Supporting information

Biosynthesis of 8-hydroxyquinoline-2-carboxylic acid, an iron chelator from the gut of the lepidopteran *Spodoptera littoralis*

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Identification of 8-HQA

Derivatization of regurgitate with diazomethane and analysis by GC-MS showed a compound with an expected molecular ion with m/z=203. (LC-MS $(M+H)^+$: m/z=190; methylation should result in m/z=189+14=203). High resolution MS suggested a molecular formula of $C_{11}H_9NO_3$ for the methyl ester. UV-spectrum (λ_{max} = 258 nm) and MS-fragmentation pattern (171: $M^{+\bullet}$ - MeOH; 143: 171 – CO; 143: 115: 143 – CO; 89: 115 - CN) suggested a heterocycle with carboxylic acid function and an additional oxygen containing substituent like indole, quinolone or iso-quinoline derivatives. Comparison of retention time, co-injection and GC-MS spectrum of methylated, commercially available 8-hydroxy-quinoline-2-carbocylic acid (Sigma-Aldrich) confirmed the identity. LC-MS retention time and mass spectrum of the substance from regurgitate and the commercially available 8-HQA were identical.

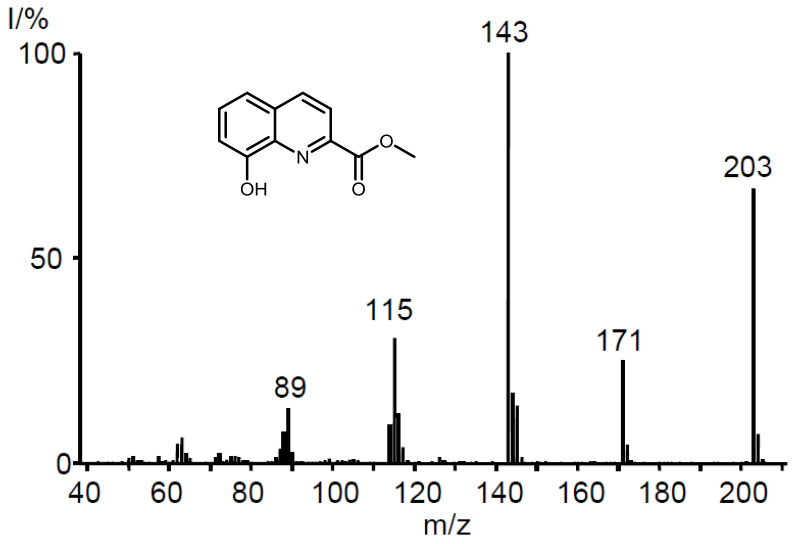


Fig S1: EI mass spectrum of methyl 8-hydroxy-quinoline-2-carboxylate

GC-MS:
Column: Alltech EC5 15 m x 0.25 mm, 0.25 µm; carrier gas: helium; constant pressure: 30 kP; injector temperature: 280 °C; split less mode; temperature program: 50 °C with 10 °C min^{-1} to 100 °C with 5 °C min^{-1} to 300 °C, kept at this temperature for 5 min.

Retention time: 16.32 min
EI-MS (70eV) *m/z* (%): $M^{+\bullet}$ 203 (87), 171 (53), 143 (100), 115 (58), 89 (36), 63 (23).
HR-EI-MS: C11H9NO3 found: $M^{+\bullet}$ 203.0573 calculated: 203.0582.

Quantification of 8-HQA, [$^2H_3$]-HQA and 3-hydroxy-kynurenine in biological samples

Biological samples (regurgitate, whole insects, dissected organs) were diluted or homogenized, respectively in methanol containing 10 µg ml^{-1} kynurenic acid (quantification of 8-HQA, [$^2H_3$]-HQA) or 10 µl ml^{-1} kynurenine (quantification of 3-hydroxy-kynurenine) as internal standard. Quantification by LC-MS was achieved by referencing the peak area of the ion-trace of 8-HQA ($[M+H]^+$ *m/z* =190) or [$^2H_3$]-HQA ($[M+H]^+$ *m/z* =193) to the peak area of kynurenic acid ($[M+H]^+$ *m/z* =190). For 3-hydroxy-kynurenine ion-traces of 3-hydoxy-kynurenine ($[M+H]^+$ *m/z* =225) and kynurenine ($[M+H]^+$ *m/z* =209)

were used. All values were corrected to amount regurgitate collected, amount of methanol used, number of larvae used for pooled regurgitate samples, amount of hemolymph collected or weight of insects extracted, respectively. Detailed information on sample collection, work-up prior to HPLC-MS measurement and HPLC conditions can be found in the particular sections of the article or supporting information.

Insect species tested for 8-HQA

8-HQA not found		8-HQA found
Coleoptera	**Lepidoptera** [l]	**Lepidoptera** [l]
Chrysomelidae (leaf beetles)	Sphingidae (hawk moths)	Noctuidae (owlet moths)
Crioceris asparagi [a*]	*Manduca sexta*	*Spodoptera exigua*
Linaeidea aenea [l]	Yponomeutidae (ermine moths)	*Spodoptera frugiperda*
Plagiodera versicolora [l]	*Yponomeuta evonymellus**	*Spodoptera littoralis*
Gastrophysa viridula [al]	Plutellidae	*Spodoptera eridania*
Phaedon cochleariae [al]	*Plutella xylostella*	Heliothis armigera
Epilachna varivestis [l]	Lasiocampidae (snout moths)	*Heliothis subflexa*
Dytiscidae (diving beetles)	*Malacosoma* sp.*	*Heliothis virescens*
Agabus sp.[a*]	Nymphalidae (brush footed butterflies)	*Orthosia cerasi**
	*Aglais urticae**	

[l]larva, [a]adult, *tentative speciation

Table S1. Insect species tested for 8-HQA. Whole insects have been extracted with methanol in cases where analysis of regurgitate was not possible. Species are grouped by order and family

Feeding of larvae

Minimal diet

A defined minimal-diet was used containing casein as the only protein source.[1] In 800 ml hot water 30 g Agar was dissolved and boiled. Casein (40 g), L-cysteine (1 g), saccharose (40 g), cellulose (50 g), ethyl 4-hydroxybenzoat (2.5 g, Alfa Aesar), sorbic acid (1.5 g), Wesson-salt mixture (10 g , MP), cobalt chloride (2.5 mg), sodium molybdate (2.5 mg), zinc acetate (5 mg) KOH solution(3 ml, 10M) were added and stirred thoroughly. A solution consisting of cooking oil (5 g), cholesterol (0.5 g, Acros), β-carotene (0.25 g), cholecalciferol (5 mg, Enzo Life Sciences), Vanderzant vitamin-mixture (10 g) and menadione (5 mg) in 10 ml ethanol was added after cooling down.

Diet with defined tryptophan concentrations

Casein in minimal diet was replaced by bacterial casein hydrolizate resulting in a tryptophan free diet (Trp-free). This diet was supplemented with 1.95 mmol g^{-1} (normal Trp diet) or 19.5 mmolg^{-1} (Trp-rich) tryptophan.

Reduction of larval gut bacteria

A mixture of erythromycin (73 µg ml^{-1}, Sigma-Aldrich), polymyxin (500 ng ml^{-1}, Sigma-Aldrich), tetracycline (25 µgml^{-1}, Carl Roth) und vancomycin (10 µg ml^{-1}, Sigma-Aldrich) was used. For each larva 100 µl were applied daily onto 1.0 g chemically defined normal-Trp diet.

Antibiotic	Effect	Bacteria affected
Erythromycin	bacteriostatic	Gram-positive
Vancomycin	bacteriostatic bactericidal	Gram-positive
Polymyxin	bactericidal	Gram-negative
Tetracycline	bacteriostatic	Gram-positive Gram-negative

Table S2. Spectrum of antibiotic mixtures applied.

Synthesis of labeled precursors

[^{2}H]-Kynurenic acid

Kynurenic acid (76 mg, 0.4 mmol, Sigma-Aldrich), palladium/charcoal (23 mg), sodium borodeuteride (10 mg, 0.24 mmol) were suspended in 3 ml D_2O. The slurry was heated to mild reflux for two days. The catalyst was filtered through Celite and the filtrate was evaporated *in vacuo* to yield the yellow-colored product (30 mg, 38%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 6.53 (s, 0.54 H) 7.27 (ddd, *J*=8.05, 6.96, 1.09 Hz, 1 H) 7.59 (ddd, *J*=8.42, 6.93, 1.37 Hz, 1 H) 7.92 (d, *J*=8.48 Hz, 1 H) 8.04 (dd, *J*=8.13, 1.49 Hz, 1 H)

^{1}H NMR of [^{2}H]-kynurenic acid indicates deuteration in position 3 with 46% labelling.

LC–MS : retention time 11.1 min (column: Purospher Star RP-18 end- capped, 5 µm, 250×4 mm, mobile phase A : quartz-distilled water with 0.5% v/v acetic acid, mobile phase B : acetonitrile with 0.5% v/v acetic acid, gradient : 10% B for 3 min, up to 100% B in 27 min, 100% B for 17 min, down to 10% B in 1 min, 10% B for 9 min, flow : 0.65 mL ml^{-1}, detection : DAD and ESI^-),

MS (ESI^-): *m/z* = 188.5 (37%, $[M - H]^-$), 189.9 (100%, $[M + 1 - H]^-$), 377.9 (82%, $[2 M + 1 - H]^-$), 379.0 (28%, $[2 M + 2 - H]^-$), where M corresponds to the non-deuterated starting material.

[$^2H_3$]-Xanthurenic acid

Xanthurenic acid (86 mg, 0.42 mmol, Sigma-Aldrich), palladium/charcoal (17 mg), sodium borodeuteride (15 mg, 0.36 mmol) were suspended in 3 ml D_2O. The slurry was heated to mild reflux overnight. The catalyst was filtered through Celite and the filtrate was evaporated *in vacuo* to yield the yellow-colored product (37 mg, 42%).

^{1}H NMR (500 MHz, D_2O) δ ppm 6.72 (s, 0.79 H) 7.04 (d, *J*=7.56 Hz, 0.39 H) 7.18 (dd, *J*=8.25, 7.79 Hz, 1 H) 7.44 (d, *J*=8.25 Hz, 0.53 H)

^{1}H NMR of [$^2H_3$]-xanthurenic acid does not allow for direct evaluation of the deuteration grade. Setting the deuteration of the least reactive hydrogen at C-6 (7.18 ppm) to 0%, the degree of deuteration at C-3 is 22%, and at C-5,7 61% and 47%.

LC–MS : retention time : 9.7 min (column : Purospher Star RP-18 end- capped, 5 µm, 250×4 mm, mobile phase A : quartz-distilled water with 0.5% v/v acetic acid, mobile phase B : acetonitrile with 0.5% v/v acetic acid, gradient 10% B for 3 min, up to 100% B in 27 min, 100% B for 17 min, down to 10% B in 1 min, 10% B for 9 min, flow : 0.65 mL ml^{-1}, detection : DAD and ESI^-)

MS (ESI^-): m/z = 204.6 (34%, $[M - H]^-$), 205.6 (100%, $[M + 1 - H]^-$), 206.6 (43%, $[M + 2 - H]^-$), 207.3 (25%, $[M + 3 - H]^-$), 208.3 (5%, $[M + 4 - H]^-$), where M corresponds to the non-deuterated starting material.

[$^2H_3$]-3-Hydroxy-kynurenine

3-Hydroxykynurenine (9 mg, 0.35 mmol; Sigma-Aldrich) was dissolved in concentrated deuterium chloride in deuterium oxide (340 µl) and the solution was stirred at 100 °C in a closed vial for 12 h. Evaporation of the solvent *in vacuo* yielded the colorless product.

^{1}H NMR (500 MHz, DCl in D_2O, 333K) δ ppm 4.54 (s, 1 H) 7.36 (s, 1 H) 7.65 (s, 1 H)

^{1}H NMR (DCl in D_2O) indicates complete exchange of the enolic protons at C-3, and complete exchange of H-5' in the ring, while H-4' and H-6' were not affected by the reaction.

Retention time : 3.9 min (column : Purospher Star RP-18 endcapped, 5 µm, 250×4 mm, mobile phase A : quartz-distilled water with 0.5% v/v acetic acid, mobile phase B : acetonitrile with 0.5% v/v acetic acid, gradient : 10% B for 3 min, up to 100% B in 30 min, 100% B for 17 min, down to 10% B in 1 min, 10% B for 9 min, flow : 0.65 ml min^{-1}, detection: ESI^-).

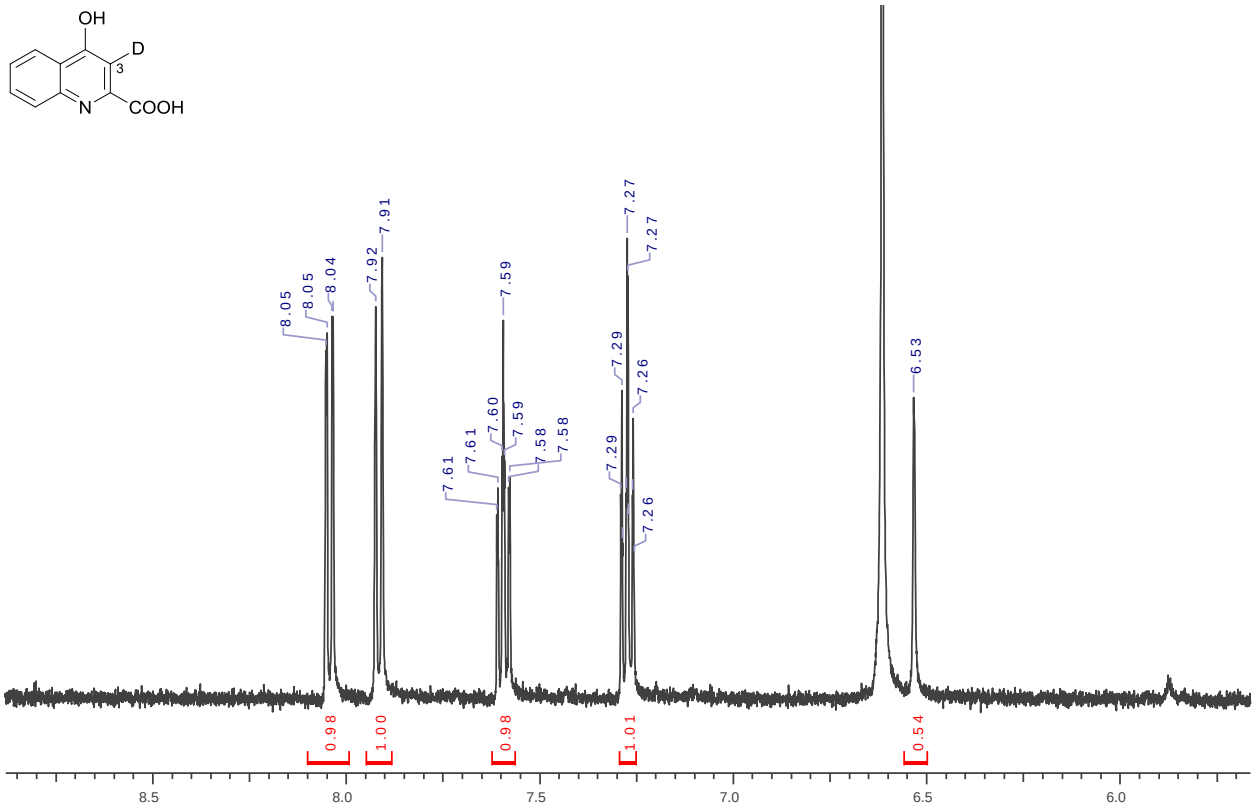
MS (ESI^-): *m/z* = 224.5 (9%, $[M + 2 - H]^-$), 226.0 (100%, $[M + 3 - H]^-$), 227.0 (91%, $[M + 4 - H]^-$), 228.0 (43%, $[M + 5 - H]^-$), where M denotes the un-deuterated molecule.

References:

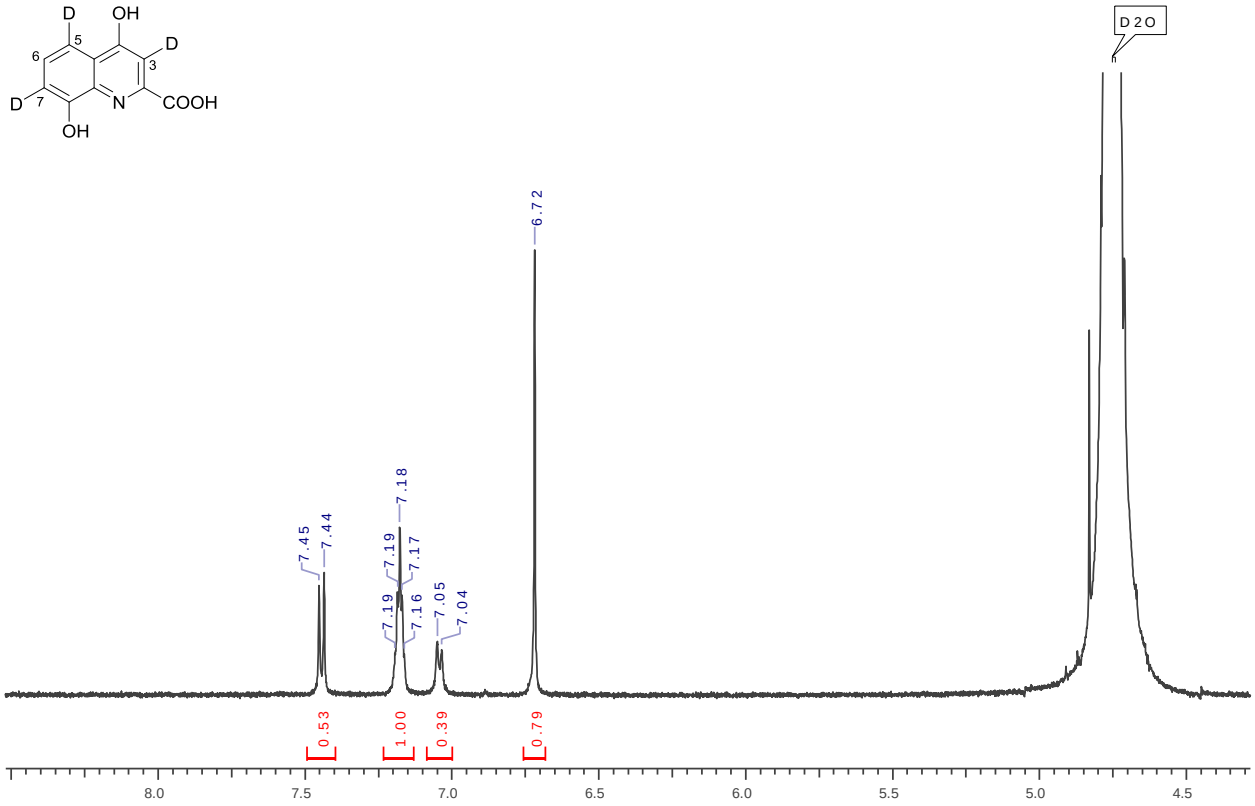
1. E. S. Vanderzant, Ann Entomol Soc Am, 1968, 61, 120-125.

^{1}H-NMR spectra of deuterated precursors

[^{2}H]-Kynurenic acid



[$^2H_3$]-Xanthurenic acid



[$^2H_3$]-3-Hydroxy-kynurenine