Sterols in Sponges

Scope and Rationale I

(aka why we care about sponges)

- Earliest widely accepted evidence for metazoa in the geological record - sponge fossils from the class Demospongia
- Geological context: Upper Phosphorite bed of the Doushantuo Formation (China, 600-570 Ma)

From a geobiology perspective the evolutionary transition from unicellular to complex mulitcellular animals is an important but poorly understood event in life's history

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Goal: Understanding evolution at the base of the metazoan tree

Sponges:

- Phylum Porifera
- Most primitive metazoa (multicellular animals)
- Classes: *Hexactinellida* (glass sponges), *Calcarea* (calcareous sponges,) and *Demospongia* (demosponges)
- *Demospongia* and *Hexactinellida* synthesize siliceous spicules; *Calcarea* synthesize spicules from calcium carbonate
- *Hexactinellida* have partial radial symmetry
- Invertebrate, sessile, primarily marine, filter-feeders that have partially differentiated tissues but without muscles, nerves, digestive systems, or internal organs
- Poor preservation and no trace fossil record
- Excellent candidate for application of biomaker techniques to more accurately constrain the first appearance of animals in the Neoproterozoic geological record (1000 to 543 Ma).

Biomarkers I

- Fossil organic compound derived from a natural product that can be assigned to particular biosynthetic origin(s)
- Provide greater insight into evolutionary transitions than possible by the traditional study of morphological fossils
- Most valuable biomarkers have well-constrained biological sources and those that remain largely structurally intact over geological time periods
- Compared to other biochemicals lipids are much more recalcitrant to dedragation
- Under optimal preservation conditions functionalized lipids are reduced to hydrocarbon skeletons that can be stable for up to billions of years

Biomarkers II

- Significant molecular information can be lost in diagenetic transformation to hydrocarbon fossils, but
- Many retain an arrangement based on a particular C skeleton whose biological precursor can sometimes be identified in extant organisms
- The presence of a particular biomarker in ancient sediments is not necessarily correlated to the presence of a particular type of organism
- Instead it may also reflect the evolution of a metabolic pathway which may be common to a diversity of organisms
- Study of living counterparts allows greater understanding of biomarkers indicative of different types of organisms, environmental conditions, or metabolic pathways in the past

Sterols I

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Cholesterol

General Structure

- Fundamental lipids present in all eukaryotes
- Serve to regulate permeability and rigidity
- Can be classed as triterpenes as they are derived from squalene

- Despite their ubiquity among eukaryotes, they, and their diagenetic alteration products, steranes, can be diagnostic for specific environmental conditions and/or taxonomic groups

E.g., one of the most specific indicators for marine conditions is the C_{30} sterane hydrocarbon, 24-*n*-propylcholestane, produced from marine chrysophyte algae

Sterols II

- $C_{27\text{-}29}$ sterols are ubiquitously and abundantly present throughout Eukarya but, consequently, are not distinctive of any particular taxon
- **Generally** . . .

Cholesterol ($C_{27:1}$) is found in animals and red algae

Ergosterol (C_{28}) is common in yeast, fungi, diatoms, and microalgae

Stigmasterol (C_{29}) is widespread in higher plants

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Sterol Synthesis I

- Sterols are the end product of isoprenoid biosynthesis in which a diversity of cyclic and acyclic compounds are built up from a universal C_5 building block isopentenyl diphosphate (IPP or IDP) leading to the C_{30} oxygenated isoprenoid oxidosqualene

- IPP can be formed by 2 different pathways, leads to different stable isotope patterns:

(1) **MVA pathway**: IPP is formed from 3 molecules of acetyl-CoA via mevalonate (found in non-photosynthetic eukaryotes)

(2) **MEP pathway**: IPP is formed from pyruvate and glyceraldehyde to form 1-deoxyxylulose 5-phosphate (DOXP) flollowed by intramolecular rearrangement and reduction to 2-C-methylerythritol 4-phosphate (MEP)

Sterol Synthesis II

- Animals, fungi, dinoflagellates cyclize **oxidosqualene to lanosterol** as the first cyclic intermediate in sterol biosynthesis
- Higher plants, most microalgae and many Protozoa convert **oxidosqualene to cycloartenol**
- This distinction is often used to infer the evolutionary history of the organism and phylogenetic relationships
- Cycloartenol and lanosterol are formed by different enzymes, each of which protonates, cyclizes, and rearragnes oxidosqualene to lanosteryl cation, but abstracts a different proton to terminate the reaction
- Cycloartenol results from ring closure and deprotonation from **C-19**
- Lanosterol is formed by deprotonating the lanosteryl cation from **C-8**

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Sponge Sterols I

- C_{30} sponge steranes (diastereoisomers of 24-isopropylcholestane) represent the only conclusive specific molecular biomarker evidence for animals in the geological record

- Sterols containing the 24-isopropylcholestane hydrocarbon skeleton have only been found in **Demosponges**; thus its presence in Neoproterozoic and early Cambrian sediments and oils is widely considered to be the result of sponges

- 24-isopropylcholestane is particularly dominant from the Neoproterozoic to Ordovician periods, most likely reflects rapid radiation, and consequent increasing abundances of sponges throughout this period

Sponge Sterols II

- Sponges potentially possess the most diverse and unique collection of sterols present in the entire metazoan kingdom

- Typically originate by:

(1) ***de novo* synthesis**

(2) dietary intake

(3) transformation of dietary sterols

(4) interaction with associated microorganisms

- Unique sterols emerge primarily due to modifications (oxygenation, alkylation, degradation) to either/both the nucleus and side chains or fundamental structural transformations of the basic skeleton

Scope and Rational II
(aka why we care about sponge sterols)

- Study of sponges offers an opportunity to evaluate both organisms (sponges) and biomarkers (sterols) and a metabolic pathway ubiquitously present throughout the animal kingdom since its origin

- Studies have shown that ability to resolve the phylogenetic histry of animals may not be possible using molecular sequences alone

- Studies specifically concerned with the abundance and distribution of 24-isopropylcholestrol (the biological precursor of 24-isopropylcholestane) among modern sponges may yield insight into the distribution of this signature biomarker among modern taxa, and possibly help to resolve evolution at the base of the metazoan tree

- In turn such study would hopefully lead to a greater understanding of the distribution of its hydrocarbon fossil (24-isopropylcholestane) in ancient counterparts

Precambrian Sponges with Cellular Structures

Li, et al., (1998)

- Earliest widely accepted evidence for metazoa in the geological record
- Geological context: Upper Phosphorite bed of the Doushantuo Formation (China, 600-570 Ma)
- Skeletons consist of siliceous, monaxonal spicules and preserved larvae
- Sponge fossils from the class *Demospongia*
- Presence of larvae suggest that calcareous sponges have an extended history in the Late Precambrian
- Data imply that *Demospongia* are ancestral form of sponges
- Evidence that animals lived 40 to 50 million years before the Cambrian Explosion

24-Isoprophylcholesterol and 22-Dehydro-24-isopropylcholesterol, Novel Sterols from a Sponge

Hofheinz, W., and G. Oesterhelt (1979)

- Report the isolation of two novel sterols (24-Isopropylcholesterol (**1)** and 22-deyhdro-24-isopropylcholesterol **(2)**) from an Austrialian sponge of the genus *Pseudaxinyssa* (class *Demospongia*, order *Axinellida)*

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- Both sterols possess normal cholesterol-like structures with 3 extra carbon atoms in the side chain
- 24-Isopropylsterols have no precedent
- Their presence in *Pseudaxinyssa* together with the unusual simplicity of the sterol mixture implies that this sponge has a highly specified ability to synthesize its sterols either *de novo* or by transformation of dietary sterols
- Specificity is not subject to environmental factors, as the authors examined 3 different samples from different reefs at different seasons, and observed similar sterol compositions
- Reasonable to consider these sterols as useful chemotaxonomic markers

Minor and Trace Sterol in Marine Invertebrates

Kokke et al., (1979)

- Report the isolation of a minor and trace sterols in the sponge *Verongia cauliformis* (Class *Demonspongiae*, Order *Verongida*)
- Isolated 24-Isopropenylcholesterol (**9**) and 24-Isopropylcholesterol (**10**) (found in Hofheinz and Oesterhalt)
- To confirm the assigned structure of **9**, a partial synthesis using fucosterol **(4)** was conducted

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- A slight modification allowed preparation of **10; 9** or **9`** appear to be plausible intermediates for **10**
- Biosynthetic implications: **9** and **10** are unique branched side chain sterols which almost certainly arise from biological methylation of fucosterol via S-adenosylmethionine (SAM); ambiguous if double bond of **9** terminates at C-28 or -25

Paleoenvironmental implications of novel C_{30} steranes in Precambrian to Cenozoic age petroleum and bitumen

McCaffrey et al., (1994)

- Found that 24-isopropylcholestanes are the most abundant C_{30} steranes in numerous Late Proterozoic and/or Early Cambrian-sourced samples
- Post-Cambrian marine oils were typically dominated by 24-*n*-propylcholestanes (molecular markers for marine chrysophtye algae), but also contained small amounts of 24-isopropylcholestanes

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- Abundance of 24-isopropylcholestanes relative to 24-*n*-propylcholestanes varies with source age rock (potential as age-diagnostic marker) :
- Late Proterozoic and Early Cambrian: high ratio (≥1)
- Younger and older samples have a lower ratio (≤0.4)
- Use this ratio because provides a benchmark for inputs of sponge biomass, relative to chrysophyte algae in sediments throughout geological time

Earlier samples - sponges not as prevalent due to predation, competition, etc.,

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Deep water sample - sponges not as prevalent compared to shelf

Older samples - before sponge radiation

INTERPRETATION:

- Temporal changes in this parameter may reflect the relative abundance of specific sponges and marine algae through geological time

- Early Cambrian abundance of certain Porifera, and much lower abundance of these organisms during the rest of the Phanerozoic suggests higher relative abundance of 24-isopropylcholestanes in Late Proterozoic and Early Cambrian sources may reflect higher sedimentary input from specific Porifera

- Consistent with the lower ratios in the older samples (e,g, B361, B453, B457) which may predate the Porifera radiation at the end of the Proterozoic

- Consistent with low ratio for a deep water Vendian calcareous shale (B455) since these Porifera would be most abundant in a shelf environment

- **Conclusion:** The 24-isopropylcholestane/24-*n*-propylcholestane ratio has excellent potential as an indicator for Later Proterozoic and/or Early Paleozoic, shallow-marine-sources

Biosynthetic Studies of Marine Lipids: *de novo* sterol biosynthesis in sponges

Kerr, et al., (1989)

- Investigated the incorporation and subsequent transformation of radiolabeled (tritium) lanosterol and cycloartenol in a variety of *Demospongiae*; sponges with and without photosynthetic and nonphotosynthetic symbionts were examined
- The precursor (titium labeled lanosterol and cycloartenol) were either transformed into 4,4,14-demethyl sterols or recovered unchanged
- Incoroporation of the precursors and transformation into sterols indicates a sponge's ability to conduct *de novo* sterol biosynthesis
- Noteworthy that the precursors are incorporated into unusual, highly alkylated sterols - not just into conventional sterols (e.g. cholesterol)
- Transformation of cycloartenol could conceivably be due to photosynthetic symbionts (e.g. cyanobacteria, zooanthellae, and zoochlorellae)
- Parallel feeding experiments were preformed with both precursors on sponge fragments with and without symbionts - in both cases both precursors were incorporated and transformed into sponge sterols to the same degree
- Similar results (i.e. lanosterol and cycloartenol effectively metabolized) for zooaxnthellae, zoochlorellae, and nonphotosynthetic bacteria

- **CONCLUSIONS:**

- Cycloartenol and lanosterol (the sterol precursors in photosynthetic (plants) and non-photosynthetic (animals) organims were transformed into 4,4,14-demethyl sterols by a variety of marine and freshwater sponges

- Symbiotic cyanobacteria, zooxanthellae, and zoochlorellae, and nonphotosynethic bacteria are apparently not involved in the observed biosynthesis

- Successful lanosterol and cycloartenol incorporation can be equated to *de novo* sterol biosynthesis

- **Conclusion:** while some sponges obtain sterols from diet, most synthesize them *de novo*

Isolation and structure elucidation of azoricasterol, a new sterol of the deepwater sponge *Macandrewia azorica*

Gross et al., (2004)

- Isolation of a new sterol (azoricasterol) (C_{29}) **(1),** with an ususual side chain, along with S-methylergothioneine (SAM) (**2**), the methylated derivative of the unusual AA ergothioneine
- Structures of compounds **1** and **2** were established via spectroscopic techniques
- The microbial biomass of the sponge is more than 60% of the entire biomass of the sponge
- Ergothioneine or its derivatives are ubiquitously present in plant and mammalian cells and tissues, but it is exclusively biosynthesized in fungi and mycobacteria
- Reasonable that SAM was assimilated by the sponge through dietary intake or is present in the microbial symbionts of the sponge

Biosynthesis of Azoricastrol Formation

Azoricastrol (C_{29}) is an unusual structure in that it contains two additional methyl groups in its side chain when compared with cholesterol

These two methyl groups form an unusual and rare elongated side chain for sterols w/ a quaternary C

Proposed biosynthesis through repeated methylation from S-adenosylmethionine (SAM)

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Mid-chain branched alkanoic acids from "living fossil" demosponges: a link to ancient sedimentary lipids?

Thiel, et al., (1999)

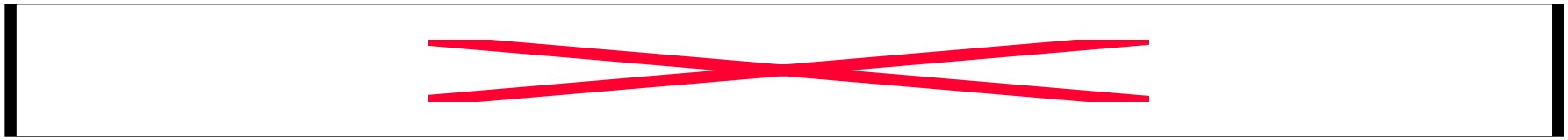
- Investigated the lipid assemblages of the the living fossil stromatoporoid *Astrosclera willeyana* and the demosponge *Agelas oroides*

- *A. willeyana* exhibits close morphological characteristics with now extinct reef building stromatopoids widespread in the Mesozoic and Palaeozoic - represents an ancient line in Porifera

- Exact systematic position of *A. willeyana* among Porifera not known; additional goal of this study is to help resolve this issue

Relative concentration of carboxylic acids in *A. oroides* and *A. willeyana* (% of total carboxylic acid fractions)

A. oroides ***A. willeyana***



1. Linear short-chain carboxylic acids

Comprise 17.8% and 26.0% of carboxylic acid fractions

Characterized by straight chain, even carbon-numbered alkanoic acids with $C_{16:0}$ (palamitic acid) and $C_{18:0}$ (stearic acid) predominating

Linear unsaturated acids are less abundant - mainly comprised of $C_{16:1}$ and $C_{18:1}$, homologues

Origin of these compounds cannot be conclusively assigned to the sponge

2. Long chain unsaturated (demospongic) acids

Comprise 12.3% and 39.2%); primary demospongic acids found: $C_{24:2}$, $C_{25:2}$, and $C_{26:2}$ acids

Demospongic acids are part of the sponge cell membranes and synthesized *de novo* via elongation of external CA precursors followed by desaturation

The presence of the C_{24}, C_{25}, and C_{26} acids appear to be a typical feature of Agelasida; rarely observed in other sponges, thus the presence of this pattern in *A. willevana* along with other biomarkers places *A. willevana* in Agelasida

3. Isoprenoic acids

Have been reported for several demosponges but not ubiquitiously observed in Porifera - often present as phytanic acid

Phytanic acid present at 2.0% in *A. willevana* and 7.1% in *A. oroides*; presence appears to be biomarker for Agelasida

Prominent occurrence of isoprenoic acids implies the potential of primitive metazoans as a biological source for funtionalized isoprenoids

Unknown if isoprenoic acids produced via *de novo* or are metabolites from modification of dietary precursors

4. *Iso-/anteiso* carboxylic acids

Carboxylic acids showing methyl branching at the ω2 and ω3 positions are *iso*- and *anteiso*-acids (*i/ai)*

Comprise 10.1% and 14.1% of *A. oroides* and *A. willeyana*

Large amounts observed for the *i/ai*- C_{15} and *i/ai*-C_{17} homologues

Widespread lipid constituents of anoxygenic bacteria; regarded as molecular markers for bacterially derived

5. MBCA

Most intriguing result of the study was the abundance of MBCA (mid-chain monomethylated carboxylic acids)

Account for 48.9% and 17.5% of *A. oroides* and *A. willeyana*

Cover a carbon number range from C_{15}-C_{25}, with methyl branching between the ω5 and ω9 positions

A. Oroides has a particularly broad spectrum of branching sites and presence of significant concentrations of MBCA over the entire carbon number range from C_{15}-C_{25}

Table removed due to copyright considerations.

MBCA origin:

Most likely from heterotrophic, symbiotic bacteria in demosponges

Generally attributed to nonphototrophic symbiont lipids rather than direct synthesis by the sponge

Conclude that demosponges have hosted their specific bacterial symbionts over geological timescales

Geological and paleobiological implications

***Iso-/anteiso* carboxylic acids**

An origin of fossil monomethylalkanes from nono-methyl branched carboxylic acid precursors is particularly evident for ancient sediments where 2- and 3-methylalkanes are observed.

These compounds are most likely derived from *iso*- and *anteiso*- branched carboxylic acids which may have been contributed by anaerobic bacteria

MBCA

In modern organisms MBCA are characteristic constituents of cyanobacterial lipids (occur in 2/3 of species so far, but other groups lack MBCAs); also occur in naturally grown cyanobacterial mats

With respect to fossil samples, the assignment of MBCA as definite cyanobacteria markers is tenuous

Instead results verify that marine bacteria are capable of producing a complex suite of structural isomers of mid-chain branched alkanoic acids which can be considered as potential precursors for complex branched alkane patterns found in ancient sediments

Interesting point is that MBCA distributions occur in bacterial symbionts of recent sponges which can be regarded as “living fossils” - since they represent the base of the metazoa tree

CONCLUSIONS:

- Found large amounts of branched carboxylic acids: including terminally branched carboxylic acids (*iso/anteiso*-) and abundant mid-chain branched carboxylic acids (MBCA) which are characterized by a variety of structural isomers present in the C_{15-25} range (so called “demospongic” acids)

- Isomeric mixtures most likely derived from hterotrophic, symbiotic bacteria living exclusively in demosponges - bacteria account for up to 60% of total sponge biomass

- The MBCA found are potential lipid precursors of mid-chain branched alkanes often present in fossil sediments and oils.

- The demospongic acid distribution and the presence of phytanic acid in *A. willeyana* correspond with the patterns found in *A. oroides* and other members of the taxon Agelasida, therefore confirming the systematic position of *A. willeyana* within this demosponge taxon

- **Working Hypothesis**: bacterial source organisms have been widespread in the geological past and are found inherited in their sponge hosts which offer a protective environment

A chemical view of the most ancient metazoa - a biomarker chemotaxonomy of hexactinellid sponges

Thiel et al., (2002)

Objectives:

- Often considered to be the most ancient metazoans - this is an ongoing debate compounded by conflicting genetic data:
- A study of the gene for Hsp70 indicated that Demospongiae form a clade with the Calcarea, excluding Hexactinellida, but recent gene sequence data support a common Demospongiae-Hexactinellida taxon
- So sequence data not conclusive! Use biomarkers!
- Investigated lipid biomarkers from 23 sponge species representing all 3 classes (Hexactinellida, Demospongia, and Calcarea) to gain insight into their phylogenetic properties, and the relationship of Hexactinellida to Demospongia and Calcarea
- Specifically targeted unique long-chain fatty acids ($>C_{24}$) (LCFA) characteristic to demosponges ("demospongic acids") to conduct these class level chemotaxonomic analyses

Results:

- Most prominent lipids were C_{28-32} polyenoic fatty acids: similar structures to demosponge membrane fatty acids ("demospongic acids"), thus supporting a close phylogenetic association between Demospongia and Hexactinellida

Note: Whereas most demosponges produce LCFA with 24-28 C atoms, the hexactinellid LCFA generally have 30 C atoms; also most hexactinellids lack methyl branched C chains among their LCFA

- Samples from Demospongia and Hexactinellida also have significant amounts of MBFA, which are characterized by isomeric mixtures of C_{15-25} homologues with single methyl groups located between ω5 and ω9 positions
- Microscopic analyses revealed good correlation between MBFA abundance and eubacteria abundance in demosponges; so MBFAs are biomarkers for eubacteria symbionts in sponges;
- The MBFAs are also present in hexactinellids, but in smaller amounts

- Samples from Demospongia and Hexactinellida also have irregular C_{25} isoprenoid hydrocarbons (which are biomakers for Archaea), with PMI skeletons
- All hexactinellids containing the C_{25} isoprenoids also contained the structurally related C_{40} homologue lycopane in even higher abundance

INTERPRETATIONS:

- LCFA:
- Hexactinellid patterns are identical to those found in demospongic acids;
- These were classically thought to be exclusive to demosponges, which have an active fatty acid elongation system and synthesize these LCFA by C_2 elongation of short-chain precursors followed by unsaturation at $\Delta^{5,9}$
- A similar mechanism for LCFA synthesis can be proposed for Hexactinellids - which have a similar unsaturation pattern
- Such co-occurrence implies very similar or identical enzyme systems for biosynthesis -
- Evidence for close phylogenetic association of Demospongiae and Hexactinellida

- MBFA:
- Can be considered plausible precursors to LCFA
- Common compounds in eubacteria, specifically non-photosynthetic microorganisms - MBFA presence attributed to these microbes and not the sponge
- The range of MBFA typically found in sponges has not been reported elsewhere in other organisms, marine sediments, or sea water
- MBFA serve as a biomarker for distinctive eubacteria highly adapted to the sponge internal environment

- PMI:
- Biomarkers for Archaea
- Co-occurrence in sponges serves as a biomarker for deepwater sponges that would lack photosynthetic symbionts

Presence of LCFA, MBFA, and PMI in Hexactinellid and Demospongiae to the exclusion of Calcarea, suggest that the former classes share a similar enzymatic system for biosynthesis of LCFA, and share common microbial communities (with a greater affinity for specific Archaea in Hexactinellids due to the more pronounced presence of isoprenoid biomarkers in these sponges; and a greater affinity for specific Eubacteria in Demosponges due to the more pronounced presence of MBFA biomarkers in these sponges)

CONCLUSIONS:

- The membrane fatty acids of Hexactinellida preserve an ancestral strategy of lipid biosynthesis, different from those found in extant organisms that are not sponges

- These unique lipid characteristics strongly support the notion that Hexactinellids are in the same group as the Demosponges

- Additional support for this phylogenetic grouping comes from the fact that both Hexactinellids and Demosponges share similar symbiont distributions relative to the Calcarea species examined; these distributions are dominated by Archaea in the Hexactinellida and eubacteria in the Demospongiae

- Lack of these biomarkers in Calcarea samples contradict the common view that Demospongia are more closely related to Calcarea than Hexactinellida

The steroids of hexactinellid sponges
Blumenberg et al., (2002)

- Follow-up to Thiol et al., 2002
- Habitat: marine, particularly remote, deeper shelf and slope environments

- Rationale: lipid constituents of these ultra-conservative animals could provide insight into the biosynthetic strategies prevailing at the very early dawn of metazoan evolution

- Investigated steroids in 20 species of Hexactinellida sponges found worldwide

- All samples had contained cholesterol (cholest-5-en-3ß-ol) and /or its saturated derivative 5α(H)-cholestan-3ß-ol, along with their C-24 alkylated (methyl- or ethyl-) homologues
- Unconventional modifications of the ring or side-chain C skeletons, common in demosponges, did not occur here
- Known intermediates occurring during *de novo* sterol synthesis from squalene (e.g. C-4 dimethylated sterols) were not observed
- 5α(H)-stanols are found in abundance among the samples
- In accordance with previous work that proposes the origin of stanols from dietary Δ^5 sterols, the authors suggest a dietary uptake of 5α(H)-stenols and transformation into 5α(H)-stanols via 3-keto intermediates
- Carbon number distributions observed resemble those from sterol mixtures of marine particulate matter

- **Conclusion: No evidence for *de novo* sterol synthesis in Hexactinellida**

Project Objectives:

- Investigated the lipid composition of 7 different modern sponges from classes *Demospongia* and *Calcarea* in order to elucidate:
- (a) the primary sterol compositions of modern sponges, particularly the distribution of 24-isopropylcholesterol among different sponge classes
- (b) whether or not there is a structural aspect preserved within sponges over geological time periods
- While we were generally interested in the sterol compositions of modern sponges, we were particularly interested in the distribution of 24-isopropylcholestrol since its hydrocarbon fossil, 24-isopropylcholestane, is an established biomarker for sponges
- More specifically, we were trying to ascertain if 24-isopropylcholestrol was present in the Calcarea group in addition to Demospongia, where it has been previously documented

Samples:

*provided by Prof. Kevin Peterson (Dartmouth)

1. *Halichondria: Demospongia* (Marine)
2. *Suberites: Demospongia* (Marine)
3. *Cliona: Demospongia* (Marine)
4. *Microciona: Demospongia* (Marine)
5. *Leucosolenia: Calcarea* (Marine)
6. *Clypeatula: Demospongia* (Freshwater)
7. *Seypha: Calcarea* (Marine)

- Total lipid extracts were separated into 5 product fractions by silica gel column chromatography and quantified by GC-MS

Sterols in Modern Sponges

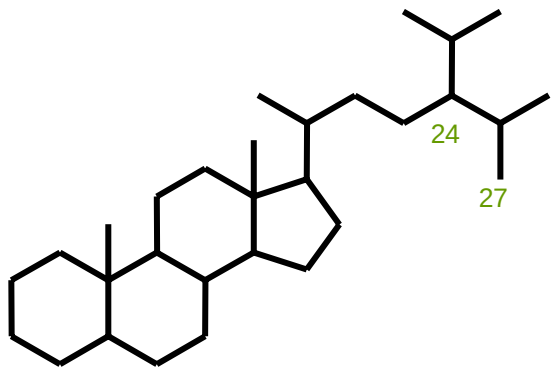
Genus	Class	Environment	C_{26} Sterols	C_{30} Sterols
Halichondria	Demospongia	Marine	Yes	No
Suberites	Demospongia	Marine	Yes	No
Xestospongia	Demospongia	Marine	Yes	No
Cliona	Demospongia	Marine	Yes	No
Microciona	Demospongia	Marine	Yes	No
Dysidea	Demospongia	Marine	Yes	Yes
Clypeatula	Demospongia	Freshwater	No	No
Leucosolenia	Clacarea	Marine	No	No
Seypha	Clacarea	Marine	No	No

Slide courtesy of G. Love

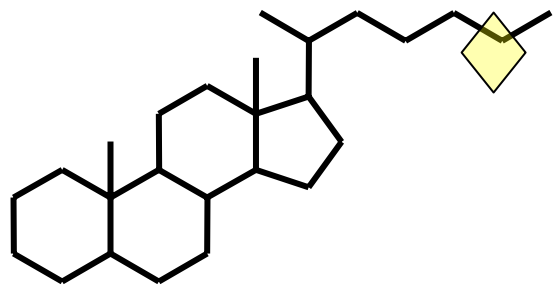
Sponges analysed in this study

Results and Discussion:

- Evident that abundant sterols were present in each of the sponges analyzed
- Unfortunately no C_{30} sterols!
- The sterols detected primarily consisted of a variety of $C_{27\text{-}29}$ desmethylsterols (not methylated at C-4)
- Cholesterol ($C_{27:1}$) is typically the most abundant sterol component of sponges
- Concurrent analysis of oils and sediments from the South Oman Salt Basin indicate that certain C_{26} steranes (27-norsteranes) may also constitute sponge markers - so these were of interest as well.
- **Trace levels of C_{26} sterols were detected in all four marine demosponges, these compounds offered promise as precursors of molecular markers of marine sponges**



24-isopropylcholestane- (C_{30})



27-norcholestane- (C_{26})

Thank you!

If you would like more information, the following reviews are very good:

1. **Steroids from sponges: Recent reports**

   Aiello, A., et al., (1999) in Steroids 64 (687-714)

2. **Sterols and other triterpenoids: Source specificty and evolution of biosynthetic pathways**

   Volkman, J.K., (2005) in Organic Geochemistry 36 (139-159)

3. **Sterols in microorganisms**

   Volkman, J.K., (2003) in Applied Microbiologial Biotechnology 60 (495-506)

EXTRA SLIDES

Scope and Rational III
(aka why we care about sponge sterols)

- The presence of unusual steroids in abundance in sponges is significant because they likely have a functional (rather than metabolic) purpose in sustaining membrane structure

- The distinctive nature of sponge sterols may result from structural integration with other membrane components, specifically phospholipids, which have head groups and fatty acids distinct from those found in higher animals

- Thus the structural variations creating the vast array of sponge sterols may arise from modifications intended to enhance the incorporation with other membrane parts

Sedimentary 24-*n*-Propylcholestanes, Molecular Fossils Diagnostic of Marine Algae

Moldowan et al., (1990)

- C_{30} sterane biomakers in sedimentary rocks and crude oils have a marine origin
- Appeared in the early Paleozoic
- Algae that biosynthesize their precursor sterols evolved between the Early Ordovidican - Devonian
- Steranes typically found as a mixture of stereoisomers at the C-5,14,17,20, and 24 positions