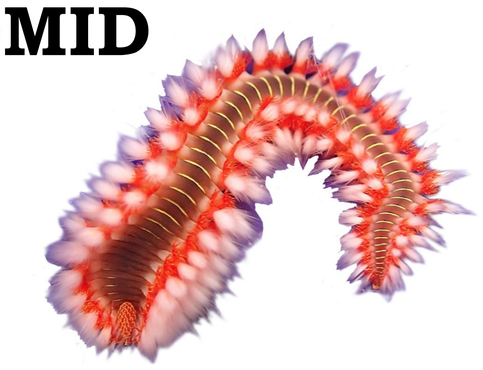
NEW SECONDARY METABOLITES IN THE AMPHINOMID FIREWORM *HERMODICE CARUNCULATA*



A. Mucci,[1] L. Forti,[2] R. Simonini,[3] V. Ferrari, [3] D. Prevedelli,[3] S. Righi[3]

[1] *Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia, Via Campi 103, 41125 Modena, Italy*
[2] *Department of Life Sciences, University of Modena and Reggio Emilia, Via Campi 103, 41125 Modena, Italy*
[3] *Department of Life Sciences, University of Modena and Reggio Emilia, Via Campi 213/D, 41125 Modena, Italy*
E-mail: adele.mucci@unimore.it

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 ^{1}H and H,C-HSQCed spectra (Fig. 1 and 2), **was wrong**. The sign of H,C correlations in HSQCed spectrum is deceiving for cyclopropanes (^{1}J(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 CH_3 signals were abnormally high and with a too symmetric shape… (Fig. 3) and ^{13}C chemical shift (around 10 ppm) was too low.

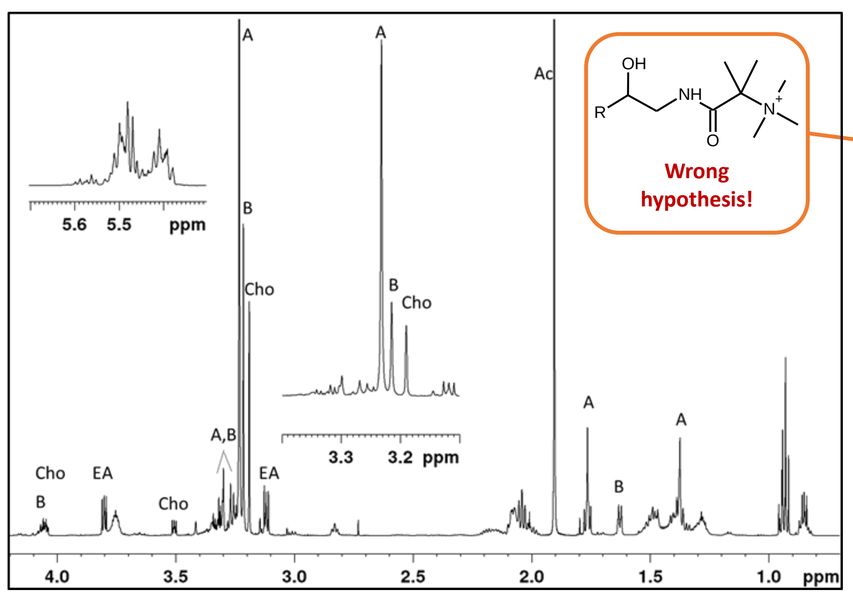


Figure 1. ^{1}H spectrum in D_2O of *H. carunculata* extract. Diagnostic signals of carunculines are marked with A and B. Cho = choline, EA = ethanolamine. Modified from [3].

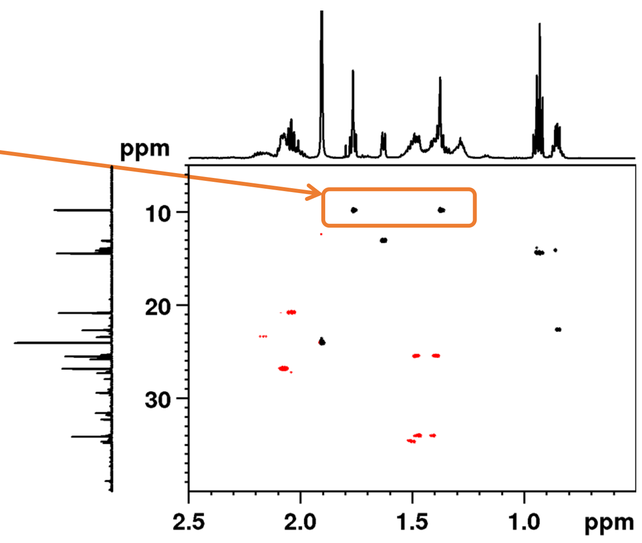


Figure 2. Enlarged regions of the H,C-HSQCed NMR spectrum. In the black rectangle, the correlation between the protons and the carbon of the cyclopropane ring, with the same sign of methyl and methyne correlations. Modified from [3].

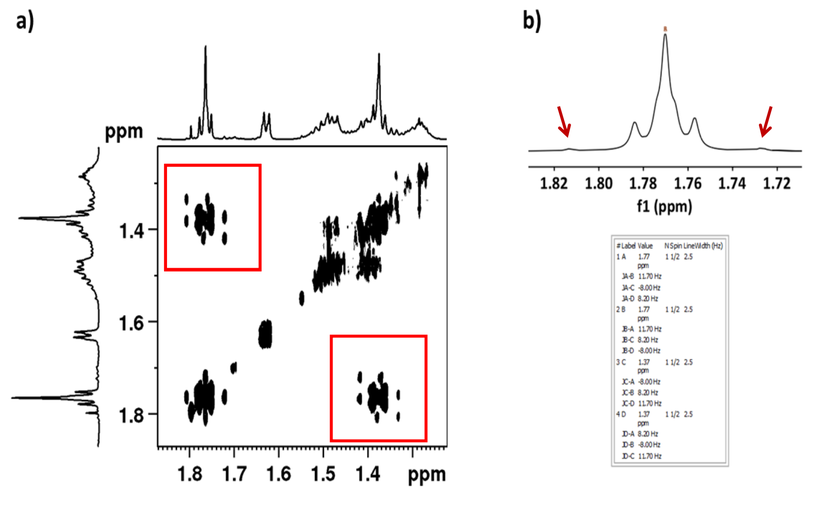


Figure 3. Enlarged region of the H,H-COSY NMR spectrum of carunculines. a) The red square shows the correlation between the AA' and BB' protons of the cyclopropane ring. b) spin-system simulation showing the shape of one of the two multiplets of the AA'BB' system (MestReNova v 14.1.2-25024). Modified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

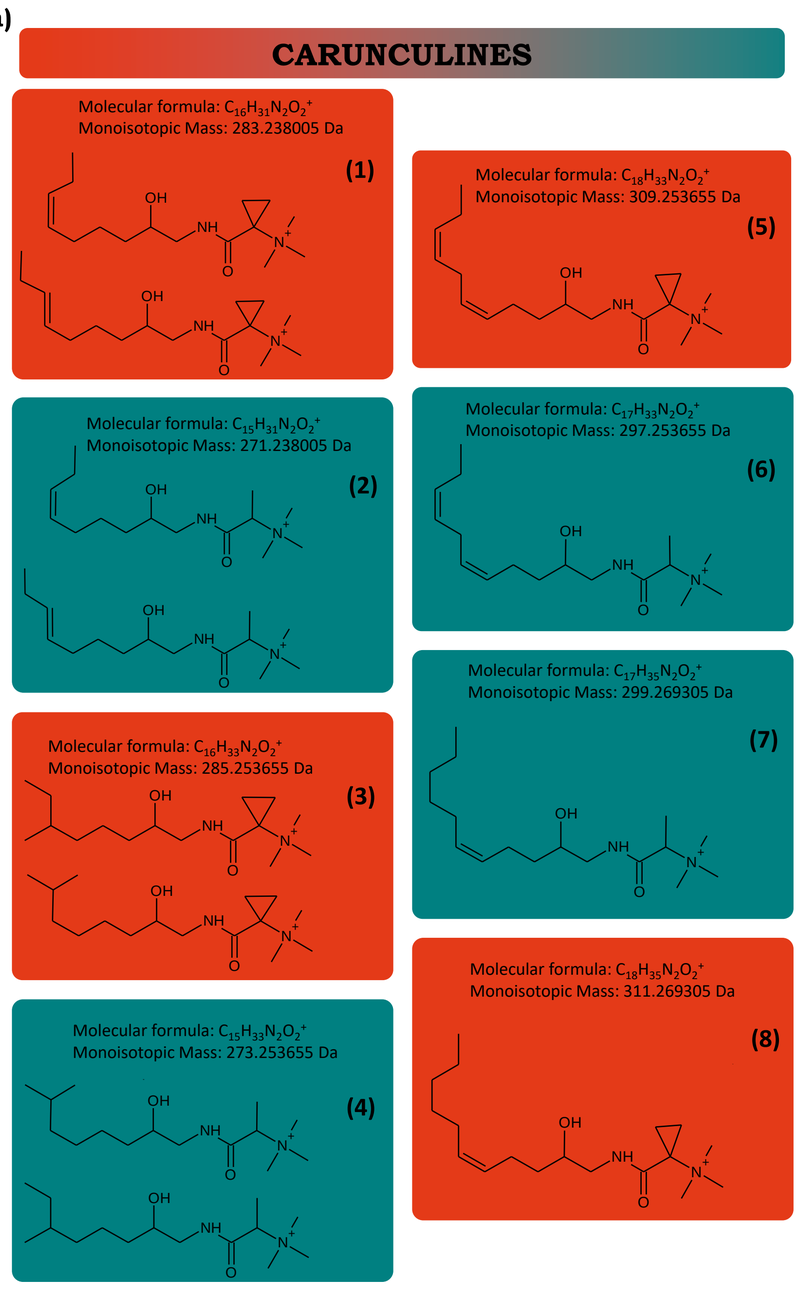


Figure 4. Proposed structures for carunculines and molecular structures of complanines. **a**) Proposed molecular structures for carunculines (**1**–**8**) and their isomers derived by matching the structures obtained by NMR spectra and the formulae obtained by HPLC-ESI/HRMS data. **b**) terminal ammonium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

Terminal ammonium portions of carunculines

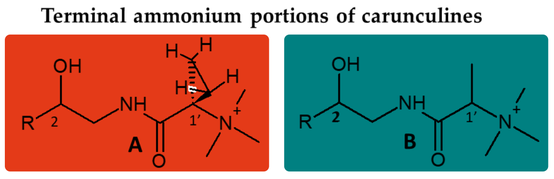


Figure **4b**

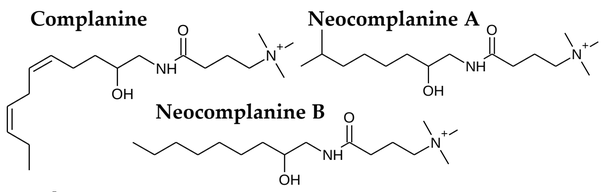


Figure **4c**

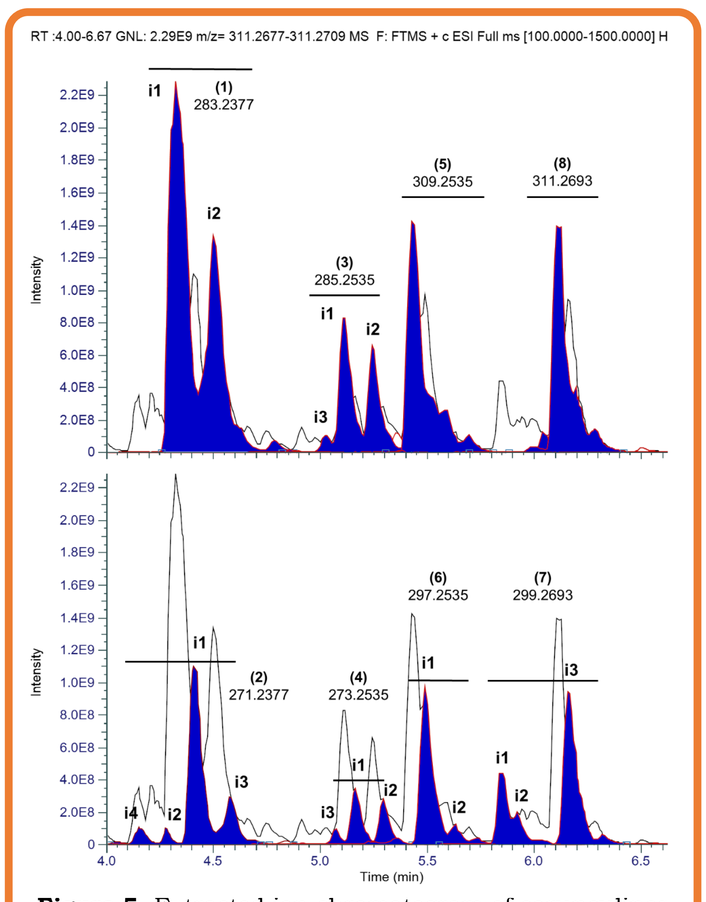


Figure 5. Extracted ion chromatogram of carunculines (**1**–**8**) from *H. carunculata*: the peaks of compounds **1**,**3**,**5**,**8** (up) and **2**,**4**,**6**,**7** (down) and related isomers (i1,i2,i3,i4) are filled in blue. Modified from [3].

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)

Carunculine **1** (*m/z* 283.2377)		Carunculine **3** (*m/z* 285.2535)		Carunculine **5** (*m/z* 309.2535)		Carunculine **8** (*m/z* 311.2693)		Proposed molecular structure / formula
MS/MS fragments		*MS/MS fragments*		*MS/MS fragments*		*MS/MS fragments*		
58.0659		*58.0659*		*58.0659*		*58.0659*		$CH_2{=}N(CH_3)_2^+$
60.0815		*60.0815*		*60.0815*		*60.0815*		$NH(CH_3)_3^+$
67.0549		*67.0549*		*67.0549*		*67.0549*		$C_5H_7^+$
84.0813		*84.0813*		*84.0813*		*84.0813*		
95.0859		*95.0859*		*95.0859*		*95.0859*		$C_7H_{11}^+$
98.0967		*98.0967*		*98.0968*		*98.0967*		
								$C_6H_{14}NO^{+}$*
116.1071		*116.1071*		*116.1071*		*116.1071*		
123.1168		*123.1168*		/		*123.1169*		$C_9H_{15}^+$
143.1177		*143.1178*		*143.1178*		*143.1178*		
	neutral loss, *m*		neutral loss, *m*		neutral loss, *m*		neutral loss, *m*	
170.1536	113.084	*172.1693*	113.084	*196.1692*	113.0843	*198.1850*	113.0843	-
198.1850	85.053	*200.2006*	85.0529	*224.2007*	85.0528	*226.2163*	85.0530	-
265.2270	18.011	*267.2426*	18.0107	*291.2426*	18.0109	*293.2582*	18.0111	- H_2O

*tentative structure

Terminal ammonium portion (B)

Carunculine **2** (*m/z* 271.2377)		Carunculine **4** (*m/z* 273.2535)		Carunculine **6** (*m/z* 297.2535)		Carunculine **7** (*m/z* 299.2693)		Proposed molecular structure / formula
MS/MS fragments		*MS/MS fragments*		*MS/MS fragments*		*MS/MS fragments*		
58.0659		*58.0659*		*58.0659*		*58.0659*		$CH_2{=}N(CH_3)_2^+$
60.0815		*60.0815*		*60.0815*		*60.0815*		$NH(CH_3)_3^+$
67.0549		*67.0549*		*67.0549*		*67.0549*		$C_5H_7^+$
81.0704		*81.0704*		*81.0704*		*81.0704*		$C_6H_9^+$
95.0859		*95.0859*		*95.0859*		*95.0859*		$C_7H_{11}^+$
123.1168		*123.1168*		*123.1170*		*123.1169*		$C_9H_{15}^+$
	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	-
166.159	105.0787	*168.1745*	105.079	*192.1741*	105.0794	*194.1902*	105.0791	- H_2O - $N(CH_3)_3$ - CO
184.1694	87.0683	*186.1851*	87.0684	*210.1848*	87.0687	*212.2007*	87.0686	- $N(CH_3)_3$ - CO
194.1539	77.0838	*196.1694*	77.0841	*220.1649*	77.0886	*222.1851*	77.0842	- H_2O - $N(CH_3)_3$
253.2271	18.0106	*255.2427*	18.0108	*279.2428*	18.0107	*281.2583*	18.0110	- H_2O

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.