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-β-amyrin (**1**), 21-dehydrodesmosterol (**2**), 3,4-dihydroxy-*cis*-cinnamic acid (**3**), and *p*-hydroxy-benzaldehyde (**4**) by high field NMR analyses as well as by comparison with structurally related compounds.

Key words: *Bryophyllum daigremontianum*, Crassulaceae, 11-oxo-epi-β-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,[1] antinociceptive, anti-inflammatory, antidiabetic[2] and antimicrobial activities.[3] 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[4], bryophyllin B[5], bryotoxin B, bryotoxin C, and 3,5,11,14-tetrahydroxy-12,19-dioxobufa-20,22-dienolide.[6]

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Dhaka Univ. J. Pharm. Sci. **7**(2): 141-144, 2008 (December)

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

MATERIALS AND METHODS

General experimental procedure. The ^{1}H NMR spectra were recorded using a Bruker AMX-400 (400 MHz) instrument in deuterated chloroform and the δ values for ^{1}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

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[7] 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_{254}) 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-amyrin (**1**). (3.0 mg, 0.06% yield): White amorphous powder; ^{1}H NMR (400 MHz, $CDCl_3$): 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; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.34 (1H, br. *d*, *J*= 6.0 Hz, H-6), 5.20 (1H, *m*, H-24), 4.71 (1H, br. *s*, H_b-21), 4.68 (1H, br. *s*, H_a-21), 4.63 (1H, br. *s*, OH-3), 3.51 (1H, *m*, H-3), 1.63 (3H, *s*, H_3-26), 1.55 (3H, *s*, H_3-27), 1.00 (3H, *s*, H_3-18), 0.69 (3H, *s*, H_3-19).

3,4-Dihydroxy-*cis*-cinnamic acid (**3**). (2.5 mg, 0.05% yield): Amorphous powder; ^{1}H NMR (400 MHz, $CDCl_3$): 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; ^{1}H NMR (400 MHz, $CDCl_3$): 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).

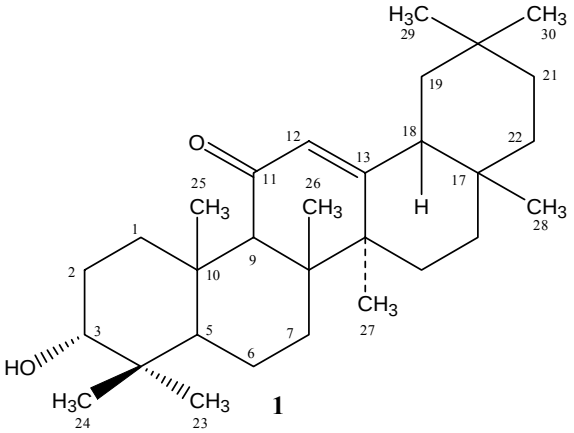
RESULTS AND DISCUSSION

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 ^{1}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 ($W_{1/2}$) of the signal suggested that this proton was at the β-position (-OH at α position).[8] The ^{1}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-amyrin (**1**). The identity of this compound was further confirmed by comparison of its spectral data with reported values.[9] Although, 11-oxo-epi-β-amyrin (**1**) has previously been reported from many plants,[9] 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*.[9]

The ^{1}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 ^{1}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 δ 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*.[9]



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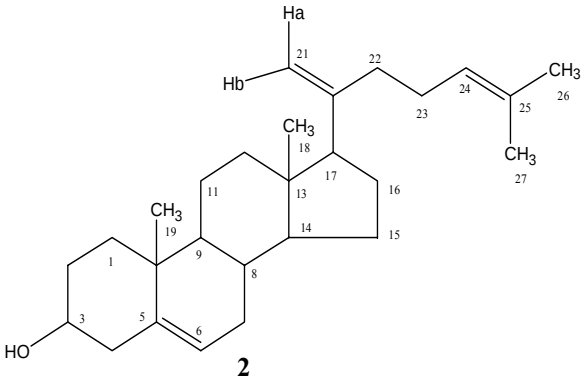

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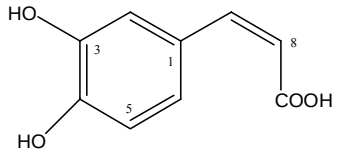

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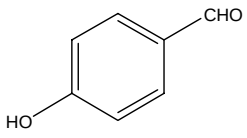

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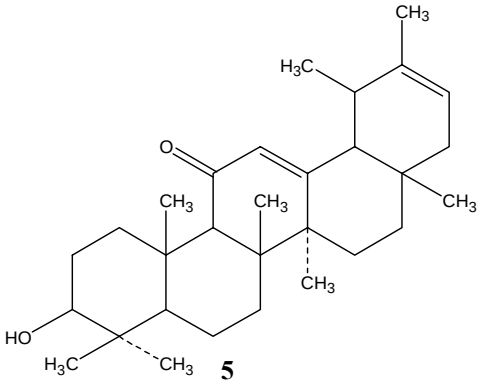

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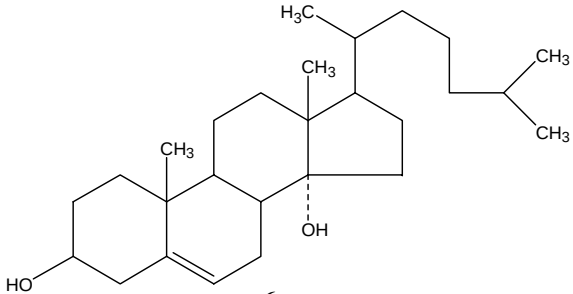

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The ^{1}H NMR spectrum of compound **3** exhibited well resolved signals for five protons between 6.0 and 8.0 ppm at δ 6.27 (1H, *d*, *J*=10 Hz), 7.36 (1H, *d*, *J*=1.5 Hz), 7.51 (1H, *d*, *J*=8 Hz), 7.64 (1H, *dd*, *J*=8.0, 1.5 Hz) and 7.70 (1H, *d*, *J*=10.0 Hz). The signals at δ 6.27 and 7.70 were attributed to the *cis* olefinic protons, H-8 and H-7, respectively while the resonances at δ 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.[10]

The ^{1}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 δ 7.00 (2H, *d*, *J*=8.4 Hz), 7.82 (2H, *d*, *J*=8.4 Hz) and a broad singlet for hydroxyl proton at δ 4.36 (1H). Comparison of these data with published values allowed to characterize this compound as 4-hydroxybenzaldehyde (**4**).[11]

ACKNOWLEDGEMENTS

The authors wish to thank the Ministry of Science and Information & Communication Technology, Government of the Peoples' Republic of Bangladesh for partial financial support for carrying out the research, and Bangladesh Council for Scientific and Industrial Research (BCSIR) for assisting with the NMR studies.

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