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LINEAR SESTERTERPENES FROM THE CARIBBEAN SPONGE *THORECTA HORRIDUS* WITH INFLAMMATORY ACTIVITY

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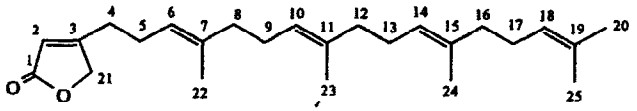
Abstract : Two new linear sesterterpenes **1** and **2**, containing an α,β-unsaturated γ-lactone ring, have been isolated from the Caribbean sponge *Thorecta horridus*, Hyatt 1877 (F. Thorectidae). The structures of the two new metabolites were established on the basis of spectral data including 2D NMR experiments. Compound **1** exhibits inflammatory activity.

Among the various classes of polyisoprenoids, sesterterpenes seem to be the most unusual metabolites from the marine environment[2]. In fact, the majority of these relatively rare secondary metabolites have been isolated from marine invertebrates. As far as the structures of the marine-derived sesterterpenes is concerned, most of them are based on a polycarbocyclic skeleton, while a limited number of linear furanoid compounds are also known. All the isolated compounds appear to be biosynthesized through rather elaborated pathways starting from the common precursor geranylfarnesol. This elaboration also comprises drastic modifications with the loss or addition of one or more carbon units.

During our investigation into bioactive secondary metabolites from marine invertebrates we have identified in the Caribbean sponge *Thorecta horridus* two new linear sesterterpenes (**1** and **2**) which hypothetically derive from the precursor through a simple oxidation at C-1 and C-21 carbon atoms. These new compounds represent the closest analogues of geranylfarnesol which have been so far observed in marine sources. The most abundant compound **1** possesses inflammatory activity.



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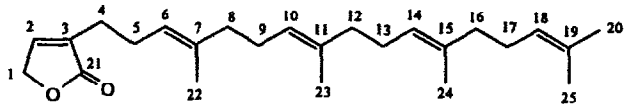

2

Specimens of sponge *Thorecta horridus*, Hyatt 1877 (F. Thorectidae) were collected by scuba at a depth of 30 m off Grand Bahama Island, during an expedition in July 1990. The MeOH/ Toluene (3:1) extract was initially fractionated by MPLC over silica-gel (230-400 mesh), with the final purification by RP-18 HPLC (100% MeOH) yielding compounds **1** and **2** (0.43 and 0.02 % dry weight of the sponge, respectively).

The molecular formula for the major compound **1**, $C_{25}H_{38}O_2$, was determined from interpretation of the M^+ (*m/z* 370.2878) ion in the HREIMS[3] in conjunction with ^1H and ^{13}C NMR data (Table 1). The molecular formula indicated 7 degrees of unsaturation. Ten olefinic resonances and 1 carbonyl resonance in the ^{13}C NMR spectrum accounted for 6 unsaturations, leaving 1 ring in compound **1**. The nature of this ring was suggested by the FT-IR bands at 1783 and 1749 cm^{-1}, characteristic of β-substituted α,β-unsaturated γ-lactones and by the ^1H NMR spectrum of **1** containing two long-range coupled (J = 1.3 Hz) signals at δ 4.73 (2H, d) and δ 5.85 (1H, quintet) attributable to the allylic methylene protons at C-21 and the lactone olefinic proton at C-2, respectively.

One- (spin-decoupling and nOe difference) and two-dimensional (COSY and long range COSY) ^1H NMR experiments allowed us to extend the lactone ring up to C-8. Useful information arose from long range couplings observed between H-2 and H_2-4, H-6 and H_3-22, H-6 and H_2-8, indicative of their allylic relationship and between the homoallylic H_2-5 and H_2-8, and from a nOe enhancement of the H_2-4 signal observed when H_2-21 was irradiated, which proved the spatial proximity of these two methylene groups.

The proposed substructure was further supported by the assignment of the resonance of the carbon atoms of the above fragment in the ^{13}C NMR spectrum of **1** (Table 1) based on DEPT and 2D heteronuclear correlation via ^1J (HETCORR) and $^{2,3}J$ (COLOC). Particularly, the COLOC experiment displayed significant correlations between C-1 and

Table 1 . ^{13}C and ^{1}H NMR assignment for compounds 1 and 2[a]

	1			2		
carbon	DEPT	^{13}C	^{1}H	DEPT	^{13}C	^{1}H
1	C	170.17		CH_2	70.10	4.76, 2H, bd (2.0)
2	CH	115.55	5.85, 1H, quintet(1.3)	CH	144.0	7.10, 1H, narrow muluplet
3	C	135.28[e]		C	137.25	
4	CH_2	28.71	2.45, 2H, bt (7.5)	CH_2	25.45[b]	2.32,2H, bt (7.5)
5	CH_2	25.65	2.30, 2H, bq (7.5)	CH_2	25.69[b]	2.27,2H, bq (7.5)
6	CH	121.75	5.12[g]	CH	122.59	5.12[l]
7	C	137.58		C	135.15[c]	
8	CH_2	39.69[b]	2.07[h]	CH_2	39.70[d]	2.07[m]
9	CH_2	26.71[c]	1.98[i]	CH_2	26.73[e]	1.98[n]
10	CH	124.32[d]	5.12[g]	CH	124.35[f]	5.12[l]
11	C	134.92[e]		C	134.86[c]	
12	CH_2	39.64[b]	2.07[h]	CH_2	39.70[d]	2.07[m]
13	CH_2	26.58[c]	1.98[i]	CH_2	26.62[e]	1.98[n]
14	CH	124.11[d]	5.12[g]	CH	124.14[f]	5.12[l]
15	C	134.92[e]		C	134.86[c]	
16	CH_2	39.57[b]	2.07[h]	CH_2	39.70[d]	2.07[m]
17	CH_2	26.43[c]	1.98[i]	CH_2	26.62[e]	1.98[n]
18	CH	123.78[d]	5.12[g]	CH	124.00[f]	5.12[l]
19	C	131.26		C	130.50	
20	CH_3	25.65	1.68, 3H, s	CH_3	25.69	1.68, 3H, s
21	CH_2	73.18	4.73, 2H, d (1.3)	C	174.50	
22	CH_3	16.15	1.62, 3H, bs	CH_3	16.10	1.59[p]
23	CH_3	15.98	1.59[o]	CH_3	16.00	1.59[p]
24	CH_3	15.98	1.59[o]	CH_3	16.00	1.59[p]
25	CH_3	17.65	1.59[o]	CH_3	17.70	1.59[p]

[a] Assignments determined by analogy to model compounds and by the 1D and 2D NMR experiments reported in the text. ^{1}H (500MHz) and ^{13}C (125MHz) NMR spectra were recorded in $CDCl_3$ with TMS as internal standard. Proton coupling constants ( ) are given in Hertz.

[b-f] Values with identical superscript within each column may be interchanged. [g-n] Values with identical superscript within each column are mutually overlapped.

[o] Broad singlet, 9H. [p] Broad singlet, 12H.

H-2, C-1 and H_2-21, C-2 and H_2-21, C-2 and H_2-4, C-5 and H_2-4, C-6 and H_3-22, C-7 and H_3-22.

The E-configuration of the C-6 double bond was inferred from the ^{13}C chemical shifts of the allylic methyl and methylenes and confirmed by the nOe enhancement of H_3-22 on irradiation at δ 2.30 (H_2-5).

The rest of the ^1H NMR spectrum of **1** consists of four methyl, five methylenes and three olefinic methine signals whose chemical shifts and multiplicities strongly suggest that an all-trans C-15 prenyl chain is linked to C-8. ^{13}C NMR spectrum, which displays all the resonances for the pertinent carbon atoms, corroborates the presence of the polyprenyl chain with E-stereochemistry of the C-10 and C-14 double bonds (Table 1).

The minor component **2** possesses a molecular formula $C_{25}H_{38}O_2$ determined on the basis of HREIMS[3] (m/z 370.2869), thus being an isomer of **1**. Its structure was determined by direct comparison of the ^1H and ^{13}C NMR spectra (Table 1) of the two compounds which illustrated that **2** also contained an all-trans polyprenyl chain with a terminal α,β-unsaturated γ-lactone ring.

However, there were relevant differences in the NMR resonances of the methine proton of the α,β-unsaturated lactone which are shifted downfield from δ 5.85 in **1** to δ 7.10 in **2** in the proton spectrum and from δ 115.55 in **1** to δ 144.0 in **2** in the carbon-13 spectrum. These data required that the methine proton must be β-positioned to the carbonyl function in **2**. This was substantiated by the carbonyl band at 1754 cm^{-1} in the FT-IR spectrum which did not display the characteristic splitting due to the presence of an α-proton.

The above data led us to the conclusion that the difference between the two isomers consisted in the position of the carbonyl and the oxymethylene groups which in **1** were located at C-1 and C-21, respectively whereas in **2** were inversely positioned.

An excellent confirmation of this assumption arose from the homoallylic relationship between H_2-1 and H_2-4, illustrated by an ^1H-^1H COSY long range experiment and from the observed chemical shifts of both ^1H and ^{13}C signals of the methylene group at C-4, which were upfield-shifted when compared with the corresponding CH_2 in **1**. An assignment of the resonances in the ^{13}C NMR spectrum of **2**, which were based on selective decouplings, DEPT experiments and comparison with model compounds supported the proposed structure.

Compound **1** exhibited inflammatory activity both *in vivo* (paw oedema) and *in vitro* (release of histamine) experiments. Injection of 0.5 - 1- 2 mg into the rat paw induced a dose-dependent oedema with a peak response occurring 1 h after the injection (Fig. A). When compound **1** was injected in rats pre-treated with mepyramine (2.5 mg/Kg) and methysergide (3 mg/Kg) the oedema induced by 1 mg compound **1** was suppressed by about 65% (Fig. A), suggesting that the inflammatory response was brought about by the release of histamine and/or 5-hydroxytryptamine. This was confirmed by the ability of **1** (0.1-1-10 μg/ml) to induce the release of histamine from rat peritoneal cells (Fig.B) in a

concentration-dependent fashion (4%, 11% and 30%). The degree of histamine release was not significantly affected by phosphatidylserine (Fig.B).

These results suggest that **1** is able to induce the release of histamine both *in vivo* and *in vitro* by a mechanism which is unaffected by phosphatidylserine and therefore possibly depends on mobilization of intracellular calcium stores.

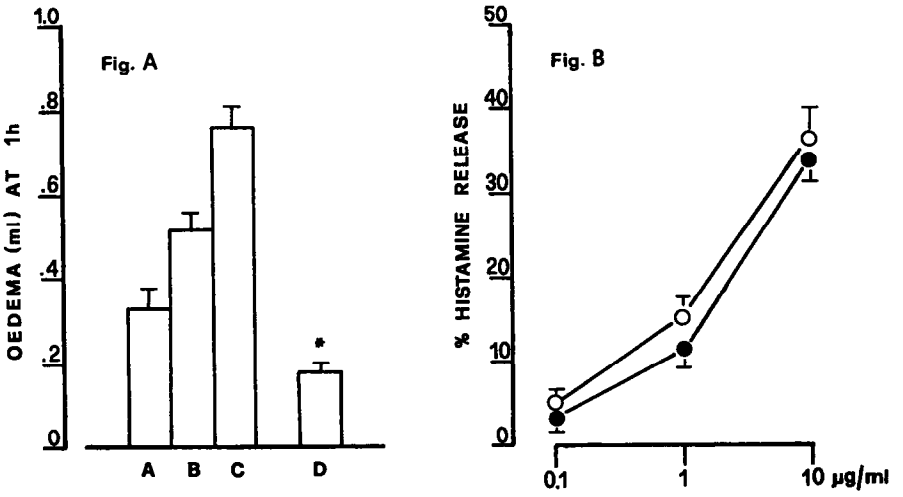

Fig. A. Oedema of the hind paw of male Wistar rats (120-140g) induced by subplantar injection of 0.5(A)-1(B)-2(C) mg compound **1**. Inhibition (D) by mepyramine (2.5 mg/Kg) and methysergide (3 mg/Kg) both given intraperitoneally 30 min before the paw injection of **1** (1 mg). The volume of the paw was measured by plethismometer[4] every 30 min for 2 h and compared to the initial one. Each bar represents the mean ± s.e.m. for n = 5 rats of the oedema ccurring at 1 h. * Shows a significant difference from untrated rats ($P < 0.05$).

Fig.B. Effect of various concentrations of **1** on the histamine release from rat peritoneal cells in absence (solid circles) or presence (open circles) of phosphatidylserine (50 µg/ml). Each point is an average of two triplicate experiments.[5]

Acknowledgements

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3. Additional spectral data : For **1**, HREIMS m/z (assignment relative intensity) 370.2878 (M^+, $C_{25}H_{38}O_2$ requires 370.2872, 26), 355.2621 (M^+ - Me, $C_{24}H_{35}O_2$, 1), 327.2322 (M^+ - 43 , $C_{22}H_{31}O_2$,7), 301.2222 (M^+ - 69 , $C_{20}H_{29}O_2$,1), 205.1973 (M^+ -165 , $C_{15}H_{25}$, 10), 204.1890 (M^+ - 166 , $C_{15}H_{24}$,2), 191.1075 (M^+ -179 , $C_{12}H_{15}O_2$, 4), 166.0995 (M^+ -204 , $C_{10}H_{14}O_2$, 22), 149.1336 (M^+ -221 , $C_{11}H_{17}$, 13), 136.1256 (M^+ -234 , $C_{10}H_{16}$, 25), 121.1015 (M^+ -249 , C_9H_{13} , 16), 98.0336 (M^+ - 272 , $C_5H_6O_2$, 68), 69.0702 (M^+ -301 , C_5H_9 , 100) ; FT-IR ($CHCl_3$) 1783, 1749, 1639, 1447, 1435 cm -1 : For **2**, HREIMS m/z (assignment relative intensity) 370.2869 (M^+,$C_{25}H_{38}O_2$ requires 370.2872 , 29), 355.2621 (M^+ - Me , $C_{24}H_{35}O_2$, 2), 327.2322 (M^+ - 43 , $C_{22}H_{31}O_2$, 6), 301.2222 (M^+- 69,$C_{20}H_{29}O_2$, 14), 205.1973 (M^+ -165 , $C_{15}H_{25}$, 10), 204.1890 (M^+ - 166 , $C_{15}H_{24}$, 13), 191.1075 (M^+ -179 , $C_{12}H_{15}O_2$, 16), 166.0995 (M^+ -204 , $C_{10}H_{14}O_2$, 3), 165.0914 (M^+ -205, $C_{10}H_{14}O_2$, 6), 149.1336 (M^+ -221, $C_{11}H_{17}$, 13), 136.1256 (M^+ -234 , $C_{10}H_{16}$, 32), 121.1015 (M^+ - 249, C_9H_{13} , 15), 98.0336 (M^+ - 272 , $C_5H_6O_2$, 11), 69.0702 (M^+ -301 , C_5H_9 , 100) ; FT-IR ($CHCl_3$) 1754, 1635, 1440, 1435 cm^{-1}.

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5. Additional data : Mixed peritoneal cells (approx. 5% mast cells) were recovered from male Wistar rats (200-220g) according to Atkinson et al.[6] Cells were washed and suspended in Tyrode solution (pH 7.2). Aliquots of cells suspension (to a final volume of 1ml) were allowed to equilibrate at 37°C in a metabolic shaker with a gentle agitation and histamine release was initiated by the addition of 0.1-1-10 µg/ml of **1** (final concentration). Some experiments were carried out in presence of phosphatidylserine (50µg/ml). The release was terminated after 10 min by addition of 2 ml ice-cold Tyrode solution. Cells and supernatants were recovered by centrifugation and histamine concentrations in solution and cells were quantified fluorimetricaly.[7] Histamine release was calculated as a percentage of the total cellular content of the amine. All values were corrected for the spontaneous release occurring in the absence of the inducer (approx. 4-5%)

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