



## Structural determination of 1 triacylglycerol isolated from the sea urchin *Echinometra lucunter*

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### ABSTRACT

Usually we worked on marine organisms like algae and invertebrates of senegalese coasts but it's the first time we investigate sea urchin species. Specimens of the sea urchin *Echinometra lucunter* were collected in December 2002 at Dakar (Senegal). Fresh sea urchins were exhaustively treated with  $\text{CHCl}_3/\text{MeOH}$  1/1 (V/V). After evaporation of solvent the oily residue (1, 92 g) was passed through an RP 18 column. The  $\text{CHCl}_3$  soluble fraction has been analyzed through spectroscopic means (NMR, Electro Spray Ionization mass spectrometry). It has been shown to contain the triacylglycerol B.

**Keywords:** Sea urchin, reverse phase chromatography (R P), NMR, Electro Spray Ionization (ESI), triacylglycerol.

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### INTRODUCTION

Marine organisms from the coast of Senegal have been subjected to many studies on algae and invertebrates such as sponges and cnidarians [1-3].

The investigation, however broad it was, did not touch the echinoderms. In order to make our contribution to scientific research a bit more complete and balanced, we undertook the study of metabolites of the sea urchin *Echinometra lucunter*.

After soaking in the mixture  $\text{CHCl}_3/\text{CH}_3\text{OH}$  (1 / 1, V / V) phase obtained is evaporated to dryness to give a residue. The crude extract was subjected to reverse phase chromatography and RP 18: fractions 6A5 eluted with methanol and chloroform contain triacylglycerol B.

With the exception of cycles, the numbering is unconventional.

It is based on the chemical shifts of NMR signals of the same nature in order to make spectroscopic interpretation easier.

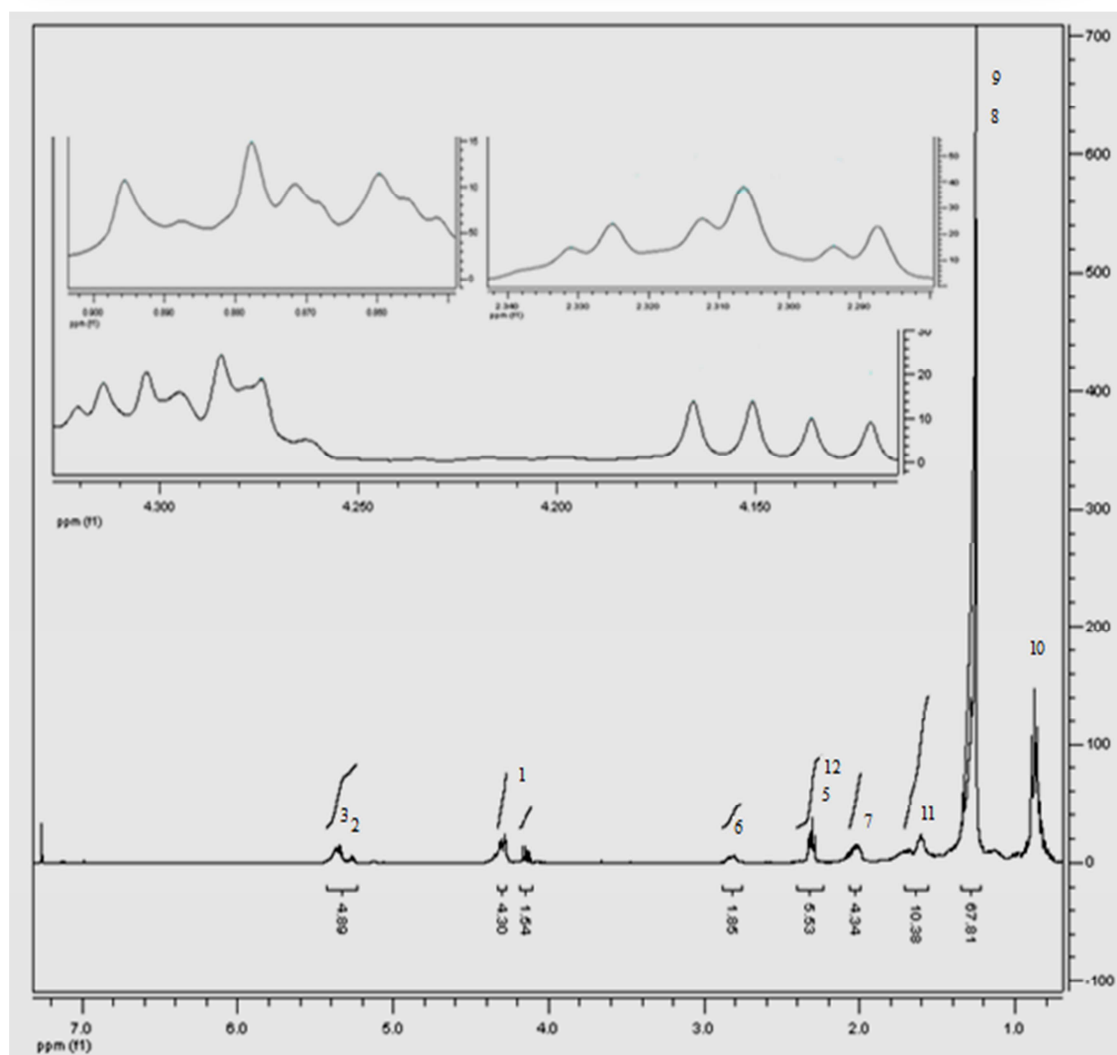
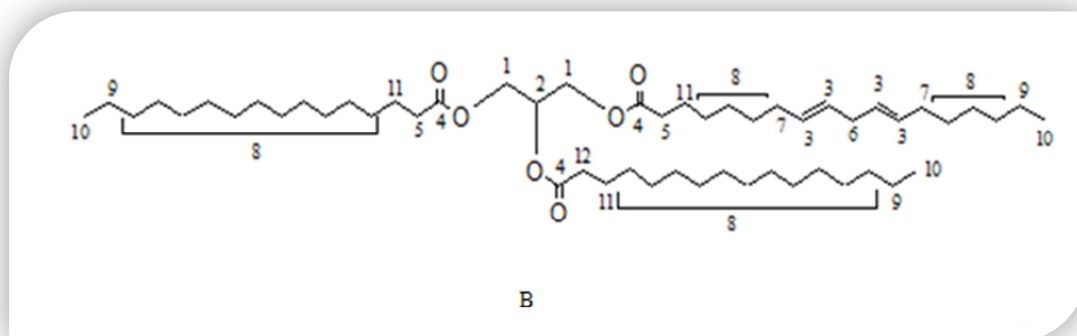


Figure 1:  $^1\text{H}$  NMR spectrum with spread region (0.90 to 0.84; 2.33 to 2.29 and 4.30 to 4.15) of the compound B

## EXPERIMENTAL SECTION

### General Methods:

The NMR spectra were measured on a Varian BRUKER ARX 400 MHz using deuterated chloroform ( $\text{CDCl}_3$ ) as an internal standard. Two-dimensional (2D) NMR was performed with  $^1\text{H}$ - $^1\text{H}$  COSY. ESI-MS spectra were obtained using a FISONG VG Autospec M. Thin-layer chromatography (T.L.C.) was performed using silica gel 60 F254 and silica gel 60 RP-18 F254.

### Urchin Material:

Specimens of the sea urchin *Echinometra lucunter* collected in December 2002 at Dakar (Sénégal), were deposited

at the Laboratory of Natural Products, Cheikh Anta Diop University.

#### Extraction and Isolation:

The sea urchin *Echinometra lucunter* (393g) was extracted with MeOH /Chloroform (1 L /1L) at room temperature. The concentrated MeOH /Chloroform fraction (1.92g) was subjected to silica gel (SiO<sub>2</sub>) column chromatography and eluted with mixtures water-methanol (9/1, 7/3, 4/6, 2/8), methanol 100%, methanol/chloroform (9/1) and finally chloroform 100%. Each eluant was monitored by thin layer chromatography (TLC), and 20 fractions (C1 to C20) were obtained. Fraction C5 (83, 2 mg) was subjected to SiO<sub>2</sub> column chromatography and was eluted with n-hexane –EtOAc (9:1→8:2→7:3→6:4→5:5→4:6→2:8→1:9, v/v) and MeOH (100%), to give 12 subfractions (1C5 to 12C5). Subfraction 4C5 ultimately furnished a new compound named B [10.2 mg, Rf 0.91 in TLC (plate RP-18 F254) in n-hexane–EtOAc (9:1), v/v].

**Triacylglycerol (B) C<sub>57</sub> H<sub>106</sub> O<sub>6</sub>.** ESI-MS m/z: 665 [M - C<sub>16</sub>H<sub>29</sub>]<sup>+</sup>; <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>, δ, ppm, J/Hz): 4.31 (2H, dd, J = 5.97; 11.89 H-1a), 4.15 (2H, dd, J = 4.31; 11.89 H-1b), 5.25 (1H, m, H-2), 2.30 (4H, t, J = 7.45, H-5), 1.29-1.33 (54 H, bs, H-8, 9), 2.02 (4H, m, H-11, J = 7.52), 1.98-2.10 (4H, m, H-7), 2.31 (2H, t, J = 7.54, H-12), 5.33 (4H, m, H-3), 2.81 (2H, dd, H-6), 0.83 (9H, t, J = 6.60, H-10).

<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, δ): 62.08 (C-1), 68.85 (C-2), 172.85 (C-4), 34.04 (C-5), 29.10 - 29.69 (C-8), 68.18 (C-13), 27.20 (C-7), 34.06 (C-12), 128.80 -130.87 (C-3), 31.91(C-6), 14.11 (C-11).

The count used is based on the chemical shifts of the NMR signals of the same nature in order to make interpretation more readable.

As a result of the superposition of some signals, the corresponding areas were plotted for better dispersion and easier allocation of signals considered.

The multiplet at δ = 5.26 ppm and the doublets split at δ = 4.31 ppm and 4.15 ppm are characteristic of a glycerol rest [4 - 8].

Analysis of the <sup>13</sup>C NMR spectrum indicates the presence of two signals at δ = 173.29 ppm attributed to two carbonyl (C-13) and δ = 172.88 ppm to the carbonyl (C-12) [9- 10].

Signals 14.12 and 14.07 ppm respectively are assigned to the two methyl groups of saturated acyl chains and that of the unsaturated acyl chain triacylglycerol [11- 12].

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