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12α-Hydroxystigmast-4-en-3-one: a new bioactive steroid from *Toona ciliata* (Meliaceae)

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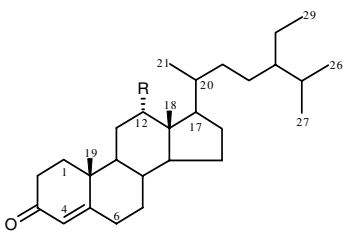
In a bioassay guided phytochemical investigation of *Toona ciliata* (Fam. Meliaceae), a new hydroxy steroidal ketone, 12α-hydroxystigmast-4-en-3-one (**1**) was isolated from the petroleum ether extract of the plant together with two known steroids and three *C*-methyl coumarins. The structure of **1** was established by means of spectroscopic analyses. Compound **1** was found cytotoxic in a brine shrimp lethality bioassay with LC_{50} of 9.9 μg/ml and it also showed significant antitumor activity with Ti_{50} value of 14.1 μg/ml in a potato disc bioassay.

1. Introduction

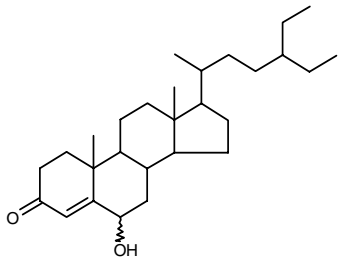
Toona ciliata M. J. Roem., syn. *Cedrela toona* Roxb. (Bengali – Toon) is a tall tree widely distributed in south and southeast Asia. The bark of the plant is used in Bengali traditional medicine as cardiotonic and also useful in ulcers, leprosy, and rheumatism. Chemical investigations of *T. ciliata* revealed a number of terpenoids [1], limonoids [1, 2] campesterol [3] and a coumarin [4]. The antifeedant activity of some isolated limonoids toward the Mexican bean beetle *Epilachna varivestis* [2, 5], antifungal activity of cedrelone against *Puccinia arachidis* [6] and feeding deterrence of its photooxidation products [7] have been reported earlier. As a part of continuing search for novel bioactive principles from the medicinal plants of Bangladesh, we studied the petroleum ether extract of the stem bark of *T. ciliata* and recently reported two known steroids and three *C*-methyl coumarins [8]. This paper describes the isolation and structure determination of a new steroid (**1**) from the same plant.

2. Investigations, results and discussion

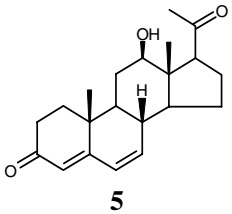
The concentrated petroleum ether extract, initially fractionated by column chromatography over Si gel followed by preparative TLC yielded compound **1**. FABMS displayed $[M + Na]^+$ ion at *m/z* 451 and the ^{13}C spectrum exhibited 29 carbon resonances. These suggested the molecular formula of **1** to be $C_{29}H_{48}O_2$ which was accounted for six degrees of unsaturation. The IR spectrum displayed bands corresponding to hydroxyl (3402 cm^{-1}) and carbonyl (1723 cm^{-1}) functionalities.



1 R = OH
2 R = H



3 β-OH
4 α-OH



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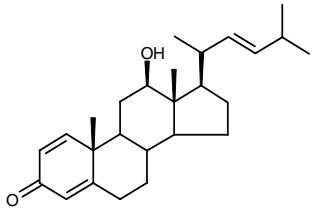

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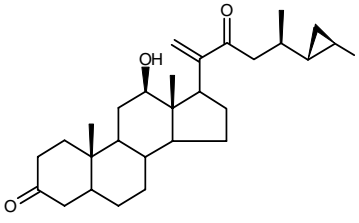

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The ^{1}H NMR spectrum of **1** was in close correspondence to that of stigmast-4-en-3-one (**2**) suggesting a close structural similarity [9]. The spectrum showed an olefinic proton as a singlet at δ 5.74 as well as three methyl doublets at δ 0.85, 0.86, 0.93, a methyl triplet at δ 0.82, and two methyl singlets at δ 0.73 and 1.19. The occurrence of a one-proton triplet at δ 3.66 rendered the compound as an interesting one indicating the presence of an oxymethine proton in the molecule. Coupling with two other protons restricted the oxymethine to C-1, C-2, C-6 or C-12. The proton would have been more deshielded if it were attached to either of C-1 or C-2 due to magnetic anisotropy of carbonyl group [10]. Considering the assignment at C-6, Della Greca et al. [9] reported that the 6β-OH in compound **3** caused a downfield shift of 0.2 ppm for H-19 methyl group owing to 1,3 diaxial interaction [11]. Again, the stereoisomeric 6α-hydroxy enone (**4**) showed the H-4 proton shifted downfield at δ 6.20 due to the spatial proximity of the 6α-OH group and the H-6 as a broad multiplet at δ 4.32 due to its β-axial orientation. These data precluded the possibility of OH assignment at C-1, C-2, or C-6 of **1**.

In 1999, Huq et al., [12] reported neridienone A (**5**) from *Nerium oleander*, which showed δ 3.71 for the oxymethine proton at H-12. Kingston and Fallis [13] reported a chemical shift value of 3.40 for C-12 proton in compound **6** while Iguchi et al. [14] described the structure of aragusterol D (**7**) isolated from a marine sponge with an oxymethine proton (H-12) at δ 3.61. Therefore, the oxymethine proton (δ 3.66) was assigned to C-12 of compound **1**. The stereochemistry of the hydroxyl group was ascertained from the ^{1}H NMR data. The small coupling constant (J = 6.5 Hz) of the proton signal at δ 3.66 due to one axial-equatorial coupling and one equatorial-equatorial coupling with the C-11 protons established that this proton was equatorial and hence the hydroxyl group was axial [15]. Me-13 was biogenetically established as beta-axial, therefore, the axial hydroxyl group must be attached alpha to C-12. From the above spectral data and by comparison with those of the relevant compounds, the structure of compound **1** was determined as 12α-hydroxystigmast-4-en-3-one. The reaction product of **1** after an exposure to mild heat and air appeared as a poorly resolved multiplet at δ 3.66 in the ^{1}H NMR spectrum and also clearly displayed the absence of carbon doublet at δ_C 72.6 (C-12) in ^{13}C NMR spectrum. This demonstrated that compound **1** could be converted into **2** at elevated temperatures.

Compound **1** was evaluated for its cytotoxicity and antitumor activity by the well-known brine shrimp lethality bioassay and potato disc bioassay [16]. In the brine shrimp lethality bioassay, the crude petroleum ether extract and the corresponding column fraction 11 from which **1** was obtained showed LC_{50} values of 5.3 μg/ml and 3.2 μg/ml while compound **1** demonstrated an LC_{50} of 9.9 μg/ml. In the potato disc bioassay, petroleum ether extract, petroleum ether column fraction 11 and compound **1** demonstrated dose-dependency and Ti_{50} values of 22.9 μg/disc, 22.3 μg/disc and 14.1 μg/disc, respectively. Compound **1** was also tested against 19 bacterial strains by the standardized disc diffusion method [17] but demonstrated only mild antibacterial activity *in vitro*.

3. Experimental

3.1. General

The ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) spectra were obtained in $CDCl_3$ on a Varian VXR-500S spectrometer and the chemical shifts are reported in ppm relative to the residual nondeuterated solvents. IR and Mass spectra were obtained on a Perkin-Elmer 1600 FTIR spectrometer and a JEOL SX 102 mass spectrometer (resolving power = 10,000) using *m*-nitrobenzyl alcohol (NBA) or polyethylene glycol as matrix. Si gel 60 (70–230 mesh) was used for CC; TLC was carried out on Kieselgel PF_{254} plates (Merck), and the spots were visualized under UV (254 and 366 nm), and by spraying the plates with vanillin (1%)-H_2SO_4 (10%) in EtOH, followed by heating.

3.2. Plant material

The stem bark of *T. ciliata* was collected from Comilla in August 2000. The plant was identified by M. Salar Khan, Ph.D., Ex-Research Consultant, Bangladesh National Herbarium. Voucher specimens have been deposited in Bangladesh National Herbarium (DACB accession no. 28,926) and Dhaka University Herbarium (DUH accession no. 18).

3.3. Extraction and isolation

The air-dried and pulverized plant material (150.0 g) was extracted in a Soxhlet at elevated temperature with 0.5 l of light petroleum ether (40–80 °C). The extract was filtered and then evaporated under reduced pressure at 40 °C using a Büchi rotary evaporator to have a gummy concentrate (1.5 g). An aliquot of the extract (1.2 g) was subjected to column chromatography over Kieselgel 60, mesh 70–230, and the column was eluted with petroleum ether, petroleum ether-EtOAc, EtOAc, EtOAc-MeOH mixtures of increasing polarity, with 56 fractions collected. Fraction 11 was subjected to preparative TLC over Silica gel PF_{254}, using toluene-EtOAc-acetic acid (97 : 3 : few drops) as developing solvent which afforded 13.6 mg of **1**.

12α-Hydroxystigmast-4-en-3-one (**1**) : yellow gum; CIMS : *m/z* $[M + H]^+$ 429 (appropriate for $C_{29}H_{48}O_2 + H^+$); FABMS : *m/z* $[M + Na]^+$ 451; IR (film): 3402, 1723, 1639 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.73 (3 H, *s*, H-18), 0.83 (3 H, *d*, J = 6.5 Hz, H-27), 0.85 (3 H, *d*, J = 6.5 Hz, H-26), 0.86 (3 H, *t*, J = 8.0 Hz, H-29), 0.93 (3 H, *d*, J = 6.5 Hz, H-21), 1.19 (3 H, *s*, H-19), 3.66 (1 H, *t*, J = 6.5 Hz, H_{eq}-12), 5.74 (1 H, *s*, H-4); ^{13}C NMR (125 MHz, $CDCl_3$): 11.95 (C-29), 11.97 (C-18), 123.74 (C-4), 17.39 (C-19), 171.70 (C-5), 18.70 (C-21), 19.02 (C-27), 19.81 (C-26), 199.65 (C-3), 21.04 (C-11), 23.08 (C-28), 24.19 (C-15), 26.09 (C-23), 28.19 (C-16), 29.16 (C-25), 32.06 (C-7), 32.96 (C-6), 33.99 (C-2), 33.99 (C-22), 35.64 (C-1), 35.70 (C-8), 36.12 (C-20), 38.61 (C-10), 42.39 (C-13), 45.84 (C-24), 53.82 (C-9), 55.82 (C-14), 56.02 (C-17), 72.63 (C-12).

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