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SMENOSPONGINE: A CYTOTOXIC AND ANTIMICROBIAL AMINOQUINONE ISOLATED FROM SMENOSPONGIA SP.

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Summary: Smenospongine, a cytotoxic and antimicrobial sesquiterpene-aminoquinone was isolated from *Smenospongia* sp. Structure elucidation was achieved by spectral analysis.

The sponge *Smenospongia* sp., a bright yellow species, was collected in the Red Sea (1), near Djibouti by Scuba diving between 20-25 m. The two specimens found were immediately immersed in methanol, and further extracted by a 1/1 methanol-chloroform mixture. The combined extracts were evaporated under reduced pressure, and the aqueous suspension extracted by dichloromethane. This dichloromethane extract exhibited marked antimicrobial (*S.aureus*) and cytotoxic activity (L 1210 leukemia cells). Fractionation was monitored by an antimicrobial bioassay using *S.aureus*. Two successive chromatographic separations on silicagel columns 230-400 mesh were performed. A first silica gel column eluted with chloroform-methanol 98/2, offered a fraction retaining the maximum activity. This fraction, separated on a second column using hexane with increasing amounts of ethyl acetate led to isolation of several compounds. The major one, a yellow compound, mp: 48-49°C; $[\alpha]_D^{29}$=-24° (c 1.10;$CHCl_3$); (0.005 % wet weight) obtained from the fraction eluted by 10 % ethyl acetate, was identified with the previously described ilimaquinone **1** (2), by its formula $C_{22}H_{30}O_4$ obtained by HRMS (3), and by ^1H (4) and ^{13}C NMR data (Table 1).

The fraction eluted with 30 % ethyl acetate furnished another active product, smenospongine **2**, obtained pure after filtration on a LH 20 column (chloroform-methanol: 40/60) as red crystals, mp 153-155°C (0.0025 % wet weight).

HMRS furnished the formula $C_{21}H_{29}NO_3$ by the molecular ion M^+ 343.2144 (5) and composition of caracteristic ions: *m/e* 191.1792, $C_{14}H_{23}$ and 153.043, $C_7H_7NO_3$; UV λ_{max}: 209 nm (ε 16150) and 317 nm (ε 15600) and IR: 1565 cm^{-1} were indicative of a quinone ring. Moreover, IR showed the presence of a hydroxy: ν_{OH} 3260 cm^{-1} and an amino group (ν_{NH_2} 3480 cm^{-1}) and in ^1H NMR (6), the upfield chemical shift of the ethylenic proton δ 5.62 ppm, suggested a position to be *ortho* to an amino group (7).

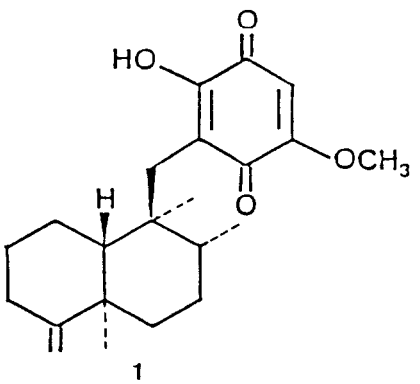
Smenospongine was further treated with Ac_2O-Pyr (24 h, room temp.), and gave a diacetyl derivative **2a** (mp: 118-120°C, M^+ 437). ^1H NMR of **2a** (δ ppm) showed, by comparison with data of acetyl ilimaquinone (O-CO-CH_3: 2.32), the presence of an O-CO-$C\underline{H}_3$ (2.33), a NH-CO-$C\underline{H}_3$ (2.24), a $N\underline{H}$-CO-CH_3 (7.55). The upfield shift of the ethylenic proton in **2a** (δ 5.30 ppm) confirmed its position *ortho* to the amino group (8).

The fragmentation *m/e* 191 ($C_{24}H_{23}$) and ^{1}H NMR (500 MHz) (6) showed that this product also possessed a drimane skeleton. The axial conformation of the H-10 proton can be deduced from its coupling constants with the two C-1 protons (J = 11.2, 2 Hz). Selective irradiation of the methyl (CH_3-13) allowed determination of the coupling constants of the H-8 proton with the C-7 protons (J = 7.8, 9.6 Hz) and hence led to infer that H-8 is axial.

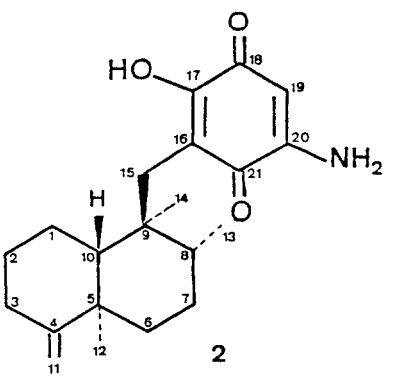
Table 1 - ^{13}C NMR ($CDCl_3$, 20 MHz, δ ppm) of ilimaquinone **1** and smenospongine **2**

1				2			
182.57 s	C-18	40.65 s	C-5	183.20 s	C-18	40.59 s	C-5
182.11 s	C-21	38.52 d	C-8	179.82 s	C-21	38.24 d	C-8
162.13 s	C-20	36.92 t	C-3	160.53 s	C-4	36.81 t	C-3
160.59 s	C-4	33.09 t	C-6	157.96 s	C-17	33.09 t	C-6
153.43 s	C-17	32.80 t	C-15	150.80 s	C-20	32.69 t	C-15
117.82 s	C-16	28.74 t	C-7	114.50 s	C-16	28.74 t	C-7
102.59 s	C-11	28.16 t	C-2	102.65 t	C-11	28.10 t	C-2
102.13 d	C-19	23.36 t	C-1	95.89 d	C-19	23.30 t	C-1
59.79 q	C-22	20.60 q	C-12	50.38 d	C-10	20.55 q	C-12
50.72 d	C-10	17.80 q	C-13	43.11 s	C-9	17.80 q	C-13
43.51 s	C-9	17.17 q	C-14			17.17 q	C-14

Comparison of the ^{13}C data of smenospongine and ilimaquinone confirmed identity of the drimane moiety of **1** and **2**: of particular help was the chemical shift of methyl-12 (δ 20.55 ppm) which allowed to conclude that the decalin junction (9) was of the *trans*-type. The lack of signals near 153 ppm in **2**, in spite of evidence for an exomethylene group supported by ^{1}H and ^{13}C NMR, led us to assign the signal at δ 160.53 for C-4.



1



2

Determination of the absolute configuration of such compounds is usually performed by CD analysis of the keto-4 terpene obtained by ozonolysis as described in (2). In our case, however, the small quantities of pure compound isolated precluded such an investigation owing to the low yields of ozonolysis.

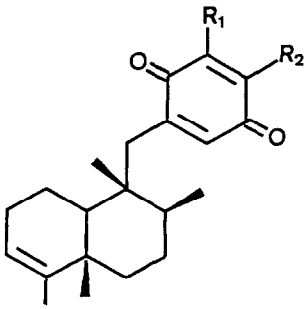
Hence we attempted to obtain this information by direct comparison of the CD spectra of **1** and **2**. Both products exhibit a negative Cotton effect in the 300 nm region in relation with the quinone chromophore. **1**: $[\theta]_{290}$ -3860; **2**: $[\theta]_{315}$ -3600 (MeOH).

These results suggest that the drimane skeleton in smenospongine possess the same absolute configuration as ilimaquinone, and thus this new derivative was assigned structure **2**.

Smenospongine exhibited promising biological activities: cytotoxicity against L 1210 leukemia cells: LD_{50} 1 µg/ml, estimated by inhibition of DNA synthesis and antibacterial activity against *S.aureus* (CMI < 5µg /ml and some *Pseudomonas aeruginosa* strains (Pyo 9 and 8203 S CMI: 25 µg/ml), but weak activity against *E.coli* (CMI: 70 µg/ml).

Experiments are currently in progress to explain the mechanism of the cytotoxicity.

Aminoquinones are very uncommun in marine and others natural products. The only examples from marine origin were derivatives of avarol **3a** and **3b**, isolated from the sponge *Dysidea avara* (10), which exhibit inhibitory activity in the sea urchin test. These derivatives like avarone and avarol are antipodal with **1** and **2**.Authors suppose that these products could be artefacts, owing to the fact than their relative concentrations differ if sponges are quickly extracted, or not. We have no reason to suppose that smenospongine should be an artefact, since our specimens have been immediatly taken up in MeOH. But, indeed, some reactions are likely to take place during extraction, since *Smenospongia* sp. is yellow-colored but immediately turns violet when plunged in MeOH. We rather suppose that these colored compounds exist in the sponge in a conjugated form, perhaps linked to proteins, probably in relation to their possible physiological role in the sponge. This fact should be further investigated.



3a: R_1 = H R_2 = NH-CH_3

3b: R_1 = NH-CH_3 R_2 = H

Other quinone derivatives from marine origin were described to exhibit interesting cytotoxic properties, such as avarone isolated from *Dysidea avara* (11), and arenarone from *Dysidea arenaria* (12), and the o-quinone: stypoldione isolated from the tropical marine algae *Stypodium zonale* (13). Avarone and stypoldione have been shown to act as inhibitors of tubulin polymerization (14).

On the other hand, the antimicrobial 5-bromo-N,N-dimethyl tryptamine and 5,6-dibromo-N,N-dimethyl tryptamine were isolated from *Smenospongia aurea* and *Smenospongia echina* beside an inactive sesquiterpene-hydroquinone: aureol (15).

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3 - Found 358.214, calc. for $C_{22}H_{30}O_4$ 358.2143.

4 - ^{1}H-NMR ($CDCl_3$, 80 MHz, δ ppm) of ilimaquinone 1: 5.85 s, H-19; 4.45 d and 4.43 d (J = 1.6 Hz), H-11; 3.86 s (3H), OCH_3; 2.51 br.s (AB syst), H-15; 1.04 s, CH_3-12; 1.00 d (J = 6 Hz), CH_3-13; 0.85 s, CH_3-14.

5 - Calc. for $C_{21}H_{29}NO_3$: 343.2147, for $C_7H_7NO_3$: 153.0426; for $C_{14}H_{23}$: 191.180.

6 - ^{1}H-NMR ($CDCl_3$, 500 MHz, δ ppm) of smenospongine 2: 8.12 s (exch.D_2O) OH; 5.62 s, H-19; 4.44 s and 4.40 s, CH_2-11; 2.51 d and 2.52 d (J = 14 Hz, AB syst), CH_2-15; 2.33 dt, H-3a; 2.10 br.dd, H-1e; 2.07 br.dd, H-3e; 1.86 m, H-2e; 1.52 ddd, H-6e; 1.45-1.36 m and 1.25 m, H-6a, H-7a, H-7e, H-2a, H-1a; 1.18 m, H-8; 1.05 s 3H, CH_3-12; 0.98 d, CH_3-13; 0.84 s 3H, CH_3-14; 0.79 dd (J = 11.2, 2 Hz), H-10.

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