Supporting Information for

HPLC-HRMS Quantification of the Ichthyotoxin Karmitoxin from *Karlodinium armiger*

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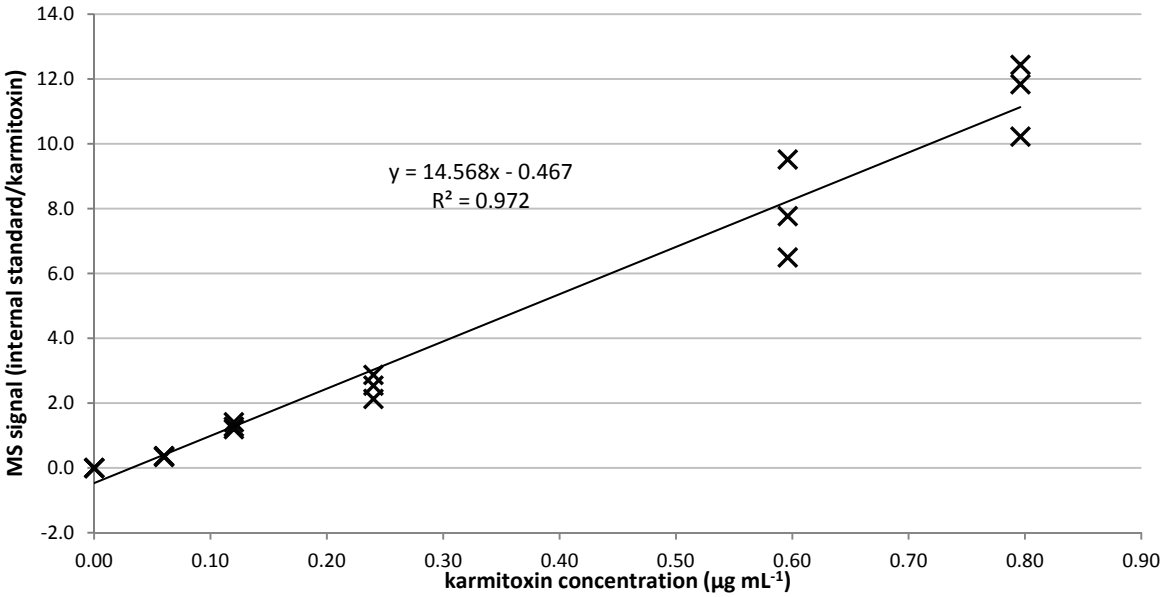


Figure S1: *Calibration curve of karmitoxin in methanol*

Calibration curve used for the quantification of karmitoxin in experimental cultures

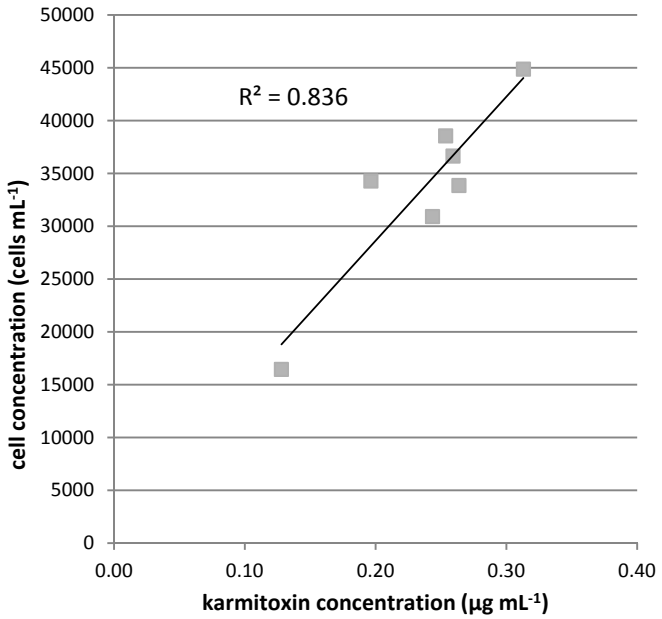


Figure S2: *Relationship between karmitoxin and phototrophic cell density*

Karmitoxin concentration compared to cell concentration in cultures grown in ammonium substituted f/2 media.

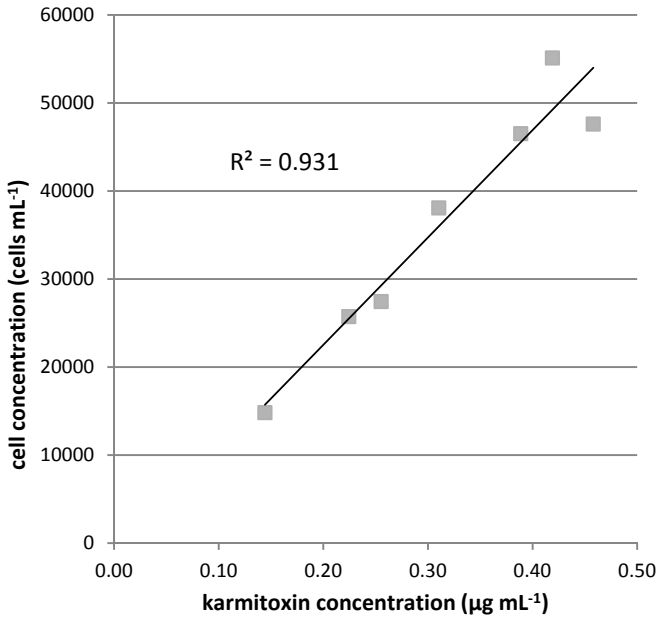


Figure S3: *Relationship between karmitoxin and mixotrophic cell density*

Karmitoxin concentration compared to cell count in cultures grown in f/2 media and fed *Rhodomonas salina*.

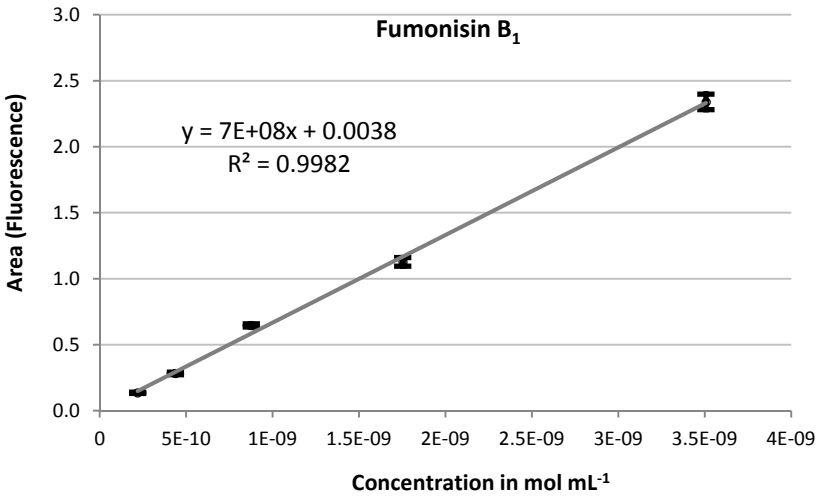


Figure S4: *Calibration curve of fumonisin B1*

Calibration curves used for the quantification of karmitoxin standard.

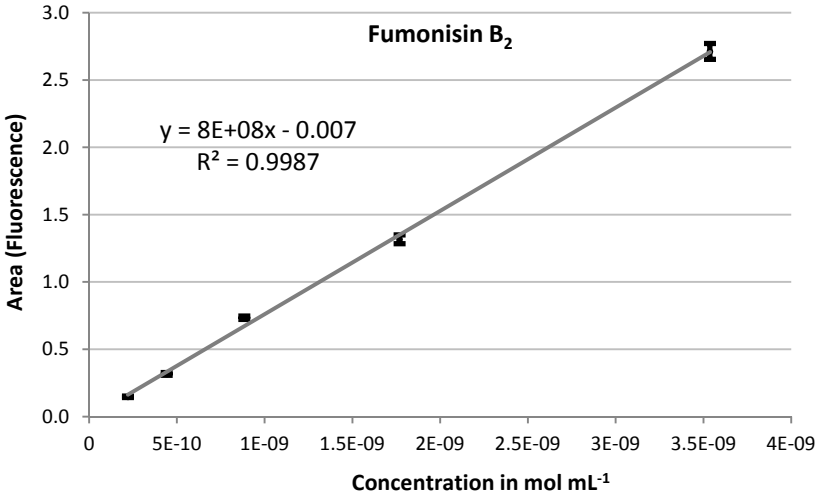


Figure S5: *Calibration curve of fumonisin B_2*

Calibration curves used for the quantification of karmitoxin standard.

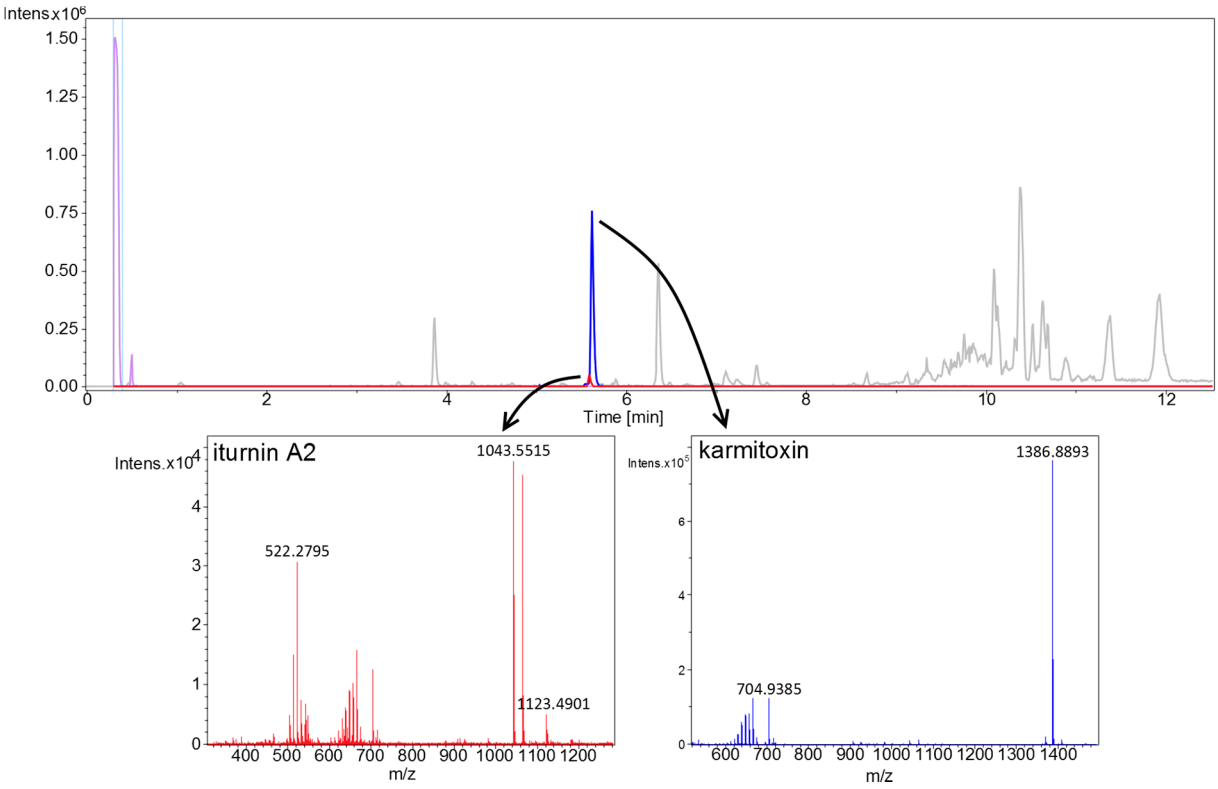
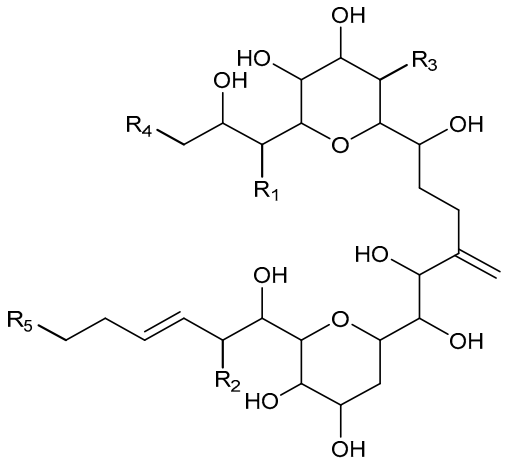


Figure S6: *Strata-X prepared sample with Bruker QTOF analysis*

This figure illustrates an example base peak chromatogram (grey line) of a Strata-X SPE prepared sample of *K. armiger* with an internal standard iturin A2 (indicated by the red extracted ion chromatogram). The blue extracted ion chromatogram indicates the $[M+H]^+$ ion of karmitoxin, and the purple extracted ion chromatogram indicates the internal calibrant.



Name	Molecular Formula	Monoisotopic Mass	Source	R_1	R_2	R_3	R_4 (polar arm)			R_5 (non-polar arm)			Reference
							Formula	backbone	methyl	Formula	backbone	triene	
Karlotoxin 9	$C_{65}H_{117}ClO_{24}$	1316.7623	*Karlodinium sp.*	H	OH	OH	$C_{34}H_{67}O_{12}$	C_{31}	3	$C_{14}H_{22}ClO_2$	C_{14}	No	(Waters et al. 2015)
Karlotoxin 8	$C_{65}H_{117}ClO_{24}$	1316.7623	*Karlodinium sp.*	H	OH	OH	$C_{34}H_{67}O_{12}$	C_{31}	3	$C_{16}H_{26}ClO_2$	C_{16}	No	(Waters et al. 2015)
Symbiopolyol	$C_{60}H_{100}NaO_{23}S$	1243.6274	*Amphidinium sp.*	OH	OH	H	$C_{27}H_{49}O_{12}S$	C_{25}	2	$C_{16}H_{23}O_2$	C_{16}	Yes	(Hanif et al. 2010)
Amphidinol 17	$C_{63}H110O24S$	1282.7108	*Amphidinium carterae*	OH	OH	H	$C_{30}H_{59}O_{13}S$	C_{27}	3	$C_{16}H_{23}O_2$	C_{16}	Yes	(Meng et al. 2010)
Amphidinol 11	$C_{71}H_{122}NaO_{28}S$	1477.7741	*Amphidinium carterae*	OH	OH	H	$C_{38}H_{71}O_{17}S$	C_{33}	5	$C_{16}H_{23}O_2$	C_{16}	Yes	(Echigoya et al. 2005)
Lingshuiol B	$C_{60}H_{100}NaO_{23}S$	1243.6274	*Amphidinium sp.*	OH	OH	H	$C_{27}H_{49}O_{12}S$	C_{25}	2	$C_{16}H_{23}O_2$	C_{16}	Yes	(Huang et al. 2004)
Carteraol E	$C_{74}H_{126}O_{24}$	1398.8639	*Amphidinium carterae*	OH	OH	H	$C_{41}H_{75}O_{13}$	C_{37}	4	$C_{16}H_{23}O_2$	C_{16}	Yes	(Huang et al. 2009)
Amphidinol 7	$C_{59}H_{100}O_{23}S$	1208.6376	*Amphidinium klebsii*	OH	OH	H	$C_{26}H_{49}O_{12}S$	C_{23}	3	$C_{16}H_{23}O_2$	C_{16}	Yes	(Morsy et al. 2005)
Amphidinol 12	$C_{68}H_{116}O_{26}S$	1380.7476	*Amphidinium carterae*	OH	OH	H	$C_{35}H_{65}O_{15}S$	C_{33}	2	$C_{16}H_{23}O_2$	C_{16}	Yes	(Echigoya et al. 2005)
Amphidinol 2	$C_{71}H_{122}O_{25}$	1374.8275	*Amphidinium klebsii*	OH	OH	H	$C_{38}H_{71}O_{14}$	C_{33}	5	$C_{16}H_{23}O_2$	C_{16}	Yes	(Paul et al. 1995)
Amphidinol 4	$C_{68}H_{116}O_{23}$	1300.7907	*Amphidinium carterae and A. klebsii*	OH	OH	H	$C_{35}H_{65}O_{12}$	C_{33}	2	$C_{16}H_{23}O_2$	C_{16}	Yes	(Houdai et al. 2001)

Name	Molecular Formula	Monoisotopic Mass	Source	R_1	R_2	R_3	R_4 (polar arm)			R_5 (non-polar arm)			Reference
							Formula	backbone	methyl	Formula	backbone	triene	
Amphidinol 6	$C_{70}H_{120}O_{24}$	1344.8170	*Amphidinium klebsii*	OH	OH	H	$C_{37}H_{69}O_{13}$	C_{35}	2	$C_{16}H_{23}O_2$	C_{16}	Yes	(Paul et al. 1997)
Amphidinol 10	$C_{66}H_{112}O_{23}$	1272.7594	*Amphidinium spp.*	OH	OH	H	$C_{33}H_{61}O_{12}$	C_{31}	2	$C_{16}H_{23}O_2$	C_{16}	Yes	(Echigoya et al. 2005)
Lingshuiol A	$C_{66}H_{112}O_{23}$	1272.7594	*Amphidinium sp.*	OH	OH	H	$C_{33}H_{61}O_{12}$	C_{31}	2	$C_{16}H_{23}O_2$	C_{16}	Yes	(Huang et al. 2004)
Amdigenol G	$C_{60}H_{102}NaO_{25}S$	1277.6329	*Amphidinium sp.*	OH	OH	H	$C_{27}H_{49}O_{12}S$	C_{25}	2	$C_{16}H_{25}O_4$	C_{16}	No	(Inuzuka et al. 2014)
Amdigenol A	$C_{104}H_{178}NaO_{43}S$	2170.1360	*Amphidinium sp*	OH	OH	H	$C_{71}H_{125}O_{30}S$	C_{66}	5	$C_{16}H_{25}O_4$	C_{16}	No	(Inuzuka et al. 2012)
Amphidinol 14	$C_{59}H_{102}NaO_{25}S$	1265.6329	*Amphidinium klebsii*	OH	OH	H	$C_{26}H_{49}O_{12}S$	C_{23}	3	$C_{16}H_{25}O_4$	C_{16}	Yes	(Morsy et al. 2006)
Luteophanol A	$C_{60}H_{102}O_{25}S$	1254.6431	*Amphidinium sp.*	OH	H	H	$C_{27}H_{49}O_{12}S$	C_{25}	2	$C_{16}H_{25}O_4$	C_{16}	No	(Doi et al. 1997)
Luteophanol D	$C_{67}H_{116}O_{25}$	1320.7806	*Amphidinium sp.*	OH	OH	H	$C_{34}H_{63}O_{12}$	C_{32}	2	$C_{16}H_{25}O_4$	C_{16}	No	(Kubota et al. 2005)
Amphidinol 15	$C_{59}H_{102}O_{22}$	1162.6863	*Amphidinium klebsii*	OH	OH	H	$C_{26}H_{49}O_9$	C_{23}	3	$C_{16}H_{25}O_4$	C_{16}	Yes	(Morsy et al. 2006)
Karlotoxin 2	$C_{67}H_{121}ClO_{24}$	1344.7936	*Karlodinium veneficum*	H	OH	OH	$C_{34}H_{67}O_{12}$	C_{31}	3	$C_{16}H_{26}ClO_2$	C_{16}	No	(Peng et al. 2010)
4,5-dihydro-karlotoxin 2	$C_{67}H_{123}ClO_{24}$	1346.8093	*Karlodinium veneficum*	H	OH	OH	$C_{34}H_{69}O_{12}$	C_{31}	3	$C_{16}H_{26}ClO_2$	C_{16}	No	(Cai et al. 2016)
4,5-dihydro-dechloro-karlotoxin 2	$C_{67}H_{124}O_{24}$	1312.8483	*Karlodinium veneficum*	H	OH	OH	$C_{34}H_{69}O_{12}$	C_{31}	3	$C_{16}H_{27}O_2$	C_{16}	No	(Cai et al. 2016)
Karatungiol B	$C_{73}H_{130}O_{27}$	1438.8799	*Amphidinium sp.*	OH	OH	OH	$C_{40}H_{73}O_{15}$	C_{37}	3	$C_{16}H_{29}O_2$	C_{16}	No	(Washida et al. 2006)
Lingshuiol	$C_{69}H_{122}O_{25}$	1350.8275	*Amphidinium sp.*	OH	OH	H	$C_{36}H_{65}O_{14}$	C_{33}	3	$C_{16}H_{29}O_2$	C_{16}	No	(Huang et al. 2004)
Karatungiol A	$C_{73}H_{132}O_{28}$	1456.8905	*Amphidinium sp.*	OH	OH	OH	$C_{40}H_{75}O_{16}$	C_{37}	3	$C_{16}H_{29}O_2$	C_{16}	No	(Washida et al. 2006)
Luteophanol C	$C_{67}H_{116}O_{25}$	1320.7806	*Amphidinium sp.*	OH	OH	H	$C_{33}H_{61}O_{12}$	C_{31}	2	$C_{17}H_{27}O_4$	C_{17}	No	(Kubota et al. 1998)
Luteophanol B	$C_{67}H_{116}O_{25}$	1320.7806	*Amphidinium sp.*	OH	OH	H	$C_{33}H_{61}O_{12}$	C_{31}	2	$C_{17}H_{27}O_4$	C_{17}	No	(Kubota et al. 1998)
64E-Chlorokarlotoxin 3	$C_{68}H_{123}ClO_{24}$	1358.8093	*Karlodinium veneficum*	H	OH	OH	$C_{34}H_{67}O_{12}$	C_{31}	3	$C_{17}H_{28}ClO_2$	C_{17}	No	(Van Wagoner et al. 2010)
10-O-Sulfokarlotoxin 3	$C_{68}H_{124}O_{27}S$	1404.8051	*Karlodinium veneficum*	H	OH	OH	$C_{34}H_{67}O_{15}S$	C_{31}	3	$C_{17}H_{29}O_2$	C_{17}	No	(Van Wagoner et al. 2010)
Karlotoxin 3	$C_{68}H_{124}O_{24}$	1324.8483	*Karlodinium veneficum*	H	OH	OH	$C_{34}H_{67}O_{12}$	C_{31}	3	$C_{17}H_{29}O_2$	C_{17}	No	(Van Wagoner et al. 2010)

Name	Molecular Formula	Monoisotopic Mass	Source	R_1	R_2	R_3	R_4 (polar arm)			R_5 (non-polar arm)			Reference
							Formula	backbone	methyl	Formula	backbone	triene	
Amphidinol 19	$C_{71}H_{122}NaO_{27}S$	1461.7792	*Amphidinium carterae*	OH	OH	H	$C_{36}H_{69}O_{16}S$	C_{33}	3	$C_{18}H_{25}O_2$	C_{18}	Yes	(Nuzzo et al. 2014)
Amphidinol 13	$C_{70}H_{118}NaO_{26}S$	1429.7530	*Amphidinium carterae*	OH	OH	H	$C_{35}H_{65}O_{15}S$	C_{33}	2	$C_{18}H_{25}O_2$	C_{18}	Yes	(Echigoya et al. 2005)
Amphidinol (Amphidinol 1)	$C_{73}H_{126}O_{27}S$	1466.8207	*Amphidinium klebsii*	OH	OH	H	$C_{38}H_{73}O_{16}S$	C_{35}	3	$C_{18}H_{25}O_2$	C_{18}	Yes	(Satake et al. 1991)
Amphidinol 18	$C_{71}H_{122}O_{24}$	1358.8326	*Amphidinium carterae*	OH	OH	H	$C_{36}H_{69}O_{13}$	C_{33}	3	$C_{18}H_{25}O_2$	C_{18}	Yes	(Nuzzo et al. 2014)
Amphidinol 9	$C_{70}H_{118}O_{23}$	1326.8064	*Amphidinium carterae*	OH	OH	H	$C_{35}H_{65}O_{12}$	C_{33}	2	$C_{18}H_{25}O_2$	C_{18}	Yes	(Echigoya et al. 2005)
Amphidinol 5	$C_{72}H_{122}O_{24}$	1370.8326	*Amphidinium klebsii*	OH	OH	H	$C_{37}H_{69}O_{13}$	C_{35}	2	$C_{18}H_{25}O_2$	C_{18}	Yes	(Paul, et al. 1997)
Amphidinol 3	$C_{70}H_{118}O_{23}$	1326.8064	*Amphidinium klebsii*	OH	OH	H	$C_{35}H_{65}O_{12}$	C_{33}	2	$C_{18}H_{25}O_2$	C_{18}	Yes	(Murata et al. 2010)
65E-Chlorokarlotoxin 1	$C_{69}H_{125}ClO_{24}$	1372.8249	*Karlodinium veneficum*	H	OH	OH	$C_{34}H_{67}O_{12}$	C_{31}	3	$C_{18}H_{30}ClO_2$	C_{18}	No	(Van Wagoner et al. 2010)
karmitoxin	$C_{73}H_{127}NO_{23}$	1385.8799	*Karlodinium armiger*	H	OH	OH	$C_{38}H_{68}NO_{11}$	$C_{36}N$	2	$C_{18}H_{31}O_2$	C_{18}	No	(Rasmussen et al. 2017)
10-O-Sulfokarlotoxin 1	$C_{69}H_{126}O_{27}S$	1418.8207	*Karlodinium veneficum*	H	OH	OH	$C_{34}H_{67}O_{15}S$	C_{31}	3	$C_{18}H_{31}O_2$	C_{18}	No	(Van Wagoner et al. 2010)
Karlotoxin 1	$C_{69}H_{126}O_{24}$	1338.8639	*Karlodinium veneficum*	H	OH	OH	$C_{34}H_{67}O_{12}$	C_{31}	3	$C_{18}H_{31}O_2$	C_{18}	No	(Van Wagoner et al. 2008)
Ostreol A	$C_{67}H_{112}N_2O_{23}$	1312.7656	*Ostreopsis cf. ovata*	OH	OH	H	$C_{36}H_{61}N_2O_{12}$	$C_{34}N_2$	2	$C_{14}H_{23}O_2$	C_{14}	No	(Hwang et al. 2013)
2-desulfo-amphidinol 7	$C_{59}H_{100}O_{20}$	1128.6808	*Amphidinium klebsii*	OH	OH	H	$C_{26}H_{49}O_9$	C_{23}	3	$C_{16}H_{23}O_2$	C_{16}	Yes	(Morsy et al. 2006)
Amdigenol E*	$C_{82}H_{138}NaO_{37}S$	1769.8535	*Amphidinium sp.*	NA	NA	NA	NA	NA	-	$C_{16}H_{25}O_4$	C_{16}	No	(Inuzuka et al. 2014)
Prorocentrol*	$C_{68}H_{114}O_{34}$	1474.7192	*Prorocentrum hoffmannianum*	NA	NA	NA	$C_{31}H_{57}O_{17}$	C_{28}	3	$C_{22}H_{33}O_6$	C_{22}	Yes	(Sugahara et al. 2011)

Table S1: *Details of the amphidinol-like metabolites from dinoflagellates*

Details of the amphidinol-like metabolites from dinoflagellates, and compounds (2-desulfo-amphidinol 7) and metabolites (amdigenol E, prorocentrol) closely related to these. * = these metabolites do not share the common core structure, but do have structural similarities.

pg cell^{-1}	
Fed	**NH_4^+**
9.36	7.03
7.46	6.58
10.15	8.45
7.95	4.65
7.65	6.11
8.28	4.97
8.61	5.79
8.65	8.18
7.85	5.52
7.60	6.18
7.41	6.30
7.08	6.73
6.97	4.53
8.04	6.60
8.09	6.65
9.05	6.48
9.17	6.49
7.91	8.63
7.86	6.19
6.96	6.78
6.16	8.48

Table S2: *Concentration of karmitoxin per cell*

Comparison of karmitoxin concentration in picograms per cell in cultures grown in f/2 media and fed *Rhodomonas salina* (left column) and cultures grown in ammonium substituted f/2 media (right column).

References for Supporting Information

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