

## Two New Isoquinoline Alkaloids from *Litsea cubeba*

Tao Feng<sup>a,b</sup>, Rong-Ting Zhang<sup>a,b</sup>, Qin-Gang Tan<sup>a,b</sup>, Xiang-Yun Zhang<sup>a,b</sup>, Ya-Ping Liu<sup>a</sup>, Xiang-Hai Cai<sup>a</sup>, and Xiao-Dong Luo<sup>a</sup>

<sup>a</sup> State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, 132 Lanhei Road, Kunming 650204, P. R. China

<sup>b</sup> Graduate School of Chinese Academy of Sciences, Beijing 100039, P. R. China

Reprint requests to Dr. Xiao-Dong Luo. Fax: +86-871-5150227. E-mail: xdluo@mail.kib.ac.cn

*Z. Naturforsch.* **2009**, *64b*, 871–874; received March 6, 2009

Two new aporphine-type isoquinoline alkaloids, (+)-*N*-(methoxy-carbonyl)-*N*-norlauroschooltzine (**1**) and (+)-*N*-(methoxy-carbonyl)-*N*-norglaucine (**2**), were isolated from *Litsea cubeba* and identified by spectroscopic techniques (NMR, MS, UV, and IR). Their structures contain an *N*-(methoxy-carbonyl) moiety, which has seldomly been found in the natural products of these analogs. Both compounds **1** and **2** showed no antibacterial activity against *Staphylococcus aureus*.

**Key words:** *Litsea cubeba*, Isoquinoline Alkaloids, (+)-*N*-(Methoxy-carbonyl)-*N*-norlauroschooltzine, (+)-*N*-(Methoxy-carbonyl)-*N*-norglaucine, Antibacterial Activity

### Introduction

Plants of the genus *Litsea* are rich in isoquinoline alkaloids, and more than 40 isoquinoline alkaloids have been isolated and reported so far. *Litsea cubeba* (Lour.) Pers., a tree or shrub belonging to the Lauraceae family, is widely distributed in China, Indonesia, and other parts of Southeast Asia [1]. It has been historically used as a folk remedy in 'dai' ethnopharmacy for the treatments of cold and bellyache in the southwest of China [2]. As part of our work to search novel and active compounds from folk medicinal plants [3], we discovered two new aporphine alkaloids from *L. cubeba*, (+)-*N*-(methoxy-carbonyl)-*N*-norlauroschooltzine (**1**) and (+)-*N*-(methoxy-carbonyl)-*N*-norglaucine (**2**). Their structures were elucidated by extensive NMR and MS techniques. Both **1** and **2** possess an *N*-(methoxy-carbonyl) moiety in their structures, which was seldomly found in the natural products of these analogs (Fig. 1). Compounds **1** and **2** were evaluated regarding their antibacterial activity against *Staphylococcus aureus*. This paper describes the isolation, structure elucidation, and bioactivity of two new isoquinoline alkaloids.

### Results and Discussion

Compound **1** was obtained as a white amorphous powder (CH<sub>3</sub>COCH<sub>3</sub>). Its positive color reaction with Dragendorff's reagent indicated that **1** was likely to

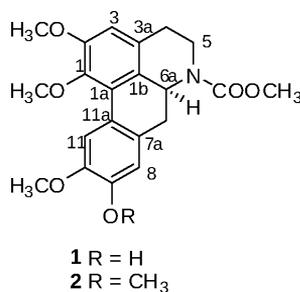


Fig. 1. Chemical structures of **1** and **2**.

be an alkaloid. The EI-MS afforded a molecular ion peak at  $m/z = 385$ , corresponding to a molecular formula C<sub>21</sub>H<sub>23</sub>NO<sub>6</sub>, which was supported by the [M+H]<sup>+</sup> peak at  $m/z = 386.1594$  (calcd. 386.1603 for C<sub>21</sub>H<sub>24</sub>NO<sub>6</sub>) in the FAB-HRMS. The <sup>1</sup>H and <sup>13</sup>C NMR data showed the characteristic pattern of an aporphine alkaloid, and the UV absorption bands at  $\lambda_{\max} = 303, 283, \text{ and } 241 \text{ nm}$  showed an aporphine alkaloid skeleton with substituents at C-1, C-2, C-9, and C-10 [4, 5]. The <sup>1</sup>H NMR spectrum of **1** showed three arene singlets at  $\delta_{\text{H}} = 8.12$  (H-11), 6.83 (H-8), and 6.62 (H-3), four methoxy signals at  $\delta_{\text{H}} = 3.90, 3.89, 3.76, \text{ and } 3.64$ , and one OH signal at  $\delta_{\text{H}} = 5.79$ , also confirmed by the IR band at  $\nu = 3506 \text{ cm}^{-1}$ . Its <sup>13</sup>C NMR spectrum indicated 21 carbons, including four methoxy ( $\delta_{\text{C}} = 59.9, 56.0, 55.8, 52.7$ ), three *sp*<sup>2</sup> methine ( $\delta_{\text{C}} = 114.4, 111.3, 110.4$ ), one *sp*<sup>3</sup> methine ( $\delta_{\text{C}} = 51.8$ ), three *sp*<sup>3</sup> methylene ( $\delta_{\text{C}} = 38.9, 34.5, 30.2$ ) and ten *sp*<sup>2</sup> quaternary carbons ( $\delta_{\text{C}} = 155.9,$

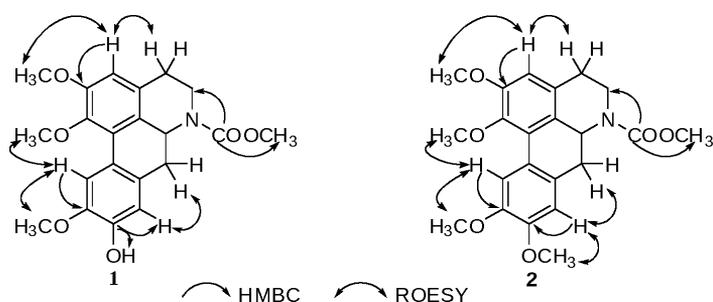


Fig. 2. Key HMBC and ROESY correlations of **1** and **2**.

151.9, 145.3, 145.1, 144.5, 130.4, 129.8, 127.7, 125.3, 123.5). A sharp absorption band at  $\nu = 1704 \text{ cm}^{-1}$  in the IR spectrum and a quaternary carbon at  $\delta_{\text{C}} = 157.5$  in the  $^{13}\text{C}$  NMR spectrum indicated the presence of a carbamate moiety [6]. The mass fragment at  $m/z = 298$  [ $\text{M}-(\text{CH}_2\text{-N-COOCH}_3)^+$ ] in the EI-MS spectrum supported the *N*-carbamate group [7], and the HMBC correlations (Fig. 2) of  $\delta_{\text{C}} = 157.5$  with  $\delta_{\text{H}} = 3.75$  (MeO), 2.88 (H-5a), and 4.43 (H-5b) confirmed the *N*-(methoxy-carbonyl) group. The positions of three other methoxy groups were decided on the basis of an ROESY experiment (Fig. 2). The ROESY correlations of H-11 with signals of two MeO groups ( $\delta_{\text{H}} = 3.90$  and 3.64, respectively) located these two methoxy groups at C-1 and C-10, the ROESY correlation of H-1 with the signal of one MeO ( $\delta_{\text{H}} = 3.89$ ) indicated that this MeO is placed at C-2. The HMBC correlations of C-10 ( $\delta_{\text{C}} = 145.3$ ) with MeO ( $\delta_{\text{H}} = 3.90$ ), and H-11 ( $\delta_{\text{H}} = 8.12$ ) indicated that this MeO should be connected to C-10 (Fig. 2), so the other MeO unit ( $\delta_{\text{H}} = 3.64$ ) should be connected to C-1. Besides, the key HMBC correlations of C-9 with H-8 ( $\delta_{\text{H}} = 6.83$ ) and the signal of the OH group ( $\delta_{\text{H}} = 5.79$ ) indicated that the OH should be placed at C-9 (Fig. 2). The above data have shown that compound **1** is very similar to laurosoltzine [8]. Since the absolute configuration of aporphine alkaloids was determined by the specific rotation [9], the positive specific rotation ( $[\alpha]_{\text{D}}^{20} = +108.3$ ) of **1** determined the *S*-form of C-6a. Compound **1** was finally identified as (+)-*N*-(methoxy-carbonyl)-*N*-norlaurosoltzine.

Compound **2** was isolated as a colorless, amorphous powder. A molecular ion peak at  $m/z = 399$  in the EI-MS and an  $[\text{M}+\text{H}]^+$  peak at  $m/z = 400.1753$  in the FAB-HRMS afforded a molecular formula  $\text{C}_{22}\text{H}_{25}\text{NO}_6$ . The UV absorptions at  $\lambda_{\text{max}} = 305$ , 285, and 241 nm displayed a 1,2,9,10-tetrasubstituted aporphine alkaloid. All the NMR data indicated that the structure of **2** was closely relative to that of **1**, ex-

cept that the OH-9 was replaced by a methoxyl group ( $\delta_{\text{H}} = 3.91$ ;  $\delta_{\text{C}} = 55.8$ ). Thus, compound **2** was suggested to be a 1,2,9,10-tetramethoxy aporphine alkaloid, similar to glaucine [10]. The HMBC correlation of  $\delta_{\text{H}} = 3.70$  (MeO) with  $\delta_{\text{C}} = 156.2$  (C=O) and the EI-MS fragment at  $m/z = 312$  [ $\text{M}-(\text{CH}_2\text{-N-COOCH}_3)^+$ ] also suggested one *N*-(methoxy-carbonyl) moiety in the structure of **2**. The positions of the methoxy groups were located by ROESY and HMBC correlations (Fig. 2). The ROESY correlation of  $\delta_{\text{H}} = 6.63$  (H-3) with 3.89 (MeO) located this MeO at C-2. The ROESY correlation of  $\delta_{\text{H}} = 6.78$  (H-8) with 3.91 (MeO) positioned this MeO at C-9. The HMBC correlation of  $\delta_{\text{C}} = 148.1$  (C-10) with  $\delta_{\text{H}} = 8.15$  (H-11) and 3.91 (MeO) indicated that this MeO group was located at C-10, and the last MeO group ( $\delta_{\text{H}} = 3.65$ ) thus must be at C-1. The positive specific rotation data ( $[\alpha]_{\text{D}}^{20} = +97.4$ ) indicated the *S*-form of C-6a. Compound **2** was finally determined as (+)-*N*-(methoxy-carbonyl)-*N*-norglaucine.

Compounds **1** and **2** were tested for their antibacterial activity against *Staphylococcus aureus*. However, they exhibited no activity.

## Experimental Section

### General

Melting points were obtained on an X-4 micro melting point apparatus. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy using KBr pellets. 1D and 2D NMR spectra were run on Bruker DRX-500 and AM-400 spectrometers with TMS as internal standard. Chemical shifts ( $\delta$ ) were expressed in ppm with reference to the solvent signals. EI-MS spectra were obtained on a VG Autospec-3000 spectrometer. HRMS ((+)-FAB) spectra were measured on an API-Qstar-Pulsar-1 spectrometer. Column chromatography was performed on silica gel (200–300 mesh, Qingdao Haiyang Chemical Co.

Table 1. <sup>1</sup>H and <sup>13</sup>C NMR data of **1** and **2** in CDCl<sub>3</sub> (δ in ppm, J in Hz).

Position	<b>1</b>		<b>2</b>	
	δ <sub>H</sub>	δ <sub>C</sub>	δ <sub>H</sub>	δ <sub>C</sub>
1		144.5 (s)		144.6 (s)
1a		127.7 (s)		127.6 (s)
1b		129.8 (s)		129.6 (s)
2		151.9 (s)		151.9 (s)
3	6.62 (1H, s)	114.4 (d)	6.63 (1H, s)	110.4 (d)
3a		125.3 (s)		125.3 (s)
4	2.62 (1H, d, 15.2)	30.2 (t)	2.63 (1H, d, 14.8)	30.2 (t)
	2.86 (1H, m)		2.89 (1H, m)	
5	2.99 (1H, m)	38.9 (t)	3.00 (1H, m)	39.0 (t)
	4.43 (1H, brs)		4.43 (1H, brs)	
6a	4.70 (1H, m)	51.8 (d)	4.71 (1H, m)	51.8 (d)
7	2.74 (1H, m)	34.5 (t)	2.74 (1H, m)	34.9 (t)
	2.81 (1H, m)		2.85 (1H, m)	
7a		123.5 (s)		124.0 (s)
8	6.83 (1H, s)	110.4 (d)	6.78 (1H, s)	111.0 (d)
9		145.1 (s)		147.3 (s)
10		145.3 (s)		148.1 (s)
11	8.12 (1H, s)	111.3 (d)	8.15 (1H, s)	111.6 (d)
11a		130.4 (s)		129.8 (s)
1-OCH <sub>3</sub>	3.64 (3H, s)	59.9 (q)	3.65 (3H, s)	59.9 (q)
2-OCH <sub>3</sub>	3.89 (3H, s)	55.8 (q)	3.89 (3H, s)	55.7 (q)
9-OH	5.79 (1H, s)			
9-OCH <sub>3</sub>			3.91 (3H, s)	55.8 (q)
10-OCH <sub>3</sub>	3.90 (3H, s)	56.0 (q)	3.93 (3H, s)	55.9 (q)
COOCH <sub>3</sub>		155.9 (s)		156.2 (s)
COOCH <sub>3</sub>	3.75 (3H, s)	52.7 (q)	3.70 (3H, s)	52.6 (q)

Ltd.) and RP-18 gel (20–45 μm, Fuji Silysia Chemical Ltd.). Fractions were monitored by TLC (GF 254, Qingdao Haiyang Chemical Co., Ltd.), and spots were visualized by heating silica gel plates sprayed with 10% H<sub>2</sub>SO<sub>4</sub> in EtOH or with Dragendorff's reagent.

#### Plant material

The aerial parts of *L. cubeba* were collected in Yunnan Province, P. R. China, in April 2007, and identified by Mr. Jing-Yun Cui, Xishuangbanna Tropic Botanical Garden, Chinese Academy of Sciences. A voucher specimen (no. 20070428) has been deposited in Kunming Institute of Botany, Chinese Academy of Sciences.

#### Extraction and isolation

An ethanol extract (30 L × 4) of the aerial parts of *L. cubeba* (10 kg) was concentrated to dryness, the residue dissolved in 5% HCl (1 L × 2), and filtered. The filtrate

was basified using 1% ammonia water to pH = 9–10, then the basic solution was partitioned with EtOAc to give a total alkaloidal fraction (47 g). The latter was chromatographed on a silica gel column (CHCl<sub>3</sub> : CH<sub>3</sub>COCH<sub>3</sub> = 1 : 0 → 1 : 1) to give three fractions (1–3). Fraction 1 (17.8 g) was further separated by silica gel chromatography to afford five subfractions (1a–1e). Fraction 1a (2.2 g) was subjected to a RP-18 column (MeOH : H<sub>2</sub>O = 8 : 2) to afford **2** (7 mg). Fraction 1b (2.2 g) was subjected to a RP-18 column (MeOH : H<sub>2</sub>O = 6 : 4) to afford **1** (134 mg).

#### (+)-*N*-(Methoxy-carbonyl)-*N*-norlauroschoztzine (**1**)

Brown, amorphous powder. – UV (CHCl<sub>3</sub>): λ<sub>max</sub> (log ε) = 303 (4.25), 283 (4.26), 241 (4.35), 230 (4.01), 215 (4.00) nm. – [α]<sub>D</sub><sup>20</sup> = +108.3 (c = 0.20, CHCl<sub>3</sub>). – IR (KBr): ν = 3506 (OH), 1701 (C=O), 1509, 1461, 1403, 1199, 1016, 768 cm<sup>-1</sup>. – <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, 20 °C, TMS) and <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, 20 °C) spectral data: see Table 1. – MS (EI, 70 eV): *m/z* (%) = 385 (85), 298 (30), 297 (100), 283 (40), 267 (15), 251 (10). – HRMS ((+)-FAB): *m/z* = 386.1594 (calcd. 386.1603 for C<sub>21</sub>H<sub>25</sub>NO<sub>6</sub>, [M+H]<sup>+</sup>).

#### (+)-*N*-(Methoxy-carbonyl)-*N*-norglaucine (**2**)

White, amorphous powder. – UV (CHCl<sub>3</sub>): λ<sub>max</sub> (log ε) = 305 (4.09), 285 (4.07), 241 (4.25), 216 (3.91), 202 (3.89) nm. – [α]<sub>D</sub><sup>20</sup> = +97.4 (c = 0.20, CHCl<sub>3</sub>). – IR (KBr): ν = 1704 (C=O), 1515, 1452, 1253, 1200, 1113, 1014, 768 cm<sup>-1</sup>. – <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, 20 °C, TMS) and <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, 20 °C) spectral data: see Table 1. – MS (EI, 70 eV): *m/z* (%) = 399 (100), 312 (20), 311 (87), 297 (26), 281 (12), 265 (15). – HRMS ((+)-FAB): *m/z* = 400.1573 (calcd. 400.1760 for C<sub>22</sub>H<sub>27</sub>NO<sub>6</sub>, [M+H]<sup>+</sup>).

#### Bioassay

The microtiter plate-based antibacterial activity assay was tested as described in the literature [11]. The bacterial strain was *Staphylococcus aureus* CMCC26001 (CMCC, National Center for Medical Culture Collections, Beijing, China). The final concentration of the test compounds was 1 μg mL<sup>-1</sup>.

#### Acknowledgement

The authors are grateful to the National Basic Research Program of China (973 Program 2009CB522300), the National Natural Science Foundation of China (C30670214), and the Chinese Academy of Sciences (*XiBuZhiGuang* Project) for partly financial support.

- [1] X.W. Li, *Flora Republicae Popularis Sinicae, Tomus 31*, Science Press, Beijing, **1982**, pp. 271–272.  
[2] Kunming Institute of Botany, Chinese Academy of

Sciences, *Flora Yunnanica, Tomus 3*, Science Press, Beijing, 1983, p. 23.

- [3] T. Feng, X.H. Cai, Z.Z. Du, X.D. Luo, *Helv. Chim. Acta* **2008**, *91*, 2247–2251.

- [4] M. Shamma, *The Isoquinoline Alkaloids*, Vol. 25, Academic Press, New York, **1972**, p. 221.
- [5] B. Tantisewie, T. Pharadai, M. Pandhuganont, H. Guinaudeau, A. J. Freyer, M. Shamma, *J. Nat. Prod.* **1989**, 52, 652–654.
- [6] Y. Y. Chen, F. R. Chang, Y. C. Wu, *J. Nat. Prod.* **1996**, 59, 904–906.
- [7] F. R. Chang, C. Y. Chen, P. H. Wu, R. Y. Kuo, Y. C. Chang, Y. C. Wu, *J. Nat. Prod.* **2000**, 63, 746–748.
- [8] K. G. R. Pachler, R. R. Arndt, W. H. Baarschers, *Tetrahedron* **1965**, 21, 2159–2167.
- [9] C. T. Montgomery, A. J. Freyer, H. Guinaudeau, *J. Nat. Prod.* **1985**, 48, 833–834.
- [10] S. R. Johns, J. A. Lambertson, C. S. Li, A. A. Sioumis, *Aust. J. Chem.* **1970**, 23, 423–426.
- [11] S. D. Sarker, L. Nahar, Y. Kumarasamy, *Methods* **2007**, 42, 321–324.