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

Jian-Qiang Zhao [a,1], Yan-Ming Wang [b,1], Yan-Long Yang [c], Ying Zeng [c], Qi-Lan Wang [a], Yun Shao [a], Li-Juan Mei [a,*], Yan-Ping Shi [b], Yan-Duo Tao [a,*]

[a] Key Laboratory of Tibetan Medicine Research, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810008, PR China
[b] Key Laboratory of Chemistry of Northwestern Plant Resources of CAS and Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, PR China
[c] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, PR China

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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-

* Corresponding authors.
E-mail addresses: meilijuan111@163.com (L.-J. Mei), soqiang.happy@163.com (Y.-D. Tao).
[1] These authors contributed equally.

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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

Table 1
^{1}H NMR and ^{13}C NMR Spectroscopic Data of Compounds **1**–**3b** in DMSO-d_6.

	1		**2**		**3a**		**3b**	
No.	δ_H, mul. (*J* in Hz)	δ_C	δ_H, mul. (*J* in Hz)	δ_C	δ_H, mul. (*J* in Hz)	δ_C	δ_H, mul. (*J* in Hz)	δ_C
1		134.6, C		147.8, C		146.9, C		147.9, C
3		136.7, C		135.6, C		135.3, C		135.5, C
3a		166.2, C		166.9, C		166.6, C		166.2, C
4	9.17, s	121.0, CH	8.83, s	115.8, CH	8.85, s	116.8, CH	8.83, s	115.9, CH
5		131.5, C		128.6, C		128.6, C		128.5, C
6		120.2, C		120.9, C		120.8, C		120.8, C
7	8.45, d (7.5)	122.2, CH	8.37, d (7.7)	121.8, CH	8.37, d (7.7)	121.9, CH	8.38, d (7.8)	121.8, CH
8	7.36, t (7.1)	120.9, CH	7.30, t (7.4)	120.1, CH	7.30, t (7.3)	120.1, CH	7.30, t (7.4)	120.1, CH
9	7.64, t (7.2)	129.3, CH	7.59, t (7.5)	128.5, CH	7.59, t (7.2)	128.8, CH	7.59, t (7.4)	128.4, CH
10	7.83, d (7.9)	113.4, CH	7.76, d (8.0)	112.7, CH	7.76, d (6.7)	112.8, CH	7.78, d (7.8)	112.9, CH
11		142.3, C		141.0, C		141.0, C		140.9, C
12	12.25, s		11.78, s		11.76, s		11.72, s	
13		135.0, C		134.0, C		134.3, C		133.9, C
14		201.5, C	5.11, brs	73.0, CH	5.38, brs	71.6, CH	5.37, d (8.8)	69.9, CH
15	2.94, brs	32.3, CH_2	1.85, m 2.06, m	33.4, CH_2	2.01, m 2.36, d (12.4, 6.3)	40.0, CH_2	1.79, t (12.0) 2.19, t (12.0)	39.9, CH_2
16	3.69, brs	31.5, CH_2	1.32, m1.72, m	30.0, CH_2	3.53, overlapped	69.9, CH	3.86, d (6.2)	68.3, CH
17		210.2, C	3.42, brs	71.5, CH	3.35, overlapped	75.1, CH	3.39, overlapped	75.4, CH
18	4.20, s	67.8, CH_2	3.25, dt (10.3, 7.8)	66.0, CH_2	3.35, overlapped 3.53, overlapped	63.2, CH_2	3.40, overlapped 3.59, d (7.9)	63.5, CH_2

Table 1; HRESIMS *m/z*: 327.0978 $[M+H]^+$ (calculated for $C_{17}H_{15}N_2O_5^+$ 327.0975).

Tangutorid B (**2**). Light brown solid. $[\alpha]_D^{25.3}$ + 14.9 (*c* 0.27, H_2O); UV (MeOH) λ_{max} nm (log ε): 192 (4.12), 208 (4.04), 237 (3.96), 271 (4.18), 340 (3.31); IR (KBr) ν_{max}: 3243, 3088, 2935, 1629, 1599, 1379, 1310, 1248, 1063 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data see Table 1; HRESIMS *m/z* 331.1296 $[M+H]^+$ (calculated for $C_{17}H_{19}N_2O_5^+$ 331.1288).

Tangutorid C (**3a**). Light brown solid. $[\alpha]_D^{24.3}$ −15.7 (*c* 0.10, MeOH); UV (MeOH) λ_{max} nm (log ε): 202 (4.02), 215 (4.02), 238 (4.01), 270 (4.19), 339 (3.39); IR (KBr) ν_{max}: 3417, 2923, 1629, 1601, 1376, 1307, 1248, 1076 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data see Table 1; HRESIMS *m/z* 347.1263 $[M+H]^+$ (calculated for $C_{17}H_{19}N_2O_6^+$ 347.1238).

Tangutorid D (**3b**). Light brown solid. $[\alpha]_D^{25.2}$ −17.1 (*c* 0.11, H_2O); UV (MeOH) λ_{max} nm (log ε): 192 (3.98), 212 (3.84), 232 (3.73), 270 (3.85), 287 (3.57), 340 (3.11); IR (KBr) ν_{max}: 3440, 3232, 2922, 1639, 1600, 1376, 1322, 1232, 1077, 1051, 1029 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data see Table 1; HRESIMS *m/z* 347.1252 $[M+H]^+$ (calculated for $C_{17}H_{19}N_2O_6^+$ 347.1238).

Tangutorid E (**4a**). Light brown solid. $[\alpha]_D^{24.2}$ −3.1 (*c* 0.70, MeOH); UV (MeOH) λ_{max} nm (log ε): 211 (4.26), 238 (4.40), 289 (4.05), 340 (3.58); IR (KBr) ν_{max}: 3410, 2923, 1630, 1592, 1498, 1454, 1429, 1325, 1234, 1061, 744 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data see Table 2; HRESIMS *m/z* 303.1348 $[M+H]^+$ (calculated for $C_{16}H_{19}N_2O_4^+$ 303.1339).

Tangutorid F (**4b**). Light brown solid. $[\alpha]_D^{24.0}$ −45.2 (*c* 0.30, MeOH); UV (MeOH) λ_{max} nm (log ε): 211 (4.26), 237 (4.41), 289 (4.05), 339 (3.57); IR (KBr) ν_{max}: 3411, 2924, 1631, 1592, 1500, 1452, 1429, 1325, 1234, 1055, 745 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data see Table 2; HRESIMS *m/z* 303.1357 $[M+H]^+$ (calculated for $C_{16}H_{19}N_2O_4^+$ 303.1339).

(3*E*,5*E*)-7-*O*-β-glucosyl-4-(2-methoxy-2-oxoethyl)hepta-3,5-die noic acid (**15**). White powder. $[\alpha]_D^{24.5}$ −45.5 (*c* 0.12, MeOH); UV (MeOH) λ_{max} nm (log ε): 203 (3.83), 223 (3.74), 278 (3.20); IR (KBr) ν_{max}: 3428, 2927, 1720, 1638, 1387, 1267, 1076, 1042 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data see Table 2; HRESIMS *m/z* 399.1247 $[M+H]^+$ (calculated for $C_{16}H_{24}NaO_{10}^+$ 399.1262).

2.4. Assay of ABTS and DPPH radical-scavenging

Radical scavenging activity against ABTS and DPPH was performed according to previously reported protocols (Gao, Xu, Yang, Xu, & Zhang, 2010; Liang, Xue, Kennepohl, & Kitts, 2016). Ascorbic acid (VC) was used as positive control. In DPPH radical scavenging assay, DPPH was dissolved in ethanol to a concentration of 250 μM and allowed the mixture to stand in the dark at room temperature for 12–16 h. The DPPH stock solution was diluted with ethanol to an absorbance of 0.700 (±0.020) at 517 nm before use. The absorbance reading was taken after mixing for 30 min. The optical density was measured at 517 nm on a microplate reader. In ABTS radical scavenging assay, ABTS solution

Table 2
^{1}H NMR and ^{13}C NMR spectroscopic data of compounds **4a**, **4b**, and **15** in CD_3OD.

	4a		**4b**			**15**	
No.	δ_H, mul. (*J* in Hz)	δ_C	δ_H, mul. (*J* in Hz)	δ_C	*No.*	δ_H, mul. (*J* in Hz)	δ_C
1		145.5, C		145.9, C	1		175.3, C
3	8.18, brs	131.9, CH	8.18, brs	131.4, C	2	3.10, overlapped	35.0, CH_2
4	8.13, brs	116.4, CH	8.10, brs	116.2, CH	3	5.80, t (7.4)	129.2, CH
5		133.3, C		133.9, C	4		134.2, C
6		121.2, C		121.2, C	5	6.28, d (15.9)	136.3, CH
7	8.06, d (7.4)	123.4, CH	7.98, brs	123.3, CH	6	5.70, dt (15.9, 6.1)	125.8, CH
8	7.22, brs	122.2, CH	7.18, brs	122.2, CH	7	4.12, dd (12.8, 6.5) 4.32, dd (12.3, 5.5)	70.5, CH_2
9	7.56, overlapped	131.6, CH	7.51, overlapped	132.0, CH	8	3.25, d (1.1)	33.5, CH_2
10	7.68, overlapped	113.7, CH	7.53, overlapped	113.5, CH	9		173.0, C
11		144.2, C		144.3, C	1′	4.20, d (7.8)	103.1, CH
13		133.9, C		132.6, C	2′	3.11, overlapped	75.1, CH
14	5.69, brs	70.1, CH	5.67, brs	67.8, CH	3′	3.25, overlapped	78.1, CH
15	2.20, brs 2.44, d (12.7)	40.4, CH_2	2.04, brs 2.19, brs	40.7, CH_2	4′	3.19, t (8.4)	71.7, CH
16	3.94, brs	71.1, CH	4.15, brs	70.0, CH	5′	3.16, m	78.0, CH
17	3.63, overlapped	76.4, CH	3.70, overlapped	76.6, CH	6′	3.57, overlapped 3.77, m	62.7, CH_2
18	3.64, d (11.3) 3.74, d (9.3)	64.4, CH2	3.72, overlapped 3.82, brs	64.6, CH_2	$-OCH_3$	3.56, s	53.4, CH_3

was diluted with ethanol to an absorbance of 0.700 (±0.020) at 734 nm. 20 μL of compounds was added into each well of 96-well cell culture plates. And then 180 μL of diluted ABTS solution (A_{734nm} = 0.700 ± 0.020) was added to the plates. The absorbance reading was taken exactly after initial mixing for 5 min. The optical density was measured at 734 nm on a microplate reader. The radical scavenging activity was determined by the following equation: % scavenging activity = $[A_{blank\ control} - A_{sample}]/A_{blank\ control} \times 100\%$. The SC_{50} value was obtained through GraphPad Prism 6.02. All determinations were carried out in triplicate.

2.5. α-Glucosidase assay

The α-glucosidase inhibitory activity assay was adapted as described previously (Yilmazer-Musa, Griffith, Michels, Schneider, & Frei, 2012). α-Glucosidase (0.26 U) from *Saccharomyes cerevisiae*

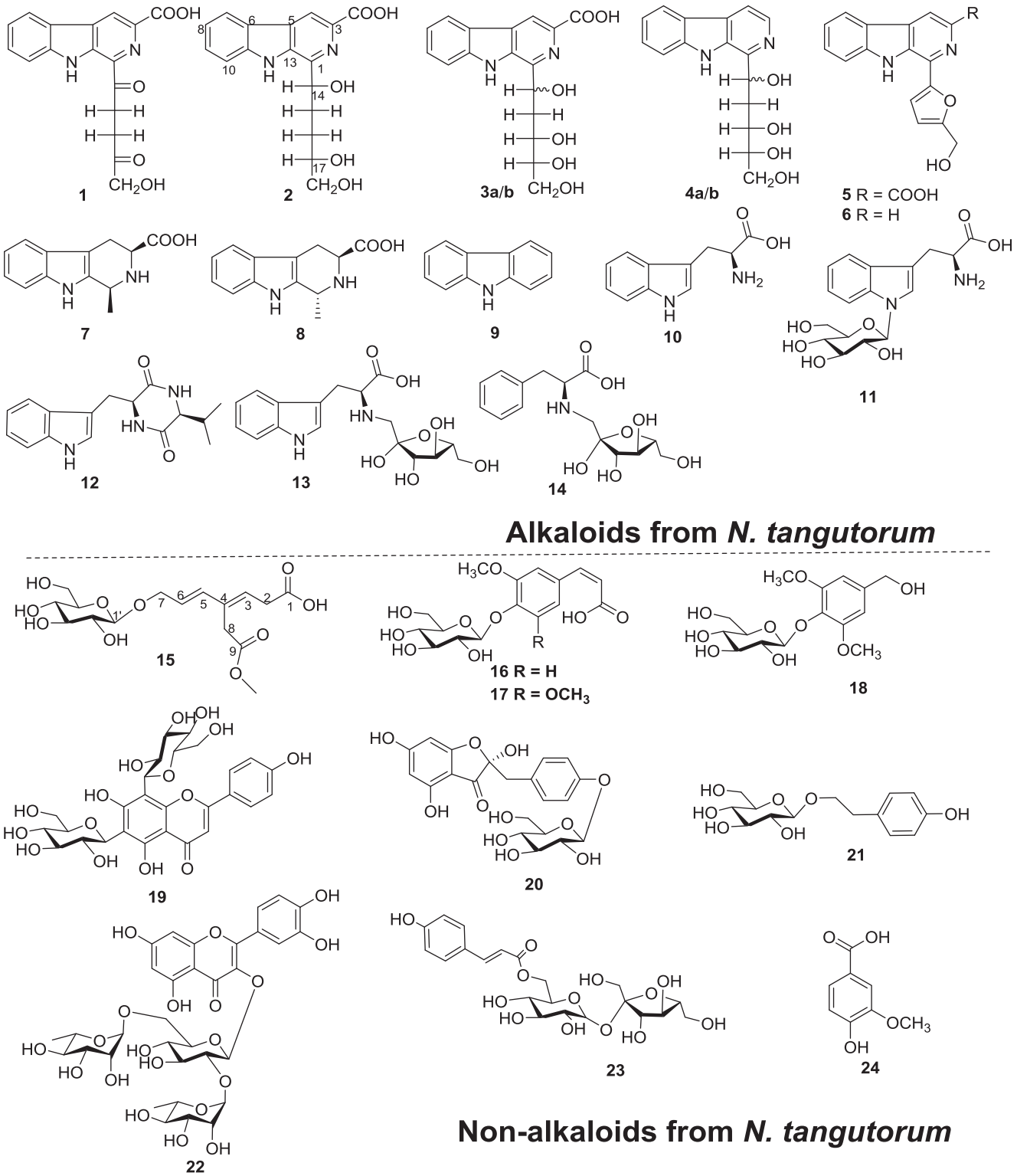

Fig. 1. Chemical structures of compounds **1**–**24**.

were diluted in 0.1 M phosphate buffer (Na_2HPO_4 and KH_2PO_4, pH 6.5). 25 μL of the test compound and 25 μL of α-glucosidase were preincubated in 96-well plates at 37 °C for 15 min. The reaction was initiated by adding 50 μL of 0.3125 mM pNPG (4-nitrophenyl α-D-glucopyranoside) as substrate. The plate was incubated for an additional 15 min at 37 °C, which was followed by the addition of 50 μL of 0.2 M Na_2CO_3 to stop the reaction. All test compounds were prepared in DMSO, and then diluted with the incubated buffer. Final concentrations of the tested compounds were between 6.25 and 250 μg/mL. The reaction was monitored by change of absorbance at 405 nm using a Dimension RxL Max clinical chemistry system Enspire MP150 (Siemens Healthineers).

2.6. Statistical analyses

Statistical elaborations were performed on Microsoft excel 2010 and GraphPad Prism 6.02 (GraphPad Software Inc., San Diego, CA, USA). All analyses were carried out in triplicate and the results are expressed as a mean ± standard deviation (SD). Significant differences were determined by one-way ANOVA, using statistical package SPSS (Version 22.0, SPSS Inc., Chicago, IL, USA). The results were regarded as significantly different at $p < 0.05$.

3. Results and discussion

The fresh fruit juice of *N. tangutorum* was subjected to the column chromatography over D101, MCI gel CHP 20P, silica gel, and preparative HPLC to afford twenty-six compounds, including four new alkaloids (**1**, **2**, **3a**, and **3b**) and a new non-alkaloid component (**15**) (Fig. 1).

Tangutorid A (**1**) was isolated as a light brown solid, and its molecular formula was determined as $C_{17}H_{14}O_5$ with 12 degrees of unsaturation by HRESIMS spectrum. The UV spectrum of **1** displayed absorption maxima at 213, 237, 271, 287, and 343 nm, suggesting the presence of β-carboline-3-carboxylic acid moiety (Jiao et al., 2010). The IR absorption bands at 3426 and 1716 cm^{-1} were assignable to hydroxy and carbonyl functional groups, respectively. The ^1H NMR spectrum in DMSO-d_6 showed resonances of five aromatic protons between δ_H 9.17 and 7.36, three methylene groups [δ_H 4.20 (2H, s), 3.69 (3H, s) and, 2.94 (2H, brs)], and a broad NH singlet at δ_H 12.25. The ^{13}C NMR and HSQC spectra revealed 17 resonances between δ_C 210.2 and 31.5, which were attributed to nine quaternary carbons, five methines and three methylene groups. Base on the ^1H–^1H COSY, HMBC (Fig. 2), and HSQC spectra, a set of four mutually coupled aromatic protons (δ_H 8.45–7.36) were assigned to the unsubstituted A ring of a β-carboline-3-carboxylic acid skeleton. The singlet proton at δ_H 9.17 was assigned as H-4 of a β-carboline-3-carboxylic acid by the HMBC correlations from the isolated aromatic proton to δ_C 166.2 (C-3a), 120.2 (C-6), and 135.0 (C-13). The above information confirmed the existence of β-carboline-3-carboxylic acid moiety. In addition, the correlations due to a $-CH_2-CH_2-$ moiety could be observed in the ^1H–^1H COSY spectrum. Combined with ^2J and ^3J correlations from δ_H 3.69 (H_2-16) to δ_C 201.5 (C-14), from δ_H 2.94 (H-15) to δ_C 210.2 (C-17), and from δ_H 4.20 (H-18) to δ_C 210.2 (C-17) in HMBC experiment, a $-CO-(CH_2)_2-CO-CH_2OH$ fragment was constructed. Since the two separate moieties, β-carboline-3-carboxylic acid and $-CO-(CH_2)_2-CO-CH_2OH$ were fully defined by the NMR data. C-1 must connect with the carbonyl group (C-14), which was also confirmed through the shielded C-1 signal at δ_C 134.6. In conclusion, the structure of **1** was fully established as shown in Fig. 1 and named as tangutorid A.

Tangutorid B (**2**) was yielded as a light brown solid. Its molecular formula was determined as $C_{17}H_{18}N_2O_5$ on the basis of its NMR and HRESIMS spectra. It was found to possess similar UV and IR spectroscopic characteristics to those of **1**. Moreover, the ^1H and ^{13}C NMR spectra of compound **2** also resembled those of **1**, except that the resonances of two carbonyl signals (C-14 and C-17) in **1** were replaced by two oxymethines (δ_H 5.11, brs; δ_C 73.0, C-14 and δ_H 3.42, brs; δ_C 71.5, C-17) in **2**. All NMR signals of **2** can be identified by HSQC, ^1H–^1H COSY and HMBC experiments (Table 1 and Fig. 2). Therefore, the structure of **2** was determined as shown in Fig. 1 and named as tangutorid B.

Tangutorids C and D (**3a** and **3b**) were both yielded as light brown solid and had the same molecular weight. Their molecular formulas were determined as $C_{17}H_{18}N_2O_6$ on the basis of NMR and HRESIMS spectra. Through extensive comparison of NMR spectroscopic data and molecular weight of **3a** and **3b** with those of **2**, we speculated that a methylene in **2** was substituted by a hydroxyl group in **3a** and **3b**. 2D NMR spectra confirmed that the hydroxyl group was located at C-16 (Table 1 and Fig. 2). Thus, compounds **3a** and **3b** had the same planar structure. But significant variations

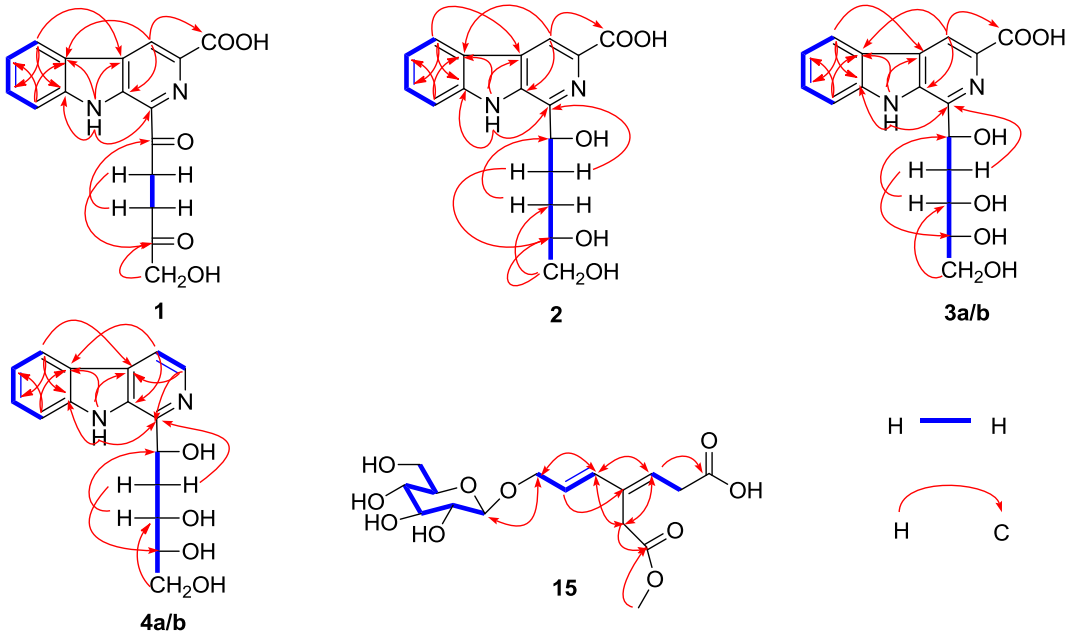

Fig. 2. Key HMBC and ^1H–^1H COSY correlations of **1**–**4** and **15**.

of the coupling constant for $^{3}J_{H\text{-}14,H\text{-}15}$ (brs for **3a** and 8.8 Hz for **3b**) and the chemical shifts of C-14 (δ_C 71.6 for **3a** and δ_C 69.9 for **3b**) could be observed. Therefore, **3a** and **3b** were evidently diastereomers with different configurations of the hydroxyl group at C-14. But there was no method to determine the exact configurations of C-14 for **3a** and **3b**, respectively. Similar diastereomers had been identified and quantitatively determined in many foodstuffs by means of HPLC analysis and chemosynthesis method (Diem & Herderich, 2001). Thus, the structures of compounds **3a** and **3b** were determined as shown in Fig. 1 and named as tangutorids C and D, respectively.

Tangutorids E and F (**4a** and **4b**) had the same molecular formula $C_{16}H_{18}N_2O_4$ determined by NMR and HRESIMS spectra. They were unequivocally identified as diastereomeric 1-(1,3,4,5-tetrahydroxypent-1-yl)-9*H*-pyrido[3,4-*b*]indoles by comparison of their ^{1}H, ^{13}C NMR, and MS data with those reported in the literature (Diem & Herderich, 2001). It is worth noting that although the existence of **4a** and **4b** in various food samples has been proved by HPLC means (Diem & Herderich, 2001), they were isolated from natural products for the first time. Therefore, we reported their structures determined by comprehensive spectroscopic analyses (Table 2 and Fig. 2) and named them as tangutorids E and F, respectively.

Compound **15** was obtained as a white powder. Its molecular formula was established as $C_{16}H_{24}O_{10}$ by HRESIMS and NMR data (Table 2). IR spectrum of **15** displayed absorption bands for hydroxyl (3428 cm^{-1}), alkyl (2927 cm^{-1}), and carboxyl (1720 cm^{-1}) groups. In the ^{1}H NMR spectrum, three olefinic protons [δ_H 6.28 (d, J = 15.9 Hz), 5.70 (dt, J = 15.9, 6.1 Hz), 5.80, (t, J = 7.4 Hz)], three methylene groups [δ_H 3.10 (2H, overlapped), 3.25 (2H, d, J = 1.1 Hz), 4.12 (1H, dd, J = 12.8, 6.5 Hz), 4.32 (1H, dd, J = 12.3, 5.5 Hz)], and a methoxy group [δ_H 3.56 (3H, s)] were observed, in addition to a typical anomeric proton signal at δ_H 4.20 (d, J = 7.8 Hz) indicating a β-orientation of the glucopyranose moiety. The ^{13}C NMR and HSQC spectra displayed 16 carbon resonances due to three quaternary carbons [including two carboxyl groups (δ_C 173.0 and 175.3)], one methoxy group (δ_C 52.4), four methylenes, and eight methines. Apart from characteristic signals of a glucopyranose moiety and a methoxy group, the remaining 9 carbons were determined to be an unsaturated fatty acid by 2D-NMR experiments. Three spin systems labeled bold in Fig. 2 were determined by ^{1}H–^{1}H COSY correlations. The HMBC correlations (Fig. 2) from anomeric proton signal at δ_H 4.20 to C-7 (70.5), from methoxyl group at δ_H 3.56 to C-9 (173.0), from H_2-8 at δ_H 3.25 to C-9, C-3 (129.2) and C-5 (136.3), from H-3 at δ_H 5.80 to C-1 (175.3) and C-5 (136.3), and from H-6 at δ_H 5.70 to C-4 (134.2) constructed the unsaturated fatty acid moiety and confirmed the location of the β-glucopyranose moiety and methoxy at C-7 and C-9, respectively. Thus, the structure of **15** was determined as shown in Fig. 1 and named as (3*E*,5*E*)-7-*O*-*β*-glucosyl-4-(2-methoxy-2-oxoethyl)hept a-3,5-dienoic acid.

The other known compounds were identified as flazin (**5**) (Nakatsuka, Feng, Goto, & Kihara, 1986), perlolidin (**6**) (Nakatsuka, Feng, Goto, & Kihara, 1986), 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**7**) (Goda et al., 1999), (1*R*,3*S*)-1-methyltetrahydro-β-carboline-3-carboxylic acid (**8**) (Piacente,

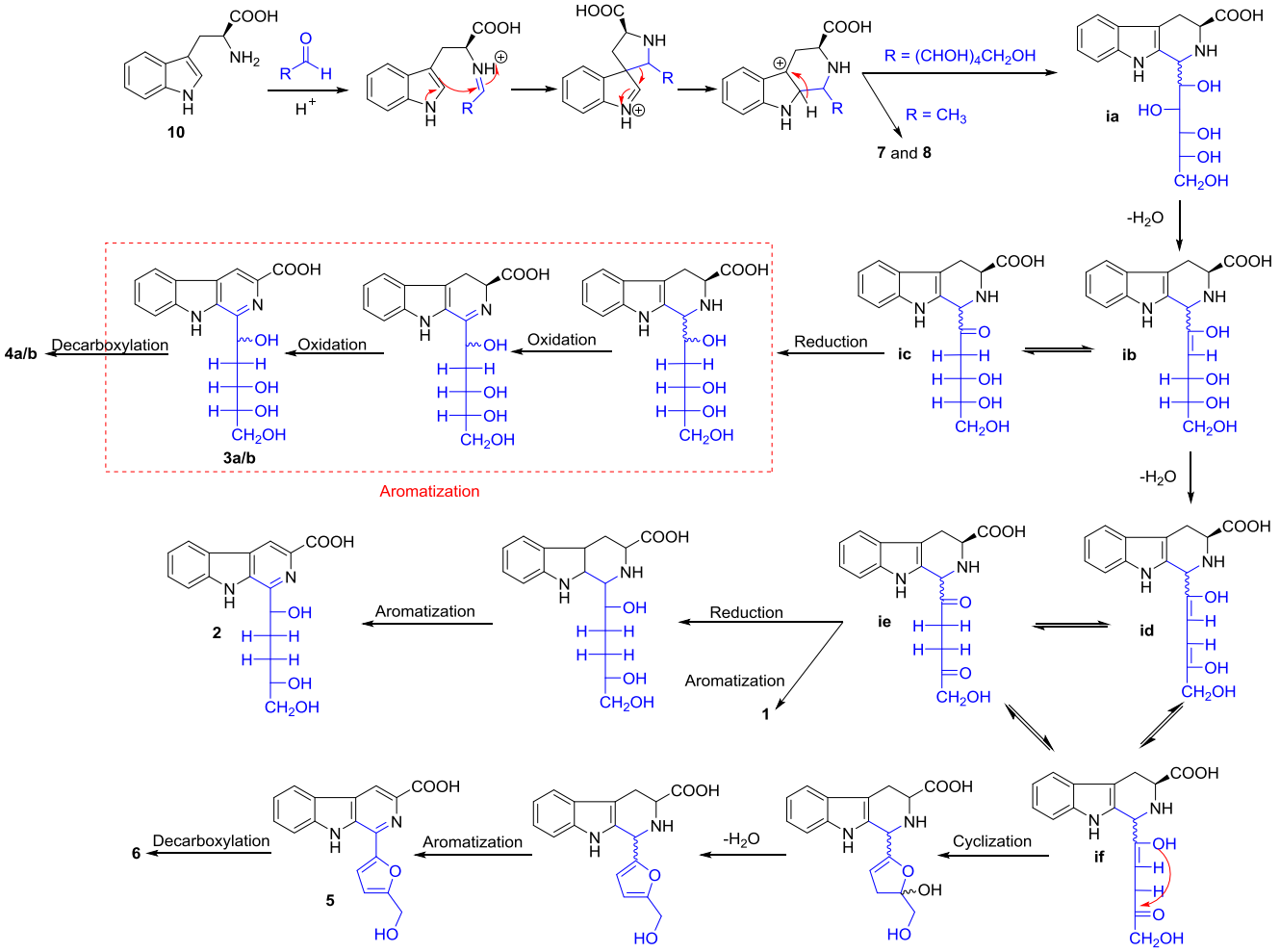

Fig. 3. Plausible biogenetic pathway for compounds **1**–**8**.

Carbone, Plaza, Zampelli, & Pizza, 2002), 9H-carbazole (**9**) (Suzuki, Hirano, Satoh, & Miura, 2015), tryptophan (**10**) (Lee & Pbillips, 1992), N^1-(β-D-glucopyranosyl-$^4C_1$)-L-tryptophan (**11**) (Diem, Bergmann, & Herderich, 2000), L-valyl-L-tryptophan anhydride (**12**) (Pedras, Smith, & Taylor, 1998), *N*-(1-deoxy-α-D-fructos-1-yl)-L-tryptophan (**13**) (Sayed et al., 2008), *N*-(1-deoxy-D-fructos-1-yl)-L-phenylalanine (**14**) (Röper, Röper, & Heyns, 1983), (2Z) 3-[4-(β-D-glucopyranosyloxy)-3,5-dimethoxyphenyl]-2-propenoic acid (**15**) (Uda, Ozawa, Takayama, Suzuki, & Maeda, 1998), *cis*-feruloyl-4-β-glucoside (**16**) (Park & Tomohiko, 2011), 3,5-dimethoxy-4-hydroxybenzyl alcohol 4-*O*-β-D-glucopyranoside (**18**) (Kitajima et al., 1998), apigenin 6,8-di-C-β-D-glucopyranoside (**19**) (Hu et al., 2006), maesopsin (**20**) (Li, Cai, & Wu, 1997), tyrosol 8-*O*-β-D-glucopyranoside (**21**) (Landtag et al., 2002), querceitn 3-*O*-(2G-rhamnosylrutinoside) (**22**) (Kazuma, Noda, & Suzuki, 2003), 6-*O*-*p*-coumaroyl-β-fructofuranosyl-(2 → 1)-α-L-glucopyranoside (**23**) (Georgopoulou, Aligiannis, Fokialakis, & Mitaku, 2005), vanillic acid (**24**) by comparison of their NMR and MS data with those reported in the literatures.

Tangutorids A–F (**1**, **2**, **3a**, **3b**, **4a**, and **4b**) are the first examples of glucose-derived β-carbolines isolated from natural product. The biosynthetic pathways for **1**–**8** were proposed starting from the co-isolated tryptophan (**10**) and corresponding aldehydes through Pictet–Spengler reaction. (Fig. 3) In the acidic environment of fruit, tryptophan (**10**) and acetaldehyde generate compounds **7** and **8** via formation of an iminium cation intermediate and cyclization probably through spiroindolenium species. Through the same mechanism, the intermediate **ia** was formed by the condensation between tryptophan (**10**) and glucose. Intermediate **ia** underwent dehydration reaction to produce **ib**. Then **ib** proceeded through

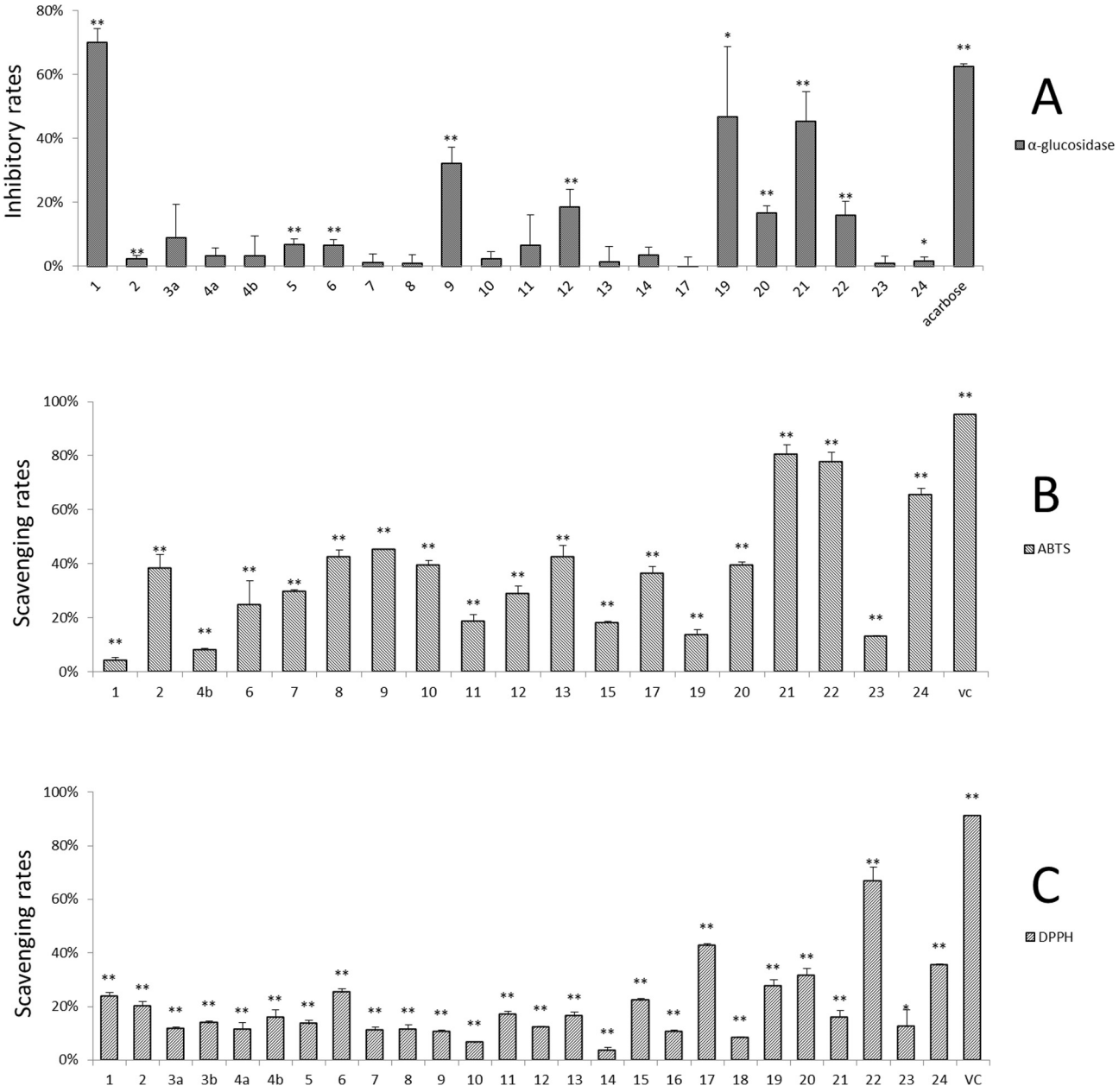

Fig. 4. (A) Inhibition rates of compounds **1**–**3a**, **4a**–**14**, **17**–**24**, and positive control acarbose against the α-glucosidase at a concentration of 250 μg/mL; Compounds **3b**, **15**, and **16** showed no activity at a concentration of 250 μg/mL; (B) ABTS free radical scavenging rates of compounds **1**, **2**, **4b**, **6**–**13**, 15, 17, **19**–**24** and positive control ascorbic acid (VC) at a concentration of 50 μg/mL; Compounds **3a**–**4a**, **5**, **14**, **16** and **18** showed no activity at a concentration of 50 μg/mL; (C) DPPH free radical scavenging rates of compounds **1**–**24** and VC at a concentration of 50 μg/mL; $^{*}p < 0.05$ and $^{**}p < 0.01$ versus blank control.

enolization to the ketone **ic**. Compounds **3a** and **3b** were generated from **ic** though reduction reaction followed by double oxidation (aromatization) reactions. Compounds **3a** and **3b** underwent decarboxylation reaction to give compounds **4a** and **4b**, respectively. On the other hand, **ib** was further dehydrated to yield another intermediate **id**. Intermediate **id** was transformed into **ie** or **if** in solution via enolization. Subsequently, compound **1** was formed through aromatization of **ie**. Compound **2** was yielded from intermediate **ie** through the hydrogenation reduction followed by aromatization reaction. Moreover, compound **5** was obtained from intermediate **if** by cyclization, dehydration and aromatization reactions in sequence. Finally, compound **5** proceeded with a decarboxylation reaction to give compound **6**. Thus, β-carboline derivatives in many fruits may be produced through Pictet–Spengler reactions using naturally occurring tryptophan (**10**) and corresponding aldehydes including glucose as precursor substance.

Nowadays, more and more people believe that the consumption of vegetables and fruits have the potential ability to protect against diseases such as cancer, cardiovascular and metabolic diseases. This is attributed to antioxidants such as vitamins C and E, carotenoids, polyphenols, and flavonoids in vegetables and fruits. (Herraiz & Galisteo, 2003) The ripe fruit of *N. tangutorum* has been used as treatment for dyspepsia and hyperglycemia by folks. Herein, all isolates from the fruit juice of *N. tangutorum* were evaluated in vitro for their antioxidant and α-glucosidase inhibitory activities with ascorbic acid and acarbose as positive control, respectively. Results of the primary screening assay showed that compounds **21**, **22** and **24** exhibited relatively better radical scavenging effect at the concentration of 50 μg/mL and compound **1** revealed the highest inhibition rate against α-glucosidase at the concentration of 250 μg/mL. (Fig. 4) These compounds were further assayed to obtain the SC_{50} and IC_{50} values for antioxidant and α-glucosidase inhibitory activities, respectively. In the ABTS radical scavenging test, compounds **21**, **22** and **24** showed potent antioxidant activity with SC_{50} values of 12.2 ± 1.9, 24.6 ± 1.2 and 30.4 ± 2.7 μg/mL, respectively. In the DPPH radical scavenging assay, compound **22** demonstrated strong antioxidant activity with SC_{50} value of 16.7 ± 2.3 μg/mL. And the positive control ascorbic acid showed antioxidant activity against ABTS and DPPH radical with SC_{50} values of 7.2 ± 0.5 and 6.3 ± 0.3 μg/mL, respectively. In addition, compound **1** showed strong α-glucosidase inhibitory activity with IC_{50} value of 63.3 ± 4.6 μg/mL, and acarbose was used as positive control with IC_{50} value of 118.4 ± 12.7 μg/mL. According to results, we find that the glucose-derived β-carboline derivative with ketone groups showed better α-glucosidase inhibitory activity than others and the α-glucosidase activity will decline a lot when the ketone groups were replaced by hydroxyl groups. This might indicate that the ketone group is an important functional group which could enhance the α-glucosidase inhibitory activity.

4. Conclusions

In summary, five new compounds, including four glucose-derived β-carbolines, 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-die noic acid (**15**) were isolated from fruit juice of *N. tangutorum* together with twenty-one known ones. Tangutorids A–F stand for a class of glucose-derived β-carboline derivatives isolated from natural products. Moreover, the antioxidant and α-glucosidase inhibitory activities of all isolated compounds were evaluated, and the results showed that some of them revealed potential bioactivity. Based on the compounds isolated in this study, the biogenetic pathways of β-carboline derivatives in *N. tangutorum* were proposed to involve Pictet–Spengler reactions and described starting from tryptophan and corresponding aldehydes. In short, the current study adds to understanding of the chemical composition and biological effects of this popularly consumed fruit juice.

Notes

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.01.031.

References

Adachi, J., Mizoi, Y., Naito, T., Ogawa, Y., Uetani, Y., & Ninomiya, I. (1991). Identification of tetrahydro-β-carboline-3-carboxylic acid in foodstuffs, human urine and human milk. *JN The Journal of Nutrition, 121*, 646–652.

Di Giorgio, C., Delmas, F., Ollivier, E., Elias, R., Balansard, G., & Timon-David, P. (2004). In vitro activity of the β-carboline alkaloids harmane, harmine, and harmaline toward parasites of the species *Leishmania infantum*. *Experimental Parasitology, 106*, 67–74.

Diem, S., Bergmann, J., & Herderich, M. (2000). Tryptophan-*N*-glucoside in fruits and fruit juices. *Journal of Agricultural and Food Chemistry, 48*, 4913–4917.

Diem, S., & Herderich, M. (2001). Reaction of tryptophan with carbohydrates: Identification and quantitative determination of novel β-carboline alkaloids in food. *Journal of Agricultural and Food Chemistry, 49*, 2486–2492.

Du, X. P., Xin, H. L., & Peng, C. (2015). Pharmacology and phytochemistry of the *Nitraria* genus. *Molecular Medicine Reports, 11*, 11–20.

Duan, J. A., Williams, I. D., & Chen, C. T. (1999). Tangutorine: A novel β-carboline alkaloid from *Nitraria tangutorum*. *Tetrahedron Letters, 40*, 2593–2596.

Gao, D. F., Xu, M., Yang, C. R., Xu, M., & Zhang, Y. J. (2010). Phenolic antioxidants from the leaves of *Camellia pachyandra* Hu. *Journal of Agricultural and Food Chemistry, 58*, 8820–8824.

Georgopoulou, C., Aligiannis, N., Fokialakis, N., & Mitaku, S. (2005). Acretoside, a new sucrose ester from *Aristolochia cretica*. *Journal of Asian Natural Products Research, 7*, 799–803.

Goda, Y., Hoshino, K., Akiyama, H., Ishikawa, T., Abe, Y., Nakamura, T., ... Toyoda, M. (1999). Constituents in watercress: Inhibitors of histamine release from RBL-2H3 cells induced by antigen stimulation. *Biological & Pharmaceutical Bulletin, 22*, 1319–1326.

Herraiz, T. (1999). Ethyl 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate: a novel β-carboline found in alcoholic beverages. *Food Chemistry, 66*, 313–321.

Herraiz, T. (2004). Tetrahydro-β-carboline bioactive alkaloids in beverages and foods. In F. Shahidi & D. K. Weerasinghe (Eds.), *Nutraceutical beverages* (pp. 405–426). Washington, DC: American Chemical Society.

Herraiz, T. (2009). β-Carboline alkaloids. In J. Gilbert & H. Z. Senyuva (Eds.), *Bioactive compounds in foods* (pp. 199–223). Oxford: Blackwell Publishing Ltd.

Herraiz, T., & Galisteo, J. (2002). Identification and occurrence of the novel alkaloid penthydroxypentyl-tetrahydro-β-carboline-3-carboxylic acid as a tryptophan glycoconjugate in fruit juices and jams. *Journal of Agricultural and Food Chemistry, 50*, 4690–4695.

Herraiz, T., & Galisteo, J. (2003). Tetrahydro-β-carboline alkaloids occur in fruits and fruit juices. Activity as antioxidants and radical scavengers. *Journal of Agricultural and Food Chemistry, 51*, 7156–7161.

Herraiz, T., & Galisteo, J. (2015). Hydroxyl radical reactions and the radical scavenging activity of β-carboline alkaloids. *Food Chemistry, 172*, 640–649.

Hu, N., Zheng, J., Li, W. C., & Suo, Y. R. (2014). Isolation, stability, and antioxidant activity of anthocyanins from *Lycium ruthenicum* Murray and *Nitraria tangutorum* Bobr of qinghai-tibetan plateau. *Separation and Purification Technology, 49*, 2897–2906.

Hu, Y. M., Ye, W. C., Li, Q., Tian, H. Y., Wang, H., & Du, H. Y. (2006). C-glycosylflavones from *Stellaria media*. *Chinese Journal of Natural Medicines, 4*, 420–424.

Jiao, W. H., Gao, H., Li, C. Y., Zhao, F., Jiang, R. W., Wang, Y., ... Yao, X. S. (2010). Quassidines A-D, bis-β-carboline alkaloids from the stems of *Picrasma quassioides*. *Journal Natural Products, 73*, 167–171.

Kazuma, K., Noda, N., & Suzuki, M. (2003). Malonylated flavonol glycosides from the petals of *Clitoria ternatea*. *Phytochemistry, 62*, 229–237.

Kitajima, J., Ishikawa, T., Tanaka, Y., Ono, M., Ito, Y., & Nohara, M. (1998). Water-soluble constituents of fennel. V. Glycosides of aromatic compounds. *Chemical & Pharmaceutical Bulletin, 46*, 1587–1590.

Kusurkar, R. S., & Goswami, S. K. (2004). Efficient one-pot synthesis of anti-HIV and anti-tumour β-carbolines. *Tetrahedron, 60*, 5315–5318.

Landtag, J., Baumert, A., Degenkolb, T., Schmidt, J., Wray, V., Scheel, D., ... Rosahl, S. (2002). Accumulation of tyrosol glucoside in transgenic potato plants expressing a parsley tyrosine decarboxylase. *Phytochemistry, 60*, 683–689.

Lee, M., & Pbillips, R. S. (1992). Fluorine substituent effects for tryptophan in ^{13}C Nuclear magnetic resonance. *Magnetic Resonance in Chemistry, 30*, 1035–1040.

Li, X. C., Cai, L., & Wu, C. D. (1997). Antimicrobial compounds from *Ceanothus americanus* against oral pathogens. *Phytochemistry, 46*, 97–102.

Liang, N., Xue, W., Kennepohl, P., & Kitts, D. D. (2016). Interactions between major chlorogenic acid isomers and chemical changes in coffee brew that affect antioxidant activities. *Food Chemistry, 213*, 251–259.

Lu, H. L., Ouyang, F., Su, Y. L., Ji, T. F., & Liu, H. (2013). Study on chemical constituents of the fruits of *Nitraria tangutorum* Bobr. *Northwest Pharmaceutical Journal, 28*, 221–223.

Nakatsuka, S. I., Feng, B. N., Goto, T., & Kihara, K. (1986). Structures of flazin and YS, highly fluorescent compounds isolated from japanese soy sauce. *Tetrahedron Letters, 29*, 3399–3402.

Papavergou, E., & Herraiz, T. (2003). Identification and occurrence of 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid: The main β-carboline alkaloid in smoked foods. *Food Research International, 36*, 843–848.

Park, B. J., & Tomohiko, M. (2011). Feruloyl, caffeoyl, and flavonol glucosides from *Equisetum hyemale*. *Chemistry of Natural Compounds, 47*, 363–365.

Pedras, M. S. C., Smith, K. C., & Taylor, J. L. (1998). Production of 2,5-dioxopiperazine by a new isolate type of the blackleg fungus *Phoma lingam*. *Phytochemistry, 49*, 1575–1577.

Piacente, S., Carbone, V., Plaza, A., Zampelli, A., & Pizza, C. (2002). Investigation of the tuber constituents of Maca (*Lepidium meyenii* Walp.). *Journal of Agricultural and Food Chemistry, 50*, 5621–5625.

Röper, H., Röper, S., & Heyns, K. (1983). N.m.r. spectroscopy of N-(1-deoxy-d-fructos-1-yl)-l-amino acids (“fructose-amino acids”). *Carbohydrate Research, 116*, 159–183.

Sayed, H. M., Mohamed, M. H., Farag, S. F., Mohamed, G. A., Omobuwajo, O. R. M., & Proksch, P. (2008). Fructose-amino acid conjugate and other constituents from *Cyperus rotundus* L. *Natural Product Research, 22*, 1487–1497.

Sen, N. P., Seaman, S. W., Lau, B. P.-Y., Weber, D., & Lewis, D. (1995). Determination and occurrence of various tetrahydro-β-carboline-3-carboxylic acids and the corresponding *N*-nitroso compounds in foods and alcoholic beverages. *Food Chemistry, 54*, 327–337.

Suzuki, C., Hirano, K., Satoh, T., & Miura, M. (2015). Direct synthesis of N-H carbazoles via iridium (III)-catalyzed intramolecular C-H amination. *Organic Letters, 17*, 1597–1600.

Uda, Y., Ozawa, Y., Takayama, M., Suzuki, K., & Maeda, Y. (1998). Free and soluble bound phenolic acids in some cruciferous vegetables. *Journal of the Japanese Society for Food Science and Technology, 35*, 360–366.

Wu, Z. B., Yao, X. Y., Wang, W., Li, Y. T., Xu, Y. H., & Chen, W. (2014). Flavonoids from *Nitraria tangutorum*. *Chemistry of Natural Compounds, 49*, 1125–1126.

Yang, Y., Wei, X., Shi, R., Fan, Q., & An, L. (2010). Salinity induced physiological modification in the callus from halophyte *Nitraria tangutorum* Bobr. *Journal of Plant Growth Regulation, 29*, 465–476.

Yang, Y., Yang, F., Li, X., Shi, R., & Lu, J. (2013). Signal regulation of proline metabolism in callus of the halophyte *Nitraria tangutorum* Bobr. grown under salinity stress. *Plant Cell, Tissue and Organ Culture, 112*, 33–42.

Yilmazer-Musa, M., Griffith, A. M., Michels, A. J., Schneider, E., & Frei, B. (2012). Grape seed and tea extracts and catechin 3-gallates are potent inhibitors of α-amylase and α-glucosidase activity. *Journal of Agricultural and Food Chemistry, 60*, 8924–8929.

Zhao, M., Bi, L. R., Wang, W., Wang, C., Baudy-Floc'h, M., Ju, J. F., & Peng, S. Q. (2006). Synthesis and cytotoxic activities of β-carboline amino acid ester conjugates. *Bioorganic & Medicinal Chemistry, 14*, 6998–7010.