Chemical Investigation of *Mesua nagassarium* (Burm. f.) Kosterm

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Abstract: Repeated chromatographic separation and purification of pet-ether and carbon tetrachloride soluble fractions of a methanol extract of stem bark of *Mesua nagassarium* (Burm. f.) Kosterm yielded five compounds. Extensive spectroscopic studies, including high field NMR analyses was conducted to identify these compounds which resulted to be friedelin (**1**), 3β-friedelanol (**2**), lupeol (**3**), 3-oxo-betulin (**4**) and spinasterol (**5**). Although compounds **1**-**3** have been reported from various plant species, but 3-oxo-betulin and spinasterol have been discovered from *M. nagassarium* (Burm. f.) Kosterm for the first time.

Keywords: *Mesua nagassarium*, Clusiaceae, 3-oxo-betulin, spinasterol.

INTRODUCTION

Mesua nagassarium (Burm. f.) Kosterm, (Bengali name- Nageswar; Family- Clusiaceae), is a medium-sized evergreen tree up to 36 m tall abundant in tropical Sri Lanka and also cultivated in Assam, southern Nepal, Indochina, and the Malay Peninsula [1]. *M. nagassarium* is a medicinal plant, whose various parts are found to possess antimicrobial [2], anti-inflammatory, haemostatic and astringent activities [3]. Essential oil from stamens showed anthelmintic activity against hookworm and tapeworm [4]. Previously some coumarin, xanthone, flavonoid and cyclohexadiene derivatives were isolated from plants belonging to the Clusiaceae family [5-8]. In continuation to research on medicinal plants for discovery of bioactive compounds, our study on *M. nagassarium* reveals five compounds from the stem bark of this plant; four triterpenoids and one steroid.

EXPERIMENTAL

General Experimental Procedures

^{1}H NMR spectra were recorded using a Bruker AMX-400 (400 MHz) and Varian VXR-500S (500 MHz) instruments and the ^{13}C NMR spectrum of friedelin (**1**) was obtained on Bruker AMX-400 at 100 MHz. The δ values for ^{1}H and ^{13}C data were corrected with reference to the residual non-deuterated solvent signals. The identity of the compounds was further established by co-TLC with authentic samples provided by the Phytochemistry laboratory, University of Dhaka.

Extraction and Isolation

The air dried and powdered stem bark (1000 g) was soaked in 3.0 L of methanol for 15 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was concentrated with a rotary evaporator and a portion (40 g) of the concentrated methanol extract was fractionated by the modified Kupchan partitioning protocol [9] into pet-ether (5.5 g), carbon tetrachloride (4.5 g), chloroform (4.0 g) and aqueous (14.0 g) soluble materials.

The pet-ether and carbon tetrachloride soluble partitionates were separately chromatographed over silica gel (Kiesel gel 60H, mesh 70-230) and the columns were eluted with pet-ether followed by mixtures of pet-ether and ethyl acetate in order of increasing polarities. Compound (**1**) was isolated as yellowish amorphous powder from the column fractions of the pet-ether soluble materials eluted with 10% ethyl acetate in pet-ether while fractions eluted with 15% ethyl acetate in pet-ether provided compound (**2**). Column chromatographic separation of the carbon tetrachloride soluble materials eluted with 20% ethyl acetate in pet-ether yielded compound (**4**) as brownish

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white gum. Similar column chromatographic separation of the carbon tetrachloride soluble partitionate eluted with 15% and 25% ethyl acetate in pet-ether afforded compound (**3**) and (**5**), respectively.

Properties of Isolated Compounds

Friedelin (**1**) (16 mg, 0.32% yield): yellowish amorphous powder; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.70 (2H, dd, *J*= 5.5, 3.0 Hz, H-1a, H-1b), 2.30 (1H, m, H-2b), 2.40 (1H, m, H-2a), 2.10 (1H, m, H-4), 1.30-1.50 (1H, m, H-6), 0.86 (3H, br. s, Me-4), 0.71 (3H, s, Me-5), 0.86 (3H, s, Me-9), 1.04 (3H, s, Me-13), 0.99 (6H, s, Me-14 and Me-20a), 1.17 (3H, s, Me-17), 0.94 (3H, s, Me-20b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.3 (C-1), 41.5 (C-2), 213.2 (C-3), 58.2 (C-4), 42.1 (C-5), 41.3 (C-6), 18.2 (C-7), 53.1 (C-8), 37.4 (C-9), 59.5 (C-10), 35.6 (C-11), 32.4 (C-12), 38.3 (C-13), 39.7 (C-14), 30.5 (C-15), 36.0 (C-16), 30.0 (C-17), 42.8 (C-18), 35.3 (C-19), 28.1 (C-20), 32.8 (C-21), 29.6 (C-22), 6.8 (C-23), 14.7 (C-24), 18.3 (C-25), 18.7 (C-26), 20.3 (C-27), 32.1 (C-28), 31.8 (C-29), 35.0 (C-30) [10].

3β-friedelanol (**2**) (14 mg, 0.26% yield): white amorphous powder; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.91 (H-2a, dt, *J*=10.0, 2.5 Hz), 3.74 (H-3, br.s), 1.75 (H-6a, dt, *J*=12.8, 3.2 Hz), 0.93 (3H, d, *J*=6.8 Hz, Me-23), 0.96 (3H, s, Me-24), 0.86 (3H, s, Me-25), 0.98 (3H, s, Me-26), 1.00 (3H, s, Me-27), 1.17 (3H, s, Me-28), 0.94 (3H, s, Me-29), 0.99 (3H, s, Me-30).

Lupeol (**3**) (11 mg, 0.24% yield): white amorphous powder; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.60 (1H, m, H-2a), 1.55, (1H, m, H-2b), 3.20 (H-3, dd, *J*=11.3 Hz, 4.8 Hz), 1.65 (1H, m, H-13), 0.90 (1H, m, H-18), 2.20 (H-19, m), 1.92 (1H, m, H-21), 0.96 (3H, s, Me-23), 0.75 (3H, s, Me-24), 0.82 (3H, s, Me-25), 1.02 (3H, s, Me-26), 0.94 (3H, s, Me-27), 0.78 (3H, s, Me-28), 4.68 (H_b-29, br.s), 4.56 (H_a-29, br.s), 1.67 (3H, s, Me-30).

3-Oxo-betulin (**4**) (9 mg, 0.20% yield): brownish-white gum; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.60 (2H, m, H-2), 3.10 (1H, m, H-19), 0.93 (3H, s, Me-23), 0.75 (3H, s, Me-24), 0.82 (3H, s, Me-25), 1.02 (3H, s, Me-26), 0.96 (3H, s, Me-27), 3.63 (H_b-28, s), 3.58 (H_a-28, s), 4.67 (H_a-29, s), 4.55 (H_b-29, s), 1.67 (3H, s, Me-30).

Spinasterol (**5**) (7 mg, 0.1556% yield): colorless gum; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.40 (2H, m, H-2), 3.60 (1H, m, H-3), 1.25 (2H, m, H-4), 5.16 (1H, brs, H-7), 1.20 (2H, m, H-16), 0.55 (3H, s, Me-18), 0.80 (3H, s, Me-19), 1.05 (3H, d, *J*=6.4 Hz, Me-21), 5.15 (1H, dd, *J*=14.0 Hz, 8.0 Hz, H-22), 5.05 (1H, dd, *J*=14.0 Hz, 8.0 Hz, H-23), 1.55 (2H, m, H-24, H-25), 0.86 (3H, d, Me-26), 0.85 (3H, d, Me-27), 0.81 (3H, t, *J*=6.5 Hz, Me-29).

RESULTS AND DISCUSSION

The five compounds were isolated from the pet-ether and carbon tetrachloride soluble fractions of methanol extract of the stem bark of *M. nagassarium* by repeated chromatographic separation and purification over silica gel. The structures of the isolated compounds were resolved by comparing NMR data available in literature.

The ^{1}H NMR spectrum of compound (**1**) revealed presence of eight methyl group signals at δ 0.71, 0.86, 0.94, 0.99, 1.04, and 1.17 including an unresolved doublet at δ 0.86 (Me-4). These were attributed to Me-5, Me-9, Me-20b, Me-14, Me-20a, Me-13, and Me-17, respectively. The ^{1}H NMR spectrum of the compound also exhibited a double doublet (*J*= 5.5 Hz and 3.0 Hz) of two proton intensity at δ 1.70 which could be ascribed to protons at C-1 position and multiplets of one proton intensity at δ 2.30, 2.40 and 2.10 indicating the presence of C-2 and C-4 protons.

The ^{13}C NMR spectrum displayed 30 carbon resonances, including a carbonyl carbon at δ 213.2. The DEPT experiment indicated that 23 out of the 30 carbon atoms in (**1**) had attached protons. Thus, it exhibited signals for 8 methyl, 11 methylene, 4 methine and 7 quaternary carbons. These ^{1}H and ^{13}C NMR spectral data are in close agreement to those published for friedelin [11-13].

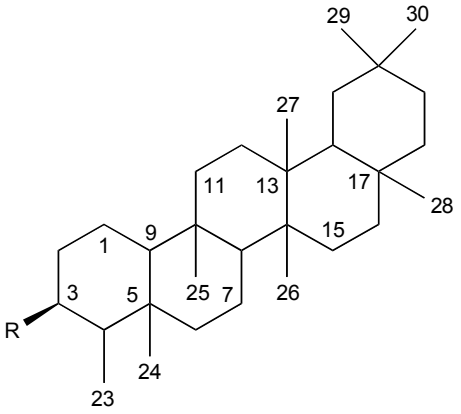
On this basis, compound (**1**) was identified as friedelin, the identity of which was further substantiated by direct comparison of its ^{1}H and ^{13}C NMR spectra with those of friedelin as well as by co-TLC with an authentic sample provided by the Phytochemistry laboratory of University of Dhaka.

The ^{1}H NMR spectrum of compound (**2**) revealed two doublet of triplets centered at δ 1.91 (1H, *J*= 10.0, 2.5 Hz) and 1.75 (1H, *J* = 12.8, 3.2 Hz), which could be attributed to H-2a and H-6a, respectively in a friedelin type triterpene. The presence of a one proton broad singlet at δ 3.74 indicated the typical oxymethine proton at C-3. The chemical shift and splitting pattern of this signal was characteristic for 3β–friedelanol type triterpenoid skeleton. The ^{1}H NMR spectrum also showed a three proton doublets (*J*= 6.8 Hz) at δ 0.93 which could be assigned to the methyl group protons at C-4. In addition, the ^{1}H NMR spectrum displayed seven

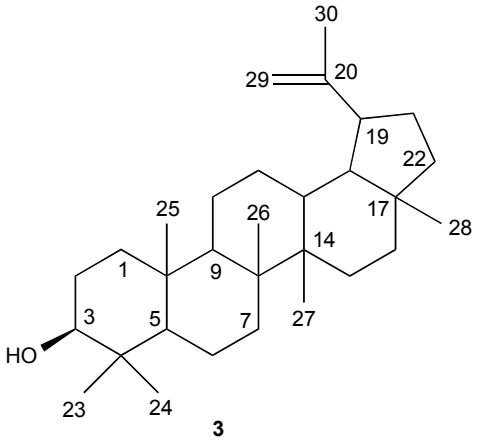
three proton singlets at δ 0.96, 0.86, 0.98, 1.00, 1.17, 0.94 and 0.99, attributable to the methyl group protons at C-5, C-9, C-13, C-14, C-17, C-20b and C-20a, respectively. Thus, it was identified as 3β-friedelanol. The identity of compound **2** as 3β-friedelanol was further confirmed by comparison of its ^{1}H NMR data with reported values [14] as well as co-TLC with an authentic sample preserved in the Phytochemistry laboratory of University of Dhaka.

The ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound (**3**) exhibited a double doublet (*J*= 11.3 Hz and 4.8 Hz) of one proton intensity at δ 3.20, typical for an oxymethine proton at C-3 in a triterpene type carbon skeleton [15-18]. The splitting pattern (as double doublet) and coupling constant values of this proton confirmed the β orientation of the C-3 oxygenated substituent. The spectrum also displayed two broad singlets at δ 4.68 and 4.56 (1H each) assignable to the vinylic protons at C-29 [17-18]. The ^{1}H NMR spectrum displayed a characteristic multiplet of one proton intensity at δ 2.20 which could be ascribed to the proton at C-19. Multiplets of one proton intensity at δ 1.60 and 1.55 could be assigned to the protons at C-2 position. Six singlets at δ 0.96, 0.75, 0.82, 1.02, 0.94 and 0.78 (3H each) were attributable to the methyl group protons at C-4 (H_3-23, H_3-24), C-10 (H_3-25), C-8 (H_3-26), C-14 (H_3-27), C-17 (H_3-28) and C-20, respectively. In addition multiplets observed at δ 1.65, 0.90 and 1.92 was assigned to protons at 13, 18 and 21 positions, respectively [17]. The downfield methyl group resonance at δ 1.67 could be ascribed to the vinylic methyl at C-20 (H_3-30). On this basis, it could be inferred that compound (**3**) was lupeol. This was further confirmed by comparing its ^{1}H NMR spectral data with the published values [15-18] as well as by co-TLC with an authentic lupeol provided in the Phytochemistry laboratory of University of Dhaka.

The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound (**4**) clearly revealed a triterpene type carbon skeleton of the lupane series with two exomethylene proton resonances at δ 4.67 (1H, s, H_a-29) and 4.55 (1H, s, H_b-29), which together with an allylic methyl group of three proton intensity at δ 1.67 (s, H_3-30) confirmed an isoprenyl functionality. It displayed five



1. R = O **2.** R = -OH



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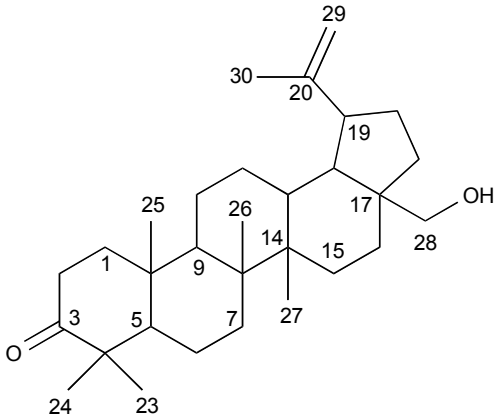

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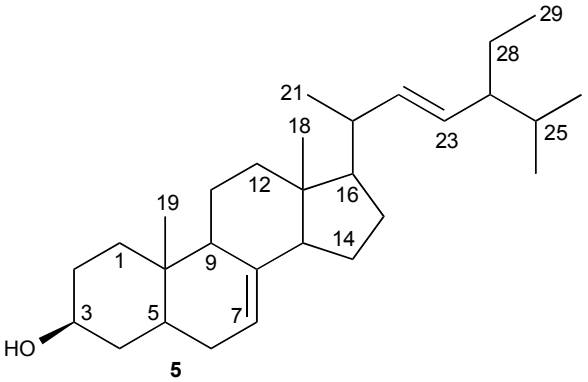

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tertiary methyl singlets at δ 0.75, 0.82, 0.93, 0.96, and 1.02 which were assigned to the methyl groups at C-24, C-25, C-23, C-27 and C-26, respectively. The ^{1}H NMR resonances at δ 3.58 and 3.63 demonstrated the presence of a hydroxymethyl (-CH_2OH) group at C-17. Signals of multiplets at δ 2.60 and 3.10 are attributable to protons at C-2 and C-18 position. The absence of any oxymethine proton resonance around δ 3.20 suggested that the C-3 in compound (**4**) was ketonic rather than alcoholic. Comparing the ^{1}H spectral data obtained for compound (**4**) and reported ^{1}H spectral data of betulin [19-22] compound (**4**) was identified as 3-oxo-betulin or betulone. This is the first report of occurrence of 3-oxo-betulin (**4**) from *M. nagassarium*.

The ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound (**5**) revealed 6 methyl resonances including two tertiary methyl groups as singlet around δ 0.55 and 0.80 for Me-18 and Me-19, respectively. The signals of three secondary methyl groups were observed as doublets at δ 1.05, 0.80 and 0.85 which were assigned to Me-21, Me-26 and Me-27, respectively. A signal for primary Me-29 was evident as a triplet (J = 6.5 Hz). Two downfield resonances at δ 5.15 and 5.05 were assigned to the trans olefinic protons at H-22 and H-23, respectively. The remaining olefinic proton appeared at δ 5.16 (br s), which could be assigned to C-7. The characteristic oxymethine proton at C-3 in a steroidal nucleus as multiplet was observed at δ 3.60. Three multiplets of two proton intensity each at δ 1.40, 1.25 and 1.20 could be ascribed to H-2, H-4 and H-16 protons and a multiplet of two proton intensity at δ 1.55 could be assigned to H-24 and H-25 [23].

The identity of compound (**5**) as spinasterol was confirmed by comparison of these data with published values [23-24] as well as by co-TLC with an authentic spinasterol provided by Phytochemistry laboratory of University of Dhaka. This is the first report of isolation of spinasterol (**5**) from this plant.

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