Petrosaspongiolides M−R: New Potent and Selective Phospholipase A_2 Inhibitors from the New Caledonian Marine Sponge *Petrosaspongia nigra*

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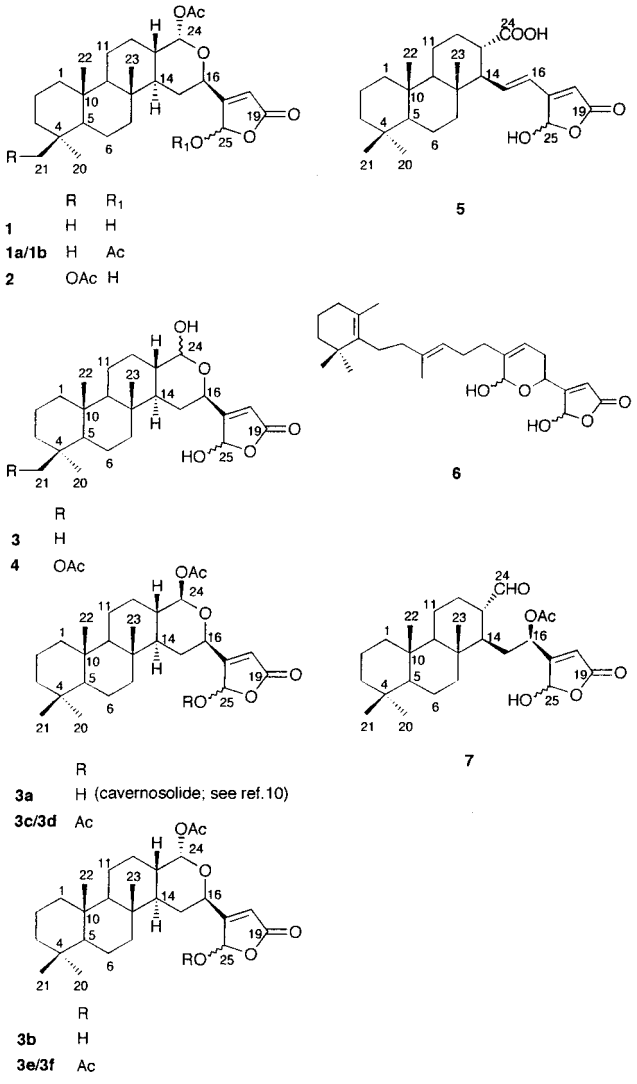
Five new bioactive sesterterpenes (**1**−**5**) have been isolated from the New Caledonian marine sponge *Petrosaspongia nigra* Bergquist and named petrosaspongiolides M−R. Their chemical structures were determined from 1D and 2D NMR studies and MS data. All compounds inhibited different preparations of phospholipase A_2 (PLA_2) by irreversibly blocking these enzymes (particularly human synovial and bee venom, see Table 3), with IC_{50} values in the micromolar range. Interestingly, these compounds displayed a much lower activity (or no activity at all) toward porcine pancreas and *Naja naja* venom PLA_2 enzymes. The most potent compound, **1** (IC_{50} 1.6 and 0.6 μM for human synovial and bee venom PLA_2 enzymes, respectively), was slightly more active than manoalide (**6**) (IC_{50} 3.9 and 7.5 μM) under our experimental conditions. Compound **3** is more selective, inhibiting human synovial PLA_2 to a greater extent than bee venom PLA_2.

Sesterterpenes, a group of pentaprenyl terpenoids quite common in marine sponges of the order Dictyoceratida[1] have received special attention from the scientific community after the discovery of manoalide[2] (**6**), a potent antiinflammatory monocarbocyclic derivative (its in vitro activity is nearly equivalent to that of hydrocortisone), that binds and blocks the enzyme phospholipase A_2 (PLA_2) through the formation of a covalent inhibitor-enzyme adduct.[3] More recently, several other sesterterpenes, like luffolide[4] and cacospongionolide,[5] have shown similar activities. All these antiinflammatory compounds share some structural features, like the presence of a γ-hydroxybutenolide moiety and often an additional masked or free aldehyde functionality, and several investigations have been devoted to the identification of a common pharmacophore in these derivatives.[6,7]

In our continuing search for bioactive substances from New Caledonian marine organisms, we have had occasion to analyze the extracts of the sponge *Petrosaspongia nigra* Bergquist 1995 sp.nov. (Dictyoceratida, Spongidae) collected off New Caledonia, from which we isolated five new sesterterpenes (**1**−**5**) named petrosaspongiolides M−R, along with a number of other cytotoxic sesterterpenes (petrosaspongiolides A−L),[8] all possessing a cheilantane skeleton. Herein we report their isolation, structure elucidation (see Tables 1 and 2 for NMR assignments), and the data from their in vitro tests on the inhibition of human sinovial, *Naja naja* venom, bee venom, and porcine pancreas PLA_2 (Table 3).



Results and Discussion

Freeze-dried specimens (1 kg, 12 kg fresh), collected off the southern coral reef of New Caledonia were

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Chemical Shifts and HMBC Correlations of the Sestertepenes **1**, **3**, and **5**

	1 ($CDCl_3$)			**3** (CD_3OD)			**5** (CD_3OD)		
position	δ_H[a]	δ_C	HMBC[b]	δ_H[a]	δ_C	HMBC[b]	δ_H[a]	δ_C	HMBC[b]
1	1.72−1.09	40.3		1.77−1.88	41.0		0.8−1.7	41.1	
2	1.53−1.29	18.6		1.29−1.47	19.5		1.32−1.56	19.5	
3	1.22−1.39	42.0		1.22−1.41	43.1		1.39−1.19	43.2	
4		33.4			34.3			34.2	
5	0.89	56.6		0.90	57.8		0.89	57.9	
6	1.40−1.61	18.6		1.43−1.50	19.5	C5	1.52−1.42	19.6	
7	0.83−1.98	39.9		1.81	41.6	C6, C8,	1.21	43.2	C5, C15
				1.11		C9, C23	1.60		
8		36.6			37.7			38.8	
9	0.90	59.5		0.92	60.9		0.96	60.1	
10		37.5			38.8			39.0	
11	1.29−1.63	19.5		1.37	20.5	C9	1.65−1.40	21.1	
				2.09		C9			
12α	1.08	27.8		1.03	29.4	C11	1.57	31.9	C9
12β	1.63			2.07		C24	2.02 dd (4.3;16.8)		
13	1.47 ddd (13.6;13.6;4)	46.3		1.33	41.8		2.46 ddd (10.7;10.7;6.1)	49.8	C12, C14
14	1.75	37.1		1.18	53.5		2.11 t (10.7)	60.4	C8, C13, C23
15α	1.85 br d (13.0)	28.4	C16	1.87	30.0	C16	6.47 dd (10.7; 17.1)	146.1	C16, C17
15β	1.33			1.24					
16	4.72 ddd (12.0; 1.5; 1.5)	67.3		4.39 ddd (11.1; 2.2; 2.2)	72.2	C13, C14	6.36 d (17.1)	122.9	C14, C15, C17, C18, C25
17		166.7			163.7			164.4	
18	6.05 d (1.5)	118.6	C16, C19	5.05 d (3.1)	117.8		5.86 s	115.5	C16, C17, C19
19		170.5			173.1			174.1	
20	0.85 s	33.4		0.85 s	33.7		0.88 s	33.8	C3, C4, C5, C20
21	0.82 s	21.4		0.92 s	21.8		0.94 s	21.9	C3
22	0.84 s	14.6		0.94 s	16.8		0.79 s	16.4	
23	0.83 s	16.4		0.93 s	15.3		1.03 s	16.9	
24	6.01 d (4.0)	93.9	C13, C16	4.37 d (8.1)	101.7	C12, C16		183.5	
25	6.20 br s	102.7		6.15 br s	94.0		6.24 s	100.1	C16, C19
CH$_3$CO	2.11 s	21.1							
CH$_3$*CO*		170.5							

[a] Coupling costants 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.

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 Si gel flash chromatography (MPLC) eluting with $CHCl_3$−MeOH mixtures with increasing amounts of MeOH, followed by reversed-phase HPLC to yield petrosaspongiolides M−R (**1**−**5**) (yields: 58.3 mg, 2.7 mg, 47.8 mg, 2.5 mg, and 3.2 mg, respectively).

Petrosaspongiolide M (**1**), a white amorphous solid, showed a weak parent peak at *m*/*z* 460 (M^+) in the EIMS together with two intense fragment peaks at *m*/*z* 400 ($C_{25}H_{36}O_4$, $M^+ - CH_3COOH$) and *m*/*z* 191 ($C_{14}H_{23}^+$). The latter fragment, typically observed in terpenoids that possess a tricyclic carbon framework in their structure,[8] suggested that **1** is a terpene. ^{1}H and ^{13}C NMR spectra[9] in $CDCl_3$ also confirmed the terpenoid nature of **1**, with four methyl singlet signals (δ_H 0.82, 0.83, 0.84, and 0.85), a complex array of overlapping signals between δ_H 1.0 and 2.0, and an acetyl group (δ_H 2.11, 3H, s; δ_C 21.1 and 170.5). Inspection of COSY, HMQC, and HMBC spectra allowed us to obtain full assignments for **1** in $CDCl_3$ solution (Table 1). COSY correlations showed connectivities for the fragment C-9/C-18 (with H-16 and H-18 sharing a long-range coupling through the quaternary carbon C-17) and C-16/C-24 through C-15/C-14/C-13, indicating that a fourth cycle (ring D) was fused to the tricyclic terpenoid system. A key HMBC correlation between H-24 (δ_H 6.01) and the oxygen-bearing C-16 (δ_C 67.3), together with the lowfield chemical shift of C-24 (δ_C 93.9), completed ring D characterization. Thus, an *O*-acetyl-hemiacetal functionality was present at C-24 with one of the two hemiacetal oxygens connecting C-24 and C-16. The presence of γ-hydroxybutenolide moiety was indicated by the proton signals at δ_H 6.20 (1H, br s, H-25) and δ_H 6.05 (1H, d, H-18) and by the broad carbon resonances[9] at δ_C 102.7 (d, C-25), 118.6 (d, C-18), 166.7 (s, C-17), and 170.5 (s, C-19). The connection of the quaternary C-17 of the γ-hydroxybutenolide portion to ring D was supported by an intense HMBC cross peak between H-18 and C-16 and by the long-range coupling between H-18 and H-16. The *S** configuration at C-24 (24α acetoxy group) was assigned on the basis of the small coupling constant observed between the axial H-13 and H-24 (J = 4.0 Hz), implying that the latter is an equatorial proton. In the same way, the shape of H-16 (dt, J = 12.0, 1.5 Hz) indicated that it must be an axial proton. Finally, these relative stereochemical assignments (13*S**, 14*R**, 16*R**, 24*S**) were also corroborated by NOE effects observed between H-16 and H-14 and between H-13 and H-24, hence completing the structure elucidation of **1**. During the preparation of this paper we found that De Rosa et al. had isolated the 24-epimer of petrosaspongiolide M, named cavernosolide, from the Mediterranean sponge *Fasciospongia cavernosa*.[10]

Petrosaspongiolide N (**2**), had the composition $C_{29}H_{42}O_8$ (HREIMS). Its ^{1}H and ^{13}C NMR spectra ($CDCl_3$, 500 and 125 MHz) clearly showed that **2** is closely related to **1**, with an additional acetyl group (δ_H 2.01, 3H, s; δ_C 20.9 and 171.3) and one tertiary methyl proton signal replaced by an AB system at δ_H 3.92 and 4.20 (2H, AB system, J = 9.2 Hz, H_2-21). These data can be explained by **2** being an acetoxy-derivative of **1**, in agreement with the molecular weight difference of 58 mass units observed between **1** and **2**. The stereospecific location of

Table 2. Selected ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Chemical Shifts and HMBC Correlations of the Sestertepenes **2**, **3c**, and **4**

	2 ($CDCl_3$)	**4** (CD_{3OD})	**3c** ($CDCl_3$)		
position	δ_C	δ_C	δ_H[a]	δ_C	HMBC[b]
1	39.9	40.6	1.77−0.75	39.9	
2	18.1	20.8	1.29−1.54	18.5	
3[c]	**36.6**	**37.4**	1.10−1.41	42.0	
4	**37.0**	**37.6**		33.3	
5	**57.2**	**58.4**	0.80	56.6	
6	18.6	20.8	1.43−1.50	18.3	
7	40.7	42.0	0.80−1.65	40.2	
8	37.1	36.6		37.6	
9	59.6	61.1	0.81	59.6	
10	37.5	38.2		36.4	
11	19.7	61.1	1.37	19.5	
			2.09		
12	27.7	19.2	1.03	27.7	
			2.07		
13	46.3	41.0	1.47	46.2	
14	37.5	53.7	1.20	52.1	
15	28.4	29.6	1.33	28.9	C17
			1.75		
16	67.2	72.1	4.38	71.2	C17
17	166.7	166.1		165.7	
18	118.0	117.8	6.13 d (3.8)	118.4	C19, C25
19	172.1	173.0		169.6	
20	**27.3**	**27.6**	0.85 s	33.3	
21	**66.9**	**68.3**	0.80 s	21.4	
22	14.5	15.4	0.82 s	14.5	
23	16.9	17.3	0.79 s	16.4	
24	93.7	101.9	5.35 d (8.3)	93.4	C13, C16, CH_3CO
25	97.0	95.8	6.90 s	92.4	C19
CH_3CO	170.6			169.3	
CH_3CO	21.1		2.17	20.7	
CH_3CO	171.3	173.3		168.8	
CH_3CO	20.9	20.8	2.12	21.0	

[a] Coupling costants 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. [c] ^{13}C NMR chemical shifts in bold are referred to the positions that are directly affected by the presence of a 21-acetoxyl group.

the acetoxy group at C-21 (i.e., the β methyl carbon attached to C-4) was inferred by the replacement of the C-21 methyl signal at δ_C 21.4 with a CH_2O- carbon resonance at δ_C 66.9 and by the chemical shift differences observed in the ^{13}C NMR spectrum for C-3, C-4, C-5, and Me-20 (Table 2)—all pointing to an oxidation of the β geminal methyl group at C-4.[8,11] Further evidence for this stereospecific assignment comes from ROESY (τ_m = 400 ms) data that showed dipolar coupling between H_2-21 and the angular Me-22.

Petrosaspongiolide P (**3**), had the composition $C_{25}H_{38}O_5$ (HREIMS and negative ion FAB). Analysis of the ^{1}H and ^{13}C NMR spectra of **3** revealed the presence of at least two interconverting species. Unfortunately, in this case, variable temperature experiments did not help because, in contrast with what was observed for **1**,[9] no significant sharpening of proton NMR peaks could be observed even at 330 K. Acetylation of **3** in pyridine at room temperature yielded two monoacetylated (**3a** and **3b**) and four diacetylated (**3c**, **3d**, **3e**, and **3f**) derivatives (see Experimental Section). The diacetylated products displayed no resonance broadening in their NMR spectra at room temperature, and therefore the major compound **3c** was selected for spectral studies (Table 2). The behavior of **3** in solution could then be explained by assuming that, besides the typical hemiacetal functionality of the γ-hydroxybutenolide ring, an additional hemiacetal function was present in the molecule. NMR data also supported this view with two lowfield proton (δ_H 4.37 and 6.15 ppm) and carbon resonances (δ_C 101.7 and 94.0 ppm) in the spectrum of **3**. Analysis of both proton−proton and proton−carbon connectivities of **3** and **3c** through the aid of COSY, HMQC, and HMBC experiments (Table 1) revealed close structural similarities between **3** and **1**. The free hemiacetal function (the masked aldehyde) was thus located at C-13, as expected, and C-16 was an oxygenated methine (δ_C 72.2) connected to the usual γ-hydroxybutenolide unit. Interestingly, this system gave rise to an HMBC cross peak between H-16 (δ_H 4.39) and C-13 (δ_C 41.8), indicating the presence of an unusual $^4J_{CH}$ coupling.

These data are consistent with **3** being the 24-*O*-desacetyl derivative of **1**, as confirmed by acetylation of **1** and **3** in dry pyridine (room temperature) and subsequent comparative ^{1}H NMR analysis of the acetates (see Experimental Section). Concerning the stereochemistry of **3**, both 24-epimers were present in solution (as in the case of the 25-epimers in the butenolide portion of these molecules). NMR analysis of intact **3** indicated that the 24*S** epimer (24β hydroxy group) is the major form present at the equilibrium.[12] This latter stereochemistry was assigned on the basis of the large proton−proton coupling constant (J = 8.1 Hz) between the axial H-13 and H-24 (H-24 is axial) and was supported by an intense ROESY (τ_m = 400 ms) cross peak between H-24 and H-12α (δ_H 1.03 ppm). Similar arguments led to assignment of the stereochemistry of C-16 as 16*R**. In fact, the pattern of dipolar effects around rings C/D (i.e., ROESY cross peaks H-14/H-24, H-14/H-12α, H-24/H-12α, H-16/H-14) indicated the axial nature of H-16.

Petrosaspongiolide Q (**4**), had the composition $C_{27}H_{40}O_7$ (HREIMS and negative ion FAB). Both NMR and MS spectral data indicated that **4** is a 21-acetoxy-derivative of **3**. As already discussed for **2**, the stereospecific location of this additional acetoxy group at C-21 was evident from the ^{13}C NMR data of **4** by comparison with those observed for compound **3** (chemical shift values for C-3, C-4, C-5, Me-20, and C-21 are particularly diagnostic; Table 2).[11] As in **2**, this stereochemical assignment was confirmed by a ROESY peak between H_2-21 and the angular Me-22.

Petrosaspongiolide R (**5**) had the composition $C_{25}H_{36}O_5$ as determined by negative-ion mode FABMS *m*/*z* 415, ^{13}C- and DEPT-135 NMR measurement. The strong peak at *m*/*z* 415 [$(M - H)^-$] observed in the FABMS (negative ion mode), together with a quaternary carbon resonance at δ_C 183.5 in the ^{13}C NMR spectrum (CD_3OD, 125 MHz), indicates that **5** is a carboxylic acid. COSY, HMQC, and HMBC data (see Table 1) were consistent with the presence of the usual tricyclic carbon system and the γ-hydroxybutenolide ring. In particular, the α,β-unsaturated γ-lactone ring was further conjugated in **5** with a C-15/C-16 trans double bond [UV (MeOH) λ_{max} (log ϵ) 264 (4.15) and NMR δ_H 6.47, dd, J = 17.1 and 10.7 Hz; 6.36, d, J = 17.1 Hz for H-15 and H-16 and δ_C 146.1, 122.9 for C-15, C-16, respectively]. The location of the carboxylic functionality at C-13 was documented by the chemical shifts of H-13 at δ_H 2.46 and C-13 at δ_C 49.8. Finally, the stereochemistry at C-13 and C-14 (13*S**, 14*R**) was based on the large

Table 3. Effect of Compounds **1**−**5** on Secretory PLA_2[a]

	bee venom		*N. naja* venom	porcine pancreas	human synovial	
	% I (10 μM)	IC_{50} (μM)	% I (10 μM)	% I (10 μM)	% I (10 μM)	IC_{50}(μM)
1	71.0 ± 1.8[c]	0.6	11.5 ± 3.0	12.3 ± 6.0	68.6 ± 2.7[c]	1.6
2	43.9 ± 2.2[c]	N.D.[d]	6.8 ± 3.0	11.6 ± 1.8	44.0 ± 2.7[c]	N.D.
3	37.9 ± 3.2[c]	N.D.	3.0 ± 1.3	0.0 ± 0.0	60.9 ± 4.4[c]	3.8
3a	34.8 ± 2.5[b]	N.D.	5.6 ± 2.8	0.0 ± 0.0	59.0 ± 2.4[c]	3.7
3c	10.6 ± 3.5	N.D.	6.9 ± 3.2	0.0 ± 0.0	32.1 ± 2.4[b]	N.D.
4	12.5 ± 2.1	N.D.	4.2 ± 2.7	0.0 ± 0.0	30.1 ± 3.7[b]	N.D.
5	18.8 ± 5.6	N.D.	1.0 ± 0.8	0.8 ± 0.8	7.1 ± 3.2	N.D.

[a] Mean ± S.E.M. (n = 6). [b] $p < 0.05$. [c] $p < 0.01$. [d] N.D. = not determined for those compounds that did not reach 50% inhibition at 10 μM. Manoalide showed IC_{50} values of 7.5 and 3.9 μM for bee venom and human synovial PLA_2, respectively.

proton−proton coupling constant between H-13 and H-14 (J = 10.7 Hz).

A thorough literature seach revealed that pharmacological data relative to compounds structurally related to manoalide, luffolide, and their analogues are often not easily comparable, due to the high variability of experimental conditions used in different laboratories for measuring inhibition of PLA_2 enzymes.[13] In our case, the IC_{50} values obtained for compounds **1**−**5** can be compared with those that we measured for manolide (**6**) under the same experimental conditions.

As already pointed out, extensive studies led to the identification of a pharmacophore model that relates chemical functionalities of these compounds with their antiinflammatory properties.[6,7] Our biological data are in agreement with what has been observed for similar compounds, both active[2,4,5] and inactive[14] and bring some new information to the current pharmacophoric model. For example, the importance of the tricyclic system (corresponding to the linear portion in manoalide) that allows a large hydrophobic area of contact in the enzyme−inhibitor recognition process[7] is confirmed by the significantly lower activity of our 21-acetoxy derivatives **2** and **4**. This result would suggest that substitution in this position leads to a fall of bioactivity, presumably because of the increased steric demand or the greater polarity of such compounds or both. The aldehyde functionality at C-24, either free or masked (hemiacetal), is also known to be a key structural feature for the bioactivity. This is indicated not only by the high activity of luffolide (**7**) possessing a free aldehyde at C-24, and correspondingly of petrosaspongiolide M (**1**), but also by the significant fall of activity of the acid petrosaspongiolide R (**5**) and the spongionolides A, B, and C,[14] which possess an alcoholic function at C-24. Such a pharmacophore model, though, does not explain the high activity of cacospongionolide,[5] a molecule characterized by an ethereal functionality replacing the usual hemiacetal. In addition, the higher degree of selectivity displayed by compounds **3** and **3a** toward human synovial PLA_2 (they inhibit human synovial PLA_2 to a greater extent than they do bee venom PLA_2) is also difficult to explain in terms of this model.

The low (but still measurable) activity of petrosaspongiolide R (**5**) could be interpreted on the basis of the known fact that the γ -hydroxybutenolide moiety alone (which, interestingly is also a masked aldehyde) does not allow a covalent ligand−enzyme interaction.[6] In addition, the blockade of the γ-hydroxyl group in the γ-lactone ring results in a lower activity (see, for example, compound **3c**), confirming again the participation of this portion in the inhibition of PLA_2.

Experimental Section

General Experimental Procedures. NMR spectra: Bruker AMX-500 (^{1}H at 500 MHz, ^{13}C at 125 MHz), δ (ppm), J in Hz, spectra referred to $CDCl_3$ and CD_3OD as internal standard; EIMS and FABMS [in glycerol or glycerol−thioglycerol (3:1) matrix; Cs^+ ions bombardment] were obtained on VG AUTOSPEC mass spectrometer; optical rotations were measured on a Perkin−Elmer 141 polarimeter; reversed-phase HPLC, C_{18} μ-Bondapak column (300 × 7.8 mm i.d.; flow rate 5 mL min^{-1}) Waters model 6000 A or 512 pump equipped with U6K injector and a differential refractometer, model 401.

Biological Material. *Petrosaspongia nigra* (order Dictyoceratida, family 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 (ref 321) at the ORSTOM Center of Nouméa. A specimen of *Petrosaspongia nigra* is kept at the Queensland Museum in Brisbane (QMG 304685).

Isolation. The organism was freeze-dried and the lyophilized material (1.0 kg) was extracted with *n*-hexane and CH_2Cl_2 in a Soxhlet apparatus, and then with MeOH (3 × 2 L) at room temperature. The CH_2Cl_2 extracts were filtered and concentrated under reduced pressure to give 30.0 g of a brown oil. The crude CH_2Cl_2 extract was chromatographated by MPLC on a Si gel column (150 × 2 g) using a solvent gradient from $CHCl_3$ to $CHCl_3$−MeOH 9:1. MPLC fractions were further purified by HPLC on a semipreparative (7.8 × 300 mm) μ-bondapak-C18 column (flow rate 5 mL/min) eluting with MeOH−H_2O mixtures to afford pure compounds **1**−**5**. The purity of each compound was judged to be > 90% by HPLC and ^{1}H NMR.

Petrosaspongiolide M (1): white amorphous solid, $[\alpha]_D$ −28.8° (c 0.02, $CHCl_3$); UV (CH_3OH) λ_{max} (log ϵ) 224 (3,58); IR ($CHCl_3$) ν_{max} 3330 (br), 1785, 1750, 1740, 1230 cm^{-1}; t_R 14.3 min, eluting with MeOH−H_2O 85:15; ^{1}H and ^{13}C NMR, see text and Table 1; EIMS m/z 460 (M^+, $C_{27}H_{40}O_6$), 400 (M^+ − CH_3COOH, $C_{25}H_{36}O_4$), m/z 191 ($C_{14}H_{23}^+$); HREIMS m/z 400.2623 (M^+ − CH_3COOH; calcd for $C_{25}H_{36}O_4$, 400.2613).

Petrosaspongiolide N (2): white amorphous solid, $[\alpha]_D$ −23.0° (c 0.001, $CHCl_3$); t_R 5.7 min, eluting with MeOH−H_2O 85:15; ^{1}H and ^{13}C NMR, see text and Table 2; HREIMS m/z 518.2863 (M^+, calcd for $C_{29}H_{42}O_8$, 518.2879).

Petrosaspongiolide P (3): white amorphous solid, $[\alpha]_D$ +13.8° (c 0.001, CH_3OH); t_R 14.8 min, eluting with

MeOH−H_2O 80:20; ^1H and ^{13}C NMR, see text and Table 1; HREIMS *m/z* 418.2734 (M^+; calcd for $C_{25}H_{38}O_5$, 418.2719).

Petrosaspongiolide Q (4): white amorphous solid, $[\alpha]_D$ +5.8° (*c* 0.001, CH_3OH); t_R 14.0 min, eluting with MeOH−H_2O 70:30 (analytical column); ^1H and ^{13}C NMR, see text and Table 2; HREIMS *m/z* 476.2787 (M^+, calcd for $C_{27}H_{40}O_7$, 476.2774).

Petrosaspongiolide R (5): white amorphous solid, $[\alpha]_D$ −15.6° (*c* 0.003, CH_3OH); UV (MeOH) λ_{max} (log ϵ) 264 (4.15); t_R 3.8 min, eluting with MeOH−H_2O 85:15; ^1H and ^{13}C NMR, see text and Table 1.

Acetylation of Petrosaspongiolide M (1). A solution of petrosaspongiolide M (**1**, 6 mg) was kept in dry pyridine (0.5 mL) and Ac_2O (0.1 mL) with stirring at room temperature for 12 h. After quenching with CH_3OH, the excess reagents were removed under reduced pressure to obtain a crude oil containing two products, **1a** and **1b**, which were separated by HPLC on diol Si gel column (Phenomenex Spherex 5 diol), petroleum ether−ethylic ether 80:20 as eluent, to obtain acetates **1a** (3.9 mg) and **1b** (2.4 mg).

Acetate 1a: ^1H NMR ($CDCl_3$) δ 6.93 (1H, s, H-25), 6.12 (1H, br s, H-18), 6.07 (1H, d, J = 4.0, H-24), 4.59 (1H, br d, H-16), 2.11 (3H, s, $COCH_3$), 2.18 (3H, s, $COCH_3$), 0.87 (3H, s, Me-23), 0.86 (3H, s, Me-20), 0.86 (3H, s, Me-22), 0.84 (3H, s, Me-21).

Acetate 1b: ^1H NMR ($CDCl_3$) δ 6.98 (1H, s, H-25), 6.05 (1H, br s, H-18), 5.93 (1H, d, J = 4.0, H-24), 4.61 (1H, br dd, H-16), 2.12 (3H, s, $COCH_3$), 2.10 (3H, s, $COCH_3$), 0.86 (3H, s, Me-23), 0.86 (3H, s, Me-22), 0.82 (3H, s, Me-20), 0.80 (3H, s, Me-21).

Acetylation of Petrosaspongiolide P (3). A solution of petrosaspongiolide P (**3**, 23.0 mg) was kept in dry pyridine (1 mL) and Ac_2O (0.4 mL) with stirring at room temperature for 12 h. After quenching with CH_3OH, the excess reagents were removed under reduced pressure to obtain a crude oil containing two monoacetates, **3a** and **3b**, and four diasteromeric diacetates, **3c**, **3d**, **3e** and **3f**, which were separated by HPLC on diol Si gel column (Phenomenex Spherex 5 diol), petroleum ether−ethylic ether 80:20 as eluent, to obtain acetates **3a** (1.5 mg), **3b** (0.5 mg), **3c** (12.0 mg), **3d** (6.1 mg), **3e** (1.9 mg), and **3f** (0.3 mg). The monoacetates **3a** and **3b** (24 epimers) possess 24*R** and 24*S** configurations, respectively. These compounds still have a hemiacetal function at C-25, so the configuration at this carbon cannot be assigned. As for the diacetates, each pair of compounds (**3c**/**3d** and **3e**/**3f**) contains derivatives with configurations that are homogeneous at C-24 (24*R** and 24*S* *, respectively) and epimeric at C-25.

Acetate 3a: ^1H NMR ($CDCl_3$) δ 6.11 (1H, br s, H-25), 6.02 (1H, br s, H-18), 5.32 (1H, d, J = 8.6, H-24), 4.45 (1H, br d, H-16), 2.13 (3H, s, $COCH_3$), 0.85 (3H, s, Me-21), 0.85 (3H, s, Me-23), 0.83 (3H, s, Me-22), 0.80 (3H, s, Me-20).

Acetate 3b: ^1H NMR ($CDCl_3$) δ 6.20 (1H, br s, H-25), 6.06 (1H, d, J = 1.5, H-18), 6.01 (1H, d, J = 4.0, H-24), 4,73 (1H, ddd, J = 1.5, 1.5, 12.5, H-16), 2.10 (3H, s, $COCH_3$), 0.85 (3H, s, Me-20), 0.84 (3H, s, Me-22), 0.83 (3H, s, Me-23), 0.82 (3H, s, Me-21).

Acetate 3d: ^1H NMR ($CDCl_3$) δ 7.02 (1H, s, H-25), 6.02 (1H, br s, H-18), 5.22 (1H, d, J = 8.7, H-24), 4.35 (1H, dd, J = 11.4, 1.5, H-16), 2.13 (3H, s, $COCH_3$), 2.10 (3H, s, $COCH_3$), 0.86 (3H, s, Me-23), 0.86 (3H, s, Me-20), 0.83 (3H, s, Me-22), 0.80 (3H, s, Me-21).

Acetate 3e: For ^1H NMR data ($CDCl_3$), see acetate **1a**.

Acetate 3f: For ^1H NMR data ($CDCl_3$), see acetate **1b**.

Pharmacological Assays. For pharmacolocical assays see R. Cholbi et al.[15]

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