How wine polyphenols evolve during wine ageing ?

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Outline

I. Introduction to phenolic compounds of grapes and wine and their evolution during wine ageing

II. Example of degradation reaction involving Malvidine-3-*O*-glucoside

III. Example of Polymerization of tannins involving acetaldehyde

IV. Conclusion

Storage of wine (O_2, H^+, T°)

Phenolic compounds

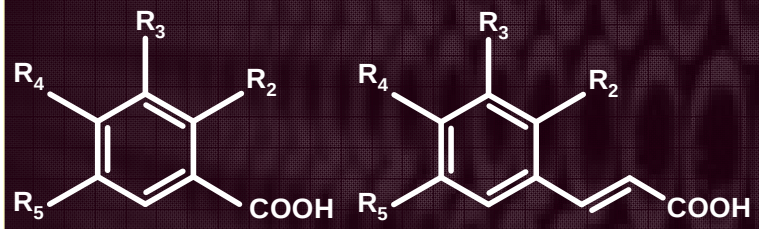
Physico-chemical transformations

Taste and color changes

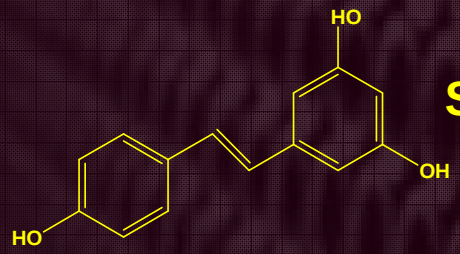
Phenolic compounds in grape and wine

Non-flavonoids

Phenolic acids

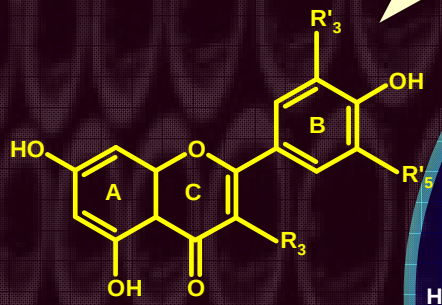


Benzoic acids Cinnamic acids

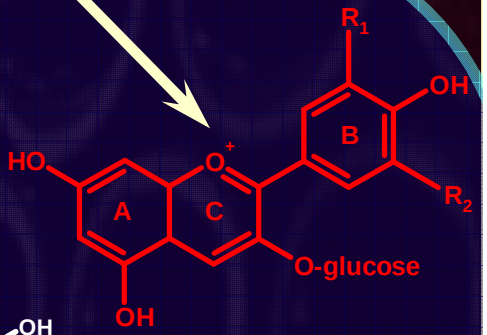


Stilbens

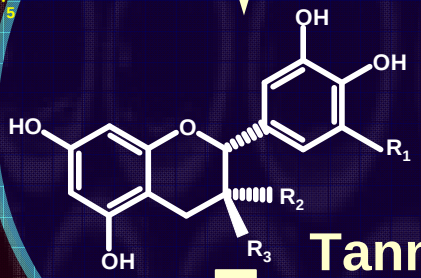
Flavonoids



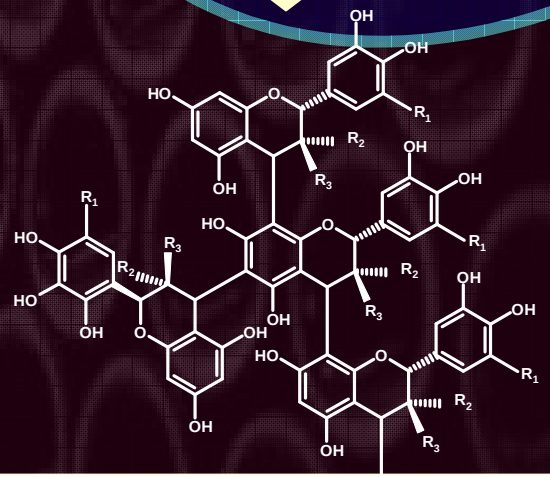
Flavonols



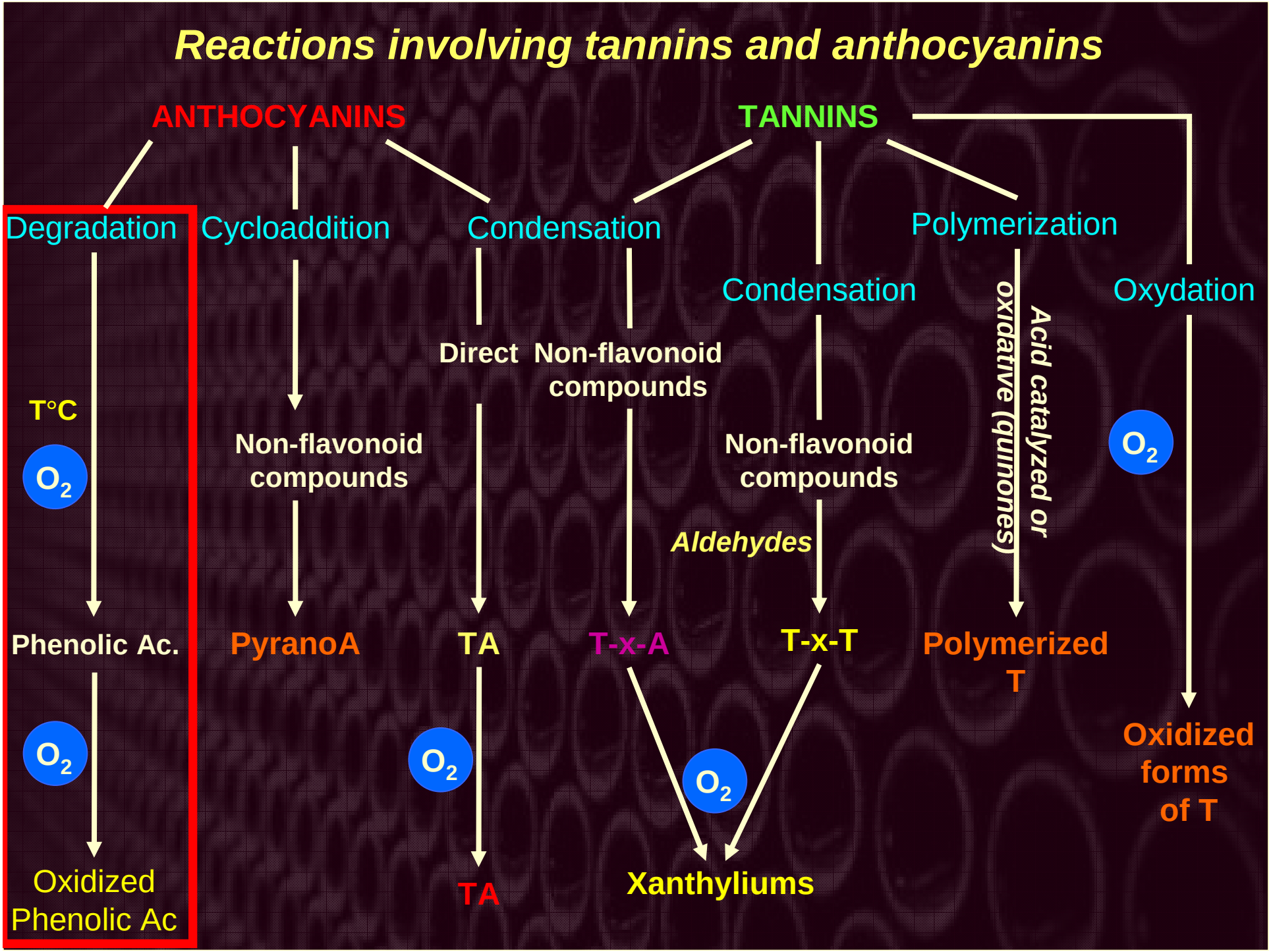
Anthocyanins

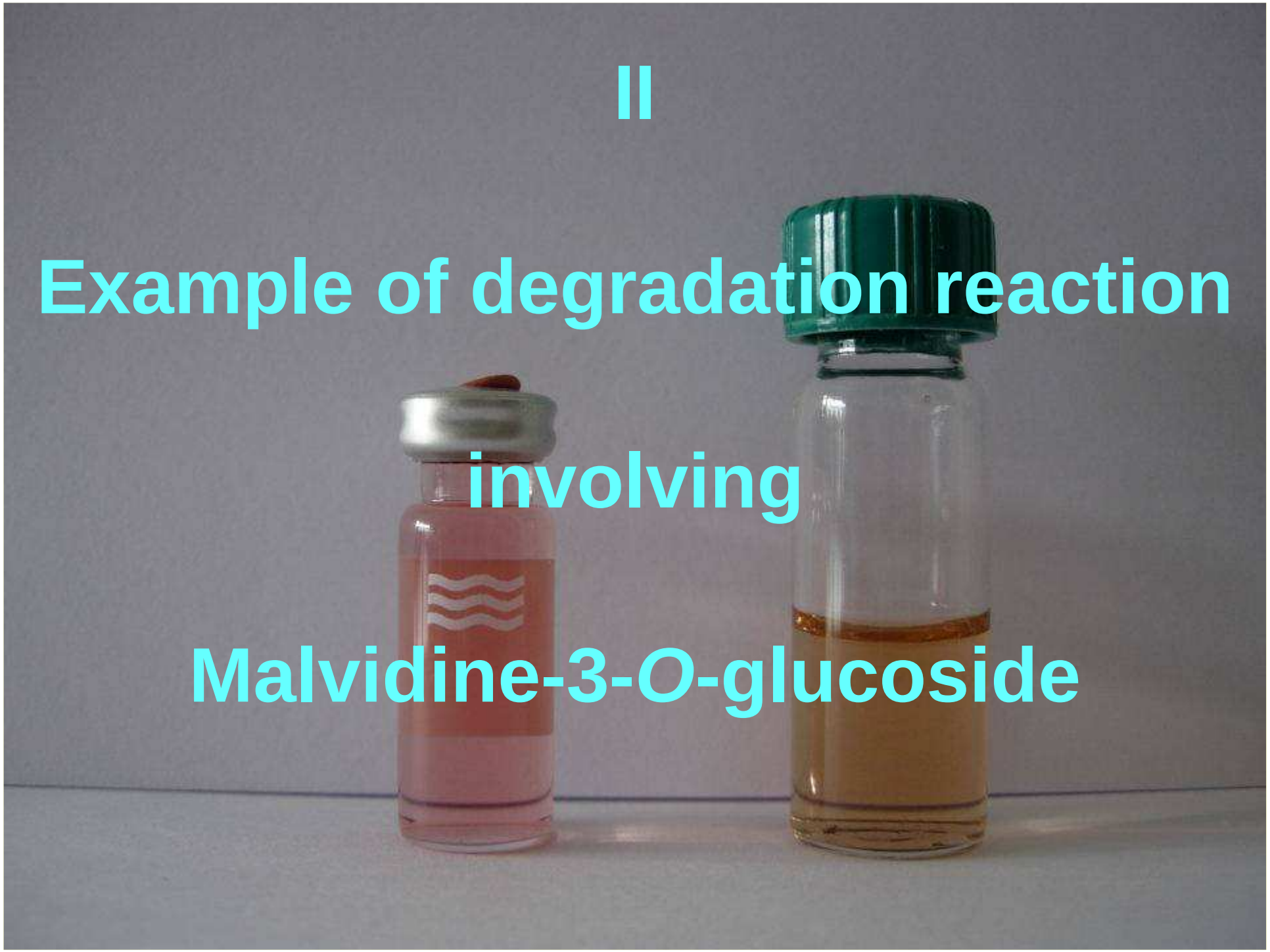


Tannins



➢ Taste and color of wines


Reactions involving tannins and anthocyanins
ANTHOCYANINS
TANNINS
Degradation
Cycloaddition
Condensation
Condensation
Polymerization
Oxydation
T°C
O2
Phenolic Ac.
O2
Oxidized Phenolic Ac
Non-flavonoid compounds
PyranoA
Direct
Non-flavonoid compounds
TA
O2
TA
T-x-A
O2
Xanthyliums
Non-flavonoid compounds
Aldehydes
T-x-T
Acid catalyzed or oxidative (quinones)
Polymerized T
O2
Oxidized forms of T



II

Example of degradation reaction involving Malvidine-3-*O*-glucoside

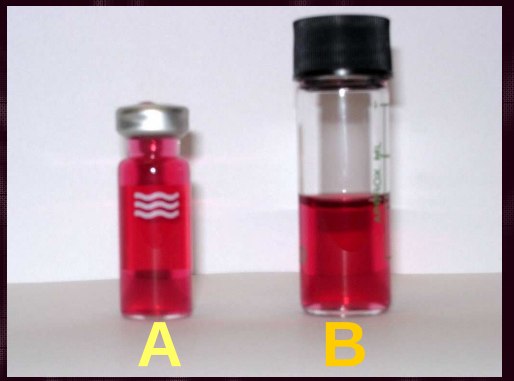
Objective:

1. Effect of O_2:

- Study of oxidoreduction state markers

Protocole:

- Model wine solution (12% EtOH, 5 $g.L^{-1}$ Tartaric acid, pH 3,4)
- Malvidine-3-*O*-glucoside (250 mg/L)
  - Low **O_2**, (control flushed with Nitrogen)(A)
  - **O_2** rich (excess 450 mg/L d' O_2) (B)
- 90℃ during 24 hours



HPLC/UV-Vis/MS

NMR

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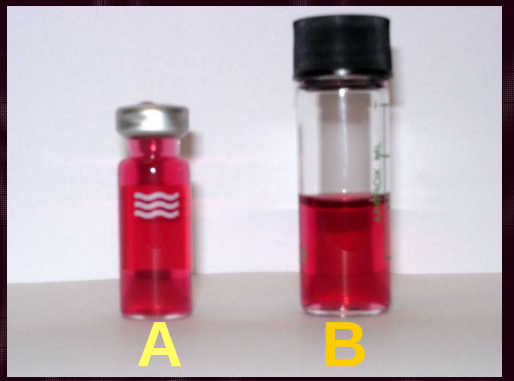
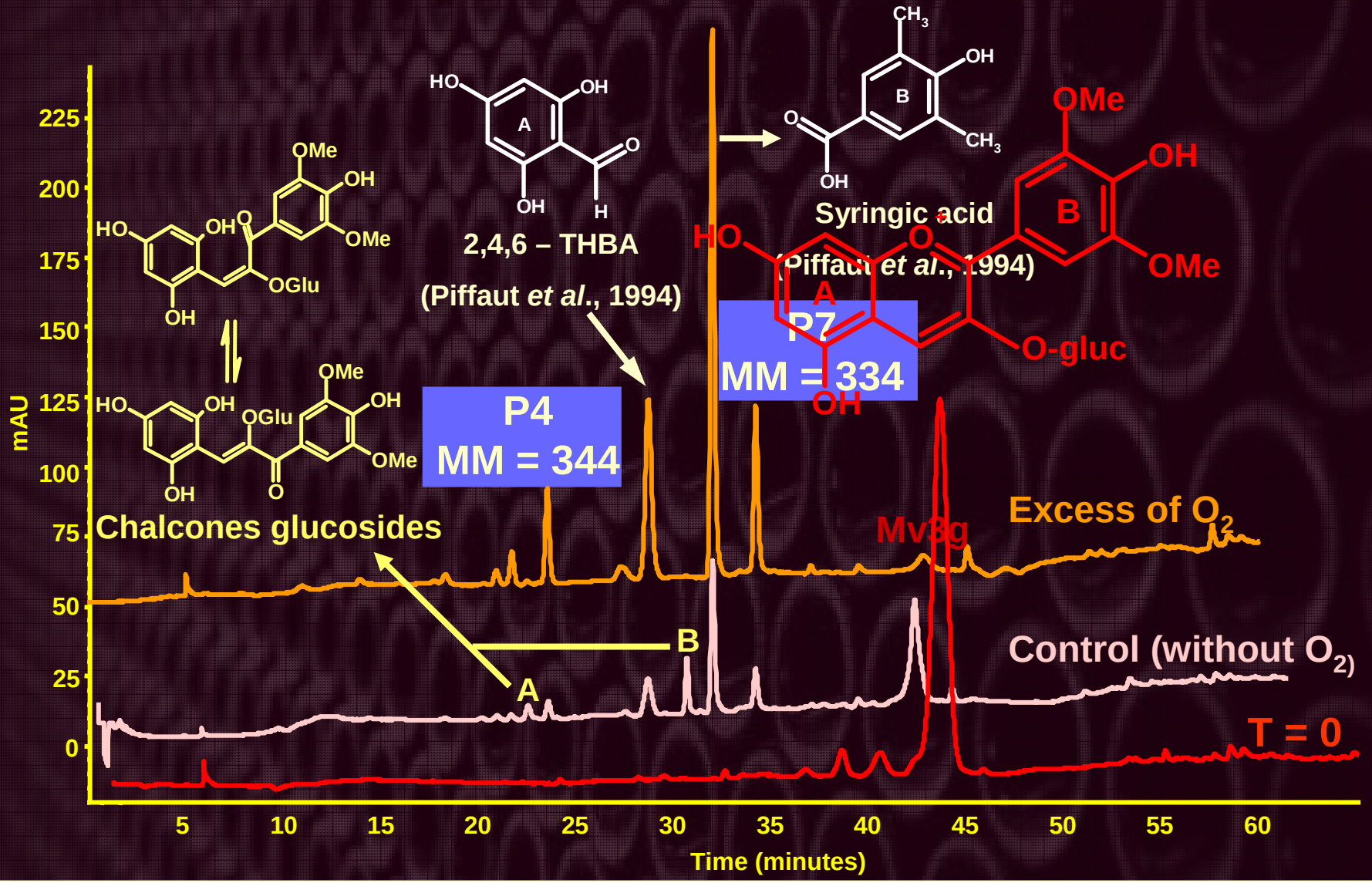


HPLC/UV-Vis/MS

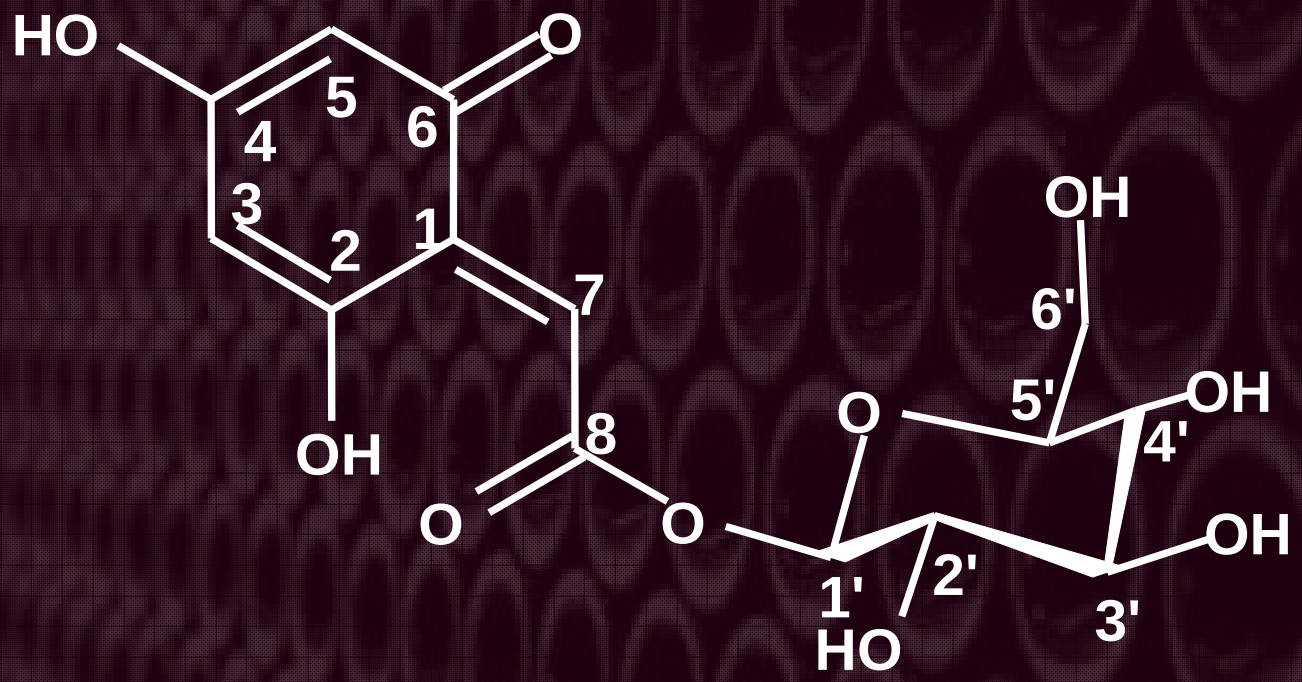
NMR

1. Effect of O2 on Mv3g degradation (pH 3,4)

- HPLC / UV (280 nm) follow up during 24 hours



- **P4 by NMR (^{1}H, Cosy ^{1}H- ^{1}H, HSQC ^{1}H- ^{13}C)**

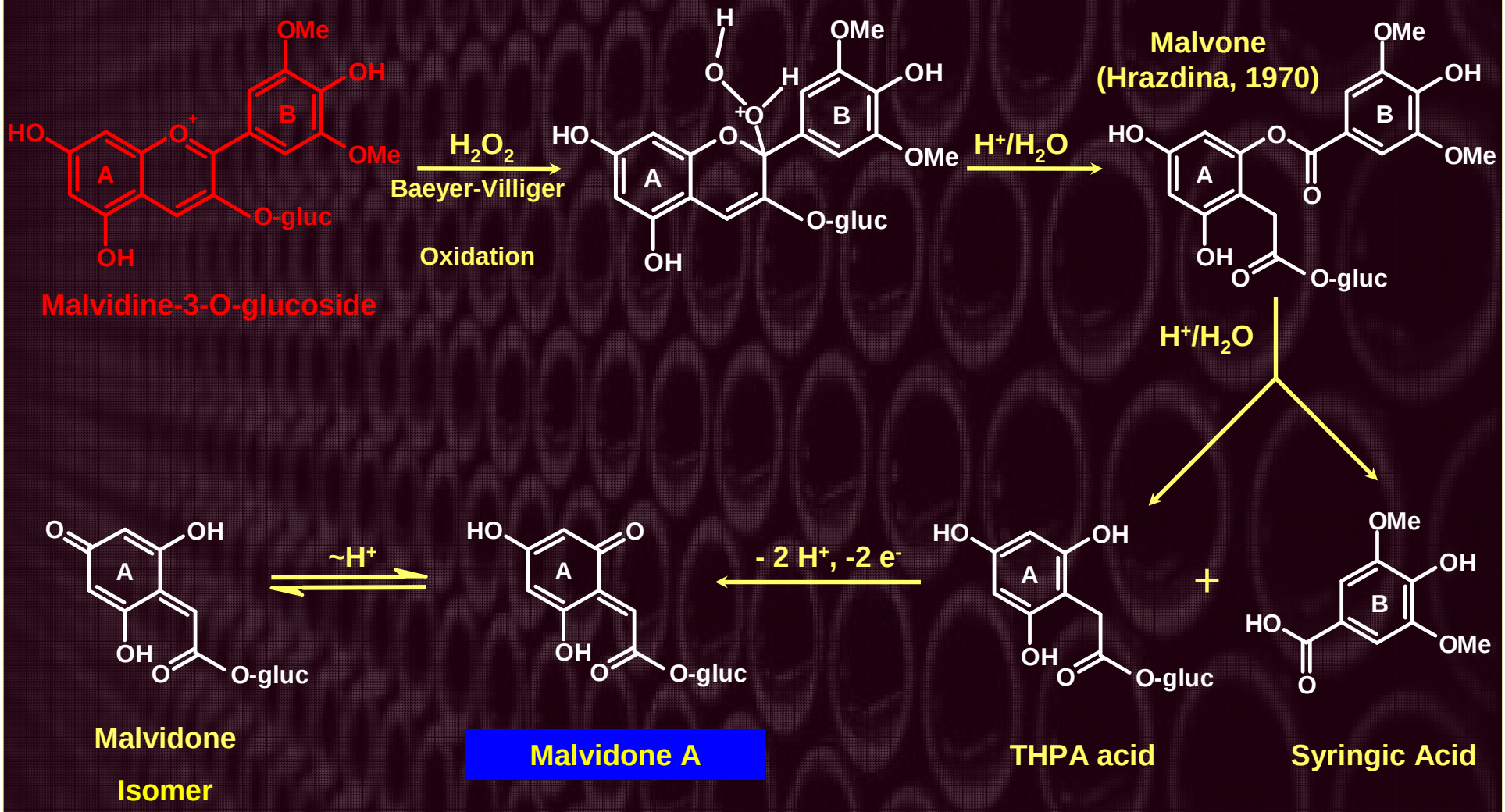


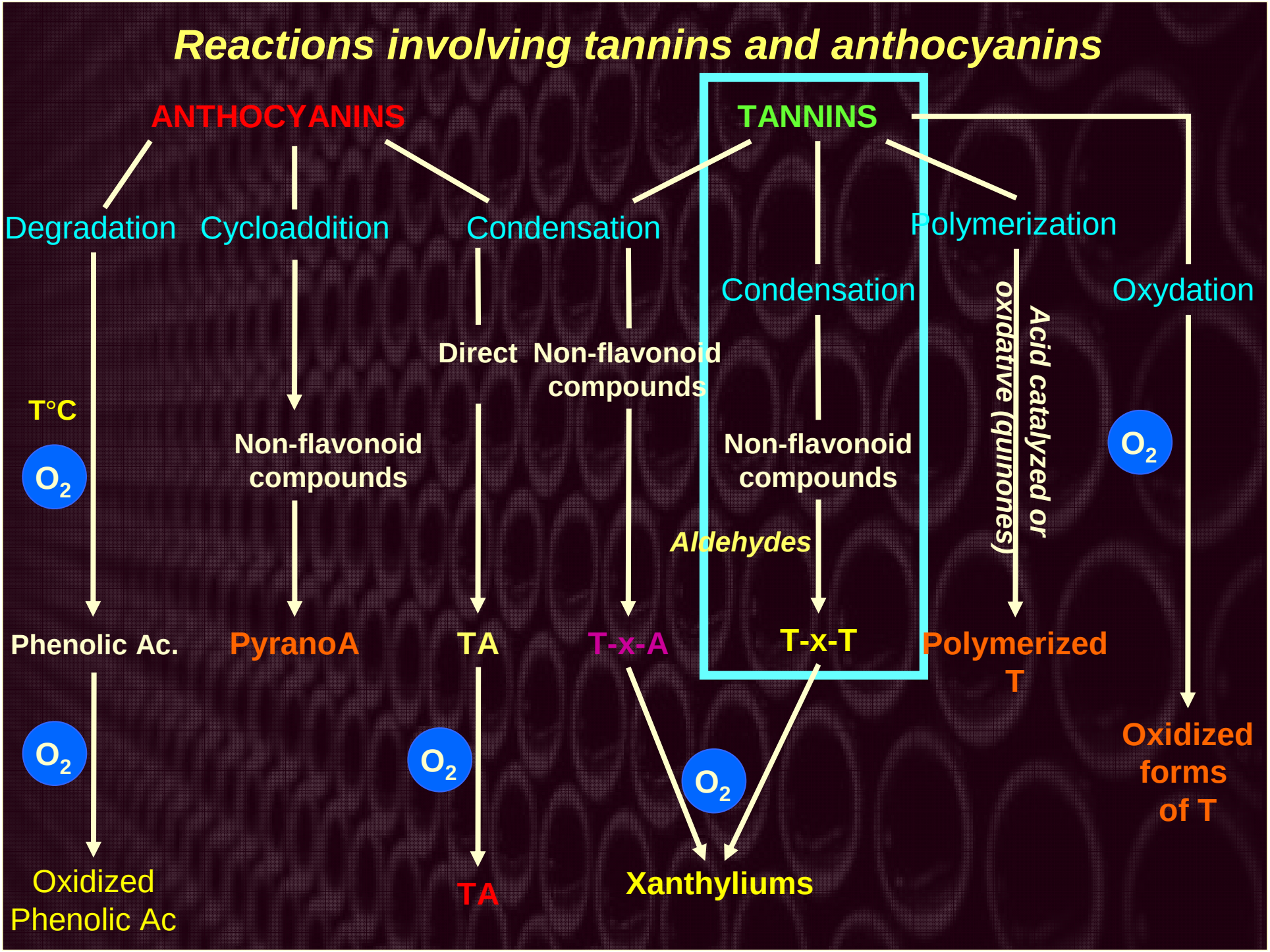
8-ß-D-glucopyranosyl-2,4-dihydroxy-6-oxo-cyclohexa-2,4-dienylidene acid

Anthocyanidone A

Lopes, P.; Richard, T.; Saucier, C.; Teissedre, P.-L.; Monti, J.-P.; Glories, Y.
Anthocyanone A: A Quinone Methide Derivative Resulting from Malvidin 3-O-Glucoside Degradation
***J. Agric. Food Chem*, 2007, 55, 2698-2704.**

▪ Proposed mechanism for Anthocyanidone A formation

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ANTHOCYANINS
TANNINS
Degradation
Cycloaddition
Condensation
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Oxydation
Direct
Non-flavonoid compounds
Non-flavonoid compounds
Non-flavonoid compounds
Aldehydes
Acid catalyzed or oxidative (quinones)
T°C
O2
O2
O2
O2
O2
Phenolic Ac.
PyranoA
TA
T-x-A
T-x-T
Polymerized T
Oxidized forms of T
Oxidized Phenolic Ac
TA
Xanthyliums

III

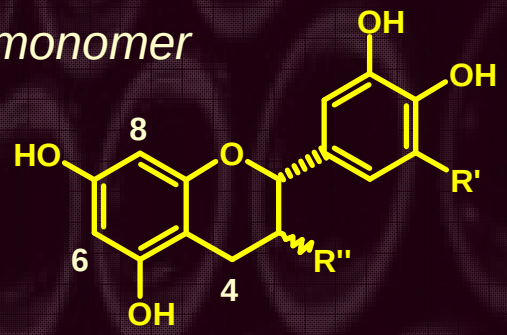
Example of Polymerization of tannins involving acetaldehyde

Tannin evolution: Example of acetaldehyde

Flavan-3-ols

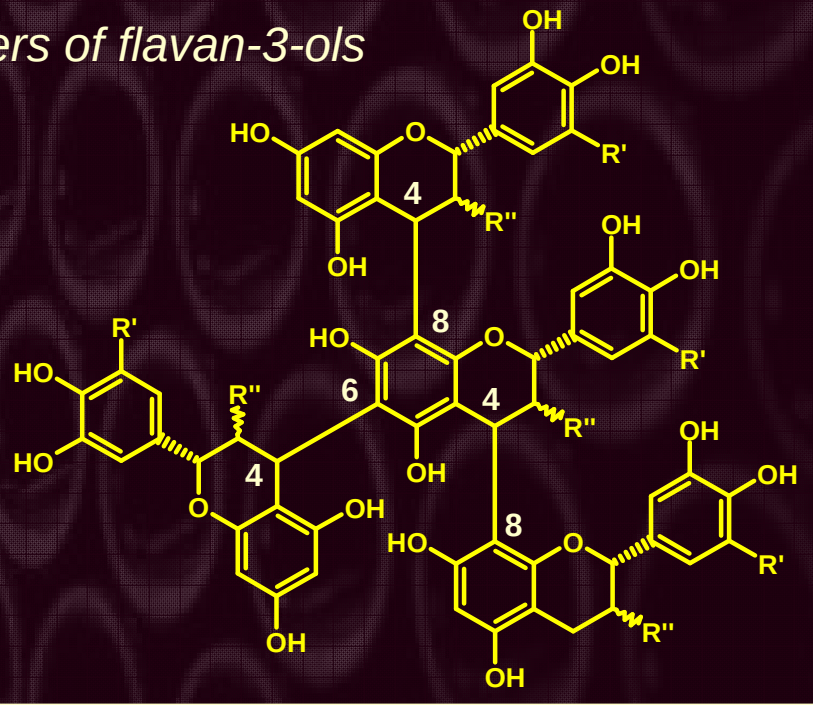
- 4 monomers
  - (+)-catechin
  - (-)-epicatechin
  - (-)-epigallocatechin
  - (-)-epicatechin gallate
- Flavan-3-ol polymers
- Wine quality
  - Taste
  - Color
- Biological properties
  - Antioxidants
  - « French Paradox »

Flavan-3-ol monomer



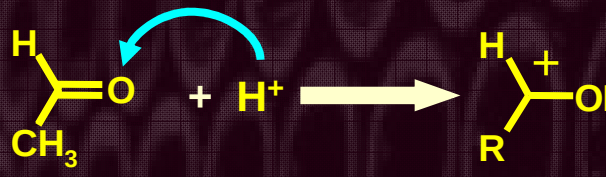
R'=H, R''=OH: **(+)-catechin, (-)-epicatechin**
R'=OH, R''=OH: **(-)-epigallocatechin**
R'=H, R''=O-gallate: **(-)-epicatechin gallate**

Polymers of flavan-3-ols

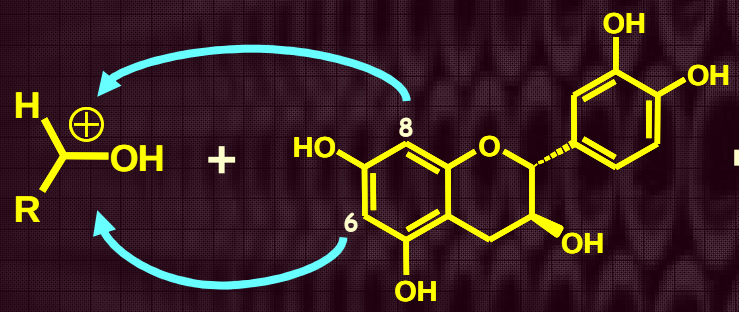


Mechanism of flavanol-Acetaldéhyde polymerization

Mechanism is known and proven to occur in wine (Saucier *et al.*, 1997; Fulcrand *et al.*, 1997; …)

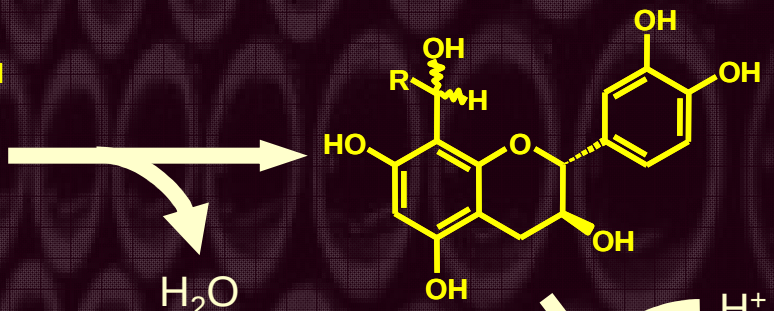


Carbocation (C^+)



C^+

(+)-Catechin



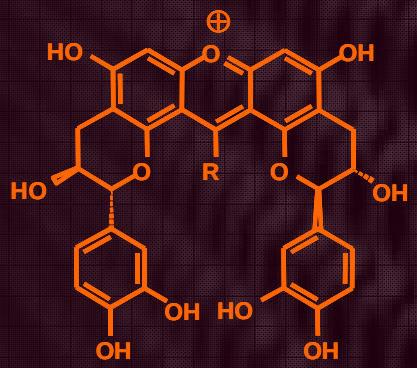
H_2O

IR 1

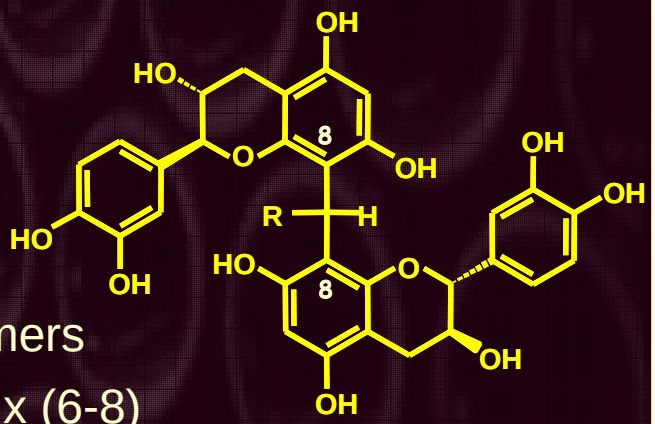
H^+ / **(+)-Catechin**

H_2O

Polymers



Xanthyliums

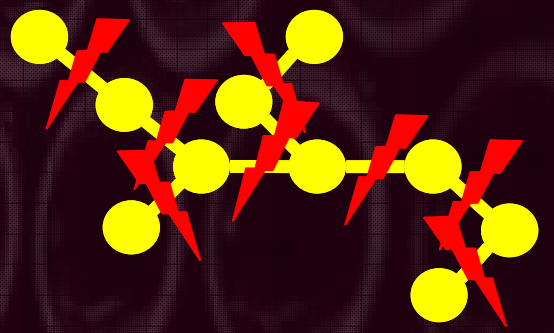


Bridged dimers

(8-8), (6-6), 2 x (6-8)

Flavan-3-ol quantification

- Flavan-3-ol polymers
  - Structural complexity
  - Analytical technique limited



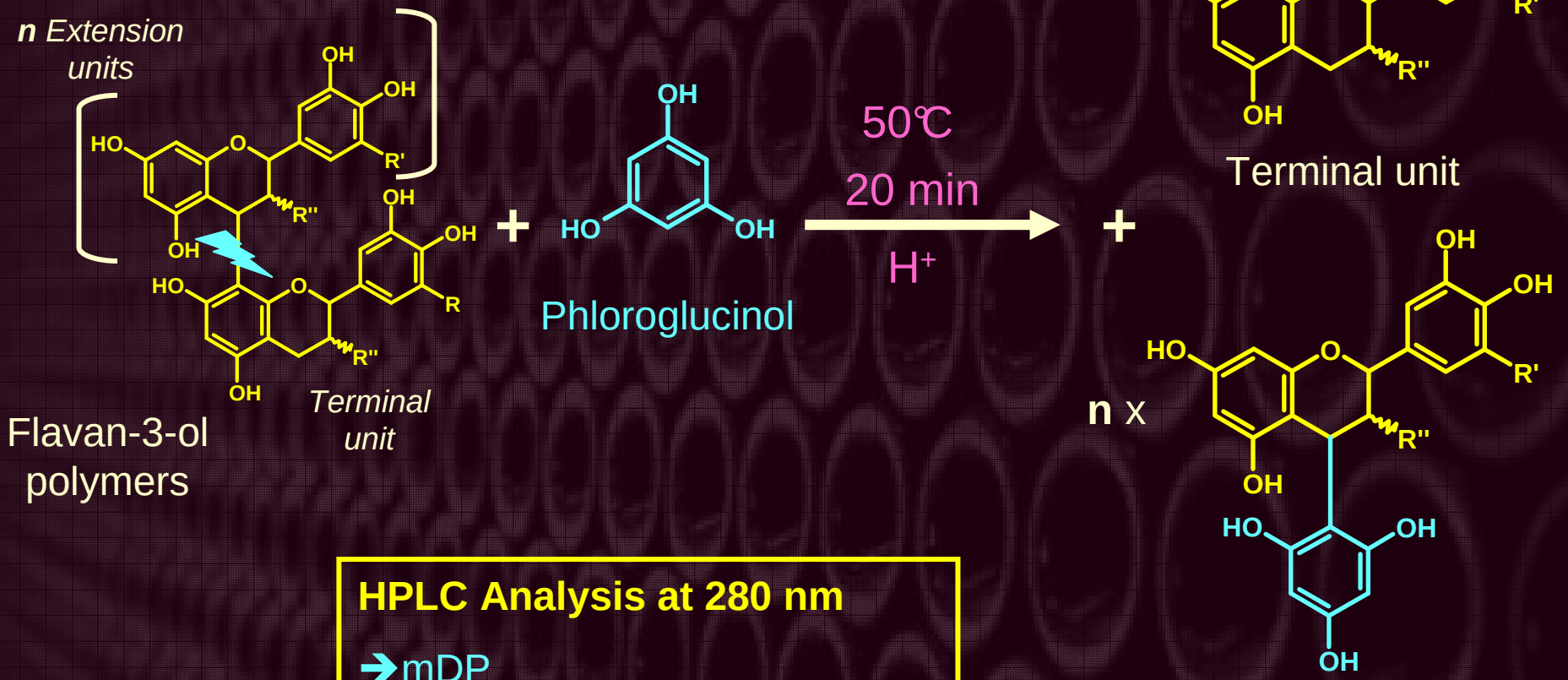
- Quantification based on polymer cleavage
- 2 methods
  - Thiolysis (Betts *et al.*, 1967; Rigaud *et al.*, 1991)
  - Phloroglucinolysis (Kennedy *et al.*, 2001; Peyrot des Gachons *et al.*, 2003)

Work choice

Phloroglucinol odorless and non-toxic / benzylmercaptan

Analysis of ethylidene-bridged flavan-3-ols in red wine

Phloroglucinolysis reaction



HPLC Analysis at 280 nm

➔mDP

➔Monomer quantification

- Terminal units
- Extension units

Phloroglucinolysis of (+)-catechin ethylidene-bridged oligomers

1°- (+)-catechin ethylidene-bridged oligomer synthesis

➔ (+)-catechin-acetaldehyde condensation

- Excess of aldehyde
- Winelike solution and 40°C

➔ Ethylidene-bridged oligomers

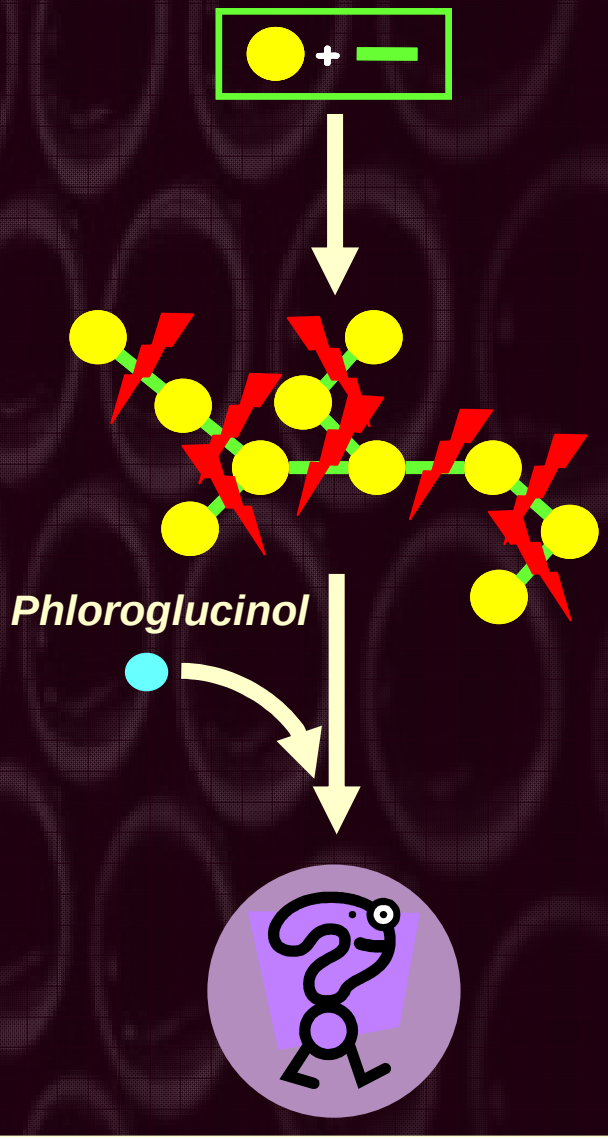
➔ Monomer

➔ Dimers, Trimers

2°- Phloroglucinolysis

- Oligomers in phloroglucinolysis reactant
- 20 min at 50°C

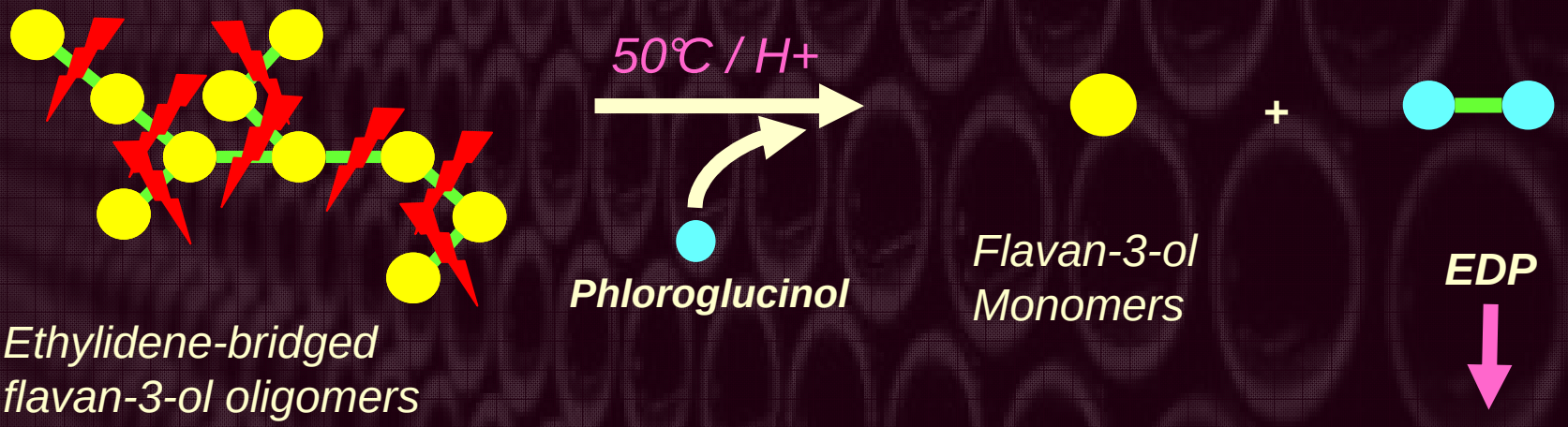
➔ LC-MS and 280 nm analyses



Ethylidene-bridged flavan-3-ol phloroglucinolysis

➔ Phloroglucinolysis

- Flavan-3-ol monomers released
- Ethylidene bridges captured by phloroglucinol : EDP



All flavan-3-ol ethylidene bridges quantified by EDP

Quantification of flavan-3-ol ethylidene bridges in wine

1°- SPE C_{18}: Purification / Concentration

2°- Phloroglucinolysis

3°- LC-MS Analysis

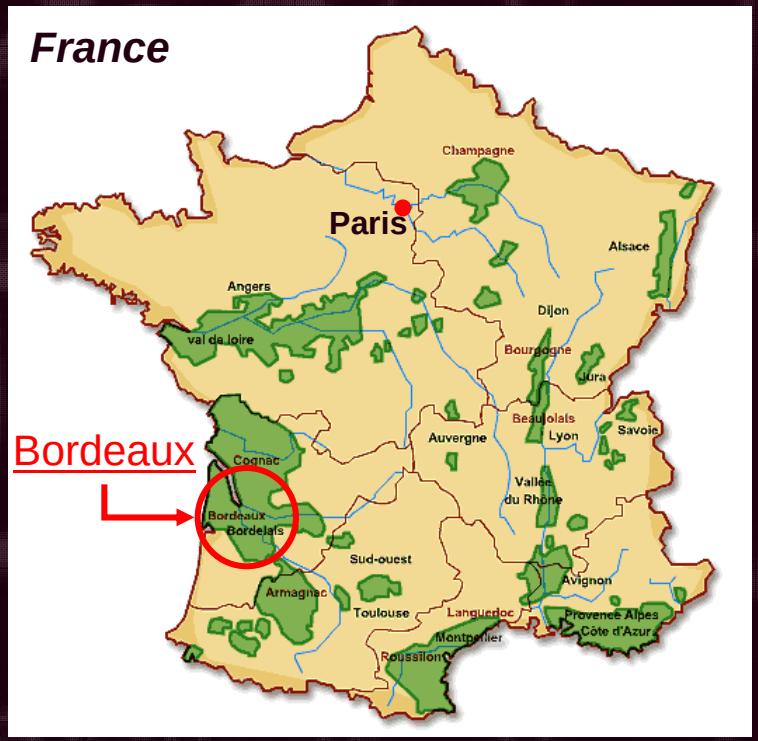
→ [EDP] → [Ethylidene bridges]

Method repeatability: 9.2%

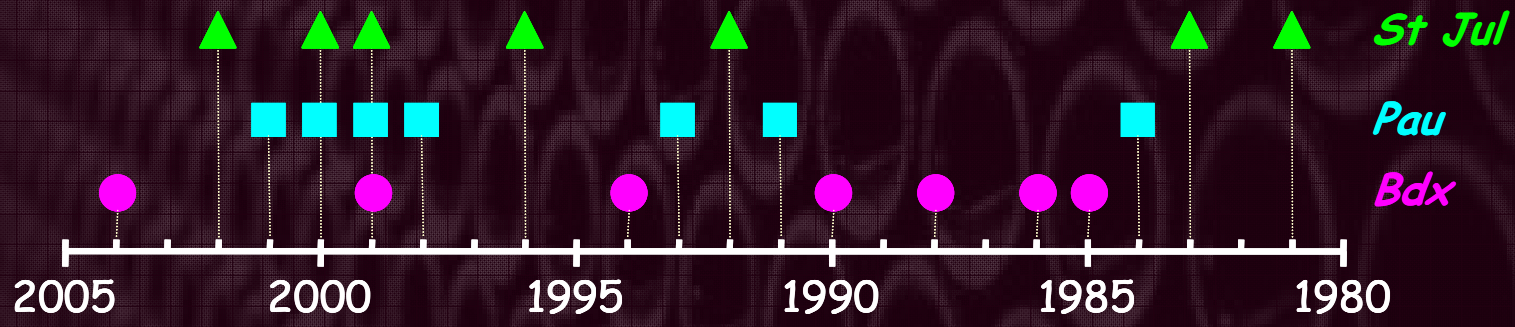
Method recovery: 83% to 90% depending on ethylidene bridges conc.

Application to 21 red wines from Bordeaux area

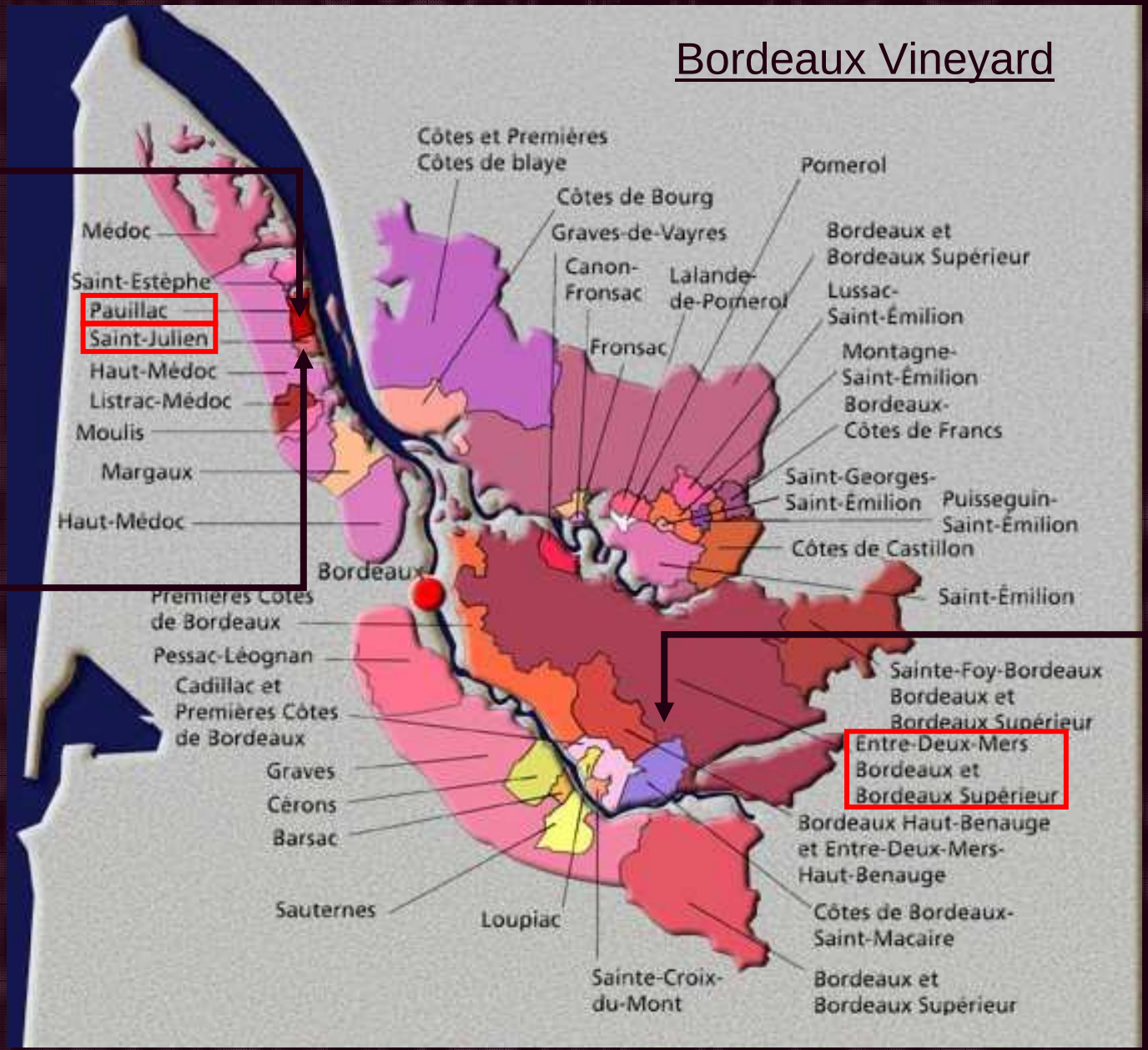
- 3 Wineries in
  - ● Bordeaux (Bdx)
  - ■ Pauillac (Pau)
  - ▲ Saint Julien (St Jul)



- Different vintages



Analysis of ethylidene-bridged flavan-3-ols in red wine

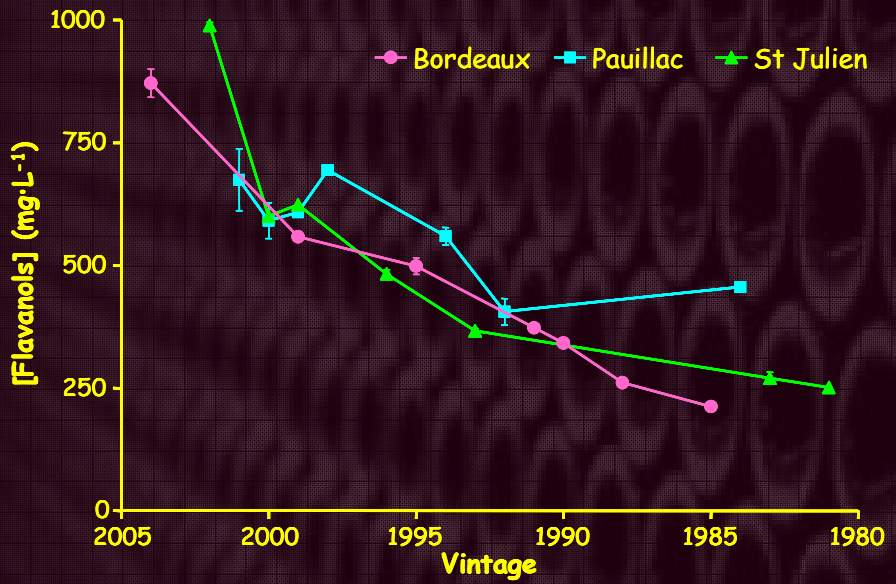


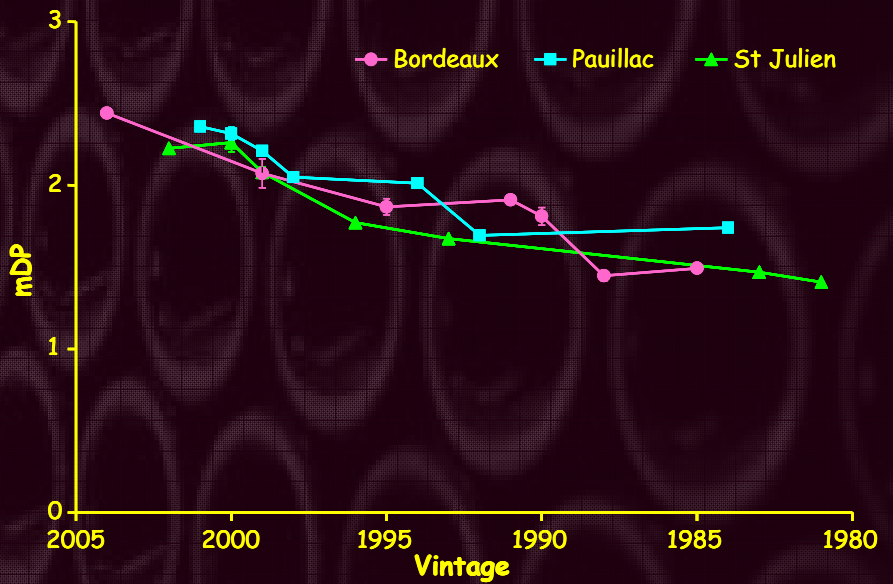
Analyses

- Flavan-3-ol contents
  - ↘↘ Flavan-3-ols (Fl)
  - ↘ mDP

↘↘ *With wine age*

➔ *Rearrangement reactions with other wine compounds*





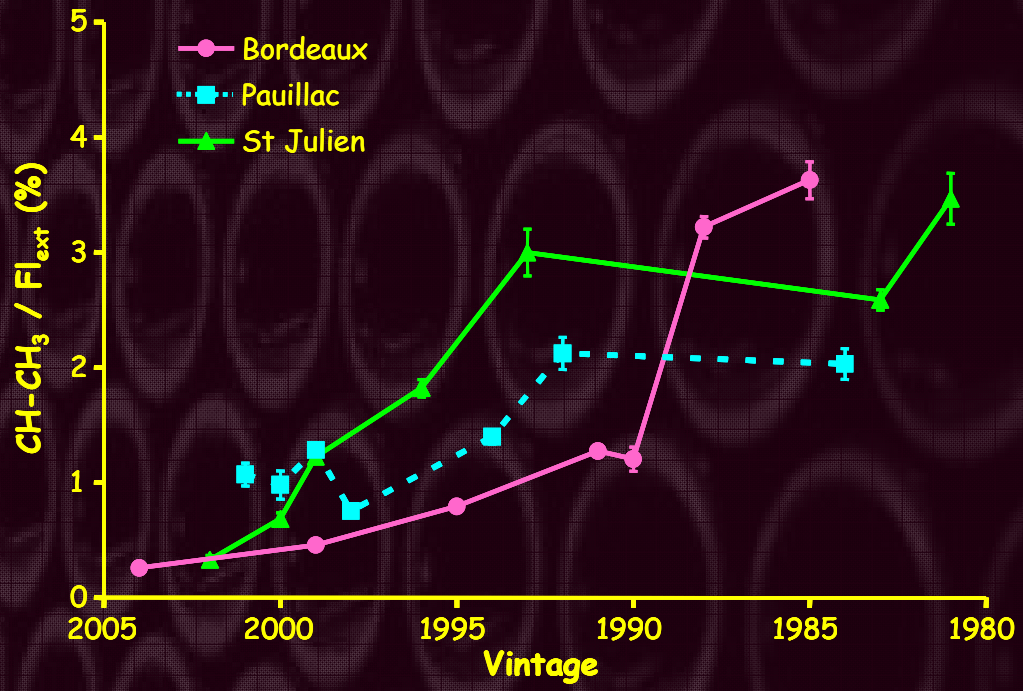
Analyses

- % of Ethylidene bridges
  - $CH\text{-}CH_3/Fl_{ext}$

➔ *Low proportion*

➔ *But ↗ with wine age*

➔ New index of wine aging ?



Conclusions

- First quantification of flavan-3-ol ethylidene bridges
  - EDP phloroglucinolysis
  - LC-MS analysis

- Proportion of Flavan-3-ol ethylidene bridges
  - Correlated with wine age

➔ Could be a new marker for wine age or help in wine oxidation management (O_2 management)

Conclusions

– Drinkine, J.; Lopes, P.; Kennedy, J. A.; Teissedre, P.-L.; Saucier, C. Analysis of ethylidene-bridged flavan-3-ols in wine. *J. Agric. Food Chem.,* **2007** ,55, 1109 -1116

– Drinkine, J.; Lopes, P.; Kennedy, J. A.; Teissedre, P.-L.; Saucier, C. Ethylidene-bridged flavan-3-ols in red wine and correlation with wine age. *J. Agric. Food Chem.,* **2007** , 55, 6292 - 6299.

Acknowledgments

Collaborators:

J. DRINKINE, P. LOPES, J.A KENNEDY, PL TEISSEDRE, Y GLORIES

Fundings:

Yvon Mau SA, Amorim, CIVB, ANRT (Gov.)


THANK YOU !