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LAWSONOL, A NEW BIOACTIVE NAPHTHOQUINONE DIMER FROM THE LEAVES OF *Lawsonia alba*

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*A new bioactive naphthoquinone dimer, lawsonol[2-(1,4-dioxo-1,4-dihydronaphthalen-2-yloxy)-3-hydroxynaphthalene-1,4-dione] (**1**), was isolated from the leaves of* Lawsonia alba. *Its structure was established on the basis of spectral data, including 2D NMR and HR-MS. Compound **1** exhibited* in vitro *antibacterial activity against* Bacillus megaterium*,* Staphylococcus aureus*,* Escherichia coli*, and* Pseudomonas aeruginosa.

Keywords: *Lawsonia alba*, Lythraceae, lawsonol, antibacterial activity.

Medicinal plants are ideal targets for the discovery of potential bioactive compounds or lead structures for new drug development [1]. *Lawsonia* is a monotypic genus represented by *Lawsonia alba* (synonym *Lawsonia inermis* L.) [2]. The plant belongs to the Family Lythreaceae and is native to Southwest Asia and North Africa; it is cultivated in many districts of Bangladesh [3]. It contains a wide range of phytochemicals, including lawsone, isoplumbagin lawsoniaside, lalioside, lawsoniaside B, syringinoside, daphneside, daphnorin, agrimonolide-6-*O*-β-D-glucopyranoside, (+)-syringaresinol-*O*-β-D-glucopyranoside, (+)-pinoresinol di-*O*-β-D-glucopyranoside, isoscutellarin-3β, hennadiol, (20*S*)-3β-30-dihydroxylupane, lawnermis acid, 3-methylnonacosan-1-ol, laxanthones I, II, III, lacoumarin, etc. [4–10]. The key coloring agent present in leaves is lawsone [8].The various *in vitro* and *in vivo* studies of *L. alba* reported that the plant possesses antifungal, antiparasitic, antiviral, anticancer, antidiabetic, tuberculostatic, anti-inflammatory, antifertility, and wound healing properties [2]. Traditionally, a paste of leaves is used to prevent skin inflammation, as well as to cure ulcers and wounds. The leaves have also been used to treat constipation and as a hematinic and febrifuge, and to treat cough, burning sensation, hemicranias, cephalagia, diarrhea, dysentery, leucoderma, leprosy, boils, scabies, hepatopathy, anemia, hemoptysis, ophthalmia, etc. [2].

Compound **1** (Fig. 1) was obtained as light orange crystals. Accurate mass measurements of **1** obtained by FT-ESI-MS yielded a parent mass at *m*/*z* 345.0408 in the negative ionization mode (calcd mass 345.0405), corresponding to the $[M - H]^-$ of a compound with a molecular formula of $C_{20}H_{10}O_6$, accounting for 16 degrees of unsaturation.

The ^{13}C NMR spectrum (Table 1) displayed 20 carbon resonances, while the HSQC experiment indicated that nine out of the 20 carbons were attached to protons. The ^1H NMR (Table 1) and DEPT 135 spectra revealed the presence of nine aromatic/olefinic methines.The one four-proton doublet at δ 8.05 ppm and two two-proton triplets at δ 7.74 and 7.67 ppm indicated the presence of two disubstituted aromatic rings. The coupling pattern suggested that **1** is likely a dimer.

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TABLE 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data for **1** ($CDCl_3$–1% CD_3OD, δ, ppm, J/Hz)

C atom	δ_H	δ_C	COSY	HMBC
1	–	185.5	–	–
2	–	157.6	–	–
3	–	157.6	–	–
4	–	185.5	–	–
4a	–	132.7	–	–
5	8.05 (1H, d, J = 7.6)	126.5	H-6	C-4, C-7, C-8a
6	7.67 (1H, t, J = 7.4)	133.1	H-7	–
7	7.74 (1H, t, J = 7.4)	134.9	H-6	–
8	8.05 (1H, d, J = 7.6)	126.5	H-7	C-1, C-6
8a	–	133.9	–	–
1′	–	182.0	–	–
2′	–	157.6	–	–
3′	6.28 (1H, s)	111.1	–	C-2, C-1′, C-2′, C-4′, C-4′a
4′	–	182.0	–	–
4′a	–	129.9	–	–
5′	8.05 (1H, d, J = 7.6)	126.4	H-6′	C-4′, C-7′
6′	7.67 (1H, t, J = 7.4)	133.1	H-7′	–
7′	7.74 (1H, t, J = 7.4)	134.9	H-6′	–
8′	8.05 (1H, d, J = 7.6)	126.4	H-7′	C-1′, C-6′, C-4′a
8′a	–	126.3	–	–

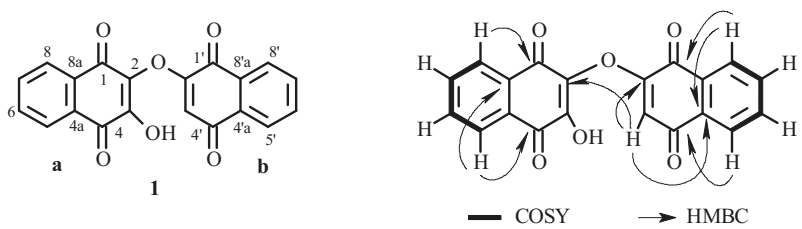

Fig. 1. Structure of **1** and its 2D-connectivity.

This is further evident from the two resonances at δ 185.5 and 182.0 ppm in the ^{13}C NMR spectrum of **1** for the carbonyl carbons at C-1 and C-4 and C-1′ and C-4′, respectively. The presence of relatively shielded resonances for carbonyl groups, in conjunction with the two disubstituted aromatic rings, suggested the presence of two naphthoquinone moieties. The analysis of one- and two-dimensional NMR spectra, including COSY, HSQC, and HMBC, led to the assignment of the partial structures **a** and **b**, as shown in Fig. 1. In structure **a**, the C5-C6-C7-C8 fragment was assigned by the tracing of cross peaks in the COSY spectrum. A naphthoquinone ring moiety was disclosed by HMBC correlations (H-5/C-4 and C-8a; H-8/C-1) and the chemical shift of C-1 and C-4 (δ 185.5 ppm). In the partial structure **b** the C5′-C6′-C7′-C8′ portion was assigned by the tracing of cross peaks in the COSY spectrum. Another naphthoquinone ring moiety was disclosed by HMBC correlations (H-3′/C-2′ and C-4′a; H-5′/C-4′; H-8′/C-1′) and the chemical shift of C-1′ and C-4′ (δ 182.0 ppm). H-3′ gave a HMBC cross-peak with C-2, indicating a connection between the partial structures **a** and **b**. The connectivity of the olefinic proton H-3′ with C-2, C-2′, and C-4′ and the chemical shifts of C-2 and C-2′ (δ 157.6 ppm) confirm the connection between the partial structures **a** and **b** through an ether linkage, as shown in Fig. 1. On the basis of the spectral data, **1** was characterized as 2-(1,4-dioxo-1,4-dihydronaphthalen-2-yloxy)-3-hydroxynaphthalene-1,4-dione, a naphthoquinone dimer for which we have given the trivial name lawsonol. A literature survey showed that **1** is a new natural product.

The *in vitro* antibacterial activity of **1** was tested against a panel of standard pathogenic control strains. Compound **1** exhibited mild to moderate activities against *Pseudomonas aeruginosa* ATCC 27833, *Bacillus megaterium* ATCC18, and *Staphylococcus aureus* ATCC 25923 with 14, 12, and 14 mm zones of inhibition, respectively. The compound showed poor activity against *Escherichia coli* ATCC 8739, and the zone of inhibition was only 8 mm (Table 2).

TABLE 2. Antibacterial Activity of Compound **1** (diameter of zone of inhibition, mm)

Bacteria	**1**	Azithromycin
Gram-negative bacteria		
Escherichia coli (ATCC 8739)	8	27
Pseudomonas aeruginosa (ATCC 27833)	14	28
Gram-positive bacteria		
Bacillus megaterium (ATCC 18)	12	50
Staphylococcus aureus (ATCC 25923)	14	27

EXPERIMENTAL

General Experimental Procedures. Accurate mass measurements were determined on a Thermo Scientific Exactive Orbitrap mass spectrometer at London King′s College, London, UK, and the data were processed by Thermo XCalibur 2.2 software. NMR spectra (both 1D and 2D) were recorded on a Bruker spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) using $CDCl_3$/1% CD_3OD. A residual solvent peak was used as an internal standard for NMR spectroscopy. TLC was conducted on normal-phase Merck Si gel 60 PF_{254} plates. Spots on TLC were visualized under UV light (at 254 and 365 nm) and by spraying with vanillin-sulfuric acid spray reagents. TLC plates were purchased from Merck, Germany.

Plant Material. The leaves of *Lawsonia alba* were collected from the garden of the Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka. A voucher specimen for this collection is maintained at the Department of Botany, University of Dhaka, under accession No. DUSH 10780.

Extraction and Isolation. Fresh matured leaves (90 gm) of *Lawsonia alba* were blended with water to prepare the juice of the plant material. The juice was filtered through a fresh cotton bed and then filtered with Whatman No. 1 filter paper. The residual plant material was suspended in 200 mL of a dichloromethane–methanol (1:1) solvent system for 5 days for cold extraction. The extract was filtered through a fresh cotton bed and then filtered with Whatman No. 1 filter paper. The whole process was repeated twice, and the combined filtrates were concentrated using a rotary evaporator machine at low temperature (40–50°C) and under reduced pressure. Compound **1** was isolated as light orange crystals (4.9 mg) from this crude extract by treatment with different solvents of varying polarities. It was visible as an orange spot on a TLC plate (R_f 0.50, toluene–40% EtOAc) and appeared as a dark quenching spot under UV light at 254 nm. Spraying the developed plate of **1** with vanillin–H_2SO_4, followed by heating, gave a purple color. It is sparingly soluble in CH_2Cl_2, $CHCl_3$, and MeOH.

Lawsonol[2-(1,4-dioxo-1,4-dihydronaphthalen-2-yloxy)-3-hydroxynaphthalene-1,4-dione] (1). Light orange crystals; molecular formula $C_{20}H_{10}O_6$. For ^{1}H NMR (400 MHz, $CDCl_3$–1% CD_3OD) and ^{13}C NMR (100 MHz, $CDCl_3$–1% CD_3OD), see Table 1. FT-ESI-MS $[M - H]^-$ *m*/*z* 345.0408 in negative ionization mode (calcd for $C_{20}H_9O_6$, 345.0405).

Antibacterial Assay. The antibacterial activity of compound **1** was tested against a panel of four pathogenic bacterial strains, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 8739, *Bacillus megaterium* ATCC 18, and *Pseudomonas aeruginosa* ATCC 27833. The cultures of the microorganisms were obtained from ICDDR, B, Bangladesh. The bacterial cultures were subcultured every two weeks on fresh nutrient agar (NA) slants and incubated at 37°C. The spectrum of antibacterial activity was studied using the technique described by Bauer et al. [11]. Azithromycin disc (30 μg/disc) was used as a positive control, and solvents were used as negative controls. The sensitivities of the bacteria to **1** (100 μg/disc) was determined by measuring the diameter of inhibitory zones in millimeters after 18 to 24 h of incubation at 37°C.

From the leaves of *Lawsonia alba*, a new bioactive naphthoquinone dimer, lawsonol (**1**), was isolated and characterized as 2-(1,4-dioxo-1,4-dihydronaphthalen-2-yloxy)-3-hydroxynaphthalene-1,4-dione on the basis of spectral data, including 2D NMR and HR-MS. Lawsonol (**1**) exhibited *in vitro* antibacterial activity against *Bacillus megaterium, Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The pipeline of antibiotics is essentially empty, and very few compounds are even in the early stages of clinical trials. Therefore, development of novel antimicrobial agents for the treatment of infections caused by pathogens is an urgent priority. Lawsonol is druglike and can be used as scaffolds for medicinal-chemistry optimizations to develop new antibacterial agents.

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