Tetrahedron Letters, Vol.30, No.1, pp 133-136, 1989 0040-4039/89 $3.00 + .00
Printed in Great Britain Pergamon Press plc

ISOSARAIN-1: A NEW ALKALOID FROM THE MEDITERRANEAN SPONGE *RENIERA SARAI* [1]

G. CIMINO, A. SPINELLA and E. TRIVELLONE

Istituto per la Chimica di Molecole di Interesse Biologico del CNR
Via Toiano n. 6, 80072, Arco Felice, Napoli, Italy

Abstract - The structural elucidation of isosarain-1 *(1)*, a minor component from the alkaloid fraction of *Reniera sarai* is reported; the previously partially characterized structures of sarains 1-3 *(3-5)* are further clarified.

Recently, the literature on marine alkaloids has received a great deal of contributions [2,3]. Many new structures have been reported. Among these, some nitrogenous macrocyclic systems seem to be characteristic metabolites of the sponges belonging to the closely related orders Nepheliospongida [4,5,6,7,8] and Haplosclerida [9,10,11].

The sponge *Reniera sarai*, order Haplosclerida, is widely present in the Bay of Naples. Its metabolic pattern is heavily characterized by a series of macrocyclic alkaloids, named sarains, possessing interesting 'phase transfer catalytic properties and which have resisted to every structural investigation [12] for a long time. Only recently [13] the structures of sarains 1-3 have been partially determined mainly by an extensive 500 MHz NMR study. They belong to a new class of alkaloids showing in their skeletons a *trans*-quinolizidine system linked to an unsaturated piperidine moiety. The two heterocyclic systems are further jointed by two linear alkyl chains undetermined in the relative length.

In this paper we report the isolation and the structure determination of isosarain-1 *(1)*, a minor component of *R. sarai* which, being closely related to sarain-1, offers key-arguments to complete the structural study of sarains 1-3.

Collection, extraction and isolation followed the published [13] procedure to afford after a series of chromatographic steps sarains 1-3 along with a compound slightly less polar[14] of sarain-1, isosarain-1 *(1*, 60 mg from 600 g of dry sponge).

Isosarain-1 *(1)* is an optically active amorphous solid: $[\alpha]_D$-23.1° (c 1.2; $CHCl_3$); ν_{max} ($CHCl_3$) 2930, 2859, 2809, 2760, 1711 cm^{-1}; EIMS m/z (%): 466 (M^+, 100), 327 (17), 232 (5); HREIMS: M^+ found 466.3916, $C_{31}H_{50}N_2O$ requires 466.3923; m/z 327 found 327.2449, $C_{21}H_{31}N_2O$ requires 327.2436; the NMR data are reported in Table 1.

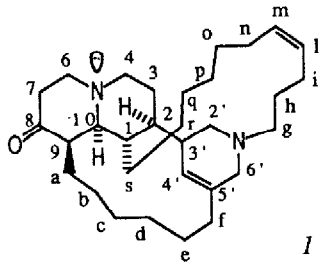
All the above data suggested a structure closely related to that of sarain-1. In fact, the two compounds have the same molecular formula and both possess a pentacyclic skeleton containing a ketone carbonyl group and two double bonds; furthermore, both display in their mass spectra a relevant fragment at m/z 327, originating from M^+ by loss of the alkyl chain $C_{10}H_{19}$. The

proton decoupled ^{13}C-NMR (Table 1) spectrum of *1* showed 29 distinct resonances with only two superimpositions at δ 27.16 and δ 25.87. The ^{1}H-NMR (Table 1) of *1* was complex but its ^{1}H-^{1}H COSY interpretation, facilitated by a 2D ^{1}H-^{13}C shift heterocorrelation and supported by a series of mono-dimensional spin decouplings, led to structure *1*.

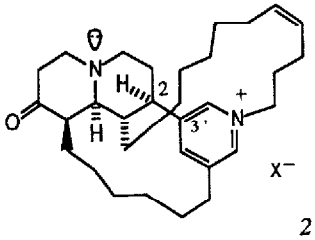
TABLE I - 500 MHz ^{1}H-NMR and 125.7 MHz ^{13}C-NMR data for isosarain-1 *(1)*[a]

N°	δ ^{13}C (m)	δ^{1}H (m, J in Hz)	N°	δ ^{13}C (m)	δ^{1}H (m, J in Hz)
1	34.59 (d)	1.70 (br ddd; 9.2, 9.2, 6)	2'	55.73	2.73 (dd; 11.7, 1.8)
2	41.79 (d)	1.52 (m)			2.33 (dd; 11.7, ~ 11[d])
3	31.82 (t)	1.75 (dddd; 11.8, 11.8, 11.8, 2)	3'	42.38	2.22 (m)
		1.62 (br d; 11.8)	4'	119.46	5.48 (br d, 4.3)
4	53.52 (d)	2.79 (ddd; 11.8, 3, 2)	5'	138.19	-
		1.96 (ddd; 11.8, 11.8, 2)	6'	54.91	3.14 (d, 15.9)
6	55.92 (t)	3.02 (br dd; 11.4, 6.9)			2.44 (d, 15.9)
		2.22 (br dd; 11.4, 15.1)	g	58.01	2.35 (m)
7	37.98 (t)	2.79 (ddd; 15.1, 15.1, 6.9)	h	28.29	1.54 (m)
		2.16 (br d; 15.1)			1.46 (m)
8	212.53 (s)	-	i	25.87	2.09 (m)
9	53.92 (d)	2.29 (br m)			1.94 (m)
10	68.65 (d)	2.08 (dd; 9.2, 2)	h	129.25	5.54 (dt; 10.8, 8.1)
a	28.55 (t)	2.01 (m)	m	130.80	5.26 (dt; 10.8, 7.9)
		1.18 (m)	n	24.94	2.14 (m)
b	27.49[b] (t)	1.48[c] (m)			2.08 (m)
c	27.16 (t)	1.25 (m)	o	27.49[b]	1.43[c] (m)
d	25.87 (t)	1.80 (m)	p	27.16	1.25 (m)
		1.35 (m)	q	29.16	1.15 (m)
e	26.83 (t)	1.55 (m)	r	26.37	1.24 (m)
		1.07 (m)			1.09 (m)
f	33.59 (t)	1.89 (m)	s	32.81	2.21 (m)
		1.83 (m)			1.25 (m)

[a] The spectra were recorded in $CDCl_3$, TMS was used as internal reference.
[b,c] Values are interchangeable.
[d] Value deduced by analysis of the H 2'eq - H 2'ax COSY cross peak.



1



2

Starting from the methylene and the methine linked to the ketone group, all the connectivities of the quinolizidine system were pointed out. In fact, the resonances at δ 2.79 (H-7 ax) and δ 2.16 (H-7 eq) together with the signals at δ 3.02 (H-6 eq) and δ 2.22 (H-6 ax) were

assigned to two methylenes between a ketone group and a nitrogen atom. Furthermore, starting from the signal at δ 2.29 (H-9) all the protons of the chain C-9, C-10, C-1, C-2, C-3, C-4 were assigned. The upshifted resonance of H-10 at δ 2.08 suggested a *trans* fused junction [15], which was confirmed by the presence in the IR spectrum of the typical Bohlmann bands[16] at 2809 and 2760 cm^{-1}.The coupling pattern of H-10 (dd, $J_{9\text{-}10}$ 2.0 Hz, $J_{1\text{-}10}$ 9.2 Hz) allowed to assign the relative stereochemistry to the adjacent chiral centres C-1 and C-9. The axial orientation of H-2 (δ 1.52) was suggested by the large coupling (11.8 Hz) with H-3 ax (δ 1.75) and confirmed by the ^{13}C-NMR resonance value of C-4 at δ 53.5 which excludes γ-shielding effects of a C-2 axial substituent.

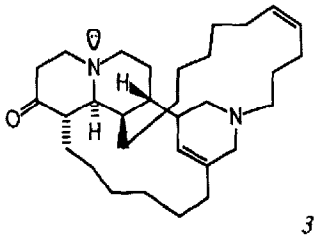
The partial piperidine structure was strongly supported by the coupling pattern of H-4' which , in the ^1H-^1H COSY spectrum, exhibited a vicinal coupling with H-3' (δ 2.22), allylic coupling with the protons at C-6' (δ 3.14 and 2.44) and, also, a W coupling with H-2' eq (δ 2.73) which in turn was further coupled with H-6' eq (δ 3.14). The stereochemistry at C-3' remains undetermined even though the H-2'ax - H-3' coupling value (~ 11 Hz), deduced by the analysis of the COSY cross peaks, could suggest an axial orientation for H-3'.

Regarding the alkyl chain between C-9 and C-5', starting from the proton value (δ 2.29) assigned to H-9, the ^1H-^1H COSY spectrum and decoupling experiments indicated the presence of six consecutive methylene units, the last one (δ ^1H-NMR 1.89 and 1.83) being linked to C-5'. Analogously, starting from δ 1.70 (H-1) a series of subsequent correlations led to identify an alkyl chain containing 11 carbons the last one being linked to a nitrogen atom (C-g, δ ^{13}C-NMR 58.01). It is relevant to note that, analogously to sarain-1[13], in this alkyl chain there is a *cis* oriented double bond separated from the nitrogen atom by three methylenes.

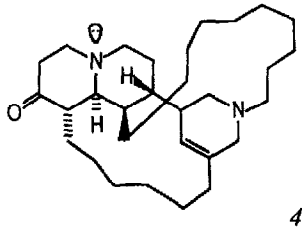
All the above evidence led to the structure *1* characterized by a linkage between the carbons 2 and 3', even though couplings between the protons at these positions were not observed in the ^1H-^1H COSY spectrum. However, the suggested structure was supported by the obtainment of a derivative of *1 (2)* by an unusual procedure .

The sample of *1* stored in $CDCl_3$ [17] showed in its ^1H-NMR some additional low-field singlets (δ 9.40, 8.62 and 8.86) increasing with time. The $CDCl_3$ solution was partitioned with water and then the water extract was partitioned with n-butanol. All the spectral data [18] of the n-butanol soluble fraction (7 mg) were in agreement with the structure *2* exhibiting a pyridinium ring. In particular, the NMR spectra provided evidence for a methylene group (C-g, δ 61.20; H-g's, δ 5.17 and 4.85) linked to a quaternary nitrogen[19]. The ^1H and ^{13}C NMR signals[18] were carefully assigned by two dimensional ^1H-^1H and ^1H-^{13}C shift correlation experiments. The linkage between the carbons 2 and 3' was confirmed by the ^1H-NMR resonance value of H-2 (δ 2.98).

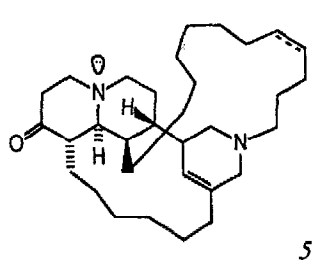
The structural analogies between *1* and sarain-1 *(3)* suggest that the two compounds have to be stereoisomers differing only for the stereochemistry at the chiral centres C-1, C-2, C-9 and perhaps C-3'. Sarain-2 *(4)* and sarain-3 *(5)* should be respectively the inferior and the superior homolog of *3*, differing only for the characteristics of the longest alkyl chain. The position of the double bond in *5* remains to be determined.



3



4



5

The skeletons of sarains are unique among the natural alkaloids. However, they could be biogenetically linked to other macrocyclic alkaloids recently found in sponges. In particular, analogously to petrosins[5,7] and xestospongins[6] ,sarains could be obtained by oxidative coupling of two 3-alkylpiperidine precursors[13]. It is interesting to observe that also xestospongins and petrosins are present in *Xestospongia exigua* and in *Petrosia seriata*, respectively, as a mixture of stereoisomers.

Acknowledgements. We thank Mr. Gennaro Scognamiglio for the skilful technical assistance. Mass spectral data were provided by the "Servizio di Spettrometria di Massa del CNR e dell'Università di Napoli". The assistance of the staff is acknowledged. The secretarial help of Mrs. Maria Rosaria Vaccaro is also appreciated.

REFERENCES AND NOTES

1.- This work was in part presented at the 16th International Symposium on the Chemistry of Natural Products, Japan 1988. G. Cimino, R. Puliti, G. Scognamiglio, A. Spinella, E. Trivellone, C. Mattia, L. Mazzarella, Abstracts, SL a2, p. 53.

2.- C. Cristophersen, in "The Alkaloids" A. Brossi, ed. Academic Press, New York, *24*, 25 (1985).

3.- D. J. Faulkner, *Nat. Prod. Rep., 4, 539 (1987) and previous reports.*

4.- J. C. Braekman, D. Daloze, P. Macedo de Abreu, C. Piccinni-Leopardi, G. Germain, M. Van Meerssche, *Tetrahedron Lett. 23*, 4277 (1982).

5.- J. C. Braekman, D. Daloze, N. Defay, D. Zimmermann, *Bull. Soc. Chim. Belg. 93*, 941 (1984).

6.- M. Nakagawa, M. Endo, N. Tanaka, L. Gen-pei, *Tetrahedron Lett. 25,* 3227 (1987).

7.- J. C. Braekman, D. Daloze, G. Cimino, E. Trivellone, *Bull. Soc. Chim. Belg. 97*, 519 (1988).

8.- H. Nakamura, S. Deng, J. Kobayashi, Y. Ohizumi, Y. Tomotake, T.Matsuzaki, Y. Hirata, *Tetrahedron Lett. 28*, 621 (1987).

9.- R. Sakai, T. Higa, C. W. Jefford, G. Bernardinelli, *J. Am. Chem. Soc. 108*, 6404 (1986).

10.- R. Sakai, S. Lohmoto, T. Higa, C. W. Jefford, G. Bernardinelli, *Tetrahedron Lett. 28,* 5493 (1987).

11.- B. J. Baker, P. J. Scheuer, J. N. Shoolery, *J. Am. Chem. Soc. 110*, 965 (1988).

12.- G. Cimino, S. De Rosa, S. De Stefano, G. Sodano, *Pure Appl. Chem. 58, 375 (1986).*

13.- G. Cimino, S. De Stefano, G. Scognamiglio, G. Sodano, E. Trivellone, *Bull. Soc. Chim. Belg. 95*, 783 (1986).

14.- *1* showed a SiO_2 t.l.c. Rf (0.8, $CHCl_3$: CH_3OH 9:1) identical to that of sarain-1; however, it was eluted under pressure on LC Lichoprep Si60 (0.025-0.40 nm) column slightly before sarain-1.

15.- H. P. Haenlow, S. Okuda, N. Nakagawa, *Tetrahedron Lett.*, 2553 (1964).

16.- F. Bohlmann, *Chem. Ber. 91*, 2157 (1958).

17.- The NMR resonances of *1* were strongly influenced by the concentration and by the presence of salts. All the NMR experiments were performed on the same sample (60 mg, 0.5 ml $CDCl_3$) washed with water and hermetically closed in a 5 mm NMR tube.

18.- FAB/MS m/z 463 (M^+, 100%); UV (CH_3OH) λ_{max} 274 nm; IR (liquid film) ν_{max} 2926, 1702, 1640 cm^{-1}; NMR ($CDCl_3$) δ ^{13}C - δ ^1H correlated by 2D experiments: 210.52 (s, C-8); 149.56 (s); 144.23 (s); 143.56 (d) - 9.40; 143.49 (d) - 8.62; 140.04 (d) - 8.86; 131.84(d, C-l) - 5.47; 127.24 (d, C-m) - 5.39; 67.95 (d, C-10) - 2.15; 61.20 (t, C-g) - 5.17, 4.85; 55.41 (t, C-6) - 2.35; 3.10; 53.07 (d, C-9) - 2.29; 51.62 (t, C-4) - 2.89, 2.15; 42.15 (d, C-1) - 1.86; 40.43 (d, C-2) - 2.98; 39.10 (t, C-7) - 2.76, 2.32; 33.59 (t, C-s) - 1.18; 33.15 (t, C-3) - 2.30, 1.83; 31.70 (t, C-h) -2.20, 2.13; 29.58 (t, C-a) - 2.10, 1.38; 29.49 (t, C-f) - 3.10, 2.90; 27.98 (t) - 1.76, 1.48; 27.88 (t) - 1.18; 27.76 (t) - 1.22; 27.59 (t) - 1.98, 1.62; 27.36 (t) - 1.43; 27.22(t) - 1.20; 26.65 (t, C-i) - 1.86; 26.33 (t) - 1.69, 1.40, 25.23 (t) - 0.94; 23.37 (t, C-n) - 2.12, 1.98.

19.- F. J. Schmitz, K. H. Hollenbeak and D. C. Campbell, *J. Org. Chem. 43*, 3916 (1978).

(Received in UK 18 November 1988)