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The biosynthetic products of chinese insect medicine, *Aspongopus chinensis*

Xiao-Hong Luo^a, Xiao-Zheng Wang^a, Hai-Long Jiang^a, Jun-Li Yang^a, Phillip Crews^b,
Frederick A. Valeriote^c, and Quan-Xiang Wu^{a,b,*}

^aState Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, PR China

^bDepartment of Chemistry and Biochemistry, University of California Santa Cruz, Santa Cruz, CA 95064, United States

^cHenry Ford Health System, Detroit, MI 48202, United States

Abstract

A new oxazole (**1**) was obtained from chinese insect medicine *Aspongopus chinensis*, along with three known *N*-acetyldopamine derivatives (**2–4**). Their structures were determined on the basis of NMR and ESI-MS analyses. The possible biosynthetic pathways of the isolated compounds are discussed. Cytotoxicities of those compounds against 10 selected cancer cells were measured *in vitro*.

Keywords

Pentatomidae; *Aspongopus chinensis*; Oxazole; *N*-acetyldopamine; Cytotoxicity

1. Introduction

Insects are a class of living creatures within the arthropods. The number of extant species is estimated at between six and ten million [1]. Insects may be found in nearly all environments, although only a small number of species occur in the ocean. Most are regarded as pests by humans. A lot insect damage crops, a few bite humans and livestock, and some are capable of transmitting diseases to humans, pets and livestock. Although pest insects attract the most attention, many insects are beneficial to the environment and to humans. Some insects can pollinate flowers. Many other insects are considered ecologically beneficial as predators and a few provide direct economic benefit.

About 150 thousand insect species have been found and more than 1000 species used as officinal and edible natural resources due to their medicinal and trophic value in China mainland. In recent years, a lot of scientists focused on the crude drugs and health products from insects. A certain scale of the extract, the peptides, proteins, and toxins from the

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*Corresponding author. Fax: +86 931 891 2582., wuqx@lzu.edu.cn (Q.X. Wu).

Supporting information

NMR spectra of compound **1** are available as Supporting Information.

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insects, has been test for the treatment of AIDS, cancer, inflammation and infectious diseases. *Aspongopus chinensis*, one of insects in Pentatomidae family, is mainly distributed in southern China. It has been employed as traditional chinese medicine to relieve pain and treat nephropathy for a long time. The extracts of *A. chinensis* possess strong anti-tumor and anti-bacterial activities which have been demonstrated by pharmacological studies [2]. *A. chinensis* also is edible in China because of its nutrient-rich and delicious taste [3]. The protein, fat, and chitin have been previously reported from *A. chinensis*. However, they are not fully exploited [4].

The potential to obtain new biosynthetic insights have stimulated our efforts to re-prioritize chemical study on the chinese medicines. We began a campaign to emphasize chemical study on the chinese insect medicine *A. chinensis* housed in our repository. The petroleum ether (60–90 °C), ethyl acetate and *n*-butanol extracts from the whole polypides of *A. chinensis* were submitted to a cytotoxicity soft agar-based disk diffusion assay against selected cancer cells L1210 (murine lymphocytic leukemia), Colon 38 (murine colon adenocarcinoma), HCT-116 (human colorectal carcinoma), H-125 (human lung adenocarcinoma), MCF-7 (human breast adenocarcinoma), LNCaP (human prostate adenocarcinoma), OVC-5 (human ovarian carcinoma), U251N (human glioma cells), MDA (human melanoma cells), PANC-1 (human pancreatic carcinoma), CEM (human leukemic lymphoid), and murine normal cells CFU-GM (granulocyte macrophage colony forming units) [5]. They exhibited significant activity against those cell lines (Table 1). The extracts were therefore analyzed based on high-performance liquid chromatography coupled to evaporative light scattering detector, photo-diode array detector and electrospray positive ionization ion trap mass spectrometry (HPLC ELSD PDA ESIMS), as an effective tool to rapidly identify and guide the isolation of target compounds from the extracts [6, 7]. And the ethyl acetate extracts of *A. chinensis* were chosen for further analysis on potential active constituents.

The ethyl acetate extract of *A. chinensis* was subjected to prep-HPLC chromatographic purification to afford a new oxazole (**1**), a known 1,2-dehydro-*N*-acetyldopamine (**2**) and two *N*-acetyldopamine dimers (**3** and **4**) [8] (Fig. 1). Their structures were elucidated on the basis of NMR and HR-ESI-MS analyses and by comparison with the literature data. 1,2-dehydro-*N*-acetyldopamine (**2**) is an important catecholamine derivative involved in the cross-linking of insect cuticular components during sclerotization, which is a vital process for the survival of insects [9]. Compounds **1–4** are phenol derivatives. So the possible biosynthetic pathways from the same precursor are discussed. The anticancer potential of phenolic compounds attracts broad attention. So the cytotoxicities of those compounds against 10 selected cancer cells were also measured *in vitro*.

2. Experimental

2.1. General

Optical rotations were determined on a Jasco DIP 370 digital polarimeter and UV data were obtained on an Agilent 8453 UV/Vis spectrophotometer. All NMR spectra were recorded in CD₃OD with 5 mm resonance (HCN) probe. Chemical shifts are reported in ppm relative to CD₃OD (δ_{H} 3.31, 4.87). A marine ESI mass spectrometer was used for low- and high-resolution mass measurements. Preparative reversed-phase (RP) separation was carried out utilizing a Waters 600E system controller and pumps. Both ELSD and UV (254 nm) were used for peak detection. Semipreparative RP HPLC used a Phenomenes Synergi Column and UV peak detection. Compound purity (> 95%) was confirmed using both ¹H and ¹³C NMR and LCMS (PDA and ELSD detection) experiments.

2.2. LC-DAD-ELSD-MS analysis

LC-PAD-ELSD-MS data were obtained with a Waters 600E series HPLC system consisting of an auto sampler, high-pressure mixing pump, column oven and PAD detector connected to a Perkin Elmer API 165 single quadrupole instrument equipped with a PE Sciex Turbo ion probe.

HPLC-PAD conditions—C18 Nova-Pak HR16 (6 μ m) cartridges, (5 μ m, 5 $\times$ 100 mm; Phenomenes Synergi), solvent system: A-0.1% formic acid water (FAW), B-methol; isocratic 10% B for 5 min, 10–90% B over 30 min, 100% B for 15 min; Flow rate: 1 mL/min; Injection volume: 15 μ L; Sample concentration: 10 mg/mL in methol. PAD conditions: 230, 254, 360 nm.

ESI-MS conditions—Positive ion mode; Split ratio: 1: 4; Scan range: 140–1000 amu; Source temperature: 350 $^{\circ}$ C; Ion spray voltage 5kV; Focussing potential: 230 V; Declustering potential: 20 V.

Prep-HPLC-UV conditions—C18 Nova-Pak HR16 cartridges, (4 μ m, 10 $\times$ 250 mm; Phenomenes Synergi), solvent system: A-FAW, B-acetonitrile (ACN); the ratio gradient and time see the specific compounds; Flow rate: 2 mL/min; Injection volume: 50 μ L; Sample concentration: 100 mg/mL in methol. UV conditions: 254 nm.

2.3. Insects material

The insects of *Aspongopus chinensis* were purchased from Lanzhou Fuxinghou Medical Materials Co., Ltd in 2009 and identified by Shi-Jian Xu, School of Life Sciences, Lanzhou University, China. The voucher specimen (KC2009002) was deposited in State Key Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou 730000, P. R. China.

2.4. Extraction and isolation

The air-dried whole insects of *Aspongopus chinensis* (100 g) were extracted exhaustively with 95% aq. EtOH (3 $\times$ 280 mL, 3 $\times$ 45 mins) at 60 $^{\circ}$ C. The extracts were evaporated to dryness under reduced pressure. The residue (104 mg) was extracted with petroleum ether (3 $\times$ 100 mL), EtOAc (3 $\times$ 100 mL), and n-BuOH (3 $\times$ 100 mL), respectively. After LCMS analysis, EtOAc fraction (61 mg) was subjected to prep-RP-HPLC (35–45% ACN / 65–55% FAW, 40 mins, 4400 mL), and 14 fractions (F1 F14) were collected. Further purification of F7 (4.8 mg) via prep-RP-HPLC (15% ACN / 85% FAW, 26 mins, 720 mL) yielded **2** (3.2 mg), F9 (14.4 mg, 15% ACN / 85% FAW, 18 mins, 1120 mL) yielded **3** (10.7 mg), F11 (11.6 mg, 25% ACN / 75% FAW, 11 mins, 1260 mL) yielded **4** (7.4 mg) and F12 (5.9 mg, 25% ACN / 75% FAW, 24 mins, 660 mL) yielded **1** (3.2 mg).

2.5. Spectroscopic data of Aspongopusin (1)

White amorphous powder; $[\alpha]_D^{24} +28$ (c 0.1, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ): 208.0 (5.98), 275.0 (5.83) nm; HR-ESI-MS m/z 192.06502 $[M+H]^+$ (calcd for $C_{10}H_{10}NO_3$, 192.06552); ^1H NMR (600MHz, MeOH- d_4) δ_H : 7.09 (s, H-4), 2.48 (s, H-6), 7.06 (d, J = 1.8 Hz, H-8), 6.81 (d, J = 8.4 Hz, H-11), 7.01 (dd, J = 7.8, 1.8 Hz, H-12); ^{13}C NMR (150MHz, MeOH- d_4) δ_C : 153.6 (s, C-2), 120.0 (d, C-4), 162.0 (s, C-5), 13.7 (q, C-6), 121.2 (s, C-7), 112.4 (d, C-8), 146.9 (s, C-9), 147.4 (s, C-10), 116.8 (d, C-11), 117.3 (d, C-12).

2.6. Disk diffusion soft agar colony formation assay

An *in vitro* cell-based assay was employed to identify solid tumor selectivity for extract partition fractions and pure compounds. The differential cytotoxicity is expressed by observing a zone differential between any solid tumor cells (Colon38, HCT-116, H-125, MCF-7, LNCaP, OVC-5, U251N, MDA, PANC-1, and CEM), leukemia cells (L1210) and murine normal cells (CFU-GM). The sample is designated as solid tumor selective if zone units of solid tumor-normal cells or leukemia cells is greater than 250 units (200 zone units = 6 mm). The activity results appear in Table 1.

3. Results and discussion

A new oxazole (**1**), a known 1,2-dehydro-*N*-acetyldopamine (**2**) and two *N*-acetyldopamine dimers (**3** and **4**) (Fig. 1) were isolated from the ethyl acetate extract of *A. chinensis*. Their structures were elucidated on the basis of NMR and HR-ESI-MS analyses and by comparison with the literature data.

Compound **1**, white amorphous powder, was assigned the molecular formula C₁₀H₉NO₃ (seven unsaturations) on the basis of HR-ESI-MS analysis (found *m/z* 192.06502 [M+H]⁺, calcd 192.06552) and NMR data. The ¹H, ¹³C NMR and DEPT experiments showed the presence of a methyl, four methines and five quaternary carbons. The ¹H and ¹³C NMR spectra also indicated the presence of a dihydroxybenzene unit (δ_H 7.06 (H-8), 6.81 (H-11), 7.01 (H-12); and δ_C 121.2, 112.4, 146.9, 147.4, 116.8, 117.3 (C-7 C-12), and a 2,5-disubstituted 1,3-oxazole (δ_H 7.09 (H-4) and δ_C 153.6 (C-2), 120.0 (C-4), 162.0 (C-5) [10].

Analysis of the ¹H ¹H COSY data (Fig. 2) led to the identification of two protons spin-systems corresponding to the C-11 C-12. gHMBC correlations (Fig. 2) of the isolated methyl protons (CH₃-6) with the oxygen-bearing quaternary carbon C-5 and correlations from H-4 to C-5 led to the 2,5-disubstituted 1,3-oxazole. Further gHMBC correlations from H-8 and H-12 to C-2 revealed the connection of oxazole and benzene units. Herein, the structure was elucidated and named as aspongopusin because it was isolated from *Aspongopus* genus.

The oxazole ring is usually encountered as a 2,4-disubstituted oxazole within the structures of complex natural products. A large number of oxazole containing natural products have been isolated from marine invertebrates and microorganisms, many of which exhibit potent biological activities [11]. This is the first time to find a 2,5-disubstituted oxazole derivative as a natural product in our best knowledge [12].

The new oxazole (**1**) and three known *N*-acetyldopamine analogues (**2–4**) are phenol derivatives. Extensive analyses of them led to the possible biosynthetic pathways (Fig. 3) from the same probable parent compound, shikimate [13]. Aminoisopropanol was derived from threonine by decarboxylation reaction and 3,4-dihydroxybenzoic acid from shikimate. The ester was afforded by 3,4-dihydroxybenzoic acid and aminoisopropanol. After carbonyl nucleophilic addition with azyl, dehydration, and dehydrogenation reactions, compound **1** could be created [10]. Tyrosine could be transformed to dopa by the enzyme tyrosine hydroxylase. Dopa is enzymatically decarboxylated to yield dopamine, which could be enzymatically *N*-acylated and dehydrogenated to generate 1,2-dehydro-*N*-acetyldopamine (**2**) [14]. *N*-acetyldopamine dimers (**3** and **4**) could be derived from polymerized **2** and *N*-acetyldopamine by the polymerase [15].

The cytotoxicity of the extract partition fractions encouraged our view that it might have strong activity against 10 cancer cell lines (Table 1). Further testing showed that compounds **1** and **4** were weak active against Colon 38 (zone units = 200), compound **2** was weak

active against L1210, compounds **3** and **4** were very weak active against U251N (zone unites 150), and compound **1** was also very weak active against MDA. Unfortunately, all of those compounds were inactive against HCT-116, H-125, MCF-7, LNCaP, OVC-5, and PANC-1 at the test concentration in the disk diffusion assay (zone unites = 0). The activity of traditional Chinese medicine is caused by the multiple chemicals. Therefore, it is reasonable that the cytotoxicities of the pure compounds were not strong like the extracts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.
The structures for compounds 1–4.

1

— ¹H-¹H COSY

→ gHMBC (from H to C)

Fig. 2.
Key ¹H ¹H COSY and gHMBC correlations for 1.

Fig. 3.
Plausible biosynthetic pathways for compounds **1–4** from shikimate.

Table 1

Disk diffusion assay data for crude extracts and pure compounds 1–4.

Collections ^a	Quality ^b	Zone unites											
		L1210	Colon 38	murine CFU-GM	H-116	H-125	MCF-7	LNCaP	OVC-5	U251N	MDA	PANC-1	CEM
DMSO		0	0	0	0	0	0	0	0	0	0	0	0
AC1	37.2 mg	200	200	- ^c	200	150	200	150	350	150	200	150	450
AC2	66.6 mg	300	150	-	100	100	100	100	150	150	100	100	-
AC3	57.8 mg	200	400	450	150	100	50	50	150	100	200	100	-
1	0.7 mg	0	200	-	0	0	0	0	0	0	100	0	-
2	1.7 mg	200	0	-	0	0	0	0	0	0	0	0	-
3	1.7 mg	0	0	-	0	0	0	0	0	150	0	0	-
4	1.5 mg	0	200	-	0	0	0	0	0	100	0	0	-

^aAC1, AC2 and AC3 mean the extracts of petroleum ether, ethyl acetate and *n*-butanol from *Aspongopus chinensis*.

^bThey were all prepared in 0.5 mL DMSO and 15 µL applied to the disk.

^c-,^d means not test.