Two Dibenzopyrrocoline Alkaloids from *Litsea cubeba*

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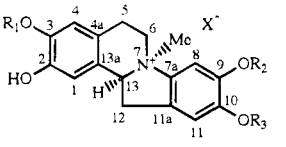
Further investigation of quaternary alkaloids from *Litsea cubeba* led to the isolation of two novel dibenzopyrrocoline alkaloids, (−)-litcubine (**1**) and (−)-litcubinine (**2**). Their structures and 7*R*,13*S* stereochemistry were elucidated by spectral analysis and chemical correlation. Including these two, only six dibenzopyrrocoline alkaloids have been isolated from natural sources.

The quaternary alkaloids in Lauraceous plants have been rarely explored despite the intensive investigation of the free bases. Recently we applied centrifugal partition chromatography, Sephadex LH-20, and ion-pair reversed-phase LC to separate these polar substances. These techniques have improved the recovery and resolution of the polar compounds and have led to the isolation of (+)-(1a*R*,1*R*)-1a-hydroxymagnocurarine from *Cryptocarya konishii* Kayata ex Kawakami and four quaternary alkaloids—(−)-magnocurarine, (−)-oblongine, (−)-8-*O*-methyl-oblongine, and xanthoplanine—from the stems of *Litsea cubeba* (Lour.) Persoon.[1] Further study of *L. cubeba* has resulted in the isolation of two novel dibenzopyrrocoline alkaloids, the isolation and structural characterization of which are presented herein.

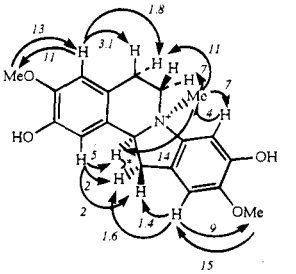
Compound **1**, isolated as the perchlorate salt, showed in the HREIMS spectrum $[M - HClO_4]^+$ at *m/z* 327.1473, corresponding to a formula of $C_{19}H_{21}NO_4$ (calcd 327.1471). The IR absorption at 3420 cm^{-1} and a bathochromic shift in the UV under alkaline conditions indicated the presence of phenolic functions. The ^{1}H-NMR spectrum (in CD_3OD) showed four singlets at δ 7.05, 6.95, 6.82, and 6.74 in the aromatic region; two MeO singlets at δ 3.87 and 3.85; one N^+-Me signal at δ 3.45; and an AMX spin system at δ 5.18 (X), 3.71 (M), and 3.13 (A) (J_{AX} = 9.2 Hz, J_{AM} = 15.6 Hz, and J_{MX} = 7.6 Hz), identified by a COSY-45 spectrum. These ^{1}H-NMR data, the presence of three methylene signals in the ^{13}C-NMR spectrum, and the ring and double-bond equivalent (10 in total) suggested **1** to be a 2,3,9,10-tetrasubstituted *N*-methyl dibenzopyrrocolinium salt.[2,3] This skeleton for **1** was confirmed by NOE studies (Chart 1).



1 R_1=R_3=Me, R_2=H, ClO_4^- salt
2 R_1=Me, R_2=R_3=H, ClO_4^- salt
3 R_1=R_2=R_3=Me, Cl^- salt
4 R_1=R_2=R_3=H, Br^- salt

The signals at δ 6.74 and 6.95 were assigned to H-1 and H-11, respectively, from their common NOE relationship to H-12's (δ 3.13 and 3.71) and the NOE of H-1 to H-13 (δ 5.18). The signals for H-12α (δ 3.71) and H-12β (δ 3.13) were distinguished by means of a NOESY spectrum, which revealed an NOE between H-13 and H-12α. The signals of H-4 and 3-OMe were designated at δ 6.82 and 3.85, respectively, from the observation of NOEs of H-4 to H-5's and 3-OMe. It was noted that the signal of H-5α (δ 3.19, ddd) was also enhanced upon irradiation of Me-7 singlet. This result would dispose H-5α at pseudoaxial position from a chemical model study. The NOEs of Me-7 to H-13 (14%) established the *cis* B/C ring junction. The striking NOEs between Me-7 and the aromatic proton H-8 (δ 7.05) established the direct linkage of the nitrogen to the bottom aromatic ring. These data consequently indicated **1** to be 2,9-dihydroxy-3,10-dimethoxy-*N*-methyldibenzopyrrocolinium perchlorate.

Chart 1. NOEs (%) of **1** (CD_3OD)



* observed in a NOESY spectrum

It has been shown that (−)-cryptaustoline chloride (**3**) possesses a 13-*S* stereochemistry.[4,5] In addition, the *cis* relationship of H-13 and *N*-Me in **3**, leading to the assignment of a *R*-configuration for *N*-7, was recently established by NOE studies.[2] Accordingly, the levorotatory optical property and *cis* B/C ring junction allow for the designation of 7-*R*,13-*S* stereochemistry for **1**, which is (7*R*,13*S*)-2,9-dihydroxy-3,10-dimethoxy-*N*-methyl-dibenzopyrrocolinium perchlorate.

The proposed structure for **1** was further supported by analysis of an HMBC spectrum optimized for J = 8 Hz. This inverted 2D NMR spectrum revealed the three-bond coupling of Me-7 protons to a quaternary aromatic carbon (C-7a, δ 140.9), which also coupled to H-11 (3-bond) and H-8 (2-bond), confirming further the skeleton for **1**. The analysis of the HMBC and HMQC spectra allowed the complete assignment of the ^{1}H- and ^{13}C-NMR data of **1** (Table 1). Among these, the signal of H-6β was designated at δ 3.62 from its direct coupling to C-6 (δ 59.7) in the HMQC. The close signals for non-oxygenated quaternary carbons, C-4a (δ 120.9), C-11a (δ 121.9), and C-13a (δ 123.4), were distinguished from

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Table 1. ^{1}H- and ^{13}C-NMR Data for Compounds **1**, **2**, and **4** (δ in ppm, CD_3OD)

	compound					
	1		**2**		**4**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	6.74 s	114.2 d	6.72 s	114.1 d	6.70 s	114.2 d
2		148.1 s		147.7 s		146.8 s
3		149.7 s		149.6 s		147.3 s
4	6.82 s	112.3 d	6.83 s	112.4 d	6.68 s	115.8 d
4a		120.9 s		121.0 s		120.9 s
5	3.19 ddd[a] (α)	25.2 t	3.23 ddd (α)	25.2 t	3.16 ddd (α)	25.0 t
	2.99 ddd (β)		3.00 ddd (β)		2.92 ddd (β)	
6	3.80 ddd (α)	59.7 t	3.80 ddd (α)	59.9 t	3.79 ddd (α)	60.0 t
	3.62 ddd (β)		3.66 ddd (β)		3.64 ddd (β)	
Me-7	3.45 s	49.9 q	3.45 s	49.8 q	3.46 s	49.9 q
7a		140.9 s		139.8 s		139.9 s
8	7.05 s	104.8 d	7.09 s	104.8 d	7.12 s	104.9 d
9		152.3 s		147.9 s		147.9 s
10		152.7 s		149.6 s		149.6 s
11	6.95 s	109.0 d	6.82 s	112.8 d	6.83 s	112.8 d
11a		121.9 s		124.5 s		124.5 s
12	3.71 dd (α)	37.6 t	3.67 dd (α)	37.3 t	3.67 dd (α)	37.5 t
	3.13 dd (β)		3.10 dd (β)		3.10 dd (β)	
13	5.18 dd	76.2 d	5.16 dd	76.2 d	5.15 dd	76.3 d
13a		123.4 s		123.2 s		121.9 s
MeO-3	3.85 s	56.5 q	3.85 s	56.5 q		
MeO-10	3.87 s	56.7 q				

[a] Proton coupling constants (J, in Hz) of **1**: $J_{5\alpha-5\beta} = 18.0$, $J_{5\alpha-6\alpha} = 3.7$, $J_{5\alpha-6\beta} = 12.0$, $J_{5\beta-6\alpha} = 3.7$, $J_{5\beta-6\beta} = 4.6$, $J_{6\alpha-6\beta} = 12.0$, $J_{12\alpha-12\beta} = 15.6$, $J_{12\alpha-13} = 7.5$, $J_{12\beta-13} = 9.2$. The corresponding proton coupling constants in **2** and **4** are similar to those in **1**.

the three-bond coupling to H-1 (C-4a), H-4 (C-13a), and H-8 (C-11a). Likewise, the oxygenated C-2 (δ 148.1), C-3 (δ 149.7), C-9/ (δ 152.3), and C-10 (δ 152.7) were assigned from their respective three-bond coupling to H-4, H-1, H-11, and H-8. This unambiguous assignment revises several ^{13}C NMR data (C-1, C-2, C-3, C-4a, C-7a, C-10, C-11, C-11a, and C-13a) assigned for cryptowoline.[3]

Compound **2**, isolated as the perchlorate salt, had a molecular formula of $C_{18}H_{19}NO_4$ assigned from EIMS $[M\text{-}HClO_4]^+$ at m/z 313, being 14 mass units fewer than that of **1**. Its ^{1}H-NMR spectrum (Table 1) was very similar to that of **1** except for the presence of only one MeO singlet instead of two. The MS and ^{1}H-NMR data indicated **2** to be simple a mono *O*-demethylated derivative of **1**. This suggestion was confirmed by NOE studies. Except for the lack of an enhancement for a MeO signal upon irradiation of H-11 (δ 6.82), the remaining NOEs for **2** are almost identical to those of **1**. The optical property and CD spectrum of **2** are also similar to those of **1**. Hence, **2** is (−)-(7*R*,13*S*)-2,9,10-trihydroxy-3-methoxy-*N*-methyldibenzopyrrocolinium perchlorate.

The ^{13}C-NMR data of **2** (Table 1) were assigned from correlation with those of **1** and analysis of an HMBC spectrum.

To confirm the stereochemistry of **1** and **2**, (−)-(7*R*,-13*S*)-2,3,9,10-tetrahydroxy-*N*-methyldibenzopyrrocolinium bromide **4** was prepared from (+)-(1*S*)-reticuline by *O*-demethylation with 47% HBr to give (+)-(1*S*)-laudanosoline hydrobromide, followed by enzymatic oxidative coupling with peroxidase.[5] The NOEs of *N*-Me to H-13 in **4** were also observed. The CD data of **4** displayed three negative Cotton effects at 299, 236, and 207 nm and a positive Cotton effect at 280 nm. The CD curves of **1** and **2**, being similar in shape to that of **4**, further confirmed their stereochemistry. The ^{1}H- and ^{13}C-NMR data of **4** (Table 1) were assigned from analysis of HMBC and HMQC data.

To our knowledge, compounds **1** and **2** are novel dibenzopyrrocolines. They are named (−)-litcubine and (−)-litcubinine, respectively.

Dibenzopyrrocolines are very rarely present in nature. Only four of such compounds—namely, (−)-cryptaustoline, (−)-cryptowoline, (−)-cryptowolidine, and (−)-cryptowolinol—have been previously isolated from the Lauraceous plants (*Cryptocarya bowiei*,[6] *C. phyllostemon*, and *C. oubatchensis*[3]). Hence, the isolation of two new members (**1** and **2**) from the same family may be meaningful from the chemotaxonomic point of view.

Experimental Section

General Experimental Procedures. Mps were measured on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were recorded on a JASCO DIP-181 digital polarimeter. IR spectra were recorded on a JASCO A-100 infrared spectrometer. UV spectra were measured on a Hitachi 150-20 spectrophotometer. CD spectra were measured on a JASCO J-710 spectropolarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AM-300 or AMX-400 spectrometer in CD_3OD using residual solvent peak as a reference; 2D NMR spectra were recorded using Bruker's standard pulse program: in the HMQC and HMBC experiment Δ = 1 s and J = 140 Hz and 8 Hz, respectively. The correlation maps consisted of 256 × 1 K data points per spectrum, each composed of 32 to 64 transients. MS spectra were recorded on a JEOL JMS-HX 110 (HREIMS) and a Finnigan TSQ-700 (EIMS) mass spectrometer.

Plant Material. See the work of Lee *et al.*[1]

Extraction and Isolation. This part of the work is the extension of study on isolation of quaternary alkaloids from the roots of *Litsea cubeba*. Fraction II from the previous CPC fractionation of the crude quaternary alkaloids[1] yielded compound **2** (17 mg) eluted out after magnocurarine from the Sephadex LH-20 column (300 mL) eluted with MeOH and MeOH-H_2O (1:1). Fraction

III from the same CPC fractionation gave two fractions (A, 0.31 g; B, 0.27 g) by Sephadex LH-20 column.[1] Further separation of fraction A by repeated Lobar RP-18 (10 mm × 240 mm) CC eluted with MeOH-0.1 M $HClO_{4(aq)}$ (13:27) gave four fractions of which fraction 2 yielded **1** (27 mg) after separation on a Sephadex LH-20 column eluted with MeOH.

(−)-Litcubine (1). Amorphous solid; $[\alpha]^{25.5}_D$ −111.0° (*c* 1.0, MeOH): IR (KBr) ν max 3420 (br s, OH), 2960 (m), 2940 (m), 1610 (m), 1518 (s), 1450 (s), 1240 (s), 1120 (s), 840 (w) cm^{-1}; UV (MeOH) λ max (log ϵ) 233 (sh, 4.06), 287 (3.96) nm; λ max (MeOH + NaOH) (log ϵ) 249 (4.14), 304 (3.99) nm; CD (*c* 2.34 × 10^{-5} M, MeOH) $[\theta]_{295}$ 0°, $[\theta]_{277}$ +2070 °, $[\theta]_{261}$ 0 °, $[\theta]_{251}$ +2040 °, $[\theta]_{248}$ +0 °, $[\theta]_{238}$ −31 630 °, $[\theta]_{217}$ −7820 °, $[\theta]_{206}$ −122 800 °; ^{1}H- and ^{13}C-NMR data, see Table 1; HMBC data H-1 to C-2, C-3, C-4a, and C-13; H-4 to C-2, C-3, C-5, and C-13a; Me-7 to C-6, C-7a, and C-13; H-8 to C-7a, C-9, C-10, and C-11a; H-11 to C-7a, C-9, C-10, C-11a, and C-12; H-12α to C-7a and C-11a; H-12β to C-7a, C-11a, C-13, and C-13a; H-13 to C-1, C-4a, Me-7, C-12, and C-13a; HREIMS *m/z* $[M - HClO_4]^+$ 327.1473 (calcd for $C_{19}H_{21}NO_4$, 327.1471); EIMS (20 eV) *m/z* $[M - HClO_4]^+$ 327 (90), 313 (100), 312 (80), 298 (72), and 284 (7).

(−)-Litcubinine (2). Amorphous solid; $[\alpha]^{25.5}_D$ −144.0° (*c* 0.5, MeOH); IR (KBr) ν max 3412 (br s, OH), 2960 (m), 2940 (m), 1618 (w), 1518 (m), 1450 (m), 1270 (m), 1242 (m), 1120 (s), 1088 (s), 870 (w), 838 (w) cm^{-1}; UV (MeOH) λ max (log ϵ) 234 (sh, 4.03), 290 (3.99) nm; λ max (MeOH+NaOH) (log ϵ) 248 (4.10), 305 (4.08) nm; CD (*c* 2.42 × 10^{-5} M, MeOH) $[\theta]_{328}$ 0 °, $[\theta]_{299}$ −1,800 °, $[\theta]_{293}$ 0 °, $[\theta]_{281}$ +2,410 °, $[\theta]_{268}$ 0 °, $[\theta]_{252}$ −5,000 ° (sh), $[\theta]_{237}$ −29,570 °, $[\theta]_{222}$ −19,640 °, $[\theta]_{207}$ −124,900 °; ^{1}H- and ^{13}C-NMR data, see Table 1; NOE data (CD_3OD) H-1 to H-12α (2%), H-12β (1%), and H-13 (5%); MeO-3 to H-4 (11%); H-4 to MeO-3 (8%), H-5α (1%), and H-5β (3%); Me-7 to H-5α (15%), H-6α (8%), H-8 (8%), and H-13 (15%); H-8 to Me-7 (5%); H-11 to H-12α (3%) and H-12β (1%); EIMS (20 eV) *m/z* $[M - HClO_4]^+$ 313 (47), 311 (90), 297 (96), 296 (100), 268 (45), 190 (95), 175 (28).

Preparation of (−)-(7*R*,13*S*)-2,3,9,10-Tetrahydroxy-*N*-methyldibenzopyrrocolinium Bromide (4). The solution of (+)-reticuline (300 mg), isolated from *Neolitsea konishii*,[7] in 47% HBr (3 mL) was stirred under reflux for 3 h. The reaction solution was kept at 4 °C overnight, and the resultant crystals (275 mg) were collected by suction. To this essentially pure (+)-(1-*S*)-laudanosoline hydrobromide (250 mg) dissolved in phosphate buffer (12.5 mL, pH 5) in a 50-mL flask was added horseradish peroxidase (1.5 mg, type VI-A, Sigma) and 3% H_2O_2 (1.5 mL).[5] The solution was stirred at 25 °C for 7 h, then was adjusted to pH 0.5 with 24% HBr, and was kept at 4 °C overnight to give 203 mg of precipitate. The pure product **4** (102 mg) was obtained by passing the precipitate through a Sephadex LH-20 column eluted with MeOH. Physical data of **4**: mp 273 °C (color change), >300° (melted); $[\alpha]^{22}_D$ −207° (*c* 0.43, H_2O) (lit.[5] −170 °C (*c* 0.5, H_2O)); CD (*c* 2.63 × 10^{-5} M, MeOH) $[\theta]_{313}$ 0 °, $[\theta]_{299}$ −2570 °, $[\theta]_{291}$ 0 °, $[\theta]_{280}$ +2520 °, $[\theta]_{265}$ 0 °, $[\theta]_{250}$ −3030 ° (sh), $[\theta]_{236}$ −39 200 °, $[\theta]_{219}$ −18 200 °, $[\theta]_{207}$ − 181 000 °; ^{1}H- and ^{13}C-NMR data, see Table 1; NOE data (CD_3OD) H-1 to H-13 (5%); Me-7 to H-5α (10%), H-6α (6%), H-8 (16%), and H-13 (17%); HMBC data H-1 to C-2, C-3, and C-4a; H-4 to C-2, C-3, C-5, and C-13a; H-5α to C-4α; Me-7 to C-6, C-7a, and C-13; H-8 to C-7a, C-9, C-10, and C-11a; H-11 to C-7a, C-9, C-10, and C-12; H-12α to C-7a and C-11a; H-12β to C-11a, C-13, and C-13a; H-13 to C-12 and C-13a; EIMS (20 eV) *m/z* $[M\text{-}HBr]^+$ 299 (49), 297 (100), 285 ($[M - MeBr]^+$, 73), 283 (87), 178 (44), 162 (38).

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