Accumulation of Tilianin and Rosmarinic Acid and Expression of Phenylpropanoid Biosynthetic Genes in *Agastache rugosa*

Pham Anh Tuan,[†,‡] Woo Tae Park,[†,‡] Hui Xu,[†] Nam Il Park,[§] and Sang Un Park*,[†]

[†]Department of Crop Science, College of Agriculture & Life Sciences, Chungnam National University, 220 Gung-dong, Yuseong-gu, Daejeon 305-764, Korea

[§]Wildlife Genetic Resources Center, National Institute of Biological Resources (NIBR), Gyungseo-dong, Seo-gu, Incheon 404-708, Korea

Ⓢ *Supporting Information*

ABSTRACT: Korean mint (*Agastache rugosa*), a perennial, medicinal plant of the Labiatae family, has many useful constituents, including monoterpenes and phenylpropanoids. Among these, tilianin and rosmarinic acid, 2 well-known natural products, have many pharmacologically useful properties. Chalcone synthase (CHS) and chalcone isomerase (CHI) catalyze the first and second committed steps in the phenylpropanoid pathway of plants, leading to the production of tilianin. In this study, cDNAs encoding CHS (ArCHS) and CHI (ArCHI) were isolated from *A. rugosa* using rapid amplification of cDNA ends (RACE)-PCR. Amino acid sequence alignments showed that ArCHS and ArCHI shared high sequence identity and active sites with their respective orthologous genes. Quantitative real-time PCR analysis was used to determine the expression levels of genes involved in tilianin and rosmarinic acid biosyntheses in the flowers, leaves, stems, and roots of *A. rugosa*. High-performance liquid chromatography (HPLC) revealed that the accumulation pattern of tilianin matched the expression patterns of *ArCHS* and *ArCHI* in different organs of *A. rugosa*. Moreover, acacetin, the precursor of tilianin, also demonstrated an accumulation pattern congruent with the expression of these 2 genes. The transcription levels of *ArPAL*, *ArC4H*, and *Ar4CL* were the highest in the leaves or flowers of the plant, which also contained a relatively high amount of rosmarinic acid. However, the roots showed a significant content of rosmarinic acid, although the transcription of *ArPAL*, *ArC4H*, and *Ar4CL* were low. The findings of our study support the medicinal usefulness of *A. rugosa* and indicate targets for increasing tilianin and rosmarinic acid production in this plant.

KEYWORDS: *Agastache rugosa*, gene expression, phenylpropanoid, rosmarinic acid, tilianin

■ INTRODUCTION

Agastache rugosa, belonging to the mint family (Labiatae), is a perennial medicinal plant widely distributed throughout East Asian countries. In Korea, this plant is a ubiquitous herb and has been used as a wild vegetable and an herbal drug in traditional therapies.[1] Studies have indicated that *A. rugosa* has a variety of pharmacological activities such as anti-HIV integration activities and antifungal effects.[2,3] Extracts of *A. rugosa* are also believed to be valuable in the treatment of inflammatory and oxidative stress-induced disorders.[4,5]

A. rugosa contains several types of essential oils, sesquiterpenes, diterpenes, triterpenes, flavonoids, and carotenoids.[6−8] Among these, tilianin, which is considered to be the main flavonoid, and rosmarinic acid have been proven to contribute to the medicinal potential of *A. rugosa*.[2,9] Tilianin is found in various plants as a glucose-glycoside compound of a flavonoid, acacetin. Natural product chemists and clinicians have been interested in tilianin because of its important biological activities, for example, its anti-inflammatory, antiatherogenic, antihypertensive, and vasorelaxant effects.[4,10,11] Rosmarinic acid, a well-known hydroxycinnamic acid ester, was identified as an active component in several medicinal plants.[12] The biosynthesis and production of rosmarinic acid in plants have been extensively studied, because it exhibits various pharmacological properties, including antiviral, antibacterial, anti-inflammatory, and antioxidant effects.[13,14] In the plant kingdom, rosmarinic acid is thought to act as a preformed, constitutively accumulated defense compound.[12]
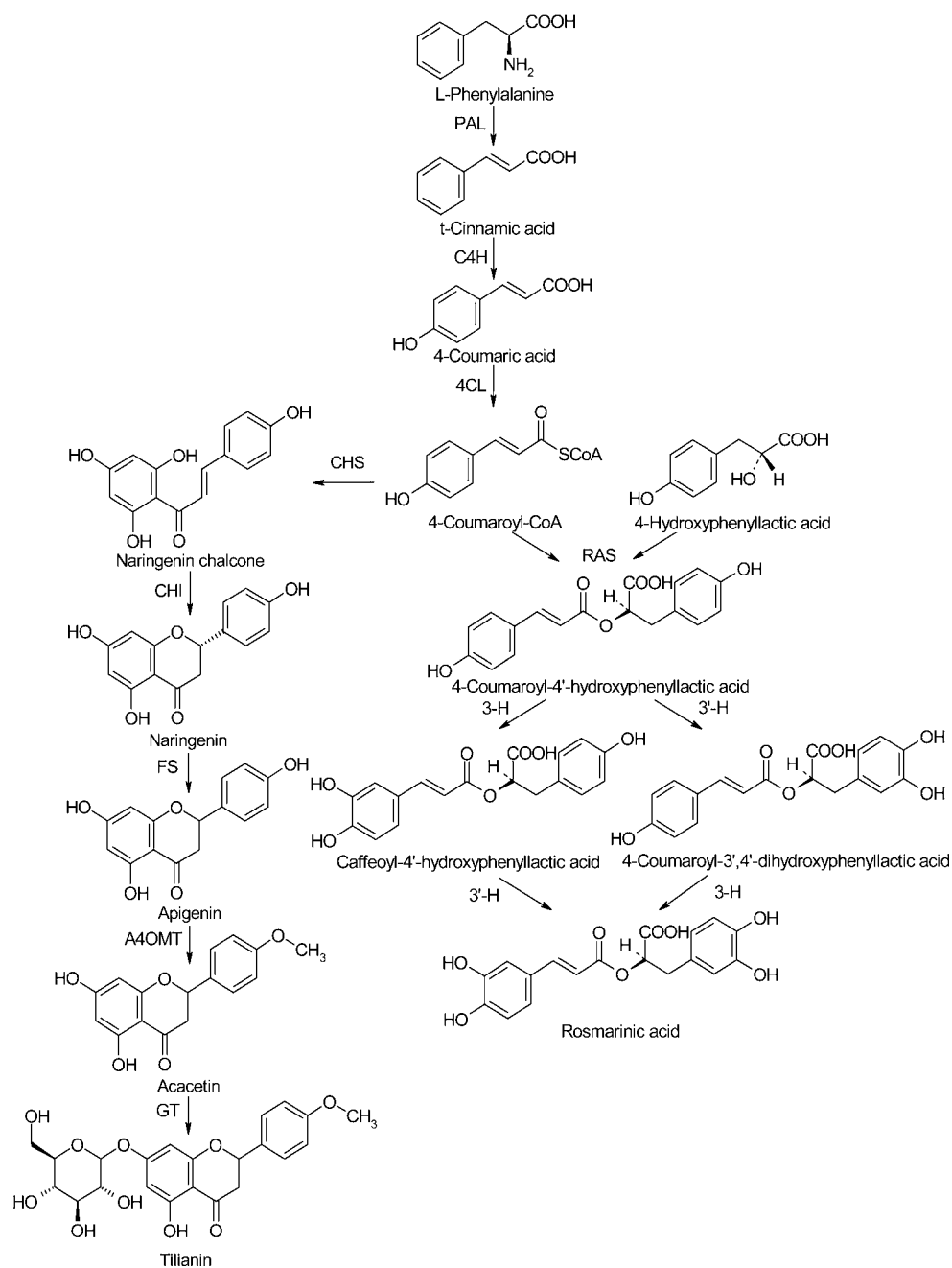
The proposed biosynthetic pathway of tilianin and rosmarinic acid in plants is shown in Figure 1. The amino acid L-phenylalanine is the starting compound for the biosynthesis of both compounds. Phenylalanine is transformed to 4-coumaroyl-CoA by 3 enzymes in the general phenylpropanoid pathway, viz., phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), and 4-coumarate:CoA ligase (4CL).[15] 4-Coumaroyl-CoA is the precursor for many secondary plant products, such as flavonoids, stilbenes, coumarins, and lignin. In the section of the pathway leading to tilianin, chalcone synthase (CHS) catalyzes the condensation of 4-coumaroyl-CoA and 3 malonyl-CoA molecules to produce naringenin chalcone.[16] Chalcone isomerase (CHI) converts naringenin chalcone to naringenin via a stereospecific isomerization.[17] In the next step, flavone synthase (FS) catalyzes the desaturation of naringenin to derive apigenin.[18] Then, apigenin 4′-O-methyltransferase (A4OMT) transfers a methyl group to apigenin to yield acacetin.[19] Finally, glucosyltransferase (GT) catalyzes the transfer of glucose to acacetin to synthesize tilianin.[20] In the branch of the pathway leading to rosmarinic

Figure 1. Proposed biosynthetic pathway of tilianin and rosmarinic acid. PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumaryl-CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; FS, flavone synthase; A4OMT, apigenin 4′-O-methyltransferase; GT, glucosyltransferase; RAS, hydroxycinnamoyl-CoA:hydroxyphenyllactate hydroxycinnamoyl transferase; 3-H, hydroxycinnamoyl; 3′-H, hydroxycinnamoyl.

acid, condensation of 4-hydroxyphenyllactic acid with 4-coumaroyl-CoA is catalyzed by hydroxycinnamoyl-CoA:hydroxyphenyllactate hydroxycinnamoyl transferase (RAS) to form 4-coumaroyl-4′-hydroxyphenyllactic acid. 4-Coumaroyl-4′-hydroxyphenyllactic acid is then converted to rosmarinic acid by 2 consecutive hydroxylation steps.[21] These reactions are catalyzed by 2 distinct cytochrome P450s, viz., 3-hydroxycinnamoyl (3-H) and 3′-hydroxycinnamoyl (3′-H).[22]

In this study, cDNAs encoding chalcone synthase (ArCHS) and chalcone isomerase (ArCHI), which are related to tilianin biosynthesis in *A. rugosa*, were isolated. The expression patterns of genes involved in tilianin and rosmarinic acid biosyntheses (*ArPAL*, *ArC4H*, *Ar4CL*, *ArCHS*, and *ArCHI*) were analyzed by

real-time PCR. The concentrations of tilianin and rosmarinic acid in different plant organs were also determined by HPLC to investigate the biosynthetic mechanisms of these compounds in *A. rugosa*.

■ MATERIALS AND METHODS

Plant Materials. *A. rugosa* plants were grown at the experimental farm of Chungnam National University (Daejeon, Korea). The flowers, leaves, stems, and roots were excised from mature plants. The samples were immediately frozen in liquid nitrogen and then stored at −80 °C and/or freeze-dried for RNA isolation and/or HPLC analysis.

RNA Isolation and cDNA Synthesis. Total RNA was isolated from different organs of *A. rugosa* separately using Plant total RNA mini kit (Geneaid, Taiwan) under the manufacturer's instruction. The quality and concentration of total extracted RNA were determined by 1% agarose gel electrophoresis and spectrophotometer analysis, respectively. For RACE-PCR, 3 μg of total RNA was used to synthesize first-strand cDNA using GeneRacer Kit (Invitrogen, Carlsbad, CA, USA) and a 10-fold dilution of the resulting cDNA was used as template. For quantitative real-time PCR, 1 μg of total RNA from different organs was used for reverse transcription using the ReverTra Ace-R kit (Toyobo, Osaka, Japan) and a 20-fold dilution of the resulting cDNA was used as template.

Cloning of the cDNA Encoding Chalcone Synthase and Chalcone Isomerase. Degenerate primers for *CHS* and *CHI* were designed based on homologies found in genes isolated earlier (Table

Table 1. Primers Used in This Study

primer	sequence (5′ to 3′)	amplicon (base pairs)
	Use: Partial Sequence	
CHS F	CCVGGDGGGAAVKYTTSRTYYTTGAA	492
CHS R	CMTCTTTBGARAANGMAAYCGTVA	
CHI F	CCTCGGYGGYGCAGGNGWNMGAGG	250
CHI R	GGNGAGACDCCRTDCTTBCCRATGTA	
	Use: RACE-PCR	
ArCHS_3	TGAAGCGCTTCATGATGTACCAGCA	970
ArCHS_5	CTCGGAGCAGACGACCAAAACCCTA	650
ArCHI_3	TCGAGTTCATCGCCGAAATCAT	681
ArCHI_5	CTTTCTCAGAGTATTGTTGCCCAGTC	426
	Use: Real-Time PCR	
ArActin_RT F	ACCTCAAAATAGCATGGGGAAGT	151
ArActin_RT R	GGCCGTTCTCTCACTTTATGCTA	
ArPAL_RT F	ACGGCTCCAACGGTCATAATAAT	108
ArPAL_RT R	ATCCGCTTTACCTCCTCAAGGT	
ArC4H_RT F	GTTCGAGAGTGAGAATGATCCGT	157
ArC4H_RT R	ATAATCCTTGAACAATTGCAGCC	
Ar4CL_RT F	ACATCTACTCGTTGAATTCGGTGC	162
Ar4CL_RT R	AGTCGAAATTATCCACCAATGGA	
ArCHS_RT F	GACCAAAGCACCTATCCCGATTA	151
ArCHS_RT R	TTGGGTTCTCCTTCAGGTACTCC	
ArCHI_RT F	GCCTTCTCCAAAGATGGTTCTGT	194
ArCHI_RT R	TCTTGATTCAGTTTTGCCTCAGC	

1). The amplified products were purified and cloned into T-blunt vector (Solgent, Daejeon, Korea) followed by sequencing. BLAST results confirmed those fragments were partial of CHS and CHI from *A. rugosa*. Using the obtained partial sequences, gene specific primer was designed to amplify the 5′-end and 3′-end of CHS and CHI. 5′-RACE-PCR and 3′-RACE-PCR were performed following the manufacturer's protocol. Degenerate primers CHS F and CHS R amplified a 492-bp fragment, of which the sequence showed similarity to other *CHSs* according to a BLAST search. Subsequently, the 5′-end and 3′-end of the *ArCHS* were generated using RACE-PCR primers. The expected PCR product was purified and cloned into T-blunt vector for sequencing.

Sequence Analysis. The deduced amino acid sequences of ArCHS and ArCHI were analyzed for homology using BLAST at the NCBI Genbank database (http://www.ncbi.nlm.nih.gov/BLAST). Sequencing alignments were carried out by BioEdit Sequence Alignment Editor, version 5.0.9 (Department of Microbiology, North Carolina State University, Raleigh, NC). Phylogenic tree was constructed by the online Web site (http://www.genebee.msu.su).

Quantitative Real-Time PCR. Base on the sequences of *ArPAL* (accession number AF326116), *ArC4H* (accession number AY616436), *Ar4CL* (accession number AY587891), *ArCHS* (accession

number JQ314450) and *ArCHI* (accession number JQ314451), real-time PCR primers were designed using the Primer3 Web site (http://frodo.wi.mit.edu/primer3/) (Table 1). Expressions of these genes were calculated by the method of relative quantification using *actin* gene as the reference.[23] Partial cDNA encoding actin was cloned in this study (accession number JX087435). Real-time PCR reaction was carried out in a 20 μL reaction mix containing 5 μL of template cDNA, 10 μL of 1X SYBR Green Realtime PCR Master Mix (Toyobo, Osaka, Japan), 0.5 μL of each primer (10 μM), and DEPC water. Thermal cycling conditions were as follows: 95 °C for 5 min; 40 cycles for 95 °C for 15 s, 56 °C for 15 s, 72 °C for 20 s. PCR products were analyzed by Bio-Rad CFX Manager 2.0 software. Three replications on each sample were used for real-time analysis.

Extraction and High-Performance Liquid Chromatography Analysis. Samples (0.1 g) were frozen in liquid nitrogen, ground to a fine powder using a mortar and pestle, and then extracted with 10 mL of 70% ethanol for 1 h at 60 °C. After centrifugation, the supernatant was filtered through a 0.45 μm poly filter (Acrodisc Syringe Filters, Pall, Port Washington, NY) and analyzed by HPLC. The analysis was performed with a C_{18} column (250 mm × 4.6 mm, 5 μm; RStech, Daejon, Korea) at 30 °C. The mobile phase was a gradient mixture of acetonitrile, methanol, and 0.2% acetic acid. The flow rate was maintained at 1.0 mL·min^{-1}, the injection volume was 20 μL, and the detection wavelength was 275 nm. The concentration of flavones in the samples was calculated by using a standard curve. Mean values were obtained from three independent replicates.

RESULTS AND DISCUSSION

Cloning CHS and CHI from *A. rugosa*. The full-length of *ArCHS* was 1501 bp in length, including a 75-bp 5′-untranslated region (UTR), an 1176-bp open reading frame, and a 250-bp 3′-UTR with a poly-A tail. The open reading frame of *ArCHS* encodes a protein of 391 amino acids with a predicted molecular mass of 42.61 kDa.

Similarly, a 903-bp full-length cDNA of *ArCHI*, with a 62-bp 5′-UTR and a 726-bp open reading frame, encoding a protein of 241 amino acids (molecular mass 25.78 kDa), as well as a 115-bp 3′-UTR with a poly-A tail, was obtained.

Sequence Analyses of ArCHS and ArCHI. A BLAST search at the amino acid level showed that ArCHS exhibited high homology with other CHSs (Figure 2). ArCHS of *A. rugosa* shares 95% identity and 98% similarity with *Perilla frutescens* CHS, 95% identity and 98% similarity with *Solenostemon scutellarioides* CHS, 93% identity and 96% similarity with *Misopates orontium* CHS, and 92% identity and 96% similarity with *Mazus pumilus* CHS. This homology search revealed that there are 4 residues (Cys164, Phe215, His303, Asn336) that are conserved in all known CHSs; these define the active sites.[24] The close relationship between ArCHS and the other CHSs observed in phylogenic analysis indicates that ArCHS is a member of the CHS family (Figure S1 in the Supporting Information).

The closest homologue of ArCHI was CHI from *Perilla frutescens* (79% identity and 88% similarity), followed by CHI from *Scutellaria baicalensis* (78% identity and 89% similarity), CHI from *Camellia sinensis* (75% identity and 88% similarity), and CHI from *Dianthus caryophyllus* (74% identity and 87% similarity). Four residues (Thr60, Tyr118, Asn125, Ser202), known to be the active sites of CHIs, were also found to be present in ArCHI of *A. rugosa* (Figure 3). Leguminous plants, on the other hand, differed from nonleguminous plants; the corresponding active site residues in leguminous plants are Thr48, Tyr106, Asn116, and Thr190.[25,26] The difference of CHS between nonleguminous and leguminous plants was also
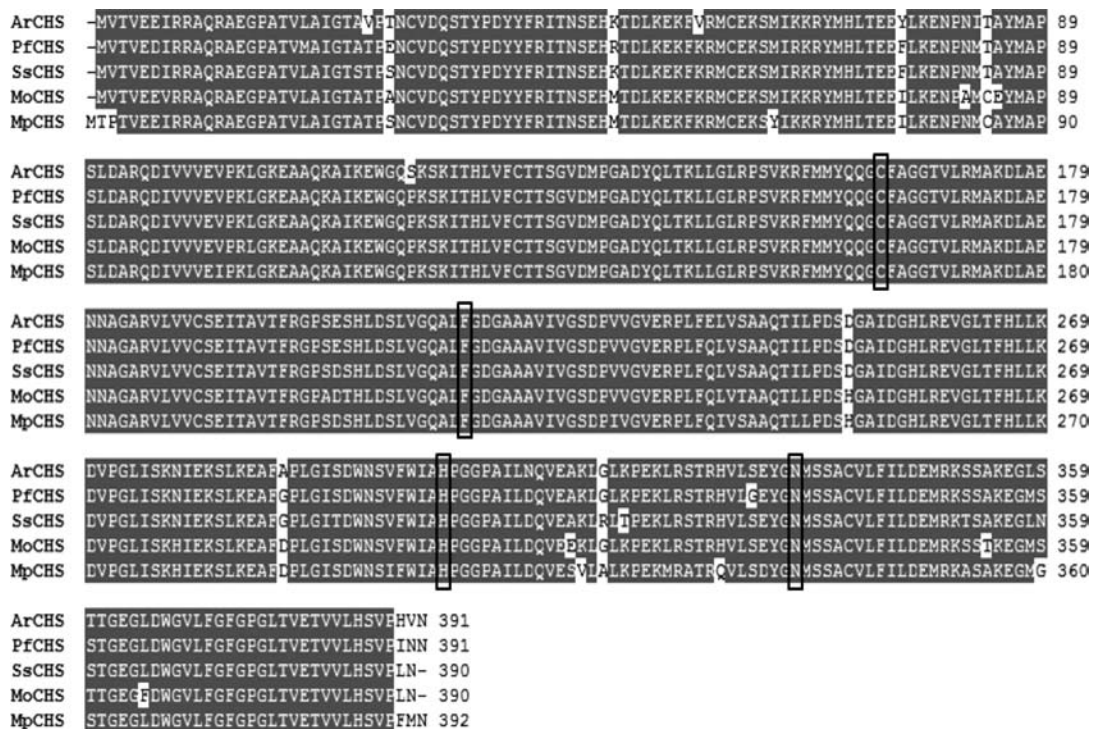
Figure 2. Multiple alignment of amino acid sequences of ArCHS with other CHSs. Highly conserved residues in all sequences are indicated in white color with black background. The black boxes are the active sites of the CHS genes. PfCHS, *Perilla frutescens* (AB002815); SsCHS, *Solenostemon scutellarioides* (EF522149); MoCHS, *Misopates orontium* (AM162205); MpCHS, *Mazus pumilus* (AY131328).


Figure 3. Multiple alignment of amino acid sequences of ArCHI with other CHIs. Highly conserved residues in all sequences are indicated in white color with black background. The black boxes are the active sites of the CHI genes. PfCHI, *Perilla frutescens* (AB362192); SbCHI, *Scutellaria baicalensis* (HQ153041); CsCHI, *Camellia sinensis* (DQ120521); DcCHI, *Dianthus caryophyllus* (Z67989).

observed in the phylogenic analysis (Figure S2 in the Supporting Information).

Expression Levels of Genes Involved in Tilianin and Rosmarinic Acid Biosyntheses in Different Organs of *A. rugosa*. Quantitative real-time PCR analysis was used to investigate the expression patterns of *ArPAL*, *ArC4H*, *Ar4CL*, *ArCHS*, and *ArCHI* in the flowers, leaves, stems, and roots of *A. rugosa*. As shown in Figure 4, the expression of *ArPAL* was the highest in the leaves, where the relative quantification to *actin* (RQ-value) was 0.14. *ArPAL* was expressed at a moderate level in the flowers (RQ 0.06) and stems (RQ 0.06) and was the lowest in the roots (RQ 0.02). In contrast to this first enzyme, the transcription level of the second enzyme, *ArC4H*, was the most abundant in the flowers (RQ 30.52), lower in the leaves (RQ 17.5) and stems (RQ 11.9), and the lowest in the root

(RQ 2.14). The level of *Ar4CL* was the highest in the leaves, with an RQ of 17.98, higher than the RQ of 11.87 in the flower. A lower expression of *Ar4CL* was found in the stems (RQ 8.66) and roots (RQ 5.45). *ArCHS* transcription was abundant in the flowers (RQ 2.9), intermediate in the leaves (RQ 1.28), and poor in the stems (RQ 0.2) and roots (RQ 0.18). The expression pattern of *ArCHI* was fairly similar to that of *ArCHS*, with the highest expression in the flowers (RQ 4.28), moderate expression in the leaves (RQ 1.24), and weak expression in the stems (RQ 0.26) and roots (RQ 0.16).

Analysis of Rosmarinic Acid and Tilianin Contents in Different Organs of *A. rugosa*. The same plant materials as those used for quantitative real-time PCR were used for HPLC analysis of tilianin and rosmarinic acid in *A. rugosa*. Tilianin, its precursor acacetin, and rosmarinic acid were measured in the
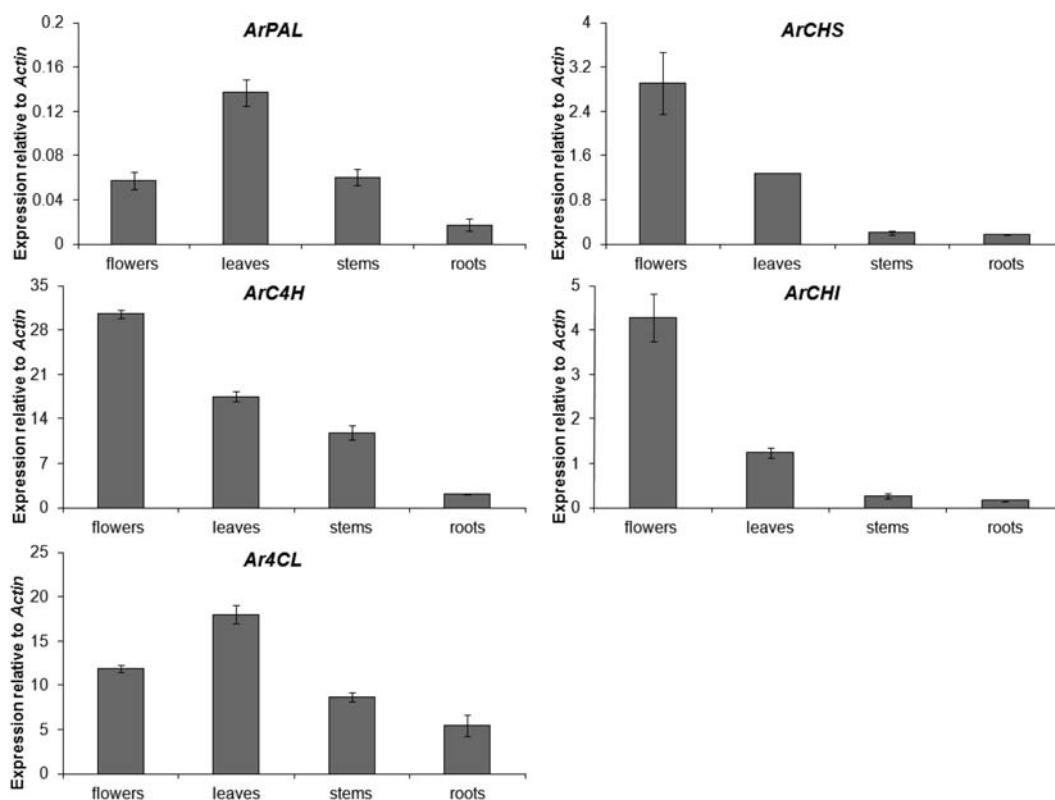
Figure 4. Expression of *ArPAL, ArC4H, Ar4CL, ArCHS,* and *ArCHI* in different organs of *A. rugosa.* The values and error bars represent the mean and standard error of 3 independent reactions, respectively.
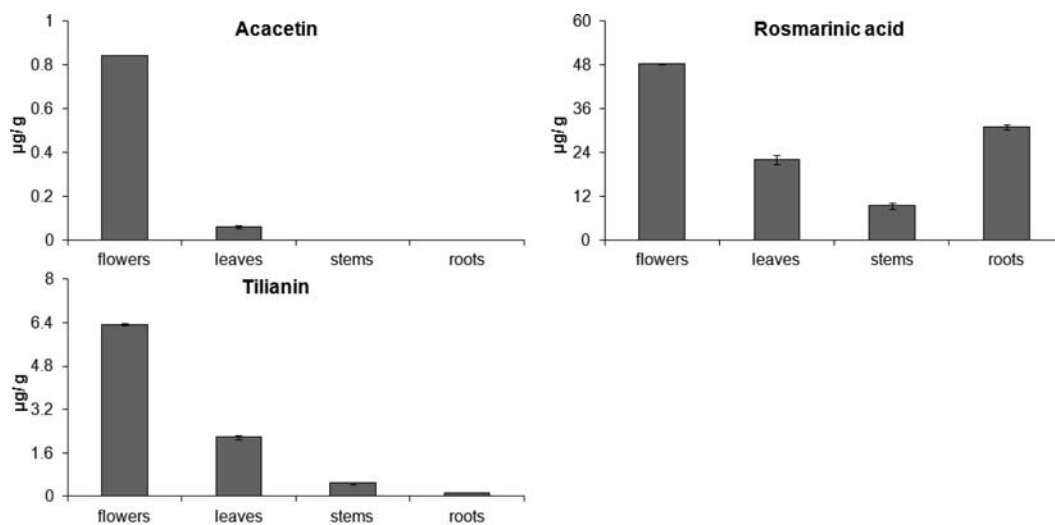
Figure 5. Accumulation of acacetin, tilianin, and rosmarinic acid in different organs of *A. rugosa.* The values and error bars represent the mean and standard error of 3 independent measurements, respectively.

flowers, leaves, stems, and roots of *A. rugosa* (Figure 5). Accumulation of acacetin was the highest in the flower, where its concentration was 0.84 μg/g of dry weight. The concentration of acacetin was very low in the leaves (0.06 μg/g) and was undetectable in the stems and roots. Moreover, the stems and roots also contained only a miniscule amount of tilianin, viz., 0.49 μg/g and 0.14 μg/g, respectively. However, tilianin levels in the leaves and flowers were markedly higher than acacetin levels. Tilianin content in the leaves was 2.18 μg/g, while an appreciable content, 6.33 μg/g, was found in the flowers. The accumulation patterns of acacetin and tilianin

correlated with the expression patterns of *ArCHS* and *ArCHI*, as shown in Figure 4.

Compared to acacetin and tilianin, rosmarinic acid was more abundantly synthesized in *A. rugosa.* Specifically, levels of rosmarinic acid were the highest in the flowers (48.43 μg/g), significant in the roots (30.97 μg/g) and leaves (22.14 μg/g), and the lowest in the stems (9.14 μg/g).

Although tilianin and rosmarinic acid have been detected and studied for a long time in *A. rugosa,* there are no reports on their biosyntheses in this plant. In the present study, CHS and CHI, which relate to the formation of tilianin, were first cloned

in *A. rugosa*. Transcription of genes involved in tilianin and rosmarinic acid biosynthetic pathways (*ArPAL*, *ArC4H*, *Ar4CL*, *ArCHS*, and *ArCHI*) were analyzed in different organs of *A. rugosa*. They were expressed constitutively with the highest levels in the flowers or leaves and the lowest levels in the roots.

The expression of PAL, C4H, and 4CL were previously found to correlate with the flavonoid contents in various plants.[27−29] The high transcription levels of *ArPAL*, *ArC4H*, and *Ar4CL* may explain the high levels of the flavonoid, acacetin, and its derivative, tilianin, in the flowers and leaves of *A. rugosa* (Figures 4 and 5). Similarly, the low expression levels of *ArPAL*, *ArC4H*, and *Ar4CL* may be responsible for the trace amounts of acacetin and tilianin detected in the roots.

The pattern of expression of *ArCHS* and *ArCHI* matched the accumulation patterns of acacetin and tilianin in different organs of *A. rugosa* conspicuously. CHS and CHI catalyze the first and the second committed steps in the phenylpropanoid pathway of plants, leading to acacetin, tilianin, and other flavonoids. Earlier studies found that *CHS* and *CHI* are the rate-limiting enzymes of the flavonoid biosynthetic pathway in some plants.[30−32] This indicates that *ArCHS* and *ArCHI* may regulate the biosyntheses of acacetin and tilianin as well as other flavonoids in *A. rugosa*.

Three enzymes of the general phenylpropanoid pathway (PAL, C4H, and 4CL) have been proven to be important for rosmarinic acid biosynthesis in some plants. In *Salvia miltiorrhiza*, PAL was demonstrated to be a key enzyme in the rosmarinic acid biosynthetic pathway; the fluctuation of rosmarinic acid content directly correlated with *PAL* expression.[33] Moreover, the downregulation of *PAL* affected the expression of *C4H* and *4CL* and caused a reduction in rosmarinic acid content in *S. miltiorrhiza*.[34] The expression patterns of *PAL* and *4CL* correlated with the enzyme activities and with the rosmarinic acid content of *Melissa officinalis* in a suspension culture.[35] In *A. rugosa*, 3 enzymes of the general phenylpropanoid pathway had the highest expression in the leaves (*ArPAL* and *Ar4CL*) or flowers (*ArC4H*), which also contained a relatively high content of rosmarinic acid. However, the roots showed a significant content of rosmarinic acid, although the transcription levels of *ArPAL*, *ArC4H*, and *Ar4CL* were low. These results contrast with previous results found in *S. miltiorrhiza*, in which the expression of *PAL* was abundant in the roots, which had a low content of rosmarinic acid.[33] This suggests that the biosynthesis of rosmarinic acid in plants is controlled by a complex mechanism.

The total concentration of tilianin was considerably higher than that of acacetin in *A. rugosa*. This is in accordance with a previous study in which tilianin was the major flavonoid in *A. rugosa*.[4] Furthermore, tilianin and acacetin were distributed mostly in the flowers and leaves, the organs of maximum interest in *A. rugosa* plants. The high amounts of tilianin and acacetin in flowers and leaves would result from a combination of endogenous biosynthesis, on one hand, and transport from the stems and roots, where only small amounts were detected, on the other. Rosmarinic acid, which is a potentially useful medicinal compound, was found abundantly in the flowers (48.43 μg/g) and roots (30.97 μg/g) of *A. rugosa*. This indicates that *A. rugosa* might be a valuable medicinal plant.

In conclusion, the sequences of CHS and CHI, together with the tilianin and rosmarinic acid analyses, may broaden our understanding of the molecular mechanisms involved in their biosynthetic pathways occurring in *A. rugosa*. Moreover, our

study also indicates targets for increasing the production of tilianin and rosmarinic acid in *A. rugosa*.

ASSOCIATED CONTENT

Supporting Information
Two figures showing phylogenic tree of ArCHS and ArCHI and some of their homologues. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
*Department of Crop Science, College of Agriculture & Life Sciences, Chungnam National University, 220 Gung-dong, Yuseong-gu, Daejeon, 305-764, Korea. Phone: +82-42-821-5730. Fax: +82-42-822-2631. E-mail: supark@cnu.ac.kr.

Author Contributions
‡These authors contributed equally to the paper.

Notes
The authors declare no competing financial interest.

ABBREVIATIONS USED
DEPC, diethylpyrocarbonate; HPLC, high-performance liquid chromatography; RACE, rapid amplification of cDNA ends; PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumaryl-CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; FS, flavone synthase; A4OMT, apigenin 4′-*O*-methyltransferase; GT, glucosyltransferase; RAS, hydroxycinnamoyl-CoA:hydroxyphenyllactate hydroxycinnamoyl transferase; 3-H, 3-hydroxycinnamoyl; 3′-H, 3′-hydroxycinnamoyl

REFERENCES
(1) Ahn, B.; Yang, C. B. Volatile flavor components of Bangah herb. *Korean J. Food Sci. Technol.* **1991**, *23*, 582−586.
(2) Kim, H. K.; Lee, H. K.; Shin, C. G.; Huh, H. HIV integrase inhibitory activity of *Agastache rugosa*. *Arch. Pharm. Res.* **1999**, *22*, 520−523.
(3) Shin, S. Essential oil compounds from *Agastache rugosa* as antifungal agents against Trichophyton species. *Arch. Pharm. Res.* **2004**, *27*, 295−299.
(4) Hong, J.-J.; Choi, J.-H.; Oh, S.-R.; Lee, H.-K.; Park, J.-H.; Lee, K.-Y.; Kim, J.-J.; Jeong, T.-S.; Oh, G. T. Inhibition of cytokine-induced vascular cell adhesion molecule-1 expression; possible mechanism for anti-atherogenic effect of *Agastache rugosa*. *FEBS Lett.* **2001**, *495*, 142−147.
(5) Lee, C.; Kim, H.; Kho, Y. Agastinol and agastenol, novel lignans from *Agastache rugosa* and their evaluation in an apoptosis inhibition assay. *J. Nat. Prod.* **2002**, *65*, 414−416.
(6) Choi, K. S.; Lee, H. Y. Characteristics of useful components in the leaves of baechohyang (*Agastache rugosa*, O. Kuntze). *J. Korean Soc. Food Sci. Nutr.* **1999**, *28*, 326−332.
(7) Lee, H.-K.; Oh, S.-R.; Kim, J.-I.; Kim, J.-W.; Lee, C.-O. Agastaquinone, a new cytotoxic diterpenoid quinone from *Agastache rugosa*. *J. Nat. Prod.* **1995**, *58*, 1718−1721.
(8) Han, D. S. Triterpenes from the root of *Agastache rugosa*. *Korean J. Pharmacogn.* **1987**, *18*, 50−53.
(9) Oh, H. M.; Kang, Y. J.; Lee, Y. S.; Park, M. K.; Kim, S. H.; Kim, H. J.; Seo, H. G.; Lee, J. H.; Chang, K. C. Protein kinase G-dependent heme oxygenase-1 induction by *Agastache rugosa* leaf extract protects

RAW264.7 cells from hydrogen peroxide-induced injury. *J. Ethnopharmacol.* **2006**, *103*, 229−235.

(10) Nam, K. H.; Choi, J. H.; Seo, Y. J.; Lee, Y. M.; Won, Y. S.; Lee, M. R.; Lee, M. N.; Park, J. G.; Kim, Y. M.; Kim, H. C.; Lee, C. H.; Lee, H. K.; Oh, S. R.; Oh, G. T. Inhibitory effects of tilianin on the expression of inducible nitric oxide synthase in low density lipoprotein receptor deficiency mice. *Exp. Mol. Med.* **2006**, *38*, 445−452.

(11) Hernández-Abreu, O.; Castillo-España, P.; León-Rivera, I.; Ibarra-Barajas, M.; Villalobos-Molina, R.; González-Christen, J.; Vergara-Galicia, J.; Estrada-Soto, S. Antihypertensive and vasorelaxant effects of tilianin isolated from *Agastache mexicana* are mediated by NO/cGMP pathway and potassium channel opening. *Biochem. Pharmacol.* **2009**, *78*, 54−61.

(12) Petersen, M.; Simmonds, M. S. J. Rosmarinic acid. *Phytochemistry* **2003**, *62*, 121−125.

(13) Swarup, V.; Ghosh, J.; Ghosh, S.; Saxena, A.; Basu, A. Antiviral and anti-inflammatory effects of rosmarinic acid in an experimental murine model of Japanese encephalitis. *Antimicrob. Agents Chemother.* **2007**, *51*, 3367−3370.

(14) Gao, L. P.; Wei, H. L.; Zhao, H. S.; Xiao, S. Y.; Zheng, R. L. Antiapoptotic and antioxidant effects of rosmarinic acid in astrocytes. *Pharmazie* **2005**, *60*, 62−65.

(15) Vogt, T. Phenylpropanoid biosynthesis. *Mol. Plant* **2010**, *3*, 2−20.

(16) Jiang, C.; Schommer, C. K.; Kim, S. Y.; Suh, D.-Y. Cloning and characterization of chalcone synthase from the moss, Physcomitrella patens. *Phytochemistry* **2006**, *67*, 2531−2540.

(17) Li, F.; Jin, Z.; Qu, W.; Zhao, D.; Ma, F. Cloning of a cDNA encoding the *Saussurea medusa* chalcone isomerase and its expression in transgenic tobacco. *Plant Physiol. Biochem.* **2006**, *44*, 455−461.

(18) Martens, S.; Mithöfer, A. Flavones and flavone synthases. *Phytochemistry* **2005**, *66*, 2399−2407.

(19) Kuroki, G.; Poulton, J. E. The para-O-methylation of apigenin to acacetin by cell-free extracts of *Robinia pseudoacacia* L. *Z. Naturforsch., C: Biosci.* **1981**, *36*, 916−920.

(20) Ogata, J.; Itoh, Y.; Ishida, M.; Yoshida, H.; Y., O. Cloning and heterologous expression of cDNAs encoding flavonoid glucosyltransferases from *Dianthus caryophyllus*. *Plant Biotechnol.* **2004**, *21*, 367−375.

(21) Petersen, M. S. Characterization of rosmarinic acid synthase from cell cultures of *Coleus blumei*. *Phytochemistry* **1991**, *30*, 2877−2881.

(22) Petersen, M. Cytochrome P450-dependent hydroxylation in the biosynthesis of rosmarinic acid in *Coleus*. *Phytochemistry* **1997**, *45*, 1165−1172.

(23) Xu, H.; Park, N. I.; Li, X.; Kim, Y. K.; Lee, S. Y.; Park, S. U. Molecular cloning and characterization of phenylalanine ammonia-lyase, cinnamate 4-hydroxylase and genes involved in flavone biosynthesis in *Scutellaria baicalensis*. *Bioresour. Technol.* **2010**, *101*, 9715−9722.

(24) Ferrer, J.-L.; Jez, J. M.; Bowman, M. E.; Dixon, R. A.; Noel, J. P. Structure of chalcone synthase and the molecular basis of plant polyketide biosynthesis. *Nat. Struct. Mol. Biol.* **1999**, *6*, 775−784.

(25) Jez, J. M.; Bowman, M. E.; Dixon, R. A.; Noel, J. P. Structure and mechanism of the evolutionarily unique plant enzyme chalcone isomerase. *Nat. Struct. Mol. Biol.* **2000**, *7*, 786−791.

(26) Gensheimer, M.; Mushegian, A. Chalcone isomerase family and fold: No longer unique to plants. *Protein Sci.* **2004**, *13*, 540−544.

(27) Xu, F.; Cai, R.; Cheng, S.; Du, H.; Wang, Y; Cheng, S. Molecular cloning, characterization and expression of phenylalanine ammonia-lyase gene from *Ginkgo biloba*. *Afr. J. Biotechnol.* **2008**, *7*, 721−729.

(28) Singh, K.; Kumar, S.; Rani, A.; Gulati, A.; Ahuja, P. *Phenylalanine ammonia-lyase* (PAL) and *cinnamate 4-hydroxylase* (C4H) and catechins (flavan-3-ols) accumulation in tea. *Funct. Integr. Genomics* **2009**, *9*, 125−134.

(29) Rani, A.; Singh, K.; Sood, P.; Kumar, S.; Ahuja, P. *p-Coumarate:CoA ligase* as a key gene in the yield of catechins in tea [*Camellia sinensis* (L.) O. Kuntze]. *Funct. Integr. Genomics* **2009**, *9*, 271−275.

(30) Knogge, W.; Schmelzer, E.; Weissenböck, G. The role of chalcone synthase in the regulation of flavonoid biosynthesis in developing oat primary leaves. *Arch. Biochem. Biophys.* **1986**, *250*, 364−372.

(31) Li, F.-X.; Jin, Z.-P.; Zhao, D.-X.; Cheng, L.-Q.; Fu, C.-X.; Ma, F. Overexpression of the *Saussurea medusa* chalcone isomerase gene in *S. involucrata* hairy root cultures enhances their biosynthesis of apigenin. *Phytochemistry* **2006**, *67*, 553−560.

(32) Muir, S. R.; Collins, G. J.; Robinson, S.; Hughes, S.; Bovy, A.; Vos, C. H. R. D.; Tunen, A. J. v.; Verhoeyen, M. E. Overexpression of petunia chalcone isomerase in tomato results in fruit containing increased levels of flavonols. *Nat. Biotechnol.* **2001**, *19*, 470−474.

(33) Hu, Y.-S.; Zhang, L.; Di, P.; Chen, W.-S. Cloning and induction of phenylalanine ammonia-lyase gene from *Salvia miltiorrhiza* and its effect on hydrophilic phenolic acids levels. *Chin. J. Nat. Med.* **2009**, *7*, 449−457.

(34) Song, J.; Wang, Z. RNAi-mediated suppression of the phenylalanine ammonia-lyase gene in *Salvia miltiorrhiza* causes abnormal phenotypes and a reduction in rosmarinic acid biosynthesis. *J. Plant Res.* **2011**, *124*, 183−192.

(35) Weitzel, C.; Petersen, M. Enzymes of phenylpropanoid metabolism in the important medicinal plant *Melissa officinalis* L. *Planta* **2010**, *232*, 731−742.

5951

dx.doi.org/10.1021/jf300833m | *J. Agric. Food Chem.* **2012**, *60*, 5945−5951