NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2026.2709593

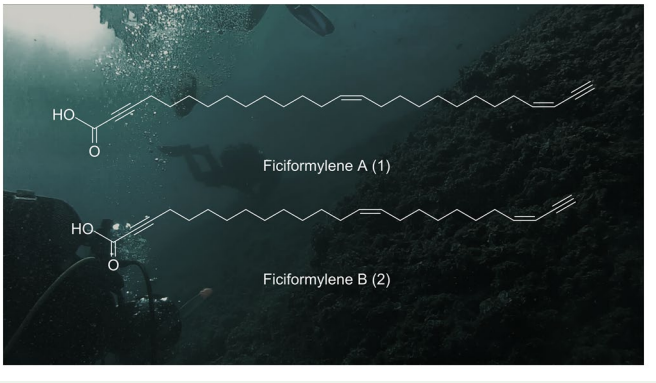
LC/MS-based metabolomics guided isolation of new polyacetylenes ficiformylenes A and B, from the mediterranean Marine sponge *Petrosia ficiformis*

Yasumoto Oyadomari[a], Leontine E. Becking[b,c], Giorgio Bavestrello[d,e], Maria Valeria D' Auria[f], Angela Zampella[f], Nobuhiro Fusetani[g], Koshi Machida[a] and Yoichi Nakao[a,g]

[a]Department of Chemistry and Biochemistry, Graduate School of Advanced Science and Engineering, Waseda University, Shinjuku, Japan; [b]Aquaculture & Fisheries Group, Wageningen University & Research, Netherlands; [c]Naturalis Biodiversity Center, Netherlands; [d]Dipartimento di Scienze della Terra, dell'Ambiente e della Vita (DiSTAV), University of Genova, Italy; [e]Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa), Italy; [f]Department of Pharmacy, University of Naples "Federico II", Italy; [g]Research Institute for Science and Engineering, Waseda University, Japan

ABSTRACT

LC/MS-based metabolite profiles of closely related sponge samples collected from Italy and Japan were analysed. Characteristic ion peaks observed in the MS data were used to guide the search for new metabolites, leading to the isolation of two new compounds, ficiformylenes A (**1**) and B (**2**). Their structures were elucidated by spectroscopic analyses, including 1D and 2D NMR and ESIMS/MS experiments, as well as ESIMS analysis of the products obtained by ozonolysis. These results demonstrate the utility of LC/MS-based comparative analysis of marine sponge secondary metabolites as an effective strategy for the discovery of novel natural products.



ARTICLE HISTORY
Received 19 March 2026
Accepted 22 July 2026

KEYWORDS
Marine sponge; *Petrosia*; LC/MS; metabolomics; polyacetylene

CONTACT Yoichi Nakao ayocha@waseda.jp

Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2026.2709593.

1. Introduction

Marine-derived natural products are an important source of compounds that exhibit unique chemical structures and potent biological activities (Blunt et al. 2018). However, with the progress of natural product discovery, the rediscovery of known compounds and the increasing costs associated with isolation and structure elucidation have become major challenges, highlighting the need for methodologies that improve the efficiency of discovering new compounds (Newman and Cragg 2007, 2012, 2016, 2020; Gaudêncio et al. 2023).

Marine sponges can show quantitative and qualitative variation in secondary-metabolite profiles even among conspecific or closely related taxa, not only across geographically distinct habitats but also at more local scales and among individual specimens (Noyer et al. 2011; Rohde et al. 2012; De Caralt et al. 2013). This property—namely, that organisms that are taxonomically identical (or similar) can be chemically distinct—makes it difficult to decide which samples or fractions should be prioritised in natural product discovery. At the same time, if comparative study designs are appropriately constructed, such differences can serve as useful clues for efficiently reaching previously unknown components.

Sponges of the genus *Petrosia* are widely distributed from tropical to temperate regions and across habitats ranging from intertidal zones to deep waters, and a diverse array of secondary metabolites has been reported from this genus, including polyacetylenes and sterols (Lee et al. 2021). Therefore, a framework that compares sets of closely related specimens, including *Petrosia* spp., and extracts candidate novel compounds based on differences in chemical profiles is considered a promising approach for improving the efficiency of new-compound discovery.

In this study, we focus on LC/MS, which enables sensitive acquisition of chemical information at an early stage of the extraction process. Using multiple sponge samples, including *Petrosia* spp., we examine a practical workflow in which multivariate analysis is applied to feature sets defined by retention time (RT) and *m/z* values to visualise inter-sample differences and enable the extraction of candidate novel compounds. This approach is expected to reduce redundant re-identification of known compounds and rationalise investment in isolation and structure elucidation, thereby improving the efficiency of sponge-derived natural product discovery.

2. Results and discussion

2.1. LC/MS-based metabolomics

This study was conducted on eight sponge specimens collected from Italy and Japan. Six specimens belonged to the genus *Petrosia*, comprising three specimens of *P. ushitsuensis* (Japan) and three specimens of *P. ficiformis* (Italy), which were selected to investigate the similarity and variability of metabolite profiles within the genus across geographically distant populations. In addition, *Chondrilla oxyastera* (Japan) and *Chondrosia reniformis* (Italy) were included as phylogenetically distinct non-*Petrosia* sponge species to provide a comparative reference for evaluating whether the metabolite profiles observed were characteristic of the genus *Petrosia* rather than general features shared among marine sponges (SI Figures S1 and S2). Each sample was kept

frozen until it was analysed. Approximately 5 g of tissue from each sample was extracted with CH_3OH. The methanolic extracts were concentrated and partitioned between H_2O and $CHCl_3$. The $CHCl_3$ layer was desalted using a reversed-phase ODS (octadecylsilyl) column, and the methanol-eluting fractions were analysed by LC/MS (positive ion mode). The resulting LC/MS data were analysed using the multivariate analysis software *Reifycs* Signpost MS™. After excluding 159 peaks detected in the CH_3OH blank, a total of 3,012 peaks (alignment ID: 1–3,350) were aligned based on a combination of RTs and *m/z* values (SI Table S1 and Alignment Results). The number of unique ion peaks for each sample is listed in SI Table S1. In total, out of the 3,012 detected peaks, approximately two-thirds (2,020) were unique to individual samples. Additionally, 76 ion peaks were shared exclusively among the three *P. ushitsuensis* specimens (SI Table S1), whereas 33 ion peaks were exclusive to the three *P. ficiformis* specimens. Notably, only six ion peaks were shared among all six *Petrosia* specimens and were undetected in the *Chondrilla* and *Chondrosia* specimens. Based on the above results, it was concluded that each sponge sample produced a greater number of unique compounds than compounds commonly shared within the same genus or species. Although many ion peaks were unique to individual samples, principal component analysis (PCA) based on RTs and *m/z* values was performed to assess the overall similarity in metabolite profiles (SI Figure S3 and S4). Interestingly, peak clusters from the three *P. ficiformis* and three *P. ushitsuensis* specimens were localised in positive regions of both the first and second principal components (PC1 and PC2, SI Figure S3), suggesting that the metabolite compositions of the six *Petrosia* specimens were similar. Moreover, comparison of the ion peak distributions between *P. ushitsuensis* and *P. ficiformis* revealed that their score plots were closely clustered, indicating a high degree of similarity in RTs and *m/z* values among respective species. Consistent with the PCA results, hierarchical clustering analysis visualised as a heatmap (SI Figure S5) also demonstrated strong similarities among samples from the same species or genus. In contrast, *Chondrilla oxyastera* and *Chondrosia reniformis* were clearly separated from the six *Petrosia* specimens, supporting their role as comparative references for assessing genus-level metabolic relationships.

Further detailed analysis of the LC/MS data revealed four characteristic ion peaks (SI Table S2). To prioritise metabolites for isolation and structural characterisation, ion peaks were comprehensively evaluated based on their occurrence patterns among sponge specimens, LC/MS signal intensities, and potential novelty inferred from their molecular formulae. The ion peaks with alignment IDs 2,508 and 2,339 were prioritised because their estimated molecular formulae did not correspond to any metabolites previously reported from marine sponges of the genus *Petrosia*, suggesting that they might represent new compounds. In contrast, alignment IDs 1,076 and 1,109 were selected because they exhibited relatively high LC/MS signal intensities and showed large loading values in the PCA loading plot [ID 1,109: (PC1, PC2) = (0.0169, 0.0222); ID 1,076: (PC1, PC2) = (0.0137, 0.0204)], indicating that they contributed substantially to the metabolomic characteristics of the genus *Petrosia*.

The ion peak of alignment ID 2,508 (RT: 19.135 min, *m/z*: 399.3260, **1**) was detected in two *P. ficiformis* samples (S14105 and S14111). Based on the chemical formula $C_{27}H_{42}O_2$ (calcd for $C_{27}H_{43}O_2^+$, 399.3258) estimated from the *m/z* value, it was highly likely to be a compound that has not been reported from marine sponges of the

genus *Petrosia*. The ion peak of alignment ID 2,339 (RT: 19.154 min, *m/z*: 371.2947, **2**) was commonly detected in three of the *P. ficiformis* samples (S14105, S14111, and S14112), and the putative chemical formula $C_{25}H_{38}O_2$ (calcd for $C_{25}H_{39}O_2^+$, 371.2945) has also not been reported previously.

The ion peak of alignment ID 1,076 (RT: 22.488 min, *m/z*: 677.5244, **3**), which was estimated to be $C_{46}H_{70}O_2$ (calcd for $C_{46}H_{70}O_2Na^+$, 677.5268), was a common ion peak detected in the three Japanese *P. ushitsuensis* samples (S14006, S14023, and S14025). Several C-46 linear acetylene compounds have been reported from *Petrosia* samples collected around Japan (Lee et al. 2021). Therefore, this ion peak may be attributable to one of them. Hierarchical clustering analysis revealed that the ion peak of alignment ID 1,109 (RT: 11.560 min, *m/z*: 275.1678, **4**) was detected in five *Petrosia* sp. samples (S14006, S14023, S14025, S14111, and S14112). Extracted ion chromatograms (*m/z* 275.168 ± 0.005) prepared from the LC/MS raw data detected the same ion peak at approximately 11.48 min in the remaining *Petrosia* sample (S14105) (SI Figure S6). Therefore, the compound giving this peak, with an estimated formula of $C_{15}H_{24}O_3$ (calcd for $C_{15}H_{24}O_3Na^+$, 275.1618), was present in all six *Petrosia* samples.

Based on these observations, compounds **1**–**4**, responsible for the four ion peaks, were isolated, and their structures were determined by ESIMS/MS analysis (Figure 1). Compounds **1** and **2** were isolated from *P. ficiformis* (S14105), whereas compounds **3** and **4** were isolated from *P. ushitsuensis* (S14023). After performing spectroscopic analyses (NMR and MS experiments, SI Figures S7–S15 and S17–S25), compounds **1** and **2** were identified as new compounds and named ficiformylenes A and B,

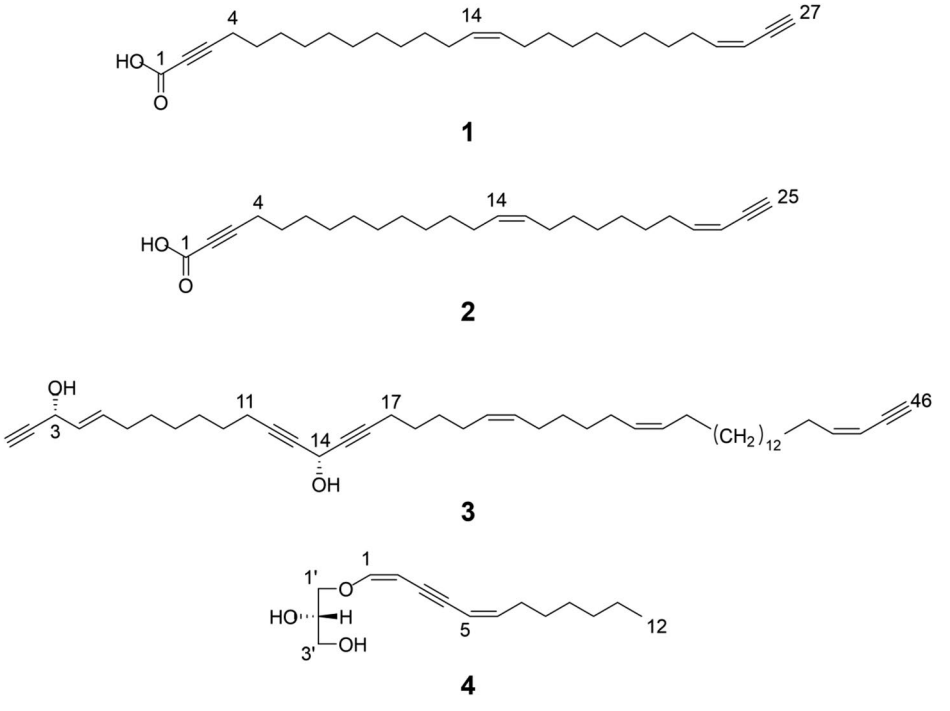


Figure 1. Structures of ficiformylenes A (**1**) and B (**2**), petrocortyne A (**3**), and petroraspailyne A1 (**4**).

respectively. Compounds **3** and **4** were identified as petrocortyne A (**3**) (Seo et al. 1998) and petroraspailyne A1 (**4**) (Seo et al. 1999), respectively, based on comparison with literature data (SI Figure S27-S30).

2.2. Structure elucidation of ficiformylenes A (1) and B (2)

Ficiformylene A (**1**) was obtained as a white amorphous solid. The molecular formula was established as $C_{27}H_{42}O_2$ by HRESIMS analysis: *m/z* 399.3256 $[M+H]^+$ (calcd for $C_{27}H_{43}O_2^+$, 399.3258), *m/z* 397.3082 $[M-H]^-$ (calcd for $C_{27}H_{41}O_2^-$, 397.3112) and *m/z* 421.3080 $[M+Na]^+$ (calcd for $C_{27}H_{42}O_2Na^+$, 421.3077). The ^{1}H NMR spectrum of ficiformylene A (**1**) measured in CD_3OD exhibited signals of four olefin protons [δ_H 6.00, 5.45, 5.34 (2H)], one terminal alkyne proton (δ_H 3.41), and several aliphatic methylene protons (δ_H 1.25–1.40). The ^{13}C spectrum (in CD_3OD) revealed the existence of one carbonyl carbon (δ_C 162.4) and four olefin carbons [δ_C 146.6, 131.0 (2C), 109.5]. Analysis of 2D ^{1}H-^{1}H COSY,^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC spectra identified three substructures **a** (C-1–C-6), **b** (C-12–C-17), and **c** (C-22–C-27) (SI Figure S31). The geometry at $\mathbf{\Delta}^{24}$ was determined to be *Z* based on the coupling constant between H-24 and H-25 (11.0 Hz). The allyl positions (C-13 and C-16) were more shielded (δ_C 28.30 and 28.24, respectively) than the aliphatic methylene carbons (δ_C 29.6–31.3), indicating that the geometry at $\mathbf{\Delta}^{14}$ was *Z* (SI Figure S31).

The number of methylene carbons among the substructures **a**–**c** was revealed by the ESIMS/MS experiment (positive mode, precursor ion: *m/z* 399.3 $[M+H]^+$) (SI Figure S32). Fragment ions at *m/z* 135.1164, and 149.1325 were observed, reflecting the presence of a C=C double bond between C-14 and C-15. The location of this double bond was further confirmed by oxidative ozonolysis of **1**, followed by ESIMS analysis in the negative mode, which gave characteristic product ions at *m/z* 201.1121 and 229.1434 (SI Figure S15) (Seo et al. 1998). According to the results above, ficiformylene A (**1**) is a new acetylene compound as expected.

Ficiformylene B (**2**) was obtained as a white amorphous solid. The molecular formula was established as $C_{25}H_{38}O_2$ by HRESIMS [*m/z* 371.2945 $[M+H]^+$ (calcd for $C_{25}H_{39}O_2^+$, 371.2945), *m/z* 369.2777 $[M-H]^-$ (calcd for $C_{25}H_{37}O_2^-$, 369.2799) and *m/z* 393.2766 $[M+Na]^+$, (calcd for $C_{25}H_{38}O_2Na^+$, 393.2764)]. The ^{1}H NMR spectrum of ficiformylene B (**2**) (in CD_3OD) was almost superimposable to that of **1**, indicating that **2** shares the same substructures as **1**; however, the length of the carbon chain differs. The position of the C=C double band was determined to be between C-14 and C-15 on the basis of the ESIMS/MS experiments (positive mode, precursor ion: *m/z* 371.3 $[M+H]^+$), which showed characteristic fragment ions at *m/z* 107.0847, 121.1008, and 175.1469, and was further confirmed by oxidative ozonolysis, followed by ESIMS analysis (negative mode) of the degradation products, which showed characteristic ions at *m/z* 173.08 and 229.14 (SI Figure S24 and S25) (Seo et al. 1998). Based on this observation, the overall structure of ficiformylene B (**2**) was elucidated.

2.3. Cytotoxicity of ficiformylenes A (1) and B (2) against HeLa and P388 cells

Ficiformylenes A (**1**) and B (**2**) were tested for cytotoxicity against HeLa and P388 cells, and **1** showed weak cytotoxicity against P388 cells (IC_{50} = 11.2 μM), whereas **2** was non-cytotoxic.

3. Experimental

See Supplementary Material.

4. Conclusions

In this study, an LC/MS-guided targeted search enabled the isolation of four acetylene compounds from a sponge extract, including two new metabolites, ficiformylenes A (**1**) and B (**2**). This outcome highlights the utility of LC/MS-driven prioritisation for capturing structurally distinctive, low-abundance constituents that may be missed by conventional workflows. Ficiformylenes A (**1**) and B (**2**) showed only weak cytotoxicity and were obtained in very low yields; therefore, their ecological or biological roles remain unclear based on the present data. Considering that sponges function as holobionts, and that sponge-derived specialised metabolites have been proposed to participate in host–microbe and microbe–microbe interactions, these compounds may warrant further investigation in a holobiont context (Taylor et al. 2007; Pita et al. 2018). However, such possible roles, including effects on symbiotic microorganisms or community structure, remain speculative and require experimental validation in future studies.

Acknowledgements

This work was inspired by the International and Interdisciplinary Environments of the JSPS Core-to-Core Program, "Asian Chemical Biology Initiative". We thank Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

Author contributions

CRediT: **Yasumoto Oyadomari**: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft; **Leontine E. Becking**: Formal analysis, Investigation, Writing – review & editing; **Giorgio Bavestrello**: Formal analysis, Investigation, Resources, Writing – original draft; **Angela Zampella**: Formal analysis, Investigation, Resources, Validation, Writing – review & editing; **Nobuhiro Fusetani**: Investigation, Resources, Supervision; **Koshi Machida**: Data curation, Investigation, Resources, Writing – original draft; **Yoichi Nakao**: Conceptualization, Data curation, Funding acquisition, Investigation, Supervision, Validation, Writing – review & editing.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This paper is a part of the outcome of research performed under a Grant of JSPS KAKENHI Grant Number 25303008, Waseda Research Institute for Science and Engineering, Grant-in-Aid for Young Scientists (Early Bird), and the Strategic Research Platforms for Private University.

Data availability statement

NMR data for the following compounds have been deposited in the Natural Products Magnetic Resonance Database (NP-MRD; www.np-mrd.org) and can be found at NP0351263 (Ficiformylene A) and NP0351264 (Ficiformylene B).

References

Blunt JW et al. 2018. Marine natural products. Nat Prod Rep. 35(1):8–53. https://doi.org/10.1039/C7NP00052A

De Caralt S et al. 2013. Sources of secondary metabolite variation in *Dysidea avara* (Porifera: demospongiae): the importance of having good neighbors. Mar Drugs. 11(2):489–503. https://doi.org/10.3390/md11020489

Gaudêncio SP et al. 2023. Advanced methods for natural products discovery: bioactivity screening, dereplication, metabolomics profiling, genomic sequencing, databases and informatic tools, and structure elucidation. Mar Drugs. 21(5):308. https://doi.org/10.3390/md21050308

Lee Y-J, Cho Y, Tran HNK. 2021. Secondary metabolites from the marine sponges of the genus *Petrosia*: a literature review of 43 years of research. Mar Drugs. 19(3):122. https://doi.org/10.3390/md19030122

Newman DJ, Cragg GM. 2007. Natural products as sources of new drugs over the last 25 years. J Nat Prod. 70(3):461–477. https://doi.org/10.1021/np068054v

Newman DJ, Cragg GM. 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod. 75(3):311–335. https://doi.org/10.1021/np200906s

Newman DJ, Cragg GM. 2016. Natural products as sources of new drugs from 1981 to 2014. J Nat Prod. 79(3):629–661. https://doi.org/10.1021/acs.jnatprod.5b01055

Newman DJ, Cragg GM. 2020. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod. 83(3):770–803. https://doi.org/10.1021/acs.jnatprod.9b01285

Noyer C, Thomas OP, Becerro MA, Franke A, Hentschel U. 2011. Patterns of chemical diversity in the Mediterranean sponge *Spongia lamella*. PLoS One. 6(6):e20844. https://doi.org/10.1371/journal.pone.0020844

Pita L, Rix L, Slaby BM, Franke A, Hentschel U. 2018. The sponge holobiont in a changing ocean: from microbes to ecosystems. Microbiome. 6(1):46. https://doi.org/10.1186/s40168-018-0428-1

Rohde S et al. 2012. Spatial variability in secondary metabolites of the Indo-Pacific sponge *Stylissa massa*. J Chem Ecol. 38(5):463–475. https://doi.org/10.1007/s10886-012-0124-8

Seo Y, Cho KW, Rho J-R, Shin J, Sim J. 1998. Petrocortynes and petrosiacetylenes, novel polyacetylenes from a sponge of the genus *Petrosia*. Tetrahedron. 54(3-4):447–462. https://doi.org/10.1016/S0040-4020(97)10290-3

Seo Y, Cho KW, Lee H-S, Rho J-R, Shin J. 1999. New acetylenic enol ethers of glycerol from the sponge *Petrosia* sp. J Nat Prod. 62(1):122–126. https://doi.org/10.1021/np9803575

Taylor MW, Radax R, Steger D, Wagner M. 2007. Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. Microbiol Mol Biol Rev. 71(2):295–347. https://doi.org/10.1128/mmbr.00040-06