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Marine natural products: new results from Red Sea invertebrates

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Abstract - New metabolites isolated from soft corals and sponges of the Red Sea are discussed.

It is the aim of this report to describe several marine natural products which have recently been isolated and identified by us. The report will include metabolites of sponges and soft corals collected in the straits of the Gulf of Eilat and the Gulf of Suez, The Red Sea(Ref.1).

Diterpenoids from *Spongia arabica* and *Dysidea* spp. From the sponge Spongia arabica (Spongia officinalis var. arabica, Vacelet) we have isolated several diterpenes. The major two compounds were the known 3α,17,19-trihydroxyspongia-13(16),14-dien-2-one(1) and the corresponding 3β,17,19-trihydroxy spongian derivative (2) (Ref.2), and as minor constituents the new 19-acetoxy, 3α-hydroxyspongia-13(16),14-dien-2-one (3) and compound 4 (Fig.1). The structure of 4 designated spongialactone A, $C_{20}H_{26}O_5$, was mainly elucidated by ^1H and ^{13}C NMR data including 2D NMR experiments. Spongialactone A is the first A-seco spongian in which ring A closed up to a ξ-lactone.

Rearranged spongians in which all four rings have been changed are well known both from sponges (Ref. 3) and from Dorid nudibranches which feed on sponges (Ref.4). Ten new rearranged spongian-type diterpens have been isolated by us from two Red Sea Dysidea sponges. All new compounds embody as a carbobicyclic portion either a perhydroazulene, shahamins A-E (5-9), or a $\Delta^{5(10)}$ octalin system, shahamins F-J(11-15), and carry one out of four heterocycles, that is, a disubstituted dihydro furan, a trisubstituted γ-lactol a trisubstituted δ-lactone or a 2,7-dioxabicyclo-[3.2.1]octane (Fig.1). The latter moiety is part of macfarlandin E (10)(Ref.3) which was the major diterpene in the examined Dysidea sponges. The structure of all the compounds were elucidated from spectral data, mainly by 1D and 2D NMR techniques, by comparison with other related known diterpenes (Ref.3 & 4) and by mass spectra.

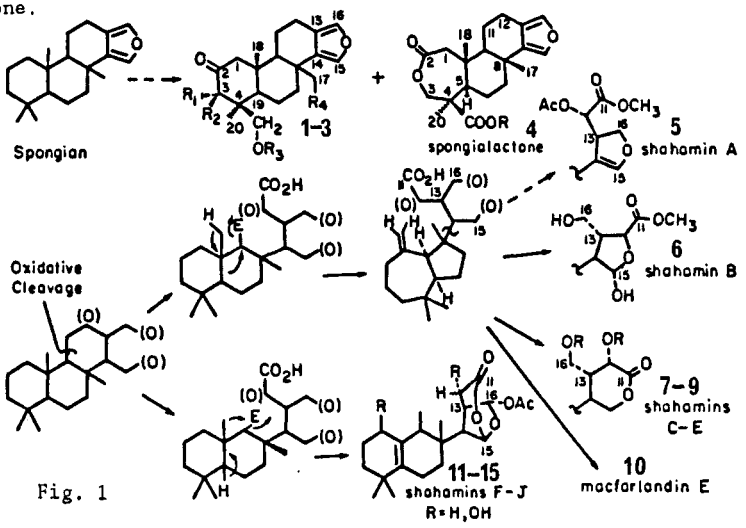


Fig. 1

Diterpenes and novel C_{24} metabolites of *Anthelia glauca* The genus Xenia family Xeniidea has earlier been shown by us and others (Ref.5) to contain three classes of compounds; the xenicins, xeniolides and xeniaphyllanes. In addition, other closely related compounds have also been disclosed from both Xenia and other organisms, e.g. gorgonians (Ref.6). Recently we have examined the secondary metabolite content of the soft coral Anthelia glauca which belongs to the Xeniidea family and, indeed, we have isolated xenia diterpenes. Thus, we found xeniculin, xeniolide A and B, isoxeniolide A, 9-desacetoxy-xenicin (Ref.6) and two other novel compounds 19 and 20 (Fig.2).

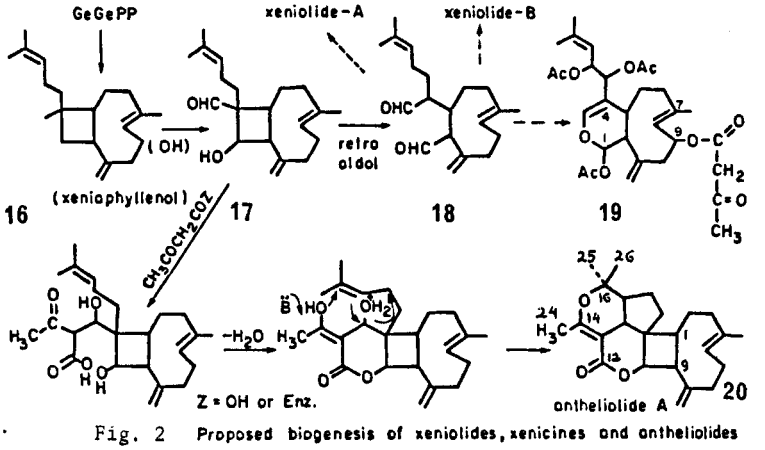
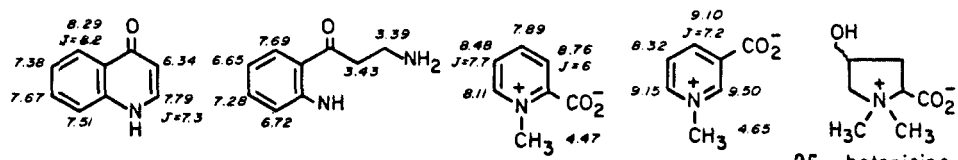


Fig. 2 Proposed biogenesis of xeniolides, xenicines and antheliolides

Compound 19 showed NMR data indicative of the xeniane skeleton and very similar to xenicin. NMR spectra (1D&2D) established the replacement of the 9-OAc by an acetoacetate group (δ_H 2.39s (3H) and 3.40s (2H)). The least polar material isolated from A. glauca, $C_{24}H_{32}O_3$, designated antheliolide A, was found to exist in two conformations in a ratio of 1:4. Intensive NMR studies including homo and hetero nuclear correlations (COSY, RELAY, short and long-range CH-correlations) proposed a penta cyclic structure (Fig.2). Embodied in this structure was the bicyclo[7.2.0]undecane system of the xeniaphyllanes which explained the observed two conformers due to restricted rotation of the nine membered ring. The cyclobutane was also in full agreement with its characteristic (Ref.5) cleavage in the mass spectrometer (m/e 148,100%, $C_{11}H_{16}$ and M-148, 31%). On the grounds of NOE experiments (1D-dNOE and 2D CONOESY) we suggested the stereochemistry of 20 which was confirmed by X-ray. Based on the chemical shift assignments (δ_C 167 ppm for the CO and 162 for the vinyl enol ether) we first suggested for 20 the structure in which Me-24 is on C-12 and the lactone vicinal to the gem dimethyl (C-25,26). A most recent X-ray diffraction analysis (Ref.7) has shown the lactone to be at C-12 and the enol ether next to C-16 (Fig.2). The biogenesis of 20 starting from GeGePP via the xeniaphyllanes is shown in Fig.2. The proposal suggests the antheliolides to be acetoacetylated diterpenes. Isolating of 19 together with the antheliolides supports the availability of the acetoacetate building block in the soft coral.

N-containing compounds from soft corals Many of the first marine natural product studies of soft corals and other organisms dealt with the relatively less polar compounds which were isolated together with large amounts of glycerides and sterols from non-polar extracts. Recently attention has been paid to the more polar extracts of complex mixtures which often possess interest-

Fig. 3

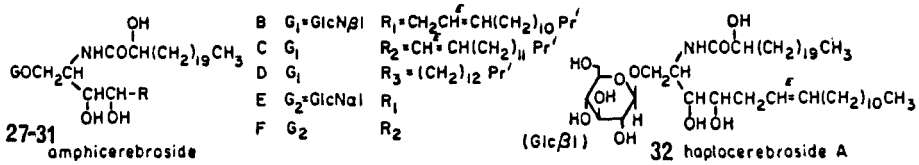


21 4-quinolone 22 kynuramine 23 homarine 24 trigonelline 25 + 26 betonicine turicine

ing bioactivities. As a result more nitrogen containing metabolites have been disclosed. It was from methylene chloride-methanol extracts of soft corals that we have isolated several N-containing compounds. Thus, from Dendronephthea sp. we have isolated homarine (23) and trigonelline (24) and from Heteroxenia ghardaquensis 4-quinolone (21) and kynuramine (22) (Fig.3). From the latter animal and from Lithophyton sp. we isolated an amide of erythro-docosasphinga-4,8-dienine which turned out to be the same compound isolated most recently from the sea anemone Anemonia sulcata (Ref.8).

Cerebrosides from the sponges *Haplosclerides* sp. and *Amphimedon viridis* Many of the investigated polar extracts of sponges contain complex mixtures of N-containing metabolites (positive ninhydrin, Dragendorf or Barollie tests). Identified were free amino acids, nucleosides, a variety of betains, like compounds 23 - 26 (Ref.9) (Fig.3) and other simple biogenic amines (Ref.10). Among others we have also isolated ceramides and cerebrosides. Thus, compound 32 (Fig. 4) was isolated from Haplosclerides sp. The latter compound

Fig. 4



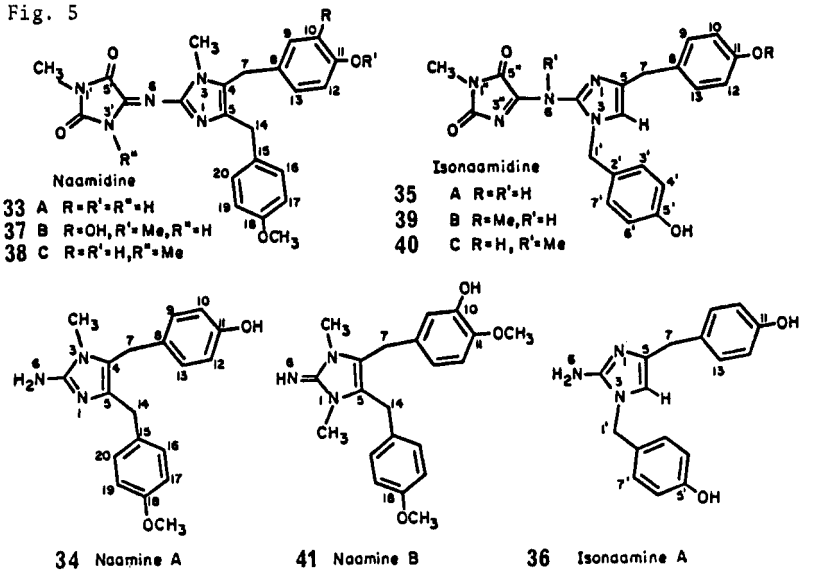
27-31 amphicerebroside 32 haplocerebroside A

afforded upon acidic hydrolysis glucose and α-hydroxy nC_{22}-carboxylic acid as well as a sphingosine, erythro-octadecasphinga-6-enine, which was identified by NMR and by mass spectra of its ozonolysis products. Compound 32, designated haplocerebroside A, is a new cerebroside (Fig.4). Amphimedon viridis another very sticky sponge, immediately after taking out from the sea, afforded in its methylene chloride-methanol extract a highly viscous mixture of compounds. The latter mixture which was separated after acetylation, contained at least five compounds which were identified upon hydrolysis,ozonolysis, NMR and mass spectrum measurements as cerebrosides 27-31 (Fig.4). The compounds named amphicerebrosides B-F, belong to two groups, B-D and E,F according to their α or β linkage to a glucosamine unit. The compounds differ further in the double bond location in the sphingosine chain (Fig.4). Similar compounds were also identified in quite a few other sponges.

Imidazole alkaloids from *Leucetta chagosensis* From the bright yellow calcareous sponge Leucetta chagosensis we have isolated, besides the more common above mentioned polar metabolites, four new groups of compounds. Representatives of these novel compounds are naamidine A(33), naamiine A(34), isonaamidine A(35) and isonaamine A(36) (Fig.5). Compounds 33-36 are the more abundant among these new imidazole alkaloids. Compound 33, $C_{23}H_{23}N_5O_4$, a yellow gum, is the major alkaloid (0.15% dry wt.). Its structure (Fig.5) was proposed on the basis of intensive NMR work (a COSY and mainly all kinds of CH-correlation experiments), mass spectroscopy as well as on the acidic degradation and reduction products (Ref.11).

Under the acidic conditions naamidine-A(33) cleaved to naamine A(34). Isomeric in their structure are isonaamidine A(35) and isonaamine A(36) which possess the N(1), 4-(rather than 4,5-) dibenzyl structure (Fig.5). Additional novel alkaloids belonging to the above groups and differing in the substitutions of the benzyl(s) and/or part of the nitrogen atoms are compounds 37-41 (Fig.5).

Fig. 5



Latrunculins: ichthyotoxic metabolites of *Latrunculia magnifica* The latrunculins (Lat A(1) and Lat B(2)) are marine toxins isolated from the sponge L. magnifica (Ref.12). Previously these compounds were found to disrupt microfilament organization and to have profound effects on the morphology of non-muscle cells (Ref.13). A recent research (Ref.14) demonstrates that the effects of Lat A&B on normal fibroblast cells and on transformed neuronal cells in culture differ from those of cytochalasin D, a potent F-actin capping agent. Both Lat A and cytochalasin D disrupt actin organization and inhibit cell growth in a permanent but different way. Lat A was recently found to affect the polymerization of pure actin in a manner consistent with the formation of a 1:1 molar complex with G-actin (Ref.15).

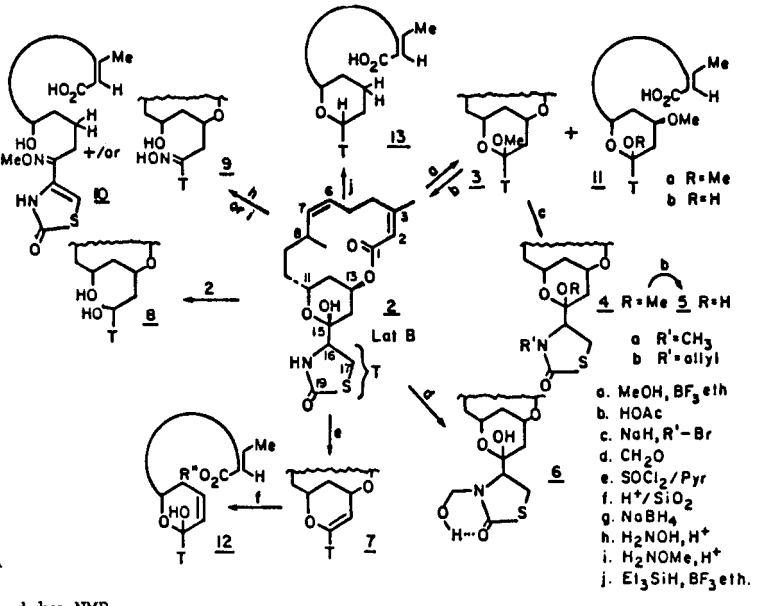
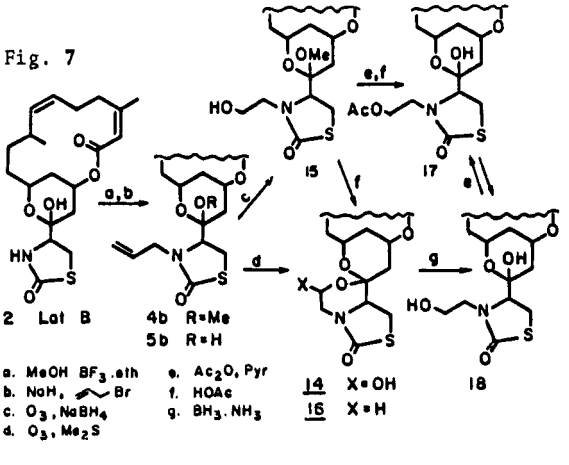


Fig. 6

The structure elucidation of Lat A was achieved by spectral methods; the functionalities were established by NMR spectroscopy and finally the complete structure was determined by an X-ray diffraction analysis (Ref.12). The absolute configuration of the molecule was established by Lat A degradation to the known L-cysteine derived ethyl 2-thiazolidinone-4-carboxylate (Ref.12). The structure of Lat B, possessing the smaller 14-membered macrolide, first suggested on the basis of ^{1}H and ^{13}C 1D-NMR data comparisons, with the data of Lat A, was later unequivocally confirmed by 2D NMR homo and hetero correlations (Ref.12) and recently also by total synthesis (Ref.16). Following the discovery of the interesting bio activity of the Lats by I. Spector (Ref.13) we undertook a structure activity relationship study. For that purpose the chemical alterations of the four major functional sites of the Lats were undertaken. That is, changes of the thiazolidinone, the tetrahydropyrane ring, the double bonds and the macrolide. The chemistry involved in these structure modifications follows.

Fig. 7



Methylation of 2 with a methanol borontrifluoride etherate mixture afforded in ca. 80% the bio inactive methyl lactol 3, which by avoiding undesirable side reactions, was a good starting material for many derivatives. Thus, compound 3 could be N-alkylated with sodium hydride and the required alkylhalide. A series of 15-OMe-N-alkyl derivatives (4) were prepared in such a manner (Fig.6 4a-b). Both series 4 and 5, which were prepared by acidic dealkylation of the lactol, were not active anymore.

Conversion of the NH to the N-formyl derivative, on the other hand, maintained the activity (compound 6). Water elimination from Lat B was achieved with thionylchloride-pyridine affording the Δ^{14}- derivative -7. Although no substantial amount of the open lactol could have been detected in solution (in contrast to 5-10% in case of the THP-thiazolidinone model (Ref.12)) Lat B could have been reduced with sodium borohydride to the 15-epimeric pair (8,Ref.12). One out of the two reduced alcohols was identical with the natural Lat C. The lactol could also be opened, although in low yields, with acidic t-butanol solution of hydroxylamine to afford oxime 9. Methoxyl amine under similar conditions furnished product 10 in which the macrolide opened up and, most likely, the intially obtained 13(14) double bond migrated into the thiazolidinone ring (Fig.6) (Ref.12b). Opening of the macrolide was also observed in compound 11- a side product of compound 3. Compound 11a was converted, by acid, to lactol 11b which most interestingly was biologically active. Both NH-signals in compounds 10 and 11b appear around δ_H 8.5ppm due to strong hydrogen bonds with, most likely, the carboxylic group. Esterification of the latter group brought the NH-signal back to around 6ppm as in Lat B by itself. Mild acidic conditions opened also the macrolide of 7 with an allylic rearrangement to give compound 12 (Fig.6). In purpose to remove the 15-OH lactol group we submitted compound 2 to a triethylsilane-borontrifluoride etherate complex reduction (Ref.17). Conditions under which the reduced open macrolide -13 (Fig.6) was obtained. Compound 13 was not active anymore (reduction of 3 on the other hand, reduces the 13-OH and maintaines the lactol). The high activity of the N-formyl derivative (6) triggered us to try and synthesize the higher N-ethylhydroxy homologue (18) (Fig.7).

Ozonolysis studies of Lat A and Lat B have shown the 6(7) double bond of Lat B to be relatively stable towards ozone. Indeed, ozonolysis of 5b with one equivalent of ozone at -78° followed by dimethylsulfide reduction left the macrolide intact and afforded compound 1 in which the newly formed aldehyde closed a lactol with the 15-OH group (Fig.7). A similar ring system, as in 14, could have been prepared by reductive (sodium borohydride) ozonolysis of 4b to 15 followed by acid cleavage of the 15-methoxy group to afford 16. Borane-amine reduction of 16 furnished the desired N-hydroxyethyl derivative of Lat B - compound 18 (Fig.7). Acetylation of 18 gave compound 17. Deeper insight into the mode of action of the Lats on actin revealed clear differences between Lat A and B initiating the prepartion of more derivatives of both toxins.

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