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OCCURRENCE OF *ERYTHRO*-DOCOSASPHINGA-4,8-DIENINE, AS AN ESTER, IN *ANEMONIA SULCATA*.

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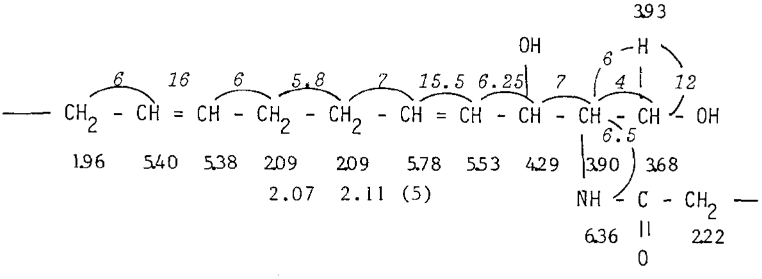
Summary : A new sphingosine derivative was isolated from *A.sulcata* ; ^{1}H and ^{13}C NMR spectra are described.

Anemonia sulcata is a largely distributed Coelenterate. Its polypeptidic toxins were extensively studied, with those of others sea anemones, since they are among the most interesting tools for analysis of the properties of the Na^+ channel (1), and potent cardiotonic compounds (2).

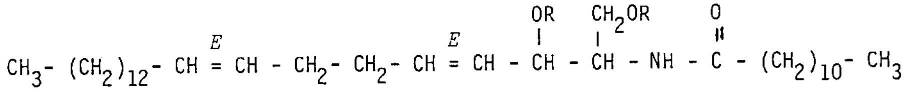
We have investigated the tentacles of *A.sulcata* collected near Sousse (Tunisia). The methylene chloride extract of the lyophilized tentacles was separated on silicagel using as eluent $CHCl_3$ with increasing amounts of acetone. The fraction obtained with chloroform-acetone,70/30 (v/v) contains the previously described peridinin (3) and $\underline{1}$, separated from peridinin by successive crystallizations in acetone (0.05 % dry weight), m.p. 297-298°C, $[\alpha]_D$ + 2.94° ($CHCl_3$). IR (KBr) : 3400, 1630, 970 cm^{-1}. $\underline{1}$ gave as a single peak in C.I.M.S. : 536 $(M+H)^+$; E.I.M.S. shown the highest mass peak at *m/e* 352 ($C_{22}H_{42}NO_2$) (4). Treatment of $\underline{1}$ with Ac_2O-pyridine gave a diacetate $\underline{2}$: 619 (M^+) ($C_{38}H_{69}NO_5$), *m/e* 499 (M-2AcOH) ($C_{34}H_{61}NO$). Hence, the formula of $\underline{1}$ was $C_{34}H_{65}NO_3$.

The ^{1}H NMR spectrum of $\underline{1}$ (250 MHz, $CDCl_3$, δ ppm, *J* Hz) displayed signals at 6.36 (d,1H,*J* = 6.5) slowly exchangeable by D_2O indicating N$\underline{H}$-C=O, 5.78 (dt,1H,*J*=15.5, 7), 5.53 (dd,1H,*J*=15.5, 6.25), 5.40 (dt,1H,*J*=16, 6), 5.38 (dt,1H,*J*=16, 6), 4.29 (br dd,1H,*J*=6.25, 3), 3.93 (dd,1H,*J*= 12, 6), 3.90 (m,1H), 3.68 (dd,1H,*J*=12, 4), 2.22 (t,2H,*J*=7.5), 2.09 (m,4H), 1.96 (br q,2H), 1.62 (br t,2H), 1.25 (br s,42H), 0.8 (t,6H).

Selective decoupling experiments showed the following sequence, indicative of a sphinga-4,8-dienine ester (5). The double bonds were *E* according to the coupling constant values 15.5 and 16 Hz, respectively. Permanganate-periodate oxidation (6) after simplified work-up gave a mixture, which analysed by E.I.M.S. shows ions at *m/e* 229 and 302, corresponding to cleavage at C_8-C_9 and C_2-C_3 of the sphingosine.



The relative stereochemistry at C-2/C-3 was determined to be *erythro* by the coupling constants (7 Hz) of H-2 and H-3 (6). The lengths of the acid and amine parts of the molecule were deduced from the peak at *m/e* 352 ($C_{22}H_{42}NO_2$) as follows :



$$CH_3-(CH_2)_{12}-CH\overset{E}{=}CH-CH_2-CH_2-CH\overset{E}{=}CH-\overset{\overset{OR}{|}}{CH}-\overset{\overset{CH_2OR}{|}}{CH}-NH-\overset{\overset{O}{\|}}{C}-(CH_2)_{10}-CH_3$$

$\underline{1}$: R = H $\underline{2}$: R = OAc

The ^{13}C NMR spectrum confirmed the proposed structure (20.15 MHz, $CDCl_3$, δ ppm) : 174.3 s, 133.20 d, 131.10 d, 129.10 d, 128.71 d, 74.55 ($\underline{C}HOH$), 62.60 t (CH_2OH), 54.93 d (CH-NH), 37.12, 32.79, 32.67, 32.40, 32.17, 29.95, 29.78, 29.61, 26.08, 22.95, 14.36.

If sphingolipids are present in especially large amounts in brain and nerve tissues, few reports on the occrrence of sphingadienine derivatives have been made so far. Dienic long chain bases from human plasma sphingomyelin were characterized as sphinga-4,14 dienines (7). In a scorpion : *Centruroides sculpuratus*, an eicosasphinga-4,11-dienine was found along with an eicosasphinga-11-enine (8).

The only report of a sphinga-4,8-dienine (C_{18}) in animals came from analysis of oyster glycolipids (9) whereas another one was suspected in a sea anemone : *Metridium senile* by Karlsson (10); in these works, structure elucidation was performed by GC-MS analysis of the TMS derivatives obtained after periodate oxidation, and the stereochemistry of the double bonds was only substantiated by IR. On the other hand, a linear terpenoid sphingosine has been found in a Tunicate (11).

To our knowledge, this is the first report of a sphinga-4,8 dienine as an ester, and the fact that it occurred as the sole component of this type is rather unexpected and allowed extensive study by NMR.

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4 - Calculated for $C_{22}H_{42}NO_2$: 352.3215, found 352.3215 ; calculated for $C_{34}H_{61}NO$: 499.4752 found 499.475 ; calculated for $C_{38}H_{69}NO_5$: 619.5157, found 619.517.

5 - In 500 MHz ^1H NMR a) the system 5.38-5.40 ppm is clarified b) signal at 2.09 ppm gave two coupled t : δ 2.11 and 2.07 (2D) and no residual coupling with others CH_2.

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