Antibacterial and Cytotoxic Limonoids from the Seeds of *Swietenia mahagony*

Md. Abdul Kader[1], Md. Ekramul Haque[1], Proma Khondkar[1], Md. Monirul Islam[2] and M. Mukhlesur Rahman[1,3]

[1]Phytochemistry Research Laboratories, Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh
[2]Department of Pharmacy, Atish Dipankar University of Science and Technology, Dhaka, Bangladesh
[3]Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29–39 Brunswick Square, London WC1N 1AX, UK

ABSTRACT: Two limonoids, swietenine (**1**) and 3-*O*-tigloylswietenolide (**2**), were isolated from the chloroform soluble fraction of an ethanolic extract of *Swietenia mahagony* seed. The structures of these compounds were confirmed by spectroscopic methods, including both 1D and 2D NMR spectroscopy. The chloroform extract and the limonoids (**1** and **2**) exhibited moderate antibacterial activity against a series of Gram positive and Gram negative bacteria. The minimum inhibitory concentrations (MICs) of **1** and **2** were found to be in the range of 32-64 µg/ml against *Bacillus megatorium* and *Escherichia coli*. Compounds **1** and **2** were also assessed for preliminary cytotoxicity against brine shrimp and the LC_{50} values were found to be 14.6 and 12.5 µg/ml, respectively.

Key words: *Swietenia mahagony*; Meliaceae; Limonoids; Swietenine; 3-tigloylswietenolide; Antibacterial activity; Cytotoxicity.

INTRODUCTION

Swietenia mahagoni (L.) Jacq., locally known as the Mahogany**,** belonging to the family Meliaceae, is a medium-sized evergreen to semi-evergreen tree having a height of 30-35 m. The plant is native to USA, Haiti, Antilles, Jamaica and the Bahamas and is cultivated in Bangladesh, India, Sri Lanka, Philippines, Indonesia, China and Malaysia.[1,2] This economically important timber tree is traditionally used for the treatment of a number of diseases including diabetes, malaria, skin diseases, fever, hypertension and tuberculosis.[2] It is also as purgative, astringent, depurative, purgative, and tonic.[2] The oil of the seeds is used in chronic skin diseases, in ulcer, in rheumatism and as antiseptic.[2] The seed extracts of *S. mahagoni* has also been found to inhibit platelet activating factor (PAF)-induced platelet aggregation.[3] The plant extract has also anti-human immunodeficiency virus activities.[4] This plant has been reported to produce a wide range of limonoids,[5-9] terpene[9-11] and polyacetylene.[12] Here, we report the isolation and identification of two limonoids, swietenine (**1**) and 3-*O*-tigloylswietenolide (**2**) from *Swietenia mahagoni* seed as well as their antibacterial activity and cytotoxicity.

Correspondence to: M. Mukhlesur Rahman
Tel: +44-207-7535800/4853. Fax : +44-207-7535964.
E-mail: mukhlesurrah@yahoo.co.uk

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MATERIALS AND METHODS

General experimental procedures. NMR spectra (both 1D and 2D) were obtained on a Bruker Avance (500 MHz for ^1H and 125 MHz for ^{13}C) spectrometer in $CDCl_3$, using the residual solvent

peaks as internal standard. HMBC spectra were optimized for a long range J_{H-C} of 7Hz (d_6=0.07s). 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. Spots on TLC and PTLC plates were visualized under UV light (254 and 366 nm) and spraying with 1% vanillin-H_2SO_4 followed by heating at 110° C for 5-10 min.

Plant material. The matured fruits of *Swietenia mahagony* were collected in December 2006 from the adjoining areas of Rajshahi University Campus, Bangladesh and were identified by Prof. Naderuzzaman, Department of Botany, Rajshahi University, Bangladesh, where a voucher specimen (Voucher number 48) of this collection has been deposited.

Extraction and isolation. The seeds from the matured fruits were separated, washed, chopped, sun dried and ground. The ground seeds (1 kg) were cold extracted with ethanol (3 L) followed by solvent-solvent partitioning with petroleum ether (60-80°C), chloroform and methanol. The chloroform soluble extract (5 gm) was further fractionated by column chromatography over silica gel (Merck) eluting with petroleum ether and ethyl acetate of increasing polarity. Preparative TLC (mobile phase- hexane: EtOAc = 2:1) on CC fractions eluted with 30-60% ethyl acetate in petroleum ether yielded **1** (28 mg; R_f = 0.53 in 25% EtOAc in hexane) and **2** (30 mg; R_f = 0.62 in 25% EtOAc in hexane) as white amorphous powder.

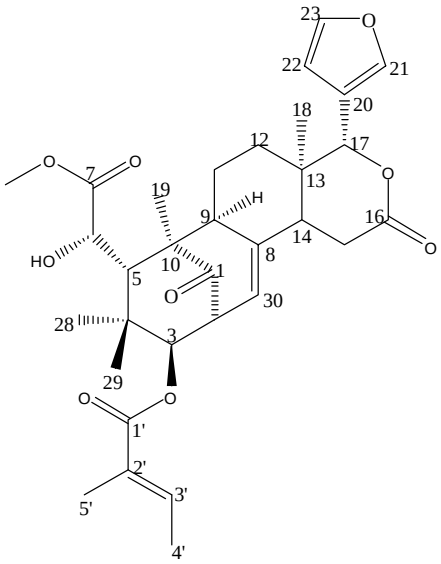
Antibacterial activity. Antibacterial assay was performed by disc diffusion technique.[13,14] The samples solution of the compounds and extracts were prepared by dissolving a definite amount of material in appropriate solvent to give the desired concentration and then applied on sterile disc (6 mm diameter, filter paper) followed by drying off the solvent in an aseptic hood. To compare the activity with standard antibiotics, kanamycin (30 µg/disc) was used. As negative control, a blank disc impregnated with 10µl of solvent followed by drying off, was used.

The minimum inhibitory concentrations (MICs) of the compounds **1** and **2** against *B. megatorium* and *E. coli* were also determined by serial dilution technique.[15]

Cytotoxicity. For cytotoxicity screening, DMSO solutions of the compounds were applied against *Artemia salina* for 24 hrs *in vivo* assay.[16] The eggs of the brine shrimp, *Artemia salina*, were collected from a local aquarium shop and hatched for 48 hr to mature shrimp called nauplii. The test samples were prepared by dissolving them in DMSO to attain concentrations - 5 µg/ml, 10 µg/ml, 20 µg/ml, 40 µg/ml and 80 µg/ml. Then 20 matured shrimps were applied to each of all experimental vials and control vial. The number of the nauplii that died after 24 hr was counted. The findings were presented graphically by plotting log of concentration versus percentage of mortality of nauplii from which LC_{50} was determined by extrapolation. The assay was performed in duplicate and the result was calculated as an average of two determinations.

RESULTS AND DISCUSSION

Column chromatography followed by preparative TLC of the chloroform soluble fraction of an ethanol extract of *S. mahagony* seed yielded two limonoids (**1** and **2**). The molecular formula of **1** was established as $C_{32}H_{40}O_9$ from HREISMS. The ^{1}H NMR spectrum (500 MHz, $CDCl_3$, Table 1) showed resonances for a β-substituted furan ring at δ 7.56 (*s*, H-21), 6.39 (*d, J* =1.5 Hz, H-22) and 7.45 (*d, J* = 1.5 Hz, H-23), an olefinic proton signal at δ 5.34 (*dt, J* =7.5, 1.5 Hz, H-30), oxymethine proton signals at 4.63 (*d, J* =8.5 Hz, H-3), 4.56 (*d, J* =1.5 Hz, H-6) and 5.55 (*s*, H-17) and resonance at 0.89, 0.98, 1.14, 1.47 for four methyl groups. The proton signals at 6.88 (*q, J* =7.0 Hz, H-3'), 1.76 (3H, *d*, *J* =7.0 Hz, H-4') and 1.82 (3H, s, H-5') indicated the presence of a tigloyl group in the molecule. The ^{13}C NMR (125 MHz, $CDCl_3$, Table 1) displayed a total of 32 carbons including four carbonyl resonances at 216.8 (C-1), 176.2 (C-7), 168.7 (C-16) & 167.1 (C-1′) and a methoxyl group at 53.5 (δ_H 3.77). A ^3J correlation by the olefinic proton at 5.34 to the carbonyl at 216.8



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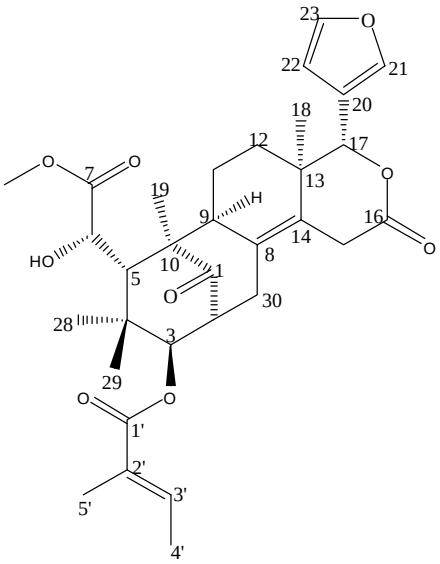

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of 1 and 2 in $CDCl_3$

Position	δ_H		δ_C	
	1	**2**	**1**	**2**
1	-	-	216.8	218.2
2	3.53, *dd, J* =8.5, 1.5 Hz	3.21, *dd, J* =8.5, 1.5 Hz	49.1	48.3
3	4.63, *d, J* =8.5 Hz	4.72, *d, J* =8.5 Hz	78.7	80.8
4	-	-	39.3	39.4
5	3.51, *s*	3.39, *s*	45.6	45.3
6	4.56, *d, J* =1.5 Hz	4.56, *d, J* =1.5 Hz	73.0	73.5
7	-	-	176.2	175.8
8	-	-	138.5	128.7
9	2.31, *m*	2.28, *m*	57.7	53.7
10	-	-	50.6	53.4
11	1.82, *m*; 2.05, *m*	1.76, *m*; 1.89, *m*	21.5	19.1
12	1.47, *m*; 1.76, *m*	1.17, *m*; 1.76, *m*	34.7	30.0
13	-	-	36.9	38.3
14	2.24, *m*	-	45.3	131.8
15	2.89, *m*; 2.76, *m*	3.24, *m*; 3.54, *m*	29.9	33.4
16	-	-	168.7	168.5
17	5.55, *s*	5.43, *s*	76.9	81.3
18	0.98	0.99	21.4	17.9
19	1.47	1.44	16.7	17.8
20	-	-	121.5	121.1
21	7.56, *s*	7.50, *s*	140.7	141.3
22	6.39 *d, J* =1.5 Hz	6.41, *d, J* =1.5 Hz	109.4	109.9
23	7.45, *d, J* = 1.5 Hz	7.44, *d, J* = 1.5 Hz	143.4	143.3
28	1.14, s	1.10, s	23.2	23.3
29	0.89, s	0.86, s	23.9	23.9
30	5.34, *dt, J* =7.5, 1.5 Hz	2.24, m	123.8	34.3
7-OMe	3.77, s	3.87, s	53.5	53.7
1′	-	-	167.1	167.4
2′	-	-	127.9	129.3
3′	6.88, *q, J* =7.0 Hz	6.94, *q, J* =7.0 Hz	139.2	139.2
4′	1.76, *d*, *J* =7.0 Hz	1.84, *d*, *J* =7.0 Hz	14.8	14.8
5′	1.82, s	1.89, s	11.9	12.5

Table 2. Antibacterial activity of petroleum ether, chloroform and methanol soluble fractions of ethanol extract and purified compounds (1 and 2) of *S. mahagony* seed

Bacteria	Diameter of the zone of inhibition (mm)					
	PE	CH	ME	**1**	**2**	K
	300 µg/disc	300 µg/disc	300 µg/disc	300 µg/disc	300 µg/disc	30 µg/disc
Gram positive						
Bacillus subtilis	8	11	9	16	13	27
B. megaterium	7	13	7	14	13	28
Sarcina lutea	8	12	7	11	15	31
Staphylococcus aureus	10	14	6	12	13	34
Gram negative						
Esherichia coli	9	14	10	12	8	28
Psedomonas aeruginosa	10	13	6	10	13	33
Salmonella typhi	13	11	7	9	12	29
Shigella boydii	7	14	6	8	10	32
Sh. dysenteriae	8	13	7	7	11	29
Sh. sonnei	6	11	9	9	12	34
Sh. shiga	8	10	8	8	13	27
Klebsiella species	7	9	7	11	9	28

PE = Petroleum ether; CH= Chloroform; ME= Methanol; K= Kanamycin

(C-1) confirmed its placement at position 30. The connectivity of tigloyl group at C-3 was confirmed by the HMBC correlation between H-3 to C-1′. Another ^3J HMBC correlation by H-5 (δ_H 3.51) to the carbonyl at 176.2 (C-7) confirmed the connectivity of the other side chain at C-5. Accordingly, compound **1** was identified as swietenine. Its ^{1}H and ^{13}C NMR data are in good agreement to those published in literature.[8] The molecular formula of **2** was established as $C_{32}H_{40}O_9$ from the [M+Na] peaks at 591. 2566 in the HREISMS. Its NMR spectra were almost identical to those of **1** except the ^{1}H and ^{13}C resonances at positions 8, 14 and 30. The methylene protons at 2.24 (δ_C 34.3) showed a ^3J correlation to the carbonyl at 218.2 (C-1) and thereby confirmed its placement at position 30. A double bond existed between C-8 and C-14 and both of these appeared as quaternary carbons (128.7 and 131.8). Thus compound **2** was identified as 3-*O*-tigloylswietenolide.[8]

The results of antibacterial activity are presented in Table 2. The fractions and purified compounds showed moderate antibacterial activity against the test organisms. The minimum inhibitory concentrations (MICs), determined by serial dilution technique,[15] for compound **1** were found to be 32 µg/ml against *B. megatorium* and 64 µg/ml against *E. coli* while the MIC of **2** was recorded as 64 µg/ml against both organisms.

In the brine shrimp lethality bioassay, the LC_{50} values of the compounds **1** and **2** were found to be 14.6 and 12.5 µg/ml, respectively.

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