Novel azaspiracids produced by Amphidomataceae

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introduction: structural variants

To date more than 20 structural azaspiracid variants are known
1. *Azadinium cf. poporum* Korea

*Azadinium cf. poporum*


Shiwha Bay (37° 18’N, 126° 36’E)
1. *Azadinium cf. poporum* Korea

Test for AZAs:

A. *poporum* Korea

858 > 840 (mass of AZA-7/8/9/10)

Unexpected result, because AZA-7/8/9/10 are known as shellfish metabolites
1. Azadinium cf. poporum Korea

AZA-1

362-type AZA

-14 (CH₂) AZA-858 (Korea) -14 (CH₂)

348-type AZA
2. *Azadinium poporum* C5 North Sea

A. *poporum* C5 North Sea

Negative for known AZAs


Danish Coast at
56° 14.52’ N, 07° 27.54’ E
2. *Azadinium poporum* C5 North Sea

precursor of m/z 348
2. Azadinium poporum C5 North Sea

A. poporum C5 North Sea

A. poporum cf. Korea

348-type AZA
2. *Azadinium poporum* C5 North Sea

A. *cf poporum* (Korea)

6 $\times$ -18 (=-H$_2$O)

1 $\times$ -44 (=-CO$_2$) + 5 $\times$ -18 (=-H$_2$O)

1 $\times$ -78 (=-C$_2$H$_6$O$_3$) + 2 $\times$ -18 (=-H$_2$O)

686.4 694.4 704.2 712.3 714.5 720.4 730.4 748.5 766.4 774.5 784.4 802.3 811.4 829.6 846.5
2. *Azadinium poporum* C5 North Sea

Toxin cell quota:
~20 fg/cell

AZA-846; structure (without stereochemistry) elucidated by NMR
(Krock et al. in preparation)

AZA-846: 39-desmethyl-7,8-dihydro-3-hydroxy-AZA-1
2. *Azadinium poporum* C5 North Sea

Fragmentation pattern for the cleavage of m/z 78 (= C$_2$H$_6$O$_3$)

![Chemical structure diagram showing the fragmentation pattern of m/z 78](image)
1. Azadinium cf. poporum Korea

6 × -18 (= -H₂O)

1 × -44 (= -CO₂) + 5 × -18 (= -H₂O)

1 × -78 (= -C₂H₆O₃) + 2 × -18 (= -H₂O)

3-hydroxy-AZA
1. *Azadinium cf. poporum* Korea

Toxin cell quota:
~2 fg/cell

sum formulas as determined by HRMS:

AZA-1: $\text{C}_{47}\text{H}_{71}\text{NO}_{12}$
AZA-858: $\text{C}_{47}\text{H}_{71}\text{NO}_{13}$

AZA-858 = 39-desmethyl-3-hydroxy-AZA-2

Structure confirmed by NMR
3. *Amphidoma languida* sp. nov., Bantry Bay, Ireland

Bantry Bay

$51^\circ 39' 4.7''\ N, 9^\circ 35' 11''\ E$

3. *Amphidoma languida* sp. nov., Bantry Bay, Ireland

![Graph showing azaspiracid levels](attachment:graph.png)

- **816 > 798**
  - AZA-816
- **830 > 812**
  - AZA-830

**830 > 812**
AZA-816 methyl ester
3. *Amphidoma languida* sp. nov., Bantry Bay, Ireland

*A. poporum* C5: C$_{46}$H$_{71}$NO$_{13}$

AZA-846

AZA-830

C$_{46}$H$_{71}$NO$_{12}$

348-type AZA
4. *Amphidoma languida* sp. nov., Bantry Bay, Ireland

A. *poporum* C5
North Sea (AZA-846)

6 x -18 (= -H₂O)
4. *Amphidoma languida* sp. nov., Bantry Bay, Ireland

Fragmentation pattern for the cleavage of m/z 78 (= C$_2$H$_6$O$_3$)

3-hydroxy-AZA

C$_2$H$_6$O$_3$ + H$_2$O

keto-enol tautomery
4. *Amphidoma languida* sp. nov., Bantry Bay, Ireland

**A. languida:**

AZA-830

Toxin cell quota:

~6 fg/cell

Sum formulas as determined by HRMS:

AZA-1: $\text{C}_{47}\text{H}_{71}\text{NO}_{12}$
AZA-846: $\text{C}_{46}\text{H}_{71}\text{NO}_{13}$
AZA-830: $\text{C}_{46}\text{H}_{71}\text{NO}_{12}$

AZA-830 = 39-desmethyl-7,8-dihydro-AZA-1

Hypothesized structure!
3. *Amphidoma languida* sp. nov., Bantry Bay, Ireland

*A. languida* AZA-830: $C_{46}H_{71}NO_{12}$

$$AZA-816$$

$$C_{45}H_{69}NO_{12}$$

348-type AZA

no A-Ring cleavage
3. *Amphidoma languida* sp. nov., Bantry Bay, Ireland

AZA-816  Toxin cell quota:
~11 fg/cell

\[ 8 \times 10^{-18} (= -\text{H}_2\text{O}) \]

\[ 1 \times 44 (= -\text{CO}_2) + 5 \times 18 (= -\text{H}_2\text{O}) \]

no M -78 (= -C\textsubscript{2}H\textsubscript{6}O\textsubscript{3})

Structure: ?
4. *Azadinium poporum*, China

Gu et al. 2011. *Harmful Algae*, accepted manuscript.
4. *Azadinium poporum* G 42, G 64, G 68, East China Sea, China

Toxin cell quotas:
2 – 23 fg/cell

AZA-2

362-type AZA
4. *Azadinium poporum* G 42, G 64, G 68, East China Sea, China

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Isolation of azaspiracid-2 from a marine sponge *Echinoclathria* sp. as a potent cytotoxin

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4. *Azadinium pororum* G 42, G 64, G 68, East China Sea, China

*A. pororum* strains G 42, G 64 and G 68 are probably the source of the sponge contamination with AZA-2
5. Azadinium poporum G 66, East China Sea, China

AZA-1

AZA-872

362-type AZA

Identical CID spectra up to m/z = 672
5. *Azadinium poporum* G 66, East China Sea, China

AZA-858

$6 \times -18 (= -\text{H}_2\text{O})$

$1 \times -44 (= \text{-CO}_2) - 5 \times 18 (= \text{-H}_2\text{O})$

$1 \times -78 (= \text{-C}_2\text{H}_6\text{O}_3) - 2 \times 18 (= \text{-H}_2\text{O})$

3-hydroxy-AZA

AZA-872

AZA-846
5. *Azadinium poporum* G 66, East China Sea, China

AZA-1

AZA-858

AZA-872
5. *Azadinium poporum* G 66, East China Sea, China

Toxin cell quota:
~1.5 fg/cell

sum formulas as determined by HRMS:

AZA-1: \( C_{47}H_{71}NO_{12} \)
AZA-872: \( C_{48}H_{73}NO_{13} \)

AZA-872 = 3-hydroxy-8-methyl-AZA-1 = AZA-11

confirmed by retention time and CID spectra comparison
introduction: structural variants

![Structural variants diagram]

<table>
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<tr>
<th>Toxin</th>
<th>$R_1$</th>
<th>$R_2$</th>
<th>$R_3$</th>
<th>$R_4$</th>
<th>[M+H]$^+$</th>
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<td>CH$_3$</td>
<td>H</td>
<td>H</td>
<td>842</td>
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<td>CH$_3$</td>
<td>H</td>
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<td>CH$_3$</td>
<td>CH$_3$</td>
<td>OH</td>
<td>H</td>
<td>872</td>
</tr>
</tbody>
</table>
6. *Azadinium poporum* G 25, Bohai Sea, China

AZA-858
AZA-858 producing strains *A. poporum* G25 and *A. cf. poporum* (Korea) are from proximate geographic regions.
6. *Azadinium poporum* G 25, Bohai Sea, China

**Toxin cell quota:**

- **AZA-858:** ~1.4 fg/cell
- **AZA-920:** ~0.02 fg/cell
- **AZA-928:** ~0.14 fg/cell
Summary

1. A new 39-desmethyl-AZA-family (348-type AZA) was found

2. 7 new AZAs were detected in *Azadinium poporum* and *Amphidoma languida*

3. The structure of AZA-846 has been fully elucidated by NMR as 39-desmethyl-7,8-dihydro-3-hydroxy-AZA-1

4. *A. poporum* seems to be the species with highest variability in AZA-production

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Thank you for your attention!