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BIOLOGICALLY ACTIVE QUINONE AND HYDROQUINONE SESQUITERPENOIDS FROM THE SPONGE *SMENOSPONGIA* sp.

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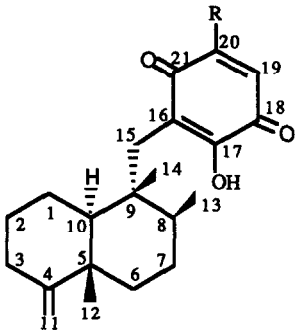
Abstract: This work describes isolation, structure and biological activity of quinone- and hydroquinone sesquiterpenoids biosynthesized by a sponge *Smenospongia* sp. Structures were established by spectral methods. All the products exhibited antimicrobial and cytotoxic activities.

In a program devoted to the search of biologically active components of marine invertebrates, we investigated the fauna of gulf of Aden near Djibouti (1). Collection of sponge samples was monitored by an on site antimicrobial bioassays on the chloroform-methanol extracts. A sponge, *Smenospongia* sp., a new species (2), exhibited marked antimicrobial activity. This activity was subsequently confirmed in a laboratory bioassay against Gram (+) and Gram (-) strains. Dichloromethane, methanol and aqueous extracts were tested. The dichloromethane extract appears the most promising against *S. aureus* and *E. coli* (Ø inhibition 23 mm and 11mm at 500 µg/disc), and this extract was also cytotoxic against the L 1210 leukemia cell line (ID_{50}: 3 µg/ml). The methanolic extract exhibited weaker antimicrobial activity (Ø inhibition 20 mm/disc against *S. aureus*) and was not cytotoxic towards L 1210 leukemia cells until 8 µg/ml. Aqueous extract showed no activity.

These results prompted us to investigate the dichloromethane extract of this species from both chemical and biological points of view. Two methods were used for extraction. Two specimens were immersed in methanol immediately after collection and subsequently extracted with a 1/1 methanol-chloroform mixture. The combined extracts were concentrated under reduced pressure and were extracted with dichloromethane (Extract A). A third specimen was air-dried and directly extracted twice with dichloromethane (Extract B). Some differences in the composition of these two dichloromethane extracts were visible in tlc analysis, hence we decided to examine them separately. Thus, eight active substances were isolated by successive chromatographies and gel filtrations, monitored by an antimicrobial bioassay.

Ilimaquinone 1

From IR ($\upsilon_{C=O}$ 1642 and 1610 cm^{-1}) and UV data (λ_{max} 209, 286 nm), the major component was a quinone. HRMS furnished the formula $C_{22}H_{30}O_4$ from the molecular ion M^+ 358. ^1H and ^{13}C NMR data (table1) suggested identity with the previously described ilimaquinone 1 (3) substantiated by optical activity: $[\alpha]_D^{29}$ = - 24°(c = 1.10; $CHCl_3$), ilimaquinone: $[\alpha]_D^{23}$= -23.2° (c =1.12; $CHCl_3$)(3). The absolute configuration of ilimaquinone was recently revised (4) as shown in 1.



ILIMAQUINONE 1 R = OCH_3

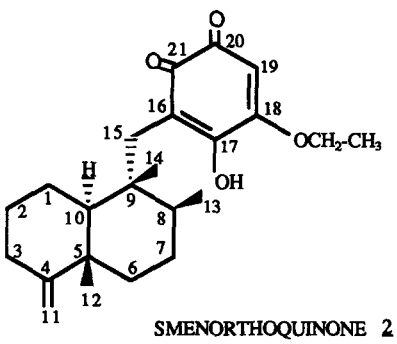
SMENOQUINONE 3 R = OH

SMENOSPONGINE 6 R = NH_2

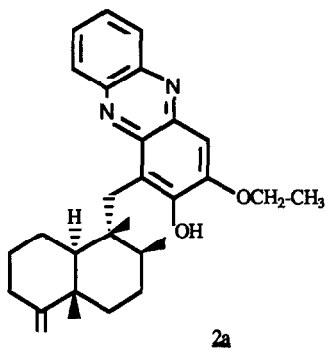
SMENOSPONGIDINE 5 R = NH - CH_2 - CH_2 - C_6H_5

SMENOSPONGIARINE 7 R = NH - CH_2 - CH_2 - CH(CH_3 4', CH_3 5') (1' 2' 3')

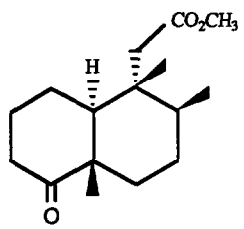
SMENOSPONGORINE 8 R = NH - CH_2 - CH(CH_3, CH_3)



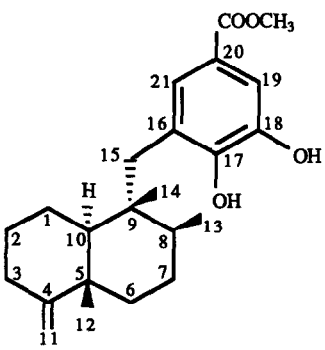
SMENORTHOQUINONE 2



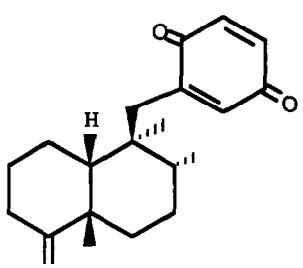
2a



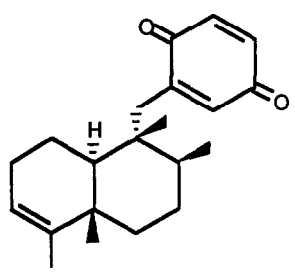
4a



SMENOSPONDIOL 4



ARENARONE 9



AVARONE 10

Smenorthoquinone 2

A less polar compound was isolated as a yellow oil which crystallized as yellow needles (MeOH). HRMS furnished the formula $C_{23}H_{32}O_4$ from the molecular ion M^+372 and composition of some important fragments *m/e* 191 ($C_{14}H_{23}$) and 182 ($C_9H_{10}O_4$). IR ($\upsilon_{C=O}$ 1658 and 1630 cm^{-1}) and UV (λ_{max} 209, 283 nm) suggested a quinone.

In ^1H NMR the signals corresponding to the terpene moiety were similar to those found in the NMR of ilimaquinone 1; a quadruplet at δ 4.04 ppm and a triplet at 1.49 ppm suggested an ethoxy group. However, the difference observed in polarity with regard to ilimaquinone (*Rf* respectively 0.59 for 2 and 0.44 for 1 in hexane-ethyl acetate 7/3) led us to consider the possibility of an *ortho* -quinone skeleton rather than a *para* -quinone one. Since spectral methods did not afford enough reliability to decide between these structural moieties, we condensed 2 with *o*-diamino benzene as described in (5). The product 2a thus obtained exhibited a molecular ion M^+ 444, showing condensation of one molecule of quinone with one molecule of *o*-diamino benzene, and therefore demonstrated the existence of an *o*-quinone. Hence we assigned this quinone structure 2 and named it smenorthoquinone.

Table 1 - ^{13}C NMR (δ ppm; $CDCl_3$; 20.115 MHz)

	1	3(CD_3OD)	2	4	6(80 MHz)	7
1	23.36 t	24.49 t	23.26 t	23.18 t	23.30 t	23.20 t
2	28.16 t	29.48 t	28.09 t	27.76 t	28.10 t	27.98 t
3	36.92 t	38.30 t	36.81 t	37.09 t	36.81 t	36.85 t
4	160.59 s*	162.35 s	160.60 s	160.18 s	160.53 s	160.33 s
5	40.65 s	41.61 s	40.58 s	40.24 s	40.59 s	40.41 s
6	33.09 t	33.70 t	33.04 t	36.63 t	33.09 t	32.97 t
7	28.74 t	30.05 t	28.68 t	27.93 t	28.74 t	28.63 t
8	38.52 d	39.20 d	38.46 d	36.52 d	38.24 d	37.96 d
9	43,51 s	43,65 s	43,41 s	42,19 s	43,11 s	42,89 s
10	50.72 d	51.46 d	50.72 d	48.26 d	50.38 d	50.01 d
11	102.59 t	102.58 t	102.51 t	102.76 t	102.65 t	102.39 t
12	20.60 q	21.06 q	20.55 q	20.55 q	20.55 q	20.48 q
13	17.80 q	18.88 q	17.78 q	17.51 q	17.80 q	17.80 q
14	17.17 q	17.85 q	17.13 q	17.51 q	17.17 q	17.16 q
15	32.80 t	33.94 t	32.81 t	33.03 t	32.69 t	32.57 t
16	117.82 s	114.45 s	117.65 s	125.32 s	114.50 s	113.53 s
17	153.43 s*	188.82 s*	153.18 s	149.13 s	157.96 s	157.06 s
18	182.57 s	179.65 s*	161.31 s	120.22 s	183.20 s	182.77 s
19	102.13 d	101.79 d	102.33 d	114.21 d	95.89 d	91.48 d
20	162.13 s	174.72 s*	184.99 s*	142.66 s	150.80 s	150.21 s
21	182.11 s	166.80 s*	182.69 s*	127.38 d	179.82 s	177.97 s
22	56.79 q		65.86 t	168.20 s		
23			13.72 q	52.09 q		
1'						41.11 t
2'						36.66 t
3'						25.92 d
4'						22.24 q
5'						22.24 q

*may be reversed

Smenoquinone 3

The third product isolated appears from IR; UV; ^1H and ^{13}C NMR (table1) to be a quinone closely related to ilimaquinone. CIMS furnished the pseudomolecular peak $[M+H]^+$ 345 and EIMS only the fragment ions *m/e* 191 and 153. The sole difference was the absence of the methoxy group in ^1H and ^{13}C NMR. Thus, this quinone appeared to be the demethyl derivative of ilimaquinone 1. Methylation of 3 and 1 afforded the corresponding permethylated compounds 3b and 1b which were identical (NMR spectra and $[\alpha]_D$). Hence we proposed structure 3 for smenoquinone.

Smenospondiol 4

Smenospondiol is the sole hydroquinone obtained from *Smenospongia* sp..The molecular formula $C_{23}H_{32}O_4$ was deduced from HRMS data and the structure elucidation achieved by spectral analysis (6): ^1H NMR, ^{13}C NMR, NOE-DIFFERENCE experiments. We used a 2D COSY LR to establish the relative configuration of the substituents.

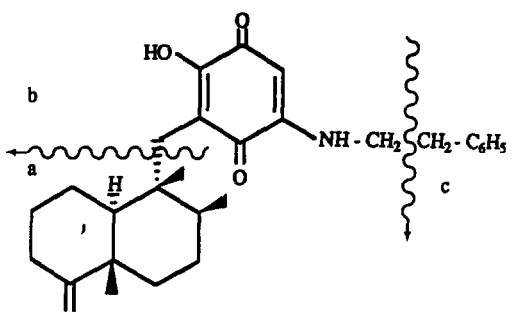
Smenospongidine 5

This product crystallized in methanol, m.p. 168-170 °C, IR: 3265, 1600, 1395 cm^{-1}

EIMS furnished a weak molecular peak M^+447, confirmed by chemical ionisation MH^+448. The molecular formula $C_{29}H_{37}NO_3$ was obtained by HRMS of the fragment ions: *m/e* 257.104 ($C_{15}H_{15}NO_3$) and *m/e* 191.179 ($C_{14}H_{23}$).

In the ^1H NMR spectrum we observed signals corresponding to the rearranged drimane skeleton and additional signals for five aromatic protons (δ 7.24 ppm), one D_2O exchangeable proton (6.47, t), an ethylenic proton at 5.41 and two methylenes (3.43, q and 2.87, t).

When selective decoupling experiments were performed, irradiation of the signal at δ 3.43 caused simplification of the signals at 6.47 and 2.87 to singlets. These results combined with HRMS analysis allowed us to propose structure 5 for smenospongidine. ^{13}C NMR (table 1), compared with the previously described smenospongine 6 (7), confirmed this structure. Of particular help was the chemical shift of CH_3-12, which was indicative of a *trans* -decaline junction (8).



a	: *m/e* 191	$C_{14}H_{23}$	
b	: *m/e* 257	$C_{15}H_{15}NO_3$	
b - c	: *m/e* 166	$C_8H_8NO_3$	
c	: *m/e* 91	C_7H_7	

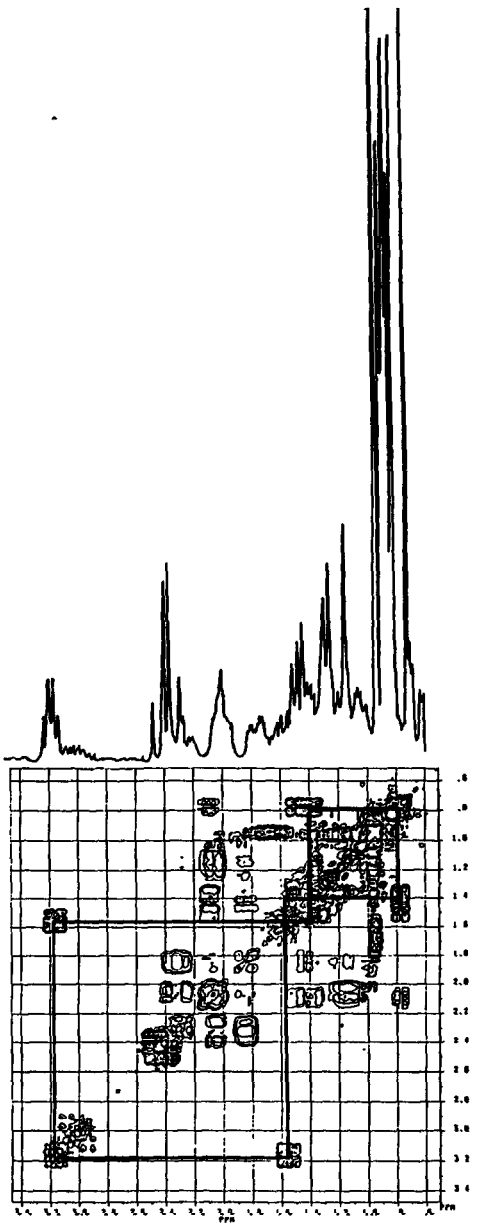
Smenospongine 6.

Smenospongine 6 was previously described (7). ^{13}C NMR data are listed in table 1 for comparison with other amino derivatives isolated from *Smenospongia* sp.

Smenospongiarine 7

Molecular composition $C_{26}H_{39}NO_3$ of this compound was deduced from HRMS analysis of the fragment ions *m/e* 223.119 ($C_{12}H_{17}NO_3$) and 191.179 ($C_{14}H_{23}$), combined with CIMS, which gave the pseudomolecular peak MH^+ 414.

In ^1H NMR we observed signals corresponding to the rearranged drimane skeleton, and additional signals at δ 6.41 ppm (1H, t, exchangeable by D_2O), 5.36 (s,1H), 3.20 (dt,2H) and further two ill-defined methyl doublets at 0.95. A 2D COSY NMR allowed correlation between these signals (scheme 1) and we propose structure 7 for smenospongiarine. ^{13}C NMR data (table 1) confirmed this structure.



Scheme 1 - 2D COSY of smenospongiarine 7
Sequence R- NH- CH_2- CH_2- CH-$(CH_3)_2$

Smenospongorine 8

Pure smenospongorine was isolated by preparative tlc on silicagel (hexane-ethyl acetate 7/3). CIMS gave the pseudomolecular peak $(M+H)^+$ 400 and EIMS characteristic ions *m/e* 191 and 209 ($C_{11}H_{15}NO_3$). ^1H NMR data are very similar to those of smenospongiarine 7 particularly by the presence of three methyl doublets at δ 0,97 ppm. The ^1H NMR spectrum, however, presents a sole methylene (dd) at δ 2.95 ppm, which according to selective decoupling experiments was correlated with an

exchangeable doublet at 6.53 and an ill-defined proton at 1.8 ppm. These data, compared with those of smenospongiarine led to structure 8 for smenospongorine.

In order to determine the absolute configuration of these sesquiterpene quinones, the classical process would be the ozonolysis leading to the ketone 4a. The small amounts of pure products isolated and the low yields of ozonolysis (or other degradative oxidation methods) precluded such a process, which turn to be of poor value (4). Hence we choose to compare CD spectra of our compounds with CD of ilimaquinone 1. CD spectra of quinones 1, 2, 5, 6 performed in methanol, all exhibited negative Cotton effect in the 300 nm region, associated with the quinone chromophore. Althought the chromophores were not quite identical, these new sesquiterpene quinones may have the same configuration as ilimaquinone 1. θ values are listed in table 2.

Table 2 - Cotton effect in the 300 nm region in relation with the quinone chromophore

Ilimaquinone 1	$[\theta]_{290} = -2376$	Smenospongidine 5	$[\theta]_{300} = -2716$
Smenorthoquinone 2	$[\theta]_{290} = -1381$	Smenospongine 6	$[\theta]_{300} = -1914$

If quinone composition of extract A obtained from wet sponges and extract B obtained from dry ones were compared, differences were found (table 3) mainly in the yields of alkylated amine derivates which appear more abundant in the extract obtained from dry animals. Thus we can deduce that the highest yields of smenospongine 6 in the extract A could be ascribed to a degradative process of the alkylated amino quinones. We noticed the absence of smenorthoquinone 2 in extract B, this quinone, a minor compound was probably lost during the purification. These results illustrate of the difficulties encountered in isolation of sensitive compounds in marine animals, and stress how carefull one must be in establishing evaluation concerning species.

Table 3 - Yields of substances 1 - 8 in extract A and B

CH_2Cl_2 extract A		CH_2Cl_2 extractB	
substance	yield (mg)	substance	yield (mg)
1	120	1	100
2	30	2	0
3	20	3	0
4	100	4	30
5	3	5	10
6	40	6	10
7	3	7	20
8	3	8	5

Biological activity

All the compounds described exhibited antimicrobial and cytotoxic activity. MIC was evaluated first towards *S.aureus* , a classical strain, and towards other strains. Results are summarized in table 4. Significant activity of smenospondiol 4 and smenospongine 6 can be observed towards *S.aureus* , and also antimicrobial activity of smenospongine 6 on *P.morganii* which belongs to the *Pseudomonas* family, resistant to most antibiotics.

Cytotoxicity was evaluated towards L 1210 leukemia cells by measurement of DNA synthesis inhibition estimated by incorporation of ^3H thymidine. The most active compounds were smenospongine 6, smenorthoquinone 2 and smenoquinone 3 for which ID_{50}: 1.5, 1.5, 2.5 µg/ml respectively could be estimated. Smenospondiol 4, ilimaquinone 1 and smenospongiarine 7 displayed an $ID_{50} = 4$ µg/ml. Further investigations concerning biological activities of these products are currently in progress.

Table 4 - Antimicrobial activity: CMI (µg/ml).

Strain Subst.	*S.aureus* 209 P	*E.coli* RL 92	*E.cloacal* P 99	*P.morganii* 1510	*K.oxytoca* R 30	*Ps.aeruginosa* PYO 9	8203 S
1	25	>50	>50	50	>50	50	50
1b	>50	>50	>50	>50	>50	25	25
2	10	>50	>50	50	>50	25	25
3	50	>50	>50	>50	>50	25	50
3b	>50	>50	>50	>50	>50	>50	>50
4	2	>50	>50	50	>50	25	50
6	7	>50	>50	25	>50	25	50
5	50						
7	50						

Other biologically active sesquiterpene quinones from a marine source were described previously. Arenarone 9 and arenarol from the sponge *Dysidea arenaria* (9) were cytotoxic against P 388 lymphocytic leukemia cells. Activity of avarone 10 and avarol (10), isolated from another sponge (*Dysidea avara*), were extensively investigated against L 1210 leukemia cells (11), lymphocytes (12) and cytotoxicity could be in relation with inhibition of tubuline polymerisation (13).

Aminoquinones are very uncommon in marine and other natural products. The only examples from marine origin were avarol derivatives isolated from *Dysidea avara* (14). Authors suppose that these products could be artefacts, since their relative concentrations differ if sponges are quickly extracted, or not.

We noticed differences in relative yields of aminoquinones according to the extraction process, and we suppose that alkylaminoquinones are partially destroyed by storage in methanol. In contrast, non alkylated aminoquinones and dihydroquinones are sensitive to air drying.

Taxonomic discussion

The genus *Smenospongia* is a new one (Wiedenmayer 1977) established by histological considerations (15). The only *Smenospongia* described so far were *S.equina* and *S.aurea* (16). In these species bromotryptamine derivatives were present, but comparison of two specimens of *S.aurea* revealed that one contained the hydroquinone sesquiterpene aureol and a bromotryptamine, and the other furnished 8-epichromazonarol and 6-bromo-aplysinopsine. From these results authors postulate that bromotryptamine derivatives are characteristic of the genus *Smenospongia* and include *Polyfibrospongia maynardii* in *Smenospongia*.

The *Smenospongia* sp. studied here was devoid of bromotryptamine derivatives and the sesquiterpene quinones described were structurally close to sesquiterpene quinones encountered in *Dysidea* or *Hippospongia*. Hence, taxonomic considerations in the genus *Smenospongia* (and other *Porifera*) need further investigations.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on Bruker WP 80, Bruker WM 200 and 500 instruments (TMS internal reference). Infrared and ultraviolet spectra were recorded on Perkin-Elmer 157G and Uvikon 810 Kontron spectrophotometers, respectively. Low resolution mass spectra were recorded on a Thomson THN 208 mass spectrophotometer. High resolution mass spectra were supplied by a Kratos MS 50 spectrophotometer. C.I.M.S. on a Nermag U.3.0. All solvents were redistilled prior to use. Melting points are uncorrected.

Collection and extraction of *Smenospongia* sp.: The sponge *Smenospongia* sp., a new species, was collected by SCUBA diving (-20 to -25 m) in the gulf of Aden, near Djibouti, in January 1985, during the Ardoukoba expedition. A sample of fresh sponge (2 kg) was immediately immersed in methanol, and further extracted with a $CHCl_3$/MeOH: 1/1 mixture. The combined extracts were evaporated under reduced pressure, and the aqueous suspension extracted by CH_2Cl_2 (extract A).

Another specimen was air-dried (44 g) and extracted first by CH_2Cl_2 (extract B), then by MeOH.

Extract A (8 g) was chromatographed on a silicagel ($CHCl_3$ / increasing amounts of MeOH). Fractions were screened for antimicrobial activity against *Staphylococcus aureus*. Two active fractions were studied: fraction a eluted with 2 %-, and fraction b eluted with 5 % MeOH in $CHCl_3$.

Fraction a eluted with 30 % AcOEt in hexane gave a mixture of 2, 1 and 3.
Smenorthoquinone 2 was separated by on a Sephadex LH 20 column, (MeOH/$CHCl_3$: 60/40).
Ilimaquinone 1 and smenoquinone 3, further chromatographed on a Florisil column (Hex/AcOEt: 70/30 and then MeOH), were separated.
Smenoquinone 3 was insoluble in the solvent Hexane/AcOEt: 70/30 and soluble in MeOH.
The respective yields were: - smenorthoquinone 2 30 mg - 0.38 % dry weight
- ilimaquinone 1 120 mg - 1.5 % dry weight
- smenoquinone 3 20 mg - 0.25 % dry weight

Fraction b, chromatographed on a silicagel column (Hex/AcOEt: 70/30), contained smenospondiol 4 (100 mg - 1.25 % dry weight), then smenospongidine 5 (3 mg - 0.03 % dry weight), and smenospongine 6 (40 mg - 0.5 % dry weight). All were further purified on a column of Sephadex LH 20 (MeOH/$CHCl_3$: 60/40).
Smenospongiarine 7 and smenospongorine 8 were present in trace amounts in extract A.

Extract B (4 g) was chromatographed on a silica gel column ($CHCl_3$/ increasing amounts MeOH). The active fractions were recombined in 2 fractions a' and b', respectively eluted with 2 % and 5 % MeOH in $CHCl_3$.

Fraction a' was filtered through a column of Sephadex LH 20 (MeOH/$CHCl_3$: 60/40) and furnished 7 and 8 as a mixture:
- smenospongiarine 7 20 mg - 0.5 % dry weight
- smenospongorine 8 5 mg - 0.125 % dry weight
- ilimaquinone 1 100 mg - 2.5 % dry weight

Smenospongiarine 7 and smenospongine 8 were separated by tlc on silicagel (Hexane/AcOEt: 70/30).

Fraction b' contained: - smenospondiol 4 30 mg - 0.75 % dry weight
- smenospongine 6 10 mg - 0,25 % dry weight

Both were separated by chromatography by a silica gel column (Hex/AcOEt:70/30), and purified on silica gel columns ($CHCl_3$/acetone: 95/5) and then on a column of Sephadex LH 20 (Hex/AcOEt: 60/40).

Methylation of ilimaquinone and smenoquinone

Methyl iodide in excess was added to 3 mg ilimaquinone in dry acetone containing potassium carbonate. The mixture was heated under reflux for 36 hr. The solvent was evaporated, and the dimethyl derivative further purified on a silicagel column (hexane-AcOEt: 80/20). The same procedure was performed with smenoquinone.

Ilimaquinone 1
$C_{22}H_{30}O_4$; *m/e* found 358.2140.
SM *m/e* (%): 358 (M^+-5), 191 (62), 168 (99), 135 (34), 121 (47), 109 (48), 107 (39), 95 (100).
$[\alpha]_D^{29}$ = -24° (c = 1.10; $CHCl_3$).
UV (EtOH) λ_{max} nm (ε): 209 (14200), 286 (14800).
IR (KBr) υ cm^{-1}: 3324, 2923, 1642, 1610, 1225.
^1H NMR ($CDCl_3$, 200 MHz) δ ppm: 5.85 (s, H-19), 4.45-4.43 (2 s, H-11), 3.86 (s, OCH_3), 2.51 (d) and 2.48 (d) (AB syst., *J* = 13 Hz, H-15), 1.04 (s, CH_3-12),1.00 (d, CH_3-13), 0.85 (s, CH_3-14), 0.76 (dd, *J* = 13 and 3.6 Hz, H-10).

Smenorthoquinone 2
$C_{23}H_{32}O_4$ (*m/e* found: 372.229; calc.: 372.230).
SM *m/e* (%): 374 (2), 372 (20), 191 (15), 182 (73), 135 (27), 121 (42), 109 (33), 107 (27), 95 (100).
UV (EtOH) λ_{max} nm : 209 (24000), 283 (18400).
IR (KBr) υ cm^{-1}: 3300, 1658, 1630.
^1H NMR ($CDCl_3$, 80 MHz) δ ppm: 7.48 (1H, br s), 5.81 (1H, s), 4.43 (2H, br s), 4.04 (2H, q), 2.16 (m),1.57 (m),1.49 (t, 3H),1.26 (m),1.04 (3H, s),1.00 (3H, br s), 0.83 (3H, s).

Smenoquinone 3
$C_{21}H_{28}O_4$; m.p. > 350°C
SM *m/e* (%): 191 (40), 154 (12), 135 (44), 121 (65), 109 (56), 107 (87), 95 (100).
UV (EtOH) λ_{max} nm (ε): 214, 286.
IR (KBr) υ cm^{-1}: 3324, 2940, 1645, 1535.
^1H NMR (MeOD, 80 MHz) δ ppm: 5.71 (1H, s), 4.76 (2H, br s), 2.40 (2H, br s), 1.01 (3H, s), 0.92 (3H, d, *J* =7 Hz), 0.78 (3H,s).

Dimethyl smenoquinone and methyl ilimaquinone
$[\alpha]_D^{29}$ = - 14.5° (c = 0.9; $CHCl_3$).
SM *m/e* (%): 372 (6), 191 (52), 182 (59), 168 (97), 135 (47), 121 (71), 109 (71), 107 (48), 95 (100).
^1H NMR ($CDCl_3$, 80 MHz) δ ppm: 5.71 (1H, s), 4.45-4.44 (2H, br s), 4.01 (3H, s), 3.80 (3H, s), 2.49 (2H, br s), 2.16 (m), 1.56 (m), 1.39 (m), 1.26 (m), 1.04 (3H, s), 0.94 (3H, br s), 0.83 (3H, s).

Smenospondiol 4
$C_{23}H_{32}O_4$; m.p. = 180-182°C.
SM *m/e* : 372, 191, 182, 175, 149, 135, 121, 109, 107, 95.
m/e 182.0575, calc. 182.0058 for $C_9H_{10}O_4$; *m/e* 191.1792, calc. 191.1800 for $C_{14}H_{23}$; *m/e* 372.2289, calc. 372.2300 for $C_{23}H_{32}O_4$.
UV (EtOH) λ_{max} nm (ε): 219 (27250), 269 (15600).
IR (KBr) υ cm^{-1}: 3310, 1700, 1602.
^1H NMR (80 MHz, $CDCl_3$) δ ppm: 7.52 (1H, d, *J* =1.8 Hz), 7.40 (1H, d, *J* =1.8 Hz), 6.25* (1H, br s), 5.97* (1H, br s), 4.39 (2H, br s), 3.87 (3H, s), 2.67 (2H, br s), 1.07 (3H, s), 1.05 (3H, d, *J* =7 Hz), 0.88 (3H, s).
^1H NMR (80 MHz, DMSO) δ ppm: 9.69 (1H, br s), 8.91 (1H, br s),7.24 (1H, d, *J* = 1.8 Hz), 7.16 (1H, d, *J* = 1.8 Hz), 4.36 (2H, br s), 3.75 (3H, s), 2.58 (2H, br s), 1.07 (3H, s), 1.01 (3H, d, *J* = 7 Hz), 0.81 (3H, s). * exchangeable D_2O.
^1H NMR (500 MHz, C_6D_6) δ ppm: 7.65 (d, *J* = 1.8 Hz, H-21), 7.38 (d, *J* =1.8 Hz, H-19), 5.98 (s,OH), 5.23 (s, OH), 4.50-4.48 (2s, =CH2), 3.45 (s, OCH3), 2.77-2.67 (dd, AB syst, *J* =14.1 Hz, C_6H_5-CH_2), 2.36 (ddd, H-3a), 2.15 (ddd, H-3e), 2.05 (br d, H-1e), 1.91 (m, H-2e), 1.61 (m, H-2a), 1.50-1.39 (m, H-1a,6a,8,7), 1.31 (m, H-7), 1.23 (ddd, H-6a), 1.19 (dd, H-10), 1.11 (d, *J* =7 Hz, CH_3-13), 1.01 (s, CH_3-14), 0.81 (s, CH_3-12).

Smenospongidine 5
$C_{29}H_{37}NO_3$; m.p. : 168-170°C.
SM *m/e* (%): 447 (7), 257 (64), 191 (11), 166 (59), 152 (25), 135 (16), 121 (23), 109 (23), 107 (20), 95 (100).
m/e 166.0495, calc. 166.0504 for $C_8H_8NO_3$; *m/e* 191.1795, calc. 191.1799 for $C_{14}H_{23}$; *m/e* 257.104, calc. 257.105 for $C_{15}H_{15}NO_3$.
IR (KBr) υ cm^{-1}: 3265, 1600, 1395.
^1H NMR ($CDCl_3$, 250 MHz) δ ppm: 6.47 (1H exch., s), 5.41 (1H, s), 4.45 (2H, br s), 3.43 (2H, q), 2.87 (2H, t), 2.52-2.51 (dd, AB syst., *J* = 14 and 2 Hz), 1.05 (3H, s), 0.98 (3H, d, *J* = 7.5 Hz), 0.84 (3H, s), 0.79 (1H, dd, *J* = 11.2 and 2 Hz).

Smenospongine 6
$C_{21}H_{29}NO_3$ m.p.: 153-155°C.
SM *m/e* (%): 343 (5), 191 (13), 153 (98), 135 (22), 121 (32), 109 (35), 107 (26), 95 (100).
m/e 343.2144, calc. 343.2147 for $C_{21}H_{29}NO_3$; *m/e* 153.0430, calc. 153.0426 for $C_7H_7NO_3$; *m/e* 191.1792, calc. 191.1800 for $C_{14}H_{23}$.
UV (EtOH) λ_{max} nm (ε): 209 (16150), 317 (15600).
IR (KBr) υ cm^{-1}: 3477, 3280, 2921, 2858, 1568, 1375, 1333, 1203.
^1H NMR ($CDCl_3$, 500 MHz) δ ppm: 8.12 (1H exch.,s, OH), 5.62 (s, H-19), 4.44 and 4.40 (s, CH_2-11), 2.57 (d) and 2.54 (d) (AB syst., *J* = 14 Hz, 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, 7a, 7e, 2a, 1a), 1.18 (m, H-8), 1.05 (3H, s, CH_3-12), 0.98 (d, *J* = 7.5 Hz, CH_3-13), 0.84 (3H,s, CH_3-14), 0.79 (dd, *J* = 11.2 and 2 Hz, H-10).

Smenospongiarine 7
$C_{26}H_{39}NO_3$; m.p.: 170-172°C
SM *m/e* (%): 413 (4), 311 (8), 283 (12), 223 (100), 191 (11), 167 (22), 153 (27), 149 (15), 135 (14), 121 (16), 109 (18), 107 (12), 95 (79).
m/e 191.179, calc.191.179 for $C_{14}H_{23}$; *m/e* 223.119, calc. 223.120 for $C_{12}H_{17}NO_3$.
UV (EtOH) λ_{max} nm (ε): 204 (27230), 324 (14070).
IR (KBr) υ cm^{-1}: 3417, 3275, 1640, 1592.
^1H NMR ($CDCl_3$, 200 MHz) δ ppm: 8.41 (1H exch., s), 6.41 (1H exch., t), 5.36 (1H, s), 4.43 (br s), 3.20 (2H, dt), 2.48 (d)-2.37 (d) (AB syst.), 2.31 (dt), 2.07 (2 H, m), 1.85 (1H, m), 1.80-1.05 (11H, m), 1.04 (3H, s), 0.95 (9H, 3d overlaped), 0.83 (3H, s), 0.78 (1H, dd).

Smenospongorine 8
$C_{25}H_{37}NO_3$
SM *m/e* (%): 399 (5), 209 (100), 191 (17), 166 (36), 152 (18), 135 (11), 121 (15), 109 (15), 107 (12), 95 (66).
UV (EtOH) λ_{max} nm (ε): 210 (14000), 329 (20150).
IR (KBr) υ cm^{-1}: 3417, 3275, 1640, 1592.
^1H NMR ($CDCl_3$, 200 MHz) δ ppm: 6.53 (1H, s), 5.41 (1H, s), 4.45 (2H, br s), 2.95 (2H, dt), 2.48 (1H, d), 2.45 (1H, d, *J* =13 Hz),1.03 (3H, s), 0.97 (9H, 3d overlaped), 0.82 (3H, s), 0.76 (1H, dd).

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