Amazing new alkaloid skeletons from the marine sponge *Reniera sarai*

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Abstract - *Reniera sarai* possesses two homologous series of
alkaloids, sarains 1-3 and sarains A-C, along with some rela-
ted minor metabolites which include isosarain-1 (1). We now
report the complete structural elucidation of 1, which suppor-
ts and further clarifies the previously only partially charac-
terized structures of sarains 1-3. X-ray structure of an ace-
tate derivative of sarain-A has discovered an amazing new ma-
crocyclic alkaloid skeleton. All the sarains have been charac-
terized by an extensive use of 2D NMR methods.

INTRODUCTION

The sponge *Reniera sarai* (order Haposclerida) is widely present in the Bay of
Naples. Its metabolic pattern is heavily characterized by a series of nitroge-
nous metabolites, named sarains, belonging to new classes of alkaloids and ex-
hibiting interesting chemical and biological properties (ref. 1). In spite of
our great interest to characterize these molecules, for a long time their
structural elucidation failed. In fact, the difficulty in obtaining single pu-
re compounds and the complex and rarely reproduceable NMR spectra make the
structural elucidation of sarains exceptionally challenging.

Our research on *R. sarai* started in 1970 but only recently we have obtained
the first conclusive results (ref. 2) which led to the partial characteriza-
tion of three new alkaloids, sarains 1-3. All the suggested structures, pos-
sess a trans-fused quinolizidine system jointed to an unsaturated piperidine
ring. The two heterocyclic systems are further linked by two alkyl chains,
undetermined in the relative length. Now, the full characterization (ref. 3)
of isosarain-1 (1), a minor component of *R. sarai*, offers further structural
elucidation to this new class of alkaloids. The very abundant UV absorbing frac-
tion of the sponge likewise resulted to be a mixture of three homologs, sara-
ins A-C. The structural peculiarities of sarains A-C are remarkably different
from those of sarains 1-3. However, all the sarains should have a related bio-
genetic origin which could be common to many macrocyclic alkaloids recently
reported (refs.4,5,6,7,8) from sponges belonging to the closely related orders
Nepheliospongida and Haposclerida.

ISOLATION OF SARAINS

The n-butanol soluble fraction (6% of the dry sponge) from the defatted aceto-
ne extract of the sponge was fractionized according to the described procedure
(ref. 2), first by short overloaded silica gel columns and then submitting
the enriched fractions to a series of repetitive chromatographic steps on
Jobin-Yvon Miniprep LC instrument. This procedure afforded the three previo-
usly described sarains along with a new compound slightly less polar of sara-
in-1, isosarain-1 (1), and three UV absorbing compounds named in order of in-
creasing molecular weight, sarain-A, sarain-B and sarain-C. After every chro-
matographic step the recovered fractions were dissolved in chloroform and wa-
shed with water to remove all traces of salts.

STRUCTURE OF ISOSARAIN-1

Isosarain-1 displayed a mass spectrum almost identical to that of sarain-1 (ref. 2) characterized by the same parent peak at m/z 466 ($C_{31}H_{50}N_2O$) and the same prominent fragment at m/z 327 ($M^+-C_{10}H_{19}$). A 500 MHz NMR study of $\underline{1}$, with an extensive use of 2D shift correlated methods, allowed to individualize all the connectivities of the partial structures A-D. Many NMR assignments were confirmed by traditional mono-dimensional decoupling experiments.
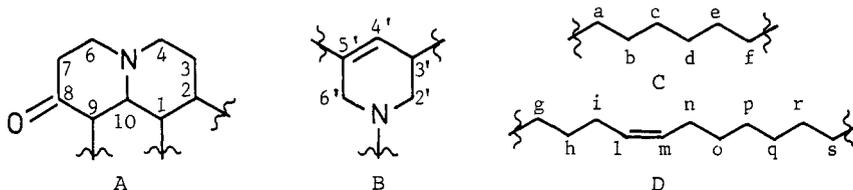
The relative stereochemistry at the chiral centres C9, C10, C1 and C2 of the partial structure A was ascertained on the basis of the following evidence: the ^{1}H-NMR chemical shift of H-10 (δ 2.08) suggested a <u>trans</u>-fused quinolizidine junction (ref. 9) which was confirmed by the presence of the typical Bohlman bands (ref. 10) in the IR spectrum; the coupling pattern of H-10 (dd; $J_{1,10}$ 9.2 Hz; $J_{9,10}$ 2 Hz) was consistent with axial and equatorial orientations of the substituents at C-9 and C-1, respectively; the C-2 substituent was equatorially oriented on the basis of the ^{13}C-NMR resonance value of C-4 (δ 53.42) not influenced by γ shielding effects. The partial structures A-D were combined placing C between C9 and C5' and D between C1 and N1' on the basis of unequivocal NMR evidence. The H2-H3' coupling was not observed in the COSY spectrum. However, at this point the only proposable structure is that depicted in formula $\underline{1}$ characterized by a C2-C3' linkage. This assignment was confirmed by the observation that $\underline{1}$ in deuterochloroform solution is slightly transformed in the compound $\underline{2}$, characterized by a trisubstituted pyridinium ring linked to C-2 (^{1}H-NMR, H-2, δ 2.98).
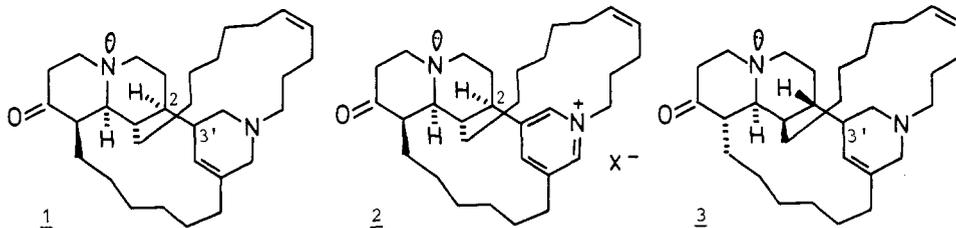
The analogy of the spectral data of $\underline{1}$ with those of sarain-1 ($\underline{3}$) suggested that both the metabolites have to possess the same alkyl chains and differ only for the relative stereochemistry at the chiral centres C-1, C-2, C-9 and perhaps C-3'. Analogously, sarain-2 and sarain-3 differ from sarain-1 ($\underline{3}$) only for the structural peculiarities of the longest alkyl chain, $C_{10}H_{20}$ in sarain-2 and $C_{12}H_{22}$ in sarain-3 with undetermined position of the double bond.

SARAINS A-C

Sarains-A, B and C, single t.l.c. UV absorbing spot (chloroform:methanol 9:1, silica gel), displayed molecular formulae ($C_{32}H_{50}N_2O_3$, $C_{33}H_{50}N_2O_3$ and $C_{34}H_{52}N_2O_3$, respectively) suggesting, analogously to sarains 1-3, their belonging to a homologous series. This was confirmed by some common spectral data: IR $\simeq$ 1650 cm^{-1}; UV $\simeq$ 238 mμ; MS, loss of the same fragment $C_{14}H_{22}NO_3$. The complex NMR spectra were seldom reproduceable being strongly influenced by traces of salts or acids and, also, by the concentration of the sample. However, working on selected hermetically closed samples, we have been able to submit all the sarains to an exhausting NMR analysis. It probably would have been unsuccessful without the luck of obtaining a crystalline acetate derivative of sarain-A ($\underline{4}$) suitable for a resolutive diffractometric study. The single crystal X-ray analysis (fig. 1) discovered an incredible structure characterized by a central tetracyclic cage in the middle of two cyclic alkyl chains. Surprisingly, a charged pseudobase moiety is present in the central cage structure; probably it is stabilized by the strong hydrogen bond (2.567 Å) between the hydroxyl group and the acetate anion.
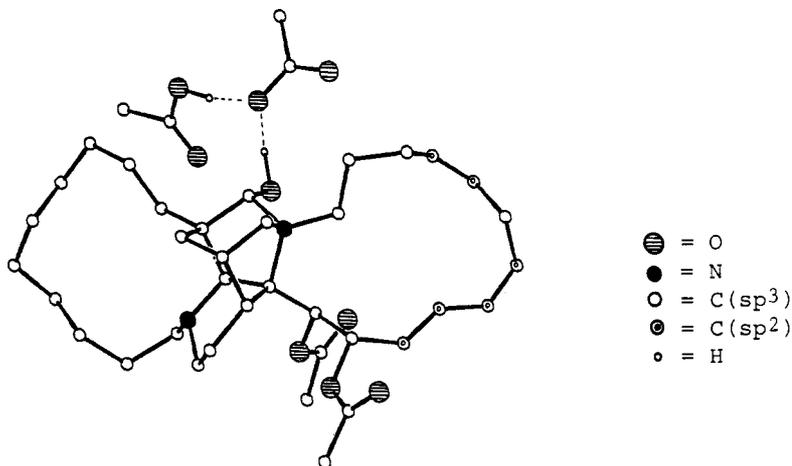
Fig. 1 - Perspective drawing of the acetate derivative of
sarain-A (only hydroxylic hydrogens are reported)

The NMR spectra of the crystalline compounds were uninterpretable and strongly influenced by the temperature, by the acidity and by the concentration of the solution.

Sarain-A was recovered unmodified after methanolysis of the acetate. The analysis of the NMR spectra led to the assignment of almost all the resonances, however the signals attributable to the methine group CH-2 were absent. In fact, no signals were recorded in the ^{13}C-NMR δ 110-90 region and in ^{1}H-NMR δ 5.5-4.5 region. However, after addition of equimolecolar amounts of deuteroacetic acid to the deuterochloroform solution of sarain-A, the ^{1}H-NMR spectrum was characterized by a new signal at δ 4.95 correlated with a carbon resonating at δ 96.6, as expected for the methine group between two heteroatoms. Probably in deuterochloroform solution sarain-A either displays a "zwitterion" structure or exhibits a strong polarization between the N and O atoms, which evolves in the presence of acetic acid to 5. Bearing in mind the anomalous IR absorption at 1650 cm^{-1} we prefer this latter hypothesis which could be due to a strong proximity interaction between an aldehyde group and a tertiary amine moiety, remembering the transannular effect described by Leonard et al. (ref. 11) on cyclic 8-aminoketones.
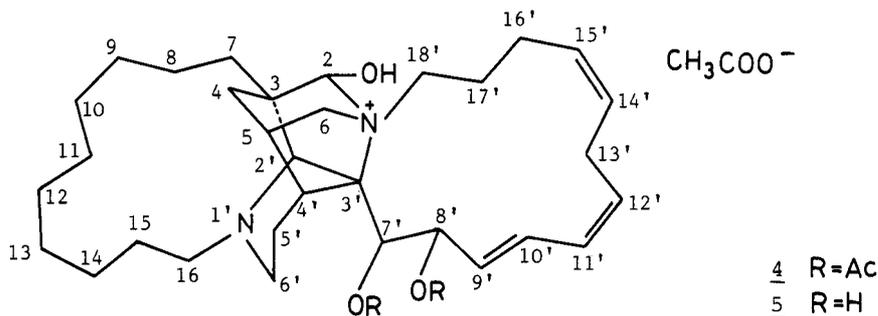
These structural peculiarities can explain the ability exhibited by sarains to link inorganic salts and the strong pH dependence of their NMR spectra. Analogously to sarains 1-3, sarain-B differs from sarain-A for the presence of an additional carbon atom and of a double bond in the alkyl chain between N1' and C3. Whereas sarain-C, in turn, is the superior homolog of sarain-B.

Apparently, there is a great structural difference between the two series of sarains . However, we would try to suggest a common biogenetic origin which derives from the observation that, breaking the 9-10 and 2-3' carbon linkages of 1 and the 1-3', 5,4' and 3,2' carbon linkages of 5, we obtain two macrocyclic skeletons which formally could be obtained by coupling of two 3-alkylpiperidine units. This biogenetic hypothesis could link in an unique scheme other macrocyclic alkaloids found in marine sponges, petrosins (ref. 4, 5),

xestospongins (ref. 6), halitoxins (ref. 12) and, also, it is supported by the recent finding (ref. 13) of two 3-alkyne pyridine derivatives, niphatyne A and B, from a Niphates sponge.

We retain that even though only recently (ref. 14) macrocyclic alkaloids have been found in sponges, they could be widely present in sponges of the orders Haposclerida and Nepheliospongida, but their structural complexity has until now prevented any extensive study. However they deserve great attention becau- se almost all these compounds exhibited interesting biological and chemical properties.

Sarains are moderately toxic to mice, toxic to Macrosiphum euphorbiae aphid and to Tetranychus urticae mite, highly toxic in the brine shrimps bioassay with Artemia salina and, furthermore, they are efficient phase transfer catalysts (ref. 2), favouring the displacement in organic solvents of chloride from ben- zylchloride by acetate ion with a yield comparable to that obtained using 18- crown-6 as catalyst (ref. 15).

Acknowledgements

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