

# Chemical analysis of the Mediterranean gorgonians and structural characterization of bio-active metabolites

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## Introduction

Cnidarians are marine invertebrate animals. They are known for their metabolic wealth which includes diterpene and sesquiterpene molecules. *Eunicella cavolini* species yields these molecules. A number of these secondary metabolites develop antibacterial, antiviral, anti-inflammatory and anti-fouling activities. A chemical signature is a chromatographic profile that characterizes the species studied. It is based on a standardized protocol in order to compare different signatures. The objective of this work, was to purify and identify most secondary metabolites produced by *Eunicella cavolini* in order to incorporate these molecular structures to the chemical signatures.

## Experimental Conditions

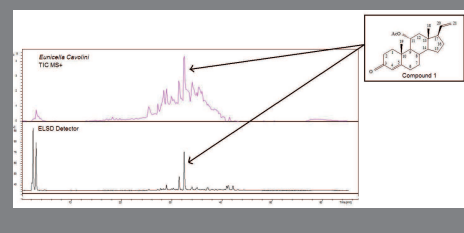
One chemical signature was performed with three samples of *Eunicella Cavolini* collected in Marseille. This chemical signature was made according to a standardized protocol (method ECIMAR) on a triple detection HPLC: UV detector, Evaporative Light Scattering Detectors (ELSD) and a mass spectrometer (source: electrospray, Analyzer: Ion trap). This analysis was achieved on a 250 x10 mm column composed of a reverse stationary phase : Gemini C6 Phenyl.

The samples were extracted with a mixture CH<sub>2</sub>Cl<sub>2</sub>/MeOH (50: 50, v/v) and ultrasounds. The organic extract was separated by flash chromatography (Armen Instrument Spot Flash) in 116 tubes, assembled in 65 fractions. Fraction 38 contained 36.82 mg of extract. It was purified by HPLC (Varian Prostar) on a column composed of a reverse stationary phase: Gemini C6 Phenyl. The single chromatographic peak was characterized using NMR mono (<sup>1</sup>H,<sup>13</sup>C) and bi-dimensional (COSY, HSQC, HMBC), mass spectrometry, infrared, UV and polarimetry. Fractions 34, 35 and 36 were purified by HPLC (Varian Prostar) on a column composed of a reverse stationary phase: C18 grafted silica. Chromatographic peaks were analyzed using <sup>1</sup>H-NMR.

## Results

Thanks to the chemical signature, the chromatographic profile of the species studied was obtained. The chromatographic peak of fraction 38 was identified as compound 1 (11 $\alpha$ -acetoxy-pregn-4,20-en-3-one). It was integrated into the chemical signature (Figure 1). This compound had already been isolated [Cimino, 1978] and characterized [Ioannou, 2008] in this species of gorgonian. This compound is the family of pregnane. The 32.4 minutes retention time highlights the apolar character of this molecule. Furthermore, ELSD detector shows that compound 1 is the majority compound because its response is proportional to the quantity of products (Figure1). Two other compounds of fraction 34, 35 and 36 were isolated, but they could not be identified.

Figure 1 : Chemical signature performed with a LC-MS.



## Conclusion

Now, compound 1 can be recognized in a single injection in HPLC by using the standardized method whereas before this compound was not recognizable. Moreover, when all the peaks of this signature will be identified, the signature may be used as a standard one.



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