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Suberin fatty acids and triterpenoids from birch outer bark

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Webinar Luke-URCA #3: biosourced molecules
30.5.2023

Outline

- Hardwood consumption in Finland
  - Amount of bark generated
  - Combustion
- Chemical composition of bark
- Suberin and betulin isolation from outer bark
- Summary
- Projects related to birch bark utilisation

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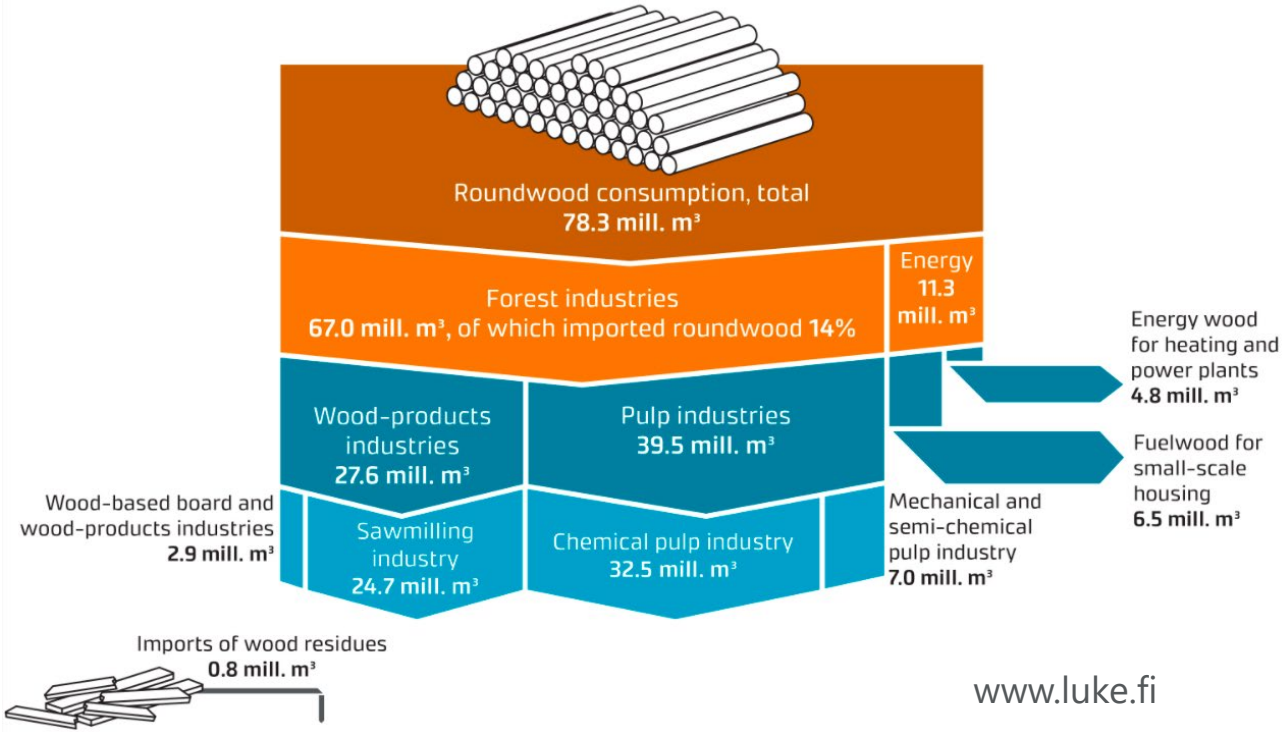
Roundwood consumption in Finland, 2020



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Finnish forest industries' roundwood consumption

2020	Logs*	Pulpwood**	Bark***
	Million m^3		
Hardwood (mainly birch)	1.04	13.42	1.74

- Logs* mainly for plywood and veneer production
- Pulpwood** for chemical pulp industry (bleached kraft pulp, dissolving pulp and high yield BCTMP)

- Tree bark*** makes ~12 % of the birch roundwood volume

stat.luke.fi/en

Björklund, L. Bark på massaved – en studie över barkhalten i travar med massaved. The Swedish Timber Measurement Council (VMR) virkesmätning och redovisning, Reports, Sundsvall, Sweden, 2004. 17 pp.

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Uses for birch bark

- Mainly combustion!!!
  - Energy production
  - Pulp mills and plywood mills
- Decorative products
  - Marginal use
- Our philosophy
  - Extract **valuable compounds** from bark and use the **residue** for energy production

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Combustion of birch bark

- Effective heating value of birch bark 21–23 MJ/kg (dry matter)
- 1 MJ = 1/3600 MWh
- 0.0058–0.0064 MWh/kg

- Forest chips price 23.2 €/MWh
- 0.135–0.148€/kg

Pulp mill example

- Annual hardwood consumption 0.9 Mm^3
- Bark generated 0.108 Mm^3
- Bark density 550 kg/m^3
- Bark generated 59.4 Mkg
- **8.0–8.8 M€**

Tilasto: Energian hinnat [verkkojulkaisu].
ISSN=1799-7984. 2. Vuosineljännes 2021, Liitetaulukko 2. Energian hintoja lämmöntuotannossa kesäkuussa 2021 . Helsinki: Tilastokeskus [viitattu: 8.11.2021].
Saantitapa: http://www.stat.fi/til/ehi/2021/02/ehi_2021_02_2021-09-09_tau_002_fi.html

Alakangas, Eija. Suomessa käytettyjen polttoaineiden ominaisuuksia. Properties of fuels used in Finland. Espoo, Finland. Valtion teknillinen tutkimuskeskus, VTT Tiedotteita – Meddelanden – Research Notes. 2045. 172 pp.

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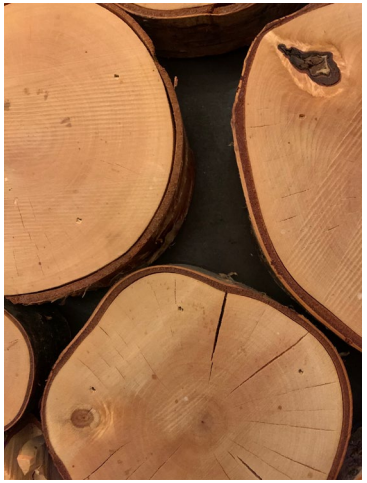
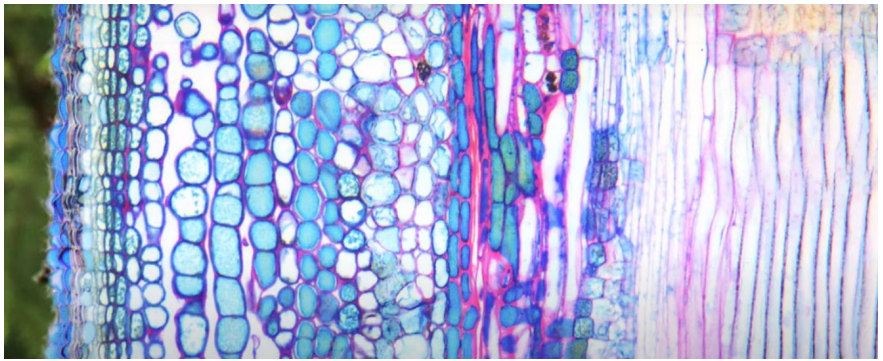
Birch bark

- Birch roundwood contains approximately 12 % (v/v) bark
- Birch bark can be divided into two layers
  - ~25-33 % outer bark
  - ~67-75 % inner bark





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Analysis of bark fractions (manual separation of outer and inner bark)

Outer bark

Inner bark

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Chemical analysis of birch bark

- **Extractives**
  - ASE 350 extraction unit
  - Lipophilic compounds extracted with hexane at 90 °C, 3 x 15 min cycles
  - Hydrophilic compounds extracted with acetone:water (95:5 v/v) at 100 °C, 3 x 15 min cycles
- **Cellulose (glucose)**
  - Acid hydrolysis -GC
- **Hemicelluloses and pectins**
  - Acid methanolysis -GC
- **Suberin and polymeric phenolic acids**
  - Hydrolysis with 3 % (w/v) KOH in EtOH at 70 °C for 2 hours. Filtration, washing and drying
- **Lignin**
  - Two-stage sulfuric acid hydrolysis
  - Acid-insoluble (gravimetric) lignin and acid-soluble lignin (UV)

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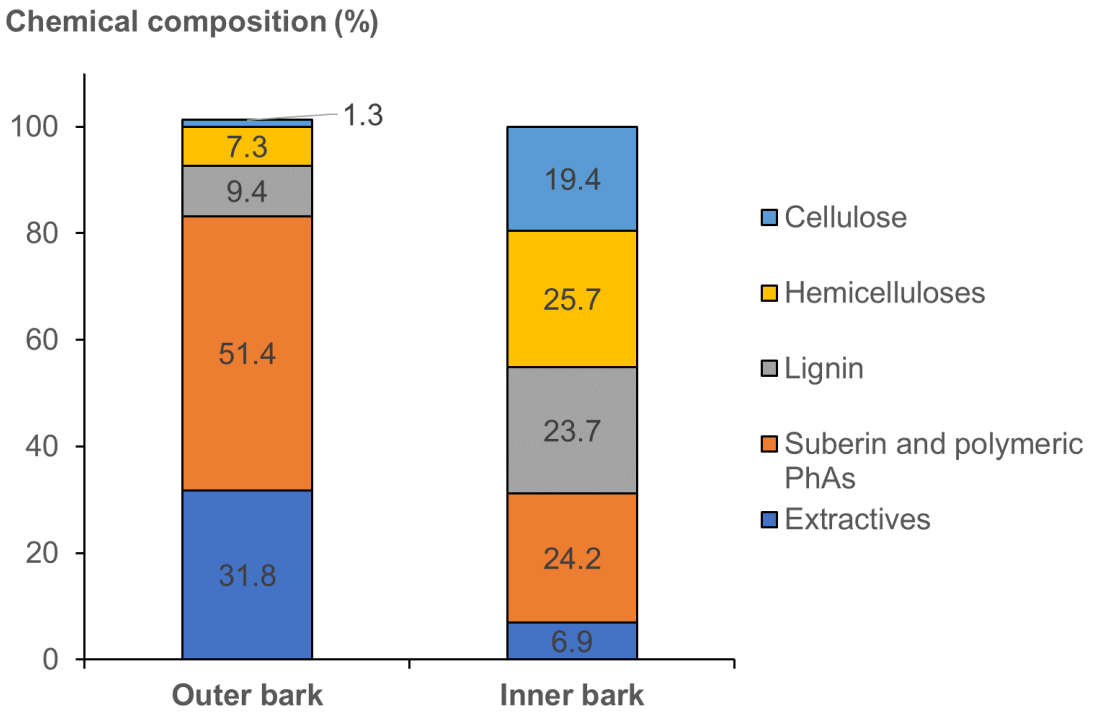
Gross chemical composition of outer and inner bark



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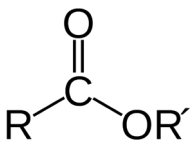
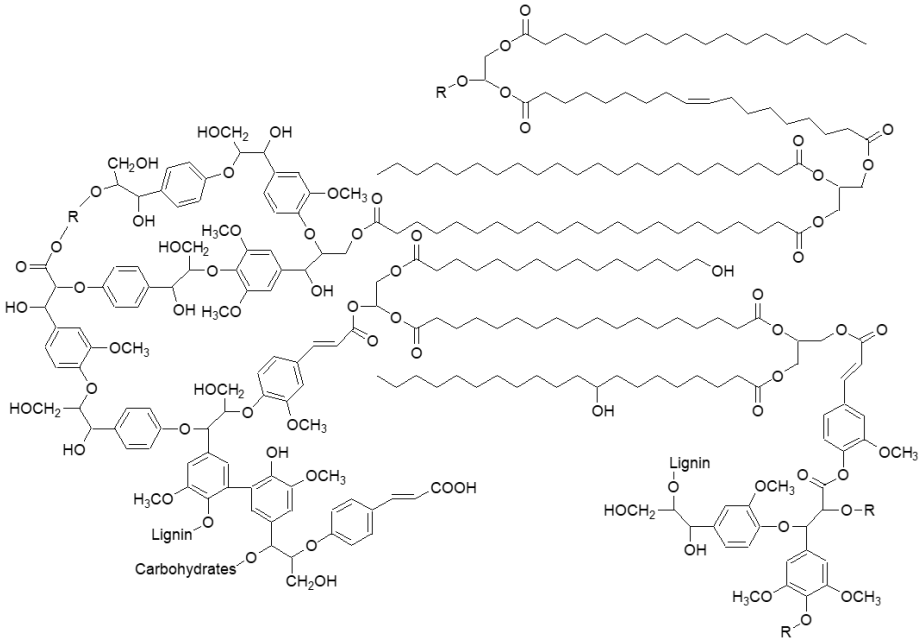
Suberin

- Mainly in birch **outer** bark
  - Suberin content up to 40-50 %
- Natural hydrophobic poly**ester**



- Suberin is believed to form partly orderly arranged lamellar structures



Monica Ek, KTH

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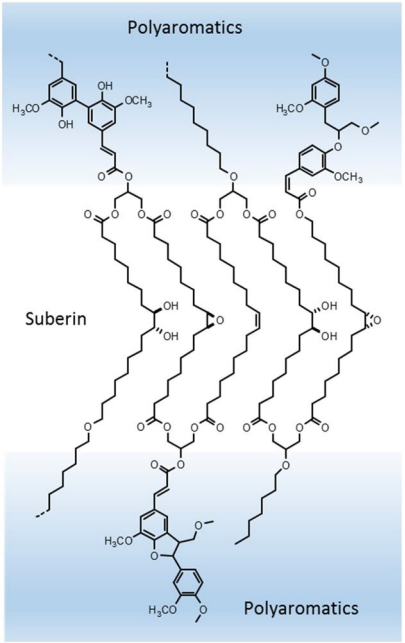
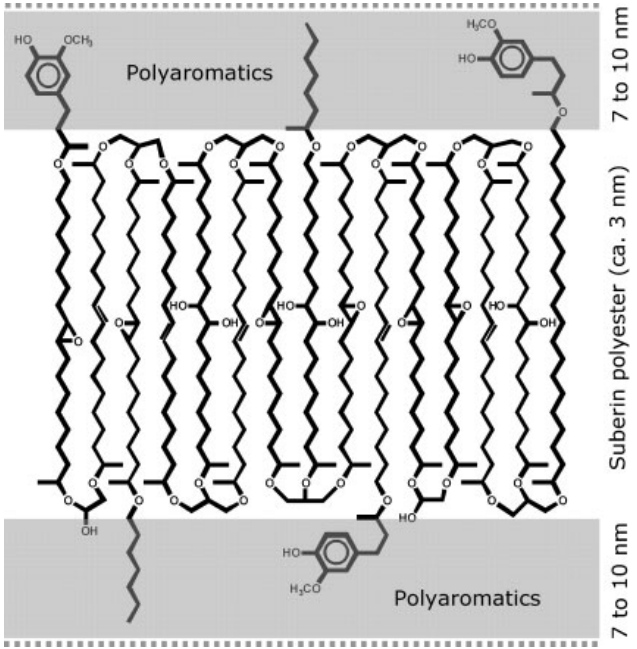
Proposed lamellar structures



Graça J (2015) Suberin: the biopolyester at the frontier of plants. Front. Chem. 3:62



Graça & Santos (2007) Suberin: A Biopolyester of Plants' Skin. Macromol. Biosci. 7:128–135

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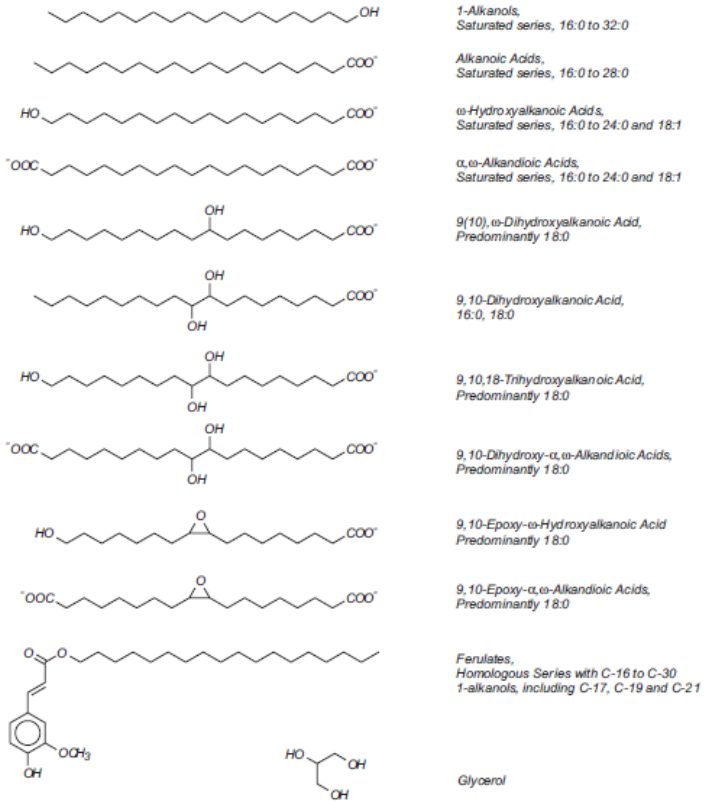
Suberin monomers

- Fatty alcohols
- Fatty acids
- Dicarboxylic acids
- ω-hydroxyacids and α, ω-diacids
- 9,10-epoxy-18-hydroxyoctadecanoic acid



Bernards, M. Demystifying suberin. Can. J. Botany 2002, 80, 227–240

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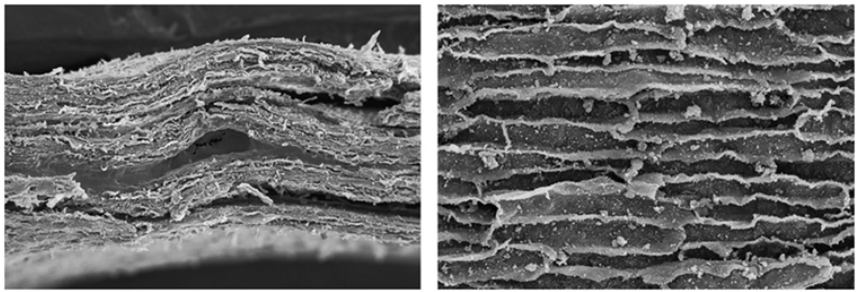
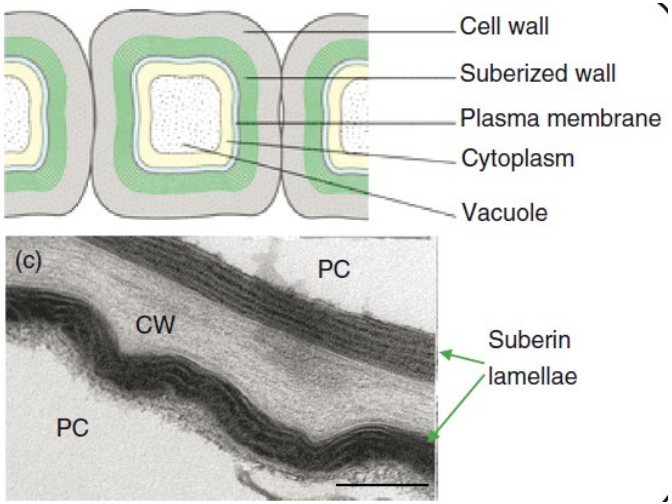
Suberin location

- Typically found in plant periderm
- The birch periderm contains alternate layers of strongly suberized cells
    - Five layers of cells with tangential walls up to 2 µm thickness
    - A variable number of layers with little or no suberization





Leite C and Pereira H (2017) Cork-Containing Barks—A Review. Front. Mater. 3:63.

Li-Beisson, Yonghua; Verdier, Gaëtan; Xu, Lin; and Beisson, Fred (May 2016) Cutin and Suberin Polyesters. In: eLS. John Wiley & Sons, Ltd: Chichester.

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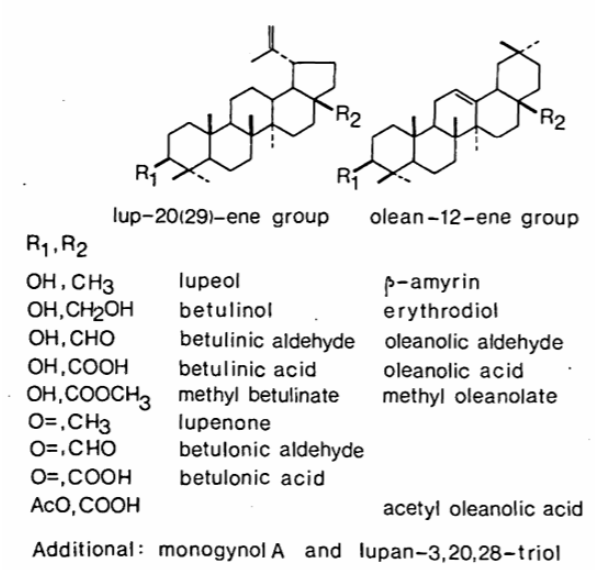
Betulin and other triterpenoids

- Mainly in birch **outer** bark
- Betulin is pentacyclic alcohol
  - Not soluble in water
  - Slightly soluble in ethanol

Triterpenoids	(mg/g outer bark)
Betulin	244.8
Lupeol	20.2
Methyl betulinate	17.3
Erythrodiol	10.9
Methyl oleanolate	9.9
Betulinic aldehyde	3.6
Other	2.3
SUM	315



Betulin is antiseptic, antiviral, antioxidant and has anti-inflammatory properties

Ekman, R. The Suberin Monomers and Triterpenoids from the Outer Bark of *Betula verrucosa* Ehrh. Holzforschung 1983, 37, 205–211.

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Extraction of betulin

- Betulin has poor solibility in many organic solvents
    - Insoluble in water
- Solubility increases with increased temperature

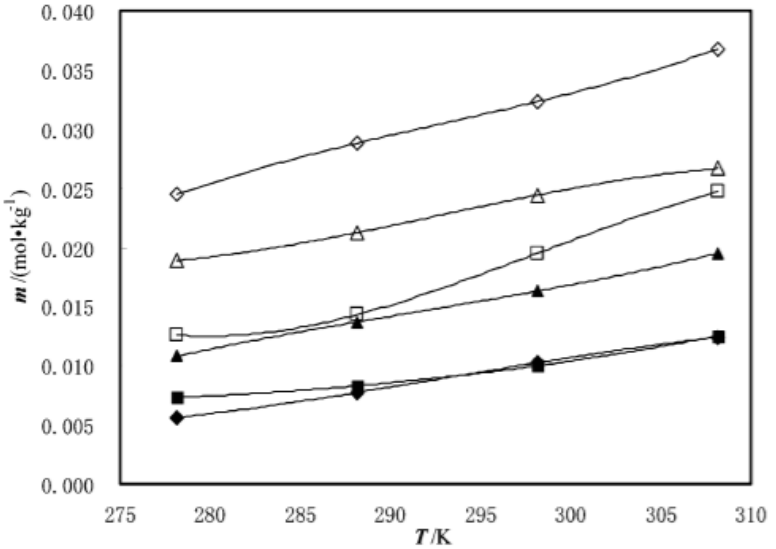

Figure 2. Solubility of betulin in different alcohol solvents: ◆, methanol; □, ethanol; △, 1-pentanol; ◇, 1-butanol; ▲, 1-propanol; ■, 1-hexanol; line,

Cao, D., Zhao, G., Yan, W. Solubilities of Betulin in Fourteen Organic Solvents at Different Temperatures. J. Chem. Eng. Data 2007, 52, 1366-1368

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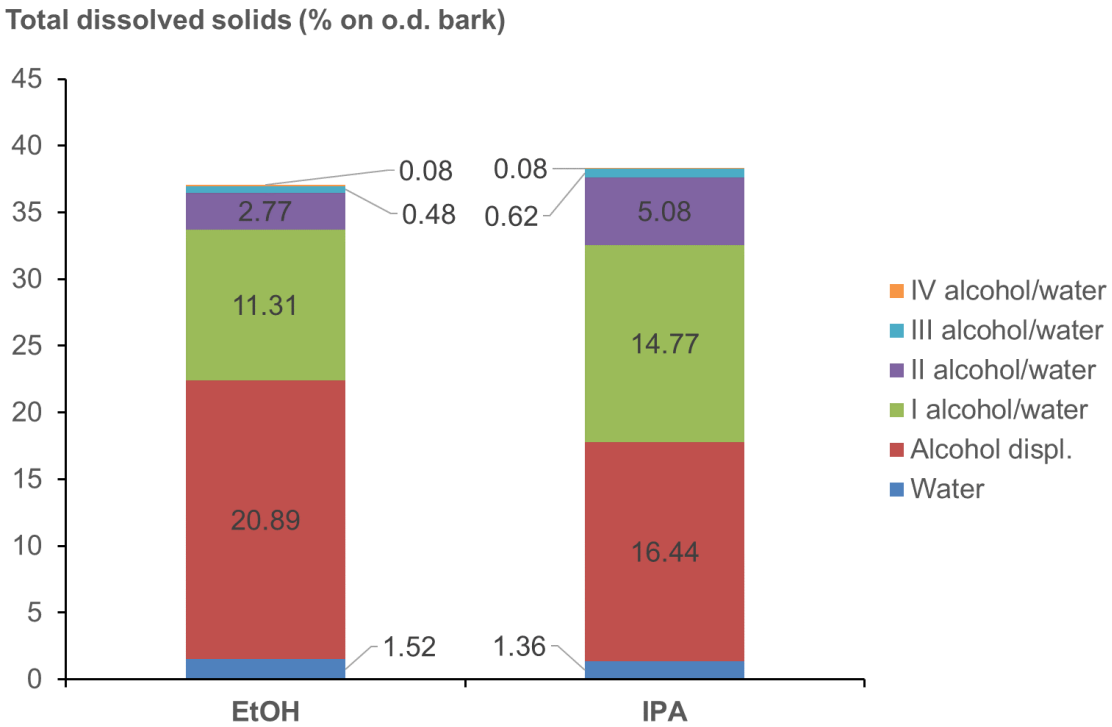
Dissolved solids after sequential extractions



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Isolation of suberin from outer bark

- Due to its complex structure, intact suberin is very difficult to obtain (compared to lignin)
- Depolymerisation into suberin fatty acids
    - Alkaline hydrolysis
    - Alkaline alcoholysis
- Partial preservation of native three-dimensional structure
    - Ionic liquids (1-ethyl-3-methylimidazolium hexanoate, cholinium hexanoate, cholinium octanoate and cholinium decanoate)
    - Recovery issues???

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Suberin and betulin (triterpenoid) isolation from birch outer bark

- Suberin content up to 40-50 % and betulin and other triterpenoids ~ 32 %

- Aim is to obtain suberin (as fatty acids) and triterpenoids in **two separate** fractions
    - **Alkaline alcoholic hydrolysis**
    - **Combined pre-extraction & hydrolysis**

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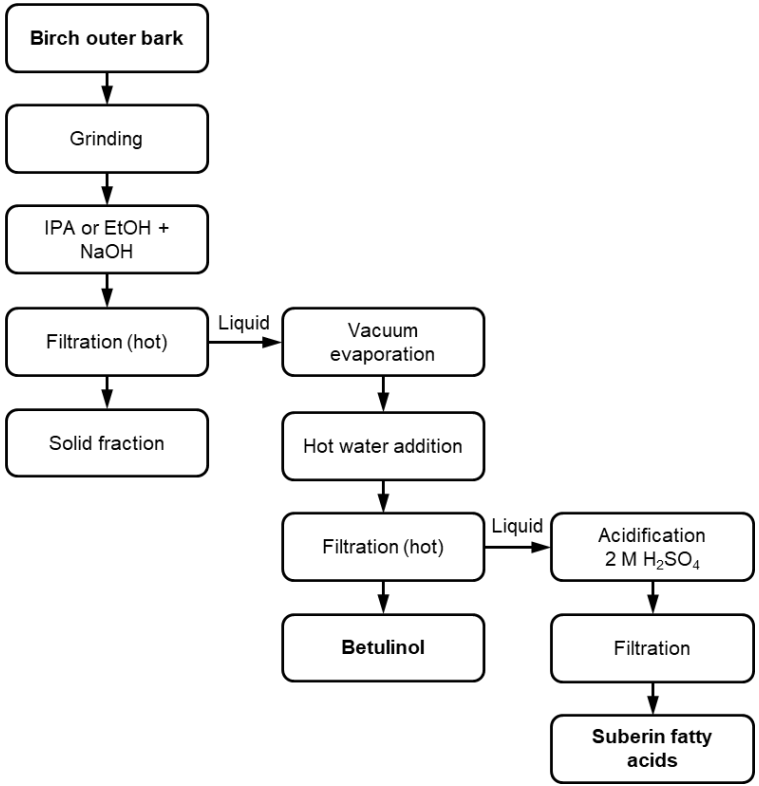
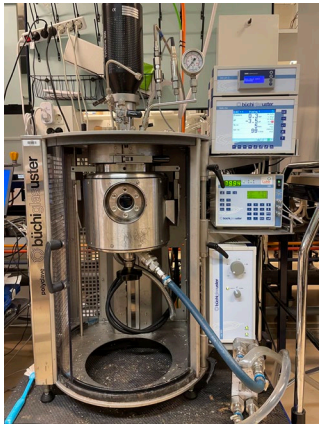
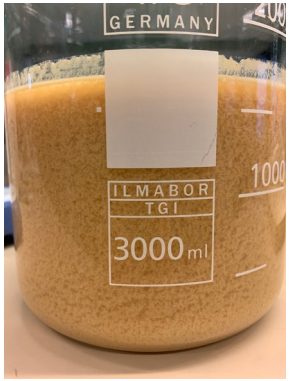
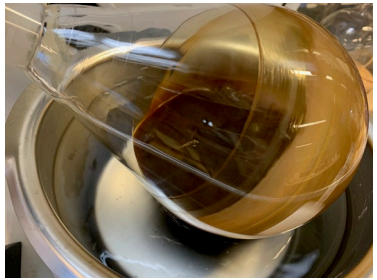
Hydrolysis scheme









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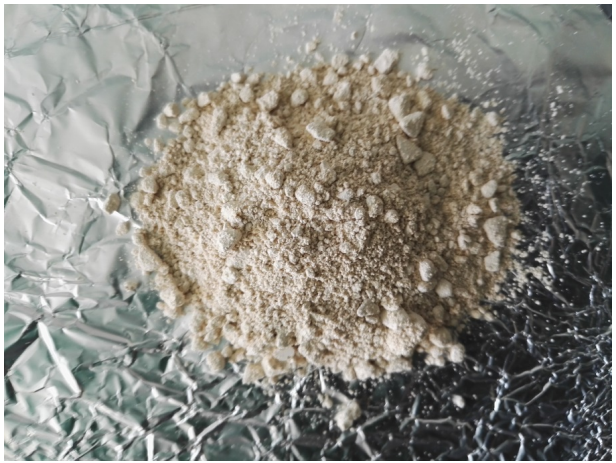
Obtained fractions

Betulin fraction



EtOH: 33.5 % (w/w) on bark
IPA: 26.5 % on bark

Suberin fatty acid fraction

EtOH: 28.1 % (w/w) on bark
IPA: 26.1 % on bark

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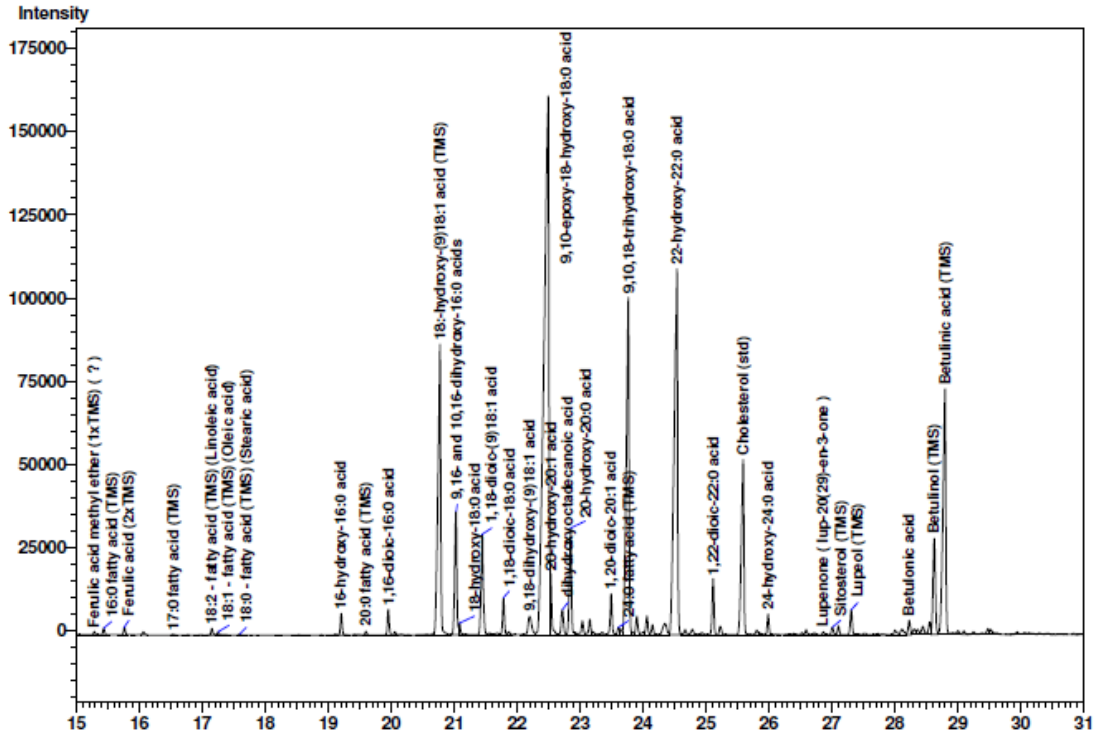
Chromatogram of suberin fatty acid fraction



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Challenges using industrial bark

- All the debarking processes are developed and optimised for using the **wood** (pulp, plywood etc.)
    - Bark is combusted (water content, impurities are controlled)
    - Bark contains also wood
- Efficient fractionation of bark is needed
    - Screening
    - Air separation (outer bark is less dense)
    - Water separation

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Industrial bark

Manually separated bark

Bark samples after grinding

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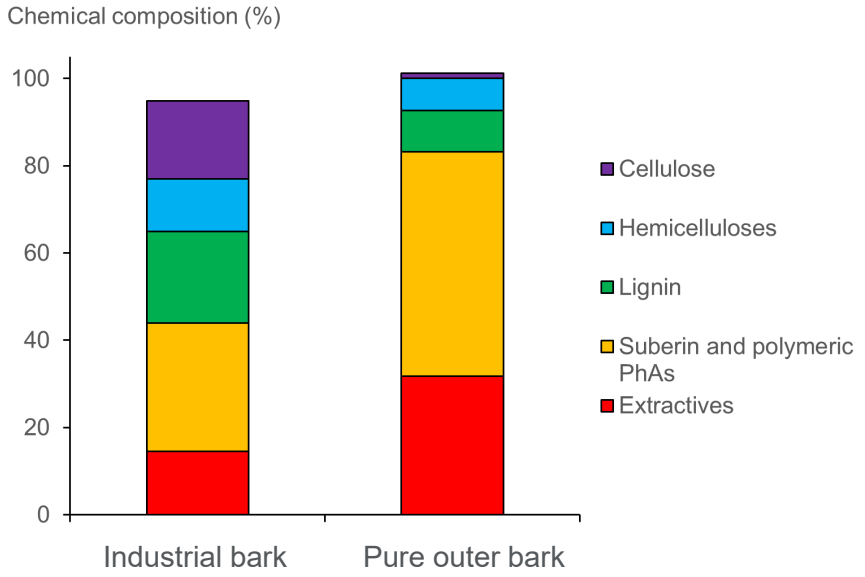
Hydrolysis of industrial bark



- Betulin, 6.5 % on o.d. outer bark
- Suberin fatty acids, 7.1 % on o.d. outer bark
- Pure outer bark
  - 5.3 times more betulin
  - 4.4 times more suberin fatty acids

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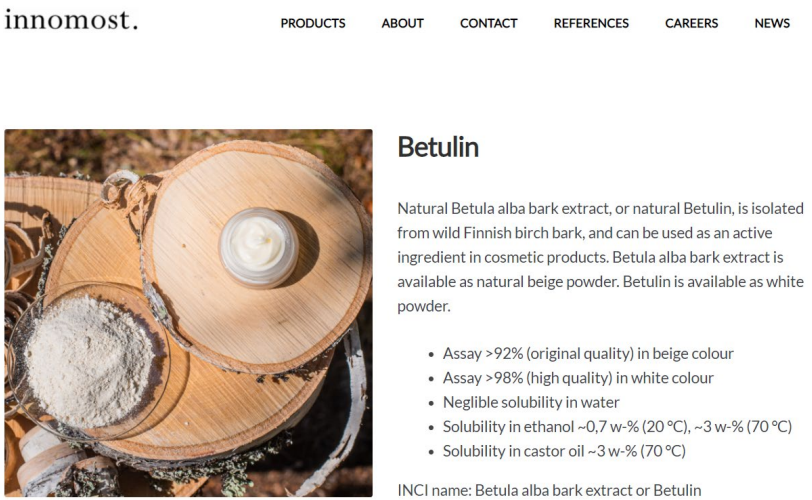
Applications

Betulin

- Can be converted to betulinic acid
- Betulinic acid prevents malaria and inflammations, and its derivatives can prevent HIV and different cancers
- Betulin is very hydrophobic and its derivatives can be used to produce water-repellent textiles
- Farmaceutical and feed applications



https://www.innomost.com/

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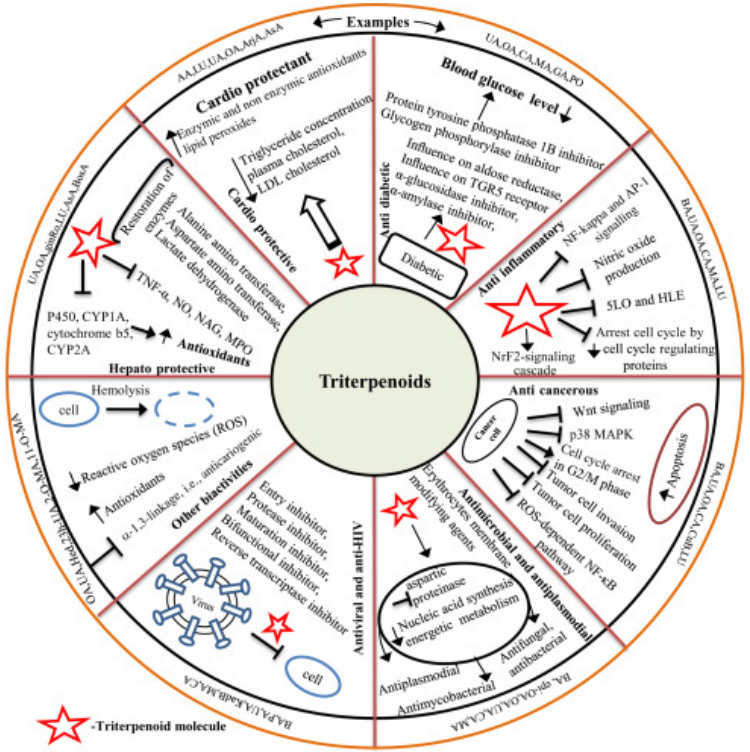
Bioactivities of triterpenoids



Sandeep, Sumit Ghosh, Chapter 12 - Triterpenoids: Structural diversity, biosynthetic pathway, and bioactivity, Editor(s): Atta-ur-Rahman, Studies in Natural Products Chemistry, Elsevier, Volume 67, 2020, Pages 411-461

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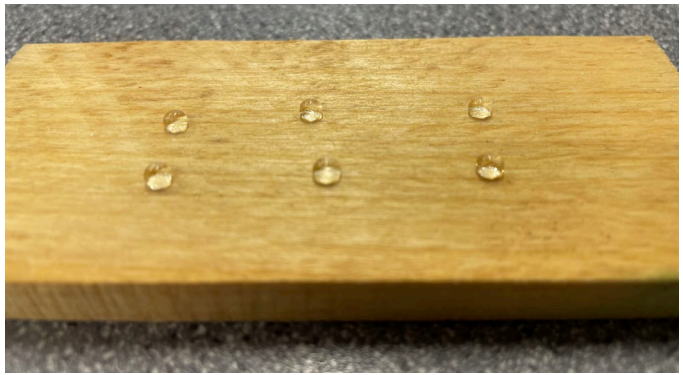
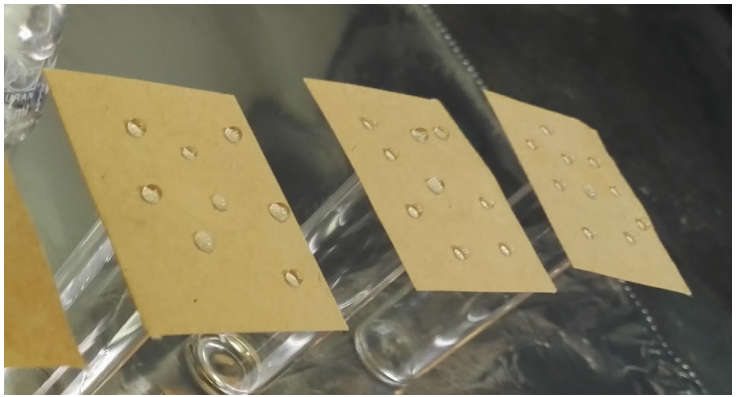
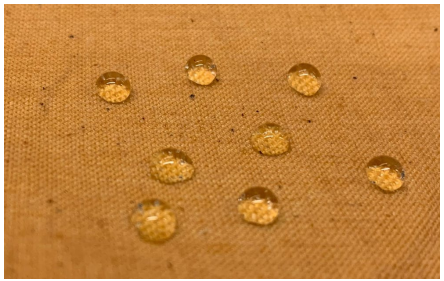
Suberin fatty acids

- Hydrophobic surfaces (packaging, textiles, wood)
- Cosmetics
- Dispersants
- Emulgators







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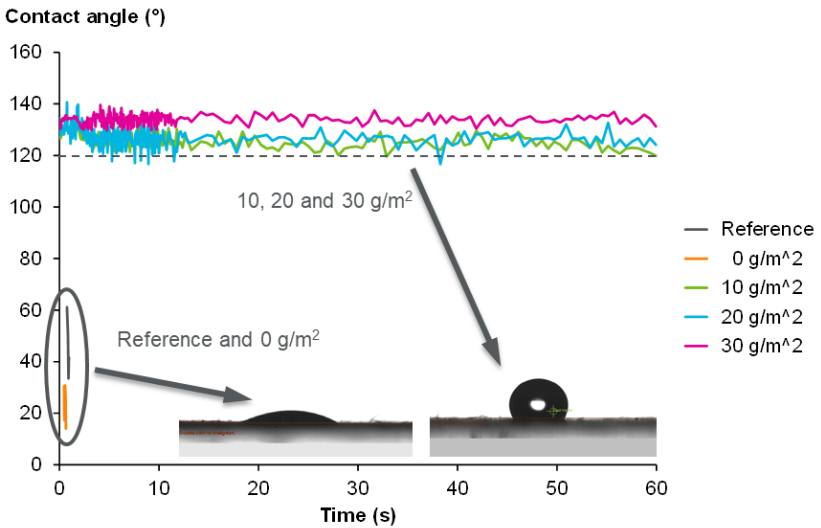

molecules

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Article

The Hydrophobicity of Lignocellulosic Fiber Network Can Be Enhanced with Suberin Fatty Acids

Risto I. Korpinen [1,*], Petri Kilpeläinen [1], Tytti Sarjala [1], Maristiina Nurmi [2], Pauliina Saloranta [2], Thomas Holmbom [3], Hanna Koivula [4], Kirsi S. Mikkonen [4], Stefan Willför [5] and Pekka T. Saranpää [1]

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Summary

- Substantial amount of bark is generated annually
- Bark is mainly combusted
- Birch bark contains valuable components which can be isolated using different methods
    - Suberin fatty acids
    - Betulin and triterpenoids
- Efficient separation of inner and outer bark is needed

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Projects related to utilisation of birch bark

- Green chemistry from forest: innovative processes and products, Luke funded project, 2015–2018
- PolyCoat - Biopolyester based hydrophobic and antimicrobial coating of lignocellulosic materials, Luke funded project, 2019–2021
- OptiBark - Biobased materials and chemicals from industrial bark and willow by optimized processes, “Key Enabling Technologies for Biobased Products”, joint program between Sweden (Vinnova) and Finland (Business Finland) 2019–2022
- SUSBINCO - Sustainable binders and coatings , Business Finland Co-innovation 2021–2023
- ENZYFUNC - Enzyme-mediated attachment and detachment of multifunctional and biobased coating aided by digital material design, Academy of Finland 2022–2024

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