Fumagillin and Dicyclohexylamine (DCH) in Apiculture

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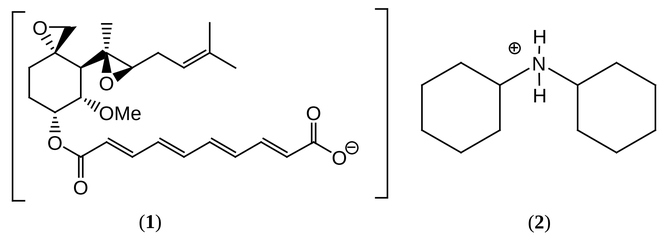
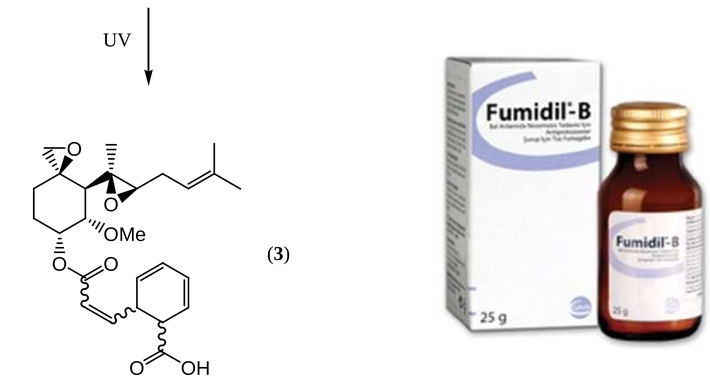
Background

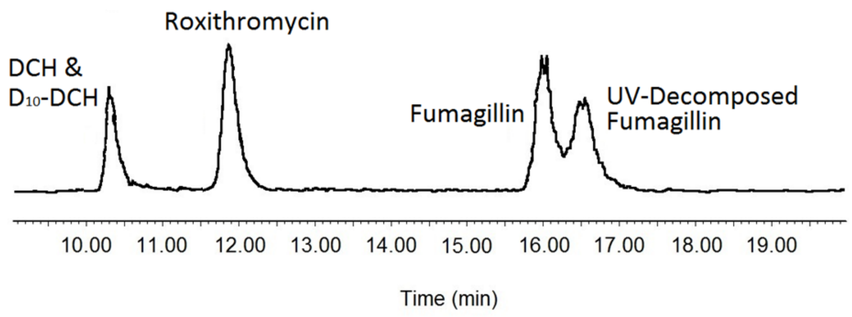
- *Nosema* disease, caused by the microsporidian fungi, *Nosema apis* and/or *Nosema ceranae* is one of the most prevalent diseases plaguing honey bees (*Apis mellifera*) today.
- *N. ceranae* has been implicated in the large scale loss of bee colonies in Europe as well as in North America.
- The only effective current chemical treatment against *Nosema* disease in honey bees is fumagillin (**1**).
- Fumagillin is obtained *Aspergillus sp*., and has been in use for an extended period of time in apiculture dating back to the early 1950's.
- Fumagillin is commercially sold as Fumidil-B® (Europe) and Fumagilin-B® (North America).
- The commercial formulation contains fumagillin as a salt, with dicyclohexylamine (**2**) being the counter ion of fumagillin in this salt
- DCH is therefore being applied in a theoretical 1:1 ratio with fumagillin.
- DCH is reportedly 5 times more toxic than fumagillin, when comparing the MSDS's of the two compounds.
- DCH is therefore a contaminant of concern in honey destined for human consumption.





Fumagillin(**1**) as its DCH (**2**) salt, and the biologically active fumagillin UV degradation products (**3**)

Analytical Method (LC-MS/MS)



Sample Preparation

- Dilution of honey with water
- Followed by Solid Phase Extraction (SPE) using Strata X (Part 8B-S100-FCH; 33 µm; 200 mg; 6 mL) polymeric reversed phase extraction cartridges (Phenomenex)
- 10µL injection volume

Standards

- Matrix Matched calibration standards of fumagillin (free from DCH) and DCH nitrite salt (both from Aldrich)
- Roxithromycin and in house prepared D_{10}-DCH were used as internal standards for fumagillin and DCH respectively

LC-MS/MS Equipment

- Waters 2695 separations module
- Waters Quattro Ultima Pt mass spectrometer
- Waters Xterra MS-C18 with column 4.6x100mm (3.5 µm packing particle size)
- Guard column 4x2.0 mm (Phenomenex)
- Column temperature maintained at 30°C

Mobile phase and Elution Gradient

- Mobile phase A: 2.0 mL ammonium formate solution (1.0 M) and 100 µL 98 % formic acid diluted to 1000 mL with water
- Mobile phase B: 2.0 mL ammonium formate solution (1.0 M), 100 µL 98 % formic acid and 100 mL of methanol diluted to 1000 mL with acetonitrile
- The mobile phase flow was kept constant at 0.250 mL min^{-1}
- The initial conditions were 90% mobile phase A (10% mobile phase B), linearly changing to 25% A (75% B) over 5 min. This condition was maintained for a further 15 min, followed by a linear return to 90% A over 5 min. The final condition was held for 5 min, for a total runtime of 30 min.

MRM Transitions

Fumagillin and UV decomposed fumagillin	459.20>177.00 (Q), 459.20>102.80 (C)
Dicyclohexylamine (DCH)	182.00>83.00 (Q), 182.00 >100.00 (C)
D_{10}-DCH	192.20>83.00 (Q), 192.20>100.00 (C)
Roxithromycin	837.40>158.00 (Q)

Q = Quantitation
C = Confirmation

Results

Honey Sample	Fumagillin (ng g^{-1})	DCH (ng g^{-1})	UV-decomposed fumagillin (ng g^{-1})
1	11.6	234.6	ND
2	<LOD	20.0	ND
3	Trace	116.4	ND
4	Trace	72.8	ND
5	11.9	124.4	ND
5	Trace	76.8	ND
7	Trace	116.0	ND
8	<LOD	Trace	ND
9	Trace	59.6	ND
10	<LOD	21.7	ND
11	<LOD	39.1	ND
12	<LOD	28.8	ND
13	Trace	64.5	ND
14	Trace	28.9	ND
15	Trace	27.5	ND
16	Trace	25.9	ND

LOD = Limit of Detection (Fumagillin 1.2 ng g^{-1}, DCH 0.24 ng g^{-1})
LOQ = Limit of Quantitation (10 ng g^{-1} for all)
ND = Not Detected (< LOD)
TRACE = Greater than LOD, but smaller than LOQ

Conclusions

- DCH was detected in all samples, even when no fumagillin was detected
- UV-Decomposed Fumagillin compounds were not detected
- DCH levels ranged from trace to 234.6 ng g^{-1}
- DCH is more stable than fumagillin in honey
- DCH residues are of greater concern than fumagillin residues in honey
- DCH is an important marker residue when determining Fumidil-B® and Fumagilin-B® usage in apiculture

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