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Compounds from the insect *Blaps japanensis* with COX-1 and COX-2 inhibitory activities

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ABSTRACT

Blapsols A–D (**1**–**4**), four new compounds possessing a 2,3-dihydrobenzo[*b*][1,4]dioxin group, together with five known *N*-acetyldopamine dimers (**5**–**9**), were isolated from *Blaps japanensis*. Their structures including the absolute configuration of (+)-**1** were determined by means of spectroscopic and X-ray crystallographic methods. Chiral HPLC was used to separate (−)- and (+)-enantiomers of compounds **1**–**4**, which were isolated from this insect as racemic mixtures. All the compounds were found to have inhibitory effects towards COX-2 with IC_{50} values in the range of 1.3–17.8 μM.

Insects, accounting for a great majority of animal species on earth, have a wide variety of predators including birds, reptiles, amphibians, mammals, and other arthropods. To survive in this ongoing escapist battle, insects have evolved various defense mechanisms. Among these, chemical defense plays an essential role for insects especially those tend to be relatively large, long-lived, and frequently aggregate.[1] Investigation of chemical defense mechanisms used by insects has been underwent for decades. In addition to proteins or peptides, a few of non-peptide defensive small molecules with intriguing chemical structures have also been characterized, demonstrating their potential in drug discovery.[2,3] However, in contrast to metabolites from plants or microorganisms, insect-derived substances and their potential roles in drug discovery have been largely ignored.

Blaps japanensis (Tenebrionidae), a medicinal insect in Yi nationality of Yunnan Province, China, has been used for the treatment of fever, cough, rheumatism, cancer, and inflammatory disorders.[4] Our previous work led to the characterization of phenolic compounds and *N*-containing compounds.[5–7] However, chemical and biological profiling of this insect is so far largely undisclosed. In the course of our continuing search for bioactive compounds from insects, the title insect was further investigated, which resulted in the isolation of four new dimeric compounds, blapsols A–D (**1**–**4**) and five known dopamine dimers (Fig. 1).[8] Below, we describe the isolation, structure identification, and anti-inflammatory effects of these compounds.

Racemic blapsol A (**1**)[9] was found to have the molecular formula $C_{18}H_{20}O_7$ (9 degrees of unsaturation) derived by analysis of its HRESIMS, ^{13}C NMR and DEPT spectra. The ^1H NMR spectrum (Table 1) of **1** contains two typical ABX spin systems, suggesting the presence of two 1,2,4-trisubstituted benzene rings. The ^{13}C NMR and DEPT spectra contain resonances for 18 carbons including one methyl, three aliphatic methylene, six methine (one sp^3, six sp^2), and seven quaternary carbons (one oxygenated sp^3, six olefinic including four oxygenated). These analyses indicated that **1** is likely a dopamine dimer derivative like compound **7**. The planar structure of **1** was established by extensive analyses of its HSQC, ^1H–^1H COSY, and HMBC spectra (Fig. 2). The ^1H–^1H COSY spectrum showed correlations between H-7/H-8, H-5′/H-6′, H-1″/H-2″, and H-3/3-OH. The HMBC experiment which shows correlations from H-1″ to C-5, C-6, and C-7, from H-2″ to C-6, from H-6′, H-1′ to C-2, and from H-3, 3-OH to C-2 constructed two phenyl alcohol frameworks, which was comparable to that of **7**. The ethyloxy group at C-2 was identified by the key HMBC correlation from OCH_2CH_3 to C-2. In addition, the HMBC correlations from H-3 to C-4a indicate that C-3 is connected to C-4a via an oxygen bridge.

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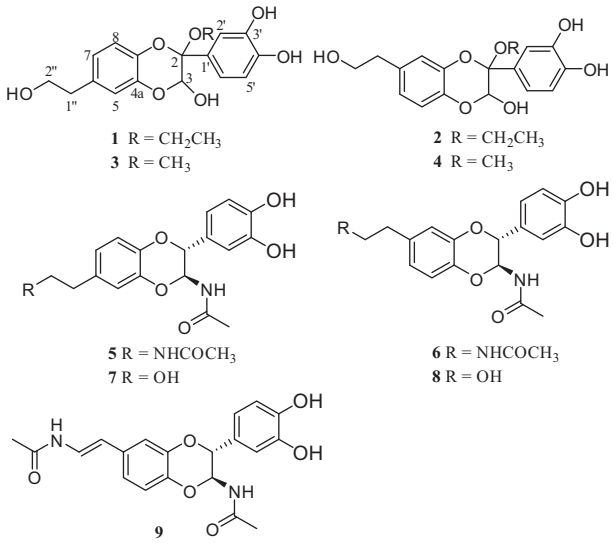

Figure 1. The structures of compounds **1**–**9**.

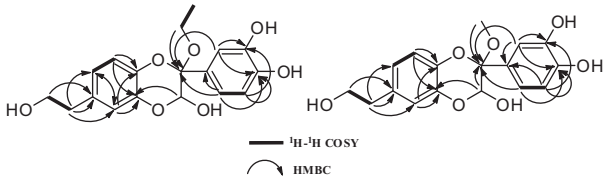

Figure 2. ^{1}H–^{1}H COSY and key HMBC correlations of compounds **1** and **3**.

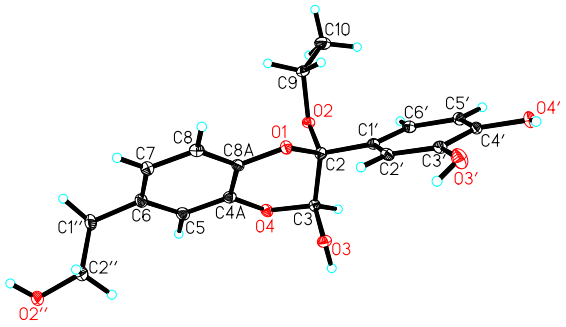

Figure 3. The X-ray structure of (+)-**1**.

Table 1
The ^{1}H NMR data of **1** and **2** (δ in ppm)

Position	**1**[a]	**2**[a]
3	5.36 d (5.3)	5.36 d (5.3)
5	6.77 d (2.1)	6.78 d (8.0)
6		6.76 dd (8.0, 2.0)
7	6.78 dd (8.6, 2.1)	
8	6.90 d (8.6)	6.90 d (2.0)
2′	7.13 d (2.1)	7.13 d (2.0)
5′	6.87 d (8.3)	6.87 d (8.2)
6′	6.98 dd (8.3, 2.1)	6.98 dd (8.2, 2.0)
1″	2.74 t (7.2)	2.75 t (7.0)
2″	3.73 t (7.2)	3.74 t (7.0)
OCH_2CH_3	3.31 q (7.0)	3.30 q (7.0)
OCH_2CH_3	0.93 t (7.0)	0.93 t (7.0)
3-OH	5.94 d (5.3)	5.96 d (5.3)

[a] 500 MHz in acetone-d_6.

Moreover, the remaining one degree of unsaturation and chemical shift of C-2 implied that C-2 and C-8a were connected by an oxygen atom to form a benzodioxane ring. The relative configurations at C-2 and C-3 could not be determined by ROESY experiments. The lack of an optical rotation indicates that **1** is racemic. Separation by using chiral HPLC yielded two enantiomers (+)- and (−)-blapsol A (**1**). As expected, (+)-**1** and (−)-**1** exhibit mirror-like CD curves (Supplementary material, Figs. S1 and S2), and the absolute configuration of (+)-**1** was finally determined to be 2*S*,3*S* (Fig. 3) by a single-crystal X-ray diffraction.[10]

Racemic blapsol B (**2**)[11] has the molecular formula $C_{18}H_{20}O_7$ by analysis of its HRESIMS, ^{13}C NMR and DEPT spectra. The ^{1}H and ^{13}C NMR spectra of **2** are similar to those of **1**. A detailed interpretation of 2D NMR data indicates that the planar structures of **1** and **2** differ only in a substitute position at the benzene ring. The HMBC correlations of H-1″, H-2″/C-7, H-8/C-1″, and H-6/C-4a indicate that **2** is the 7-positioner of **1**. The lack of an optical rotation of **2** indicates that it is racemic. Separation by using chiral HPLC yielded two enantiomers, whose absolution configurations were determined by comparing with experiment CD data of **1** (Supplementary material), therefore, the absolute configurations of (+)-**2** and (−)-**2** was defined as 2*S*,3*S* and 2*R*,3*R*, respectively.

Blapsol C (**3**),[12] obtained as a yellowish solid, has the molecular formula $C_{17}H_{18}O_7$ (9 degrees of unsaturation) based on analysis of its HRESIMS, ^{13}C NMR and DEPT spectra. The ^{1}H and ^{13}C NMR spectra of **3** are similar to those of **1**. Their difference is that a ethyloxy group in **1** is replaced by an methoxy group in **3**, which is supported by the HMBC correlation of OCH_3/C-2. Detailed analysis of its HSQC and HMBC correlations (Fig. 2) revealed that compound **3** had the planar structure as shown (Fig. 1). In the same manner, racemic **3** was separated by using chiral HPLC to obtain (+)-**3** and (−)-**3**. Eventually, the absolute configurations of (+)-**3** and (−)-**3** was determined to be 2*S*,3*S* and 2*R*,3*R*, respectively, by comparison of their CD data with those of **1** (Supplementary material).

Blapsol D (**4**)[13] was obtained as a yellowish solid. The molecular formula of **4** was found to be same as that of **3** ($C_{17}H_{18}O_7$), as deduced from its HRESIMS, ^{13}C NMR and DEPT spectra. The ^{1}H and ^{13}C NMR spectra of **4** are similar to those of **3**. Detailed analysis of its HSQC and HMBC correlations revealed that compound **4** is a 7-positioner of **3**, in accordance with a key HMBC correlation of H-6/C-4a. Therefore, the planar structure of **4** was determined as shown (Fig. 1). Likewise, compound **4** was separated by using chiral HPLC to obtain (+)-**4** and (−)-**4**, whose absolute configurations were determined to be 2*S*,3*S* and 2*R*,3*R*, respectively, according to comparison their CD data with those of **1** (Supplementary material).

Five known compounds were isolated and identified as *trans*-2-(3′,4′-dihydroxy-phenyl)-3-acetylamino-6-(*N*-acetyl-2″-aminoethyl)-1,4-benzodioxane (**5**),[14] *trans*-2-(3′,4′-dihydroxy-phenyl)-3-acetylamino-7-(*N*-acetyl-2″-aminoethyl)-1,4-benzodioxane (**6**),[14] *trans*-2-(3′,4′-dihydroxyphenyl)-3-acetylamino-6-hydroxyethyl-1,4-benzdioxane (**7**),[15] *trans*-2-(3′,4′-dihydroxyphenyl)-3-acetylamino-7-hydroxyethyl-1,4-benzdioxane (**8**),[15] and *trans*-2-(3′,4′-dihydroxyphenyl)-3-acetylamino-7-(*N*-acetyl-2″-aminoethylene)-1,4-benzodioxane (**9**)[16] by comparing their spectroscopic data with those previously reported for these substances.

Of note, the known compounds **5**–**9** are *N*-acetyldopamine derivatives, this class of compounds have also been found in the other insect species and are considered to be components responsible for cuticle sclerotization.[17–19] From a biogenic point of view, compounds **1**–**4** are in fact derivatives of *N*-acetyldopamine dimers. Interestingly, all the compounds were isolated as racemic mixtures, this phenomenon was also observed from the other insect-derived dopamine oligomers,[20] which allows us to tentatively hypothesize that these substances may simply be the

Table 2
The ^{1}H NMR data of **3** and **4** (δ in ppm)

Position	**3**[a]	**4**[b]
3	5.38 s	5.31 s
5	6.77 d (2.0)	6.92 d (2.1)
6		6.80 dd (8.2, 2.1)
7	6.78 dd (8.7, 2.0)	
8	6.92 d (8.7)	6.82 d (8.2)
2′	7.11 d (2.0)	7.04 d (2.1)
5′	6.88 d (8.2)	6.83 d (8.3)
6′	6.96 dd (8.2, 2.0)	6.94 dd (8.3, 2.1)
1″	2.73 t (7.0)	2.76 t (7.2)
2″	3.72 t (7.0)	3.74 t (7.2)
OCH_3	2.98 s	3.02 s

[a] 500 MHz in acetone-d_6.
[b] 500 MHz in methanol-d_4.

Table 3
The ^{13}C NMR data of **1**–**4** (δ in ppm)

Position	**1**[a]	**2**[a]	**3**[a]	**4**[b]
2	98.6	98.6	98.6	99.1
3	92.5	92.4	92.4	92.7
4a	141.5	140.4	141.5	140.3
5	118.4	118.4	118.4	118.9
6	134.2	123.2	134.4	123.8
7	122.6	133.5	122.6	133.8
8	117.7	117.6	117.7	118.1
8a	140.0	141.4	139.7	141.5
1′	130.0	129.9	129.1	129.0
2′	115.4	115.3	115.4	115.5
3′	146.2	146.2	146.3	146.9
4′	145.6	145.6	145.6	146.2
5′	119.6	119.5	119.6	119.8
6′	115.7	115.7	115.7	116.0
1″	39.6	39.5	39.6	39.5
2″	64.0	64.0	63.9	64.4
OCH_2CH_3	58.3	58.2		
OCH_2CH_3	15.5	15.4		
OCH_3			49.5	49.9

[a] 125 MHz in acetone-d_6.
[b] 125 MHz in methanol-d_4.

Table 4
COX-1 and COX-2 inhibitory activities of the compounds

Compd	IC_{50} (μM)	
	COX-1	COX-2
(+)-**1**	NA[a]	2.52
(−)-**1**	NA	6.04
(+)-**2**	NA	8.24
(−)-**2**	NA	6.02
(+)-**3**	NA	13.7
(−)-**3**	NA	8.6
(+)-**4**	NA	17.8
(−)-**4**	NA	9.7
5	NA	2.6
6	NA	3.6
7	NA	1.3
8	NA	2.5
9	13.8	2.7
Celecoxib	81.7	0.016

[a] NA: no activity.

early-stage intermediates of random oxidation by enzyme prior to formation of high-molecular weight melanin-like molecules.

In consideration of the traditional usage of *B. japanensis*, anti-inflammatory activities of all the compounds were evaluated by using cyclooxygenase-1 (COX-1) and COX-2 inhibitory assays.[21] The results showed that all the isolates exhibit COX-2 inhibitory activity with IC_{50} values ranging from 1.3 to 17.8 μM (Table 4). However, among all the compounds, only **9** is active towards COX-1 with IC_{50} value of 13.8 μM (Table 4). It is well known that inhibition of COX-2 but not COX-1 is beneficial for pathogenic inflammation. In this study, although the biological activities of these compounds are moderate towards COX-2, this finding in part reveals the traditional medical application of *B. japanensis* for the treatment of inflammatory disorders. In addition, this study might provide a new structure scaffold for the design of non-steroidal anti-inflammatory drugs.

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Supplementary data

Supplementary data (experimental section, CD, NMR, and HRMS spectra of **1**–**4**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.04.085.

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8. *Isolation:* The air-dried powder of *B. japanensis* bodies (5 kg) was soaked with 50% aqueous EtOH (3 × 30 L) at room temperature. The combined extracts were concentrated to obtain a crude extract (500 g), which was partitioned with BuOH in H_2O to obtain a BuOH extract (150 g). The BuOH extract was divided into 6 parts (Fr. 1–Fr. 6) by using silica gel column chromatography eluted with a gradient of $CHCl_3$/MeOH (100:1–0:1). Fr. 3 (8 g) was separated by using a MCI gel CHP 20P column eluting with gradient aqueous MeOH (10:90–80:20) to provide eight portions (Fr. 3.1–Fr. 3.8). Fr. 3.4 (1.1 g) was separated by using Sephadex LH-20 (MeOH) followed by a RP-18 column (MeOH/H_2O, 20:80–50:50) to give **1** (10 mg) and **2** (9.5 mg). Fr. 3.5 (2.8 g) was separated by using Sephadex LH-20 (MeOH) followed by a RP-18 column (MeOH/H_2O, 20:80–50:50), and semi-preparative HPLC (MeOH/H_2O, 30:70) to obtain **3** (5.5 mg) and **4** (5.5 mg). Compounds **1**–**4**, which are racemic, were subjected to chiral HPLC to yield (+)-**1** (3.7 mg) and (−)-**1** (3.9 mg) (*n*-hexane/2-propanol, 82:18), (+)-**2** (3.3 mg) and (−)-**2** (3.4 mg) (*n*-hexane/2-propanol, 65:35), (+)-**3** (1.8 mg) and (−)-**3** (1.4 mg) (*n*-hexane/2-propanol, 65:35), and (+)-**4** (1.5 mg) and (−)-**4** (1.4 mg) (*n*-hexane/2-propanol, 65:35). Fr. 4 (20 g) was separated by using MCI gel CHP 20P eluted with a gradient of aqueous MeOH (10:80–60:40) to yield five fractions (Fr. 4.1–Fr. 4.5). Fr. 4.2 (1.5 g) was subjected to gel filtration on Sephadex LH-20 (MeOH) followed by chromatography on a RP-18 column (MeOH/H_2O, 10:65–50:50) and semi-preparative HPLC (MeOH/H_2O, 20:80) to yield **7** (6 mg) and **8** (6 mg). Fr. 4.4 (2.5 g) was subjected to gel filtration on Sephadex LH-20 (MeOH) followed by chromatography on a RP-18 column (MeOH/H_2O, 15:65–55:45) to yield **5** (320 mg), **6** (200 mg), **9** (20 mg).
9. Blapsol A (**1**): Yellowish crystal; $\{[\alpha]_D^{22}$ +131.9 (*c* 0.16, MeOH); CD (MeOH) $\Delta\varepsilon_{205}$ +39.74, $\Delta\varepsilon_{228}$ +11.46; (+)-**1**$\}$; $\{[\alpha]_D^{22}$ −141.8 (*c* 0.15, MeOH); CD (MeOH) $\Delta\varepsilon_{205}$ −45.27, $\Delta\varepsilon_{228}$ −11.74; (−)-**1**$\}$; UV (MeOH) λ_{max} (log ε) 280 (3.83), 204 (4.74) nm; ESIMS *m/z* 347 $[M-H]^-$; HRESIMS *m/z* 347.1134 $[M-H]^-$ (calcd for $C_{18}H_{19}O_7$, 347.1131). ^{1}H and ^{13}C NMR data, see Tables 1 and 3.
10. *Crystal data for* (+)-**1**: $C_{18}H_{20}O_7$, *M* = 348.34, orthorhombic, *a* = 9.0373(2) Å, *b* = 9.9958(2) Å, *c* = 18.3179(4) Å, α = 90.00°, β = 90.00°, γ = 90.00°, *V* = 1654.75(6) Å^3, *T* = 100(2) K, space group *P*212121, *Z* = 4, μ(CuKα) = 0.909 mm^{-1}, 15658 reflections measured, 3017 independent reflections (R_{int} = 0.0424). The final R_1 values were 0.0420 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1062 ($I > 2\sigma(I)$). The final R_1 values were 0.0423 (all data). The final $wR(F^2)$ values were 0.1064 (all data). The goodness of fit on F^2 was 1.146. Flack parameter = 0.01(19). The Hooft parameter is 0.03(5) for 1243 Bijvoet pairs. Crystallographic data of (+)-**1** have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC 1045655). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html.

11. *Blapsol B* (**2**): Yellowish solid; {$[\alpha]_D^{22}$ +114.3 (*c* 0.06, MeOH); CD (MeOH) $\Delta\varepsilon_{205}$ +55.64, $\Delta\varepsilon_{228}$ +13.87; (+)-**2**}; {$[\alpha]_D^{22}$ −145.9 (*c* 0.05, MeOH); CD (MeOH) $\Delta\varepsilon_{205}$ −13.36, $\Delta\varepsilon_{228}$ −52.32; (−)-**2**}; UV (MeOH) λ_{max} (log ε) 280 (3.79), 204 (4.81) nm; ESIMS *m/z* 347 $[M-H]^-$; HRESIMS *m/z* 347.1137 $[M-H]^-$ (calcd for $C_{18}H_{19}O_7$, 347.1131). ^1H and ^{13}C NMR data, see Tables 1 and 3.
12. *Blapsol C* (**3**): Yellowish solid; {$[\alpha]_D^{22}$ +94.8 (*c* 0.16, MeOH); CD (MeOH) $\Delta\varepsilon_{205}$ +97.87, $\Delta\varepsilon_{228}$ +8.65; (+)-**3**}; {$[\alpha]_D^{22}$ −168.5 (*c* 0.13, MeOH); CD (MeOH) $\Delta\varepsilon_{205}$ −38.93, $\Delta\varepsilon_{228}$ −9.65; (−)-**3**}; UV (MeOH) λ_{max} (log ε) 280 (3.81), 204 (4.79) nm; ESIMS *m/z* 333 $[M-H]^-$; HRESIMS *m/z* 333.0976 $[M-H]^-$ (calcd for $C_{17}H_{17}O_7$, 333.0974). ^1H and ^{13}C NMR data, see Tables 2 and 3.
13. *Blapsol D* (**4**): Yellowish solid; {$[\alpha]_D^{22}$ +83.9 (*c* 0.08, MeOH); CD (MeOH) $\Delta\varepsilon_{205}$ +44.65, $\Delta\varepsilon_{228}$ +10.58; (+)-**4**}; {$[\alpha]_D^{22}$ −62.9 (*c* 0.09, MeOH); CD (MeOH) $\Delta\varepsilon_{205}$ −32.33, $\Delta\varepsilon_{228}$ −7.45; (−)-**4**}; UV (MeOH) λ_{max} (log ε) 280 (3.83), 204 (4.82) nm; ESIMS *m/z* 333 $[M-H]^-$; HRESIMS *m/z* 333.0978 $[M-H]^-$ (calcd for $C_{17}H_{17}O_7$, 333.0974). ^1H and ^{13}C NMR data, see Tables 2 and 3.
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21. *Cyclooxygenase (COX) inhibitory assay:* All the compounds were evaluated for their COX inhibitory activities in vitro by using a Cayman's COX Fluorescent Inhibitor Screening Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA).[22] Briefly, ovine COX-1 and human recombinant COX-2 enzymes were pre-incubated with serially diluted test compounds for 15 min at room temperature, and then heme and the fluorometric substrate were added and incubated for another 15 min at room temperature. The reaction was started by the addition of arachidonic acid and allowed to proceed for 2 min. The fluorescence intensity was measured at 595 nm using 530 nm excitation on a micro plate reader (Envision, PerkinElmer). The data were analyzed using Graphpad Prism 5 (Graphpad Software Inc.). Celecoxib (Sigma, St. Louis, MO, USA) was used as the positive control.
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