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FIREWORM HERMODICE CARUNCULATA / Mucci, A.; Forti, L.; Simonini, R.; Ferrari, V.; Prevedelli, D.; Righi,
S.. - (2022). (Intervento presentato al convegno Italian-French International Conference on Magnetic
Resonance tenutosi a Milano nel 27-30 settembre 2022).

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NEW SECONDARY METABOLITES IN THE AMPHINOMID FIREWORM *HERMODYCE CARUNCULATA*

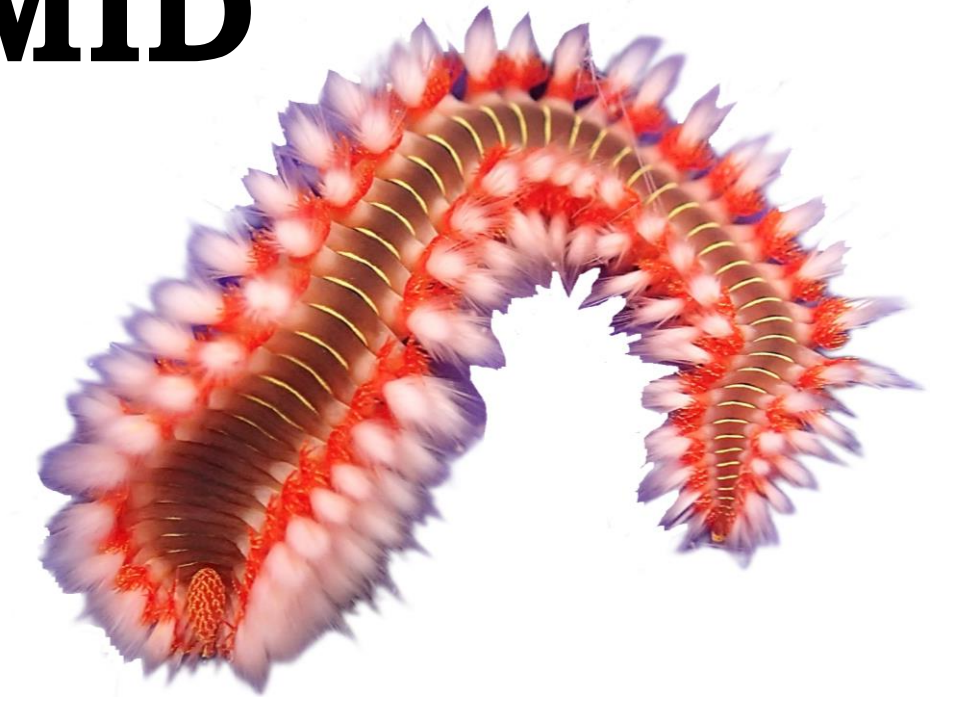
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Eight betaine-derived novel compounds were found in extracts of the Mediterranean stinging fireworm *Hermodice carunculata*. The identification of their structures relies on 1D and 2D NMR (Fig. 1-3) and HPLC-ESI/HRMS spectra. Two types of terminal ammonium portions A and B and a series of different alkyl chains were identified (Fig. 4a,b). Their matching provides the structures of uncharacterized secondary metabolites, named **carunculines**, and their related isomers. These molecules differ from already known trimethylammonium inflammatory compounds (i.e. complanines) isolated from another amphinomid species, for the structures of the terminal ammonium groups (Fig. 4c) [1]. **Carunculine** anatomical distribution within *H. carunculata* was assessed by screening through HPLC-ESI/HRMS (Fig. 5, Table 1): their occurrence was revealed in all the body parts analyzed, both involved in predator-prey interactions [2], and mainly in the digestive apparatus. The results achieved reveal an array of different novel compounds from a chemically unknown species, improving knowledge on Marine Animal Products with chemical and biological potential for bioprospection [3]. Overall, these data reinforce the necessity of studying poorly-investigated taxa to expand knowledge on animal venom biology, their mechanisms of action and exploitation as promising source of drug molecules.

- Pitfalls of NMR spectra -

Our **first hypothesis**, based on ¹H and H,C-HSQCed spectra (Fig. 1 and 2), **was wrong**. The sign of H,C correlations in HSQCed spectrum is deceiving for cyclopropanes (¹J(H,C) around 160 Hz): methylenes seem methyl signals but in the COSY spectrum the intensity of the “long range” correlations between what should have been geminal CH₃ signals were abnormally high and with a too symmetric shape... (Fig. 3) and ¹³C chemical shift (around 10 ppm) was too low.

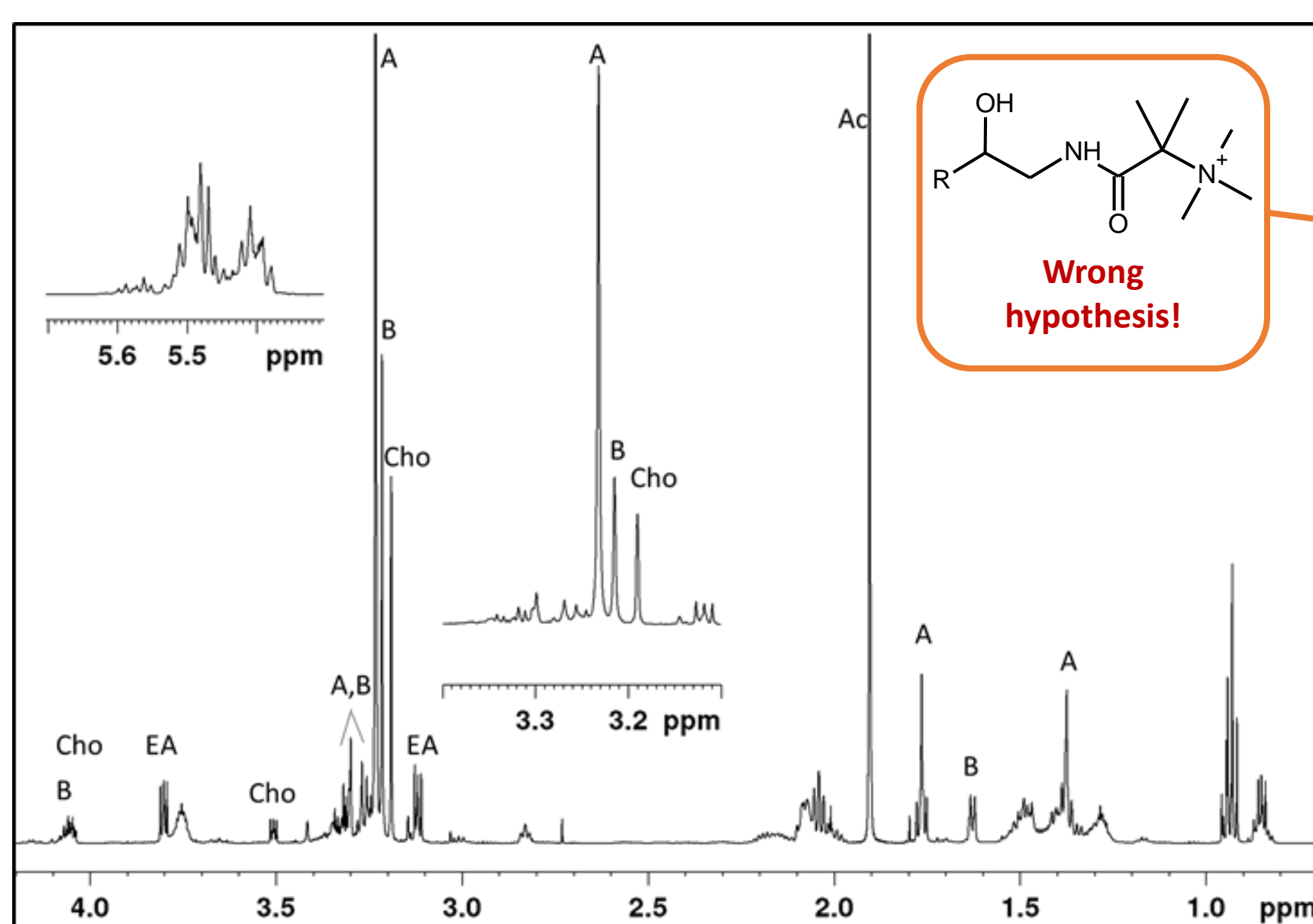


Figure 1. ¹H spectrum in D₂O of *H. carunculata* extract. Diagnostic signals of carunculines are marked with A and B. Cho = choline, EA = ethanolamine. Modified from [3].

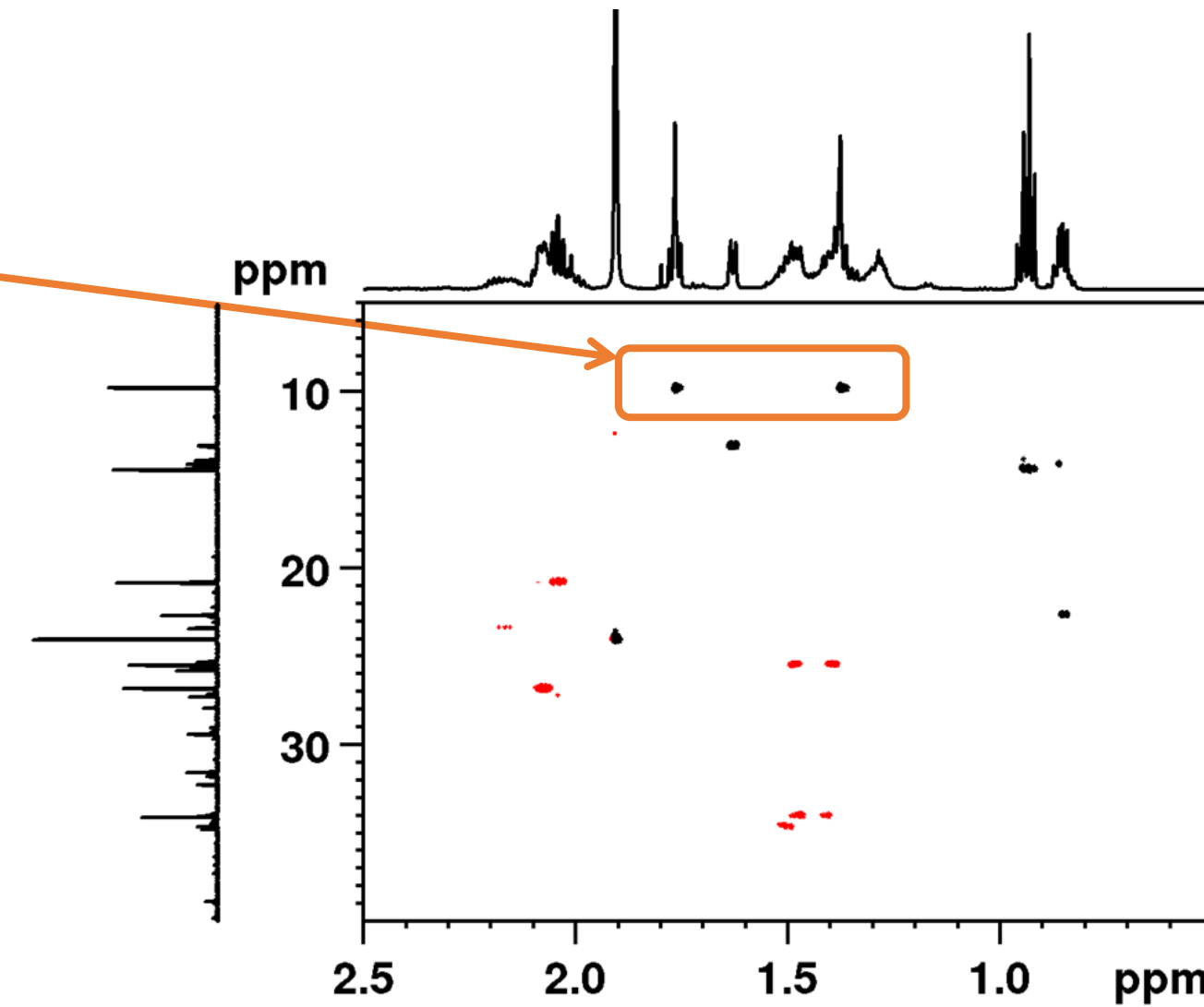


Figure 2. Enlarged regions of the H,C-HSQCed NMR spectrum. In the black rectangle, the correlation between the protons and the carbon of the cyclopropane ring, with the same sign of methyl and methyne correlations. Modified from [3].

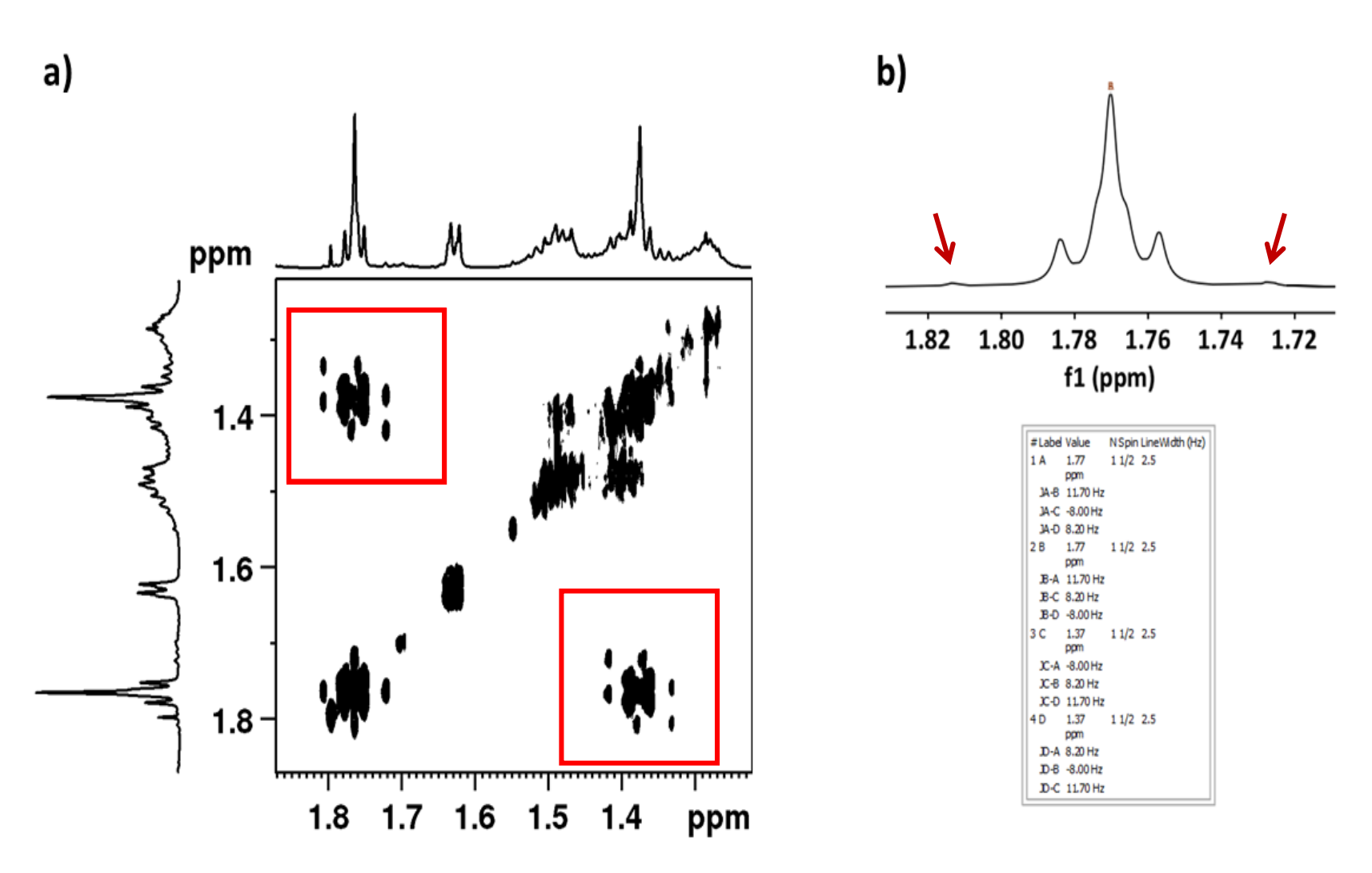


Figure 3. Enlarged region of the H,H-COSY NMR spectrum of carunculines. a) The red square shows the correlation between the AA' and BB' protons of the cyclopropane ring. b) spin-system simulation showing the shape of one of the two multiplets of the AA'BB' system (MestReNova v 14.1.2-25024). Modified from [3].



Figure 4. Proposed structures for carunculines and molecular structures of complanines. **a)** Proposed molecular structures for carunculines (1-8) and their isomers derived by matching the structures obtained by NMR spectra and the formulae obtained by HPLC-ESI/HRMS data. **b)** terminal ammonium portion (A) or (B); **c)** molecular structures of complanine and neocomplanines. Modified from [3].

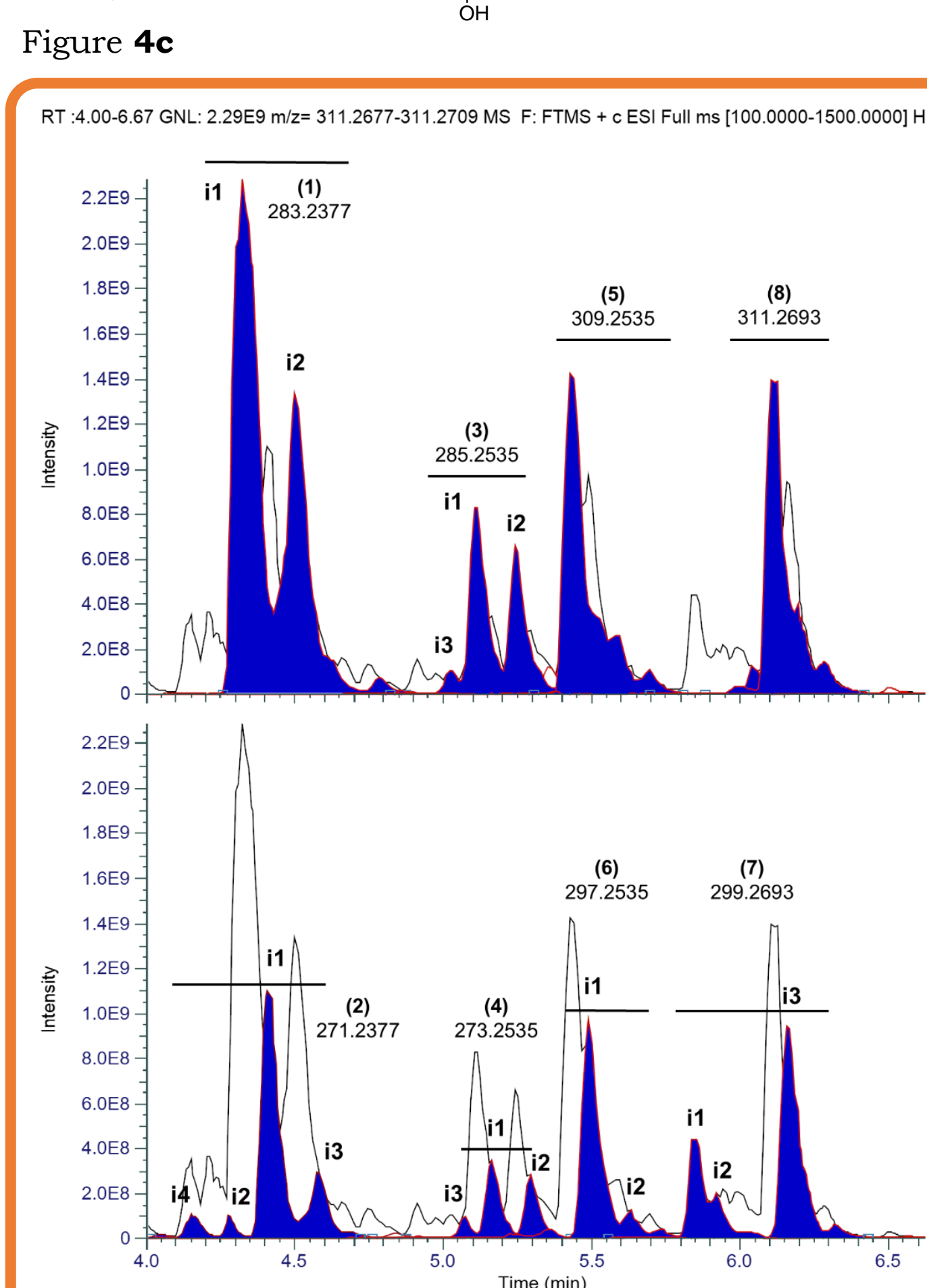
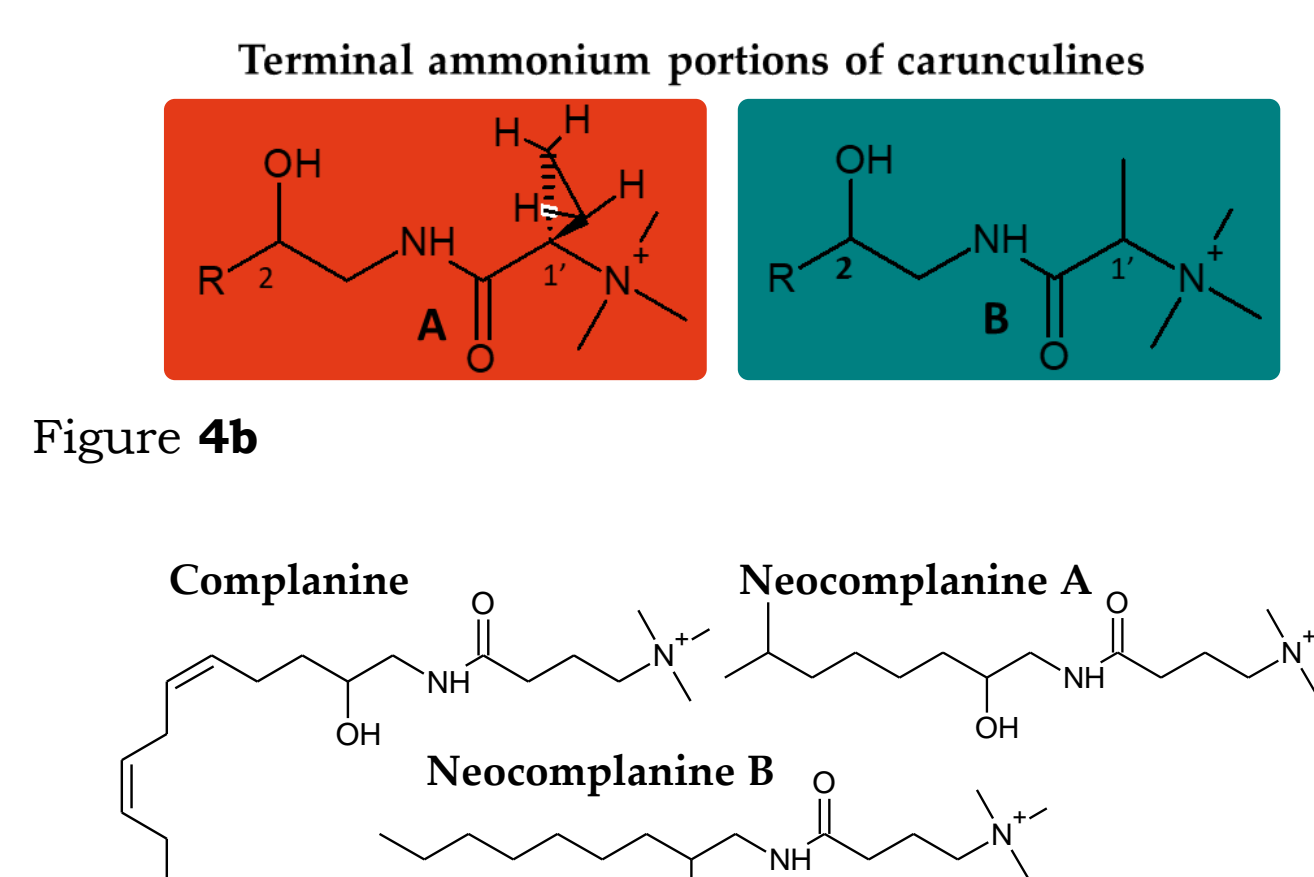


Figure 5. Extracted ion chromatogram of carunculines (1-8) from *H. carunculata*: the peaks of compounds 1, 3, 5, 8 (up) and 2, 4, 6, 7 (down) and related isomers (i1, i2, i3, i4) are filled in blue. Modified from [3].

Table 1. Relevant MS/MS fragments identified in carunculines with A (1, 3, 5, 8) and B (2, 4, 6, 7) using HPLC-ESI/HRMS. The fragmentation pathways are completely different, corroborating the proposed structures. Modified from [3].

Terminal ammonium portion (A)					Proposed molecular structure / formula			
Carunculine 1 (m/z 283.2377)	Carunculine 3 (m/z 285.2535)	Carunculine 5 (m/z 309.2535)	Carunculine 8 (m/z 311.2693)	MS/MS fragments				
58.0659	58.0659	58.0659	58.0659	58.0659	CH ₂ =N(CH ₃) ₂ ⁺			
60.0815	60.0815	60.0815	60.0815	60.0815	NH(CH ₃) ₂ ⁺			
67.0549	67.0549	67.0549	67.0549	67.0549	C ₃ H ₇ ⁺			
84.0813	84.0813	84.0813	84.0813	84.0813	C ₇ H ₁₁ ⁺			
95.0859	95.0859	95.0859	95.0859	95.0859	C ₈ H ₁₄ N ₂ O ⁺			
98.0967	98.0967	98.0968	98.0967	98.0967	C ₈ H ₁₄ N ₂ O ⁺			
116.1071	116.1071	116.1071	116.1071	116.1071	C ₈ H ₁₄ N ₂ O ⁺			
123.1168	123.1168	/	123.1169	123.1169	C ₇ H ₁₁ ⁺			
143.1177	143.1178	143.1178	143.1178	143.1178	H ₂ N-C(=O)-C ₃ H ₇			
neutral loss, m	neutral loss, m	neutral loss, m	neutral loss, m	neutral loss, m				
170.1536	113.084	172.1693	113.084	198.1850	113.0843	-N(CH ₃) ₂		
198.1850	85.053	200.2006	85.0529	224.2007	85.0528	226.2163	85.0530	-N(CH ₃) ₂
265.2270	18.011	267.2426	18.0107	291.2426	18.0109	293.2582	18.0111	-H ₂ O

Terminal ammonium portion (B)					Proposed molecular structure / formula			
Carunculine 2 (m/z 271.2377)	Carunculine 4 (m/z 273.2535)	Carunculine 6 (m/z 297.2535)	Carunculine 7 (m/z 299.2693)	MS/MS fragments				
58.0659	58.0659	58.0659	58.0659	58.0659	CH ₂ =N(CH ₃) ₂ ⁺			
60.0815	60.0815	60.0815	60.0815	60.0815	NH(CH ₃) ₂ ⁺			
67.0549	67.0549	67.0549	67.0549	67.0549	C ₃ H ₇ ⁺			
81.0704	81.0704	81.0704	81.0704	81.0704	C ₅ H ₉ ⁺			
95.0859	95.0859	95.0859	95.0859	95.0859	C ₇ H ₁₁ ⁺			
123.1168	123.1168	123.1170	123.1169	123.1169	C ₇ H ₁₁ ⁺			
neutral loss, m	neutral loss, m	neutral loss, m	neutral loss, m	neutral loss, m				
140.1432	131.0945	142.1589	131.0946	166.1591	131.0944	168.1746	131.0947	-H ₂ O-N(CH ₃) ₂
166.159	105.0787	168.1745	105.079	192.1741	105.0794	194.1902	105.0791	-CO
184.1694	87.0683	186.1851	87.0684	210.1848	87.0687	212.2007	87.0686	-N(CH ₃) ₂ -CO
194.1539	77.0838	196.1694	77.0841	220.1649	77.0886	222.1851	77.0842	-H ₂ O-N(CH ₃) ₂
253.2271	18.0106	255.2427	18.0108	279.2428	18.0107	281.2583	18.0110	-H ₂ O

References

[1] K. Nakamura, Y. Tachikawa, M. Kitamura, O. Ohno, M. Suganuma and D. Uemura, *Org. Biomol. Chem.* **6**, 2058-2060 (2008); [2] R. Simonini, F. Maggioni, F. Zanetti, S. Fai, L. Forti, D. Prevedelli and S. Righi, *J. Exp. Mar. Biol. Ecol.* **534**, 151487 (2021); [3] S. Righi, L. Forti, R. Simonini, V. Ferrari, D. Prevedelli and A. Mucci, *Mar. Drugs* (2022), accepted.