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Secondary Metabolites from *Melocanna baccifera* (Roxb.)

M.R. KUDDUS[1], F. RUMI[1], M.A. KAISAR[1], M.S. RAHMAN[1], C.M. HASAN[1], M.A. HASSAN[2] and M.A. RASHID[1,3*]

[1]Phytochemical Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh
[2]Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh
[3]Centre for Biomedical Research, University of Dhaka, Dhaka-1000, Bangladesh

*Corresponding author: Fax: +880 2 8612069; Tel: +880 2 8612069, 9661900-73, Extn: 4363, 4364, 8137; E-mail: rashidma@univdhaka.edu

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A total of 6 compounds were isolated from the carbon tetrachloride soluble fraction of a methanol extract of the fruits of *Melocanna baccifera*. The structures of the isolated compounds were elucidated as isochavicinic acid (**1**), olean-12-en-28-carboxy-3β-acetate (**2**), 3-oxo-olean-12-en-28-al (**3**), eugenol (**4**), β-sitosterol glucoside (**5a**) and stigmasterol glucoside (**5b**) by high field NMR (^{1}H NMR and ^{13}C NMR, DEPT, ^{1}H-^{1}H COSY, HSQC and HMBC) analyses as well as by comparison with structurally related compounds.

Key Words: *Melocanna baccifera*, Gramineae, Eugenol, Isochavicinic acid, β-Sitosterol glucoside, Stigmasterol glucoside, Olean-12-en-28-carboxy-3β-acetate, 3-Oxo-olean-12-en-28-al.

INTRODUCTION

Melocanna baccifera (Roxb.) (Bengali name-muli bash, Family- Gramineae) is a thin-walled, small diameter and non-clump forming bamboo. It naturally grows all over Bangladesh[1]. This plant is known to flower gregariously at intervals of 40-44 years[2]. The seeds of this plant are reported to increase the fertility in rats or enhance human libido[3].

Bamboo leaves are reported to have flavone, phenolic acid, anthracene, quinone, lactone, amylose, amino acid and trace elements which possess antioxidant, antiaging, antifatigue and anticancer activities. They are also responsible for preventing cardio-cerebro-vascular diseases, protecting the liver, widening capillary vessels, smoothing micro-circulation, vitalizing the brain, improving memory and sleep and the texture of skin[4].

We, herein, report the first time isolation of isochavicinic acid (**1**), olean-12-en-28-carboxy-3β-acetate (3β-acetoxy oleanolic acid) (**2**), 3-oxo-olean-12-en-28-al (oleanonic aldehyde) (**3**), eugenol (**4**), β-sitosterol glucoside (**5a**) and stigmasterol glucoside (**5b**) from the methanolic extract of fruits of *M. baccifera*.

EXPERIMENTAL

NMR spectra (both 1D and 2D) were obtained on a Bruker Avance (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer, using the residual solvent peaks as internal standard. HMBC spectra were optimized for a long range $J_{H\text{-}C}$ of 7 Hz (d_6 = 0.07 s). Column chromatography (CC) was conducted on Si gel (Merck, mesh 70-230). TLC and PTLC were carried out using Merck Si gel 60 PF_{254} on glass plates at a thickness of 0.5 mm and spots were visualized under UV light (254 and 366 nm) and spraying with 1 % vanillin-H_2SO_4 followed by heating at 110 ºC for 5-10 min.

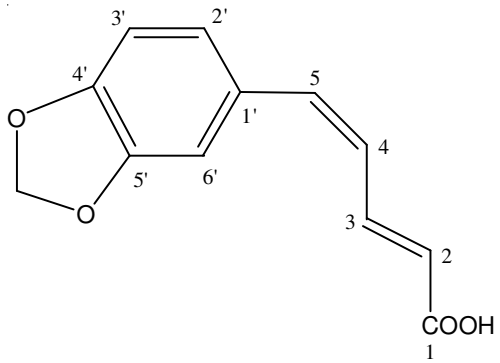
The fruits of *M. baccifera* were collected from Dhaka in June 2008. They were cut into small pieces and sun dried for 7 days followed by oven drying for 24 h at 40 ºC to facilitate proper grinding.

Extraction and isolation: The powdered material (533 g) was soaked in 1.5 L of methanol 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 methanol extract was fractionated by the modified Kupchan partitioning protocol[5] which afforded *n*-hexane (650 mg), carbon tetrachloride (750 mg), dichloromethane (350 mg) and aqueous (3.05 g) soluble materials.

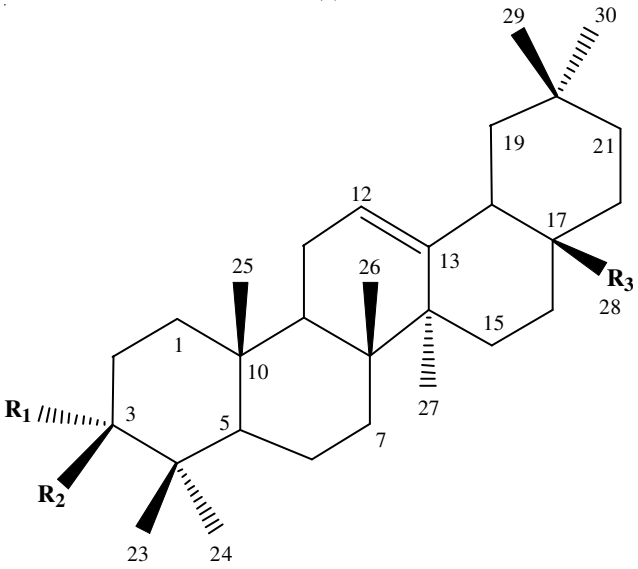
An aliquot of the carbon tetrachloride soluble partitionate (650 mg) was fractionated by column chromatography over silica gel (Kieselgel 60, mesh 70-230) using pet-ether and ethyl acetate mixture in order of increasing polarities. A total of 141 fractions were collected, each 20 mL. Preparative thin layer chromatography (PTLC) of column fractions eluted with 40 % ethyl acetate in pet-ether over silica gel using toluene-ethyl acetate (85:15) afforded compound **1** (15 mg). Again,

PTLC of column fractions eluted with 10 % ethyl acetate in pet-ether over silica gel using 10 % ethyl acetate in toluene as the developing solvent gave compound **2** (10 mg). On the other hand, column fraction 36-38, upon preparative TLC over silica gel using toluene-ethyl acetate (90:10) yielded compound **3** (8.5 mg).

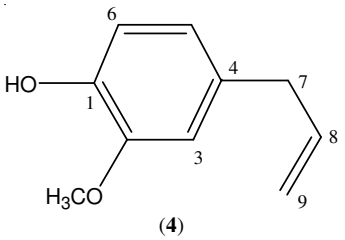
Evaporation of solvents from column fraction 22 obtained with 12 % ethyl acetate in pet-ether gave compound **4** (3.5 mg) whereas a mixture of two compounds (**5a, 5b**) were obtained from the column fraction 138 eluted with 30 % methanol in ethyl acetate.



(**1**)



Compound	R_1	R_2	R_3
2	H	CH_3-COO-	-COOH
3	O	O	-CHO



(**4**)

Isochavicinic acid (1): (15.0 mg, 0.3 % yield); yellow crystalline mass; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 (1H, ddd, *J* = 14.5, 8.4, 1.6 Hz, H-3), 6.97 (1H, d, *J* = 1.6 Hz, H-6'), 6.88 (1H, dd, *J* = 8.0, 1.6 Hz, H-2'), 6.77 (1H, d, *J* = 8.0 Hz, H-3'), 6.74 (1H, br. s, H-5), 6.73 (1H, br.s, H-4), 6.42 (1H, d, *J* = 14.5 Hz, H-2), 5.94 (2H, s, -OCH_2O–). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.5 (C-1), 120.1 (C-2), 142.5 (C-3), 125.4 (C-4), 138.2 (C-5), 131.1 (C-1'), 122.5 (C-2'), 108.5 (C-3'), 148.2 (C-4'), 148.1 (C-5'), 105.7 (C-6'), 101.3 (-OCH_2O-).

Olean-12-en-28-carboxy-3β-acetate (2): 10.0 mg, 0.2 % yield; amorphous white powder; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.23 (1H, br. s, H-12), 4.49 (1H, dd, *J* = 8.8, 4.2 Hz, H-3α), 2.03 (3H, s, -OAc), 1.07 (3H, s, H_3-27), 0.95 (6H, s, H_3-25, H_3-26), 0.86 (6H, s, H_3-23, H_3-24), 0.84 (6H, s, H_3-29, H_3-30); ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.3 (C-1), 28.0 (C-2), 81.0 (C-3), 37.7 (C-4), 55.4 (C-5), 18.2 (C-6), 30.6 (C-7), 39.6 (C-8), 47.5 (C-9), 37.0 (C-10), 23.3 (C-11), 125.8 (C-12), 138.0 (C-13), 42.0 (C-14), 24.1 (C-15), 23.3 (C-16), 48.0 (C-17), 52.6 (C-18), 38.3 (C-19), 37.7 (C-20), 32.9 (C-21), 36.8 (C-22), 28.1 (C-23), 16.7 (C-24), 15.6 (C-25), 17.0 (C-26), 23.6 (C-27), 182.7 (C-28), 17.1 (C-29), 21.2 (C-30), 171.0 ($CH_3\underline{C}OO$-), 21.3 ($\underline{C}H_3COO$-).

3-Oxo-olean-12-en-28-al (3): 8.5 mg, 0.17 % yield; amorphous white powder; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.39 (1H, s, H-28), 5.36 (1H, t, *J* = 4.0 Hz, H-12), 1.14 (3H, s, H_3-27), 1.08 (3H, s, H_3-23), 1.03 (6H, s, H_3-24, H_3-25), 0.91 (6H, s, H_3-26, H_3-30), 0.79 (3H, s, H_3-29); ^{13}C NMR (100 MHz, $CDCl_3$): δ 45.6 (C-1), 33.2 (C-2), 207.3 (C-3), 47.5 (C-4), 55.4 (C-5), 19.6 (C-6), 32.3 (C-7), 39.6 (C-8), 46.9 (C-9), 36.8 (C-10), 22.1 (C-11), 123.0 (C-12), 143.1 (C-13), 42.0 (C-14), 26.7 (C-15), 23.6 (C-16), 49.2 (C-17), 55. 4 (C-18), 39.2 (C-19), 38.2 (C-20), 27.7 (C-21), 34.2 (C-22), 25.5 (C-23), 23.4 (C-24), 21.5 (C-25), 17.1 (C-26), 15.1 (C-27), 201.2 (C-28), 33.1 (C-29), 26.1 (C-30).

Eugenol (4): 3.5 mg, 0.07 % yield; amorphous white powder; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.83 (1H, d, *J* = 8.4 Hz, H-6), 6.68 (1H, d, *J* = 2.0 Hz, H-3), 6.67 (1H, dd, *J* = 8.4, 2.0 Hz, H-5), 5.99 (1H, m, H-8), 5.45 (1H, s, OH-1), 5.04 (1H, br. s, Ha-9), 5.03 (1H, br. s, Hb-9), 3.87 (3H, s, OMe-2), 3.30 (2H, d, *J* = 6.8 Hz, H_2-7).

β-Sitosterol glucoside (5a) + stigmasterol glucoside (5b): 12.5 mg, 0.25 % yield; white powder; ^{1}H NMR (400 MHz, CD_3OD + few drops $CDCl_3$) was identical to published values[6,7].

RESULTS AND DISCUSSION

A total of 6 compounds were isolated from a carbon tetrachloride soluble fraction of the methanol extract of fruits of *M. baccifera* by repeated chromatographic separation and purification over silica gel. The structures of the isolated compounds were solved by high field NMR (^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H-^{1}H COSY, HSQC and HMBC) analyses as well as by comparison with structurally related compounds.

The ^{13}C NMR spectrum of compound **1** displayed 12 carbon resonances, while the HSQC and DEPT experiments indicated that 8 out of the 12 carbons were attached to protons. The DEPT 135 spectral data allowed to define these signals into 1 methylene (δ 101.3), 7 methines (δ 142.5, 138.2, 125.4, 122.5, 120.1, 108.5, 105.7) and 3 quaternary olefinic carbons (δ 148.2, 148.1, 131.1) and a carboxylic acid (δ 165.5) group. The ^{1}H NMR spectral data of compound **1** indicated the presence of a 1,3,4-trisubstituted aromatic ring with resonances at δ 6.77 (1H, d, *J* = 8.0 Hz), 6.88 (1H, dd, *J* = 8.0, 1.6 Hz) and 6.97

(1H, d, J = 1.6 Hz) and a singlet of two proton intensity at δ 5.94 typical for a methylenedioxy group. In addition, the ^{1}H NMR spectrum demonstrated four olefinic proton signals at δ 6.42 (1H, d, J = 14.5 Hz), 6.73 (1H, br. s), 6.74 (1H, br. s) and 7.38 (1H, ddd, J = 14.5, 8.4, 1.6 Hz). The large coupling (J = 14.5 Hz) between the proton resonating at δ 6.42 and 7.38 suggested the *trans* coupling, while the downfield shift of the latter proton allowed to place it at beta (β) position to a carboxylic acid group. In the ^{1}H-^{1}H COSY spectrum, the proton at δ 7.38 exhibited correlations with the protons resonating at δ 6.42 and 6.73, which established them to be a part of the same spin system. However, no coupling was observed between the protons at δ 6.73 and 6.74. This suggested a *cis* relationship between these two protons, as cis coupled protons are known to exhibit coupling constant in the range of 0-12 Hz. The assignment of the ^{1}H and ^{13}C resonances in **1** was achieved by careful analyses of the ^{1}H-^{13}C correlated HSQC and HMBC data (Fig. 1). The linkage of the side chain with the aromatic ring was established by HMBC correlations from δ 6.88 (H-2') and 6.97 (H-6') to δ_C 138.2 (C-5), 6.74 (H-5) to δ_C 105.7 (C-6') and 6.73 (H-4) to δ_C 131.1 (C-1'). On this basis, compound **1** was characterized as isochavicinic acid (**1**). Although, isochavicinic acid has previously been reported from *Piper nigrum* (Family-Piperaceae)[8], this is the first report of its isolation from a *Melocanna* species.

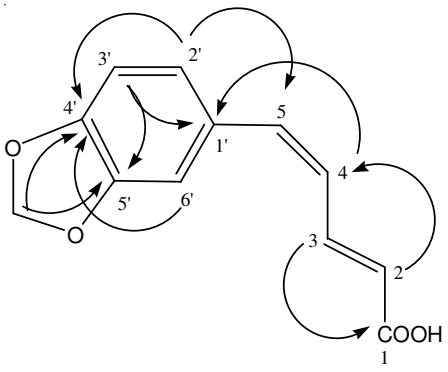


Fig. 1. Key HMBC correlations observed in **1**

The ^{13}C NMR spectrum of compound **2** displayed 32 carbon resonances while the DEPT experiments indicated that 23 out of the 32 carbon atoms in **2** had attached protons. Thus, it exhibited signals for 8 methyls (δ_C 28.1, 23.6, 21.2, 21.3, 17.1, 17.0, 16.7, 15.6), 10 methylenes (δ_C 38.3, 38.3, 36.8, 32.9, 30.6, 28.0, 24.1, 23.3, 23.3, 18.2), 5 methines (δ_C 125.8, 81.0, 55.4, 52.6, 47.5) and 9 quaternary carbons (δ_C 182.7, 171.0, 138.0, 48.0, 42.0, 39.6, 37.7, 37.7, 37.0). The ^{1}H NMR spectrum of compound **2** displayed a one proton broad singlet at δ 5.23 for an olefinic proton at C-12. The spectrum also displayed a double doublet (J = 8.8 and 4.2 Hz) of one proton intensity centered at δ 4.49 indicative of H-3 proton in a triterpene nucleus. The downfield shift of H-3 suggested that it was esterified. A singlet integrating for three protons at δ 2.03 revealed the presence of an acetyl group at C-3. The ^{1}H NMR spectral data of compound **2** demonstrated seven three proton singlets at δ 1.07, 0.95, 0.95, 0.86, 0.86, 0.84, 0.84 for methyl groups. On this basis, compound **2** was characterized as olean-12-en-28-carboxy-3β-acetate; a triterpene known to occur in *Serissa serissoides*[9], *Lanata camara*[10] and *Spathodea campanulata*[11], the identity of which was further confirmed by comparison of its spectral data with previously reported values[9-11]. This is the first report of its occurance from a *Melocanna* species.

The ^{13}C NMR spectrum of compound **3** displayed 30 carbon resonances. The DEPT experiments indicated that 22 out of the 30 carbons in compound **3** were protonated. Thus, it exhibited signals for 7 methyls (δ_C 33.1, 26.1, 25.5, 23.4, 21.5, 17.1, 15.1), 10 methylenes (δ_c 45.6, 39.2, 34.2, 33.2, 32.3, 27.7, 26.7, 23.6, 22.1, 19.6), 5 methines (δ_C 201.2, 123.0, 55.4, 55.4, 46.9) including an aldehyde group (δ_C 201.2) and 8 quaternary carbons (δ_C 207.3, 143.1, 49.2, 47.5, 42.0, 39.6, 38.2, 36.8) including a ketone group (δ_C 207.3). The ^{1}H NMR spectrum of compound **3** displayed the typical olefinic proton signal at δ 5.36 for H-12 in oleanane type triterpenoids[12]. The spectrum also showed a sharp downfield singlet at δ 9.39 for an aldehydic group proton. The ^{1}H NMR spectrum exhibited seven signals for methyl protons at δ 1.14, 1.08, 1.03, 1.03, 0.91, 0.91, 0.79. The absence of oxymethine proton and carbon signal in the ^{1}H and ^{13}C NMR spectra of compound **3** and the presence of a carbonyl group resonance at δ 207.3 suggested that C-3 was a ketonic functionality instead of a carbinol. On this basis, compound **3** was characterized as 3-oxo-olean-12-en-28-al/ oleanonic aldehyde, which was further substantiated by comparison with published values[12]. Although, oleanonic aldehyde has been reported from the galls of *Pistacia terebinthus* (family-Anacardiaceae)[12], this is the first report of its occurance from a *Melocanna* species.

The ^{1}H NMR spectrum of **4** displayed signals at δ 6.67 (1H, dd, J = 8.4, 2.0 Hz), 6.68 (1H, d, J = 2.0 Hz) and 6.83 (1H, d, J = 8.4 Hz) appropriate for a 1,3,4-trisubstituted benzene, in addition to an olefinic proton (δ 5.99 m), a benzylic methylene (δ 3.30, 2H, d, J = 6.8 Hz), an exomethylene (δ 5.03, 5.04, each 1H, br. s), a methoxyl δ 3.87 (3H, s) and a hydroxyl (δ 5.45, 1H, br. s) proton resonances. These spectral features allowed to characterize compound **4** as eugenol, which was further confirmed by comparison of its spectral data with previously reported values[13].

The ^{1}H NMR spectrum of compound **5** revealed it to be a mixture of two closely related compounds. However, careful analysis of the ^{1}H NMR spectrum demonstrated that both the oxymethine proton resonance at 3.52 (m) and the olefinic signal at 5.32 (m) were characteristic for H-3 and H-6 of a steroidal skeleton that integrated for two protons each whereas the remaining olefinic protons at δ 5.00 (1H, dd, J = 5.4, 6.5 Hz) and 5.08 (1H, dd, J = 15.0, 6.5 Hz) could be assigned to H-22 and H-23, respectively. This clearly suggested that the spectrum was acquired from a mixture of two steroidal compounds. Comparison of the spectrum with reported values as well as by co-TLC with authentic samples allowed to characterize compound **5** as a mixture of β-sitosterol glucoside (**5a**)[6] and stigmasterol glucoside (**5b**)[7].

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