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Chiral resolution and bioactivity of enantiomeric benzofuran neolignans from the fruit of *Rubus ideaus* L.

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ABSTRACT

Rubus ideaus L., a member of the Rosaceae family, is popular for its distinctive flavor and attractive red color. In order to explore the functional factors possibly presented in the fruit, seven new benzofuran neolignans (**1a/1b–3a/3b** and **4b**), together with one known neolignan **4a**, were isolated from the fruit of *R. ideaus*. Compounds **1a/1b–4a/4b** as four pairs of enantiomers were separated on a chiral chromatographic column. Their structures including absolute configurations were elucidated by extensive spectroscopic data analyses, including UV, IR, HRESIMS, NMR, and comparing their experimental electronic circular dichroism (ECD) spectra with calculated ECD spectra. Additionally, all these enantiomeric compounds were evaluated for their antioxidant, neuroprotective, and anti-Aβ_{1-42} aggregation activities.

1. Introduction

Red raspberry (*Rubus ideaus* L.), belonging to the family Rosaceae, is grown as a commercial fruit crop mainly distributed in Eastern Asia, North America and Eastern Europe [1,2]. It has become one of the most popular fruits in the world due to its delicious flavor and abundant nutrition [3]. Previous phytochemical investigations on *R. ideaus* led to the isolation and identification of several flavonoids, tannins, and phenolic acid derivatives, which have potential health-promoting effects, including antioxidative [4], anti-obesity, anti-neurodegenerative and antitumor activities [5,6]. However, to the best of our knowledge, the pharmacological studies mainly focused on the flavonoids and phenolic acids [7,8], and few bioactivity assays of lignans have been reported from this plant. Besides, recent researches suggested that lignans exhibited significant activities, especially antitumor, anti-inflammatory, antioxidative, and anti-neurodegenerative [9,10].

In the course of our survey on biologically active substances in medicinal plants, considerable attention has been given to the occurrence of compounds with antioxidant effects, since these substances are expected to be potentially useful for the treatment of neurodegenerative diseases. As part of our ongoing search for new bioactive substances from the traditional Chinese herbal medicines or medicinal food [11,12], chemical studies of the fruit of *R. ideaus* was carried out, leading to the discovery of four pairs of enantiomeric benzofuran neolignans (**1a/1b–4a/4b**, Fig. 1) including seven new compounds (**1a/1b–3a/3b** and **4b**). The enantioseparations of these compounds were achieved using chiral HPLC approaches, and their structures including absolute configurations were elucidated by extensive spectroscopic analyses and ECD calculations. In addition, the antioxidant activities by DPPH and ABTS assay, neuroprotective activities, as well as anti-Aβ_{1-42} aggregation activities of compounds **1a/1b–4a/4b** were evaluated.

2. Materials and methods

2.1. General experimental procedures

Optical rotations were obtained by using a JASCO DIP-370 digital polarimeter. The UV spectra were measured on an AOE UV-1800 spectrophotometer. The spectra CD were obtained using MOS 450 detector from Bio-Logic. The FT-IR spectra were obtained on a Bruker IFS-55 spectrometer. ^{1}H NMR, ^{13}C NMR, HMBC and HSQC were recorded

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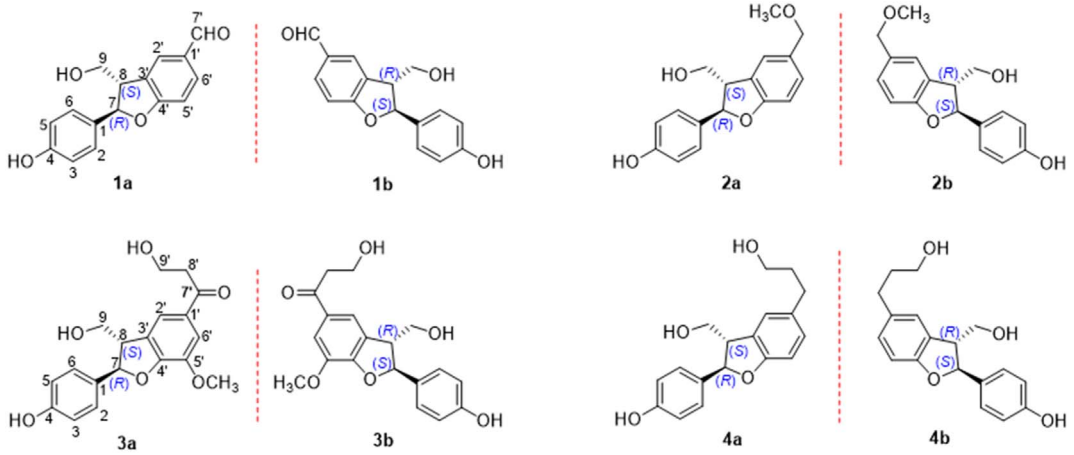

Fig. 1. Chemical structures of compounds **1a/1b–4a/4b**.

on Bruker ARX-400 and AV-600 spectrometers with TMS as an internal standard. HRESIMS experiments were performed on an Agilent G6520 Q-TOF spectrometer. The chromatographic silica gel (200–300 mesh) was produced from Qingdao Marine Chemical Company, and ODS (50 μm) was produced by YMC Co. Ltd. Macroporous adsorption resin D101 was purchased from Cangzhou Bon Adsorber Technology Co. Ltd. Semi-preparative RP-HPLC isolation was achieved with an Agilent 1100 instrument using YMC C_{18} column (250 × 10 mm, 5 μm). Peak detection was made with a refractive index detector (RID). A chiral column Daicel Chiralpak AD-H (250 × 4.6 mm, 5 μm) was used for chiral HPLC separation. DPPH, ABTS, HFIP, MTT, Aβ_{1-42}, Trolox and curcumin were purchased from Sigma-Aldrich (St. Louis, MO). The OD value was performed on Varioskan Flash Multimode Reader (Thermo scientific).

2.2. Plant material

The fruit of *Rubus ideaus* L. were collected from Shenyang, Liaoning province, P. R. China, in June 2015, and were identified by Prof. J.C. Lu (Department of Natural Products Chemistry, Shenyang Pharmaceutical University, P. R. China.). A voucher specimen (No. 20150601) has been deposited in the herbarium of Shenyang Pharmaceutical University, Liaoning, P. R. China.

2.3. Extraction and isolation

The air-dried fruit of *Rubus ideaus* L. (20 kg) were crushed and refluxed with 70% EtOH (3 × 50 L) for 3 h. The extract (1750 g) was suspended in H_2O and then chromatographed on a D101 macroporous resin column using H_2O/EtOH (from 100:0 to 5:95) as eluents, yielding four fractions (fractions I ~ IV). Fraction III was subjected to silica gel CC and eluted with CH_2Cl_2/CH_3OH to yield seven fractions (III_a ~ III_g). Among them, III_b (26.5 g) was further purified by ODS CC using H_2O/EtOH as a mobile phase gradient (from 90:10 to 30:70) to afford six fractions (A − F) on the basis of HPLC analysis. Fr.A (4.1 g) was subjected to further silica gel column chromatography and eluted with CH_2Cl_2/CH_3OH (from 90:10 to 50:50) to yield five fractions (A1 ~ A5) on the basis of silica gel TLC analysis. Fr.A2 was subjected to preparative HPLC and eluted with CH_3OH/H_2O (18:82) to yield six fractions (A2–1 ~ A2–6). A2–2 was subjected to semi-preparative HPLC and with CH_3CN/H_2O (10:90) as eluents to afford **1** (8 mg, t_R 29.6 min, 3.5 mL/min) and **2** (6 mg, t_R 39.1 min, 3.5 mL/min). Similarly, Fr·B (5.4 g) was also submitted to separation over a silica gel column chromatography using CH_2Cl_2/CH_3OH (from 90:10 to 55:45) to yield Fr. B1 ~ Fr. B5 on the basis of silica gel TLC analysis. Subsequently, B3 was purified by preparative HPLC with the mobile phase 40% MeOH/H_2O (v/v) to obtain five fractions B3–1 ~ B3–5. Then, B3–4 was subjected to semi-preparative HPLC and eluted with CH_3CN/H_2O (12:88, v/v) to yield **3** (5 mg, t_R 24.4 min, 3.5 mL/min) and **4** (42 mg, t_R 27.0 min, 3.5 mL/min). In addition, direct enantiomeric separation of compounds **1** and **4** were achieved by preparative chiral HPLC with 2-propanol-*n*-hexane (20:80, v/v), affording **1a** (1.7 mg) and **1b** (1.5 mg), **4a** (4.1 mg) and **4b** (4.6 mg). Additionally, compounds **2a** (1.9 mg) and **2b** (1.4 mg), **3a** (1.6 mg) and **3b** (1.8 mg) were obtained by preparative chiral HPLC with 2-propanol-*n*-hexane (15:85, v/v), respectively.

2.4. Spectroscopic data

2.4.1. Idaeusin A (1)

Colorless oil; $[\alpha]_D^{20}$ + 2.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 225.9 (3.13), 285.6 (0.99) nm; IR (KBr) ν_{max} 3421, 2939, 2253, 1677, 1517, 1454, 1384, 1134, 1028, 824, 762 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS: *m/z* 293.0795 $[M + Na]^+$ (calcd for $C_{16}H_{14}O_4Na$, 293.0784).

(7R,8S)-idaeusin A (**1a**). $[\alpha]_D^{20}$ + 26.8 (*c* 0.10, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 212 (+6.13), 241 (+0.02), 263 (+2.27), 292 (+0.94) nm.

(7S,8R)-idaeusin A (**1b**). $[\alpha]_D^{20}$–34.7 (*c* 0.10, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 212 (−0.80), 238 (+2.25), 264 (−1.23), 287 (−0.02) nm.

2.4.2. Idaeusin B (2)

Colorless oil; $[\alpha]_D^{20}$ + 6.6 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 206.7 (3.55), 226.2 (2.56), 282.8 (0.82) nm; IR (KBr) ν_{max} 3381, 2938, 1614, 1517, 1498, 1452, 1328, 1213, 1141, 1056, 1031, 953, 834 cm^{-1}; ^1H and ^{13}C NMR data see Table 1. HRESIMS: *m/z* 309.1170 $[M + Na]^+$ (calcd for $C_{17}H_{18}O_4Na$, 309.1103).

(7R,8S)-idaeusin B (**2a**). $[\alpha]_D^{20}$ + 51.2 (*c* 0.10, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 236 (−2.50), 283 (+0.30) nm.

(7S,8R)-idaeusin B (**2b**). $[\alpha]_D^{20}$–46.3 (*c* 0.10, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 235 (+4.33), 289 (−0.02) nm.

2.4.3. Idaeusin C (3)

Colorless oil; $[\alpha]_D^{20}$–3.4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 222.8 (3.30), 285.6 (1.18) nm; IR (KBr) ν_{max} 3373, 3021, 2935, 1702, 1613, 1597, 1447, 1363, 1264, 1229, 1171, 1104, 1033, 822 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS: *m/z* 367.1192 $[M + Na]^+$ (calcd for $C_{19}H_{20}O_6Na$, 367.1158).

(7R,8S)-idaeusin C (**3a**). $[\alpha]_D^{20}$ + 48.6 (*c* 0.10, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 234 (−6.31), 243 (+0.46), 279 (+4.81) nm.

(7S,8R)-idaeusin C (**3b**). $[\alpha]_D^{20}$–51.3 (*c* 0.10, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 232 (+4.13), 246 (−2.25), 284 (−5.92) nm.

2.4.4. Idaeusin D (4)

Colorless oil; $[\alpha]_D^{20}$–2.6 (*c* 0.11, MeOH);UV (MeOH) λ_{max} (log ε) 220.4 (4.19), 284.8 (1.90) nm; IR (KBr) ν_{max} 3353, 2939, 1614, 1517,