

# Secondary Metabolites from *Bryophyllum daigremontianum*

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**ABSTRACT:** A total of four compounds were isolated from the *n*-hexane soluble fraction of a methanolic extract of the whole plant of *Bryophyllum daigremontianum*. The structures of the isolated compounds were elucidated as 11-oxo-epi- $\beta$ -amyrin (**1**), 21-dehydrodesmosterol (**2**), 3,4-dihydroxy-*cis*-cinnamic acid (**3**), and *p*-hydroxybenzaldehyde (**4**) by high field NMR analyses as well as by comparison with structurally related compounds.

**Key words:** *Bryophyllum daigremontianum*, Crassulaceae, 11-oxo-epi- $\beta$ -amyrin, 21-dehydrodesmosterol, 3,4-dihydroxy-*cis*-cinnamic acid, *p*-hydroxybenzaldehyde.

## INTRODUCTION

*Bryophyllum daigremontianum* (Bengali name- Pathorkuchi, Family- Crassulaceae) is a perennial, glabrous herb with simple, opposite, oblong-lanceolate, serrate, obtuse, purple blotched beneath, petiole long leaves found in Bangladesh to a limited extent. *Bryophyllum* is reputed for antitumor,<sup>1</sup> antinociceptive, anti-inflammatory, antidiabetic<sup>2</sup> and antimicrobial activities.<sup>3</sup> Previous phytochemical studies with *Bryophyllum* revealed the occurrences of bryophollenone, bryophollone, cholestane-3,6,14-triol, 3,3',4',5,5',7-hexahydroxyflavan, 3-hydroxy-12,20-ursadien-11-one, 2-(9-decenyl) phenanthrene, bryophyllin-A<sup>4</sup>, bryophyllin B<sup>5</sup>, bryotoxin B, bryotoxin C, and 3,5,11,14-tetrahydroxy-12,19-dioxobufa-20,22-dienolide.<sup>6</sup>

We, herein, report the isolation of 11-oxo-epi- $\beta$ -amyrin (**1**), 21-dehydrodesmosterol (**2**), 3,4-dihydroxy-*cis*-cinnamic acid (**3**) and *p*-hydroxybenzaldehyde (**4**) from *B. daigremontianum*.

## MATERIALS AND METHODS

**General experimental procedure.** The <sup>1</sup>H NMR spectra were recorded using a Bruker AMX-400 (400 MHz) instrument in deuterated chloroform and the  $\delta$  values for <sup>1</sup>H spectra were referenced relative to the residual non-deuterated solvent signal.

**Plant material.** The whole plant of *B. daigremontianum* was collected from Savar, Dhaka in January 2004. A voucher specimen has been deposited in Dhaka University Herbarium (Accession no.-01).

**Extraction and isolation.** The powdered material (533 gm) was soaked in 1.5 liter of methanol

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in a large flask and was kept for 7 days with occasional shaking and stirring. The whole mixture was then filtered off through a filter paper and the filtrate thus obtained was concentrated at 40°C with a rotary evaporator. A portion (5.0 gm) of the concentrated methanol extract was fractionated by the modified Kupchan partitioning protocol<sup>7</sup> which afforded of *n*-hexane (750 mg), carbon tetrachloride (550 mg), chloroform (450 mg) and aqueous (3.05 gm) soluble materials.

A portion of the *n*-hexane soluble partitionate (650.0 mg) was chromatographed over Sephadex (LH-20) using *n*-hexane-dichloromethane-methanol (2.5:1). A total of 35 fractions (each 10 ml) were collected. Preparative thin layer chromatography (PTLC) of column fraction 10, over silica gel (Silica gel PF<sub>254</sub>) with 5% and 25% ethyl acetate in toluene provided compound **1** (3.0 mg). Again, PTLC of column fraction 14 with 20% ethyl acetate in toluene gave compounds **2** (2.5 mg). On the other hand, column fraction 22, upon preparative chromatography over silica gel using toluene-ethyl acetate (70:30) provided compounds **3** (2.5 mg) and **4** (2.5 mg).

**11-Oxo-epi-β-amyirin (1).** (3.0 mg, 0.06% yield): White amorphous powder; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 5.63 (1H, *t*, *J*=1.0 Hz, H-12), 3.46 (1H, br. *s*, H-3), 2.37 (1H, *t*, *J*=7.5 Hz, H-18), 1.15 (3H, *s*), 1.13 (3H, *s*), 1.08 (3H, *s*), 1.03 (3H, *s*), 0.99 (3H, *s*), 0.98 (3H, *s*), 0.94 (3H, *s*), 0.84 (3H, *s*).

**21-Dehydrodesmosterol (2).** (2.5 mg, 0.05% yield): Amorphous powder; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 5.34 (1H, br. *d*, *J*= 6.0 Hz, H-6), 5.20 (1H, *m*, H-24), 4.71 (1H, br. *s*, H<sub>b</sub>-21), 4.68 (1H, br. *s*, H<sub>a</sub>-21), 4.63 (1H, br. *s*, OH-3), 3.51 (1H, *m*, H-3), 1.63 (3H, *s*, H<sub>3</sub>-26), 1.55 (3H, *s*, H<sub>3</sub>-27), 1.00 (3H, *s*, H<sub>3</sub>-18), 0.69 (3H, *s*, H<sub>3</sub>-19).

**3,4-Dihydroxy-cis-cinnamic acid (3).** (2.5 mg, 0.05% yield): Amorphous powder; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.70 (1H, *d*, *J*=10.0 Hz, H-7), 7.64 (1H, *dd*, *J*=8.0, 1.5 Hz, H-6), 7.51 (1H, *d*, *J*=8 Hz, H-5), 7.36 (1H, *d*, *J*=1.5 Hz, H-2), 6.27 (1H, *d*, *J*=10 Hz, H-8).

**4-Hydroxybenzaldehyde (4).** (2.5 mg, 0.05% yield): Amorphous powder; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 9.8 (1H, *s*), 7.82 (2H, *d*, *J*=8.4 Hz), 7.00 (2H, *d*, *J*=8.4 Hz), δ 4.36 (1H).

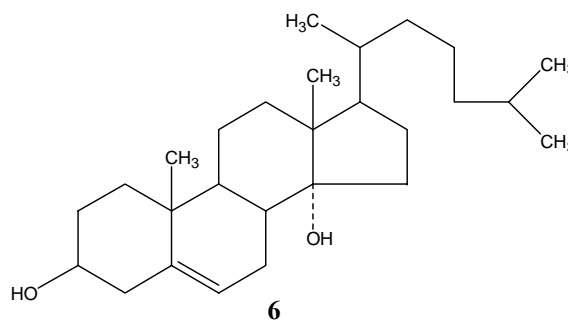
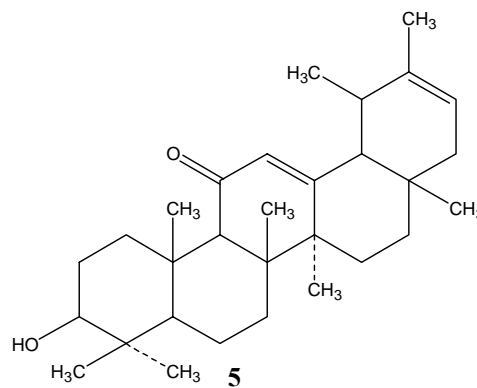
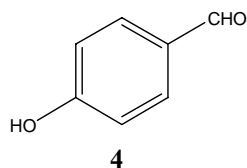
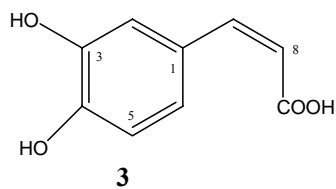
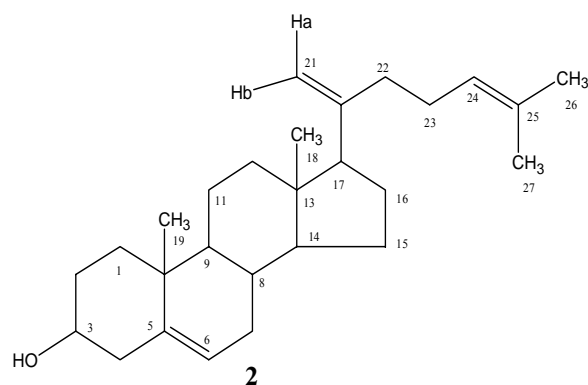
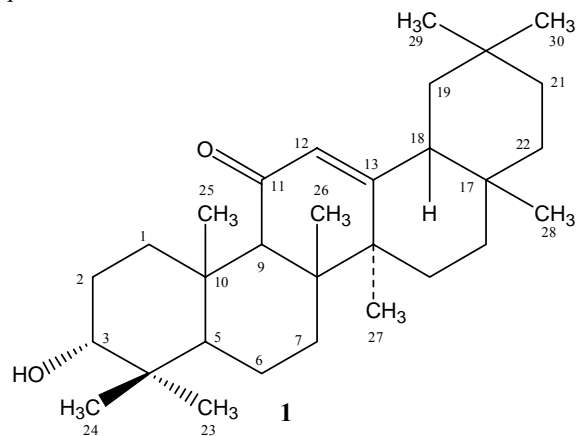
## RESULTS AND DISCUSSION

A total of four compounds were isolated from *n*-hexane soluble fraction of a methanolic extract of *B. daigremontianum* by repeated chromatographic separation and purification over silica gel. The structures of the isolated compounds were solved by NMR data analysis as well as by comparison with related compounds.

The <sup>1</sup>H NMR spectrum of compound **1** displayed an olefinic proton signal at δ 5.63 (*d*, *J*=1.0 Hz). The chemical shift of this proton suggested its placement at C-12, adjacent to a carbonyl group. The broad singlet at δ 3.46 could be assigned to an oxymethine proton at C-3. The absence of strong coupling and a low width half (*W*<sub>1/2</sub>) of the signal suggested that this proton was at the β-position (-OH at α position).<sup>8</sup> The <sup>1</sup>H NMR spectrum also showed eight methyl singlets at δ 0.84, 0.94, 0.98, 0.99, 1.03, 1.08, 1.13 and 1.15. On the basis of the above spectral data, compound **1** was characterized as 11-oxo-epi-β-amyirin (**1**). The identity of this compound was further confirmed by comparison of its spectral data with reported values.<sup>9</sup> Although, 11-oxo-epi-β-amyirin (**1**) has previously been reported from many plants,<sup>9</sup> this is the first report of its occurrence from *B. daigremontianum*. However, a closely related compound bryophynol (**5**) has previously been isolated from *B. pinnatum*.<sup>9</sup>

The <sup>1</sup>H NMR spectrum of compound **2** displayed a one proton multiplet at δ 3.51 and a doublet at δ 5.34 (*J*=6.0 Hz), both of which are typical for H-3 and H-6 of a steroidal carbon skeleton. In addition, the spectrum showed two methyl singlets at δ 0.69 and 1.00 and two methyl resonances in the downfield region at δ 1.63 and 1.55. These were assigned to the methyls at C-10 and C-13 and the *gem* dimethyls at C-25, respectively. Two broad one proton broad singlets were also seen in the <sup>1</sup>H NMR spectrum at δ 4.68 and δ 4.71, which were characteristic of an

exomethylene proton at C-21. The multiplet of one proton intensity centered at  $\delta$  5.20 could be assigned to H-24. On the basis of the above spectral data, compound **2** was tentatively identified as 21-dehydrodesmosterol (**2**), which is structurally related to bryophyllol (**6**) previously isolated from *B. pinnatum*.<sup>9</sup>



The <sup>1</sup>H NMR spectrum of compound **3** exhibited well resolved signals for five protons between 6.0 and 8.0 ppm at  $\delta$  6.27 (1H, *d*, *J*=10 Hz), 7.36 (1H, *d*, *J*=1.5 Hz), 7.51 (1H, *d*, *J*=8 Hz), 7.64 (1H, *dd*, *J*=8.0, 1.5 Hz) and 7.70 (1H, *d*, *J*=10.0 Hz). The signals at  $\delta$  6.27 and 7.70 were attributed to the *cis* olefinic protons, H-8 and H-7, respectively while the resonances at  $\delta$  7.36, 7.51 and 7.64 could be assigned to a 1,3,4-trisubstituted benzene moiety. Comparison of these data with published values led to identify compound **3** as 3,4-dihydroxy-*cis*-cinnamic acid.<sup>10</sup>

The <sup>1</sup>H NMR spectrum of compound **4** showed signals for an aldehydic proton at 9.8 (1H, *s*) and a *para* disubstituted aromatic proton resonances at  $\delta$  7.00 (2H, *d*, *J*=8.4 Hz), 7.82 (2H, *d*, *J*=8.4 Hz) and a broad singlet for hydroxyl proton at  $\delta$  4.36 (1H). Comparison of these data with published values allowed to characterize this compound as 4-hydroxybenzaldehyde (**4**).<sup>11</sup>

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