Phytochemistry, Vol. 22, No. 10, pp. 2189–2191, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00

ENDOGENOUS GIBBERELLINS IN ENDOSPERMS AND COTYLEDONS OF *SECHIUM EDULE* DURING SEED GROWTH AND MATURATION

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(*Revised received* 18 *February* 1983)

Key Word Index—*Sechium edule*; Cucurbitaceae; gibberellins; identification; plant hormones; GA_9; GA_4/GA_7.

Abstract—Endosperm and cotyledons from seeds of *Sechium edule* were analysed for gibberellins by HPLC and GC/MS. GA_4 and GA_7 were the major gibberellins present in both tissues in all stages of seed growth. GA_9 was also present in endosperm and cotyledons but at lower levels than GA_4/GA_7. GA_1 and GA_3 were identified only in cotyledon extracts. A dramatic decrease of the total amount of GAs was observed in cotyledons at the last stage of maturation. The amount of gibberellins detected in endosperm is among the highest found in plant organs.

INTRODUCTION

Seeds of many dicotyledon species contain large amounts of gibberellins; this has resulted in the identification of most higher plant gibberellins from seeds [1]. Several correlations have been reported between the gibberellin content of seeds and their development. However, to attempt to assess how gibberellins may participate in seed development a knowledge of the distribution of individual gibberellins in seed tissues and their qualitative and quantitative changes during seed growth is essential. Qualitative and quantitative analyses of endogenous gibberellins during seed growth have been reported [2, 3] but very few reports concerning the occurrence of gibberellins in the individual components the seed are available [4–6].

We are currently involved in research concerned with the presence and metabolism of growth regulators in developing seeds of *Sechium edule*. The choice of this material was based on preliminary observations that immature fruits of *Sechium* contained very high levels of growth regulators, particularly gibberellins and cytokinins. This confirmed a previous report [7] on the presence of gibberellin-like substances in this seed. We report here on gibberellin analyses on cotyledons and endosperm during seed growth while in the accompanying paper [8] we report on gibberellin biosynthesis in the same tissues.

RESULTS AND DISCUSSION

To investigate gibberellin content during seed development, the material was classified as shown in the footnote to Table 1. For each class of seeds, the cotyledons and endosperm were analysed separately. Preliminary results obtained by using TLC and bioassay had indicated the occurrence of relatively non-polar gibberellin-like substances in endosperm extracts while in cotyledon extracts biologically active more polar products were also detected. We therefore developed HPLC conditions which gave a good separation of gibberellin standards, GA_9, GA_4 and GA_7, GA_1 and GA_5, which have polarities similar to the predominant gibberellin-like substances present in the seed extracts. The acidic ethyl acetate extracts were purified by HPLC, using the procedure developed with the standards. A good separation of zones of biological activity was achieved.

In Fig. 1 the elution profile of gibberellin standards on HPLC is shown. Figure 2 shows the HPLC separation of the extract of endosperms from class C seeds. The shaded areas of Fig. 2 represent the biological activity of one twentieth of each fraction, corresponding to 0.2 ml of

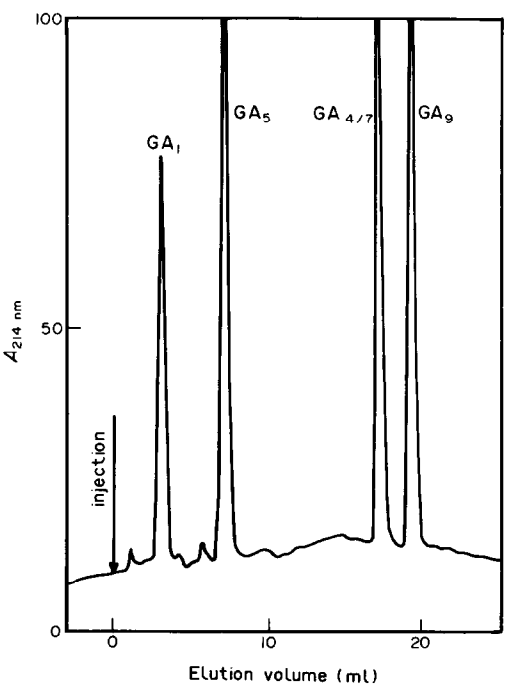

Fig. 1. HPLC of GA_1, GA_5, $GA_{4/7}$ and GA_9 standards. Conditions as in Experimental.

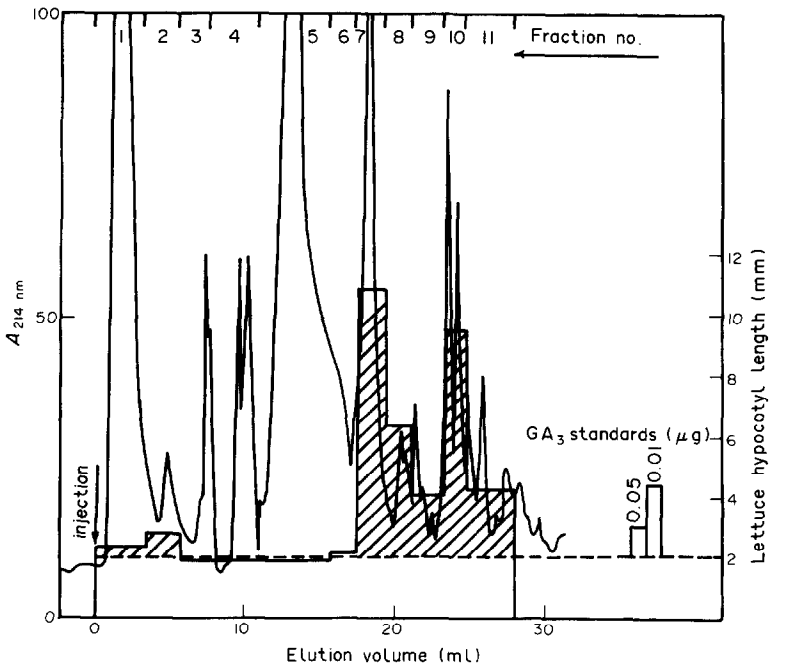

Fig. 2. HPLC of endosperm extract from seeds of class C. Conditions as in Experimental. Dashed areas indicate biological activity.

endosperm, as shown by the lettuce hypocotyl test. The major zones of gibberellin activity corresponded to the elution volumes of GA_4/GA_7 and GA_9.

After silylation, the biologically active fractions were analysed by GC and GC/MS. The major peak present in the GC trace of fraction 7 showed the same retention time as the TMS GA_4/GA_7 standards. Repetitive scans were taken in this peak. The spectra taken at the leading edge of the peak showed predominantly ions characteristic of TMS GA_4 whereas the spectra taken in the trailing edge of the peak showed predominantly ions characteristic of TMS GA_7. GC analysis of fraction 8 showed a series of peaks, one of which cochromatographed with the TMS GA_9 standard. Analysis of this peak by GC/MS gave a spectrum with all the ions characteristic of TMS GA_9. Fraction 10, which also showed gibberellin-like activity was further investigated but conclusive identification of the active substances was not achieved.

The biologically active fractions from HPLC analysis of all endosperm and cotyledon samples were analysed by the above procedure. The compounds identified conclusively in each extract are listed in Table 1. As shown in this table, endosperm from seeds of all sizes contained: GA_4/GA_7 and GA_9. A major difference between endosperm and cotyledons was the presence in the latter of a more polar group of gibberellins together with GA_4, GA_7 and GA_9. Among these more polar compounds GA_1 and GA_3 were identified conclusively by HPLC and GC/MS. No trace of GA_9 was found in class D cotyledons, although a specific search was conducted with the peakfinder program of the MS focused on the single ion at m/z 270.

The absence of internal standards does not permit a true quantitative determination of substances present in the extracts. Estimates were made, on the basis of GC peak areas, of the relative quantities of GAs present at

Table 1. Gibberellins identified and their approximate amounts (μg/g fr. wt) in endosperm and cotyledons from seeds of the various classes

	Class*			
	A	B	C	D
Endosperm	GA_4+GA_7 (2.5) GA_9 (0.2)	GA_4+GA_7 (15) GA_9 (0.2)	GA_4+GA_7 (19.5) GA_9 (0.2)	GA_4+GA_7 (19.5) GA_9 (0.2)
Cotyledons	Not extracted	GA_1 (0.02) GA_3 (0.02) GA_4+GA_7 (4.5) GA_9 (0.05)	GA_1 (0.08) GA_3 (0.08) GA_4+GA_7 (7) GA_9 (0.05)	GA_1 (trace) GA_3 (trace) GA_4+GA_7 (0.3)

*Classification of seeds according to lengths (mm) of seed and cotyledons. A: seeds, 35–38; cotyledons, 5–10; B: seeds 35–38; cotyledons 11–20; C: seeds, 38–40; cotyledons 20–30; D: seeds, 40–45; cotyledons, 31–37.

different developmental stages. Endosperm tissues generally contained larger amounts of gibberellins than cotyledons.

As can be seen from Table 1, aside from the absence of GA_9 in class D cotyledons, there was little qualitative change in the GAs in seed parts at different developmental stages. However, the relative amounts of these compounds changed markedly during development. Endosperm from seeds of class C and D, representing the most advanced maturation stages of the seed, contained the greatest amount of gibberellin, the largest part of which was accounted for by GA_4 and GA_7. The amount of GA_9 did not change considerably in endosperm through all developmental stages of the seed, being always present in much smaller amounts than GA_4/GA_7.

In cotyledons of class B GA_4/GA_7 were the main gibberellins present. GA_1 and GA_3, also detected in cotyledons, were both present. Cotyledons from seeds of class C contained the largest amount of gibberellins among the three classes of cotyledons examined, with GA_4 and GA_7 as the major forms present. The more polar gibberellins, GA_1 and GA_3, were present in greater amounts than in class B cotyledons. By contrast, GA_9 was detected at the same level as the preceding stage. In mature cotyledons (class D), a dramatic decrease of the total amount of gibberellins occured leading to the disappearance of GA_9 and to a dramatic reduction of GA_4 and GA_7. GA_1 and GA_3 were present only in trace amounts.

The gibberellin levels found in *Sechium* endosperm are among the highest found in plant organs. Most of the papers published on gibberellins in seeds refer to the gibberellin content of the whole seed [4, 5]. These results demonstrate that the distribution of gibberellins among the different tissues of the seed varies in a qualitative and quantitative way and show that particular tissues contain extremely high levels of hormones. It is interesting to note that the gibberellin content of endosperm does not change qualitatively in all the classes of seeds studied while only a quantitative increase was observed in more mature seeds. Therefore, whatever is the function of gibberellins in the endosperm during seed development, it seems to be constant throughout the lifetime of this tissue.

On the other hand, the qualitative differences between endosperm and cotyledons may reflect different functions of gibberellins in the two organs. The identification and approximate quantitation of GAs in endosperm and cotyledons of *Sechium* was carried out as a necessary prerequisite for detailed studies into the biosynthesis of these compounds in this tissue. The results of these studies will be reported in a separate paper.

EXPERIMENTAL

Plant material. Plants of *S. edule* were grown in the field during the period July–November 1981. Seeds taken from fruits at different stages of maturation were dissected and cotyledons free of embryo and endosperms were collected separately. The material was classified in relation to seed and cotyledon length and stored at −24° prior to extraction.

Extraction and purification. Cotyledons (7 g) were homogenized at 4° with 80% MeOH in a blender. Cotyledons from seeds of class A were not extracted owing to the very limited amount of material available. The homogenate was stirred for 12 hr at 4° and then centrifuged at 2000 *g* for 15 min. The pellet was then re-extracted twice with 0.5 vol MeOH. The combined extracts were reduced under vacuum at 35° and the aq. residue partitioned against EtOAc at pH 2.8. The EtOAc extract was stored at −24°, frozen H_2O was separated and the EtOAc removed by evaporation. Endosperm (4 ml) was extracted by using the same procedure.

HPLC. This was performed on a LDC instrument equipped with a UV absorbance detector operating at 214 nm. A column (15 cm × 1/4 in o.d.) packed with Lichrosorb RP18, 5 μ was used. The solvent flow rate was 1 ml/min. Samples were applied to the column after dissolving in 5% aq. MeCN and fractions were collected while the column was being eluted as follows: 5% MeCN in H_2O for 8 min; linear gradient from 5–20% over 20 min; linear gradient from 20–100% of MeCN in H_2O over 8 min.

GC and GC/MS analysis. HPLC fractions showing gibberellin-like activity in the lettuce hypocotyl bioassay were dried and trimethyl silylated with pyridine–hexamethyldisilazane–trimethylchlorosilane (5:1:1). The silylated fractions were chromatographed on a PYE UNICAM 104 gas chromatograph equipped with a dual FID: 3% OV 1 on a GC Q 100–200 mesh (150 cm × 4 mm i.d.); N_2 40 ml/min; temp. programme 200–300° at 4°/min. GC/MS spectra were obtained on a Hewlett–Packard instrument (5992 B) equipped with GC, jet separator and operating at 70 eV. The GC column (190 cm × 2 mm i.d.) was packed with 2% OV 1. Scans were taken at 380 amu/sec over the range 70–600 amu. The following compounds were identified in the TMSi derivatized fractions by comparison of their mass spectra with reference spectra: (a) endosperm: GA_9, GA_4 and GA_7; (b) cotyledons: GA_9, GA_4, GA_7, GA_1 and GA_3.

Acknowledgements—The authors thank Dr. R. Horgan for his constructive criticism of the manuscript. This work was supported by a CNR, IPRA grant. Additional funds were provided by CNR grant number 82.02704.06.

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