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Chemical Investigation of the Stem Barks of *Aporosa roxburghii*

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Abstract

Two compounds were isolated from the dichloromethane crude extract of the stem barks from *Aporosa roxburghii*. Based on the spectral evidence, their structures were determined to be friedelan-3-one (**1**) and *β*- sitosterol (**2**) This is the first report of occurrence of these compounds from *A. roxburghii.*

Key words: *Aporosa roxburghii*, Euphorbiaceae, friedelan-3-one and *β*- sitosterol.

I. Introduction

Aporosa roxburgii locally known as pata kharalla, kasua is an evergreen tree belonging to the family Euphorbiaceae. The synonyms of this plant are *Aporosa octandra*, *Aporosa dioica*, *Alnus dioica* Roxb, The plant is widely distributed in the coastal forest and hill tracts of Bangladesh and also found outside of Bangladesh such as Pakistan, North India, and Nepal to South China, South-east Asia and West Malaysia[1, 2] and has been used as a folk medicine for treatment of jaundice, stomach ulcers [3] and colic fever [4]

As part of our chemical investigation on Bangladeshi medicinal plants, we reported here in the isolation and structure elucidation of one triterpenoid and one steroid from dichloromethane extract. The structures of these compounds were determined to be friedelan -3-one (**1**) and *β*-sitosterol (**2**) based on the spectral and chemical evidences.

II. Materials And Methods

General experimental procedure The ^{1}H-NMR spectrum were recorded using a Bruker DPX-400 (400 MHz) instrument. For NMR studies deuterated chloroform was used and the δ values for ^{1}H were referenced to the residual nondeuterated solvent signal. Culumn chromatography (CC) was performed on a silica gel column (Kieselgel 60, 70-230 mesh). Precoated plates of silica gel 60 PF_{254} were used for analytical purposes.

Plant material The stem barks of *A. roxburghii* were collected from Modhupur, Tangail during March 2008 and identified by Bangladesh National Herbarium. A voucher specimen has been deposited in Bangladesh National Herbarium (DACB accession no-34384) Dhaka, Bangladesh.

Extraction and isolation of compounds About 400 gm of the powdered material was taken in a clean, round bottomed flask and soaked in 1.5 liter of dichloromethane. The container with its content was sealed by foil and kept for a period of 15 days. It was filtered through filter paper and the filtrate thus obtained was concentrated at 50 °C with a rotary evaporator. Crude dichloromethane extract (1.5gm) was subjected to column chromatography for fractionation on silica gel (Kieselgel 60, 70-230 mesh) and eluted with gradients of petroleum ether/dichloromethane, dichloromethane, dichloromethane/methanol and finally with methanol to afford 30 fractions (each 100 mL). Fractions 7,8 and 14 upon washing with petroleum ether gave compounds- **1** (10 mg), and **2** (12 mg) respectively.

Friedelan-3-one (1): White crystals; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.74 (3H,s,H-24), 0.88 (3H, s, H-25), 0.89 (3H, d, J=2.7 Hz, H-23), 0.96 (3H, s, H-30), 1.01(3H, s, H-26), 1.0 2 (3H, s, H-27), 1.06(3H, s, H-28), 1.19(3H, s, H-29), 1.26 (3H,s, H-30), 1.97(1H, m, H-1a),2.28 (2H, m, H-2b, H-4), 2.40 (1H, m, H-2a),1.29-1.78 (m, rest of the protons); ^{13}C NMR (400 MHz, $CDCl_3$): δ 22.3 (t, C-1),41.5 (t, C-2), 213.1 (s, C-3), 58.3 (d, C-4), 42.2 (s, C-5),41.5 (t, C-6), 18.3 (t, C-7), 53.1 (d, C-8), 37.5 (s, C-9),59.5 (d, C-10), 36.1 (t, C-11), 30.0 (t, C-12), 39.7(s, C-13), 38.3 (s, C-14), 32.5(t, C-15), 36.1 (t, C-16), 30.5(s,C-17), 42.8 (d, C-18), 35.7 (t, C-19), 28.2 (s, C-20), 32.8 (t, C-21), 39.3 (t, C-22), 6.8 (q, C-23), 14.7 (q, C-24), 18.0(q, C-25), 20.3 (q, C-26), 18.7 (q, C-27), 32.5 (t, C-28), 35.4 (t, C-29), 32.1 (d, C-30).

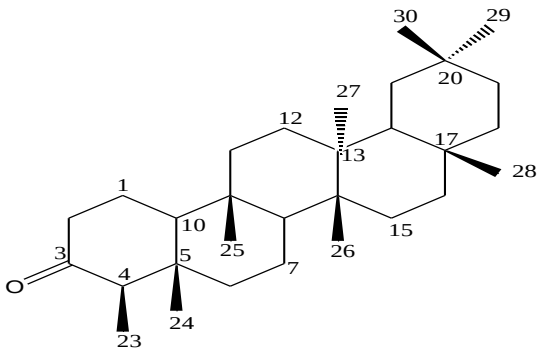
***β*-sitosterol (2):** Colorless needles; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.51(1H, m,H-3),5.34 (1H, m, H-6), 1.00 (3H, s, 10-CH_3), 0.67 (3H,s, 13-CH_3), 0.91 (3H, d , J = 6.8, 20-CH_3), 0.81 (3H, d , J = 7.6, 25-CH_3), 0.82(3H, d , J = 7.6, 25-CH_3).

III. Results And Discussion

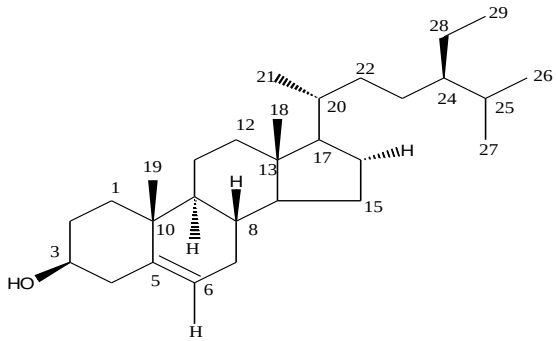
Two compounds were isolated from dichloromethane extract of stem barks of A. *roxburghii* by repeated chromatographic separation over silica gel. The structures of the isolated compounds were elucidated by extensive NMR spectral analyses. The presence of triterpenoid skeleton in both of compounds **1** & **2** was suggested by the vanillin sulfuric acid test.

The ^{1}H NMR spectra of compound-**1** revealed signal for two double doublet of one proton intensity at δ 2.23 typical for H-1a and at δ 2.23 (1H, dd, J=13.2, 7.2Hz) typical for H-2b and 4H of the pentacyclic triterpinoid and one equitorial proton at δ 2.38 (1H, m) for Heq-2.The spectrum displayed one multiplate at δ 1.95 for H-1a. eight singlet signals in the regions δ 0.72 ppm, δ 0.86 ppm, δ 0.87 ppm, δ 0.95ppm , δ 0.99 ppm, δ 1.00 ppm, δ 1.04 ppm and δ 1.17 ppm each integrating for three protons, assignable to eight tertiary methyl groups at C-24, C-25, C-23, C-30, ,C-26,C-27, C-28 and C-29 respectively.^{13}C NMR also exhibited resonances of 30 carbon atoms which were assigned after examination of the DEPT spectra as eight methyl groups, 11 methylene, 4 methine and seven quaternary carbon atoms, The presence of a keto group was evident in the ^{13}C NMR spectrum from the appearance of a keto carbon signal at δ 213.1 Finally, the structure was identified Friedelan-3-one. The identity of which was further substantiated by comparison of its spectroscopic data with those reported previously for compound-**1**[5-10].

The ^{1}H NMR spectra of compound-**2** showed two one-proton multiplets at δ 3.51 and δ 5.33 typical for H-3 and H-6 of a steroidal nucleus. The spectrum further revealed two singlets at δ 0.67 and δ1.00 each integrating for three protons, assignable to two tertiary methyl groups at C-13 and C-10 respectively. The ^{1}H NMR spectrum also showed two doublets centered at δ 0.81(J = 7.6 Hz) and 0.83 (J = 8.0 Hz) which could be attributed to two methyl group at C-25. The doublet at δ 0.92 (J = 6.0 Hz) was demonstrative of a methyl group at C-20. These NMR spectral features are characteristics of a steroidal carbon skeleton of *β*-sitosterol. Finally, the structure of was identified as *β*-sitosterol by comparing its reported ^{1}H NMR data [11-13].



Friedelan-3-one (1)



***β*- sitosterol (2)**

This is the first report of occurrence of compounds **1** and **2** from *A. roxburghii.*

IV. Acknowledgements

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