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SUPPLEMENTARY DATA

A ^{1}H NMR based study of metabolites profiling of garden snail *Helix lucorum* hemolymph

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EXPERIMENTAL

^{1}H NMR spectroscopy

Each sample, consisting of typically 50 mg of lyophilized hemolymph from *Helix lucorum* (< 1kD or < 3kD) was extracted using 1 ml phosphate buffer (pH was adjusted to 7.35 ± 0.05) under stirring for 2 h. The solution was centrifugated for 10 min and 540 μl of natant was transferred into a 5 mm NMR tube and 60 μl of D_2O containing 1.86 mM 3-(trimethylsilyl)-propionic-d4 acid sodium salt (TSP-d4) was added to yield a final TSP concentration of 0.186 ± 0.05 mM. The sample was allowed to equilibrate at 298 K for 15 min prior to data acquisition. ^{1}H NMR spectra were acquired on a Bruker Avance II+ spectrometer operating at 14.1 T (corresponding to a proton Larmor frequency of 600 MHz), equipped with an Z-gradient 5 mm BBO probe. Temperature was set to 298.0 K, and controlled within ±0.1 K by means of the B-VT 3000 VTU system. All NMR spectra were acquired with water suppression by on-resonance pre-irradiation of the water signal using 150 Hz bandwidth of the water suppression pulse. The carrier frequency SFO1 was adjusted sample-by-sample within 0.15 Hz precision for optimal

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water suppression. The ^{1}H-NMR spectra were acquired with the 1D-noesy pulse sequence. Typical acquisition parameters included a 5 s relaxation delay, 256 scans, 4 dummy scans, 16 ppm (9615 Hz) spectral width, 64 K complex points, 3.41 s acquisition time, 10 ms mixing time, and 150 Hz bandwidth of the water suppression pulse, total acquisition time about 30 min. Data were multiplied by an exponential decay function with a line-broadening factor of 0.3 Hz, prior to Fourier transformation (FT) and phase correction. Several experiments were acquired for unambiguous assignment of the metabolites: 1D sequence with presaturation using composite pulse and spoil gradient [1] in order to achieve better solvent suppression, 1D sequence with water suppression using excitation sculpting with gradients using perfect echo [2, 3] in order to get superior suppression of the water signal and to increase the sensitivity, 1D selective TOCSY, 2D J-resolved (JRES), 2D COSY and 2D HSQC.

Homonuclear 2D-COSY and heteronuclear 2D ^{1}H,^{13}C HSQC experiments were measured as well on most of the samples to assign the metabolite resonances. Two-dimensional ^{1}H,^{13}C HSQC NMR experiments were acquired using the hsqcetgpprsisp.3 Bruker pulse sequence (HSQC with sensitivity improvement, echo/anti-echo-TPPI gradient selection, decoupling during acquisition, and presaturation during the relaxation delay). Typical acquisition parameters included: 3.0 s relaxation delay, 64 scans, 16 dummy scans, 0.085 s acquisition time, 145 Hz for direct XH coupling constant, 1024 × 200 complex data point, and 6009 Hz (10 ppm) and 26410 Hz (175 ppm) spectral widths in F2 and F1. Data were zero-filled to a 1024 × 1024 data matrix and treated with squared cosine window function (along F2 and F1) prior to FT in phase-sensitive mode. The 2D COSY NMR spectra were acquired using the cosygpmfqf Bruker pulse programme (gradient selected, double quantum filter, magnitude mode, adapted with presaturation during the relaxation delay). Acquisition parameters included: 3 s relaxation delay, 16 scans, 16 dummy scans, 0.322 s acquisition time, 4096 × 256 data points, and 6356 Hz (10 ppm) spectral width (in F2 and F1). Data were treated with sine window function (along both F2 and F1) prior to FT.

1D selective TOCSY experiments with water suppression were measured as well on most of the samples to identify the metabolites and unambiguously assign the resonances. 1D selective TOCSY experiments were acquired using the seldigpzs Bruker pulse sequence (1D homonuclear Hartman-Hahn transfer using DIPSI2 sequence for mixing using selective refocussing with a shaped pulse with zero quantum suppression with presaturation during the relaxation delay).

Typical acquisition parameters included a 2 s relaxation delay, 1024 scans, 4 dummy scans, 9615 Hz (16 ppm) spectral width, 64 K complex points, 3.41 s acquisition time, 80 ms 180 degree Gaussian refocusing pulse, total acquisition time about 30 min.

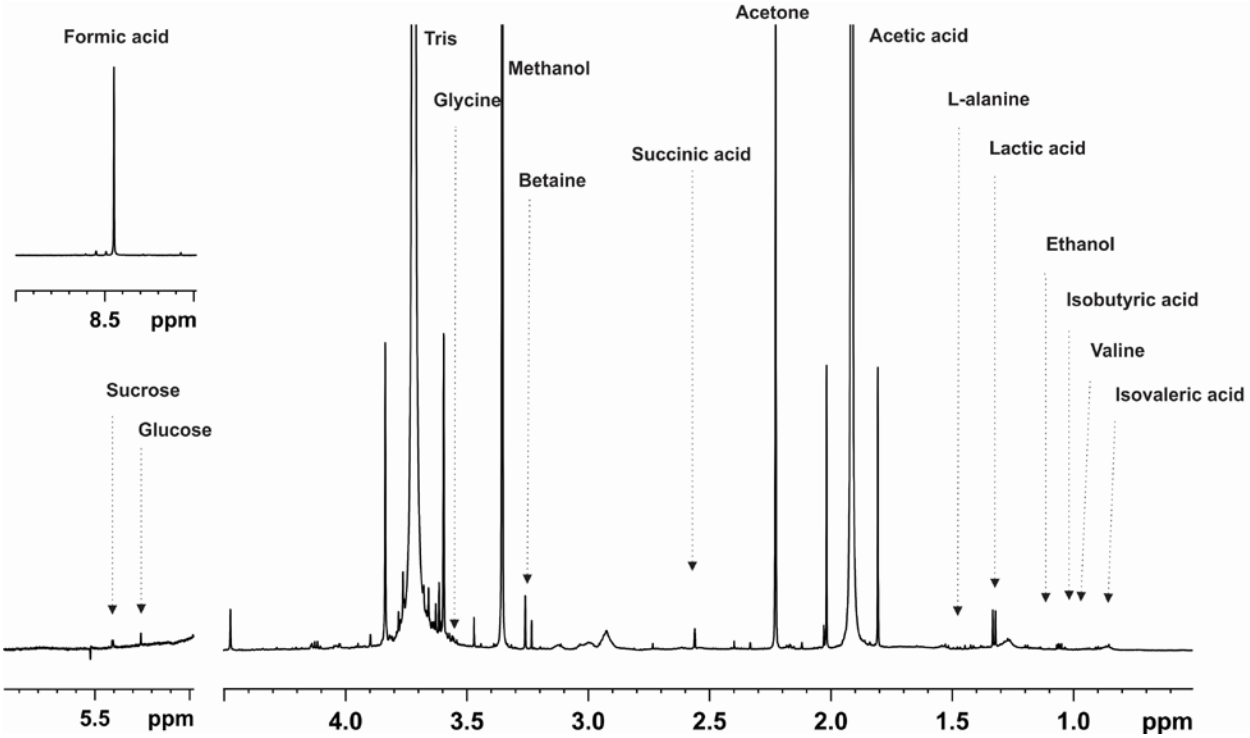

Fig. S1. Typical ^{1}H-NMR spectrum of lyophilized hemolymph from *Helix lucorum* (< 3 kD). (1D-noesy pulse sequence, with pre-irradiation of water, pH=7.35, 298.0 K), with resonance assignment. The spectral regions devoid of signals, that contain the residual water signal, and that of reference TSP as well have been cut away.

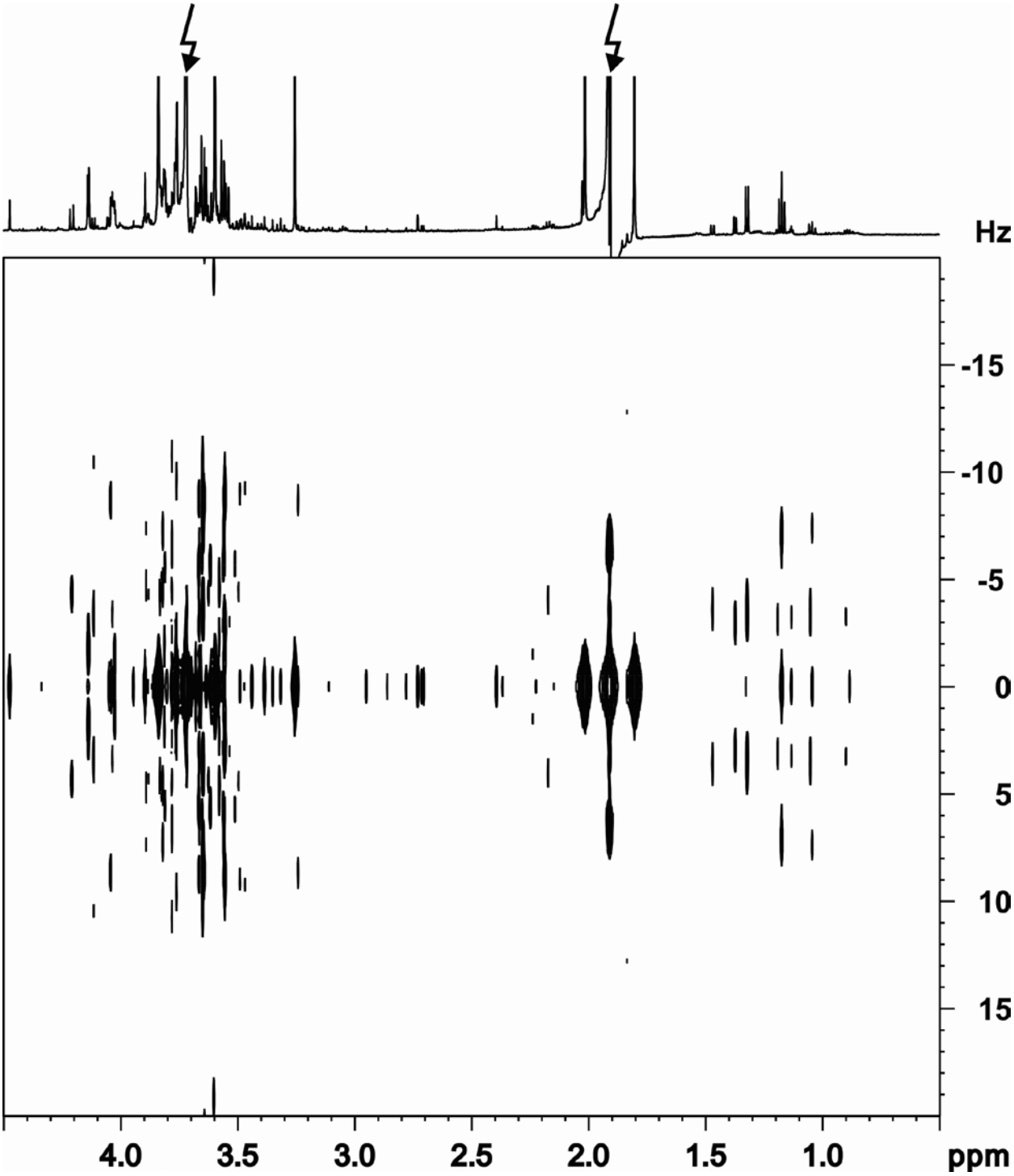

Fig. S2. Typical 2D JRES spectrum of lyophilized hemolymph from *Helix lucorum* (< 1 kD) in the range from 4.5 to 0.5 ppm, pH=7.35, 298.0 K, with simultaneous suppression of signals of water, Tris and Acetic acid.

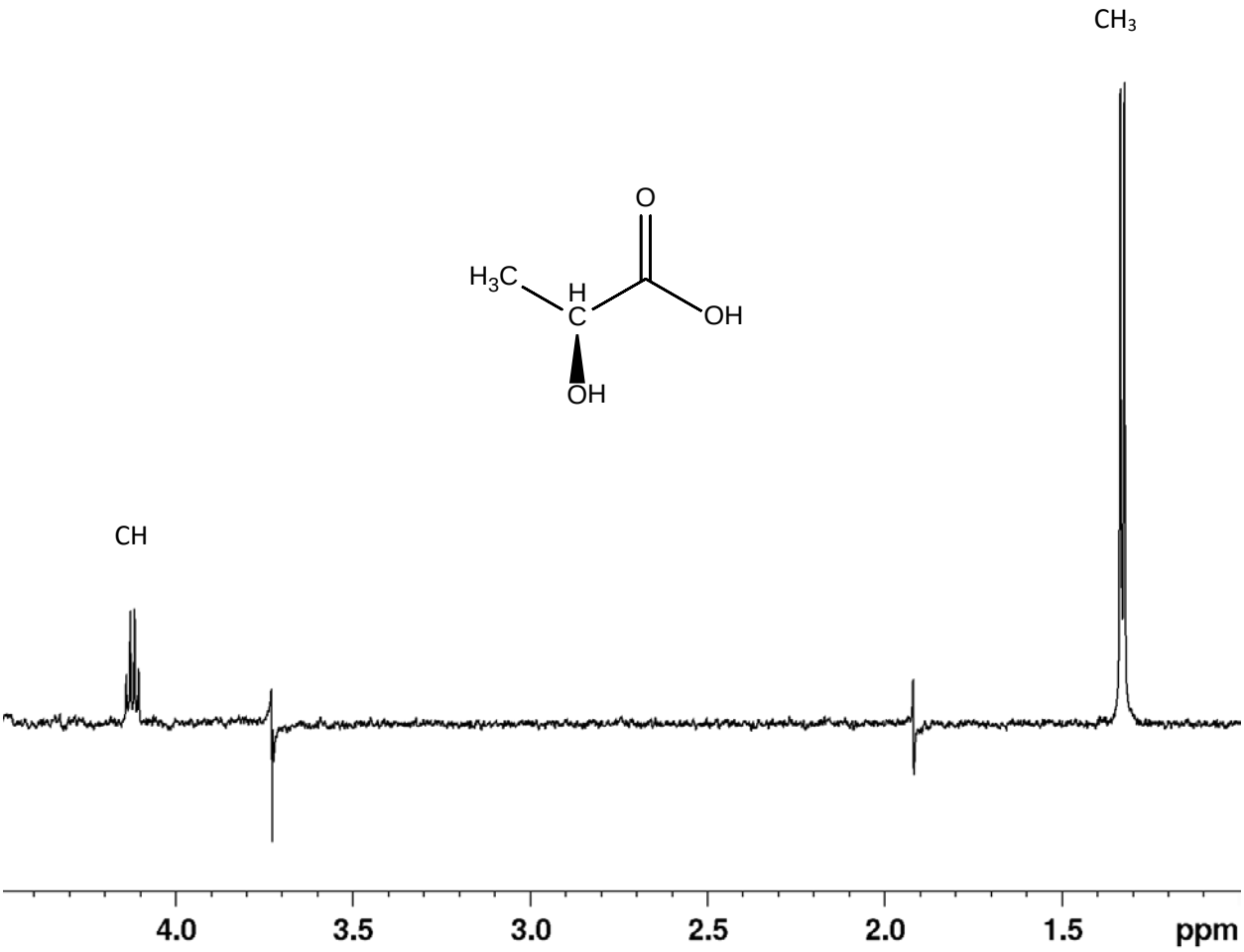

Fig. S3. Typical 1D selective TOCSY with water suppression of lyophilized hemolymph from *Helix lucorum* (< 1kD) revealing the metabolite lactic acid (lactate).

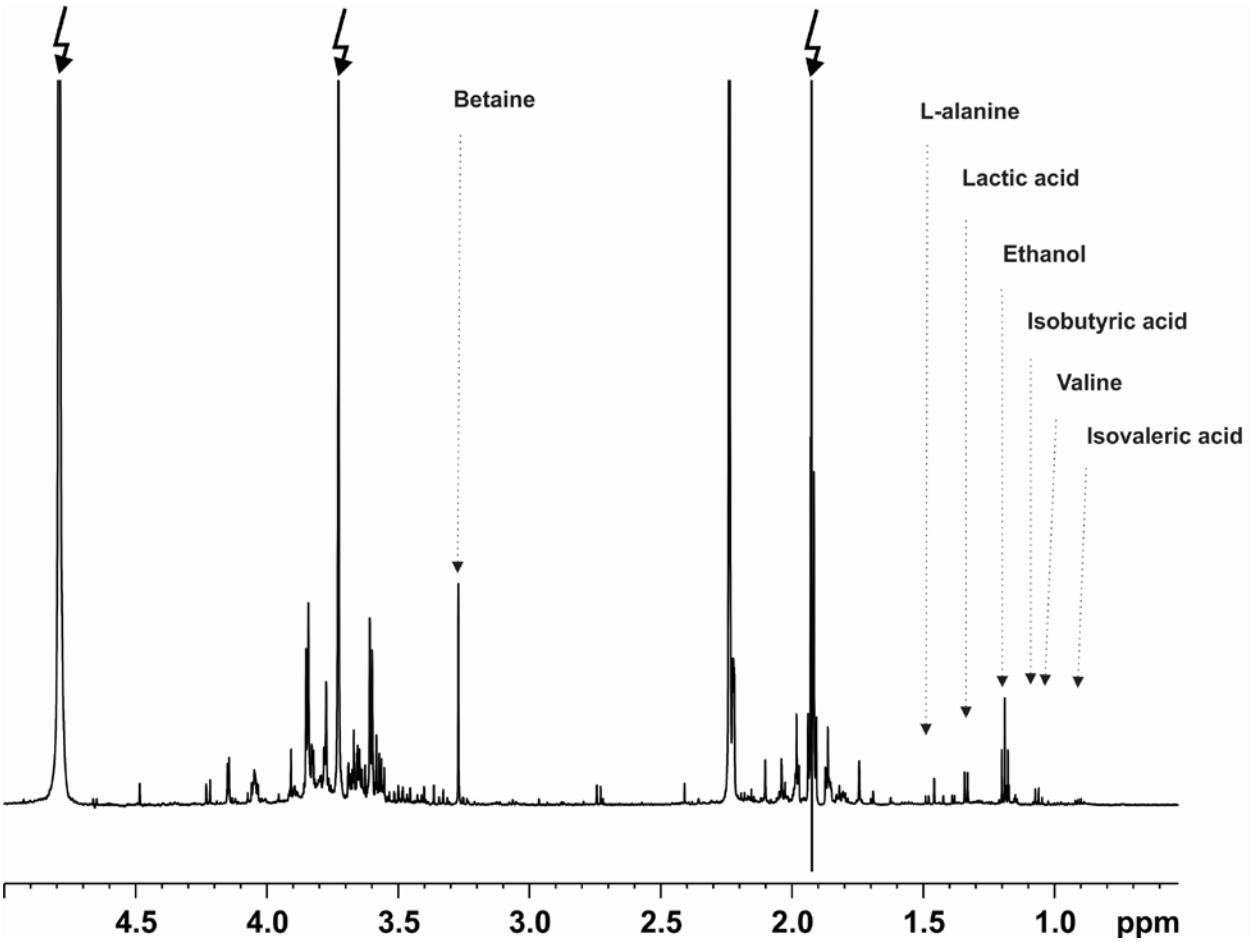

Fig. S4. Typical ^{1}H 1D NOESY NMR spectrum of lyophilized hemolymph from *Helix lucorum* (< 1kD) in the range from 4.5 to 0.5 ppm, pH=7.35, 298.0 K, with simultaneous suppression of signals of water, Tris and Acetic acid.

REFERENCES

1. A. Bax, *Journal of Magnetic Resonance (1969)*, **65**, 142 (1985).
2. T.L. Hwang, A.J. Shaka, *Journal of Magnetic Resonance, Series A*, **112**, 275 (1995).
3. R.W. Adams, C.M. Holroyd, J.A. Aguilar, M. Nilsson, G.A. Morris, *Chemical Communications*, **49**, 358 (2013).