

# GlycoStil ANR project: Accessing stilbene oligomers through Green Chemistry

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# Aim of GlycoStil project

- » Producing new stilbenes derivatives for the cosmetic industry
- » Private-Public partnership



Stilbene library by  
green chemistry

Stilbenes  
production through  
cell culture

URD ABI  
by AgroParisTech

Purification and  
chemo-typing



Downstream  
processing



Scale-up  
LCA and TEA

novéal  
INGREDIENTS FOR BEAUTY



Evaluation of  
biological properties

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ICMR  
Institut de Chimie Moléculaire de Reims  
UMR CNRS 7312

Downstream  
processing

RIBP  
Résistance Induite et  
Bio-protection des Plantes

Scale-up  
LCA and TEA

novéal  
INGREDIENTS FOR BEAUTY

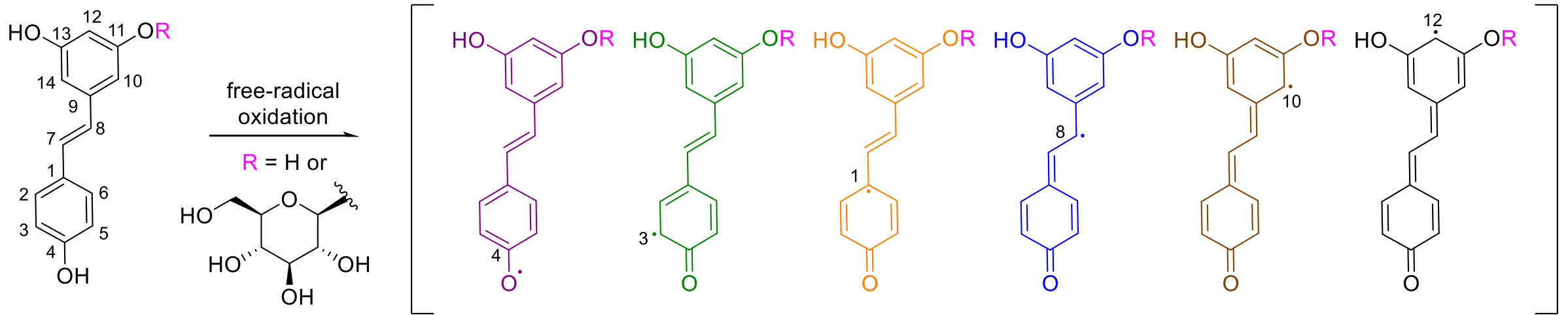
MEDyC

Evaluation of  
biological properties

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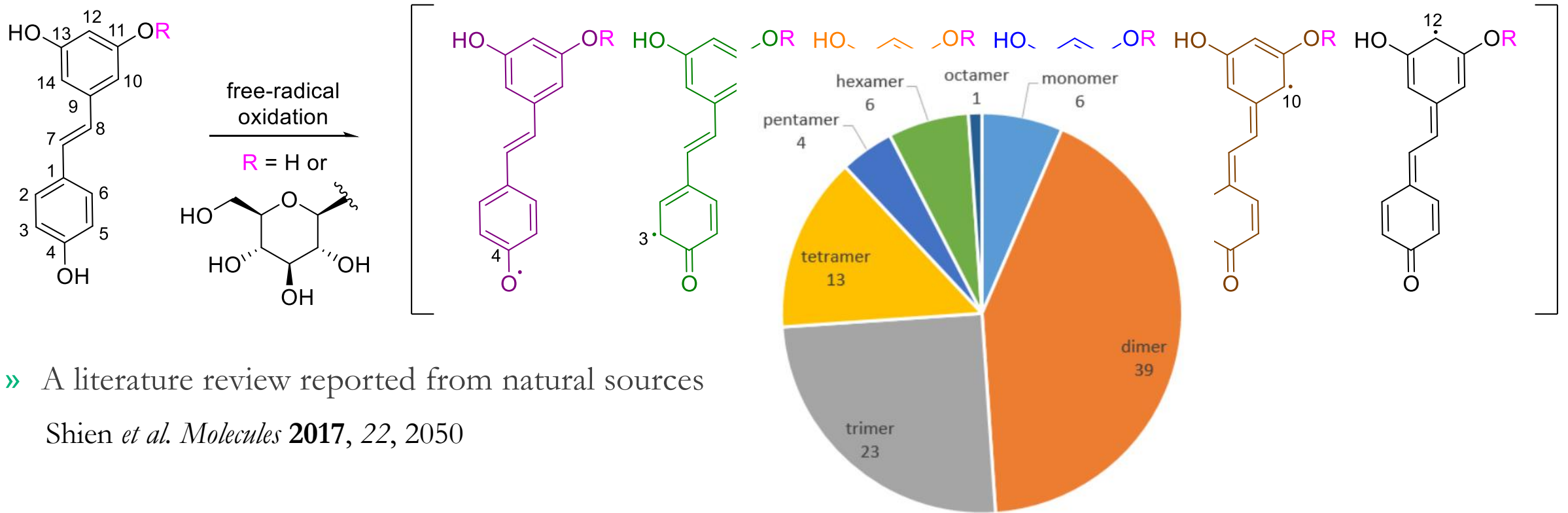
# Oxidative coupling: generation of radicals

- » Radical can be generated by oxidases (*i.e.* laccase, peroxidase) or metal (*e.g.* silver acetate)
- » 6 radicals from the *para*-phenol



# Oxidative coupling: generation of radicals

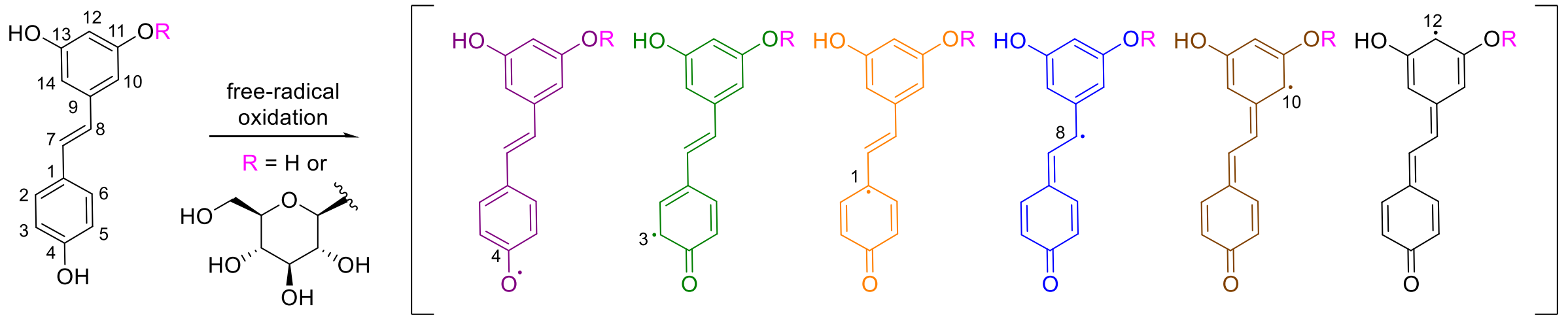
- » Radical can be generated by oxidases (*i.e.* laccase, peroxidase) or metal (*e.g.* silver acetate)
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- » A literature review reported from natural sources  
Shien *et al. Molecules* **2017**, *22*, 2050

# Oxidative coupling: generation of radicals

- » Radical can be generated by oxidases (*i.e.* laccase, peroxidase) or metal (*e.g.* silver acetate)
- » 6 radicals from the *para*-phenol



- » A literature review reported from natural sources: 6 monomers, 39 dimers, 23 trimers and 13 tetramers  
Shien *et al. Molecules* **2017**, *22*, 2050
- » How influence the reaction to increase selectivity and yield?

# Focus on Labruscol

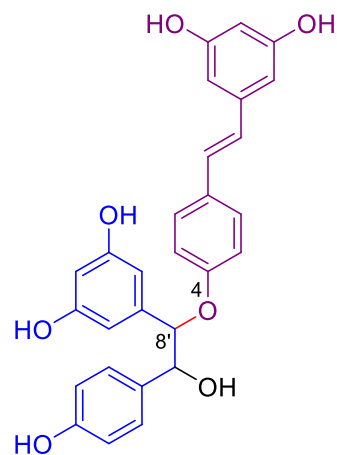
Sursin *et al.* *ACS Sustainable Chemistry & Engineering* **2023**, 11, 31, 11559-11569, 10.1021/acssuschemeng.3c01997

## » Enzymatic coupling with commercial laccase from *Trametes versicolor*

- Only one example in the literature reported presence of labruscol from laccase-mediated oxidation of resveratrol (less than 5% yield)

Ponzoni *et al.* *Adv Synth Catal* **2007**, 349 (8–9), 1497–1506. 10.1002/adsc.200700043

- After OVAT screening, a DOE was performed



Labruscol

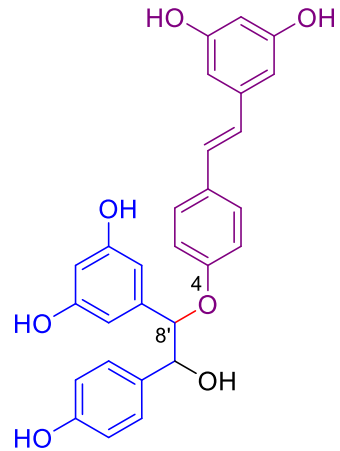
Parameters	Name	Abbr.	Units	Levels			Precision
				-1	0	1	
X <sub>1</sub>	%Acetone	%Ace	%	20	45	70	1.25
X <sub>2</sub>	Enzyme/substrate ratio	E/S	U/mmol	6	33	60	0.45
X <sub>3</sub>	Concentration	Conc	mmol/L	20	50	80	1.5
X <sub>4</sub>	Enzyme addition rate	rate	mL/h	7	28.5	50	1.08
X <sub>5</sub>	Temperature	Temp	°C	25	35	45	0.5

Amount of radical at each time influences the radical coupling

# Focus on Labruscol

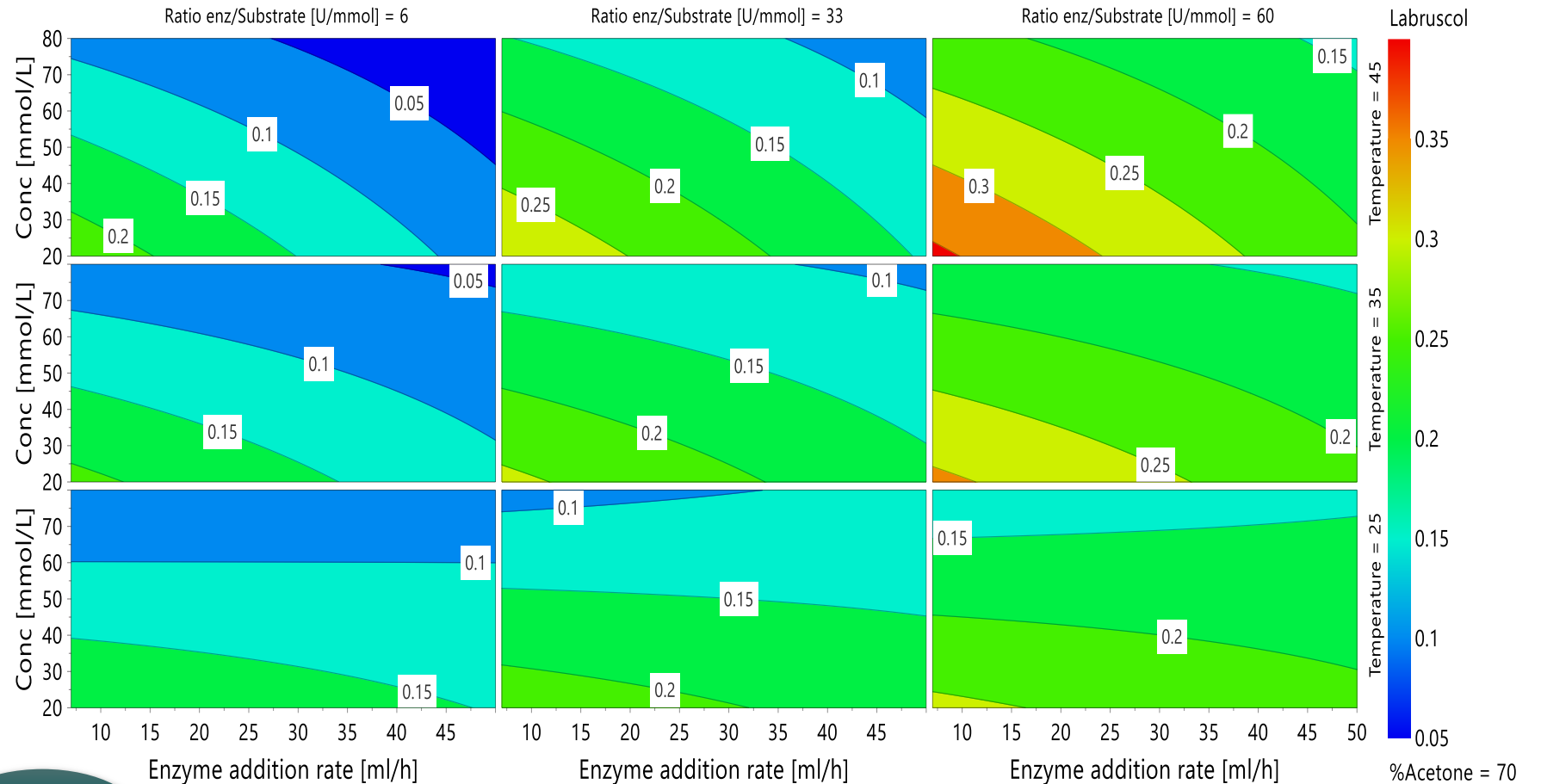
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» Enzymatic coupling with commercial laccase from *Trametes versicolor*



Labruscol

Optimized conditions  
70% Acetone-MilliQ water  
20 mM of resveratrol  
Enzyme 60 U.mmol<sup>-1</sup>  
6 mL.h<sup>-1</sup>  
Temperature 45°C



37.2%



# Focus on Labruscol

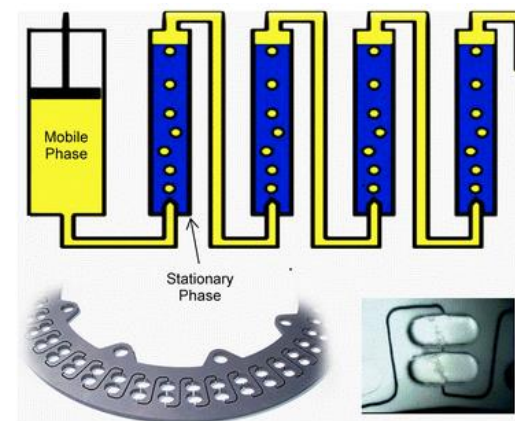
Sursin *et al.* *ACS Sustainable Chemistry & Engineering* **2023**, *11*, 31, 11559-11569, 10.1021/acssuschemeng.3c01997

- » Scale from 114 mg to 2 g of resveratrol
  - Increased reaction time from 1.5 to 6 hours to achieve 95% conversion
- » Purification by two methodologies: flash chromatography *vs* centrifugal partition chromatography

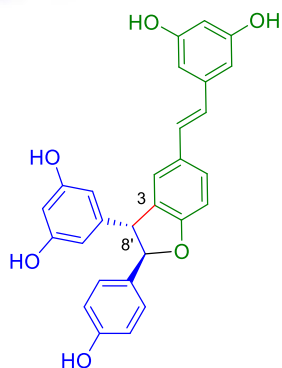


2 separations required

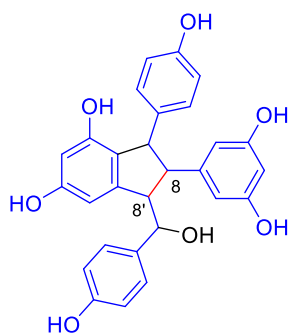
- 1) Normal phase to recover  $\delta$ -viniferin (57%)
- 2) Reverse phase to recover labruscol (21%) and Leachianol (10%)



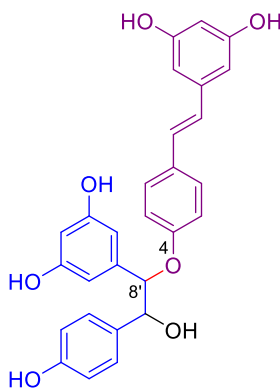
1 run  
 Leachianol (6%)  
 Pallidol (1.4%)  
 Labruscol (26%)  
 $\delta$ -viniferin (63%)  
 +unknown compounds



$\delta$ -viniferin



Leachianol



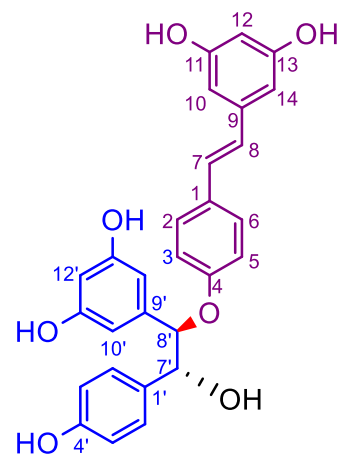
Labruscol

Solvent systems	n-heptane (% v/v)	Ethyl acetate (% v/v)	Methanol (% v/v)	Water (v/v)
System L	47.73	48.10	3.04	1.12
System J	33.09	62.42	2.61	1.87
System G	22.65	72.84	2.1	2.2

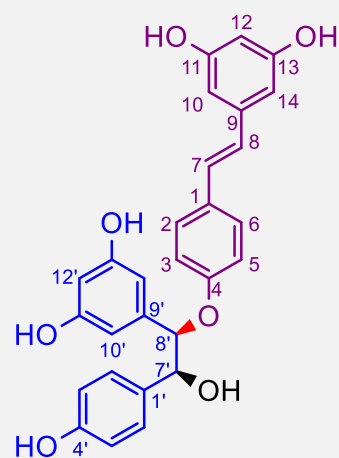
# Identification of new compounds

Sursin *et al.* *ACS Sustainable Chemistry & Engineering* **2023**, 11, 31, 11559-11569, 10.1021/acssuschemeng.3c01997

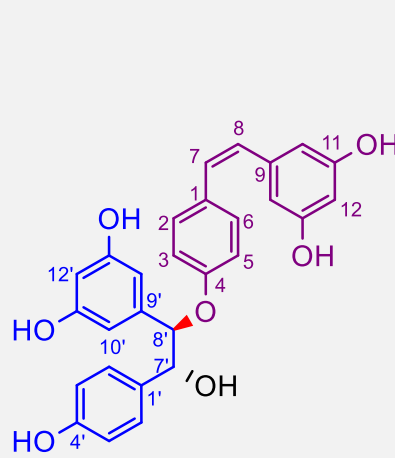
» CPC fractions containing unknown product were repurified by HPLC-prep to afford pure sample for NMR analysis



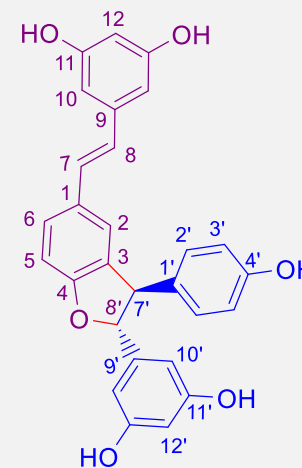
*trans-E-Labruscol*



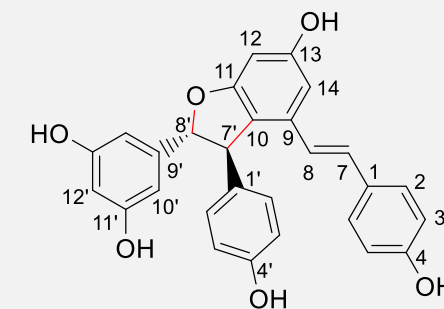
*cis-E-Labruscol*



*Z-Labruscol*



*iso-δ-viniferin*



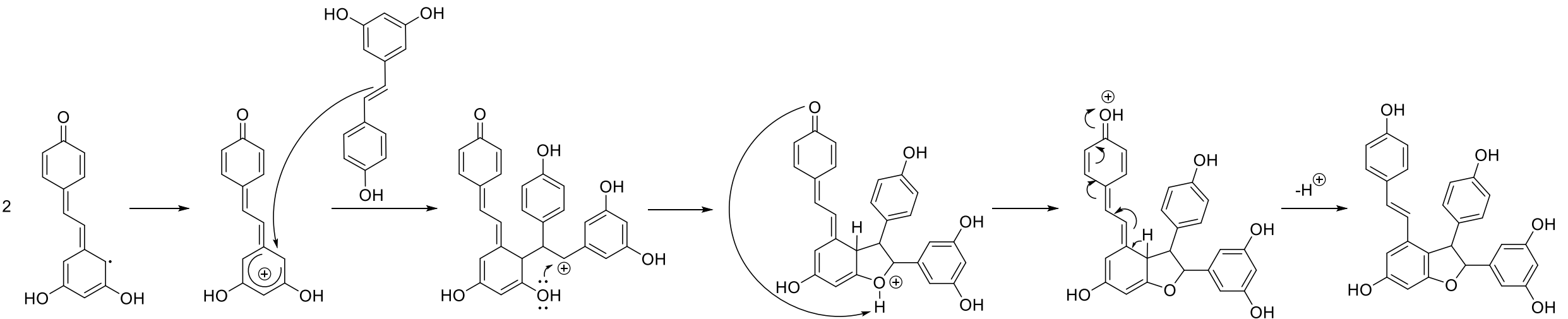
*iso-ε-viniferin*

4 new compounds

# Hypothesized mechanism for iso-e-viniferin

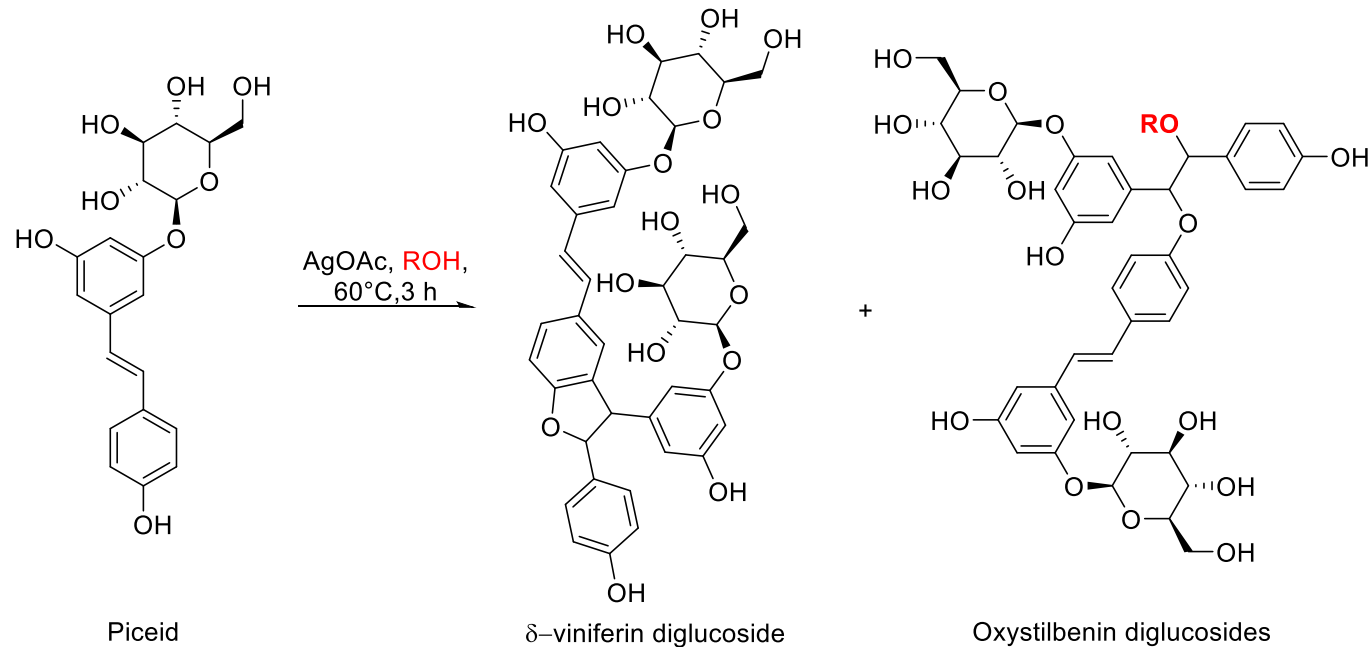
Sursin *et al.* *ACS Sustainable Chemistry & Engineering* **2023**, *11*, 31, 11559-11569, 10.1021/acssuschemeng.3c01997

» Dismutation as key event



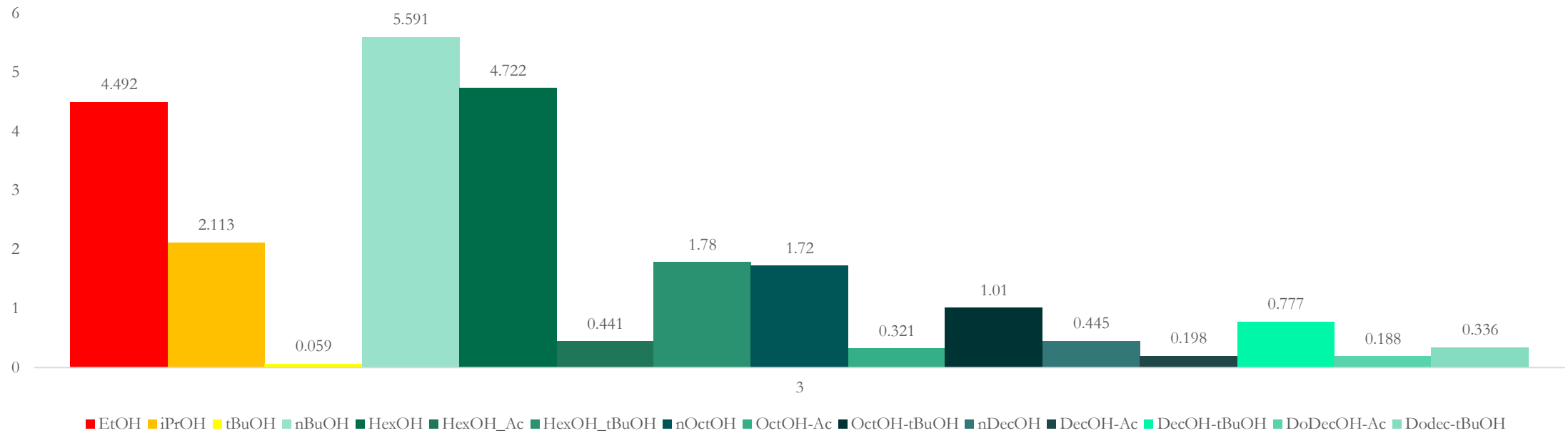
# Expanded family: Oxystilbenin

- » Production of glucosylated stilbenes in alcoholic solvent
  - Previous work to synthesis  $\delta$ -viniferin diglucoside (63%) + Presence of ethyl oxystilbenin diglucoside (up to 26%)  
*Vinet et al. ACS Sustainable Chemistry and Engineering* **2022**, 10, 28, 9166-9175, 10.1021/acssuschemeng.2c02010
  - Extension to other alcohol
- » Aims: Expand the library of compounds and optimized production of  $\delta$ -viniferin diglucoside



# Expanded family: Oxystilbenin

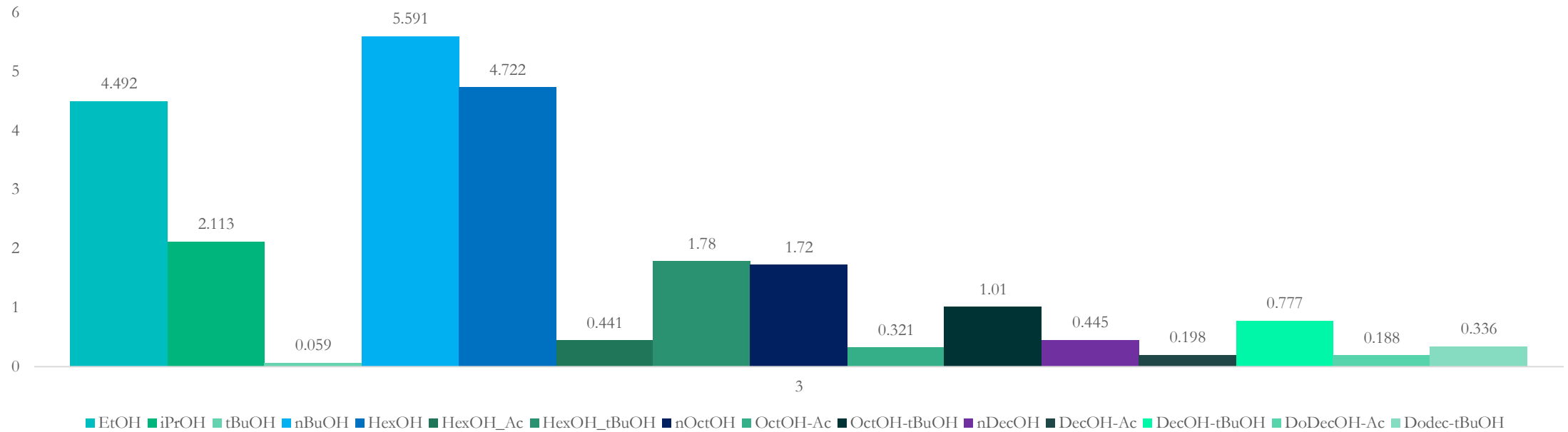
- » Impact of substitution degree
  - Decreasing with increase of the alcohol substitution



# Expanded family: Oxystilbenin

## » Impact of the chain length of the alcohol

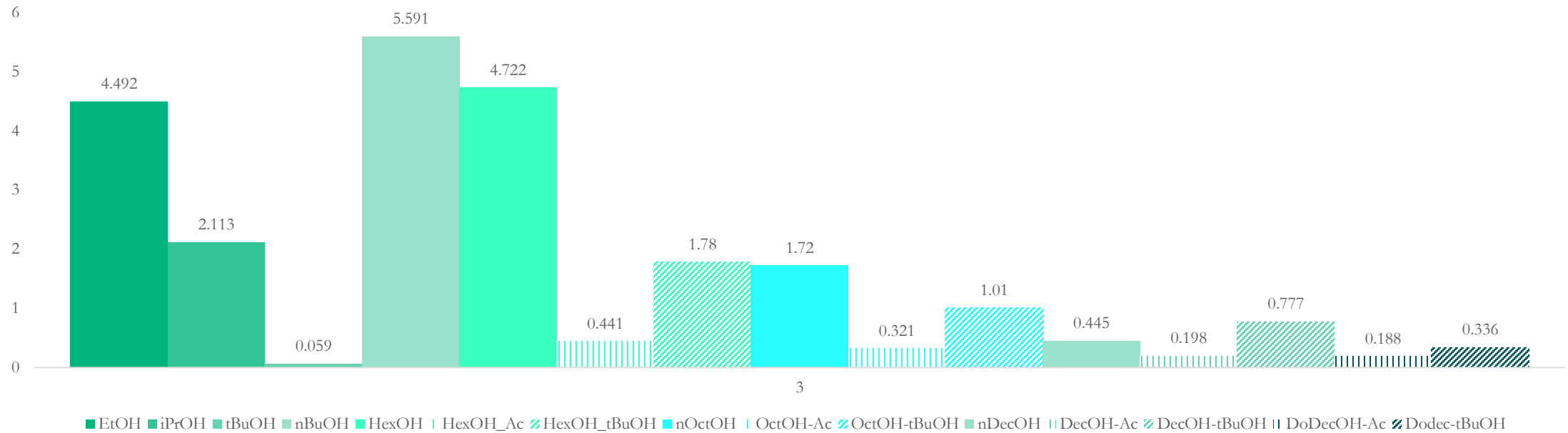
- Maximal production at C4 and drastic decrease from C8 probably due to solubility issues



# Expanded family: Oxystilbenin

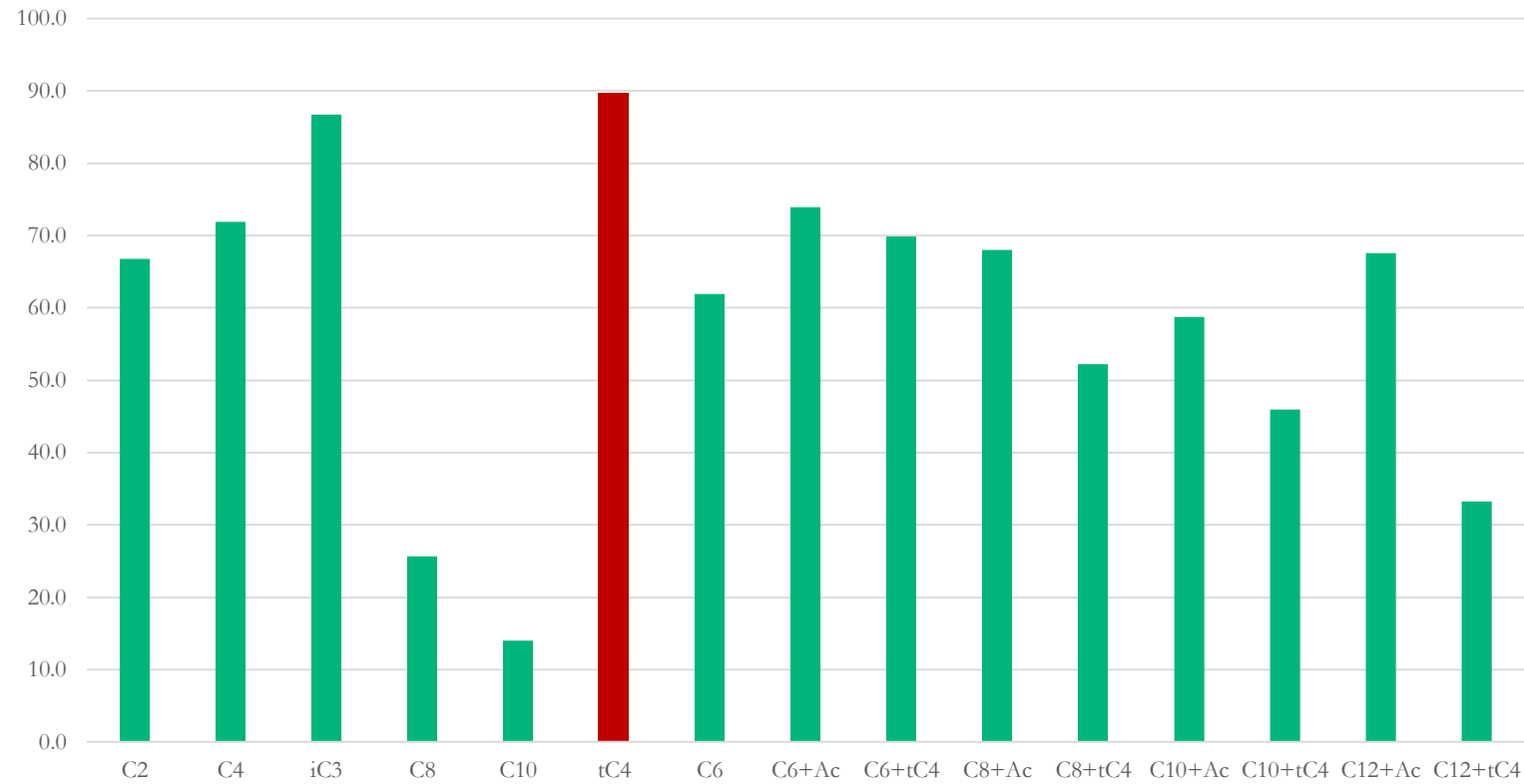
## » Impact of using a co-solvent

- Acetone decreased drastically the selectivity toward oxystilbenin forms
- *Tert*-butanol allowed a better production of oxystilbenin from C10



# Increasing amount of $\delta$ -viniferin diglucoside

» Inhibition of oxystilbenin increased yield of  $\delta$ -viniferin diglucoside





# Conclusion & Perspectives

## *Take home message*

- » 11 new compounds
- » Improved synthesis of Labruscol (37%) and  $\delta$ -viniferin diglucoside (89%)
- » Alternative and efficient purification by centrifugal partition chromatography (CPC)

## *Perspectives*

- » Test biological activities of these new compounds
- » Find alternative to silver acetate
- » Expand further more the diversity of structure

# Acknowledgement



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