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BIOLOGICALLY ACTIVE GIBBERELLINS IN IMMATURE SEEDS OF *PYRUS SEROTINA*

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Key Word Index—*Pyrus serotina*; Rosaceae; pear; gibberellins; bioassay; rice seedling test; barley half-seed test; thin layer chromatography; mass spectrometry; selected ion monitoring.

Abstract—Biologically active gibberellins were isolated from immature seeds of Japanese pear (*Pyrus serotina*). The major one was tentatively identified as GA_4 by selected ion monitoring. Evidence for the possible presence of GA_7 in the seeds was inconclusive but GA_3 was not detected by either the rice seedling or barley half-seed bioassay.

INTRODUCTION

In earlier unpublished experiments, biologically active gibberellin-like substances were detected in the immature seeds of *Pyrus serotina* by several bioassay tests. They were presumed to be GA_4 (**1**) and/or GA_7 (**2**). Recently, Bearder *et al.* [1] identified GA_{25} and GA_{45} (**3**), and Martin *et al.* [2] identified GA_{17} and an unidentified gibberellin, possibly 3β-hydroxy GA_{45} in addition to the above GAs, in extracts of immature seeds of *Pyrus communis*. GA_{45} (**3**) was the only biologically active compound of those GAs that they identified, and interestingly GA_{45} (**3**) differs from GA_4 (**1**) merely by the position of the hydroxyl group.

This study was, therefore, carried out to establish whether or not the GAs which we isolated in seeds of *Pyrus serotina* were the same as those isolated in those of *Pyrus communis*.

RESULTS AND DISCUSSION

Although Hirata *et al.* [3] reported the presence of GA_3, GA_4 and GA_7 in extracts of immature seeds of Japanese pear (*Pyrus serotina* cv Nijisseiki = maternal parent of Shinseiki), GA_3-like activity was not detected in our sample extracted from the immature seeds of Japanese pear either by the rice seedling test (Fig. 1) or the barley half-seed test. Therefore, the presence of GA_4 and/or GA_7 was presumed in seeds of Shinseiki pear. As in many other plant tissues, gibberellin-like substances were present in seeds of Japanese pear in extremely small quantity. However, GC–MS was sensitive enough to determine such small amounts of GAs by monitoring for selected specific ions [4].

In the case of GA_4 (**1**), *m/e* 418, 386, 372 and 358 were selected as characteristic ions for the GA_4 Me TMS, and the ion peaks of the methylated and trimethylsilylated

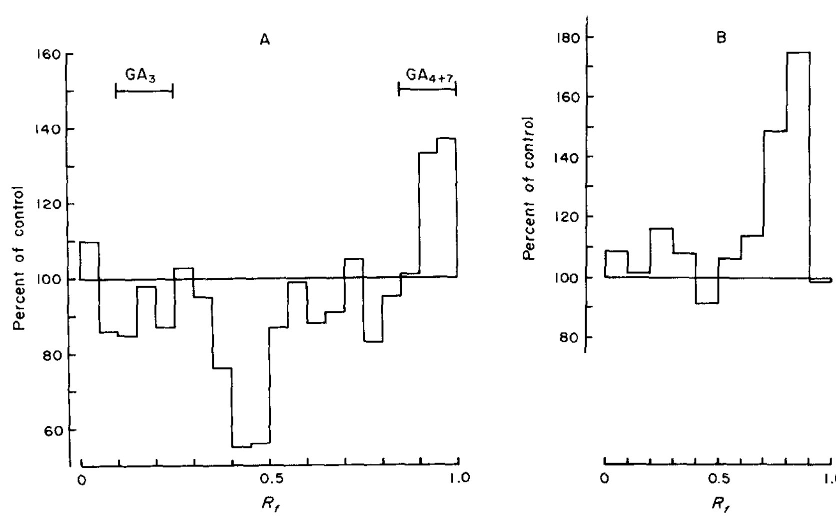
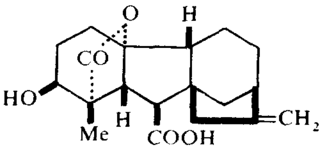


Fig. 1. Histograms depicting the rice seedling responses to gibberellin-like activity separated by paper chromatography of the ethyl acetate fraction of Japanese pear seeds. Developing solvents: A = $CHCl_3$–HOAc–H_2O (8:3:5), B = *iso*-PrOH–H_2O(4:1).



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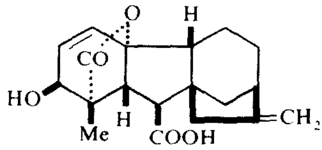

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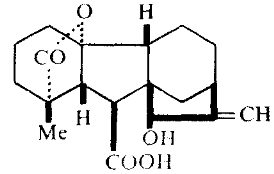

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unknown appeared at the same retention time and similar relative ion intensity as the authentic GA_4 (Fig. 2).

From these data it is tentatively concluded that GA_4 (1) is present in seeds of Shinseiki pear. In the same way ions at m/e 416, 384, 372 and 356 present in the MS of GA_7 Me TMS were monitored in the derivatized extract. The results (Fig. 3) were, however, inclusive for the presence of GA_7 (2).

We were also interested in checking on the presence of GA_{45} (3) in the extract. From the GLC traces which we received from Dr. MacMillan, Bristol, England, we assumed that the peak for GA_{45} Me TMS determined through total ion monitoring should appear at a slightly earlier retention time than that of GA_4 Me TMS using either an SE-33 or a QF-1 column.

Near the position of the specific ion peaks of GA_4 Me TMS, there were no signs of other peaks with the ions m/e 418 (M^+) and 358 (M − 60). Therefore, GA_{45} (3) must be present at an extremely low level in Japanese pear seeds compared to GA_4 (1). The most characteristic ion of GA_{45} Me TMS at m/e 156 was not chosen for monitoring, because analysis of any fragment ion having a molecular weight more than 30% different from that of the molecular ion is not considered suitable for this multiple ion detection technique. Single ion monitoring at m/e 156 for GA_{45} Me TMS would have been possible, but we did not pay attention to this ion because the molecular ion of the derivative did not seem to be present in our pear extract.

It is interesting to note that GA_4 and GA_7 were in immature seeds of apples, which are taxonomically very closely related to pear. Together with such evidence that exogenously applied GA_4 was very effective in inducing parthenocarpy in Japanese pear [5], and lanolin paste of this gibberellin applied to fruit pedicel at 1600 ppm around the end of Stage I resulted in about 30% increase

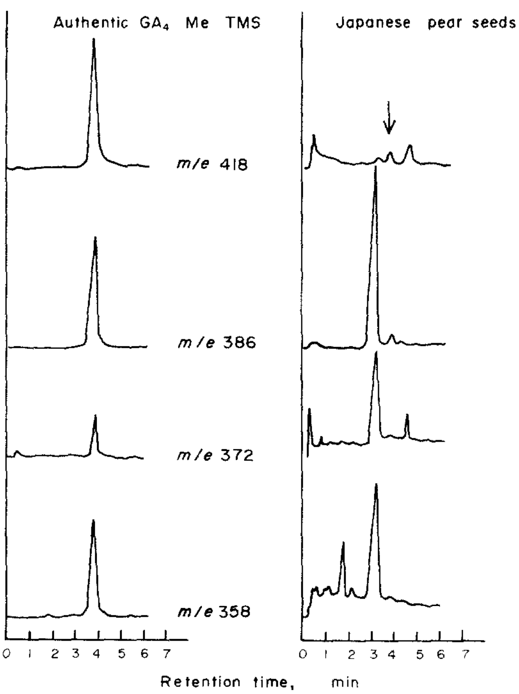

Fig. 2. Selected ion current traces of the methylated and trimethylsilylated derivative of gibberellin (GA_4) present in a partially purified ethyl acetate fraction of Japanese pear seeds. Monitoring was conducted at m/e 418, 384, 372 and 356. An arrow points to the peaks with a retention time near 3.9 that represent GA_4 Me TMS.

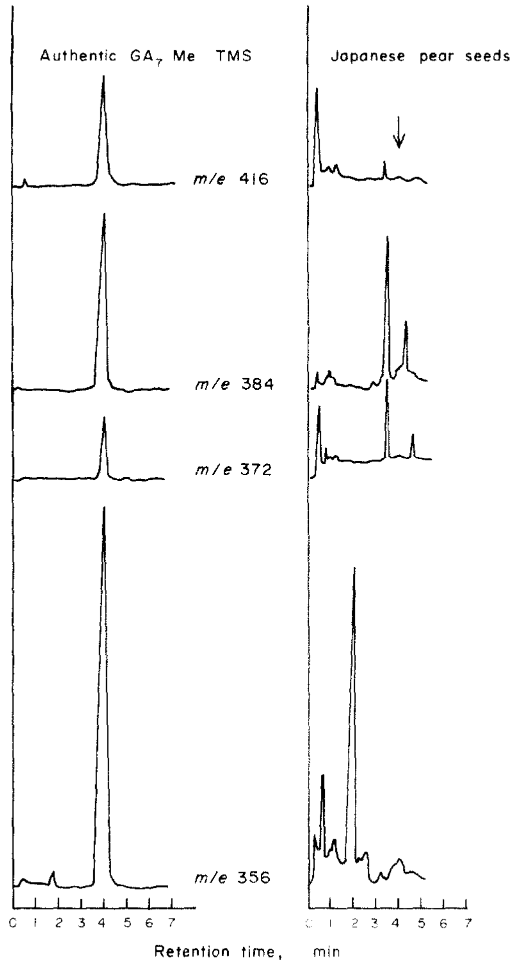

Fig. 3. Selected ion current trace of methylated and trimethylsilylated derivatives of gibberellin (GA_7) present in a partially purified ethyl acetate fraction of Japanese pear seeds. Monitoring was conducted at m/e 416, 384, 372 and 356. An arrow points to the peaks with a retention time near 4.0 that represent GA_4 Me TMS.

in fruit size of Japanese pear at harvest [6], we have confirmed that GA_4 is one of the most physiologically significant GAs in this species.

EXPERIMENTAL

Seeds (50 g) were collected from immature Japanese pear fruits (*Pyrus serotina* cv Shinseiki) 35 days after bloom and immediately frozen in dry ice. The frozen seeds were extracted in 3 l. of cold 80% MeOH for 24 hr, and homogenized in 200 ml absolute MeOH after filtration. The homogenate was re-extracted in 3 l. cold 80% MeOH for another 24 hr. The combined MeOH solns were evapd *in vacuo* to leave an aq. soln which was adjusted to pH 2.5 with 1 N HCl and extracted 3 × with EtOAc. The EtOAc extract was partitioned 3 × against satd $NaHCO_3$ soln and the aq. phase adjusted to pH 2.5 with 6 N HCl. Extraction 3 × with EtOAc gave an extract containing gibberellins.

The EtOAc fraction was concd and streaked on 6 sheets of Whatman 1 MM paper (45 × 48 cm) and developed (ascending) for *ca* 16 hr (*ca* 20 cm in height) with H_2O. Authentic GA_3 and GA_{4+7} were co-chromatographed as references. Since biological activity of GA-like substances (the rice seedling bioassay) appeared above R_f 0.9, in the region of authentic GA_3 and GA_{4+7}, the zone from R_f 0.8 to 1.0 was eluted with MeOH and dried *in vacuo*. The eluate was then redissolved in MeOH and streaked on 2 sheets each of the Whatman paper and developed (ascending) for *ca* 16 hr (*ca* 20 cm in height) with 2 solvent systems, A = $CHCl_3$–HOAc–H_2O (8:3:5) and B = *iso*-PrOH–H_2O (4:1). Judging from the biological activity (Fig. 2) the zone from R_f 0.8 to 1.0 of system A was eluted with MeOH.

The eluate was firstly subjected to TLC (Merck, 20 × 20 cm, Kieselgel 60, 0.25 mm thickness) developed with $CHCl_3$–MeOH–H_2O–HOAc (20:10:2:1). Biological activity in each R_f zone was checked with the barley half-seed bioassay [4] after elution with Me_2CO–MeOH (2:1). The biologically active zone (R_f 0.8–1.0) corresponding to authentic GA_4 or GA_7 was further subjected to TLC with C_6H_6–Me_2CO–HOAc (15:4:1) together with authentic GA_4 or GA_7 markers. The zone corresponding to these GAs was eluted with Me_2CO–MeOH (4:1) and reduced to dryness. The residue was dissolved in MeOH, methylated with CH_2N_2, trimethylsilylated, and subjected to selected ion monitoring using a Hitachi 063 gas chromatograph coupled to a RMU-6 MG mass spectrometer equipped with a multiple ion detector. Instrument conditions were: glass column (1 m × 3 mm i.d.) packed with 3% OV-101 on Gas Chrom-Q (80–100 mesh). Column, injection port and separator temp. were 260, 280 and 300°, respectively. The ionization voltage was 20 eV. Selected ion monitoring was conducted at *m/e* 418 (M^+), 386 (M − 32), 372 (M − 46), 358 (M − 60) for GA_4 Me TMS, and *m/e* 416 (M^+), 384 (M − 32), 372 (M − 44), 356 (M − 60) for GA_7 Me TMS.

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