Marine Drugs

Review

Sterol Ring System Oxidation Pattern in Marine Sponges

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Abstract: The marine sponges (Porifera) are a unique group of sedentary organisms from which several novel natural products are reported, many of which have useful biological activities. In producing unusual sterols, they occupy a preeminent position among the various groups of organisms. The polar sterols of sponges reported as at the end of the year 2002 number about 250; their ring structure changing a hundred times. The oxidation pattern in the sterol ring system, from the point of view of biogenesis seems to be mainly of four types. Each sponge species is able to produce sterols fitting into one of the four main biogenetic pathways viz., (i) 3β-hydroxy-Δ^5-sterol pathway, (ii) 3β-hydroxy-Δ^7-sterol pathway, (iii) 3β-hydroxy-$\Delta^{5,7}$-sterol pathway, and (iv) 3α-hydroxy sterol pathway.

Keywords: Marine sponges, Polar sterols, Unusual sterols, Ring system, Oxidation pattern

Introduction

The 'usual' sterols have the 3β-hydroxy-Δ^5 (or Δ^0)-cholestane (**I**) nucleus and a C_8-C_{10} side chain [1]. There are over 200 such sterols, occurring in marine organisms as complex inseparable mixtures and their identification is usually done by GC-MS. The 'unusual' sterols [2] have either or both of the characteristics of: (i) side chains ranging from C_0 to C_{12} involving loss or addition of carbon atoms at positions other than C-24, and (ii) (multiple) oxygenation of the nucleus and/or the

side chain. These sterols, by virtue of their greater spread on the polarity scale, can be isolated in pure condition by liquid chromatography. But, many of them are very unstable and should be handled at very mild conditions so that artifacts are not mistaken as natural products.

The polar sterols of sponges, particularly the sulfate esters (Schemes 12-14) have interesting and useful biological activities that make them targets of biological evaluation and synthesis.

Although polyhydroxy sterols have been found in various groups of marine organisms e.g., algae, porifera, coelenterata, bryozoa, molluska, echinodermata, arthropoda, tunicata and chordata, a preeminent position is occupied by porifera, i.e., sponges. A full review of the marine polyhydroxy steroids was published in 1993 [3]. The reviews that appeared since [4,5] discuss briefly on sponge sterols. The present review's purpose is besides giving the account as of date, to describe for the first time the biogenetic relationships that possibly exist in the sterol nuclear structure. This aspect may have a bearing on sponge classification, and the geographic occurrence of the organism, and be of great utility in chemotaxonomic studies. For brevity, the structures of sterols are presented as part structures, focusing on the oxidation pattern occurring in the sterol ABCD ring system (alone). A single sterol nucleus, as shown in various schemes (Schemes 3-14) may stand for a number of individual steroids with structural changes occurring in the side chain that are, however, not shown.

In order to propose biosynthetic relationships, as marine biosynthetic studies are few [6,7], clues are taken from the pathways operating in the terrestrial plants and animals, which are documented quite well, and since the pathways operating in marine organisms should essentially be similar to those operating in terrestrial organisms [8]. In Schemes 1-14 are presented sequential oxidations within the sterol ABCD ring system that should be taking place as part of biogenesis within marine sponges. In each product structure, the center where the structural change has resulted compared to the precursor is shown in red color. The biogenic connectivity between various sterol ring structures although hypothetical is depicted with the arrow ($\rightarrow$) sign for clarity although this sign is usually reserved for chemical conversions that actually take place. Most often, each sponge species contains a particular group of polar sterols dominated by a set of closely related biogenetic mechanisms as presented in each scheme. However, since the schemes are formulated basing on the 'reported' sterol composition, and since there is occasionally a lack of information on the total sterol composition of the sponge (often, it is only the new compounds that are described), the schemes are subject to refinement.

Several novel sterols containing extra oxygen substitution and side chain modified by alkylations/dealkylations have been reported from marine sponges. In quite a few species, novel sterols are the (single) major components of their extracts. Typical examples are aplysterol (**II**) and 24(28)-didehydro aplysterol (**III**), the first sterols [9] with a methyl group at C-26, which have been found as the major sterols of the sponges of the genus *Aplysia (Verongia),* calysterol (**IV**), the major sterol (90% of the sterol mixture) of the sponge *Calyx nicaensis* [10], petrosterol (**V**) of the sponge *Petrosia ficiformis* [11,12], strongylosterol (**VI**), the sole sterol of *Strongylophora durissima* [13], and xestosterol (**VII**) and sutinasterol (**VIII**) isolated as the predominant sterols of *Xestospongia muta* [14], and *Xestospongia sp*. [15] respectively.
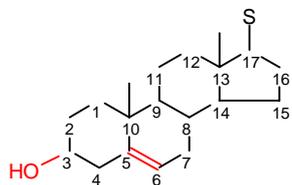
Cholesterol Ring System (N)   IX, lanosterol


I   II   III   IV

V   VI   VII   VIII
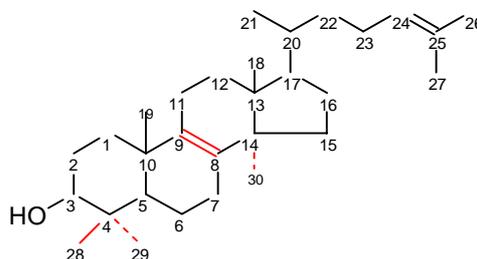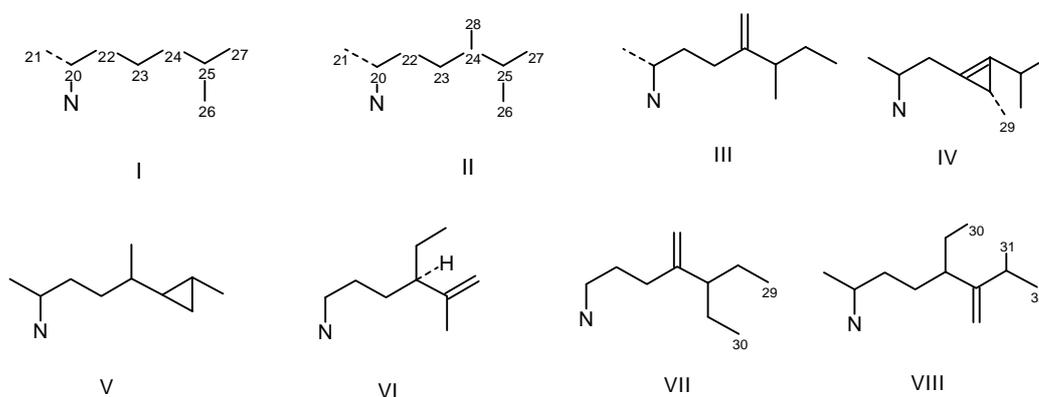
General biosynthetic Reactions in marine sponges

Working on the usual cholesterol skeleton, sponges are capable of performing enzymatic oxidation around the active sites, 3β-OH and Δ^5 functionalities.

1. epoxidation (generally αα and rarely ββ) followed by its opening in different pathways,
2. oxidation of the allylic C-7 and C-4 carbons to give simple alcohols of the preferred configuration, and
3. isomerisation of the double bond(s).

The reactions that take place on the 3β-OH and the new OH groups that are introduced (Scheme 2) are:

1. oxidation to a carbonyl,
2. dehydration producing unsaturation which will create new active allylic positions for further oxidation,
3. retro Diels-Alder reaction in the case of vicinal diols, and
4. condensation reactions involving OH, CH₂OH, CHO, and COOH groups at appropriate locations.

These reactions centering the Δ^5 and the 3β -OH are shown in Schemes 1 and 2 respectively.

Scheme 1.Significant biosynthetic reactions caused by Δ^5 (S = side chain; see also scheme 10)
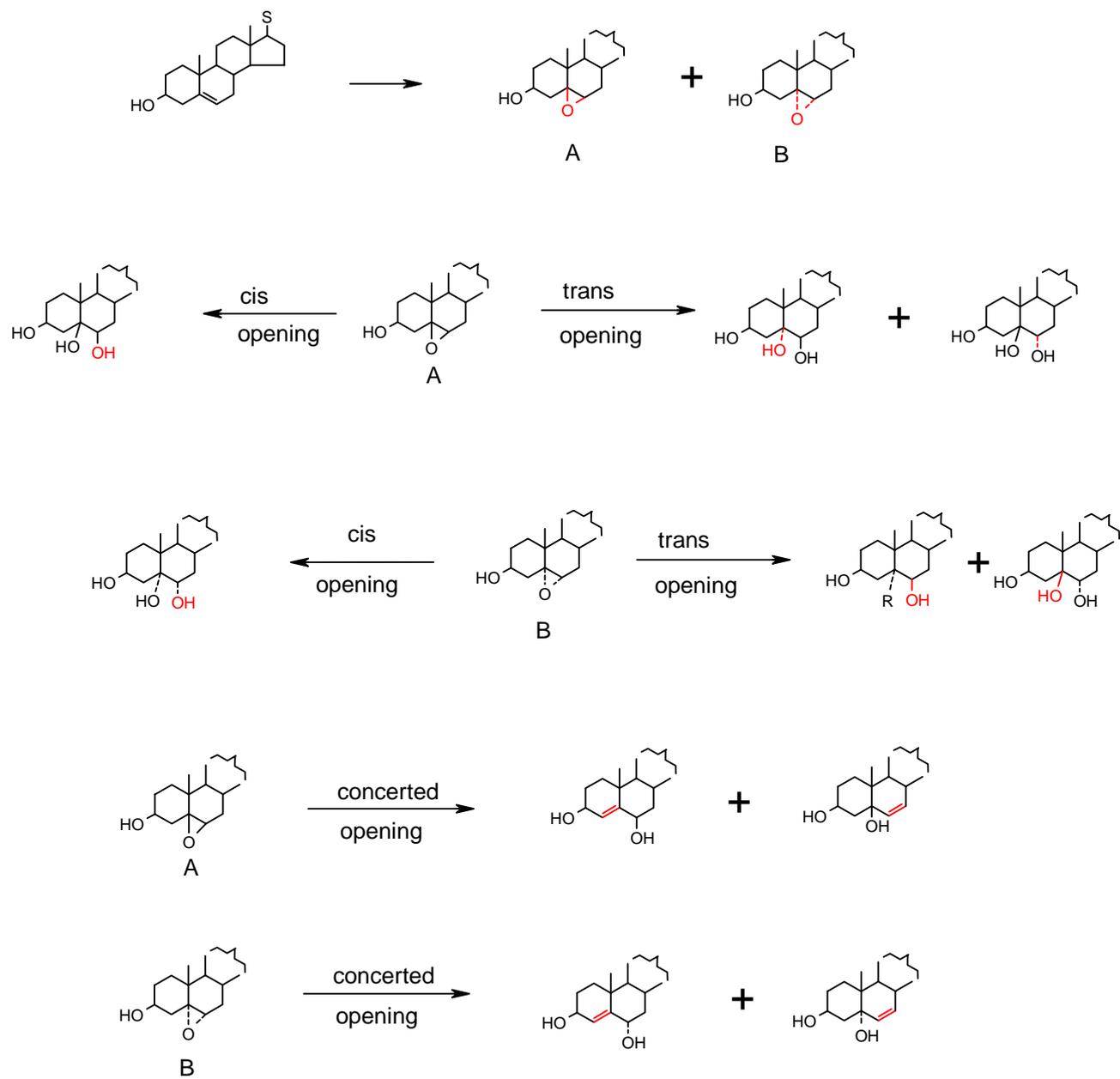
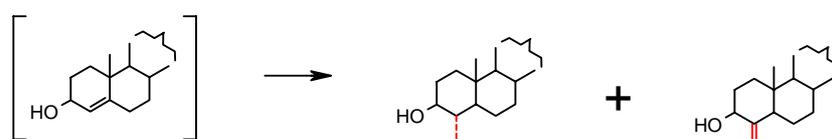**Scheme 2.** Significant biosynthetic reactions caused around 3β-OH; [   ] intermediate.

(i)   The 6β–OH substituent present in a cis 1,3–diaxial manner to the C-10 Me group can oxidize it in a step-wise fashion.
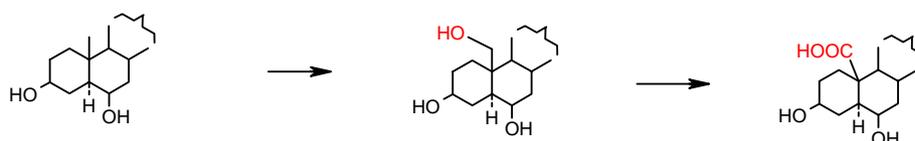
(ii)   Epimerisation of the 3β-OH can occur if activated by migration of Δ^5 to Δ^4.
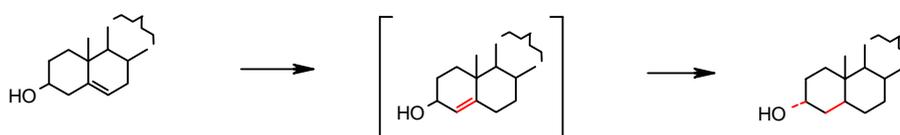
(iii)   The Δ^4 is amenable for alkylation.

In the following account, the progression of biosynthetic oxidative reactions that should be operating on the sterol ring system is presented. The pathways shown in the Schemes and discussed in text refer only to the ring system and not the complete structure of the steroid, as the structure of the side chain is not considered due to space constraint.
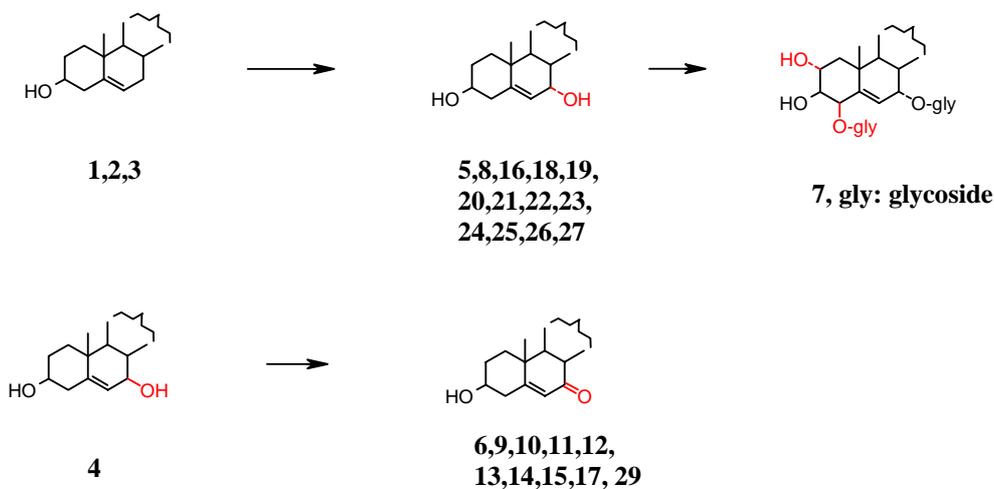
I. Δ^5-3β-Hydroxy Steroids

Polar sterols in which the parent sterol nucleus is retained are **1** and **2** from *Calyx nicaensis* [16] and *C. podatypa* [17], and **3** recently from an Indian sample of *Petrosia testudinaria* [18].

1. *Oxidation at C-7 (Scheme 3)*: The epimeric alcohols **4** and **5** and their ketone **6** are from *Corallistes undulatus* [19] and *Cliona copiosa* [20] by the allylic C-7 oxidation. It was for some time suspected that the C-7 oxidation might be resulting from auto-oxidation during isolation procedure. Hence, the isolation of the 7α-glycoside **7** (paschastrelloside A) from *Paschastrella sp*. confirms a biotic origin of the 7α oxygen [21]. The novel feature of **7** is its 2α-OH; the sterol inhibits cell division of fertilized starfish eggs. The presence of the

7α-OH and 7-keto group naturally in the sponge has received further proof from the recent isolation of a number of steroids **8-11** (gelliusterols A-D) from *Gellius sp*. of the Panaman Caribbean coast [22]. Sterol **12** was isolated from *Polymastia sobustia* from South China Sea [23], as well as **14** [24], **13** from *Geodia japonica* also from South China Sea [25]. Sterols **15** and **16** are from a Japanese specimen of *Strongylophora corticata* [26], and **17** (polysterol A) from *Epipolasis sp*. [27], and **18-27** topsentinols A to J from an Okinawan species of *Topsentia* [28]. The sterol containing the nucleus **28** (polysterol B belonging to another sterol subclass, the 3α-hydroxy sterol sulfates: Scheme 13) co-occurs with **29** (polysterol A). Although for the 3α-oxygenated sterols also, the parent is the 3β-Δ^5-sterol nucleus, the biogenetic pathway is somewhat different. The isolation of sterols belonging to different biogenetic pathways may be due to symbionts causing species heterogeneity or artifact formation on preservation and the subsequent isolation procedure. Hence, it is necessary to know these factors well for rationalizing the co-occurrence of sterols belonging to different biogenetic classes. The *Strongylophora corticata* sterols may exemplify this dimension.

Scheme 3. Oxidations at C-7 of Δ^5-3β-hydroxy steroid skeleton

1,2,3

5,8,16,18,19,
20,21,22,23,
24,25,26,27

7, gly: glycoside

4

6,9,10,11,12,
13,14,15,17, 29

2. ***Oxidation confined to Ring A (Scheme 4):*** The alcoholic C-3 and allylic C-4 are active sites for oxidation. The formation of 3-ketone can facilitate migration of the Δ^5 to the conjugated Δ^4 position, as found in **30**, mycalone from *Mycale sp*. of Southern Australia [29]. The chloroketones **31** and **32**, kiheisterones C and E present in *Strongylacedon sp*. from Maui along with the chlorohydrin D **33** are the only halogenated sterols isolated from sponges even though halogenated, particularly brominated natural products are common in marine sponges being derived from red algal symbionts. The products of C-2 activation are the diosphenols **34** (kiheisterone A) and **35** (kiheisterone B) of the same sponge [30]. A hydroxylation of the allylic C-4 is demonstrated by the 3β-sulfoxypregnane **36** isolated

Scheme 4. Δ^5 sterols: Oxidations in the ring A/B system; [   ] : not isolated

38, 39, 40
xyl: xylose; gal: galactose
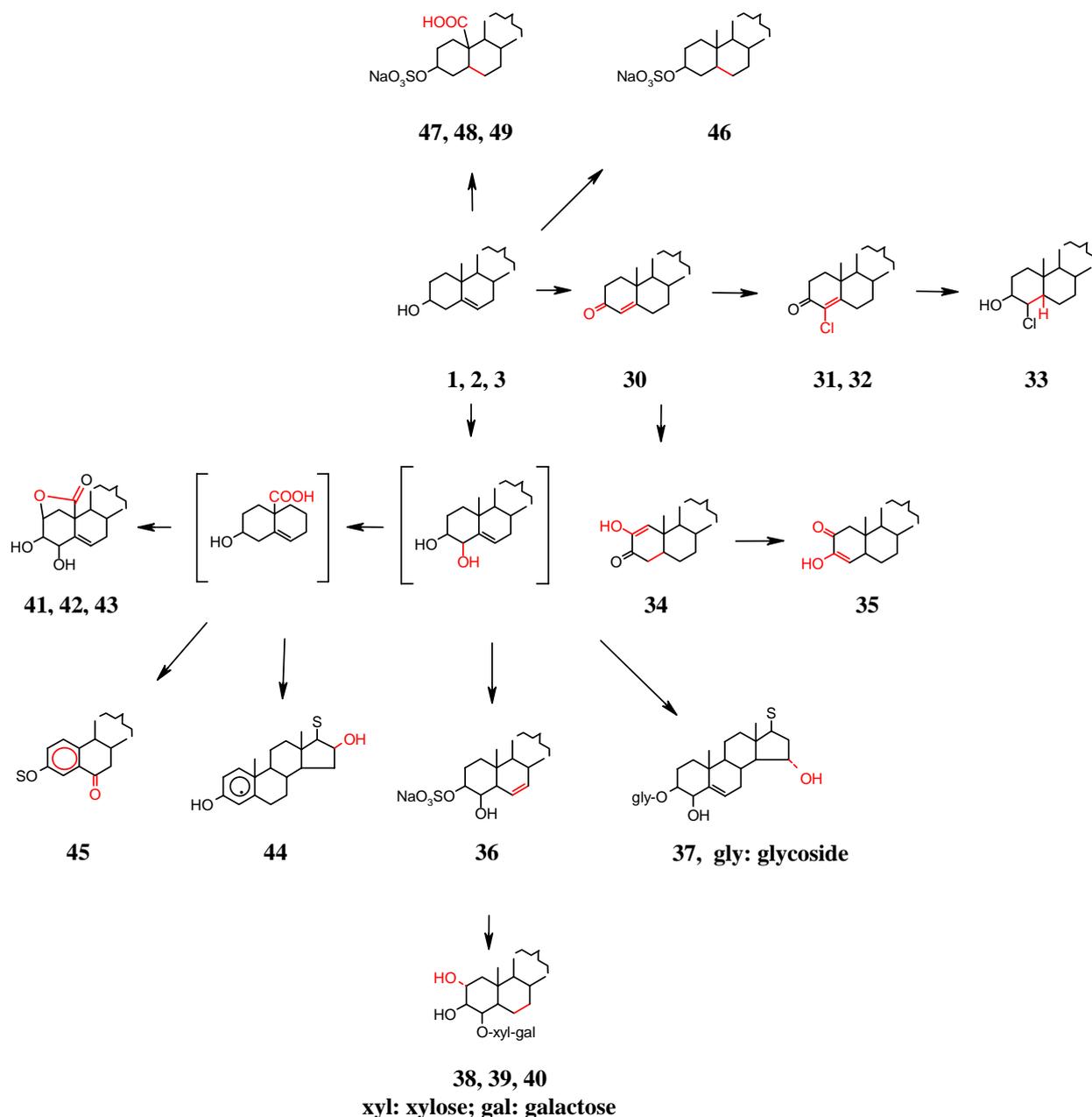
from *Stylopus australis* [31], and the glycoside **37** from *Mycale laxissima* [32]. The C-1 is activated *via* the Δ^2 formation by dehydration of the 3β-OH. The Δ^2, not so far observed in sponge sterols is a routine feature in the highly oxygenated sterol classes of withanolides and physalins from land plants belonging to the Solanaceae family [33]. The intermediate Δ^2 can then indulge in vicinal 2α,3β-diol and 2β,3β-diol formation, e.g., the glycosides **38-40**, the wondosterols A, B and C isolated from a two sponge association of *Poecillastra*

wondoensis and *Japsis wondoensis* [34]. With the ring A becoming oxygen rich, the 19β-Me becomes amenable for oxidation to –COOH group and consequent lactonisation with the 2β-OH, as seen in the pregnane γ-carbolactones **41-43** isolated from the Hawaiian sponge *Strongylophora sp.* [35]. The free COOH group can also disappear by loss of CO_2 leading to ring-A aromatisation found in the sterols **44** geodisterol isolated from *Geodia sp.* from Papua New Guinea [36], and the 19-nor pregnane glycoside **45** from *Cribrochalina olemda* from Pohnpei, Micronesia [37]. Oxidative elimination of the 19-Me takes place rather easily in sponges belonging to Axinellideae, e.g., *Axinella polypoides*, which contains **46** as the important sterol [38-40]. Its precursor A/B ring structure containing the 19-COOH group is present in the sterols **47-49** isolated from *Toxadocia zumi* [41].

3. ***Oxidations and rearrangements in the* A/B ring system (Scheme 5):** In sponges, ring A-rearranged sterols cooccur with 3-keto sterols, and 3,6-diones, a phenomenon that is particularly unique in sponges belonging to the families Axinellideae and Hymeniacidonidae. This is attributed to an efficient enzyme system due to which the A/B ring reaction precedes oxidation at other centres, e.g., Δ^{15} introduction in **50**, and Δ^{14}-16α-OH system in **51** found in the sterols of *Axinella proliferans* from Reunion island in the Indian Ocean [42]. The biosynthesis of the unique **52** and **53** (anthosterones A and B respectively) of *Anthoracurata gracia* is suggested to take place by a benzilic acid rearrangement of a 2,3-diketo precursor as a new type of ring A contraction step [43].

Scheme 5. Δ^5 sterols: 3- Ketosterols and rearranged sterols; [ ] : not isolated; S : side chain
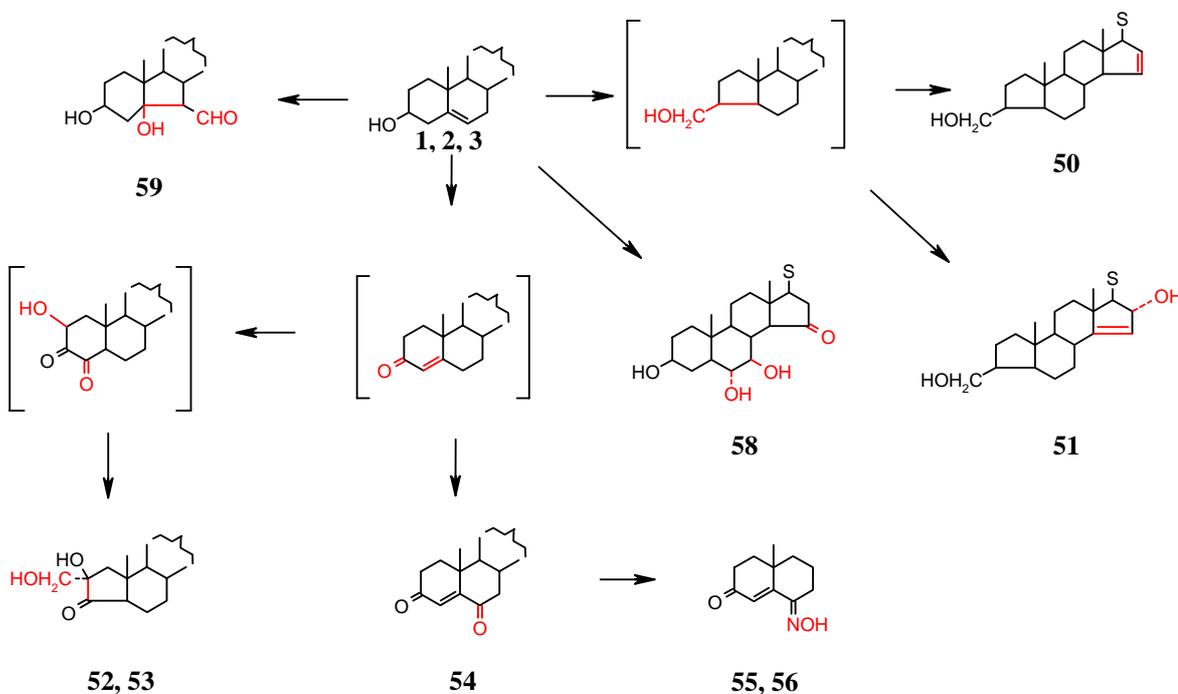
The Δ⁴-3,6-diketosterols **54**, with several conventional side chains are also from *Anthoracurata gracia*, the sponge from which anthosterones **52** and **53** are isolated [43]. The 3,6-diketones of *Geodia cydonium* [44] and *Cinachyra tarentina* [45] co-occur with the more common 3-ketones [46]. The 6-oximino-3-ketones **55** and **56** were obtained from a mixture of *Cinchyrella alloclada* and *C. apion* [47]. The 5α,6α-dihydroxylation is seen in **57** from *Spirastrella inconstans* from India [48], and the 6α,7β-dihydroxylation is seen in **58** clathriol from *Clathria lissosclera* [49] of New Zealand. The former seems to be the precursor of ring B rearranged **59** orostanal isolated from *Stellata hiwasaensis* of Japan [50]. The sterol **59** is cytotoxic and apoptosis-inducing.

4. ***Ring C oxidation (Scheme 6):*** The ring C site of oxidation at C-12 may not be requiring activation offered by a Δ⁵, a Δ⁷ or a 3β-OH. The saturated sterol **60** is in fact isolated in this group from *Rhizochalina incrustata* [51]. The activation seems to be coming from the heavily oxygenated (cyclopropane ring containing) side chains, c.f., the potent antitumour **61** [52], and **62-64** [53] from *Xestospongia sp.*, which are named aragusterols A to D, and **65** and **66** [54] and **67** [55] named as xestosterol A, xestosterol B and aragusterol E respectively, from another *Xestospongia sp.* collected from Okinawa. In rare cases, a further hydroxylation occurs at C-7, e.g., **68** xestokerol B; [54] isolated along with xestokerols A, B and D and C-16, e.g., **69** [55], another aragusterol (aragusterol F) of the *Xestospongia sp* from Okinawa. The skeletons **70** and **71** are of aragusterols G and H respectively, also isolated from this collection [55]. The sterols **72**, and **73** are aragusteroketals A and C respectively that are also from the same sponge [56], and perhaps artifacts of the isolation procedure.

**Scheme 6**. Δ⁵ sterols: Ring C oxidation in saturated sterols ; [   ] : not isolated; S : side chain
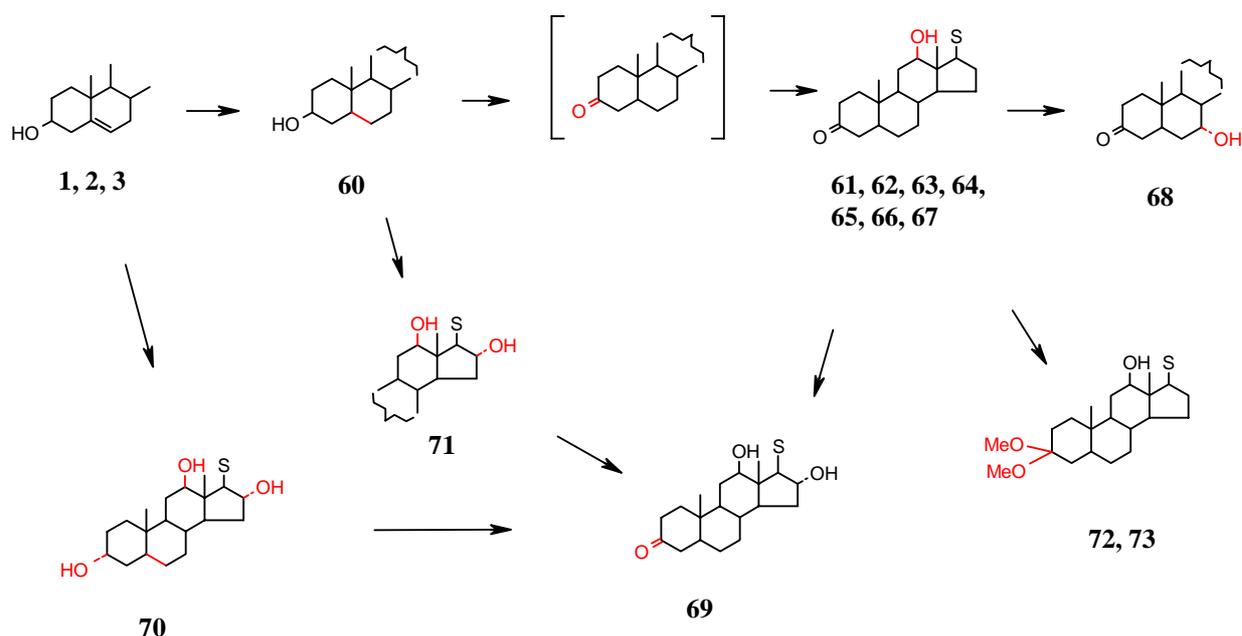
II. Δ⁷-Sterols

The parent 3β-hydroxy-Δ⁷-sterol nucleus is present in **74** thymosiosterol and **75** (24,27-didehydrothymosiosterol) isolated from *Thymosiopsis sp.* from France [57], and **76** isolated from a Caribbean sponge *Scleritoderma sp.* cf. *paccardi* [58].

1. ***Oxidation involving C-7, C-8, C-9, C-11 and C-14 (Scheme 7)***: The 3β-hydroxy-5,6-dihydro-Δ⁷ sterol nucleus seems to be undergoing allylic C-9 and C-14 (of the isomerised Δ⁸ nucleus) oxidation pathways. The C-9 oxidized **77** from *Jericopsis graphidiophora* [59] co-occurs with the C-14 oxidized **78** and **79** [60].

Scheme 7. Δ⁷ sterols: Oxidation involving C-7, C-8, C-9, C-11, and C-14 ;
[ ]: not isolated: S : side chain
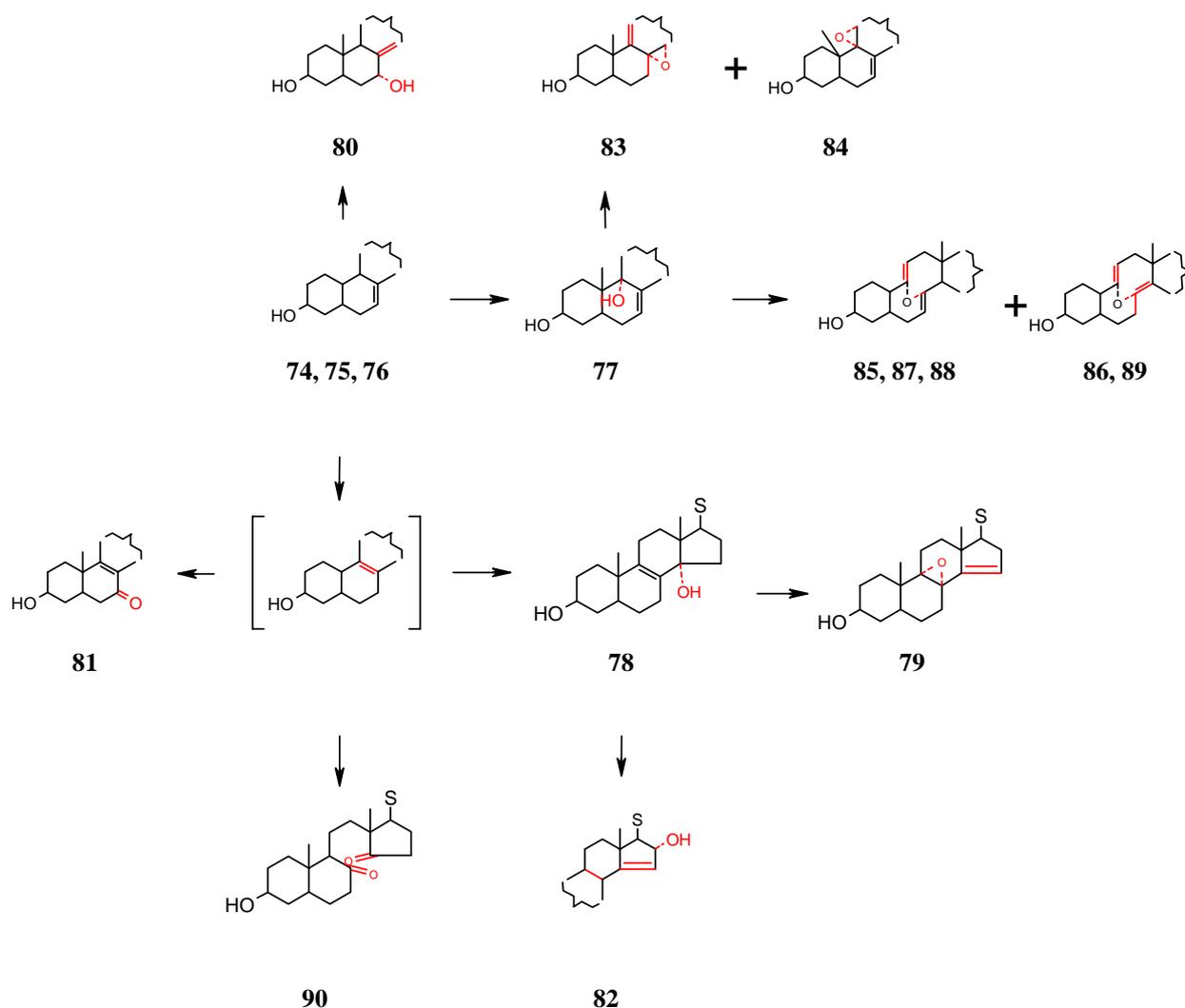
The Δ^8 migrated to $\Delta^{8(14)}$ while 8α-OH is formed in **80** isolated from *Pellina semitubulosa* [20]. The Δ^8-7-ketone **81** is from *Jereicopsis graphidiophora* [59]. The Δ^{14}-16-α-hydroxy sterol **82** is from the Mediterranean sponge *Topsentia aurantiaca* [61]. Extension of unsaturation to $\Delta^{9(11)}$ followed by epoxidation is behind **83** and **84** [62]. The products of retro Diels-Alder reaction followed by cyclic ether formation, viz., **85-87**, and their 3-methyl ethers **88**, and **89** are from *Microscleroderma spirophora* from Senegal [60] that co-occur with the 8,14-seco-8,14-dione **90**.

2. ***Sterol amines (Scheme 8):*** The steroidal alkaloids, plakinamines **91-95** are α-amino ketones that are significantly cytotoxic from a *Corticium sp.* from Vanuatu [63]. Recently, it is found that the aminoketones (e.g., **96** plakinamine F) cooccur with the aminohydrins, e.g., **97** (plakinamine E) in the *Corticium sp.* of Guam [64], and **98** in a Vanuatuan collection of the same sponge [65]. The amines **96** and **97** have moderate cytotoxicity and antifungal activity, and nucleic acid-cleaving property. These aminohydrins probably formed *via* the addition of the elements of $(CH_3)_2NOH$ across a Δ^3 which may be responsible for the aminoketones cited above. The 3-amino steroids **99** and **100** that result from the addition of NH_3 across Δ^3 are also isolated from the Vanuatuan collection [65].

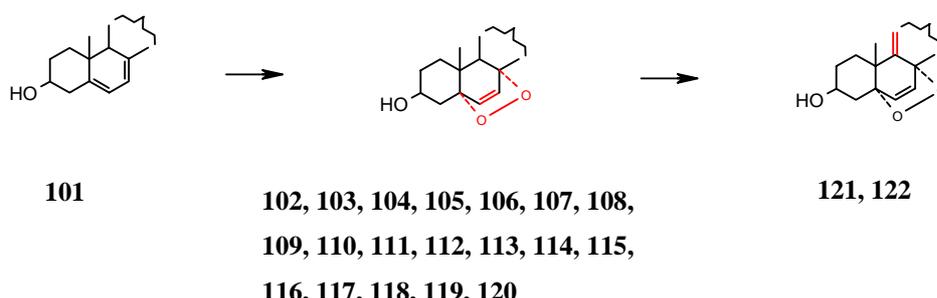**Scheme 8.** Δ^7 sterols: Sterol amines; [ ] : not isolated
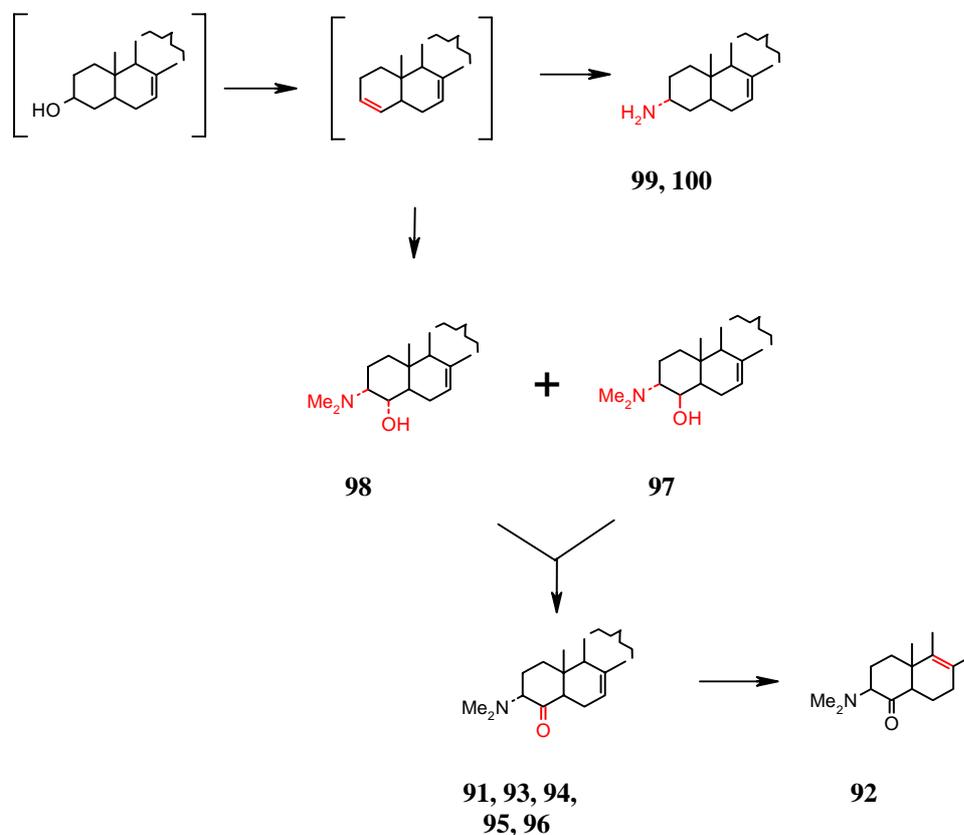
III. $\Delta^{5,7}$-Sterols

Many sponge sterols are derived by oxidation of the $\Delta^{5,7}$-sterol nucleus. An intact 3β-hydroxy-$\Delta^{5,7}$ nucleus is present in the recently isolated **101** from the Jamaican sample of *Agelas sceptrum* [66].

1. ***Epidioxides (Scheme 9):*** Endoperoxides are routinely prepared in the laboratory by the action of singlet oxygen on cyclic conjugated dienes. Hence, when the endoperoxides **102-106** were isolated from *Tethya aurantia* [67] and **102**, **107** and **108** from *Axinella cannabina* [68], it was suspected that they might be artifacts. However, such epidioxides continue to be isolated even when extreme care is taken to prevent their possible formation during extraction and isolation procedure. Thus, the Okinawan sponge *Axinyssa sp*. gave **109** axinysterol [69], and *Lendenfeldia chondroides* from Palau gave the antifouling sterols **110** and **111** [70]. The sponge species *Luffariella* cf. *variabilis* of Japan gave a mixture of the sterol epidioxides **112-120**, accompanied with the cytotoxic **121**, possessing extra $\Delta^{9(11)}$ double bond [71], which system is also present in **122**, recently isolated [72] from the same *Axinyssa sp*. that earlier gave **109** axinysterol [69] and which inhibits the growth of several human cancer cell lines.

Scheme 9. $\Delta^{5,7}$ sterols: Epidioxides

101   **102, 103, 104, 105, 106, 107, 108,**   **121, 122**

109, 110, 111, 112, 113, 114, 115,

116, 117, 118, 119, 120

2. ***Epoxy derivatives of*** $\Delta^{5,7}$***system (Scheme 10):*** The 1,2-oxides of the $\Delta^{5,7}$ sterols are predominantly α,α. The intact epoxide **123** and **124** its $\Delta^{8(14)}$ isomer, both having cytotoxicity to a range of human and murine cell lines are isolated recently [73] from *Polymastia tenax*. These 7α-alcohols are associated with the dienone **125** in the sponge. This typical dienone structure containing steroids were earlier isolated as **126**, **127** and **128** from *Clathrina clathrus* [74]. The 5α,6α-epoxy-7α-hydroxy-$\Delta^{8(14)}$ system is also present in **129** isolated from an Indian specimen of *Ircinia fasciculata* [75] which should be the biogenetic precursor of **130** [76]. The 5α,6α-epoxy group opens up in a number of possible ways (see also Scheme 1), producing 5α,6α-dihydroxy system, 5α,6β-dihydroxy system, and the 5α-H,6α-hydroxy system. The 5β,6β-epoxide system also occurs in which the 3β-OH had epimerised to 3α-OH. The opening of this epoxide also proceeds in a number of ways, e.g., 5α,6β-dihydroxy system, 5β,6α-dihydroxy system and 5β-H,6β-hydroxy sterols. In each case, the Δ^7 causes activation of sites for further modification of the sterol structure.

Scheme 10. $\Delta^{5,7}$ sterols: Epoxides and derivatives; [ ] : not isolated
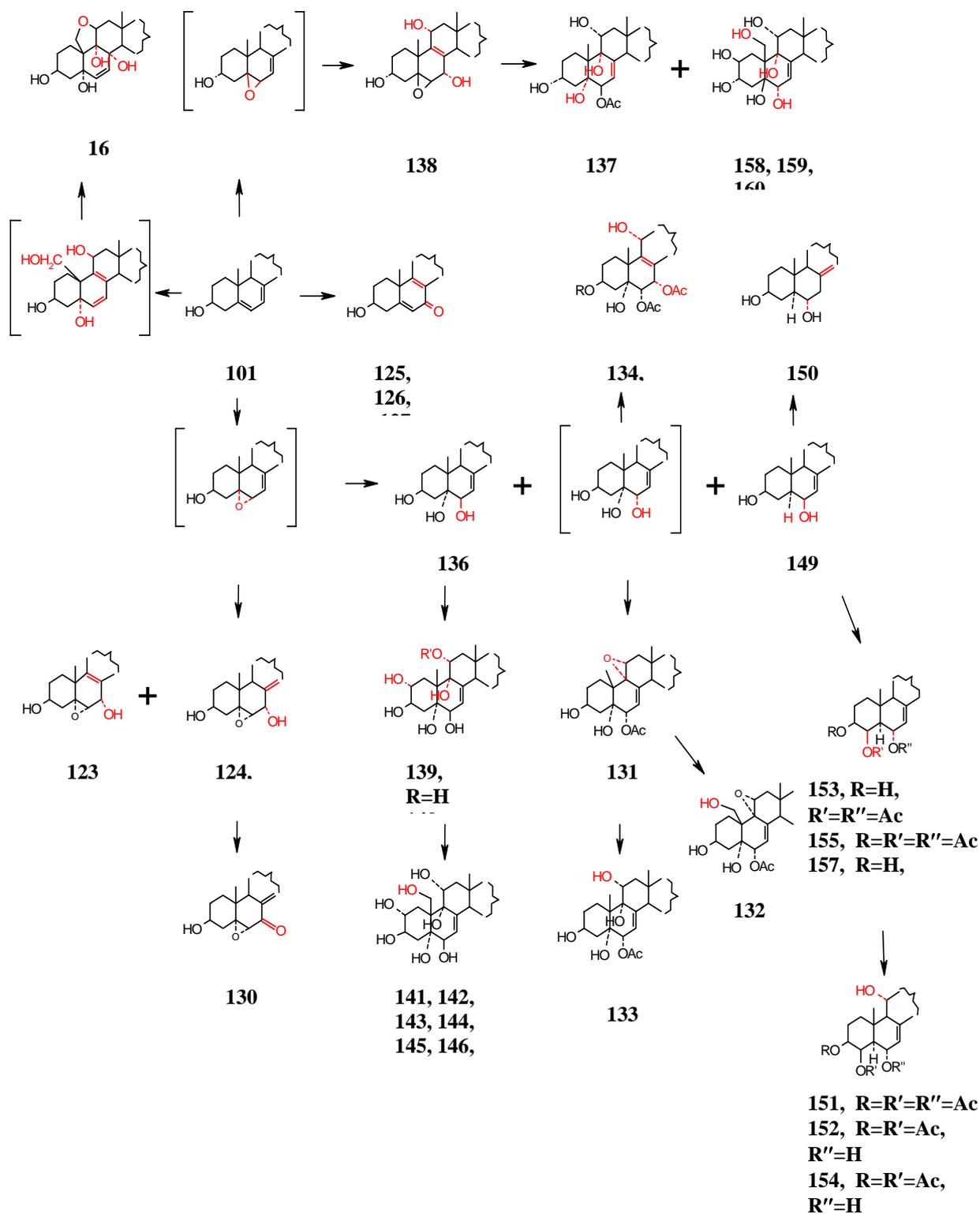
The 5α,6α-dihydroxy system is evidenced in sterols **131** [77], **132** [78], and **133** [79] which are products of oxidation at extended sites. The sterol **131** is from *Dysidea sp.* from Northern Australia, and contains the additional 9α,11α-epoxide of a $\Delta^{9(11)}$, itself made possible by action from Δ^8. The sterols that co-occur with **131** in the sponge are **134** and **135**, in which the C-11 activation is in evidence. The sterols **134** and **135** inhibit the binding of IL-8 to the human recombinant IL-8 receptor type A. The sterol **132**, also containing the 9α,11α-epoxide is from an unidentified species of *Dysidea* collected from Guam [78]. In this sterol, the 19-Me is additionally hydroxylated. The sterol **133** is from *D. herbaceae* [79] from Ethiopia. This sponge is unique since each of the four sterols **136, 133, 137** and **138** isolated from it represents one type of 5,6-epoxide (or its opening), viz., a *trans* opening of the 5α,6α-epoxide, a *cis* opening of the 5α,6α-epoxide, a *trans* opening of the 5β,6β-epoxide of the 3α-hydroxy sterol and the 5β,6β-epoxy-4α-hydroxy sterol itself respectively.

The 5α,6β-dihydroxy system is shown in addition to **136**, in **139-148**. The sterol **139** and **140** are from *D. fragilis* [80] collected in the Black Sea. The eight sterols **141-148** are from *D. etheria* from Bermuda [81]. The 5α-H,6α-hydroxy system is present in **149** [82] and **150** [83]. It is also present in **151** obtained from a Japanese *Spongia sp.* [84] which also gave **152-157** [85]. The unique feature of these six sterols is the presence of 4β-oxygen function. Further products of the 5β,6β-epoxide opening, in addition to **137** of *Dysidea herbaceae* [79] are the A/B cis **158-160** obtained from the same species of *D. etheria* that gave the A/B trans **141-148**; hence, the unique ability of the two species of *Dysidea*. *D. herbaceae* is further unique for its **161** herbasterol [86], a 5β-H-9(11)-seco steroid, which is ichthyotoxic and antimicrobial. The cyclic ether **162** is from *D. tupha* of the Mediterranean [87].

3. *9(11)-Seco Steroids***:** A $\Delta^{9(11)}$ activation produces the 9α,11α-vicinal diol system which in turn appears to be responsible for the producion by retro-Diels Alder reaction, the 9,11-seco ketoaldehydes **163-165** luffasterols A, B and C present in *Luffariella sp.* from Palau [88], **166** [45] and **167** [89] isolated from the Mediterranean sponge *Spongia officinalis*. The keto aldehyde **166** goes to the keto alcohols **168** and **169** [45] in the sponge. The epoxy keto alcohol **170** glaciasterol B-3-acetate of *Fasciospongia cavernosa* which is toxic to brine shrimp, also from the Mediterranean [90], is however not associated with its corresponding aldehyde as also in the case of **171** blancasterol from the NE Pacific sponge *Pleraplysilla sp.* [91] from Vancouver and **172** from a Japanese species of *Stelletta* [92]. In the antihistaminic secosterols **173-182** of *Euryspongia sp.* from New Caledonia [93]; the 2-OH which is usually β in this series is epimerised to α-OH.

9(11)- Seco Steroids:
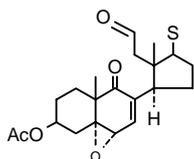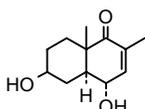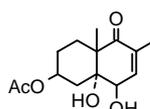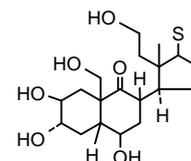

163, 164, 165,   **166**   **167**   **161**
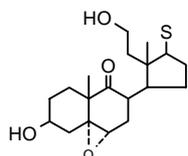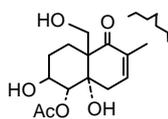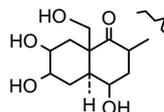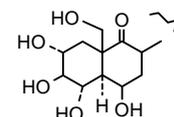
168, 169, 170   **171**   **172**   **173, 174, 175, 176,**
                                                                            177 178, 179, 180,
                                                                            181, 182

4. *Oxidation not involving 5α,6α-epoxide (Scheme 11)*: The reactions of the $\Delta^{5,7}$ system without the mediation of the 5α,6α-epoxide come under this group, e.g., **183** from an Indian specimen of *Suberites carnosus* [94]. Of particular significance is the methylation at C-4, activated by Δ^5, as indicated by the occurrence of **184** polymastiamides A [95], and **185-189** polymastiamides B to F in *Polymastia baletiformis* from Norway, of which A, C, D and F have the 4α-Me substituent and B and E do not have substitution at C-4 [96]. The mildly cytotoxic **190** from *Theonella swinhoei* from Phillippines, has instead a C-4 methylene group, a group that also occurs in the sterols **191-193** from *T. swinhoei* from Okinawa [97]. In **191** and **193**, the $\Delta^{8(14)}$ underwent oxidation to give the 8-14 seco-8,14-dione. The C-4 activation leading to a 4α-oxysulfate substitution is noticed in the ten sterols **194-203** acanthosterol sulfates A to J from *Acanthodendrilla sp.* from Japan [98]. Of these, **202** (acanthosterol sulfate I) and **203** (acanthosterol sulfate J) showed antifungal activity and cytotoxicity.

Scheme 11. $\Delta^{5,7}$ sterols: Oxidation not involving epoxides; [ ] : not isolated; S : side chain

125   **101**   **183**

184, R=Me
185, R=H
186, R=Me
187, R=Me
188, R=H
189, R=Me

194, 195, 196, 197, 198,
199, 200, 201, 202, 203

190, 192

191, 193
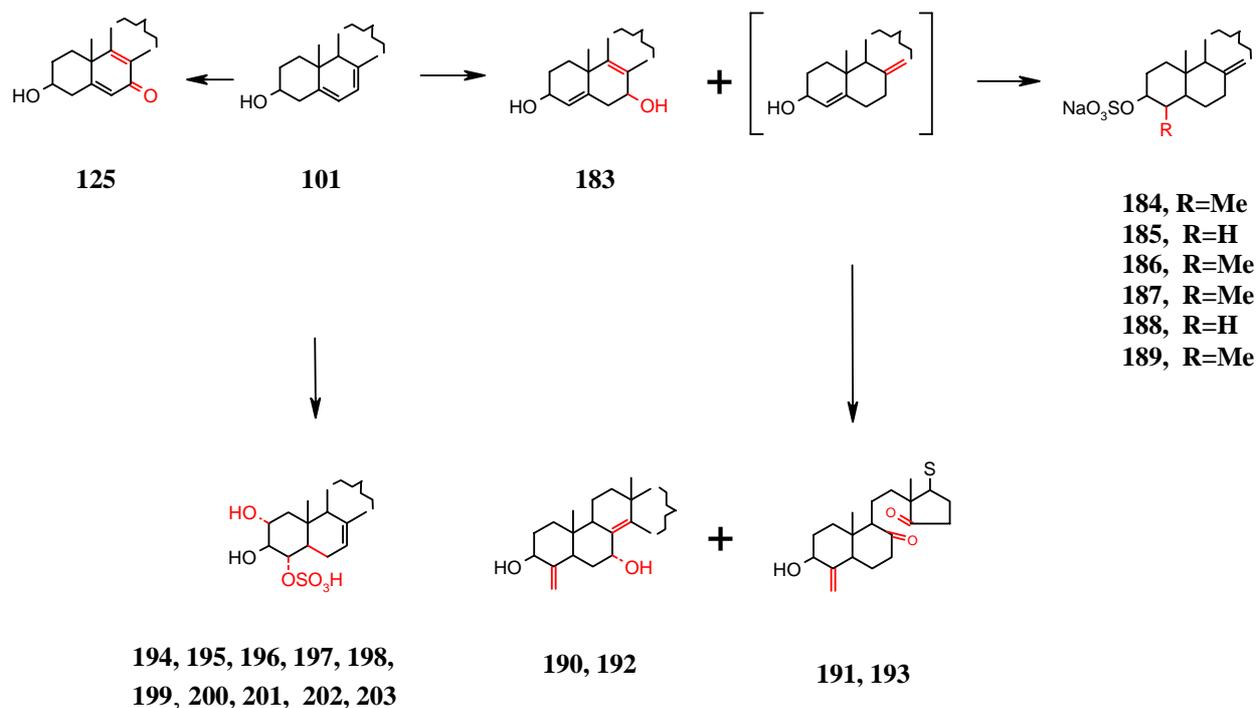
IV. 3α-Hydroxy Steroids

The mandatory configuration of the 3-OH is β_{eq} for the basic sterol skeleton. However, the shifting of Δ^5 to Δ^4 can induce epimerization of the 3-OH to α_{ax}, a cofiguration that gets stabilized by sulfate ester formation and Δ^4 reduction in the sponge sterols.

> *1.*   *Δ^5-Origin (Scheme 12)*: The ring system of the sulfated steroids has a lone representative containing unsaturation in **204** [99]; all others are saturated, cf., **205** halistanol B sulfate from *Pachastrella sp*. [100] that inhibits endothelium converting enzyme. Weinbergsterols **206** (A) and **207** (C), have hydroxylation at C-16 while weinbergsterol B **208** has further hydroxylation at C-18; they are isolated from *Petrosia weiinbergii* [101,102]. The disulfates **209**, **210** and **207** are sterol orthoesters involving 16β-OH (and 20-OH and 22-O-butyrate of the regular side chain), isolated from the same sponge. In this group, the 15α,16β-dihydroxylation is seen in **211** clathsterol with

anti HIV-1 reverse transcriptase activity from an Eritrean sponge of genus *Clathria* [103]. The cytotoxic and antifungal **212** echinoclasterol with heavily oxygenated ring E is from the south Australian sponge *Echinoclathria subhispida* [104].

Scheme 12. 3α- oxysteroids: Δ^5 origin; [ ] : not isolated, S = side chain

211

204

212   **208**   **206, 207,**   **205**
                            209, 210
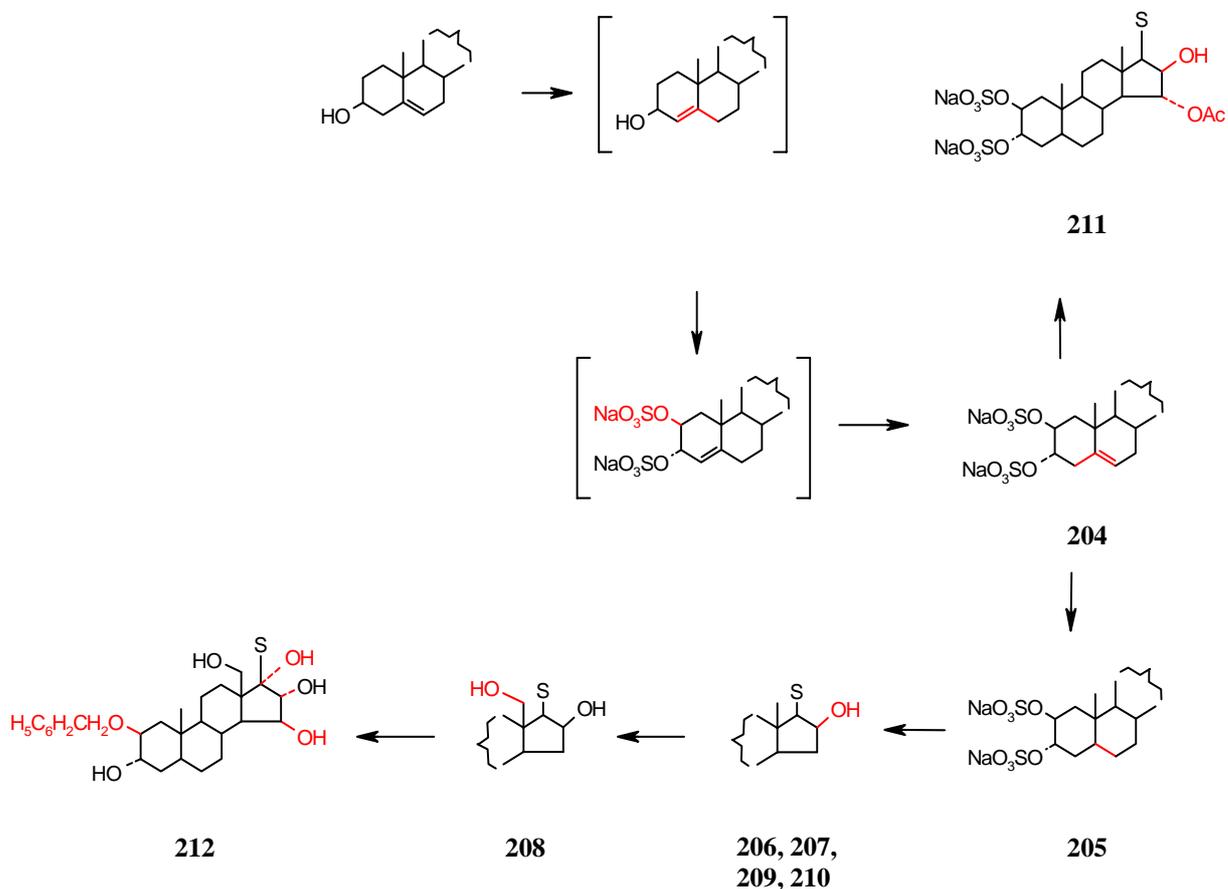
3. ***$\Delta^{5,7}$-Origin (Scheme 13)***: The 3α-sulfate esterification is more prolific when the genesis is from the $\Delta^{5,7}$ sterol skeleton. The activation of ring carbons by Δ^7 seems to extend to C-15α by migration of Δ^7 to $\Delta^{8(14)}$. In this group, **213** is halistanol sulfate from *Halichondria moorei* [99] which has potential activity against HIV virus. It is the forerunner of several halistanol sulfates, e.g., **214-217** halistanols A to D from *Epipolasis sp.* [105], and **218** to **220**, *in vitro* HIV inhibiting halistanol sulfates F to H from *Pseudoaxynissa digitata* [106]. The sterol **221** which showed inhibition in guanosine diphosphate/G protein RAS exchange assay is ophirapstanol trisulfate from *Topsentia ophiraphidites* [107]. The sterol **222** is sokotrasterol sulfate isolated from two Halichondriidea species [108], and **223** is norsokotrasterol sulfate from *Trachyposis halichondroides* [109]. The sterol **224** is from a Japanese specimen of *Topsentia sp.* [110]. The trioxysulfate **29** polysterol B sulfate of a

Japanese specimen of *Epipolasis sp.* is accompanied in the sponge with **28** polysterol A[27], a sterol that belongs to group 1 as mentioned earlier (Scheme 3).

Scheme 13. 3α-oxysteroids:Δ^{5,7} origin; [ ] : not isolated, S : side chain

R=Na; **28, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223.**
R=tris-(2 amino-imidazolium); **224**

225

229

R=H, 14β-H; 228
R=SO₃Na, 14β-H; 231
R=SO₃Na, 14α-H; 232

226

230

227

R=H, R′= cyclic with S; 233
R=PO₂HNa,
R′= cyclic with S; 234
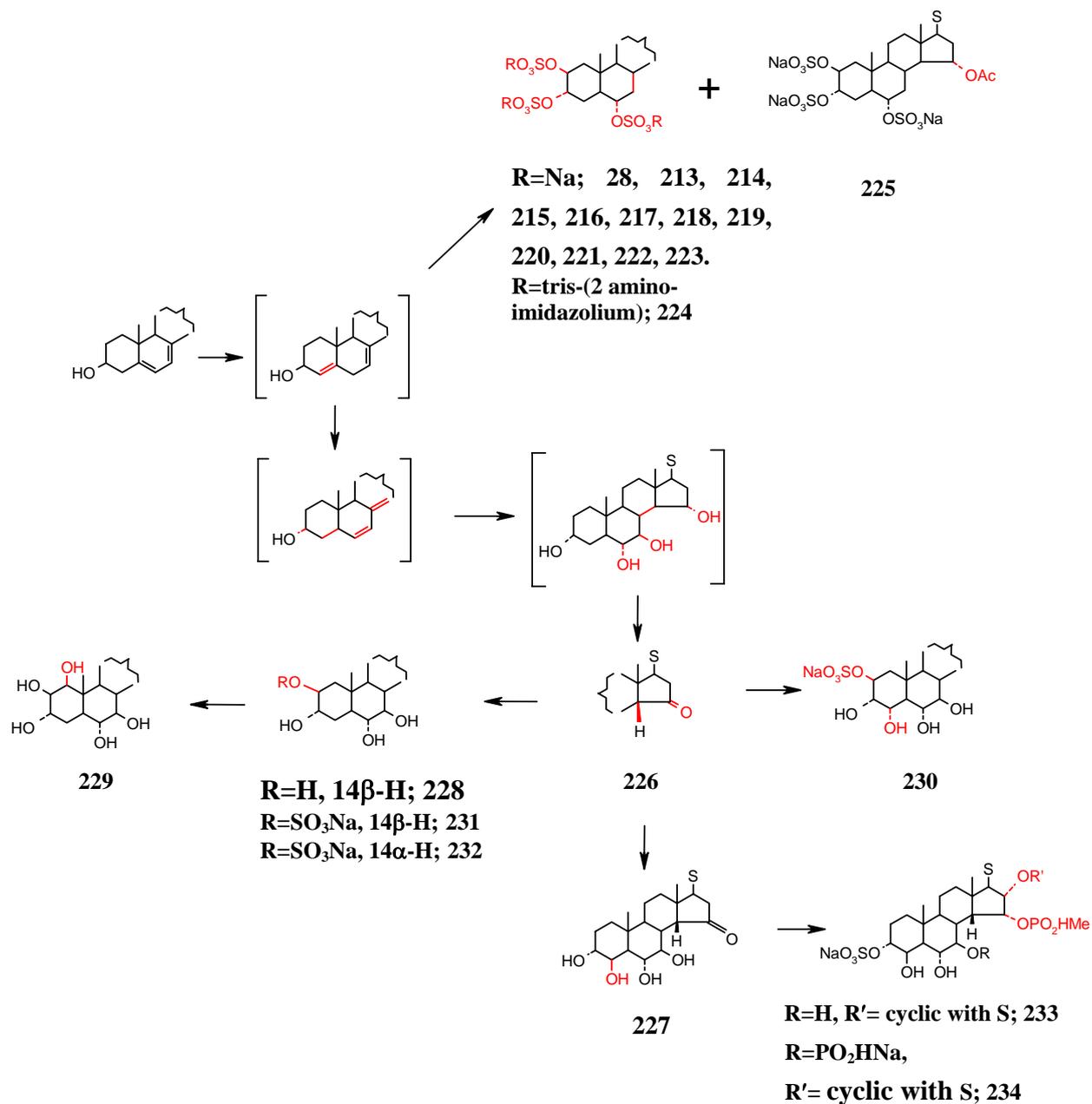
In *Epipolasis sp.* [105], the trioxysulfates are associated with the product **225** of further hydroxylation at C-15. The 15-ketosterol **226** xestobergsterol A, that inhibits the release of histamine from rat mast cells was isolated from *Xestospongia bergquistii* [111]. It was earlier isolated from *Ircinia sp.* from Okinawa [112]. The simultaneous activation of 6α by Δ^4 and 7β and 15α by $\Delta^{8(14)}$ followed by oxidation of the 15-OH to 15-ketone appears to be taking place in **227** contignasterol [113]. In these 15-keto sterols, the configuration at C-14 is 14βH as opposed to the usual 14αH configuration. The same 14βH configuration is noticed in **228** xestobergsterol C and the further 1β-hydroxylated steroid **229** xestobergsterol B from the above *Ircinia sp.* The sterol **230** with an additional 4α-OH from the Malaysian *Haliclona sp.* [114] and **231** from a new species of *Oceanapia* [115] also contain this ring structure. The sterol **231** is in fact accompanied with its 14αH epimer **232** in the sponge. Hence, a switchover of the original 14αH configuration to the more stable 14βH is indicated in these ketones. The reduction thereafter of the 15-ketone to 15β-OH should be responsible for **233** and **234** of two Philippine unidentified Haplosclerid sponges [116].

3. ***14α-Methylation (Scheme 14)***: The 14α-methylation is more common among tetracyclic triterpenes of land plants, e.g., lanosterol (**VIII**). This feature, together with the 4,4-dimethylation over the C_{19} cholesterol nucleus gives the usual C_{22} tetracyclic triterpene nucleus.

Scheme 14. 14α-Methylation; [ ] : not isolated, S : side chain
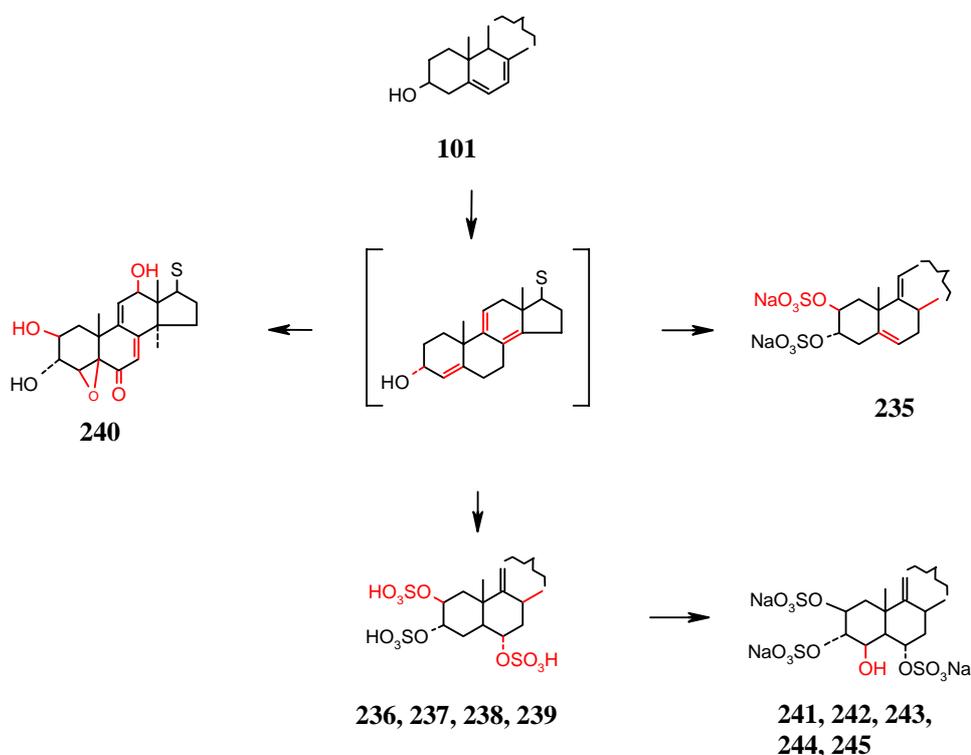
The 14α-sterols of sponges all possess a $\Delta^{9(11)}$-unsaturation indicating that biological methylation in these sterols by 1,2-addition is facilitated in a homoannular-1,3-diene ring C as shown in the Scheme 14, cf., **235** lembehsterol B with Δ^5 retained from the Indonesian *Petrosia strongylata* isolated together with the 6-O-sulfate ester viz., **236** lembehsterol A [117]. This steroid ring system was earlier found in **237** ibisterol sulfate (which is cytoprotective against the HIV-1 virus) from *Topsentia sp*. [118] and later also in **238** and **239**, ibisterols B and C of a Phillippine sponge *Xestospongia sp*. These two sterols are associated with the ketoepoxide **240** [119]. The sterols **238**, **239** and **240** are inhibitors of HIV-I integrase. In **241** to **245**, topsentiasterol sulfates A to E isolated from an Okinawan *Topsentia sp*. have the additional 4β-OH group [120].

Conclusions

As at the end of the year 2002, there are about 250 polar sterols from marine sponges that contain features of oxidation in the ring structure following a set pattern; the ring structure changing a hundred times. From this pattern, the sponges are inferred to follow pathways that appear to be distinct and characteristic of the individual sponge species. The marine sponges, in terms of their ability to produce polar sterols appear to be working on one of the four types of the sterol A/B ring system viz., (i) Δ^5-3β-hydroxy system, (ii) Δ^7-3β-hydroxy system, (iii) $\Delta^{5,7}$-3β-hydroxy system and (iv) 3α-oxy-Δ^5 and 3α-oxy-$\Delta^{5,7}$ sterol systems. In a few exceptional cases, a sponge may, however, contain sterols belonging to different classes, e.g., *Dysidea herbaceae*. Since the observed chemical composition of a sponge may have been, in addition to the intrinsic nature of the sponge itself, due to symbionts, ecological variations, and isolation procedure, these changes should be carefully considered in trying to infer biogenetic relationships. Once this is done, it may become possible to predict new structures that can perhaps fit into the gaps of the biogenetic sequence of a given sponge, before they are actually isolated as natural products.

Acknowledgements

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