COPYRINE ALKALOIDS FROM THE STEMS OF *Orophea kerrii*

Abdul-Wahab Salae,* Arunyanee Kuichan, Uma Prawat, and Pittaya Tuntiwachwuttikul
Laboratory of Natural Products Chemistry, Faculty of Science and Technology, Phuket Rajabhat University, Muang, Phuket 83000, Thailand
*e-mail: wahab_a@pkru.ac.th

Abstract: A new copyrine alkaloid, 8,9-dimethoxysampangine (**1**), together with three known copyrine alkaloids, eupomatidine-1 (**2**), eupolauridine (**3**), and 8-methoxyeupolauridine (**4**) were isolated from the stems of *Orophea kerrii*. Their structures were determined by spectroscopic methods.

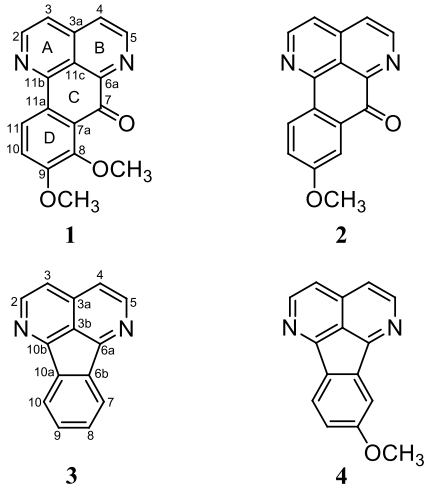

Figure 1. Copyrine alkaloid isolated from *Orophea kerrii*.

Introduction: *Orophea kerrii* (Anonaceae family), locally named "prik nok morcar", is distributed in the southern part of Thailand. *Orophea* plants have been used widely as traditional medicine, for example, the root of *O. setosa* was used to cure coughs or remove fever.[1] Phytochemical studies on several *Orophea* species have been reported, resulting in the isolation of alkaloids, flavonoids, polyacetylenes, lignans, and tocopherol derivatives.[2-8] However, the chemical constituents of *O. kerrii* and their biological activities have not been reported. In the present study, the isolation, purification, and structural elucidation of alkaloids from *O. kerrii* are reported.

Methodology:

General Experimental Procedures

Melting points were determined on a Buchi melting point B-540 apparatus. Ultraviolet (UV) absorption spectra were measured with a SPECORD® 210 PLUS analytik Jena spectrophotometer. Infrared spectra (IR) were recorded with a Shimadzu FTIR-8900 IR spectrophotometer. The 1D and 2D NMR spectra (^{1}H, ^{13}C, COSY, HSQC, and HMBC) were measured in $CDCl_3$ solution on a Bruker AVANCE 400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C NMR spectra). Chemical shift values are reported in δ (ppm), relative to internal TMS or residual solvent peak; coupling constants (*J*) are in hertz (Hz). Vacuum liquid chromatography (VLC) was performed on silica gel 60H (Merck, 5-40 μm) and RP-18 (Merck, 15-25 μm).

Plant material

The stems of *O. kerrii* were collected from Phangnga Province, Thailand, in January 2016. The plant was identified by Dr. Piya Chalermglin, Thailand Institute of Scientific and

Technological Research, Thailand. A voucher specimen (PKRU2 0 1 60 02) was deposited at the Laboratory of Natural Products Chemistry, Faculty of Science and Technology, Phuket Rajabhat University, Phuket, Thailand.

Extraction and Isolation

The stems of *O. kerrii* (3.4 kg) were extracted with EtOH (15 L) in percolators four times. The EtOH extract was filtered and evaporated to give a dark brown residue (115.4 g), which was partitioned between H_2O and EtOAc.

The EtOAc extract (44.2 g) was separated by VLC over silica gel using a gradient of EtOAc–*n*-hexane (0–100%, stepwise) to give thirteen fractions (OK1–OK13). Compound **3** (32.3 mg) was recrystallized from fraction OK6 to give yellow solid. Fraction OK7 (2.4 g) was subjected to VLC over silica gel using a gradient of acetone–*n*-hexane to afford six subfractions (OK7-1–OK7-6). Subfraction OK7-6 (467.2 mg) was further purified by VLC over silica gel to give compound **4**, which was recrystallized from CH_2Cl_2–hexane to obtain yellow solid (21.5 mg). Fraction OK10 (3.2 g) was recrystallized from EtOAc–hexane to obtain compound **2** as yellow needles (18.0 mg). Fraction OK12 (5.2 g) was separated by VLC over reversed-phase silica gel using 50% H_2O–MeOH to give eight subfractions (OK12-1–OK12-8). Subfraction OK12-6 (209.8 mg) was subjected to VLC over silica gel to afford three subfractions (OK12-6-1–OK12-6-3). Compound **1** (3.0 mg) was obtained from subfraction OK12-6-3 after purification by VLC over reversed-phase silica gel using 50% H_2O–MeOH.

8,9-Dimethoxysampangine **(1)**: yellow solid; m.p. 195-197 ^{o}C; UV (MeOH) λ_{max} nm (log ε) 274 (3.43) nm; IR ν_{max} 2781, 1678, 1616, 1570, 1450, 1408, 1385, 1362, 1312, 1258, 1238, 1076, 1018, 987 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1.

Eupomatidine-1 **(2)**: yellow needles; m.p. 198-200 ^{o}C (lit. m.p. 195-197 ^{o}C)[9]; UV (MeOH) λ_{max} nm (log ε) 274 (3.43) nm; IR ν_{max} 2326, 1655, 1547, 1481, 1427, 1400, 1373, 1327, 1269, 1234, 1211, 1165, 1076, 1026, 980 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1.

Eupolauridine **(3)**: yellow solid; m.p. 150-152 ^{o}C (lit. m.p. 150-152 ^{o}C)[10]; UV (MeOH) λ_{max} nm (log ε) 274 (3.43) nm; IR ν_{max} 2785, 1624, 1600, 1515, 1438, 1416, 1366, 1296, 1242, 1060, 995, 948 cm^{-1}; ^{1}H and ^{13}C NMR see Table 2.

8-Methoxyeupolauridine **(4)**: yellow solid; m.p. 137-138 ^{o}C (lit. m.p. 137-138 ^{o}C)[11]; UV (MeOH) λ_{max} nm (log ε) 274 (3.43) nm; IR ν_{max} 2815, 1632, 1609, 1512, 1470, 1420, 1358, 1292, 1246, 1219, 1184, 1103, 1065, 1034, 995 cm^{-1}; ^{1}H and ^{13}C NMR see Table 2.

Results and Discussion: The stems of *O. kerrii* were extracted with EtOH and the extract was partitioned between EtOAc and H_2O. Chromatographic separation of the EtOAc fraction on silica gel led to the isolation of compounds **1-4** (**Figure 1**).

Compound **1** was isolated as yellow solid, m.p. 113-114 ^{o}C. The IR spectrum of **1** showed absorptions for conjugated carbonyl group (1678 cm^{-1}), and benzene rings (1616, 1570, and 1450 cm^{-1}). The UV spectrum showed absorption bands at 228, 233, 350 nm indicated the presence of an extended aromatic copyrine alkaloid.[12-13] The ^{1}H and ^{13}C NMR spectra of **1** (Table 1) together with DEPT, HSQC, ^{1}H–^{1}H COSY and HMBC experiments were very similar to those of sampangine, which was previously isolated from the stem bark of *Cananga odorata*.[14] The ^{13}C NMR spectrum showed seventeen carbons, including a carbonyl carbon (δ_C 180.9), eight quaternary carbons, six methine and two methyl. The ^{1}H NMR spectrum of **1** showed signals for second pair of α and β pyridine protons at δ_H 8.80 and 7.63 (each 1H, d, J = 5.6 Hz) and 9.08 and 7.85 (each 1H, d, J = 5.2 Hz). Their multiplicities and coupling constants are distinguishing the pyridine ring protons from other aromatic protons.[14] Signals for two *ortho*-coupled aromatic protons at δ_H 7.34 (d, J = 8.8 Hz, H-10) and 8.68 (d, J = 8.4 Hz, H-11). In the HMBC experiments showed correlations from two doublets at δ_H 7.85 and 9.08 were assigned to H-4 and H-5 (ring B),

respectively, which showed ^{3}J correlation between H-4 (δ_H 7.85) to C-3 (δ_C 118.1) and C-11c (δ_C 118.7) in HMBC spectrum. ^{3}J HMBC correlations were observed from the aromatic protons at δ_H 7.34 (H-10) to C-8 (δ_C 151.5) and C-11a (δ_C 128.0), and δ_H 8.68 (H-11) to C-7a (δ_C 126.3), C-9 (δ_C 156.8), and C-11b (δ_C 151.6), indicating the tetrasubstituted aromatic ring. The signal resonating at δ_C 151.5 (C-8) and 156.8 (C-9) indicated the presence of methoxyl groups at δ_H 4.04 (s) and 4.01 (s), respectively. Compound **1** was thus identified as 8,9-dimethoxysampangine.

Compound **2** was obtained as yellow needles, m.p. 198-200 ^{o}C (lit. m.p. 195-197 ^{o}C),[9] and identified as eupomatidine-1 by comparing its spectral data with the literature data for **2**. This compound was previously isolated from *Eupomatia bennettii*.[9] The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**, except for the absence of the methoxyl group at δ_H 4.04 and δ_C 61.5 in **1**. The ^{1}H NMR spectrum of the D ring of **2** (Table 1) showed signals for trisubstituted aromatic ring [(δ_H 7.85 (d, J = 2.8 Hz, H-8), 7.31 (dd, J = 8.8, 2.8 Hz, H-10), 8.66 (d, J = 8.8 Hz, H-11)] instead of signals for the tetrasubstituted aromatic ring in **1**. This is the first report of the isolation of **2** from the genus *Orophea* as well as its complete ^{1}H and ^{13}C NMR (Tables 1) assignments on the basis of the COSY and HMBC spectra (**Figure 2**).

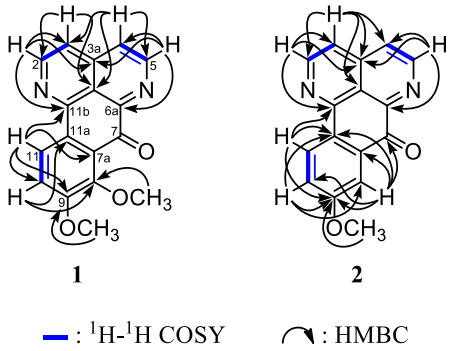

Figure 2. ^{1}H-^{1}H COSY and HMBC correlations for compounds **1**-**2**.

Table 1. ^{1}H and ^{13}C NMR data of compounds **1**-**2** in $CDCl_3$

	1		**2**	
Position	δ_H	δ_C	δ_H	δ_C
2	8.80 (d, 5.6)	147.0	8.78 (d, 5.6)	147.4
3	7.63 (d, 5.6)	118.1	7.60 (d, 5.6)	118.2
3a		138.8		138.7
4	7.85 (d, 5.2)	122.8	7.87 (d, 5.6)	123.6
5	9.08 (d, 5.2)	148.7	9.08 (d, 5.6)	148.4
6a		148.9		148.0
7		180.9		181.9
7a		126.3		133.9
8		151.5	7.85 (d, 2.8)	110.6
9		156.8		162.3
10	7.34 (d, 8.8)	117.4	7.31 (dd, 8.8, 2.8)	122.6
11	8.68 (d, 8.8)	122.6	8.66 (d, 8.8)	127.4
11a		128.0		128.7
11b		151.6		151.3
11c		118.7		119.1
8-OCH_3	4.04 (s)	61.5		
9-OCH_3	4.01 (s)	56.4	3.99 (s)	55.9

Compound **3** was obtained as yellow solid, m.p. 150-152 ºC (lit. m.p. 150-152 ºC),[10] The ^{1}H and ^{13}C NMR data of **3** (Table 2) were closely related to those of **1** and **2** (Table 1), except for the presence the carbonyl group in ring C. The above data, suggested a copyrine alkaloid with eupolauridine skeleton.[13] The ^{1}H NMR spectrum of **3** showed an AA′BB′ coupling system at δ_H 7.88 (2H, d, J = 5.6 Hz, H-7/10) and 7.36 (2H, d, J = 5.6 Hz, H-8/9) in ring D. The structure assignment was confirmed by the detailed analysis of COSY and HMBC experiments (**Figure 3**). Based on these correlations and literatures,[14] the structure of **3** was determined to be eupolauridine.

Compound **4** was obtained as yellow solid, m.p. 137-138 ºC (lit. m.p. 137-138 ºC).[11] The UV and IR spectra were similar to those of **3**. The appearances of a methyl proton singlet at δ_H 3.95 and a methyl carbon at δ_C 55.9 in the ^{1}H and ^{13}C NMR spectra, respectively, suggested an additional methoxy group. The positions of a methoxy group at C-8 (δ_C 163.1) on the D ring was further confirmed by HMBC correlations between δ_H 3.87 (8-OCH$_3$) to C-8 (δ_C 163.1) (**Figure 3**). Compound **4** was therefore confirmed as 8-methoxyeupolauridine.[11]

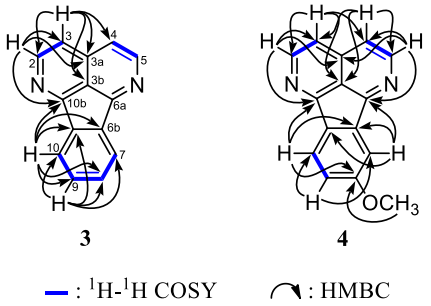

Figure 3. ^{1}H-^{1}H COSY and HMBC correlations for compounds **3-4**.

Table 1. ^{1}H and ^{13}C NMR data of compounds **3-4** in $CDCl_3$

	3		**4**	
Position	δ_H	δ_C	δ_H	δ_C
2	8.59 (d, 6.0)	149.8	8.60 (d, 6.0)	148.5
3	7.31 (d, 6.0)	117.5	7.39 (d, 6.0)	116.7
3a		135.1		135.4
3b		120.8		121.7
4	7.31 (d, 6.0)	117.5	7.44 (d, 6.0)	118.0
5	8.59 (d, 6.0)	149.8	8.71 (d, 6.0)	150.0
6a		162.7		162.4
6b		139.7		142.1
7	7.88 (d, 5.6)	122.7	7.59 (d, 2.4)	109.7
8	7.36 (d, 5.6)	131.2		163.1
9	7.36 (d, 5.6)	131.2	6.95 (dd, 8.0, 2.4)	115.8
10	7.88 (d, 5.6)	122.7	7.99 (d, 8.0)	124.9
10a		139.7		131.1
10b		162.7		162.5
8-OCH$_3$			3.95 (s)	55.9

Conclusion: A new alkaloid, 8,9-dimethoxysampangine (**1**), together with three known alkaloids, eupomatidine-1 (**2**), eupolauridine (**3**), and 8-methoxyeupolauridine (**4**), were isolated for the first time from *Orophea* genus. Their structures were identified by analysis of spectroscopic data and comparison of the NMR data with those previously reported.

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