The Enzymic Preparation of $^{14}$C-Kaurene

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Summary. Endosperm from immature seeds of *Cucurbita pepo* L. converts $2\cdot^{14}$C-DL-mevalonate to $^{14}$C(-)-kaurene with a yield of nearly 40% of the active isomer. Kaurene is the main product and the only diterpene hydrocarbon which is formed from mevalonate in the system and is therefore easily obtained radiochemically pure. The product was identified by thin-layer chromatography and recrystallization with authentic (-)-kaurene to constant specific radioactivity.

Introduction

Kaurene is a diterpene precursor of gibberellic acid (Cross et al., 1964; Graebe et al., 1965) and probably of other gibberellins as well. A supply of highly labeled $^{14}$C-kaurene was desired for an investigation of its further transformations in higher plants at different stages of development. The chemical preparation of $^{14}$C-kaurene requires unlabeled kaurene, which is difficult to obtain, as a starting material (Cross et al., 1964). The enzymic formation of $^{14}$C-kaurene has, however, been accomplished from the commercially available $2\cdot^{14}$C-mevalonic acid by the use of cell-free systems from immature seeds of *Echinocystis macrocarpa* (Graebe et al., 1965) and *Pisum sativum* (Anderson and Moore, 1967; Graebe, 1968) and from germinating seeds of *Ricinus communis* (West et al., 1967). Kaurene has also been obtained by feeding mevalonate to the dicotyledoneous plant *Stevia rebaudiana* in vivo (Hanson and White, 1968). Of these systems, the one from *Echinocystis* is by far the most efficient in converting mevalonic acid to kaurene, yields of up to 40% (of the active isomer) having been obtained according to reported information (West et al., 1967). Also the *Ricinus* system appears to be very active although no detailed data are available. In comparison, the highest yield from the *Pisum* system that can be calculated from published figures (Anderson and Moore, 1967) is less than 4%. Since immature fruits of *Echinocystis* are difficult to obtain and the plant is difficult to cultivate, a more generally available source was sought and found in another plant of the same family. This paper reports a cell-free system from immature seeds of *Cucurbita pepo*, which also converts up to 40% of the added mevalonic acid to kaurene.

Materials and Methods

Fully grown but still very pale fruits of *Cucurbita pepo* L. were purchased from local dealers or harvested from plants cultivated in the Botanical Garden. The
immature seeds were removed, their tips were cut off, and the semi-liquid endosperm was squeezed out. Five to twelve ml of endosperm were obtained per fruit. The endosperm was homogenized lightly in a glass homogenizer and centrifuged at 35,000 × g for 20 min. The supernatant was used as enzyme preparation. The standard incubation mixture was based on experience with terpenoid biosynthesis in cell-free systems from pea plants (GRAEBE, 1968). In a total volume of 0.2 ml it contained (μmoles):

- 2-14C-DL-mevalonate, 0.04 (spec. act. 4.82 μμc/μmole, 173 × 10² count/min of active isomer);
- MgCl₂, 1;
- MnCl₂, 0.2;
- ATP, 1;
- phosphoenol pyruvate, 2;
- pyruvate kinase, 5 μg;
- and enzyme preparation, 0.15 ml.

Phosphoenol pyruvate and pyruvate kinase were added to regenerate ATP from ADP. The incubation was carried out at 30° for 3.5 hours under a continuous stream of nitrogen to prevent further conversion of the generated kaurene (DENNIS and WEST, 1967). After the incubation, neutral lipids were extracted with acetone and petroleum ether, separated by two-directional thin-layer chromatography (TLC), located by scanning for radioactivity and counted by liquid scintillation with an average counting-yield of 81%, all as previously described (GRAEBE, 1968).

Results and Discussion

The incubation yielded radioactive products, which migrated like kaurene, squalene, geranylgeraniol and farnesol upon TLC (Table 1). It is seen that the major product, in this case 32% of the added mevalonate, migrated like kaurene. Some more (780,000 count/min) of this product was obtained from a 4-ml incubation and identified as (−)-kaurene by the following criteria:

1. Two samples of the radioactive fraction containing approximately 70,000 and 7,000 count/min gave only one radioactive peak each when they were re-chromatographed on silica gel G impregnated with AgNO₃ developed with n-hexane/benzene (7:3). This chromatographic system is known to separate kaurene from several closely related diterpene hydrocarbons (WEST et al., 1967). The peaks of radioactivity coincided perfectly with co-chromatographed authentic (−)-kaurene.

Table 1. Fractionation of radioactive products obtained from mevalonate with the Cucurbita system. The standard incubation mixture (0.2 ml) contained cofactors and 0.04 μmoles of 2-14C-DL-mevalonate (173,000 count/min of the active isomer). The products were separated by two-directional TLC according to GRAEBE (1968)

<table>
<thead>
<tr>
<th>Chromatographic fraction</th>
<th>Incorporation (count/min)</th>
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</thead>
<tbody>
<tr>
<td>Kaurene</td>
<td>56,208</td>
</tr>
<tr>
<td>Squalene</td>
<td>107</td>
</tr>
<tr>
<td>Geranylgeraniol</td>
<td>5,773</td>
</tr>
<tr>
<td>Geranylgeraniol a</td>
<td>6,207</td>
</tr>
<tr>
<td>Farnesol</td>
<td>642</td>
</tr>
</tbody>
</table>

a Second crop, released upon acidification.