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Chemical components of the roots of Noni (*Morinda citrifolia*) and their cytotoxic effects

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ABSTRACT

Roots of *Morinda citrifolia* (Noni or Yor in Thai) have been used traditionally for thousands of years to treat chronic diseases such as cancer and heart disease. In this study, three new anthraquinones together with 15 known compounds were isolated from the roots of *M. citrifolia* (Rubiaceae). Their structures were established by spectroscopic methods, particularly 1D and 2D NMR techniques. Six known compounds, together with two new compounds (**2** and **3**) showed significant inhibitory effects on the proliferation of human lung and colon cancer cells.

1. Introduction

Morinda citrifolia L. (Rubiaceae), known as "Noni", is widely distributed in tropical Asia, India, and the Pacific Islands. Almost all parts of this plant including fruits, flowers, leaves, bark, stem, and roots have been used as food, medicine, and fabric dyes for more than 2000 years by the Polynesian people [1]. Noni juice from *M. citrifolia* fruit has become a popular tonic in recent years since it is reputed to prevent lifestyle-related diseases. The plant has displayed antibacterial, antiviral, antifungal, antitumor, anthelminthic, analgesic, hypotensive, anti-inflammatory, and immune-enhancing activities in pharmacological studies although their efficacies are largely unproven [2]. To date, some of its major biologically active components, which may contribute to its reputed and diversified health benefits, have been investigated. About 200 phytochemicals have been identified from the noni plant, including anthraquinones, flavonoids, polysaccharides, glycosides, iridoids, lignans, and triterpenoids [3]. Compounds such as scopoletin, rutin, ursolic acid, β-sitosterol, asperuloside, and damnacanthal are considered as key components of noni fruits [3–5]. In our previous study, four new glycosides [6,7] and four new iridoids, as well as two known iridoids and five known flavonoids were identified from noni leaves [8–10]. The two new iridoids showed significant inhibition of UVB-induced Activator Protein-1 (AP-1) activity. Upon continuation, three new anthraquinones together with 15 known compounds were isolated from the roots of noni. We present herein the isolation, structural elucidation, and cytotoxicity of these compounds.

2. Material and methods

2.1. General experimental procedures

All ^{1}H, ^{13}C, and 2D NMR were obtained on a VXR-600 (Varian Inc., Palo Alto, CA) operating at 600 and 150 MHz. Compounds were analyzed in $CDCl_3$, CD_3OD and DMSO-d_6 with tetramethylsilane (TMS) (Aldrich Chemical Co., Allentown, PA) as an internal standard. APCI-MS was obtained on a Fisons/VG Platform II mass spectrometer. Preparative thin-layer chromatography was performed on Sigma-Aldrich TLC

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plates (1000 μm thickness, 2–25 μm particle size), Thin-layer chromatography was performed on Sigma-Aldrich TLC plates (250 μm thickness, 2–25 μm particle size), with compounds visualized by spraying with 10% (v/v) H_2SO_4 in ethanol solution.

2.2. Chemicals

Silica gel (130–270 mesh), Sephadex LH-20 (Sigma Chemical Co., St. Louis, MO) and a Lichroprep RP-18 column were used for column chromatography. All solvents used for chromatographic isolation were analytical grade and purchased from Fisher Scientific (Springfield, NJ). Dimethylsulphoxide (DMSO) and phenazine methosulfate (PMS) were purchased from Sigma Chemical Co.

2.3. Plant material

The air-dried roots of *M. citrifolia* (Rubiaceae) were collected from Hawaii in 2004 and identified by Dr. Robert T. Rosen. A voucher specimen (RUCAFT033) was deposited in the Herbarium of the Center for Advanced Food Technology, Rutgers University.

2.4. Extraction and isolation procedure

The dried powders of noni roots (1.5 kg) were extracted with 95% ethanol (4 L) at 50 °C for 1 day. The extract was concentrated to dryness under reduced pressure and the residue was dissolved in water (500 mL) and partitioned successively with hexane (3×500 mL), ethyl acetate (3×500 mL), and *n*-butanol (3×500 mL). The ethyl acetate-soluble portion (31.1 g) was subjected to a silica gel column using an *n*-C_6H_{12}/$CHCl_3$/MeOH gradient system (5:1:0, 2:1:0, 1:1:0, 1:2:0, 0:1:0, 0:3:1, and 0:0:1; 2.0 L for each gradient system), yielding twenty nine fractions. Fraction 5 (0.6 g) was separated on a silica gel column (*n*-C_6H_{12}/$CHCl_3$, 5:1), yielding compounds **4** (91 mg) and **5** (46 mg). Fraction 8 (0.7 g) was filtered, yielding compound **8** (800 mg). Fraction 9 was separated first by a normal phase silica gel column (*n*-C_6H_{12}/$CHCl_3$, 10:1), followed by a Sephadex LH-20 column (95% EtOH), yielding compound **7** (30 mg). Fractions 10–12 were combined (5.2 g) and rechromatographed on a silica gel column (*n*-C_6H_{12}/$CHCl_3$, 10:1 to 5:1), yielding 13 subfractions. Compound **9** (1.5 g) was crystallized from subfraction 12. Subfractions 4, 9 and 6 were subjected to a Sephadex LH-20 column with 95% EtOH, yielding compounds **7** (35 mg), **3** (22 mg), **2** (18 mg) and **18** (100 mg, crystallized). Fraction 14 (0.9 g) was subjected to a Sephadex LH-20 column and eluted with 95% ethanol, yielding three subfractions (1–3). Subfraction 2 was separated by an RP C-18 column (MeOH, 75% to 80%), yielding compounds **15** (40 mg), **1** (24 mg), and **13** (50 mg). Subfraction 3 was subjected to a normal phase silica gel column and eluted with hexane/chloroform (1:1.5) to obtain compound **10** (80 mg). Fraction 16 (1.1 g) was applied to an RP C-18 column and eluted with 75% MeOH, yielding compounds **16** (30 mg) and **13** (25 mg). Fraction 21 (0.7 g) was purified by column chromatography. RP C-18 (MeOH 65–85%) and Sephadex LH-20 (95% EtOH) columns were used to acquire compounds **17** (20 mg) and **11** (20 mg), respectively. Subfraction 1 was applied to preparative TLC (20×20 cm) with hexane/ethyl acetate (1.5:1) (RF: 0.48) to yield compound **14** (20 mg).

2.5. Growth inhibition against human lung and colon cancer cells

Cell growth inhibition was determined by the MTT assay [11]. The cells were plated in 96-well microtiter plates and allowed to attach for 24 h at 37 °C. The test compounds in DMSO were added to cell culture medium to desired final concentrations (final DMSO concentrations for control and treatment groups were 0.1%). After the cells were cultured for 24 h, the medium was aspirated and the cells were treated with 100 μL of fresh medium containing 2.4 mmol/L MTT. After incubation for 1–3 h at 37 °C, the MTT-containing medium was aspirated, 100 μL of DMSO was added to solubilize the formazan precipitate, and the plate was read at 550 nm on a microtiter plate reader. The reading reflected the number of viable cells and was expressed as a percentage of viable cells in the control. Both H1299 (human lung cancer cells) and HCT116 (human colon cancer cells) were cultured in McCoy's 5A medium. All of the above media were supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, and 1% glutamine and the cells were kept in a 37 °C incubator at 95% humidity and 5% CO_2.

3. Results

From the combination of solvent extraction, partition and modern chromatography methods, including normal and reversed phase silica gel column chromatography, preparative thin-layer chromatography, and Sephadex LH-20 column chromatography, a total of 18 compounds were isolated from noni roots, three of which are new compounds. The structures were elucidated by spectral methods that include APCI-MS, ^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}H COSY, HMQC, and HMBC.

3.1. Structure elucidation

The known compounds 1-hydroxy-2-methylanthraquinone (**4**) [12], tectoquinone or 2-methylanthraquinone (**5**) [13,14], 2-formyl-1-hydroxyanthraquinone (**6**) [15], 2-formylanthraquinones (**7**) [16], nordamnacathal, or 1,3-dihydroxy-2-formylanthraquinone (**8**) [17] (Zhou et al., 1994), damnacanthal, or 1-methoxy-3-hydroxy-2-formylanthraquinones (**9**) [17], alizarin 1-methyl ether, or 1-methoxy-2-hydroxyanthraquinone (**10**) [18], 1-methoxy-3-hydroxyanthraquinone (**11**) [19], 1-methyl-3-hydroxyanthraquinone (**12**), rubiadin, or 1,3-dihydroxy-2-methylanthraquinone (**13**) [12,20], 1,3-dimethoxy-2-methoxymethylanthraquinone (**14**) [12], ibericin (**15**) [21,22], monndone-5-methylether, or 1,7-dihydroxy-8-methoxy-2-methylanthraquinone (**16**) [20], decumbic acid (**17**) [23] and cholest-22-en-3-ol (**18**) [24] were identified by comparison of their spectroscopic data with those reported in the literature.

Nonin A (compound **1**) had a molecular formula of $C_{17}H_{16}O_5$ as determined by APCI-MS, ^{1}H and ^{13}C NMR data. The ^{1}H and ^{13}C NMR data (Table 1) exhibited the presence of two tertiary methyls (one of them is methoxyl group), one oxygenated methylene, six olefinic methines and eight

Table 1
^{1}H and ^{13}C NMR chemical shift data (600 MHz and 150 MHz, respectively) of compounds **1**–**3** (δ in ppm, J in Hz).

	1 [a]		**2** [a]		**3** [a]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		157.0 s		192.0 s		192.0 s
2		102.3 s	3.82 d, 13.8	59.4 d	4.01 d, 13.8	56.3 d
3		112.6 s	3.17 dd, 4.8, 13.8	43.4 d	3.62 brd, 14.4	45.2 d
4		140.9 s		104.2 s		103.6 s
4a		128.8 s		131.		131.2 s
5	8.10 d, 7.8	121.7 d	7.72 d, 7.2	126.4 d	7.77 d, 7.2	123.4 d
6	7.58 t, 7.8	129.7 d	7.51 t, 7.2	130.3 d	7.49 dd, 7.2, 9.6	130.0 d
7	7.48 t, 7.8	126.7 d	7.69 dd, 7.2, 9.6	135.1 d	7.65 dd, 7.2, 9.6	134.9 d
8	8.33 d, 7.8	124.4 d	7.96 d, 9.6	127.5 d	8.03 d, 7.2	128.0 d
8a		125.4 s		140.7 s		140.3 s
CO		172.5 s		169.2 s		169.6 s
OMe	3.99 s	52.5 q	3.83	52.6 q	3.82 s	52.7 q
1′	7.19 d, 10.2	124.8 d	5.68 dd, 4.8, 9.6	126.8 d	5.52 d, 9.6	125.2 d
2′	5.64 d, 10.2	125.3 d	6.01 d, 9.6	133.0 d	5.98 d, 9.6	133.5 d
3′		78.1 s		80.7 s		80.3 s
4′	3.76 d, 12.0	68.1 t	4.16 d, 7.2	79.9 t	3.94 d, 7.2	77.8 t
	3.70 d, 12.0		3.81 d, 7.2		3.56 d, 7.2	
5′	1.37 s	21.6 q	1.52 s	19.1 q	1.59 s	19.1 q

[a] Data was recorded in CD_3OD.

quaternary carbons. The ^{1}H and ^{13}C NMR data of **1** were very similar to those of mollugin [12], indicating that they are structurally related analogs. As compared with mollugin, the major difference is compound **1** has one methyl group (($\delta_H = 1.37$, s; and $\delta_C = 21.6$, q) and one oxygenated methene ($\delta_H = 3.76$, d, $J = 12.0$ Hz, 1 H; $\delta_H = 3.70$, d, $J = 12.0$ Hz, 1 H; and $\delta_C = 68.1$, t) (Table 1) instead of two methyl groups in mollugin, clearly indicating that one of the methyl groups in mollugin was oxygenated to form the hydroxymethyl. This was further supported by the correlations between H-2′ and C-4′, H-5′ and C-4′, and H-4′ and C-3′ in the HMBC spectrum (Fig. 1). The structure of **1** was identified as shown in Fig. 2 and named as nonin A. Full assignments of the ^{1}H and ^{13}C NMR signals of **1** were made by analyzing the signals in HMBC and HMQC spectra (Table 1).

Nonin B (compound **2**) had a molecular formula of $C_{17}H_{16}O_5$ with 10° of unsaturation, as determined by the APCI-MS, ^{1}H and ^{13}C NMR data. In accordance with its molecular formula, all of the 17 carbons were further classified by the chemical shift and HMQC spectrum as two methyls, one oxygenated methylene, eight methines (six of which were olefinic carbons), and six quaternary carbons (one ester carbonyl, one ketone carbonyl, two oxygenated and two olefinic carbons). These above functionalities accounted for six degrees of unsaturation, and the remaining four required compound **2** to be tetracyclic. A detailed account of the structural assignment of **2** is given below. The NMR spectra of compound **2** showed an AA′BB′ coupling system (δ 7.77 d, $J = 7.2$ Hz, 7.49 dd, $J = 7.2$, 9.6 Hz, 7.65 dd, $J = 7.2$, 9.6 Hz, and 8.03 d, $J = 7.2$ Hz), suggesting an ortho-substituted benzene ring (A ring). The HMBC correlations between H-3 ($\delta_H = 3.17$, dd, $J = 13.8$, 4.8 Hz) and C-1 ($\delta_C = 192.0$), C-2 ($\delta_C = 59.4$), and C-4 ($\delta_C = 104.2$) constructed the structure of the B ring, which was further confirmed by the HMBC correlations between H-2 ($\delta_H = 3.82$, d, $J = 13.8$ Hz) and C-1 and H-5 ($\delta_H = 7.72$, d, $J = 7.2$ Hz) and C-4. The C ring was constructed by the chemical shifts of C-4 ($\delta_C = 104.2$) and C-3′ ($\delta_C = 79.9$) and the HMBC correlations of H-3/C-4, H-3/C-1′, and H-2′/C-3′. The methyl ($\delta_H = 1.52$, s, 3 H; $\delta_C = 19.1$) attached to C-3′ was assigned as C-5′ by the HMBC correlations of H-5′/C-4′, H-5′/C-3′, and H-5′/C-2′. These functionalities and rings

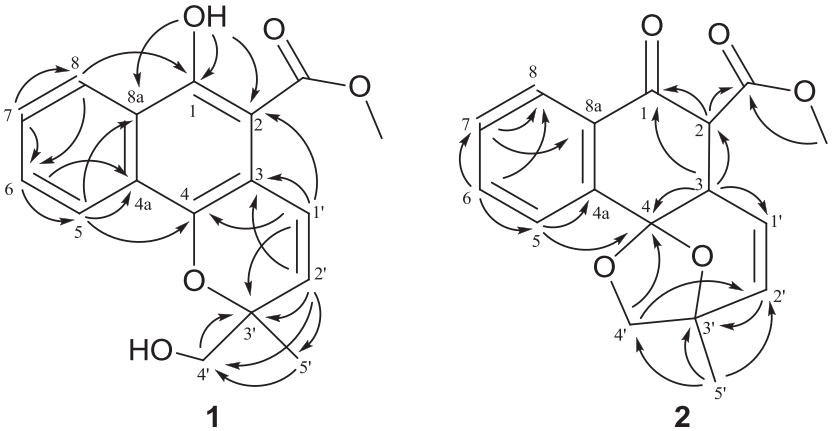

Fig. 1. Significant HMBC (H→C) correlations of compounds **1** and **2**.

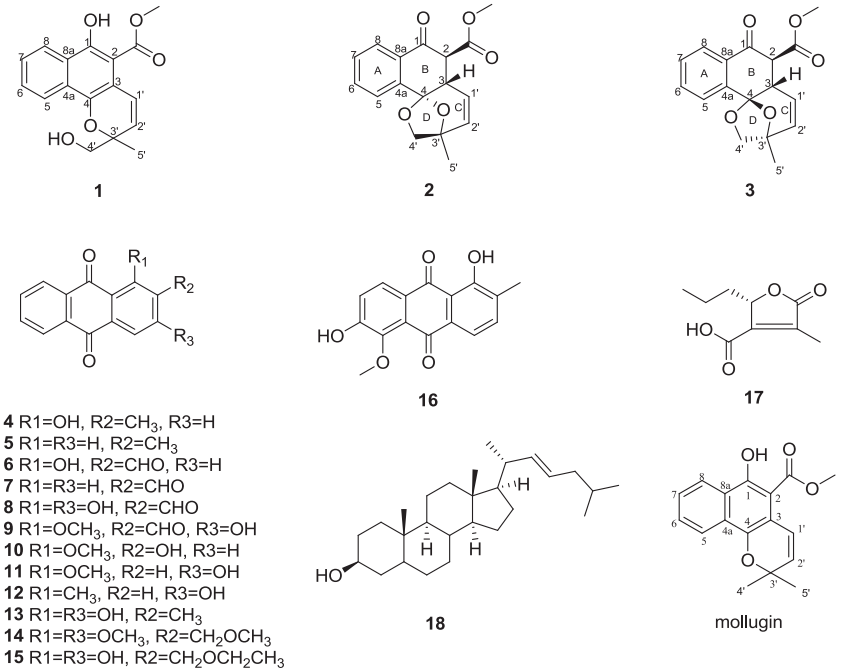

Fig. 2. Structures of compounds **1**–**18** and mollugin.

accounted for 9° of unsaturation, and the remaining 1° of unsaturation required the presence of one additional ring in **2**. Analysis of the chemical shift of C-4 (δ_C 104.2 s) and C-4′ (δ_C 79.9 t) suggested that this ring (D ring) was likely to be formed by the connection of C-4 and C-4′ via an ether linkage, which was further confirmed by an HMBC correlation between H-4′ (δ 4.16 d, $J = 7.2$ Hz) and C-4.

The relative stereochemistry of **2** was tentatively determined by the ^{1}H NMR data and a computer modeled 3D structure (Fig. 3). The coupling constant ($J_{2,3} = 13.8$ Hz) between H-2 and H-3 indicated that they both possessed an a-axis in the B ring, in which H-2 was arbitrarily assigned as an α-orientation leading to the β-orientation of H-3. The stereochemistry of C-4 and C-3′ was also determined by the computer modeled 3D structure. A computer modeled 3D structure of **2** was generated by using MM2 force field calculations for energy minimization with the molecular modeling program Chem3D Ultra 8.0, which showed that there are two different configurations in the D ring for compound **2** (Fig. 3). The two different configurations led to a different dihedral angle between H-3 and H-1′ (Fig. 3). In the ^{1}H NMR spectrum, the coupling constant between H-3 and H-1′ was 4.8 Hz, which indicated that the relative stereochemistry of compound **2** should be the same as that of 2a in Fig. 3. Therefore, the structure of **2** was tentatively identified as shown in Fig. 2 and named as nonin B.

Nonin C (Compound **3**) was shown to have the molecular formula $C_{17}H_{16}O_5$ based on APCI-MS, ^{1}H and ^{13}C NMR data, suggesting that it is an isomer of **2**. As compared with compound **2**, the ^{1}H and ^{13}C NMR data of **3** (Table 1) indicated that their structures are closely related. The differences were likely due to the configuration of the D ring. As shown in the ^{1}H NMR spectrum, there was no coupling between H-3 and H-1′, suggesting that the dihedral angle between H-3 and H-1′ is ca. 90° in its preferred conformation. The relative stereochemistry of compound **3** should be the same as that of 2b in Fig. 3. Therefore, the structure of **3** was tentatively identified as shown in Fig. 2 and named as nonin C. Further analysis is needed to confirm the stereochemistry of nonin B and C.

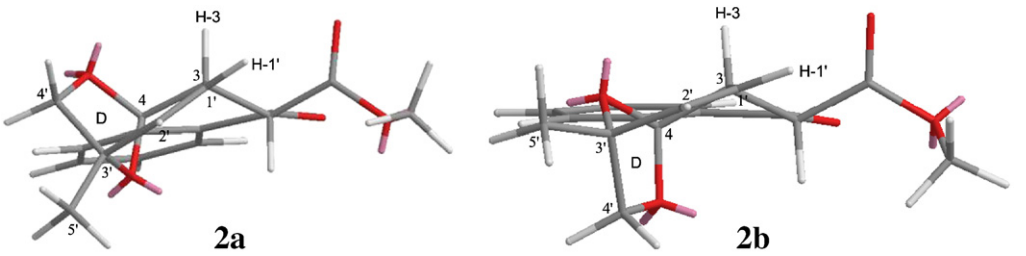

Fig. 3. Two plausible stereochemical configurations of compound **2**.

Table 2
Growth inhibitory effects (IC_{50}, ug/mL) of compounds **2**–**5**, **7**, **10**, **15** and **16** on H1299 human lung cancer cells and HCT116 human colon adenocarcinoma cells (each value represents the mean ± standard deviation, n = 6–8).

	2	3	4	5	7	10	15	16
H1299	24.8 ± 2.1	>50	4.1 ± 1.2	6.1 ± 1.6	4.9 ± 1.2	4.3 ± 1.5	>50	>50
HCT116	32.8 ± 1.9	>50	6.9 ± 1.7	9.8 ± 1.7	5.9 ± 1.5	31.8 ± 2.1	>50	>50

3.2. Growth inhibition against human lung and colon cancer cells

The growth inhibitory activities of compounds **2**–**5**, **7**, **10**, **15**, and **16** were tested by treating human lung cancer cells and human colon cancer cells with them for 48 h. The results indicated that these compounds showed effective inhibition of HCT116 with **7**>**4**>**5**>**2**>**10**>**3**, **15** and **16**, which was similar to the results obtained from H1299 cells (Table 2). Compound **7** exhibited the highest inhibitory activities on H1299 and HT116 cell lines with IC_{50} values of 4.9 ± 1.2 μg/mL and 5.9 ± 1.5 μg/mL, respectively. For the new compounds, compound **2** showed inhibition of H1299 and HCT116 cell lines with IC_{50} values of 24.8 ± 2.1 μg/mL and 32.8 ± 1.9 μg/mL, respectively, while compound **3** had IC_{50} values greater than 50 μg/mL for both cell lines.

4. Discussion

In this research, we systematically studied the chemical components of Tahitian noni roots. A total of 18 compounds were isolated, three of which are new compounds. The structures of the isolated compounds were elucidated by spectral methods. Most of these compounds showed effective inhibitory activity against both human lung and colon cancer cells. Numerous biological activities have been reported for anthraquinons, which are the major constituents of plants of this family. For example, damnacanthal (**10**), the major constituent of noni root, was reported to be a potent and selective inhibitor of p56 (lck) tyrosine kinase in a variety of tissues [25,26]. It is also an inhibitor of ras function [27] and shows an intensive inhibitory effect against topoisomerase II [28]. In addition, it acts as an immunomodulatory agent [29]. Compounds **14**–**16** were reported to have mutagenic activity [1]. Thus, noni roots are a rich source of bioactive anthraquinons and further studies on their bioactivities will be warranted.

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