Cucullamide, a New Putrescine Bisamide from *Amoora cucullata*

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Received April 30, 2010; accepted May 21, 2010; published online May 21, 2010

A new putrescine bisamide derivative named cucullamide (1) was isolated from the leaves of *Amoora cucullata*, together with five known natural products, dasyclamide (2), ent-2β-hydroxymanool (3), chrysin (4), apigenin (5), and kaempferol-3-*O*-β-D-glucopyranoside (6). The structure of the new isolated compound was elucidated on the basis of 1D and 2D NMR as well as high resolution-electrospray ionization (HR-ESI)-MS spectroscopic analysis.

Key words *Amoora cucullata*; putrescine; bisamide; spectroscopy

Amoora cucullata (Meliaceae) is a tall tree, grown in the coastal areas of Southeast Asia and the Indian Ocean. This plant has been used as a folk medicine for treatment of inflammation, marrow and diarrhea.[1)] Additionally, juice of the leaves has antibacterial activity and extensively used for the treatment of dysentery, skin and cardiac diseases.[2)] The crude methanolic extracts of leaves were reported to show anti-inflammatory, antinociceptive, diuretic, and central nervous system (CNS) depressant activities.[3,4)] Phytochemical analysis of the stem bark of *Amoora cucullata* led to the isolation of fridelin, stigmasterol, β-sitosterol, betulinic acid and caffeic acid.[5)] Furthermore, analysis of the leaves showed the presence of several polyphenols and tannins, whether from its fruits, several rocagloic acid derivatives were isolated.[6)] In continuation of our phytochemical screening of *A. cucullata* leaves, we report the isolation and structure elucidation of one new putrescine bisamide, cucullamide (**1**), along with five known natural compounds (**2**—**6**) (Fig. 1). The new putrescine bisamide derivative (**1**) and dasyclamide (**2**) were evaluated for their Wnt signal inhibitory activities, since we are recently interested in screening studies targeting signaling molecules related to cancer diseases.[7)]

Results and Discussion

The fresh leaves of *Amoora cucullata* were collected from the Sundarbans Mangrove Forests, Bangladesh.[8)] The methanolic extract of *A. cucullata* was partitioned successively with ethyl acetate, *n*BuOH, and water. The ethyl acetate fraction was then subjected to repeated column chromatography on Sephadex LH20, silica gel 60N, octadecylsilyl (ODS) gel, and preparative thin layer chromatography (PTLC) to obtain dasyclamide (**2**),[9)] ent-2β-hydroxymanool (**3**),[10)] chrysin (**4**),[11)] apigenin (**5**),[12)] and kaempferol-3-*O*-β-D-glucopyranoside (**6**).[13)] All of the known compounds were identified by spectroscopic data including 1D and 2D NMR and by comparison with those published in the literature. All these five known compounds are isolated and reported for the first time from this plant.

Compound **1** was isolated as a white solid. On the TLC plate after developing compound **1**, it gave a positive color reaction after spraying with Dragendorff reagent and dark fluorescence under UV light at 254 nm. The electrospray ion-

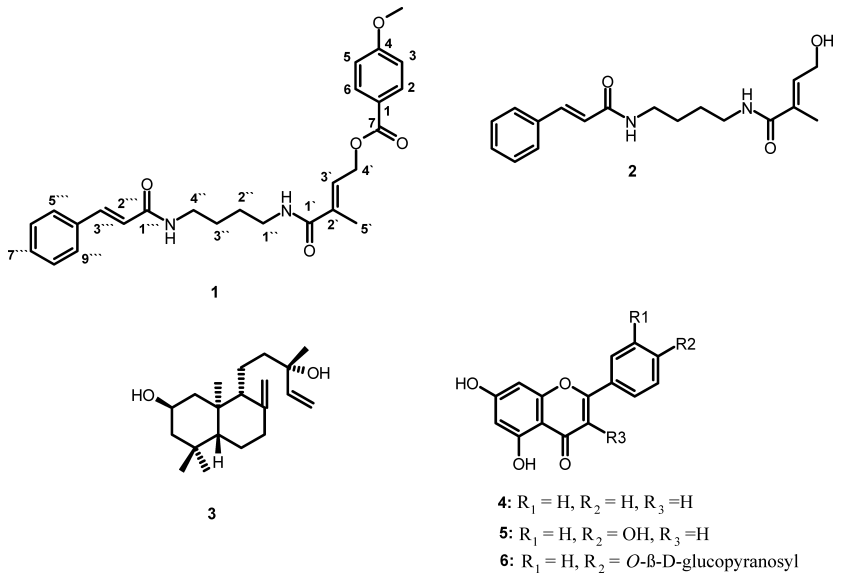

Fig. 1. Structures of Isolated Compounds

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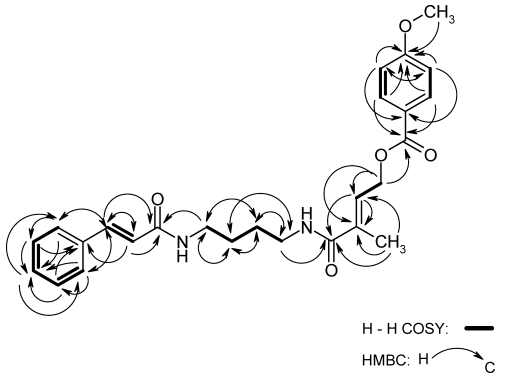

Fig. 2. Structure of Compound **1** with Key 2D-NMR Correlations

ization (ESI)-MS of **1**, displaying in the positive mode ion signals at *m/z* 473 $[M+Na]^+$, suggested a molecular weight of 450 Dalton. The molecular formula was determined as $C_{26}H_{30}N_2O_5$ by high resolution (HR)-ESI-MS *m/z* 473.20880 (Calcd for $[C_{26}H_{30}N_2O_5+Na]^+$ 473.20524). The IR spectrum of **1** showed absorption bands at 3289 cm^{-1} (NH), typical for secondary amides, and 1658, 1605, 1541 cm^{-1} (>N–C=O stretching region). The ^{1}H-NMR spectrum of **1** in CD_3OD (Table 1) exhibited chemical shifts similar to those of dasyclamide (**2**). It showed a sharp set of aromatic protons [δ_H 7.98 (2H, d, J=7.9 Hz) and 6.97 (2H, d, J=7.9 Hz)], which characterize AA′ BB′ system of *p*-disubstituted benzene ring. Additionally, multiplet signals with integration of five protons [δ_H 7.52 (2H, m) and 7.36 (3H, m)] were observed indicating the presence of a monosubstituted phenyl system. A typical *trans* pair of doublet protons [δ_H 7.50 (1H, d, J=15.8 Hz) and 6.58 (1H, d, J=15.8 Hz)] and one doublet of triplet signal [δ_H 6.39 (1H, dt, J=1.6, 6.0 Hz)] were identified. The aliphatic region indicated the presence of five methylene groups [δ_H 4.95 (2H, d, J=6.0 Hz), 3.31 (2H, m), 3.28 (2H, m), and 1.60 (4H, m)], one methoxy group [δ_H 3.85 (3H, s)], and methyl signal [δ_H 1.95 (3H, s)]. All assignments were clearly derived from 2D NMR spectra (HH-correlation spectroscopy (COSY), ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC)). The ^{13}C-NMR spectrum **1** revealed three carbonyls (δ_C 171.6, 168.6, 167.6), one oxygenated sp^2 carbons (δ_C 165.3), other three sp^2 methine carbons (δ_C 141.6, 136.1, 121.9), one quaternary carbon (δ_C 132.7), and signals assignable to *p*-disubstituted and monosubstituted benzene ring. Furthermore, one methoxy carbon (δ_C 56.0), five methylenes (δ_C 62.2, 40.4, 40.2, 27.9, 27.8), and one methyl group (δ_C 13.3) were delivered. When the NMR data of **1** were compared with those of dasyclamide (**2**), major differences were observed in the presence of *p*-disubstituted benzene ring and methoxy group in **1**. Additionally, the H_2-4′ in compound **1** was shifted downfield 0.72 ppm to δ 4.95 and resonated as a doublet instead of doublet of doublets as found in **2**. The corresponding change in the ^{13}C-NMR spectrum was shifting from δ 62.2 to 59.5 in case of **2**. These comparison gave an evidence to the presence of *p*-disubstituted benzene moiety connected to C-4′ of the terminal hydroxyl tiglic acid unit in dasyclamide (**2**). The HMBC correlations of H-3/H-5 at δ_H 6.97 and H-2/H-6 δ_H 7.98 with δ_C 123.3 (C-1) and δ_C 165.3 (C-4), and the OCH_3 group at δ_H 3.85 with δ_H 165.3 (C-4) revealed the existence of *p*-methoxy benzoic acid (Fig. 2). Connectivity of the latter to the C-4′ was confirmed from the HMBC correlations of the methylene protons at δ_H 4.95 (H_2-4′) and the aromatic proton at δ_H 7.98 (H-2/H-6) with the carbonyl group at δ_H 167.6 (C-7). By combination of the above evidence and ^{1}H- and ^{13}C-NMR spectroscopic analysis, it was concluded that the structure of cucullamide is **1**. Several putrescine bisamides such as aglaiduline, leptagline, gigantamide A, grandiamide D were isolated from different *Aglaia* plants.[14,15] This is the first report of the isolation of putrescine bisamide possessing *p*-methoxy benzoic acid substituent, *via* ester link, from *A. cucullata* leaves. We examined the Wnt signal inhibitory activity of the cucullamide (**1**) and dasyclamide (**2**) using a luciferase reporter gene assay.[16] The results showed that both compounds had no effect at a lower concentration of 22.2 μM.

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for Cucullamide (**1**) in CD_3OD

Position	δ_H (ppm)	δ_C (ppm)	HMBC (^{1}H to ^{13}C)
1		123.3	
2/6	7.98 d (7.9)	129.9	1, 4, 7
3/5	6.97 d (7.9)	114.8	1, 4
4		165.3	
7		167.6	
1′		171.6	
2′		132.7	
3′	6.39 qt (1.6, 6.0)	136.1	1′, 2′
4′	4.95 d (6.0)	62.2	2′, 3′, 7
5′	1.95 s	13.3	1′, 2′, 3′
1″	3.28 m	40.4	1′,2″, 3″
2″	1.60 m	27.9	1″, 3″, 4″
3″	1.60 m	27.8	1″, 2″, 4″
4″	3.31 m	40.2	1‴, 2″, 3″
1‴		168.6	
2‴	6.58 d (15.8)	121.9	1‴, 3‴, 4‴
3‴	7.50 d (15.8)	141.6	1‴, 2‴, 5‴, 9‴
4‴		136.3	
5‴/9‴	7.52 m	128.8	3‴,4‴, 7‴
6‴/8‴	7.36 m	130.2	4‴, 7‴
7‴	7.36 m	130.8	5‴, 9‴
4-OCH_3	3.85 s	56.0	4

Experimental

General Experimental Procedures The NMR data were measured on a JEOL JNM ecp600 spectrometer. Mass spectra were recorded on AccuTOF-T100LP (JOEL) mass spectrometer. IR spectra were recorded on ATR in a Jasco FT-IR 230 spectrophotometer, and UV spectra were obtained on a Shimadzu UV mini-1240 spectrometer.

Plant Material Fresh leaves of *A. cucullata* were collected from the Sundarbans' Mangrove Forests, Bangladesh in November, 2008 and were taxonomically identified by Prof. A. K. Fazlul Huq, Forestry and Wood Technology Discipline, Khulna University, Bangladesh. A voucher specimen was also deposited there for future reference (F018). The air-dried plants were subjected to grinding before extraction.

Extraction and Isolation The dried, ground leaves of *A. cucullata* (280 g) was extracted with MeOH for 2 d at room temperature followed by homogenization and filtration, which then underwent evaporation and vacuum desiccation to get the crude extract (26 g). The MeOH extract of leaves of *A. cucullata* was dissolved in 10% aqueous MeOH, and partitioned successively with ethyl acetate (100 ml×2) and *n*BuOH (100 ml×2) to give three fractions. The ethyl acetate extract (3.3 g) was subjected to silica gel PSQ100B Column chromatography (CC) to afford fractions 2A—2N. Fraction 2I (193 mg) was subjected to Silica gel 60N CC to afford three fractions. The middle fraction (23 mg) was subjected to ODS flash CC to get compounds **4** (5.6 mg) and **5** (3.8 mg). Compounds **1** (25 mg) and **2** (1.9 mg) were purified from fraction 2J (256 mg) by PTLC (6 plates, 20×20 cm,

$CHCl_3$/15% MeOH) followed by Sephadex LH-20 (CH_2Cl_2/MeOH, 3 : 2). Fraction 2K (198 mg) was applied to Sephadex LH20 column (MeOH) to obtain compound **3** (31.5 mg). Fraction 2L (217 mg) was subjected to PTLC (5 plates, 20×20 cm, $CHCl_3$/20% MeOH) to get compound **6** (35.8 mg).

Cucullamide (**2**): White solid; UV λ_{max} (MeOH) 213 (ε 15521) and 249 (6647) nm; IR (ATR) ν_{max} 3289, 1658, 1605, 1541 and 751 cm^{-1}; ^{1}H- and ^{13}C-NMR (Table 1); Positive ESI-MS *m*/*z*: 473.2 $[M+Na]^+$ ($C_{26}H_{30}N_2O_5$); HR-ESI-MS *m*/*z*: 473.20880 (Calcd for $[C_{26}H_{30}N_2O_5+Na]^+$: 473.20524).

Acknowledgments This work was supported by Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS). The research described in this paper was supported by JSPS postdoctoral fellowship (ID No. P09042).

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