Secondary metabolites from marine microorganisms*

ALPHONSE KELECOM

Departamento de Biologia Geral, Universidade Federal Fluminense, Cx. Postal 100.436, 24001-970 Niterói, RJ

Manuscript received on September 24, 2001; accepted for publication on October 16, 2001;
presented by Affonso Guidão Gomes

ABSTRACT

After 40 years of intensive research, chemistry of marine natural products has become a mature field. Since 1995, there are signals of decreased interest in the search of new metabolites from traditional sources such as macroalgae and octocorals, and the number of annual reports on marine sponges stabilized. On the contrary, metabolites from microorganisms is a rapidly growing field, due, at least in part, to the suspicion that a number of metabolites obtained from algae and invertebrates may be produced by associated microorganisms. Studies are concerned with bacteria and fungi, isolated from seawater, sediments, algae, fish and mainly from marine invertebrates such as sponges, mollusks, tunicates, coelenterates and crustaceans. Although it is still to early to define tendencies, it may be stated that the metabolites from microorganisms are in most cases quite different from those produced by the invertebrate hosts. Nitrogenated metabolites predominate over acetate derivatives, and terpenes are uncommon. Among the latter, sesquiterpenes, diterpenes and carotenes have been isolated; among nitrogenated metabolites, amides, cyclic peptides and indole alkaloids predominate.

Key words: bacteria, fungi, alkaloids, despsipeptides, terpenoids, acetogenins, biological activities.

INTRODUCTION

In the early sixties, the increasing needs for drugs able to control new illnesses or resistant strains of microorganisms stimulated to look for unconventional new sources of bioactive natural products. The oceans turned out to be an attractive field. Since then, giant efforts have been accomplished worldwide aiming the isolation of new metabolites from marine organisms. Pioneer research was mainly concerned with marine toxins in part because of the numerous poisoning and feeding intoxications suffered by American soldiers in the Pacific, during World War II (Halstead 1965). Most of the marine toxins appeared to be protein mixtures or highly polar compounds with very complex chemical structures that were, at that time, extremely difficult to handle. Thus, the interest moved rapidly from toxins to terpenes that could be obtained from invertebrates (principally coelenterates) and from red or brown benthic macroalgae (Kelecom 1999).

At the beginning, most of the chemical studies were conducted randomly as a result of poor available ethnopharmacologic information. In the seventies, the interest for bioactive compounds and later on for chemical ecology introduced new driving forces that would not only organize, but also allow an extraordinary expansion of the whole field of marine natural products research, that must be considered now as a “mature field” (Faulkner 1998).

The search for new biomedicals from marine organisms resulted in the isolation of more or less...
10,000 metabolites (Fusetani 2000), many of which endowed of pharmacodynamic properties. A broad spectrum of biological activities has been detected, such as: antibiotic, antifungal, toxic, cytotoxic, neurotoxic, antimitotic, antiviral, antineoplastic and CV activities. In more recent years, new targets have been added to the general screening, for example: AIDS, immunosuppression, anti-inflammation, Alzheimer disease, ageing processes and some tropical diseases (Kelecom 1999).

However, since 1995, it became evident that all classical algae sources began to be much less studied than in the past; similarly, studies on coelenterates were also declining and the annual number of papers on sponge metabolites reached a maximum (Kelecom 1999 – Table X), although continuing to dominate the reports of new compounds (Faulkner 1999). In contrast, metabolites from marine microorganisms is a rapidly growing field as can be best observed from the number of reviews dedicated to this topic (Fenical 1993, Kobayashi and Ishibashi 1993, Jensen and Fenical 1994, 1996, 2000, Davidson 1995, Liberra and Lindquist 1995, Pietra 1997, Berman et al. 1997, Faulkner et al. 2000).

In a recent paper, the amazing increase in the number of reports on new metabolites from marine microorganisms has been emphasized (Kelecom 1999). Two years later, this tendency is even more evident, and it may be asked why? This review intends to answer this question and to describe the state-of-art of secondary metabolites from marine microorganisms. Dinofagellates, Cyanophyceae and other microalgae will not be covered here. Information on these groups can be found elsewhere (Faulkner 1984-1999).

WHY MARINE MICROORGANISMS?
The importance of terrestrial bacteria and fungi as sources of valuable bioactive metabolites has been very well established for more than half a century. As a result, over 120 of the most important medicines in use today (penicillins, cyclosporin A, adriamycine, etc.) are obtained from terrestrial microorganisms. At first sight thus, the expectable enormous biodiversity of marine microorganisms might have been the reason for the interest in their study. An additional possible explanation should be that marine microorganisms constituted the ultimate “inviolated” frontier for the search of marine natural products. But although valid, these were not the true starting reasons.

Hence, when, how and why did such studies started?
The isolation by Brotzu, in the late forties, of the antibiotics cephalosporins C (\textbf{1}) and P\textsubscript{1} (\textbf{2}), together with other metabolites, from the fungi \textit{Cephalosporium} sp cultivated from seawater collected near a sewage outlet off the coast of Sardinia (Burton and Abraham 1951) seems well to be the first conclusive work in this area, but it remained an isolated fact and the marine ancestry of such compounds was even claimed to be “dubious” (\textit{e.g.} Scheuer 1963). Undoubtedly more important was the suspicion that a number of metabolites obtained from algae and invertebrates could be produced by associated microorganisms. Indeed, it has been frequently suggested, but seldom demonstrated, that microorganisms should be in some instances the true producers of a number of secondary marine metabolites. Di-bromotyrosines from \textit{Aplysia} sponges, halogenated metabolites from \textit{Dysidea} spp, macrolactones and sulfur containing compounds were claimed to be probably produced by associated organisms. Aryl carotenoids in sponges were suspected to be originated from inhabiting bacteria (Liaaen-Jensen 1967). Similarly, it was stated that “\textit{there is strong circumstantial evidence that the alkaloids from a species of the genus Reniera may be fabricated by a symbiotic microorganism}” (Faulkner 1984, p. 558), since mimosamycine (\textbf{3}) obtained from the sponges \textit{Reniera} sp (Frincke and Faulkner 1982) and \textit{Xestospongia} sp (McKee and Ireland 1987) had previously been isolated from the fungi \textit{Streptomyces lavendulae} No. 314 (Fukumi et al. 1977). Such considerations stimulated some very interesting works and resulted in important contributions that will be commented in the section “Results and Discussion”.

\textit{An. Acad. Bras. Cienc.}, (2002) 74 (1)
TABLE I
Evolution of the marine literature.

| Decade        | No. publications | No. substances |
|---------------|------------------|----------------|
|               | total microorg.  | % total microorg. |
| 1978-1987     | 2070 21         | 1.0 22 0.7      |
| 1988-1997     | 4791 151        | 3.2 246 3.5     |
| variation (%) | 131 619         | – 131 1016 –    |

*Transition zone between the Amazonian dense forests and central Brazilian drier cerrados.

CURRENT STATUS OF THE FIELD

A quantitative analysis of the marine literature over the past twenty years is reported in Table I (Chemical Abstracts; Faulkner 1984-1999). It appears that during the period 1978-1987, 2070 papers were published on marine natural products. Among them, only 21 (1%) were dedicated to microorganisms. These publications described a total of 3076 metabolites, 22 of which (0.7%) originated from bacteria and fungi. Along the next decade (1988-1997), there was an increase of 131% both in the total number of publications (4791) and in the number of isolated metabolites (7099). During the same period, however, the evolution in the research effort on microorganisms has been much more impressive: 151 reports appeared describing 246 compounds, corresponding to increases of 619% and 1016% respectively, and it may be stated that the plenary lecture held by William Fenical at the VI International Symposium on Marine Natural Products, in 1989, undoubtedly catalyzed research in the field (Fenical et al. 1989).

Considering the annual productions, it can be seen that the field is growing up in an exponential way (Figures 1a and 1b). However, the mean number of products described per publication is low: ~ 1.5 (Figure 1c), and the mean number of products isolated per organism is <3, except in 1997 (Figure 1d). These are characteristics of almost unexplored and very competitive research areas where each isolated metabolite (or couple of metabolites) can be published due to novelty, and where the organisms are only submitted to preliminary investigation.

Studies on marine microorganisms are facing some expected but also unexpected problems. First, the taxonomy of marine bacteria and marine fungi is very poorly defined, so that binomial identifications are frequently uneasy to be carried out, and many papers describe metabolites isolated from numbered strains of otherwise partially or totally undefined organisms. This situation results in the impossibility to use chemotaxonomic leads for future research or even for comparative work; worse, studies may be duplicated without detecting it. The second question is almost philosophic in nature: which are the requirements for a microorganism to be considered a "marine species"? (MacLeod 1965). Bacteria often need seawater or chlorine to grow, but most of the fungi have no requirements at all. There are strictly marine species; other species that have special requirements to grow, but only during part of their living cycle, and there are also species that have been isolated from the marine environment but that are taxonomically identical to terrestrial well defined species. The third point refers to technical problems that arise to grow up marine microorganisms. Marine bacteria, much more than fungi, have proven to be uneasy to culture. Special media had to be developed, and it seems logical to admit that the most difficult organisms to grow might well be the strictly marine ones, and thus the ones that might produce the most original new metabolites.
Many of these organisms are usually lost, and so are their chemicals. In addition, on isolating a microorganism from its natural environment (or host) and culturing it, metabolic changes may occur probably due to partially unsatisfied micronutrients in the culture medium. This eventually will result in the obtainment of quantitatively and/or qualitatively modified natural products. The fourth and final point that will be discussed here call the attention to the high unpredictability of expected results. Thus, very closely related chemicals can be produced by quite distant microorganisms; conversely, a single species may produce substances originated from unrelated biosynthetic pathways, depending on the origin of the microorganisms. Examples of these situations will be given below (see Tables IX and X).

So far, studies have been concerned with bacteria and fungi, isolated from seawater, sediments, algae, fish and mainly from marine invertebrates such as sponges, mollusks, tunicates, coelenterates

Fig. 1 – Tendencies of the literature on marine microorganisms.
and crustacean (Table II). Two opposite approaches can be observed. A random approach that uses any available material for the isolation of microorganisms (seawater, sediments, gastrointestinal content of fish or even peaces of floating wood). The second approach uses algae, invertebrates and fish with well defined purposes, such as the obtainment of target metabolites or the study of the chemistry involved in the host/microorganisms associations. It appears, from Table II, that 33% of studied microorganisms have been isolated from sponges. This is obviously related to the plethora of bioactive compounds isolated from that phylum. Marine sediments have also been extensively used. Superficial waters and estuarine sediments have yielded microorganisms closely related to terrestrial ones. Thus deep waters and non-estuarine sediments have been preferred, and have yielded some highly unexpected metabolites.

### TABLE II

| Origin | %  |
|--------|--|
| • seawater | 2 |
| • sediment | 23 |
| • algae | 10 |
| • fish | 9 |
| • invertebrates | 47 |
| ○ sponges | 33 |
| ○ mollusks | 5 |
| ○ tunicates | 5 |
| ○ coelenterates | 2 |
| ○ crustacean | 2 |
| • other: worms, reefs, wood,... | 9 |
| Total | 100 |

Although it is somewhat early to define a general picture of the field, some trends can already be observed. It appears that 56% of the isolated substances (100% n=258) are nitrogenated compounds. Acetate-derived metabolites amount to 30%, and the terpenoids reach only 13% (Table III). This distribution is very different from the one observed for marine invertebrates where the mevalonate-derived metabolites are the most abundant (Kelecom 1999). Sulfur is identified in 13% of the 258 compounds considered in the present study. This frequency is somewhat higher than for marine invertebrates (Kelecom 1999). Halogenation has been reported in 14 substances (8%); chlorine rather than bromine is the dominating halogen. Nitrogenated and acetate-derived products constitute the first five principal classes of metabolites found in marine microorganisms (Table IV). This contrasts drastically with the situation reported for the invertebrates where the top-5 classes of metabolites all belong to the mevalonate pathway (Kelecom 1999). Thus, diterpenes that occupy the first position of the ranking in invertebrates appear only in the ninth position. Moreover, some classes of compounds common in invertebrates are so far absent from microorganisms (see below).

Terpenes are basically represented by sesquiterpenes and diterpenes, but some carotenes, prenylquinones and one steroid have also been described (Table V). Among the carotenes, a rare C_{50} homologue, okadaxanthine (4), has been isolated from the bacteria *Pseudomonas* sp, strain KK 10206C obtained from an homogenate of the sponge *Halichondria okadai* (Miki et al. 1994), the well known source of the cytotoxic polyketide okadaic acid (5), a selective inhibitor of the serine/threonine protein phosphatase 1 and 2A (Fujiki and Suganuma 1993). Figure 2 represents terpenoids skeletons found in microorganisms. When compared to invertebrates or algae, the diversity of skeleton is extremely low. Indeed, monoterpenes, sesterterpenes, triterpenes, steroid and triterpene saponines and polyoxygenated steroids have not yet been found in marine microorganisms.

Malonate-derived metabolites include aliphatic, alicyclic or lactonized acetogenins, metabolites from mixed acetate-propionate origin and macrolides (Table VI). Although showing structural variations, all these classes of natural products have also been found in invertebrates. Prostaglandins, com-

*An. Acad. Bras. Cienc.*, (2002) 74 (1)
### TABLE III

**Biosynthetic pathways.**

| Source       | Acetate % | Acetate no. | Mevalonate % | Mevalonate no. | Nitrogenated % | Nitrogenated no. | Chiquimate % | Chiquimate no. |
|--------------|-----------|-------------|--------------|----------------|----------------|------------------|--------------|----------------|
| microorganisms | 30 (78)  | 13 (35)     | 56 (145)     | <1 (1)         |                |                  |              |                |
| invertebrates  | 15 (309) | 62 (1325)   | 18 (390)     | 0.1 (2)        |                |                  |              |                |

### TABLE IV

**Ranking of the most frequent chemical classes in microorganisms compared to the invertebrates.**

| Chemical classes | microorganisms | invertebrates |
|------------------|----------------|--------------|
| no.              | %             | no.          | %             |
| 1 amines and amides | 43 (16.7)     | 101 (4.8)    |               |
| 2 indole alkaloids  | 36 (13.9)     | 65 (3.1)     |               |
| 3 acetogenines    | 33 (12.8)     | 30 (1.4)     |               |
| 4 cyclic peptides | 25 (9.7)      | 48 (2.3)     |               |
| polypropionates   | 25 (9.7)      | 57 (2.7)     |               |
| 6 macrolides      | 17 (6.6)      | 21 (1.0)     |               |
| 7 sesquiterpenes  | 16 (6.2)      | 393 (18.8)   |               |
| 8 phenazines      | 11 (4.3)      | n.r.         | -             |
| 9 diketopiperazines | 10 (3.9)     | n.r.         | -             |
| ones              | 10 (3.9)      | 496 (23.4)   |               |
| 11 carotenes      | 4 (1.5)       | n.r.         | -             |
| guanidines        | 4 (1.5)       | 23 (1.1)     |               |
| benzothiazoles    | 4 (1.5)       | n.r.         | -             |
| (iso)quinolines   | 4 (1.5)       | 23 (1.1)     |               |
| 15 dihalotyrosines| 3 (1.2)       | 48 (2.3)     |               |
| 16 macrolactames  | 2 (0.8)       | n.r.         | -             |
| 17 diphenylethers | 1 (0.4)       | 13 (0.6)     |               |
| 18 steroids       | 1 (0.4)       | (>250)       | 11.8          |
| others            | 9* (3.5)      | 546** (25.6) |               |

General total: 258 (100) 2114 (100)

n.r. = not reported in Kelecom (1999); *prenylated thiolenes, 1 isoflavonoid, triglycerides and miscellaneous metabolites; **polyoxygenated steroids, sesiterpenes, eudistamines, polyacetylenics, steroid and triterpene saponines, prostaglandines and triterpenes.
mon in octocorals, polyacetylenics from Porifera and polyethers from sponges and coelenterates (Kelecom 1999) have so far not been found in microorganisms.

Nitrogenated compounds are dominated by amines and amides, indole alkaloids and cyclic peptides (Table VII), but many other heterocyclic systems have been encountered, some of them being unknown from other marine sources (Figure 3). Halogenated amines, nucleosides, mycosporines and alkaloids of the eudistomine group, that are well known constituents of marine invertebrates are so far absent from microorganisms, and this is particularly surprising in the case of the mycosporines that are characteristic constituents of terrestrial fungi.

As mentioned above, the first goal of the studies on bacteria and/or fungi was to prove which from the marine invertebrates or their associated microorganisms are the true sources of isolated metabolites. In the case of microbial origin, it should be possible
to obtain reasonable amounts of valuable substances through large scale fermentation. Besides this major reason, the study of the associated organisms should help to solve relevant questions related to the biology and ecology of marine invertebrates and algae. Finally, being an unexplored field, a random search may afford unexpected new metabolites that might eventually be endowed of interesting pharmacological properties. In this respect, anti-tumor and antibacterial activities have been most frequently observed. This results from the enormous interest for new chemicals highly efficient against all kinds of cancers or against the very resistant strains of bacteria such as *Staphylococcus aureus*, that constitute a major problem in hospital infections.

The principal investigated biological activities are reported in Table VIII. The number and percentages of active compounds are shown for bacteria and...
SECONDARY METABOLITES FROM MARINE MICROORGANISMS

TABLE VII
Number of nitrogenated metabolites.

| Chemical class       | microorganisms | invertebrates |
|----------------------|----------------|---------------|
|                      | no.  | %    | no.  | %    |
| 1 amines and amides  | 43   | 29.6 | 101  | 25.9 |
| 2 indoles            | 36   | 24.7 | 65   | 16.7 |
| 3 cyclic peptides    | 25   | 17.5 | 48   | 12.3 |
| 4 phenazines         | 11   | 7.6  | n.r. | –    |
| 5 diketopiperazines  | 10   | 6.9  | n.r. | –    |
| 6 guanidines         | 4*   | 2.8  | 23   | 5.9  |
| 7 benzothiazoles     | 4    | 2.8  | n.r. | –    |
| 8 (iso)quinolines    | 4    | 2.8  | 29   | 7.4  |
| 9 dihalotyrosines    | 3    | 2.1  | 48   | 12.3 |
| 10 macrolactames     | 2    | 1.4  | n.r. | –    |
| others               | 3*   | 2.1  | 76** | 19.5 |
| Total                | 145  | 100  | 390  | 100  |

n.r. = not reported in Kelecom (1999); *2 pyroles and 1 adenine-like; **halogenated amines, nucleosides, mycosporines, eudistomines.

fungi, and the original sources of microorganisms are given. It appears that from a total of 258 reported substances, 79 (31%) have shown some biological activity; 47 metabolites were isolated from bacteria and 32 from fungi. The numbers of antitumor and antibacterial compound from bacteria are almost the same (c.a. 20), but fungi are more efficient sources of anti-cancer (57%) rather than antibacterial (22%) compounds. Another striking difference is related to the source of the microorganisms (Table VIII, sub-totals). Thus 40% of the active principles from bacteria came from sediment bacteria, but only 11% from sponge bacteria, and even less from algal bacteria (9%). A different situation is observed for fungi, with 62% of active substance isolated from sponge fungi, 22% from algal fungi and only 16% from sediment fungi. Thus when antitumor principles are desired, sediment bacteria, algal fungi or fungi from sponges should be the preferred sources. If antibacterial metabolites are aimed, one should prefer bacteria over fungi, and better sediment bacteria. It should be emphasized, however, that these predictions were deduced from the reduced set of available data.

Notwithstanding the starting character of the studies on marine microorganisms, it may already be deduced that the chemistry of both fungi and bacteria is hard to predict. Tables IX and X show, for a selected set of organisms, the chemical classes of isolated metabolites, their metabolic origin (PW) and the eventual associated biological activity. It is clear that most of the microorganisms have yielded very different classes of chemicals, through distant biosynthetic pathways, and that such strong chemical variations seem to be related to the origin of the microorganisms rather than to the microorganisms themselves. Thus, all the metabolites isolated from bacteria of the genus Bacillus are nitrogenated, but depending on the origin of the microorganisms (deep water, sediment, mollusk, sponge or worm), the metabolites will be aminoglicosides, cyclic peptides, depsipeptides or even amino-isocoumarins.

An. Acad. Bras. Cienc., (2002) 74 (1)
On the contrary, fungi of the genus *Aspergillus* furnished fumiquinazolines, indole alkaloids, halogenated acetogenins and sesquiterpenes, again depending on the origin of the fungus (Table IX). As expected, a very large array of pharmacological activities have been found associated to such a chemodiversity, and the general chemical picture is presently rather complex.

*An. Acad. Bras. Cienc.*, (2002) 74 (1)
TABLE VIII
Distribution of the biological activities in marine microorganisms.

| Biological activities | Bacteria | | Fungi |
|-----------------------|----------|-----------------|--------|
|                       | no.      | %   | origin | no. | %   | origin |
| antitumor             | 17       | 37  | alcyonarian (2); alga (1); | 18 | 57  | alga (6); fish (2); |
|                       |          |     | fish (1); mollusk (2); | | | mollusk (1); reef (1); |
|                       |          |     | sediment (8); sponge (1); | | | sediment (2); sponge (5); |
|                       |          |     | tunicate (1); wood (1) | | | wood (1) |
| antibacterial         | 21       | 45  | alga (3); mollusk (1); | 7 | 22  | sediment (2); sponge (5) |
|                       |          |     | sediment (8); sponge (4); | | | |
|                       |          |     | tunicate (1); water (3); | | | |
|                       |          |     | worm (1) | | | |
| antiviral             | 2        | 4   | sediment (2) | 1 | 3   | phanerogame (1) |
| antifungal            | –        | –   | – | 2 | 6   | alga (1); wood (1) |
| anti-inflammatory     | 1        | 2   | jellyfish (1) | 1 | 3   | crab (1) |
| enzymatic inhibition  | 4        | 8   | mollusk (1); sediment(1); | 2 | 6   | sponge (10); wood (1) |
|                       |          |     | undefined (1); water (1) | | | |
| others                | 2        | 4   | mollusk(1); undefined (1) | 1 | 3   | sediment (1) |
| sub-total             | 19       | 40  | sediments only | 5 | 16  | sediments only |
| sub-total             | 5        | 11  | sponges only | 20 | 62  | sponges only |
| sub-total             | 4        | 9   | algae only | 7 | 22  | algae only |
| Total                 | 47       | 100 | – | 32 | 100 | – |

RESULTS AND DISCUSSION

In what follows, we will discuss briefly results obtained with microorganisms isolated from some of the most remarkable marine species. Metabolites are usually extracted either from the organism or from the culture medium.

The fire sponge Tedania ignis has been studied since the beginning of marine natural products chemistry because of its stinging properties. This sponge and related species of the same genus have been shown to produce the aryl carotenoid tedanin (6), suspected to be of bacterial origin (Liaaen-Jensen 1967), atisanediol, a diterpene of the atisane skeleton (Schmitz et al. 1983) together with the bicyclic tedanalactame (Cronan and Cardellina 1994), two pyrazoles (Parameswaran et al. 1997) and three diketopiperazines (7-9) (Schmitz et al. 1983). An unidentified bacterium of the genus Micrococcus could be isolated from T. ignis. Surprisingly, the cultured bacteria did not furnished the expected carotene, but yielded four benzothiazoles (unknown in Tedania spp) in addition to the three diketopiperazines cited above that were already known from terrestrial sources (Chen 1960, Kodaira 1961). This is one of the very few examples proving the production of sponge metabolites by an associated microorganism (Stierle et al. 1988).

One of the most fascinating works in chemical ecology involving microorganisms is the isolation of isatin (10) from the shrimp Palaemon macrodactylus. Microscopic analysis of embryos of the shrimp showed that their surface was consistently covered by a bacteria of the genus Alteromonas that turned out to be the true producer of isatin. Treatment of the embryos by antibacterial agents inhibited the growth of the bacteria, but all the embryos died from infec-
TABLE IX
Chemical classes and activities observed in some selected marine fungi.

| Microorganism (● origin) | PW | chemical class      | activity      |
|--------------------------|----|---------------------|---------------|
| Aspergillus              |    |                     |               |
| ● fish                   | N  | fumiquinazoline      | cytotoxic     |
| ● algae                  | Ter| sesquiterpene nitrobenzoate | –          |
| ● sponge                 | N  | indole diketopiperazine | antitumoral  |
|                          | Ac | chlorolactone        | –             |
| Leptosphaeria            |    |                     |               |
| ● brown alga             | N  | indole diketopiperazine | cytotoxic   |
| ● sea-grass              | Ac | naphtoquinone        | antidopamine  |
| Penicillium              |    |                     |               |
| ● fish                   | N  | acyclic peptide      | cytotoxic     |
| ● green alga             | N  | indole               | cytotoxic     |
|                          | Ac | acyl polycetide      | cytotoxic     |
| ● offshore sediment      | Ac | aromatic lactone     | inhibitor of cellular growth |
| ● undefined sediment     | N  | lactame              | neuritogenic  |
| Phoma                    |    |                     |               |
| ● crab shell             | Ter| diterpene           | PAF antagonist |

PW = pathway; N = nitrogenated compound; Ac = acetate-derived compound; Ter = terpenoid.

Dibromotyrosines are common metabolites from Aplysina sponges (Minale et al. 1976) that have been proposed as chemotaxonomic markers of the order Verongida (Berquist and Wells 1983). Aerothionin (11) and homoaerothionin (12) were first isolated from Aplysina (Verongia) aerophoba and Verongia thiona (Fattorusso et al. 1970, Moody et al. 1972), but were absent from a Brazilian sample of Aplysina fistularis forma fulva (Kelecom and Kannengiesser 1979, Kelecom 1997). Several reports published in the early eighties, suspected of the bacterial origin of these metabolites. In a study of the localization of 11 and 12 in Aplysina fistularis forma fulva from the intertidal zone at La Jolla, California, Faulkner and co-workers measured the rate of exudation of these metabolites. They found that A. fistularis, when molested, released 100 times more bromotyrosines (Walker et al. 1985). They also observed that sponge spherulous cells, located near the exhalant canals, contained very high concentrations of bromine (Thompson et al. 1983), and concluded that 11 and 12 were thus not produced by bacteria. This is consistent with the fact that bacteria are present in low amounts in the sponge tissues (Faulkner et al. 2000).

A more complex situation has been observed for the sponge Dysidea herbacea that has been studied in several parts of the world. This species has yielded sesquiterpenes, like spirodysin (13) (Kazlauskas et al. 1978b) and herbadysidolide (14) (Charles et al. 1978), brominated biphenyl ethers, e.g. (15) (Norton and Wells 1980), several hexachlorinated amino acid derivatives such as 13-demethylisodysidenin (16) (Erickson and Wells 1982) and...
## TABLE X
Chemical classes and activities observed in some selected marine bacteria.

| Microorganism (• origin) | PW chemical class | activity               |
|--------------------------|------------------|------------------------|
| Actinomycete             |                  |                        |
| • coastal sediment       | Ac lactone       | –                      |
| • coelenterate           | N depsipeptide   | antiinflammatory       |
| • deep-sea sediment      | Ac bromonaphthoquinone | antibiotic     |
| • sediment (undefined)   | Ac lactonized FA | –                      |
| • shallow water sediment | Ac bromonaphthoquinone and lactone | antibiotic and –   |
| • undefined              | Ac sesquiterpene | –                      |
| Alteromonas              |                  |                        |
| • crustacean             | N indole         | antifungal             |
| • open sea               | N cyclic peptide | –                      |
| • sponge                 | N macrolactame and amide ester | cytotoxic and –   |
| • undefined              | N dipyrrrole     | antibiotic             |
| • undefined              | N guanidine      | toxic                  |
| • undefined              | N amide ester    | antimicrobial           |
| • undefined              | Ac cyclic aromatic FA | bronchodilator |
| Bacillus                 |                  |                        |
| • deep waters            | N aminoglycoside | antimicrobial           |
| • mollusk                | N depsipeptide   | cytotoxic              |
| • polychae               | N cyclic peptide | antimicrobial           |
| • sediment               | N N-isocoumarine and cyclic peptide | antitumor and –   |
| • sponge                 | N cyclic depsipeptide | –              |
| Bacteria Gram +          |                  |                        |
| • deep sea sediment C-237| Ac macrolide    | antiviral               |
| • undefined              | N cyclic lysine  | cytotoxic               |
| Pseudomonas              |                  |                        |
| • fish skin              | N guanidine      | toxic                  |
| • polychae               | N cyclic peptide | antimicrobial           |
| • red alga               | N cyclic peptide | antimicrobial           |
| • sponge                 | Ter C50 carotene | –                      |
| • sponge                 | N diketopiperazine and phenazine | –          |
| • tunicate               | N amide         | –                      |
| • undefined              | N indole and quinolinol | antimicrobial and antibiotic |
| • undefined              | N guanidine diketopiperazine | chitinase inhibitor |
| Streptomyces             |                  |                        |
| • estuarine sediment     | N N-glycosylated flavonoid | –               |
| • fish                   | N peptide       | –                      |
| • gorgonian              | Ac FA lactone   | cytotoxic              |
| • mollusk                | N macrolactame  | superoxide inhibitor   |
| • shallow water sediment | Ac phenazine    | antimicrobial           |
| • sediment               | N diketopiperazine, pyrrole | – and enzyme inhibition |
| • sponge                 | N phenazine and lactone amide | antimicrobial and antibiotic |
| • undefined              | Ac naphtoquinone | –                      |
| Vibrio                   |                  |                        |
| • fish                   | N indole        | –                      |
| • fish pathogen          | N amide        | –                      |
| • sponge                 | Ac bromo diphenyl ether | antimicrobial       |
| • undefined              | N indole and lactame | antimicrobial and – |

PW = pathway; N = nitrogenated compound; Ac = acetate-derived compound; Ter = terpenoid; FA = fatty acid.

diketopiperazine (17) (Kazlauskas et al. 1978a). Examination of the sponge established that it contains up to 50% of cellular volume of the cyanobacteria symbiont Oscillatoria spongelliae (Faulkner et
al. 2000). From a very delicate process of sponge and symbionts cells separation, it could be shown that sponge cells contained the sesquiterpenes 13 and 14, and that the symbiont O. spongelliae contained the halogenated metabolites 15, 16 or 17, depending on the collecting place of the sponge (Faulkner et al. 2000). Moreover, an unidentified bacterium of the genus Vibrio could be isolated from a Dysidea species. When cultured, this bacteria produced biphenyl 15 in very low amounts (Elyakov et al. 1991). This result contrasts with the amazingly high amounts of that compound (from 5 to 12% dry weight) usually found in the sponge (Utkina et al. 1988) and may be related to metabolism depletion on isolating the bacteria from its host. Anyway, it results that the terpenes of Dysidea seem to be produced by the sponge and that the halogenated compounds are bacterial metabolites (Unson and Faulkner 1993). Moreover, very distant bacteria such as Vibrio and Oscillatoria seem both to be able to produce the same diphenyl ether 15.

The sponge Theonella swinhoei is a still more complex situation. The sponge produces the macro-lactone swinholide (Carmely and Kashman, 1985), a powerful cytotoxic and antifungal compound whose structure was revised to dimer 18 (Kitagawa et al. 1990). The genus Theonella was then intensively studied and several other bioactive substances have been obtained, such as onnamide A, an antiviral guanidine derivative from mixed biosynthetic origin (Sakemi et al. 1988), macrocyclic tridecadepsipeptides, e.g. theonellamine B that inhibits the Na/K-transporting ATPase (Kitagawa et al. 1986, Nakamura et al. 1986), and the cyclic glycopeptide theopalauamide 19. It has been shown that at least three different microorganisms are associated to T. swinhoei (Faulkner et al. 2000). Thus glycopeptide 19 has been found in filamentous bacteria.
and swinholide (18) in unicellular bacteria. Neither the sponge cells, nor the associated cyanobacteria (*Aphanocapsa feldmannii*) contained 18 or 19 (Faulkner et al. 2000).

Many other examples could be described here, but the hitherto commented results already allow to conclude that, in most cases, the metabolites from microorganisms are quite different from those produced by the algal or invertebrate hosts.

Thus, the sponge *Halichondria okadai* furnishes okadaic acid (6) (Tachibana et al. 1981) and the antitumor principle norhalichondrine A (20) (Uemura et al. 1985). Okadaic acid was thought to be produced by a microbial or microalgal symbiont of the sponge, since 6 was also obtained from the dinoflagellate, *Prorocentrum lima* (Murakami et al. 1982). Two unidentified bacteria, *Pseudomonas* sp and *Alteromonas* sp, have been isolated from *H. okadai* homogenates. None of these bacteria yielded compounds 6 or 20: *Pseudomonas* produced a C50 carotenoid (5) (Miki et al. 1994), and *Alteromonas* furnished alteramide A (21), a macrolactame that exhibits powerful cytotoxicity against P388 leukemia, lymphoma L1210 and KB carcinoma with IC50 values of 0.5, 1.7 and 5.0 μg/ml respectively (Shigemori et al. 1992).

The obligate marine fungus *Leptosphaeria* sp collected on the brown alga *Sargassum tortile* is a rich source of several diketopiperazines, the leptosines, *e.g.* (22a-c) (Takahashi et al. 1994). These metabolites that are endowed of cytotoxic, antileukemic and antineoplastic properties, have never been observed in the brown alga the which affords chromenol meroditerpenes (Kikuchi et al. 1983; Numata et al. 1991). Similarly, sponges of the genus *Hyrtios* have furnished a potent cytotoxic macrolide, altohyrtine A (23) (Kobayashi et al. 1993), and several terpenoids, one of them, manoalide (24), is an
an interesting anti-inflammatory agent (Kobayashi et al. 1994) first isolated from the sponge \textit{Luffariella variabilis} (Silva and Scheuer, 1980). Again, none of these compounds was obtained from the fungus \textit{Aspergillus niger} isolated from a \textit{Hyrtios} species. This microorganism yielded, however, asperazine (25), a diketopiperazine that exhibits selective antileukemic activity (Varoglu et al. 1997).

A number of other bacteria and fungi have also been isolated from the sea hare \textit{Aplysia kurodai}, the gorgonian \textit{Pacifigorgia} sp, and sponges of the genera \textit{Ircinia}, \textit{Jaspis}, \textit{Mycale} and \textit{Xestospongia}. Although these studies did not yield the expected bioactive compounds observed in the invertebrate hosts, an array of completely new bioactive metabolites could be isolated that opens new perspectives in the field of marine natural products chemistry.

**CONCLUSIONS**

Since the historical isolation of the prostanoids 26 and 27 from the Caribbean gorgonian \textit{Plexaura homomalla} (Weinheimer and Spraggins 1969), innumerable bioactive compounds have been described and some of them have reached the clinical assays. However, it has often proven extremely difficult, and in some cases impossible, to provide from invertebrates or macroalgae sufficient amounts of many of these substances due to limited amounts found in the producing organism, or to limited quantity of the organism itself, or to geographic, seasonal or sexual variations in the amounts and in the nature of produced secondary metabolites. On the other hand, the structural complexity of most of the interesting bioactive substances precluded the development of commercially viable syntheses.

New ways had to be found.

Direct isolation of target compounds from bacterial or fungal symbionts has not proven to work well. Although fermentation of microorganisms is a possible way, it does not apply to metabolites produced strictly by macroorganisms. Thus, genetic engineering complemented by bacterial fermentation is probably the future in MNP research for biomedicines, as already anticipated by Elyakov (1998).
ACKNOWLEDGMENTS
The author thanks the Scientific Research Council (CNPq) for a research fellowship and Prof. Roberto Gomes de Souza Berlinck (USP/São Carlos, Brazil) for providing abundant literature and for critical ideas on the field.

RESUMO
Após 40 anos de intensos esforços, a química de produtos naturais do mar tornou-se um campo maduro. Desde 1995, existem sinais de queda de interesse pela busca de novos metabólitos a partir das fontes tradicionais como macroalgas e octocorais, e uma estabilização do número anual de trabalhos sobre esponjas marinhas. Em franca expansão estão as pesquisas com microorganismos, devido em grande parte às suspeitas de que muitos dos metabólitos de algas e invertebrados poderiam ser produzidos, de fato, pelos microorganismos associados. Estudam-se bactérias e fungos, isolados da água do mar, de sedimentos, algas, peixes e principalmente de invertebrados marinhos como esponjas, moluscos, tunicados, celen- terados e crustáceos. Embora seja cedo ainda para definir tendências, pode-se dizer que, na maioria dos casos, os microorganismos produzem metabólitos e até classes de metabólitos bem diferentes daqueles encontrados nos invertebrados hóspedes. Analisando as rotas biogenéticas, observa-se predominância de substâncias nitrogenadas, muitos derivados da via do acetato e poucos terpenóides. Estes são representados por sesquiuperpenos, diterpenos e alguns carotenos. Substâncias nitrogenados incluem principalmente amidas, peptídeos cíclicos e alcalóides indolícicos.

Palavras-chave: bactérias, fungos, alcalóides, despsi-peptídeos, terpenóides, acetogéninas, atividades biológicas.

REFERENCES
BERNAN VS, GREENSTEIN, M AND MAISE, WM. 1997. Marine microorganisms as a source of new natural products. Adv Appl Microbiol 43: 57-90.
BERQUIST PR AND WELLS RJ. 1983. Chemotaxonomy of the Porifera: the development and current status of the field. In: Marine Natural Products: Chemical and Biological Perspectives, SCHEUER, PJ (editor), Academic Press, vol. V, pp. 17-22.
BURTON HS AND ABRAHAM EP. 1951. Isolation of antibiotics from a species of Cephalosporium. Cephalosporins P1, P2, P3, P4 and P5. Biochem J 50: 168-174.
CARMELY S AND KASHMAN Y. 1985. Structure of swinholide-A, a new macrolide from the marine sponge Theonella swinhoei. Tetrahedron Lett 26: 511-514.
CHARLES C, BRAEKRMAN JC, DALOZE D, TURSCH B, DECLERCQ JP, GERMAIN G AND VAN MEERSCHE M. 1978. Chemical studies of marine invertebrates XXXIV. Herba-dysidolide and herbasolide, two unusual sesquiuperpenos from the sponge Dysidea herbacea. Bull Soc Chim Belg 87: 481-486.
CHEN YS. 1960. Studies of the metabolic products of Rosellinia necatrix I. Isolation and characterization of several physiologically active neutral substances. Bull Agr Chem Soc Jpn 24: 372-381.
CRONAN JR JM AND CARDELLINA II JH. 1994. A novel δ-lactam from the sponge Tedania ignis. Nat Prod Letters 5: 85-88.
DAVIDSON BS. 1995. New dimensions in natural products research: cultured marine microorganisms. Curr Opin Biotechnol 6: 284-291.
ELYAKOV GB. 1998. Horizons in marine bioorganic chemistry, in: IX Int. Symposium on Marine Natural Products, Symposium Proceed., Townsville, Australia, Abstract OR-28.
ELYAKOV GB, KUZNETSOVA T, MIKHAILOV VV, MALTSIEV II, VOINOV VG AND FEDOREYEV SA. 1991. Brominated diphenyl ethers from a bacterium associated with the sponge Dysidea sp. Experientia 47: 632-633.
ERICKSON KL AND WELLS RJ. 1982. New polychlorinated metabolites from a Barrier Reef collection of the sponge Dysidea herbacea. Aust J Chem 35: 31-38.
FAULKNER DJ. 1984-1999. Marine Natural Products, Nat Prod Rep 1: 251-280, 1: 551-598, 3: 1-33, 4: 539-576, 5: 613-663, 7: 269-309, 8: 97-147, 9: 323-364, 10: 497-539, 11: 355-394, 12: 223-269, 13: 75-125,
FAULKNER DJ, HARPER MK, HAYGOOD MG, SALOMON CE AND SCHMIDT EW. 2000. Symbiotic bacteria in sponges: sources of bioactive substances. In: FUSETANI N (Ed.), Drugs from the Sea, Basel: Karger, p. 107-119.

Fenical W. 1993. Chemical studies of marine bacteria: developing a new resource. Chem Rev 93: 1673-1683.

Fenical W, Jensen P, Tapiolas D, Gustaison K, Roman M AND Pathirana C. 1989. Natural products chemistry of sediment-derived marine bacteria. Proceed. VI Int. Symp. Marine Natural Products, Dakar, Senegal.

Frincke JM AND FAULKNER DJ. 1982. Antimicrobial metabolites of the sponge Reniera sp. J Am Chem Soc 104: 265-269.

Fujiki H and Suganuma M. 1993. Tumor promotion by inhibitors of protein phosphatases 1 and 2A: the okadaic class of compounds. Adv Cancer Res 61: 143-194.

Fukumi H, Kurihara H, Hata T, Tamura C, Mishima H, Kubo A AND Arai T. 1977. Mimosamycin, a novel antibiotic produced by Streptomyces lavendulae n 314: structure and synthesis. Tetrahedron Lett, 3825-3828.

Fusetani N. 2000. Introduction. In: FUSETANI N (Ed.), Drugs from the Sea, Basel: Karger, p. 1-5.

Gil-Turnes MS, Hay ME AND Fenical W. 1989. Symbiotic marine bacteria chemically defend crustacean embryos from a pathogenic fungus. Science 246: 116-118.

Halstead BW. 1965. Poisonous and Venomous Marine Animals of the World, vol. I, chap. 1, pp. 1-155, US Government Printing Office, Washington DC.

Jensen PR AND Fenical W. 1994. Strategies for the discovery of secondary metabolites from marine bacteria. Ann Rev Microbiol 48: 559-584.

Jensen PR AND Fenical W. 1996. Marine bacterial diversity as a resource for novel microbial products. J Ind Microbiol 17: 346-351.

Jensen PR AND Fenical W. 2000. Marine microorganisms and drug discovery: current status and future potential. In: FUSETANI N (Ed.), Drugs from the Sea, Basel: Karger, p. 6-29.

Kazlauskas R, Murphy PT AND Wells RJ. 1978a. A diketopiperazine derived from trichloro-leucine from the sponge Dysidea herbacea. Tetrahedron Lett, 4945-4948.

Kazlauskas R, Murphy PT AND Wells RJ. 1978b. A new sesquiterpene from the sponge Dysidea herbacea. Tetrahedron Lett, 4949-4950.

Kelecom A. 1997. Marine natural products in Brazil. Part 1. Isolation and structure determination. Ciênc Cult – J Braz Adv Sci 49: 321-330.

Kelecom A. 1999. Chemistry of Marine Natural Products: Yesterday, Today and Tomorrow. An Acad Bras Cienc 71: 249-263.

Kelecom A AND KANNENGIESSER GJ. 1979. Chemical Constituents of Verongia Sponges I – A Comparison Between Brazilian and Mediterranean Species. An Acad Bras Cienc 51: 633-637.

Kitagawa I, Kobayashi M, Lee NK, Shibuya H, Kawata Y AND Sakiyama F. 1986. Structure of theonellapeptolide Id, a new bioactive peptolide from an Okinawan marine sponge, Theonella sp (Theonelliae). Chem Pharm Bull 34: 2664-2667.

Kitagawa I, Kobayashi M, Katori T, Yamashita M, Tanaka J, Doi M AND Ishida T. 1990. Absolute stereostructure of swinholide A, a potent cytotoxic macrolide from the Okinawan marine sponge Theonella swinhoei. J Am Chem Soc 112: 3710-3712.

Kobayashi J AND Ishibashi M. 1993. Bioactive metabolites of symbiotic marine microorganisms. Chem Rev 93: 1753-1769.

Kobayashi M, Aoki S, Sakai H, Kihara N, Sasaki T AND Kitagawa I. 1993. Altohyrtins B and C and 5-desacetylaltohyrtin A, potent cytotoxic macrolide congeners of altohyrtin A, from the Okinawan marine sponge Hyrtios alatum. Chem Pharm Bull 41: 989-991.

Kobayashi M, Okamoto T, Hayashi K, Yokoyama N, Sasaki T AND Kitagawa I. 1994. Absolute config-
uration of C-4 of the manoolide family, biologically active sesterterpenes from the marine sponge *Hyrtios erecta*. Chem Pharm Bull 42: 265-270.

Kodaira Y. 1961. Toxic substances to insects, produced by *Aspergillus ochraceus* and *Oospora destructor*. Agr Biol Chem 25: 261-262.

Liaaen-Jensen S. 1967. Recent advances in the chemistry of natural carotenoids. Pure Appl Chem 14: 227-244.

Liberra K and Lindquist U. 1995. Marine fungi: a prolific source of biologically active natural products? Pharmazie 50: 583-588.

MacLeod RA. 1965. The question of the existence of specific marine bacteria. Bacteriol Rev 29: 9-23.

McKee TC and Ireland CM. 1987. Cytotoxic and antimicrobial alkaloids from the Fijian sponge *Xestospongia caycedoi*. J Nat Prod 50: 754-756.

Miki W, Otaki N, Yokoyama A, Izumida H and Shimizu N. 1994. Okadaxanthin, a novel C50-carotenoid from a bacterium, *Pseudomonas* sp. KK 10206C associated with a marine sponge, *Halichondria okadai*. Experientia 50: 684-686.

Minale L, Cimino G, De Stefano S and Sodano G. 1976. Natural products from *Porifera*. Fort Chem Org Naturstoffe 33: 1-72.

Moody K, Thomson RH, Fattorusso E, Minale L and Sodano G. 1972. Aerotherixin and homoaerothxin: two tetrabromo spirohexadienylisoxazoles from *Verongia* sponges. J Chem Soc Perkin I: 18-24.

Murai K, Oshima Y, Ichiba T, Kohmoto S, Saucy G and Higa T. 1988. Isolation and structure elucidation of onnamide A, a new bioactive metabolite of a marine sponge, *Theonella* sp. J Am Chem Soc 110: 4851-4853.

Nakamura H, Kobayashi JI, Nakamura Y, Ohizumi Y, Kondo T and Hrata Y. 1986. Theonellamine B, a novel peptidal Na,K-ATPase inhibitor from an Okinawan marine sponge of the genus *Theonella*. Tetrahedron Lett 27: 4319-4322.

Norton RS and Wells RJ. 1980. Use of $^{13}$C spin-lattice relaxation measurements to determine the structure of a tetrabromo diphenyl ether from the sponge *Dysidea herbacea*. Tetrahedron Lett 21: 3801-3804.

Numata A, Kanbara S, Takahashi C, Fuiki R, Yoneda M, Fujita E and Nabeshima Y. 1991. Cytotoxic activity of marine brown algae and cytotoxic principle of the brown alga *Sargassum tortile*. Chem Pharm Bull 39: 2129-2131.

Parameswaran PS, Naik CG and Hegde VR. 1997. Secondary metabolites from the sponge *Tedania anhelans*: isolation and characterization of two novel pyrazole acids and other metabolites. J Nat Prod 60: 802-803.

Pietra F. 1997. Secondary metabolites from marine microorganisms – bacteria, protozoa, algae and fungi – achievements and prospectives. Nat Prod Rep 14: 453-464.

Sakemi S, Ichiba T, Kohimoto S, Saucy G and Higa T. 1988. Isolation and structure elucidation of onnamide A, a new bioactive metabolite of a marine sponge, *Theonella* sp. J Am Chem Soc 110: 4851-4853.

Scheuer PJ. 1973. Marine Natural Products, p. 38, Academic Press, NY and London.

Schmitz FJ, Vanderah DJ, Hollenbeck KH, Enwall CEL, Gopichand Y, Sengupta PK, Hossain MB and Van Der Helm D. 1983. Metabolites from the marine sponge *Tedania ignis*. A new atisanediol and several known diketopiperazines. J Org Chem 48: 3941-3945.

Shigemori H, Bae MA, Yazawa K, Sasaki T and Kobayashi J. 1992. Alteramida A, a new tetracyclic alkaloid from a bacterium *Alteromonas* sp associated with the marine sponge *Halichondria okadai*. J Org Chem 57: 4317-4320.

Silva ED de and Scheuer PJ. 1980. Manoolide, an antibiotic sesterterpenoid from the marine sponge *Luffariella variabilis*. Tetrahedron Lett 21: 1611-1614.

Stierle AC, Cardellina II JH and Singleton FL. 1988. A marine *Micrococcus* produces metabolites ascribed to the sponge *Tedania ignis*. Experientia 44: 1021.

Tachibana K, Scheuer PJ, Tsukitani Y, Kikuchi H, Van Engen D, Clardy J, Gopichand Y and Schmitz FJ. 1981. Okadaic acid, a cytotoxic polyether from two marine sponges of the genus *Halichondria*. J Am Chem Soc 103: 2469-2471.

Takahashi C, Numata A, Ito Y, Matsumura E, Minoura K, Eto H, Shingu T, Ito T and Hasegawa T. 1994. Leptosins I and J, cytotoxic substances...
produced by a *Leptosphaeria* sp. Physico-chemical properties and structures. J Antibiot 47: 1242-1249.

**Thompson JE, Barrow KD and Faulkner DJ.** 1983. Localization of two brominated metabolites, aerothionin and homoaerothionin, in spherulous cells of the marine sponge *Aplysina fistularis* (= *Verongia thiona*). Acta Zool 64: 199-210.

**Uemura D, Takahashi K, Yamamoto T, Katayama C, Tanaka J, Okumura Y and Hirata Y.** 1985. Norhalichondrin A: an antitumor polyether macrolide from a marine sponge. J Am Chem Soc 107: 4796-4798.

**Unson MD and Faulkner DJ.** 1993. Cyanobacterial symbiont biosynthesis of chlorinated metabolites from *Dysidea herbacea* (Porifera). Experientia 49: 349-353.

**Utkina NK, Kazantseva MV and Denisenko VA.** 1988. Brominated diphenyl ethers from the marine sponge *Dysidea fragilis*. Chem Nat Prod 23: 508-509 [translation of the paper published in Khim Prirod Soed (1987) no. 4, 603-605.

**Varoglu M, Corbett TH, Valeriote FA and Crews P.** 1997. Asperazine, a selective cytotoxic alkaloid from a sponge-derived culture of *Aspergillus niger*. J Org Chem 62: 7078-7079.

**Walker RP, Thompson JE and Faulkner DJ.** 1985. Exudation of biologically active metabolites in the sponge *Aplysina fistularis*. II. Chemical evidences. Mar Biol 88: 27-32.

**Weinheimer AJ and Spraggins RL.** 1969. The occurrence of two new prostaglandin derivatives (15-epi-PGA2 and its acetate, methyl ester) in the gorgonian *Plexaura homomalla*. Tetrahedron Lett, 5185-5188.