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Abstract

One new p-quinonoid aporphine alkaloid, obtusipetadione (1), and eleven known compounds (2-12) were isolated from the acetone extract of the twigs of Dasymaschalon obtusipetalum. Their structures were elucidated by spectroscopic methods. The cytotoxic and antimalarial activities of the isolated compounds were evaluated. Compound 1 showed significant in vitro antiplasmodial activity against the P. falciparum strains TM4 and K1 (multidrug resistant strain) with IC50 values of 2.46 0.12 and 1.38 0.99 μg/mL, respectively with no cytotoxicity. Compound 9 had more modest antiplasmodial activity, but significant cytotoxicity.

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Cytotoxic and Antimalarial Alkaloids from the Twigs of *Dasymaschalon obtusipetalum*

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One new *p*-quinonoid aporphine alkaloid, obtusipetadione (**1**), and eleven known compounds (**2-12**) were isolated from the acetone extract of the twigs of *Dasymaschalon obtusipetalum*. Their structures were elucidated by spectroscopic methods. The cytotoxic and antimalarial activities of the isolated compounds were evaluated. Compound **1** showed significant *in vitro* antiplasmodial activity against the *P. falciparum* strains TM4 and K1 (multidrug resistant strain) with IC_{50} values of 2.46±0.12 and 1.38±0.99 μg/mL, respectively with no cytotoxicity. Compound **9** had more modest antiplasmodial activity, but significant cytotoxicity.

Keywords: *Dasymaschalon obtusipetalum*, *p*-Quinonoid aporphine alkaloid, Cytotoxic activity, Antimalarial activity.

Dasymaschalon is a small genus in the Annonaceae family. This genus comprises about 40 species distributed in Africa and Southeast Asia, particularly in Thailand and the Malaysian Peninsular. Twelve species are found in Thailand [1-2]. These plants have been reported to contain various types of secondary metabolites, including, alkaloids [3a-b,4], acetogenin [5], xanthones [6] and flavonol glycosides [7]. Previous research on the phytochemical constituents of *D. blumei* [3a], *D. sootepense* [3b], *D. dasymaschalum* [1] and *D. trichophorum* [8] indicated that some of these were cytotoxic to cancer cell lines, including mouse lymphoid neoplasma (P-388), human epidermoid carcinoma of the mouth (KB), human colon cancer (Col-2), human lung cancer (Lu-1), rat glioma (ASK) and human breast cancer (MCF-7), without affecting the viability of the noncancerous human embryonic kidney cell line (Hek 293). There is no previous study on the phytochemistry of *D. obtusipetalum*.

This paper reports on the examination of the acetone extract of the twigs of *D. obtusipetalum*. One new *p*-quinonoid aporphine alkaloid (**1**) was isolated, along with eleven known compounds (**2-12**): sinactine (**2**) [9], epicatechin (**3**) [10], 3'-*O*-methyl-(-)-epicatechin (**4**) [10], goniopedaline (**5**) [11], aristolactam BII (**6**) [11], piperolactam A (**7**) [12], aristolactam AII (**8**) [11], 10-amino-3,6-dihydroxy-2,4-dimethoxyphenanthrene-1-carboxylic acid lactam (**9**) [13], 3,5-dihydroxy-2,4-dimethoxyaristolactam (**10**) [3a], piperolactam C (**11**) [12] and crotepoxide (**12**) [14] (Figure 1). The structures of the compounds were elucidated using spectroscopic methods, especially 1D and 2D NMR spectroscopy, and confirmed by comparison of their ^1H and/or ^{13}C NMR spectroscopic data with those reported in the literature. The cytotoxic and antimalarial activities of the isolated compounds were also evaluated.

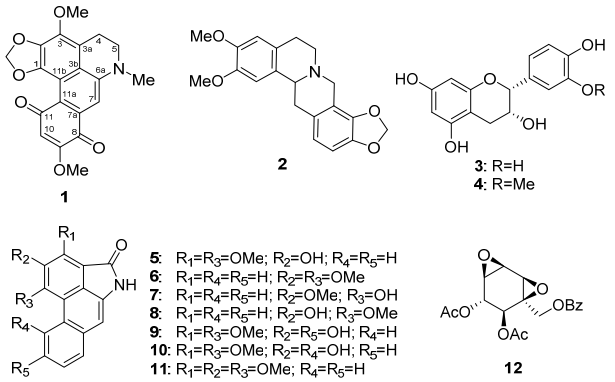

Figure 1: Structures of the isolated compounds **1-12** from *D. obtusipetalum* twigs.

Obtusipetadione (**1**) was obtained as a blue solid with mp 255-256 °C. It displayed a pseudomolecular ion peak $[M+H]^+$ at *m/z* 368.1124 (calcd. for $C_{20}H_{18}NO_6$, 368.1134) in the ESI-TOF-MS, corresponding to the molecular formula of $C_{20}H_{17}NO_6$. The UV spectrum displayed maximal absorption bands at λ_{max} 224, 276, 322 and 590 nm, suggesting the presence of a *p*-quinonoid type, aporphine nucleus [15]. The IR spectrum revealed the absorption bands of a conjugated carbonyl (1671 cm^{-1}) group and an aromatic (1626 and 1524 cm^{-1}) ring system. The ^1H NMR spectrum (Table 1) displayed signals for one singlet aromatic proton (δ 6.98, s, 1H), and one singlet alkene proton (δ 6.02, s, 1H), and resonances for two methylenedioxy protons (δ 6.14, s, 2H), two methoxy groups (δ 4.07 and 3.85, each s, 3H), one *N*-methyl group (δ 3.21, s, 3H) and two sets of methylene protons [δ 3.45 and δ 3.12, each t, J = 6.4 Hz, 2H]. Compound **1** showed resonances for twelve quaternary

Table 1: NMR spectroscopic data of obtusipetadione (**1**) ($CDCl_3$ at 400 MHz).

Position	obtusipetadione (**1**)			
	δ_H (mult., *J* in Hz)	δ_C		HMBC ($^1H \rightarrow ^{13}C$)
1	-	141.8		-
2	-	138.6		-
3	-	140.4		-
3a	-	116.8		-
3b	-	120.2		-
4	3.12 (t, 6.4)	22.5	CH_2	C-3, C-3a, C-3b, C-5
5	3.45 (t, 6.4)	49.5	CH_2	C-3b, C-4, *N*-Me, C-6a
Me-6	3.21 (s)	40.2	CH_3	C-5, C-6a, C-7
6a	-	149.7		-
7	6.98 (s)	98.4	CH	C-3b, C-7a, C-8, C-11, C-11a, C-11b
7a	-	132.3		-
8	-	181.9		-
9	-	157.2		-
10	6.02 (s)	111.0	CH	C-8, C-9, C-11, C-11a, C-11b
11	-	184.0		-
11a	-	116.7		-
11b	-	113.1		-
OMe-3	4.07 (s)	59.9	CH_3	C-3
OMe-9	3.85 (s)	55.7	CH_3	C-9
OCH_2O	6.14 (s)	101.0	CH_2	C-1, C-2, C-3

(δ 184.0, 181.9, 157.2, 149.7, 141.8, 140.4, 138.6, 132.3, 120.2, 116.8, 116.7 and 113.1), two methine (δ 111.0 and 98.4), three methylene (δ 101.0, 49.5 and 22.5), two methoxy (δ 59.9 and 55.7), and one *N*-methyl (δ 40.2) carbons in the ^{13}C NMR and DEPT 135 spectra (Table 1). In the ^1H-^1H COSY spectrum, the methylene protons, H-5 (δ 3.45) coupled with the other methylene protons, H-4 (δ 3.12). These latter correlated with the ^{13}C NMR signals for C-3 (δ 140.4), C-3a (δ 116.8), C-3b (δ 120.2) and C-5 (δ 49.5) in the HMBC spectrum.

The methoxy group resonating at δ 4.07 was located at C-3 on the basis of the HMBC cross peak between this signal and that for C-3 (δ 140.4). In the HMBC spectrum the ^1H NMR signal of the *N*-Me group (δ 3.21) correlated with the ^{13}C NMR signals of C-5 (δ 49.5), C-6a (δ 149.7) and C-7 (δ 98.4). The aromatic proton signal at δ 6.98 was assigned to H-7 due to its HMQC cross peak with C-7 (δ 98.4), and HMBC correlations with C-3b (δ 120.2), C-7a (δ 132.3), C-8 (δ 181.9) and C-11a (δ 116.7). The downfield singlet resonance at δ 6.02 was assigned to H-10 according to its HMQC cross peak with C-10 (δ 111.0), as well as the HMBC correlations with C-8 (δ 181.9), C-9 (δ 157.2), C-11 (δ 184.0), C-11a (δ 116.7) and C-11b (δ 113.1). In addition, the remaining methoxy group resonance, H_3-9 (δ 3.85), showed a HMBC cross peak with C-9 (δ 157.2). These data together with the ^{13}C NMR chemical shifts of C-8 and C-11 indicated a *p*-benzoquinone moiety with a methoxy group at C-9. The methylenedioxy moiety was linked at C-1 (δ 141.8) and C-2 (δ 138.6) of the core structure due to its HMBC correlations between these protons and C-1 and C-2. Consequently, obtusipetadione was assigned the structure shown in Figure 1.The NMR spectroscopic data of fissilandione [15] and that of **1** are very similar, except for the additional methoxy group at C-3 in **1**.

All isolated compounds, except for **11**, were evaluated for their cytotoxicity against the KB (oral cavity cancer) human and Vero cell lines (African green monkey kidney, normal cells) and antimalarial activities against the *Plasmodium falciparum* strains, TM4 and K1 (multidrug resistant strain) (Table 2). Compound **1** exhibited significant antimalarial activities with IC_{50} values of 2.46±0.12 and 1.39±0.99 μg/mL against the *P. falciparum* TM4 and K1 strains, respectively with no cytotoxicity to mammalian cells, Vero and KB cells. In addition, compounds **2**, **5** and **9** showed moderate activities, with IC_{50} values in the range of 18.2±8.93-33.2±7.41 and 18.9±2.03-27.9±7.38 μg/mL against the TM4 and K1 strains, respectively. Compound **9** displayed cytotoxic activity against the KB cell line with an IC_{50} value of 4.61±0.02 μg/mL, and was toxic against the Vero cell line with an IC_{50} value of 1.83±0.57 μg/mL. Compounds **3-4**, **6-8**, **10** and **12** were inactive.

Table 2: Cytotoxic and antimalarial activities of the isolated compounds (IC_{50}, μg/mL).

Compounds	Cytotoxic activity		Antimalarial activity against *P. falciparum*	
	KB	Vero cells	TM4	K1
1	-[a]	-[a]	2.46±0.12	1.38±0.99
2	-[a]	-[a]	18.2±8.93	27.2±8.09
3	-[a]	-[a]	-[a]	-[a]
4	-[a]	-[a]	-[a]	-[a]
5	-[a]	44.0±6.70	33.2±7.41	27.9±7.38
6	-[a]	-[a]	-[a]	-[a]
7	-[a]	-[a]	-[a]	-[a]
8	-[a]	-[a]	-[a]	-[a]
9	4.61±0.02	1.83±0.57	33.2±7.41	18.9±2.03
10	-[a]	-[a]	-[a]	-[a]
11	Nt	Nt	Nt	Nt
12	-[a]	-[a]	-[a]	-[a]
Doxorubicin[b]	0.56			
Ellipticine[b]		0.093		
Chloroquine[c]			0.0096	0.089±0.012
Cycloguanil[c]			0.0095±0.003	0.81±0.19
Pyrimethamine[c]			0.019±0.003	7.7±2.1

[a]Inactive at 50 μg/mL. [b]Positive control for cytotoxic assay. [c]Reference drugs for antiplasmodial activity. Nt: Not tested.

Experimental

General: The specific rotations were measured in MeOH at the sodium D-line on a Bellingham & Stanley ADP220 polarimeter. UV-vis absorption spectra were recorded in MeOH with a Perkin-Elmer UV-vis spectrophotometer. The infrared (IR) spectra were determined of neat samples using a Perkin-Elmer FTS FT-IR spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a 400 MHz Bruker FT NMR Ultra Shield spectrometer. Chemical shifts are expressed in δ (ppm) referring to the tetramethylsilane (TMS) peak at δ 0.00 ppm. Mass spectrometric data were obtained on a Micro TOF, Bruker Daltonics mass spectrometer. Melting points were measured on a SANYO Gallenkamp melting point apparatus. Thin-layer chromatography (TLC) was performed on silica gel 60 GF_{254} (Merck). Column chromatography (CC) was performed on Sephadex LH-20, and silica gel (Merck) type 100 (63-200 μm) and type (5-40 μm for Quick column chromatography; QCC). All solvents for extraction and chromatography were routinely distilled prior to use.

Plant material: The twigs of *D. obtusipetalum* Botanical authority were collected at Doi Tung, Chiang Rai Province, Thailand in March 2013. The plant was identified by Mr James Maxwell from the CMU herbarium, Chiang Mai University, where a voucher specimen has been deposited (specimen no. Maxwell 07-351).

Extraction and isolation: The air dried twigs of *D. obtusipetalum* (7.1 kg) were extracted with acetone (15L) over a period of 3 days at room temperature and 3 times. Removal of the solvent under reduced pressure provided the acetone extract (120.8 g) as a dark brown gum. This was separated by QCC over silica gel and eluted with a gradient of hexanes-ethyl acetate-methanol to give 5 fractions (A–E). Fraction B (2.04 g) was further separated by Sephadex LH-20 CC with 100% MeOH to afford 4 sub-fractions (B1-B4). Sub-fraction B2 (69.9 mg) was fractionated by CC over silica gel with acetone/hexanes (3:7) to afford 3 sub-fractions (B2A-B2C). Compound **12** (10.9 mg) was contained in the second sub-fraction (32.5 mg) as colorless crystals after washing with MeOH [14]. Fraction D (4.0 g) was separated by Sephadex LH-20 CC with 100% MeOH to afford 8 sub-fractions (D1-D8). Sub-fraction D2 (285.8 g) was washed with MeOH to give compound **2** (17.7 mg) as a light orange solid [16]. Sub-fraction D4 (102.0 mg) was further purified by CC over silica gel with a gradient of $MeOH/CH_2Cl_2$ (1:99-20:80) giving 5 sub-fractions (D4A-D4E). Compound **4** (1.6 g) was obtained as a yellow solid, mp 198-200°C (lit. mp not reported) and compound **3** (34.6 mg) as a white solid [17] from sub-fractions D4B and D4D, respectively. Purification of sub-fraction

D5 (1.1 g) by Sephadex LH-20 CC with 100% MeOH gave 3 sub-fractions (D5A-D5C). Compounds **11** (2.1 mg), as a yellow viscous oil and **1** (6.0 mg), as a blue solid, mp 255-256 °C, were obtained from sub-fraction D5B (352.3 mg) after purification with CC over silica gel using acetone/hexanes (2:3) as a mobile phase. Sub-fraction D6 (881.5 mg) was separated by silica gel CC with acetone/hexanes (3:7) to afford 3 sub-fractions (D6A-D6C). Sub-fraction D6B (73.0 mg) was further isolated by CC over silica gel with MeOH/CH_2Cl_2 (2:98) to provide 3 fractions (D6B1-D6B3). The second sub-fraction (16.6 mg) was fractionated by CC over silica gel with EtOAc/hexanes (2:3) to provide compounds **6** (3.5 mg) as yellow needles [18], and **5** (2.5 mg) as yellow needles [19]. Sub-fraction D7 (156.9 mg) was separated by CC over silica gel with a gradient of MeOH/CH_2Cl_2 (2:98-5:95) to afford 5 sub-fractions (D7A-D7E). Purification of sub-fraction D7B (23.1 mg) by CC over silica gel with EtOAc/hexanes (2:3) provided compounds **10** (4.0 mg) as brownish yellow needles [3a], and **8** (6.3 mg) as yellow needles [19]. Compound **7** (9.0 mg), obtained as light green needles, mp 280-282°C (lit. 303-306°C, decomposed [12]), was isolated from sub-fraction D7D (19.1 mg) after purification by CC over silica gel with MeOH/CH_2Cl_2 (2:98). Sub-fraction D7E (37.0 mg) was further purified by silica gel CC using acetone/hexanes (3:7) to give compound **9** (8.1 mg) as yellow needles, mp 195-196°C (lit. mp not reported).

Obtusipetadione (1)
Blue solid
MP: 255-256ºC.
IR (neat): 1671, 1626, 1524, 1458, 1312, 1234, 1122, 1056 cm^{-1}.
UV/Vis λ_{max} (MeOH) nm (log ε): 590 (3.02), 322 (3.84), 276 (3.83), 224 (4.01).
^1H NMR and ^{13}C NMR: Table 1
HRMS-ESI-TOF: *m/z* $[M + H]^+$ calcd for $C_{20}H_{18}NO_6$, 368.1134; found: 368.1124.

***Bioactivity assays*:** For the cytotoxicity assays, KB cells (human oral cavity cancer cells) and normal Vero cells from African green monkey kidney [20] were evaluated using sulforhodamine B (SRB) assay [21]. Ellipticine and doxorubicine were used as the standard compounds. The *in vitro* antimalaria activity against *Plasmodium falciparum* (TM4 and K1, multidrug resistant strains) was carried out using as described by Wangchuk *et al*. [21].

Supplementary data: Spectral data are available.

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