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A New Aporphine Alkaloid from *Illigera aromatic*

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Abstract: Chemical investigation of the aerial part of *Illigera aromatic* S.Z. Huang & S.L. Mo has resulted in the isolation and characterization of one new aporphine alkaloid, illigerine B (**1**), along with three known analogues laurodionine B (**2**), *N*-formyl-laurolitsine (**3**) and illigerine A (**4**). Their structures were established by spectrometric means and physico-chemical properties. The *in vitro* cytotoxic activities of compound **1** against Hela, SMMC7721, and Bcap37 cell lines were evaluated. Compound **1** exhibited moderate cytotoxic activity against the three tumor cell types, with IC_{50} values of 12.40 ± 0.78, 32.61 ± 2.05, and 28.69 ± 1.80 μg/mL. This work shown that aporphine alkaloids might be useful as characteristic markers in chemotaxonomic research of the genus *Illigera*.

Keywords: Hernandiaceae; *Illigera aromatic;* aporphine alkaloid; Illigerine B; cytotoxicity.

1. Introduction

Aporphines derivatives are widely distributed in plants of the family Hernandiaceae. Many of these isolates exhibit diversified biological activities, including cytotoxic, vasorelaxing, anti-platelet aggregation, antioxidant, and antiplasmodial properties [1-7]. *Illigera aromatica* S. Z. Huang & S. L. Mo (Hernandiaceae) is a small liana distributed mainly in Guangxi and Yunnan provinces, P. R. China. The stems are used medicinally to treat coughs, rheumatic arthralgia, indigestion, and injuries from falls [8]. Previous chemical research on this plant led to the isolation of some aporphines and oxoaporphines [6, 7]. In this study, we reinvestigated the aerial part of the plant *I. aromatica*, which was collected in Nanning, Guangxi Province, P. R. China. One new compound aporphine alkaloid, illigerine B (**1**) (Figure 1), along with three known compounds laurodionine B (**2**), *N*-formyl-laurolitsine (**3**) and illigerine A (**4**) were isolated from this species. The *in vitro* cytotoxic activities of the new compound against Hela, SMMC7721, and Bcap37 cell lines were also reported.

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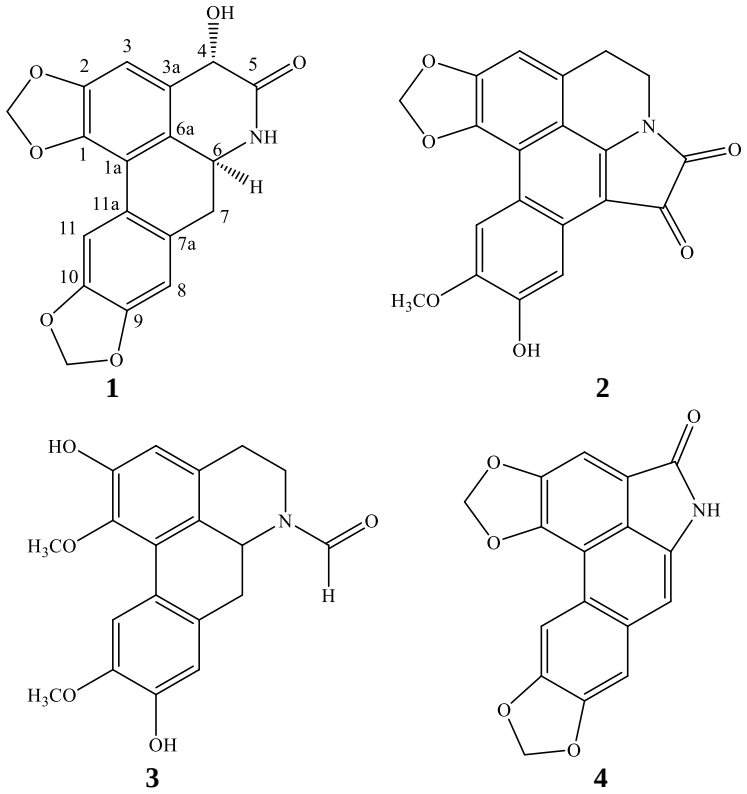

Figure 1. The chemical structures of compounds **1-4.**

2. Materials and Methods

2.1. Plant Material

The aerial part of the plant *Illigera aromatica* S.Z. Huang & S.L. Mo was collected on Oct. 2015 in Nanning, Guangxi Province, P. R. China. A voucher specimen (IA-20151001) was identified by Prof. Bin Wu of Zhejiang University, and maintained in the School of Food Science and Biotechnology, Zhejiang Gongshang University, Hangzhou, P. R. China.

2.2. General Experimental Procedures

Melting point (uncorrected), BUCHI M565 instrument; IR spectrum (KBr), NicoletAvatar-360 FT-IR spectrometer; 1D, 2D NMR, Bruker AVANCE DMX 500 NMR spectrometer (^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) with TMS as internal standard, 25^{o}C); HR-ESI-MS, Agilent 6210 TOF-MS spectrometer equipped with an ESI source; ESI-MS, Thermo LCQ Fleet ion trap mass spectrometer. TLC was performed using Merck pre-coated plates (Si gel 60 F_{254}) of 0.25 mm thickness.

2.3. Extraction and Isolation

The shade-dried, powdered aerial part of the plant *Illigera aromatica* (25 kg) were extracted at room temperature three times with methanol (3 × 50 L). The extracts were evaporated *in vacuo* to afford a gummy residue (1200 g). This residue was partitioned in H_2O and extracted with EtOAc (3 × 10 L) and *n*-butanol (3 × 10 L), successively. The EtOAc extract (293 g) was adsorbed onto silica gel (300 g) and subjected to chromatography over silica gel (80 × 1000 mm, 100-200 mesh), eluting with petroleum ether (PE)/EtOAc gradient mixtures. Eleven main fractions (*Fr.* 1 ~ *Fr.* 11) were obtained by checking with TLC and combined. Small samples of the fractions were detected by Dragendorff's reagent, with gum obtained from the *Fr.* 7 and *Fr.* 8 showing positive reaction. *Fr.* 8 was subjected to

chromatography over silica gel (40 ×300 mm, 300 g, 200-300 mesh), eluting with CH_2Cl_2/MeOH gradient mixtures to afford 14 subfractions (S-*Fr.* 8-1 ~ S-*Fr.* 8-14). S-*Fr.* 8-14 was subjected to Sephadex LH-20 (20 × 1000 mm, Amersham) column and eluted with MeOH to yield **1** (4.7 mg). *Fr.* 7 was further-separated on a silica gel column (45 × 600 mm, 200-300 mesh), eluted with PE-EtOAc (1:1) to give 3 sub-fractions (S-*Fr.* 7-1 ~ S-*Fr.* 7-3). S-*Fr.* 7-1 and S-*Fr.* 7-2 were re-purified to obtain compounds laurodionine B (**2**, 5.8 mg) and *N*-formyl-laurolitsine (**3**, 4.5 mg). S-*Fr.* 7-3 was re-purified on silica gel column (25 × 500 mm, 200-300 mesh), eluted with PE-EtOAc (5:1-1:1) to give compound illigerine A (**4**, 1.8 mg).

2.4. Spectroscopic Data

*Illigerine B (**1**):* Yellowish-orange powder. MP: 246-250 °C. UV (MeOH): λ_{max} (log ϵ): 241 (4.38), 285 (3.31), 312 (4.25) nm. IR (KBr): υ_{max}^{KBr} 3431, 3176, 2926, 1664, 1502, 1460, 1400, 1246, 1061, 1039, 941, 852, 569 cm^{-1}. ^1H and ^{13}C NMR (DMSO-d_6): Table 1. ESI-MS: *m/z* = 362 $[M + Na]^+$, 338 $[M - H]^-$, 320 $[M-H-H_2O]^-$ (MS^2), 290 $[M-H-H_2O-CH_2O]^-$ (MS^3), 260 $[M-H-H_2O-CH_2O-CH_2O]^-$ (MS^4), 232 $[M-H-H_2O-CH_2O-CH_2O-CO]^-$ (MS^5). HR-ESI-MS: *m/z* $[M + Na]^+$ calcd for $C_{18}H_{13}NO_6 + Na^+$, 362.0635; found: 362.0627. Yield: 0.002 %.

2.5. Cytotoxic Assay

The *in vitro* bioactivity of compounds **1-4** against three tumor cell lines: human cervical carcinoma cells (Hela), human hepatic carcinoma cells (SMMC7721), and human breast cancer cells (Bcap37) were assayed by the MTT method, and cisplatin was used as a positive control (Table 2). The tumor cells were cultured at 37°C under a humidified atmosphere of 5% CO_2 in RPMI-1640 medium supplemented with 10% fetal calf serum, and dispersed in replicate 96-well plates (1×10^4 cells/well) for 48 h. Compounds **1-4** (10-200 μM), or cisplatin (DDP positive control), were then added. After 72 h of exposure to the testing agents, the cell viability was determined by the MTT by recording the absorbance at λmax 570 nm with an ELISA reader. Each test was performed in triplicate (n = 3). The dose resulting in 50% inhibition of cell growth, IC_{50}, was calculated by NDST software. The data were expressed as mean ± standard deviation (S.D.) [10, 11].

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was obtained as a yellowish-orange powder and its molecular formula was deduced to be $C_{18}H_{13}NO_6$ by HR-ESI-MS at *m/z* 362.0627 $[M + Na]^+$ (calcd. for $C_{18}H_{13}NO_6 + Na^+$, 362.0635), with an unsaturation degree of thirteen. The IR absorption band at 3424, 3176, 1664, and 1400 cm^{-1} suggested the presence of hydroxyl and amide groups. The UV spectrum showed absorption maxima at 241, 285 and 312 nm. The ^1H, ^{13}C NMR and HSQC spectra of **1** (Table 1) displayed the characteristic NMR features for an aporphine alkaloid bearing two benzene rings (δ_H 7.52 (s), 6.97 (s), and 6.85 (s); δ_C 143.5 (s), 115.3 (s), 147.8 (s), 107.7 (d), 129.0 (s), 126.0 (s), 128.7 (s), 109.5 (d), 147.2 (s), 146.9 (s), 107.0 (d) and 124.0 (s)), two -OCH_2O- units (δ_H 6.19 (s), 6.06 (s) corresponding to δ_C 101.7 (t); δ_H 6.05 (s), 6.04 (s) corresponding to δ_C 101.8 (t)), an amide group (δ_H 8.35 (s, NH); δ_C 170.2 (s)), an methylene (δ_H 2.56 (dd, *J* = 14.15, 14.55 Hz), 3.04 (dd, *J* = 5.20, 14.55 Hz); δ_C 36.3 (t)), an methine (δ_H 4.50 (dd, *J* = 5.20, 14.15 Hz); δ_C 50.1 (d)), and an oxysubstituted methine (δ_H 4.61 (d, *J* = 5.90 Hz); δ_C 68.3 (d)). Thus, **1** was deduced to have an aporphine skeleton [7, 9], with two methylenedioxy and one hydroxyl substituent groups. The low resolution negative electrospray ionization tandem mass spectrometry spectra (S3-S7) of compound **1** showed *m/z* 338 $[M-H]^-$ and fragment ions at *m/z* 320 $[M-H-H_2O]^-$ (MS^2), 290 $[M-H-H_2O-CH_2O]^-$ (MS^3), 260 $[M-H-H_2O-CH_2O-CH_2O]^-$ (MS^4), 232 $[M-H-H_2O-CH_2O-CH_2O-CO]^-$ (MS^5), confirming these substituent groups.

The HMBC spectrum of **1** showed correlations (Figure 2A) from δ_H 4.61 (H-4) to δ_C 107.7 (C-3), 129.0 (C-3a), 170.2 (C-5), and 126.0 (C-6a), from δ_H 8.35 (NH) to δ_C 68.3 (C-4), 170.2 (C-5), 50.1 (C-6), 126.0 (C-6a), and 36.3 (C-7), suggested the hydroxyl group was linked to C-4 and the amine unit at

C-5. The HMBC correlations from δ_H 6.19, 6.06 to δ_C 143.5 (C-1), 147.8 (C-2), from δ_H 6.05, 6.04 to δ_C 147.2 (C-9), 146.9 (C-10) determined that the two methylenedioxy groups link at C-1/C-2 and C-8/C-9, respectively. Furthermore, in the NOESY experiment (Figure 2B), the NOE correlations between H-4 (δ_H 4.61) and O-H (δ_H 5.90), between H-6 (δ_H 4.50) and NH (δ_H 8.35) were observed, but no correlation was observed between H-4 and H-6 (α-configuration) [2, 4, 5], suggesting that H-4 was β-configuration and 4-OH was α-configuration. Therefore, the chemical structure of compound **1** is elucidated as shown in Figure 1, a new natural aporphine alkaloid and named illigerine B.

Furthermore, the three known compounds were isolated and identified as laurodionine B (**2**), *N*-formyl-laurolitsine (**3**) and illigerine A (**4**) [6] base on the NMR and MS data and corresponding with those from literatures.

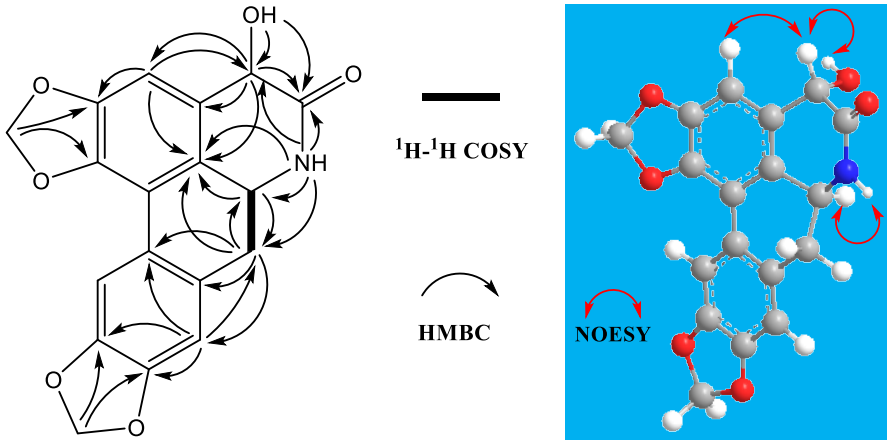

Figure 2. The selective key HMBC (A) and NOESY (B) correlations of compound **1**

Table 1. NMR data of compound **1** (at 600 MHz in DMSO-d_6, δ in ppm, J in Hz)

Position	δ_C (ppm)[ab]	δ_H (ppm)[c]	HMBC[d]
1	143.5 (C)	-	-
1a	115.3 (C)	-	-
2	147.8 (C)	-	-
3	107.7 (CH)	6.85 (s)	2, 4, 6a
3a	129.0 (C)	-	-
4	68.3 (CH)	4.61 (d, J = 5.90)	3, 3a, 5, 6a
5	170.2 (C)	-	-
6	50.1 (CH)	4.50 (dd, J = 5.20, 14.15)	6a, 7
6a	126.0 (C)	-	-
7	36.3 (CH_2)	2.56 (dd, J = 14.15, 14.55) 3.04 (dd, J = 5.20, 14.55)	6, 6a, 7a, 8, 11a
7a	128.7 (C)	-	-
8	109.5 (CH)	6.97 (s)	7, 11a, 9, 10
9	147.2 (C)	-	-
10	146.9 (C)	-	
11	107.0 (CH)	7.52 (s)	1a, 7a, 9, 10
11a	124.0 (C)	-	-
-OCH_2O-	101.7 (CH_2)	6.19 (s), 6.06 (s)	1, 2
-OCH_2O-	101.8 (CH_2)	6.04 (s), 6.05 (s)	9, 10
-NH	-	8.35 (s)	4, 5, 6, 6a,7
-OH	-	5.90 (d, J = 5.90)	3a, 4, 5

[a] Recorded at 125 MHz; [b] Multiplicities inferred from DEPT and HSQC experiments;
[c] Recorded at 500 MHz; [d] Proton showing long range correlation with indicated carbons.

3.2. Cytotoxicity Activity

Compound **1** exhibited moderate cytotoxic activity against the three tumor cell types, with IC_{50} values of 12.40 ± 0.78, 32.61 ± 2.05, and 28.69 ± 1.80 μg/mL. Compounds **2** and **4** also shown moderate activity against these three tumor cell lines. Compound **3** had activity against Hela and SMMC7721 with IC_{50} values of 21.45 ± 1.56 and 18.31 ± 2.11 μg/mL, but no inhibit activity against Bcap37 (IC_{50} > 40 μg/mL).

Table 2. Cytotoxic effects of compounds **1-4** against tumor cell lines (72h)

Compound	**IC_{50} (μg/mL) ± SD**		
	Hela	**SMMC7721**	**Bcap37**
Illigerine B (**1**)	12.40 ± 0.78	32.61 ± 2.05	28.69 ± 1.80
Laurodionine B (**2**)	11.77 ± 1.02	20.83 ± 1.80	32.89 ± 2.84
N-formyl-laurolitsine (**3**)	21.45 ± 1.56	18.31 ± 2.11	> 40
Illigerine A (**4**)	18.39 ± 2.06	15.62 ± 1.75	38.21 ± 4.27
DDP (Positive control)	5.70 ± 0.37	6.40 ± 0.41	8.90 ± 0.57

We reported the isolation, chemical structure characterization and cytotoxic activity of the new aporphine alkaloid, illigerine B (**1**). This work demonstrated that aporphine alkaloids are typical bioactive compounds of the genus *Illigera*, and might be useful as characteristic markers in chemotaxonomic research.

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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References

[1] L. M. Conserva, C. A. B. Pereira and J. M. Barbosa-Filho (2005). Alkaloids of the Hernandiaceae: Occurrence and a compilation of their biological activities, The Alkaloids, vol. **54**. Academic Press, San Diego, pp. 175-243.
[2] J. J. Chen, H.C. Hung, P. J. Sung, I. S. Chen and W. L. Kuo (2011). Aporphine alkaloids and cytotoxic lignans from the roots of *Illigera luzonensis*, *Phytochemistry* **72**, 523-532.
[3] V. Lakshmi, K. Pandey, S. K. Mishra, S. Srivastava, M. Mishra and S. K. Agarwal (2009). An overview of family Hernandiaceae, *Rec. Nat. Prod.* **3**(**1**), 1-22.
[4] K. S. Chen, Y. C. Wu, C. M. Teng, F. N. Ko and T. S. Wu (1997). Bioactive alkaloids from *Illigera luzonensis*, *J. Nat. Prod.* **60**, 645-647.
[5] I. S. Chen, J. J. Chen, C. Y. Duh, I. L. Tsai and C. T. Chang (1997). New aporphine alkaloids and cytotoxic constituents of *Hernandia nymphaeifolia*, *Planta Med.* **63**,154-157.
[6] Y. C. Ge, H. J. Zhang, K. W. Wang and X. F. Fan (2018). Aporphine alkaloids from *Illigera aromatica* from Guangxi Province, China, *Phytochemistry* **154**, 73-76.

[7] J. W. Dong, L. Cai, X. J. Li, J. P. Wang, R. F. Mei and Z. T. Ding (2017). Monoterpene esters and aporphine alkaloids from *Illigera aromatica* with inhibitory effects against cholinesterase and NO production in LPS-stimulated RAW264.7 macrophages, *Arch. Pharm. Res.* **40**(**12**), 1394-1402.
[8] Flora of China Editorial Committee (2008). Flora of China, vol. **7**. Science Press, Beijing, pp. 255-260.
[9] Z. F. Guo, X. B. Wang, J. G. Luo, J. Luo, J. S. Wang and L. Y. Kong (2011) A novel aporphine alkaloid from *Magnolia officinalis*, *Fitoterapia* **82**, 637-641.
[10] K. W. Wang, D. Li, B. Wu and X. J. Cao (2016). New cytotoxic dimeric and trimeric coumarins from *Chimonanthus salicifolius*, *Phytochem. Lett.* **16**, 115-120.
[11] Y. C. Ge, Y. C. Cheng, K. W. Wang and H. Wang (2017). Unusual 28, 29-nor-9, 19 cycloartane triterpenoids from Chinese medical plant *Streptocaulon griffithii* Hook, *Phytochem. Lett.* **22**, 185-188.