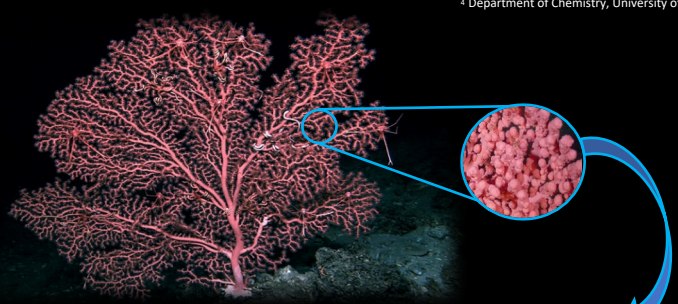
Deep-Sea Diterpenoids Isolated from the Bubble Gum Coral (*Paragorgia arborea*)

Sam Afoullouss[1,2], John H. Adams[3], Bill J. Baker[4], A. Louise Allcock[2], Olivier. P. Thomas[1]

[1] Marine Biodiscovery, School of Chemistry and Ryan Institute, National University of Ireland Galway (NUI Galway), University Road, H91TK33 Galway, Ireland
[2] School of Natural Sciences and Ryan Institute, National University of Ireland Galway (NUI Galway), University Road, H91TK33 Galway, Ireland
[3] College of Public Health, University of South Florida, Tampa, FL 33612, USA
[4] Department of Chemistry, University of South Florida, Tampa, FL 33620, USA

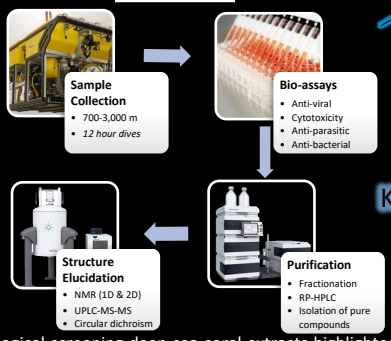


In-situ photograph of *Paragorgia arborea,* at a depth of 1,450 m

Introduction

Deep-sea coral reefs are among the most unique and complex ecosystems on our planet.[1] Ranging in depth from 300 – 3,000 m, not only do they host a diverse range of unique species from a broad range of phyla, but they do so under intense physio-chemical conditions including high pressure, low temperatures and an absence of light. Driven by metabolic adaptations required to thrive in these extreme environmental conditions, the fauna which inhabit these reefs have proven to be a rich source of secondary metabolites with novel chemical scaffolds and bioactivity. With an aim to discover new bioactive metabolites from the Irish deep-sea coral reefs, our sample collection expeditions used a Remotely Operated Vehicle (ROV) to sample at depths of 3,000 m, along the Celtic continental margin. We targeted a diverse range of soft corals (Order: Alcyoncea), for their abundance of bioactive metabolites.[2] An extensive bioactive screening process, combined with NMR based prioritization, led our attention to focus on the bubble gum coral, *Paragorgia arborea,* the organic extract of which showed antiplasmodial activity and cytotoxicity. An in-depth chemical investigation of this species resulted in the isolation of eight xenicane diterpenoids, three of which are new; two xeniaphyllanes, miolenols A(**1**) and B(**2**), both containing a rare cyclobutanol ring; a new xeniolide, epoxy-coraceniolide A (**3**), and five known xeniolides, coraxeniolide A and C,[3] 9-deoxyxeniolide A,[4] acalycigorgin E,[5] and acalycixeniolide F.[6]

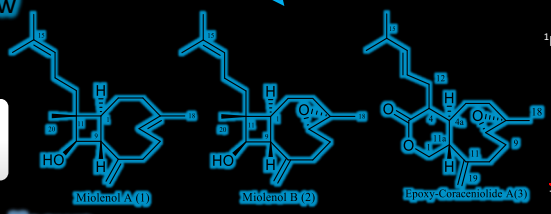
Workflow



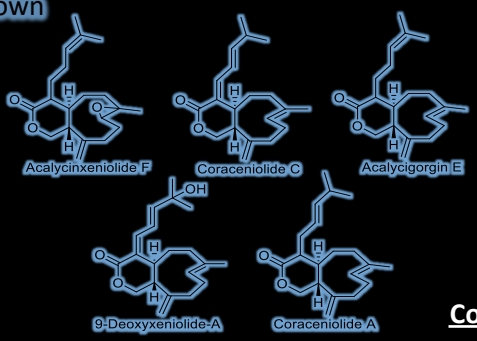
Biological screening deep-sea coral extracts highlighted the soft coral, *Paragorgia arborea,* as having potent activity in antiplasmodial and cytotoxicity assays.

Chemical profiling using ^{1}H-NMR and UPLC-DAD-ESLD indicated the presence of xenicane class of diterpenes. This class of diterpenes have known antiplasmodial activity.[3]

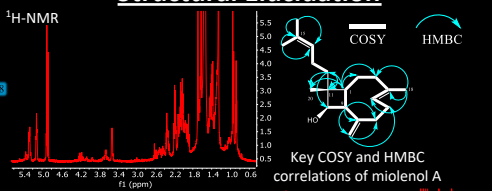
New



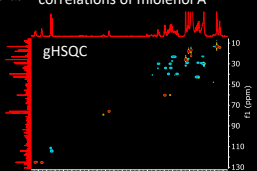
Known



Structural Elucidation

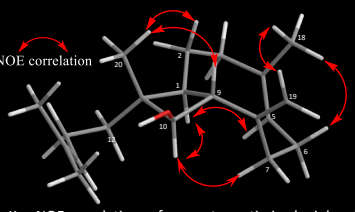


Structures of (**1**)-(**3**) were determined using 1D/ 2D NMR experiments, which revealed three new xenicane class diterpenes with miolenol A & B containing a rare cyclobutanol ring.



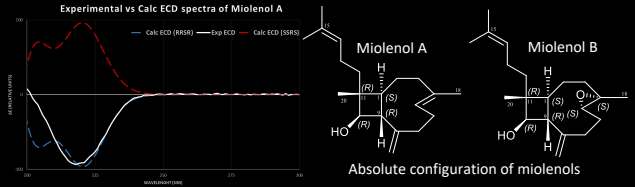
Mass spectra of miolenol A (M+H$^+$ 289.25 Da)

Stereochemistry



Key NOE correlations of geometry optimized miolenol A

Relative stereochemistry of (**1**)-(**3**) was determined by NOESY correlations

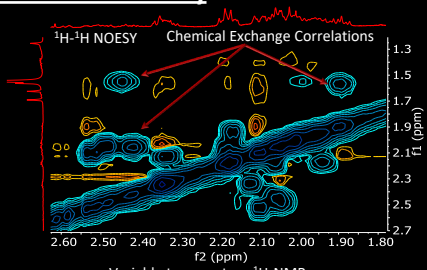


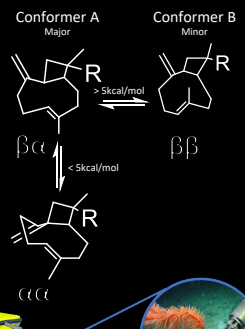
Absolute configuration of miolenols

Absolute stereochemistry of (**1**)-(**3**) was determined by comparison of experimental and calculated ECD spectra, using M062X/Def2TZVP and b3lyp/6-311G(2d,p).

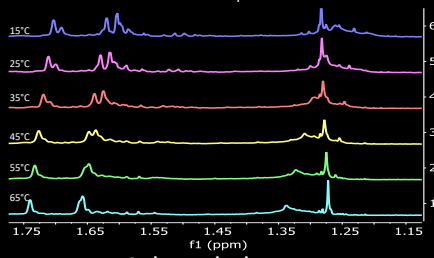
Conformational flexibility

Chemical exchange correlations between major and minor conformers observed in miolenol A NOE spectra indicates interconversion between conformers. This was confirmed by variable temperature proton NMRs and analysis of NOE correlations. Major conformer A consists of βα and αα conformations and minor conformer B contains ββ conformation.[9]



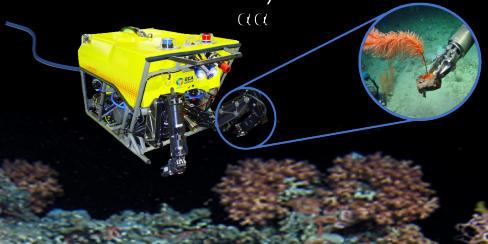


Variable temperature ^{1}H-NMR



References

1.Henry, L.-A.; Roberts, J. M., Global Biodiversity in Cold-Water Coral Reef Ecosystems. In *Marine Animal Forests: The Ecology of Benthic Biodiversity Hotspots*, Rossi, S.; Bramanti, L.; Gori, A.; Orejas, C., Eds. Springer International Publishing: Cham, 2017; pp 235-256.
2. Berrue, F.; Kerr, R. G., Diterpenes from gorgonian corals. *Nat. Prod. Rep.* **2009**, *26* (5), 681-710.
3. Ospina, C. A.; Rodriguez, A. D.; Sanchez, J. A.; Ortega-Barria, E.; Capson, T. L.; Mayer, A. M. S., Caucanolides A-F, unusual antiplasmodial constituents from a Colombian collection of the gorgonian coral Pseudopterogorgia bipinnata. *J. Nat. Prod.* **2005**, *68* (10), 1519-1526.
4. Ospina, C. A.; Rodriguez, A. D.; Sanchez, J. A.; Ortega-Barria, E.; Capson, T. L.; Mayer, A. M. S., Caucanolides A-F, unusual antiplasmodial constituents from a Colombian collection of the gorgonian coral Pseudopterogorgia bipinnata. *J. Nat. Prod.* **2005**, *68* (10), 1519-1526.
5.Ochi, M.; Kataoka, K.; Tatsukawa, A.; Kotsuki, H.; Shibata, K., BIOLOGICALLY-ACTIVE XENICANE DITERPENOIDS FROM THE GORGONIAN ACALYCIGORGIA SP. *Heterocycles* **1994**, *38* (1), 151-158.
6. Rho, J.-R.; Lee, H.-S.; Seo, Y.; Cho, K. W.; Shin, J., New Xenicane Diterpenoids from the Gorgonian Acalycigoria inermis. *J. Nat. Prod.* **2000**, *63* (7), 1045-1046.
7. Hubner, M.; Rissom, B.; Fitjer, L., Conformation and dynamics of (-)-beta-caryophyllene. *Helv. Chim. Acta* **1997**, *80* (6), 1972-1982.



Acknowledgements

This research was carried out with the support of the Marine Institute under the Marine Research Programme and the support of the Irish Government. This publication has emanated from research supported in part by a research grant from Science Foundation Ireland (SFI) and the Marine Institute under the Investigators Programme Grant Number SFI/15/1A/3100, co-funded under the European Regional Development Fund 2014-2020. Bioassays were carried out, in collaboration, with the Sun Lab (C. diff), Shaw Lab (ESKAPE), Rodhe Lab (TB), Adams Lab (malaria), Kee Lab (cervical cancer) and the Rice Lab (parasitic amoeba). The authors would also like to thank Dr. Ryan Young for sample collection, extraction and prioritisation, Dr. Laurence Jennings for aiding in ECD calculations, Karen Croke for DNA sequencing the speciman and Dr. Nicole E. Avalon for arranging sample distribution for biological screening.