Anti-inflammatory metabolites from marine sponges

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Marine sponges are a rich source of biologically active secondary metabolites with novel chemical structures. Eighty four anti-inflammatory compounds have been isolated from marine sponges. This is the first comprehensive review presenting the structures and anti-inflammatory activities of marine sponge metabolites. (100 references)

Introduction

Marine sponges continue to be a virtual cornucopia of novel natural products, varying widely in both chemical structure and biological activity.[1–3] There have been several recent reviews detailing the different classes of novel marine natural products, or the various types of biological activities reported for these compounds.[4,5] Although the major phospholipase A_2 (PLA_2) inhibitors isolated from marine sources have been reviewed *per se*,[6,7] this review provides the first holistic overview of the anti-inflammatory metabolites isolated from marine sponges. Only the structures of anti-inflammatory sponge metabolites published before August 1st, 2004, are reviewed here.

As the secondary metabolite composition of sponges is dominated by terpenoid compounds,[1] it is not surprising that anti-inflammatory sponge natural products are also dominated by isoprenoid derived metabolites, especially sesterterpenes. This review will initially present the structures of anti-inflammatory terpenoids, followed by those metabolites of other biogenetic origin.

As well as variation in the classes of sponge natural products that possess anti-inflammatory activity, there is also great variation in the assays that are used to detect these activities. The most commonly used assay to assess anti-inflammatory activity is the inactivation of PLA_2. PLA_2 enzymes hydrolyze phospholipids at the *sn*-2 position of the glycerol backbone, generating arachidonic acid which is then metabolized *via* several different pathways to give inflammatory compounds such as prostaglandins, thromboxanes and leukotrienes. Inhibition of the various PLA_2 enzymes can therefore prevent initiation of the arachidonic acid cascade.[6,7]

Another assay that has fallen from favor because of the ethical implications of using live animal models, is the mouse paw or mouse ear oedema assay. In this assay, mouse ears or paws are either exposed to an inflammatory agent such as carrageenan or 12-*O*-tetradecanoylphorbol acetate (TPA), or to a mixture of the inflammatory agent and the potential anti-inflammatory compound to be tested. After sacrificing the animal, the inflamed area is weighed. The weight of treated tissue is compared to that of the inflammatory control to determine the level of anti-inflammatory activity.[3]

Finally, superoxides are precursors of various lethal oxidants and have been implicated in the biosynthesis of inflammatory prostaglandins. Inhibition of superoxide production can be measured *in vitro* using visible spectrophotometry where changes in superoxide production by cell neutrophils (stimulated to produce superoxides by the addition

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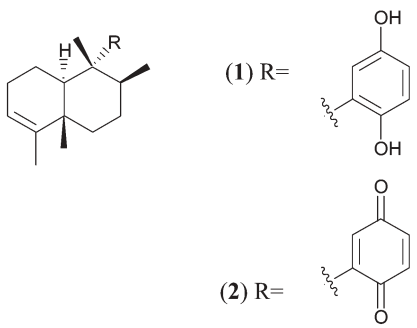
of *N*-formyl-methionine-leucine-phenylalanine [fMLP] or phorbol-12-myristate acetate [PMA]) are monitored in the presence of anti-inflammatory compounds.[8,9]

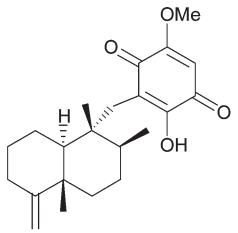
Terpenoid anti-inflammatory compounds

Sesquiterpenes

Thirteen anti-inflammatory sesquiterpenes have been reported from marine sponges. The first is the hydroquinone avarol (**1**), originally isolated from the sponge *Dysidea avara*, and its quinone isomer avarone (**2**).[10] The original isolation of **1** was followed by the publication of its absolute configuration two years later by the same group.[11] Both **1** and **2** inhibit mouse paw (**1**: ED_{50} = 9.2; **2**: ED_{50} = 4.6 mg kg^{-1}) and ear oedemas (**1**: ED_{50} = 97, **2**: ED_{50} = 397 μg per ear) induced by carrageenan and TPA respectively.[11,12] Compound **1** also inhibited recombinant synovial PLA_2 activity (IC_{50} = 158 μM),[11,12] while both **1** and **2** inhibited superoxide production in rat peritoneal leukocytes stimulated with fMLP [**1**: IC_{50} = 96.6 nM; **2**: IC_{50} = 123.2 nM] or TPA (**1**: IC_{50} = 567.2 nM; **2**: IC_{50} = 1226.1 nM).[11,12]



The quinone sesquiterpene ilimaquinone (**3**) was first reported from *Hippospongia metachromia* in 1979 after the initial crude extract of the sponge was shown to exhibit mild anti-microbial activity.[13] The absolute stereochemistry of **3** was initially proposed from a weak positive CD Cotton effect of a degradation product,[1,13] but was subsequently reassigned after **3** was chemically converted into aureol possessing known absolute configuration.[14] Several syntheses of **3** have been reported since its initial isolation.[15,16] Unfortunately, no further details have been published describing the anti-inflammatory activity of **3** originally alluded to by Faulkner and co-workers.[17]



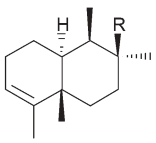
(**3**)

Bolinaquinone (**4**), a hydroxyquinone sesquiterpene, was isolated from a *Dysidea* sp. collected in the Philippines.[18] Subsequently, **4** has been re-isolated with the sesquiterpenes dysidotronic acid (**5**),[19] dysidenones A (**6**) and B (**7**), and dysidine (**8**), from a species of *Dysidea* collected off the Vanuatu Islands.[20] All of these metabolites posses anti-inflammatory activity against four different secretory PLA_2 enzymes, belonging to groups I (*Naja naja* venom and porcupine pancreatic enzymes), II (human synovial recombinant enzyme) and III (bee venom enzyme) (Table 1).[19,20] Bolinaquinone (**4**) has also been shown to inhibit mouse ear oedema induced by TPA *via* both topical and oral administration (ID_{50} = 76.7 μg per ear and 5.6 mg kg^{-1} respectively).[21]

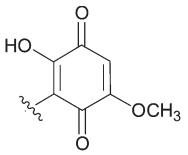
Table 1 Inhibitory effects of various compounds against different secretory PLA_2 activities using manoalide (**24**) as a control[19,20,65,67,69,70]

	N. naja venom	Pancreas		Human synovial		RAP + zymosan		Bee venom	
Compound	% Inhib. (10 μM)	% Inhib. (10 μM)	IC_{50} (μM)[a]	% Inhib. (10 μM)	IC_{50} (μM)[a]	% Inhib. (10 μM)	IC_{50} (μM)[a]	% Inhib. (10 μM)	IC_{50} (μM)[a]
4	26.0 ± 1.6	86.4 ± 1.9	0.4	100.0 ± 0.0	0.2			99.1 ± 0.6	0.1
5	1.8 ± 1.1	18.8 ± 2.9		80.6 ± 3.3	2.6			26.0 ± 4.1	
1:1 (**6**) and (**7**)	11.0 ± 2.0	23.5 ± 2.5		45.7 ± 6.3				1.9 ± 1.7	
8	0.7 ± 0.7	1.2 ± 1.2		73.8 ± 2.4	2.0			33.4 ± 6.3	
24	17.0 ± 1.7	14.3 ± 6.8		93.2 ± 0.2	3.9	38.4 ± 0.5		62.5 ± 3.8	7.5
36	0.0 ± 0.0	64.2 ± 2.1	4.0	86.7 ± 2.5	4.3	36.9 ± 1.4		35.4 ± 1.2	
37	3.5 ± 1.6	14.4 ± 1.2		90.7 ± 1.3	3.0	21.8 ± 3.0		96.3 ± 0.6	2.3
38	0.0 ± 0.0	5.3 ± 4.4		96.7 ± 0.4	1.4	65.1 ± 4.2	7.8	94.8 ± 1.1	2.8
39	11.5 ± 3.0	12.3 ± 6.0		68.6 ± 2.7	1.6	—		71.0 ± 0.6	0.6
40	6.8 ± 3.0	11.6 ± 1.8		44.0 ± 2.7		—		43.9 ± 2.2	
41	3.0 ± 1.3	0.0 ± 0.0		60.9 ± 4.4	3.8	—		37.9 ± 3.2	
42	4.2 ± 2.7	0.0 ± 0.0		30.1 ± 3.7		—		12.5 ± 2.1	
43	1.0 ± 0.8	0.8 ± 0.8		7.1 ± 3.2		—		18.8 ± 5.6	
44	1.3 ± 0.8	14.3 ± 6.8		34.4 ± 6.5		18.8 ± 3.2		37.1 ± 6.3	
45	8.7 ± 3.9	19.5 ± 3.6		87.2 ± 2.1	5.8	25.6 ± 1.9		5.4 ± 2.1	
64	0.5 ± 0.5	18.0 ± 8.1		40.1 ± 77		17.1 ± 4.6		33.1 ± 6.0	
65	0.4 ± 0.4	14.2 ± 5.1		34.6 ± 5.8		17.9 ± 4.2		32.2 ± 6.0	
66	3.1 ± 2.2	9.1 ± 3.5		40.4 ± 5.7		30.9 ± 5.3		36.2 ± 5.4	
67	0.0 ± 0.0	7.6 ± 4.0		48.2 ± 3.8		19.6 ± 5.4		37.6 ± 6.5	

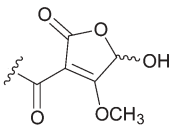
[a] IC_{50} values only calculated for those compounds that reached greater than 50% inhibition.



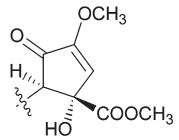
(**4**) R=



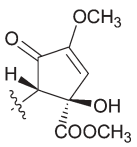
(**5**) R=



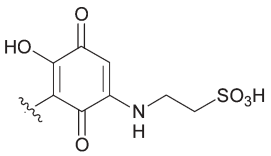
(**6**) R=



(**7**) R=



(**8**) R=



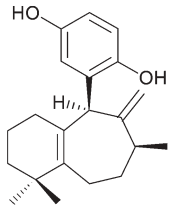
Pro-inflammatory cytokines, such as interleukins 1, 2, 6 and 8 (IL-1, IL-2, IL-6 and IL-8) have been implicated in inflammatory disorders such as psoriasis, rheumatoid arthritis and osteoarthritis.[22] For example, IL-8 acts as an chemoattractant and activator of neutrophils, thus promoting superoxide production.[23] Inhibition of the various IL receptors, therefore, can provide a method of reducing inflammation. The five hydroquinone sesquiterepenes frondosins A–E (**9–13**), isolated from the Micronesian sponge *Dysidea frondosa*, inhibit two different IL-8 receptors (IL-8Rα and 8Rβ) and the protein kinase C enzyme with IC_{50} values in the μM range (Table 2).[23]

Diterpenes

Ten anti-inflammatory diterpenes have been reported from sponges, all of which are members of the spongian class of compounds. All known spongian diterpenes have been isolated from sponges of the Dendroceratid or Dictyoceratid classes, or from nudibranchs that prey upon these sponges. Gracilin A (**14**)[24] and 12-acetoxytetrahydroaplysulfurin-1 (**15**), both from Californian *Aplysilla* sp.,[6] aplyroseols-1 (**16**), -5 (**17**) and -6 (**18**) from the Australian sponge *Aplysilla rosea*,[25] norrisolide (**19**) from the nudibranch *Chromodoris norrisi* and the Palauan sponge *Dendrilla* sp.,[26,27] and dendrillolide A (**20**) from a Palauan *Dendrilla* sp.[27] all inhibit PLA_2 at a concentration of 2 μg mL^{-1} (Table 3).[6]

Table 2 Interleukin inhibition by frondosins A–E (**9–13**) IC_{50} (μM)[23]

Compound	IL-8 Rα	IL-8 Rβ	PKC-α
9	3.4	3.2	1.8
10	9.6	10.8	4.8
11	84	26.3	20.9
12	98	10.8	26
13	64	37.1	30.6



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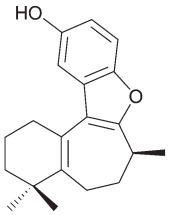

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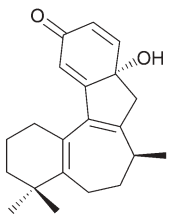
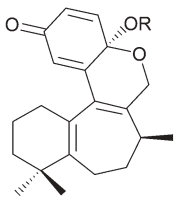
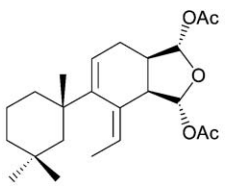


(**11**)

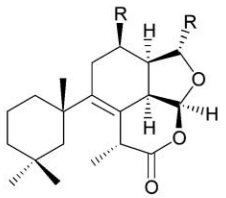


(**12**) R=H

(**13**) R=Ac

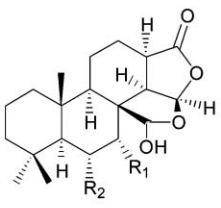


(**14**)



(**15**) R=OAc

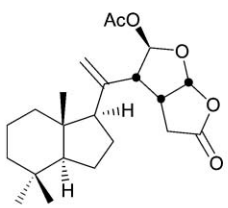
(**23**) R=H



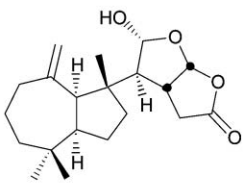
(**16**) R_1= OCOPr, R_2=H

(**17**) R_1=OH, R_2=OCOPr

(**18**) R_1=OAc, R_2=OCOPr



(**19**)

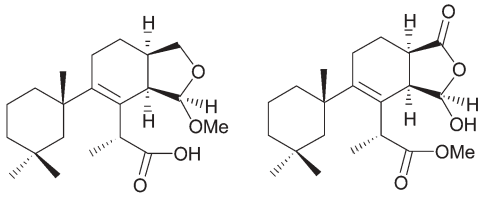


(**20**)

Table 3 Inhibitory effects of various diterpenes against PLA_2[6,24–27]

Compound	% Inhib. (2 μg mL^{-1})
14	69
15	60
16	65
17	44
18	50
19	66
20	48

In early 2004, two separate groups simultaneously published the structures of several rearranged spongian diterpenes from two separate species of Dendroceratid sponges.[28,29] Of the eight compounds isolated from *Chelonaplysilla violacea*, pourewic acid A (**21**), methylpourewate B (**22**) and cadlinolide C (**23**) inhibited the production of superoxide in human peripheral blood neutrophils stimulated with either fMLP (IC_{50} = 74, 58, 13 μM respectively) or PMA (IC_{50} = 77, 58, 13 μM respectively).[9,28] Unfortunately, the paucity of the remaining five rearranged spongian diterpenes prevented a comparative structure–activity study for this group of compounds.[28]

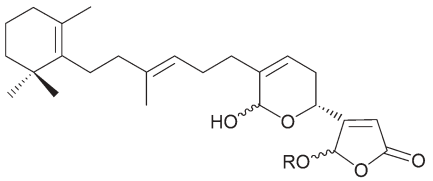


(**21**) (**22**)

Sesterterpenes

Anti-inflammatory metabolites from sponges are dominated by sesterterpenes, which can be subdivided into three broad categories: manoalide, scalaradial and cacospongionolide type compounds.

Manoalide (**24**) is probably the most well known of all anti-inflammatory sponge metabolites and was originally isolated by de Silva and Scheuer in 1980 from the sponge *Luffariella variabilis*.[30] Rapid tautomerisation prevents the determination of the stereochemistry at the two hemi-acetal centers of **24** while the *R* configuration at the third asymmetric center was established after comparison of the CD spectrum of the reduction product of **24** with that of a synthetic analogue.[31] A comprehensive review of the isolation, biosynthesis, synthesis and biological activity of **24** and its analogues has been published.[32] There has been much interest in the biological activity of manoalide (**24**). An early study of the pharmacology of **24** noted that this compound prevented the irreversible action of the PLA_2 containing neurotoxin β-bungarotoxin.[33] Other studies have indicated that **24** and analogues inhibited bee venom (IC_{50} = 0.1 μM) and cobra venom (IC_{50} = 1.9 μM) PLA_2 enzymes.[32,34–36] The chemical mechanism by which **24** inhibits bee venom PLA_2 was described in 1992,[37] and this was followed by similar studies of the mechanisms of action of **24** against a variety of molecular targets.[37–42] The irreversible inactivation of bee venom PLA_2 is caused by Schiff base formation involving the masked α,β-unsaturated aldehyde of the γ-hydroxybutenolide of **24** and the Lys-94 residue of the PLA_2 protein.[6,32,43,44]

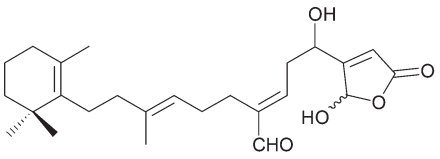


(**24**) R=H

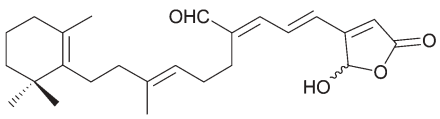
(**25**) R= Ac

Other examples of anti-inflammatory manoalide-related compounds include manoalide monoacetate (**25**) isolated from the sponges *Thorectandra excavatus* and *Hyrtios* sp.,[45,46] secomanoalide (**26**) from *Luffariella variabilis* possessing similar potency and efficacy as **24**,[38,47] 4*E*, 6*E*-dehydromanoalide from *Luffariella variabilis* (**27**) which shows reduced potency but similar efficacy to that of **24** against bee venom PLA_2 (IC_{50} = 0.28 μM),[38,48] variabilin (**28**) from *Ircinia variabilis*[49] (IC_{50} = 6.9 μM against human synovial secretory PLA_2; IC_{50} = 7.9 μM against U937 cytosolic PLA_2),[50] thorectolide monoacetate (**29**) from *Hyrtios* sp. (inhibits cobra venom PLA_2 up to concentration of 2 μM),[46] luffariellolide (**30**) from *Luffariella* sp. (reversible bee venom PLA_2 inactivation IC_{50} = 0.23 μM, antagonist against PMA induced inflammation in the mouse ear [PMA {T/C-1} = 0.929 ± 0.200, PMA+**30** {50 μg per ear}{T/C-1} = 0.221 ± 0.068])[51] and finally, luffariellins A (**31**) and B (**32**) isolated from *Luffariella variabilis* (reversible bee venom PLA_2 inactivation IC_{50} = 56 and 62 nM for **31** and **32** respectively, antagonists against PMA induced inflammation in the mouse ear [PMA {1-T/C} = 0.550 ± 0.035, PMA+**31** {50 μg per ear}{1-T/C} = 0.330 ± 0.016, PMA+**32**{50 μg per ear}{1-T/C} = 0.230 ± 0.007).[52] There are many other manoalide (**24**) analogues known that have not been reported to possess anti-inflammatory activity.[32]

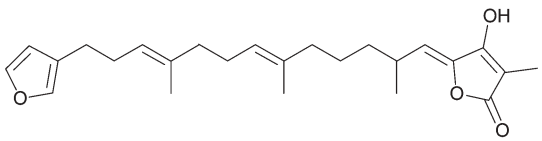
Scalaradial (**33**) is a tetracyclic sesterterpene with the scalarane carbon skeleton, which was originally isolated from the marine sponge *Cacospongia mollior* by Cimino *et al.* in 1974.[53] The absolute stereochemistry of **33** was reported several years later following re-isolation along with several other isomers from the sponge *Spongia nitens*.[54] Since then, many more analogues of **33** have been reported.[55] Scalaradial (**33**) is a potent bee venom PLA_2 inhibitor (IC_{50} = 0.07 μM),[56] as well as an inhibitor of PMA-induced arachidonic acid release *in vivo* in the mouse peritoneal macrophage (IC_{50} = 0.05 μM). Scalaradial (**33**) was also shown to inhibit hydrolysis of mixed phosphatidylcholine micelles (IC_{50} = 0.07 μM).[57] The effects of **33** on human neutrophils[58] and experiments to investigate the structure–activity relationships of **33** using suitable analogues have been performed.[59] The nor-sesterterpene hyrtial (**34**) was isolated from the sponge *Hyrtios erecta*, along with several analogues of **33**.[60] This compound was later re-isolated with a further five novel scalarane-type sesterterpenes.[55] It was during this latter study that **34** was shown to



(**26**)



(**27**)



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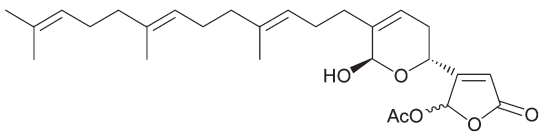

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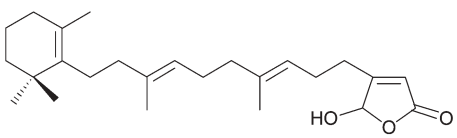
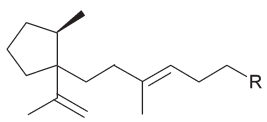
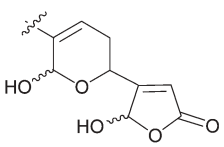


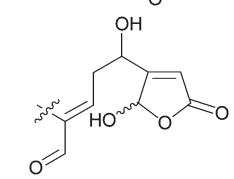
(**30**)



(**31**) R=

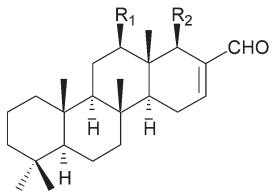


(**32**) R=



decrease by 43% the weight of mouse ear oedema induced by PMA at a concentration of *ca.* 50 μg per ear.[55]

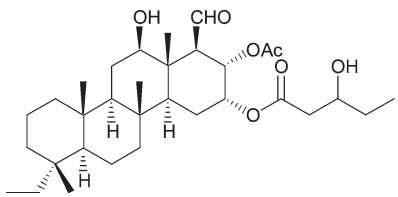
The scalarane-type bishomosesterterpene foliaspongin (**35**) was isolated from the Okinawan marine sponge *Phyllospongia foliascens*, although it was two years later before the final structural elucidation was completed.[61,62] The latter



(**33**) R_1=H, R_2=CHO

(**34**) R_1=OAc, R_2=H

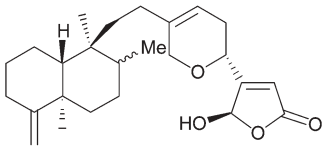
publication also detailed the anti-inflammatory activity of **35** (18.1% inhibition at 10 μg per disk utilizing the chorio-allantoic chick embryo membrane assay).[61] The chick embryo chorio-allantoic membrane assay is used to screen for anti-angiogenesis (inhibition of the growth of blood vessels) and is applicable for the discovery of compounds active against several pathological conditions including rheumatoid arthritis and psoriasis.[63]



(**35**)

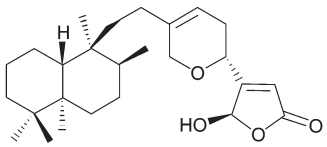
Cacospongionolide B (**36**) is a tetracyclic sesterterpene originally obtained from *Fasciospongia cavernosa*.[64] Studies have shown that **36** possesses anti-inflammatory activity on TPA induced mouse ear oedema after oral doses of 5, 10 or 20 mg kg^{-1} and also inhibits secretory PLA_2 *in vitro* (Table 1).[65] Models show that **36** suppresses the expression of inflammatory enzymes by inhibiting nuclear factor-κB activation.[66] It has been noted that **36** is a congener of manoalide (**24**) and that it shows enhanced stability over this archetypical compound.[67] The absolute stereochemistry of **36** was published after its re-isolation along with the anti-inflammatory sesterterpenes cacospongionolide (**37**) and cacospongionolide E (**38**) (Table 1).[67] A recent preliminary study of **36** and **38** and their analogues has suggested that the reported $sPLA_2$ activity of these compounds is enantioselective and is, unlike manoalide (**24**), independent of the γ-hydroxybutenolide moiety.[68]

A series of novel sesterterpenes possessing a cheilantane skeleton, petrosaspongionolides M (**39**), N (**40**), P (**41**), Q (**42**) and R (**43**), were isolated from *Petrosaspongia nigra* in addition to the known compounds petrosaspongionolides A–L.[69] Compounds **39–43** were tested for activity against a panel of PLA_2 enzymes (Table 1). Two related sesterterpenes were later isolated from a sample of *Spongia* sp. collected from the Vanuatu Islands, along with four pyridinium alkaloids (see alkaloid section below).[70] 21-Hydroxypetrosaspongiolide K (**44**) and 21-hydroxypetrosaspongiolide P (**45**) inhibited four secretory PLA_2 enzymes (Table 1).[70] Neither exhibited cytotoxic effects on human neutrophils at the concentrations tested.[70]

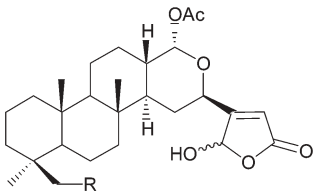


(**36**) R= α-Me

(**38**) R= β-Me

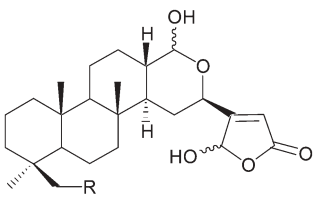


(**37**)



(**39**) R=H

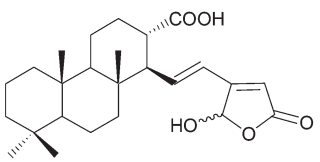
(**40**) R=OAc



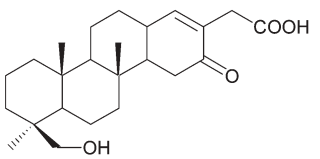
(**41**) R=H

(**42**) R=OAc

(**45**) R=OH

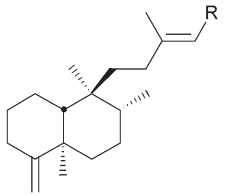


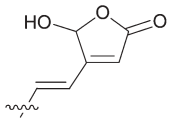
(**43**)

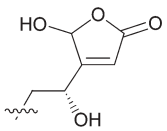


(**44**)

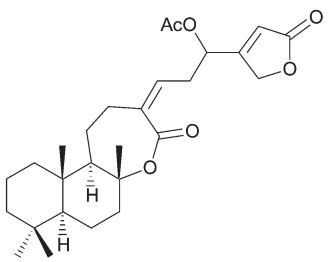
A sesterterpene containing a drimane bicyclic motif was isolated from an extract of three sponges that were inadvertently combined together during the collection trip.[71] The sesterterpene, palauolide (**46**), was initially isolated as an antimicrobial metabolite.[71] It was subsequently re-isolated together with its presumed biosynthetic precursor, palauolol (**47**), from the Palauan sponge *Fascaplysinopsis* sp.[72] Both **46** and **47** inactivate bee venom PLA_2 (**46**: 85%; **47**: 82% inhibition at 0.8 μg mL^{-1}).[72]



(**46**) R= 

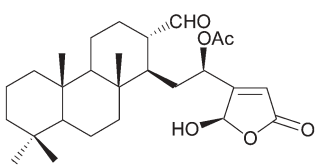
(**47**) R= 

Luffalactone (**48**) was isolated during the same study that yielded 4*E*, 6*E*-dehydromanoalide from *Luffariella variabilis* (**27**).[48] This study was part of a large-scale targeted collection of the sponge to generate significant quantities of manoalide (**24**) for clinical evaluation. The authors noted significant variation in the secondary metabolic composition of the sponges collected.[48] The carbon skeleton of **48** had previously been observed from various species of *Salvia* but was unprecedented from the marine environment. Luffalactone (**48**) showed 52% inhibition of oedema in the mouse ear assay at 50 μg per ear.[48]



(**48**)

Investigation of a *Luffariella* sp. collected from Palau yielded the novel compound luffolide (**49**), the structure of which was determined by X-ray analysis.[73] Although limited by a paucity of material, biological evaluation of **49** indicated that its topical application reduced inflammation of mouse ear oedema by 55% (50 μg per ear). Luffolide (**49**) also completely inhibited the hydrolysis of phosphatidylcholine by bee venom PLA_2 at a concentration of 3.5 μM.[73]

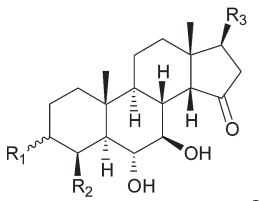


(**49**)

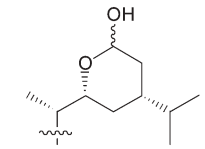
Steroids

Very few anti-inflammatory steroids have been reported from marine sponges. The first was contignasterol (**50**), isolated from the sponge *Petrosia contignata*, collected from Papua New Guinea.[74] Contignasterol (**50**) is of note as it was the first naturally occurring steroid to be isolated with H-14 in the ''unnatural'' β configuration.[74] Since then, several more 14β sterols have been isolated, all of which possess a ketone functionality at C-15.[8,75–77] The absolute stereochemistry of **50** has been reported recently.[78] Contignasterol (**50**) was found to inhibit the anti-immunoglobulin E (anti-Ige) stimulated release of histamine from sensitized rat mast cells in a dose dependent manner.[79] As this activity was deemed promising,[80] several derivatives of **50** have been prepared and one compound, IPL576-092, has advanced into clinical trials as an anti-inflammatory/anti-asthma agent.[81]

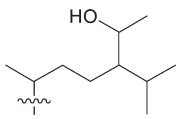
A recent novel development has been the synthesis of hybrid structures of IPL576-092 and either manoalide (**24**) or contignasterol (**50**). The IPL576-092-manoalide hybrid inhibited human synovial $sPLA_2$ by 63% at 100 μM and was able to reduce nitric oxide and PGE_2 (64 and 72% respectively) at a concentration of 10 μM using stimulated human monocytes.[82]



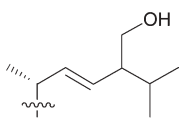
(**50**) R_1= α-OH, R_2=OH, R_3=



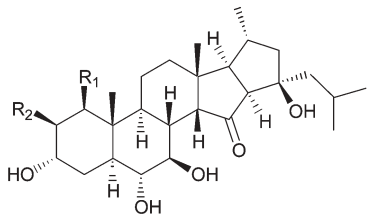
(**54**) R_1= β-OH, R_2=H, R_3=



(**55**) R_1= β-OH, R_2=H, R_3=



The structures of two further 14β sterols, xestobergsterols A (**51**) and B (**52**), were isolated from the Okinawan marine sponge *Xestospongia bergquista* in the same year as the publication of the structure of contignasterol (**50**).[76] Several years later, the stereochemistry of these two metabolites was revised after they were re-isolated, along with xestobergsterol C (**53**), from an Okinawan *Ircinia* sp..[77] Xestobergsterols A (**51**) and B (**52**) were found to be potent inhibitors of histamine release from rat peritoneal mast cells induced by anti-Ige (**51** IC_{50} = 0.05 μM, **52** IC_{50} = 0.10 μM).[76] No anti-inflammatory activity was reported for **53** although both **51** and **53** were shown to be cytotoxic, whilst surprisingly **52** was not.[77]



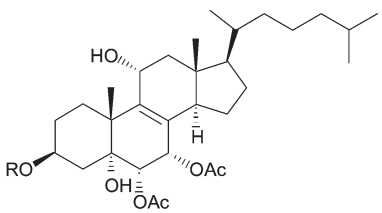
(**51**) R_1=H, R_2=OH

(**52**) R_1=R_2=H

(**53**) R_1=R_2=OH

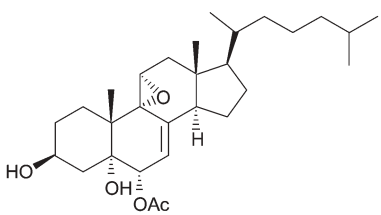
NMR based screening of the sponge *Clathria lissosclera*, collected by dredging from 100 m off the Three Kings Islands, New Zealand, highlighted the presence of a polyoxygenated compound. Further partitioning using polymeric reverse-phased stationary phase led to the isolation of the 14β sterol, clathriol A (**54**).[75] Later studies of this sponge resulted in the eventual isolation of a second related sterol, clathriol B (**55**).[8] Both **54** and **55** were found to inhibit the production of superoxide from human peripheral blood neutrophils stimulated with either fMLP (**54**: IC_{50} = 33 μM; **55**; IC_{50} = 27 μM) or PMA (**54**: 140 μM; **55**: 130 μM).[8,75]

Three polyoxygenated sterols were isolated from a new species of *Dysidea* sponge collected in Queensland, Australia. Sterols **56–58** were isolated using bioassay guided fractionation and all three inhibit IL-8Ra with IC_{50} values of 20, 5.5 and 4.5 μM respectively.[83]



(**56**) R=H

(**57**) R=Ac



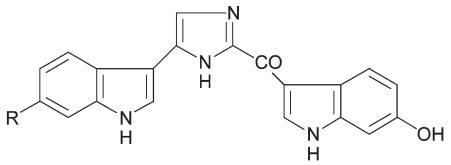
(**58**)

Nitrogenous anti-inflammatory compounds

Alkaloids

Four classes of anti-inflammatory alkaloid have been reported from marine sponges. The first are the bis(indoyl)imidazole alkaloids topsentin (**59**) and bromotopsentin (**60**) from the

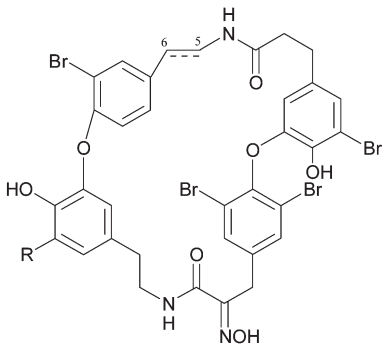
Mediterranean shallow-water sponge *Topsentia genitrix*.[84,85] Both **59** and **60** have been shown to inactivate PLA_2 (IC_{50} = 0.5 and 6.0 μM respectively) and to inhibit PMA-induced mouse ear oedema (ED_{50} = 15 and 30 μg per ear respectively), both of which are comparable or better than the anti-inflammatory effects of standards such as manoalide (**24**), hydrocortisone or indomethacin.[7,86]



(**59**) R=H

(**60**) R=Br

Bastadins-4 (**61**), -8 (**62**) and -9 (**63**) were isolated from the marine sponge *Ianthella basta*, collected in both Australia and Guam.[87,88] As well as exhibiting cytotoxic activity against the murine P388 cell line, all three metabolites inhibited inflammation in the mouse ear assay (**61**: 89%; **62**: 93%; **63**: 94% inhibition at 50 μg per ear).[87]



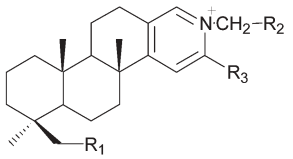
(**61**) R=Br, Δ^5

(**62**) R=Br, 6-OH

(**63**) R=H, Δ^5

A series of four anti-inflammatory pyridinium alkaloids were isolated from the same *Spongia* sp. collected from the Vanuatu Islands that yielded 21-hydroxypetrosaspongiolide K (**44**) and 21-hydroxypetrosaspongiolide P (**45**) (see sesterterpene section above).[70] Spongidines A–D (**64–67**) inhibited four secretory PLA_2 enzymes (Table 1).[70] None of the compounds exhibited cytotoxic effects on human neutrophils at the concentrations tested.[70]

The brominated alkaloid hymenialdisine (**68**) was identified by X-ray crystallography following its isolation from the sponges *Axinella verrucosa* and *Acanthella aurantiaca*, along with substantial quantities of the alkaloid oroidin.[89] Since the initial publication, there have been several reports of the re-isolation of **68**, including one with its geometrical isomer.[90] Hymenialdisine (**68**) has been found to inhibit IL-1 induced prostaglandin E_2 production with IC_{50} = 0.6 μM.[91]

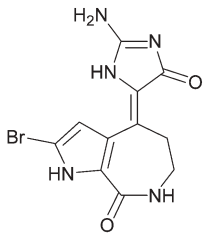


(**64**) R_1=R_3=H, R_2=COOH

(**65**) R_1=OAc, R_2=COOH, R_3=H

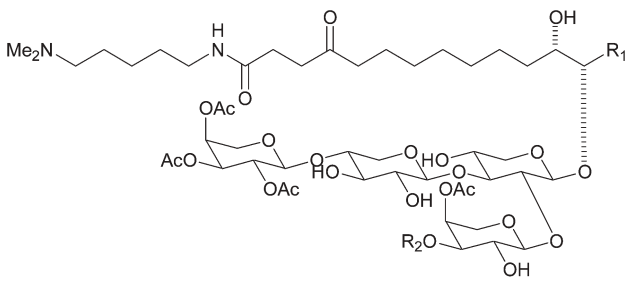
(**66**) R_1=H, R_2=COOH, R_3=CH_2CH_2COOH

(**67**) R_1=H, R_2=CH_2SO_3H, R_3=H



(**68**)

A series of long-chain dihydroxyketo-fatty acid amide tetrasaccharides were isolated from the Japanese sponge *Erylus placenta* collected from two different localities.[92,93] Eryusamines B–E (**69–72**) were all found to inhibit the binding of IL-6 to its receptor with IC_{50} = 66, 33, 37 and 17 μg mL^{-1} respectively.[93]



(**69**) R_1=$CH_2(CH_3)_2$, R_2=H

(**70**) R_1=$CH_2(CH_3)_2$, R_2=Ac

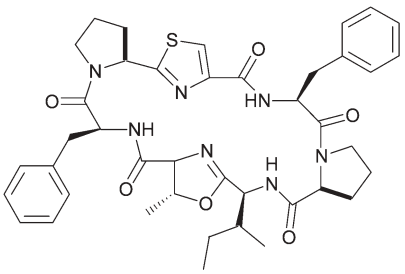
(**71**) R_1=$CH_2CH_2CH_2CH_3$, R_2=Ac

(**72**) R_1=$CH_2CH_2CH_2CH_2CH_3$, R_2=Ac

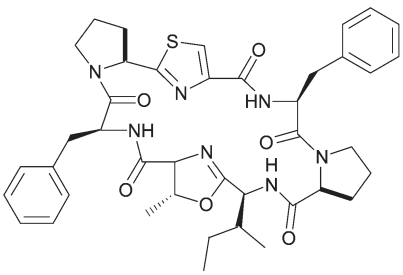
Peptides

There are very few reported peptide-derived anti-inflammatory compounds from marine sponges. Two stable conformers of a cyclic heptapeptide, *cis*, *cis*- (**73**) and *trans*, *trans*-ceratospongamide (**74**) were isolated from both the red macroalga *Ceratodictyon spongiosum* and the sponge *Sigmadocia symbiotica* which exist together in an unusual symbiotic relationship.[94] The thallus of the alga consists of a reticulated filamentous meshwork which is entirely covered by the sponge.[94] The anti-inflammatory activity of both **73** and **74** was examined by measuring the inhibition of $sPLA_2$ by hepatocellular carcinoma cells stimulated with the pro-inflammatory

cytokine IL-1β. The minor *trans*, *trans*- conformer (21% of total isolated ceratospongamide), **74**, inhibited expression of secretory PLA_2 (a key enzyme in the arachidonic acid cascade) with ED_{50} of 32 nM, whilst no inhibitory effect was noted for **73** up to a similar concentration in the same assay.[94]

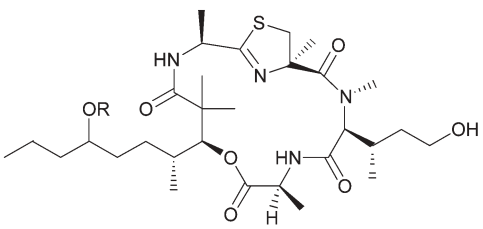


(**73**)



(**74**)

A sample of *Haliclona* sp. collected off the Vanuatu Islands yielded the two novel peptides halipeptins A (**75**) and B (**76**) of mixed biogenesis. Halipeptin A (**75**) was inactive in anti-fungal, anti-viral (HSVS1 and HIV1) and anti-microbial assays but was found to inhibit mouse paw oedema in a dose dependent manner with 60% inhibition at 300 μg kg^{-1}. This compared favorably with the standards indomethacin and naproxen (ED_{50} = 12 and 40 mg kg^{-1} respectively), indicating that **76** is 40 and 130 times more potent than these commercially available anti-inflammatory pharmaceuticals respectively.[95] A structural revision of **75** and **76** followed after the publication of halipeptin Cs structure along with the synthesis of the parent compounds.[96]

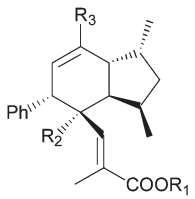


(**75**) R=Me

(**76**) R=H

Other anti-inflammatory compounds

Only two other groups of compounds possessing anti-inflammatory activity have been reported from marine sponges. The cytotoxic carboxylic acid plakotenin (**77**), possessing an unprecedented carbon skeleton, was isolated from the Okinawan marine sponge *Plakortis* sp.[97] Compound **77** was subsequently re-isolated along with its sodium salt (**78**) and with the free acid (**79**) and sodium salt of *homo*-plakotenin (**80**), and the sodium salt of *nor*-plakotenin (**81**), following a bioassay guided isolation targeting compounds that inhibit proliferation of arthritic cells.[98] At a concentration of 1 μg mL^{-1}, several of the compounds strongly inhibited the proliferation of rheumatoid synovial fibroblasts in response to platelet-derived growth factor-BB (PDGF-BB), as measured by the lack of [^{3}H]thymidine incorporation when compared to controls (% inhibition in rheumatoid synovial fibroblasts: **77**: 76.8%; **78**: 35.7%; **79**: 73.5%; **80**: 27.8%; **81**: 0.0%).[98] None of the compounds were able to inhibit proliferation at 0.1 μg mL^{-1}, suggesting a very restricted dynamic range of activity for the plakotenin metabolites.[98]



(**77**) R_1=H, R_2=Et, R_3=Me

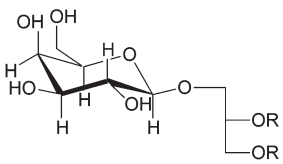
(**78**) R_1=Na, R_2=Et, R_3=Me

(**79**) R_1=H, R_2=Et, R_3=Et

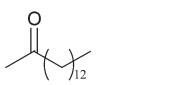
(**80**) R_1=Na, R_2=Et, R_3=Et

(**81**) R_1=Na, R_2=Me, R_3=Me

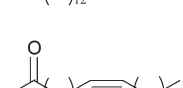
Finally, a mixture of anti-inflammatory glycolipids referred to as M-5 have been isolated from the Okinawan sponge *Phyllospongia foliascens*.[99] The M-5 complex (**82–84**) comprises a mixture of glycolipids with three different side chains (methyl myristate (**82**), methyl 8-hexadecenoate (**83**) and methyl palmitate (**84**) chains) attached to the glycosidated-glycerol portion of the molecule.[99] The anti-inflammatory activity of **82–84** is mentioned although no experimental



(**82**) R=



(**83**) R=



(**84**) R=

details are noted. A patent covering the use of **82–84** as an anti-allergy and anti-inflammatory agent has been filed.[100]

Concluding remarks

In conclusion, marine sponges have provided many examples of novel secondary metabolites that possess varied chemical structures and potent anti-inflammatory activity. The various compounds reported vary in both their modes of action and the level of activity noted. This field promises to be an active area of research for some time.

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