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Gastrointestinal Absorption of Ternatins, Polyacylated Anthocyanins Derived from Butterfly Pea (*Clitoria ternatea* L.) Petals in Rats

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Ternatins, polyacylated anthocyanins that contain two or more aromatic acyl groups, are found in the petals of butterfly pea (*Clitoria ternatea* L.). We examined the gastrointestinal absorption of ternatins in rats after oral administration of the extract of the butterfly pea petals. Ingested ternatins were absorbed rapidly in the gastrointestinal tract in their original acylated forms. Nine ternatins were detected, together with preternatin A3, in rat blood plasma at 15 min after oral administration. After a single oral dose of 0.0527 mmol/kg ternatin, the maximum plasma concentration and area under the plasma concentration curve for total ternatin was 0.141 ± 0.035 μM and 16.398 ± 1.542 μM·min, respectively, during the 8-h period post-administration. The absorption of ternatins in blood plasma tended to negatively correlate with increasing order of molecular weight; however, ternatins carrying symmetrical substitution patterns and glucosyl terminals on the both side chains at the 3′ and 5′ positions of the aglycone were exceptionally bioavailable.

Key words ternatin, anthocyanin, absorption, butterfly pea petal, side-chain, terminal structure

INTRODUCTION

In the past several decades, anthocyanins have attracted much attention because of their abundance in a balanced daily diet[1)] and their multiple biological functions that benefit human health.[2)] Studies suggest that anthocyanins help to prevent chronic diseases[3)] because they can accumulate in the body during long-term ingestion.[4)] However, anthocyanin bioavailability is generally considered low, based on its absorption profiles in plasma.[5)]

Available data have shown that the biological fates of anthocyanins, such as gastrointestinal absorption,[6,7)] metabolism,[8)] and tissue accumulation,[9)] are influenced largely by the types of anthocyanin ingested. We have discovered that pelargonidin-3-*O*-β-D-glucopyranoside, which is the dominant anthocyanin in strawberries, is highly bioavailable (> 13%),[10)] whereas other anthocyanins, like cyanidin-3-*O*-β-D-glucopyranoside (Cy3G)[7)] and delphinidin-3-*O*-β-D-glucopyranoside (Dp3G),[8)] are poorly bioavailable (< 1%). The instability of Cy3G and Dp3G in intestinal conditions, which are weakly acidic to alkaline, could partly explain the low absorption profile of anthocyanins from the gastrointestinal tract.

On the other hand, acylated anthocyanins are relatively stable because of intramolecular hydrophobic stacking between the anthocyanidin (aglycone of anthocyanin) and the aromatic ring on the acyl moiety.[11)] The side chain acyl and sugar groups of an acylated anthocyanin protect the anthocyanin, increasing its molecular stability and biological functions.[12)] Studies on the absorption of acylated anthocyanins with a molecular weight range of 817–1185 from various plant materials have provided detailed insight into the biological functions of these molecules *in vivo*.[13–16)] Despite their high molecular weight, acylated anthocyanins are absorbed to a similar extent as non-acylated anthocyanins,[14,15)] although peonidin-3-caffeoylsophoroside-5-glucopyranoside (Pn3CafSop5G) showed a specifically low absorption profile.[13)]

Edible flowers also contain various structures of polyacylated anthocyanins with repeating glycosyl and acyl groups attached to the anthocyanidin.[11)] Ternatins are a group of polyacylated anthocyanins found in butterfly pea (*Clitoria ternatea* L.) petals (BPP).[17–19)] These pigments are exceptionally stable in weak acidic and neutral aqueous solution because of their unique sandwich-type intramolecular hydrophobic stacking between the anthocyanidin and aromatic rings attached to the two side chains via the hydroxyl groups of the anthocyanidin. This property facilitates their application as stable, natural food colorants.[11)] Moreover, ternatins show potent antioxidant activities *in vitro*,[11)] suggesting their possible contribution to a health-promoting effect. However, the intestinal absorption of ternatins, which proves the expression of *in vivo* function, has seldom been examined to date.

In the present study, we evaluate the gastrointestinal absorption of ternatins in rats after oral administration of BPP extract containing 10 ternatin analogues, including preternatin A3 (Fig. 1). The molecular weight and structural difference in the acyl moieties, such as the symmetries of substitution patterns and the combinations of terminal structures of both side

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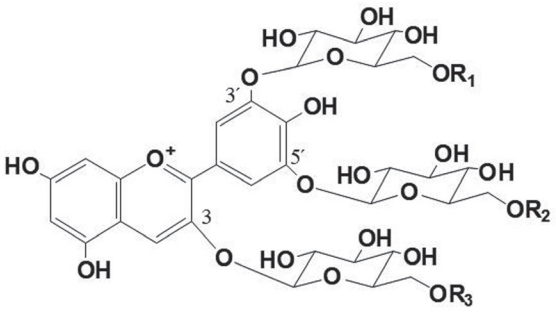

Anthocyanin	R_1	R_2	R_3	Molecular weight
Ternatin A1 (1)	−CGCG	−CGCG	−Ma	2109
Ternatin A2 (2)	−CGCG	−CG	−Ma	1801
Ternatin A3 (3)	−CG	−CG	−Ma	1492
Ternatin B1 (4)	−CGCG	−CGC	−Ma	1947
Ternatin B2 (5)	−CGC	−CG	−Ma	1638
Ternatin B3 (6)	−CGCG	−C	−Ma	1638
Ternatin B4 (7)	−CG	−C	−Ma	1330
Ternatin D1 (8)	−CGC	−CGC	−Ma	1785
Ternatin D2 (9)	−CGC	−C	−Ma	1476
Preternatin A3 (10)	−CG	−CG	−H	1406

Fig. 1. Structures of Ternatins in BPP Extract

C, *p*-coumaroyl; G, D-glucosyl; Ma, malonyl residue.

chains, are discussed in the context of the ternatin amounts in blood plasma.

MATERIALS AND METHODS

Reagents HPLC-grade acetonitrile was obtained from Kanto Chemical Co., Ltd. (Tokyo, Japan). All other reagents, including trifluoroacetic acid (TFA) and polyclar AT, polyvinylpyrrolidone (PVP), were purchased from Wako Pure Chemical Industry (Osaka, Japan) and used without further purification. HP-20 resin was purchased from Mitsubishi Chemical Co. (Tokyo, Japan). Sephadex LH–20 (25–100 μm) was obtained from Amersham Biosciences, Inc. (Buckinghamshire, UK).

Plant Materials *Clitoria ternatea* L. were grown in a farm of Minami-Kyushu University and the mature stage flower petals were collected during July and October 1996. They were dried at 45°C overnight, and stored in a silica gel desiccator until use for extraction.

Preparation of BPP Extract BPP extract containing 10 ternatin analogues was obtained according to a previous method.[17] Briefly, 200 g of dried BPP were immersed in 300 mL of 80% aqueous methanol solution overnight and filtered. The extraction procedure was repeated four times. The combined crude extract containing polyacylated anthocyanins was evaporated to dryness *in vacuo* at 40°C. 0.6 g of the crude extract was obtained from 200 g of dried BPP. Individual ternatins in the extract were quantified by HPLC.

HPLC Analysis HPLC conditions were as described in our previous report with modification.[15] Briefly, aliquots (100 μL) of sample solutions were injected into an HPLC system (Hitachi 7200) equipped with a Develosil ODS-HG 5 column (1.0 mm × 150 mm) using 0.5% aqueous TFA (solvent A) and acetonitrile containing 0.5% TFA (solvent B) in linear gradient elution. The solvent gradient was as follows: from 87.5% A/12.5% B (v/v) to 73.5% A/26.5% B (v/v) for 40 min, and 67.5% A/32.5% B (v/v) for a further 20 min at a flow rate of 0.1 mL/min. The elution profile was monitored at 530 nm with a UV–VIS detector (Hitachi L-7000).

Purification of Ternatin A2 Ternatin A2 was isolated for quantitative purposes by following a previous method.[19] Briefly, BPP extract containing ternatins (prepared above) was dissolved in 300 mL of 1% aqueous acetic acid, adsorbed on an HP-20 resin column (60 mm × 450 mm), washed with 1% aqueous acetic acid, and eluted with 70% aqueous ethanol containing 1% acetic acid. After evaporation, the residue was dissolved in 0.1 N HCl:methanol (3:7, v/v) and chromatographed on a PVP column (45 mm × 100 mm) in the same solvent. The elute containing ternatin A2 was applied to an HP-20 column to remove HCl, washed with 1% aqueous acetic acid, eluted with 70% aqueous ethanol containing 1% acetic acid and then evaporated to dryness *in vacuo*. Finally, ternatin A2 was isolated by repeating semi-preparative HPLC using an Inertsil ODS-2 column (20 mm × 250 mm) under isocratic elution conditions. The mobile phase was a mixture of solvent A (15% aqueous acetic acid) and solvent B (30% aqueous acetonitrile containing 15% acetic acid) in various ratios (65% A/35% B to 90% A/10% B (v/v)). The flow rate was 7 mL/min and the effluent was monitored at 530 nm. After the eluent was evaporated to dryness, the isolated pigment was dissolved in a small amount of TFA and precipitated with the excess diethyl ether to obtain the TFA salts as a reddish-purple powder. The structure of the pigment was identified by FAB–MS and NMR spectrometry, as described previously.[19]

Animals and Diets SPF male Wistar ST rats (aged 5 weeks and body weight of 160 g) purchased from Japan SLC, Inc. (Hamamatsu, Japan) were housed individually in stainless-steel wire-mesh cages at 23 ± 1°C for conditioning under a 12-h light/dark cycle. Rats were allowed *ad libitum* access to tap water and a controlled diet for 7 d before the experiment. Animal experiments were performed in accordance with the Guidelines on the Care and Use of Laboratory Animals issued by Niigata University of Pharmacy and Applied Life Sciences. The protocol was approved by the Committee on the Ethics of Animal Experiments of Niigata University of Pharmacy and Applied Life Sciences (approval number 17-1). All efforts were made to minimize suffering.

Experimental Design After 7 d of adaptation, three rats were cannulated with a polyethylene tube (PE-50) into a neck vein under anesthesia with a mixed solution of medetomidine hydrochloride (0.15 mg/kg), midazolam (2 mg/kg), and butorphanol tartrate (2.5 mg/kg) by intraperitoneal injection as described in our previous method with modifications.[15] Briefly, the neck vein was isolated, and a small hole cut with scissors to insert the PE 50 tube. Once the tube was inserted, the vein and tube were occluded, and the tube was guided out from the back of the rats. After starving for 24 h, BPP extract (ternatin mixtures) dissolved in 0.1% aqueous citric acid was administered orally (400 mg BPP extract/kg body weight) to three rats, according to our previous research.[15] During the experiment, the rats were allowed to move freely in the cages. Blood samples were collected *via* the cannulated tube using a heparinized syringe at 0, 15, 30, 60, 120, 240, and 480 min. Donor blood was collected from other healthy rats under anes-

thesia by venipuncture of the inferior vena cava into a sodium citrate solution (500 μL of 10% aqueous sodium citrate per 8 mL of blood). After the blood was withdrawn (600 μL), the same volume of donor blood was injected through the cannulated vein tube. Each blood sample was immediately centrifuged at 3,000 *g*, 4°C for 5 min to prepare plasma samples for HPLC analysis.

Plasma Preparation Ternatins were extracted from blood plasma using a Sep-Pak C_{18} Light cartridge (Waters, Milford, MA, USA) based on our previously reported method.[15] Briefly, plasma (300 μL) samples were applied to the Sep-Pak C_{18} Light cartridges conditioned with methanol (2 mL) and 3% aqueous TFA (2 mL). The cartridges were then washed successively with 2 mL of 3% aqueous TFA, dichloromethane, and benzene, and ternatins were eluted with 50% aqueous acetonitrile containing 1% TFA. The eluent was evaporated to dryness *in vacuo* and dissolved in 150 μL of 0.5% aqueous TFA. The dissolved sample was passed through a Centricut ultramembrane filter (0.45 μM; Kurabou Co. Ltd., Osaka, Japan) before HPLC injection. Recovery of the sample by this method was 89.1%, as verified by spiking purified ternatin A2 into normal blood plasma.

Statistical Analysis Significant differences among concentrations of ternatin in blood plasma were determined by one-way ANOVA, followed by Tukey's multiple comparison test ($P < 0.05$). All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria, version 4.0.3). More precisely, it is a modified version of R commander (version 2.7-1) designed to add statistical functions frequently used in biostatistics.

RESULTS

Determination of Ternatins in Rat Blood Plasma Figure 2A shows a typical HPLC chromatogram of the BPP extract detected at 530 nm. Fifteen individual pigments were separated, of which 10 were determined to be ternatin analogues from the retention time of the purified samples obtained from previous reports (Fig. 1).[17–19] Four out of 10 pigments (peaks 11–14) in the BPP extract were predicted to be preternatins from the HPLC retention time. Due to the insufficient amounts of purified samples of individual ternatins for quantitative purposes, the orally administered dose of each ternatin in the BPP extract and the plasma concentration of each ternatin were quantified as ternatin A2[19] equivalents (Table 1). Figure 2B shows a typical HPLC chromatogram of rat blood plasma at 15 min after oral administration of the BPP extract. Nine ternatins were detected together with preternatin A3. The peak intensity of each ternatin in the blood plasma of rats that received the BPP extract orally differed from that seen in the original BPP extract. Other pigments (peaks 11–14) in the BPP extract were also observed in rat blood plasma; however, only peak 15 was not detected in any plasma samples. Furthermore, the new peaks were not observed at 530 nm under the HPLC conditions utilized during the 8-h period after administration, presumably due to the metabolites of ternatins.

Plasma Parameters of Ternatins Figure 3 shows the plasma concentration profiles of ternatins versus time after oral administration of BPP extract. Using the profiles, we determined the maximum plasma concentration (C_{max}), the time at which the maximum plasma concentration was reached (t_{max}), and the area under the plasma concentration curve during the 8-h period after oral administration (AUC_{0-8}) of individual ternatins (Table 1). The C_{max} of total ternatin reached 0.141 ± 0.035 μM at 15 min after oral administration; the t_{max} of each ternatin varied from 15 to 30 min; and the AUC_{0-8} of total ternatin was 16.398 ± 1.542 μM·min (see Table 1).

Because the dose of each ternatin in the BPP extract varied from 0.0007 to 0.0228 mmol/kg, both the AUC_{0-8} and the C_{max} of the individual ternatins were normalized by an orally administered dose of the corresponding ternatins (AUC_{0-8}/D and C_{max}/D) for comparison of their blood plasma amounts. The AUC_{0-8}/D, indicating the bioavailability of the individual ternatins, varied from 204.01 ± 19.06 to 653.42 ± 99.59 μM·min/mmol/kg, and the C_{max}/D values varied from 1.82 ± 0.4 to 6.71 ± 2.16 μM/mmol/kg. As shown in Fig. 4, AUC_{0-8}/D and C_{max}/D values of ternatins overall tended to be correlated negatively with increasing order of molecular weight. Thus, to clarify the effect of side chain structures on ternatin absorption, both AUC_{0-8}/D and C_{max}/D results are compared for symmetrical ternatins and asymmetrical ternatins, as defined in the Discussion. The AUC_{0-8}/D values of symmetrical ternatins are well correlated with increasing order of molecular weight with some exceptions, whereas the AUC_{0-8}/D values of asymmetrical ternatins are not correlated with molecular weight (Fig. 4A). The C_{max}/D values of symmetrical terna-

Table 1. Orally Administered Dose and Plasma Parameters of Ternatins

Anthocyanin	C_{max} (μM)	t_{max} (min)	AUC_{0-8} (μM min)	*Dose* (mmol/kg)
Ternatin A1 (1)	0.0076 ± 0.0017	15	0.790 ± 0.074	0.0021
Ternatin A2 (2)	0.0182 ± 0.0039	15	1.671 ± 0.170	0.0046
Ternatin A3 (3)	0.0043 ± 0.0001	30	0.504 ± 0.028	0.0008
Ternatin B1 (4)	0.0483 ± 0.0145	15	4.643 ± 0.434	0.0228
Ternatin B2 (5)	0.0252 ± 0.0063	15	3.027 ± 0.386	0.0063
Ternatin B3 (6)	0.0083 ± 0.0019	15	1.089 ± 0.125	0.0028
Ternatin B4 (7)	0.0039 ± 0.0004	30	0.447 ± 0.071	0.0010
Ternatin D1 (8)	0.0134 ± 0.0030	15	2.115 ± 0.310	0.0074
Ternatin D2 (9)	0.0082 ± 0.0018	15	1.642 ± 0.245	0.0042
Preternatin A3 (10)	0.0048 ± 0.0016	15	0.470 ± 0.072	0.0007
Total ternatin	0.1414 ± 0.0352	15	16.398 ± 1.542	0.0527

Values are means ± SEM of three rats.
C_{max}, maximum plasma concentration; t_{max}, time at which the maximum plasma concentration was reached; AUC_{0-8}, area under the plasma concentration curve during the 8-h period after oral administration.

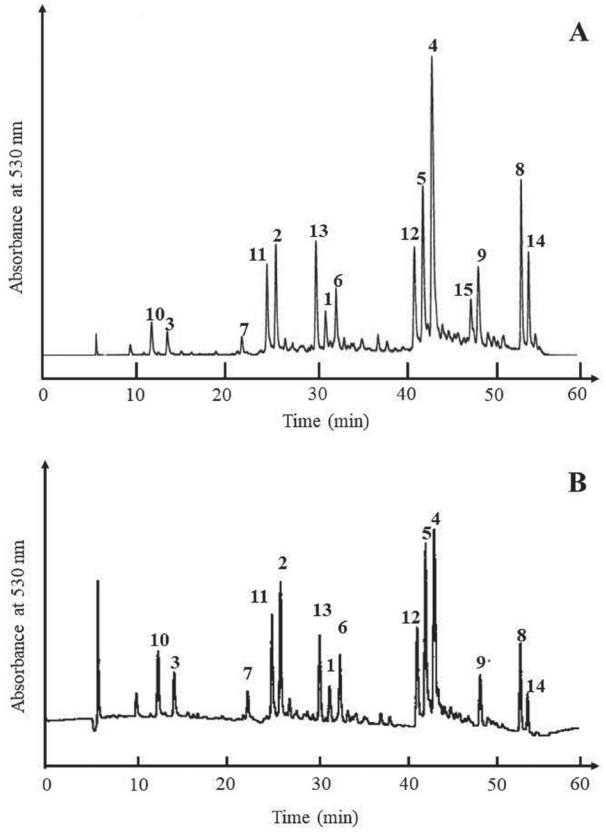

Fig. 2. Typical HPLC Chromatogram of Ternatin in BPP and Rat Blood Plasma

A: Original BPP extract. **B**: Rat blood plasma 15 min after oral administration of the BPP extract. Peak numbers correspond to the compounds as follows: 1, ternatin A1; 2, ternatin A2; 3, ternatin A3; 4, ternatin B1; 5, ternatin B2; 6, ternatin B3; 7, ternatin B4; 8, ternatin D1; 9, ternatin D2.

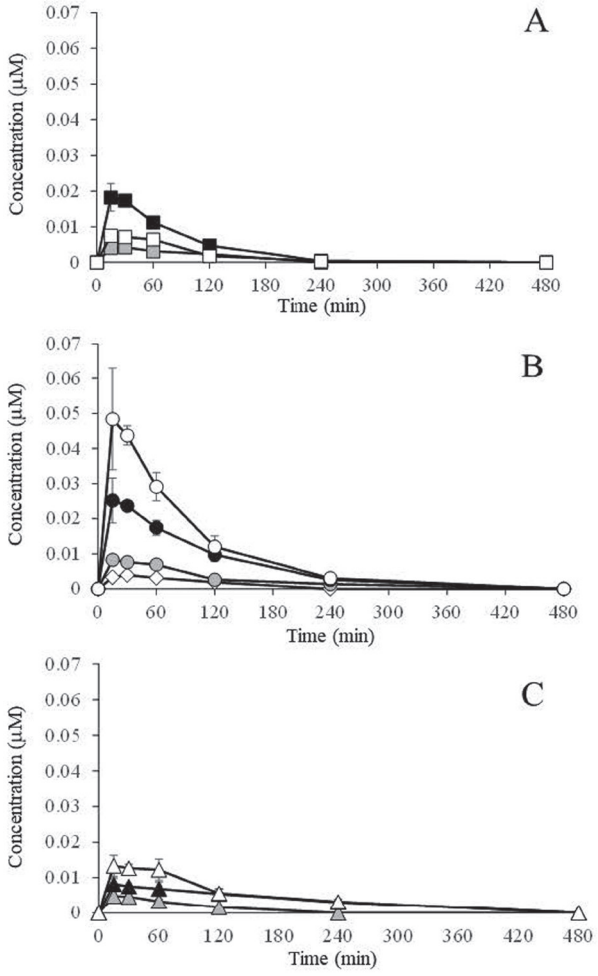

Fig. 3. Plasma Concentration Profiles of Ternatins After Oral Administration of BPP Extract

A: Ternatin A series. Open squares, closed squares and gray squares indicate ternatin A1, A2 and A3, respectively. **B**: Ternatin B series. Open circles, closed circles, gray circles and open diamonds indicate ternatin B1, B2, B3 and B4, respectively. **C**: Ternatin D series and preternatin A3. Open triangles, closed triangles and gray triangles indicate ternatin D1, D2 and preternatin A3, respectively. Values are means ± SEM of three rats.

tins appear well correlated with increasing order of molecular weight with some exceptions, and the C_{max}/D values of asymmetrical ternatins, unlike the AUC_{0-8}/D values of asymmetrical ternatins, were positively correlated with increasing order of molecular weight (Fig. 4B). The AUC_{0-8}/D of total ternatin was 311.15 ± 29.26 μM·min/mmol/kg, and C_{max}/D was 2.68 ± 0.67 μM/mmol/kg at 15 min after oral administration of the BPP extract.

DISCUSSION

Ternatins are characterized as malonylated delphinidin 3,3′,5′-triglucosides (Dp3G3′G5′G: deacylternatin) having 3′,5′-side chains with alternating D-glucose (G) and *p*-coumaric acid (C) units.[17–20] Although the biosynthetic pathways of ternatins remain unclear, preternatins, precursors of ternatins have been suggested.[17–20] It is expected that BPP contain various types of preternatins, although only preternatin A3 has been elucidated structurally.[20] Ternatins from BPP are categorized by the terminal residues of the 3′- and 5′-side chains (R_1 and R_2) into four series: ternatin A, where both terminal residues of R_1 and R_2 are –G; ternatin B, where the terminal residues of R_1 and R_2 are –G and –C; and ternatin D, where the terminal residues of R_1 and R_2 are a series of –C. The structure of nine ternatin analogues were determined in BPP, encompassing a molecular weight range of 1330 to 2109.[17–19] Among ternatin analogues, ternatin A1 is the largest anthocyanin found in nature to date.[11] However, the large molecular weight of ternatins may inhibit their absorption from the gastrointestinal tract. This work aimed to clarify whether large molecule ternatins were directly absorbed from the gastrointestinal tract. The intravenous administration of BPP extract was not examined here, thus, the pharmacokinetic parameters such as volume of distribution were not obtained. Instead of these parameters, absorption of ternatin was evaluated with AUC_{0-8}/D values in blood plasma.

In the present study, minimum numbers of rats (3 individual rats) were examined to summarize plasma concentration profiles of ternatins. The relative standard errors of ternatins versus mean values obtained were 5.46 to 15.93% for *AUC* and 3.01 to 32.28% for C_{max}. These values were similar to anthocyanin absorption levels obtained from 4 or 5 rats (8.00 to 28.67% for *AUC* and 1.00 to 32.00% for C_{max}) in our previous research.[6–10,15,21] Additionally, results of the AUC_{0-8}/D values were significantly different among several ternatins, therefore, *AUC* values obtained in the present study using minimum numbers of rats were trustworthy to overview absorption profiles

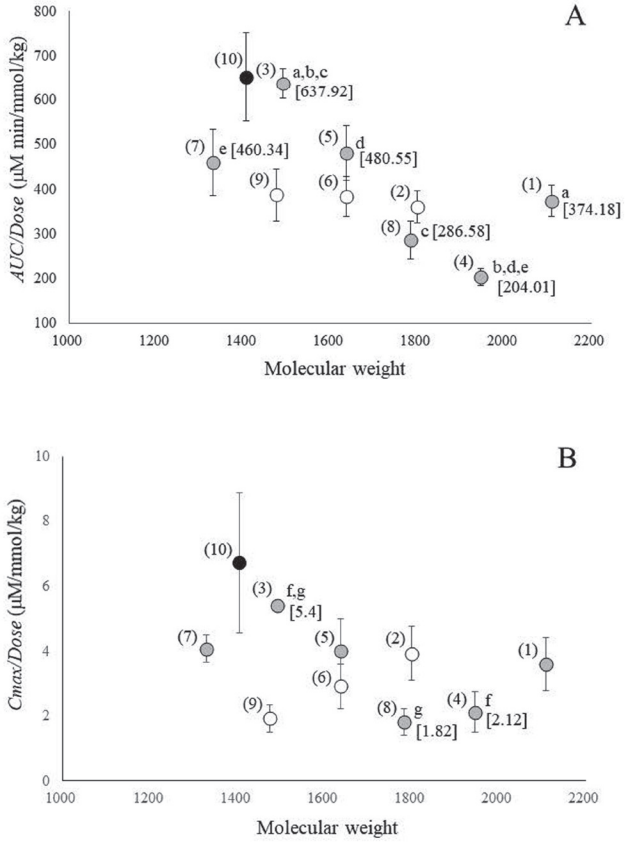

Fig. 4. Correlation between Molecular Weight and Absorption Parameters of Ternatins

A: AUC_{0-8}/D. **B:** C_{max}/D. Gray circles, open circles, and closed circles indicate symmetrical ternatin, asymmetrical ternatin, and preternatin A3, respectively. Values are mean ± SEM of three rats. The same lowercase letters on the circles indicate a significant difference ($P < 0.05$). Values in square brackets show mean of AUC_{0-8}/D or C_{max}/D. Numbers in parentheses correspond to the compounds as follows: 1, ternatin A1; 2, ternatin A2; 3, ternatin A3; 4, ternatin B1; 5, ternatin B2; 6, ternatin B3; 7, ternatin B4; 8, ternatin D1; 9, ternatin D2.

of ternatins from gastrointestinal tract. The C_{max} vales were not suitable for the comparison because of the large variances.

HPLC analysis revealed that nine ternatin analogues, together with preternatin A3, were absorbed from the gastrointestinal tract and detected in rat blood plasma 15 min after oral administration of the BPP extract at a dose of 400 mg/kg (0.0527 mmol/kg as total ternatin). Four unknown pigments (peaks 11–14) in the original BPP extract were also found in blood plasma. These pigments are predicted to be preternatin analogues not structurally elucidated in BPP to date. Interestingly, only peak 15 was not detected in rat blood plasma during the 8-h period after oral administration. Some researchers reported poor absorption of Pn3CafSop5G, a dominant anthocyanin in purple sweet potato, in both rats[13] and humans[16] after oral administration and concluded that the low absorption profile of Pn3CafSop5G was due to its large molecular weight of 949. However, we previously reported that *trans*-nasunin, whose molecular weight is 919, was absorbed to an extent similar to that of the non-acylated anthocyanin, Dp3G.[15] In the present study, the AUC_{0-8}/D value of total ternatin was 311.15 ± 29.26 μM·min/mmol/kg and was similar to that obtained in our previous research with *trans*-nasunin (280.9 μM·min/mmol/kg) and Dp3G (255.3 μM·min/mmol/kg).[15] Together with our previous results,[13,15,16] the present results demonstrate that molecular weight is not the sole factor determining the bioavailability of anthocyanins.

The peak intensities of the individual ternatins detected in the HPLC chromatogram of rat blood plasma were different from those of the original BPP extract, indicating that the chemical structure of the ternatins, including the molecular weight, influences the absorption profile of ternatins. The orally administered dose of individual ternatins in the BPP extract varied from 0.0007 to 0.0228 mmol. Hence, the AUC_{0-8} values of each ternatin were normalized to the orally administered dose of the corresponding ternatin in the BPP extract. The AUC_{0-8}/D values of the ternatins did not correlate precisely with the order of molecular weight, suggesting that the terminal structures and symmetries of substitution patterns of both side chains are associated with the bioavailability of ternatins.

When the difference in molecular weight between ternatins was more than one CG unit (molecular weight of 308), the effect of molecular weight was expressed as the difference in AUC_{0-8}/D values, such that B2 (–CGC, –CG) > B1 (–CGCG, –CGC), where the entries inside the parentheses indicate the R_1 and R_2 residues of each ternatin. However, the effects of the side-chain structures between ternatins with molecular weight differences of more than one CG unit were not well correlated with bioavailability. Therefore, ternatins with molecular weight differences of C (molecular weight of 146) or G (molecular weight of 162) were compared below (rule 1). Additionally, we classified the ternatins by the extent of the symmetry of substitution patterns of both the 3′ and 5′ side chains of malonylated Dp3G3′G5′G. When the difference of substitution patterns of both side chains was none, –C, or –G, the ternatin was classified as a symmetrical type, whereas when the difference of substitution patterns of both side chains was more than one –CG unit, the ternatin was classified as an asymmetrical type (rule 2).

Based on the two rules defined above, we compared AUC_{0-8}/D values between symmetrical ternatins showing molecular weight differences of C or G: A1 (–CGCG, –CGCG), B1 (–CGCG, –CGC), D1 (–CGC, –CGC), B2 (–CGC, –CG), A3 (–CG, –CG), and B4 (–CG, –C). Four out of six ternatins showed a negative correlation between bioavailability and increasing order of molecular weight (A3 > B2, B2 > D1, and D1 > B1), whereas the other ternatins showed a positive correlation with the order of molecular weight (A1 > B1 and A3 > B4). When the terminal structures in both side chains are considered, symmetrical ternatins carrying –G terminals on both side chains (ternatin A1 and A3) showed specifically higher AUC_{0-8}/D values than symmetrical ternatins having –C and –G terminals on both side chains (ternatin B1 and B4). Contrary to this, the AUC_{0-8}/D values of asymmetrical ternatins (A2, B3, D2) were not influenced by the terminal structures of the side chains and showed almost similar values: D2 (–CGC, –C) = B3 (–CGCG, –C) = A2 (–CGCG, –CG). Collectively, these observations demonstrate that molecular weight partly modulates ternatin bioavailability, and that symmetrical A series are exceptionally bioavailable.

The C_{max} of ternatin was normalized to the orally administered dose (C_{max}/D), and the values were compared among ternatins. The results indicate that the C_{max}/D values of ternatins show a trend similar to the AUC_{0-8}/D values discussed above. However, almost all the results were not significant because of large variances (significant difference between A1 and B1, and A3 and D1). The C_{max}/D of total ternatin (2.68 ± 0.67

μM/mmol/kg) was similar to that obtained in our previous research: 1.39 μM/mmol/kg for Dp3G and 3.16 μM/mmol/kg for *trans*-nasunin.[15]

When we focused on the metabolism of anthocyanins, the *O*–methyl metabolites,[7,8] anthocyanidin glucuronides[7] and the anthocyanin glucuronides (glucoside-glucuronides)[21] were produced normally as major metabolites of non-acylated anthocyanins and were the dominant distributed forms in the peripheral tissues.[9] These observations suggest that anthocyanin metabolites are critically involved in the health-promoting effect of anthocyanin, especially for disease prevention. In the present study, no ternatin metabolites were observed at 530 nm in rat blood plasma under the HPLC conditions utilized. We previously made a similar observation that colored metabolites of nasunin were seldom detected in rat blood plasma when purified nasunin was administered orally.[15] We hypothesize that acylated anthocyanins are difficult to hydrolyze in small intestine, are not distributed in hepatic cells, and thereby avoid *O*–methylation and glucuronidation because of their relatively large molecular weight and acyl moieties attached to anthocyanidin. This property of avoiding hepatic and intestinal metabolism may partly explain why acylated anthocyanins show similar plasma concentration profiles despite their large molecular weight compared to non-acylated anthocyanins, in addition to their relatively more stable nature. As discussed in the present study, the plasma concentration profiles of ternatins differed depending on the side-chain structures. However, we did not determine whether differences in plasma profiles resulted from differences in direct intestinal absorption or from deacylation and deglycosylation in the small intestine; for example, ternatin B2 (–CGC, –CG) was metabolized to ternatin A3 (–CG, –CG), then absorbed and detected in blood plasma as ternatin A3. Precise studies on the intestinal metabolism of purified ternatins, including the production of ring fission metabolites, are required to clarify these points.

We examined, for the first time, the gastrointestinal absorption of polyacylated anthocyanins, ternatins, in rats. The results demonstrate that 10 ternatin analogues derived from BPP, including preternatin A3, are absorbed in their original acylated forms to the same extent as those of other acylated and non-acylated anthocyanins. The results also indicate that ternatins may contribute to health-promoting effects *in vivo* in their polyacylated forms. Further studies on the metabolism and tissue accumulation of ternatins are now in progress.

Conflict of interest The authors declare no conflict of interest.

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