380–405 nm and they are shifted hypsochromic in the N-heteroaromatic substituted derivatives compared with other N-cycloalkyl compounds [2]. An important fact is that aromatic heterocycles alone have no significant inhibitory effect on mutagenicity of UV (inhibition 0–3%).

Experimental

1. Chemicals

The synthesis of the studied benzothiazolium salts was described previously [1]. DMSO was used as a solvent in all biological experiments.

2. Microorganisms

Euglena gracilis (strain Z) was maintained on a Cramer-Myers medium [3] supplemented with sodium acetate (0,5%) under static conditions at 26 ± 2 °C and with the permanent lighting.

3. Mutagenicity and antimutagenicity assays

3.1. Preparation of cells

E. gracilis cells in the exponential growth phase in the C. M. medium were centrifuged, washed and resuspended in a phosphate buffer (pH 6.2), preincubated for 16–18 h under permanent illumination at 26 °C and then used in the experiments.
Cell suspensions (about $0.8–1.0 \times 10^5$ cells/ml) were supplemented with tested substances in appropriate concentrations (2–100 μg/ml), incubated 8–10 h at 26 °C (co-treatment) and then UV irradiated. In the experiments with the post-treatment the cell suspensions (without benzothiazoles) were irradiated and then inoculated and incubated in medium containing corresponding benzothiazoles.

3.2. UV irradiation

2 ml of *E. gracilis* cells were transferred into Petri-dishes (7 cm diameter), inserted into a UVC 508 crosslinker and exposed to UV irradiation (200 J/m^2, 254 nm). After 24 h in dark irradiated cells were diluted and plated onto agar plates. All procedures were carried out under green safe-light to prevent photoreactivation. After 10–14 days of cultivation (2 days in the dark, remaining days in the light) growning green and white (bleached) colonies were counted and bleaching effect (mutagenicity) was determined.

3.3. Antimutagenicity

The mutagenicity inhibition percentage (MI%) was calculated for all samples, the method has been described previously [4]. The IC_{50} values (μg/ml) were interpolated from the cubic function for a relation between the concentration used and the MI %.

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A new sterol from the pseudobulb of *Desmotrichum fimbriatum* Blume

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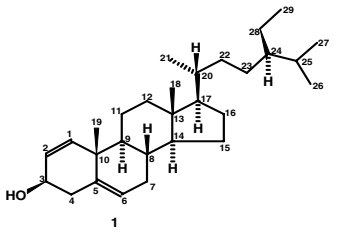
Desmotrichum fimbriatum Blume (Orchidaceae), popularly known as 'Jivanti', is an epiphytic orchid distributed up to 2,700 m. It is an important crude drug used in the indigenous system of medicine as astringent, aphrodisiac, expectorant, stimulant, cardio tonic, asthma, bronchitis, and throat infections [1, 2]. The presence of α- and β-jivantic acids from the aerial part of the plant has been reported [3]. The present paper describes the isolation and characterization of a new sterol.
Desmosterol (**1**) was isolated from petroleum ether-chloroform (55 : 45) mixture as colourless crystalline solid. It responded positively to the Lieberman-Burchard test for sterols. The IR spectrum contained absorption bands for hydroxyl group (3450 cm^{-1}), unsaturation (1640 cm^{-1}) and gem dimethyl isopropyl group (1380, 1308, 1080 cm^{-1}). The EIMS of **1** exhibited a molecular ion peak at m/z 412 as the base peak corresponding to the molecular formula $C_{29}H_{48}O$, which is supported by ^{13}C and DEPT NMR spectra. It indicated six degrees of unsaturation, four of which fully adjusted to the four rings of the carbocyclic nucleus and the remaining two to the olefinic bonds.
The EIMS exhibited diagnostically important fragment ions at m/z 226, 197 due to C_8/C_{14} and C_{11}/C_{12} fission and the ions at 248 and 164 arose due to C_8/C_{14} and C_9/C_{11} fission. These fragments suggested that the compound was a C_{29}- sterol [4, 5] possessing one hydroxyl group at in ring A/B which was placed at C-3 on the basis of biogenetic consideration, a C-10 saturated side chain and a diunsaturated steroidal skeleton of which one of the unsaturation was at C-2. The presence of a prominent peak at m/z 69 and 83 were suggestive of the existence of one of the double bond at C-5 and confirmation of the location of the hydroxyl group at C-3 in the ring.
The ^1H NMR spectrum of **1** showed the presence of three downfield one-proton doublets at δ 5.35 (J = 5.1 Hz) assigned to H-6, at δ 5.14 (J = 8.4 Hz) attributed to H-1 and another at δ 5.02 (J = 8.4, 8.4 Hz) associated with H-2. A broad multiplet at δ 3.52 ($w_{1/2}$ = 15.6 Hz) integrating for one proton was ascribed to H-3 axial carbinolic proton. Four doublets integrating for three proton each at δ 0.97 (J = 6.60 Hz), 0.84 (J = 6.0 Hz), 0.82 (J = 6.0 Hz) and 0.88 (J = 6.50 Hz) were due to three secondary methyl functionalities at C-21, C-26, C-27 one primary methyl group and C-29 respectively. The remaining two methyls resonated as three proton broad singlets at δ 0.68 and 1.00 and were correspondingly assigned to the two tertiary methyls at C-18 and C-19. The appearance of

all the methyls in the region δ 0.68–1.00 suggested that these groups were attached to saturated carbons [6]. The remaining two methylene and methine groups appeared in the region δ 2.28–1.05.

The ^{13}C NMR spectrum of **1** exhibits the presence of 29-carbons atom. The DEPT NMR spectrum exhibited six methyl, nine methylene, eleven methine and three quaternary carbon atoms. The signals at δ 140.8, 121.8, 129.3 and 138.41 were assigned to olefinic carbons at C-5, C-6, C-2 and C-1, respectively. The C-3 carbinol carbon resonated at δ 71.7. The degree of protonation of each carbon atom was determined by DEPT experiments and the assignment of carbon chemical shifts was made by comparison of δ values in the corresponding carbon atoms in the structurally similar sterols [7, 8]. The 24 R-configuration of the ethyl group was confirmed by comparison of the chemical shifts of carbons and protons of the side chain in ^{13}C and ^{1}H NMR spectra of **1** with a series of sterols having similar configuration at C-24 (δ 45.9), particularly β-sitosterol [9] and stigma-4-en-6β-ol-3-one [7].

The COSY 45^0 experiments were performed to confirm the structure which with long range CH correlations established the complete substitution pattern of **1**. The connectivity's between H-3/H-1, H-1/H-4, H-6/H-2, H-14/H-16 and Me-21/H-24 were observed.

On the basis of these spectral evidences compound **1** was characterized as (24R) stigmast-1,5-dien-3β-ol.



1

Experimental

1. General procedure

Melting point is uncorrected. IR spectra were recorded in CCl_4 on a Perkin-Elmer-377 spectrophotometer. ^{1}H (300 MHz), ^{13}C (75 MHz) and 2D NMR spectra were screened by Brucker Spectrospin NMR instrument in $CDCl_3$ using TMS as internal standard. EIMS was run at 70 ev on a Joel D-300 spectrometer. Column chromatography was performed on silica gel (Merck, 60–120 mesh) and thin layer chromatography on silica gel G (Merck).

2. Plant material

Pseudobulbs of *D. fimbriatum* Blume were purchased from the local market of Khari Baoli, Delhi, India, in October 1999. The specimen were identified by Dr. M. P. Sharma (Taxonomist), in the Department of Botany, Jamia Hamdard. A voucher specimen is deposited in the herbarium of the Phytochemistry Research Laboratory, Jamia Hamdard.

3. Extraction

The pseudobulbs of *D. fimbriatum* were extracted with petroleum ether, chloroform and then finally with MeOH. The chloroform fraction was concentrated and chromatographed on silica gel (60–120 mesh). The column was eluted with petroleum ether and chloroform in order of increasing polarity.

4. Isolation and characterization of 1

Elution of the column with petroleum ether-chloroform (55 : 45), (fraction 95–115) afforded colourless crystals of **1**, which were recrystallized from chloroform-acetone as colourless crystalline solid, 80 mg, m.p. 126–130 °C, R_f 0.52 (hexane-acetone-4 : 1); UV λ_{max} ($CHCl_3$) 248 nm (log ε 6.7); IR ν_{max} (KBr) 3450, 2957, 1640, 1463, 1380, 1308, 1080, 1035, 795 cm^{-1}; ^{1}H NMR: δ 5.35 (1 H, d, J = 5.1 Hz, H-6), 5.14 (1 H, d, J = 8.4 Hz, H-1), 5.02 (1 H, dd, J = 8.4, 8.4 Hz, H-2), 3.52 (1 H, brm, $w_{1/2}$ J = 15.6 Hz, H-3β), 1.00 (3 H, brs, Me-19), 0.97 (3 H, d, J = 6.6 Hz, Me-21), 0.88 (3 H, d, J = 6.5 Hz, Me-29), 0.84 (1 H, d, J = 6.0 Hz, Me-26), 0.82, (3 H, d, J = 6.0 Hz, Me-27), 0.68 (3 H, brs, Me-18), 1.00 (3 H, brs, Me-19); ^{13}C NMR; δ 138.41 (C-1), 129.3 (C-2), 71.84 (C-3), 42.35 (C-4), 140.8 (C-5), 121.8 (C-6), 29.76 (C-7), 31.71 (C-8), 50.21 (C-9), 36.21 (C-10), 21.15 (C-11), 37.33 (C-12), 39.85 (C-13), 56.93 (C-14), 24.34 (C-15), 28.31 (C-16), 56.13 (C-17), 11.92 (C-18), 19.45 (C-19), 34.01 (C-20), 18.85 (C-21), 31.97 (C-22), 26.16 (C-23), 45.90 (C-24), 29.23 (C-25), 19.11 (C-26), 19.88 (C-27), 23.18 (C-28), 12.04 (C-29); EIMS (rel. int. %): m/z 412 $[M]^+$ ($C_{29}H_{48}O$)(100), 397 (23.1), 394 (33.7), 379 (18.0), 327 (25.7), 303 (27.8), 248 (26.7), 226 (14.4), 197 (8.8), 164 (17.1), 144 (27.0), 94 (27.2), 83 (26.1), 81 (33.5), 70 (23.6), 69 (29.1), 55 (70.3).

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