PLANT BIOCHEMISTRY

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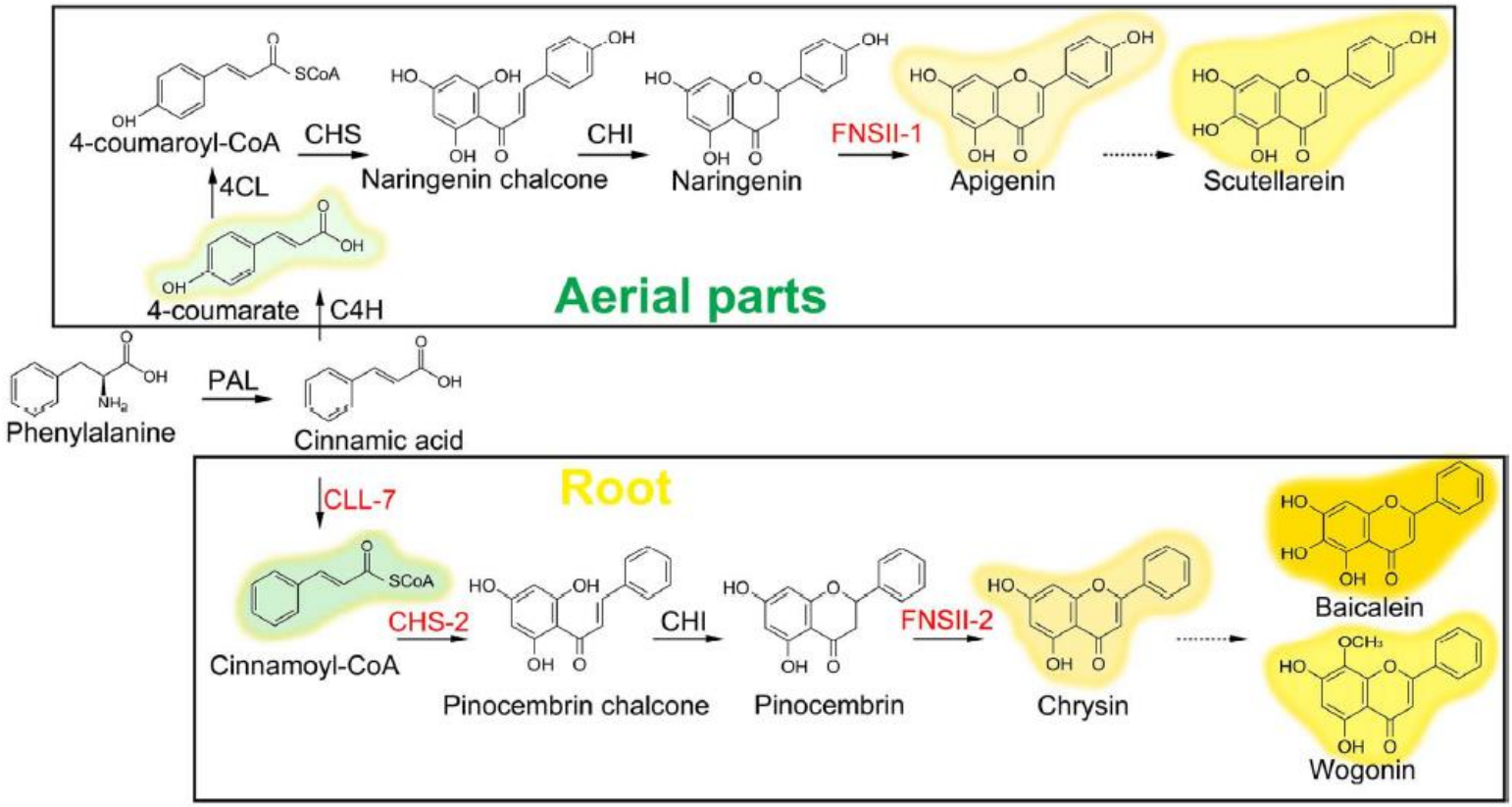
A specialized flavone biosynthetic pathway has evolved in the medicinal plant, *Scutellaria baicalensis*

Qing Zhao,[1,2,3,4] Yang Zhang,[2]* Gang Wang,[3] Lionel Hill,[2] Jing-Ke Weng,[5] Xiao-Ya Chen,[1,4,6] Hongwei Xue,[1,4] Cathie Martin[2,4]†



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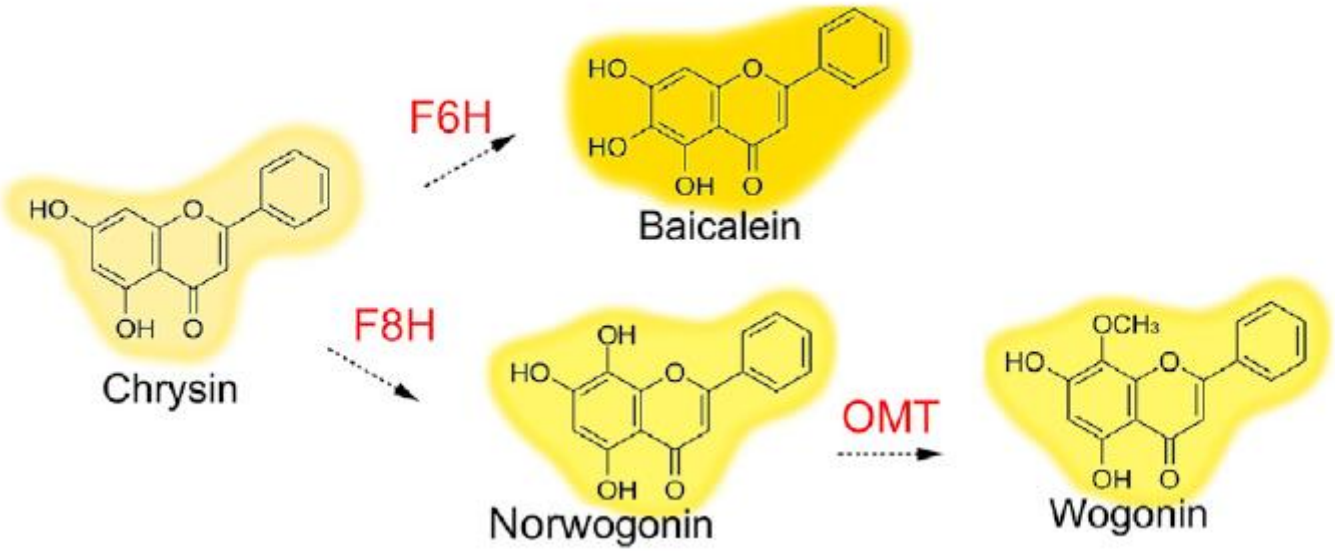
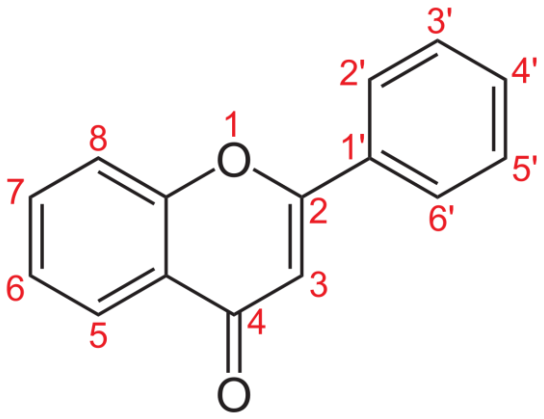
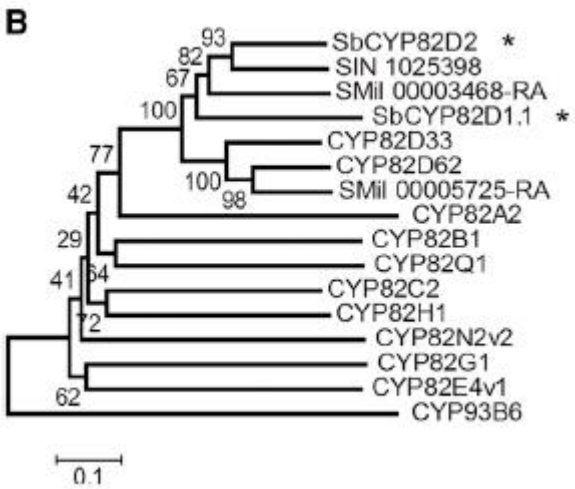
Two CYP82D Enzymes Function as Flavone Hydroxylases in the Biosynthesis of Root-Specific 4′-Deoxyflavones in *Scutellaria baicalensis*

Qing Zhao[1,2,7], Meng-Ying Cui[1,7], Olesya Levsh[3,4], Dongfeng Yang[1,5], Jie Liu[1], Jie Li[2], Lionel Hill[2], Lei Yang[1], Yonghong Hu[1], Jing-Ke Weng[3,4], Xiao-Ya Chen[1,6] and Cathie Martin[1,2,*]

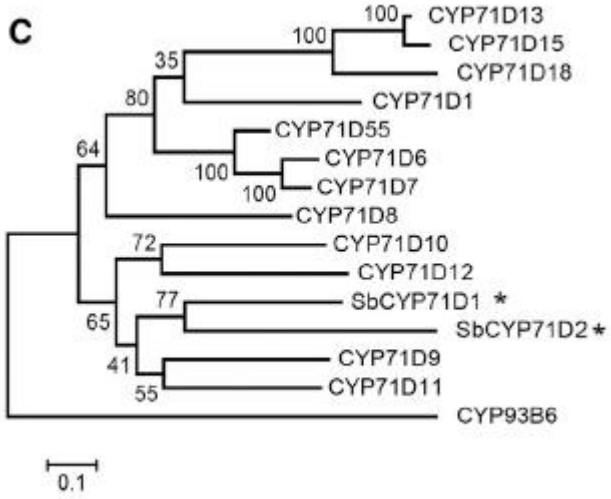




F6H in basil + mint



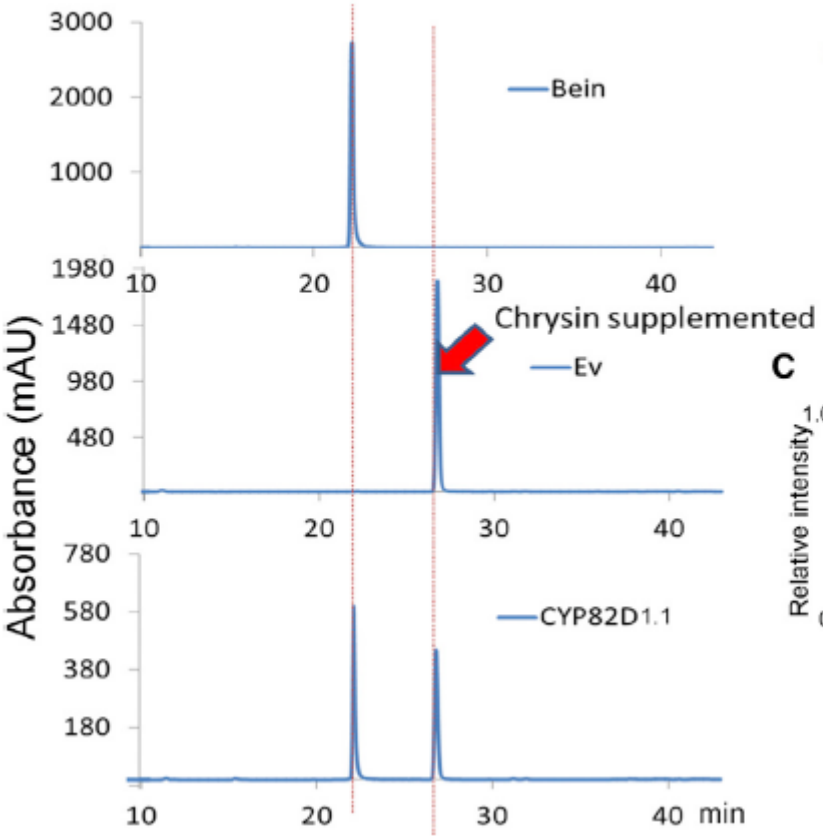
F6H in soybean



Four candidates:
SbCYP82D2
SbCYP82D1.1
SbCYP71D1
SbCYP71D2

Each transformed into yeast and fed with chrysin
Only yeast transformed with SbCYP82D1.1 produces baicalein

A



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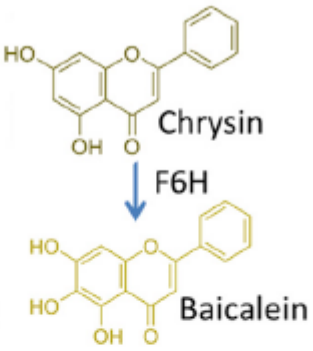

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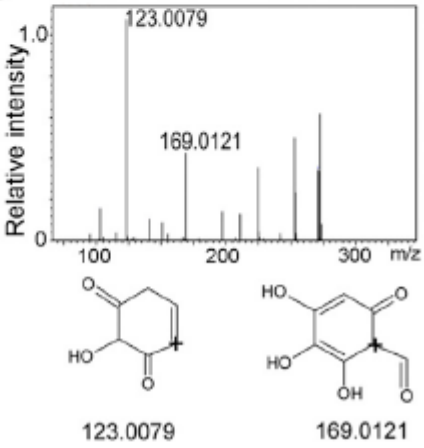


D

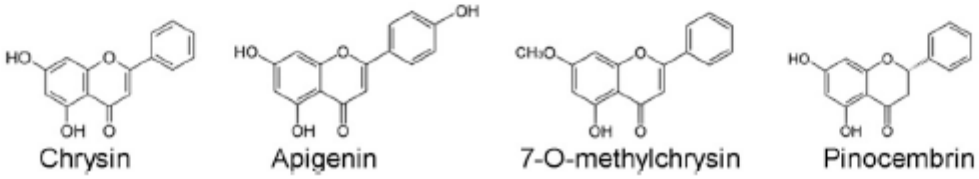
Enzyme	Substrate	Km (μM)	Vmax (pkar mg^{-1} protein)	Vmax/Km
SbCYP82D1.1	chrysin	0.121±0.002	733.047±10.014	6013.508

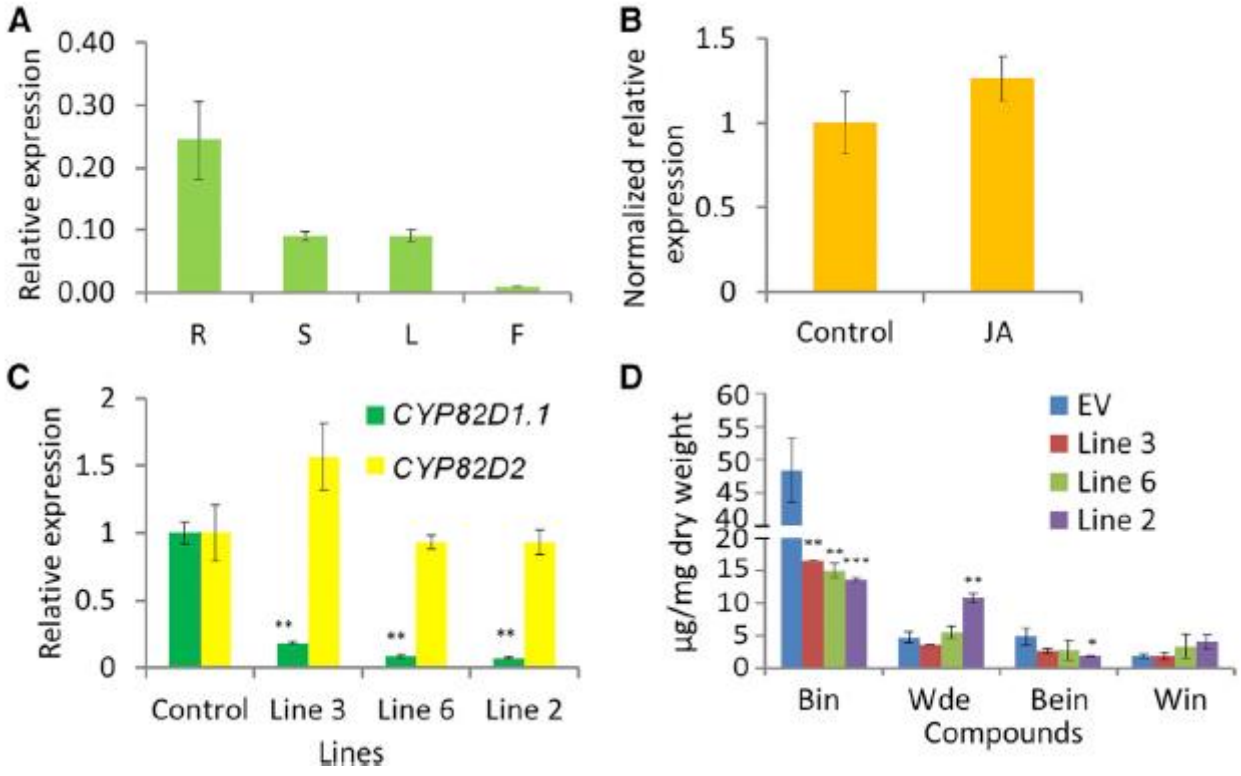
E

Substrates	Chr	Api	7-O-Mechr	Pin
SbCYP82D1.1a	100±0.54	90.43±1.95	85.85±1.52	ND
SbCYP82D1.1b	100±0.56	92.32±1.42	87.94±1.44	ND

[a]Substrates supplied at 5 μM; [b]Substrates supplied at 2.5 μM; ND, not detectable.

F





RNAi silencing

Baicalin

Wogonoside

Baicalein

Wogonin

the plant journal

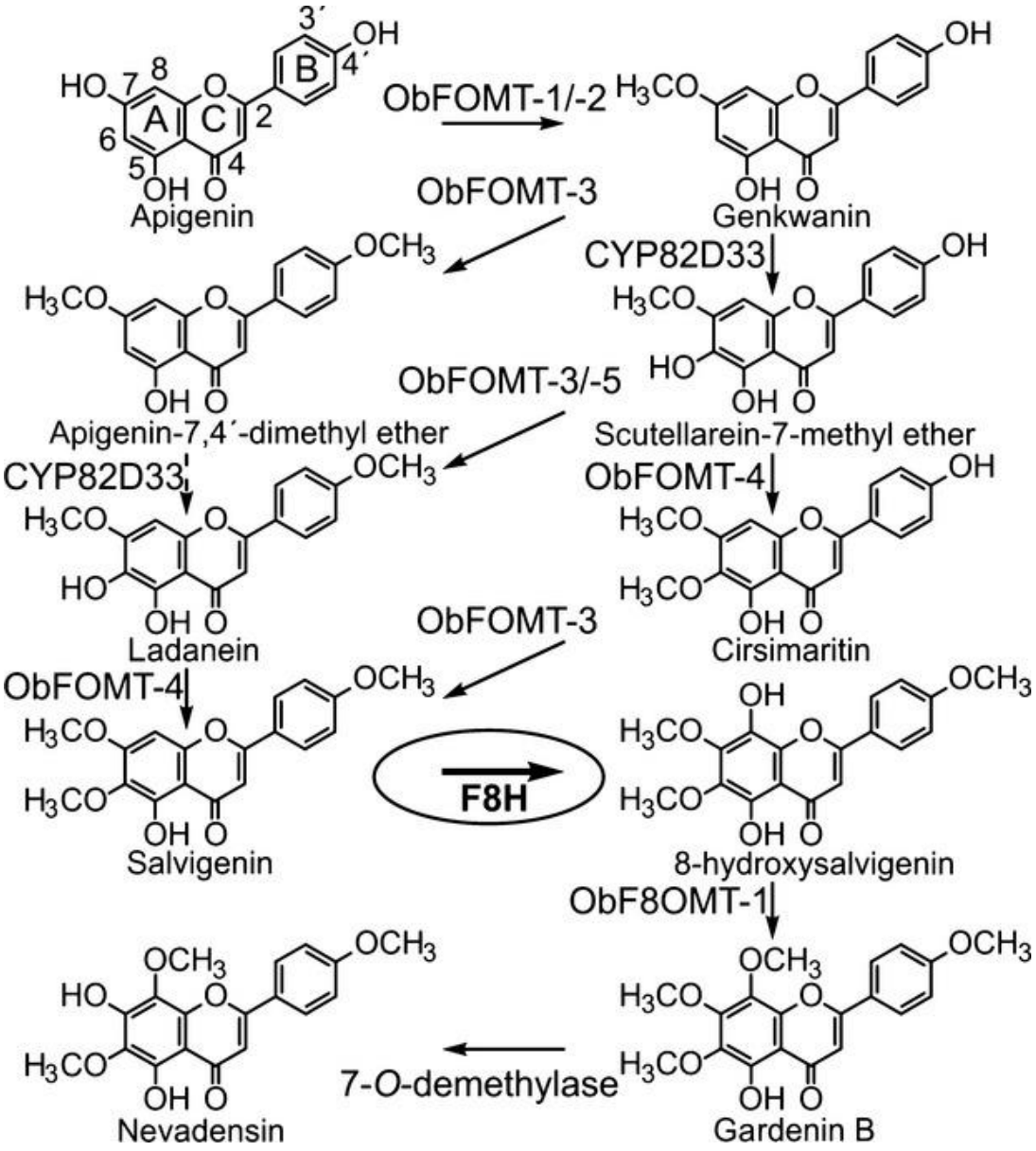
SEB SOCIETY FOR EXPERIMENTAL BIOLOGY

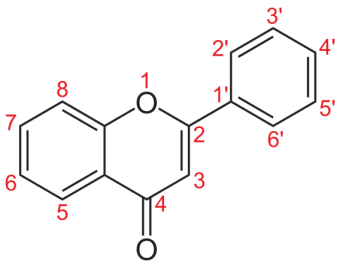
Featured Article | Free Access

Unexpected roles for ancient proteins: flavone 8-hydroxylase in sweet basil trichomes is a Rieske-type, PAO-family oxygenase

Anna Berim, Jeong-Jin Park, David R. Gang

First published: 19 August 2014 | https://doi.org/10.1111/tpj.12642 | Citations: 16





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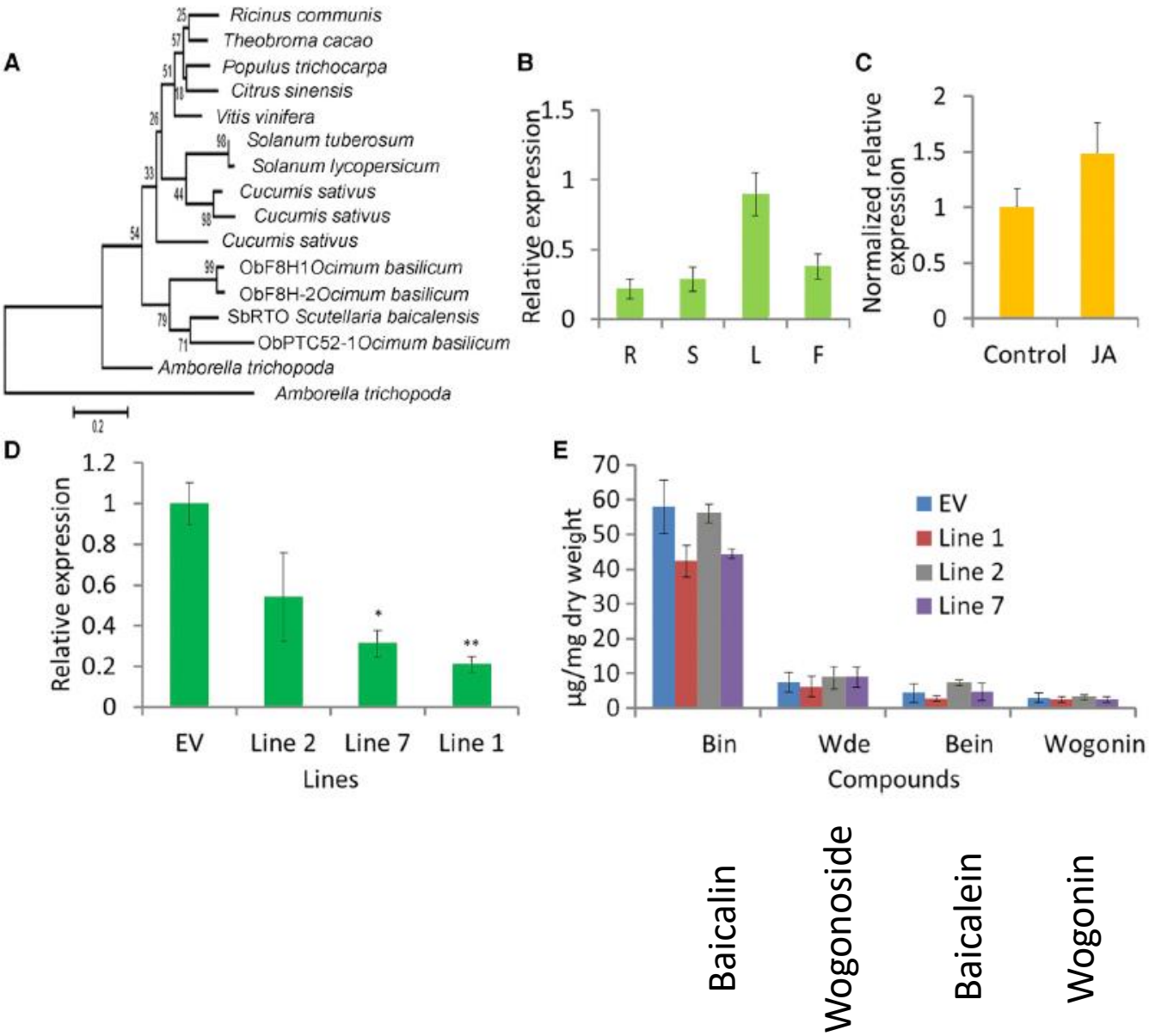
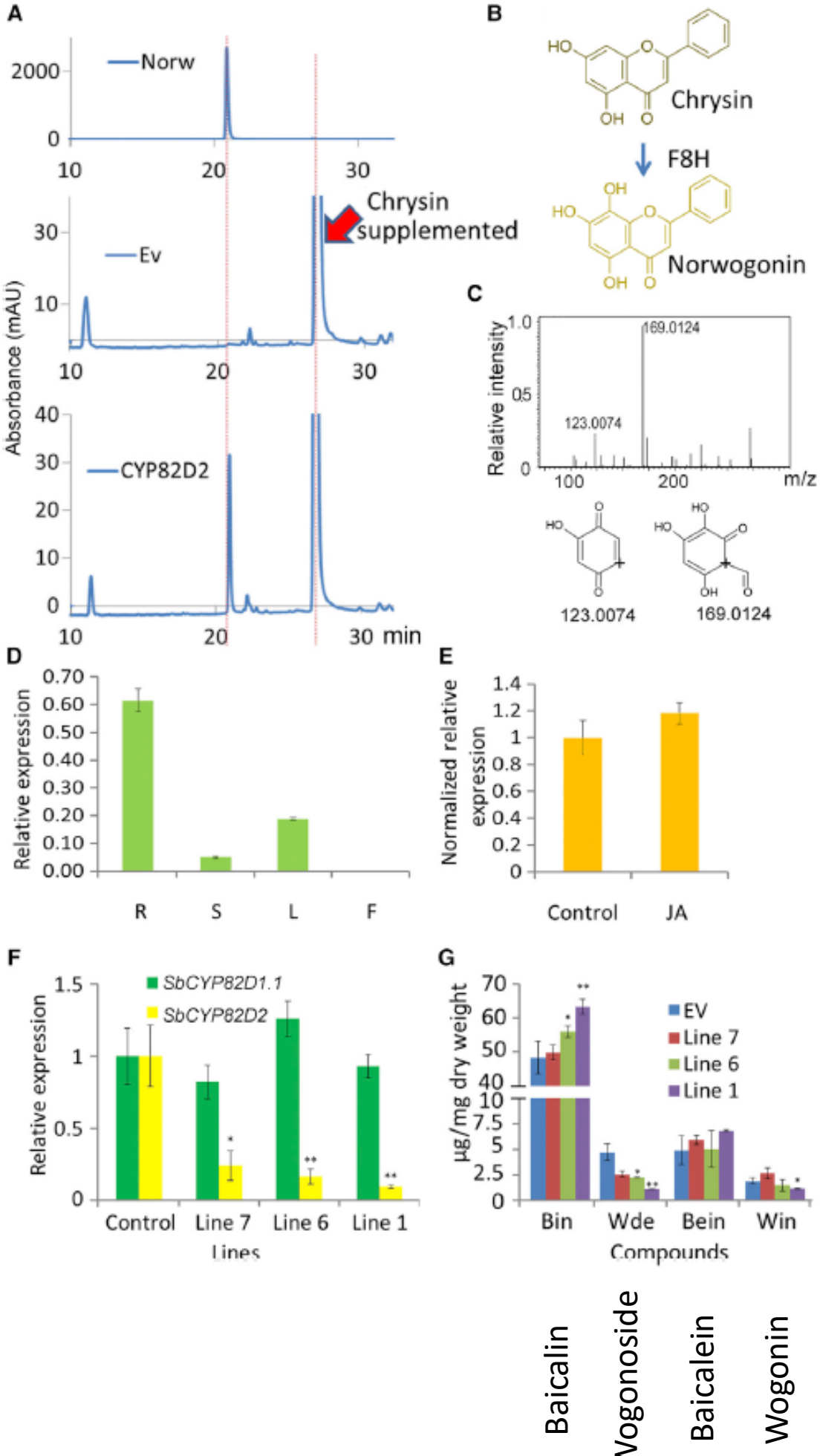
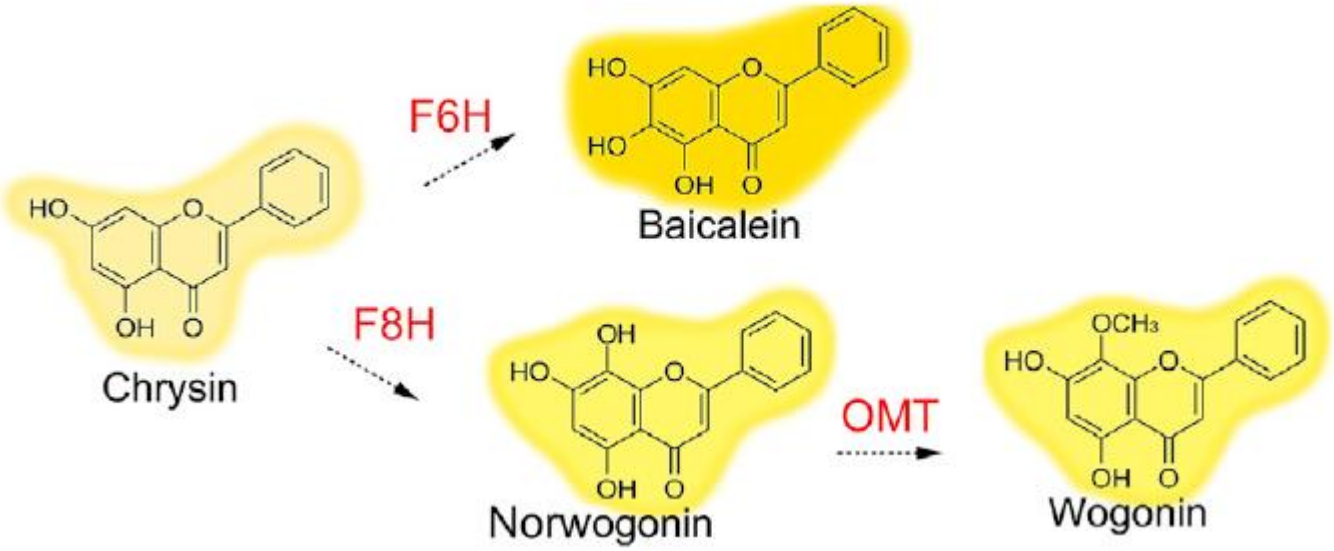
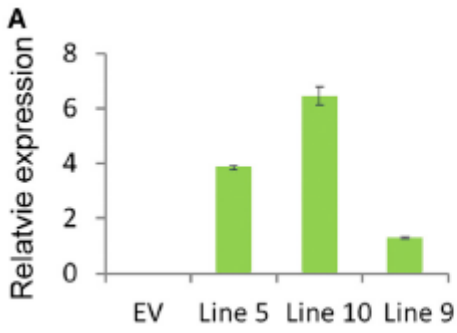


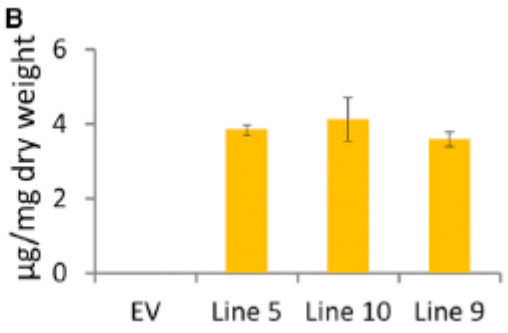

A
Norw
Ev
CYP82D2
Chrysin supplemented
Absorbance (mAU)
min
B
Chrysin
F8H
Norwogonin
C
Relative intensity
169.0124
123.0074
m/z
123.0074
169.0124
D
Relative expression
R
S
L
F
E
Normalized relative expression
Control
JA
F
SbCYP82D1.1
SbCYP82D2
Relative expression
Control
Line 7
Line 6
Line 1
Lines
G
µg/mg dry weight
EV
Line 7
Line 6
Line 1
Bin
Wde
Bein
Win
Compounds
Baicalin
Wogonoside
Baicalein
Wogonin



Overexpression in *Arabidopsis*

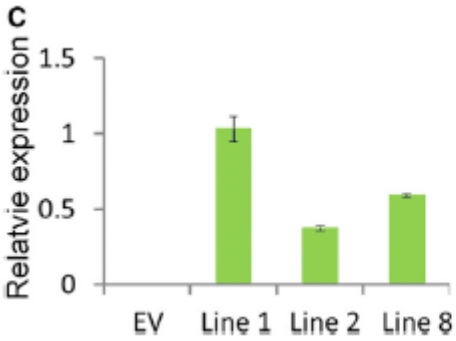
SbCYP82D1.1 (F6H)

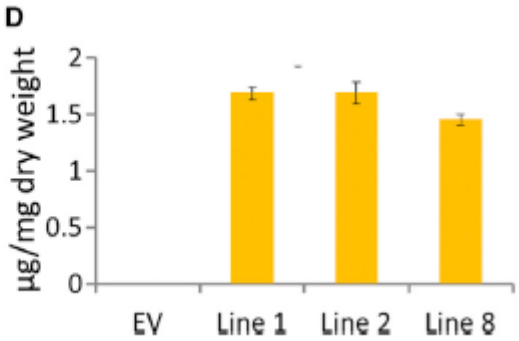




Baicalein

SbCYP82D2 (F8H)





Norwogonin

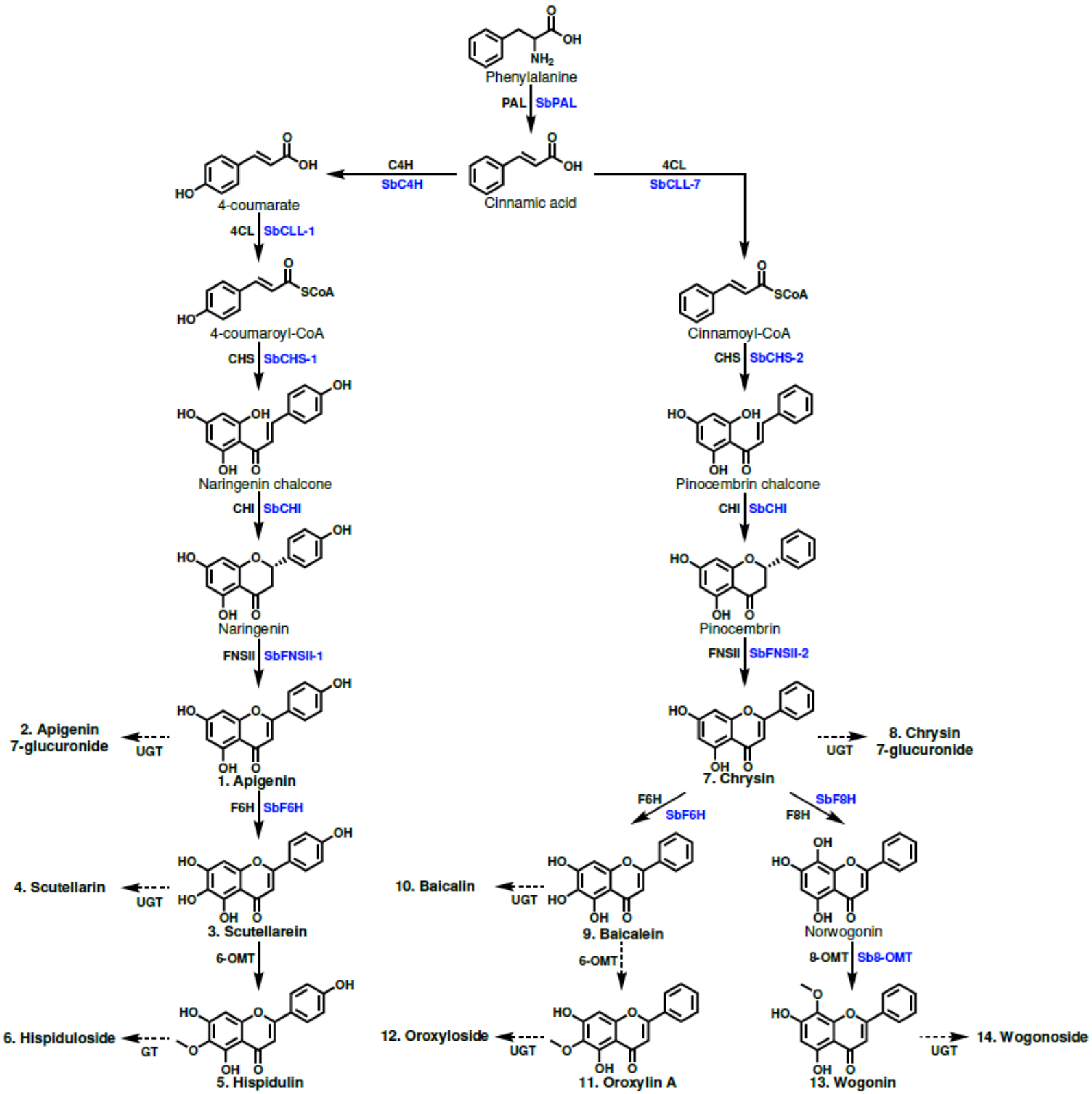


Figure 1. Proposed 4´-hydroxyflavone and 4´-deoxyflavone pathway. Structures of glycosylated metabolites are not shown to save space but are included in Appendix S1. Enzyme names in blue are specific isoforms that have been identified in *S. baicalensis*, and enzyme names in black are general names. Metabolites that were quantified have names in bold and are numbered to match the labeling of Figure 2.

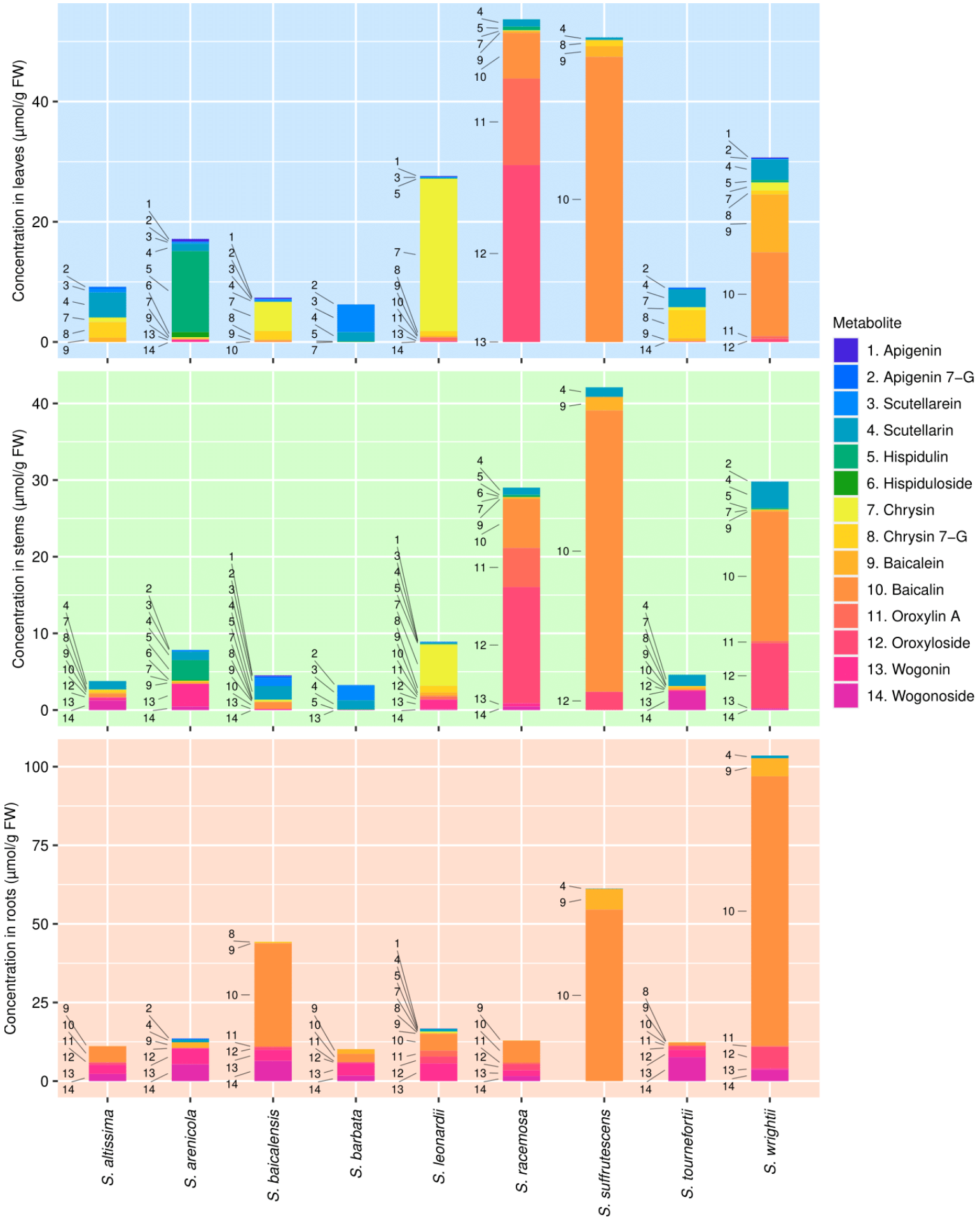


Figure 2. Organ-specific metabolite data collected from 9 *Scutellaria* species via High Performance Liquid Chromatography (HPLC). Samples were taken in biological triplicate, and the average concentration of each metabolite calculated. Metabolites are numbered to match their order of occurrence in the flavone pathway, shown in Figure 1.

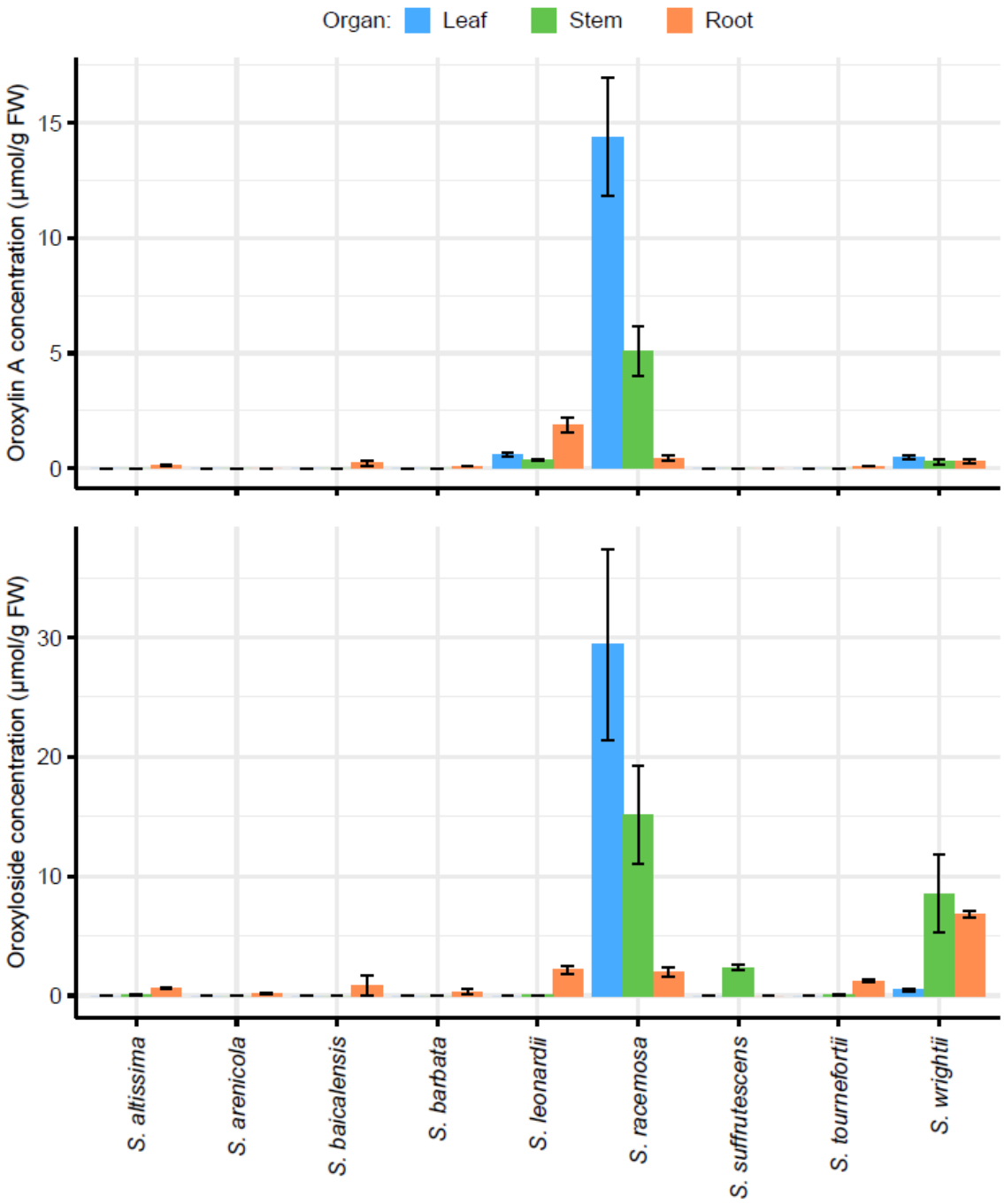
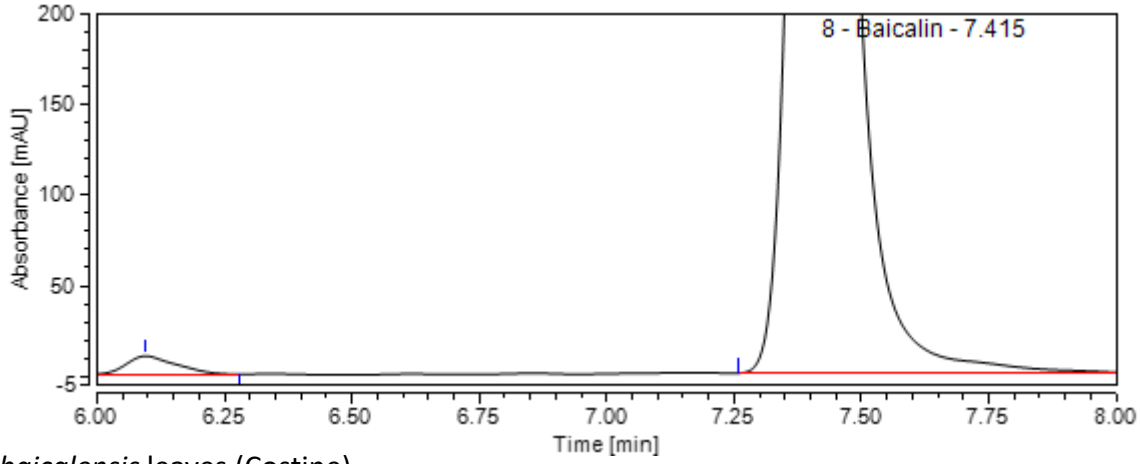
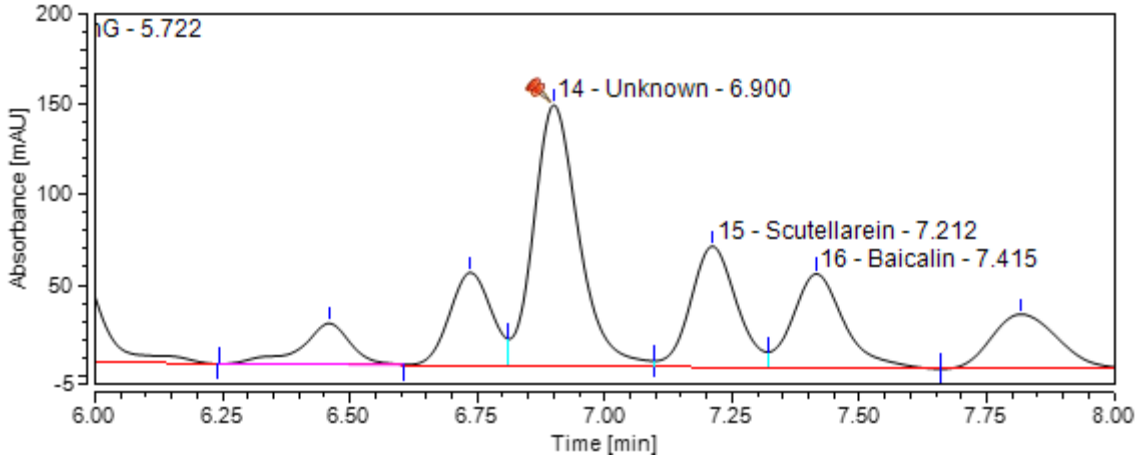


Figure 3. Organ-specific oroxylin A (top) and oroxyloside (bottom) concentrations in 9 *Scutellaria* species, as determined via High Performance Liquid Chromatography (HPLC). Concentrations were averaged from tissue samples taken from 3 biological replicates, and error bars represent standard error.

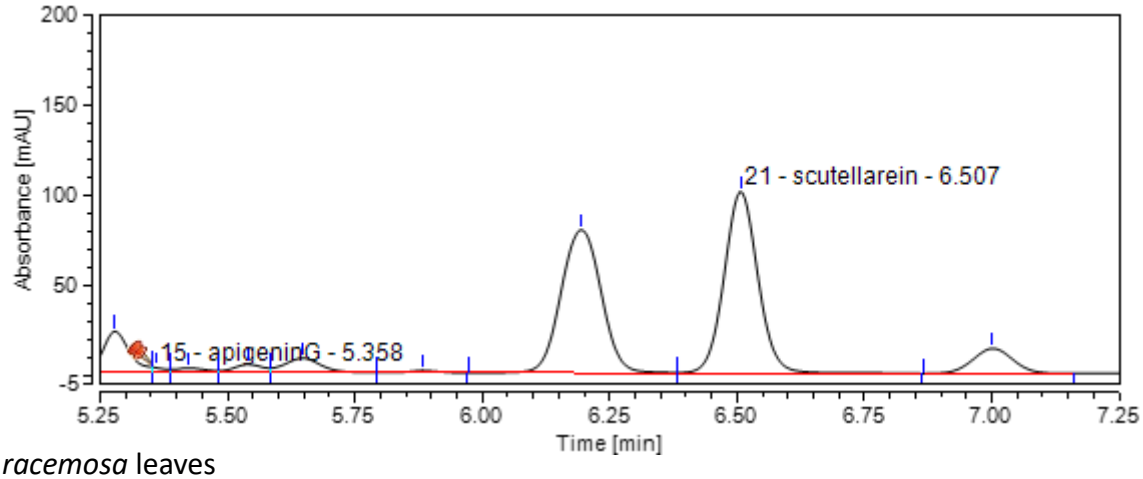
S. baicalensis roots (Costine)



S. baicalensis leaves (Costine)



S. barbata leaves



S. racemosa leaves

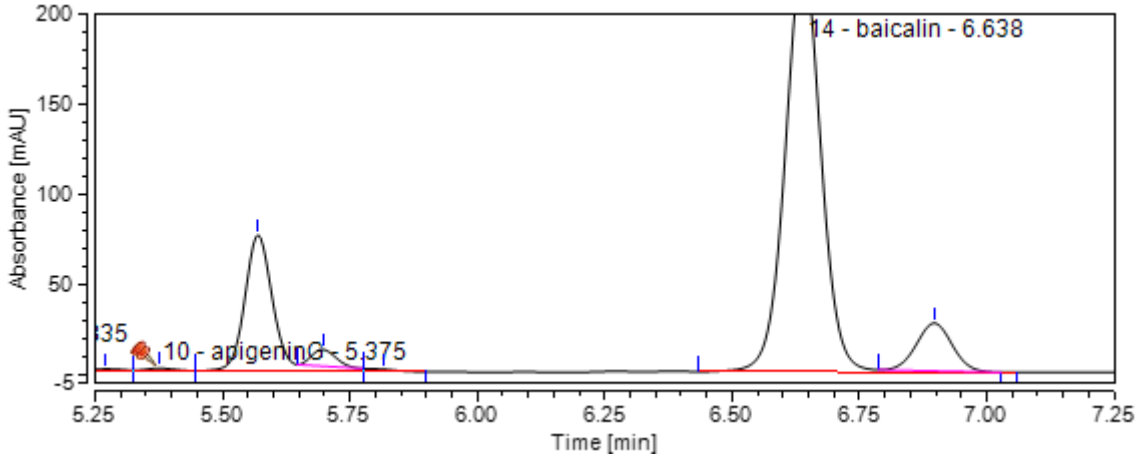
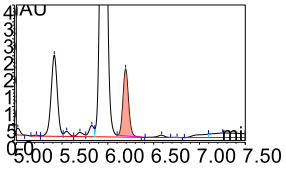
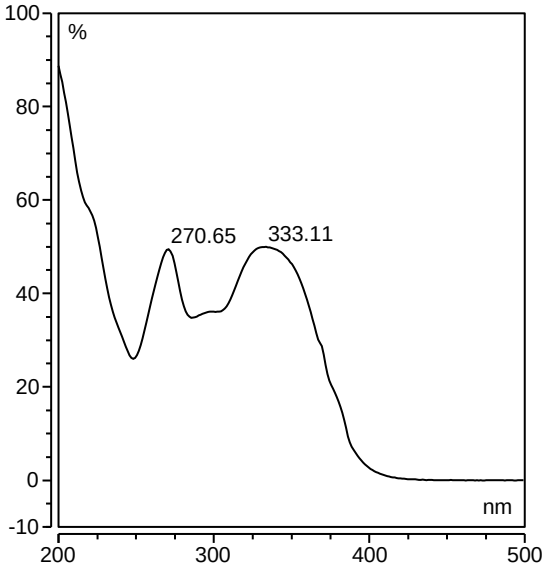


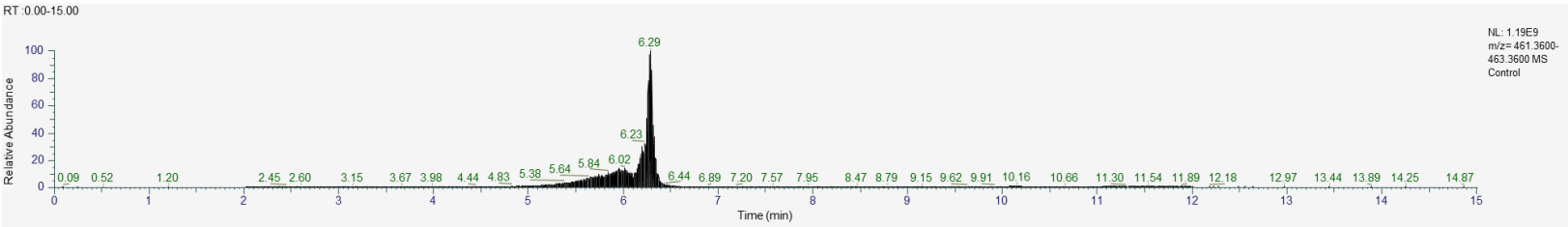
Figure 4. Comparison of chromatograms collected via HPLC from (A) *S. baicalensis* roots, (B) *S. baicalensis* leaves, (C) *S. barbata* leaves, and (D) *S. racemosa* leaves. Time interval displayed was selected to center the unknown peak in the chromatograms.


AU
5.00 5.50 6.00 6.50 7.00 7.50

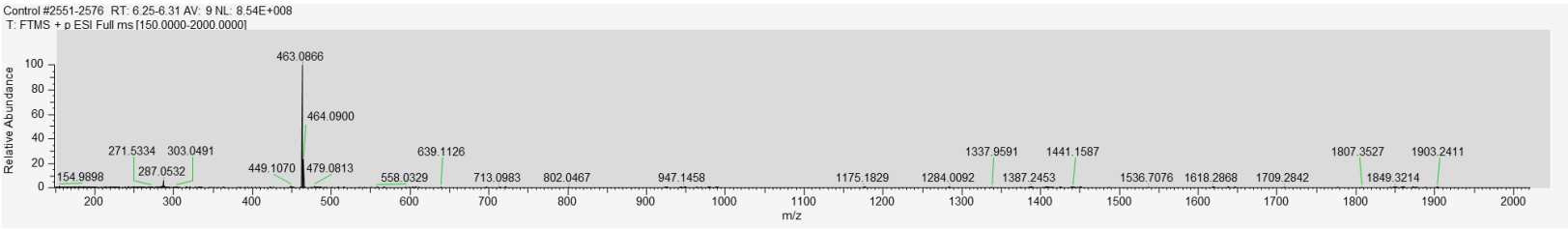

%
270.65
333.11
nm
100
80
60
40
20
0
-10
200
300
400
500

Scutellarin

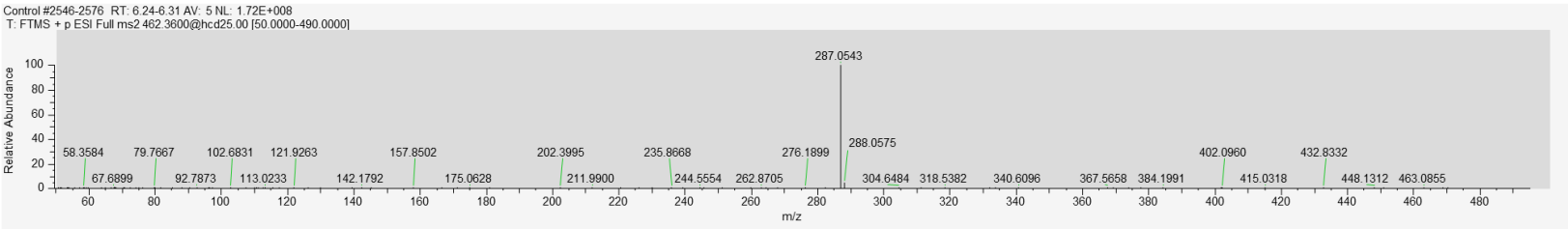
EIC



M/Z

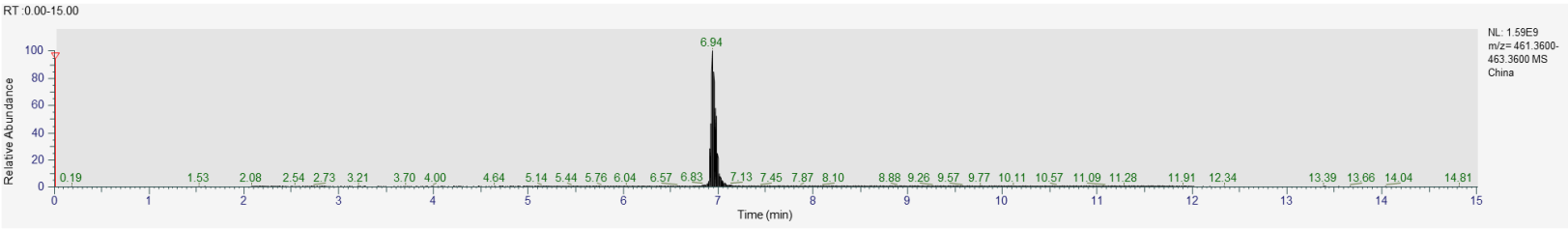


MS/MS



Unknown

EIC



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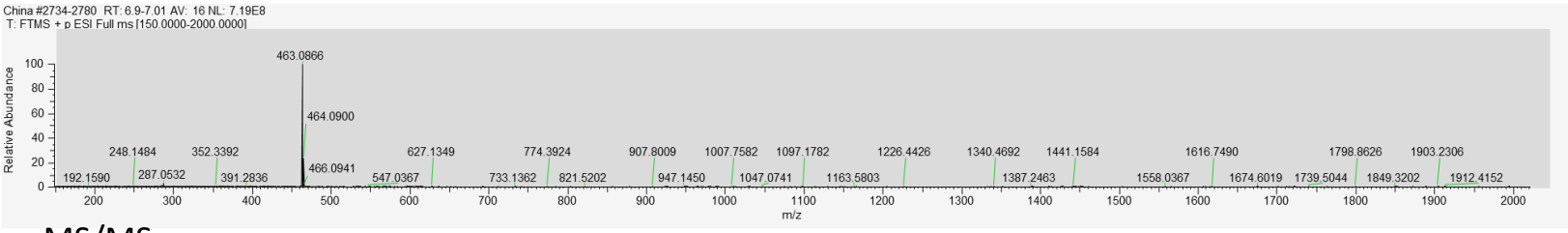

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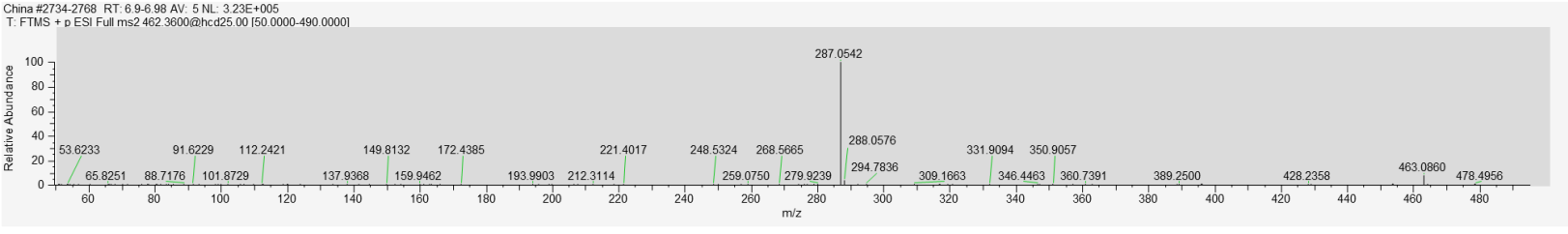
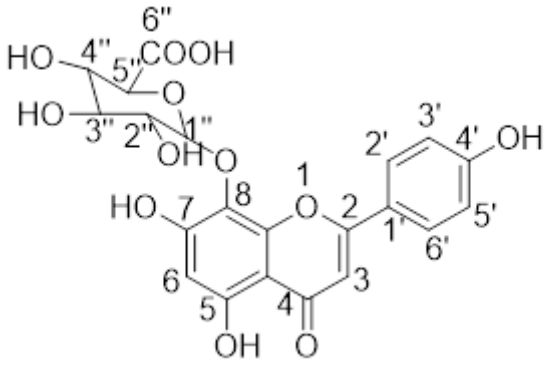
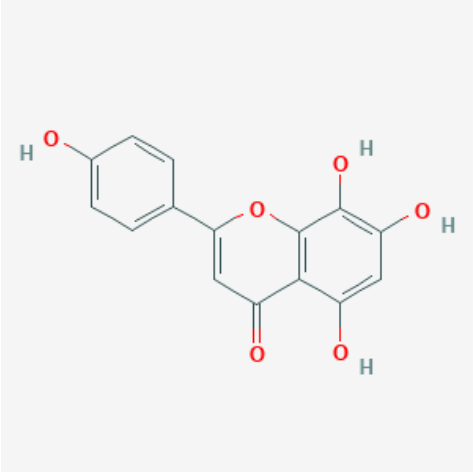


Figure 5. LC-MS + NMR data used to elucidate identity of unknown metabolite.

Predicted structure from NMR data:



Structure from PubChem: Isoscutellarein



Identity of unknown: Isoscutellarein 8-glucuronide

TODO:

Quantify isoscutellarein 8-glucuronide in all extractions

Apigenin feeding

Yeast activity test with Rieske-type oxygenase from *S. baicalensis*

Tobacco infiltration with RTO

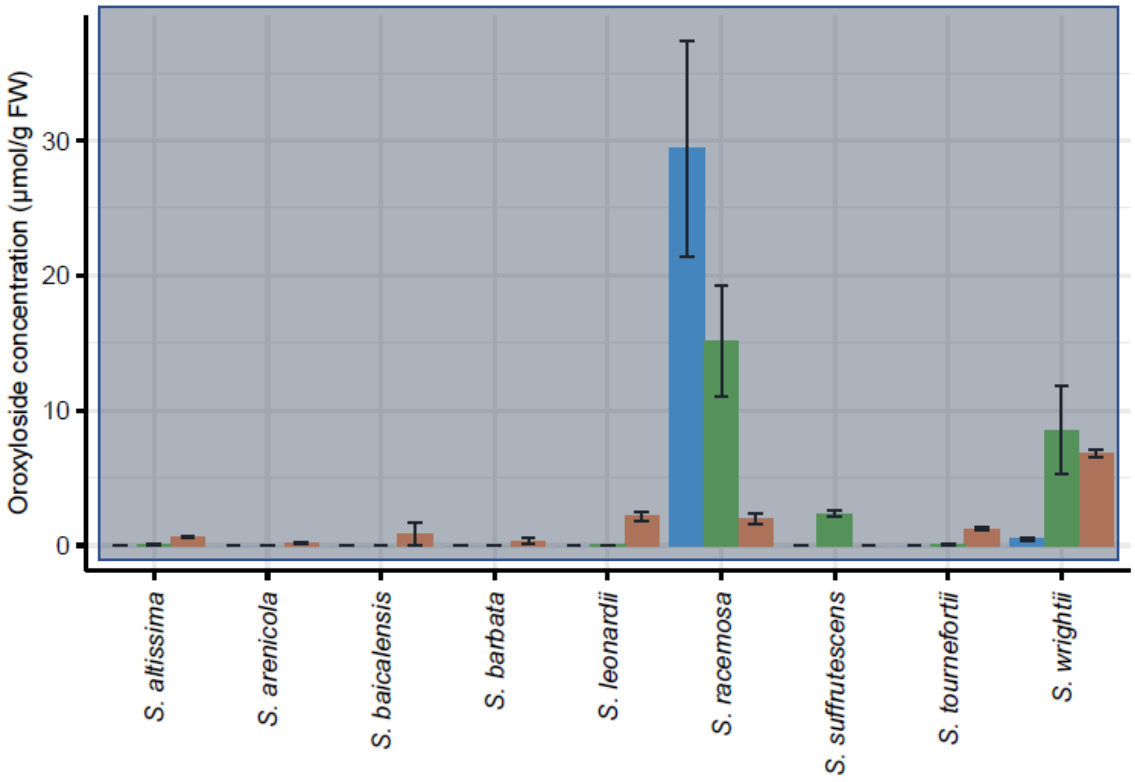


Figure 6. Organ-specific isoscutellarein 8-glucuronide concentrations in 9 *Scutellaria* species, as determined via High Performance Liquid Chromatography (HPLC). Concentrations were averaged from tissue samples taken from 3 biological replicates, and error bars represent standard error.

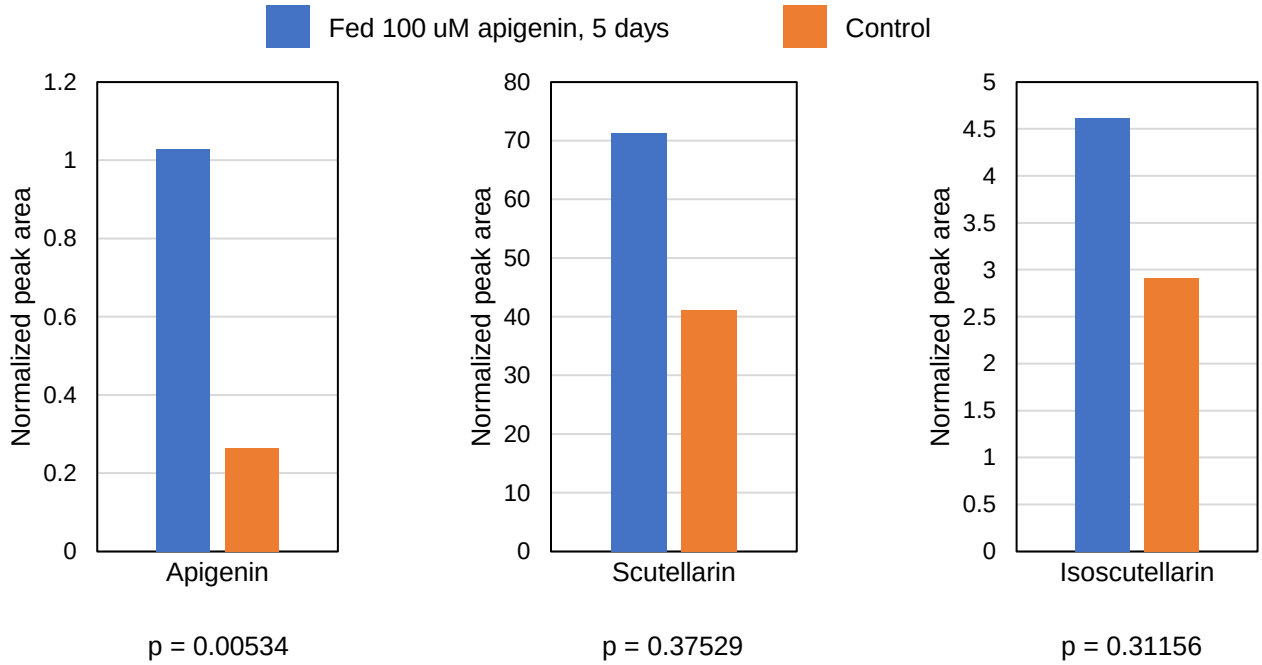


Figure 7. *S. barbata* apigenin feeding results / Yeast activity test / *N. benthamiana* infiltration

Proposed pathway:

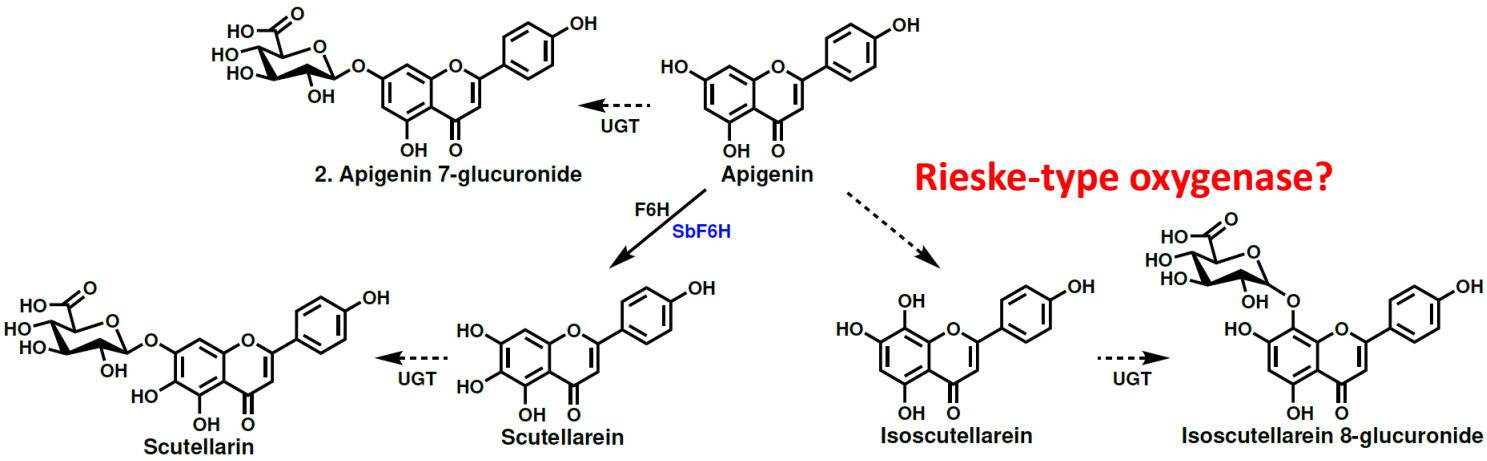


Figure 8. Proposed pathway for biosynthesis of isoscutellarein 8-glucuronide in *Scutellaria*.