Secondary compounds of lichens: identification and uses

Introduction…

Lichens produce an unique variety of extra cellular secondary metabolites known as lichen substances.

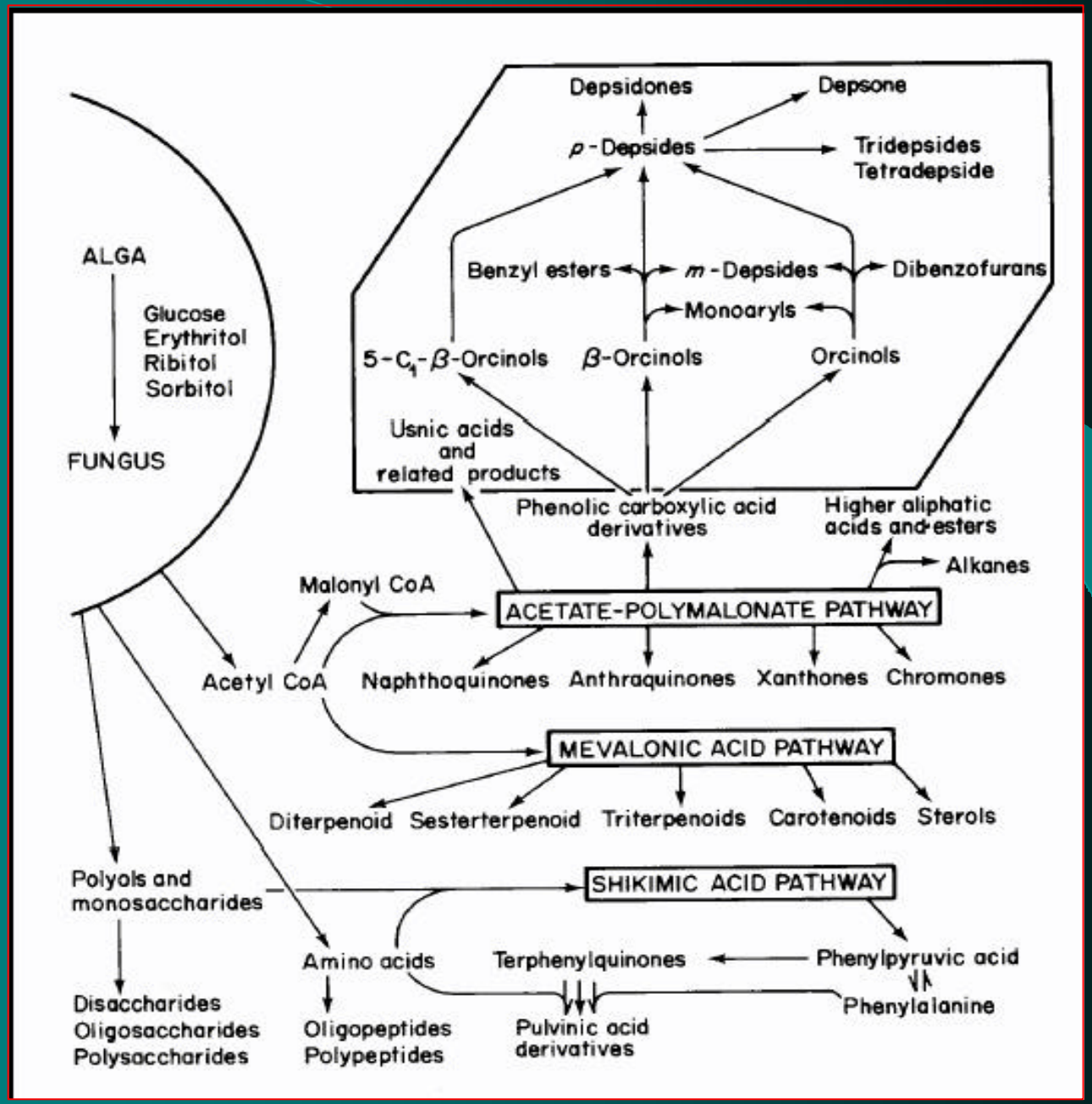
These compounds exist within the thalli either in an amorphous form or as crystals.

The quantities of these lichen substances were up to 30% of the dry weight of the lichen thalli in certain lichen species.

More than 700 such compounds have now been isolated from nearly 5,000 lichen species.

These compounds are very important in the lichen systematics and phylogeny.

Secondary Metabolite Production in Lichens : The Important Pathways



Biochemical pathways of lichens

Polyketide Pathway

Dibenzofurans

Depsidones

Depsides

Depsones

Xanthones

Chromones

Usnic acid

Anthraquinones

Mevalonate Pathway

Steroids

Diterpenes

Triterpenes

Shikimic acid pathway

Pulvinic acid

Terphenylquinones

Primary metabolites

Carbohydrates

Polysaccharide cell wall compounds
eg. lichenan, isolichenan

Proteins and Amino acids

Hydroproteins
Oligopeptides
Polypeptides

Fatty acids

Aliphatic acids
Lactonecarboxylic acid
Cycloaliphatic compounds

Mevalonic Acid Pathway……

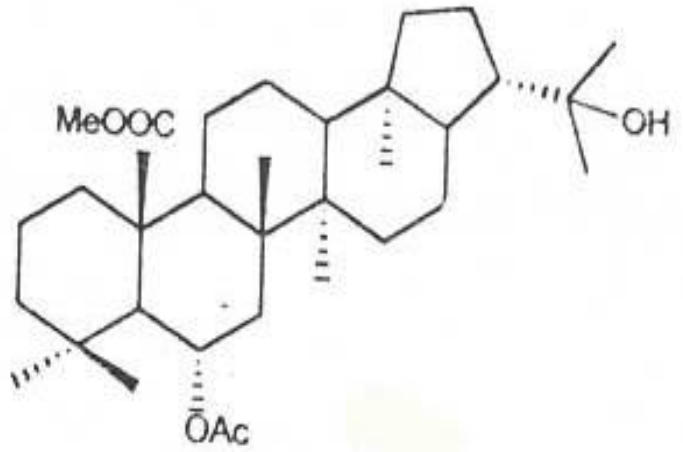
Compounds such as steroids and triterpenoids are formed through this pathway.

Eg. Methyl aipolate isolated from *Physcia aipolia*

Wilkins *et al*. (1989): *Aust J Chem* 42:1415.

•Lichen *Physcia aipolia* is widely distributed in India and in Tamil Nadu

•Habitat of *P. aipolia* ranges from Coastal – 800 m alt. in the Western and Eastern Ghats of Tamil Nadu.



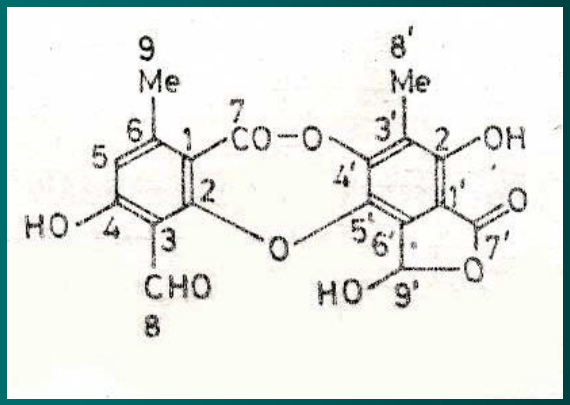
Methyl aipolate

Shikimic Acid Pathway……

Pulvinic acid derivatives are most common (K-Yellow pigments).

Eg. Norstictic and Salazinic acid isolated from *Sticta* and *Pseudocyphellaria*

Microcrystals of Norstictic acid in GAW reagent



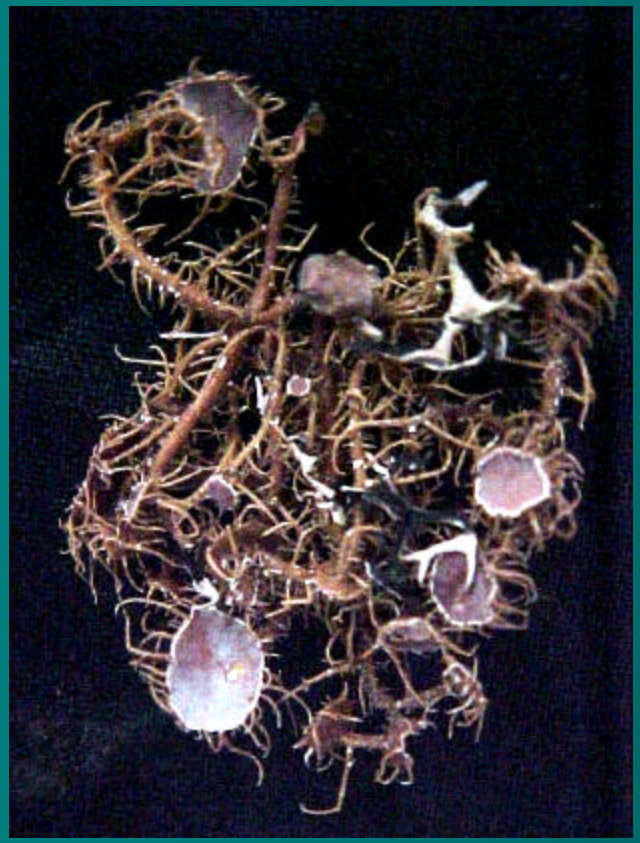
Norstictic acid

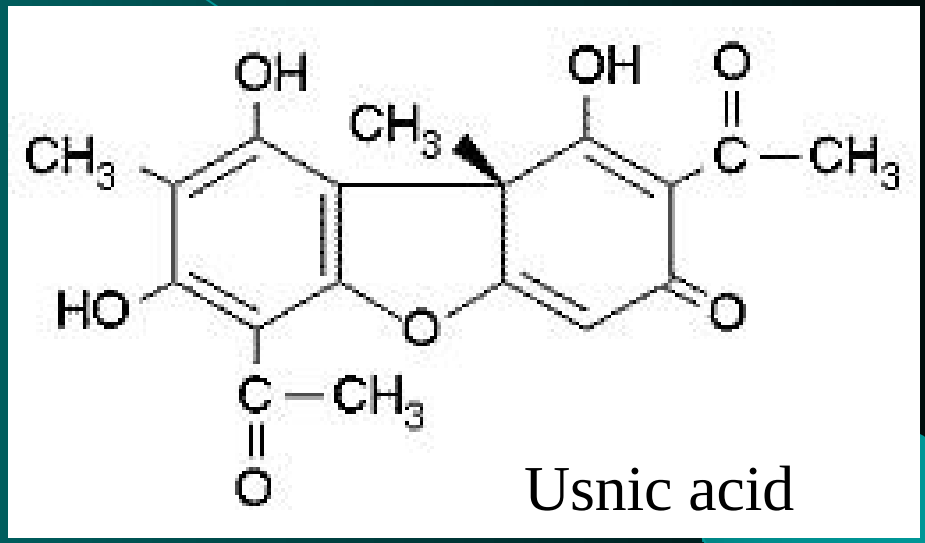
8 *Sticta* & 4 ***Pseudocyphellaria*** species occur in Tamil Nadu mostly in places above 850 m altitude

Rao *et al*. (1966) *Curr. Sci*., **35**: 147-148

Acetate-polymalonate Pathway……

Usnic acid a yellow- green cortical pigment



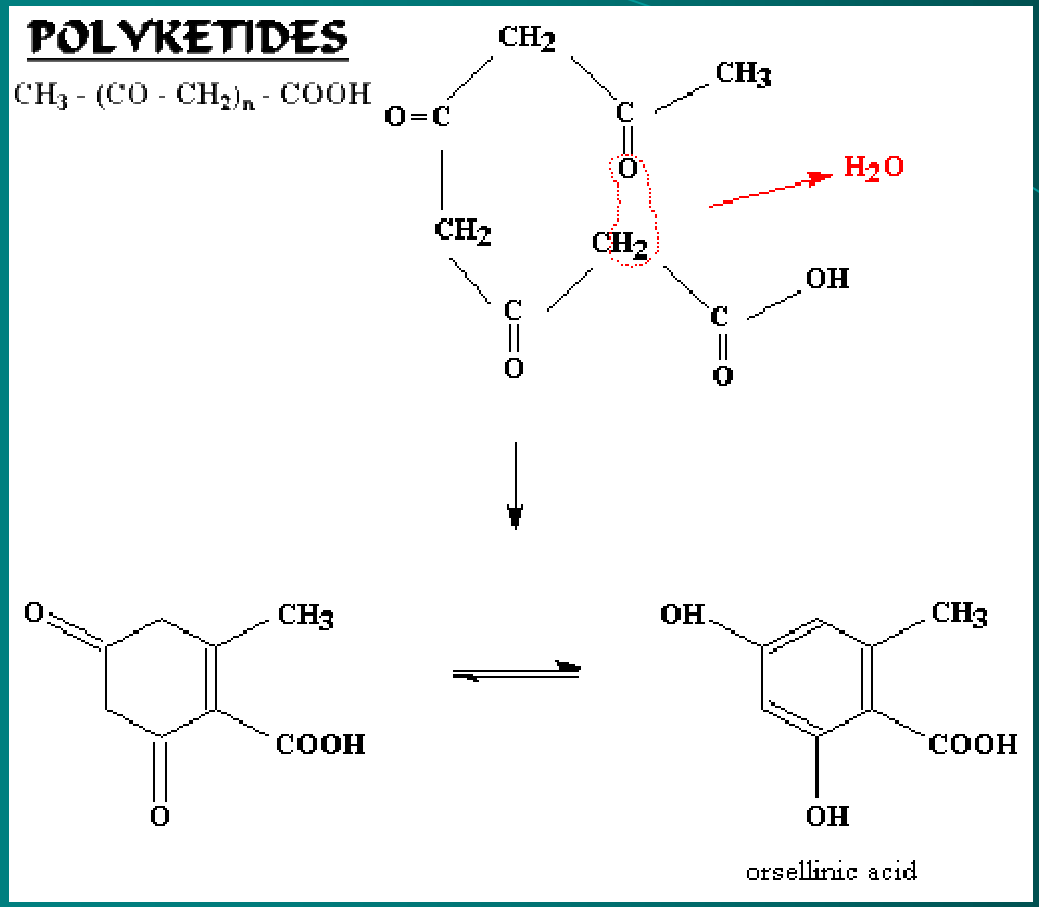


Usnic acid

30 *Usnea* species occur in Tamil Nadu mostly in places above 850 m altitude

Ref: Ragnaswami, S. and Rao, V.S. (1955) *Indian Jour. Pharm.* **17**: 70.

Lichen compounds through Polyketide biosynthesis



Monocyclic phenols

Depsides

Depsidones

Depsones

Dibenzofurans

Usnic acid and related compounds

Chromones

Xanthones

Naphthaquinones

Anthraquinones

The thumb rule may be that 1 compound in 5000 becomes a drug,

But among the compounds called polyketides, drug prospecting odds have been much better—around 1 in 100.

Polyketides are small, cyclized molecules produced by sharing biosynthetic pathways that produce a common ketone structure.

Between 5000 and 10,000 are known, and about 1% of them possess drug activity.

Sales of the more than 40 polyketide drugs—including antibiotics,immunosuppressantscholesterol-lowering agents, antifungals, & cancer chemotherapeutics

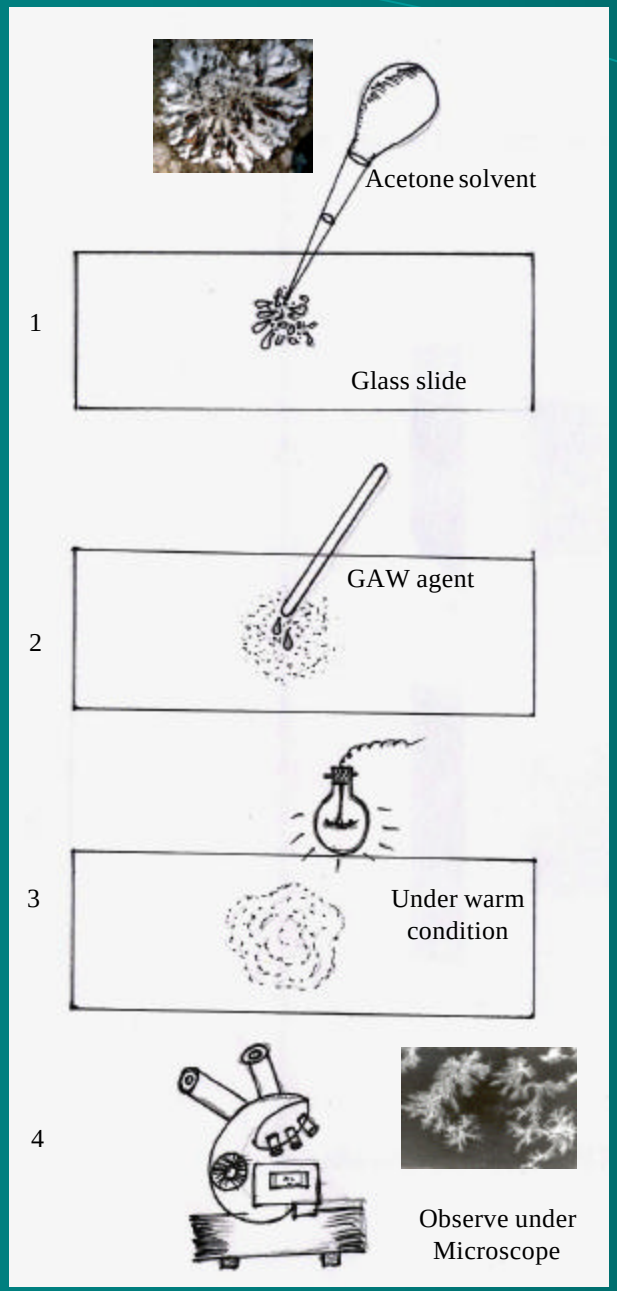
Sales exceed $15 billion a year

Lichen Spot tests Procedure (Huneck and Yoshimura, 1996)

1. Apply the reagent solutions* to the lichen's cortex or medulla (or both) using a small glass needle. Keep a separate needle for each reagent.
2. Observe the colour.
3. Wait at least half a minute before concluding that the test is negative.

Test	Reagent*
K test	10% water solution of potassium hydroxide
C Test	a solution of commercial bleach
P Test	a saturated alcohol solution (95% ethanol) of *p*-phenylenediamine (1,4-diamiobenzene) or (Steiner's solution) 1 g of *p*-phenylenediamine, 10g of sodium sulphite, and 5 ml liquid detergent to 100ml distilled water
KC	K and C are applied together (that's called the KC test when the k is applied first
CK	C is applied first

Microcrystal Test



1. Place a small fragment of the lichen thallus over a slide. Add few drops of acetone-leave to evaporate-remove the thallus fragments

2. Add few drops of crystalization agent like GAW * to the residue

3. Keep the slide on a warm place

4. Place the cover slip and observe it under the compound microscope for crystal formation. Compare the crystal types with published literature for identification.

Acronym	Solvent mixer	Proportion
GAW*	Glycerol: ethanol:water	1:1:1
GE	Glycerol: acetic acid	1:3
An	aniline: glycerol: ethanol	1:2:2
$_{O}$T	o-toluidine: glycerol: ethanol	1:2:2
Py	pyridine: glycerol: water	1:3:3
Q	quinoline: ethanol: glycerol	1:2:2

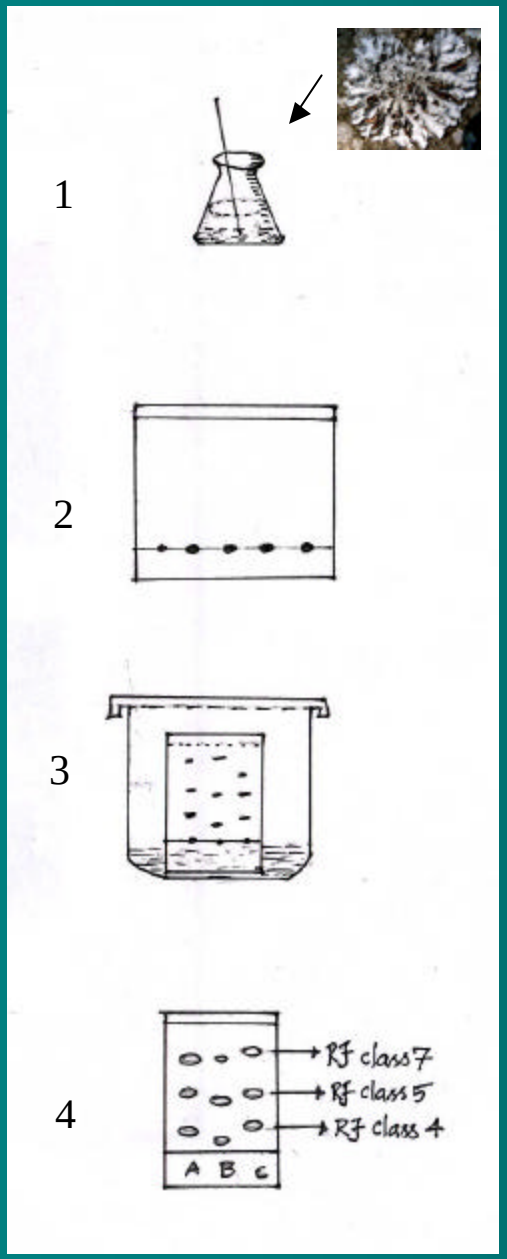
Thin Layer Chromatography (TLC) is employed in qualitative and quantitative separation of compounds in a mixture.

This procedure has a stationary phase (silica gel, cellulose, etc.) coated as a layer on to a solid support (glass or aluminum sheets) on which the compound mixer is loaded and run in mobile phase (solvents ranging from non polar (hexane, ether) to polar (water)).

The developed plate is known as a chromatogram.
A suitable reagent sprayed over the chromatogram to identify by colour, the spots representing compounds and its Rf value (the ratio of the distance moved by the compound to the solvent is called retention factor (Rf) which is characteristic of a compound in a particular solvent system) is determined.

This value and colour of the compound should be compared with a standard for confirmation.

Thin Layer Chromatography (TLC)



1. Extraction of secondary metabolite from the lichen thalli (minimal quantity) using acetone (50 ml) in a Conical flask/glass vial.

2. Using capillary tube load few drops of extract in acetone, and let the solvent of the extract evaporated (mark a loading line on the plate, 2cm from one edge of the plate).

3. Run the chromatogram in a suitable solvent system* in a TLC tank (use separate tanks for different solvent systems) until the solvent covers 90% of the plate.

4. Take the chromatogram out of the tank and evaporate the solvent. View the chromatogram and mark any coloured spots.

5.Note down the colour and Rf value of the spot. Compare with the standard and identify the compound.

Solvents systems*

Name	Acronym	Basic solvent systems	Proportion
A	T.D.A.	Toluene: dioxan: acetic acid	180:60:8 ml
B	H.E.F.	Hexane: diethyl ether: formic acid	130:100:20 ml
C	T.A.	Toluene: acetic acid	200:30 ml
G	G	Toluene: ethylacetate: formic acid	139:83:8 ml

Nowadays advanced analytical techniques such as High-performance liquid chromatography (HPLC), UV, IR and magnetic resonance spectroscopy, mass spectrometry and x-ray crystallography are used to identify and isolate the lichen compounds.

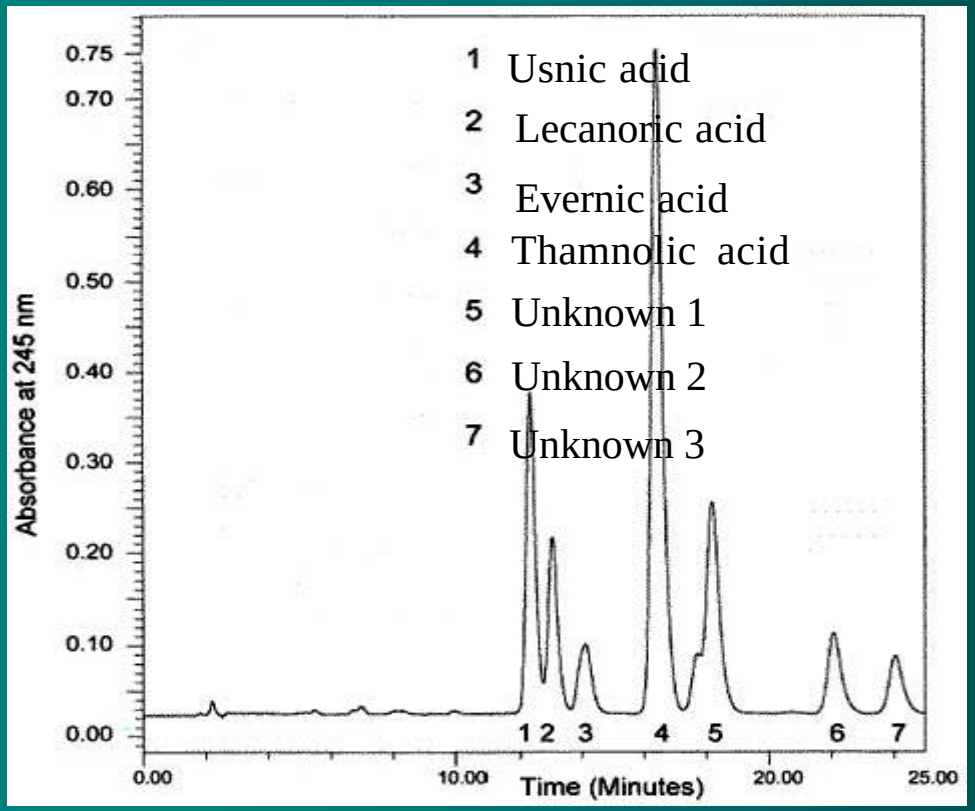
Since the studies on the diversity of lichen secondary metabolites still remain under explored, these organisms may serve as the future potential source for novel compounds and genetic materials.

The Rf class values for each lichen substance is given in the following table:

Rf Class	Colour of spot after H_2SO_4 spray and heat	Lichen Substance	K,C,P spot tests on thallus/ medulla or UV exposure
1	rose-orange	consalazinic acid	-
1-2	dark grey	fumarprotocetraric acid	P + yellow-red
1-2	grey-orange	erythrin	C + red
1-2	dark grey	protocetraric acid	P + yellow-red
1-2	grey-orange	thamnolic acid	K + yellow-orange, P + orange
1-2	yellow-orange	constictic acid	K + yellow-red, P + yellow-red
2	-	caperatic acid	-
2	yellow-orange	salzinic acid	K + yellow-orange, P + red
2	bluish yellow	squamatic acid	UV + yellow
2	pale violet grey	pannaric acid	C + green
2	dark grey to black	physodalic acid	P + red
2-3	yellow	barbatolic acid	K + yellow-red, P + orange
2-3	-	diploschistesic acid	C + blue
3	orange	stictic acid	K + yellow, P + orange
3	pale	physodic acid	KC + orange-red
3	yellow or grey	gyrophoric acid	C + red
3	olive-yellow	hypoprotocentraric acid	-
3	yellow-grey	lecanoric acid	C + red
3	pale green to grey	lobaric acid	KC + red
3	dull yellow to brown	psoromic acid	P + yellow-red
3-4	pale straw	olivetoric acid	C + red

3-4	straw colour	strepsilin	C + green
3-4	yellow	evernic acid	-
3-4	grey black	virensic acid	P + yellow-red
3-4	brownish	grayanic acid	-
3-4	orange-yellow	baeomycesic acid	-
4	broght yellow	norstictic acid	K + red, P + orange
4	colourless	a-collatolic acid	KC + pink
4	orange	sekikaic acid	-
4	yellow	diffractiac acid	-
4	yellow	barbatic acid	-
4	yellow grey margin	divaricatic acid	-
4-5	yellow-orange	perlatolic acid	-
4-5	pink-orange	homosekikaic acid	-
5	greenish	norlobaridone	KC + red
5	violet	zeorin	-
5	dusky orange	cryptochlorophaeic acid	C + yellow, KC + reddish
6	yellow	rhizocarpic acid	UV + yellow-orange
6-7	pale	lichexanthone	UV + yellow-orange
7	greenish-grey	usnic acid	UV + quench
7	dark green	pannarin	P + orange
7	yellow	parietin	K + violet-purple, UV + orange
7	yellow-orange	atranorin	K + yellow

Isolation of lichen compounds using High Performance Liquid Chromatography (HPLC)



Isolate warm Methanol Extract using Clean and dried lichen fragments.

Add standards (Benzoic acid and Anthracene internal solvent controls) and inject extract

Identify Compounds with retention index values (RI) calculated using standard Retention time (RT) values. Samples are collected using an automated fraction collector and absorption peaks graphically represented.

For HPLC a spectrophotometric detector operating at 254 nm with a flow rate of 1 ml/min is used.

Two solvent systems can be used: 1% aqueous orthophosphoric acid and methanol in the ratio 7:3 (A) and methanol (B).

Ecological functions of Lichen Secondary metabolites (Rundel, 1978)

Ecological function	Compound
Light-Screening (to protect photobiont from excess light)	Usnic acid, Parietin
Anti-herbivore defense	Pulvinic acid derivatives
Anti microbial	Usnic acid
Allelopathic (including antibiotic)	Psoromic, Lecanoric, Usnic and Gyrophoric acids

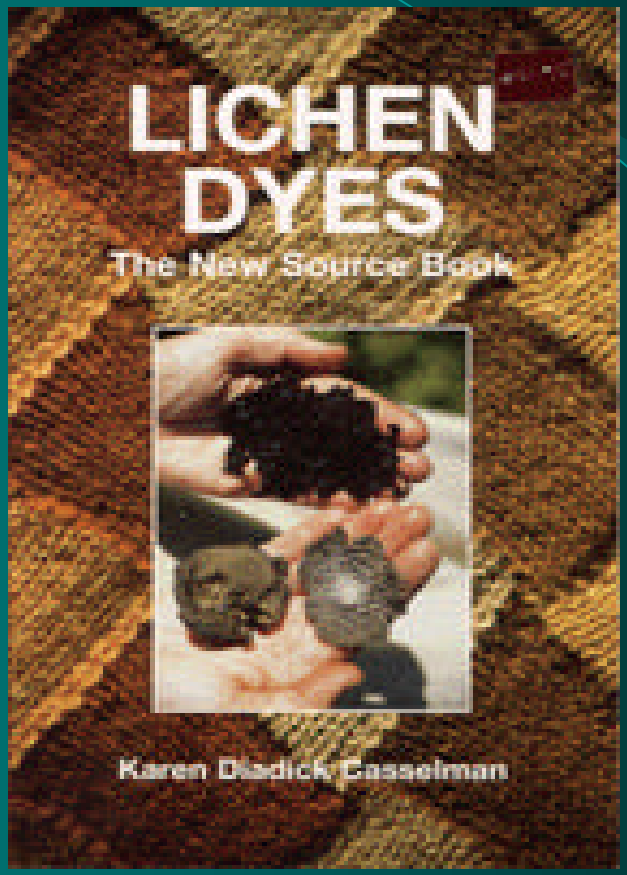
Economic importance of lichen secondary compounds

Dyes

Litmus

Perfumes

Medicines



http://www.dyeman.com/bbook4.GIF

Lichen acids were the source of important dyes for cotton and wool in medieval Europe.

Two purple and red dyes, orchil and cudbear, were obtained from the lichens *Roccella* and *Ochrolechia*.

Lichen dyes were dissolved in human urine, and the yarns were immersed in this mixture.

Ammonia salts in the urine functioned as mordants to make the dyes permanent

Litmus (an acid/base indicator) from *Roccella montagnei*

The tinctoral properties of lichens are due to the presence of lichen secondary metabolites, some of which contain chromogens from which the colouring matter is derived.

Under the combined influence of ammonia and oxygen, lecanoric acid and erythrin in *Roccella montagnei* give orcin and subsequently orcein, which are the colouring matters of orchil and which, in the presence of sodium or potassium carbonates, form azolitmin and erythrolitmin (colouring matters of litmus)

Wealth of India

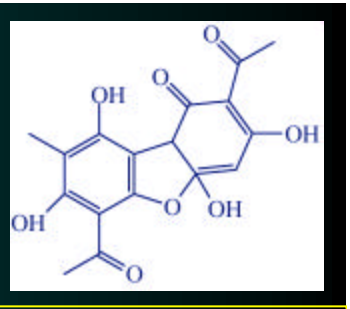
"Oakmoss lichen"

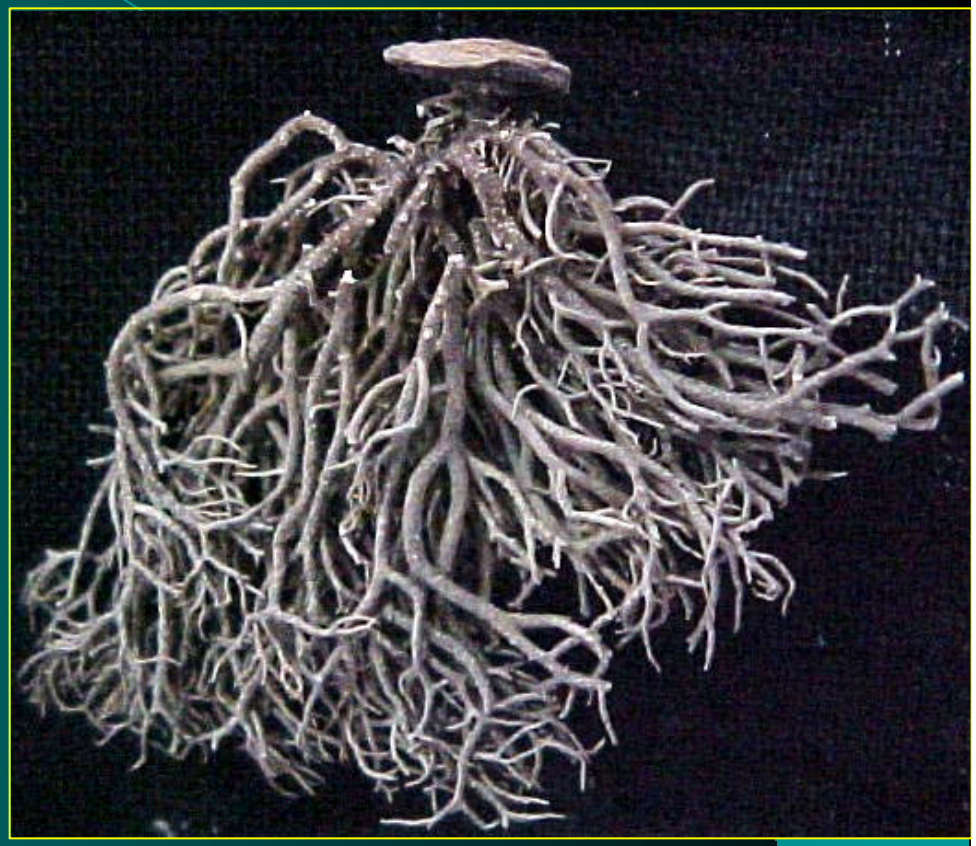
(*Evernia prunastri*)

This species is harvested commercially in south-central Europe, and then sent to France where it is used in the manufacture of fine perfumes.

The lichen acts as a fixative for other scents, and also adds a subtle herbal fragrance of its own.

Usnea spp.





Antifungal, Antibacterial properties

Important References……

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MIETZSCH E., LUMBSCH, H.T. & ELIX, J.E. (1994): WINTABOLITES (Mactabolites for Windows). - Users manual and computer program, 2nd ed. (Universitat Essen) 54p.