

Two New Isoquinoline Alkaloids from *Litsea cubeba*

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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 (CH₃COCH₃). Its positive color reaction with Dragendorff’s reagent indicated that **1** was likely to

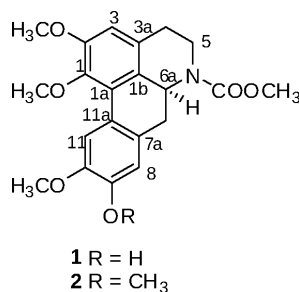


Fig. 1. Chemical structures of **1** and **2**.

be an alkaloid. The EI-MS afforded a molecular ion peak at $m/z = 385$, corresponding to a molecular formula C₂₁H₂₃NO₆, which was supported by the [M+H]⁺ peak at $m/z = 386.1594$ (calcd. 386.1603 for C₂₁H₂₄NO₆) in the FAB-HRMS. The ¹H and ¹³C 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 ¹³C NMR spectrum indicated 21 carbons, including four methoxy (δ_C = 59.9, 56.0, 55.8, 52.7), three sp² methine (δ_C = 114.4, 111.3, 110.4), one sp³ methine (δ_C = 51.8), three sp³ methylene (δ_C = 38.9, 34.5, 30.2) and ten sp² quaternary carbons (δ_C = 155.9,

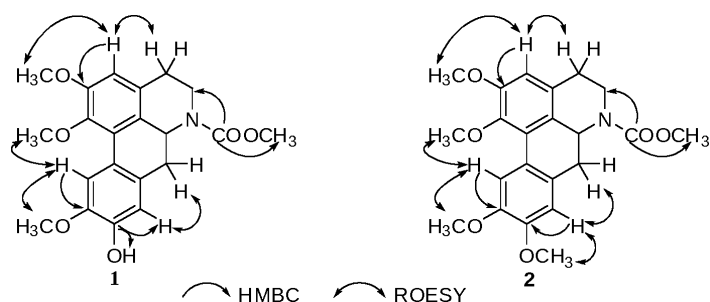


Fig. 2. Key HMBC and ROESY correlations of **1** and **2**.

151.9, 145.3, 145.1, 144.5, 130.4, 129.8, 127.7, 125.3, 123.5). A sharp absorption band at $\nu = 1704\text{ cm}^{-1}$ in the IR spectrum and a quaternary carbon at $\delta_{\text{C}} = 157.5$ in the ^{13}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 singal 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 lauroschoztine [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*-(methoxycarbonyl)-*N*-norlauroschoztine.

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*-(methoxycarbonyl)-*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. ^1H and ^{13}C NMR data of **1** and **2** in CDCl_3 (δ in ppm, J in Hz).

Position	1 δ_{H}	δ_{C}	2 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), 2.86 (1H, m)	30.2 (t)	2.63 (1H, d, 14.8), 2.89 (1H, m)	30.2 (t)
5	2.99 (1H, m), 4.43 (1H, brs)	38.9 (t)	3.00 (1H, m), 4.43 (1H, brs)	39.0 (t)
6a	4.70 (1H, m)	51.8 (d)	4.71 (1H, m)	51.8 (d)
7	2.74 (1H, m), 2.81 (1H, m)	34.5 (t)	2.74 (1H, m), 2.85 (1H, m)	34.9 (t)
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 $\times$ 4) of the aerial parts of *L. cubeba* (10 kg) was concentrated to dryness, the residue dissolved in 5% HCl (1 L $\times$ 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 $\rightarrow$ 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*-norlauroschoitzine (**1**)

Brown, amorphous powder. – UV (CHCl_3): λ_{max} ($\log \epsilon$) = 303 (4.25), 283 (4.26), 241 (4.35), 230 (4.01), 215 (4.00) nm. – $[\alpha]_{\text{D}}^{20}$ = +108.3 (c = 0.20, CHCl_3). – IR (KBr): ν = 3506 (OH), 1701 (C=O), 1509, 1461, 1403, 1199, 1016, 768 cm^{-1} . – ^1H NMR (400 MHz, CDCl_3 , 20 $^\circ\text{C}$, TMS) and ^{13}C NMR (100 MHz, CDCl_3 , 20 $^\circ\text{C}$) 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 $\text{C}_{21}\text{H}_{25}\text{NO}_6$, $[\text{M}+\text{H}]^+$).

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

White, amorphous powder. – UV (CHCl_3): λ_{max} ($\log \epsilon$) = 305 (4.09), 285 (4.07), 241 (4.25), 216 (3.91), 202 (3.89) nm. – $[\alpha]_{\text{D}}^{20}$ = +97.4 (c = 0.20, CHCl_3). – IR (KBr): ν = 1704 (C=O), 1515, 1452, 1253, 1200, 1113, 1014, 768 cm^{-1} . – ^1H NMR (400 MHz, CDCl_3 , 20 $^\circ\text{C}$, TMS) and ^{13}C NMR (100 MHz, CDCl_3 , 20 $^\circ\text{C}$) 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 $\text{C}_{22}\text{H}_{27}\text{NO}_6$, $[\text{M}+\text{H}]^+$).

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 $\mu\text{g mL}^{-1}$.

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.

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