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.

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).

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 anticonvulsant, 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 CHCl3/MeOH to afford 14 fractions (100–0:0–100) that were designated A–O.

Fraction F (CHCl3/MeOH, 100:5 vol/vol; 3.4834 g) was further subjected to ODS column elution with MeOH/H2O (40:60 vol/vol), after which a fraction (107.6 mg) of the eluted material was purified by preparative RP-HPLC (MeOH/H2O, 30:70 vol/vol) to obtain compound 2 (13.1 mg). Fraction G (CHCl3/MeOH, 100:8 vol/vol; 5.9045 g) was further purified by ODS column elution with MeOH/H2O (50:50 vol/vol) to yield 3 fractions. Fraction D2 (1.7607 g) was purified by preparative RP-HPLC (MeOH/H2O, 40:60 vol/vol) to obtain compounds 1 (40 mg), 3 (40.6 mg), 4 (11.0 mg), and 5 (159.3 mg). Fraction D2 (0.4297 g) was purified by preparative RP-HPLC (MeOH/H2O, 42:58) to obtain compounds 9 (13.0 mg) and 10 (15.0 mg). Fraction H (CHCl3/MeOH, 100:10, 3.047 g) was further purified by ODS column elution with MeOH/H2O (40:60 vol/vol) to yield a fraction (0.2313 g) that was purified by preparative RP-HPLC (MeOH/H2O, 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.
Ten N-acetyldopamine dimers from Periostracum Cicadae

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_{3}O_{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_{3}O_{9}). The UV spectrum of 1 exhibited λ_{max} at 280.6 nm (MeOH).

The 1H-NMR (600 MHz, CD_{3}OD) 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-3-1), 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, J=7.2 Hz, H-1′′′), and δ 3.39 (t, J=7.2 Hz, H-2′′′); and 4 methine protons at δ 4.76 (d, J=7.2 Hz, H-2′), δ 4.77 (d, J=7.2 Hz, H-3′), and δ 5.72 (d, J=7.2 Hz, H-3′) (Table I). The UV spectrum of 1 (Fig. 1) 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-3-1), 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, J=7.2 Hz, H-1′′′), and δ 3.39 (t, J=7.2 Hz, H-2′′′); and 4 methine protons at δ 4.76 (d, J=7.2 Hz, H-2′), δ 4.77 (d, J=7.2 Hz, H-3′), and δ 5.72 (d, J=7.2 Hz, H-3′) (Table I). The UV spectrum of 1 (Fig. 1) 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-3-1), 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, J=7.2 Hz, H-1′′′), and δ 3.39 (t, J=7.2 Hz, H-2′′′); and 4 methine protons at δ 4.76 (d, J=7.2 Hz, H-2′), δ 4.77 (d, J=7.2 Hz, H-3′), and δ 5.72 (d, J=7.2 Hz, H-3′) (Table I).

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 proton signal at δ 5.77 (H-2′), 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 the carbon signal at δ 144.2 (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. 1H-NMR, 13C-NMR, and HMBC spectral data of compound 1.

| Posi- | 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=7.2 Hz, 1H) | 78.3 | C-6, 7, 8, 8a, 3′ |
| 3′    | 5.72 (d, J=7.2 Hz, 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, 8a |
| 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, 8a, 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=7.2 Hz, 2H) | 35.7 | C- 6′, 7′, 8′, 2′ |
| 2″″   | 3.39(t, J=7.2 Hz, 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 |
nm (λL) 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 (2S,3R,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. 1H-NMR, 13C-NMR, and HMBC spectral data of compound 2.

| Position | δ(H) (J in Hz) | δ(C) | HMBC |
|----------|---------------|------|------|
| 2        | 4.71 (d, J=7 Hz, 1H) | 78.2 | C-1″, 2″, 6″, 3, 8a |
| 3        | 5.75 (d, J=7 Hz, 1H) | 78.4 | C-1″, 2″, 3-1, 4a |
| 5        | 7.05 (brs. 1H) | 117.3 | C-6, 7, 4a, 2′ |
| 7        | 6.99 (d, J=7 Hz, 1H) | 122.4 | C-5, 4a, 2′ |
| 8        | 7.05 (brs. 1H) | 117.3 | C-5, 6, 8a |
| 4a       | 144.5 |       |      |
| 8a       | 144.3 |       |      |
| 2′       | 4.79 (d, J=7 Hz, 1H) | 78.2 | C-6, 7, 8, 3′, 8′a |
| 3′       | 5.73 (d, J=7 Hz, 1H) | 77.9 | C-6, 2′, 3′-1, 4a |
| 5′       | 6.83 (dd, J=7.2,13.8 Hz, 1H) | 118.1 | C-6′, 4a |
| 6′       | 6.78 (dd, J=7,13.8 Hz, 1H) | 123.4 | C-5′, 8′, 4′, 1″ |
| 7′       | 134.3 |       |      |
| 8′       | 6.85 (dd, J=7,13.8 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 Hz, 1H) | 115.5 | C-1″, 4″, 6″, 2 |
| 3″       | 146.5 |       |      |
| 4″       | 147.2 |       |      |
| 5″       | 6.79 (d, J=7 Hz, 1H) | 116.2 | C-1″, 3″ |
| 6″       | 6.77 (d, J=7 Hz, 1H) | 120.6 | C-2″, 4″, 2 |
| 1‴       | 2.73 (t, J=7 Hz, 2H) | 35.7 | C-6′, 7′, 8′, 2″ |
| 2‴       | 3.38 (t, J=7 Hz, 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 C30H31N3O9 on the basis of its HR-ESI-MS data (m/z 578.2114 [M+H]+; calcld. 578.2139 for C30H32N3O9). The 1H-NMR and 13C-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 (λL). Therefore, based on the basis of its HR-ESI-MS data (Wu, 2009; Yang et al., 2012) and comparison with 1. Thus, compound 2 was identified as (2R,3S,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 C30H31N3O9 on the basis of its HR-ESI-MS data (m/z 578.2111 [M+H]+; calcld. 578.2139 for C30H32N3O9). The 1H-NMR and 13C-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 (λL) were observed. The absolute stereochemistry of 3 was determined to be (2S,3R,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 3 was identified as (2S,3R,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 C3 (3).

Compound 4 was obtained as a white powder. The molecular formula of 4 was determined to be C30H31N3O9 on the basis of its HR-ESI-MS data (m/z 578.2114 [M+H]+; calcld. 578.2139 for C30H32N3O9). The 1H-NMR and 13C-NMR spectral data of 4 (Table IV) indicated that its structure was closely related to that of 3. The CD spectrum of 4, positive Cotton effects at 235 nm and 280 nm (λL) were observed (Wu, 2009; Yang et al., 2012). The absolute stereochemistry of 4 was determined to be (2R,3S,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 (2R,3S,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 C4 (4).
6-(N-acetyl-2-aminoethyl)-1,4-benzodioxan-2-yl)-1,4-benzodioxane (Fig. 2) and named Cicadamide C4 (4).

Table III. ¹H-NMR, ¹³C-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 (brs, 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- 4a, 3″-1, 2″ |
| 5″       | 6.78 (d, J=7.2Hz, 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 (brs, 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 (brs, 1H) | 120.6 | C- 1″′, 4″, 2 |
| 1″′′     | 2.71 (t, J=7.2 Hz, 2H) | 35.7 | C- 6′, 7″, 2″′, 5′ |
| 2″′′     | 3.37 (t, J=7.2 Hz, 2H) | 42.1 | C- 6′, 2″′-1, 1″′′ |
| 2″′′-1   | | 173.2 | |
| 2″′′-1   | 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 C₁₉₆H₃₁N₃O₁₀ on the basis of its HR-ESI-MS data (m/z 594.2054 [M+H]+; calcd. 594.2088 for C₁₉₆H₃₁N₃O₁₀). The ¹H-NMR and ¹³C-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-acetamino-6-(3-acetamino-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 C₁₉₆H₃₂N₃O₉ on the basis of its HR-ESI-MS data (m/z 594.2054 [M+H]+; calcd. 594.2088 for C₁₉₆H₃₂N₃O₉). The ¹H-NMR and ¹³C-NMR spectral data of 6 (Table VI) were similar to those of 5, with the exception of the absence of the hydroxyl group located at δ 4.81(C-2″′) in compound 5. The HMBC experiment suggested that the planar structure of 6 was identical to that of 5. The methylene protons at δ 3.37(C-1″′) and δ 3.34 (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-acetamino-2-glyoxyl group. In the CD spectrum of 6, positive Cotton effects at 235 nm and 280 nm (Lα) were observed. The absolute stereochemistry of 6 was determined to be (2R,3S,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 (2R,3S,2′R,3′S)-2-(3′,4′-dihydroxyphenyl)-3-acetamino-6-(3-acetamino-6-(N-acetyl-2-amino-1-hydroxyethyl)-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 C₁₉₆H₃₂N₃O₁₀ on the basis of its HR-ESI-MS data (m/z 594.2082 [M+H]+; calcd. 594.2088 for C₁₉₆H₃₂N₃O₁₀). The ¹H-NMR and ¹³C-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-acetyl-2-ethyl group. The HMBC data showed that the methylene proton signal at δ 3.37(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 6 and at the 6′-position in 7. The CD spectrum of 6 showed a negative Cotton effect at 235 nm and a positive Cotton effect at 280 nm (Lα). The absolute stereochemistry of 7 was determined to be (2R,3S,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 (2R,3S,2′R,3′S)-2(3′,4′-dihydroxyphenyl)-3-acetamino-6-(3-acetamino-7-(N-acetyl-2-amino-1-hydroxyethyl)-1,4-benzodioxan-2-yl)-1,4-benzodioxane (Fig. 2) and named Cicadamide C7 (7).
1,4-benzodioxan-2-yl)-1,4-benzodioxide (Fig. 2) and named Cicadamide C7 (7).

Table IV. 1H-NMR, 13C-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 (brs, 1H) | 117.3 | C-6, 7, 4a, 8a |
| 4a       |             | 144.1 |       |
| 8a       |             | 144.3 |       |
| 2"       | 4.79 (d, J=7.2 Hz, 1H) | 78.3 | C-8'a, 6, 7, 8', 3' |
| 3"       | 5.75 (d, J=7.2 Hz, 1H) | 78.1 | C-4'a, 3', 1', 2' |
| 5"       | 6.76(brs, 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(brs, 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" |
| 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_{18}H_{15}NO_{10} on the basis of its HR-ESI-MS data (m/z: 594.2012 [M+H]⁺; calculated 594.2088 for C_{18}H_{15}NO_{10}). The 1H-NMR and 13C-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 (Lα). The absolute stereochemistry of 8 was determined to be (2R,3S,2'R,3'S)-2-(3',4'-dihydroxyphenyl)-3-acylamino-7-(3-acylamino-7-(N-acetyl-2-amino-1-hydroxyethyl)-1,4-benzodioxan-2-yl)-1,4-benzodioxide and named Cicadamide C8 (8).

Table V. 1H-NMR, 13C-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=7.2 Hz, 1H) | 76.5 | C-8'a, 5, 6, 7, 3' |
| 3"       | 5.68 (d, J=7.2 Hz, 1H) | 76.3 | C-2', 3'-1 |
| 5"       | 6.77(brs, 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(brs, 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. ¹H-NMR, ¹³C-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(brs, 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|                   |
| 1"      |                | 128.5|                   |
| 2"      | 6.83(brs, 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) | 48.1 | C-6", 1", 2", 2"" |
| 3.43 (dd, J=3.9, 13.5 Hz, 1H) | 173.6|                   |

Compound 9 was obtained as a white powder. The molecular formula of 9 was determined to be C₁₉ₒ₆H₂₅₉N₂O₉ on the basis of its HR-ESI-MS data (m/z 576.1982 [M+H]+; calcd. 576.1982 for C₁₉ₒ₆H₂₅₉N₂O₉). 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 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 and a negative Cotton effect at 280 nm (l.). Thus, compound 9 was identified as (2S,3R,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. ¹H-NMR, ¹³C-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 (brs, 1H)      | 117.4| C-4a, 8a, 7      |
| 6       |                | 131.0|                   |
| 7       | 6.97(brs, 1H)      | 122.0| C-5, 6, 8, 2'    |
| 8       | 6.97 (brs, 1H)      | 118.1| C-8a, 7, 6       |
| 4a      |                | 145.0|                   |
| 8a      |                | 143.6|                   |
| 1"      |                | 128.5|                   |
| 2"      | 6.83(brs, 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) | 48.1 | C-6", 1", 2", 2"" |
| 3.43 (dd, J=3.9, 13.5 Hz, 1H) | 173.6|                   |

Ten N-acetyldopamine dimers from Periostracum Cicadae
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"m |
| 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, 7.2Hz, 1H) | 73.0 | C-6', 7', 8' |
| 2""      | 3.42(dd, J=4.2, 13.8Hz, 1H) | 48.1 | C-1"", 2""-1, 7' |
| 3.45(dd, J=4.2, 13.8Hz, 1H) | 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$_{3}$O$_{9}$ on the basis of its HR-ESI-MS data (m/z 576.1982 [M+H]$^+$; calcd. 576.1982 for C$_{30}$H$_{29}$N$_{3}$O$_{9}$). 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 (1L$_b$) were observed. The absolute stereochemistry of 10 was determined to be (2R,3S,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 (2R,3S,2'R,3'S)-2-(3',4'-dihydroxyphenyl)-3-acetylamino-7-(3-acetylamino-7-(N-acyl-2-aminoethylen)-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 (brs, 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 15.0Hz, 1H) | 113.9 | C-2"", 8', 6' |
| 2""      | 7.35(dd, J=3.4, 15.0Hz, 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 | $\delta$(H)(J in Hz) | $\delta$(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", 6", |
| 1"""     | 6.11 ($d,J=14.4$Hz, 1H) | 113.9      | C-2"", 7", 6", 8" |
| 2"""     | 7.33 ($d,J=14.4$Hz, 1H) | 123.0      | C-2"", 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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Identification of Ten New N-acetyldopamine Dimers from Periostracum Cicadae

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Supplementary Table S1. The detailed information of Cicadamide C1 (1)–C10 (10).

| Compound   | Detailed information                                                                                                                                 |
|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|
| Cicadamide C1 (1) | White powder; MP: 180.6–181.2 °C; [α]20D +7.9° (c =0.003, MeOH)                                                                                      |
|            | IR (vKBr, cm⁻¹): 3278, 3067, 1506, 1371, 1263, 1031                                                                                                     |
|            | UV (MeOH) λmax(log ε): 280.6 nm                                                                                                                         |
|            | 1H NMR (600 MHz, CD3OD): see Table I.                                                                                                                    |
|            | 13C NMR (150 MHz, CD3OD): see Table I.                                                                                                                   |
|            | HMBC: see Figure 2.                                                                                                                              |
|            | ESI-TOF-HR-MS: m/z 578.2160 [M+H]+; calcd. 578.2139 for C30H32N3O9 (2S,3R,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 |

Cicadamide C2 (2) | White powder; MP: 184.5–187.3 °C; [α]20D +24.3° (c =0.009, MeOH)                                                                                      |
|            | IR (vKBr, cm⁻¹): 3257, 3047, 1649, 1502, 1437, 1373, 1269, 1020                                                                                         |
|            | UV (MeOH) λmax(log ε): 280.3 nm                                                                                                                         |
|            | 1H NMR (600 MHz, CD3OD): see Table II.                                                                                                                   |
|            | 13C NMR (150 MHz, CD3OD): see Table II.                                                                                                                   |
|            | HMBC: see Figure 2.                                                                                                                              |
|            | ESI-TOF-HR-MS: m/z 578.2141 [M+H]+; calcd. 578.2139 for C30H32N3O9 (2R,3S,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 |

Table continued on next page..................

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Compound C3 (3) White powder
MP: 181.3–198.9 °C
[α]_D^20 -26.1° (c 0.03, MeOH)
IR (ν_{max}, cm\(^{-1}\)): 3269, 3047, 1678, 1506, 1437, 1373, 1267, 1213
UV (MeOH) λ_{max}(log ε): 280.8 nm
1H NMR (CD_{3}OD): see Table III.
13C NMR (CD_{3}OD): see Table III.
HMBC: see Figure 2.
ESI-TOF-HR-MS: m/z 578.2111 [M+H]+; calcd. 578.2139 for C_{30}H_{32}N_{3}O_{9} (2S,3R,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

Compound C4 (4) White powder
MP: 179.7–182.7 °C
[α]_D^20 +13.4° (c 0.006, MeOH)
IR (ν_{max}, cm\(^{-1}\)): 3398, 3062, 1654, 1506, 1438, 1373, 1267, 1028
UV (MeOH) λ_{max}(log ε): 280.8 nm
1H NMR (CD_{3}OD): see Table IV.
13C NMR (CD_{3}OD): see Table IV.
HMBC: see Figure 2.
ESI-TOF-HR-MS: m/z 578.2114 [M+H]+; calcd. 578.2139 for C_{30}H_{32}N_{3}O_{9} (2R,3S,3′R,2′S)-2-(3′,4′-dihydroxyphenyl)-3-acetylamino-7-(3-acetylamino-6-(N-acetyl-2-aminoethyl)-1,4-benzodioxan-2-yl)-1,4-benzodioxane

Compound C5 (5) White powder
MP: 226.3–228.7 °C
[α]_D^20 +15.2° (c 0.006, MeOH)
IR (ν_{max}, cm\(^{-1}\)): 3062, 1653, 1508, 1438, 1373, 1253, 1027
UV (MeOH) λ_{max}(log ε): 280.8 nm
1H NMR (CD_{3}OD): see Table V.
13C NMR (CD_{3}OD): see Table V.
HMBC: see Figure 2.
ESI-TOF-HR-MS: m/z 578.2144 [M+H]+; calcd. 578.2139 for C_{30}H_{32}N_{3}O_{9} (2R,3S,2′R,3′S)-2-(3′,4′-dihydroxyphenyl)-3-acetylamino-6-(3-acetylamino-7-(N-acetyl-2-amino-1-hydroxyethyl)-1,4-benzodioxan-2-yl)-1,4-benzodioxane

Compound C6 (6) White powder
MP: 185.7–188.3 °C
[α]_D^20 +18.1° (c 0.006, MeOH)
IR (ν_{max}, cm\(^{-1}\)): 3423, 1510, 1267, 1213, 1029
UV (MeOH) λ_{max}(log ε): 280.6 nm
1H NMR (CD_{3}OD): see Table VI.
13C NMR (CD_{3}OD): see Table VI.
HMBC: see Figure 2.
ESI-TOF-HR-MS: m/z 594.2054 [M+H]+; calcd. 594.2088 for C_{30}H_{32}N_{3}O_{10} (2R,3S,2′R,3′S)-2-(3′,4′-dihydroxyphenyl)-3-acetylamino-6-(3-acetylamino-6-(N-acetyl-2-amino-1-hydroxyethyl)-1,4-benzodioxan-2-yl)-1,4-benzodioxane

Compound C7 (7) White powder
MP: 186.4–188.2 °C
[α]_D^20 +12.9° (c 0.005, MeOH)
IR (ν_{max}, cm\(^{-1}\)): 3466, 2314, 1269, 1028
UV (MeOH) λ_{max}(log ε): 280.4 nm
1H NMR (CD_{3}OD): see Table VII.
13C NMR (CD_{3}OD): see Table VII.
HMBC: see Figure 2.
ESI-TOF-HR-MS: m/z 594.2082 [M+H]+; calcd. 594.2088 for C_{30}H_{32}N_{3}O_{10} (2R,3S,2′R,3′S)-2-(3′,4′-dihydroxyphenyl)-3-acetylamino-6-(3-acetylamino-7-(N-acetyl-2-amino-1-hydroxyethyl)-1,4-benzodioxan-2-yl)-1,4-benzodioxane

Table continued on next page................
Cicadamide C8 (8)  White powder
MP: 186.6–187.4°C
\([\alpha]_{D}^{20} +4.6^\circ (c 0.002, \text{MeOH})\)
IR \((\nu_{\text{KBr}}, \text{cm}^{-1}): 3273, 3074, 1664, 1508, 1436, 1371, 1263, 1035\)
UV (MeOH) \(\lambda_{\text{max}}(\log \varepsilon): 280.8 \text{ nm}\)
1H NMR (600 MHz, CD3OD): see Table VIII.
13C NMR (150 MHz, CD3OD): see Table VIII.
HMBC: see Figure 2.
ESI-TOF-HR-MS: \(m/z\) 594.2012 \([\text{M+H}]^+\); calculated 594.2088 for \(\text{C}_{30}\text{H}_{32}\text{N}_{3}\text{O}_{10}\) \((2\text{R},3\text{S},2'\text{R},3'\text{S})-2-(3',4'-\text{dihydroxyphenyl})-3-\text{acetylamino}-7-(3-\text{acetylamino}-7-\text{N}-\text{acetyl}-2-\text{amino}-1-\text{hydroxylethyl})-1,4-\text{benzodioxan-2-yl})-1,4-\text{benzodioxane}\)

Cicadamide C9 (9)  White powder
MP: 163.9–165.5°C
IR \((\nu_{\text{KBr}}, \text{cm}^{-1}): 3269, 1649, 1508, 1271, 1033\)
UV (MeOH) \(\lambda_{\text{max}}(\log \varepsilon): 279.6 \text{ nm}\)
1H NMR (600 MHz, CD3OD): see Table IX.
13C NMR (150 MHz, CD3OD): see Table IX.
HMBC: see Figure 2.
ESI-TOF-HR-MS: \(m/z\) 576.1982 \([\text{M+H}]^+\); calculated 576.1982 for \(\text{C}_{30}\text{H}_{30}\text{N}_{3}\text{O}_{9}\) \((2\text{S},3\text{R},2'\text{R},3'\text{S})-2-(3',4'-\text{dihydroxyphenyl})-3-\text{acetylamino}-7-(3-\text{acetylamino}-7-\text{N}-\text{acetyl}-2-\text{aminoethylethene})-1,4-\text{benzodioxan-2-yl})-1,4-\text{benzodioxane}\)

Cicadamide C10 (10)  White powder
MP: 226.8–230.4°C
\([\alpha]_{D}^{20} -26.3^\circ (c -0.01, \text{MeOH})\)
IR \((\nu_{\text{KBr}}, \text{cm}^{-1}): 3275, 3061, 1649, 1502, 1437, 1373, 1269, 1020\)
UV (MeOH) \(\lambda_{\text{max}}(\log \varepsilon): 281.6 \text{ nm}\)
1H NMR (600 MHz, CD3OD): see Table I0.
13C NMR (150 MHz, CD3OD): see Table I0.
HMBC: see Figure 2.
ESI-TOF-HR-MS: \(m/z\) 576.1982 \([\text{M+H}]^+\); calculated 576.1982 for \(\text{C}_{30}\text{H}_{30}\text{N}_{3}\text{O}_{9}\) \((2\text{R},3\text{S},2'\text{R},3'\text{S})-2-(3',4'-\text{dihydroxyphenyl})-3-\text{acetylamino}-7-(3-\text{acetylamino}-7-\text{N}-\text{acetyl}-2-\text{aminoethylethene})-1,4-\text{benzodioxan-2-yl})-1,4-\text{benzodioxane}\)