ORIGINAL ARTICLE

Chemical composition investigation of the *Clinacanthus nutans* Lindau leaves

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ABSTRACT

The leaves of *Clinacanthus nutans* Lindau have long been traditionally used in Thailand as an anti-inflammatory drug for the treatment of insect bites, herpes infection and allergic responses. A crude chloroform extract was separated by means of chromatographic and bioactivity-guided fractionation techniques to give eight pure compounds. Structure elucidation of the isolated compounds was carried out on the basis of spectral analyses, including DEPT, COSY, NOESY, HMQC and HMBC. These eight compounds were related to chlorophyll a and chlorophyll b namely 13^2-hydroxy-(13^2S)-chlorophyll b (**1**), 13^2-hydroxy-(13^2R)-chlorophyll b (**2**), 13^2-hydroxy-(13^2S)-phaeophytin b (**3**), 13^2-hydroxy-(13^2R)-phaeophytin b (**4**), 13^2-hydroxy-(13^2S)-phaeophytin a (**5**), 13^2-hydroxy-(13^2R)-phaeophytin a (**6**), purpurin 18 phytyl ester (**7**) and phaeophorbide a (**8**). Five of these (compounds 1, 2, 4, 5, 6) were identified as novel compounds. These compounds have not been previously reported in this species. Further studies on the antiviral activity of the isolated compounds are in progress.

Keywords: *Clinacanthus nutans* Lindau, chlorophyll a and chlorophyll b related compounds, 13^2-hydroxy-(13^2S)-chlorophyll b, 13^2-hydroxy-(13^2R)-chlorophyll b, 13^2-hydroxy-(13^2S)-phaeophytin b, 13^2-hydroxy-(13^2R)-phaeophytin b, 13^2-hydroxy-(13^2S)-phaeophytin a, 13^2-hydroxy-(13^2R)-phaeophytin a, purpurin 18 phytyl ester, phaeophorbide a.

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องค์ประกอบทางเคมีของใบพญายอ

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บทคัดย่อ

ใบพญายอเป็นพืชสมุนไพรที่นิยมใช้กันในอดีตจนถึงปัจจุบัน มีสรรพคุณในการรักษาการอักเสบ อันเนื่องมาจากพิษสัตว์แมลงกัดต่อย โรคเริม โรคงูสวัด และอาการแพ้ต่างๆ เมื่อนำส่วนสกัดด้วยคลอโรฟอร์มของใบพญายอมาแยกด้วยวิธีคอลัมน์โครมาโทกราฟีได้สารบริสุทธ์ 8 ชนิด จากการวิเคราะห์โครงสร้างของสารทั้งหมดที่แยกได้โดยใช้ข้อมูลทางสเปคโทรสโกปี (DEPT, COSY, NOESY, HMQC และ HMBC) พบว่าเป็นสารประกอบที่มีสูตรโครงสร้างคล้ายคลึงกับคลอโรฟิลล์ เอ และคลอโรฟิลล์ บี คือ 13^2-hydroxy-(13^2S)-chlorophyll b (1), 13^2-hydroxy-(13^2R)-chlorophyll b (2), 13^2-hydroxy-(13^2S)-phaeophytin b (3), 13^2-hydroxy-(13^2R)-phaeophytin b (4), 13^2-hydroxy-(13^2S)-phaeophytin a (5), 13^2-hydroxy-(13^2R) phaeophytin a (6), purpurin 18 phytyl ester (7) and phaeophorbide a (8) ซึ่งสารประกอบหมายเลข 1, 2, 4, 5, 6 ยังไม่มีรายงานว่าพบในพืชนี้มาก่อน และการทดสอบฤทธิ์ต้านไวรัสของสารเหล่านี้ กำลังอยู่ในระหว่างทำการวิจัยทดสอบฤทธิ์อยู่

INTRODUCTION

Clinacanthus nutans (Burm.f.) Lindau is an often cultivated small shrub, native to tropical Asia. Fresh leaves of *C. nutans* has long been used in Thailand by traditional doctors to treat skin rashes, insect and snake bite as well as herpes simplex virus (HSV), and varicella-zoster virus (VZV) lesions. Extracts from the leaves were reported to possess analgesic and anti-inflammatory activities (Satayavivad, *et al*., 1996), antiviral activities against varicella-zoster virus (Thawaranantha, *et al*., 1992) and herpes simplex virus type-2 (Jayavasu, *et al*., 1992a). Clinical trials in patients with genital herpes are also reported (Jayavasu, *et al*., 1992b) and (Sangkitjaporn, *et al*., 1995). Clinical trials have shown the successful use of a *C. nutans* preparation (cream or lotion) for the relief of minor skin inflammation, insect bites, treatment of genital herpes and varicella-zoster lesions in patients (Charuwichitratana, *et al*., 1996), however, negative results have also been reported (Yoosook, *et al*., 1999).

C. nutans has been phytochemically and chemically investigated previously for stigmasterol (Dampawan, 1976), lupeol, β-sitosterol (Dampawan, *et al*., 1977), belutin (Lin, *et al*., 1983). Six known C-glycosyl flavones, vitaxin, isovitexin, shaftoside, isomollupentin-7-*O*β-glucopyranoside, orientin, isoorientin (Teshima, *et al*., 1997), five sulfur-containing glycosides (Teshima, *et al*., 1998), two glycoglycerolipids (Satakhun, *et al*., 2001), a mixture of nine cerebroside and a monoacylmonogalatosylglycerol (Tuntiwachwuttikul, *et al*., 2004), have been isolated. However, only the two glycoglycerolipids have been shown to exhibit antiviral activity.

This present article deals with the preliminary study at the Medicinal Plant Research Institute, Department of Medical Science, Ministry of Public Health on antiviral compounds isolated from this plant using bioassay-guided fractionation. The most antiviral active fractions were selected for further antiviral-guided fractionation by means of chromatographic techniques. This led to the isolation of eight pure compounds which were identified as chlorophyll a and chlorophyll b related compounds by spectroscopic methods. Chlorophyll related compounds isolated from plants and marine organisms have been shown to possess antioxidative activity (Sakata, *et al*., 1990; Watanabe, *et al*., 1993). Further studies on the antiviral activity of the isolated compounds are in progress.

MATERIAL AND METHODS

General

The ^{1}H NMR data for compounds 1-8 are shown in Table 1 and the ^{13}C-NMR data compounds 1-8 are shown in Table 2. The NMR spectra were run in C_5D_5N at room temperature on a Brucker 500 MHz spectrometer. HMBC and HMQC spectra were recorded on a Brucker 500 MHz spectrometer.

Silica gel 60 for column chromatography, 70-230 mesh, silica gel GF_{254} for thin layer chromatography, silica gel 60 PF_{254} for preparative layer chromatography (E. Merck, Germany) and solvents of technical grade were used. Anisaldehyde-sulfuric acid spraying reagent (modification b) was prepared according to the method of Stahl (1965).

Plant material

Fresh aerial parts of *C. nutans* (Burm.f.) Lindau (Family Acanthaceae) were collected during October to December 1998, from Bangkok, Chanthaburi and Nakhon Pathom Provinces of Thailand. The specimens were authenticated by the Botanical Section, Medicinal Plant Research Institute, Department of Medical Sciences, where a voucher specimens (Bansiddhi 432) was deposited. The leaves were separated from the stems, washed thoroughly and dried in an oven at 50 ^{0}C. The dried sample was ground to powder.

Extraction and Isolation

The dried powdered leaves (4.9 kg) were sequentially extracted with hexane and chloroform, respectively. The chloroform extract was concentrated in vacuo to give a residue (90.5 g) which was chromatographed on a silica gel 60 column. The column was eluted successively with hexane-ethyl acetate (1:1), ethyl acetate, chloroform-ethanol (1:1), and ethanol. Four major fractions (I, 32.92 g; II, 6.50 g; III, 30.51 g and IV, 4.90 g) were obtained by monitoring with TLC (toluene-petroleum ether 35-60 ^{o}C-methanol-methyl ethyl ketone 30:60:5:5). All fractions were examined for anti-herpes simplex virus activities by plaque reduction method. Fraction I and II, were selected for further purification. A portion of **Fraction I** (1.0017 g) was further separated by preparative thin-layer chromatography (hexane-ethyl acetate 7:3) to afford five fractions (A, 0.0450 g; B, 0.0406 g; C, 0.0791 g; D, 0.0697 g and E, 0.1469 g). Fraction A (0.0450 g) was further purified by preparative thin-layer chromatography using the same developing solvent to give crude **compound 1** (0.0105 g) and **compound 2** (0.0098 g), which were recrystallized from methanol (0.0052 g) and (0.0048 g), respectively (^{1}H- and ^{13}C-NMR: Table 1 and 2). Fraction B (0.0406 g) was further purified by preparative thin-layer chromatography using the same developing solvent to give crude **compound 3** (0.0105 g), which was recrystallized from methanol (0.0047 g) (^{1}H- and ^{13}C-NMR: Table 1 and 2). Fraction C (0.0791 g) was further purified by preparative thin-layer chromatography using the same developing solvent to give crude **compound 4** (0.0136 g) (^{1}H- and ^{13}C-NMR: Table 1 and 2). Fraction D (0.0791 g) was further purified by preparative thin-layer chromatography using the same developing solvent to give crude **compound 5** (0.0117 g) and **compound 6** (0.0113 g), which was recrystallized from methanol (0.0061 g) and (0.0057 g), respectively (^{1}H- and ^{13}C-NMR: Table 1 and 2).

Fraction III (30.51 g) was chromatographed on a silica gel 60 column (855 g), eluting successively with chloroform, followed by chloroform-ethanol gradient. Monitoring by TLC using the same solvent system as mentioned above, five fractions (A, 1.39 g; B, 0.80 g; C, 0.49 g; D, 6.13 g and E, 2.55 g) were obtained. Fraction A (1.39 g) was purified by preparative thin-layer chromatography using chloroform-methanol (9:1) as the developing solvent to give crude **compound 7** (0.3503 g). The crude compound was further purified by preparative thin-layer chromatography using hexane-ethyl acetate (7:3) as the solvent system to provide pure **compound 7** (0.0285 g) (^{1}H- and ^{13}C-NMR: Table 1 and 2). Purification of fraction B (0.80 g) by the preparative thin-layer chromatography, developing with chloroform-methanol (9:1) and recrystallization from chloroform-ethanol yielded **compound 8** (0.0136 g) (^{1}H- and ^{13}C-NMR: Table 1 and 2). The structures of compounds isolated from the chloroform extract of *C. nutans* leaves were elucidated on the basis of spectral analysis, including DEPT, COSY, NOESY, HMQC and HMBC. The compounds 1-8 were identified as chlorophyll a and chlorophyll b derivatives.

RESULTS AND DISCUSSION

The dried powdered leaves were extracted in a soxhlet apparatus. The isolation of compounds was performed by column chromatography on a silica gel 60 column eluted with appropriate solvents. Collection of fractions was monitored by TLC on silica gel 60 GF_{254} using anisaldehyde-sulfuric acid as a spraying reagent. Further purification of isolated compounds were carried out by PLC on silica gel 60 PF_{254}, 1.00 mm thickness to give eight pure compounds (**Compounds 1-8**). Structures of isolated compound were elucidated on the basis of spectral analysis, including DEPT, COSY, NOESY, HMQC and HMBC. By comparison of their NMR data with literature values, compounds 1-8 were identified as chlorophyll a and chlorophyll b related compounds.

Compound **1** was obtained as a bright green powder. The MS (ESI-TOF) mass spectrum of compound **1** showed a molecular ion peak at m/z 923.6 (M+1)+; $C_{55}H_{70}N_4O_7Mg$ requires 922.5. The ^{1}H-NMR and ^{13}C-NMR data of compound 1 were found to be closely similar to those of compounds **3** and **4**. It was therefore proved to have a chlorin ring system like compounds 3 and 4, except for the lack of two NH protons for chlorin (dihydroporphine) ring. Furthermore, the ^{1}H-NMR and ^{13}C-NMR spectroscopic properties established the presence of three downfield methine protons (δ 11.01 for H-5, δ 10.16 for H-10, δ 8.92 for H-20), methyls attached to C-2, C-12, and C-18, an ethyl at C-8 and an aldehyde at C-7^1 (Tables 1 and 2). The ^{13}C-NMR spectrum of compound **1** displayed four carbonyl carbon signals at δ 194.9, 188.0, 174.0 and 173.5. The absolute configuration at C-13^2 of compound **1** was further confirmed by the observed correlations of H-18^1 to H-13^4 and H-17 to H-13^4 in the NOESY spectrum of compound 1 (Fig. 2). Direct comparison of the ^{1}H-NMR and ^{13}C-NMR data of compound **1** (Table 1 and 2) with those of the known compound 13^2-hydroxy-(13^2*S*)-chlorophyll b (Watanabe, *et al*., 1993) showed that they were closely equivalent indicating that compound **1** is 13^2-hydroxy-(13^2*S*)-chlorophyll b (Fig. 1).

Compound **2** was obtained as a bright green powder. The MS (ESI-TOF) mass spectrum. The ^{1}H-NMR and ^{13}C-NMR data of compound **2** were found to be closely similar to those of compound 1. Direct comparison of the ^{1}H-NMR and ^{13}C-NMR data of compound **2** (Table 1 and 2) with those of the known compound 13^2-hydroxy-(13^2*R*)-chlorophyll b (Watanabe, *et al*., 1993) showed that they were closely equivalent indicated that compound **2** is 13^2-hydroxy-(13^2*R*)-chlorophyll b (Fig. 1).

Compound **3** was obtained as a green powder. The IR spectrum present of amine, hydroxyl, and ester functional groups. The absolute configuration at C-13^2 of compound **3** was further confirmed by the observed correlations of H-17^1 to H-13^4 and H-17^2 to H-13^4 in the NOESY spectrum of compound 3 (Fig. 2). These features indicated the structure of compound **3** was similar to the known compound phaeophytin a (Dechatiwongse, *et al*., 2001) except for the presence of an aldehyde group at C-7^1. Thus compound **3** was identified as 13^2-hydroxy-(13^2*S*)-phaeophytin b (Fig. 1).

Compound **4** was obtained as a dark green amorphous solid. The IR, ^{1}H-NMR and ^{13}C-NMR data of compound **4** were found to be closely similar to those of compound 3. Direct comparison of the ^{1}H-NMR and ^{13}C-NMR data of compound 4 (Table 1 and 2) with those of the known compound 13^{2}-hydroxy-(13^{2}*R*)-phaeophytin b (Matuo, *et al*., 1996) showed that they were closely equivalent indicated that compound **4** is 13^{2}-hydroxy-(13^{2}*R*)-phaeophytin b (Fig. 1).

Compound **5** was obtained as a green powder. The IR spectrum showed the presence of amine, hydroxyl, and ester functional groups. The absolute configuration at C-13^{2} of compound **5** was further confirmed by the observed correlations of H-17^{1} to H-13^{4} and H-17^{2} to H-13^{4} in the NOESY spectrum of compound **5** (Fig. 2). These features indicated the structure of compound **5** was similar to the known compound phaeophytin a (Matuo, *et al.*, 1996). Thus compound **5** was identified as 13^{2}-hydroxy-(13^{2}*S*)-phaeophytin a (Fig. 1).

Compound **6** was obtained as a green powder. The IR, ^{1}H-NMR and ^{13}C-NMR data of compound **6** were found to be closely similar to those of compound 5. Direct comparison of the ^{1}H-NMR and ^{13}C-NMR data of compound **6** (Table 1 and 2) with those of the known compound 13^{2}-hydroxy-(13^{2}*R*)-phaeophytin a (Matuo, *et al*., 1996) showed that they were closely equivalent indicated that compound **6** is 13^{2}-hydroxy-(13^{2}*R*)-phaeophytin a (Fig. 1).

Compound **7** was isolated as a grayish green solid. The ^{1}H-NMR and ^{13}C-NMR spectra of compound **7** (Tables 1 and 2) closely matched with those of compound 3 and purpurin 18 (Dechatiwongse, *et al*., 2001). The ^{1}H-NMR data comparison showed similarity to purpurin 18 with an extra phytyl ester proton side chain. Thus compound **7** was identified as purpurin 18 phytyl ester (Fig. 1).

Compound **8** was isolated as dark green powder. The ^{1}H-NMR and ^{13}C-NMR spectra of compound **8** (Tables 1 and 2) showed similarity to that of the known compound phaeophorbide a methyl ester (Dechatiwongse, *et al.*, 2001). The ^{1}H-NMR spectrum of compound **8** however, lacked the methyl ester signal at δ 3.57 (Table 1). The HMBC spectrum of compound **8** (Fig. 3) showed the interaction via multiple bonds between C and H giving the support to the assignments. Thus compound 8 was identified as phaeophorbide a (Fig. 1).

In conclusion, we have discovered the antiviral chlorophyll a and chlorophyll b related compounds from *C. nutans*, an important Thai medicinal plant used for herpes infections in primary health care. By using suitable analytical methods, these compounds will be further used as markers for qualitative control of the preparations made from the plant extract. Further studies on the antiviral activity and the use of these compounds as markers are being conducted at the Department of Medical Science Ministry of Public Health.

Table 1 ^{1}H-NMR chemical shifts of the compounds **1-8**

Proton	**Chemical Shifts in ppm (Coupling Constant in Hz)**								
	1		2	3	4	5	6	7	8
2^1	3.27	*s*	3.27	3.45	3.35	3.61	3.40	3.32	3.38
3^1	8.26	*dd, J* = 11.65,10.9	8.26	8.18	8.19	7.85	8.10	8.10	8.22
3^2 (*E*)	6.49	*dd, J* = 17.8, 2.5	6.49	6.54	6.54	6.41	6.37	6.41	6.40
3^2 (*Z*)	6.06	*dd, J* = 12.6, 2.5	6.06	6.21	6.21	6.25	6.18	6.20	6.21
5	10.91	*s*	10.91	11.01	11.00	9.91	9.70	9.66	9.73
7^1	11.57	*s*	11.57	11.48	11.50	3.29	3.19	3.17	3.26
8^1	4.18	*q, J* = 7.65	4.18	4.19	4.22	3.76	3.66	3.71	3.75
8^2	1.75	*t, J* = 7.55	1.75	1.81	1.83	1.73	1.68	1.69	1.71
10	10.06	*s*	10.06	10.16	10.16	9.76	9.88	9.89	9.90
12^1	3.68	*s*	3.68	3.72	3.70	3.87	3.73	3.84	3.69
13^2-H		*s*							6.90
13^2-OH	6.48	*s*	6.46	6.52	6.20	5.53	5.35		
13^4-OMe	3.74	*s*	3.74	3.85	3.85	3.69	3.71		3.93
17	5.14	*m*	5.54	5.23	5.41	5.24	5.77	5.46	4.59
18	4.49	*dq, J* = 7.3	4.50	4.67	4.60	4.41	4.60	4.67	4.66
18^1	1.56	*d, J* = 6.95	1.56	1.74	1.71	1.61	1.74	1.74	1.86
20	8.55	*s*	8.57	8.92	8.89	8.74	8.93	8.84	8.86
21-NH		*(br,s)*		-1.33	-1.32	-0.89	-1.48	0.11	-1.30
23-NH		*(br,s)*		0.66	0.68	0.78	0.67	0.38	0.89

Table 2 ^{13}C-NMR chemical shifts of the compounds **1-8**

Carbon	1	2	3	4	5	6	7	8
1	148.7	148.6	144.0	150.0	142.6	142.6	144.8	142.7
2	149.5	149.5	133.5	135.5	132.8	132.8	133.0	133.0
2^1	12.8	12.8	12.4	12.1	12.4	12.5	12.3	12.5
3	140.9	140.8	137.5	135.5	136.8	136.7	137.9	137.8
3^1	130.9	130.9	129.5	124.3	129.9	129.8	129.3	129.9
3^2	120.8	120.7	123.0	121.5	123.4	123.3	123.0	123.0
4	137.3	137.1	137.4	136.0	136.3	136.8	138.0	137.3
5	104.5	104.2	102.6	104.9	98.7	98.6	104.0	90.3
6	156.6	156.4	160.2	152.0	155.9	156.1	157.2	156.4
7	132.2	131.9	138.8	134.3	137.4	137.4	137.5	137.3
7^1	188.8	188.7	188.0	191.7	11.5	11.5	11.4	11.6
8	143.3	143.5	148.2	149.7	146.2	146.0	147.0	146.1
8^1	19.9	19.8	19.6	18.7	19.1	19.3	20.0	20.0
8^2	19.9	19.6	19.6	18.7	18.0	18.0	20.0	20.0
9	156.4	156.2	151.8	150.2	152.0	151.9	151.4	152.0
10	110.5	109.9	108.2	114.6	105.2	105.1	108.9	105.5
11	139.4	139.3	138.1	135.9	137.9	138.6	132.5	138.8
12	131.4	131.0	138.8	135.7	129.7	129.9	140.8	129.7
12^1	12.8	12.9	12.5	12.3	12.5	12.4	12.7	12.3
13	149.0	149.1	128.8	135.8	128.3	128.1	112.7	130.1
13^1	195.4	195.3	194.9	196.5	194.6	194.7	160.1	190.3
13^2	92.2	92.4	91.3	92.1	91.1	91.2	165.0	65.9
13^3	175.0	174.4	174.0	172.8	174.2	173.5		170.8
13^4	53.3	53.4	53.6	54.9	53.1	53.1		53.1
14	163.2	163.8	151.1	150.4	150.7	150.7	140.0	150.5
15	110.7	109.2	111.0	106.0	111.7	111.2	94.0	106.9
16	160.1	160.4	166.8	162.5	163.5	163.5	178.3	163.0
17	50.8	50.4	51.5	51.8	51.7	51.3	56.0	52.5
17^1	31.1	31.0	32.4	31.1	31.1	32.4	32.4	31.2
17^2	31.6	31.0	32.7	31.6	32.3	32.7	33.6	32.9
17^3	173.5	173.3	173.5	173.6	173.9	173.9	173.6	177.8
18	50.6	50.3	51.1	50.3	51.1	51.1	49.8	50.9
18^1	23.3	23.3	23.3	22.7	23.1	23.1	24.4	23.6
19	170.4	171.7	175.6	178.7	173.5	173.0	177.7	173.8
20	94.1	94.2	95.1	95.1	94.9	91.8	96.2	94.6

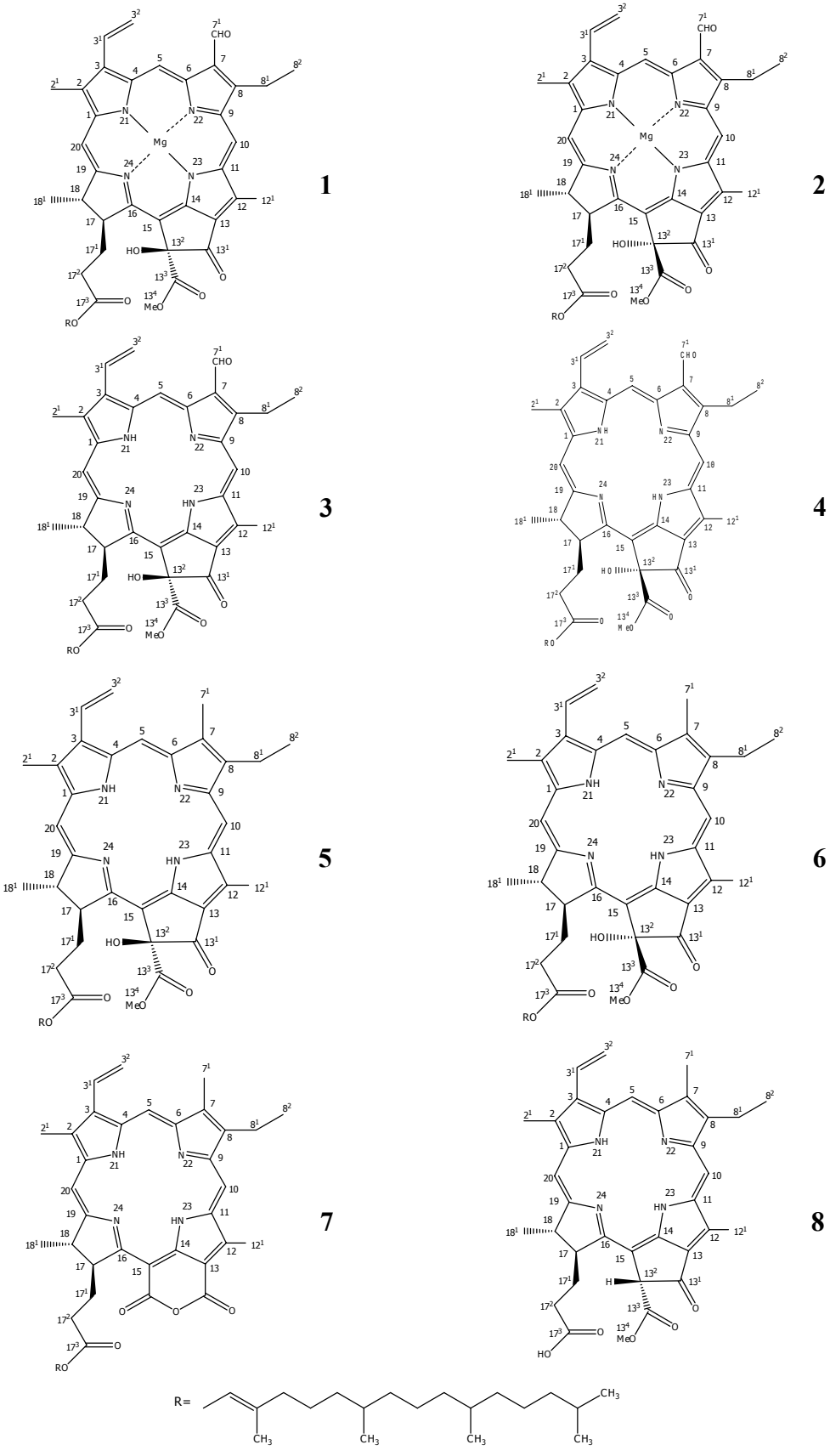
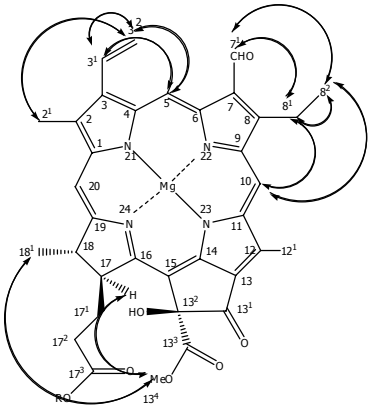


Fig. 1 Structure of compounds **1-8**



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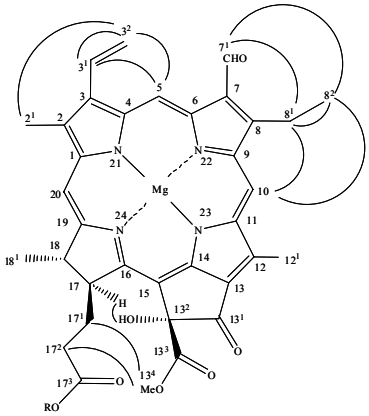

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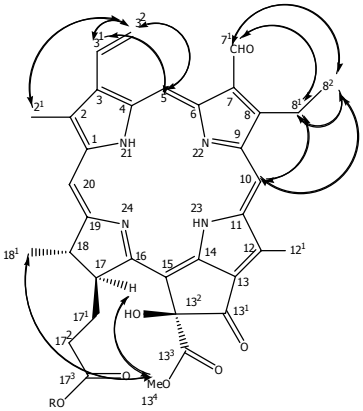

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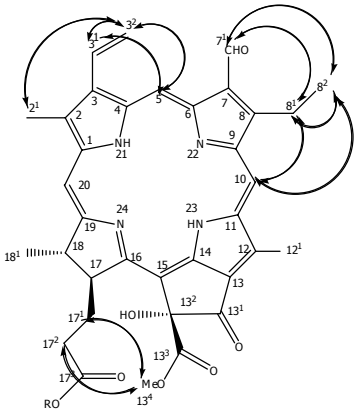

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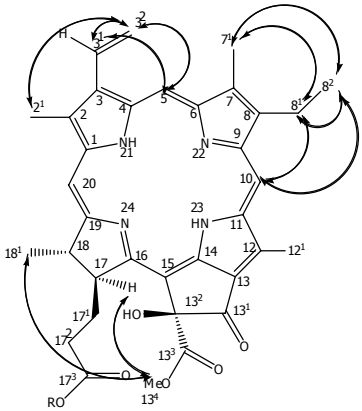

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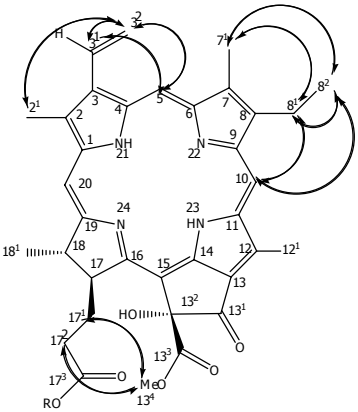

6

Fig. 2 The NOESY correlations of compounds **1, 2, 3, 4, 5** and **6**

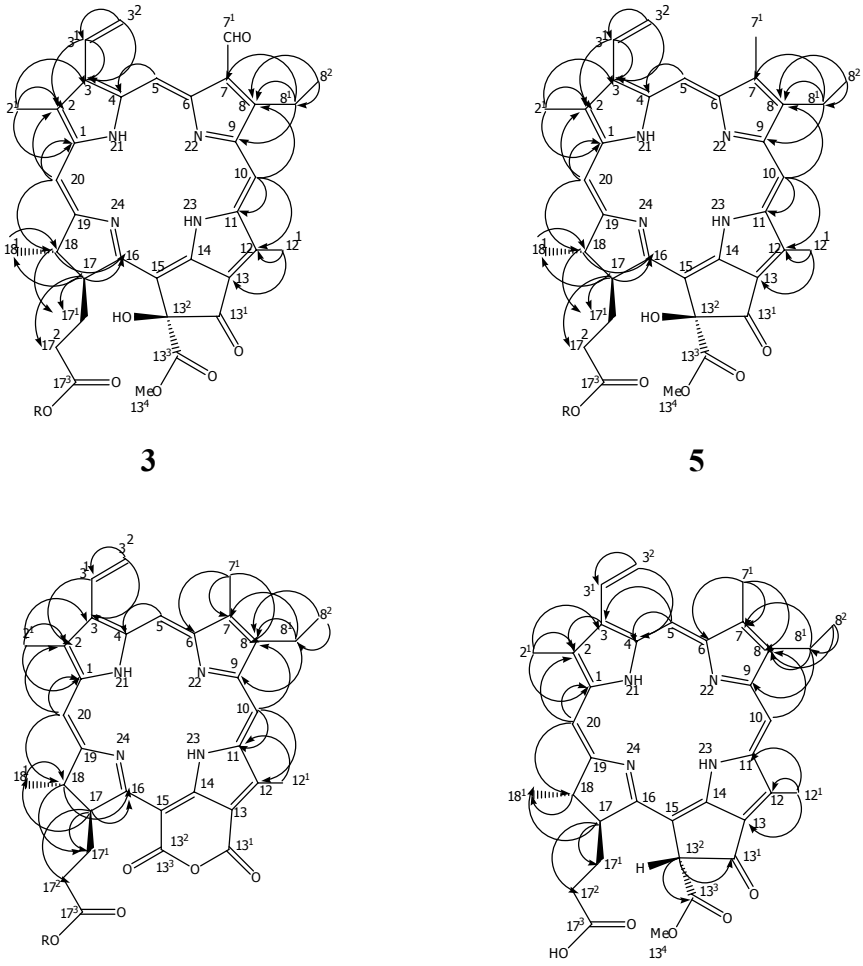

Fig. 3 The HMBC correlations of compounds **3, 5, 7** and **8**

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References

Charuwichitratana S, Wongrattanapasson N, Timpatanapong P, Bunjob M. *Herpes zoster* : treatment with *Clinacanthus nutans* cream. Int J Dermatal 1996;35: 665-6.

Dampawan P, Huntrakul C, Reutrakul V. Constituents of *Clinacanthus nutans* and the crystal structure of LUP- 20(29)-ene-3-one. J Sci Soc Thailand 1977;3:14-26.

Dampawan P. Studies of the chemical constituents of *Clinacanthus nutans* (Acanthaceae) and *Zingiber cassumunar* Roxb. Master thesis, Mahidol University, Bangkok, Thailand. 1976.

Dechatiwongse Na Ayudhya T, Sakdarat S, Shuyprom A, Pattamadilok D, Bansiddhi J, Waterman PG, Karagianis G. Chemical constituents of the leaves of *Clinacanthus nutans* Lindau. Thai J Phytopharm 2001; 8(1):1-8.

Hynninen PH, Leppakases TS, Mesilaakso M. The enolate anions of chlorophylls a and b as ambident nucleophiles in oxidations with (L)- or (D)-(10-camphorsulfonyl)oxaziridine. Synthesis of 132(S/R)-hydroxychlorophylls a and b. Tetrahedron 2006;62:3412-22.

Jayavasu C, Balachandra K, Sangkitjaporn S. Clinical trial in the treatment of genital herpes patients with *Clinacanthus nutans* extract. Com Dis J 1992;18:152-61.

Jayavasu C, Dechatiwongse T, Balachandra K. The virucidal activity of *Clinacanthus nutans* Lindau extract against *Herpes simplex* virus type-2: an in vitro study. Bull Dept Med Sci 1992;34:153-8.

Lin J, Li HM., Yu JG. Studies on the chemical constituents of niu xu hua (*Clinacanthus nutans*). Zhongcaoyao 1983;14:337-8.

Matuo A, Ono K, Hamasaki K, Nozaki, H. Phaeophytins from cell suspension culture of the liverwort *Plagiochila ovalifolia*. Phytochemistry 1996;42(2):427-30.

Sakata K, Yamamoto K, Ishikawa H, Yagi A, Etoh H, Ina K. Chlorophyyone-a, a new pheophorbide-A related compound isolated from Ruditapes Philippinarum as an antioxidative compound. Tetrahedron Lett 1990;31:1165-8.

Sangkitporn S, Balachandra K, Bunjob M, Chaiwat S, Dechatiwongse Na Ayudhya T, Jayavasa C. Treatment of *Herpes zoster* patients with *Clinacanthus nutans* (bi phyya yaaw). J Med Assoc Thailand 1995;78: 624-7.

Satakhun S. Chemical Constituents of *Clinacanthus nutans* leaves. Master thesis, Chulalongkorn University, Bangkok, Thailand. 2001.

Satayavivad J, Bunyaoraphatsara N, Kitisiripornkul S, Tanasomwang W. Analgesic and anti-inflammatory activities of extract of *Clinacanthus nutans* Lindau. Thai J Phytopharm 1996;3:7-17.

Teshima K, Kaneko T, Ohtani K. C-glycosyl flavones from *Clinacanthus nutans*. Natural Medicines 1997; 51:557.

Teshima K, Kaneko T, Ohtani K. Sulfur-containing glucosides from *Clinacanthus nutans*. Phytochemistry 1998;48:831-5.

Thawaranantha D, Balachandra K, Jongtrakulsiri S, Chavalittumrong P, Bhumiswasdi J, Janyavasu C. *In vitro* antiviral activity of *Clinacanthus nutans* on varicella-zoster virus. Siriraj Hosp Gaz 1992;44:285-91.

Tuntiwachwuttikul P, Pootaeng-on Y, Phansa P, Taylor WC. Cerebrosides and a monoacylmonogalactosyglycerol from *Clinacanthus nutans*. Chem Pharm Bull 2004;52:27-32.

Watanabe N, Yamamoto K, Ihshkawa H, Yagi A. New chlorophyll-a related compounds isolated as antioxidants from Marine Bivalves. J Nat Prod 1993;56:305-17.

Yoosook C, Panpisutchai Y, Chaichana S, Santisuk T, Reutrakul V. Evaluation of anti-HSV-2 activities of *Barleria lupulina* and *Clinacanthus nutans*. J Ethnopharmacol 1999;67:179-87.