Two New Isoquinoline Alkaloids from *Litsea cubeba*

Tao Feng[a,b], Rong-Ting Zhang[a,b], Qin-Gang Tan[a,b], Xiang-Yun Zhang[a,b], Ya-Ping Liu[a], Xiang-Hai Cai[a], and Xiao-Dong Luo[a]

[a] 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
[b] 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

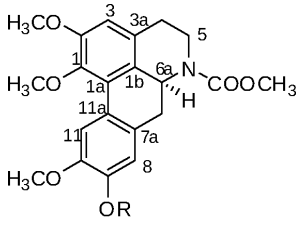
Z. Naturforsch. **2009**, *64b,* 871 – 874; received March 6, 2009

Two new aporphine-type isoquinoline alkaloids, (+)-*N*-(methoxy-carbonyl)-*N*-norlauroscholtzine (**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*-norlauroscholtzine, (+)-*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*-norlauroscholtzine (**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.



1 R = H
2 R = CH_3

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

Results and Discussion

Compound **1** was obtained as a white amorphous powder (CH_3COCH_3). Its positive color reaction with Dragendorff's reagent indicated that **1** was likely to be an alkaloid. The EI-MS afforded a molecular ion peak at *m/z* = 385, corresponding to a molecular formula $C_{21}H_{23}NO_6$, which was supported by the $[M+H]^+$ peak at *m/z* = 386.1594 (calcd. 386.1603 for $C_{21}H_{24}NO_6$) in the FAB-HRMS. The ^1H and ^{13}C NMR data showed the characteristic pattern of an aporphine alkaloid, and the UV absorption bands at λ_{max} = 303, 283, and 241 nm showed an aporphine alkaloid skeleton with substituents at C-1, C-2, C-9, and C-10 [4, 5]. The ^1H NMR spectrum of **1** showed three arene singlets at δ_H = 8.12 (H-11), 6.83 (H-8), and 6.62 (H-3), four methoxy signals at δ_H = 3.90, 3.89, 3.76, and 3.64, and one OH signal at δ_H = 5.79, also confirmed by the IR band at ν = 3506 cm^{-1}. Its ^{13}C NMR spectrum indicated 21 carbons, including four methoxy (δ_C = 59.9, 56.0, 55.8, 52.7), three sp^2 methine (δ_C = 114.4, 111.3, 110.4), one sp^3 methine (δ_C = 51.8), three sp^3 methylene (δ_C = 38.9, 34.5, 30.2) and ten sp^2 quaternary carbons (δ_C = 155.9,

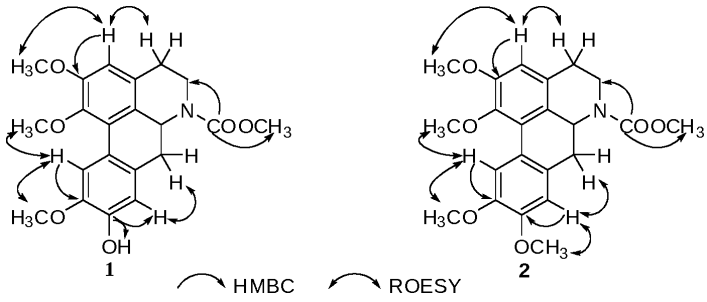


Fig. 2. Key HMBC and ROESY correlations of **1** and **2**.

151.9, 145.3, 145.1, 144.5, 130.4, 129.8, 127.7, 125.3, 123.5). A sharp absorption band at ν = 1704 cm^{-1} in the IR spectrum and a quaternary carbon at δ_C = 157.5 in the ^{13}C NMR spectrum indicated the presence of a carbamate moiety [6]. The mass fragment at *m/z* = 298 [M–(CH_2-N-$COOCH_3$)]$^+$ in the EI-MS spectrum supported the *N*-carbamate group [7], and the HMBC correlations (Fig. 2) of δ_C = 157.5 with δ_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 (δ_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 (δ_H = 3.89) indicated that this MeO is placed at C-2. The HMBC correlations of C-10 (δ_C = 145.3) with MeO (δ_H = 3.90), and H-11 (δ_H = 8.12) indicated that this MeO should be connected to C-10 (Fig. 2), so the other MeO unit (δ_H = 3.64) should be connected to C-1. Besides, the key HMBC correlations of C-9 with H-8 (δ_H = 6.83) and the singal of the OH group (δ_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 lauroscholtzine [8]. Since the absolute configuration of aporphine alkaloids was determined by the specific rotation [9], the positive specific rotation ($[\alpha]_D^{20}$ = +108.3) of **1** determined the *S*-form of C-6a. Compound **1** was finally identified as (+)-*N*-(methoxy-carbonyl)-*N*-norlauroscholtzine.

Compound **2** was isolated as a colorless, amorphous powder. A molecular ion peak at *m/z* = 399 in the EI-MS and an [M+H]$^+$ peak at *m/z* = 400.1753 in the FAB-HRMS afforded a molecular formula $C_{22}H_{25}NO_6$. The UV absorptions at λ_{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**, except that the OH-9 was replaced by a methoxyl group (δ_H = 3.91; δ_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 δ_H = 3.70 (MeO) with δ_C = 156.2 (C=O) and the EI-MS fragment at *m/z* = 312 ([M–(CH_2-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 δ_H = 6.63 (H-3) with 3.89 (MeO) located this MeO at C-2. The ROESY correlation of δ_H = 6.78 (H-8) with 3.91 (MeO) positioned this MeO at C-9. The HMBC correlation of δ_C = 148.1 (C-10) with δ_H = 8.15 (H-11) and 3.91 (MeO) indicated that this MeO group was located at C-10, and the last MeO group (δ_H = 3.65) thus must be at C-1. The positive specific rotation data ($[\alpha]_D^{20}$ = +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 (δ) 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. ^{1}H and ^{13}C NMR data of **1** and **2** in $CDCl_3$ (δ in ppm, *J* in Hz).

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
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_3	3.64 (3H, s)	59.9 (q)	3.65 (3H, s)	59.9 (q)
2-OCH_3	3.89 (3H, s)	55.8 (q)	3.89 (3H, s)	55.7 (q)
9-OH	5.79 (1H, s)			
9-OCH_3			3.91 (3H, s)	55.8 (q)
10-OCH_3	3.90 (3H, s)	56.0 (q)	3.93 (3H, s)	55.9 (q)
$COOCH_3$		155.9 (s)		156.2 (s)
$COOCH_3$	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_2SO_4 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 $^0/_{00}$ 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 ($CHCl_3 : CH_3COCH_3$ = 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 ($MeOH : H_2O$ = 8 : 2) to afford **2** (7 mg). Fraction 1b (2.2 g) was subjected to a RP-18 column ($MeOH : H_2O$ = 6 : 4) to afford **1** (134 mg).

*(+)-N-(Methoxy-carbonyl)-N-norlauroscholtzine (**1**)*

Brown, amorphous powder. – UV ($CHCl_3$): λ_{max} (log ε) = 303 (4.25), 283 (4.26), 241 (4.35), 230 (4.01), 215 (4.00) nm. – $[\alpha]_D^{20}$ = +108.3 (*c* = 0.20, $CHCl_3$). – IR (KBr): ν = 3506 (OH), 1701 (C=O), 1509, 1461, 1403, 1199, 1016, 768 cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$, 20 °C, TMS) and ^{13}C NMR (100 MHz, $CDCl_3$, 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 $C_{21}H_{25}NO_6$, $[M+H]^+$).

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

White, amporhous powder. – UV ($CHCl_3$): λ_{max} (log ε) = 305 (4.09), 285 (4.07), 241 (4.25), 216 (3.91), 202 (3.89) nm. – $[\alpha]_D^{20}$ = +97.4 (*c* = 0.20, $CHCl_3$). – IR (KBr): ν = 1704 (C=O), 1515, 1452, 1253, 1200, 1113, 1014, 768 cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$, 20 °C, TMS) and ^{13}C NMR (100 MHz, $CDCl_3$, 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 $C_{22}H_{27}NO_6$, $[M+H]^+$).

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^{-1}.

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 Insititute 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. Lamberton, 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.