- THE CHEMISTRY of Lichens -

… emphasizing unique features

Photo © F. Bungartz 2002

Lecture for PLB 400
- Lichenology-

by F. Bungartz
Arizona State University

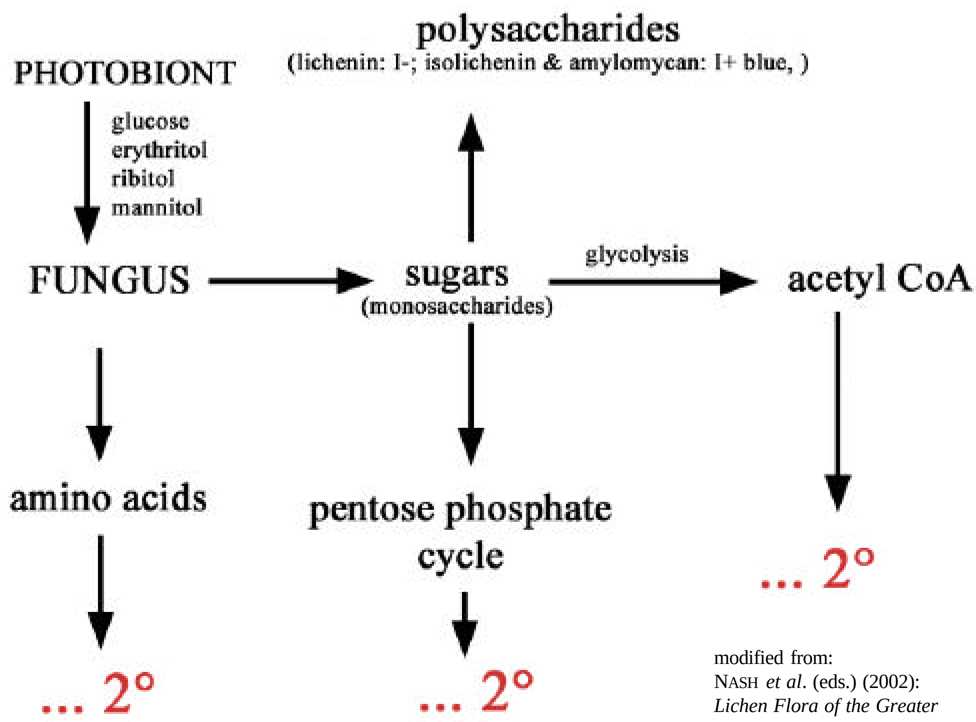
A bit of history...

- 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

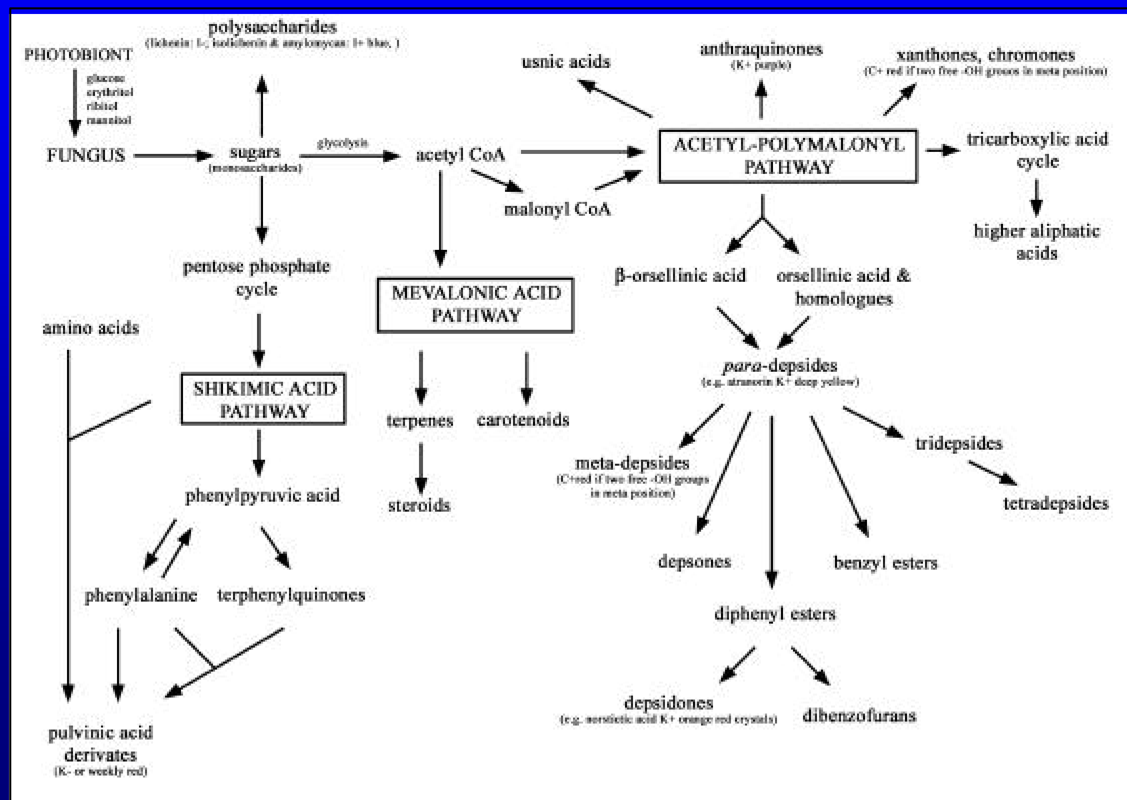


modified from:
NASH *et al*. (eds.) (2002):
Lichen Flora of the Greater Sonoran Desert Region

Major Pathways

- Shikimic Acid Pathway
- Mevalonic Acid Pathway
- Acetyl Polymalonyl Pathway

Major Pathways - Overview

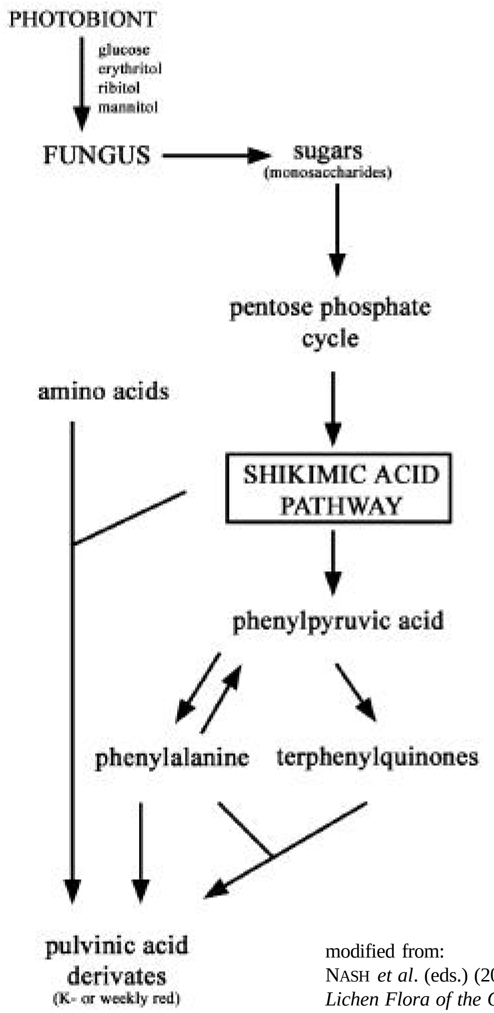


from:
NASH *et al*. (eds.) (2002): *Lichen Flora of the Greater Sonoran Desert Region*

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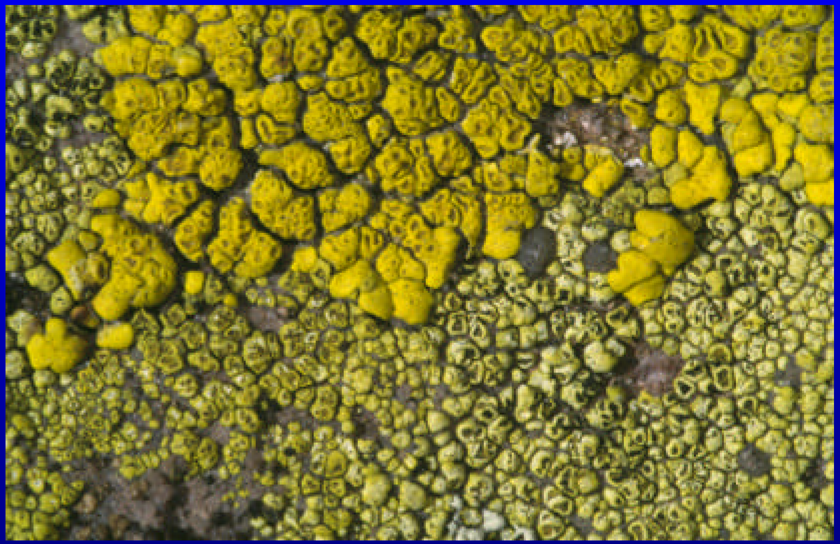
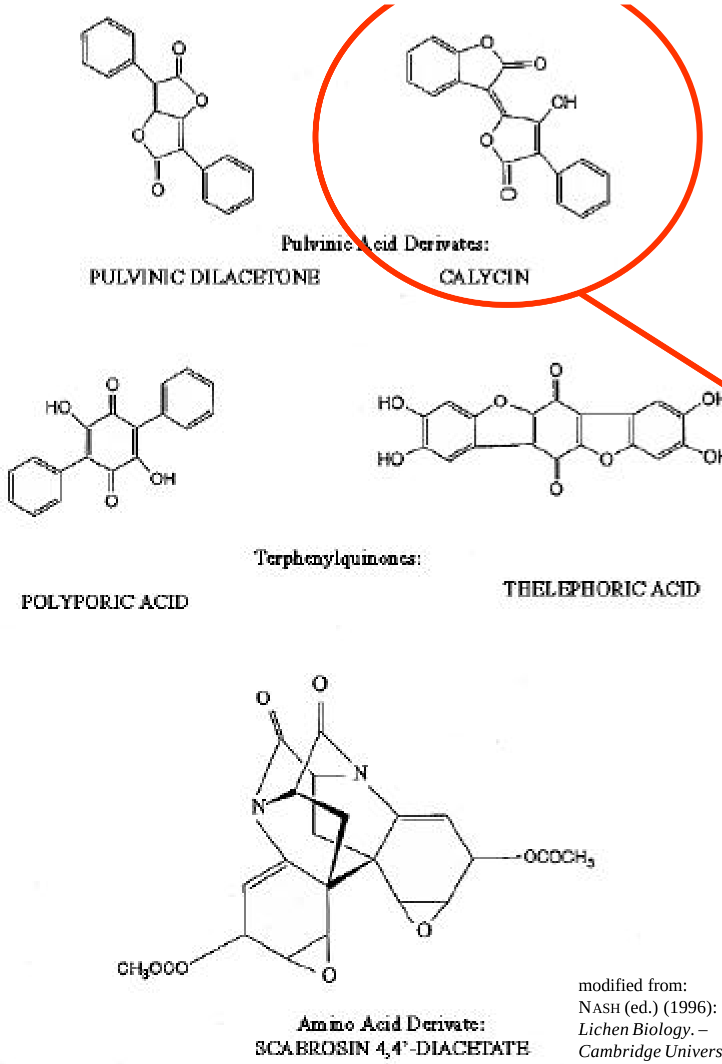
NASH *et al*. (eds.) (2002):
Lichen Flora of the Greater Sonoran Desert Region

Shikimic Acid Pathway

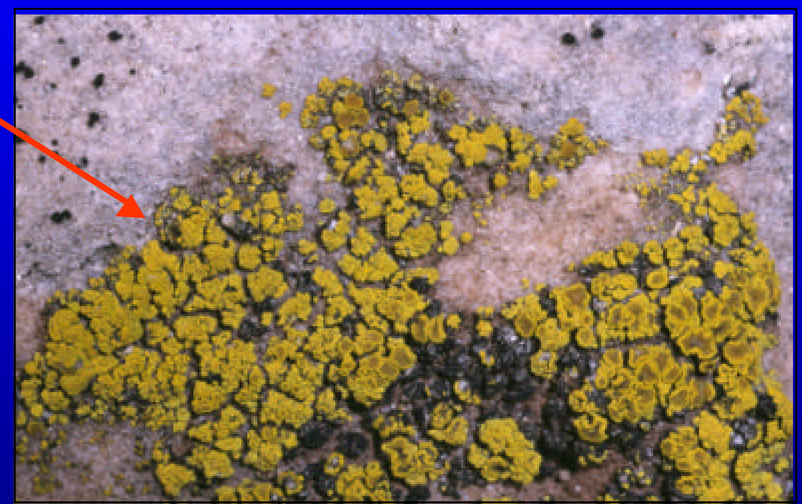


bright yellow color because of rhizocarpic acid in *Acarospora* subg. *Xanthothallia*



modified from:
NASH (ed.) (1996):
Lichen Biology. – *Cambridge University Press*

Shikimic Acid Pathway



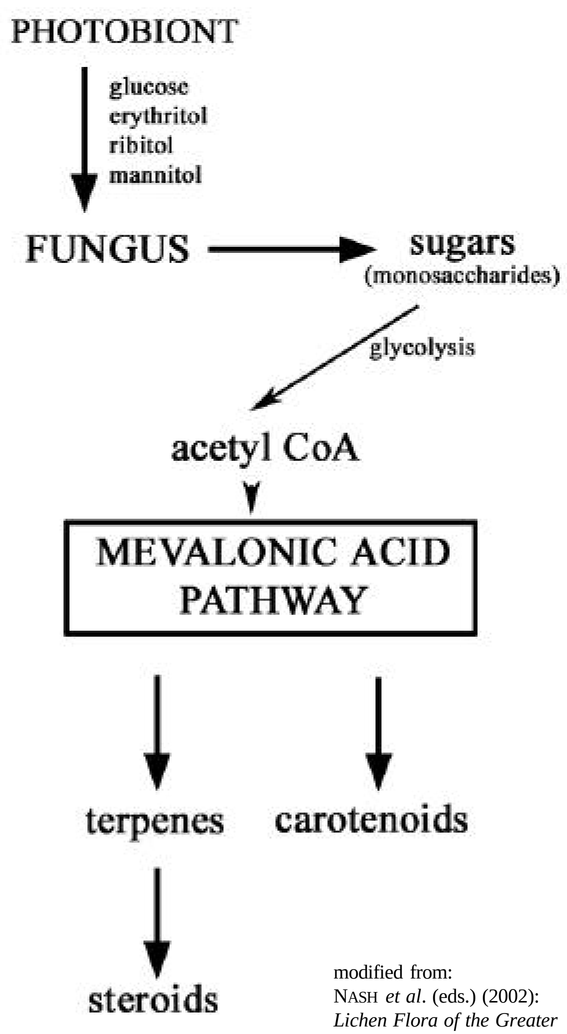
egg-yolk color caused by Calycin
in *Candelariella rosulans*

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*)



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NASH *et al*. (eds.) (2002):
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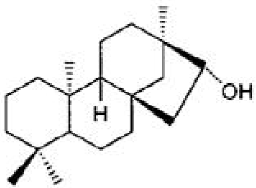
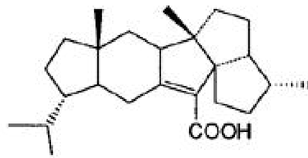
Mevalonic Acid Pathway

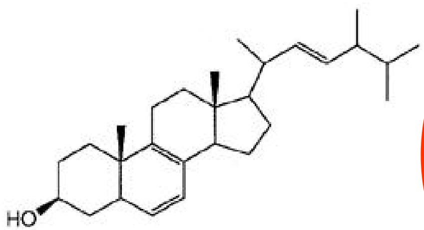
Mevalonic Acid Pathway



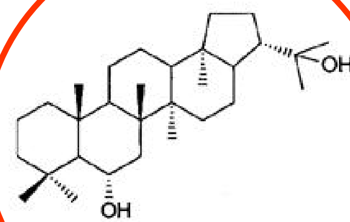
Diterpene:
16α-HYDROXYKAURANE



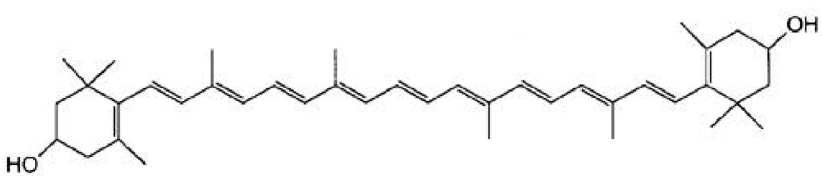
Sesterpene:
RETIGERANIC ACID



Steroid:
ERGOSTEROL

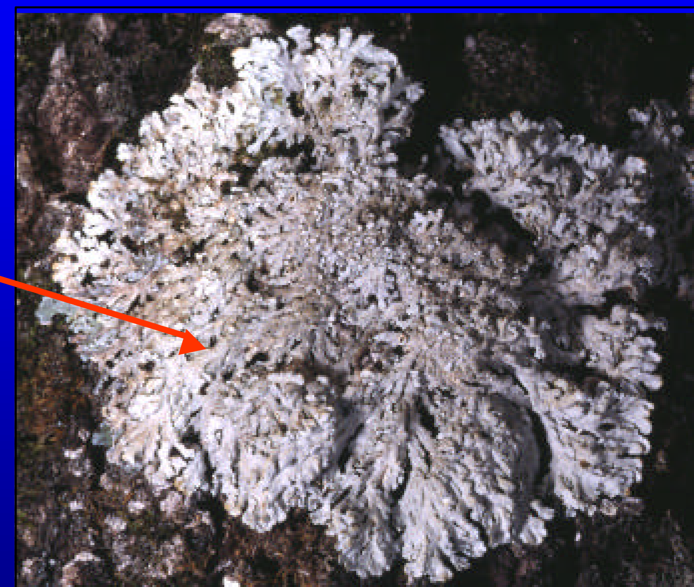


Triterpene:
ZEORIN



Carotenoid:
ZEAXANTHIN

modified from:
NASH (ed.) (1996): *Lichen Biology*. – *Cambridge University Press*



the colorless substance zeorin occurs in the medulla of *Heterodermia rugulosa*,

Photos © F. Bungartz 2002

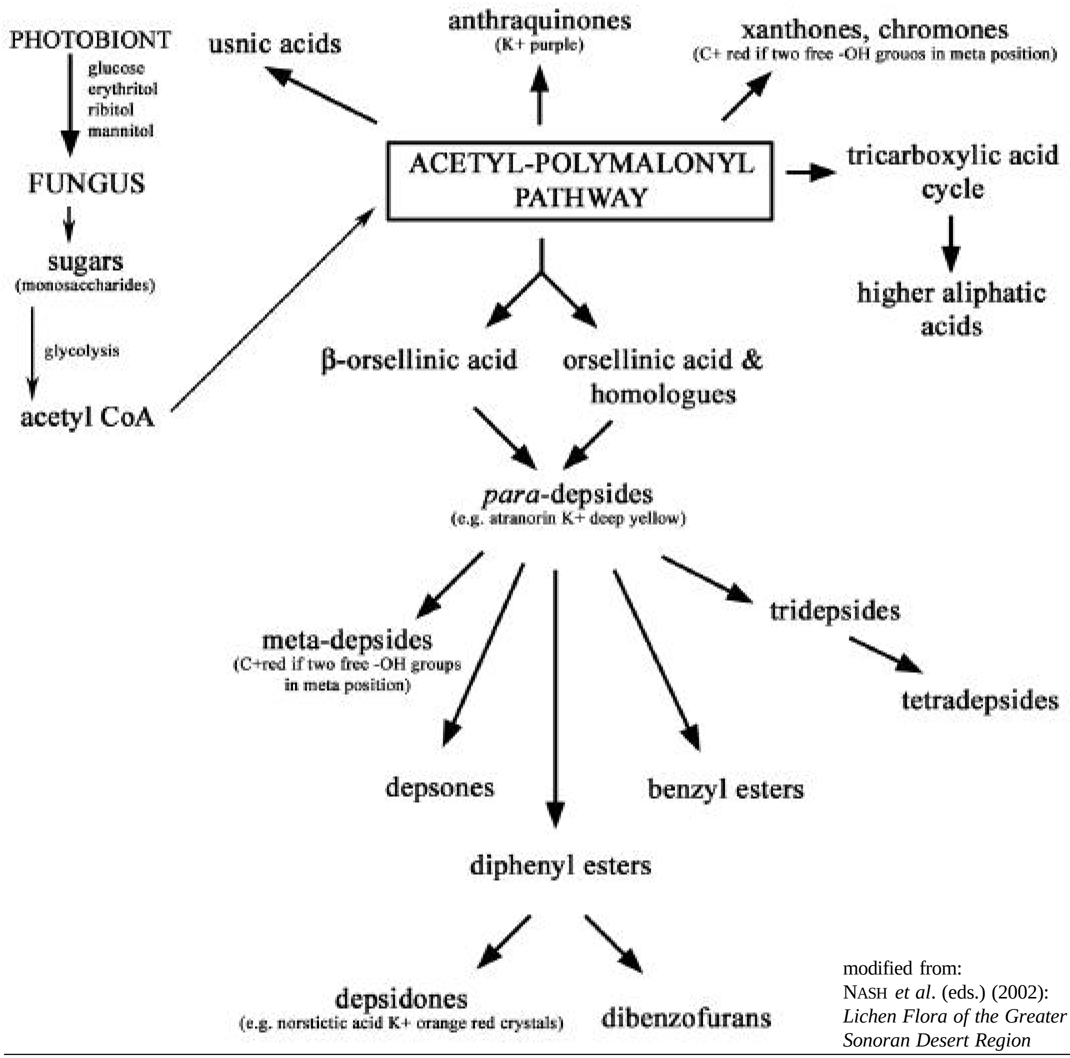
Acetyl Polymalonyl Pathway

- 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



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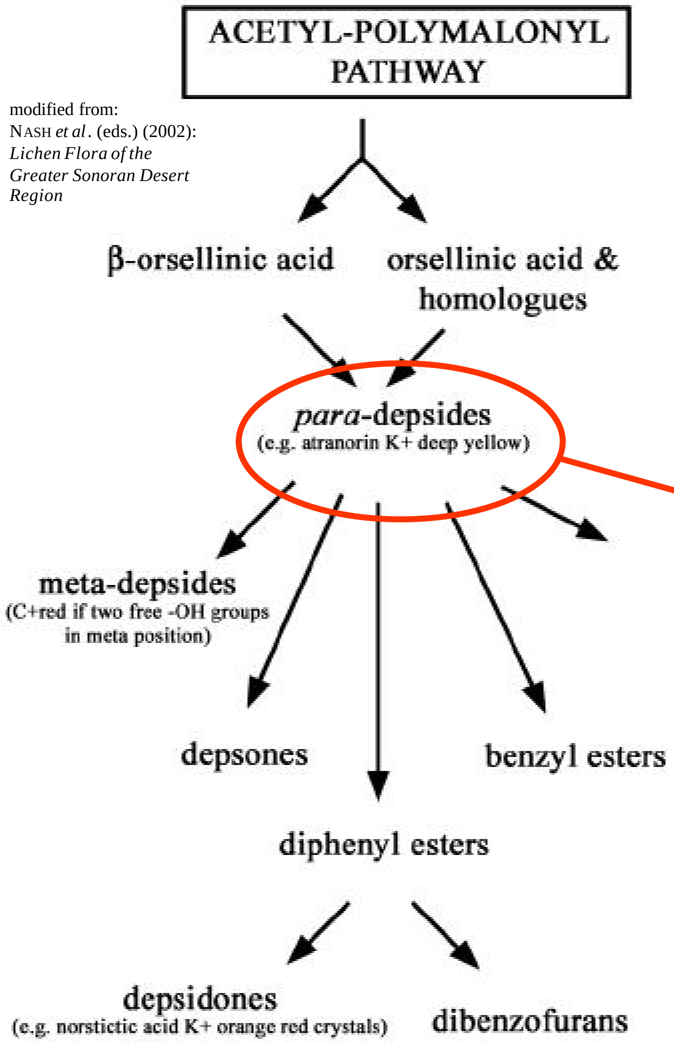
Lichen Flora of the Greater Sonoran Desert Region

Acetyl Polymalonyl Pathway

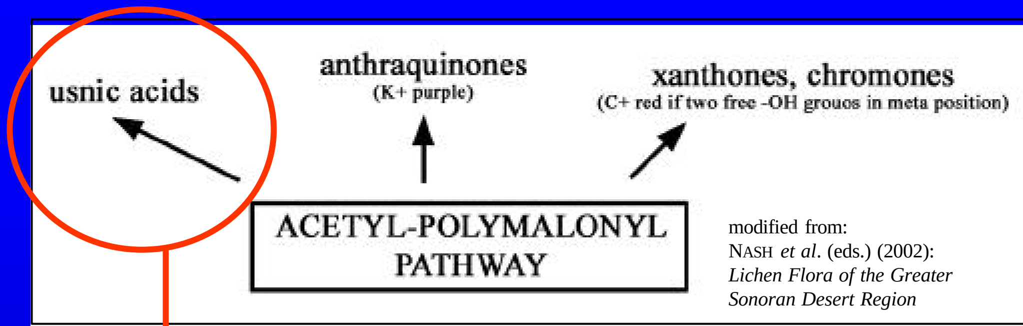


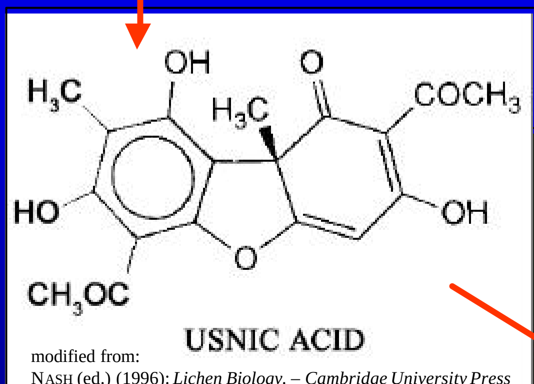
modified from:
NASH *et al*. (eds.) (2002):
Lichen Flora of the Greater Sonoran Desert Region

atranorin as colorless sunscreen pigment in Heterodermia ciliatomarginata,

Photo © F. Bungartz 2002

Acetyl Polymalonyl Pathway





yellow lime-green color of usnic acid in Usnea arizonica

Photo © F. Bungartz 2002

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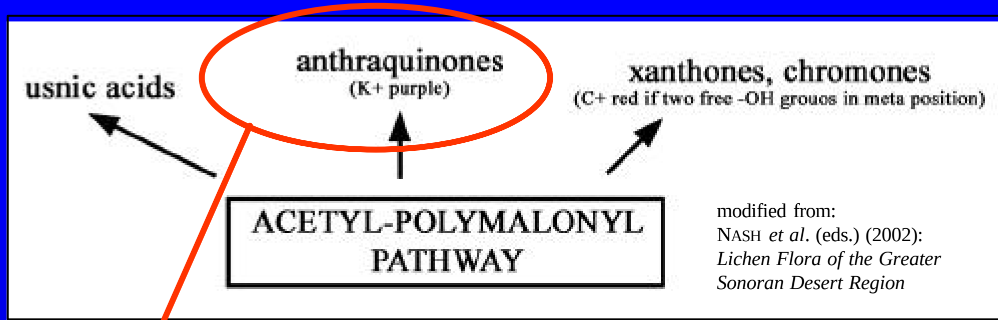
Acetyl Polymalonyl Pathway



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

Chemotaxonomy

from
CULBERSON, W.L.
& CULBERSON, C.F. (1970):
A phylogenetic view of chemical evolution in lichens.
The Bryologist 73(1): 1-31

	Orcinol			β-Orcinol											
	para-depsides, tridepsides	meta-depsides	depsidones	para-depsides	meta-depsides	depsidones	dibenzofurans	usnic acids	fatty acids	anthraquinones	xanthones	chromones	terpenes, etc.	pulvinic acid derivatives	saccharides
Number of compounds	23	9	11	7	4	14	6	4	21	25	16	4	32	9	24
Class Ascomycetes															
Order Lecanorales															
Lichinaceae	—	—	—	—	—	—	—	—	—	—	—	—	—	—	11+
Collemataceae	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—
Pannariaceae	—	—	—	1	—	1	—	—	—	—	—	—	—	—	—
Peltigeraceae	1	—	—	—	—	—	—	—	+	2	—	—	2	—	5
Nephromataceae	—	—	—	—	—	—	—	+	—	6	—	—	2	—	2
Stictaceae	5	1	—	1	—	4	—	+	—	+	—	—	6+	5	4
Graphidaceae	1	—	—	1	—	4	—	—	—	—	1	—	1	—	—
Thelotremataceae	—	—	—	—	—	4	—	—	—	—	1	—	—	—	—
Lecideaceae	6	—	1	3	—	6	1	+	2	5	6	—	1	6	—
Stereocaulaceae	2	—	2	2	1	5	1	+	1	1	—	—	4	—	1
Cladoniaceae	3	6	1	4	3	4	2	+	4+	—	—	—	6+	—	6+
Umbilicariaceae	3	—	—	1	—	2	—	—	1	3	—	—	1	1	11
Diploschistaceae	2	—				1	—	—	—	—		—	—	—	—
Pertusariaceae	2	—	1	1	2	4	—	—	—	—	6	—	—	—	2
Acarosporaceae	1	—		—		1		+	2	—				4	—
Lecanoraceae	5	—	3	3	2	8	1	+	4+	—	12	2	4	2	8
Candelariaceae	—	—	—	—	—	—	—	—				—		3	1
Parmeliaceae	11	1	6	5	1	9	—	+	10+	1	1	—	13	2	14
Anziaceae	2	1	1	2	—	1	—	—	1	—	—	—		—	—
Ramalinaceae	5	5		4	—	6	—	+	1+	—	—	—	1	—	4
Usneaceae	5	—	2	8	3	11	2	+	4+	+		1	4	1	7
Buelliaceae	1		1	2	—	2	—	+	—	—	4	—	1	2	1
Physciaceae	1	2	—	2	—	2	—	—	—	8	1	—	2	—	3
Teloschistaceae	—	—	—	1	—	1	—	—	—	6	—	—	3	2	9
Order Sphaeriales															
Verrucariaceae	—	—	—	—	—	—	—	—	—	—	—	—	1	—	4
Order Caliciales															
Caliciaceae	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—
Cypheliaceae	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—
Sphaerophoraceae	3	—	—	2	—	2	—	+	—	4	—	—	1	1	2
Order Myriangiales															
Arthoniaceae	—	—	—	1	1	1	—	+	—	—	1	—	—	—	—
Order Pleosporales															
Arthopyreniaceae	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—
Pyllopyreniaceae	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
Order Hysteriales															
Opegraphaceae	4	—	—	—	—	1	—	—	—	—	—	—	—	—	—
Roccellaceae	3	—	—	—	—	4	1	—	2+	—	—	1	5	—	7+
Class Fungi Imperfecti															
Lepraria	—	—	—	1	—	1	1	—	1	—	—	1	1	5	2

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.:

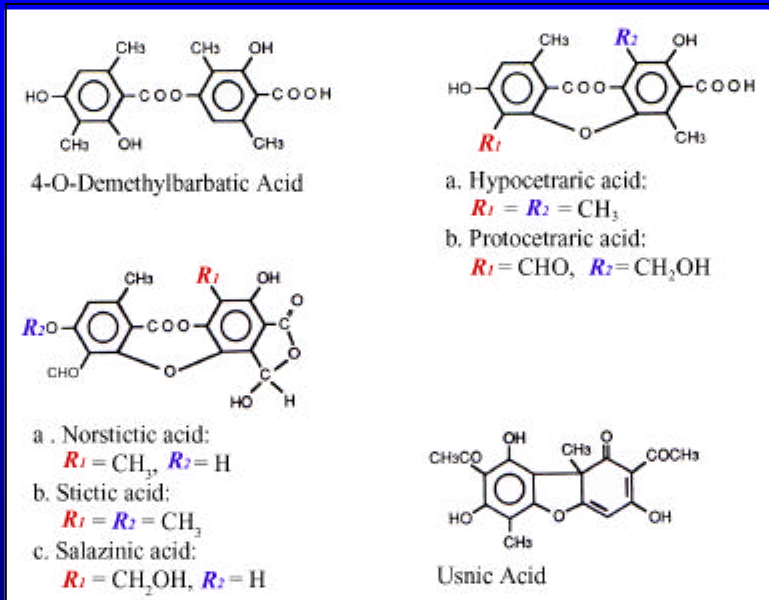
  Zoology: Similar birds with different mating songs being reproductively isolated

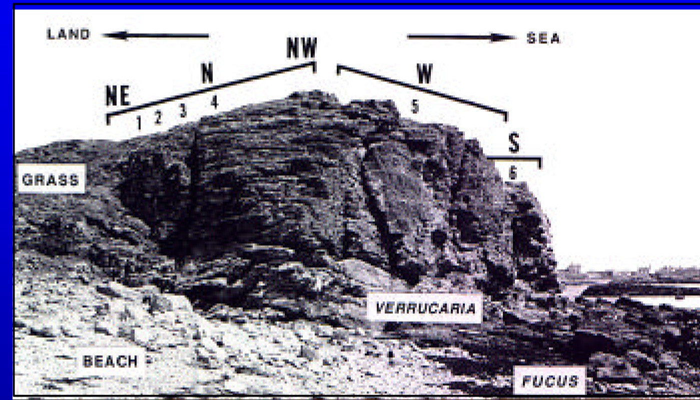
- CULBERSON & CULBERSON: Chemical diversity as evidence for ”Sibling Species” in Lichens, e.g.:

Ramalina & *Cladonia*

Sibling species of *Ramalina siliquosa* agg.

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





from CULBERSON, W.L., CULBERSON, 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 acid	stictic
Ecology	**sheltered** →					**exposed**

Taxonomy:

Fries (1831)	*R. siliquosa* s. l.					
Sheard (1978)	*R. siliquosa* s. str.			*R. cuspidata* s. str.		
Culberson (1967)	*R. siliquosa* s.str.	*R. druidarum*	*R. crassa*	*R. stenoclada*	*R. atlantica*	*R. curnowii*
Culberson et al. (1993)	not enough data	hybridization ?		hybridization ?		

Sibling Species of *Cladonia chlorophaea* agg.

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

Cladonia pyxidata (L.) Hoffm.

	Fumarproto-cetraric Acid	Grayanic Acid	Merochloro-phaeic Acid	Cryptochloro-phaeic Acid	4-0-methyl-cryptochloro-phaeic Acid	Per-latolic Acid
C. chlorophaea	+					
C. grayi	+/-	+				
C. merochlorophaea	+		+	trace	+	
C. cryptochlorophaea	+/-			+		
C. perloma			+	trace	+	+

Analysis of podetia and sporelings

- frequent hybridization:
  sporelings of ***C. grayi*** with chemistry of
  C. merochlorophaea *a*nd 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

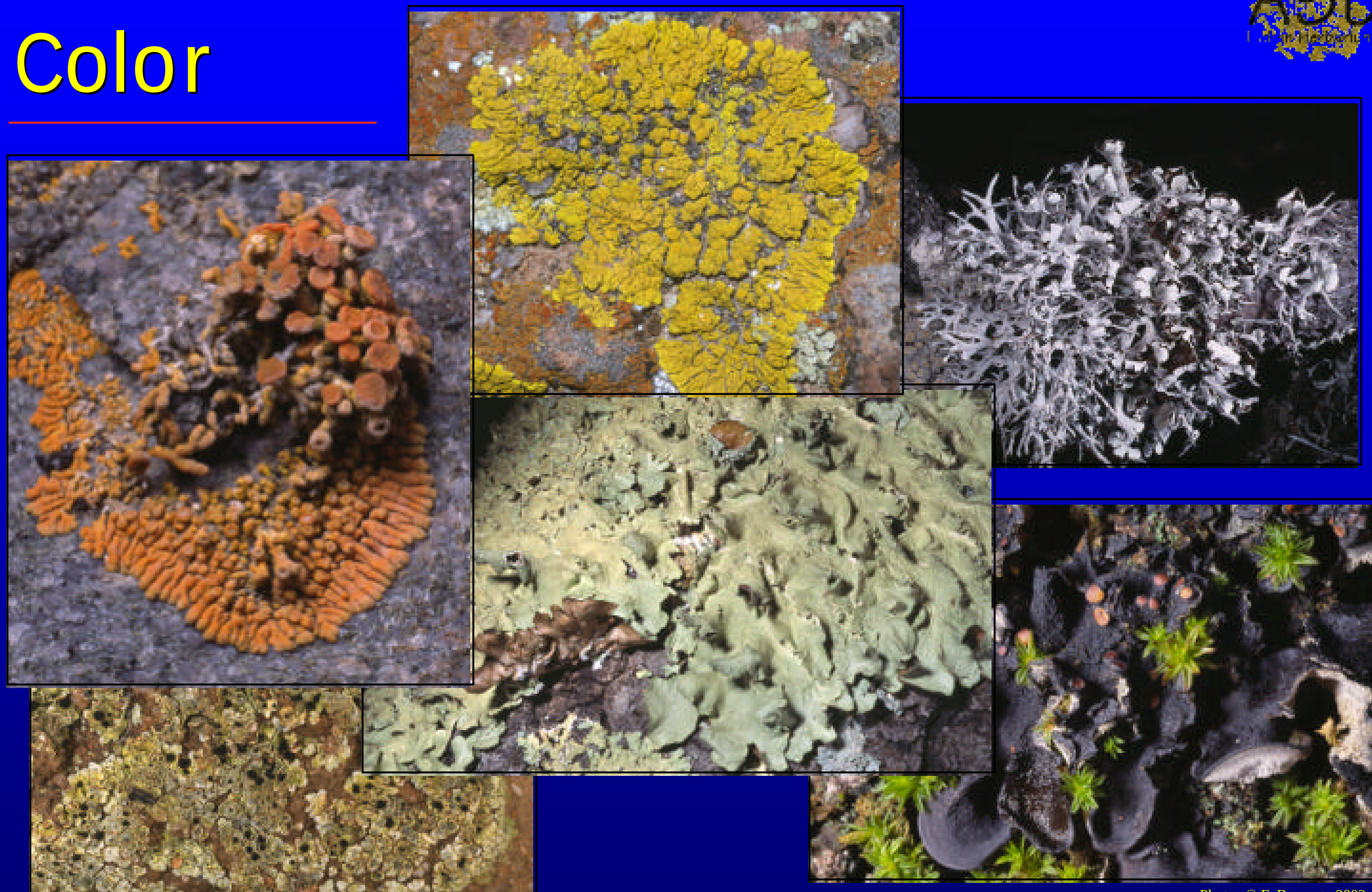
- cortical pigments (for UV-protection) e.g. atranorin, usnic acid, lichexanthone (ivory UV+ yellow), various brown pigments
- allelopathic:
  - protection against herbivores
  - antibiotic properties
- may have some effect on wheathering of rock substrates,

  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



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Spot Tests ...

Photo © F. Bungartz 2002

- P (para-phenylendiamine):

  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)

...

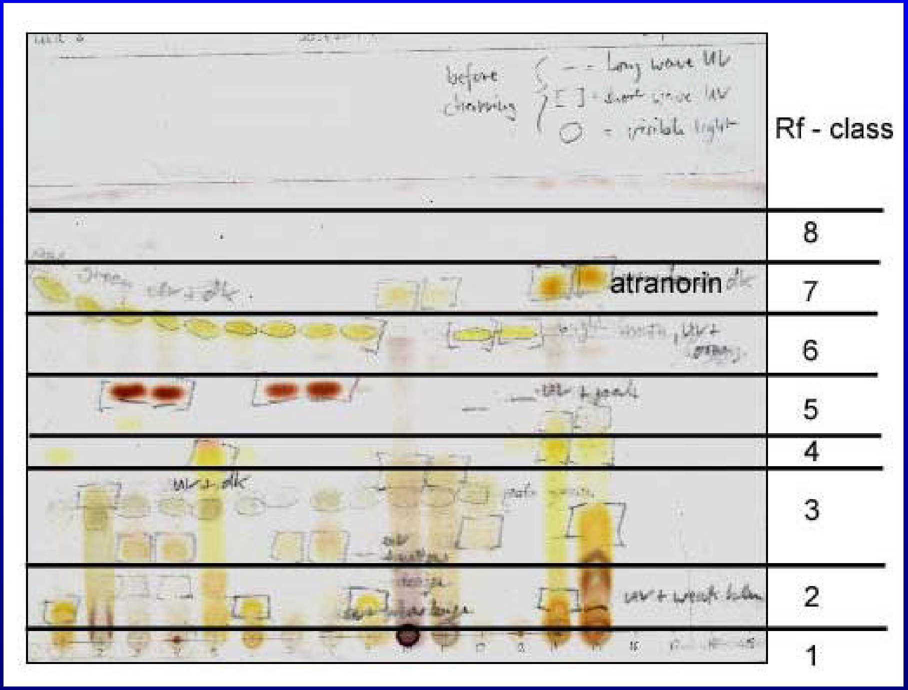
UV-Fluorescence

various color reactions, e.g.:

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

etc.

Thin-Layer Chromatography



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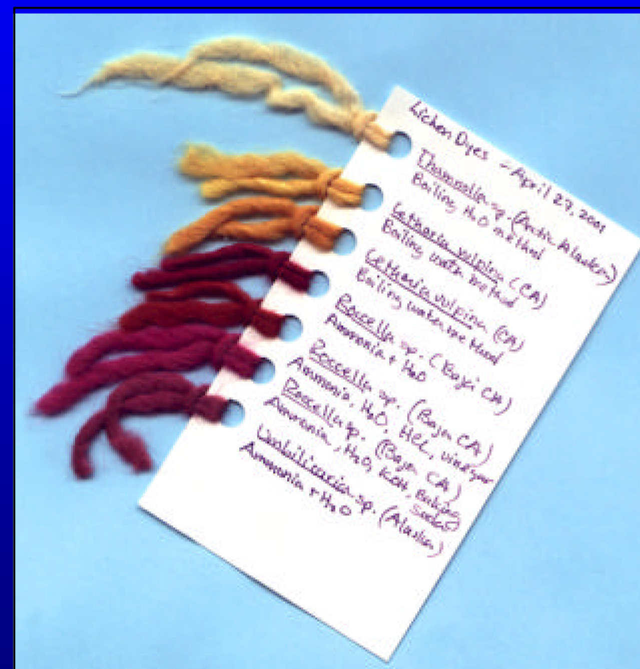
the use of lichen substances

- colors and dyes
- perfumes
- pharmaceuticals

from SCHÖLLER (ed.) (2002): Flechten.
Kleine Senckenberg Reihe 27



© **Karen Dillman**, ASU Graduate Student
Lichenology Class, Spring 2001

And if you didn‘t like all the chemistry talk ...

Photo © F. Bungartz 2002

... you may want to try some *Cetraria islandica*-Schnapps