Glutinosine A: A New Morphinandienone Alkaloid from *Litsea glutinosa*

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**Abstract:** A new morphinandienone alkaloid, named glutinosine A was 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 (HRR-ESI-MS). The effects of the new compound on glucose consumption in HepG2 cells were evaluated. Whereas, the result showed that this compound displayed no activity in stimulating glucose consumption.

**Keywords:** *Litsea glutinosa*; Glutinosine A; morphinandienone; glucose consumption activities. © 2018 ACG Publications. All rights reserved.

1. **Plant Source**

The root barks of *Litsea glutinosa* were collected from Wenchang City of Hainan Province, China, in October 2017. The plant was identified by Prof. Niankai Zeng (School of Pharmaceutical Science, Hainan Medical University), and a voucher specimen was deposited at the herbarium of School of Pharmaceutical Science, Hainan Medical University for future reference (No.LG201710).

2. **Previous Studies**

*L. glutinosa* is an evergreen medium-size tree, which is widely distributed in subtropical and tropical regions. This plant has been used as traditional medicine for treatment of many diseases [1,2]. Pharmacological investigations indicated that the leaves and barks of *L. glutinosa* possessed antibacterial, anti-inflammatory, anti-nociceptive, analgesic and anti-diabetic properties [3,4]. Up to now, aporphine alkaloid, benzo[furan neolignans as the characteristic chemical constituents have been
obtained from this plant [5,6,7]. Previously, we have isolated six alkaloids of N-trans-feruloyltyramine, N-cis-feruloyltyramine, N-trans-sinapoyltyramine, boldine, laurolitsine, litsine A [8, 9].

3. Present Study

The root barks of L. glutinosa (7.0 kg) were air-dried, cut into pieces and were extracted with 95% 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$_2$SO$_4$. 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 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 (Frac. 1-Frac. 5). Frac. 4 was further purified by Sephadex LH-20 eluting with methanol to afford four fractions (Subfrac. 1-Subfrac. 4). Subfrac. 2 was purified by HPLC using a mixture of methanol-water (35:65) to afford glutinosine A (15 mg). HPLC was performed on a Shimadzu LC-6AD system using a column of Agilent ZORBAX SB-PHENYL (250 × 9.4 mm, 5 μm) equipped with an SPD-10A detector.

Glutinosine A (1): Yellow powder (MeOH), $[\alpha]_{D}^{25} = -20$ (c 0.05), UV (MeOH) $\lambda_{max}$ (log ε) 238 (3.90), 280 (3.88) nm. $^1$H-NMR (600 MHz, CD$_3$OD) $\delta$ (ppm) = 7.12 (1H, s, H-4), 6.92 (1H, s, H-5), 6.64 (1H, s, H-1), 6.44 (1H, s, H-8), 4.30 (1H, d, $J = 6.0$ Hz, H-9), 3.89 (3H, s, 3-OCH$_3$), 3.83 (3H, s, 6-OCH$_3$), 3.48 (1H, d, $J = 18.0$ Hz), 3.41 (1H, dd, $J = 18.0$, 6.0 Hz), 3.36 (3H, s, O-N-CH$_3$), 3.28 (1H, dd, $J = 18.0$, 6.0 Hz, H-16a), 3.17 (1H, m, H-16b), 2.45 (1H, dd, $J = 18.0$, 6.0 Hz, H-15a), 2.02 (1H, m, H-15b). $^{13}$C-NMR (150 MHz, CD$_3$OD) $\delta$ (ppm) = 115.8 (C-1), 148.3 (C-2), 153.1 (C-3), 111.1 (C-4), 121.6 (C-5), 158.4 (C-6), 182.4 (C-7), 127.9 (C-8), 77.7 (C-9), 36.2 (C-10), 130.5 (C-11), 126.3 (C-12), 42.8 (C-13), 149.7 (C-14), 38.2 (C-15), 61.3 (C-16), 57.3 (C-17), 58.3 (O-N-CH$_3$). HR-ESI-MS: m/z 344.1478 ([M + H]$^+$, calcd. C$_{19}$H$_{22}$NO$_5$ for 344.1498).

Glucose consumption assay: HepG2 cells were incubated with the serum-free high glucose DMEM containing the different concentration of 1 (10 μM) in the presence or absence of insulin (100 nM). After incubation for 24 h, the medium glucose concentration was measured by glucose kit according to the operation manual.

Compound 1 was obtained as an amphorous powder. Its molecular formula C$_{19}$H$_{22}$NO$_5$ was determined by HRESIMS at m/z 344.1478 [M + H]$^+$ (calcd. 344.1498). The $^1$H-NMR data displayed signals for three methoxy groups [$\delta_H$ 3.83 (3H, s, 3-OCH$_3$), 3.89 (3H, s, 6-OCH$_3$), 3.36 (3H, s, O-N-CH$_3$)], a set of aromatic protons at [$\delta_H$ 7.12 (1H, s, H-4), 6.64 (1H, s, H-1)], two olefinic protons [$\delta_H$ 6.92 (1H, s, H-5), 6.44 (1H, s, H-8)], an methine proton [$\delta_H$ 3.59 (1H, m, H-9)], three methene protons [$\delta_H$ 3.48 (1H, d, $J = 18.0$ Hz, H-10a), 3.41 (1H, dd, $J = 18.0$, 6.0 Hz, H-10β), 3.28 (1H, dd, $J = 18.0$, 6.0 Hz, H-16a)], 3.17 (1H, m, H-16b), 2.45 (1H, dd, $J = 18.0$, 6.0 Hz, H-15a), 2.02 (1H, m, H-15b). The $^{13}$C-NMR spectrum and HSQC experiments resolved 19 carbon resonances attributable to
one carbonyl (δC 182.4), three methyls (δC 57.3, 56.4, 58.3), three methylenes (δC 36.2, 38.2, 61.3), an methine (δC 77.7), one quaternary carbon (δC 42.8), a four-substituted phenyl-ring (δC 115.8, 148.3, 153.1, 111.1, 121.6, 158.4) and two double bond (δC 121.6, 158.4, 127.9, 149.7). All these spectroscopic data indicated that I possessed a morphinandienone skeleton [10].

The 1H-1H COSY correlations between the H-15 and H-16 and between H-9 and H-10 revealed the existence of two units of -CH2-CH2 and -CH-CH2 as shown in Figure 1. The HMBC spectrum of I exhibited correlations from the aromatic proton at H-1 (δH 6.64) to carbons at δC 36.2 (C-10), δC 148.3 (C-2) and 130.5 (C-11) suggested C-10 was attached to C-11 of the phenyl ring. HMBC correlations from the methene proton at δH 2.45 (H-15) to carbons at δC 42.8 (C-13), δC 149.7 (C-14), δC 126.3 (C-12), δC 128.7 (C-16), δC 121.6 (C-5) and correlations from methene carbon proton at δH 4.30 (H-9) to δC C-8 (127.9), δC 42.8 (C-13), δC 149.7 (C-14), δC 58.3 (O-N-CH3) confirmed that I had the morphinandienone skeleton. The ROESY correlations between δH 3.83 (3-OCH3) and δH 7.12 (H-4), and between δH 3.89 (6-OCH3) and δH 6.92 (H-5) verified the positions of the two methoxyl groups. Further checking its NMR data, I was similar to those of pallidine except for the characteristic downfield shifts of the carbon resonances at δC 77.7, 61.3, 58.3 for C-9, C-16, and O-N-CH3 [10]. By comparing the 13C-NMR data of I with those of pallidine, the carbon signals at C-9, C-16, and O-N-CH3 were relatively deshielded (ΔδC +17.0, 15.6, 16.7) suggesting that I was N-oxide. The coupling constants of 3.48 (1H, d, J = 18.0 Hz), 3.41 (1H, dd, J = 18.0, 6.0 Hz) could be applied to assign 10α and 10β, respectively [11]. In the ROESY spectrum, correlations between δH 4.30 and δH 3.48 (10α) indicted that H-9 was α orientated. Thus, compound I was established as a new morphinandienone alkaloid with a given name glutinosine A.

Figure 2. Key HMBC correlations of compound 1

The new compound was tested for its effect on stimulating glucose consumption in HepG2 cells. The result showed that the new compound exhibited no activity.

Aporphine alkaloids have been deemed as the characteristic constituents. Till now, no morphinandienone alkaloids have been obtained from this plant. Therefore, the new compound isolated in present study displayed chemotaxonomical significance, which should be highlighted.

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products
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**References**


