LC-MS/MS analysis of lipophilic toxins in Japanese *Dinophysis* species

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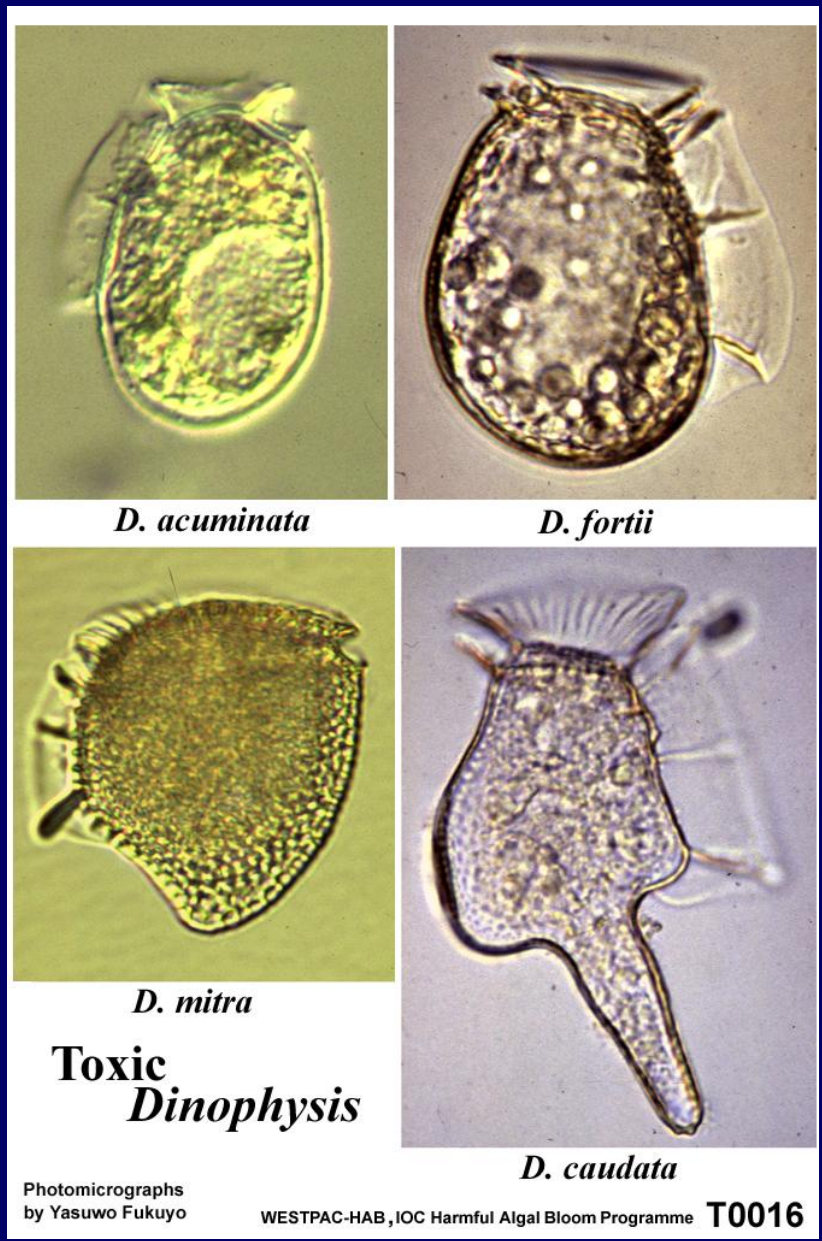
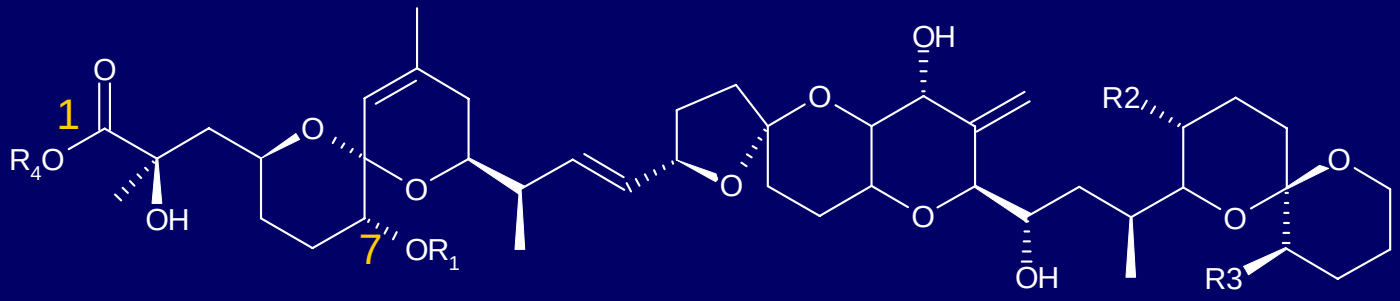
Dinophysis species in Japan



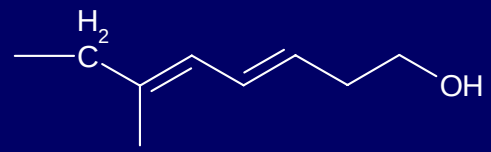
- *D. fortii, D. acuminata, D. mitra, D. tripos, D. rotundata* etc.
- Carrier species: Bivalves (Scallops, Mussels, etc.)



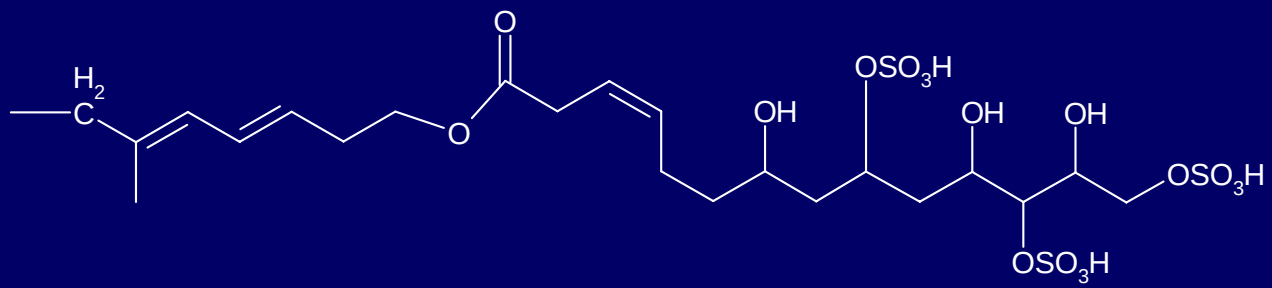
		R1	R2	R3	R4
free toxins	okadaic acid (OA)	H	CH_3	H	H
	dinophysistoxin-1 (DTX1)	H	CH_3	CH_3	H
	dinophysistoxin-2 (DTX2)	H	H	CH_3	H
7-*O*-acyl-esters	dinophysistoxin-3 (DTX3)	acyl	CH_3	CH_3	H

1-diol-esters	OA diol esters	R1	R2	R3	R4
	OA D8	H	CH3	H	a
	DTX4	H	CH3	H	b

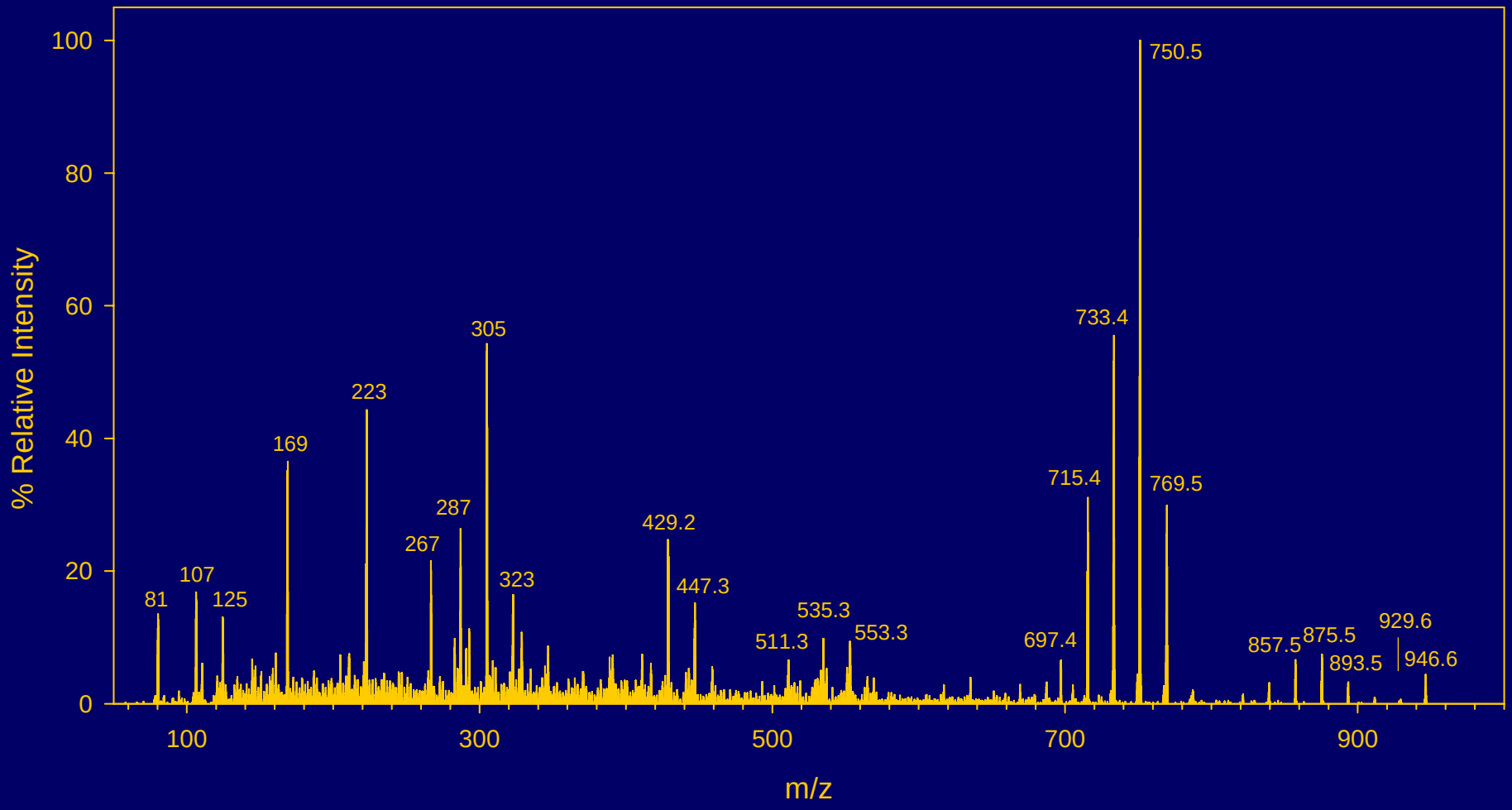
a



b

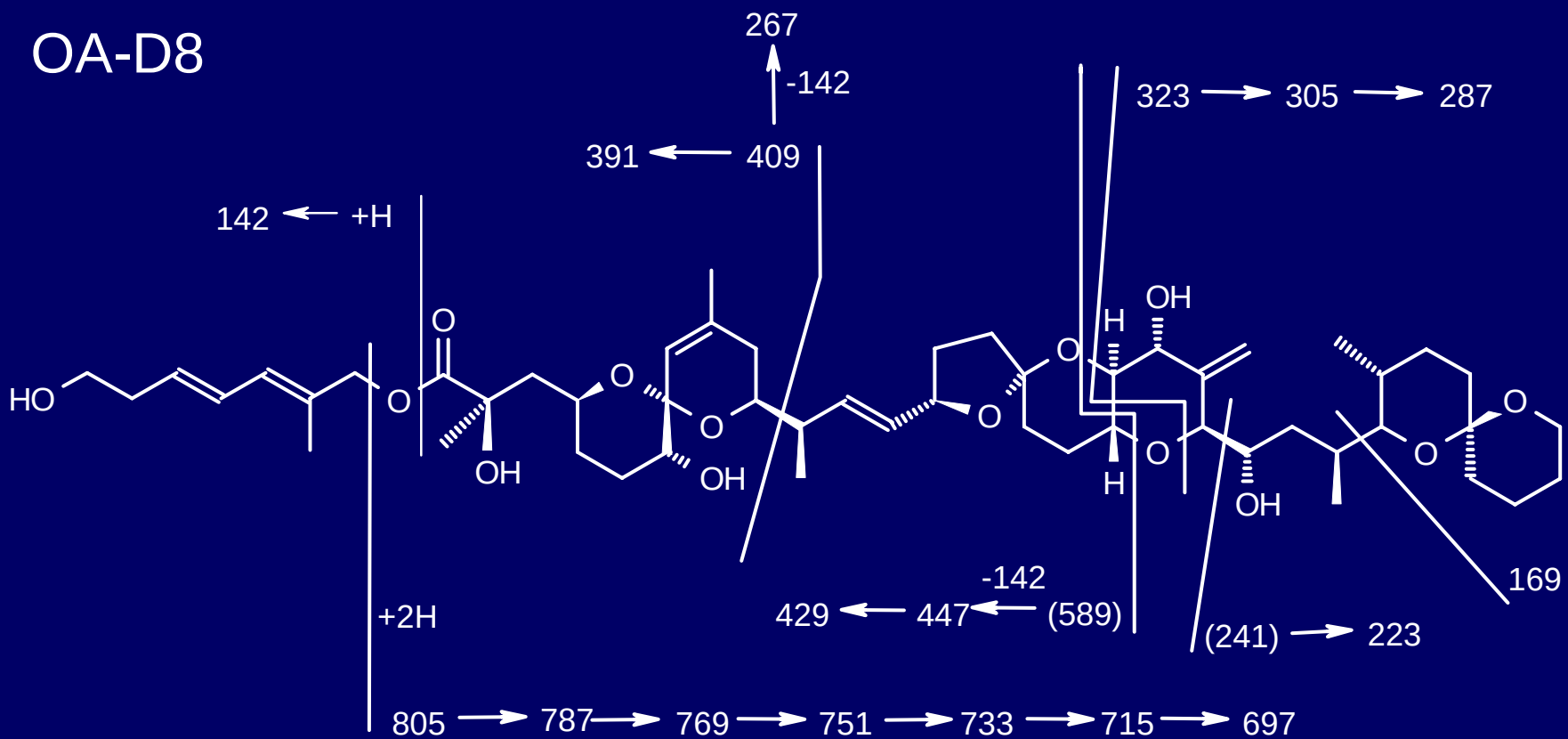


LC-MS/MS of peak #1 (OA D8)



T. Suzuki et al., Rapid Comm. Mass Spectrom. 18, 1131-1138, 2004

Proposed MS/MS fragmentation for OA-diol ester



$[M+NH_4]^+$ m/z 946 $\xrightarrow{-NH_3}$ $[M+H]^+$ 929 → 911 → 893 → 875 → 857 → 839

T. Suzuki et al., Rapid Comm. Mass Spectrom. 18, 1131-1138, 2004

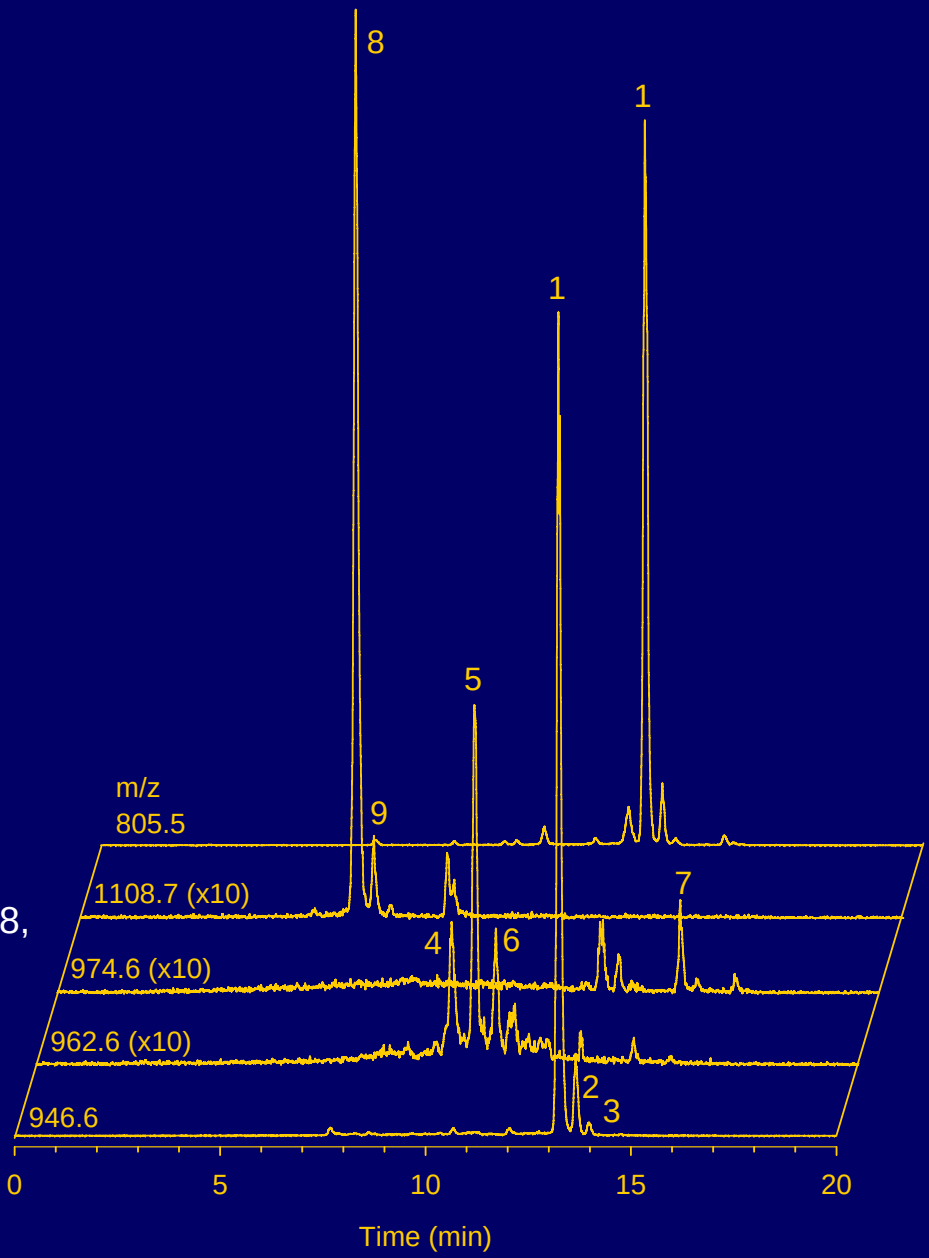
LC-MS chromatogram of all the OA diol esters obtained from D. acuta in New Zealand

LC-MS conditions

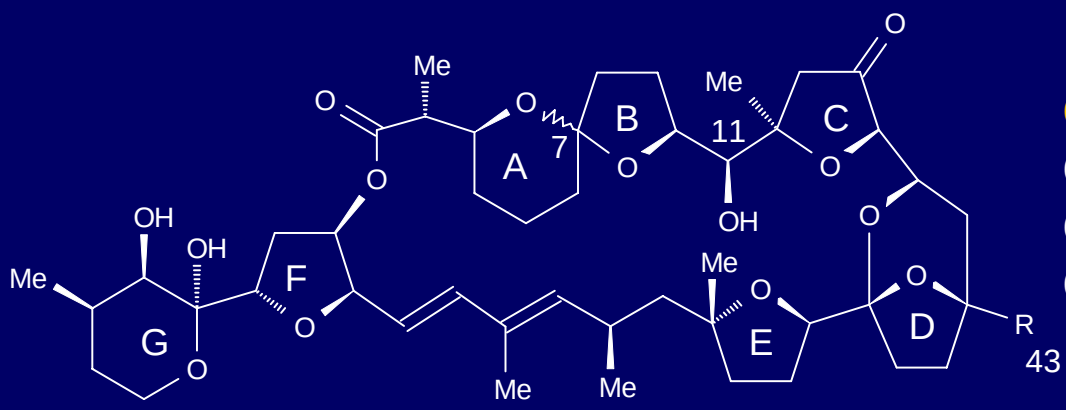
Column: Hypersil-BDS-C8
(150 mm x 2 mm i.d)
Flow rate: 0.2 mL/min
Mobile phase: A water, B 95% MeCN both containing 2 mM $HCOONH_4$ and 50 mM HCOOH

Step 1: 40 % B 100%B for 20 min
Step 2: 100 % B for 20 min

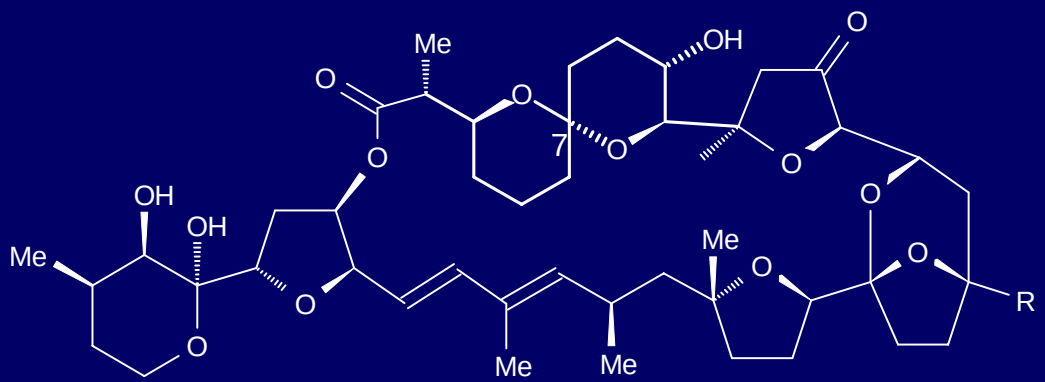
T. Suzuki et al., Rapid Comm. Mass Spectrom. 18, 1131-1138, 2004



Pectenotoxins (PTX)



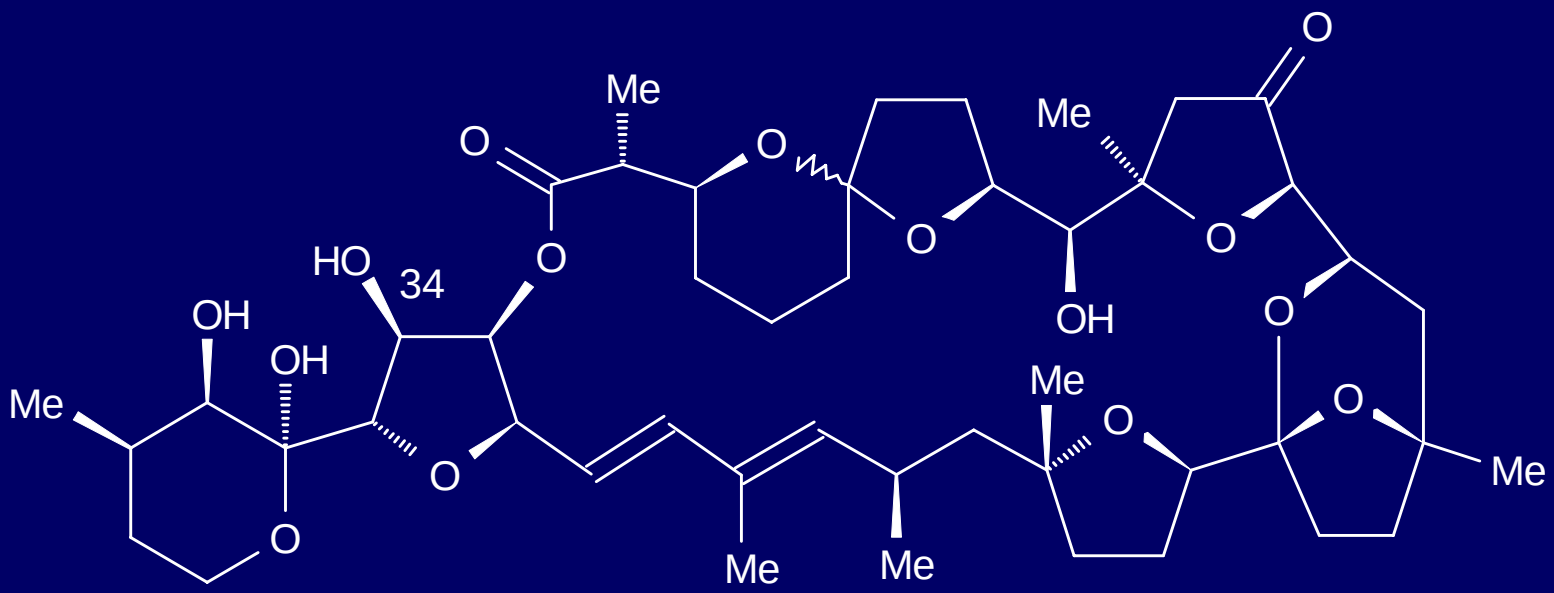
R	C7		
	R	S	MW
CH_3	PTX2		858.5
CH_2OH	PTX1, PTX4		874.5
CHO	PTX3		872.5
COOH	PTX6, PTX7		888.5



R	C7		
	R	S	MW
CH_2OH		PTX8	874.5
COOH		PTX9	888.5

- *Hepatotoxic*
- *Depolymerization of actin*

Structure of a novel PTX elucidated by NMR



PTX11 (34-OH PTX2)

T. Suzuki et al., Chem. Res. Toxicol. 19, 310-318, 2006

Lipophilic toxin profiles in Dinophysis species in Japan

Species	Toxins
D. fortii	DTX1, PTX2
D. acuminata	OA
D. mitra	DTX1
D. tripos	DTX1
D. rotundata	DTX1

J.S. Lee et al., J. Appl. Phycol. 1, 147-152, 1989
T. Suzuki et al., J. Appl. Phycol. 8, 509-515, 1997
T. Suzuki et al., Toxicon 37, 187-198, 1999
T. Suzuki et al., J. Chromatogr. A 815, 155-160, 1998

Dinophysis samples

No	Date	Site	Species	Cell numbers
1	2005/ 1/ 18	Yakumo	D. tripos	12
2	2005/ 1/ 18	Yakumo	D. ovum	2
3	2005/ 4/ 26	Yakumo	D. norgevica	100
4	2005/ 4/ 26	Yakumo	D. norvegica	111
5	2005/ 4/ 26	Yakumo	D. acuminata or ovum	35
6	2005/ 4/ 26	Yakumo	D. norvegica	120
7	2005/ 4/ 26	Yakumo	D. acuminata or ovum	56
8	2005/ 5/ 10	Yakumo	D. acuminata or ovum	53
9	2005/ 5/ 10	Yakumo	D. acuminata or ovum	153
10	2005/ 5/ 19	Abashiri	D. acuminata	223
11	2005/ 5/ 19	Abashiri	D. acuminata	200
13	2005/ 6/ 20	Yakumo	D. acuminata	18
14	2005/ 6/ 20	Yakumo	D. fortii	7
15	2005/ 6/ 20	Yakumo	D. acuminata	142
16	2005/ 6/ 20	Yakumo	D. fortii	32
17	2005/ 6/ 20	Yakumo	D. rudgei	11
18	2005/ 6/ 20	Yakumo	D. infundibulus	92
19	2005/ 7/ 20	Yakumo	D. fortii	149
20	2005/ 7/ 20	Yakumo	D. rudgei	16
21	2005/ 8/ 22	Yakumo	D. mitra	100
22	2005/ 8/ 22	Yakumo	D. mitra	100
23	2005/ 8/ 22	Yakumo	D. rotundata	88
24	2005/ 8/ 22	Yakumo	D. tripos	73
25	2003/ 5/ 20	Yamada	D. fortii	200

Solid phase extraction (SPE) of toxins

Dinophysis spp. (30-200 cells) / 1mL DW

↓

Samples were kept in a freezer

↓

Sep Pak C18 Plus

← 5 mL distilled water

← 5 mL methanol

↓

Methanol eluate was evaporated

← 200 uL methanol

↓

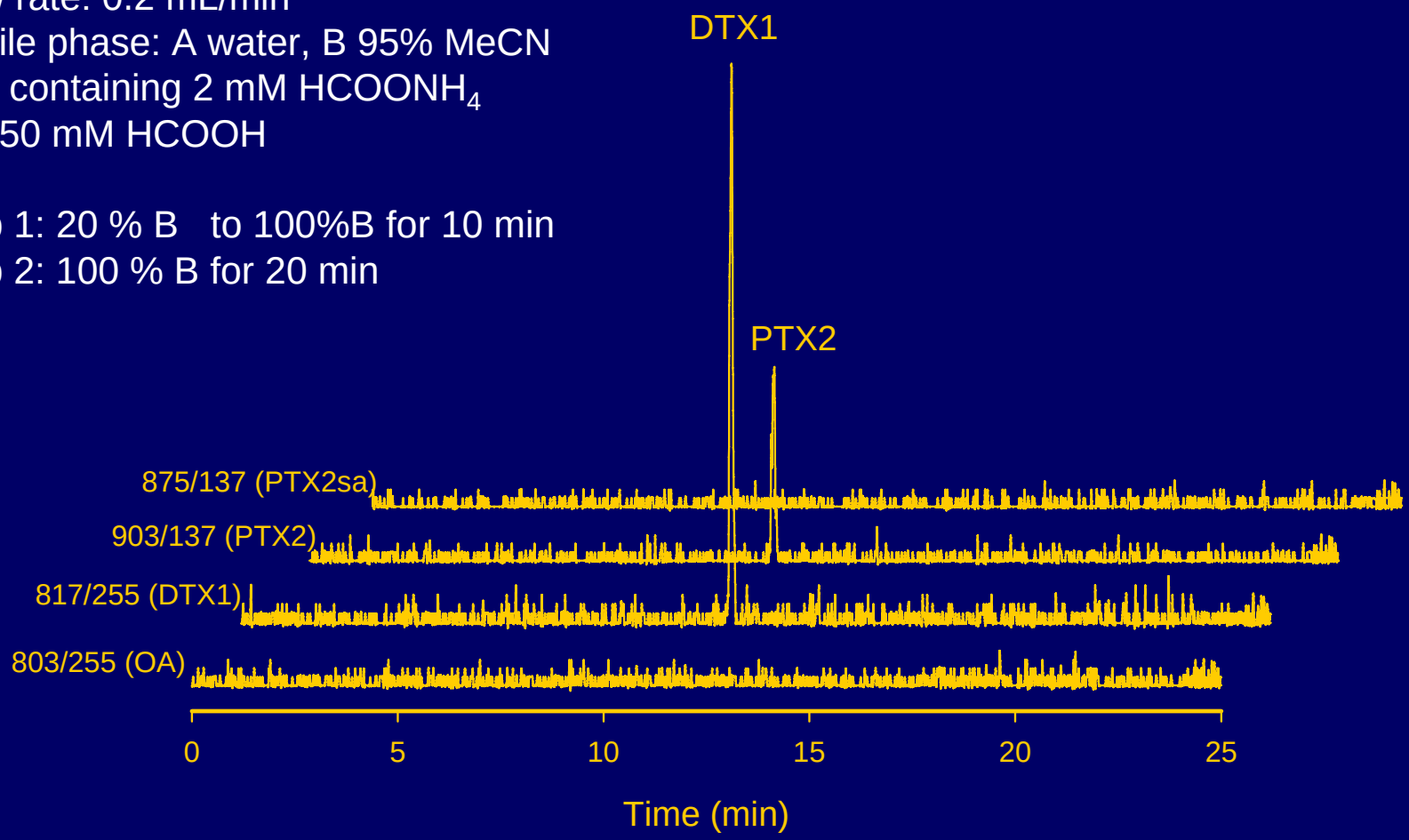
10 uL injection into LC-MS

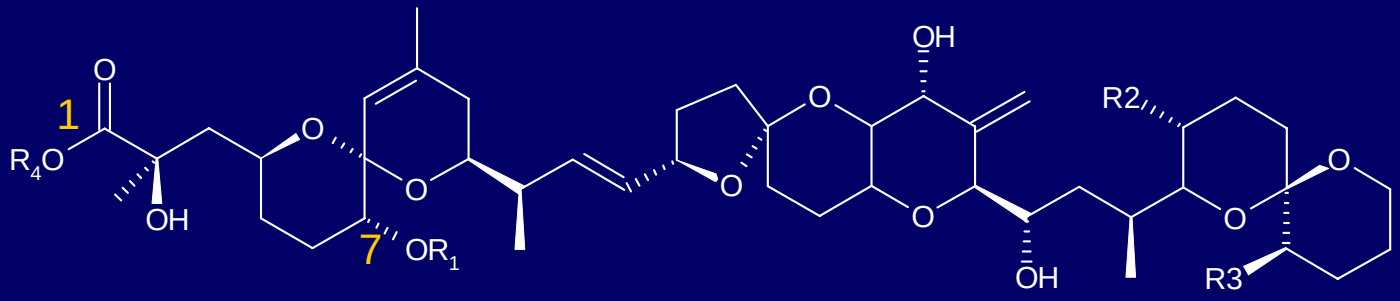
T. Suzuki et al., J. Appl. Phycol. 8, 509-515, 19

LC-MS/MS chromatogram of lipophilic toxins obtained from D. fortii in Hokkaido Japan

Column: Hypersil-BDS-C8
(50 mm x 2 mm i.d)
Flow rate: 0.2 mL/min
Mobile phase: A water, B 95% MeCN
both containing 2 mM $HCOONH_4$
and 50 mM HCOOH

Step 1: 20 % B to 100%B for 10 min
Step 2: 100 % B for 20 min

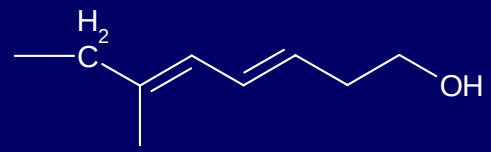




		R1	R2	R3	R4
free toxins	okadaic acid (OA)	H	CH_3	H	H
	dinophysistoxin-1 (DTX1)	H	CH_3	CH_3	H
	dinophysistoxin-2 (DTX2)	H	H	CH_3	H
7-*O*-acyl-esters	dinophysistoxin-3 (DTX3)	acyl	CH_3	CH_3	H

1-diol-esters	OA diol esters	R1	R2	R3	R4
	OA D8	H	CH3	H	a
	DTX4	H	CH3	H	b

a



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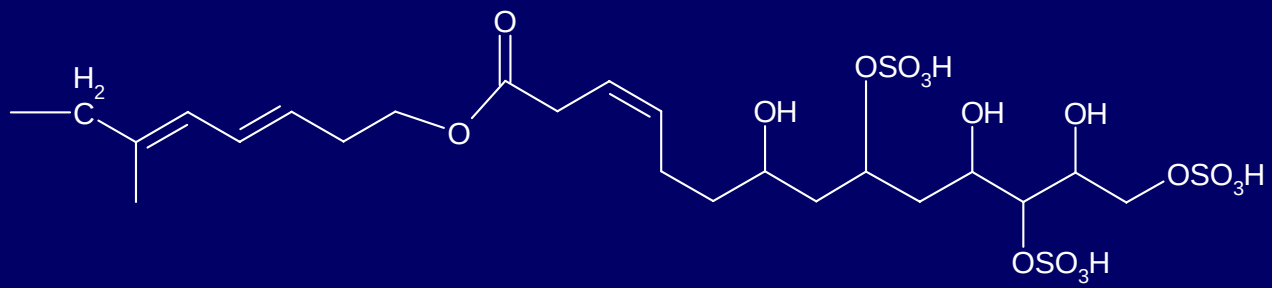
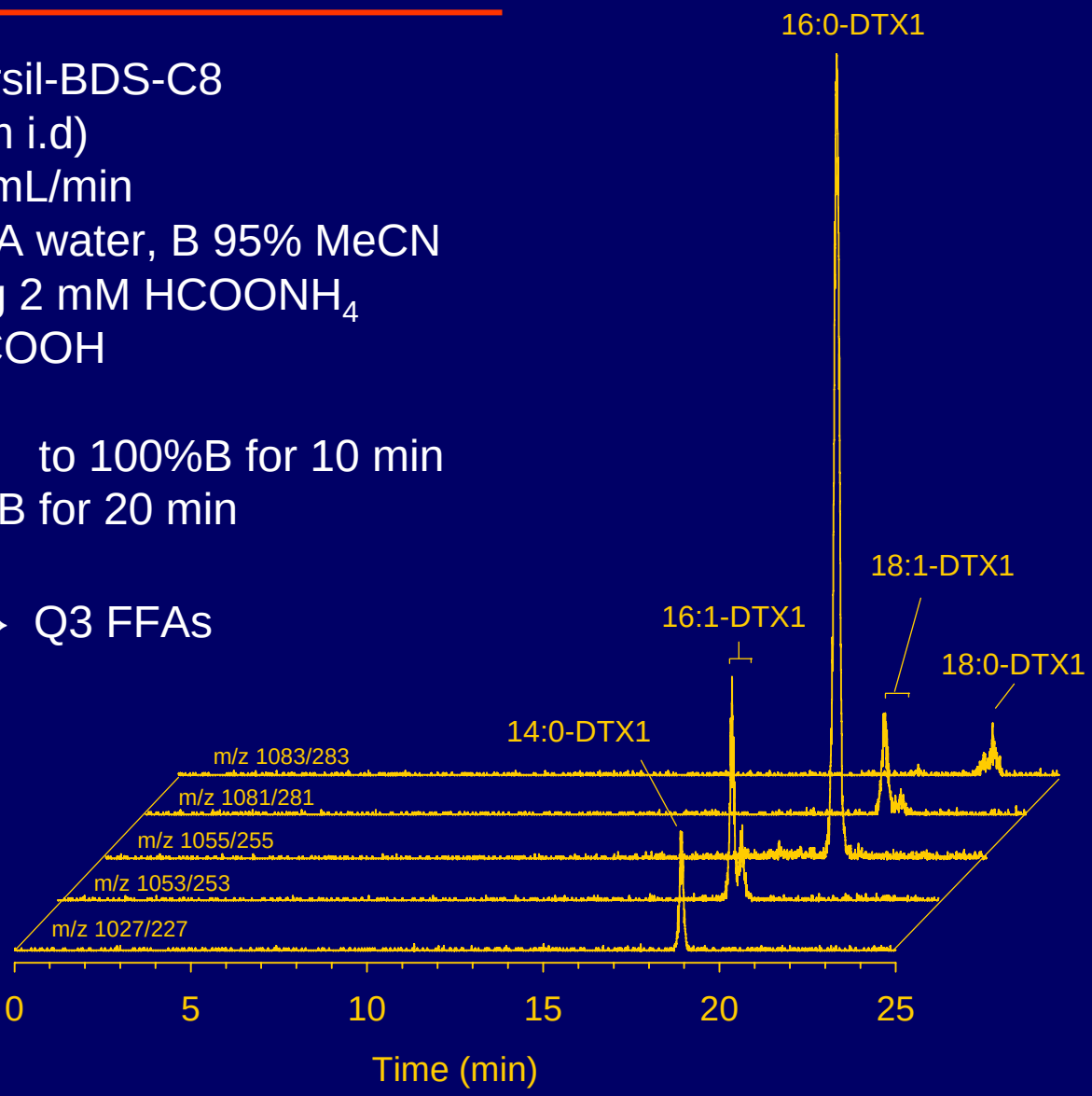


LC-MS/MS chromatogram of 7-O-acyl-DTX1 esterified with several fatty acids detected in scallops

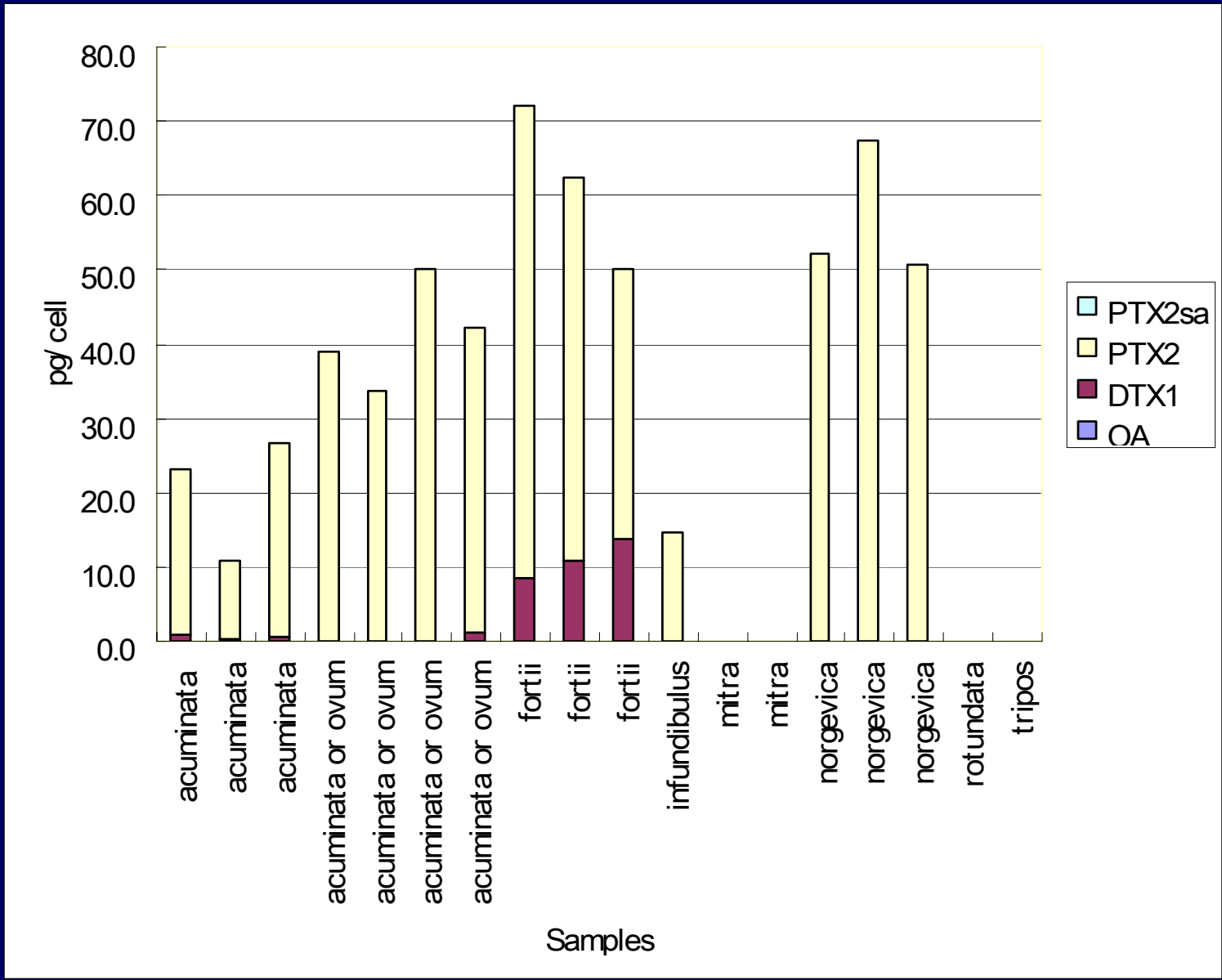
Column: Hypersil-BDS-C8
(50 mm x 2 mm i.d)
Flow rate: 0.2 mL/min
Mobile phase: A water, B 95% MeCN
both containing 2 mM $HCOONH_4$
and 50 mM HCOOH

Step 1: 20 % B to 100%B for 10 min
Step 2: 100 % B for 20 min

(-) Q1 $[M-H]^-$ → Q3 FFAs



Toxin profiles of Dinophysis species in Japan



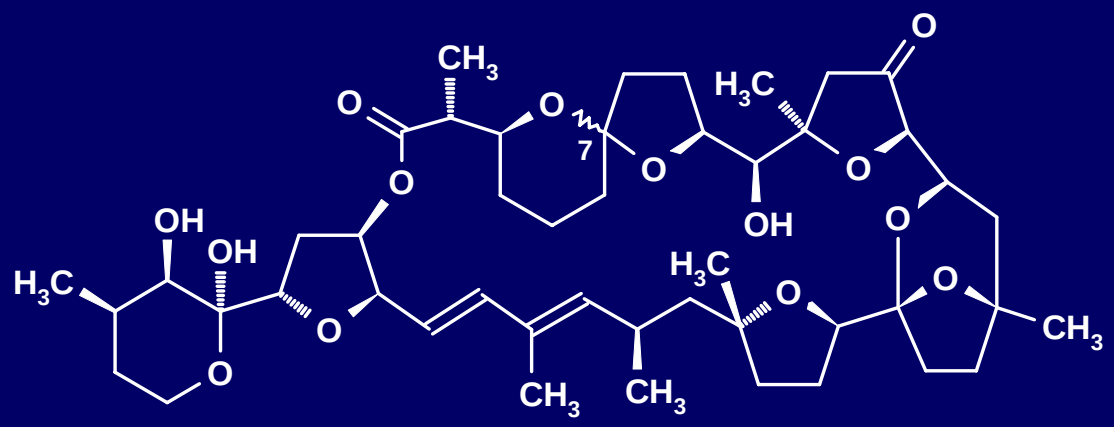
Conversion of PTX2 in bivalves

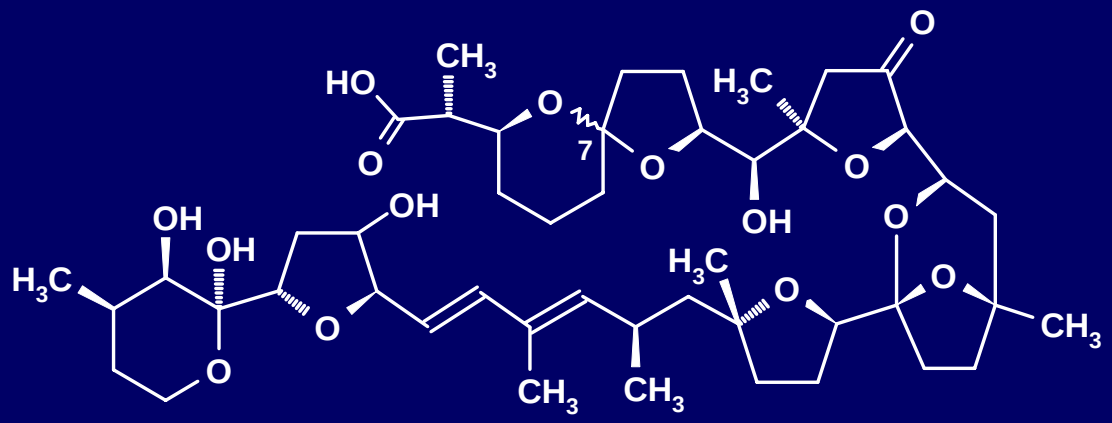
PTX2

Mussels etc.

PTX2sa

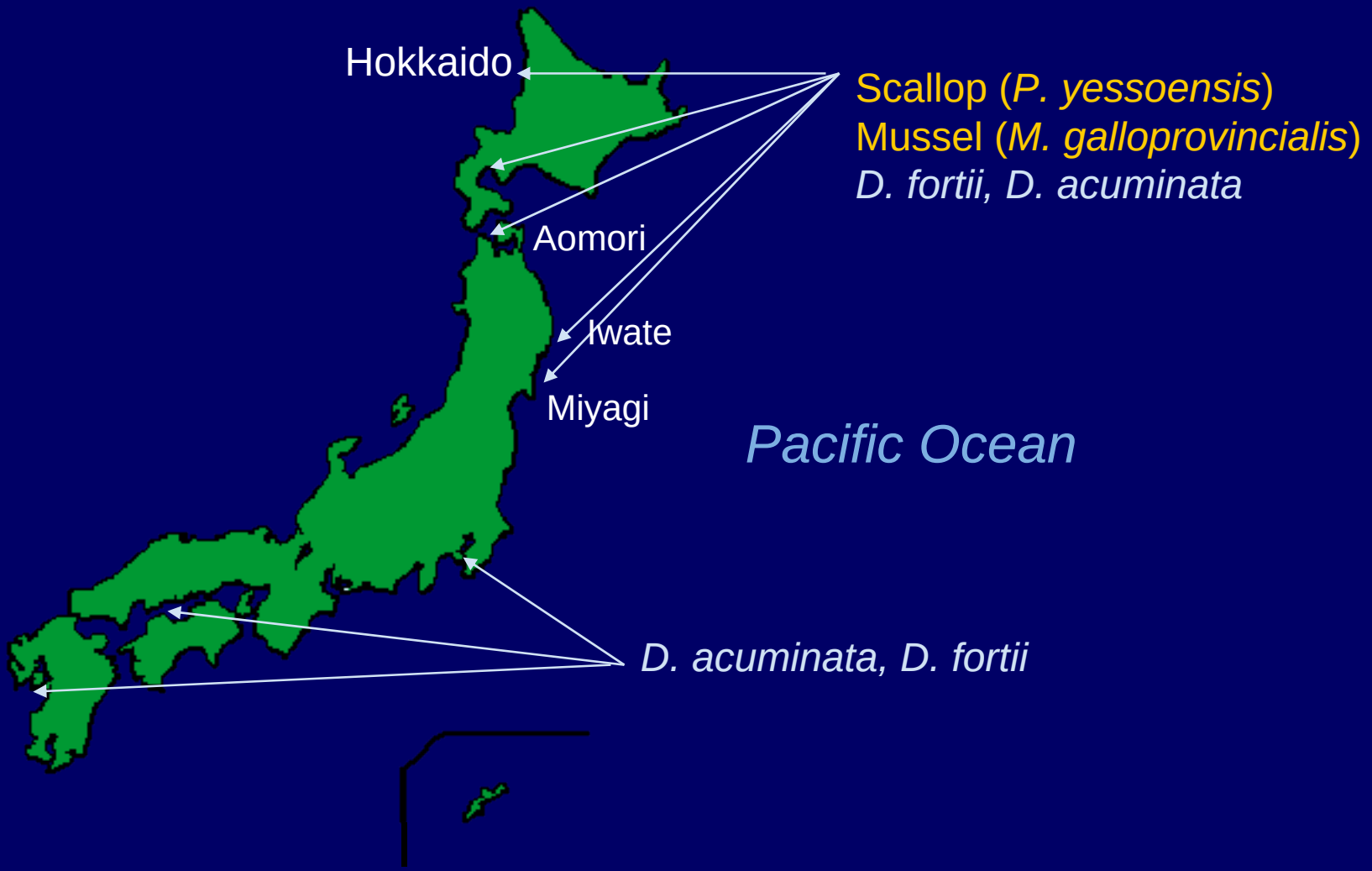
7-*epi*-PTX2sa





Suzuki, T., Mackenzie, L., Stirling, D., & Adamson, J. (2001). Toxicon 39, 507-514.

Production areas where harvesting was ceased due to contamination of shellfish with lipophilic toxins in 2005



Summary

- In our previous study, several OA diol-esters and a novel pectenotoxin, PTX11, were discovered in *D. acuta* collected in New Zealand. These toxins were not detected in any *Dinophysis* strains in Japan.
- PTX2 was the dominant toxin in *D. acuminata, D. norvegica* and *D. infundibulus* whereas both DTX1 and PTX2 were the principal toxins in *D. fortii.*
- *D. mitra* and *D. tripos* did not produced any toxins.