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Isolation and identification of antioxidant and α-glucosidase inhibitory compounds from fruit juice of *Nitraria tangutorum*

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ABSTRACT

Nitraria tangutorum Bor., having edible berries, is valued for reputed health benefits in Qinghai-Tibet plateau. The phytochemical research on the fruit juice of *N. tangutorum* led to the isolation of twenty-six compounds including five new compounds, tangutorids A–D (**1**, **2**, **3a**, and **3b**), and (3*E*,5*E*)-7-*O*-β-glucosyl-4-(2-methoxy-2-oxoethyl)hepta-3,5-dienoic acid (**15**). The structures of these compounds were elucidated through comprehensive spectroscopic analyses. Tangutorids A–F were the first examples of glucose-derived β-carbolines from natural products. The biogenetic pathways of **1**–**8** were proposed to involve Pictet–Spengler reactions and described starting from the co-isolated tryptophan (**10**) and corresponding aldehydes. All isolates were evaluated for their antioxidant and α-glucosidase inhibitory activities. Compounds **21**, **22**, and **24** showed antioxidant activity with SC_{50} values ranging from 12.2 ± 1.9 to 30.4 ± 2.7 μg/mL, and compound **1** showed strong α-glucosidase inhibitory effect with IC_{50} value of 63.3 ± 4.6 μg/mL.

1. Introduction

β-Carbolines are a kind of naturally-occurring pyridoindole alkaloids produced from tryptophan and aldehydes and widespread in commercial food and beverages (Herraiz, 2004). It has become clear that these alkaloids occur in fruits, fruits processed products (Herraiz & Galisteo, 2002), smoked foods (Papavergou & Herraiz, 2003), fermented products (Adachi et al., 1991; Herraiz, 1999; Sen, Seaman, Lau, Weber, & Lewis, 1995), and are produced during food production processing and storage (Herraiz, 2009). In addition, these alkaloids also can be found in the human biological tissues and fluids, which illustrates that they probably come from the diet (Adachi et al., 1991). Previous studies showed that β-carboline alkaloids frequently exhibit extensive biological activities such as neuroactive, antioxidant, antimicrobial, antithrombotic, antiparasiticidal, antimalarial, antiviral, and antitumor effects (Di Giorgio et al., 2004; Herraiz & Galisteo, 2015; Kusurkar & Goswami, 2004; Zhao et al., 2006). These molecules could possess both biological and toxicological effects when they are ingested from foods or beverages, which will be potentiated further if they are accumulated within body tissues (Herraiz, 2009). Therefore, the analysis, isolation and identification of β-carboline alkaloids in foodstuffs are of great interests.

Nitraria tangutorum Bobr, a shrub belonging to the *Nitraria* genus (Zygophyllaceae), is mainly grown in desert and semidesert regions of Qinghai-Tibet plateau. It is a typical desert plant with strong resistance to drought, salinity, alkalinity, and high temper-

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ature and has been used as an ideal material for studying the adaptability and response of plants to salinity stress (Yang, Wei, Shi, Fan, & An, 2010; Yang, Yang, Li, Shi, & Lu, 2013). The fruit of *N. tangutorum*, called "desert cherry", has a high potential economic value as a source of edible berries and has been traditionally used as healthy food and folk medicine to treat abnormal menstruation, heart disease, neurasthenia, and dyspepsia in western China (Hu, Zheng, Li, & Suo, 2014). Besides, the fruit was also wildly used to extract pigments as natural food colorants and make wine by locals. In previous studies, numerous compounds, such as alkaloids and flavonoids had been isolated from the plants of *Nitraria* genus, and many of them showed exert antitumor or anti-oxidative activities. (Du, Xin, & Peng, 2015) And a few β-carboline alkaloids and phenols have been identified from the leaves, seeds, and dry fruit of *N. tangutorum* (Duan, Williams, & Chen, 1999; Lu, Ouyang, Su, Ji, & Liu, 2013; Wu et al., 2014). However, there is little information about the chemical constituents of the fresh fruit juice of *N. tangutorum*. In order to identify the chemical constituents of the fresh fruit juice of *N. tangutorum*, we carried out the phytochemical investigation on it. Herein, we report the isolation and structural identification of new compounds, as well as the probable biosynthetic pathway of β-carboline alkaloids from the fruit juice of *N. tangutorum*. The antioxidant and α-glucosidase inhibitory activities of the isolates are also tested.

2. Material and methods

2.1. General experimental procedure

A Horiba SEPA-300 high-sensitive polarimeter was used to measure optical rotations. (Horiba, Kyoto, Japan) Ultraviolet absorption (UV) spectra were acquired on a Shimadzu UV2401A ultraviolet–visible spectrophotometer (Shimadzu Co., Tokyo, Japan). IR spectra were performed on a Bio-Rad FTS-135 series spectrometer (Bio-Rad, Hercules, CA, USA) with KBr pellets. 1D and 2D NMR spectra were measured on a Bruker Avance III-600 spectrometer, using tetramethylsilane as an internal standard. Chemical shifts were reported in units of δ (ppm) and coupling constants (J) were expressed in Hz. High resolution mass spectra were performed on an API QSTAR Pulsar-1 spectrometer (Advanced Biomics, Los Angeles, CA, USA) tandem an Agilent 6230 Accurate-Mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). Column chromatography (CC) were carried out over D101 risen (Tianjin Agricultural Chemical Co. Ltd., Tianjin, China), silica gel (100–200 mesh) (Qingdao Haiyang Chemical Co., Qingdao, China) and MCI gel CHP 20P (75–150 μm, Tokyo, Japan). Pre-coated silica gel plates (Qingdao Haiyang Chemical Co., Qingdao, China) were used for thin-layer chromatography (TLC). Detection was done under UV light (254 nm and 365 nm) and by spraying the plates with 10% sulfuric acid ethanol solution followed by heating. An Agilent series 1200 (Agilent Technologies, Palo Alto, CA, USA) was used for analysis HPLC. Semi-preparative HPLC was done on a Hanbon series NP7005C (Hanbon Sci & Tech, Jiangsu, China), the column used was XCharge C18 (10 μm, 100 Å, 250 mm × 20 mm, Acchrom, Beijing, China). Preparative HPLC was done on a Hanbon series NP7010C equipped with a dynamic axial compression column DAC-HB50 C18 (10 μm, 100 Å, 400 mm × 50 mm, Hanbon Sci & Tech, Jiangsu, China)

2.2. Plant material

The ripe fruit of *N. tangutorum* was manually collected in Qaidam Basin, Qinghai Province, People's Republic of China, and identified by Professor Lijuan Mei (Northwest Institute of Plateau Biology, Chinese Academy of Science). The fruit was washed and ground by hydraulic laboratory-scale juice extractor. The cloudy juice obtained was filtered through degreasing cotton to remove the solid residue. According to this method, two liters of fresh fruit juice was obtained and used as experimental material.

2.3. Extraction and isolation

The fresh fruit juice (2 L) of *N. tangutorum* was subjected to D101 Risen column chromatography (CC), eluting successively with water, 40% ethanol and 90% ethanol aqueous solution (each for 4 L) to give three fractions (Fr1−3).

The 40% ethanol aqueous fraction (Fr2, 40 g) was submitted to MCI gel CHP 20P CC eluting with gradient methanol aqueous (0%, 20%, 40%, 60%, 80% and 100% of methanol, each for 2L) to afford six portions Fr21−26 by TLC analysis. Compound **13** (339 mg) was acquired by re-precipitation from Fr21 (2.1 g) in methanol and the filtrate was further separated by prep-HPLC through an XCharge C18 column (10%–30% MeCN with 0.2% formic acid, for 40 min, with a flow rate of 15 mL/min) to give compounds **16** (t_R of 12.4 min, 21 mg), **17** (t_R of 14.0 min, 13 mg), and **15** (t_R of 16.4 min, 17 mg). Fr22 (7.0 g) was subjected to a silica gel column, eluting with a $CHCl_3$-MeOH-H_2O gradient system (9:1:0.1–6:4:1, v/v/v), to give three fractions (Fr221–223) based on TLC analysis. Fr221 (1.1 g) was subjected to an XCharge C18 column (5%–45% MeCN with 0.2% formic acid, for 40 min, with a flow rate of 15 mL/min) to give compound **14** (t_R of 19.1 min, 117 mg). With the same method, compound **18** (t_R of 14.3 min, 9 mg) was obtained from Fr222 (136 mg). Fr223 (3.1 g) was separated through an XCharge C18 column (5%–25% MeCN with 0.2% formic acid, for 20 min, with a flow rate of 15 mL/min) to give a Fr2231 (2.7 g) and compounds **8** (t_R of 16.7 min, 13 mg) and **11** (t_R of 18.2 min, 6 mg). Fr2231 was further subjected to an XCharge C18 column (5%–35% MeCN with 0.2% formic acid, for 60 min, with a flow rate of 15 mL/min) to yield compounds **10** (t_R of 15.7 min, 18 mg), **7** (t_R of 21.1 min, 20 mg), and **9** (t_R of 21.1 min, 73 mg). Fr23 (7.2 g) was separated by a silica column eluting with a $CHCl_3$-MeOH-H_2O gradient system from 9:1:0.1 to 7:3:0.5 to yield compound **12** (5 mg) and Fr231 (3.4 g). Fr231 was separated over an XCharge C18 column (5%–55% MeCN with 0.2% formic acid, for 50 min, with a flow rate of 15 mL/min) to give compounds **23** (t_R of 17.1 min, 13 mg), **20** (t_R of 19.0 min, 88 mg), **19** (t_R of 19.9 min, 56 mg), **21** (t_R of 22.5 min, 37 mg), and **22** (t_R of 19.0 min, 113 mg). Fr24 (4.2 g) was separated through a DAC-HB50 C18 column (10%–50% MeOH with 0.2% formic acid, for 40 min, with a flow rate of 60 mL/min) to yield three fractions (Fr241−243). Fr241 (244 mg) was further purified by an XCharge C18 column (8% MeCN with 0.2% FA, for 30 min, with a flow rate of 15 mL/min) to give compounds **4a** (t_R of 18.7 min, 26 mg) and **4b** (t_R of 19.4 min, 21 mg). Fr242 (1.3 g) was purified by an XCharge C18 column (5%–35% MeCN with 0.2% FA, for 50 min, with a flow rate of 15 mL/min) to yield compounds **3a** (t_R of 21.1 min, 57 mg), **3b** (t_R of 22.2 min, 31 mg), **24** (t_R of 25.0 min, 4 mg), and **2** (t_R of 31.9 min, 12 mg). Fr25 (2.7 g) was subjected to a silica column eluting with a $CHCl_3$-MeOH-H_2O gradient system (9:1:0.1–7:3:0.5, v/v/v) to yield compound **6** (67 mg) and Fr251 (1.2 g). Fr251 was further purified by an XCharge C18 column (10%–60% MeCN with 0.2% formic acid, for 50 min, with the follow rate of 15 mL/min) to give compounds **5** (t_R of 29.7 min, 77 mg) and **1** (t_R of 31.4 min, 109 mg). Fig. S43 showed the extraction and separation processes associated with the fresh fruit juice (2 L) of *N. tangutorum*.

Tangutorid A (**1**). Light brown solid. UV (MeOH) λ_{max} nm (log ε): 213 (4.14), 237 (4.00), 271 (4.27), 287 (3.98), 343 (3.45), 393 (3.06); IR (KBr) ν_{max}: 3426, 1716, 1694, 1670, 1625, 1366, 1305, 1276, 1135, 1021 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data see