Chemistry of Natural Compounds, Vol. 47, No. 6, January, 2012 [Russian original No. 6, November–December, 2011]

STRUCTURAL DETERMINATION OF TWO LIPIDS ISOLATED FROM THE SEA URCHIN *Echinometra lucunter*

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UDC 547.918

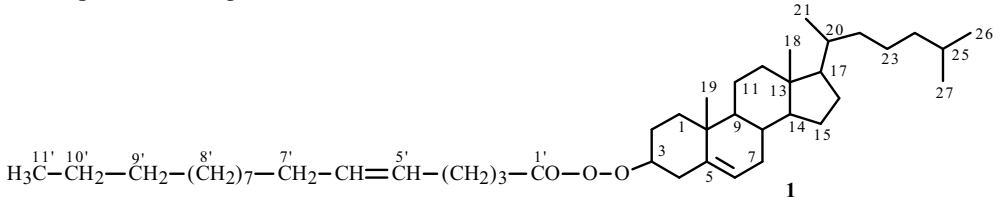
Specimens of the sea urchin Echinometra lucunter *were collected at Dakar (Senegal). Fresh sea urchins were exhaustively treated with $CHCl_3$–MeOH 1:1 (v/v). After evaporation of solvent the oily residue (1, 92 g) was passed through an RP 18 column. The $CHCl_3$ soluble fraction has been analyzed through spectroscopic means (NMR, electrospray ionization mass spectrometry). It has been shown to contain the two sterol derivatives lucunterperacetate (**1**) and peroxylucunterine (**2**).*

Keywords: Sea urchin, reverse phase chromatography (RP), sterol.

Marine organisms from the coast of Senegal have been the subject of many studies, such as algae and invertebrates such as sponges and cnidarians [1–3]. The investigations, however broad they were, did not touch on 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 $CHCl_3$–CH_3OH (1:1), the phase obtained is evaporated to dryness to give a residue. The crude extract was subjected to reverse phase chromatography and RP 18. Fractions A4 and B2, respectively eluted with methanol and chloroform, contain sterol derivatives lucunterperacetate (**1**) and peroxylucunterine (**2**). 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.

The resonance multiplet δ 3.52 ppm is typical of proton of 3β-hydroxysterol. The multiplet at δ 0.67 ppm is typical of tertiary methyl C-18 of sterol skeletons [4, 5]. The two triplets at 0.88, and 2.33 ppm, associated with the broadened singlet between 1.24 to 1.29 ppm, indicate a set of signals characteristic of fatty acid spectra [6–8].

In ^{1}H NMR spectra of the compound **2**, the two doublets at δ 6.20 and δ 6.50 ppm show protons of Csp^2 bound to the peroxide bridge. Also, the chemical shifts of C-5 and C-8 at 81.73 and 79.41 ppm conform to the presence of oxygen on the corresponding carbons. The ion fragment at *m/z* 365 ($C_{27}H_{43}O_2 - H_2O_2$) on the ESI-mass spectra indicates the sterol skeleton associated with the peroxide bridge.



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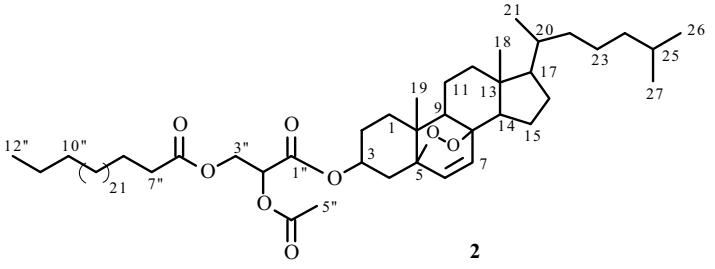

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 0009-3130/12/4706-0932

EXPERIMENTAL

General Methods. The NMR spectra were measured on a Varian Bruker ARX 400 MHz using deuterated chloroform ($CDCl_3$) as an internal standard. Two-dimensional (2D) NMR was performed with ^1H–^1H COSY. ESI-MS spectra were obtained using a Fisong VG AutoSpec M. Thin-layer chromatography (TLC) 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 (Senegal) were deposited at the Laboratory of Natural Products, Cheikh Anta Diop University.

Extraction and Isolation. The sea urchin *Echinometra lucunter* (393 g) was extracted with MeOH–chloroform (1 L:1 L) at room temperature. The concentrated MeOH–chloroform fraction (1.92 g) was subjected to silica gel (SiO_2) column chromatography and eluted with mixtures water–methanol (9:1, 7:3, 4:6, 2:8), 100% methanol, methanol–chloroform (9:1), and finally 100% chloroform. Each eluant was monitored by thin-layer chromatography (TLC), and seven fractions (A1 to A5, B and C) were obtained. Fraction A4 (160 mg) was subjected to SiO_2 column chromatography and 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 20 subfractions (1A4 to 20A4). Subfraction 3A4 ultimately furnished a new compound lucunterperacetate (**1**) [4.4 mg, R_f 0.73 in TLC (plate RP-18 F254) in *n*-hexane–EtOAc (7:3), v/v]. Fraction B2 (332 mg) was subjected to SiO_2 column chromatography and 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 20 subfractions (1B2 to 20B2). Subfraction 4B2 ultimately produced a new compound peroxylucunterine (**2**) [2.9 mg, R_f 0.64 in TLC (plate RP-18 F254) in *n*-hexane–EtOAc (8:2), v/v.

Lucunterperacetate (1). $C_{44}H_{76}O_3$. ESI-MS *m*/*z*: 457 $[M - C_{14}H_{27}]^+$. NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.40–5.36 (2H, m, H-5′, 6′), 5.34 (1H, m, H-6), 3.52 (1H, m, H-3), 2.36 (2H, t, J = 7.46, H-2′), 2.27 (2H, m, H-4), 2.10, 2.02 (4H, m, H-7′, 4′), 2.00, 1.15 (2H, m, H-12a, 12e), 1.99, 1.51 (2H, m, H-7a, 7e), 1.82 (2H, m, H-2) 1.81, 1.22 (2H, m, H-16a, 16e), 1.57, 1.10 (2H, m, H-15a, 15e), 1.56 (2H, m, H-3′), 1.53 (H, m, H-25), 1.50, 1.07 (2H, m, H-1a, 1e), 1.49 (2H, m, H-11), 1.37 (1H, m, H-20), 1.33–1.26 (18H, m, H-10′, 9′, 8′), 1.34, 1.08 (2H, m, H-22a, 22e), 1.19 (2H, m, H-23), 1.12 (2H, m, H-24), 1.12 (1H, m, H-8), 1.08 (1H, m, H-17), 1.00 (3H, s, H-19), 0.98 (1H, m, H-14), 0.93 (1H, m, H-9), 0.92 (3H, d, J = 6.53, H-21), 0.88 (3H, t, J = 7.06, H-11′), 0.86, 0.85 (3H, d, J = 6.60, H-27), 0.86, 0.85 (3H, d, J = 6.62, H-26), 0.67 (3H, s, H-18). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 177.81 (C-1′), 140.76 (C-5), 131.39–128.12 (C-5′, 6′), 121.74 (C-6), 71.85 (C-3), 56.79 (C-14), 56.18 (C-17), 50.16 (C-9), 42.34 (C-13), 42.31 (C-4), 39.81 (C-12), 39.54 (C-24), 37.72 (C-1), 36.52 (C-10), 36.21 (C-22), 35.80 (C-20), 33.06 (C-2′), 31.93 (C-9′), 31.93 (C-7), 31.67 (C-8), 29.78 (C-2), 29.71–29.08 (C-n7), 28.25 (C-16), 28.03 (C-25), 27.26 (C-4′), 26.45 (C-7′), 24.65 (C-3′), 24.31 (C-15), 23.85 (C-23), 22.81 (C-27), 22.71 (C-10′), 22.57 (C-26), 21.11 (C-11), 19.41 (C-19), 18.74 (C-21), 14.13 (C-11′), 11.88 (C-18).

Peroxylucunterine (2). $C_{59}H_{102}O_8$. ESI-MS *m*/*z*: 940 $[M + 2H]^+$. NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.50 (1H, d, J = 8.53, H-7), 6.20 (1H, d, J = 8.47, H-6), 5.29 (1H, m, H-2″), 4.92 (1H, m, H-3), 4.31 (1H, dd, J = 5.96, 11.88, H-3″a), 4.15 (1H, dd, J = 4.31, 11.88, H-3″b), 2.30 (2H, t, J = 7.58, H-7″), 2.24 (2H, m, H-4), 2.01 (3H, s, H-12″), 2.02, 1.10 (2H, m, H-12a, 12e), 1.92 (2H, m, H-2), 1.80, 1.22 (2H, m, H-16a, 16e), 1.65–1.49 (2H, m, H-3″), 1.62, 1.36 (2H, m, H-11a, 11e), 1.58 (1H, m, H-25), 1.57, 1.49 (2H, m, H-15a, 15e), 1.43 (1H, m, H-9), 1.33–1.25 (42H, m, H-9″), 1.26 (1H, m, H-20), 1.26–1.02 (2H, m, H-22a, 22e), 1.19, 1.08 (2H, m, H-23), 1.08 (1H, m, H-17), 1.01 (1H, m, H-14), 0.92 (3H, d, J = 6.53, H-21), 0.88 (3H, t, J = 7.06, H-12″), 0.87 (3H, s, H-19), 0.85 (6H, d, H-27, 26), 0.84, 0.81 (2H, m, H-24a, 24e), 0.67 (3H, s, H-18). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 170.11 (C-1″, C-4″, C-6″), 135.05 (C-6), 130.94 (C-7), 81.73 (C-5), 79.41 (C-8), 69.50 (C-3, 2″), 62.08 (C-3″), 56.40 (C-17), 51.50 (C-14), 51.01 (C-9), 44.73 (C-13), 39.40 (C-12, C-24), 36.95 (C-10), 35.94 (C-22), 35.23 (C-20), 34.31 (C-1), 34.04 (C-7″), 33.20 (C-4), 31.94 (C-10″), 29.71 (C-2), 29.71–29.13 (C-9″), 28.24 (C-16), 27.99 (C-25), 26.28 (C-15), 28.25 (C-16), 28.03 (C-25), 26.45 (C-7″), 24.65 (C-3″), 24.31 (C-15), 23.79 (C-8″), 23.40 (C-23), 22.81 (C-27), 22.70 (C-11″), 22.55 (C-26), 21.33 (C-5″), 20.61 (C-11), 18.58 (C-19), 18.07 (C-21), 14.13 (C-12″), 12.64 (C-18).

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