Fatty Hormones in Pollen and Immature Seeds of Bean

Pinto bean pollen contains an ether-soluble hormone(s) chromatographically similar to brassins, which are fatty hormones. Immature seeds of Pinto bean contain several ether-soluble hormones. Of the two studied, both behaved alike chromatographically and one has fatlike characteristics. Both are unlike other known plant hormones. The evidence indicates that some fatty hormones in the bean pollen are qualitatively different from those in the immature bean seed.

Various indole compounds, abscisic acid, phaseolic acid, cytokinins, gibberellins, and gibberellin-like substances have been isolated from pollen, ovaries, immature seeds, and other parts connected with sexual reproduction of plants (Gupta and Maheshwari, 1970; Khalifah *et al.*, 1965; Kimura, 1969; Kirtoka, 1968; Muir, 1951; Murofushi *et al.*, 1969; Redemann *et al.*, 1968; Smith, 1969, and bibliographies in these references).

Recently brassins, characterized as fatty compounds with hormone activity, were isolated from rape (*Brassica napus* L.) and alder (*Alnus glutinosa* L. Gaertn.) pollen (Mitchell *et al.*, 1970). We now report the presence of similar fatty hormones in pollen and immature seeds of bean (*Phaseolus vulgaris* L. var. Pinto). Furthermore, we found the hormones from the pollen to be qualitatively different from those in intact seeds collected 6 days after pollination.

We examined bean pollen for the presence of hormones by collecting it from open anthers and stigmatic surfaces of individual flowers and extracting these samples with ether. The small amount of extract obtained (less than 10 μg/pollen sample) induced only slight acceleration of internode growth when assayed with the bean second internode method (Mitchell and Livingston, 1968).

We then collected a composite sample (1.5 mg) from open anthers and the stigmatic surfaces of about 30 bean flowers which yielded 170 μg of ether extract. This crude extract accelerated elongation of the second internodes an average of 18.6-fold using 10 μg/plant and four replications.

To determine whether this extract contained brassin-like compounds, we chromatographed 130 μg of it using silica gel and benzene-methanol-acetic acid (45:8:4). An area of silica gel bounded by R_f 0.35 and 0.45 was extracted with absolute ethyl alcohol, a procedure used for the purification of brassins (Mitchell *et al.*, 1970). Gibberellic acid shows R_f 0.6 under these conditions. The purified material from the pollen (less than 10 μg) was divided into three equal portions and each portion was assayed. Marked acceleration of internodal elongation resulted (15-fold), indicating the presence of a fatty hormone or a group of fatty hormones with an R_f value the same as that of brassins (Figure 1). The type of growth induced by the purified bean pollen extract appeared, however, to involve only elongation, rather than elongation and cell division as reported for brassins (Worley and Mitchell, 1970). This difference in response may have been due to differences in dosages used, or to structural differences in the chromatographically similar fatty hormones obtained from the two kinds of pollen. A rough survey of remaining areas on the plate showed that one area bounded by R_f 0.05–0.35 also contained activity.

Extracts of stigmas were made to determine whether hormone activity in these may have contaminated some of the pollen collected from the stigmatic surfaces. Hormone activity was not detected in pollen-free stigmas collected 1 to 2 days prior to the time when the pollen was shed. On the other hand, an extract prepared from unopened anthers collected at the same time and from some of the same flowers accelerated elongation an average of 6.7-fold (10μg/plant).

Previously, hormone activity was detected in ether extracts of immature bean seeds of the Black Valentine variety (Mitchell *et al.*, 1951). In the present study, an ether extract of intact Pinto seeds in a comparable stage of development (6 days after pollination) accelerated second internode elongation an average of 14.4-fold (20 μg/plant). This crude extract was purified in the same manner as that used for brassins (Mitchell *et al.*, 1970). The material obtained was inactive (10 μg/plant) indicating that brassin activity is not present in readily detectable amounts.

We then chromatographed approximately 1 g of crude extract obtained from the immature seeds using silica gel, and the benzene-methanol-acetic acid solvent system. Fluorescent spots were apparent under uv at the following R_f values: 0.08, 0.26, 0.37, 0.60, 0.80, 0.85, and 0.98. The largest spot (R_f 0.26) was visible in white light. This R_f 0.26 area of silica gel was extracted with absolute ethyl alcohol. The alcohol was evaporated and the residue extracted five times with ether resulting in 140 μg of ether-soluble material. The residual ether-insoluble portion was extracted with absolute ethyl alcohol in which practically all of it dissolved, thus making 1300 μg of alcohol-soluble material available. This alcohol-soluble portion increased growth approximately 16-fold, while the ether-soluble portion increased growth an average of 3.6-fold (10 μg/plant). This experiment was repeated five times, during which R_f values of the seed hormones varied from 0.24 to 0.28. We used a typical plate from this series, and the alcohol-soluble portion of each of the remaining minor spots was assayed (10 μg/plant). A relatively small

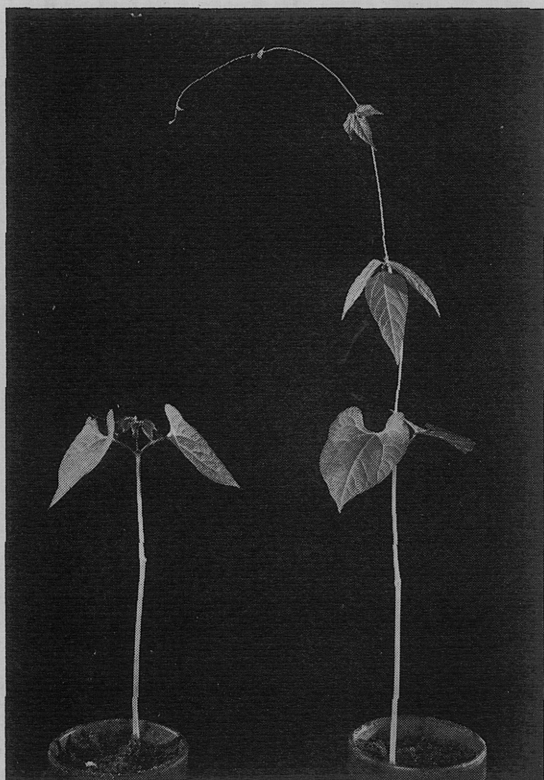

Figure 1. Elongation of plant treated in the second internode assay with less than 3 μg of a chromatographically purified fatty hormone preparation obtained from bean pollen (right), compared with that of plant treated with the fractionated lanolin carrier alone

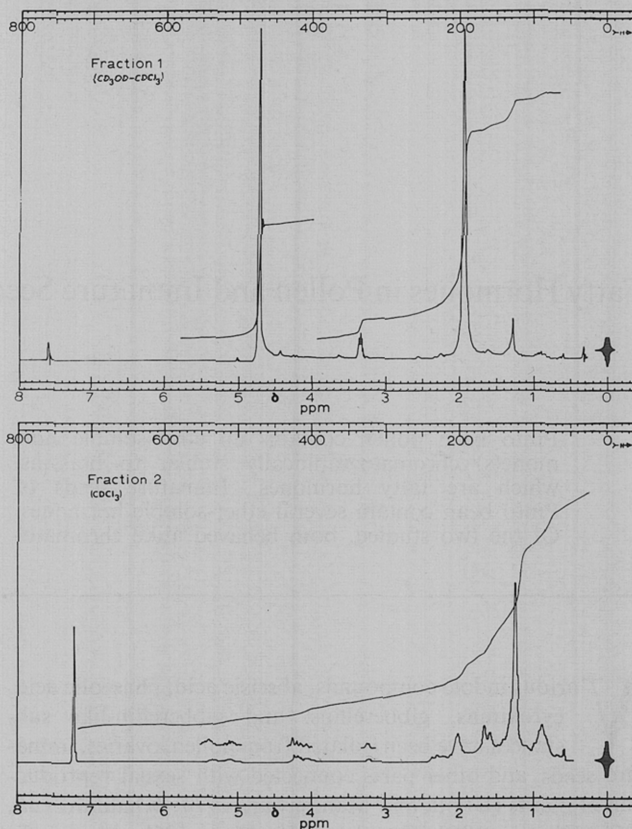

Figure 2. Nuclear magnetic resonance spectra of the two seed hormone fractions

spot at R_f 0.9 showed moderate activity (6-fold increase) while extracts from the other spots induced relatively slight change in elongation of the second internode (0.7–2.7-fold increase).

Chromatographically, the brassins obtained from bean and rape pollen (R_f 0.4) were unlike the major bean seed hormones (R_f 0.26). The area of silica gel containing the brassins was invisible in white light. In contrast, the area containing the seed hormones was readily visible (light brown). Chromatographed separately, the brassins were not visible under uv; the seed-hormones fluoresced. With respect to solubility, the brassins from both rape and bean pollen were readily soluble in ether. In contrast, the purified R_f 0.26 seed hormones were practically insoluble in ether but readily soluble in absolute ethyl alcohol. Because of the availability of the R_f 0.26 seed hormones and their relatively high specific activity, these were subjected to chemical examination.

Regarding physical measurements, the R_f 0.26 seed hormones showed light absorption at 280 and 435 mμ (in ethanol). Infrared spectrum (potassium bromide pellet) exhibited strong absorption bands 3400 (broad), 1560 and 1410 cm^{-1}, in addition to medium intensity bands at 1240, 1140, 1100, 1050, and 650 cm^{-1}. The bands at 1560 and 1410 cm^{-1} appear to be characteristic of amide absorption. The nuclear magnetic resonance (nmr) spectrum in methanol-d_4 showed signals, from internal tetramethyl silane (δ scale), at 1.87 ppm as a singlet; multiplet at 1.08–1.28 ppm, and another multiplet at 2.10–3.0 ppm. The spectrum is complicated by additional resonance signals in the regions 3.43–4.02, 4.4–5.2, and 5.8–6.8 ppm. Analysis by combined gas chromatography and mass spectrometry indicated that the extract of the R_f 0.26 spot on the silica gel consisted of two components with characteristic and intense peaks at *m/e* 279 and 368, which might be due to either the appearance of molecular ions or the loss of fragments from the parent peaks. The fragmentation pattern does not correspond to that of gibberellins, or indoles, or to that of phaseolic acid (Redemann *et al.*, 1968; Brinks *et al.*, 1969). Separation of the R_f 0.26 seed hormones was also achieved by gel chromatography using Bio-beads (S-X4, 200–400 mesh, Bio-Rad Laboratories, Richmond, Calif.) with a mixture of methylene dichloride-methanol (80:20). Both fractions proved to be moderately active (10 μg/plant).

The nmr spectra of Fractions 1 and 2 from the seed hormone are illustrated in Figure 2. Fraction 1 in a mixture of methanol-d_4 and chloroform-d showed a singlet absorption at 1.9 ppm, in addition to other weak resonance signals (Figure 2). Fraction 2 exhibited a singlet at 1.24 ppm for methylene protons in a long-chain hydrocarbon derivative, and an unresolved triplet for terminal methyl attached to methylene groups at 0.9 ppm. The absorption between 1.4–2.2 ppm was attributed to protons of methyl and methylene groups attached to double bonds. The multiplicity character at about 4.1 and also 5.2 ppm suggests that there are some methylene and methine protons that are attached to oxygen functions in Fraction 2. The absorption at 5.2 ppm might also arise from olefinic protons. These results clearly indicate that Fraction 2 is a fatty acid derivative, while Fraction 1 is not, except for some contamination by Fraction 2.

These spectroscopic data suggest that one of these R_f 0.26 seed hormones (Fraction 2) is fatty material; they both differ from brassins, and they do not belong to any of the known types of hormones.

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