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Review article

Lipid compounds of freshwater sponges: family Spongillidae, class Demospongiae

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Abstract

More than 100 novel, unusual and rare fatty acids, lipids and sterols have been isolated from freshwater sponges. The structures, biogenesis, synthesis and bioactivity of some lipid compounds of freshwater sponge species are reviewed.

Keywords: Sponges; Lipids; Phosphonolipids; Fatty acids; Synthesis; Biosynthesis; Sterols; Spongillidae

1. Introduction

Porifera is an early branching event in the history of animals and separated the sponges from other metazoans. As one would expect based on their phylogenetic position, fossil sponges are among the oldest known animal fossils, dating from the Late Precambrian. The approximately 5000 living sponge species are classified in the phylum Porifera, which is composed of three distinct groups: the Hexactinellida (glass sponges), the Demospongiae, and the Calcarea (calcareous sponges). The class Demospongiae is by far the most diverse sponge group. Greater than 90 percent from all known living sponge species are demosponges. Sponges are multicellular animals consisting of masses of cells embedded in a gelatinous matrix. The matrix is bound together by minute, spine-like structures of calcium or silica called spicules and spongy organic fibers called spongin. Sponges are sessile organisms with no bilateral symmetry, no clear anterior/posterior axis, limited differentiation of tissues into organs

Abbreviations: TLC, thin-layer chromatography; NMR, nuclear magnetic resonance spectroscopy; UV, ultraviolet spectroscopy; IR, infrared spectroscopy; HPLC, high resolution liquid chromatography; HRMS, high resolution mass spectrometry; VLCFA, very long-chain fatty acids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid; CPE, cyclic acetal PE; LPC, lysophosphatidylcholine PC; LPE, lysophosphatidylethanolamine PE; Sph, sphingomyelin; GL, glycolipids; PL, phospholipids; NL, neutral lipids; PAF, platelet activating factor; PR, products of saponification and hydrolysis; MMBFA, multimethyl-branched fatty acids.

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and no nervous system (Hooper and Van Soest, 2002).

Although most of the more than 5000 known sponges are found in marine environments, more than 150 species live in freshwater. Freshwater sponges are pitted with pores and often are yellow, brown or greenish. Sponges filter large volumes of water through their pores, capturing tiny particles for food. Freshwater sponges vary in colour from pale cream to green. Sponge reproduction is by gemmules, and gemmule characters are much used in identification at the genus and species level. All freshwater sponges are classified in the Desmospongiae, family Spongillidae (Racek, 1969). Molecular systematics of sponges has been reported using 18S rRNA, 28S rRNA and Hsp70 allozyme divergence (Borchiellini et al., 2000). The authors concluded that whatever the genes, the phylogenies obtained suggest that Porifera could be paraphyletic and that the phylogenetic relationships of most of the families and orders of the Demospongiae have to be reassessed. Classification and habitat of investigated freshwater sponges show in Table 1.

The freshwater Spongillidae undergo a slightly different gemmulation. Gemmules consist of aggregates of archaeocytes laden with reserve granules. In addition, however, they are surrounded by protective membranes formed by the archaeocytes. The protective covering is generally reinforced by spicules, which vary in shape according to the species and are useful in classification (Hooper and Wiedenmayer, 1994).

The Baikalian sponges are arborescent encrusting or have the form of small cushion-shaped masses. The skeleton comprises ascending multispicular tracts joined by isolated spicules or paucispicular tracts. Spongin is generally more abundant in the skeleton than in spongillids. The spicules may be completely embedded in spongin. Gemmules are not formed in these sponges. There are three genera: *Lubomirskia, Baicalospongia* and *Swartschewskia* that occur in Lake Baikal (Russia). These sponges are believed to be of haploscleridan affinities but evolved independently of the family Spongillidae. Four additional genera, *Ochridaspongia, Cortispongilla, Pachydictyum*, and *Nudospongilla* are found in other deep lakes and are similar in structure and may belong here (Masuda et al., 1999).

Both marine (Wilkinson, 1983) and freshwater (Wilkinson, 1980; Berner and Titlyanov, 1993; Corallini and Gaino, 2001) sponges contain a wide variety of microbial symbionts, often with many different symbionts in the same species. Some bacterial symbionts appear to have evolved with sponges since Precambrian times, and symbiosis has continued because masking capsules have obscured the symbionts from sponge phagocytic cells. The role of bacterial symbionts is obscure. Symbiont bacteria, cyanobacteria and/or algal species are capable of metabolizing a wide range of organic compounds, including fatty acids, thereby adding to the nutrition of the sponge/symbiont system (Wilkinson, 1987). Both symbiont types can occupy up to 40% of the sponge tissue volume, and play an important role in sponge ecology and biotransformation of the organic matter.

Freshwater and marine sponges (Bergquist, 1978) are the most primitive multicellular animals and possess unique sponge biomembranes (Litchfield and Morales, 1976). It has been known for some time that these membranes contain a high proportion of unusual fatty acids (Bergquist, et al., 1984; Dembitsky and Chelomin, 1985; Joseph, 1979; Djerassi and Lam, 1991). Since 1950, over 3000 papers have been published reporting the structures of more than 5500 metabolites unique to marine sponge species (Urban et al., 2000) some of which show high biological activity. Sponge lipids differ markedly in their chemical and biochemical properties from lipids obtained from other living organisms (Bergquist, 1978; Dembitsky et al., 1989; Dembitsky and Srebnik, 2002; Djerassi and Lam, 1991; Rezanka, 1989). Two classes of sponges: Demospongiae and Calcarea contain very long-chain (C_{24}–C_{38}) fatty acids (Bergquist, et al., 1984; Rezanka, 1989).

A comprehensive chemotaxonomic investigation on sponges was reported in the 1950s (Bergquist, 1978) and several wide-ranging studies have been made utilizing fatty acids (Bergquist, et al., 1984), sterols (Djerassi and Silva, 1991), carotenoids (Minale, 1978), free amino acids (Bergquist and Hartman, 1969), and acid mucopolysaccharides

Table 1
Classification, collected places of the investigated freshwater sponges

Classification	Collected place, data, depth	Reference
Phylum Porifera		
Class Demospongiae		
Order Haplosclerida		
Family Spongillidae		
Genus *Baicalospongia*		
B. bacilifera	Lake Baikal, Russia, July 1979, 6m	Dembitsky, 1981a,b
B. bacillifera	Lake Baikal, Russia, August 1992, 11 m	Dembitsky et al., 1993
B. bacilifera	Lake Baikal, Russia, June 1997, 25 m	Imbs and Latyshev, 1998
B. bacilifera	Lake Baikal, Russia, August–October, 1989, 20–50 m	Latyshev et al., 1992
B. intermedia	Lake Baikal, Russia, July 1979, 6m	Dembitsky, 1981a,b
B. intermedia	Lake Baikal, Russia, August 1992, 11 m	Dembitsky et al., 1993
B. intermedia	Lake Baikal, Russia, June 1997, 25 m	Imbs and Latyshev, 1998
B. intermedia	Lake Baikal, Russia, August–October, 1989, 20–50 m	Latyshev et al., 1992
Genus *Cortispongilla*		
C. barroisi	Lake Tiberias, Israel, July 1994, 3 m	Dembitsky and Rezanka, 1996
Genus *Ephydatia*		
E. fluviatilis	Lake Lagunita, Stanford, USA, July 1988	Hahn et al., 1989
E. syriaca	Jordan River, Israel, August 1994, 2 m	Dembitsky and Rezanka, 1996
Genus *Eunapis*		
E. fragilis	Lake Michigan, open water, IL, USA, July 1994, 10 m	Early et al., 1996
E. fragilis	Calumet River, IL, USA, July 1994, 8.5 m	Early et al., 1996
E. fragilis	Hammond Harbor, Lake Michigan, USA, July 1994, 4 m	Early et al., 1996
Genus *Euspongilla*		
E. lacustris	Volga River Estuary, Russia, August, 1990, 2.5 m	Dembitsky et al., 1991
Genus *Heterororotula*		
H.multidentata	Doro River, Nagano Prefecture, Japan, 2001	Sata et al., 2002
Genus *Lubomirskia*		
L. abietina	Lake Baikal, Russia, July 1979, 9 m	Dembitsky, 1981a,b
L. abietina	Lake Baikal, Russia, August–October, 1989, 20–50 m	Latyshev et al., 1992
L. baicalensis	Lake Baikal, Russia, July 1990, 8–20 m	Kolesnikova et al., 1992
L. baicalensis	Lake Baikal, Russia, July 1979, 6 m	Dembitsky, 1981a,b
L. baicalensis	Lake Baikal, Russia, August 1992, 11 m	Dembitsky et al., 1993
L. baicalensis	Lake Baikal, Russia, June 1997, 25 m	Imbs and Latyshev, 1998
L. baicalensis	Lake Baikal, Russia, August–October, 1989, 20–50 m	Latyshev et al., 1992
Genus *Nudospongilla*		
Nudospongilla sp.	Lake Hula, Israel, May 1994, 3 m	Dembitsky and Rezanka, 1996
Genus *Swartschewskia*		
S.bacillifera	Lake Baikal, Russia, July 1979, 10 m	Dembitsky, 1981a,b
S.bacillifera	Lake Baikal, Russia, August–October, 1989, 20–50 m	Latyshev et al., 1992
Genus *Spongilla*		
S.alba	Lake Shuinji, Shimane Prefecture, Japan, 2001	Sata et al., 2002
S.wagneri	Louisiana, NO, USA, 1969, fall waters	Laseter and Poirrier, 1970

(Bergquist, 1979). Given the growth of the literature on sponge secondary metabolites, there is now clear opportunity for further studies.

There are many reviews which detail the structure, distribution and biosynthesis of fatty acids found in marine sponge species (Dembitsky and Srebnik, 2002; Djerassi and Lam, 1991; Rezanka, 1989; Joseph, 1979; Dembitsky and Chelomin, 1985) but none deals with the lipid compounds isolated from freshwater sponge species. This is the first attempt to review the published literature on the variability of lipid structures of unusual and rare fatty acids found in freshwater sponges and not isolated from marine sources.

2. Fatty acids

It has been reported that both silicious freshwater *Spongilla lacustris* (Clarke and Mazur, 1941) and marine *Spheciospongia vesparia* and *Suberites compata* (Bergman and Swift, 1951) sponges contain significant amounts of free and very long-chain (C_{24}–C_{28}) fatty acids, respectively. Many publications reported the presence of unusual, branched, halogenated and other fatty acids in marine sponge species (Joseph, 1979; Dembitsky and Chelomin, 1985; Rezanka et al., 1987; Rezanka, 1989; Djerassi and Lam, 1991; Dembitsky and Srebnik, 2002) but none deals with fatty acids and other lipid compounds isolated from the freshwater sponges.

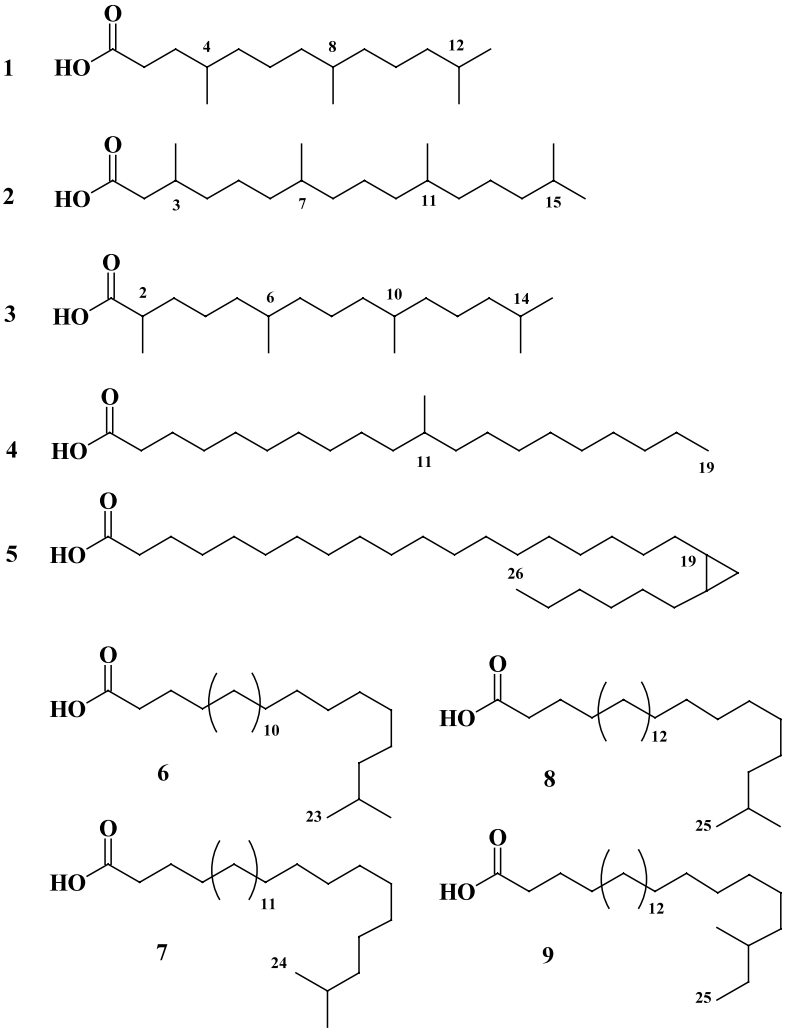
The first detailed GC analysis of freshwater sponge *Spongilla wagneri* was reported by Laseter and Poirrier, 1970. The authors discovered the presence of $C_{24:0}$ (5.7%) and $C_{24:1}$ (3.2%) fatty acids in the gemmule outer coat. The presence of very long-chain fatty acids (VLCFA) (C_{24}–C_{28}) in freshwater sponges was reported for the first time in 1981 by Dembitsky (1981a,b). These VLCFA have been isolated from Baikal sponges belonging to the genera Lubomirskia and Baicalospongia. Further investigation of different freshwater sponge species showed that these organisms contain a number of new, unusual and rare fatty acids.

2.1. Saturated fatty acids

Among saturated fatty acids that show a considerable variation in different lipid fractions, e.g. neutral, glycolipid (GL) and phospholipid, sponges from Lake Baikal which contain 46 saturated (including 'classic' fatty acid) and also *iso*- and *anteiso*-fatty acids (Dembitsky et al., 1993, 1994) are the most interesting. Freshwater sponges contain three polymethyl branched fatty acids such as 4,8,12-trimethyltridecanoic **1**, phytanic **2** and pristanic **3** acids. These acids may have chemotaxonomical significance for both marine and freshwater sponges (Carballeira et al., 1987, 1989; Hahn et al., 1989). Also among the saturated fatty acids from $C_{12:0}$ to $C_{32:0}$, two unusual acids, 11-Methyl-$C_{19:0}$ **4** and cyclopropyl-$C_{26:0}$ **5** have been found in *Baicalospongia bacillifera*, *B. intermedia*, *Lubomirskia baicalensis* (Dembitsky et al., 1993, 1994). Rare *iso*-$C_{23:0}$–$C_{25:0}$ **6**–**8** and *anteiso*-$C_{25:0}$ **9** VLCFA acids have also been found in sponges from Baikal Lake (Dembitsky et al., 1993, 1994) and in sponges from Middle East Lakes (Rezanka and Dembitsky, 2002, see supporting information) sponges. High levels of two isoprenoid fatty acids, **1** (36.7, 24.4 and 8.8% in three sponge species, respectively) and unusual and rare 5,9,13-trimethyltetradecanoic acid (1.5, 0.6 and 0.5%, respectively) were identified from the sponges *Cinachyrella alloclada* and *C. kukenthali* collected on the Senegal coastal waters, near Dakar (Barnathan et al., 1993), and from the sponge *Cinachyrella* aff. *schulzei* collected in waters of New Caledonia (Barnathan et al., 1994). Sponges from Senegal coastal waters contained up to 36.7% of **1** in the phospholipid fraction. The latter plays a role as a major cell membrane component (Gillan et al., 1988). But results obtained by Barnathan et al. (1993, 1994) show that large concentrations of this acid are not characteristic of just the sponge families Spirastrellidae and Cliobidae, as previously suggested (Carballeira et al., 1987, 1989). 4,8,12-Trimethyltridecanoic acid is probably derived by degradation of phytol, which is itself derived from chlorophyll (Barnathan et al., 1993). The origin of 5,9,13-trimethyltetradecanoic acid is uncertain, and it is probable that this acid is also derived

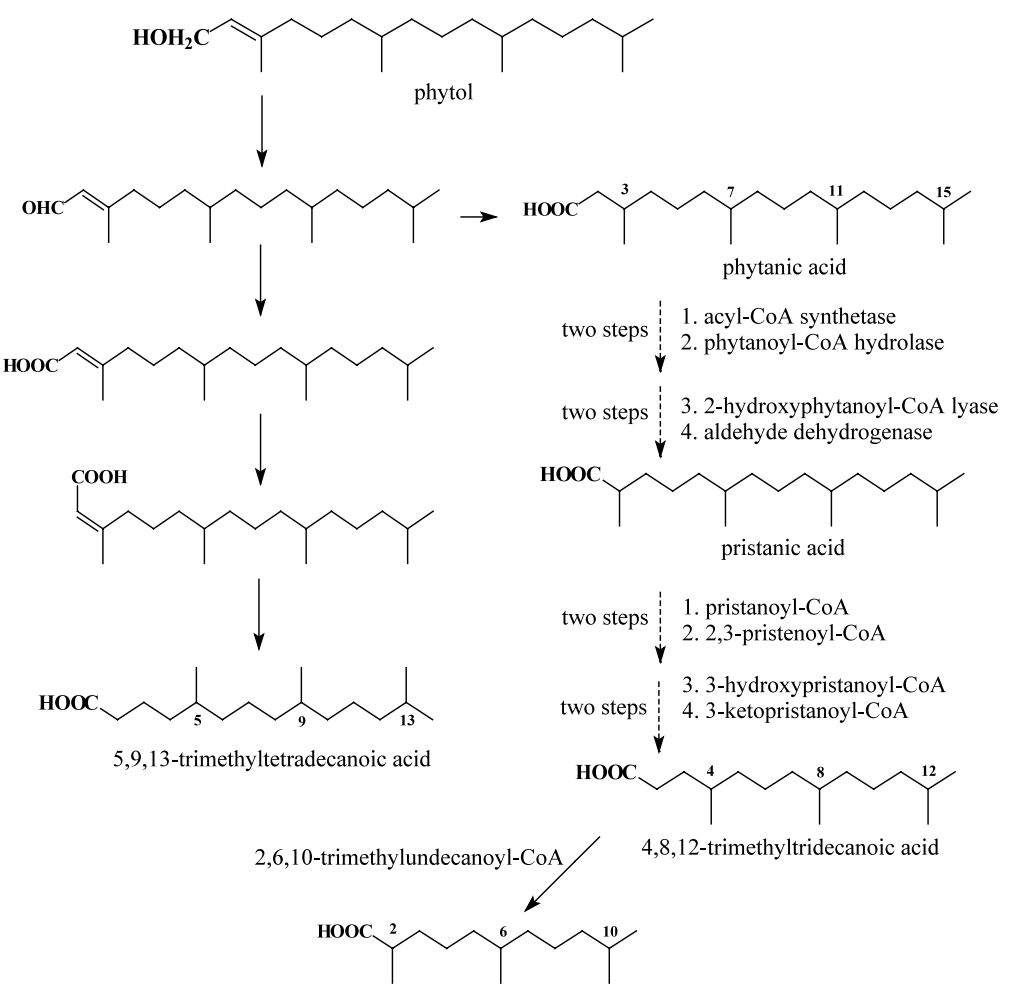
from phytol having a different biosynthetic pathway (Barnathan et al., 1993).

gradation pathway of phytol. Scheme 1 shows the possible pathway of formation this rare acid.



Known isoprenoid fatty acids were reviewed in 1973 (Lough, 1973), but their biosynthetic origin and relationship remains unclear. The presence of 5,9,13-TM-14:0 in sponges indicated another de-

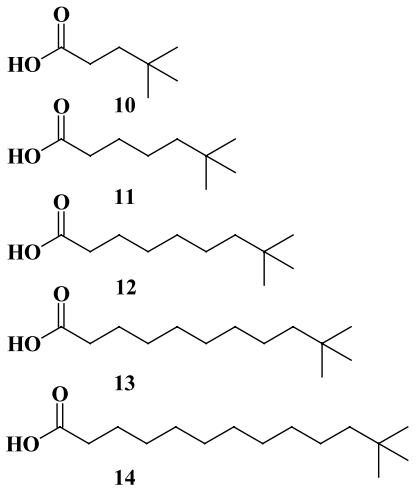
Phytanic acid is derived from phytol, the side chain of chlorophyll, the mechanism of degradation of this acid has long remained unknown, but recently the degradation process has been partly

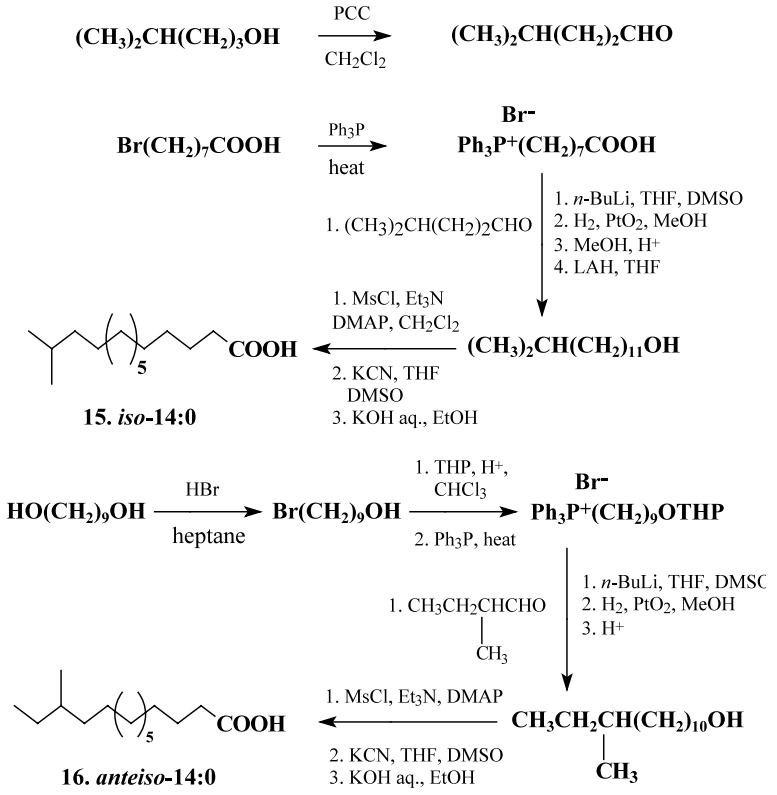


Scheme 1. The mechanism of phytanic acid α- and β-oxidation (right part), and proposed pathway for the production of rare 5,9,13-trimethyltetradecanoic acid during the metabolism of phytol in marine bacteria (left part).

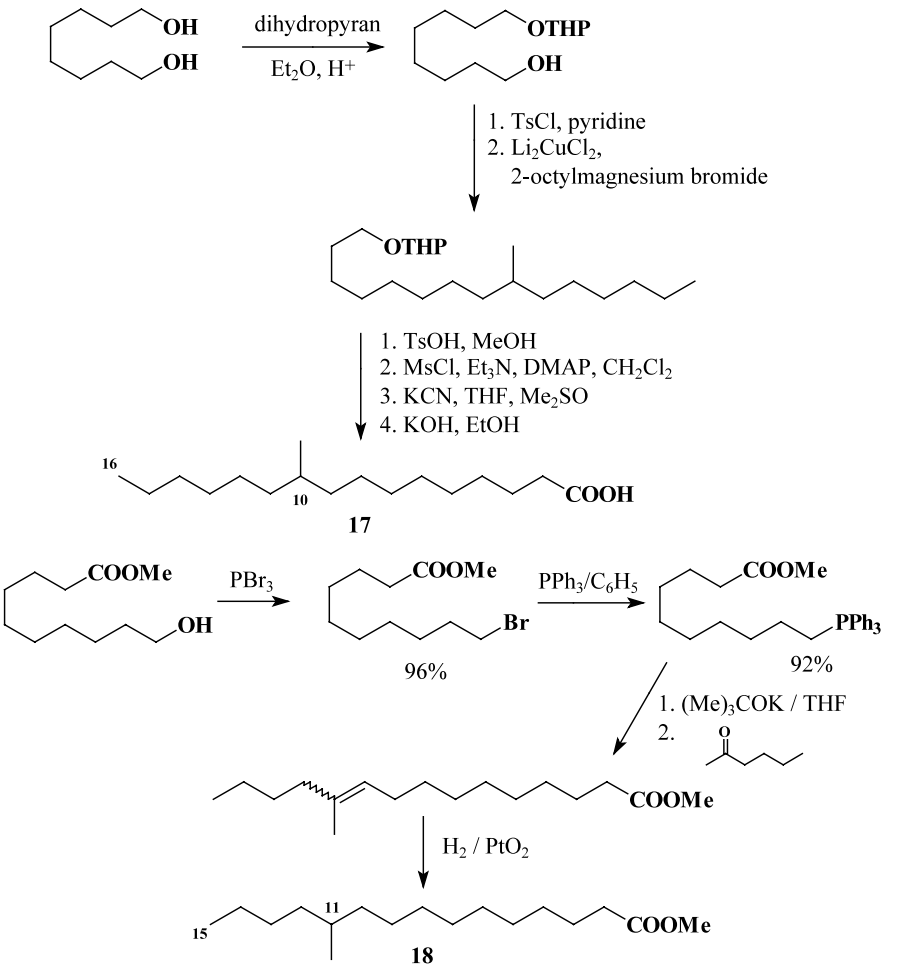
elucidated (see review Verhoeven and Jakobs, 2001). Pristanic and 4,8,12-TM-13:0 **1** and other isoprenoid acids are derived from phytol according Scheme 1. 5,9,13-TM-14:0 acid is formed from phytol by some marine bacteria such as *Acinobacter* sp., *Pseudomonas nautica*, *Marinobacter* sp., and *M. hydrocarbonoclasticus* (Rontani et al., 1999). It is possible that the 5,9,13-TM-14:0 acid found in sponge species could be of bacterial origin. Synthesis of 5,9,13-TM-14:0 has recently been reported (Section 2.1.4).

The unusual neo fatty acids **10**–**14** have been isolated as minor components from the glycolipids (GL) fraction of freshwater sponges, *Ephydatia syriaca*, *Nudospongilla* sp. and *Cortispongilla barroisi* (Dembitsky, 1996). Previously, the amide of **11** was found in cyanobacterium *Lyngbya majuscula* (Nagle et al., 1996), and also as a lipid side chain incorporated into cyclic lipopeptides from a Curacao strain of *L. majuscula* (Orjala et al., 1995). It is possible that these neo acids **10**–**14** could be of cyanobacterial origin.





Scheme 2. Synthesis of *iso*- and *anteiso*-tetradecanoic acids.



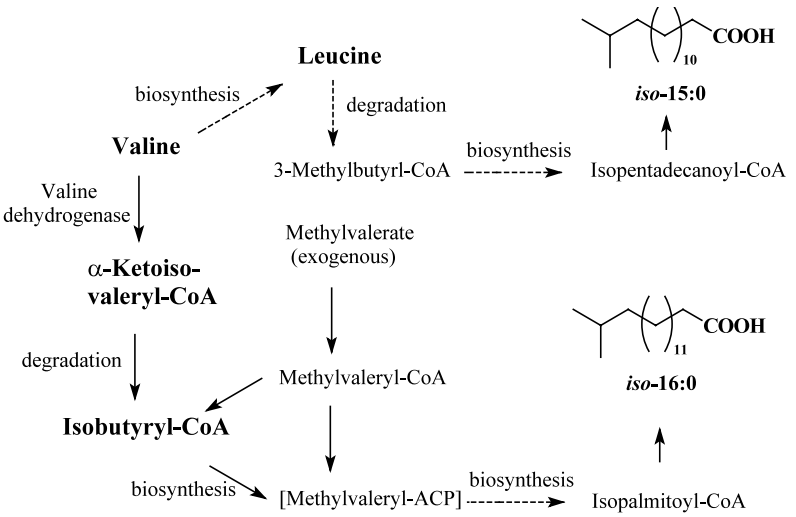
Scheme 3. Synthesis of mid-chain branched saturated fatty acids.

2.1.1. Synthesis of iso- and anteiso-saturated fatty acids

Freshwater as well as marine sponges contain many different *iso*- and *anteiso*-saturated fatty acids. Some of them have been synthesized. Scheme 2 shows the synthesis of *iso*-tetradecanoic **15** and *anteiso*-tetradecanoic **16** acids (Carballeira et al., 1986). Using this methodology the *iso*- and *anteiso*-fatty acids with different long-chains could be synthesized.

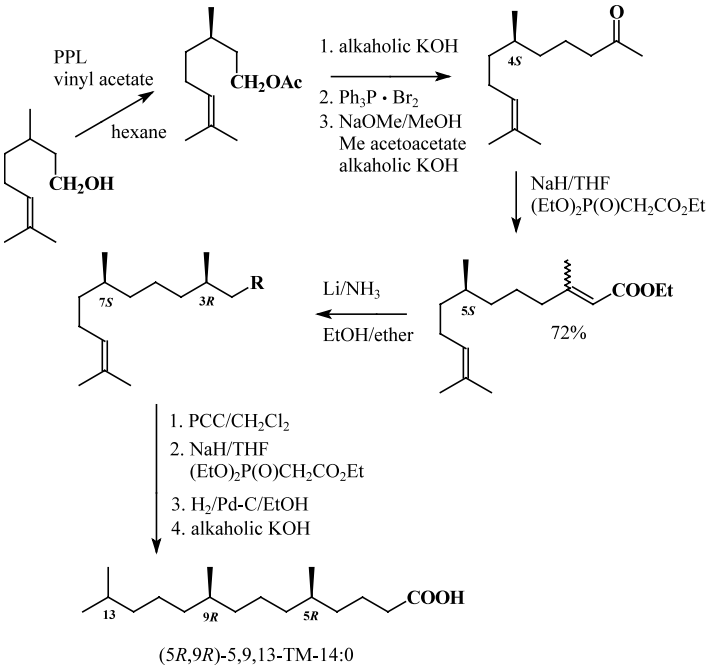
2.1.2. Synthesis of mid-chain branched saturated fatty acids

Mid-chain branched alkanoic acids have been isolated from both marine and freshwater sponges (Djerassi and Lam, 1991; Thiel et al., 1999; Dembitsky, 1996; Dembitsky et al., 1993, 1994) and some from them such as 13-methylhexadecanoic acid display antimicrobial activity (MIC = 1.56 g/ml) against the cariogenic bacterium *Streptococcus mutans* (Stierle and Faulkner, 1991).



Scheme 4. Proposed pathways of *iso*- fatty acids and amino acid metabolism in Actinomycete, *Streptomyces collinus* (Wallace et al., 1995). Adapted by authors.

Short mid-chain 10-methyl-hexadecanoic **17** acid (Raederstorff et al., 1987) and a novel 11-methyl-pentadecanoic **18** acid which isolated from sponge *Calyx podatypa* (Carballeira et al., 1998) have been synthesized by different methods (Scheme 3). 11-Methylheptadecanoic and 13-methyl-nonadecanoic acids have also been synthesized (Walkup et al., 1981).



Scheme 5. Synthesis of (5*R*,9*R*)-5,9,13-trimethyltetradecanoic acid.

Table 2
New, unusual and rare dienoic fatty acids isolated from freshwater sponges

Fatty acid	Sponge	Reference
19. 7-Dodecenoic (Δ^7-12:1)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Cortispongilla barroisi	Rezanka and Dembitsky, 2002
	Ephydatia syriaca	Rezanka and Dembitsky, 2002
	Lubomirskia baicalensis	Dembitsky et al., 1994
	Nudospongilla sp.	Rezanka and Dembitsky, 2002
20. 8-Tridecenoic (Δ^8-13:1)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Cortispongilla barroisi	Rezanka and Dembitsky, 2002
	Ephydatia syriaca	Rezanka and Dembitsky, 2002
	Lubomirskia baicalensis	Dembitsky et al., 1994
	Nudospongilla sp.	Rezanka and Dembitsky, 2002
21. 5-Tetradecenoic (Δ^5-14:1)	*Baicalospongia bacilifera*	Imbs and Latyshev, 1998
22. 14-Methyl-4-pentadecenoic (14-Me-Δ^4-15:1)	*Baicalospongia bacilifera*	Imbs and Latyshev, 1998
23. 13-Methyl-4-pentadecenoic (13-Me-Δ^4-15:1)	*Baicalospongia bacilifera*	Imbs and Latyshev, 1998
24. 4-Hexadecenoic (Δ^4-16:1)	*Baicalospongia bacilifera*	Imbs and Latyshev, 1998
25. 16-Methyl-4-heptadecenoic (16-Me-Δ^4-17:1)	*Baicalospongia bacilifera*	Imbs and Latyshev, 1998
26. 15-Methyl-4-heptadecenoic (15-Me-Δ^4-17:1)	*Baicalospongia bacilifera*	Imbs and Latyshev, 1998
27. 9-Methyl-6-heptadecenoic (9-Me-Δ^6-17:1)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Cortispongilla barroisi	Rezanka and Dembitsky, 2002
	Ephydatia syriaca	Rezanka and Dembitsky, 2002
	Lubomirskia baicalensis	Dembitsky et al., 1994
	Nudospongilla sp.	Rezanka and Dembitsky, 2002
28. 7-Methyl-12-heptadecenoic (7-Me-Δ^{12}-17:1)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Cortispongilla barroisi	Rezanka and Dembitsky, 2002
	Ephydatia syriaca	Rezanka and Dembitsky, 2002
	Lubomirskia baicalensis	Dembitsky et al., 1994
	Nudospongilla sp.	Rezanka and Dembitsky, 2002
36. 5,8-Tetradecadienoic ($\Delta^{5,8}$-14:2)	*Baicalospongia bacilifera*	Imbs and Latyshev, 1998
37. 6,9-Tetradecadienoic ($\Delta^{6,9}$-14:2)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Cortispongilla barroisi	Rezanka and Dembitsky, 2002
	Ephydatia syriaca	Rezanka and Dembitsky, 2002
	Lubomirskia baicalensis	Dembitsky et al., 1994
	Nudospongilla sp.	Rezanka and Dembitsky, 2002
38. 5,9-Octadecadienoic ($\Delta^{5,9}$-18:2)	*Baicalospongia bacilifera*	Imbs and Latyshev, 1998
39. 6,11-Octadecadienoic ($\Delta^{6,11}$-18:2)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Cortispongilla barroisi	Rezanka and Dembitsky, 2002
	Ephydatia syriaca	Rezanka and Dembitsky, 2002
	Lubomirskia baicalensis	Dembitsky et al., 1994
	Nudospongilla sp.	Rezanka and Dembitsky, 2002
40. 6,11-Eicosadienoic ($\Delta^{6,11}$-20:2)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Cortispongilla barroisi	Rezanka and Dembitsky, 2002
	Ephydatia syriaca	Rezanka and Dembitsky, 2002
	Lubomirskia baicalensis	Dembitsky et al., 1994
	Nudospongilla sp.	Rezanka and Dembitsky, 2002

Table 2 (*Continued*)

Fatty acid	Sponge	Reference
41. 8,11-Eicosadienoic ($\Delta^{8,11}$-20:2)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Cortispongilla barroisi	Rezanka and Dembitsky, 2002
	Ephydatia syriaca	Rezanka and Dembitsky, 2002
	Lubomirskia baicalensis	Dembitsky et al., 1994
	Nudospongilla sp.	Rezanka and Dembitsky, 2002
42. 11,15-Eicosadienoic ($\Delta^{11,15}$-20:2)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Lubomirskia baicalensis	Dembitsky et al., 1994

2.1.3. Biosynthesis of iso-fatty acids

Modern sponges contain a wide variety of prokaryotic and eukaryotic symbionts including bacteria, cyanobacteria, and algae (Wilkinson, 1987). Actinomycetes have recently been found to be a significant component of sponge associated microbiota (Webster et al., 2001) and are of particular interest considering the excellent track-record of these microbes in production of bioactive compounds (Yousaf et al., 2002). The cellular fatty acids streptomycetes are known to consist primarily of branched chain fatty acids with minor straight chain components (Saddler et al., 1987). A similar combination of branched chain fatty acids were observed by Kaneda (1963) forty years ago. Research on *Bacillus* has shown that branched acids are likely produced using the amino acid catabolites, isobutyryl-CoA, 2-methylbytyryl-CoA and 3-methylbutyryl-CoA, as biosynthetic starter units (Kaneda, 1991). Wallace et al. (1995) has demonstrated biosynthesis in three Actinomycetes: *Streptomyces collinus*, *S. cinnamonensis*, and *Saccharopolyspora* (Sp.) *erythraea*. The authors showed that from 81 to 92% of total fatty acids were found as *iso*-acids. The proposed mechanism of biosynthesis branched chain fatty acids show in Scheme 4.

2.1.4. Synthesis of (5R,9R)-5,9,13-trimethyltetradecanoic acid

A synthesis of (5*R*,9*R*)-5,9,13-trimethyltetradecanoic acid has been developed (Scheme 5) starting from (±)-citronellol via a sequential acetylation protocol using two porcine pancreatic lipase (PPL) (Sankaranarayanan et al., 2002).

2.2. Monoenoic fatty acids

Usually, monoenoic fatty acids of freshwater as well as marine sponges contain one double bond in positions 5, 7, or 9, but the double bond in VLCFAs is found also in 13, 15, 17 and 19. More than 50 monoenoic acids have been isolated from sponges collected in Baikal Lake (Dembitsky et al., 1993, 1994) and sponges from Middle East Lakes and Jordan River (Rezanka and Dembitsky, 2002). Some new monoenoic acids have identified from freshwater sponges **19**–**28** and not reported previously in marine sponges (Table 2). The composition of new Δ4 monoenoic acids has been identified by GC–MS from lipids of sponge *Baicalospongia bacilifera* (Imbs and Latyshev, 1998). As previously reported, mainly phospholipids (PL) of most marine sponge species contain various Δ5 olefinic fatty acids including demospongic acids (Litchfield and Marcantonio, 1978; Rezanka, 1989). Mass spectrum of the fatty acid **26** show in Fig. 1.

Six novel polymethyl branched fatty acids **29**–**34** with Δ^5 position of the double bond have been isolated from the GL fraction of *Baicalospongia intermedia*, *B. bacilifera* and *Lubomirskia baikalensis* belonging to family Luborskiidae (Rezanka and Dembitsky, 1993). These fatty acids have unusual structures including two fragments: one from the carboxyl end belonging to demospongic acids, and the second belonging to typical iso-

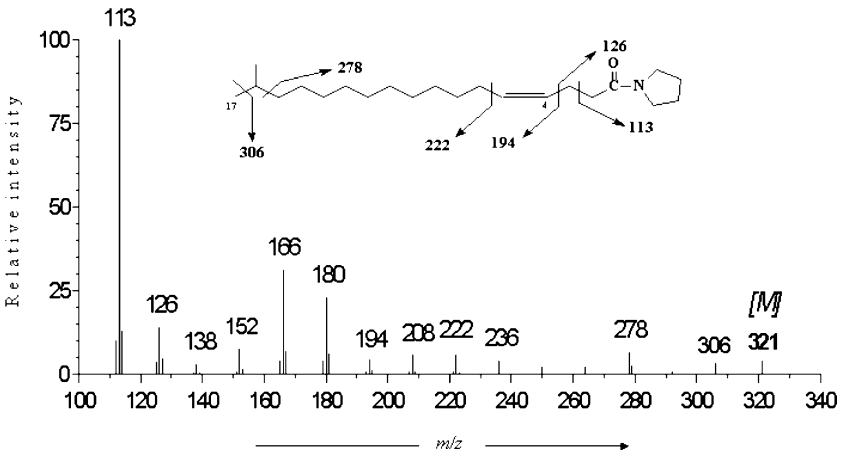
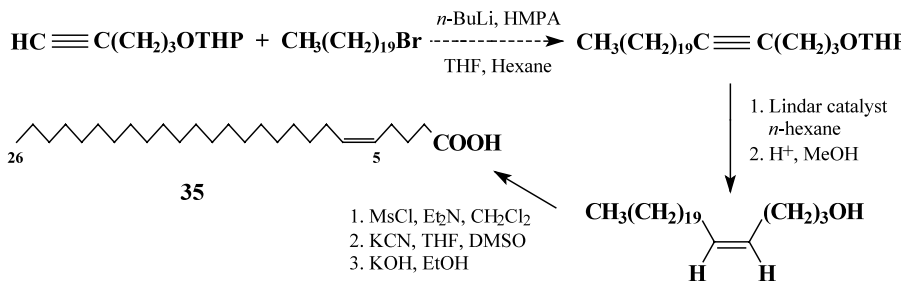


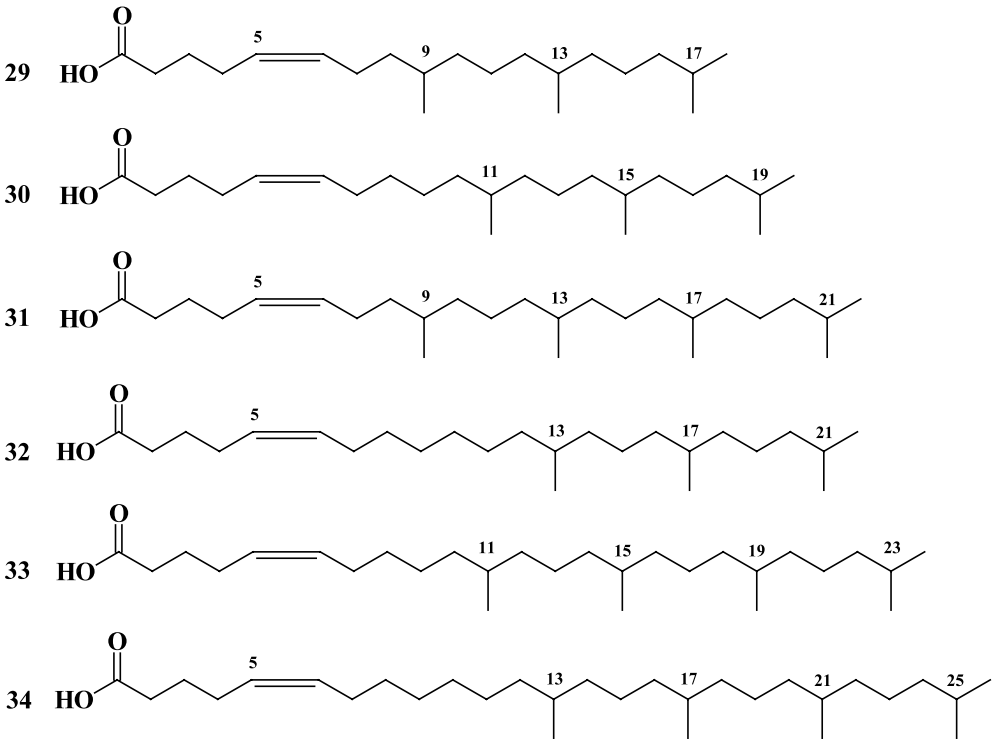
Fig. 1. Part of mass spectrum of (*Z*)-*N*-16-methylheptadec-4-enoylpyrrolidine isolated from sponge *Baicalospongia bacilifera* (Imbs and Latyshev, 1998). Adapted by authors.

prenoid acids such as **1**–**3**. The mechanism of biosynthesis these unusual acids has been elucidated.

Separation and isolation of fatty acids was done according to following procedure. Total methyl esters from the GL fraction were development with hexane–Et_2O (80:20, v/v) on 20×20 cm^2 thin-layer chromatography (TLC) plates coated with a 0.5 mm layer of silica gel G with 10% $AgNO_3$. After visualization with 2′,7′-dichlorofluprescein (under ultraviolet (UV)), the band corresponding to components having one to three double bounds was scraped and extracted with Et_2O. The extract was dissolved in 3 ml MeOH, and urea (500 mg) was added and dissolved by warming. The solution crystallized at −15 °C, 3 h. After filtration, the filtrate was acidified and extracted by hexane to isolate the methyl-branched fatty acid esters. An aliquot of this extract was identified by GC–MS as the picolinyl esters, prepared and described by Harvey (1984). The remainder of this extract was used for the isolation of pure compounds. Elution was performed with a gradient of MeCN–H_2O (90:10) to pure MeCN during 50 min. The effluent (single peaks) was manually collected. Pure fatty acids **29**–**34**, **43**–**48**, **50**–**56** were obtained at 92–94% purity (checked by GC–MS). The structure of these fatty acids was established according to ^{13}C NMR, high resolution mass spectrometry (HRMS), UV, and infrared (IR) data (Rezanka and Dembitsky, 1993).



Scheme 6. Synthesis of (*Z*)-5-hexacosenoic acid, **35**.



2.2.1. Synthesis of demospongic acid with Δ^5 double bond

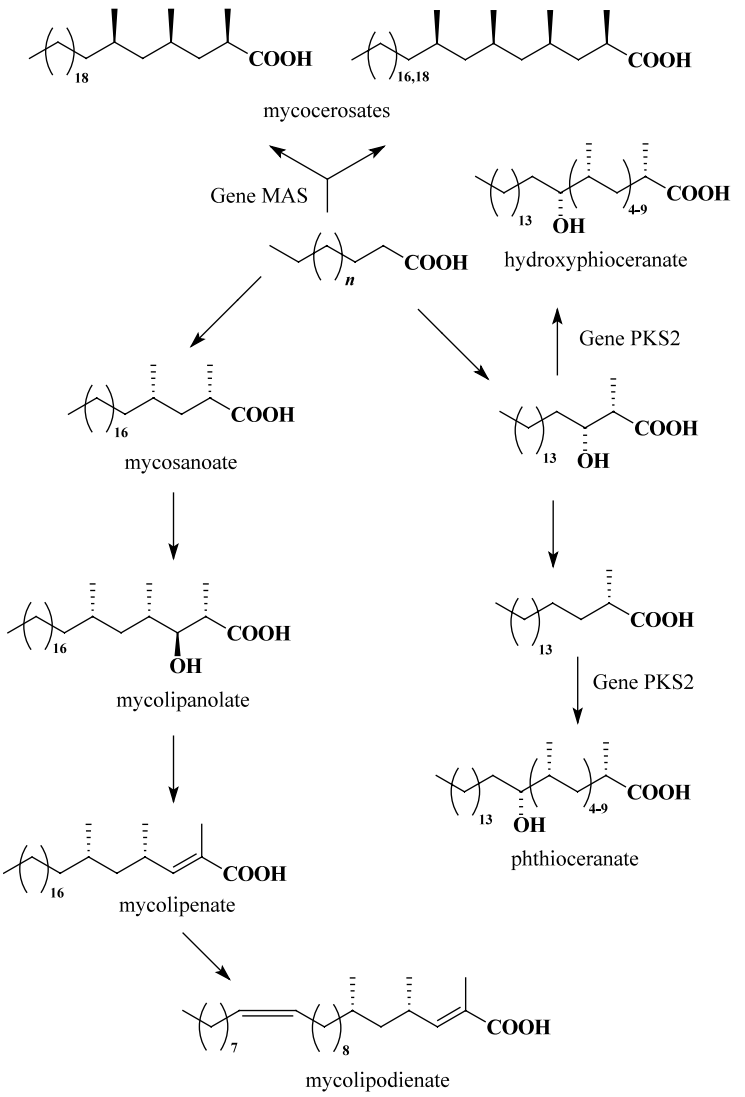
In the course of studying sponge fatty acids, Hahn et al. (1988) synthesized the 5–26:1 hexacosenoic acid **35**, which is the common intermediate in the biosynthesis of demospongic polyunsaturated fatty acids (Scheme 6).

2.2.2. Biosynthesis of multimethyl-branched fatty acids

Biosynthesis of multimethyl-branched fatty acids (MMBFA) in sponge species has not yet beenproven, but the biosynthesis of MMBFA by some microorganisms was recently reported (Minnikin et al., 2002). The unusual lipophilic nature of the cell envelope tubercle *Bacillus* has high proportions of unique long-chain compounds, and the so-called mycolic acids. Their structures were established and have been reviewed by Brennan (1988) and Besra and Chatterjee (1994). Unique MMBFA incorporated into structure GL of *Mycobacterium tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, and *M. bovis* BGG variant produced a phenolic GL, the so-called mycoside B (Minnikin et al., 1986). Some of the MMBFA contain one or two double bonds (mycolipanate and mycolipodiene). The structure of MMBFA and their biosynthesis is shown in Scheme 7. Structures of fatty acids discovered from freshwater sponges and MMBFA probably have partly common biosynthetic pathways?

2.3. Dienoic fatty acids

More than 30 dienoic fatty acids were identified in glyco- and phospholipid fractions in sponges from Lake Baikal (Dembitsky et al., 1993, 1994) and sponges from Middle East Lakes (Rezanka and Dembitsky, 2002). Some unusual acids **40**–**42** which have been isolated from Baikalian sponges are shown in Table 2. These acids have not been reported from marine sources. Most of the dienoic compounds are from freshwater sponges, including conventional methylene-interrupted fatty acids of the n−6 and n−3 families, such as 9,12–18:2, and 12,15–18:2. Demospongic dienoic acids



Scheme 7. Structures and proposed biosynthetic pathways leading to the complex MMBFA from *Mycobacterium tuberculosis* (Minnikin et al., 2002). Adapted by authors.

usually have the $\Delta^{5,9}$ structure, including VLCFAs such as 29:2, 30:2 and 31:2. A high percentage of demospongic acids such as 5,9–24:2 (in separated fractions up to 34%), 5,9–25:2 (11%) and 5,9–26:2 (35%) have recently been isolated from freshwater sponges *Heterorotula multidentata* and *Spongilla alba*, Lake Shinji, Shimane prefecture, Japan (Sata et al., 2002), and the sponge *Ephydatia fluviatilis* (32% in PS) from Lake Lagunita, Stanford, USA (Hahn et al., 1989).

The novel (*E,E*)-4,6-dimethyl-2,4-hexadecadienoic acid **43** has been isolated from *Ephydatia syriaca* (Rezanka and Dembitsky, 2002), (*Z,Z*)-22-methyl-5,9-nonacosadienic acid **44** was isolated in sponges from Lake Baikal (Dembitsky et al., 1993, 1994). This acid **44** was also found in the

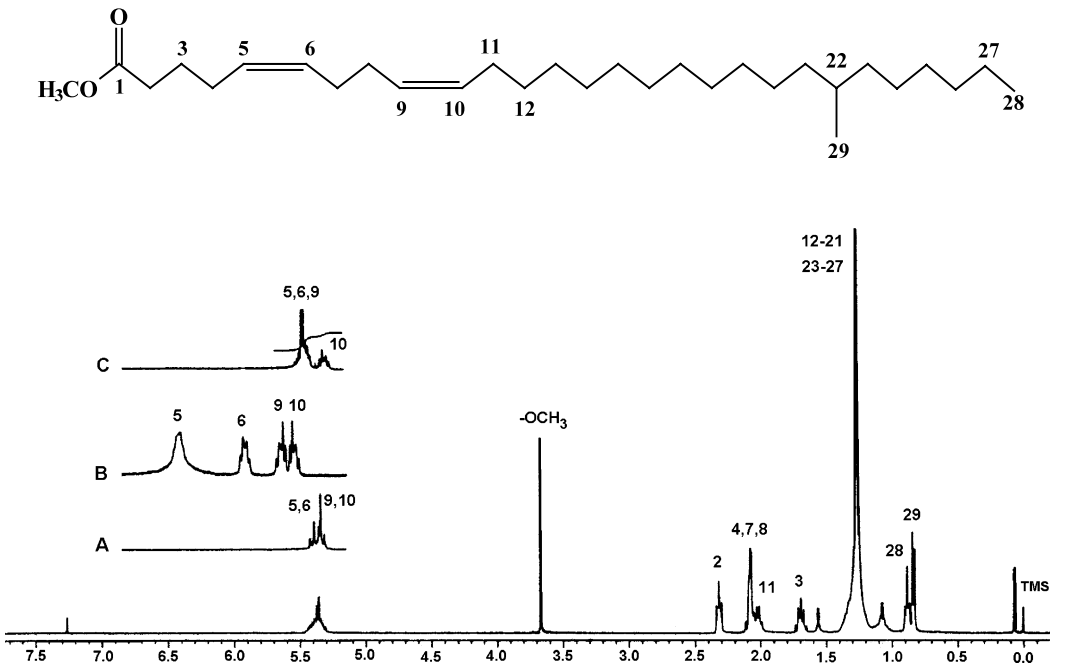


Fig. 2. 360 MHz ^{1}H NMR spectrum of methyl 22-methyl-5,9-octacosadienoate measured in $CDCl_3$ (TMS reference). Part A, olefinic proton signals upon irradiation of 2.0–2.2 ppm region. Part B, olefinic protons in presence of 0.5 equiv of Yb $(fod)_3$. Part C, olefinic protons from spectrum measured in d_6-benzene. Numbers given above peaks indicated bearing the protons responsible for those signals (Walkup et al., 1981). Adapted by the authors.

marine sponge *Myrmekioderma styx* (2%) (Carballeira et al., 1993). Fig. 2 shows the structure and ^{1}H-NMR spectrum of 22-methyl-5,9-28:2 acid isolated from *Aplysina fistularis* and also found in other sponge species.

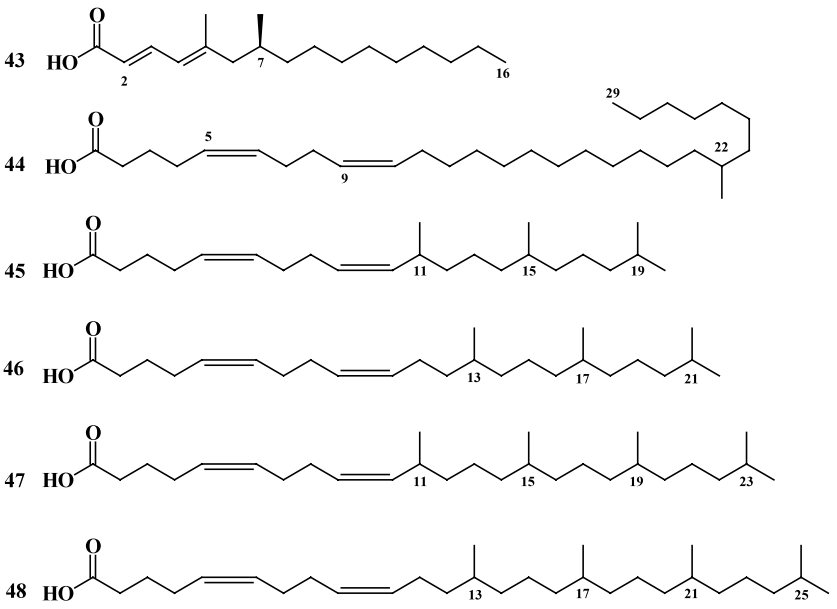
Four new isoprenoid dienoic acids with the $\Delta^{5,9}$ structure **45**–**48** have been isolated from *Baicalospongia intermedia*, *B. bacilifera* and *Lubomirskia baikalensis*, all belonging to the family Lubomirskiidae (Rezanka and Dembitsky, 1993). These acids have also not been reported from marine sponge species.

2.3.1. Synthesis of $\Delta^{5,9}$ dienoic acids

Unusual branching in the central portion of the chain of 22-methyl-5,9-octadecanoic acid (22-Me-$\Delta^{5,9}$-28:2) isolated from the marine sponge *Aplysina fistularis* (Walkup et al., 1981) similar to structure **44** has been synthesized by Raederstorff et al. (1987) who obtained the ethyl esters of (22*R*)-(−)-22-Me-$\Delta^{5,9}$-28:2 **49a** and (22*S*)-(+)-22-Me-$\Delta^{5,9}$-28:2 **49b** (Scheme 8).

The $\Delta^{5,9}$ fatty acids (*Z,Z*)-5,9-hexadecadienoic acid, (*Z,Z*)-5,9-nonadecadienoic acid, and (*Z,Z*)-5,9-eicosadienoic acid were synthesized for the first time in four steps (9–12% overall yield, Scheme 9) starting from 2-(2-bromoethyl)-1,3-dioxolane (Carballeira et al., 1998).

Unusual ethylene-interrupted $\Delta^{5,9}$ dienoic acids have been isolated mainly from marine and freshwater sponges (Djerassi and Lam, 1991). Several of these fatty acids are known to arise from a symbiotic relationship of associated bacteria with the host cells of marine invertebrates. Recently, it was demonstrated that sponges are not the only source of these $\Delta^{5,9}$ dienoic acids, but other marine organisms, such as zoanthids and anemone, can metabolize, although in trace amounts, intermediate chain-length $\Delta^{5,9}$ acids such as 5,9-heneicosadienoic acid, isolated from marine anemone *Stoichactis helianthus* (Carballeira and Medina, 1994). New fatty acid (*Z,Z*)-14-methyl-5,9-pentadecadienoic acid has been isolated from the Caribbean gorgonian *Eunicea succinea* (Carbal-



leira et al., 1997). Interestingly, this acid was particularly active against Gram-positive bacteria such as *Staphylococcus aureus* (MIC = 0.24 mol/ml) and *S. faecalis* (MIC = 0.16 mol/ml). Therefore, this acid could have arisen from associated bacteria within *E. succinea.*

Naturally occurring $\Delta^{5,9}$ fatty acids include the shorter-chain analog (*Z,Z*)-5,9-hexadecadienoic acid, which was originally reported from the cellular slime mold *Dictyostelium discoideum* (Davidoff and Korn, 1962), and 5,9–16:2 and 5,9–18:2 acids have been determinated in slime molds *Trichia favoaiena* and *T. varia* (Rezanka, 1993). Also unusual examples from terrestrial sources include the (*Z,Z*)-5,9-octadecadienoic acid isolated from the seed oils of the Gymnospermae (Madrigal and Smith, 1975; Wolff and Bayard, 1995; Gunstone et al., 1995). Recently, two $\Delta^{5,9}$ dienoic acids have found in plants (Carballeira and Cruz, 1998). 16-methyl-5,9-octadecadienoic (0.2%), 5,9-nonadecadienoic (0.2%), 5,9-eicosadienoic (0.2%), and 5,9-tetracosadienoic (0.3%) acids were isolated from plant *Malvaviscus arboreus* (Malvaceae), and 17-methyl-5,9-octadeca-dienoic acid was isolated from flowers of *Allamanda cathartica* (Apocynaceae) and *M. arboreus*.

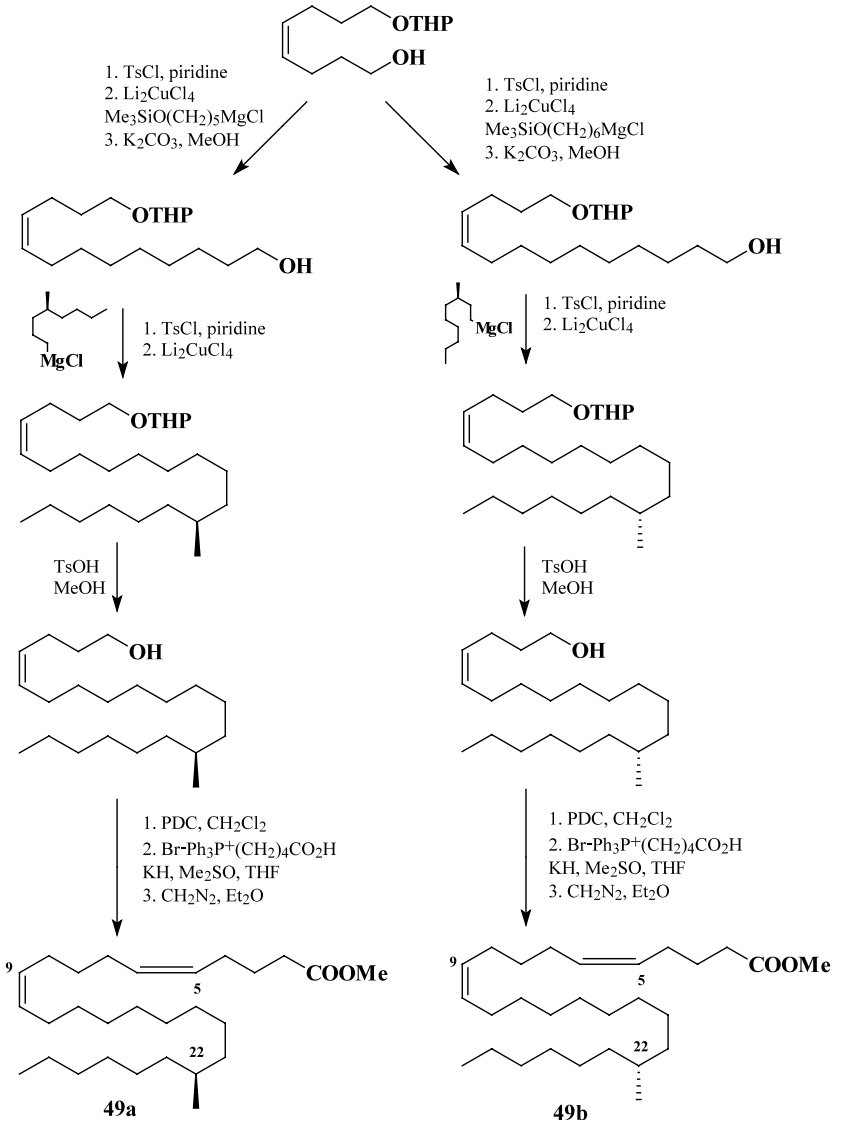
Based on these findings we may conclude that the biosynthetic pathways of invertebrates, sponges, myxomycetes, and some plants have a common enzymatic system to synthesize $\Delta^{5,9}$ ethylene-interrupted dienoic acids.

2.3.2. Biosynthesis of unusual dienoic acids

The unusual and novel 6,11-octadecadienoic (6,11–18:2) **39** and 6,11-eicosadienoic (6,11–20:2) **40** acids have also been isolated from the marine sponge *Euyspongia rosea* (Carballeira and Maldonado, 1989). The isolation of these 6,11-dienoic acids **39**,**40** reveals the presence of new biosynthetic possibilities in sponges and suggests the presence of an active Δ^{6} and Δ^{12} desaturases in *E. rosea* (Scheme 10).

2.4. Trienoic fatty acids

Previously, investigations of the fatty acid profile of nearly 200 marine species of the Porifera has confirmed the occurrence of high percentages of both chain demospongic C_{24}–C_{38} (up to 79%) and polyunsaturated acids (Bergquist et al., 1984; Rezanka, 1989; Dembitsky and Srebnik, 2002). The study of the distribution of demospongic acids



Scheme 8. Synthesis of two isomers of 22-methyl-5,9-28:2 acid, **49**a,b.

in freshwater sponges also has confirmed the presence of high concentration of trienoic acids: *Heterorotula multidentata*, 5,9,19–26:3 (62% in separated fraction) (Sata et al., 2002); *Baicalospongia bacilifera*, 5,9,19–26:3 (26%) (Imbs and Latyshev, 1998); *Swartschewska papyracea*, 26:3 (28%) (Latyshev et al., 1992). Unusually high levels of eicosatetraenoic (10%), eicosapentaenoic (11.6%), and docosahexaenoic (11.8%) acids have been found in freshwater sponges from Middle East (Dembitsky and Rezanka, 1996). Some novel and unusual trienoic acids have also been isolated **50**–**56** (Table 3). GC–MS separation VLCFA of total lipid extract show in Figs. 3 and 4.

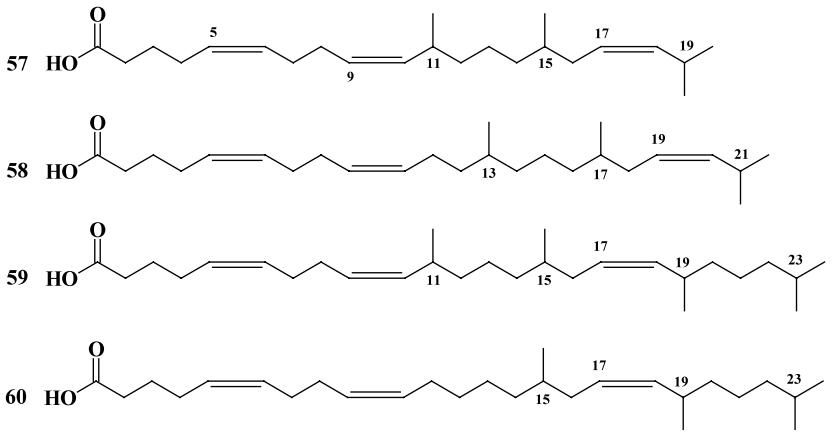
A novel *trans*-olefinic VLCFA **52** was found in sponges from Baikal Lake with abundance up to 10% of the total sponge fatty acids (Vysotskii et al., 1990). Compound **52b** was obtained by iodolactone formation of pyrrolidine derivatives to determine the trans-double bond position (Scheme

Table 3
New, unusual and rare trienoic fatty acids isolated from freshwater sponges

Fatty acid	Sponge	Reference
50. 5,9,12-Octadecatrienoic ($\Delta^{5,9,12}$-18:3)	*Baicalospongia bacilifera*	Imbs and Latyshev, 1998
51. 5,8,11-Eicosatrienoic ($\Delta^{5,8,11}$-20:3)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Cortispongilla barroisi	Rezanka and Dembitsky, 2002
	Ephydatia syriaca	Rezanka and Dembitsky, 2002
	Lubomirskia baicalensis	Dembitsky et al., 1994
	Nudospongilla sp.	Rezanka and Dembitsky, 2002
52. 5c,9c,19t-Hexacosatrienoic ($\Delta^{5,9,19}$-26:3)	*Baikalospongia bacillifera*	Vysotskii et al., 1990
	Lubomirskia baicalensis	Vysotskii et al., 1990
	Swartschewskia papyracea	Vysotskii et al., 1990
53. 15,18,21-Tetracosatrienoic ($\Delta^{15,18,21}$-24:3)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Lubomirskia baicalensis	Dembitsky et al., 1994
	Cortispongilla barroisi	Rezanka and Dembitsky, 2002
	Ephydatia syriaca	Rezanka and Dembitsky, 2002
	Nudospongilla sp.	Rezanka and Dembitsky, 2002
54. 5,9,20-Heptacosatrienoic ($\Delta^{5,9,20}$-27:3)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Cortispongilla barroisi	Rezanka and Dembitsky, 2002
	Ephydatia syriaca	Rezanka and Dembitsky, 2002
	Lubomirskia baicalensis	Dembitsky et al., 1994
	Nudospongilla sp.	Rezanka and Dembitsky, 2002
55. 5,9,23-Nonacosatrienoic ($\Delta^{5,9,23}$-29:3)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Cortispongilla barroisi	Rezanka and Dembitsky, 2002
	Ephydatia syriaca	Rezanka and Dembitsky, 2002
	Lubomirskia baicalensis	Dembitsky et al., 1994
	Nudospongilla sp.	Rezanka and Dembitsky, 2002
56. 5,9,23-Tricosatrienoic ($\Delta^{5,9,23}$-30:3)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Cortispongilla barroisi	Rezanka and Dembitsky, 2002
	Ephydatia syriaca	Rezanka and Dembitsky, 2002
	Lubomirskia baicalensis	Dembitsky et al., 1994
	Nudospongilla sp.	Rezanka and Dembitsky, 2002

10). **52b** had a *trans*-olefinic absorption band on IR spectrum at 978 cm^{-1}, that indicated the presence of a *trans* double bond in the Δ^{19} position of trienoic 26:3 acid isolated from *B. bacillifera*, *L. baicalensis* and *S. papyracea* Scheme 11.

Four new isoprenoid fatty acids **57**–**60** were identified from three freshwater sponge species *Baicalospongia intermedia*, *B. bacilifera* and *Lubomirskia baikalensis* and their structures were elucidated by means ^{1}H, ^{13}C NMR, HRMS, reversed-phase high resolution liquid chromatography (HPLC) (Rezanka and Dembitsky, 1993). The mass spectrum of a representative compound is shown in Fig. 2.



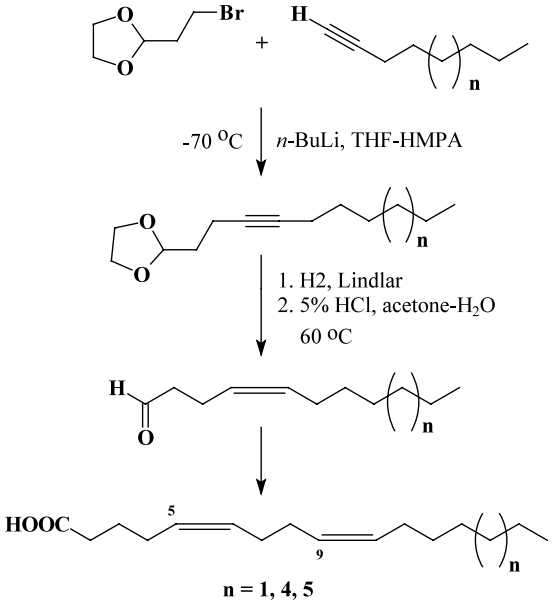
The sponge's *Lumorskia baicalensis* gammarid parasite *Brantia (Spinacantus) parasitica* t living in the sponge body also contain all the demospongic acids, mainly in phospho- and GL fractions and does not differ substantially from the host sponge (Dembitsky et al., 1994). Fatty acid diversity present in the parasite and in host is also very similar. Among trienoic demospongic acids in sponge's parasite mainly presence of 5,9,17–26:3 (in PL, 3.98%, in GL, 5.09%), 5,9,19–26:3 (1.67 and 1.30%, respectively), 5,9,19–28:3 (1.18 and 1.51%), 5,9,21–28:3 (1.42 and 1.82%).

2.4.1. Biosynthesis of trienoic fatty acids

Biosynthesis of demospongic acids such as (5*Z*,9*Z*)-5,9-hexacosadienoic and (5*Z*,9*Z*,19*Z*)-5,9,19-hexacosatrienoic acids by freshwater sponge *Ephydatia fluviatilis* has been reported (Hahn et al., 1989). In the freshwater sponge *Ephydatia fluviatilis*, all the precursors (16:0, Δ^9-16:1, 26:0, Δ^5-26:1, Δ^9-26:1 and $\Delta^{5,9}$-26:2) were converted to the $\Delta^{5,9,19}$-26:3 acid, clearly showing the existence of Δ^{19}-desaturase activity on the $\Delta^{5,9}$-26:2 fatty acid. By contrast, the trienoic acid (26:3) is biosynthesized by marine sponge *Microciona prolifera* from exogenous 9–16:1 acid. This marine sponge can occur at either the Δ^5 or Δ^9 stages with a preference ratio of 1/5 (Δ^5/Δ^9 desaturase activity). Results of the biosynthesis both freshwater and marine sponge species are summarized in Scheme 12.

Fatty acid desaturases are highly stereo and regioselectives, and oxygen depending within transformation stearyl-CoA to its oleyl counterpart (Behrouzian and Buist, 2002). Research on membrane-bound desaturases showed that they focused on pinpointing the site of initial oxidative attack involved in double bond formation. Δ^5 and Δ^9 desaturases which are involved in desaturation reactions in sponges, also have been found in some organisms. Thus, Δ^5 desaturase *Bacillus subtilis*, and Δ^9 desaturase in green algae *Chlorella vulgaris*, yeast *Saccharomyces cerevisiae*, and in cyanobacteria *Spirulina platensis*, and mechanism of fatty acid desaturation has recently been in detail reported (Behrouzian and Buist, 2002).

Examination of fractionated sponge *Halichondria moorei* (Lawson et al., 1986) tissue shows that demospongic acids occur in high proportions in cell membranes, *Reniera* sp. and *Pseudaxinyssa* sp. (Lawson et al., 1988), and membranes display classical trilaminar structure in four marine and in freshwater sponge *Ephydatia mülleri* (Lethias et



Scheme 9. Synthesis of $\Delta^{5,9}$ dienoic fatty acids (Carballeira et al., 1998).

al., 1983). These results challenge the hypothesis of a unique structure for sponge cell membranes. Most demospongic acids were constituents of the PL, which are typical membrane lipids, and in particular the amino-PL, e.g. phosphatidylserine (PS) and phosphatidylethanolamine (PE) (Lawson et al., 1988).

2.5. Polyenoic fatty acids

New, unusual and rare tetra-, penta- and hexanoic compounds, the least abundant fatty acids, have been found in some freshwater sponges from Siberia (Lake Baikal, Russia) and Middle East (Lake Hula, Sea of Galilee, spring of these fatty acids **61**–**70** are shown in Table 4.

Very long-chain and multibranched polyunsaturated fatty acids **71**–**74** of the three freshwater sponges, *Ephydatia syriaca*, *Nudospongilla* sp., and *Cortispongilla barroisi* have been found (Rezanka and Dembitsky, 2002). The toxicity of

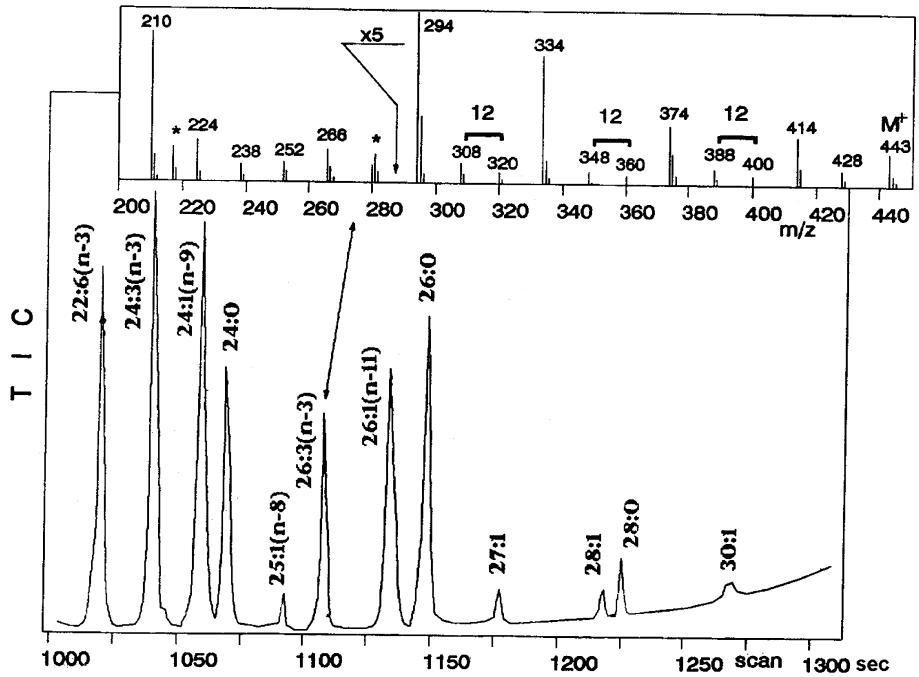


Fig. 3. Partial GC–MS chromatogram separation VLCFA of the total lipid extract of sponge *Euspongilla lacustris* without preliminary development on silica gel with $AgNO_3$ and urea purification.

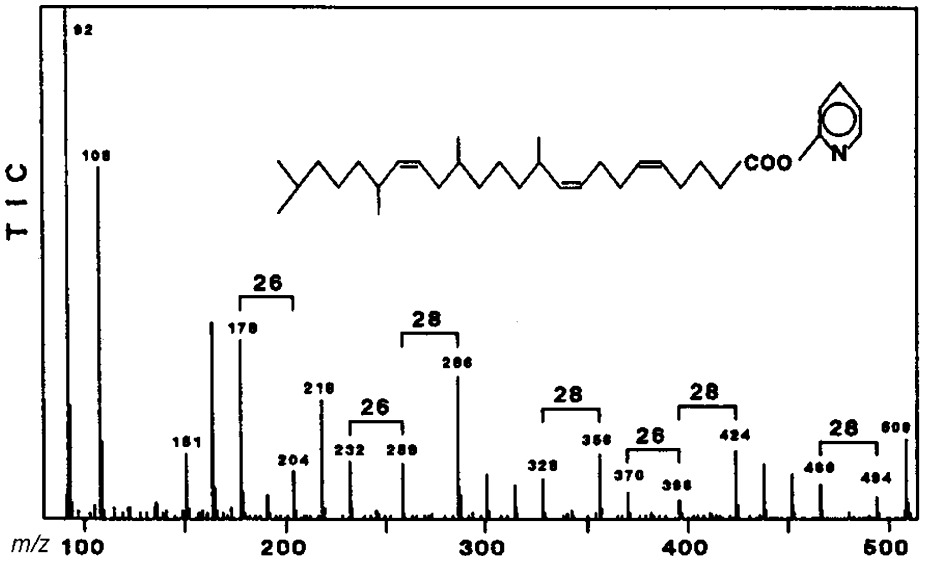
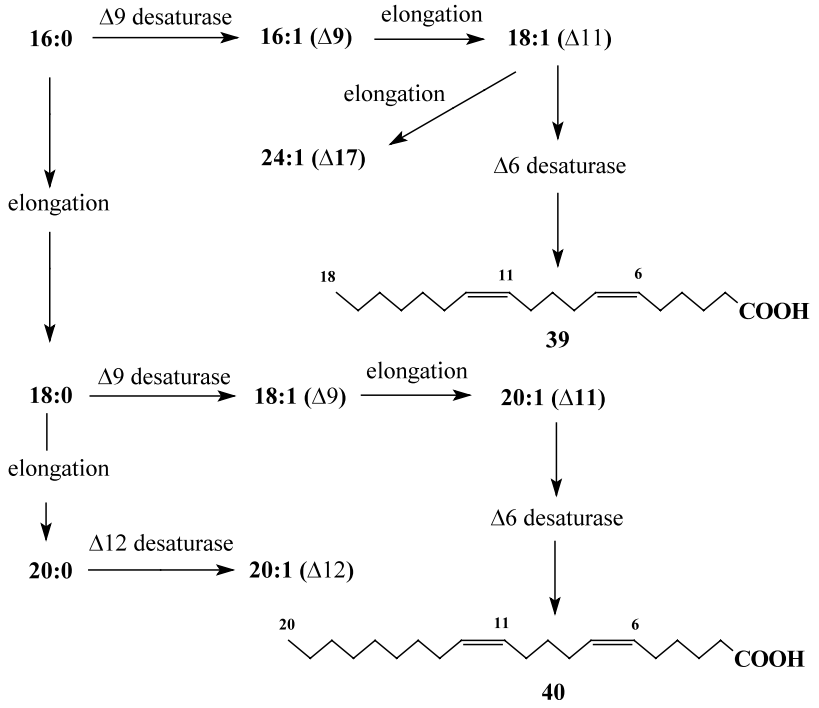


Fig. 4. Structure and mass spectrum of picolinate of 11,15,19,23-tetramethyl-5,9,17-tetracosatrienoic acid, **59**.

compounds **71**–**74** was tested in the *Artemia salina* shrimp bioassay. The methyl esters (**71**–**74**) showed bioactivity against *Artemia salina*, and these compounds also showed high antimicrobial activities against *Staphylococcus aureus* and *Bacilius subtius*.



Scheme 10. Possible biosynthetic pathways for the 6,11–18:2 **39** and 6,11–20:2 **40** acids isolated from *Eurypongia rosea*.

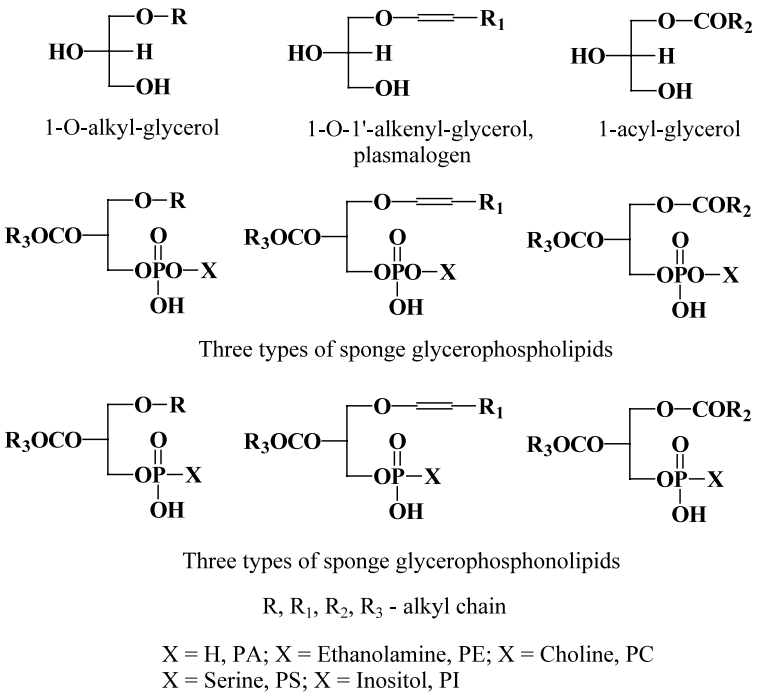


Fig. 5. Main groups of ether and diacyl glycerophospho- and glycerophosphonolipids isolated from freshwater sponges.

Oxidative splitting and chromatography of appropriate methyl esters on a chiral capillary column determined the absolute configuration of **71**–**74**. Non-chiral and chiral compounds were isolated from the reaction mixture, i.e., (2*S*)-2-methylsuccinic acids dimethyl ester (from **74**), (2*S*)-2-succinic acid dimethyl ester and (2*S*)-2-methylbutyric acid methyl ester (from **72**), and (2*R*)-2-methylsuccinic acid dimethyl ester and (4*R*)-4-methylhexanoic acid methyl ester (from **71**) (Rezanka and Dembitsky, 2002). Acid **74** was determined to be a novel hexane-type compound.

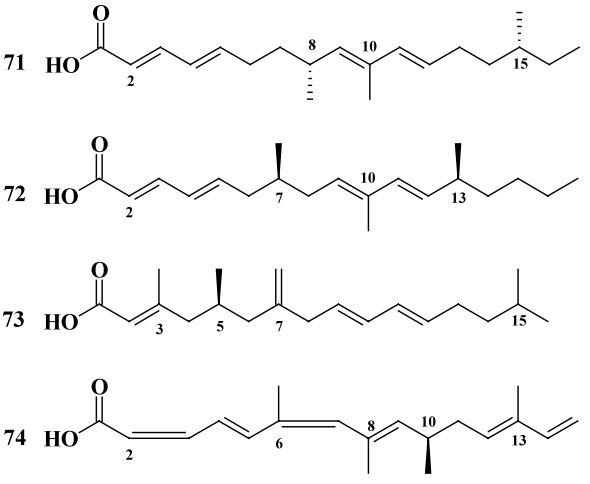


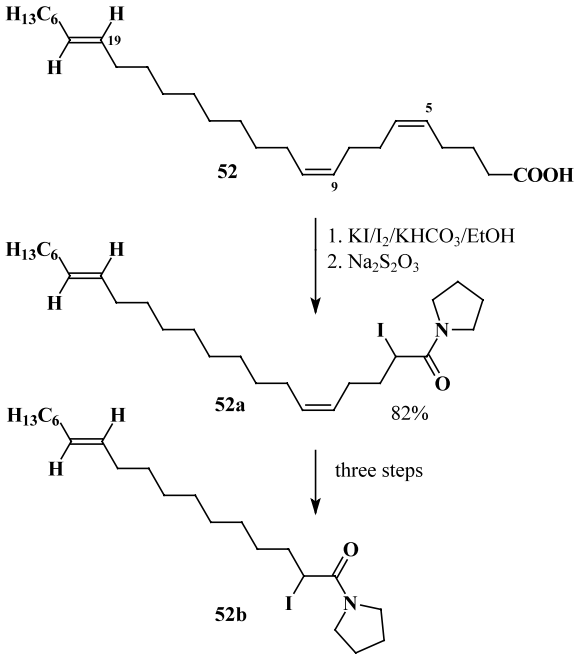
Table 4
New, unusual and rare polyenoic fatty acids isolated from freshwater sponges

Fatty acid	Sponge	Reference
61. 4,7,10,13-Hexadecatetraenoic ($\Delta^{4,7,10,13}$-16:4)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Lubomirskia baicalensis	Dembitsky et al., 1994
62. 6,9,12,15-Hexadecatetraenoic ($\Delta^{6,9,12,15}$-16:4)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Lubomirskia baicalensis	Dembitsky et al., 1994
63. 5,9,12,15-Octadecatetraenoic ($\Delta^{5,9,12,15}$-18:4)	*Baicalospongia bacilifera*	Imbs and Latyshev, 1998
64. 3,6,9,12,15-Octadecapentaenoic ($\Delta^{3,6,9,12,15}$-18:5)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
65. 6,9,12,15,18-Heneicosapentaenoic ($\Delta^{6,9,12,15,18}$-21:5)	*Lubomirskia baicalensis*	Dembitsky et al., 1994
	Baikalospongia bacillifera	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Lubomirskia baicalensis	Dembitsky et al., 1994
66. 9,12,15,18-Tetracosatetraenoic ($\Delta^{9,12,15,18}$-24:4)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Lubomirskia baicalensis	Dembitsky et al., 1994
67. 6,9,12,15,18-Tetracosapentaenoic ($\Delta^{6,9,12,15,18}$-24:5)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Lubomirskia baicalensis	Dembitsky et al., 1994
68. 6,9,12,15,18,21-Tetracosahexaenoic ($\Delta^{6,9,12,15,18,21}$-24:6)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Lubomirskia baicalensis	Dembitsky et al., 1994
69. 15,18,21,24-Tricontatetraenoic ($\Delta^{15,18,21,24}$-30:4)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Lubomirskia baicalensis	Dembitsky et al., 1994
70. 15,18,21,24,27-Tricontapentaenoic ($\Delta^{15,18,21,24,27}$-30:5)	*Baikalospongia bacillifera*	Dembitsky et al., 1993
	Baikalospongia intermedia	Dembitsky et al., 1993
	Lubomirskia baicalensis	Dembitsky et al., 1994

3. Sponge lipids

Lipids from sponges as well as in both freshwater and marine invertebrates consist mainly of saponifiable compounds, such as glycerides, along with some unsaponifiable lipids, some of which are ether lipids (alkoxylipids) (Mangold, 1979; Dembitsky et al., 1989; Dembitsky, 1996). Typical ether lipids are monoalkyl ethers of glycerol, also called alkyl or alkenyl glyceryl ethers (Mangold and Paltauf, 1983). Many of derivatives of natural and synthetic ether glycerophospholipids show high biological activity (Braguet et al., 1988), and may become one of most important and useful lipids in the pharmaceutical industry (Urata and Takaishi, 1996). Three types of monoether lipids have been recognized depending on whether they are related to neutral (mono-, di- and/or triacylglycerols) or glycerophospholipids and on whether the ether group contains or does not contain a Δ^1 *trans* double bond (Fig. 5). The ether group is generally in the *sn*-1 position though some diethers (*sn*-1 and *sn*-2) are also isolated from sponges.

Rare phosphonolipids from sponge species have also been isolated from both animal and plant Kingdoms (Mukhamedova and Glushenkova, 2000). The structures of three types of phospho-



Scheme 11. Confirmation and determination of *trans* double bond in Δ^{19} position of 5c,9c,19t-26:3 acid **52** isolated in freshwater sponges from Lake Baikal (Vysotskii et al., 1990). Adapted by authors.

nolipids are shown in Fig. 5. The natural distribution, chemistry, analysis and metabolism of phosphonolipids, as well as their possible biochemical function have been reviewed (Hori and Nozawa, 1982). Phosphonolipids, like PL, are developed on TLC by Dittmer's reagent, which turns PL brown and phosphonolipids blue. The IR spectra of phosphonolipids lack an absorption band characteristic of the P–O–C bond and contain bands at 730 and 1180 cm^{-1}. The difference between phospho- and phosphonolipids is more evident in nuclear magnetic resonance spectroscopy (NMR) spectra (Fig. 6A and B). Benezra et al. (1970) used ^{1}H-NMR shown the difference in absorption of P–C and P–O–C bonds in *N*-methyl-2-aminoethylphosphonate and L-α-glyceryl-2-aminoethyl phosphate. The P–CH_2 protons usually appear at stronger field than those of the P–O–CH_2 group. NMR spectra of L-α-glyceryl-*N*-methyl-2-aminoethylphosphonate were compared with those of synthetic L-α-glyceryl-*N*-methyl-2-aminoethylphosphate. It was found that the methylene H atoms of the phosphate and phosphonate appear in different regions. The P–CH_2 protons give a multiplet at 2.72 ppm; the P–O–CH_2 protons, a singlet at 0.89 ppm (water was used as an internal standard). The similar results for determination of P–C bond by ^{1}H NMR were also obtained (Adosraku et al., 1996; Early and Glonek, 1996).

The phosphonolipid content in biological samples is calculated in various ways. The P–C bond is very strong and stable to prolonged treatment with HCl (6M, 110 °C, 48 h) and HBr (conc., 125 °C, 10 h). This property of these compounds is also used to determine phosphonates. A sample is subjected to strong acid hydrolysis. The content of phospholipid phosphorus in the hydrolysate is determined. The residue is combusted by HOCl. The phosphonic phosphorus is determined by spectrophotometrical methods. The phosphonolipid content is also determined from the total phosphorus content relative to the phospholipid phosphorus and from the content of in the polar lipids (Horiguchi, 1972).

Composition information of lipids, GL, and PLs of freshwater sponges is rather sparse and involves only a few representatives of this animal group. Published results are summarized in Table 5. The main phospholipid classes are: PC **84**, PE **75** and PS **83**. Rare lipids such as 2-aminoethylphosphonates **82**, *N*-methyl- **78** and *N,N*,-dimethyl-PE **79** have been isolated from *Eunapius fragilis* (Early et al., 1996) and *Euspongilla lacustris* (as well as marine sponges (Dembitsky et al., 1989) contain alkyl- and alkenyl-forms of the two main PL—PC **84** and PE **75**. Also, a biological active lipid—1-alkyl-2-acetyl-PC **87**, known as platelet activating factor (PAF) (Mangold and Paltauf, 1983) was found in *Eunapius fragilis* (Early et al., 1996). Rare lipids of 1,2-di-O-alkyl-glycerophospholipids and 1,2-di-O-alkyl-glycerophosphonolipids have been isolated from sponge *Ectyodoryx kovdaicum* (Dembitsky, 1988b). Using ^{31}P-NMR a number of unusual lipids and phosphorus-containing metabolites have been found in *Eunapius fragilis* from Lake Michigan (Early and Glonek, 1996) such as 2-*N,N,N*-trimethylaminoethylphosphonate, 2-aminoethylphosphonate **82**, glyceryl-phosphoryl(glycerylphosphoryl)glycerol, serine

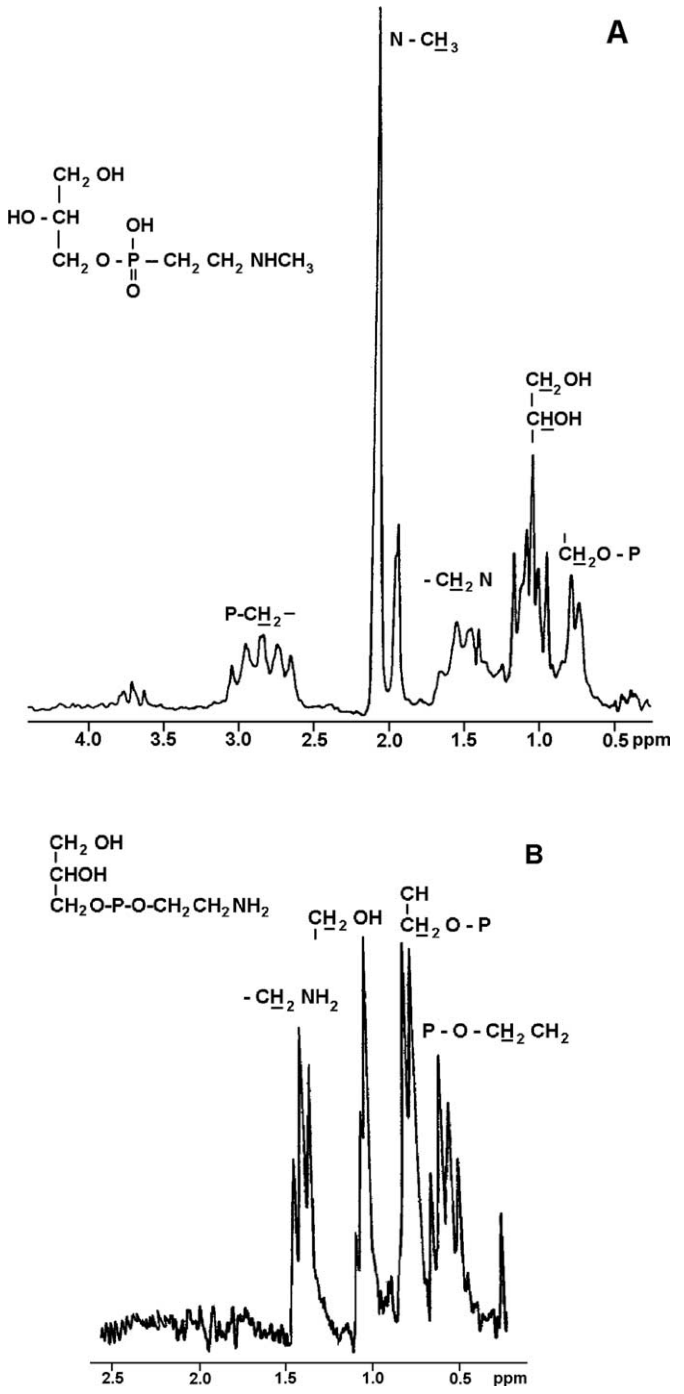
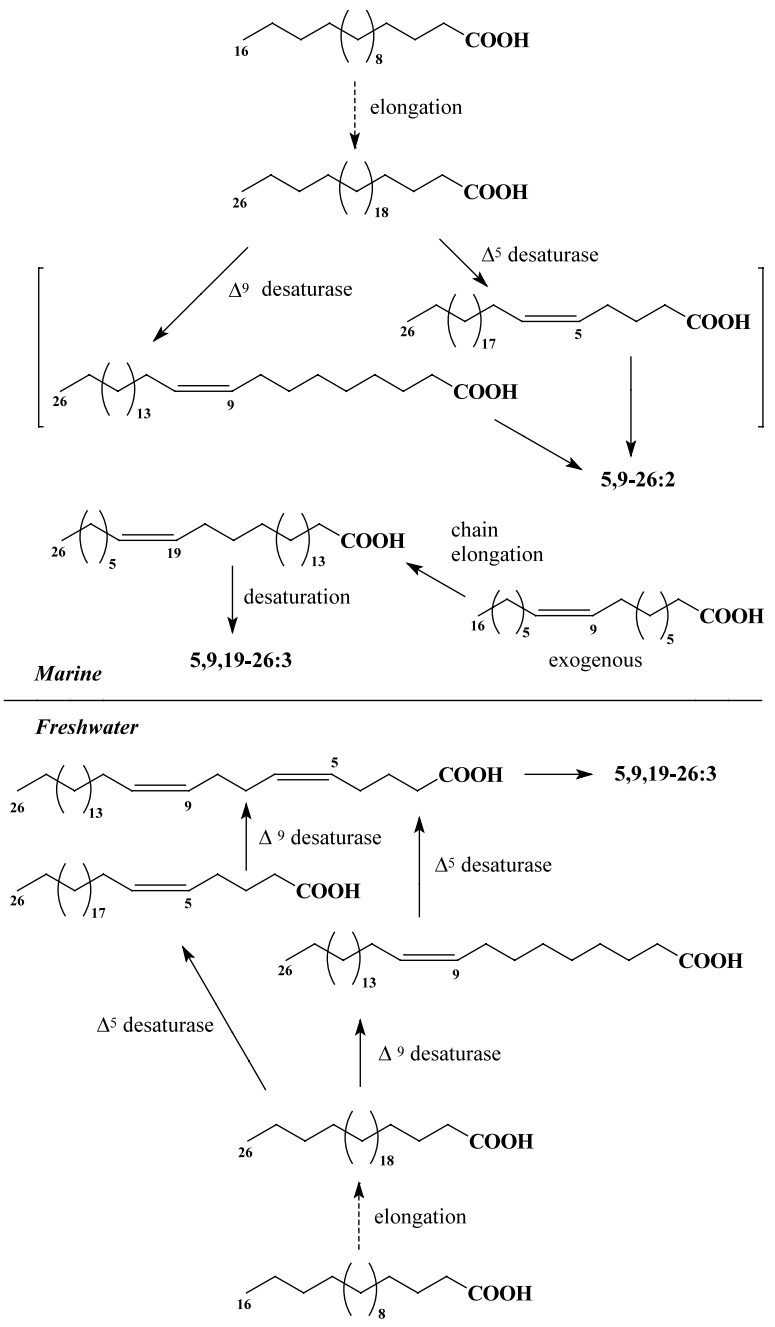


Fig. 6. ^{1}H-NMR spectra of L-α-glyceryl-(*N*-methyl-2-aminoethyl) phosphonate (**A**) and L-α-glyceryl-2-aminoethyl phosphate (**B**). Both compounds dissolved in $^{2}H_2O$.Compound A, 2.72 ppm (2H, P–CH_2–, *m*), 2.09 ppm (3H, N–CH_3, *s*), 1.54 ppm (2H, CH_2–N, *m*). Compound B, 1.44 ppm (2H, CH_2N, *t*), 1.10 ppm (CH_2(OH), *d*), 0.81 ppm (3H, CH(OH)–CH_2–OP), 0.61 ppm (2H, P–O–CH_2, *d*). Chemical shift in δ ppm units relative to H_2O can be conveniently converted to δ ppm units relative to TMS by using the relation: $\delta_{TMS} = 5.20 - \delta_{H2O}$ (Benezra et al., 1970). Adapted by authors.



Scheme 12. Proposed biosynthetic pathways of 5,9,19-hexacosatrienoic acid ($\Delta^{5,9,19}$-26:3) by freshwater sponge *Ephydatia fluviatilis* and marine sponge *Microciona prolifera*. Adapted by authors.

ethanolamine phosphodiester, and also phosphorylated glycones and amino acids.

Comparative investigation of the lipids of the native (**IVa**, Table 5) and washed (**IVb**) sponge

Table 5
Lipid compositions of some freshwater sponges

Sponge species	I	II	III	IVa	IVb	Va	Vb	Vc	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Lipid classes																	
Total lipids[a]	57.6	3.7	13.6	12.5	1.6				26.8	36.1	52.4						
Neutral lipids[b]	38.7	59.6	54.5	64.9	59.3				38.1	25.9	40.2						
GL[b]	6.6	12.0	18.9	11.8	18.7				9.7	11.2	14.6						
PL[b]	54.7	28.4	26.6	23.3	22.0				52.2	62.9	45.2						
75. PE[c]				23.5	42.6												
(Sum of diacyl- and alkyl-acyl forms)	14.8	5.8	8.9			14.9	15.7	16.2									
76. PE plasmalogen	17.3	13.4	8.7	8.2	18.8	21.1	20.6	19.3	19.2	11.6	12.6	25.6	19.9	23.9	21.4	28.2	26.1
77. Alkylacylphosphatidylethanolamine				7.7	17.8				6.3	2.8	4.6	7.1	4.7	4.8	5.1	6.6	3.2
78. Phosphatidyl-*N*-monomethylethanolamine	3.4								2.1								
79. Phosphatidyl-*N*,*N*-dimethylethanolamine	2.2									1.6	2.1						
80. Lysophosphatidylethanolamine (LPE)	3.6	4.8	10.0	13.3	7.5	0.6	0.7	0.5	0.6	2.2	0.5	3.6	4.2	2.0		1.7	1.2
81. LPE plasmalogen						1.1	1.0	1.2									
82. (2-aminoethyl)-phosphonate						0.6	0.7	0.8		1.2							
83. PS	10.6	9.5	10.1	5.9	4.8	11.6	12.4	11.8	10.2	15.2	9.5	10.7	17.1	14.1	16.3	13.0	19.0
Ninhydrine positive phospholipid	2.1	13.7	9.5														
84. Phosphatidylcholine (PC)				25.1	27.1				32.6	35.9	34.8						
(Sum diacyl- and alkyl-acyl- forms)	31.5	25.0	21.2			10.7	8.9	9.2				37.3	39.9	37.9	38.4	44.3	43.2
85. PC plasmalogen	4.6	6.8	7.5	1.6	3.7				3.5	4.2	6.1	4.2	1.9	3.2	2.2	3.0	1.9
86. Lysophosphatidylcholine (LPC)	2.8	11.1	11.9						0.5	2.1	4.2	1.8	5.3	4.1		0.8	
87. Alkylacylphosphatidylcholine				5.2	9.9	20.8	22.7	19.2	15.3	17.2	19.1						
88. Lysoalkylphosphatidylcholine						3.4	2.8	5.2									
89. PI	2.5	5.2	5.7	7.1	5.0	3.3	3.1	2.7	3.3	2.2	1.6	2.9	3.0	2.4	2.6	2.0	3.5
90. PA	0.9	4.7	6.5	5.3	2.2	0.1	0.1	0.1	0.9	0.4	0.3	0.9		0.6	0.2		
91. Lysophosphatidic acid						1.9	1.7	1.7									
92. Glycerol plasmalogen						0.9	1.1	1.1									
93. Dihydrosphingomyelin						2.0	1.6	1.9									
94. Phosphatidylglycerol						0.7	0.2	1.2	2.1	1.2	3.2						
95. Diphosphatidylglycerol	2.6					4.1	4.9	4.6	3.4	2.2	1.4	1.2	0.9	1.9	1.4	0.4	1.0
Unidentified phospholipid1	0.8														1.2		
Unidentified phospholipid2	0.3											2.7	3.1				
Unidentified phospholipid3						0.6	0.7	1.2									

(I) *Euspongilla lacustris*, Volga River Estuary, Russia, Dembitsky et al., 1991; **(II)** *Baicalospongia bacilifera*, Lake Baikal, Russia, Dembitsky et al., 1993; **(III)** *Baikalospongia intermedia*, Lake Baikal, Russia, Dembitsky et al., 1993; **(IVa)** *Lubomirskia baicalensis,* native, Lake Baikal, Russia, Dembitsky et al., 1994; **(IVb)** *Lubomirskia baicalensis,* washed, Lake Baikal, Russia, Dembitsky et al., 1994; **(Va)** *Eunapius fragilis*, Hammon Harber, Laggon, Southwestern Lake Michigan, USA, Early et al., 1996; **(Vb)** *Eunapius fragilis*, Open waters, Lake Michigan, USA, Early et al., 1996; **(Vc)***Eunapius fragilis*, Calumet River, USA, Early et al., 1996; **(VI)** *Ephydatia syriaca*, Spring of the Jordan River, Banias, Middle East, Dembitsky, 1996; **(VII)** *Nudospongilla* sp., Lake Hula, Middle East, Dembitsky, 1996; **(VIII)** *Cortispongilla barroisi*, Sea of Galilee (Lake Tiberias–Lake Kinneret), Middle East, Dembitsky, 1996; **(IX)** *Swartschewskia papyracea*, Lake Baikal, Russia, Dembitsky, 1982; **(X)** *Lubomirskia abietina*, Lake Baikal, Russia, Dembitsky, 1982; **(XI)** *Lubomirskia baicalensis*, Lake Baikal, Russia, Dembitsky, 1982; **(XII)** *Lubomirskia fusifera*, Lake Baikal, Russia, Dembitsky, 1982; **(XIII)** *Baicalospongia bacilifera*, Lake Baikal, Russia, Dembitsky, 1982; **(XIV)** *Baicalospongia intermedia*, Lake Baikal, Russia, Dembitsky, 1982.

[a] Total lipids, mg/g dry wt.

[b] Percentage of total lipids.

[c] Percentage of lipid phosphorus.

Lubomirskia baicalensis has been described (Dembitsky et al., 1994). The results of lipid analysis showed that the total lipid content in the unwashed sample was 12.5 mg/g dry wt, while that from the washed one was 1.6 mg/g dry wt (Table 5); thus, microalgae contained 87% total lipids from the native sponge. Also, the varying amounts of alkyl, alkenyl and diacyl forms of the main phospholipid classes, PC **84** and PE **75**, in native and washed sponge were demonstrated (Dembitsky, 1988a; Dembitsky et al., 1989). The alkenyl form (plasmalogen) is dominant in PE **76**, and alkyl form usually is dominant in PC **84** of both marine and freshwater sponge species (Dembitsky et al., 1989; Early et al., 1996). In Fig. 7 is shown the separation of two main ether lysophospholipids, PE and PC. Early et al. (1996) also studied phospholipid compositions in *Eunapius fragilis* collected in different places of Lake Michigan (USA). They found that sponge phospholipid composition changed with habitat, for instance, in lagoon (**Va**, Table 5), Lake (**Vb**), and river (**Vc**).

3.1. Synthesis of phosphonolipids

Synthesis of PL, including diacyl- and ether lipids have been reported in many papers and reviewed in some books (Mangold and Paltauf, 1983; Braguet, 1991; Braguet et al., 1988; Gunstone, 1999) and, therefore, has not been discussed in this review. The chemical synthesis of phosphonolipids with different sets of hydrophobic and hydrophilic constituents is rather simple (Engel, 1988). As a rule, the reaction of glycerides or ceramides with substituted phosphonic acid chlorides is used. Depending on the resulting phosphonolipid, various phosphorylating agents such as *N*-phthalimido-ethylphosphonic acid chloride, bromoethylmetaphosphonate, (2-trimethylammonium-methyl)phosphonate, *N*,*N*-dibenzylaminoethylphosphonic acid, and others are used. Synthesis of 1,2-diacyl- **96a–c** (Baer and Stanacev, 1965a) and 1,2-dialkyl-L-α-glycerol ethers **97a–c** (Baer and Jagannadha Rao, 1967), phosphonic acid analogues of PC is shown in Scheme 13.

The same reaction was used for synthesis of 1,2-diacyl- **98a–c** (Baer and Stanacev, 1964) and 1,2-dialkyl L-α-glyceryl-(2-aminoethyl)phosphonates

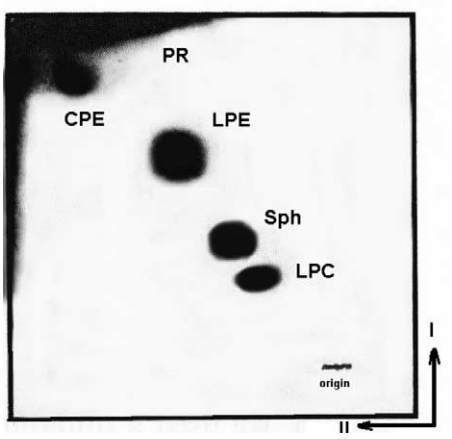
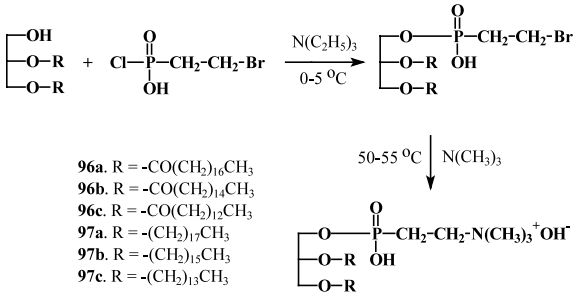


Fig. 7. 2D-TLC chromatogram of separation of ether lysophospholipids from freshwater sponge *Eunapius fragilis*. LPE, 1-alkyl-2-lyso-PE, LPC, 1-alkyl-2-lyso-PC, cyclic acetal PE (CPE) and Sph, standard PL. Glass plate (10 × 10 cm) pre-coated with KSK Silica gel mixed with 10% of gypsum and developed in the first direction with $CHCl_3$–MeOH-*n*-PrOH-28% ammonia solution (40:30:20:10, v/v). Second direction: $CHCl_3$–Acetone–Formic acid–MeOH-*n*-PrOH (60:30:20:20:10:5, v/v).

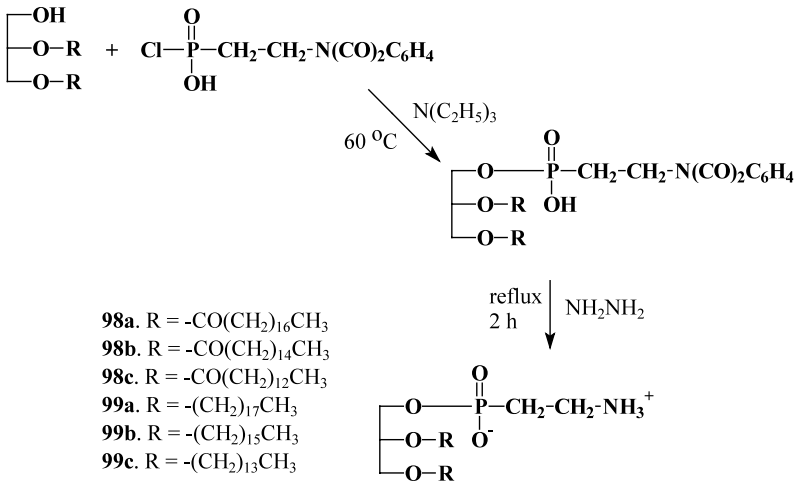


Scheme 13. Synthesis of diacyl and dialkyl phosphonic acid analogues of PC.

99a–c (Baer and Stanacev, 1965b) analogues of PE show in Scheme 14.

L-α-glyceryl-(2-aminoethyl)phosphonate **82** as a polar moiety of amino phosphonolipids has also been synthesized by Baer and Sarma (1967), by the reaction of D-acetone glycerol and (2-phthalimidoethyl)metaphosphonate in boiling benzene (Scheme 15).

Other phosphonolipids, which were detected in marine invertebrates, including sponges (Moschidis, 1985a), termed as ceramide aminoethylphosphonate **100** (Scheme 16) were synthesized (Baer and Sarma, 1969). The *erythro*-*N*-palmitoyl-*D*-

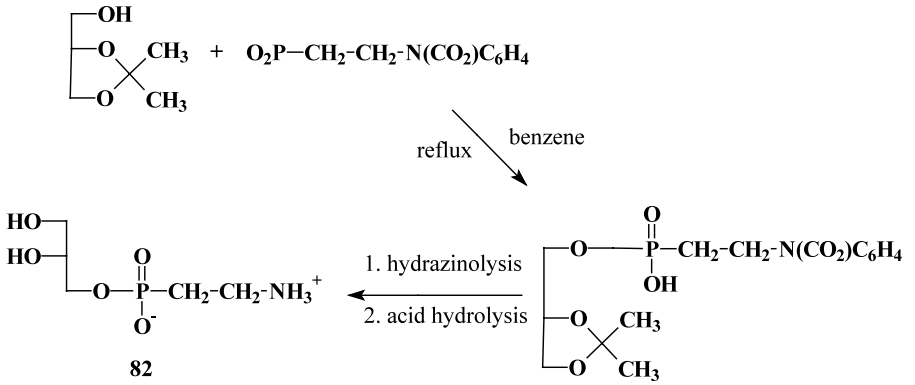


Scheme 14. Synthesis of diacyl and dialkyl phosphonic acid analogues of PE.

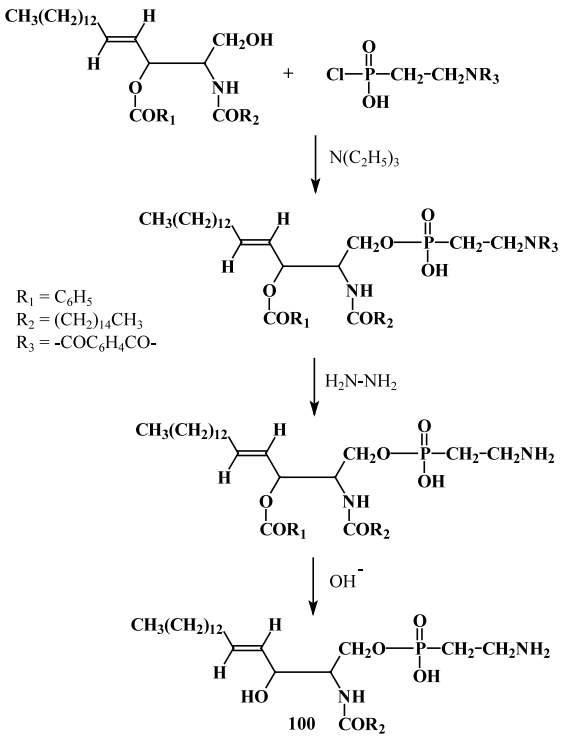
sphingosyl-1-(2-aminoethyl)phosphonate was obtained by phosphonylation of *erythro*-*N*-palmitoyl-3-O-benzoyl-*D*-sphingosyl with (2-phthalimidoethyl)phosphonic acid chloride and triethyl-amine, and consecutive removal of the protective phthaloyl and benzoyl groups, followed hydrazinolysis and mild alkaline hydrolysis, respectively.

Synthesis of other phosphonate analogues PLs has also been reported (Moschidis, 1985a) including, a *myo*-inositol (Dreef et al., 1991), phosphatidylinositol (PI) (Pratt et al., 1992), isosteres of the PAF (Li et al., 1993), PAF (Liu et al., 1993), α-hydroxy-phosphonolipid analogues of PA (Schwartz et al., 1988a), chiral vinylic phosphonolipid analogues of PA and PC (Schwartz et al., 1988b), racemic *N*-substituted diether phosphonolipids (Turcotte et al., 1991a,b), L-α-(*N*-methyl)cephalines (Baer and Pavanaram, 1970), and 1-hydroxy-3-acyloxypropylphosphonic acids and their derivatives (Alekseichuk et al., 1992). Phosphonolipids have a high biological activity and can be used as potential therapeutic agents (Krise and Stella, 1996). Comparative 2D-TLC separation of synthetic phosphonolipids and native PL and phosphonolipids isolated from *Tetrahymena pyriformis* show in Fig. 8.

Phosphonolipids with a C–P bond between the phosphorus part and the polar lipid head are found in nature (Moschidis, 1985a). However, phosphonolipids formed from 1,2-propanediol and phosphonic acid, i.e., 1,2-diacyloxypropyl-3-



Scheme 15. Synthesis of L-α-glyceryl-(2-aminoethyl)phosphonate.



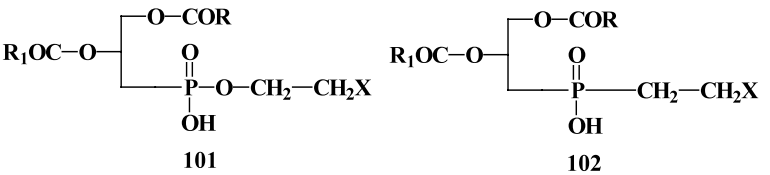
Scheme 16. Synthesis of *erythro*-*N*-palmitoyl-*D*-sphingosyl-1-(2-aminoethyl)phosphonate.

phosphonates **101** and phosphonic acid analogs with two P–C bonds, i.e., 1,2-diacyloxypropylphosphinates **102** can theoretically exist. Phosphonolipids of type **101** and **102** have not yet been found in nature. However, they have been synthesized (Disselnkoetter et al., 1985; Moschidis, 1985b) because they are interesting as inhibitors of various phospholipases and as analogues of thrombocyte activating factors (Cambillau et al., 1996).

3.2. Biosynthesis of phosphonolipids

The biosynthesis of phosphonolipids has been studied using cell free homogenates of ciliated protozoan *Tetrahymena pyriformis* (Horiguchi, 1972a). This organism contains all phosphononate analogues of main phospholipid classes PC, PE, PS, PI (Adosraku et al., 1996). Incorporation of radioactivity from [^{32}P]orthophosphate and from [3-^{14}C]-phosphoenolpyruvate into 2-aminoethylphosphonic acid in free cell preparations from *Tetrahymena pyriformis* was demonstrated. These experiments established the pathway to 2-aminoethylphosphonic acid through decarboxylation and amination (as a bypass). But thus it appears that the major biosynthetic pathway to 2-aminoethylphosphonic acid involves amination by way of phosphonoacetaldehyde (Scheme 17).

According to other experiments with *Tetrahymena*, which used [2-^{12}C]ethanolamine and [3-^{14}C]serine, the labeling pattern suggests that 2-aminoethylphos-phonate phosphorus may originate from phosphotidase or its derivatives, and that the C–P bond may be formed by reaction of the carbon precursor with lipid-bound phosphorus (Liang and Rozenberg, 1968). These results are compatible with the assumption that the precursor of 2-aminoethylphosphonate is a glycolytic intermediate, and that the phosphoenol-pyruvate is the most likely precursor. Phosphatidic acid (PA) and phosphoenol-pyruvate may react according to Scheme 18. Amination of the product lead to PS **103**, and decarboxylation of PS **103** by PS



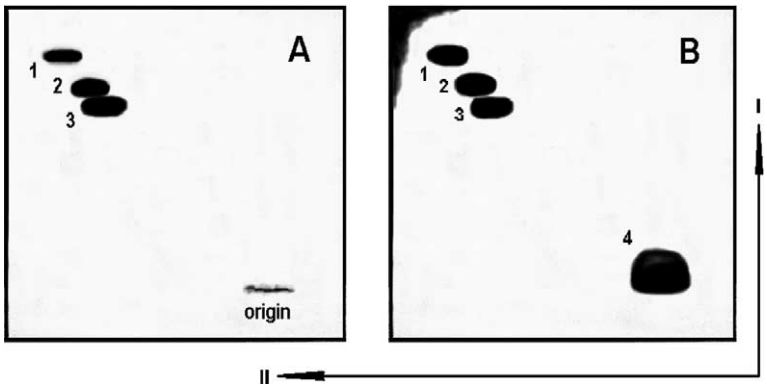


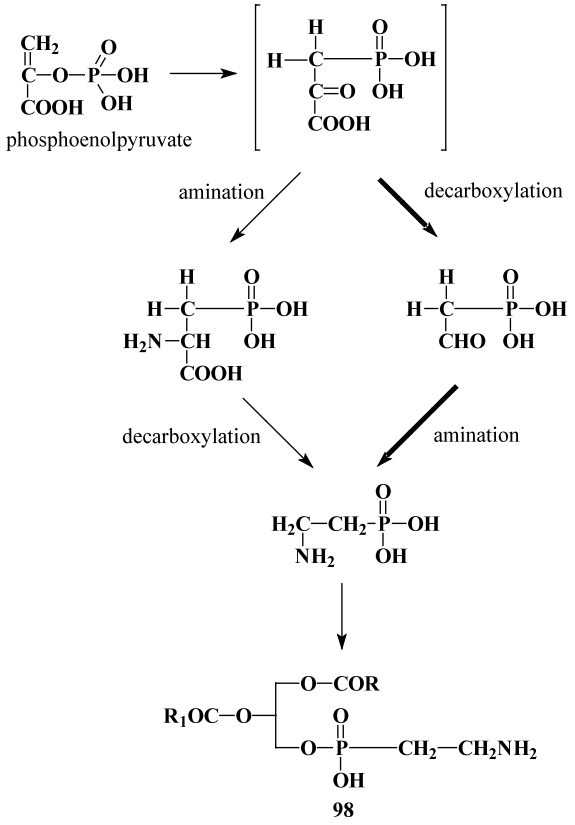
Fig. 8. 2D-TLC separation of various synthetic and origin phosphonolipids. Glass plate (10 × 10 cm^2) with Silica gel G and developed in the first and second directions with mixture of MeOH–H_2O (65:35, v/v). **A**, separation of synthetic phosphonic analogues: 1, Sph; 2, PE, and 3, PC. **B**, separation of phospho- and phosphonolipids isolated from *Tetrahymena pyriformis*: 1, phosphonic analogue of Sph; 2, phosphonic analogue of PE; 3, phosphonic analogue of PC; 4, mixture of origin PL of *T.pyriformis*, which including of PE, PC, PS, PI, and LPC.

decarboxylase (Voelker, 1997) forms the phosphonate analogue of PE **98**.

4. Sponge steroids

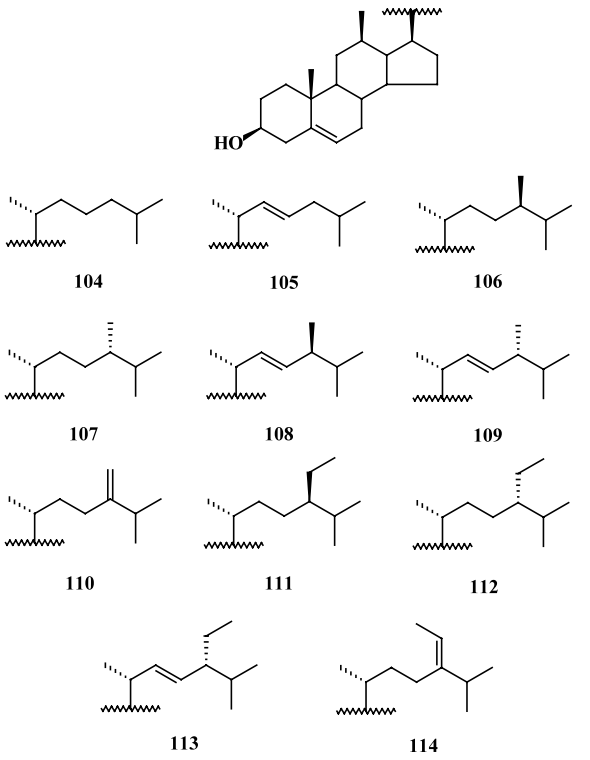
Modern reinvestigation of sponge sterols dates from 1972 and since that time sterols of several novel types have been discovered. Interest in this area centers on the search for novel modifications of conventional sterol structures, both in side chain and nuclear (Walton and Pennock, 1972). However, in this case several curious structures so far encountered have already raised questions about the presumed course of side chain biosynthesis. Efforts to elucidate the biosynthetic pathways continues. Later studies show that marine sponges produce the greatest variety of secondary metabolites, including many of steroids (see reviews: Stonik, 2001; Aiello et al., 1999; D'Auria et al., 1993; Djerassi and Silva, 1991; Kerr and Baker, 1991). However, little work has been reported on natural products of freshwater sponges. Thus, Mazur (1941) more than 60 years ago isolated from the sponge *Spongilla lacustris* 5,6-dihydrostigmasterol. A series of steroids **104**–**114** have been found in the freshwater sponges *Ephydatia fluviatilis* and *Spongilla lacustris* (Mansoni et al., 1988). These sponges have rather similar sterol composition and contain almost exclusively Δ^5-sterols. Cholesterol **104** was the major sterol in both sponge species, 74 and 66%, respectively. *Spongilla lacustris* contained 22-dehydrocholesterol **105**, campesterol **106**, 22,23-dihydrobrassicasterol **107**, 24-epibrassicasterol **108**, brassicasterol **109**, clionasterol **110**, and poriferasterol **113**. The same sterols were isolated from *Ephydatia fluviatilis* and, in addition sitosterol **111** and the stanol of 24-methyl-5α-cholestan-3β-ol (0.6%) were found.

In general, sponges which have moderate to high proportion of lipid in the form of sterol, contain only small amounts of terpenes. The reverse frequently applies, sponges low in sterol can contain considerable quantities of terpenes. These compounds utilize the same precursors and thus appear to be metabolic alternatives. It is possible that sponges high in terpene and low in sterol lack an efficient enzymatic mechanism for converting two farnesyl pyrophosphate units tail

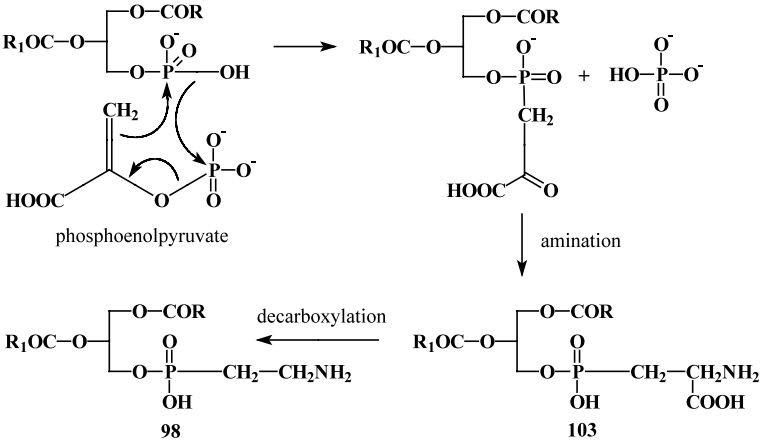


Scheme 17. Proposed pathway of the biosynthesis phosphonate analogue of PE.

to tail to form sterol precursor. Instead terpenes are formed by the continued head to tail fusion of isoprene units.

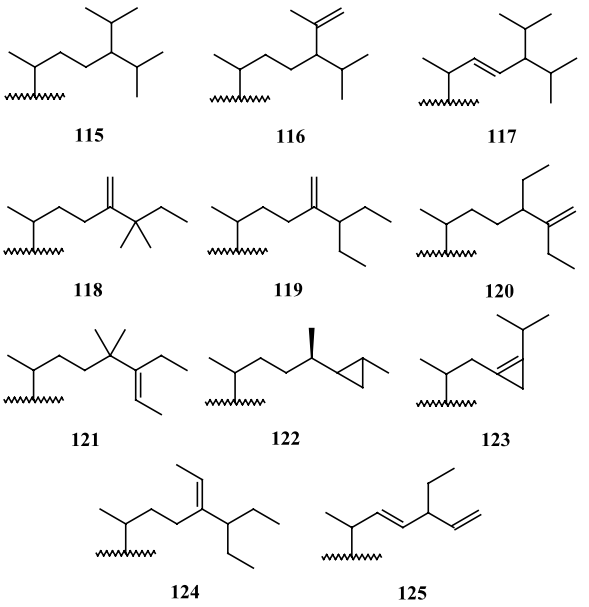


Kerr et al. (1989) studied the biosynthesis of sterols in marine and two freshwater sponges *Ephydata fluviatilis* and *Eunapius fragilis*, and showed that cycloartenol and lanosterol were transformed into the 4,4,14-demethyl sterols in most but not all, in both marine and freshwater sponges. Also it was shown that while some



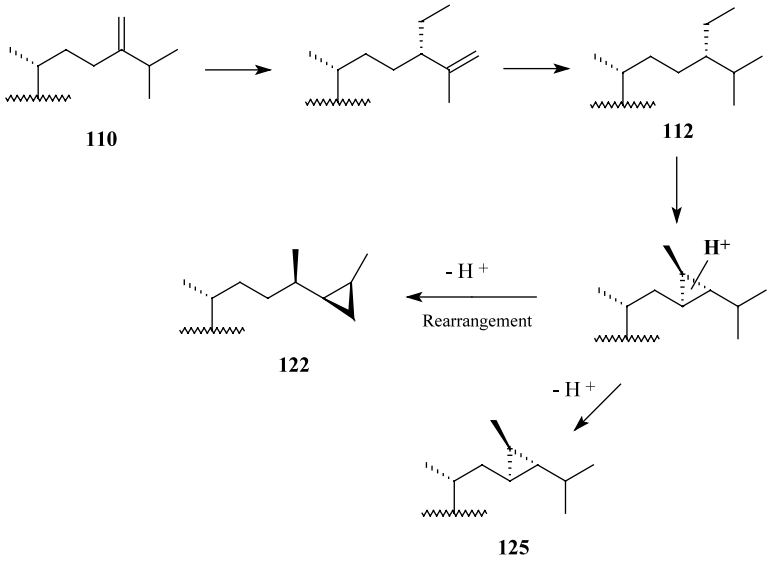
Scheme 18. Proposed pathway of the biosynthesis of phosphonate analogues of amino PL, PS and PE.

sponges obtained sterols from the diet, the majority synthesize them *de novo*. It is known that many sponges contain symbiotic cyanobacteria, however, they are apparently not involved in the observed sterol biosynthesis. Steroids **115**–**125** have been isolated from marine and freshwater sources. A new minor sterol 24-ethyl-26-norcholesta-5,22*E*,25-trien-3β-ol **125**, named baikalosterol, has been isolated from *Baicalospongia bacilifera* (Makarieva et al., 1991). Major steroids of *Baicalospongia bacilifera* include cholesterol (39.2%), (24*S*)-ethylcholest-5,22*E*-dien-3β-ol (23.8%), 24ξ-methylcholesta-5,22*E*-dien-3β-ol (10.8%), and (24*S*)-methylcholest-5-en-3β-ol (10.1%). Baikalosterol **125**, and fourteen known steroids were found in *Lubomirskia baicalensis* (Kolesnikova et al., 1992). Some typical 'sponge' sterols have been determined in freshwater algae. Thus, poriferasterol (24*R*)-24-ethylcholesta-5,22-dien-3β-ol) **113** was found in freshwater green algae *Hydrodictyon reticulatum* (Akihisa et al., 1991), in the unicellular algae *Nematochrysopsis roscoffensis* and *Chrysotila lamellose* (Raederstorff and Rohmer, 1984), in a cell-free homogenate of the algae *Trebouxia* sp. (Wilkomirski and Goad, 1983), and in the culture of the chrysophyte algae *Ochromonas malhamensis* (Knapp et al., 1971). Possibly, that some sterols isolated from freshwater sponges could be algal origin.



4.1. Sterol biosynthesis

Although a multitude of unusual sterols have been isolated from sponges, most sponges, in fact, have the mundane sterols found in animals, plants, and fungi., i.e. cholesterol and sterols bearing one or two extra carbon atoms at C-24. The biosyn-



Scheme 19. Biosynthesis of petrosterol 122 and dihydrocalysterol 125.

thetic study of such sterols in sponges should be of interest for comparative purposes. However, very few studies have concerned themselves with the biosynthesis of these compounds because they were believed to be diet derived and lack the glamour of the multiply methylated or cyclopropyl sterols (Giner, 1993).

De novo sterol biosynthesis in the sponges *Aplysina fistularis* and *Tethya aurantia* has been studied (Silva et al., 1991). It was shown that [3-^{3}H]-squalene was the best precursor for demonstrating *de novo* biosynthesis in a wide range of sponges. Petrosterol **110** and dihydrocalysterol **125** are derived from a common intermediate (Doss et al., 1990; Giner et al., 1990) (Scheme 19) and are the major component of the sterol mixture of the two sponges *Pretrocia ficiformis* (Mattia et al., 1978; Silva and Zollo, 1978) and *Cribrochalina vasculum* (Doss et al., 1990). Petrosterol **102** also was found in the freshwater sponges *Ephydata fluviatilis* and *Eunapius fragilis* (Kerr et al., 1989). More depth analysis of sterol biosynthesis in sponges, algal species, and dinoflagellate was reported (Djerassi and Silva, 1991; Giner, 1993).

5. Concluding remarks

Freshwater as well as marine sponges have been examined. It has been established that the fatty acids, and possibly other lipid compounds display diversity in number and type greater than that seen in any other phylum. The presence of the demospongic C_{24}–C_{32} acids is remarkable. Most organisms terminate their fatty acid elongation at C_{22} or lower. It was shown that freshwater and marine sponge species contain many common lipid compounds: sterols, PL, and fatty acids. But many novel fatty acids and lipids isolated from freshwater sponges have not been found in marine species. It is reasonable to assume that long-chain fatty acids are involved in membrane structure and the same applies to the diverse sterols which are present in sponges. Support for this idea is found in the discovery that unusual fatty acids from sponges are derived from PL which are themselves major membrane components. The presence of long-chain acids and extended side chain sterols must have an effect on sponge membrane characteristics. Little is known of the details of membrane structure and lipid composition of freshwater sponges. This could prove an interesting field for further investigation along biochemical, chemical and physiological lines. For example, recently a new species of sponge of the genus Hererorotula was discovered in the Horomatangi geothermal system of Lake Taupo, New Zealand (De Ronde et al., 2002). The habitat of the Lake Taupo deep sponges is extreme in terms of depth and substrate chemistry. The Taupo sponges live in water with relatively high concentrations of toxic metals such as mercury, arsenic, and native sulfur. Investigation of the lipid compounds of Taupo sponges should lead to the discovery of new fatty acids, sterols and other unusual natural products.

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