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Steroids

Steroids 64 (1999) 687–714
Review article

Steroids from sponges: Recent reports

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Received 10 December 1998; received in revised form 15 February 1999; accepted 23 February 1999

Keywords: Steroids; Sponges; Polyhydroxysteroids; Sulfate steroids; Marine sterols; Review

1. Introduction

All marine organisms have been proven to be a veritable cornucopia of unusual steroid metabolites, but some believe that marine sponges may provide the most diverse and biogenetically unprecedented array of unconventional steroids in the entire animal kingdom [1]. The steroids isolated from sponges are sometimes very complex mixtures of highly functionalized compounds, many of which have no terrestrial counterpart. Commonly encountered features in these compounds include additional oxygenation of both the nucleus and the side-chain, side-chains extensively modified by alkylation and degradation, and structural modification of the basic carbon skeleton. The occurrence of sulfate esters of polyoxygenated sterols in sponges has also been well documented [2,3].

Unconventional steroids often co-occur with conventional ones and are sometimes present in small amounts; however, many exceptions are reported for some sponges that are found with unusual structures as the predominant steroids rather than cholesterol or the conventional 3β-hydroxy sterols [4–6]. It is, therefore, particularly interesting when a sponge contains unusual steroids in large quantities, as these very likely play a functional (rather than metabolic) role in maintaining the integrity of membranous structures. It has been hypothesized and, to some extent, documented that the uniqueness of sterols in cell membranes of sponges is related to the other membrane components, particularly the phospholipids. These latter compounds seem to have head groups and fatty acids very different from those of higher animals; therefore, the structural modifications exhibited by the sponge sterols may be a sort of structural adjustments for a better fit with other membrane components [7–10].

The highly functionalized steroids have recently attracted considerable attention because of their biological and pharmacological activities. Remarkable examples are herbasterol [11], which is ichthyotoxic, xestobergsterols [12,13], and contignasterol [14], potent inhibitors of histamine release from rat mast cells induced by anti-IgE, and halistanol disulfate B [15], an endothelin-converting enzyme inhibitor. Most of the oxygenated cholesterol derivatives have been shown to exert a cytotoxic activity on human cancer cells lines in vitro. Also, enzymatic transformations leading to secosterols, i.e. ring cleavage products of cholesterol, may result in the formation of products with cell division-inhibitory properties [16]. Finally, the recent discovery of the antiviral properties of sulfated polyhydroxysterols has increased interest in these compounds [17].

Up to the present, several excellent reviews have been published on the structure and distribution of the steroids of marine invertebrates, including the sponges, the last one dating back to 1991 [2]. A study dedicated exclusively to polyoxygenated sterols was also published in 1993 [3]. Furthermore, recent literature in this area demonstrates that marine sponges continue to be a rich source of interesting new steroids.

This review was intended to be a survey on the new spongal steroids recently reported in the literature (1991–1997). The material is organized on the basis of the level of deviation respect to a common steroid compound (e.g. cholesterol); this arrangement led to the following five classes of compounds: (1) polyoxygenated steroids, (2) steroid sulfates, (3) steroids with unconventional side-chain, (4) unconventional nuclei, and (5) miscellaneous. Of course, some compounds may belong to more than one of the above classes; so, the following criteria have been used for their classification. Section 2 includes polyoxygenated steroids with conventional side-chain and nucleus; Section 3 contains all the steroids possessing sulfate functions; Section 4 includes steroids with unconventional side-chain and con-

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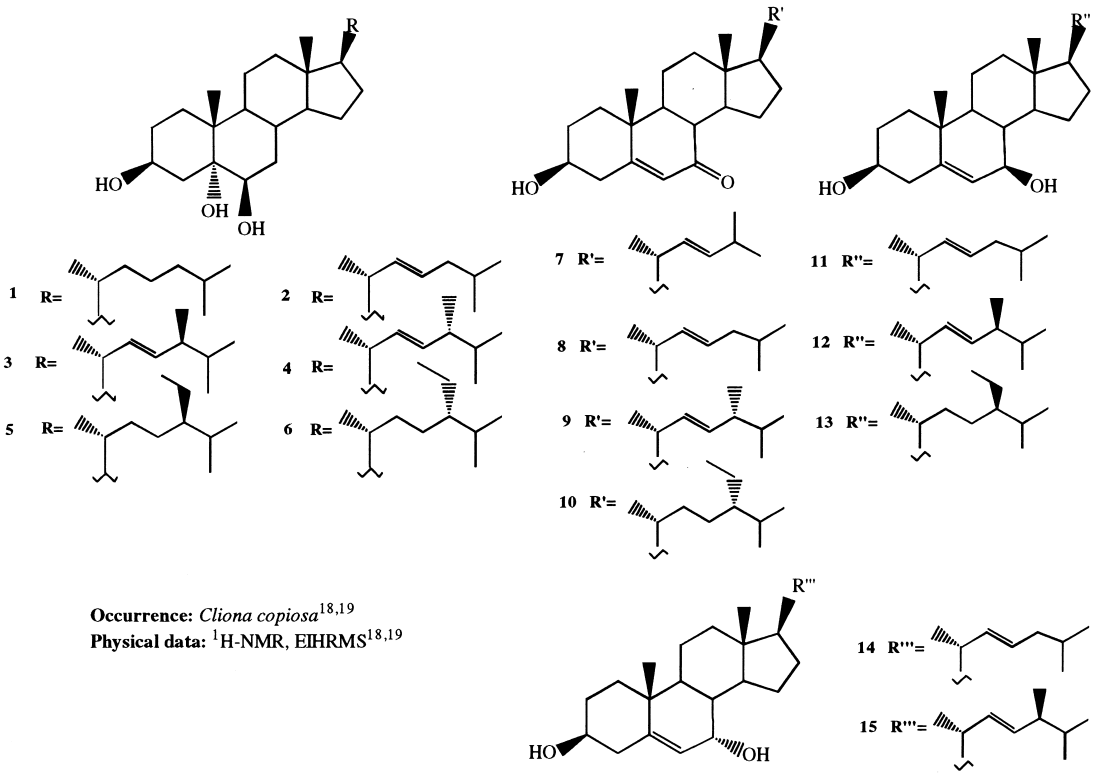
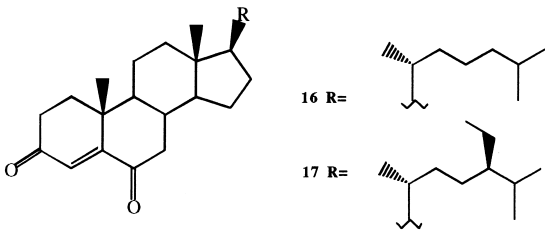


Occurrence: *Cliona copiosa*[18,19]
Physical data: ^{1}H-NMR, EIHRMS[18,19]



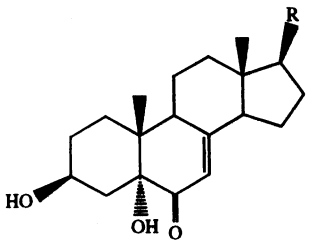
Occurrence: *Cinachyra tarentina*[20]
Physical data: ^{1}H and ^{13}C-NMR (complete assignment)[20]

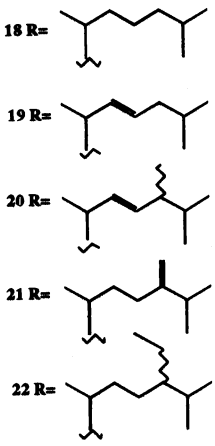
ventional nucleus; and Section 5 reports all the steroids possessing a modified nucleus. Occasionally, a few marine sponges were found to contain atypical steroids, which cannot be included in the above classes; they are reported in Section 6 (miscellaneous) of the present review.

2. Polyoxygenated steroids

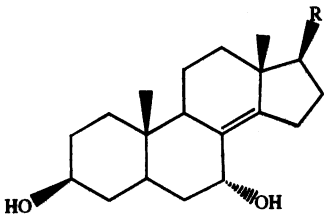
Six new 3β,5α,6β-trihydroxysterols (**1–6**) with a saturated nucleus have been isolated from two different populations of the sponge *Cliona copiosa*, collected from two different sites of the bay of Naples [18]. Partial synthesis of compounds **1**, **5**, and **6** confirmed the structures of these compounds. Successively, several new Δ^5-3β-hydroxy-7-ketosteroids and the related Δ^5-3β,7β-, and Δ^5-3β,7α-dihydroxysterols (**7–15**) have been described as constituent of the same species [19].

Cholest-4-ene-3,6-diones (**16** and **17**) were isolated from *Cinachyra tarentina* [20]. Compound **16** had been previously synthesized starting from cholesterol; structure **17** has been confirmed by its synthesis starting from sitosterol through a Jones oxidation that allowed also to assign the *R* chirality at C-24.

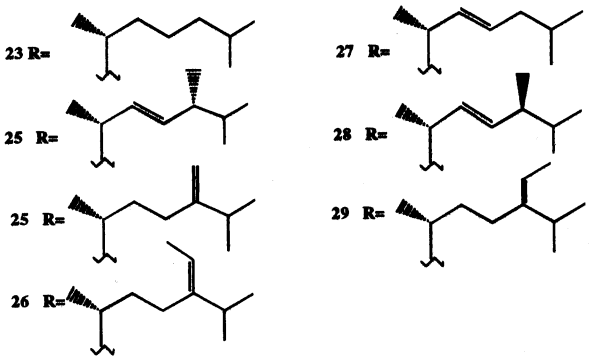




Occurrence: *Oscarella lobularis*[21]
Physical data: ^{1}H-NMR (complete assignment), ^{13}C-NMR of **18**[21]



Occurrence: *Pellina semitubulosa*[23]
Physical data: ^{1}H-NMR[23]

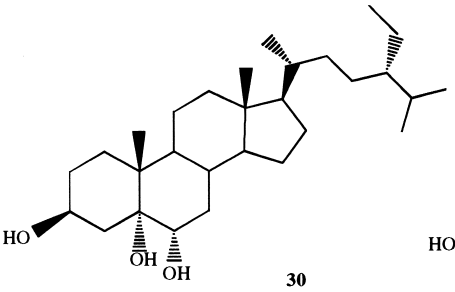


The five novel 5α-hydroxy-6-keto-Δ^{7}-sterols (**18–22**) isolated from *Oscarella lobularis* [21] may be considered the 'missing links' in the hypothesized biosynthetic pathway leading to the incisterols, a class of C_{18} compounds deriving from the biodegradation of the steroidal nucleus, isolated from the sponge *Dyctionella incisa* [22].

Seven new $\Delta^{8(14)}$-3β,7α-dihydroxysterols (**23–29**) were isolated from *Pellina semitubulosa*. Compound **23** has been synthesized starting from 5α-cholest-7-en-3β-ol, following a previously reported synthetic procedure [23].

Two clionasterol derivatives, (24*S*)-24-ethylcholesta-3β,5α,6α-triol (**30**) and (24*S*)-24-ethylcholesta-3β,5α-diol-6-one (**31**), have been isolated from *Spirastrella incostans* [24,25]. The structure **31** has been confirmed by semisynthesis starting from clionasterol.

Extensive investigations performed on *Dysidea* species showed that highly oxygenated sterols are widespread in this genus. All the isolated sterols are characterized by oxygenated functions exclusively on rings A and B, as well as at C-11. Significant variations in the sterol profile have been observed within the genus and even for specimens of the same species coming from different habitats. Sterols **32** through **34**, all bearing a 24-methylene side-chain, have been isolated as their triacetates from *D. herbacea* [26]. The sterol acetates were found to possess antitumor activity against P388 cell line. A novel polyhydroxylated sterol with $\Delta^{8(14),24}$ unsaturations (**35**) has been isolated from a *Dysidea* species [27]. Incrustasterols A and B (**36** and **37**) have been isolated from a sample of *D. incrustans* collected in the Mediterranean sea along the coasts of Tunisia [28]. They



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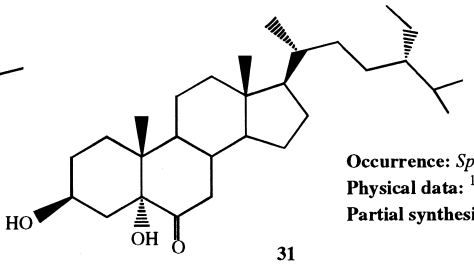

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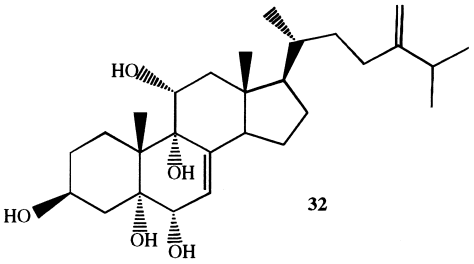
Occurrence: *Spirastrella incostans*[24,25]
Physical data: ^{1}H and ^{13}C-NMR.
Partial synthesis[24,25]



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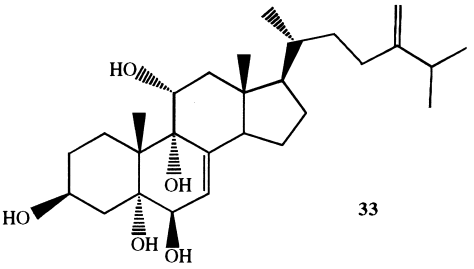

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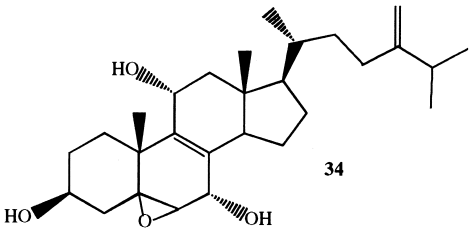

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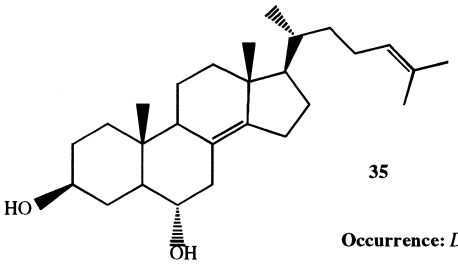
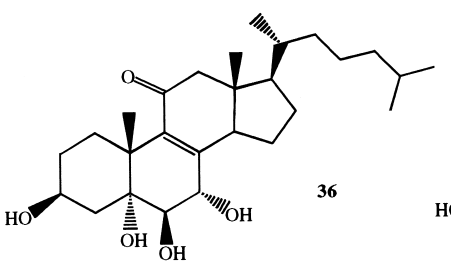
Occurrence: *Dysidea herbacea*[26]
Physical data: ^{1}H and ^{13}C-NMR of their acetyl derivatives[26]



35

Occurrence: *Dysidea* sp.[27]

contain the rare $\Delta^{8(9)}$-11-keto functionality and were shown to be cytotoxic against human non-small-cell lung, renal carcinoma, and melanoma cell lines. Because very limited amounts of the incrustasterols had been isolated from the natural source, their synthesis has been performed starting from the easy available Δ^{7}-cholesterol [29]. A variable sterol pattern has been described for the same species, *D. fragilis*, coming from different geographical areas. Two highly hydroxylated sterols (**38** and **39**) were isolated from the Black Sea sponge *D. fragilis* [30]. Biosynthetic experiments performed on the sponge fed with [4-^{14}C]cholesterol showed that dietary cholesterol suffers biological oxidation leading to **38**. 5α-Cholesta-8(14),24-diene-3β,6α-diol (**40**) has been isolated from a *D. fragilis* collected along the South China coasts [31]. Finally, the steroid composition of *D. fragilis* collected in the lagoon of Venice differed remarkably from the aforementioned specimens of the same species. The lagoonal sample contained large amounts of polar steroids, comprising 13 polyhydroxysterols; eight of them (**36, 88–90**, and **174–177**) were novel compounds; as reported above, compound **36** was independently found as constituent of *D. incrustans* [28]. Compounds **88** through **90**



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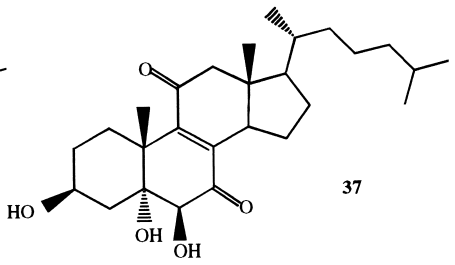
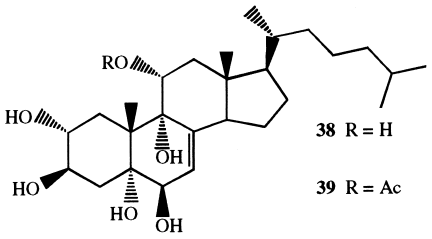


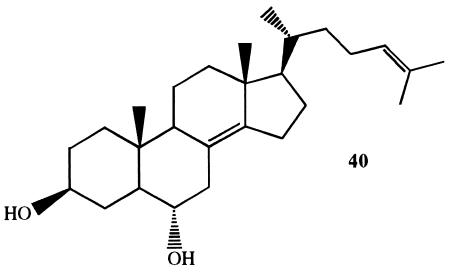
37

Occurrence: *Dysidea incrustans* (**36, 37**)[28], *Dysidea fragilis* (**36**)[6]
Physical data: ^{1}H and ^{13}C-NMR (complete assignment)[6,28]
Synthesis of **36**[29]



38 R = H

39 R = Ac



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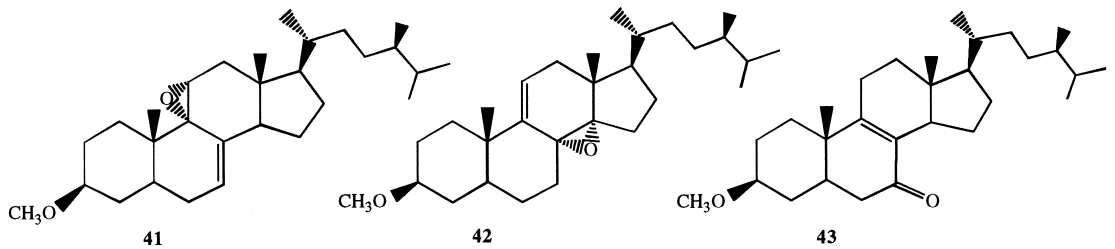
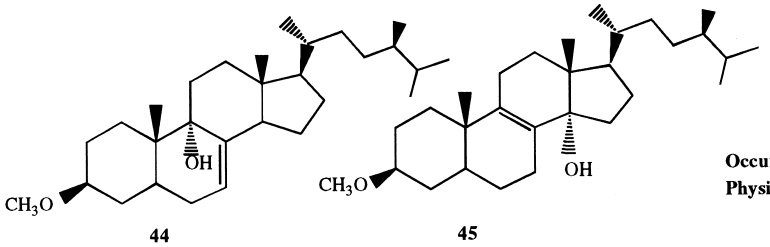
Occurrence: *Dysidea fragilis*[30,31]
Physical data: ^{1}H- and ^{13}C-NMR (complete assignment).[30,31]



41 **42** **43**



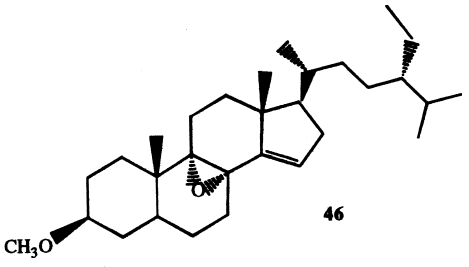
44 **45**

Occurrence: *Jereicopsis graphidiophora*[32]
Physical data: ^{1}H and ^{13}C-NMR[32]

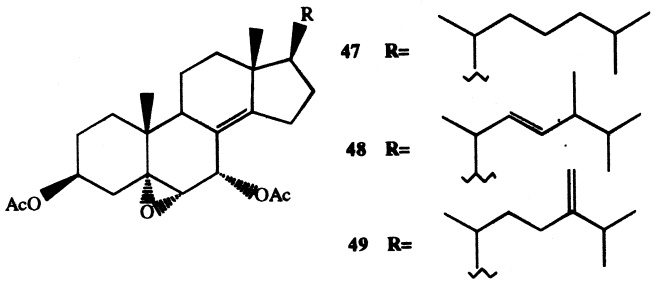
and **174** and **175** are described in sections 3 and 5, respectively; 3β,5α,6β,7α-tetrahydroxy-cholest-8(9)-en-11-one (**36**), was proved to be cytotoxic on two different cell lines in vitro [6].

The lipid fraction of the Pacific sponge *Jereicopsis graphidiophora* yielded several 3β-methoxysteroids, whereas the conventional 3β-hydroxysterols were totally absent; five of them (**41–45**) were novel compounds containing an additional oxygenated function such as an epoxy, hydroxy, or ketone group. Compound **42** is the first finding of a $\Delta^{9(11)}$-8,14-epoxide function in a naturally occurring steroid [32].

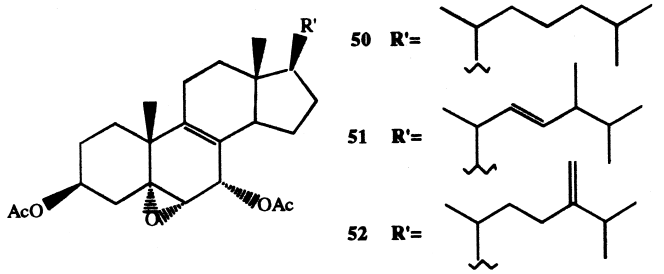
Analogously to *J. graphidiophora*, the Senegalese sponge *Microscleroderma spirophora* elaborates large quantities of 3β-methoxysteroids, whereas no significant amounts of 3β-hydroxysteroids are present in its extract. Six different methoxysteroids have been isolated and three of them (**46**, **166**, and **167**) were novel compounds [33]. Com-



Occurrence: *Microscleroderma spirophora*[33]
Physical data: ^{1}H and ^{13}C-NMR (complete assignment)[33]



Occurrence: *Spongia officinalis*[36]
Physical data: ^{1}H and ^{13}C-NMR; X-ray analysis of **47**[36]



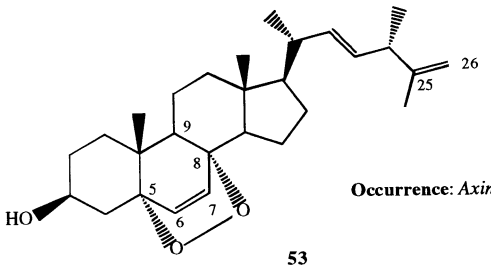
pounds **167** and **168** are described in section 5; **46** contains a double bond at position 14 and a tetrasubstituted oxirane ring located at position 8,9. The occurrence of 3β-methoxysteroids has an interesting chemotaxonomic significance. Recently, Levi suggested to assign the genera *Microscleroderma* and *Jereicopsis* to the same order (Rhizomorina), based on interpretation of their morphologic attributes [34]. In addition to *M. spirophora* and *J. graphidiophora*, only one species of this order, *Aciculites pulchra*, has been analyzed for steroids and resulted to elaborate usual 3β-hydroxysterols [35]. So, the ability of producing methoxysteroids seems not to be a general feature of the order, but appears limited to some species. Therefore, the presence of methoxysterols could be a useful chemical marker for an improved classification of Rhizomorina sponges.

Two groups of new 5α,6α-epoxysterols with an unsaturation either at position 8(14) (**47–49**) or 8(9) (**50–52**) occur in *Spongia officinalis*, which is a rich source of diverse steroidal metabolites. The new products have been isolated as diacetates and their structures determined by spectral analysis; an X-ray diffraction experiment, performed on compound **47,** gave conclusive information on its stereochemistry at C-7 [36]. Successively, compound **47** has been found in the sponge *Ircinia fasciculata* [37].

Axinysterol (**53**), occurring in an Okinawan sponge of the *Axinyssa* genus, is the first example of a 5α,8α-epidioxy sterol with a 24-methyl-22,25-diene system in the side-chain [38]. Its structure has been determined on the basis of spectroscopic evidences and chemical transformation; particularly, the catalytic hydrogenation of axinysterol and ergosterol peroxide gave the same 3β,5α-diol, allowing to confirm the full structure of **53** including the stereochemical details. Two axinysterol analogues, differing only in the nature of the side-chain (**54** and **55**), have been isolated from *Suberites carnosus* along with (22*E*,24*R*)-24-methylcholest-4,8(9),22-triene-3α,7β-diol (**56**) [39].

(24*R*)-24-Methyl-5α-cholest-7-enyl-3β-methoxymethyl ether (**57**), isolated from a deep-water marine sponge *Scleritoderma* sp. cf. *paccardi*, is the first report of a methoxymethyl ether sterol from a natural source. It was shown to be cytotoxic against P-388 tumor cell lines [40].

Three novel sterols with a rare D-ring unsaturation and bearing a 16α-hydroxyl group (**58–60**) have been isolated from the Mediterranean sponge *Topsentia aurantiaca* [41]. Previously, the only marine sterols possessing this unique unsaturation had been isolated from three taxonomically very different species, the Pacific sponge *Homaxinella trachys* [42], the starfish *Echinaster sepositus* [43], and some cultured *Dinoflagellates* [44].



Occurrence: *Axinyssa* sp.[38]

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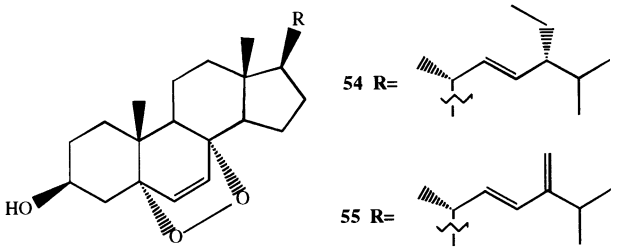

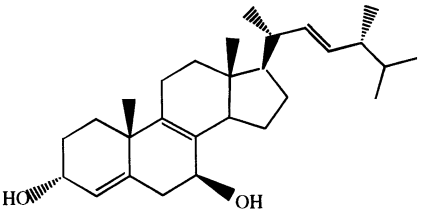

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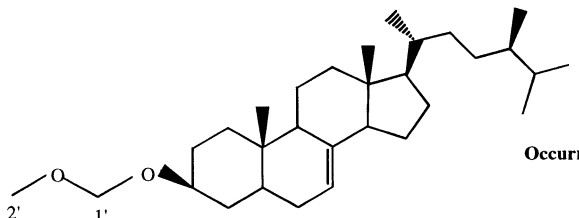
Occurrence: *Suberites carnosus*[39]



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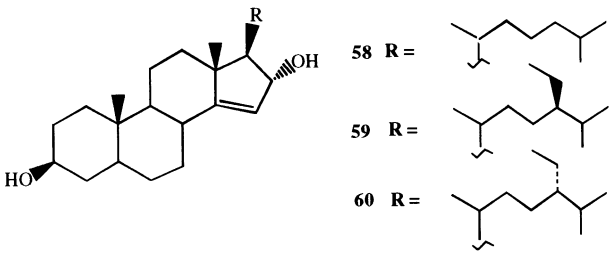
Occurrence: *Scleritoderma* sp. cf. *paccardi*[40]

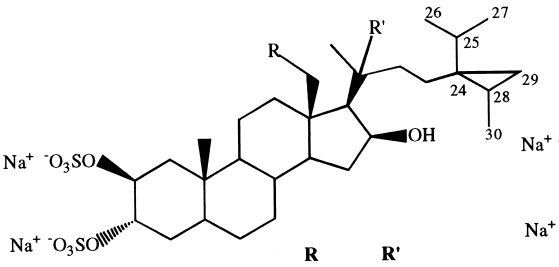


Occurrence: *Topsentia aurantiaca*[41]
Physical data: ^{1}H and ^{13}C-NMR[41]

3. Steroid sulfates

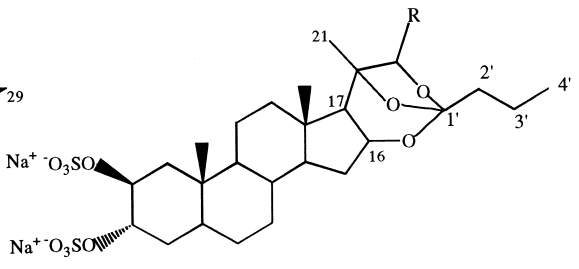
Several sulfated polyhydroxysterols have been recently isolated from sponges. Most of these natural products are characterized by the 2β,3α,6α-tri-*O*-sulfate functions together with additional alkylation in the side-chain. These steroids are of interest not only because of their structures but also because of their physiological activities, which include an anti-human immunodeficiency virus (HIV) effect and a high inhibitory action on some enzymes.

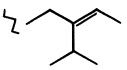
Weinbersterols A and B (**61** and **62**) and orthoesterol disulfates A, B, and C (**63–65**) are new antiviral steroid sulfates isolated from *Petrosia weinbergi* [45,46]. They exhibited in vitro activity against the feline leukemia virus (FeLV), mouse influenza virus (PR8), and mouse coronavirus (A59). Weinbersterol sulfates A and B are the second group of steroids found to contain this unusual cyclopro-

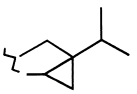


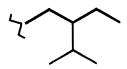
	R	R'
61	H	OH
62	OH	H

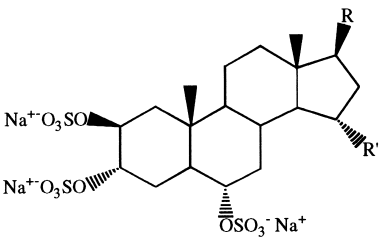
Occurrence: *Petrosia weinbergi*[45,46]
Physical data: ^{1}H and ^{13}C-NMR, HRFABMS[45,46]



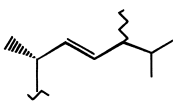
63 R= 

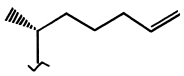
64 R= 

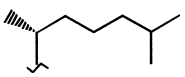
65 R= 

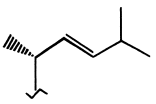


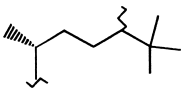
Occurrence: *Epipolasis* sp.[48]
Physical data: ^{1}H and ^{13}C-NMR (complete assignment)[48]

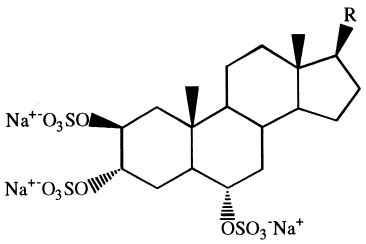
66 R=  **R'=H**

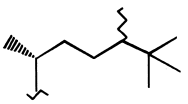
67 R=  **R'=H**

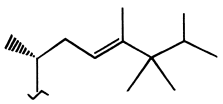
68 R_1=  **R'=H**

69 R=  **R'=H**

70 R=  **R'=OH**

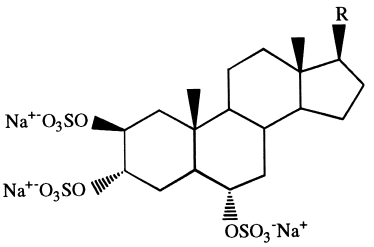


Halistanol R= 

Sokotrasterol R= 

pane-containing side-chain and the first from a sponge. Compound **61** is also active in vitro against HIV.

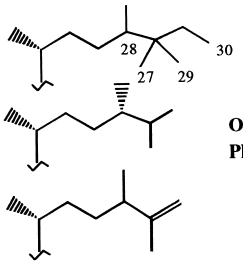
Halistanol sulfate, present in *Halichondriidae* sponges and characterized by the 2β,3α,6α-trisulfoxy functionalities, is the first example of sulfated sterol isolated from Porifera [47]. Successively, several new halistanol sulfate analogues, differing only in their side-chains, have been reported. Halistanol sulfates A through E (**66–70**) have been isolated from *Epipolasis* sp. together with halistanol sulfate, whose absolute stereochemistry was determined by



71 R=

72 R=

73 R=



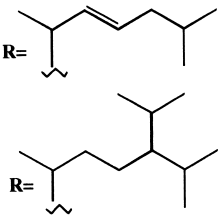
Occurrence: *Pseudoaxinyssa digitata*[49]
Physical data: ^{1}H and ^{13}C-NMR[49]

74 R=

75 R=

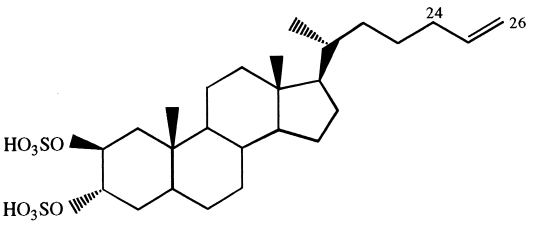
76 R=

77 R=

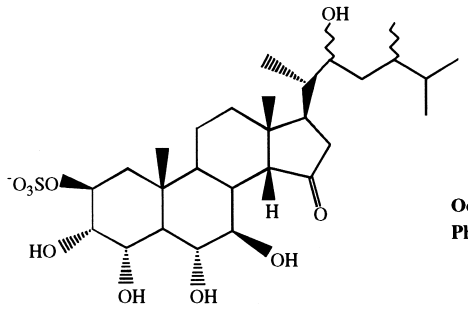


Occurrence: *Trachyopsis halicondroides, Cymbastela coralliophila*[50]
Physical data: ^{1}H and ^{13}C-NMR, EIMS[50]

Occurrence: *Pachastrella* sp.[15]
Physical data: $^{1}H^{13}C$-NMR (complete assignment)[15]



78



Occurrence *Haliclona* sp.[52]
Physical data: ^{1}H and ^{13}C-NMR (complete assignment)[52]

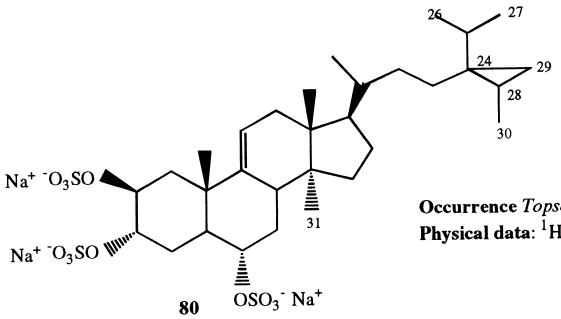
79

application of the modified Mosher method on the 2,3-diol system generated by acid hydrolysis [48]. Halistanol sulfates F through H (**71–73**) have been isolated from *Pseudoaxinissa digitata* [49]; they were proved to be cytoprotective against HIV. Four new steroids (**74–77**), all possessing identical nuclei containing the 2β,3α,6α-trisulfoxy substitution, have been isolated from *Trachyopsis halichondroides* and *Cymbastela coralliophila* [50]. Besides, halistanol sulfate was found to be the main steroid constituent of *C. coralliophila*, whereas the known sokotrasterol sulfate was predominant in *T. halichondroides* [51].

Halistanol disulfate B (**78**), present in *Pachastrella* sp., was found to be active at a micromolar concentration in the endothelin-converting enzyme assay [15].

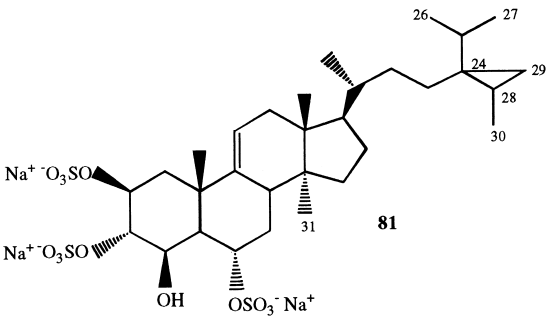
Haliclostanone sulfate (**79**) and, once more, halistanol sulfate have been isolated from a *Haliclona* sponge [52]. Compound **79** is unique in that it is a member of the rare class of naturally occurring sterols sharing a *cis* C/day ring junction, a 14β proton, and a C-15 ketone. This is the first case of isolation of halistanol sulfate from a *Haposclerida* sponge rather than a *Halichondrida* or *Axinellida* sponge.

A further member of the halistanol series is ibisterol sulfate (**80**), discovered from a *Topsentia* species [53]. It combines a $\Delta^{9(11)}$ olefin and a methyl group at C-14 with the 2β,3α,6α-trisulfoxy functionalities and a cyclopropane-containing side-chain. The presence of a $\Delta^{9(11)}$ double bond with methyl substitution at position 14 is inter-

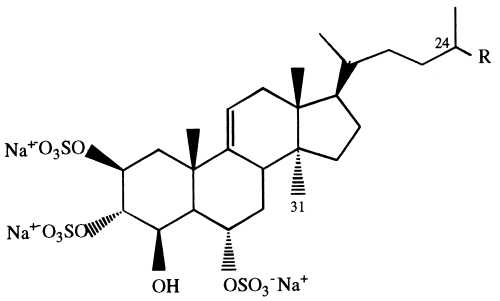


80

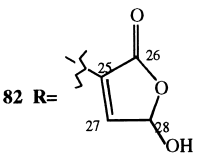
Occurrence *Topsentia* sp[53]
Physical data: ^{1}H and ^{13}C-NMR (complete assignment)[53]

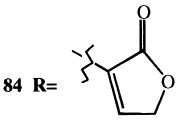


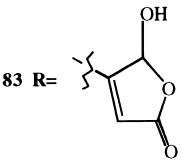
81

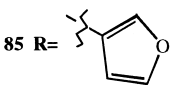


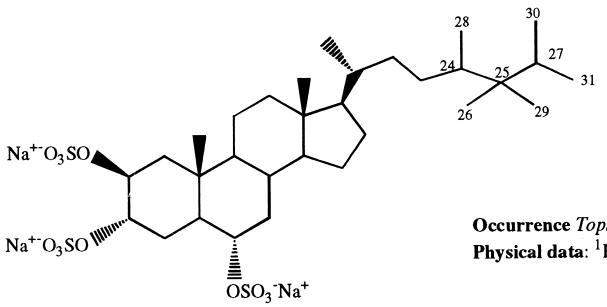
Occurrence *Topsentia* sp.[56]
Physical data: ^{1}H and ^{13}C-NMR (complete assignment); HRFABMS[56]









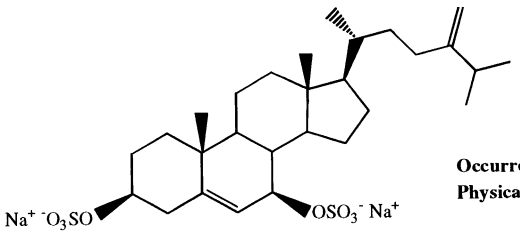


86

Occurrence *Topsentia ophiraphidites*[57]
Physical data: ^{1}H and ^{13}C-NMR[57]

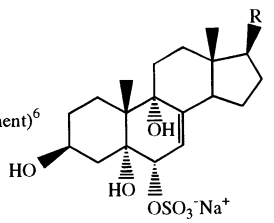
esting from a biosynthetic point of view. This pattern is quite rare, having been observed among the marine organisms only in sterols from olothurians [54]. It, according to Cordeiro and Djerassi [55], is biosynthesized in sea cucumber directly from parkenol ($\Delta^{9(11)}$ isomer of lanosterol), rather than from lanosterol or cycloartenol. However, this pathway has not been observed in sponges and, therefore, ibisterol sulfate could be a target for new biosynthetic studies [53].

The 4β-hydroxy derivative of ibisterol (topsentiasterol E; **81**) has been isolated from another *Topsentia* species together with four compounds (topsentiasterol sulfates A–D; **82–85**) that represent the first isolation of polysulfated steroids possessing a butenolide or a furan functionality at the end of the



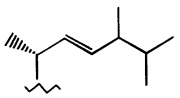
87

Occurrence *Poecillastra laminaris*[58]
Physical data: ^{1}H and ^{13}C-NMR[58]

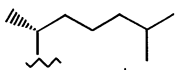


Occurrence *Dysidea fragilis* sp.[6]
Physical data: ^{1}H and ^{13}C-NMR (complete assignment)[6]

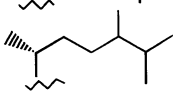
88 R=

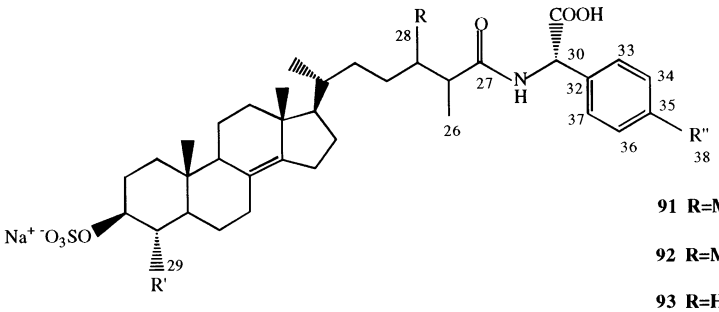


89 R=



90 R=





Occurrence: *Polymastia boletiformis*[59,60]
Physical data: HREIMS, ^{1}H and ^{13}C-NMR (complete assignment)[59,60]

91 R=Me R'=Me R''=OMe $\Delta^{25,26}$

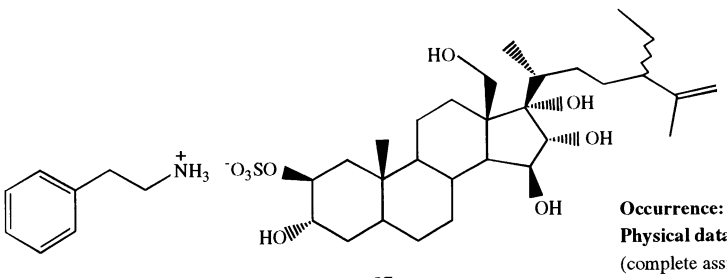
92 R=Me R'=H R''=OMe $\Delta^{25,26}$

93 R=H R'=Me R''=OMe $\Delta^{24,25}$

94 R=H R'=H R''=OMe $\Delta^{24,25}$

95 R=Me R'=Me R''=H $\Delta^{25,26}$

96 R=H R'=Me R''=H $\Delta^{24,25}$

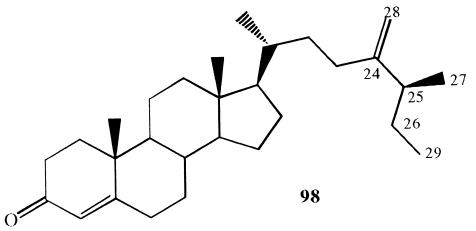


97

Occurrence: *Echinoclathria subhispida*[61]
Physical data: HRFABMS, ^{1}H and ^{13}C-NMR (complete assignment)[61]

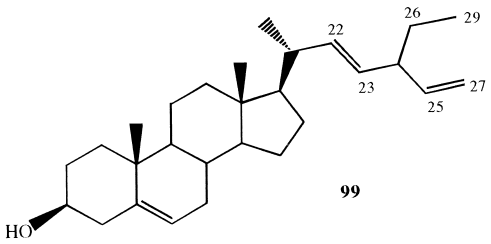
side-chain [56]. Topsentiasterols A–E were shown to be antibacterial against *Pseudomonas aeruginosa* and *Escherichia coli*, but only topsentiasterols D and E exhibited antifungal activity against *Mortierella ramannianus* and *Candida albicans*. Ophirapstanol trisulfate (**86**), isolated from *Topsentia ophiraphidites*, is the 23,24-dihydro derivative of sokotrasterol. It exhibited significant inhibition in the guanidine diphosphate/G protein RAS exchange assay [57].

Annasterol sulfate (**87**) is the first representative of a new structural series of spongal steroid sulfates. It has been isolated from the sponge *Poecillastra laminaris*, collected at a depth of 750 m, and is the first naturally occurring derivative of 3β,7β-O-disulfated diols of the ergostane series



98

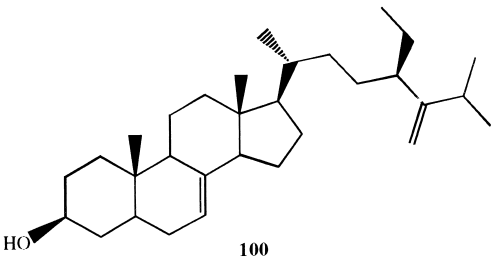
Occurrence: *Neosiphonia supertes*[64]
Physical data: ^{1}H and ^{13}C-NMR[64]



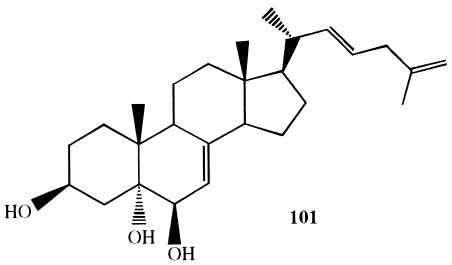
99

Occurrence: *Baicalospongia bacilifera*[65]
Physical data: EIMS,^{1}H-NMR[65]

Occurrence: *Xestospongia* sp[5]
Physical data: HREIMS,^{1}H and ^{13}C-NMR, X-ray studies[5]



100



101

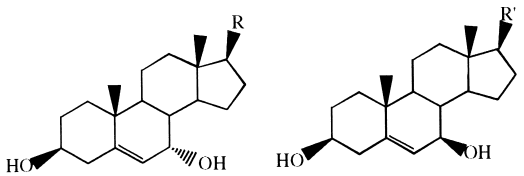
Occurrence: *Bienma* sp[66]
Physical data: HRFABMS,^{1}H and ^{13}C-NMR[66]

[58]. This compound inhibits the enzyme endo-β-1,3-glucanase activity.

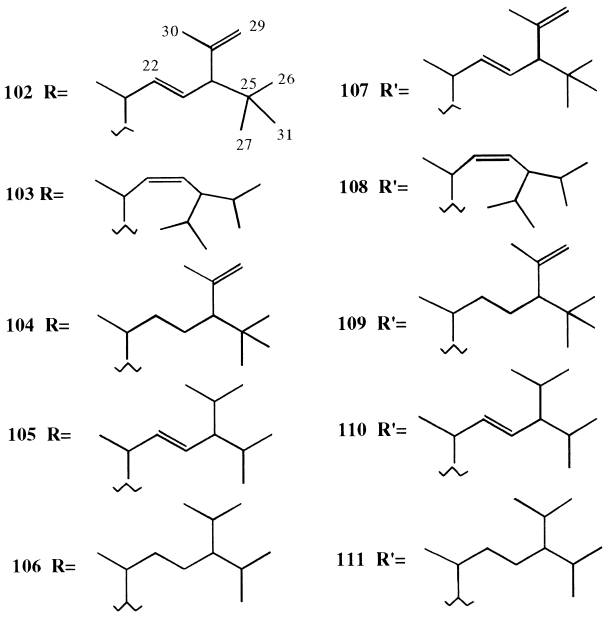
A 6α-sulfoxy function characterizes the steroids **88** through **90,** isolated from *Dysidea fragilis* collected in the lagoon of Venice, which were proved to be cytotoxic on two different tumor cell lines in vitro [6].

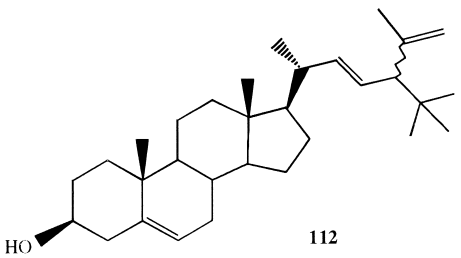
Polymastiamides A through F (**91–96**) are 3β-*O*-sulfated sterols occurring in the sponge *Polymastia boletiformis* [59, 60]. The minor polymastiamides B through F were initially obtained as an inseparable mixture because of the extreme polarity of these molecules; once the structure of the first isolated polymastiamide A was determined and the presence of the sulfate and carboxylic acid functionalities revealed, they were isolated as their desulfated methyl esters after acidic methanolysis. During this treatment, polymastiamides were found to undergo double-bond migration to give the Δ^{14} artifacts. The interesting side-chain modification in the polymastiamide series, which involves the linkage to a nonprotein amino acid via an amide bond, is without literature precedent.

Sulfated steroids are usually isolated as sodium or potassium salts. Bioassay-guided fractionation of the water-soluble portion of the MeOH extract of *Echinoclathria subhispida*, collected in South Australia, afforded a unique salt of a novel polyoxygenated sterol sulfate, echinoclasterol sulfate (**97**) [61]. It contains a phenethylammonium ion as a counter ion, which is the first example for marine natural products. Echinoclasterol sulfate is a hexahydroxy sterol, rarely encountered among sponge steroidal metabolites; the OH group at C-17 is particularly noteworthy. The compound exhibited antifungal activity

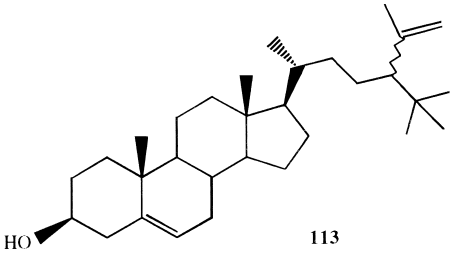


Occurrence: *Topsentia* sp.[69]
Physical data: HREIMS,[1]Hand [13]C-NMR[69]





112



113

Occurrence: *Epipolasis* sp.[70]

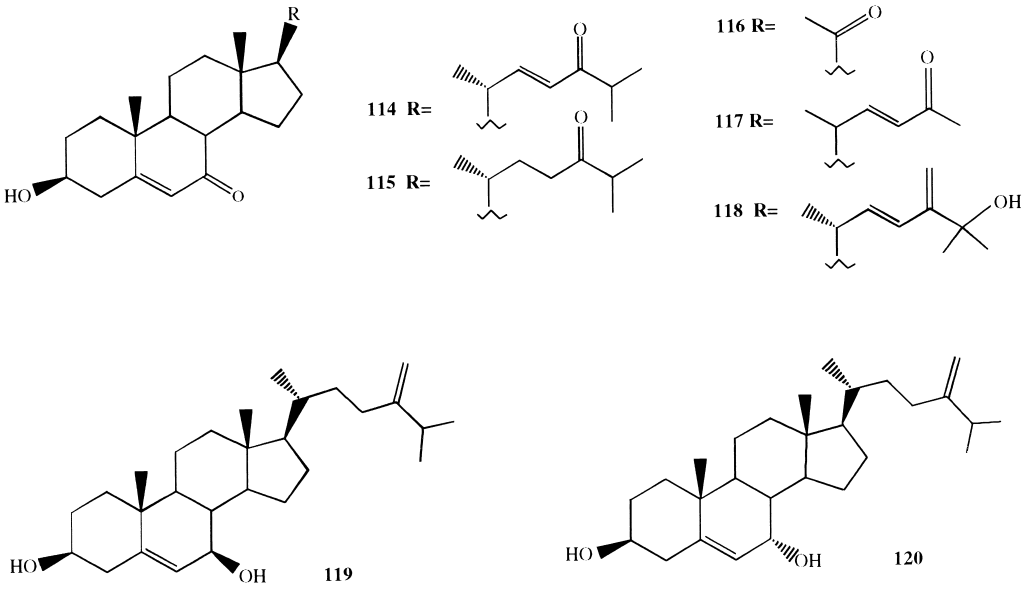
against *Mortierella ramannianus* and moderate cytotoxicity against PC-9 human lung cancer cells.

4. Steroids with unconventional side-chain

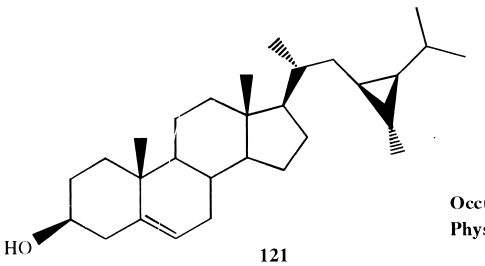
Steroids isolated from sponges frequently contain substantially modified side-chains, such as those with high degrees of alkylation and/or other unusual functionalization. Biosynthetic studies have been performed on mechanism and scope of sterol side-chain dealkylation in sponges [62], and a detailed review has been published as a comprehensive summary of biosynthesis of sterol side-chain in marine organisms [63].

(25*S*)-26-Methyl-24-methylenecholest-4-en-3-one (**98**) has been isolated from the marine fossil sponge *Neosiphonia supertes* [64]. The unambiguous confirmation of this structure was obtained through an Oppenauer oxidation of the known 24(28)-dehydroaplysterol, also isolated from the sponge, which led to a keto compound whose spectroscopic properties were identical to those of compound **98**.

Baikalosterol (**99**) is a novel steroid with a 24-ethyl-26-nor-22,25-diene group in the side-chain isolated from the sponge *Baicalospongia bacilifera*, a common species of the Baikal lake [65]. This unusual side-chain suggests interesting questions with respect to the biosynthesis of **99**, which is probably a product of dealkylation of a C_{29} sterol. This is supported by the composition of the total sterol fraction of



Occurrence: *Stelodoryx chlorophylla*[71]
Physical data: EIMS, ^{1}H-NMR[71]



Occurrence: *Cribrochalina vasculum*[75]
Physical data: HREIMS, ^{1}H-NMR[75]

B. bacilifera, whose 30% is represented by C_{29} sterols. In particular, (22*E*,24*S*)-methylcholesta-5,22,25-trien-3β-ol, a minor 25-unsaturated C_{29} sterol also isolated from the sponge, could be the precursor of **99**.

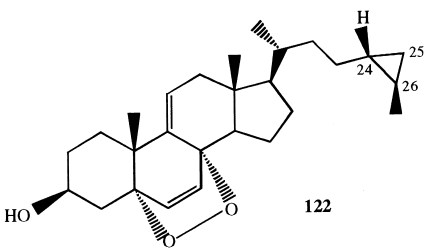
Sutinasterol (**100**) contains a C_{12} side-chain that is presumably the product of quadruple bioalkylation. It represents the 94% of the steroid fraction of a *Xestospongia* sponge. An X-ray crystallographic structure study of sutinasterol was performed to confirm its structure and to determine the stereochemistry at C-24 [5].

A new cytotoxic sterol, bienmasterol (**101**), possessing the rare 22,25-diene side-chain, has been isolated from the Okinawan sponge *Bienma* sp. [66]. This feature, previously found in the major component of the sterol mixture of the Hawaiian sponge *Ciocalypta* sp. [67], provided strong evidence for the proposed intermediacy of a 22,25-diene in the biosynthesis of sterols [68]. Bienmasterol exhibited cytotoxicity against murine lymphoma L1210 and human epidermoid carcinoma KB cells in vitro.

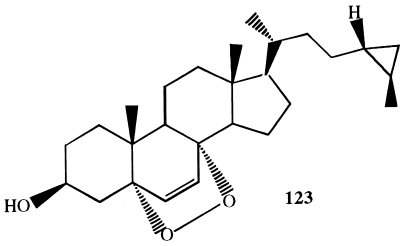
Topsentinols A through J (**102–111**), 10 new 7-hydroxysterols with unusual polyalkylated side-chains, have been isolated from the Okinawan marine sponge *Topsentia* sp. [69]. Side-chains containing the 24-isopropenyl-25-methyl-22*E*-ene group (in **102** and **107**) and the 24-isopropyl-22*Z*-ene group (in **103** and **108**) are unprecedented. Topsentinol B (**103**) showed antifungal activity against *Trichophyton mentagrophytes*.

Two new sterols, epipolasterol and 22,23-dihydroepipolasterol (**112** and **113**), have been found in *Epipolasis* sp. [70]. These are unusual metabolites, as they both contain a *t*-butyl group as well as two degrees of unsaturation in the side-chain.

Twenty-two steroid components present in the deep-water sponge *Stelodoryx chlorophylla*, from New Caledonia, have been identified; among them, seven products (**114–120**) were new compounds [71]. With the exception of **119** and **120**, they are steroids with oxygenated or short oxygenated side-chains. These compounds appear to be

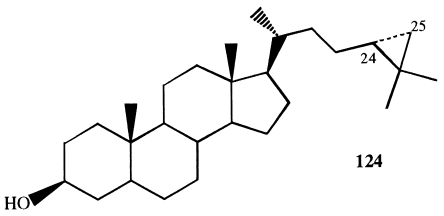


122



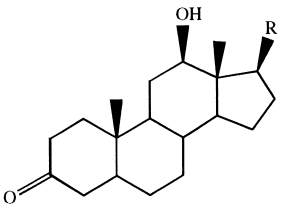
123

Occurrence: *Tethya* sp[77]



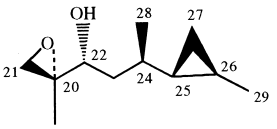
124

Occurrence: *Rizochalina incrustata*[78]

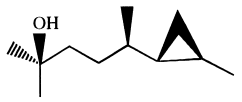


Occurrence: *Xestospongia* sp.[79-82]
Physical data: ^{1}H and ^{13}C-NMR
Total synthesis of **125**[83]

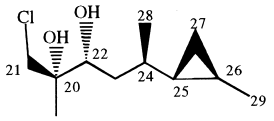
125 R=



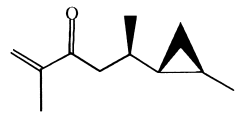
126 R=



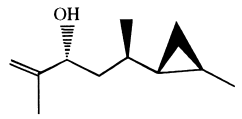
127 R=



128 R=

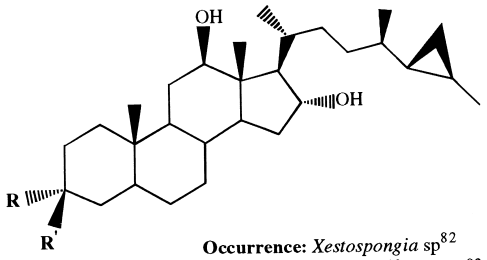


129 R=



autoxidation products of the corresponding Δ^5 sterols. Their presence in the steroid mixture could be the consequence of the storage of the sponge for a long time, even if the quantities of some steroids are much higher than expected for autoxidation products.

The cyclopropane and cyclopropene sterols found in marine sponges of the order Haposclerida are very interesting and, recently, a biosynthetic pathway leading to spongal cyclopropyl sterols has been shown starting from clionasterol through a novel biochemical desaturation reaction [72–74]. Several new examples of this class of sterols have been recently reported. A new cyclopropane sterol, **121**, has been isolated from the sponge *Cribrochalina vasculum* [75]; it differs from the known (23*S*,24*S*,28*R*)-dihydrocalysterol only in the configuration at C-23 [76]. Its discovery points to new biosynthetic implications; in addition to the mechanistic interest of this compound in the biosynthetic cyclopropane-forming

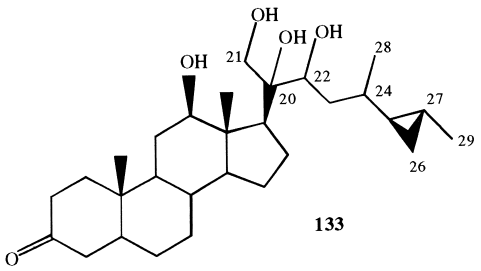


130 R, R'=O

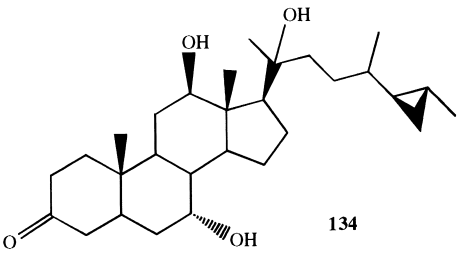
131 R=OH, R'=H

132 R=H, R'=OH

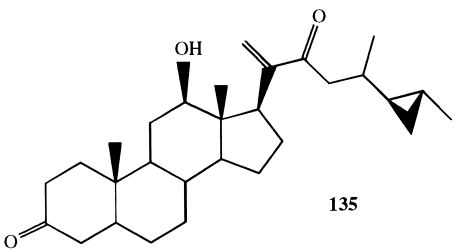
Occurrence: *Xestospongia* sp[82]
Physical data: ^{1}H and ^{13}C-NMR[82]



133

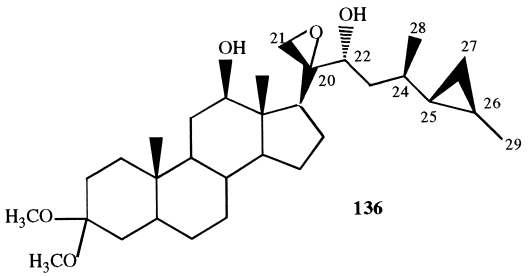


134



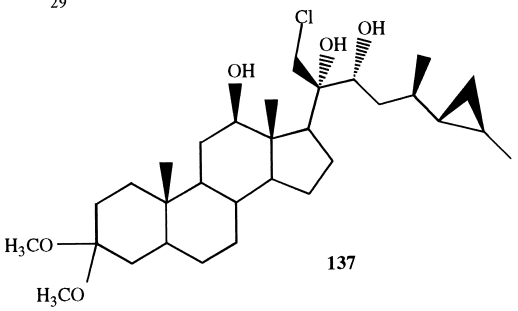
135

Occurrence: *Xestospongia* sp[85]
Physical data: ^{1}Hand ^{13}C-NMR (complete assignment)[85]



136

Occurrence: *Xestospongia* sp[86]
Physical data: ^{1}H and ^{13}C-NMR (complete assignment)[86]



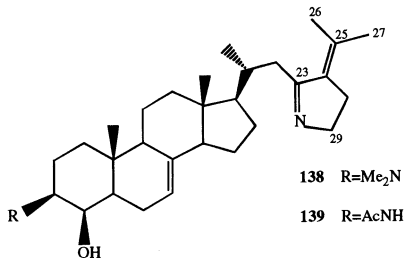
137

reaction, it could serve as precursor to calysterol via *cis* desaturation.

A 5α,8α-epidioxy functionality characterizes the two sterols, **122** and **123**, isolated from a sponge of the genus *Tethya* possessing a cyclopropyl ring at C-24 [26] of the side-chain [77].

(24*R*)-24,25-Methylene-5α-cholestan-3β-ol (**124**) has been isolated from *Rhizochalina incrustata* and its structure has been determined on the basis of spectral data and chemical transformations [78].

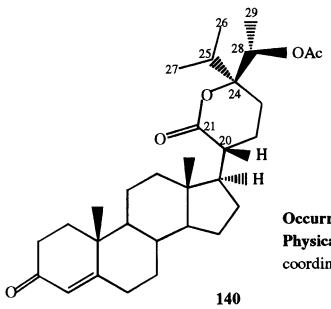
Aragusterols A through H (**125–132**) are additional cyclopropane-containing sterols isolated from Okinawan *Xesto-*



138 R=Me_2N

139 R=AcNH

Occurrence: *Corticium* sp.[87]
Physical data: ^1H and ^{13}C-NMR (complete assignment[87]



Occurrence: *Mycale* sp.[89]
Physical data ^1H and ^{13}C-NMR, crystal data, atomic coordinates [89]

140

spongia species; they are characterized by the rare 26,27-cyclopropane group [79–82]. Moreover, aragusterol C is the first marine steroid found to have a chlorinated side-chain. Its structure was determined by X-ray analysis. Aragusterols are important not only for their unique structures but also for their biological activity. Aragusterols A and C very strongly inhibit the proliferation of KB cells at IC_{50} 0.042 and 0.041 μg/ml, respectively, and express potent in vivo antitumor activity against L1210 leukemia in mice. To have these compounds available in greater amounts for more detailed pharmacological research, the synthesis of aragusterols A, B, C, and D has been successfully performed. The synthesis involves the enantioselective formation of a side-chain segment and its stereoselective coupling with a 20-keto steroid obtained from (+)-hecogenin [83]. The synthesis of 5-epiaragusterol A from deoxycholic acid, whose natural abundance permits synthesis on a large scale, has also been achieved; 5-epiaragusterol A showed an antiproliferative activity comparable with that of aragusterol A [84].

Xestosterols A–C (**133–135**) [85] and aragusteroketals A and C (**136** and **137**, respectively) [86], strictly related to aragusterols, have also been reported from an Okinawan *Xestospongia*. Xestokerols A and B are rare C-20 oxidized steroids from marine origin; biogenetically, they may have been generated through oxidation of C-20/C-21 double bond of xestokerol C. Compounds **133** through **135** also exhibited in vitro cytotoxicity and antimicrobial activity against Gram-positive bacteria. Xestokerol C has a structure similar to that of aragusterol D, but the absolute configurations at C-24, C-25, and C-27 were undetermined.

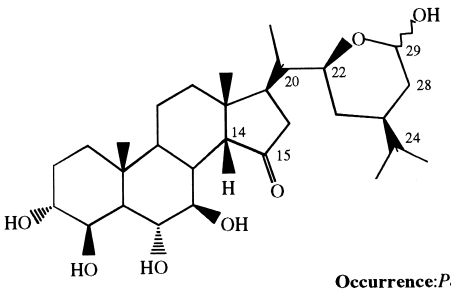
Lokysterolamines A and B (**138** and **139**, respectively) are two steroidal alkaloids isolated from an undescribed *Corticium* species [87]. The compounds bear a skeletal relationship to the previously described plakinamine A [88]; compound **138** is *N,N*-dimethyl-4β-hydroxy-3-*epi*-plakinamine A and compound **139** is *N*-acetyl-4β-hydroxy-3-*epi*-plakinamine A. They possess antimicrobial and antifungal activity and in vitro activity in P-388, A-549, HT-29, and MEL-28 cell assays.

Mycalone (**140**), isolated from a southern Australian *Mycale* species, is a new steroid possessing a six-membered lactone side-chain [89]. Its stereostructure has been determined by spectroscopic methods and X-ray crystallographic structure analysis.

5. Unconventional nuclei

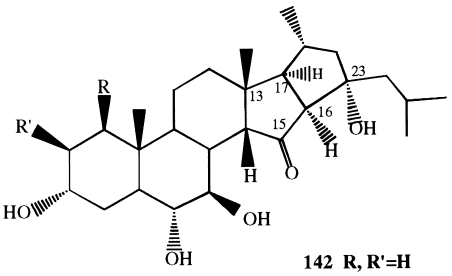
One of the significant features of steroids as a class of natural products is the almost universal occurrence of one particular set of configurations at the ring junction carbon atoms of the nucleus. Contignasterol (**141**) is the first marine steroid found to have a *cis* C/day ring junction as well as a cyclic hemiacetal functionality at C-29 in the side-chain, which is without precedent. It has been isolated from *Petrosia contignata* [14]; because of a slow spontaneous epimerization observed in contignasterol, a result of the presence of the hemiacetal functionality, the structural analysis was better performed on contignasterol tetraacetate and on the pentaacetate of its reduction product. This sterol represents the first example of a naturally occurring steroid with the 'unnatural' 14β proton orientation, although steroids with a 14β-hydroxyl functionality (i.e. digitoxin) are well known from nature. Because inverted ring junction configurations at any center in the steroidal nucleus are rare, biogenetic origin of the unnatural H-14 orientation in contignasterol is of considerable interest.

The same *cis* C/day ring junction has been found in (23*S*)-16β,23-cyclo-3α,6α,7β,23-tetrahydroxy-5α,14β-cholestan-15-one (xestobergsterol A; **142)** and (23*S*)-16β,23-cyclo-1β,2β,3α,6α,7β,23-hexahydroxy-5α,14β-cholestan-15-one (xestobergsterol B; **143**) isolated from the methanol/toluene extract of the Okinawan *Xestospongia bergquistia* [12]. They are the first report of steroids possessing five carbocyclic rings and the *cis* C/day ring junction. Biogenetically, they are considered most likely to be the products of an intramolecular aldol-type reaction in the organism. Xestobergsterols A and B strongly inhibited histamine release from rat peritoneal mast cells induced by anti-IgE in a dose-dependent manner. Successively, xestobergsterols A and B have been reisolated from an *Ircinia* species together with another analogous sterol, xestobergsterol C (**144**) [13]. In this study, the stereochemistry at C-23 (β-OH rather than α-OH, as initially assigned) and the conformation of the



141

Occurrence:*Petrosia contignata*[14]
Physical data: HREIMS, ^{1}H and ^{13}C-NMR (complete assignment)[14]

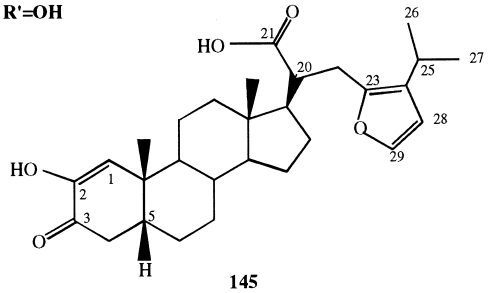


142 R, R'=H

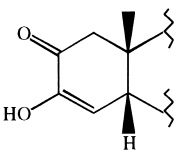
143 R, R'=OH

144 R=H, R'=OH

Occurrence:*Xestospongia bergquistia* (**142**, **143**)[12], *Ircinia* sp.(**142**-**144**)[13]
Physical data: HREIMS, CD, ^{1}H and ^{13}C-NMR (complete assignment)[12,13]

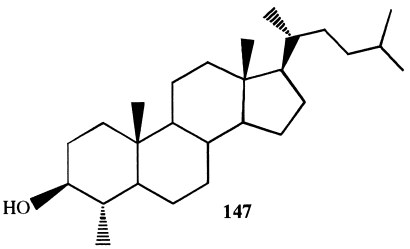


145



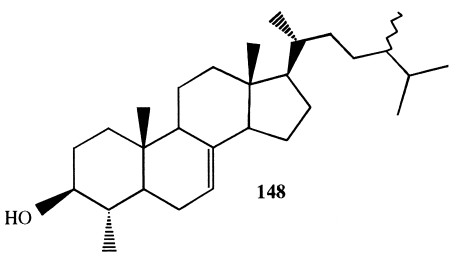
146

Occurrence: Poecilosclerida sponge[90]
Physical data ^{1}H and ^{13}C-NMR (complete assignment)[90]



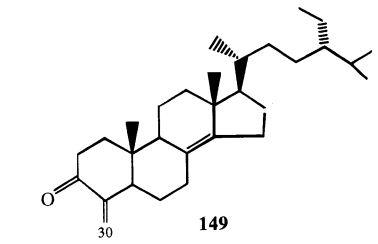
147

Occurrence: *Haliclona cinerea, Haliclona flavescens*[92]

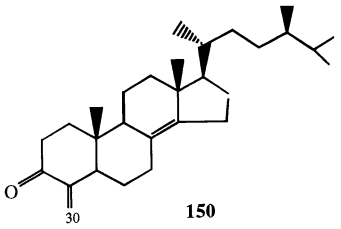


148

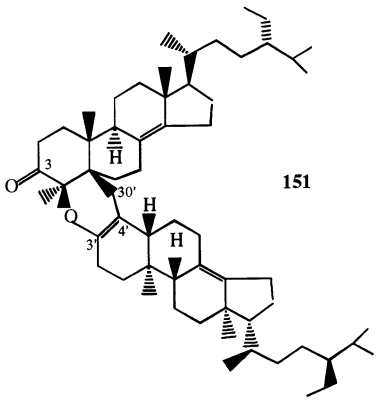
ring C (chair form) for xestobergsterol A and B have been revised on the basis of reexamination of the nuclear magnetic resonance data; furthermore, the CD exciton chirality method was applied to determine the absolute stereochemistries at C-6 and C-7 in xestobergsterol A. Xestobergsterols B and C were assumed to possess the



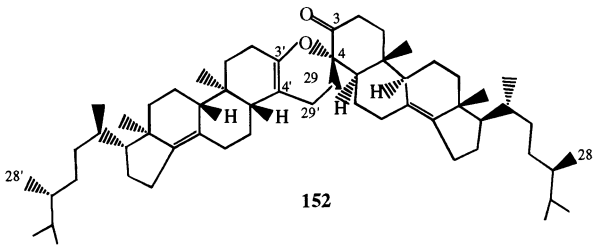
149



150

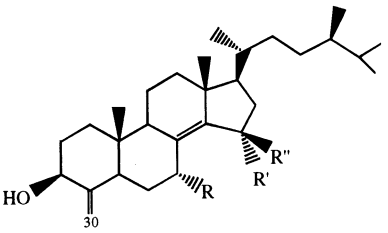


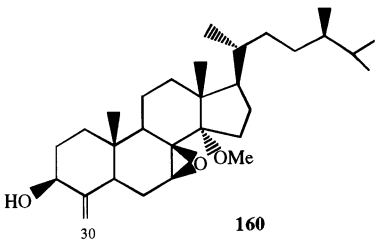
151



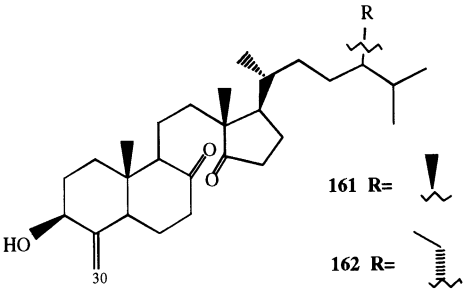
152

153 R=OH R'=H R''=H
154 R=OMe R'=H R''=H
155 R=OH R'=OMe R''=H
156 R=OH R'=H R''=OMe
157 R=OH R'=OH R''=H
158 R=OH R'=OCOR R''=H
159 R=H R'=OH R''=H



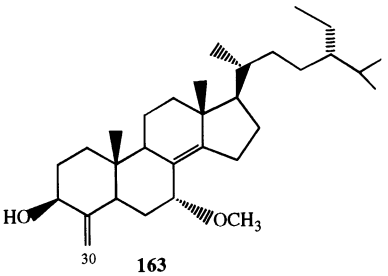


160



161 R=

162 R=

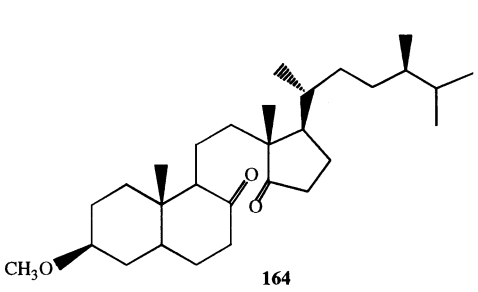


163

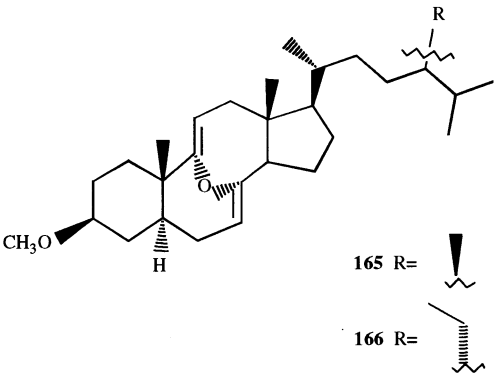
Occurrence: *Theonella swinhoei*[94-97]
Physical data: X-ray studies of **152**[95], ^{1}H and ^{13}C-NMR[94-97]

same absolute configurations (both *R*) because these sterols may be generated through the same biosynthetic pathway. Xestobergsterol C and A were shown to be cytotoxic against murine leukemia cells L1210, whereas xestobergsterol B was not significantly cytotoxic.

Kiheisterones A and B (**145** and **146**, respectively) were isolated from a sponge of the order Poecilosclerida, collected along the coast of the island of Maui (Hawaii) [90]. Each sterol contained an α,β-disubstituted furan in the side-chain, *cis*-fused A/B ring, a monoenolized α-di-

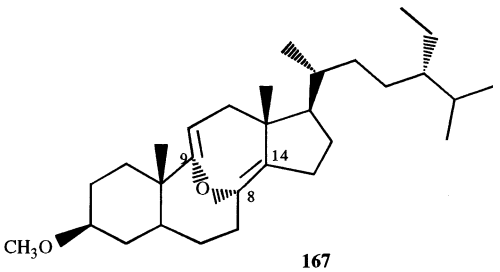


164



165 R=

166 R=



167

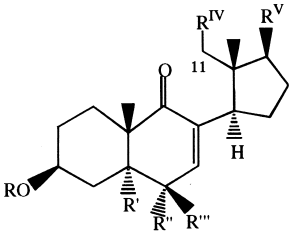
Occurrence: *Jereicopsis graphidiophora* (**164, 165**)[98], *Microscleroderma spirophora* (**166,167**)[33]
Physical data: ^{1}H and ^{13}C-NMR (complete assignment)[33,98]

ketone in the A ring, and a C-21 carboxyl group. **145** and **146** are stable to acid, but both equilibrate to a 1:1 mixture in base. Oxidation of C-21 to an alcohol is not uncommon in marine sterols, but this is the first case of sponge sterols with a C-21 carboxylic acid. The occurrence of a furan in the side-chain of a sterol is also unprecedented; the heterocyclic was supposed to be biogenetically derivable from oxidation of C-23 and C-29 to carbonyl groups followed by condensation. Kiheisterones A and B exhibit mild cytotoxicity against several human tumor cell lines (A-549 lung carcinoma and HT-29 colon adenocarcinoma) and against the P-388 murine lymphocytic leukemia cell line. Both compounds are more cytotoxic against nontumorous monkey kidney cells.

4-Methyl sterols, mainly distributed in plants and dinoflagellates [91], have also been found sometimes in gorgonians and tunicates, but such compounds are rare in sponges. Thirteen 4α-methyl sterols have been identified from *Haliclona cinerea* (two samples) and *Haliclona flavescens* in the frame of a study aimed to establish whether these sterols are derived from the diet or are synthesized by the sponges [92]. Among these, two sterols (**147** and **148**) resulted in new compounds.

Two biosynthetically unusual 4-methylene steroids, theonellasterone and conicasterone (**149** and **150,** respectively), along with the previously described corresponding alcohols theonellasterol and conicasterol [93], have been isolated from the Okinawan *Theonella swinhoei*. A dimeric steroid, bistheonellasterone (**151**), considered to be biosynthesized through a Diels-Alder cycloaddition of theonellasterone (**149**) and its Δ^4-isomer, has also been found in the same sponge [94]. It is noteworthy that theonellasterone and conicasterone were seen under an optical microscope as crystals deposited in the tissue of fresh marine sponge. The analysis of a *T. swinhoei* specimen, collected off the Hachijo-jima island, afforded conicasterol and a new dimeric steroid, bisconicasterone (**152**). Its structure was established as a Diels-Alder-type dimer of conicasterone [95]. It is noteworthy that no trace of theonellasterol, previously reported as the major component of the same species collected at Okinawa, has been found in this sample of *T. swinhoei*, which suggests a site-depending biochemistry of this species. A further investigation of the Hachijo specimen yielded seven oxygenated conicasterol derivatives (**153–159**) along with (24*R*)-7β,8β-epoxy-24-methyl-4-methylene-14α-methoxy-cholestan-3β-ol (**160**) and (234*R*)-3β-hydroxy-24-methyl-4-methylene-8,14-secocholestane-8,14-dione (**161**) [96]. Compound **161** has been isolated successively from an Okinawan *T. swinhoei* (swinhosterol B) along with swinhosterol A (**162**), which had an ethyl group at C-24 instead of a methyl group, and the new methoxysterol (24*S*)-24-ethyl-7-methoxy-4-methylene-5α-cholest-8(14)-en-3β-ol (swinosterol C; **163**) [97].

Swinosterols A and B (**162** and **161**, respectively) are the second example of the unique 8,14-seco structure. This feature has been first encountered in jereisterol B (**164**) isolated from *Jereicopsis graphidiophora* [98]. Compound **164** combine the rare 3β-methoxy and seco features; its structural determination was confirmed by the synthesis of the model 3β-acetoxy-8,14-secoergostane-8,14-dione by oxidation with ruthenium tetroxide of 3β-acetoxy-ergost-8(14)-ene. Jereisterol A, (**165**), also isolated from *J. graphidiophora*, had the unique 8α,9α-oxido-8,9-seco-5α-cholesta-7,9(11)-diene feature [98]. This structure is without precedent in the steroid literature; the 8α,9α-oxido stereochemistry has been suggested by the chemical shift of the H-5 proton, downfield shifted, implying the location of the oxygen function and H-5 on the same face of the molecule.



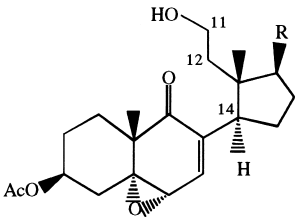
168 R, R', R'''=H; R''=OH; R^{IV}=CHO; R^{V}=

169 R=Ac; R', R'''=OH; R''=H; R^{IV}=CHO; R^{V}=

170 R, R', R'''=H; R''=OH; R^{IV}=CH_2OH; R^{V}=

171 R, R', R'''=H; R''=OH; R^{IV}=CH_2OH; R^{V}=

Occurrence: *Spongia officinalis*[99-101]
Physical data: ^{1}H and ^{13}C-NMR[99-101]



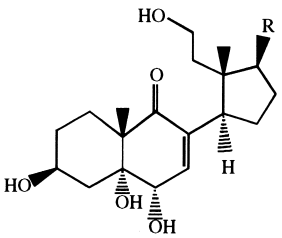
172 R=

173 R=

174 R=

175 R=

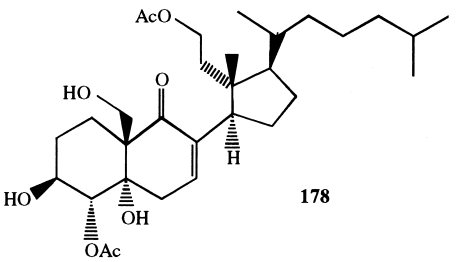
Occurrence: *Aplysilla glacialis* (**172,173**)[102], *Dysidea fragilis* (**173-175**)[6]
Physical data: ^{1}H and ^{13}C-NMR[102,6]



176 R=

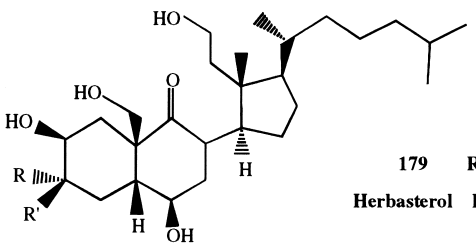
177 R=

Occurrence: *Dysidea fragilis*[6]
Physical data: ^{1}H and ^{13}C-NMR (complete assignment)[6]



178

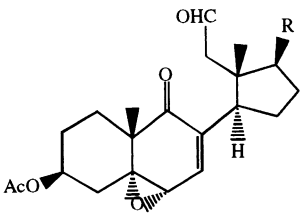
Occurrence: *Pteraplysilla* sp.[103]
Physical data: ^{1}H and ^{13}C-NMR[103]



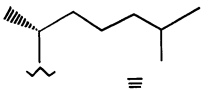
179 R=H, R'=OH
Herbasterol R=OH, R'=H

Occurrence: *Stelletta* sp. (**179**)[104]
Physical data: ^{1}H and ^{13}C-NMR (complete assignment)[104]

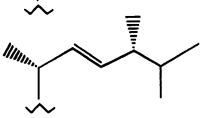
A homologue of jereisterol A (**165**), possessing the unique 8α,9α-oxido-8,9-secocholesta-7,9(11)-diene feature and a 24-ethyl side-chain (**166**) has been isolated from *Microscleroderma spirophora* along with its $\Delta^{8(14),9(11)}$ isomer (**167**) [33]. The 24*S* stereochemistry for compounds **166** and **167** has been proposed on the basis of the chemical shift values of H-28_a and H-28_b, which showed a diagnostic dependence on the configuration at C-24



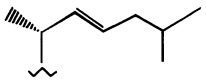
180 R'=Ac, R=



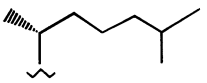
181 R'=Ac, R=



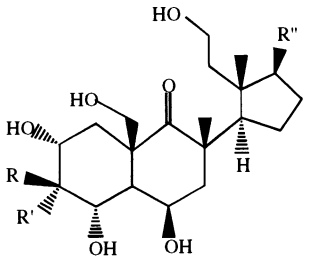
182 R'=Ac, R=



183 R'=H, R=



Occurrence: *Luffariella* sp. (**180-182**)[105], *Spongia matamata* (**183**)[106]
Physical data: ^{1}H and ^{13}C-NMR (complete assignment)[105, 106]



Occurrence: *Eurispongia* sp.[107]
Physical data: ^{1}H and ^{13}C-NMR[107]

184 R=OH, R'=H, R''=a

185 R=OH, R'=H, R''=b

186 R=OH, R'=H, R''=c

187 R=OH, R'=H, R''=d

188 R=OH, R'=H, R''=e

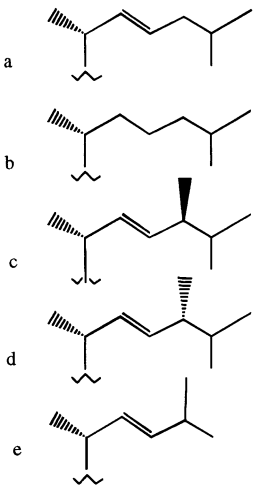
189 R=H, R'=OH, R''=a

190 R=H, R'=OH, R''=b

191 R=H, R'=OH, R''=c

192 R=H, R'=OH, R''=d

193 R=H, R'=OH, R''=e



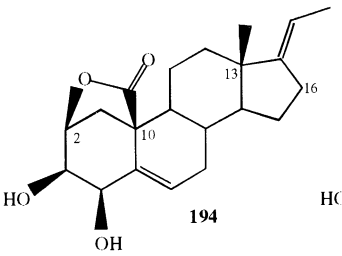
when compared with those of sitosterol (24*R*-ethylcholesterol) and clionasterol (24*S*-ethylcholesterol). This analysis has been proposed as a new method for the determination of the configuration at C-24 of saturated 24-ethyl side-chains.

A large number of new 9,11-secosterols have been recently isolated; structurally, all have a keto group at C-9 and a side-chain like those usually found in 'normal' sterols, differences residing in the A/B ring (*cis* or *trans* junction or Δ^5) and in the type and degree of oxygenation.

Four novel 9,11-secosterol (**168–171**) have been isolated from *Spongia officinalis* [99–101]. They could be derived from a 5,7,9(11)-triene sterol through oxidation at the C-5 and C-6 carbons, to give **169**, or only at C-6, to give **168**, **170**, and **171**, with concomitant oxidative cleavage of the 9(11) double bond. Partial synthesis confirmed the proposed structures of the sterols **168** through **171**.

Glaciasterols A and B (**172** and **173**, respectively) have been found in the Northeastern Pacific sponge *Aplysilla glacialis* [102]. Their structures have been clarified by a combination of spectroscopic analysis and chemical conversions on the native steroid **172** and the diacetate of **173**. Glaciasterols A and B showed an interesting in vitro cytotoxic activity in murine leukemia L1210 and human breast cancer cell line assays.

Two glaciasterol analogues (**174** and **175**), present in a specimen of *Dysidea fragilis* coming from the lagoon of Venice, possess the same nuclear structure of **172** and **173,** differing only in the nature of their side-chains. In addition, the sponge has been also found to produce the new 9,11-



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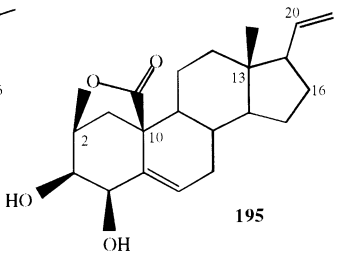

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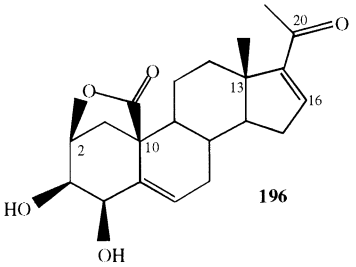
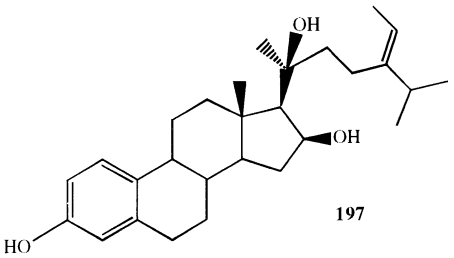


196

Occurrence: *Strongilophora* sp.[108]
Physical data: ^{1}H and ^{13}C-NMR (complete assignment), X-ray studies on **194**.[108]



197

Occurrence: *Geodia* sp.[109]
Physical data ^{1}H and ^{13}C-NMR .[109]

secosterols, **176** and **177**; compound **177** exhibited cytotoxic activity in vitro on two different cell lines [6].

A cytotoxic 9,11-secosteroid, blancasterol (**178**), bearing two acetate functionalities located at C-4 and C-11, has been isolated from a Northeastern Pacific *Pleraplysilla* species [103]. Blancasterol showed in vitro cytotoxicity against L1210 murine leukemia, drug-sensitive MCF-7 human breast cancer, and drug-resistant MCF-7 Ad^{r} human breast cancer cell lines. The cytotoxicity of blancasterol parallels that of the glaciasterol diacetates, demonstrating that the epoxide functionality in the B ring of glaciasterols is not required for the toxicity.

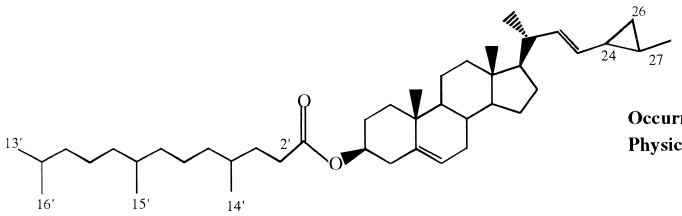
Stellettasterol (**179**), a new antifungal 9,11-secosterol isolated from *Stelletta* sp. [104], differs from herbasterol, an ichthyotoxic secosterol previously isolated from *Dysidea herbacea* [11], only in the absolute configuration at C-3. It is noteworthy that two species taxonomically remote, belonging to the genera *Dysidea* (Dyctioceratida) and *Stelletta* (Christida), contained secosterols differing only in the stereochemistry at C-3.

Luffasterols A through C (**180–182**) are 9,11-secosterols elaborated from the Palauan sponge *Luffariella* [105] species; they contain a 3β-acetoxy-5α,6α-epoxy-9-oxo-9,11-secocholest-7-en-11-al ring system joined to three different side-chains. The 3-deacetyl derivative of luffasterol A (**183**) has been isolated from *Spongia matamata* [106].

Eurypongiols A1 through A5 and B1 through B5 (**184–188** and **189–193**, respectively) have been isolated from *Eurypongia* sp. [107]. Eurypongiols A1 through A5 differ in their side-chain but have the same 2α,3β,4α,6β,11,19-hexahydroxy-9,11-secocholestane skeleton. Eurypongiols B1 through B5 are the corresponding 3α-epimers. They are the most highly hydroxylated secosteroids isolated so far from sponges and are the first hydroxylated at C-4. Compounds **184** and **185** strongly inhibit the release of histamine from rat mastocysts.

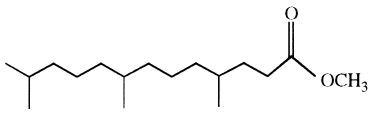
Three new pregnanes (**194–196**) with the unusual 10,2-carbolactone structural feature have been found in *Strongylophora* species [108]. Their structures, confirmed by X-ray crystallography, derive from 2,3,4-trihydroxy-5-pregnenes differing in D ring and/or their side-chain.

Geodisterol (**197**), the major component of the polar extract of the sponge *Geodi*a sp. [109], represents the first polyoxygenated sterol with an aromatic A ring isolated from marine organisms. Steroids with aromatic A ring have been obtained from terrestrial plants and animals as hormones (e.g. estradiol), but all those steroids have a small side-chain in comparison with **197**.

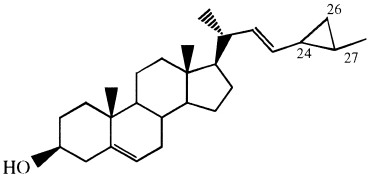


198

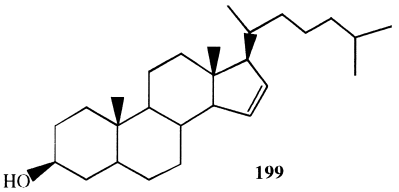
Occurrence: *Xestospongia* sp.[110]
Physical data: HREIMS, ^{1}H and ^{13}C-NMR[110]



a

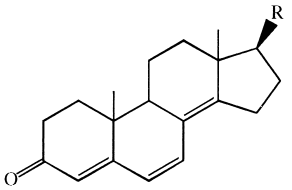


b

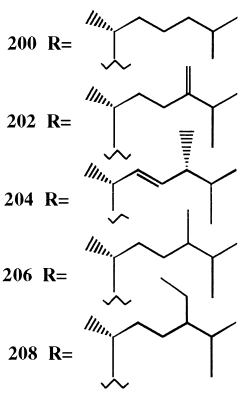


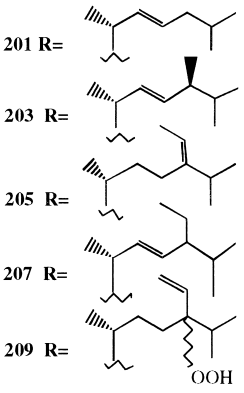
199

Occurrence: *Topsentia aurantiaca*[41]
Physical data: HREIMS, ^{1}H and ^{13}C-NMR (complete assignment)[41]



Occurrence: *Dysidea herbacea*[112]
Physical data: ^{1}H and ^{13}C-NMR[112]

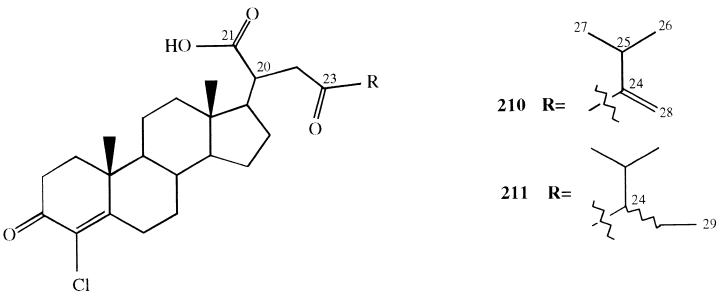


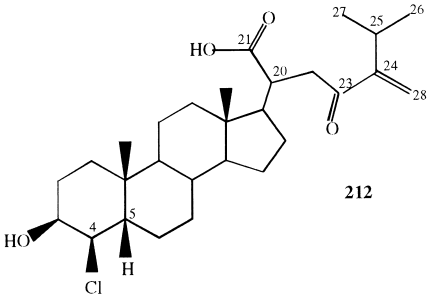


6. Miscellaneous

(22*E*)-24,26-Cyclo-5α-cholest-22-en-3β-yl 4′,8′,12′-trimethyltridecanoate (**198**) is a sterol ester isolated from a deep water marine sponge, *Xestospongia* sp. [110]. The sterol ester **198** was hydrolyzed and successively methylated with diazomethane to give the methyl ester **a** and the sterol **b,** which were identified by analysis of their spectroscopic data. The sterol **b** was previously reported from the soft coral *Sarcophyton glaucum* [111].

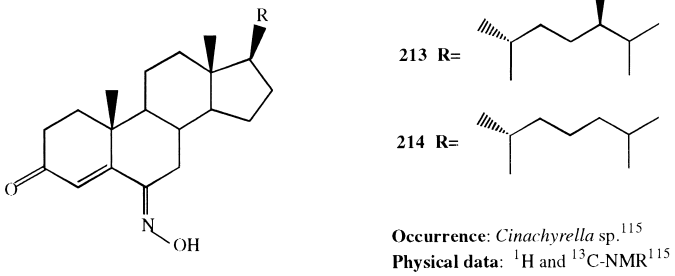
5α-Cholest-15-en-3β-ol (**199**), isolated from *Topsentia aurantiaca* along with compounds **58** through **60** (see section 2), contains an uncommon unsaturation in the D ring. This is the first report of **199** as a naturally occurring





212

Occurrence: *Strongylacidon* sp.[114]
Physical data: HRFABMS, ^{1}H and ^{13}C-NMR (complete assignment)[114]



Occurrence: *Cinachyrella* sp.[115]
Physical data: ^{1}H and ^{13}C-NMR[115]

compound; it was previously described as a synthetic product [41].

Ten 3-oxo-4,6,8(14)-triunsaturated steroids with cholestane (**200** and **201**), ergostane (**202–204** and **206**), and stigmastane skeletons (**205** and **207–209**) were found in the lipid extract of *Dysidea herbacea* [112]. Of these, **206** through **209** were obtained as C-24 epimeric mixtures. Compounds **200** through **209** are the second example of steroids isolated from a marine source having the conjugated 3-oxo-4,6,8(14)-triene system. Previously, compound **201** had been isolated from *Dyctionella incisa* [113]. The hydroperoxide **209** was shown to be an artifact derived from **205** during storage.

Halogenated steroids are very rare in nature. Kiheisterones C, D, and E (**210–212**) are three unprecedented halogenated marine steroids found in the 2-propanol/CH_2Cl_2 extract of the sponge *Strongylacidon* sp. [114], whose major cytotoxic constituents are kiheisterones A and B (**145** and **146,** respectively; see section 5). The existence in many organisms of reductase capable of converting 4-en-3-one steroids to the corresponding 3β-hydroxy-5β(H) compounds is well documented and kiheisterone D with its *cis*-chlorohydrin functionality may have arisen from enzymatic reduction of kiheisterone C.

Steroids **213** and **214**, isolated from *Cinachyrella* sp. [115], are the first natural 6-hydroximino-4-en-3-one steroids occurring in nature. This new class of steroids, which has been recently synthesized, shows a high affinity for human placental aromatase and functions as a competitive inhibitor of this enzyme [116].

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