CHEMICAL CONSTITUENTS FROM THE ASCOMYCETOUS FUNGUS *Tuber indicum*

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Truffles, also known as "black diamonds", are generally hypogeous or underground mushrooms which grow in symbiosis with certain trees. There are more than a hundred different kinds of truffles around the world, most of which grow in various parts of Europe, in particular in France. There are 25 species of the genus *Tuber* (Tuberaceae) in China. *Tuber indicum* Cooke et Massee is distributed mainly in the provinces of Yunnan and Sichuan of China [1].

The dried fruiting bodies of *T. indicum* were purchased from Yunnan Province in April 2000 and identified by Prof. P. G. Liu and X. H. Wang, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, P. R. China, where a voucher specimen (HKAS 22914) is deposited.

Extraction and Isolation. The dried and powdered fruiting bodies (4.7 kg) were extracted successively three times with $CHCl_3$ and four times with $CHCl_3$–MeOH (1:1) at room temperature, which were concentrated to dryness *in vacuo*, respectively, to give both $CHCl_3$ extracts (154 g) and $CHCl_3$–MeOH extracts (122 g). The $CHCl_3$ extract was subjected to silica gel CC using a gradient elution of petroleum ether–Me_2CO; the obtained fractions (19:1, 15:1, and 9:1) gave compounds **3** (185 mg) and **2** (36 mg), respectively. The $CHCl_3$–MeOH (1:1) extract was chromatographed over silica gel using $CHCl_3$ and increasing concentrations of MeOH in $CHCl_3$ as eluents. The resulting fractions (8:2, 7:3, 6:4) furnished compounds **1** (42 mg), **4** (30 mg), **6** (29 mg), and **7** (300 mg), respectively. The fraction containing **5** was chromatographed over a RP-8 column [eluted using MeOH–H_2O (50%), rate of flow: 1 mL/min, HPLC pump64], followed by gel permeation through Sephadex LH-20 in MeOH–H_2O (8:2) to afford **5** (41 mg).

5ξ-Methoxypyrrolidin-2-one was prepared by the method previously described.

We continue our further research into the bioactive constituents of the ascomycete *Tuber indicum* [1–4], from which seven compounds, 5ξ-hydroxypyrrolidin-2-one (**1**), ergosterol peroxide (**2**), ergosterol (**3**), uracil (**4**), adenosine (**5**), azelaic acid (**6**), and D-allitol (**7**), have been isolated and structurally elucidated. The *in vitro* inhibitory activity toward phospholipase A2 (PLA_2) was tested for these compounds.

Compound 1, yield 0.00089%, colorless crystals, mp 147–149°C, $[\alpha]_D^{25}$ ±0 (*c* 0.2, H_2O) [5]. The molecular formula $C_4H_7NO_2$ was determined by HR-EI-MS at *m/z* 101.0471 $[M]^+$ (calc. for $C_4H_7NO_2$, 101.0476). Its ^1H NMR spectrum showed the presence of an amide NH [δ 8.61 (1H, br.s)], a methine [δ 5.04 (1H, m, H-5)], a methylene [δ 2.18–2.30 (2H, m, H-3ax and H-3eq)], and the other methylene [δ 1.99–2.06 (1H, m, H-4ax), and δ 1.78–1.86 (1H, m, H-4eq)]. The ^{13}C NMR (DEPT) spectrum of **1** furnished one quaternary carbon, one methine, two methylenes, in which one quaternary carbon [δ 177.3] attributed to an amide carbonyl carbon and a methine [δ 82.6] assigned to a hemiaminoacetal carbon were recognized. The optical rotation of **1** in water indicated it to be racemic. Methylation of **1** gave a crystalline 5ξ-methoxypyrrolidin-2-one with mp 54–56°C [5], whose spectral data were in agreement with those in the literature [5]. Consequently, the above evidence established **1** as 5ξ-hydroxypyrrolidin-2-one. This substance was prepared as a racemate form [5], but it was isolated from a natural source for the first time. Although the natural (*R*)-5-hydroxypyrrolidin-2-one as an antibacterial agent was isolated previously from the leaves of *Hyptis verticillata* [6], there was no report in the literature concerning the isolation of this alkaloid from higher fungi previously.

Compound 2, yield 0.00076%, colorless crystal, mp 182–184°C, $[\alpha]_D$ –34° (*c* 0.6, $CHCl_3$) [7, 8], was identified as ergosterol peroxide by comparison of physicochemical data and spectral data with those in the literature [7, 9–11].

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Compound 3, yield 0.00393%, colorless crystal, mp 152–154°C, ergosterol [11, 12].

Compound 4, yield 0.00063%, white amorphous powder, mp>300°C, uracil [9, 12].

Compound 5, yield 0.00087%, white powder, mp 233–235°C, $[\alpha]_D^{25}$ –57.8° (*c* 0.6, H_2O) [9], which was characterized as adenosine [9, 12, 13].

Compound 6, yield 0.00061%, white crystal, mp 106–107°C, azelaic acid [14].

Compound 7, yield 0.00638%, colorless needles, mp 154.5–156°C, $[\alpha]_D^{25}$ ±0° (*c* 0.36, H_2O) [9], D-allitol [12].

Anti-phospholipase A2 (PLA_2) bioassay showed sterol **2** alone had *in vitro* selectively inhibitory activity against PLA_2 from *Crotalus adamenteus* venom with an ED_{50} value of 100 μg/mL, but inactive against *Apis mellifcra* bee venom PLA_2. In this study, **2** are structurally related fungal lanostane derivatives such as ganoderic acids isolated from *Ganoderma lucidum* [15], and pachymic acid and dehydrotumulosic acid from *Poria cocos* [16] that have previously been described as inhibitors of different forms of phospholipase A_2. The evidence appears to support further the anti-inflammatory effect of **2**. Of these isolates, others except for **3** were obtained for the first time from the genus *Tuber*.

General Experimental Procedures. Melting points were obtained on an XRC-1 apparatus and uncorrected. Optical rotations were measured on a Horiba SEPA-300 polarimeter. NMR spectra were recorded on Bruker AM-400 and Bruker DRX-500 instruments with TMS as an internal standard. MS were recorded on a VG Auto Spec-3000 mass spectrometer. IR spectra were obtained in KBr pellets with a Bio-Rad FTS-135 infrared spectrophotometer. CC was performed over silica gel (200–300 mesh), LiChroprep RP-8 gel (40–63 μm, Merck). TLC was carried out on plates precoated with RP-18 (Merck) and silica gel F_{254} (Qingdao Marine Chemical Ltd., P.R. China).

Anti-PLA_2 Assay. Inhibitory activity on phospholipase A_2 (PLA_2) test was performed as described previously [12, 17].

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