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Identification of Ten New *N*-acetyldopamine Dimers from Periostracum Cicadae

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ABSTRACT

Periostracum Cicadae is the cast-off shell of the cicada *Cryptotympana pustulata* Fabricius, it is a widely used animal based traditional folk medicine, it is found to have many effects including antipyretic, antiallergic and antioxidant activities. In this study, ten *N*-acetyldopamine dimers, named Cicadamide C1–C10 (compounds **1–10**), were isolated from Periostracum Cicadae. One-dimensional NMR, two-dimensional NMR, mass spectrometry, CD spectroscopy, and chemical evidence were performed to further determine their structures. In the results, ten *N*-acetyldopamine dimers were isolated and their structures were elucidated. This study provides a basic reference for further biological effects study on Periostracum Cicadae.

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Key words
***N*-acetyldopamine dimers, Periostracum cicadae, Traditional Chinese medicine, Cicadamide**

INTRODUCTION

Periostracum Cicadae is a well-known animal based traditional folk medicine. It is the cast-off shell of *Cryptotympana pustulata* Fabricius, commonly known as the black cicada, which is mainly distributed in Shandong, Henan, Hubei, and Sichuan Provinces of China. In traditional Chinese medicinal practice, Periostracum Cicadae, is considered to be cold-natured and have a sweet flavor, and is used for its therapeutic effect against vitiligo (Zhang and Che, 2004), anti-type IV allergic activity (Lin *et al*., 2001), an inhibitory effect on diabetic retinopathy (Xing, 2010), and anticonvulsant activity (An, 2008).

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The clinical efficacy of Periostracum Cicadae is a consequence of its chemical constituents. Its crude extract has been reported to exhibit a variety of biological activities when it was administered by various routes. Hsieh *et al*. (1991) demonstrated that the water extract of Periostracum Cicadae had anticonvulsitant, sedative and hypothermic effects through pharmacological research. Shin *et al*. (1999) summarized the effects of oriental medicines including one from *Cryptotympana* on the systemic anaphylactic reactions induced by compound 48/80, and demonstrated that *Cryptotympana atrata* could significantly inhibit the rate of mast cells degranulation and systemic anaphylactic reaction, indicating that it may be beneficial to treat nonspecific anaphylaxis. Yang *et al*. (2013) explored the method of analyzing trace elements from Periostracum Cicadae. Liu *et al*. (2004) researched the effects of Periostracum Cicadae water extract on hemorheology in hyperlipidemic rats. The results showed that Periostracum Cicadae could significantly improve its hemorheology, which was reflected in the significant reduction of whole blood and plasma viscosity, thrombosis *in vitro*, erythrocyte aggregation index, serum triglyceride and total cholesterol levels. Wang *et al*. (2010) used

different extraction methods and solvents to preliminarily isolate and study the antibacterial activity of the active ingredients of Periostracum Cicadae. It was found that the extracts obtained by different extraction methods had obvious antibacterial effects, but the differences between them were not significant, which showed that the active ingredients of Periostracum Cicadae had strong antibacterial activities.

Previous reports on its biological components have revealed that it is rich in dopamine (Noda *et al*., 2000; Yang *et al*., 2016; Liu *et al*., 2019). Oxenkrug and Requintina (2005) studied the effect of *N*-acetyldopamine on lipopolysaccharide (LPS) induced lipid peroxidation in the form of malondialdehyde (MDA) by measuring the thiobarbituric acid (TBA) reactive substances in rat brain homogenates *in vitro*, and found that *N*-acetyldopamine inhibited the formation of MDA in a concentration dependent manner and its effect was stronger than that of melatonin. Xu *et al*. (2006) isolated two *N*-acetyldopamine dimers from the methanolic extracts of Periostracum Cicadae and showed that they both exhibited antioxidant and anti-inflammatory activities in LPS induced RAW264.7 cells. Lu *et al*. (2015) identified three new *N*-acetyldopamine dimers from Dung Beetle *Catharsius molossus*, a similar traditional Chinese Medicine from insects.

In this study, we further investigated the phytochemistry of Periostracum Cicadae, with the aim of identifying the previously unknown phthalides with biologically activity from this folk medicine, the structures of new identified compounds were established using spectroscopic methods. In the result, 10 new compounds (**1–10**) were isolated from Periostracum Cicadae (Fig. 1). Herein, we describe the isolation and structural elucidation of compounds **1–10**.

MATERIALS AND METHODS

Materials

The dried Periostracum Cicadae in this study was purchased from a traditional medicine market in Urumuqi, Xinjiang, China. It was identified by Prof. Jincai Lu from School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University. The sample of Periostracum Cicadae was further deposited at Research Department of Natural Medicine of Shenyang Pharmaceutical University, with a voucher specimen (No. 20081001). The others relevant chemical reagents were analytical pure.

General experimental procedures

HR-ESI-MS was performed on a waters LCT Premier KE399 mass spectrometer (Waters Corp., Milford, MA, USA). A model MOS-450 Chiral Detector (Bio-Logic SAS, Caix, France) was used for CD analysis. The one and two-dimensional NMR spectra were recorded in CD3OD on a Bruker AV-600 spectrometer (1H, 600 MHz; 13C, 150 MHz) (Bruker Corp., Billerica, MA, USA) using tetramethylsilane (TMS) as the internal standard. Preparative HPLC was carried out using a waters 2998 photodiode array detector at 220 nm with a waters 2695 separation module (Waters Corp., Billerica, MA, USA) and a Shim-pack CLC-ODS reversed-phase column (No. 61514407B; Shimadzu Corp., Kyoto, Japan). Silica gel for chromatography was obtained from Oceanview Chemical Group Co. Ltd. (Qingdao, China).

Extraction and isolation

The powder of dried Periostracum Cicadae (5 kg) was extracted with EtOH (50 L) for 3 times under reflux conditions, each time for 3 h. The combined EtOH extracts (76 g) were concentrated *in vacuo*. A part of the EtOH fraction (70 g) was subjected to silica gel column chromatography (250 g) with a gradient of $CHCl_3$/MeOH to afford 14 fractions (100:0–0:100) that were designated A–O.

Fraction F ($CHCl_3$/MeOH, 100:5 vol/vol; 3.4834 g) was further subjected to ODS column elution with MeOH/H_2O (40:60 vol/vol), after which a fraction (107.6 mg) of the eluted material was purified by preparative RP-HPLC (MeOH/H_2O, 30:70 vol/vol) to obtain compound **2** (13.1 mg). Fraction G ($CHCl_3$/MeOH, 100:8 vol/vol; 5.9045 g) was further purified by ODS column elution with MeOH/H_2O (50:50 vol/vol) to yield 3 fractions. Fraction D_1 (1.7607 g) was purified by preparative RP-HPLC (MeOH/H_2O, 40:60 vol/vol) to obtain compounds **1** (40 mg), **3** (40.6 mg), **4** (11.0 mg), and **5** (159.3 mg). Fraction D_3 (0.4297 g) was purified by preparative RP-HPLC (MeOH/H_2O, 42:58) to obtain compounds **9** (13.0 mg) and **10** (15.0 mg). Fraction H ($CHCl_3$/MeOH, 100:10, 3.047 g) was further purified by ODS column elution with MeOH/H_2O (40:60 vol/vol) to yield a fraction (0.2313 g) that was purified by preparative RP-HPLC (MeOH/H_2O, 37:73 vol/vol) to obtain compounds **6** (25.0 mg),**7** (35.5 mg), and **8** (13.1 mg). The detailed compound characterization and related information of **1-10** were listed in Supplementary Table S1.

RESULTS AND DISCUSSION

Ten isolated compounds

As a result of our investigation, 10 new compounds (**1–10**) were isolated from Periostracum Cicadae (Fig. 1). NMR and CD spectra (Figs. 2 and 3) were used to further identify the structures of Compounds **1–10**.

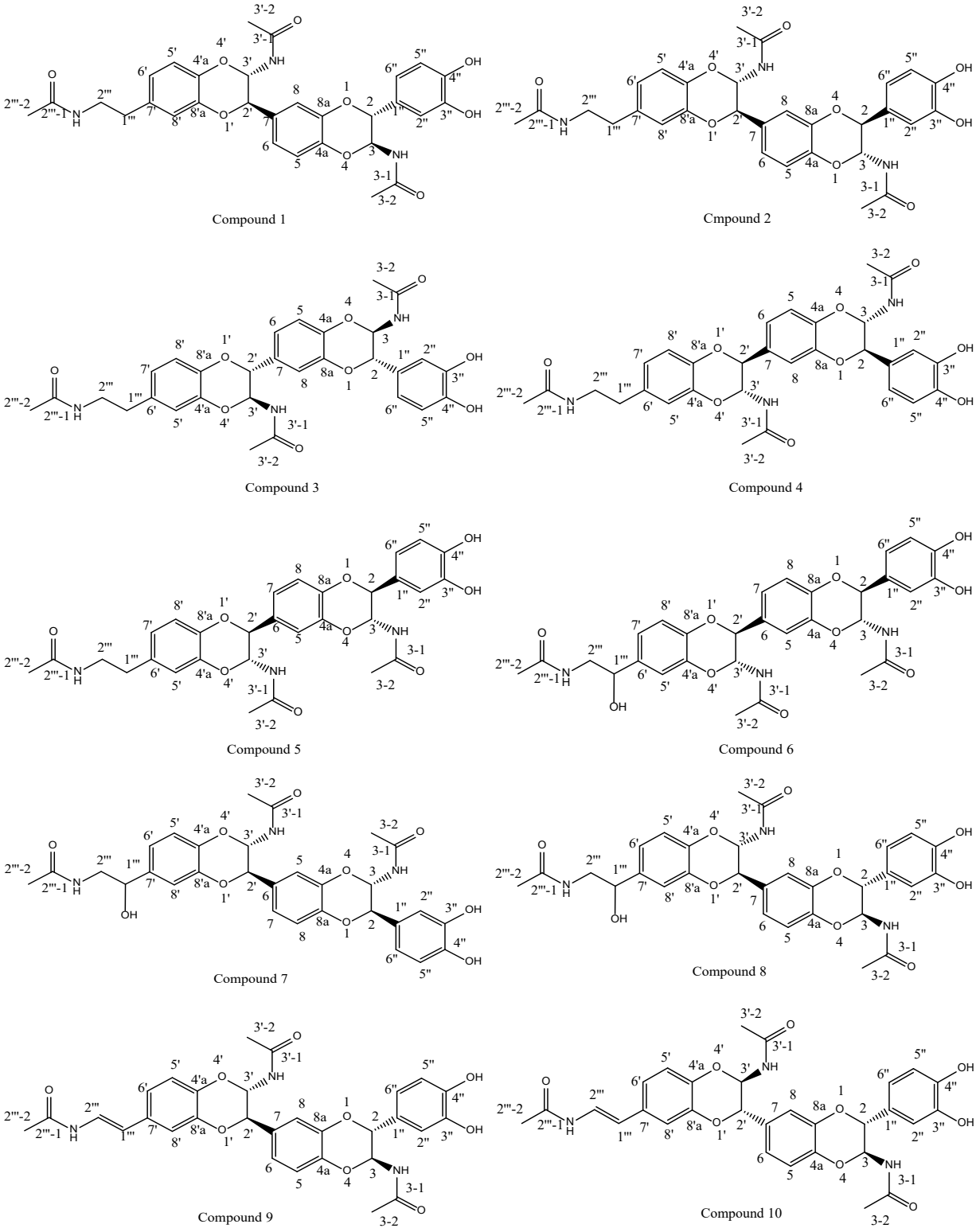

Fig. 1. Structures of compounds **1–10**.
Note: The structural diagrams of these compounds were drawn based on the analysis of ^{13}C-NMR, ^{1}H-NMR, HMBC and HMQC data, but not every compound needs to do HMQC.

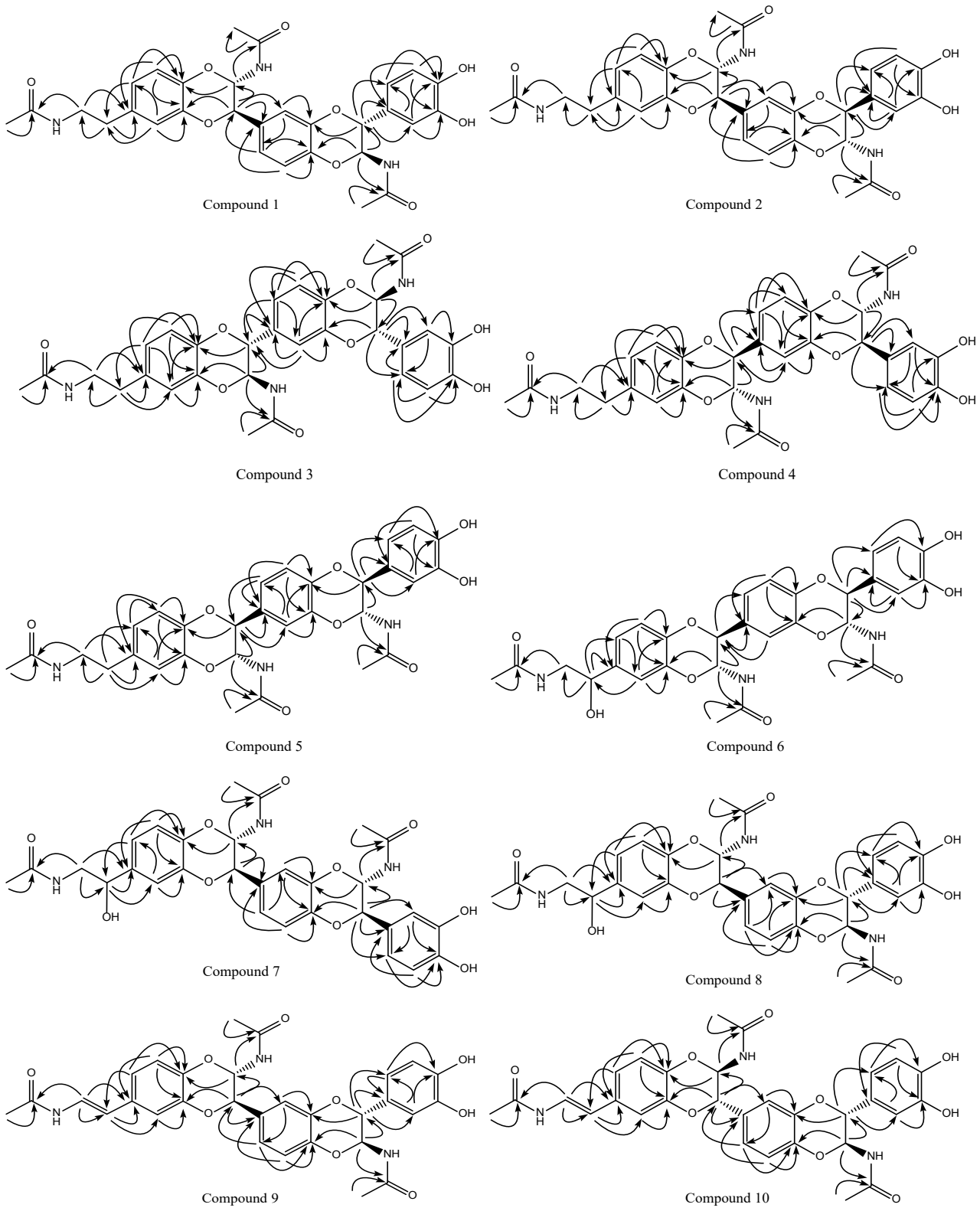

Fig. 2. Key HMBC correlations of compounds **1–10**.

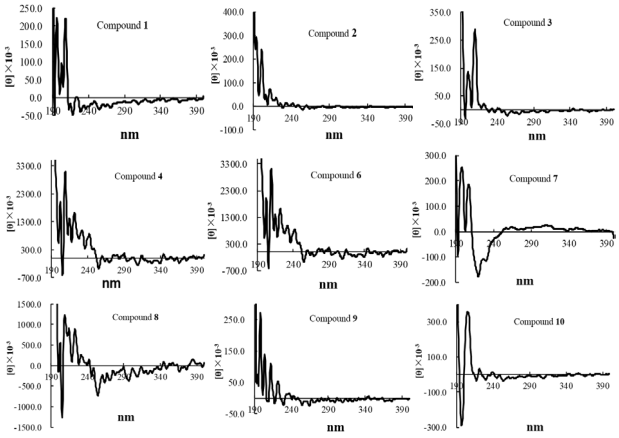

Fig. 3. CD spectral data of compounds **1–10**.
Note: After spectrum analysis based on ^{13}C-NMR, ^{1}H-NMR and HMQC data, compound **5** and compound **3** were found to be isomers, so the CT scan images of compound 5 is not given here.

Structure elucidation of the compounds

Compound **1** was obtained as a white powder. The molecular formula of **1** was established as $C_{30}H_{31}N_3O_9$ on the basis of its HR-ESI-MS data (*m/z* 578.2160 $[M+H]^+$; calcd. 578.2139 for $C_{30}H_{32}N_3O_9$). The UV spectrum of **1** exhibited λ_{max} at 280.6 nm (MeOH).

The ^{1}H-NMR (600 MHz, CD_3OD) spectrum of **1** (Table I) showed 3 signals of ABX-type spin systems in the aromatic region (δ 6.77–7.05); 3 singlet peaks at δ 1.90 (*s*, 3H, H-3-2), δ 1.94(*s*, 3H, H-3′-2), and δ 1.92(*s*, 3H, H-2‴-2); signals ascribable to 2 methylenes at δ 2.73(*t*, 2H, *J* = 7.2 Hz, H-1‴) and δ 3.38(*t*, 2H, *J* = 7.2 Hz , H-2‴); and 4 methine protons at δ 4.76(*d*, 1H, *J*=7.2 Hz, H-2), δ 4.80(*d*, 1H, *J*=7.2 Hz, H-2′), δ 5.77(*d*, 1H, *J*=7.2 Hz, H-3), and δ 5.72(*d*, 1H, *J*=7.2 Hz, H-3′). The ^{13}C-NMR (150 MHz, CD3OD) spectrum (Table I) of 1 exhibited 30 signals and an acetamide structure of the carbonyl carbon signals at δ 173.3(C-3-1), δ 173.3(C-3′-1), and δ 173.3(C-2‴-1), in addition to 18 signals in the aromatic region and signals ascribable to 3 methyl groups.

Analysis of HMBC (and HMQC, but not every compound needed to do HMQC) spectrum was performed to allot the H-atoms to their bonded C-atoms (Fig. 1 and Table I). The information concerning the location of these units was obtained from the HMBC experiment (Fig. 2). The HMBC correlations (Fig. 2 and Table I) indicated long-range couplings between the methine protonsignal at δ 5.77(H-3) and both the carbon signal at δ 144.4(C-4a) and the *N*-acetylamino carbon signal at δ 173.3 (C-3-1), as well as between the proton signal at δ 4.76(H-2) and both the carbon signal at δ 144.2(C-8a) and the 3, 4-substituted benzene carbon signal at 128.5(C-1″), suggesting the presence of unit A (Fig. 2). The methine proton signal at δ 5.72(H-3′) was correlated with the carbons at δ 142.1(C-4′a) and δ 173.3(C-2‴-1), and the methylene proton signal at δ 3.39(H-2‴) was correlated with the 1, 4-benzodioxane moiety carbon signal at δ 134.3(C-7′), δ 2.73(H-1‴), and δ 173.3(C-2‴-1). According to these results, the *N*-acetylamino and *N*-acetylamino-2-ethyl groups were located at the 3′ and 7′ positions of the 1,4-benzodioxane moiety, indicating the presence of unit B (Fig. 2). Finally, the HMBC correlations from 4.80(H-2′) to δ 130.9(C-7), showed a linkage among units A and B.

Table I. ^{1}H-NMR, ^{13}C-NMR, and HMBC spectral data of compound 1.

Position	δ(H)(J in Hz)	δ(C)	HMBC
2	4.76 (*d*, *J*=7.2 Hz, 1H)	78.2	C-8a, 1″, 2″, 6″, 3
3	5.77 (*d*, *J*=7.2 Hz, 1H)	77.8	C-4a, 1″, 2, 3-1
5	6. 93 (*dd*, *J*=4.2, 8.4 Hz, 1H)	118.0	C-6, 7, 4a
6	6.98 (*dd*,*J*=1.8, 8.4 Hz, 1H)	122.3	C-8, 4a, 2′
7		130.9	
8	7.05 (*dd*,*J*=1.8, 8.4 Hz, 1H)	117.4	C-6, 7, 8a, 2′
4a		144.4	
8a		144.2	
2′	4.80 (*d*, *J*=7.2Hz, 1H)	78.3	C-6, 7, 8, 8′a, 3′
3′	5.72 (*d*, *J*=7.2Hz, 1H)	77.9	C-4′a, 7′, 2′, 3′-1
5′	6.85 (*dd*, *J*=2.4, 8.4 Hz, 1H)	118.1	C-6′, 7′, 4′a, 8′a
6′	6.77 (*dd*, *J*=8.4 Hz, 1H)	123.3	C-5′, 8′, 4′a, 1‴
7′		134.3	
8′	6.84 (*dd*, *J*=2.4, 8.4 Hz, 1H)	118.1	C-7′, 4′a, 8′a, 1‴
4′a		142.1	
8′a		144.1	
1″		128.5	
2″	6.87 (*d*, *J*=7.8 Hz, 1H)	115.6	C- 3″, 4″, 6″, 2
3″		146.5	
4″		147.2	
5″	6.79 (*d*, *J*=8.4 Hz, 1H)	116.2	C-1″, 3″, 4″
6″	6.78(*d*, *J*=8.4 Hz, 1H)	120.6	C- 2″, 4″, 2
1‴	2.73(*t*, *J*=7.2Hz, 2H)	35.7	C- 6′, 7′, 8′, 2‴
2‴	3.39(*t*, *J*=7.2Hz, 2H)	42.1	C-7, 1‴, 2″-1
2‴-1		173.3	
2‴-2	1.92 (*s*, 3H)	22.6	C- 2″-1
3-1		173.3	
3-2	1.91 (*s*, 3H)	22.6	C- 3 -1
3′-1		173.3	
3′-2	1.91 (*s*, 3H)	22.5	C- 3′ -1

The trans-configuration of the 1,4-benzodioxane moiety of **1** was confirmed by the coupling constants (*J* = 7.2 Hz) between protons H-2 and H-3. In the CD spectrum of **1** (Fig. 3), a negative Cotton effect at 235 nm and 280

nm (^1L_b) was observed (Wu, 2009). Therefore, based on existing reports (Yang *et al*., 2012; Noda *et al*., 2000), the absolute stereochemistry of **1** was determined to be (2*S*,3*R*,2′*R*,3′*S*). Thus, **1** was identified as (2*S*,3*R*,2′*R*,3′*S*)-2-(3′,4′-dihydroxyphenyl)-3-acetylamino-7-(3-acetylamino-7-(*N*-acetyl-2-aminoethyl)-1,4-benzodioxan-2-yl)-1,4-benzodioxane (Fig. 2) and named Cicadamide C1 (**1**).

Table II. ^{1}H-NMR, ^{13}C-NMR, and HMBC spectral data of compound 2.

Position	δ(H)(J in Hz)	δ(C)	HMBC
2	4.71 (*d*, *J*=7.2 Hz, 1H)	78.2	C-1″, 2″, 6″, 3, 8a
3	5.75 (*d*, *J*=7.2 Hz, 1H)	78.4	C-1″, 2, 3-1, 4a
5	7.05 (*br.s*. 1H)	117.3	C-6, 7, 4a, 2′
6	6.99 (*d*,*J*=7.8 Hz, 1H)	122.4	C-5, 4a, 2′
7		130.9	
8	6.93 (*d*,*J*=7.8 Hz, 1H)	118.0	C-5, 6, 8a
4a		144.5	
8a		144.3	
2′	4.79 (*d*, *J*=7.2Hz, 1H)	78.2	C-6, 7, 8, 3′, 8′a
3′	5.73 (*d*, *J*=7.2Hz, 1H)	77.9	C-6, 2′, 3′-1, 4′a
5′	6.83 (*dd*, *J*=7.2,13.8 Hz, 1H)	118.1	C-6′, 4′a,
6′	6.78 (*dd*, *J*=7.2,13.8 Hz, 1H)	123.4	C-5′, 8′, 4′a, 1‴
7′		134.3	
8′	6.85 (*dd*, *J*=7.2,13.8 Hz, 1H)	118.1	C-7′, 6′, 8′a, 1‴
4′a		142.1	
8′a		144.1	
1″		128.5	
2″	6.86 (*d*, *J*=7.2 Hz, 1H)	115.5	C- 1″, 4″, 6″, 2
3″		146.5	
4″		147.2	
5″	6.79 (*d*, *J*=7.2 Hz, 1H)	116.2	C-1″, 3″
6″	6.77 (*d*, *J*=7.2 Hz, 1H)	120.6	C- 2″, 4″, 2
1‴	2.73 (*t*, *J*=7.2Hz, 2H)	35.7	C-6′, 7′, 8′, 2″
2‴	3.38 (*t*, *J*=7.2Hz, 2H)	42.1	C-7′, 1‴, 2″-1
2‴-1		173.3	
2‴-2	1.92 (*s*, 3H)	22.6	C- 2″-1
3-1		173.3	
3-2	1.91 (*s*, 3H)	22.6	C- 3 -1
3′-1		173.3	
3′-2	1.91 (*s*, 3H)	22.5	C- 3′ -1

Compound **2** was obtained as a white powder. The molecular formula of **2** was determined to be $C_{30}H_{31}N_3O_9$ on the basis of its HR-ESI-MS data (*m/z* 578.2141 $[M+H]^+$; calcd. 578.2139 for $C_{30}H_{32}N_3O_9$). The ^{1}H-NMR and ^{13}C-NMR spectra (Table II) of **2** were similar to those of **1**. The HMBC data suggested that the planar structure of **2** was identical to that of **1**. Therefore, **2** may be a diastereomer of **1** at chiral centers C-2 and C-3. The CD spectrum of **2** showed a negative Cotton effect at 235 nm and a positive Cotton effect at 280 nm (^1L_b). The absolute stereochemistry of **2** was determined to be (2*R*,3*S*,2′*R*,3′*S*) by the 2-phenyl-1,4-benzodioxane CD rule (Wu, 2009; Yang *et al*., 2012) and comparison with **1**. Thus, compound **2** was identified as (2*R*,3*S*,2′*R*,3′*S*)-2-(3′,4′-dihydroxyphenyl)-3-acetylamino-7-(3-acetylamino-7-(*N*-acetyl-2-aminoethyl)-1,4-benzodioxan-2-yl)-1,4-benzodioxane (Fig. 2) and named Cicadamide C2 (**2**).

Compound **3** was obtained as a white powder. The molecular formula of **3** was determined to be $C_{30}H_{31}N_3O_9$ on the basis of its HR-ESI-MS data (*m/z* 578.2111 $[M+H]^+$; calcd. 578.2139 for $C_{30}H_{32}N_3O_9$). The ^{1}H-NMR and ^{13}C-NMR spectral data of **3** indicated that its structure was closely related to that of **1**, and suggested that **3** was a positional isomer of **1** (Table III). The HMBC analysis of **3** revealed that **1** and **3** differed only in the position of the *N*-acetylamino-2-ethyl group (Fig. 2). The HMBC correlations from the methylene proton signal at *δ* 3.37(H-2‴) correlated with the 1,4-benzodioxane moiety carbon signal at *δ* 134.4(C-6′), which was located at the 6′-position in **3** and at the 7′-position in **1**. In the CD spectrum of **3**, negative Cotton effects at 235 nm and 280 nm (^1L_b) were observed. The absolute stereochemistry of **3** was determined to be (2*S*,3*R*,2′*S*,3′*R*) by the 2-phenyl-1,4-benzodioxane CD rule (Wu, 2009; Yang *et al*., 2012), and comparison with **1**. Thus, compound **3** was identified as (2*S*,3*R*,2′*S*,3′*R*)-2-(3′,4′-dihydroxyphenyl)-3-acetylamino-7-(3-acetylamino-6-(*N*-acetyl-2-aminoethyl)-1,4-benzodioxan-2-yl)-1,4-benzodioxane (Fig. 2) and named Cicadamide C3 (**3**).

Compound **4** was obtained as a white powder. The molecular formula of **4** was determined to be $C_{30}H_{31}N_3O_9$ on the basis of its HR-ESI-MS data (*m/z* 578.2114 $[M+H]^+$; calcd. 578.2139 for $C_{30}H_{32}N_3O_9$). The ^{1}H-NMR and ^{13}C-NMR spectral data of **4** (Table IV) indicated that its structure was closely related to that of **3**. The HMBC data suggested that the planar structure of **4** was identical to that of **3**. Therefore, **4** may be a diastereomer of **3** at chiral centers C-2 and C-3, C-2′, and C-3′. In the CD spectrum of **4**, positive Cotton effects at 235 nm and 280 nm (^1L_b) were observed (Wu, 2009; Yang *et al*., 2012). The absolute stereochemistry of **4** was determined to be (2*R*,3*S*,2′*R*,3′*S*) by the 2-phenyl-1,4-benzodioxane CD rule (Wu, 2009; Yang *et al*., 2012) and comparison with **2.** Thus, compound **4** was identified as (2*R*,3*S*,2′*R*,3′*S*)-2-(3′,4′-dihydroxyphenyl)-3-acetylamino-7-(3-acetylamino-

6-(*N*-acetyl-2-aminoethyl)-1,4-benzodioxan-2-yl)-1,4-benzodioxane (Fig. 2) and named Cicadamide C4 (**4**).

Table III. ^{1}H-NMR, ^{13}C-NMR, and HMBC spectral data of compound 3.

Position	δ(H)(J in Hz)	δ(C)	HMBC
2	4.76 (*d*, *J*=7.2 Hz, 1H)	78.3	C- 1″, 2″, 6″, 4a
3	5.73 (*d*, *J*=7.2 Hz, 1H)	77.7	C- 8a, 3-1, 2
5	6. 92 (*s*, 1H)	118.1	C- 4a, 6, 7
6	6. 97 (*dd*, *J*=3.0, 7.8 Hz, 1H)	122.2	C- 4a, 8, 2′
7		130.9	
8	7.03(*br.s*, 1H)	117.4	C-4a, 8a, 6, 7, 2′
4a		144.2	
8a		143.2	
2′	4.79 (*d*, *J*=7.2Hz, 1H)	78.2	C- 6, 7, 8, 8′a
3′	5.73 (*d*, *J*=7.2Hz, 1H)	78.2	C- 4′a, 3′-1, 2′
5′	6.78 (*d*, *J*=7.2 Hz, 1H)	118.2	C- 4′a, 8′a, 7′
6′		134.4	
7′	6.75 (*dd*, *J*=1.8, 8.4 Hz, 1H)	123.2	C- 8′a, 5′, 1‴
8′	6.91 (*d*, *J*=7.2 Hz, 1H)	117.9	C-4′a, 8′a, 6′, 7′
4′a		144.3	
8′a		142.7	
1″		128.5	
2″	6.79(*br.s*, 1H)	116.1	C- 1″, 3″, 6″
3″		146.4	
4″		147.2	
5″	6.88 (*d*, *J*=4.2 Hz, 1H)	115.6	C- 4″, 6″
6″	6.77 (*br.s*, 1H)	120.6	C- 1″, 4″, 2
1‴	2.71(*t*, *J*=7.2Hz, 2H)	35.7	C- 6′, 7′, 2‴, 5′
2‴	3.37(*t*, *J*=7.2Hz, 2H)	42.1	C- 6′, 2‴-1, 1‴
2‴-1		173.2	
2‴-2	1.92 (*s*, 3H)	22.6	C- 2‴-1
3-1		173.2	
3-2	1.91 (*s*, 3H)	22.5	C- 3-1
3′-1		173.3	
3′-2	1.90 (*s*, 3H)	22.6	C- 3′-1

Compound **5** was obtained as a white powder. The molecular formula of **5** was determined to be $C_{30}H_{31}N_3O_{10}$ on the basis of its HR-ESI-MS data (*m/z* 578.2144 $[M+H]^+$; calcd. 578.2139 for $C_{30}H_{32}N_3O_{10}$). The ^{1}H-NMR and ^{13}C-NMR spectral data of **5** (Table V) indicated that its structure was closely related to that of **3**, and suggested that **5** was a positional isomer of **3**. The HMBC analysis of **5** (Fig. 2) revealed that **3** and **5** differed only in the position of the A group. The HMBC correlations from δ 4.86(H-2′) to δ 129.7(C-6) demonstrated the linkage among units A and B, which was located at the 6-position in **5** and at the 7-position in **3.** Thus, compound **5** was identified as 2-(3′,4′-dihydroxyphenyl)-3-acetylamino-6-(3-acetylamino-6-(*N*-acetyl-2-aminoethyl)-1,4-benzodioxan-2-yl)-1,4-benzodioxane (Fig. 2) and named Cicadamide C5 (**5**).

Compound **6** was obtained as a white powder. The molecular formula of **6** was determined to be $C_{30}H_{31}N_3O_9$ on the basis of its HR-ESI-MS data (*m/z* 594.2054 $[M+H]^+$; calcd. 594.2088 for $C_{30}H_{32}N_3O_9$). The 1H-NMR and 13C-NMR spectra data of **6** (Table VI) were similar to those of **5**, with the exception of the absence of the hydroxyl group located at δ 48.1(C-2‴) in compound **5**. The HMBC experiment suggested that the planar structure of **6** was identical to that of **5**. The methine protons at δ3.37(C-1‴) and δ 3.43 (C-1‴) were correlated with δ 73.0(C-6) and δ 173.6(C-2‴-1), and δ 4.65 (H-2‴) was correlated with δ 48.1(C-2‴) and δ 137.9(C-6′), indicating an *N*-acetylamino-2-glyoxyl group. In the CD spectrum of **6**, positive Cotton effects at 235 nm and 280 nm ($^{1}L_b$) were observed. The absolute stereochemistry of **6** was determined to be (2*R*,3*S*,2′*R*,3′*S*) by the 2-phenyl-1,4-benzodioxane CD rule (Wu, 2009; Yang *et al*., 2012) and comparison with **3**. Thus, compound **6** was identified as (2*R*,3*S*,2′*R*,3′*S*)-2-(3′,4′-dihydroxyphenyl)-3-acetylamino-6-(3-acetylamino-6-(*N*-acetyl-2-amino-1-hydroxylethyl)-1,4-benzodioxan-2-yl)-1,4-benzodioxane (Fig. 2) and named Cicadamide C6 (**6**).

Compound **7** was obtained as a white powder. The molecular formula of **7** was determined to be $C_{30}H_{31}N_3O_{10}$ on the basis of its HR-ESI-MS data (*m/z* 594.2082 $[M+H]^+$; calcd. 594.2088 for $C_{30}H_{32}N_3O_{10}$). The ^{1}H-NMR and ^{13}C-NMR spectral data of **7** (Table VII) indicated that its structure was closely related to that of **6**, and suggested that **7** was a positional isomer of **6**. The HMBC analysis of **7** (Fig. 2) revealed that **6** and **7** differed only in the position of the *N*-acetylamino-2-ethyl group. The HMBC data showed that the methylene proton signal at δ3.37(C-2‴) and δ 3.45(C-2‴) was correlated with the 1,4-benzodioxane moiety carbon signal at δ 137.7(C-7′), which was located at the 7′-position in **7** and at the 6′-position in **6**. The CD spectrum of **7** showed a negative Cotton effect at 235 nm and a positive Cotton effect at 280 nm ($^{1}L_b$). The absolute stereochemistry of **7** was determined to be (2*R*,3*S*,2′*R*,3′*S*) by the 2-phenyl-1,4-benzodioxane CD rule (Wu, 2009; Yang *et al*., 2012) and comparison with **1**. Thus, compound **7** was identified as (2*R*,3*S*,2′*R*,3′*S*)-2-(3′,4′-dihydroxyphenyl)-3-acetylamino-6-(3-acetylamino-7-(*N*-acetyl-2-amino-1-hydroxylethyl)-

1,4-benzodioxan-2-yl)-1,4-benzodioxane (Fig. 2) and named Cicadamide C7 (**7**).

Table IV. ^{1}H-NMR, ^{13}C-NMR, and HMBC spectral data of compound 4.

Position	δ(H)(J in Hz)	δ(C)	HMBC
2	4.77 (*d*, *J*=7.2 Hz, 1H)	78.1	C-8a, 1″, 2″, 6″,3
3	5.76 (*d*, *J*=7.2 Hz, 1H)	77.7	C-4a, 2,3-1
5	6. 90 (*d*, *J*=8.4 Hz, 1H)	118.0	C-7, 8a
6	6.97 (*dd*,*J*=1.8, 8.4 Hz, 1H)	122.3	C-5, 8, 4a
7		130.9	
8	7.04 (*br.s*, 1H)	117.3	C-6, 7, 4a, 8a
4a		144.1	
8a		144.3	
2′	4.79 (*d*, *J*=7.2Hz, 1H)	78.3	C-8′a, 6, 7, 8, 3′
3′	5.75 (*d*, *J*=7.2Hz, 1H)	78.1	C-4′a, 3′-1, 2′
5′	6.76(*br.s*, 1H)	118.2	C-4′a, 8′a, 7′, 1‴
6′		134.3	
7′	6.72 (*dd*, *J*=1.8, 8.4 Hz, 1H)	123.1	C-5′, 8′a, 1‴
8′	6.87 (*d*, *J*=8.4 Hz, 1H)	117.9	C-8′a, 6′, 7′
4′a		143.3	
8′a		142.7	
1″		128.5	
2″	6.88 (*d*, *J*=7.8 Hz, 1H)	115.5	C- 4″, 6″
3″		146.4	
4″		147.1	
5″	6.80 (*d*, *J*=8.4 Hz, 1H)	116.2	C-6″, 4″, 3″
6″	6.76(*br.s*, 1H)	120.6	C-2″, 4″
1‴	2.71(*t, J*=7.2Hz, 2H)	35.7	C-5′, 6′, 7′, 2‴
2‴	3.37(*t, J*=7.2Hz, 2H)	42.1	C-6′, 1‴, 2‴-1
2‴-1		173.2	
2‴-2	1.92 (*s*, 3H)	22.7	
3-1		173.2	
3-2	1.90 (*s*, 3H)	22.6	
3′-1		173.2	
3′-2	1.86 (*s*, 3H)	22.6	

Compound **8** was obtained as a white powder. The molecular formula of **8** was determined to be $C_{30}H_{31}N_3O_{10}$ on the basis of its HR-ESI-MS data (*m/z* 594.2012 $[M+H]^+$; calcd. 594.2088 for $C_{30}H_{32}N_3O_{10}$). The ^{1}H-NMR and ^{13}C-NMR spectral data of **8** (Table VII) indicated that its structure was closely related to that of **7**, and suggested that **8** was a positional isomer of **7**. The HMBC analysis of **8** (Fig. 2) revealed that **7** and **8** differed only in the position of the A group. The HMBC correlations from δ 4.82(H-2′) to δ 130.9(C-7) demonstrated the linkage among units A and B, which was located at the 6-position in **7** and at the 7-position in **8**. The CD spectrum of **8** showed a positive Cotton effect at 235 nm and a negative Cotton effect at 280 nm ($^{1}L_b$). The absolute stereochemistry of **8** was determined to be (2*R*,3*S*,2′*R*,3′*S*) by the 2-phenyl-1,4-benzodioxane CD rule (Wu, 2009; Yang *et al*., 2012) and comparison with **1**. Thus, compound **8** was identified as (2*R*,3*S*,2′*R*,3′*S*)-2-(3′,4′-dihydroxyphenyl)-3-acetylamino-7-(3-acetylamino-7-(*N*-acetyl-2-amino-1-hydroxylethyl)-1,4-benzodioxan-2-yl)-1,4-benzodioxane and named Cicadamide C8 (**8**).

Table V. ^{1}H-NMR, ^{13}C-NMR, and HMBC spectral data of compound 5.

Position	δ(H)(J in Hz)	δ(C)	HMBC
2	4.79 (*d*, *J*=7.2 Hz, 1H)	76.7	C-8a, 1″, 2″, 6″, 3
3	5.66 (*d*, *J*=7.2 Hz, 1H)	75.7	C-2,3-1
5	6. 99 (*d*, *J*=7.2 Hz, 1H)	116.2	C-8a, 4a, 7
6		129.7	
7	6. 90 (*d*, *J*=9.0 Hz, 1H)	120.7	C-6, 2′
8	6. 97 (*d*, *J*=3.6 Hz, 1H)	116.8	C-4a, 8a, 6
4a		142.0	
8a		143.0	
2′	4.86 (*d*, *J*=7.2Hz, 1H)	76.5	C-8′a, 5, 6, 7, 3′
3′	5.68 (*d*, *J*=7.2Hz, 1H)	76.3	C-2′, 3′-1
5′	6.77(*br.s*, 1H)	116.8	C-8′a, 4′a, 7′
6′		133.3	
7′	6.71 (*d*, *J*=8.4 Hz, 1H)	121.7	C-5′, 8′
8′	6.89 ((*d*, *J*=2.4, 8.4 Hz, 1H))	116.1	C-4′a, 8′a, 6′
4′a		141.9	
8′a		141.0	
1″		126.6	
2″	6.81(*br.s*, 1H)	114.9	C-4″, 6″
3″		145.2	
4″		145.9	
5″	6.74 (*d*, *J*=7.8 Hz, 1H)	115.4	C-1″, 3″
6″	6.72 (*d*, *J*=7.8 Hz, 1H)	119.1	C-2″, 4″
1‴	2.62(*t, J*=7.2Hz, 2H)	34.4	C-2‴, 5′, 6′, 7′
2‴	3.24(*t, J*=7.2Hz, 2H)	40.3	C-1‴, 2‴-1, 6′
2‴-1		169.2	
2‴-2	2.07 (*s*, 3H)	22.7	C-2‴-1
3-1		169.2	
3-2	1.92 (*s*, 3H)	22.7	C-3-1
3′-1		169.7	
3′-2	1.91 (*s*, 3H)	22.7	C-3′-1

Table VI. ^{1}H-NMR, ^{13}C-NMR, and HMBC spectral data of compound 6.

Position	δ(H)(J in Hz)	δ(C)	HMBC
2	4.73 (*d*, *J*=7.2 Hz, 1H)	78.3	C-2″, 1″, 6″, 3
3	5.69 (*d*, *J*=7.2 Hz, 1H)	77.8	C-2, 3-1, 4a
5	6.96(*br.s*, 1H)	117.4	C-2′
6		131.1	
7	6. 94 (*d*, *J*=8.4 Hz, 1H)	122.4	C-8a
8	6. 91 (*d*, *J*=8.4 Hz, 1H)	117.8	C-6, 7, 4a, 8a
4a		143.7	
8a		145.0	
2′	4.79 (*d*, *J*=7.2Hz, 1H)	78.3	C-7, 6, 5
3′	5.72 (*d*, *J*=7.2Hz, 1H)	78.2	C-2′, 3′-1, 4′a
5′	6.90 (*d*, *J*=8.4 Hz, 1H)	115.9	C-4′a, 8′a, 1‴
6′		137.9	
7′	6.74 (*d*, *J*=8.4 Hz, 1H)	120.7	C-5′, 8′
8′	6.91 (*d*, *J*=8.4 Hz, 1H)	118.1	C-7′, 6′, 4′a
4′a		143.4	
8′a		143.7	
1″		128.5	
2″	6.83(*br.s*, 1H)	115.6	C-1″, 3″, 2
3″		146.5	
4″		147.2	
5″	6.79 (*d*, *J*=8.4 Hz, 1H)	116.2	C-2″, 4″
6″	6.88 (*d*, *J*=8.4 Hz, 1H)	120.7	C-5″, 6″, 3″
3-1		173.2	
3-2	1.87 (*s*, 3H)	22.5	
3′-1		173.2	
3′-2	1.89 (*s*, 3H)	22.5	
1‴	4.65 (*dd*, *J*=7.2, 4.5 Hz, 1H)	73.0	C-5′, 6′, 7′, 2‴
2‴	3.37 (*dd*, *J*=3.9, 13.5 Hz, 1H) 3.43 (*dd*, *J*=3.9, 13.5 Hz, 1H)	48.1	C-6′, 1‴, 2‴-2
2‴-1		173.6	
2‴-2	1.97 (*s*, 3H)	22.6	

Compound **9** was obtained as a white powder. The molecular formula of **9** was determined to be $C_{30}H_{29}N_3O_9$ on the basis of its HR-ESI-MS data (*m/z* 576.1982 $[M+H]^+$; calcd. 576.1982 for $C_{30}H_{30}N_3O_9$). Comparison of the NMR data of **9** (Table IX) with those of **8** showed that the compounds were very similar, with the exception of the presence of 2 olefinic protons at *δ*6.15(C-1‴) and *δ* 7.35(H-2‴) in **9** in place of the hydroxyl group present in **8**. The absolute stereochemistry of **9** was determined by its CD spectrum (Wu, 2009; Yang *et al*., 2012), which showed a positive Cotton effect at 235 nm ($^{1}L_b$). Thus, compound **9** was identified as (2*S*,3*R*,2′*R*,3′*S*)-2-(3′,4′-dihydroxyphenyl)-3-acetylamino-7-(3-acetylamino-7-(*N*-acetyl-2-aminoethylene)-1,4-benzodioxan-2-yl)-1,4-benzodioxane and named Cicadamide C9 (**9**).

Table VII. ^{1}H-NMR, ^{13}C-NMR, and HMBC spectral data of compound 7.

Position	δ(H)(J in Hz)	δ(C)	HMBC
2	4.76 (*t*, *J*=7.2 Hz, 1H)	78.3	C-1″, 2″, 6″, 3, 8a
3	5.74 (*d*, *J*=7.2 Hz, 1H)	77.8	C-2, 4a 3-1,
5	6. 99 (*br.s*, 1H)	117.4	C-4a, 8a, 7
6		131.0	
7	6.97(*br.s*, 1H)	122.0	C-5, 6, 8, 2′
8	6.97(*br.s*, 1H)	118.1	C-8a, 7, 6
4a		145.0	
8a		143.6	
2′	4.81 (*t*, *J*=7.2Hz, 1H)	78.3	C-5, 6, 7, 3′, 8′a
3′	5.75 (*d*, *J*=7.2Hz, 1H)	78.2	C-2′, 3′-1, 4′a
5′	6.90 (*d*, *J*=8.4 Hz, 1H)	117.9	C-8′a, 4′a, 6′, 7′
6′	6.94 (*d*, *J*=8.4 Hz, 1H)	120.8	C-4′a, 8′a, 1‴
7′		137.7	
8′	7.02(*br.s*, 1H)	115.8	C-4′a, 8′a, 5′, 6′
4′a		143.0	
8′a		144.0	
1″		128.5	
2″	6.88(*br.s*, 1H)	115.6	C-2, 3″, 4″, 6″
3″		146.5	
4″		147.2	
5″	6.79 (*d*, *J*=7.8 Hz, 1H)	116.1	C-1″, 3″, 4″, 6″
6″	6.77 (*d*, *J*=7.8 Hz, 1H)	120.6	C-2, 2″, 4″
3-1		173.2	
3-2	1.90 (*s*, 3H)	22.5	C-3-1
3′-1		173.3	
3′-2	1.91 (*s*, 3H)	22.6	C-3′-1
1‴	4.69(*dd*, *J*=4.8, 7.2Hz, 1H)	73.0	C-6′, 7′, 8′
2‴	3.37(*dd*, *J*=4.8, 7.2Hz, 1H) 3.45(*dd*, *J*=4.8, 7.2Hz, 1H)	48.1	C-1‴, 7, 2‴-1
2‴-1		173.6	
2‴-2	1.96 (*s*, 3H)	22.6	C-2‴-1

Table VIII. ^{1}H-NMR, ^{13}C-NMR, and HMBC spectral data of compound 8.

Position	δ(H)(J in Hz)	δ(C)	HMBC
2	4.75 (*d*, *J*=7.2 Hz, 1H)	78.3	C-8a, 1″, 2″, 6″, 3
3	5.77 (*d*, *J*=7.2 Hz, 1H)	77.9	C-4a, 2
5	6.94 (*d*, *J*=8.4 Hz, 1H)	118.1	C-4a, 8a, 7
6	6.99 (*dd*, *J*=1.8, 8.4 Hz, 1H)	122.2	C-4a, 8
7		130.9	
8	7.05 (*d*, *J*=7.8 Hz, 1H)	117.3	C-8a, 6
4a		144.2	
8a		144.4	
2′	4.82 (*d*, *J=7.2*Hz, 1H)	78.3	C-8′a, 6, 7, 8, 3′
3′	5.74 (*d*, *J=7.2*Hz, 1H)	78.2	C-4′a, 2′
5′	6.89 (*dd*, *J*=2.4, 8.4 Hz, 1H)	117.9	C-4′a, 8′a, 7′
6′	6.92 (*d*, *J*=8.4 Hz, 1H)	120.9	C-4′a, 1‴
7′		137.8	
8′	7.04(*d*, *J*=7.8 Hz, 1H)	115.8	C-8′a, 6′
4′a		143.0	
8′a		144.1	
1″		128.5	
2″	6.86(*d*, *J*=7.2 Hz, 1H)	115.6	C-3″, 4″, 6″, 2
3″		146.5	
4″		147.2	
5″	6.94 (*d*, *J*=8.4 Hz, 1H)	118.1	C-1″, 3″, 4″
6″	6.77 (*d*, *J*=8.4 Hz, 1H)	120.6	C-1″, 4″
3-1		173.3	
3-2	1.90 (*s*, 3H)	22.5	C-3-1
3′-1		173.3	
3′-2	1.91 (*s*, 3H)	22.6	C-3′-1
1‴	4.68 (*dd, J=5.4, 7.2*Hz, 1H)	73.0	C-6′, 7′, 8′
2‴	3.42(*dd, J=4.2, 13.8*Hz, 1H) 3.45(*dd, J=4.2, 13.8*Hz, 1H)	48.1	C-1‴, 2‴-1, 7′
2‴-1		173.6	
2‴-2	1.94 (*s*, 3H)	22.6	C-2‴-1

Compound **10** was obtained as a white powder. The molecular formula of **10** was determined to be $C_{30}H_{29}N_3O_9$ on the basis of its HR-ESI-MS data (*m/z* 576.1982 $[M+H]^+$; calcd. 576.1982 for $C_{30}H_{30}N_3O_9$). The ^{1}H-NMR and ^{13}C-NMR spectra of **10** (Table X) were similar to those of **9**. The HMBC experiment suggested that the planar structure of **10** was identical to that of **9**. Therefore, **10** may be a diastereomer of **9** at chiral centers C-2′ and C-3′. In the CD spectrum of **10**, negative Cotton effects at 235 nm and 280 nm ($^{1}L_b$) were observed. The absolute stereochemistry of **10** was determined to be (2*R*,3*S*,2′*R*,3′*S*) by the 2-phenyl-1,4-benzodioxane CD rule (Wu, 2009; Yang *et al*., 2012) and comparison with **9**. Thus, compound **10** was identified as (2*R*,3*S*,2′*R*,3′*S*)-2-(3′,4′-dihydroxyphenyl)-3-acetylamino-7-(3-acetylamino-7-(*N*-acetyl-2-aminoethylene)-1,4-benzodioxan-2-yl)-1,4-benzodioxane (Fig. 2) and named Cicadamide C10 (**10**). Overall, the 10 compounds were obtained and elucidated.

Table IX. ^{1}H-NMR, ^{13}C-NMR, and HMBC spectral data of compound 9.

Position	δ(H)(J in Hz)	δ(C)	HMBC
2	4.78 (*d*, *J*=7.2 Hz, 1H)	78.4	C-8a, 1″, 6″ 2″, 3
3	5.76 (*d*, *J*=7.2 Hz, 1H)	78.2	C-4a, 2, 3-1
5	6. 96 (*d*, *J*=7.2 Hz, 1H)	118.2	C-4a, 8, 7
6	6.99 (*d*,*J*=7.2 Hz, 1H)	122.4	C-4a, 2′
7		130.9	
8	7.06 (*br.s*, 1H)	117.3	C-8a, 6, 2′
2′	4.81 (*d*, *J=7.2*Hz, 1H)	78.3	C-8′a, 6, 7, 8, 3′
3′	5.75 (*d*, *J=7.2*Hz, 1H)	78.0	C-4′a, 2′, 3′-1
5′	6.93 (*d*, *J*=8.4 Hz, 1H)	118.0	C-4′a, 6′, 7′
6′	6.90 (*d*, *J*=8.4 Hz, 1H)	120.2	C-7′, 8′, 4′a, 1‴
7′		132.2	
8′	6.88 (*d*, *J*=8.4 Hz, 1H)	114.8	C-8′a, 4′a, 6′, 1‴
4a		144.3	
8a		144.4	
4′a		143.7	
8′a		143.2	
1″		128.5	
2″	6. 86 (*br.s*, 1H)	115.5	C-4″, 6″, 2
3″		146.3	
4″		147.2	
5″	6.78 (*d*, *J*=8.4 Hz, 1H)	116.1	C-3″, 6″
6″	6.76(*d*, *J*=8.4 Hz, 1H)	120.5	C-1″, 2″, 4″
1‴	6.15(*dd, J=4.2 15.0*Hz,1H)	113.9	C-2‴, 8′, 6′
2‴	7.35(*dd, J=3.4, 15.0*Hz, 1H)	122.9	C-2‴-1, 1‴, 7′
2‴-1		170.6	
2‴-2	2.07 (*s*, 3H)	22.6	C-2‴-1
3-1		173.3	
3-2	1.92 (*s*, 3H)	22.6	C-3 -1
3′-1		173.3	
3′-2	1.91 (*s*, 3H)	22.2	C-3′ -1

Table X. ^{1}H-NMR, ^{13}C-NMR, and HMBC spectral data of compound 10.

Position	δ(H)(J in Hz)	δ(C)	HMBC
2	4.73 (*d*, *J*=7.2 Hz, 1H)	78.3	C-8a, 2″, 6″
3	5.72 (*d*, *J*=7.2 Hz, 1H)	78.3	C-4a, 2, 3-1
5	6. 94 (*d*, *J*=7.2 Hz, 1H)	118.2	C-4a, 7
6	6.94 (*d*,*J*=7.2 Hz, 1H)	123.0	C-4a, 8, 2′
7		132.1	
8	6.96 (*br.s*, 1H)	118.1	C-8a, 6, 2′
2′	4.79 (*d*, *J=7.2*Hz, 1H)	78.3	C-8′a, 6, 7, 8
3′	5.73 (*d*, *J=7.2*Hz, 1H)	78.3	C-4′a, 2′, 3′-1
5′	6.85 (*d*, *J*=8.4 Hz, 1H)	118.1	C-4′a, 8′a, 7′
6′	6.85 (*d*, *J*=8.4 Hz, 1H)	120.2	C-7′, 8′, 4′a, 1‴
7′		132.1	
8′	6.83 (*d*, *J*=8.4 Hz, 1H)	114.9	C-8′a, 6′, 1‴
4a		145.0	
8a		145.0	
4′a		143.7	
8′a		143.1	
1″		128.5	
2″	6.83 (*d*, *J*=8.4 Hz, 1H)	115.6	C-1″, 3″, 6″
3″		146.5	
4″		146.5	
5″	6.78 (*d*, *J*=8.4 Hz, 1H)	116.2	C-1″, 3″, 6″
6″	6.75 (*d*, *J*=8.4 Hz, 1H)	120.6	C-2″, 4″, 5″,
1‴	6.11 (*d, J*=14.4Hz, 1H)	113.9	C-2‴, 7′, 6′, 8′
2‴	7.33 (*d, J*=14.4Hz, 1H)	123.0	C-2‴-1, 1‴, 7′
2‴-1		170.6	
2‴-2	2.03 (*s*, 3H)	22.6	C-2‴-1
3-1		173.2	
3-2	1.89 (*s*, 3H)	22.6	C-3 -1
3′-1		173.2	
3′-2	1.87 (*s*, 3H)	22.6	C-3′ -1

CONCLUSION

In this study, the previously unknown phthalides with biologically activity from Periostracum Cicadae were identified. The structures of new identified compounds were established using spectroscopic methods. In the result, 10 new compounds (**1–10)** were isolated, the structural elucidation of compounds **1–10** were described. This study aims to provide a reference for the further functional research and the development and utilization of Periostracum Cicadae.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20210513050500

Statement of conflict of interest

The authors have declared no conflict of interest.

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