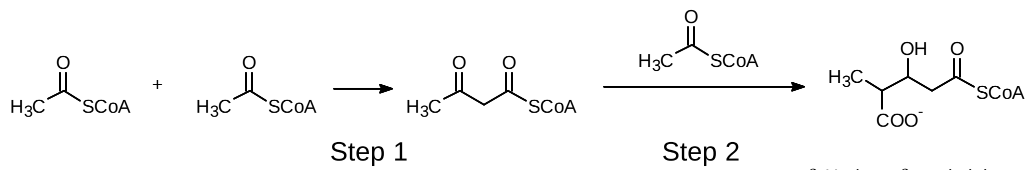
Biology 407b Plant Secondary Metabolism
Assignment No. 2 Biosynthesis of Secondary Metabolites

Solutions

1. In the mevalonate pathway for the biosynthesis of isopentenyl pyrophosphate, 3 units of acetyl-CoA are combined to make the 6-carbon intermediate 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). (Refer to Figure 24-4, Buchanan et al, 2000, Biochemistry and Molecular Biology of Plants). Knowing that this sequence of reactions involves both aldol and Claisen reactions, draw a complete set of reactions illustrating the biosynthesis of HMG-CoA. Be sure to include double-barbed arrows to indicate e$^-$ movement. (Hint: Don't worry about how all the Coenzyme A groups are removed, except the one lost during the Claison reaction involved in the sequence.)

[2 Marks]

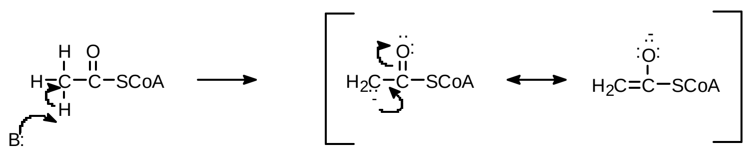
Overall reaction:



β-Hydroxyβ-methylglutaryl-CoA

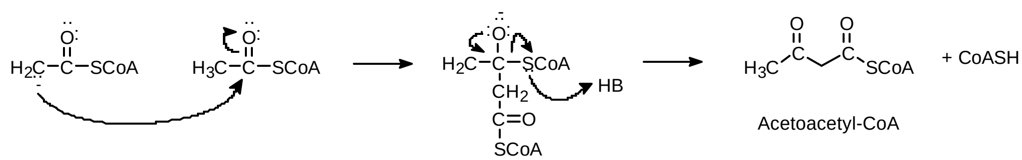
Step 1: Claisen Reaction:

i)



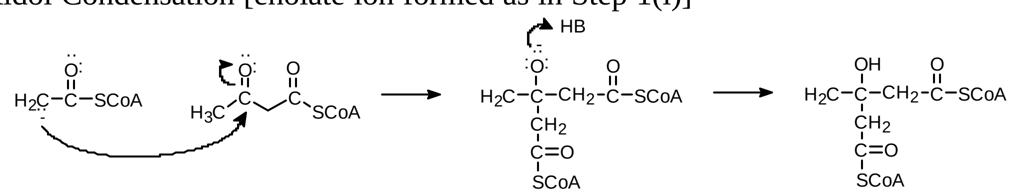
Resonance-stabilized enolate ion

ii)



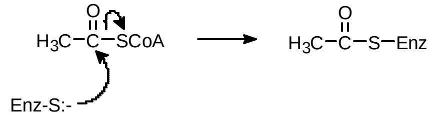
Acetoacetyl-CoA

Step 2: Aldol Condensation [enolate ion formed as in Step 1(i)]

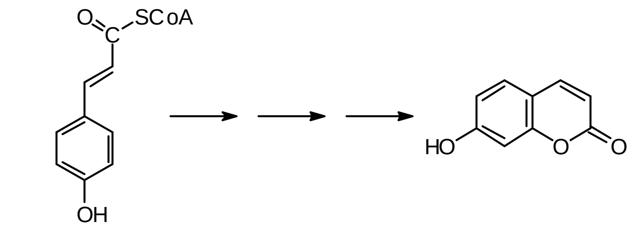


β-Hydroxyβ-methylglutaryl-CoA
(shown as di-CoA ester)

Note: The enzyme catalyzing step 2, HMG-CoA synthase, has an active site cysteine that displaces the CoASH of acetyl-CoA, prior to enolate ion formation (see below). Consequently, after condensation with acetoacetyl-CoA, the product released is the mono-CoA ester HMG-CoA as shown above in the overall reaction.

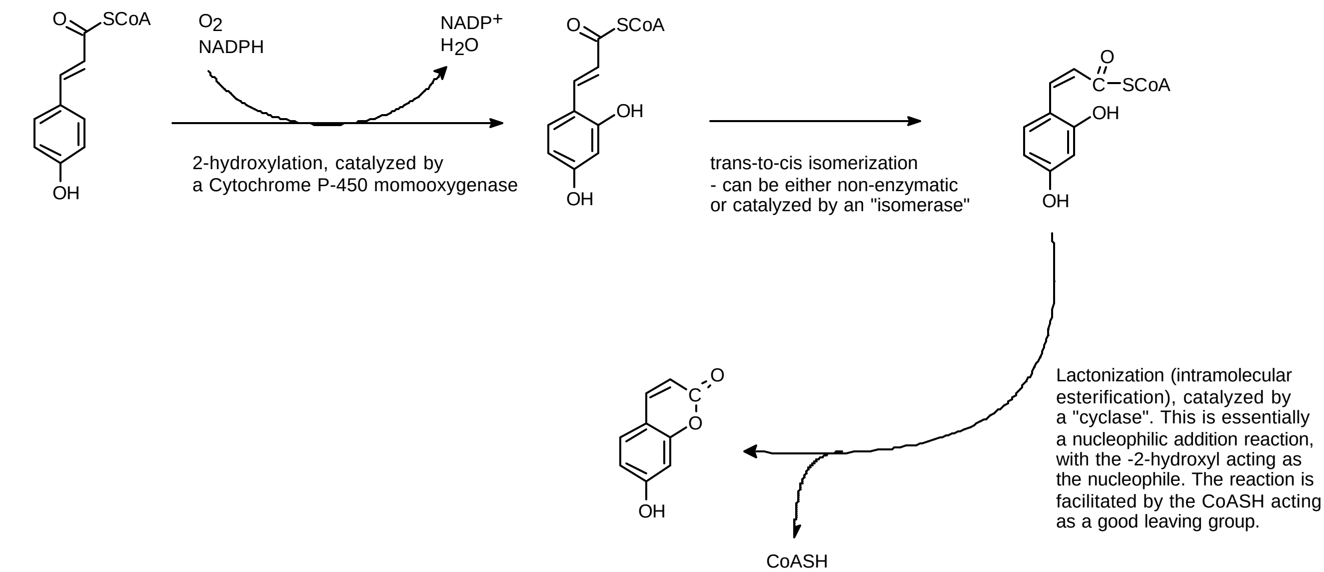


2. For the natural product umbelliferone (a coumarin), suggest a plausible biosynthetic route, starting with *p*-coumaroyl-CoA. You should only need 3 steps. (Hint: *trans* to *cis* isomerization can occur non-enzymatically.) For each step, describe what kind of reaction is taking place, what kind of enzyme is involved and what coenzymes or cofactors are required. (You may need to look beyond your class notes to do this…)

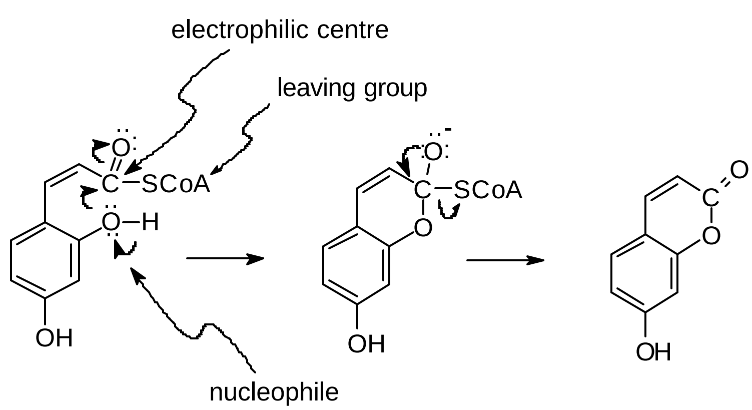


p-Coumaroyl-CoA Umbelliferone

[2 Marks]
Solution:



[Bonus: Predict the e$^-$ flow (using double-barbed arrows) for the cyclization reaction that must take place, and label the reactive components (e.g., electrophile, neutrophile, etc).] [1 Mark]



3. Briefly explain how enzymes are able to catalyze complex chemical reactions under mild conditions (e.g., ambient temperature, neutral pH, dilute substrate concentrations, etc.).

[1 Mark]

Enzymes are able to catalyze complex chemical reactions under mild conditions for several reasons. These include:

a) binding site residues create a 3-D site that allows only certain molecules to bind, sometimes a specifically as a single isomer (e.g., stereo isomer) of a single chemical compound.
b) Once bound, substrates are fixed in a specific orientation, usually with the appropriate reactive groups in close proximity. This has the same affect as increasing the substrate concentration. In addition, the bound substrates are held fast, and have no significant mobility (i.e., they are immobilized by the (usually) much larger enzyme). This has the affect of taking the randomness out of collisions between reactive molecules
c) Acidic and basic amino acid residues within the active site, and in close proximity to bound substrates have the affect of creating an environment with extremes of pH. These side chains can react with bound substrates, effectively removing or donating protons from specific sites.
d) Enzymes can either bind prosthetic groups or recruit co-substrates that enable catalysis of specialized reactions (e.g., FAD, FMN or heme groups)
e) Enzymes can associate with one another, forming a complex (either transiently or more permanently) through which a series of sequential reactions can take place without any of the intermediates being released into the cytoplasm.