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Review

Phenolic derivatives from *Blaps japanensis* and their biological evaluation

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ABSTRACT

This study was designed to characterize compounds from two thousands of insects of *Blaps japanensis*. With this purpose, pipajiains A–C (**1**–**3**), three novel phenolic derivatives, pipajiains D (**4**) and E (**5**), two new natural occurring compounds, and four known substances were isolated thereof. Their structures were identified by spectroscopic methods. Biological activities of all these compounds towards EV71, tuberculosis, COX-2, ROCK1/2, and JAK3 kinases were evaluated.

1. Introduction

Insects are the most diverse group of animals on earth. To survive in the complex environment, insects have evolved various defense mechanisms. Of which, chemical defense may be one of the most effective ways, exemplified by species of Carabidae and Tenebrionidae which have the ability to spray chemicals from their abdomen to repel predators [1]. Previous studies revealed that the defensives molecules tend to be low molecular weight or volatile such as acids, aldehydes, aromatic ketones, quinones, terpenes [2]. Due to the nature of chemical weapons of defensive substances, chemicals from insects are probably potential sources for modern drug discovery. Actually, hundreds of insects are medicinally used worldwide. *Blaps japanensis* is an insect species belonging to the family Tenebrionidae. Previous reports demonstrated that quinone and quinol derivatives from the extract or defensive sprays of *Blaps* species exhibit significant antibacterial activities against several microbial strains [3–6]. In recent years, we have become in particular interested in *B. japanensis* due to its wide medical applications in Yi-nationality medicine of Yunnan province of People's Republic of China for the treatment of cancer and inflammation associated disorders [7]. As a consequence, our efforts led to the isolation of several phenolic compounds and *N*-containing compounds thereof [8–10]. However, our previous study focused on compounds from the dried insect bodies. To get a deep insight into the chemical profiling of living *B. japanensis*, we undertook a re-investigation on this insect, which accordingly resulted in the isolation of nine phenolic derivatives, resembling those sprays from *Blaps* (Fig. 1). In this paper, we describe their isolation, structure characterization and biological evaluation.

2. Experimental

2.1. General

UV spectra were obtained on a Shimadzu UV-2401PC spectrometer. NMR spectra were recorded on a Bruker Avance III 600 MHz spectrometer, with TMS as an internal standard. EIMS and HREIMS were collected on Autospec Premier P776 spectrometer. ESIMS and HRESIMS were measured on an API QSTAR Pulsar 1 spectrometer. C-18 silica gel (40–60 μm; Daiso Co., Japan), MCI gel CHP 20P (75–150 μm, Mitsubishi Chemical Industries, Tokyo, Japan), Sephadex LH-20 (Amersham Pharmacia, Uppsala, Sweden), and silica gel (200–300 mesh; Qingdao Marine Chemical Inc., PR China) were used for column chromatography. Silica gel GF_{254} (Qingdao Marine Chemical Inc., PR China) was used for preparative TLC. Semi-preparative HPLC was carried out using an Agilent 1200 liquid chromatograph with a YMC-Pack ODS-A column (250 mm × 10 mm, i.d., 5 μm).

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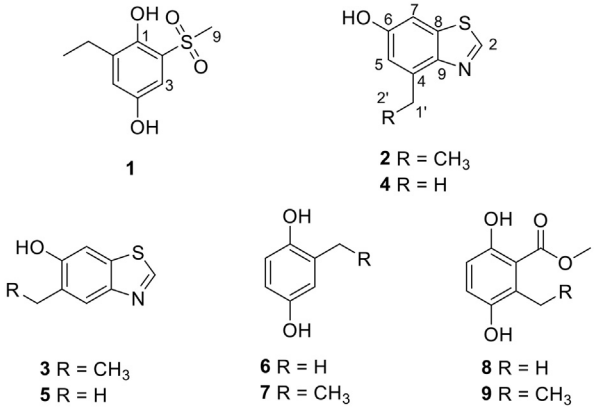

Fig. 1. Structures of compounds **1**–**9**.

2.2. Insect material

B. japanensis were purchased from Kunming Market of Flowers and Birds (collected from Honghe Country, Yunnan Province, People's Republic of China) in October 2015 and authenticated by Professor Dazhi Dong at Kunming Institute of Zoology, Chinese Academy of Science, People's Republic of China. A voucher specimen (CHYX-0604) was deposited at the State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, Chinese Academy of Science, People's Republic of China.

2.3. Extraction and isolation

The living *B. japanensis* (1.8 kg, 2000 insects) was executed by 80% EtOH overnight. The bodies of *B. japanensis* were homogenized followed by ultrasonic extraction with aqueous EtOH (80%, 4 × 3 L × 2 h) at room temperature and subsequent concentration to afford a crude extract (103.5 g), which was divided into six fractions (A–F) by a MCI gel CHP 20P column eluted with aqueous MeOH (5%–100%). Of which, Fr. A (2.3 g) was further fractionated into 5 parts (Frs. A1–A5) by Sephadex LH-20 (MeOH/H_2O, 80:20). The Fr. A3 (250 mg) was submitted to preparative RP-HPLC (MeOH/H_2O, 10:90) to yield 4 parts (Frs. A3.1-A3.4). Fr. A3.3 (75 mg) was further chromatographed over semi-preparative HPLC (CH_3CN/H_2O, 8:92) to yield compound **6** (10.8 mg, t_R = 24 min). Likewise, Fr. D (4.8 g) was passed through Sephadex LH-20 (MeOH) to afford Frs. D1-D6. Of which, Fr. D4 (400 mg) was purified by semi-preparative HPLC (CH_3CN/H_2O, 25:75) to yield compounds **1** (1.2 mg, t_R = 26 min), **7** (10.8 mg, t_R = 14.5 min), **8** (6.6 mg, t_R = 22 min), and **9** (6.4 mg, t_R = 36 min), respectively. Fr. E (5.0 g) was separated by Sephadex LH-20 (MeOH) follow by semi-preparative HPLC (CH_3CN/H_2O, 30:70) to give **4** (6.2 mg, t_R = 14.1 min), **5** (3.3 mg, t_R = 14.8 min), **2** (2.8 mg, t_R = 23.4 min), and **3** (2.2 mg, t_R = 25.8 min).

Pipajiain A (**1**). Yellowish solid; UV (MeOH) λ_{max} (log ε) 305 (3.45), 263 (3.63), 206 (4.22) nm; ESIMS *m/z* 215 $[M - H]^-$; HRESIMS *m/z* 215.0380 $[M - H]^-$ (calcd for $C_9H_{11}O_4S$, 215.0384). ^1H and ^{13}C NMR data, see Table 1.

Pipajiain B (**2**). Yellowish solid; UV (MeOH) λ_{max} (log ε) 277 (4.24), 239 (4.41), 219 (4.62), 207 (4.73) nm; ESIMS *m/z* 180 $[M + H]^+$; HRESIMS *m/z* 180.0480 $[M + H]^+$ (calcd for $C_9H_{10}NOS$, 180.0478). ^1H and ^{13}C NMR data, see Table 1.

Pipajiain C (**3**). Yellowish solid; UV (MeOH) λ_{max} (log ε) 275 (3.91), 243 (3.99), 219 (4.36), 206 (4.43) nm; ESIMS *m/z* 180 $[M + H]^+$; HRESIMS *m/z* 180.0477 $[M + H]^+$ (calcd for $C_9H_{10}NOS$, 180.0478). ^1H and ^{13}C NMR data, see Table 1.

Pipajiain D (**4**). Yellowish solid; ESIMS *m/z* 166 $[M + H]^+$; HRESIMS *m/z* 166.0319 $[M + H]^+$ (calcd for C_8H_8NOS, 166.0321). ^1H NMR (600 MHz, CD_3OD) δ_H: 8.90 (1H, s, H-2), 7.17 (1H, d, *J* = 2.1 Hz, H-7), 6.84 (1H, d, *J* = 2.1 Hz, H-5), 2.64 (3H, s, H-1′); ^{13}C NMR (150 MHz, CD_3OD) δ_C: 157.0 (C-6), 152.2 (C-2), 147.3 (C-9), 136.2 (C-8), 134.7 (C-4), 117.8 (C-5), 104.9 (C-7), 18.4 (C-1′).

Pipajiain E (**5**). Yellowish solid; ESIMS *m/z* 166 $[M + H]^+$; HRESIMS *m/z* 166.0315 $[M + H]^+$ (calcd for C_8H_8NOS, 166.0321). ^1H NMR (600 MHz, CD_3OD) δ_H: 8.91 (1H, s, H-2), 7.74 (1H, s, H-4), 7.30 (1H, s, H-7), 2.33 (3H, s, H-1′); ^{13}C NMR (150 MHz, CD_3OD) δ_C: 156.1 (C-6), 153.0 (C-2), 147.8 (C-9), 133.3 (C-8), 127.2 (C-5), 124.5 (C-4), 106.3 (C-7), 16.8 (C-1′).

Table 1
^1H (600 MHz) and ^{13}C NMR (150 MHz) data of **1**–**3** (δ in ppm, *J* in Hz, methanol-d_4).

No	1		2		3	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		147.3				
2		129.2	8.91, s	152.1	8.92, s	153.0
3	7.01, d, 2.8	112.2				
4		151.8		141.0	7.75, s	123.0
5	6.92, d, 2.8	123.9	6.86, d, 2.4	116.0		133.3
6		136.8		157.3		155.8
7	2.64, q, 7.5	24.0	7.18, d, 2.4	105.0	7.31, s	106.5
8	1.20, t, 7.5	14.3		136.4		133.3
9				146.7		147.9
1′			3.07, q, 7.2	26.5	2.75, q, 7.2	24.7
2′			1.32, t, 7.2	15.4	1.27, t, 7.2	14.6
S-CH_3	3.19,s	44.0				

2.4. Procedure for biological evaluation

All the isolates were evaluated for their inhibitory effects against EV71 [11], COX-2 [12], ROCK1/2 [8], JAK3 [12], and tuberculosis [11] as previously described methods.

3. Results and discussion

Pipajiain A (**1**) was found to have the molecular formula $C_9H_{12}O_4S$ (4 degrees of unsaturation) derived by analysis of its HRESIMS, ^{13}C NMR, and DEPT spectra. The ^1H NMR spectrum (Table 1) of **1** shows two aromatic protons resonated at δ_H 7.01 (H-3, d, *J* = 2.8 Hz) and δ_H 6.92 (H-5, d, *J* = 2.8 Hz), corresponding to a 1,2,4,6-tetrasubstituted benzene ring. The ^{13}C NMR and DEPT spectra of **1** show nine carbons attributed to two methyl, one sp^3 methylene, two sp^2 methine and four quaternary carbons (including two oxygenated).The signals of **1** imply that the presence of 2-ethylbenzene-1,4-diol motif. This conclusion is further confirmed by the chemical shifts of C-1 and C-4 and detailed 2D NMR experiments (Fig. 2), which show ^1H-^1H COSY correlation of H_2-7/H_3-8 and HMBC correlations of H_3-8/C-6, H-7/C-1, C-5, C-6, H-3/C-1, C-5, and H-5/C-1, C-3. In addition to a substituted benzene ring, in consideration of the molecular composition of **1** and chemical shifts of H-9 (δ_H 3.19) and C-9 (δ_C 44.0), the remaining signals should be ascribed to a methylsulfonyl group, such a group has been actually found in polyrhadopamine C [13], an ant-derived product. As far as the position of the methylsulfonyl group is concerned, the HMBC correlation of H-9/C-2 is missing due to the limited amount of material. However, the coupling pattern in the aromatic ring unambiguously clarifies the

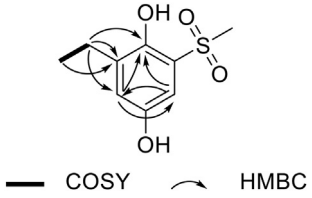

Fig. 2. ^1H-^1H COSY and key HMBC correlations of **1**.

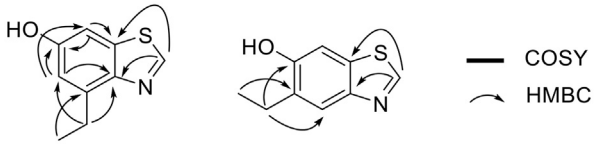

Fig. 3. ^{1}H-^{1}H COSY and key HMBC correlations of **2** and **3**.

location of the methylsulfonyl group at C-2 (δ_C 129.2). In this way, the structure of **1** was identified and named pipajiain A.

Pipajiain B (**2**) has the molecular formula C_9H_9NOS (6 degrees of unsaturation) by analysis of its HRESIMS, ^{13}C NMR, and DEPT spectra. The ^{1}H NMR spectrum of **2** indicates two *meta* aromatic protons respectively at δ_H 7.18 (d, J = 2.4 Hz, H-7) and δ_H 6.86 (d, J = 2.4 Hz, H-5), indicative of a 1,2,3,5-tetrasubstituted benzene ring, one downfield shifted proton at δ_H 8.91 (s, H-2), and one ethyl group [δ_H 3.07 (q, J = 7.2 Hz, H-1′); δ_H 1.32 (t, J = 7.2 Hz, H-2′)]. The ^{13}C NMR and DEPT spectra (Table 1) give 9 signals classified into one methyl, one methylene, three aromatic methine and four aromatic quaternary carbons. The architecture of **2** was mainly identified by detailed interpretation of 2D NMR data (Fig. 3). The ^{1}H-^{1}H COSY spectrum of **2** shows correlation of H_2-1′/H_3-2′ in accordance with the presence of one ethyl group. The HMBC spectrum gives correlations of H_3-2′/C-4 (δ_C 141.0), H_2-1′/C-4, C-5 (δ_C 116.0), C-9 (δ_C 146.7), H-5/C-6 (δ_C 157.3), C-7, C-9, H-7/C-5, C-6, C-8 (δ_C 136.4), and C-9, in consideration of the diagnostic chemical shift of C-6, suggesting the positions of ethyl and hydroxyl groups and substituted pattern in the benzene ring. The diagnostic chemical shifts of C-2 (δ_C 152.1), C-8, C-9, and H-2 (δ_H 8.91, s) indicate that **2** contains a thiazole group resembling that of 5/6-hydroxy benzothiazole [14–16]. Further HMBC correlation of H-2/C-8, C-9 and the remaining one degree of unsaturation support our conclusion. Therefore, the structure of **2** was determined and named pipajiain B.

Analysis of the HRESIMS, ^{13}C NMR, and DEPT data of pipajiain C (**3**) shows that it has the same molecular formula and similar NMR data as does **2**. The only difference between **2** and **3** is that the ethyl group of **3** is located at C-5 gaining supports from the HMBC correlations (Fig. 3) of H-2′/C-5, H-1′/C-4, C-5, and C-6. This conclusion is in accordance with the observation of H-4 and H-7 respectively as singlet in the ^{1}H NMR spectrum. As a consequence, the structure of compound **3** were established and named pipajiain C.

The NMR data of compound **4** (pipajiain D) are similar to those of **2** differing in that the ethyl group in **2** is replaced by a methyl in **4**, HMBC correlations of H-1′/C-4, C-5, C-9 support our conclusion. Likewise, detailed NMR interpretation shows that the only change between compounds **5** (pipajiain E) and **3** is that the ethyl group in **3** is substituted by a methyl in **5**, corresponding to the observations of HMBC correlations of H-1′/C-4, C-5, C-6. Notably, compounds **4** and **5** have been synthesized by Mitchell and colleagues [17]. however, they were isolated from natural sources for the first time. In addition, the NMR data of **4** and **5** were also firstly assigned in the present study.

Four known compounds were identified as 2-methyl-1,4-benzenediol (**6**) [18], 2-ethyl-1,4-benzenediol (**7**) [18], 2-methyl-3,6-dihydroxybenzoic acid methyl ester (**8**) [19], and 2-ethyl-3,6-dihydroxybenzoic acid methyl ester (**9**) [19], respectively, by comparing their spectroscopic data with those literature data.

B. japanensis has been used to treat cough, fever, rheumatism, cancer, and inflammatory disorders [7]. To fully explore biological activities of *B. japanensis* derived compounds, multiple assays in hand including EV71, tuberculosis, COX-2, ROCK1/2, and JAK3 kinases were utilized. As shown in Table 2, unfortunately, compounds **3–5** are inactive in all the assays. In contrast, compound **1** is active towards ROCK1, ROCK2, and JAK3 with respective IC_{50} values of 24.3 μM, 7.26 μM, and 2.26 μM. The COX-2 inhibitory activities of compounds **2**, **8**, and **9** with respective IC_{50} values of 8.91 μM, 11.50 μM, and 7.70 μM. In addition, the potency of compounds **6–9** against ROCK2 is stronger than ROCK1, indicating the selectivity of these isolates. The present findings not only unveil the chemical profiling of non-peptide insect natural products but also imply that benzenediol derivatives might be a promising scaffold for structural optimization.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2017.05.010.

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