

UNPRECEDENTED ALKALOID SKELETON FROM  
THE MEDITERRANEAN SPONGE *RENIERA SARAI*:  
X-RAY STRUCTURE OF AN ACETATE DERIVATIVE OF SARAIN-A<sup>1</sup>

G. CIMINO\*, C. A. MATTIA<sup>o</sup>, L. MAZZARELLA<sup>o</sup>, R. PULITI\*,  
G. SCOGNAMIGLIO\*, A. SPINELLA\*, E. TRIVELLONE\*

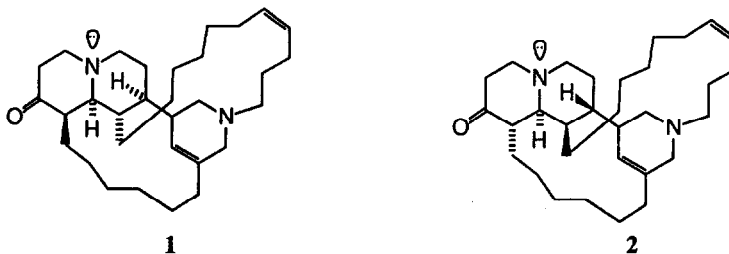
\* Istituto per la Chimica di Molecole di Interesse Biologico del C. N. R.  
Via Toiano n.6, 80072, Arco Felice, Napoli, Italy  
<sup>o</sup> Dipartimento di Chimica, Università  
Via Mezzocannone n. 4, 80134, Napoli, Italy

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**Abstract.**- The Mediterranean sponge *Reniera sarai* is a rich source of new alkaloids. Until now the study has been limited to the less polar compounds. In this paper we report the first studies on the more polar UV absorbing alkaloid fraction, which resulted a mixture of three homologs, sarains A-C. A resolute X-ray study on an acetate derivative of sarain-A has discovered an unprecedented alkaloid skeleton exhibiting a central cage structure, characterized by a charged pseudobase moiety, in the middle of two cyclic alkyl chains.

The metabolic pattern of the Mediterranean sponge *Reniera sarai* is heavily characterized by the presence of a series of unique complex polycyclic alkaloids, named sarains<sup>2,3,4</sup>. In fact, the SiO<sub>2</sub> t.l.c. analysis (CHCl<sub>3</sub>: CH<sub>3</sub>OH, 9:1) of the *n*-butanol soluble fraction from the defatted acetone extracts of *R. sarai* revealed, after exposition to iodine vapours, four main well resolved spots of which only the most polar was UV absorbing. The compounds were named in order of increasing polarity sarain-1, sarain-2 and sarain-3 while the UV absorbing fraction resulted a mixture of three homologs named in order of increasing molecular weight sarain-A, sarain-B and sarain-C. Recently<sup>3</sup>, the structures of sarains 1-3 have been partially characterized. Subsequently<sup>4</sup>, the structure elucidation of a minor component of *R. sarai*, isosarain-1 (1) stereoisomer of sarain-1 (2), enabled us to definitively assign to all these alkaloids a pentacyclic skeleton characterized by a *trans*-quinolizidine moiety linked to an unsaturated piperidine ring. The two heterocyclic systems were further jointed by two alkyl chains accounting for the remaining two cycles. Sarain-2 and sarain-3 are closely related to 2 exhibiting differences only in the longest alkyl chain.

The authors dedicate this paper to the memory of Professor Edgar Lederer, President of the Scientific Council of ICMIB from 1968 to 1982.



The study of the UV absorbing fraction led, after a series of tedious and repetitive steps, to sarains A-C which, analogously to sarains 1-3, resulted to belong to a homologous series. In fact the molecular formulae, deduced by accurate mass measurements (HRMS), were  $C_{32}H_{50}N_2O_3$  (sarain-A),  $C_{33}H_{50}N_2O_3$  (sarain-B) and  $C_{34}H_{52}N_2O_3$  (sarain-C). Furthermore, sarains A-C exhibited a series of common peculiarities. In their mass spectra a relevant peak (at  $m/z$  258, 270 and 284, respectively), resulting from loss of the same fragment  $C_{14}H_{22}NO_3$ , has always been recorded. Sarains A-C showed UV maxima at  $\sim 238$  nm characteristic of a conjugated diene chromophore. The  $^1H$ -NMR (experimental) and  $^{13}C$ -NMR (Table 1) spectra of these alkaloids were closely related. In particular the analysis of the  $^1H$ -NMR spectra, by spin decoupling and by  $^1H$ - $^1H$  COSY experiments, led to the common partial structure:



In fact, all the  $^1H$ -NMR spectra of sarains A-C showed a sharp doublet at  $\sim \delta$  4.10 assigned to the methine proton of a secondary alcoholic group. This methine was linked, on the basis of spin decoupling evidence, to a 1,4 disubstituted diene system jointed to a bisvynilic methylene by  $^1H$ - $^1H$  COSY evidence. All the  $^1H$ -NMR spectra exhibited two characteristic singlets at  $\sim \delta$  4.20 and  $\sim \delta$  3.50, while signals attributable to two further olefinic protons were present only in the spectra of sarain-B ( $\delta$  5.37 and 5.25) and sarain-C ( $\delta$  5.37 and 5.20). Strangely the IR spectra of sarains A-C showed a large band at  $1650\text{ cm}^{-1}$ , while the  $^{13}C$ -NMR spectra were devoid of signals in the region between  $\delta$  220 and 150. On the basis of these conflicting data, the cycles in sarains A-C should be either 5 plus a carbonyl group or 6. Most likely this problem would have remained unresolved without the resolute aid of the crystallographic analysis.

In fact, during the chromatographic purification of an acetylated derivative of sarain-A, we obtained a crystalline product which, after recrystallization from ethyl acetate, provided us crystals suitable for the X-ray study.

The structure of di-acetylated derivative of sarain-A (3) was solved using direct methods and difference Fourier syntheses and was refined to a crystallographic R factor of 0.044. A view of the final X-ray model is shown in Fig. 1 and the atomic parameters for non-hydrogen atoms are reported in Table 2. The structure, which emerged from the electron density map, has a totally new architecture and presents several interesting features. The molecule is characterized by a charged tetracyclic nucleus, which includes a quaternary nitrogen atom and is neutralized by an acetate-acetic acid system. The central nucleus is formed by two condensed piperidine rings surrounded by two alkyl chains which form a thirteen and fourteen membered cycle respectively. The fully saturated thirteen membered macrocycle (a) embodies two carbon atoms of the central cage and the piperidine nitrogen N1'. The other macrocycle (b) contains three double bonds and embodies the quaternary nitrogen N1 and one carbon atom of the cage. In the cage the eight C-C bond lengths range from 1.501(4) to 1.555(3)Å, with a mean value of 1.535Å,

and the distances involving the quaternary nitrogen are  $N1-C6 = 1.534(3)$ ,  $N1-C2 = 1.548(2)$  and  $N1-C3' = 1.630(3)\text{\AA}$ . The long value of the last bond distance is probably caused by the geometrical strain in the cage structure which requires the substituents at  $N1$  and  $C3'$  in an eclipsed conformation [ $C2-N1-C3'-C2' = -2.6^\circ$ ,  $C6-N1-C3'-C4' = -1.5^\circ$ ,  $C18'-N1-C3'-C7' = -7.3^\circ$ ]. In contrast the  $N1-C18'$  bond length, external to the cage, is only  $1.504(3)\text{\AA}$ . The valency angles around the C atoms, not involving heteroatoms, can be divided into three subgroups with average values of  $109.9^\circ$  (six-membered rings),  $101.0^\circ$  (five-membered rings) and  $114.2^\circ$  (external to rings). In the uncharged piperidine ring, the C-C and C-N distances are on average  $1.537$  and  $1.473\text{\AA}$  respectively, while the valency angle at the nitrogen  $N1'$  is  $116.4(4)^\circ$  and the mean values of the bond angles C-C-C and N-C-C are  $112.3^\circ$  and  $113.8^\circ$  respectively.

Table 1 - 125.8 MHz  $^{13}\text{C}$ -NMR data of sarains A-C <sup>a</sup>

Sarain-A	Sarain-B	Sarain-C
$\delta$ (m) <sup>b</sup>	$\delta$ (m) <sup>b</sup>	$\delta$ (m) <sup>b</sup>
135.3 (d)	134.5 (d)	135.1 (d)
129.9 (d)	131.6 (d)	130.0 (d)
129.6 (d)	129.8 (d)	129.7 (d)
129.0 (d)	129.4 (d)	129.5 <sup>c</sup> (d)
127.6 (d)	128.8 (d)	128.9 (d)
125.3 (d)	128.1 (d)	127.4 (d)
79.0 (d)	127.1 (d)	125.2 (d)
70.9 (d)	125.5 (d)	79.1 (d)
65.7 (d)	77.7 (d)	70.9 (d)
64.0 (s)	70.6 (d)	66.9 (d)
57.0 (t)	67.3 (s)	63.6 (s)
55.9 (t)	66.1 (d)	56.4 (t)
54.0 (t)	57.0 (t)	56.2 (t)
51.3 (s)	56.0 (t)	53.9 (t)
44.0 (t)	54.2 (t)	51.8 (s)
38.9 (d)	50.4 (s)	44.3 (t)
38.6 (d)	43.4 (t)	38.7 (d)
37.4 (t)	39.0 (d)	38.5 (d)
33.3 (t)	38.9 (d)	37.1 (t)
28.0 (t)	36.4 (t)	32.7 (t)
27.6 (t)	32.9 (t)	29.6 (t)
27.5 (t)	27.1 (t)	28.1 (t)
25.8 (t)	26.9 (t)	28.0 (t)
25.6 (t)	26.4 (t)	27.7 (t)
25.5 (t)	26.1 (t)	27.2 (t)
25.2 (t)	25.9 <sup>c</sup> (t)	26.3 (t)
25.0 (t)	25.5 (t)	25.8 (t)
24.9 (t)	24.7 <sup>c</sup> (t)	25.6 (t)
24.5 (t)	24.1 (t)	25.5 (t)
24.0 (t)	20.9 (t)	25.3 (t)
19.5 (t)		24.6 (t)
		24.2 (t)

<sup>a</sup> The spectra were recorded in  $\text{CDCl}_3$ , TMS was used as internal reference.

<sup>b</sup> Determined by DEPT sequence.

<sup>c</sup> Two resonances superimposed.

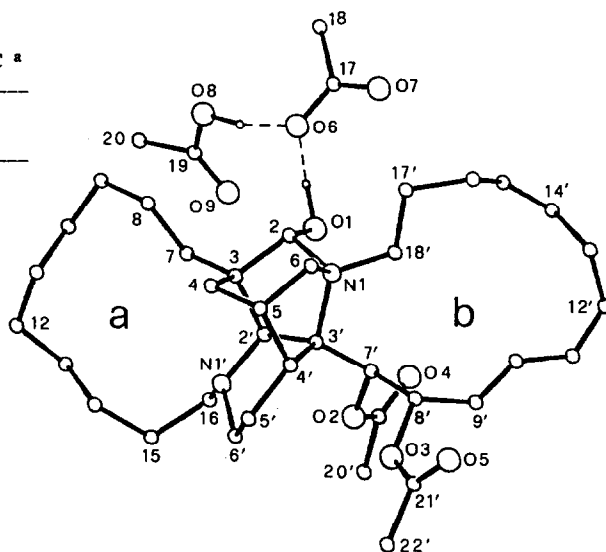
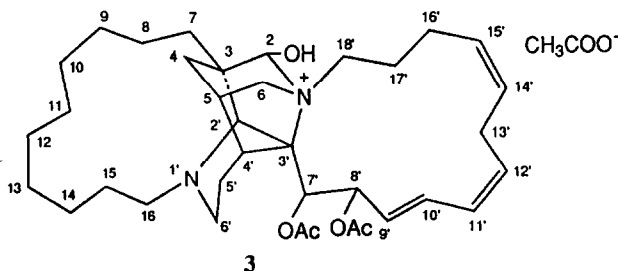


Fig. 1 - Perspective view of the X-ray structure of diacetyl sarain-A and acetic acid system with atomic labelling, numbers denote C atoms unless indicated otherwise. For clarity the H-atoms are omitted except those which take part in hydrogen bonding.



**Table 2** - Positional parameters and equivalent isotropic thermal parameters ( $A^2$ )  
with e.s.d.'s in parentheses for non-hydrogen atoms  
 $B_{eq} = 4/3 \sum_i \sum_j b_{ij} a_i \cdot a_j$

*Sarain-A di-acetylated derivative*

Atom	x	y	z	$B_{eq}$	Atom	x	y	z	$B_{eq}$
O1	0.9698(1)	0.3587(0)	0.4332(1)	3.58(3)	C2'	0.8146(2)	0.4058(2)	0.5264(2)	3.56(5)
O2	0.8841(1)	0.2996(2)	0.7195(1)	4.18(4)	C3'	0.9036(2)	0.4565(2)	0.6177(2)	3.37(4)
O3	0.9395(1)	0.4421(2)	0.8657(1)	4.64(4)	C4'	0.8600(2)	0.5629(2)	0.6484(2)	4.12(5)
O4	0.9844(2)	0.1618(2)	0.6941(2)	7.01(6)	C5'	0.7484(2)	0.5536(3)	0.6664(2)	5.48(6)
O5	1.0506(2)	0.5541(3)	0.9758(2)	7.49(6)	C6'	0.6963(2)	0.4457(4)	0.6405(2)	5.68(7)
N1	0.9876(1)	0.4995(2)	0.5566(1)	3.24(4)	C7'	0.9594(2)	0.3758(2)	0.7027(2)	3.53(4)
N1'	0.7065(2)	0.4043(2)	0.5403(2)	4.68(5)	C8'	1.0192(2)	0.4235(2)	0.8101(2)	3.97(5)
C2	0.9391(2)	0.4635(2)	0.4413(2)	3.31(4)	C9'	1.1046(2)	0.3487(3)	0.8691(2)	4.59(6)
C3	0.8212(2)	0.4742(2)	0.4315(2)	3.62(5)	C10'	1.2056(2)	0.3610(3)	0.8678(2)	5.00(6)
C4	0.7924(2)	0.5921(2)	0.4507(2)	4.37(6)	C11'	1.2918(3)	0.2840(3)	0.9083(2)	5.48(7)
C5	0.8634(2)	0.6348(2)	0.5564(2)	4.33(6)	C12'	1.3823(3)	0.2852(4)	0.8834(3)	6.27(8)
C6	0.9780(2)	0.6223(2)	0.5583(2)	4.14(5)	C13'	1.4128(2)	0.3639(4)	0.8113(3)	6.70(9)
C7	0.7504(2)	0.4307(3)	0.3267(2)	4.29(5)	C14'	1.3729(3)	0.3248(4)	0.6971(3)	6.09(8)
C8	0.7363(2)	0.5078(3)	0.2324(2)	5.35(7)	C15'	1.3216(2)	0.3806(3)	0.6135(3)	5.76(7)
C9	0.6681(3)	0.4604(4)	0.1278(2)	5.95(8)	C16'	1.2923(2)	0.4965(3)	0.6061(4)	5.62(7)
C10	0.5695(3)	0.4033(5)	0.1332(3)	7.9(1)	C17'	1.1763(2)	0.5190(3)	0.5430(2)	4.72(6)
C11	0.4925(3)	0.4693(7)	0.1752(3)	9.9(2)	C18'	1.1025(2)	0.4668(2)	0.5992(2)	3.70(5)
C12	0.4082(3)	0.3989(9)	0.2014(4)	12.5(3)	C19'	0.9089(2)	0.1931(3)	0.7187(2)	4.83(6)
C13	0.4489(3)	0.3242(7)	0.2924(4)	11.7(2)	C20'	0.8324(4)	0.1265(3)	0.7553(3)	6.68(9)
C14	0.4752(3)	0.3759(7)	0.4011(3)	9.6(2)	C21'	0.9668(3)	0.5085(3)	0.9490(2)	5.13(6)
C15	0.5328(2)	0.3021(5)	0.4932(3)	8.3(1)	C22'	0.8798(3)	0.5169(4)	1.0005(2)	7.40(8)
C16	0.6570(2)	0.2988(4)	0.5147(3)	6.47(8)					

*Acetate-acetic acid system*

O6	1.0127(2)	0.3256(2)	0.2587(1)	5.27(5)	C17	1.1060(2)	0.3338(2)	0.2508(2)	4.26(5)
O7	1.1827(2)	0.3618(2)	0.3232(2)	6.05(5)	C18	1.1217(3)	0.3046(4)	0.1468(2)	6.03(8)
O8	0.8568(2)	0.2661(2)	0.1062(2)	5.95(6)	C19	0.7999(3)	0.1855(3)	0.1234(3)	5.56(7)
O9	0.8179(2)	0.1386(2)	0.2055(2)	6.77(6)	C20	0.7122(3)	0.1539(5)	0.0280(3)	7.90(9)

The conformation of the saturated alkyl chain, which forms the thirteen membered ring (a), can be described by the sequence of the conformational states *tggtggtgg*. The values of the endocyclic torsion angles are  $178^\circ$ ,  $44^\circ$ ,  $57^\circ$ ,  $-167^\circ$ ,  $68^\circ$ ,  $76^\circ$ ,  $-171^\circ$ ,  $85^\circ$  and  $60^\circ$ , starting from the torsion angle around the bond C7-C8. The regular increase of  $B_{eq}$ 's (Table 2) starting from C7 and C16 toward C12 is dominated by the increase of the  $B_{22}$  component of the anisotropic thermal parameters (deposited material), which indicates a large oscillation of this part of the structure in a direction almost normal to the average plane of the ring. The large thermal motion of the flexible unsubstituted part of the saturated ring is clearly correlated to the looseness of the packing in this region (Fig. 2).

The fourteen membered ring (b) includes one *trans* and two *cis* double bonds involving the atoms C9', C10', C11' C12', C14' and C15'. In the diene system, the torsion angle about the C10'-C11' bond, which separates the *trans* and the *cis* double bond, is  $162.6(7)^\circ$  so that the structure formed by the six carbon atoms from C8' to C13' is approximately planar. In the macrocycles the C(sp<sup>3</sup>)-C(sp<sup>3</sup>) bonds are on average  $1.530\text{\AA}$  and the corresponding values for the C(sp<sup>3</sup>)-C(sp<sup>2</sup>) and C(sp<sup>2</sup>)=C(sp<sup>2</sup>) bonds are  $1.505$  and  $1.324\text{\AA}$ . The two acetoxy groups linked

to C7' and C8' of the macrocycle **b** are planar within the experimental errors and their geometrical parameters have the expected values. The chiral centres at C7' and C8' have the same configuration. The configuration presented in this paper was chosen arbitrarily as the absolute configuration was not established.

Molecular packing is shown in Fig. 2. Each molecule of sarain is linked to the acetate ion by a strong hydrogen bond between the hydroxyl group O1-H and the carbonylic oxygen O6: [O1.....O6=2.567(2)Å]. The oxygen O6 is also involved in a second H bond with the hydroxyl group O8-H of the acetic acid molecule at a distance of 2.542(2)Å. The hydrogen atoms, involved in hydrogen bonds, stand out clearly from the difference Fourier map and are properly placed for the H-bonding interaction; the D-H.....A angle values are 168° and 166° respectively.

The bond distances observed in the acetate-acetic acid system are also correlated to the hydrogen bonding network. The acetic acid molecule shows a large difference in the values of the two C-O bond lengths [C19-O8 = 1.308(4)Å and C19-O9 = 1.199(3)Å]. In the acetate moiety these bond lengths are more similar, although the distance involving O6, which acts as acceptor in two strong hydrogen bonds, is significantly longer than the other [C17-O6 = 1.260(3), C17-O7 = 1.226(3)Å].

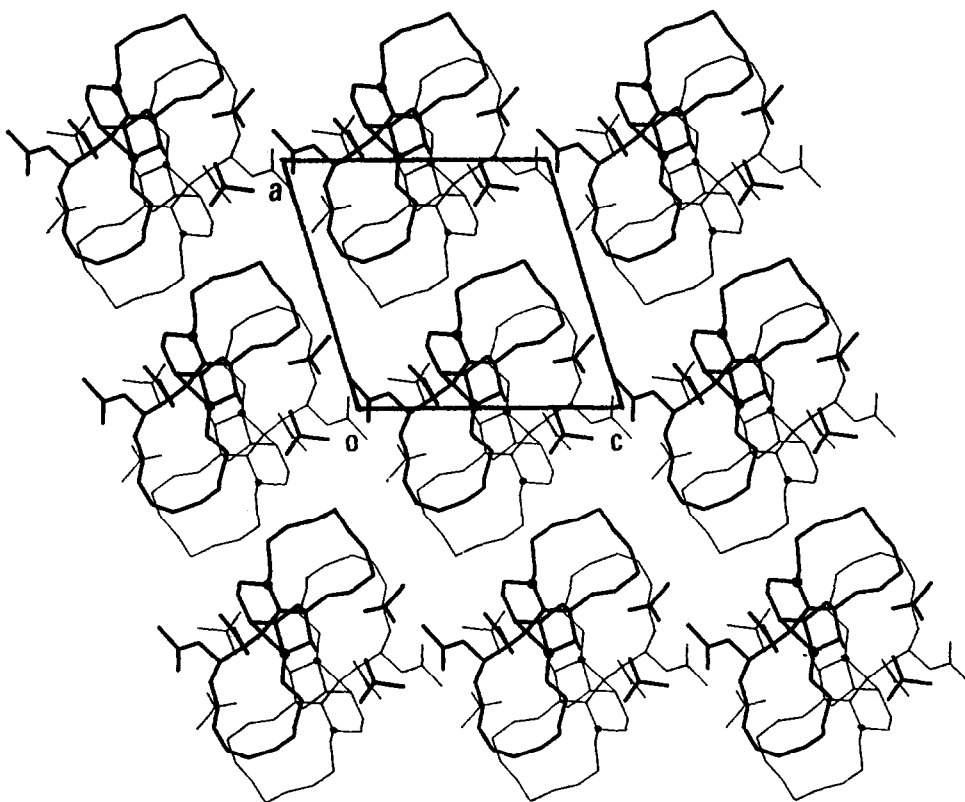


Fig. 2 - Crystal packing along the **b** direction of diacetyl derivative of sarain-A: filled circles indicate nitrogen atoms.

**Table 3** - NMR data of **3** after treatment with 0.1M NaHCO<sub>3</sub><sup>a</sup>

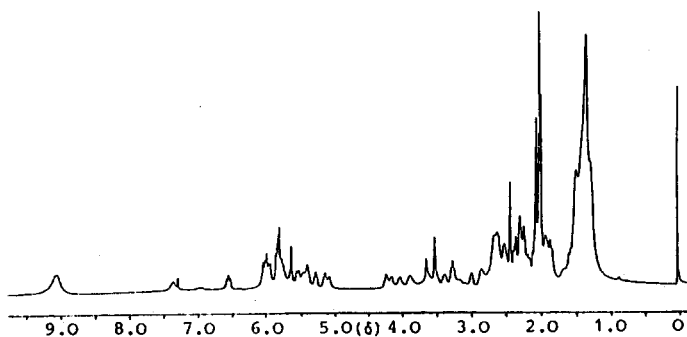
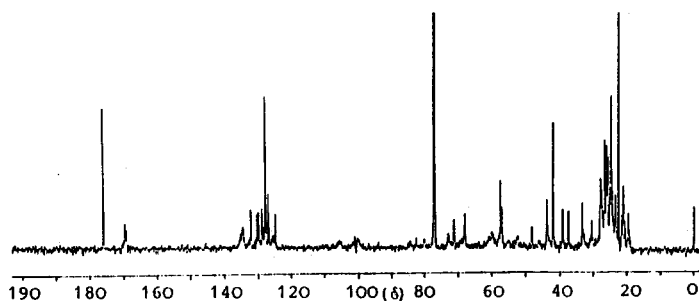
$\delta^{13}\text{C}$	(m) <sup>b</sup>	$\delta^1\text{H}^c$
170.1	(s)	-
169.4	(s)	-
132.8	(d)	5.74
131.3	(d)	7.00
130.2	(d)	5.43
126.9	(d)	5.43
126.8	(d)	6.05
125.7	(d)	5.68
78.5	(d)	*
74.7	(d)	6.10
68.9	(s)	-
64.9	(d)	3.61
58.3	(t)	2.90
57.7	(t)	2.62
54.7	(t)	2.76
51.8	(s)	-
43.5	(t)	3.12-2.69
40.5	(d)	1.86
38.6	(d)	2.03
36.8	(t)	1.38
32.0	(t)	1.55
28.8	(t)	1.50
27.2	(t)	1.40-1.26
26.6	(t)	3.09-2.78
25.8	(t)	1.34-1.47
25.1	(t)	2.04
24.9	(t)	1.26-1.43
24.7	(t)	1.34
22.9	(t)	2.10-1.64
21.3	(q)	2.09
21.2	(q)	2.09
19.6	(t)	1.40-1.20

<sup>a</sup> Spectra recorded in CDCl<sub>3</sub>, TMS was used as internal reference.

<sup>b</sup> Determined by DEPT sequence.

<sup>c</sup> Correlated by <sup>1</sup>H-<sup>13</sup>C 2D experiments.

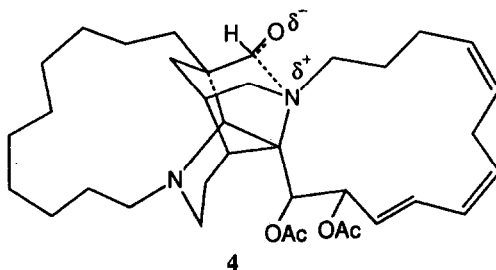
\* No correlation observed.

**Fig. 3a** - 500 MHz <sup>1</sup>H-NMR spectrum of **3** (CDCl<sub>3</sub>, TMS = 0).**Fig. 3b** - 125.8 MHz <sup>13</sup>C-NMR spectrum of **3** (CDCl<sub>3</sub>, TMS = 0).

The <sup>1</sup>H-NMR spectra (Fig. 3a) of the crystalline compound (**3**) were uninterpretable and strongly influenced by the temperature. In fact, relevant differences were detected varying the temperature of the probe from 223K to 323K. However, the signals were always highly broadened suggesting that **3** in CDCl<sub>3</sub> solution adopts different conformations. The <sup>13</sup>C-NMR spectra (Fig. 3b) were also uninterpretable and confirmed the presence of conformers or isomers showing more signals than those expected. Washing the chloroformic solution of **3** with 0.1 M NaHCO<sub>3</sub> it was possible to obtain quite well-resolved <sup>1</sup>H and <sup>13</sup>C NMR spectra which, even though some <sup>13</sup>C resonances were very low and broad, were correlated by <sup>1</sup>H-<sup>13</sup>C 2D experiments (Table 3). However, even bearing in mind the X-ray structure of **3**, it was not possible to rationalize these data. In particular, no resonances attributable to the methine adjacent to the quaternary nitrogen were observed. In fact, the expected regions, <sup>13</sup>C  $\delta$  110-90 and <sup>1</sup>H  $\delta$  5.0-4.5, were

completely devoid of signals. Simultaneously, no aldehydic resonances were observed. The apparent anomaly could be rationalized supposing that the treatment of 3 with  $\text{NaHCO}_3$ , subtracting the acetic counterion, delivers a neutral molecule ( $M^+$  at  $m/z$  594 and not 595) 4 containing an aldehydic function and a tertiary amine group, which for a proximity effect, analogous to the transannular one described by Leonard<sup>5</sup>, strongly interact altering the spectral properties of the carbonyl group. Studies are in progress on this particular aspect.

The carbon skeleton of sarain-A is unprecedented among the natural alkaloids and it is completely different from those exhibited by sarains 1-3 and by other polycyclic alkaloids found recently in marine sponges petrosins<sup>6,7,8</sup>, xestospongins<sup>9</sup>, manzamines<sup>10,11,12</sup>, keramines<sup>13</sup>, papuamine<sup>14</sup> and haliclونadamine<sup>15</sup>. However, it is worth noting that a saturated linear alkyl chain containing 10 carbons is present in both the minor members of the two series, sarain-2 and sarain-A.



## EXPERIMENTAL

$^1\text{H}$  and  $^{13}\text{C}$ -NMR spectra were measured on a WM 500 Bruker spectrometer ( $\delta$  ppm/TMS). The 2D NMR spectra were obtained using Bruker's microprograms. Mass spectra were taken on AEI MS-30 (EIMS) and Kratos MS 50 (HREIMS and FAB/MS) instruments. UV spectra were recorded on a Shimadzu-Bausch and Lomb Spectronic 210 apparatus. IR spectra were recorded in liquid film with a Nicolet DX FT spectrometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. Melting points were determined on a Kofler hot-stage microscope and are uncorrected.

Pre-coated Kieselgel 60 F254 plates (Merck) were used for TLC; spots were detected by exposing to iodine vapour. Column chromatography was carried out on Merck Kieselgel 60 powder (0.063-0.200 mm). Lichoprep Si60 (0.025-0.040 mm) was used for under pressure column chromatography on the LC Jobin Yvon apparatus.

### Isolation procedure:

*R. sarai* (520 g, dry weight after extraction) was collected in the Bay of Naples and extracted as previously described<sup>3</sup>. The n-butanol soluble fraction (35 g) from the defatted acetone extract was chromatographed on an overloaded Si gel column (300 g, step gradient elution from  $\text{CHCl}_3$  :  $\text{CH}_3\text{OH}$  99 : 1 to  $\text{CHCl}_3$  :  $\text{CH}_3\text{OH}$  30 : 70) collecting, along with the previously reported<sup>3</sup> sarains 1-3, an UV absorbing fraction (8.2 g) which resulted by TLC (BAW, n-butanol-acetic acid-water, 60-15-25) analysis to be a mixture of three compounds sarain-B ( $R_f$  = 0.8), sarain-A ( $R_f$  = 0.7) and sarain-C ( $R_f$  = 0.65). This mixture was rechromatographed on Si gel column (80 g, step gradient elution from  $\text{CHCl}_3$  :  $\text{CH}_3\text{OH}$  98 : 2 to  $\text{CHCl}_3$  :  $\text{CH}_3\text{OH}$  80 : 20) obtaining in order of elution: a mixture of sarain-A and sarain-B (6.451 g), a mixture of sarains A-C (1.140 g) and sarain-C (400 mg). A fraction (1.5 g) of the mixture of sarain-A and sarain-B was

partially resolved using under pressure (15 at) chromatography on Miniprep (60 g Lichoprep Si 60, BAW). 36 fractions (~ 10 ml) were collected: 9-12, sarain-B (170 mg); 13-21; sarain-A + sarain-B (600 mg); 22-36, sarain-A (220 mg).

After every chromatographic separation the recovered fractions were dissolved in  $\text{CHCl}_3$  and washed with water to remove all traces of salt. It has to be pointed out that the strong tendency of sarains to be absorbed irreversibly to solid phases makes every chromatographic procedure seldom reproduceable.

#### Sarain-A:

Amorphous powder;  $[\alpha]_D = 66.0^\circ$  (c 1.3,  $\text{CHCl}_3$ ); IR  $\nu_{\text{max}}$  (liquid film): 2940, 2860, 1660  $\text{cm}^{-1}$ ; UV  $\lambda_{\text{max}}$  ( $\text{CH}_3\text{OH}$ ): 238 ( $\epsilon$  14.939) nm; EIMS, m/z (%): 510 ( $\text{M}^+$ , 60), 492 (38) 481 (100), 463 (42), 258 (70); HREIMS: m/z 510.3795 ( $\text{C}_{32}\text{H}_{50}\text{N}_2\text{O}_3$  requires 510.3821), m/z 258.2194 ( $\text{C}_{18}\text{H}_{28}\text{N}$  requires 258.2222).

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 6.46 (1H, dd, J = 10.5 and 15.5 Hz) 6.03 (1H, t, J = 10.5 Hz), 5.72 (2H, m), 5.20 (1H, m), 5.15 (1H, m), 4.22 (1H, s), 4.10 (1H, d, J = 7.9 Hz), 3.59 (1H, s), other resonances between 3.14 and 1.21  $\delta$ . Resonances assigned to the bisvynilic methylene by  $^1\text{H-}^1\text{H}$  COSY:  $\delta$  3.13 and 2.50.

$^{13}\text{C-NMR}$ : the data are reported in Table 1.

#### Sarain-B:

Amorphous powder;  $[\alpha]_D = 76.3^\circ$  (c 1.2,  $\text{CHCl}_3$ ); IR  $\nu_{\text{max}}$  (liquid film): 2940, 2860, 1660  $\text{cm}^{-1}$ ; UV  $\lambda_{\text{max}}$  ( $\text{CH}_3\text{OH}$ ): 238 ( $\epsilon$  13.415); EIMS, m/z (%): 522 ( $\text{M}^+$ , 65) 504 (32), 493 (100), 475 (30), 270 (45); HREIMS m/z 522.3830 ( $\text{C}_{33}\text{H}_{50}\text{N}_2\text{O}_3$  requires 522.3821); m/z 270.2235 ( $\text{C}_{19}\text{H}_{28}\text{N}$  requires 270.2222).

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 6.42 (1H, dd, J = 10.6 and 15.8 Hz), 6.00 (1H, t, J = 10.6), 5.69 (2H, m), 5.37 (1H, m), 5.25 (1H, m), 5.18 (1H, m), 5.14 (1H, m), 4.26 (1H, s) 4.13 (1H, d, J = 7.5 Hz), 3.59 (1H, s), other resonances between 3.16 and 1.11  $\delta$ . Bisvynilic methylene:  $\delta$  3.04 and 2.56.

$^{13}\text{C-NMR}$ : the data are reported in Table 1.

#### Sarain C:

Amorphous powder;  $[\alpha]_D = 67.4^\circ$  (c 1.1,  $\text{CHCl}_3$ ); IR  $\nu_{\text{max}}$  (liquid film): 2940, 2860, 1660  $\text{cm}^{-1}$ ; UV  $\lambda_{\text{max}}$  ( $\text{CH}_3\text{OH}$ ): 238 ( $\epsilon$  13.582); EIMS, m/z (%): 536 ( $\text{M}^+$ , 60), 518 (30), 507 (100), 489 (75), 284 (40); HREIMS: m/z 536.3941 ( $\text{C}_{34}\text{H}_{52}\text{N}_2\text{O}_3$  requires 536.3978); m/z 284.2408 ( $\text{C}_{26}\text{H}_{30}\text{N}$  requires 284.2378).

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 6.46 (1H, dd, J = 10.7 and 15.8 Hz), 6.03 (1H, t, J = 10.4 Hz), 5.74 (2H, m), 5.37 (2H, m), 5.20 (1H, m), 5.16 (1H, m), 4.20 (1H, s), 4.10 (1H, d, J = 8.3 Hz), 3.6 (1H, s), other resonances between 3.19 and 1.16  $\delta$ . Bisvynilic methylene:  $\delta$  3.20 and 2.49.

$^{13}\text{C-NMR}$ : the data are reported in Table 1.

#### Acetylation of sarain-A and sarain-B:

A fraction (3 g) of the mixture of sarain-A and sarain-B was acetylated with  $\text{Ac}_2\text{O}$  (20 ml) in pyridine (20 ml) at r.t. for 24 h. The excess of reagents was removed by treatment with  $\text{H}_2\text{O}$  and extraction with  $\text{CHCl}_3$ . After removing *in vacuo* of the solvent, the residue (3.2 g) was chromatographed on a Si gel column (30 g) by stepwise elution from  $\text{CHCl}_3$ - $\text{CH}_3\text{OH}$  98 : 2 to  $\text{CHCl}_3$ - $\text{CH}_3\text{OH}$  80 : 20, 9 fractions were collected. After evaporation of the solvent the fraction 3 (320 mg) revealed a crystalline structure. Crystallization from ethyl acetate yielded plated prisms of 3: m.p. 152-154 $^\circ\text{C}$ ,  $[\alpha]_D$  150 $^\circ$  (c 0.7,  $\text{CHCl}_3$ ); IR  $\nu_{\text{max}}$  (liquid film) 1730, 1660  $\text{cm}^{-1}$ ; UV



$\lambda_{\max}$  (CH<sub>3</sub>OH); 242 ( $\epsilon$  16.530); FAB/MS *m/z* (%): 595 (M<sup>+</sup>, 100); EIMS *m/z* (%): 595 (M<sup>+</sup>, 55), 535 (75), 505 (78), 475 (100), 445 (95), 258 (80); HREIMS: *m/z* 595.4068 (C<sub>36</sub>H<sub>55</sub>N<sub>2</sub>O<sub>5</sub> requires 595.4111), *m/z* 258.2202 (C<sub>18</sub>H<sub>28</sub>N requires 258.2222).

<sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra are reported in fig. 3a and 3b, respectively.

3 after treatment with 0.1 M NaHCO<sub>3</sub>: <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra are reported in table 3; FAB/MS *m/z* (%): 595 (100); EIMS *m/z* (%): 594 (15), 534 (10), 505 (18), 475 (100), 445 (38), 258 (20).

### Crystal structure determination:

Single crystals of di-acetylated derivative of sarain-A were obtained, by careful recrystallization from ethyl acetate, in the form of colourless prisms elongated along *b*. Crystal of size 0.85x0.38x0.25mm was selected for the crystallographic study. Accurate cell parameters were obtained by least-squares refinement of the setting angles of 24 reflections at medium  $\theta$  ( $24^\circ \leq \theta \leq 30^\circ$ ), using Ni-filtered CuK $\alpha$  radiation and Enraf-Nonius CAD-4F diffractometer on line with a PDP11/34 Digital computer. Crystal data: C<sub>36</sub>H<sub>55</sub>N<sub>2</sub>O<sub>5</sub><sup>+</sup>·CH<sub>3</sub>COO<sup>-</sup>·CH<sub>3</sub>COOH, Mw = 714.95: monoclinic, space group P2<sub>1</sub> with Z = 2, a = 13.084(1), b = 12.450(1), c = 13.277(1)Å,  $\beta$  = 106.53(2)°, V = 2073.4(5) Å<sup>3</sup>, D<sub>c</sub> = 1.145 g/cm<sup>3</sup>. 3957 independent reflections ( $\theta_{\max}$  = 68°) were collected at room temperature, using  $\omega$ - $\theta$  scan mode, as suggested by peak-shape analysis. During the data collection the intensities of three standard reflections were monitored every 5 h (4% variation) in order to check the crystal and equipment stability. The intensities were corrected for Lorentz and polarization factors, but not for the absorption effect ( $\mu$  = 6.15 cm<sup>-1</sup>). The structure was solved by direct methods (MULTAN<sup>16</sup>) and difference Fourier syntheses. The positional and anisotropic temperature parameters for the 51 non-hydrogen atoms (the y coordinate of O1 was fixed to define the origin) were refined by full-matrix (on F) least-squares method to a value of the discrepancy index  $R = \sum ||F_o| - |F_c|| / \sum |F_o|$  of 0.08. At this stage all H atoms, on the basis of difference Fourier synthesis and geometrical considerations, were included in the last refinement as fixed atoms with the isotropic thermal parameters set equal to B<sub>eq</sub> of the parent atoms. At convergence the final value of R was 0.044 for the 3693 observed reflections with I  $\geq$  3.  $\sigma$ (I) (100, 020 and 143 excluded from final refinement for asymmetric background): R<sub>w</sub> = 0.066 with  $w^{-1} = \sigma^2(F_o)$ .

For this crystallographic work, the equipment of the "Centro di Metodologie Chimico-fisiche dell'Università di Napoli" and SDP package<sup>17</sup> was used. Scattering factors were taken from Cromer and Waber<sup>18</sup>.

For non-H atoms the final atomic parameters with esd in parentheses are given in Table 2. Structure factors, hydrogen atoms parameters and anisotropic thermal parameters have been deposited together with a list of the geometrical internal parameters.

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## REFERENCES AND NOTES

- 1.- This work was in part presented at the 16<sup>th</sup> International Symposium on the Chemistry of Natural Products, IUPAC, Japan 1988. Cimino, G.; Puliti, R.; Scognamiglio, G.; Spinella, A.; Trivellone, E.; Mattia, C. A.; Mazzarella, L., Abstracts SL a2, p. 53.
- 2.- Cimino, G.; De Rosa, S.; De Stefano, S.; Sodano, G. 1986, *Pure Appl. Chem.* 58, 375-386.
- 3.- Cimino, G.; De Stefano, S.; Scognamiglio, G.; Sodano, G.; Trivellone, E. 1986, *Bull. Soc. Chim. Belg.* 95, 783-800.
- 4.- Cimino, G.; Spinella, A.; Trivellone, E. *Tetrahedron Lett.* , in press.
- 5.- Leonard, N. J.; Adamick, J. A.; Djerassi, C.; Halpern, O. 1958, *J. Am. Chem. Soc.* 80, 4858-4862 and references therein.
- 6.- Braekman, J. C.; Daloze, D.; Macedo de Abreu, P.; Piccinni-Leopardi, P.; Germain, G.; Van Meerssche, M. 1982, *Tetrahedron Lett.* 23, 4277-4280.
- 7.- Braekman, J. C.; Daloze, D.; Defay, N.; Zimmermann, D. 1984, *Bull. Soc. Chim. Belg.* 93, 941-944.
- 8.- Braekman, J. C.; Daloze, D.; Cimino, G.; Trivellone, E. 1988, *Bull. Soc. Chim. Belg.* 97, 519522.
- 9.- Nakagawa, M.; Endo, M.; Tanaka, N.; Gen-pei, L. 1984, *Tetrahedron Lett.* 25, 3227-3230.
- 10.- Sakai, R.; Higa, T.; Jefford, W. C.; Bernardinelli, G. 1986, *J. Am. Chem. Soc.* 108, 6404-6405.
- 11.- Sakai, R.; Kohmoto, S.; Higa, T.; Jefford, C. W.; Bernardinelli, G. 1987, *Tetrahedron Lett.* 28, 5493-5496
- 12.- Ichiba, T.; Sakai, R.; Kohmoto, S.; Sancy, G.; Higa, T. 1988, *Tetrahedron Lett.* 29, 3083-3086.
- 13.- Nakamura, H.; Deng, S.; Kobayashi, J.; Ohizumi, Y.; Tomotake, Y.; Matsuzaki, T.; Hirata, Y. 1987, *Tetrahedron Lett.* 28, 621-624.
- 14.- Baker, B. J.; Scheuer, P. J.; Shoolery, J. N. 1988, *J. Am. Chem. Soc.* 110, 965-966.
- 15.- Fahy, E.; Molinsky, T. F.; Harper, M. K.; Sullivan, B. W.; Faulkner, D. J.; Parkanyi, L.; Clardy, J. 1988, *Tetrahedron Lett.* 29, 3427-3428.
- 16.- Main, P.; Fiske, S. J.; Hull, S. E.; Lessinger, L.; Germain, G.; Declercq, J. P.; Woolfson, M. M. 1980, *MULTAN-80*. A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data, Univ. of York, England, and Louvain, Belgium.
- 17.- Enraf-Nonius. 1979, *Structure determination package*, Delft, The Netherlands.
- 18.- Cromer, D. T.; Waber, J. T. 1974, *International Tables for X-ray Crystallography*, vol. IV. Birmingham, Kynoch Press, England.