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NOTE

Isolation and Characterization of (22*E*,24*S*)-Stigmasta-5,22,25-trien-3β-ol from *Clerodendrum viscosum* Vent.

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(22*E*,24*S*)-Stigmasta-5,22,25-trien-3β-ol was isolated from the petroleum-ether soluble fraction of a methanol extract of the whole plant of *Clerodendrum viscosum* Vent. (Family-Verbenaceae). The structure of the isolated compound was elucidated by comprehensive analysis of spectroscopic data. To our best of knowledge, this is probably the first report of its occurrence from this plant.

Key Words: *Clerodendrum viscosum* Vent., Verbenaceae, Stigmasta-5,22,25-trien-3β-ol.

Clerodendrum viscosum Vent. (Bengali name-Bhat, Ghetu) is a flowering shrub, about 4 feet high, with broadly ovate leathery leaves, pinkish white flowers and small fruits enclosed in red bracts[1]. The leaf and root are widely used as antidandruff, antipyretic, ascaricide, laxative, vermifuge and in treatments of convulsion, diabetes, gravel, malaria, scabies, skin diseases, sore, spasm, scorpion sting, snake bite and tumor[2,3]. Previous phytochemical investigation of this plant led to the isolation of sterols, sugars, flavonoids and saponins[4]. Scutellarin and hispidulin-7-O-glucuronide are present in the leaf[5]. Poriferasterol and stigmasterol are the components of the aerial parts[6]. As a part of our ongoing investigations on local medicinal plants of Bangladesh[7,8], in this paper, we report the first time isolation of (22*E*,24*S*)-stigmasta-5,22,25-trien-3β-ol from the ethanol extract of *Clerodendrum viscosum*.

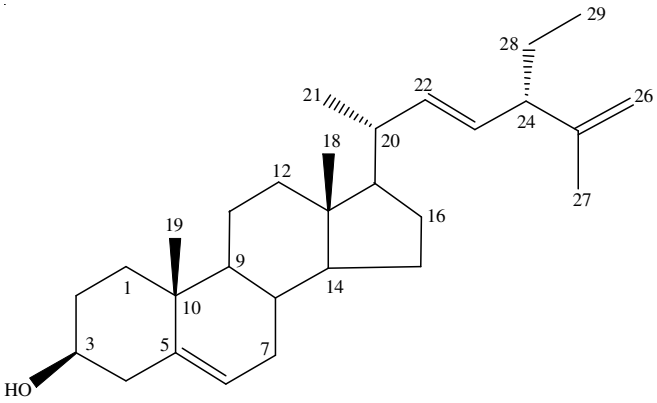
The ^{1}H NMR spectrum was recorded using a Bruker AMX-400 (400 MHz) instrument and the spectrum was referenced to the residual nondeuterated solvent signal. Preparative thin layer chromatography (PTLC) was carried out using Merck Si gel 60 F_{254} on glass plates (20 cm × 20 cm) at a thickness of 0.5 mm. TLC was conducted on normal-phase Merck Si gel 60 F_{254} on glass plates and the spots on thin layer chromatography and preparative thin layer chromatography plates were visualized under UV light at 254 nm as well as by spraying with vanillin sulfuric acid followed by heating for 5 minutes at 110 ºC.

Whole plant parts of *Clerodendrum viscosum* were collected from Savar, Dhaka in August 2010. A voucher specimen for this collection has been deposited in the herbarium of the Department of Botany, University of Dhaka. The samples were cut into small pieces and sun dried for 7 days followed by oven drying for 24 h at 40 ºC to facilitate grinding.

Extraction and isolation: The powdered material (900 g) was soaked in 2.5 L of ethanol in a large conical flask for 7 days with occasional shaking and stirring. The whole mixture was then filtered off through a cotton plug followed by Whatman filter paper No.1 and the filtrate thus obtained was concentrated at 40 ºC with a rotary evaporator. A portion (5 g) of the concentrated ethanol extract was fractionated by the modified Kupchan partitioning protocol[9] to yield petroleum ether (1 g), carbon tetrachloride (1.1 g), chloroform (0.85 g) and aqueous (1.65 g) soluble materials.

An aliquot of the petroleum-ether soluble partitionate (650 mg) was fractionated by column chromatography over silica gel (Kiesel gel 60, mesh 70-230) using pet-ether and ethyl acetate mixture in order of increasing polarities. A total of 138 fractions were collected, each 20 mL. Preparative thin layer chromatography (PTLC) of column fractions eluted with 30 % ethyl acetate in pet-ether over silica gel using toluene-ethyl acetate (90:10) afforded compound **1** (10 mg).

Detection method: (22*E*,24*S*)-Stigmasta-5, 22, 25-trien-3β-ol (**1**): 12.0 mg; white needle; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.68 (3H, s, H-18), 0.83 (3H, t, *J* = 7.6 Hz, H-23), 1.00 (3H, s, H-19), 1.01 (3H, d, *J* = 6.4 Hz, H-21), 1.68 (1H, s, H-27), 3.53 (1H, m, H-3), 4.70 (2H, bs, H-26), 5.17 (1H, dd, *J* = 15.6, 7.2 Hz, H-23), 5.24 (1H, dd, *J* = 15.6, 7.6Hz, H-22), 5.34 (1H, d, *J* = 5.6 Hz, H-6).



Compound **1**

Repeated chromatographic separation and purification of the petroleum-ether soluble partitionate of a methanol extract of the whole plant of *Clerodendrum viscosum* provided (22*E*, 24*S*)-stigmasta-5,22,25-trien-3β-ol, the structure of which was resolved by NMR analysis and by comparison with published values[10].

The ^{1}H NMR spectrum of compound **1** displayed signals at δ 0.68 and 1.00 (3H, each) assignable to two tertiary methyl groups at C-13 and C-10, respectively. The C-21 secondary methyl group appeared as a doublet (J = 6.4 Hz) at δ 1.01. On the other hand, a triplet (J = 6.5 Hz) of the three proton intensity at δ 0.83 could be accounted for the primary methyl group attached to C-28.

The ^{1}H NMR spectrum further revealed a one proton multiplet at δ 3.53, the position and multiplicity of which was indicative of H-3 proton. The typical signal for the olefinic proton, H-6 of the steroidal skeleton was evident from a doublet from at δ 5.34 integrating one proton. The olefinic protons (H-22 and H-23) appeared as characteristic downfield signals at δ 5.24 and δ 5.17, respectively. Each of these signal was observed as double doublets (J = 15.6, 7.2 Hz) showing their coupling with the neighbouring olefinic (*trans*) and methine protons. The spectrum also revealed a two proton broad singlet at δ 4.70 and a deshielded proton at δ 1.68 is due to the presence of isopropene group at C-24. On the basis, the compound **1** was characterized as (22*E*,24S)-stigmasta-5,22,25-trien-3β-ol.

Conclusion

The present phytochemical study of the petroleum-ether soluble fraction of the ethanol extract of *Clerodendrum viscosum* afforded a stigmasterol derivative, the structure of which was established as (22*E*,24*S*)-stigmasta-5,22,25-trien-3β-ol by extensive spectroscopic studies.

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