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Further Studies of Alkaloids from *Reniera sarai*: Structures of Saraine-3 and Isosaraine-3; Absolute Stereochemistry of Saraine-1 and Saraine-2

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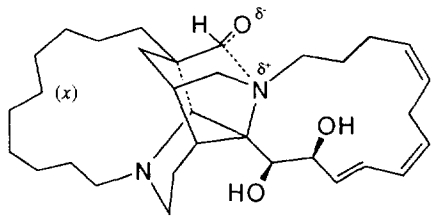
Abstract: A new macrocyclic diamine alkaloid, named isosaraine-3 (**9**), has been isolated from the Mediterranean sponge *Reniera sarai*. The structures of both this new compound and saraine-3 (**6**), until now only partially clarified, have been completely elucidated by extensive spectroscopic studies. The absolute stereochemistry of the quinolizidone systems present in the co-occurring major alkaloids, saraine-1 (**4**) and saraine-2 (**5**), has been determined by applying advanced Mosher's method to the alcohol derivatives (**10-13**).

Macrocyclic diamine alkaloids represent an emerging group of natural products from marine organisms. Reports of these alkaloids from marine sponges have become more numerous over recent years.[2-39] Up to now, more than ten classes of polycyclic alkaloids belonging to this group have been discovered. They are saraines,[2-7] haliclamines,[8] xestospongins,[9-11] petrosins,[12-15] papuamines,[16,17] manzamines,[18-27] cyclostellettamines,[28] mandangamine,[29] xestocyclamines,[30,31] ircinols,[32-34] halicyclamine-A,[35] ingenamines,[36-38] halitoxins.[39] A common feature among them is that all of them, in spite of exhibiting formally quite different frameworks, could biogenetically derive from *bis*-3-alkylpyridine or reduced *bis*-3-alkylpyridine units as suggested first for the *Reniera sarai* alkaloids[2] and then by many reports.[7,15,25,30,33,35,37,38,40]

Saraines are characteristic secondary metabolites of the Mediterranean sponge *R. sarai* and possess a series of promising bioactivities.[41] Until now, nine macrocyclic alkaloids, named saraines A-C (**1-3**), saraines 1-3 (**4-6**), and isosaraines 1-3 (**7-9**), have been isolated from *R. sarai*. Distinct skeletons are exhibited by saraines A-C and saraines 1-3 whereas some minor co-occurring alkaloids, isosaraines 1-3, are characterized by the same framework of saraines 1-3 but displaying inverted relative stereochemistry at the chiral centers C-1, C-2, and C-9. In our previous work, we have reported the structural studies on saraines 1-2,[2] saraine-A,[3,4] isosaraine-1,[5] -2.[6] More recently, the structures of saraine-B (**2**), -C (**3**) and the absolute stereochemistry of saraines A-C have also been determined.[7] In the present paper we report the full structural characterization of saraine-3 (**6**) and isosaraine-3 (**9**) and the absolute stereochemistry of the quinolizidone system present in saraine-1 (**4**) and saraine-2 (**5**). This work further supports the previously reported structures of saraines 1-2 and isosaraines 1-2 which were characterized mainly by extensive spectroscopic studies.



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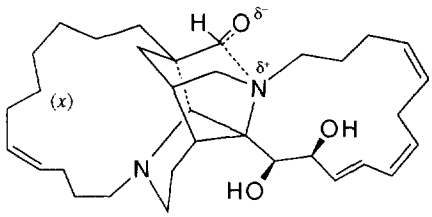

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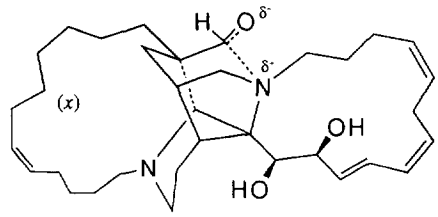

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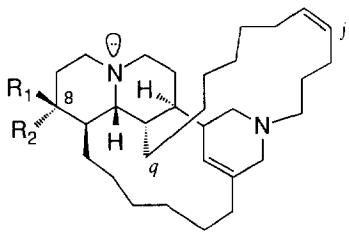
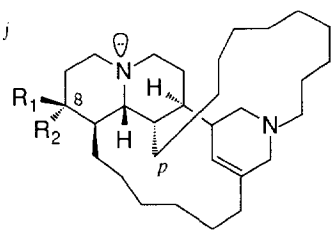
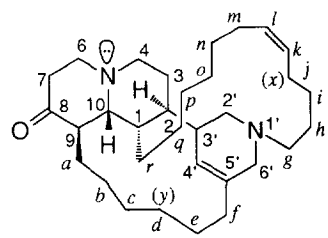


4: R_1=R_2=O
10: R_1=H, R_2=OH
10S: R_1=H, R_2=(*S*)-OMTPA
10R: R_1=H, R_2=(*R*)-OMTPA
11: R_1=OH, R_2=H
11S: R_1=(*S*)-OMTPA, R_2=H
11R: R_1=(*R*)-OMTPA, R_2=H



5: R_1=R_2=O
12: R_1=H, R_2=OH
12S: R_1=H, R_2=(*S*)-OMTPA
12R: R_1=H, R_2=(*R*)-OMTPA
13: R_1=OH, R_2=H
13S: R_1=(*S*)-OMTPA, R_2=H
13R: R_1=(*R*)-OMTPA, R_2=H



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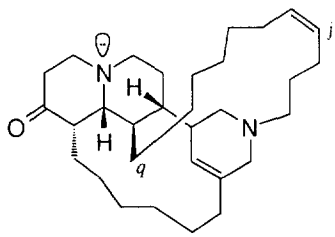

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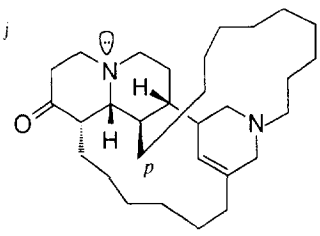

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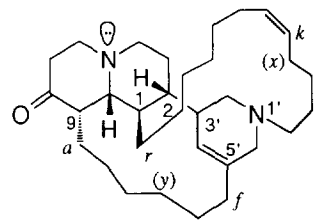


9

The usual work-up[2,3] of the *n*-butanol-soluble fraction from the defatted acetone extract of *R. sarai* yielded, together with the previously described alkaloids,[2-6] a minor component, named isosaraine-3 (**9**), slightly more polar than saraine-3 (**6**) on TLC (SiO_2; $CHCl_3$/MeOH, 9:1; **6**, *Rf* 0.53; **9**, *Rf* 0.50).

Table 1. Comparison of ^{13}C-NMR Data[a] of Saraines 1-3 (**4**-**6**) and Isosaraines 1-3 (**7**-**9**)

C	**4**[2] mult.	**5**[2] mult.	**6**[*] mult.	**7**[5] mult.	**8**[6] mult.	**9** mult.
1	36.3 d	37.5 d	36.8 d	34.6 d	34.1 d	34.1 d
2	37.2 d	37.1 d	38.9 d	41.8 d	38.5 d	35.9 d
3	24.5 t	24.7 t	26.2 t	31.8 t	31.6 t	30.6 t
4	46.3 t	46.1 t	46.0 t	53.5 t	53.9 t	53.7 t
6	54.6 t	55.8 t	53.8 t	55.9 t	55.9 t	55.8 t
7	38.7 t	39.6 t	37.8 t	38.0 t	38.2 t	38.4 t
8	210.8 s	210.8 s	211.2 s	212.5 s	212.5 s	212.2 s
9	52.9 d	52.5 d	53.6 d	53.9 d	52.9 d	52.6 d
10	68.8 d	70.2 d	66.7 d	68.7 d	67.3 d	67.1 d
2'	54.8 t	55.6 t	53.6 t	55.7 t	55.1 t	51.3 t
3'	40.4 d	40.6 d	40.9 d	42.4 d	42.5 d	40.5 d
4'	121.8 d	121.7 d	121.6 d	119.5 d	119.6 d	121.3 d
5'	135.9 s	136.2 s	135.6 s	138.2 s	137.9 s	134.6 s
6'	55.0 t	56.5 t	54.3 t	54.9 t	54.2 t	53.5 t
a	21.2 t	21.4 t	22.0 t	28.5 t	28.4 t	28.3 t
b	26.3[b] t	26.9[c] t	26.3[d] t	27.5 t	27.8 t	27.7[f] t
c	24.3[b] t	26.6[c] t	24.3[d] t	27.2 t	28.1 t	28.5[f] t
d	27.3[b] t	26.6[c] t	27.3[d] t	25.9 t	26.5 t	26.8[f] t
e	26.3[b] t	26.3[c] t	24.2[d] t	26.8 t	27.3 t	27.2[f] t
f	31.6 t	31.5 t	32.9 t	33.6 t	33.4 t	32.9 t
g	56.6 t	57.3 t	56.8 t	58.0 t	57.1 t	56.5 t
h	26.5 t	26.2[c] t	22.8 t	28.3 t	23.6 t	21.6 t
i	24.3 t	25.6[c] t	26.6 t	25.9 t	27.2[e] t	26.3[f] t
j	129.3 d	25.2[c] t	26.4 t	129.3 d	26.7[e] t	26.4 t
k	130.3 d	23.9[c] t	129.6 d	130.8 d	26.4[e] t	129.4 d
l	24.8 t	23.6[c] t	131.1 d	24.9 t	26.0[e] t	131.7 d
m	27.1[b] t	23.6[c] t	27.1 t	27.5 t	25.4[e] t	26.4 t
n	29.2[b] t	27.1[c] t	29.2[d] t	27.2 t	25.3[e] t	29.3[f] t
o	28.4[b] t	29.1[c] t	28.4[d] t	29.2 t	22.8 t	27.2[f] t
p	28.3[b] t	37.3 t	26.3[d] t	26.4 t	29.8 t	27.2[f] t
q	36.8 t		28.3[d] t	32.8 t		26.8[f] t
r			37.4 t			37.5 t

[a] Bruker AMX 500 MHz; δ values are reported in ppm referenced to $CHCl_3$ (δ 77.0); The multiplicity was determined by DEPT technique.

[b-f] Values with same superscripts in same column are tentatively assigned and may be interchangeable.

[*] The present data are slightly different from those reported in ref. 1 due to the different sample.

The previous[2] partial characterization of saraine-3 (**6**) led to a structure, closely related to those of saraine-1 (**4**) and saraine-2 (**5**), with a pentacyclic skeleton containing a quinolizidone system linked to a piperidine ring both directly (linkage between C-2 and C-3') and through two linear alkyl chains (from C-9 to C-5' and from C-1 to N-1'). Saraine-3 (**6**) is a superior homologue of saraine-1 (**5**) containing an additional methylene in the alkyl chain (*x*). The location in the longer alkyl chain (*x*) of a double bond was undetermined as well as the relative length of the two alkyl chains. In the present study, saraine-3 has been again isolated and reanalyzed. The ^{1}H- and ^{13}C-NMR data (**Table 1, 2**) were similar but not identical to those previously reported. This is a general peculiarity of saraines, probably due to the easy adoption of different conformations. However, the relative stereochemistry around the quinolizidone system was confirmed. The proton H-9 was assigned as axial on the basis of the large coupling (J=10.5 Hz) with the adjacent axial proton at C-10 while the equatorial orientation of H-1 was clarified by its small coupling with H-10 (J=1.9 Hz). The proton H-2 was also equatorially oriented on the basis of the high field ^{13}C-NMR resonance of C-4 (δ 46.0) due to the presence

of a 2-axial substituent; finally, the shape of the protons at C-2' (H-2'$_{eq}$:δ 2.87, dd, J=10.5, 5.5 Hz; H-2'$_{ax}$:δ 2.35, dd, J=10.5, 10.5 Hz) substantially supported an axial orientation of H-3'. In addition, other 2D experiments, in particular ^{1}H-^{13}C long-range HETCOR and TOCSY, were highly diagnostic to support all assignments of the heterocyclic systems and also to correctly locate the double bond in the alkyl chain (*x*). All the ^{1}H-^{13}C heterocorrelations are reported in **Table 2**.

Table 2. Comparison of ^{1}H-NMR Data[a] of Saraine-3 (**6**) and Isosaraine-3 (**9**)

H	**6** (δ [b,c])		Long-range ^{1}H-^{13}C correlations	**9** (δ [b])		Long-range ^{1}H-^{13}C correlations
1	1.51		C2, C3', C*r*	1.82		C10
2	1.63		C3, C2', C4', C*r*	1.62		C1
3	1.92	1.66	C2, C4	1.77	1.66	
4	3.02	2.88	C3	2.83	1.95	C2, C10
6	3.16	2.87	C7, C8, C10	3.03	2.22	C10
7	2.69	2.20	C8, C9	2.75	2.21	C6
8	-			-		
9	2.17		C1, C8, C10, C*a*	2.31		
10	2.33		C1, C2, C4, C6, C*a*, C*r*	2.05		
2'	2.87	2.35	C3', C4', C6'	3.09	2.68	
3'	2.65		C2'	2.86		
4'	5.46		C*f*	5.69		C2, C2', C5', C6'
5'	-			-		
6'	3.06	3.02	C2', C4', C5'	3.32	3.17	C5', C*g*
a	1.64	1.21	C8, C9, C10	1.92	1.32	
*b**	1.52			1.63	1.15	
*c**	1.19			^		
*d**	1.14			1.34		
*e**	1.57	1.41	C5'	1.63	1.17	
f	2.22	1.98	C4', C5'	2.13	1.92	C5', C6'
g	2.79	2.57	C2'	3.11	2.72	C2'
h	1.55	1.48	C*g*, C*j*	1.70	1.53	C*g*, C*j*
i	1.44		C*g*, C*k*	1.34		C*g*, C*k*
j	2.12		C*k*	2.21	2.10	C*k*
k	5.28		C*l*	5.27		C*l*
l	5.40		C*k*	5.34		C*k*
m	2.08		C*k*, C*l*	2.14	2.06	C*k*, C*l*
n	1.38	1.36		1.44	1.26	
*o**	1.22	1.18		^		
*p**	1.52			^		
*q**	1.16	1.44		^		
r	1.44	1.25	C1, C10	1.53	1.25	

[a] Bruker AMX 500 MHz; δ values are reported in ppm referenced to $CHCl_3$ (δ 7.26).
[b] The assignments were aided by ^{1}H-^{1}H COSY, TOCSY, and ^{1}H-^{13}C-HETCOR.
[c] The present data are somewhat different from those reported in ref. 1 due to the different sample.
* The ^{1}H-NMR resonances at these positions were tentatively assigned.
^ The ^{1}H-NMR resonances at these positions were indistinguishable.

Moreover, the cross peaks observed for H-10 (with C-4, C-6, C-*a* and C-2) and C-8 (with H_2-6, H_2-9, H_2-*a*) supported the assignments of the atoms of both the quinolizidone ring and the substituents at C-9 and C-1. Analogously, the ^{1}H-^{13}C long-range cross peaks of C-4' with the protons at C-2', C-6' and C-*f* confirmed the piperidine assignments with the substitution at C-5'. The linkage (C-2, C-3') between the two heterocyclic systems was, finally, confirmed by the correlations of both C-2' and C-4' with H-2. The position of the additional double bond in the longer alkyl chain (*x*) spanning from C-1 to N-1' was unambiguously determined

at Δ^k by analysing the TOCSY, HETCOR-TOCSY and ^1H-^{13}C long-range HETCOR spectra of **6**. In fact, long-range HETCOR experiments connected C-*g* (δ 56.8) to H_2-*h* (δ 1.55, 1.48) and H_2-*i* (δ 1.44); C-*k* (δ 129.6) to H_2-*j* (δ 2.12) and H_2-*i*. TOCSY experiment connected H_2-*g* (δ 2.79, 2.57) to H_2-*h* and H_2-*i* and also to H_2-*j* according to structure **6**. The chemical shifts of the vinyl carbons C-*j* (δ 26.4) and C-*m* (δ 27.1) were consistent with the *Z* configuration for the Δ^k alkene.[42] Like saraine-1 and -2,[2] the relative length of the two alkyl chains (*x* and *y*) was tentatively suggested by the common strong fragment at *m/z* 327 ($M^+ - C_{11}H_{21}$) in the EIMS spectrum and by biogenetic analogy with saraines A-C (**1-3**)[3,4,7] that contain in the alkyl chain (*x*) 10, 11, and 12 carbons, respectively.

Isosaraine-3 (**9**) was an optically active amorphous solid. HREIMS data of **9** (*m/z* 480.3933, required 480.3923) coincided with the molecular formula of saraine-3 ($C_{32}H_{52}N_2O$). Indeed, the spectral properties of **6** and **9** are very similar. For example, the ^{13}C-NMR spectrum of **9**, like that of saraine-3, exhibited also a bisubstituted double bond (δ 129.4 d, 131.7 d), a trisubstituted double bond (δ 134.6 s, 121.3 d), and a ketone functionality (δ 212.2, s). The main differences between **6** and **9** happened in quinolizidone moiety where the resonances of C-4 (δ 53.7) and C-3 (δ 30.6), in the ^{13}C-NMR spectrum of **9**, were downshifted according to the absence of the γ-gauche effects[43] caused by the axial substituents at C-2 and C-1, respectively. Since three of the eight unsaturations present in the molecule were thus accounted for, compound **9** was assumed to possess five rings which included two nitrogen atoms. Detailed NMR spectra studies of isosaraine-3 (**Table 1** and **2**) confirmed the presence of the same heterocyclic core as other saraines.[5,6]

All the connectivities of the quinolizidone system from H-1 to H_2-4, from H_2-6 to H_2-7 and from H-9 to H-10 were recognized by COSY and TOCSY experiments. The nature of the *trans*-fused quinolizidone moiety was put in evidence by the presence of IR Bohlmann bands at 2760 and 2805 cm^{-1}[44] and by the chemical shift of H-10 (δ 2.05)[45] along with the comparison with model compounds.[2,5,6] The relative stereochemistry at the chiral centers C-1 and C-9, opposite to those of saraines 1-3, was deduced by analysing the coupling pattern of H-10. The value of $J_{9\text{-}10}$=1.8 Hz was indicative of an equatorial-axial coupling while that of $J_{1\text{-}10}$=10.5 Hz implied the coupling of axial-axial oriented protons.

The unsaturated piperidine moiety was confirmed by the detailed interpretation of 2D NMR spectra and by comparison with the NMR data of isosaraine-1 (**7**) , and -2 (**8**) (**Table 1**). The location of the olefin was easy because the olefinic proton (δ 5.69, H-4') displayed in the COSY spectrum, in addition to the vicinal coupling with H-3', a series of significant allylic couplings with H_2-2' (δ 3.09, 2.68), H_2-6' (δ 3.32, 3.17), and H_2-*f* (δ 2.13, 1.92). The carbon at position *g* was attached to the nitrogen atom on the basis of its downfield ^{13}C-NMR resonance (δ 56.5). Furthermore, H-4' displayed a series of highly diagnostic ^1H-^{13}C long-range correlations with C-2 (δ 35.9), C-2' (δ 51.3), C-6' (δ 53.5) suggesting the linkage between C-2 and C-3'. The proton H-3' was axially oriented according to the coupling pattern of $H\text{-}2'_{ax}$ which showed two large coupling constants (*J*=10.5 and 10.5 Hz) due to the vicinal coupling with H-3' and the geminal coupling with $H\text{-}2'_{eq}$.

The comparison of the ^{13}C-NMR resonances of C-2, C-2' and C-3' of isosaraine-3 with those of isosaraines 1-2 put in evidence notable differences (see **Table 1**). Considering that compounds **7-9** possess the same stereochemistry at C-2, probably, these differences could be rationalized either by different conformations of the piperidine ring or by different configurations at C-3'.

The assignment of the two alkyl chains that complete the structure **9** was mainly deduced by the interpretation of 2D NMR. The relative lengths of the two chains were suggested by both EIMS spectra and

biogenetic analogy with saraines A-C.[3,4,7] More exactly, the relevant fragment at *m/z* 327 ($C_{21}H_{31}N_2O$), which is present in the EIMS spectra of all saraines **4-9**,[2,5,6] is probably due to the loss of the linear fragment $C_{11}H_{21}$ from the alkyl chain (*x*).[2] Because of this, one chain (*y*) should contain six methylenes (C-*a* to C-*f*) and another one, analogously with saraine-C (**3**), should consist of ten methylenes and an alkene (C-*g* to C-*r*), respectively. However, as mentioned above, the relative length of the two alkyl chains is uncertain. Finally, the double bond in the longer alkyl chain (*x*) was placed at Δ^k by analysis of the NMR spectra and by comparison with saraine-3. In fact, TOCSY and HETCOR-TOCSY experiments connected H_2-*g* (δ 3.01, 2.72) to H_2-*h* (δ 1.70, 1.53), to H_2-*i* (δ 1.46); H-*k* (δ 5.27) to H_2-*j* (δ 2.21, 2.10), to H_2-*i* supporting this assignment. The *Z* geometry of the double bond was determined by the ^{13}C-NMR chemical shifts of the vinyl carbons (C-*j*, C-*m* δ 26.4).

Table 3. Selected ^{13}C-NMR Data[a,b] for the Alcohol Derivatives of Saraines 1-2 (**10-13**) and the Corresponding MTPA Esters (**10S**, **10R**, **11S**, **11R**, **12S**, **12R**, **13S**, **13R**)

C	**10**	**10S**	**10R**	**11**	**11S**	**11R**	**12**	**12S**	**12R**	**13**	**13S**	**13R**
1	34.5	34.9	34.9	35.3	35.5	35.2	36.9	36.2	36.8	37.8	37.6	37.6
2	36.3	37.4	37.4	35.3	35.4	35.3	36.1	36.8	36.4	36.9	36.7	36.8
3	24.3	24.0	24.0	2.50	23.4	23.3	2.88	24.0	24.1	24.0	23.9	23.9
4	47.2	47.2	47.2	50.5	50.3	50.3	46.7	47.1	47.5	50.3	50.0	49.9
6	53.3	52.7	52.7	49.7	50.3	50.3	53.4	53.2	53.1	49.7	50.0	50.3
7	31.7	24.3	24.3	32.5	29.2	29.5	31.1	28.1	28.1	31.6	29.1	29.3
8	78.7	83.7	83.7	64.8	72.0	71.7	78.3	83.8	83.8	64.7	72.0	71.7
9	44.5	42.3	42.3	41.9	40.9	40.6	44.6	41.3	41.3	41.4	39.9	40.0
10	67.6	67.6	67.6	64.7	64.9	65.0	67.6	68.3	68.4	64.4	64.7	64.6
2'	54.2	55.6	55.6	54.5	54.5	54.4	55.2	55.1	55.1	56.1	56.1	55.5
3'	40.7	41.0	41.0	41.9	41.8	41.8	40.5	40.7	40.9	42.3	42.3	42.1
4'	122.2	121.9	121.9	124.6	124.3	124.3	122.1	122.1	122.2	125.0	124.6	124.5
5'	135.6	135.9	135.9	134.3	135.9	135.9	135.6	135.9	135.9	134.2	135.8	135.7
6'	55.4	55.6	55.6	56.4	56.1	56.3	56.5	56.5	56.8	56.1	55.4	56.0
a	28.1	28.4	28.4	20.3	20.3	20.4	28.9	28.2	28.2	20.4	20.6	20.2
f	31.6	31.7	31.7	31.5	31.5	31.5	31.5	31.4	31.4	32.3	31.6	31.6
g	55.9	56.8	56.8	56.1	56.3	56.1	57.3	57.2	57.2	57.6	57.6	57.5
p	-	-	-	-	-	-	37.6	37.1	36.8	36.8	36.5	36.5
q	37.3	37.2	37.2	36.5	36.4	36.3						

[a] Bruker AMX 500 MHz; $CDCl_3$; δ values referenced to $CHCl_3$ (δ 77.0 ppm).

[b] The multiplity was determined by DEPT technique. It is the same of the corresponding carbons reported in **Tab.1** except C-8 (d).

On the basis of the knowledge of the relative relationships of the chiral centers around the quinolizidone system, advanced stereochemical studies had been performed. In order to utilize Mosher's method,[46,47] saraine-1 (**4**) and -2 (**5**), co-occurring major metabolites of the sponge, were reduced with $NaBH_4$ affording two pairs of corresponding 8-OH epimers: **10** (equatorial 8-hydroxy-saraine-1), **11** (axial 8-hydroxy-saraine-1), and **12** (equatorial 8-hydroxy-saraine-2), **13** (axial 8-hydroxy-saraine-2). The two pairs of epimers were fully characterized by detailed analysis of their COSY, HMQC, HMBC and TOCSY spectra. The orientation of the

hydroxy group at C-8, in compounds **10** and **12**, was assigned as equatorial according to the coupling pattern of H-8 (see experimental) which showed two large coupling constants due to the vicinal couplings with H-7_{ax} and H-9_{ax}, respectively. On the contrary, the axial orientation of 8-OH in compounds **11** and **13** was supported by the ^{13}C-NMR resonances of C-6 (δ 49.7) which resonated at a higher field respect to the same carbon of compounds **10** and **12** (see **Table 3**) due to γ-gauche effect caused by the axial 8-OH. In addition, the shape of H-8 (δ 3.96, br s) is also coherent with its equatorial orientation.

Having these compounds in hand we began to study their absolute stereochemistry. First, 8-OMTPA esters of **10** were synthesized since **10** is the main reductive product of saraine-1. The ^{1}H-NMR resonances of the (*S*)- and (*R*)- MTPA esters of **10** (**10S**, **10R**) prepared by treatment with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenyl acetyl (MTPA) chloride in dry pyridine at room temperature, respectively, were assigned by extensive analysis of their 1D and 2D NMR spectra. The results of ^{1}H-NMR of **10S**, **10R** and the $\Delta\delta$ values ($\Delta\delta=\delta_{S\text{-MTPA-ester}}-\delta_{R\text{-MTPA-ester}}$) for the protons near to the chiral center C-8 were listed in **Table 4**.

Table 4. Selected ^{1}H-NMR Chemical Shifts[a] for the MTPA Esters of the Alcohol Derivatives of Saraines 1-2 (**10**-**13**) and $\Delta\delta$ ($\delta_{S\text{-MTPA ester}}$ - $\delta_{R\text{-MTPA ester}}$)[b]

H	**10S**	**10R**	$\Delta\delta$	**11S**	**11R**	$\Delta\delta$	**12S**	**12R**	$\Delta\delta$	**13S**	**13R**	$\Delta\delta$
4a	2.88	2.87	+5	2.77	2.80	-15	2.87	2.85	+10	2.81	2.83	-10
4b	2.35	2.35	0	2.10	2.11	-5	2.22	2.20	+10	2.05	2.08	-15
6a	2.88	2.87	+5	2.50	2.58	-40	2.88	2.86	+10	2.51	2.60	-45
6b	2.34	2.34	0	2.03	2.16	-65	2.39	2.35	+20	2.05	2.19	-70
7a	1.80	1.70	+50	1.93	1.87	-30	1.92	1.90	+10	1.91	1.92	-5
7b	1.61	1.60	+5	1.90	1.78	-60	1.76	1.65	+54	1.85	1.90	-25
8	4.72	4.73	-5	5.31	5.35	-20	4.70	4.71	-5	5.31	5.34	-15
9	1.60	1.68	-40	1.90	1.89	+5	1.71	1.73	-10	1.88	1.86	+10
10	-[c]	-[c]		1.60	1.56	+20	1.71	1.71	0	1.64	1.63	+5
1	1.95	2.01	-30	1.98	1.96	+10	1.87	1.90	-15	1.81	1.78	+15
2	1.83	1.87	-20	1.89	1.90	-5	1.92	1.92	0	1.88	1.88	0

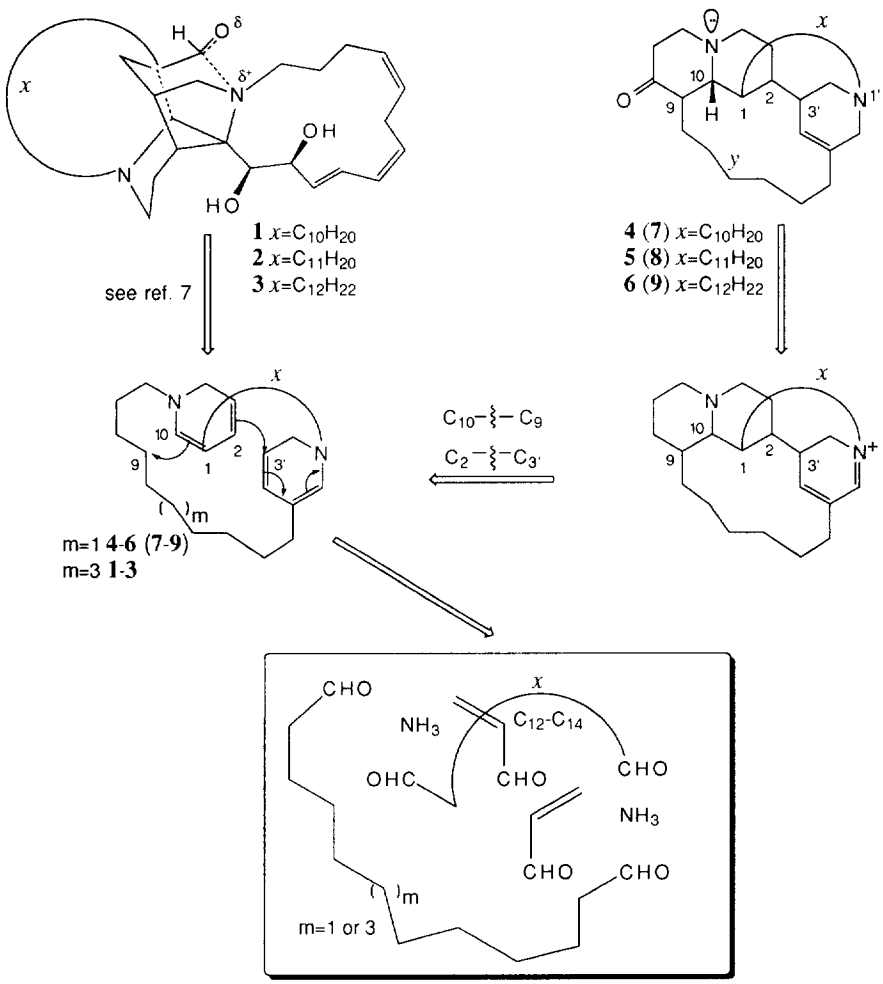
[a] Bruker AMX 500 MHz; $CDCl_3$; δ values referenced to $CHCl_3$ (δ 7.26 ppm); The assignments were aided by ^{1}H-^{1}H COSY, TOCSY and ^{1}H-^{13}C HETCOR.

[b] $\Delta\delta$ values are given in Hz.

[c] δ values could not be unambiguously assigned.

Due to the anisotropic effect of the benzene rings, the signals attached to the carbons at C-4, C-6 and C-7 in **10R** were observed at a higher field than those of **10S**, while the signals due to the protons at C-9, C-10, C-1, and C-2 in **10R** were observed at a lower field as compared to those of the **10S**. According to the MTPA determination rule,[46] the absolute stereochemistry at C-8 was assigned as *R*. Although significant $\Delta\delta$ values near C-8 were observed, the arrangement of the protons on the right side and left side of the "MTPA plane" having trifluromethyl, ester carbonyl, and carbinol methine proton coplanar was not systematical. In particular, the signals due to the protons at C-4 and C-6 were observed at virtually the same chemical shifts in both (*R*)- and (*S*)-MTPA esters. Inspection of the Drieding models of the MTPA esters of **10** indicated that there are no steric impediments to the MTPA group adopting the "ideal conformation", but revealed that the phenyl group was

located quite far away from H_2-6 and H_2-4. Probably this is the reason why the $\Delta\delta$ values in these positions were so small. However, it raised a necessity for checking the MTPA esters of the epimeric alcohol **11** in order to obtain more reliable stereochemical assignments. Hence, 8-OMTPA esters of **11** were also prepared by using the same procedure. In this case, as expected, the $\Delta\delta$ (δ_S-δ_R) values (see **Table 4**) were better arranged on both sides of MTPA plane but with a little anomaly of the methine at C-2 which resonated at the almost same field in **11S** and **11R**. Of course, in contrast to 8-OMTPA esters of **10**, positive $\Delta\delta$ values were recorded for protons at C-9, C-10, and C-1, whereas negative $\Delta\delta$ values were observed for H_2-6, H_2-4 and H_2-7 according to the absolute configuration *S* at C-8.



Scheme 1. Possible retrosynthetic pathway of saraines **1-9** according to ref. 40

The inverted configuration at C-8 displayed in **10** and **11**, a pair of epimeric alcohols, verified the validity of the Mosher ester methodology for our system. On the basis of above results and because the relative stereochemistry at other centers of quinolizidone system has been assigned by NMR techniques, we propose the

1*S*, 2*S*, 9*R*, 10*R* configuration for saraine-1 (**4**). Of course, it is impossible to determine the absolute stereochemistry at C-3' bearing the two independent heterocyclic systems.

In order to reconfirm the above absolute stereochemistry assignments, we also applied the same method to the Mosher esters of **12** (**12**S, **12**R) and **13** (**13**S, **13**R). Similar results like those of Mosher esters of **10**, **11** were observed. Some selected $\Delta\delta$ values are listed in **Table 4**. Once again, 8*R* configuration was assigned to **12**, whereas an opposite configuration (8*S*, as expected) was suggested for **13**. It is clear that saraine-2 (**5**), bearing in mind its relative stereochemistry, possesses at C-1, C-2, C-9 and C-10 the same absolute stereochemistry suggested for saraine-1. The limited amounts of saraine-3 (**6**) prevented from establishing its absolute stereochemistry by using the same procedure as mentioned above. However, considering that **6** is a superior homologue of **4** and **5**, it probably has the same absolute configurations (1*S*, 2*S*, 9*R*, 10*R*).

Saraines **4-9** formally displayed a completely different skeleton from that of co-occurring saraines A-C (**1-3**). However, they are structurally analogous. By analogy with Baldwin's proposal,[40] a similar biogenetic pathway like that of saraines A-C[7] could be extended also to explain the origin of compounds **4-9**. **Scheme 1** outlined how these alkaloids were biogenetically synthesized, *in vivo*, using almost the same building blocks as those of saraines A-C. Probably, some stereoselective enzymes are capable of catalyzing the intramolecular condensation of achiral partially reduced *bis*-3-alkylpyridine macrocycles, which contain 10 carbons in an alkyl chain and 10, 11, and 12 carbons in another alkyl chain (*x*), to form saraines 1-3 (**4-6**) and isosaraines 1-3 (**7-9**). This hypothesis links also the origin of saraines to other macrocyclic diamine alkaloids.[8-39] Three recent reports[32,34,37] about the ability of the sponge to biosynthesize both the stereoisomeric metabolites give further support to above hypothesis. However, the true biosynthetic origin of saraines is still a matter of discussion. According to the biogenetic hypothesis reported for petrosins in ref. 48, isosaraines 1-3 could be products of some post-biosynthetic equilibrations that, opening the quinolizidone ring of saraines 1-3, epimerize some stereocenters through retro-Mannich-Mannich and immonium ion-enamine equilibria.

EXPERIMENTAL SECTION

General Procedures. ^{1}H- and ^{13}C-NMR spectra were measured on a Bruker AMX 500 spectrometer. 2D experiments were performed using standard micro programs of Bruker software. AEI MS-30 (EIMS), Kratos MS-50 (HREIMS) instruments were used for obtaining mass spectra. IR spectra were recorded in liquid film with Nicolet DX FT spectrometer. Optical rotations were measured with a Perkin Elmer 141 polarimeter. Normal-phase HPLC purification was performed on a Waters liquid chromatograph using a R. I. detector. The column for semi-preparation was Spherisorb-S5N analytical column [5 μm, 4.6 mm (i.d.)x25 cm].

Merck precoated Si gel plates and Kieselgel 60 F_{254} plates were used for TLC; Spots were detected by exposing the plates to iodine vapour. Commercial Merck Si gel 60 (70-230 mesh ASTM) was used for column chromatography.

Collection of animal material. The sponge was collected in the Bay of Naples by Dr. E. Mollo. A voucher specimen is available for inspection at the ICMIB.

Isolation procedure. All the experiments were carried out on saraines 1-3 (**4-6**) obtained following the isolation procedure described in previous papers.[2,3] Isosaraine-3 (**9**) was obtained by the following procedure:

Fresh sponge (dry wt. 125 g), after a usual work-up,[2,3] afforded a fraction (1.1 g) containing a mixture of saraines 1-3 together with some minor related compounds. The mixture was subjected to second silica gel column, and eluted by light petroleum with increasing amounts of Et_2O. The fractions 17-19 eluted with light petroleum/Et_2O (7:3) yielded crude isosaraine-3 (55 mg) which was further purified by chromatography in a Pasteur pipette [SiO_2; $CHCl_3$/MeOH (99:1)]. Pure isosaraine-3 (**9**, 13.4 mg) was obtained from fractions 21-75.

Saraine-3 (6): Amorphous powder, $[\alpha]_D$ -27.4° (*c* 0.8, $CHCl_3$); IR ν_{max} (liquid film): 2928, 2857, 2805, 2760, 1703, 1456, 756 cm^{-1}; EIMS, *m/z* (%): 480 (M^+, 100), 327 (32); HREIMS: *m/z* 480.4073 ($C_{32}H_{52}N_2O$ requires 480.4079); *m/z* 327.2441 ($C_{21}H_{31}N_2O$ requires 327.2436).

A sample of 10 mg of **6** in 0.5 ml $CDCl_3$ (TMS as internal reference), placing in a sealed NMR tube, was used for NMR experiments; ^{13}C- and ^1H-NMR data are listed in **Table 1, 2**, respectively.

Isosaraine-3 (9): Amorphous powder, $[\alpha]_D$ -16.3° (*c* 1.34, $CHCl_3$); IR ν_{max} (liquid film): 2928, 2857, 2805, 2760, 1703, 1456, 756 cm^{-1}; EIMS, *m/z* (%): 480 (M^+, 100), 327 (32); HREIMS: *m/z* 480.3933 ($C_{32}H_{52}N_2O$ requires 480.3923); *m/z* 327.2439 ($C_{21}H_{31}N_2O$ requires 327.2447).

A sample of 13.4 mg of **9** in 0.5 ml $CDCl_3$ (TMS as internal reference), placing in a sealed NMR tube, was used for NMR experiments; ^{13}C- and ^1H-NMR data are listed in **Table 1, 2**, respectively.

NaBH4 reduction of Saraine-1 (4): Following the procedure described in previous work,[2] saraine-1(**4**) (260 mg) was reduced to corresponding reductive products (a pair of 8-OH epimers) (252.4 mg) showing, in TLC [SiO_2, $CHCl_3$/MeOH/NH_3 (9:1:0.05)], two spots with almost equal R_f values (**10**, 0.33, **11**, 0.32). Further purification of this mixture was achieved by using normal phase HPLC with an eluent of *n*-Hexane/Me_2CO/Et_2NH (95:5:1) to afford pure compounds **10** (25.4 mg), **11** (15.4 mg), respectively.

NaBH4 reduction of Saraine-2 (5): Following the same procedure as described above, saraine-2 (240 mg) was reduced to corresponding alcohols (155.3 mg). The mixture was chromatographed on a Silica gel column using gradient elution ($CHCl_3$/MeOH, 99:1 to 91:9) to yield pure epimers **12** (33.4 mg), **13** (15.5 mg), respectively.

Spectral data of equatorial 8-hydroxy-saraine-1 (10): Amorphous powder, $[\alpha]_D$ -62.3° (*c* 2.54, $CHCl_3$); EIMS, *m/z* (%): 468 (M^+, 100), 329 (35); ^1H-NMR ($CDCl_3$) δ (ppm): 2.06 (m, H-1), 1.92 (br s, H-2), 1.67 (m, H_2-3), 2.87 (m, H-4a), 2.28 (m, H-4b), 2.84 (m, H-6a), 2.30 (m, H-6b), 1.71, 1.67 (m, H_2-7), 3.30 (br dd, *J*=8.5, 10.5 Hz, H-8_{ax}), 1.46 (m, H-9), 1.68 (m, H-10), 2.66 (br d, *J*=10.5 Hz, H-$2'_{eq}$), 2.09 (m, H-$2'_{ax}$), 2.26 (m, H-3'), 5.50 (br s, H-4'), 3.04 (d, *J*=16.5 Hz, H-6'a), 2.36 (m, H-6'b), 1.58, 1.20 (m, H_2-*a*), 2.15, 1.83 (m, H_2-*f*), 2.36, 2.26 (m, H_2-*g*), 1.68, 1.28 (m, H_2-*h*), 2.46, 1.80 (m, H_2-*i*), 5.42 (m, H-*j*), 5.42 (m, H-*k*), 2.24, 1.80 (m, H_2-*l*), 1.61, 1.32 (m, H_2-*q*); ^{13}C-NMR (CDCl3): see **Table 3**.

Spectral data of axial 8-hydroxy-saraine-1 (11): Amorphous powder, $[\alpha]_D$ -37.7° (*c* 0.24, $CHCl_3$); EIMS, *m/z* (%): 468 (M^+, 100), 329 (32); ^1H-NMR ($CDCl_3$) δ (ppm): 2.02 (d, *J*=1.8 Hz, H-1), 1.92 (m, H-2), 1.72, 1.65 (m, H_2-3), 2.81 (m, H-4a), 2.14 (m, H-4b), 2.53 (m, H-6a), 2.26 (m, H-6b), 1.84, 1.71 (m, H_2-7), 3.96 (br s, H-8_{eq}), 1.78 (m, H-9), 1.58 (m, H-10), 2.62 (br d, *J*=10.5 Hz, H-$2'_{eq}$), 2.03 (m, H-$2'_{ax}$), 2.32 (m, H-3'), 5.61 (br d, H-4'), 3.04 (d, *J*=16.5 Hz, H-6'a), 2.33 (m, H-6'b), 1.55, 1.37 (m, H_2-*a*), 2.16,

1.81 (m, H_2-*f*), 2.38, 2.24 (m, H_2-*g*), 1.63, 1.30 (m, H_2-*h*), 2.29, 1.69 (m, H_2-*i*), 5.45 (m, H-*j*), 5.39 (m, H-*k*), 2.50, 1.78 (m, H_2-*l*), 1.49, 1.16 (m, H_2-*q*); ^{13}C-NMR ($CDCl_3$): see **Table 3**.

Spectral data of equatorial 8-hydroxy-saraine-2 (12): Amorphous powder, $[\alpha]_D$ -44.3° (*c* 0.65, $CHCl_3$); EIMS, *m/z* (%): 456 (M^+, 100), 329 (32); ^{1}H-NMR ($CDCl_3$) δ(ppm): 1.95 (m, H-1), 1.93 (m, H-2), 1.84, 1.62 (m, H_2-3), 2.94 (m, H-4a), 2.37 (m, H-4b), 2.92 (m, H-6a), 2.53 (m, H-6b), 1.71, 1.68 (m, H_2-7), 3.35 (ddd, *J*=8.5, 10.7, 3.1 Hz, H-8_{ax}), 1.47 (m, H-9), 1.91 (m, H-10), 2.53 (br d, *J*=10.5 Hz, H-$2'_{eq}$), 2.21 (m, H-$2'_{ax}$), 2.19 (m, H-3'), 5.39 (br s, H-4'), 3.04 (d, *J*=16.5 Hz, H-6'a), 2.45 (m, H-6'b), 1.70, 1.18 (m, H_2-*a*), 2.13, 1.84 (m, H_2-*f*), 2.37, 2.41 (m, H_2-*g*), 1.52, 1.28 (m, H_2-*p*); ^{13}C-NMR ($CDCl_3$): see **Table 3**.

Spectral data of axial 8-hydroxy-saraine-2 (13): Amorphous powder, $[\alpha]_D$ -32.2° (*c* 0.41, $CHCl_3$); EIMS, *m/z* (%): 456 (M^+, 100), 329 (32); ^{1}H-NMR ($CDCl_3$) δ(ppm): 1.85 (m, H-1), 1.90 (m, H-2), 1.82, 1.62 (m, H_2-3), 2.82 (m, H-4a), 2.12 (m, H-4b), 2.54 (m, H-6a), 2.34 (m, H-6b), 1.87, 1.69 (m, H_2-7), 3.96 (br s, H-8_{eq}), 1.75 (m, H-9), 1.63 (m, H-10), 2.52 (br d, *J*=10.5 Hz, H-$2'_{eq}$), 2.12 (m, H-$2'_{ax}$), 2.23 (m, H-3'), 5.58 (br s, H-4'), 3.08 (d, *J*=16.5 Hz, H-6'a), 2.37 (m, H-6'b), 1.52 (m, H_2-*a*), 2.20, 1.84 (m, H_2-*f*), 2.52, 2.12 (m, H_2-*g*), 1.32 (m, H_2-*p*); ^{13}C-NMR ($CDCl_3$): see **Table 3**.

Preparation of MTPA esters of reductive products of saraines 1-2 (10-13): To a solution of 6 mg equatorial 8-hydroxy-saraine-1 (**10**) in 0.5 ml dry pyridine was added 0.05 ml of (*R*)-(-)-α-methoxy-α-trifluoromethylphenyl acetyl (MTPA) chloride. The mixture was allowed to stir at room temperature for about 15 hours during which time the solution gradually became dark brown. Evaporation of the solvent under reduced pressure gave a residue which was resolved in $CHCl_3$ and was washed 3 times by using same volume distilled water. Evaporation of the $CHCl_3$ gave the crude (*S*)-MTPA ester. Further purification by chromatography in a Pasteur pipette (SiO_2; $CHCl_3$/MeOH) yielded pure (*S*)-MTPA ester of **10** (**10**S) (6.2 mg). Following the identical procedure with (*S*)-(+)-α-methoxy-α-trifluoromethylphenyl acetyl (MTPA) chloride gave the (*R*)-MTPA ester of **10** (**10**R). The (*S*)- and (*R*)-MTPA esters of **11** (**11**S and **11**R), **12** (**12**S and **12**R) and **13** (**13**S and **13**R) were prepared as described above.

10S: obtained as colourless liquid; EIMS, *m/z* 684 (M^+); ^{13}C-NMR ($CDCl_3$) : see **Table 3**; ^{1}H-NMR ($CDCl_3$): see **Table 4**.

10R: obtained as colourless liquid; EIMS, *m/z* 684 (M^+); ^{13}C-NMR ($CDCl_3$) : see **Table 3**; ^{1}H-NMR ($CDCl_3$): see **Table 4**.

11S: obtained as colourless liquid; EIMS, *m/z* 684 (M^+); ^{13}C-NMR ($CDCl_3$) : see **Table 3**; ^{1}H-NMR ($CDCl_3$): see **Table 4**.

11R: obtained as colourless liquid; EIMS, *m/z* 684 (M^+); ^{13}C-NMR ($CDCl_3$) : see **Table 3**; ^{1}H-NMR ($CDCl_3$): see **Table 4**.

12S: obtained as colourless liquid; EIMS, *m/z* 672 (M^+); ^{13}C-NMR ($CDCl_3$) : see **Table 3**; ^{1}H-NMR ($CDCl_3$): see **Table 4**.

12R: obtained as colourless liquid; EIMS, *m/z* 672 (M^+); ^{13}C-NMR ($CDCl_3$) : see **Table 3**; ^1H-NMR ($CDCl_3$): see **Table 4**.

13S: obtained as colourless liquid; EIMS, *m/z* 672 (M^+); ^{13}C-NMR ($CDCl_3$) : see **Table 3**; ^1H-NMR ($CDCl_3$): see **Table 4**.

13R: obtained as colourless liquid; EIMS, *m/z* 672 (M^+); ^{13}C-NMR ($CDCl_3$) : see **Table 3**; ^1H-NMR ($CDCl_3$): see **Table 4**.

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