

Lignan, Neolignans and Feruloyl Ester from Stems of *Piper suiipigua*

ลิกแนน นีโอลิกแนนและเฟอร์รูลอยเอสเทอร์จากลำต้นจ๊กค้ำนหัววอก

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ABSTRACT

Investigation of the chemical constituents from the stems of Chak khan hua wok (*Piper suiipigua*) by chromatographic separation led to the isolation of four secondary metabolites. Their structures were determined by using spectroscopic evidence (IR, UV, ¹H NMR, ¹³C NMR and 2D NMR) as well as optical rotation to be one lignan, (-)-veraguensin (1), two neolignans, piperbonin A (2) and piperbonin B (3), and one feruloyl ester, eicosyl ferulate (4).

บทคัดย่อ

การศึกษาองค์ประกอบทางเคมีจากลำต้นของจ๊กค้ำนหัววอก (*Piper suiipigua*) ด้วยการแยกสารโดยวิธีทางโครมาโทกราฟี ได้สารเมแทบอไลต์ทุติยภูมิทั้งหมด 4 สาร และได้พิสูจน์โครงสร้างทางเคมีโดยวิธีทางสเปกโทรสโกปี (IR, UV, ¹H NMR, ¹³C NMR และ 2D NMR) รวมทั้ง optical rotation พบว่าเป็นสารในกลุ่มลิกแนน 1 สาร คือ (-)-veraguensin (1) เป็นสารในกลุ่มนีโอลิกแนน 2 สาร คือ piperbonin A (2) และ piperbonin B (3) และเป็นสารกลุ่มเฟอร์รูลอยเอสเทอร์ 1 สาร คือ eicosyl ferulate (4)

Keywords: *Piper suiipigua*, Lignan, Neolignan

คำสำคัญ: จ๊กค้ำนหัววอก ลิกแนน นีโอลิกแนน

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Introduction

Lignans and neolignans are a class of plants secondary metabolites which are biochemically related to the shikimate pathway (Saleem et al., 2005). They are formed in nature by oxidative coupling of two phenylpropanoid units, whose site of connection determines their classification into lignans (8,8'-linkage), neolignans (all other C,C'-linkages), and oxylignans (C-O-C'-linkages) (Curti et al., 2006). Lignans and neolignans are very important natural products of vast structural diversity with possess significant biological activities, such as antitumor, anti-inflammatory, antioxidant, antiviral, antimalarial, antifungal, anticancer, and antimicrobial (Saleem et al., 2005; Li et al., 2007; Li et al., 2011). Lignans and neolignans widely distributed in several families such as the Piperaceae family and one of its members is *Piper suiipigua* which easily found in the north of Thailand. Herein, we report the isolation of one lignan, (-)-veraguensin (**1**); two neolignans, piperbonin A (**2**) and piperbonin B (**3**); and one feruloyl ester, eicosyl ferulate (**4**) from the stems of *P suiipigua*.

Objectives of the study

To isolate and determine the chemical constituents from the stems of *P. suiipigua*.

Methodology

Air-dried powder stems of *P. suiipigua* (1.4 kg) were extracted successively three times with *n*-hexane (3x 3L), EtOAc (3x 3L) and MeOH (3x 3L) at room temperature. Removal of solvents from the extracts by using rotary evaporator under reduced pressure gave crude *n*-hexane, EtOAc, and MeOH extracts, respectively. The *n*-hexane extract was separated on silica gel column chromatography (CC) and eluted with a gradient solvent system of *n*-hexane:EtOAc and EtOAc:MeOH to give 12 fractions (EF₁-EF₁₂). Fraction HF₇ was separated on silica gel CC, eluted with a gradient system of *n*-hexane:EtOAc (90:10 to 70:30) to give 17 subfractions (HF_{7,1}-HF_{7,17}). Subfraction HF_{7,13} was purified by silica gel CC to give a pale yellow oil of compound **2** (51 mg). Fraction HF₉ was further separated on silica gel CC to give 16 subfractions (HF_{9,1}-HF_{9,16}). Subfraction HF_{9,7} was purified by silica gel CC, eluted with an isocratic system of CH₂Cl₂:EtOAc (99:1) to provide a white solid of compound **1** (9.5 mg). Subfraction HF_{9,8} was purified by silica gel CC to give a pale yellow oil of compound **3** (5.1 mg). The EtOAc extract was applied on silica gel CC and eluted with a gradient solvent system of *n*-hexane:EtOAc and EtOAc:MeOH to give 12 fractions, (SEF₁-SEF₁₂). Fraction EF₆ was separated on silica gel CC to give 10 subfractions (EF_{6,1}-EF_{6,10}). Subfraction EF_{6,3} was purified by silica gel CC to afford a white solid of compound **4** (6.6 mg). The isolation and separation of the stems of *P. suiipigua* are shown in Scheme 1.

Results and Discussion

Separation of crude extracts from stems of *P. suiipigua* mainly by chromatographic methods afforded four compounds, (-)-veraguensin (**1**), piperbonin A (**2**), piperbonin B (**3**), and eicosyl ferulate (**4**). The

structures of the isolated compounds were identified by analysis of physical and spectroscopic data (UV, IR, ^1H and ^{13}C NMR and 2D NMR) and optical rotation.

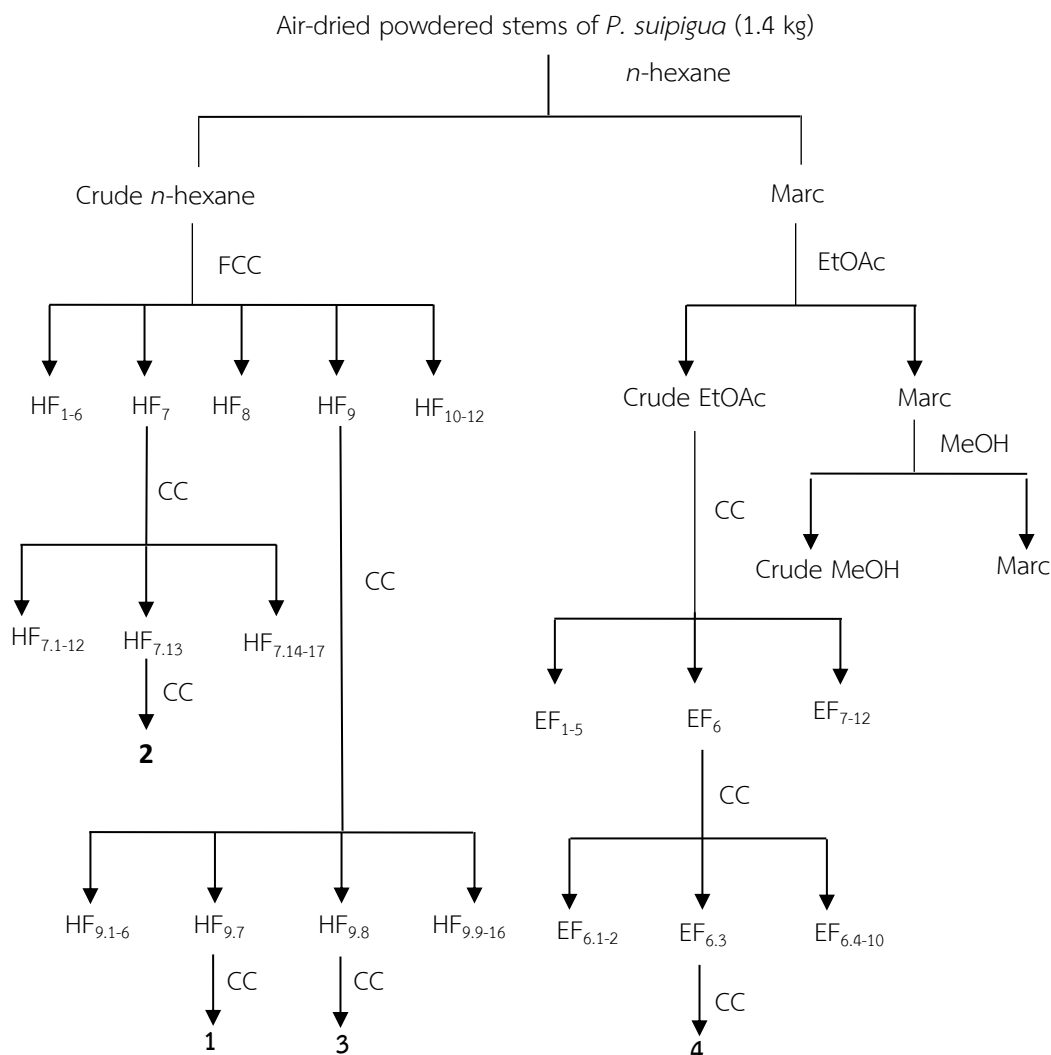


Figure 1 Separation and isolation scheme of the stems of *P. suipigua*.

Compound **1** was obtained as a white solid with a specific rotation of $[\alpha]_D^{25} -40.2$ (c 0.10, CHCl_3). mp 127-129°C (128-129 °C (Kim et al., 2007)). The IR spectrum showed absorption bands of aromatic at 1515 and 1463 cm^{-1} and ether group at 1264 cm^{-1} . The ^{13}C NMR and HMQC spectra data of **1** (Table 1) contained 22 carbons, including six sp^2 quaternary, six sp^2 methine, two sp^3 methine, two sp^3 oxybenzylic, two sp^3 methyl and four methoxy groups. The ^1H NMR spectra of **1** (Table 1) showed characteristic signals of two oxybenzylic protons at δ_{H} 5.07 (d, J = 8.6 Hz, C-2) and δ_{H} 4.35 (d, J = 9.3 Hz, C-5), two methyl groups at δ_{H} 1.00 (d, J = 7.0 Hz, C-6) and δ_{H} 0.59 (d, J = 6.6 Hz, C-7), two 3,4-dimethoxy phenyl groups at δ_{H} 7.00 (d, J = 2.0 Hz, C-2'), δ_{H} 6.79 (d, J = 8.0 Hz, C-5'), δ_{H} 6.97 (d, J = 8.0, 2.0 Hz, C-6'),

δ_{H} 6.81 (d, $J = 1.6$ Hz, C-2'') δ_{H} 6.79 (d, $J = 8.0$ Hz, C-5'') and δ_{H} 6.81 (d, $J = 8.0$ Hz, C-6''), four methoxy groups at δ_{H} 3.84 (s), 3.82 (s), 3.80 (s) and 3.80 (s). The COSY spectrum showed correlations of the tetrahydrofuran unit, H-2 \leftrightarrow H-3 \leftrightarrow H-4 \leftrightarrow H-5. The HMBC spectrum displayed correlations of H-2 to C-5, C-3, C-4, C-7, C-1', C-2' and C-6'; H-3 to C-2, C-4 and C-7; H-4 to C-3, C-5 and C-6; H-5 to C-3, C-4, C-6, C-1'', C-2'' and C-6''; H-6 to C-3, C-4 and C-5; H-7 to C-2, C-3 and C-4 confirming the tetrahydrofuran unit linked to two aromatic rings at C-1 and C-1'' respectively. The relative configuration of **1** was established on the basis of NOESY correlations between H-2 and H-3, H-5; H-3 and H-2, H-7. Based on the comparison of ^1H and ^{13}C NMR spectral data with those reported in the literature, compound **1** was deduced to be a lignan derivative, (-)-veraguensin (Kim et al., 2007).

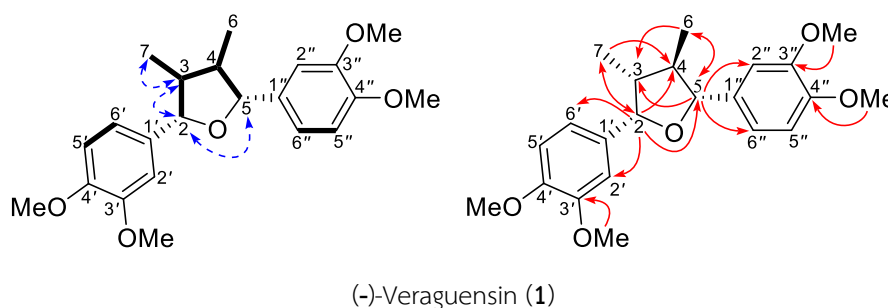


Figure 2 HMBC (→), COSY (↔), NOESY (↔) correlations of (-)-veraguensin (**1**).

Compound **2** was obtained as a pale yellow oil with a specific rotation of $[\alpha]_{\text{D}}^{25} +72.4$ (c 0.10, MeOH). The IR spectrum showed absorption bands of ester carbonyl group at 1734 cm^{-1} and aromatic group at 1592 , 1509 and 1462 cm^{-1} . The ^{13}C NMR and HMQC spectral data of **2** (Table 2) displayed 23 carbon signals attributable to eight sp^2 methine, seven sp^2 quaternary (including one carbonyl), two sp^3 oxymethines and six sp^3 methyl (including methoxy groups). The ^1H NMR spectral data of **2** (Table 2) showed characteristic signals of an *E* propenyl group at δ_{H} 6.33 (dd, $J = 15.8, 1.7$, H-7'), 6.13 (m, H-8') and 1.86 (dd, $J = 6.6, 1.7$ Hz, H-9'), two trisubstituted phenyl rings at δ_{H} 6.94 (dd, $J = 8.3, 1.9$ Hz, H-6), 6.91 (d, $J = 1.9$ Hz, H-2) and 6.82 (d, $J = 8.3$ Hz, H-5), δ_{H} 6.88 (d, $J = 1.9$ Hz, H-2'), 6.88 (dd, $J = 8.3$ Hz, H-5') and 6.83 (dd, $J = 8.3, 1.9$ Hz, H-6'). Three singlet signals of methoxy protons showed at δ_{H} 3.88, 3.86 and 3.84 respectively, a methyl of acetoxyl group at H-7 (δ_{H} 2.00, s) and a methyl group at H-9 (δ_{H} 1.17 (d, $J = 6.3$ Hz). The COSY spectrum showed correlations of H-7 \leftrightarrow H-8 \leftrightarrow H-9 and correlations of the *E* propenyl unit, H-7' \leftrightarrow H-8' \leftrightarrow H-9'. The HMBC spectrum displayed correlations of H-7 to C-1, C-2, C-6; H-8 to C-7 and C-4' confirmed two oxymethine groups linked to aromatic carbons at C-1 and C-8' respectively. The correlations of H-7' to C-2', and C-8'; H-8' to C-1' and C-7'; H-9' to C-8' confirming the *E* propenyl group linked to the aromatic carbon at C-1'. The NOESY spectrum showed correlations

between H-7 and H-8. The C-7, C-8 relative configurations of **2** was determined as threo configuration according to the $J_{7,8}$ value of 7.1 Hz. Based on the comparison of ^1H and ^{13}C NMR spectral data with those reported in literature, compound **2** was deduced to be a known neolignan derivative, piperbonin A, which was previously been isolated from *Piper bonii* (Ding et al., 2016).

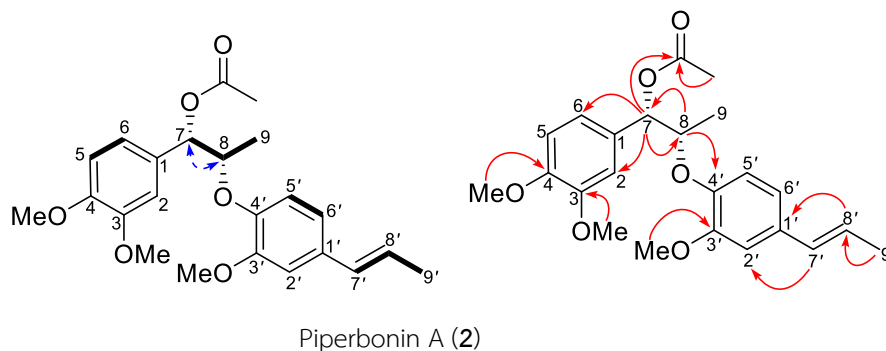


Figure 3 HMBC (→), COSY (—), NOESY (←-→) correlations of piperbonin A (**2**).

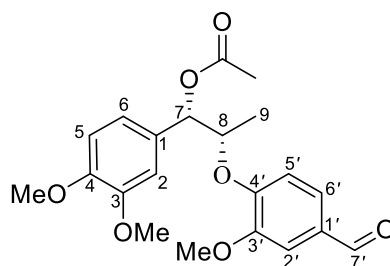
Table 1 ^1H and ^{13}C NMR spectral data of **1** (400 MHz, CDCl_3)^a and (-)-veraguensin (400 MHz, CDCl_3)^a

Position	1					(-)-veraguensin	
	δ_{H}	δ_{C}	COSY	HMBC	NOESY	δ_{H}	δ_{C}
2	5.07, d (8.6)	83.0	3	3, 7, 1', 2', 6'	3, 5	5.14, d (8.3)	83.0
3	2.18 m	45.9	2, 4, 7	2, 4, 7	2, 7	2.24 m	45.9
4	1.70 m	47.8	3, 5, 6	3, 5, 6		1.79 m	48.0
5	4.35, d (9.3)	87.2	4	3, 4, 6, 1'', 2'', 6''	2	4.42, d (9.2)	88.3
6	1.00, d (6.6)	15	4	3, 4, 5		1.07, d (6.8)	15.2
7	0.59, d (7.0)	14.9	3	2, 3, 4	3	0.66, d (7.2)	14.9
1'		133.4					133.4
2'	7.00, d (2.0)	110.3				7.07 s	110.3
3'		148.5					148.5
4'		148					147.9
5'	6.79, d (8.0)	110.9	6'			6.89, d (8.4)	110.9
6'	6.97, dd (8.2, 2.0)	119.2	5'			7.04, d (8.4)	119.5
1''		133.8					133.7
2''	6.81, d (1.6)	109.9				6.88 s	109.8
3''		148.5					148.5
4''		148.9					148.9
5''	6.79, d (8.0)	110.6	6'			6.84, d (8.4)	110.6
6''	6.81, d (8.0)	118.6	5'			6.89, d (8.4)	118.5

3'-OMe	3.84 s	55.9	3'	3.91 s	55.8
4'-OMe	3.82 s	55.8	4'	3.89 s	55.7
3''-OMe	3.80 s	55.8	3'	3.86 s	55.7
4''-OMe	3.78 s	55.7	4'	3.86 s	55.7

^oChemical shift values are in ppm, and *J* values (in Hz) are presented in parentheses.

Compound 3 was obtained as a pale yellow oil with a specific rotation of $[\alpha]_D^{25} +58.2$ (c 0.10, MeOH). The IR spectrum showed absorption bands of an ester carbonyl group at 1734 cm^{-1} , aromatic formyl group at 1688 cm^{-1} and aromatic group at $1592, 1511$ and 1465 cm^{-1} . The ^1H and ^{13}C NMR spectral data of **3** (Table 3) were similar to those of **2**, except for a propenyl group at C-1' which was displaced by a formyl group, C-1' $\delta_{\text{H/C}}$ 9.78 (s)/190.8. The relative configuration of **3** was also determined as a threo ($J_{7,8} = 7.1\text{ Hz}$). Based on the comparison of ^1H and ^{13}C NMR spectral data with those reported in literature, compound **3** was deduced to be a known neolignan derivative, piperbonin B, which was previously been isolated from *Piper bonii* (Ding et al., 2016).



Piperbonin B (**3**)

Compound 4 was obtained as a white solid, mp $59\text{-}62\text{ }^\circ\text{C}$ ($57\text{-}59\text{ }^\circ\text{C}$ (Chang et al., 2011)). Its molecular formula $\text{C}_{30}\text{H}_{50}\text{O}_4$, was determined from the HRESITOFMS observed m/z 475.3787 (M+H)^+ . The IR spectrum showed absorption bands of CH_3 stretching at 2917 cm^{-1} , CH_2 stretching vibration at 2850 cm^{-1} , ester carbonyl group at 1714 cm^{-1} and aromatic group at $1594, 1512$ and 1463 cm^{-1} . The ^{13}C NMR spectral data of **4** (Table 4) contained 30 carbons, including five sp^2 methine carbons, four sp^2 quaternary carbons (one carbonyl carbon) nineteen sp^3 methylenes, a sp^3 methyl and one methoxy group. The ^1H NMR spectra of **4** (Table 4) showed characteristic signals of olefinic protons at δ_{H} 7.61 (d, $J = 15.9\text{ Hz}$, H-7) and δ_{H} 6.29 (d, $J = 15.9\text{ Hz}$, H-8), a 3,4-disubstituted benzene ring at δ_{H} 7.03 (dd, $J = 2.0\text{ Hz}$, H-5), δ_{H} 6.91 (d, $J = 8.1\text{ Hz}$, H-5) and 7.07 (d, $J = 8.2, 1.8\text{ Hz}$, H-6), oxymethylene protons at δ_{H} 4.18 (t, $J = 6.8\text{ Hz}$, H-1'), seventeen methylenes groups at δ_{H} 1.26 (brs) and δ_{H} 1.69 and a methyl groups at δ_{H} 0.88 (t, $J = 6.7\text{ Hz}$). The COSY spectrum showed correlations of H-5 \leftrightarrow H-6; H-7 \leftrightarrow H-8; H-1' \leftrightarrow H-2' \leftrightarrow H-3'. The HMBC spectrum displayed correlations of H-6 to C-2, C-7; H-7 to C-2, H-6; H-8 to C-1 confirming the double bond linked to the aromatic ring and correlation of H-8 to C-9 confirming the double bond linked to acetoxy group. Based on the comparison of ^1H and ^{13}C NMR spectral data with those reported in the

literature, compound **4** was deduced to be a known long-chain aliphatic feruloyl ester, eicosyl ferulate (Baldé et al., 1991; Chang et al., 2011).

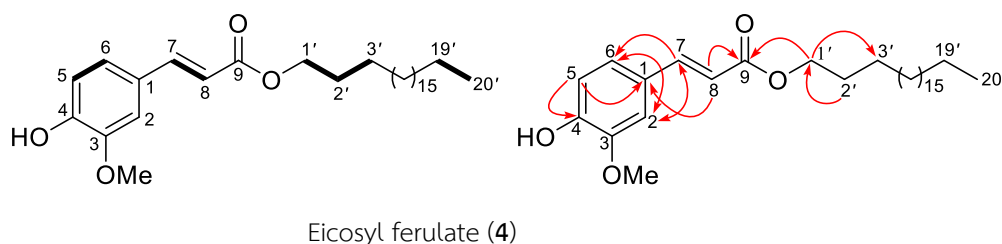


Figure 4 HMBC (—→), COSY (—) correlations of eicosyl ferulate (**4**).

Table 2 ¹H and ¹³C NMR spectral data of **2** (400 MHz, CDCl₃)^a and piperbonin A (400 MHz, CDCl₃)^a

Position	2					piperbonin A	
	δ_H	δ_C	COSY	HMBC	NOESY	δ_H	δ_C
1		129.9					129.8
2	6.91, d (1.9)	110.7		3		6.91, d (1.9)	110.6
3		148.9					148.8
4		149					148.9
5	6.82, d (8.3)	110.9	6	1, 4		6.82, d (8.3)	110.8
6	6.94, dd (8.3, 1.9)	120	5	1, 5		6.94, dd (8.3, 1.9)	120
7	5.89, d (7.1)	78.2	8	1, 2, 6, OAc	8	5.89, d (7.1)	78.2
8	4.55, m	78.1	7, 9	4', 7	7	4.55, m	78.1
9	1.17, d (6.3)	16.8	8			1.17, d (6.3)	16.8
1'		132.4					132.3
2'	6.88, d (1.9)	109.7		1', 3'		6.88, d (1.9)	109.2
3'		150.5					150.5
4'		147					147
5'	6.88, dd (8.3)	117	6'	4', 6'		6.88, d (8.3)	117
6'	6.83, dd (8.3, 1.9)	118.6	5'	1', 2', 4', 7'		6.83, dd (8.3, 1.9)	118.6
7'	6.33, dd (15.8, 1.7)	130.5	8'	2', 8'		6.32, dd (15.7, 1.5)	130.5
8'	6.13, m	124.2	7', 9'	1', 7'		6.11, m	124.2
9'	1.86, dd (6.6, 1.7)	18.4	8'	8'		1.85, dd (6.7, 1.5)	18.4
3-OMe	3.88, s	55.9		3		3.87 s	55.9
4-OMe	3.86 s	55.8		4		3.85 s	55.8
3'-OMe	3.84 s	55.8		3'		3.83 s	55.8
OAc		170.1					170
	2.00 s	21.1		OAc		1.99 s	21.4

^aChemical shift values are in ppm, and *J* values (in Hz) are presented in parentheses.

Table 3 ^1H and ^{13}C NMR spectral data of **3** (400 MHz, CDCl_3)^a and piperbonin B (400 MHz, CDCl_3)^a

Position	3		piperbonin B	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1		129.2		129.3
2	6.85, d (1.9)	110.7	6.91, d (1.9)	110.7
3		149.0		149.0
4		149.2		149.3
5	6.78, dd (8.2, 1.7)	111.0	6.84, d (8.2)	111.0
6	6.88, dd (8.2, 1.9)	120.2	6.95, dd (8.2, 1.9)	120.2
7	5.87, dd (7.1, 1.7)	77.7	5.93, d (7.1)	77.8
8	4.66, m	77.4	4.74, m	77.6
9	1.17, d (6.6, 1.7)	16.4	1.24, d (6.4)	16.6
1'		130.5		130.6
2'	7.36, d (1.7)	110.1	7.42, d (1.8)	110.1
3'		150.6		150.7
4'		153.5		153.6
5'	7.01, d (8.0)	114.2	7.08, d (8.1)	114.2
6'	7.33, dd (8.0, 1.7)	126.3	7.44, dd (8.1, 1.8)	126.5
7'	9.79, s	190.9	9.85, s	191.0
3-OMe	3.82, s	56.0	3.89, s	56.0
4-OMe	3.80, s	56.0	3.88, s	56.0
3'-OMe	3.84, s	56.0	3.91, s	56.1
OAc		170.0		170.1
	1.93, s	21.1	2.00, s	21.2

^aChemical shift values are in ppm, and *J* values (in Hz) are presented in parentheses.

Table 4 ^1H and ^{13}C NMR spectral data, of **4** (400 MHz, CDCl_3)^a and eicosyl ferulate (400 MHz, CDCl_3)^a

Position	4				eicosyl ferulate	
	δ_{H}	δ_{C}	COSY	HMBC	δ_{H}	δ_{C}
1		127				127
2	7.03, d (2.0)	109.3		2, 3, 7	7.02, d (2.0)	109.2
3		147.8				147.9
4		146.7				146.7
5	6.91, d (8.2)	115.7	6	4, 1'	6.96, d (8.4)	115.6
6	7.07, dd (8.2, 1.8)	123	5	2, 7	7.06, d (8.4, 2.1)	123
7	7.61, d (15.9)	144.6	8	2, 6	7.59, d (15.9)	144.6
8	6.29, d (15.9)	114.7	7	1, 9	6.27, d (15.9)	114.7
9		167.4				167.4
3-OMe	3.92 s	55.9		4	3.90 s	55.9

1'	4.18, t (6.8)	64.6	2'	9, 3'	4.16, t (6.6)	64.5
2'	1.69, m	31.9	1', 3'	1'	1.67, m	31.9
3'-19'	1.26, br s	29.7- 22.7			1.23, br s	29.7-22.7
20'-Me	0.88, t (6.7)	14.1	19'		0.86, t (6.0)	14.1

^aChemical shift values are in ppm, and *J* values (in Hz) are presented in parentheses.

Conclusions

Chromatographic separation of the extracts from stems of *P. suipigua* afforded four compounds, including (-)-veraguensin (**1**), piperbonin A (**2**), piperbonin B (**3**) and eicosyl ferulate (**4**). The structures of the isolated compounds were identified by analysis of physical and spectroscopic data and optical rotation as well as compared with those data previously reported in literature.

Acknowledgements

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