Aristolactams and Sterols from *Piper wallichii*

อริสโตแลคแตมและสเตอรอลจากต้นจะค้าน

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ABSTRACT

The *piper* is one of the genera in the family Piperaceae. Many species are known as spices and herb, which are used in the cuisine of many cultures and in traditional medicine around of the world. In this work, phytochemical investigation from the stems and leaves of *Piper wallichii* (Piperaceae) was presented. Crude extracts of stems and leaves were separated by chromatographic method and led to the isolation of two aristolactams, stigmalactam (**1**) and piperolactam A (**2**) together with two sterols, ***β***-sitosterol (**3**) and stigmasterol (**4**). Their structures were elucidated primarily by analysis of their spectroscopic 1D and 2D NMR and IR data as well as comparison with those reported in the literature.

บทคัดย่อ

พืชกลุ่มพริกไทยจัดเป็นพืชหนึ่งในวงศ์ Piperaceae ซึ่งเป็นพืชที่ใช้เป็นเครื่องเทศในการปรุงอาหารของหลายวัฒนธรรมและเป็นส่วนผสมในยาแผนโบราณในหลายประเทศทั่วโลก จากการศึกษาองค์ประกอบทางเคมีจากจะค้าน (*Piper wallichii* (Miq.) Hand.-Mazz) โดยแยกจากส่วนสกัดหยาบลำต้นและใบ ด้วยวิธีทางโครมาโทกราฟี ได้สารในกลุ่มอริสโตแลคแตม 2 สาร คือ stigmalactam (**1**) และ piperolactam A (**2**) สารกลุ่มสเตอรอล 2 สาร คือ ***β***-sitosterol (**3**) และ stigmasterol (**4**) โดยได้วิเคราะห์โครงสร้างของสารที่แยกได้ด้วยเทคนิคทางสเปกโทรสโคปี ได้แก่ 1D และ 2D NMR และ IR รวมทั้งนำข้อมูลที่ได้มาเปรียบเทียบกับในรายงานวิจัยที่ตีพิมพ์แล้ว

Keywords: *Piper wallichii*, aristolactams, sterols

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Introduction

A plant in the genus *Piper* of the family Piperaceae are composed of an approximately 2000 species widely distributed in tropical and subtropical regions of the world (Gutierrez et al., 2013). *Piper* plants are also known under the common name "pepper" (Salehi et al., 2019). They have been employed for the production of pepper in spice markets and their secondary metabolites have also been used in traditional medicine for thousands of years, including China, India and Thailand. Therefore, it is interesting to study the chemical components of *piper* species. Many structurally diverse amides with the wide range of bioactivities such as cytotoxic, stomach aches, insect repellents, anti-inflammatory, insecticidal, and antifeedant activities was isolated from the genus *Piper* (Shingala et al., 2011). A part of these, aristolactam, a large and important group of naturally occurring alkaloids possessing the phenanthrene lactam skeleton, was frequently reported with biologically properties such as anti-inflammatory, treating of arthritis, gout, rheumatism, antiPAF, antimycobacterial, and neuro-protective (Marques et al., 2011). In addition, sterols, essential component of eukaryotic cell membranes playing an important role in regulating the physicochemical properties of cell membranes, were also reported. They are mainly classified into phytosterols (plant origin), zoosterols (animal origin), and mycosterols or ergosterol (fungal origin). All types of sterols share a structure of similar chemical backbone and differ in the number and position of double bonds or lengths of side carbon chains. Moreover, they also exhibited various other health benefits including anticancer, anti-inflammation, antioxidation, neuroprotection and cardiovascular protection (He et al., 2018).

This work investigated the phytochemical constituents of a Thai herb, Cha Khan (*P. wallichii* (Miq.) Hand.-Mazz). It can be found in the northern, north-eastern and south-western parts of Thailand, as well as China, Nepal, India, Bengal and Indonesia. This plant possesses multiple activities such as hepatoprotective, antioxidant, vasodilator, antiarrhythmic and anticancer effects. The stems of *P. wallichii* are medicinally used by the local people in China to treat rheumatoid arthritis, inflammatory diseases, cerebral infarction and angina. The fruits and leaves are used medicinally against cold, cough and fever (Salehi et al., 2019).

Objectives of the study

The aim of this study was to isolate and characterize the chemical constituents from stems and leaves of the *P. wallichii*.

Materials and methods

The stems and leaves of *P. wallichii* (Miq.) Hand.-Mazz were collected from Mae Rim district, Chiang Mai province, Thailand. The voucher specimens, C. Suwanphakdee 291 (BK, BKF, KKU) were deposited at Department of Botany, Faculty of Science, Kasetsart University.

Extraction and isolation

Air dried stems of *P. wallichii* were ground and extracted with EtOAc and MeOH 10L for three time each at room temperature, respectively. Removal of solvents from each extract gave crude EtOAc and MeOH extracts. The crude EtOAc extract was separated on silica gel column chromatography (CC), eluted with a gradient system of *n*-hexane, EtOAc and MeOH to give 14 fractions (SE_1-SE_{14}). The 5.1 mg of brownish yellow needles of compound **1** was obtained from fraction SE_9. The filtrate was evaporated using rotary evaporator at 40 °C to dryness and then the crude extract was separated by flash column chromatography (FCC), eluted with an isocratic system of *n*-hexane:acetone (90:10) to give 7 subfractions ($SE_{9.1}$-$SE_{9.7}$). The 68.4 mg of yellowish needles of **2** was isolated from subfraction $SE_{9.5}$. Air dried powder of leaves was extracted with *n*-hexane 10L for three time at room temperature. The *n*-hexane extract was separated by CC, eluted with a gradient system of *n*-hexane and EtOAc to give 8 fractions (LH_1-LH_8). Fraction LH_5 was further separated by CC, eluted with a gradient system of *n*-hexane: acetone (95:5 to 50:50) to give colorless needles of compound **3** (12.40 mg) and compound **4** (9.70 mg).

Results and discussion

Compound 1 was obtained as brownish yellow needles, mp 276-277 °C (276-278 °C (Chia et al., 2000)). The IR spectrum exhibited the amide absorption band at 3368 cm^{-1}, hydroxyl group at 3132 cm^{-1}. The amide carbonyl absorption band appeared at 1654 cm^{-1}. The absorption bands of the C-H stretching appeared at 2923 and 2848 cm^{-1}, while the bending vibration appeared at 1474 and 1442 and 1389 cm^{-1}. The absorption bands at 1234, 1121, and 1023 cm^{-1} were assigned to C-O stretching and the absorption band at 1605 cm^{-1} indicated the C=C stretching. The ^{13}C NMR, DEPT and HSQC spectral data of compound **1** (Table 1) displayed 18 carbon signals attributable to three methoxy, one carbonyl, four sp^2 methine, ten sp^2 quaternary carbons. The ^1H NMR spectral data of compound **1** (Table 1) shows three methoxy groups as three singlet signals at δ_H 4.43 (3H, s, 2-OCH_3), 3.96 (3H, s, 3-OCH_3), and 4.15, (3H, s, 4-OCH_3), four aromatic protons appear at δ_H 8.62 (1H, d, H-5), 7.09 (1H, dd, H-7), 7.66, d, H-8), and 7.11 (1H, s, H-9). The HMBC spectrum displayed the correlations of the methoxy protons at 2-O*Me* to C-2 (153.9), 3-O*Me* to C-3 (145.8) and 4-O*Me* to C-4 (157.2) indicating the location of these methoxy groups in the structure. The correlations of aromatic protons H-5 to C-4a (127.0), C-4b (115.9), C-6 (155.0), C-7 (116.4); H-7 to C-6 and C-8 (129.9); H-8 to C-4b, C-8a (128.0), C-9 (107.0); H-9 to C-10 (131.3) and C-10a

(127.9) confirmed the connections in the molecule. The COSY spectrum of compound **1** clearly showed correlations between H-8/H-7 and H-8/H-9 to support the aristolactam skeleton. Therefore, these ^{1}H and ^{13}C NMR spectroscopic data were confirmed by comparison with those data reported for stigmalactam (Chia et al., 2000) as shown in Table 1. Thus, compound **1** was identified as a stigmalactam (Figure 1).

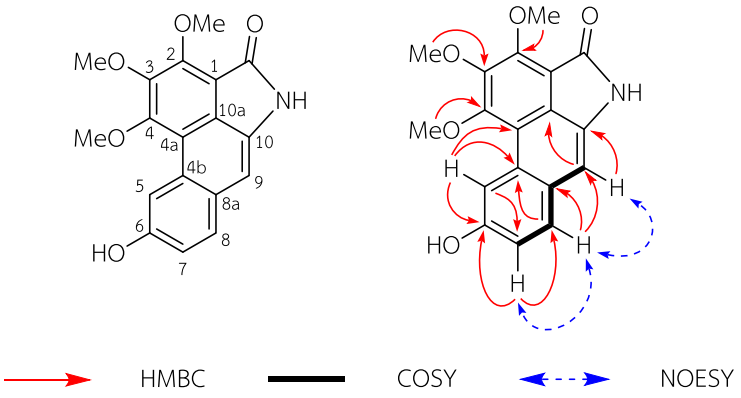

Figure 1 Key HMBC, COSY and NOESY correlations of stigmalactam (**1**)

Table 1 ^{1}H and ^{13}C NMR spectral data of compound **1** (400 MHz, CD_3OD+$CDCl_3$) and stigmalactam (400 MHz, CD_3OD)

Position	Stigmalactam		**1**				
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	HMBC	COSY	NOESY
1		126.8		126.0			
2		156.9		153.9			
3		147.5		145.8			
4		158.7		157.2			
4a		127.2		127.0			
4b		117.2		115.9			
5	8.64 d (2.8)	112.2	8.62 d (2.5)	111.1	4a, 4b, 6, 7		
6		151.2		155.0			
7	7.09 dd (8.0, 8.8)	117.5	7.09 d (8.7)	116.4	6, 8	8	8
8	7.72 d (8.8)	131.1	7.66 d (8.6)	129.9	4b, 8a, 9	7, 9	7, 9
8a		129.3		128.0			
9	7.17 s	108.0	7.11 s	107.0	10, 10a	8	8

10		133.0		131.3	
10a		128.6		127.9	
2-OMe	4.41 s	63.2	4.43 s	62.7	2
3-OMe	3.99 s	63.1	3.96 s	61.5	3
4-OMe	4.19 s	61.4	4.15 s	60.7	4
C=O		167.9		167.7	

Compound 2 was obtained as yellowish needles, mp 271-272 °C (271-273 °C (Sun et al., 1987)). The IR spectrum exhibited the amide absorption band at 3446 cm^{-1}, hydroxyl group at 3180 cm^{-1}. The amide carbonyl absorption band appeared at 1691 cm^{-1}, while the absorption band of C-O stretching displayed at 1230, 1130, 1031 cm^{-1}. The absorption bands of C=C stretching appeared at 1616 and 1501 cm^{-1}, and the C-H stretching found at 1373 and 1325 cm^{-1}. The ^{1}H and ^{13}C NMR spectroscopic data of compound **2** (Table 2) showed the typical pattern of an aristolactams skeleton similar to that of stigmalactam (**1**), relating to six aromatic protons at δ_H/δ_C 7.69 (1H, s)/108.0 (C-2), 9.25 (1H, m)/127.9 (C-5), 7.50 (1H, m)/126.7 (C-6), 7.50 (1H, m)/124.8 (C-7), 7.80 (1H, m)/128.7 (C-8), and 7.08 (1H, s)/105.3 (C-9), together with a methoxy group attached to C-3 of aromatic ring at δ_H/δ_C 4.06 (3H, s)/56.7. In addition, other seven quaternary carbons were one carbonyl (δ_C 170.2) and six carbons at δ_C 116.0 (C-1), 114.7 (C-4a), 134.5 (C-5a), 127.3 (C-9a), 134.9 (C-10), and 125.2 (C-10a), respectively. Based on the comparison of ^{1}H and ^{13}C NMR spectral data with those reported in the literature (Ee et al., 2008), compound **2** was identified as a piperolactam A (Figure 2).

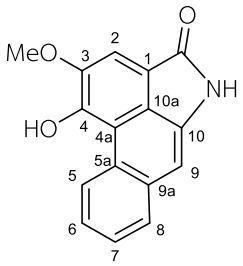

Figure 2 Structure of piperolactam A (**2**)

Compound 3 was obtained as colorless needles, mp 136-138 °C (135-137 °C (Eck et al.,1939)). The IR spectrum showed a broad band of O-H stretching at 3355 cm^{-1}. The absorption bands of the C-H stretching appeared at 2934 and 2866 cm^{-1}, while the C-H bending vibrations appeared at 1462, 1377, 1330 and 1242 cm^{-1}. The medium absorption band of C-O stretching displayed at 1108 cm^{-1}. The ^{1}H NMR spectrum of **3** (Table 3) showed the presence of two methyl singlet protons at δ_H 0.73 (H-18), and

0.93 (H-19), three methyl doublet protons at δ_H 0.94 (H-21), 0.82 (H-26), and 0.84 (H-27) and methyl triplet protons at δ_H 0.82 (H-29) together with one olefinic proton at δ_H 5.28 (H-6) which suggested the sterol structure. Based on the above evidence and the comparison of ^{1}H NMR spectral data with those reported for phytosterols (Ododo et al., 2016), compound **3** was a plant sterol, ***β***-sitosterol. Finally, mixed-mp of compound **3** with the authentic ***β***-sitosterol confirm that, compound **3** was ***β***-sitosterol as shown in Figure 3.

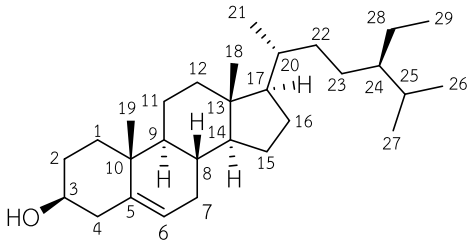

Figure 3 Structure of ***β***-Sitosterol (**3**)

Table 2 ^{1}H and ^{13}C NMR spectral data of compound **2** (400 MHz, CD_3OD) and piperolactam A (400 MHz, Acetone-d_6)

Position	Piperolactam A		**2**	
	δ_H	δ_C	δ_H	δ_C
1		117.3		116.0
2	7.75 s	108.8	7.69 s	108.0
3		150.3		150.0
4		149.1		148.4
4a		115.5		114.7
5a		135.5		134.5
5	9.31 m	128.7	9.25 m	127.9
6	7.52 m	127.5	7.50 m	126.7
7	7.25 m	125.8	7.50 m	124.8
8	7.86 m	129.4	7.80 m	128.7
9a		128.0		127.3
9	7.15 s	105.5	7.08 s	105.3
10		136.1		134.9
10a		125.8		125.2

NH	9.83 *br*, s			
C=O		169.9		170.2
OMe	4.09 s	57.6	4.06 s	56.7

Table 3 ^{1}H and ^{13}C NMR spectral data of compound **3** (400 MHz, $CDCl_3$) and ***β***-sitosterol (600 MHz, $CDCl_3$)

Position	***β***-Sitosterol	**3**
	δ_H (*J* in Hz)	δ_H (*J* in Hz)
1	1.46 m	1.46 m
2	1.56 m	1.55 m
3	3.54 m	3.44 m
4	2.32 m	2.32 m
6	5.37 t (6.4)	5.28 t (7.0)
7	2.04 m	2.10 m
8	1.69 m	1.79 m
9	1.55 m	1.55 m
11	1.52 m	1.52 m
12	1.51 m	1.51 m
14	1.50 m	1.50 m
15	1.58 m	1.58 m
16	1.85 m	1.83 m
17	1.45 m	1.44 m
18	0.70 s	0.73 s
19	1.03 s	0.93 s
20	1.60, m	1.60 m
21	0.94 d (6.4)	0.94 d (6.2)
22	0.93 m	0.93 m
23	1.15 m	1.15 m
24	1.38 m	1.38 m
25	1.57 m	1.58 m
26	0.84 d (6.4)	0.82 d (6.2)
27	0.86 d (6.4)	0.84 d (6.2)
28	1.10, m	1.10, m

29	0.82 t	0.82 t
OH	1.98 s	2.10 s

Compound 4 was obtained as colorless needles, mp 167-169 °C (170-171 °C (Eck et al., 1939)). The IR spectrum showed a broad band of O-H stretching at 3359 cm^{-1}. The absorption bands of the C-H stretching appeared at 2975, 2964, and 2867 cm^{-1}, while the bending vibrations appeared at 1460, 1438, and 1382 cm^{-1}. The medium absorption band of C-O stretching displayed at 1109 cm^{-1}. The ^{1}H NMR spectrum of compound **4** (Table 4) showed the presence of two methyl singlet protons at δ_H 0.62 (H-18) and 0.94 (H-19), three methyl doublet protons at δ_H 1.00 (H-21), 0.84 (H-26), and 0.78 (H-27), together with three olefinic protons δ_H 5.28 (H-6), 5.10 (H-22), and 4.97 (H-23) which showed characteristic signals similar to ***β***-Sitosterol (**3**). Based on the above evidence and on the comparison of ^{1}H NMR spectral data with those of plant sterols reported in the literature (Forgo et al., 2004), compound **4** was a stigmasterol. To conclude this, compound **3** was mixed-mp with the authentic stigmasterol and the result confirmed that compound **4** was a stigmasterol (Figure 4).

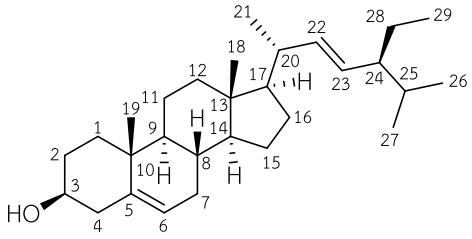

Figure 4 Structure of stigmasterol (**4**)

Table 4 ^{1}H and ^{13}C NMR spectral data of compound **4** (400 MHz, $CDCl_3$) and stigmasterol (600 MHz, $CDCl_3$)

Position	Stigmasterol	**4**
	δ_H (*J* in Hz)	δ_H (*J* in Hz)
1	1.84 m	1.78 m
2	1.83 m	1.77 m
3	3.51 m	3.45 m
4	2.30 m	2.20 m
6	5.34 m	5.28 m
7	1.97 m	1.92 m
8	1.46 m	1.42 m

9	0.94 m	0.85 m
11	1.50 m	1.45 m
12	2.00 m	1.90 m
14	1.01 m	0.94 m
15	1.56 m	1.50 m
16	1.72 m	1.60 m
17	1.15 q (9.9)	1.10 m
18	0.70 s	0.62 s
19	1.01 s	0.94 s
20	2.06 m	1.95 m
21	1.03 d (6.2)	1.00 d (6.2)
22	5.17 dd (15.2, 8.6)	5.10 dd (15.1, 8.6)
23	5.04 dd (15.2, 8.6)	4.97 dd (15.1, 8.6)
24	1.54 m	1.48 m
25	1.55 m	1.49 m
26	0.85 d (6.4)	0.84 (6.4)
27	0.80 d (6.4)	0.78 (6.4)
28	1.43 m	1.40 m
29	0.81 t (7.3)	0.79 m
OH		2.10 s

Conclusions

Chromatographic separation of the crude *n*-hexane, EtOAc and MeOH extracts from stems and leaves of *P. wallichii* contains four compounds (**1**-**4**): two aristolactams, stigmalactam (**1**) and piperolactam A (**2**) together with two well-known sterols, *β*-sitosterol (**3**) and stigmasterol (**4**). Their structures were identified by using spectroscopic evidence (IR, ^{1}H NMR, ^{13}C NMR,and 2D NMR) as well as comparison data with those reported in the literature.

Acknowledgements

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