Gibberellin-like Substances from the Developing Banana Fruit

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Summary. The occurrence of 2 gibberellin-like substances was demonstrated in the developing banana fruit, *Musa sapientum*, Linn. Chemical and biological evidence led to the tentative identification of the 2 compounds as GA7 and GAx (previously isolated from citrus fruits). Support for such identification was obtained from thin layer chromatography, gradient elution column chromatography, spectrofluorometry, the dwarf maize test, and the cucumber hypocotyl test. Significance of the GAx-designated compound increased since it is believed to occur in the fungus *Fusarium moniliforme*, Sheld, in addition to 2 different species of higher plants. It does not resemble any of the known gibberellins as far as chromatography is concerned.

The naturally occurring hormones of the banana fruit have received less attention than those of other fruits. The work of Steward and Simmonds (13), Nichols (11), and that of Khalifah (7) represents the only literature concerned with natural hormones of bananas. Simmonds (12) suggested a role of auxin in the development of parthenocarpic banana fruit on the basis of his exogenous application of α-naphthylacetic acid. A cell division factor, presumably a cytokinin (13), and an auxin, identified as indoleacetic acid (7), were extracted from young banana fruit. Based on indirect evidence from paper chromatography, IAA or an indole compound very closely allied to it was postulated in seeded banana fruit (11). No work has been done on naturally occurring gibberellins or gibberellin-like compounds in bananas. This paper reports the existence of 2 gibberellin-like substances in the developing banana fruit. Data from thin-layer chromatography, fluorometric assay, column chromatography, and biological assays provided tentative identification of the 2 compounds.

Materials and Methods

Young fruit of the parthenocarpic banana *Musa sapientum*, Linn, was sampled when it reached about 6.5 cm long. The fruit was frozen immediately in dry ice and was thawed in absolute methanol just prior to analysis. Extraction was carried out by macerating the whole fruit in 80% methanol with a Waring blender and shaking the mixture for 3 hours. All subsequent steps were identical with those described previously (7), except for the use of ethyl acetate instead of ether. The final ethyl acetate extract was fractionated by thin-layer chromatography (9) and gradient elution column chromatography (5).

Prospective gibberellin-like substances were examined fluorometrically and biologically. An Amin-Bowman spectrophotofluorimeter was used for fluorometric assay of the extracted compounds dissolved in 85% sulfuric acid. The dwarf maize test (d₈) as described by Most and Vlitos (10) and the cucumber hypocotyl test as described by Brian and Hemming (1) were employed for biological assay. The material used for bioassay was obtained by fractionation of the fruit extract by gradient elution column chromatography using the benzene: acetonitrile system (5). Five replications of each bioassay were done and the data were analyzed statistically.

Results and Discussion

Thin-layer Chromatography. When the fruit extract was fractionated on silica gel G with isopropyl ether:acetic acid (95:5), 2 fluorescent spots appeared under ultraviolet light after spraying with 5% sulfuric acid in ethanol and heating. One spot was located at Rₚ 0.41 and gave a purple fluorescence; the other gave blue fluorescence at Rₚ 0.48. The substance with lower Rₚ value corresponded exactly to marker spots of standard GA₇ and GA₅ developed on the same plate. When the spray solution consisted of 70% sulfuric acid in water, the extracted substance (Rₚ 0.41) was visible in the ultraviolet light even before heating the chromatoplate. This is characteristic of GA₇ and provided the first indication that this compound is probably GA₇ rather than GA₅. Gibberellin A₅ requires heating on the sprayed chromatoplate for 10 minutes at 120° for it to become visible under ultraviolet light (9). When the solvent system used was benzene:acetic acid:water (8:3:5), this substance appeared at Rₚ 0.53. This corresponded to the GA₇ marker spot but not to the GA₅ marker spot (Rₚ 0.73). These Rₚ values are lower than those of MacMillan and Suter (9), but this could be due to different operation conditions.

The spot with Rₚ 0.48 in the isopropyl ether
solvent system had similar properties to the gibberellin GA, which was isolated from citrus (6) and the gibberellin isolated from the fungus *Fusarium moniliforme* (4). Repeated thin layer chromatography indicated that this compound has *R* is 0.66 in the benzene:acetic acid:water solvent system. GA has a unique property that differentiates it from other known gibberellins; it becomes visible under ultraviolet light as a blue spot following spray with 5% sulfuric acid in ethanol and prior to heating. After the chromatoplate is heated this substance becomes visible as a blue spot under ultraviolet light of short wavelength (253.7 nm); it is almost invisible when the ultraviolet wavelength is 366 nm.

**Fluorometric Assay.** Examination of the 2 gibberellin-like substances in the spectrofluorometer added further support to their tentative identification as GA and GA. The spot with respective *R* values of 0.41 and 0.53 had a maximum excitation wavelength of 450 nm with maximum emission at 470 nm. This is typical of GA; no other known gibberellin exhibits similar fluorometric maxima (3, 6). The second gibberellin-like substance had fluorescence properties similar to GA, GA, GA, GA, and GA (3, 6, Table 1). Chromatographic evidence, however, excluded all but GA as a possible identity for this substance.

**Column Chromatography.** The 2 isolated gibberellin-like substances were expected to follow a certain fractionation pattern in column chromatography, since they were tentatively identified as GA and GA. According to the technique described by Khalifah et al. (5), GA was eluted in fractions 24 to 29 in the chloroform:methyl acetate:methanol system, and in fractions 40 to 46 in the benzene:acetoniitrile:methanol system. When the plant extract was fractionated by the chloroform system, a gibberellin-like material was detected in fractions 26 to 37 and behaved like GA in TLC and fluorometry. When the benzene gradient elution system was employed, this material was found in fractions 39 to 48 (Table 1). The 2 column chromatography systems, therefore, constituted additional evidence that GA occurs naturally in the developing banana fruit.

Column chromatography predictions concerning the compound designated GA turned out as expected. This substance was detected in fractions 31 to 42, overlapping the GA and GA areas in the chloroform system (5). Fractions 31 to 37 contained a mixture of both gibberellin-like substances of the banana fruit as indicated by thin layer chromatography. The benzene system, as expected, successfully separated the 2 compounds from each other (Table 1). GA was found in fractions 52 to 60, compared to the other compound in fractions 39 to 48. This behavior is characteristic of GA of citrus (5, 6, 8).

**Biological Assays.** The gibberellin-like nature of the 2 extracted substances was confirmed by the biological assay. Both compounds were active in the dwarf maize (*d* ) test. In the cucumber hypocotyl test, the GA-like material was obviously active, while the activity of the GA-like designated compound was barely significant at the 5% level. The latter bioassay is known to be almost specific for GA, GA, and GA (2, 14, 15). The high activity of the extracted GA-like substance supports such identification. Elson et al. (3) were first to report the occurrence of GA in the seeds of a flowering plant, *Echinocystis macrocarpa*.

The compound designated GA is of special interest since it does not resemble any of the known gibberellins. It was first isolated independently and almost simultaneously from the fungus *Fusarium moniliforme* Sheld. (4) and from a higher plant, *Citrus sinensis*, Linn. (6). The extensive work done by Jones (4) on the fungus gibberellin should be done with GA from citrus or banana fruits before reaching a conclusive identification.

**Table I. Properties of the Gibberellin-like Substances from Young Banana Fruit.**

<table>
<thead>
<tr>
<th>Property</th>
<th>Substance 1</th>
<th>Substance 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thin-layer chromatography (<em>R</em> ):</td>
<td>Isopropyl ether:acetic acid (95:5)</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>Benzene:acetic acid:water (8:3:5)</td>
<td>0.53</td>
</tr>
<tr>
<td>Column chromatography (fraction numbers):</td>
<td>Chloroform: ethyl acetate system</td>
<td>28–37</td>
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<tr>
<td></td>
<td>Benzene:acetoniitrile system</td>
<td>39–48</td>
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<tr>
<td>Fluorometric properties:</td>
<td>Excitation wavelength (nm)</td>
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<tr>
<td></td>
<td>Emission maximum (nm)</td>
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<tr>
<td>Biological activity: Maize test</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>Cucumber test</td>
<td>+ +</td>
<td>+</td>
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<tr>
<td>Identification</td>
<td>GA</td>
<td>GA (4, 6)</td>
</tr>
</tbody>
</table>

**Literature Cited**

5. **Khalifah, R. A., L. N. Lewis, and C. W. Cogins, Jr.** 1965. Gradient elution column chromatography systems for the separation and iden-


