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Two new cytotoxic isomeric indole alkaloids from the roots of *Nauclea orientalis*

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ABSTRACT

A pair of new isomeric indole alkaloids, naucleaorals A (**1**) and B (**2**) were isolated from the roots of *Nauclea orientalis*. The structures of compounds 1 and 2 were fully characterized using spectroscopic data, and were tested for their cytotoxicity (HeLa and KB cells) and antimalarial activity. Compound 1 showed significant cytotoxicity to HeLa cells with an IC_{50} value of 4.0 μg/mL, while compound 2 exhibited very modest cytotoxicity to both cell lines with IC_{50} values of 7.8 and 9.5 μg/mL, respectively. Both compounds proved to be inactive in antimalarial assays (IC_{50} > 10.00 μg/mL).

1. Introduction

The genus *Nauclea* (Rubiaceae) is comprised of about 35 species, including *Nauclea officinalis*, *Nauclea latifolia* and *Nauclea diderrichhii*, which are used in folk medicine. *Nauclea orientalis* is a large fruit-bearing tree with large glossy leaves often found in the Northern, Northeastern and central parts of Thailand. It has been used as a traditional medicine, with the bark and leaves of this plant being used as a pain reliever for abdominal pains, animal bites and wounds [1]. Previous phytochemical investigations on this plant have resulted in the isolation all characterization of a series of indole alkaloids, [2] with some of these having promising anticancer and antimalarial activities [3,4]. In our continuing search for anticancer agents from Thai medicinal plants,[5,6] the bioassay-guided fractionation of a CH_2Cl_2 extract from roots of *N. orientalis* led to the isolation of two new isomeric indole alkaloids, naucleaorals A (**1**) and B (**2**). Herein we report the isolation and structural elucidation of these new compounds (**1** and **2**) (Fig. 1), and include the results of their cytotoxicity and antimalarial activity assays.

2. Experimental

2.1. General

Melting points were determined on a Fisher–Johns Melting Point Apparatus and are uncorrected. ^1H, ^{13}C and 2D NMR spectra were recorded on a Varian model Mercury$^+$ 400 spectrometer and the chemical shifts are reported in parts per million (ppm), referenced to solvent residues (δ_H 7.25 and δ_C 77.0 ppm for $CDCl_3$-d_1). Adsorbents such as silica gel 60 Merck Nos. 7729 and 7734 were used for column chromatography, and silica gel 60 Merck 7735 was used for

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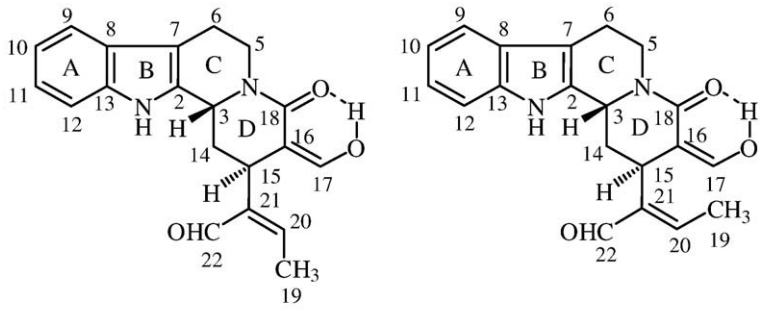

Fig. 1. Structures of alkaloids **1** and **2**.

preparative TLC. Merck silica gel 60 F_{254} plates were used for analytical TLC. ESIMS and HRESIMS were obtained using model VG TRIO 2000 and Micromass LCT mass spectrometers. IR data was obtained in KBr discs using a Nicolet 6700 FT-IR spectrometer. Optical rotations were measured on a Jasco P-1010 polarimeter. UV–visible absorption spectra were carried out using a UV-2552PC UV–Vis spectrometer (Shimadzu, Kyoto, Japan).

2.2. Plant material

The roots of *N. orientalis* were collected from Mahasarakham province of Thailand in April 2008. The plant material was identified by Ms. Suttira Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, where a voucher specimen (Khumkratok no. 89-08) is deposited.

2.3. Extraction and isolation

The air-dried roots of *N. orientalis* (4.5 kg) were successively extracted in a Soxhlet with CH_2Cl_2, EtOAc and MeOH. The solvents were evaporated in vacuo to afford crude CH_2Cl_2 (50.7 g), EtOAc (96.4 g) and MeOH (48.3 g) extracts, respectively. The CH_2Cl_2 extract was subjected to vacuum liquid chromatography (VLC) over silica gel (Merck Art 7730), using successive elutions of hexane, CH_2Cl_2, EtOAc and MeOH with increasing polarity to afford five fractions (A–E). The VLC fraction B was chromatographed on a silica gel column using a stepwise gradient elution of MeOH in CH_2Cl_2 to yield three fractions (B1–B3). Fraction B1 was subjected to preparative TLC using a gradient of EtOAc/hexane (40:60) to furnish naucleaorals A (1, 5.3 mg) and B (2, 10.6 mg), respectively.

Naucleaoral A (**1**) was obtained as an orange amorphous powder; mp 165–167 °C; $[\alpha]_D^{20} = -74°$ (c 0.005, MeOH); UV (MeOH) λ_{max} (log ε): 264 (2.65), 272 (2.69), 278 (2.66) nm;

Table 1
^{1}H and ^{13}C NMR data of **1** and **2** in $CDCl_3$ (400 MHz for ^{1}H, 100 MHz for ^{13}C).

Position	**1**		**2**	
	δ_C	δ_H (mult, *J*)	δ_C	δ_H (mult, *J*)
1-NH	–	7.71 (1H, s)	–	7.95 (1H, s)
2	132.0	–	132.0	–
3	49.1	4.43 (1H, d, *J* = 11.6 Hz)	50.3	4.58 (1H, d, *J* = 10.0 Hz)
5a	39.0	2.79 (1H, m) [a]	39.2	2.91 (1H, m) [a]
5b	–	5.06 (1H, m)	–	5.17 (1H, m)
6a	21.0	2.82 (1H, m)	21.0	2.84 (1H, m)
6b	–	2.78 (1H, m) [a]	–	2.90 (1H, m) [a]
7	110.0	–	110.0	–
8	127.0	–	126.7	–
9	118.3	7.43 (1H, d, *J* = 7.2 Hz)	118.3	7.51 (1H, d, *J* = 7.2 Hz)
10	119.9	7.05 (1H, dd, *J* = 7.2,7.6 Hz)	119.9	7.12 (1H, dd, *J* = 7.2,7.6 Hz)
11	122.3	7.12 (1H, dd, *J* = 7.2,7.6 Hz)	122.3	7.18 (1H, dd, *J* = 7.2,7.6 Hz)
12	111.0	7.24 (1H, d, *J* = 7.6 Hz)	111.0	7.31 (1H, d, *J* = 7.6 Hz)
13	136.0	–	136.2	–
14a	33.1	1.83 (1H, dt, *J* = 3.2,13.6 Hz)	33.6	1.87 (1H, dt, *J* = 3.0,13.2 Hz)
14b	–	2.43 (1H, dt, *J* = 3.2,13.6 Hz)	–	2.34 (1H, dt, *J* = 3.0,13.2 Hz)
15	32.6	3.65 (1H, br s)	30.7	3.83 (1H, br s)
16	101.1	–	101.1	–
17	161.6	6.90 (1H, d, *J* = 10.1 Hz)	160.3	6.97 (1H, d, *J* = 10.4 Hz)
18	169.1	–	169.5	–
19	13.2	2.16 (3H, d, *J* = 7.6 Hz)	15.0	2.03 (3H, d, *J* = 7.2 Hz)
20	147.9	6.50 (1H, q, *J* = 7.6 Hz)	153.2	6.78 (1H, q, *J* = 7.2 Hz)
21	142.0	–	145.6	–
22	190.4	10.23 (1H, s)	195.2	9.39 (1H, s)
-OH	–	13.85 (1H, d, *J* = 10.1 Hz)	–	13.80 (1H, d, *J* = 10.4 Hz)

[a] Data can be interchanged.

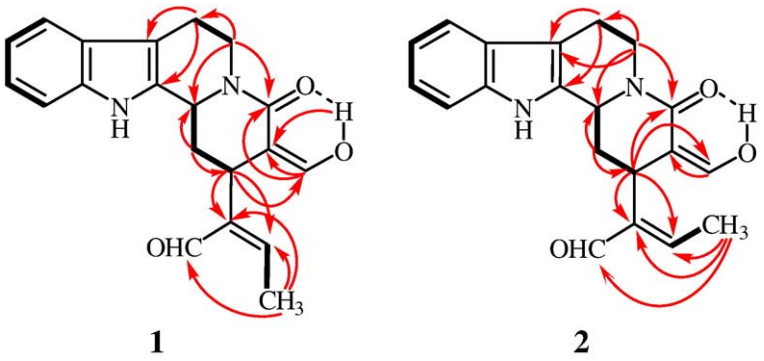

Fig. 2. Selected HMBC (arrow curves) and COSY (bold lines) correlations in **1** and **2**.

IR bands (KBr): 3427, 2925, 2856, 1719, 1659, 1630, 1444, 1381 1264, 1164, 1111, 752, 674 cm^{-1}; HRESIMS m/z: $[M+Na]^+$ 359.1367 (calcd. for $C_{20}H_{20}N_2O_3Na$, 359.1367); CD ($c = 0.30\times10^{-3}$, MeOH) $\delta\varepsilon^{20^\circ}$ (nm): 0(275), −3.57(244), −3.92(238), −0.91(208) −2.12(203); ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectral data see Table 1.

Naucleaoral B (**2**) was obtained as an orange amorphous powder; mp 177–179 °C; $[\alpha]_D^{20} = -81^\circ$ (*c* 0.010, MeOH); UV (MeOH) λ_{max} (log ε): 263 (2.71), 272 (2.69), 278 (2.70) nm; IR bands (KBr): 3413, 2927, 2850, 1707, 1657, 1631, 1442, 1382, 1198, 1116, 752, 672 cm^{-1}; positive ion HRESIMS m/z: $[M+Na]^+$ 359.1377 (calcd. for $C_{20}H_{20}N_2O_3Na$, 359.1367); CD($c = 0.30\times10^{-3}$, MeOH) $\delta\varepsilon^{20^\circ}$(nm):0(265), −7.04(2340, −5.86(229), −0.87(210), −3.14(200); ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectral data see Table 1.

2.4. Cytotoxicity assay

The isolated compounds (**1** and **2**) were subjected to cytotoxic evaluation against KB (human epidermoid carcinoma) and HeLa (human cervical carcinoma) cell lines employing the colorimetric method [7,8]. Adriamycin was used as the reference substance which exhibits activity against KB and HeLa cell lines. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma Chemical Co., USA) was dissolved in saline to make a 5 mg/mL stock solution. Cancer cells (3×10^3 cells) suspended in 100 μg/wells of MEM medium containing 10% fetal calf serum (FCS, Gibco BRL, Life Technologies, NY, USA) were seeded onto a 96-well culture plate (Costar, Corning Incorporated, NY 14831, USA). After 24 h pre-incubation at 37 °C in a humidified atmosphere of 5% CO_2/95% air to allow cellular attachment, various concentrations of test solution (10 μL/well) were added and then these were incubated for 48 h under the above conditions. At the end of the incubation, 10 μL of tetrazolium reagent was added into each well followed by further incubation at 37 °C for 4 h. The supernatant was decanted, and DMSO (100 μL/well) was added to allow formosan solubilization. The optical density (OD) of each well was detected using a Microplate reader at 550 nm and for correction at 595 nm. Each determination represented the average mean of six replicates. The 50% inhibition concentration (IC_{50} value) was determined by curve fitting.

2.5. Antimalarial assay

The antimalarial activity of **1** and **2** was evaluated against the parasite Plasmodium falciparum (K1, multidrug-resistant strain), using the method of Trager and Jensen [9]. Quantitative assessment of activity in vitro was determined by means of the microculture radioisotope technique based upon the method described by Desjardins et al [10]. The inhibitory concentration (IC_{50}) represented the concentration that causes 50% reduction in parasite growth as indicated by the in vitro uptake of [^{3}H]-hypoxanthine by *P. falciparum*. The standard reference compound employed was artemisinin.

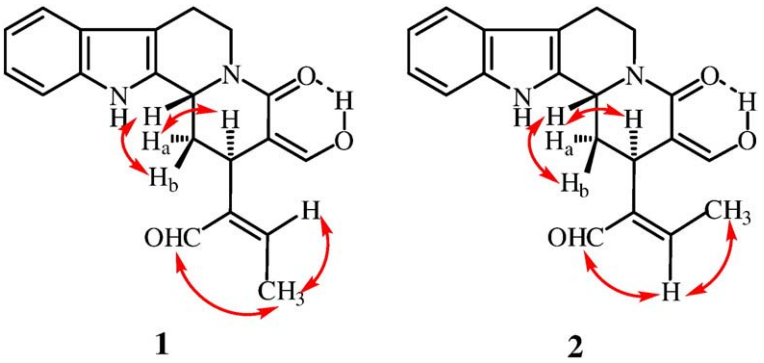

Fig. 3. Relative stereochemistries in alkaloids **1** and **2**.

Table 2
In vitro cytotoxicity of **1** and **2** against HeLa and KB cells.

Compounds	IC_{50} (μg/mL)	
	HeLa cells	KB cells
Naucleaoral A (**1**)	4.0	38.9
Naucleaoral B (**2**)	7.8	9.5
Adriamycin (standard)	0.018	0.018

3. Results and discussion

Naucleaoral A (**1**) was isolated as a orange amorphous solid, with its molecular formula determined as $C_{20}H_{20}N_2O_3$ on the basis of its $[M+Na]^+$ peak in the HRESIMS spectrum at m/z 359.1367.

The ^1H NMR spectrum of 1 displayed aromatic proton signals characteristic of an unsubstituted indole nucleus (ring A) [two doublet of doublets at δ_H 7.05 (1H, dd, $J=7.2$, 7.6 Hz, H-10) and 7.12 (1H, dd, $J=7.2$, 7.6 Hz, H-11), two doublets at δ_H 7.24 (1H, d, $J=7.6$ Hz, H-12) and 7.43 (1H, d, $J=7.2$ Hz, H-9) and the singlet at δ_H 7.71 (1H, s, 1-NH)]. Besides the indole ring signals, compound 1 also showed the presence of a δ-lactam ring (ring D) and hydroxymethylene group at C-16 (δ_C 101.1) and signal attributed to the hydrogen-bond chelated hydroxyl proton at δ_H 13.85 (1H, d, $J=10.1$ Hz), which was elucidated by HMBC correlations of H-17 at δ_H 6.90 (1H, d, $J=10.1$ Hz) to C-16 (δ_C 101.1) and C-18 (δ_C 169.1). The interpretation of the HMBC correlations of H-5b at δ_H 5.06 (1H, m) to C-3 (δ_C 49.1) and C-18 (δ_C 169.1), H-6a at δ_H 2.82 (1H, m) to C-2 (δ_C 132.0) and C-7 (δ_C 110.0) and H-14a at δ_H 1.83 (1H, m) to C-2 (δ_C 132.0) confirmed that ring B was fused to the indole nucleus at C-2 and C-7, and a δ-lactam ring (ring D) at C-3. In addition, the attachment of a –(CHO)C $CHCH_3$ group to C-15 (δ_C 32.6) was inferred from the methyl group signal at δ_H 2.16 (3H, d, $J=7.2$ Hz, C-19 and δ_C 13.2) allocated to C-20 (δ_C 147.9), while a singlet at δ_H 10.23 (1H, s, H-22) was assigned to an aldehyde proton also connected to C-22. These assignments were deduced by HMQC and HMBC spectral correlations (Fig. 2). The NOESY interaction between H-22 (–CHO) and the methyl C-19 revealed the Z configuration of this group. Additionally, the ambiguous relative configurations of the two chiral carbon centers, C-3 and C-15 were also confirmed by a NOESY spectrum (Fig. 3) and CD spectral analysis. The CD spectra of **1** showed a negative Cotton effect at the longest wavelength absorption at 238 nm compared with vincoside [11] proving that proton attached to C-3 was in the β configuration. The complete assignment of ^1H and ^{13}C NMR data for compound **1** is shown in Table 1. On the basis of this spectral evidence, we propose the structure of **1**, the new alkaloid, naucleaoral A, as being that shown in Fig. 1.

Naucleaoral B (**2**) was afforded as an orange amorphous solid, whose molecular formula was established as $C_{20}H_{20}N_2O_3$ by means of the $[M+Na]^+$ peak in the HRESIMS spectrum at m/z 359.1377, identical to that of **1**.

The ^1H and ^{13}C NMR spectra of compound **2** were very similar to those of **1**, except for the down field shift of the methyl protons at δ_H 2.03 (3H, d, $J=7.2$ Hz), the aldehyde proton at δ_H 10.23 (1H, s), and an upfield shift for the methine proton (H-20) of the –(CHO)C $CHCH_3$ group at δ_H 6.78 (1H, q, $J=7.2$ Hz) , connected to C-15 (δ_C 30.7). After complete assignment of the planar structure of **2**, we turned our attention to the stereochemistry of the –(CHO)C $CHCH_3$ group. The NOESY spectrum of 2 (Fig. 3) showed a through-space interaction between H-22 (–CHO) and H-20 supporting an E-type stereochemistry of the double bond between C-20 (δ_C 147.9) and C-21 (δ_C 142.0). Moreover, the absolute configurations of stereocenters C-3 and C-15 were also confirmed by means of the NOESY and CD spectra. The complete assignments of **2** are shown in Table 1, which confirm **2** (naucleoral B) to be a structural isomer of **1**.

Compounds **1** and **2** were tested for cytotoxicity (HeLa and KB cells) (Table 2) and antimalarial activity. Compound **1** showed significant cytotoxicity against HeLa cells with an IC_{50} value of 4.0 μg/mL, while compound **2** exhibited only very modest cytotoxicity against both cell lines with IC_{50} values of 7.8 and 9.5 μg/mL, respectively. On the other hand, both compounds showed little potential as antimalarials due to their inactivity in antimalarial assays ($IC_{50}>10.00$ μg/mL).

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