- THE CHEMISTRY of Lichens -

... emphasizing unique features

Lecture for PLB 400
- Lichenology-

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Arizona State University
A bit of history...

- first isolation of lichen substances:
  - **Bebert** (1831) *Vulpinic Acid*
  - **Alms** (1832) *Picrolichenic Acid*
  - **Knop** (1844) *Usnic Acid*
- classical period (early 1900’s):
  - **Zopf** (1907): *Die Flechtenstoffe in chemischer, botanischer pharmakologischer und technischer Beziehung*
  - **Hesse** (1912): *Flechtenstoffe in Biochemisches Handlexikon*
- **Asahina** (1954): *Chemistry of Lichen Substances*
- Chicita F. **Culberson** & William L. **Culberson** (1970’s)
- John A. **Elix**
Lichen Substances

- ca. 600 - 700 secondary metabolites currently known
- only 60 - 80 also occur in other organisms like vascular plants
- extracellular
- often crystalline on lichen hyphae
- usually complex organic acids
- many phenolic substances
- large amounts: usually 1-5% (rarely up to 36% of the dry weight)!
Secondary Metabolism

modified from:
Nasht et al. (eds.) (2002): Lichen Flora of the Greater Sonoran Desert Region
**Major Pathways**

- Shikimic Acid Pathway
- Mevalonic Acid Pathway
- Acetyl Polymalonyl Pathway
Major Pathways - Overview

from:
NASH et al. (eds.)
Shikimic Acid Pathway

- rather **small group** of lichen secondary metabolites
- **often unique to lichens**
- derived from **pentose phosphate cycle** and **amino-biosynthesis**
- i.e. pulvinic acid derivates,
  e.g. vulpinic acid, rhizocarpic acid (bright yellow pigments, K-)

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Shikimic Acid Pathway
Shikimic Acid Pathway

bright yellow color because of rhizocarpic acid in Acarospora subg. Xanthothallia

modified from:
NASH et al. (eds.) (2002): Lichen Flora of the Greater Sonoran Desert Region

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Shikimic Acid Pathway

Pulvinic Acid Derivatives:
- PULVINIC DILACTONE
- CALYCIN

Terphenylquinones:
- POLYPORIC ACID
- THELEPHORIC ACID

Amino Acid Derivative:
- SCABROSIN 4,4'-DIACETATE

egg-yolk color caused by Calycin in *Candelariella rosulans*


Photo © F. Bungartz 2002
Mevalonic Acid Pathway

- a large group of secondary metabolites
- **usually not restricted to lichens** but also common in other organisms
- derived from **Acetyl CoA**
- i.e. terpenes, carotenoids and steroids
  
e.g. Zeorin (= hopane-6a, 22-diol)
Mevalonic Acid Pathway

modified from:
NASH et al. (eds.) (2002):
Lichen Flora of the Greater Sonoran Desert Region
Mevalonic Acid Pathway

the colorless substance zeorin occurs in the medulla of Heterodermia rugulosa,
Photos © F. Bungartz 2002
Acetyl Polymalonyl Pathway

- the largest group of lichen secondary metabolites
- usually unique to lichens, but a few also found in other organisms
- derived from:
  - polymalonyl pathway without pre-cursors,
    e.g. usnic acids, anthraquinones, xanthones & chromones
  or
  - via orsellinic acid as a pre-cursor
    i.e. depsides (e.g. atranorin), depsidones (e.g. norstictic acid),
    dibenzofuranes (e.g. pannaric acid) ...
Acetyl Polymalonyl Pathway

modified from:
NASH et al. (eds.) (2002):
*Lichen Flora of the Greater Sonoran Desert Region*
Acetyl-Polymalonyl Pathway

β-orsellinic acid
orsellinic acid & homologues

para-depsides
(e.g. atranorin K+ deep yellow)

meta-depsides
(C-red if two free -OH groups in meta position)

depsides

benzyl esters
diphenyl esters
diphenyl esters
diphenyl esters
depsidones
(e.g. norstictic acid K+ orange red crystals)
dibenzofurans

depsidones

Atranorin as colorless sunscreen pigment in Heterodermia ciliatomarginata,
Photo © F. Bungartz 2002

modified from:
NASH et al. (eds.) (2002):
Lichen Flora of the Greater Sonoran Desert Region
**Acetyl Polymalonyl Pathway**

Yellow lime-green color of usnic acid in *Usnea arizonica*

*modified from: Nasit et al. (eds.) (2002): Lichen Flora of the Greater Sonoran Desert Region*

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**ACETYL-POLYMALONYL PATHWAY**

- **anthraquinones** (K+ purple)
- **xanthones, chromones** (C+ red if two free -OH groups in meta position)
Acetyl Polymalonyl Pathway

modified from:
Nash et al. (eds.) (2002): Lichen Flora of the Greater Sonoran Desert Region

anthraquinones (K+ purple)
xanthones, chromones (C+ red if two free -OH groups in meta position)

usnic acids

orange red anthraquinones in Caloplaca ignea
Photo © F. Bungartz 2002
Chemotaxonomy

from

CULBERSON, W.L.
A phylogenetic view of chemical evolution in lichens.
The Bryologist 73(1): 1-31
Chemotypic variation in lichens

• replacement substances:
  one substance replaced by a closely related substance

• chemosyndromes:
  several substances regularly occur together, e.g.
  stictic acid complex

• accessories:
  substances may or may not occur, of little taxonomic value
Sibling Species Concept *(sensu CULBERSON)*

- **Cryptic Species:**
  ... species with identical morphology, but nevertheless genetically isolated & distinct, e.g.:
  - Zoology: Similar birds with different mating songs being reproductively isolated

- **CULBERSON & CULBERSON:** Chemical diversity as evidence for ”Sibling Species” in Lichens, e.g.:

  *Ramalina* & *Cladonia*
Sibling species of Ramalina siliquosa agg.

- Ecological variation along the shore of Anglesy, Wales, Great Britain

from Culberston, W.L., Culberston, C.F. & Johnson A. (1993);
Speciation of lichens of the Ramalina siliquosa complex...
Am. J. Bot. 80(12): 1472-1481
### Challenging the Sibling Species Concept

**Substances**
- protocetraric
- hypocetraric
- salazinic
- norstictic
- no stictic acid

**Ecology**
- sheltered
- exposed

**Taxonomy:**

<table>
<thead>
<tr>
<th>Author</th>
<th>Substances</th>
<th>Ecology</th>
<th>R. siliquosa s. l.</th>
<th>R. siliquosa s. str.</th>
<th>R. cuspidata s. str.</th>
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</thead>
<tbody>
<tr>
<td>Fries (1831)</td>
<td>protocetraric</td>
<td>exposed</td>
<td><strong>R. siliquosa</strong></td>
<td><strong>R. siliquosa</strong></td>
<td><strong>R. cuspidata</strong></td>
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<tr>
<td>Sheard (1978)</td>
<td>hypocetraric</td>
<td>sheltered</td>
<td></td>
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<td></td>
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<tr>
<td>Culberson (1967)</td>
<td>salazinic</td>
<td>sheltered</td>
<td><strong>R. siliquosa</strong> s.str.</td>
<td><strong>R. druidarum</strong></td>
<td><strong>R. crassa</strong></td>
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<tr>
<td>Culberson et al. (1993)</td>
<td>norstictic</td>
<td>exposed</td>
<td></td>
<td><strong>R. stenoclada</strong></td>
<td><strong>R. atlantica</strong></td>
</tr>
</tbody>
</table>

- not enough data
- hybridization ?

- **R. cernoscula**
- **R. cernoscula**
Sibling Species of *Cladonia chlorophaea* agg.

- Chemical variation within a population of *Cladonia chlorophaea* agg.

*Cladonia pyxidata* (L.) Hoffm.

Photo © F. Bungartz 2002
<table>
<thead>
<tr>
<th></th>
<th>Fumarprotocetraric Acid</th>
<th>Grayanic Acid</th>
<th>Merochloro-phaeic Acid</th>
<th>Cryptochloro-phaeic Acid</th>
<th>4-0-methyl-cryptochloro-phaeic Acid</th>
<th>Per-latolic Acid</th>
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<tbody>
<tr>
<td><strong>C. chlorophaea</strong></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>C. grayi</strong></td>
<td>+/-</td>
<td>+</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>C. merochlorophaea</strong></td>
<td>+</td>
<td>+</td>
<td></td>
<td>trace</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>C. cryptochlorophaea</strong></td>
<td>+/-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>C. perloma</strong></td>
<td>+</td>
<td>trace</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>
Analysis of podetia and sporelings

- frequent hybridization:
  sporelings of *C. grayi* with chemistry of
  *C. merochlorophaea* and vice versa

- frequent hybridization:
  sporelings of *C. chryptochlorophaea* with
  chemistry of *C. perlomera* and vice versa

- hybridization extremely rare:
  *C. chlorophaea* produced only one single sporeling with
  cryptochlorophaeic acid (i.e. the chemotype of *C. cryptochlorophaea*)
possible function of lichen substances

- cortical pigments (for UV-protection) e.g. atranorin, usnic acid, lichexanthone (ivory UV+ yellow), various brown pigments
- allelopathic:
  - protection against herbivores
  - antibiotic properties
- may have some effect on wheathering of rock substrates, as complexing agents (chelates), but largely insoluble under natural conditions...
Analysis of Lichen Substances

- observational: color
- spot tests, UV - fluorescence
- microcrystallization techniques
- thin-layer chromatography (TLC)
- high performance liquid chromatography (HPLC)
- mass spectroscopy (structural analysis)
Color
Spot Tests ...

- **P** (para-phenylenediamine): yellow – orange – red with depsides & depsidones containing aldehyde groups (-CHO)
- **K** (potassium hydroxide KOH): K+ purple with orange anthraquinones, e.g. Caloplaca, Xanthoria, Teloschistes; K- with yellow pulvinic acids, e.g. Candelaria, Candelariella, Candelina, Acarospora, Letharia
- **C** (Ca-hypochlorite): pink with depsides and xanthones with two free hydroxyl groups (-0H)
various color reactions, e.g.:

- **Lichexanthone**: UV+ yellow
- **Squamatic Acid**: UV+ white
- **Barbatic Acid**: UV+ whitish blue
- **Arthothelin**: UV+ orange

etc.
Thin-Layer Chromatography

Photo © F. Bungartz 2002
the use of lichen substances

- colors and dyes
- perfumes
- pharmaceuticals


© Karen Dillman, ASU Graduate Student
Lichenology Class, Spring 2001
And if you didn't like all the chemistry talk ... 

... you may want to try some

*Cetraria islandica*-Schnapps