Two New Aporphine Alkaloids from *Litsea glutinosa*

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Chemical examination of the BuOH extract of the leaves and twigs of *Litsea glutinosa* collected from Xishuangbanna resulted in the isolation of two new aporphine alkaloids, namely litseglutine A (**1**) and B (**2**), along with two known aporphine alkaloids, boldine (**3**) and laurolitsine (**4**). The structures of the new alkaloids have been elucidated on the basis of spectra analysis as 6-demethyl-9-methoxy-1,2-(methylenedioxy)aporphin-10-ol (=6,7,7a,8-tetrahydro-10-methoxy-5*H*-benzo[*g*]-1,3-benzodioxolo[6,5,4-*de*]quinolin-11-ol; **1**) and 1,10,11-trimethoxyaporphin-2-ol (=5,6,6a,7-tetrahydro-1,10,11-trimethoxy-6-methyl-4*H*-dibenzo[*de*,*g*]quinolin-2-ol; **2**).

1. Introduction. – The genus *Litsea* is a member of the Lauraceae and comprises nearly 200 species, which are distributed widely throughout tropical and subtropical Asia, North America, and subtropical South America. *Litsea glutinosa* is an evergreen medium-sized tree. Its barks and leaves are used as a demulcent and mild astringent for diarrhoea and dysentery, the roots are used for poulticing sprains and bruises, and the oil extracted from the seeds is used in the treatment of rheumatism [1]. Recently, research disclosed that the MeOH extract of *Litsea glutinosa* bark effectively inhibited both Gram-positive and Gram-negative bacteria. The results justify the reported uses in diarrhoea and dysentery [2]. A previous paper reported the presence of some alkaloids from the barks of *Litsea glutinosa* [3]. In our further biological evaluation, the BuOH extract of the leaves and twigs of *Litsea glutinosa* were shown to exhibit significant cytotoxic activity against human Hela cell lines *in vitro*. We report herein on the isolation and characterization of two new aporphine alkaloids, litseglutine A (**1**) and B (**2**).

2. Results and Discussion. – Compound **1** was obtained as optically active brown solid. Its molecular formula $C_{18}H_{17}NO_4$ based on HR-ESI-MS (*m*/*z* 312.1233 ($[M+1]^+$) indicated that **1** possessed eleven degrees of unsaturation. Compound **1** was positive to *Dragendorff*'s test and $FeCl_3$ solution. The UV absorption maxima at 218, 282, and 306 nm were characteristic of a 1,2,9,10-tetrasubstituted aporphine [4]. Based on further spectroscopic data (see *Table* and *Fig. 2*), the structure given in *Fig. 1* was assigned to **1** which was named litseglutine A.

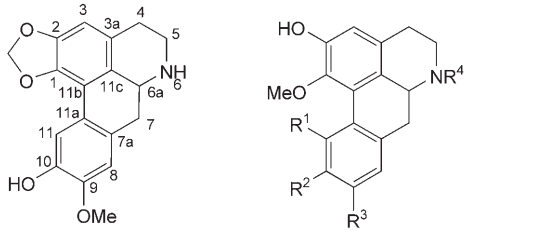

Fig. 1. *Structures of compounds* **1**–**4**

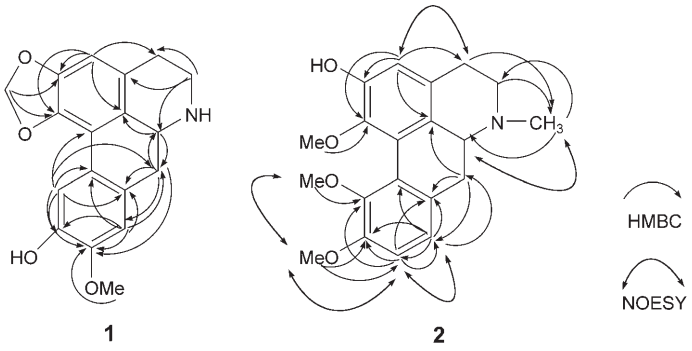

Fig. 2. *Selective HMBC and NOESY correlations of* **1** *and* **2**

The IR spectrum (KBr) of **1** showed characteristic absorption bands at 3430, 1136 (Ar–OH), 1609, 1515, 1462, 1410 (skeleton of aporphine alkaloids) cm^{-1}. The EI-MS fragments at *m/z* 311 (M^+), 310 ($[M-1]^+$), 295, 282, 281, 279, 267, 251, and 237 (see *Exper. Part*) are consistent with those of aporphine alkaloids [5]. The ^{13}C- and DEPT-NMR spectra (*Table*) revealed the presence of 18 C-signals which were attributed to 1 OCH_2O (*t* at δ(C) 102.1), 1 MeO (*q* at δ(C) 56.6), 3 CH_2 (*t* at δ(C) 43.8, 36.1, and 28.9), and 4 CH groups (*d* at δ(C) 116.1, 112.3, 107.8, and 49.5), and 9 quaternary C-atoms (*s* at δ(C) 148.7, 147.9, 147.5, 143.7, 129.1, 127.3, 126.4, 123.7, and 117.8). In the ^1H-NMR spectrum (*Table*), one *s* due to a MeO group at δ(H) 3.74 was evident. A low-field one-proton *s* at δ(H) 7.54 (δ(C) 112.3 (*d*)) exhibited the characteristics of the deshielded H–C(11)[1]) of the aporphine system. The other two aromatic protons at δ(H) 6.59 and 6.42 (2*s*), suggested that no adjacent aromatic protons were present in rings A or D. In the HMBC experiment (*Fig. 2*), δ(H) 6.59 correlated with δ(C) 123.7(C(11a)), 147.5 (C(10)), 147.9 (C(9)), and 36.1 (C(7)); and δ(H) 6.42 with δ(C) 126.4 (C(11c)), 143.7 (C(1)), 148.7 (C(2)), 28.9 (C(4)), and 117.8 (C(11b)), allowing the assignment of the two aromatic protons at δ(H) 6.59 and 6.42 to H–C(8) and H–C(3), respectively (*Fig. 2*)[1]). The signals at δ(H) 5.95 and 5.81 (2 br. *s*, each 1 H; δ(C) 102.1 (*t*)) indicated the presence of a OCH_2O group. The location of this OCH_2O group at C(1) and C(2) was established by the HMBC correlations δ(H) 5.95, 5.81/δ(C) 143.7 (C(1)), and 148.7 (C(2)). Therefore, the phenolic OH and MeO group must be located at C(9) and C(10). The HMBC experiment (*Fig. 2*) confirmed the position of MeO at C(9) by the correlations δ(H) 2.52 (H_β–C(7))/δ(C) 49.5 (C(6a)), 123.7 (C(11a)), 126.4 (C(11c)), 116.1 (C(8)), 129.1 (C(7a)), and 147.9 (C(9)), and δ(H) 3.74 (MeO–C(9))/δ(C) 147.9 (C(9)).

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[1]) For convenience, the atom numbering of **1** and **2** are the same; for systematic names, see *Exper. Part.*

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

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
C(1)	–	143.7 (*s*)	–	146.2 (*s*)
C(2)	–	148.7 (*s*)	–	150.8 (*s*)
H–C(3)	6.42 (*s*)	107.8 (*d*)	6.69 (*s*, 1 H)	115.9 (*d*)
CH_2(4)	2.91, 2.58 (*m*, each 1 H)	28.9 (*t*)	2.82, 2.76 (*m*, each 1 H)	28.2 (*t*)
CH_2(5)	3.26, 2.91 (*m*, each 1 H)	43.8 (*t*)	3.21, 2.80 (*m*, each 1 H)	53.9 (*t*)
MeN	–	–	2.70 (*s*)	43.2 (*q*)
CH_2(7)	2.70 (*dd*, J = 14.1, 4.9, H_α), 2.52 (*dd*, J = 14.1, 4.9, H_β)	36.1 (*t*)	3.18, 2.45 (*dd*, J = 13.8, 4.3, each 1 H)	35.6 (*t*)
H–C(8)	6.59 (*s*)	116.1 (*d*)	7.05 (*d*, J = 8.2)	123.5 (*d*)
H–C(9)	–	147.9 (*s*)	6.95 (*d*, J = 8.2)	113.4 (*d*)
C(10)	–	147.5 (*s*)	–	153.6 (*s*)
H–C(11)	7.54 (*s*)	112.3 (*d*)	–	148.6 (*s*)
C(3a)	–	127.3 (*s*)	–	128.1 (*s*)
H–C(6a)	3.80 (*dd*, J = 9.7, 4.6)	49.5 (*d*)	3.31(*dd*, J = 9.5, 4.3)	64.6 (*d*)
C(7a)	–	129.1 (*s*)	–	130.5 (*s*)
C(11a)	–	123.7 (*s*)	–	126.3 (*s*)
C(11b)	–	117.8 (*s*)	–	125.6 (*s*)
C(11c)	–	126.4 (*s*)	–	127.0 (*s*)
*Me*O–C(1)	–	–	3.44 (*s*)	60.9 (*q*)
*Me*O–C(9)	3.74 (*s*)	56.6 (*q*)	–	–
*Me*O–C(10)	–	–	3.88 (*s*)	56.7 (*q*)
*Me*O–C(11)	–	–	3.61 (*s*)	61.1 (*q*)
OCH_2O	5.95, 5.81 (br. *s*, each 1 H)	102.1 (*t*)	–	–

[1]) For convenience, the atom numbering of **1** and **2** are the same; for systematic names, see *Exper. Part.*

To compound **2**, the molecular formula $C_{20}H_{23}NO_4$ was attributed from its HR-ESI-MS (*m*/*z* 342.1707 ($[M+1]^+$)). Compound **2** was positive to *Dragendorff*'s test. The UV absorptions at 278 and 306 nm revealed that **2** was a tetrasubstituted aporphine. The MS fragmentation pattern of **2** was identical with that of aporphine alkaloids [5]. The IR and NMR data (*Table*, *Fig. 2*) established the structure of **2** (*Fig. 1*) which was named litseglutine B. Litseglutin B(**2**) is substituted at C(11), which is rare among aporphine alkaloids.

The IR spectrum of **2** showed absorption bands at 3400, 1410, 1462, 1515, and 1609 cm^{-1}, indicating the presence of a phenolic OH group and the skeleton of an aporphine alkaloid. The ^{1}H-NMR spectrum exhibited the presence of three aromatic MeO groups at δ(H) 3.88, 3.61, and 3.44, and 1 MeN group at δ(H) 2.70. Two *d* of aromatic protons at δ(H) 7.05 (J = 8.2 Hz, 1 H), and 6.95 (J = 8.2 Hz, 1 H) suggested that these protons were adjacent. The HMBC experiment (*Fig. 2*) established their positions at C(8) and C(9) by the correlations H–C(8) (δ(H) 7.05)/C(7) (δ(C) 35.6), C(10) (δ(C) 153.6), C(11) (δ(C) 148.6), and C(11a) (δ(C) 126.3), and H–C(9) (δ(H) 6.95)/C(8) (δ(C) 123.5), C(7a) (δ(C) 130.5), C(10) (δ(C) 153.6), and C(11) (δ(C) 148.6). Thus, C(10) and C(11) were substituted. The NOESY plot confirmed these assignments by the *H*–C(8)/*H*–C(9) correlation. The aromatic MeO groups at δ(H) 3.88 and 3.61 showed HMBC correlations with C(10) (δ(C) 153.6) and C(11) (δ(C) 148.6) respectively, which supported the positions of the two MeO groups at C(10) and C(11). Moreover, the *s* of an aromatic proton at δ 6.69 was assigned to H–C(3), similarly to compound **1**. Thus, the third MeO group must be located at C(1)or C(2). In the HMBC experiment, δ(H) 3.44 (MeO) correlated with δ(C) 146.2 (C(1)), indicating the location of this MeO group at C(1).

The identification of compounds **3** and **4**, the major constituents from *Litsea glutinosa*, were established as boldine (**3**) and laurolitsine (**4**) by direct comparisons (co-TLC, UV, IR, MS, and NMR) with the authentic samples [6]. Compound **3** was reported to possess antiplatelet aggregation and vasorelaxing activity [6]. Compound **4** showed blood-pressure-lowering activity [6].

Experimental Part

General. TLC: commercial silica gel plates (*Qing Dao Marine Chemical Group Co.*). CC = Column chromatography. $[\alpha]_D$: *Horiba SEAP-300* spectropolarimeter. UV-Spectra: *Shimadzu UV-2401PC* spectrophotometer; λ_{max} in nm. IR Spectra: *Bio-Rad FTS-135-IR* spectrophotometer; ν_{max} in cm^{-1}. ^1H-, ^{13}C-NMR, and 2D-NMR Spectra: *Bruker AV-500* spectrometer; chemical shifts δ in ppm relative to $SiMe_4$ as internal standard, and coupling constant J in Hz. EI-MS: *VG-Autospec-3000* mass spectrometers.

Plant Material. The leaves and twigs of *Litsea glutinosa* were collected in October 2002 in Xishuangbanna Country of Yunnan Province, P. R. China, and identified by Professor *Zhi-Hao Hu* of the department of botany, Yunnan University. A voucher specimen (No. 200305) is deposited in the School of Pharmacy, Yunnan University.

Extraction and Isolation. The powdered plant material of *Litsea glutinosa* (12.0 kg) was repeatedly extracted with EtOH at r.t. The extract was then evaporated and the obtained brown syrup partitioned in H_2O and separated by extraction with solvents into a petroleum ether fraction (80 g), AcOEt fraction (54 g), and BuOH fraction (108 g). The BuOH extract was subjected to CC (silica gel, $CHCl_3$/MeOH (99:1 to 1:1), then MeOH): *Fractions I–X*. *Fr. I* and *II* were resubmitted to CC (silica gel, then *Sephadex LH-20*): **1** (5 mg), **2** (10 mg), **3** (106 mg), and **4** (50 mg).

Litseglutine A (=*6-Demethyl-9-methoxy-1,2-(methylenedioxy)aporphin-10-ol*[1]),(=*6,7,7a,8-Tetrahydro-10-methoxy-5*H-*benzo[*g*]-1,3-benzodioxolo[6,5,4-*de*]quinolin-11-ol*; **1**): Brown solid. $[\alpha]_D^{24} = 36.56$ ($c = 0.13$, MeOH). UV(MeOH): 218, 282, 306. IR (KBr): 3430, 1609, 1515, 1462, 1410, 1276. ^1H- and ^{13}C-NMR: *Table*. EI-MS: 311 (40, M^+), 310 (100, $[M-1]^+$), 295 (5, $[M-OH]^+$), 282 (15, $[M-29\,(CH{=}NR)]^+$), 279 (25), 267 (9, $[M-CO_2]^+$), 251 (10, $[M-CO_2-OH]^+$), 237 (3, $[M-CO_2-MeO]^+$). HR-ESI-MS: 312.1233 ($[M+1]^+$, $C_{18}H_{17}NO_4^+$; calc. 312.1235).

Litseglutine B (=*1,10,11-Trimethoxyaporphin-2-ol* = *5,6,6a,7-Tetrahydro-1,10,11-trimethoxy-6-methyl-4*H-*dibenzo[*de,g*]quinolin-2-ol*; **2**): Light brown solid. $[\alpha]_D^{24} = 30.5$ ($c = 0.13$, MeOH). UV(MeOH): 278, 306. IR (KBr): 3400, 1410, 1462, 1515, 1609. ^1H- and ^{13}C-NMR: *Table*. EI-MS: 341 (30, M^+), 340 (15, $[M-1]^+$), 326 (35, $[M-15]^+$), 310 (40, $[M-31]^+$), 253, 149, 123, 109 (100), 97, 81, 69. HR-ESI-MS: 342.1707 ($[M+1]^+$, $C_{20}H_{23}NO_4^+$; calc.342.1705).

Cytotoxicity Assay. The cytotoxic activitity against human Hela cell lines were assayed using the standard MTT method *in vitro*.

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