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Bioactive metabolites from marine invertebrates

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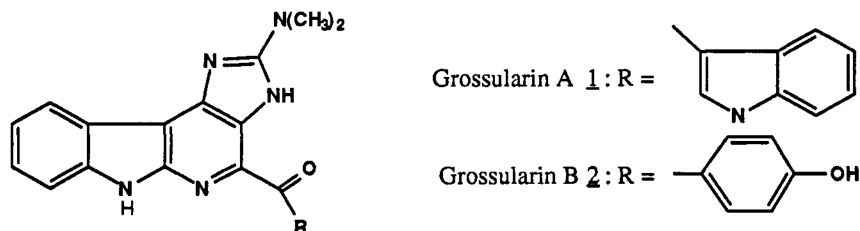
Abstract. Recent results in the chemistry of bioactive marine products are presented. Concerning the cytotoxic α-carbolines : grossularins A and B, the mechanism of the toxicity using supercoiled DNA and synthesis of the imidazolo-α-carboline moiety are presented. In the polysulfur compounds series, the structure of lissoclinotoxin B is proposed as well as a new synthesis of pentathiepins. Novel sesquiterpene sulfates from a *Spongia* sp. are described as $p80^{cdc25}$ inhibitors.

Our program devoted to search of bioactive compounds in marine invertebrates involves two important steps: first, screening of extracts and pure compounds and, second, synthesis of active compounds.

Concerning the first point we developed simple tests directed towards specific targets : DNA, enzymes involved in the cell cycle or cause of disorders (Na^+/K^+ATPase, phospholipase A2, elastase) which offered the advantage of direct elucidation of the mechanism of the activity. We also engaged synthesis of the most active compounds, because availability of a molecule is a perequisite condition for a possible development. Moreover, synthesis may afford interesting analogues.

From a tunicate *Dendrodoa grossularia* we previously isolated a series of original indolic derivatives, and among them the grossularines A and B, the first α–carbolines from a natural source. In a cloning system bioassay they proved to be cytotoxic for human solid tumor cells (1,2).



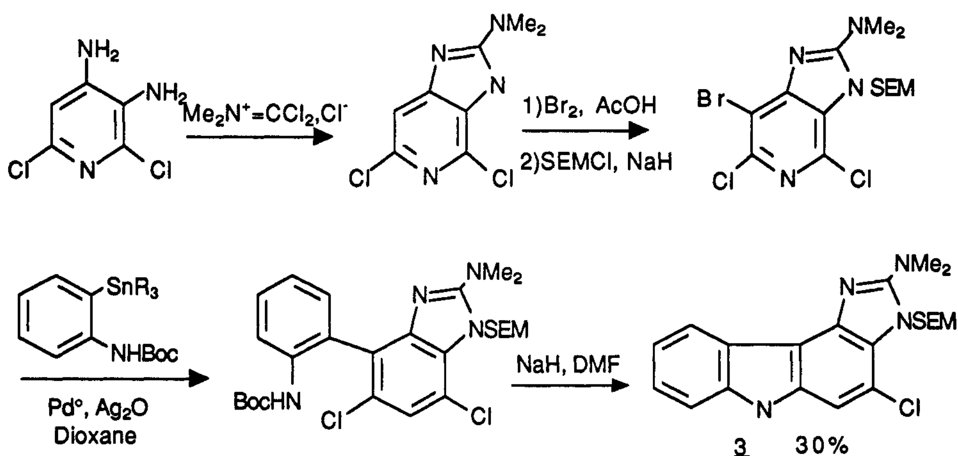
Experiments using viscosimetric titration performed on calf thymus DNA and supercoiled DNA (pBR 322) showed that helix lenghtening and unwinding angle induced by grossularin B were similar to those provoked by the standard ethydium bromide. The classical intercalators stabilized DNA helix against thermal denaturation; in such an experiment 2 showed a typical bimodal transition behaviour typical reflecting the stabilization induced by this compound. In contrast, grossularin A appeared to be an helix destabilizer (3). Gel electrophoresis separation is an easy method to perform in view to appreciate DNA strand scission (4). Electrophoresis migration of pBR 322 in presence of both grossularins, in presence or absence of $CuCl_2$, was performed. We can observe that with grossularine A, in presence of $CuCl_2$,

only relaxed DNA is present. Grossularine A treated with 0.5 M $CuCl_2$ led to a stable complex (visualized by U.V. spectrum and CCM), likely by the indolic NH of the side-chain of two molecules of 1 with one of $CuCl_2$.

Owing to the potential interest of these α–carbolines, we engaged there synthesis. Methods to prepare α-carbolines exist in the litterature. However, because of the complexity of our molecules which possessed an imidazole ring, we search for more convenient methods.

We investigated the possibility of a cross-coupling between suitable anilines and pyridins catalyzed by Pd^o, followed by a base catalysed cyclisation. This new strategy proved to be very efficient and a number of substituted α-carbolines were prepared in good yields (5).

Access to the grossularins themselves requires condensation of the imidazole pyridin (obtained in two steps according to sheme 1). Cross-coupling of the imidazolo-pyridin was tedious, however using stoechiometric silver oxide in combination with Pd^o (5) led to the desired compound. Intramolecular cyclisation furnished the tetracyclic moiety 3 of the grossularins.



Scheme 1.

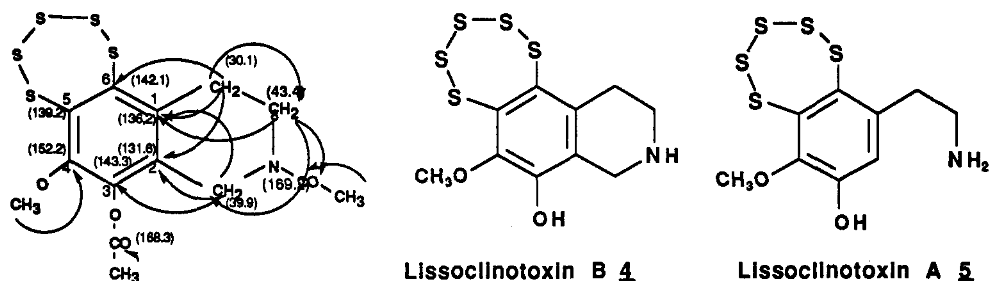
Among the synthetic products obtained (not shown) some displayed activity against bacteria and are currently assayed towards tumor cells.

The tunicate *Lissoclinum perforatum* was also studied: the methanolic extract was highly antibiotic and moderately cytotoxic. The major active product was lissoclinotoxin A we described as a trithiane (6). As the same time we proposed this structure, the group of Ireland published the structure of a close congener, varacin, a benzopentathiepin isolated from *Lissoclinum varau.*(7). Varacin was described as highly cytotoxic and any antimicrobial activity was mentionned.

The question about the number of sulfur atoms in our products puzzeled us over a long time. On the basis of a prominent peak at m/z 261 in MS we concluded to a trithian base, but minute peaks corresponding to five sulfur atoms and even 7 sulfur atoms are visible.

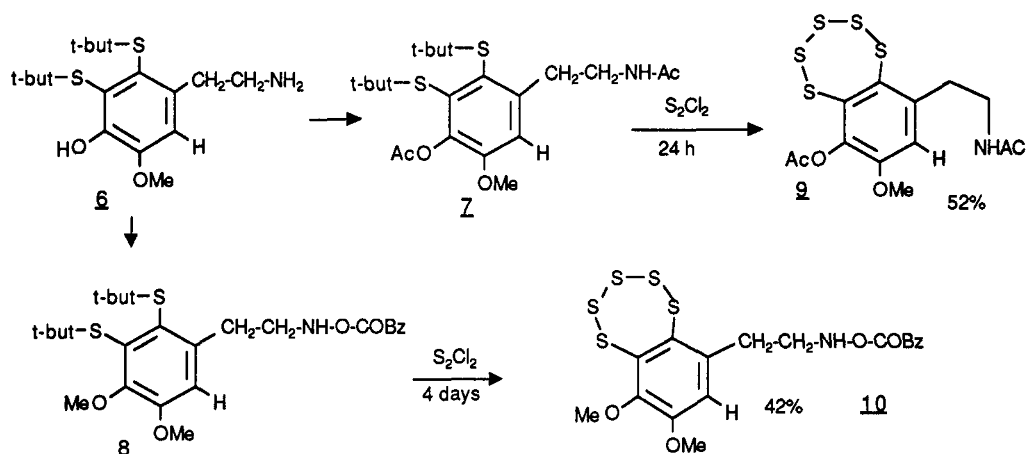
Later on, we purified a minor component, also strongly antibiotic for which mass spectra (EI and CI) confidently indicate the presence of five sulfur atoms. Long range heteronuclear correlations, allowed to establish the structure of lissoclinotoxin B as 4. (Scheme 2)

We therefore reexamined structure of lissoclinotoxin A and compared it with synthetic pentathiepins and trithianes. Finally electron spray MS furnished the formula of lissoclinotoxin A by the base peak at m/z 326 corresponding to a pentathiepin structure. Hence we concluded that structures 5 and 4 should be assigned to lissoclinotoxin A and B respectively (8).



Scheme 2.

Meanwhile, needing reference compounds, we engaged synthesis in this series. Only few methods exist in the literature concerning preparation of pentathiepins and trithianes.



In our approach, we first introduced the sulfur atoms as sulfides on a dibromoderivative. The first step, i.e., bromination of vanillin, was easy to achieve but bromination of isovanillin, which is to yield lissoclinotoxin proved unsuccessfull. So we went on with the synthesis of isolissoclinotoxin.

Introduction of the side-chain was easy. Access to the pentathiepin or the trithiane rings by the classical methods revealed inefficient in our hands. Since it was previously demonstrated that S_2Cl_2 is able to cleave some thioethers especially trityl ones, we investigated the behaviour of t-butylthioethers towards this reagent. From a commun intermediate 6 we prepared 7 and 8. When treated with two equivalents of sulfure chloride, disulfides 7 and 8 furnished the pentathiepins as the major product. After purification we obtained diacetyl isolissoclinotoxin and benzoyl varacin in good yields (respectively 55% and 42%)(9).

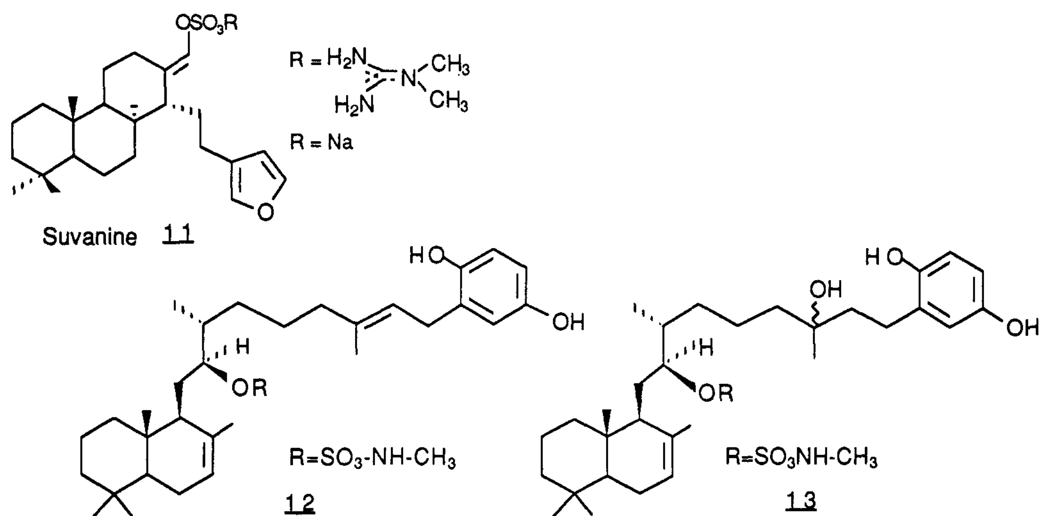
As in the case of lissoclinotoxin, the molecular ion of the synthtic pentathiepins 9 and 10 was very weak and pentathiepin structures was confidenlty secured by analysis.

Lissoclinotoxin A exhibits antimocrobial activity that they be compared to that of antibiotic such as cefotoxin. Lissoclinotoxin A was also toxic for L1210 Leukemia cells and more interestingly is toxic for resistant strains of *Plasmodium falciparum* (more potent than quinine and chloroquine).

In disk bioassays lissoclinotoxin B revealed even more potent than lissoclinotoxin A towards Gram(+) and Gram(-) strains. Synthetic diacetyl isolissoclinotoxin 9, is strongly active against Gram(+) but inactive towards Gram(-) bacteria. Benzoyl varacin 10 is devoied of antibacterial activity. Study of biological activity in this series is in progress.

Other active compounds came from *Spongia sp* collected in New Caledonia. The methanolic and dichloromethane extracts inhibited p80cdc2. $P80^{cdc2}$ is a tyrosine phosphatase which allows cells to enter phase M and can thus be used to detect antimitotic compounds and discloses the mechanism of the activity. The phosphatase activity can be monitored by a simple colorimetric assay (10). Bioassay-guided purification led to three active substances. The major one proved to be suvanine 11 previously isolated for *Coscinoderma* sp.(11). The other active compounds were sulfated sesterpene hydroquinones: spongiasulfate 12 and 13. The structures were solved by extensive 1D and 2D NMR and comparison with sesterpene sulfates isolates from a sponge of the family Halichondriidae (12). Stereochemistry was assigned by comparison with known labdanes (13).

In the inhibition of p80cdc2 the IC_{50} of 11, 12 and 13 were respectively 1, 1.5 and 5 µg/ml.



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