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KAURENE AND KAURENOL BIOSYNTHESIS IN CELL-FREE SYSTEM OF *PHASEOLUS COCCINEUS* SUSPENSOR

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Key Word Index—*Phaseolus coccineus*; Leguminosae; suspensor: gibberellic acid biosynthesis; *ent*-kaur-16-ene; *ent*-kaur-16-en-19β-ol; squalene.

Abstract—It is shown that suspensor tissue of *Phaseolus coccineus* can biosynthesize *ent*-kaur-16-ene and *ent*-kaur-16-en-19β-ol, two key precursors in the biosynthesis of gibberellins.

INTRODUCTION

Recent studies indicate that the suspensor plays a fundamental role during early embryo development [1, 2]. The suspensor, which originates from the first division of the zygote, is present in the seed until it reaches 12 mm in length; it then undergoes a degenerative process ending with its disappearance in 16 mm seeds. The seeds may then grow in the absence of suspensor. Studies performed with *Phaseolus coccineus* embryo–suspensor system cultivated *in vitro* showed that when the early embryo was deprived of suspensor it died [2].

One of the physiological functions of the suspensor could be the synthesis of growth substances [3–5]. Most of the work on identification and metabolism of gibberellic acids in whole seeds of *P. coccineus* was carried out in the period 1960–1970 [6–9]. More recently, however, the presence of these hormones and of cytokinin-like substances in the embryo–suspensor system was ascertained [10, 11] and work in progress shows that only one gibberellin-like substance is present in suspensor taken from 4 mm seeds.

From the above evidence, the suspensor seems to carry out the vital function of hormonal biosynthesis, but as present knowledge on GA biosynthesis in higher plants is based almost exclusively on investigations of immature seeds, it is not known whether the suspensor is a site of GA biosynthesis. Therefore we decided to perform some experiments in order to assess the GA biosynthetic potential of suspensor.

RESULTS

A cell-free extract was prepared from 1000 suspensors (200 mg fr. wt) of *P. coccineus* taken from 5–10 mm seeds and incubated with mevalonic acid-[2-^{14}C] (4×10^6 dpm). The EtOAc-soluble fraction extracted from the incubation medium was purified by TLC using hexane as solvent. Scanning for radioactive substances after hexane development showed two radioactive peaks co-chromatographing with squalene and *ent*-kaur-16-ene standards (respectively R_f 0.6–0.8). Of the total incorporation of 8×10^5 dpm, 2.7×10^5 dpm co-chromatographed with *ent*-kaur-16-ene and 2×10^4 dpm with squalene. The *ent*-kaur-16-ene band, after elution with hexane from the Si gel, was silylated and analysed by preparative GLC. The distribution of radioactivity in the preparative GLC chromatogram was determined and it was found that all the labelling was associated with a peak co-chromatographing with *ent*-kaur-16-ene. The radioactive peak was well separated on the GLC analysis and this fact allowed us to obtain a clear MS spectrum. On GC–MS analysis, the MS taken on this peak was identical to that of *ent*-kaur-16-ene run under the same conditions and also showed ^{14}C isotopic abundance at M + 2, M − 15 + 2 and M − 43 + 2.

Redevelopment of the Si gel plate, where the *ent*-kaur-16-ene band had been scraped off, with $CHCl_3$–EtOAc–HOAc (85:15:1), showed two other radioactive peaks. These two peaks were eluted and put through the same analytical procedure as for *ent*-kaur-16-ene. The GC–MS analysis of the more polar peak gave a spectrum which, when compared with the spectrum of standard *ent*-kaur-16-en-19β-ol TMSi ether, showed the same characteristics. The ^{14}C isotopic abundance was particularly evident at M + 2, M − 15 + 2, M − 90 + 2 and M − 103 + 2.

Suspensor enzymatic preparation incorporated MVA-[2-^{14}C] into *ent*-kaur-16-ene at high rate while the squalene biosynthesis was lower. In contrast, endosperm and seed coat cell-free preparation incorporated MVA-[2-^{14}C] only into the squalene-like peak.

DISCUSSION

The above experiments conclusively show that suspensor tissue can biosynthesize *ent*-kaur-16-ene and *ent*-kaur-16-en-19β-ol during the very stage when it supports growth of the embryo [2]. This present work has thus shown the biosynthesis of GA precursors in a single, well defined tissue of the seed.

ent-Kaur-16-ene and *ent*-kaur-16-en-19β-ol are specific

precursors of gibberellins. Although their synthesis does not prove GA biosynthesis, it indicates the potential for it. GA biosynthesis has been investigated extensively in immature seeds but the efforts have been concentrated on the activity in endosperm of *Marah, Cucurbita* and *Ricinus* [12–14] or whole seeds were used as in *Pisum* [15–17]. In contrast, with *P. coccineus* endosperm preparation a high synthesis of squalene and no synthesis of *ent*-kaur-16-ene was found.

EXPERIMENTAL

Cell-free extract. Suspensors or other seed tissue (endosperm and seed coat) were removed from the same batch of seeds (5–10 mm length) of *P. coccineus*, homogenized with 0.05 M phosphate buffer pH 8 with 2.5 mM $MgCl_2$ (1:1 fr. wt/vol.) and centrifuged at 10 000 *g* for 30 min. The supernatant, stored under liquid N_2, was used as the enzyme source throughout. The incubation mixture contained 5 mM $MgCl_2$, 1 mM $MnCl_2$, 5 mM ATP, 10 mM phosphoenol pyruvate, 0.5 mM NADPH, 1 mM MVA-[2-^{14}C] (13 μC/μmol) and cell-free extract which constituted 75% of the total vol. (1.4 ml). The incubation was carried out at 30° for 2 hr. Extraction of the incubation mixture by partitioning 3× against EtOAc was done after stopping the reaction with Me_2CO and adjusting the pH to 3. Purification of extracted products was performed on 0.5 mm TLC plates of Si gel pre-washed with EtOH–HOAc (70:30).

Preparative GLC. Derivatization procedures were used as previously described [18]. Temp. was programmed from 180° (2 min) to 300° at 4°/min. A portion of the column eluate (80%) was collected using a micropreparative attachment [18] and counted for the radioactivity.

GC–MS. Performed on a glass column (4 mm × 1.5 m) packed with 3% OV-1. MS were obtained at 70 eV, a source temp. of 230° and a separator temp. of 250°.

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