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Inhibitory activity of *Filipendula ulmaria* constituents on recombinant human histidine decarboxylase

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ABSTRACT

Histidine decarboxylase (HDC) catalyses the formation of histamine, a bioactive amine. Agents that control HDC activity are beneficial for treating histamine-mediated symptoms, such as allergies and stomach ulceration. We searched for inhibitors of HDC from the ethyl acetate extract of the petal of *Filipendula ulmaria*, also called meadowsweet. Rugosin D, rugosin A, rugosin A methyl ester (a novel compound), and tellimagrandin II were the main components; these 4 ellagitannins exhibited a non-competitive type of inhibition, with K_i values of approximately 0.35–1 μM. These K_i values are nearly equal to that of histidine methyl ester (K_i = 0.46 μM), an existing substrate analogue inhibitor. Our results show that food products contain potent HDC inhibitors and that these active food constituents might be useful for designing clinically available HDC inhibitors.

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1. Introduction

Histamine is a bioactive amine that is a chemical mediator of allergic reactions, neurotransmitter, and inducer of gastric acid secretion (Shahid, Khardori, Khan, & Tripathi, 2011). Histidine decarboxylase (HDC, E.C. 4.1.1.22) is the sole enzyme involved in histamine synthesis in mammals. Inhibiting HDC activity lowers histamine levels in the body; therefore, agents that inhibit HDC activity would be useful for treating histamine-mediated symptoms, such as allergies, gastric ulcers, and inflammation. However, despite receiving great attention, the search for HDC inhibitors has not been sufficiently successful, probably because of difficulties handling enzymes and the low amounts of HDC in the examined tissues.

Histidine is a substrate of HDC, whereas pyridoxal 5′-phosphate is a cofactor for mammalian HDC. The substrate analogues histidine methyl ester and α-fluoromethyl histidine have been known as specific HDC inhibitors for longer than three decades. However, neither agent has been used clinically. Pyridoxyl-histidine methyl ester was recently synthesised as an intracellular HDC inhibitor (Wu, Yu, & Gehring, 2008). From natural products epigallocatechin gallate and epicatechin gallate of green tea were reported to inhibit HDC activity (Nitta, Kikuzaki, & Ueno, 2007; Rodriguez-Caso, Rodriguez-Agudo, Sanchez-Jimenez, & Medina, 2003). Two quercetin glycosides from *Pimenta dioica* (also called allspice), quercetin 3-*O*-β-D-glucuronide 6″-methyl ester and quercetin 3-*O*-(2-*O*-galloyl)glucoside, were also identified as potent HDC inhibitors (Nitta, Kikuzaki, & Ueno, 2009). However, these compounds from natural products do not resemble histidine or pyridoxyl histidine and their inhibitory mechanisms remain unclear. In our recent study, we successfully crystallised HDC; the structural analysis revealed that natural compounds might be able to inhibit histamine synthesis by binding to specific regions of HDC (Komori, Nitta, Ueno, & Higuchi, 2012b). Additional examples of potent HDC inhibitors are necessary to further understand structure-inhibition relationships. Food components would be good sources for obtaining safe and potent HDC inhibitors (Nitta et al., 2009).

Filipendula ulmaria, often called meadowsweet, is an herb widely used not only as a food flavouring agent but also as an herbal medicine. It protects and soothes the mucous membranes of the digestive tract, reduces excess acidity, and eases nausea (Haslam, 1996). It is used to treat heartburn, hyperactivity, diarrhoea, gastritis, and peptic ulceration and to relieve the pain of rheumatism (Haslam, 1996). In our screening study, meadowsweet extract was one of the three most effective extracts for HDC inhibition

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from among 122 plant samples (Nitta, Ito, & Ueno, 2008). In the present study, the active constituents of meadowsweet were identified and the characteristics of HDC inhibition were investigated.

2. Material and methods

2.1. General

Optical rotations were measured using a JASCO Polarimeter P-1030 (JASCO International Co., Ltd., Tokyo, Japan). NMR spectra were obtained from a Varian Unity plus 500 spectrometer (500 MHz, Varian Inc., Palo Alto, CA). FAB-MS (matrix: 3-nitrobenzyl alcohol) were measured on a JEOL JMS 700T mass spectrometer (JEOL Ltd., Tokyo, Japan). HPLC analysis was carried out with a JASCO PU-980 intelligent pump equipped with a JASCO MD-910 multi-wavelength detector. The column for HPLC was a Mightysil RP-18 GP (5 μm, 250 × 4.6 mm; Kanto Chemical Co., Inc., Tokyo, Japan). Sephadex LH-20 (Pharmacia, Uppsala, Sweden) and Chromatorex ODS DM1020T (100–200 mesh, Fuji Silysia Chemical, Aichi, Japan) were used for column chromatography. Chemicals were of analytical reagent grade.

2.2. Extraction and isolation

Flowers of meadowsweet (*F. ulmaria*) were a kind gift from Ichimaru Pharcos Ltd. (Gifu, Japan). The dried flowers (1 kg) were pulverised and successively extracted with *n*-hexane (5 L × 5 times), CH_2Cl_2 (5 L × 5 times) and 70% aqueous Me_2CO (5 L × 5 times) at room temperature. For each extraction, the plant material was soaked in the solvent and allowed to stand overnight. The combined *n*-hexane extract and combined CH_2Cl_2 extract were evaporated under reduced pressure to afford the *n*-hexane extract (15.0 g) and CH_2Cl_2 extract (11.7 g), respectively. Acetone from the combined 70% aqueous Me_2CO extract was concentrated under reduced pressure at 40 °C, and the resulting aqueous residue was partitioned with EtOAc to yield EtOAc-soluble (120.6 g) and H_2O-soluble (206.5 g) fractions. An aliquot of the total EtOAc-soluble fraction (21.7 g) was subjected to Sephadex LH-20 column chromatography (CC) using CH_3OH as an eluent to give eight fractions. Fraction 4 (0.22 g) was recrystallised with CH_3OH to afford quercetin (108 mg). Fraction 6 (0.75 g) and fraction 7 (0.38 g) were individually rechromatographed over ODS gel (CH_3CN:H_2O, 3:17) to give tellimagrandin II (28.7 mg) (Wilkins & Bohm, 1976) and rugosin A (29.4 mg) (Hatano, Ogawa, Yasuhara, & Okuda, 1990b; Okuda, Hatano, Yazaki, & Ogawa, 1982b) from fraction 6 and tellimagrandin II (6.3 mg) and rugosin A (24.0 mg) from fraction 7. Fraction 8 (1.51 g) was subjected to ODS CC (CH_3OH:H_2O, 1:3) followed by ODS CC (CH_3OH:H_2O, 3:7), to give tellimagrandin II (22.6 mg), rugosin A (19.6 mg), rugosin D (7.6 mg) (Hatano, Ogawa, Shingu, & Okuda, 1990a; Okuda, Hatano, & Ogawa, 1982a) and **1** (93.4 mg).

Compound **1**: A light-brown powder, $[\alpha]_D^{21}$ +63 (*c* 0.45, acetone); ^{1}H NMR (acetone-d_6): [glucose moiety] δ_H 6.17 (1H, d, *J* = 8.3 Hz, H-1), 5.58 (1H, dd, *J* = 8.3, 9.5 Hz, H-2), 5.82 (1H, dd, *J* = 9.5, 9.8 Hz, H-3), 5.17 (1H, dd, *J* = 9.8, 10.0 Hz, H-4), 4.51 (1H, brdd, *J* = 6.6, 10.0 Hz, H-5), 5.29 (1H, dd, *J* = 6.6, 13.4 Hz, H-6a), 3.79 (1H, brd, *J* = 13.4 Hz, H-6b); [galloyl group I] δ_H 7.10 (2H, s, H-2, 6); [galloyl group II] δ_H 7.00 (2H, s, H-2, 6); [galloyl group III] δ_H 6.98 (2H, s, H-2, 6); [valoneoyl group] δ_H 6.49 (1H, s, H-3), 6.27 (1H, s, H-3′), 7.06 (1H, s, H-6″), 3.68 (3H, s, $COOC\underline{H}_3$); ^{13}C NMR (125 MHz, acetone-d_6): [glucose moiety] δ_C 93.6 (C-1), 71.7 (C-2), 73.2 (C-3), 70.7 (C-4), 72.9 (C-5), 63.0 (C-6); [galloyl group I] δ_C 119.7 (C-1), 110.3 (C-2, 6), 146.2 (C-3,5), 139.8 (C-4), 164.9 (C-7); [galloyl group II] δ_C 120.3 (C-1), 110.2 (C-2, 6), 146.0 (C-3,5), 139.4 (C-4), 165.6 (C-7); [galloyl group III] δ_C 120.6 (C-1), 110.1 (C-2, 6), 145.8 (C-3,5), 139.2 (C-4), 166.3 (C-7); [valoneoyl group] δ_C 116.0 (C-1), 125.5 (C-2), 107.7 (C-3), 145.3 (C-4), 136.8 (C-5), 144.8 (C-6), 167.7 (C-7), 117.8 (C-1′), 125.9 (C-2′), 105.7 (C-3′), 146.9 (C-4′), 137.4 (C-5′), 144.7 (C-6′), 167.8 (C-7′), 115.2 (C-1″), 143.4 (C-2″), 140.5 (C-3″), 137.0 (C-4″), 139.7 (C-5″), 109.6 (C-6″), 165.9 (C-7″), 52.0 ($COO\underline{C}H_3$). FAB-MS (negative-ion mode) *m/z* 1119 $[M-H]^-$.

2.3. HPLC analysis of isolated compounds and meadowsweet extracts

Dried flowers (0.5 g) of meadowsweet were extracted with 50 mL of 70% aqueous acetone for 15 min with stirring before filtration. For preparation of an infusion, the plant material (0.5 g) was extracted with 50 mL of boiling water for 5 min with stirring before filtration. The 70% aqueous acetone extract, the infusion and four isolated compounds were analysed by HPLC. A Mightysil RP-18 GP column (250 × 4.6 mm, 5 μm particle size, Kanto Chemical) was used. Solvent A was 0.3% (v/v) trifluoroacetic acid (TFA) in CH_3CN and solvent B was 0.3% (v/v) TFA in water. The linear gradient elution used was as follows: 10–30% A in B over 30 min; 30–40% A in B over 15 min; 40% A in B held for 5 min. The flow rate was 1.0 mL/min. The detection wavelength was 280 nm. Tellimagrandin II, rugosin A, rugosin D and compound **1** were detected with t_R of 16.2, 16.0, 17.9 and 18.9 min, respectively (see Supplementary Fig. 1).

2.4. HDC inhibition assay

A test meadowsweet sample was dissolved in 50 v/v% EtOH. An active form of recombinant human HDC, that is a C-terminal truncated form (Furuta, Nakayama, Sugimoto, Ichikawa, & Tanaka, 2007; Nitta, Ohshita, Liu, Kuronuma, & Ueno, 2010), was prepared as described previously (Komori, Nitta, Ueno, & Higuchi, 2012a). K_m of HDC examined for histidine was 0.1 mM. HDC inhibition assay mixture contained 0.1 mM dithiothreitol, 0.01 mM PLP, a test meadowsweet sample and 0.9 μg/ml enzyme (with specific activity of approximately 1500 nmol/min/mg) in 100 mM potassium phosphate buffer (pH 6.8) and reaction was initiated by addition of L-histidine at 37 °C. The final volume of the assay was 400 μl, including 5 μl meadowsweet sample so as to give a final concentration of 0.1% for *n*-hexane and CH_2Cl_2 extracts and the EtOAc- and H_2O-soluble fractions, or 0.2–1 μM for isolated compounds. After 20 min incubation the reaction was terminated by addition of 20 μl of 60% perchloric acid. For a time-dependent test, HDC was pre-incubated with tellimagrandin II, rugosin A, rugosin D and compound **1** for a set time period at 37 °C. Incubation was initiated by addition of L-histidine and the mixture was reacted for 20 min at 37 °C. Histamine produced in the assays was injected onto an HPLC system equipped with a histamine pak column (Tosoh, Tokyo, Japan). Separated histamine was fluorometrically measured by the *o*-phthalaldehyde method, which was described previously (Nitta et al., 2007). Duplicate samples were analysed in each experiment.

2.5. Kinetic data analysis

For determining inhibition constant, the concentration of L-histidine was set to a range from 0.1 to 0.8 mM. The kinetic data was analysed by using the SigmaPlot Enzyme Kinetics Module (Systat Software, San Jose, CA). The velocities were fitted to the equations and the best-fit inhibition model was identified from competitive, non-competitive, uncompetitive and mixed models. K_i and αK_i were used as the EI (enzyme-inhibitor) and ESI (enzyme–substrate-inhibitor) dissociation constants, respectively, in the Enzyme Kinetics Module. The Akaike criterion was used to determine which equation fit the data best according to the instructions of the Enzyme Kinetics Module.

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