety of vitamins and minerals. Moderate intake of *P. Ostreatus* can regulate human metabolism, enhance physical fitness and immunity.

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In this paper, two new terpenoid indole alkaloids (terpendoles) were obtained by studying the chemical constituents of solid fermentation mycelium of *P. Ostreatus*, laying a foundation for understanding the material basis of its traditional medical usage.

2. Materials and Methods

2.1. Materials and Instruments

The NMR spectra were measured on a Bruker AV-400 NMR spectrometer with TMS as an internal standard (BRUCK USA Inc., Houston, TX). HR-ESI-MS data were measured on an Agilent 6530 Q-TOF LC-MS system (USA), Shimadazu LC-10A and Shimadazu LC-8A were the pressure pumps for the HPLC for analysis and preparative liquid chromatography with UV detector, respectively. The chromatographic column for HPLC is RP-18 (250 mm × 10 mm, 5 μm, YMC). Sample separation was performed on silica gel (200-300 mesh) (Marine Chemical Factory, Qingdao, China) and ODS (YMC Co., Ltd., Japan) chromatography. All the reagents were of analytical grade of HPLC grade and purchased from Yuwang Chemical Co. Ltd. (Shandong, China).

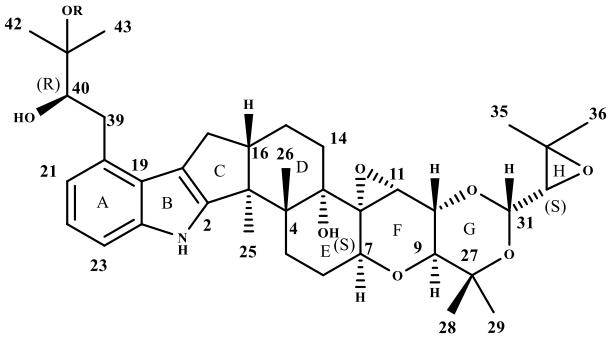
Pleurotus ostreatus (Jacq. :Pers.) Roll is provided by the Research institute of edible fungi of Shenyang Agricultural University. The strains are now preserved in the Wenzhou Medicual University.

2.2. Fermentation

The selected strains was put into the constant temperature incubator and cultivated in the dark for 10 days at 25 ℃until the whole plate was full. The 0.5 cm^2 mycelium of vigorous growth from the plate was selected and inoculated into a 500 mL conical flask filled with 120 mL seed culture fluid, and was subsequently cultured in a shaker at 25 ℃for 7 days. Each bottle of seed culture medium was put into 6 to 8 bottles of 500 mL conical flask containing rice culture medium, and was cultured at 25 ℃for 25 days.

2.2. Extraction and Isolation

The fermented product of *Pleurotus ostreatus* (Jacq. :Pers.) Roll mycelium was mashed and extracted by ethyl acetate for four times under the ultrasonic (1h for each time). The extract was concentrated in *vacuo* to dryness to yield obtain about 50 g of crude extract. The crude extract was separated by silica gel column chromatography and gradient eluted with dichloromethane-methanol (v/v, 100:0-0:100) to obtain thirteen main fractions. The second fraction (C:M, 60:1-40:1) was further separated by ODS chromatography to give five fraction. The fourth fraction (80% MeOH-H_2O) was subsequently purified by preparative HPLC eluted with MeOH-H_2O (76%, v/v) to yield compound **1** (13.5 mg) and **2** (11.2 mg).



1 R = H
2 R = OCH_3

Figure 1. Chemical structural of compounds **1-2**

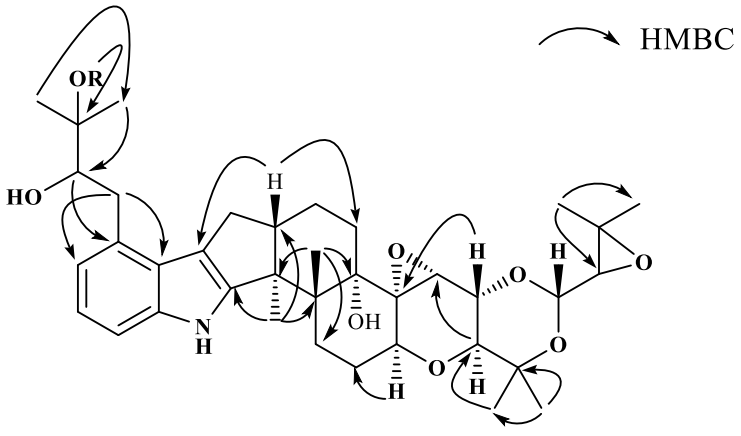
2.3 Spectroscopic Data

*Terpendole N (**1**):* White amporphous powder; $[\alpha]_D^{20}$ = -30.3 (*c* 0.5, MeOH); UV (MeOH): λ_{max} = 208, 229, and 283 nm; ^{1}H-NMR and ^{13}C-NMR (400/100 MHz, DMSO-d_6) see Table 1; HR-ESI-MS calcd for $C_{37}H_{51}NO_8Na$ $[M+Na]^+$ 660.3512, found 660.3509.

*Terpendole O (**2**):* White amporphous powder; $[\alpha]_D^{20}$ = -25.3 (*c* 0.5, MeOH); ^{1}H-NMR and ^{13}C-NMR (400/100 MHz, DMSO-d_6) see Table 1; HR-ESI-MS calcd for $C_{38}H_{53}NO_8Na$ $[M+Na]^+$ 674.3669, found 674.3661.

3. Results and Discussion

Compound **1** was obtained as a white amorphous powder. $[\alpha]_D^{20}$ -30.3 (*c* 0.5, MeOH). The molecular formula was determined as $C_{37}H_{51}NO_8$ based on the quasi-molecular peak given by HR-ESI-MS at *m/z* 660.3509 [*M* + Na]$^+$, suggesting that the unsaturation degree was 13. UV spectrum gave the absorption peaks at λ_{max} 208, 229, and 283 nm that are characteristic absorption peaks for indole rings. ^{1}H-NMR (400 MHz, DMSO- d_6) gave one active proton signal at δ_H 10.57 (1H, s, NH-1) and revealed the existence of one *ortho*-tri-substituted benzyl group by giving proton signals at δ_H 6.76 (1H, d, *J* = 7.0 Hz, H-21), 6.82 (1H, t, *J* = 7.8, 7.0 Hz, H-22), and 7.07 (1H, d, *J* = 7.8 Hz, H-23). Furthermore, proton signals at δ_H 1.24 (3H, s, H-35), 1.23 (3H, s, H-36), 1.19 (6H, s, H-28, 29), 1.16 (3H, s, H-25), 1.14 (3H, s, H-42), 1.12 (3H, s, H-43), and 1.04 (3H, s, H-26) indicated the presence of eight angular methyl groups. In the ^{13}C-NMR (100 MHz, DMSO- d_6) spectrum, all the data assignable to ring A to H were almost identical to those of the terpendole A and tolypocladin L [8, 9]. Thus, compound **1** was elucidated as a new terpendole bearing terpendole A as the main skeleton. Besides the NMR signals ascribable to the terpendole A moiety, one isopentyl group with two oxygen substituents was determined by the HMBC correlations from H-42 (δ_H 1.14) to C-43 (24.6), from H-43 (δ_H 1.12) to C-40 (δ_C 77.8) and C-41 (δ_C 71.9), and from H-40 (δ_H 3.42) to C-39 (δ_C 34.8) as shown in Figure 2. And the location of the isopentyl linked to C-20 of the terpendole A skeleton was determined based on HMBC correlations from H-40 (δ_H 3.42) to C-20 (δ_C 131.1), from H-39 (δ_H 2.53) to C-19 (δ_C 124.5), C-20 (δ_C 131.1), and C-21 (δ_C 119.6).



1 R = H
2 R= CH_3

Figure 2. Key HMBC correlations of compounds **1**-**2**

The relative configuration of **1** was established by NOESY spectrum analysis, which gave the correlations between H-16 and H-26, H-26 and H-10, H-10 and H-28, H-9 and H-7, H-25 and H-5. Thus relative configurations of ring C-G were established as shown in Figure 3. Due to that the stereo-

configurations for C-40, C-32, and C-33 could not be determined by NOESY method, the X-ray method was adopted to determine the absolute configuration of **1**. Therefore, the final structure of **1** was determined as shown in Figure 4 and named Terpendole N.

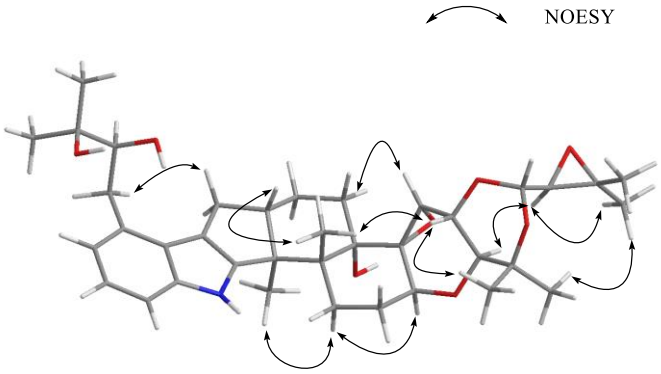


Figure 3. NOESY correlations of compounds **1**-**2**

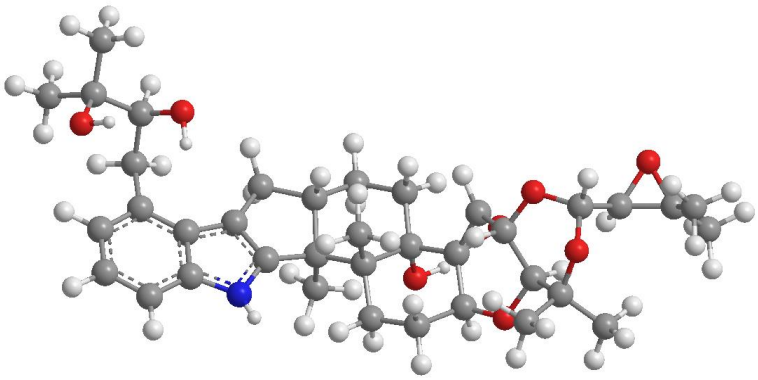


Figure 4. X-crystal results for compounds **1**

Compound **2** was also obtained as a white amorphous powder. $[\alpha]_D^{20}$ -25.3 (*c* 0.5, MeOH). The molecular formula was determined as $C_{38}H_{53}NO_8$ based on the quasi-molecular peak given by HR-ESI-MS at *m/z* 674.3661 $[M + Na]^+$, which means compound **2** possessed the same unsaturation degree as compound **1**. By comparing the ^{1}H-NMR NMR data to those of **1**, we found that **2** gave one extra methoxyl proton signal at δ_H 3.17 compared with **1**. Apart from the extra methoxyl group, signals for the eight angular methyls [δ_H 1.24 (3H, s, H-35), 1.23 (3H, s, H-36), 1.19 (6H, s, H-28, 29), 1.16 (3H, s, H-25), 1.12 (3H, s, H-42), 1.13 (3H, s, H-43), and 1.04 (3H, s, H-26)] were almost identical to those of **1**. In the ^{13}C-NMR spectrum, all the carbon signals of **2** were almost identical to those of **1**, except for one methoxyl group at δ_C 48.8, and slight differences at C-41, C-42, and C-43, which implied that **2** was the -OH (C-40)-methoxylated derivative of **1**. Furthermore, the HMBC correlation for the methoxyl group [from δ_H 3.17 with the corresponding δ_C 48.8 to δ_C 77.2 (C-41)] substantiated that the methoxyl group was link to C-40 hydroxyl. Thus, the planar structure of **2** was established as shown in Figure 1. Since the ^{13}C-NMR NMR data of **2** for ring A-H were identical to those of **1** (see Table 1), the relative configuration of **2** was considered to be the same as **1**, being further confirmed by NOESY correlations shown in Figure 3. Subsequently, the stereo-configuration of **2** was also determined to be identical to that of **1** since both **1** and **2** showed the same experimental CD curves around 250, 275, and 325 nm (Figure 5). Thus, the structure of **2** was determined as shown in Figure 1 and named Terpendole O.

Table 1. ^{1}H-NMR and ^{13}C-NMR data for compounds **1-2** (400 MHz in DMSO-d_6, δ in ppm, J in Hz)

Position	Compound 1		Compound 2	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
2	151.7	-	151.6	-
3	49.8	-	49.8	-
4	42.2	-	42.2	-
5a	25.5	1.70 (1H, m)	25.5	1.70 (1H, m)
5b		2.43 (1H, m)		2.43 (1H, m)
6a	28.4	1.64 (1H, m)	28.4	1.64 (1H, m)
6b		2.16 (1H, m)		2.16 (1H, m)
7	70.7	4.29 (1H, t, J = 9.0 Hz)	70.7	4.29 (1H, t, J = 9.0 Hz)
9	71.1	3.47 (1H, d, J = 10.0 Hz)	71.1	3.47 (1H, d, J = 10.0 Hz)
10	70.1	4.07 (1H, d, J = 10.0 Hz)	70.1	4.07 (1H, d, J = 10.0 Hz)
11	58.7	3.61 (1H, s)	58.7	3.61 (1H, s)
12	67.1	-	67.1	-
13	76.5	-	76.5	-
14	28.6	1.46-1.55 (2H, m)	28.6	1.46-1.55 (2H, m)
15a	20.4	1.48 (1H, m)	20.4	1.48 (1H, m)
15b		1.78 (1H, m)		1.78 (1H, m)
16	49.6	2.68 (1H, m)	49.6	2.68 (1H, m)
17a	28.9	2.41 (1H, m)	28.9	2.41 (1H, m)
17b		2.80 (1H, m)		2.80 (1H, m)
18	114.7	-	114.7	-
19	124.5	-	124.5	-
20	131.1	-	131.1	-
21	119.6	6.76 (1H, d, J = 7.0 Hz)	119.6	6.76 (1H, d, J = 7.0 Hz)
22	119.2	6.82 (1H, t, J = 7.8, 7.0 Hz)	119.2	6.82 (1H, t, J = 7.8, 7.0 Hz)
23	109.3	7.07 (1H, d, J = 7.8 Hz)	109.3	7.07 (1H, d, J = 7.8 Hz)
24	139.8	-	139.8	-
25	16.0	1.16 (3H, s)	16.0	1.16 (3H, s)
26	18.0	1.04 (3H, s)	18.0	1.04 (3H, s)
27	74.7	-	74.7	-
28	28.1	1.18 (3H, s)	28.1	1.18 (3H, s)
29	16.6	1.19 (3H, s)	16.6	1.19 (3H, s)
31	94.7	4.68 (1H, d, J = 6.7 Hz)	94.7	4.68 (1H, d, J = 6.7 Hz)
33	61.8	2.76 (1H, d, J = 6.7 Hz)	61.8	2.76 (1H, d, J = 6.7 Hz)
34	56.9	-	56.9	-
35	18.8	1.24 (3H, s)	18.8	1.24 (3H, s)
36	24.1	1.23 (3H, s)	24.0	1.23 (3H, s)
39a	34.8	2.53 (1H, m)	34.8	2.53 (1H, m)
39b		3.16 (1H, d, J = 10.5 Hz)		3.16 (1H, d, J = 10.5 Hz)
40	77.8	3.42 (1H, d, J = 10.5 Hz)	77.7	3.40 (1H, d, J = 10.5 Hz)
41	71.9	-	77.2	-
42	26.7	1.14 (3H, s)	19.3	1.12 (3H, s)
43	24.6	1.12 (3H, s)	21.8	1.13 (3H, s)
1-NH	-	10.58 (1H, s)	-	10.59 (1H, s)
13-OH	-	4.57 (1H, s)	-	4.56 (1H, s)
40-OH	-	3.60 (1H, s)	-	3.60 (1H, s)
41-OH	-	3.48 (1H, s)	-	3.48 (1H, s)
-OCH_3	-	-	48.8	3.17 (3H, s)

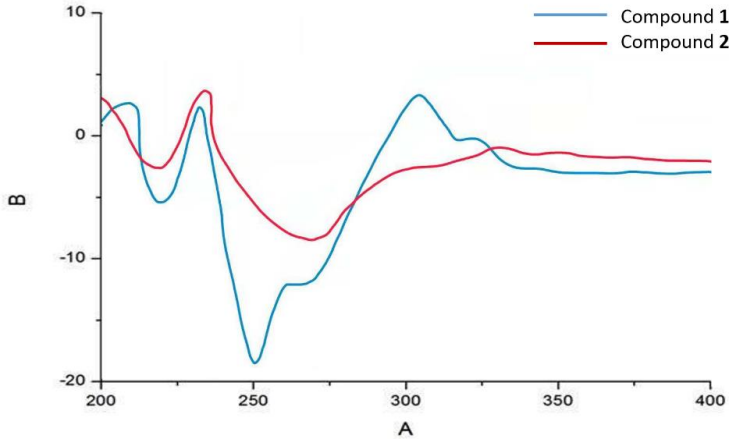


Figure 5. Experimental CD curves for compounds **1** and **2**

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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