“Simplakidine A, An Unique Pyridinium Alkaloid From the Caribbean Sponge *Plakortis simplex*” by C. Campagnuolo, C. Fattorusso, E. Fattorusso, A. Ianaro, B. Pisano, and O. Tagliatela-Scafati.

Supporting Information

Isolation procedure. A specimen of *Plakortis simplex* was collected in Summer 1999 along the coasts of Berry Island (Bahamas), and identified by Prof. M. Pansini (Università di Genova). A voucher specimen has been deposited with the ref. number 2006. The organism was immediately frozen after collection and kept frozen until extraction when the sponge (57 g, dry weight after extraction) was homogenized and extracted with methanol (4 × 500 mL) and with chloroform (4 × 500 mL). The methanol extract was initially partitioned between H₂O and *n*-BuOH and then the organic phases were combined and concentrated *in vacuo* affording 29.3 g of a brown-colored viscous oil. This was subjected to chromatography on a column packed with RP18 silica gel and eluted with H₂O/MeOH 9:1 (A₁), H₂O/MeOH 7:3 (A₂), H₂O/MeOH 4:6 (A₃), H₂O/MeOH 2:8 (A₄), H₂O/MeOH 1:9 (A₅), MeOH/CHCl₃ 9:1 (B), CHCl₃ (C). Fraction A₃ (442 mg) was further chromatographed by MPLC (SiO₂ 230-400 mesh; solvent gradient system of increasing polarity from EtOAc to MeOH). Fractions eluted with EtOAc/MeOH 3:7 were re-chromatographed by HPLC (eluent MeOH/H₂O 1:1) affording pure simplakidine A (**1**, 1.4 mg).

Simplakidine A (1)-Physical Data. Colorless amorphous solid. $[\alpha]_D^{25} - 21.7^\circ$ (c = 1.2 mg/mL in MeOH); IR (KBr): $\nu_{\max}$ 3540, 1710, 1625, 1068, 915 cm⁻¹; UV (MeOH): $\lambda_{\max}$ 272 (log ϵ 3.55); FAB-MS (negative ions): m/z 434 (M-H)⁻. Nanospray-MS: m/z 436 (M+H)⁺, 481 (M+2Na-H)⁺. HR-Nanospray-MS analysis: m/z 436.2687 (M+H)⁺, calcd. for C₂₄H₃₈NO₆ m/z 436.2699; m/z 481.2630 (M+2Na-H)⁺, calcd. for C₂₄H₃₆NO₆Na₂ m/z 481.2625. ¹H and ¹³C NMR (500 and 65 MHz, respectively): Table 1.

Preparation of the (R)- and (S)-MTPA esters of 1. Simplakidine A (**1**, 0.4 mg) was dissolved in anhydrous pyridine (50 μ L), treated with 7 μ L of (*R*)-MTPA chloride and then maintained at room temperature overnight under stirring. The obtained mixture, after evaporation of the solvent, showed to contain the (*S*)-MTPA ester of **1**. The use of (*S*)-MTPA chloride in the same procedure led to the (*R*)-MTPA ester of **1**.

(S)-MTPA ester. Selected ¹H NMR chemical shifts (CD₃OD): δ 5.75 (H-3, m); 2.53 (H-2a, dd, J = 14.8, 3.0 Hz); 2.38 (H-2b, dd, J = 14.8, 10.0); 1.78 (H-5a, dd, J = 14.7, 7.1 Hz); 1.47 (H-4, m); 1.35 (H₂-13, m); 1.32 (H-5b, overlapped); 0.97 (H₃-17, t, J = 7.3 Hz).

(R)-MTPA ester. Selected ¹H NMR chemical shifts (CD₃OD): δ 5.75 (H-3, m); 2.47 (H-2a, dd, J = 14.8, 3.0 Hz); 2.31 (H-2b, dd, J = 14.8, 10.0); 1.84 (H-5a, dd, J = 14.7, 7.1 Hz); 1.55 (H-4, m); 1.39 (H-5b, overlapped); 1.36 (H₂-13, m); 0.97 (H₃-17, t, J = 7.3 Hz).

Selected differences $\Delta\delta_{(S-R \text{ MTPA esters})}$: H-2a (+ 0.06 ppm); H-2b (+ 0.07 ppm); H-5a (- 0.06 ppm); H-5b (- 0.07 ppm); H-4 (- 0.08 ppm); H₂-13 (- 0.01 ppm).

Molecular mechanics. All the calculations were performed on a Indigo2 Silicon Graphics workstation using the InsightII (Accelrys, San Diego) molecular modeling software package. The two possible diastereomers (3*R*, 4*R*, 6*R*, 8*R*, 9*R*, 10 *S* and 3*R*, 4*R*, 6*R*, 8*R*, 9*R*, 10 *R*) of simplakidine A (**1**) were built (Builder module) assigning atomic charges and potentials derived from the esff force field. The structures were then subjected to a preliminary energy minimization procedure ($\epsilon = 32.70$ *r; BFGS minimization algorithm; maximum RMS derivative less than 0.001 Kcal/mol) to obtain the conformers to be used as starting points for the subsequent conformational analysis. The torsional angles H-9/C-9/C-10/H-10 and H-10/C-10/C-4'/C-5' have been systematically rotated with an angle increment of 10° within a 60-120° range for the former and a 0-359° range for the latter. The obtained conformations were used as starting points for geometrical optimization using the same energy parameters above described. The violations from experimentally observed dipolar couplings (a cut-off value of 3.5 Å has been used as the maximum consistent distance for H-5'/H-9 H-5'/H-11, and H-5'/H-17) and $^3J_{\text{H-9/H-10}}$ coupling constant were checked on all the resulting conformers.

Figure 3. A 3*R*, 4*R*, 6*R*, 8*R*, 9*R*, 10 *S* conformer of simplakidine A (**1**). Atoms are coloured by element type (H = grey; C = green; N = blue; O = red). Hydrogens, except those involved in NOEs, are omitted for clarity.

Summary of 1D and 2D NMR Data of Simplakidine A (spectra recorded in CD₃OD)

Pos.	δH , mult., J in Hz	δC , mult.	COSY (H→H)	g-HMBC (H→C)	ROESY (H→H)
1		181.6, C			
2a	2.41, dd, 14.8, 3.0	42.5, CH ₂	2b, 3	1	2b, 3
2b	2.27, dd, 14.8, 10.2		2a, 3	1	
3	4.39, ddd, 10.2, 3.0, 1.5	72.1, CH	2a, 2b, 4	1	2a, 2b, 4
4	1.50, overlapped	45.1, CH	3, 5a, 5b, 16a, 16b		3, 5a, 5b
5a	1.85, dd, 14.1, 7.4	43.1, CH ₂	5b, 4	3, 6, 15	4, 8, 16a
5b	1.49, overlapped		5a, 4	3, 6, 15	4
6		83.9, C			
7a	2.00, overlapped	46.9, CH ₂	7b, 8	6, 15	8, 10
7b	1.56, overlapped		7a, 8	6, 15	
8	2.14, overlapped	42.7, C	7a, 7b, 9, 13a, 13b	14	5a, 7a
9	3.72, dd, 8.1, 1.3	86.0, CH	8, 10	6	13, 15, 5'
10	3.95, ddd, 10.6, 3.9, 1.3	46.4, CH	9, 11a, 11b	12, 5'	7a
11a	2.12, overlapped	21.6, CH ₂	10, 11b, 12	9, 10, 12	12, 5'
11b	1.99, overlapped		10, 11a, 12	9, 10, 12	12, 5'
12	0.80, t, 7.3	12.6, CH ₃	11a, 11b	10, 11	
13a	1.57, overlapped	24.0, CH ₂	8, 13b, 14	8, 14	9, 15
13b	1.32, overlapped		8, 13a, 14	8, 14	9, 15
14	0.97, t, 7.3	12.7, CH ₃	13a, 13b	8, 13	13a, 13b
15	1.11, s	26.3, CH ₃		5, 6, 7	9, 13a, 13b
16a	1.69, m	27.2, CH ₂	4, 16b, 17	4, 17	5a
16b	1.31, overlapped		4, 16a, 17	4, 17	
17	1.00, t, 7.3	13.1, CH ₃	16a, 16b	5, 16	
2'	8.80, s	144.6, CH		3', 7'	7'
3'		142.7, C			
4'		163.0, C			
5'	8.20, d, 6.2	129.6, CH	6'	10, 3', 6'	9, 11, 6'
6'	8.68, d, 6.2	144.4, CH	5'	5', 7'	5', 7'
7'	4.34, s	48.0, CH ₃		2', 6'	2', 6'
8'		169.3, C			

 Comparison of selected NMR data of simplakidine A (**1**) with those of plakortether B (**2**) (in CD₃OD)

Pos.	1		2	
	δH , mult., J in Hz	δC , mult.	δH , mult., J in Hz	δC , mult.
2a	2.41, dd, 14.8, 3.0	42.5, CH ₂	2.47, dd, 12.9, 2.8	38.4, CH ₂
2b	2.27, dd, 14.8, 10.2		2.15, dd, 12.9, 9.3	
3	4.39, ddd, 10.2, 3.0, 1.5	72.1, CH	4.27, ddd, 9.3, 2.8, 1.5	71.6, CH
4	1.50, overlapped	45.1, CH	1.52, overlapped	43.3, CH
5a	1.85, dd, 14.1, 7.4	43.1, CH ₂	1.78, dd, 14.1, 8.2	42.8, CH ₂
5b	1.49, overlapped		1.46, dd, 14.1, 2.8	
6		83.9, C		82.9, C
15		26.3, CH ₃		26.3, CH ₃

16a	1.69, m	27.2, CH ₂	1.64, m	26.8, CH ₂
16b	1.31, overlapped		1.33, overlapped	
17	1.00, t, 7.3	13.1, CH ₃	0.97, t, 7.3	12.8, CH ₃

¹H NMR spectrum of Simplakidine A in CD₃OD

^{13}C NMR spectrum of Simplakidin A in CD_3OD

HMQC spectrum of simplakidine A