

Biogenesis of sotolon in *Laetiporus sulphureus*

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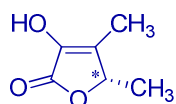
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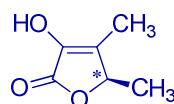
Regionalverbandstagung Nord der LChG, Hamburg, 25.03.2014

Introduction

- caramel, nutty, curry, seasoning-like odour ^{1,2}
- occurrence: fenugreek seeds, lovage, soy sauce, wine



R-(-)-sotolon 89 µg L⁻¹



S-(+)-sotolon 0.8 µg L⁻¹

perception threshold
(model wine solution)³

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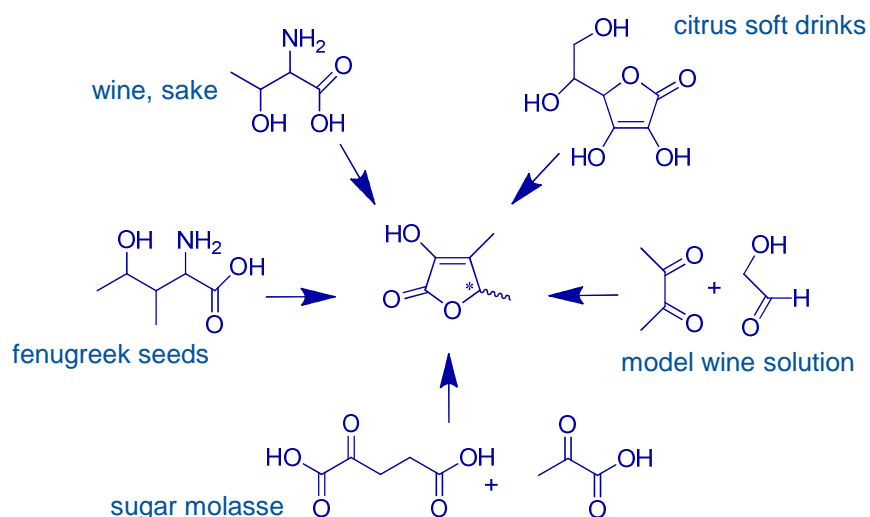
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¹ Blank et al. (1993) FFJ, 8, 191-195, ² Sulser et al. (1972), ZLUF, 148, 215-221, ³ Pons et al. (2008) JAF, 56, 1606-10

Introduction

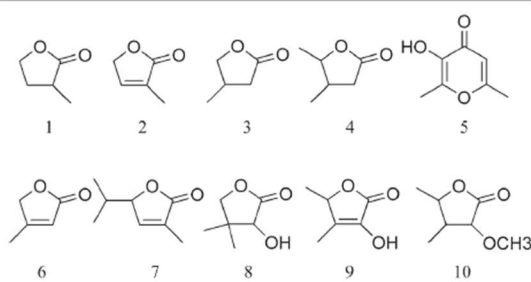
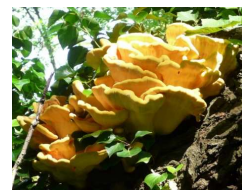
Putative building blocks of sotolon



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Retrospect:

Volatiles responsible for the seasoning-like flavour of *L. sulphureus* ⁴:



- (1) 3-methyl-dihydrofuran-2-one
- (2) 3-methyl-5H-furan-2-one
- (3) 4-methyl-dihydrofuran-2-one
- (4) 4,5-dimethyl-dihydrofuran-2-one
- (5) 6-methylmaltol
- (6) 4-methyl-5H-furan-2-one
- (7) 5-isopropyl-3-methyl-5H-furan-2-one
- (8) pantolactone
- (9) **sotolon**
- (10) 4,5-dimethyl-3-methoxy-5H-furan-2-one

- cultivation on wheat gluten
- putative precursor: 4-hydroxyisoleucine
- chemical reaction or enzyme catalysis?

⁴ Krings et al. (2011), FFJ, 26, 174-9

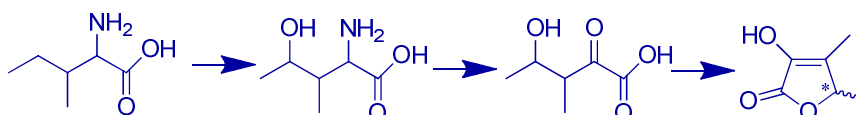
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Retrospect:

- Volatiles responsible for the seasoning-like flavour of *L. sulphureus*: 4



Hypothesis:



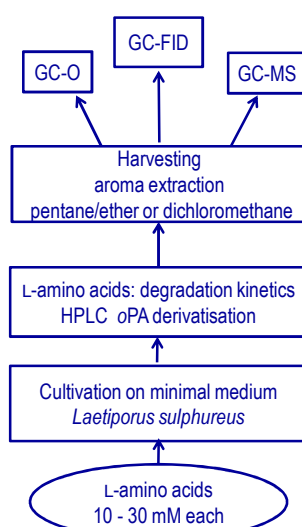
→ Cultivation with concerted supplementation of amino acids

- putative precursor: 4-hydroxyisoleucine
- chemical reaction or enzyme catalysis?

2-one

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Bottom up experimental design

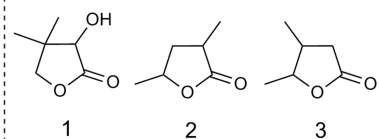


Submerged cultures
of *L. sulphureus*

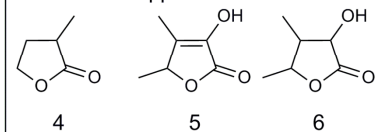
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O-heterocycles produced in cultures of *L. sulphureus* supplemented with amino acids

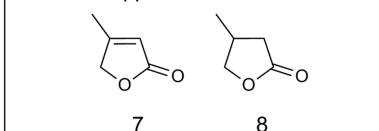
independent of supplementation



L-isoleucine supplementation



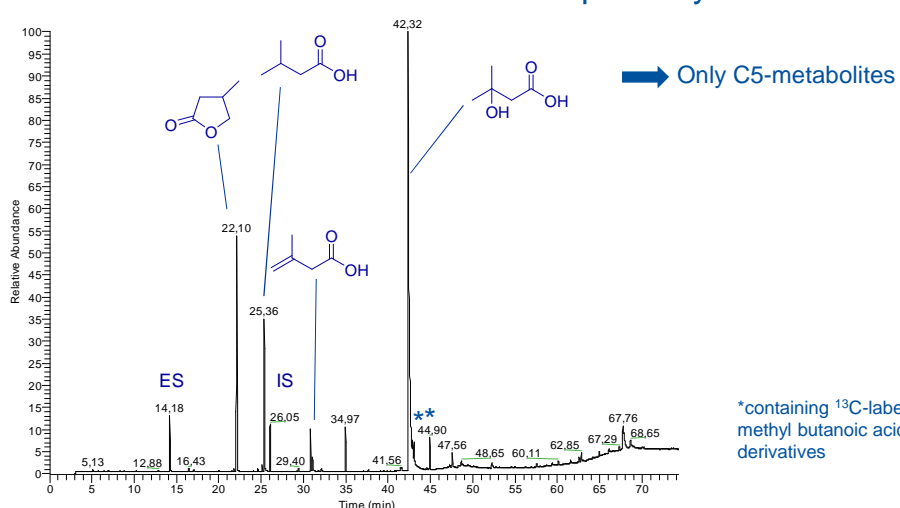
L-leucine supplementation



- (1) pantolactone
- (2) 3,5-dimethyl-dihydro-2(3H)-furanone
- (3) 4,5-dimethyl-dihydro-2(3H)-furanone
- (4) 3-methyl-dihydro-2(3H)-furanone
- (5) **sotolon**
- (6) 3-hydroxy-4,5-dimethyl-dihydro-2(5H)-furanone
- (7) 4-methyl-2(5H)-furanone,
- (8) 4-methyl-dihydro-2(3H)-furanone

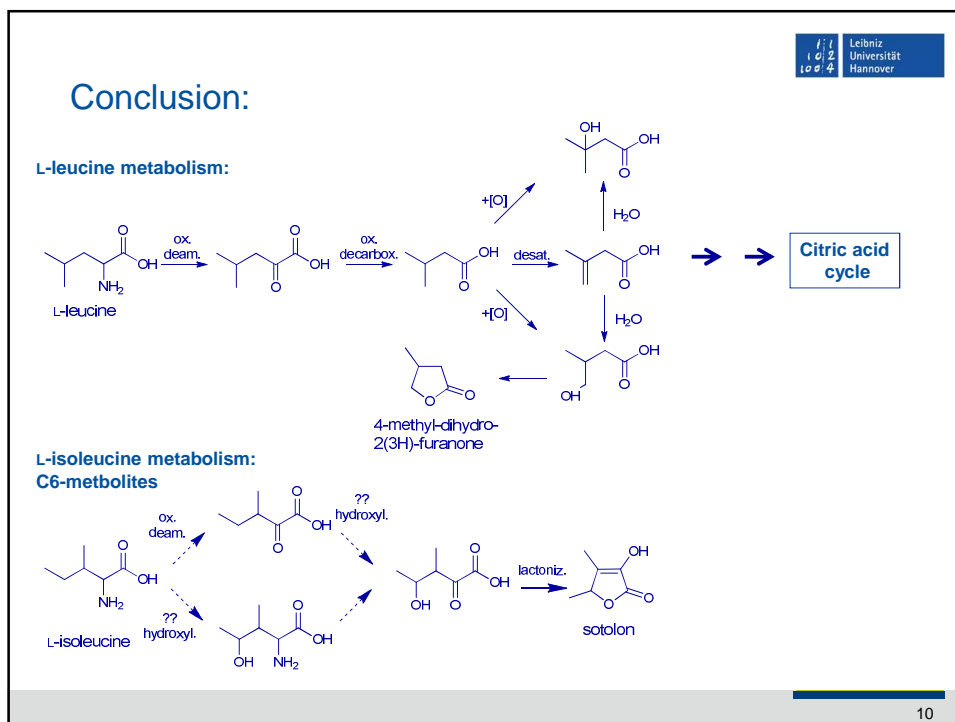
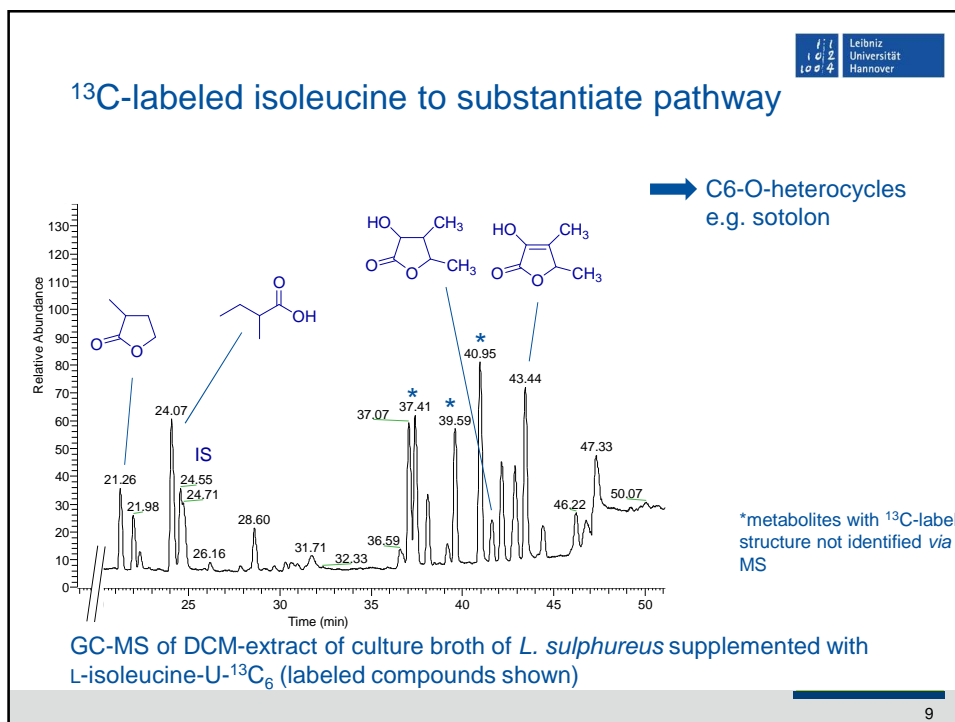
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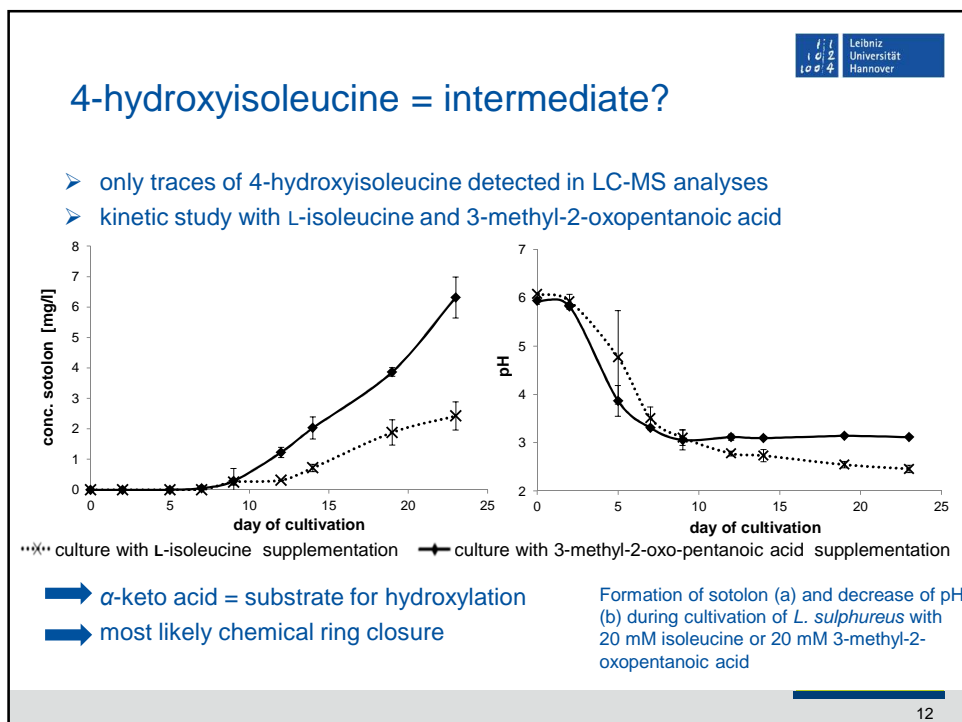
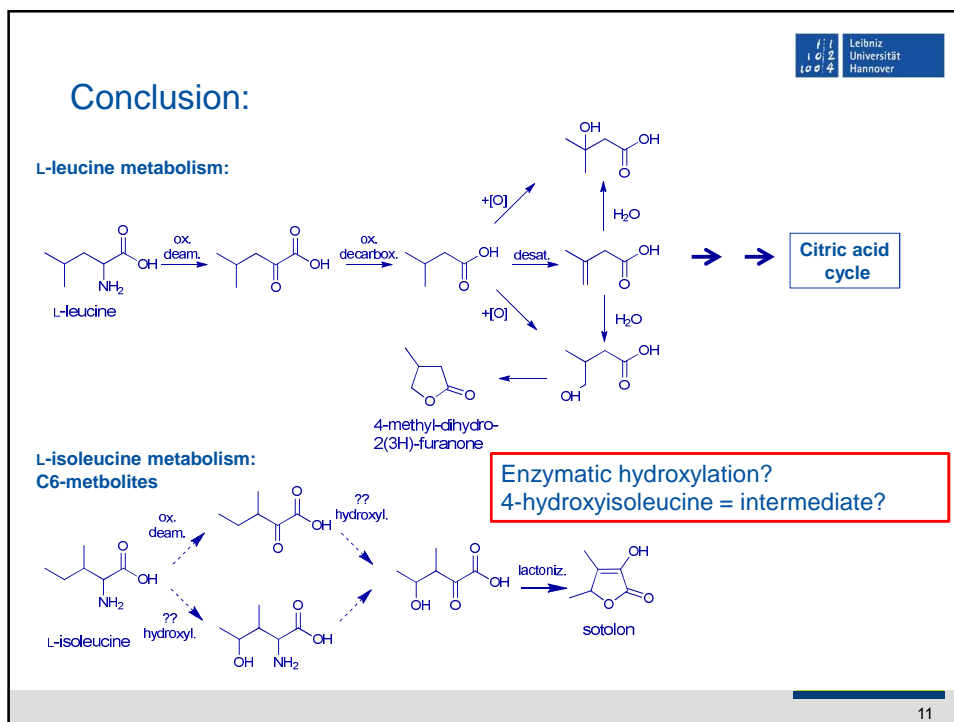
^{13}C -labeled leucine to substantiate pathway



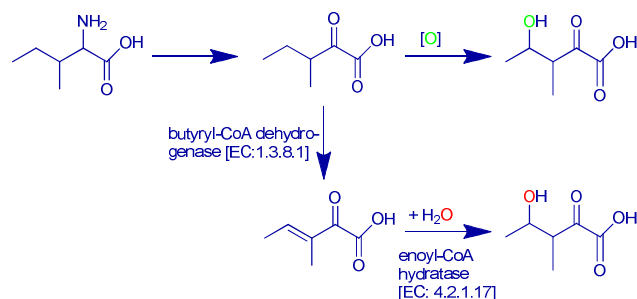
GC-MS of P/E-extract of culture broth of *L. sulphureus* supplemented with L-leucine- $^{13}\text{C}_2$ (labeled compounds shown)

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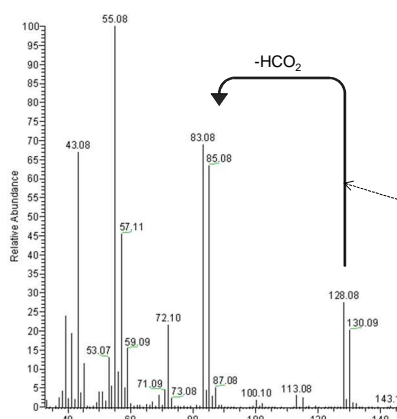
Enzymatic hydroxylation? Degradation of L-isoleucine in H₂¹⁸O



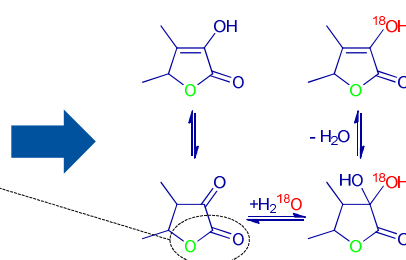
- submerged cultivation of *L. sulphureus* with 50 % H₂¹⁸O in minimal medium
- supplementation with 20 mM 3-methyl-2-oxo-pentanoic acid
- incorporation of H₂¹⁸O in sotolon = hydroxylation through water addition to double bond

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Enzymatic hydroxylation? Degradation of L-isoleucine in H₂¹⁸O



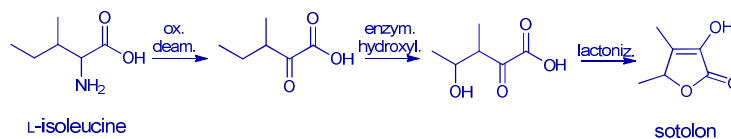
EI-MS spectrum of sotolon with the ¹⁸O-label created in cultures of *L. sulphureus* supplemented with ¹⁸O-water (50 %); neutral loss of HCO₂ highlighted.



- water incorporation only at former C2 of amino acid
- oxygenase activity on former C4 of amino acid
→ hydroxyl group

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Final conclusion



- L-leucine and L-isoleucine are precursors of O-heterocyclic compounds in cultures of *L. sulphureus*
- Decarboxylation of L-isoleucine is slowed down → C6-compounds
- Sotolon is a direct C6-metabolite of L-isoleucine degradation
 - α -keto acid is the preferred substrate
 - Oxygenase introduces hydroxyl group at C4

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Thank you very much for your attention!

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