UNUSUAL STEROL COMPOSITION AND CLASSIFICATION OF THREE MARINE SPONGE FAMILIES

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ABSTRACT

Five sponge species belonging to the family Suberitidae are shown to contain 80-95 % of 5α-stanols, mainly cholestanol (51 - 74 %), confirming the data already known for this family. Three *Ciocalypta* species (Halichondriidae) contained four quite unusual 24-isopropyl and 24-isopropenyl Δ5 sterols, accounting for 70 - 80 % of the total sterol mixture, mainly 24-isopropylcholesterol (41 - 59 %). Fourteen A-*nor*-sterols were found as major sterols (66 - 72 % of the total sterol fraction) in *Stylissa carteri* (family Dictyonellidae). These results confirm that sterols can provide useful chemotaxonomic data for the classification of these sponge families.

KEY WORDS

Sterols, Suberitidae, Halichondriidae, Dictyonellidae, Axinellidae, *Ciocalypta, Acanthella, Stylissa,* chemotaxonomy.

INTRODUCTION

Sponges, which occupy a low position in the evolutionary scale, are difficult to classify due to their few available useful morphological characteristics (BERGQUIST, 1978). Thus, sponges often pose a difficult identification task for systematists. Among the various sponge metabolites, sterols have been well-documented since the pioneer studies (BERGMANN *et al.,* 1950). In contrast to the terrestrial sterols, sponge sterols show complex variations in both the tetracyclic nucleus and the side chain. Over 100 unprecedented sterols have been found to date from marine sponges.

Taxonomy is essential to the development of marine natural products. The use of taxonomic knowledge makes it possible to focus investigation on sponge genera which offer the greatest potential for biologically active compounds. Sterols were the first group of sponge metabolites to be used in biochemical taxonomic studies. Sterols are present in almost all species and show great variation in composition. Certain sponges possess very low sterol content or may be devoid of sterols. Several extensive studies have been achieved (BERGQUIST*,* 1978; BERGQUIST *et al.,* 1980,

1986, 1991), and helpful data have been obtained by comparing the complete sterol composition of different sponge species. The genus *Acanthella,* is well-known to contain a lot of nitrogen-containing terpenes but little is known about the membrane constituents of sponge species belonging to the family Dictyonellidae.

As part of our investigations on sponge sterols (SJOSTRAND *et al.,* 1981; BARNATHAN *et al.,* 1992, 2000; BARNATHAN, 1993), we conducted a comparative chemical study of sterols. A sufficient number of different sponge specimens was needed to allow group characteristics of families to be appraised. This work deals with sterol composition from sponge species belonging to the families Suberitidae (*Pseudosuberite*s, *Suberites, Rhizaxinella*), Halichondriidae (*Ciocalypta*) and Dictyonellidae (*Stylissa*).

MATERIAL AND METHODS

Sponges were collected along the Senegalese coast by hand at low tide (*Pseudosuberites* sp., **A**, and *Suberites massa,* **B**) or by Scuba diving at depths of 15 - 35 m (*Suberites* sp_1, **C**, *Suberites* sp_2, **D**, and *Rhizaxinella elongata*, **E**) (1987-1997). The three *Ciocalypta* species, namely sp_1, sp_2 and sp_3, were collected off Dakar, at depth of 20 - 30 m. *Stylissa carteri,* **L,** was collected by Scuba diving in the Red Sea off Jeddah, Saudi Arabia, at depth of 10 - 20 m. For all specimens, vouchers have been deposited at the Centre d'Océanologie de Marseille, Station marine d'Endoume.

Sterols were isolated during lipid class separation from fractions eluted by dichloromethane. Sterol mixtures were analysed by GC (OV-17) and by gas chromatography-mass spectrometry (GC/MS) in free form, or as acetates. GC/MS experiments: HP-1 column (12 m, 0.2 mm i.d., 0.33 μm phase thickness). Temperature programmed at 7° C x min^{-1}, 150 - 300° C. A 30 m x 0.32 mm ID fused silica capillary column coated with DB-1 (0.25 μm phase thickness) was used to analyse sterol fractions from sponges **D**, **E** and **L**. The carrier gas was helium. Column temperature was programmed for steryl acetates from 170 to 300° C at 3° C x min^{-1}. Unusual 24-alkyl sterols were isolated and purified by high performance liquid chromatography HPLC (RP-18 column, MeOH) and studied by ^{1}H-nuclear magnetic resonance ^{1}H-NMR (Bruker AMX-500).

RESULTS

For some sponge species, several samples have been studied at different times of year. As shown below, a number of genera have not yet been investigated in the families Suberitidae, Halichondriidae and Dictyonellidae. The families Suberitidae, Halichondriidae and Dictyonellidae have been recently revised (respectively: VAN SOEST, 2002; ERPENBECK & VAN SOEST, 2002; VAN SOEST *et al.,* 1990, 2002).

Tab. I shows sponge families for which sterol composition has been reported. Among genera belonging to the family Axinellidae, Tab. I indicates those containing A-*nor*-sterols. The genus *Homaxinella* formerly classified as an axinellid sponge, is now assigned to Suberitidae and one species contains A-*nor*-sterols.

Tab. I. Classification of genera of four sponge families indicating those for which sterol composition is available. (Classification: HOOPER & VAN SOEST, 2002).

Tetractinomorpha	**Hadromerida**	**Suberitidae**
Aaptos[a] *Caulospongia Homaxinella*[c] *Plicatellopsis Prosuberites Protosuberites Pseudospongorites Pseudosuberites*[b] *Rhizaxinella*[b] *Suberites*[b] *Terpios*[a]		
Ceractinomorpha	**Halichondrida**	**Halichondriidae**
Amorphinopsis Axinyssa Ciocalapata Ciocalypta[b] *Epipolasis Halichondria Hymeniacidon*[a] *Laminospongia Spongosorites Topsentia Vosmaeria*		
Ceractinomorpha	**Halichondrida**	**Axinellidae**
Auletta Axinella[c] *Cymbastela Dragmacidon Dragmaxia Pararhaphoxia Reniochalina Phakellia*[c] *Phycopsis Ptilocaulis*		
Ceractinomorpha	**Halichondrida**	**Dictyonellidae**
Acanthella Dictyonella Liosina Lipastrotethya Phakettia Rhaphoxya Scopalina Stylissa[b] *Svenzea Tethyspira*		

[a] Data available on sterol composition. [b] This study. [c] Sponge containing A-*nor*-sterols

Suberitidae sterols

Tab. II gives data available on sterols from suberitid sponges (VAN SOEST, 2002). Major stanol content was formerly found in *Aaptos* sp., *Suberites compacta*, *S. domuncula*, *S. suberea*, *Terpios fugax* and *T. zeteki* (= *Suberites aurantiaca*) (BERGMANN *et al.,* 1950).

Tab. II. Reported 5α-stanol content (%) in Suberitidae.

Aaptos sp.	New Zealand	90.0	BERGQUIST *et al.,* 1980
Aaptos aaptos	Mediterranean	99.5	DINI *et al.,* 1984
Suberites carnosus	Mediterranean	86.0	DE SIMONE *et al.,* 1980
Suberites domuncula	Mediterranean	98.0	BERGQUIST *et al.,*1980
Suberites domuncula	Mediterranean	95.1	DINI *et al.,* 1984
Terpios zeteki (= *S. aurantiaca)*	Hawaii	90.8	DELSETH *et al.,* 1979

Tab. III shows the simplified sterol composition of our suberitid sponges. Sterol components were readily identified by GC-MS as recently reported (BARNATHAN *et al.,* 2000). Mass spectra of stanol acetates exhibited molecular ion peak M^+, and some diagnostic fragment ions, mainly m/z 215 as base peak (GOAD & AKIHISA, 1997). The level of only the major component of each sterol class is given. The complete composition has been given (BARNATHAN *et al.,* 2000). Four stanols occurred in each sponge accounting for 80 - 90 % of the total sterol mixture, including cholestanol as major component (51 - 74 %). Five usual Δ5-sterols accounted for 50 - 60 %, including sitosterol as major component. In addition, six Δ7-sterols were present at low levels except for lathosterol.

Tab. III. Sterol composition in suberitid sponges (for the meaning of **A** to **E**, see Materials and methods). All data are expressed as % of total sterol mixture.

Sterols	**A**	**B**	**C**	**D**	**E**
5α-Stanols	84.5	85.1	86.3	83,6	96.0
cholestanol	65.6	72.2	71.7	12,6	51.2
Δ5-Stenols	10.8	10.1	5.2	2.7	4.0
sitosterol	9.3	6.3	3.0	0.8	4.0
Δ7-Stenols	3.6	4.8	9.2	13.2	--
lathosterol	2.4	2.1	9.2	12.6	--

Halichondriidae sterols (*Ciocalypta*)

Tab. IV shows that the three species of brush-shaped *Ciocalypta* possessed similar sterol composition. 24-Isopropyl and 24-isopropenyl Δ5-sterols accounted for 75 - 89 % of the total sterol mixture. Mass spectra of the acetates displayed the $(M\text{-}AcOH)^+$ and $(M\text{-}AcOH\text{-}iPr)^+$ ions as expected for Δ5 sterols. These four unusual sterols were isolated by HPLC and identified by NMR.

Table 4. Sterol composition of three unidentified *Ciocalypta* species.

Sterols	*Ciocalypta*		
	sp_1	sp_2	sp_3
24-isopropenyl-22*E*-dehydrocholesterol	2.8	--	--
24-isopropenylcholesterol	9.7	--	--
24-isopropyl-22*E*-dehydrocholesterol	21.2	33.1	30.0
24-isopropylcholesterol	41.5	47.7	58.8
Other Δ5 sterols (5 to 9 compounds)	24.8	19.2	11.2

24-Isopropylcholesterol and 24-isopropyl-22*E*-dehydrocholesterol were initially identified as the only sterols in the Australian sponge *Pseudaxynissa* sp. (HOFHEINZ & OESTERHELT, 1979). 24-Isopropenyl sterols were identified in *Verongia* (= *Aplysina*) *cauliformis* and in further specimens of *Pseudaxynissa* sp. (TAM HA *et al.,* 1985). Structure of the side chain and of the nucleus (Fig. 2) of the major compounds are given below on Fig. 1 and Fig. 2.

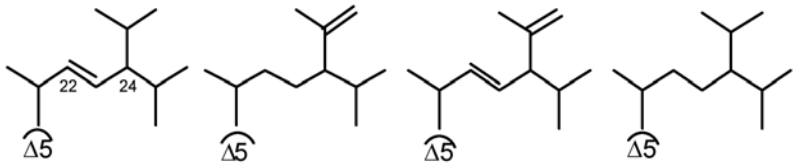


Fig. 1. Side chains of major 24-alkyl-sterols from *Ciocalypta* sponges.

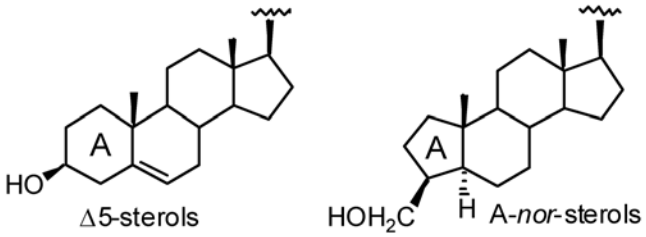


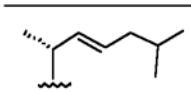
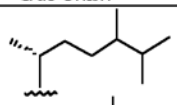
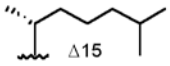
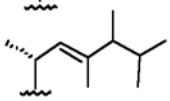
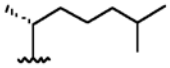
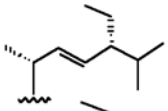
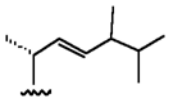
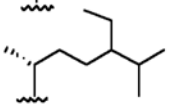
Fig. 2. Tetracyclic nucleus of usual Δ5 sterols and A-*nor*-sterols.

Dictyonellidae sterols (*Acanthella, Stylissa*)

The Saudi Red Sea sponge *Stylissa carteri* (**L**) contained mostly very uncommon A-*nor*-sterols associated with common sterols at low level (26 - 33 %). All sterol components were identified by GC-MS. Spectra of free A-*nor*-sterols display the molecular ion peak M^+ and the peak $(M\text{-}OCH_3)^+$. The base peak $(M\text{-}155)^+$ corresponds to the opening of cycle D and loss of $C_{11}H_{22}$ fragment. A rare Δ15 A-

nor-sterol was identified from diagnostic ions, mainly *m/z* 206 (GOAD & AKIHISA, 1997). Tab. V gives the composition of A-*nor*-sterols for *Stylissa carteri* (this study), *Acanthella aurantiaca (=Stylissa carteri)* collected in the Red Sea (BÖHLIN *et al.,* 1982), and *Acanthella cristagalli (=Stylissa carteri)* collected in Okinawa (TESHIMA *et al.,* 1984).

Tab. V. Major A-nor-sterols from *Stylissa carteri*[a], **L**; *Acanthella aurantiaca*[b], **M**; *Acanthella cristagalli*[c], **N***.

Side Chain	L	M	N	Side Chain	L	M	N
	1.7	5	4.2		13.5	9.0	20.7
 Δ15	7.5	6	5.7		2.1	-	-
	29.2	20	32.4		9.7	5.0	6.3
	24.2	28	18.1		10.5	15.0	10.7

[a] This study; [b] BÖHLIN *et al.,* 1982; [c] TESHIMA *et al.,* 1984. *Probably the same species *Stylissa carteri.*

DISCUSSION AND CONCLUSIONS

Only species of Suberitidae possess very high levels of 5α-stanols. All other sponge families investigated by us to date (Tetillidae, Axinellidae, Desmoxyidae, Euryponidae, Tedaniidae, Myxillidae) in the same collection sites along the Senegalese coast had usual sterol compositions, with 15 - 20 usual Δ5 3β-hydroxysterols including cholesterol, brassicasterol, campesterol, poriferasterol as major components (SJOSTRAND *et al.,* 1981; BARNATHAN *et al.,* 1992; BARNATHAN, 1993; BARNATHAN *et al.,* unpubl. data). It should be noted that a species *Homaxinella balfourensis* (genus now assigned to the Suberitidae) mainly contained 5α-stanols confirming our results (SELDES *et al.,* 1986). In the contrary, *Homaxinella trachys* that contains almost only A-*nor*-sterols does not seem to be a suberitid sponge but rather an axinellid sponge (EGGERSDORF *et al.,* 1982).

However stanols are present as major sterol components in some sponge species not belonging to Suberitidae. Thus, *Polymastia fusca*, also from the order Hadromerida but belonging to the family Polymastiidae (BOURY-ESNAULT *et al.,* 1990, BOURY-ESNAULT, 2002a), contained 96 % of stanols (DELSETH *et al.,* 1979). *Chondrilla nucula* (order Chondrosida, family Chondrillidae) (BOURY-ESNAULT & LOPES, 1985; BOURY-ESNAULT, 2002b) had 80 % of stanols (DELSETH *et al.,* 1979). *Axinella damicornis* belonging to the family Axinellidae (order Halichondrida) was reported to contain 99 % of stanols (DINI *et al.,* 1984). *Hymeniacidon perlevis*, belonging to the

family Halichondriidae, does not possess these 24-branched sterols, but appears to be close to the Suberitidae since it contains 73 % of stanols (ERDMAN & THOMSON, 1972).

About thirty years ago, A-*nor*-sterols were first isolated from *Axinella verrucosa* (MINALE & SODANO, 1974). A series of five 3β-hydroxymethyl-A-nor-steranes were identified in *Acanthella aurantiaca* (= *Stylissa carteri*) (BÖHLIN *et al.,* 1982) and four new A-nor-sterols were found in *Acanthella cristagalli* (= *Stylissa carteri*) (TESHIMA *et al.,* 1984). A-*Nor*-sterols were also identified in *Axinella proliferans* (AKNIN *et al.,* 1996) and in *Pseudaxynissa cantharella* (= *Cymbastela cantharella*), (DE NANTEUIL *et al.,* 1985). A-*Nor*-sterols have been found in sponges mainly from the family Axinellidae. Surprisingly, they have been found also in a Halichondriidae species *Hymeniacidon perlevis* (TESHIMA *et al.,* 1980). Thus, more work is required on other Halichondriidae using different chemotaxonomic markers or sequencing studies.

Furthermore, it should be noted that some Senegalese sponges, namely *Ptilocaulis* sp., *Axinella* sp., *Pseudaxinella lunaecharta* and *P.* cf. *lunaecharta* had usual sterols and no A-*nor*-sterols (SJOSTRAND *et al.,* 1981; BARNATHAN *et al.,* 1992; BARNATHAN, 1993; BARNATHAN *et al.,* unpubl. data). *Ciocalypta* sponges contained high amounts of 24-isopropyl Δ5 sterols and 24-isopropenyl Δ5 sterols. This feature was only found in *Pseudaxynissa* sp. (HOFHEINZ & OSTERHELT, 1979).

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