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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 (HR-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.

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 anti-bacterial, anti-inflammatory, anti-nociceptive, analgesic and anti-diabetic properties [3,4]. Up to now, aporphine alkaloid, bnenzofuran neolignans as the characteristic chemical constituents have been

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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_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 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) λ_{max} (log ε) 238 (3.90), 280 (3.88) nm. ^{1}H-NMR (600 MHz, CD_3OD) δ (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, H-10α), 3.41 (1H, dd, *J* = 18.0, 6.0 Hz, H-10β), 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_3OD) δ (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 (3-OCH_3), 56.4 (6-OCH_3), 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.

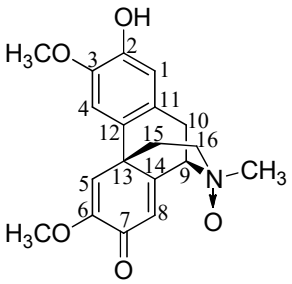

Figure 1. Structure of compound **1** isolated from *L. glutinosa*

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 [δ_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 [δ_H 7.12 (1H, s, H-4), 6.64 (1H, s, H-1)], two olefinic protons [δ_H 6.92 (1H, s, H-5), 6.44 (1H, s, H-8)], an methine proton [δ_H 3.59 (1H, d, *J* = 6.0 Hz)], three methene protons [δ_H 3.48 (1H, d, *J* = 18.0 Hz, H-10α), 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 (δ_C 182.4), three methyls (δ_C 57.3, 56.4, 58.3), three methylenes (δ_C 36.2, 38.2, 61.3), an methine (δ_C 77.7), one quaternary carbon (δ_C 42.8), a four-substituted phenyl-ring (δ_C 115.8, 148.3, 153.1, 111.1, 121.6, 158.4) and two double bond (δ_C 121.6, 158.4, 127.9, 149.7). All these spectroscopic data indicated that **1** possessed a morphinandienone skeleton [10].

The ^{1}H-^{1}H COSY correlations between the H-15 and H-16 and between H-9 and H-10 revealed the existence of two units of -CH_2-CH_2 and -CH-CH_2 as shown in Figure 1. The HMBC spectrum of **1** exhibited correlations from the aromatic proton at H-1 (δ_H 6.64) to carbons at δ_C 36.2 (C-10), δ_C 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 δ_H 2.45 (H-15) to carbons at δ_C 42.8 (C-13), δ_C 149.7 (C-14), δ_C 126.3 (C-12), δ_C 128.7 (C-16), δ_C 121.6 (C-5) and correlations from methene carbon proton at δ_H 4.30 (H-9) to δ_C C-8 (127.9), δ_C 42.8 (C-13), δ_C 149.7 (C-14), δ_C 58.3 (O-N-CH_3) confirmed that **1** had the morphinandienone skeleton. The ROESY correlations between δ_H 3.83 (3-OCH_3) and δ_H 7.12 (H-4), and between δ_H 3.89 (6-OCH_3) and δ_H 6.92 (H-5) verified the positions of the two methoxyl groups. Further checking its NMR data, **1** was similar to those of pallidine except for the characteristic downfield shifts of the carbon resonances at δ_C 77.7, 61.3, 58.3 for C-9, C-16, and O-N-CH_3 [10]. By comparing the ^{13}C-NMR data of **1** with those of pallidine, the carbon signals at C-9, C-16, and O-N-CH_3 were relatively deshielded ($\Delta\delta_C$ +17.0, 15.6, 16.7) suggesting that **1** 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 δ_H 4.30 and δ_H 3.48 (10α) indicted that H-9 was α orientated. Thus, compound **1** was established as a new morphinandienone alkaloid with a given name glutinosine A.

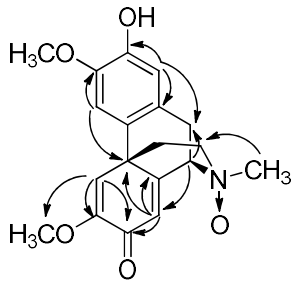

Figure 2. Key HMBC correlations of compound **1**

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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