Occurrence of Gibberellin A<sub>3</sub> in Parthenocarpic Apple Fruit†

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The gibberellins have been established as effective fruit setting agents (5). In the apple, where auxins are generally inactive, the gibberellins induce parthenocarpy and sustain fruit growth to maturity (2, 6, 10). This pronounced fruit-setting activity and the established observation that asymmetric growth of apple fruit is related to incomplete seed development, has encouraged investigations on the relationship of endogenous seed gibberellins to fruit-set and growth.

Gibberellin-like activity has been demonstrated in endosperm (11) and GA<sub>4</sub> and GA<sub>7</sub> identified in immature apple seed (8). The biosynthesis of gibberellins by seeds is viewed as a specific role of seeds in fruit-set of the apple (7). However, some cultivars of Malus are prone to produce seedless fruit after exposure to low temperature during the fruit-setting period. The presence of gibberellin-like substances in such parthenocarpic fruit is, herein, reported.

Immature seedless apple fruits (Malus sylvestris Mill., selection New Jersey 12) developing subsequent to a severe frost, 4 days after full bloom, were harvested (6-wk post-bloom), frozen immediately and stored at −30° until used. Frozen fruits (6.9 kg) were immersed in 100% acetone (7.6 liters), then each fruit was halved to check for seedlessness (no seeds were found), homogenized in acetone and extracted twice by stirring under nitrogen for 24 hours at 2°.

The filtrates were combined and concentrated to an aqueous solution (3.5 liters, pH of 2.8) in vacuo. The pH of the filtrate was increased to 6.0 with 10% KOH (13) and centrifuged to remove proteins and other insoluble substances. The supernate was washed with n-hexane and adjusted to pH 2.0, stirred for 2 hours after addition of 70 g of activated charcoal and filtered. The charcoal was washed several times with distilled water and eluted with acetone. The eluate (acetone) was evaporated to dryness, the residue (2.62 g) suspended in phosphate buffer (0.2 m, pH 6.2) and extracted with chloroform (9). The aqueous phase was adjusted to pH 2.5 with sulfuric acid and extracted with ethyl acetate. The chloroform and ethyl acetate extracts were dried over anhydrous sodium sulfate and evaporated to dryness yielding residues of 0.108 and 0.690 g, respectively. These residues were taken up in ethanol and lanolin paste preparations of either fraction were found to induce parthenocarpy in tomato. The chloroform fraction was more active than the ethyl acetate fraction. Considerable quantities of inhibitors were present in the ethyl acetate fraction as was evidenced by injury of treated ovaries.

Further purification was achieved by streaking the extracts on Whatman 3 MM paper (57 × 46 cm) and developing with isopropanol:ammonium hydroxide:water (10:1:1 v/v). The chromatograms were cut transversely, at intervals of 0.1 R<sub>P</sub>, and eluted with 80% ethanol. The gibberellin activity of each eluate was assessed using dwarf pea (9) (cv. Morse's Progress No. 9) and cucumber seedling (4) (cv. Burpee Hybrid) assays.

The chloroform fraction contained an active component(s) migrating to R<sub>P</sub> 0.4 to 0.7 and which was similar in activity and R<sub>P</sub> with authentic GA<sub>3</sub> treated under identical conditions (fig 1). A greater quantitative response was obtained from the chloroform than ethyl acetate fraction (approx 10-fold) in the dwarf pea assay. Little or no activity was observed for both fractions in the cucumber assay.

Further purification was limited to the active component(s) of the chloroform fraction migrating to R<sub>P</sub> 0.4 to 0.7. The eluate was streaked on silica gel-G plates (E. Merck F-254, 250 μ). Plates were developed with the lower phase of carbon tetrachloride:acetic acid:water (8:3:5, v/v) plus 20% ethyl acetate. The active component(s) did not migrate, thus it was eluted from the origin with ethanol and rechromatographed using benzene: n-butanol:acetic acid (70:25:5 v/v) as the developing solvent. The active component(s) was identical to GA<sub>3</sub> and/or GA<sub>7</sub> with respect to R<sub>P</sub> (0.54) and color of fluorescence and differed in both respects.

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from GA₂, GA₄, and GA₇ (respective Rₚ values 0.35, 0.80, and 0.82).

The biological activity of the active component(s) was similar to GA₁ and/or GA₃ in the dwarf pea, cucumber seedling, and dwarf corn assays (Table I).

To distinguish between GA₁ and GA₃, the residue from the chloroform fraction was treated with diazomethane (1) or trimethyl silyl chloride (12) to prepare the methylated and silylated derivatives for gas chromatography. Retention times on a SE-30 (3%) column (0.5 cm diam, and 100 cm long) at 200°C using a mixture composed of argon (75 ml min⁻¹), hydrogen (60 ml min⁻¹) and oxygen (50 ml min⁻¹) as the carrier gas were established employing a F & M Model 402 GLC equipped with a flame ionization detector. The retention time for the major component (27.8 min) of the chloroform fraction was similar to GA₃ and the chloroform fraction labeled with authentic GA₃ (Fig. 2). The retention time for GA₁ was slightly shorter (25.5 min) than that of the chloroform fraction or authentic GA₃. Therefore, we have concluded that this gibberellin (retention time 27.8 min) is most

![Figure 1: Histogram illustrating the biological activity of eluates from paper chromatograms of the chloroform fraction, chloroform fraction plus GA₃, and GA₃ as indexed by the dwarf pea (cv. Morse’s Progress No. 9) assay.](image1)

![Figure 2: Gas-liquid chromatography of the methylated chloroform fraction (CF) of parthenocarpic apple fruit and GA₁, GA₃, and GA₄ + GA₇ on 3.0% SE-30.](image2)

**Table I. Activity of the Chloroform Fraction, GA₃, and GA₇ in Selected Bioassays**

<table>
<thead>
<tr>
<th>Bioassay</th>
<th>0.4–0.5</th>
<th>0.5–0.6</th>
<th>0.6–0.7</th>
<th>GA₃ 0.01 μg/plant</th>
<th>GA₇</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dwarf pea</td>
<td>300</td>
<td>522</td>
<td>355</td>
<td>477</td>
<td>...</td>
</tr>
<tr>
<td>Cucumber seedling</td>
<td>117</td>
<td>117</td>
<td>108</td>
<td>96</td>
<td>156</td>
</tr>
<tr>
<td>Dwarf corn, d₅</td>
<td>120</td>
<td>262</td>
<td>129</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Dwarf corn, d₇</td>
<td>120</td>
<td>305</td>
<td>148</td>
<td>123</td>
<td></td>
</tr>
</tbody>
</table>

¹ Paper chromatogram was developed with isopropanol:ammonium hydroxide:water (10:1:1, v/v). Strips of indicated Rₚ were eluted with ethanol, reduced to dryness, taken up in 50% ethanol containing 0.05% Tween 80 and 10 μl (equivalent to 3.46 g fr tissue) were applied per plant.
likely GA₃. There is also an additional component in the chloroform fraction with a retention time similar to GA₄ and/or GA₅ (fig 2). Similar results were obtained with the silylated derivatives.

Based on the biological activity (dwarf corn) observed, the amount of GA₂ recovered in the chloroform fraction represented a concentration calculated to be approximately 143 μg/kg of fresh tissue. Since gibberellin-like activity was also present in the ethyl acetate fraction, the total gibberellin concentration may be slightly higher.

Recovery of GA₃ in the chloroform fraction from seedless apple fruit is of interest for purified GA₃ has been reported as being insoluble in chloroform (9). Perhaps in the presence of other natural products GA₃ solubility in chloroform is modified.

The role of gibberellins in growth and development of parthenocarpic fruit is not clear. GA₄ and GA₅ are more active than GA₃ in inducing parthenocarpy (3) and they have been found in seeds of immature apple fruit (8). To what extent these gibberellins occurred in other fruit tissue and whether or not they participate in fruit growth is not known. Our data demonstrate the presence of GA₃ in apple fruit tissue and suggest that fruit tissue other than seeds may participate in the biosynthesis of GA. Other interpretations are also possible: namely, that GA is translocated to fruits subsequent to synthesis in other plant tissue, but no direct evidence is available.

In summary GA₃ was isolated from frost-induced parthenocarpic apple fruit and its identity was established by biological response, thin layer, paper and gas liquid chromatography.

**Literature Cited**


