Mevalonate Kinase from Etiolated Cotyledons of French Beans

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From a comparison of the pH optima of mevalonate kinase activity in extracts of French-bean tissues, Rogers, Shah & Goodwin (1966) concluded that isoenzyme forms existed in the chloroplast and cytoplasm. The pH optimum of the chloroplast enzyme (pH 7.5) was similar to that of the *Hevea* enzyme (Williamson & Kekwick, 1965) and to those of enzymes from animal sources, but the pH optimum of the cytoplasmic enzyme (pH 5.5) was quite different, though similar to that of etiolated pumpkin cotyledons (Loomis & Battaile, 1963).

Preliminary studies with acetone-dried powder preparations of etiolated cotyledons of French beans (Rogers *et al.* 1966) demonstrated the presence of a phosphatase acting on ATP with an activity 3000 times that of the mevalonate kinase. This phosphatase activity also hydrolysed ADP and mevalonate 5-phosphate. Cotyledon mevalonate kinase was therefore purified, with a view to removing the phosphatase activity. The properties of the cotyledon enzyme were investigated preliminary to a study of the properties and development of the chloroplast enzyme.

Macerated cotyledons from 4–5-day-old dark-grown French-bean seedlings suspended in an equal volume of 0.08M-phosphate buffer, pH 7.0, were centrifuged at 105000g for 75 min. at 2°. The fraction from the scum-free supernatant soluble in 20%-satd. and precipitated by 45%-satd. ammonium sulphate was dissolved in and dialysed against the phosphate buffer, applied to a Sephadex G-200 column and eluted with the same buffer. The active fractions were concentrated, applied to a DEAE-Sephadex column equilibrated with the same buffer and eluted with potassium chloride increasing in concentration linearly to 0.5M; the mevalonate kinase was present in fractions containing 0.1–0.2M-potassium chloride. These combined fractions had a specific activity about 40 times that of the starting material, with a phosphatase activity only 25% of that of the mevalonate kinase.

These preparations had a pH optimum at 7.0 assayed by both spectrophotometric and radiochromatographic procedures (Williamson & Kekwick, 1965) and a K_m for mevalonate of 0.04 mM. The product of the reaction was chromatographically indistinguishable from authentic mevalonate 5-phosphate (Williamson & Kekwick, 1965), and was converted into mevalonate by calf intestinal alkaline phosphatase and into mevalonate 5-pyrophosphate by *Hevea* phosphomevalonate kinase (Skilleter, Williamson & Kekwick, 1966).

In contrast with the results of Rogers *et al.* (1966) the mevalonate kinase from etiolated French-bean cotyledons appears to resemble the *Hevea* enzyme in its kinetic properties more closely than that from pumpkin cotyledons.

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Effect of SKF7997 and SKF525 on Diterpene and Sterol Biosynthesis in *Gibberella fujikori* from [2-¹⁴C]Mevalonate

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The compounds SKF7997 [tris(2-diethylaminoethyl) phosphate] and SKF525 (2-diethylaminoethyl 2,2-diphenylvalerate), which were shown to inhibit phytosterol biosynthesis in *Nicotiana* (Reid, 1968a) and β -amyrin and phytosterol biosynthesis in *Pisum* (Reid, 1968b), were examined for their effect on shake cultures of *Gibberella fujikori* fed on [2-¹⁴C]mevalonate.

Four fractions were isolated: culture acids (A), culture neutrals (B), mycelium acids (C) and mycelium neutrals (D). Fractions (C) and (D) were derived by saponification of the lipids of the mycelium, which contained esters and glycerides; these were greatly increased in the presence of SKF525 and the formation of red pigment also increased with SKF525.

The total incorporations of the precursor were: control, 66.7%; with SKF7997, 60.9%; with SKF525, 54.3%. The largest incorporation was into fraction (A) (47.0%, 44.5% and 26.5% respectively) and fraction (D) (12.8%, 8.3% and 20.0%).

The major labelled components in fraction (D) were kaurene, kaurenol, epimanoyl oxide and material that chromatographed with the methyl sterols and sterols. Mycelia grown with SKF525 showed in addition the accumulation of radioactivity in kaurenol, enhanced accumulation in the sterol groups and in a fraction more polar than the sterols that could be resolved into a major component with the properties of a diol, and in 7-hydroxykaurenolide and 7,18-dihydroxykaurenolide as minor components. The control and

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SKF7997-treated samples contained no kaurenal, but in the SKF7997-treated one the presumed diol accumulated. This compound and the components of the sterol fraction are under investigation.

Fraction (B) contained small amounts of kaurene, epimanol oxide, kaurenol, fujenal and 7-hydroxy-kaurenolide, but the major component was 7,18-dihydroxykaurenolide.

Fraction (A) was a mixture of gibberellins in which gibberellic acid predominated. The results with SKF525 showed that this compound inhibited the conversion of kaurenal into kaurenoic acid, with the accumulation of kaurene, kaurenol and kaurenal in the mycelium; Dennis & West (1967) showed that SKF525 inhibited the conversion of kaurenol into kaurenoic acid in *Echinocystis macrocarpa* Greene. The present studies indicate the importance of examining the total metabolites of *G. fujikori*, not only those in the culture fluid but also those in the mycelium.

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Monoterpene β -D-Glucosides in Roses: Isolation and Formation from DL-[2- 14 C]Mevalonate

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It has been reported (Staikov & Zolotovitch, 1960) that petals of *Rosa damascena* var. Mill contain monoterpenes in non-steam-distillable forms ('bound monoterpenes'). We have shown that the petals of *Rosa damascena* var. Versicolora and of *Rosa dilecta* cultivar Lady Seton also contain 'bound monoterpenes'.

The non-volatile monoterpene derivatives have been isolated from petals of the *Rosa dilecta* cultivar Lady Seton and purified by silica gel chromatography and preparative thin-layer electrophoresis. On hydrolysis either with sulphuric acid or with β -glucosidase, monoterpene alcohols and a monosaccharide were released in equimolar proportions. The monoterpene alcohols have been shown to comprise 80–85% of geraniol, with traces of nerol and citronellol, and the monosaccharide moiety to be D-(+)-glucose. The purified glucosides had identical chromatographic and electrophoretic properties with both enzymically and chemically synthesized geraniol β -D-glucoside. We conclude that the petals of *Rosa dilecta* cultivar Lady Seton contain geraniol β -D-glucoside together with smaller amounts of nerol and citronellol β -D-glucosides.

The formation of free monoterpenes and monoterpene β -D-glucosides has been investigated as a function of flower maturation in the *Rosa dilecta* cultivar. Both free monoterpene and monoterpene β -D-glucoside concentrations increased rapidly when the flowers were opening. The maximum concentration of monoterpene β -D-glucoside occurred approx. 2 days before that of the free monoterpenes, the latter occurring when the flowers were fully open.

Studies on the biosynthesis of free monoterpenes and of monoterpene β -D-glucosides have been carried out on isolated flowers near the stage when they contained maximum concentrations of monoterpene β -D-glucosides. Radioactivity, applied to the peduncles of harvested flowers as dibenzyl-ethylenediamine DL-[2- 14 C]mevalonate, appeared in both free monoterpenes and monoterpene β -D-glucosides within 15 min. About 0.8% of the radioactivity was incorporated into free monoterpenes and 10–11% into monoterpene β -D-glucosides. The flux of carbon atoms derived from mevalonic acid through the monoterpene β -D-glucosides was rapid, reaching a maximum 1 hr. after addition of label and returning to a minimum after 3 hr.

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Isolation of Ubiquinone-11 and Ubiquinone-12 from Green Peppers

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It is well established that ubiquinones-1 to -10 occur in biological materials (Crane, 1965; Daves, Muraca, Whittick, Friis & Folkers, 1967). In the course of investigating the ubiquinone complements of higher-plant tissues, we have isolated ubiquinones-11 and -12 from green peppers.

Green peppers (2 kg.) were deseeded and the lipid was extracted by a routine procedure (Whistance, Threlfall & Goodwin, 1967). The lipid was then fractionated by chromatography on a column of Brockmann grade III acid-washed alumina (Woelm) developed by stepwise elution with light petroleum (b.p. 40–60°) containing increasing amounts of diethyl ether (Whistance *et al.* 1967). The ubiquinone-containing fractions (eluted from the column by 3% and 5% diethyl ether in light petroleum) were examined by our routine thin-layer chromatographic procedures (Whistance *et al.* 1967; Whistance, Dillon & Threlfall, 1969), when it was found that, in addition to ubiquinones-8 (6 μ g.), -9 (94 μ g.) and -10 (1012 μ g.), compounds with the