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Discovery of Plant Growth Stimulants by C−H Arylation of 2-Azahypoxanthine

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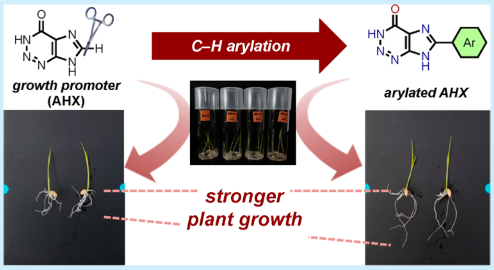
Supporting Information

ABSTRACT: A series of new AHX derivatives were synthesized by Pd-catalyzed C−H arylation. Their rice-growth-promoting activity was evaluated in vivo. Among the synthesized compounds, C8 phenyl-substituted AHX showed remarkable growth-promoting activity on rice. The present study shows the power and significant opportunity of C−H functionalization chemistry to rapidly transform biologically active natural products into more active compounds.



Fairy rings are naturally occurring ring-shaped thick growth or necrotic spots of grasses, which are seen on the lawn (Figure 1). Sometimes fruiting bodies of fungi are also seen in the fairy rings. The term "fairy rings" has its origin in the myths and superstitions associated with their occurrence in the Middle Ages. The first scientific article about this phenomenon was reported in 1675.[1] In 1995, Couch reported that this mysterious ring is due to the interaction between a fairy-ring-forming fungus and a plant.[2] More recently, we disclosed that molecular entities of this interaction are plant-growth-stimulating compound 2-azahypoxanthine (AHX) and plant-growth inhibitor imidazole-4-carboxamide (ICA) identified from one of the fairy-ring-forming fungi, *Lepista sordida*.[3] These compounds have been named "fairy chemicals" (FCs).[4] Regulation of plant growth by FCs was observed in all the plants tested, regardless of their species. Furthermore, treatment of FCs increased the yields of rice, wheat, and other various types of crops in greenhouse and/or field experiments.[3,5] We also reported that AHX and AOH are endogenously produced in plants by a new purine metabolic pathway.[3b,6] Based on the results above, we have hypothesized that FCs are a new family of plant hormone.[7]

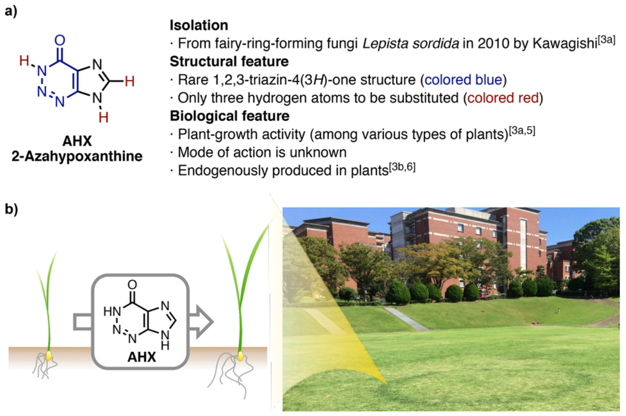


Figure 1. Fairy chemical AHX. (a) Structure and features of AHX. (b) Plant growth caused by AHX. Picture: fairy ring on the turfgrass field, University of Shizuoka, Japan. Reprinted with permission by Dr. Makoto Inai. Copyright Dr. Makoto Inai.

Although AHX has an interesting plant-growth activity, the mode of action of how AHX enhances plant growth has been poorly understood. Even the structure−activity relationship (SAR) of AHX derivatives has not yet been defined due to the difficulty in chemical derivatization of AHX. AHX has a 1,2,3-triazin-4(3*H*)-one structure that is rarely seen in natural products. There are only three hydrogen atoms to be substituted in AHX (Figure 1a). Initially, we synthesized *N,N*′-dialkyl AHX by a simple alkylation reaction and evaluated their plant-growth-promoting activity (Figure 2). However, most *N,N*′-dialkyl AHX did not show noticeable

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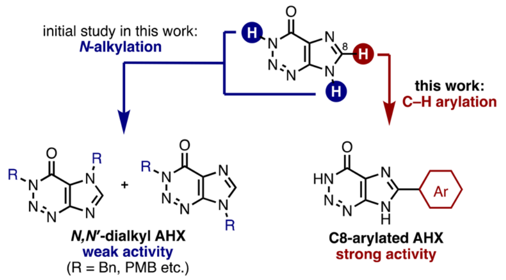
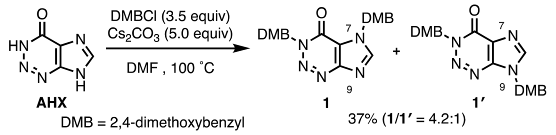


Figure 2. Brief summary of SAR of AHX derivatives.

growth-promoting activity (see Supporting Information (SI) for details). From these results, we decided to focus on the functionalization of the remaining C–H bonds of the imidazole ring. Despite several preliminary attempts at C–H functionalizations, such as halogenation and alkylation, the instability of the AHX skeleton and the lower reactivity at a C8 position disturbed the derivatization of AHX. Therefore, an alternative appropriate synthetic method was required for obtaining C8-substituted AHX derivatives. Herein, we report the application of C–H arylation chemistry to synthesize AHX derivatives and the evaluation of their plant-growth-promoting activity.[8] The easy access to these derivatives allowed us to rapidly discover a novel plant-growth stimulator that shows activity stronger than that of the original AHX (Figure 2). This compound might be a key for identifying the target of AHX.

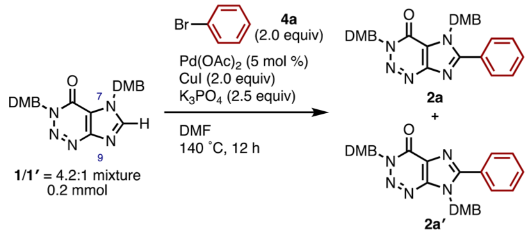
Although extensive screening of reaction conditions was conducted to synthesize arylated AHX in one step by metal-catalyzed C–H arylation,[9] none of the examined conditions was successful (see SI for details). Thus, we decided to use a protecting group for N–H moieties to accomplish the synthesis (Scheme 1). We chose 2,4-dimethoxybenzyl (DMB) as a protecting group because it can be deprotected easily under mild oxidative conditions. First, we protected the two nitrogen atoms of AHX by the DMB group. This protection reaction afforded a separable mixture of regioisomers **1** and **1′** in a ratio of 4.2:1. The structures of these isomers were identified by the heteronuclear multiple bond coherence and heteronuclear multiple quantum correlation (see SI for details). The low yield of **1**/**1′** is attributed to the lower stabilities of both protecting-group-free AHX and DMBCl.

Scheme 1. Protection of AHX with a DMB Group



Next, we examined the palladium-catalyzed C–H arylation of DMB-protected AHX (**1** and **1′**) with bromoarenes. Based on the C–H arylation reactions of related imidazole substrates reported by Miura[10] and Rossi,[9a] we identified reaction conditions for the coupling of DMB-protected AHX and bromoarenes using a Pd/Cu catalytic system. For example, treatment of DMB-protected AHX (**1**/**1′** = 4.2:1) with bromobenzene (2.0 equiv) in the presence of $Pd(OAc)_2$ (5 mol %), CuI (2.0 equiv), and K_3PO_4 (2.5 equiv) in DMF at 140 °C for 12 h afforded the expected C–H arylation products (**2a**/**2a′** = 1.4:1) in 50% yield (Table 1, entry 1). The ratio of DMB regioisomers changed during the reaction. The independent reactions using isomerically pure **1** and **1′** revealed that both isomers have almost the same reactivity, giving a mixture of **2a** and **2a′** (**1**: 56% yield (**2a**/**2a′** = 1.4:1), **1′**: 38% yield (**2a**/**2a′** = 1.2:1); see SI for details). These results suggest that migration of DMB group between N7 and N9 positions occurs during the reaction.

Table 1. Pd/Cu-Mediated C–H Phenylation of DMB-Protected AHX



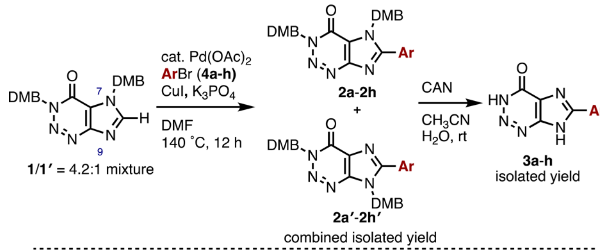
entry	deviation from standard conditions	NMR yield (%)[a]
1		50, **2a**/**2a′** = 1.4:1
2	$PCy_3·HBF_4$ (10 mol %) was added	52, **2a**/**2a′** = 1.4:1
3	$P^tBu_3·HBF_4$ (10 mol %) was added	49, **2a**/**2a′** = 1.1:1
4	BINAP (5 mol %) was added	43, **2a**/**2a′** = 1.4:1
5	Na_2CO_3 instead of K_3PO_4	46, **2a**/**2a′** = 1.3:1
6	K_2CO_3 instead of K_3PO_4	21, **2a**/**2a′** = 2.5:1
7	Cs_2CO_3 instead of K_3PO_4	44, **2a**/**2a′** = 1.9:1
8	DMA instead of DMF	43, **2a**/**2a′** = 1.7:1
9	1,4-dioxane instead of DMF	17, **2a**/**2a′** = 3.3:1
10	toluene instead of DMF	17, **2a**/**2a′** = 2.4:1
11	iodobenzene instead of **4a**	37, **2a**/**2a′** = 1.3:1
12	without CuI	1, **2a**
13	without $Pd(OAc)_2$	11, **2a**/**2a′** = 1.8:1
14	1.1 mmol scale, reaction time 15 h	71[b], **2a**/**2a′** = 2.6:1

[a] Determined by ^1H NMR using CH_2Br_2 as an internal standard. [b] Isolated yield.

Listed in Table 1 are the effects of reaction parameters in the Pd-catalyzed C–H arylation of DMB-protected AHX. The addition of ligands such as PCy_3, P^tBu_3, and BINAP had no beneficial effect on the yield of **2a**/**2a′** (43–52% yield; entries 2–4). The product yield depends on the base employed, increasing in the order of $K_2CO_3 < Cs_2CO_3 < Na_2CO_3 < K_3PO_4$ (entries 1, 5, 6, and 7). The polarity of solvents is also important. Whereas DMF and DMA resulted in moderate yields (50 and 43% yields; entries 1 and 8), the reactions in 1,4-dioxane and toluene showed significant decrease in yield (entries 9 and 10). The use of iodobenzene instead of bromobenzene resulted in the decrease of the yield of **2a**/**2a′** (37% yield; entry 11). Whereas the absence of CuI almost shut down the reaction (entry 12), the absence of $Pd(OAc)_2$ still gave the target material in 11% yield (entry 13), suggesting that copper-mediated (non-palladium-catalyzed) C–H arylation also occurs in this reaction. Notably, conducting the reaction in larger scale increased the yield (71% yield; entry 14).

Using the optimized conditions, we synthesized a range of arylated AHX derivatives via Pd-catalyzed arylation with bromoarenes **4a**–**h** (Scheme 2). The reactions with sterically hindered bromoarenes, such as 2-bromotoluene (**4b**) and 1-bromonaphthalene (**4f**), gave the corresponding products in good yields (92 and 78%). The reactions with bromobenzene (**4a**), 3-bromotoluene (**4c**), 4-bromotoluene (**4d**), 4-bromobiphenyl (**4e**), 1-bromo-2-fluorobenzene (**4g**), and 1-bromo-3-chlorobenzene (**4h**) resulted in moderate yields of C–H

Scheme 2. Synthesis of Arylated AHX 3 through C−H Arylation and Deprotection[a]



2a/2a′: Ar = Ph, 71%[b] (**2a/2a′** = 2.6:1)	**3a**: Ar = Ph, 42%
2b/2b′: Ar = 2-tolyl, 92% (**2b/2b′** = 4.2:1)	**3b**: Ar = 2-tolyl, 21%
2c/2c′: Ar = 3-tolyl, 65% (**2c/2c′** = 1.5:1)	**3c** Ar = 3-tolyl, 31%
2d/2d′: Ar = 4-tolyl, 69% (**2d/2d′** = 1.5:1)	**3d**: Ar = 4-tolyl, 18%
2e/2e′: Ar = 4-Ph-C_6H_4, 64% (**2e/2e′** = 1.6:1)	**3e**: Ar = 4-Ph-C_6H_4, 25%
2f/2f′: Ar = 1-naphthyl, 78% (**2f/2f′** = 2.8:1)	**3f**: Ar = 1-naphthyl, 8%
2g/2g′: Ar = 2-fluorophenyl, 56%[c] (**2g/2g′** = 1.9:1)	**3g**: Ar = 2-fluorophenyl, 25%[d]
2h/2h′: Ar = 3-chlorophenyl, 35%[c] (**2h/2h′** = 1.3:1)	**3h**: Ar = 3-chlorophenyl, 19%

[a]Reaction conditions for C−H arylation: Pd(OAc)$_2$ (5 mol %), ArBr (2.0 equiv), CuI (2.0 equiv), K_3PO_4 (2.5 equiv), DMF, 140 °C, 12 h. Reaction conditions for deprotection of DMB group: CAN (8.0 equiv), CH_3CN, H_2O, rt. [b]Reaction time was 15 h. [c]**1**/**1′** = 4.4:1. [d]Single isomer **2g** was used as a starting material. CAN = Ce(NH$_4$)$_2$(NO$_3$)$_6$. DMB = 2,4-dimethoxybenzyl.

arylation products (35−71%). These results suggest that the influence of the steric hindrance on the aryl group could affect the ratio of DMB regioisomers.

Next, we examined the deprotection of DMB groups from the C−H arylated AHX derivatives **2** (Scheme 2). Treatment of **2** with ceric ammonium nitrate led to the formation of the corresponding deprotected compounds (**3a**−**h**) in moderate to low yield (8−42%). In addition, we also conducted the deprotection reaction of isomerically pure **2a** and **2a′** independently. Both isomers gave the desired compound **3a** in similar yields (35 and 20%) (see SI for details).

With the newly synthesized compounds (**3a**−**h**) in hand, we investigated the SAR of AHX analogues and derivatives in terms of growth-promoting activity. The growth-promoting activity was investigated using rice (*Oryza sativa* L. *cv.* Nipponbare). The activities of reported AHX-related compounds (Figure 3a) showed good agreement with the previously reported results.[11] Xanthine (Xan), hypoxanthine (HX), imidazole (IMI), ethyl imidazole-4-carboxylate (EtC), and 5-aminoimidazole-4-carboxamide (AICA) did not show shoot or root-growth-regulating activity. ICA exhibited growth-inhibitory activity in both shoot and root, and AHX and 2-aza-8-oxohypoxanthine (AOH) showed growth-promoting activity in roots (Figure 3b).

The plant-growth-regulating activities of the arylated AHX derivatives **3**, synthesized in this study, are summarized in Figure 4 and Figure 5. To our delight, all the arylated AHXs tend to have a root-growth-promoting activity stronger than that of the original AHX. The introduction of a phenyl (**3a**), *o*-methylphenyl (**3b**), 4-biphenylyl (**3e**), 1-naphthyl (**3f**), or *m*-chlorophenyl (**3h**) moiety at the C8 position of AHX was found to be highly effective for the rice root growth activity. Substitutions on the phenyl group with the *m*-methyl (**3c**), *p*-methyl (**3d**), or *o*-fluoro (**3g**) group resulted in activity weaker than that of **3a**. Although further studies toward the mode of action of AHX is necessary, it is obvious that C−H functionalization chemistry[12] disclosed a novel chemical space of biological importance which is only accessible by synthetic chemistry.

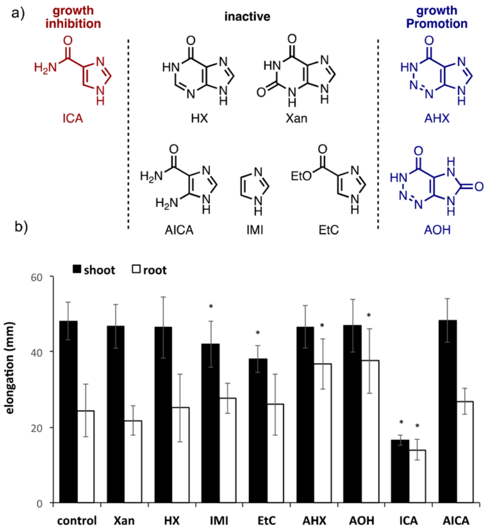


Figure 3. Plant-growth-regulating activities of AHX-related compounds. (a) Chemical structures of the reported AHX-related compounds. (b) Effect of Xan, HX, IMI, EtC, AHX, AOH, ICA, and AICA on the growth of rice. Germinated seeds were treated with 0.1 mM solution of Xan, HX, IMI, EtC, AHX, AOH, ICA, and AICA and incubated for a week in a test tube. Results are the mean ± standard deviation ($n = 12$). Asterisk indicates a value that is significantly different from that of the control (Student's *t*-test, $p < 0.05$).

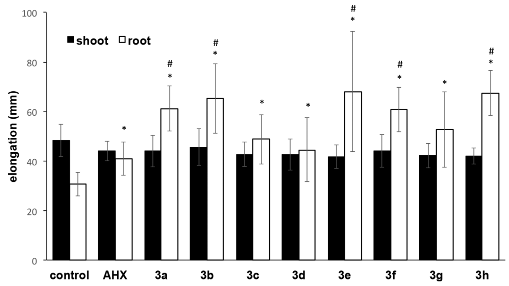


Figure 4. Effect of arylated AHXs **3a**−**h** on the growth of rice. Germinated seeds were treated with 0.1 mM solution of **3a**−**h** and incubated for a week in a test tube. Results are the mean ± standard deviation ($n = 12$). Asterisk indicates a value that is significantly different from that of the control (Student's *t*-test, $p < 0.05$). Number sign indicates a value that is significantly different from that of the AHX-treated group (Student's *t*-test, $p < 0.05$).

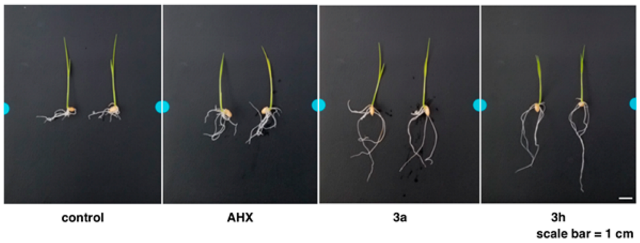


Figure 5. Images of rice seedlings after the growth-promotion assay. Germinated seeds were treated with 0.1 mM solution of compounds.

In summary, C−H arylation reaction of DMB-protected AHX made it possible to discover novel plant-growth-promoting compounds. Moreover, the newly synthesized arylated AHX derivatives showed growth-promoting activity stronger than that of the natural plant growth promoter AHX. These series of compounds have immense potential for

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applications in agriculture. Future identification of target(s) of AHX and its arylated derivatives may provide the molecular basis of their interesting plant-growth activity including fairy-ring formation. Moreover, the present work emphasizes that, with the recent advent of game-changing C−H functionalization chemistry,[12] there are significant opportunities to use these technologies to accelerate agricultural science.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b02407.

> Detailed experimental procedures for chemical synthesis and growth-promotion assay, spectral data for all compounds including scanned images of ^{1}H, ^{13}C NMR spectra (PDF)

Accession Codes

CCDC 1845158−1845159 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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