Triterpenoids from the Stem Bark of *Avicennia officinalis*

Md. Enamul Haque, Hussain Uddin Shekhar, Akim Uddin Mohamad, Hafizur Rahman, AKM Mydul Islam and M. Sabir Hossain

Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka 1000, Bangladesh

ABSTRACT: The triterpinoids, betulinic acid, lupeol and betulinaldehyde, were isolated from the ethyl acetate extract of the stem bark of *Avicennia officinalis* (Avicenniaceae) by a combination of column and preparative thin-layer chromatography over silica gel. The structures of these compounds were determined by spectroscopic analysis (UV, IR, ^{1}H NMR, ^{13}CNMR and EIMS). This is the first report of a systematic phytochemical investigation and the presence of these triterpoids from this plant.

Key words: Triterpenoid, Avicenniaceae, Betulinic acid, Lupeol and Betulinaldehyde

INTRODUCTION

Avicennia officinalis is a medium-sized tree growing in brackish water. The 15 species in the single genus of Avicenniaceae family are found on tropical coasts as constituents of mangrove vegetation.[1] Previous Phytochemical investigations on the different species of *Avicennia* resulted in the isolation of essential oil and sugars like arabinose, glucose and ribose. Among other compounds alkaloids, flavonoids, steroids, terpenoids and iridoids are most considerable components.[2] In Bangladesh, *Avicennia officinalis* is widely distributed in Sundarban and locally it is known as Baen. This plant is used for thrush in children. The heartwood is rubbed against a course stone. The tree oils of this plant exhibited cytotoxic activity.[2] The earlier studies on this plants resulted in the isolation of C iridoid glucoside, 7-O-*trans* cinnamoyl-4-epilogenin, geniposidic acid, 2-cinnamoyl-mussaenoside.[2] So far no detail phytochemical and biological studies have been carried out on this plant. Since this plant has good medicinal properties, the present work has been undertaken to isolate, purify and identify secondary metabolites. In this paper the isolation and structural elucidation of the betulinic acid **(1)**, lupeol **(2)** betulinaldehyde **(3)** by using spectroscopic techniques like UV, IR, ^{1}H NMR, ^{13}CNMR and EIMS are being reported.

Correspondence to: Md. Enamul Haque
Phone: 9661920-59 Ext. 7949 (Off)
9661256 (Res.), 01716-034465 (Mobile)

MATERIALS AND METHODS

General. Melting points were determined on a kolfer hot-stage apparatus and are uncorrected. UV spectrum was taken in MeOH solution using a Perkin-Elmer lambda 9UV/Vis./NIR Spectrometer. IR spectra were recorded on $CHCl_3$ solutions on either a Perkin-Elmer 580 or Philips 9800 FTIR Spectrometer. ^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker WP 200 SY and AM 200 SY instruments (^{1}H, 200. 132 MHz; ^{13}C, 50.32 MHz) using TMS as internal standard and $CDCl_3$ as solvent. Electron impact mass spectra (EIMS) were recorded using a VG updated MS 12 Spectrometer and optical rotations were measured on an optical activity AA-

100 Polarimeter in $CHCl_3$ solutions at 20^0C. Petroleum ether Specifically refers to the bp $40\text{-}60^0$ fractions.

Plant materials. The stem bark of *Avicennia officinalis* Gaertn was collected from Khulna district of Bangladesh. A voucher specimen has been deposited at the Herbarium of the University of Glasgow, Glasgow, U.K.

Extraction and isolation. The Sun-dried stem bark powder (500 g) of *A. officinalis* was extracted in a Soxhlet apparatus for three days with EtOAc. This extract was concentrated *in vacuo* and subjected to flash column chromatography over silica gel (Merck Kieselgel GF_{254}). Elution of the column first with petroleum ether, increasing amounts of EtOAc in petroleum ether and finally with methanol yielded a number of fractions. The proportion of solvent systems used to obtain fraction 5, 7 and 15 were petroleum ether-EtoAc (95 : 5), (92 : 8) and (77 : 23) respectively. Fraction 5 gave betulinic acid (**1**, 20 mg) and fraction 7 gave lupeol (**2**,10 mg) upon multiple pTLC using petroleum ether-EtOAc (95 : 5) and (90 : 10) respectively. pTLC of fraction 15 using petroleum ether-EtOAc (80 : 20) afforded betulinaldehyde (**3**,15 mg).

Betulinic acid (**1**) ν_{max}: 3060, 1630, 880 cm^{-1}. EIMS *m/z* (rel. Int.): 456 [M^+] (5), 441[M^+ - CH_3] (10), 438 [M^+ - H_2O] (20), 426 [M^+ - (15-15)] (10), 415 [M^+ - C_3H_5] (25), 208 (10), 206 (8), 163 (80), 135 (63), 107 (60), 105 (40), 79 (53), 41 (100). The ^{1}H NMR [δ_H: 0.65, 0.75, 0.90, 0.96, 0.98 and 1.65], vinyl methyl [δ_H: 1.67 (br d, *J*=0.5 Hz)], a secondary carbinol [δ_H: 3.16 (dd, *J*=9.5, 6.0 Hz)] and [δ_H: 2.95 (ddd, *J*=9.5, 6.0 Hz, 0.5 Hz)], an exomethylene group [δ_H: 4.55 (1H, d, *J*=0.4 Hz)] and [δ_H: 4.65 (1H, d, *J*=0.4 Hz)]. ^{13}C NMR: 39.0 (C-1); 27.6 (C-2); 78.2 (C-3); 39.0 (C-4); 55.5 (C-5); 18.4 (C-6); 34.5 (C-7); 40.8 (C-8); 50.7 (C-9); 37.3 (C-10); 21.0 (C-11); 25.6 (C-12); 38.2 (C-13); 42.5 (C-14); 30.4 (C-15); 32.6 (C-16); 56.3 (C-17); 47.1 (C-18); 49.4 (C-19); 150.0 (C-20); 29.9 (C-21); 37.3 (C-22); 27.9 (C-23); 15.4 (C-24); 16.2 (C-25); 16.3 (C-26); 14.6 (C-27); 180.6 (C-28); 108.8 (C-29); 19.6 (C-30).

Lupeol (**2**), white crystals (MeOH), mp 210-212^0; $[\alpha]_D$ + 30.4^0 (C, 0.58 in $CHCl_3$); IR ν_{max}: 3610, 3070, 3015, 1640, 1520, 1380, 1217, 1020, 887 cm^{-1}; EIMS *m/z* (rel. int.): 426 [M^+] (2), 411 [M^+ - CH_3] (3), 408 [M^+ -H_2O] (3), 218 (5), 207 (6), 189 (58), 163 (80), 135 (57), 107 (68), 105 (55), 79 (54), 41 (100); ^{1}H NMR: δ_H: 0.75, 0.78, 0.81, 0.92, 0.94, 1.02 (Me-28, Me-23, Me-24, Me-25, Me-26, Me-27), 1.67 (3H, br d, *J*=0.5 Hz, Me-30), 3.18 (1H, dd, *J*=9.6, 6.2 Hz, *H*α-3), 4.56 (1H, d, *J*=0.4 Hz, *Ha*-29), 4.67 (1H, dq, *J*=0.4, 0.5 Hz, *Hb*-29); ^{13}C NMR: δ_C: 38.0 (C-1), 27.4 (C-2), 79.0 (C-3), 38.7 (C-4), 55.3 (C-5), 55.3 (C-5), 18.3 (C-5), 18.3 (C-6), 34.2 (C-7), 40.1 (C-8), 50.4 (C-9), 37.7 (C-10), 20.9 (C-11), 25.1 (C-12), 38.0 (C-13), 42.8 (C-14), 27.4 (C-15), 35.6 (C-16), 42.8 (C-17), 48.2 (C-17), 48.2 (C-18), 48.0 (C-19), 150.9 (C-20), 28.5 (C-21), 40.0 (C-22), 28.1 (C-23), 15.4 (C-24), 16.1 (C-25), 15.9 (C-26), 14.6 (C-27), 18.0 (C-28), 109.5 (C-29), 19.4 (C-30).

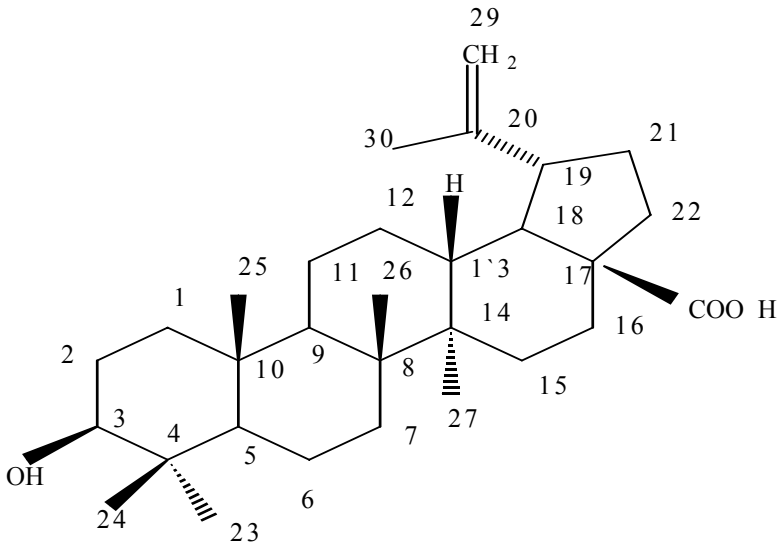
Betulinaldehyde (**3**), white crystals (MeOH), mp $188\text{-}190^0$ ν_{max}: 3300, 2890, 1700, 1640, 885 cm^{-1}, $C_{30}H_{48}O_2$ *m/z* 440 [M^+] (10), 425 [M^+ -15] (20), 422 [M^+ -18] (55); 411 [M^+-CHO] (15), 407 [M^+ -18-15] (20), 309 (10), 302 (15), 220 (15), 163 (80), 135 (63), 107 (60), 105 (40), 79 (53), 41 (100). ^{1}H NMR: δ_H: 0.70, 0.80, 0.85, 0.90, 1.20 and 1.60, [δ_H: 1.67 (br d, *J*=0.5 Hz)], [δ_H: 3.17 (dd, *J*=9.5, 6.1 Hz)] and [δ_H: 2.95 (ddd, *J*=9.5, 6.0 Hz, 0.5 Hz)], an exomethylene group [δ_H: 4.55 (1H,d, *J*=0.4 Hz)] and [4.65 (1H, d, *J*=0.4 Hz)]. ^{13}C NMR δc: 39.1 (C-1); 27.6 (C-2); 79.0 (C-3); 39.1 (C-4); 55.4 (C-5); 18.3 (C-6); 34.4 (C-7); 40.7 (C-8); 50.6 (C-9); 37.7 (C-10); 20.9 (C-11); 25.5 (C-12); 38.1 (C-13); 42.4 (C-14); 30.5 (C-15); 32.5 (C-16); 56.2 (C-17), 47.0 (C-18); 49.3 (C-19); 150.0 (C-20); 29.8 (C-21); 37.2 (C-22); 27.9; (C-23), 15.4 (C-24); 16.2 (C-25); 16.3 (C-26); 14.6 (C-27); 180.0 (C-28); 108.8 (C-29); 19.6 (C-30).

RESULTS AND DISCUSSION

The ethyl acetate extract of the stem bark of *A. officinalis* afforded three triterpenoids **(1-3).** The isolated compounds were identified by spectroscopic analysis as well as by comparision of their spectral data with previously reported values.

Betulinic acid (**1**) was isolated as white crystal (MeOH). IR spectrum exhibited hydroxyl [νmax: 3610, 1020 cm^{-1}] and exomethylene [νmax: 3060, 1630, 880]. It mass spectrum displayed an [M^+] peak at *m/z* 456 corresponding to $C_{30}H_{48}O_3$, together with fragments at *m/z* 441 [M^+-15] and 438 [M^+-18] and a base peak at *m/z* 43 [$C_3H_7^+$].

The ^{1}H NMR spectrum of (**1**) revealed signals for five tertiary methyl. [δ_H: 0.65, 0.75, 0.90, 0.96, 0.98], a vinyl methyl [δ_H: 1.97 (br d, *J*=0.5 Hz)], a secondary carbinol [δ_H: 3.16 (dd, *J*=9.5 and 6.0 Hz)] and [δ_H: 2.95 (ddd, *J*=9.0,6.0 and 0.5 Hz)] an exomethylene group [δ_H: 4.55 (1H, d, *J*=0.4 Hz)] and [δ_H: 4.65 (1H, d, *J*=0.4 Hz). These data indicated a pentacyclic triterpinoid of betulinic acid and comparation with published data[3] confirmed the indentify of (**1**) as betulinic acid.



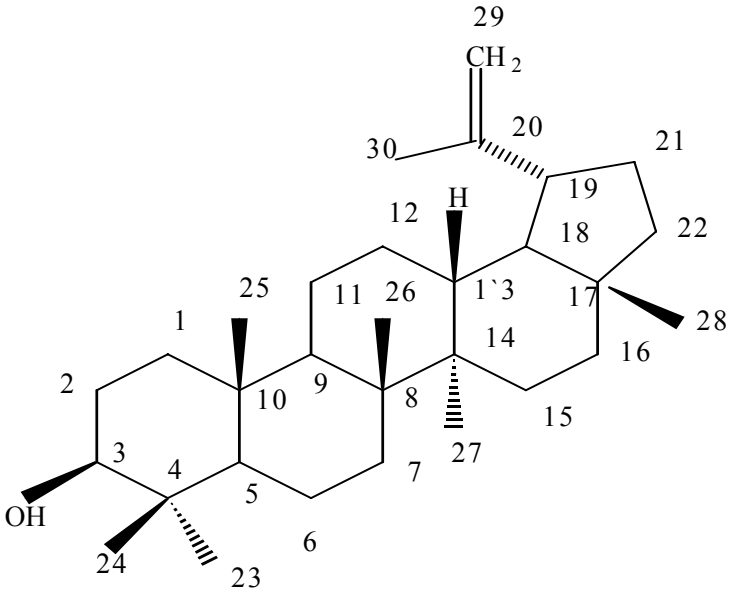
Betulinic acid as AO-2

The ^{13}C NMR spectrum of (**3**) showed six methyl group [δ_C: 27.9 (C-23), 15.4 (C-24), 16.2 (C-25), 16.3 (C-26), 14.6 (C-27), 19.6 (C-30)] and exomethylene group [δ_C: 150.0 (C-30), 108.8 (C-29)] and a secondary hydroxyl bearing carbon [δ_C: 79.0 (C-3) and an carboxyl group at δ_C: 180.6 (C-28) in addition to ten methylene, five methine and five quaternary carbons. These data were identical to those reported betulinic acid.[3]

Lupeol (**2**) was isolated as white crystals from methanol and gave mp 210-212^o [α] $_D$ + 30.4 ^{o} (C, 0.58 in $CHCl_3$). Its IR spectrum exhibited hydroxyl [ν_{max}: 3610, 1020 cm^{-1}] and exomethylene [ν_{max}: 3070, 1640, 887 cm^{-1}] absorption. The mas spectrum displayed a molecular ion [M^+] peak at *m/z* 426 corresponding to $C_{30}H_{50}O$ together with fragments at *m/z* 411 [M^+–15] and 408 [M^+–18] which were due to the loss of methyl group and a molecule of water from the molecular ion peak. The mass spectrum also showed a base peak at *m/z* 41 [$C_3H_5^+$] arising from the loss of the side chain of lupeol. The ^{1}H NMR spectrum exhibited six tertiary methyl singlets at [δ_H: 0.75, 0.77, 0.80, 0.92, 0.94 and 1.02], a methine group at [δ_H: 1.66 (br d, *J*=0.5 (Hz)], a secondary carbinol group at [δ_H: 3.20 (dd, *J*=9.6 and 6.2 Hz)] and an exomethylene group at [δ_H: 4.58 (1H, d, *J*=0.4 Hz) and [δ_H: 4.65 (1H, dq, *J*=0.4 and 0.5 Hz)] typical of pentacyclic triterpenoid [4,5] of the lupeol (**1**).

The structural assignment of (**2**) was further substantiated by its ^{13}C NMR spectrum which showed seven methyl groups at [δc: 28.0 (C-23), 19.3 (C-30), 18.0 (C-28), 16.1 (C-25), 15.9 (C-26), 15.4

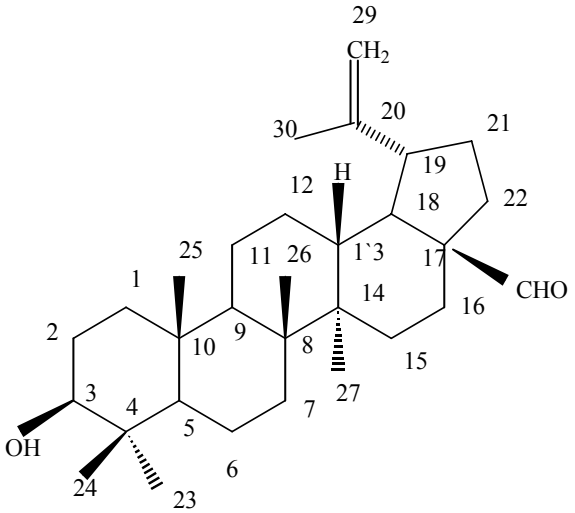
(C-24), 14.5 (C-27)], an exomethylene group at [δc: 150.8 (C-20), 109.3 (C-29)] and a secondary hydroxyl bearing carbon at [δc: 78.9 (C-3)], in addition to ten methylene, five methine and five quaternary carbons. The shielding of C-23 methyl of (**2**) could be due to the influence of the adjacent C-3 hydroxyl group.[3,6] These data were in close agreement with those reported for lupeol (**2**)[3,6] and further confirmed the indentity of (**2**) as lupeol.



Lupeol as AO-1

Betulinaldehyde (**3**) was isolated as crystals (MeOH), mp188-190º. It IR spectrum displayed absorption at ν_{max}: 3300, 2890, 1700, 1640, 885 cm^{-1}. It's exhibited a [M^+] peak at *m/z* 440 corresponding to $C_{30}H_{48}O_2$, together with fragments at *m/z* 425. [M^+-15] and 410 [M^+ -18] and a base peak at *m/z* 41 [$C_3H^+_5$] corresponding to a lupeol type triterpinoid.

The ^{1}H NMR spectrum of (**3**) revealed signals for five tertiary methyl. [δ_H: 0.70, 0.80, 0.85, 0.90, 1.20 and 1.60] a vinyl methyl [δ_H: 1.67 (br d, J=0.5 Hz)] a secondary carbinol [δ_H: 3.17 (dd, J=9.5 and 6.1 Hz)] and [δ_H: 2.95 (ddd, J=9.5,6.0 and 0.5 Hz)] an exomethylene group [δ_H: 4.55 (1H, d, J=0.4 Hz)] and [δ_H: 4.65 (1H, d, J=0.4 Hz)]. These data indicated a pentacyclic triterpinoid of lupeol type with an aldehyde group and comparation with published data[6] confirmed the identity of (**3**) as betulinaldehyde.



Betulinaldehyde as AO-3

The ^{13}C NMR spectrum of (**3**) showed six methyl groups [δc: 27.9 (C-23), 15.4 (C-24), 16.2 (C-25), 16.3 (C-26), 14.6 (C-27), 19.6 (C-30)] and an

exomethylene group [δ_C: 150.0 (C-20), 108.8 (C-29) and a secondary hydroxyl bearing carbon [δ_C: 79.0 (C-3), and an aldehyde group at [δ_C: 180.0 (C-28)], in addition to ten methylene, five methine and five quaternary carbons. These data were identical to those of betulinaldehyde.[6] This is the first report of the isolation of these triterpinoids from *Avicennia Officinalis*. Further analysis may result in the isolation of more biologically active compounds.

ACKNOWLEDGEMENT

One of us thanks the Association of Commonwealth Universities for a research Fellowship to MEH and Professor G. Webbs, Department of Chemistry, University of Glasgow, Scotland, U.K. for providing NMR facilities.

REFERENCES

1. Chopra, R.N., Nayar, S.L. and Chopra, I.C. 1956. *Antidesma (Euphorbiaceae). Glossary of Indian Medicinal Plants.* p. 21, CSIR, New Delhi.
2. Ghani, A. 1998. *Medicinal Plants of Bangladesh: Chemical constituents and uses*, 1st edn, Asiatic Society of Bangladesh, p. 212-216.
3. Sholichin, M., Yamasaki, K., Kasal, R. and Tanaka, O. 1980. Carbon 13- nuclear magnetic resonance of lupane-type triterpene, lupeol, betulin and betulinic acid. *Chem. Pharam. Bull.* **28**, 1006-1008.
4. Gunasekera, S.P., Cordell, G.A. and Farnsworth, N.R. 1982. Constituents of *Pithecellobium multiflorum*. *J. Nat Prod.* **45**, 651.
5. Garcia, B., Alberto Macro, J. Seoane, E. and Tortajada, A. 1981. Triterpinoids, waxes and tricin in Phoenix canariensis. *J. Nat. Prod*. **44**, 111-113.
6. Wenkert, E, Baddeley, G.V., Burfitt, I.R. and Moreno, L.N. 1978. Carbon-13 nuclear magnetic resonance spectroscopy of naturally-occurring substances, LVII. Triterppenes related to lupane and hopene. *Org. Mag. Res.* **11**, 337-343.