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Biologically active compounds from marine organisms

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Abstract - In our search for cytotoxic compounds from marine animals we have isolated representatives from various classes of nitrogen-containing compounds, most of which show cytotoxic activity. Structure elucidation of these compounds is discussed. Included among the isolates are new acylated spermidines, polycyclic aromatic alkaloids related to amphimedine and 2-bromoleptoclinidinone, and a representative of a novel sulfur containing 3,5,8-isoquinolinetrione.

A wide variety of novel alkaloids have been reported from marine organisms in recent years[1] in contrast to the paucity of such metabolites described in the early years of marine natural products research.[2] Among the new type of alkaloids reported in the past few years are a series of polycyclic aromatic alkaloids such as amphimedine (**1**),[3] 2-bromoleptoclinidinone (**2**),[4] ascididemim (= leptoclinidinone , **3**),[5] and shermilamine (**4**)[6] which have in common the B,C,D,E-ring portion of **1**. Until a few years ago when convenient NMR experiments for determining long range H/C correlations such as the one-dimensional selective inept (INAPT)[7] and the long range 2D H/C correlation[8] became commonly available, unambiguous structure elucidation of these rather simple looking compounds was quite difficult by spectroscopic methods. Because of the remoteness of some of the quaternary carbons from any protons, 2D ^{13}C-^{13}C coupling experiments[8] are also needed to help trace out the entire skeleton. The fact that many of these metabolites are sparingly soluble makes it difficult to carry out all the desired NMR experiments.

We have recently isolated additional members of this class of aromatic alkaloids in the course of our continuing search for potential antitumor compounds. The source of two of the new alkaloids is the ascidian *Amphicarpa meridiana* which was collected at Stenhouse Bay, South Australia. The chloroform-methanol extracts yielded meridine, **5**, as a yellow, non-crystalline solid either via conventional successive chromatographies on silica gel or via centrifugal countercurrent chromatography (CCCC) using a chloroform-methanol-5% aqueous HCl (5:5:3) solvent system. Meridine, $C_{18}H_9N_3O_2$ (HRMS), showed signals in the ^1H NMR spectrum for one exchangeable proton (15.26 ppm) and eight aromatic protons which were arranged as three isolated spin systems as shown in rings A, D and E in formula **5**. The NOE shown on the formula provided evidence for the location of the OH and comparison of proton and carbon NMR data with that of related compounds suggested **5** as a likely structure. A fully coupled carbon spectrum and several long range H/C correlations led to the carbon assignments shown at the lower right hand corner of the structure page. Eventually crystals of a trifluoroacetate salt were obtained by slow evaporation at 5° C of a solution of meridine in $CDCl_3$ plus a few drops of CF_3CO_2D. X-ray analysis confirmed structure **5** with the added proton at the nitrogen of ring-A. A strong hydrogen bond was evident between the OH and the neighboring nitrogen.

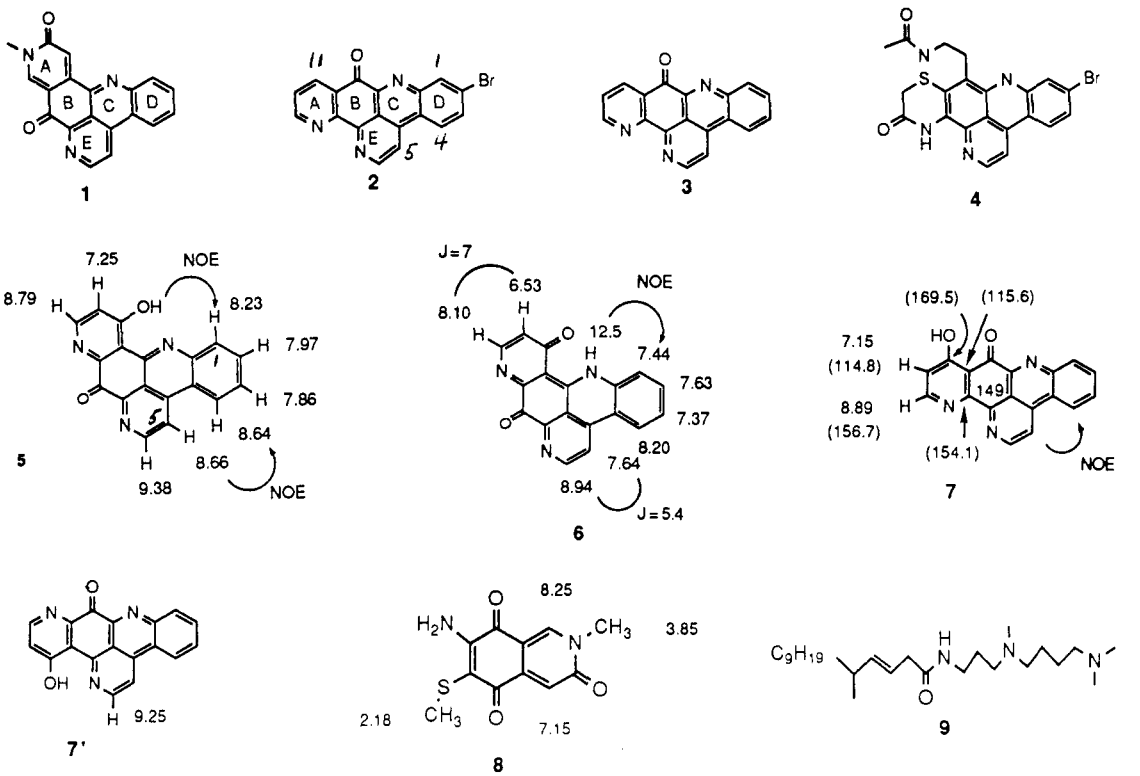
During successive isolations of meridine via silica gel chromatography, a second alkaloid, isomeridine (**6**), was isolated, also $C_{18}H_9N_3O_2$. However, after isomeridine had been stored in $CDCl_3$ for 1-2 days, the ^1H NMR spectrum of the sample was indistinguishable from that of meridine, indicating that the two alkaloids were tautomers. Three possible tautomeric structures were considered in which ring A was a pyridone and the exchangeable proton was, respectively, at each of the different nitrogens. Detection of a NOE between the exchangeable proton (12.51 ppm) and the proton resonating at 7.44 ppm (H-1) in the proton NMR spectrum of isomeridine confirmed that **6** was the correct structure.

In the course of reisolating 2-bromoleptoclinidinone (**2**) for biologically testing purposes from a repeat collection of an ascidian, *Leptoclinides* sp.,collected in Truk Lagoon, Federated States of Micronesia, an additional alkaloid, **7**, was obtained by conventional chromatographic methods. The formula $C_{18}H_9N_3O_2$ was confirmed by HRMS and the IR confirmed the presence of a hydrogen-bonded OH (3067 cm^{-1}).Comparison of the proton and carbon-13 data of **3** and **7** revealed that these two compounds had identical B,C,D,E-ring moieties. Differences between the compounds thus apparently was confined to ring A. The chemical shifts and small coupling constants of the pair of aromatic protons on this ring were indicative of the α,β-protons of a pyridine ring, cf. **5**. An INAPT experiment revealed that the proton signal at 8.89 ppm was long-range coupled to the hydroxyl bearing carbon and hence structures **7** and **7'** were inferred. No NOE was observed between the exchangeable proton and and any other signals, in contrast to the case for meridine, see above. This provided further evidence that the OH is hydrogen bonded to the carbonyl oxygen and not to a nitrogen as would occur in **7'**. In **7'** an NOE would be expected between the OH and the 9.25 ppm proton. Hence structure **7** is proposed for this alkaloid. This is also consistent with the fact that **7** and **2** occur in the same organism.

Alkaloids **1, 2, 3, 5 and 7** all exhibit cytotoxicity to cultures of murine leukemia cells (P388) at 0.3-0.4 mg/mL. Compounds **3** and **5** inhibit topoisomerase II at 30 and 75 μM concentrations, respectively.

Ascididemin and meridine embody the key features of the set of four alternative structures I-IV proposed for neocalliactine acetate.[9] Neocalliactine acetate is a derivative prepared from calliactine, a pigment isolated from the sea anemone *Calliactis parasitica*. No definitive structure has been determined for calliactine. Possible neocalliactine acetate structures I and II have the overall skeletal outline of ascididemin, while III and IV have the meridine ring array and within each of these pairs the difference lies in the orientation of the left-most pyridine ring. Hence it appeared that the confirmed ^{13}C NMR data for meridine and ascididemin could be used to deduce an unambiguous structure assignment for neocalliactine acetate and, by inference, that of its precursor, calliactine.

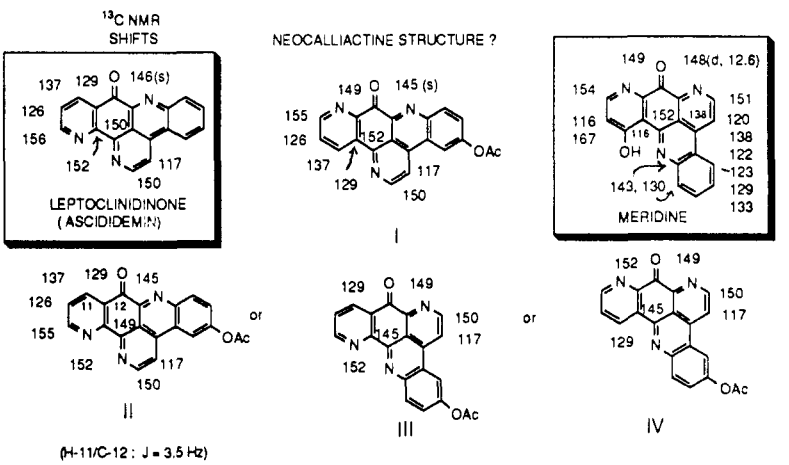
Comparison of the ^{13}C NMR shifts of 11-hydroxyascididemin (**7**) with those of ascididemin (**3**) confirmed that the hydroxyl group has very little effect on the shifts of carbons meta to the hydroxyl-substituted position as might have been expected from data for simple substituted pyridines.[10] Hence the shifts of the carbons adjacent to the carbonyl group in meridine should be excellent models for any deoxy analogs thereof such as I and IV.



Scheme I shows the key ^{13}C NMR chemical shifts for ascididemin, meridine, and I-IV. Shifts around structure I (the "slightly preferred" structure) are those assigned in the literature (the 149 and 152 ppm assignments may be reversed). Only <u>one</u> signal, 145 ppm, is reported to be a singlet. Shifts specified on structures II-IV are transpositions of the assigned shifts for I to like carbons. The data allow III and IV to be ruled out unequivocally. In IV the signals assignable to the quaternary carbons flanking the carbonyl group all occur at quite different chemical shifts and this is incompatible with the meridine model. Also, the 145 ppm signal should be a doublet.

In structure III the 145 ppm singlet signal must be assigned to the position "para" to the carbonyl group to avoid 3-bond couplings. This shift

Scheme 1



is inconsistent with either the asciddemin or the meridine models. Also, the 117 and 150 ppm signals of neocalliactine acetate fit well for ring D of asciddemin, but not the analogous ring C of meridine.

For both I and II the carbon-13 NMR shifts of rings A and B fit their respective models well and hence no differentiation can be made from this data. The multiplicities of carbon signals 149 and 152 ppm (d, 10.8 Hz; mult., respectively) are in accord with expectations for structure II whereas in I the 149 ppm signal would be expected to be dd (or m). The coupling to the carbonyl carbon signal (3.5 Hz) is appropriate for the anticipated 3-bond coupling in II, but not in I as already noted in the literature.[9] Conversely, I, but not II, is compatible with a NOE observed[9] between the signals assigned as H-6 and H-8 (in I). Although these data still do not allow an equivocal choice to be made between I and II, we slightly prefer II, placing greater weight on the carbon coupling data than on the NOE result.

From the methanol and chloroform-methanol extracts of a bryozoan, *Membranipora perfragilis* collected at Stenhouse Bay, South Australia, a low molecular weight cytotoxic metabolite designated perfragilin, **8**, was isolated in trace quantities. Its structure was determined by x-ray analysis. Perfragillin bears a resemblance to mimosamycin which is produced by *streptomyces lavendulae*, but which has also been isolated from a sponge.[11] This raises the possibility that perfragillin is also produced by a microorganism.

A cytotoxic acylated spermidine derivative (ED_{50} = 0.04 μg/mL vs P388), **9**, was isolated from a *Sinularia* species of soft coral collected at the island of Nauru. Mass spectral analysis and NMR data confirmed the structure.

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