Anais da Academia Brasileira de Ciências (2002) 74(1): 151–170
(Annals of the Brazilian Academy of Sciences)
ISSN 0001-3765
www.scielo.br/aabc

Secondary metabolites from marine microorganisms*

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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

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*Presented as an invited talk in XXII RESEM (2000).

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, Bernan 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 **(1)** and P_1 **(2)**, together with other metabolites, from the fungi *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'' (*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. Dibromotyrosines from *Aplysia* sponges, halogenated metabolites from *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 ''*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 **(3)** obtained from the sponges *Reniera* sp (Frincke and Faulkner 1982) and *Xestospongia* sp (McKee and Ireland 1987) had previously been isolated from the fungi *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''.

TABLE I

Evolution of the marine literature.

Decade	No. publications			No. substances		
	total	microorg.	%	total	microorg.	%
1978-1987	2070	21	1.0	3076	22	0.7
1988-1997	4791	151	3.2	7099	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.

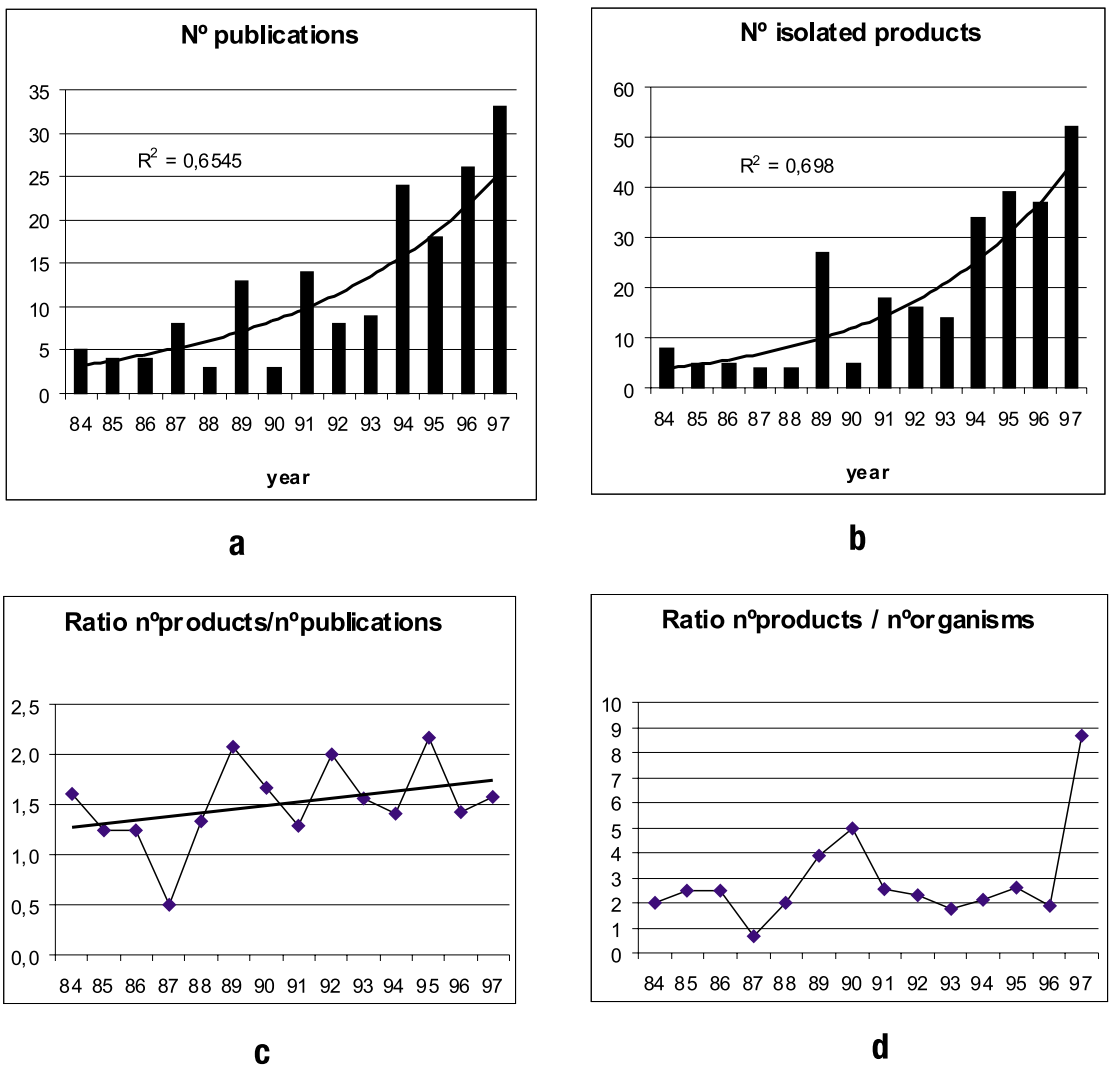

Fig. 1 – Tendencies of the literature on marine microorganisms.

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

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 of the microorganisms.

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-

TABLE III

Biosynthetic pathways.

Source	Acetate		Mevalonate		Nitrogenated		Chiquimate	
	%	no.	%	no.	%	no.	%	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.	-
	diterpenes	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 thiolanes, 1 isoflavonoid, triglycrides and miscellanous metabolites; **polyoxygenated steroids, sesterterpenes, eudistomines, polyacetylenics, steroid and triterpene saponines, prostaglandines and triterpenes.

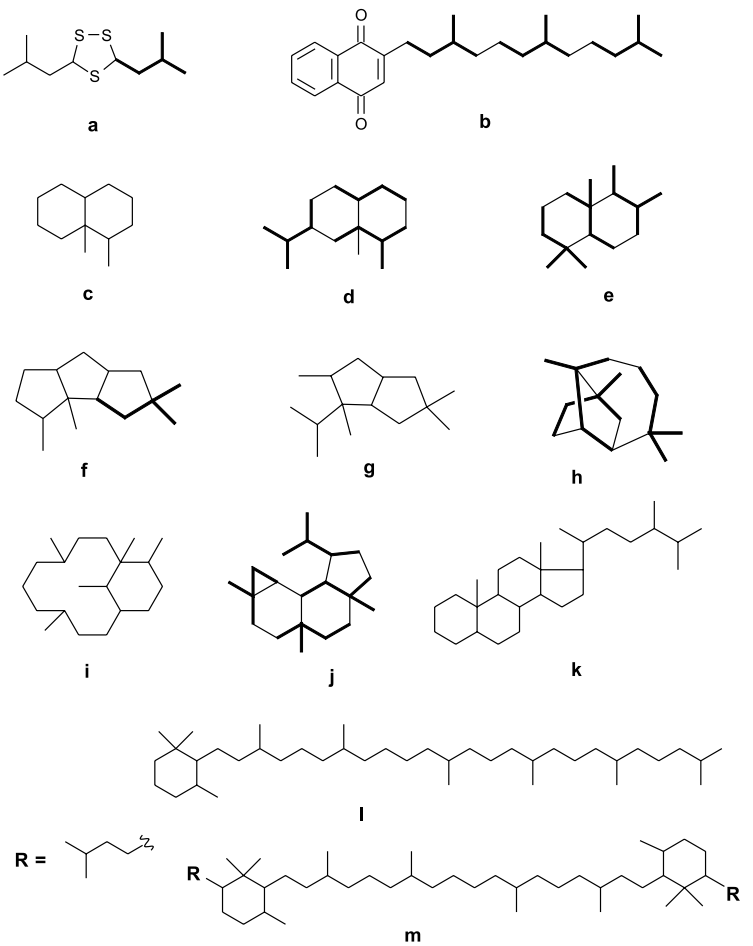

Fig. 2 – Terpenoid diversity in natural products from marine microorganisms. a=prenylated thiolane; b=polyprenylated naphtoquinone; c=tri-*nor*eremophilane; d=eremophilane; e=drimane; f=coriolane; g=seco-coriolane; h=unnamed; i=phomactane; j=neoverrucosane; k=ergostane; l=β, Ψ-carotene; m=extended β, β-carotene.

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

TABLE V

Number of mevalonate-derived metabolites.

	Chemical class	microorganisms		invertebrates	
		no.	%	no.	%
1	sesquiterpenes	16	45.7	393	29.7
2	diterpenes	10	28.6	496	37.4
3	carotenes	4	11.4	n.r.	–
4	prenyl-quinones	2	5.7	18	1.4
5	isoprene units	2	5.7	n.r.	–
6	simple sterol	1	2.9	n.r.	–
	others	–	–	418*	31.5
	Total	35	100	1325	100

n.r. = not reported in Kelecom (1999); *monoterpenes, sesterterpenes, triterpenes, triterpene and steroid saponines, polyoxygenated steroids.

TABLE VI

Number of malonate-derived metabolites.

	Chemical class	microorganisms		invertebrates	
		no.	%	no.	%
1	acetogenines	33	42.3	30	9.7
2	acetate-propionate	25	32.1	57	18.5
3	macrolides	17	21.7	21	6.8
4	diphenyl-ethers	1	1.3	13	4.2
	others	2*	2.6	188*	60.8
	Total	78	100	309	100

*triglycerides; **polyacetylenics, prostaglandines and polyethers.

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

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

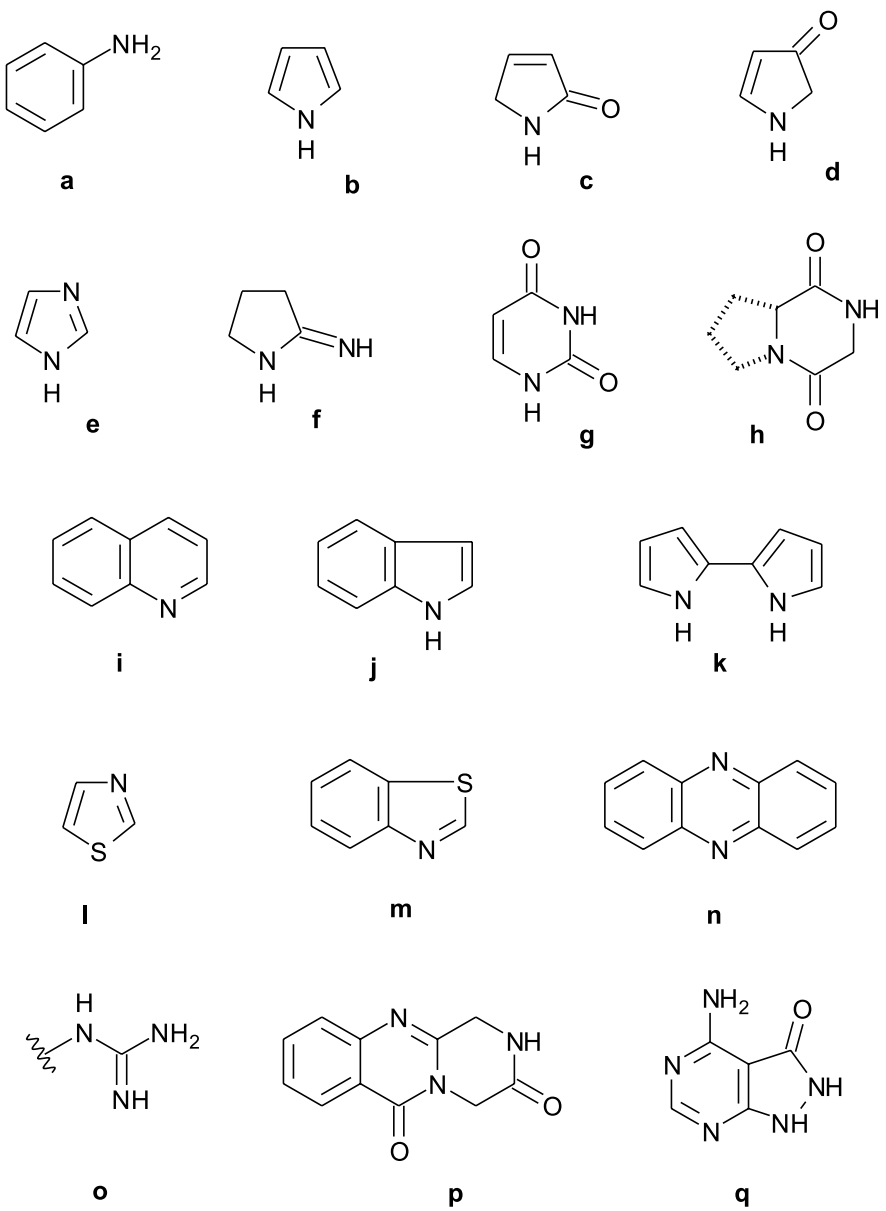

Fig. 3 – Nitrogenated systems in natural products from marine microorganisms. a=aniline; b=pyrrole; c=γ-lactame; d=β-oxo-pyrrole; e=imidazole; f=unnamed; g=diketopyrimidine; h=diketopiperazine; i=quinoline; j=indole; k=dipyrole; l=thiazole; m=benzothiazole; n=phenazine; o=guanidine; p=fumiquinazoline; q=adenine-like.

(Table X). 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 expectable, a very large array of pharmacological activities have been found associated to such a chemodiversity, and the general chemical picture is presently rather complex.

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); fish (1); mollusk (2); sediment (8); sponge (1); tunicate (1); wood (1)	18	57	alga (6); fish (2); mollusk (1); reef (1); sediment (2); sponge (5); wood (1)
antibacterial	21	45	alga (3); mollusk (1); sediment (8); sponge (4); tunicate (1); water (3); worm (1)	7	22	sediment (2); sponge (5)
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); undefined (1); water (1)	2	6	sponge (10); wood (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	neuritrogenic
Phoma			
• crab shell	Ter	diterpene	PAF antagonist

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

tion by the fungus *Lagenidium callinectes*. In fact, the bacterial metabolite isatin protects the shrimp embryos against fungal overgrowth (Gil-Turnes et al. 1989).

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	bromonaphtoquinone	antibiotic
• sediment (undefined)	Ac	lactonized FA	–
• shallow water sediment	Ac	bromonaphtoquinone and lactone	antibiotic and –
	Ter	sesquiterpene	–
• undefined	Ac	glycosylated macrolide	
Alteromonas			
• crustacean	N	indole	antifungal
• open sea	N	cyclic peptide	–
• sponge	N	macrolactame and amide ester	cytotoxic and –
• undefined	N	dipyrrole	antibiotic
	N	guanidine	toxic
	N	amide ester	antimicrobial
	Ac	cyclic aromatic FA	bronchodilatator
Bacillus			
• deep waters	N	aminoglycoside	antimocrobial
• mollusk	N	despsipeptide	cytotoxic
• polychaete	N	cyclic peptide	antimocrobial
• sediment	N	N-isocoumarine and cyclic peptide	antitumor and –
• sponge	N	cyclic despsipeptide	–
Bacteria Gram +			
• deep sea sediment C-237	Ac	macrolide	antiviral
• undefined	N	cyclic lysine	cytotoxic
Pseudomonas			
• fish skin	N	guanidine	toxic
• polychaete	N	cyclic peptide	antimicrobial
• red alga	N	cyclic peptide	antimicrobial
• sponge	Ter	C50 carotene	–
	N	diketopiperazine and phenazine	–
• tunicate	N	amide	–
• undefined	N	indole and quinolinol	antimicrobial and antibiotic
	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	N	phenazine	antimicrobial
	Ac	FA lactone	–
• 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
	N	indole and lactame	antimicrobial and –
• undefined	N	guanidine and lactame	toxic and –

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

the 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

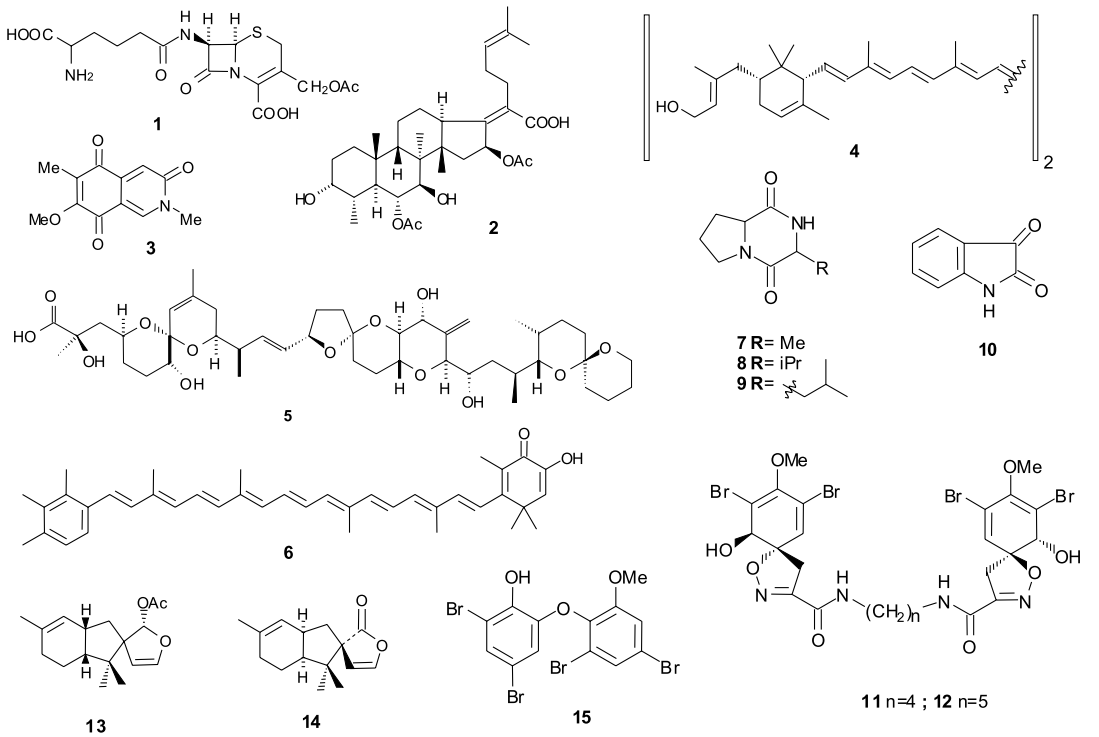

Fig. 4.

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 macrolactone 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

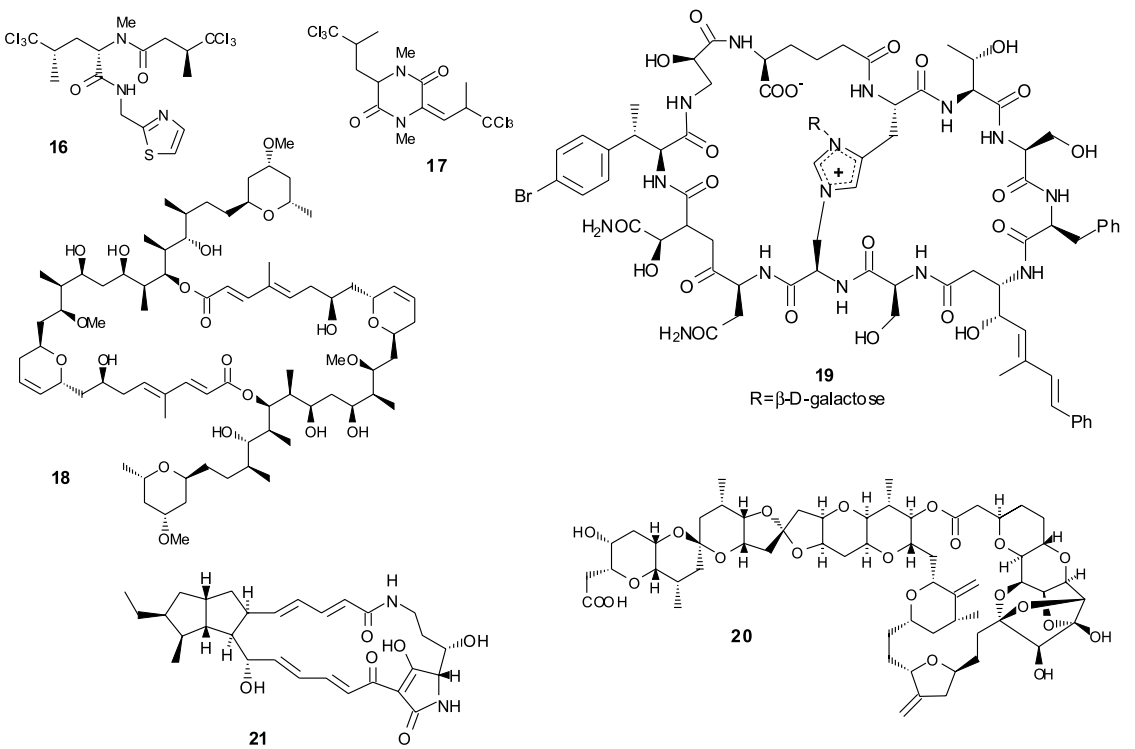

Fig. 5.

and swinholide **(18)** in unicellular bacteria. Neither the sponge cells, nor the associated cyanobacteria (*Aphanocapsa feldmanni*) 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 C_{50} 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 IC_{50} 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 antineoplasic 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

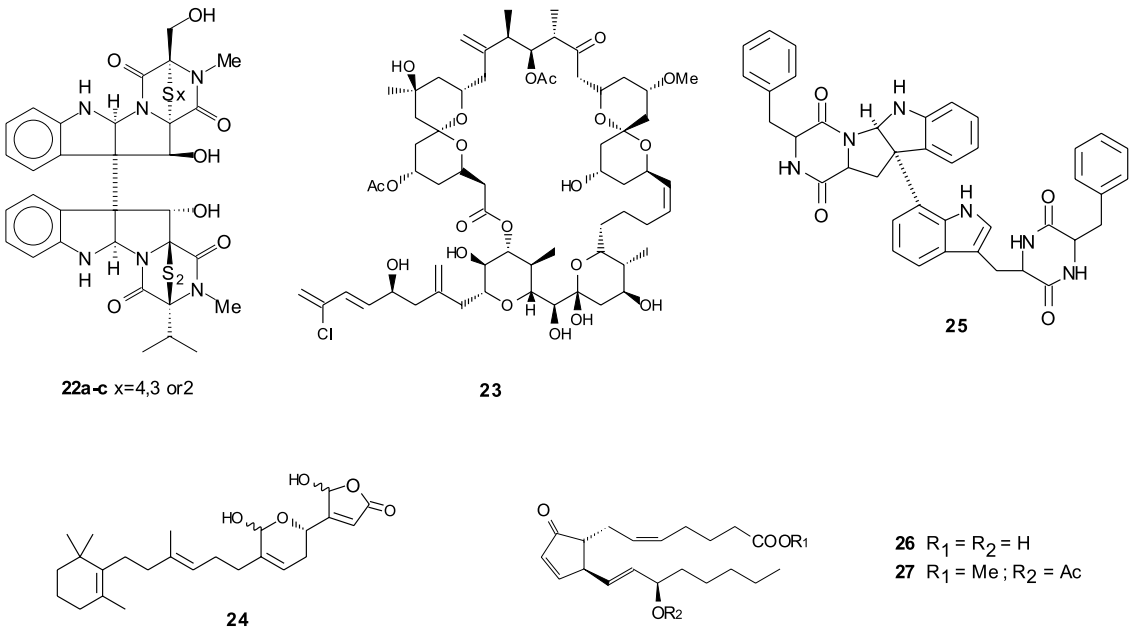

Fig. 6.

interesting anti-inflammatory agent (Kobayashi et al. 1994) first isolated from the sponge *Luffariella variabilis* (Silva and Scheuer, 1980). Again, none of these compounds was obtained from the fungus *Aspergillus niger* isolated from a *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 *Aplysia kurodai*, the gorgonian *Pacifigorgia* sp, and sponges of the genera *Ircinia*, *Jaspis*, *Mycale* and *Xestospongia*. Although these studies did not yielded 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 *Plexaura homomalla* (Weinheimer and Spraggins 1969), innumerous bioactive compounds have been described and some of them have reach 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, celenterados 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 sesquiterpenos, diterpenos e alguns carotenos. Substâncias nitrogenados incluem principalmente amidas, peptídeos cíclicos e alcalóides indólicos.

Palavras-chave: bactérias, fungos, alcalóides, despsipeptídeos, terpenóides, acetogeninas, atividades biológicas.

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