Intricate aspects of sponge chemistry

Michèle GUYOT
Laboratoire de Chimie du Muséum national d'Histoire naturelle,
associé au CNRS,
63 rue Buffon, F-75231 Paris (France)
guyot@mnhn.fr

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ABSTRACT
Sponges are able to biosynthesize original secondary metabolites which present promising pharmacological activity especially in cancer chemotherapy. The role played by symbiotic microorganisms in the biosynthesis of sponge secondary metabolites is currently a subject of intensive studies, in the hope that these structurally-complex metabolites may be obtained by biotechnological processes. Some highly cytotoxic, antimicrobial, repellent compounds are found in other marine animals, especially molluscs. Some shell-less molluscs prey on sponges and use these bioactive products for their own defense. This demonstrates the close relationships between marine organisms and the difficulties encountered to find the true producers of marine natural products.

KEY WORDS
Porifera,
antitumour,
antiviral,
HIV,
cytotoxic,
antimicrobial,
immunosuppressive,
anti-inflammatory,
associated microorganisms,
predator.

RÉSUMÉ
Aspects complexes de la chimie des éponges.
Les éponges biosynthétisent des métabolites secondaires originaux d'un grand intérêt pharmacologique, tout particulièrement dans le domaine de la chimiothérapie anticancéreuse. Le rôle joué par des micro-organismes symbiotes dans la biosynthèse de certains de ces métabolites secondaires fait l'objet de recherches intensives en raison de la possibilité ainsi offerte d'obtenir ces métabolites, de structure souvent complexe, par voie biotechnologique. Certains métabolites d'éponges, toxiques, antimicrobiens, répulsifs, sont trouvés dans d'autres organismes marins et particulièrement les mollusques. Certains mollusques dépourvus de coquilles consomment des éponges, souvent une espèce spécifique, et utilisent les métabolites élaborés par leur hôte pour leur propre défense. Cela montre les relations étroites existant entre les organismes marins et les difficultés rencontrées pour établir quels organismes produisent réellement les produits naturels d'origine marine.

MOTS CLÉS
Porifera,
antitumoral,
antiviral,
VIH,
cytotoxique,
antimicrobien,
immunosuppresseur,
anti-inflammatoire,
micro-organismes associés,
prédateurs.

INTRODUCTION

Marine natural products chemistry has experienced explosive growth over the past twenty years beginning with Werner Bergmann's pioneer work in the 1950's (Bergmann & Burke 1955). Ocean biodiversity began to be appreciated from the beginning of this century, and estimates of the number of species range from 1.5 to 4.5 million species, with the majority of these being, to date, undescribed. Thus the potential of the oceans to provide new bioactive metabolites is enormous. The main interest in marine natural products, and especially in sponge metabolites, is the high incidence of anticancer and cytotoxic metabolites (Schmitz *et al.* 1993).

Among marine invertebrates, demosponges originated the interest in marine chemistry for discovery of new drugs, and remain the most prolific phylum concerning new and pharmacologically active compounds. However, some metabolites ascribed to sponges are now suspected to be actually biosynthesized by microorganisms frequently present in symbiotic association. Moreover, whatever the actual producer, sound experimental evidences support the defensive role of sponge secondary metabolites for molluscs, and notably nudibranchs.

SPONGES SOURCE OF PHARMACOLOGY ACTIVE SUBSTANCES

Until the beginning of the century, only terrestrial plants were investigated for drug discovery. Interest in marine chemistry started with the work of W. Bergmann (Bergmann & Feeney 1951) who isolated three nucleosides: spongouridine, spongothymidine and spongosine from the Caribbean sponge *Cryptotethya crypta* Laubenfels, 1949. The two pyrimidine nucleosides contained arabinose instead of the classical ribose and spon-

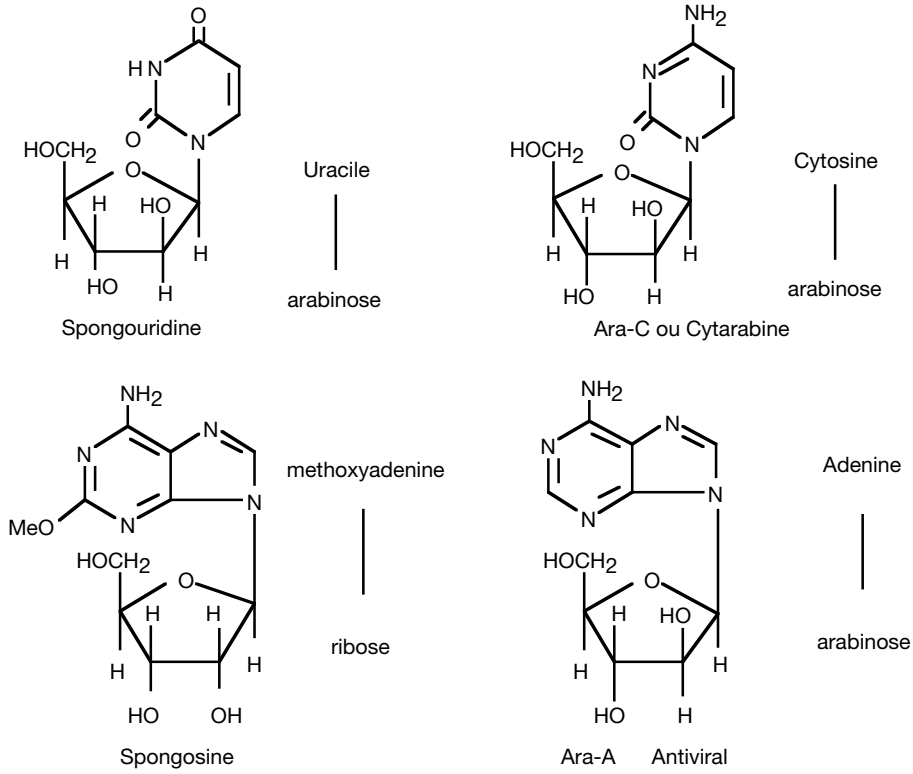


FIG. 1. — Structure of natural and synthetic nucleosides.

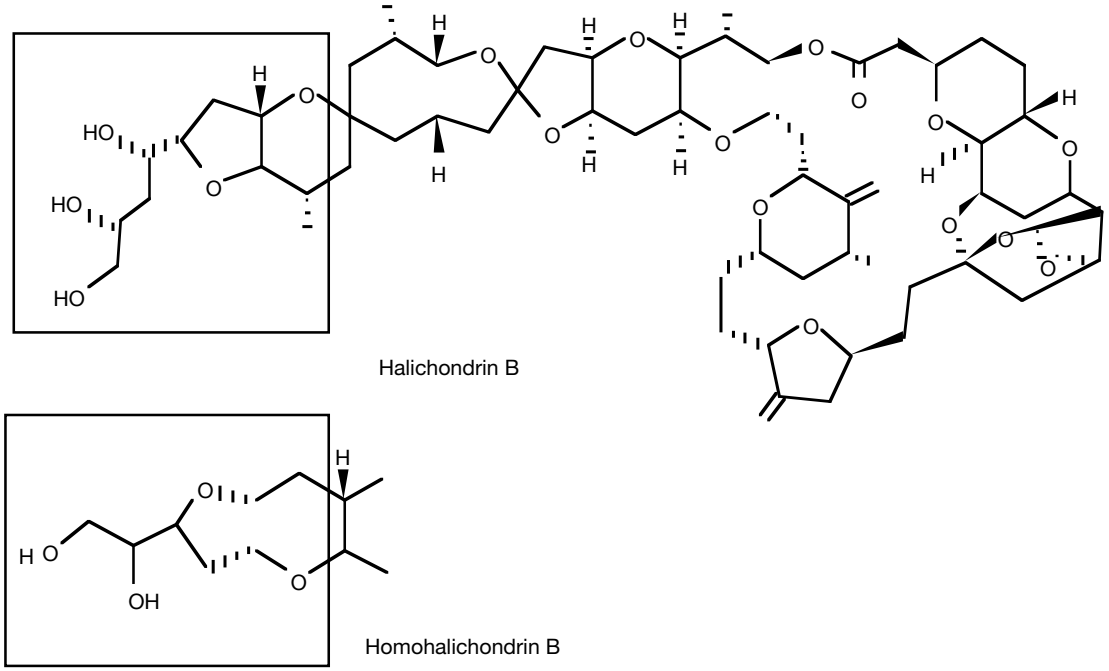


FIG. 2. — Structure of halichondrin B and homohalichondrin B.

gosine combined ribose with a novel base, methoxyadenine. Because of widespread interest in nucleosides at that period, due to the discovery of the anticancer activity of 5-fluorouracyl, the biological properties of these new nucleosides were widely studied. Antiviral properties of these nucleosides were demonstrated (Privat de Guarilhe & Rudder 1964) and initiated the synthesis of analogues which led to the first antiviral compound, Ara-A (Vidarabine) active against Herpes viruses, and to an antitumor compound, Ara-C (Cytarabine) effective in acute lymphoid leukemia (Fig. 1). Currently these are the only marine related compounds in clinical use, with cephalosporins, since the first one, cephalosporin C, was isolated from the marine fungus *Cephalosporium acremonium* Corda (Newton & Abraham 1955).

Since this pioneering work, a number of structurally original and biologically active compounds have been discovered from sponges, most of them with potential application as anticancer agents. The Japanese sponge *Halichondria okadai* (Kadota, 1922) provided the halichondrins, a new class of polyethers (Hirata & Uemura 1986). Halichondrin B and homohalichondrin B (Fig. 2), the most potent in the series, are highly cytotoxic *in vitro* and *in vivo* towards P388 and B16 melanoma (ID_{50} 0.093 10^{-3}µg/ml for halichondrin B and 0.1 10^{-3}µg/ml for homohalichondrin B). They are active *in vivo* against P388 leukemia (T/C: 323% at 10 µg/kg) and B16 melanoma (T/C 233 at 60 µg/kg). They are also potently active *in vivo* against lung tumor (Fodstad *et al.* 1996). Both inhibit microtubule assembly dependent on microtubule-associated proteins (Bai *et al.* 1991). Unfortunately, the development of halichondrins is hampered by limited supplies of material. Numerous synthetic approaches have been devoted to this class of compounds, however due to the complexity of the structures, these approaches are not economically profitable. Members of the halichondrins family were subsequently isolated from other sponges: *Axinella* sp. (Pettit *et al.* 1991), *Phakellia* sp. (Pettit *et al.* 1993c) and *Lissodendoryx* sp. in New Zealand (Litaudon *et al.* 1997) suggesting that they can be biosynthesized by an associated microorganism. Alternative routes to obtain enough supply of halichondrins are investigated: the search for

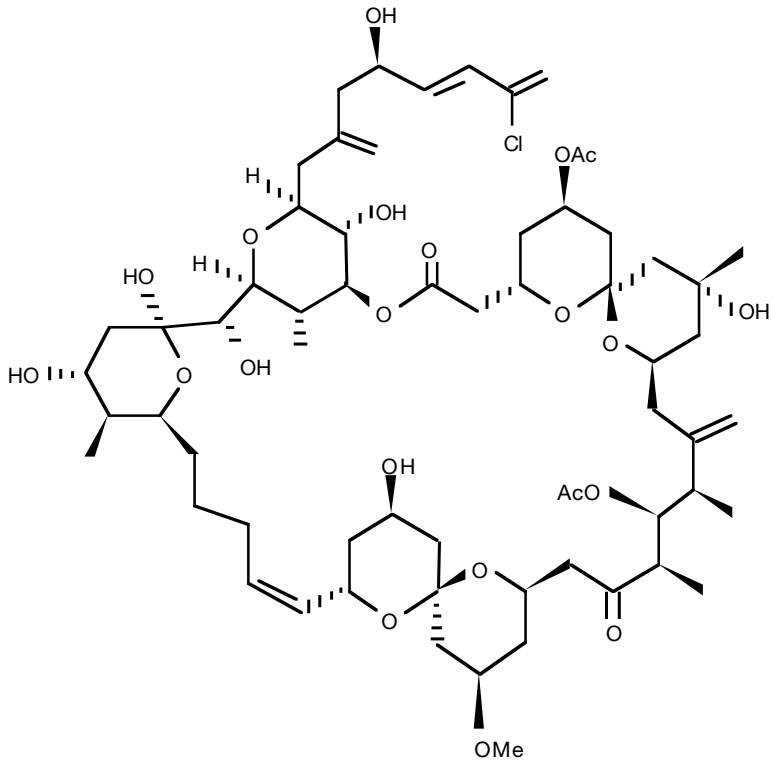


FIG. 3. — Structure of spongistatin 1.

cultivable microorganisms eventually responsible for their biosynthesis, sponge culture, tissue culture (Munro *et al.* 1994).

Another original class of cytotoxic polyether derivatives are spongistatins, isolated from *Spongia* sp. (Pettit *et al.* 1993a) and *Spirastrella spinispirulifera* (Carter, 1879) (Pettit *et al.* 1993b). In addition, some presumably identical compounds, the altohyrtins have been isolated from the Okinawan sponge *Hyrtios altum* (Poléjaeff, 1884) (Kobayashi *et al.* 1993) and cinachyrolide A (identical to spongistatin 4) from a sponge of the genus *Cinachyra* Sollas, 1886 (Fusetani 1993). The most potent is spongistatin 1 (Fig. 3) (absolute configuration from Guo 1988), highly cytotoxic (ID50 2.5-4 10^{-11} M) against a subset of highly chemoresistant tumor cells comprising the NCI panel of 60 human cancer cell lines (lung, brain, colon) and B16 melanoma. The spongistatins are inhibitors of tubulin polymerization and bind in a distinct region of the vinca domain (Bai *et al.* 1995).

Jasplakinolide (jaspamide) (Fig. 4) was isolated independently by two groups (Crews *et al.* 1986) (Zabriskie *et al.* 1986) from an Indo-Pacific sponge *Jaspis* sp., later identified as *J. johnstoni* (Schmidt, 1862). Jasplakinolide was found anti-

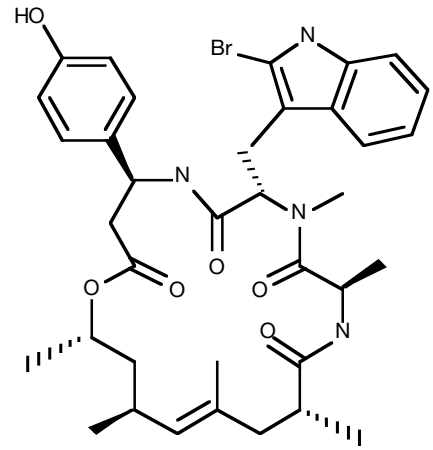


FIG. 4. — Structure of jasplakinolide (jaspamide).

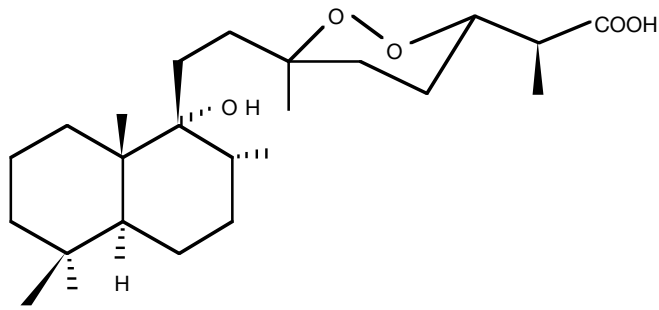


FIG. 5. — Structure of mycaperoxide B.

fungal and cytotoxic (Crews *et al.* 1986) and insecticide (Zabriskie *et al.* 1986). Further investigations revealed that Jasplakinolide was active against 36 solid tumor cell cultures in the NCI human tumor panel, acting by disruption of the actin cytoskeleton in mammalian cells. Jasplakinolide is a potential candidate for further preclinical development and a lead structure for a novel class of therapeutic agents (Senderowicz *et al.* 1995).

Mycaperoxide B (Fig. 5) obtained from a New Zealand sponge *Mycale* sp. (Tanaka *et al.* 1993) exhibits antiviral activity (Herpes simplex virus-1 and vesicular stomatitis virus) and anticancer activity *in vitro* and *in vivo* (ovarian and lung cancer) and is presently in advanced preclinical trials (Pharmamar).

The same species, collected from a different location contained pateamine A (Fig. 6) (Northcote *et al.* 1991). Total synthesis of pateamide A (Romo *et al.* 1998) allowed to evaluate the biological activity of this promising immunosuppressive agent isolated from the New Zealand sponge *Mycale* sp.

Another promising candidate for immunosuppressive therapy is discodermolide (Fig. 7) isolated from the sponge *Discodermia dissoluta* (Schmidt, 1880) (Gunasekera *et al.* 1990) which shows cytotoxic and immunosuppressive activities. Discodermolide blocks cellular proliferation in lymphoid and non-lymphoid cells and this blocking action is not due to cytotoxicity (Longley *et al.* 1993). It also exhibits *in vivo* immunosuppressive properties, being effective in suppressing (93%) the graft-versus-host splenomegaly response of grafted mice at 1.25 mg/kg. In comparison, mice treated with 150 mg/kg of cyclosporine A showed 80% suppression of the splenomegaly response. More recently it was demonstrated that discodermolide competitively inhibits the binding of paclitaxel (Taxol) to tubulin polymers and inhibits the growth of paclitaxel-resistant cells. (Kowalski *et al.* 1997).

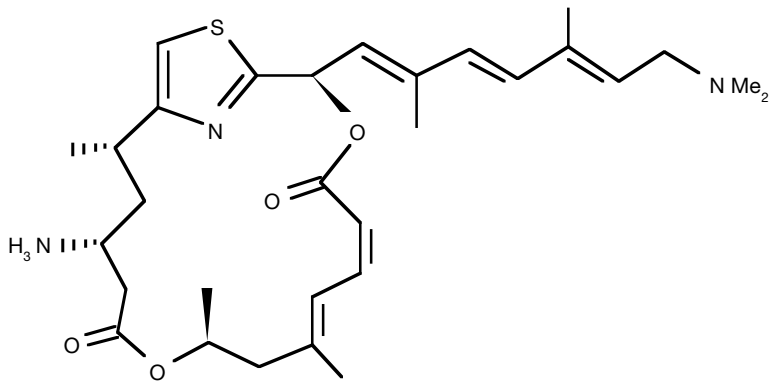


FIG. 6. — Structure of pateamine A.

New antiviral compounds were recently isolated from sponges. The Caribbean sponge *Batzella* sp. (Patil *et al.* 1995) contains the new batzelladines along with crambescin (Fig. 8) (Berlinck *et al.* 1990) and crambescidin 800 (Jares-Erijman *et al.* 1991), previously isolated from the Mediterranean sponge *Crambe crambe* (Schmidt, 1862). These alkaloids share the same guanidine-

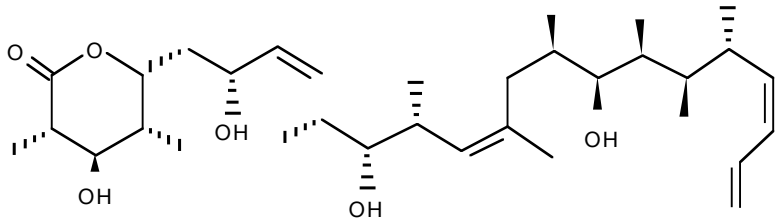
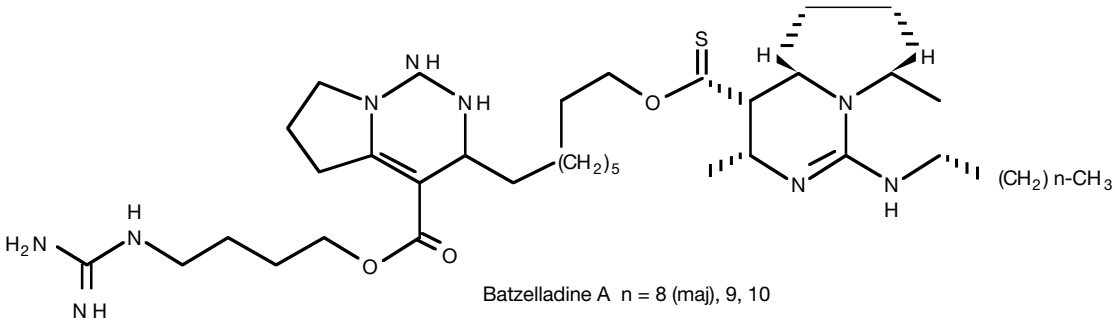
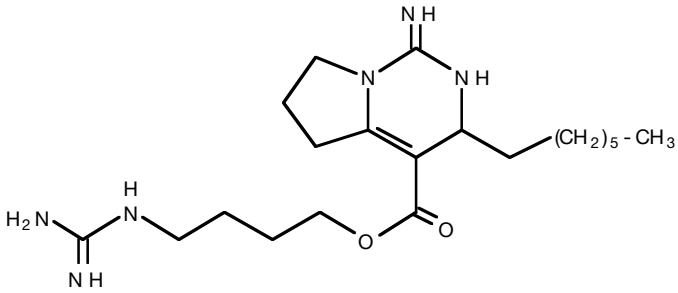


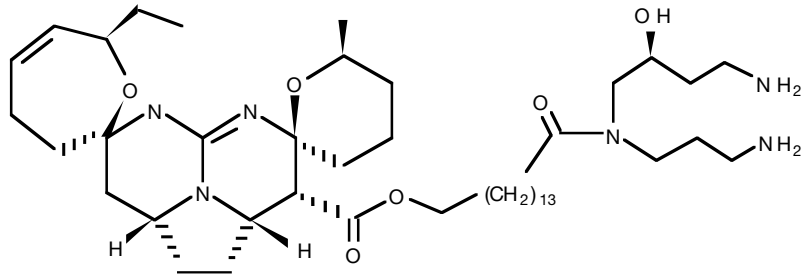
FIG. 7. — Structure of discodermolide.



Batzelladine A n = 8 (maj), 9, 10



Crambescin A



Crambescidin 800

FIG. 8. — Structure of batzelladine A and crambescidins.

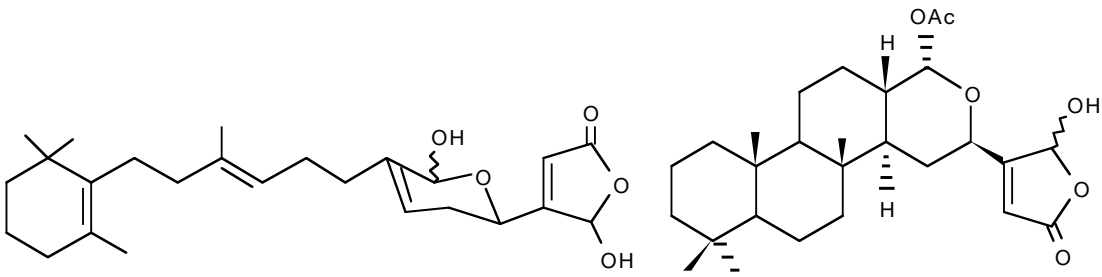


FIG. 9. — Structure of manoalide and petrosaspongiolide.

containing heterocyclic system. They displayed *in vitro* anti-HIV activity and batzelladines A and B inhibit HIV gp12-human CD4 binding. They are the first natural products of small molecular weight that have been shown to inhibit the gp120-CD4 interaction. However all are toxic for Vero cells and the design of less toxic analogues is required.

Luffariella variabilis (Poléjaeff, 1884) provided manoalide (Fig. 9) (Silva & Scheuer 1980), the first substance ever observed to selectively inhibit phospholipase A2, the main enzyme involved in the inflammatory response (Potts *et al.* 1992). Although this compound ultimately failed as a drug, its use as a model for designing inhibitors of this important enzyme will have lasting effect in medicinal research.

Other potent PLA2 inhibitors, petrosaspongiolides were obtained from the New Caledonian sponge *Petrosaspongia nigra* (Bergquist, 1995) (Randazzo *et al.* 1998), the most active being petrosiaspongiolide M (Fig. 9), a potential anti-inflammatory compound.

ROLE OF SYMBIOTIC MICROORGANISMS IN THE BIOSYNTHESIS OF SPONGE METABOLITES

Marine plants and animals are well known to have developed highly specific relationships with numerous microorganisms (Jensen & Fenical 1994).

Sponges, perhaps to a greater extent than any other marine invertebrates, harbor extraneous organisms on their surfaces, in their canal systems, and in the intercellular matrix which constitutes a large part of the body. The volume of bacteria in some species can reach 40% of the total cellular content (Vacelet & Donadey 1977; Wilkinson 1978).

The question of the role of these microorganisms in the synthesis of compounds of biological interest is currently the subject of intensive research efforts. We have underlined the difficulties encountered in total synthesis of complicated metabolites such as halichondrins and spongistatins, and the possibility of finding cultivable microorganisms is of considerable interest for development of bioactive molecules. Many examples bolster this hypothesis; the first one was okadaic acid, first isolated from the sponge *Halichondria okadai* (Kadota, 1922) (Tachibana *et al.* 1981) as the main cytotoxic compound of the extract, and later demonstrated as the toxin responsible for the intoxications due to the marine dinoflagellates (*Prorocentrum lima* Dodge, 1975, *Dinophysis fortii* (Pavillard, 1916) concentrated in a variety of filter feeders. Okadaic acid (Fig. 10) is a selective inhibitor of alkaline phosphatase PPA2, allowing this product to be used as a probe for the study of basic cellular phosphorylation processes. Okadaic acid, like manoalide is now commercially available for biochemical research.

Other previously ascribed to invertebrates were later demonstrated to be biosynthesized by bacteria. *Dysidea herbacea* (Keller, 1889) is a common shallow-water sponge which has been extensively studied. All samples contain terpenoids, and either polychlorinated or polybrominated compounds, but not both (Unson *et al.* 1994). The dominant endosymbiont is the filamentous cyanobacterium *Oscillatoria spongeliae* (Schulze,

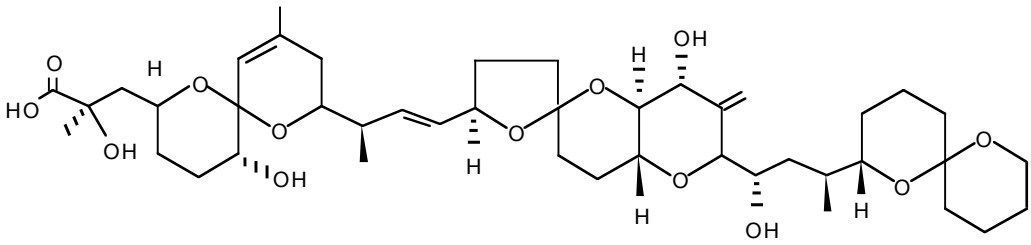
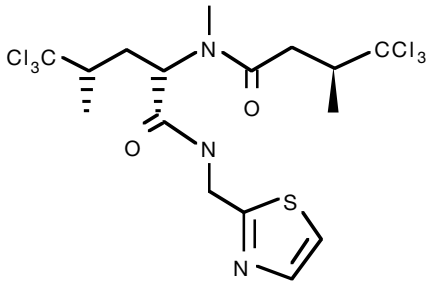
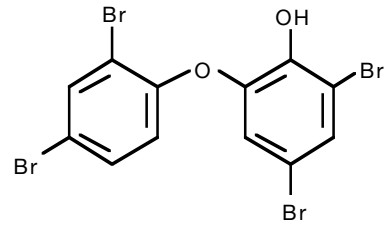


FIG. 10. — Structure of okadaic acid.

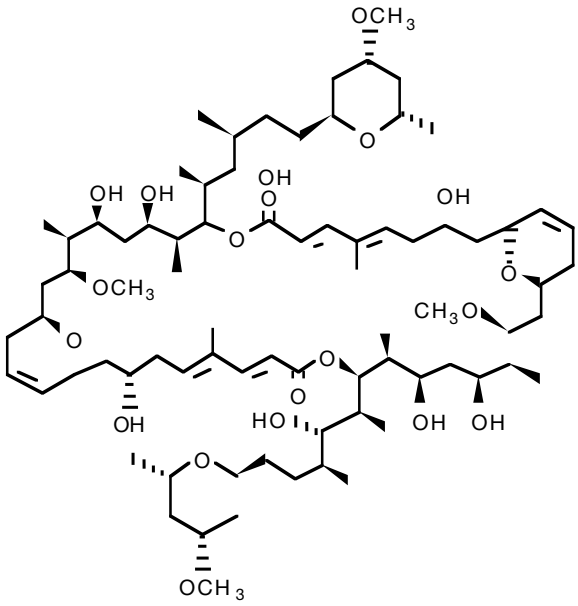


13 - demethylisodysidenin

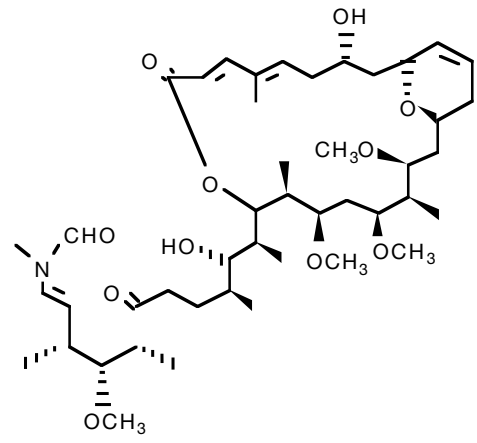


Brominated diphenyl ether 1

FIG. 11. — Structure of 13-demethylisodysidein and brominated ether.



Swinholide A



Scytophycin C

FIG. 12. — Structure of swinholide A and scytophycin C.

1892) Gomont, but heterotrophic bacteria are also present. The flow-cytometric separation of the symbionts from the sponge cells showed that chlorinated metabolites such as 13-demethylisodysidenin are located in the cyanobacterial filaments (Unson & Faulkner 1993). Another study revealed that the bromophenylether 1 also occurs exclusively in the Cyanobacteria (Fig. 11).

Another example is swinholide. The two monomeric units of the dimeric lactone swinholide, a potent cytotoxic macrolide isolated from the sponge *Theonella swinhoei* Gray, 1868 (Kobayashi *et al.* 1989) are obviously similar to scytophycin C obtained from the Cyanobacteria *Scytonema pseudohofmanni* Bharadwaja, 1934 (Ishibashi *et al.* 1986). This similarity has suggested the possibility that swinholide may be produced by a cyanobacteria (Fig. 12).

The presence of filamentous bacteria, thought to be cyanobacteria by the authors, was reported for the Okinawan collection of *T. swinhoei*, and it was suggested that these microorganisms might produce swinholide and bioactive peptides (Kitagawa *et al.* 1990). However four distinct cell populations were found to be consistently present in *T. swinhoei*: sponge cells, heterotrophic bacteria, cyanobacteria and filamentous bacteria. Chemical analyses of each cell type showed the macrolide swinholide A to be limited to the heterotrophic bacteria and a cyclic peptide in the filamentous bacteria. No major metabolites were located in the cyanobacteria or in sponge cells (Bewley *et al.* 1996).

Furthermore bacteria collected from sponges (Stierle and Stierle 1992; Shigemori *et al.* 1992; Imamura *et al.* 1993; Jayatilake *et al.* 1996), have allowed the isolation of antimicrobial compounds which could indicate that these bacteria may also play a role in the defense mechanism of these invertebrates.

TRANSFER OF SPONGE METABOLITES TO OTHER INVERTEBRATES

If the ecological role of the majority of these secondary metabolites has not yet been clarified, it seems obvious that highly toxic, antimicrobial or deterrent products contribute to their defense (Pawlik 1993). However the ecological role of sponge metabolites have often been demonstrated through their role in molluscs' defense.

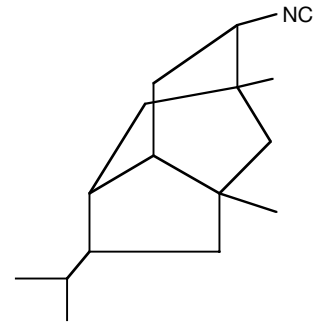


FIG. 13. — Structure of 9-isocyanopupukeanane.

Relationships between molluscs and sponges have been early reported: nudibranchs, shellless molluscs, have developed the ability to use sponge metabolites for their own defense (Schulte & Scheuer 1982; Thompson *et al.* 1982; Faulkner 1992). The first evidence that a defense allomone of a nudibranch derived from a sponge dates from the study on *Phyllidia varicosa* Lamarck, 1801. The observation that crustaceans kept in an aquarium with *P. varicosa* are killed within 30 min prompted Scheuer's group to isolate the toxin, obtained in minute amounts. Fortunately they observed that *P. varicosa* fed on a *Hymenacidon* sp. which had the same characteristic odor, and they succeeded to elucidate the structure as 9-isocyanopupukeanane (Fig. 13) (Schulte & Scheuer 1982). Later this metabolite was found in other nudibranchs.

Latrunculin A (Fig. 14), one of the most toxic compounds isolated from sponges, was first isolated from *Latrunculia magnifica* Keller, 1889 (Kashman *et al.* 1980). Latrunculin A causes fish disorientation, hemorrhage and eventually death of fish.

This compound has been found in the nudibranchs *Chromodoris elizabethina* Bergh, 1877 (Okuda & Scheuer 1985), *Chromodoris williani* Rudman, 1982 and *Chromodoris* sp., *Glossodoris quadricolor* (Rüppell & Leuckart, 1828) (Cimino *et al.* 1993), *Chromodoris lochi* Rudman, 1892 (Corley *et al.* 1988).

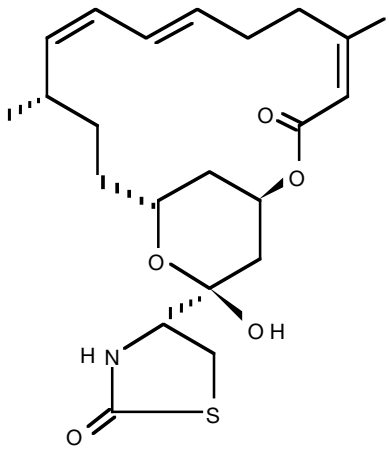


FIG. 14. — Structure of latrunculin A.

Scalaradial, a terpenoid present as the major metabolite in several sponges, *Cacospongia scalaris* Schmidt, 1862, *Cacospongia mollior* Schmidt, 1862, *Spongia oceania* Laubenfels, 1950, *Hyrtios erectus* (Keller, 1889), is transformed by mollusc predators and the resulting metabolites are concentrated into dorsal glands where they are used for defensive purposes.

Hypselodoris orsini (Verany, 1846), a mollusc which preys specifically on the sponge *Cacospongia mollior*, converts scalaradial into deoxyscalarin and 6-keto-deoxyscalarin (Cimino *et al.* 1993). *Glossodoris pallida* (Rüppell & Leuckart, 1828) preys on *Hyrtios erectus* and transforms scalaradial only in deoxyscalarin (Rogers *et al.* 1991), when *Chromodoris youngbleuthi* Kay & Young, 1969 which lives on *Spongia oceania* is able to oxidize scalaradial in the position 14 (Terem & Scheuer 1986). Of these compounds, deoxyscalarin and 6-keto-deoxoscarin, less toxic than scalaradial itself, are still feeding inhibitors (Fig. 15).

In a similar manner, the opistobranch mollusc *Hypselodoris webbi* (d'Orbigny, 1839) is able to select, among its potential prey, sponges chemically rich in deterrent furanosesquiterpenoids. (Fontana *et al.* 1994).

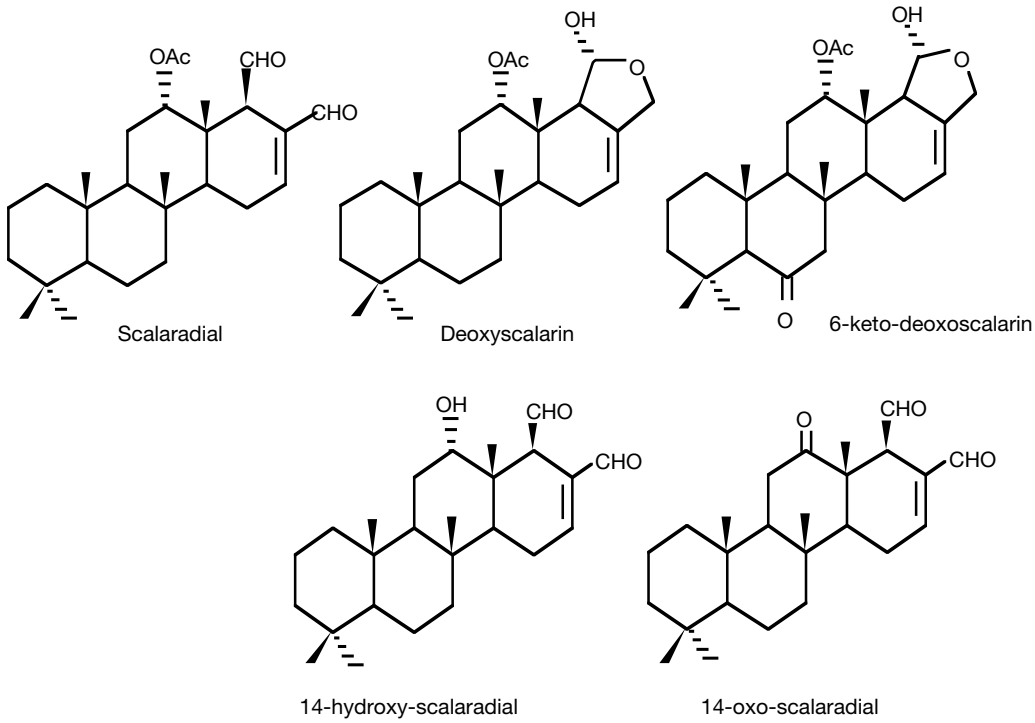


FIG. 15. — Structure of scalaradial and transformation products.

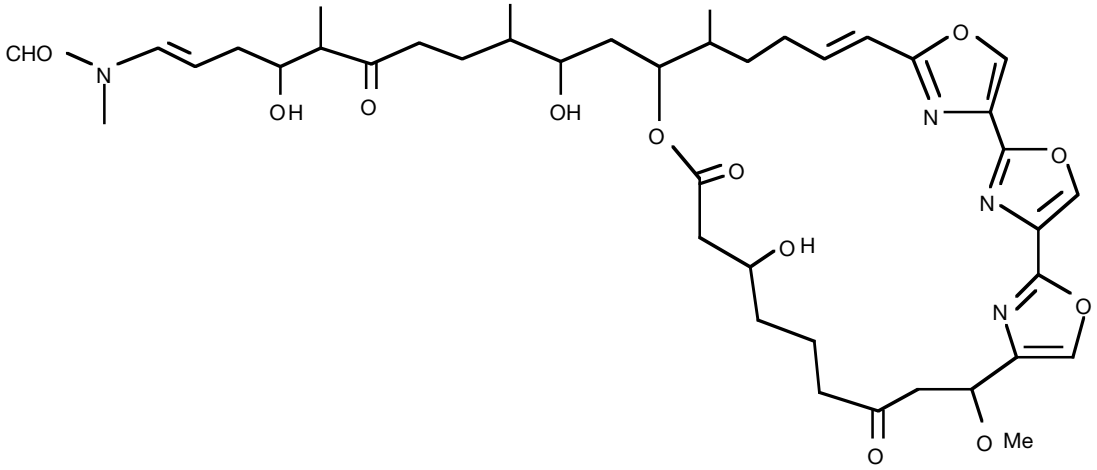


FIG. 16. — Structure of dehydrohalichondramide.

The origin of unusual macrolides such as dehydrohalichondramide (Fig. 16) found in the nudibranch *Hexabranchus sanguineus* (Rüppell & Leuckart, 1828) (Spanish dancer) and its egg masses was clarified by Pawlik (Pawlik *et al.* 1988) who demonstrated that these macrolides are the major metabolites of a *Halichondria* sp. sponge, the usual prey of the nudibranch. These strong fish antifeedant compounds are concentrated in the mantle and the egg masses, presumably to protect them from the attacks of predators.

CONCLUSION

The few examples given in this paper illustrate the interest of sponge metabolites for pharmaceutical and ecological purposes and also the close relationships that exist between marine organisms. Sponges metabolites have been recognized as useful chemical defense by other marine organisms: the majority of the defensive substances isolated from nudibranchs comes from predation on sponges. Furthermore, some sponge metabolites are likely biosynthesized by associated or symbiotic microorganisms. This suggests close association and perhaps co-evolution between sponges and microorganisms and sponges and nudibranchs.

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