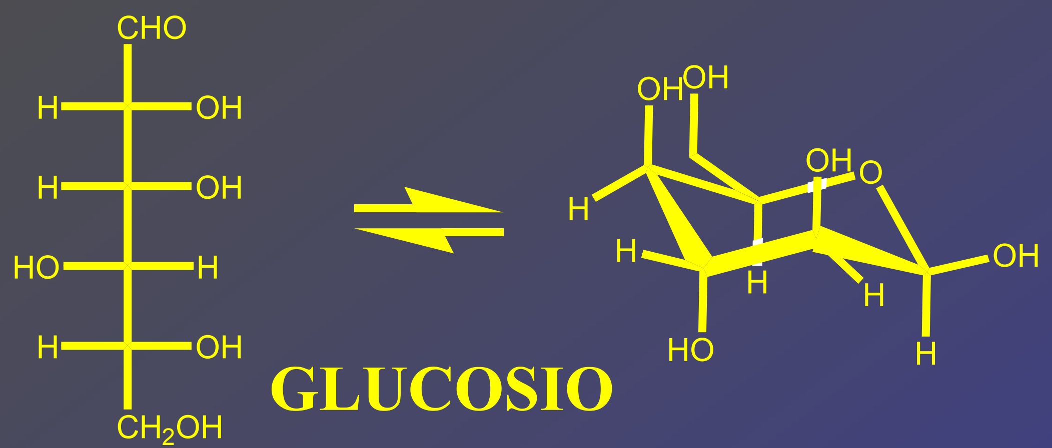
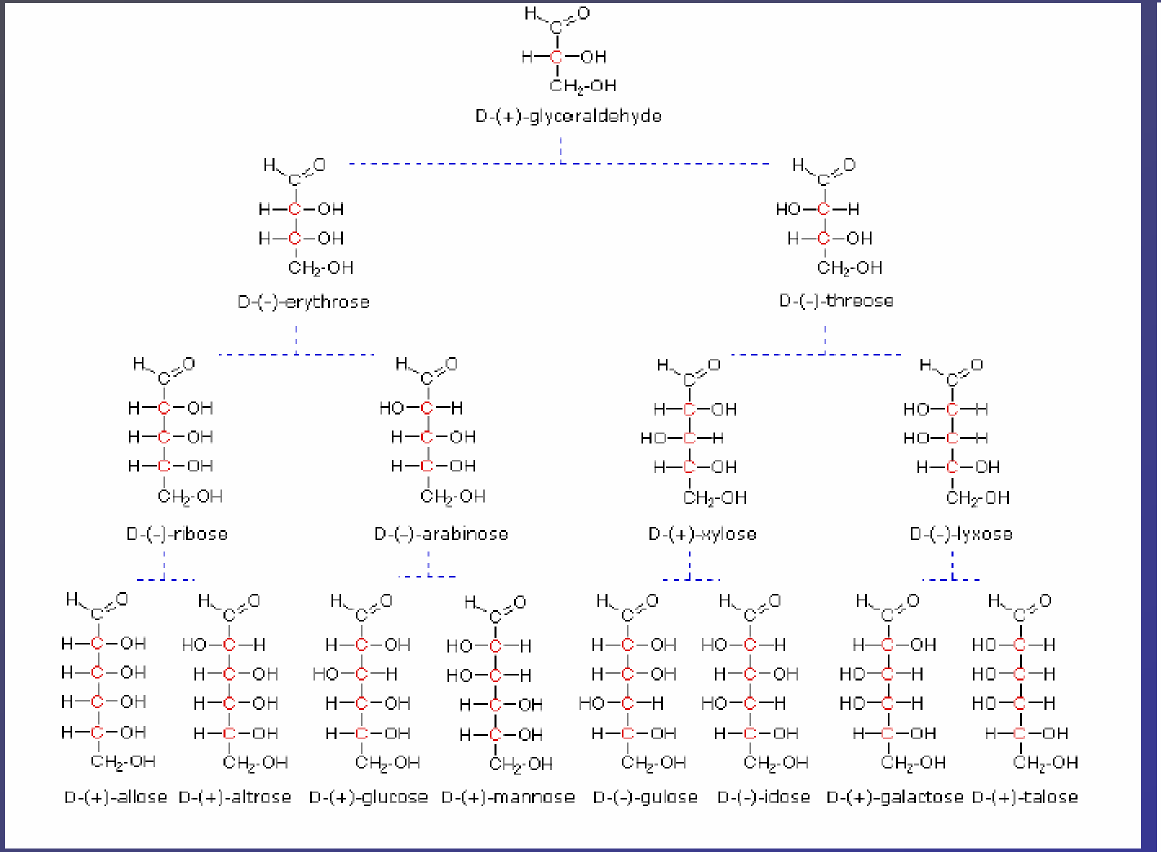
Natural Products Chemistry of Marine Organisms

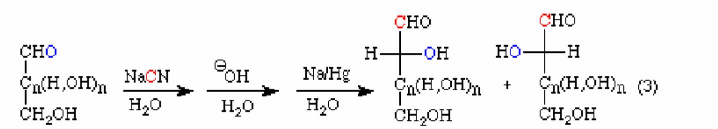
afontana@icmib.na.cnr.it



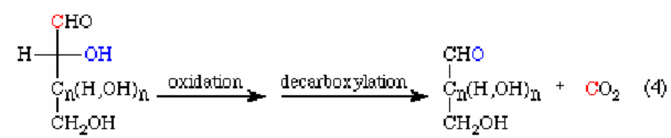
GLUCOSIO

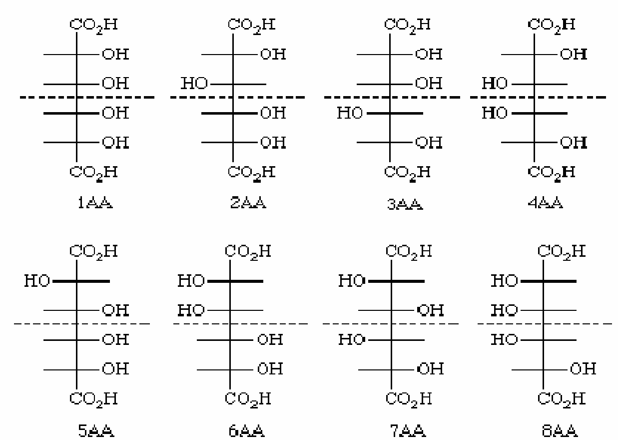
Emil Fisher
Premio Nobel 1902

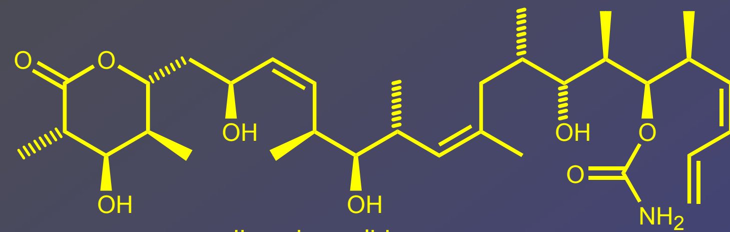




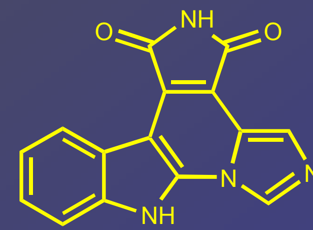
Epimers – diastereomers that differ only at one stereocenter.



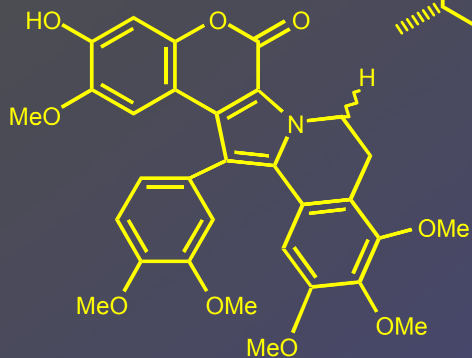




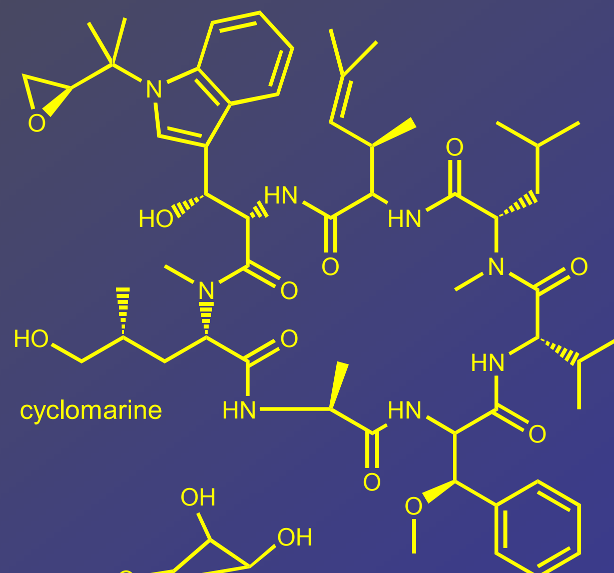
discodermolide



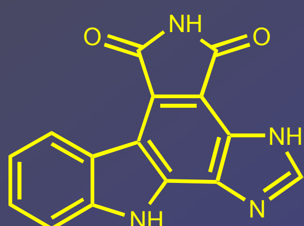
granulatimide



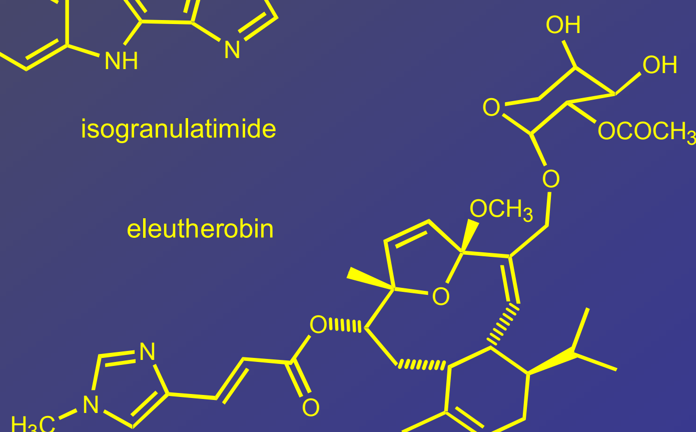
Lamellarin I



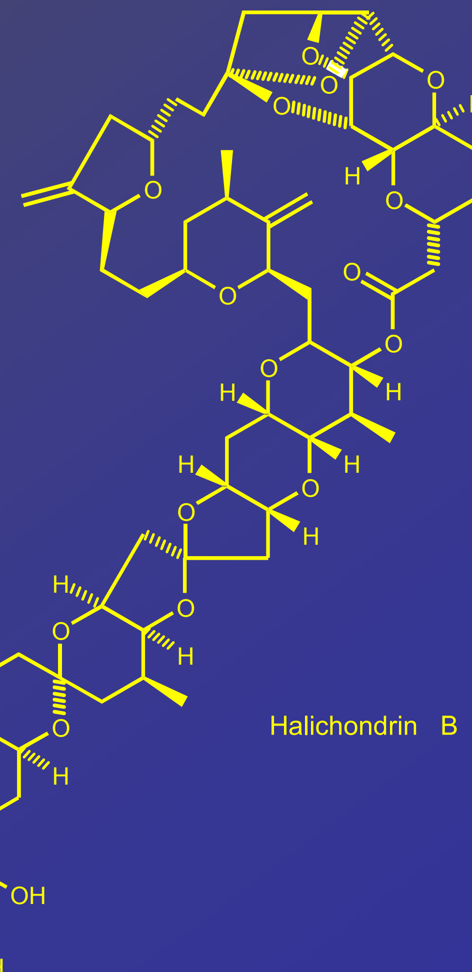
cyclomarine



isogranulatimide



eleutherobin



Halichondrin B

Drugs from the Sea

Marine Natural Products

From 1967: [1]

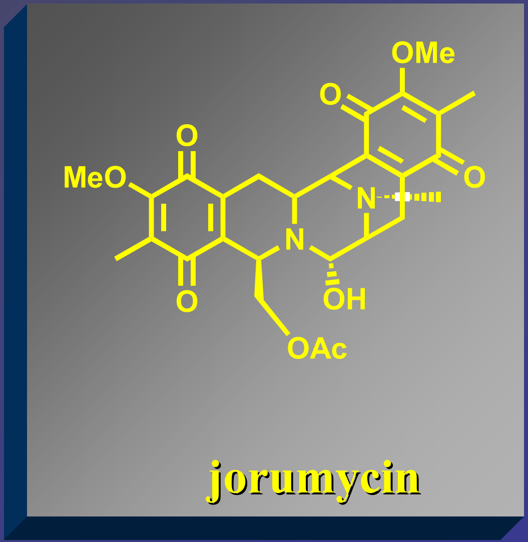
- **More than 18.000 New metabolites**
- **0,1% lead structures**
- **More than 400 patents**

↓

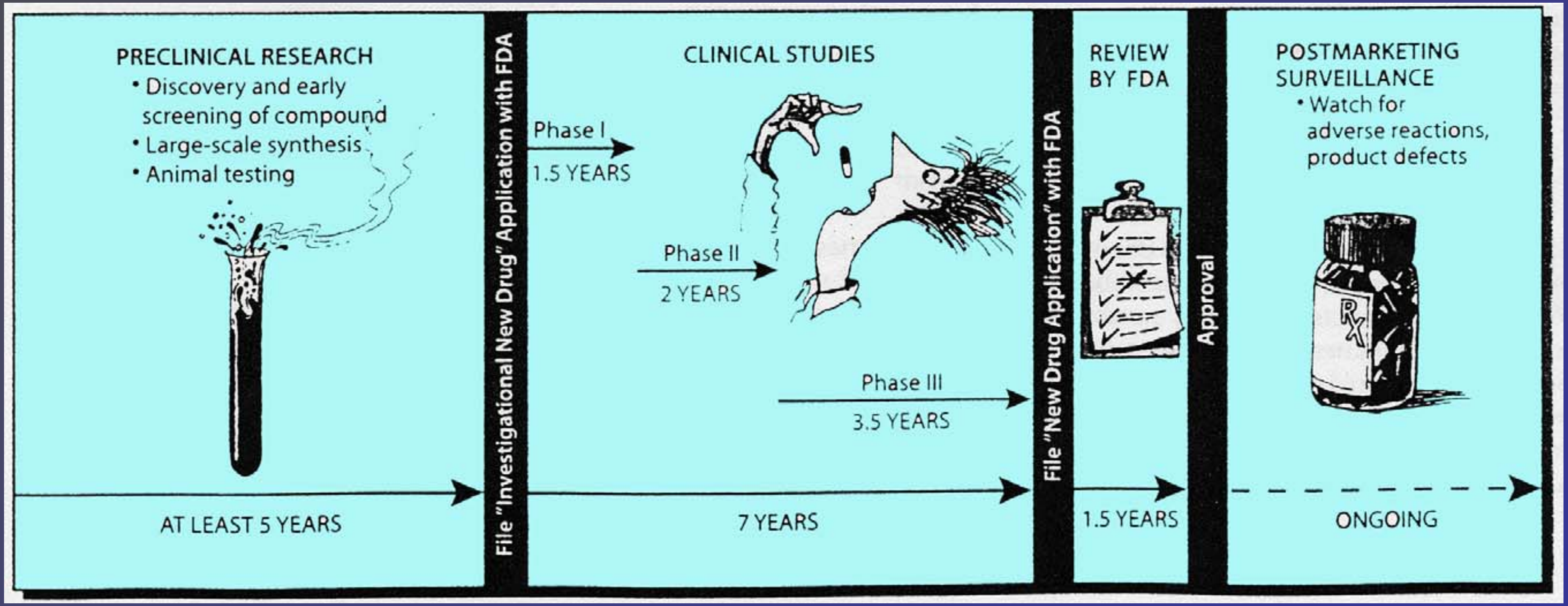
Therapeutical Potential

(1) FUSETAMI N (ed), DRUGS FROM THE SEA, Basel, Karger, 2000, 1-15

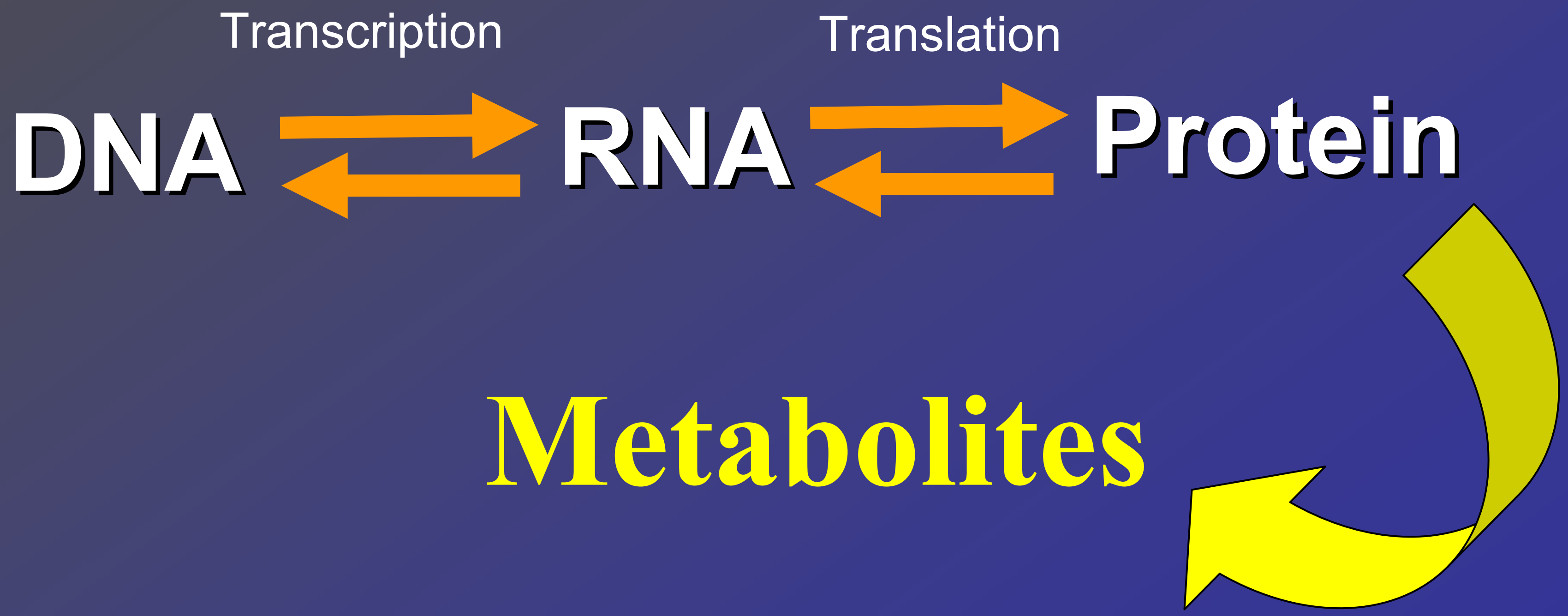
Zalypsis®



- Zalypsis® (PM00104/50) is PharmaMar's fifth new marine derived compound that has begun Phase I clinical trials for the treatment of solid tumours.
- Zalypsis® is a novel chemical entity related to the marine natural compounds Jorumycin and the family of Renieramycins, obtained from molluscs and sponges, respectively. Zalypsis binds to DNA and is cytotoxic; however, it does not activate the "DNA damage checkpoint" response. Thus, Zalypsis has cytotoxic effects dependent on DNA binding that are not associated with DNA damage.
- In pre-clinical trials, Zalypsis demonstrated strong in vitro and in vivo antitumoural activity in a wide variety of solid and haematological tumour cell lines and human transplantable breast, gastric, prostate and renal xenografted tumours. Zalypsis also demonstrated a manageable and reversible preclinical toxicology profile.

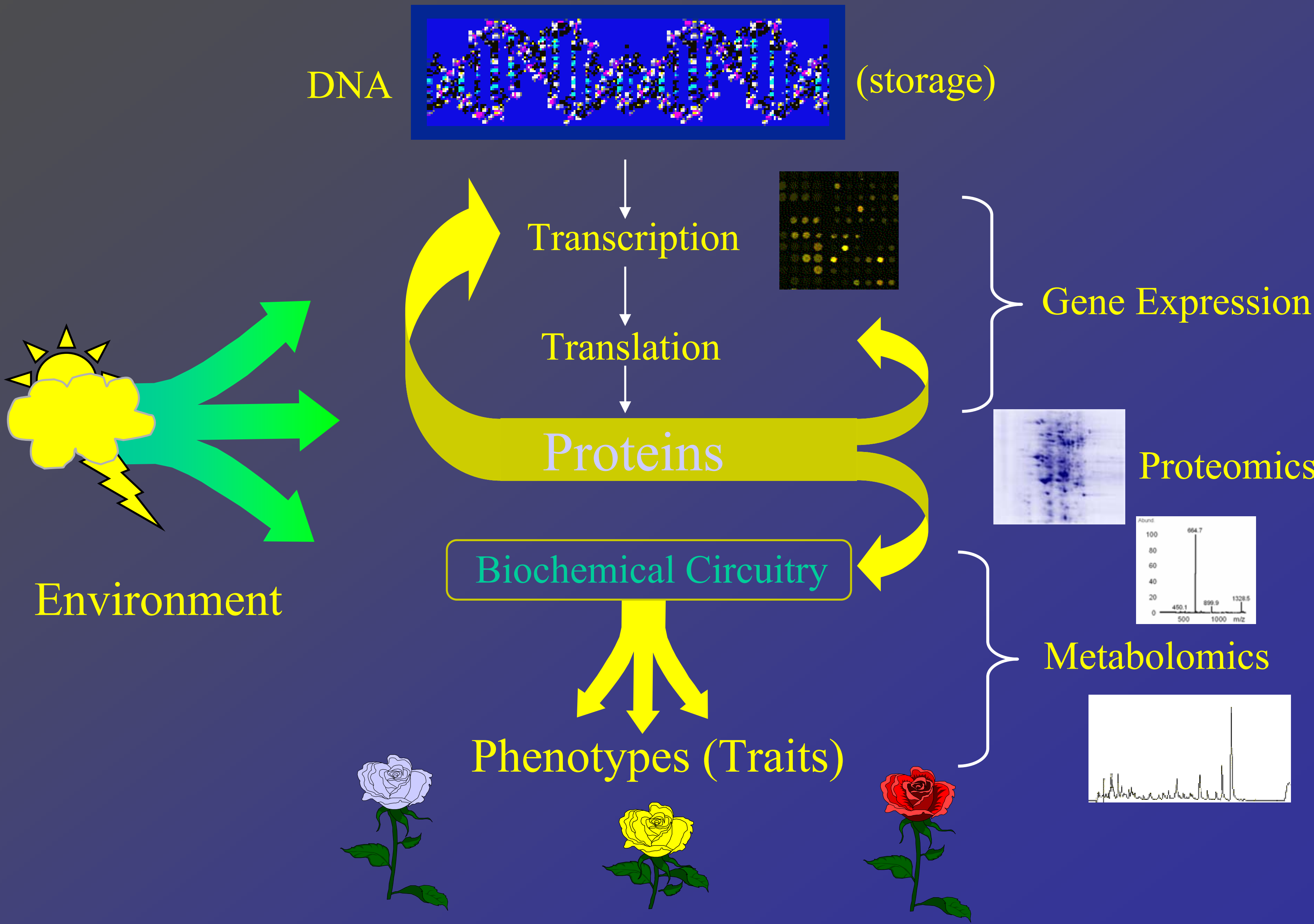

PRECLINICAL RESEARCH
• Discovery and early screening of compound
• Large-scale synthesis
• Animal testing
AT LEAST 5 YEARS
File "Investigational New Drug" Application with FDA
CLINICAL STUDIES
Phase I
1.5 YEARS
Phase II
2 YEARS
Phase III
3.5 YEARS
7 YEARS
File "New Drug Application" with FDA
REVIEW BY FDA
1.5 YEARS
Approval
POSTMARKETING SURVEILLANCE
• Watch for adverse reactions, product defects
RX
ONGOING

$$\textbf{DNA} \underset{}{\overset{\text{Transcription}}{\rightleftarrows}} \textbf{RNA} \underset{}{\overset{\text{Translation}}{\rightleftarrows}} \textbf{Protein}$$


Transcription
Translation
DNA
RNA
Protein
Metabolites

The metabolite pattern (metabolome) reflects the cell's status. It is the totality of metabolic processes including anabolism and catabolism, as well as all the related cellular processes such as absorption, distribution, and detoxification of natural and xenobiotic materials, energy utilization, signal transduction, and regulation. It results from the expression of the genome and proteome in response to the cellular environment. While the Genome is representative of what might be, and the Proteome is what is expressed, it is the Metabolome that represents the current status of the cell or tissue.

Predicting Life Processes: Reverse Engineering Living Systems



Guido Cimino

Margherita Gavagnin
Emiliano Manzo
Maria Letizia Ciavatta
Marianna Carbone (PhD Student)

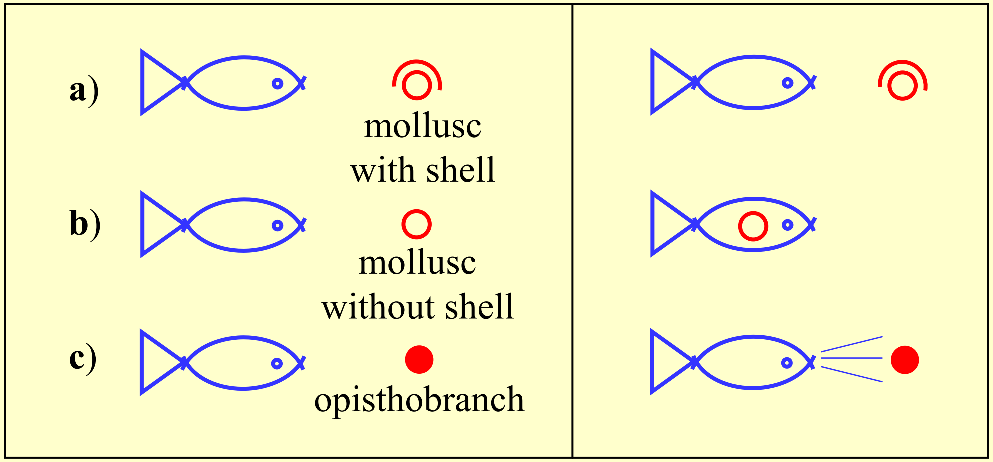
Guido Villani
Ernesto Mollo

Adele Cutignano
Giuliana d'Ippolito (PhD student)
Antonio Maiello
Angelo Fontana

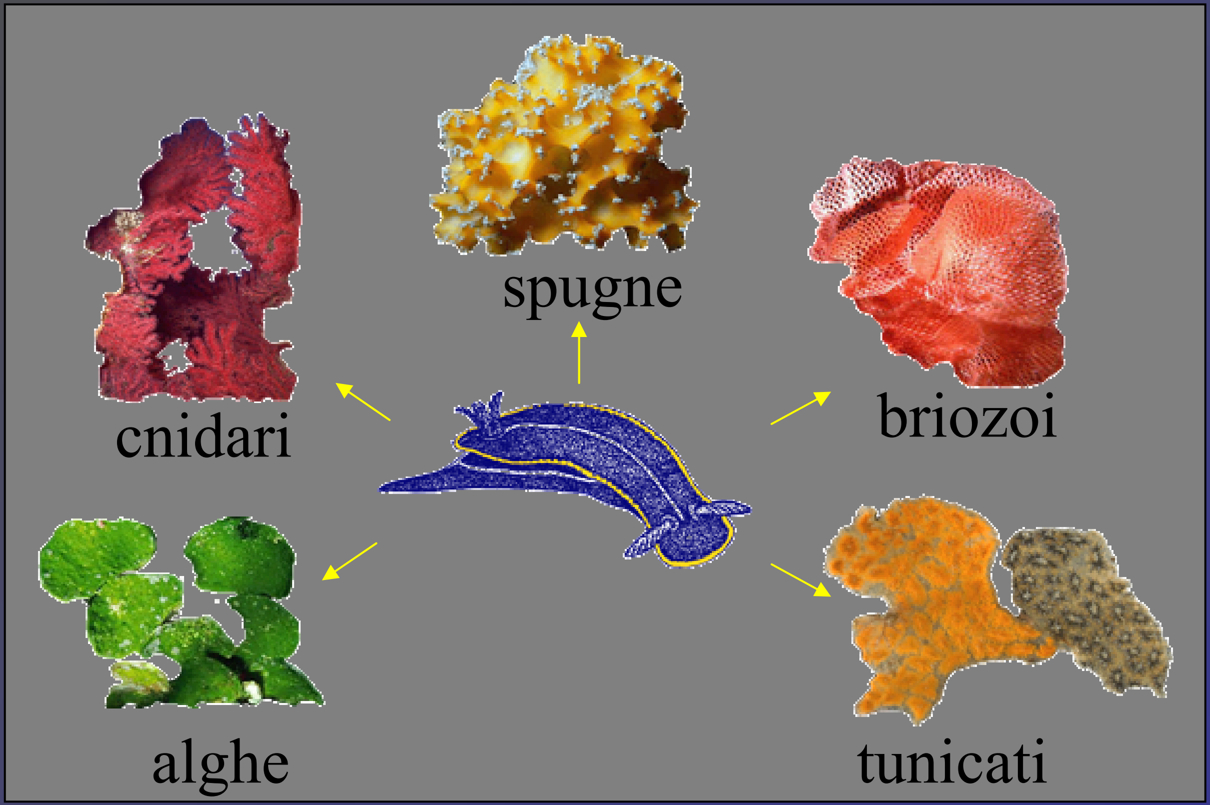
Memorie storiche sugli opistobranchi

1890 Il prof. Herdman pose alcuni esemplari vivi di *Ancula cristata* sulla lingua del suo assistente dr. Moore

1960 T.E. Thompson J. Mar. Biol. U.K. **39**, 115



1976 J. Ros *Oecologia aquatica* **3**, 153
"bad taste of the nudibranchs"
"ignorance of the chemical mechanisms"

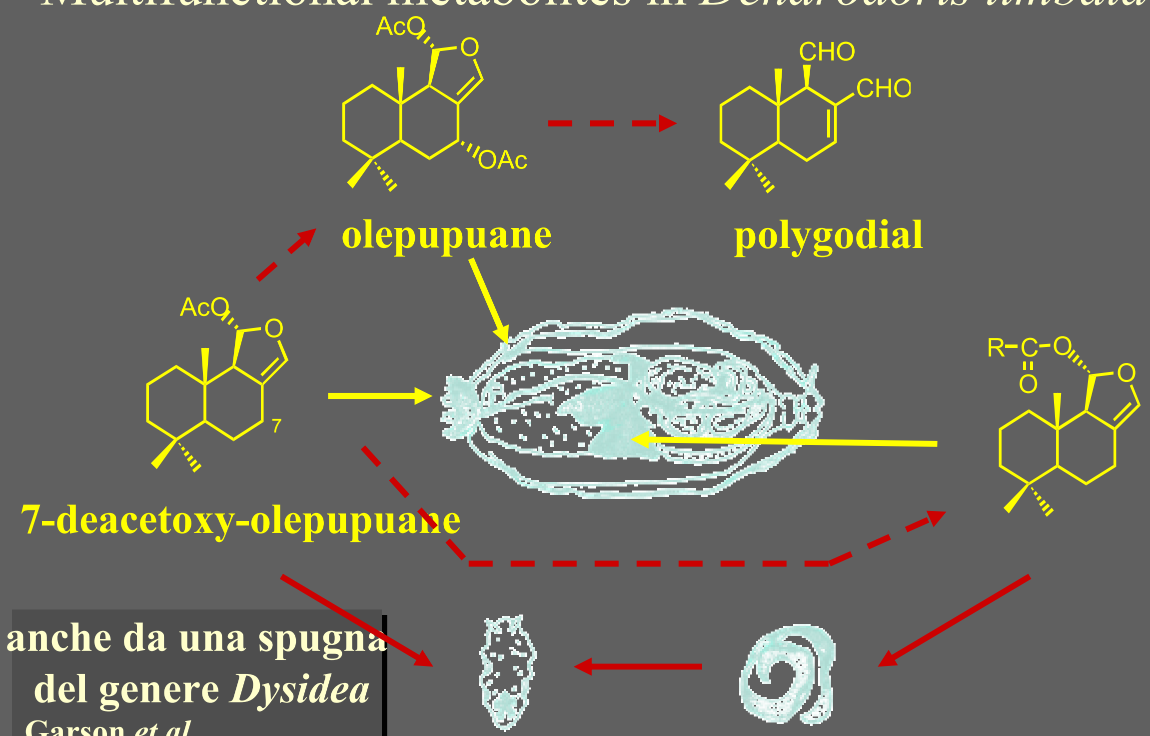


- **bio-accumulation**

- **bio-transformation**

- **bio-synthesis**

Multifunctional metabolites in *Dendrodoris limbata*



anche da una spugna del genere *Dysidea*
Garson *et al.*
***J. Nat. Prod.*. 55, 364, 1992**

Experientia **47**, 56, 1991

Ecological activity: aposematic colours of marine opisthobranchs

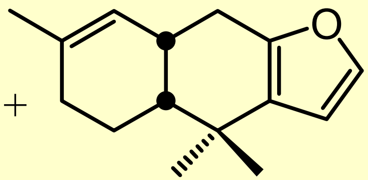
Ecologica activity: aposematic colours in marine opisthobranchs

Thalassoma pavo
10 esemplari

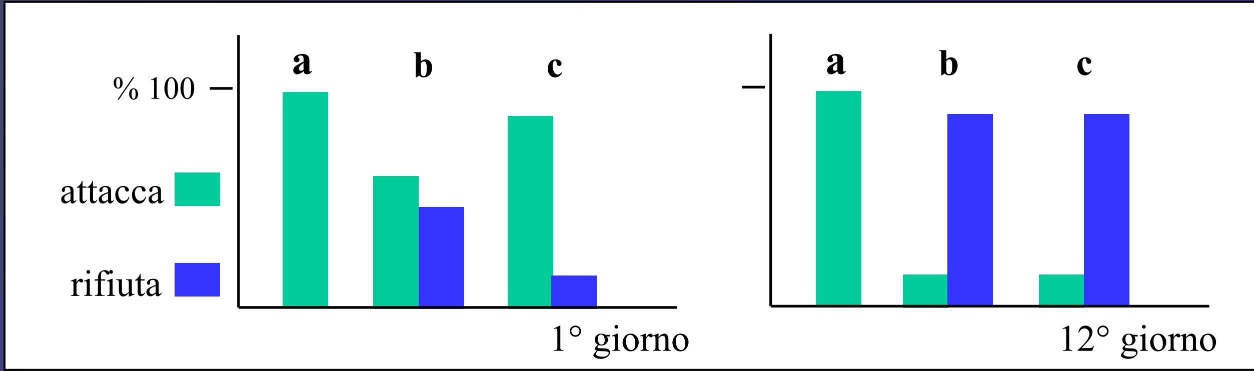
→

25 modelli
- **a) non aposematici**
- **b) aposematici con tossine**
- **c) aposematici senza tossine**

Hypselodoris +



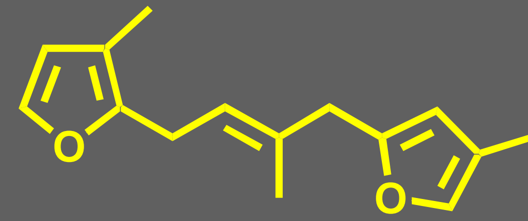
furodysinin



T. pavo remembers the external features of ptentially dangerous preys

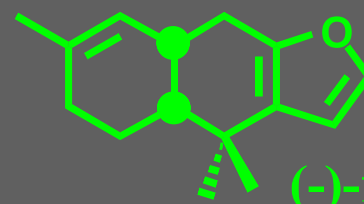
Dietary origin of bioactive molecules

Hypselodoris webbi
(nudibranco)



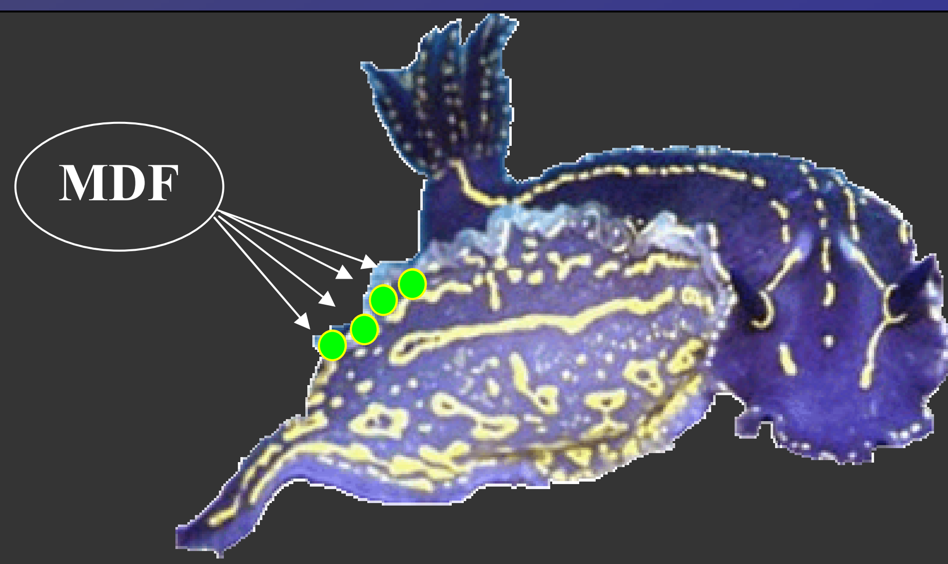
longifolin

Dysidea fragilis
(spugna)



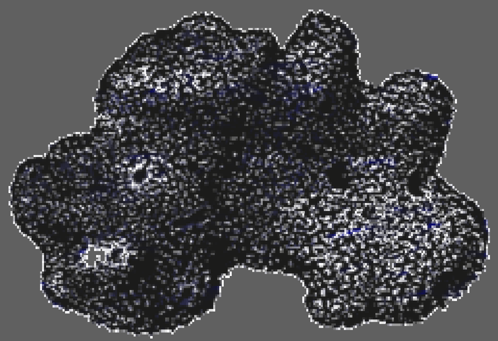
(-)-furodysinin

gg.	(-)-furodysinin / longifolin
0	0 (pura longifolin)
7	3,8
35	13,0

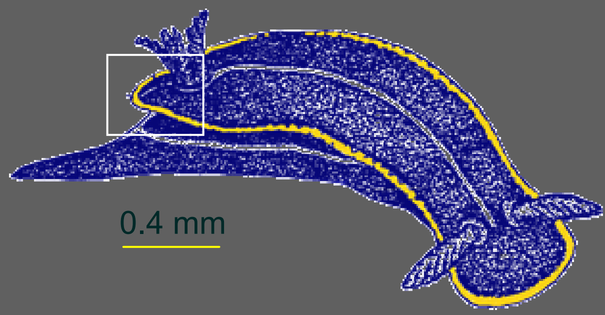


Experientia **50**, 510, 1994

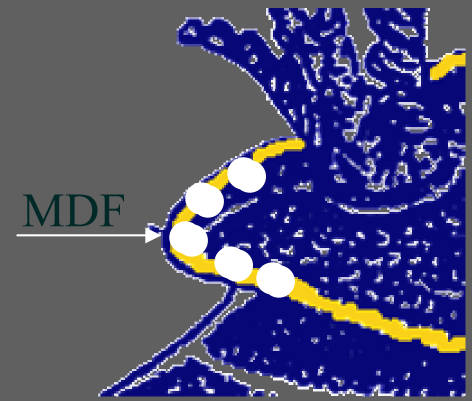
Bio-transformation of dietary metabolites



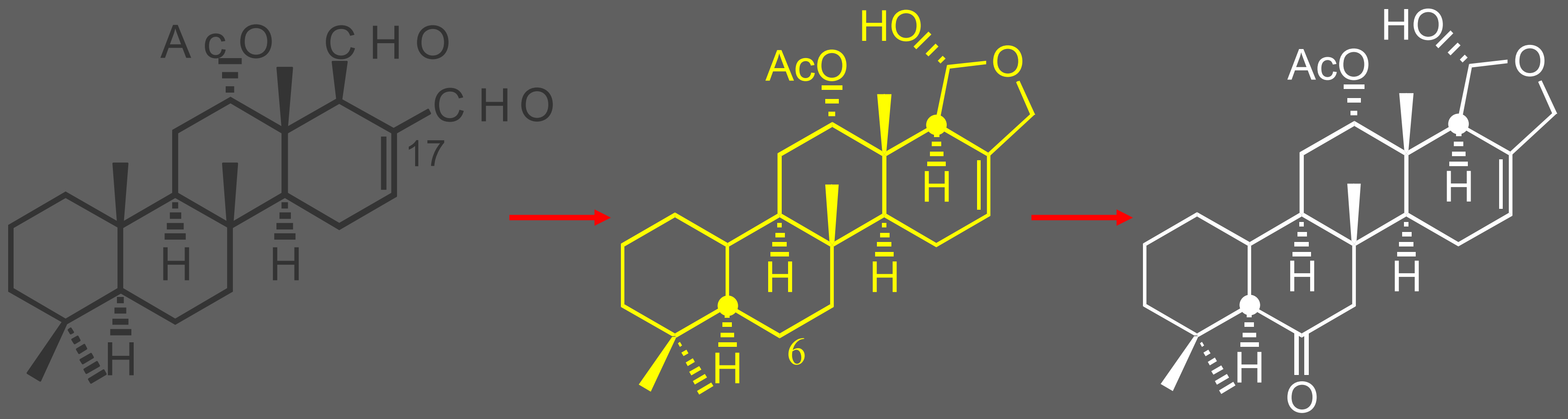
C. mollior



H. orsini

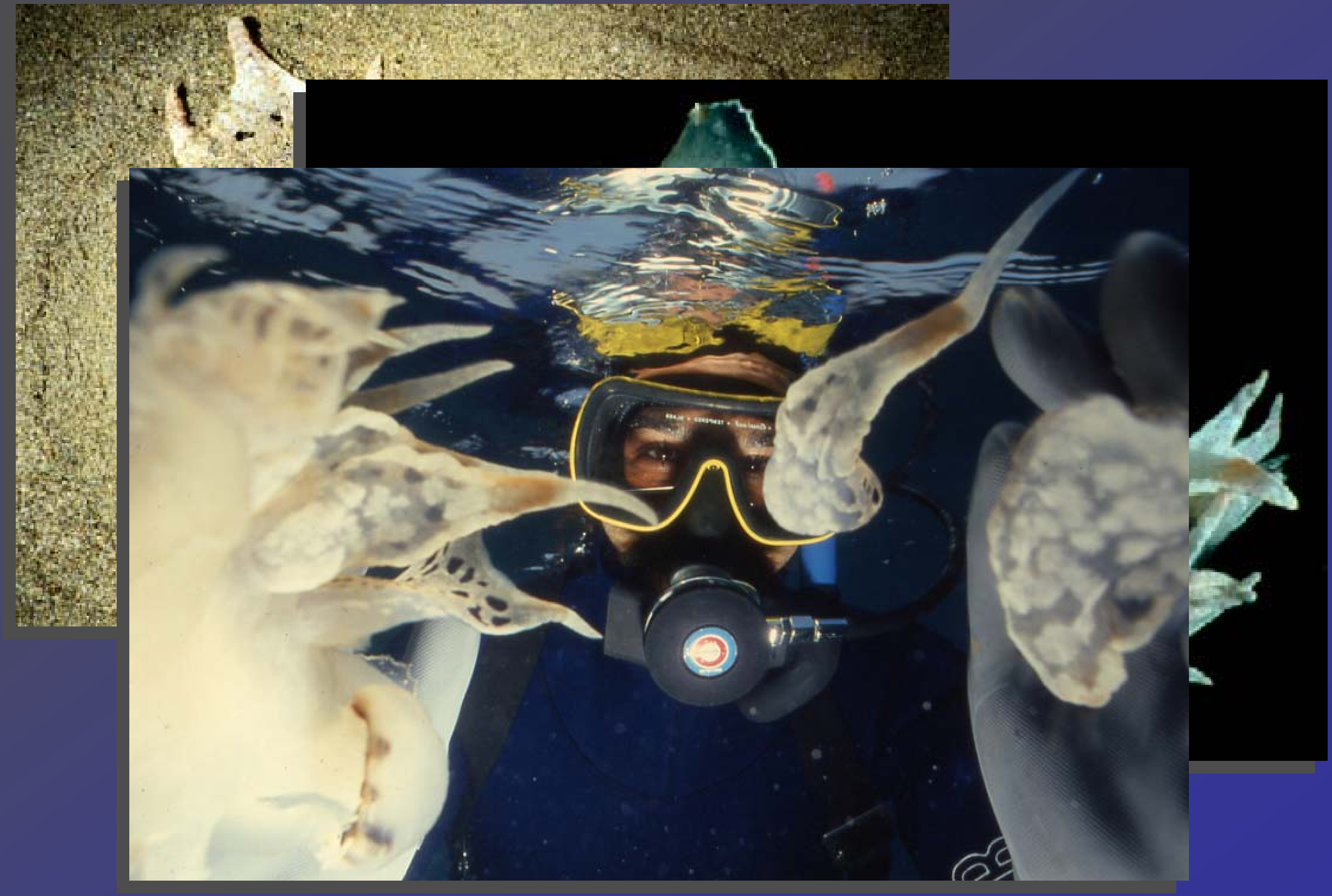


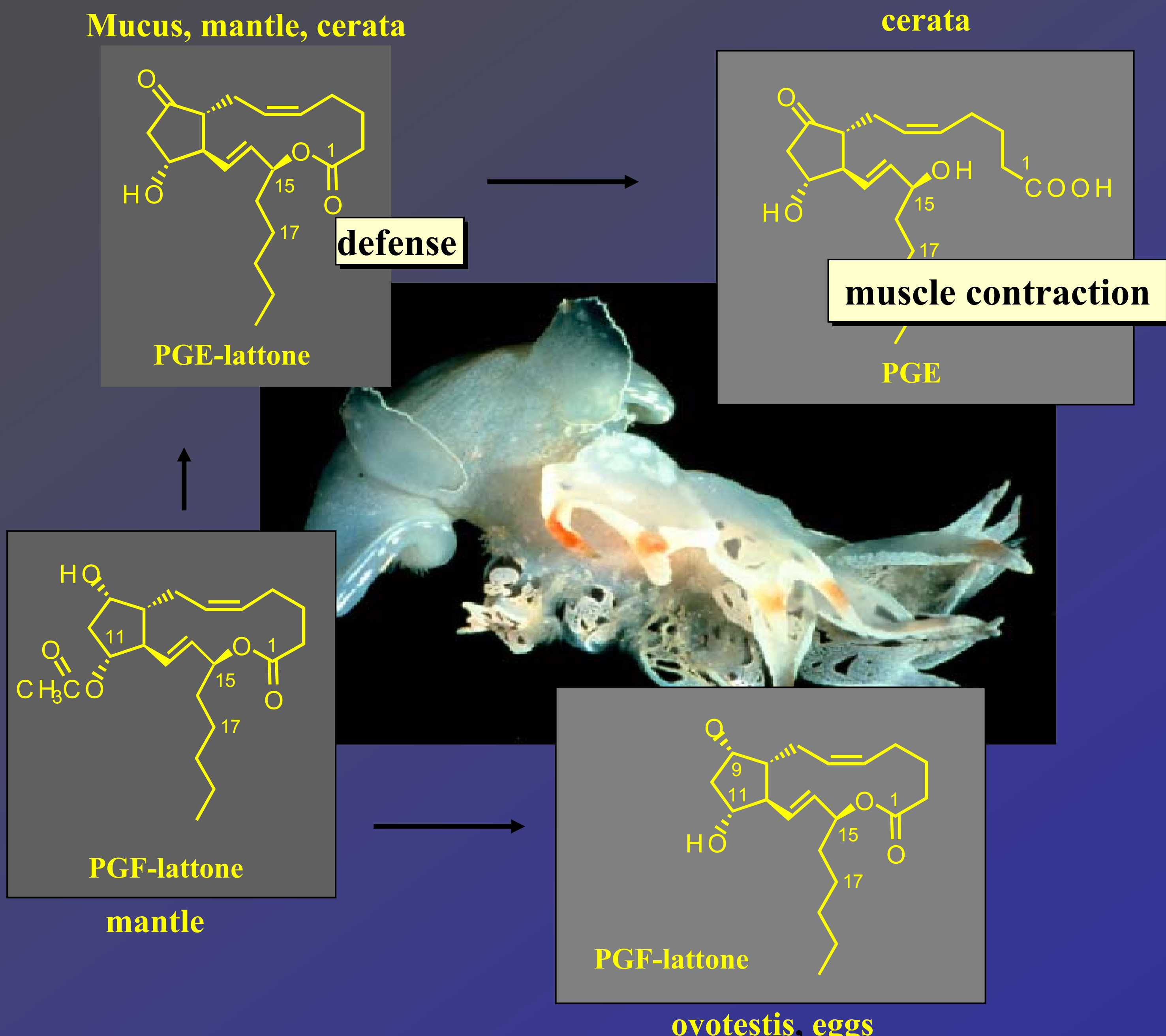
***H. orsini* MDF**



Experientia **49**, 482, 1993

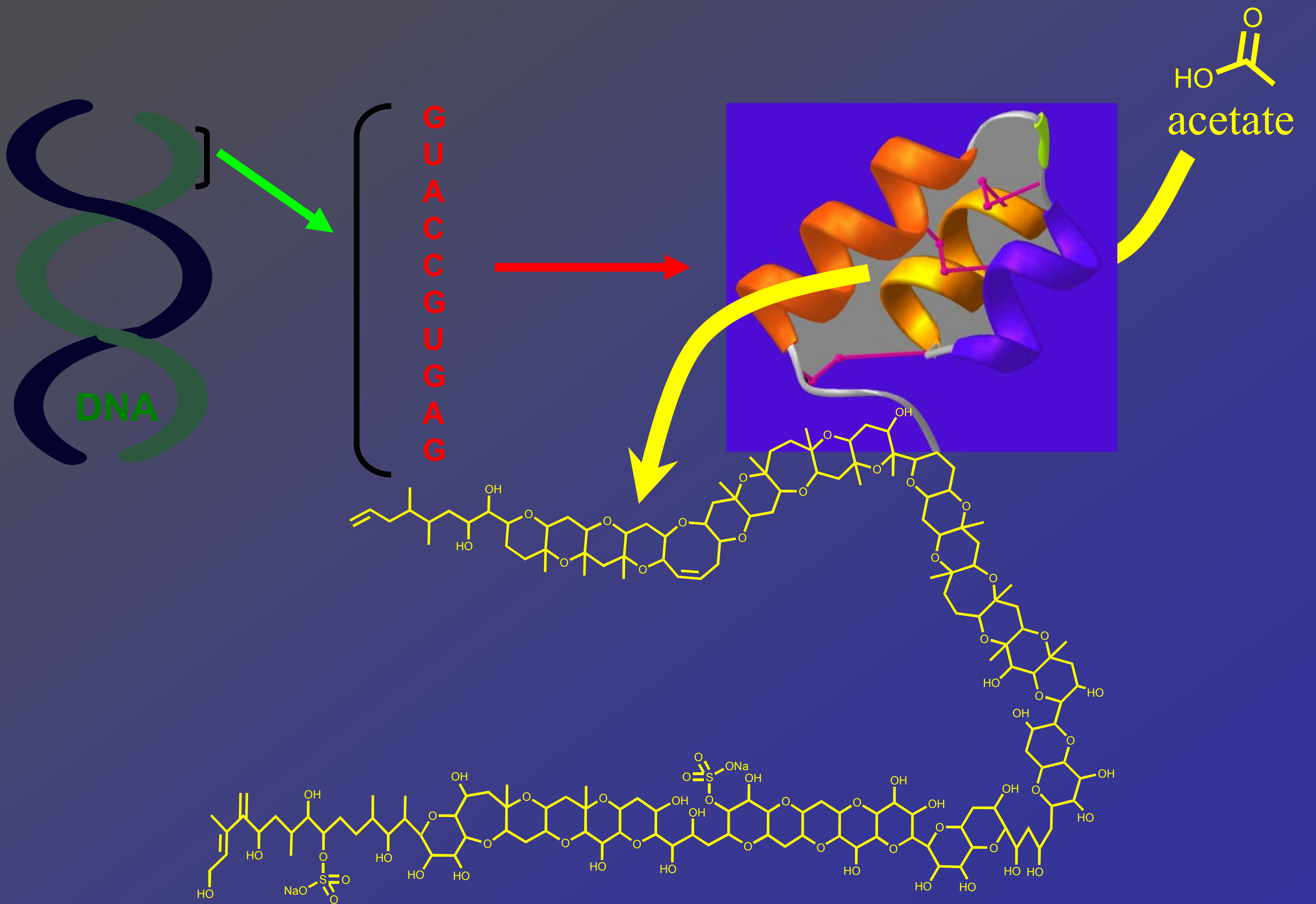
Biosynthesis in *Tethys fimbria*



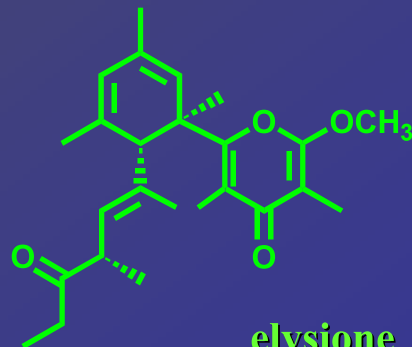
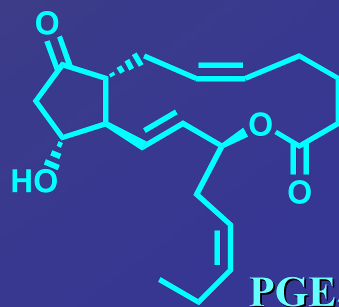
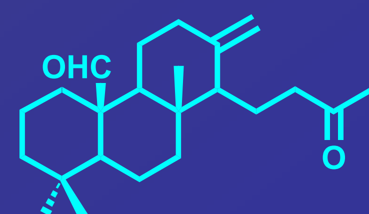

Mucus, mantle, cerata
PGE-lattone
defense
cerata
PGE
muscle contraction
PGF-lattone
mantle
PGF-lattone
ovotestis, eggs

Biosynthesis

the way in which a natural product is produced by in a living organism



Biosynthetic Capacity and Chemical Markers

order	chemical class	chemicals
Cephalaspidea	ω-aryl-methylchetones	 navenone-B
Sacoglossa	polypropionates	 elysione
Nudibranchia	acetogenins	 PGE_2- 1,15-lactone
	terpenes	 luteone

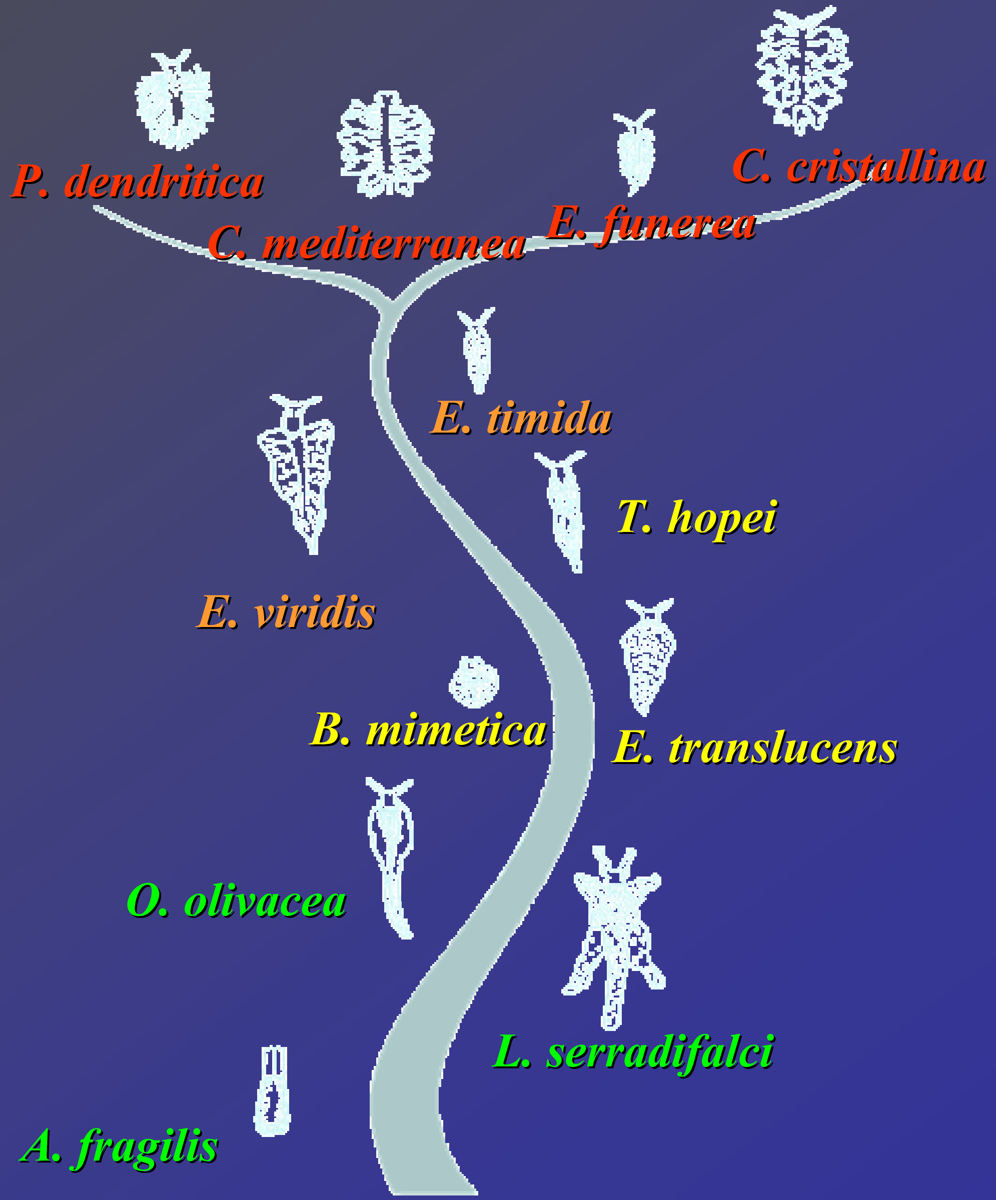
Biosynthetic Capacity and Evolutionary Scenario

polypropionates

polypropionates

diterpenoids

sesquiterpenoids



Cimino & Ghiselin, Chemoecology, 8, 51, 1998

- Isolation and structure elucidation

  - **Faulkner DJ Stallard MO Fayos J Clardy J 1973 A novel monoterpene from the sea hare; Aplysia californica. J. Am. Chem. Soc. 95 3413**
  - **Kato Y Scheuer PJ 1974 Aplysiatoxin and debromoaplysiatoxin; constituents of the marine mollusk Stylocheilus longicauda. J. Am. Chem. Soc. 96 2245**

- Chemical ecology

  Scheuer PJ 1977 Chemical communication of marine invertebrates. Bioscience 27 664

  - **Kinnel RB Dieter RK Meinwald J Van Engen D Clardy J Eisner T Stallard MO Fenical W 1979 Brasilenyne and cis-dihydrorhodophytin; antifeedant medium-ring haloethers from a sea hare (Aplysia brasiliana) Proc. Natl. Acad. Sci. U. S. A. 76 3576**

- Biosynthetic studies

  - **Ireland CM Scheuer PJ 1979 Photosynthetic marine mollusks: in vivo 14C incorporation into metabolites of the sacoglossan Placobranchus ocellatus Science (Washington DC) 205 922**
  - **Sleeper HL Fenical W 1977 Navenones A-C: trail-breaking alarm pheromones from the marine opisthobranch Navanax inermis J. Am. Chem. Soc. 99 2367**

Biosynthesis of Secondary Metabolites in Opisthobranchs

Phylum **Mollusca**

Class **Gastropoda**

Subclass **Opisthobranchia**

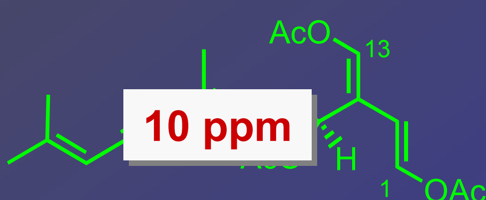
Order

Gymnosomata **Acochlidea** **Thecosomata** **Rhodopemorpha**

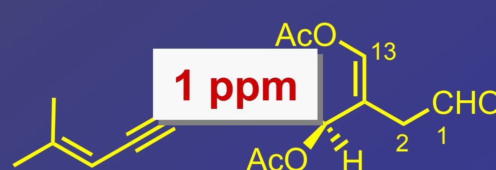
Cephalaspidea **Sacoglossa** **Anaspidea** **Notaspidea** **Nudibranchia**

Cephalaspidea	Sacoglossa
Navanax inermis	***Placobranchus ocellatus***
Haminoea cymbalum	***Elysia viridis***
Bulla striata	***Cyerce cristallina***
	Ercolania funerea
	Oxynoe viridis

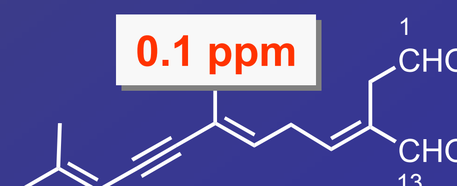
Biotransformation of Caulerpenyne?



caulerpenyne



oxytoxin-1



oxytoxin-2

toxicity to *G. affinis*

Experientia **46**, 767, 1990

Could the Direct Observations be Misleading?

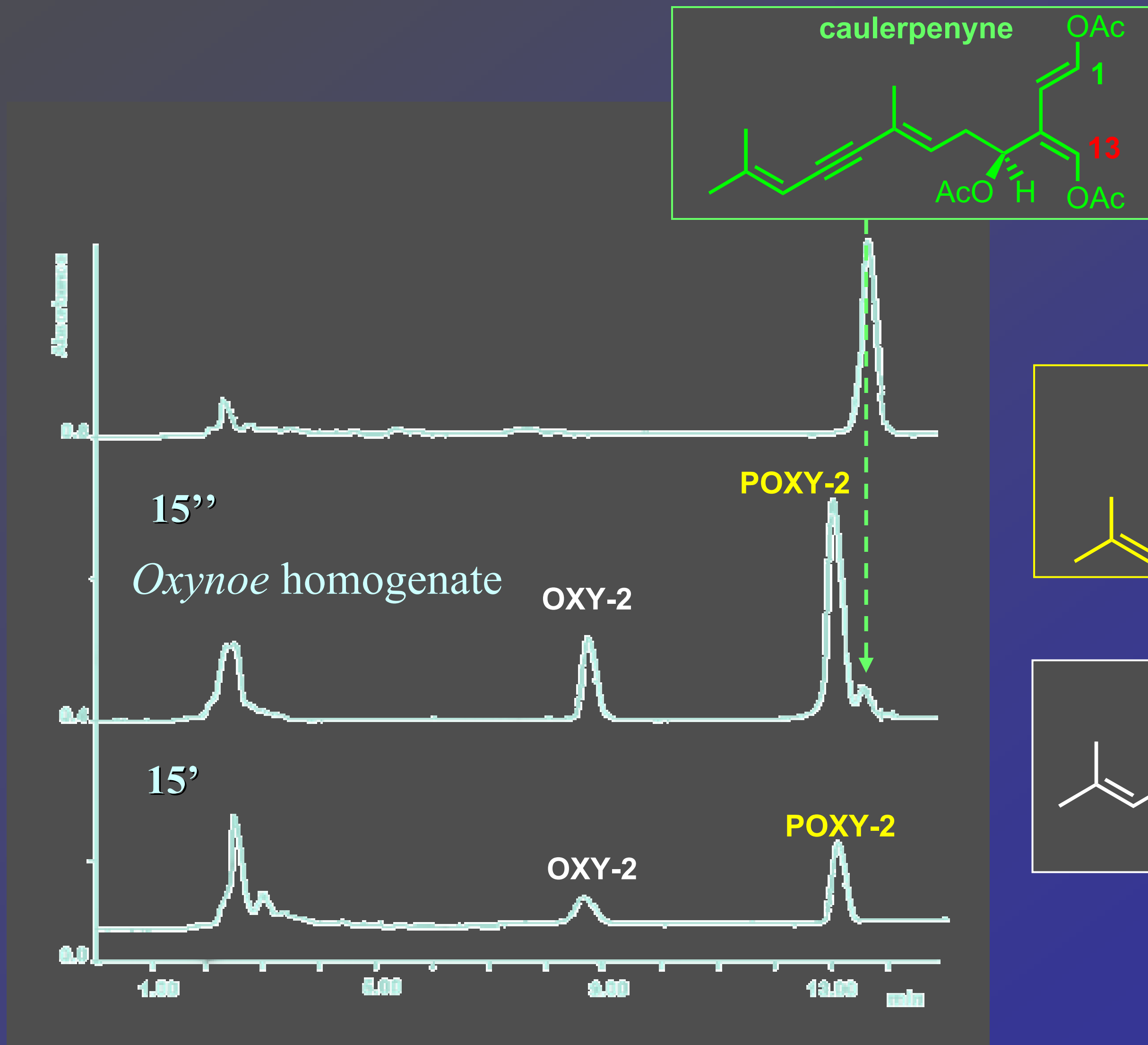
• selective bio-accumulation of minor dietary metabolites

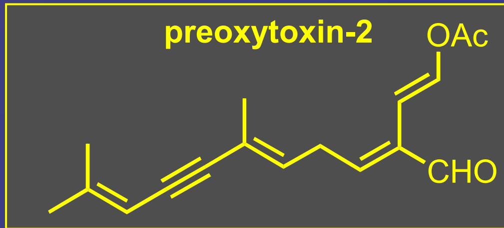
Pietra *et al*., *Helv. Chim. Acta* **78**, 1759, 1995

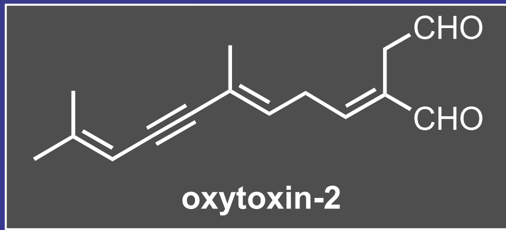
• enzymatic structural modifications in the injuried tissues of the prey

Pohnert and Jung, *Org. Lett.* **26**, 509, 2003

Addition of Caulerpenyne to *Oxynoe* homogenate

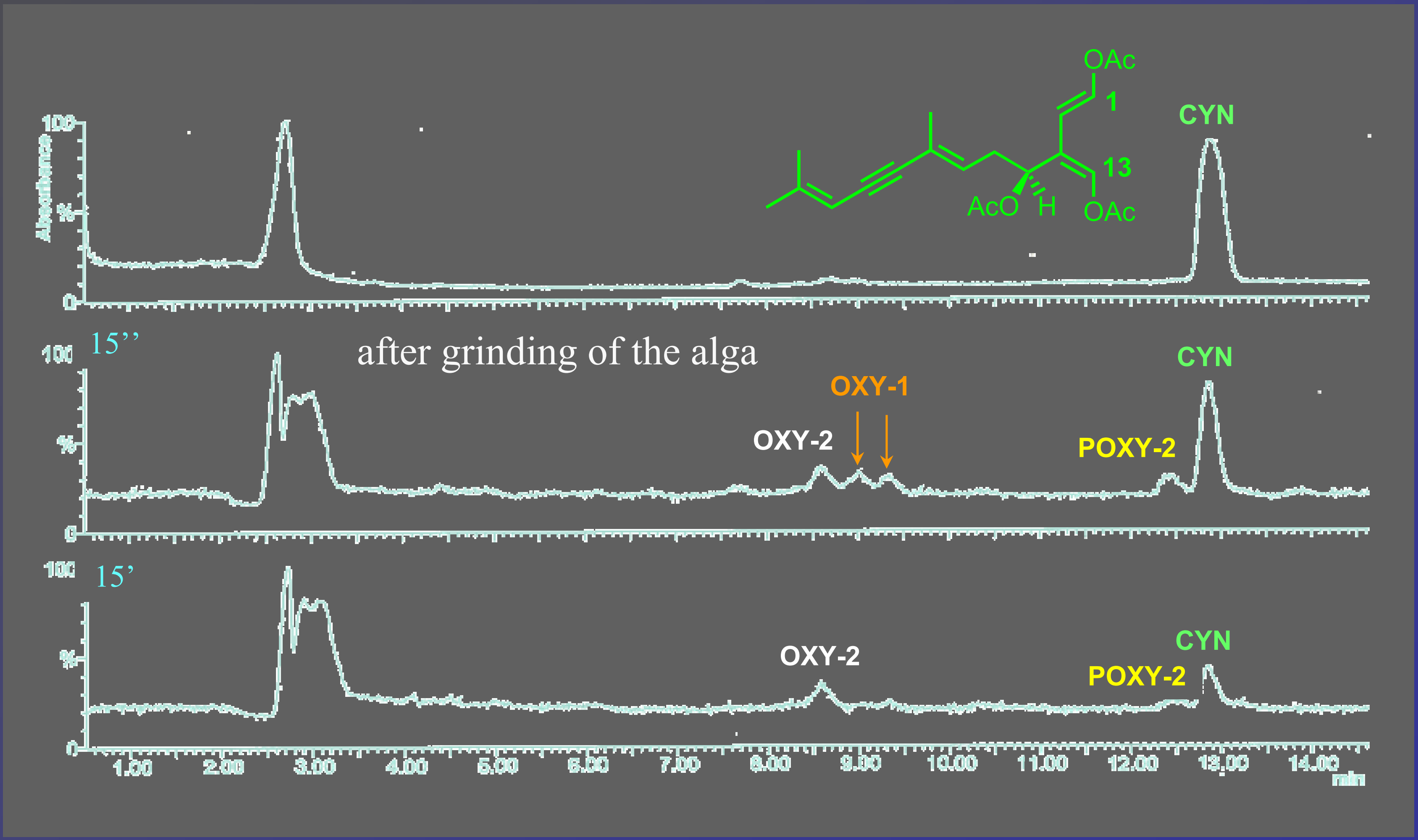




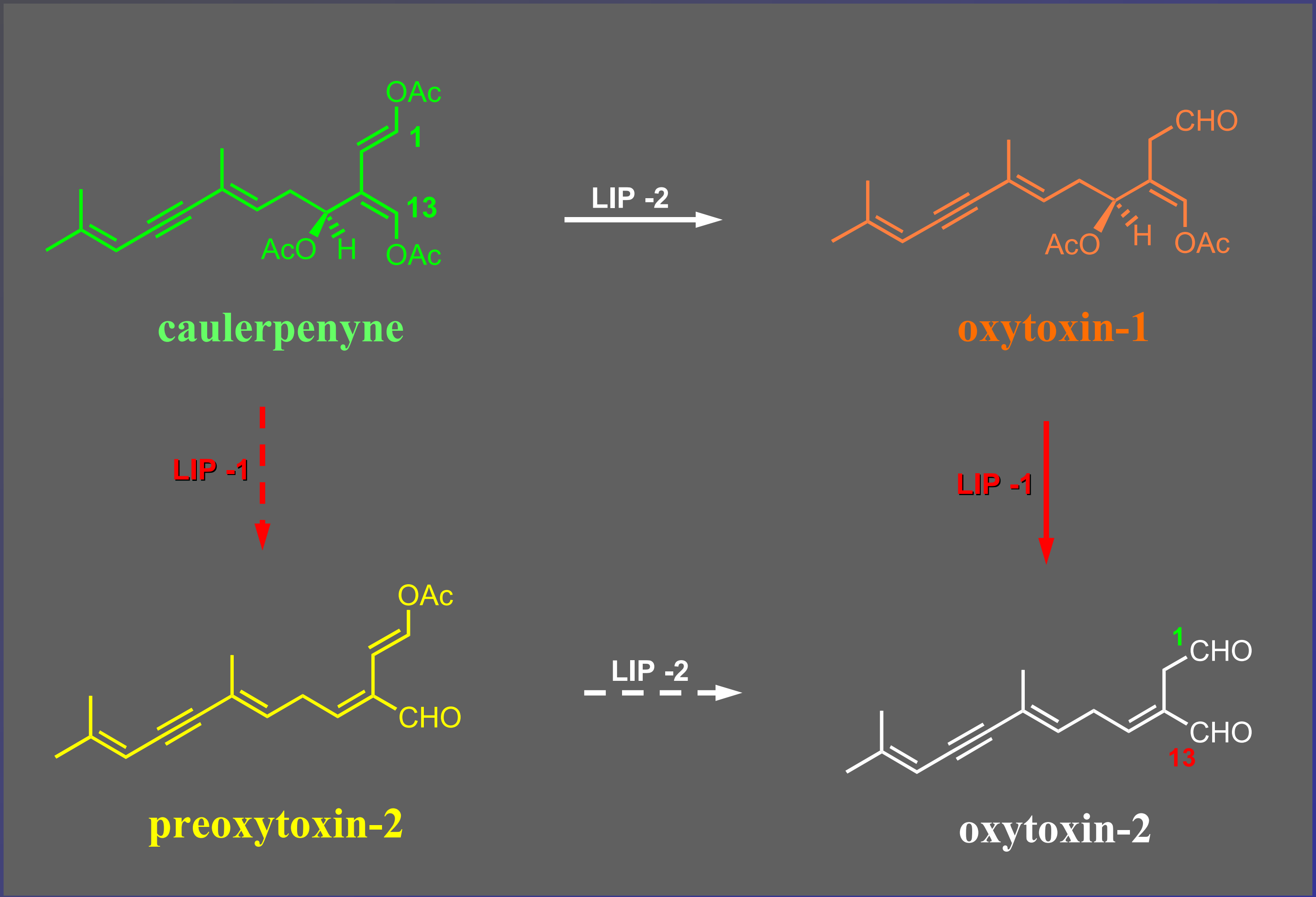


Org. Biomol. Chem. 2004, **2**, 001 - 005

Wound-activated Hydrolysis in ***Caulerpa prolifera***



Selective Lipases are Active in *Oxynoe olivacea*

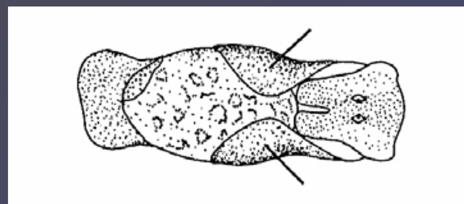


Org. Biomol. Chem., 2004, **2**, 001-005

Metabolite Compartmentalization in Cephalaspideans

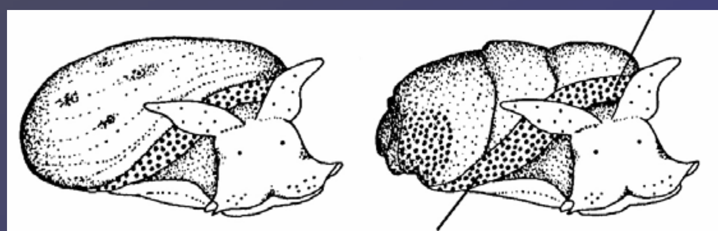
Les glandes palléales de défense chez le *Scaphander lignarius*

Perrier and Fisher
Séanc. Ac. Sci. Paris 146, 1163, 1908

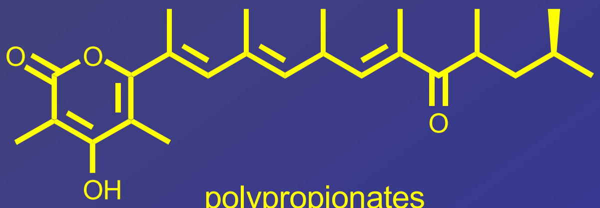


Haminoea fusari

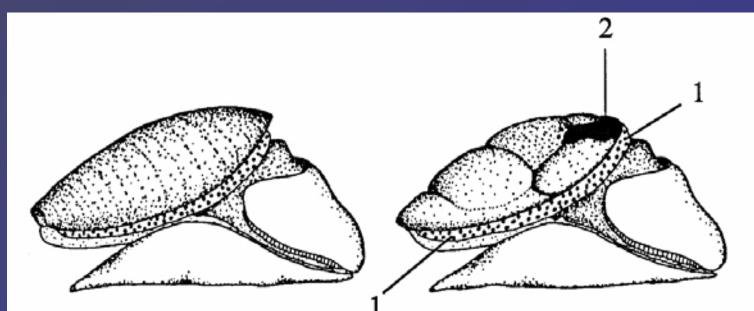
3-alkyl-pyridines



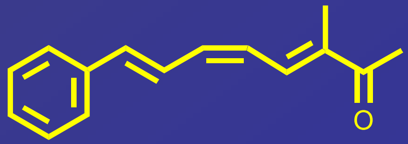
Bulla striata



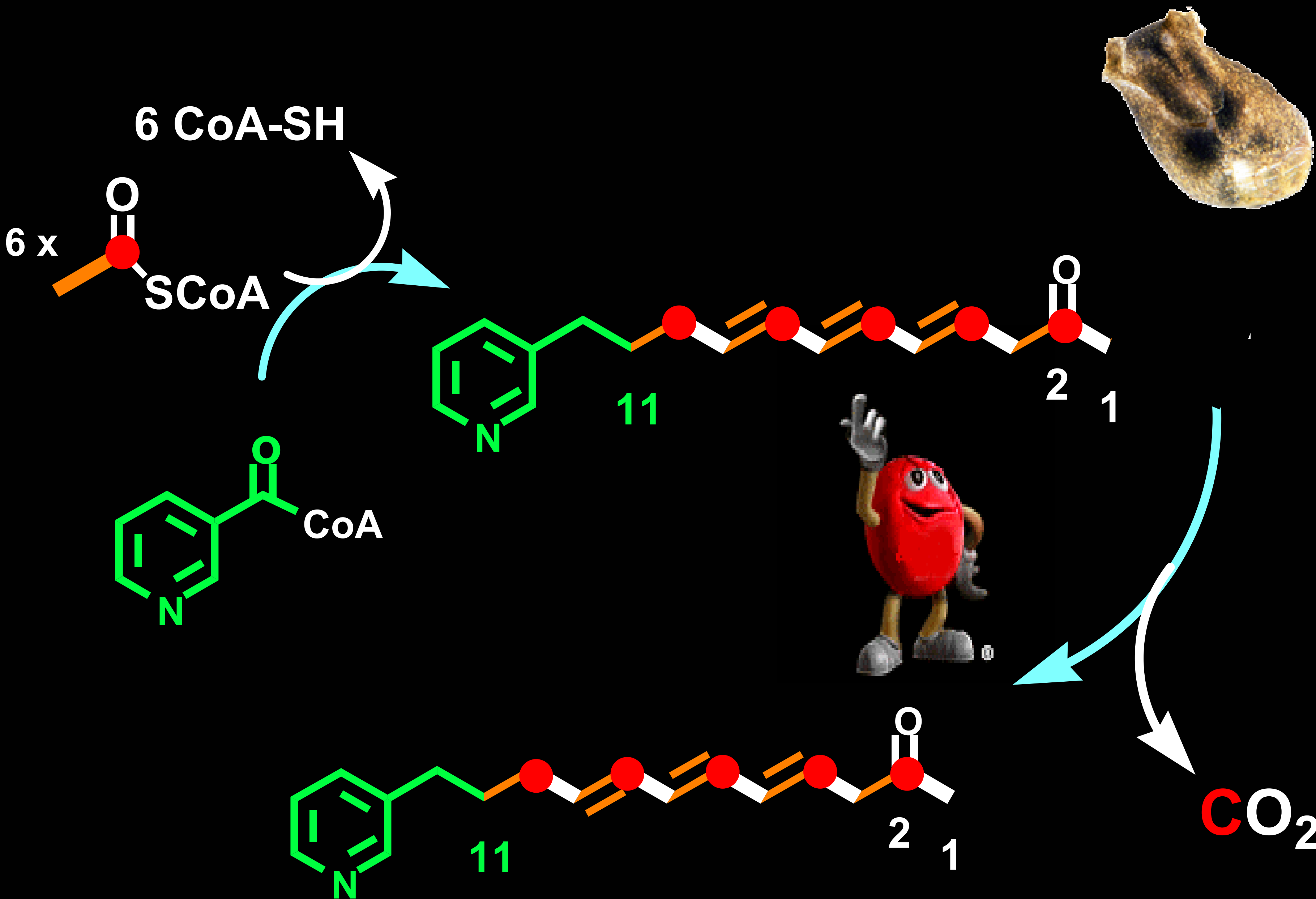
polypropionates



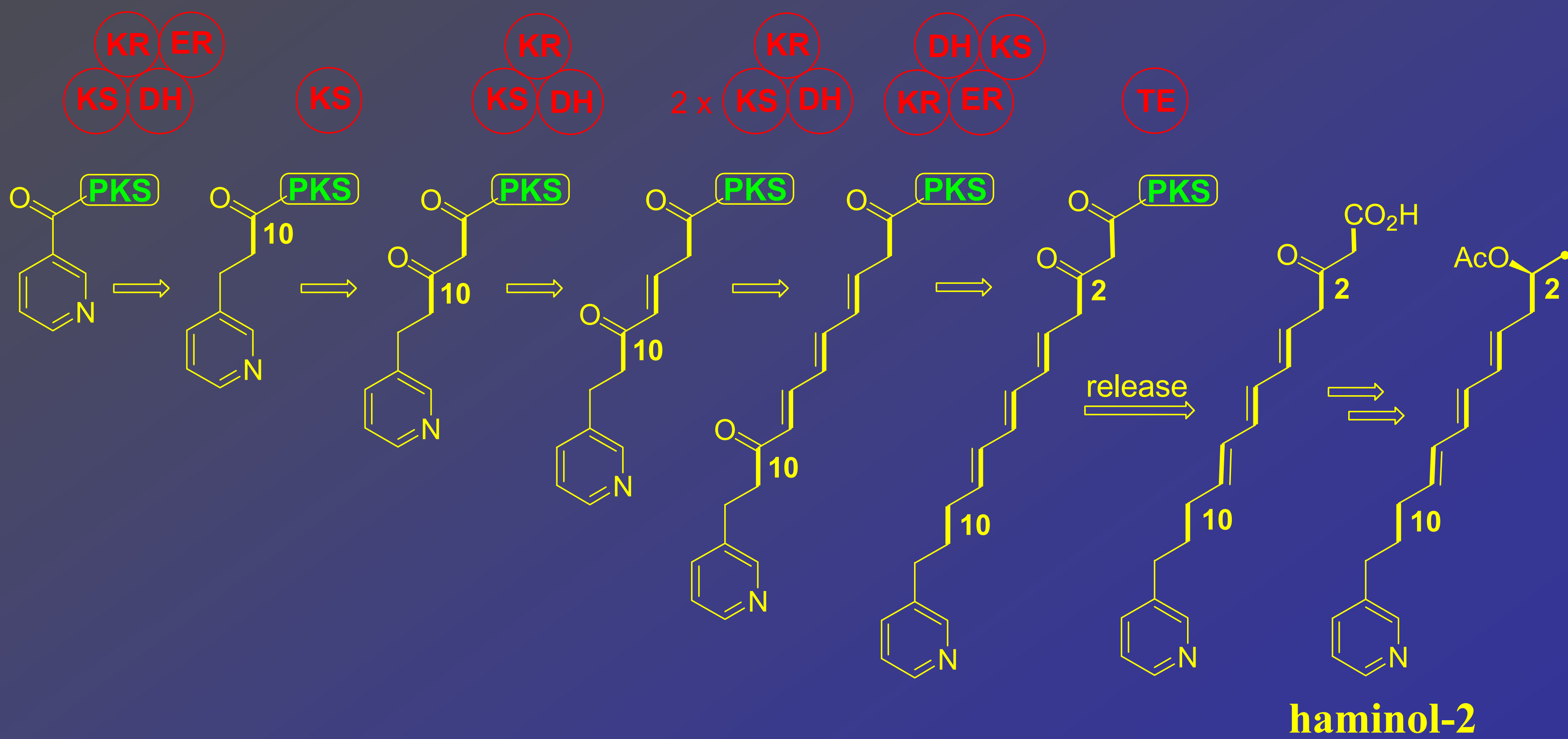
Scaphander lignarius

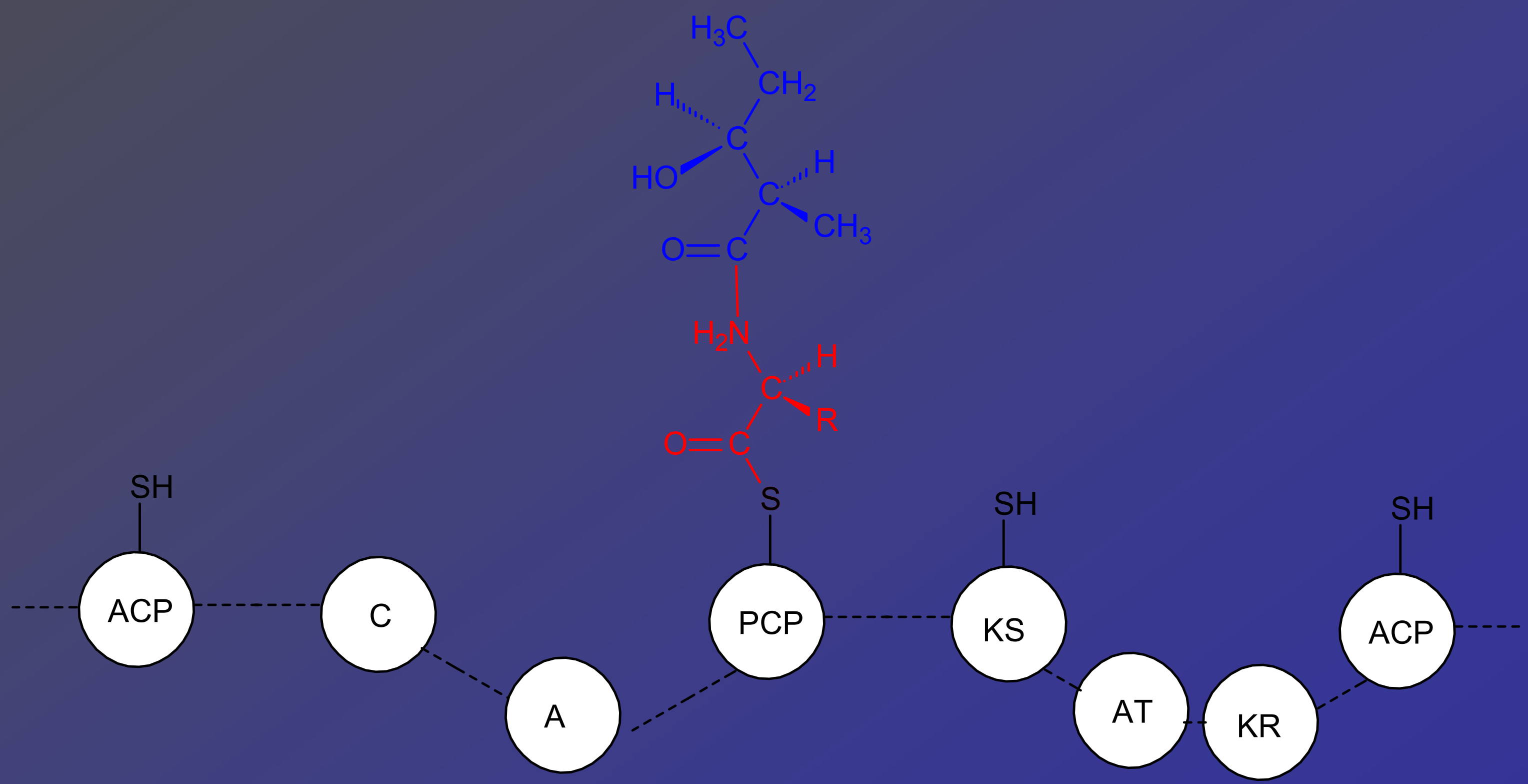


ω-aryl-methyl-chetones

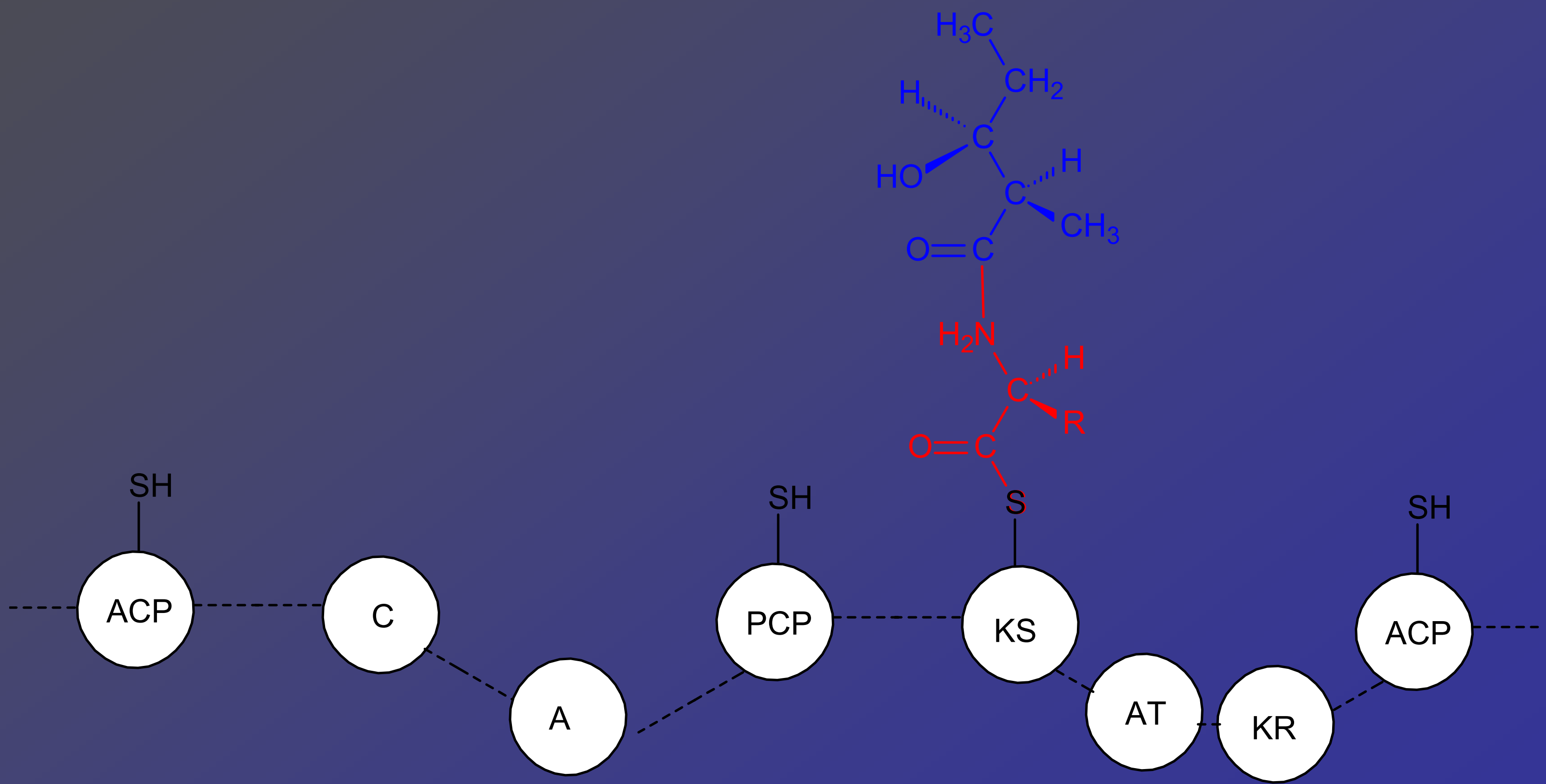

6 CoA-SH
6 x
O
SCoA
O
CoA
N
O
11
2
1
N
O
11
2
1
N
CO_2

Assembly of haminol on *Haminoea's* modular enzyme

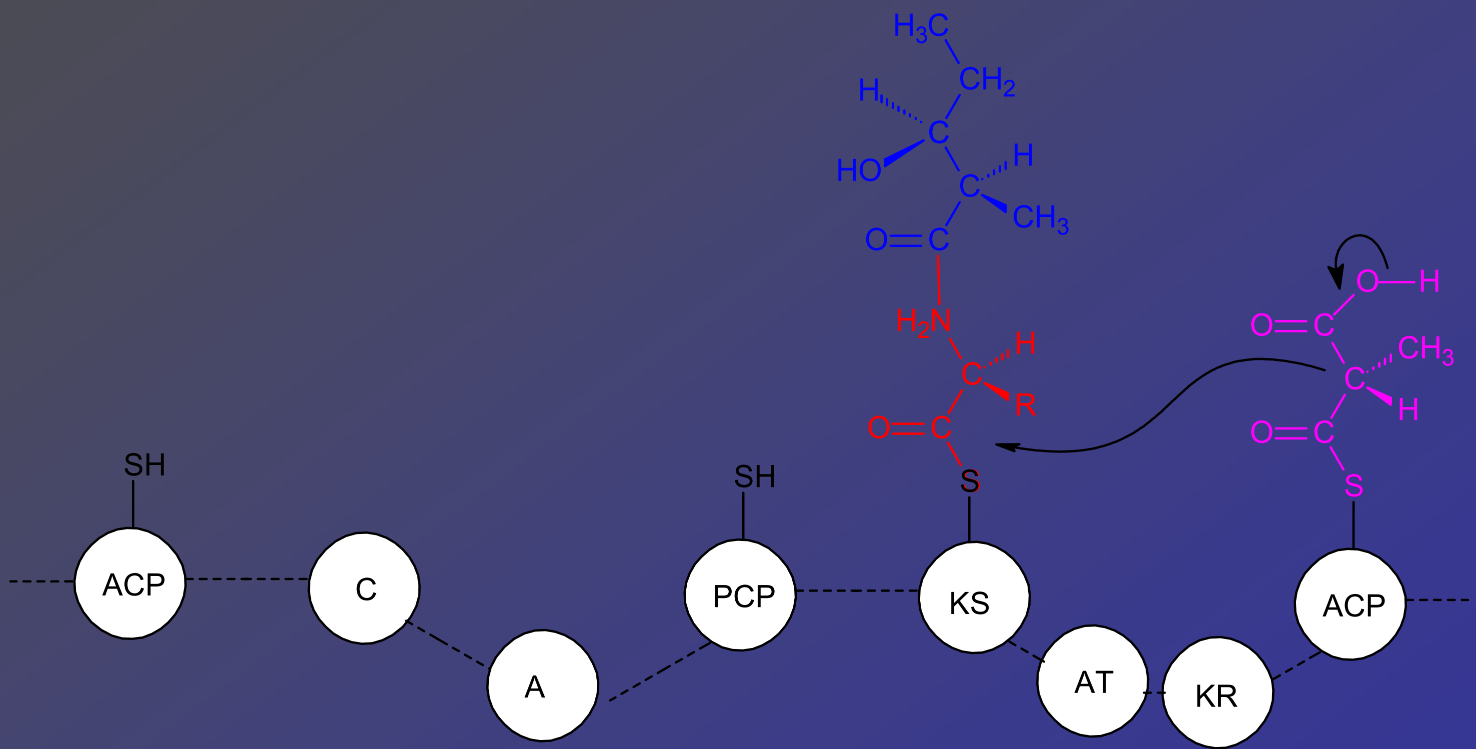




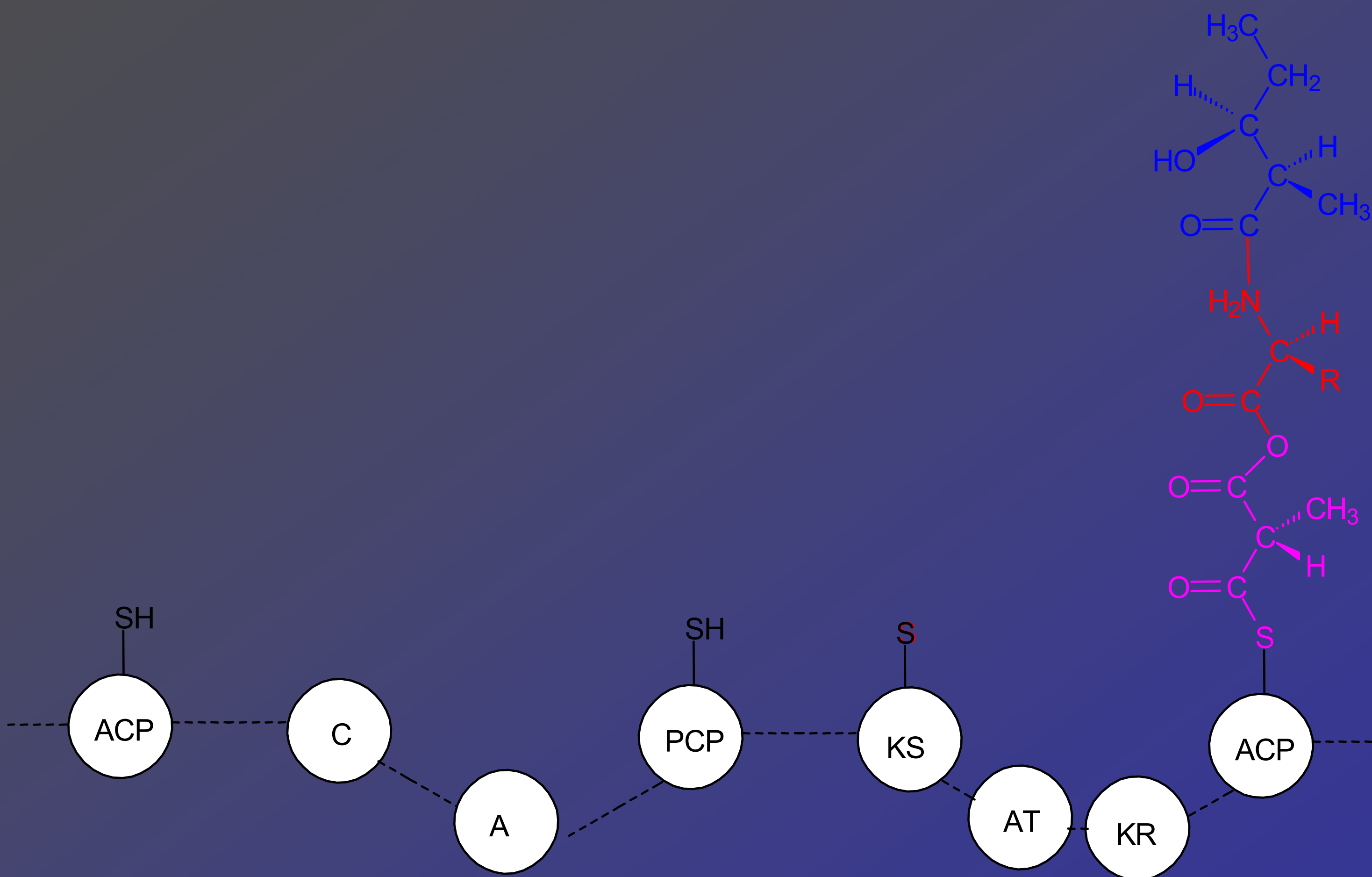
H3C
CH2
H
C
HO
H
C
CH3
O=C
H2N
H
C
R
O=C
S
SH
ACP
C
A
PCP
SH
KS
AT
KR
SH
ACP



SH
ACP
C
A
SH
PCP
KS
AT
KR
SH
ACP



H3C
CH2
H
C
HO
C
H
CH3
O=C
H2N
C
H
R
O=C
S
O-H
O=C
C
CH3
H
O=C
S
SH
SH
ACP
C
A
PCP
KS
AT
KR
ACP



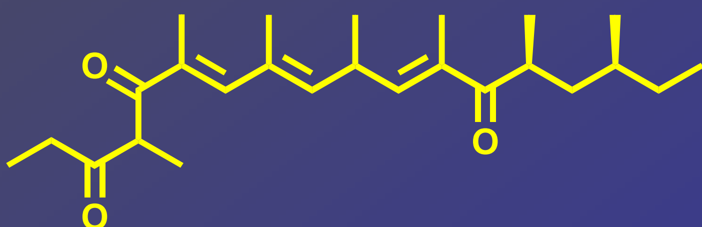
H3C
CH2
H
C
HO
H
C
CH3
O=C
H2N
H
C
R
O=C
O
O=C
CH3
C
H
O=C
S
SH
SH
S
ACP
C
A
PCP
KS
AT
KR
ACP

Biosynthesis in *Bulla striata*

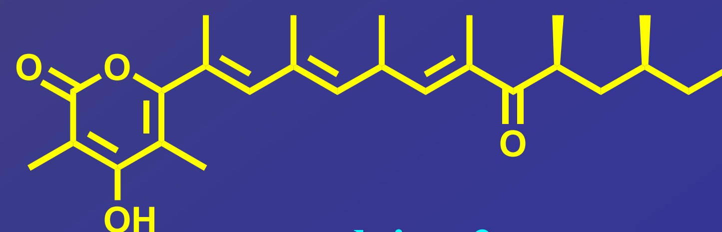
9μCi [1-^{14}C]-propionic acid

3 specimens – 3 days

3 chromatographic steps



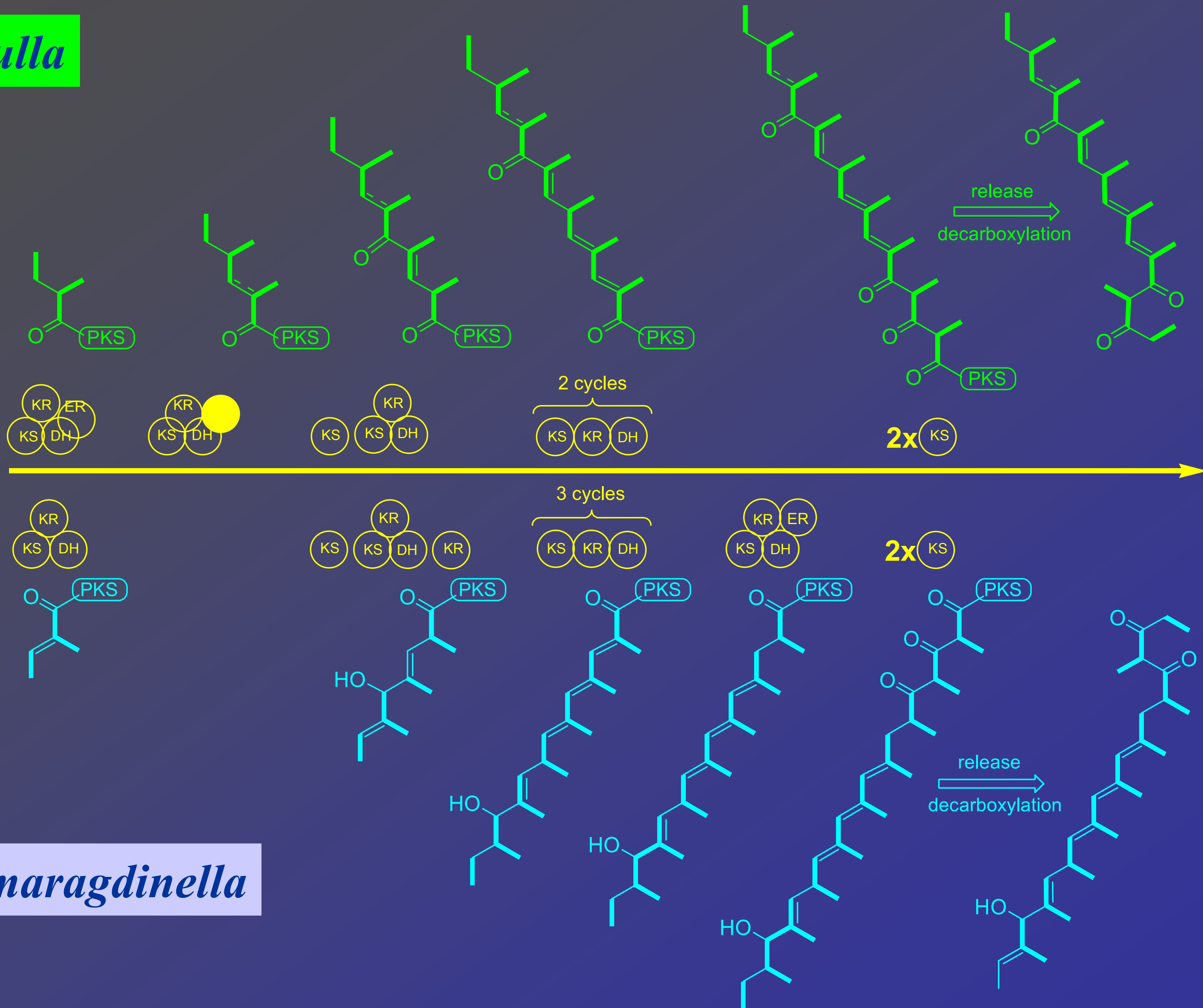
aglajne-1
520 cpm/mg



aglajne-3
394 cpm/mg

Tetrahedron Lett 45, 6847, 2004

Bulla



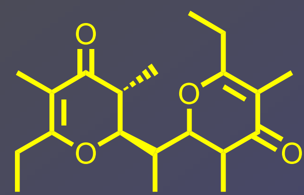
Smaragdinella

Current Org. Chem., 2005, in press

Metabolites from:

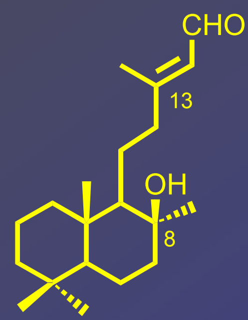
Mediterranean Pleurobranchoidea

Pleurobranchus membranaceous
Tetrah. Lett.
34, 6791, 1993



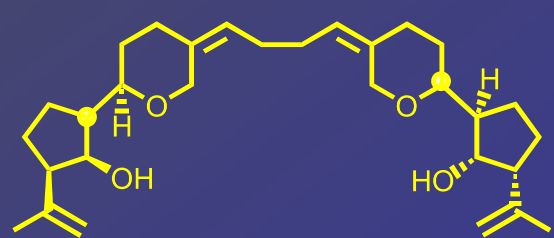
membrenone-C

Pleurobranchaea meckelii
Tetrahedron
36, 8673, 1995



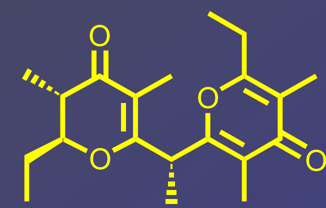
8β-hydroxy- labd-13*E*-en-15-al

Pleurobranchus testudinarius



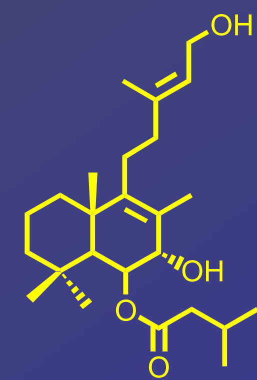
testudinariol-A

Intertidal Molluscs

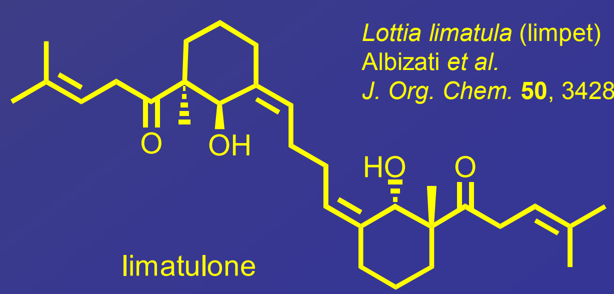


vallartanone-B

Siphonaria maura
Manker and Faulkner
J. Org. Chem. **54**, 5374, 1989



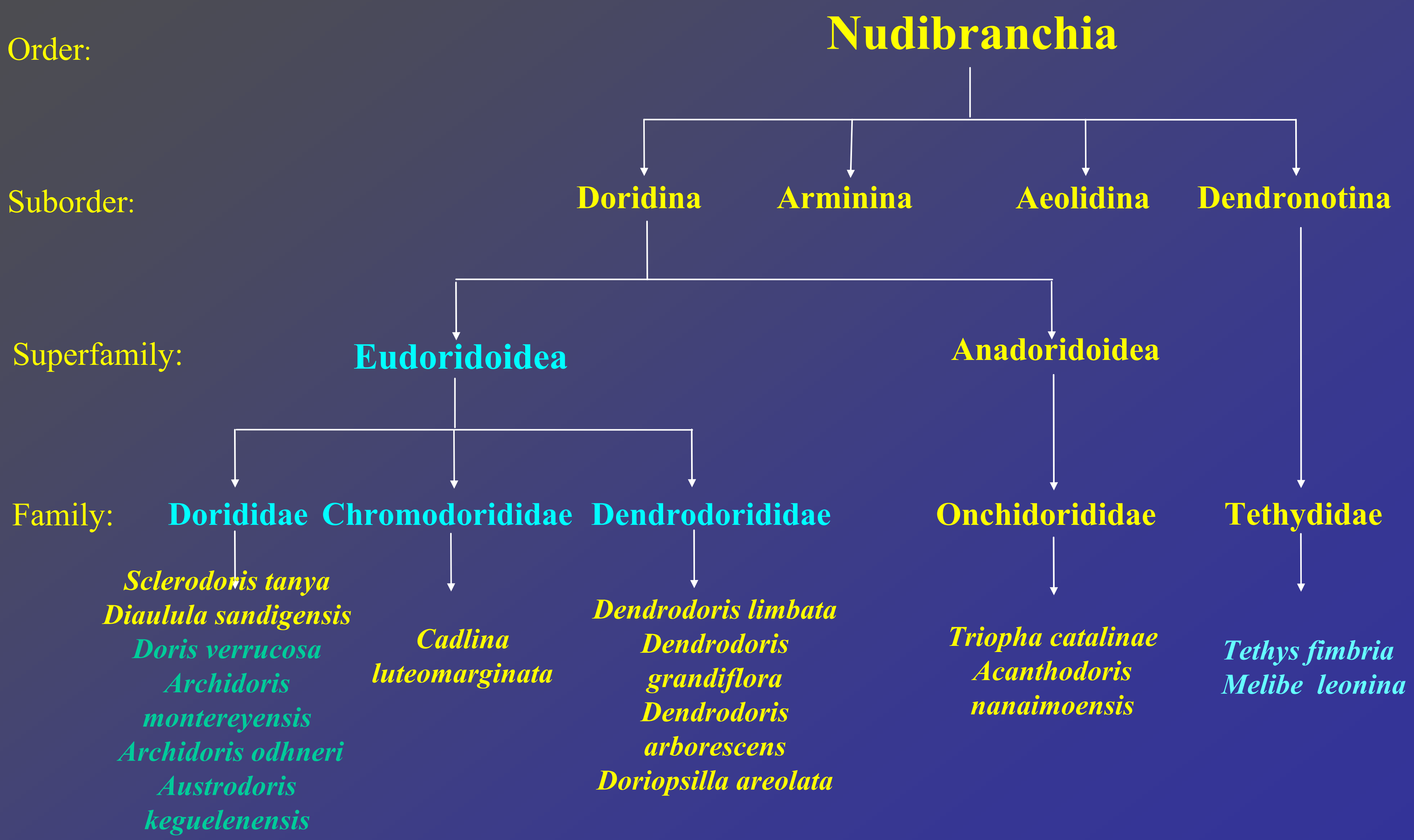
Trimusculus reticulatus
Rice
J. Exp. Mar. Biol. **93**, 83, 1985



limatulone

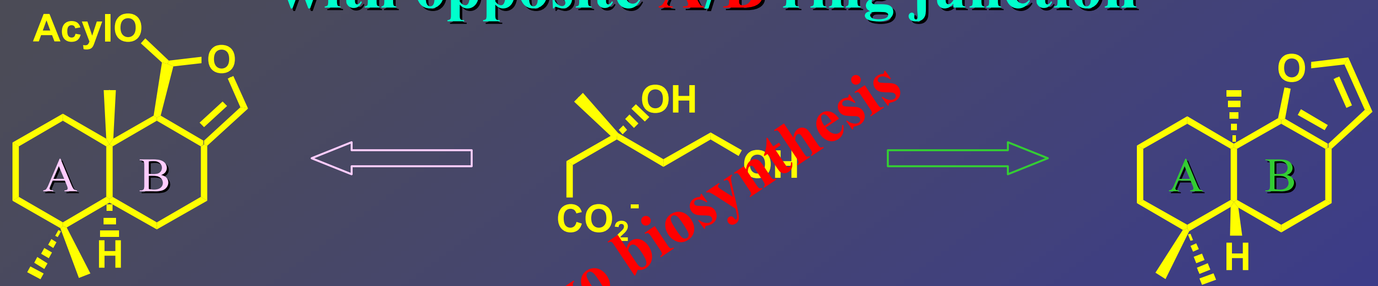
Lottia limatula (limpet)
Albizati *et al.*
J. Org. Chem. **50**, 3428, 1985

Biosynthetic studies of Nudibranchs



Doriopsilla areolata

biosynthetizes typical sponge sesquiterpenoids with opposite A/B ring junction



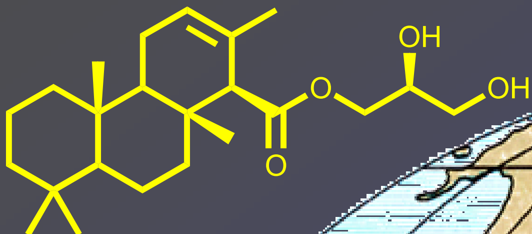
drimane esters　　　　mevalonate　　　　(-)-pallescensin-A

Tetrahedron 57: 8913, 2001
J Org Chem *68*, 2405, 2003

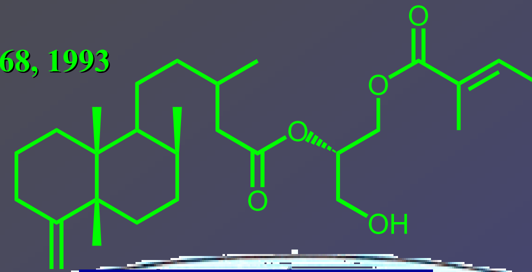
- lateral gene transfer (Faulkner)
- retrosynthetic mode (Ghiselin)

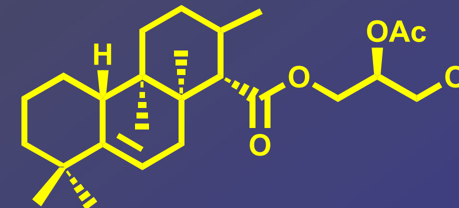
Purification of the enzyme is currently planned in order to address the molecular aspects of the process in the nudibranch (and in the sponge)

Tetrahedron 21, 797, 1980;
Tetrahedron 25, 11, 1984;

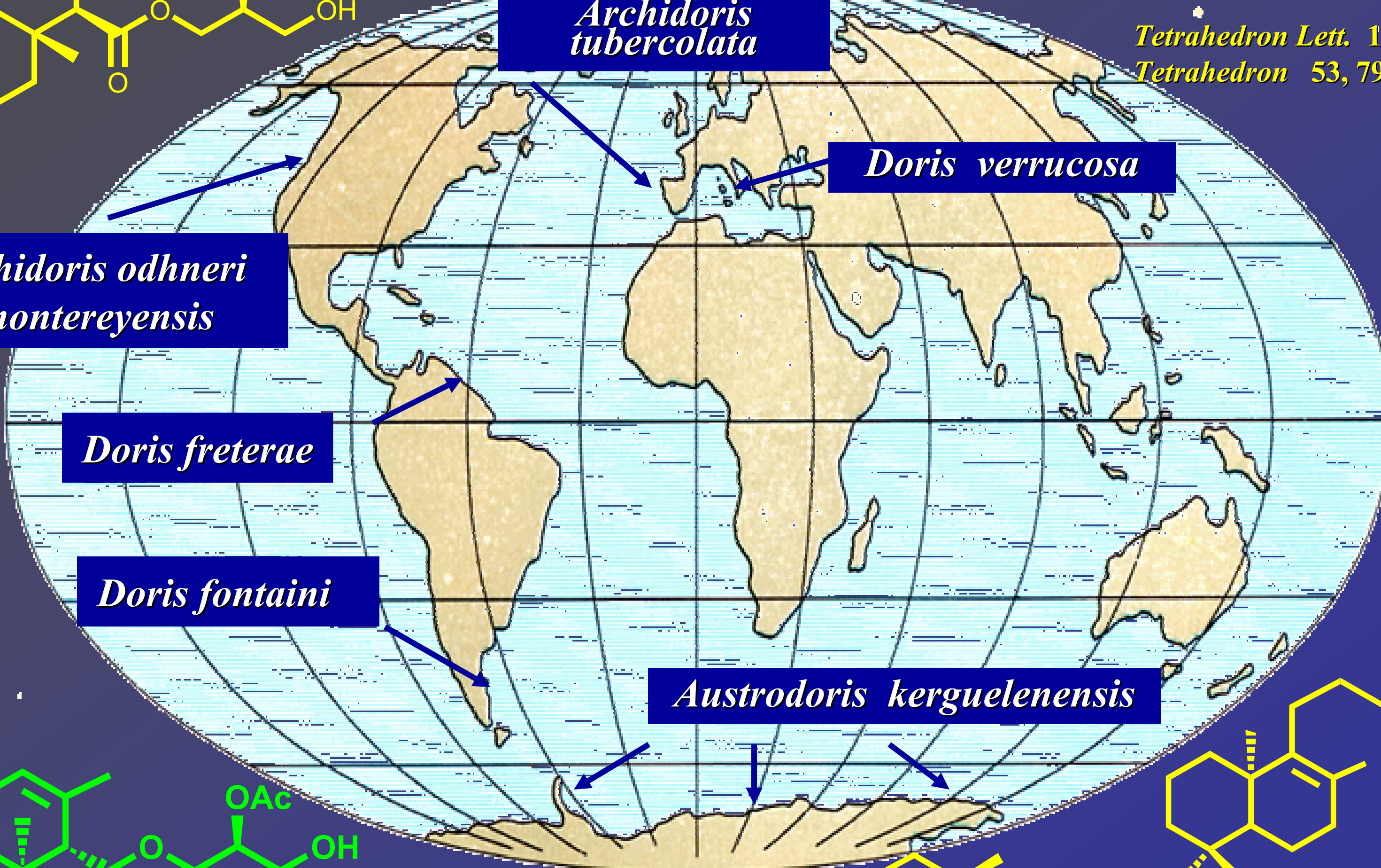


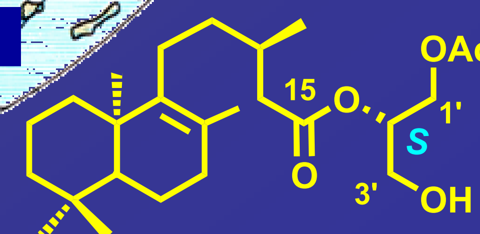
Experientia 49, 268, 1993

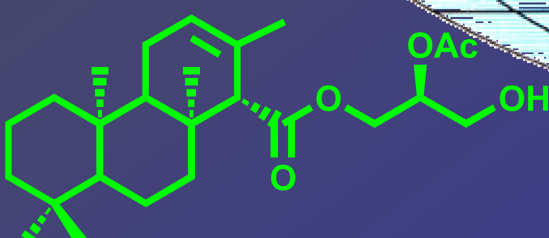




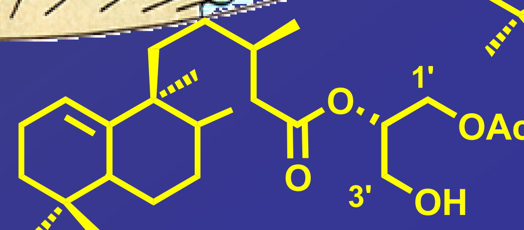
Tetrahedron Lett. 11, 6093, 1990;
Tetrahedron 53, 797, 1491;



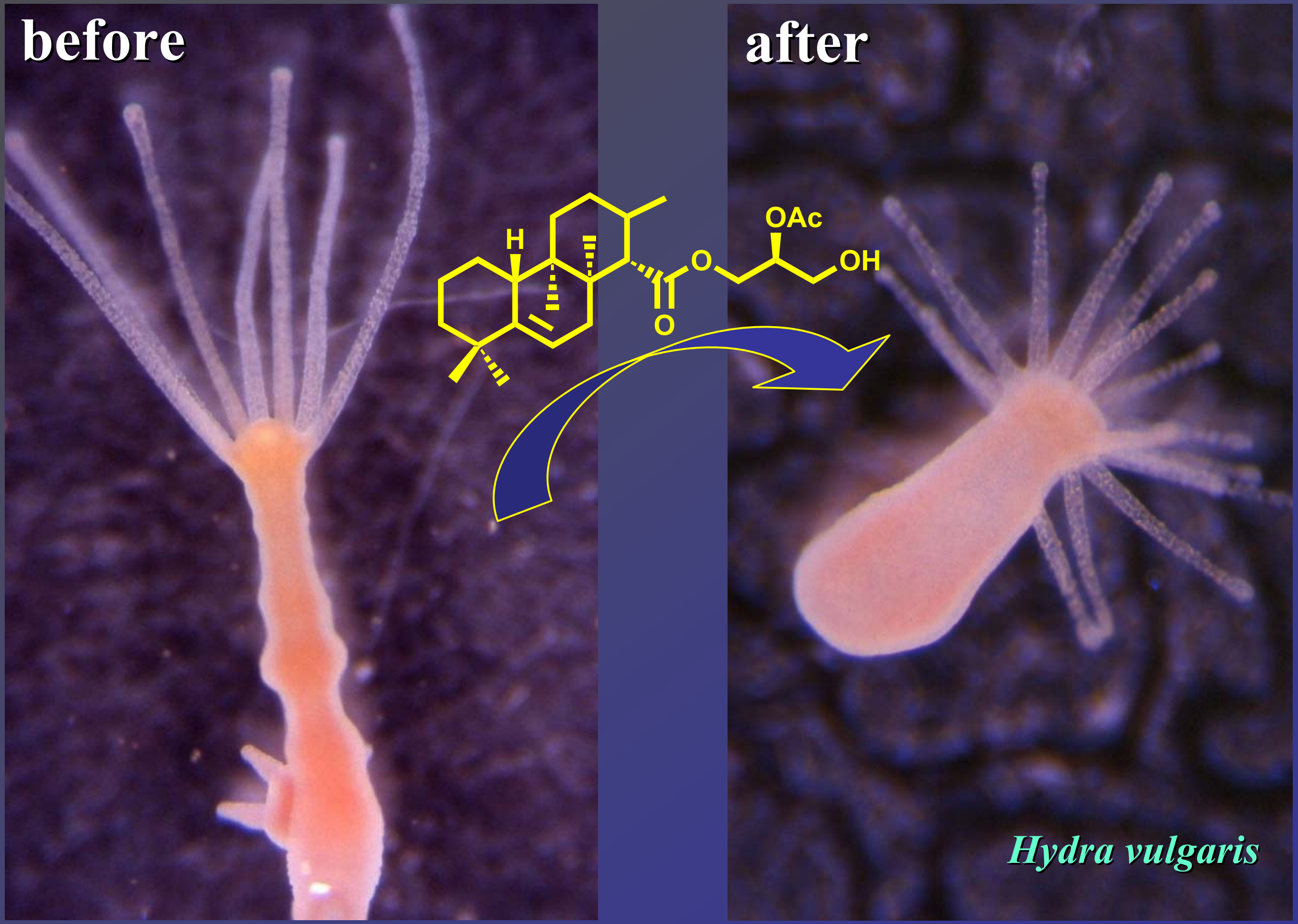




Experientia 49, 268, 1993



Tetrahedron 47, 9743, 1991;
Tetrahedron 59, 5579, 2003

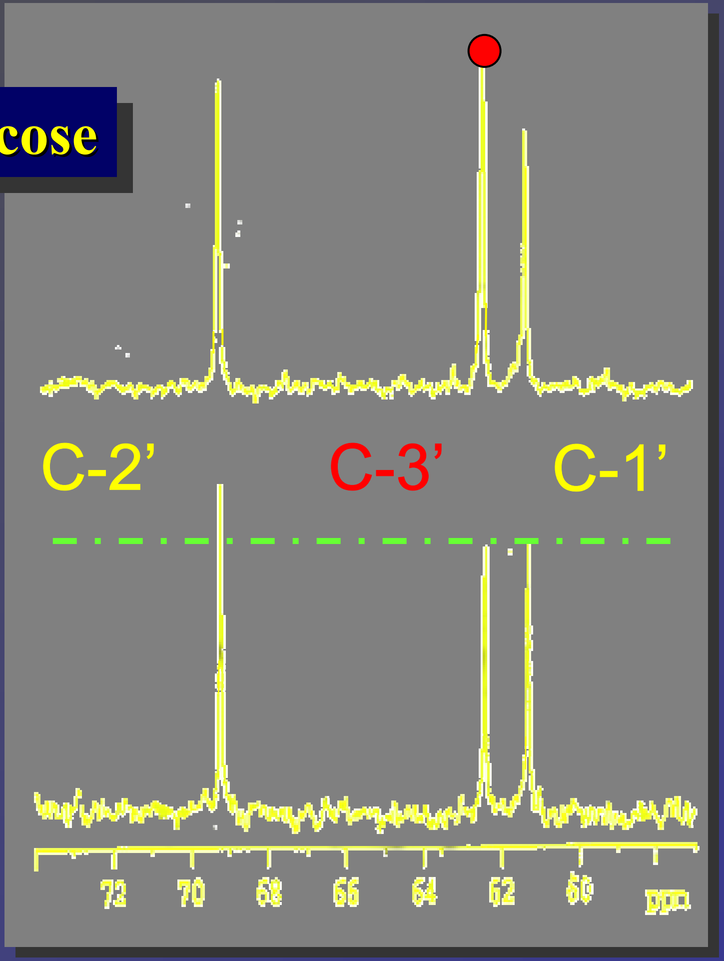


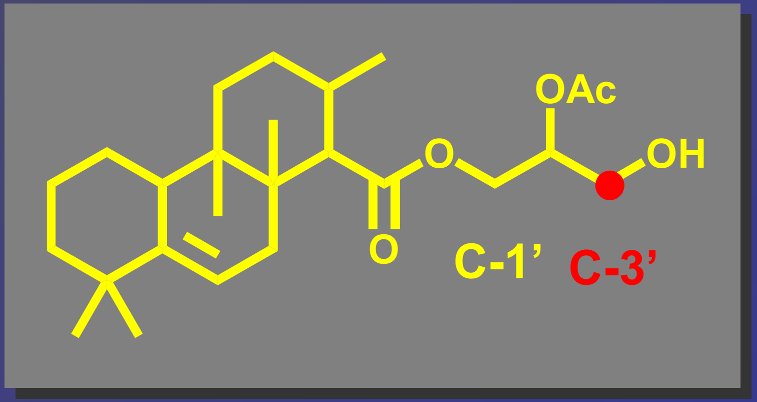
Terpenoid acyglycerols are feeding-deterrent and ichthyotoxic. Like the classical diacyglycerols, these compounds are activators of protein kinase C and, in vivo, show morphogenic effects

Experientia, 52, 874, 1996

Glycerol Origin in Verrucosins

[6-^{13}C]-glucose

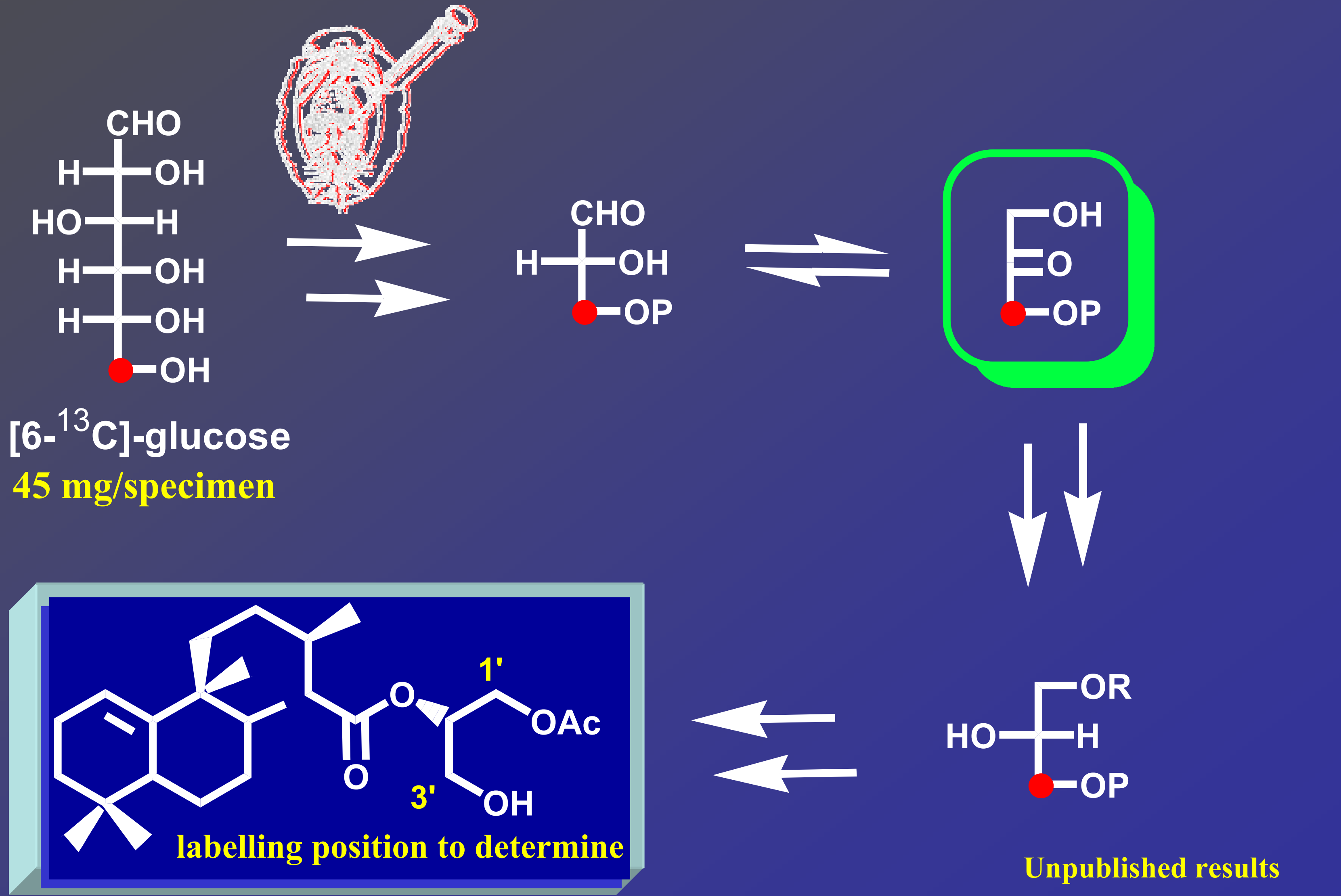




Natural intensity of glycerol ^{13}C signals

Eur J Org Chem 16, 3104, 2003

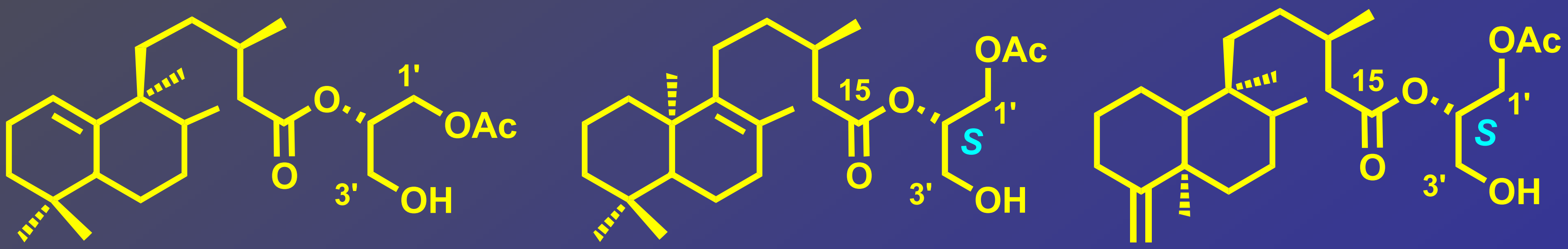
Experiment with Antarctic *Austrodoris kerguelenensis*



Unpublished results

Experiment with Antarctic *Austrodoris kerguelenensis*

Despite the *de novo* biosynthesis, specimens of the same population show different metabolites



Such a diversity is analogous to that described in a restricted group of terrestrial plants (e.g., *Polyathia longifolia*), thus suggesting a similar enzymatic arsenal

Tetrahedron, 59, 5579, 2003

- **Opisthobranchs possess the ability to produce al large number of unique secondary metabolites, some of which (e.g. 3-alkylpyridine and polypropionates) are unlike those found in any terrestrial species. ;**

- **Our knowledge of the biosynthetic processes leading to these products is still in its infancy – only a few genera have been investigated;**

- **The tremendous progress of genetic and genomic techniques is expected to bring a crucial contribution, although these studies alone will not provide all the answers since the expression of a gene may have no definitive relationship to the ultimate production of the metabolites. This emphasizes the need for traditional biosynthetic work as prerequisite for the full exploration of the biochemical aspects of the secondary metabolism in these organisms;**

- **Characterization of enzymes involved in synthesis of ecological mediators is likely the next step for the comprehension of secondary metabolic pathways and ecological interactions (Biochemical Ecology) in marine organisms, either betnhic and planktonic;**