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豺皮樟和圆叶豺皮樟中的阿朴啡生物碱成分

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摘要：从豺皮樟（*Litsea rotundifolia* var. *oblongifolia*）和圆叶豺皮樟（*Litsea rotundifolia*）的茎中分离得到三个阿朴啡型生物碱成分，通过光谱解析及化学方法，三个化合物分别鉴定为降波尔定（1）、波尔定（2）和氮乙酰降波尔定（3）。其中氮乙酰降波尔定系首次直接从植物中得到。

关键词：豺皮樟；圆叶豺皮樟；阿朴啡型生物碱；氮乙酰降波尔定
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APORPHINE ALKALOIDS OF *LITSEA ROTUNDIFOLIA* AND *L. ROTUNDIFOLIA* VAR. *OBLONGIFOLIA*

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Abstract: Three aporphine alkaloids, laurolitsine, boldine and N-acetyllaurolitsine were isolated from the stems of both *Litsea rotundifolia* and *L. rotundifolia* var. *oblongifolia*. Their structures were identified on the basis of spectral analysis and chemical correlation. Among them, N-acetyllaurolitsine was found as a naturally occurring compound for the first time.

Key words: *Litsea rotundifolia*; *Litsea rotundifolia* var. *oblongifolia*; Aporphine alkaloids; N-acetyllaurolitsine

Both *Litsea rotundifolia* and *L. rotundifolia* var. *oblongifolia* are used in folk medicine as analgesic and anti-inflammatory drugs in Guangdong Province of China. No chemical work has previously been reported on them. Under a program of screening for South China medicinal plants with cardiovascular activities, we found that the alkaloidal fractions of the ethanolic extracts from stems of the two plants showed blood pressure lowering activity. The constituents of the alkaloidal fractions were, therefore, investigated. In this paper, we describe the isolation and the identification of three aporphine alkaloids from the stems of the plants.

Results and discussions

The alkaloidal mixtures from ethanolic extracts of the entitled plants were obtained by

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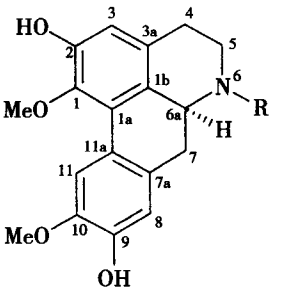
the usual acid-base treatment. Repeated column chromatography on silica gel of the alkaloidal mixtures led to the isolation of three aporphine alkaloids, laurolitsine (also known as norboldine) (1), boldine (2) and N-acetyllaurolitsine (3). The structures of these compounds were identified based on spectral analysis and chemical correlation. Among them, N-acetyllaurolitsine, which was previously prepared by semisynthesis[1], was found as a naturally occurring product for the first time.



The UV spectra of 1 and 2 showed maximal absorption at 218, 282 and 303 nm, suggesting that these compounds belonged to 1, 2, 9 and 10 tetrasubstituted group of aporphine alkaloids[2]. The MS fragmentation pattern of 1 and 2 was identical with that of aporphine alkaloids[3]. In ^{1}H NMR spectra of 1 and 2, a low field one-proton singlet characteristic of deshielded proton at C-11 of the aporphine system was present at δ7.87 and 7.96, respectively. The presence of two three-proton singlets at δ3.55 and 3.76 in 1, and δ3.58 and 3.85 in 2 showed the substitution of methoxyl groups at C-1 and C-10[4]. A three-proton singlet at δ2.53 in the spectrum of 2 indicated the presence of an N-methyl group in 2. This signal was absent in that of 1. In the remaining proton signals, two aromatic proton singlets at δ6.66 and 6.49 in 1, δ6.79 and 6.57 in 2 could be assigned to the protons at C-8 and C-3. The spectral evidences above indicated that compound 1 was laurolitsine and compound 2 was boldine[2, 5]. and this was supported by their ^{13}C NMR data (see Table 1) which were in good agreement with those in literature [6].

The UV spectrum of compound 3 also indicated an aporphine alkaloid substituted at

Table 1 ^{13}C NMR chemical shifts of 1, 2 and 3

C	1	2	3*	C	1	2	3*
1	142.5s	143.8s	144.8s	8	115.0d	115.7d	*117.0*, 116.6d
1a	126.9s	127.3s	128.4s	9	145.9s[b)]	146.9s[b)]	147.6s[b)]
1b	125.7s	125.7s	n.d.	10	145.7s[b)]	147.1s[b)]	148.2s[b)]
2	149.0s	150.3s	151.1s	11	112.1d	112.4d	113.4d
3	114.6d	114.7d	*115.5*, 115.7d	11a	123.0s	124.0s	124.8s
3a	129.3s[a)]	129.6s[a)]	*130.6*, 131.0s[a)]	N-Me		43.5q	
4	28.6t	28.8t	*30.7*, 30.0t	NCOMe			*169.2*, 169.6s
5	42.6t	53.7t	*42.2*, 36.7t	NCOMe			*22.5*, 21.6q
6a	53.5d	63.3d	*51.4*, 54.5d	1-OMe	59.2q	60.1q	59.8q
7	36.2t	34.2t	*34.1*, 36.2t	10-OMe	55.7q	56.4q	56.4q
7a	129.7s[a)]	130.3s[a)]	*131.6*, 131.3s[a)]				

*The values in italics of the pairs were those of the major conformer.

n. d.: Not detected.

[a, b)] The assignments may be interchangeable.

positions 1, 2, 9 and 10. The positive FAB mass spectrum of 3 gave a base ion peak at m/z 356 $[M+H]^+$ indicating the molecular weight of 355, and major fragment ions at m/z 296 $[M-HNC(OH)Me]^+$ and m/z 283 $[M-H_2CN(OH)Me]^+$ suggesting that an acetyl group was substituted at N atom[7]. The ^1H NMR spectrum (see Experiment) of 3 resembled that of compound 1, except that a three-proton singlet at δ2.18 for an acetyl methyl group was present in 3 and the signals of H-11 and H-8 in the spectrum of 3 showed two sets of resonances, indicating that compound 3 was an N-acetyl derivative of compound 1[1,7] and the compound existed in equilibrium of two E/Z isomers due to its amide C-N bond with partial double bond character like other aporphine N-acetyl derivatives[7]. The ^{13}C NMR spectrum of 3 (Table 1) also showed that the compound was in existence of two geometric isomers in equilibrium. In conclusion, 3 was determined as N-acetyllaurolitsine, which was finally confirmed by treatment of laurolitsine (1) with Ac_2O in DMF as described in literature [1] to afford compound 3.

Compound 1, the major constituent of the alkaloidal fractions from the two plants, was pharmacologically tested and showed blood pressure lowering activity. Compounds 2 and 3 were reported to possess antiplatelet aggregation and vasorelaxing activity[8].

Experiment

General Mps: uncorr. UV: MeOH as solvent. IR: KBr discs. NMR: 400 MHz (^1H) or 100 MHz (^{13}C), chemical shifts as δ values (ppm) relative to TMS, DMSO-d_6 as solvent in compound 1, acetone-d_6 as that in 2 and C_5D_5N as that in 3. FAB-MS: positive ion mode with m-nitrobenzyl alcohol as a matrix. EIMS: direct inlet and 70 eV. TLC: silica gel 60 F_{254}, $CHCl_3$-MeOH (9:1 or 8:2), spray reagent Dragendoff of H_2SO_4 (10%) in EtOH followed by heating. CC: silica gel 60 (100−200 mesh).

Plant Material The stems of *Litsea rotundifolia* and *L. rotundifolia* var. *oblongifolia* were collected at Dinghushan Mountain, Zhaoqing City, Guangdong, China in Oct., 1997, and identified by Prof. Z. L. Huang. A voucher specimen of each species has been deposited at the Herbarium of South China Institute of Botany, the Chinese Academy of Sciences.

Extraction and isolation The powdered air-dried stems (3.1 kg) of *Litsea rotundifolia* were extracted by percolation with 90% EtOH three times at room temperature. The EtOH extracts were concentrated to syrup under reduced pressure, and the syrup was dissolved with 5% hydrochloric acid. The aqueous acidic solution was defatted with petroleum ether (3× 200 ml) and then basified to pH 9 with aqueous Na_2CO_3. The basic aqueous solution was extracted 6 times with $CHCl_3$. The combined $CHCl_3$ extract gave, after concentration, an alkaloidal mixture (7.0 g) which was then subjected to a silica gel CC, eluted with

$CHCl_3$-MeOH mixtures of increasing polarity [(19:1) to (8:2)], yielding five fractions (I-V). Faction IV was rechromatographed on a silica gel column eluting with $CHCl_3$-MeOH (85: 15) to give compound 1 (2.9 g, 0.0935%). Fraction II was further subjected to a silica gel CC, eluted with $CHCl_3$-MeOH (95:5), followed by recrystallization from MeOH, to afford compound 3 (20 mg, 0.000645%). Fraction III was subjected to preparative TLC [plate: silica gel 60 F254, solvent $CHCl_3$-MeOH (9:1)] to afford compound 2 (20 mg, 0.000645%).

Under a similar procedure, the powdered air-dried stems (4.0 kg) of *Litsea rotundifolia* var. *oblongifolia* afforded compound 1 (3.3 g, 0.0825%), 2 (20 mg, 0.0005%) and 3 (25 mg, 0.000625%).

Laurolitsine (1)　$C_{18}H_{19}NO_4$, M 313; light brown amorphous powder, mp. 134-136 ℃; $[\alpha]_D^{25}$ +87.9° (c 0.008, MeOH); UV λ_{max}^{MeOH} (logε): 218 (4.41), 282 (4.08) and 303 (4.09); IR V_{max}^{KBr} cm^{-1}: 3532, 3480, 1608, 1506, 1454, 1080; EIMS m/z (%): 313 $[M]^+$(84), 284 $[M-CH_2NH]^+$ (100), 283 $[M-H-CH_2NH]^+$(48), 269 $[M-CH_2NH-Me]^+$(60), 268 (49), 240 (28); ^1H NMR: 7.87 (1H, s, H-11), 6.66 (1H, s, H-8), 6.49 (1H, s, H-3), 3.76 (3H, s, 10-OMe), 3.55 (3H, s, 1-OMe), 3.51 (1H, dd, J=14.0 and 4.4 Hz, H-6a); ^{13}C NMR: see Table 1.

Boldine (2)　$C_{19}H_{21}NO_4$, M 327; white amorphous powder, mp. 157−159 ℃; $[\alpha]_D^{25}$+ 85.6° (c 0.56, MeOH); UV λ_{max}^{MeOH} (logε): 218 (4.46), 282 (4.18) and 303 (4.20); IR v_{max}^{KBr} cm^{-1}: 3481, 1606, 1504, 1454, 1076; EIMS m/z (%): 327 $[M]^+$ (74), 326 $[M-H]^+$ (85), 312 $[M-Me]^+$ (17), 284 $[M-CH_2NMe]^+$ (100), 283 $[M-H-CH_2NMe]^+$ (30), 269 $[M-CH_2NMe-Me]^+$ (55), 268(34), 240 (18); ^1H NMR: 7.96 (1H, s H-11), 6.79 (1H, s, H-8), 6.57 (1H, s, H-3), 3.85 (3H, s, 10-OMe), 3.58 (3H, s, 1-OMe), 2.53 (3H, s, N-Me); ^{13}C NMR: see Table 1.

N-Acetyllaurolitsine (3)　$C_{20}H_{21}NO_5$, M355; colorless needles (MeOH), mp. 271−274 ℃; $[\alpha]_D^{25}$ +32.9 ° (c 0.42, C_5H_5N); UV λ_{max}^{MeOH} (logε): 218 (4.53), 283 (4.11) and 303 (4.12); IR V_{mzx}^{KBr} cm^{-1}: 3527, 3477, 1637, 1608, 1506, 1454, 1270, 1084; FABMS m/z (%): 356 $[M+H]^+$ (100), 355 $[M]^+$ (96), 339 $[M+H-OH]^+$(3), 296 $[M-HNC(OH)Me]^+$(7), 283 $[M-H_2CNC(OH)Me]^+$(5); ^1H NMR: *8.48* and 8.50 (1H, s, H-11), *7.22* and 7.25 (1H, s, H-8), 7.01 (1H, s, H-3), 3.85 (6H, s, 10-OMe and 1-OMe), 2.18 (3H, s, N-Me) [the δ values in italics were those of the major conformer]; ^{13}C NMR: see Table 1.

Conversion of compound 1 to compound 3　The mixture of compound 1 (200 mg), DMF (3 ml), and Ac_2O (0.2 ml) was stirred at room temperature for 48 hours in a sealed tube, and the solvent was evaporated under reduced pressure to give an amorphous residue. The residue, on recrystallization from MeOH, afforded colorless needles (180 mg) which were confirmed as compound 3 by direct comparison (mp, co-TLC, UV, IR, ^1H NMR and ^{13}C NMR).

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