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BIOACTIVE MARINE SESTERTERPENOIDS

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ABSTRACT: Terpenoids are by far the largest class of natural products. Within this class of compounds, the sesterterpenes form a rare group of isoprenoids, which occur in widely differing source. Marine organisms have provided a large number of sesterterpenoids, possessing novel carbon skeleton and a wide variety of biological activities. The more significant sesterterpenoids from marine organisms, which show biological activities, are reported and they are grouped in a biogenetic sequence. Natural products that do not contain 25 carbon atoms but are obviously sesterterpene derivatives are also included. The anti-inflammatory activity is the most relevant among the biological activities observed for marine sesterterpenoids. The high potential for some of these products suggested that they could be developed as drugs for the treatment of inflammation. The different directions that can be taken to obtain quantities of secondary metabolites are reported.

INTRODUCTION

Natural products play a dominant role in the discovery of leads for the development of drugs for the treatment of human diseases. It should be realised that the bioactive compounds, which are synthesised in nature to protect a given organism, had been selected from a wide variety of possibilities and were under the pressure of evolution for several hundreds of million years to reach an optimal activity.

The terpenoids (isoprenoids) are a class of secondary metabolites that may be formally considered to be constructed from the five-carbon isoprene unit [1]. The terpenes have been classified primarily on the basis of their number of isoprene units (monoterpenes C_{10}, sesquiterpenes C_{15}, diterpenes C_{20}, sesterterpenes C_{25}, triterpenes C_{30} and carotenoids C_{40}) and then on their carbon skeleton. The monoterpenes, sesquiterpenes, diterpenes and sesterterpenes contain the isoprene units linked head to tail, while the triterpenes and carotenoids contain two C_{15} and C_{20} units, respectively linked in the middle tail to tail. Several thousand terpenes

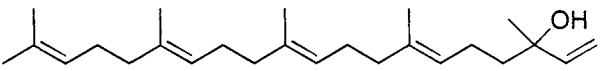
have been isolated and they are by far the largest class of natural products. Within this class of compounds, the sesterterpenes form a rare group of isoprenoids, which occur in widely differing source and have been isolated from terrestrial fungi [2], plants [3] and insects [4] as well as from marine organisms [5,6], mainly from sponges and nudibranches. Marine organisms have provided a large number of sesterterpenoids, possessing novel carbon skeletons different from those present in terrestrial species. Several sesterterpenoids isolated from marine organisms have shown a wide variety of biological activities.

The aim of this contribution is to review the more significant sesterterpenoids from marine organisms, which show biological activities, emphasising those compounds with a potential industrial application. In this review the structures will be covered in a biogenetic sequence and also include natural products that do not contain 25 carbon atoms but are obviously sesterterpene derivatives, such as degraded sesterterpenes with 21-24 carbon atoms, and alkylated sesterterpenes with 26-27 carbon atoms. In addition, some of our own results on anti-inflammatory activity of marine metabolites have been reported. Likewise, some data on the different directions that can be taken to obtain secondary metabolites have been included in the final section to suggest alternative production of marine metabolites and to highlight the possible future importance of marine biotechnology in the production of large quantities of marine secondary metabolites. Previous specific reviews on sesterterpenoids have been published [7-10]. Furthermore, several reviews on terpenoids [11-13] and on marine organisms [5-6,14-16] all contain material on sesterterpenoids.

LINEAR SESTERTERPENOIDS

After the isolation of all-*trans*-geranylnerolidol (**1**) and geranylfarnesol (**2**) from the phytopathogenic fungus *Cochliobolus* [17] and from the wax of the insect *Ceroplastse albolineatus* [18], a large number of acyclic (absence of formation of any additional carbon-carbon bonds compared with a linear combination of isoprene units) sesterterpenoids were isolated from marine organisms. Furanosesterterpenes are a prominent class of metabolites mainly isolated from marine sponges of the family Thorectidae. The less elaborate component of this interesting group is furospinosulin-1 (**3**), first isolated from *Ircinia spinosula* [19] and later

from several Dictyoceratida species, including the South African *Fasciospongia* sp. [20], the Australian *Thorecta* sp. [21] and Californian *Spongia idia* [22] that contains also the oxidized derivative, idiadione (**4**).



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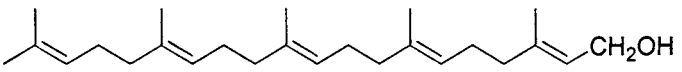

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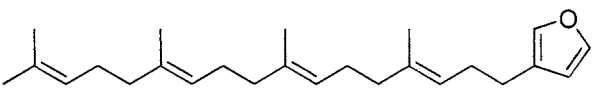

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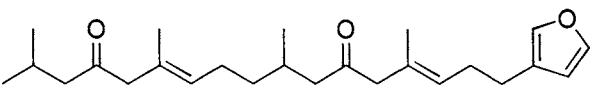

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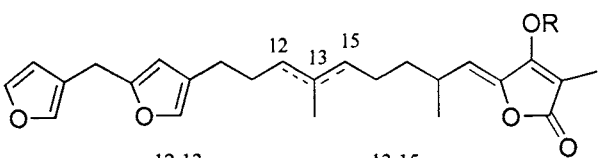


5a $\Delta^{12,13}$ R = H; **6a** $\Delta^{13,15}$ R = H

5b $\Delta^{12,13}$ R = SO_3H; **6b** $\Delta^{13,15}$ R = SO_3H

Both compounds **3** and **4** showed activity at10 μg/ml [22] in the *Artemia salina* bioassay, which gives results that correlate well with cytotoxicity in cancer cell lines such as human epidermoid carcinoma KB, murine lymphoma P388 [23], mouse lymphoma L5178y and murine lymphoma L1210 [24]. Minale and co-workers [25] reported in 1972 the isolation from the sponge *I. oros* of ircinin-1 (**5a**) and ircinin-2 (**6a**), both containing two furan rings and a conjugated tetronic acid moiety. Before the isolation of ircinins, only four others sesterterpenoids were known. Subsequently, it was reported that the mixture of both ircinins inhibited potently mouse ear oedema after topical application, with an ID_{50} of 51

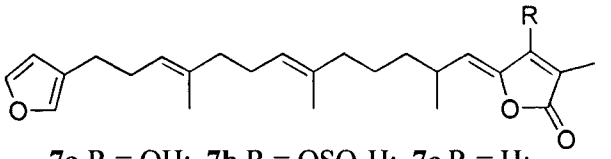
μg/ear. Ircinin was found to be a good inhibitor of human recombinant synovial phospholipase A_2 (PLA_2) (IC_{50} 3.1 μM) and 5-lipoxygenase (IC_{50} 1.3 μM), and it was not cytotoxic on human neutrophils at all concentrations tested (0.1-100 μM) [26]. These results demonstrate that ircinin is a novel marine inhibitor of PLA_2 with a potent topical anti-inflammatory profile and they suggest a potential role of ircinin as an inhibitor of inflammatory processes.

Recently, the anti-inflammatory activity has been recorded in many sesterterpenes, with a mechanism of action different from those of non-steroidal, anti-inflammatory drugs (NSAID). The inflammatory response has been shown to be involved in a diverse array of pathological conditions such as arthritis, gout, psoriasis, bee stings and many chemically induced oedemas. The inflammatory response is mediated by the biosynthesis of eicosanoids from arachidonic acid (arachidonic acid cascade), as well as other autacoids released locally in response to an irritant. Arachidonic acid is primarily stored in the *sn*-2 position of membrane phospholipids. The hydrolysis of the ester at this position is specifically catalysed by PLA_2. Lipoxygenase, cycloxygenase and cytochrome P-450 are enzymes from arachidonic acid cascade [27]. The most commonly used non-steroidal, anti-inflammatory agents, indomethacine and the salicylate, inhibit the cycloxygenase pathway but the use of these inhibitors is associated with an increased risk of gastrointestinal bleeding and renal complications. Thus, the inhibition of release of arachidonic acid by PLA_2 has become an attractive target for investigation. The development of marine specific inhibitors of PLA_2 offers the prospect for a new generation of anti-inflammatory drugs without side effects, derived from non-selective inhibition of constitutive enzymes.

From the sponge *I. variabilis* was isolated variabilin (**7a**), a furanosesterterpene containing a tetronic acid moiety, which showed antimicrobial activity against Gram-positive bacteria *Staphylococcus aureus* [28] and *Sarcina lutea* [29].

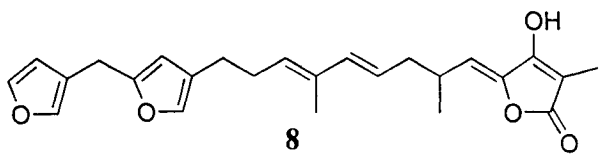
After the preliminary observation of Tiberio in 1895 [30] and Fleming in 1929 [31] that a metabolic product of the mould *Penicillium notatum* inhibited the growth of a staphylococcal culture, and the introduction of penicillin in the treatment of bacterial infections several antimicrobial drugs were produced. The introduction of antimicrobial drugs for the control of infection is the biggest achievement in the history of medicine.

Unfortunately, many bacteria acquire resistance to one or more of the antibiotics to which they were formerly susceptible. Since most antibacterial agents interact with a specific protein or cellular component, modification of the target is a common means by which resistance can be conferred. Pharmaceutical companies are actually developing new antimicrobial agents against resistant bacteria.



7a R = OH; **7b** R = OSO_3H; **7c** R = H;

Later was reported that variabilin (**7a**) is a good inhibitor of human secretory and cytosolic PLA_2 with anti-inflammatory activity [32] and shows *in vitro* antiviral activity against *Herpes simplex* (HSV) and *Polio vaccine* (PV1) viruses [33]. The high cytotoxicity against the BSC cell line exhibited by variabilin severely limits its potential usefulness as antiviral agent [33].



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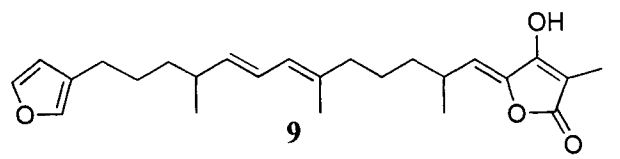

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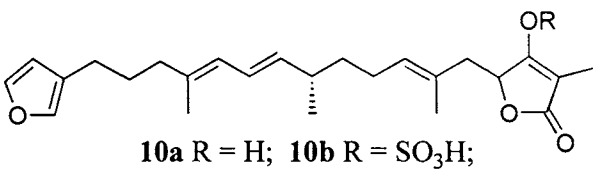
Thereafter, several compounds closely related to ircinins and variabilin have been isolated [5,6]. Fusetani and co-workers [34] reported the isolation of two compounds, dehydroderivative of ircinin (**8**) and an isomer of variabilin (**9**) from the Japanese sponge *Cacospongia scalaris*. Both compounds inhibited the cell division of fertilised starfish eggs at a concentration of 1.0 μg/ml. This assay is a variation on the test with sea urchin embryos, which can detect DNA and RNA synthesis inhibitors, microtubule assembly and protein synthesis inhibitors, the common leads

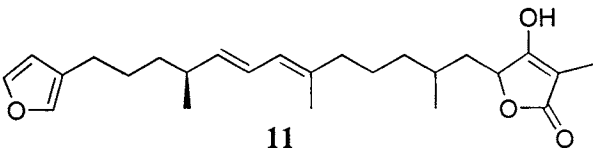
for the development of anticancer drugs [35]. From the Australian sponge, *Thorecta* sp. was isolated the rare 22-deoxy-variabilin (**7c**) that inhibited the growth of *S. aureus* at 100 μg/disk, *Bacillus subtilis* at 50 μg/disk and *Candida albicans* at 100 μg/disk in a standard agar plate assay [21].

From sponges of the genus *Ircinia*, collected in the Northern Adriatic Sea, together with the previously described ircinin-1 (**5a**), ircinin-2 (**6a**) and variabilin (**7a**), the corresponding sulphates **5b-7b** were isolated. The sulphated derivatives **5b-7b** showed greater activity in the *A. salina* bioassay (LC_{50}: 1.72 and 1.22 μg/ml, ircinins and variabilin sulphated, respectively), than the corresponding non-sulphated compounds **5a-7a** (LC_{50}: 2.38, 2.73 and 2.10 μg/ml), being less toxic in the fish (*Gambusia affinis*) lethality test (LC_{50}: **5b-6b** 5.09, **7b** 9.50, **5a** 3.35, **6a** 3.03 and **7a** 3.15 μg/ml) [36].

Less common are those examples of this class of compound in which the tetronic acid moiety is nonconjugated. Palinurin (**10a**) was the first example of this class of compound, isolated from *I. variabilis* [37]. Subsequently, from an Australian *Ircinia* sp. was isolated a hydro derivative (**11**) of palinurin, which inhibits the growth of *B. subtilis* [38].



10a R = H; **10b** R = SO_3H;



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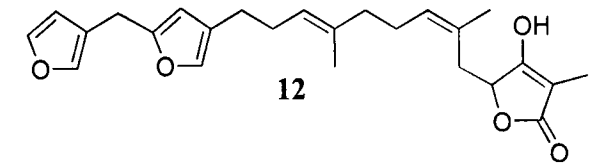
Additionally, three metabolites of this class of compounds, spongionellin (**12**), dehydrospongionellin (**13**) [39] and okinonellin B (**14**) [40], were isolated from a Japanese sponge of genus *Spongionella* and were shown to inhibit the cell division of fertilised starfish eggs at 2.0-5.0 μg/ml. From an Australian *Dysidea* sp. was isolated isopalinurin (**15**) that possessed inhibitory properties against the protein phosphatase enzyme in chicken forebrain [41].



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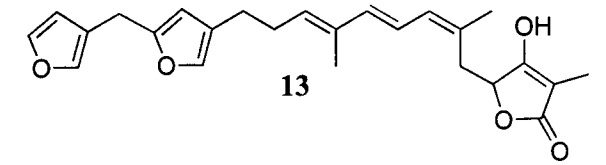

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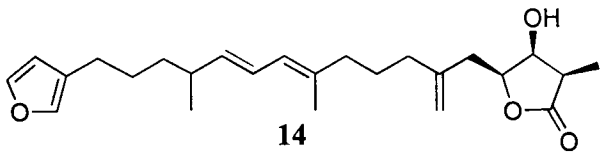

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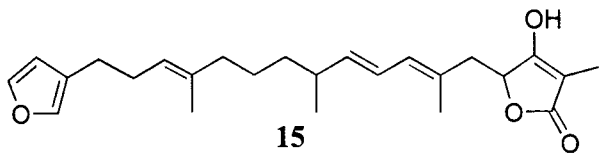

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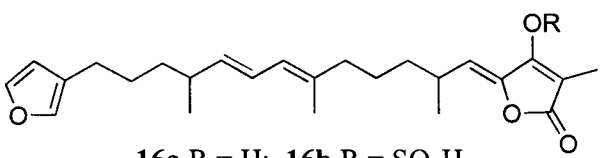
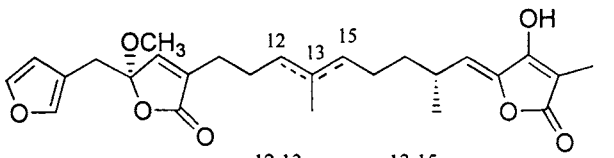
Palinurin (**10b**) and fasciculatin (**16b**) sulphates, together with the previously described palinurin (**10a**) and fasciculatin (**16a**), were isolated from the Tyrrhenian sponges *I. variabilis* and *I. fasciculata,* respectively. Yet again the sulphated derivatives were more active in *A. salina* bioassay (LC_{50}: **10b** 3.04, **16b** 1.44, **10a** 7.56, **16a** 2.03 μg/ml) and less toxic in the fish lethality test (LC_{50}: **10b** 2.30, **16b** 2.20, **10a** 1.67, **16a** 1.04 μg/ml) [42].



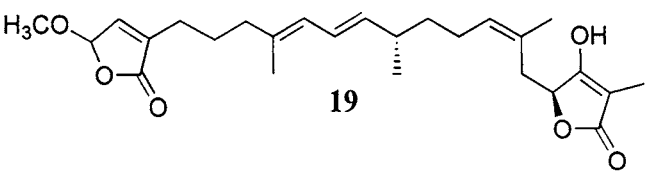
16a R = H; **16b** R = SO_3H

Several compounds closely related to palinurin and spongionellin have been isolated [5,6] that showed moderate antimicrobial and cytotoxic activities.

Sponges of the genus *Sarcotragus* are a rich source of sesterterpenes with both conjugated and nonconjugated tetronic acid moieties [43,44]. Sarcotins G (**17**) and H (**18**) are closely related to ircinin-1 (**5a**) and –2 (**6a**), except that a furan ring is replaced by a 5-methoxy-2(5H)-furanone moiety. These compounds showed cytotoxic activity with IC_{50} values 5.0-10.0 μg/ml against five human tumour cell lines (lung carcinoma A549, ovarian carcinoma SK-OV-3, skin carcinoma SK-MEL-2, central nervous system carcinoma XF498 and colon carcinoma HCT15) [44], while sarcotin F (**19**), an oxidised derivative of palinurin (**10a**) showed less cytotoxic activity (IC_{50} 7.6-24.1 μg/ml) in the same panel of cell lines [44].



17 $\Delta^{12,13}$; **18** $\Delta^{13,15}$



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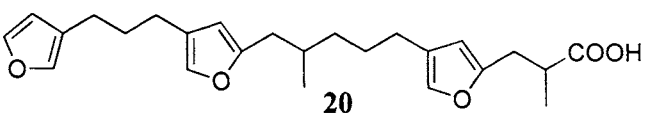

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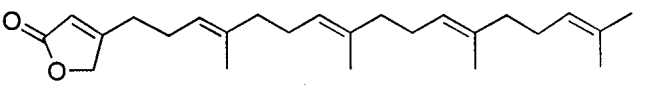
Unusual trifuranosesterterpene acids, hippospongins A-C, were isolated from the Australian *Hippospongia* sp., which are speculated to be biosynthetically related to the C_{25} tetronic acids and the C_{21} furanoterpenes. Only hippospongin A (**20**) showed a mild antimicrobial activity, inhibiting the growth of *S. aureus* at a concentration of 200 μg/disk [45].

An α,β-unsaturated, γ-lactone linear sesterterpene (**21**) was isolated from the Caribbean sponge *Thorecta horridus* that exhibited inflammatory activity both *in vivo* inducing paw oedema and *in vitro* inducing release of histamine [46].

From a sample of the sponge *Fasciospongia cavernosa*, collected in the bay of Naples (Italy), were isolated two linear sesterterpenes, cacospongionolide D (**22**) with a γ-hydroxybutenolide moiety, and luffarin-V (**23**) with two γ-butenolide functionalities in the molecule. Cacospongionolide D showed a potent activity (LC_{50} 0.1 μg/ml) [47] in the *A. salina* bioassay, and a moderate ichthyotoxicity to *G. affinis* (LC_{50} 2.54 μg/ml) in the fish lethality assay. Luffarin-V was less active (LC_{50} 1.72 μg/ml) in the *A. salina* bioassay.



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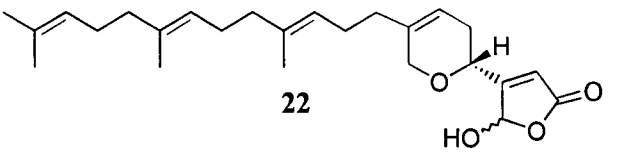

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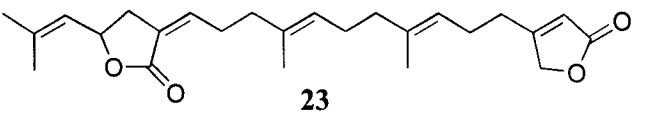

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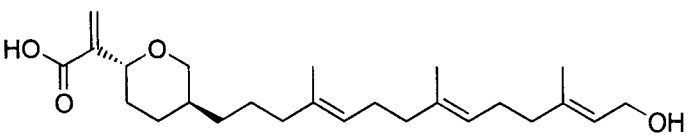

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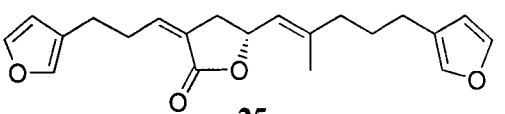
An unusual sesterterpenoid acid (**24**) with a tetrahydropyran ring was isolated from the Indonesian sponge *Hippospongia* sp. that inhibited the human Ras-converting enzyme (hRCE), with an IC_{50} value of 10 μg/ml [48]. The Ras signalling pathway has emerged as an important target for the development of anticancer drugs. Ras is a membrane bound G protein that functions as a molecular switch in a network of signalling pathways, controlling cell differentiation and proliferation. Mutated Ras genes, encoding activated Ras proteins, have been identified in approximately 30% of all human cancers. The approaches to therapeutic intervention in

the Ras signalling have focussed on the development of inhibitors that block the lipid modification needed for proper Ras membrane localisation (farnesyl transferase inhibitors) or to finding inhibitors of proteolytic processing of Ras (RCE protease inhibitors) [49].

Linear, closely related difuranoterpenes containing 21 carbon atoms have been found to occur in large amounts in the sponge of the genus *Spongia*. All of them possess the same carbon skeleton, and oxidation in the central chain accounts for all their differences. The first two C_{21} compounds, ninetin (**25**) and dihydroninetin (**26**) isolated from *S. nitens* [50] possess a γ-lactone ring in the central part of the chain. *S. officinalis* and *Hippospongia communis* contain several C_{21} furanoterpenes in large amounts [51,52]. From a specimen of *S. officinalis*, collected in the Northern Adriatic Sea, together with furospongin-2 (**27**), previously reported from the same sponge collected in the Tyrrhenian Sea [51], its three isomers (**28-30**) were isolated. These C_{21} furanoterpenes (**27-30**) showed high activity (LC_{50} 0.09-1.60 μg/ml) in the *A. salina* bioassay [53].



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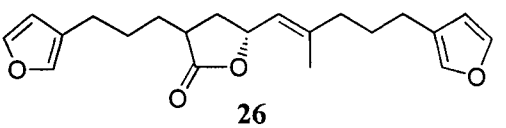

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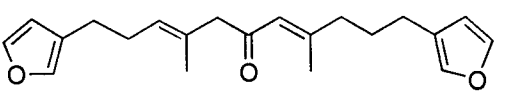
The most widely distributed component of this group, furospongin-1 (**31**), which possesses interesting biological activities, was first isolated from the Mediterranean *S. officinalis,* and the closely related *H. communis* [51], few years later, was isolated from two Australian sponges, *Phyllospongia radiata* and *P. foliescens* [54]. Furospongin-1 showed analgesic [55] and antispasmodic activities [56]. Furthermore, furospongin-1 reduced, at μM concentration, the tissue levels of ATP and had no significant effect on ATP breakdown in bovine mitochondria, showing that its antispasmodic action has a mechanism different from that of the mitochondrial ATP synthase inhibitor, oligomycin [56].



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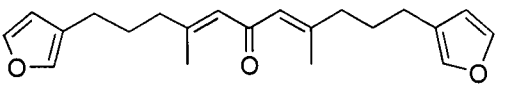

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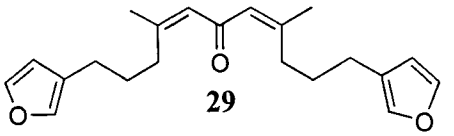

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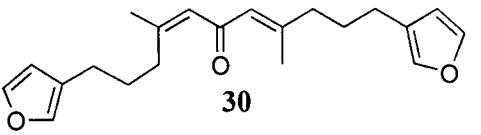

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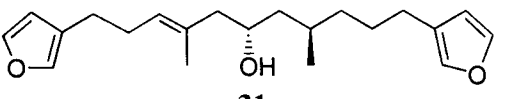
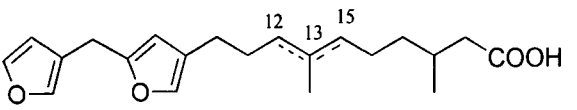


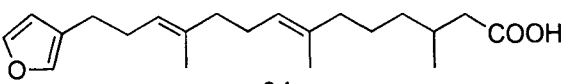
31

Linear C_{21} furanoterpenes commonly occur in Spongiidae or Thorectidae sponges with structurally-related sesterterpenic tetronic acids from which they are biosynthesised by the loss of a four-carbon fragment [14]. This hypothesis has raised considerable interest [15] and has received some experimental support from the degradation of linear conjugated furanosesterterpenic tetronic acids to C_{21} furanoterpenic carboxylic acids by oxidation in basic aqueous solution [57]; and from the isolation of C_{21} furanoterpenic carboxylic acids, ircinin-3 (**32**) and ircinin-4 (**33**), related to ircinin-1 and ircinin-2, from the sponge *I. Oros* [58]; the acid **34** related to variabilin (**7a**) isolated from sponge of genus *Sarcotragus* [59]; and the acid **35** together with the C_{20} aldehyde **36** both related to fasciculatin (**16a**) [57]. The C_{21} furanoterpene acid **34** showed antiviral (HSV and PV1) and cytotoxic activities comparable with that of variabilin [33]. Further evidences of the hypothesis of Minale were obtained by the isolation of three chlorinated C_{24} norsesterterpenes (**37-39**), closely related to ircinin-1 (**5a**) and ircinin-2 (**6a**), from the sponge *I. oros*, collected in the Northern Adriatic Sea [60]. In fact, we may suppose

that **37** and **39** are the first stage of degradation of the tetronic acid, through introduction of a chlorine atom by action of a chloroperoxidase, then hydrolysis of the lactone ring and subsequent decarboxylation to produce keto-chlorohydrins, which can easily be degraded giving the C-21 terpenes. The norsesterterpenes, **37-39,** showed less cytotoxic activity (LC_{50} 8.2-8.6 μg/ml) than ircinin-1 (**5a**) and ircinin-2 (**6a**) (LC_{50} 2.4 and 2.7 μg/ml), in the *A. salina* bioassay [60].



32 $\Delta^{12,13}$; **33** $\Delta^{13,15}$



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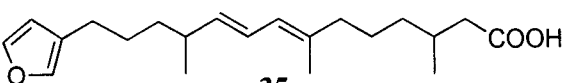

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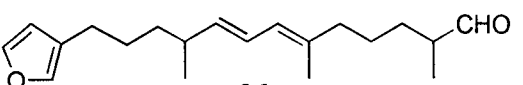

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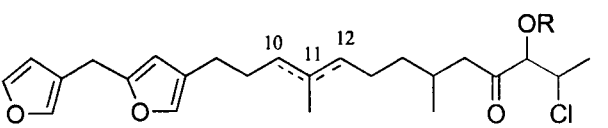
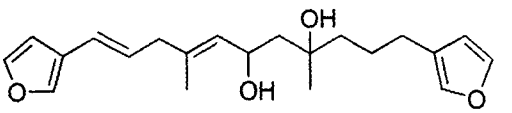


37 $\Delta^{10,11}$ R = H; **38** $\Delta^{10,11}$ R = Ac;
39 $\Delta^{11,12}$ R = H

Untenospongins A (**40**) and B (**41**), isolated from an Okinawan species of *Hippospongia* exhibited potent coronary vasodilating activity, markedly inhibiting KCl induced contraction of rabbit coronary artery with IC_{50} values of 10^{-6} and 2 x 10^{-6} M, respectively [61].

The norsesterterpenoids, sarcotins N (**42**), O (**43**) and *ent*-kurospongin (**44**), isolated from *Sarcotragus* sp., showed moderate cytotoxicity, with

IC_{50} values 3.0-30.0 μg/ml, against a panel of five human tumour cell lines (A549, SK-OV-3, SK-MEL-2, XF498 and HCT15) [62].



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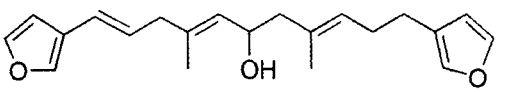

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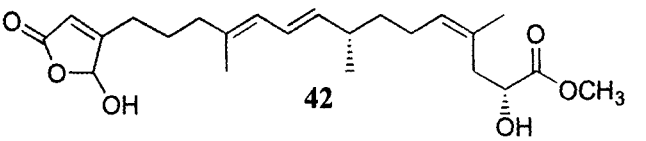

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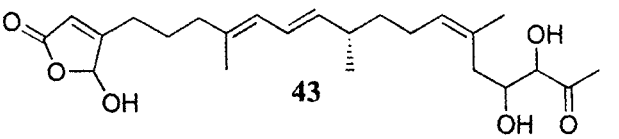

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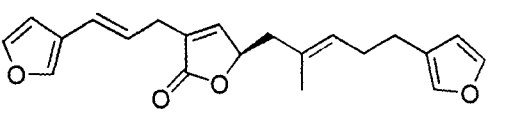
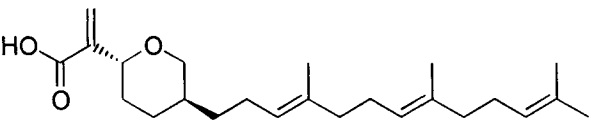


44

Rhopaloic acids A-C (**45-47**), three unusual norsesterterpenes isolated from a Japanese species of *Rhopaloeides,* selectively inhibited the gastrulation of fertilised eggs of the Starfish *Asterina pectinifera* at μM level. The minimum inhibitory concentrations of **45-47** were 0.5, 0.4 and 0.2 μM, respectively [63]. Rhopaloic acid A, which was synthesised [64], exhibited also potent cytotoxicity against human myeloid K562 cells (IC_{50} 0.04 μmol/l), human leukaemia MOLT4 cells (IC_{50} 0.05 μmol/l), and L1210 cells (IC_{50} 0.10 μmol/l) [65]. Furthermore, rhopaloic acids A-C together with rhopaloic acids D-G (**48-51**) were isolated from an Indonesian *Hippospongia* sp.. Rhopaloic acids A-E showed a RCE protease inhibitory activity with IC_{50} values of ≈ 10 μg/ml [48]. Compounds **45-49** were more active in the cell-based assay against colon tumour LoVo cells (IC_{50} ≈ 1 μg/ml) than in the enzyme assays, suggesting

that the cytotoxic effect of the compounds might result from hitting more than one molecular target [48].



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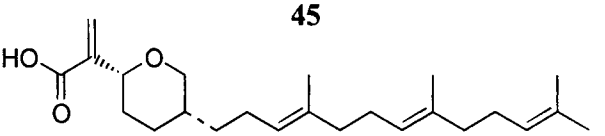

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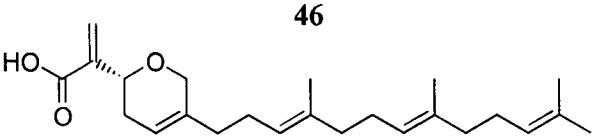

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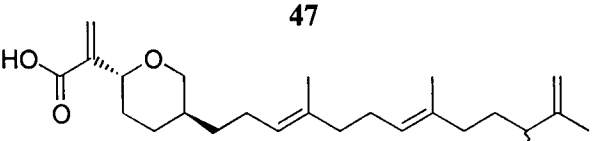

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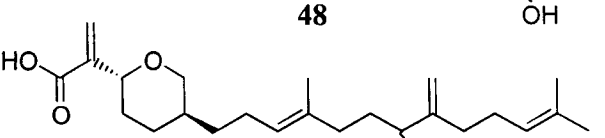

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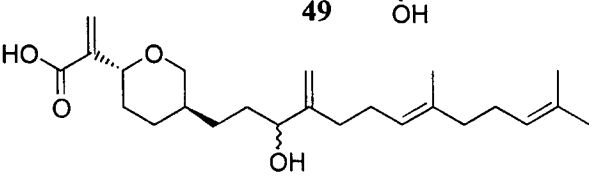

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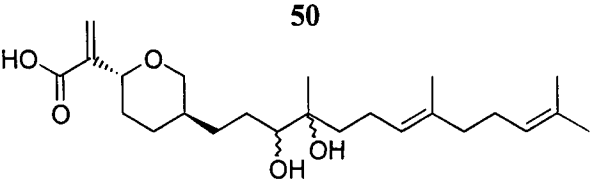
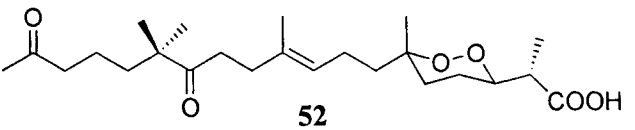


51

Muqubilone (or aikupikoxide A) (**52**), a norsesterterpene peroxide acid, isolated from the Red Sea sponge *Diacarnus erythraeanus* showed *in vitro* antiviral activity against herpes simplex virus type 1 (HSV-1) with IC_{50} of 30.0 μg/ml [66], and cytotoxic activity with an IC_{50} > 1 μg/ml, against three type of cancer cells, including P388, A549 and human colon carcinoma HT29 [67].

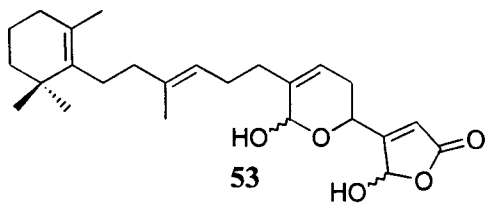


52

MONOCARBOCYCLIC SESTERTERPENOIDS

Marine sponges of genus *Luffariella* (Thorectidae; Dictyoceratida) are a rich source of monocarbocyclic sesterterpenoids and most of them possess interesting bioactivities. In 1980 and 1981, Scheuer and co-workers [68,69] reported the isolation of manoalide (**53**), seco-manoalide (**54**), (6*E*)- (**55**) and (6*Z*)-neomanoalide (**56a**) from the Palauan sponge *L. variabilis*, which showed interesting antimicrobial activity against Gram positive bacteria *Streptomyces pyogenes*, *S. aureus* and *B. subtilis* [68,69]. Later, Kobayashi and co-workers [70] reported, from the Okinawan sponge *Luffariella* sp., the isolation of manoalide (**53**), (6*E*)- (**55**) and (6*Z*)-neomanoalide (**56a**) that showed cytotoxic activity against L1210 cells (IC_{50} 0.032, 9.8 and 5.6 μg/ml for **53**, **55** and **56a**, respectively), and only manoalide was active against KB cells with an IC_{50} value of 0.3 μg/ml [70,71]. Manoalide is the first compound of this group to be reported, characterised by cyclisation that is reminiscent to those of the carotenoids and one or two potentially reactive rings, γ-hydroxybutenolide ring and a δ-lactol ring (α-hydroxy-dihydropyran ring) or its derivative. Subsequently, it was found that manoalide showed molluscicidal activity towards *Biomphalaria glabrata* at 1.5 ppm [72], analgesic activity at 50 mg/kg in the phenylquinone test, and anti-inflammatory activity in the induced inflammation of the mouse ear, with a potency greater than that of indomethacine and less than that of hydrocortisone [73]. The most important finding has been that manoalide is the inhibitor of various secreted forms of PLA_2 at nM concentration

[74-77]. It was suggested that the binding of manoalide to PLA_2 is irreversible and involves initial formation of a Schiff base (imine) between a lysine residue on PLA_2 and the aldehyde group of γ-hydroxy-butenolide, than a second lysine reacts with the aldehyde group of α-hydroxy-dihydropyran ring to produce an adduct in which the manoalide is irreversibly bound to PLA_2 [75,78]. Over 140 citations concerning manoalide recorded in MEDLINE show the high interest pointed to this compound. Eight total syntheses have been reported [79-86]. Seco-manoalide (**54**), which is the geometrical isomer of manoalide, has similar potency and efficacy in the inhibition of bee venom PLA_2, suggesting that the inhibition reaction is not dependent on a rigid geometrical relationship between the aldehyde group and the second lysine residue [75].



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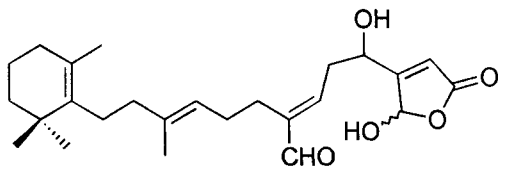

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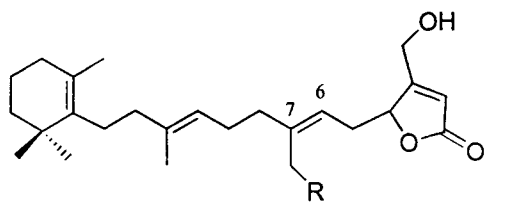
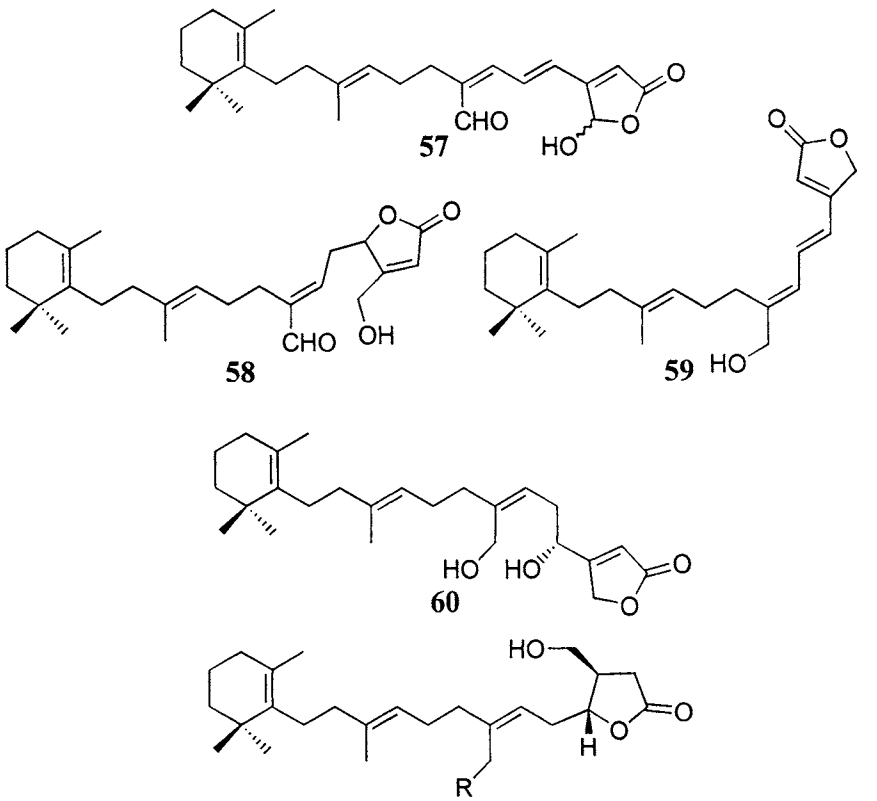


55 $\Delta^{6,7}E$, R = OH

56a $\Delta^{6,7}Z$, R = OH; **56b** $\Delta^{6,7}Z$, R = OAc

From the Western Pacific sponge *L. variabilis* was isolated dehydro-manoalide (**57**) that showed a marked decrease in inhibition of bee venom PLA_2 (IC_{50} 0.28 μM) [76, 87].

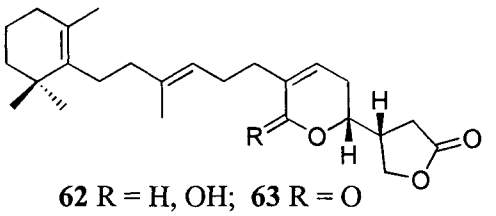
In 1992, König and co-workers [72] reported the isolation of *Z*-2,3-dihydro-neomanoalide (or luffariolide C) [88] (**61a**), its 24-acetyl derivative (**61b**), 6*Z*-24-acetoxy-neomanoalide (**56b**) and *E*-neomanoalide-24-al (**58**), from an Australian sponge of genus *Luffariella*. All these compounds showed antibacterial activity against *Escherichia coli*, *B. subtilis* and *Micrococcus luteus*, in a TLC bioautographic test [72].



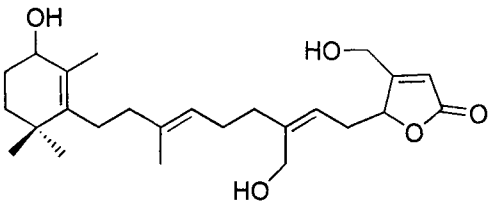
61a R = OH; **61b** R = OAc

Kobayashi and co-workers [70] reported, from the Okinawan sponge *Luffariella* sp., the isolation of several sesterterpenoids related to manoalide, named luffariolides A-J (**59-67**). All luffariolides showed cytotoxic activity against L1210 cells (IC_{50} 1.1-4.5 μg/ml) and only luffariolides F (**64**) and G (**65**) exhibited weak activity also against KB

cells [70,71,88]. Luffariolides H (**66**) and J (**67**) showed antimicrobial activity against *S. aureus,* with minimum inhibitory concentrations (MIC) of 16.7 and 33.3 μg/ml, respectively, *B. subtilis* (MIC, both 8.4 μg/ml) and *M. luteus* (MIC, both 8.4 μg/ml) [88].



62 R = H, OH; **63** R = O



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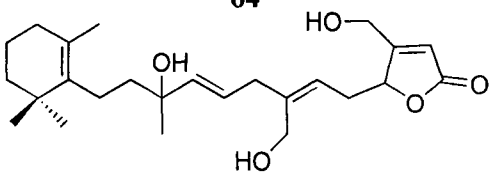

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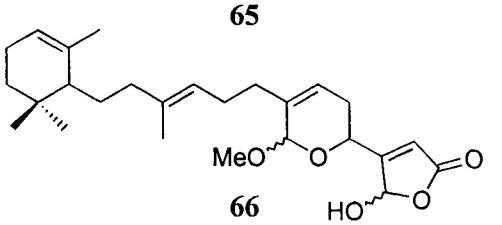

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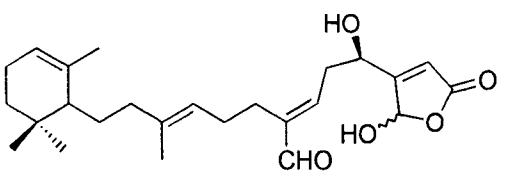
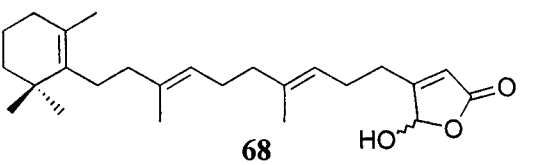


67

Faulkner and co-workers reported the isolation of luffariellolide (**68**) from a Palauan sponge *Luffariella* sp., which was a potent antagonist of topical induced inflammation in the mouse ear, but it was less potent than manoalide (**53**) inhibitor of bee venom PLA_2 with an IC_{50} value of 1.6 x 10^{-7} M. Luffariellolide is a partially reversible inhibitor of bee venom PLA_2, because it lacks one of the two masked aldehyde groups that appears to be responsible for the irreversible reaction of manoalide with lysine residue of PLA_2 [89].



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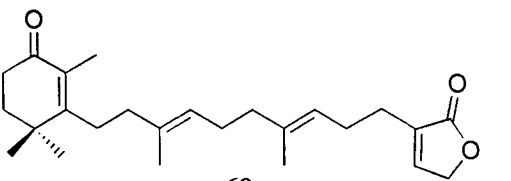

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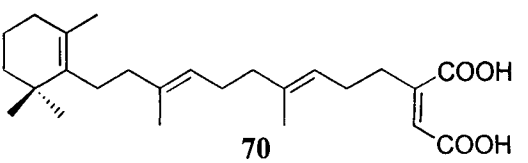

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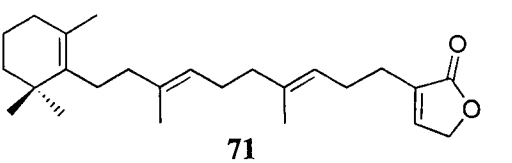
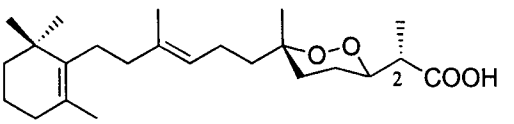


71

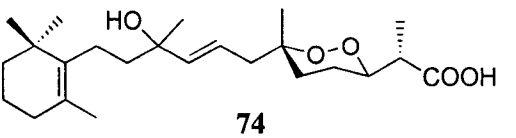
From the Fijian sponge *Fascaplysinopis reticulata* were isolated two sesterterpenoids related to luffariellolide, *iso*-dehydro-luffariellolide (**69**) and dehydro-luffariellolide diacid (**70**). *Iso*-dehydro-luffariellolide inhibited at 1 mg/ml 81% of the HIV-1 reverse transcriptase activity [90] and reduced the activity of $p56^{lck}$ tyrosine kinase at 0.5 mM to 45% in ELISA based assays [91]. Hyrtiolide (**71**) was isolated from the Fijian sponge *Hyrtios erecta* together with its correlated *iso*-dehydro-

luffariellolide. Hyrtiolide showed weak antifungal activity towards *Ustilago violaceae* [91].

Muqubilin [92] (or prianicin A) [93] (**72**), a norsesterterpene peroxide acid, isolated from the Red Sea sponges, *Prianos* sp. [92-94] and *Diacarnus erythraeanus* [66] showed antimicrobial activity against *Streptococcus beta haemolytic* (MIC 2.5 μg/ml), *S. aureus* (MIC 12.0 μg/ml) and *Corynebacterium diphteriae* (MIC 3.0 μg/ml) [93], and it displayed potent *in vitro* activity against *Toxoplasma gondii* at a concentration of 0.1 μM without significant toxicity [66]. Furthermore, muqubilin totally inhibited the cell division of fertilised sea urchin eggs at 16 μg/ml [94]. *Ent*-muqubilin (**72**), 2-*epi*-muqubilin (**73**) and deoxy-diacarnoate B (**121**) (see bicyclic section) were isolated from the New Caledonian sponge *Diacarnus levii* [95]. The mixture of all three compounds showed cytotoxicity against both chloroquine sensitive and resistant strains of *Plasmodium falciparum*, the human parasite responsible for the most severe cases of malaria [95].



72; **73** 2-epi



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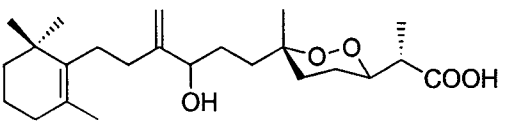

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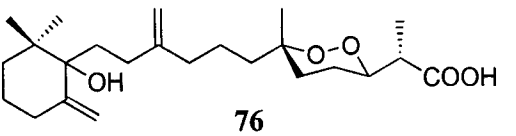


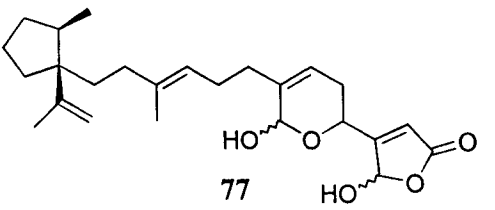
76

The finding of new antimalarial drugs, particularly those against multiresistant *P. falciparum*, is extremely important, because in the last

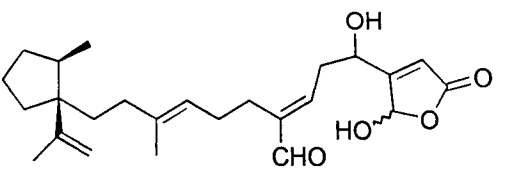
years the malaria has regained its status as an extremely important threat to the human health. It is estimated that, in regions where malaria is endemic, each year about 1.5 million of people die from this disease.

Tasnemoxides A-C (**74-76**), closely related to muqubilin, were isolated from the Red Sea *D. erythraeanus*, and showed moderate cytotoxicity (IC_{50} > 1 μg/ml) against three cancer cell lines including P388, A549 and HT29 [96].

In order to provide sufficient manoalide for continued pharmacological evaluation, Faulkner and coworkers made an extensive collection of *L. variabilis*, from different locations in Palau. From a small number of specimens of *L. variabilis* were isolated two new metabolites, luffariellin A (**77**) and Luffariella B (**78**) in place of manoalide and seco-manoalide [97]. Despite the different carbon skeleton, the functional groups in luffariellins A and B are identical with those in manoalide and seco-manoalide, respectively, and they showed almost identical anti-inflammatory properties. Both luffariellins were potent antagonists of topical induced inflammation in the mouse ear, and inhibitors of bee venom PLA_2, with an IC_{50} value of 5.6 x 10^{-8} M and 6.2 x 10^{-8} M, for luffariellins A and B, respectively [97].



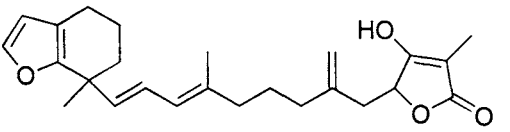
77



78

Hippospongin (**79**), isolated from the Okinawan sponge *Hippospongia* sp., is an unusual sesterterpene containing an isolated cyclohexenofuran ring and a tetronic acid moiety, which showed antispasmodic activity (5 x 10^{-6} M), abolishing the contractile responses to carbachol and histamine on the guinea-pig ileum [98]. Further sesterterpenes (**80** and **81**) and two

norsesterterpene (**82** and **83**), related to hippospongin, were isolated from the Okinawan sponge *Ircinia* sp.. The norsesterterpenes **82** and **83** were more cytotoxic (IC_{50} < 1 μg/ml) than the sesterterpenes **80** and **81** ($IC_{50} \geq$ 1 μg/ml) against KB cells [99]. An additional norsesterterpene, untenic acid (**84**) was isolated from an Okinawan sponge *Spongia* sp., which activates sarcoplasmic reticulum Ca^{2+}-ATPase [100].



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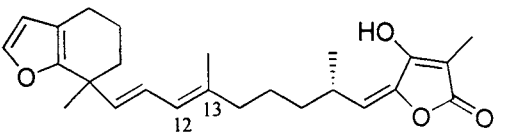
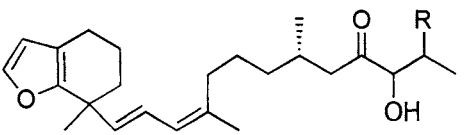
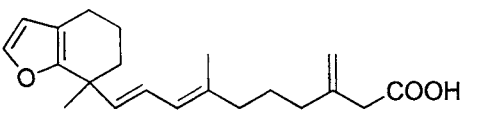


80 $\Delta^{12,13}E$; **81** $\Delta^{12,13}Z$



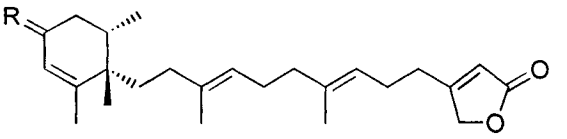
82 R = H; **83** R = Cl



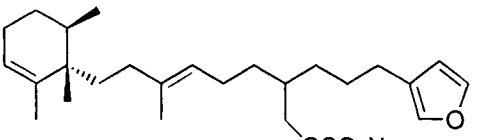
84

From the Caribbean sponge *Cacospongia linteiformis* were isolated cyclolinteinone (**85**) [101] and its 3-deoxy derivative (**86**) [102] with a novel rearranged monocarbocyclic skeleton, 1-alkyl-1,2,6-trimethyl-2-cyclohexene ring system. Both compounds were ichthyotoxic at 10 ppm to *G. affinis*, and showed antifeedant activity at a concentration of 30 μg per cm^2 of food pellets against the fish *Carassius aurantus* [101,102]. Furthermore, cyclolinteinone showed anti-inflammatory activity, inhibiting the nuclear transcription factor-κB binding activity, inducible nitric oxide synthase (iNOS) and cyclo-oxygenase-2 (COX-2) enzymes,

and it was capable of controlling the excessive production of both prostaglandin (PGE2) and nitric oxide (NO) [103].



85 R = O; **86** R = H_2



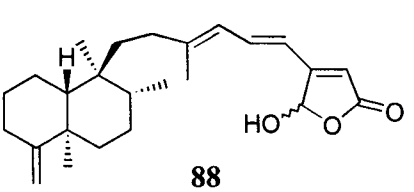
87

Halisulphate 2 (**87**), a sulphated sesterterpene with a monocarbocyclic skeleton related to cyclolinteinone, was isolated from the Californian sponge *Halichondria* sp. [104]. Halisulphate 2 showed anti-microbial activity against *S. aureus, C. albicans* and *B. subtilis* at 20 μg/disk, it inhibited mouse ear oedema after topical application and was an inhibitor of PLA_2 [104].

BICARBOCYCLIC SESTERTERPENOIDS

Sesterterpenoids with a bicarbocyclic skeleton in many instances show structures reminiscent of the clerodane and labdane diterpenoids. Palauolide (**88**), isolated from an unidentified Palauan sponge, is structurally a classical example of clerodane type [105]. From the Palauan sponge *Fascaplysinopsis* sp. was isolated palauolol (**89**) that maybe a biosynthetic precursor of palauolide [106]. Palauolide (**88**) and palauolol (**89**), both containing a functional group γ-hydroxybutenolide ring, inactivate bee venom PLA_2 with 85% and 82% inhibition for **88** and **89**, respectively, at 0.8 μg/ml [27,106], and showed anti-microbial activity against *B. subtilis* and *S. aureus* at 10 μg/disc [105,106]. From the Palauan sponge *Thorectandra* sp. were isolated palauolide (**88**), palauolol (**89**) together with their derivatives, named thorectandrols A-E (**90-94**). Compounds **88-94** were tested for antiproliferative and cytotoxic activities against 12 human tumour cell lines originated from breast, CNS,

colon, lung, ovarian and renal carcinoma, leukaemia and melanoma. Palauolol (**89**) was active in all the cell lines with IC_{50} in the range 0.5-7.0 μg/ml, while palauolide (**88**) showed a decrease in activity in all the cell lines with IC_{50} 7.7-53 μg/ml. Thorectandrols A-D were weakly active with IC_{50} over 30 μg/ml, whereas thorectandrol E was not cytotoxic to any of the cell lines at the maximum dose tested [107,108].



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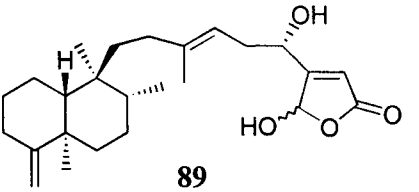

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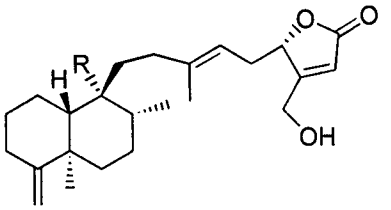
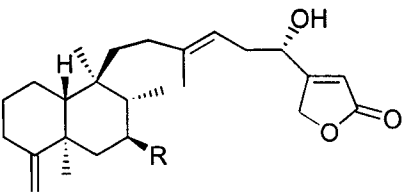
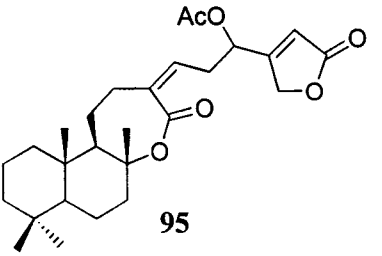


90 R = H; **91** R = OAc



92 R = OAc; **93** R = H
94 R = OH

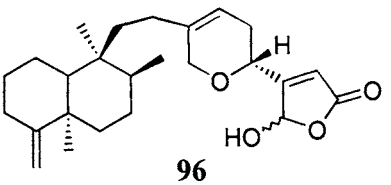


95

Luffalactone (**95**) from the Pacific *Luffariella variabilis* is a sesterterpene with a labdane type skeleton, related to manoalide (**53**). Luffalactone showed 52% inhibition of oedema in the mouse ear assay at 50 μg/ear [87].

In order to find compounds related to cacospongionolide (**155**) (see tricyclic section) [109], we have investigated other Mediterranean horny sponges belonging to the family Thorectidae. From a specimen of *Fasciospongia cavernosa*, we isolated, in good yields, an isomer of cacospongionolide, named after for uniformity cacospongionolide B (**96**)

[110]. Structural differences between the two compounds are due to the absence of the cyclopropane ring and the presence of an exomethylene group. There are two varieties of *F. cavernosa*, one is massive, and the second is encrusted. The massive form is very common along the Adriatic coasts and Aegean Sea, while the encrusted form is distributed in all the Mediterranean Sea. Normally, from specimen of the massive form were isolated only one or two correlated metabolites, while from specimen of the encrusted form were isolated a complex mixture of cacospongionolides: cacospongionolides D (**22**) [47], E (**97**) [111] and F (**98**) [112] that was recently synthesised [113], and related metabolites, such as 25-deoxycacospongionolide B (**99**) [114] and cavernosolide (**151**) [115].



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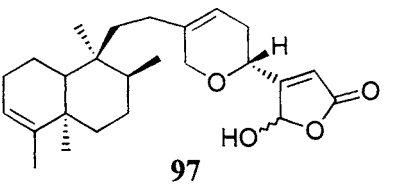

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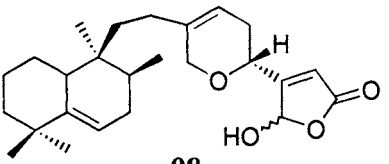

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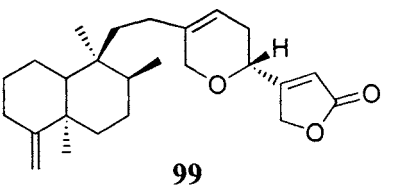


99

The isolation of several related constituents from individual specimens of *F. cavernosa* confirms the peculiarity of the sponges belonging to the family Thorectidae. In fact, similar variation of related metabolites was observed for the sponges *Luffariella variabilis*, *L. geometric* and *Thorectandra excavatus* [5]. The structures of cacospongionolides are similar to that of manoalide (**53**) (see monocyclic section). The differences between the two compounds, apart the non-polar region, are due to the lack of the hydroxyl group at C-24 in cacospongionolide. This lack renders the cacospongionolides more stable than manoalide. Despite the absence of the C-24 hemiacetal function, cacospongionolides showed potent inhibitory activity on recombinant human synovial PLA_2 similar to that of manoalide, while a lower inhibitory activity was shown on other secretory PLA_2s [116].

As cacospongionolide (**155**), cacospongionolide B (**96**) showed a high cytotoxicity (LC_{50} 0.25 μg/ml), in the *A. salina* bioassay. It was moderately ichthyotoxic to *G. affinis* (LC_{50} 1.05 μg/ml) and showed a high antibacterial activity against the Gram-positive bacteria *B. subtilis* and *M. luteus*, with an MIC value of 0.78 μg/ml, comparable with that of gentamycin [110]. Further pharmacological screening revealed that cacospongionolide B is a new inhibitor of PLA_2, preferentially inhibiting the human synovial PLA_2 (IC_{50} 4.3 μM), and pancreatic PLA_2 (IC_{50} 4.0 μM), and its potency on the human synovial enzyme was comparable to that of the reference inhibitor, manoalide (IC_{50} 3.9 μM). This activity was confirmed *in vivo* on a model of chronic inflammation, the established adjuvant-induced arthritis. Cacospongionolide B was less active than indomethacine, an NSAID. Nevertheless, the stomachs of the animals treated with this NSAID showed redness and perforations, while these toxic effects were absent in the rats treated with cacospongionolide B [116]. Furthermore, it has been shown that cacospongionolide B inhibited nuclear factor-*k*B (NF-*k*B)-DNA binding activity and nuclear translocation of this transcription factor. The NF-*k*B pathway has emerged as an important target for the development of drugs against chronic inflammatory disorders and cancer. Moreover, cacospongionolide B is able to downregulate the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), resulting in decreased production of the two important mediators of inflammation process detected in high levels in rheumatoid synovial tissues, nitric oxide (NO) and prostaglandin E_2 (PGE_2). Cacospongionolide B also reduced the mRNA expression of the major factor in the development of chronic inflammatory conditions, tumour necrosis factor- α (TNF-α) [117]. The use of cacospongionolide B as inhibitor of the PLA_2 is covered by patents [118]. Recently, Snapper and co-workers reported the total synthesis of cacospongionolide B and its enantiomer [119]. The examination of $sPLA_2$ inhibition with synthetic variants of cacospongionolide B revealed that the inhibition is enantioselective, i.e. the natural product is a more potent inhibitor of bee venom $sPLA_2$ (IC_{50} 49 μM) than the unnatural enantiomer (IC_{50} 106 μM). Moreover, the inhibition is notable for synthetic precursor possessing the furan group (IC_{50} 76 μM) in place of γ-hydroxybutenolide moiety. These results suggest that the γ-hydroxybutenolide moiety is not the sole structural feature of the natural product involved in $sPLA_2$ inhibition [119].

All cacospongionolides isolated showed more or less similar biological activities. In particular, as anti-inflammatory agents, they preferentially inhibited bee venom and human synovial PLA_2 in the μM range (Table 1). Cacospongionolide E (**97**), however, was the most potent inhibitor towards human synovial PLA_2, showing higher potency than the referenced compound monoalide [111]. Our results confirmed the suggestion [76] that the pyranofuranone part interacts with PLA_2 enzymes, but that the hydrophobic region of the molecule, which can be partially linear (manoalide) or cyclic (cacospongionolides), may facilitate this interaction. These results demonstrate that cacospongionolides are a novel class of marine metabolite inhibitors of PLA_2 with a potent topical anti-inflammatory profile and a high antimicrobial activity and this suggests a potential role of cacospongionolides as drugs.

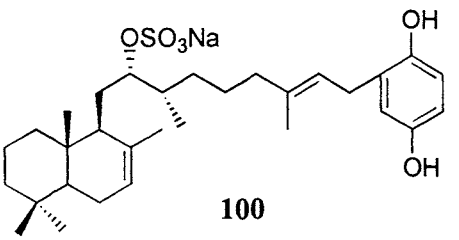
Table 1. Effect of Different Cacospongionolides on a Panel of Secretory PLA_2[a] [111]

PLA_2 Enzymes	*N. naja* venom %I (10 μM)	Pancreas %I (10 μM) IC_{50} (μM)	Human synovial %I (10 μM) IC_{50} (μM)	RAP+zymosan %I (10 μM) IC_{50} (μM)	Bee venom %I (10 μM) IC_{50} (μM)
Cacospongionolide (**155**)	3.5	14.1 N.D.	90.7 3.0	21.8 N.D.	96.3 2.3
Cacospongionolide B (**96**)	0.0	64.2 4.0	86.7 4.3	36.9 7.8	35.4 N.D.
Cacospongionolide E (**97**)	0.0	5.3 N.D.	96.7 1.4	65.1 N.D.	94.8 2.8
Manoalide (**53**)	17.0	32.3 N.D.	93.2 3.9	38.4 N.D.	62.5 7.5

[a] IC_{50} values were determined for those compounds that reach 50% inhibition at 10 μM; N.D. = not determined.

A number of carbobicyclic sesterterpenoid sulphates were found, including halisulfate 1 (**100**), isolated from *Halichondria* sp. [104]; halisulfates 8-10 (**101-103**), isolated from the Australian sponge *Darwinella australensis* [120]; hipposulfates A (**104**) and B (**105**), isolated from the Okinawan *Hippospongia metachromia* [121] and sulfircin (**106**) that was isolated as its N,N-dimethylguanidinium salt, from a deep-sea member of the genus *Ircinia* [122]. Halisulfate 1 (**100**) is an inhibitor of human 12-lipoxygenase (12-HLO) (IC_{50} 1.0 μM) and 15-HLO (IC_{50} 0.9 μM) [123]. 12-HLO is involved in the development of

psoriasis and controlling cancer cell proliferation, while 15-HLO in the development of atherosclerosis and tumourigenesis. Halisulfates 9 (**102**) and 10 (**103**) inhibited cell division of the fertilised eggs of the sea urchin *Strongylocentrotus intermedius* (IC_{50} 50 and 35 μg/ml for **102** and **103**, respectively) [120]. Hipposulfates B (**105**) showed cytotoxic activity with an IC_{50} of 2.0 μg/ml against four human tumour cell lines, A549, P388, melanoma MEL28 and HT29 [121].



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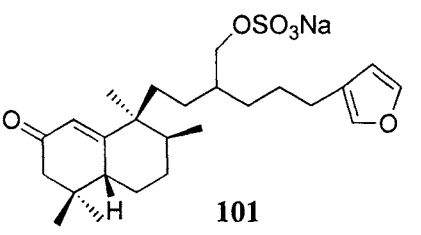

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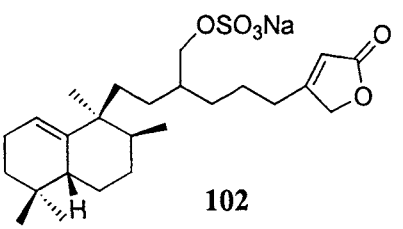

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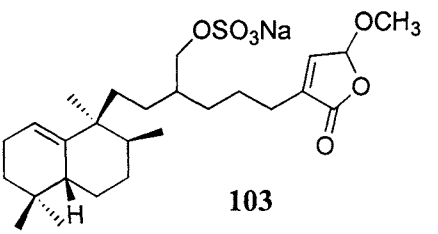

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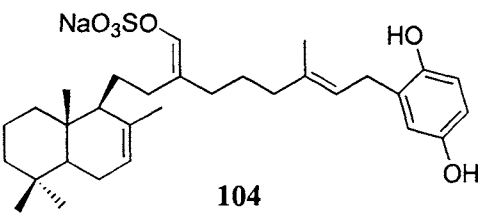

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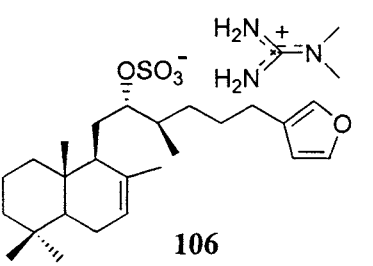

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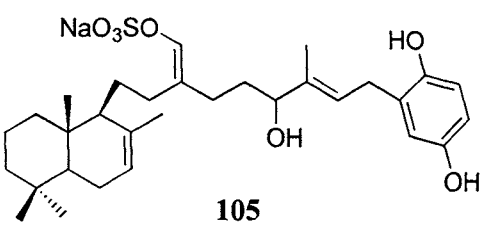
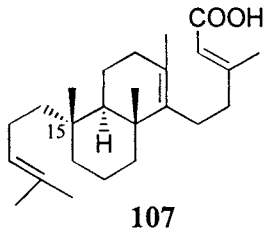


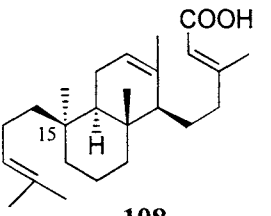
105

Sulfircin (**106**) showed activity against the fungal pathogen *C. albicans* with a MIC of 25 μg/ml [122].

A new class of sesterterpenes in which the middle three units of a penta-isoprenoid chain cyclised into a bicyclic system, leaving the first and the last isoprenoids to substitute the decaline moiety, was isolated from sponges of genus *Dysidea* and *Ircinia*. From the Palauan *Dysidea* sp. was isolated dysideapalaunic acid (**107**) that inhibited the aldose reductase [124]. An inhibitor of aldose reductase is expected to prevent neuropathy or cataract as a complication of diabetes. These diseases are caused by the accumulation of sorbitol in the peripheral nerve or the crystalline lens, as a result of enzymatic reduction of glucose by the aldose reductase in the sorbitol cycle [125,126]. The absolute stereochemistry of dysideapalaunic acid was established by its total synthesis [127,128].



107



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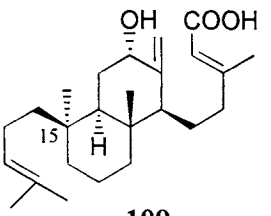

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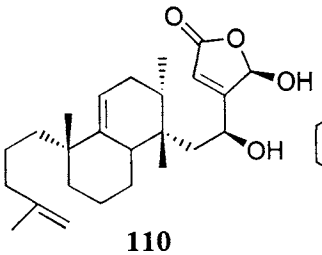

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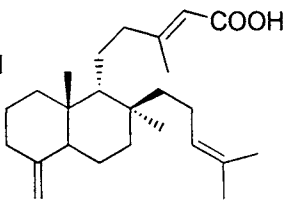
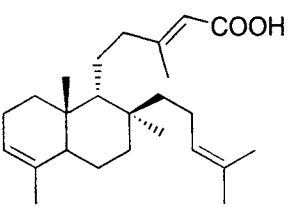


111

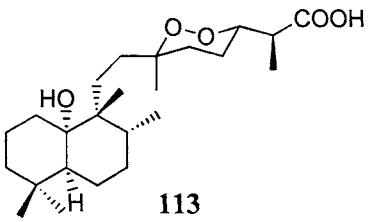


112

Kohamaic acids A (**108**) and B (**109**) were isolated from the Okinawan *Ircinia* sp.. They exhibited cytotoxicity against P388 cells, with IC_{50} values of >10 (32%) and 2.8 μg/ml, respectively. Kohamaic acids are closely related to dysideapalaunic acid (**107**), but they have different stereochemistry at C-15 [129]. Dysidiolide (**110**), isolated from the Caribbean sponge *D. etheria*, is a potent inhibitor of the human cdc25A protein phosphatase (IC_{50} 9.4 μM), a potential target for anticancer therapy. Moreover, dysidiolide inhibited growth of the A549 (IC_{50} 4.7 μM) and P388 (IC_{50} 1.5 μM) cells [130]. The interesting biological activities and the rare structural features of dysidiolide prompted several

researchers to undertake its total synthesis [131-139]. From *D. cinerea* were isolated two new inseparable metabolites, bilosespens A (**111**) and B (**112**). The mixture of both bilosespens showed cytotoxic activity with an IC_{50} of 2.5 μg/ml against four human tumour cell lines (A549, P388, MEL28 and HT29) [140].

Carbobicyclic norsesterterpenoids, containing cyclic peroxides were isolated from four sponge genera, *Mycale, Latrunculia, Sigmosceptrella* and *Diacornis*. From a Thai *Mycale* sp. were isolated two related norsesterterpenoids 1,2-dioxanes, mycaperoxides A (**113**) and B (**114**), which showed significant cytotoxicity (IC_{50} 0.5-1.0 μg/ml) against the cell lines P388, A549 and HT29 and displayed antiviral activity (IC_{50} 0.25-1.0 μg/ml) against vesicular stomatitis virus and herpes simplex virus type-1 [141].



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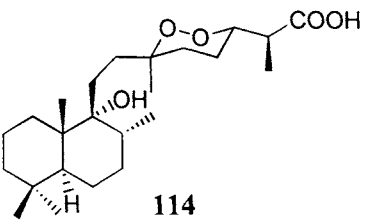
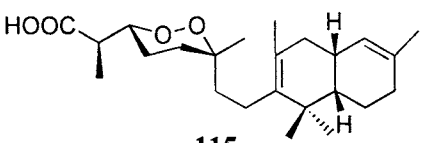


114



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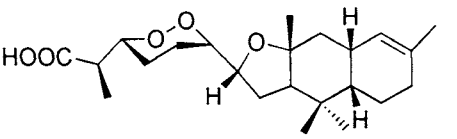
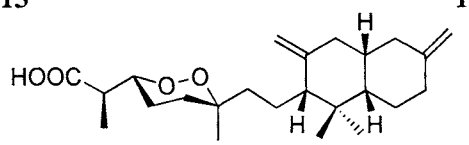


116

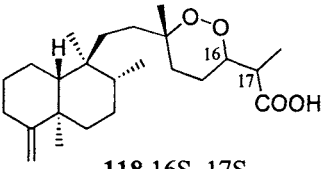


117

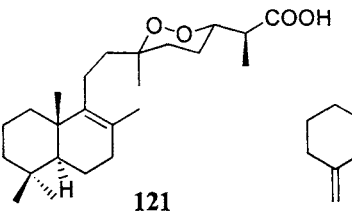
Trunculins A-E are norsesterterpene peroxides isolated from *Latrunculia brevis* [142,143]. Only trunculins A (**115**), B (**116**) [142] and E (**117**) [143] inhibited the growth of *S. aureus, B. subtilis* and *C. albicans* when tested at 100 mg/disk in the standard disk assay. From *Sigmosceptrella laevis* were isolated sigmosceptrellins A-C (**118-120**) that were ichthyotoxic (LD 5 μg/ml) against *Lebistes reticulatus* [144]. Together with *ent*-muqubilin (**72**) and 2-*epi*-muqubilin (**73**) (see

monocyclic section), from the New Caledonian sponge *Diacornus levii* was isolated the antimalarial agent deoxy-diacarnoate B (**121**) [95].

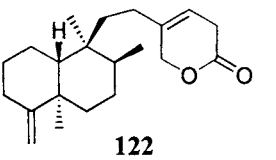
From a specimen of *F. cavernosa* collected in the Aegean Sea, together with cacospongionolides B (**96**) and F (**98**), was isolated a new C_{21} terpene δ-lactone (**122**), closely related to the cacospongionolide B, by the loss of four C atoms, through an oxidative rupture of the γ-hydroxy-butenolide ring [145].



118 16S, 17S
119 16R, 17S
120 16S, 17R



121



122

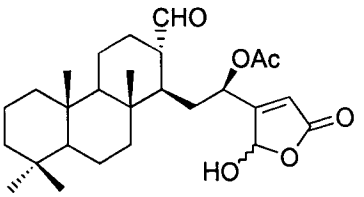
This new compound, named cavernolide (**122**), showed anti-inflammatory activity and exhibited specific inhibition of human synovial PLA_2 in a concentration-dependent manner with an IC_{50} value of 8.8 μM. Cavernolide was less potent in this assay than the referenced inhibitor manoalide (IC_{50} 3.9 μM). In addition, this compound reduces TNF-α production, iNOS and COX-2 expressions [146].

TRICARBOCYCLIC SESTERTERPENOIDS

Marine sponges are a rich source of tricarbocyclic sesterterpenoids with a cheilanthane skeleton, which seems to be derived from geranylfarnesol by a cyclisation initiated at the isopropylidene group that is typical of triterpenes. Luffolide (**123**), an anti-inflammatory compound, is a classic example of this class of compounds. The hydrolysis of phosphatidyl choline by bee venom PLA2 is completely inhibited by luffolide at a concentration of 3.5 μM [147]. Further bioactive metabolites with cheilanthane skeleton were isolated from sponges of genus *Spongia, Cacospongia, Petrosaspongia, Fasciospongia,* and *Ircinia* and from the nudibranch *Chromodoris*.

Spongianolides A-F (**124-129**) possessing a γ-hydroxybutenolide moiety, were isolated from a *Spongia* sp. [148]. The absolute

stereochemistry of spongionolide A was established by its total synthesis [149].



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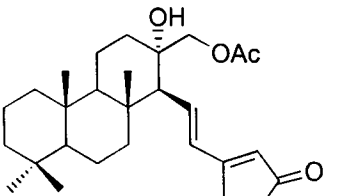
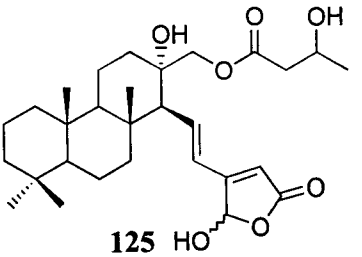
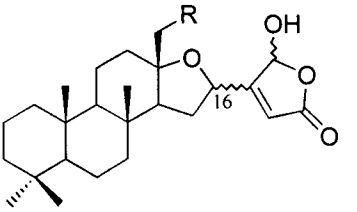


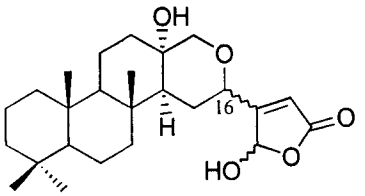
124



125



126 16R, R = $OCOCH_3$
127 16S, R = $OCOCH_3$
128 16R, R = $OCOCH(OH)CH_3$
129 16S, R = $OCOCH(OH)CH_3$
130 16R, R = H
133 16R, R = OH
134 16S, R = OH



131 16R
132 16S

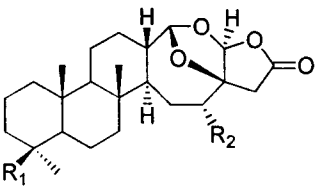
Spongianolides A-E inhibited protein kinase C (PKC) at IC_{50} 20-30 μM, moreover, compounds **124-127** potently inhibited (IC_{50} 0.5-1.4 μM) the proliferation of the mammary tumour cell line MCF7 [148]. Simultaneously, from the Caribbean sponge *Cacospongia linteiformis* were isolated the spongianolides C and D (**126** and **127**) designated as lintenolides A and B, which showed high antifeedant activity against the fish *C. aurantus* (30 μg per cm^2 of food pellets) and ichthyotoxicity to *G. affinis* (10 ppm) [150]. Further, lintenolides C-G (**130-134**) were isolated from the Caribbean sponge *Cacospongia* cf. *linteiformis* [151,152]. All lintenolides A-G inhibited the growth of murine fibrosarcoma WEHI 164, murine monocyte/macrophage J774, bovine endothelial GM7373 and P388 cell lines (Table 2) [152].

Table 2. Cytotoxicity of Different Lintenolides Against a Panel of Tumour Cells [152]

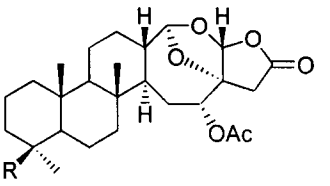
	Mean IC_{50} (μg/ml)			
Cell line:	**WEHI 164**	**J774**	**P388**	**GM7373**
Lintenolide A (**126**)	0.92	0.36	0.098	0.085
Lintenolide B (**127**)	3.1	0.71	0.30	0.34
Lintenolide C (**130**)	50.0	23.4	2.7	0.22
Lintenolide D (**131**)	46.5	10.9	19.0	25.0
Lintenolide E (**132**)	53.3	30.7	125.0	0.021
Lintenolide F (**133**)	8.8	0.94	0.90	1.6
Lintenolide G (**134**)	3.2	1.70	0.037	0.30

From the New Caledonian sponge *Petrosaspongia nigra* were isolated several tricarbocyclic sesterterpenoids petrosaspongiolides A-J (**135-144**) [153,154] and M-R (**145-149**) [155]. From a Vanuatu *Sponge sp.*, a 21-hydroxy derivative of petrosaspongiolide P (**150**) was isolated [156]. All these compounds are biogenetically derived from luffolide (**123**). Petrosaspongiolides A-J exhibited cytotoxicity (IC_{50} 0.5-14.8 μg/ml) against human bronchopulmonary non-small-cell-lung carcinoma cell line (NSCLC-N6) [154]. Petrosaspongiolides M-R (**145-149**) inhibited different preparations of PLA_2 by irreversibly blocking these enzymes, particularly human synovial and bee venom, with IC_{50} values in the micromolar range. These compound displayed a much lower activity (or no activity at all) towards porcine and *Naja naja* PLA_2 enzymes. The most potent compound, petrosaspongiolide M (**145**) (IC_{50} 1.6 and 0.6 μM for human synovial and bee venom PLA_2 enzymes), was slightly more active than manoalide (**53**) (IC_{50} 3.9 and 7.5 μM) under the same experimental conditions. Petrosaspongiolide P (**147**) was more selective, inhibiting human synovial PLA_2 (IC_{50} 3.8 μM) to a greater extent that bee venom PLA_2 (37.9% inhibition at 10 μM) [155]. Furthermore, petrosaspongiolide M was able to reduce in a dose-dependent fashion,

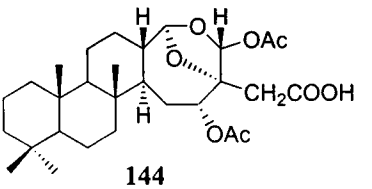
PGE_2, TNFα, LTB_4 levels [157], and it has shown to modulate the expressions of COX-2 and iNOS by interfering with NF-kB [158].



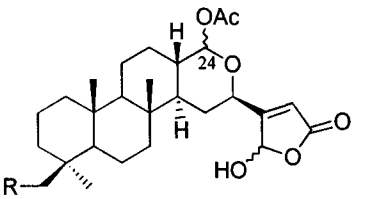
135 $R_1 = CH_3$, $R_2 = OAc$
137 $R_1 = CH_2OAc$, $R_2 = OAc$
138 $R_1 = CH_2OH$, $R_2 = OAc$
139 $R_1 = CHO$, $R_2 = OAc$
140 $R_1 = COOH$, $R_2 = OAc$
141 $R_1 = CH_2OH$, $R_2 = OH$
142 $R_1 = COOH$, $R_2 = OH$



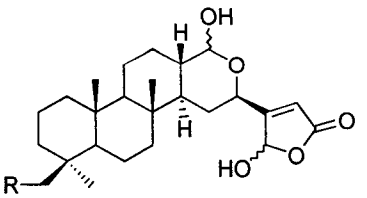
136 R = CH_3
143 R = CH_2OAc



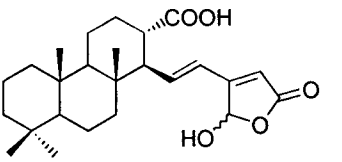
144



145 24S, R = H
146 24S, R = OAc
151 24R, R = H



147 R = H
148 R = OAc
150 R = OH

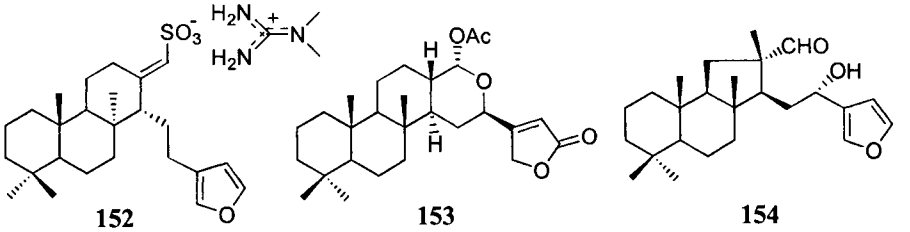


149

Besides, petrosaspongiolide M was capable of reducing the morphine withdrawal at 10^{-8} M [159]. The 21-hydroxy derivative of petrosaspongiolide P (**150**) inhibited human synovial PLA_2 at 10 μM with a value of IC_{50} 5.8 μM, showing a slightly lower potency but higher selectivity towards this enzyme than the referenced inhibitor manoalide [156].

Cavernosolide (**151**), isolated from the Tyrrhenian sponge *Fasciospongia cavernosa*, is the 24 epimer of petrosaspongiolide M (**145**)

and showed high cytotoxicity (LC_{50} 0.37 μg/ml) in the *A. salina* bioassay and a moderate ichthyotoxicity (LC_{50} 0.75 μg/ml) to *G. affinis* [115].



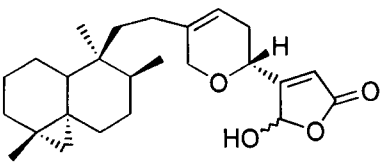
152 **153** **154**

Suvanine (**152**), isolated from the sponge *Coscinoderma mathewsi*, has a cheilanthane skeleton with different stereochemical features and contains both sulphate and furan rings [160-162]. Suvanine was found to facilitate neuromuscular transmission in the indirectly stimulated rat hemidiaphragm preparations. Suvanine was also an acetyl cholinesterase inhibitor, and similar properties were exhibited by the suvanine sodium salt [161]. Besides, the suvanine sodium salt showed antithrombin and antitrypsin activity with IC_{50} of 9 and 27 μg/ml, respectively [162]. Furthermore, suvanine was ichthyotoxic towards goldfish at 10 μg/ml, and exhibited 90% inhibition of sea urchin egg cell division at 16 μg/ml [160]. Inorolide C (**153**) was isolated from the nudibranch *Chromodoris inornata*. It was shown to inhibit the proliferation of KB (IC_{50} 6.4 μg/ml) and L1210 (IC_{50} 1.9 μg/ml) cells [163].

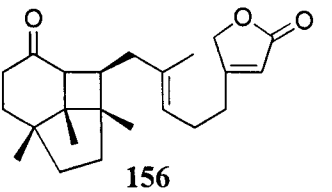
From the Okinawan sponge *Hyrtios erectus* was isolated hyrtiosal (**154**), possessing a novel rearranged tricarbocyclic skeleton (hyrtiosane) [164]. Its structure was confirmed by total synthesis [165]. This compound exhibited *in vitro* antiproliferative activity against KB cells with an IC_{50} of 3.0 μg/ml [164].

In 1988, we reported the isolation and structural elucidation of a new tricarbocyclic sesterterpene [109], bearing a γ-hydroxybutenolide moiety, from the Dictyoceratide sponge, *Fasciospongia cavernosa*, erroneously classified as *Cacospongia mollior*, collected in the North Adriatic Sea. We named this compound after cacospongionolide (**155**), on the basis of the erroneous classification of the sponge [110]. Cacospongionolide was reported as a potent inhibitor of human synovial and bee venom PLA_2 (Table 1) [111]. Besides, cacospongionolide showed high cytotoxic

activity (LC_{50} 0.1 μg/ml), in the *A. salina* bioassay, very high inhibition (75%) in the crown-gall potato disc assay, an antitumoural like test [109].

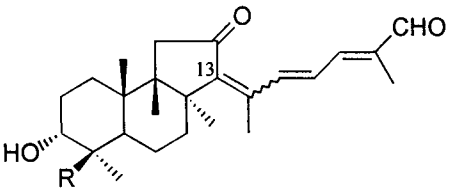


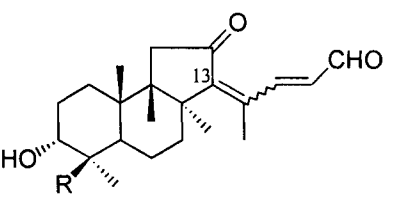
155



156

From the Caribbean sponge *Cacospongia linteiformis* was isolated lintenone (**156**) with a new tricarbocyclic skeleton, which contains fused cyclohexane, cyclopentane and cyclobutane rings. Lintenone exhibited high antifeedant activity against the fish *C. aurantus* (30 μg per cm^2 of food pellets), ichthyotoxicity to *G. affinis* (10 ppm) and moderate toxicity in *A. salina* assay (LC_{50} 109 ppm) [166].





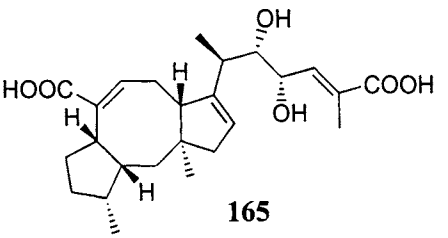
157 R = CH_2OH, $\Delta^{13} = Z$
158 R = CH_2OH, $\Delta^{13} = E$
161 R = COOH, $\Delta^{13} = Z$
162 R = COOH, $\Delta^{13} = E$

159 R = CH_2OH, $\Delta^{13} = Z$
160 R = CH_2OH, $\Delta^{13} = E$
163 R = COOH, $\Delta^{13} = Z$
164 R = COOH, $\Delta^{13} = E$

From the New Caledonian sponge *Rhabdastrella globostellata* were isolated two isomalabaricane sesterterpenes, aurorals 1 and 2 (**157** and **158**) and the corresponding trinor-sesterterpenes aurorals 3 and 4 (**159** and **160**) [167]. From the Okinawan sponges *Rhabdastrella (Jaspis) stellifera* were isolated the corresponding oxidised compounds jaspiferals C-F (**161-164**) [168]. Since jaspiferals C-F were isolated together with the related triterpenes stelliferins A-F [169] and nortriterpenes jaspiferals A-B [168], we can suppose that also aurorals 1-4 and jaspiferals C-F are degraded triterpenoids. Aurorals, which differ from jaspiferals by the presence of a primary alcohol group at C-4 position, exhibited higher

cytotoxic activity on the KB cells. The mixtures of aurorals 1-2 (**157** and **158**) and jaspiferals C-D (**161** and **162**) showed ID_{50} values of 0.2 and 5.5 μg/ml, respectively. The mixtures of aurorals 3-4 (**159** and **160**) showed moderate activity on KB cells with an IC_{50} of 8.0 μg/ml, while jaspiferals E-F (**163** and **164**) were inactive until 10 μg/ml [167]. Furthermore, the mixtures of jaspiferals C-D, and jaspiferals E-F exhibited cytotoxicity against L1210 cells with IC_{50} values of 4.3 and 3.1 μg/ml, respectively [168]. Besides, jaspiferals E-F showed antifungal activity against *Trichophyton memtagrophytes* (MIC 50 μg/ml) [168].

Halorosellinic acid (**165**) possessing an ophiobolane skeleton was isolated from the cultural broth of the marine fungus *Halorosellinia oceanica*. Compound **165** showed moderate antimalarial activity with IC_{50} value 13 μg/ml and weak antimycobacterial activity with MIC 200 μg/ml [170].



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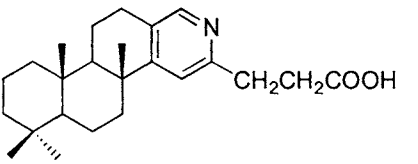

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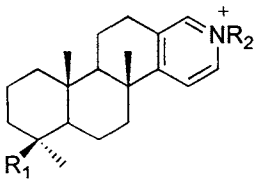
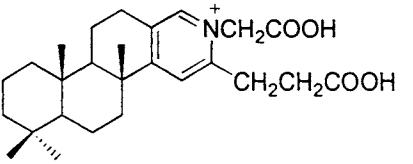


167 $R_1 = CH_3$, $R_2 = CH_2COOH$
168 $R_1 = CH_2OAc$, $R_2 = CH_2COOH$
170 $R_1 = CH_3$, $R_2 = CH_2CH_2SO_3H$



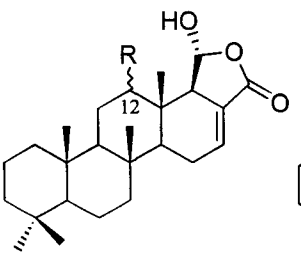
169

From the New Caledonian *Petrospongia nigra*, together with the previously reported petrosaspongiolides A-J (**135-144**) was isolated a pyridium alkaloid 23-norsesterterpene named petrosaspongiolide L (**166**) that showed cytotoxic activity against NSLC-N6 cells with IC_{50} value of 5.7 μg/ml. Petrosaspongiolide L could be considered a condensation product with ammonia of a 16-keto,18-al precursor, derived from petrosaspongiolide K (**209**) (see tetracyclic section) [154]. Four

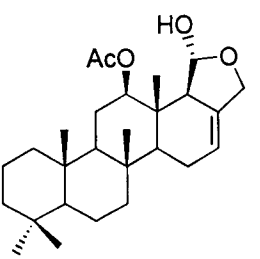
pyridinium alkaloids, spongidines A-D (**167-170**), related to petrosaspongiolide L, were isolated from the Vanuatu *Spongia* sp.. These compounds inhibited mainly the human synovial PLA_2 at 10 μM and they were devoid of significant cytotoxic effect on human neutrophils at concentration up to 10 μM [156].

TETRACARBOCYCLIC SESTERTERPENOIDS

The main group of marine tetracarbocyclic sesterterpenoids is of those with a scalarane skeleton, which appears to be of the same origin as cheilanthane and is formed by closely biosynthetic process involving additional cyclisation. Metabolites of this class have been reported from marine sponges of the order Dictyoceratida and their predator nudibranches [5, 6]. The first example of this group was scalarin (**171**), isolated from the sponge *Cacospongia scalaris* bearing a γ-hydroxybutenolide moiety. [171].



171 R = α-OAc
172 R = β-OAc
173 R = β-OH



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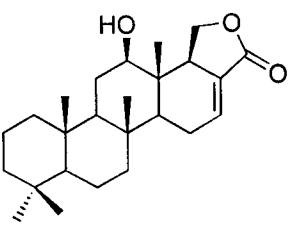
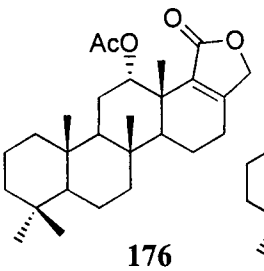


175

A number of 19-deoxy, 20-deoxo, 12-*O*-deacetyl and 12-epimers were isolated [5,6]. From the Japanese *Spongia sp.* were isolated 12-*epi*-scalarin (**172**), 12-*O*-deacetyl-12-epi-scalarin (**173**), 12-*epi*-deoxoscalarin (**174**) and 12-*O*-deacetyl-19-deoxyscalarin (**175**) [172]. These compounds exhibited selective cytotoxicity against four tumour cell lines, being more active on L1210 cell line (IC_{50} 13.2, 2.3, 2.1 and 1.6 μg/ml for **172-175**, respectively) and less active on A549, KB and HeLa cell lines with an IC_{50} of the range 14.3–29.4 μg/ml [172]. 12-*O*-deacetyl-19-deoxyscalarin (**175**), first isolated from the sponge *Hyrtios erecta*, showed also

cytotoxicity against P388 cells with IC_{50} of 2.9 μg/ml [173]. Moreover, compound **175** showed antitumour activity *in vivo* on sarcoma-180-implanted mice with an increase of lifespan (ISL) of 50.3% at 5 mg/kg intraperitoneal administrations. This activity is more potent than of a positive control, 5-fluorouracil (ISL: 32.9%) at the same dose [172]. 12-*Epi*-acetylscalarolide (**176**), isolated from the Spanish *C. scalaris*, showed significant cytotoxic activity towards a panel of four tumour cell lines (Table 3) [174]. 12-*O*-acetyl-16-*O*-methylhyrtiolide (**177**), with an additional methoxy group at C-16 exhibited cytotoxicity against L1210, A549, KB and HeLa cell lines with IC_{50} values of 2.2, 5.3, 15.6 and 5.3 μg/ml, respectively [172].



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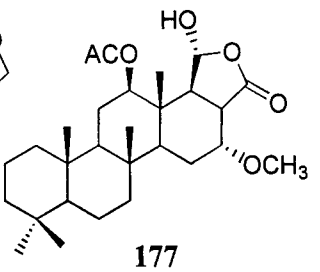

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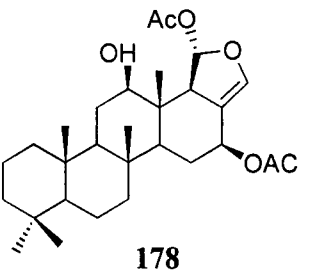
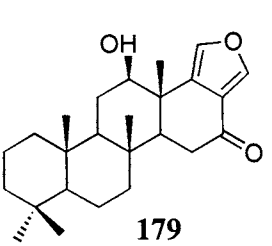


178

Heteronemin (**178**), first isolated from the sponge *Heteronema erecta* [175], was toxic to *A. salina* and gametes of the giant kelp *Macrocystis pyrifera* at 10 μg/ml and also immobilised the larvae of the red abalone *Haliotis rufescens* at 1 μg/ml [22]. Furthermore, heteronemin showed antituberculosis activity, inhibiting the growth of *Mycobacterium tuberculosis* with an MIC of 6.25 μg/ml [176]. Salmahyrtisol B (**179**), isolated from the Red Sea *Hyrtios erecta* [177], is related to scalarafuran (**180**), isolated from *Spongia idia*, a compound toxic to *A. salina* at 10 μg/ml, [22]. Salmahyrtisol B showed cytotoxic activity with an $IC_{50} \geq 1$ μg/ml against P388, A549 and HT29 cells [177].

Generally, scalarane sesterterpenoids are not functionalised on A- and B-rings. A structure-activity study showed that an oxygen-bearing substituent at C-3 of scalaranes, together with the presence of hydroxyl groups at C-12 and C-19, leads to increase of antitumour activity [178]. Accordingly, salmahyrtiol C (3-oxo-12-*O*-deacetyl-12-epi-deoxyscalarin) (**181**), first isolated from the Japanese *H. erecta* [178] and subsequently from the Red Sea *H. erecta* [177], exhibited potent cytotoxicity against P388 (IC_{50} of 14.5 ng/ml) and human gastric carcinoma MNK-1 (IC_{50} of

57.7 ng/ml), MNK-7 (IC_{50} of 56.0 ng/ml) and MNK-74 (IC_{50} of 36.8 ng/ml) cells. Intraperitoneal administration of **181** (0.5-8.0 mg/kg) on mice with P388 leukaemia increased the mean survival time (10.7-15 days) and ISL (24.4-74.4%) dose-dependently [178]. 12-Deacetoxy-21-acetoxyscalarin (**182**), isolated from the Japanese *H. erecta,* showed cytotoxic activity against P388 cells with IC_{50} value of 0.9 μg/ml [179].



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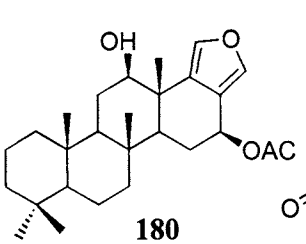

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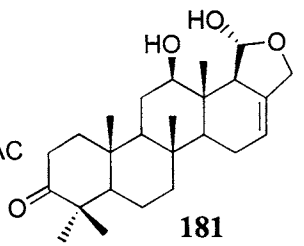

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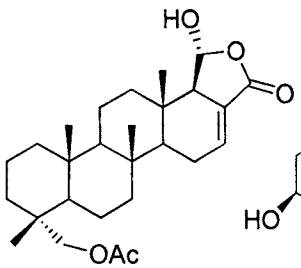

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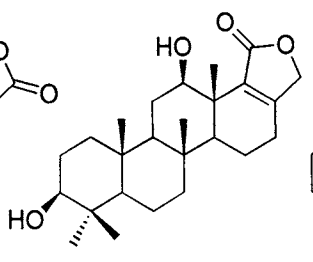

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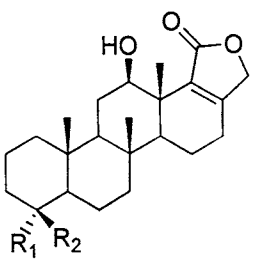
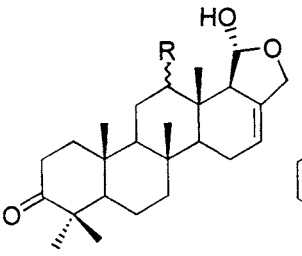


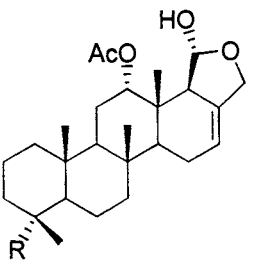
184 $R_1 = CH_3$, $R_2 = CH_2OH$
185 $R_1 = CH_2OH$, $R_2 = CH_3$

From the Maldivian *H. erecta* were isolated sesterstatins 1-3 (**183-185**) that showed cytotoxic activity against P388 cells with IC_{50} value of 0.46, 4.2 and 4.3 μg/ml, respectively [180]. Additional 3- (**186** and **187**) and 19-oxygenated scalaranes (**188** and **189**) were isolated from the nudibranch *Chromodoris inornata* that showed cytotoxic activities against L1210 (IC_{50} 6.6, 0.95, 4.1 and 0.35 μg/ml for **186-189**, respectively) and KB (IC_{50} 22.8, 5.2, 21.0 and 3.1 μg/ml for **186-189**, respectively) cell lines [163]. Scalaradial (**190**) and its 12-deacetoxy derivative (**191**) are two classical examples of compounds with a 1,4-dialdehyde moiety. Scalaradial (**190**) was isolated from two species of *Cacospongia, C. mollior* [181] and *C. scalaris* [174]; 12-deacetoxyscalaradial (**191**) was isolated from *C. mollior* [182]. The majority of terpenoids, containing an unsaturated 1,4-dialdehyde functionality, are intensely pungent [183] and

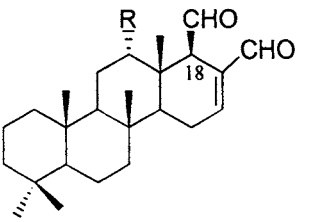
generally are very versatile repellents [184]. This activity was explained by their interaction with vanilloid receptors [185]. However, scalaradial (**190**) was tasteless and showed antifeedant activity at a concentration twice the sesquiterpene polygodial (**192**) [186]. The antifeedant activity of 12-deacetoxyscalaradial (**191**) was similar to that reported for **192**, and moreover 12-deacetoxyscalaradial was hot to the taste. These results showed that the molecular size was not a restrictive factor in these activities and pointed out the specific importance of the substituent at C-12 in **190** and **191**, or in the equivalent C-1 position of a supposed polygodial derivative [182].



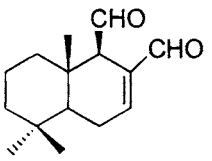
186 R = β-OAc
187 R = α-OAc



188 R = CH_2OH
189 R = CH_2OAc



190 R = OAc
191 R = H
193 R = OH
194 R = OAc, 18-*epi*



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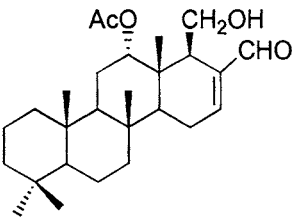

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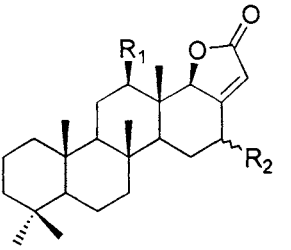


196 R_1 = OH, R_2 = βOH
197 R_1 = OAc, R_2 = βOH
198 R_1 = OH, R_2 = αOAc

In 1991, de Carvalho & Jacobs [187] reported the potent activity of scalaradial (**190**) against bee venom PLA_2 (IC_{50} 0.07 μM). They observed that scalaradial completely inactivated the enzyme by a two-step mechanism, involving apparent non-covalent binding followed by covalent modification. Subsequently, we observed that scalaradial showed

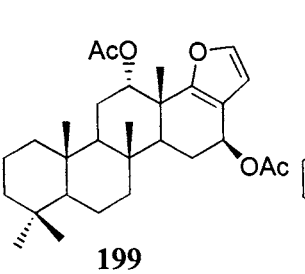
a topical anti-inflammatory activity on ear oedema in mice, with an ID_{50} of 172 μg/ear comparable with that of indomethacine. It is a potent inhibitor of several PLA_2, with a high selectivity for human recombinant synovial PLA_2 (IC_{50} 0.5 μM). Moreover, scalaradial showed cytotoxic effects on human neutrophils at concentrations of 5 μM [26]. Many other scalaranes were screened in the bee venom PLA_2 assay but all showed less activity than scalaradial. From the Japanese *C. scalaris* was isolated deacetylscalaradial (**193**) that showed interesting cytotoxic activity against L1210 cells with an IC_{50} value of 0.58 μg/ml [188]. Scalaradial (**190**) and deacetylscalaradial (**193**) were shown to act on both R- and C-type vanilloid receptors [185]. From the *C. scalaris*, collected in the Southern Coast of Spain, were isolated 18-*epi*-scalaradial (**194**) and 19-dihydroscalaradial (**195**). Both compounds showed significant cytotoxicity towards four tumour cell lines (Table 3) [174].

Table 3. Cytotoxicity of Compounds 176, 194, 195, 199, 206-208 Against a Panel of Tumour Cells [174]

	Mean IC_{50} (μg/ml)			
Cell line:	**P388**	**A549**	**HT29**	**MEL28**
12-*epi*-acetylscalarolide (**176**)	1.0	2.0	2.0	2.0
18-*epi*-scalaradial (**194**)	0.2	0.2	0.2	0.5
19-dihydroscalaradial (**195**)	2.0	2.0	2.0	2.5
16-acetylfuroscalarol (**199**)	2.5	5.0	2.5	10.0
norscalaral A (**206**)	1.0	1.0	1.0	2.0
norscalaral B (**207**)	2.0	2.0	2.0	2.0
norscalaral C (**208**)	1.2	2.5	5.0	2.5

From the Japanese *H. erecta* were isolated two sesterterpenoids (**196** and **197**) [179] related to scalarolbutenolide (**198**), isolated from the Mediterranean *Spongia nitens* [189]. Compounds **196** and **197** were cytotoxic against P388 cells with IC_{50} values of 0.4 and 2.1 μg/ml, respectively [179]. These compounds cannot strictly be considered as

scalarane, because they show different arrangements of the carbons C-24 and C-25. 16-Acetylfuroscalarol (**199**), with moderate cytotoxicity (Table 3), isolated from the Spanish *C. scalaris* [174] and 12-*O*-acetyl-16-*O*-deacetyl-12,16-episcalarolbutenolide (**200**), cytotoxic against L1210 (IC_{50} 2.4 μg/ml) and KB (IC_{50} 7.6 μg/ml) cell lines, isolated from the nudibranch *C. inornata* [163], showed the same carbon skeleton of scalarolbutenolide. From the Indonesian *Phyllospongia* sp. were isolated two sesterterpenes (**201** and **202**), which exhibited cytotoxicity against KB cells at 10 μg/ml [190].



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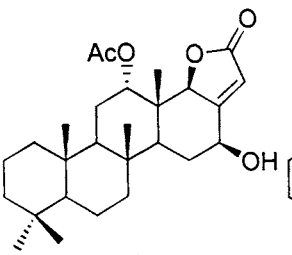

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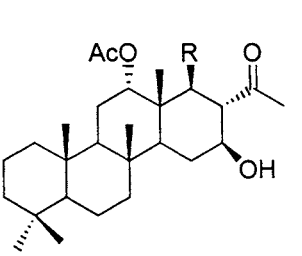
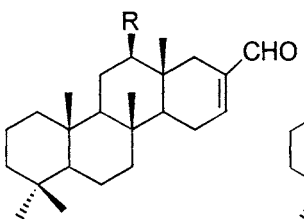
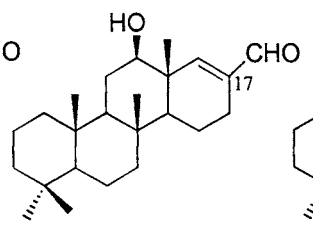


201 R = H
202 R = OH



203 R = OAc
204 R = OH



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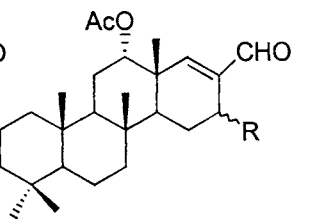
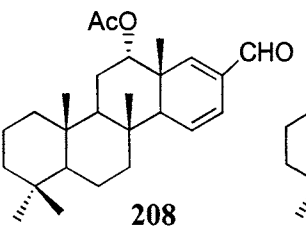


206 R = 16β-OH
207 R = 16α-OH

Tetracarbocyclic norsesterterpenoids are extremely rare and are only isolated from sponge of subclass Dictyoceratida. Hyrtial (**203**), isolated from *H. erecta*, was the first 25-norscalarane to be reported. It showed anti-inflammatory activity at 50 μg/ml close to the activity of indomethacine [191]. From the Okinawan sponge *H. erecta* were isolated 12-deacetylhyrtial (**204**) and its Δ^{17} isomer (**205**) that showed cytotoxic activity against KB cells with IC_{50} values of 10.0 and 2.82 μg/ml, respectively [192]. Norscalarals A-C (**206-208**) isolated from the Spanish *C. scalaris* showed cytotoxicity against four tumour cell lines (Table 3) [174]. Petrosaspongiolide K (**209**), isolated from the New Caledonian

Petrosaspongia nigra, was the first reported 23-norscalarane. Petrosaspongiolide K showed cytotoxic activity (IC_{50} 1.3 μg/ml) against NSCLC-N6 cells [154].

Scalarane sesterterpenoids also include alkylated derivatives, called homoscalaranes with methylations at C-20 or C-24 and bishomoscalaranes with methylations at C-20 and C-24 and rarely at C-23 and C-24 [193].



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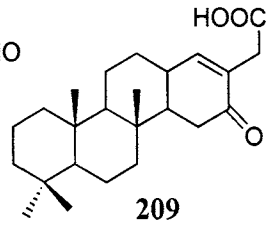

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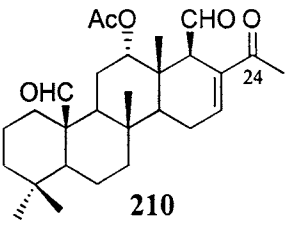
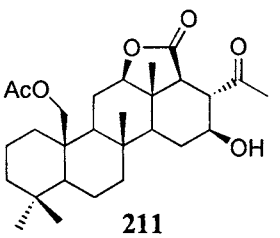


210

A series of 24-methylscalaranes were isolated from the Palauan sponges *Dictyoceratida* sp. and *Halichondria* sp. [194]. Only compound **210** was shown to have significant inhibitory activities (IC_{50} 0.5 μg/ml) on the platelet aggregations caused by adenosine 5'-diphosphate, collagen, or arachidonic acid [194]. Another group of related compounds were isolated from the Australian sponge *Lendenfeldia* sp., as only the compound **211** was the inhibitor of platelet aggregation [195]. Further 24-homoscalaranes were isolated from *L. frondosa*, and only the compound **212** exhibited moderate anti-inflammatory activity, inhibiting 35% of bee venom PLA_2 at 8 μM [196].



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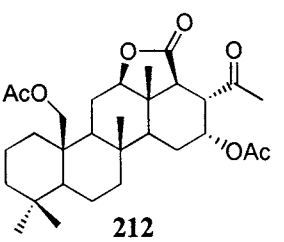

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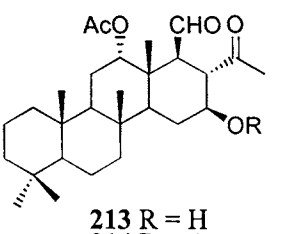
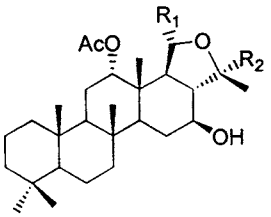


213 R = H
214 R = Ac

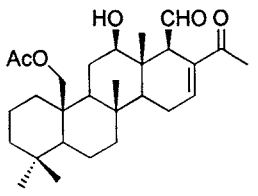
Four 24-homoscalaranes (**213-216**) that exhibited 30-95% inhibition of the growth of KB cells at 10 μg/ml were isolated from the Indonesian *Phyllospongia* sp. [190]. From the Pacific nudibranch *Glossodoris sedna* were isolated several scalarane and homoscalarane compounds, but only

compound **217** was ichthyotoxic at 0.1 ppm against *G. affinis* and inhibited mammalian cytosolic PLA_2 (IC_{50} 18.0 μM) [197].

Foliaspongin (**218**), a 20,24-dimethylscalarane derivative, isolated from the sponge *Phyllospongia (Carteriospongia) foliascens*, showed anti inflammatory activity [198,199].



215 R_1 = OH, R_2 = H
216 R_1 = OMe, R_2 = OMe



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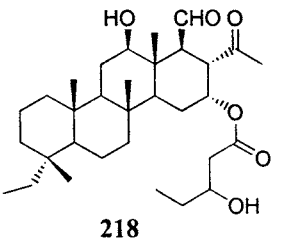

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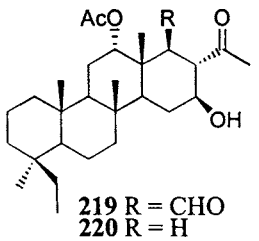
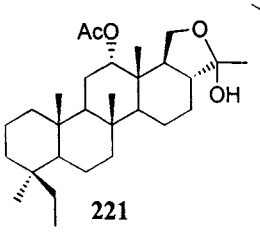


219 R = CHO
220 R = H



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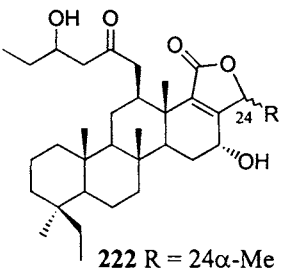
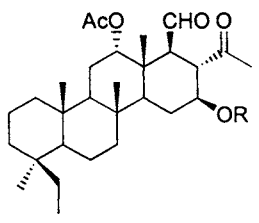
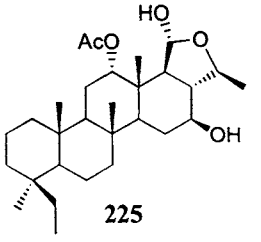


222 R = 24α-Me
223 R = 24β-Me



224 R = H
226 R = $CH_3CHOHCH_2CO$-
227 R = $CH_3CH_2CHOHCH_2CO$-
228 R = CH_3CH_2CO-
229 R = CH_3CO-



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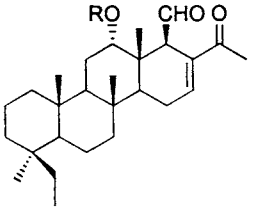


230 R = $CH_3CH_2CH(OCOCH_3)CH_2CO$-
231 R = $CH_3CH_2CH(OCOCH_2CH_3)CH_2CO$-
232 R = $CH_3CH(OCOCH_3)CH_2CO$-

Subsequently, several bishomosesterterpenoids were isolated from *P. foliascens*, collected in different seas. From the Neo Guinean sponge *C. foliascens* were isolated several bishomosesterterpenoids, but only

compounds **219-221** showed ichthyotoxic effects towards *L. reticulatus* at LD_{50} of 5, 20 and 40 mg/l, respectively [200]. Phyllactones A (**222**) and B (**223**), with moderate cytotoxicity against KB cells (IC_{50} 20.0 μg/ml), were isolated from the Chinese *P. foliascens* [201]. From the Indonesian *Phyllospongia* sp. were isolated two 20,24-dihomoscalaranes (**224** and **225**) that showed cytotoxicity against KB cells at 10 μg/ml [190]. From the Australian *Strepsichordaia lendenfeldi*, together with the alcohol **224**, were isolated four different acyl derivatives (**226-229**) and three esters with the same skeleton and different acyl groups (**230-232**). All these compounds exhibited potent cytotoxicity against both P388 and A549 cell lines (Table 4) [202].

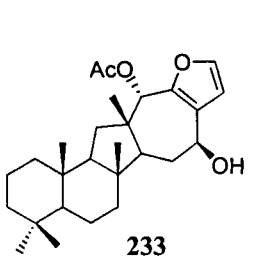
Table 4. Cytotoxicity of Compounds 224, 226-232 Against a Panel of Tumour Cells [202]

	Mean IC_{50} (μg/ml)							
Cell line:	**224**	**226**	**227**	**228**	**229**	**230**	**231**	**232**
P338	0.1	0.23	0.5	0.67	0.91	0.12	0.12	0.2
A549	0.1	0.66	0.5	0.67	0.88	0.25	0.21	0.2

From the Red Sea *Hyrtios erecta*, together with hyrtiosal (**154**), previously reported [164], was isolated salmahyrtisol A (**233**), a furan sesterterpene with a new tetracarbocyclic skeleton. The coexistence of the unusual sesterterpenes **233** and **154** is noteworthy from the biosynthetic viewpoint and maybe hyrtiosal is the logical biosynthetic intermediate for salmahyrtisol A. Salmahyrtisol A showed cytotoxic activity with an with $IC_{50} \geq 1$ μg/ml against three type of cancer cells including P388, A549 and HT29 [177].

Suberitenones A (**234**) and B (**235**), isolated from the Antarctic sponge *Suberites* sp., are two sesterterpenoids with an unprecedented carbon skeleton. Suberitenone B inhibited (IC_{50} 10 μmol/ml) the cholesteryl ester transfer protein (CETP), which mediates the transfer of cholesteryl ester and triglyceride between high-density lipoproteins and low-density lipoproteins. Many studies have found an inverse correlation between levels of high-density lipoproteins and incidence of atherosclerotic cardiovascular diseases. Therefore, CETP inhibition is considered to be a good target for the development of an effective agent against atherosclerotic diseases [203].

From the Japanese nudibranch *Chromodoris inornata* were isolated two sesterterpenes, inorolides A (**236**) and B (**237**) with a new carbon skeleton. Both compounds exhibited cytotoxic activity against L1210 (IC_{50} 1.9 and 0.72 μg/ml for **236** and **237**, respectively) and KB (IC_{50} 3.4 and 2.2 μg/ml for **236** and **237**, respectively) cell lines [163].



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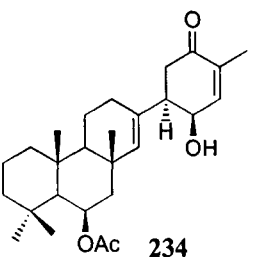

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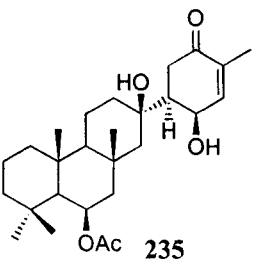

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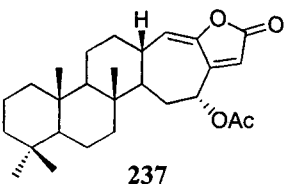

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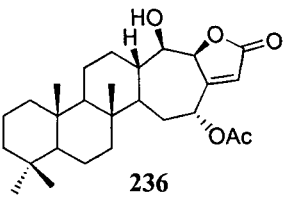

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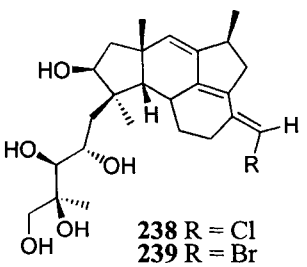
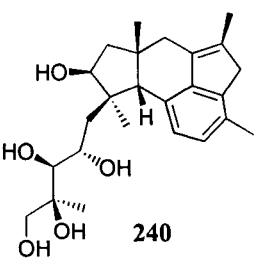


238 R = Cl
239 R = Br



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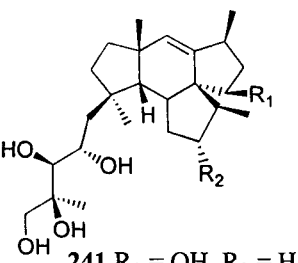
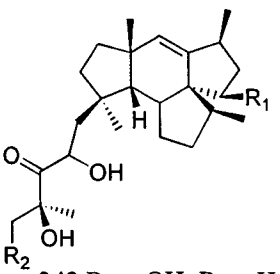


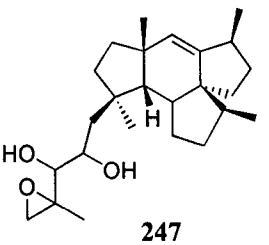
241 R_1 = OH, R_2 = H
242 R_1 = H, R_2 = OH
244 R_1 = R_2 = H

From the marine fungus *Fusarium heterosporum* were isolated two groups of sesterterpenes, neomangicols A-C (**238-240**) [204] and mangicols A-G (**241-247**) [205], both with unusual carbon skeleton that constitutes two new classes of rearranged sesterterpenes. Neomangicols A (**238**) and B (**239**) were found to be active against a variety of cancer cell lines. Neomangicol A was most active against MCF7 and human colon carcinoma CACO2 cell lines, displaying IC_{50} values of 4.9 and 5.7 μM, respectively. Neomangicol B was less active having a mean IC_{50} value of

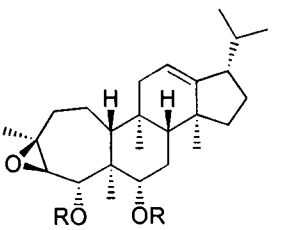
27 μM across the entire panel (versus 10 μM for neomangicol A). Neomangicol B displayed antibacterial activity similar to that of gentamycin, against the Gram-positive bacterium *B. subtilis* [204]. Mangicols A-G (**241-247**) showed weak cytotoxicity with IC_{50} values ranging from 18 to 36 μM in the 60 cell lines panel. Mangicols A and C inhibited mouse ear oedema (81 and 57% reduction in oedema, respectively) at 50 μg per ear. These values are consistent with the potencies of the anti-inflammatory agent, indomethacine [205].



243 R_1 = OH, R_2 = H
245 R_1 = H, R_2 = OH
246 R_1 = R_2 = H



247



248a R = H
248b R = Ac

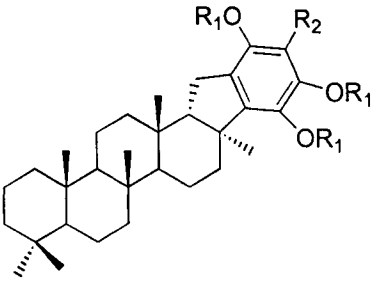
Aspergilloxide (**248a**), a sesterterpene epoxide diol with a new carbon skeleton was isolated from the marine fungus of the genus *Aspergillus*. It showed little cytotoxicity towards HCT116, but its acetate derivative (**248b**) inhibited HCT116 cell line at 61 μM [206].

PENTACARBOCYCLIC SESTERTERPENOIDS

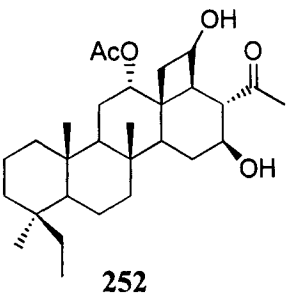
Although numerous marine sesterterpenoids have been found, only a few sesterterpenoids possessing a pentacarbocyclic skeleton have been isolated. Disidein (**249a**) and two halogenated related derivatives (**250a, 251**) were isolated from the Mediterranean sponge *Dysidea pallescens* [207-208]. The stereochemistry of disidein was determined by X-ray analysis of the acetyl derivatives of bromo-disidein (**250b**), which shows the same carbon skeleton of scalarane. The triacetyl disidein (**249b**) showed moderate analgesic activity [55].

From the Neo Guinean sponge *Phyllospongia foliascens*, together with bishomoscalarane derivatives (see tetracyclic section), was isolated a related compound (**252**) with an additional cyclobutane ring. This

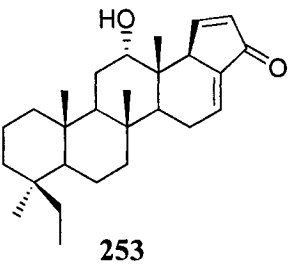
compound showed ichthyotoxic effects towards *L. reticulatus* at the LD_{50} of 5 mg/l [200].



249a $R_1 = R_2 = H$; **249b** $R_1 = Ac$, $R_2 = H$
250a $R_1 = H$, $R_2 = Br$; **250b** $R_1 = Ac$, $R_2 = Br$
251 $R_1 = H$, $R_2 = Cl$



252



253

Phyllofenone B (**253**), an additional bishomoscalarane derivative with a pentacarbocyclic skeleton was isolated from *P. foliascens*. It showed cytotoxicity against P388 cells with IC_{50} value of 5.0 μg/ml [209].

PRODUCTION OF MARINE COMPOUNDS

Although the marine environment is a plentiful source of interesting new products with pharmaceutical potential, only a few of these marine natural products have reached the stage of commercial production. Arabinofuranosyladenine (ara-A, isolated from the Gorgonian *Eunicella cavolini*) [210] is the unique marine secondary metabolite currently in clinical use and is one of most potent antiviral drugs [211]. The second one is avarol, a sesquiterpene hydroquinone isolated from the sponge

Dysidea avara [212,213] currently commercialised as a cream against skin disorder.

Words such as "promising" and "potential" dominate the literature on marine natural products, while papers describing successful application of these products remain scarce. In fact, patent applications are less than 10% of the total number of papers published on marine natural products. The number of patent applications on marine natural products is very little when compared with those of terrestrial origin. The limited availability of larger quantities of a particular organism as starting material for extraction of the compounds is one of the major causes for the low attractiveness of such secondary metabolites for commercial utilisation. Furthermore, the isolation of large quantities of these compounds from animal tissues is unacceptable because of its devastating impact on the natural environment. Four different approaches can be undertaken to obtain bioactive marine secondary metabolites in bulk amounts:

1- Chemical synthesis
2- Aquaculture
3- Cultivation of marine organisms in bioreactor
4- Cell culture.

Chemical Synthesis

Generally, pharmaceutical companies need a strong patent position before starting the long and expensive path, of a drug development, and they prefer compounds that can be synthesised. This approach has successfully been undertaken specially for those compounds with a potential industrial application, but very often, for the high structural and stereochemical complexity of the metabolite, the synthesis includes many steps with low yield and it is not commercially realistic.

Aquaculture

The first attempt for *in situ* aquaculture of commercial marine sponges (bath sponges) was made in Adriatic Sea in 1870, but no detailed statement of the methods employed was reported. Smith [214] first reported the description of cultivation of sponges in the late 19^{th} century. Subsequently, Moore [215] described the procedures for the cultivation of sponges. The technique exploits the capacity of sponges to regenerate them and to form new colonies even only by small fragments. Then, the large-scale commercial sponge aquaculture was developed in several

countries [216-218]. Farming of sponges in a sustainable manner for the production of bioactive compounds has recently been started both in New Zealand [219] and Mediterranean Sea [220].

Cultivation of Marine Organisms in Bioreactor

Aquaculture has the disadvantage that the growth rate of sponges is dependent on *in situ* conditions, which cannot be controlled. Therefore, some researchers have considered the possibility of producing sponge biomass under controlled condition. The main difficulties are the supply of an adequate food source and the accumulation of waste products. Recently, Osinga and co-workers [221] reported growth of the sponge *Pseudosuberites andrewsi* in a closed system, using the microalgae *Chlorella sorokiniana* and *Rhodomonas* sp. as food source. These two microalgae were selected, because it was microscopically observed, on fresh material, that these algae were ingested and digested by the sponge cells. The high growth rates observed for this sponge suggest a promising future for cultivation of sponges in closed systems.

Cell Culture

The high proliferation capacity of sponge cells suggests that it should be easily feasible to establish their cell cultures *in vitro*. Then, in analogy to the production of bioactive metabolites from fungi and bacteria, the production of secondary metabolites will be accomplished in a bioreactor using sponge cells in culture. In the last few years, there has been developed the production of axenic sponges cell culture, but until now, only the maintenance of sponge cells *in vitro* has been achieved [222-224]. Primary cell cultures have been obtained from several sponges, with a low cell density in the cultures. This low proliferation can be explained in the culture condition utilised and/or in the experimental approach to establish the culture condition. The lack of in-depth knowledge of the nutritional requirements of marine sponges maybe one question to settle. Recently, we have reported that by optimising some physical parameters (pH, temperature, light) and supplementing the commercial medium with different compounds, such as cholesterol, fatty acids, glucose, it was possible to promote the sponge cell proliferation [225,226].

It has been observed that the single cells in suspension did not proliferate readily [223], because they loose telomerase activity and hence

their potency for cell division [227]. The formation of multicellular aggregates from dissociated single sponge cells regain telomerase activity, and with this their growth potential. These aggregates were termed primmorphs [228,229].

Another promising method is the fragmentation of intact sponges. Brümmer and co-workers [230] reported the *in vitro* cultivation of sponge fragments without further dissociation and reaggregation. There are same limitations in the cultivation of sponge fragments. In fact, only species with high capability of wound healing can be used for fragmentation [230].

In all methods, cell culture, primmorphs and fragmentation, morphological changes indicate that the culture conditions may not be optimal. Further ecological parameters have to be involved in the optimisation of culture conditions and sponge bioreactor design.

Recent studies have demonstrated the ability of sponge cell cultures to produce secondary metabolites [231,232]. If an appropriate growth medium and bioreactor system for primmorphs can be developed, this system may have promising biotechnological potential.

ACKNOWLEDGMENTS

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