C–H arylation enables synthesis of imidazole-4-carboxamide (ICA) based fairy chemicals with plant growth-promoting activity

C–H arylation enables synthesis of imidazole-4-carboxamide (ICA) based fairy chemicals with plant growth promoting activity

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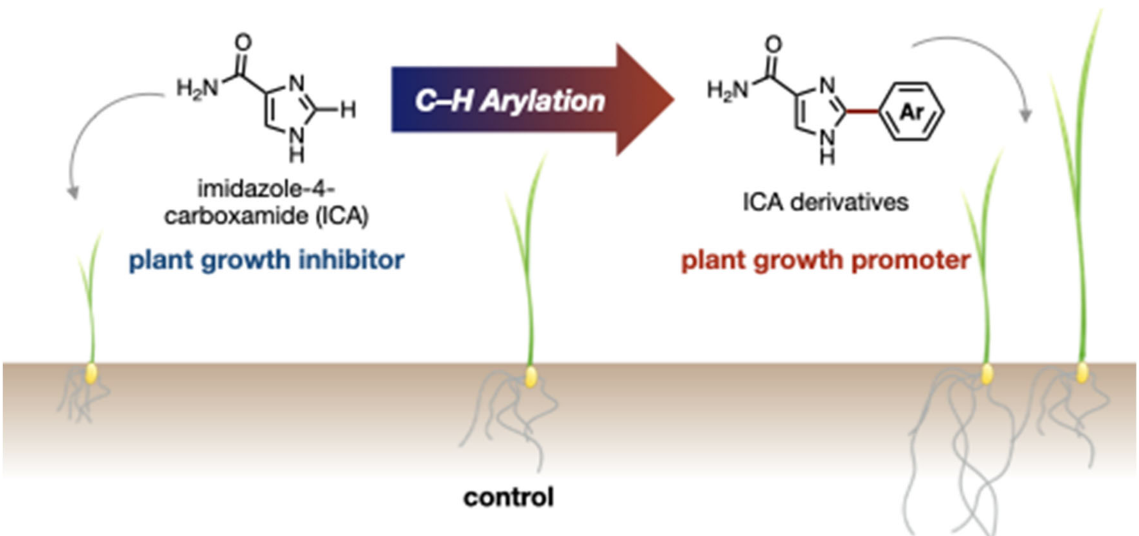

Abstract

Imidazole-4-carboxamide (ICA), which is one of a group of “fairy chemicals” (FCs) that cause the fairy ring phenomena, has plant growth inhibitory activity. FCs have the potential as candidates for a new family of plant hormones as they have been found endogenously in all plant species tested, and show growth-regulating activity against the plants. While basic research on FCs is progressing, they are also expected to be applied not only to agrochemicals but also as pharmaceuticals. Derivatization of one of FCs, 2-azahypoxanthine (AHX) and the structure-activity relationship (SAR) studies have clarified its activity as a plant growth promoter. Yet, imidazole-4-carboxamide (ICA) has not been derivatized at all and SAR regarding its activity remains unknown. In this study, we synthesized the derivatives of ICA by direct C–H arylation of ICA precursors and evaluated its activity in rice. The 12 total compounds including the arylated ICAs and their precursors were evaluated for root and shoot elongation in rice, resulting in the discovery that a number of compounds unexpectedly have an elongation activity in the root and shoot.

Keywords

C–H arylation, fairy chemicals, plant growth activity, imidazole-4-carboxamide

“Fairy rings” is a phenomenon in which ring-shaped thick growths or necrotic spots of grasses occur. Since the first publication of a scientific paper on fairy rings in 1675,[1] various reports on their causes have been published. However, no definitive conclusion on the cause of fairy rings had been reached until recently. In 2010, Kawagishi found that 2-azahypoxanthine (AHX) and imidazole-4-carboxamide (ICA), which were isolated from the fungus *Lepista sordida* that forms fairy rings, have growth-promoting and growth-inhibitory activities, respectively, resulting in the fairy ring formation.[2,3] In addition, it was found that AHX is metabolized to 2-aza-8-oxohypoxanthine (AOH), which has growth-promoting activity.[4,5] These compounds involved in the formation of fairy rings were named “fairy chemicals (FCs)”.[6,7,8]

Interestingly, FCs have been found not only in fungi but also in plants such as rice, Arabidopsis and potato.[4,9] These compounds naturally occur in plants,[4,10,11] and are shown to have activity at low concentrations,[2,3] attracting much attention as a candidate for a new family of plant hormones.[12] Plant hormones have historically contributed to agriculture, and FCs are no exception whereby AHX and ICA were shown to increase yields of wheat and rice.[13,14] More interestingly, it was recently discovered that FCs can be utilized as mammalian drugs as well as agrochemicals. For example, AHX inhibits hypoxia-inducible factor (HIF) activity, resulting in the inhibition of retinal angiogenesis in oxygen-induced retinopathy mice.[15] ICA suppresses the expression of immune checkpoints such as PD-L1 and PD-L2, improving the reactivity of cisplatin against cancer in mouse.[16] AOH activates human epidermal cells to promote the expression of genes related with cell adhesion, barrier function of skin, protease of stratum corneum differentiation, epidermal differentiation, and hyaluronan synthase 3 (HAS3).[17]

Due to interesting bioactivities of FCs beyond the formation of fairy rings, the construction of a FC-based chemical library has become increasingly important not only for acquiring highly active/selective molecules but also for elucidation of their modes of action. In particular, the elucidation of their physiological actions and targets is crucial to prove FCs as plant hormones. Identification of their target proteins using affinity probes is one of the approaches to understand their physiological actions. In 2014, we synthesized biotin-conjugated AHXs for target identification.[18] On the other hand, to develop more active FCs, we discovered a palladium-catalyzed direct C–H arylation of plant-growth promoter AHX.[19] The arylated AHXs showed a stronger growth-promoting activity than that of original AHX. To explore and deepen knowledge of FCs such as undeveloped ICA, we herein describe the derivatization of plant-growth inhibitor ICA by a C–H arylation and the effect of the thus-synthesized ICA derivatives on rice root and shoot growth (Figure 1).

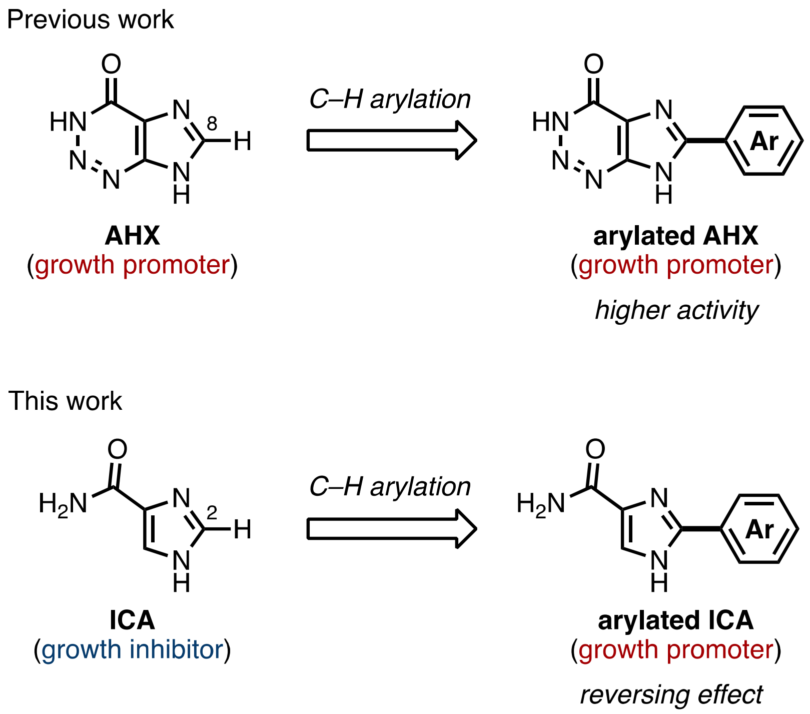

Figure 1. Synthesis of arylated fairy chemicals via C–H arylation

Following the previous report on the derivatization of AHX, we first investigated direct C–H arylation of the commercially available ethyl imidazole-4-carboxylate (**1**), an ICA precursor. As **1** has two C–H bonds on the imidazole ring, namely at the C2 and C5 positions, site selective reaction is demanded. In addition, the N–H bonds are generally needed to be protected due to their reactivity, but in terms of step economy, protection and deprotection steps should be avoided. Among the related reports on direct C–H arylation of imidazoles at the C2 position using transition metal catalysts such as palladium,[20,21,22,23] nickel,[24] copper,[25,26,27,28] and rhodium,[29,30,31,32] we found that the conditions reported by Bellina and Rossi,[22] were satisfactory for the C2-selective arylation of *N*-unprotected imidazole-4-carboxylate **1**. When **1** was reacted with iodobenzene in the presence of palladium acetate (5 mol%) and copper iodide (2.0 equiv) in DMF at 140 °C for 24 h, the C2-arylated product **2a** was obtained in 70% yield (Figure 2). The structure of **2a** was determined by X-ray crystallography.

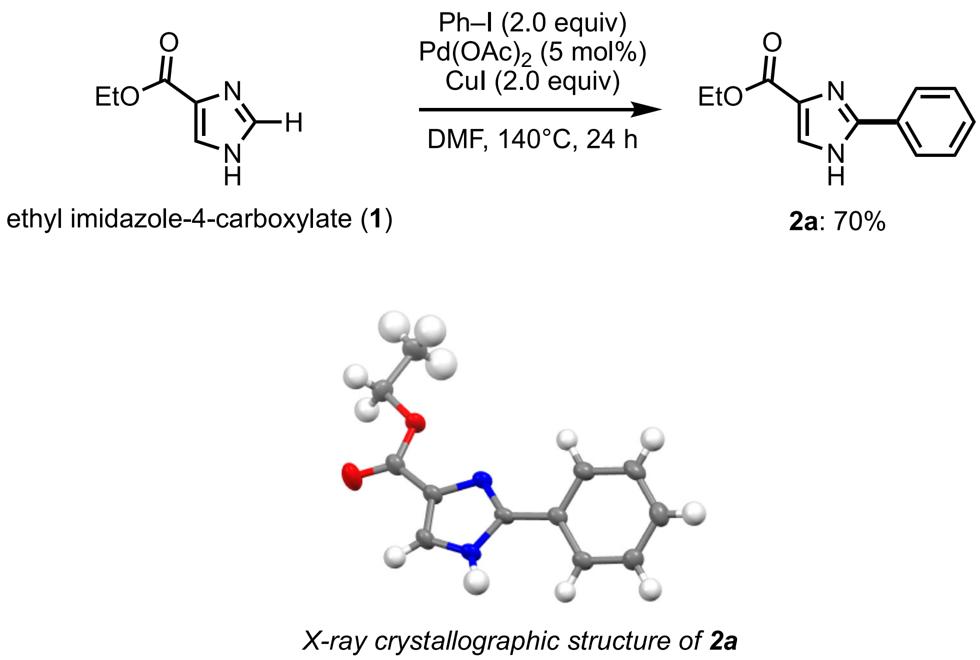

Figure 2. Palladium-catalyzed C–H arylation of an ICA precursor **1** at C2-position

Having established the optimized C–H arylation conditions for **1**, we next synthesized a range of arylated imidazole-4-carboxylates **2** via Pd-catalyzed arylation with iodoarenes (Figure 3). The reaction with *p*- and *m*-tolyl iodides gave the corresponding products **2b** and **2c** in 44% and 56% yields, respectively. With regard to the substituents at the *p*-position of the phenyl group, compounds with electron-deficient moieties such as trifluoromethyl, chloro, and acetyl groups were synthesized (**2d**–**2f**). The thus-obtained arylated products **2** were reacted with aqueous ammonia to obtain 2-arylated ICAs **3a**–**3f** in moderate to high yields (Figure 3).

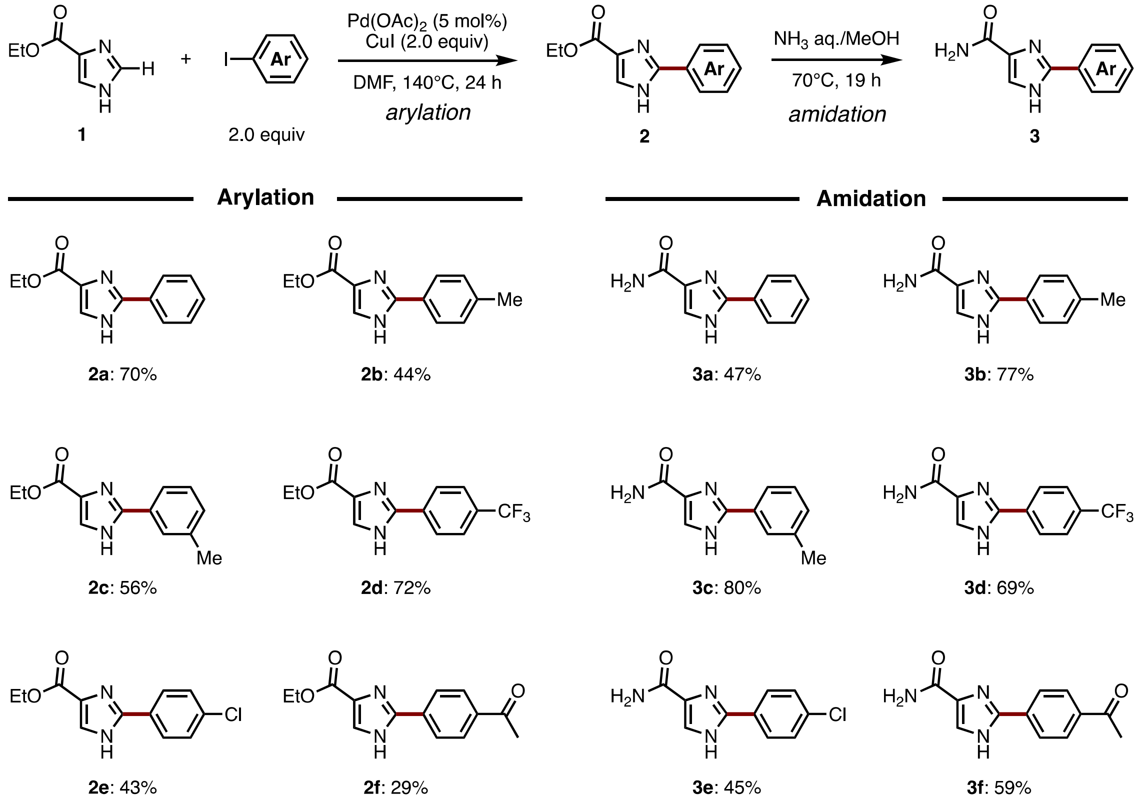

Figure 3. Synthesis of ICA derivatives **3a**–**3f** via C–H arylation of **1** and amidation of **2**.

Thanks to the power of C–H arylation methodology, a relatively small library of ICA derivatives was rapidly constructed from the readily available imidazole-4-carboxylate **1**, which allowed us to explore the previously untapped biological activity of ICAs. In this paper, we report the preliminary study on the bioactivity of ICA derivatives **2** and **3** in rice (Figure 4). Rice seeds were germinated and incubated on agar medium containing 100 μM of compound for 7 days, under long day conditions, after which the lengths of shoot and root were measured. It was found that ICA derivatives **2** as well as non-arylated **1** had no effect on root and shoot length in comparison to controls. Although the parent ICA showed growth inhibition activity in both terms of shoot and root length, all arylated compounds **3** did not. Surprisingly, unlike ICA, 2-phenyl ICA **3a** showed growth promoting activity in the shoot. Other arylated ICAs had no activity, indicating that substitution on 2-phenyl ring was restricted in regards to root/shoot length inhibition. In addition, *p*-trifluoromethyl phenyl ICA **3d** had root lengthening activity. By comparing the results of **3b** and **3d** having the similar structure yet opposite activity, fluorine atoms play an essential role for the activity switching.

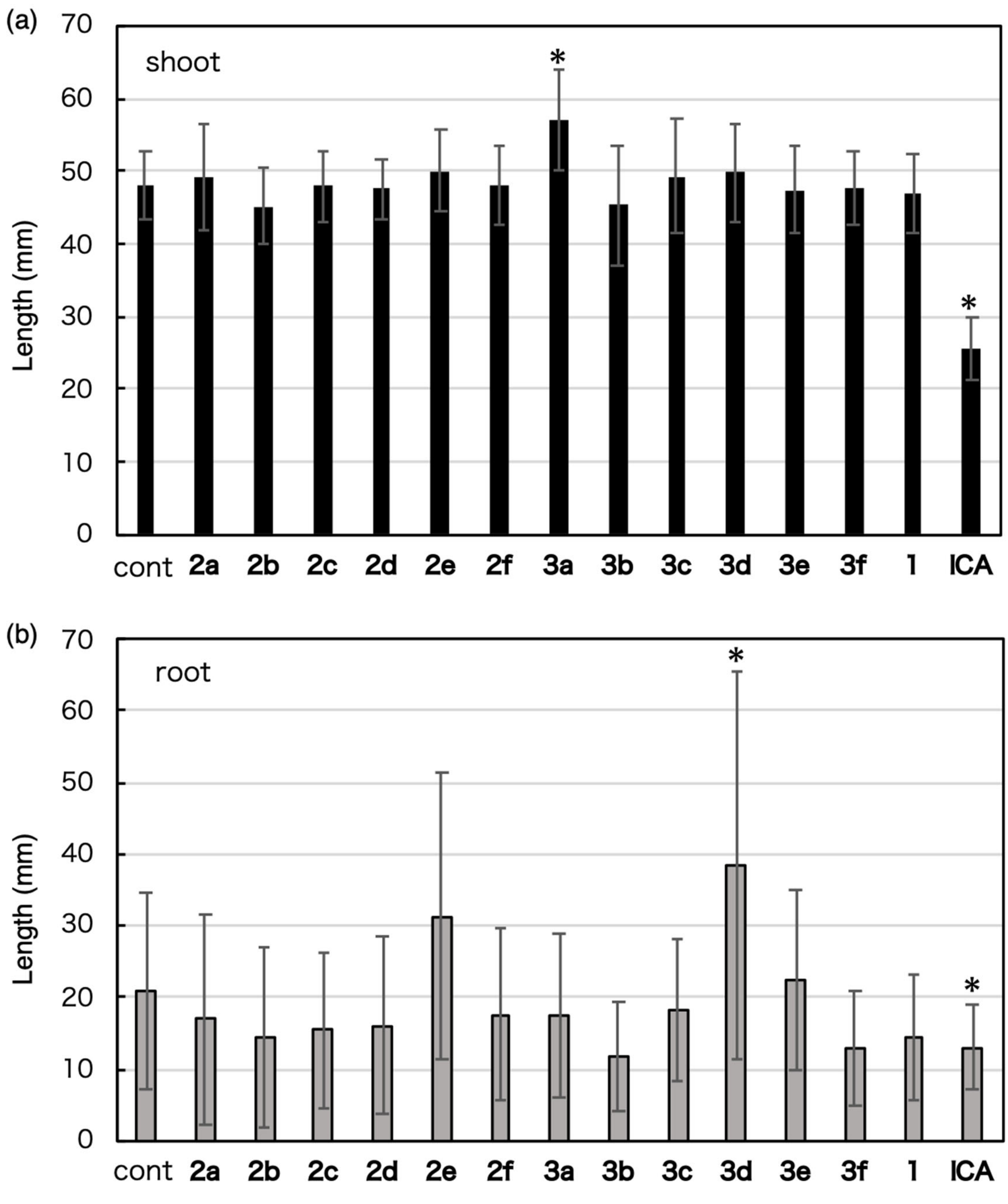

Figure 4. Evaluation on the effect of ICA derivatives in (a) shoot and (b) root growth. Germinated seeds were treated with 100 µM solution of the compounds. Results are the mean ± standard deviation (n = 16–24). Asterisk indicates a value that is significantly different from the control (Student's t-test, $p<0.05$).

In summary, a palladium-catalyzed C2-selective direct C–H arylation reaction of imidazole-4-carboxylate, an ICA precursor, has been developed. The ICA derivatives were rapidly obtained by amidation of the arylated ICA precursors. A total of 12 compounds including the arylated ICAs and their precursors were evaluated for root and shoot elongation in rice, resulting in the discovery that **3a** and **3d** unexpectedly have elongation activity in the root and shoot, respectively, in contrast to ICA which has an inhibitory effect. In the future, we will continue investigating the mechanisms of action in detail among others with the goal to determine why **3a** and **3d** have such differing activity to ICA. We also hope to do further SAR studies on ICA to determine its relationship on activity. These derivative molecules will be starting points in order to uncover the biological mechanisms of fairy chemicals and develop their use as a new plant hormone family.

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Notes

The authors declare no competing financial interest.

Data availability

Materials and methods, experimental procedures, and NMR spectra are available in the Supplementary Information or from the corresponding authors upon request. Crystallographic data generated during this study are available in the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures Service, www.ccdc.cam.ac.uk/structures, under deposition numbers 2209426.

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