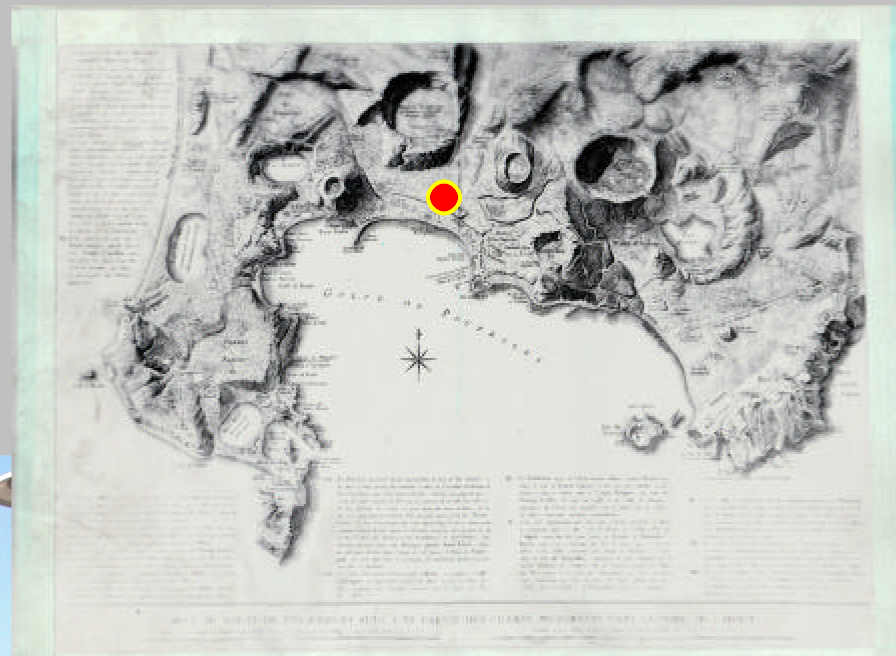
Chemistry in ecological assays

marbef

Training course on bioassays in chemical ecology

Tjarno, 9-14 septmber



CNR - Institute of Biomolecular Chemistry

afontana@icmib.na.cnr.it

Definition of Ecology for Chemists

Ecology is the branch of science that studies the distribution and abundance of living organisms, their habitats, and the interactions between them and their environment — which includes both abiotic (non-living) elements like climate and geology, and biotic ones like other species.

Interactions between different organisms:

Negative effect

for (one or both) interacting species/populations:

competition – outcome depends on innate capabilities of nutrient uptake, metabolic rates

antagonism – specific inhibitor or metabolic product may impede growth/metabolism of others (antibiotic or bacteriocin release, lactic acid production)

Positive effect

for (one or both) interacting species/populations:

cooperative interactions - interacting species must share same/nearby environment

Syntrophy/symbiosis – organisms together carry out transformation neither can conduct alone

complementary metabolic interactions – for example S cycling: anaerobic sulfate reducing bacteria (SO_4^{2-} ? H_2S) provide substrate for microaerophilic sulfide-oxidizing bacteria ($H_2S + ½ O_2$? $S^0 + H_2O$)

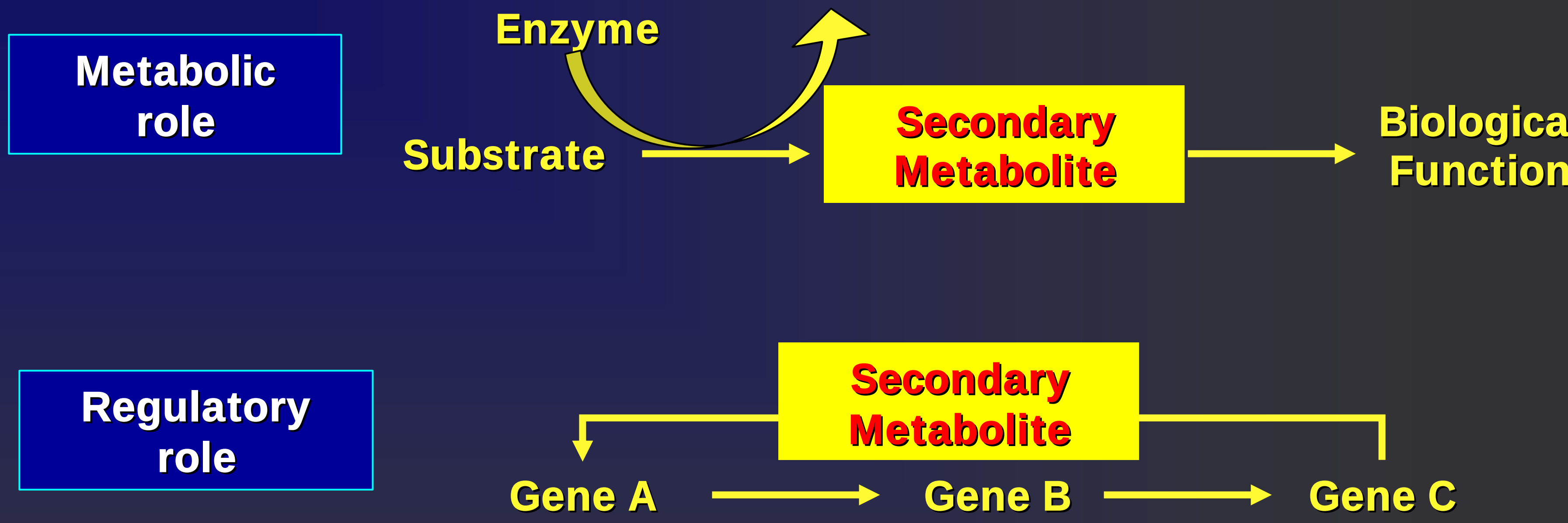
The CHEMICAL ECOLOGY concerns the identification and biological evaluation of chemical compounds (> 1000 amu) (generally named natural products or secondary metabolites) working as mediators between organisms of the same species or of different species

Natural products are defined as chemicals produced by an organism that do not play a role in the internal chemistry of the organism. It has been proposed that a natural product must serve the producing organism by enhancing its viability in nature, such as acting as an anti-feedant or antibiotic (Williams 1189). Since natural products do not play a direct role in the normal metabolic processes of an organism, natural products are often equated to secondary metabolites

(Williams & Dudley 1989)

Strictly speaking, any biological molecule is a natural product. But this term usually refers to secondary metabolites that are not involved in primary metabolism, and therefore differ from the more prevalent macromolecules such as proteins and nucleic acids that make up the basic machinery of life…. Many of these products find important biotechnological applications in biomedical research, and in the agriculture, aquaculture and chemical industries.... Aside from their biotechnological applications, many natural products play fundamental roles as defences against predators, competitors and pathogens, and are therefore driving ecosystem functionalities.

Adrianna Ianora, from "Rosemeb" Introduction



Small molecules often carry vital information.

Jerry Meinwald, PNAS 2005

With genome sequences accumulating at a rapid pace, one major goal of biology is to understand the function of genes. Many gene functions are comprehensible only within the context of chemical communication, and emerging research on genomics and chemical communication has catalyzed development of this highly productive interface. Many of the most abundantly represented genes in the genomes characterized to date encode proteins mediating interactions among organisms, enzymes involved in biosynthesis of pheromones and toxins, and enzymes catalyzing the detoxification of defense compounds. ………Across a wide range of organisms, many of the same classes of molecules perform these functions, even if the precise identity of the molecule in particular systems differs.

Berenbaum and Robinson, PNAS, 2003

What the chemist can do for chemical ecology:

- Isolation and structure elucidation (natural product chemistry);

- Identification and “certification of quality” of the natural product(s) (analysis);

- Providing information for taxonomy (Chemo-taxonomy);

- Origin and Function.

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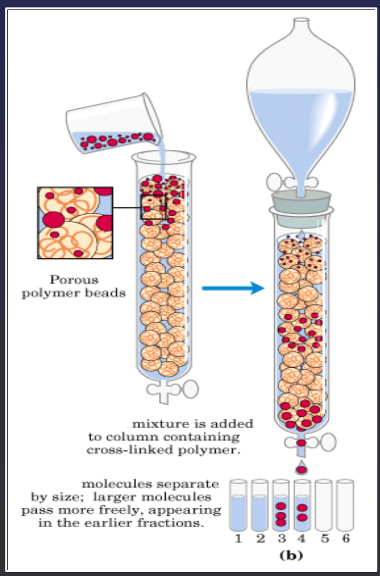
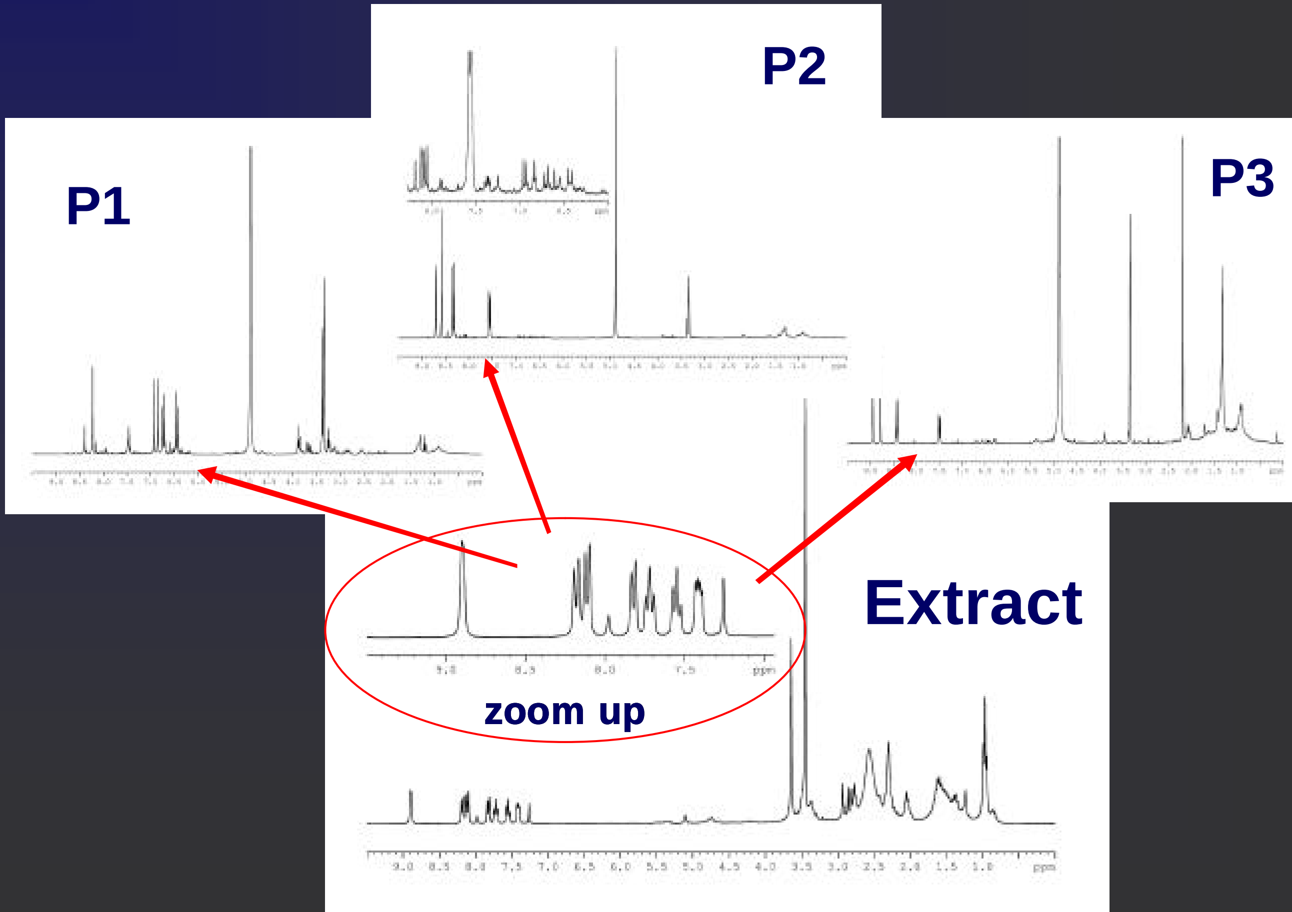
Chemist’s contribute in chemical ecology:

- Isolation and structure elucidation (natural product chemistry);





Training course on “Chemical methods in chemical ecology, Naples (Italy)
9-14 September 2OO7

5th Euroconference on Marine Natural Products, Ischia (Italy)
15-20 September 2OO7

Consider a signal compound (pheromone, allomone, kairomone, hormone, or neurotransmitter) with a modest molecular mass of ~ 300:

•Since 1850, the amount of compound required for a structure determination compared with ours has decreased at least a million-fold.

•In 1950, structure proof for such a compound might have been carried out by using a sample size of ~ 50 mg.

•Today, this task might be accomplished by using only ~ 50 µg or perhaps even 50 ng for a moderately complex unknown, which would represent a reduction of between 10^3 and 10^6 in sample size.

Furthermore, a structure determination that might have required 20 or more years, starting in 1900, might be accomplished in 1 hour today, using readily available NMR spectroscopic techniques.

Sample size, g	Sample size, no. of molecules	X-ray crystallography	NMR spectroscopy	MS
~300×10^{0}	6.23×10^{23}	+	+	+
50×10^{0}	10^{23}	+	+	+
50×10^{-3}	10^{20}	+	+	+
50×10^{-6}	10^{17}	+	+	+
50×10^{-9}	10^{14}	−	−	+
50×10^{-12}	10^{11}	−	−	+
50×10^{-15}	10^{8}	−	−	−
50×10^{-18}	10^{5}	−	−	−
50×10^{-21}	10^{2}	−	−	−

+, applicable; −, inapplicable.

50 ng of our hypothetical signal compound corresponds to a hundred trillion (10^{14}) molecules. Because 10^{2} or fewer signal molecules are certainly sufficient in many situations to trigger behavioral responses, there is still a quantitative gap of at least a factor of 10^{12} between biologically significant quantities of a molecular messenger and the quantities that a chemist skilled in the art can characterize today.

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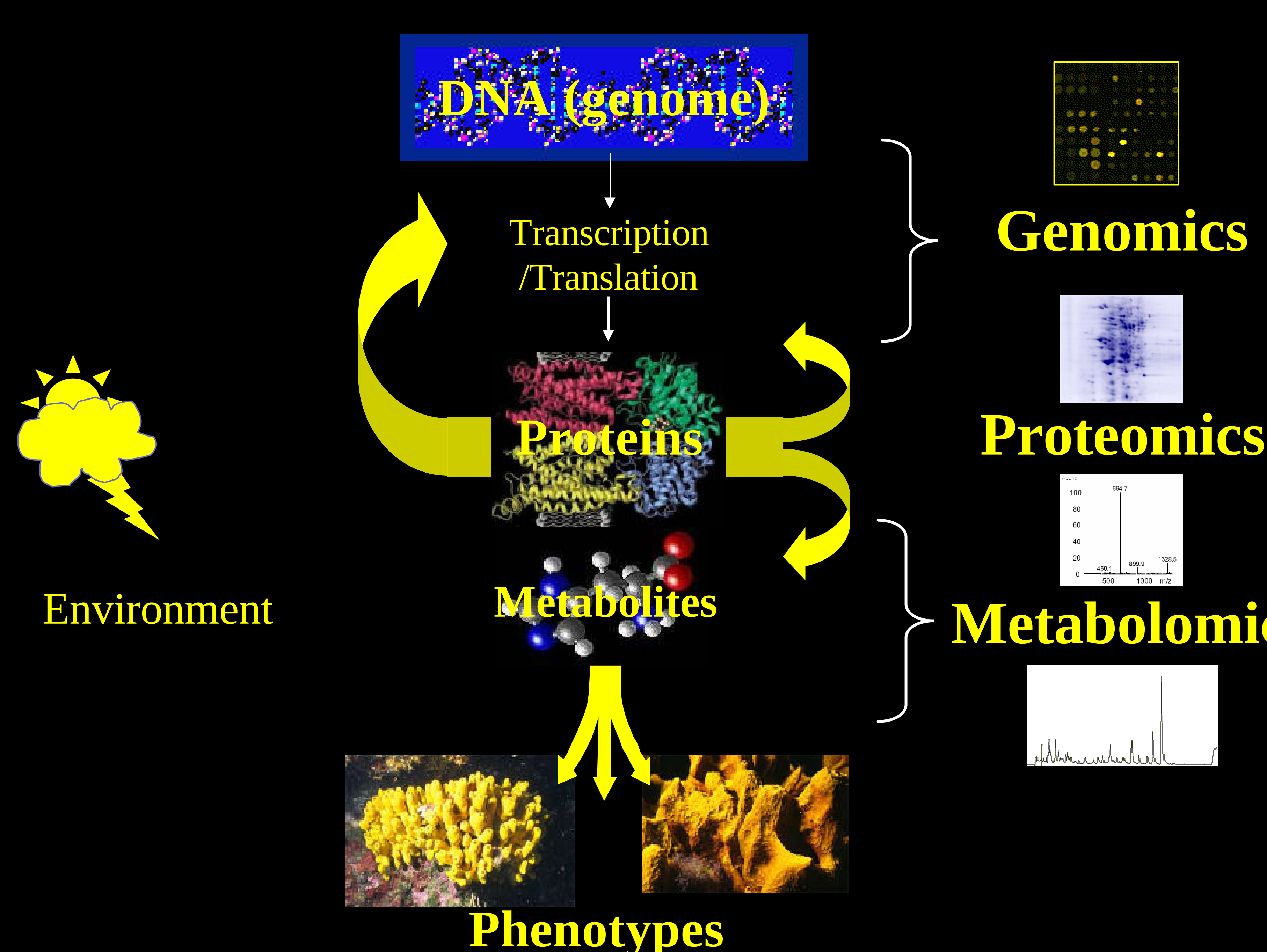
DNA (genome)
Transcription
/Translation
Proteins
Metabolites
Phenotypes
Environment
Genomics
Proteomics
Metabolomic
Abund.
100
80
60
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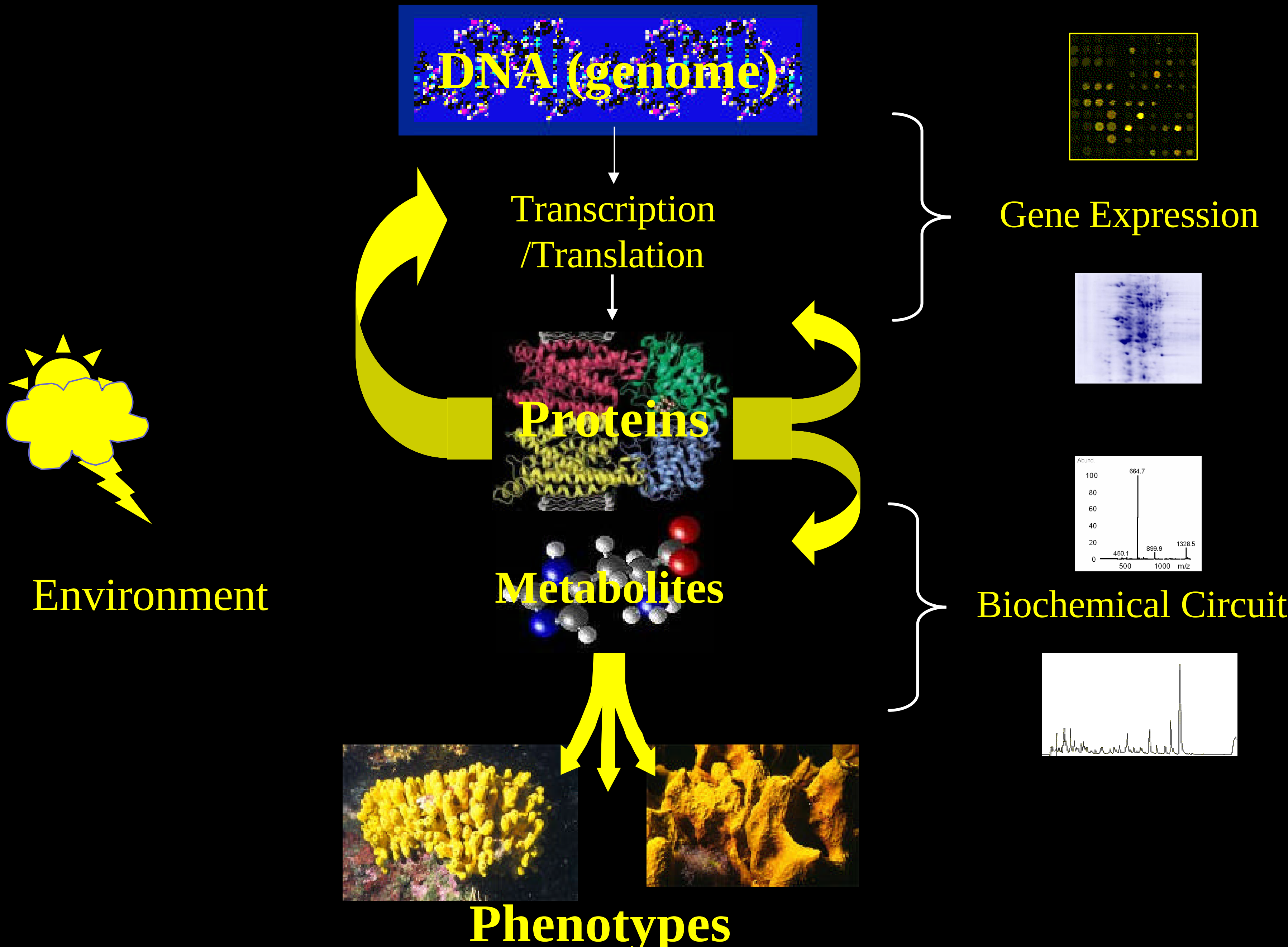
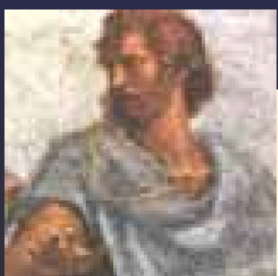
20
0
664.7
450.1
899.9
1328.5
500
1000
m/z


DNA (genome)
Transcription
/Translation
Gene Expression
Proteins
Environment
Metabolites
Abund.
664.7
100
80
60
40
20
0
450.1
899.9
1328.5
500
1000
m/z
Biochemical Circuit
Phenotypes

The metabolite content (metabolome) reflects the totality of metabolic processes. It results from expression of the genome and proteome, but also includes "independent" cellular processes such as absorption, distribution, and detoxification of natural and xenobiotic materials, energy utilization, signal transduction, and regulation. While the Genome is representative of what might be, and the Proteome is what is expressed, it is the "Metabolome" that represents the "actuality" of the cell or tissue.

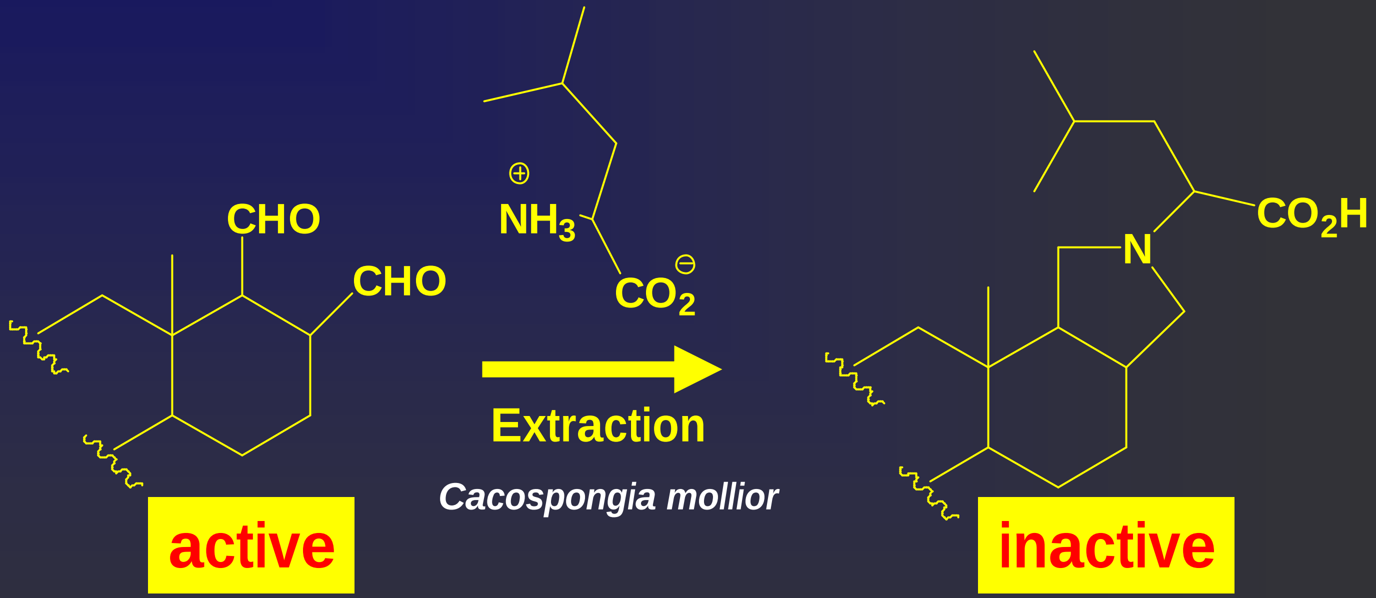


"…there must then be a principle of such a kind that its substance is activity …. the actuality (*enteleceia *-entelecheia or energeia) is the end, and it is for the sake of this that the potentiality (*dunamiV *– dynamis) is acquired..."

(Aristotle, Methaphysics, 350 B.C.)

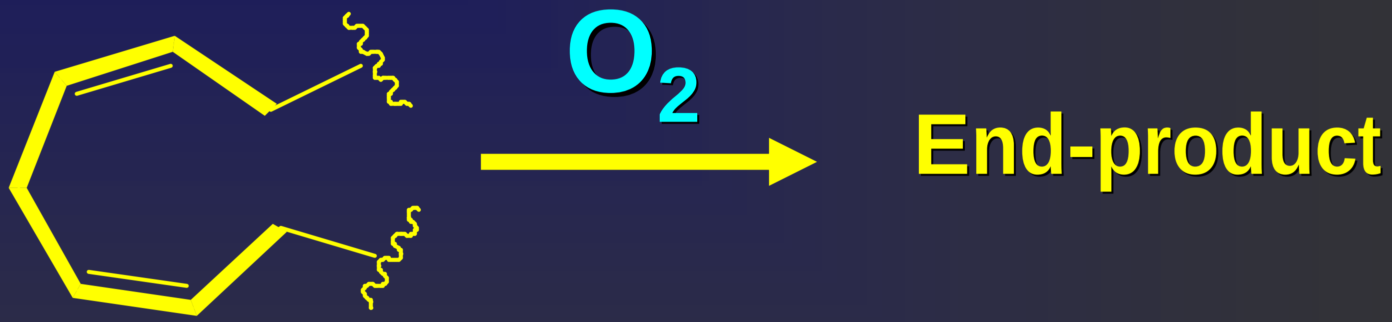
‘Quality and fidelity” of the natural product

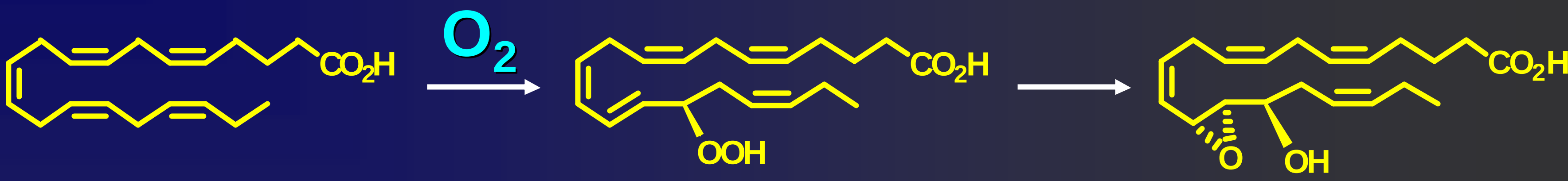
Chemical manipulation (extraction, purification, analysis ….) always produces artifacts.



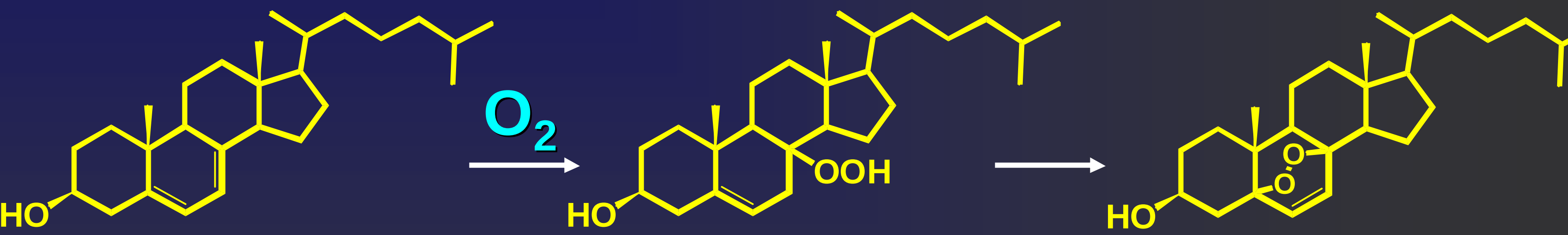
The potential effect of these products is of primary importance in the evaluation of the biological activity of extracts and pure metabolite

But not always it is so easy to establish if the product is truly “natural”…



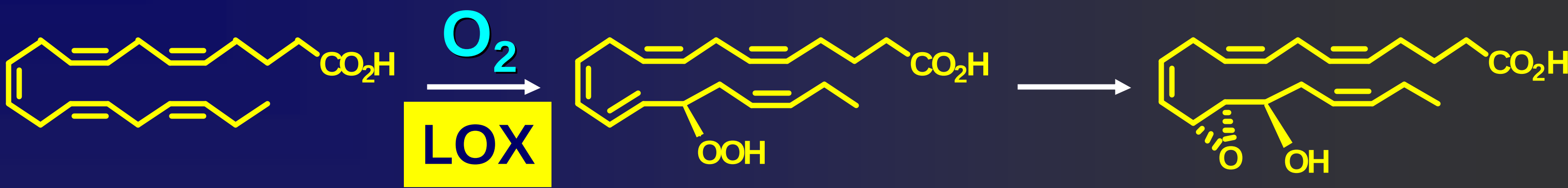


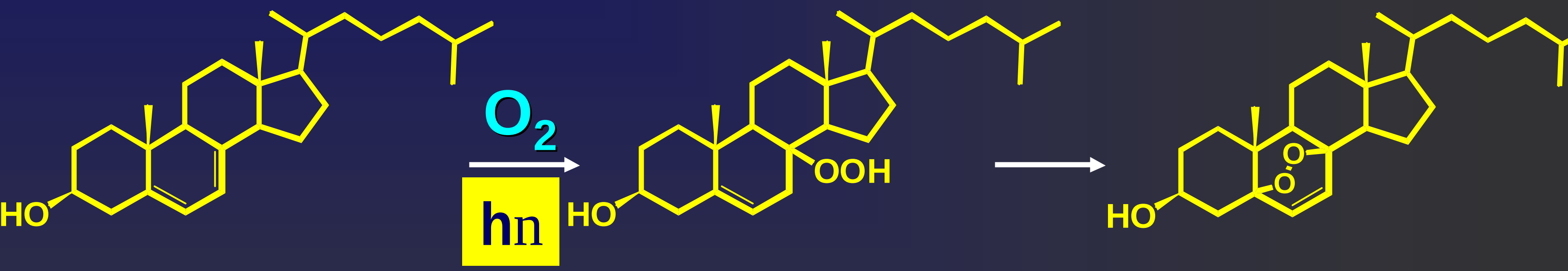
CO_2H
O_2
CO_2H
OOH
CO_2H
O
OH

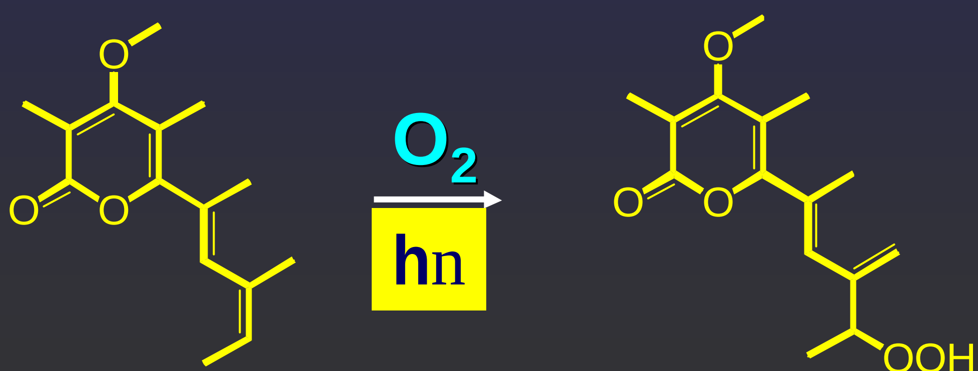


HO
O_2
HO
OOH
HO
O
O

O
O
O
O_2
O
O
O
OOH


CO2H
O2
LOX
OOH
CO2H
CO2H
O
OH


HO
O2
hn
HO
OOH
HO
O
O


O
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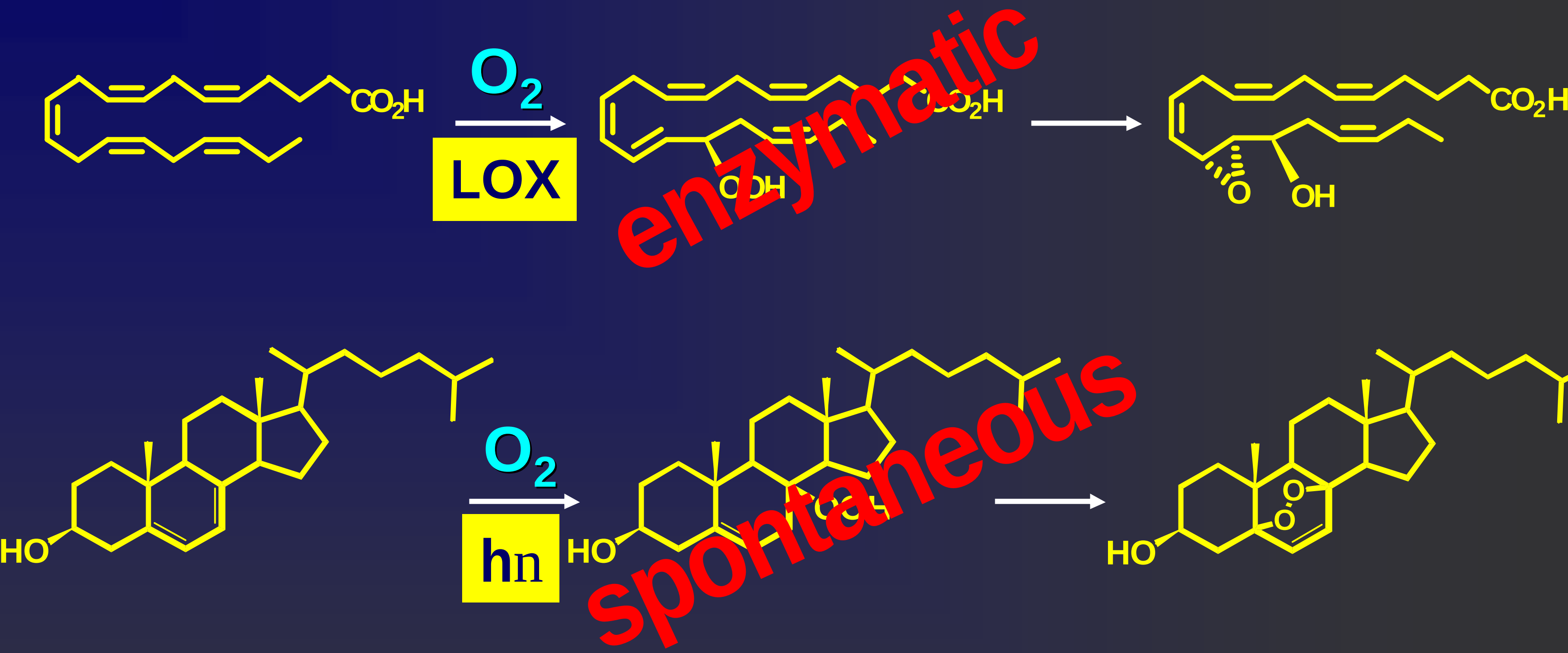
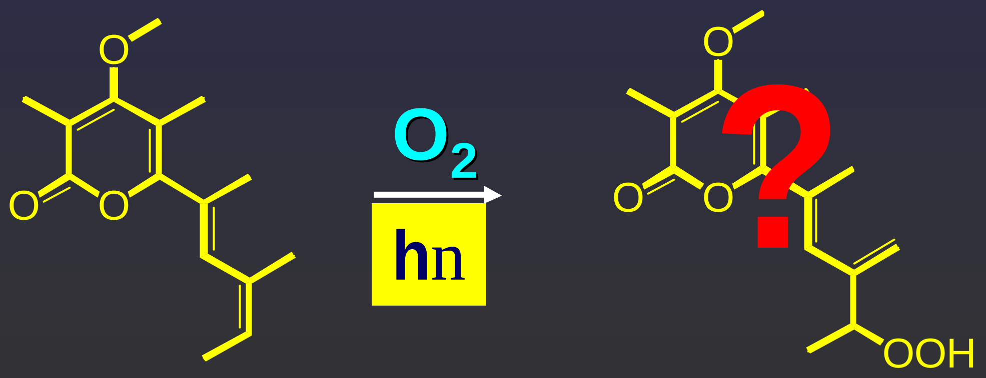
OOH





sunscreen effect

Propionates of *Placida dendritica* (sacoglossa opisthobranchia)

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Sorry , not much to say!!!!!

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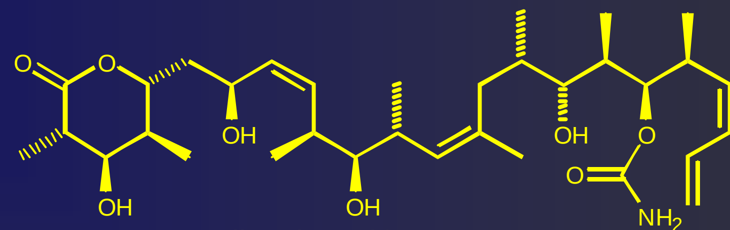
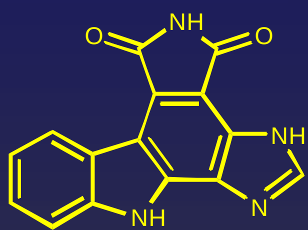
34 of the 36 phyla of our planet’s biodiversity is found in oceans

13’000 marine natural products described to date

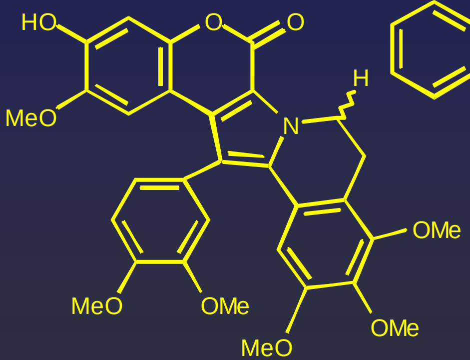
granulatimide



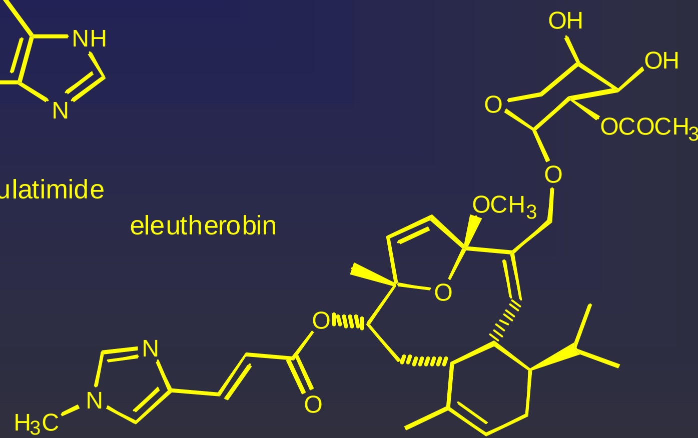
discodermolide



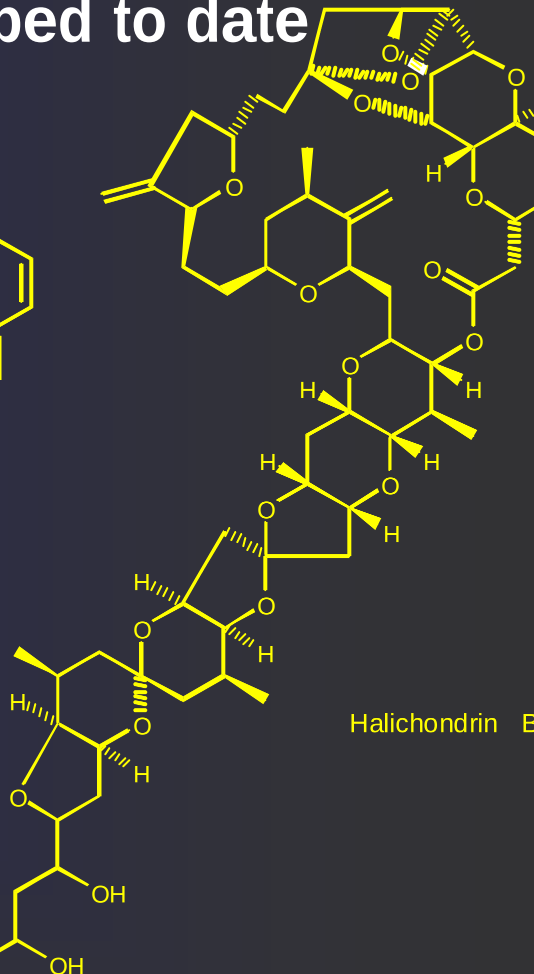
isogranulatimide



Lamellarin I



eleutherobin

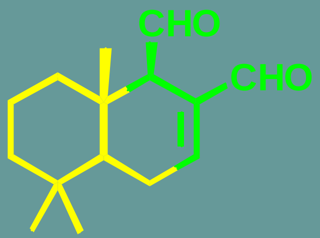


Halichondrin B

Chemical signaling shares several features irrespective of taxon. Across a wide range of organisms, many of the same classes of molecules perform this function, even if the precise identity of the molecule in particular systems differs.

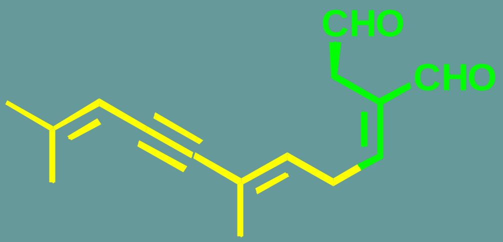
From the molluscs to mankind

Dendrodoris

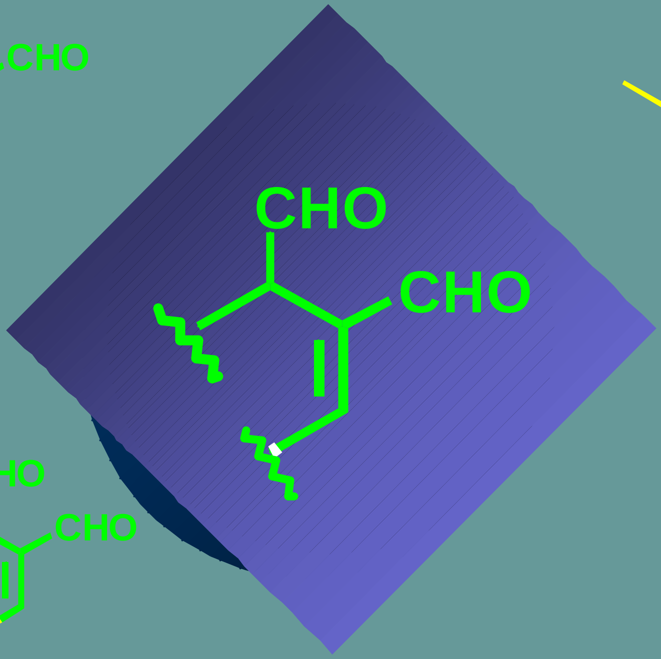


“de novo”

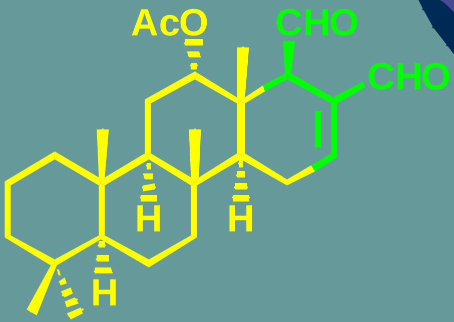
Oxynoidea



da *Caulerpa*



H. orsini

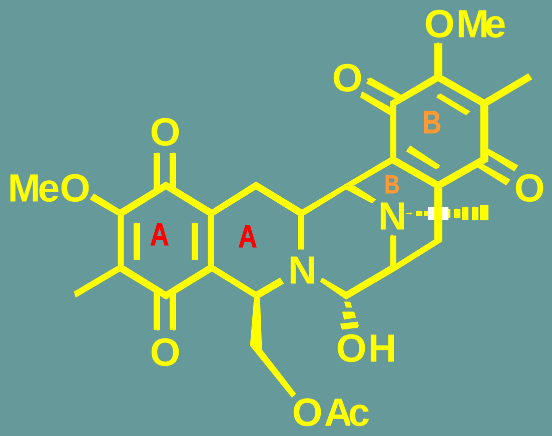


da *Cacospongia*

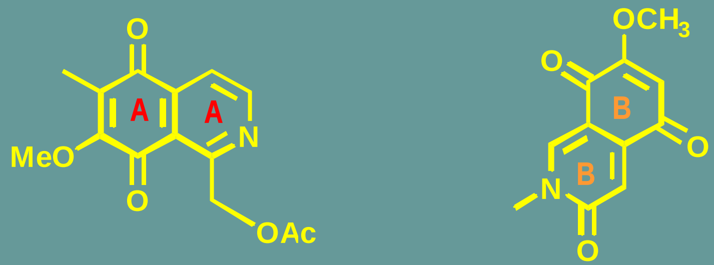
“scalaradials, are an extremely important class of anti-inflammatory compounds”

Jacobs in *Marine Biotechnology* **1**, 20, 1993

Antitumor compound from *Jorunna funebris*



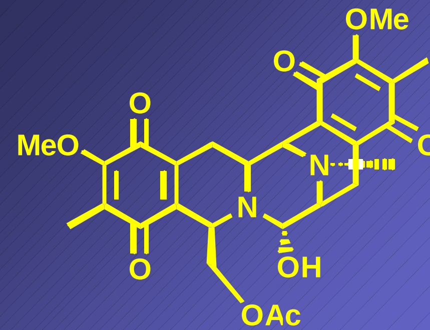
jorumycin



dalla spugna *Oceanapia* sp.

Tetrahedron **56**, 7305, 2000

Zalypsis®



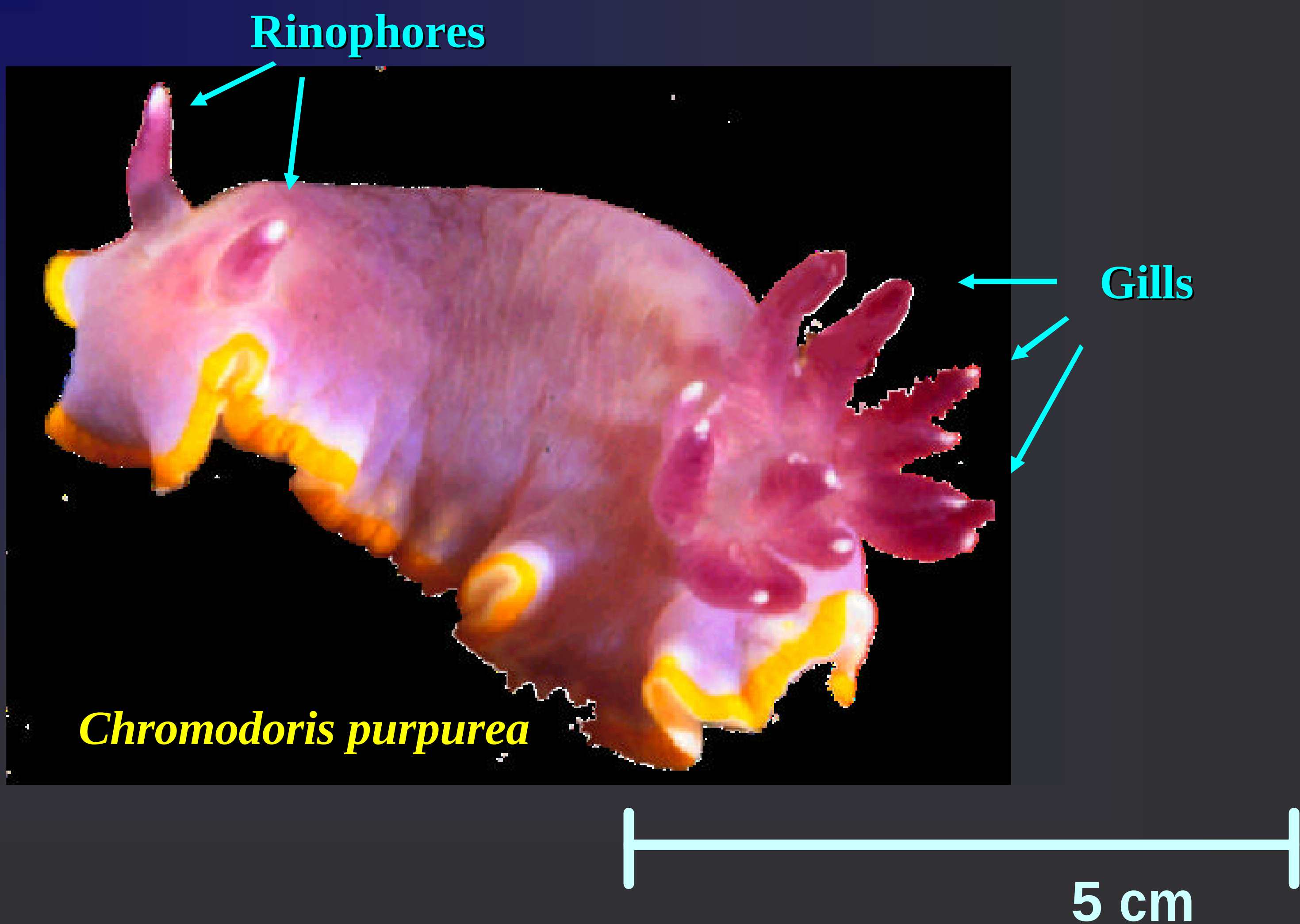
jorumycin

Zalypsis® (PM00104/50) is PharmaMar's fifth new ma… …ound that has begun Phase II clinical trials for the treatment o… …s.

•Zalypsis® is a novel chemical entity related to the… …e natural compounds Jorumycin and the family of Renieramycins, …ained from molluscs and sponges, respectively. Zalypsis binds to DNA an… 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.

Opisthobranchs



Guido Cimino

Margherita Gavagnin
Ernesto Mollo
Guido Villani
Emiliano Manzo
Antonio Maiello
Franco Castelluccio
Marianna Carbone
Adele Cutignano
Giuliana d'Ippolito

Taxonomy of Opisthobranchs

Phylum	Mollusca
Class	Gastropoda
Subclass	Opisthobranchia

Order

Acochlidea	Thecosomata	Rhodopemorpha
Gymnosomata	Anaspidea	Notaspidea
Cephalaspidea	Sacoglossa	Nudibranchia

- 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

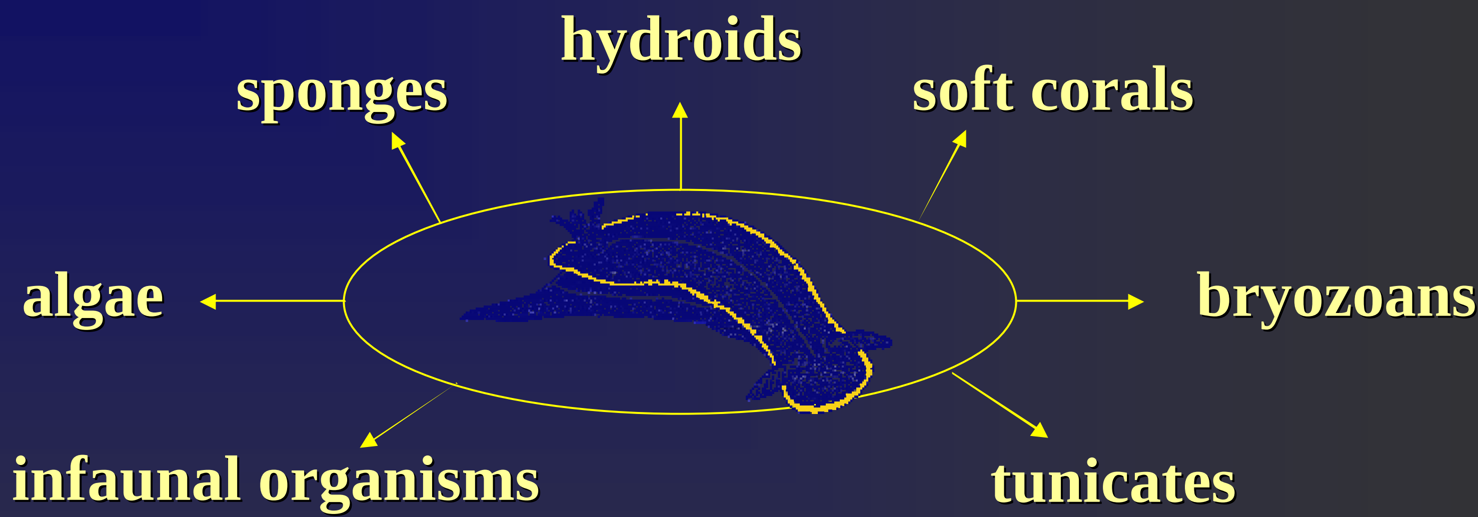
•Kinnel RB Dieter RK Meinwald J Van Engen D Clardy J Eisner T Stallard MO Fenical W 1979 Brasilenyne and cis-dihydrorhodophytin; antifeedant medium-ring haloethers from a sea hare (Aplysia brasiliana) Proc. Natl. Acad. Sci. U. S. A. 76 3576

- 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

Why the chemical interest?



ILLI IMPRUDENTES IPSI SIBI SAEPE VENENUM VERGEBANT,
NUNC DANT ALIIS SOLLERTIUS IPSI

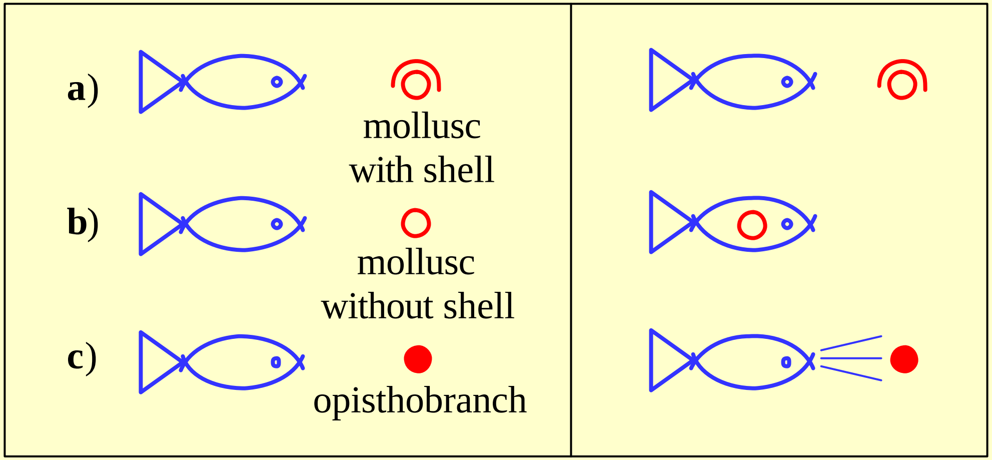
Tito Lucrezio Caro ***De Rerum Natura***

“after some intoxications, they discovered that the natural venoms could be more useful if offered to the enemies"

Hystoric milestones on Chemical Ecology of Opisthobranchs

1890 Prof. Herdman tested some alive specimens of *Ancula cristata* by putting them on the toungue of his assistant dr. Moore

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

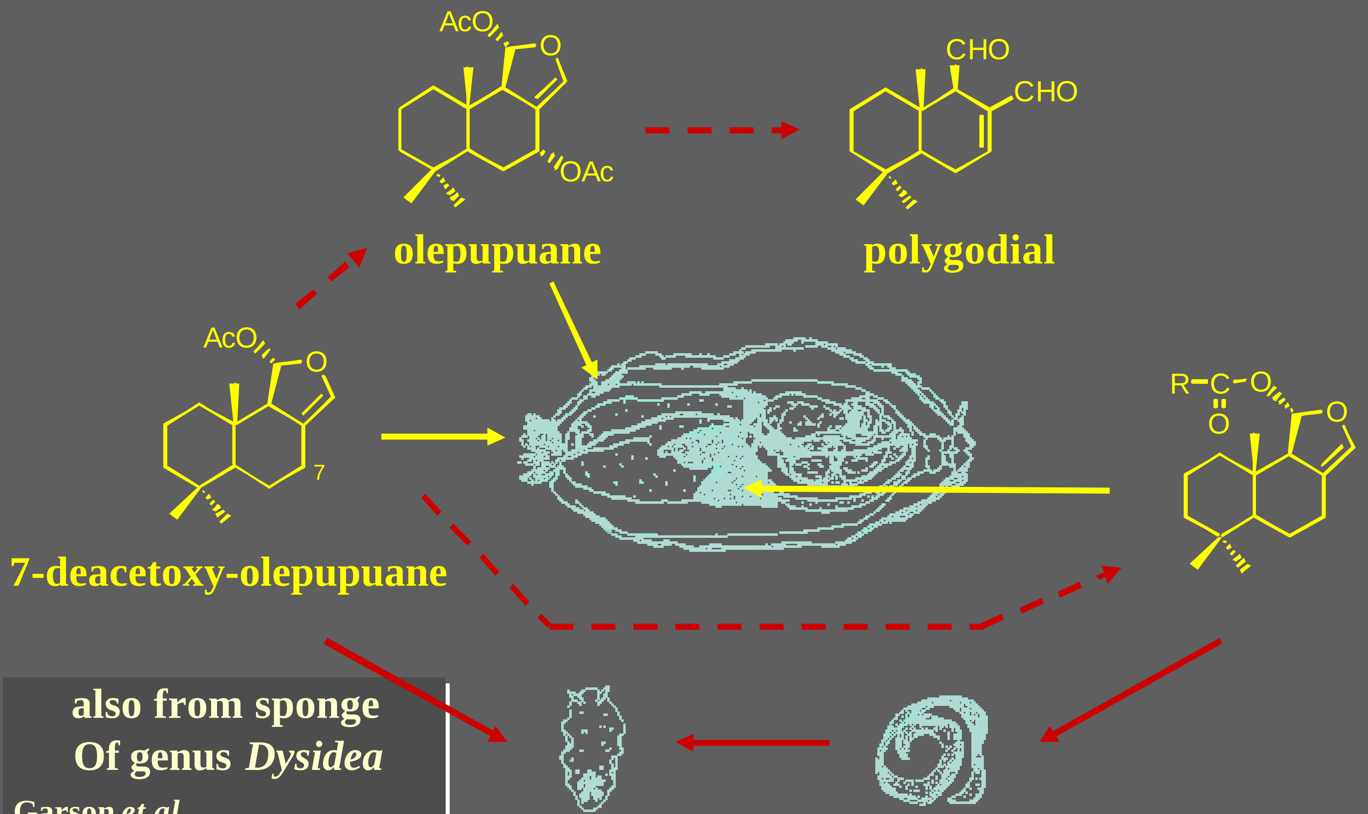


1976 J. Ros *Oecologia aquatica* **3**, 153
"bad taste of the nudibranchs"
"ignorance of the chemical mechanisms"

RESEARCH AIMS

- Dietary habits and metabolite origin
- Bioassays
- Biosynthesis
- Anatomical distribution of the active molecules

Multifunctional metabolites in *Dendrodoris limbata*



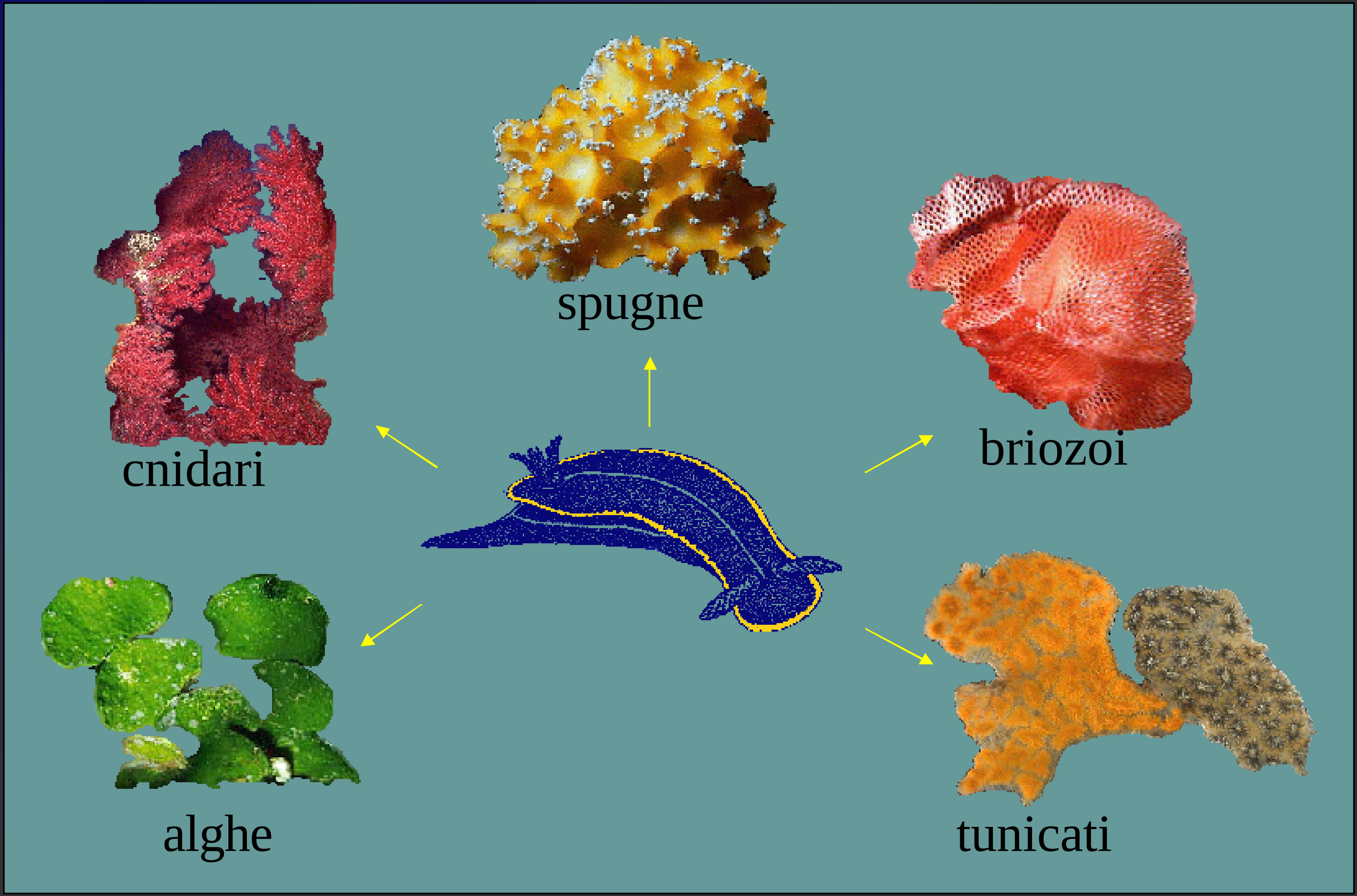
also from sponge
Of genus *Dysidea*

Garson *et al.*
***J. Nat. Prod.*. 55, 364, 1992**

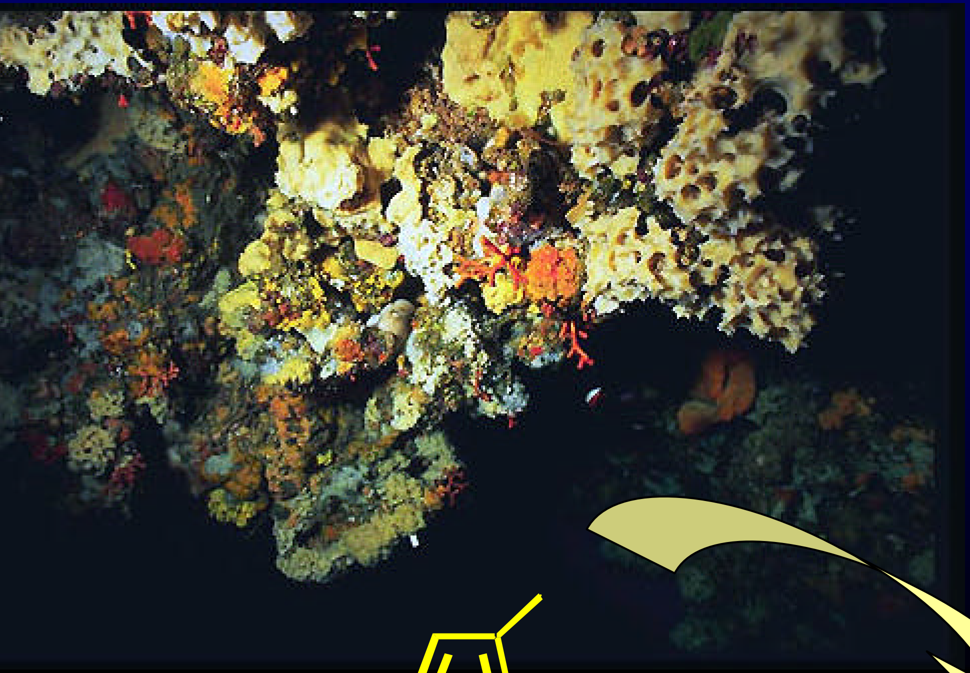
Experientia **47**, 56, 1991

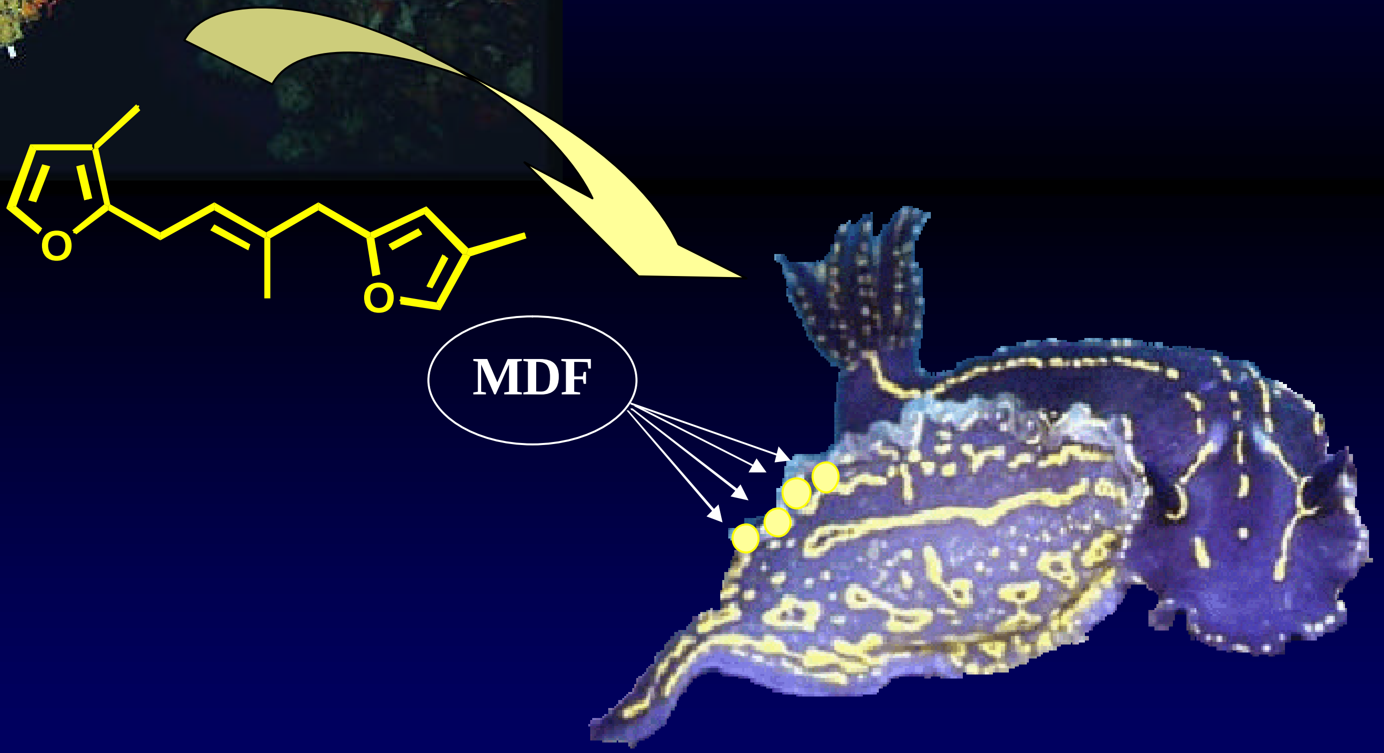
Origin of secondary metabolites

- diet
- transformation of dietary products
- biosynthesis


spugne
cnidari
briozoi
alghe
tunicati

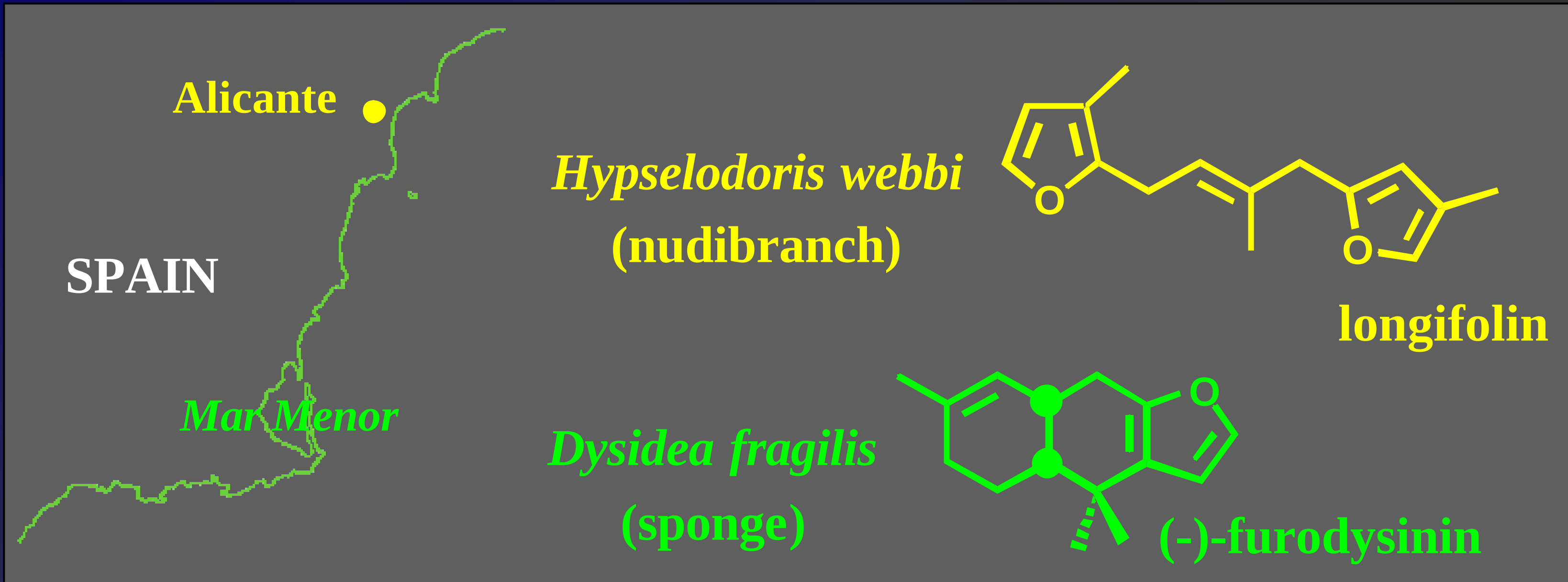
Transfer of Sponge Metabolites to the MDF's of *H. webbi*



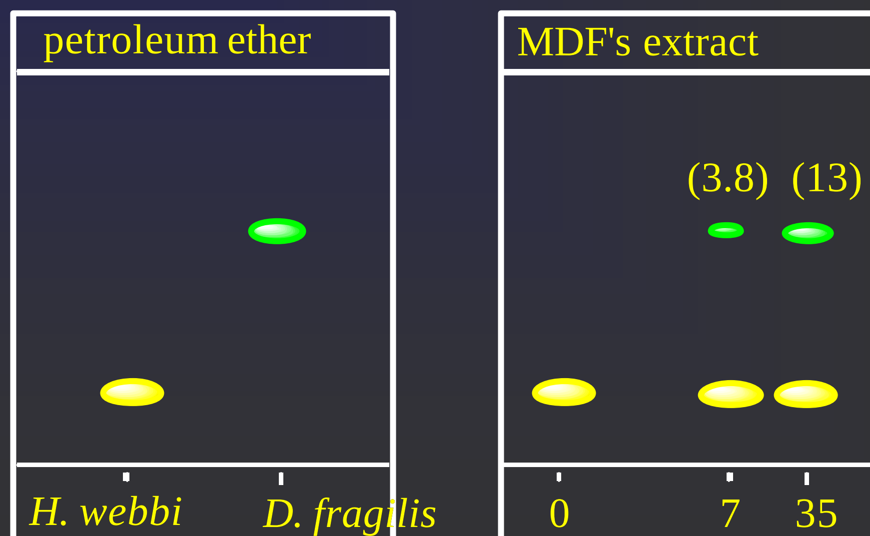


Experientia **50**, 510, 1994

Transfer of diet-derived chemicals into the MDF of *H. webbi*

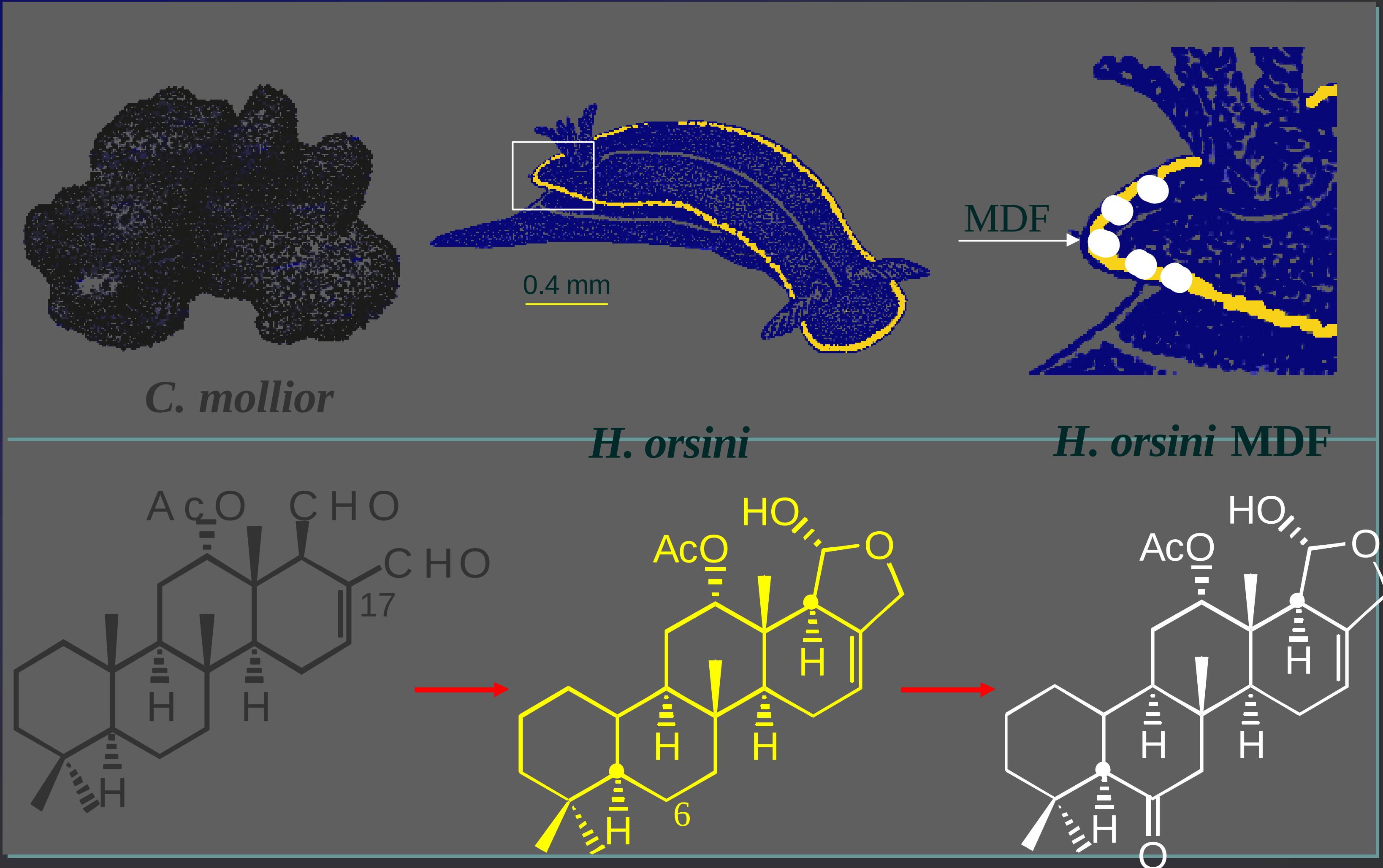


gg.	(-)-furodysinin / longifolin
0	0 (only longifolin)
7	3,8
35	13,0



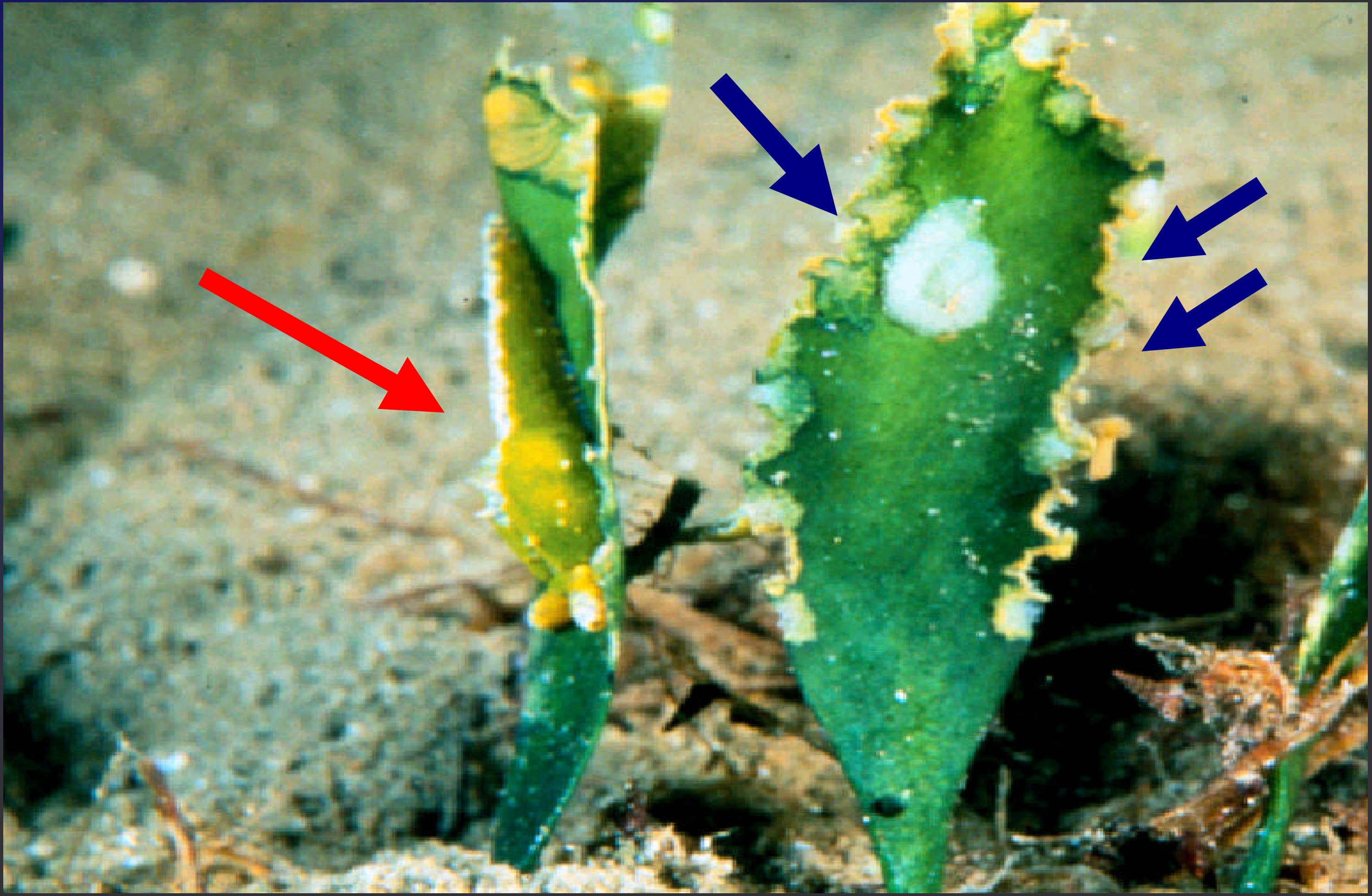
Experientia **50**, 510, 1994

Transformation of sponge-derived metabolite

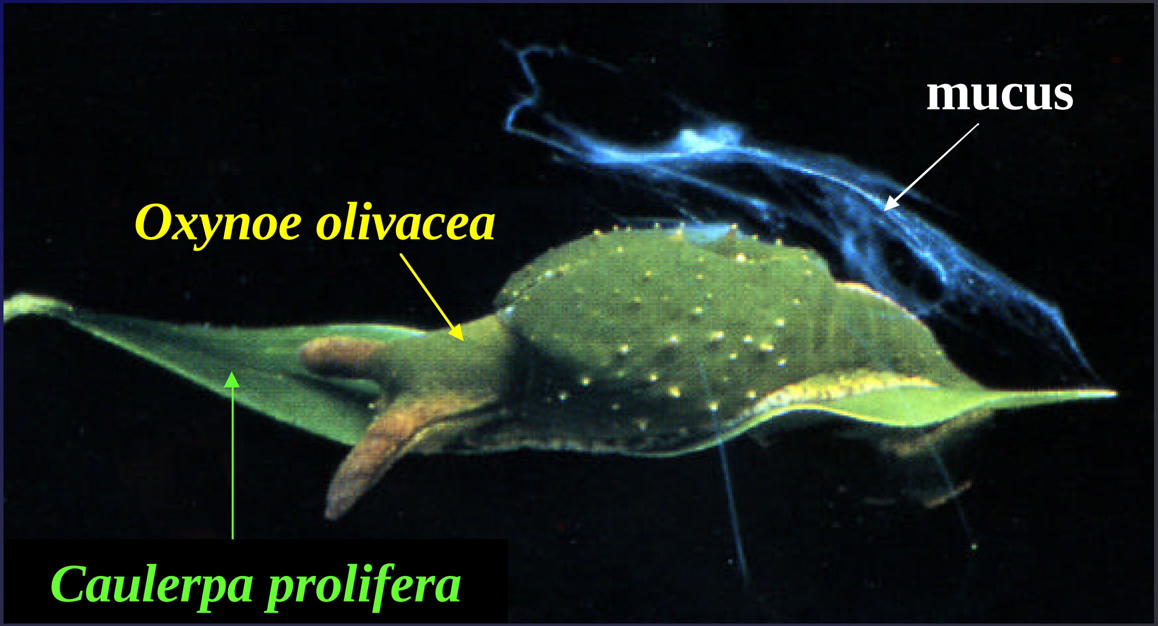


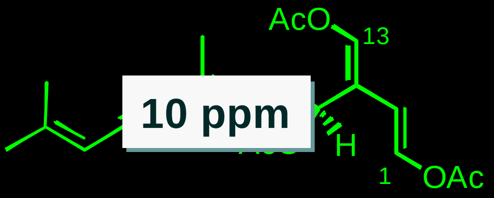
Experientia **49**, 482, 1993

Caulerpenyne-derived products in sacoglossans

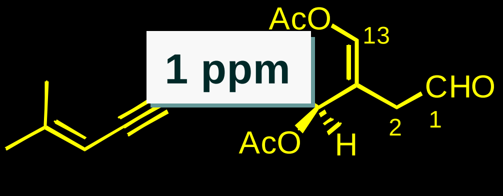


Caulerpenyne-derived products

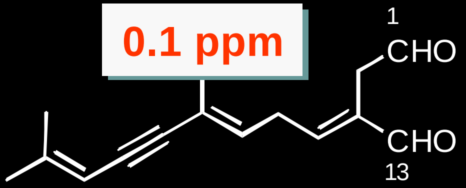




caulerpenyne



oxytoxin-1



oxytoxin-2

toxicity against *G. affinis*

Cimino, *Experientia* **46**, 767, 199

About the origin of oxytoxins……

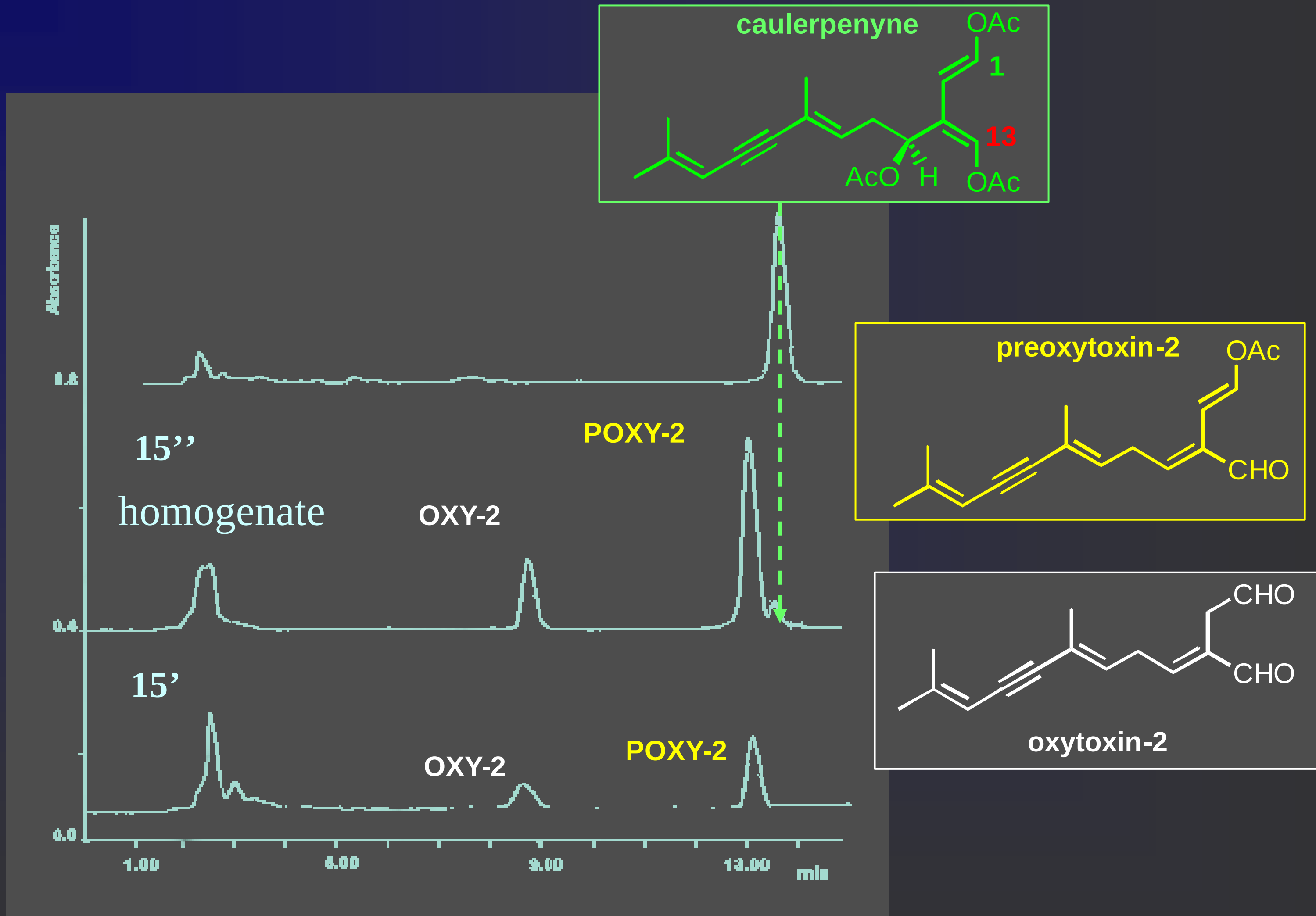
• **accumulation of toxins from the alga**

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

• **wound-induce transformation of algal metabolite**

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

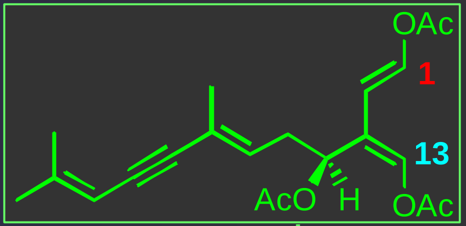
Studies with homogenates of *Oxynoe* and caulerpenyne

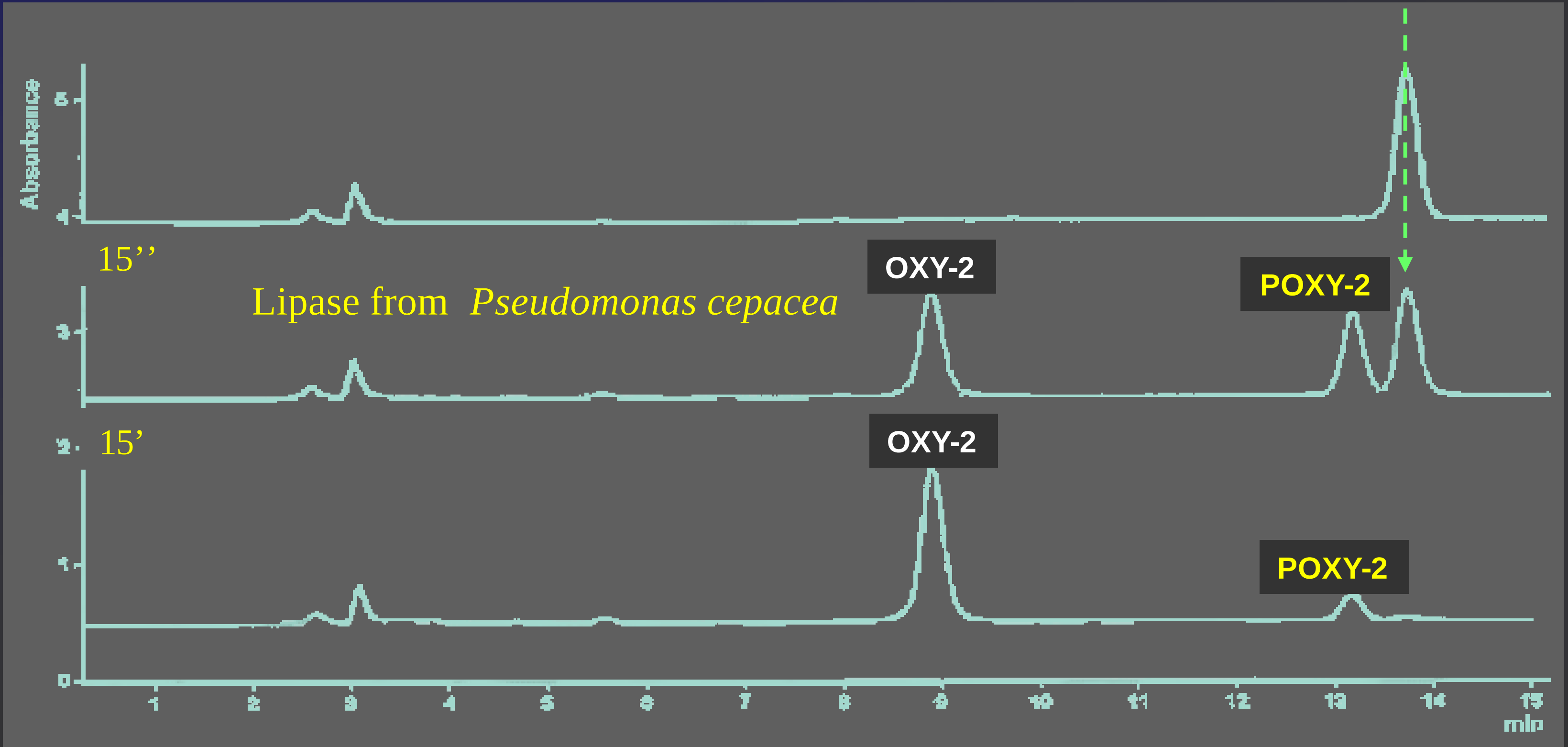


Org. Biomol. Chem. **2**, 1, 2004

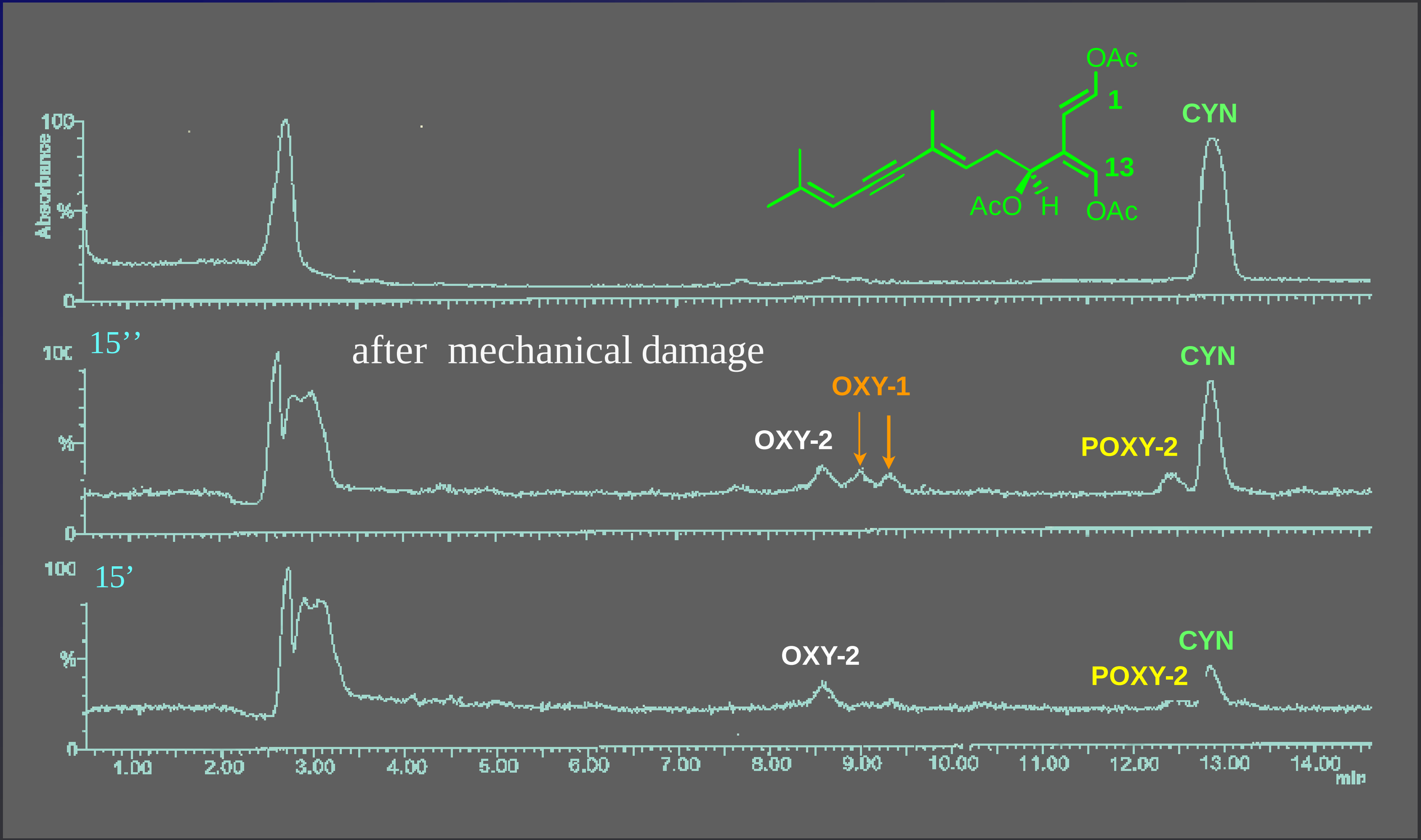
Lipase-mediated hydrolysis of caulerpenyne

Source	Reaction time	Yiekd
Aspergillus niger	90 min	80%
Aspergillus oryzae	345 min	15%
Mucor miehei	120 min	80%
Penicillium chamemberti	720 min	nessuna
Candida rugosa	90 min	95%
Pseudomonas cepacea	30 min	100%

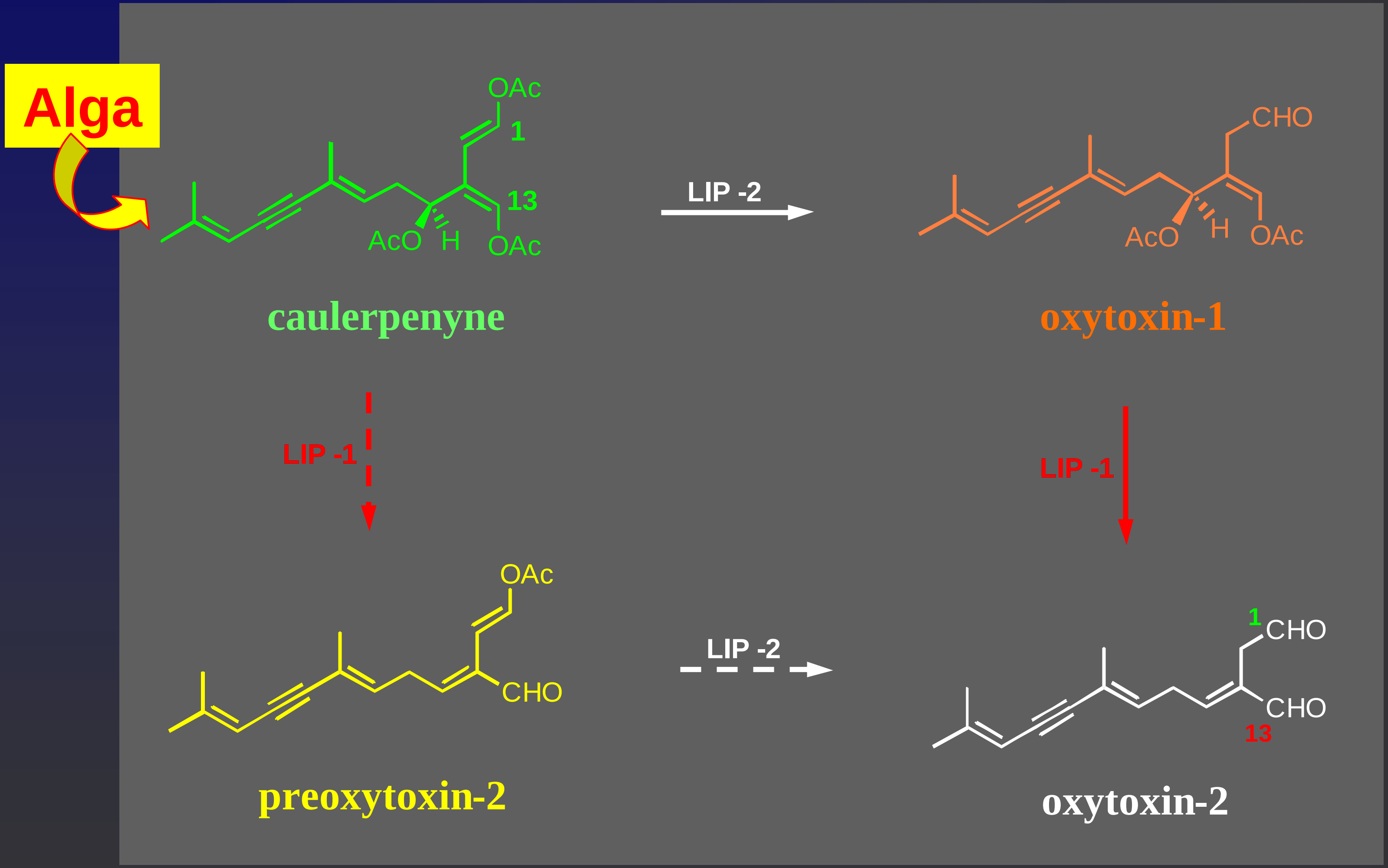




Wound-induced hydrolysis in *Caulerpa prolifera*



Oxytoxin synthesis in *Oxynoe olivacea*



Org. Biomol. Chem. 2, 1, 2004

BIOASSAYS

with freshwater organisms

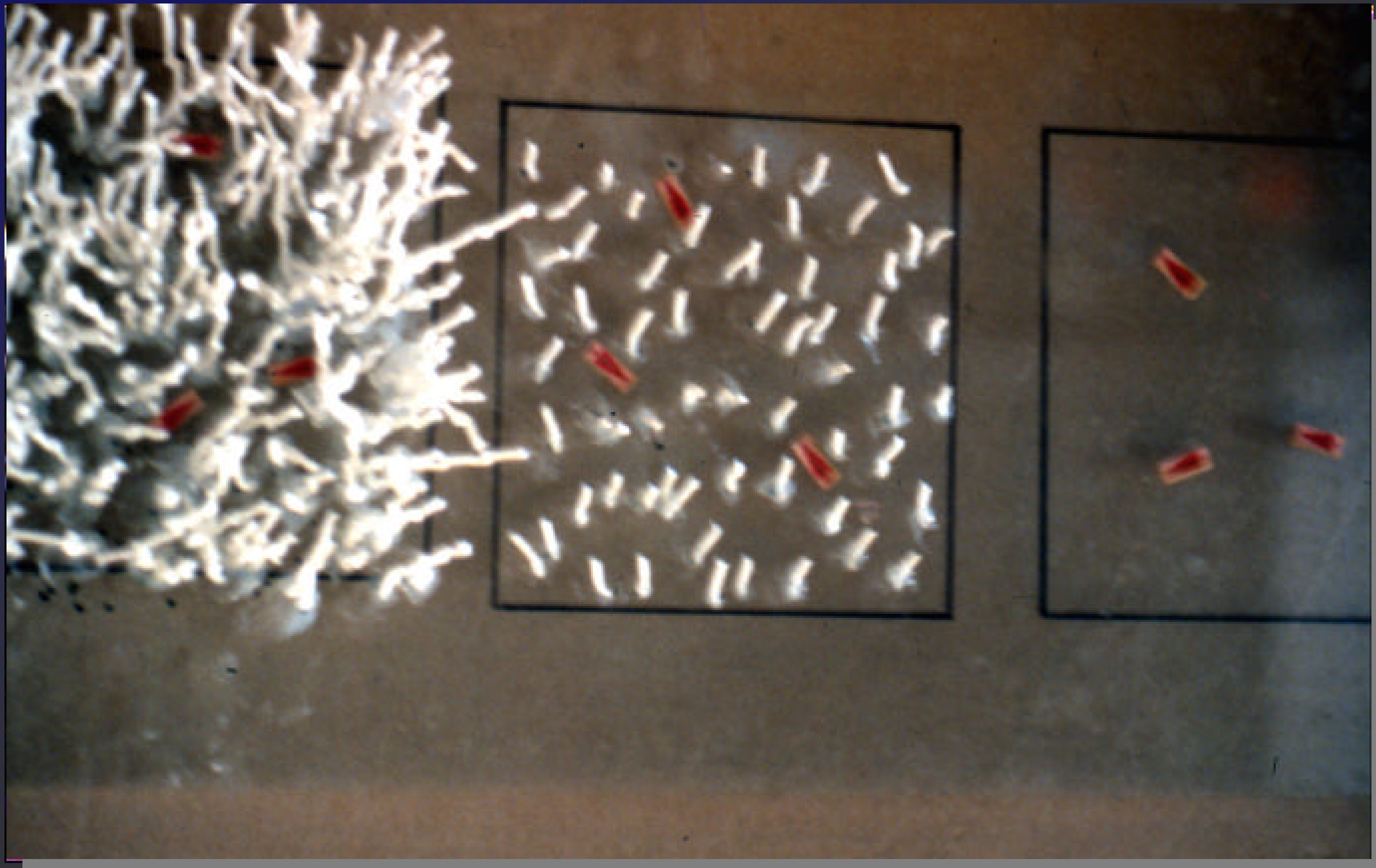
Artemia salina	*Carassius auratus*	*Gambusia affinis*
cytotoxicity	antifeedant	hycthyotoxicity

Not ecologically relevant but easy to carry out in any lab

with organisms from the same habitat

Ecologically relevant but noy easy to carry out in any lab

Marin's Experiment



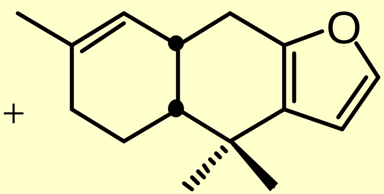
Studies on warning colours of Chromodoridid nudibranchs

Thalassoma pavo
10 specimens

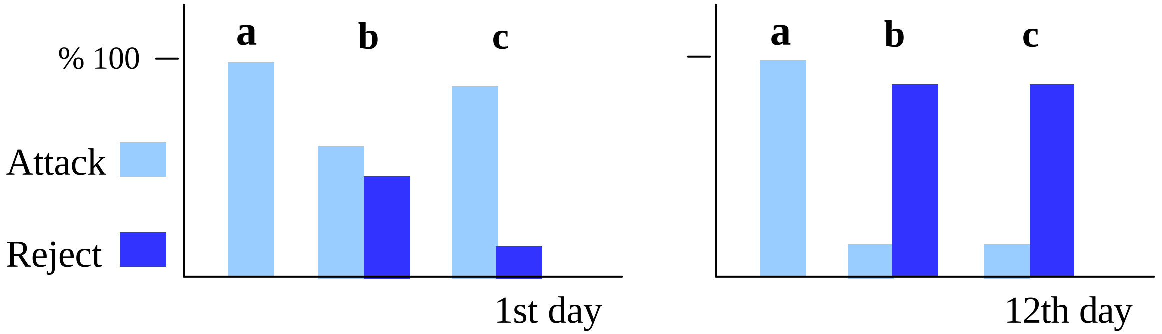
→

25 modelli
- **a) non aposematic**
- **b) aposematic + chemical**
- **c) aposematic + no chemical**

Hypselodoris +



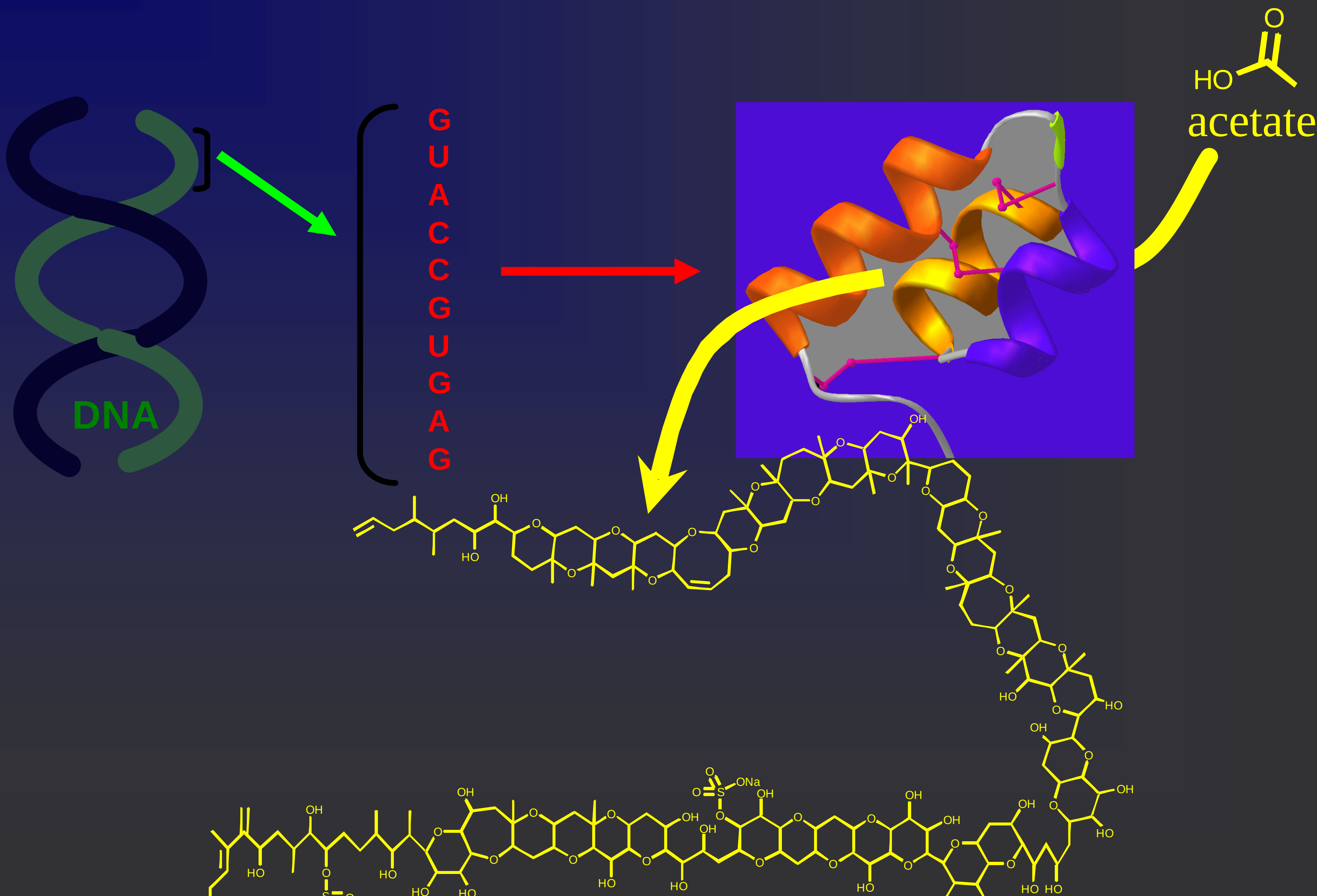
furodysinin



T. Pavo keeps memory of the potentially dangerous preys

Biosynthesis

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



Biosynthesis Determination

- Intuitive proposal of pre-cursor (Literature)
- **Feeding experiment with labelled precursors**
- Extract target-metabolite
- Determine inclusion/location of isotopic label
- **Characterization of the enzymatic activities**
- **Build-up the biosynthetic pathway**
- Molecular techniques

Biosynthesis: Unstable Isotopic Labels

Often used: ^{3}H (tritium) and ^{14}C (Half-lives: 12.3 and 5,700 years respectively)

- Unstable isotopic labels: advantages
  - **Sensitive isotope detection**
  - **Tiny amount of incorporation detected**
  - **Tiny amounts of labelled substrate required**
- Unstable isotopic labels: disadvantages
  - **Suitable laboratory and Handling difficulties**
  - **Location of incorporation difficult to determine**
  - **Tritium is poor mimic for hydrogen**

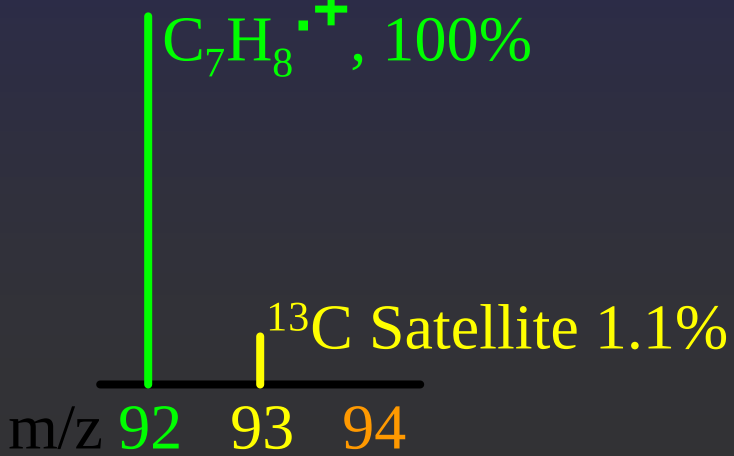
Biosynthesis: Stable Isotopic Labels

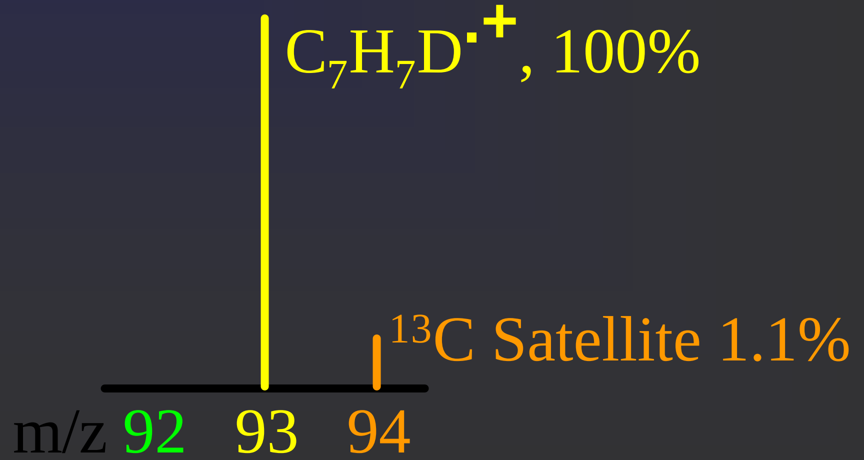
Most commonly used: ^{2}H (deuterium, D), ^{13}C, ^{15}N and ^{18}O

- **Main advantages:**
  - ✓ No handling difficulties
  - ✓ 100% enriched samples can be used
  - ✓ Multiply labelled pre-cursors available
  - ✓ Availability of simple labelling methodologies
  - ✓ Site of incorporation
  - ✓ Deuterium is better mimic for hydrogen than tritium
- **Main disadvantages:**
  - ✓ Relatively low sensitivity (6,000 times more difficult to detect ^{13}C than ^{14}C)
  - ✓ Still not a cheap process!

Biosynthesis: ^{2}H-labelled precursors

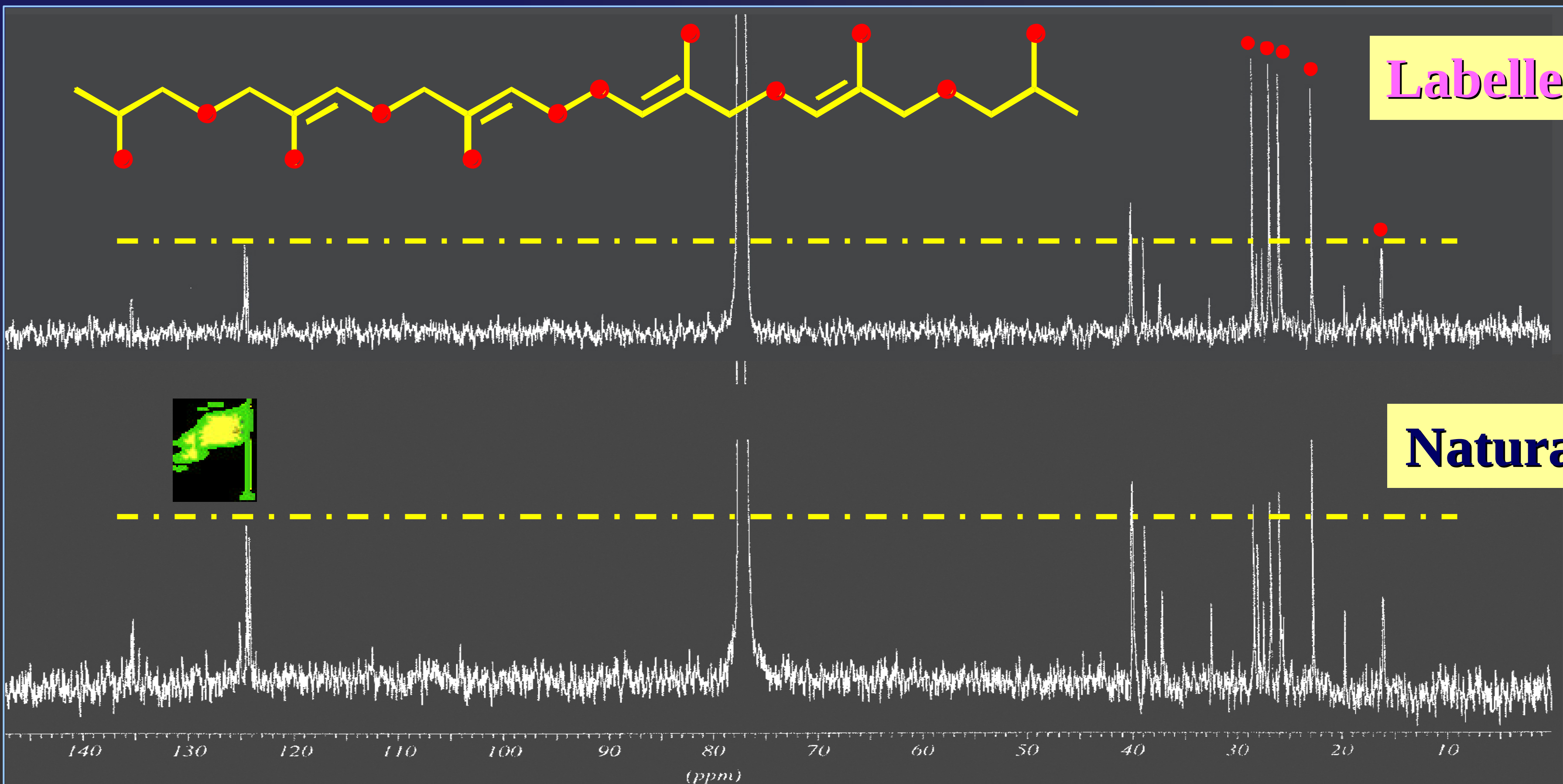
- **Detection of deuterium label:**
  - ✓**Loss of signal in ^{1}H spectrum**
  - ✓**^{2}H is NMR active: obtain spectrum**
  - ✓**Splitting in ^{1}H decoupled ^{13}C spectrum: Singlet will become a triplet as ^{2}H has *I* = 1**
  - ✓**Upfield shift in ^{13}C spectrum: ^{13}C-D resonate »0.3-0.6 ppm upfield of ^{13}C-H**
  - ➢ **Increased intensity of M+1 ion in mass spectrum**





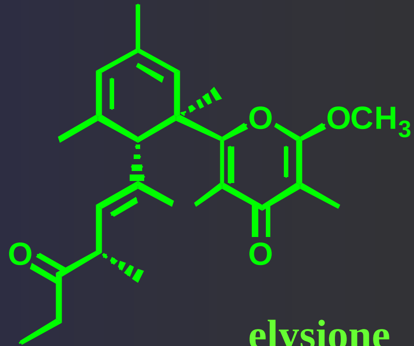
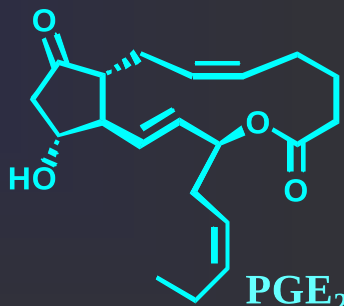
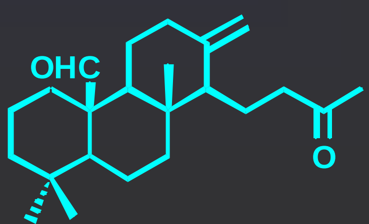
Biosynthesis: ^{13}C-labelled precursors

- **Detection of ^{13}C label:**
  - ✓ **Enhanced intensity in ^{13}C NMR spectrum (or increased intensity of M+1 ion in mass spectrum)**



J. Org. Chem., **2000**, *66*, 620

Biosynthetic Capacity and Chemical Markers

order	chemical class	chemicals
Cephalaspidea	w-aryl-methylchetones	 navenone-B
Sacoglossa	polypropionates	 elysione
Nudibranchia	acetogenins	 PGE$_2$- 1,15-lacto
	terpenes	 luteone

Biosynthesis in Cephalaspideans

Philinopsis depicta

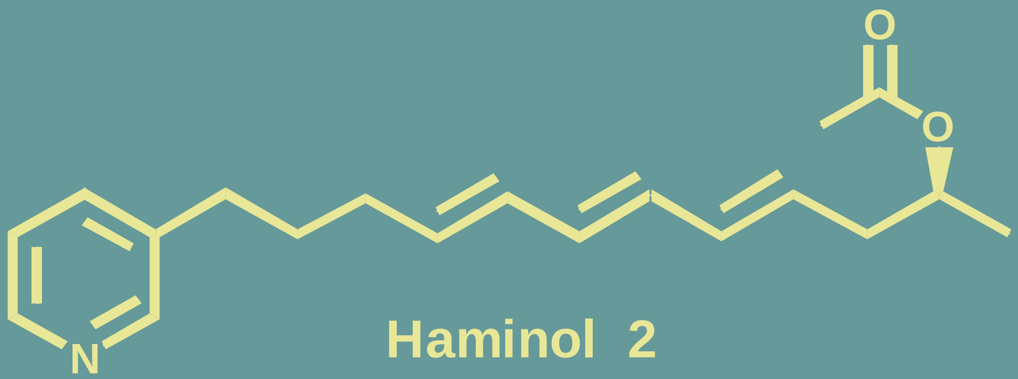
Haminoea orbignyan

Bulla striata

Haminols work as Alarm pheromones

Experientia, 47, 61, 19

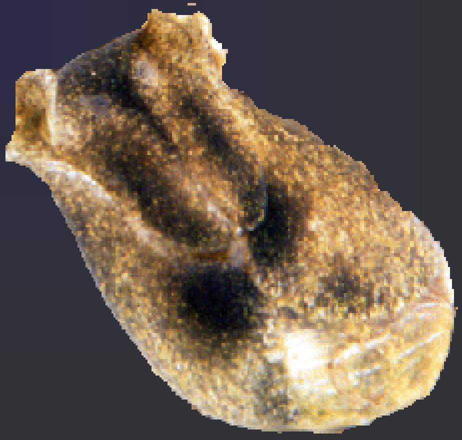
Biosynthesis in *Haminoea orbignyana*



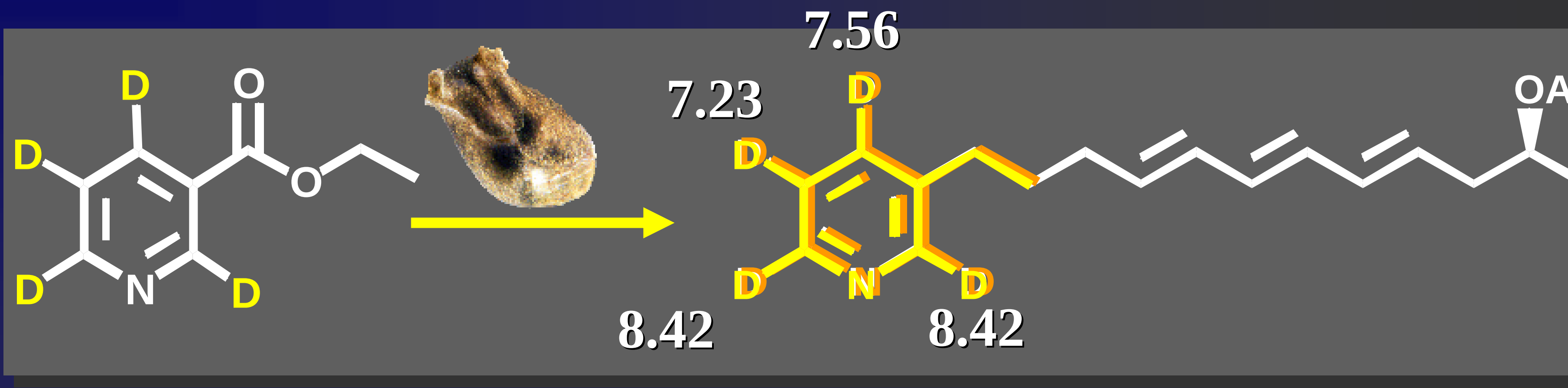
Haminol 2

d_4-nicotinic acid
[1-^{13}C]-acetate,
[1,2-$^{13}C_2$]-acetate
[2-^{14}C]-acetate

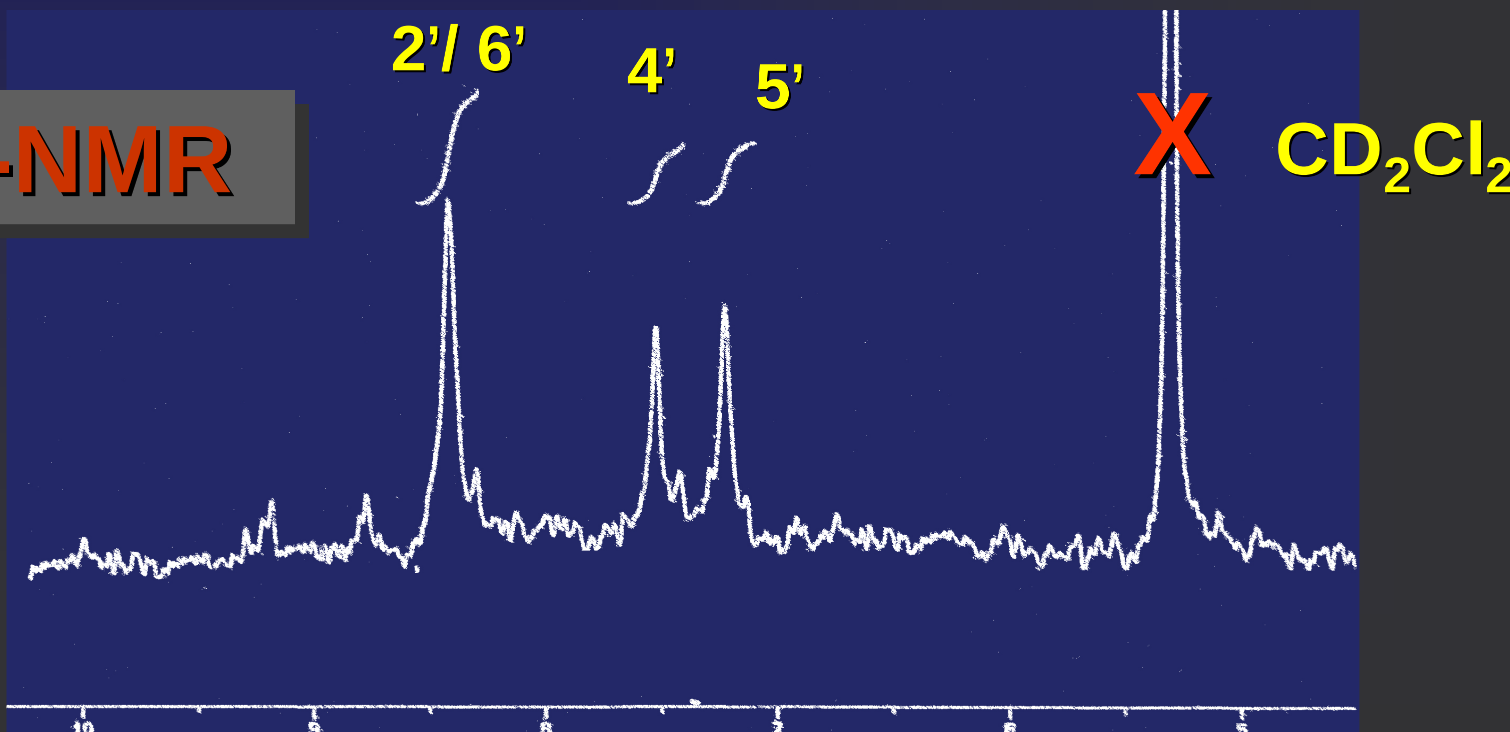
$\xrightarrow[\text{3-days}]{\text{injection}}$

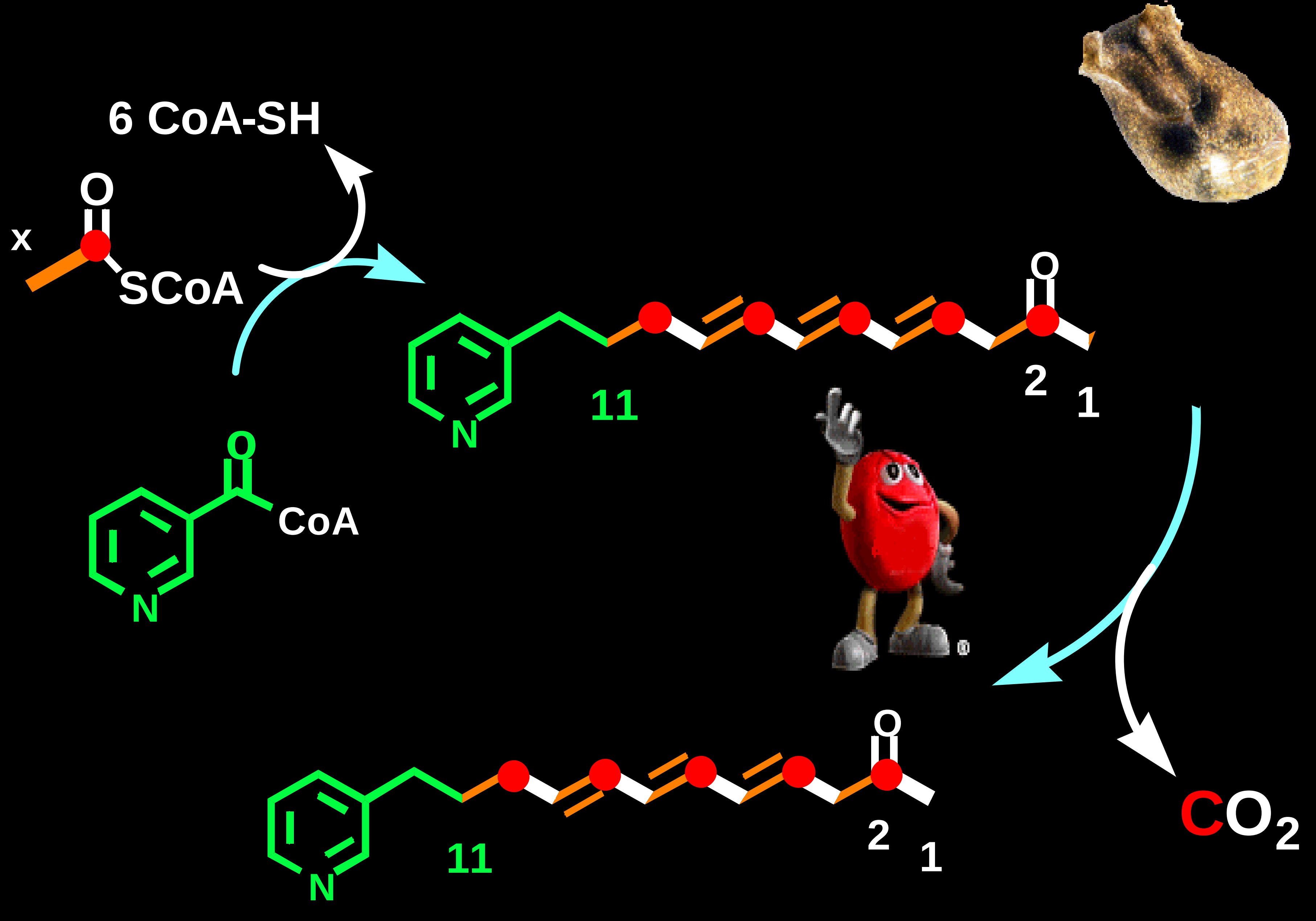


Haminoea orbignyana: Feeding d_4-nicotinic ethyl ester



^{2}H-NMR





6 CoA-SH
x
O
SCoA
O
CoA
N
O
11
2
1
N
O
11
2
1
N
CO2

Bioassays with Hydra vulgaris

PKC-mediated tissue regeneration

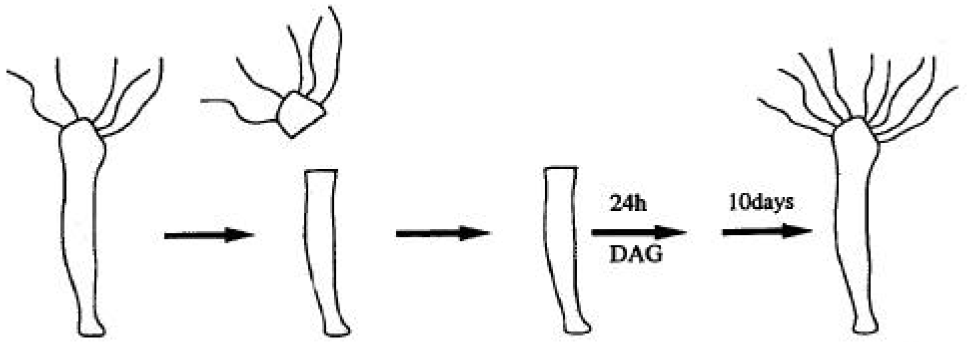
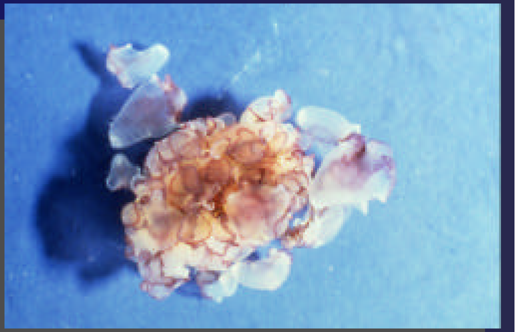
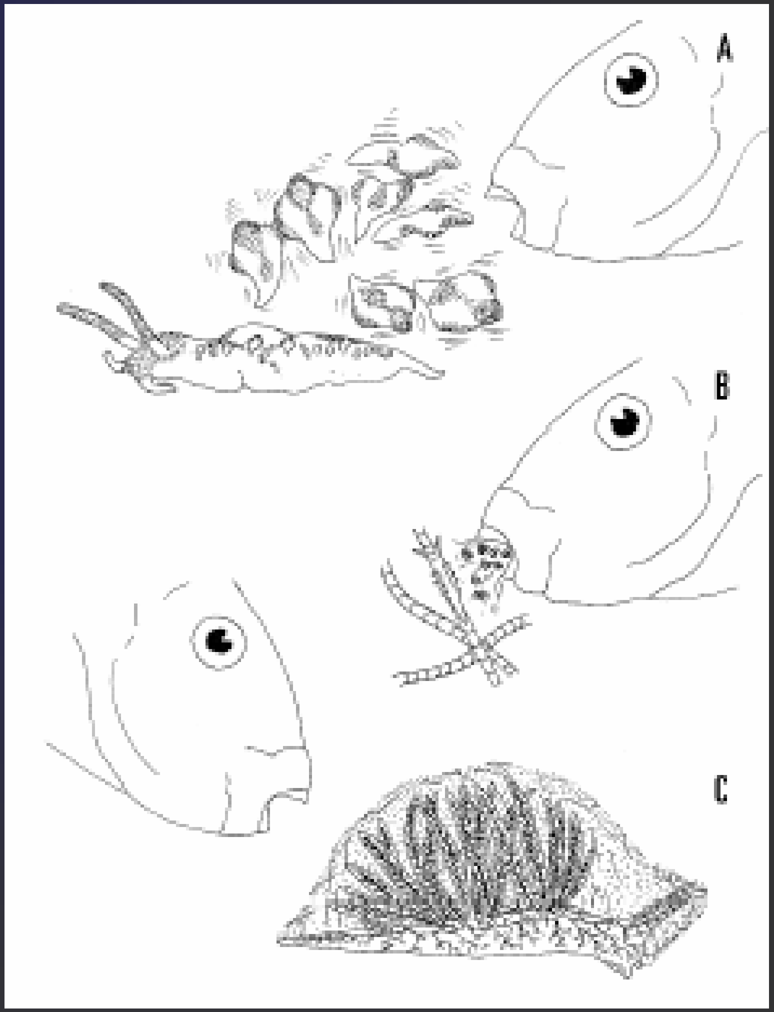


Fig. 2. Schematic representation of the *Hydra vulgaris* regeneration assay.

Autotomy in *Cyerce cristallina*





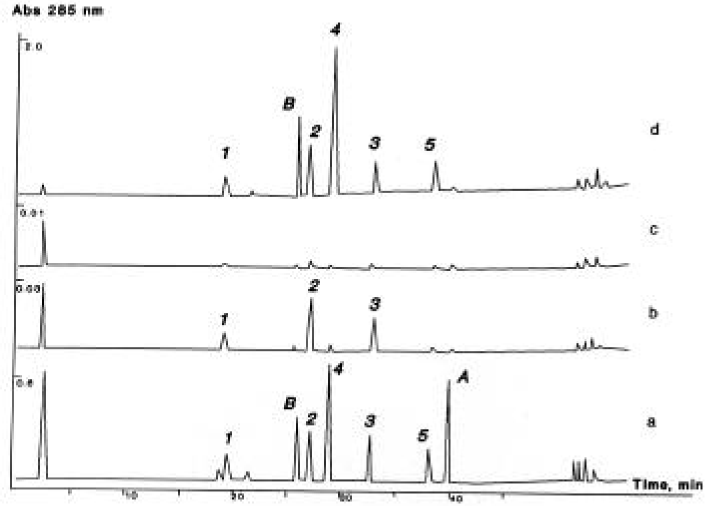
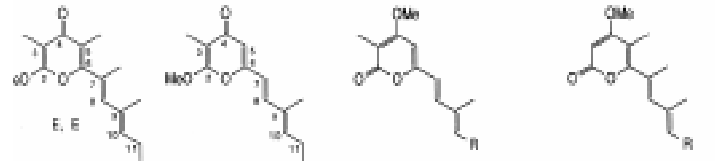


Figure 3. HPLC traces of the acetone extracts of *C. cristallina* cerata (*a*, 75 μg, 1/100 of the extract), mantle (*b*, 100 μg, 1/32 of the extract), digestive gland (*c*, 100 μg, 1/32 of the extract) and mucus (*d*, 200 μg, 1/5 of the extract). The tissues and mucus (~ 7 ml) were obtained from the same specimen. The digestive gland and cerata might be contaminated by traces of mantle tissue or mucus, respectively. This experiment was repeated three times. The amount of each metabolite varied, from specimen to specimen, from 0.02 to 0.3 mg, depending on the tissue and type of compound, but was always distributed as shown. (The various cyercenes are labeled on the diagram: *A*, *B*, 1–5, as in fig. 2.)

Cyerce cristallina



1 Cyercene-A

2 Cyercene-B

3 Cyercene-1 R = CH_3
4 Cyercene-2 R = CH_2CH_3
5 Cyercene-3 R = $CH(CH_3)_2$

6 Cyercene-4 R = CH_3
7 Cyercene-5 R = CH_2CH_3

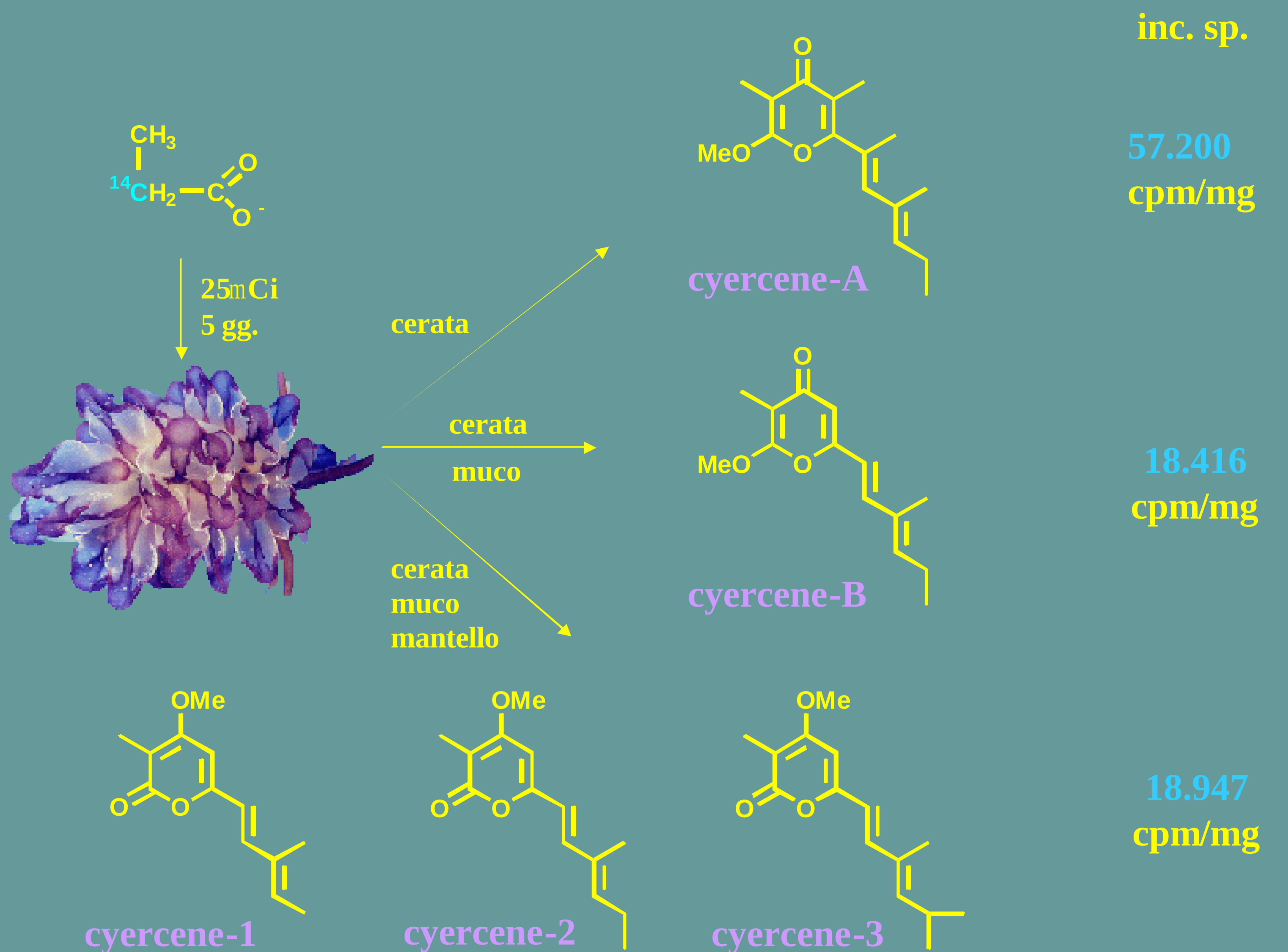
Table 3. Regenerative activity of some of the pyrones isolated from *Cyerce cristallina*, *Ercolania funerea* and *Placida dendritica* on *H. vulgaris* tentacles. Data expressed as average tentacle number (ATN) ± SEM of at least 30 hydra in three separate experiments. A regeneration index, calculated as maximal % of enhancement of control ATN divided by the dose exerting this effect, also shown for those compounds exhibiting statistically significant action. Only the dose showing the maximum effect is reported; higher doses were sometimes toxic to hydra

Compound (source)	Dose ($\mu g\ ml^{-1}$)	ATN ± SE	Significance ($P < 0.05$)	Regeneration index (% enhancement/dose)
Control	–	4.7 ± 0.1	–	–
Cyercene-A (*C. cristallina*)	15	7.9 ± 0.5	Yes	4.5
Cyercene-B (*C. cristallina*, *E. funerea*)	40	5.6 ± 0.5	No	–
Cyercene-2 (*C. cristallina*)	75	7.3 ± 0.6	Yes	0.7
Cyercene-3 (*C. cristallina*)	75	8.0 ± 0.7	Yes	0.9
Cyercene-4 (*C. cristallina*)	100	6.2 ± 0.5	No	–
7-methyl-cyercene-1 (*E. funerea*)	20	6.8 ± 0.4	Yes	2.2
7-methyl-12-norcyercene-B (*E. funerea*)	50	8.1 ± 0.2	Yes	1.4
7-methyl-cyercene-B (*E. funerea*)	70	6.6 ± 0.2	Yes	0.6
7-methyl-cyercene-2 (*E. funerea*)	100	6.0 ± 0.3	No	–
Isoplacidene-B (*P. dendritica*)	70	6.4 ± 0.3	Yes	0.5
Isoplacidene-A (*P. dendritica*)	10	5.7 ± 0.4	No	–
Placidene-B (*P. dendritica*)	50	4.7 ± 0.4	No	–
Placidene-A (*P. dendritica*)	50	5.1 ± 0.4	No	–

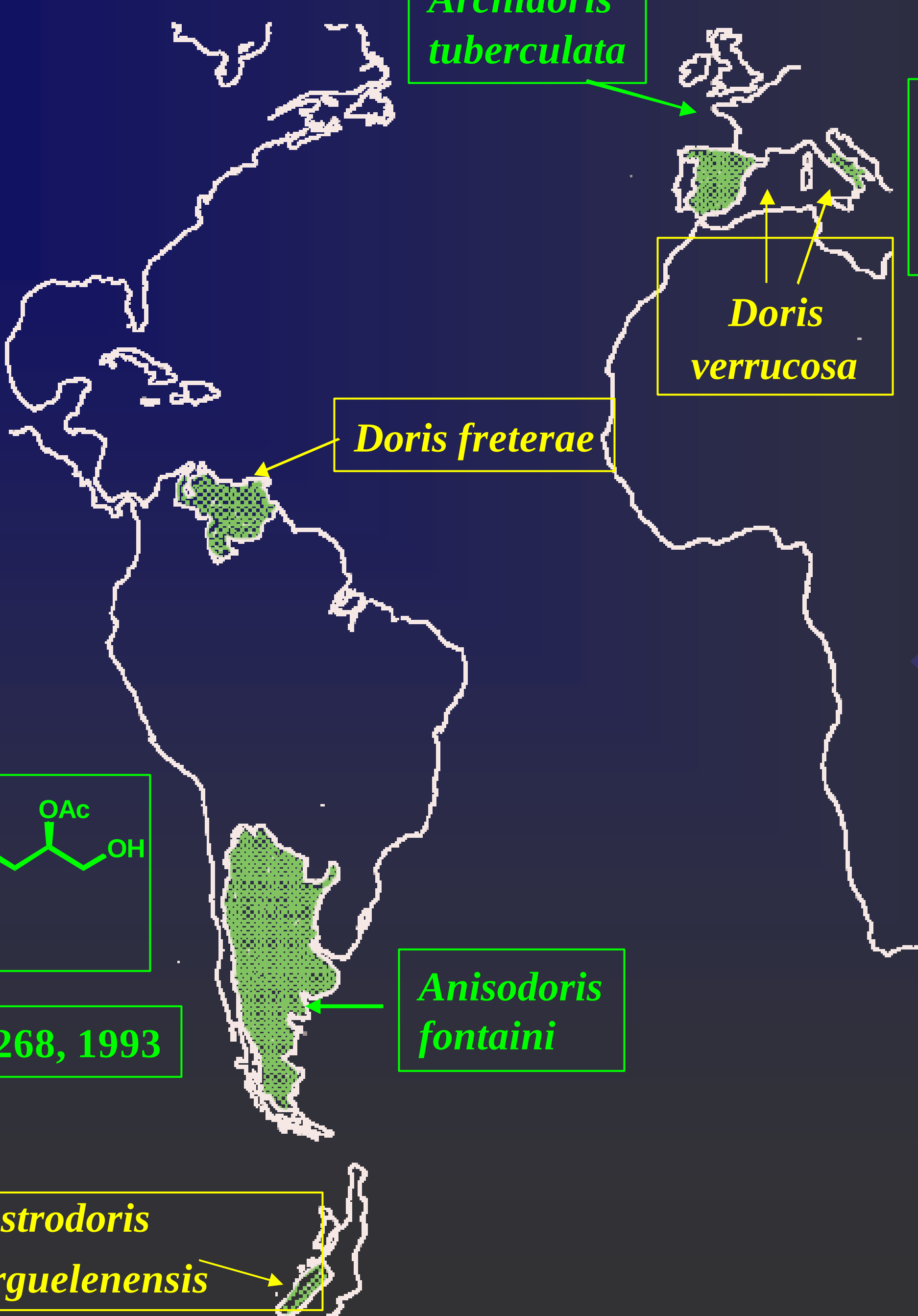
Table 4. Ichthyotoxic activity of the most abundant components of the supposedly toxic slime secreted by *Cyerce cristallina*, *Ercolania funerea*, *Caliphylla mediterranea* and *Placida dentritica*, and of the ethereal extracts from the slimes themselves on *Gambusia affinis*. Data, representative of three separate experiments, expressed in μg ml^{-1} and the toxicity ranking used (toxic, very toxic) was according to Coll et al. (1982). Only the compounds/extracts possessing activity are reported

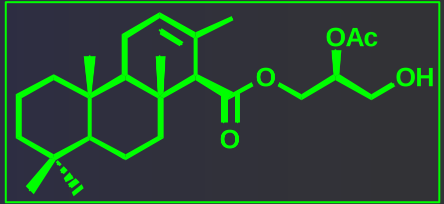
Compound or extract (Source)	Dose ($\mu g\ ml^{-1}$ water)	Toxic	Very toxic
Slime extract (*C. cristallina*)	10		Yes
Cyercene-A (*C. cristallina*)	10		Yes
Cyercene-B (*C. cristallina*, *E. funerea*)	10	Yes	No
Cyercene-2 (*C. cristallina*)	10	Yes	No
Cyercene-3 (*C. cristallina*)	10		Yes
Cyercene-4 (*C. cristallina*)	5 10	Yes	No Yes
Slime extract (*E. funerea*)	70		Yes
7-methyl-12 norcyercene-B (*E. funerea*)	20 30	Yes	No Yes
7-methyl-cyercene-B (*E. funerea*)	16	Yes	No
Slime extract (*P. dendritica*)	10		Yes
Placidene-A (*P. dendritica*)	10 20	Yes	No Yes
Placidene-B (*P. dendritica*)	10		Yes

Biosynthesis in *Cyerce cristallina*

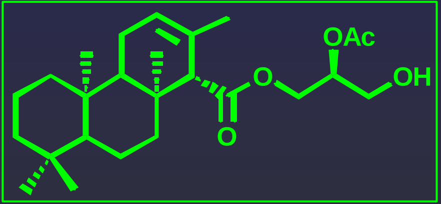


Experientia **47**, 1221, 1991





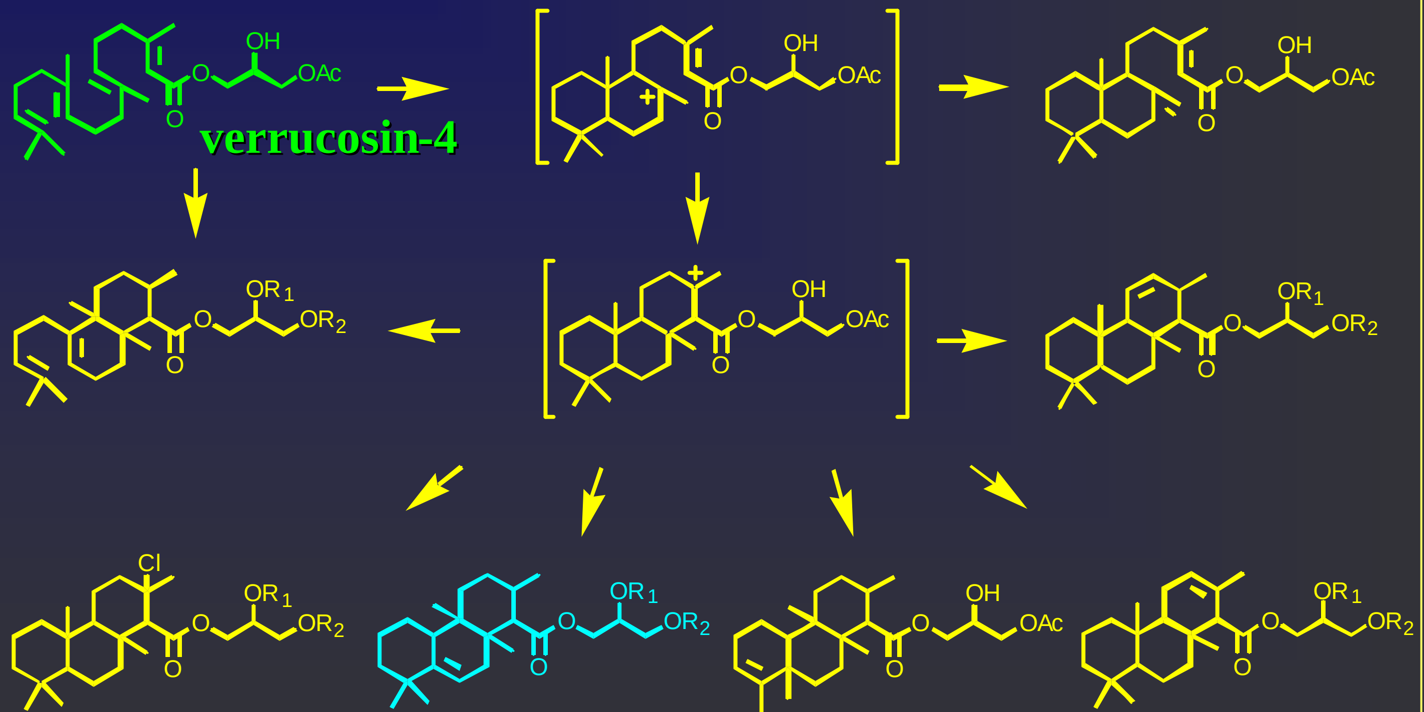
C_{20} diacylglycerols



Experientia 49, 268, 1993

Doris verrucosa

ichthyo-toxic diterpenoyl glycerides



verrucosin A R_1= H, R_2= Ac
verrucosin B R_1= Ac, R_2= H

R_1 = OH, R_2 = Ac
or *vice versa*

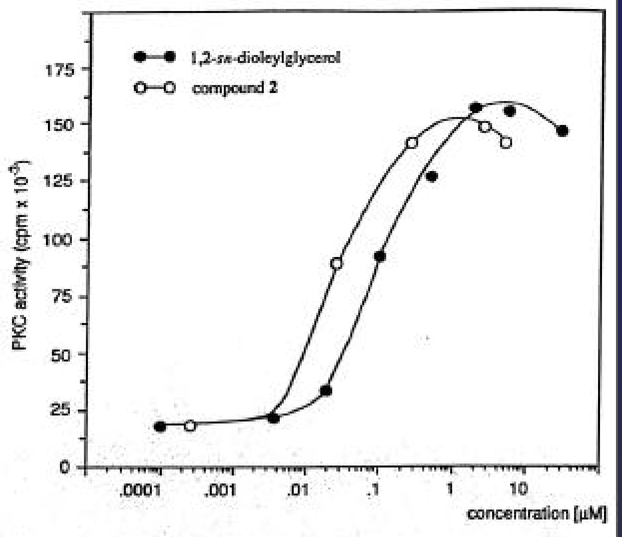


Figure 1. Dose-response curve for the effect of 1,2-*sn*-dioleoyl-glycerol and compound **2** on Ca^{2+}/PS-induced rat brain PKC activation. The effect was measured as radioactivity (^{32}P) incorporated into histone H1.

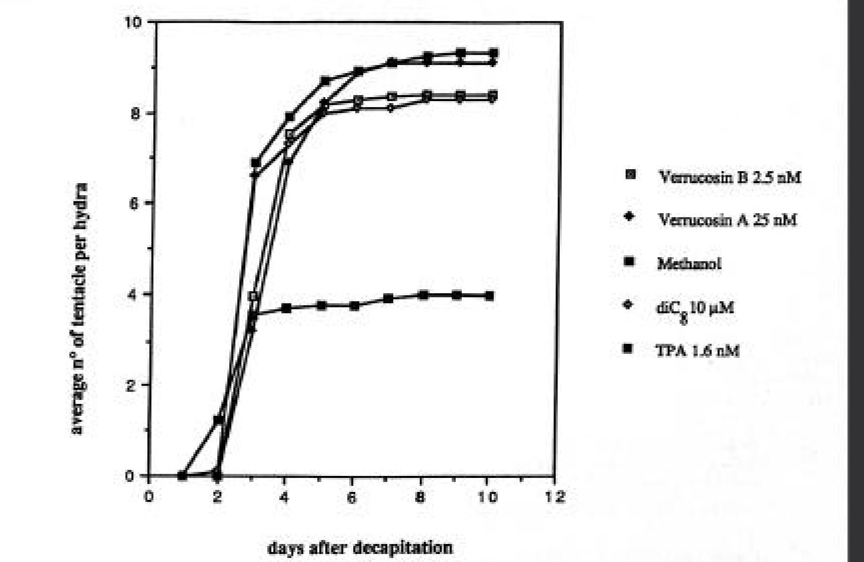


Fig. 4. Time–response curve for the effect of various substances on *Hydra vulgaris* average tentacle number. The concentrations used are shown and were those necessary to obtain a maximal effect on tentacle regeneration.

Those of us who have had the privilege of pursuing problems involving natural products chemistry and chemical communication have enjoyed the endeavor immensely. It is hard to imagine that future generations of chemiphiles will not be at least equally intrigued by the opportunities that the explorations of future biophiles are sure to provide. If one were to ask whether chemistry right now is anywhere near reaching its ultimate goal with respect to providing a full molecular understanding of chemical communication, the answer must be a resounding NO! Future opportunities far outweigh present accomplishments, which are best viewed as a promising start. So, we are not there yet, but we are certainly on our way!