Aglaiaabbrevins A–D, New Prenylated Bibenzyls from the Leaves of Aglaia abbreviata with Potent PTP1B Inhibitory Activity

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Four new prenylated bibenzyls, named aglaiaabbrevins A–D (2, 4–6), were isolated from the leaves of Aglaia abbreviata, along with two known related analogues, 3,5-dihydroxy-2-[3,7-dimethyl-2(E),6-octadecenyl]bibenzyl (7) and 3,5-dihydroxy-2-[3-methyl-2-butenyl]bibenzyl (8). The structures of the new compounds were elucidated on the basis of extensive spectroscopic experiments, mainly one and two dimensional (1D- and 2D)-NMR, and the absolute configuration of 5 was determined by the measurement of specific rotation. The isolated compounds were evaluated for their protein tyrosine phosphatase-1B (PTP1B) inhibitory activity. The results showed that compounds 5–7 exhibited more potent PTP1B inhibitory effects with IC₅₀ values of 2.58±0.52, 2.44±0.35, and 2.23±0.14µM, respectively, than the positive control oleanolic acid (IC₅₀=2.74±0.20µM). On the basis of the data obtained, these bibenzyls with the longer C-2 prenyl groups may be considered as potential lead compounds for the development of new anti-obesity and anti-diabetic agents. Also, the PTP1B inhibitory effects for prenylated bibenzyls are being reported for the first time.

Key words Aglaia abbreviata; prenylated bibenzyl; aglaiaabbrevin; protein tyrosine phosphatase-1B inhibitor; potential lead compound

Type 2 diabetes (T2D) is a chronic disorder characterized by hyperglycemia associated with a gradual decline in insulin sensitivity and/or insulin secretion, and has become one of the most serious health problems worldwide. On the other hand, obesity is one of the major risk factors for developing T2D due to insulin resistance.1) Protein tyrosine phosphatases (PTPs) are responsible for the dephosphorylation of tyrosine residues and are considered negative regulators of insulin signaling.2) Among the various members of the PTP superfamily, PTP1B plays a critical role in metabolic signaling pathways, which places it in an ideal position as a therapeutic drug target for diabetes and obesity.3) Therefore, PTP1B is a promising drug target for the treatment of T2D and obesity and is also involved in cancer. Although there have been a number of reports on the design and development of PTP1B inhibitors, new types of such compounds with suitable pharmacological properties remain to be discovered.

The plant Aglaia abbreviata C. Y. Wu (Meliaceae), which is a wild evergreen shrub found on mountain slopes at up to ca. 500–1600 m altitude, is widely distributed throughout southwestern China.4) Members of the genus Aglaia have been studied extensively, resulting in the isolation of many types of interesting secondary metabolites, particularly of various triterpenoids (e.g., cycloartanes, dammaranes, tirucallanes) and flavaglines (e.g., cyclopenta[b]benzofuran, cyclopenta[bc]benzopyrans, benzo[b]oxepines),5) while limited information is available on A. abbreviata.6–10) Previous phytochemical investigations of A. abbreviata have led to the isolation and characterization of a bisamide,10) four pregnane steroids,3) three nortriterpenoids8) and five dammarane triterpenoids.8–10) In an ongoing research for biologically active substances from various natural sources,11–15) the plant A. abbreviata attracted our attention because the ethyl acetate (EtOAc) extract of the leaves of this plant showed inhibitory activity against PTP1B with an 65.2% inhibition at the concentration of 20 µg/mL. As a result, four new (aglaiaabbrevins A–D, 2, 4–6) and two known (7, 8) prenylated bibenzyls were isolated (Fig. 1). The structures of the new compounds were elucidated by extensive spectroscopic analysis, aided by the comparison with data of related derivatives. Compounds 5–7 exhibited potent inhibitory activity against PTP1B. Herein, we report the isolation, structure elucidation, and PTP1B inhibitory activity of these

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compounds.

Results and Discussion

The air-dried and powdered leaves of *A. abbreviata* were extracted with 95% ethanol (EtOH) by percolation at room temperature. The EtOAc-soluble portion of the EtOH extract was repeatedly subjected to silica gel and Sephadex LH-20 column chromatography, followed by reversed-phase semipreparative HPLC purification, providing four new prenylated bibenzyls (2, 4–6) and two known analogues (7, 8). By comparing their observed and reported spectroscopic data, the known bibenzyls were identified as 3,5-dihydroxy-2-[3,7-dimethyl-2(E)-6-octadienyl]bibenzyl (7) and 3,5-dihydroxy-2-(3-methyl-2-butenyl)bibenzyl (8). Compound 2 was isolated as a yellow, amorphous powder, and its UV spectrum was consistent with a bibenzyl structure. The molecular formula of 2 was determined to be 

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| Position | $\delta_{c}$, Type | $\delta_{h}$ (in Hz) |
|----------|------------------|------------------|
| 1        | 145.0, C         |                  |
| 2        | 104.8, C         |                  |
| 3        | 162.0, C         |                  |
| 4        | 112.3, C         |                  |
| 5        | 159.6, C         |                  |
| 6        | 111.3, CH        | 6.32, s          |
| 7        | 22.3, CH         | 3.43, d (7.2)    |
| 8        | 121.5, CH        | 5.26, t (7.2)    |
| 9        | 135.4, C         |                  |
| 10       | 18.0, CH         | 1.82, brs        |
| 11       | 25.9, CH         | 1.76, brs        |
| 12       |                  | 27.8, CH         |
| 13       |                  | 1.42, s          |
| 14       |                  | 27.8, CH         |
| 15       |                  | 1.42, s          |
| 16       |                  | 75.8, CH         |
| $\alpha$ | 37.2, CH         | 3.07, m          |
| $\beta$ | 33.1, CH         | 2.82, m          |
| $1'$     | 127.5, C         | 128.0, C         |
| $2'$     | 154.0, C         | 153.9, C         |
| $3'$     | 115.6, CH        | 6.82, brd (8.0)  |
| $4'$     | 127.7, CH        | 7.13, td (8.0, 1.2) |
| $5'$     | 120.8, CH        | 6.88, td (8.0, 10) |
| $6'$     | 130.3, CH        | 7.12, brd (8.0)  |
| $3$-OH   |                  | 11.39, s         |
| $5$-OH   |                  | 5.87, brs        |
| $2^-$-OH |                  | 5.60, brs        |

classified with the aid of distortionless enhancement by polarization transfer (DEPT) and heteronuclear single-quantum correlation (HMQC) experiments as three methylenes (one oxygenated at $\delta_{c}$ 52.3), three methylenes, six $sp^2$ methines, and nine quaternary carbons (three oxygenated at $\delta_{c}$ 162.0, 159.6, 154.0) including one ester carbonyl carbon at $\delta_{c}$ 171.0. In the $^1$H-NMR spectrum (Table 1), an aromatic singlet at $\delta_{h}$ 6.32 (1H, s) and four ABCD-type aromatic protons at $\delta_{h}$ 7.13 (1H, td, $J$=8.0, 1.2Hz), 7.12 (1H, brd, $J$=8.0Hz), 6.88 (1H, td, $J$=8.0, 1.0Hz), and 6.82 (1H, brd, $J$=8.0Hz) clearly indicated two benzene rings were pentasubstituted and ortho-disubstituted, respectively. Two separated multiplets at $\delta_{h}$ 3.07 (2H) and 2.82 (2H) were obviously attributed to two benzylic methylenes. In addition, the $^1$H-NMR spectrum also displayed signals for an olefinic proton at $\delta_{h}$ 5.26 (1H, t, $J$=7.2Hz), two vinyl methyls at $\delta_{h}$ 1.82 (3H, brs) and 1.76 (3H, brs), and one methylene doublet at $\delta_{h}$ 3.43 (2H, d, $J$=7.2Hz), typical of a prenyl group. The above data were compatible with a prenylated bibenzyl skeleton substituted with a methyl ester [C$_2$H$_5$O$_2$] (Calcld 355.1545) in the high resolution-electrospray ionization (HR-ESI)-MS spectrum. The IR spectrum exhibited the presence of hydroxy (3212 cm$^{-1}$), an ester carbonyl (1654 cm$^{-1}$), and aromatic ring (1598, 1510, 1420 cm$^{-1}$). The $^{13}$C-NMR spectrum (Table 1) disclosed 21 signals which were supported by $^1$H–$^1$H correlation spectroscopy (COSY) and heteronuclear multiple bond correlation (HMBC) experiments.

Table 1. $^1$H- and $^{13}$C-NMR Spectroscopic Data for 2 and 4–6 in CDCl$_3$

| Position | $\delta_{c}$, Type | $\delta_{h}$ (in Hz) |
|----------|------------------|------------------|
| 1        | 145.0, C         |                  |
| 2        | 104.8, C         |                  |
| 3        | 162.0, C         |                  |
| 4        | 112.3, C         |                  |
| 5        | 159.6, C         |                  |
| 6        | 111.3, CH        | 6.32, s          |
| 7        | 22.3, CH         | 3.43, d (7.2)    |
| 8        | 121.5, CH        | 5.26, t (7.2)    |
| 9        | 135.4, C         |                  |
| 10       | 18.0, CH         | 1.82, brs        |
| 11       | 25.9, CH         | 1.76, brs        |
| 12       |                  | 27.8, CH         |
| 13       |                  | 1.42, s          |
| 14       |                  | 27.8, CH         |
| 15       |                  | 1.42, s          |
| 16       |                  | 75.8, CH         |
| $\alpha$ | 37.2, CH         | 3.07, m          |
| $\beta$ | 33.1, CH         | 2.82, m          |
| $1'$     | 127.5, C         | 128.0, C         |
| $2'$     | 154.0, C         | 153.9, C         |
| $3'$     | 115.6, CH        | 6.82, brd (8.0)  |
| $4'$     | 127.7, CH        | 7.13, td (8.0, 1.2) |
| $5'$     | 120.8, CH        | 6.88, td (8.0, 10) |
| $6'$     | 130.3, CH        | 7.12, brd (8.0)  |
| $3$-OH   |                  | 11.39, s         |
| $5$-OH   |                  | 5.87, brs        |
| $2^-$-OH |                  | 5.60, brs        |

a) $\delta$ in ppm; assignments made by DEPT, COSY, HMQC, and HMBC experiments. b) At 400MHz for $^1$H- and 100MHz for $^{13}$C-NMR experiments. c) At 600MHz for $^1$H- and 150MHz for $^{13}$C-NMR experiments. d–h) Interchangeable.
A comparison of the NMR data of 2 with those reported for the known bibenzyl 1, 2-carbomethoxy-3,5-dihydroxy-4-(3-methyl-2-butyl)bibenzyl, which was previously reported as a synthetic product, revealed that they are structural analogues, with the only difference being the presence of an additional hydroxy group at C-2' in 2. This assignment was based mainly on the HMBC correlations from H-16 (δ1.28) to C-2 (δ5 171.0). The other two hydroxy groups were attached to C-3 and C-5, respectively, on the basis of the chemical shifts of C-3 (δ6 171.0) and from both C-6 (δ6 5.32) and H-7 (δ4 3.43) to C-4 and C-5. The ester carbonyl carbon (δ5 171.0) could form hydrogen-bond with the C-3 hydroxy group and could be responsible for the downfield resonance of this hydroxy group, which placed the methyl ester moiety at C-2. This deduction was further supported by the HMBC correlations from H-6 to C-2 and the ester carbonyl carbon (δ5 171.0). The HMBC correlation from H-7 to C-4 established that the prenyl group was linked to C-4. Thus, 2 was elucidated as 2-carbomethoxy-3,5,2'-trihydroxy-4-(3-methyl-2-butyl)-bibenzyl, and was also assign the trivial name aglaiabbrevin A.

Compound 4 was obtained as a yellow, amorphous powder. The molecular formula of C19H20O3 was established by positive HR-ESI-MS at m/z 297.1476 [M+H]+ (Calcd 297.1491). The 1H- and 13C-NMR spectra (Table 1) of 4 were virtually identical to those of the known bibenzyl, 2,2-dimethyl-5-hydroxy-7-(2-hydroxyphenyl)-ethyl]chromene.

Compound 5 was found to be a yellow, amorphous powder. The negative HR-ESI-MS spectrum exhibited a pseudomolecular ion at m/z 365.2112 [M−H]− (Calcd 365.2117), indicating a molecular formula of C19H19O3. The 1H- and 13C-NMR spectra (Table 1) of 5 closely resembled those of the coexisting known bibenzyl 3,5-dihydroxy-2-[3,7-dimethyl-2(E),6-octadienyl]bibenzyl (7), with the only difference being a different prenyl chain at C-2. The two dimensional (2D)-NMR data (COSY, HMBC) established this structural fragment as shown in Fig. 2. From the COSY spectrum of 5, it is possible to establish two proton sequences from H-7 (δH 3.29) to H-8 (δH 5.13) and from H-10 (δH 2.06) to H-12 (δH 4.26) through H-11 (δH 1.49 and 1.62). The HMBC correlations from H-12 to C-11 (δC 34.0), the terminal double bond carbons C-13 (δC 149.1) and C-14 (δC 114.2), and C-15 (δC 173.3), from H-15 (δH 1.71) to C-12 (δC 75.8), C-13, and C-14, and from H-16 (δH 1.79) to C-8 (δC 123.3), C-9 (δC 135.4), and C-10 (δC 35.7) were observed in the HMBC spectrum. The E-configuration of the Δ2 double bond was suggested by the chemical shifts of C-7 (δC 24.9) and C-16 (δC 16.3),22,23 which was further confirmed by the observed rotating frame nuclear Overhauser enhancement spectroscopy (ROESY) correlation between H-7 (δH 3.29) and H16 (δH 1.79) in the ROESY spectrum. The above observations indicated the presence of a 6-hydroxy-3,7-dimethyl-2(E),7-octadienyl prenyl moiety. The absolute configuration of the only chiral center (C-12) of 5 was deduced to be R by comparing the optical rotation value of 5 [α]2θ +8.9, CHCl3 with those of [(α)-(+)-3-methyl-3-buten-2-ol [α]2θ +7.6, CHCl3]24 and (S)-17-hydroxy-18,20-ene-neogrofolin ([α]2θ −9.0, MeOH).25 Consequently, the structure of 5 (aglaiabbrevin C) was identified as (R)-(+)-3,5-dihydroxy-2-[6-hydroxy-3,7-dimethyl-2(E),7-octadienyl]bibenzyl.

Compound 6, a yellow, amorphous powder, had the same molecular formula of C20H20O3 as 5 based on the HR-ESI-MS ion peak at m/z 365.2114 [M−H]− (Calcd 365.2117). The 1H- and 13C-NMR spectra (Table 1) of 6 showed great similarity to those of 5. In fact, the structure of 6 differs from 5 only by the location of the double bond and the hydroxyl group of the prenyl group at C-2, where the hydroxyl group in 6 was shifted from C-12 to C-13 accompanied by the isomerization of the olefin from the Δ13(14) to Δ11. The assignment was confirmed by the HMBC correlations (Fig. 2) from H-14/H-15 (δH 1.28) to the olefinic carbon C-12 (δC 140.0) and the oxygen-bearing quaternary carbon C-13 (δC 69.4). The large coupling constant (J=15.0 Hz) observed for H-12 and the chemical shifts of C-7 (δC 24.9) and C-16 (δC 16.4) were indicative of a 8E,11E-configuration of the double bonds. Accordingly, the structure of 6 (aglaiabbrevin D) was established as 3,5-dihydroxy-2-[7-hydroxy-3,7-dimethyl-2(E),5(E)-octadienyl]bibenzyl.

Considering potent PTP1B inhibitory property previously reported for numerous prenylated flavonoids,26 all the isolates were subjected to testing in vitro for their inhibitory effects against PTP1B with oleic acid as the positive control (IC50=2.74±0.20 µM), which has proved to be an excellent PTP1B inhibitor.26,27 As shown in Table 2, all the compounds tested, except for 4, inhibited strongly PTP1B activity. In particular, compounds 5–7 exhibited more potent PTP1B inhibitory activity with IC50 values of 2.58±0.52, 2.44±0.35, and 2.23±0.14 µM, respectively, than the positive control, olea- nolic acid. The results obtained indicated that substitution of

Fig. 2. Selected COSY and HMBC Correlations of Compounds 2 and 4–6.

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the longer prenyl chains at C-2 might be responsible for an increase in PTP1B inhibitory activity of prenylated bibenzyls. Furthermore, these bibenzyls may be considered as potential lead compounds for the development of new anti-obesity and anti-diabetic agents. To the best of our knowledge, the PTP1B inhibitory effects of prenylated bibenzyls are being reported for the first time.

**Experimental**

**General Experimental Procedures** Optical rotation was measured in CHCl₃ on an Anton Paar MCP-200 polarimeter using a 100 mm metallic microcell. UV absorption spectra were recorded in MeOH on a Varian Cary 100 UV-Vis spectrophotometer; peak wavelengths are reported in nm. IR spectra were obtained in thin polymer films on a Shimadzu FTIR-8400 spectrometer; peaks are reported in cm⁻¹. The NMR spectra were measured at 300 K on Bruker DRX 400 and Avance 600 spectrometers. Chemical shifts are reported in parts per million (δ) with the residual CDCl₃ signal (δH 7.26 ppm; δC 77.00 ppm) as an internal standard for ¹H- and ¹³C-NMR and coupling constants (J) in Hz; assignments were supported by ¹H–¹H COSY, HSQC, and HMBC experiments. ESI-MS and HR-ESI-MS were carried out on a Bruker Daltonics Esquire2000plus instrument and a Waters Q-TOF Ultima mass spectrometer, respectively. Semipreparative HPLC was performed on an Agilent-1260 system equipped with a VWD G1341B detector using YMC-Pack ODS-A (250×10 mm, 5 µm) by eluting with a MeOH or CH₃CN–H₂O system at 3.5 mL/min. Commercial silica gel (200–300 and 400–500 mesh; Qingdao, China) was used for column chromatography (CC). Precoated SiO₂ plates (HSGF-254; Yantai, China) were used for analytical TLC. Spots were detected on TLC under UV light or by heating after spraying with anisaldehyde H₂SO₄ reagent. Sephadex LH-20 (Amersham Biosciences) was also used for CC. All solvents used for extraction and isolation were of analytical grade.

**Biological Material** The leaves of *Aglaia abbreviata* were collected from Xishuangbanna, Yunnan Province, People’s Republic of China, in May 2014, and were authenticated by Mr. Jin-Long Dong, Xishuangbanna Botanical Garden, Chinese Academy of Sciences, People’s Republic of China. A voucher specimen has been deposited in the Herbarium of School of Pharmacy, Nanchang University (accession number ZW1401).

**Extraction and Isolation** The air-dried and powdered leaves (7.8 kg) were extracted three times with EtOH–H₂O (40L×3, 95:5, v/v) for 2 d each extraction at room temperature. The EtOH extracts were combined and evaporated in vacuo to give 530 g of a residue, which was suspended in 6 L of water and then partitioned successively with petroleum ether (PE) (three times with 4 L each), EtOAc (three times with 4 L each), and n-BuOH (three times with 2 L each). The EtOAc-soluble portion (186 g) was concentrated in vacuo, and then fractionated by silica gel CC (8×85 cm, 200–300 mesh, 900 g) eluting with petroleum ether (PE)–acetone (10:0–0:10) to afford four fractions (A–D), which were combined based on the analysis of TLC. Fraction B (18 g), eluted with PE–acetone (8:2–7:3), was further subjected to a Sephadex LH-20 column (4.0×120 cm, 150 g) eluted with CHCl₃–MeOH (1:1) to six subfractions (B1–B6). Subfraction B3 (1.2 g) was separated by silica gel CC (2.5×30 cm, 400–500 mesh, 8 g) eluting with PE–acetone (7.5:2.5), and successively further purified by reversed-phase semipreparative HPLC eluting with MeOH–H₂O (80:15) to afford compounds 5 (2.3 mg, tᵣ=10.2 min), 6 (3.5 mg, tᵣ=11.5 min), 7 (32.0 mg, tᵣ=16.8 min), and 8 (51.8 mg, tᵣ=14.0 min). Subfraction B5 (530 mg) was purified by reversed-phase semipreparative HPLC (CH₃CN–H₂O, 75:25) to yield compounds 2 (6.1 mg, tᵣ=8.0 min) and 4 (12.5 mg, tᵣ=12.4 min).

Aglaiabbrevin A (2)

Yellow amorphous powder; UV λₘₐₓ (MeOH) nm (logε): 230 (3.52), 265 (3.20), 312 (2.96); IR (KBr) cm⁻¹: 3312, 1654, 1598, 1510, 1420, 1216; ¹H- and ¹³C-NMR data: see Table 1; (+) HR-ESI-MS m/z 355.1544 [M+H⁺] (Calcd for C₁₅H₁₉O₅⁺, 355.1545).

Aglaiabbrevin B (4)

Yellow amorphous powder; UV λₘₐₓ (MeOH) nm (logε): 233 (4.28), 272 (3.93), 298 (3.88); IR (KBr) cm⁻¹: 3340, 2944, 1602, 1519, 1451, 1206; ¹H- and ¹³C-NMR data: see Table 1; (+) HR-ESI-MS m/z 297.1476 [M+H⁺] (Calcd for C₁₄H₁₅O₃⁺, 297.1491).

Aglaiabbrevin C (5)

Yellow amorphous powder; [α]D²⁰ +8.9 (c=0.26, CHCl₃); UV λₘₐₓ (MeOH) nm (logε): 216 (4.38), 232 (4.01), 285 (3.60); IR (KBr) cm⁻¹: 3335, 1598, 1500, 1431, 1198; ¹H- and ¹³C-NMR data: see Table 1; (+) HR-ESI-MS m/z 365.2112 [M+H⁺] (Calcd for C₁₅H₂₃O₅⁺, 365.2117).

Aglaiabbrevin D (6)

Yellow amorphous powder; UV λₘₐₓ (MeOH) nm (logε): 216 (4.33), 232 (3.98), 285 (3.72); IR (KBr) cm⁻¹: 3320, 1612, 1515, 1420, 1203; ¹H- and ¹³C-NMR data: see Table 1; (+) HR-ESI-MS m/z 365.2114 [M+H⁺] (Calcd for C₁₅H₂₃O₅⁺, 365.2117).

**PTP1B Inhibitory Activity Bioassay** The PTP1B inhibitory activity bioassay of compounds 2 and 4–8 was carried out as described in our previous paper.²⁸ Oleanolic acid was used as the positive control.

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**Conflict of Interest** The authors declare no conflict of interest.

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**Table 2. Inhibitory Activity of Compounds 2 and 4–8 against PTP1B**

| Compound | PTP1B inhibitory activity IC₅₀ (µM) |
|----------|-----------------------------------|
| 2        | 15.51±1.54                       |
| 4        | 45.74±1.79                       |
| 5        | 2.58±0.52                        |
| 6        | 2.44±0.35                        |
| 7        | 2.23±0.14                        |
| 8        | 11.03±2.16                       |
| Oleanolic acid | 2.74±0.20                  |

a) IC₅₀ values were determined by regression analysis and expressed as the mean±standard deviation (S.D.) of three replicates. b) Positive control.
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