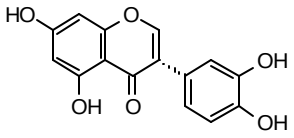
Plant Sciences 407b Plant Secondary Metabolism

Assignment No. 5 Phenolic Biosynthesis

Solutions

1. For each of the following compounds, state the class of phenolic it represents, and describe (point form) its biosynthesis from a hydroxycinnamic acid. [2 marks]



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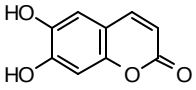

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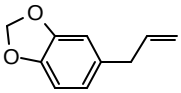

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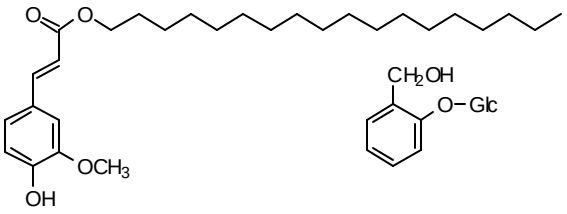
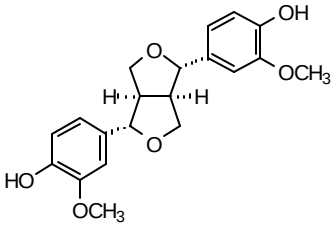


4 **5**



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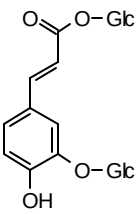

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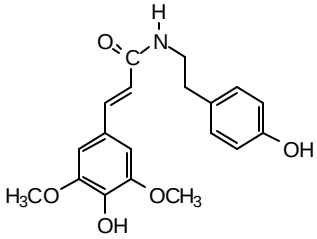

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Note: The enzymes responsible for each step are indicated in parentheses. In some cases the enzyme names are generic or even hypothetical. The enzyme names are added for information only, and are not part of the required answer.

	Compound Class	Parent Hydroxy-cinnamate	Biosynthesis
1	Isoflavonoid	*p*-Coumaric acid	• formation of Co-A ester (4CL), • condensation with 3 x malonyl-CoA (chalcone synthase), • C-ring closure (chalcone isomerase) • rearrangement (isoflavone synthase) • dehydrogenation (2-hydroxyisoflavanone dehydratase) • B-ring hydroxylation (isoflavone-3'-hydroxylase).
2	Coumarin	*p*-Coumaric acid	• formation of Co-A ester (4CL) • hydroxylation at 2-position on ring (cyt P450 monooxygenase) • lactonization (cyclase)

			• hydroxylation of the aromatic ring (cyt P450 monooxygenase)
3	Phenylpropene	Ferulic acid	• formation of Co-A ester (4CL) • side chain reduction to aldehyde (CCR) • side chain reduction to alcohol (CAD) • side chain reduction to propene (dehydratase) • ring closure via methlenedioxy bridge formation (cyclase)
4	Ferulate Ester (Hydroxycinnamoyl ester)	Ferulic acid	• formation of Co-A ester (4CL) • transfer of ferulate to fatty alcohol acceptor (feruloyl-CoA:fatty alcohol feruloyl transferase)
5	Benzyl Alcohol Glucoside	Cinnamic Acid	• formation of Co-A ester (4CL) • side chain reduction to benzoic acid (reductase) • side chain reduction to benzyl alcohol (dehydrogenases, 2-steps) • hydroxylation at position 2 of benzene ring (cyt P450 monooxygenase) • glycosylation at 2-hydroxy position (glucosyl transferase)
6	Lignan	*p*-Coumaric acid (x2)	• formation of Co-A ester (x2) (4CL) • side chain reduction to aldehyde (x2) (CCR) • side chain reduction to alcohol (x2) (CAD) • hydroxylation at 3-position of benzene ring (x2) (Cinnamate-3-hydroxylase) • *O*-methylation (x2) (Caffeoyl *O*-methyltransferase) • oxidation at phenolic hydroxyl (x2) (peroxidase or oxidase) • 8-8' coupling (dirigent protein-mediated) • ring closure to form furan (x2) (spontaneous)
7	Hydroxycinnamoyl glucoside	Caffeic Acid	• transfer of glucose from UDP-glucose to either position carboxylic acid or C-3 hydroxyl (glucosyl transferase) • transfer of glucose from UDP-glucose to either position carboxylic acid or C-3 hydroxyl (glucosyl transferase)
8	Hydroxycinnamoyl amide	Sinapic Acid*	• transfer of glucose from UDP-glucose to the carboxylic acid position (glucosyl transferase) • exchange of glucose for Coenzyme A (sinapoyl glucose:Coenzyme A sinapoyl transferase) • transfer of sinapoyl unit from sinapoyl-CoA to tyramine acceptor (sinapoyl-CoA:tyramine sinapoyl transferase)

*Note: as discussed in class, the formation of Sinapoyl-CoA from sinapic acid has yet to be demonstrated *in vitro*, let alone *in vivo*. However, the formation of tyramine amides from hydroxycinnamoyl-CoA esters has been demonstrated. This sequence is therefore hypothetical but is based on the need to "activate" the carboxylic acid of sinapic acid in order to transfer the sinapoyl unit to a tyramine acceptor. An alternative route would be to form the Co-A ester of a hydroxycinnamate earlier in the general phenylpropanoid metabolism pathway (e.g., *p*-coumarate or ferulate) followed by the appropriate hydroxylation and *O*-methylation to form the sinapoyl carbon skeleton (with a CoA already attached).

2. Describe the synthesis of *p*-coumaroyl-CoA from phenylalanine. Using examples, discuss why *p*-coumaroyl-CoA is considered to be a central intermediate in phenylpropanoid metabolism. [3 marks]

p-Coumaroyl-CoA is biosynthesized from L-phenylalanine through three enzymatic reactions. The first, catalyzed by phenylalanine ammonia-lyase, catalyzes the deamination of L-phenylalanine to form *trans*-cinnamic acid. This enzyme contains an unusual dehydroalanine residue in its active site that binds L-phenylalanine through the aromatic ring, facilitating the elimination of ammonia. Interestingly, this ammonia is recaptured by the GS/GOGAT ammonia assimilation system, and re-used to form new L-phenylalanine via the shikimate pathway. The second enzymatic step in the biosynthesis of *p*-coumaroyl-CoA involves the hydroxylation of cinnamic acid via cinnamate-3-hydroxylase, a cytochrome P-450 monooxygenase to yield *p*-coumaric acid. Finally, *p*-coumaric acid is converted into its coenzyme-A derivative via 4-coumaroyl-CoA ligase, in a reaction involving free coenzyme-A and ATP.

Once formed, *p*-coumaroyl-CoA can undergo many different metabolic fates. Historically, the hydroxylation and *O*-methylation of *p*-coumaric acid to form ferulic acid was thought to proceed via the free acids. More recently, however, evidence has been presented to support the notion that these reactions take place either at the level of *p*-coumaroyl-CoA itself or after either transfer of the *p*-coumaroyl moiety to shikimate (or quinate) or after reduction to *p*-coumaryl aldehyde. Alternatively, *p*-coumaroyl-CoA can serve as the immediate precursor to all the flavonoids, through its condensation with three malonyl-CoA units as catalyzed by chalcone synthase. Similarly, the formation of coumarins is thought to proceed via the 2-hydroxylation and subsequent cyclization of *p*-coumaroyl-CoA, with hydroxylation, methylation and/or glycosylation reactions occurring after the coumarin ring structure is formed. Also, any *p*-coumaroyl derivatives (e.g., esters, amides), or phenylpropenes such as chavicol, would likely be formed from *p*-coumaroyl-CoA.

From the examples above it can be seen that *p*-coumaroyl-CoA has several competing metabolic fates. Thus, from a biosynthetic pathway perspective, it can be argued that it is a central intermediate in phenylpropanoid metabolism. Arguably, not all of the reactions/pathways described above would be occurring in the same cell, but this does not diminish the point that *p*-coumaroyl-CoA is involved in each of them.

[Note: Your answer may differ from this while still being substantially correct, if the point is made (with examples) that *p*-coumaroyl-CoA has several different metabolic fates. A complete answer should have at least two or three examples…]