Gibberellin Biosynthesis in Bambusoideae

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Gibberellin \text{A}_\text{8} (\text{GA}_\text{8}), \text{GA}_{12}, \text{GA}_{20}, \text{GA}_{42}, \text{and GA}_{52}, \text{were} identified from the shoots of \text{Phyllostachys edulis}, together with previously identified \text{GA}_{16}, \text{GA}_{19}, \text{and GA}_{20}, \text{and the occurrence of a 2\beta-hydroxy-GA}_{12}-\text{like compound was suggested. An experiment on shoots of the bamboo species confirmed the metabolism from GA}_{12}-\text{aldehyde to GA}_{12} \text{and GA}_{13}, \text{in P. bambusoides, and from GA}_{12}-\text{aldehyde to GA}_{12} \text{and GA}_{12} \text{to GA}_{13}, \text{in Sasa kurilensis. These findings strongly suggest the participation of the early-13-hydroxylation pathway for gibberelin biosynthesis in the shoots of Bambusoideae.}

**Key words:** Bambusoideae: gibberelin; \text{Phyllostachys bambusoides}: \text{Sasa kurilensis}

In an earlier stage of research on the isolation of endogenous GAs in higher plants, \text{GA}_{19} has been isolated from the boiling-water extract of a large amount of column stalks (44 tons) of \text{Phyllostachys edulis} A. et C. Riv. ("Moso-bamboo") by Murofushi et al. More recently, endogenous GAs in the shoots of several bamboo species have been re-examined and their distribution in young vegetative tissues studied to clarify the biosynthetic pathway in Bambusoideae. As a result, three 13-hydroxylated GAs, \text{GA}_{12}, \text{GA}_{19}, \text{and GA}_{20}, \text{have been identified} from the shoots of bamboo species \text{P. edulis (Moso), P. bambusoides} Sieb. et Zucc. (Madake), and \text{Sasa kurilensis} (Rupr.) Makino et Shibata (Nemogarakide), suggesting that the early 13-hydroxylation pathway operates in these species.

In this note, we report the further identification of endogenous GAs in the shoots of \text{P. edulis}, and the conversion of \text{GA}_{12} \text{and GA}_{12}-\text{aldehyde to GA}_{12}, \text{which is the key step dividing the early-13-hydroxylation pathway from the early-non-hydroxylation pathway in a feeding experiment when using the young shoots of bamboo species.}

A young shoot of \text{P. edulis} (2 kg fr. wt.) was homogenized in methanol and then filtered. The methanol extract was concentrated and subjected to solvent fractionation to give an acidic ethyl acetate-soluble fraction which was purified by using a Bond Elut DEA cartridge (Varian) and ODS-HPLC. After ODS-HPLC, the

<p>| Table 1. Identification of Endogenous GAs in \text{P. edulis} by GC-MS* |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>\text{t}_{R} \text{ on HPLC} (min)</th>
<th>\text{t}_{R} \text{ on GC} (min)</th>
<th>\text{Principal ions and relative abundance (m/z (%))}</th>
<th>\text{GAs identified}</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-12</td>
<td>12.55</td>
<td>536 (M+, 40), 521 (10), 504 (10), 477 (15), 387 (10), 207 (100)</td>
<td>\text{2\beta-OH GA}_{12}</td>
</tr>
<tr>
<td>19-20</td>
<td>10.21</td>
<td>418 (M+, 100), 403 (15), 387 (5), 375 (30), 359 (20), 303 (25)</td>
<td>\text{GA}_{19}</td>
</tr>
<tr>
<td>20-22</td>
<td>11.46</td>
<td>462 (M+, 15), 447 (5), 434 (100), 402 (40), 375 (50), 374 (60)</td>
<td>\text{GA}_{19}</td>
</tr>
<tr>
<td>21-22</td>
<td>13.52</td>
<td>432 (M+, 70), 417 (10), 403 (5), 373 (20), 238 (40), 207 (100)</td>
<td>\text{GA}_{20}</td>
</tr>
<tr>
<td>24-26</td>
<td>11.21</td>
<td>492 (M+, 80), 477 (5), 460 (40), 432 (30), 373 (30), 208 (100)</td>
<td>\text{GA}_{12}</td>
</tr>
</tbody>
</table>

* Samples were methylated and then trimethylsilylated before the GC-MS analysis.

* HPLC conditions: Experiment 1) column, Senshu Pak ODS-4253-D (10 mm i.d. x 250 mm, Senshu Sci. Co.); elution solvent, linear gradient of 100% solvent A (30% methanol and 1% acetic acid) to 100% solvent B (methanol) in 30 min, flow rate, 3 ml/min; oven temp., 40 C. Experiment 2) elution solvent, linear gradient of 100% solvent A (20% acetonitrile and 0.5% acetic acid) to 100% solvent B (80% acetonitrile and 0.5% acetic acid) in 55 min, flow rate, 1 ml/min. The other conditions were the same as those for Experiment 1.

* GC-MS conditions: Experiment 1) instruments, JEOL AX-500 mass spectrometer coupled with a Hewlett Packard 5890 Series II gas chromatograph; column, DB-1, 0.25 mm i.d. x 15 m, 0.25 mm film thickness (J&W Scientific); carrier gas, He; injection temp., 250 C; oven temp., held at 130 C for 2 min, increased in a linear gradient from 130 C to 220 C (32 C/min), held at 220 C for 4 min, and further increased in a linear gradient of 220 C to 270 C (8 C/min). Experiment 2) instruments, JEOL JMS-AX 505W mass spectrometer coupled with a Hewlett Packard 5890 series II gas chromatograph; injection temp., 220 C; separator temp., 260 C. The other conditions were the same as those for Experiment 1.

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Abbreviations: GA, gibberelin; ODS, octadecysilane.
Table II. Identification of Metabolites from $^{13}$C, $^{3}$H-GA$_{12}$-aldehyde (GA$_{12}$-ald), and $^{14}$C$_{6}$-GA$_{13}$

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Putative metabolite</th>
<th>Radioactivity (Bq)</th>
<th>$t_{1/2}$ on GC (min)</th>
<th>Principal ions and relative abundance (m/z (%)</th>
<th>GA determined</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. bambusoides$^a$</td>
<td>GA$_{12}$</td>
<td>135</td>
<td>7.72</td>
<td>462 (9), 387 (12), 359 (100)</td>
<td>$^{13}$C-GA$_{12}$</td>
</tr>
<tr>
<td></td>
<td>GA$_{13}$</td>
<td>151</td>
<td>8.26</td>
<td>565 (14), 550 (24), 475 (25), 448 (100)</td>
<td>$^{13}$C-GA$_{13}$</td>
</tr>
<tr>
<td>S. kurilensis</td>
<td>GA$_{12}$</td>
<td>203</td>
<td>7.72</td>
<td>462 (9), 387 (12), 359 (100)</td>
<td>$^{13}$C-GA$_{12}$</td>
</tr>
<tr>
<td></td>
<td>GA$_{13}$</td>
<td>426</td>
<td>8.33</td>
<td>572 (23), 557 (32), 482 (16), 453 (100)</td>
<td>$^{13}$C-GA$_{13}$</td>
</tr>
</tbody>
</table>

$^a$ Samples were directly trimethylsilylated (without methylation) before the GC-MS analysis.
$^b$ $^{14}$C$_{6}$-GA$_{13}$ was also fed to P. bambusoides, but the amount of metabolites was insufficient for a GC-MS identification.
$^c$ $^{13}$C, $^{3}$H-GA$_{12}$-ald (17.9 kBq, a 50% methanol solution) was injected into the plant materials (6 seedlings, ca. 8 g seedling) which were then incubated for 4 h at room temperature.
$^d$ $^{13}$C, $^{3}$H-GA$_{13}$-ald (39.6 kBq; a 50% methanol solution) was injected into the plant materials (13 seedlings, ca. 2.4 g seedling) which were then incubated for 4 h at room temperature.
$^e$ $^{14}$C-GA$_{13}$ (5 kBq, 50% methanol solution) was injected into the plant materials (6 seedlings, ca. 3 g seedling) which were then incubated for 4 h at room temperature.
$^f$ Radioactivity in each GA fraction was counted after Nucleosil 5 N(CH$_3$)$_2$ purification.
$^g$ Conditions for GC-MS analysis: instruments, Incon 50 mass spectrometer (Finnigan Matt) connected with Hewlett Packard 5890 gas chromatography; column, DB-1, 0.25 mm i.d. $\times$ 15 m, 0.25 mm film thickness (J&W Scientific); carrier gas, He; injection temp., 250 C; oven temp., held at 80 C for 1 min, increased in a linear gradient from 80 C to 245 C (30 C/min), and further increased in a linear gradient from 245 C to 300 C (5 C/min).

Individual GA fractions were subjected to an analysis by full-scan GC-MS. As shown in Table I (Experiment 1), GA$_{15}$, GA$_{16}$, GA$_{18}$, and GA$_{53}$, and a 2$\beta$-hydroxy-GA$_{53}$, like compound$^h$ were identified. This is the first identification of GA$_{15}$, GA$_{44}$, and GA$_{53}$ from the Bambusoidaeae species. In addition to these GAs, GA$_{13}$ and GA$_{29}$, which had not previously been detected in Bambusoidaeae, were identified together with GA$_{15}$, in the other experiment (Experiment 2, Table I) with the same plant materials. All of these GAs identified were 3-hydroxylated GAs, and no evidence was found for the occurrence of non-3-hydroxylated GAs such as GA$_{4}$, GA$_{10}$, or GA$_{24}$. These results suggest that the early-3-hydroxylation pathway operates dominantly in the young shoots of P. edulis.

In metabolic studies, shoot tips (the youngest leaf sheaths after removing the outer sheaths with P. edulis and P. bambusoides, and the top part of the whole plant with S. kurilensis), in which the existence of a relatively high level of GA had been suggested,$^i$ were prepared from young shoots of P. edulis, P. bambusoides, and S. kurilensis. [17,13C, $^{3}$H]GA$_{12}$-aldehyde (1.28 GBq mmol)$^j$ and [17,12,18,14$^{14}$C$_{6}$]GA$_{12}$ (6.6 GBq mmol)$^k$ were injected into shoot tips, which were then incubated for 4 h at room temperature (approx. 26 C). After the incubation, they were extracted with methanol, and the extracts were subjected to the same purification steps as those already mentioned. After ODS-HPLC purification, radioactivity was found in all putative GA$_{53}$ fractions from the three species fed with GA$_{12}$-aldehyde and GA$_{13}$. GC-MS identification of 13C- or 14$^{14}$C$_{6}$-GA$_{53}$ in the ODS-HPLC fractions from P. edulis was unsuccessful, probably because the amount of GA$_{53}$ in the fractions was insufficient for identification. No further investigation of this species was conducted because of the limitation in plant materials.

The putative GA fractions by ODS-HPLC from P. bambusoides and S. kurilensis were further purified by HPLC on a Nucleosil 5 N(CH$_3$)$_2$ column$^l$ and then submitted to a GC-MS analysis. In P. bambusoides, 13C-GA$_{12}$ and 13C-GA$_{53}$ were identified by fully-scan GC-MS as metabolites of 13C, $^{3}$H-GA$_{12}$-aldehyde (Table II). The conversion of 13C, $^{3}$H-GA$_{12}$-aldehyde into 13C-GA$_{12}$, and of 14$^{14}$C$_{6}$GA$_{12}$ into 14$^{14}$C$_{6}$GA$_{53}$ was also confirmed by full-scan GC-MS in S. kurilensis. Gibberellin A$_{13}$-aldehyde was not identified in any sample. In the experiments when feeding 13C,$^{3}$H-GA$_{12}$-aldehyde to these bamboo shoots, radioactivity was detected in the putative GA$_{10}$ fraction, but was insufficient for any further analysis by GC-MS.

The present results show that the tips of Bambusoidaeae shoots had significant 13-hydroxylation activity, indicating that the early-13-hydroxylation pathway operates in these species. This
conclusion is strongly reinforced by the fact that all of the GAs identified in three Bambusoideae species were 13-hydroxylated GAs. The dominant participation of the early-13-hydroxylation pathway must be a common feature in the vegetative tissue of Gramineae. In our previous study, we have reported that the level of GAs in rapidly growing tissue (the youngest leaf sheath) of the young bamboo shoot was higher than that in the other parts. In our present study, the metabolism of GA was observed in shoot tips by injecting a substrate into these tissues. These results suggest that young developing shoot tissue is the major site for GA biosynthesis in Bambusoideae. This hypothesis is consistent with the conclusions from studies on the shoots of pea and rice.

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References