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Structural and Stereochemical Studies of Saraines: Macrocyclic Alkaloids of the Sponge *Reniera sarai*

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Abstract: The absolute stereochemistry of saraines A-C (**2**-**4**), a class of unusual polycyclic alkaloids from the Mediterranean sponge *Reniera sarai*, has been elucidated by applying high field ^{1}H-NMR to Mosher's method. The structures of saraine-B (**3**) and saraine-C (**4**), only partially clarified previously, now have been fully characterized by extensive analysis of their NMR spectra.

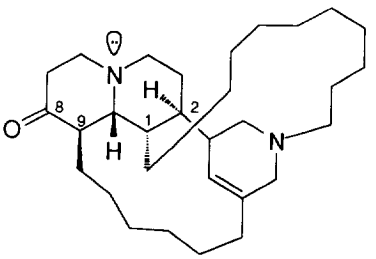
A growing collection of complex and structurally diverse macrocyclic alkaloids, that are related to each other by their apparent biogenetic origin from macrocycles composed of 3-alkylpyridine or reduced 3-alkylpyridine units, have been isolated from marine sponges in recent years. Since when our tentative biogenetic hypothesis suggested that saraines,[1-3] haliclamines,[4] xestospongins,[5] and petrosins[6] are all derived from reduced *bis*-3-alkylpyridine macrocycles, the origin of these alkaloids has been being an intriguing topic. Baldwin and Whitehead[7] recently suggested that the biosynthesis of manzamines[8] also proceeds through a *bis*-3-alkylpyridine macrocycle. More recently, Crews and his co-workers[9] reviewed the biogenetic pathway of these alkaloids and outlined how their apparently varying diamine framework are actually closely related.

Until now, more than ten related alkaloid skeletons have been reported.[1-6, 8-26] Most of them were discovered from sponges belonging to the order Haplosclerida. A few were isolated from Dictyoceratida sponges while cyclostellettamines[26] were found in the subclass Tetractinomorpha.[9] Saraines are characteristic secondary metabolites isolated from the Mediterranean sponge *Reniera sarai* Pulitzeri-Finali (Demospongiae: Haplosclerida: Renieridae). Like other macrocyclic alkaloids,[9,25] saraines possess a series of promising bioactivities.[27]

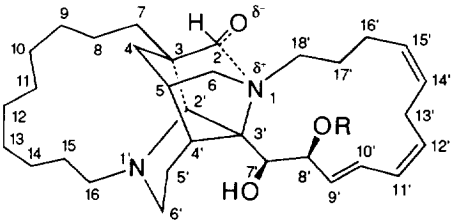
Complex separation steps led to two homologous series of saraines, exemplified by saraine-2 (**1**) and saraine-A (**2**). The former possesses a *trans*-fused quinolizidine system linked to a piperidine ring both directly and by two linear alkyl-chains. Some minor co-occurring alkaloids, named isosaraines 1-3[23,24] exhibit an inverted stereochemistry at the chiral centers C-1, C-2, and C-9. The latter, on the contrary, is characterized by a central nucleus, formally obtained by condensation of two piperidine rings, surrounded by two alkyl chains. Some saraines[1,23,24] belonging to the first group were structurally characterized mainly by an extensive 500 MHz NMR study while the skeleton of saraines belonging to the second group was established by an *X*-ray analysis on an acetate derivative of saraine-A (**2**)[2] and by following detailed spectral studies of saraine-A itself.[3] Recently, the central nucleus of saraine-A, a tricyclic core, has also been synthesized.[28,29]

Saraine-B (**3**) and saraine-C (**4**) are superior homologues of saraine-A (**1**). They exhibit a series of common peculiarities like **1**: the IR spectra of **3** and **4** showed absorptions indicative of the presence of

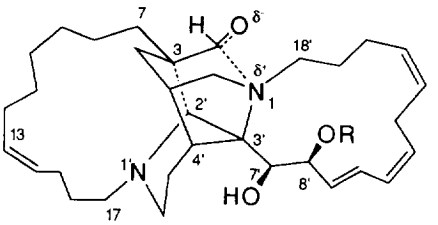
carbonyl functionality (1650 cm^{-1}) and hydroxy groups (3500 cm^{-1}); their UV spectra showed maximum absorption at 238 nm indicating the presence of a conjugated diene chromophore; a relevant peak (at *m/z* 270 and 284 respectively), resulting from loss of the same fragment $C_{14}H_{22}NO_3$, has always been recorded in their mass spectra. A careful examination of ^1H- and ^{13}C-NMR spectra of **3** and **4**, in comparison with those of saraine-A (**Table 1**), revealed that their skeletons differ only in the length of the non-oxidized alkyl chains that also contain a *cis*-oriented double bond.[3] However, their true structures, especially the location of the double bond, remain to be fully clarified. In addition, the absolute stereochemistry of saraines A-C (**2-4**) also remains unknown. For better understanding the chemistry of *Reniera sarai*, we have further studied saraines. In this report we will describe the full characterization of **3** and **4**, and, furthermore, the absolute stereochemistry of saraines A-C (**2-4**) is suggested.



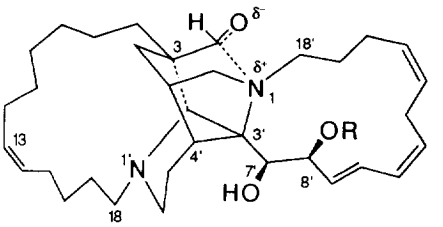
1



2 : R=H
2S: R=(*S*)-MTPA
2R: R=(*R*)-MTPA



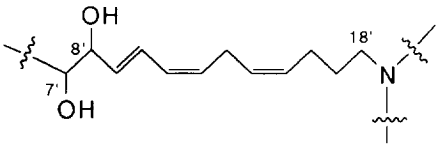
3 : R=H
3S: R=(*S*)-MTPA
3R: R=(*R*)-MTPA



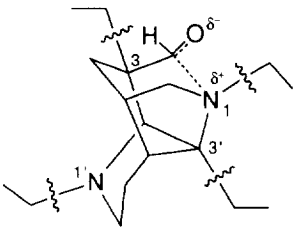
4 : R=H
4S: R=(*S*)-MTPA
4R: R=(*R*)-MTPA

Saraine-B (**3**), obtained as an amorphous powder, had a molecular formula of $C_{33}H_{50}N_2O_3$ established by HREIMS (*m/z* 522.3830 requires 522.3821). NMR spectra of **3** connected 47 hydrogen atoms to 32 carbons (17xCH_2, 13xCH, 2xC) and revealed 8 sp^2 carbons assignable to four olefins. The remaining atoms ($C_1H_3N_2O_3$), bearing in mind the structure of saraine-A (**2**), were assigned to an aldehyde group (CHO), to two hydroxy groups (OHx2) and to two tertiary nitrogen atoms. A detailed analysis of the COSY, HMBC, HMQC, and TOCSY data identified the partial structure **a** and the central pentacyclic core (partial structure **b**). For the former, significant COSY and TOCSY correlations connected all protons from H-8' to H_2-18'. Strangely, direct coupling between H-7'(δ 4.24) and H-8' (δ 4.11) was not observed. In fact, as it has already described for saraine-A, H-7' displayed a singlet peak while H-8' was a sharp doublet. In spite of this anomaly,

which was justified by assuming a value of $J_{7'\text{-}8'} \approx 0$, a linkage between 7' and 8' was suggested and unambiguously confirmed by diagnostic correlations between C-7' (δ 79.5) and H-8' (δ 4.11) and between C-8' (δ 71.0) and H-7' (δ 4.24) observed in the long-range HETCOR experiment. Probably, saraine-B displays at C-7' and C-8' the same relative stereochemistry, supported by previous diffractometric studies, of saraine-A (**2**). Furthermore, HMBC experiment also connected C-3' to C-7' and N-1 to C-18'. A series of long-range couplings were observed between C-3' (δ 62.8) and H-7' (δ 4.24); between C-4' (δ 38.4) and H-7'; between C-7'(δ 79.5) and H-2' (δ 3.57); between C-6 (δ 56.0) and H_2-18' (δ 3.01 and 2.51). Finally, the *E* stereochemistry of the alkene at $\Delta^{9'}$ was suggested by a large coupling constant between H-9' and H-10' ($J_{9'\text{-}10'}$ = 15.5 Hz) while the *Z* nature of $\Delta^{11'}$ and $\Delta^{14'}$ was indicated by the ^{13}C NMR chemical shifts of the vinyl carbons C-13' (δ 25.6) and C-16' (δ 25.5).[30]



a



b

The partial structure **b** was suggested by extensive analysis of 2D NMR spectra and by comparison with the data previously reported for **2**.[2,3] Analogously to saraine-A (**2**), we observed neither the resonance attributable to H-2 nor the resonance assignable to the carbonyl carbon (C-2). However, **3** was treated, in a similar manner like saraine-A, by adding CD_3COOD in an equimolecular ratio with **3**. The ^{1}H- and ^{13}C-NMR spectra of this sample showed the expected signal at δ 5.25, correlated, by HETCOR experiment, to a ^{13}C resonance at δ 97.1. The signals were unequivocally assigned to a methine between a quaternary nitrogen atom and a hydroxy group rationalized by an interaction of the aldehyde group and the tertiary amine moiety. This experiment confirmed the same "proximity effect" presented between carbonyl group and amine moiety as observed in saraine-A (**2**)[3] and reported many years ago by Leonard[31].

Subtraction of the atoms present in the pentacyclic core (partial structure **b**) and in the bridge from N-1 to C-3' (partial structure **a**) from the molecular formula of **3** indicated that the remaining portion, consisting of nine aliphatic methylenes (CH_2x9) and two olefinic methines (CHx2), has to form a linear chain. The ^{13}C-NMR chemical shift at δ 56.6 attached C-17 to N-1'. The HMBC correlation observed between C-17 and H-2' (δ 3.57) further supported this connectivity. The end of this alkyl chain (C-7) was linked to C-3 by a long-range coupling between C-3 (δ 51.1) and H-7 (δ 1.58) and by comparison with saraine-A (**2**). Moreover, COSY and TOCSY spectra of **3** displayed a correlation of H_2-17 with two pairs of protons (H_2-16, δ 1.79, 1.35, and H_2-15, δ 2.10, 2.07). The further coupling of H_2-15 with the olefinic proton (H-14, δ 5.29) led to unequivocally localize the double bond in the bridge at Δ^{13}. The ^{13}C NMR chemical shifts of the vinyl carbons C-12 (δ 26.1) and C-15 (δ 26.0) indicated a *Z* configuration for the double bond.[30]

Saraine-C (**4**) gave a parent ion in the HREIMS at *m/z* 536.3941 appropriate for a formula of $C_{34}H_{52}N_2O_3$. Compound **4** showed ^{1}H- and ^{13}C-NMR data (**Table 1**) very similar to those of **2** and **3**. The

differences among them were the length of the non-oxidized linear alkyl chain and the location of the alkene function in this chain. The NMR analysis immediately led to the partial structures **a** and **b**.

Table 1. Comparison of ^{1}H- and ^{13}C-NMR Data[a] of Saraine-B (**3**) and Saraine-C (**4**) with Saraine-A (**2**)

	δ ^{13}C, m			δ ^{1}H[b]					
No.	**2**[3]	**3**	**4**	**2**[3]		**3**		**4**	
2	-	-	-	8.50		8.56		8.63	
3	51.3 s	51.1 s	51.8 s	-		-		-	
4	33.3 t	33.2 t	32.7 t	1.66	2.24	1.58	2.26	1.66	2.26
5	38.9 d	38.7 d	38.7 d	2.10		2.07		2.11	
6	55.9 t	56.0 t	56.2 t	2.35	2.74	2.31	2.67	2.36	2.76
7	37.4 t	37.2 t	37.1 t	1.25	1.72	1.58	1.38	1.53	1.37
8	19.5 t	21.0 t	22.2[d] t	1.40	1.55	1.39		1.34	
9[c]	27.6 t	27.6 t	27.2 t	1.34		1.35		1.25	
10[c]	25.2 t	25.2 t	28.0 t	1.52	1.21	1.42	1.25	1.15	
11[c]	25.0 t	25.0 t	27.7 t	1.40	1.21	1.80	1.12	1.36	
12[c]	24.9 t	26.1 t	25.6 t	1.21	1.65	2.20	2.05	1.77	2.16
13	24.0 t	131.6 d	129.8 d	1.21	1.48	5.39		5.37	
14	28.0 t	127.9 d	130.1 d	1.21	1.50	5.29		5.36	
15[c]	27.5 t	26.0 t	25.3 t	1.57		2.10	2.07	2.11	1.92
16	57.0 t	26.7 t	28.1 t	2.90	3.00	1.79	1.35	1.65	1.32
17		56.6 t	26.4 t			2.91	2.76	1.68	1.57
18			56.5 t					2.94	
2'	65.7 d	67.9 d	66.9 d	3.59		3.57		3.54	
3'	64.0 s	62.8[d] s	63.2 s	-		-		-	
4'	38.6 d	38.4 d	38.5 d	2.35		2.35		2.36	
5'[c]	24.5 t	24.7 t	24.6 t	1.91	2.35	1.90	2.32	1.89	2.34
6'	44.0 t	43.3 t	44.3 t	2.81	3.13	2.87	3.06	2.77	3.16
7'	79.0 d	79.5[d] d	79.2 d	4.22		4.24		4.19	
8'	70.9 d	71.0 d	70.9 d	4.10		4.11		4.09	
9'	135.3 d	135.2 d	135.2 d	5.71		5.75		5.73	
10'	125.3 d	125.1 d	125.2 d	6.46		6.45		6.47	
11'	129.6 d	129.4 d	129.5 d	6.03		6.02		6.03	
12'	129.9 d	129.7 d	129.8 d	5.72		5.71		5.72	
13'[c]	25.8 t	25.6 t	25.6 t	2.50	3.13	2.45	3.16	2.51	3.17
14'	129.0 d	129.0 d	128.9 d	5.20		5.20		5.20	
15'	127.6 d	127.5 d	127.5 d	5.15		5.14		5.18	
16'[c]	25.6 t	25.5 t	25.6 t	2.17	2.10	2.13	2.08	2.17	2.11
17'[c]	25.5 t	25.8 t	25.8 t	1.48	1.52	1.45		1.78	1.48
18'	54.0 t	54.2 t	53.9 t	2.58	3.00	2.51	3.01	2.54	2.99

[a] In $CDCl_3$; 500MHz; δ values are reported in ppm referenced to $CHCl_3$(δ ^{1}H 7.26 and δ ^{13}C 77.0).

[b] The assignments were aided by ^{1}H-^{1}H COSY, TOCSY, and by ^{1}H-^{13}C-HETCOR.

[c] Every C-H correlation can be assigned to one of these carbons.

[d] The previously reported data[2] have been revised.

Subtraction of the atoms present in the partial structures **a** and **b** from the molecular formula of saraine-C (**4**) revealed that the bridge from N-1' to C-3 contains twelve carbons and one alkene functionality. ^{13}C NMR spectra of **4** showed that these carbons were ten methylenes and two olefinic methines. Extensive analysis of the COSY, TOCSY, HETCOR-TOCSY, HMQC, and HMBC data supported a bridge with twelve carbons spanning N-1' and C-3 as shown (**Table 1**). The correlations between H_2-7 (δ 1.37 and δ 1.53) and C-7 (δ 37.1) were observed in ^{1}H-^{13}C HETCOR experiment. HMBC and TOCSY data supported the connectivities indicated by the COSY data. For example, HMBC experiment connected C-3 (δ 51.8) to H-7 (δ 1.53); C-18 (δ 56.5) to H-2' (δ 3.54), H-17 (δ 1.68) and H-16 (δ 1.65); C-14 (δ 130.1) to H_2-15 (δ 2.11 and 1.92), H-16 and H_2-12 (δ 1.77 and 2.16); C-13 (δ 129.8) to H_2-12 and H_2-15. TOCSY and HETCOR-TOCSY experiments

connected H_2-18 (δ 2.94) to H_2-17 (δ 1.68 and 1.57), to H_2-16 (δ 1.65 and 1.32) and also to H-14 (δ 5.36). All these data led to unequivocally place the olefin in the alkyl chain at Δ^{13} according to structure **4**. Finally, the ^{13}C-NMR chemical shifts of the vinyl carbons C-12 (δ 25.6), C-15 (δ 25.3) were consistent with the *Z* orientation for the Δ^{13} alkene.

The absolute stereochemistry of saraines A-C (**2-4**) was determined by applying the modified Mosher's method.[32-38] (*S*)- and (*R*)- MTPA esters of saraines A-C (**2-4**) were prepared by treatment with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenyl acetyl (MTPA) chloride in dry pyridine at room temperature, respectively. Compounds **2-4** were converted to corresponding MTPA esters **2S**, **2R**, **3S**, **3R**, **4S**, **4R**, respectively. All the ^{1}H-NMR resonances of the esters were assigned by an extensive analysis of 1D and 2D NMR spectra. Significant $\Delta\delta$ values ($\Delta\delta=\delta_{S\text{-MTPA-ester}}-\delta_{R\text{-MTPA-ester}}$) for the protons near to the chiral center C-8' were observed. In fact, without exception among **2-4**, positive $\Delta\delta$ shifts were observed for the methine protons (H-7', H-2' and H-4') and methylene protons (H_2-5' and H_2-6') whereas negative effects were recorded for the olefinic protons (H-9' to H-11'). Inspection of the Drieding models of the MTPA esters of **2-4** indicated that there are no steric impediments to the MTPA group adopting the "ideal conformation" having trifluromethyl, ester carbonyl, and carbinol methine proton coplanar. Therefore, according to the MTPA determination rule[32-38], the *S* absolute stereochemistry at C-8' of saraines A-C (**2-4**) was suggested. Some selected $\Delta\delta$ values are listed in **Table 2**. Bearing in mind the relative stereochemistry established by *X*-ray diffraction study[2] of a diacetate derivative of saraine-A and by the comparison of the NMR data of the saraines A-C, **2-4** should have the same absolute configuration at all chiral centers (2'*S*, 3'*S*, 4'*R*, 7'*S*, 8'*S*, 3*R*, 5*S*). It may be worth pointing out that only 8'-OMTPA esters were obtained even with excess of MTPA reagent. Probably, the formation of 7'-OMTPA esters is sterically hindered.

Table 2. Selected ^{1}H-NMR Chemical Shifts[a] for the MTPA Esters of Saraines A-C (**2-4**) and $\Delta\delta$ [b]

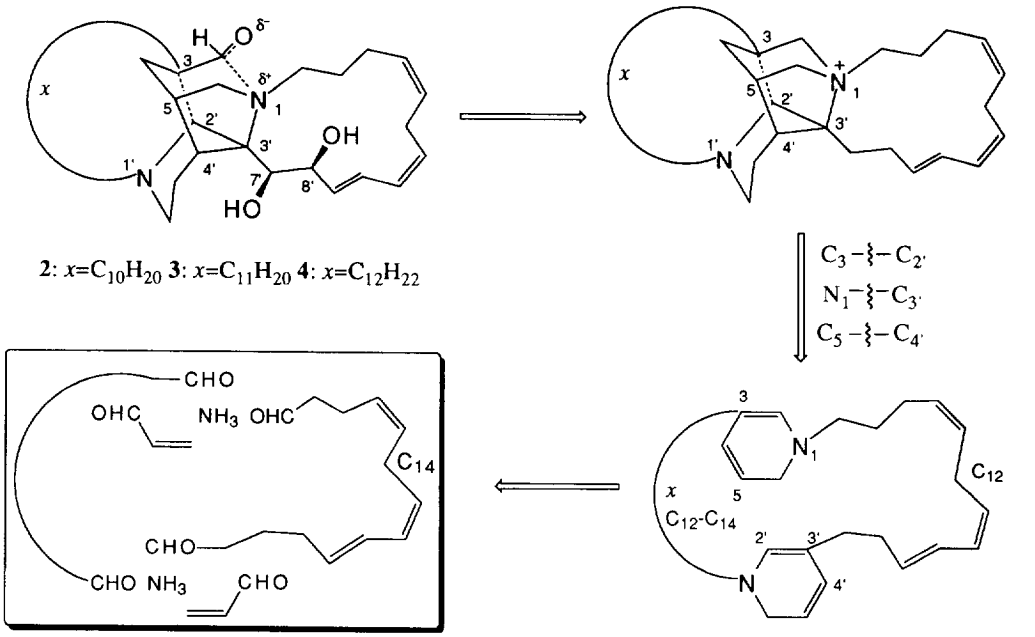
	Saraine-A(**2**)			Saraine-B(**3**)			Saraine-C(**4**)		
H	**2S**	**2R**	Δδ	**3S**	**3R**	Δδ	**4S**	**4R**	Δδ
2'	3.59	3.47	+60	3.64	3.46	+90	3.60	3.42	+90
4'	2.13	1.60	+265	-	-		-	-	
5'a	2.40	1.74	+330	2.46	1.62	+420	2.40	1.60	+400
5'b	1.75	0.82	+465	1.82	0.81	+505	1.77	0.80	+485
6'a	3.12	2.88	+120	3.06	2.80	+130	3.13	2.89	+120
6'b	2.76	2.50	+130	2.90	2.60	+150	2.75	2.52	+115
7'	4.47	4.39	+40	4.61	4.42	+95	4.44	4.35	+45
8'	5.52	5.37	+75	5.62	5.35	+135	5.52	5.34	+90
9'	5.76	5.87	-55	5.79	5.87	-40	5.75	5.85	-50
10'	6.71	6.72	-5	6.63	6.70	-35	6.68	6.72	-20
11'	6.04	6.06	-10	6.00	6.05	-25	6.03	6.06	-15

[a] 500 MHz; $CDCl_3$; δ values are reported in ppm referenced to $CHCl_3$ (δ 7.26).

[b] $\Delta\delta$ values ($\Delta\delta=\delta_{S\text{-MTPA-ester}}-\delta_{R\text{-MTPA-ester}}$) are given in Hz.

Saraines A-C (**2-4**) represent a unique group of macrocyclic alkaloids. However, it is reasonable to suggest, by analogy with the Baldwin and Whitehead proposal,[7] a similar biogenetic pathway for them.

Scheme 1 outlines the retro-biosynthesis of saraines A-C that proceeds through a partially reduced *bis*-3-alkylpyridine macrocycle which contains 12 carbons in an alkyl chain and 10, 11, and 12 carbons in another alkyl chain (*x*). These macrocycles, through some intramolecular reactions, form saraines A-C (**2-4**).



Scheme 1. Possible retrosynthetic pathway of saraines A-C (**2-4**) according to ref.7

This biogenetic hypothesis links the origin of saraines A-C to that of co-occurring saraines 1-3[1] and other marine macrocyclic diamine alkaloids.[4-6, 8-26] Much evidence supports the above biogenetic pathway; however, several recent reports[16,17,25] suggest that the real situation may be more complex.

EXPERIMENTAL SECTION

General Procedures. All the experiments were carried out on saraines A-C (**2-4**) obtained following the isolation procedure described in previous papers.[1,2] ^{1}H- and ^{13}C-NMR spectra were measured on a WM 500 Bruker spectrometer. 2D experiments were performed using standard micro programs of Bruker software. AEI MS-30 (EIMS), Karatos MS-50 (HREIMS), and ZAB VG (FABMS) instruments were used for obtaining mass spectra. Matrix used for measuring positive FABMS was glycerol (positive ion mode). UV spectra were recorded on a Shimatzu-Bausch and Lomb Spectronic apparatus. IR spectra were recorded in liquid film with nicolet DX FT spectrometer. Optical rotations were measured with a Perkin Elmer 141 polarimeter.

Merck precoated Si gel plates and Kieselgel 60 F_{254} plates were used for TLC; Spots were detected by exposing 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. see previous reports.[1,2]

Saraine-B (3): Amorphous powder, $[\alpha]_D$ +76.3° (*c* 1.2, $CHCl_3$); IR ν_{max} (liquid film): 2940, 2860, 1660 cm^{-1}; UV λ_{max} (MeOH): 238 (ε 13415) nm; EIMS, *m/z* (%): 522 (M^+, 65), 504 (32), 493 (100), 475 (30), 270 (45); HREIMS: *m/z* 522.3830 ($C_{33}H_{50}N_2O_3$ requires 522.3821); *m/z* 270.2235 ($C_{19}H_{28}N$ requires 270.2222).

A sample of 70 mg of **3** in 0.5 ml $CDCl_3$ (TMS as internal reference) was used for NMR experiments; ^1H- and ^{13}C-NMR data are listed in **Table 1**.

Influence of acidity on NMR spectral features of saraine-B: The evaluation of the influence of acidity on saraine-B (**3**) was performed following the identical procedure described in previous paper.[3]

^1H-NMR of **3** (molar ratio CD_3COOD/Saraine B = 1.0) ($CDCl_3$) δ (ppm): 5.25 (H-2), 1.96, 2.10 (H_2-3), 2.44 (H_2-4), 2.83, 4.04 (H_2-6), 1.66, 1.60 (H_2-7), 3.01, 2.84 (H_2-17), 3.85 (H-2'), 4.58 (H-7'), 4.43 (H-8'), 5.70 (H-9'), 6.49 (H-10'), 6.09 (H-11'), 5.81 (H-12'), 3.83, 3.65 (H_2-18'); ^{13}C-NMR δ (ppm): 97.1 (C-2), 46.6 (C-3), 30.9 (C-4), 39.5 (C-5), 55.0 (C-6), 33.2 (C-7), 56.7 (C-17), 55.9 (C-2'), 84.6 (C-3'), 69.6 (C-7'), 68.9 (C-8'), 130.2 (C-9'), 126.7 (C-10'), 129.3 (C-11'), 130.8 (C-12'), 54.7 (C-18').

Saraine-C (4): Amorphous powder, $[\alpha]_D$ +67.4° (*c* 1.1, $CHCl_3$); IR ν_{max} (liquid film): 2940, 2860, 1660 cm^{-1}; UV λ_{max} (MeOH): 238 (ε 13582) nm; EIMS, *m/z* (%): 536 (M^+, 60), 518 (30), 507 (100), 489 (75), 284 (40); HREIMS: *m/z* 536.3941 ($C_{34}H_{54}N_2O_3$ requires 536.3978); *m/z* 284.2408 ($C_{20}H_{30}N$ requires 284.2378).

A sample of 120 mg of **4** in 0.5 ml $CDCl_3$ (TMS as internal reference) was used for NMR experiments; ^1H- and ^{13}C-NMR data are listed in **Table 1**.

Preparation of MTPA esters of saraines A-C: To a solution of 10 mg saraine-A (**2**) 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 saraine-A (**2S**) (2.5 mg). Following the identical procedure with (*S*)-(+)-α-methoxy-α -trifluoromethylphenyl acetyl (MTPA) chloride gave the (*R*)-MTPA ester of saraine-A (**2R**). The (*S*)- and (*R*)-MTPA esters of saraine-B (**3S** and **3R**) and saraine-C (**4S** and **4R**) were prepared as described above.

2S: obtained as colourless liquid; positive FABMS, $(M+H)^+$ *m/z* 727; ^1H-NMR ($CDCl_3$): see **Table 2.**

2R: obtained as colourless liquid; positive FABMS, $(M+H)^+$ *m/z* 727; ^1H-NMR ($CDCl_3$): see **Table 2.**

3S: obtained as colourless liquid; positive FABMS, $(M+H)^+$ *m/z* 739; ^1H-NMR ($CDCl_3$): see **Table 2.**

3R: obtained as colourless liquid; positive FABMS, $(M+H)^+$ *m/z* 739; ^1H-NMR ($CDCl_3$): see **Table 2.**

4S: obtained as colourless liquid; positive FABMS, $(M+H)^+$ *m/z* 753; ^1H-NMR ($CDCl_3$): see **Table 2.**

4R: obtained as colourless liquid; positive FABMS, $(M+H)^+$ *m/z* 753; ^1H-NMR ($CDCl_3$): see **Table 2.**

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