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诺丽酵素中两个新的曲酸类化合物对胰岛素抵抗 HepG2 细胞葡萄糖摄取的影响

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摘要 从诺丽酵素中分离获得两个新的曲酸类化合物 nonikojic A (**1**)和 nonikojic B (**2**), 通过多种现代波谱技术确定了它们的化学结构. 并对这 2 个化合物进行了胰岛素抵抗 HepG2 细胞葡萄糖摄取的活性评价, 结果显示化合物 **1** 和 **2** 在 50 μmol/L 剂量下均可显著地促进胰岛素抵抗 HepG2 细胞的葡萄糖摄取, 从而改善胰岛素抵抗.
关键词 诺丽酵素; 曲酸类化合物; 葡萄糖摄取; 胰岛素抵抗 HepG2 细胞

Two New Kojic Acids from *Noni* Juice with Glucose Uptake Activity in Insulin-Resistant HepG2 Cells

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Abstract Two new kojic acids, nonikojics A and B (**1** and **2**), were obtained from the water soluble extract of the *Noni* juice, and their chemical structures were elucidated by comprehensive modern spectroscopic techniques. The effects on glucose uptake of two new compounds were evaluated in insulin-resistant HepG2 cells, and the results indicated that **1** and **2** significantly increased glucose uptake with doses of 50 μmol/L and thus improved insulin resistance.
Keywords *Noni* juice; kojic acids; glucose uptake; insulin-resistant HepG2 cells

1 Introduction

Morinda citrifolia L. (*Noni*) from the family Rubiaceae is used as a traditional medicinal herb to treat various diseases more than 2000 years in Polynesia due to its active ingredients.[1-3] Besides, it is also widely distributed in Southern China such as Hainan island and Paracel islands by reason of the favorable tropical and subtropical climate. *Noni* fruit is principally fermented into *Noni* juice for consumption to get rid of pungent and bitter taste of ripe *Noni* fresh fruit.[4-5] Phytochemical studies have showed that *Noni* juice mainly contains flavonoids, lignans and glycoside compounds,[6-11] and some of which exhibited significant ability of promoting glucose uptake in insulin resistant (IR) HepG2 cells to exert hypoglycemic activity.[12-14] In the course of exploring the structurally diverse bioactive constituents from this species, two new compounds, nonikojics A and B (**1** and **2**) were obtained from *Noni* juice (Figure 1). Moreover, activ-

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ity test results showed that the two compounds could increase glucose uptake in IR-HepG2 cells. Herein, we report the isolation, structure elucidation, and the effects on cellular glucose uptake of two new compounds.

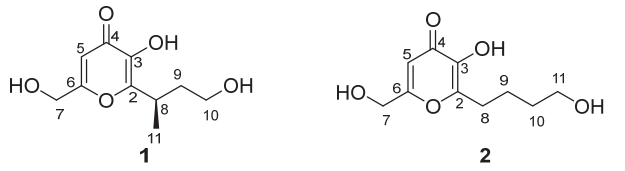

Figure 1 Structures of compounds **1** and **2** isolated from *Noni* juice

2 Results and discussion

2.1 Structure identification of compound 1

Compound **1** was isolated as yellow oiliness. Its molecular formula was determined as $C_{10}H_{14}O_5$ (4 degrees of unsaturation) on the basis of HRESIMS data ($[M+H]^+$, *m/z* 215.0901; calcd 215.0902). The ^1H NMR data of **1** (Table 1) showed one olefince proton signal at δ_H 6.43 (s, 1H, H-5), three methylene signals at δ_H 4.41 (s, 2H, H-7), 1.93 (m, 1H, H-9a), 1.79 (m, 1H, H-9b) and 3.53 (t, J=7.6 Hz, 2H, H-10), one methyl signal at δ_H 1.26 (d, J=6.8 Hz, 3H, H-11) and one methine signal at δ_H 3.39 (m, 1H, H-8). The ^{13}C NMR and DEPT spectra data (Table 1) exhibited 10 carbon resonances, including five olefinic sp^2 carbons (δ_C 176.3, 169.3, 156.7, 142.7 and 109.7), three methylenes (δ_C 61.3, 60.8 and 37.7), a methine (δ_C 30.8) and a methyl group (δ_C 17.9). Based on these findings, compound **1** has a kojic acid skeleton. The location of the hydroxymethyl at C-6 was confirmed by the HMBC correlation from H-7 to C-5/6 (Figure 2). The connectivity of C-8–C-11 was established by ^1H-^1H COSY correlations between H-8/H-9, H-9/H-10 and H-8/H-11. The HMBC correlations from H-9 and H-11 to C-2 (Figure 2) established that the C-8 linked with C-2. Thus, the planar structure of **1** was defined as depicted. The absolute configuration of **1** only holding one chiral center at C-8 was determined as *R*-configuration by comparison of the optical rotation ($[\alpha]_D^{24}$ −28.4), compared with that of standard versicolone B ($[\alpha]_D^{25}$ −33.1).[15] Thus, the structure of **1** named nonikojic A was assigned as depicted.

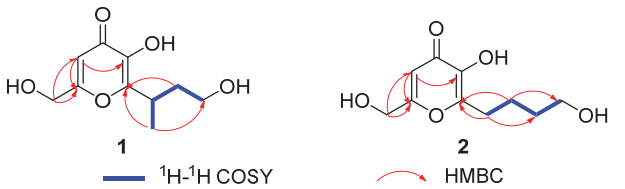

Figure 2 Key ^1H-^1H COSY and HMBC correlations of **1** and **2**

2.2 Structure identification of compound 2

Compound **2** was isolated as yellow oiliness. Its molecular formula was determined as $C_{10}H_{14}O_5$ (4 degrees of unsaturation) on the basis of HRESIMS data ($[M+H]^+$, *m/z* 215.0903; calcd 215.0902). The ^1H NMR data of **2** (Table 1) showed one olefince proton signal at δ_H 6.44 (s, 1H, H-5), five methylene signals at δ_H 4.40 (s, 2H, H-7), 2.76 (t, J=7.6 Hz, 2H, H-8), 1.75 (m, 2H, H-9), 1.59 (m, 2H, H-10) and 3.58 (t, J=7.6 Hz, 2H, H-11). The ^{13}C NMR and DEPT spectra data (Table 1) exhibited 10 carbon resonances, including five olefinic sp^2 carbons (δ_C 176.2, 169.2, 154.2, 143.3 and 109.8), and five methylenes (δ_C 62.4, 61.3, 32.9, 28.7 and 24.2). Based on these findings, compound **2** was also presumed to be a kojic acid, and the structure of **2** was found to be similar to compound **1**. The difference was with the presence of five methylenes and the absence of a methyl in **2**. The connectivity of C-8–C-11 was established by ^1H-^1H COSY correlation between H-8/H-9, H-9/H-10, and H-10/H-11 (Figure 2). The HMBC correlation from H-9 to C-2 established that the C-8 linked with C-2. The structure of **2** was defined as depicted. Thus, the structure of **2** named nonikojic B.

Table 1 ^1H NMR and ^{13}C NMR data (δ) for **1** and **2** (400, 100 MHz)

Position	**1** (in CD_3OD)		**2** (in CD_3OD)	
	δ_H (*J*/Hz)	δ_C	δ_H (*J*/Hz)	δ_C
1				
2		156.7		154.2
3		142.7		143.3
4		176.3		176.2
5	6.43 (s)	109.7	6.44 (s)	109.8
6		169.3		169.2
7	4.41 (s)	61.3	4.40 (s)	61.3
8	3.39 (m)	30.8	2.76 (t, 7.6)	28.7
9	1.93 (m) 1.79 (m)	37.7	1.75 (m)	24.2
10	3.53 (t, 7.6)	60.8	1.59 (m)	32.9
11	1.26 (d, 6.8)	17.9	3.58 (t, 7.6)	62.4

2.3 Effects on glucose uptake of compounds 1 and 2 in IR-HepG2 cells

The effects on glucose uptake of compounds **1** and **2** were evaluated in IR-HepG2 cells, and rosiglitazone (ROSI) at 25 μmol/L was used for positive control. As shown in Figure 3, the glucose uptake of the model group (MOD) incubated with 50 μmol/L high glucose was significantly lower than that of control group (CON), indicating that IR was successfully established in HepG2 cells. While the positive control ROSI reversed the decrease in glucose uptake of IR cells in MOD group (P<0.01). Furthermore, the results indicated that **1** and **2** also significantly increased glucose uptake in IR-HepG2 cells with doses of 50 μmol/L, suggesting that two compounds contributed to improving IR and thus may play the hypoglycemic effect. Moreover, it also indicates that the activities of two new compounds may have a supporting effect on the treatment of diabetes by *Noni* juice to some extent.

3 Conclusions

Two new kojic acids, nonikojics A and B (**1** and **2**), were

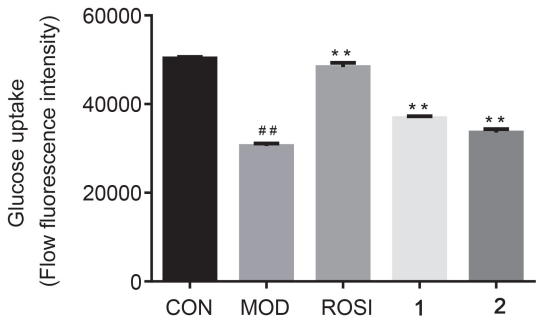

Figure 3 Effects on glucose uptake of compounds **1** and **2** in IR-HepG2 cells

obtained from the water extract of *Noni* juice, and the structures were identified by NMR and HRESIMS. Activity test results confirmed that **1** and **2** significantly increased glucose uptake with doses of 50 μmol/L and thus improved insulin resistance.

4 Experimental section

4.1 General experimental procedures

Optical rotations were measured on a JASCO P-1020 digital polarimeter (Jasco, Japan). IR spectra were recorded on a Thermo Nicolet 6700 (using KBr disks) spectrophotometer (Thermo Scientific, Madison, WI, USA). 1D and 2D NMR spectra were measured on Bruker AV-400 (Bruker Corporation, Switzerland) and JNM-ECZ600R (JEOL, Japan) instrument with TMS as the internal standard. HRESIMS spectra were made on a Bruker Daltonics Apex-Ultra 7.0 T (Bruker Corporation, Billerica, MA, USA). Semi-preparative HPLC was performed on an Agilent 1260 LC series with a DAD detector using an Agilent Eclipse XDB-C_{18} column (9.4 mm×250 mm, 5 μm). Sephadex LH-20 (Pharmacia Co. Ltd, Sandwich, UK) and Silica gel (200～300 mesh, 300～400 mesh Qingdao Marine Chemical Factory, Qingdao, China) were used for column chromatography (CC). All solvents were purchased from Xilong Chemical Reagent Factory (Guangzhou, China).

4.2 Material

Noni juice used for this investigation was supplied with Hainan Songji Yunshang Technology Limitied Company in October 2017.

4.3 Extraction and isolation

The *Noni* fruit slices were sealed into the sterile flask and fermented for 3 months to obtain *Noni* juice. And then *Noni* juice (20.0 L) was extracted with ethyl acetate (each time for 20.0 L) for 3 times at room temperature. The remaining water was evaporated in vacuum to obtain H_2O fraction (100.0 g), which was purified over the macroporous resin (D-101) by eluting with a gradient of EtOH/H_2O (20%, 40%, 50%, 80% and 100%) to give five fractions (Fr.1～Fr.5). And Fr.3 (2.3 g) was further subjected to MCI GEL CHP20p chromatography (MeOH/H_2O, 10%→100%) to yield five fractions (Fr.3A～Fr.3E). Compounds **1** (8.0 mg) and **2** (9.8 mg) were separated from Fr.3C via semi-preparative HPLC (13% CH_3OH, 2.0 mL/min, t_R=32.0 min and 35.0 min).

4.4 Spectroscopic data

Compound **1**: Yellow oiliness; $[\alpha]_D^{24}$ − 28.4 (*c* 0.1, CH_3OH); UV (CH_3OH) λ_{max} [log ε/(L•mol^{-1}•cm^{-1})]: 192 (0.95) nm; IR (KBr) ν_{max}: 3446, 2927, 1650, 1385 cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Table 1; HRESIMS (*m/z* 215.0901 [M＋H]$^+$, calcd for 215.0902).

Compound **2**: Yellow oiliness; UV (CH_3OH) λ_{max} [log ε/(L•mol^{-1}•cm^{-1})]: 192 (0.84) nm; IR (KBr) ν_{max}: 3439, 2932, 1659, 1384 cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Table 1; HRESIMS (*m/z* 215.0903 [M＋H]$^+$, calcd. for 215.0902).

4.5 Cellular glucose uptake activity assay

In this study, HepG2 cells were induced with 50 mmol/L high glucose for 48 h to establish IR cell model as previous described by Luo *et al.*[16] with some modification. Subsequently, the effects of compounds **1** and **2** on glucose uptake were tested in IR-HepG2 cells. The cells were treated with **1** and **2** at doses of 50 μmol/L for 48 h, and then incubated fluorescent-labeled glucose analogue (2-NBDG) with 25 μmol/L for another 20 min. Finally, the fluorescence signal intensity of intracellular 2-NBDG was measured by flow cytometry to obtain the liver glucose uptake. All values are expressed as means ±SD, n=6. $^{\#\#}P$<0.01 compared with the CON group, and $^{**}P$<0.01 compared with the MOD group.

Supporting Information 1D NMR and 2D NMR, HRESIMS spectra of compounds **1** and **2**. The Supporting Information is available free of charge via the Internet at http://sioc-journal.cn/.

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