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STRUCTURAL STUDIES ON SARAINE A[1,2]

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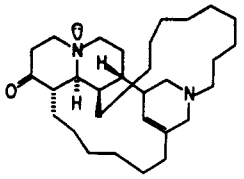
ABSTRACT.—The structure of saraine A [**3**], an unprecedented nitrogenous metabolite from the Mediterranean sponge *Reniera sarai*, has been investigated mainly by means of 2D nmr methods. Although an acetate derivative **2** of saraine A was previously described by X-ray analysis, the underivatized product showed some spectroscopic anomalies that can be explained by the existence in **3** of a strong "proximity effect" involving an aldehyde moiety and the nitrogen atom of a tertiary amine group.

The metabolic pattern of the marine sponge *Reniera sarai* Pulitzeri-Finali (Demospongiae: Haplosclerida: Renieridae) is characterized by the presence of a series of unusual alkaloids (1–4). Some of these, saraines 1–3 and isosaraine 1[3], exhibit a structure characterized by a quinolizidine system linked to a piperidine ring both directly and by two linear alkyl-chains. Others, saraines A–C, display a completely different alkaloid skeleton characterized by a central nucleus, formally obtained by condensation of two piperidine rings, surrounded by two alkyl chains. While the structure of the alkaloids belonging to the first group, such as saraine 2 [**1**], has been clarified mainly by an extensive 2D nmr study (2,3), the skeleton of saraines A–C has been suggested by an X-ray analysis (4) on an acetate derivative **2** of saraine A. In spite of this crystallographic characterization, the true structure of the underivatized saraine A [**3**] remains to be fully clarified. In fact, the spectral data of **2** were uninterpretable (4), while those of **3** showed a series of apparent anomalies. In addition, the nmr analysis of **3** was particularly complex because of the absence of related models in the literature and the difficulty of obtaining reproducible spectra; the nmr spectra were too strongly influenced by the concentration of the sample and by the presence of traces of impurities. This paper re-



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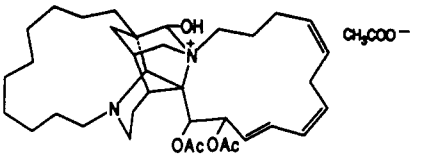

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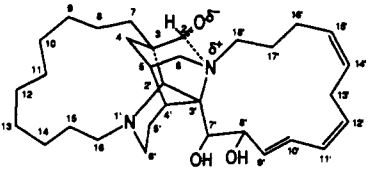

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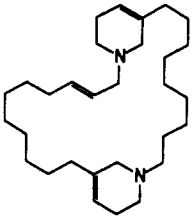

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[1]In the previous literature this compound was named sarain A, but saraine A is more appropriate for an alkaloid.

[2]Part of this work has been presented at the 16th International Symposium on the Chemistry of Natural Products, Kyoto, Japan, 29 May–3 June 1988.

[3]In the previous literature these alkaloids were named sarains and isosarains.

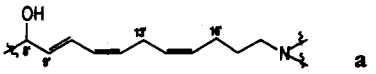
ports first an extensive analysis of the nmr spectra of saraine A [**3**], second an adequate rationalization of the unusual spectroscopic behavior, and finally a tentative biogenetic hypothesis correlating the alkaloid skeleton of saraines to those of other macrocyclic alkaloids recently found in marine organisms.

Surprisingly, this study has revealed that saraine A [**3**] displays structural peculiarities unexpected upon looking at the X-ray structure of its acetate derivative **2**. In fact, the aldehyde group and the tertiary amine moiety are not arranged in a pseudobase structure, but they are strongly interacting for a "proximity effect" which deeply modifies the spectral properties of **3**.

RESULTS AND DISCUSSION

The complex isolation procedure of saraine A is reported in previous papers (2,4). The main spectral features of saraine A led to a $C_{32}H_{50}N_2O_3$ compound containing a diene chromophore (λ max 238 nm), and a carbonyl group (ν max 1660 cm^{-1}). Of course, bearing in mind the structure of the crystalline product **2**, the presence of the carbonyl group is a matter of discussion. Another conflicting datum is the presence in the eims spectra of the molecular ion at *m/z* 510 and not at *m/z* 511 as expected by observing the molecular peak of **2** (*m/z* 595). Owing to the strong influence of both the concentration of the sample and the presence of traces of inorganic salts on the nmr spectra of **3** (Figure 1), all nmr experiments were performed on the same sample of **3** washed with H_2O and hermetically closed in a 5-mm nmr tube (50 mg in 0.5 ml of $CDCl_3$).

The nmr analysis (Table 1) immediately led to the partial structure **a**.



a

In fact, the signal resonating at δ 4.10 (H-8′, d, 7.9 Hz) coupled with one of the terminal protons (H-9′, δ 5.71, dd, 7.9 and 15.5 Hz) of a 1,4-disubstituted diene moiety linked to a bisvinylic methylene (H-13′, δ 3.13 and 2.50) on the other side. The allylic protons at C-16′ (δ 2.17 and 2.10) coupled with the protons (δ 1.52 and 1.48) of a methylene which in turn was linked to another methylene (δ ^1H 2.58 and 3.00, δ ^{13}C 54.0) showing a downshifted ^{13}C resonance value attributed to the deshielding effect of a nitrogen atom. Strangely, the ^1H-nmr signal assigned to H-8′ (δ 4.10) resonates as a sharp doublet. Bearing in mind the structure of **2**, this suggests a value of ca. 0 for the coupling constant $J_{8'-7'}$.

The ^1H- and ^{13}C-nmr chemical shifts (Table 1) of the atoms of the central nucleus were assigned by an extensive use of 2D methods: ^1H-^1H COSY, HETCOR, and long range HETCOR. We did not observe any resonance attributable to the methine corresponding to CH-2 as expected from looking at structure **2**. The singlet at δ 4.22 was strongly suspected to be H-2, but a long-range experiment unequivocally assigned this resonance to H-7′. In fact, the ^{13}C-nmr signal at 38.6 (C-4′) exhibited long-range couplings with the protons resonating at δ 4.22 (H-7′), δ 3.59 (H-2′), and δ 2.81 (H-6′). Only four resonances of the saturated alkyl chain were unambiguously assigned on the basis of general chemical shift rules (5). The remaining six methylenes showed ^{13}C-nmr resonances between δ 28.0 and 24.0 and ^1H-nmr signals within the range δ 1.60 and 1.15. Finally, the ^1H-nmr spectrum also exhibited a broad signal at a δ 8.50, which was unaffected by treatment with D_2O.

Owing to the absence of observable nmr resonances attributable to the atoms in position 2, we suspected, on the basis of the intramolecular interactions reported in the

TABLE 1. ^{1}H- and ^{13}C-nmr Data of Saraine A [3].

Carbon	δ ^{13}C[a] (m)	δ ^{1}H[a,b]	δ ^{1}H long-range coupled	δ ^{13}C[c] (m)	δ ^{1}H[b,c]
C-2	—	8.50		98.0 (d)	5.23
C-3	51.3 (s)	—	2.10 (H-5)[d]	46.5 (s)	—
C-4	33.3 (t)	1.66, 2.24	3.59 (H-2')[d]	30.9 (t)	1.98, 2.10
C-5	38.9 (d)	2.10		39.4 (d)	2.46
C-6	55.9 (t)	2.35, 2.74		54.7 (t)	4.06, 2.84
C-7	37.4 (t)	1.25, 1.72		33.2 (t)	1.67, 1.60
C-8	19.5 (t)	1.40, 1.55	1.72 (H-7)[d]	18.6 (t)	1.45
C-9[f]	27.6 (t)	1.34		29.6 (t)	1.23
C-10[f]	25.2 (t)	1.52, 1.21		27.9 (t)	1.35, 1.56
C-11[f]	25.0 (t)	1.40, 1.21		27.8 (t)	1.37
C-12[f]	24.9 (t)	1.21, 1.65		24.7 (t)	1.22, 1.46
C-13[f]	24.0 (t)	1.21, 1.48		25.8 (t)	1.25, 1.45
C-14[f]	28.0 (t)	1.21, 1.50		24.2 (t)	1.25, 1.45
C-15	27.5 (t)	1.57		25.4 (t)	1.61
C-16	57.0 (t)	2.90, 3.00		56.6 (t)	3.00, 2.84
C-2'	65.7 (d)	3.59		55.8 (d)	3.84
C-3'	64.0 (s)	—		83.6 (s)	—
C-4'	38.6 (d)	2.35	4.22 (H-7')[d], 3.59 (H-2')[d] 2.81 (H-6')[e]	40.1 (d)	2.31
C-5'	24.5 (t)	1.91, 2.35		24.7 (t)	2.31, 2.09
C-6'	44.0 (t)	2.81, 3.13		44.4 (t)	3.16, 2.84
C-7'	79.0 (d)	4.22		69.6 (d)	4.58
C-8'	70.9 (d)	4.10	6.46 (H-10')[d,e], 4.22 (H-7')[d,e]	68.7 (d)	4.43
C-9'	135.3 (d)	5.71	4.22 (H-7')[d]	130.0 (d)	5.70
C-10'	125.3 (d)	6.46		126.7 (d)	6.49
C-11'	129.6 (d)	6.03		129.3 (d)	6.09
C-12'	129.9 (d)	5.72	6.46 (H-10')[d], 2.50 (H-13')[d]	130.8 (d)	5.81
C-13'	25.8 (t)	2.50, 3.13	6.03 (H-11')[d]	26.0 (t)	3.16, 2.59
C-14'	129.0 (d)	5.20	2.10 (H-16')[d]	128.0 (d)	5.31
C-15'	127.6 (d)	5.15	2.10 (H-16')[d], 1.48 (H-17')[d]	125.7 (d)	5.28
C-16'	25.5 (t)	2.17, 2.10		25.4 (t)	2.46, 2.29
C-17'	25.6 (t)	1.48, 1.52		26.2 (t)	2.21, 1.50
C-18'	54.0 (t)	2.58, 3.00		54.7 (t)	3.84, 3.61

[a]In $CDCl_3$.
[b]The assignments were aided by ^{1}H-^{1}H COSY and by ^{1}H-^{13}C HETCOR.
[c]$CDCl_3$ and CD_3COOD equimolecular with **3**.
[d]By long-range HETCOR: $J_{C-H} = 10$ Hz.
[e]By long-range HETCOR: $J_{C-H} = 5$ Hz.
[f]Every C-H correlation can be assigned to one of these carbons.

literature, that the spectroscopic anomalies of saraine A [**3**] could be due to a strong "proximity" interaction between N-1 and C-2. In fact, as reported by Leonard *et al.* (6) in studies on cyclic aminoketones, the transannular N-C co-interactions lead to strong modifications of the spectral and chemical properties of both the carbonyl and the amine moieties. In particular, the carbonyl ir maximum is shifted lower than the normal frequency and, furthermore, disappears by acid treatment, which favors the formation of both a full N-C transannular bond and a hydroxy group. Analogously, the carbonyl ir maximum of **3** is lower (1660 cm^{-1}) than the normal value for carbonyl stretching, whereas it is not observed after treatment of **3** with HCl. The presence of a strong "proximity effect" in **3** was further supported by a series of nmr spectra of **3** in the presence of CD_3COOD (Experimental, Table 2). After addition of CD_3COOD in an equimolecular ratio with **3**, the ^{1}H- (Figure 1b) and ^{13}C-nmr (Figure 1d) spectra showed some new signals and, in particular, a sharp singlet at δ 5.23 correlated, by HETCOR experi-

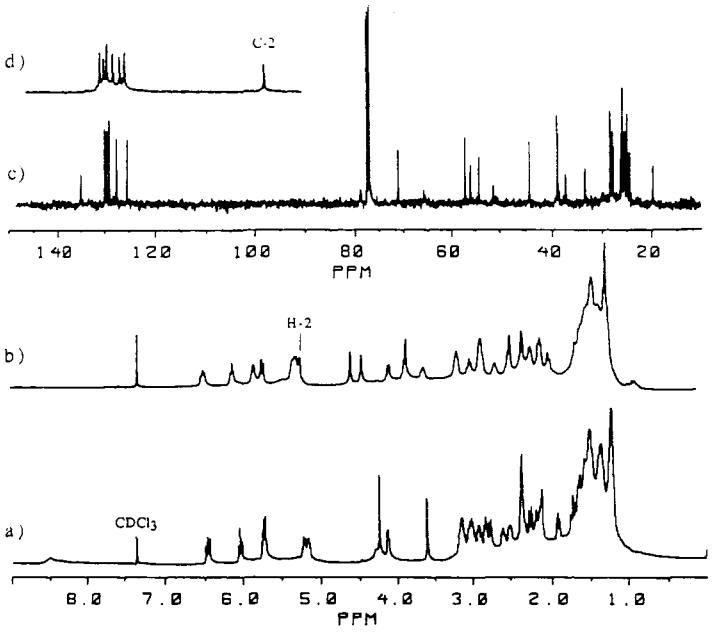
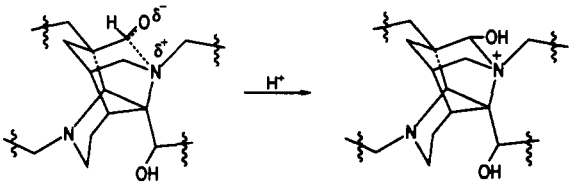


FIGURE 1. ^{1}H- and ^{13}C-nmr spectra of saraine A [**3**]: a and c in $CDCl_3$, b and d (partial ^{13}C-nmr spectrum) in $CDCl_3 + CD_3COOD$.

ments, to a ^{13}C resonance at δ 98.0. The signals were unequivocally assigned to a methine between a quaternary nitrogen and a hydroxy structure, as expected according to Leonard's theory and to the structure **2** of the acetate derivative of **3**.

We can conclude that the structure of saraine A [**3**] is characterized by a central system (partial structure **b**) containing an aldehyde group and a tertiary amine moiety, interacting for a "proximity effect," which strongly modifies the ir and nmr properties of **3**. In particular, the nmr spectra of **3** in neutral solution were devoid of the typical resonances due to the aldehydic moiety (only H-2 was observed as a broad signal at ca. δ 8.5, whereas no carbonyl resonances, probably being too broad, were detected in the 220–180 δ region of the ^{13}C-nmr spectra). However, **3** evolves to a charged pseudobase (partial structure **c**) by acid treatment. The relevant chemical shifts due to the presence of the charged nitrogen atom are reported in Table 1.



b **c**

It is relevant to observe that the first studies of Leonard *et al.* (6) followed the suggestion of Kermack and Robinson (7) that in some alkaloids the low carbonyl reactivity and the weakened basic character of the amine group could be due to an electronic nitrogen-carbonyl interaction.

We tried to reduce the aldehyde group of **3**: no reduction products were obtained with $NaBH_4$, while catalytic (C/Pd) reduction yielded a hexahydroderivative which at the nmr analysis (Experimental) confirmed the presence of the pseudobase moiety.

Saraine A [**3**] has a completely different alkaloid skeleton (**d** in Figure 2) from that of saraines 1–3. However, looking at the skeleton (**e** in Figure 2) of saraine 2 [**1**], we observe that both **1** and **3** show a saturated 10-membered alkyl chain in their skeletons. It is fascinating to suggest a related biogenetic origin for these alkaloids. The formal break of some C-C linkages of the two alkaloids skeletons, as shown in Figure 2, leads to two macrocyclic alkaloids **f** and **g**, which biogenetically could derive by oxidative coupling of either 3-alkyl or *N*-alkyl piperidine precursors. In the former case, it seems that the same precursor (**h**) could give both saraine 2 [**1**] and saraine A [**3**] by coupling with 3-alkyl piperidine containing, respectively, 10 and 12 carbons in the alkyl chain.

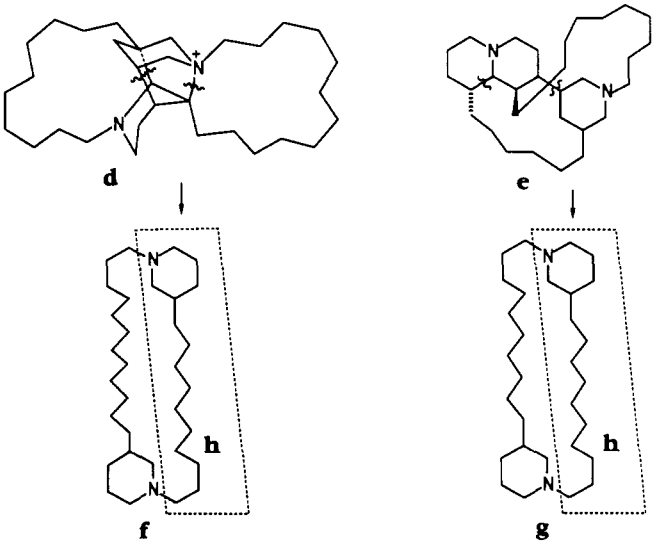


FIGURE 2. Biogenetic hypothesis correlating the alkaloid skeleton of saraine A (**d**) to that of saraine 2 (**e**).

This biogenetic hypothesis links the origin of saraines to that of other marine quinolizidine alkaloids, petrosins (8,9), and xestospongins (10), as previously suggested by our group (2) and reproposed by Kobayashi *et al.* (11) on the basis of the finding in the same sponge *Xestospongia* sp. of both quinolizidine and 1-oxa-quinolizidine alkaloids. More recently (12), two macrocyclic alkaloids found in a sponge of the genus *Haliclona*, exhibiting piperidine cycles (e.g., haliclamine B [**4**]), give further support to the above hypothesis.

Saraines fall in an only recently emerging group of new alkaloids that generally display a series of interesting biological properties (e.g., vasodilative, antineoplastic, cytotoxic). These alkaloids are stimulating interest in further studies aimed at knowing their biosynthetic origin and their effective biological role in the life cycle of the sponges and also at confirming their structural peculiarities by synthesis.[4]

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All the experiments were carried out on saraine A obtained following the isolation procedure described in previous papers (2,4). Nmr spectra were recorded on a Bruker WM-500 spectrometer. 2D experiments were performed using standard microprograms of Bruker software. AEI MS-30 (eims) and Kratos MS-50 (hreims) instruments were used for obtaining mass spectra.

[4]C.H. Heathcock, personal communication.

Uv spectra were recorded on a Varian DMS 90. Ir spectra were recorded in liquid film using a Nicolet DX FT spectrometer.

BIOLOGICAL MATERIAL.—The sponge *R. sarai* was collected in the Bay of Naples by the staff of the Stazione Zoologica. A voucher specimen is available at the "Natural Products Department" of ICMIB.

SARAINE A.—Amorphous powder: $[\alpha]_D$ +66.0° (c = 1.3; $CHCl_3$); ir ν max (liquid film) 2940, 2860, 1660 cm^{-1}; the carbonyl adsorption disappears in presence of HCl; uv λ max (MeOH) 238 (ϵ 14.939) nm; eims *m/z* (%) $[M]^+$ 510 (60), 492 (38), 481 (100), 463 (42), 258 (70); hreims *m/z* 510.3795 ($C_{32}H_{50}N_2O_3$ requires 510.3821), *m/z* 258.2194 ($C_{18}H_{28}N$ requires 258.2222).

A sample of 50 mg of **3** in 0.5 ml of $CDCl_3$ (TMS internal reference) was used for nmr experiments; nmr data are in Table 1.

INFLUENCE OF ACIDITY ON NMR SPECTRAL FEATURES OF SARAINE A.—The evaluation of the influence of acidity on saraine A was performed following the changes of nmr spectra induced by adding increasing amounts of CD_3COOD up to a molecular ratio CD_3COOD to saraine A of 2:1.

A new sample of 65 mg of **3** was used for this study, and probably because of a different degree of interaction between the carbonyl and the tertiary amine groups, some chemical shifts were slightly different from those observed for the previous sample of 50 mg. During the addition of CD_3COOD several shifts of signals were observed in ^{13}C- and ^1H-nmr spectra. When the sample contained an equimolecular amount of **3** and CD_3COOD no further relevant shifts were observed when adding acid. Due to this observation, a sample containing this ratio was used for an extensive analysis by 2D experiments (Table 1). Some selected ^{13}C-nmr shifts observed during this study are reported in Table 2.

TABLE 2. Selected Shifts Observed in the ^{13}C-nmr Spectrum of **3** after the Addition of CD_3COOD up to Molar Ratio CD_3COOD/Saraine A = 1.0.

Carbon	Molar ratio CD_3COOD/saraine A					
	0[a]	0.2	0.4[a]	0.6	0.8	1.0[a]
C-2	[b]	[b]	[b]	[b]	102.14	97.97
C-3	51.08	49.32	48.12	47.21	46.78	46.50
C-4	33.26	32.38	31.78	31.34	31.03	30.87
C-5	38.88	39.08	39.22	39.30	39.35	39.36
C-6	55.81	55.58	55.29	[b]	[b]	54.75
C-7	37.24	35.55	34.43	33.69	33.40	33.16
C-8	19.51	19.26	19.18	18.98	18.72	18.56
C-16	56.94	56.78	56.69	56.60	56.58	56.57
C-2′	65.43	61.74	59.06	[b]	[b]	55.76
C-7′	78.58	75.11	72.73	70.79	70.12	69.57
C-8′	70.76	69.93	69.45	69.08	68.82	68.69
C-9′	134.82	133.06	131.54	131.07	130.48	130.01
C-10′	125.25	125.75	126.14	126.36	126.51	126.68
C-11′	129.40	129.33	129.31	129.28	129.28	129.30
C-12′	129.79	130.22	130.51	130.70	130.80	130.80
C-14′	128.85	128.54	128.40	128.25	128.17	128.04
C-15′	127.31	126.68	126.28	125.99	125.86	125.69
C-18′	54.04	54.18	54.36	54.50	54.60	54.75

[a]Values assigned on the basis of ^1H-^{13}C-2D-COSY.

[b]Broad signal not easily assignable in the spectrum.

HYDROGENATION OF SARAINE A.—Saraine A (5 mg) was dissolved in EtOH (10 ml), and 10% Pd/C (1 mg) was added; the mixture was stirred under atmosphere of H_2 (2 atm, 60 h). The solution was filtered and the solvent removed to obtain a crude product which was chromatographed on Si gel [1 g, $CHCl_3$-MeOH (9:1)], affording two fractions containing products that from eims spectra were both shown to be the hexahydroderivative of **3**. However, one of them gave a ^1H-nmr spectrum with very broad lines while the other one afforded a well resolved spectrum that allowed the nmr analysis of the compound.

HEXAHYDRODERIVATIVE OF SARAINE A.—Eims *m/z* (%) $[M]^+$ 516 (7), 498 (20), 469 (90), 454 (31), 258 (100); nmr ($CDCl_3$) δ ^{13}C-δ ^1H (resonances assigned on the basis of 2D ^1H-^{13}C-COSY and ^1H-^1H decouplings) 97.5 (d, C-2) 4.91, 85.6 (s, C-3′) 69.2 (d, C-7′) 4.15, 68.7 (d, C-8′) 3.74; 56.8 (t, C-16) 2.89, 3.03, 54.6 (d, C-2′) 3.77, 54.0 (t, C-6) 2.95, 3.96; 54.0 (t, C-18′) 3.75, 4.05; 46.4 (s, C-3); 44.6 (t, C-6′) 2.83, 3.18; 40.8 (d, C-4′ or C-5) 2.22; 39.8 (d, C-4′ or C-5) 2.52; 33.3 (t, C-7) 1.49, 1.70; 31.6 (t, C-9′) 1.68; 31.3 (t, C-4) 2.03, 2.17; 28.0–1.56, 1.30; 27.9–1.56; 26.8–1.52, 1.33; 26.2–1.64, 1.42; 25.8–1.38, 1.56; 25.6–1.71, 1.26; 25.3–1.27, 1.52; 25.0 (t, C-5′) 2.13, 2.33; 24.8–1.25, 1.49; 24.6–1.36; 24.1–1.28; 23.7–1.48, 1.58; 18.8 (t, C-8) 1.42, 1.62.

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