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The Source of "Fairy Rings": 2-Azahypoxanthine and its Metabolite Found in a Novel Purine Metabolic Pathway in Plants**

*Jae-Hoon Choi, Toshiyuki Ohnishi, Yasuhiro Yamakawa, Shogo Takeda, Shuhei Sekiguchi, Waki Maruyama, Kimiko Yamashita, Tomohiro Suzuki, Akio Morita, Takashi Ikka, Reiko Motohashi, Yoshikazu Kiriiwa, Hiroyuki Tobina, Tatsuo Asai, Shinji Tokuyama, Hirofumi Hirai, Nobuhiro Yasuda, Keiichi Noguchi, Tomohiro Asakawa, Shimpei Sugiyama, Toshiyuki Kan, and Hirokazu Kawagishi**

Abstract: ■■*Revised title ok?*■■*Rings or arcs of fungus-stimulated plant growth occur worldwide; these are commonly referred to as "fairy rings". In 2010, we discovered 2-azahypoxanthine (AHX), a compound responsible for the fairy-ring phenomenon caused by fungus; AHX stimulated the growth of all the plants tested. Herein, we reveal the isolation and structure determination of a common metabolite of AHX in plants, 2-aza-8-oxohypoxanthine (AOH). AHX is chemically synthesized from 5-aminoimidazole-4-carboxamide (AICA), and AHX can be converted into AOH by xanthine oxidase. AICA is one of the members of the purine metabolic pathway in animals, plants, and microorganisms. However, further metabolism of AICA remains elusive. Based on these results and facts, we hypothesized that plants themselves produce AHX and AOH through a pathway similar to the chemical synthesis. Herein, we demonstrate the existence of endogenous AHX and AOH and a novel purine pathway to produce them in plants.*

"Fairy rings" are zones of stimulated grass growth owing to the interaction between a fungus and a plant.[1] Since the first scientific article about fairy rings in 1675, and subsequent studies reviewed in Nature in 1884, this phenomenon had been attributed to an unknown "fairy".[2]

In a previous report, we disclosed that this fairy is in fact 2-azahypoxanthine (AHX; **1**), a plant-growth stimulator produced by one of fairy-ring-forming fungi, *Lepista sordida*. This compound exhibits growth-regulating activity towards not only turf grass, but also towards other plants tested, regardless of their families.[3] Furthermore, this compound increased seed yields of rice and wheat in pot experiments, suggesting the possibility of its practical use in agriculture.[3] A combination of cDNA microarray, RT-PCR, and bioassays for **1** indicate that the effects of this compound on plants are independent of known plant hormones, and that it acts alone as though it is a phytohormone.[3] Compound **1** is chemically synthesized from 5-aminoimidazole-4-carboxamide (AICA; **3**);[4] **3** reacts with $NaNO_2$ to form 4-diazo-4*H*-imidazole-5-carboxamide (**4**), and treatment of **4** with NH_3, or incubating **4** in water, produces **1**. The precursor **3**, and its ribotide (AICAR; **7**) are common members of the purine metabolic pathway in animals, plants, and microorganisms, and **7** is an intermediate of IMP, inosine, hypoxanthine, **9**, and **10** in the pathway (Figure 1). However, the metabolism of **3** remains elusive.

From our findings and the facts mentioned above, we formed the hypothesis that plants themselves produce **1** through a pathway similar to the chemical synthesis. Herein, we report the finding of a novel metabolite of **1**, the existence of endogenous **1** and the metabolite, and the discovery of their biosynthetic pathway in plants.

It was first found that **1** converted into a metabolite immediately after it was absorbed into rice (Figure 2a). In order to obtain this metabolite, the extracts of seedlings treated with **1** were fractionated by silica gel flash column chromatography followed by HPLC, leading to the purifica-

[*] Dr. J.-H. Choi, Dr. T. Ohnishi, K. Yamashita, Dr. T. Suzuki, Prof. Dr. H. Kawagishi
Research Institute of Green Science and Technology, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529 (Japan)
E-mail: achkawa@ipc.shizuoka.ac.jp
Y. Yamakawa, S. Takeda, S. Sekiguchi, W. Maruyama, Prof. Dr. A. Morita, Dr. T. Ikka, Dr. R. Motohashi, Dr. Y. Kiriiwa, Dr. S. Tokuyama, Dr. H. Hirai
Graduate School of Agriculture, Shizuoka University
836 Ohya, Suruga-ku, Shizuoka 422-8529 (Japan)
Dr. H. Tobina, T. Asai
Center for Education and Research in Field Sciences, Faculty of Agriculture, Shizuoka University, 63 Kariyado, Fujieda 426-0001 (Japan)
Dr. N. Yasuda
Japan Synchrotron Radiation Research Institute
Spring-8,1-1-1 Kouto, Sayo-cho, Sayo-gun, Hyogo 679-5198 (Japan)
Dr. K. Noguchi
Tokyo University of Agriculture and Technology
Koganei, Tokyo 184-8588 (Japan)
Dr. T. Asakawa, Dr. S. Sugiyama, Prof. Dr. T. Kan
Tokyo University of Agriculture and Technology
Koganei, Tokyo 184-8588 (Japan)

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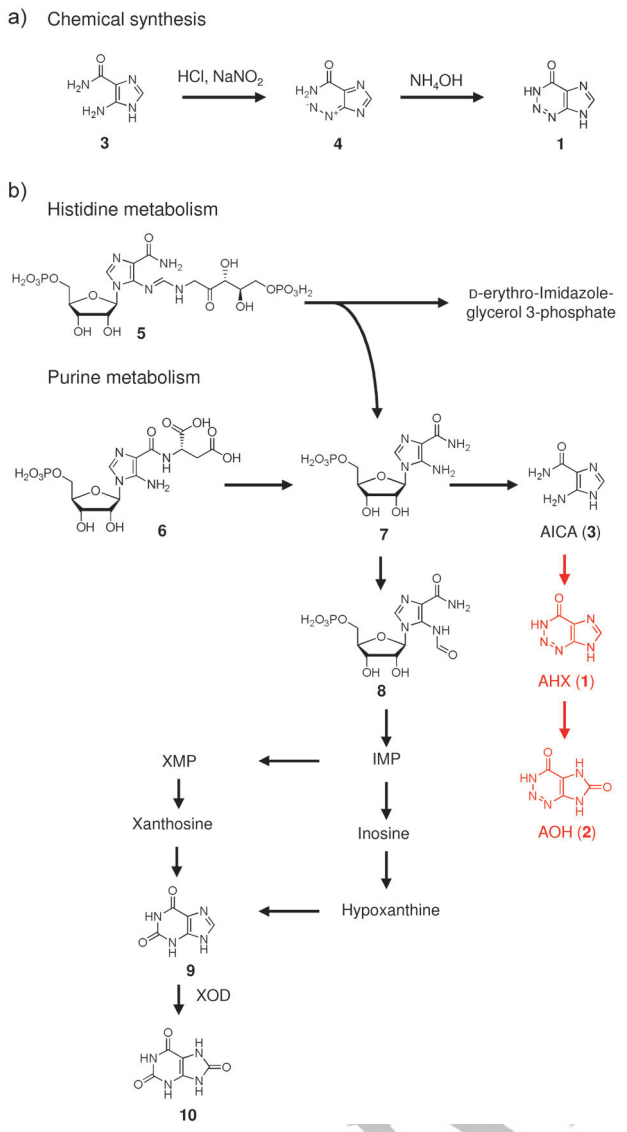

Figure 1. a) Synthetic route from AICA (**3**) to AHX (**1**). b) Purine metabolic pathway in animals, plants and microorganisms, including the novel route. The route in black in (b) was adapted from the KEGG (Kyoto Encyclopedia of Genes and Genomes). New metabolites and route are indicated in red. **1** = 2-azahypoxanthine, **2** = 2-aza-8-oxohypoxanthine, **3** = 5-aminoimidazole-4-carboxamide, **4** = 4-diazo-4*H*-imidazole-5-carboxamide, **5** = phosphoribulosylformimino-AICAR-phosphate, **6** = succino-AICAR, **7** = AICA ribotide, **8** = 5-formyl AICAR, **9** = xanthine, **10** = uric acid, IMP = inosine monophosphate, XOD = xanthine oxidase, XMP = xanthine monophosphate.

tion of a novel compound, 2-aza-8-oxohypoxanthine (AOH; **2**), as the metabolite, the structure of which was determined by X-ray crystal analysis (Figure 2b,c; see also Figure S1 in the Supporting Information). This conversion from **1** into **2** was also observed in *Arabidopsis*, tomato, and turfgrass (Figure S2), and reminded us of the reaction of xanthine oxidase (XOD) to produce **10** from **9** (Figure 1).

Therefore, **1** was treated with commercially available XOD, and **2** was quantitatively obtained (Figure S3). Compound **2** elongated the seedlings of bentgrass and rice in a fashion similar to **1** (Figures S4 and S5). In addition, **1** and **2**

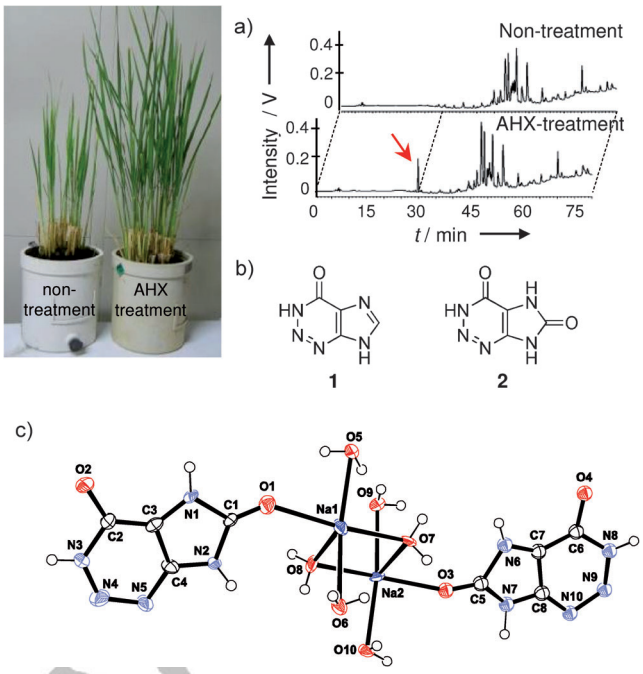

Figure 2. a) HPLC profile of the metabolite (**2**) of **1** in **1**-treated rice shoots. Extracts of rice shoots were analyzed by reverse-phase HPLC using a Develosil C30-UG-5 column with a solvent gradient (2% MeOH in 0.05% trifluoroacetic acid (TFA) for 12 min, 2–100% MeOH in 0.05% TFA for 60 min, and 100% MeOH for 20 min) at a flow rate of 0.5 mL min^{-1} at a wavelength of 254 nm. The arrow indicates the peak of **2** (29.6 min). b) Structures of AHX (**1**) and AOH (**2**). c) ORTEP drawing of **2** with ellipsoids set at 50% probability. Hydrogen atoms are shown as small spheres of arbitrary radii.

exhibited growth-yielding positive effects on rice. When rice was grown in soil supplemented with **1** or **2** (5 μM) in pot experiments, the seed yield per plant increased from 41.7 to 59.3 g (rate of increase, 42.2%) or 36.9 to 46.3 g (25.5%), respectively (Table S1).

To support the existence of **1** and **2** in rice, we quantified the endogenous **1** and **2** levels in rice cultivated under aseptic conditions by liquid chromatography/tandem mass spectrometry (LC-MS/MS) using [4-^{13}C,2-^{15}N]AHX (**17**) and [4-^{13}C,2-^{15}N]AOH (**18**) as internal standards (Scheme 1). We detected **1** (457 ± 75 ng kg^{-1} F.W. in shoot and 273 ± 52 ng kg^{-1} F.W. in root)■■please define F.W. for non-specialists■■ and **2** (1289 ± 406 ng kg^{-1} F.W. in shoot and not detected in root) (Figure S6). These results indicated that **1** and **2** are endogenously synthesized in both the shoot and root of rice. The amounts of endogenous **1** and **2** in rice were similar to those of the known plant hormones, strigolactones[5] and brassinosteroids.[6] Furthermore, **1** and/or **2** were detected in *Arabidopsis*, rice, bentgrass, zoysiagrass, tomato, potato, *Eucalyptus*, *Chlorella*, and *Parachlorella* (Figure 3; see also Table S2 and Figures S7 and S8).

To confirm the existence of our hypothetical biosynthetic route from **3** to **1** and **2** in plants (Figure 1), We performed a pulse chase labeling experiment using rice seedlings cultivated in liquid medium with [5-^{13}C]AICA (**16**; 0.1 mM) and analyzed the incorporation rate of **16** into the seedlings, and the amount of [4-^{13}C]AHX (**19**) and [4-^{13}C]AOH (**20**) in

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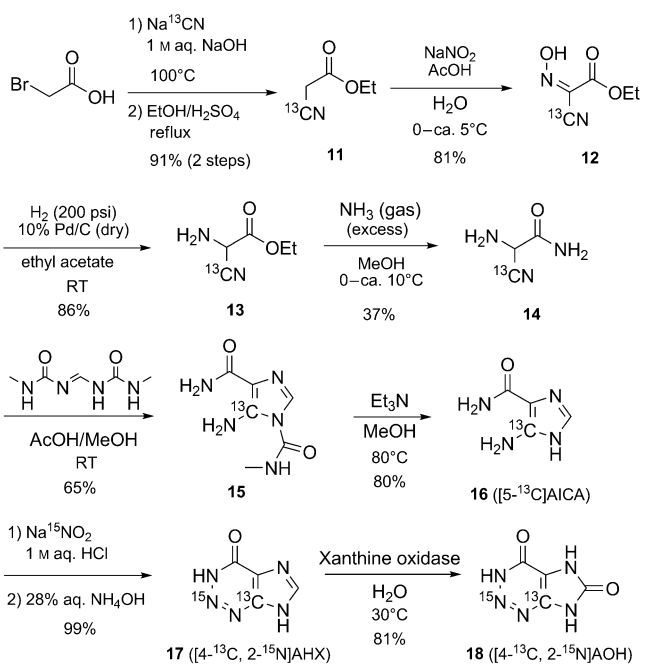

Scheme 1. Synthesis of isotope-labeled **1**, **2**, and **3**.

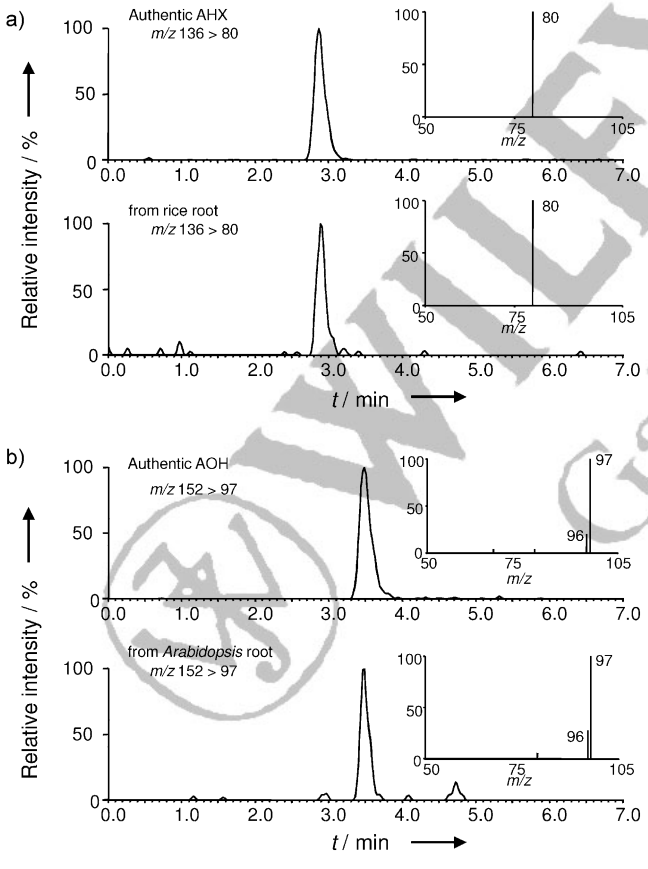

Figure 3. Identification of **1** and **2** in rice root (a) and *Arabidopsis* root (b) by negative mode of LC-MS/MS. a,b) LC-MS/MS chromatogram and MS/MS spectra of **1** and **2**, authentic sample (top), and root extracts (bottom). Characteristic transitions (precursor ion to daughter ion) for **1** and **2** were monitored.

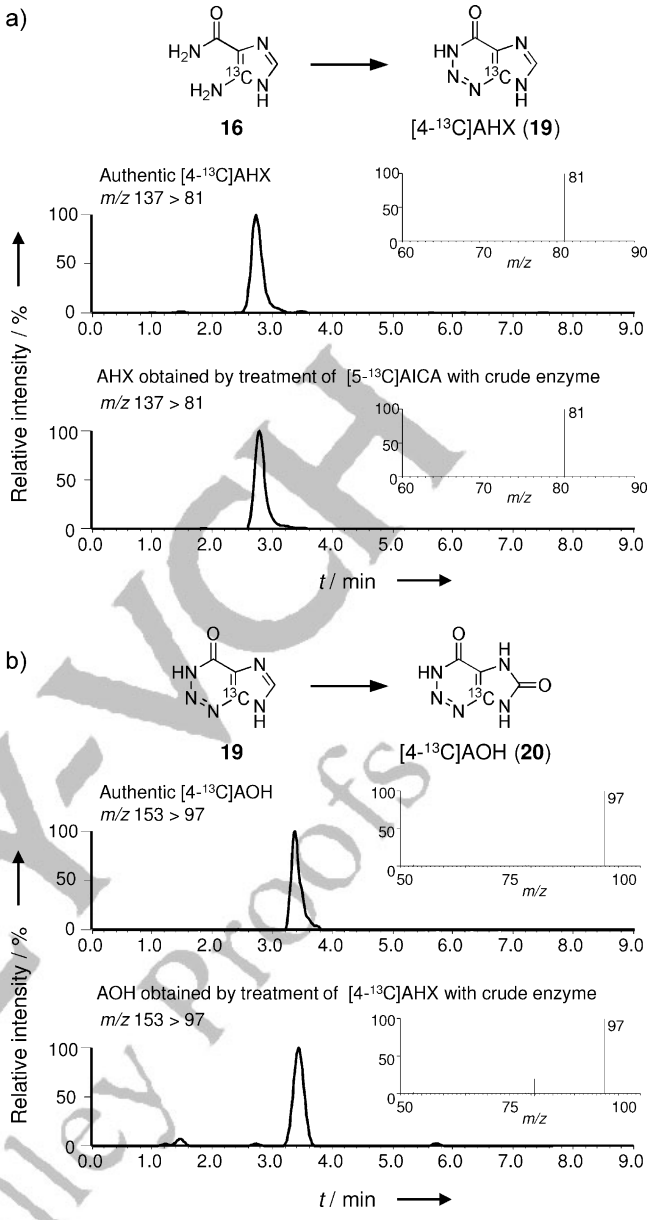

Figure 4. Detection of **19** (a) and **20** (b) in reaction mixtures treated with the crude rice enzymes. LC-MS/MS chromatogram and MS/MS spectra obtained for **19** and **20**, authentic sample (top), and root extracts (bottom). Characteristic transitions (precursor ion to daughter ion) for **19** and **20** were monitored.

the seedlings. A trace of **16** was detected with in the medium, and **20** was found in the seedlings. Compound **16** in the culture medium was incorporated at 99.98% into the rice seedlings, and this incorporated **16** was then converted into **20** through **19** (Figure S9).

Crude enzymes that catalyze the conversion from **16** into **19** and from **19** into **20** were extracted from rice and *Arabidopsis*, respectively, and fractionated by ammonium sulfate precipitation (Table S3). The supernatant obtained by ammonium sulfate precipitation of rice-root extracts, which showed the strongest activity of the reaction from **16** to produce **19**, was further separated by ultrafiltration, and the resulting fraction with a molecular weight of 10000–50000

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exhibited enzymatic activity (Figure 4a). The fraction with molecular weight over 50000, which was obtained by ultrafiltration of the precipitates from a 30% saturation of ammonium sulfate showed enzyme activity for the conversion of **19** into **20** (Figure 4b).

All of these results lead to the conclusion that **1** and **2** are new metabolites in a novel purine metabolic pathway in plants, at least in rice and *Arabidopsis*. As mentioned above, all of the plants and algae analyzed contained **1** and/or **2**, thus indicating that the pathway is conserved in other organisms. In addition, the molecular mechanism of the growth-promoting activity of the new metabolite **2** was investigated using oligo DNA microarrays of rice (*Oryza sativa* L. cv. Nipponbare). The gene expression profile of rice treated with **2** was very similar to that of **1** (Figure S10). The most conspicuous results of the microarray analysis was significant induction of glutathione *S*-transferases (GST; Os09t0367700-01, Os03t0785900-01, Os10t0528300-01), an aquaporin, OsTIP2;1 (Os02t0658100-01), and Bowman–Birk type proteinase inhibitor (Os01t0127600-01) upon treatment with **1** or **2** (Figure S10). The up-regulation was also confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR; Figure S10 and Table S4).[3] These results suggest that the mysterious fairy that stimulates the plant growth in fairy rings might not only be **1**, but also **2** (Figure S4 and S5).

It will take some time before the new purine pathway and the mechanism of action of **1** and **2** are completely elucidated. However, from all the results it is clear that the chemical "fairies" **1** and **2** are commonly biosynthesized by the novel purine metabolic pathway in plants.

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[1] a) H. B. Couch, *Diseases of turfgrasses*, Krieger, Malabar, **1995**, pp. 181–186; b) J. D. Smith, N. Jackson, A. R. Woolhouse, *Fungal diseases of amenity turf grasses*, E. & F. N. Spon, London, **1989**, pp. 339–353; c) H. L. Shantz, R. L. Piemeisel, *J. Agric. Res.* **1917**, *11*, 191–245; d) J. Ramsbottom, *Nature* **1926**, *117*, 158–159.
[2] H. Evershed, *Nature* **1884**, *29*, 384–385.
[3] J.-H. Choi, K. Fushimi, N. Abe, H. Tanaka, S. Maeda, A. Morita, M. Hara, R. Motohashi, J. Matsunaga, Y. Eguchi, N. Ishigaki, D. Hashizume, H. Koshino, H. Kawagishi, *ChemBioChem* **2010**, *11*, 1373–1377.
[4] a) K. Vaughan, D. E. V. Wilman, R. T. Wheelhouse, M. F. G. Stevens, *Magn. Reson. Chem.* **2002**, *40*, 300–302; b) Y. Wang, M. F. G. Stevens, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 185–188; c) D. R. Hannah, M. F. G. Stevens, *Chem. Res.* **2003**, 398–401.
[5] a) M. Umehara, A. Hanada, S. Yoshida, K. Akiyama, T. Arite, N. Takeda-Kamiya, H. Magome, Y. Kamiya, K. Shirasu, K. Yoneyama, J. Kyozuka, S. Yamaguchi, *Nature* **2008**, *455*, 195–200; b) V. Gomez-Roldan, S. Fermas, P. B. Brewer, V. Puech-Pagès, E. A. Dun, J.-P. Pillot, F. Letisse, R. Matusova, S. Danoun, J.-C. Portais, H. Bouwmeester, G. Bécard, C. A. Beveridge, C. Rameau, S. F. Rochange, *Nature* **2008**, *455*, 189–194.
[6] T. Ohnishi, B. Godza, B. Watanabe, S. Fujioka, L. Hategan, K. Ide, K. Shibata, T. Yokota, M. Szekeres, M. Mizutani, *J. Biol. Chem.* **2012**, *287*, 31551–31560.

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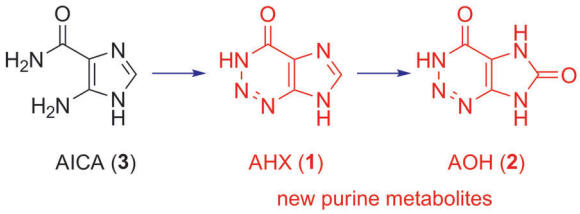

The fairy of the rings: The common metabolite, 2-aza-8-oxohypoxanthine (**2**), of the "fairy", 2-azahypoxanthine (**1**), was isolated from rice, thus supporting the existence of endogenous **1** and **2** in plants. Compound **1** is synthesized from **3** by treatment with $NaNO_2$ and then NH_3; **1** can then be converted into **2** by xanthine oxidase. The new purine pathway to form the compounds occurs in rice and *Arabidopsis* through a route similar to the chemical synthesis. ■■Revised text ok?■■

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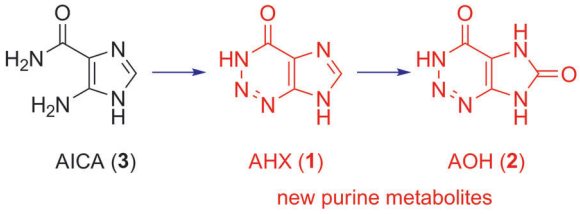

Das Stoffwechselprodukt 2-Aza-8-oxohypoxanthin (**2**) des mit Hexenringen in Verbindung stehenden 2-Azahypoxanthin (**1**) wurde aus Reis isoliert, was für das Vorliegen von endogenem **1** und **2** in Pflanzen spricht. **1** kann durch Umsetzung von **3** mit zunächst $NaNO_2$ und anschließend NH_3 synthetisiert und danach durch Xanthin-Oxidase in **2** umgewandelt werden. Der neu entdeckte Purinpfad zu diesen Verbindungen in Reis und *Arabidopsis* folgt einer ähnlichen Route wie die chemische Synthese.

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