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Supplemental Information

Ascaroside Signaling Is Widely Conserved among Nematodes

Andrea Choe, Stephan H. von Reuss, Dima Kogan, Robin B. Gasser, Edward G. Platzer, Frank C. Schroeder, and Paul W. Sternberg

Supplemental Inventory

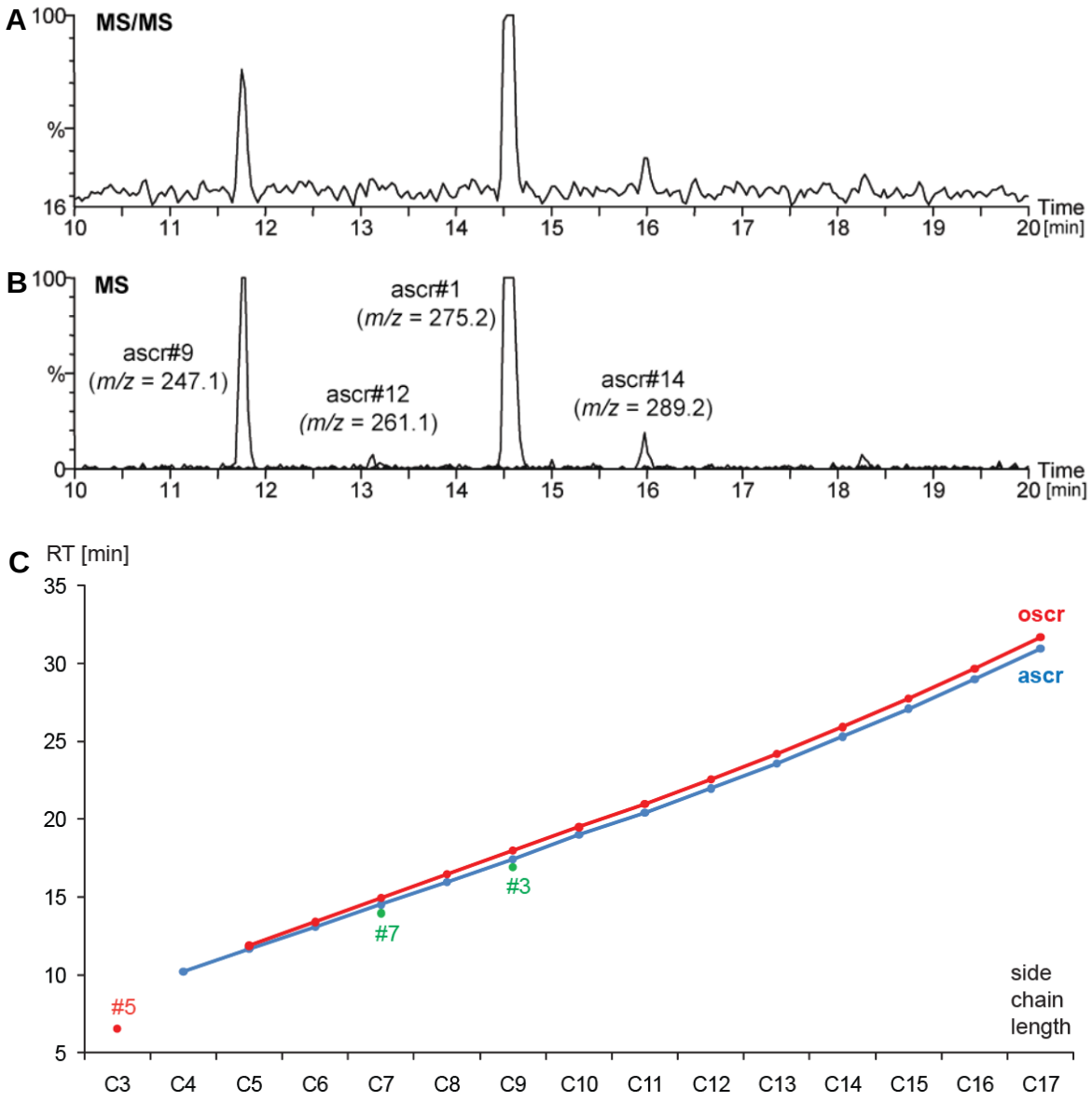


Figure S1. Identification of Ascarosides, Related to Figure 1

(A) Total ion current (TIC) chromatogram from LC-MS/MS screen for precursor ions of *m/z* 73.0 reveals ascaroside signals in worm water extract of adult *S. glaseri*.

(B) Corresponding ion traces for ascr#9, ascr#12, ascr#1, and ascr#14 from LC-MS measurement.

(C) HPLC retention times of ascarosides (ascr's) and omega-ascarosides (oscr's), side chains range from 3–17 carbons, derived from analysis of wild-type and peroxisomal beta-oxidation mutants of *C. elegans* and synthetic standards. See Figure 1.

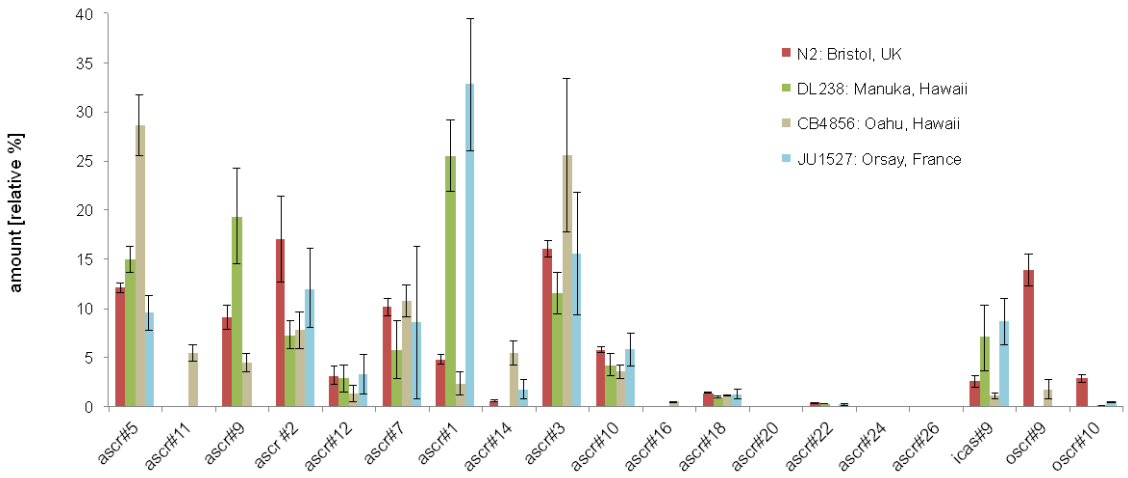


Figure S2. HPLC-MS Analysis of Worm Media Samples Obtained from Several Strains of *C. elegans*, Related to Figure 2

Error bars are S.D. See Figure 2.

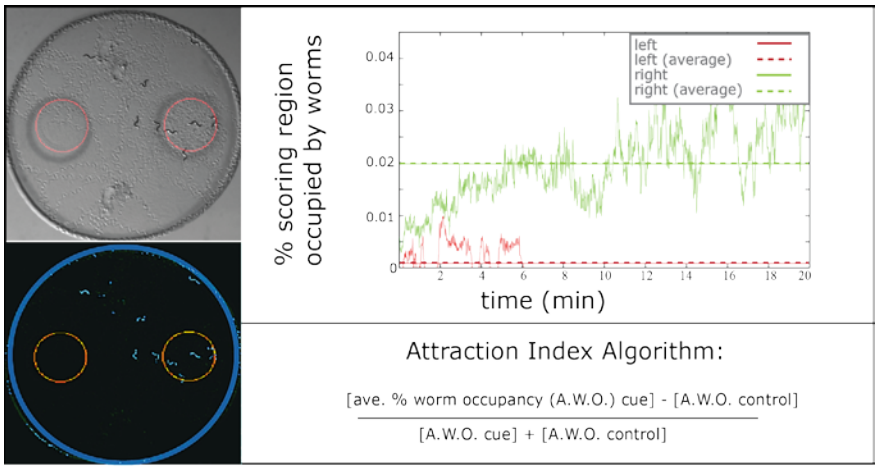


Figure S3. Automated Software Scoring Worm Retention in Areas Conditioned by Ascarosides, Related to Figure 3

Our software detects the presence of worms in both regions and provides an output of the percentage of each scoring region occupied by worms, which is calculated once per second during the entire trial. The output is a plot of worm occupancy ratio vs. time for both scoring regions (right panel). We adopted the scoring index described by Bargmann *et al.* (1993) [47]. See Figure 3.

Supplemental Experimental Procedures

2.1. Automated Software (for Retention Assay): A video camera attached to the microscope produces a digital video stream, which is then analyzed. The ratio of time the average worm spends in each region of interest is calculated for every trial. For ease of implementation, we assumed that all worms in a single experiment are roughly the same size. We thus counted worm pixels instead of whole worms, allowing us to take into account fractions of a worm in the region of interest. It also eliminated the need for a shape-based worm identification algorithm, and allowed each frame to be analyzed independently. We applied a band-pass filter to each frame to eliminate the effect of uneven lighting and also accentuate the worms against the background. The worm was then identified after thresholding the filtered image. Throughout each experiment, we know the locations and sizes of the regions of interest. Through the filtering described above, we know which pixels are occupied by worms and which ones are not. We were then able to calculate the ratio of worm-pixels to all pixels inside the region of interest to produce the worm-occupancy ratio. This calculation is done for every frame, giving us a plot output of worm-occupancy ratio vs. time for each region.

2.2. Culture of Nematodes and Collection of worm Exudates: *C. elegans, Rhabditis sp., O. tipulae, C. sp.7, P. pacificus, P. redivivus, Koernia sp.,* and *P. strongyloides* were maintained on standard 6 cm agar plates with *E. coli* OP50. To grow large amounts, we grew them in liquid culture using S complete medium with *E. coli* HB101 at 20°C at 250 rpm in an incubator shaker. The worms were exposed to several wash and filtration steps using S Basal to remove bacteria. The worms were collected between the washes by centrifugation at 3000 rpm for 5 minutes. We then incubated the worms at 1 worm/µL in S Basal for 6 hours. The worms were then filtered out and the remaining supernatant was passed through a 0.22 µm filter before being stored at -20°C.

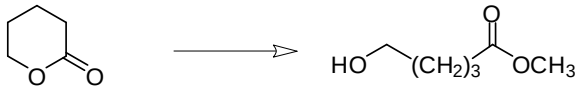
S. carpocapsae, S. glaseri, S. riobrave, and *H. bacteriophora* were maintained as described by Hallem, E.A. et al. (2011) [50]. Briefly, several last-instar *Galleria mellonella* larvae were placed on a 6 cm Petri dish with a 55 mm Whatman 1 filter paper. Approximately 500-1000 IJs were placed on this filter paper. After 7 days, the insect cadavers were placed on an inverted 6 cm Petri plate carrying a Whatman 1 filter paper and immersed in a small volume of ddH_2O in a 10 cm Petri dish. Emerging IJs were collected and washed several times as described above, before being incubated in ddH_2O at 1 worm/µL for 6 hours. Adults were dissected out of insect cadavers after 3-4 days post-infection and washed as described above and incubated at 1 worm/10 µL (given their large size) or grown on OP50 for use on the holding assay.

N. brasiliensis, A. suum, P. penetrans, and *Romanomermis spp.* were provided by collaborators (Table S1). *N. brasiliensis* was incubated in 0.85% saline at 1worm/ 10 µL (given their large size) at 30°C. *A. suum* were washed in sterile saline before being incubated in DMEM for 3, 6, and 13 hours at 37°C. The supernatants were tested individually, then subsequently pooled for combined analysis. *P. penetrans* and *Romanomermis spp.* were incubated in ddH_2O at 20°C at 250 rpm overnight.

2.3. Worm Sample Preparation: Worm water samples were lyophilized, extracted with 2 x 1-10 ml methanol, and filtered over cotton wool. Extracts were concentrated in vacuum and resulting residues were resuspended in 150 µL methanol and filtered.

2.4. Syntheses of Ascarosides:

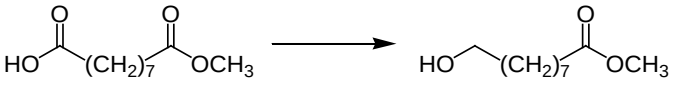
Methyl 5-hydroxypentanoate



1 **2**

Freshly distilled δ-valerolactone (**1**) (856 mg) in methanol (17 ml) was treated with conc. H_2SO_4 (100 µl) and refluxed for 12 h [51]. The solution was cooled to -20 °C, treated with $NaHCO_3$ (80 mg), and stirred for 10 min, after which the mixture was filtered and the solvent removed in vacuum. The residue was taken up in dichloromethane (10 ml), dried over Na_2SO_4, filtered, and concentrated in vacuum to give methyl 5-hydroxypentanoate (**2**) (1071 mg, 94%) as a colorless oil, which was used directly without any further purification. ^{1}H NMR (400 MHz, acetone-d_6): δ 1.55 (2H, m), 1.68 (2H, m), 2.35 (2H, t, J = 7.4 Hz), 3.57 (2H, m), 3.64 (3H, s); ^{13}C NMR (100 MHz, acetone-d_6): δ 22.2, 32.9, 34.1, 51.5, 61.9, 174.2.

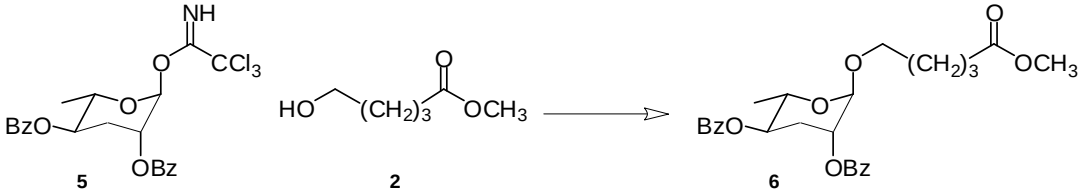
Methyl 9-hydroxynonanoate



3 **4**

A solution of nonanedioic acid monomethyl ester (**3**) (923 mg, 4.6 mmol) in dry tetrahydrofuran (3 ml) at -20 °C was treated with 1 M BH_3 in tetrahydrofuran (4.6 ml, 4.6 mmol) over 10 min [52]. After stirring at RT for 4 h, the reaction was quenched with 0.77 M aqueous K_2CO_3 solution (10 ml) at 0 °C. The product was extracted with diethyl ether (3 x 20 ml), washed with saturated aqueous NaCl solution, dried over Na_2SO_4, and concentrated in vacuum to afford methyl 9-hydroxynonanoate (**4**) (850 mg, 99% yield) as a colorless oil which was used directly without any further purification. ^{1}H NMR (400 MHz, chloroform-d_1): δ 1.27-1.37 (8H, m), 1.50-1.66 (4H, m), 2.29 (2H, t, J = 7.5 Hz), 3.62 (2H, t, J = 6.5 Hz), 3.66 (3H, s).

Methyl 5-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(*2H*)-tetrahydropyran-2-yloxy)-pentanoate

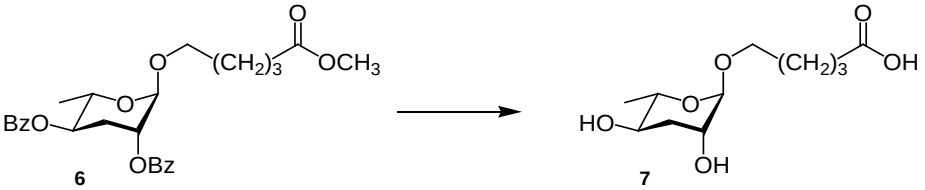


5 **2** **6**

A solution of 2,4-di-*O*-benzoyl-ascarylose-1-(2,2,2-trichloroacetimide) (**5**) (132 mg, 263 µmol, prepared according to Butcher, R.A. et al. (2007) [12]) and **2** (125 mg, 950 µmol) in dry dichloromethane (3 ml) at 0 °C was treated with trimethylsilyloxy triflate (5 µl). After 3 h the solution was washed with saturated aqueous $NaHCO_3$ solution (0.5 ml), dried over Na_2SO_4, and concentrated in vacuum. Flash column chromatography on silica gel using a 20 – 40% (*v/v*) ethyl acetate gradient in *n*-hexane afforded **6** (66.8 mg, 142 µmol, 47%) as a colorless oil. ^{1}H NMR (400 MHz, acetone-d_6): δ 1.28 (3H, d, J = 6.2 Hz), 1.67-1.80

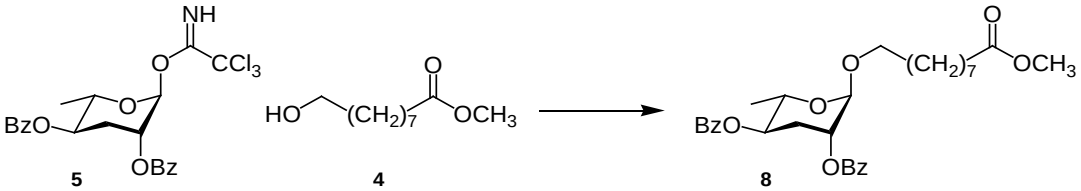
(4H, m), 2.23 (1H, m), 2.40 (2H, t, *J* = 6.9 Hz), 2.48 (1H, m), 3.58 (1H, m), 3.64 (3H, s), 3.83 (1H, m), 4.13 (1H, dq, *J* = 9.8 Hz, *J* = 6.0 Hz), 4.87 (1H, s.br), 5.15 (1H, ddd, *J* = 11.0 Hz, *J* = 10.4 Hz, *J* = 4.5 Hz), 5.18 (1H, s.br), 7.50-7.60 (4H, m), 7.62-7.72 (2H, m), 8.05 (2H, d, *J* = 7.5 Hz), 8.11 (2H, d, *J* = 7.5 Hz); ^{13}C NMR (100 MHz, acetone-d_6): δ 18.3, 22.5, 29.6, 30.4, 34.0, 51.5, 67.5, 67.9, 71.4, 71.5, 97.0, 129.4, 129.5, 130.3, 130.4, 131.0, 131.0, 134.1, 134.2, 165.9, 166.0, 174.0.

5-(3'*R*,5'*R*-Dihydroxy-6'*S*-methyl-(*2H*)-tetrahydropyran-2-yloxy)-pentanoic acid (oscr#9)



A solution of **6** (66.8 mg, 142 µmol) in dry tetrahydrofuran (0.5 ml) was added to a mixture of LiOH (28 mg, 1.4 mmol) and water (0.6 ml) in 1,4-dioxane (4 ml). After stirring at 66 °C for 2 h the solution was acidified with glacial acetic acid and concentrated in vacuum. Flash column chromatography on silica gel using a 5 – 30% (*v/v*) methanol gradient in dichloromethane containing 1% glacial acetic acid afforded oscr#9 (**7**) (26 mg, 105 µmol, 74%) as a colorless oil. ^{1}H NMR (400 MHz, methanol-d_4): δ 1.22 (3H, d, *J* = 6.0 Hz), 1.58-1.72 (4H, m), 1.77 (1H, ddd, *J* = 13.1 Hz, *J* = 11.1 Hz, *J* = 3.2 Hz), 1.95 (1H, ddt, *J* = 13.1 Hz, *J* = 3.7 Hz, *J* = 0.9 Hz), 2.33 (2H, t, *J* = 7.2 Hz), 3.43 (1H, dt, *J* = 9.6 Hz, *J* = 6.0 Hz) 3.47 – 3.59 (2H, m), 3.71 (1H, dt, *J* = 9.8 Hz, *J* = 6.2 Hz), 3.77 (1H, m), 4.50 (1H, s); ^{13}C NMR (100 MHz, methanol-d_4): δ 18.1, 23.0, 30.1, 34.7, 36.0, 67.9, 68.3, 69.4, 70.9, 100.4, 177.5; ESI-MS (negative mode) *m/z* = 247.1 [M-H].

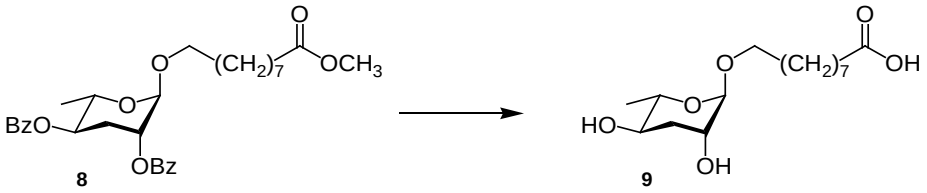
Methyl 9-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(*2H*)-tetrahydropyran-2-yloxy)-nonanoate



A solution of 2,4-di-*O*-benzoyl-ascarylose-1-(2,2,2-trichloroacetimide) (**5**) (132 mg, 263 µmol, prepared according to Butcher, R.A. et al. (2007) [12]) and **4** (112.8 mg, 600 µmol) in dry dichloromethane (3 ml) at 0 °C was treated with trimethylsilyloxy triflate (5 µl). After 3 h the solution was washed with saturated aqueous $NaHCO_3$ solution (0.5 ml), dried over Na_2SO_4 and concentrated in vacuum. Flash column chromatography on silica gel using 20–40% (*v/v*) ethyl acetate gradient in *n*-hexane afforded **8** (99.3 mg, 190 µmol, 72% yield) as a colorless oil. ^{1}H NMR (400 MHz, acetone-d_6): δ 1.28 (3H, d, 6.2 Hz), 1.30 – 1.40 (6H, m), 1.40 – 1.49 (2H, m), 1.56 – 1.72 (2H, m), 2.22 (1H, ddd, *J* = 13.6 Hz, *J* = 11.5 Hz, *J* = 3.2 Hz), 2.30 (2H, t, *J* = 7.5 Hz), 2.46 (1H, m), 3.55 (1H, dt, *J* = 9.8 Hz, *J* = 6.5 Hz), 3.60 (3H, s), 3.81 (1H, dt, *J* = 9.6 Hz, *J* = 6.6 Hz), 4.13 (1H, dq, *J* = 9.7 Hz, *J* = 6.2 Hz), 4.86 (1H, s.br), 5.15 (1H, ddd, *J* = 11.4 Hz, *J* = 9.8 Hz, *J* = 4.6 Hz), 5.18 (1H, m),

7.50 – 7.60 (4H, m), 7.63 – 7.71 (2H, m), 8.04 (2H, m), 8.11 (2H, m); ^{13}C NMR (100 MHz, acetone-d_6): δ 18.3, 25.6, 26.8, 29.7, 29.9, 30.0, 30.2, 30.4, 34.4, 51.4, 67.4, 68.2, 71.4, 71.5, 97.0, 129.4, 129.5, 130.2, 130.3, 130.9, 131.0, 134.1, 134.2, 165.9 (2C), 174.3.

9-(3'*R*,5'*R*-Dihydroxy-6'*S*-methyl-(*2H*)-tetrahydropyran-2-yloxy)-nonanoic acid (oscr#10)



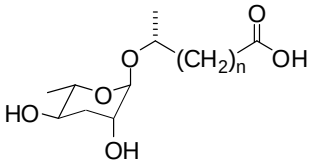
A solution of **8** (99.3 mg, 190 µmol) in tetrahydrofuran (500 µl) was added to a mixture of LiOH (38 mg, 1.9 mmol) and water (800 µl) in 5 ml 1,4-dioxane (5 ml). After stirring at 66 °C for 3 h the solution was acidified with acetic acid and concentrated in vacuum. Flash column chromatography on silica gel using a 5–30% (*v/v*) methanol gradient in dichloromethane containing 1% glacial acetic acid afforded **9** (49 mg, 161 µmol, 85%) as a colorless oil. ^{1}H NMR (400 MHz, methanol-d_4): δ 1.22 (3H, d, *J* = 6.1 Hz), 1.32-1.43 (8H, m), 1.56-1.63 (4H, m), 1.77 (1H, ddd, *J* = 13.1 Hz, *J* = 11.1 Hz, *J* = 3.2 Hz), 1.96 (1H, ddt, *J* = 13.1 Hz, *J* = 3.7 Hz, *J* = 0.9 Hz), 2.28 (2H, t, *J* = 7.4 Hz), 3.41 (1H, dt, *J* = 9.6 Hz, *J* = 6.2 Hz) 3.49 – 3.59 (2H, m), 3.68 (1H, dt, *J* = 9.8 Hz, *J* = 5.5 Hz), 3.76 (1H, m), 4.49 (1H, s); ^{13}C NMR (100 MHz, methanol-d_4): δ 17.3, 25.2, 26.4, 28.0, 29.3, 29.5, 29.6, 30.5, 34.1, 61.1, 67.4, 68.5, 69.9, 99.4, 176.8; ESI-MS (negative ion mode) *m/z* = 303.2 [M-H].

Table S1. Nematode Strains

Species	Strain	Source or References
Nippostrongylus brasiliensis	no designation	Edward G. Platzer
Heterorhabditis bacteriophora	M31e	Sternberg collection
Rhabditis sp.	AF5	Caenorhabditis Genetics Center
Oscheius tipulae	PS1305	Sternberg collection
Oscheius dolichuroides	PS1727	Sternberg collection
Oscheius carolinensis	no designation	Sternberg collection
Caenorhabditis elegans	N2	Sternberg collection
Caenorhabditis sp.7	JU1325	Marie-Anne Felix
Ascaris suum	no designation	Robin Gasser
Pristionchus pacificus	RS2333	Ralf Sommer
Koernia sp.	RS1982	Ralf Sommer
Pratylenchus penetrans	3/C	Antoon T. Ploeg
Panagrellus redivivus	PS2298	Sternberg collection
Pelodera strongyloides	DF5022	Caenorhabditis Genetics Center
Steinernema carpocapsae	ALL	Sternberg collection
Steinernema scapterisci	no designation	Sternberg collection
Steinernema riobrave	no designation	Sternberg collection
Steinernema glaseri	VII	S. Patricia Stock
Romanomermis iyengari	no designation	Edward G. Platzer
Romanomermis culicivorax	3B4	Edward G. Platzer
Caenorhabditis elegans	DL238	J. Scott Cameron
Caenorhabditis elegans	CB4856	Caenorhabditis Genetics Center
Caenorhabditis elegans	JU1527	Marie-Anne Felix

All nematode strains used in this study are listed here; species without a strain identification are labeled “no designation”.

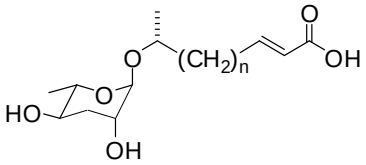
Table S2A. Structures and HPLC-ESI-MS Data of (ω-1)-Oxygenated Saturated Ascarosides



Side chain (n)	SMID[1]	Molecular formula	Molecular mass [g/mol]	*m/z* $[M-H]^-$
1	ascr#11	$C_{10}H_{18}O_6$	234.1103	233.10
2	ascr#9	$C_{11}H_{20}O_6$	248.1260	247.12
3	ascr#12	$C_{12}H_{22}O_6$	262.1416	261.13
4	ascr#1	$C_{13}H_{24}O_6$	276.1573	275.15
5	ascr#14	$C_{14}H_{26}O_6$	290.1729	289.17
6	ascr#10	$C_{15}H_{28}O_6$	304.1886	303.18
7	ascr#16	$C_{16}H_{30}O_6$	318.2042	317.20
8	ascr#18	$C_{17}H_{32}O_6$	332.2199	331.21
9	ascr#20	$C_{18}H_{34}O_6$	346.2355	345.23
10	ascr#22	$C_{19}H_{36}O_6$	360.2512	359.24
11	ascr#24	$C_{20}H_{38}O_6$	374.2668	373.26
12	ascr#26	$C_{21}H_{40}O_6$	388.2825	387.28

[1]SMID: Small Molecule IDentifier for small molecules identified from *C. elegans* and other nematodes. The Small Molecule Metabolite Identifier database (www.smid-db.org) is an electronic resource maintained by Frank Schroeder and Lukas Mueller at the Boyce Thompson Institute in collaboration with WormBase. The purpose of this database is to introduce searchable, gene-style identifiers, "SMIDs", for all small molecules newly identified from *C. elegans* and other nematodes.

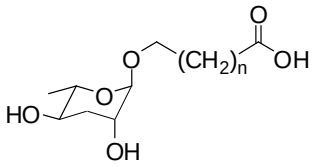
Table S2B. Structures and HPLC-ESI-MS Data of (ω-1)-Oxygenated Unsaturated Ascarosides



Side chain (n)	SMID[1]	Molecular formula	Molecular mass [g/mol]	*m/z* $[M-H]^-$
2	ascr#7	$C_{13}H_{22}O_6$	274.1416	273.13
4	ascr#3	$C_{15}H_{26}O_6$	302.1729	301.17

[1]SMID: Small Molecule IDentifier for small molecules identified from *C. elegans* and other nematodes. The Small Molecule Metabolite Identifier database (www.smid-db.org) is an electronic resource maintained by Frank Schroeder and Lukas Mueller at the Boyce Thompson Institute in collaboration with WormBase. The purpose of this database is to introduce searchable, gene-style identifiers, "SMIDs", for all small molecules newly identified from *C. elegans* and other nematodes.

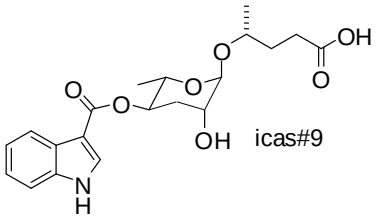
Table S2C. Structures and HPLC-ESI-MS Data of (ω)-Oxygenated Saturated Ascarosides



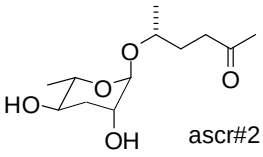
Side chain (n)	SMID[1]	Molecular formula	Molecular mass [g/mol]	*m/z* $[M-H]^-$
1	ascr#5	$C_9H_{16}O_6$	220.0947	219.09
3	oscr#9	$C_{11}H_{20}O_6$	248.1260	247.12
7	oscr#10	$C_{15}H_{28}O_6$	304.1886	303.18
9	oscr#18	$C_{17}H_{32}O_6$	332.2199	331.21

[1]SMID: Small Molecule IDentifier for small molecules identified from *C. elegans* and other nematodes. The Small Molecule Metabolite Identifier database (www.smid-db.org) is an electronic resource maintained by Frank Schroeder and Lukas Mueller at the Boyce Thompson Institute in collaboration with WormBase. The purpose of this database is to introduce searchable, gene-style identifiers, "SMIDs", for all small molecules newly identified from *C. elegans* and other nematodes.

Table S2D. Structures and HPLC-ESI-MS Data of Indole Ascaroside icas#9 and ascr#2



icas#9



ascr#2

SMID[1]	Molecular formula	Molecular mass [g/mol]	*m/z*
icas#9	$C_{20}H_{25}NO_7$	391.1631	390.16 $[M-H]^-$
ascr#2	$C_{12}H_{22}O_5$	246.1467	269.14 $[M+Na]^+$

[1]SMID: Small Molecule IDentifier for small molecules identified from *C. elegans* and other nematodes. The Small Molecule Metabolite Identifier database (www.smid-db.org) is an electronic resource maintained by Frank Schroeder and Lukas Mueller at the Boyce Thompson Institute in collaboration with WormBase. The purpose of this database is to introduce searchable, gene-style identifiers, "SMIDs", for all small molecules newly identified from *C. elegans* and other nematodes.

Supplemental References

50. Hallem, E.A., Dillman, A.R., Hong, A.V., Zhang, Y., Yano, J.M., DeMarco, S.F., and Sternberg, P.W. (2011) A sensory code for host seeking in parasitic nematodes. Curr Biol, *21(5)*, 377-383.

51. Huckstep M., Taylor R.J.K., and Caton M.P.L. (1982) A convenient method of preparing the leukotriene precursor methyl 5-oxopentanoate. Synthesis, *10*, 881-882.

52. Kai K., Takeuchi, J., Kataoka, T., Yokoyama, M., and Watanabe, N. (2008) Structure-activity relationship study of flowering-inducer FN against *Lemna paucicostata*. Tetrahedron, *64*, 6760-6769.