SHORT REPORT

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Litsine A: A New Aporphine Alkaloid from the Root Barks of *Litsea glutinosa*

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Abstract: A new aporphine alkaloid, named Litsine A, along with two known ones were isolated from the root barks of *Litsea glutinosa*. The new structure was determined by various spectroscopic techniques including 1D(^{1}H-, ^{13}C-NMR), 2D-NMR(HMBC, HSQC, COSY and ROESY) and high resolution electrospray ionization mass spectrometry (HR-ESI-MS), where as the structures of the known compounds were elucidated through 1D-NMR, mass spectrometric analysis and compared with the reported data in literature. The effects of these compounds on glucose uptake in C2C12 myotubes were evaluated at the concentration of 10μM. The three compounds exhibited potent activity, with **1** as the best one, in increasing glucose uptake.

Keywords: *Litsea glutinosa*; Litsine A; aporphine alkaloids; glucose uptake activities.

1. Plant Source

In the ongoing search of phytochemical studies of anti-diabetic plants distributed in Hainan Island, China. Herein, we report the isolation, structure elucidation and biological studies of a new aporphine alkaloid, Litsine A (**1**) along with two known compounds, boldine (**2**) [1] and laurolitsine (**3**)[2] (Figure 1), from the root barks of *L. glutinosa*. The investigated plant was collected from Wenchang City of Hainan Province, China, in October 2017, and authenticated by Prof. Niankai Zeng (School of Pharmaceutical Science, Hainan Medical University) according to the Flora of China. A voucher specimen (No.LG201710) was deposited at the herbarium of School of Pharmaceutical Science, Hainan Medical University.

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2. Previous Studies

L. glutinosa is a member of the genus *Litsea* in the Lauraceae family, which is widely distributed in subtropical and tropical regions. In China, this plant has been used as traditional medicine for treatment of many diseases [3,4]. Pharmacological investigations indicated that the leaves and barks of *L. glutinosa* possessed anti-bacterial, anti-inflammatory, anti-nociceptive, analgesic, and anti-diabetic properties [5,6]. Studies on chemical constituents have led to the isolation of lignans, alkaloids, etc [7,8]. Previously, we reported three new lignan glycosides and other known compounds from this plant [9,10].

3. Present Study

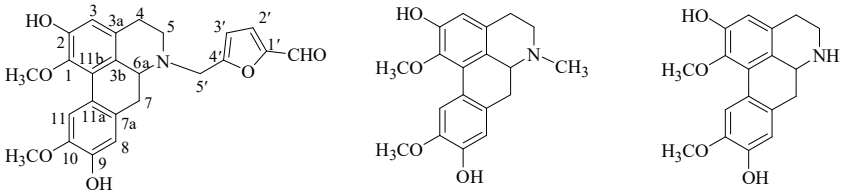
The root barks of *L. glutinosa* (7.0 kg) were air-dried, cut into pieces, and were extracted with ethanol. The ethanol extract was concentrated under reduced pressure to give a residue (660 g). The residue was dissolved in water, and then its pH value was adjusted to 2 by adding 1% H_2SO_4. The acidic solution was partitioned by chloroform to remove the lipid-soluble compounds. Then the pH value of the residue was adjusted to 10 by adding ammonium hydroxide. The pH of the ammonium hydroxide is 12, and the concentration is 25%. Then, ethyl acetate was used to extract the residue to give the alkaloid-rich extract (55 g) after concentration. The alkaloid-rich extract was separated by silica gel column chromatography using a gradient ratio of dichloromethane-acetone as the eluent to give five fractions (Fra1-Fra5). Fra.3 was further purified by Sephadex LH-20 to afford four fractions (subfra1-subfra4). Subfra.2 was purified by HPLC using a mixture of methanol-water (45:55) to afford Litsine A (**1**, 15 mg). Subfra3 was isolated by HPLC using a mixture of methanol-water (45:55) to give boldine (**2,** 10 mg) and laurolitsine (**3,** 8 mg).

*Litsine A (**1**)*: Yellow powder (MeOH), $[\alpha]^{D}_{25}$ = 35 (c 0.05), UV (MeOH) λmax (log ε) 258 nm. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm) = 9.50 (1H, s, CHO), 7.95 (1H, s, H-11), 7.37 (1H, d, J = 6.0 Hz, H-2′), 6.78 (1H, s, H-8), 6.65 (1H, d, J = 6.0 Hz, H-3′), 6.52 (1H, s, H-3), 4.18 (1H, d, J = 12.0 Hz, H-5′a), 4.01 (1H, d, J = 12.0 Hz, H-5′b), 3.86 (3H, s, 10-OMe), 3.57 (3H, s, 1-OMe), 3.18 (1H, m, H-5α), 3.16 (1H, m, H-7α), 3.15 (1H, m, H-6a), 2.98 (1H, m, H-4α), 2.62 (1H, m, H-4β), 2.60 (1H, m, H-5β), 2.49 (1H, m, H-7β). ^{13}C NMR (150 MHz, CD_3OD) δ (ppm) = 144.9 (C,C-1), 151.1 (C,C-2), 115.6 (CH, C-3), 128.7 (C, C-3a), 131.3 (C, C-3b), 30.3 (CH_2, C-4), 51.9 (CH_2, C-5), 60.8 (CH, C-6a), 35.4 (CH_2, C-7), 127.5 (C, C-7a), 116.4 (CH, C-8), 147.7 (C, C-9), 148.4 (C, C-10), 113.3 (CH, C-11), 131.4 (C, C-11a), 130.8 (C, C-11b), 154.6 (C, C-1′), 125.2 (CH, C-2′), 114.2 (CH, C-3′), 160.4 (C, C-4′), 52.0 (CH_2, C-5′), 61.1 (CH_3, 1-OMe), 57.1 (CH_3, 10-OMe), 179.8 (CH, 1′-CHO). HR-ESI-MS: *m/z* 422.1599 ($[M + H]^+$, calcd. $C_{24}H_{24}NO_6$ for 422.1604). ESI(+)MS: *m/z* 422.1 $[M+H]^+$, 297.1, 284.1, 265.1, 109.2 ($C_6H_5O_2^+$).

Cell culture: C2C12 myoblasts were maintained in DMEM supplemented with 10% FBS at 37 °C and 5% CO2. To induce differentiation, media was replaced with DMEM containing 2% horse serum when the cells reached confluence. The C2C12 cells were kept in this differentiating medium for 5 days, which allowed cells to be fully differentiated and used in the following experiments.

Glucose uptake assay: Glucose uptake assay was performed as previously reported [11]. Briefly, differentiated C2C12 myotubes were incubated with the serum-free DMEM containing the fluorescent glucose analog 2-NBDG (10 μM) and various compounds (10 μM) or insulin (100 nM). After incubation for 12 h, medium was removed and cells were washed with phosphate-buffered saline (PBS) twice. Cells were scraped out in 1 mL of PBS and transferred into 5 mL polystyrene round-bottom tubes (BD Falcon) and kept at 4 °C. The amount of 2-NBDG taken up by the cells was measured by determining the fluorescence intensities at Ex/Em = 475 nm/550 nm using a Tecan Infinite M1000Pro Microplate Reader (TECAN Group Ltd, Shanghai, China).

The root barks of *L. glutinosa* were air-dried, cut into pieces, and were extracted with ethanol-water. The ethanol extract was concentrated under reduced pressure to give a residue. The residue was dissolved in water, and then its pH value was adjusted to 2 by adding 1% H_2SO_4. The acidic solution was partitioned by chloroform to remove the lipid-soluble compounds. Then the pH value of the residue was adjusted to 10 by adding ammonia. Then, ethyl acetate was used to extract the basic solution, after concentration, to give the alkaloid-rich extract. Phytochemical investigation on alkaloid-rich extract has resulted in the isolation of one new aporphine alkaloid, Litsine A (**1**) (Figure 1).

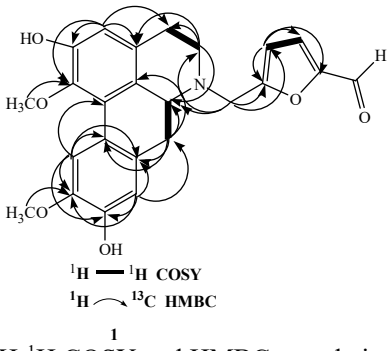


1 **2** **3**

Figure 1. Structure of compounds **1-3** isolated from *L. glutinosa*

Compound **1,** Litsine A, was obtained as yellow powder. Its molecular formula was established to be $C_{24}H_{23}NO_6$ by interpretation of HRESIMS, requiring 14 degrees of unsaturation. The ^{1}H NMR spectrum showed notable signals attributing to two methoxy protons at δ_H 3.57 (3H, s), 3.86 (3H, s). Further examination of the spectrum revealed the existence of one aldehyde proton at δ = 9.50 (1H, s) and a set of aromatic protons at δ = 6.65 (1H, d, J = 6.0 Hz, H-3′), 7.37 (1H, d, J = 6.0 Hz, H-2′), 6.52 (1H, s, H-3), 6.78 (1H, s, H-8), 7.95 (1H, s, H-11). Moreover, the two deshielded methylene protons at δ = 3.18 (1H, m, H-5α), 2.60 (1H, m, H-5β), 4.18 (1H, d, J = 12.0 Hz, H-5′a), 4.01 (1H, d, J = 12.0 Hz, H-5′b), and a methine proton at δ_H 3.16 (1H, m) were consistent with the presence of *N*-methylene and *N*-methine. Its ^{13}C NMR alongside HSQC data showed signals for two methoxy groups, four methylenes, six methines, one aldehyde carbon, and eleven non-protonated carbons.

The ^{1}H-^{1}H COSY and HSQC spectra of **1** revealed the existence of three units as shown in Figure 2. The HMBC spectrum of 1 exhibited correlations from the aromatic proton at H-3 (δ_H 6.52) to carbons at δ_C 151.1 (C-2), δ_C 144.9 (C-1), δ_C 128.7 (C-3a) and δ_C 30.3 (C-4) and from the methylene proton at δ_H 3.18 (H-5) to carbons at δ_C 30.3 (C-4), δ_C 128.7 (C-3a) and δ_C 60.8 (C-6a). The correlations from methylene protons at δ_H 3.16, 2.49 (H-7) to carbons at δ_C 60.8 (C-6a), δ_C 131.3 (C-3b), δ_C 127.5 (C-7a) and δ_C 131.4 (C-11a). The correlations from H-8 (δ_H 6.78) to C-7 (δ_C 35.4), C-7a (δ_C 127.5) ,C-9 (δ_C 147.7) and C-10 (δ_C 148.4) and the correlations from H-11 (δ_H 7.95) to C-10 (δ_C 148.4), C-9 (δ_C 147.7), C-11a (δ_C 131.4) and C-11b (δ_C 130.8) verified the connection from C-7 to C-11. These data indicated that **1** had an aporphine alkaloid skeleton similar to that of boldine [1,10]. However, some differences in the NMR signals for the side chain were observed. The HMBC spectrum of **1** exhibited correlations from H-3′ (δ_H 6.65) to C-1′ (δ_C 154.6), C-2′ (δ_C 125.2) and C-4′ (δ_C 160.4) suggested the presence of a furan moiety and the aldehyde group was attached to C-1′ (δ_C 154.6). Its ESI-MS spectrum suggested the presence of the furanoaldehyde moiety (m/z 109.2). The side-chain connectivity was deduced from the HMBC correlations from H-5′ (δ_H 4.01, 4.18) to C-5 (δ_C 51.9), C-6a (δ_C 60.8), C-4′ (δ_C 160.4) and C-3′ (δ_C 114.2). These HMBC correlations also displayed that the furan moiety was attached to C-5′ and C-5′ was connected to the *N* atom. Finally, the correlations from methoxy proton δ_H 3.57 (3H, s) to C-1 (δ_C 144.9) and correlations from the methoxyl proton δ_H 3.86 (3H, s) to C-10 (δ_C 148.4) together with the ROESY correlations between the protons at δ_H 7.95 (H-11) and δ_H 3.57 (1-OCH$_3$) and δ_H 3.86 (10-OCH$_3$) suggested the two methoxy groups attached to C-1 and C-10, respectively. Thus, compound **1** was assigned as a new aprophine alkaloid with a given name Litsine A, as illustrated in Figure 1.



1

Figure 2. Key ^{1}H-^{1}H COSY and HMBC correlations of compound **1**

Compounds **1-3** were tested for their effects on stimulating glucose uptake in C2C12 myotubes. These results showed that the three compounds increased the glucose uptake potently and **1** exhibited the best activity as shown in Figure 3.

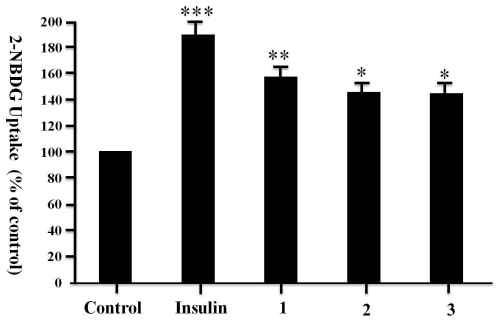


Figure 3. Effects of compounds **1-3** on glucose uptake in C2C12 myotubes

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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References

[1] S. Castro-Saavedra, G. Fuentes-Barros, C. Tirapegui, W. Acevedo-Fuentes, B. Cassels, A. Barriga and M. Vilches-Herrera (2016). Phytochemical analysis of alkaloids from the Chilean endemic tree Cryptocarya

alba, *J. Chil. Chem. Soc*. **61**, 3076-3080.
[2] D. Hughes, K. Genest and W. Skakum (1968). Alkaloids of *Peumus boldu*s. Isolation of laurotetanine and laurolitsine, *J. Pharm. Sci.* **57**, 1619-1620.
[3] G.D. Kong, Y. Zhao, H.G. Li, B. J. Chen, X.N. Wang, H.L. Zhou, X.H. Lou, M.D. Ren and T. Shen (2015). The genus *Litsea* in traditional Chinese medicine: an ethnomedical, phytochemical and pharmacological review, *J. Ethnopharmacol*. **164**, 256-264.
[4] R. Bhowmick, M.S. Sarwar, S.M. Dewan, A. Das, B. Das, M.M. Uddin and M.S. Islam (2014). In vivo analgesic, antipyretic, and anti-inflammatory potential in Swiss albino mice and in vitro thrombolytic activity of hydroalcoholic extract from *Litsea glutinosa* leaves, *Biol. Res*., **47**, 56-63.
[5] S. Mandal, C. Kumar, A. Majumder, R. Majumder and B. Maity (2000). Antibacterial activity of *Litsea glutinos*a bark, *Fitoterapia* **71**, 439-41
[6] Y.S. Wang, R. Huang, H.Lu, F.Y. Li and J.H. Yang (2010). A new 2'-oxygenated flavone glycoside from Litsea glutinosa (Lour.) C. B. Rob., *Biosci. Biotechnol. Biochem*. 74, 652-654.
[7] D. Das, S. Maiti, T. Maiti and S. S. Islam (2013). A new arabinoxylan from green leaves of *Litsea glutinosa* (Lauraeae): structural and biological studies, *Carbohydr. Polym*. 92, 1243-1248.
[8] N. Agrawal, D. Pareek, S. Dobhal, M. Sharma, Y. Joshi and M. Dobhal (2013). Butanolides from methanolic extract of *Litsea glutinosa*, *Chem. Biodivers*. 10, 394-400.
[9] Y.N. Wu, Y.Jin, L. Dong, Y.Y. Li, C.Y. Zhang, M. Gui and X.P. Zhang (2017). New lignan glycosides from the root barks of *Litsea glutinosa*, *Phytochemistry lett.* 20, 259-262.
[10] Y.N. Wu, L. Dong, Y.Y. Li, Y.F. Tan, Y. Jin, Y.B. Li and X.P. Zhang (2017). Chemical constituents from bark of *Litsea glutinosa* and their antidiabetic targets, *Mod. Chin. Med.*19: 956-959.
[11] X.P. Zhang, C.Y. Chen, Y.Y. Li, D.L. Chen, L. Dong, W. Na, C.M. Wu, J.Q. Zhang, and Y.B. Li (2016). Tadehaginosides A-J, phenylpropanoid glucosides from *Tadehagi triquetrum*, enhance glucose uptake via the upregulation of PPARγ and GLUT-4 in C2C12 myotubes, *J. Nat. Prod*. **79**, 1249-1258.