Note

Endogenous Gibberellins in *Aralia cordata*

Takaaki NISHIJIMA, Fumio MURAKAMI,* Masaji KOSHIKOA, and Hiroko YAMAZAKI

National Research Institute of Vegetables, Ornamental Plants, and Tea, Ministry of Agriculture, Forestry, and Fisheries of Japan, Kusawa, Ano-cho, Age-gun, Mie 514–23, Japan

*Kuroiso Branch, Tochigi Prefectural Agricultural Experiment Station, Sakitama, Kuroiso, Tochigi 325, Japan*

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Eight gibberellins (GAs) were identified in extracts of buds of *Aralia cordata* by full scan GC/MS and by Kovats retention indices. These GAs comprised five GAs on the early-13-hydroxylation pathway [GA_{13}, GA_{19}, GA_{20}, GA_{44}, and GA_{13}] and three other GAs [GA_{4}, GA_{15}, and GA_{37}]. The major GAs were GA_{19} and GA_{44}.

*Aralia cordata* Thunb. is a plant whose blanched sprout is commercially produced for a vegetable. For dormancy breaking and shoot elongation of *A. cordata*, a certain period of low temperature is required. In forcing culture of the plant, however, gibberellin (GA) is sometimes applied exogenously to promote dormancy breaking, suggesting involvement of endogenous GA in dormancy and stem elongation of *A. cordata*. This note deals with the identification of endogenous GAs in buds of *A. cordata*.

Basal buds of *A. cordata* (226 g fresh weight in total, about 25 to 35 mm in length) were harvested in September, 1991 in Tochigi prefecture, Japan, then immersed in methanol and stored at 5°C until extraction. The samples were extracted three times with 5-fold (v/w) methanol using a blender. The methanol extract was concentrated in vacuo and fractionated by the usual method to give an acidic ethyl acetate (AE) fraction. The AE fraction was pre-purified by polyvinylpyrrolidone (10 g), a Sep-pak C_{18} cartridge and then a Bondesil DEA (5 g) column. The purified AE fraction was then chromatographed by high-pressure liquid chromatography (HPLC) on an ODS column (ODS 5, 15 mm x 10 mm i.d., Nomura Chemical, Japan), eluted with a 60-min program of two linear gradients of methanol:0.1% acetic acid/water (45 to 50% methanol for 25 min and then 50 to 80% methanol for 25 min, after which methanol was kept at 80% for 10 min) at a flow rate of 2 ml per min. HPLC fractions were collected every 2 min. The GA-like activity of the HPLC fractions was tested using modified dwarf rice (*Oryza sativa* L.) microdrop assays. The test plants used were cv. Tan-ginbozu treated with 80 μM uniconazole (for detection of biologically active GAs) and cv. Waico-C treated with 80 μM uniconazole plus 40 mM prohexadione calcium (for specific detection of 3β-hydroxylated GAs). HPLC fractions were further analyzed by combined gas chromatography/quadrupole mass spectrometry (GC/MS, JEOL Automass 20, in full scan mode) with a J&W DB-1 fused silica column.
capillary column (0.257 mm i.d. x 15 m, 0.25 μm film thickness). Sample derivatization and GC/MS analysis were done as described by Endo et al.2)

As shown in Fig. 1, several groups of HPLC fractions showed substantial biological activity on cv. Tan-ginbozu. Out of those groups, two were active on cv. Waito-C (fractions 2 to 4 and 20 to 21), suggesting the presence of 3β-hydroxylated GAs.

From the biologically active fractions, ent-kaurenoic acid and seven GAs were identified by GC/MS (Fig. 1, Table). The presence of GA₃₃, GA₄₄, GA₁₉, GA₂₀, and GA₁ is indicative of the early-13-hydroxylation pathway operating in the buds of A. cordata (Fig. 2). Further, the presence of GA₁₅ is indicative of the early-non-hydroxylation pathway also operating. However, early-3β-hydroxylation may occur in the early-non-hydroxylation pathway as shown in Fig. 2. This is because we could not detect GA₂₄ and GA₉, which are matabolites from GA₁₅ in the early-non-hydroxylation pathway, but we detected GA₃₇, which may be produced from GA₁₅ through 3β-hydroxylation. GA₄, which may be the active GA of both the early-non-hydroxylation and early-3β-hydroxylation pathways, was not identified, probably because the quantity was too small estimated from the very low biological activity on cv. Waito-C of the HPLC fractions where GA₄ should be eluted (i.e., fractions 22 and 23). However, as shown in Fig. 2, GA₃₇ might be converted into GA₄ through GA₃₆, because we identified GA₄ in the buds harvested in November of the same year (Table).

Based on the biological activity, GA₁₉ and GA₄₄, which belong to the early-13-hydroxylation pathway, were very likely to be the most abundant GAs.

Such endogenous GAs might be involved in the dormancy and stem elongation of A. cordata.

References