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N^2-(1-Methoxycarbonylethyl)guanosine, a new nucleoside coupled with an amino acid derivative from *Amanita exitialis*

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Abstract

A new purine nucleoside coupled with an amino acid derivative, N^2-(1-methoxycarbonylethyl)guanosine **1**, along with β-carboline and russulaceramide was isolated from the fruiting bodies of *Amanita exitialis*, a newly described poisonous mushroom. Its structure was elucidated by spectroscopic methods. This is the first report of naturally occurring nucleosides in which an α-amino acid derivative is bonded through its α-amino nitrogen to a nucleobase aglycone by a C–N bond. The new compound was found to be toxic in brine shrimp lethality test (BST).

Keywords: N^2-(1-Methoxycarbonylethyl)guanosine; Guanosine; Purine alkaloid; Nucleoside; *Amanita*; *Amanita exitialis*

Amanita exitialis is a fatal mushroom occurring in Guangzhou, Guangdong Province and originally described by Yang and Li in 2001 [1]. It has caused the death of a dozen of people since 2000. A previous HPLC analysis detected toxic cyclopeptides, amatoxins and phallotoxins, from the fruiting bodies of this mushroom [2]. For better understanding of its metabolites, we carried out a chemical investigation on this mushroom and isolated a new purine nucleoside, N^2-(1-methoxycarbonylethyl)guanosine **1**, along with β-carboline [3] and russulaceramide [4]. Herein, we report the isolation and structure elucidation of this new compound.

The fresh fruiting bodies of *A. exitialis* (13.8 kg), collected at South China Botanical Garden, Guangzhou, China, during May 2008, were extracted with 95% EtOH at room temperature. The extract was suspended in H_2O and then extracted successively with petroleum ether, EtOAc and n-BuOH. The n-BuOH-soluble fraction was subjected to silica gel column chromatograph (CC), eluted with $CHCl_3$–MeOH mixtures of increasing polarities, to yield six fractions (I–VI). Fraction III, obtained on elution with $CHCl_3$–MeOH (80:20), was further separated by Sephadex LH-20 CC using MeOH to give compound **1** (16 mg). The EtOAc-soluble fraction and the petroleum ether-soluble fraction were both separated by silica gel CC using $CHCl_3$ as mobile phase to afford β-carboline (10 mg) and russulaceramide (14 mg), respectively.

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Table 1
^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data of **1** in DMSO-d_6.

Position	^{1}H (J in Hz)	^{13}C
1	10.92 br s	
2		151.8
4		150.2
5		117.4
6		156.5
8	7.92 s	136.6
1′	5.63 d (5.6)	87.2
2′	4.48 t (5.6)	70.4
3′	4.05 m	73.0
4′	3.84 ddd (4.6, 3.5, 3.5)	85.3
5′a	3.59 m	61.6
5′b	3.50 m	
2-NH	7.25 br s	
1″		172.4
2″	4.40 m	49.2
3″	1.38 d (7.2)	17.4
OCH_3	3.66 s	52.1

Compound **1**, obtained as a yellowish amorphous solid, $[\alpha]_D^{25}$ + 14.3 (c 0.021, MeOH), had a molecular formula of $C_{14}H_{19}N_5O_7$ determined from the ion peak at m/z $[M+Na]^+$ 392.1177 (calcd for $C_{14}H_{19}N_5O_7Na$, 392.1182) in the HRESIMS together with the ^{13}C and DEPT NMR data. Its UV spectrum exhibited an absorption at λ_{max} (MeOH) 254 (log ε = 3.75) nm. The ^{1}H and ^{13}C NMR spectra (Table 1) in combination with the H^{1}–H^{1} COSY and HMQC experiments showed the presence of a β-ribofuranosyl group [δ_H 5.63 (d, 1H, J = 5.6 Hz, H-1′), 4.48 (t, 1H, J = 5.6 Hz, H-2′), 4.05 (m, 1H, H-3′), 3.84 (ddd, J = 4.6, 3.5, 3.5 Hz, H-4′), 3.59 (m, 1H, H-5′a), and 3.50 (m, 1H, H-5′b); δ_C 87.2 (C-1′), 70.4 (C-2′), 73.0 (C-3′), 85.3 (C-4′), and 61.6 (C-5′)] [5], an alanine residue [δ_H 4.40 (m, 1H, H-2″) and 1.38 (d, 3H, J = 7.2 Hz, H-3″); δ_C 172.4 (C-1″), 49.2 (C-2″), and 17.4 (C-3″)] [6], and a methoxy group [δ_H 3.66 (s, 3H); δ_C 52.1]. In addition, the spectra displayed the signals for an aromatic methine [δ_H 7.92 (s, 1H, H-8); δ_C 136.6 (C-8)], four aromatic quaternary carbons [δ_C 156.5 (C-6), 151.8 (C-2), 150.2 (C-4), and 117.4 (C-5)], and two NH groups [δ_H 10.92 (br s, 1H, H-1) and 7.25 (br s, 1H, 2-NH)], indicating the presence of a guanine residue [5]. The structural residues and functional groups noted above were assembled by the HMBC spectrum (key correlations depicted in Fig. 1). The HMBC correlations from H-8 to C-1′ and from H-1′ to C-4 and C-8 showed the β-ribofuranosyl group was located at N-9 to form a guanosine residue. The correlations between the methoxy protons and C-1″ and between H-2″ and C-2 indicated that the methoxy group was linked to C-1″ to form alanine methyl ester in which the N-atom of α-amino group was bonded to C-2 of the purine nucleus. Therefore, the structure of **1** was determined as N^2-(1-methoxycarbonylethyl)guanosine (Fig. 1).

The stereochemistry of C-2″ was not assigned due to limited amount of sample. To our knowledge, compound **1** is the first naturally occurring nucleoside coupled with an α-amino acid derivative, in which the α-amino acid derivative is bonded through its α-amino nitrogen to the nucleobase aglycone by a C–N bond. In brine shrimp lethality test (BST) [7,8], compound **1** exhibited toxicity against brine shrimp larvae with an LC_{50} value of 3.25 μg/mL, less toxic than squamocin (LC_{50} = 0.11 μg/mL), a known toxic natural compound [9]. This compound was also evaluated using MTT

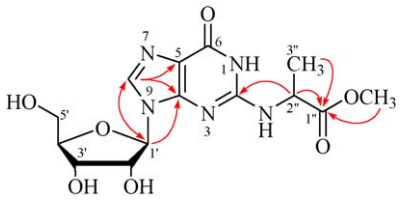

Fig. 1. Structure and key HMBC correlations of **1**.

method [10] for cytotoxicities against several human cancer cell lines including lung cancer (A549), pulmonary carcinoma (LAC), gastric carcinoma (SGC-7901), and hepatoma (HepG2) cell lines, but found to be inactive at 100 μg/mL.

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