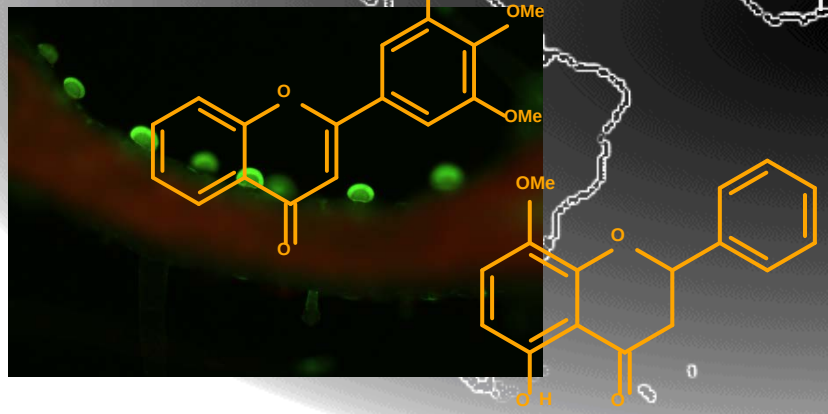
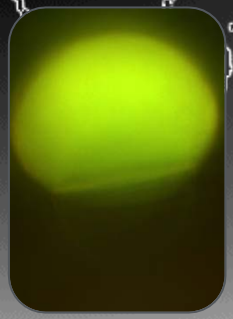
Exudate Flavonoids of *Primula*: Past, Present, Future





XXVIII[th] International Conference on Polyphenols

2016

VIENNA

July 11-15

Valant-Vetschera, K.M., Elser, D., Bhutia, T.D., Wollenweber, E. & Brecker, L.

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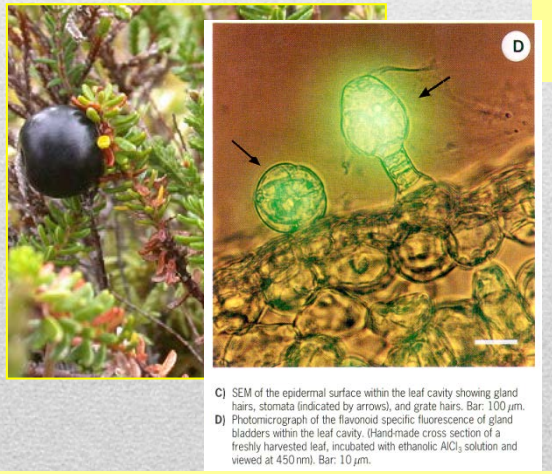
Exudate Flavonoids - Flavonoids on The Surface

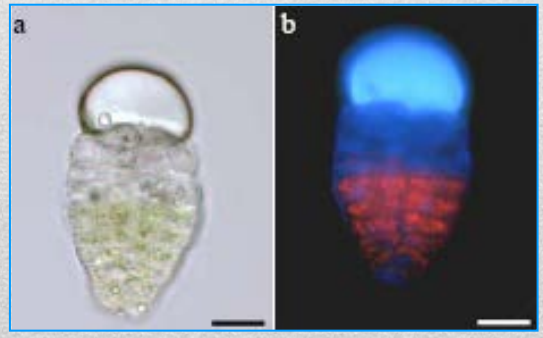
- Secreted and deposited on the epidermis of aerial parts in a resinous (lipophilic) matrix
- Habitat connected (arid/semiarid, alpine)
- Secretion: Respective mechanisms required (secreting glandular hairs, secreting cells and tissues)
- Assumed functions: UV-Protection, herbivore deterrence, antifungal and allelopathic activities
- Rinsing plant material with lipophilic solvent (acetone) >> exudate
- Standard analysis by HPLC-profiling and comparative TLC
- Structure analysis of isolated compounds by MS and NMR

Localization of Exudate Flavonoids



C) SEM of the epidermal surface within the leaf cavity showing gland hairs, stomata (indicated by arrows), and grate hairs. Bar: 100 µm.
D) Photomicrograph of the flavonoid specific fluorescence of gland bladders within the leaf cavity. (Hand-made cross section of a freshly harvested leaf, incubated with ethanolic $AlCl_3$ solution and viewed at 450 nm). Bar: 10 µm.

Wollenweber et al., 1992: Bot. Acta 105, 300-305



Göpfert, J. et al., NPR 2006, Vol. 1, 935 - 940

The genus *Primula* L.

- 450 species
- 75% East-Asia

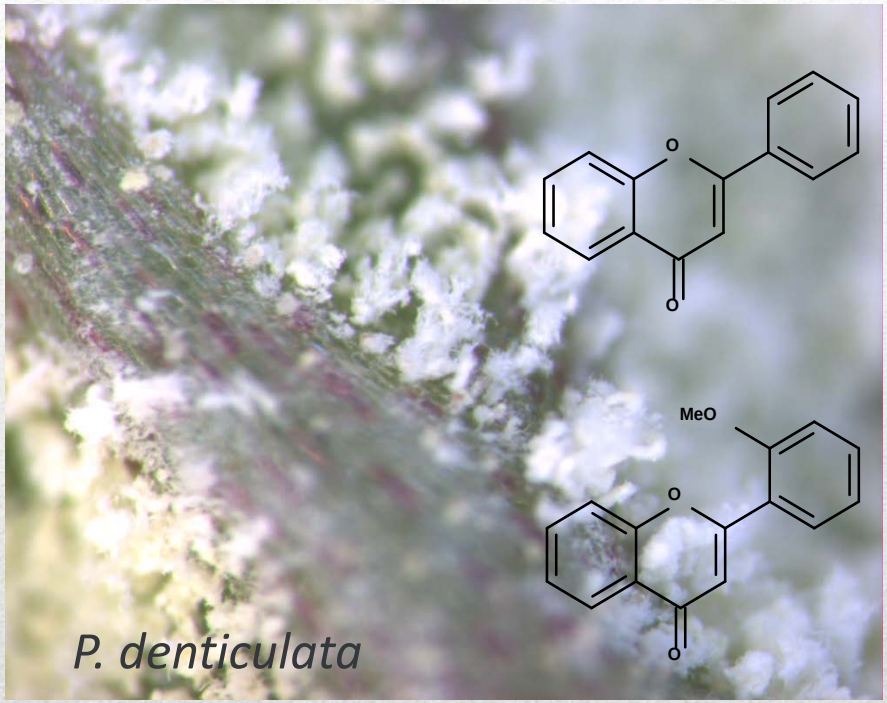
Characteristic features

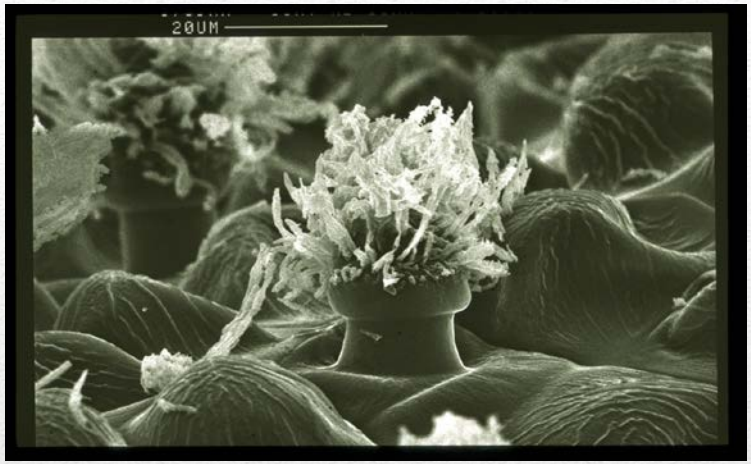
- Floral diversity incl. heterostyly
- Secretions from glandular hairs on aerial parts

Complicated molecular phylogeny not fully resolved

Approx. 150 species studied for exudate composition

Primula-type Flavones: Why and Where ?



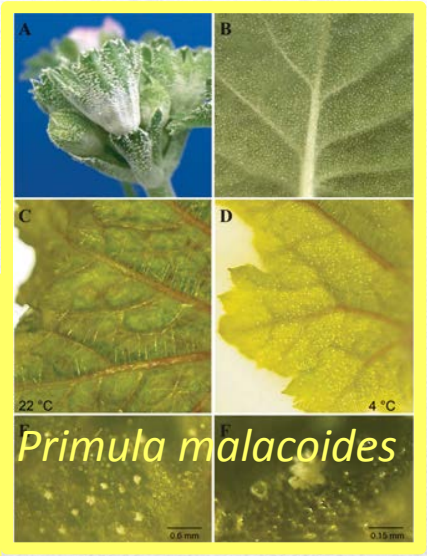


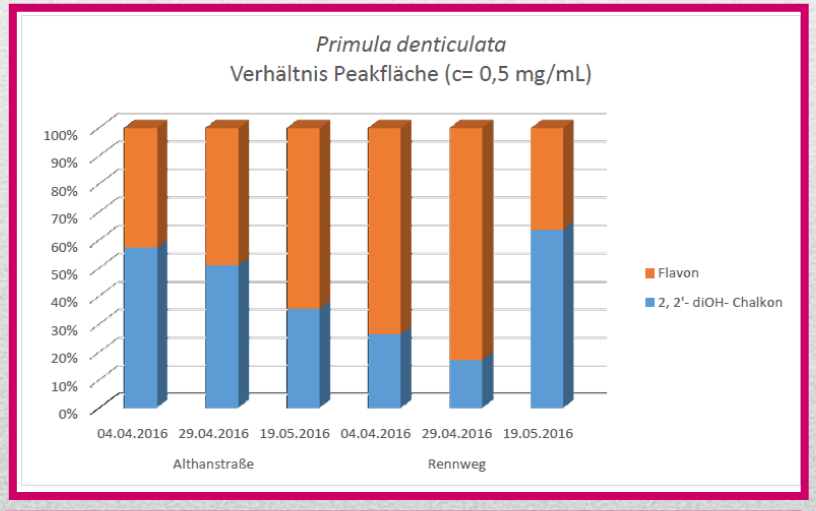
Wollenweber & Schnepf, Vergleichende Untersuchungen über die flavonoiden Exkrete von „Mehl“ und „Öl“-Drüsen bei Primeln und die Feinstruktur der Drüsenzellen. Z. Pflanzenphysiol. 64, 216 (1970)

- Farinose or oily secretions on aerial parts, consisting mainly of flavonoids in large amounts
- Unsubstituted flavone frequent, predominant
- Unusual substitution patterns in Ring A and B
- Localization of PAL in glandular hairs

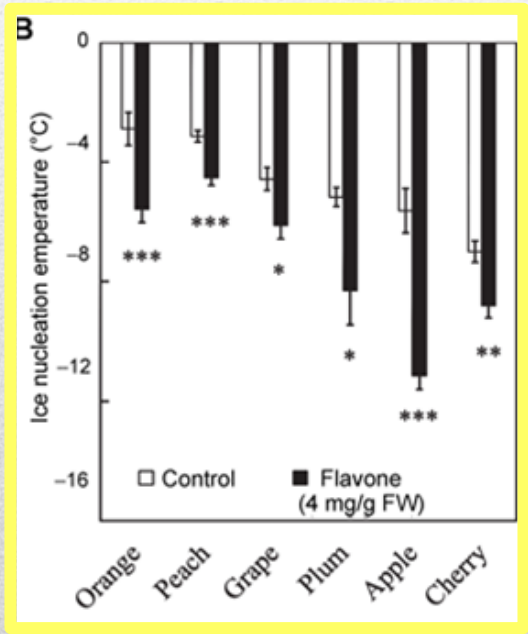
Functions of Exudate Flavonoids of *Primula*

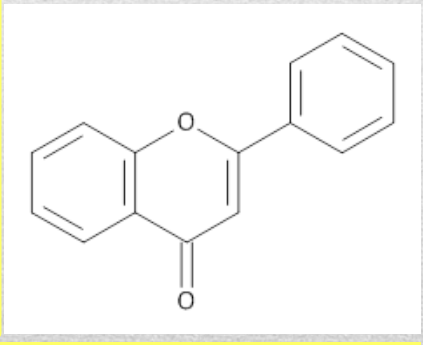
- UV-B Protection
- Antifungal: Dihydrochalcones
- Cytostatic properties: Glandular flavones of *P. denticulata*
- Freezing tolerance: Flavone





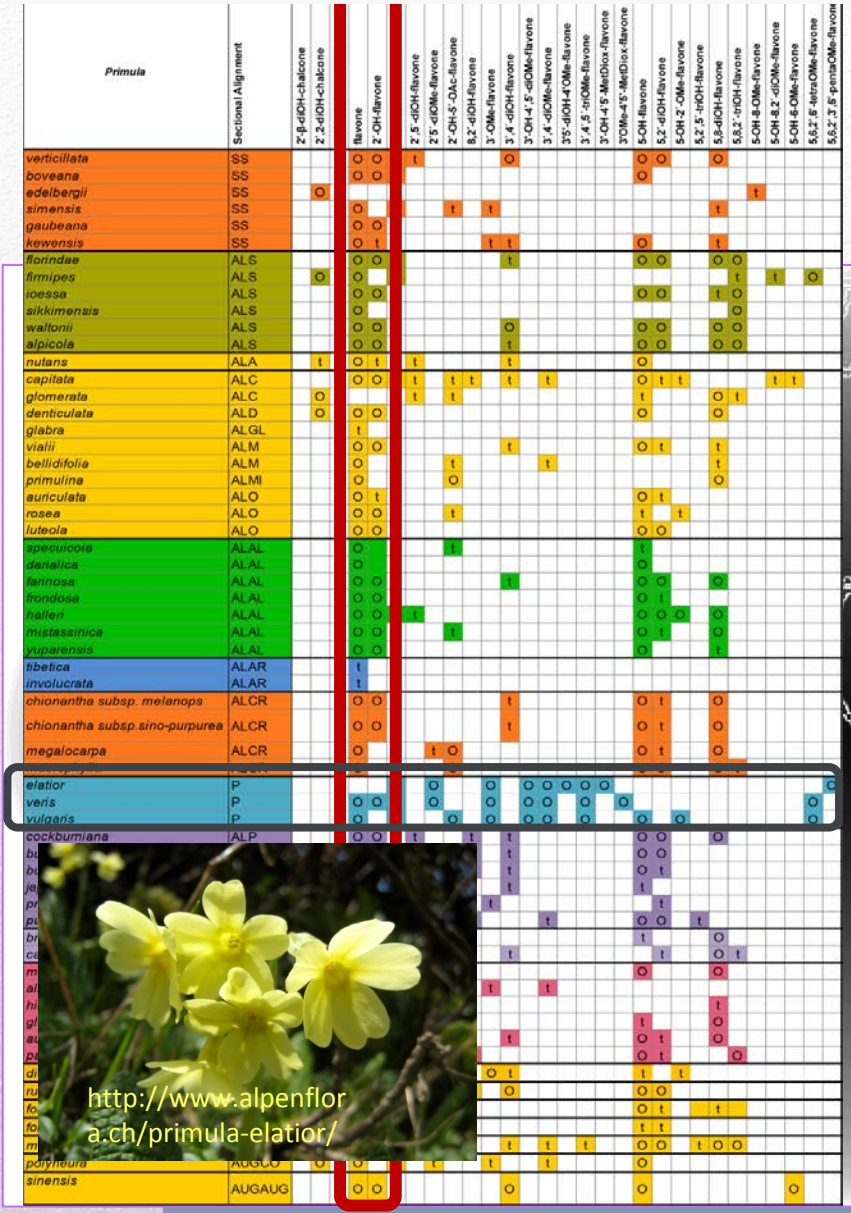
V. Reuter, Preliminary tests





Isshiki et al., 2014:
http://onlinelibrary.wiley.com/doi/10.1111/jipb.12145/full#jipb12145-fig-0004

Exudate Flavonoids of *Primula* spp: Structural and Biogenetic Chemodiversity



Examples for predominant occurrence of *Primula*-type flavones

- **Flavone (unsubstituted)**
- **2'-OH Flavone**
- 8,2'-diOH Flavone
- 5-OH Flavone
- 5,8-diOH Flavone

Examples for restricted occurrence of *Primula*-type flavones

- 3'4'5'-triOH Flavone
- 3'OH-4'5'-O_2CH_2 Flavone

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Valant-Vetschera, K.M., Bhutia, T.D., Wollenweber, E. 2009. NPC. 4, 365-370

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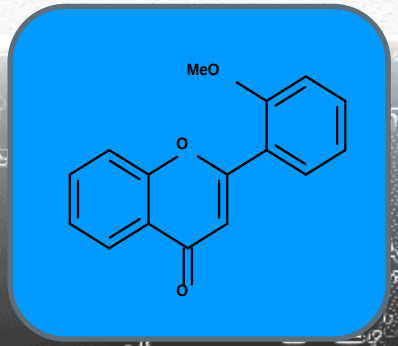
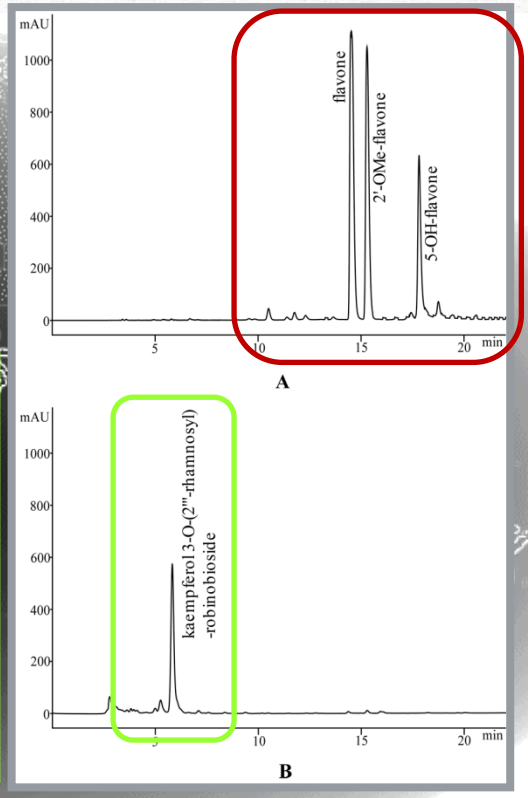
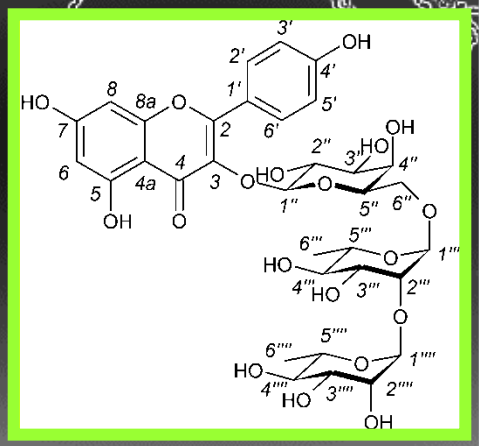
Flavonoids in Selected *Primula* spp.: Bridging Micromorphology with Chemodiversity

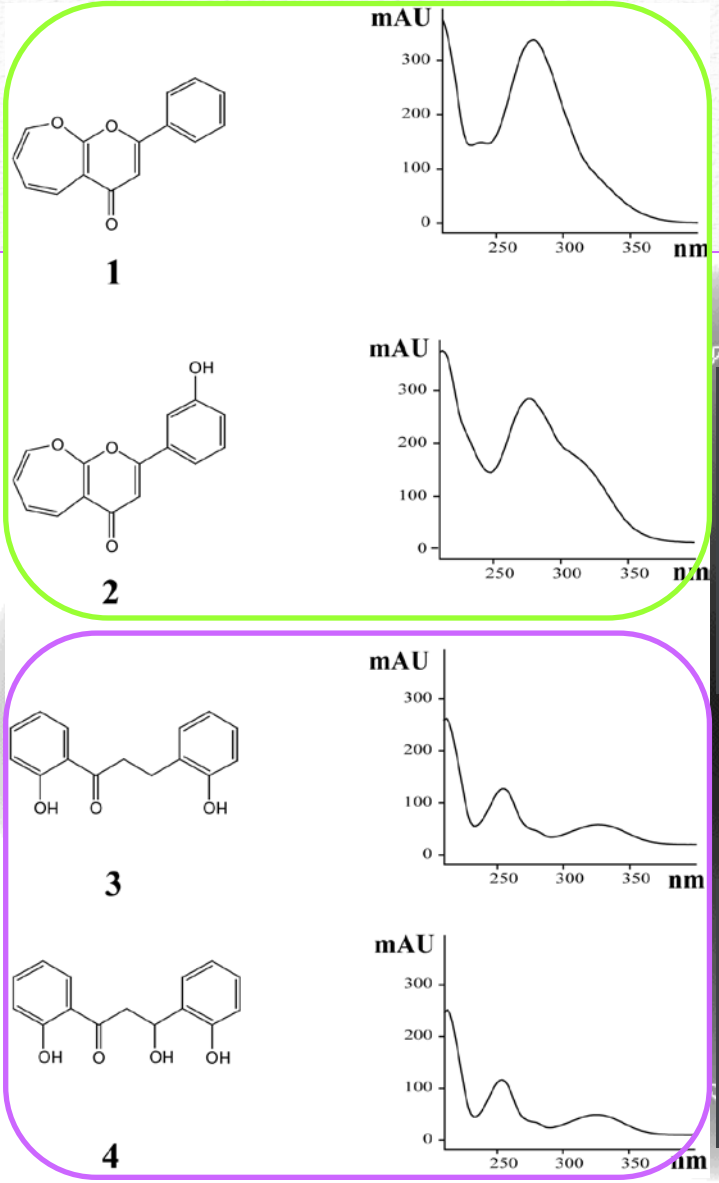






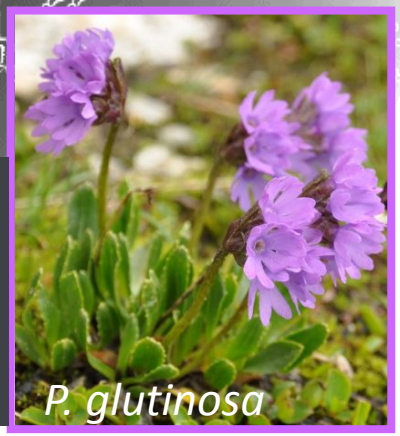
Primula vialii: Comparison between exudate and tissue flavonoid composition

Orphan Flavonoids and Dihydrochalcones from *Primula* Exudates



- Primcortusin (**1**) & 3‘-OH-der. (**2**) in all studied populations
- Co-occuring with flavone, 2',5'-diOMe-flavone and traces of 5-OH-flavone

- Dihydrochalcones 3 and 4 in all populations
- Co-occuring with 5-OH-flavone and traces of 2,2‘-diOH-chalcone



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Bhutia, et al., 2013, NPC 8, 1081

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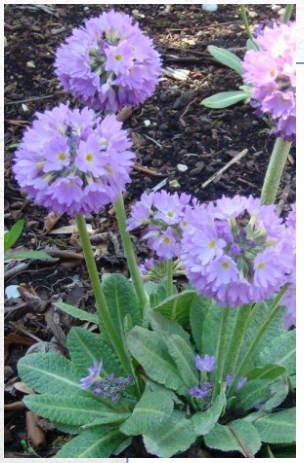
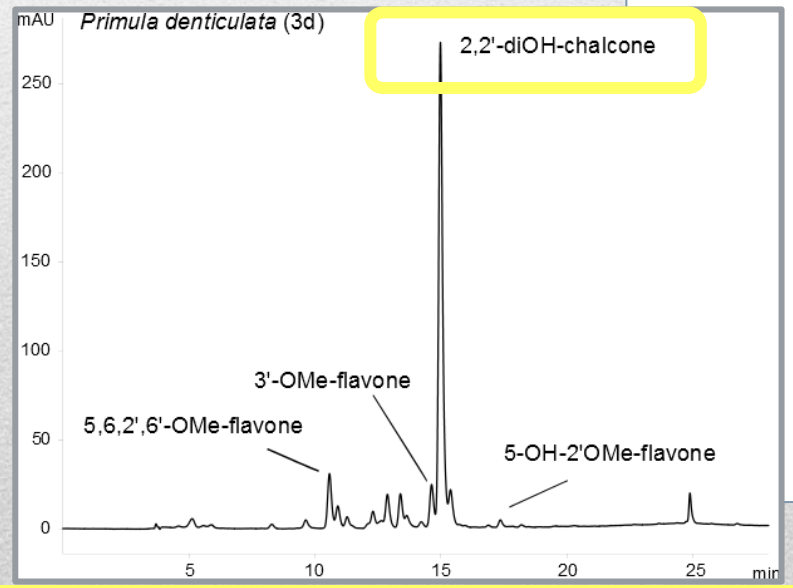
Striking Diversification of Exudate Profiles in Selected *Primula* Lineages

P. denticulata

Seasonal Variation



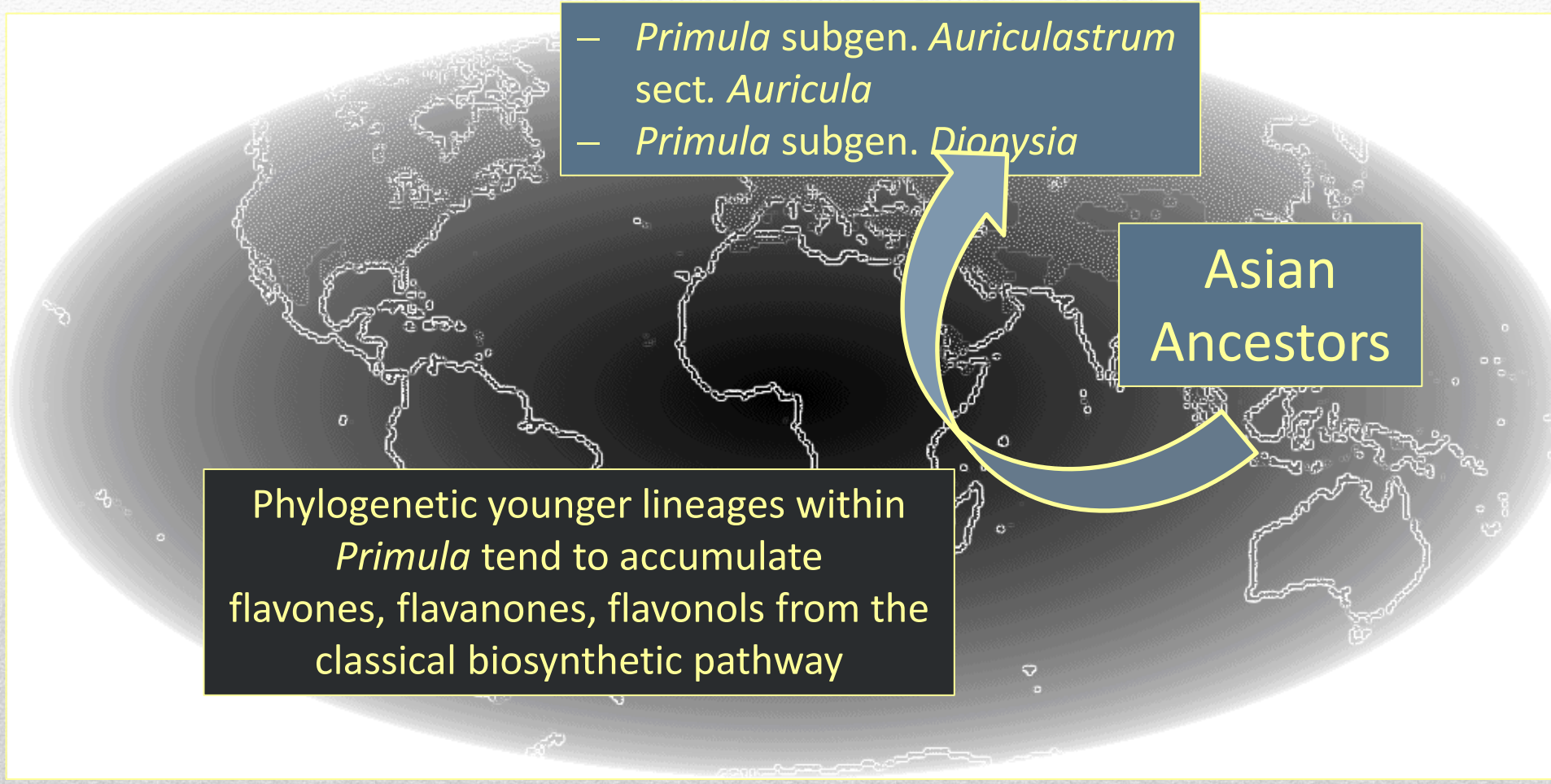


No.	*P. denticulata* Collections	2,2'-diOH-Chalcone	2-OMe-2'-OH-Chalcone	Unidentfied Dihydrochalcone	Flavone	2',5'-diOH-Flavone	2'OMe-Flavone	2'-OH-5'-OAc-Flavone	3'OMe-Flavone	5-OH-Flavone	5-OH-2'-OMe-Flavone	5,8,2'-triOH-Flavone	5,8-diOH-Flavone	5,6,2',6'OMe-Flavone	x1: Rt 24.338
1d	20.10.2014	●		●	x				x		x			x	
2d	20.10.2014	●							x		x			x	
3d	20.10.2014	●							x		x			x	
4d	20.10.2014	●		●					x		x			x	
5d	17.11.2014				●					x					
6d	10.03.2015				●			x		x		x	x		
7d	18.03.2015				●			x					x		
8d	19.03.2015				●			x					x		
9d	20.03.2015				●			x		x			x		
10d	08.04.2015				●			x		x		x			
11d*	07.05.2015				●							x	x		
12d	07.05.2015	●	x		x	x	x		x	x	x				
13d	02.06.2015	●			x		●			x					
14d	27.07.2015	●	x		x		x			x					x

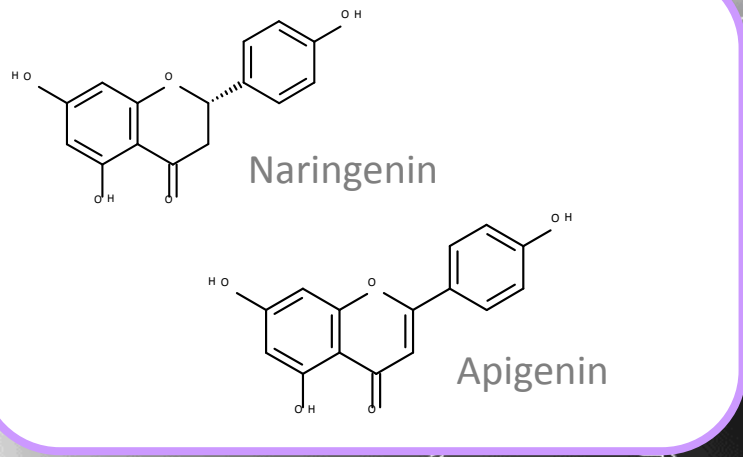
● major compound **x** minor compound *leaf with farina residues

HPLC-Chromatogram of *P. denticulata* leaf exudate dominated by 2,2'-diOH Chalcone

Unexpected Variation in Exudate Flavonoid Composition - Evolutionary Significance?



Primula hirsuta
Unexpected Variation in Exudate Flavonoid Composition



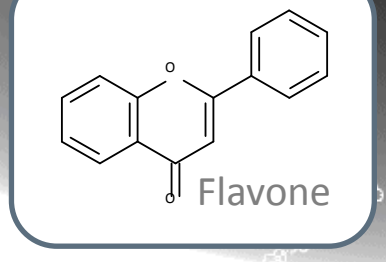
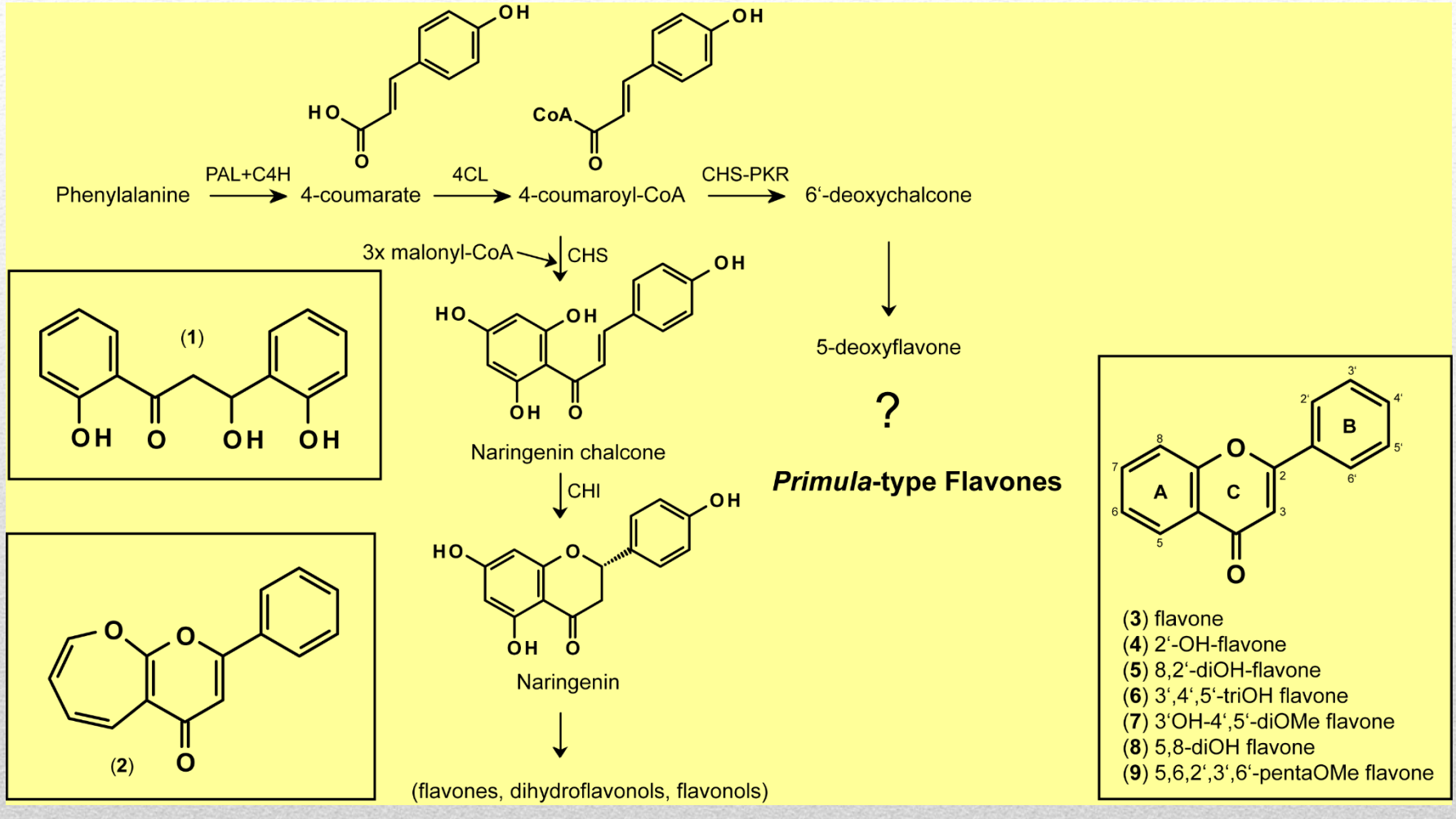


Table 2: Phytochemical diversification of exudate profiles in selected *Primula* species.

Primula		Flavone	2'-OH-Flavone	2'-OMe-Flavone	2'-OH-5'-OAc-Flavone	3'-OMe-Flavone	3',4'-diOMe Flavone	4'-OH-Flavone	5-OH-Flavone	5,2'-diOH -Flavone	5,8-diOH-Flavone	Apigenin	Apigenin-7-Me	Kaempferol	Kaempferol -3-Me	Naringenin	Naringenin 7,4'-diMe	Flavonoid derivatives	Primin	Primin derivatives	*ent*-kaur-16-en-19-oic acid (1)	Unknown compds.
daonensis[1]	EUA																			•		
daonensis[2]	EUA																			•		
pedemontana[3]	EUA																	F1		•		
villosa[4]	EUA																x		x	•		
hirsuta[5]	EUA											•	x	x	x	x				•		
hirsuta[6]	EUA											•	x	x	x	x				•		
hirsuta[7]	EUA	•			x															x		
hirsuta[a]	EUA	•	x		x						x											
albenensis[b]	EUA	•	x			x	x															
albenensis[c]	EUA	x	x					x														
latifolia[8]	EUA	•		x	x	x					x											
marginata[b]	EUA	•	•						•		•											
auricula[b]	EUA	•	•				x		•	x	•											
auricula[c]	EUA	x	x					x														
glutinosa[9]	CYA								•													
glaucescens[a]	CYA	•			x				x		•											
minima[10]	CYA	•			x				x		x											
minima[11]	CYA	•																				
minima[12]	CYA																					
minima[13]	CYA																					
minima[14]	CYA																				x	
minima[15]	CYA																					
clusiana[16]	CYA																					
integrifolia[17]	CYA																					
longipes[18]	CRY	•			x				x		•											
algida[19]	AL	•			x				x		x											
elatior subsp. *pallasii*[20]	PR	•			x				x		•											
elatior subsp. *meyeri*[21]	PR	•							x													

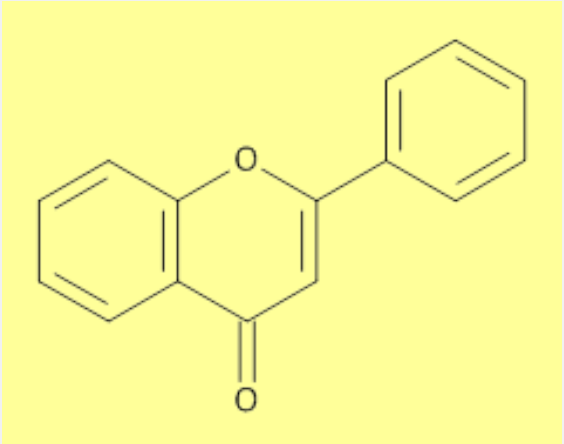
Elser, D. et al., Natural Product Communications 2016, 11, 585

Biosynthesis: Present View



Generation of the Flavone Core

- *Primula*-type flavonoids are probably not generated via the classical biosynthetic pathway
- 5-Deoxyflavonoid pathway is probably not involved



Decoration or De-Decoration?

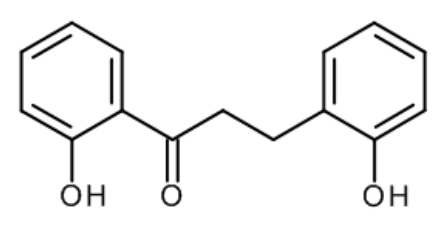
Where and how?

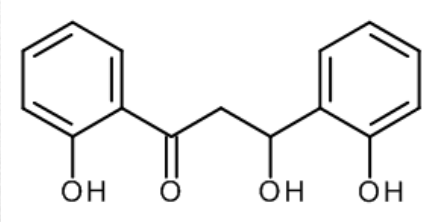
- Plastids in trichomes ?
- Mutualistic endophytes: Fungi, bacteria ?

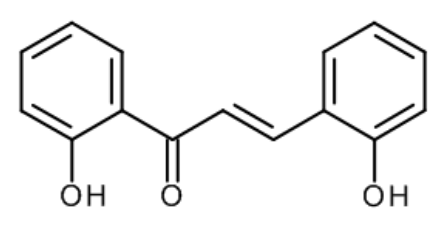
How to explain the switchover to classical flavonoid pathway in single species and groups?

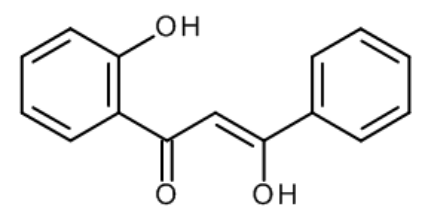
Generation of the Flavone Core

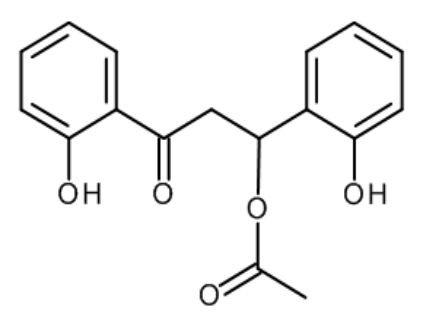
Search for Precursors I - Chalcones and Dihydrochalcones











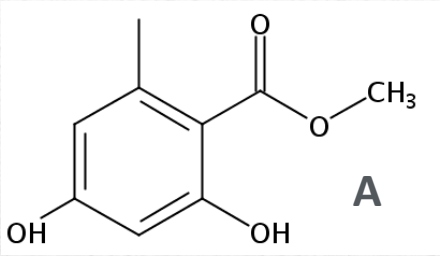
2,2‘-diOH Chalcone
→ 5,2‘diOH Flavone

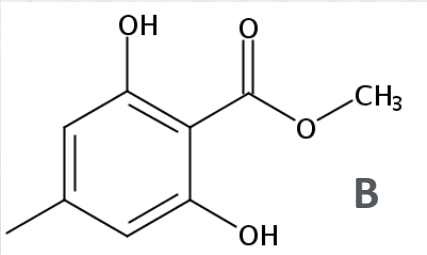
2‘ß-diOH Chalcone
→ Flavone

No corresponding flavanones in *Primula* exudates

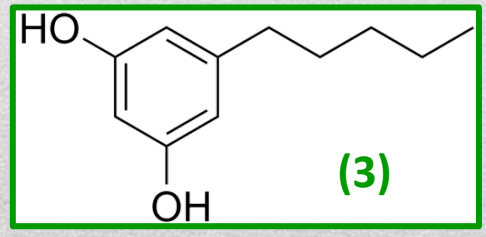
Generation of The Flavone Core

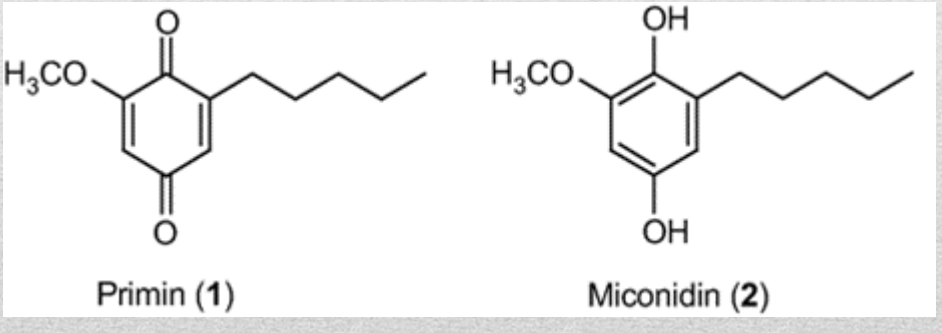
Search for Precursors II - Essential Oil Compounds from *Primula* spp.





- Benzoyl Derivatives (A), (B): 60 % in *P. obconica* oil[1]
- Benzoquinones[2]
  - Primin (1), Miconidin (2))
  - Biosynthesis from Olivetol (3)



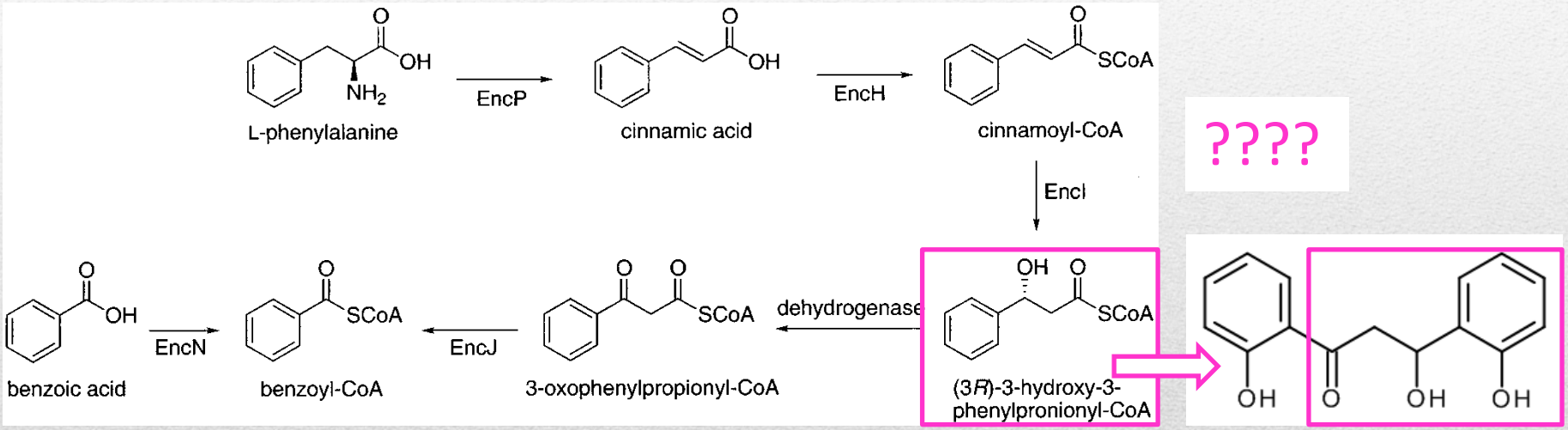


There are too many OH-groups around!

[1]Nan et al., Natural Product Letters, Vol. 16, No. 4, pp. 249–253
[2]Christensen & Larsen, Contact Dermatitis, 2000, 42, 149–153

Could Bacterial Endophytes be Involved?

From Benzoic acid to Chalcone (Moore et al., J. Nat. Prod. 2002, 65, 1956)

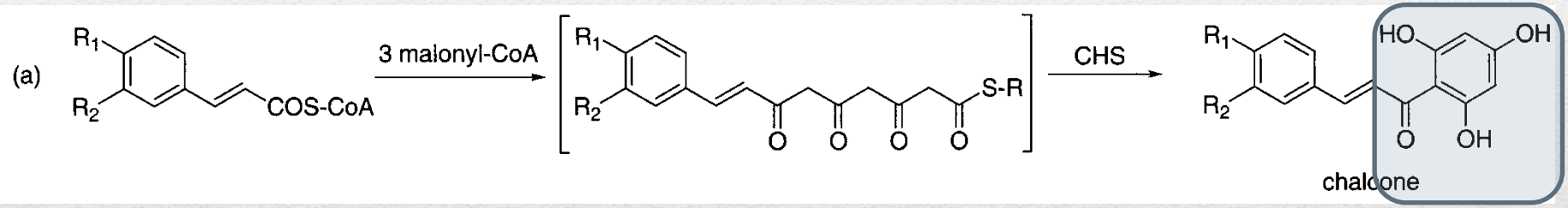


Proposed Biosynthesis of the Enterocin PKS Starter Unit Benzoyl-CoA in *Streptomyces maritimus*

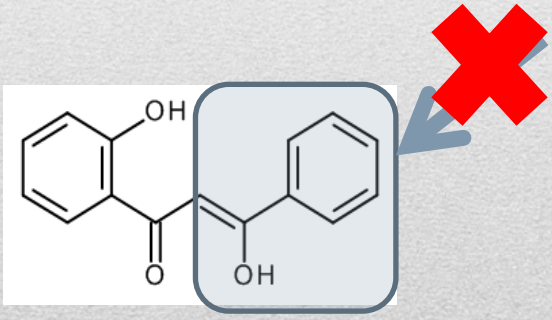
Are these enzymes also present in plant sources?

Could Bacterial Endophytes be Involved?

From Benzoic Acid to Chalcone (Moore et al., J. Nat. Prod. 2002, 65, 1956)

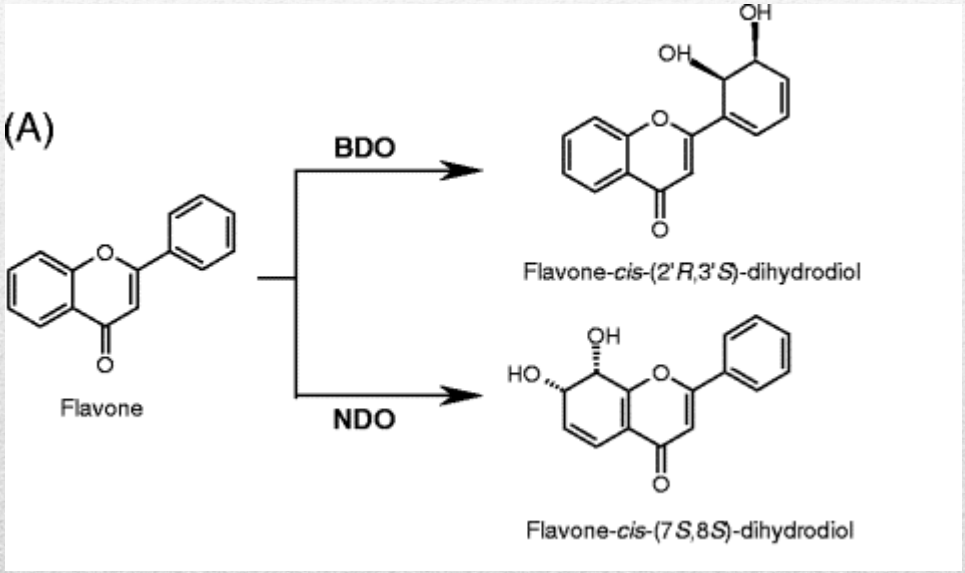


Biosynthetic precursors imply presence of more OH groups than found in *Primula* chalcones



Decoration of Flavone by Bacterial Endophytes?

Flavonoids biotransformation by bacterial non-heme dioxygenases, biphenyl and naphthalene dioxygenase (Seo et al., Appl. Microbiol. Biotechnol. 2011, 91, 219)

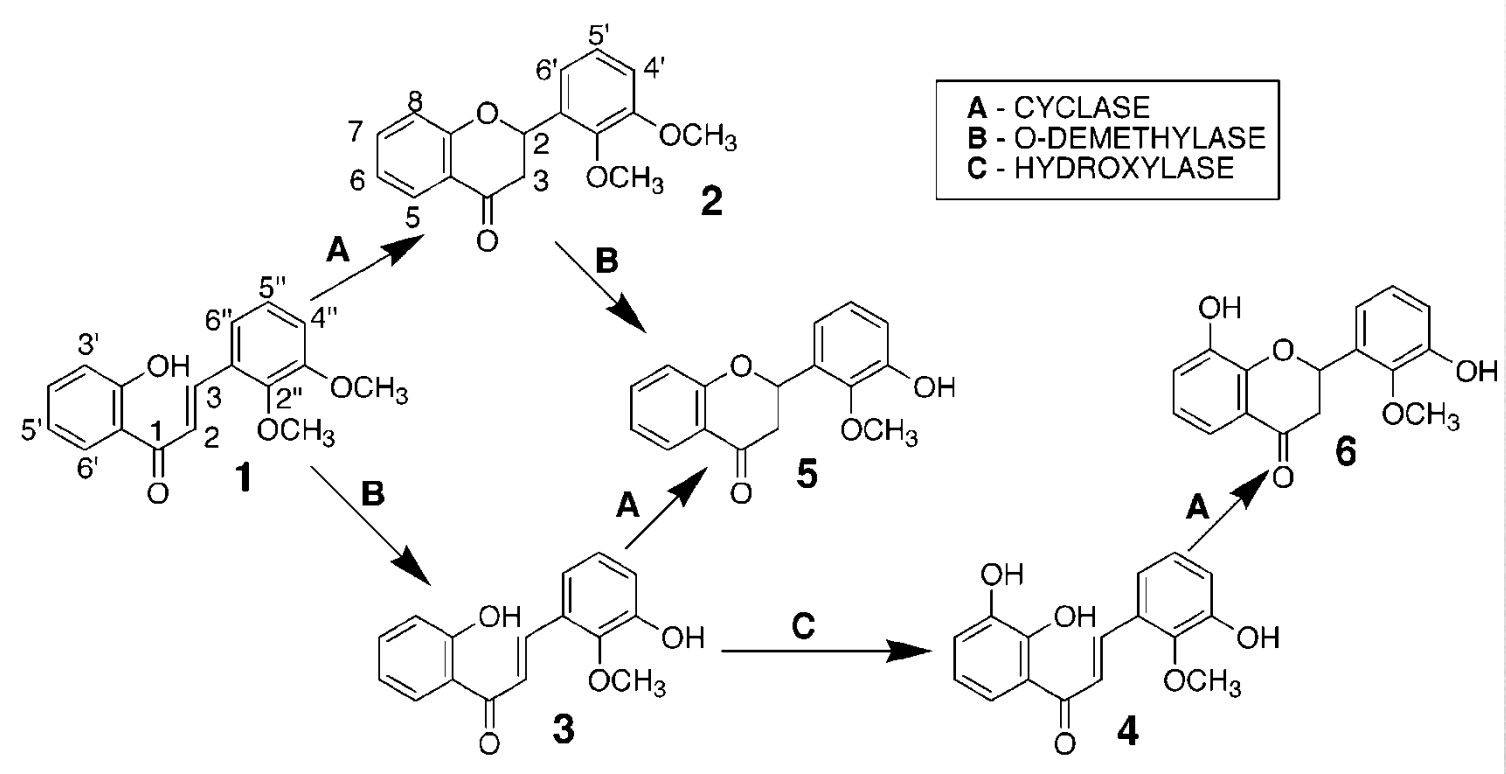


Biotransformation by Biphenyl dioxygenase (BDO) and Naphthalene dioxygenase (NDO) *Escherichia coli* strain JM 109 expressing BDO/NDO of *Pseudomonas* spp.

Wow! There is some capacity….

Decoration/Modification by Fungal Endophytes?

Fungi: Microbial transformation of chalcones: Hydroxylation, 0-Demethylation, and Cyclization to flavanones (Sanachez-Gonzalez & Rosazza, J. Nat. Prod. 2004, 67, 553).



Modifications of synthetic (1) by *Aspergillus alliaceus*

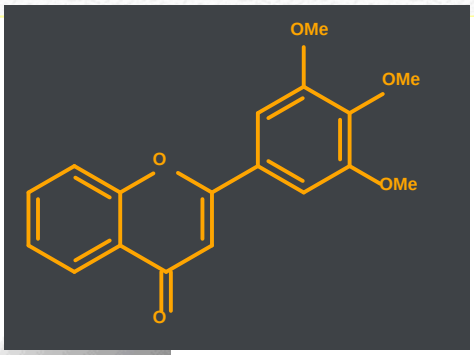
Diversification of exudate compounds in *Primula*

- Frequent occurrence
  - Flavone and unusual substituted flavone derivatives
  - Few chalcones
- Asiatic ancestors - relatively uniform flavonoid profile
- Younger phylogenetic lineages of CE Alps
  - Quantitative and qualitative variation between populations
  - Flavonoids from classical biosynthetic pathway
  - Primin and derivatives
- Diversification possibly reflects the phylogeny of this complex genus
- Variation in derived lineages warrants deeper investigations(functional?)

Testing Hypotheses

- Freezing tolerance
- Biosynthesis of *Primula*-type flavones and chalcones
  - Substrates and PKS enzymes from glandular hairs
  - Core structure biosynthesis through endophytes?
  - Decoration/Modification
    - Fungal/ microbial enzymes?
    - Flavone/Chalcones as substrate for “ordinary decoration“ enzymes?

Acknowledgements



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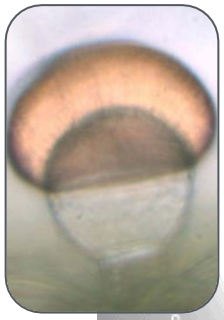

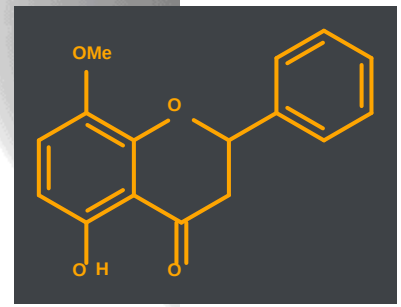

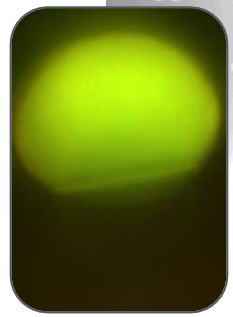

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