Natural Products Chemistry of Marine Organisms

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Emil Fisher
Premio Nobel 1902

GLUCOSIO

Epimers — diastereomers that differ only at one stereocenter.
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® (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.
DNA $\iff$ RNA $\iff$ Protein

Transcription

Translation
DNA → RNA → Protein

Transcription → Translation

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

DNA (storage)

Transcription

Translation

Proteins

Environment

Biochemical Circuitry

Phenotypes (Traits)

Gene Expression

Proteomics

Metabolomics
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  

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- a) mollusc with shell
- b) mollusc without shell
- c) opisthobranch

**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*

7-deacetoxy-olepupuane

polygodial

olepupuane

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

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<tr>
<td>1° giorno</td>
<td>% 100</td>
<td>attacca</td>
<td>rifiuta</td>
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<td>12° giorno</td>
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*T. pavo* remembers the external features of potentially dangerous preys
Dietary origin of bioactive molecules

**Hypselodoris webbi** (nudibranch) in Mar Menor, SPAIN

**Dysidea fragilis** (spugna) in Alicante, SPAIN

<table>
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<tr>
<th>gg.</th>
<th>(-)-furodysinin</th>
<th>longifolin</th>
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<tr>
<td>0</td>
<td>0 (pura longifolin)</td>
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<tr>
<td>7</td>
<td>3,8</td>
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<tr>
<td>35</td>
<td>13,0</td>
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*Experientia* 50, 510, 1994
Bio-transformation of dietary metabolites

Experientia 49, 482, 1993
Biosynthesis in *Tethys fimbria*
Mucus, mantle, cerata

PGE-lattone

PGF-lattone

cerata

PGF-lattone

ovotestis, eggs

defense

muscle contraction
Biosynthesis
the way in which a natural product is produced by in a living organism
Biosynthetic Capacity and Chemical Markers

order

Cephalaspidea

Sacoglossa

Nudibranchia

chemical class

\(\text{\(\omega\)-aryl-methylchetonnes}\)

polypropionates

acetogenins

terpenes

chemicals

navenone-B

elysione

PGE\(_2\)-1,15-lactone

luteone
Biosynthetic Capacity and Evolutionary Scenario

- P. dendritica
- C. mediterranea
- E. funerea
- C. cristallina
- polypropionates

- C. mediterranea
- E. funerea

- C. cristallina
- polypropionates

- E. timida
- T. hopei

- E. viridis
- B. mimetica

- E. translucens

- O. olivacea

- L. serradifalci

- A. fragilis

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 debromoaplysia toxin; 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

• 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

- *Navanax inermis*
- *Haminoea cymbalum*
- *Bulla striata*
- *Placobranchus ocellatus*
- *Elysia viridis*
- *Cyerce cristallina*
- *Ercolania funerea*
- *Oxynoe viridis*
Biotransformation of Caulerpenyne?

Oxy noe olivacea

Caulerpa prolifera

mucus

Oxytoxin-1

Oxytoxin-2

Caulerpenyne

10 ppm

caulerpenyne

1 ppm

oxytoxin-1

0.1 ppm

oxytoxin-2

toxicity to G. affinis

Experientia 46, 767, 1990
Could the Direct Observations be Misleading?

- selective bio-accumulation of minor dietary metabolites

- enzymatic structural modifications in the injured tissues of the prey
Addition of Caulerpenyne to *Oxy noe* homogenate
Wound-activated Hydrolysis in *Caulerpa prolifera*

After grinding of the alga

- OXY-1
- OXY-2
- POXY-2
- CYN

15''

15'

15
Selective Lipases are Active in *Oxynoe olivacea*

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*

*Bulla striata*

*Scaphander lignarius*
Assembly of haminol on *Haminoea*’s modular enzyme
Biosynthesis in *Bulla striata*

![Image showing biosynthesis process](image)

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

Metabolites from:

**Mediterranean Pleurobranchoidea**

- *Pleurobranchus membranaceous*
  - Tetrah. Lett. 34, 6791, 1993
  - Membranone-C

- *Pleurobranchaea meckelii*
  - Tetrahedron 36, 8673, 1995

- *Pleurobranchus testudinarius*
  - Testudinarioi-A

**Intertidal Molluscs**

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

- *Trimusculus reticulatus*
  - Rice
  - Limatulone

- *Lottia limatula* (limpet)
  - Albizati et al.
  - J. Org. Chem. 50, 3428, 1985
Biosynthetic studies of Nudibranchs

Order:
Nudibranchia

Suborder:

Superfamily:
Eudoridoidea

Family:
Dorididae Chromodorididae Dendro dorididae

- Sclerodoris tanya
- Diaulula sandigensis
- Doris verrucosa
- Archidoris montereyensis
- Archidoris odhneri
- Austrodoris keguelenensis
- Cadlina luteomarginata
- Dendrodoris limbata
- Dendrodoris grandiflora
- Dendrodoris arborescens
- Doriopsilla areolata

Anadoridoidea

Onchidorididae

- Triopha catalinae
- Acanthodoris nanaimoensis

Tethydidae

- Tethys fimbria
- Melibe leonina
**Doriopsilla areolata**

biosynthetizes typical sponge sesquiterpenoids

with opposite A/B ring junction

\[
\text{AcylO} \xrightarrow{\text{drimane esters}} \xrightarrow{\text{de novo biosynthesis}} \text{CO}_2^- \xrightarrow{\text{mevalonate}} (-\text{-pallescensin-A})
\]

- 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 57: 8913, 2001
J Org Chem 68, 2405, 2003
Archidoris odhneri
A. montereyensis

Doris freterae

Doris fontaini

Austrodoris kerguelenensis

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

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

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

Experiment with Antarctic *Austrodonis kerguelenensis*

[6-\(^{13}\text{C}\)]-glucose

Unpublished results

45 mg/specimen

labelling position to determine

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 a 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 benthic and planktonic.