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EMBRYO-SUSPENSOR OF *TROPAEOLUM MAJUS*: IDENTIFICATION OF GIBBERELLIN A_{63}

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Key Word Index—*Tropaeolum majus*; Tropaeolaceae; nasturtium; identification; gibberellins; gibberellin A_{63}.

Abstract—Embryos and suspensors of *Tropaeolum majus* at the same stages of seed development were analysed for gibberellins by GC-MS. GA_{63} was the only gibberellin present in both tissues. The amount of GA_{63} in the suspensor is higher than in the embryo.

INTRODUCTION

The development of a suspensor is a very common, though not universal, feature of embryogenesis in angiosperms.

An enormous variation in suspensor structure occurs both between and within species [1, 2]. The suspensor of the nasturtium, *Tropaeolum majus*, consists of three threads which radiate from a cell group (basal cell) lying outside the micropyle: one thread suspends the embryo into the endosperm cavity (suspending thread), one runs along the ovule and penetrates the carpel wall opposite the chalaza (carpel haustorium or dorsal thread) and the third grows through the integument and placenta to the point of entry of the vascular bundle of the raphe (placental haustorium) [3, 4].

Ultrastructural studies on the *Tropaeolum* suspensor indicates high synthetic activity and storage of certain substances in the suspending thread, while the basal cells develop wall ingrowths which are suggestive of transfer of nutritive material from the fruit tissues [5].

A role for the suspensor in the synthesis of hormones has been clearly indicated in the suspensor of *Phaseolus coccineus*, at least for gibberellins [6].

Przybyllok and Nagl [7] reported in the suspensor of *T. majus* the presence of auxin (IAA), significantly higher than in the embryo proper and recent results [8] indicate the presence of GA-like substances in suspensors of *T. majus* and *Cytisus laburnum*.

In this work, we report on the identification of one gibberellin with biological activity in the embryo and suspensor of *T. majus*.

RESULTS AND DISCUSSION

The bioassay data for the fractions from silica gel chromatography of extracts from the embryos and suspensors (data taken from ref. [8]) of *T. majus* are shown in Fig. 1. The active fractions (embryo, 41–44; suspensor,

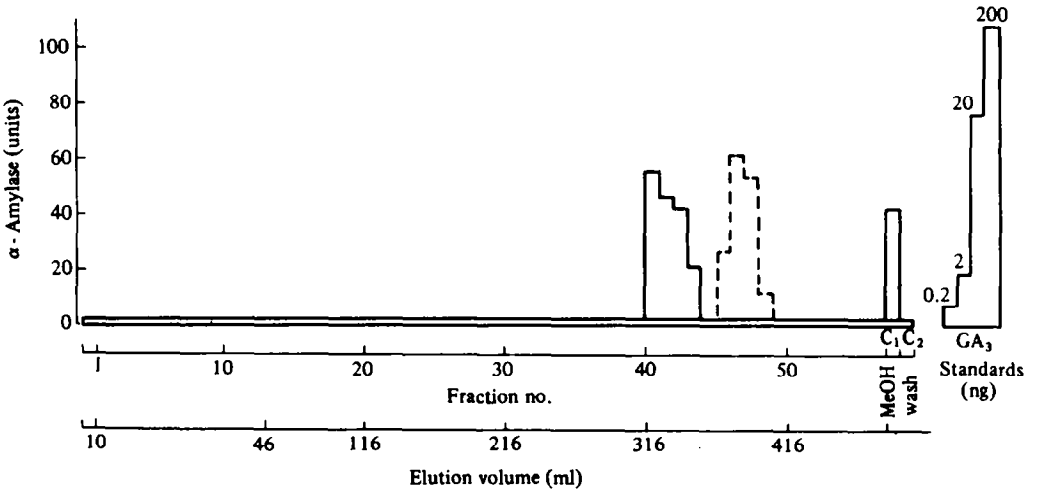
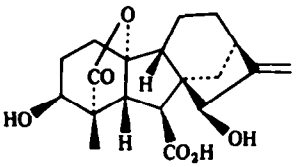


Fig. 1 Distribution of gibberellin activity (barley endosperm α-amylase bioassay) in 1/50 dilution of the *T. majus* embryo extract eluted from a silica gel column. GA, gibberellic acid; - - -, GA-activity from *T. majus* suspensor (data already published in ref. [8]).

46–49) were combined, silylated and analysed by analytical and preparative GC. In each sample, only one peak from preparative GC showed biological activity.

GC-MS analysis of the biologically active peaks showed that they both contained the same TMSi derivative. Furthermore, in each case, the spectra of the MeTMSi derivative prepared by sequential methylation and silylation of the hydrolysed TMSi derivative isolated by preparative GC was identical to that of GA_{63} (1) MeTMSi (MacMillan, J., personal communication).



1 GA_{63}

The amounts of GA_{63} in the suspensor and embryo were measured by GC (GA_1 as standard). The suspensor was found to have a higher level (22 μg/g fr. wt) than the embryo (6.4 μg/g fr. wt). These quantitative data are in accord with the results obtained for the embryo-suspensor of *P. coccineus* [9–11].

It has been observed [12] that species characterized by massive suspensors show little endosperm development; it has been suggested, therefore, that in these species the suspensor might take over the function of the endosperm.

EXPERIMENTAL

Plant material. Six hundred suspensors (236 mg fr. wt) and embryos (932 mg fr. wt) were isolated under a stereoscopic microscope from developing seeds of *T. majus* and immediately frozen at −20° prior to extraction. Extraction, solvent partitioning, silica gel partition chromatography were performed as described previously [8, 9].

GC and GC-MS analysis. Silica gel fractions showing gibberellin-like activity in the barley endosperm bioassay were dried and trimethylsilylated with pyridine–hexamethyldisilazane–trimethyl chlorosilane (5:1:1) and subjected to GC analysis. FID-GC: glass column (150 × 0.4 cm i.d.) packed with 3% OVI on chromosorb Q (100–200 mesh), N_2 40 ml min^{-1}, 200–300° at 6° min^{-1}. For preparative analysis, a portion of the column eluate (about 70%) was collected using a micropreparative attachment, whilst the remainder was detected by the FID.

GC-MS was performed on Hewlett-Packard 5992 B equipped with jet separator. MS: 70 eV; GC: 2% OVI (90 × 0.2 cm i.d.), He 25 ml min^{-1}, oven temp. 200–300° at 4° min^{-1}. GA_{63} TMSi derivative: *m/z* (rel. int.): 564 $[M]^+$ (12), 549 (6), 447 (15), 446 (42), 223 (5), 73 (100); GA_{63}MeTMSi derivative: *m/z* (rel. int.) 506 $[M]^+$ (40), 491 (14), 447 (7), 446 (16), 287 (16), 282 (12), 223 (13), 73 (100).

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