New Oxoprotoberberine and Aporphine Alkaloids from the Roots of *Amoora cucullata* with Their Antiproliferative Activites

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Abstract: Two new oxoprotoberberine alkaloids, amocurine A and B (**1** and **2**), a new aporphine alkaloid, amocurine C (**3**), along with three known compounds (**4**−**6**) were isolated from the roots of *Amoora cucullata*. Their structures were determined by analysis of spectroscopic data. The isolated compounds were evaluated for their antiproliferative activity against three human cancer cell lines (KB, oral cavity; MCF-7, breast cancer; and NCI-H187, small cell lung cancer). Compounds **3** showed the most potent activities against KB and MCF-7 cell lines with IC_{50} values 3.5 and 4.2 µM, respectively.

Keywords: Oxoprotoberberine; aporphines; antiproliferative activity; *Amoora cucullata*. © 2019 ACG Publications. All rights reserved.

1. Introduction

Amoora cucullata is a mangrove plant belonging to the family Meliaceae and occurs naturally in the coastal areas of Southeast Asia and the Indian Ocean. This plant has been used as a folk medicine for the treatment of marrow and diarrhea [1]. The leaves are traditionally used to treat inflammation, skin diseases, and cardiac diseases [2−3]. As part of our continuing research on Thai medicinal plants [4−8], a phytochemical study on the roots of *A. cucullata* was carried out. Previous investigation on the fruits of *A. cucullata* reported two new rocaglamide derivatives [9]. In continuation of our phytochemical screening of *A. cucullata* roots, we report the isolation and structure elucidation of two new oxoprotoberberine alkaloids, amocurine A and B (**1** and **2**), a new aporphine alkaloid, amocurine C (**3**), together with three known compounds, dehydrodicentrine (**4**) [10], stephanine (**5**) [11], and roemerine (**6**) [11] (Figure 1). The structures of the compounds were determined by spectroscopic data including 1D and 2D NMR and by comparison with those published in the literature [12−14]. Furthermore, all the isolated alkaloids were *in vitro* evaluated for their antiproliferative potential activities.

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Figure 1. Structures of compounds **1–6** isolated from *Amoora cucullata*

2. Materials and Methods

2.1. General Experimental Procedures

Optical rotations were determined on a Rudolph (Hackettstown, NJ, USA) Autopol II automatic polarimeter. UV spectra were measured with a UV-160A spectrophotometer (Shimadzu, Kyoto, Japan), and IR spectra were recorded on a Perkin-Elmer 1750 FTIR spectrophotometer (Perkin-Elmer, Waltham, MA, USA). The ^{1}H and ^{13}C-NMR spectra were recorded in CDCl$_3$ using a 500 MHz Varian Unity INOVA spectrometer. Chemical shifts are recorded in parts per million (δ) in CDCl$_3$. Mass spectra (EI or FAB) were recorded on a Finnigan-MAT 95 XL spectrometer. Thin-layer chromatography (TLC) was performed on silica gel 60 GF$_{254}$ (Merck). Column chromatography was carried out on silica gel 60 GF$_{254}$ (Merck, Darmstadt, Germany). All solvents for extraction and chromatography were routinely distilled prior to use.

2.2. Plant Material

The roots of *Amoora cucullata* were collected from Khanom, Nakhon Si Thammarat, Thailand, in May 2010. A voucher specimen (number WU-0145) was deposited in the herbarium of Walailak University, Thasala, Nakhon Si Thammarat, Thailand.

2.3. Extraction and Isolation

The roots of *A. cucullata* (0.8 kg) were extracted three times by maceration with acetone at room temperature. Removal of the solvent under reduced pressure provided a crude extract (8.2 g). The acetone extract was separated by column chromatography over silica gel and eluted with a gradient of hexane-EtOAc to afford nine fractions (R1-R9). Fraction R2 (860 mg) was purified by CC on silica gel using isocratic eluted with EtOAc-hexane (1:4) to give four subfractions R2A to R2D. Subfraction R2B (120 mg) was purified over Sephadex LH-20 with MeOH to provide compound **4**

(11.4 mg). Fraction R3 (650 mg) was subjected to a silica gel column eluted with EtOAc-hexane (1:9) to yield compound **3** (10.8 mg). Fraction R4 (1.6 g) was purified by CC using EtOAc-hexane (1:2) to provide five subfractions (R4A-R4E). Compounds **5** (9.8 mg) and **6** (10.5 mg) were derived from fraction R4C (380 mg) by CC using EtOAc-hexane (1:4) as eluent. Fraction R6 (1.2 g) was separated by CC on a silica gel with solvent mixtures EtOAc-hexane (1:4) to give five subfractions (R6A-R6E). Subfraction R6B (145 mg) was further purified over Sephadex LH-20 with MeOH to afford compound **1** (10.8 mg). Fraction R7 (1.1 g) was eluted with EtOAc-hexane (1:9) yielding compound **2** (15.4 mg) after purification by CC and preparative TLC.

2.4. Spectroscopic Data

Amocurine A (1): Brownish-red gum; IR ν_{max} (KBr, cm^{-1}): 3420, 1647, 1590, and 1514; UV (MeOH, nm) λ_{max} (log ε): 251 (2.95), 338 (1.90); ^{1}H (500 MHz, CDCl$_3$) and ^{13}C NMR (125 MHz, CDCl$_3$) spectral data, see Table 1 and 2; HRESIMS *m/z* 354.1339 [M+H]$^+$ (calcd. for C$_{20}$H$_{20}$NO$_5$: 354.1341).

Amocurine B (2): Brownish-red gum; [α]$_D^{28}$ −97 (*c* 0.02, MeOH); IR ν_{max} (KBr, cm^{-1}): 3421, 1635, 1590 and 1502; UV (MeOH, nm) λ_{max} (log ε): 251 (2.95), 388 (1.90); ^{1}H (500 MHz, CDCl$_3$) and ^{13}C NMR (125 MHz, CDCl$_3$) spectral data, see Table 1 and 2; HRESIMS *m/z* 356.1494 [M+H]$^+$ (calcd. for C$_{20}$H$_{22}$NO$_5$: 356.1498).

Amocurine C (3): Dark brown gum; IR ν_{max} (KBr, cm^{-1}): 1684, 1592, and 1512; UV (MeOH, nm) λ_{max}: 265 (4.76), 329 (4.21); ^{1}H (500 MHz, CDCl$_3$) and ^{13}C NMR (125 MHz, CDCl$_3$) spectral data, see Table 1 and 2; HRESIMS *m/z* 366.1345 [M+H]$^+$ (calcd. for C$_{21}$H$_{20}$NO$_5$: 366.1341).
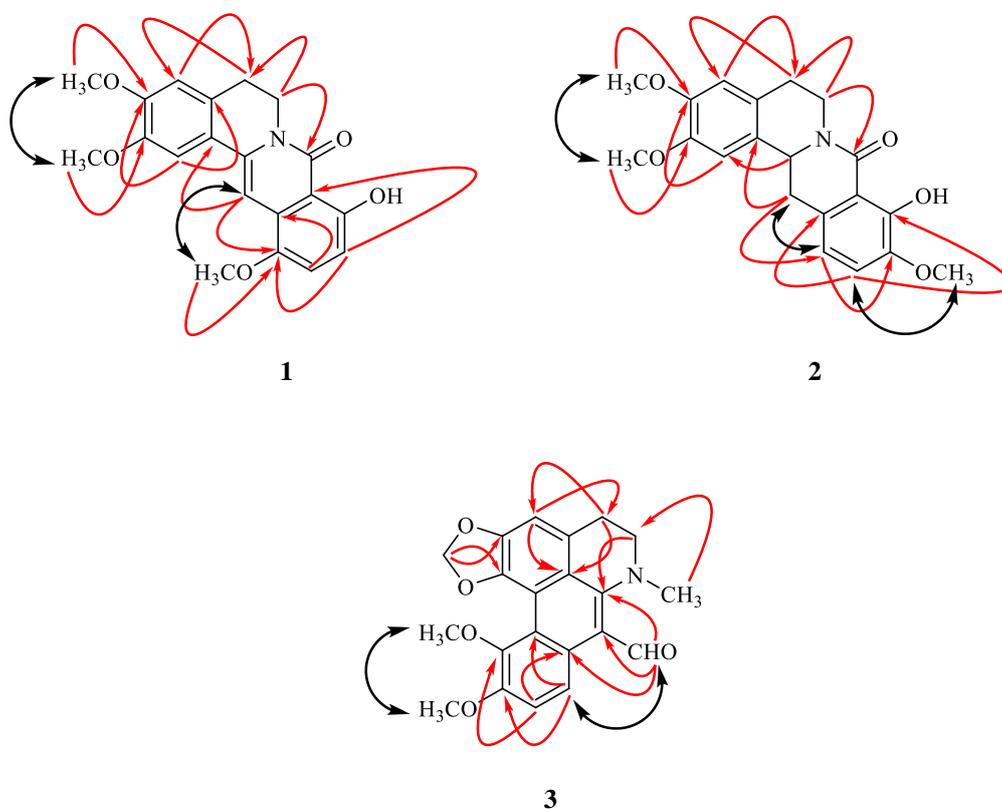
Figure 2. Key HMBC (*arrow curves*) and NOESY (↔) correlations of compounds **1**–**3**

2.5. Antiproliferative Activity Assay

Human breast carcinoma cell line (MCF-7), human lung carcinoma cell line (NCI-H187), and human oral epidermal carcinoma cell line (KB) were obtained from the Bioassay Laboratory, BIOTEC, NSTDA, Pathumthani, Thailand. The MCF-7, NCI-H187, and KB cells were maintained in RPMI 1640 and low glucose DMEM medium, respectively, supplemented with 10% fetal bovine serum and incubated under standard conditions (37 °C, 5% CO_2). The isolated compounds were diluted to the desired concentration (80, 40, 20, 10 and 5 μg/ml) in the medium. The cultures were incubated for 72 h with the test compounds.

MTT assay, as described by Vichai et al. [15], was employed to determine cell viability. After 72 h of incubation, 100 μl of 0.5 mg/ml MTT reagent was added into each well, and incubated for 30 min in a CO_2 incubator. MTT solution was then discarded and 100 μl dimethyl sulfoxide (DMSO) was added into each well to solubilize the cells and dissolve the color substance. For complete solubilization, the plates were vigorously agitated for 5 min at room temperature, and then measured on a microplate reader at a wavelength of 570 nm. MTT solution with only DMSO was used as the blank, while Ellipticine and Doxorubicin were the positive controls. Each assay was performed with three replications. A calibration curve based on optical density was constructed from which growth inhibition was determined. The 50% growth inhibition concentrations (IC_{50}) of the pure compounds were calculated from fitted response curves.

3. Results and Discussion

3.1. Structure Elucidation

Amocurine A (**1**) was isolated as a brownish-red gum with the molecular formula $C_{20}H_{20}NO_5$ as determined by HRESIMS of the $[M+H]^+$ ion at *m/z* 354.1339. The UV spectrum displayed absorption bands at 251 and 338 nm, and the IR spectrum revealed the presence of one hydroxyl group (3420 cm^{-1}), a conjugated amide (1647 cm^{-1}), and an aromatic ring system (1590 and 1514 cm^{-1}), indicating the presence of an oxoprotoberberine-type alkaloid [16]. An important feature for this oxoprotoberberine alkaloid in the ^{1}H-NMR spectrum was a downfield-shifted proton at δ_H 4.27 (t, *J* = 6.0 Hz, H-6) caused by the deshielding effect of the amide and the anisotropic effect of the C-8 carbonyl group. The ^{1}H NMR spectrum of **1** (Table 1) showed resonances for a chelated hydroxyl group (δ_H 13.15, br s), two *ortho*-coupled aromatic protons at δ_H 7.36 (d, *J* = 8.4 Hz, H-10) and 7.02 (d, *J* = 8.4 Hz, H-11), two singlet aromatic protons at δ_H 6.84 (H-1) and 6.64 (H-4), one set of coupled methylene protons at δ_H 4.27 (t, *J* = 6.0 Hz, H-6), and 2.94 (t, *J* = 6.0 Hz, H-5), and three methoxy groups at δ_H 3.96 (s, OCH$_3$-2), 3.88 (s, OCH$_3$-12), and 3.84 (s, OCH$_3$-3).

Compound **1** showed resonances for an amide carbonyl (δ_C 166.3), nine quaternary carbons (δ_C 154.3, 152.7, 148.1, 147.8, 134.5, 127.7, 126.3, 122.8, and 111.6), two methylene carbons (δ_C 39.1 and 29.4) and three methoxy carbons (δ_C 62.3, 60.8, and 56.2) in the ^{13}C-NMR and DEPT spectra (Table 2). The HMBC correlation (Figure 2) between H-6 (δ_H 4.27) and a carbon resonance at δ_C 166.3 suggested that an amide carbonyl was positioned at C-8. The chelated hydroxyl proton at δ_H 13.15 was located at C-9 (δ_C 148.1) due to being hydrogen bonded to the amide carbonyl carbon at C-8 (δ_C 166.3). Two *ortho*-coupled aromatic protons at δ_H 7.36 and 7.02 were assigned to H-10 (δ_C 123.1) and H-11 (δ_C 116.7), respectively, on the basis of the correlations of H-10 with C-8a (δ_C 111.6), C-9 (δ_C 148.1), C-11 (δ_C 116.7), and C-12 (δ_C 154.3) and H-11 with C-10 (δ_C 123.1), C-12 (δ_C 154.3), and C-12a (δ_C 122.8) in the HMBC spectrum. The olefinic proton resonating at δ_H 7.19 was assigned as H-13 based on HMQC and HMBC correlation. Further HMBC correlations were found from H-13 to C-8a (δ_C 111.6), C-12 (δ_C 154.3), C-12a (δ_C 122.8), and C-14 (δ_C 134.5). Two singlet aromatic proton resonances at δ_H 6.84 and 6.64 were assigned to H-1 (δ_C 112.1) and H-4 (δ_C 111.9), respectively, on the basis of the HMBC correlations of H-1 with C-2 (δ_C 147.8), C-3 (δ_C 152.7), and C-4a (δ_C 127.7) and H-4 with C-1a (δ_C 126.3), C-3 (δ_C 152.7), C-4a (δ_C 127.7), and C-5 (δ_C 29.4). Consequently, the structure of **1** was elucidated and it was named amocurine A.

Table 1. ^{1}H-NMR (500 MHz, CDCl$_3$) Spectroscopic data for **1−3**

position	1[a]	2[a]	3[a]
1	6.84 s	6.86 s	
1a			
1b			
2			
3			6.87 s
3a			
4	6.64 s	6.71 s	3.19 t (6.0)
4a			
5	2.94 t (6.0)	3.05 br d (15.7)	3.35 t (6.0)
		2.70 m	
6	4.27 t (6.0)	4.88 br d (13.2)	
		2.86 br d (12.2)	
6a			
7			
7a			
8			6.98 d (9.0)
8a			
9			8.64 d (9.0)
10	7.36 d (8.4)		
11	7.02 d (8.4)	7.27 d (8.1)	
11a			
12		6.99 d (8.1)	
12a			
13	7.19 s	3.14 dd (15.3, 3.1)	
		2.84 dd (15.3, 13.2)	
14		4.77 dd (13.2, 3.1)	
2-OCH$_3$	3.96 s	3.95 s	
3-OCH$_3$	3.84 s	3.84 s	
10-OCH$_3$		3.89 s	3.97 s
11-OCH$_3$			3.95 s
12-OCH$_3$	3.88 s		
9-OH	13.15 br s	13.12 br s	
7-CHO			9.52 s
NCH$_3$			3.11 s
OCH$_2$O			6.17 s

Amocurine B (**2**) was proposed to have the molecular formula C$_{20}$H$_{22}$NO$_5$ based on HRESIMS. The ^{1}H-NMR data (Table 1) revealed the presence of two *ortho*-coupled aromatic protons at δ_H 7.27 (d, J = 8.1 Hz, H-11) and 6.99 (d, J = 8.1 Hz, H-12), two singlet aromatic protons at δ_H 6.86 (H-1) and 6.71 (H-4), and two methylene protons [δ_H 3.05 (br d, J = 15.7 Hz) and 2.70 (m)] and [δ_H 4.88 (br d, J = 13.2 Hz) and 2.86 (br d, J = 12.2 Hz)], which are similar to those of amocurine A (**1**). The main difference found was the additional methine proton [δ_H 4.77 (dd, J = 13.2, 3.1 Hz, H-14)], which was coupled to two diastereotopic methylene protons [δ_H 3.14 (dd, J = 15.3, 3.1 Hz) and 2.84 (dd, J = 15.3, 13.2 Hz)] for compound **2**. This methylene group was situated at C-13 (δ_C 37.1) by the observation of its HMQC and HMBC correlations of H-13 to C-1a (δ_C 127.4), C-8a (δ_C 110.9), C-12 (δ_C 115.1), C-12a (δ_C 122.0), and C-14 (δ_C 55.7) and between the aromatic proton H-12 [δ_H 6.99 (d, J = 8.1 Hz)] and C-13. The methine proton (δ_H 4.77) was located at C-14 (δ_C 55.7) from the correlation between the singlet aromatic proton at δ_H 6.86 (H-1) and C-14 in the HMBC spectrum. In addition, the HMBC correlations from H-11 to C-9 (δ_C 144.8), C-10 (δ_C 150.3), C-12 (δ_C 115.1), and C-12a (δ_C 122.0) and from H-12 to C-8a (δ_C 110.9), C-10 (δ_C 150.3), C-11 (δ_C 119.0), and C-12a (δ_C 122.0). The absolute configuration of H-14 was determined as *S*, based on the negative of its specific rotation when compared to that of the related compound (*S*)-(−)-2,3-dimethoxy-8-oxoberbine, [α]$_D$ −413.8 (*c*

0.36, CHCl$_3$) [17], for which the structure was assigned using X-ray crystallography. Therefore, the structure of **2** was established, and it was named amocurine B.

Table 2. ^{13}C-NMR Spectroscopic data for **1–3** (125 MHz, δ in ppm)

position	1	2	3
1	112.1	110.4	141.5
1a	126.3	127.4	117.3
1b			127.5
2	147.8	148.5	144.0
3	152.7	150.2	106.7
3a			126.2
4	111.9	107.5	30.7
4a	127.7	127.9	
5	29.4	31.5	50.5
6	39.1	37.9	
6a			145.5
7			110.2
7a			129.4
8	166.3	165.5	108.4
8a	111.6	110.9	
9	148.1	144.8	123.3
10	123.1	150.3	150.1
11	116.7	119.0	146.5
11a			118.5
12	154.3	115.1	
12a	122.8	122.0	
13	103.1	37.1	
14	134.5	55.7	
2-OCH$_3$	56.2	56.3	
3-OCH$_3$	60.8	60.6	
10-OCH$_3$			56.1
11-OCH$_3$			60.5
12-OCH$_3$	62.3	62.1	
9-OH			
7-CHO			188.5
NCH$_3$			40.4
OCH$_2$O			100.8

Amocurine C (**3**) was obtained as a dark brown gum. The molecular formula was established as C$_{21}$H$_{20}$NO$_5$ by HRESIMS of the [M+H]$^+$ at *m/z* 366.1345. The UV absorption bands at 265 and 329 nm indicated the presence of a benzenoid moiety and the IR spectrum exhibited a conjugated carbonyl absorption (1684 cm^{-1}) and an aromatic ring system (1592 and 1512 cm^{-1}). The ^{1}H-NMR spectrum (Table 1) showed the characteristic signals of a tetrahydroaporphinoid alkaloid [18] of a singlet *N*-methyl proton (δ_H 3.11), a singlet aromatic proton at δ_H 6.87 (H-3), two *ortho*-coupled aromatic protons at δ_H 8.64 (d, *J* = 9.0 Hz, H-9) and 6.98 (d, *J* = 9.0 Hz, H-8), one coupled methylene protons at δ_H 3.35 (t, *J* = 6.0 Hz, H-5) and 3.19 (t, *J* = 6.0 Hz, H-4), and an aldehyde proton at δ_H 9.52 (s, H-7). In addition, the spectrum displayed resonances due to two methoxy singlets at δ_H 3.97 (OCH$_3$-10) and 3.95 (OCH$_3$-11) and a methylenedioxy proton (δ_H 6.17, s). The ^{13}C-NMR and DEPT spectra revealed the presence of 21 signals, including signals for a carbonyl carbon (δ_C 188.5), a methylenedioxy carbon (δ_C 100.8), two methylene carbons (δ_C 50.5 and 30.7), three methine carbons (δ_C 123.3, 108.4, and 106.7), eleven quaternary carbons (δ_C 150.1, 146.5, 145.5, 144.0, 141.5, 129.4, 127.5, 126.2, 118.5, 117.3, and 110.2), two methoxy carbons (δ_C 60.5 and 56.1), and *N*-methyl carbon (δ_C 40.4). The

spectroscopic data for **3** were similar to those of epiganine B [10], except that compound **3** showed two *ortho*-coupled aromatic protons (δ_H 8.64 and 6.98). These aromatic protons were located at C-9 (δ_C 123.3) and C-8 (δ_C 108.4), respectively. The assignments of H-9 and H-8 were supported by the HMBC correlations from H-9 to C-7a (δ_C 129.4), C-8 (δ_C 108.4), C-10 (δ_C 150.1), and C-11 (δ_C 146.5) and from H-8 to C-7 (δ_C 110.2), C-7a (δ_C 129.4), C-9 (δ_C 123.3), C-10 (δ_C 150.1), and C-11a (δ_C 118.5). Further HMBC correlations (Figure 2) from the methylenedioxy unit to C-1 (δ_C 141.5) and C-2 (δ_C 144.0) and from the aldehyde proton (δ_H 9.52) to C-6a (δ_C 145.5), C-7 (δ_C 110.2), and C-7a (δ_C 129.4), indicated that the methylenedioxy was positioned between C-1 and C-2 and the aldehyde group at C-7, respectively. Accordingly, the structure of **3** was elucidated, and it was named amocurine C.

3.2. Antiproliferative Activity

All the compounds were evaluated for their *in vitro* antiproliferative activities against three human cancer cells, namely KB, MCF-7, and NCI-H187, using the MTT method (Table 3). In the antiproliferative activity assay, the aporphine alkaloid **3** showed significant antiproliferative activities against KB, MCF-7, and NCI-H187 cell lines with IC_{50} values 3.5, 4.2 and 6.7 µM, respectively. Compound **4** exhibited antiproliferative activities against KB, MCF-7, and NCI-H187 cells (IC_{50} values 9.3, 10.1 and 8.5 µM, respectively), whereas compounds **1, 2, 5** and **6** displayed selective moderate antiproliferative activity against NCI-H187 cell line with IC_{50} values 20.5, 40.2, 30.4 and 25.2 µM, respectively. These results implied that the antiproliferative activity observed were related to aporphine compounds with $C_{6a}=C_7$ and methylenedioxy functions, which corresponds to the result previously described by Likhitwitayawuid et al. [19] that aporphine alkaloids containing a 1,2-methylenedioxy group are potent against cancer cell lines. Compounds **3** and **4** demonstrated stronger activity against cancer cell lines than compounds **5** and **6.** These findings suggest that the aromatic ring system, $C_{6a}=C_7$, and 1,2-methylenedioxy ring as well as the planarity of structure have powerful effects on the antiproliferative activity.

Table 3. Antiproliferative activities of compounds **3**−**6** (IC_{50}, µM)[a]

Compounds	KB	MCF-7	NCI-H187
1	>50	>50	20.5 ± 0.2
2	>50	>50	40.2 ± 0.5
3	3.5 ± 0.6	4.2 ± 1.4	6.7 ± 0.1
4	9.3 ± 0.8	10.1 ± 0.2	8.5 ± 0.5
5	>50	>50	30.4 ± 1.1
6	>50	>50	25.2 ± 0.8
Doxorubicin[b]	0.5 ± 0.1	6.8 ± 0.1	0.7 ± 0.2
Ellipticine[b]	2.1 ± 0.1		1.7 ± 0.1

[a]Values are mean ± standard deviation; mean of three assays
[b]Positive control for antiproliferative activity assay

Acknowledgments

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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