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SPONGES IN QATARI WATERS A NEW SOURCE OF MARINE NATURAL PRODUCTS FOR BIOLOGICAL APPLICATIONS

By

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ABSTRACT

Sponges are the most primitive multicellular organisms belonging to Animalia kingdom. They lack symetry and do not have differentiated tissues or organs. However, as a consequence of this primitive organization it is very likely that sponges are the richest source of marine organisms for biological properties and pharmaceutical applications. Attached for life on their substrate and devoid of mecanical protection against predators sponges have elaborated a lot of sophisticated means of chemical defence including metabolites produced by the sponge itself and by endosymbiotic microorganisms. This could explain that Porifera is the most studied phylum of marine invertebrates for both chemical and pharmacological point of view. This aspect of sponge chemistry will be presented and developed according to their biological activities.

To the best of our knowledge, no inventory of sponges from Arabian Gulf is available and very few is known about their biodiversity. Scarce data available for the whole region will be presented. Inventory of sponges from Qatari waters started two years ago and it is in progress. From preliminary results it appears that most of collected sponges belong to Haplosclerida, an order for which very few is known concerning secondary metabolites and this explains the great interest of this research program. Some original results on Sponges from Qatar will be presented in the lipid field.

INTRODUCTION - Why Sponges are so interesting ?

From a zoological point of view sponges are still a matter of controversy. Due to their position in the phylogenetic tree (Figure 1, {1}.) sponges are located between unicellular organisms (protozoa) and true pluricellular eukaryotic animals (metazoa). It is very difficult about sponges to define an « individua » as likely without any ambiguity about andy other animal. This explains why some biologists have created the term « parazoa » for sponges only {2}.

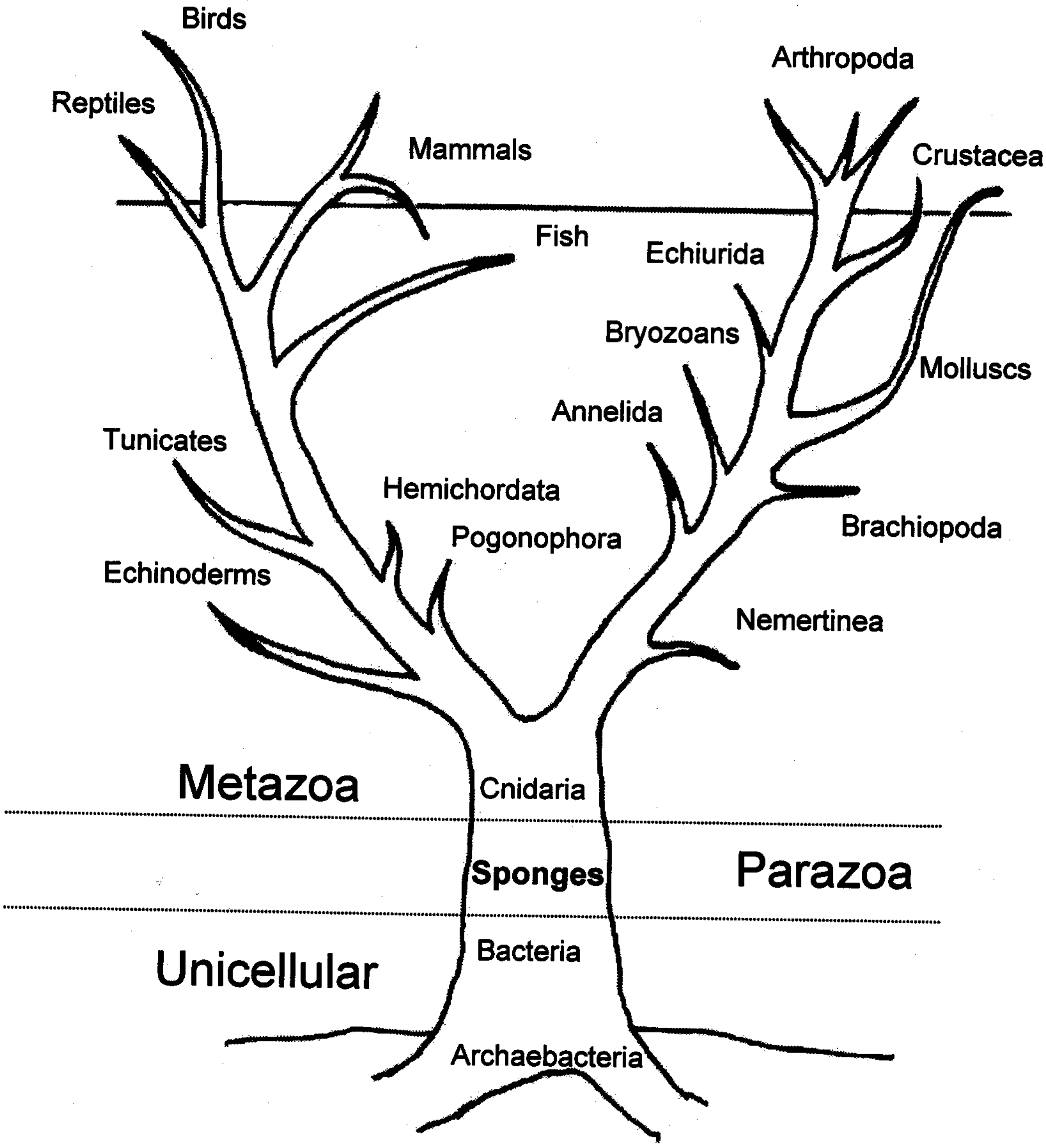


Figure 1 : Position of Sponges among other phyla (from [1]).

From a chemical point of view in the Marine Natural Products field the importance of Sponges clearly appears in the following Tables and Figures.

Table 1 : Marine Natural Products - State of the Art since 1970 [3].

Phyla	Nb. of publications	Nb. of new molecules
Bryozoans	136	112
Chlorophyceae	143	22
Tunicates	528	532
Molluscs	694	785
Echinoderms	388	825
Phaeophyceae	774	1,018
Rhodophyceae	684	1,054
Cnidaria	1,098	1,825
Sponges (Porifera)	**2,070**	**3,473**

So, among all categories of marine organisms - algae and marine invertebrates- sponges are the most studied organisms. The distribution of natural products between nitrogenous and non-nitrogenous compounds is also of interest because most of active molecules contain nitrogen atoms. This distribution is given in Figure 2 for the main classes of marine invertebrates.

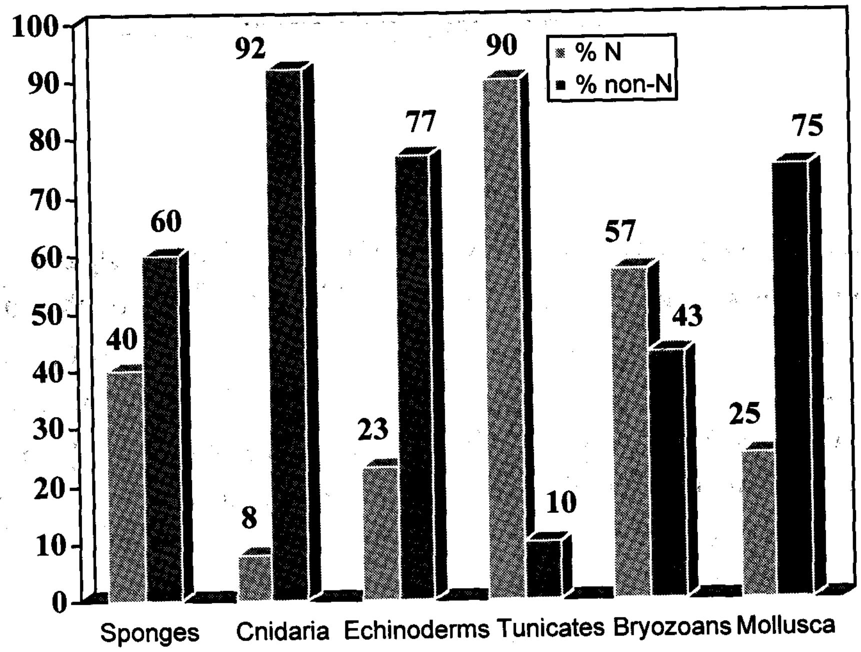


Figure 2 : Distribution of nitrogenous componuds and non-nitrogenous compounds among the main classes of marine invertebrates (from [3] and [4].

It appears from Figure 2 that only two phyla, sponges and bryozoans, have a quite similar distribution for nitrogenous and non-nitrogenous compounds. Meanwhile, bryozoans although attached like sponges are very difficult to collect in appreciable quantity and also difficult to identify due to the lack of specialized taxonomists. Thus, sponges appear as the best phylum for study of both nitrogenous and/or non-nitrogenous compounds. Furthermore, from Figures 1 and 2 it should be noticed that the content of nitrogenous compounds within a given phylum is not at all related with its position on the phylogenetic tree. All this could explain the extensive researches on sponge chemistry all over the world. Table 2 summarizes this trend in Marine Natural Products chemistry.

Table 2 : Sponge chemistry - State of the art since 1970 [3].

Period	**Nb. of publications**	**Nb. of new molecules**
1970 - 1974	63	99
1975 - 1979	164	225
1980 - 1984	208	324
1985 - 1989	450	703
1990 - 1994	770	1,379
1995 - 1997	415	743
Total	**2,070**	**3,473**

The conclusion for Table 2 is very impressive : since the 90's a new compound is isolated from a sponge every 2 days ! The discovery of biologically active compounds from sponges-some of which being already on the pharmaceutical market - is not new. To the best of our knowledge the first pharmaceutical produced thanks to sponge chemistry is the antineoplastic and antiviral Aracytine™ (UpJohne). Figure 3 displays a very short list of important compounds isolated from sponges with intense biological activity.

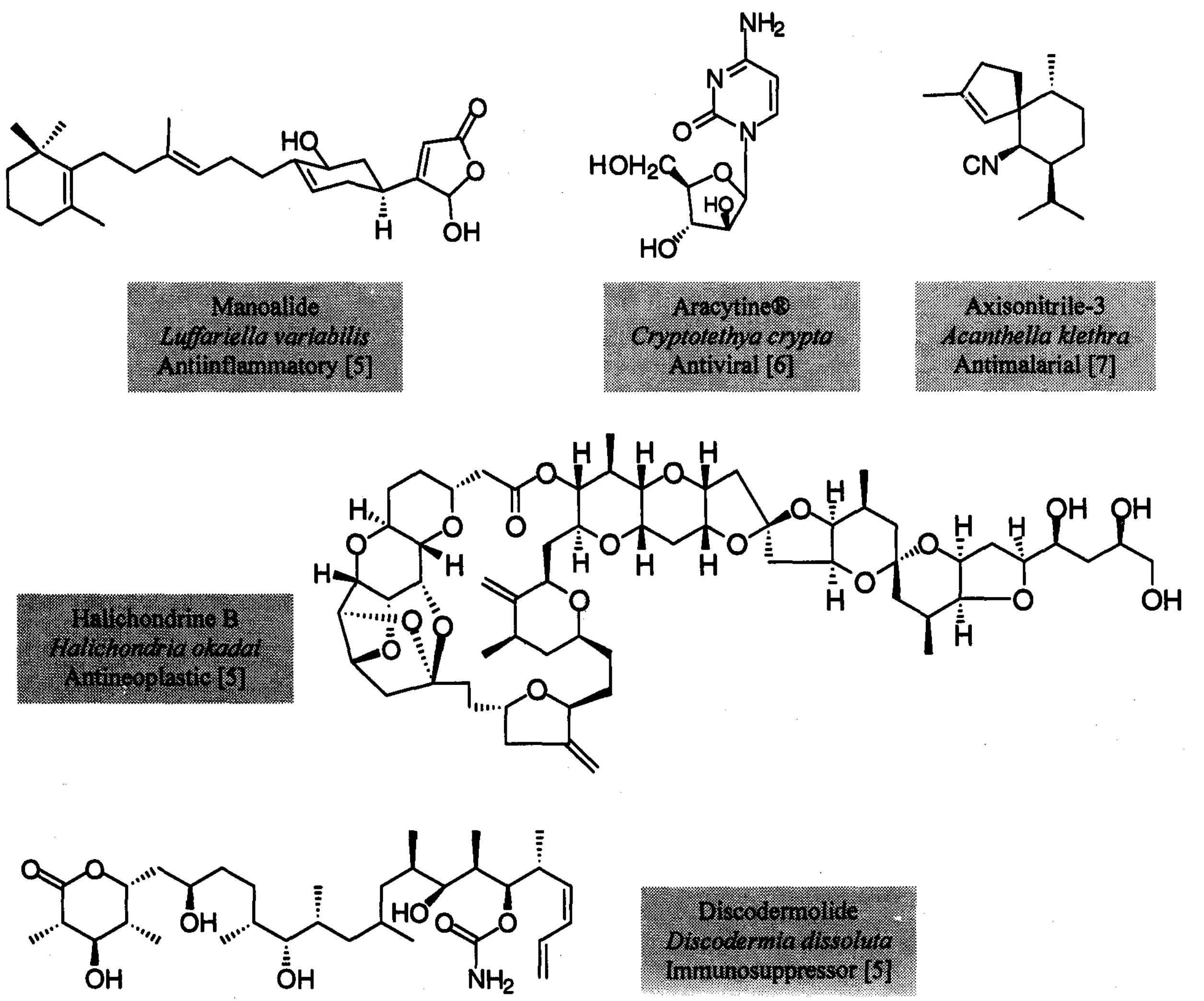


Figure 3. Some sponge metabolites with high biological activity.

Basic considerations on Sponges

Basically, *a sponge is a sedentary, filter-feeding metazoan which utilizes a single layer of flagellated cells (choanocytes) to pump a unidirectional water current through its body* (2). The filtering activity of a sponge is very high, a sponge is considered to filter its own volume within a minute and it finds oxygen and all needed nutrients in the circulating sea water. The basic organization of a sponge is given in Figure 4.

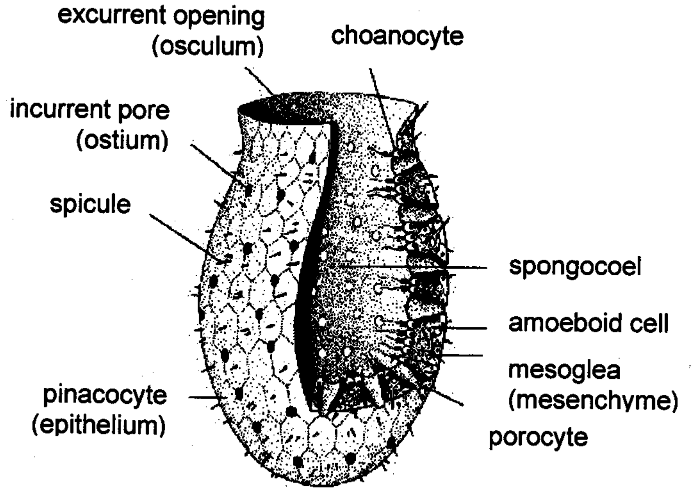


Figure 4 : Basic organization of a sponge (from [2, 5])

Sclerocytes from mesohyle produce and organize the skeleton elements called spicules (Figure 4). The spicules are either siliceous (silica) or calcareous (calcite). Two kinds of spicules are to be distinguished : the microscleres, the smaller with 2 to 100 µm size and the megascleres, the larger with 100 to 4,000 µm size. Associated with the spicules are the spongin fibers, a filamentous protein similar to the horn one, Most of sponges contain both spicules and spongin fibers, some of them only contain spongin and there is only a very few number of species that are completely devoid of skeleton.

Two main criteria are used for classification of sponges.

The first one is a biological criteria :

The mode of reproduction that can be oviparous or viviparous.

The second criteria is a physical criteria :

The chemical nature, symetry degree, size and shape of spicules along with the overall form and organization of the skeleton. According to both criteria specialists agree to classify sponges within four classes but most of sponges belong to the Class Demospongiae (Table 3).

Table 3 : The four classes of sponges

Class	Nb. of publications [3]	Nb. of compounds [3]
Calcarea	25	36
Demospongiae	2,040	3,437
Hexactinellidae	5	-
Sclerospongiae	-	-
Total	**2,070**	**3,473**

So, the chemistry of sponges is essentially the chemistry of Demospongiae and to have a good look on this very large class.

Table 4 summarizes the main features and the classification of Demospongiae.

Table 4 : Classification and main features of Demospongiae

Ceractinomorpha

* Viviparous
* Megascleres are always monaxonids
* Microscleres are often sigmoids
* 6 main orders :
  - Halichondrida, Haplosclerida and Poecilosclerida
  Contain siliceous spicules and spongin fibers
  - Dendroceratida, Dictyoceratida and Verongida
  only contain sponging fibers

Tetractinomorpha

* Oviparous
* Megascleres are monaxonids or tetraxonids
* Microscleres are often asteroids
* 5 main orders : Astrophorida, Axinellida, Desmophorida, Hadromerida, Spirophoida

Homoscleromorpha

* Sponges devoid of any skeleton *(Oscarella)* belong to this rare class

From an ecological point of view sponges are usually living on rocks or stones but some rare species have been observed on plant roots, especially in mangroves.

Being attached and completely devoid of movement sponges are chemically protected against predators. This explain why secondary metabolites from sponges are so numerous. However, all these chemicals can be elaborated either by the sponge itself or by endosymbiotic microorganisms. This is especially the case for terpenes and terpenoids, often abundant in sponges by usually synthesized by endosymbiotic chlorophyllic microorganisms.

To summarize, the chemodiversity of phylum Porifera (sponges) is very likely the largest of the living world, at least for eukaryotic pluricellular organisms. The main features of this chemodiversity are the following (8):

* All classes of chemicals have been found in sponges.
* All Demosponges are known to contain specific long-chain fatty acids, so-called demospongic acids {9}, most of them containing bromine atoms and other unusual groups.
* Sponges from Axinellida order often contain unusual nitrogen-containing terpenes and unusual sterols.
* Dictyo-and Dendroceratida (all devoid of spicules) contain high level of terpenes, especially rare sesterterpenes and low level of sterols.
* All kinds of biological activities are observed in sponges, especially antitumoral, anti viral, antiinflammatory and immunosuppressive. Several bioactive compounds isolated from sponges are good lead compounds for clinical uses.

Sponges from the Gulf and around Qatar coasts :

Sponges in the Gulf are quite unknown. To the best of our knowledge the only report of sponges in the Gulf consists in two colour pictures of unidentified sponges probably form Qatar coasts (10). Despite the fact that almost all remains to be done in the sponge field. the lack of information about it could be considered as good for scientists because sponges in the Gulf appear to be a completely new area of research. In the context of our multidisciplinary research program on marine organisms (algae and invertebrates) around Qatar coasts biologists and chemists have work for tens of years.

In this context, since 1996 we have initiated the inventory of Qatar sponges in relation with the Department of Marine Science, Faculty of Science, University of Qatar and some preliminary but interesting results were achieved. From a general point of view and despite some analogies it is too early to compare sponges from Qatar (Gulf area) and those from the Red Sea. General trends about Qatar sponges are the following :

* Demospongiae from Qatar mainly belong to the subclass Ceractinomorpha for which species from 5 out of 6 orders have been identified.
* Within this subclass order of Haplosclerida and Hadromerida are well rep-

resented . Haplosclerida is important because few is known about its chemistry but this order has recently been shown to contain straight-chain acetylenes as chemotaxonomic markers (II). Due to the fact that sponges from the whole Gulf area are completely unknown from a chemical point of view, so the study of these sponges appears to be particularly important.

* Concerning Calcareous sponges two genera of only one order have been found.

All these preliminary but however important results are displayed in Table 5.

Table 5 : Sponges from Qatar - preliminary results

Class	Sub-class	Order	Genus
Demospongiae	Ceractinomorpha	Haplosclerida	*Callyspongia, Gelliodes, Niphates*
		Poecilosclerida	*Tedania*
		Dendroceratida	*Aplysilla*
		Dictyoceratida	*Dysidea, Ircinia*
		Verongida	*Hexadella*
	Tetractinomorpha	Hadromerida	*Chondrillastra, Cliona, Cliothosa, Suberites*
	Homoscleromorpha		*Oscarella*
Calcareae	Calcinea	Clathrinida	*Clathrina, Leucetta*

It is surprising that two very common orders : Halichondrida, subclass Ceractinomorpha and Axinellida, subclass Tetractinomorpha have not yet been found in Qatari waters but researches for sponge inventory are still in the beginning. Also strange, within Verongida order we have not yet found any specimen of common and well-known genera *Verongia (Aplysna)* or *Suberea.* However a species belonging to the rare genus *Hexadella* has been collected.

Sponge lipids - basic considerations

There are many scientific reasons to study sponge lipids, some of them can be summarized as follows :

* Sponge lipids are a rich source of new chemical structures, either for simple lipids such as fatty acids or sterols or for more complex lipids such as glycolipids that contain both an aminoalcohol and carbohydrate moiety.
* The comparative chemical studies usually done on simple lipids can provide useful additional characters to evaluate existing classifications and even to suggest taxonomic relationships.
* The identification of postulated intermediates can illustrate known or new biosynthetic pathways.
* Fatty acids and sterols are biological markers for microorganisms so, it is possible to determine what part of fatty acid content could be attributed to endosymbiotic symbionts.
* It is important to understand the role of the unique phospholipid fatty acids and sterols that constitute the sponge cell membrane by comparison with other organisms.
* The global cell structure is the same for all organisms : a bilayer of amphipathic lipids (phospholipids, glycolipids, sterols) with globular proteins embedded in it (Figure 5).

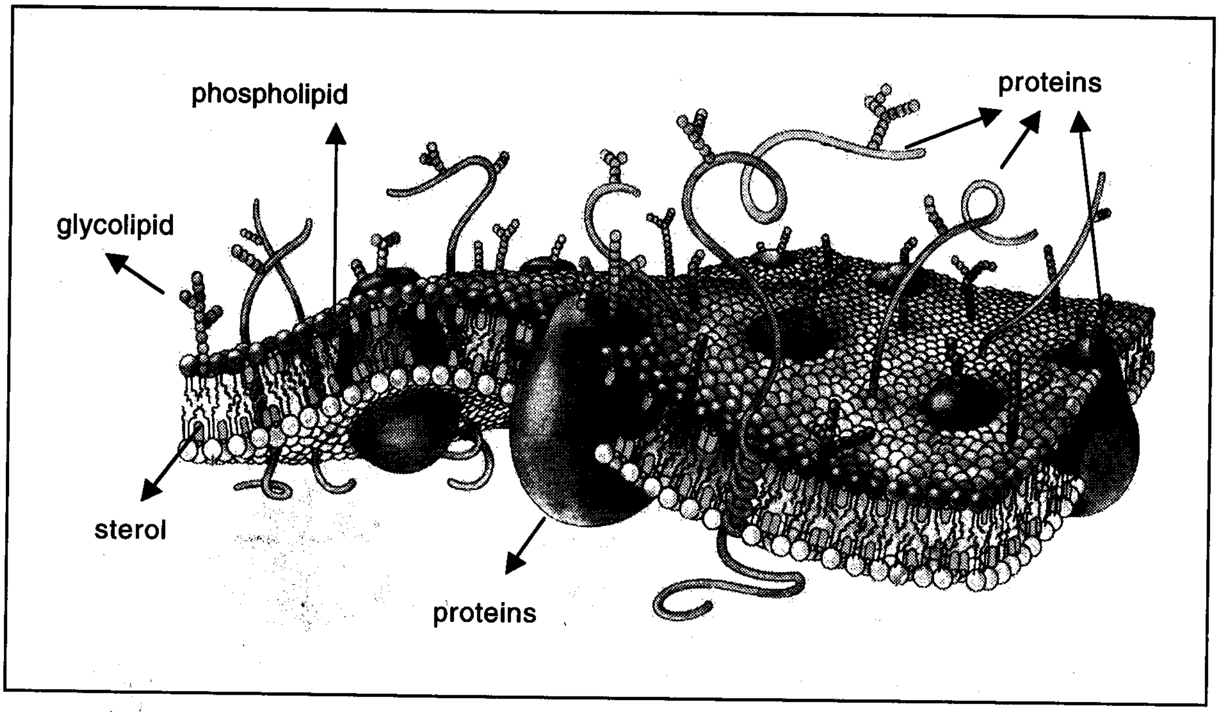


Figure 5 : Simplified scheme of a cell membrane (from [13])

Due to this general structure the physico-chemical properties of cell membranes depend on the nature of phospholipid hydrophobic chains (phospholiped fatty acids). In a famous paper Litchfield and Moralles showed that these properties are better described with the phospohlipid fatty acids fall inside region, called " region of feasible adaptation ", for all organisms (micro-organisms, plants and animals) but sponges (Figure 6, [10]).

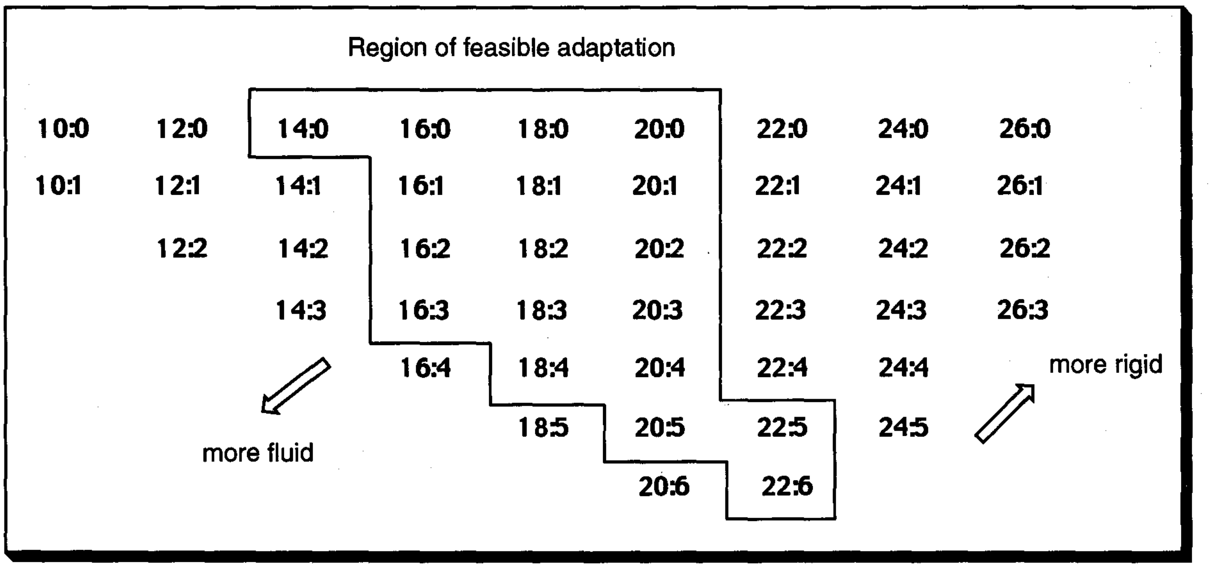


Figure 6 : Litchfield matrix, major phospholipid fatty acids (> 5%) in cell membranes [10]

This matrix was originally established with major (>5%) fatty acids occurring in more than 30 organisms, half of which were marine ones. For sponges the corresponding matrix is completely different, most of phospholipid fatty acids (demospongic acids) fall outside the central region in contrast with other organisms (Figure 7).

14:0	15:0	16:0	17:0	18:0	19:0	20:0		22:0	23:0	24:0	25:0	26:0	27:0	28:0			
		16:1	17:1	18:1	19:1	20:1		22:1		24:1		26:1	27:1	28:1			
		16:2		18:2		20:2	21:2		23:2	24:2	25:2	26:2	27:2	28:2	29:2	30:2	
				18:3								26:3		28:3	29:3	30:3	
		16:4				20:4											
																30:5	34:5
								22:6									

Figure 7 : Major phospholipid fatty acids (> 5%) for sponge-cell membranes

Thus, the fatty acids from sponge phospholipids have a series of very unusual characters :

* Very long-chain ranging up to 34 carbon atoms.
* Even and odd-number of carbon atoms in approximately the same quantity.
* Most of them contain the unusual Δ5,9 diene system and some rare monosaturation patterns such as Δ7, Δ11, Δ13, Δ17 and Δ23.
* Several fatty acids have been shown to contain vinylic bromine atoms on C6.
* Branched demospongic acids often contain methyl groups on unusual positions, especially *iso* and *antiso* pairs.
* Demospongic acids are found mostly in the aminophospholipids, phosphatidy1 ethanolamine (PE) and phosphatidylserine (PS).

Phospholiped fatty acids from Qatar sponges

The Figure 8 gives the well-known widely used and complete general procedure to isolate all classes of lipids from any kingdom of organism. According to the compounds he wants to study; the researcher will focus on a special class. Thus, for our first researches on lipids from Qatar sponges, we have isolated and studied only phospholipid fatty acids and glycolipids but, of course, other chemical classes such as sterols and apolar lipids will be investigated later.

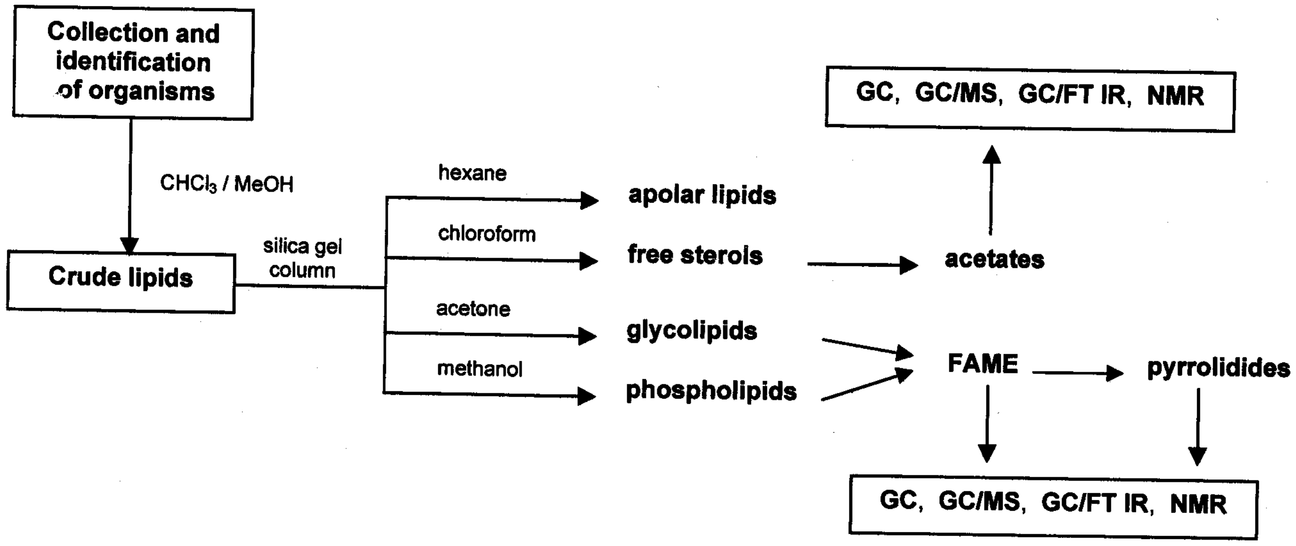


Figure 8 : General procedure for lipid analysis

The identification of every fatty acids is performed by gas-chromatography/mass spectrometry experiments (GC / MS) due to the very tiny quantities thus, 2 or 3 mg of purified *N* -acylpyrrolidides derivatives of phospholipid fatty acid mixture usually contain from 50 to 75 different compounds. The determination of the particular Δ5, 9 fatty acids can be achieved without difficulty using the base peaks *m/z* 113 (MacLafferty rearrangment of *N*-acylpyrrolidides derivatives) and other key peaks establishing the position of double bonds (*m/z* 140/152, 180, 196/208, ...) (Figure 9).

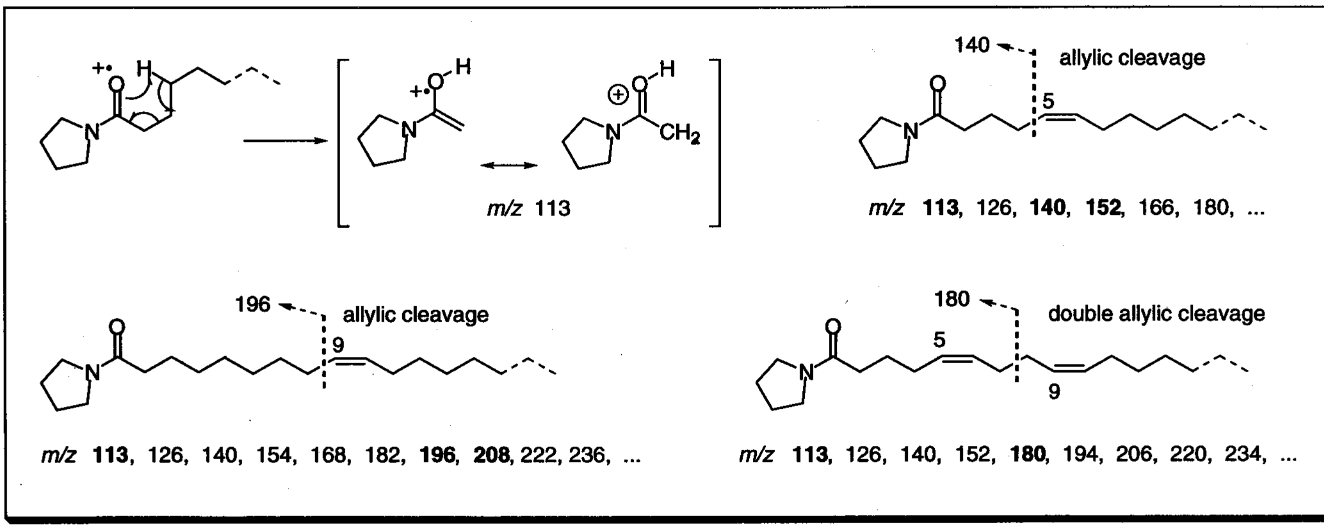


Figure 9. MacLafferty rearrangment and other key fragmentations for *N*-acylpyrrolidides.

The Table 6 gives the Δ5.9 phospholipid fatty acids composition for five sponges collected off the coasts of Qatar.

Table 6 : Δ5,9 fatty acid composition of 5 sponges from Qatar

Δ5,9-fatty acid	*Gelliodes incrustans*	*Niphates* sp.	*Callyspongia* sp.1	*Callyspongia* sp.2	*Dysidea* sp.
16 :2	-	0.3	-	-	-
17 :2	-	-	-	5.9	-
18 :2	-	1.9	-	-	-
20 :2	0.7	-	-	-	-
22 :2	0.4	-	-	-	-
Δ 5,9,17-24 :3	-	-	-	-	2.3
24 :2	0.4	0.3	-	-	19.1
25 :2	2.1	-	2.2	-	16.2
6-Br-26 :2	2.3	-	-	-	-
Δ 5,9,17-26 :3	-	-	-	-	4.0
Δ 5,9,19-26 :3	1.2	0.3	-	-	6.6
26 :2	7.7	5.3	32.4	1.8	17.9
Δ 5,9,19-27 :3	-	-	1.5	-	-
Δ 5,9,21-27 :3	-	-	0.7	-	-
27 :2	6.1	-	0.3	3.1	2.0
6-Br-27 :2	-	0.3	-	-	-
Δ 5,9,21-28 :3	0.3	0.6	-	2.8	-
28 :2	1.1	14.6	-	26.4	0.6
Δ 5,9,21-29-3	-	0.7	-	-	-
29 :2	-	0.5	-	0.3	-
30 :2	-	-	-	3.6	-
Total	**22.3**	**24.8**	**37.1**	**43.9**	**68.7**

These five sponges are especially interesting due to their very high content in Δ5,9 fatty acids. Three other demosponges were also studied but their Δ5,9 fatty acids content were less than 10% *(Callyspongia* sp. cf. *siphonella, Gelliodes* sp1. and sp2). A calcareous sponge (*Leucetta* sp.) has been shown to contain the smallest percentage of Δ5,9 fatty acids (1.1%) of the total fatty acid fraction (13).

From a biosynthetic point of view the Δ5,9 fatty acids belonging to the n-7 series are of special interest. As demonstrated by 14C short-chain precursor incorporation studies, Δ5,9 fatty acids are synthesized *via* homologation (2C) followed by a double desaturation at either the C9 or C5 loci (Figure 10 [14]).

9-16:1 ⟶ ⟶ 19-26:1 ⟶ 9,19-26:2 ⟶ 5,9,19-26:3

Figure 10 : General biosynthetic scheme for Δ5,9 (n-7) fatty acids in sponges [15]

So, it is possible to postulate a biogenesis for all fatty acids belonging to the n-7 series according to this general scheme. Figures 11-13 give these hypothesis for Qatar sponges that contain high levels of Δ5,9 fatty acids and high levels of fatty acids belonging to the n-7 series. In the three Figures fatty acids really found are bold, fatty acids already known but not found in our samples are plain and fatty acids very likely implied in the biosynthesis but not yet found in any sponge are in brackets.

In the cell membrane phospholipids are associated with glycoplipids (Figure 5). If phospholipids mainly act as structural components glycolipids play a fundamental role in molecular recognition, especially sphingolipids (e.g. blood groups in mammals). In sponges cells are not organized in differentiated tissues and organs and probably, this explains why glycosphingolipids are so numerous and important for these primitive animals.

In a first approximation two groups of glycolipids are to be considered. Glycoglycerolipids derive from glycerol and glycosphingolipids derive from sphingosine, both of them can be divided into two classes : glycoglycerolipids and sulfolipids for the first group and ceramidses and cerebrosides for the second group (Table 7). The experimental way to isolate and analyze glycolipids from any organism is summarized in Figure 14.

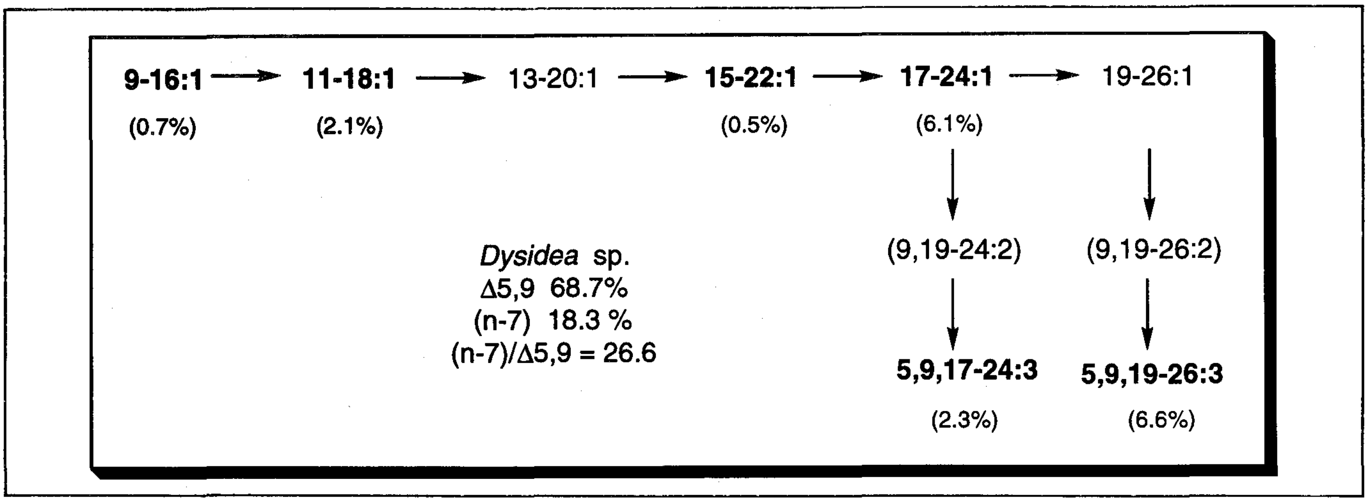


Figure 11 : Probable biosynthetic pathways for (n-7) fatty acids in *Dysidea* sp.

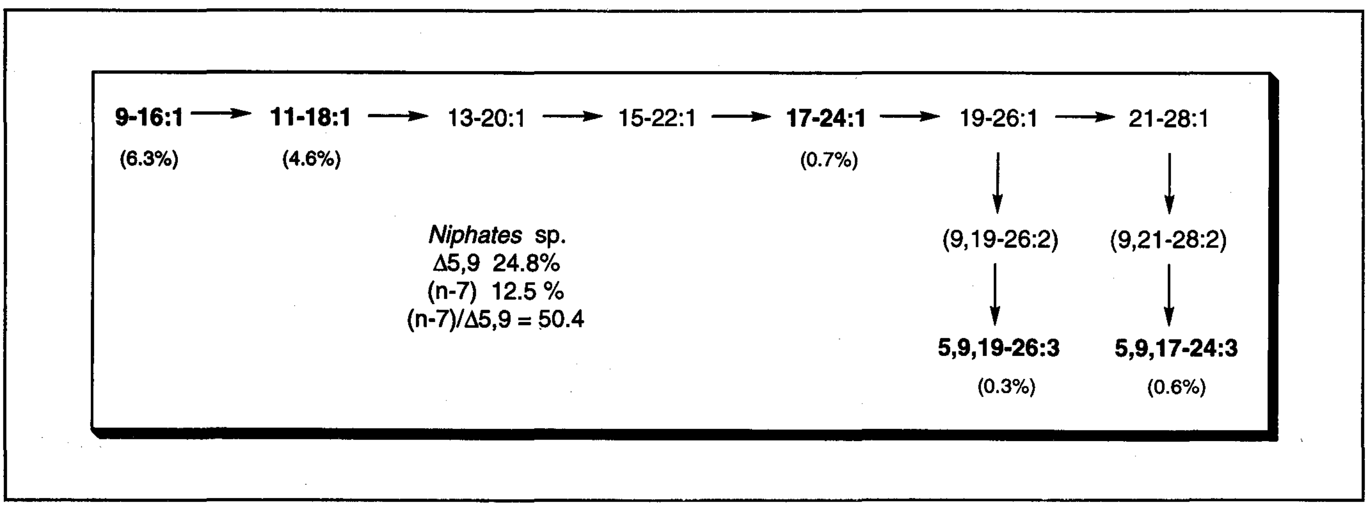


Figure 12 : Probable biosynthetic pathways for (n-7) fatty acids in *Niphates* sp.

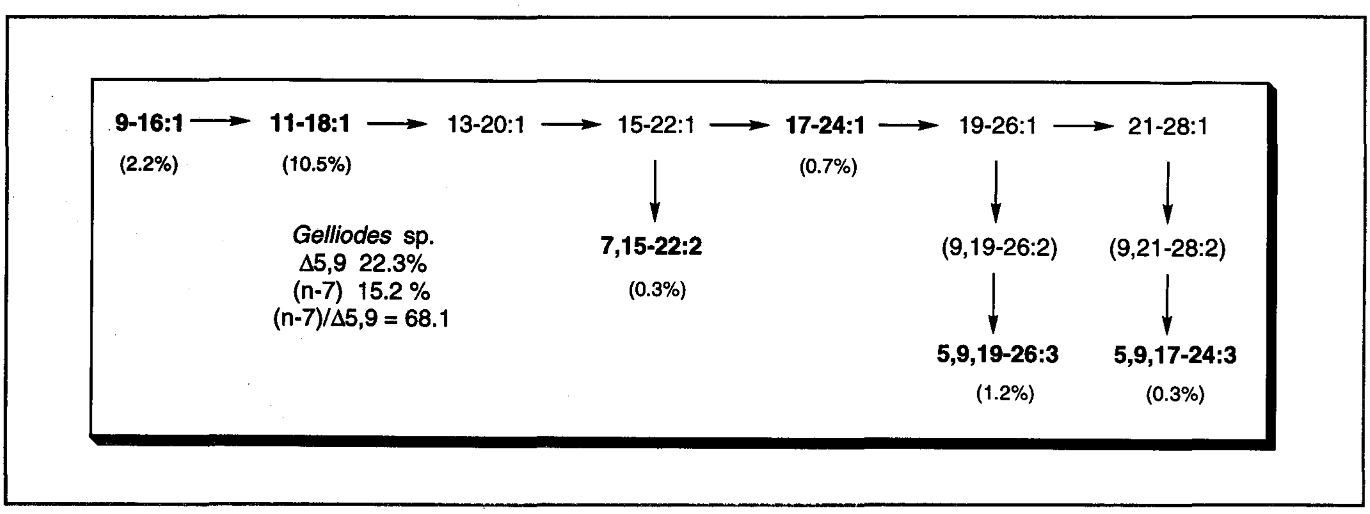


Figure 13 : Probable biosynthetic pathways for (n-7) fatty acids in *Gelliodes* sp.

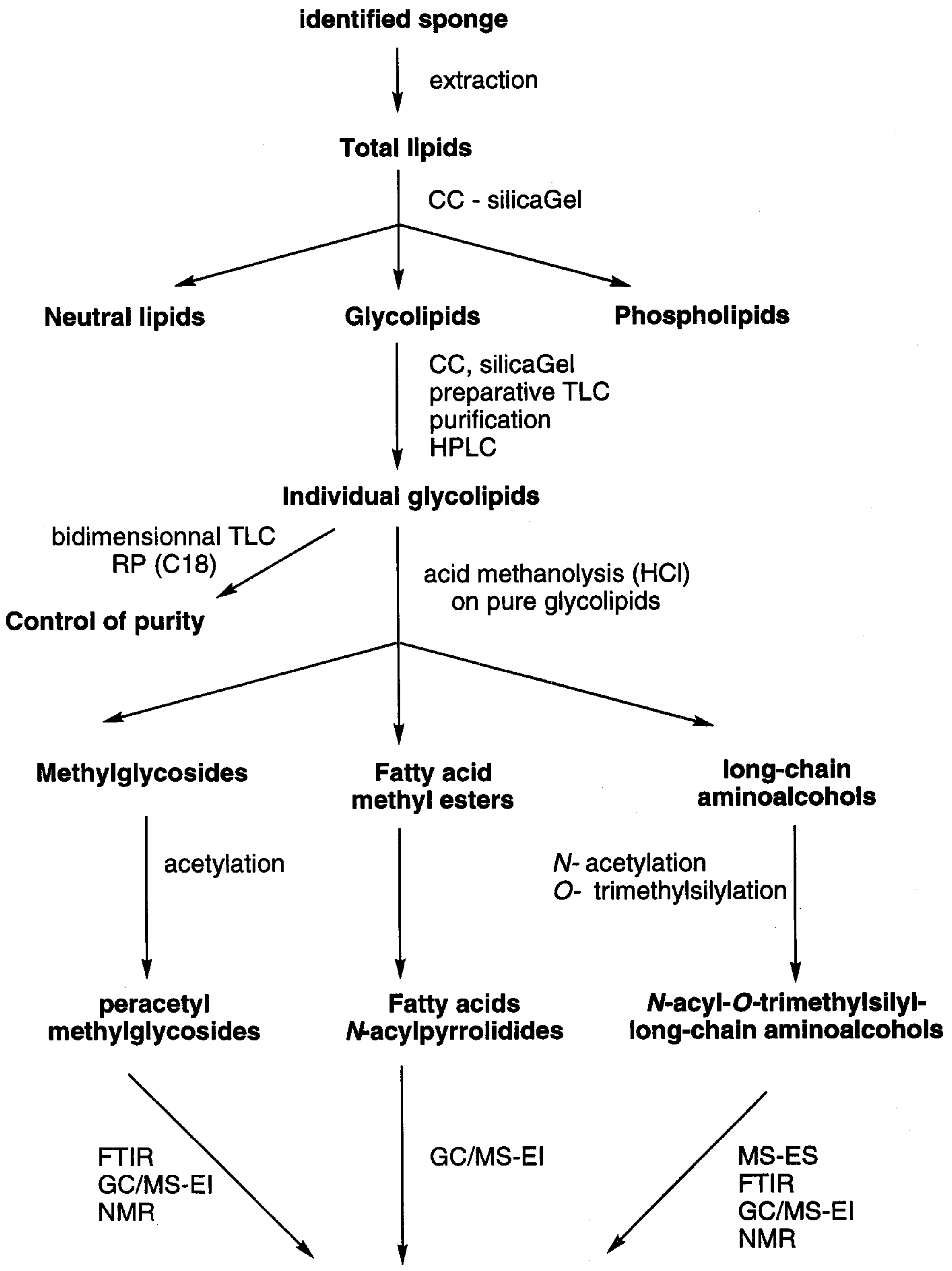
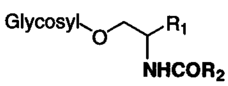


Figure 14 : Results for structural identification of glycolipids

Researches on glycolipids from Qatari sponges are in progress, preliminary results for two series of cerebrosides from *Dysidea* sp. are displayed on Table 8. The main point is the observation of a Δ5,9 long-chain fatty acid.

Table 8. Preliminary results for 2 cerebrosides from *Dysidea* sp.



Cerebroside	Glycoside	Aminoalcohol (R_1)	Fatty acids (R_2)
Qka	identification in progress	dihydroxy-C_{16}	Δ6-15:1 ; Δ6-16:1 Δ6-17:1 ; Δ6-18:1 Δ5,9-24:2
Qkb	β-galactose	identification in progress	95 % saturated fatty acids, maj. : 14:0 (10%), 16:0 (44%), 18:0 (11%) very long-chain fatty acids (> 24C)

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