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Chemical Constituents of *Tectus maximus* Koch, 1844

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Abstract: Two previously unreported compounds (**1** and **2**) together with eight known compounds (**3**-**10**) were isolated from the methanol extract of sea snail *Tectus maximus* Koch, 1844. Their chemical structures were determined to be 6-acetoxy-deoxyinosine (**1**), 6-acetoxy-inosine (**2**), deoxyinosine (**3**), inosine (**4**), adenosine (**5**), deoxyadenosine (**6**), deoxyuridine (**7**), thymidine (**8**), glycerol arachidonate (**9**), and arachidonic acid (**10**) on the basis of HR-ESI-MS and NMR spectroscopic analyses. This is the first report of those compounds from the genus *Tectus*.

Keywords: Tegulidae; *Tectus maximus*; 6-acetoxy-deoxyinosine; 6-acetoxy-inosine.

1. Introduction

Tectus maximus is a species of sea snail, known as marine gastropod mollusk, in the family Tegulidae. The *Tectus* species has been found in the South Pacific islands and used to make traditional ornaments by indigenous people. Most of *Tectus* species are edible and very nutritious, however, some of them are poisonous foods [1, 2]. Up to date, there have been no studies on the chemical compositions and bioactivity of *Tectus maximus*. Continuing our program to study Vietnamese marine organisms, now we report the isolation and determination of two previously unreported compounds (**1** and **2**) together with eight known compounds (**3**-**10**) from the methanolic extract of the sea snail *T. maximus*.

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2. Materials and Methods

2.1. General

The HR-ESI-MS was performed using an Agilent 6530 Accurate Mass Q-TOF LC/MS system. NMR spectra were obtained on a Bruker AM600 spectrometer using TMS as an internal standard. Column chromatography was carried out using silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck, Darmstadt, Germany). Thin layer chromatography was used pre-coated silica gel 60 F_{254} plates (Merck, Darmstadt, Germany). Semi-preparative HPLC was acquired on an Agilent 1100 system (Agilent technologies, Santa Clara, CA, USA), using J'sphere ODS-H80 semi-preparative column (20×250 mm, YMC, Kyoto, Japan).

2.2. Material

Snail samples of *Tectus maximus* Koch, 1844 were collected from Truong Sa archipelago, Vietnam in June 2021 (GPS coordinates: 8°38'39"N 111°55'21"E, 8°51'53"N 112°15'27"E, 8°58'30"N 113°42'23"E, 7°53'34"N 112°55'18"E, 11°25'37"N 114°19'40"E, and 10°22'30.9"N 114°28'55.4"E) and taxonomically identified by Dr Nguyen Thi Bich Nga, at the Institute of Biotechnology, VAST. Voucher specimens (TS07-04) have been deposited at Vietnam - Russia Tropical Center, Vietnam.

2.3. Extraction and Isolation

The samples of *T. maximus* (1.5 kg) was defrosted, then they were extracted with MeOH for three times in an ultrasonic bath (each: 6 L MeOH, 40 ^oC, 3hrs) and 70.8 g of the extract obtained. The extract was well mixed with water (2.0 L) and separated with CH_2Cl_2 (2.0 L) to obtained CH_2Cl_2 extract (TD1, 5.1 g). TD1 (5.0 g) was loaded on a silica gel column and eluted with a gradient solvent system of CH_2Cl_2 in MeOH (1/0, 40/1, 20/1, 0/1) to get four fractions, TD1A-TD1D. The fraction TD1B (852 mg) was purified on a semi-preparative HPLC, eluting with 95% ACN in H_2O to give **9** (5.5 mg, t_R 27.8). The fraction TD1C (905 mg) was chromatographed on a RP-18 column, eluting with acetone/water (2/1) to give 4 fractions, TD1C1-TD1C4. The fraction TD1C1 (550 mg) was chromatographed on a silica gel column using dichloromethane/acetone/water (1/3.5/0.2) as the eluent to get 5 fractions, TD1C1A-TD1C1E. The fraction TD1C1C was purified on the HPLC, eluting with 10% ACN to obtain **7** (5.2 mg, t_R 29.6) and **8** (5.5 mg, t_R 39.5). The fraction TD1C1D (75 mg) was purified on the HPLC, eluting with 15% MeOH in H_2O to obtain **6** (8.0 mg, t_R 62.3) and **5** (12.4 mg, t_R 63.5). The fraction TD1C1E (250 mg) was chromatographed on a silica gel column eluting with dichloromethane/acetone/water (1/3.5/0.2) to get 4 fractions, TD1C1E1- TD1C1E4. The fraction TD1C1E1 was purified on the HPLC, eluting with 10% ACN to obtain **2** (4.0 mg, t_R 33.4), and **1** (4.5 mg, t_R 39.2). TD1C1E3 (60.0 mg) was purified on the HPLC, eluting with 5% ACN to obtain **3** (3.5 mg, t_R 29.6). TD1C3 (102 mg) was purified on the HPLC, eluting with 95% ACN to obtain **10** (5.0 mg, t_R 44.8). The fraction TD1D (1.2 g) was chromatographed on a RP-18 column, eluting with acetone/water (2/1) to give 4 fractions, TD1D1- TD1D4. TD1D3 (58.2 mg) was purified on the HPLC, eluting with 5% ACN to obtain **4** (5.2 mg, t_R 29.4)

*6-Acetoxy-deoxyinosine (**1**):* A dark yellow solid; HR-ESI-MS *m/z* 295.1035 $[M + H]^+$ (calcd. for $C_{12}H_{15}N_4O_5$, 295.1037); ^1H NMR (DMSO-d_6, 600 MHz) δ (ppm): 7.89 (1H, s, H-2), 8.05 (1H, s, H-8), 2.09 (3H, s, $COCH_3$), 6.26 (1H, dd, J = 8.4, 6.0 Hz, H-1′), 2.19 (1H, ddd, J = 10.4, 6.0, 2.4 Hz, H_a-2′), 2.69 (1H, ddd, J = 10.4, 8.4, 5.4 Hz, H_b-2′), 4.38 (1H, ddd, J = 5.4, 2.4, 2.4 Hz, H-3′), 3.89 (1H, m, H-4′), 3.61 (1H, dd, 12.0, 4.2 Hz, H_a-5′), 3.51 (1H, dd, 12.0, 3.6 Hz, H_b-5′). ^{13}C NMR (DMSO-d_6, 150 MHz) δ (ppm) data are shown in Table 1.

*6-Acetoxy-inosine (**2**)*: A dark yellow solid; HR-ESI-MS *m/z* 311.0982 $[M + H]^+$ (calcd. for $C_{12}H_{15}N_4O_6$, 311.0986); ^1H NMR (DMSO-d_6, 600 MHz) δ (ppm): 7.90 (1H, s, H-2), 8.05 (1H, s, H-8), 2.09 (3H, s, $COCH_3$), 5.78 (1H, d, J = 6.6 Hz, H-1′), 4.59 (1H, dd, J = 6.6, 5.4 Hz, H-2′), 4.11 (1H, dd, J = 5.4, 4.30 Hz, H-3′), 3.59 (1H, m, H-4′), 3.65 (1H, dd, 12.0, 3.0 Hz, H_a-5′), 3.53 (1H, dd, 12.0, 3.0 Hz, H_b-5′). ^{13}C NMR (DMSO-d_6, 150 MHz) δ (ppm) data are shown in Table 1.

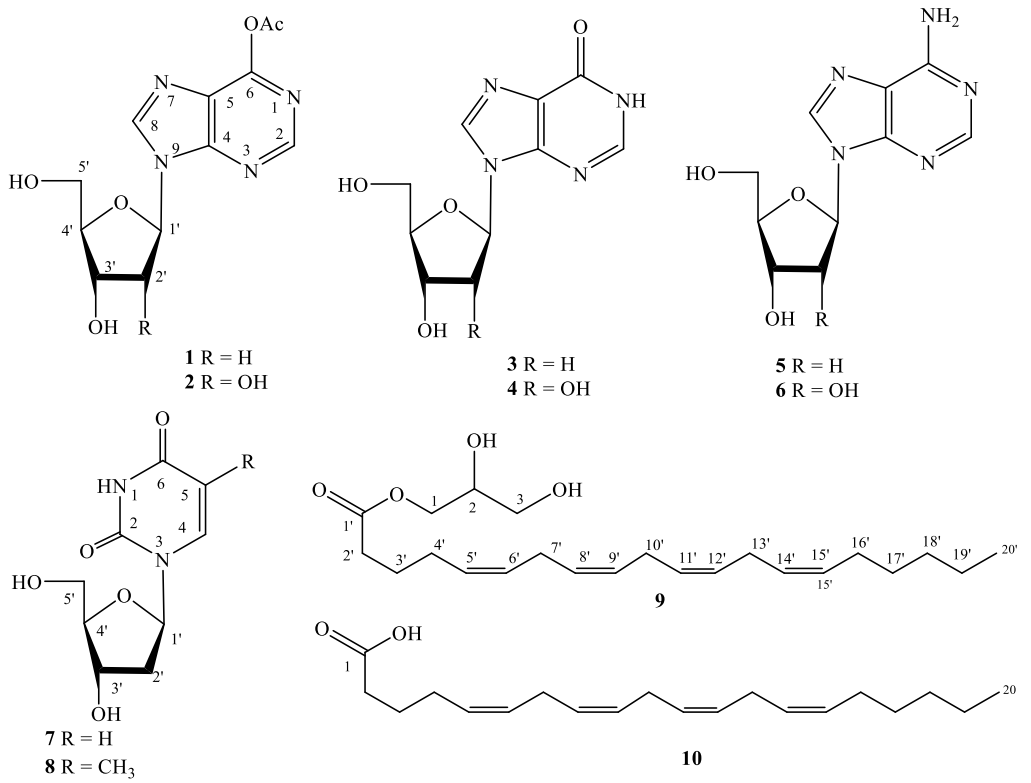

Figure 1. The chemical structures of compounds **1**-**10**

3. Results and Discussion

3.1. Structure Elucidation

Compounds **1**-**10** were isolated from the methanol extract of *T. maximus* by using combined various chromatographic methods. The known compounds were identified as deoxyinosine (**3**), inosine (**4**) [3, 4], adenosine (**5**), deoxyadenosine (**6**) [5], deoxyuridine (**7**) [4], thymidine (**8**) [6], glycerol arachidonate (**9**) [7], and arachidonic acid (**10**) [7] (Figure 1) by comparing their 1D and 2D NMR data and mass spectral data with those reported in the literature (Supplemental Figures S17-S40). To the best of our knowledge, the compounds were firstly isolated from the genus *Tectus*.

The molecular formula of **1** was determined to be $C_{12}H_{14}N_4O_5$ by the HR-ESI-MS (*m/z* 295.1035 $[M + H]^+$, calcd. for $C_{12}H_{15}N_4O_5$, 295.1037). Similar to compound **3**, the ^{1}H NMR spectrum of **1** showed two singlets of two olefinic protons bearing nitrogen atoms at δ_H 7.89 (H-2) and 8.05 (H-8), signals of a deoxyribose moiety at δ_H 6.26 (dd, 8.4, 6.0 Hz, H-1′), 2.19 and 2.69 (H-2′), 4.38 (H-3′), 3.89 (H-4′), 3.61 and 3.51 (H-5′) [8, 9]; and additional methyl protons of an acetoxy group at 2.09 (3H, s). The ^{13}C NMR spectrum of **1** exhibited three quaternary carbons at 163.5 (C-6), 148.5 (C-4), 124.7 (C-5) and two olefinic methine carbons at δ_C 150.2 (C-2) and 137.2 (C-8) assigning for the hypoxanthine unit, five carbons of the deoxyribose at δ_C 88.1, 84.3, 71.2 (3 x CH), 62.1 and 40.4 (CH_2), and signals at δ_C 167.7 and 18.7 of an acetoxy group [8, 9]. In the HSQC spectrum, protons at δ_H 6.26, 2.19/2.69, 4.38, 3.89, 3.61/3.51 showed the cross peaks with carbons at δ_C 84.3, 40.4, 71.2, 88.1, and 62.1, respectively. In addition, the ^{1}H-^{1}H COSY correlations of H-1′/H-2′/H-3′/H-4′/H-5′ were observed (Figure 2). In the NOESY spectrum, H-3′ (δ_H 4.38) exhibited cross peaks with H-5′ (δ_H 3.61 and 3.51) indicating that these protons were in the same side of the molecule as showing in the deoxyribofuranosyl group (Figure 3) which indicated the occurrence of a 2-deoxyribose moiety in the molecule (Figure 2) [8, 9]. The HMBC correlations from H-1′ (δ_H 6.26) to C-4 (δ_C 148.5) and C-8 (δ_C 137.2) showed that the sugar moiety linked

to hypoxanthine unit similar to **3**. Furthermore, NOESY correlations between H-8 (δ_H 8.05) and H-3′ (δ_H 4.38)/ H_b-2′ (δ_H 2.69) indicated that for *β*-deoxyribofuranosyl group (Figure 3). The presence of *β*-deoxyribofuranosyl group was also demonstrated by splitting pattern of H-1′ (dd, *J* = 8.4 and 6.0 Hz) in comparison with that reported in the literature [*β*-deoxyribofuranosyl: H-1′ (dd, *J* = 8.0 and 6.0 Hz) and *β*-deoxyribofuranosyl: H-1′ (dd, *J* = 8.0 and 3.1 Hz)] [7]. The down field chemical shift of C-6 (163.5) of **1** compared to that of deoxyinosine (δ_C 156.7) moiety of **3** together with the above HR-ESI-MS result suggested that the acetoxy group linked to C-6 in the structure [3,4]. Thus, the compound **1** was indicated to be 6-acetoxydeoxyinosine, a previously unreported compound (Supplemental Figures S1-S8).

Table 1. ^{13}C NMR data for compounds **1-6**

Position	1	2	3	4	5	6
2	150.2 (CH)	150.1 (CH)	145.9 (CH)	145.9 (CH)	152.4 (CH)	152.4 (CH)
4	148.5 (C)	148.6 (C)	147.8 (C)	148.2 (C)	148.9 (C)	149.1 (C)
5	124.7 (C)	124.8 (C)	124.4 (C)	124.4 (C)	119.3 (C)	119.4 (C)
6	163.5 (C)	163.4 (C)	156.7 (C)	156.6 (C)	156.1 (C)	156.2 (C)
8	137.2 (CH)	137.7 (CH)	138.4 (CH)	138.7 (CH)	139.5 (CH)	139.9 (CH)
$\underline{C}OCH_3$	167.7 (C)	167.8 (C)	-	-	-	-
$CO\underline{C}H_3$	18.7 (CH_3)	18.7 (CH_3)	-	-	-	-
1′	84.3 (CH)	88.3 (CH)	83.6 (CH)	87.5 (CH)	83.9 (CH)	87.9 (CH)
2′	40.4 (CH_2)	73.4 (CH)	39.4 (CH_2)	74.1 (CH)	39.4 (CH_2)	73.4 (CH_2)
3′	71.2 (CH)	70.9 (CH)	70.6 (CH)	70.3 (CH)	71.0 (CH)	70.7 (CH)
4′	88.1 (CH)	86.1 (CH)	87.9 (CH)	85.6 (CH)	88.0 (CH)	85.9 (CH)
5′	62.1 (CH_2)	61.9 (CH_2)	61.6 (CH_2)	61.3 (CH_2)	61.9 (CH_2)	61.7 (CH_2)

The ^{1}H NMR of the compound **2** showed two singlets at δ_H 7.90 (H-2) and 8.05 (H-8), one acetoxy methyl group at δ_H 2.09, and sugar moiety signals. In the HSQC spectrum, the cross peaks of δ_H 5.78 (H-1′)/ δ_C 88.3 (C-1′), δ_H 4.59 (H-2′)/ δ_C 73.4 (C-2′), δ_H 4.11 (H-3′)/ δ_C 70.9 (C-3′), δ_H 3.59 (H-4′)/ δ_C 86.1 (C-4′), δ_H 3.53 and 3.65 (H-5′)/ δ_C 61.9 (C-5′) were observed. In addition, the ^{1}H-^{1}H COSY cross peaks of H-1′/ H-2′/ H-3′/ H-4′/ H-5′ were clearly observed and, that suggested the sugar moiety of the compound **2** was ribose. Those data suggested that the C-2′ methylene signals in compound **1** should be replaced by a methine carbinol signals (δ_C 73.4/δ_H 4.59) in **2** (Table 1). The NOESY cross peaks from H-5′ (δ_H 3.53 and 3.65) to H-3′ (4.11) and from H-5′ to H-2′ (δ_H 4.59) suggested these protons were located at the same side of the molecule as showing in the ribofuranosyl group (Figure 3). The aglycon of the compound **2** was confirmed as very similar to compound **1** by comparison of the NMR data of both compounds (Table 1) and, these similarities were also confirmed in the HMBC spectrum as shown in Figure 2. The HR-ESI-MS of the compound **2** exhibited a quasi-molecular ion peak at *m/z* 311.0982 [M + H]$^+$ (calcd. for $C_{12}H_{15}N_4O_6$, 311.0986), indicated its molecular formula is $C_{12}H_{14}H_4O_6$. Moreover, the HMBC correlations of H-1′ (δ_H 5.78) to C-4 (δ_C 148.6) and C-8 (δ_C 137.7) confirmed the sugar linked to N-9 position. Different from the compound **1**, the *α*- and *β*-ribofuranosyl groups is not distinguished by value of $J_{H\text{-}1'/H\text{-}2'}$ coupling constant, however, carbon chemical shift of C-1′ (δ_C 88.3) is strongly indicated the presence of *β*-ribofuranosyl group (*β*-ribofuranosyl group: $\delta_{C\text{-}1'}$ 87.9; and β-ribofuranosyl group: $\delta_{C\text{-}1'}$ 83.3 [7]). Additionally, the NOESY correlations between H-8 (δ_H 8.05) and H-3′ (δ_H 4.11)/ H-2′ (δ_H 4.59) were also indicated for *β*-ribofuranosyl group in the molecule. The C-6 down field shift (δ_C 163.4) in the compound **2** compared to that of inosine (δ_C 157.2 [5] or 158.39 [6]) suggested the acetoxy group attached to C-6 as in the compound **1**. Thus, chemical structure of compound **2** was elucidated as 6-acetoxyinosine, a previously unreported compound (Supplemental Figures S9-S16).

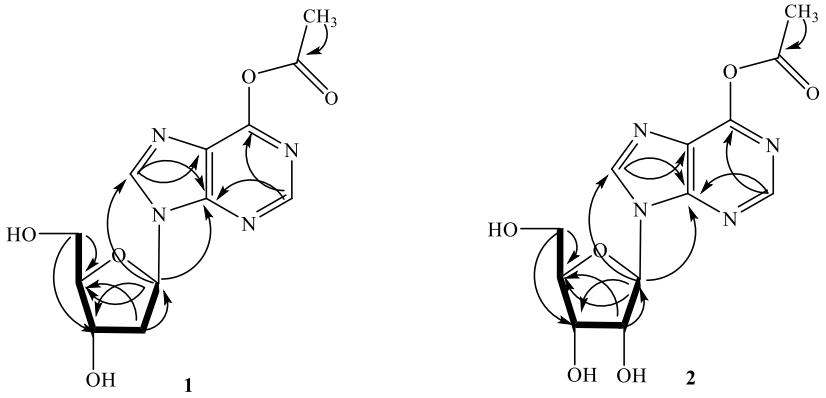

Figure 2. Key ^{1}H-^{1}H COSY (H—H) and HMBC (H→C) correlations for compounds **1** and **2**

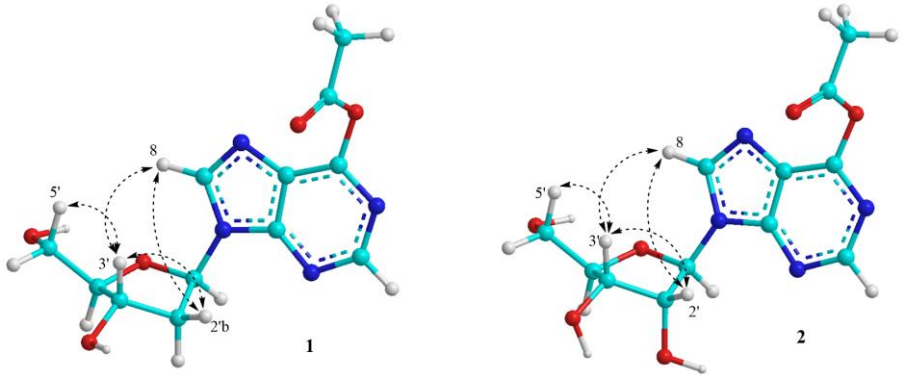

Figure 3. Important NOESY correlations for compounds **1** and **2**

The ^{13}C NMR data of compounds **1**-**6** were shown in Table 1 and they consist of a shared purine core. Compounds **1**, **3**, and **5** contained *β*-deoxyribofuranosyl group meanwhile compounds **2**, **4**, and **6** contained *β*-ribofuranosyl group. The related spectra and data NMR data of compounds **3**-**10** were given in supporting information part of the article (Figures S17-S40).

3.2. Cytotoxic Activity

Marine organisms are one of the important sources for discovery of new agents in cancer chemotherapy. Numerous marine derived compounds have been introduced for clinical studies and some of them were approved to be anti-cancer drugs such as lurbinectedin and trabectedin from tunicates, brentuximab vedotin and enfortumab vedotin from mollusks, cytarabine and eribulin mesylate from sponges [10]. Therefore, in this study, the isolated compounds **1**-**10** were evaluated for their cytotoxic activity against human lung carcinoma (SK-LU-1) and human hepatocellular carcinoma (HepG2), which are the two typical human cancerous cell lines, were selected for study. Each compound was screened its cytotoxic activity at a concentration of 4 µM using Sulforhodamine B assay (See supplementary materials) [11]. Unfortunately, all of the compounds showed weak cytotoxic effects against both SK-LU-1 and HepG2 cell lines. Percentages of dead cells induced by compounds were found less than 50% in all experiments.

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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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