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New Cytotoxic Sesterterpenes From The New Caledonian Marine Sponge *Petrosaspongia nigra* (Bergquist).

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Abstract: Along with two known cheilanthane sesterterpene lactones, **1** and **2**, eight new related sesterterpenes (**3-10**) and two new nor-sesterterpenes (**11** and **12**) have been isolated from the New Caledonian marine sponge *Petrosaspongia nigra* Bergquist 1995 (new genus, new species). Their structures were determined from 1D and 2D NMR studies and mass spectral data. They exhibited cytoxicity against the NSCLC-N6 human bronchopulmunary non-small-cell-lung carcinoma cell lines. © 1997 Elsevier Science Ltd.

Marine sponges have provided the largest number of sesterterpenes, a relatively rare group of pentaprenyl terpenoids of widespread distribution, i.e. fungi, lichens, higher plants, insects and various marine organisms, whose structures are derivable from gerany-farnesyl diphosphate[1-3]. Sesterterpenes from Dyctioceratic sponges include the linear C-25 furans and their C-21 degradation products, the group of γ-hydroxybutenolides, potent anti-inflammatory agents exemplified by the monocarbocyclic manoalide[4], the bicarbocyclic cacospongiolide[5] and the tricarbocyclic luffolide[6], that are inhibitors of the enzyme phospholipase A_2 (PLA_2)[7], and the most common tetracarbocyclic scalarane sesterterpenoids that also exhibit a variety of biological activities, including anti-inflammatory[8].

Recently, two new cheilantane sesterterpene lactones **1** and **2**[9] (the structure of **1** was determined by X-ray analysis[10]) were isolated from a sponge incorrectly assigned to the genus *Dactylospongia*[9] and then reassigned as a new genus and new species: *Petrosaspongia nigra* (Bergquist 1995)[10].

In our continuing search for cytotoxic substances form New Caledonian marine organisms[11], we found potent activity in the dichoromethane extract of the sponge *Petrosaspongia nigra* (Bergquist 1995 sp.nov., Dictyoceratida, Spongidae) collected off New Caledonia, from which we isolated **1** and **2**, eight new related sesterterpenes (**3-10**) and two new nor-sesterterpenes (**11** and **12**). Herein we report their isolation, structure elucidation and cytoxicity data.

Freeze dried specimens (2.7 Kg, 12 Kg fresh), collected off the southern coral reef of New Caledonia were sequentially extracted with *n*-hexane and dichloromethane in a Soxhlet apparatus and then with methanol at room temperature. The dichloromethane soluble material was fractionated by silica gel flash chromatography (MPLC) eluting with $CHCl_3$/MeOH mixtures with increasing amounts of MeOH, followed by reverse phase HPLC to yield **1-12**, which we named petrosaspongiolides A-L (yields : 10.1mg, 15.0mg, 3.3mg, 2.5mg, 3.4mg, 2.8mg, 2.5mg, 5.4mg, 0.9mg, 3.1mg, 5.0mg, 2.0mg, respectively).

Petrosaspongiolides A (**1**) and B (**2**) (known compounds): structure analysis was carried out mostly with **2**, *m/z* 460 (M^+) and 191 ($C_{14}H_{23}$). The ^{1}H-NMR spectrum ($CDCl_3$, 500 MHz) exhibited signals for five methyl

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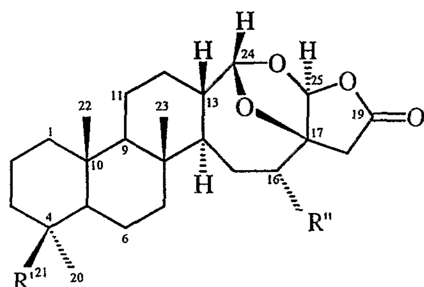
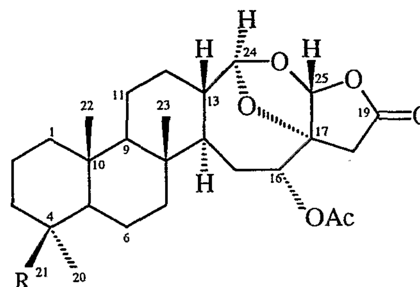
singlets (δ 0.75. 0.77, 0.80 and 0.85 ppm) including one at δ 2.18 ppm for an acetate, and three oxygenated methines (δ 5.70 s, 5.48 br s, and 5.15 dd). The ^{13}C-NMR spectrum ($CDCl_3$, 125 MHz) revealed 27 carbons including signals for an acetate unit (δ 170.1 and 21.1 ppm), an ester carbonyl at δ 171.1 ppm and three oxygenated methines, including two acetals, δ 114.1 and 102.8 ppm. Inspection of literature indicated that the same structural units were present in the structure of a sesterterpene lactone, isolated from a sponge of the genus *Dactylospongia*[9]. Identification of the major compound from the *Petrosaspongia nigra* was confirmed by comparison of ^{1}H- and ^{13}C-NMR and mass spectral data with those reported for **2**[9].

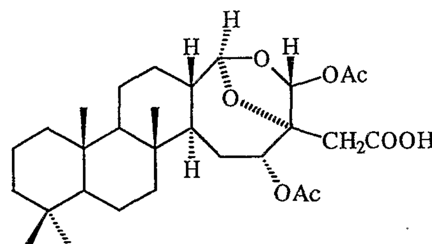
The identification of the isomeric second major compound, *m/z* 460 (M^+) and 191 ($C_{14}H_{23}$), with **1** followed in a straightforward manner. The most significant differences observed in the ^{1}H-NMR data of **1** and **2** were for protons of H25, shielded by *ca.* 0.5 ppm in **2** (δ 5.70 ppm in **2** and 6.21 ppm in **1**), and of H24 which appeared as a singlet in **2** and as a doublet (J=2 Hz) coupled to H13 in **1**.



	R'	R''
1	CH_3	OAc
3	CH_2OAc	OAc
4	CH_2OH	OAc
5	CHO	OAc
6	COOH	OAc
7	CH_2OH	OH
8	COOH	OH



	R
2	CH_3
9	CH_2OAc



10

Petrosaspongiolide C (**3**) had a molecular ion (EIMS) at *m/z* 518, 58 mass units more than the known **1** and **2**. ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz) data were very similar to those of **1**, except for the lack of a methyl group replaced by an acetoxymethyl group in **3**, δ_H 4.19 -3.87 (AB system, J=12.0 Hz, CH_2) and 2.01s (CH_3), δ_C 66.9 (CH_2), 21.0 (CH_3), 171.3 ppm (CO) (Table 1). Significant shifts of C3, C4 and C20 (δ_C 36.2, 37.0 and 27.3 ppm) in **3** relative to **2** (δ_C 42.0, 33.3 and 33.3 ppm) suggested the location of acetoxyl group at C21[12]. This assignment was substantiated by ROESY(mixing time 500ms; $CDCl_3$) data which indicated that $H_2$21 was axial, because an intense cross-peak was observed between $H_2$21 and Me22 at δ_H 0.77 ppm. Further ROESY correlations bewteen H14 (δ_H 1.01 ppm) and H25 (δ_H 6.18 ppm) and between H24 (δ_H 5.58 ppm), H13 (δ_H 1.54 ppm) and H12β (δ_H 1.66 ppm) were consistent with an α orientation for H25 and a β orientation for H24. The signals for H14 which appeared as a triplet with a large coupling constant (J=

10.0 Hz) confirmed that the C/D ring junction was *trans*. Thus, petrosaspongiolide C (**3**) is the 21-acetoxy derivative of petrosaspongiolide A (**1**).

Petrosaspongiolide D (**4**), *m/z* 476, 16 mass units more than **1** and **2**, again showed ^{1}H ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz) spectra very similar to those of **1**, except for the presence of additional primary hydroxyl group in **4**, δ_H 3.72 and 3.45 (AB system, J=12.0 Hz, CH_2). A C21 hydroxyl group followed unambigously from NMR data and was further supported by the absence in **4** of the characteristic high-field methyl carbon signal observed at δ_C 21.3 ppm in **1** and replaced by an oxygenated methylene carbon resonating at δ_C 65.4 ppm (Table 1).

Petrosaspongiolide E (**5**). NMR spectra ($CDCl_3$, 500 MHz) showed low field resonances at δ_H 9.70 (s) ppm and δ_C 205.5 ppm assigned to a formyl group. Again, comparison of spectral data makes clear that compound **5**, *m/z* 474 (14 mass units more than **1**) is related to the known **1** by oxydation of the Me21 to an aldehyde.

Petrosaspongiolide F (**6**), *m/z* 490 (EIMS), is related to **1** by oxydation of C21 methyl to a carboxyl group, followed unambigously from NMR data ($CDCl_3$, 500 MHz). The only significant differences in the data were for signals corresponding to ring A (Table 1), such as the replacement of methyl carbon signal (δ 21.4 ppm in **1**) by a carbonyl signal at δ 172.0 ppm, accompained by the downfield shift of C4, δ_C 44.9 *vs*. 33.3 ppm in **1**, and by the highfield shift of C3 and C20, δ_C 38.8 and 28.9 *vs*. 41.9 and 33.3 ppm in **1**.

Petrosaspongiolide G(**7**), *m/z* 434 (EIMS). ^{13}C-NMR (125 MHz, CD_3OD) data of **7** were very similar to those of **4** except for the absence of an acetyl group in **7**, thus indicating that **7** was the deacetyl derivative of **4**. This was substantiated by the significant high field shift of H16 (500 MHz, CD_3OD) (δ_H 4.25 vs. 5.42 ppm in **1**) and downfield shifts of C15 (δ_C 34.1 vs. 30.1ppm in **1**) and C17 (δ_C 90.4 vs. 86.9 ppm in **1**).

Petrosaspongiolide H (**8**), *m/z* 448 (EIMS), is the more polar compound of this series. The ^{1}H-NMR spectra (500 MHz, CD_3OD) readily implied that compound **8** is the 16-deacetyl derivative of **6**. Again the only significant differences in the NMR data of **8** relative to those of **6** were for signals corrisponing to H16 (δ_H 4.29 ppm) and carbons C15 and C17 (125 MHz, CD_3OD) (δ_C 34.5 and 90.2ppm, respectively).

Table 1. ^{13}C-NMR chemical shifts (ppm) of ring A of compounds **1,3-8**

	1 ($CDCl_3$)	**3** ($CDCl_3$)	**4** ($CDCl_3$)	**5** ($CDCl_3$)	**6** ($CDCl_3$)	**7** (CD_3OD)	**8** (CD_3OD)
C1	39.9	39.8	40.0	39.2	40.2	41.5	41.9
C2	18.5	18.1	18.2	18.1	19.1	19.4	20.8
C3	41.9	36.2	35.6	34.4	38.8	36.7	40.1
C4	33.3	37.0	38.6	48.3	44.9	38.4	38.1
C5	56.2	56.8	56.8	56.6	56.8	58.5	58.8
C10	37.5	37.4	37.4	37.8	38.0	39.8	38.1
C20	33.3	27.3	26.8	24.1	28.9	27.7	29.9
C21	21.4	66.9	65.4	205.6	172.0	65.2	174.7

Petrosaspongiolide I (**9**), *m/z* 518 (EIMS), is an isomer of **3**. The ^{1}H ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz) spectra was very similar with **2** except for the presence of an aditional acetoxyl group, which was unambigously located at C21, as followed from the comparison of the proton and carbon signals corrisponding to ring A with the same signals in **3**.

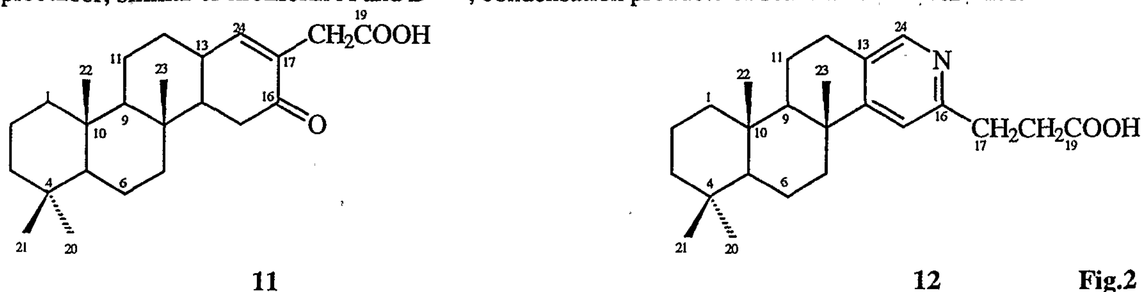
Petrosaspongiolide J (**10**). The ^{1}H (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) spectra confirmed the presence of four tertiary methyls (δ_H 0.73, 0.77, 0.78 and 0.80 ppm) two acetate function (δ_H 2.09 and 2.18; δ_C 170.2, 20.9 and 169.9, 21.15 ppm), three oxygenated methins (δ_H 6.20brs, 5.38s and 5.29dd) and one more carbonyl at 172.7 ppm. The electron impact low resolution mass spectrum of **10** showed the base peak at *m/z* 191 ($C_{14}H_{23}$), already abserved in the spectra of **1** and **2** and interpretated, after accurate measurements, as

originating from cleavage of the ring C. These spectral features indicated that **10** showed the common structure of rings A, B and C of **1** and **2**, and oxygenated functions, including two acetals, were present in the remaning part of the molecule. Interpetation of COSY cross-peaks led to the partial structure C9 to C16. HMBC data connected the oxygenated methine at C16 (δ_H 5.29 ppm) to the acetate carbonyl at d 169.9 ppm, as well as the oxygenated methine at δ_H 5.38 (H25) was weakly correlated to the second acetate carbonyl at δ 170.2 ppm; the remaning oxygenated methine at δ_H 6.20 (H24) gave HMBC cross-peaks with C13 at δ_C 44.0 and C17 at δ_C 86.6 ppm. Further HMBC correlations H16/C15, C14 and H25/C24 allowed the construction of the bicyclic oxygenated portion. The remaining CH_2-COOH structural unit was linked to C17 as substantiated by ^3J HMBC cross peaks H18/C16 and ^2J HMBC correlation H18/CO19. Thus the structure of compound **10** could be determined. The stereochemistry was assigned as in **2**, from the appearance of H24 as a singlet and supported from an intense ROESY(mixing time 500ms; $CDCl_3$) cross-peak observed between H13 and H25.

Petrosaspongiolide K (**11**). The ^1H (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) spectra and the interpretation of the HMBC data (Table 2) indicated that **11** showed a common structure of rings A,B and C of petrosaspongiolide A (**1**) and B (**2**), while the remaining portion was significantly different. In addition to fourteen signals assigned to carbons C1-C14 and to methyl carbons C20-C23 in the rings A,B and C, the ^{13}C NMR spectrum revealed six more carbons including signals for an α,β-conjugated ketone (δ_C 155.0, 131.4 and 201.3 ppm), two methylenes (δ_C 37.6 and 36.2 ppm) and one carbonyl at δ_C 174.0 ppm, which accounted in total for 24 carbons and for 35 non exchangeable protons. The presence of an α,β-unsaturated ketone was supported by UV (234 nm, ε=5545). The EI mass spectrum showed the molecular ion peak at *m/z* 372 (HREIMS: *m/z* 372.269733, required 372.266445), accompained by a prominent fragment at *m/z* 328 (loss of CO_2), thereby indicating the presence of a carboxyl group. Thus, the molecular formula $C_{24}H_{36}O_3$ could be determined. The olefinic singlet at δ_H 6.64 ppm (C24, δ_C 154.9 ppm) and the methylenes at δ_H 2.49dd-2.17t ppm (C15) and 3.27d-3.15d ppm (C18) were connected through non protonated carbons on the basis of HMBC data (Table 2), which included cross peaks: H24/C12,C14,C16,C18; H15α/C14,C16; H15β/C14; H13/C24; Me23/C14, thereby constructing the α,β-unsaturated cyclohexanone fused at C13 and C14 of ring C, carrying a side chain CH_2COOH at C17. Thus, the structure of compound **11** could be determined. Rings C/D were assigned as trans-fused junction on the basis of two high coupling costants exhibited by the allylic H14 resonating in a clear region of the ^1H-NMR spectrum at δ 2.28 (brt, J=8Hz) and indicative of the axial orientation of H14and H13. Petrosaspongiolide K (**11**) is the first example of a 25-norsesterterpene of the cheilantane group.

Petrosaspongiolide L (**12**) gave a FABMS (negative-ion) pseudo molecular ion peak at *m/z* 368 $(M-H)^-$. In the EIMS the molecular ion at *m/z* 369 (HREIMS: *m/z* 369.309780, required 369.308731) is accompained by a prominent peak (base peak) at *m/z* 324 ($M-CO_2H$) , indicative for the precence of a carboxyl group. The ^{13}C-NMR (125 MHz, CD_3OD) spectrum revealed 24 carbons including four quaternary methyls (δ_C 17.1, 21.9, 26.0 and 33.8 ppm) and three quaternary carbons at δ_C 34.3 ppm, 39.0 ppm and 39.6 ppm consistent with a tricarbocyclic skeleton with a gem-dimethyl group at C4 and two methyl groups at the ring junction C8 and C10, already observed in **1** and **2**. The high field signals at δ 18.6 ppm, 19.7 ppm and 20.0 ppm, attributed to the methylenes at C11, C6 and C2 lying in position γ with respect to the axial methyls confirmed the transA/B-transB/C skeleton. Interpretation of COSY cross-peaks identified the sequence C1-C3, C5-C7 and C9-C12 and showed that the protons of C12 were downfield shifted to δ 2.78 and 2.98 ppm, characteristic for a benzylic methylene. Also shifted downfield was the C8 methyl signals, δ_H 1.25 ppm, δ_C 26.0 ppm. Three quaternary and two CH aromatic carbons at δ_C 162.6s , 158.4s and 130.7s, 149.4d, 120.5d ppm, respectively, indicated the presence of a trisubstituted pyridine. The UV spectrum (266, ε=2375; 210, ε=5545) gave support to presence of a pyridine. Two methylene signals at δ 33.6 ppm and 36.0 ppm coupled each to the other (COSY) and one carbonyl signal at δ_C 177.7 ppm completed the ^{13}C-NMR spectrum of **11**. The two aromatic protons were observed resonating as singlets at δ_H 8.18 ppm and 7.26 ppm. These structural units were connected through non protonated carbons on the basis of HMBC data (Table 2). Cross peaks H12/C13, C14 and Me23 /C14 implied

that the pyridine was fused to ring C at C13, C14; H15/C13, C8 and C16, H17/C15 and H24/C13, C14 allowed constructing the pyridine portion with the propionic side chain at C16. Petrosaspongiolide L (**11**), which is apparently a 25-norsesterterpene, could be considered a condensation product with ammonia of a 16keto 24al precursor, similar to molliorin A and B[13-14], condensation products of scalaradial with amines.



11 **12** **Fig.2**

Table 2. ^{1}H-and ^{13}C-NMR chemical shifts and HMBC correlations of the nor-sestertepenes **11** and **12**

	11 ($CDCl_3$)			**12** (CD_3OD)		
position	δ_H[a]	δ_C	HMBC[b]	δ_H[a]	δ_C	HMBC[b]
1	1.97–0.78	39.7			40.8	
2	1.55–1.32	18.6			20.0	
3	1.38–1.12	42.0			43.2	
4		33.0			34.3	
5	0.81	56.6		0.95	57.7	
6	1.59–1.38	18.4			19.7	
7	1.69–0.81	39.8		2.48	40.0	
8		36.8			39.0	
9	0.85	59.2		1.28	56.2	
10		37.5			39.6	
11	1.69-1.39	20.6	C9	1.72-1.95	18.6	C9,C12
12	1.18-2.01dd(4.2,14.4)	32.8	C11, C13	2.78ddd(16, 11.3, 7.1)- 2.98dd(16, 7.3)	28.0	C13 C13, C14
13	2.38brt(8.0)	36.9	C24		130.7	
14	1.5	54.3			162.6	
15	2.49dd(4.2, 16.7)- 2.17t(16.7)	37.6	C16 C14, C16	7.26s	120.5	C16, C8, C13
16	-	201.3			158.4	
17		131.4		3.05t(8.9)	33.6	C15
18	3.15d(16.8)- 3.27d(16.8)	36.2		2.67t(8.9)	36.0	
19		174.0			177.7	
20	0.82s	21.0		0.92s	21.9	
21	0.86s	33.0		0.92s	33.8	
22	0.85s	16.3		1.02s	17.1	
23	0.99s	15.4	C14, C9, C8	1.25s	26.0	C14
24	6.64brs	155.0	C12, C14, C16, C18	8.18s	149.4	C13, C14

[a]Coupling constants are in parentheses and given in Hz. ^{1}H assignments aided by COSY experiments.[b]HMBC optimized for $^{2,3}J_{CH}$=10.0 Hz.

Cytotoxic activity of the sesterterpenoids **1-12** is summarized in Table 3. Petrosaspongiolide A(1) was submitted to *in vivo* tests on immunodepressed rats carrying a bronchopulmonary tumor (NSCLC-N6). It was observed an inhibition of tumoral proliferation at 20mg/Kg without significant toxicity.

Table 3. Cytotoxicity against NSLC-N6[a] tumor cells (IC_{50} expressed in μg/ml)

Compounds	IC_{50}	Compounds	IC_{50}
1	13.0	**7**	inactive
2	14.8	**8**	8.1
3	0.5	**9**	6.8
4	5.2	**10**	6.3
5	4.5	**11**	1.3
6	8.7	**12**	5.7

[a]NSCLC-N6: human bronchopulmonary non-small-cell-lung-carcinoma.

Experimental Section

General Information. For general information see: Zampella A. et. al.[15]

Isolation. The sponge *Petrosaspongia nigra.* (Dictyoceratida, Spongidae) was collected in November 1987 and December 1988 in the waters of the southern coast of New Caledonia. Taxonomic identification was performed by Dr. P.R. Bergquist, and reference specimens are on file (reference 321) at the ORSTOM Centre of Nouméa. Preliminary assays for cytotoxic activity showed marked activities for the dichloromethane extracts (P388 cells, 100% inhibition at 10 μg/ml dose, and KB cells, 78% inhibition at 10 μg/ml dose).
The organism was freeze-dried and the lyophilized matherial (1.0 Kg) was extracted with *n*-hexane and dichloromethane in a Soxhlet apparatus, then with MeOH (3x2 L) at room temperature. The dichloromethane extracts were filtered and concentrated under reduced pressure to give 30.0 g of a brown oil. The crude dichloromethane extract was chromatographated by MPLC on a silica gel column (150x2 g) using a solvent gradient from $CHCl_3$ to $CHCl_3$/MeOH 9:1. MPLC fractions were further purified by HPLC on a semipreparative (7.8x300 mm) μ-Bondapak-C18 column (flow rate 5 ml/min) eluting with MeOH:H_2O mixtures to afford pure compounds **1-12**. The purity of each compound was judged to be > 90% by HPLC and ^{1}H-NMR.

Petrosaspongiolide A (**1**) and B (**2**):($C_{27}H_{40}O_6$, M.W.=460) (**1**,$[\alpha]_D$= -15°, c=0.02, $CHCl_3$; **2**,$[\alpha]_D$= -71°, c=0.03, $CHCl_3$), t_r=24.0 and 19.2 min, respectively, eluting with MeOH/H_2O 85:15. ^{1}H and ^{13}C-NMR were virtually identical to those reported by Cambie *et al.*[9].
Petrosaspongiolide C (**3**): ($C_{29}H_{42}O_8$, M.W.=518) $[\alpha]_D$= -12.5° (c=0.003, $CHCl_3$); t_r=7.6 min, eluting with MeOH/H_2O 85:15. ^{1}H and ^{13}C-NMR see text and Table 1.
Petrosaspongiolide D (**4**): ($C_{27}H_{40}O_7$, M.W.=476) $[\alpha]_D$= -27° (c=0.003, $CHCl_3$); t_r=4.8 min, eluting with MeOH/H_2O 85:15. ^{1}H and ^{13}C-NMR see text and Table 1.
Petrosaspongiolide E (**5**): ($C_{27}H_{38}O_7$, M.W.=474) $[\alpha]_D$= -19.4° (c=0.003, $CHCl_3$); t_r=5.6 min, eluting with MeOH/H_2O 85:15. ^{1}H and ^{13}C-NMR see text and Table 1.
Petrosaspongiolide F (**6**): ($C_{27}H_{38}O_8$, M.W.=490) $[\alpha]_D$= -17.8° (c=0.002, CH_3OH); t_r=4.0 min, eluting with MeOH/H_2O 85:15. ^{1}H and ^{13}C-NMR see text and Table 1.
Petrosaspongiolide G (**7**): ($C_{25}H_{38}O_6$, M.W.=434) $[\alpha]_D$= -25.2° (c=0.002, CH_3OH); t_r=8.0 min, eluting with MeOH/H_2O 70:30 (analytical coloumn). ^{1}H and ^{13}C-NMR see text and Table 1.
Petrosaspongiolide H (**8**): ($C_{25}H_{36}O_7$, M.W.=448) $[\alpha]_D$= +4.3° (c=0.003, CH_3OH); t_r=3.6 min, eluting with MeOH/H_2O70:30 (analytical coloumn). ^{1}H and ^{13}C-NMR see text and Table 1.
Petrosaspongiolide I (**9**): ($C_{29}H_{42}O_8$, M.W.=518) $[\alpha]_D$= -28° (c=0.001, $CHCl_3$); t_r=5.2 min, eluting with MeOH/H_2O 85:15. ^{1}H and ^{13}C-NMR see text and Table 1.

Petrosaspongiolide J (**10**): ($C_{29}H_{44}O_8$) $[\alpha]_D$= -14.5° (c=0.003, $CHCl_3$); t_r=17.6 min, eluting with $MeOH/H_2O$ 80:20. White amorphous solid, EIMS *m/z* 520 (1, M^+), 460 (31, M^+ -CH_3COOH), 445 (39, M^+ -CH_3 -CH_3COOH), 400 (14, M^+ -2CH_3COOH), 191 (100, $C_{14}H_{23}$) ^{1}H-NMR ($CDCl_3$) δ : 6.20 (1H, br s, H24), 5.38 (1H, s, H25), 5.29 (1H, br d, J=10 Hz, H16), 3.06 and 2.91 (2H, AB, J=16.9 Hz, H18, 18'), 2.18 (3H, s, MeC̲O), 2.07 (3H, s, MeCO), 0.83 (3H, s, Me-20), 0.79 (3H, s, Me-21), 0.78 (3H, s, Me-23), 0.75 (3H, s, Me-22); ^{13}C-NMR ($CDCl_3$) δ: 172.7 (s, CH_2C̲OOH), 170.2 (s, MeC̲O), 169.9 (s, MeC̲O), 11.3 (d, C24), 94.0 (d, C25), 86.6 (s, C17), 71.8 (d, C16), 59.3 (d, C9), 56.6 (d, C5), 45.0 (d, C14), 44.0 (d, C13), 42.0 (t, C3), 39.9 (t, C1), 39.9 (t, C7), 37.5 (s, C10) , 37.0 (s, C8), 35.4 (t, C18), 33.3 (q, C20), 33.3 (s, C4), 31.0 (t, C12), 26.3 (t, C15), 21.4 (q, C21), 21.2 (q, M̲e̲CO), 20.9 (q, M̲e̲CO), 20.6 (t, C11), 18.6 (t, C2), 18.5 (t, C6), 16.1 (q, C23), 15.2 (q, C22).

Petrosaspongiolide K (**11**): ($C_{24}H_{36}O_3$, M.W.=372) $[\alpha]_D$= -15.4° (c=0.004, $CHCl_3$); t_r=24.8 min, eluting with $MeOH/H_2O$ 80:20. ^{1}H and ^{13}C-NMR see text and Table 2.

Petrosaspongiolide L (**12**): ($C_{24}H_{35}O_2N$, M.W.=369) $[\alpha]_D$= -33.3° (c=0.001, CH_3OH); t_r=3.2 min, eluting with $MeOH/H_2O$ 80:20 (analytical coloumn). ^{1}H and ^{13}C-NMR see text and Table 2.

Cytotoxic assays. Experiments are performed in 96 wells microtiter plates ($2x10^5$ cells/ml). Cell growth is estimated by a colorimetric assay based on the conversion of tetrazolium dye (MTT) to a blue formazan product using live mitochondria.[16] Eight determinations are performed for each concentration. Control growth is estimated for 16 determinations. Optical density at 570 nm corresponding to solubilized formazan is read for each well on Titertek Multiskan MKII.

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