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Article

S-Adenosylhomocysteine Analogue of a Fairy Chemical, Imidazole-4-carboxamide, as its Metabolite in Rice and Yeast and Synthetic Investigations of Related Compounds

Hitoshi Ouchi, Takuya Namiki, Kenji Iwamoto, Nobuo Matsuzaki, Makoto Inai, Mihaya Kotajima, Jing Wu, Jae-Hoon Choi, Yoko Kimura, Hirofumi Hirai, Xiaonan Xie, Hirokazu Kawagishi,* and Toshiyuki Kan*

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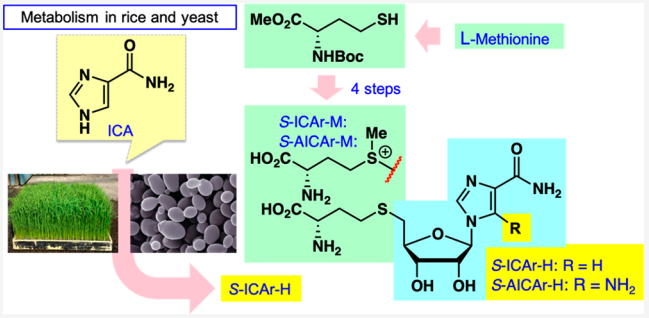
ABSTRACT: During the course of our investigations of fairy chemicals (FCs), we found *S*-ICAr-H (**8a**), as a metabolite of imidazole-4-carboxamide (ICA) in rice and yeast (*Saccharomyces cerevisiae*). In order to determine its absolute configuration, an efficient synthetic method of **8a** was developed. This synthetic strategy was applicable to the preparation of analogues of **8a** that might be biologically very important, such as *S*-ICAr-M (**9**), *S*-AICAr-H (**10**), and *S*-AICAr-M (**11**).



Fairy rings are zones of promoted or inhibited grass growth due to an interaction between a fungus and a plant.[1] This phenomenon had been caused by unknown "fairies" before our chemical discoveries. We disclosed that the fairies, the two plant-growth regulators produced by a fairy-ring forming fungus *Lepista sordida*, are 2-azahypoxanthine (**1**: AHX) and imidazole-4-carboxamide (**2**: ICA).[2] Furthermore, 2-aza-8-oxohypoxanthine (**3**: AOH) was isolated as a metabolite of AHX common in plants, and the compound also stimulated plant growth.[3] The three compounds were named fairy chemicals (FCs) after the title of the article in *Nature* that introduced our study.[4] FCs exhibited growth regulatory activity against all the plants tested, regardless of the families they belong to, and provided tolerance to various stresses to plants.[5] In addition, yields of rice, wheat, and other crops were increased by FCs treatment, suggesting that FCs can be applied to agriculture.[5,8] We have also proven the endogenous existence of FCs in plants and mushrooms and discovered a new route in the purine metabolic pathway through which FCs are biosynthesized from 5-aminoimidazole-4-carboxamide (**4**: AICA).[3,5−7]

We hypothesized that FCs are a new family of plant hormones, and the clarification of the metabolic pathways of FCs is important to substantiate the hypothesis.[7,9−11] A riboside of ICA (ICAr, **5**) and a glucoside of **5** (**6**) have been isolated from ICA-treated rice, and endogenous existence of **5** and a ribotide of ICA (**7**) in rice has been proved (Figure 1).[12] The metabolites showed no or weak activity against plant growth, suggesting that the interconversion between FCs and their glycosides regulated the homeostasis of rice. In the course of our continuing search for metabolite(s) of **2** from rice, we found a new metabolite **8a**. Herein, we describe the isolation of the compound, determination of its absolute configuration by chemical synthesis, and synthetic development of its related compounds.

RESULTS AND DISCUSSION

First of all, we investigated whether ICA (**2**) was converted to further metabolites or not. Rice seedlings were treated with **2**, and their roots were extracted with EtOH and acetone, successively. The solutions were combined and dried under reduced pressure. The extracts were analyzed by high-performance liquid chromatography (HPLC), and an unknown peak having a UV absorption pattern similar to those of **2**, **5**, **6**, and **7** was detected (Figure S1). Therefore, we tried to isolate the unidentified metabolite, and the extracts were fractionated by octadecylsilyl (ODS) gel flash column chromatography, followed by HPLC, giving the target metabolite **8a** (Figure 2).

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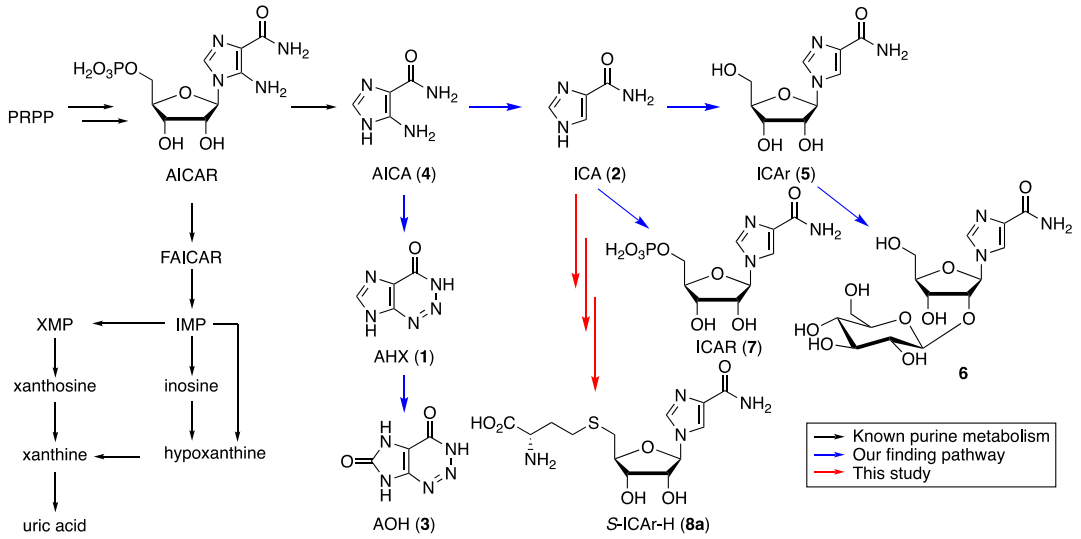

Figure 1. Metabolic pathway of purine in plants, animals, and microorganisms.

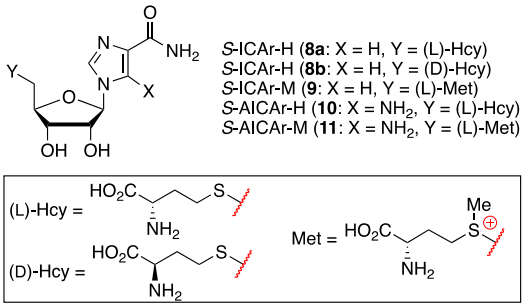

Figure 2. Structures of *S*-ICAr-H (**8a**) and its derivatives.

Compound **8a** was purified as a pale yellow, amorphous material. The molecular formula was determined as $C_{13}H_{20}N_4O_6S$ by high-resolution electrospray ionisation mass spectrometry (HRESIMS), indicating the presence of six degrees of unsaturation in the molecule. Its structure was elucidated by the interpretation of nuclear magnetic resonance (NMR) spectra including distortionless enhancement by polarization transfer (DEPT), double quantum filtered correlation spectroscopy (DQF-COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond coherence (HMBC).

The DEPT experiment indicated the presence of three methylenes, seven methines, and three nonprotonated carbons. The ^{13}C NMR data (δ_C 34.1, 72.8, 75.8, 84.8, 91.8, 121.4, 131.3, 136.9, 163.0) indicated that **8a** had the same skeleton as that of ICAr.[12] The ribose part (C-1′−C-5′) was constructed by the DQF-COSY correlations (H-1′/H-2′; H-2′/H-3′, 4′; H-3′/H-4′; H-4′/H-5′) and the HMBC correlations (H-1′/C-2′; H-2′/C-1′, C-4′; H-3′/C-1′, C-5′; H-4′/C-3′; H-5′/C-3′, C-4′) (Figure 3). The linkage position between the sugar and **2** was determined from the cross-peaks (H-1′/C-2 and H-1′/C-5) (Figure 3). This compound possessed four more carbons than ICAr. The moiety constructed with the additional four carbons, homocysteine (C-1″−C-4″), was suggested by the characteristic ^{13}C NMR chemical shifts at δ_C 28.1, 30.5, 53.1, and 173.1, the DQF-COSY correlations (H-1″/H-2″; H-2″/H-3″; H-3″/H-4″), and the HMBC correlations (H-1″/C-2″, C-3″; H-2″/C-1″, C-3″, C-4″; H-3″/C-1″, C-2″, C-4″) (Figure 3). The molecular formula of the compound and its HMBC

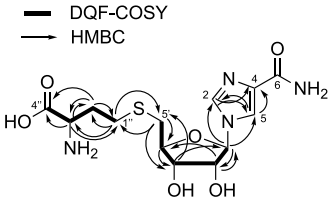

Figure 3. DQF-COSY and HMBC correlations of *S*-ICAr-H (**8a**).

cross-peaks (H-5′/C-1″; H-1″/C-5′) indicated that the homocysteine was connected to the ribose at C-5′ *via* a thioether bond. The complete assignment of all the proton and carbon signals of NMR was accomplished as shown in Table 1.

Table 1. ^{1}H and ^{13}C NMR Data for 8a

	8a (in D_2O)	
	^{13}C	^{1}H
position	δ_C, type	δ_H, mult (J in Hz)
2	136.9, CH	8.60, br.s
4	131.3, C	
5	121.4, CH	8.01, br.s
6	163.0, C	
1′	91.8, CH	5.78, d (4.6)
2′	75.8, CH	4.37, dd (4.6, 4.6)
3′	72.8, CH	4.16, dd (4.6, 5.2)
4′	84.8, CH	4.23, m
5′a	34.1, CH_2	2.90, dd (5.2, 14.3)
5′b		2.82, dd (6.4, 14.3)
1″	28.1, CH_2	2.63, dd (7.5, 7.5)
2″a	30.5, CH_2	2.11, m
2″b		2.04, m
3″	53.1, CH	3.93, dd (6.3, 6.3)
4″	173.1, C	

Compound **8a**, described as *S*-ICAr-H, was a new compound; however, its absolute configuration could not be determined by the spectroscopic data. Furthermore, very interestingly, we found that yeast (*Saccharomyces cerevisiae*) also produced **8a** by treatment with **2** (Figure S6). Therefore, we tried to clarify the absolute configuration of the compound by chemical synthesis.

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We deduced that the ribose in **8a** was D, because all the known naturally occurring ribosides have D-ribose. In order to determine the absolute configuration of the homocysteine part in **8a**, we decided to condense **5** and each of the optical isomers of homocysteine derivative **14**. However, both of the enantiomers of homocysteine are very expensive for the synthetic starting material and we needed a more practical way to synthesize the target compound.[13] Then, we came up with an idea to use both enantiomers of methionine that are much less expensive (L-form: $ 21/25 g and D-form: $ 84/25 g; TCI) for synthesis of the corresponding homocysteine.

After the selective removal of the methyl group of L-methionine (**12**) by Birch condition, without purification of the produced homocysteine, oxidative dimerization with hydrogen peroxide proceeded smoothly to provide the stable homocystine.[13] In order to accomplish the efficient C−S bond formation of **8a**,[14,15a] the protection of the carboxy and amino groups by methyl esterification and incorporation of a Boc group provided **13**. The cleavage of the disulfide bond of **13** was performed by treatment with Zn in the presence of acetic acid to give **14**, which was subjected to the coupling without purification. On the contrary, ICAr part **16** was synthesized from protected ICAr (**15**), which was readily synthesized from inosine by mesylation and iodine displacement.[15b] Upon the treatment of **14** and **16** with triethylamine in DMF, the desired condensation proceeded smoothly to afford **17**. Subsequently, the stepwise deprotection of **17** was carried out by the removal of the TBS ether and Boc groups and the hydrolysis of the methyl ester to give **8a**. Furthermore, employing the same sequence for the synthesis of **8a**, the synthesis of its diastereomer **8b** was also accomplished using D-methionine. A remarkable difference of the retention times of **8a** and **8b** was observed in HPLC analysis by using a chiral-phase column, CROWNPAK CR (+) (Figure S8). As a result, the isolated compound was determined to be **8a**.

The plant growth regulatory activities of **8a** and **8b** were examined using rice. Both diastereomers inhibited the shoot growth only at a high concentration (0.1 mM) like **2**, while they did not show any activity against the root unlike **2** (Figure S9).

The structure of **8a** is similar to that of *S*-adenosylhomocysteine (SAH). SAH is derived from *S*-adenosylmethionine (SAM) in many living organisms (Figure 4). SAM is a key

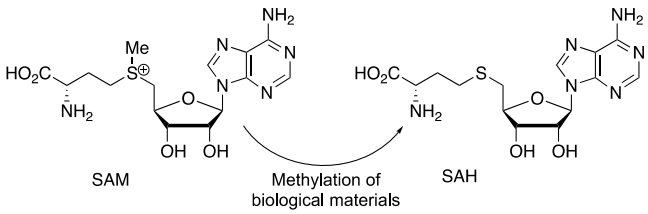
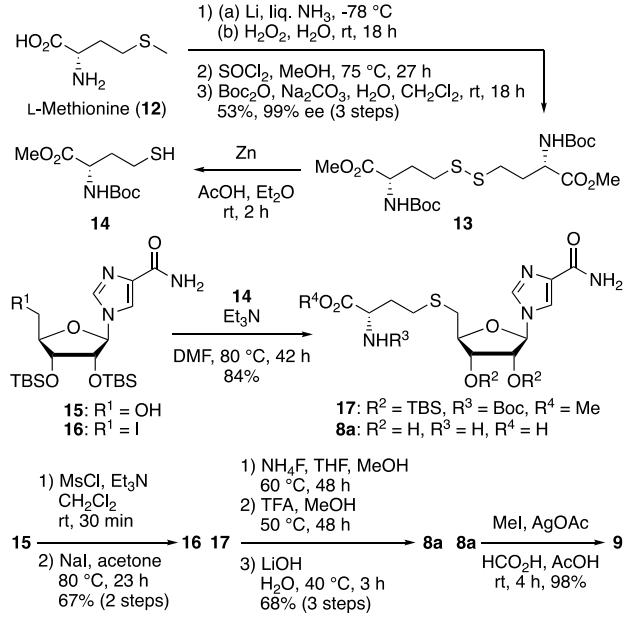


Figure 4. Structures of SAM and SAH.

compound for living organisms, because the compound serves as the methyl group donor for various biological materials such as DNA, peptide, choline, creatinine, and many other small molecules, with SAH as the byproduct. SAM is also a biosynthetic precursor of polyamines, spermidine and spermine, and a gaseous plant hormone, ethylene.[16,17] In addition, SAH is a strong inhibitor of several methyltransferases.[18] Therefore, new analogues of SAH and SAM derivatives should be expected to be significant compounds for the biological investigations.

We thought that it was highly possible that the analogue of SAH, **8a**, was generated from the analogue of SAM, *S*-ICAr-M (**9**), in plants, and therefore, we synthesized the analogue. Upon the treatment of **8a** with methyl iodide and AgOAc in HCO_2H and CH_3CO_2H, the methylation reaction proceeded smoothly to give **9**. Because the alkyl sulfide derivative possessed a labile nature, the purification of **9** by general methods was not possible. Therefore, the purification of the compound was performed according to the reported procedure[19] for the preparation of similar *S*-dimethyladenosyl methionine; briefly, 0.1% TFA in H_2O was added to the reaction mixture and resulting precipitates were removed by filtration. After washing the filtrate with Et_2O and removing the organic solvent under reduced pressure at room temperature to avoid degradation, the purification of **9** was performed by HPLC of the resulting aqueous solution (Scheme 1).

Scheme 1. Synthesis of *S*-ICAr-H (8a) and *S*-ICAr-M (9)



Next, we turned our attention to *S*-AICAr-H (**10**), as AICAr (**18**: acadesine) is known as a metabolite in the purine metabolic pathway like FCs[21] and its aglycon, AICA (**4**), is converted to ICA (**2**) in plants, and **10** has been found in the plasma and urine of homocystinurics patients.[20] However, the detailed spectroscopic data of **10** as well as its chemical synthesis have not been reported. We expected that the employment of a similar procedure for the preparation of **8a** would readily provide the desired **10** and the methionine analogue of **10**, *S*-AICAr-M (**11**). After protection of all the hydroxy groups of **18** as TBS ethers and selective hydrolysis by acidic conditions, the resultant primary alcohol was converted to the corresponding iodide derivative **19** by treatment with PPh_3 and I_2. Upon the treatment of **14** and **19** with triethylamine in DMF, the desired coupling reaction proceeded smoothly to give **20**. After the deprotection of the TBS ethers of **20**, the deprotection of the Boc group and the hydrolysis of the methyl ester provided the desired *S*-AICAr-H (**10**). Additionally, the treatment of **10**, similar to that for the

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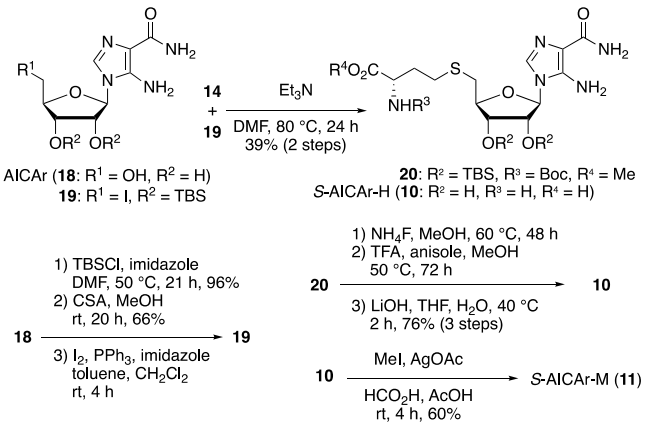
conversion of **8a** to **9**, afforded **11** without providing any *N*-alkyl derivatives (Scheme 2).

Scheme 2. Synthesis of *S*-AICAr-H (10) and *S*-AICAr-M (11)



As mentioned above, SAM is a methyl group donor in living organisms. The methylation is catalyzed by various methyl transferases. The ability of the synthesized SAM analogues, *S*-ICAr-M (**9**) and *S*-AICAr-M (**11**), as substrates of one of the enzymes, caffeic acid 3-*O*-methyltransferase, was compared with that of SAM. The transferase of rice was heterologously expressed in *Escherichia coli*, and caffeic acid was used as the acceptor. In the presence of SAM, caffeic acid was completely methylated by the enzyme to form ferulic acid. Compounds **9** and **11** also served as methyl donors; however, their conversion rates of caffeic acid to ferulic acid was much lower than that of SAM: **9**, 60% and **11**, 49% compared to SAM, 100% (Figure S10).

In summary, we discovered a unique metabolite of ICA, *S*-ICAr-H (**8a**), in rice and yeast and developed an efficient synthetic method for **8a**. The absolute configuration of **8a** was determined by comparison with synthetic epimers **8a** and **8b**. Furthermore, our synthetic strategy was applicable to the preparation of *S*-ICAr-M (**9**), *S*-AICAr-H (**10**), and *S*-AICAr-M (**11**). Our new synthesized compounds possess potential as chemical biology tools for the investigation of extremely important biomolecules, SAM and SAH. Some of the synthesized compounds might exist in nature in addition to **8a**, and we are now trying to confirm their endogenous existence.

EXPERIMENTAL SECTION

General Experimental Procedures for Isolation. The specific rotation values were measured by a Jasco DIP-1000 polarimeter, and infrared (IR) spectra were recorded on a Jasco FT/IR-4100 spectrometer. ^{1}H NMR spectra were recorded on a JEOL ECZ-500R spectrometer at 500 MHz, while ^{13}C NMR spectra were recorded on the same instrument at 125 MHz. The HRESIMS spectra were measured on a JMS-T100LC mass spectrometer. The HPLC separations were performed with a Jasco Gulliver system using reverse-phase HPLC columns (Develosil C30-UG-5, Nomura chemical and Cosmosil 5PYE waters Nacalai Tesque). An ODS gel (Cosmosil 140 C18-OPN, Nacalai Tesque) was used for flash column chromatography. All solvents used throughout the experiments were obtained from Kanto Chemical Co.

General Experimental Procedures for Synthesis. Optical rotations were measured on a JASCO P-2200 polarimeter. Infrared (IR) spectra were recorded on a SHIMADZU IRPrestige-21. NMR [^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz)] spectra were determined on a JEOL ECZ-500R instrument. Chemical shifts for ^{1}H NMR were reported in ppm relative to the singlet at 7.26 ppm for deuteriochloroform, the broad singlet at 3.30 ppm for deuteriomethanol, or the singlet at 4.65 ppm for deuterium oxide. Chemical shifts for ^{13}C NMR were reported in parts per million relative to the centerline of a triplet at 77.0 ppm for deuteriochloroform or the centerline of a septet at 49.0 ppm for deuteriomethanol. High-resolution mass spectra (HRMS) were obtained on a Bruker Daltonics micrOTOF (ESI). Analytical thin layer chromatography (TLC) was performed on Merck precoated analytical plates, 0.25 mm thick, silica gel 60 F_{254}. Preparative TLC separations were made on 7 cm × 20 cm plates prepared with a 0.25 mm layer of Merck silica gel 60 F_{254}. Compounds were eluted from the adsorbent with 10% MeOH in $CHCl_3$. Silica gel column chromatography separations were performed on Chromatorex PSQ 60B or PSQ 100B purchased from Fuji Silysia Chemical Ltd. HPLC analyses and separations were performed with a SHIMADZU HPLC Prominence system. Reagents and solvents were of commercial grade and were used as supplied with the following exceptions: (1) CH_2Cl_2, dimethylformamide, tetrahydrofuran, and toluene were dried over molecular sieves 4A; (2) MeOH was dried over molecular sieves 3A. All reactions sensitive to oxygen and/or moisture were conducted under an argon atmosphere.

Detection of ICA (2) and its Metabolite 8a by HPLC. All plants were divided into two parts: the shoot and root. The shoot and root were extracted with EtOH. After removing the solvent of each extracts under reduced pressure, they were dissolved in 5% MeOH and subjected to HPLC analysis. The extracts were analyzed by reversed-phase HPLC using a Develosil C30-UG-5 column (ϕ 4.6 mm × 250 mm, 5 μm) with a gradient manner (5% MeOH (B) in (A) for 30 min, 5%−100% B in A for 45 min, and 100% B for 30 min) at a flow rate of 0.5 mL/min at a multiple wavelength using a photodiode array detector[12] (Figure S1, Supporting Information).

Purification of 8a from ICA-Treated Rice. Rice seeds were germinated in nursery boxes, incubated for a week, and then treated with ICA (0.5 mM) in a growth chamber under a 16 h photoperiod at 28 °C for 2 weeks. The treated seedlings were divided into two parts, shoot and root. The root (1.9 kg) was extracted with EtOH and then acetone. After the solution was combined and concentrated under reduced pressure, the concentrate was extracted with CH_2Cl_2 to remove lipophilic substances and dried under reduced pressure. The residue (14.6 g) was fractionated by ODS gel flash column chromatography (Cosmosil 140 C18-OPN, Nacalai Tesque, Japan; 5%, 10%, 25%, 50% MeOH, MeOH) to obtain five fractions. Fraction 2 (263.5 mg) was further separated by HPLC with a Develosil C30-UG-5 column (ϕ 20 mm × 250 mm, 5 μm; flow rate 5 mL/min; injection volume 1 mL; 5% MeOH; UV detection 210 nm) to obtain 21 fractions (fraction 2-1 to 2-21). Fraction 2-10 was separated by HPLC with Cosmosil 5PYE (ϕ 10 mm × 250 mm, 5 μm; flow rate 2 mL/min; injection volume 0.5 mL; 2% MeOH; UV detection 210 nm) to afford **8a** (2.0 mg). **8a**: pale yellow amorphous. $[\alpha]_D^{25}$ −31 (*c* 0.31, H_2O). IR (film): 3342, 664 cm^{-1}. ^{1}H and ^{13}C NMR data: Table 1. ESIMS *m*/*z* 359 $[M - H]^-$. HRESIMS *m*/*z* 383.1022 $[M + Na]^+$ (calcd for $C_{13}H_{20}N_4NaO_6S$, 383.1001).

Yeast (*Saccharomyces cerevisiae*). *Saccharomyces cerevisiae* strains (W303) were precultured in YPAD medium [1% yeast extract, 2% peptone, 2% glucose, and 150 μM adenine] for 12 h (25 °C, 100 rpm). Then, 10 μL of the culture was diluted in 70 mL of YPAD medium. The yeast strains were cultured until the OD_{600} reached 0.5, and the cells obtained by centrifugation (10 000*g*, 5 min) were washed with distilled water. The cells were diluted with SC medium [0.5% ammonium sulfate, 2% glucose, 0.5% casamino acids, 0.17% yeast nitrogen, 0.0006% tryptophan, 0.01% uracil, and 150 μM adenine/ICA] so that the OD_{600} at the start of culture was 0.2 and were cultured for 5 days. The cells collected by centrifugation (10 000*g*, 5 min) were extracted and fractionated according to previous report.[5]

Identification and Quantification of 8a. The identification and quantification of *S*-ICAr-H (**8a**) from the ICA-treated samples was completed using a triple linear ion trap instrument (LIT)

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(QTRAP5500; AB Sciex) with an electrospray ionization (ESI) source and coupled to a UHPLC system (Nexera X2; Shimadzu) in MRM (Multiple Reaction Monitoring) analysis mood. MS/MS spectra were recorded in product ion scan mode using LIT. The MRM analysis was performed at 25 V collision energy, and the transitions of m/z 361 > 134 and 361 > 88 were monitored for *S*-ICAr-H in the ESI positive ion mode. The data acquisition and analysis were performed with the Multi Quant software (ver. 3.0.1). The quantification of *S*-ICAr-H was conducted using synthetic standards. The exudate samples were dissolved in 50% aqueous MeOH and filtered through a spin column (PVDF 0.45 μm, Millipore). An aliquot of the filtered 50% aqueous MeOH sample solution was diluted with a volume of either pure 50% MeOH or 50% MeOH containing known amounts of *S*-ICAr-H standard. The increase in peak area on the chromatogram corresponded to the amounts of *S*-ICAr-H standards added, enabling the amount of **8a** in the samples to be deduced (Figure S6).

Bioassay. Rice seeds (*Oryza sativa* L. cv. Nipponbare) were sterilized in EtOH for 5 min and then 1% sodium hypochlorite for 30 min on a plastic container (18.5 × 14.5 × 4.5 cm^3). The seeds were washed completely in sterile H_2O and germinated for 2 days at 30 °C with intensive light. The germinated seeds (n = 4) were planted onto test tube (ϕ 5.5 cm × 10 cm) containing samples (1, 10, and 100 μM) and incubated for a week at 30 °C with intensive light. The lengths of the root and shoot were measured to an accuracy of 0.01 mm with an absolute digimatic caliper (Mitutoyo Co.) (Figure S9).

Expression of Recombinant OsCOMT1. RNA was isolated from root of rice (*Oryza sativa* L. cv. Nipponbare) using an RNeasy Plant Mini Kit (QIAGEN), according to the manufacturer's protocol. The cDNA was synthesized with the PrimeScript RT Reagent Kit (TaKaRa). The full-length cDNA from rice that corresponds to caffeic acid 3-*O*-methyltransferase (*COMT1*) was amplified with the following primers: 5′-GAG CTC GAA TTC GGA CTA CTT GGT GAA CTC GAT GGC-3′ (forward) and 5′-GCG GCA GCC ATA TAA TGG GTT CTA CAG CCG CC-3′ (reverse). The cDNA sequence ligated into the pET-28a (+) vector linearized by BamHI and Pvu II to yield pET-28a (+)-*COMT1* plasmid. For the expression of the COMT1 protein, *Escherichia coli* BL21-Codon Plus (DE3) was transformed with the pET-28a (+)-*COMT1* plasmid. Recombinant *E. coli* was cultured in lysogeny broth (LB) broth containing 20 mg/L kanamycin sulfate at 37 °C overnight. Four milliliters of the overnight culture was added to 500 mL of fresh LB containing 20 mg/L kanamycin sulfate, and it continued to incubate for 3−4 h and was shaken at frequency of 150 rpm at 37 °C. Upon the culture reaching an OD_{600} of 0.5, isopropyl-β-D-thiogalactopyranoside (IPTG) was added to the culture at a final concentration of 1 mM to induce protein expression and the culture was incubated for 3 h at 37 °C. The cells were harvested by centrifugation, resuspended in lysis buffer (20 mM Tris-HCl, 100 mM NaCl, 30 mM imidazole, pH 7.4), and sonicated on ice. After centrifugation, the supernatant was loaded onto a Histrap HP column (1 mL, GE Health Sciences) equilibrated with buffer A (20 mM Tris-HCl, 100 mM NaCl, 30 mM imidazole, pH 7.4) and the His-tagged protein was eluted with buffer B (20 mM Tris-HCl, 100 mM NaCl, 500 mM imidazole, pH 7.4). The purified enzyme concentration was determined using a Pierce 660 nm Protein Assay Reagent (Thermo Fisher Scientific).

Enzyme Assay. Enzymatic reactions with purified recombinant OsCOMT1 were carried out according to Koshiba et al.[22] *S*-adenosyl-L-methionine (SAM) and the analogues, *S*-ICAr-M and *S*-AICAr-M, were used as a methyl donor. The typical reaction mixture (100 μL) contained 50 mM Tris-HCl (pH 7.4), 200 μM caffeic acid (CA), 200 μM methyl donors, and 1 μM enzyme solution. After incubation for 2 h at 30 °C, the reaction was terminated by adding 50 μL of MeCN. Following centrifugation to remove protein, the reaction products were analyzed by a Shimadzu UPLC system (Shimadzu) coupled to an LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific) equipped with an electrospray ionization probe. An ACQUITY UPLC BEH C18 column (ϕ 2.1 mm × 50 mm, 1.7 μm, Waters) was used in the analysis (injection volume, 5 μL; solvent, 30% MeCN with 0.05% formic acid; flow rate; 0.2 mL/min). MS analysis was performed in the negative FTMS mode at a resolution of 30 000 at m/z 400 with the following source parameters: sheath gas flow, 50; auxiliary gas flow rate, 10; tube lens, −63 V; capillary voltage, −16 V; ion spray voltage, 3 kV (Figure S10).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c01269.

Figures of HPLC profiles, ^{1}H and ^{13}C NMR spectra, HMQC, HMBC, and COSY spectra, MRM chromatograms and product ion spectra, growth regulatory of ICA, and LC−MS/MS chromatograms and discussions of synthetic methods (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Hirokazu Kawagishi − *Graduate School of Integrated Science and Technology, Research Institute of Green Science and Technology, and Graduate School of Integrated Science and Technology, Shizuoka University, Shizuoka 422-8529, Japan*; orcid.org/0000-0001-5782-4981; Email: kawagishi.hirokazu@shizuoka.ac.jp

Toshiyuki Kan − *School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka 422-8526, Japan*; orcid.org/0000-0002-9709-6365; Email: kant@u-shizuoka-ken.ac.jp

Authors

Hitoshi Ouchi − *School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka 422-8526, Japan*

Takuya Namiki − *School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka 422-8526, Japan*

Kenji Iwamoto − *School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka 422-8526, Japan*

Nobuo Matsuzaki − *Graduate School of Integrated Science and Technology, Shizuoka University, Shizuoka 422-8529, Japan*

Makoto Inai − *School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka 422-8526, Japan*; orcid.org/0000-0001-7074-0520

Mihaya Kotajima − *Graduate School of Integrated Science and Technology, Shizuoka University, Shizuoka 422-8529, Japan*

Jing Wu − *Research Institute of Green Science and Technology, Shizuoka University, Shizuoka 422-8529, Japan*

Jae-Hoon Choi − *Graduate School of Integrated Science and Technology, Research Institute of Green Science and Technology, and Graduate School of Integrated Science and Technology, Shizuoka University, Shizuoka 422-8529, Japan*

Yoko Kimura − *Graduate School of Integrated Science and Technology, Shizuoka University, Shizuoka 422-8529, Japan*

Hirofumi Hirai − *Graduate School of Integrated Science and Technology, Research Institute of Green Science and Technology, and Graduate School of Integrated Science and Technology, Shizuoka University, Shizuoka 422-8529, Japan*

Xiaonan Xie − *Center for Bioscience Research and Education, Utsunomiya University, Utsunomiya, Tochigi 321-8505, Japan*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jnatprod.0c01269

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